Spectroscopic Distinguishability, Forced Degradation Kinetics and LC-MS/MS degradation product characterizations involving 5-Fluorouracil and similar or affiliated compounds in relation to Environmental Concerns

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A Dissertation

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In Partial Fulfillment

Of the Requirements for the Degree

Doctor of Philosophy

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by

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MAY 2015

The undersigned, appointed by the Dean of the Graduate School,
have examined the dissertation entitled
Spectroscopic Distinguishability, Forced Degradation Kinetics and LC-MS/MS

degradation product characterizations involving 5-Fluorouracil and similar or

affiliated compounds in relation to Environmental Concerns

Presented by Eddie LaReece Pittman

A candidate for the degree of Doctor of Philosophy

And hereby certify that in their opinion it is worthy of acceptance.

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Professor Gary Baker

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TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

LIST OF SCHEMES

ACADEMIC ABSTRACT

5-Fluorouracil (5-FU) is a commonly used antiviral and anticancer drug. 5-FU is believed to exist in various tautomeric forms, which are believed to contribute to 5-FU's cytotoxicity. In order to understand the activity of 5-FU in biological and environmental settings, a combined theoretical and experimental approach was used to determine the predominant tautomer in aqueous environments. Spectral characterization of 5-FU will enable development of improved analytical methods for 5-FU. The structures of the 6 most relevant tautomers of 5-FU were optimized using DFT (B3LYP/6-311++G(d, p)) and their respective UV absorption, IR, Raman and NMR spectra calculated. The UV resonance Raman spectrum of 5-FU is also reported. The C=O (1711(cm^{-1})), C=C (1673(cm^{-1})) and C-N (1463(cm^{-1})) and N-H (1510(cm^{-1})) stretching frequencies are strongly enhanced in the deep UV resonance Raman spectrum of 5-FU. Comparison of the theoretical spectra with measured UV absorption, IR, UV Raman and NMR indicate that the 5-FU keto tautomer is the predominant species in aqueous environments.

Four applicable set of environmental conditions were applied towards 5-FU which all produced results that can be used to help further the overall agenda. Thermal/Saline and Photolytic/Alkali both completely degraded 5-FU but Thermal/Saline did it quicker. While the Thermal/Alkali caused some degradation over the course of a week, Thermal/Acidic didn't come close to matching over the same period of time. Synergism was demonstrated and definitely raises even more concerns regarding the realistic adverse potential these sort of compounds

may have once they make their way into the environment; thus, this study's statement was mad

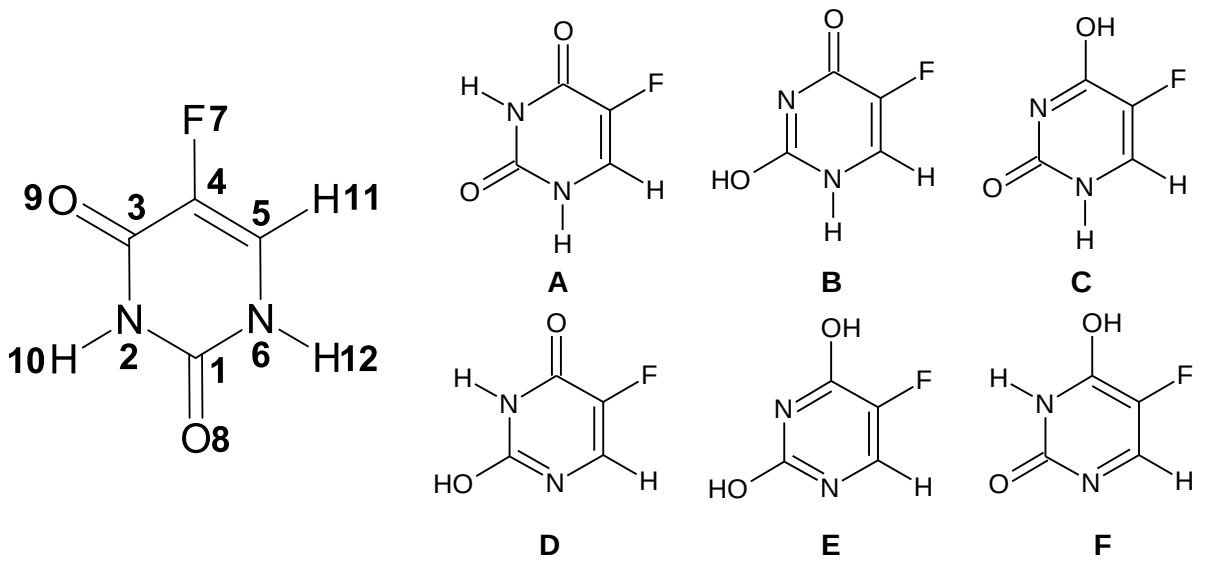
CHAPTER 1

1.1 INTRODUCTION

Until recently, the Environmental Protection Agency (EPA) and other similar authorities have been primarily concerned with monitoring and regulating a relatively small number of “priority” pollutants in the nation’s air, water and soil [1]. This effort has largely ignored Pharmaceutical and Personal Care Products (PPCPs) that may enter the environment via sewage, waste water plants and hospital estuaries making PPCPs a risk of great importance. Once exposed to the environment, they may undergo unpredictable transformations when mixed with either other PPCPs, or their metabolites. Some PPCPs alone have been shown to be endocrine disruptors [2-4], cause neurological toxicity in humans. Additionally, these agents are known to cause ecological problems [2]. Thus, there is a clear demand for increased knowledge in this area.

Miege et al.[5] reviewed 117 research papers and reports examining the removal of PPCPs at wastewater treatment plants (WWTPs). The reports largely focused (115 out of 117) on the removal of hydrophilic PPCPs from the aqueous phase. Hormones, analgesics, and antibiotics were reported with the greatest frequency. They studied the processes that are designed to remove *hydrophilic* PPCPs within WWTPs but acknowledged they only took into account a fraction of PPCPs that may be in WWTPs. Identifying and quantifying hydrophobic PPCPs first requires appropriate detection methods.

5-fluorouracil (5-FU) is a cytostatic pharmaceutical (Scheme 1) known to cause toxic reactions. It undergoes multiple reaction pathways and typically has adverse side effects when it is administered to patients [6]. 5-FU was selected for this study due to its widespread use and potentially far reaching environmental effects. It is believed that this investigation will also serve to compliment the work done [5].



5-Fluorouracil(5FU)

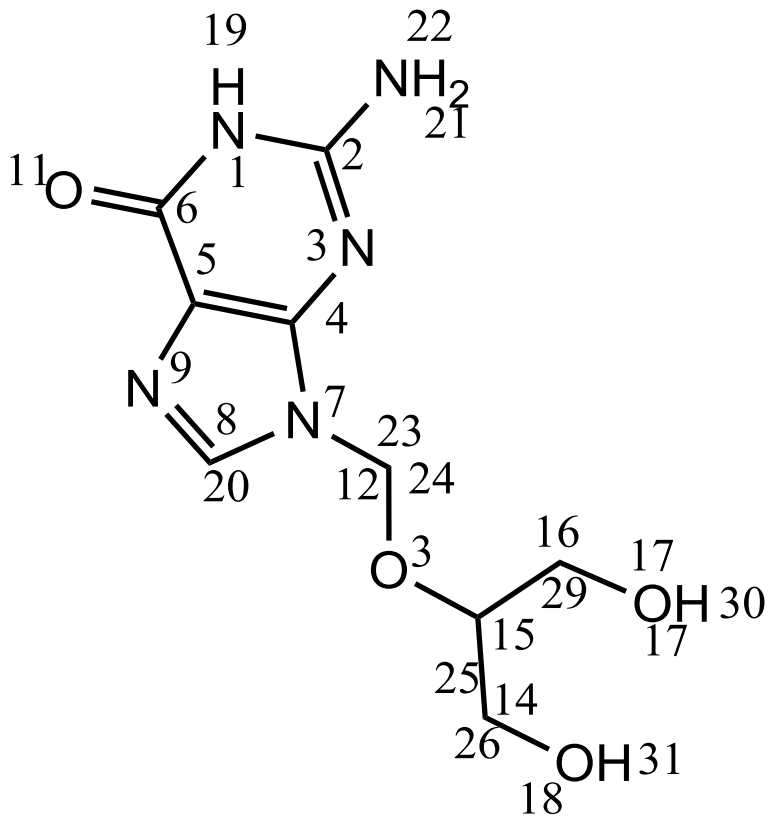
Scheme 1. Cytostatic drug 5-fluorouracil (5-FU) and it's 6 most recognized tautomers [6].

Although some investigations of this, and similar compounds have already commenced, there exists a great need for better understanding 5-FU's fate and how it degrades in the environment. Hirahara, et al. [7] performed a photodegradation study on some environmentally hazardous pharmaceuticals, including fenthion (O,O-Dimethyl O-[3-methyl-4-(methylsulfanyl)phenyl] phosphorothioate), an organothiophosphate insecticide and avicide that is structurally similar to 5-FU with a six-membered ring with three substituents. Fenthion, is also known to be neurotoxic like 5-FU and was shown to photodegrade via two pathways to yield fenthion sulfoxide and 3-methyl-4-methylthiophenol (MMTP). Hirahara et al. [7] then correlated that finding to suggest that fenthion may easily degrade in river water or an eutrophic lake. Bernadou et al. [8], used ^{19}F NMR spectroscopy to study the urinary excretion of 5-FU and found a minimum metabolite concentration of 15 μM during the first 30 minutes and 3 μM after 12 hours.

Until recently, EPA and others have been primarily concerned with monitoring and regulating a relatively small number of "priority" pollutants in the nation's air, water and soil [1]. Unfortunately, this effort has overlooked Pharmaceutical and Personal Care Products (PPCPs) that may enter the environment from a variety of sources (i.e. sewage, waste water plants and hospital estuaries) making PPCPs a risk to public health. Ganciclovir (GCV/Scheme 2) fits within the general scope of the PPCP category and is specifically an antiviral used to treat infections of the eye. These compounds are currently unregulated and their potential to interact with other agents in the

environment poses great risks to the health and safety of the general population. 5-FU is currently one of the popular anticancer agents with a usage rate of 74 to 300 kg/year for greater 23 million people[9]. On the other hand Ganciclovir is a not as popular antiviral used to treat sight disorders and infection, but it is categorized as a human carcinogen (cytoxic drug)[10]. This study is part of an overall plan set to examine the combined application of computational methods, liquid chromatography and Raman methods to study the properties of this compound in an effort to better identify and quantify it. Since non-aqueous solvents such as acetonitrile were used at various stages, this study may complement the work of Miege et al. [5] by providing data on non-water soluble compounds.

5-Fluorouracil is a commonly used cytostatic drug used to treat chemotherapy patients, neck/gastrointestinal disorders and breast cancer[11]. 5-Fluorouracil and another antineoplastic drug, tamoxifen together are the two most commonly used anticancer drugs internationally. Cytotostatic agents are indiscriminant in their arrest of the cell cycle. Therefore, the drugs intended effect in a limited population of malignant cells has wider reaching unintended negative consequences for healthy cells. Toxicity is severe, varied and can indeed be lethal. Many drugs have thus far been detected in aquatic environments but only a few cytostatic substances and no ecotoxicology data on cytostatic agents has been reported. One unique challenge in the study of toxicology

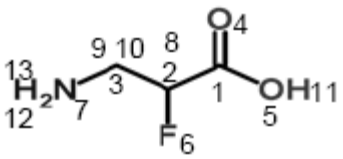


Ganciclovir(GCV) [4]

Scheme 2. Anti-Viral drug Ganciclovir [4].

presents itself as drugs are metabolized. In addition to parent compound toxicity, metabolic intermediaries, produced in vivo, may also exhibit cytotoxicity. 5-Fuorouracil is known to break down to fluroacetaldehyde (F_{acet}), fluoroacetic acid (FAC), fluoromalonaldehydic acid (FMASAld), α-Fluoro-β-Alanine (FBAL/ Scheme3) and fluoride (F^-). There is still much ambiguity regarding the in vivo metabolic breakdown of 5-FU preceding the activity against preferably cancerous cells. However, it is clear that FAC is the metabolite or chemical that directly acts on cells and it is believed FBAL may be directly involved as well. However, the scientific community has not reached consensus. Facet and FMASAID strongly influence generation of FAC which ultimately leads to apoptosis. It is generally established that potentially toxic drugs are detected within the environment within the range of µg/L – ng/L [1] and 5-FU specifically has been measured with limit of detection (LOD) and limit of quantification (LOD) at 0.02 ug/mL and 0.5 µg/mL (LOQ), respectively [2]. Since there is a chance for 5-FU or any of its metabolites to interact with each other or other compounds with similar mechanisms, there is the risk for producing synergistic effects. There is a dire need to not only detect the degradation products, but also to understand that these processes may occur within the environment. This could help pave the way for ecotoxicological data required for risk assessment plan to be published and eventual regulation of 5-FU. Therefore, the research objectives for this research agenda are to demonstrate systematic ways similar compounds may be distinguished, show the effects combinations of various conditions may have on the degradation of 5-FU

and to characterize any degradation products formed from these degradation processes.



α-fluoro-β-alanine

Scheme 3. 5-FU metabolite α-fluoro-β-alanine [6].

CHAPTER II

SPECTROSCOPIC DISTINGUISHABILITY

II.1 INTRODUCTION

This area of the research agenda may be the most fundamental because identifying compounds spectroscopically in environmental matrices can be complicated. Pharmaceuticals with similar functional groups as well as chemical reactive groups may behave similarly with respect to interactive patterns with other environmental constituents and conditions. Environmental transport and ultimate fate obviously may also be similar with respect to similar drug analytes, therefore; the ability to discern different analytes within these complicated matrices is an uphill battle.

The experimental approach here involves the use of a variety of spectroscopic techniques which may, not only confirm, but provide complementary information. For example, Raman and IR analysis may have very similar or identical patterns peak formations from identical modes or functional groups when analyzing the same sample. However, the intensity levels of each mode may be vastly different between the two techniques. The use of different techniques is beneficial where sampling matrices are different; for example water vs. sludge.

II.2 EXPERIMENTAL

II.2a) UV spectroscopy was carried out on a Cary 50 UV Varian system 50 Bio UV-Vis Spectrophotometer. 5-fluorouracil (5-FU)(99%/Aldrich Chem. Co./ Milwaukee, WI) was prepared to ~ 0.1mM in aqueous solvent for UV analysis. Ganciclovir (GCV) (InVivo Gen/ San Diego, CA) was prepared to ~ 0.0666mM in aqueous solvent for UV analysis. Spectroscopic UV-Vis measurements were recorded within the wavelength range of 190-900 nm for both prepared samples along with respective blanks (18 MΩ water).

II.2b) FT-IR spectroscopy was carried for solid state samples in form of KBr pellets which were recorded from 400-4000(cm^{-1}) on a Thermo Nicolet FTIR Spectrometer (Madison WI, 53744). The KBr (sample) pellets were made using 5-FU, GCV; α-Fluoro-β-Alanine(FBAL)(individually) solid sample and KBr which together were first finely mixed using a mortar/pestle system then an amount (only determined by the system itself and quality pellet desired through trial and error) was compressed between 2 bolts within a large nut. The large nut (bolts taken out) with the pellet still suspended in it was situated in the sample holder area of the instrument so the pellet could be analyzed with the IR beam.

II.2c) Ultra Violet resonance Raman (UVRR) analysis was carried out on a custom designed UVRR spectrometer similar to those in prior literature[12, 13]. An adjustable frequency-quadrupled titanium-sapphire laser (Coherent Inc., Santa Clara, CA) was designated as the excitation source. The Ti: sapphire laser was guided to a second harmonic generator (SHG) which doubles the frequency

via the use of a lithium triborate crystal. A dichroic mirror was then used to filter the remaining IR laser beam from the SHG produced beam. These two beams were then spatially overlapped within the third harmonic generator (THG) which provided a beam of lower wavelength. The resultant beam was split from the rest of the IR beam with another dichroic mirror. These two beams were spatially overlapped which in turn yielded a frequency mixing within the fourth harmonic generator (FHG) that produced a beam of lower wavelength. This beam which is incident was then reflected was then mirror reflected in order to irradiate the sample.

The samples circulated through a thermo-controlled chamber with kept constant temperature at 4°C. A thin layer of sample was produced as result of the solution flowing through a 19 gauge needle and between two thin nitinol wires. This layer of sample was irradiated directly by the locally reflected incidental beam while a continuous stream of nitrogen gas eliminated ambient oxygen from the sample chamber. The Raman scattering was collected within a 135° backscattering geometry and dispersed via a 1.25 meter spectrometer (Horiba Jobin Yvon Inc., Edison, NJ) equipped with a 3600 grove/mm grating. At the sample chamber, the laser power was kept below 0.5 mW by monitoring the power with a Fieldmax II-TO™ laser power meter in order to avoid degrading the sample. Each spectrum was collected over 180 minutes which produced 72 individual spectra that were averaged. The spectra were collected and exported via a CSV format using Synergy software (Horiba Jobin Yvon Inc., Edison NJ). The cyclohexane spectrum and the peak positions published by Ferraro and

Nakamoto were used for calibration of the spectra. A calibration spectrum was collected for every collection session.

For the independent analysis, a 0.60mM 5-FU sample along with a buffered blank was analyzed at an excitation wavelength of 197nm. For the Raman Excitation Profile for the 197 series, spectroscopic analyses were performed on 3-4 ml aliquots of the pre-prepared 0.50mM 5-FU and 50mM $HClO_4^-$ solution, at excitation wavelengths of 197, 199, 201, 203 and 205nm. Aliquots of the corresponding buffer solution were also analyzed at each excitation wavelength. For the Raman Excitation Profile for the 263 series, two separate analyses were performed at each wavelength of 263, 265, 267, 269 and 271nm. This two-part analysis was required in order to measure all peaks of interest because the C=O/C=C modes typically seen within one window at the higher frequencies (at your lower excitation levels, ~197nm) do not appear within one window. Since each analysis will yield a spectrum with only a window or range of frequencies, a second analysis at each wavelength was needed in order to see the adjoining spectral window range containing the C=O and C=C vibrational modes not seen within the first.

For the independent analysis, a 0.50mM GCV sample along with a buffered blank was analyzed at an excitation wavelength of 197nm. For the Raman Excitation Profile for the 197 nm series, spectroscopic analyses were performed on 3-4 ml aliquots of the pre-prepared 0.50mM GCV and 50mM $HClO_4^-$ solution, at excitation wavelengths of 197, 199, 201, 203 and 205nm.

Aliquots of the corresponding buffer solution were also analyzed at each excitation wavelength.

II.2d) NMR Spectroscopy analyses of 1.88mg of 5-FU(62.3mM) dissolved in 1ml of D_2O were carried out on a Bruker Avance model ARX-250 NMR instrument (Bruker Corporation Fremont, CA 1998) at ambient probe temperature. Shimming calibration was done on the D_2O solvent and chemical shifts were reported in units of parts-per-million (ppm). This process which was not anticipated, was implemented in order to confirm that the 5-FU sample analyzed in this study exist predominantly as the keto-tautomer.

II.2e) Computationally, six 5-FU tautomers along with a GCV model were constructed using ChemBio 3D Ultra (Version 12.0 1986-2009 CambridgeSoft) and were optimized geometrically and energetically using the DFT/B3LYP/6-311++G(d, p) method [14,15] through a GaussView 5.08 graphical interface (Gaussian, Inc./2000-2008 Semichem, Inc.) with the Gaussian 09(Gaussian, Inc. Wallington, CT 1995) program. These GaussView/Gaussian calculations were performed directly on the Clark BioInformatics Consortium applications server (The Curators of University of Missouri 2003-2012). In regards to infrared, Raman and ^{13}C NMR spectra predictions, calculations were performed using density functional theory (DFT) at the B3LYP/6-311++G(d, p) level on the most stable 5-FU tautomer structures and GCV structure optimized at this level. An additional NMR prediction was carried out through ACD CNMR Predictor (Advanced Chemistry development, Inc./Informer Technologies Inc. Toronto,

Ontario, Canada 2012) which involved uploading of the optimized 5-FU tautomer as a ".gjf" file followed by the application of a Gaussian Interface.

II.3 RESULTS

11.3a) UV Absorption Analysis of 5-FU involved theoretical optimizations was carried out on six different 5-FU tautomers (Scheme 1). The optimized structures (tautomers A through F) were then subjected to electronic transition calculations (UV-Visible predictions). Of the 6 tautomers, only two were comparable with the experimental result, tautomer A, the keto conformer, and tautomer D, one of the enol conformers. The predicted excitation maximum for both tautomers occurred at approximately 135 nm (not shown), which is in the vacuum UV region and outside the functional range of the absorption spectrometer. However, the second and third excitation maxima in the theoretical spectra (Figure 1) are qualitatively similar to the experimental absorption bands of 5-FU in the 190-300 nm region.

The experimental UV absorption spectrum (Figure 1) demonstrates that 5-FU has two absorption maxima that occur at approximately 215 and 275 nm, corresponding to two separate electronic transitions within the molecule. Qualitatively, the theoretical intensities of the two bands for tautomer A match more closely with the experimental spectrum, suggesting the predominant form is the keto form. This results are consistent with previous studies, which have also reported an absorption maxima for 5-FU around 270 nm [2] [1].

Assuming that the two experimental absorption maxima are chiefly due to excitation of the two C=O functional groups of the 5-FU molecule (the C=C functional group would give rise to excitation at lower frequencies not scanned), the UV spectra should show absorption bands as a result of π to π^* and n to π^* transitions. The π to π^* transition requires more energy than n to π^* and should be dominant over the n to π^* absorption band if there are two C=O functional groups within the molecule. Since the π to π^* transition requires more energy, its absorption band should have a lower wavelength (blue shift) than the n to π^* absorption band and as seen is more intense than the other peak. Conversely, if there were only one C=O (an enol form) then the n to π^* absorption

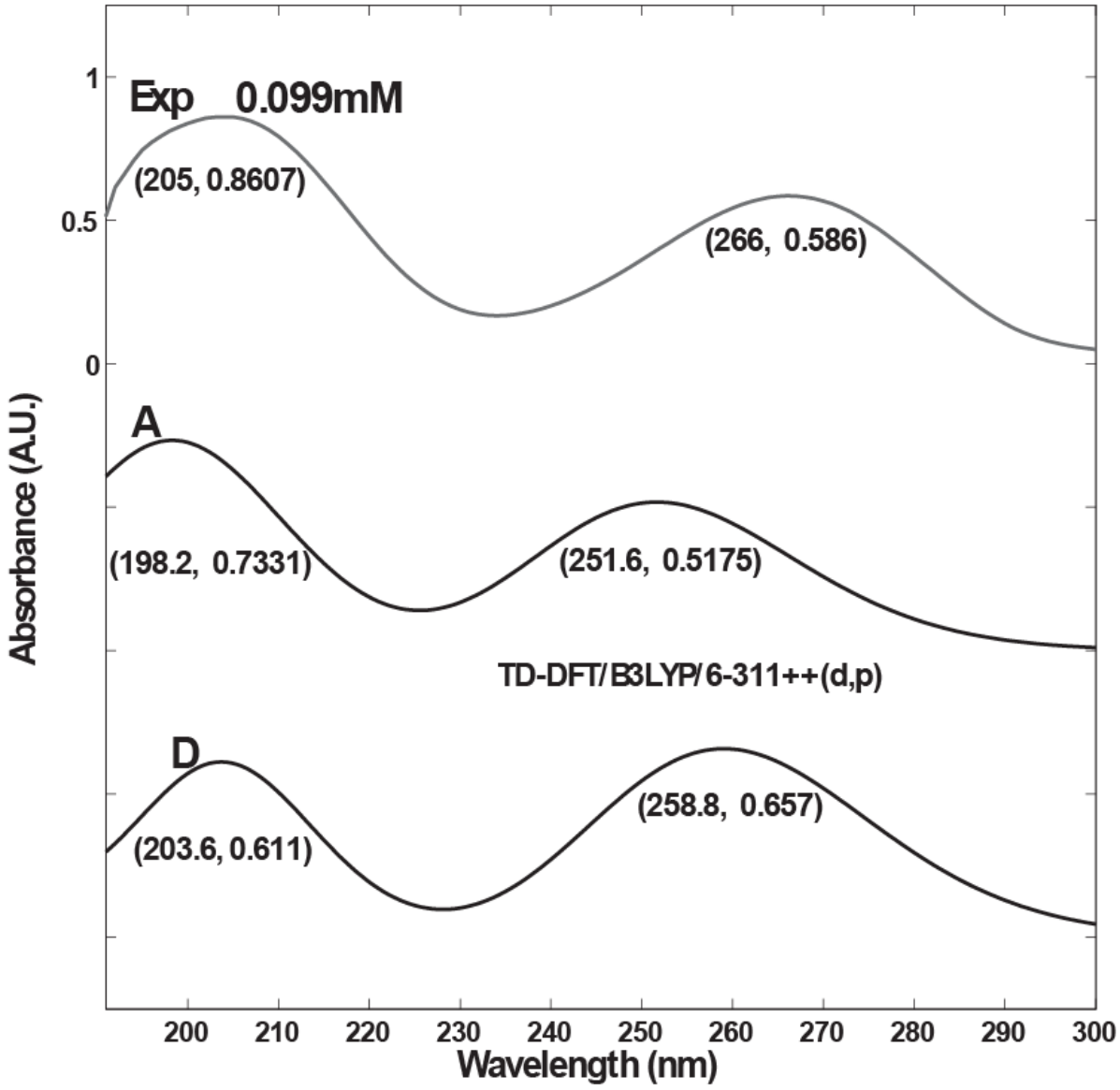


Figure 1. Theoretical (tautomerA/D) and observed UV-Vis spectra for 5-FU. The theoretical spectrum was calculated with DFT/B3LYP/6-311++G(d,p).

band (2nd band) should be more intense than the first. Figure 1 also shows that the experimental band intensities are not consistent with an enol or tautomer D form, though there are reasonable qualitative similarities.

Now in relation UV analysis of Ganciclovir (GCV), two reference UV spectra for GCV were found as seen in figure 2. Looking at the reference spectra in comparison with our experimental, qualitatively, a near perfect correlation is seen and the excitation maximums among all three spectra are roughly the same (253nm for this study, 254nm for mtnviewfarm.net[16]; 252nm from Ijcas [17]). This study's GCV experimental has a λ max of ~ 253 nm and mtnviewfarm.net[16] has λ max of ~ 254 Additionally, both experimental spectra are internal consistent with one another and qualitatively similar. Ganciclovir has many areas of its structure which can interact with a particular solvent which would yield an effect on the positioning of peaks deriving from n →π* and π → π *. GCV is a polar hydrophilic compound and polar solvents will cause the π → π * transition to shift to a greater extent in the red while the n → π * goes to a greater extent to the blue compared to the effect the solvent would have on smaller polar hydrophilic compounds. This situation may be due in part to the fact that overlapping of the two different peaks within the experimental UV spectra was observed (figure 2).

11.3b) The FTIR spectrum of 5-FU has two strong features that occurred at 1248 and 1663(cm^{-1}). A shoulder at 1722(cm^{-1}) is apparent on 1663(cm^{-1}) feature. This feature is likely a combination of the C=O and C=C stretching frequencies, which are predicted to occur at 1806, 1780 and 1711(cm^{-1}), respectively for tautomer A. However, it should be noted that bands arising from the C=O stretching frequency (1784(cm^{-1})), N=C stretching frequency (1664(cm^{-1})) and C=C stretching frequency (1624(cm^{-1})) for tautomer D are also predicted to occur in this region. The other strong feature in the FTIR spectrum of 5-FU occurs at 1248(cm^{-1}) (Figure 3), which is assigned to the C-F stretching frequency. The position of the C-F stretching frequency is predicted to occur at 1256 and 1264(cm^{-1}) for the A and D tautomers, respectively. The C=C stretching frequency is predicted to occur at lower frequency than the C=O stretching frequency. The feature at 1506(cm^{-1}) is may be the C=C stretching frequency. Unfortunately, the peak(s) in the 2900-3400(cm^{-1}) range are most likely due to moisture adsorbed by the KBr, the weaker NH stretching frequencies are arguably intertwined and overlapped within this area.

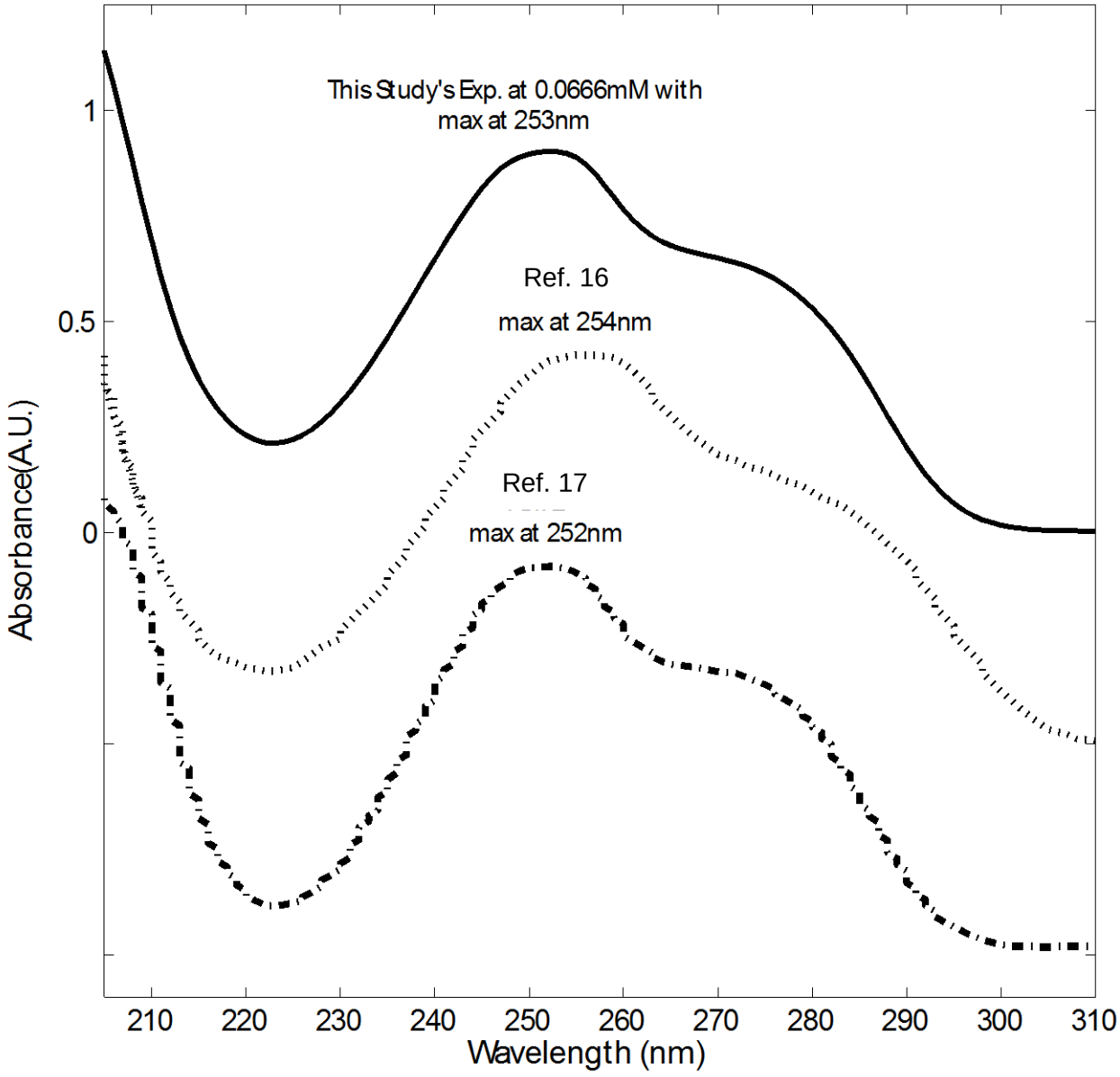


Fig. 2. This study's UV-Vis spectrum on 0.067mM 5-FU versus prior studies [16;17].

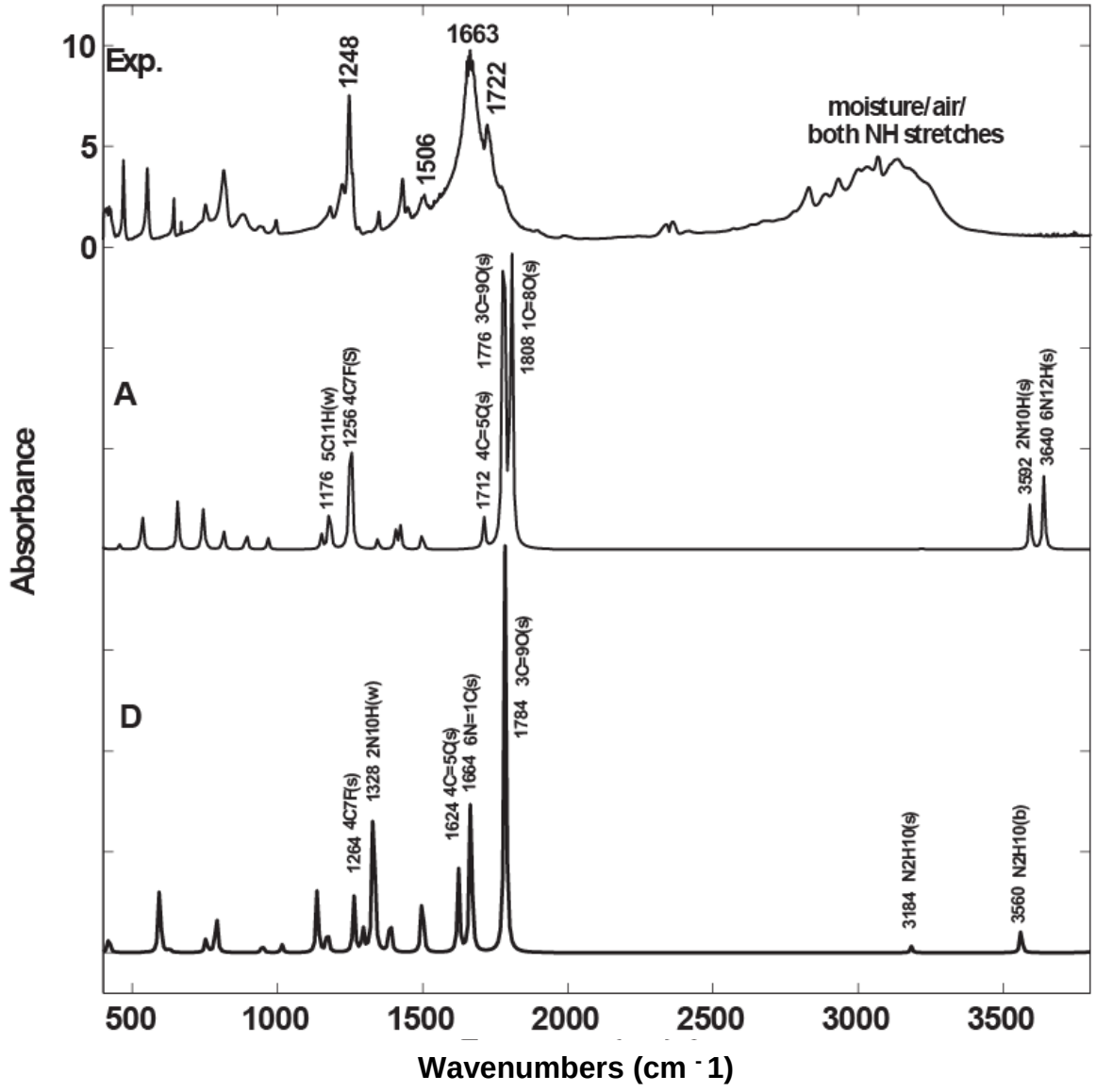


Figure 3. Theoretical and experimentally measured IR spectra of 5-FU. Experimentally measured spectrum of 5-FU in solid phase (top panel) and theoretical spectra calculated at DFT/B3LYP/6-311++G(d,p) level on tautomer A(middle panel) and tautomer D (bottom panel).

A prior study [18] identified their three IR predictions(using different methods) in support of their experimental as being appreciable and consistent with respect to 5-FU's most distinct stretching frequency peaks (C=O, C_6-H and C-F). There are three stretching frequency peaks that are consistent between the experimental IR and theoretical band positions. In Figure 7, there are arguably two NH stretching frequencies giving rise to multiple peaks in the 3500-3650(cm^{-1}) region and in the 1690-1750(cm^{-1}) vicinity (Figure 3) there are the obvious C=O stretching frequencies and the smaller partially overlapping peak to the left of which at ~1600(cm^{-1}) may be due to C_4=C_5 stretching frequency. This study's Raman results (Figure 4) have at least four modes of strong consistencies between the experimental and theoretical spectra. These scattering bands 26, 27 (1700-1750(cm^{-1})) for both C=O stretching frequencies, 17 (~1350(cm^{-1})) for C_4F_7 stretching frequency and 12 (~750(cm^{-1})) for $N_9C_1N_2$ bending/stretching frequencies. Thus, this study's significance has been demonstrated using unique methods which were validated in a similar manner as Palafox and Rastogi [18] showed.

This study's other two compounds of interest can easily be distinguished from each other and 5-FU because for one α-fluoro-β-alanine (FBAL) has only one C=O stretching frequency at ~ 1767(cm^{-1}) (figure 5) while GCV's FTIR spectrum's most significant peak is at ~ 1248(cm^{-1}) (Figure 6) which may be mainly due to the compound's CF component. The other key factors giving rise to peaks were due to ring bending at 1072(cm^{-1}) and those resulting from the OH stretching. Even though, the region where the OH stretching frequency is

observed is significantly obscured by peaks from moisture from the air, the contribution from GCV OH stretching frequencies are arguably observed with the more intense peaks(3181, 3385; 3431 (cm^{-1})).

II.3c) Resonance Raman spectroscopy has the advantage of lower detection limits than normal Raman spectroscopy when resonance enhancement occurs. Although all of the Raman modes are present in the resonance Raman spectrum, the resonance enhanced modes will dominate the spectrum. The position of the Raman bands will be the same in the normal Raman and resonance Raman spectra, though the relative intensities will differ greatly. The UV resonance Raman spectrum of 5-FU has a number of distinct features.

The lowest frequency band visible in the UVRR spectrum of 5-FU occurred at 759(cm^{-1}). This feature is relatively weak but assigned to C-F stretching frequency, though N_2-C_1-N_9 bending frequency is also predicted to contribute in this region. Above the perchlorate band (932(cm^{-1})), another weak feature occurred at 1008(cm^{-1}). This feature may be assigned to CN and CH combinatory stretching frequencies as supported by prior literature [18, 19] and theory.

Three bands were apparent in the region from 1100-1400(cm^{-1}), which occurred at 1226, 1256 and 1349(cm^{-1}) (Figure 4). Based on the IR spectrum of 5-FU (Figure 3), in which the C-F stretching frequency occurs at 1248(cm^{-1}), the band at 1256(cm^{-1}) is assigned to the C-F stretching frequency of 5-FU. The theoretical Raman spectra of tautomers A and D also have features in this region that are derived from the C-F stretching frequency (Figure 3 and Table 1). The

lower frequency band at 1226(cm^{-1}) is assigned to a combination of CH and CN stretch/bending frequencies as well.

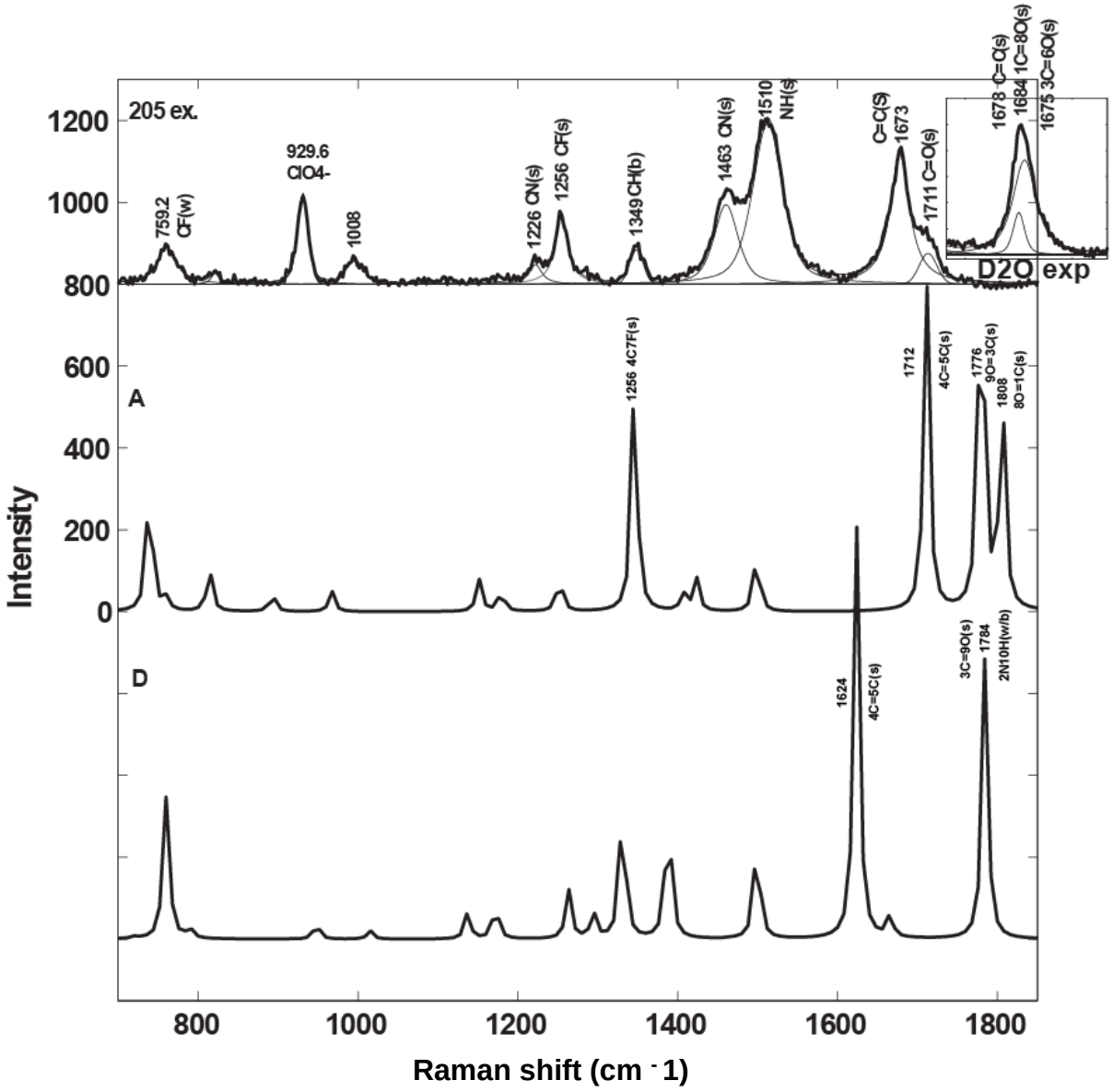


Figure 4. Theoretical (tautomer A/D) and experimentally measured Raman spectra for 5-FU.The experimental spectrum was obtained at a concentration of 600uM using an excitation wavelength of 197nm (top panel) with inset (D_2O solvent analysis). Theoretical spectra for tautomers A and D were calculated at DFT/B3LYP/6-311++G(d,p) level (bottom panel).

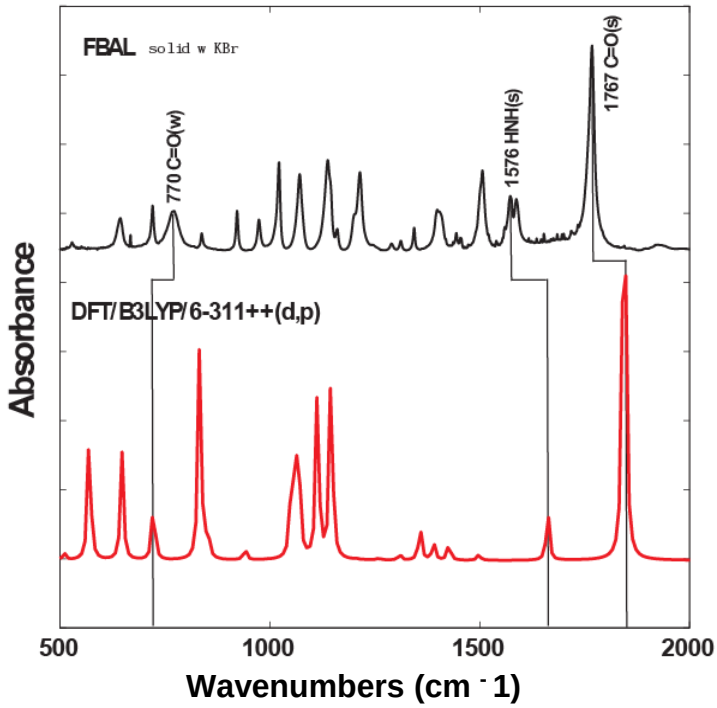


Figure 5. Theoretical and experimentally measured IR spectra of FBAL. Experimentally measured spectrum of FBAL in solid phase (top panel) and theoretical spectra calculated at DFT/B3LYP/6-311++G(d,p) level (bottom panel).

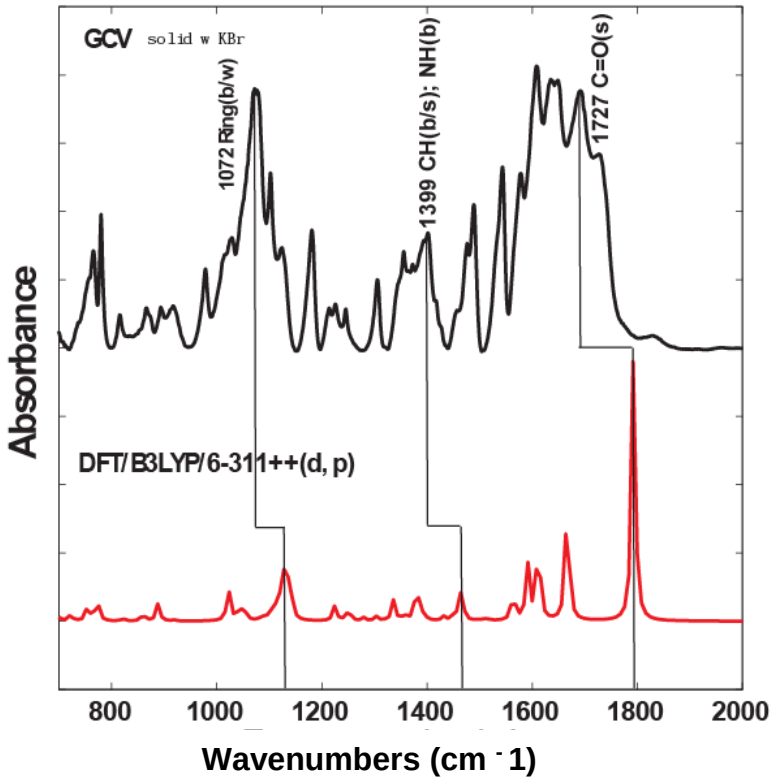


Figure 6. Theoretical and experimentally measured IR spectra of GCV. Experimentally measured spectrum of GCV in solid phase (top panel) and theoretical spectra calculated at DFT/B3LYP/6-311++G(d,p) level (bottom panel).

Two features at 1463 and 1510(cm^{-1}) can be assigned to a combination of C-N stretching and N-H bending frequency. The C_5-N_9 stretching and N_9-H_{12} bending frequency is predicted to occur at 1423(cm^{-1}). The 1600-1800(cm^{-1}) region of the UVRR spectrum of 5-FU has two features that occur at 1673 and 1711(cm^{-1}) (Figure 4). Based on the predicted Raman spectra, the C=O stretching frequency (tautomers A and D) and possibly the C=C stretching frequency (tautomer A) should occur in this region. Deuterium exchange was employed to determine which peak(s) derived from the C=O stretching frequencies as these peaks should downshift up H/D exchange. The inset in Figure 4 shows that upon H/D exchange the higher frequency peak downshifts underneath the main band at 1678(cm^{-1}). The peak appears asymmetrical and is slightly upshifted from 1673 to 1678(cm^{-1}), likely because it is a combination of two bands. The band at 1711(cm^{-1}) is assigned to C=O stretching frequency based on its downshift in D_2O. The lack of a shift of the band at 1673(cm^{-1}) suggests this band arises from C=C stretching frequency.

The experimental results from the UV Resonance Raman (UVRR) analysis are very much consistent for the most part with the theoretical results in regard to mode placement as seen in Figure 7 Preliminary analysis was performed for experimental Raman on GCV vs. the new theoretical method(B3LYP/6-311++G(d, p))(Fig. 7). No published analysis or interpretations of spectroscopic results for this compound are known. Hossenini et al. [20] have published general observations of Raman analysis done with a confocal Raman spectroscope. In that study, they revealed that ganciclovir exhilarated unique

spectral features at 631, 1263, 1302, 1448 and 1560(cm^{-1}) compared to this study's distinct features at 1250, 1340, 1380, 1430, 1510, 1550 and 1590(cm^{-1}). Qualitatively, this study's spectrum is, for most part, consistent with the spectral profile of the Hosseini's work [20]. It is hypothesized that differences in our spectra may be attributed to effects of enhanced resonance.

Table 1

Summary of theoretical (tautomer A) and experimental IR and Raman spectroscopic results for 5-FU.

Mode	Frequency (cm^{-1})	IR (Theory) (Intensity)	IR (Exp.) (Trans.)	Raman (Theory) (Intensity)	Raman (Exp./197 nm) (Intensity)	Atoms/bonds Involved
10	626.10	10.12				C3O9(b)
	656.11					C1O8(b)
12	743.87	8.12		20.01		N6C1N2(b)
13	749.43					C4C3(s)
						C4F7(s)
14	758.70				61	C4F7(s)
						N2C3C4(b)
15	811.20			1.012		C4F7(s)
16	913.96			0.512		C5H11(b)
17	968.00			1.011		C1N6(s)
18	1176.21	8.123		1.312	42	N6H12(b
						C5H11(b)
19	1256.00	22.107	31	1.019	93	C4F7(s)
						N6H12(b)
						C5H11(b)
20	1252.28					C4F7(s)
						N6C5(s)
21	1345.90			100.43	94	N2H10(s)
						C5H11(b)
						N2C1N9(s)
23	1423.08			1.001	106	N6H12(b)
						C5N6(s)
24	1498.90			2.012	194	C5N6(s)
						N6H12(b)
25	1711.20	8.12	10	190.13	252	C5=C4(s)
26	1779.82	61.05	14	115.13		C3=O9(s)
27	1805.89	65.14	10	100.00	77	C1=O8(s)
28						C5H11(s)
29	3593.23	10.41				N2H10(s)
30	3640.52	16.23				N6H12(s)

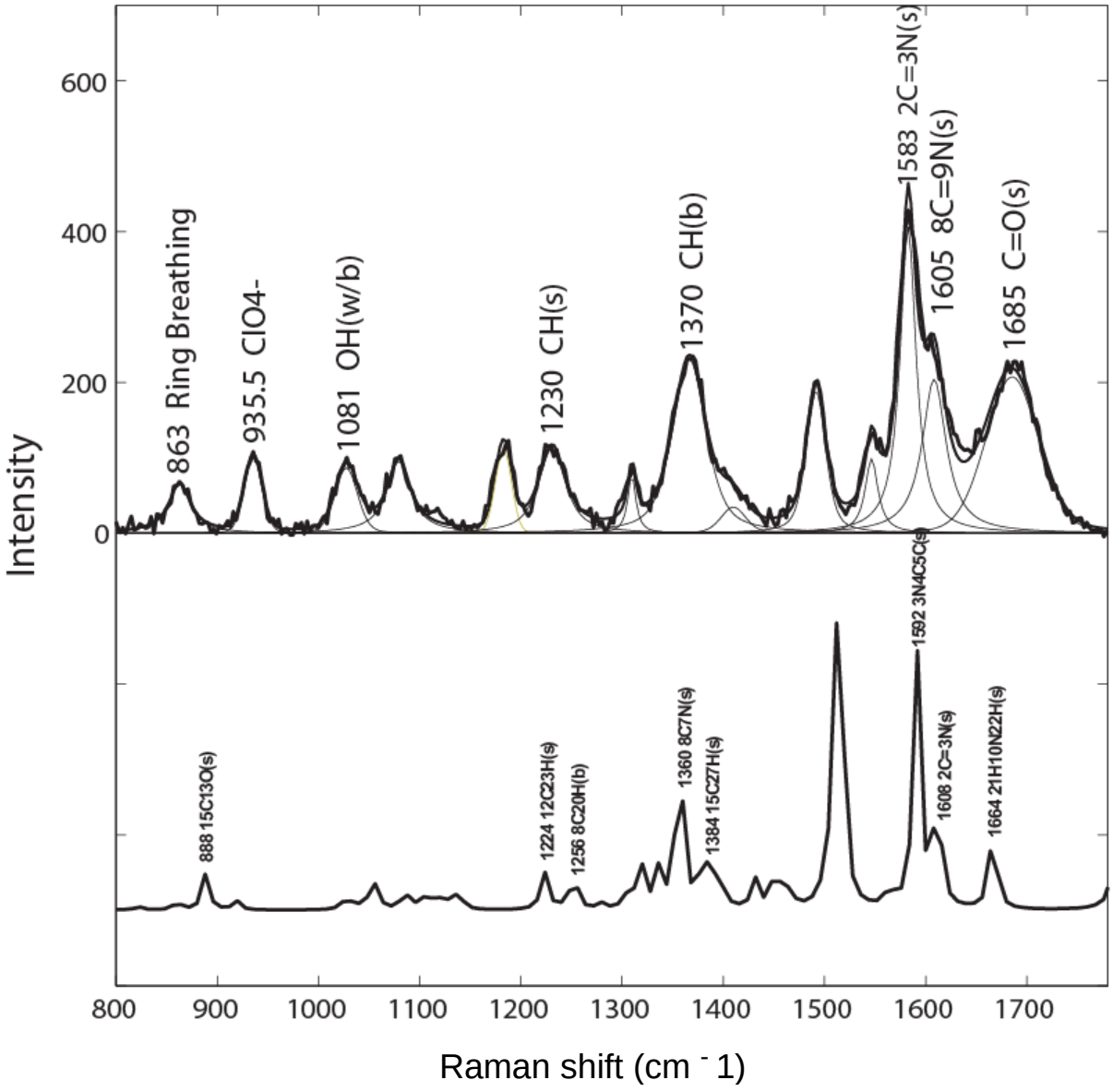


Figure 7. Theoretical and experimentally measured Raman spectra of GCV. Experimentally measured spectrum of 0.500mM GCV in aqueous solution (top panel) and theoretical spectra calculated at DFT/B3LYP/6-311++G(d,p) level (bottom panel).

The Raman cross section were determined for 5-FU at several excitation wavelengths (197, 199, 201, 203 and 205 nm) using the equation below.

$$\sigma_{\lambda,amide} = \left(\frac{\sigma_{ClO_4^-}}{n}\right)\left(\frac{I_{amide}}{I_{ClO_4^-}}\right)\left(\frac{v_{exc} - v_{ClO_4^-}}{v_{exc} - v_{amide}}\right)^4 \left(\frac{C_{ClO_4^-}}{C_{amide}}\right)\left(\frac{A_0 + A_{amide}}{A_0 + A_{ClO_4^-}}\right)$$

σ_{ClO4} = std. C/S for ClO4, I= intensity, γ= freq. of an excitation wavelength, C= concentration; A = UV absorbance

The strongest resonance enhancement was observed for the C_4=C_5 stretching frequency mode (1673(cm^{-1})) and the combination C_5-N_9 s/N_9-H12 bending frequency mode (1510(cm^{-1})) (Table 2/Figure 8). However, the cross section of combination C_5-N_9 s/N_9-H_{12} bending frequency band appeared relatively constant over range of excitation wavelengths. Similar trends were observed for the majority of the bands in the UVRR spectra of 5-FU. The exception are the features at 1673 and 1711(cm^{-1}), for which the cross sections increased with increasing excitation energy (decreasing wavelength). Furthermore, this study's Raman results in Figure 3 shows a C_1=O peak at 1711(cm^{-1}), both C_4=C_5 (predominantly) and C_3=O at 1673(cm^{-1}), N_2H_{10} at 1510(cm^{-1}), CH at 1349(cm^{-1}), CF at 1256(cm^{-1}) and ring/CF at 759.2(cm^{-1}). These findings are consistent for the most part with results from prior studies such as that by Pavel et al. [19]. However, with respect to this study, important differences exist between the C=C peak in this study overlapping the C_3=O peak at 1673(cm^{-1}) and the CF peak possibly overlapping CH peaks at 1256(cm^{-1}). Discrepancies are also observed when comparing this study's experimental and theoretical findings which mainly serve as a means to help identify experimental

modes. This is the first study to use UVRR analysis on 5-FU which generates an enhancement of certain modes not normally seen in regular Raman analysis and is likely the cause of the observed differences in spectra.

Table 2. Calculated Raman cross sections versus the 932 cm^{-1} band of perchlorate.

λ(nm)	$C_1=O_8$	$C_4=C_{5(s)}$	$N_6H_{12(b)}$	$C_1N_{6(s)}$	$C_4F_{7(s)}$	$C_1N_{6(b)}$	$C_4F_{7(s)}$
197	130	370	281	154	132	51	88
199	149	407	275	183	115	49	55
201	135	271	242	162	120	55	65
203	95	219	247	139	110	42	53
205	86	207	248	143	109	42	53

[a] λ= Wavelength of excitation energy.

[b] Millibarns/molecule steradian; 1 barn = 10^{-1}.

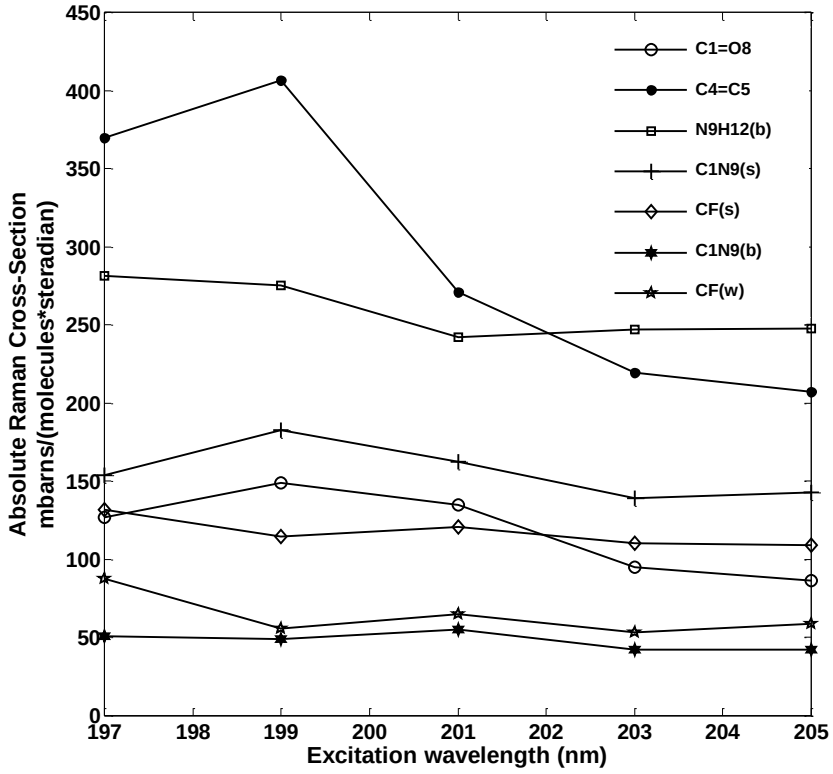


Figure 8. Experimental Raman cross sectional calculations of modes from all relevant peaks/modes from the 197 Series Raman Excitation Profile.

This study's Raman modes are closely consistent with Rastogi and Palafox's [18] assignments a C_2=O peak at 1724(cm^{-1}) (identified as C_1=O in this study) which has a lower intensity than the other C=O peak. This is due to the fact that the C_2=O functional group is blocked from influences created by the rest of the molecule. Such shielding is achieved through the ring's nitrogens on both sides of the molecule. Likewise, the other C=O (C_4=O) functional group is influenced by the neighboring electronegative fluorine atom. Rastogi and Palafox [18] made peak assignments at 1670(cm^{-1}) to C_4=O (which correlates to C_3=O in this study), C_4=C_5 at 1709(cm^{-1}), N_1H at 1503/1448(cm^{-1}), N_3H at 1348(cm^{-1}), C_6H at 1256(cm^{-1}), and CF/ring wagging at 1224(cm^{-1}) and 767(cm^{-1}). Pavel et al. [8] had similar mode assignments with C_2=O at 1786(cm^{-1}), C_4=O at 1770(cm^{-1}), C_4=C_5 at 1709(cm^{-1}), N_1H at 1487(cm^{-1}), N_3H at 1405(cm^{-1}), C_6H at 1334(cm^{-1}), CF at 1230(cm^{-1}) and ring vibrational bending at 800(cm^{-1}). Dobrosz-Teperek et al. [21] claimed that both of their C=O modes were in the 1700(cm^{-1}) vicinity, C_4=C_5 at 1649(cm^{-1}) and CF/ring at 1246(cm^{-1}).

Raman Cross Sectional Analysis(similarly done as with 5-FU) was applied towards the results from an excitation profile (197nm Series) of 500uM GCV in 50mM ClO_4^- at varying excitation energies (197, 199, 201, 203; 205 nm). As there was an increase in excitation energy there appeared to be a tendency for intensity increases with modes 1, 2, 3, 7 and 13 as seen in fig. 9. These trends are also reflected in the cross sectional results shown in table 3. Greater cross sectional value differences were observed between those at the 197nm

compared to those at 205 nm for the vibrational modes which comply with this sort of trend.

II.3d) NMR spectra was used in order to confirm that the predominant tautomer of 5-FU, a ^{13}C NMR analysis was performed. The ^{13}C NMR indicates four distinct carbons (Figure 10). The first Carbon (C_3) is double bonded with O_6. This carbon neighbors F_7 to generate a coupling pattern which gives rise to the doublet at ~ 160 ppm. Another Carbon ($C_{1,}$) bonded to O_8 gives rise to a peak at ~ 151 ppm. The third, $C_{4,}$ is bonded to F_7 which in turn is coupled to O_6 giving rise to the doublet at ~ 141 ppm. Finally, $C_{5,}$ is bonded to H_{11} giving rise to a doublet at ~ 126 ppm. This ^{13}C NMR framework is consistent with that of the keto tautomer (A) as the ACD and DFT ^{13}C NMR predictions support (Figure 10).

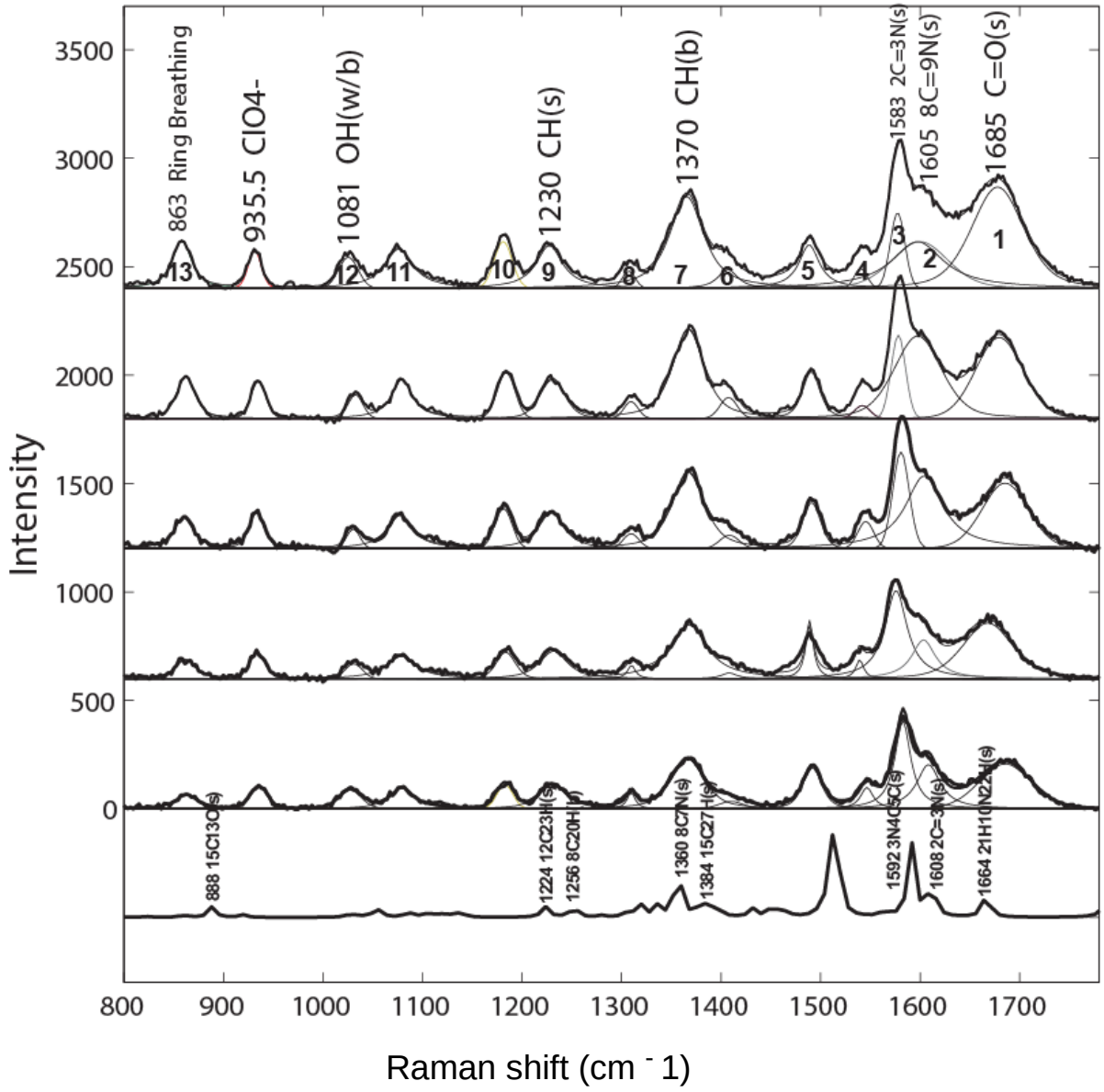


Figure 9. Raman analysis of 500uM GCV and 50 mM ClO_4^- in aqueous solution at varying excitation energies (197, 199, 201, 203; 205 nm).

TABLE 3. Raman cross-section for GCV with ~ 950/cm band of aqueous ClO_4^- (**197series**)

λ(nm)	σ01	σ02	σ03	σ04	σ05	σ06	σ07	σ08	σ09	σ10	σ11	σ12	σ13
197	403.77	466.03	819.34	236.53	355.85	134.17	410.96	155.61	200.87	213.56	177.22	173.57	117.09
199	332.18	325.18	518.86	168.17	278.27	80.86	306.39	105.16	150.40	165.93	126.18	91.86	102.73
201	245.19	256.34	426.59	119.09	162.88	94.73	258.81	68.70	118.99	144.30	113.45	72.06	101.56
203	219.97	221.44	357.72	98.79	124.03	94.99	232.29	60.23	107.18	115.01	98.01	65.93	104.02
205	210.85	191.95	277.13	79.97	98.35	78.71	183.29	49.21	85.32	99.11	74.11	67.63	85.62

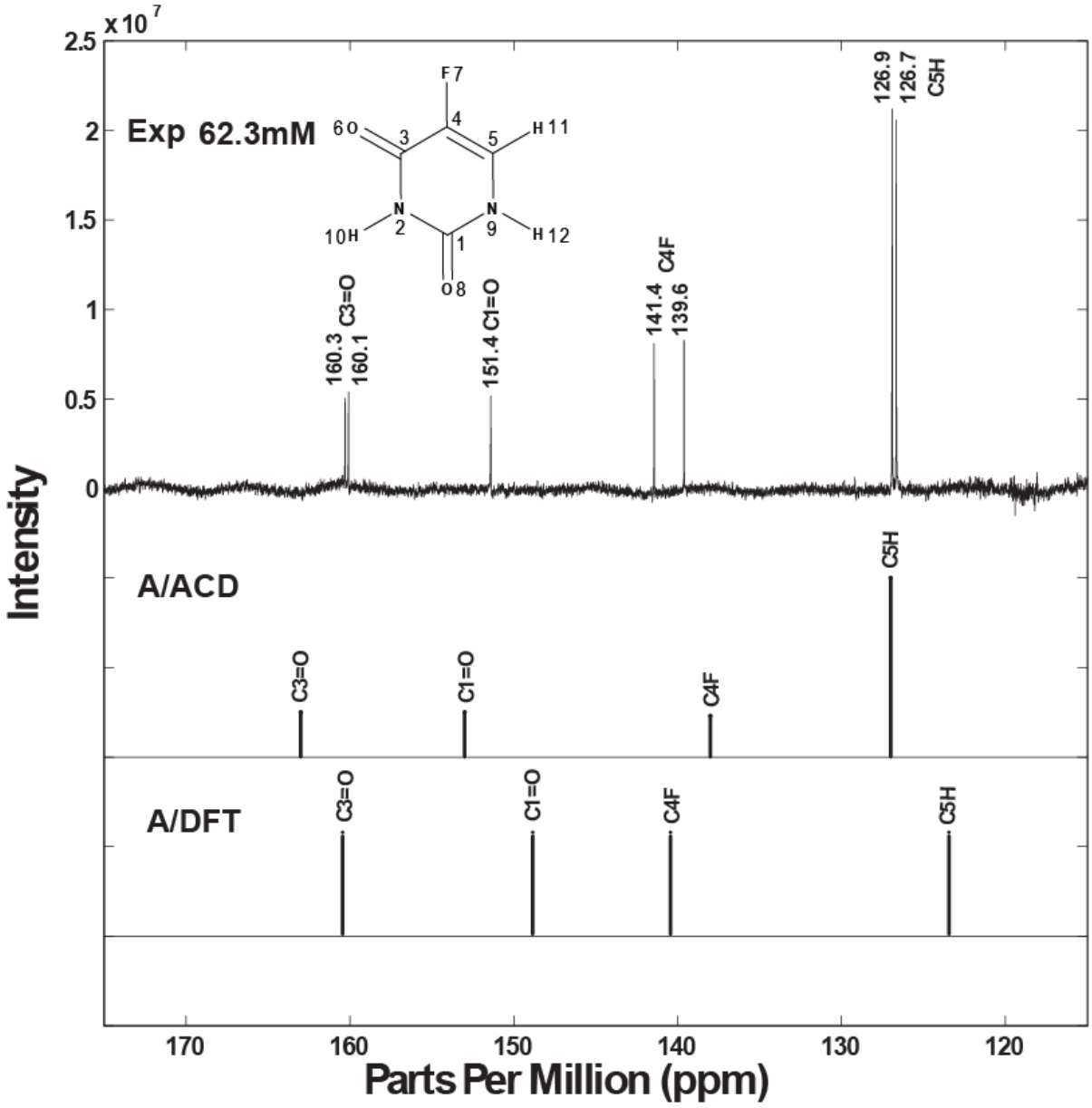


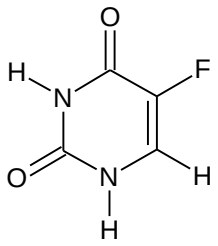
Figure 10. ^{13}C-NMR spectra of 5-FU were obtained at a concentration of 62.3 mM aqueous 5-FU (top panel). Lower panels show 5-FU tautomer A using ACD nmr predictor/theory (middle) and tautomer A using DFT/B3LYP/6- 311++ G(d,p).

II.3e) The geometric optimization process involves systematically rearranging the molecule. The atoms, angles and bonds within 5-FU were assigned a total energy value. The arrangement corresponding to the minimum energy value was taken as 5-FU's optimized conformer. The optimization process was accomplished using GaussView/Gaussian (GaussView 5.0.8, Gaussian, Inc./2000-2008 Semichem, Inc) and was applied after each molecule was put through a preliminary force field calculation process within ChemBio 3D Ultra (Ultra-Version 12.0 1986-2009 CambridgeSoft). A summary of the optimization results for all six tautomers are shown in Table 4. Similar prior studies have demonstrated the usefulness of DFT level type calculations to geometrically optimize a structure. One prior study [22] employed DFT/B3LYP/6-31+G**, HF/6-31+G** and MP2/6-31+G** independently, to optimize the six tautomeric forms of 5-FU (Table 4). It was found that each method showed tautomer A (keto) was the most geometrically relaxed (i.e. lowest Total Energy) of the six. In the current study, the six 5-FU tautomers were optimized using DFT/B3LYP/6-311++G(d, p). This study's results too show tautomer A (keto) to be the most geometrically relaxed of all six optimized tautomers (Table 4). The current study's use of DFT/B3LYP/6-311++G(d, p) level was chosen because of its success in the optimization of 2-aminoiconicotunic acid by Karabacak et al. [23]. More so, Tanak and Agar [24] also demonstrated that the DFT/B3LYP/6-311++G(d, p) method was reliable for obtaining good agreement with experimental UV-Visible absorption spectra on certain di-substituted phenols.

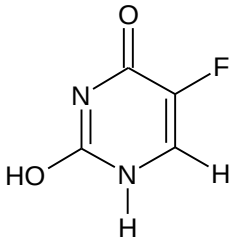
Thus, this was the first time method DFT/B3LYP/6-311++G(d, p) was applied towards 5-FU optimizations and predictions.

Table 4. Optimization of the six 5-FU tautomers shown below using DFT calculations with RB3LYP/6-311++G(d,p) level of theory.

5-FU tautomer (DFT/RB3LYP/6-311++G(d,p))	TE (au)
A	-514.20061567 a.u.(CS)
B	-514.15589791 a.u.(C1)
C	-514.17302521 a.u.(CS)
D	-514.17192224 a.u.(CS)
E	-514.17600961 a.u.(CS)
F	-514.16699519 a.u.(CS)



A



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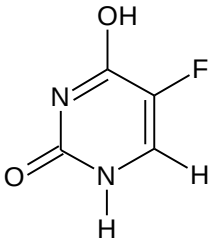
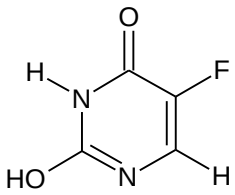


C



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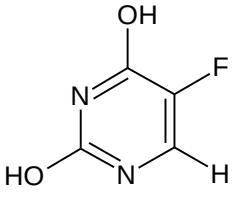

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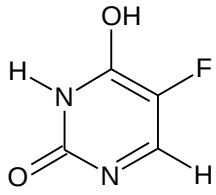

F

II.4 SUMMARY

Although this study's UV, IR and NMR results were consistent with the findings of similar literature on the subject, it has nonetheless broken new ground with respect to spectroscopic data for 5-FU tautomers. This is the first study of its kind to utilize the DFT/B3LYP/6-311++G(dlp) method and UVRR in the context of 5FU. A series of simple spectroscopic analyses and DFT calculations allowed the tautomeric forms to be easily deduced., Experimental 5-FU was easily distinguished from GCV ((UV, IR; UVRR(1^{st})) and FBAL(IR).

Chapter III

FORCED DEGRADATION KINETICS

III.1 INTRODUCTION

Simulating environmental relevant degradation conditions in order to demonstrate the effects they have on these compounds is extremely vital because it gives reason to further pursue to try and validate with the real thing, whether it be directly or indirectly. Showing various environmental conditions that may degrade these type of molecules helps in painting a picture that certain degradation may in fact take place within the environment. Even further, showing how synergism places a role with respect to multiple degradation conditions makes the overall goal in getting these pharmaceuticals regulated, more complicated, but in fact could simplify the expected requirements needed for regulation too. Condition effects on degradation may be synergistic which in reality is hard to ascertain concrete values on so more broader terms may be drawn when the time comes to regulate.

The design for these forced degradation studies involve investigating the effects that different environmental conditions may have on the degradation of 5-FU. While doing so, there was also an interest in seeing if any synergism or additive effects may be demonstrated via merely comparing results from similar experiments where only differences involves independent condition effects versus combined.

III.2 EXPERIMENTAL

III.2a) The thermal/alkaline study began with a sterilization process using a predetermined amount of 18 MΩ water further sterilized in an autoclave (Tuttnauer Sterilizer HP Model 40). Next, the sample supply prep involved the dissolution of 0.025 mM 5-FU in aqueous solvent (from the autoclaved water). The pH was then raised to ~12.0 using NaOH (50%/50%/Fisher Scientific). Preliminary studies reflected that two weeks was sufficient time required to run the experiment. After the sample supply was prepared, an aliquot (~3 mLs) was put into a 1 cm UV quartz cuvette then run on the UV instrument (Cary 50 UV Varian system/UV-Vis Spectrophotometer) for the experiment's t_0 measurement. Other aliquots were taken from the sample supply and placed into 10mL glass tubes and capped tightly. The aliquoted samples were placed into individual wells of aluminum heat blocks. These samples were then heated to 90°C using a hot plate (Corning). The temperature was constantly monitored using a standard glass thermometer (Germany/serial#A-0905000847) placed in a sample well in the heat block. Thereafter, at various times(daily for 7 days) throughout the experiment, the aliquoted samples were pulled from the heat block, one per designated time, allowed to cool, and then measured with the UV.

III.2b) The thermal/acidic study began with a sterilization process using a predetermined amount of 18 MΩ water further sterilized in an autoclave (Tuttnauer Sterilizer HP Model 40). The sample supply prep involved the

dissolution of 0.025 mM 5-FU in aqueous solvent (from the autoclaved water) The pH of the sample was lowered to ~ 3.5 using HCl(12.1 Normality/Fisher Scientific). Preliminary studies reflected that the experiment's duration be two weeks. The sample supply was prepared to form an aliquot (~3 mLs) and was placed into a 1 cm UV quartz cuvette. The sample was then run on the UV instrument (Cary 50 UV Varian system/UV-Vis Spectrophotometer) for the experiment's t_0 measurement. Other aliquots were taken from the sample supply and placed in 10mL glass tubes which capped and tightly sealed. The aliquoted samples were then placed in individual wells of aluminum heat blocks and heated to 90°C using a hot plate (Corning). The temperature was constantly monitored using a standard glass thermometer (Germany/serial#A-0905000847) placed in one of the sample wells of the heat block. Thereafter, at various times throughout the experiment, the aliquoted samples were pulled from the heat block, one per designated time, allowed to cool then measured with the UV.

III.2c) The thermal/saline study began with a sterilization process using a predetermined amount of 18 MΩ water further sterilized in an autoclave (Tuttnauer Sterilizer HP Model 40). The sample supply prep involved the dissolution of 0.025 mM 5-FU in aqueous solvent (from the autoclaved water)which was made saline through the addition of NaClO (Fisher Scientific) sufficient to make a solution with concentration of 0.005 g/mL (5.0 mg/mL). Preliminary studies reflected that the experiment's duration be from seven to nine hours. The sample supply was prepared forming an aliquot (~3 mLs) and was

placed into a 1 cm UV quartz cuvette. The sample was then run on the UV instrument (Cary 50 UV Varian system/UV-Vis Spectrophotometer) in order to determine the t_0 measurement. Other aliquots were taken from the sample supply and placed in individual 10mL glass tubes. The aliquoted samples were then placed into individual wells of aluminum heat blocks and heated to 90°C using a hot plate (Corning.) The temperature was constantly monitored using a standard glass thermometer (Germany/serial#A-0905000847) which was placed in a sample well of the heat block. Thereafter, at various times throughout the experiment, aliquoted samples were pulled from the heat block, one per designated time, allowed to cool then measured with the UV.

III.2d) The photolytic/alkaline study basically began with a sterilization process with a predetermined amount of 18 MΩ water further sterilized in an autoclave (Tuttnauer Sterilizer HP Model 40). Afterwards, the sample supply prep involved 0.025 mM 5-FU dissolved in aqueous solvent (from the autoclaved water)where pH was raised to ~ 12.0 with use of NaOH(50%/50%/Fisher Scientific). Preliminary studies reflected that the experiment's duration be ~ 1 day long so after the sample supply was prepared an aliquot (~3 mLs) was put into a 1 cm UV quartz cuvette then ran on the UV instrument (Cary 50 UV Varian system/UV-Vis Spectrophotometer) for the experiment's t_0 measurement. Afterwards, a certain amount (predetermined) was taken from the sample supply and put into 50mL glass bottle with a screw-on cap. The bottle of sample was then put on top of UV lamp (UVGL-15/Compact UV Lamp with 254/365nm UV

settings) exposing it only to the 254nm side. Thereafter, at various times throughout the duration of the ~ 1 day + experiment, ~3 mL of the sample exposed to UV light was drawn from the vessel using 3mL graduated disposable transfer pipets(Samco Scientific, Mexico), filtered with plastic syringe(BD 20 mL Syring Luer-Lok Tip), allowed to cool then measured via UV.

III.2e) A relevant preliminary experiment tested the stability 5-FU may have in various conditions where nitrogen was involved. This work called for 0.099mM 5-FU to be mixed in solution with 10mM NH_4OH, Urea and $(NH_4)_2SO_4$ independently. Standard UV analysis was set to be used to monitor any changes 5-FU may undergo immediately after being added with each. This effort was proposed after learning that a prior study [25] suggested that most microbial related degradation in nature involves a nitrogen source while another source[26] asserted that 5-FU was subjected to more degradation when dissolved in solution with a Tris buffer(has Nitrogen) than it is in alkaline solution.

III.3 RESULTS

III.3a) The thermal/alkaline experiment(s) demonstrated a trend typically related to degradation (albeit not completely) over the course of time with the application of heat (~90°C)(Figures15/16). Figure 11 shows that the initial UV spectra (grey/top) at t_0 before applying heat taking on a shape uncharacteristic of 5-FU in regular unaltered aqueous solvent. This seems to suggest that 5-FU is deprotonated or undergone a minor tautomeric change. Figure 12 reflects marginal deviations about each respective absorbance measurement mean. This

mean was taken at set times during each one week experiment. This serves as a starting point and is analogous to other degradation experiments. Thereafter UV analysis was carried out on samples subjected to no elevated temperatures (grey spectrum), one day (blue spectrum) of elevated temperatures, two days (green spectrum), three days (brown spectrum), four days (magenta spectrum), five days (cyano spectrum), six days (red spectrum) and seven days (purple spectrum). An inverse relationship was observed between the time a number of like samples subjected to elevated temperatures, and an absorbance max of approximately 266 nm consequently, this study was significantly more successful than its counterpart experiment, the Thermal/Acidic degradation study.

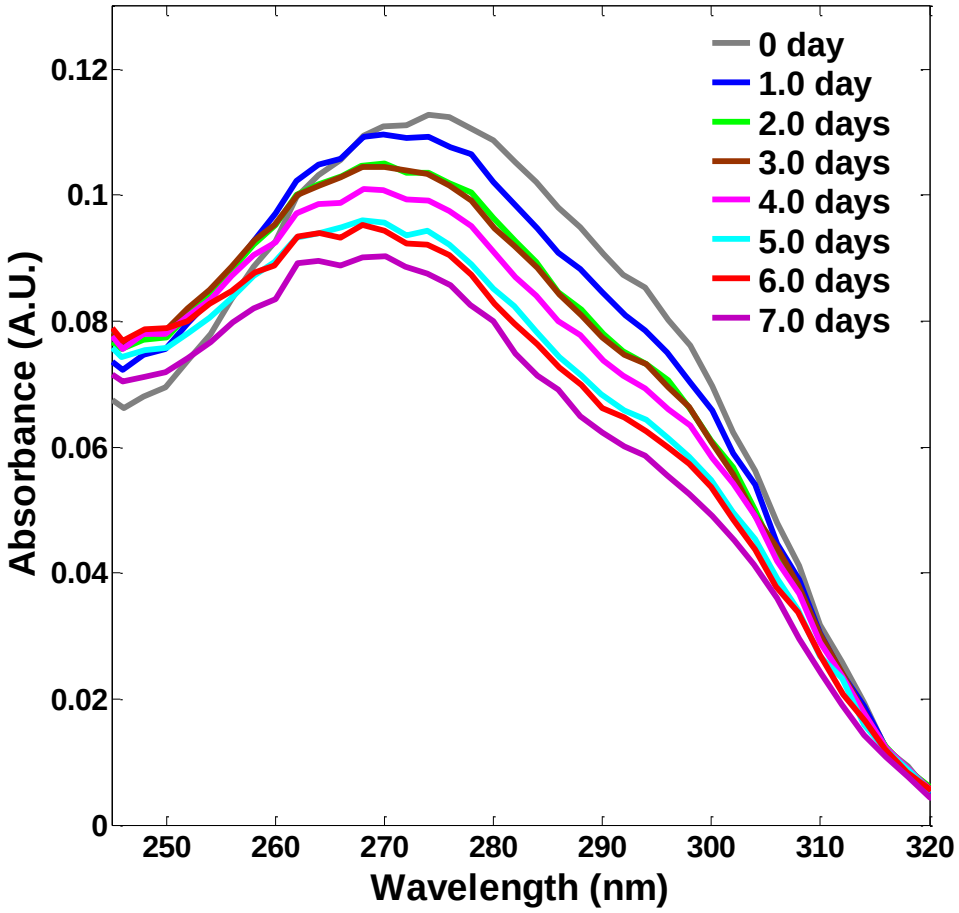

Fig. 11 UV analysis of ~ 0.025 mM FU in alkaline solvent at ~ pH 12 (n=2) at room temperature(0 day) and subsequent analyses of ~0.025 mM FU at pH 12 subjected to 90°C temperatures for 1, 2, 3, 4, 5, 6; 7 days.

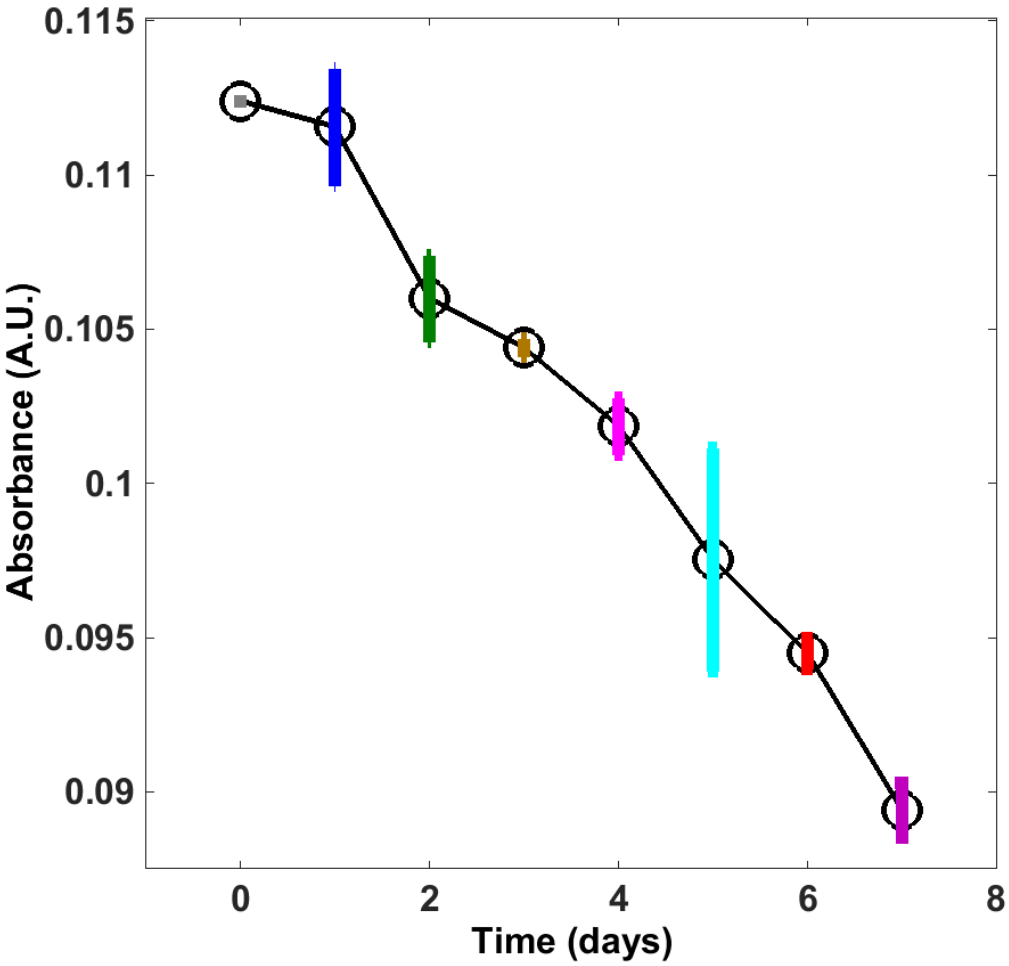

Fig. 12 UV deviation from mean spectra for thermal/alkali experiments (x3) for ~ 0.025 mM FU in alkaline solvent at ~ pH 12.

III.3b) The thermal/acidic experiment(s) as seen in figures 13/14 reflect minimal degradation as function of time similar to samples subjected to elevated temperatures. Figure 18, however, reflects great deviation about the mean between these three replicate experiments but they all balance out with the rest of the error bars from different days absorbance values measured. Prior studies have shown varying results for experiments of a similar nature. Therefore, it is likely that the compound undergoing acidic degradation stress may account for this observable difference. 5-FU is considered a weak acid and thus, less affected by thermal/acidic stress compared to thermal/alkaline stress as one might expect.

III.3c) The thermal/salinity experiment(s) analogous with prior two experimental studies with respect to the time measurements were taken. The duration of the experiment was less than 1 day while requiring elevated temperatures in order to get a gradual decrease in absorbance max with respect to the t_0 (see grey spectra/figs 15/16). Substantial degradation was interpreted after the first measurement at hour one which is seen with the gray spectrum (figure 15). The overall perceived degradation is much greater than that demonstrated in the thermal/alkaline study. The trend and spectra shapes are all different as well. Thus, it may be easy to assume that the degradation pathways between the thermal/alkaline and thermal/saline may be at least slightly different while the mechanisms may differ vastly. This thermal/saline experiment which used NaClO showed complete degradation within only nine hours and is a first with respect to studies of this kind on 5-FU.

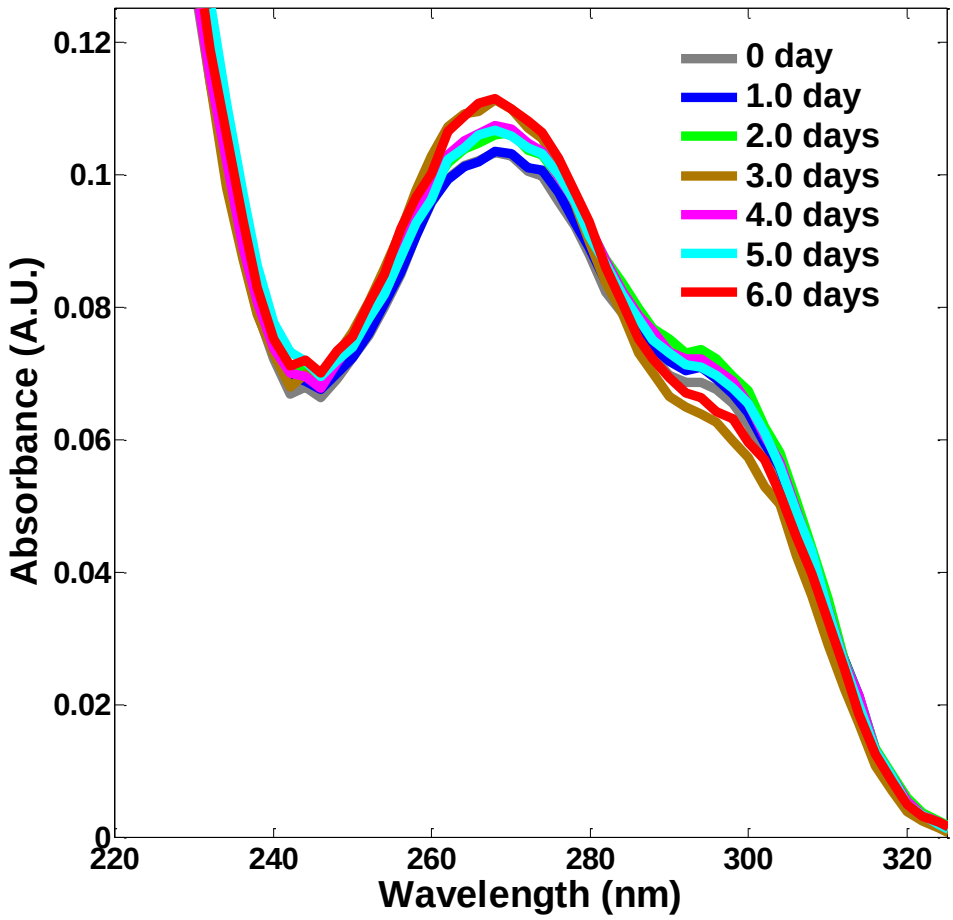

Fig. 13 UV analysis of ~ 0.025 mM FU in acidic solvent (~ pH 3.5) at lab temperature(0 day) and subsequent analyses of ~0.025mM FU at pH 3.5 subjected to 90°C temperatures for 1, 2, 3, 4, 5; 6 days.

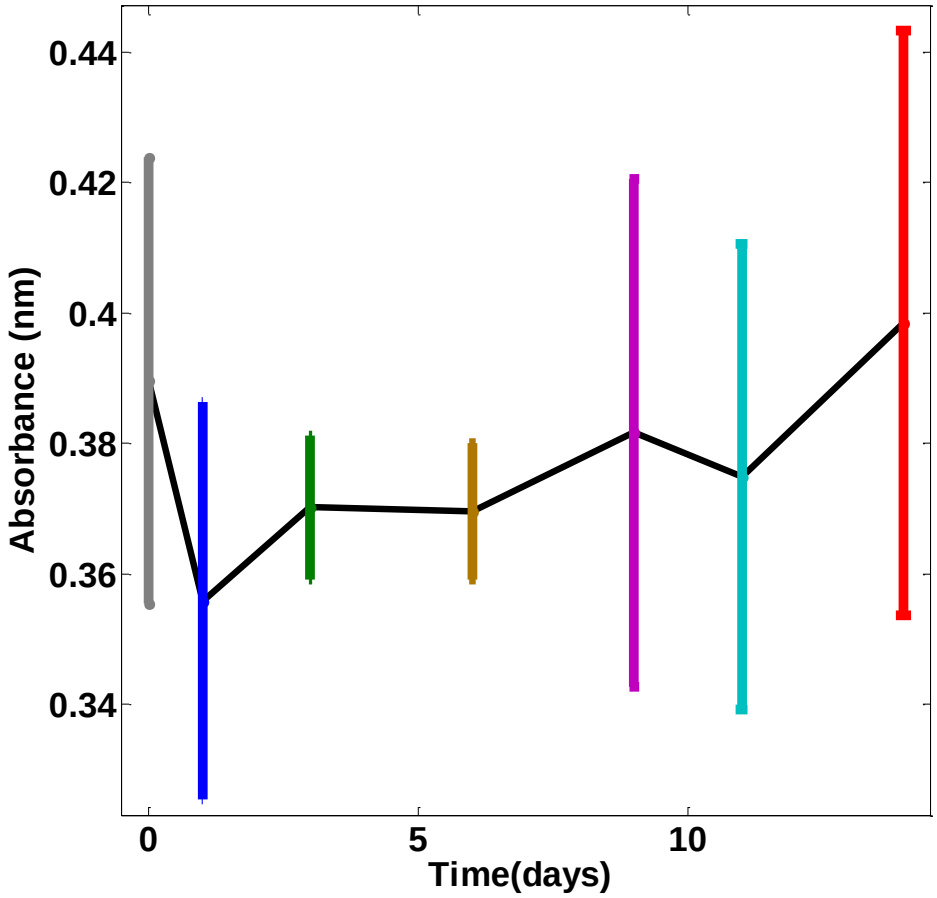

Fig. 14 UV deviation from mean spectra for thermal/acidic experiments (x3) for ~ 0.025 mM FU in acidic solvent (pH= ~3.5) at lab temperature(0 day) and subsequent analyses of ~0.025mM FU at pH 3.5 subjected to 90°C temperatures for 1, 2, 3, 4, 5; 6 days.

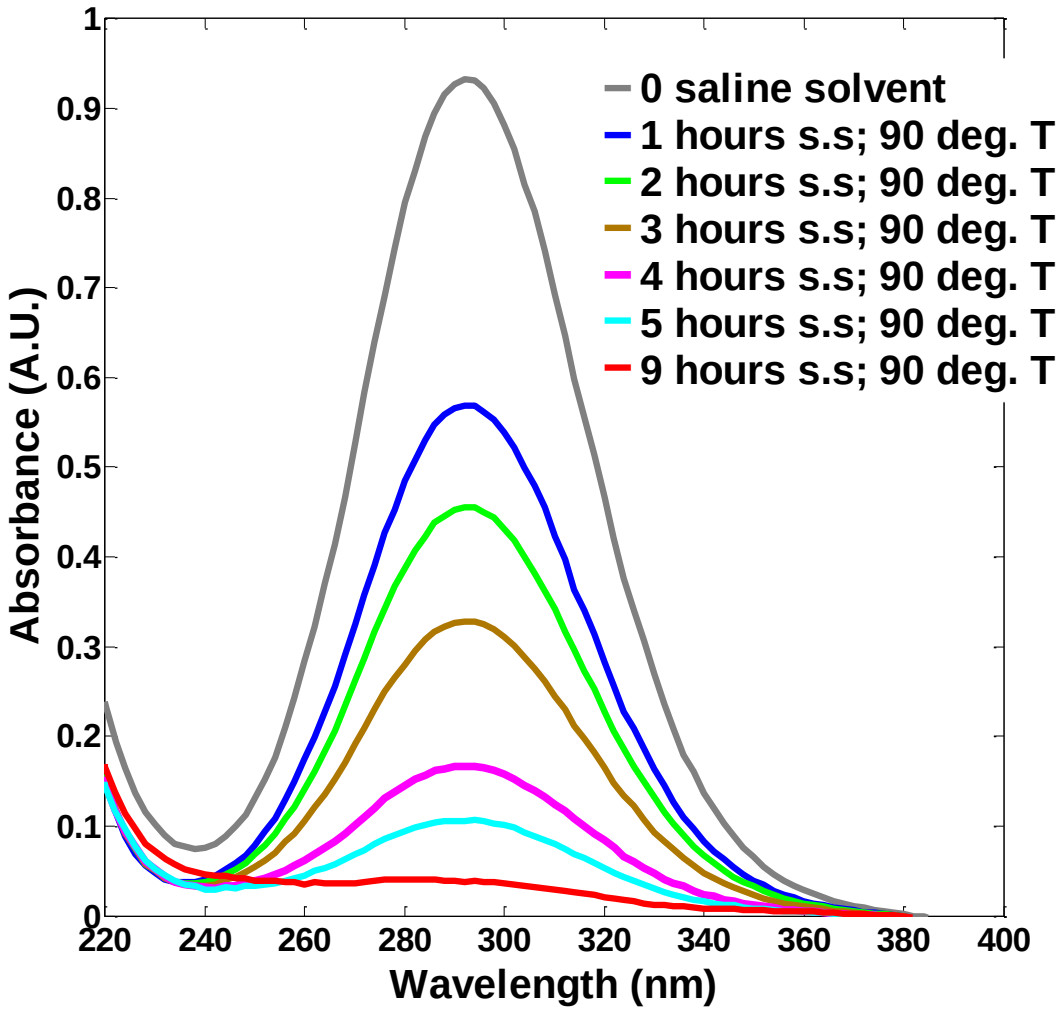

Fig. 15 UV spectra for ~0.025 mM 5-FU in saline sln. ([NaClO= 0.005g/mL]/n=3) and subsequent analyses of ~0.025mM FU in saline sln. ([NaClO= 0.005g/mL]/n=3) subjected to 90°C temperatures for 1, 2, 3, 4, 5; 9 hours.

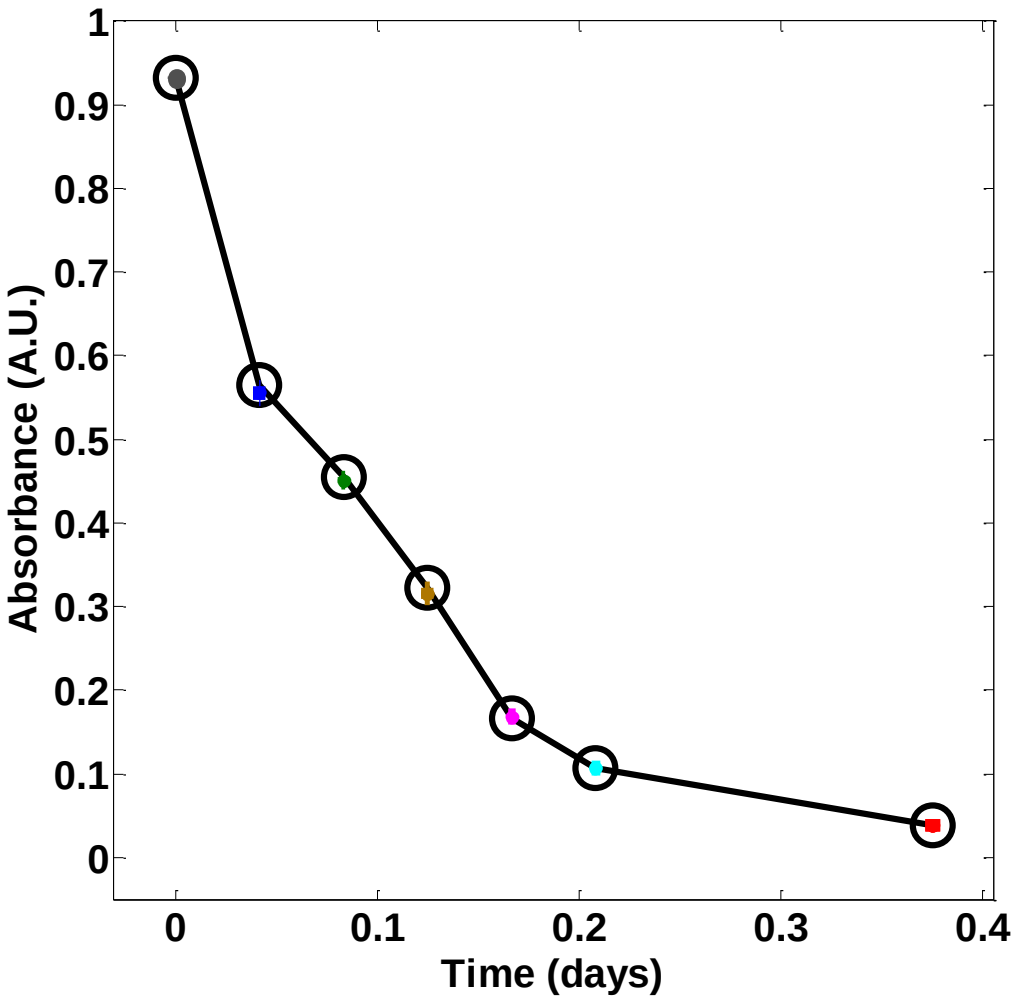

Fig. 16 UV deviation from mean spectra for Thermal/Saline experiments (x3) for ~ 0.025 mM FU in saline solvent([NaClO] = 0.005g/mL) subjected to 90°C temperatures for 0.1, 2, 3, 4, 5; 9 hours .

III.3d) Photolysis along with alkalinity with assistance of TiO_2 needed only a day or so to get 5-FU to fully degrade. Figures 17/18 indicate that taking measurements at only a few minutes after applying photolytic light of 254nm produced dramatic decrease of the absorbance max, almost spontaneously. Regardless, these measurements, do reflect a trend until the absorbance max at ~ 266nm is completely gone. This is noted after just more than a day of subjection to UV light. Aside from the kinetics being significantly faster than those of Thermal/Alkaline but comparable though slightly slower than that of Thermal/Saline, the degradation route may be most similar to Thermal/Alkaline because of the obvious initial deprotonation at t_0. Thereafter, the photolytic/alkali (PAC) study is most similar to the Thermal Saline because of the obvious mechanisms(radicals) involved which effectively facilitate complete degradation.

III.3e) Some preliminary results may be seen as very useful for future though not fully pursued here. Figure 19 shows that the 5-FU structure changes in presence of NH_4OH, urea and $(NH4)_2SO_4$. Though no further changes or degradation were observed as function of time, it would be interesting to see each combined with elevated temperatures(thermolysis). Upon carrying out such experiments, it would be even more interesting to see how expected enhanced degradation compares with thermal/alkaline results. Figures 20 and 21 are the 13C nmr spectra of the FU-tris mix that was made before 03-21-13. This particular spectra was run on, 04-19-13. It was determined that the 5-FU portion of the spectra appears as anticipated with no significant modifications. It appears to be in its keto state. However, further analysis reflects that the C5 (carbon that

was attached to hydrogen in the 5-FU only spectra) peak is significantly lower in intensity than the analogous C5 peak within the 5-FU-Tris spectra (compare fig. 12 5-FU inset with fig. 21). One can conclude that the Tris complex is bonded to C5. The absence of hydrogen means the Nuclear Overhauser Effect (NOE) is not as relevant. The C5 peak is therefore much weaker than might have been anticipated were it bonded to a hydrogen atom.

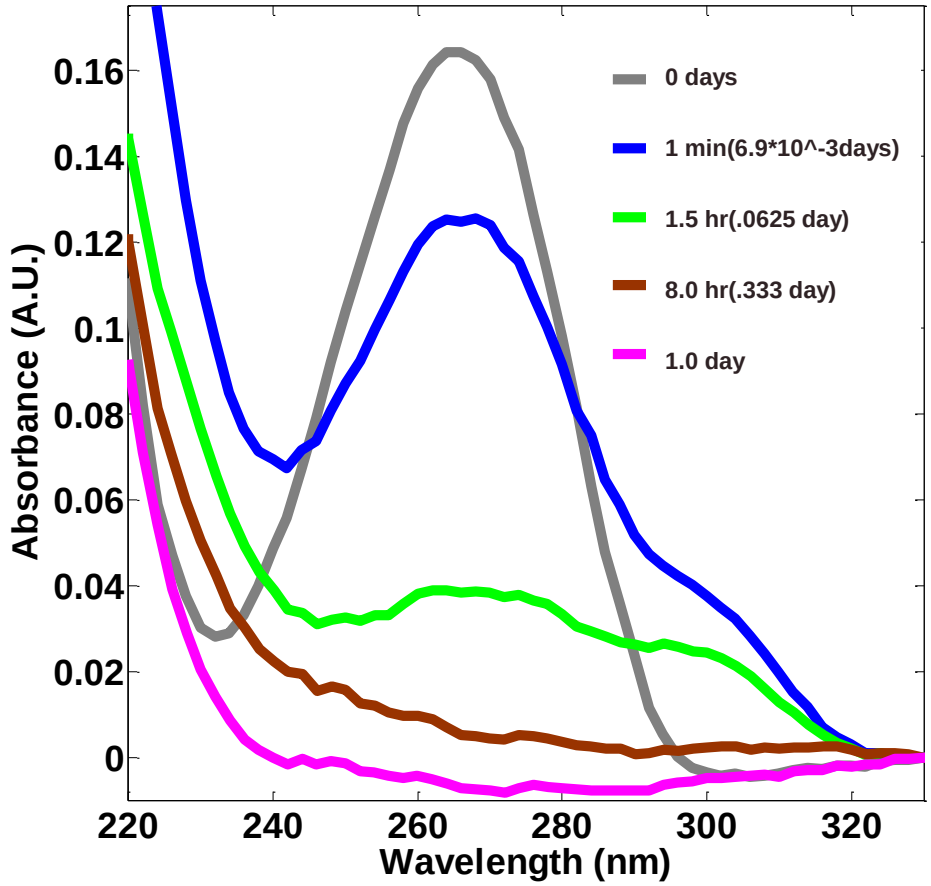

Fig. 17 UV analysis of ~ 0.025 mM FU in alkaline solvent (~ pH 12.4) at lab temperature followed by subjection to photolytic stress (254nm) w/TiO_2 (n=3) for various times leading up to one day.

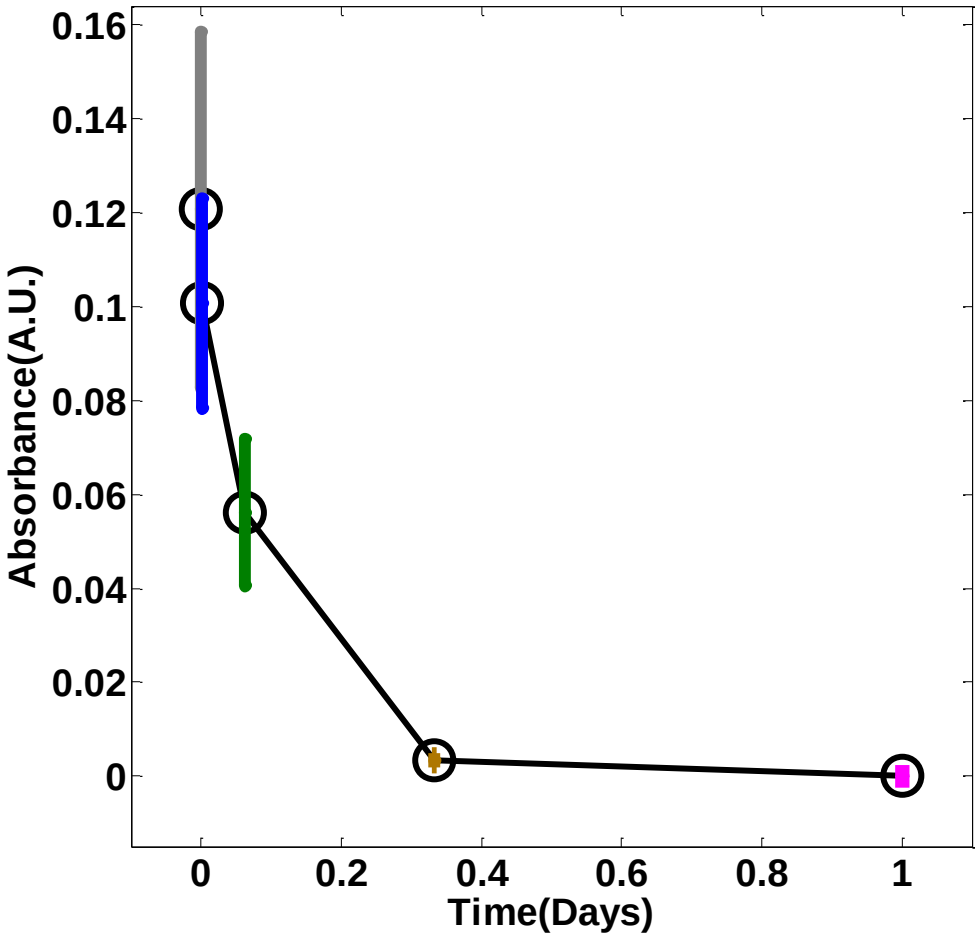

Fig. 18 UV deviation from mean spectra for photolytic/alkaline experiments (x3) for ~ 0.025 mM FU.

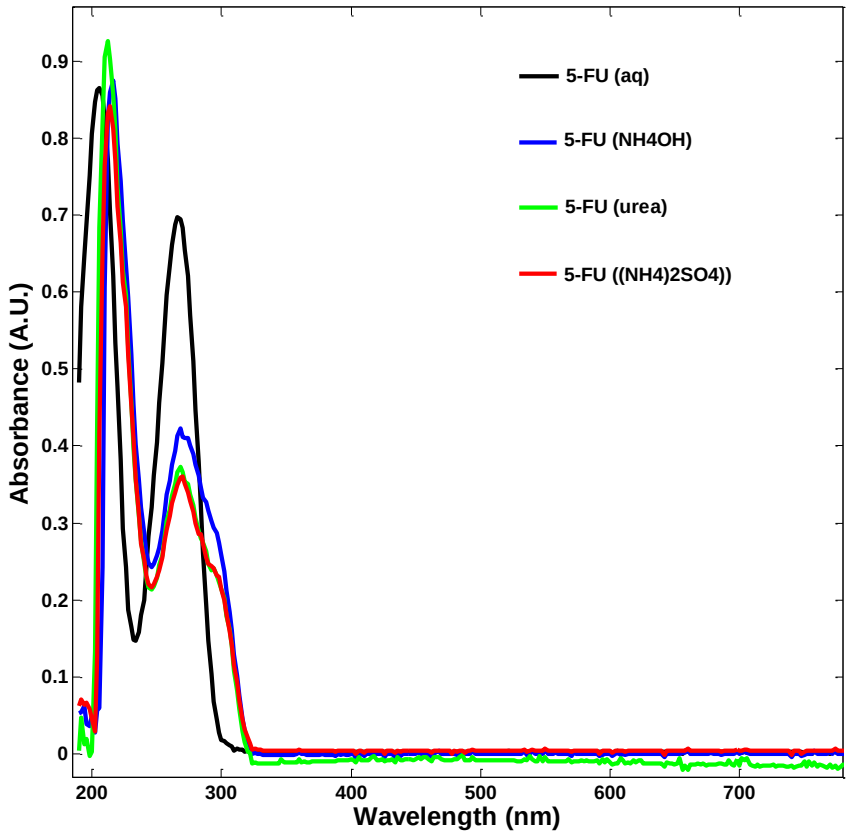

Fig. 19 UV analysis of 0.099 mM FU in 10.0 mM NH4OH, Urea; $(NH_4)_2SO_4$.

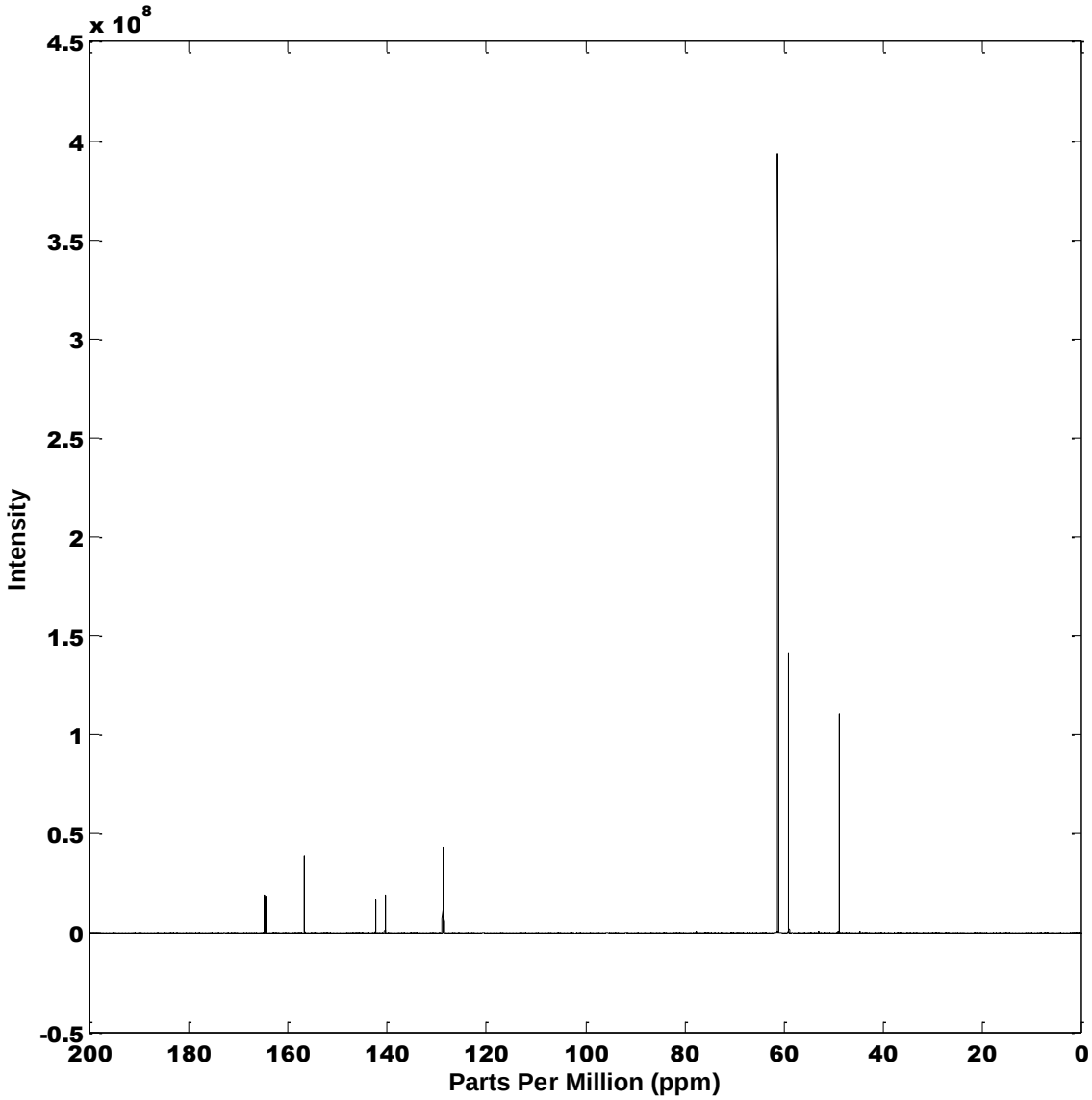

Fig. 20 ^{13}C NMR of 0.381 M 5-FU-Tris.

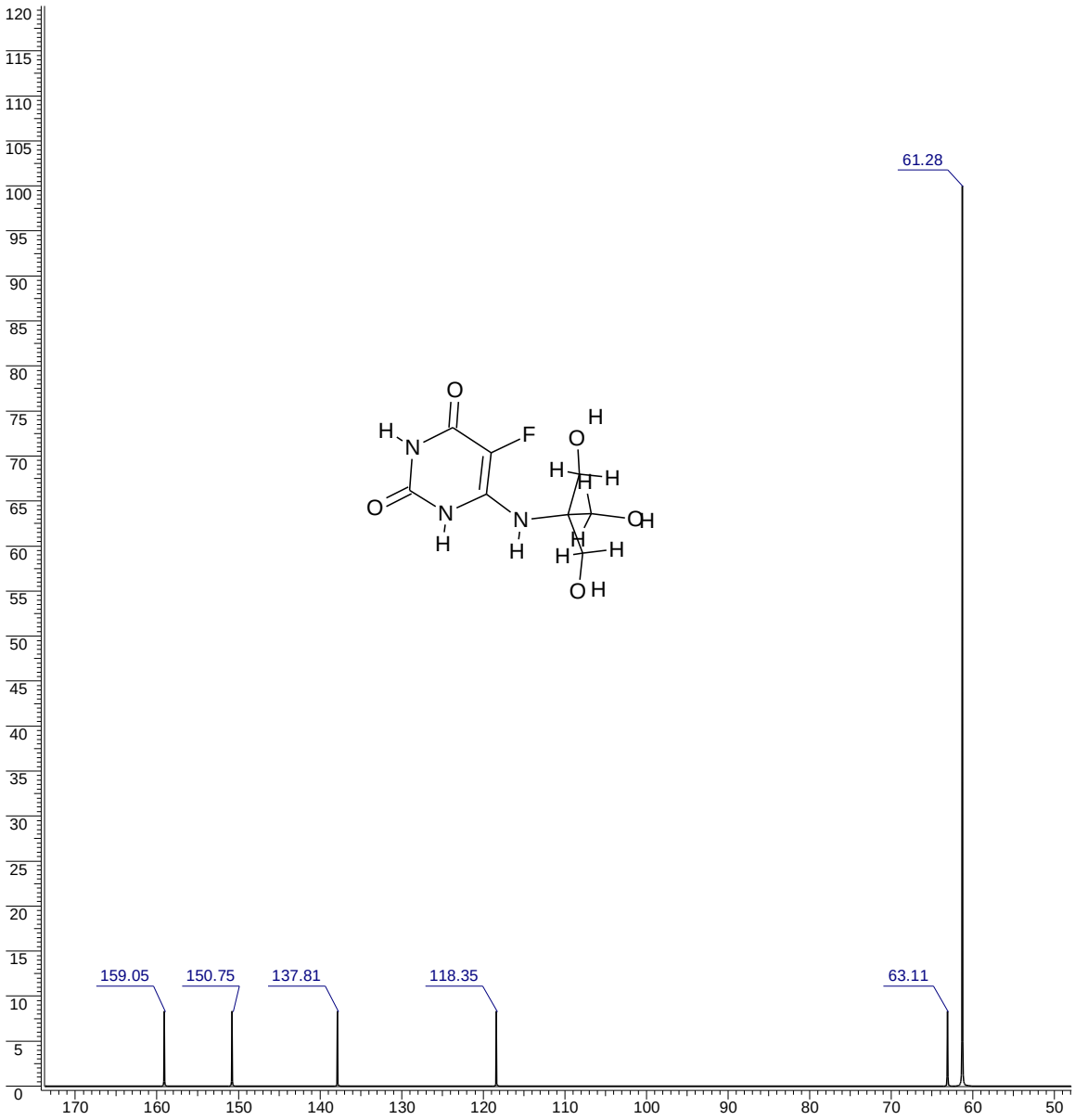

Fig.21 ^{13}C NMR prediction calculation (ACD) on the 5FU-Tris complex with connection via NH.

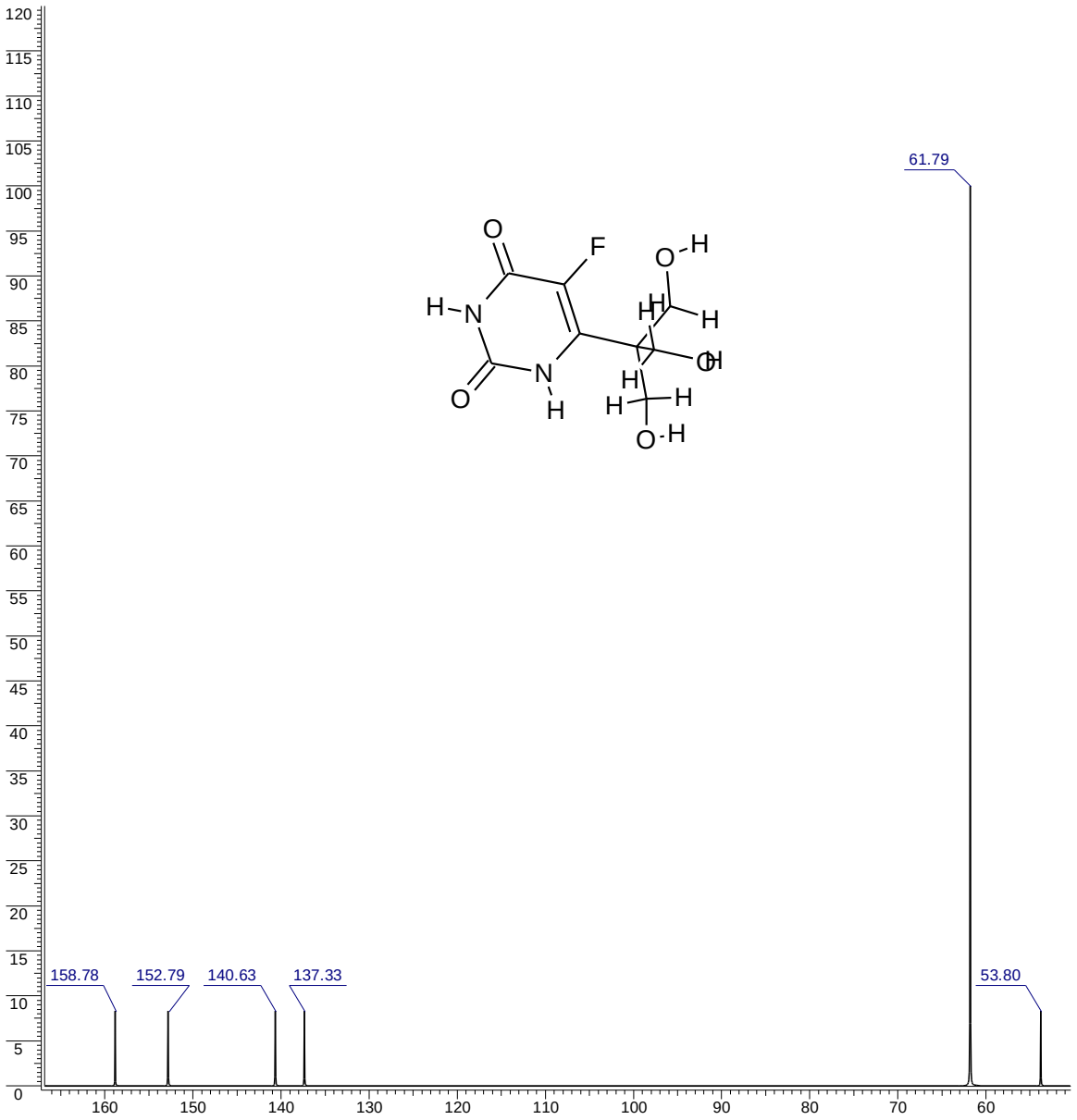

Fig. 22 ^{13}C NMR prediction calculation (ACD) on the 5FU-Tris complex with connection directly to C(4°).

Upon analysis of the Tris complex (($H_2NC(CH_2OH)_3$))component of the FU-Tris spectra(fig. 20), different peaks are observed which relate to the presence of three other types of differently situated carbon atoms.. These are not affiliated with carbons within the 5-FU complex. Turning our attention to the Tris complex, one can see only two types of differently situated carbons and that is the quaternary carbon (total of one). Additionally there are three secondary carbons. The peak of the greatest intensity corresponds to the secondary carbons. The smaller peak then corresponds to the quaternary carbon. An ACD prediction of the Tris complex confirms this . 5-FU-Tris (fig. 20), both smaller peaks are situated to the left of the larger one.. It is anticipated that Tris is bound to the C_5 is supposed to be through the Tris' NH component. The quaternary carbon which is attached to the NH should have a greater NMR shift than the secondary carbons due to lesser electron density. Clearly, there exists a departure from what is anticipated with respect to the FU-Tris complex. No NH is likely present because the smaller peak exists to the right of the larger one. It can therefore be deduced that the FU-Tris complex is bonded with C_5 of FU directly to the quaternary carbon of the Tris complex with no involvement from the complex's NH. This hypothesis was verified by performing ACD calculations on FU-Tris with NH(fig. 21) and FU-Tris without NH(fig. 22).

III.4 SUMMARY

The article by X Fei et al [27] suggest that the order of degradation on 5-FU(from best to worst) is Oxidative(1-3%) > Thermal only (2.5%) > Thermal & Humidity(2%) > Alkalinity(0.5%) > Acidic(0.1%) > Photolysis (figure 1 [27]) while F. K. Alanzi et al. [9] results suggest Oxidative(90.1%) > Thermal(87.8%) > Acidic(86.7%) > Alkalinity(83.17%) (figure 2/table 1[9]). Comparing those rankings with those here the following order emerges: Thermal/Saline > Photolytic/Alkali > Thermal/Alkali > Thermal/Acidic. The common parameter amongst all three rankings is with oxidative driven degradation. This yields the most significant form of hydrolysis on 5-FU. One can categorize these Thermal/Saline and Photolytic/Alkali studies are related due to the radical aspects during the most significant stages of the experiments. The complete hydrolysis of 5-FU was achieved by way of a unique oxidative hydrolysis method involving synergism. This will be the first of its kind to be published. Neither thermolysis, salinity, photolysis nor alkalinity by themselves would come close to completely degrading 5-FU. The reported ranking of oxidative being better than alkalinity which in term is better than acidic (omitting other conditions which are not applicable here) is most consistent with the ranking achieved here. One may say it may not be totally adequate to compare the rankings here with other rankings which did not invoke the same level of synergism because all of F. K. Alanzi et al. [9] experiments(except for Thermal) involved heating environment to only 60°C . Interesting enough, they got their acidic reactions to provide better hydrolysis than their alkali counterparts which greatly differs from what was

achieved here. Thus, the dynamics of these studies correlate with a greater emphasis of synergism than the prior studies.

CHAPTER IV

LC-MS/MS CHARACTERIZATIONS

IV.1 INTRODUCTION

Identifying degradation products that may arise from these forced degradation studies is essential because it, by itself, draws a link between the compound degrading and potential toxicity that may result thereafter. Synergism may play a major role here as well because who is to say exactly what is needed out in the environment in order for toxicity to exist with respect to human exposure, either via drinking water, eating, breathing or skin exposure. The complex ways that individual pharmaceuticals may come together and be harmful may be impossible to replicate or simulate in a lab. However, if it may be shown in very discrete ways that a small considered to be harmless amount of drug “a” exposed to condition “b” being mixed with a harmless amount of drug “c” may altogether produce a product that may have 100 or 1000x the toxicity potential than the individual constituents that produced it then there may be no need in pursuing the rest of the formalities in order curb toxicity exposures and prevent potential catastrophes.

The design of this experiment involved developing unique fragmental characteristics for each analyte to be screened. As a result, the monitoring of each analyte’s product ion characteristics facilitated identifying of degradation products within samples subjected to various degradation conditions.

IV.2 EXPERIMENTAL

IV.2 a) A method was developed in order to detect and quantitate 5-FU, FBAL, FUC and FUPA using a Waters LC system(Waters Acquity triple quadrupole mass spec/Phenomenex Kinetex C18/100mm x 4.6mm/2.6µm particle size reverse phase column) and MS/MS detector((Electrospray Ionization(EI) in the negative ion mode with multi reaction monitoring(MRM)). The mobile phase consisted of 10 mM ammonium acetate and 0.1% formic acid in water (A) and 100% acetonitrile (B). The gradient conditions were 0–0.5 min, 2% B; 0.5–7 min, 2–80% B; 7.0–9.0 min, 80–98% B; 9.0–10.0 min, 2% B; 10.0–15.0 min, 2% B at a flow rate of 0.5 ml/min. The MS/MS system was operated using electrospray ionization (ESI) in the negative ion mode with capillary voltage of 1.5 kV. The ionization source was programmed at 150 ^{o}C and the desolvation temperature was programmed at 450 ^{o}C. The MS/MS system was operated in the multi-reaction monitoring (MRM) mode and the collision energy was 30V. The molecular parent ions were screened and the product ions used for the quantifications were determined from the spectra obtained from injecting 30 µL of a standard solution containing 10mg/L of the pharmaceutical standards. The protonated molecule ion $[M - H]^-$ and its corresponding product ion spectrum are shown in Figure 23. Table 5 shows the masses of the $[M-H]^-$ parent ion and the masses of the product ions used in this study. The predominant fragment ion was selected as the product ion for quantification. Analytical data were processed using Waters software (Waters, CA, USA). The respective spectra and chromatogram for each standard were used to establish a screening mechanism

which would enable the detector to recognize if any of the corresponding analytes were recognized. These screening processes were set in a manner where for all four standards could be applied simultaneously (MRM) during each run (see table5).

Table 5 MRM analysis

Analyte	Precursor Ion ((m/z)	Product Ion (m/z)
5-FU	129	42
FUPA	149	149
FBAL	106	106
FAC	77	77

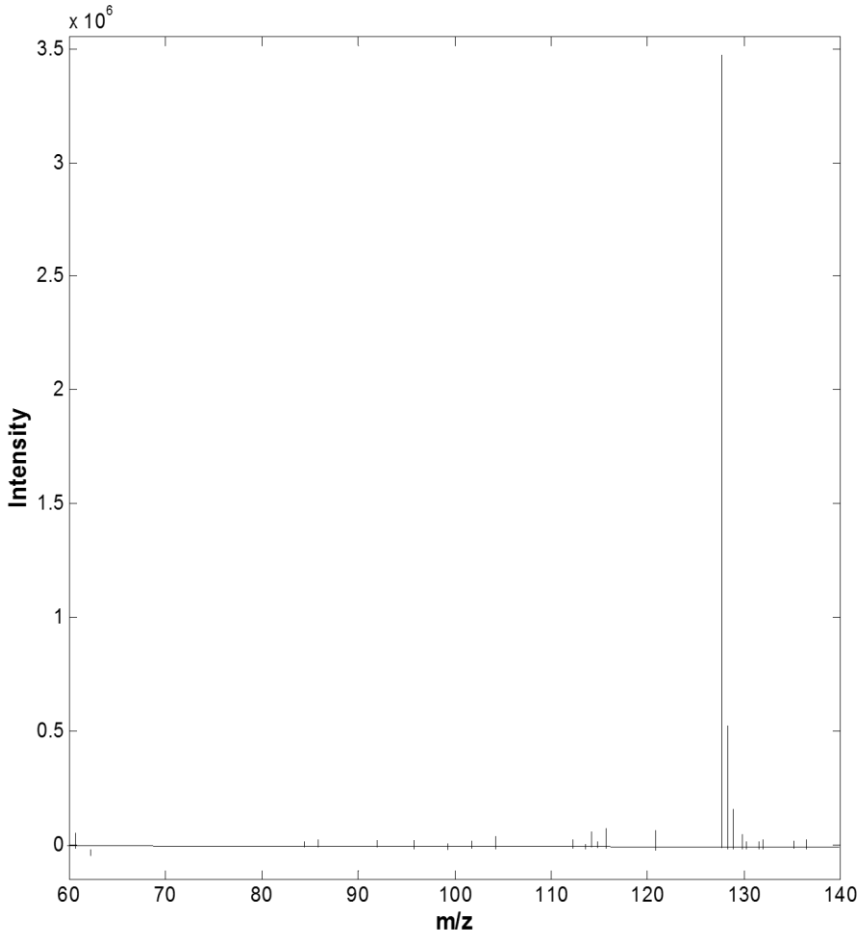

Fig. 23b **FU Mol Ion** standard method development in prep for calibrations

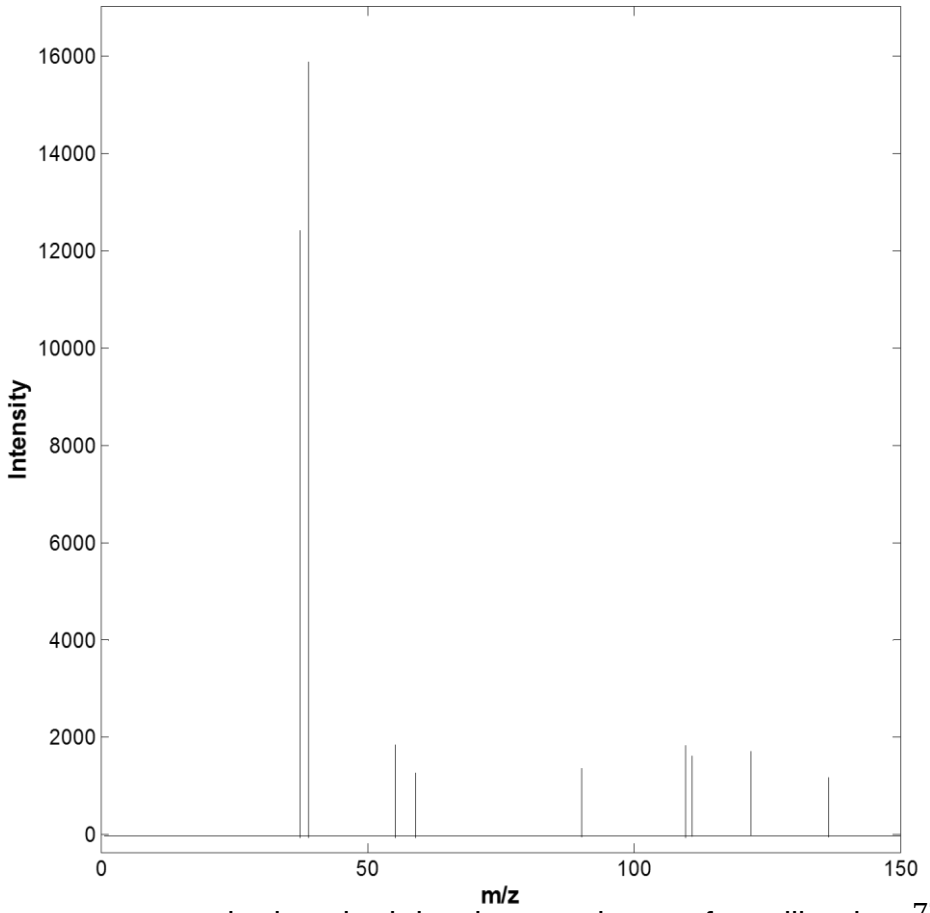

Fig. 23c **FU Product Ion S** standard method development in prep for calibrations

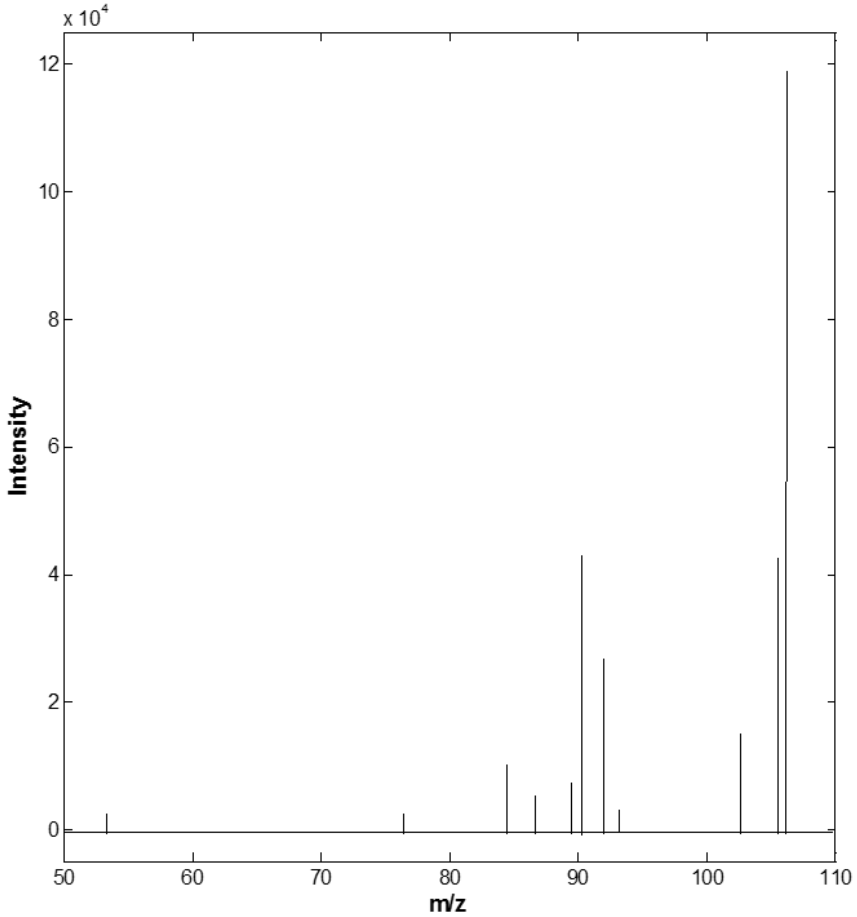

Fig. 23f **FBAL MoI Ion** standard method development in prep for calibrations

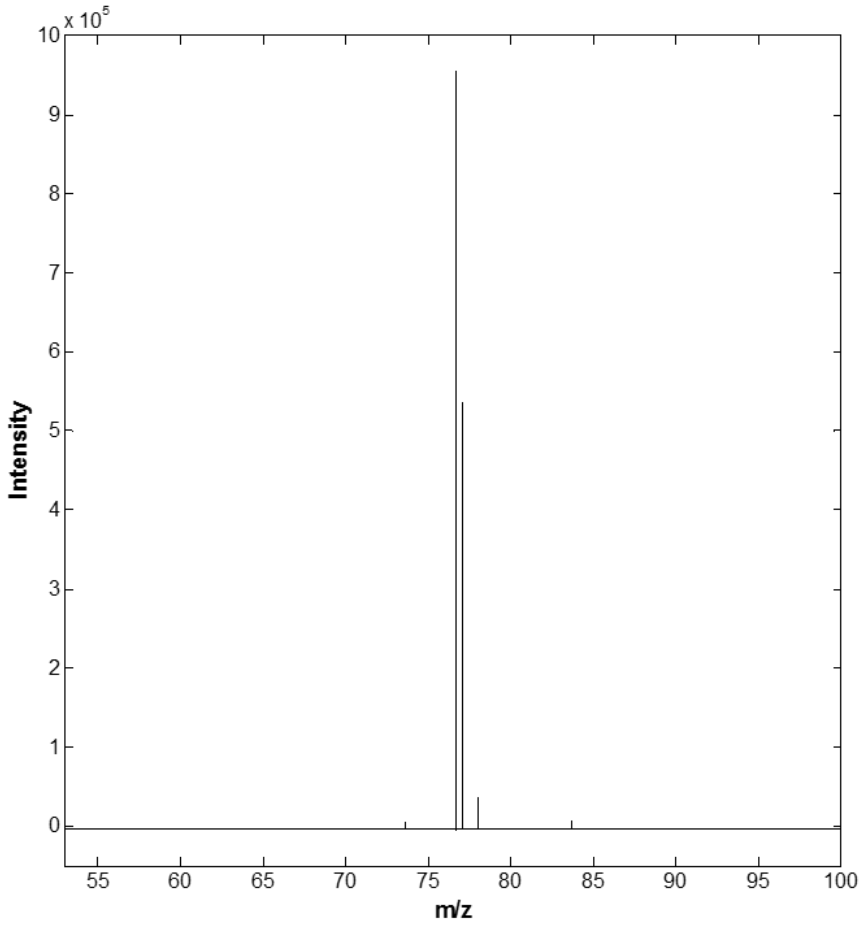

Fig. 23i **FAC MoI Ion** standard method development in prep for calibrations

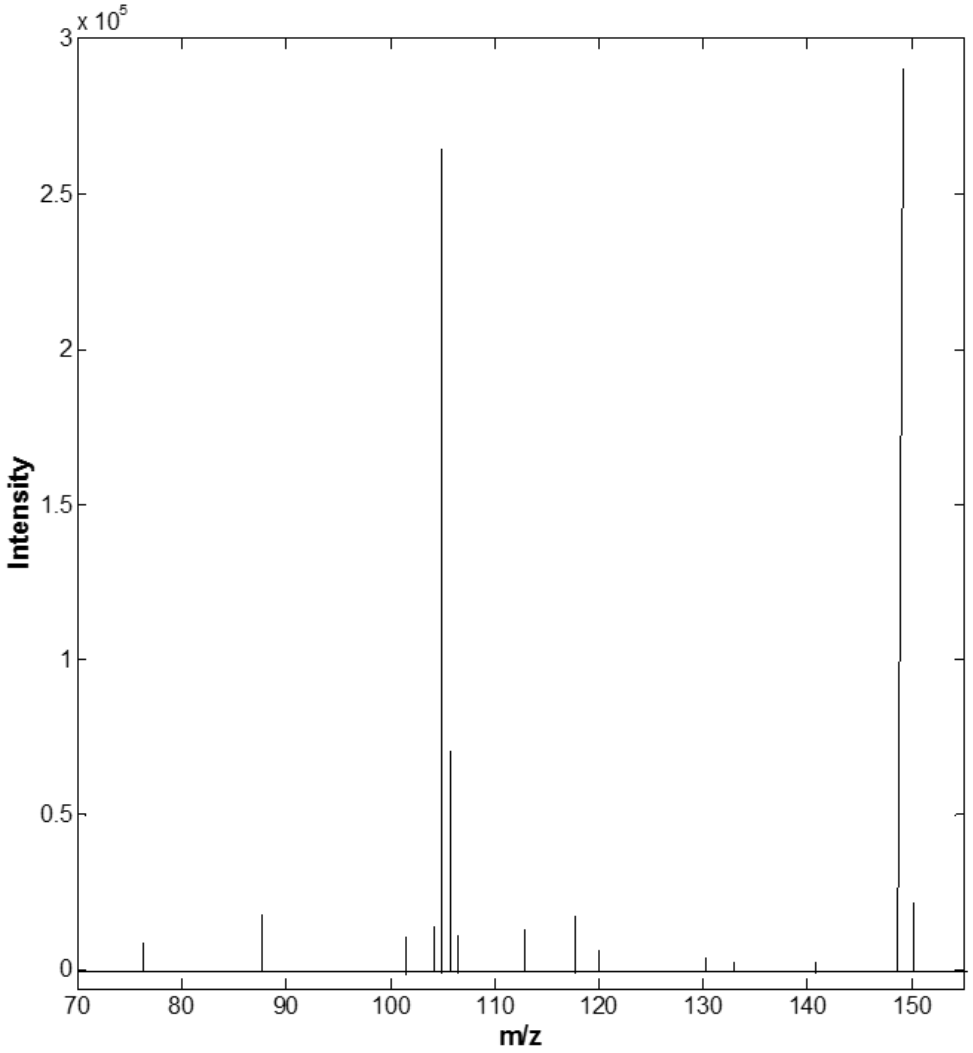

Fig. 23l FUPA Mol Ion standard method development in prep for calibrations

After the filtering processes (fingerprints) were set up for each of the standards, the instrumental setup was calibrated for those standards at concentrations of 5, 1, 0.5, 0.25, 0.10, 0.05 ppm. Mixtures for each calibration concentration were made with all the standards in the mixture for each designated concentration. This facilitated the calibration process. The construction of the calibration curves (see fig 24) served as means to be in position to quantitate those analytes detected in the samples subjected to the various degradation processes already outlined in Chapter lll.

IV.3 RESULTS

IV.3a) The thermal/alkaline study's samples were analyzed via LC-MS/MS in order to determine if 5-FU may have broken down to FUPA, FBAL or/and FAC. The same samples analyzed using UV were analyzed with LC-MS/MS. Figure 26a indicates that, 5-FU clearly degraded over time. Tandem MS analysis was capable of determining if any of the minimal degradation products screened for were produced whereas LC-UV could not. Retention times for each of the three degradation products were similar coming off the column. However, the screening for not only retention times of diagnostic ions, but the intensities of those diagnostic ions together with the molecular ions (~m/z) gives rise to a much more selective process. This enables the three compounds to be distinguished and quantitated if detected. Figures 26b, 26c; 26d reflect intensities of the respective product chromatograms which should correlate to the amount of analyte quantitated at each time of measurement. These analyses were replicated and reproducibility was confirmed. The degradation of 5-FU which

increased as a function of time was consistent with its analogous UV (figure 11) even though there was no appreciable quantitation of degradation products screened for(5-FU obviously reproducibly converts to a product this study didn't screen for). Although it could not be seen what 5-FU is breaking down to, this study demonstrated and confirmed that the Thermal/Alkaline method here may degrade the 5-FU compound significantly.

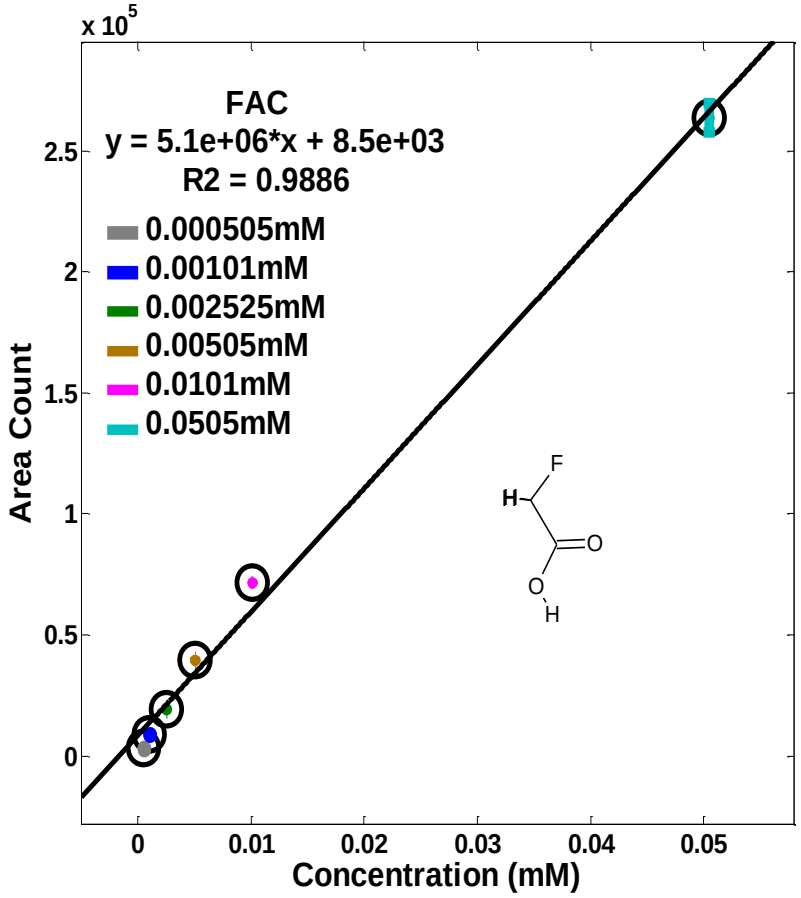

Fig. 24a FAC calibration plot

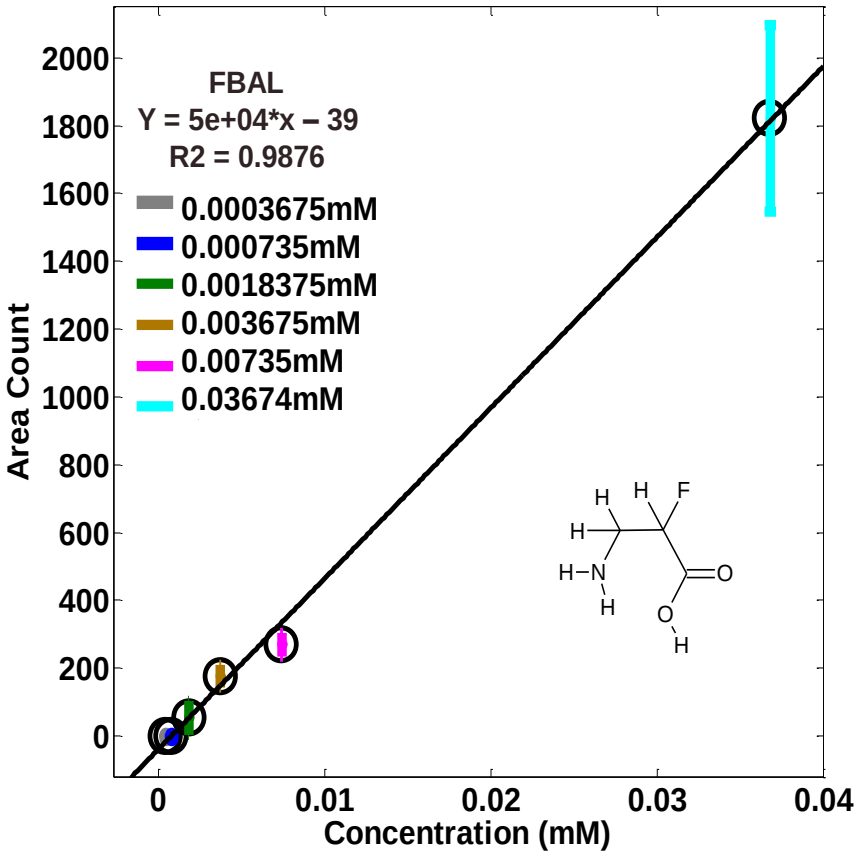

Fig. 24b FBAL calibration plot.

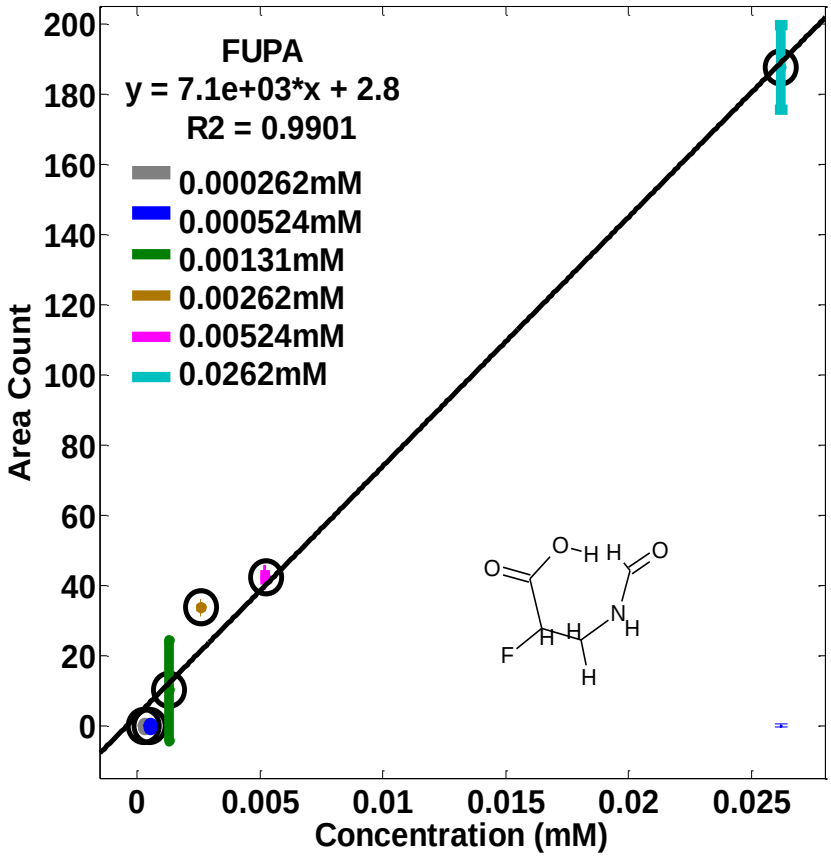

Fig. 24c FUPA calibration plot.

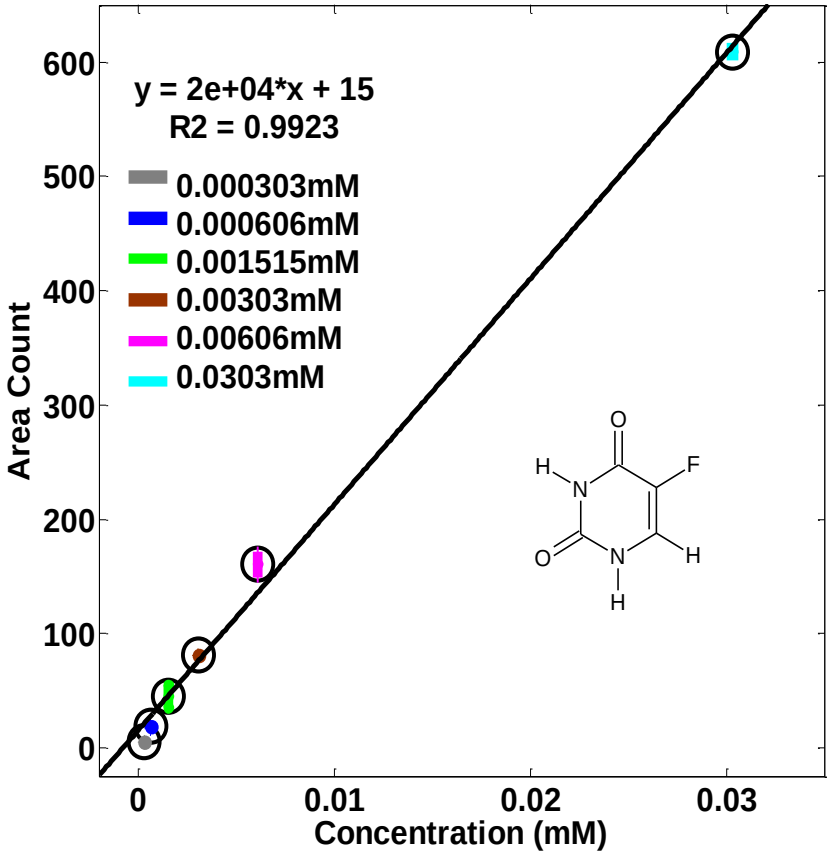

Fig. 24d 5-FU calibration plot

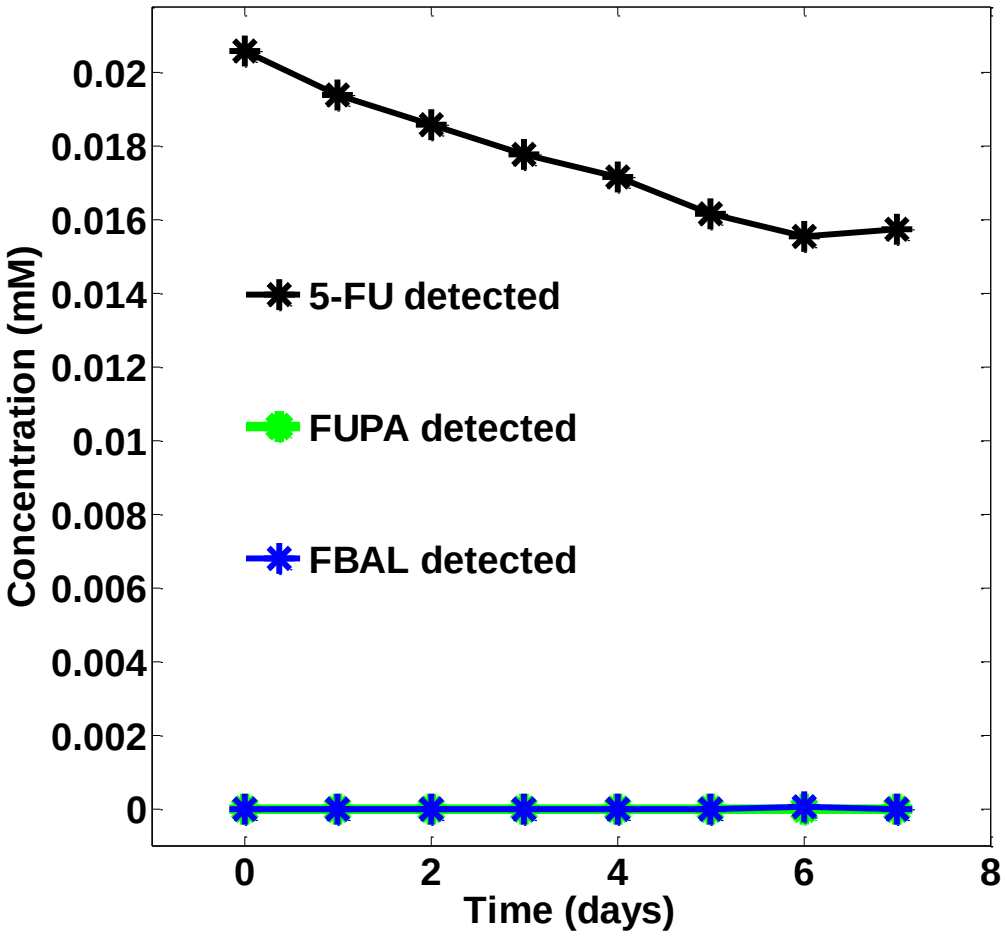

Fig. 26a Thermal/alkaline product chromatograph results in screening for 5-FU, FUPA; FBAL for the average of 3 measurements at each incremental measurement during the duration of the experiments.

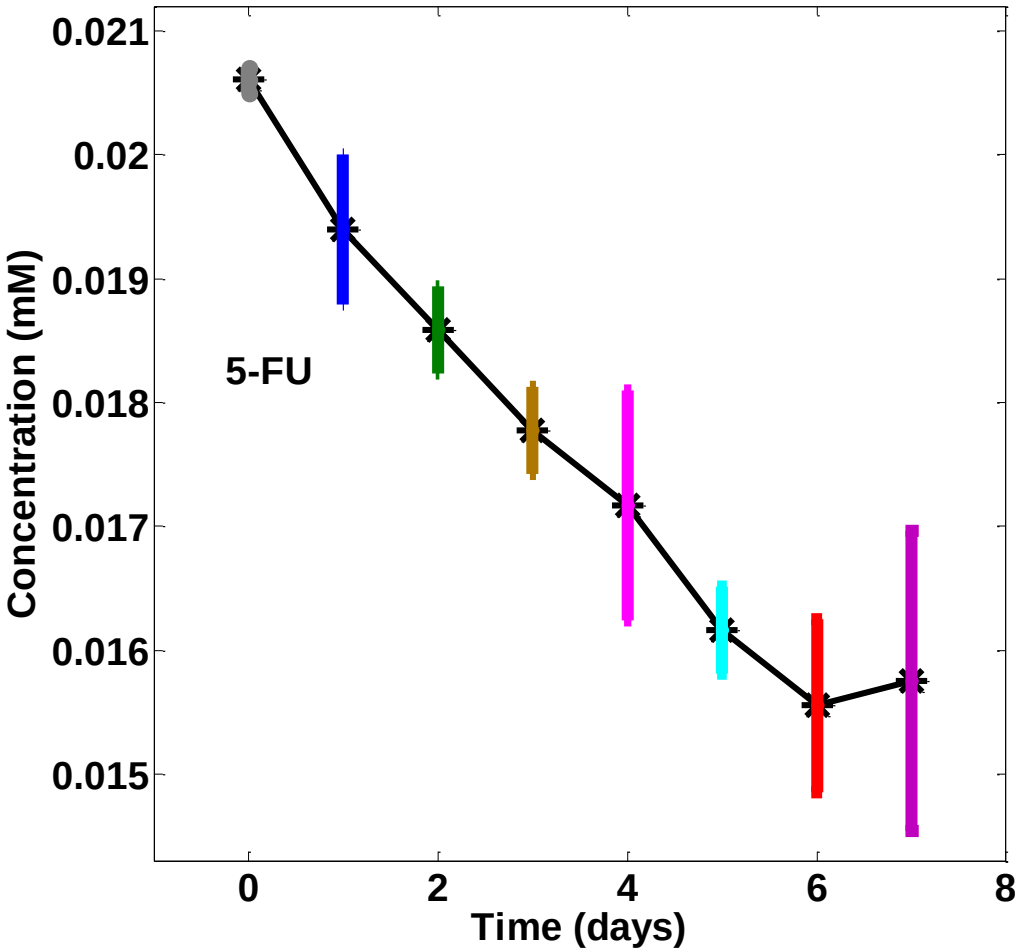

Fig. 26b Thermal/alkaline product chromatograph results in screening for 5-FU for the average and deviation of 3 measurements at each incremental measurement during the duration of the experiments.

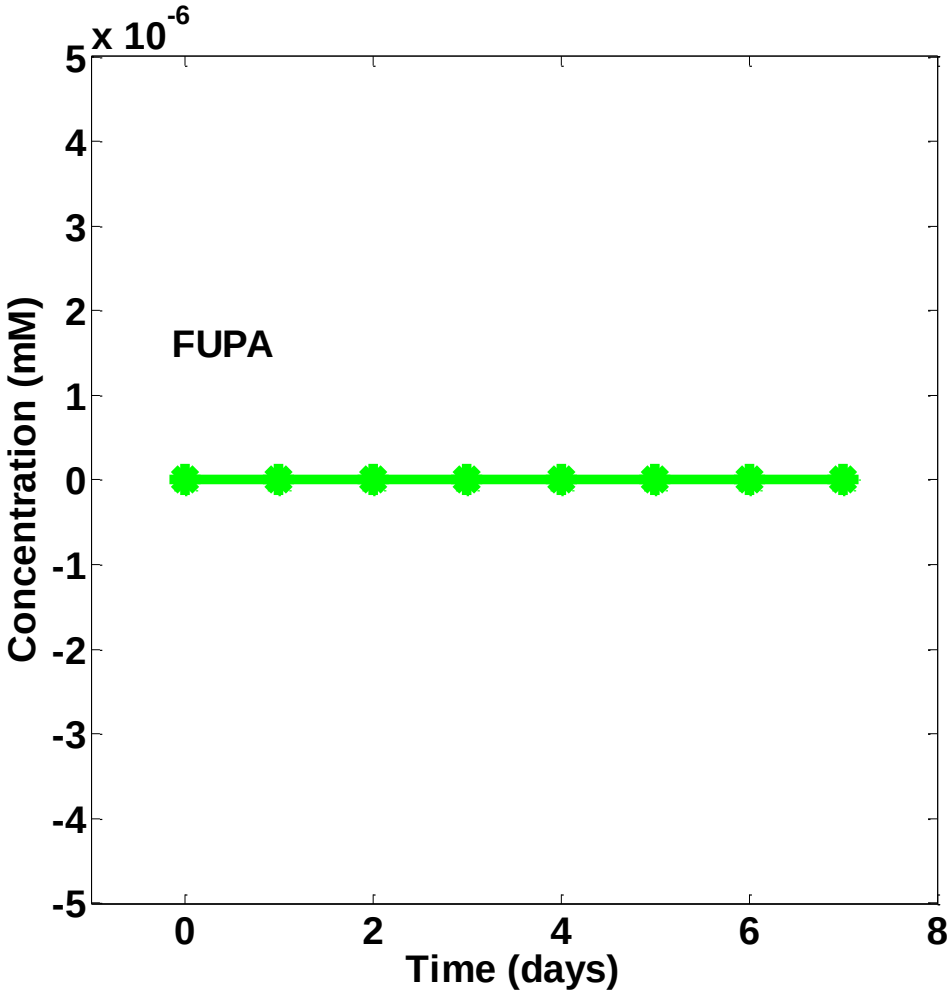

Fig. 26c Thermal/alkaline product chromatograph results in screening for FUPA for the average and deviation of 3 measurements at each incremental measurement during the duration of the experiments.

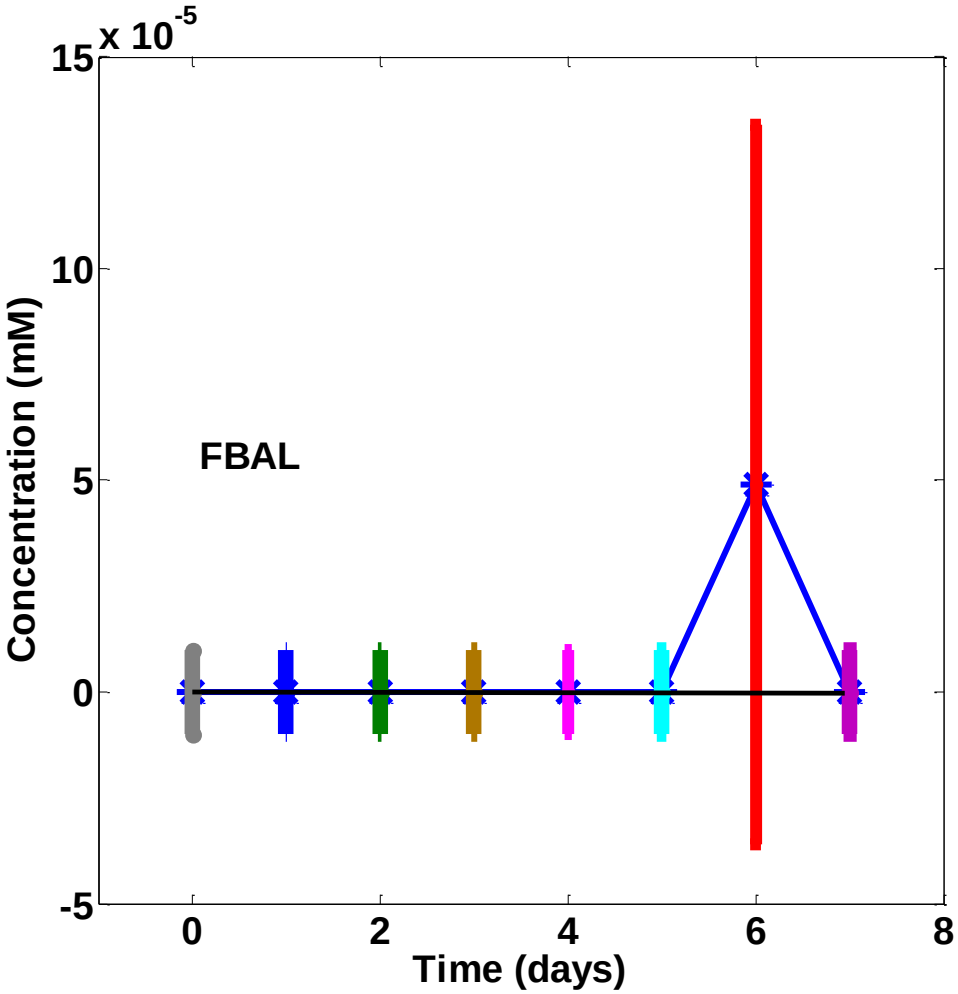

Fig. 26d Thermal/alkaline product chromatograph results in screening for FBAL for the average and deviation of 3 measurements at each incremental measurement during the duration of the experiments.

IV.3b) Figures 27(a-d) is consistent with the analogous figure 14 in regard to the thermal/acidic study. Nothing was ascertained from the UV spectra in regard to possible 5-FU degradation. However, traces of FBAL were detected which is fairly consistent with most history on these sort of degradation studies involving acidity. Thus, though not validated independently, it does affirm that the alkalinity rather than acidity induces a “synergistic” degradation effect on 5-FU when in combination with elevated temperatures.

IV.3c) Figures 28(a-d) show that the thermal/saline study’s degradation pathway may be very similar to the photolytic/alkalinity study though mechanisms are vastly different. FUPA was quantitated here too. Mechanistic-wise, the thermal/saline study should be most similar to the thermal/alkalinity study but the differences in magnitudes of degradation between the two overshadow that comparison. Other products or another major product is involved to which 5-FU converts to though it were not screened for here. Nevertheless, novelty was demonstrated with this thermal/saline study with it being the first to get 5-FU completely degraded.

IV.3d) The photolytic/alkaline study with assistance of TiO_2 (figures 29(a-d)) appears to be fairly consistent with what was seen within its analogous UV spectra (figure 21). One can see a consistent decline with respect to the amount of 5FU. Figure 22 definite reflects this more specifically. Here one will notice a substantial 5-FU analyte signal/peak (bottom/ gray) before applying photolytic light compared to no signal what so ever after 8 hours. The reanalysis of samples subjected to this study seems to indicate that 5-FU transformed into another

degradation product besides FUPA(obviously one of the several not accessible for this study). Consecutively, as 5-FU diminishes to nothing, another degradation product probably emerges as the sole analyte[25]. This supports the thinking that the degradation pathway is different from one involved with the thermal/saline and thermal/alkaline studies. The new photolytic/thermal study definitely and completely degraded the 5-FU molecule as figures 21 and 29 reflect. The degradation products screened for were not any of the major product(s) that 5-FU transforms to under these conditions. There may be only minor conversions to FBAL and FUPA during the experiment which can't be confirmed due to their such low detections. A similar study [25] got 5-FU to degrade into what they identified as a reduced(between C-F and C-NH2) analog of FBAL which would have a $[M-H]^-$ of 104 whereas FBAL's $[M-H]^-$ is 106. It is easy to assume that the mechanism behind their photodegradation process is similar to this study's with the generation of radicals such as °OH except that they used Bicarbonates and Nitrates to generate but here TiO_2 was used. Thus, novelty was still shown here with the complete degradation of 5-FU using a different environmental relevant means to generate radicals that may enhance or catalyze the photodegradation process. Arguably, the same major degradation product may have been produced here but that needs to be confirmed.

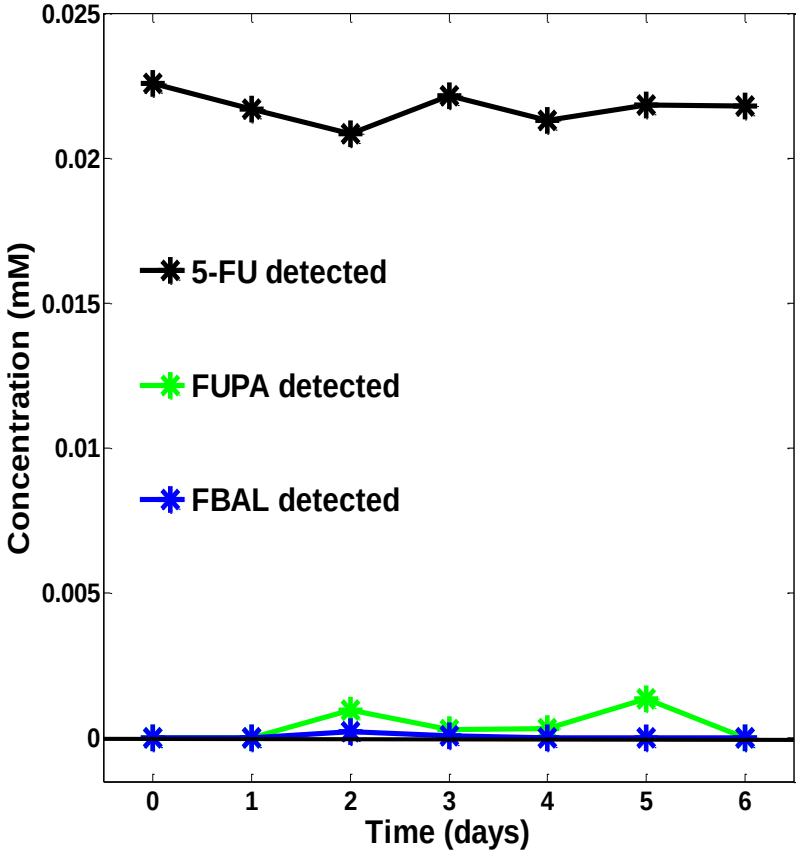

Fig. 27a Thermal/acidic product chromatograph results in screening for 5-FU, FUPA; FBAL for the average of 3 measurements at each incremental measurement during the duration of the experiments.

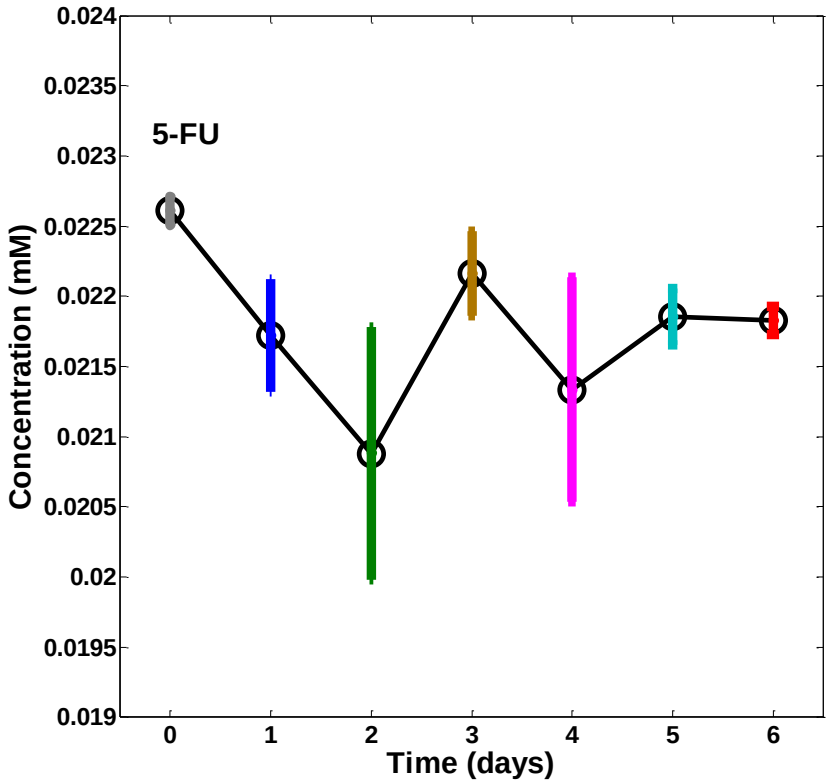

Fig. 27b Thermal/acidic product chromatograph results in screening for 5-FU for the average and deviation of 3 measurements at each incremental measurement during the duration of the experiments.

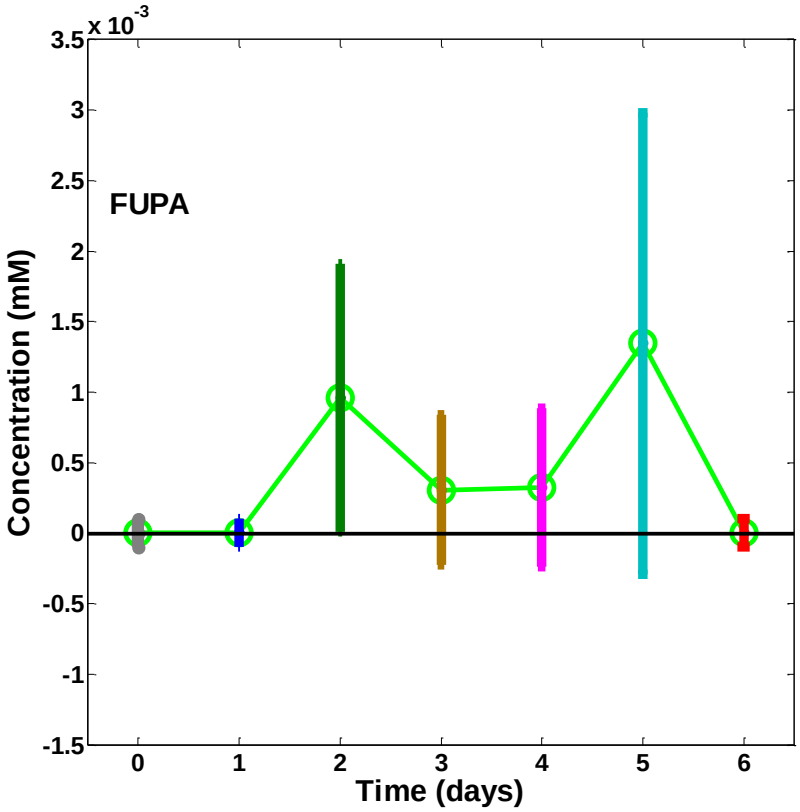

Fig. 27c Thermal/acidic product chromatograph results in screening for FUPA for the average and deviation of 3 measurements at each incremental measurement during the duration of the experiments.

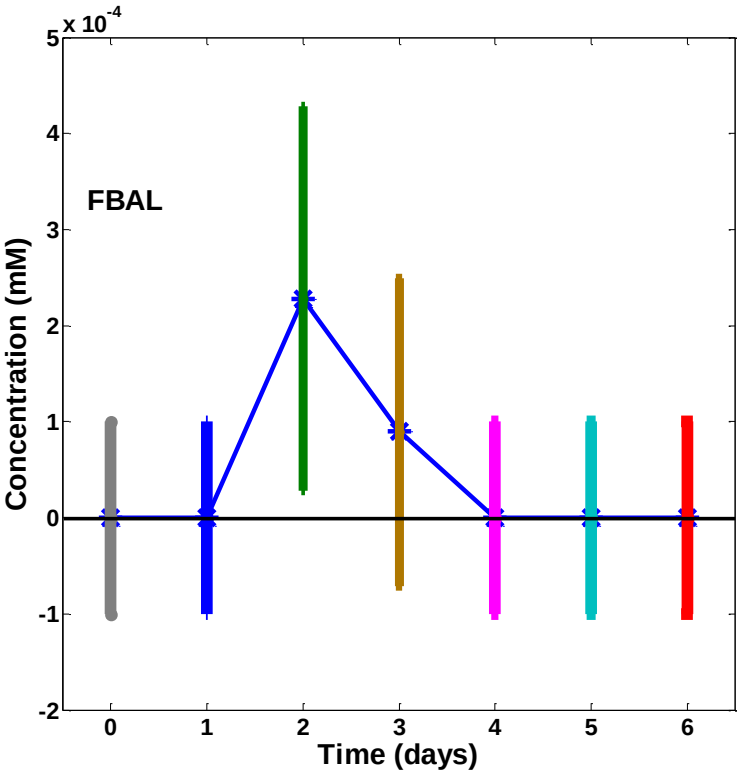

Fig. 27d Thermal/acidic product chromatograph results in screening for FBAL for the average and deviation of 3 measurements at each incremental measurement during the duration of the experiments.

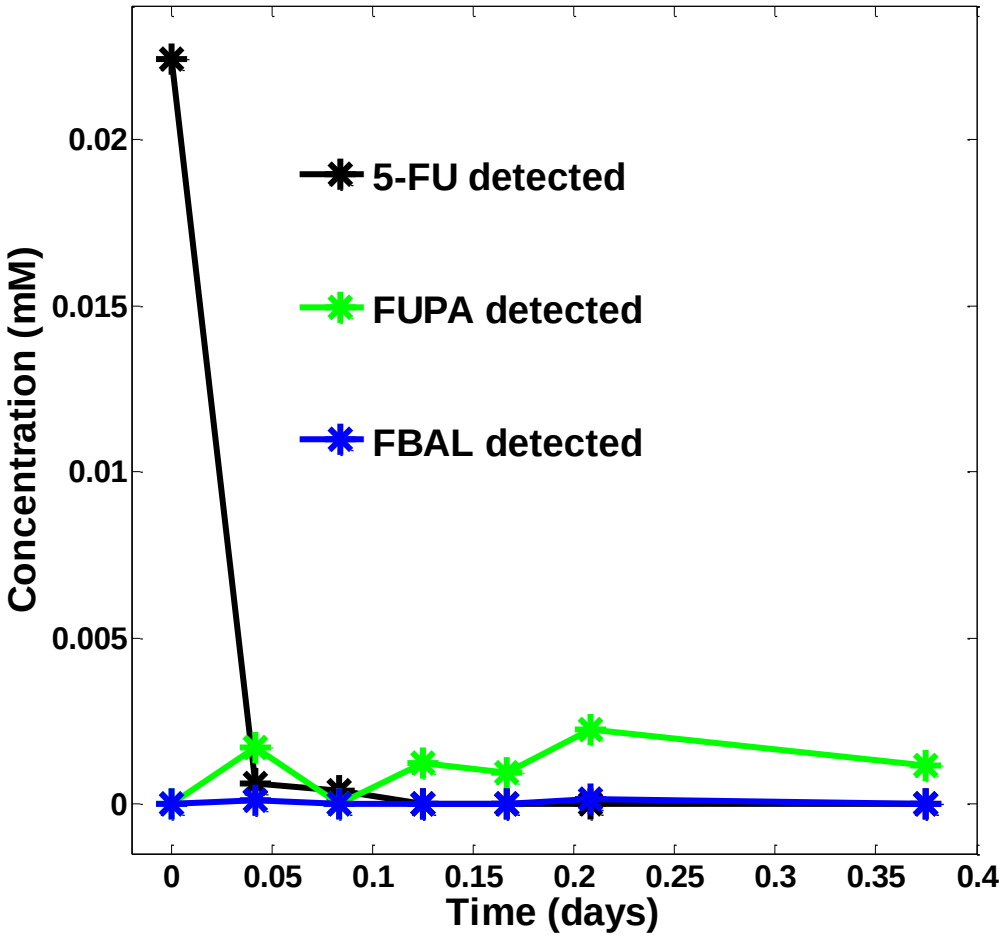

Fig. 28a Thermal/saline product chromatograph results in screening for 5-FU, FUPA; FBAL for the average of 3 measurements at each incremental measurement during the duration of the experiments.

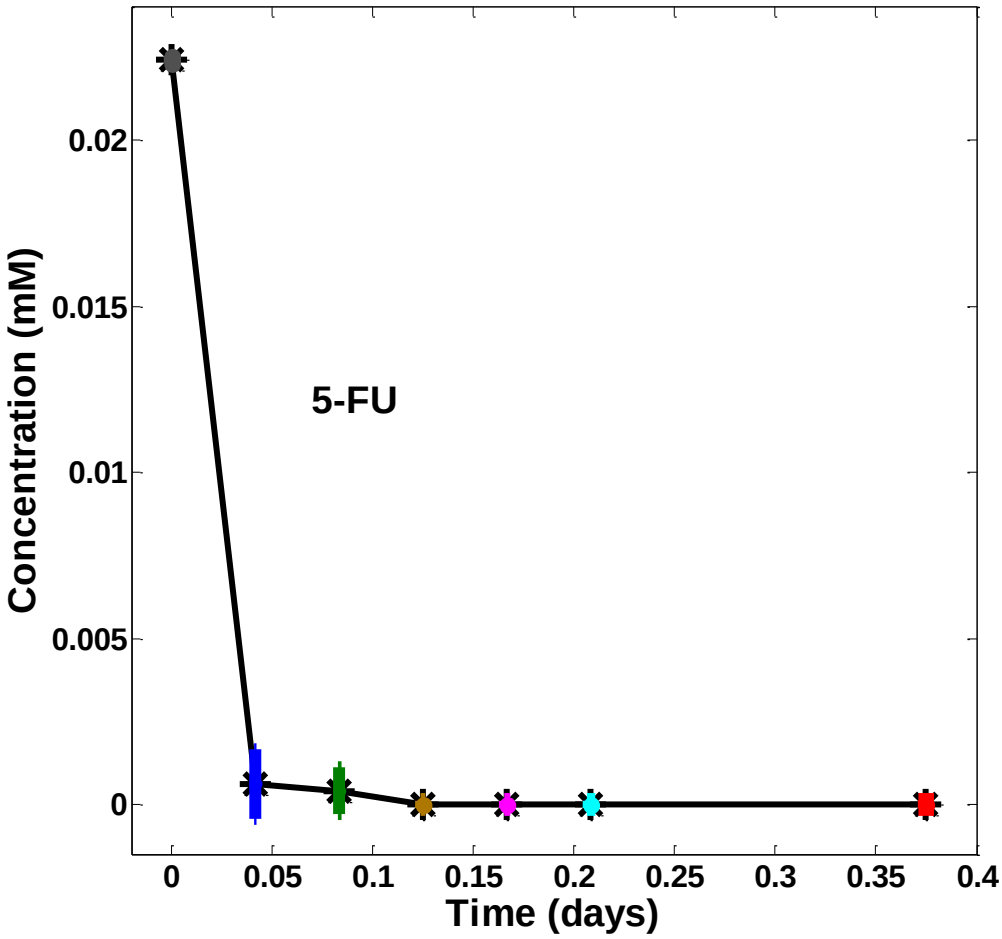

Fig. 28b Thermal/saline product chromatograph results in screening for 5-FU for the average and deviation of 3 measurements at each incremental measurement during the duration of the experiments.

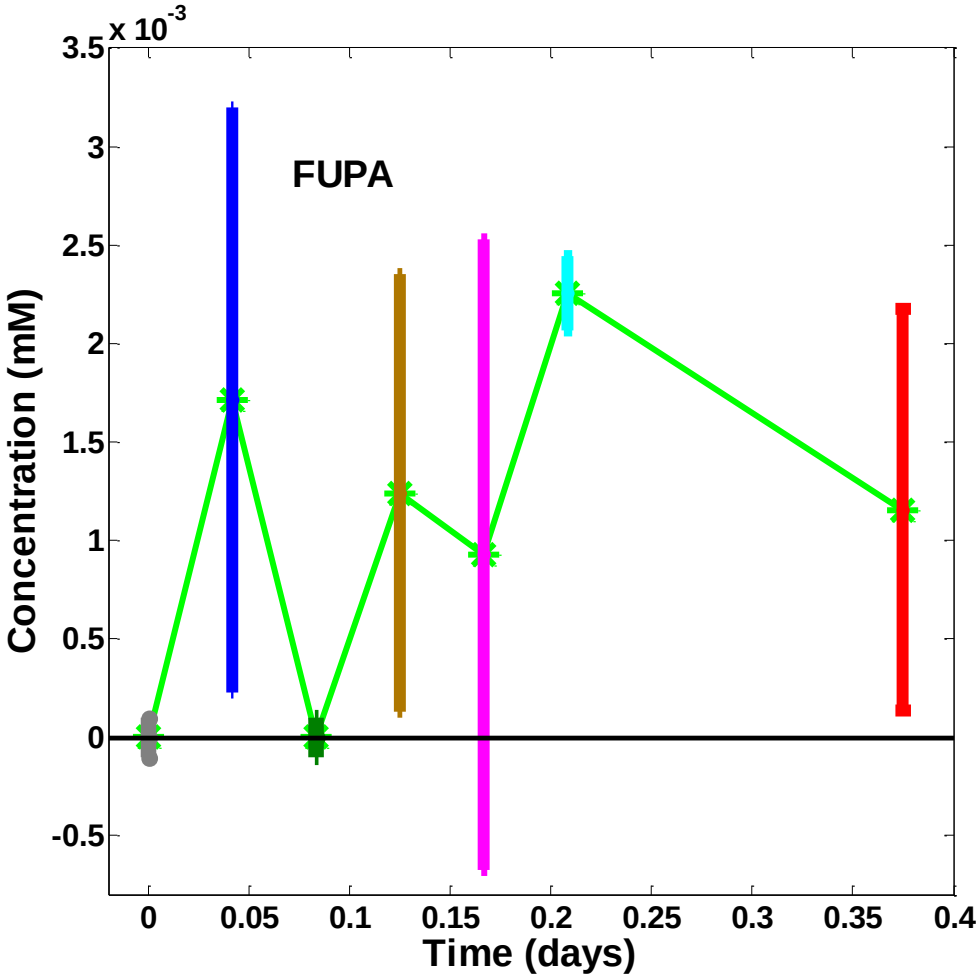

Fig. 28c Thermal/saline product chromatograph results in screening for 5-FUPA for the average and deviation of 3 measurements at each incremental measurement during the duration of the experiments.

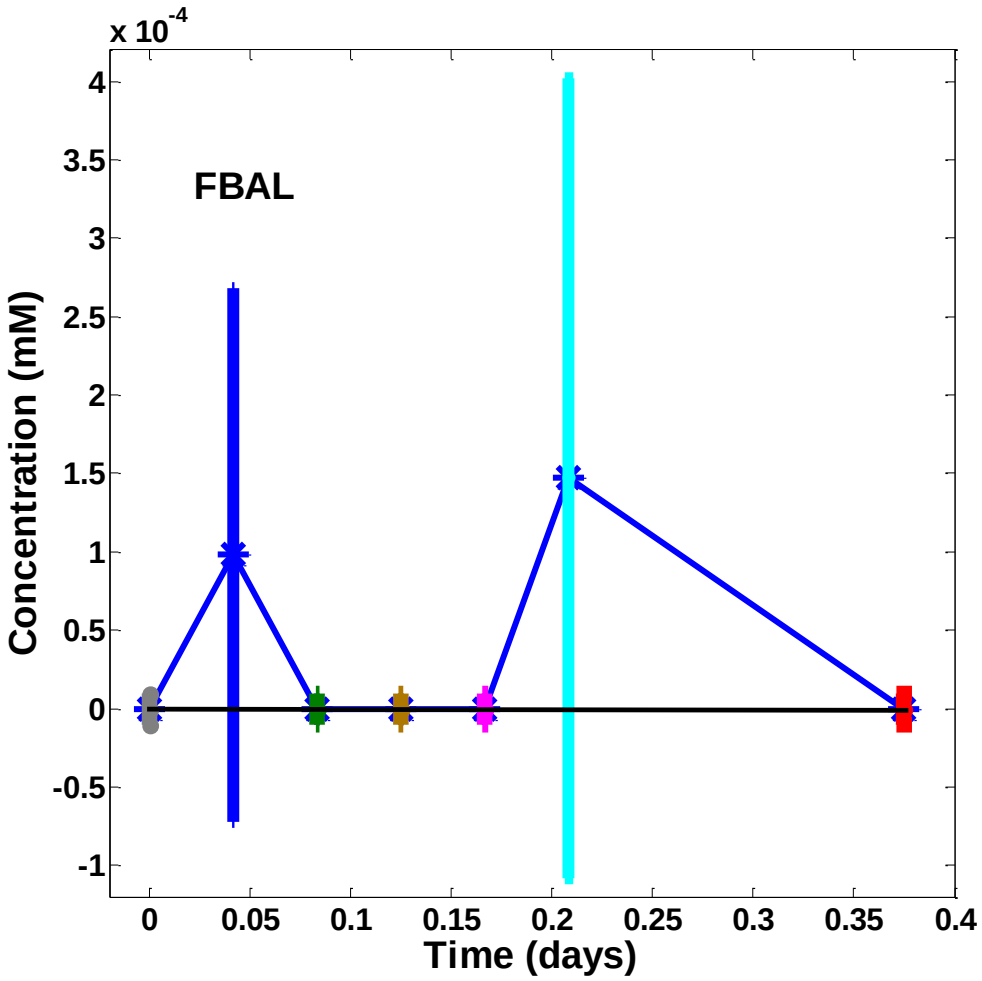

Fig. 28d Thermal/saline product chromatograph results in screening for 5-FBAL for the average and deviation of 3 measurements at each incremental measurement during the duration of the experiments.

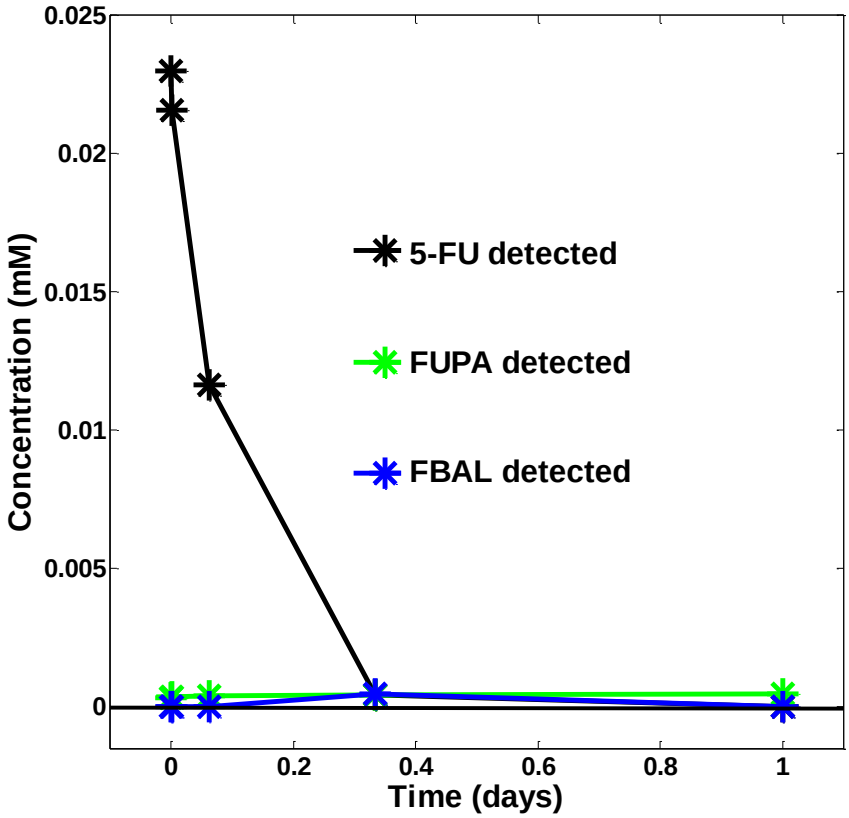

Fig. 29a Photolytic/alkaline average quantitative results over time after screening for 5-FU, FUPA; FBAL for the average of 3 measurements at each incremental measurement during the duration of the experiments.

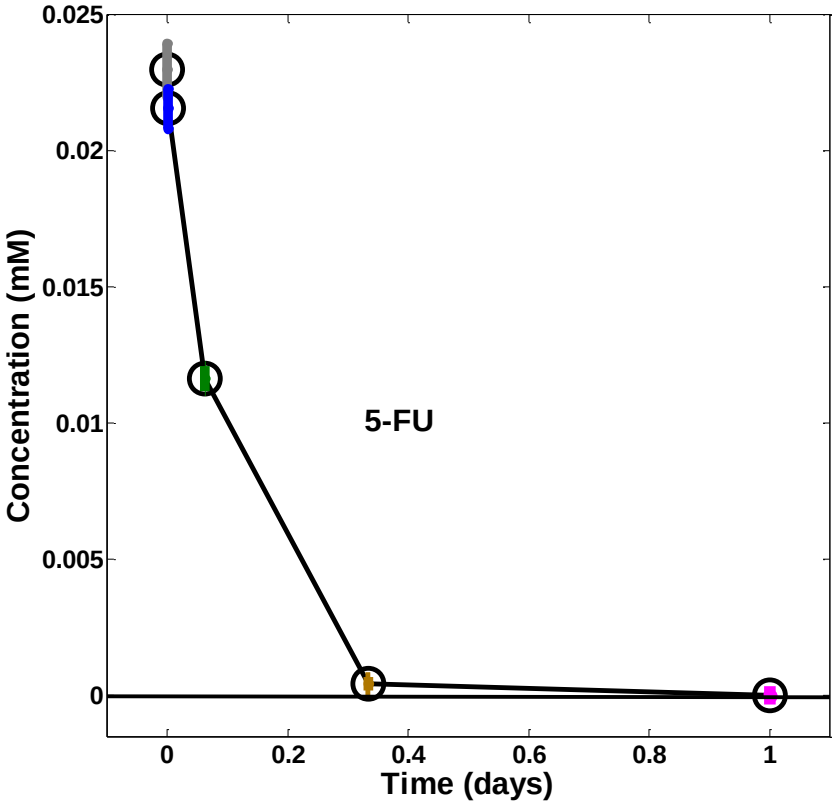

Fig. 29b Photolytic/alkaline quantitative results over time after screening for 5-FU for the average and deviation of 3 measurements at each incremental measurement during the duration of the experiments.

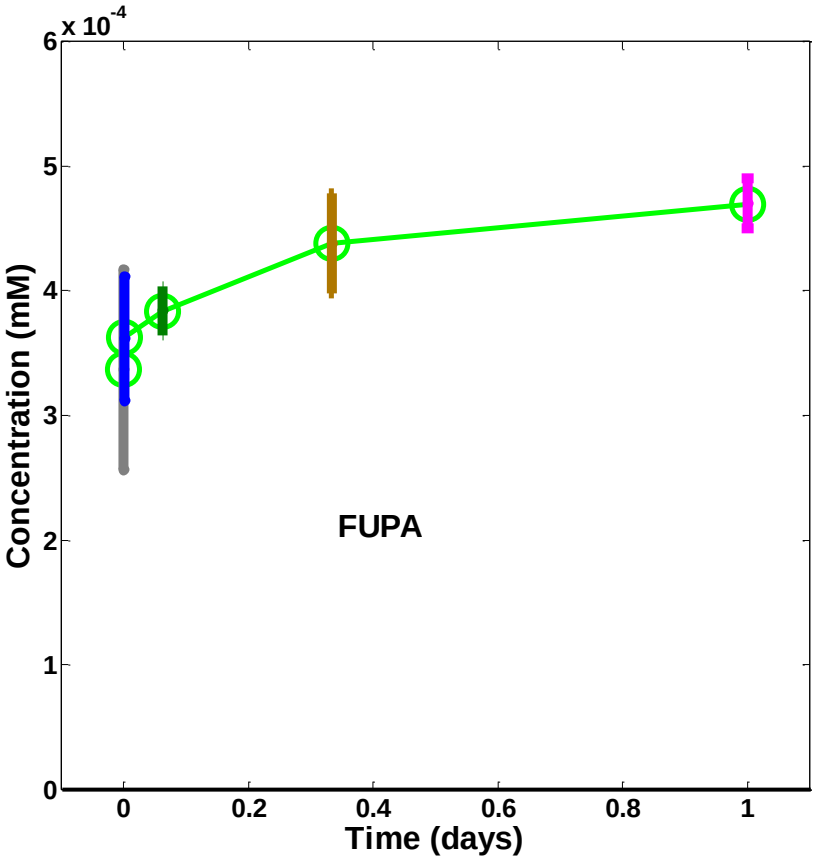

Fig. 29c Photolytic/alkaline quantitative results over time after screening for FUPA for the average and deviation of 3 measurements at each incremental measurement during the duration of the experiments.

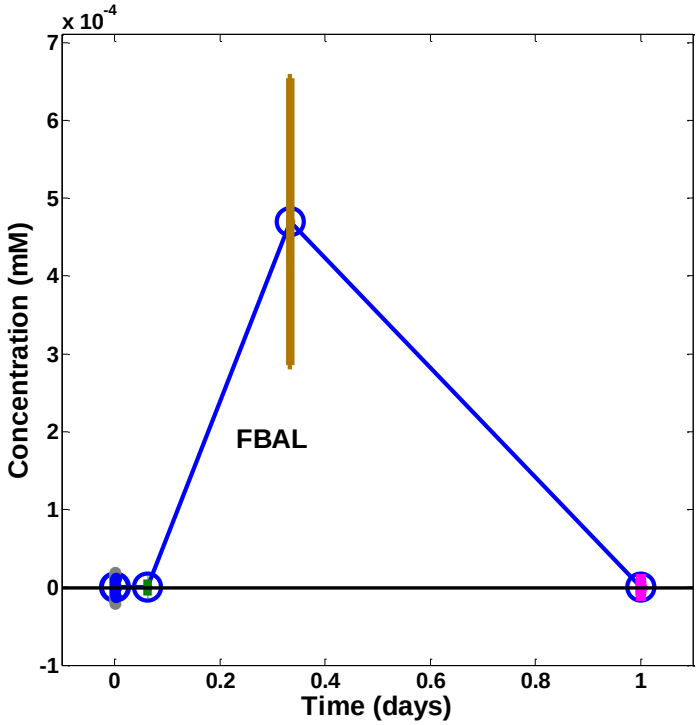

Fig. 29d Photolytic/alkaline quantitative results over time after screening for FBAL for the average and deviation of 3 measurements at each incremental measurement during the duration of the experiments.

IV.4 SUMMARY

Hydrolysis is a chemical reaction during which molecules of water (H_2O) are split into hydrogen cations (H^+) and hydroxide (OH^-) in the process of a chemical mechanism. This process is important, environmentally, because hydrolysable chemicals make their way into ground waters and streams, leaching and runoff. Though the rates are independent of microbial population and oxygen supply, they are dependent upon pH, temperature and concentrations. Understanding more about hydrolysis is useful in predicting the fate of fresh water systems. These can be determined by the derivation of kinetic data. The effects saline solution (0.005g/mL NaClO) had in this process. This particular study dealt with combining Temperature (90°C) and NaClO for degradation which demonstrated that this sort saline significantly influenced thermolysis which by itself was minimum(high temperatures alone used to force degrade). In general, saline is known to influence hydrolysis as a function of the substrate. If the substrate is an acid, one can expect an increase in hydrolysis; shown with the following equation:

$$R\text{-}X + A^- \rightarrow RA + X^-,$$
$$RA + H_2O \rightarrow ROH + HA,$$
$$(HA + X \rightleftarrows A^- + HX). \quad [28]$$

More so, since this study used NaClO this time around, the production of gas enhanced degradation even more than what would be anticipated via use of regular salt water. That difference may be reasoned here:

$HClO + H^+ + H^+ + Cl^- \rightarrow Cl_2 + H_2O$ (acidic conditions)

$H_2O = H^+ + OH^-$

$K_w = [H^+][OH^-]$

pKw = 10^-Kw

Kw is Temperature Dependent

Thus,

↑Temp ∝ ↑Cl2 (g)

↑Cl2 (g) ∝ ↑Cavitation ∝ ↑ Degradation

Photolysis may be similar to thermolysis with respect to the fact that both drive the facilitation of hydrolysis via the "Reduction" route. The UV light absorbed by the sample's solute molecules acts similar to the absorption of heat (temperature rise). This drives the initial reduction step. Thereafter, a certain amount of remaining aqueous solvent undergoes equilibrium enough to facilitate the subsequent conversion to FUPA. Importantly however, remaining solvent is not sufficiently equilibrated to cause further reduction. This is due to the fact that OH^- is more abundant than H^+ in the presence of TiO_2 and UV light which generate various radicals along with OH. This occurs via a series of mini reactions. These reactions create an imbalance within the system such that FUPA can't be further reduced to yield FBAL. Such a product may be obtained with a forced thermolysis which calls for a need for further investigations.

Thermal/acidic series (figure 27a) and as seen basically nothing is happening which confirms what was inferred from the UV analysis (fig. 13).

Researchers F. XU et al. [27] got only 0.1% degradation of their original compound (5-FU) to contribute to formation of a degradation product which they did not identify or characterize, specifically. In the present study, Le Chatelier's Principle [29] was used in order to justify any regrouping to some sort of stability when an aqueous matrix's natural equilibrium (Reaction Scheme 1: $H_2O \leftrightarrow H^+ + OH^-$) may be offset by some external influence (another substance, modifying pH, etc.). The situation here involves that natural balance being offset by heat or raise in Temperature (Rxn Scheme 2: $2H_2O + \Delta \leftrightarrow 2H_{2(g)} + O_{2(g)}$) as was previously mentioned. The external influence most greatly affecting the equilibrium can be attributed to the acidic conditions (H^+ions). However, acidic conditions have no influence on an inclination towards gaseous conditions((Rxn Scheme 3: $H^+ + H_{2(g)} + O_{2(g)} \rightarrow$ no reaction)) [23] whereas a previous discussion of thermal/alkali conditions does facilitate some equilibrium adjustments((Rxn Scheme 4: $OH^- + H_{2(g)} + O_{2(g)} \rightarrow [OH^- \bullet H_2]$)) [30] This in turn leads to an influence on the hydrolysis of 5-FU. Thus with no equilibrium of hydronium and hydroxide ions, there is no hydrolysis of 5-FU in this thermal/acidic study. Again, looking at Table 6/Degradation Summary Table, we see $k_{FU}/t_{1/2}$ for thermal/saline study is -0.0597mMol/day/~0.0624 day versus $k_{FU}/t_{1/2}$ for thermal/acidic's -0.00013mM/day/~ 86.9 days. Thermal degradation alone on 5-FU looked very similar to thermal/acidic using UV Absorption monitoring. So it is easy to assume that the little degradation that occurred for the thermal alone may be equal of magnitude to what Thermal/Acidic showed. Therefore, saline put in the midst of an ordinary thermal study enhances the degradation for the

compound of interest. Y. Yang et al [31] looked at the effects that saline could have in various processes focused on the removal(degradation) of organic contaminants from industrial and municipal wastewaters per Y. Yang et al [31].

Table 6. Degradation Study.

Parameters/ Factors, etc.	Thermal/Saline	Photolytic/Alkali	Thermal/Alkali	Thermal/Acidic
k_{fbal}	N/A	N/A	N/A	N/A
k_{FUPA}	0.00308mM/ day	0.000133/ day	N/A	N/A
$T_{1/2fbal}$	N/A	N/A	N/A	N/A
$T_{1/2fupa}$	N/A	N/A	N/A	N/A
K_{5FU}	-0.0597mM/ day	-3.7/day	-0.00069mM/ day	-0.00013mM/ day
$T1/2_{5FU}$	0.0624 day	0.187 day	14.9 days	86.9 days
rxn order	0	1st	0	~ 0

These type of scenarios are good in trying to fully understand mechanisms behind hydrolysis processes which can be related here with: Thermal/Saline > Photolytic/Alkali > Thermal/Alkali > Thermal/Acidic.

Thus, the novelty of this study can be appreciated which is a first for 5-FU and should stir up enough interests for follow up study to be pursued.

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APPENDIX

0051

77

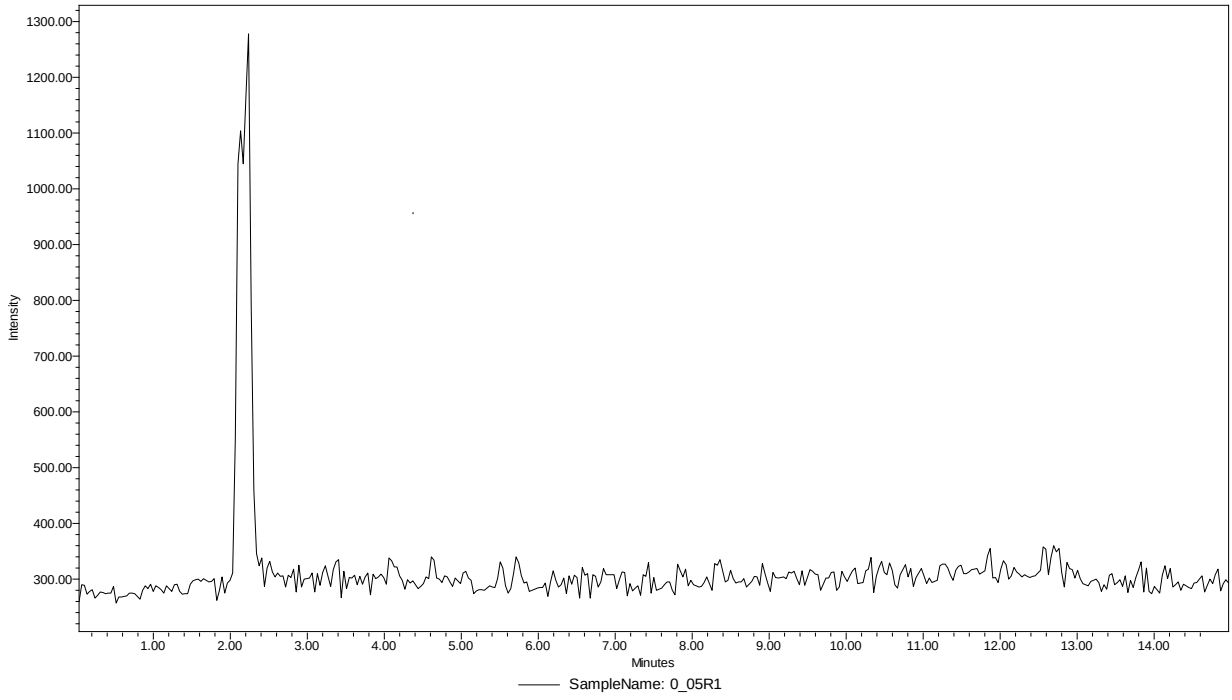

Fig. 25a product chromatographs for 0.05 ppm FAC 1st replicate.

105

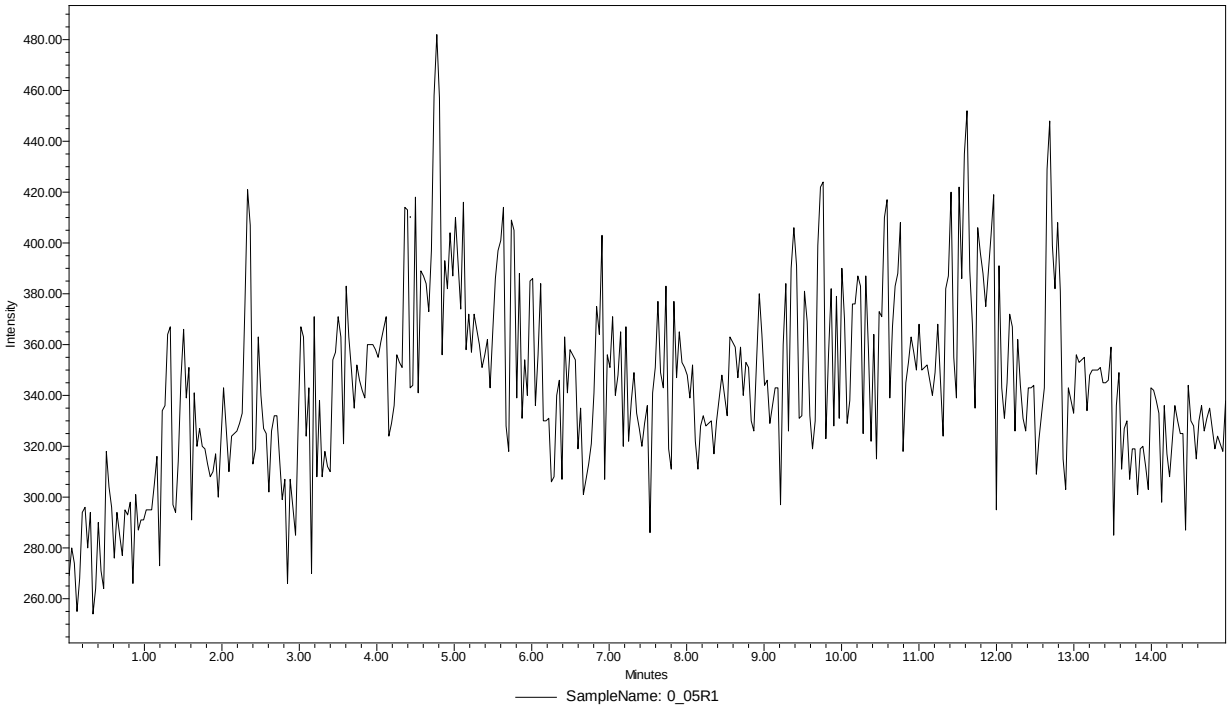

Fig. 25b product chromatographs for 0.05 ppm FMASld 1st replicate.

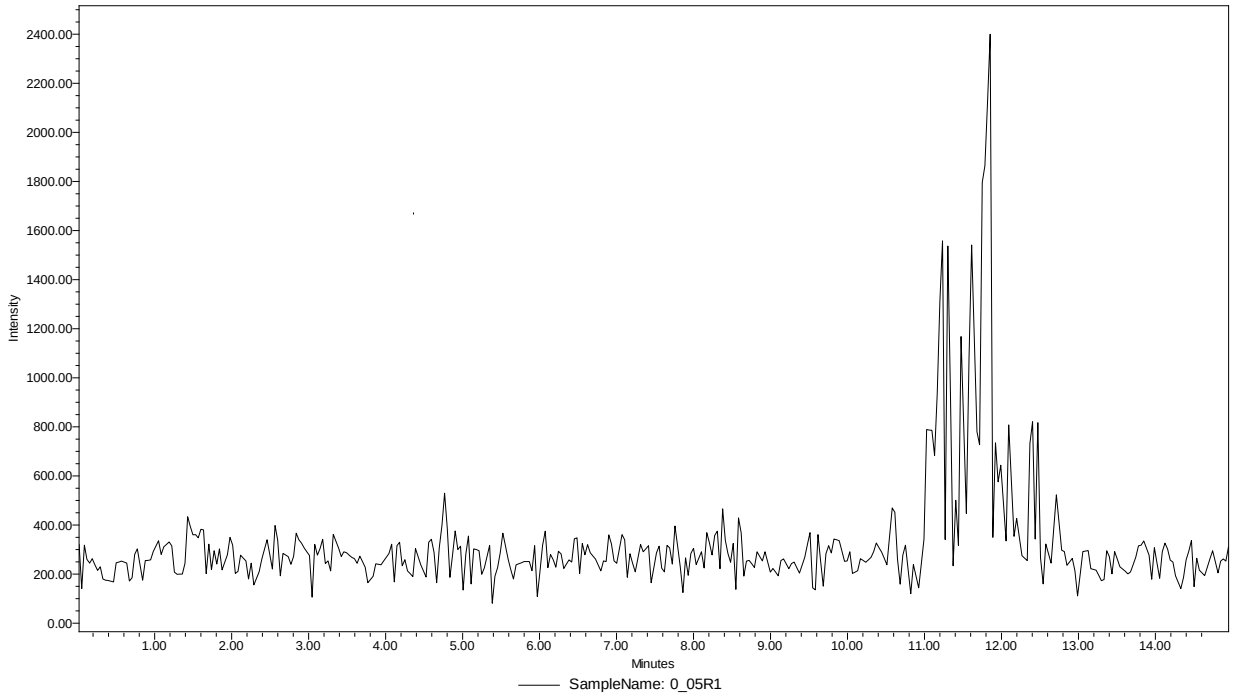

Fig. 25c product chromatographs for 0.05 ppm FBAL 1st replicate.

149

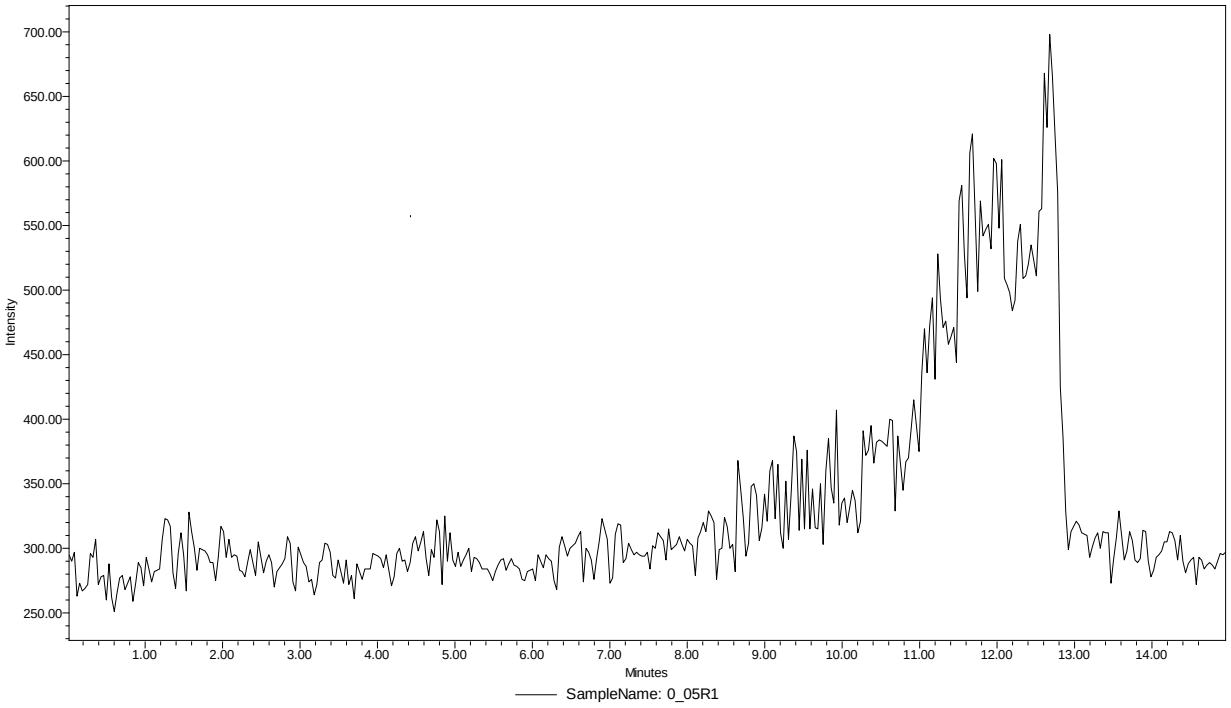

Fig. 25d product chromatographs for 0.05 ppm FUPA 1st replicate.

129

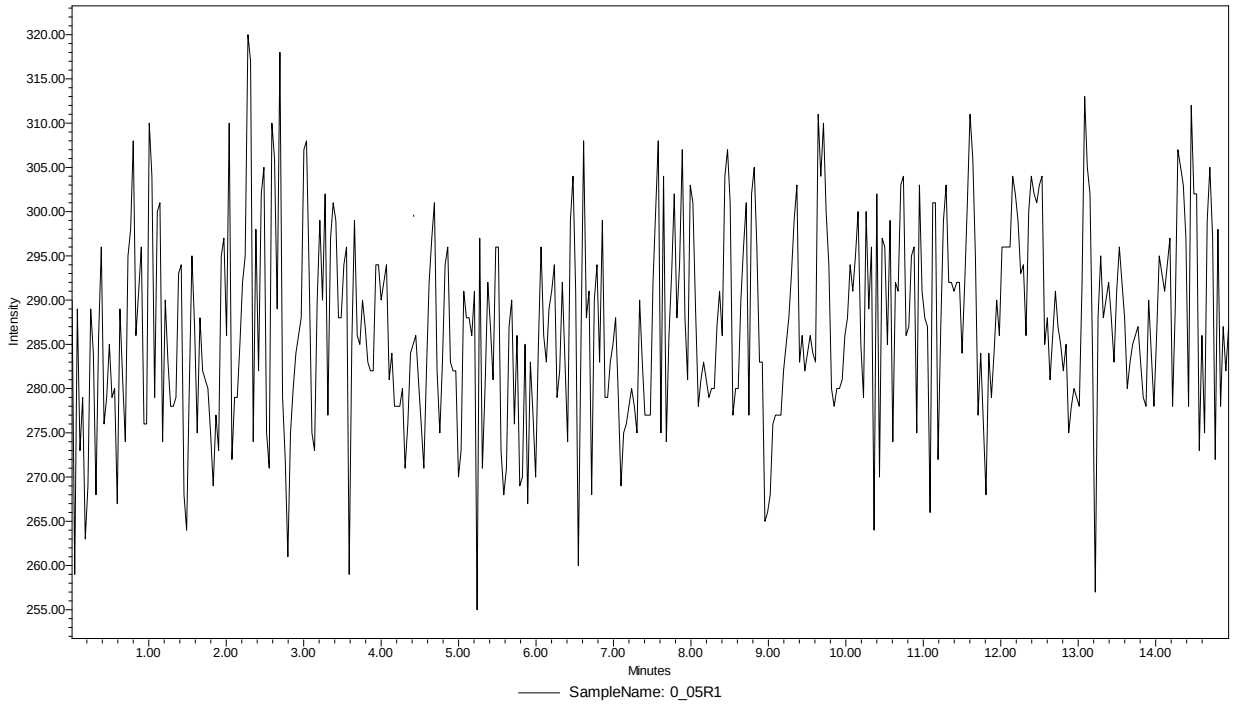

Fig. 25e product chromatographs for 0.05 ppm 5-FU 1st replicate.

0052

77

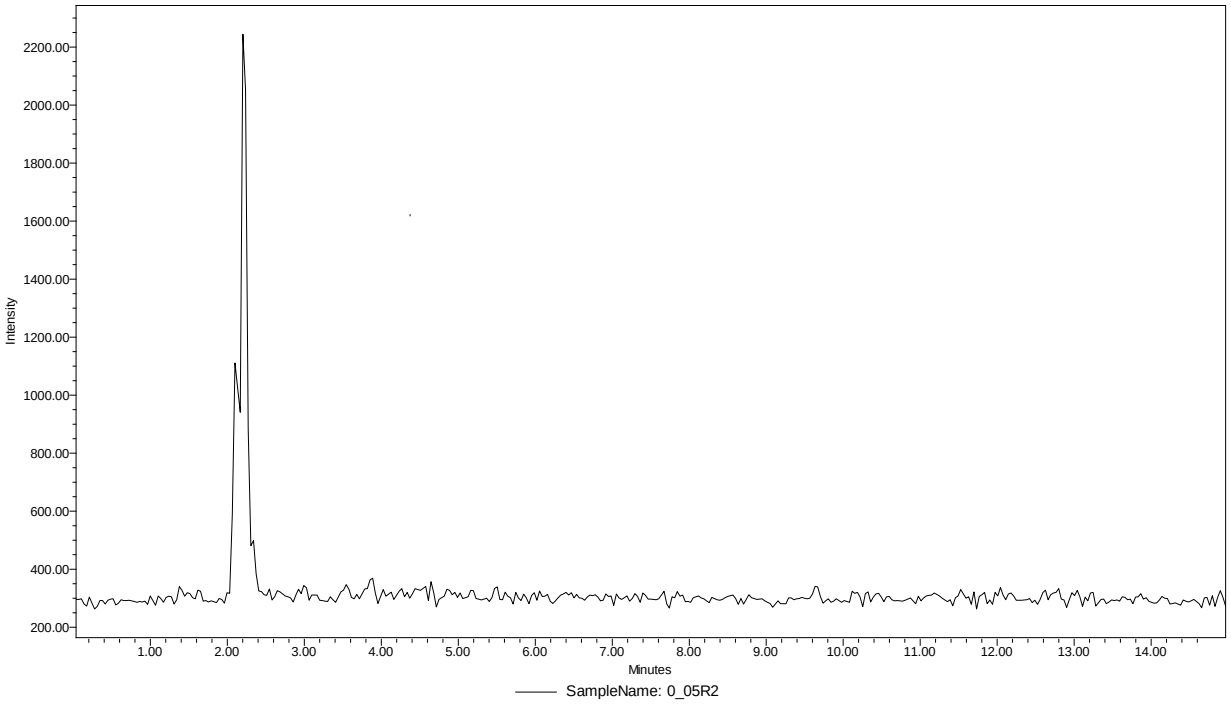

Fig. 25f product chromatographs for 0.05 ppm FAC 2nd replicate.

105

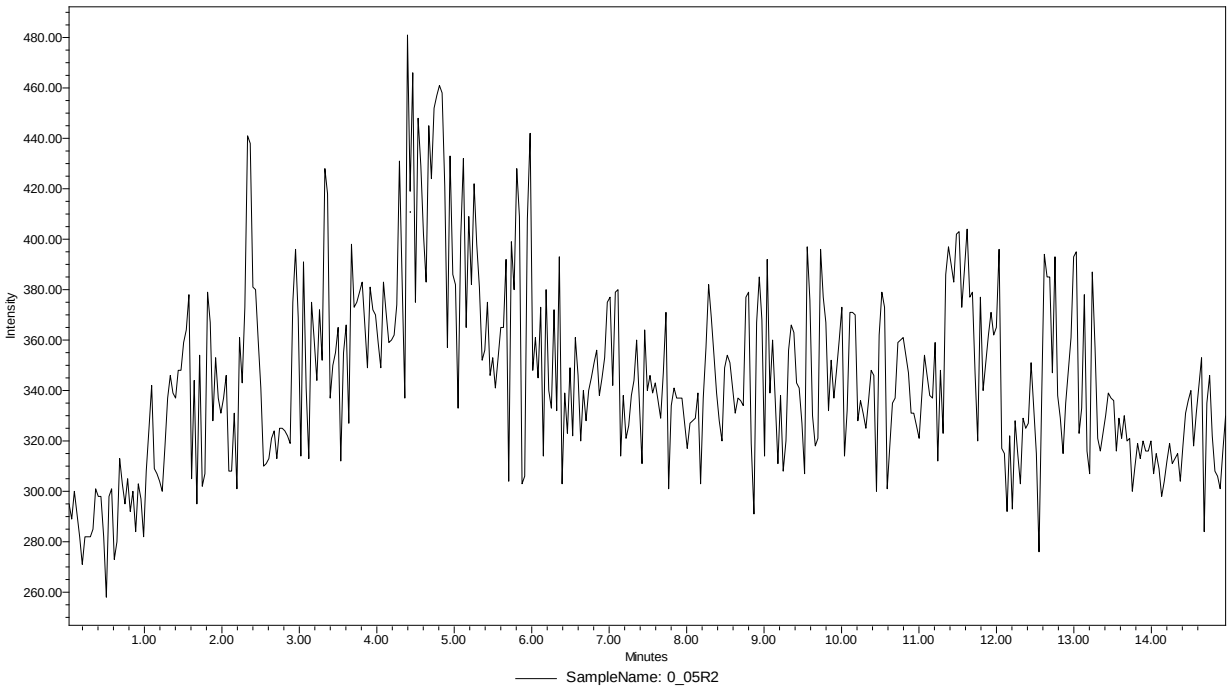

Fig. 25g product chromatographs for 0.05 ppm FMASld 2nd replicate.

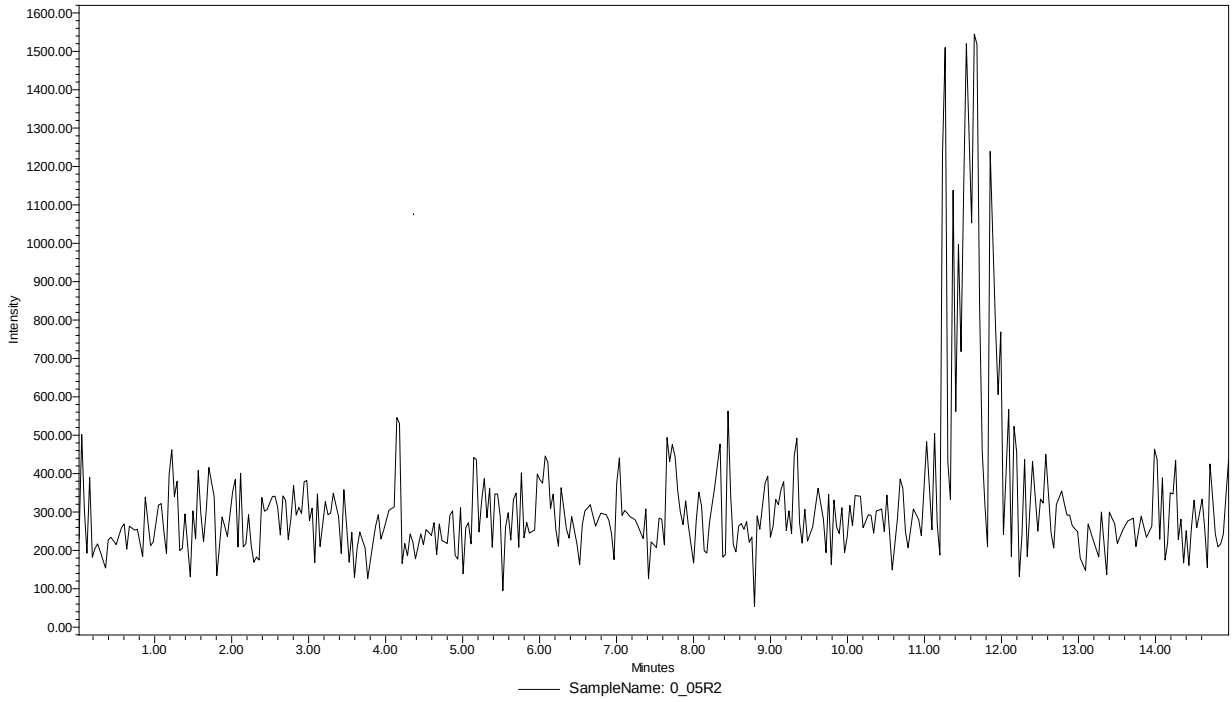

Fig. 25h product chromatographs for 0.05 ppm FBAL 2nd replicate.

149

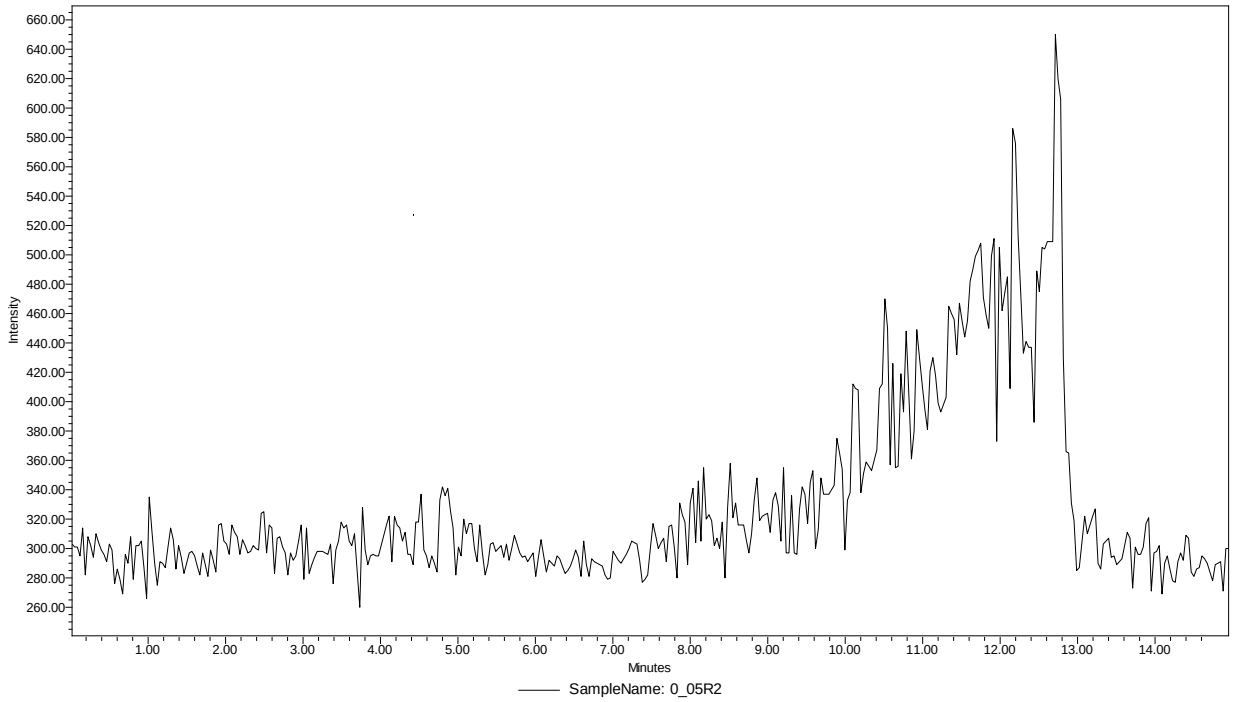

Fig. 25i product chromatographs for 0.05 ppm FUPA 2nd replicate.

129

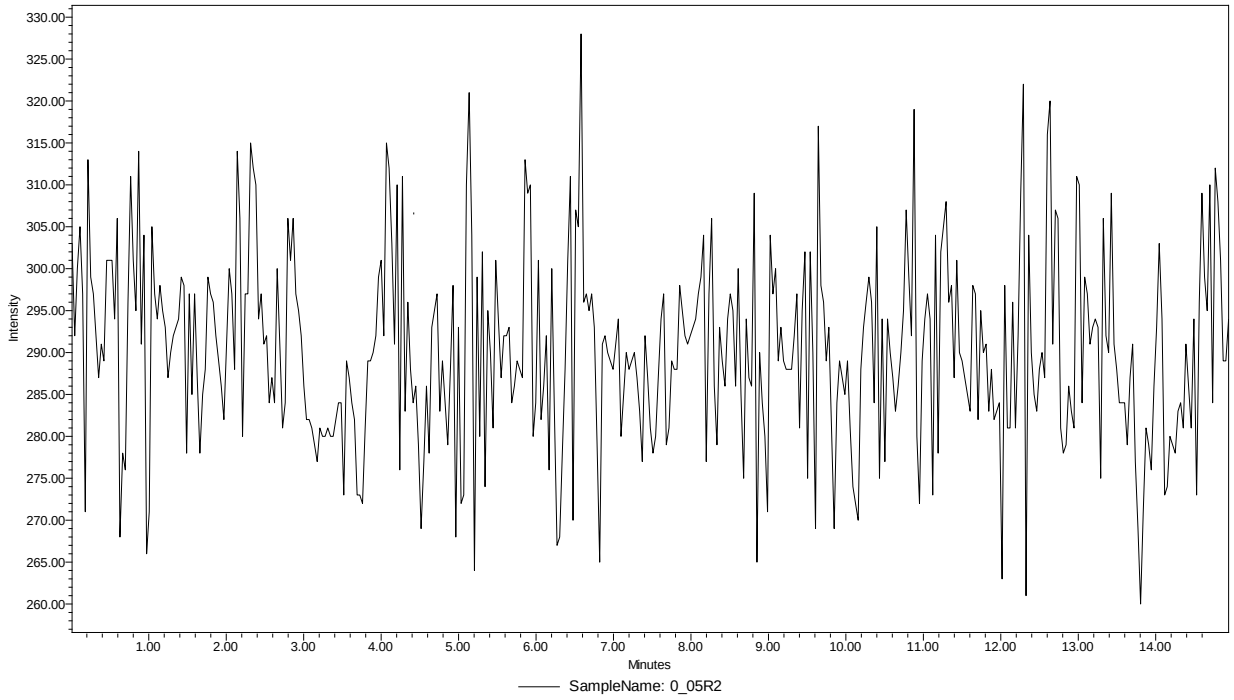

Fig. 25j product chromatographs for 0.05 ppm 5-FU 2nd replicate.

0251

77

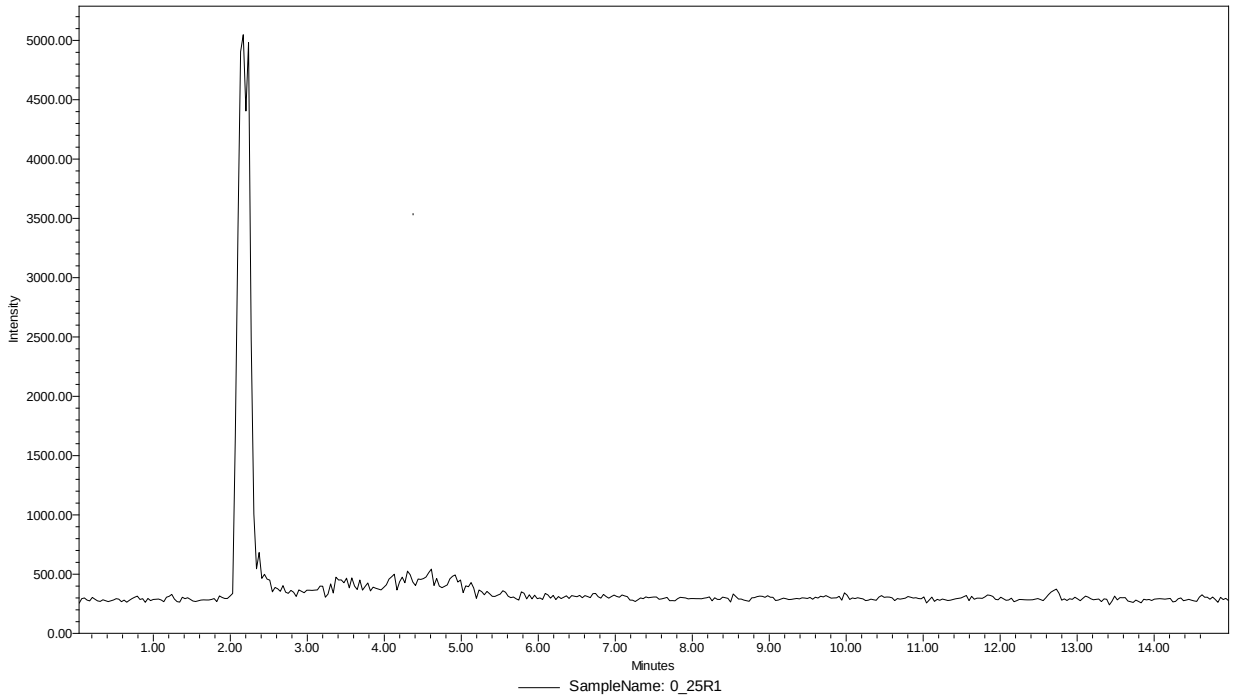

Fig. 25k product chromatographs for 0.025 ppm FAC 1st replicate.

105

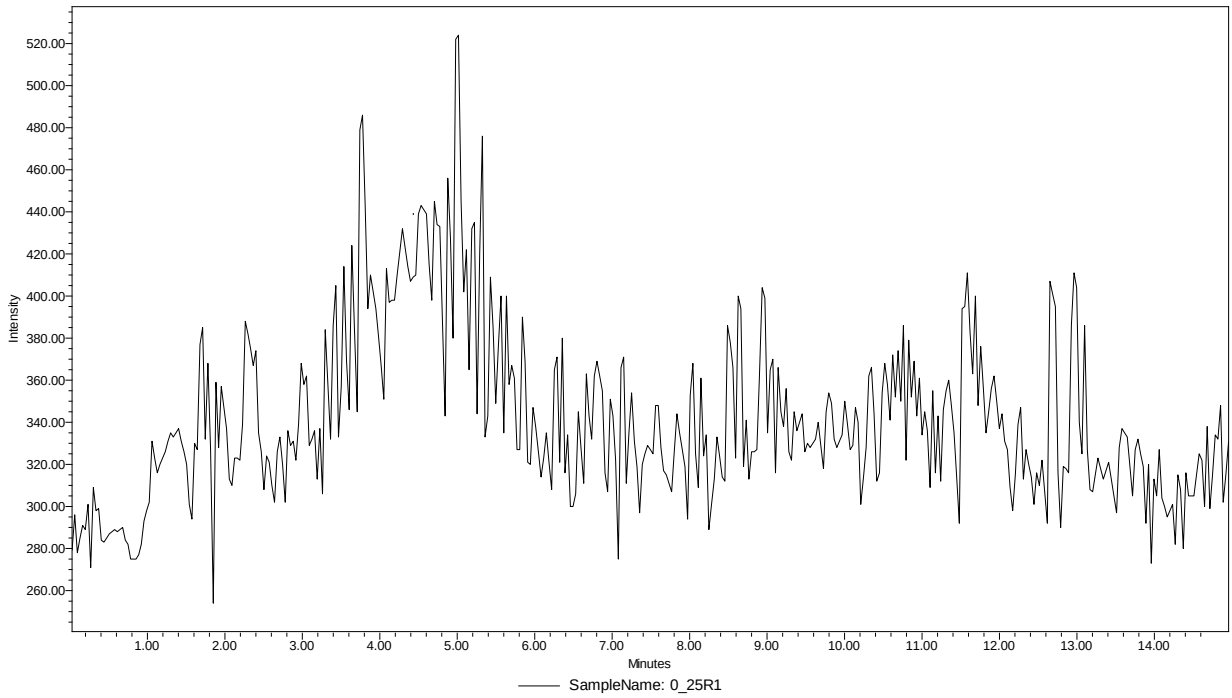

Fig. 25l product chromatographs for 0.025 ppm FMASld 1st replicate.

106

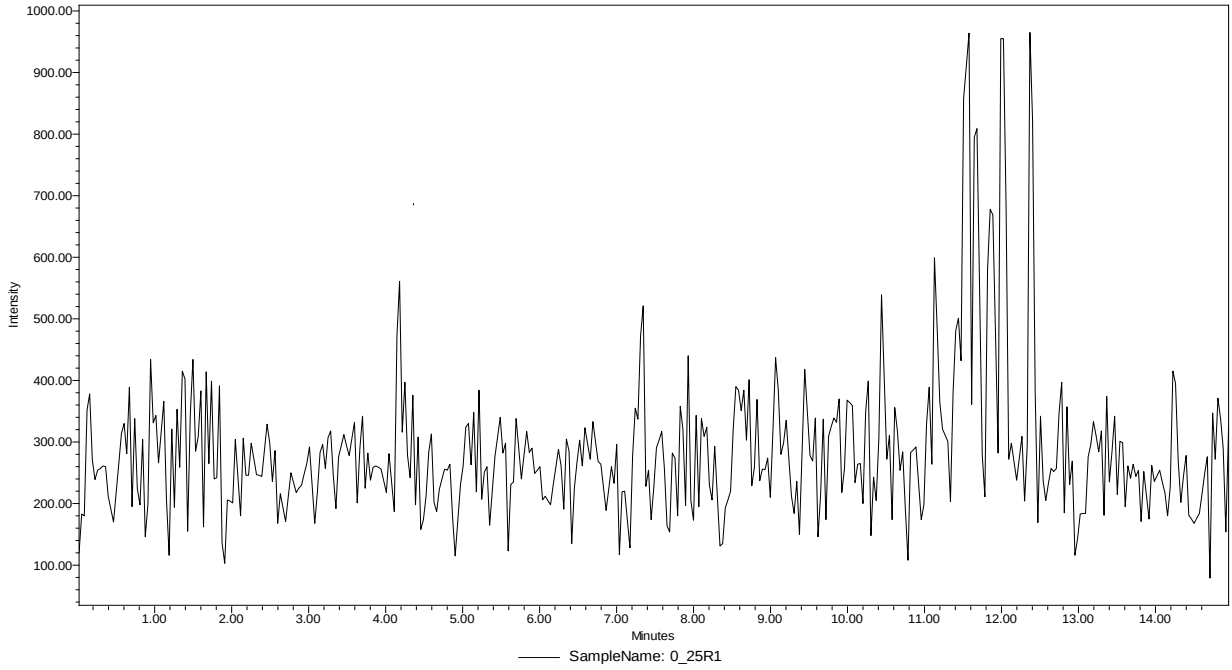

Fig. 25m product chromatographs for 0.025 ppm FBAL 1st replicate.

149

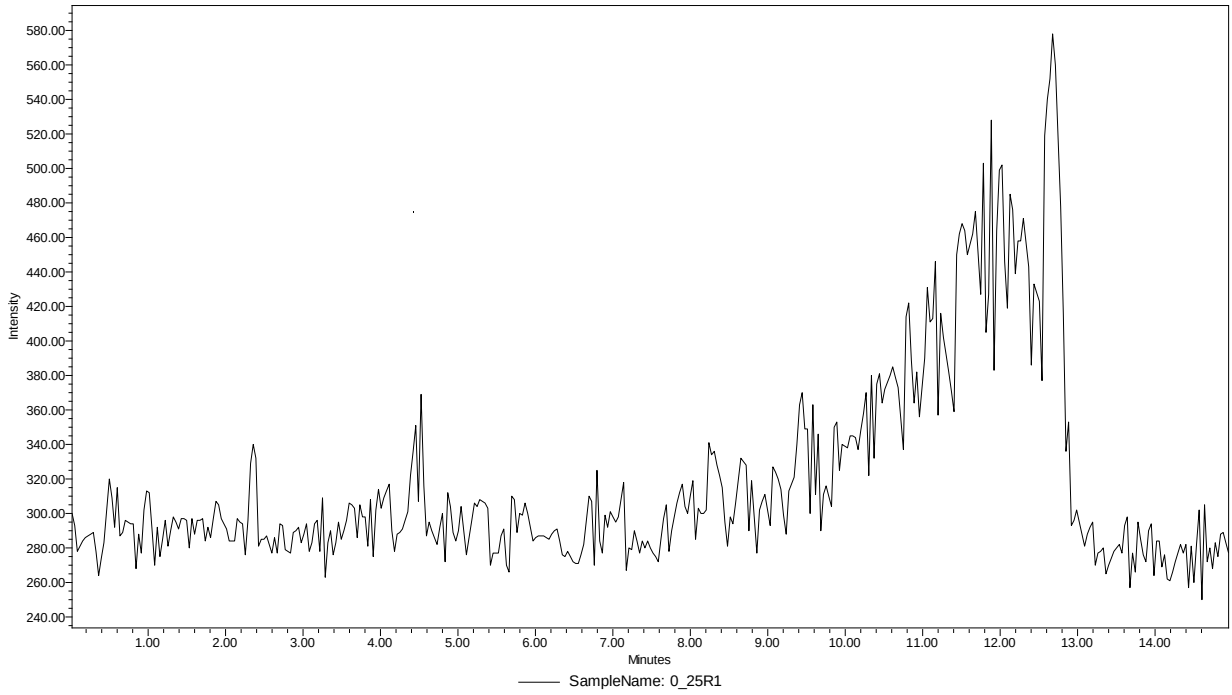

Fig. 25n product chromatographs for 0.025 ppm FUPA 1st replicate.

129

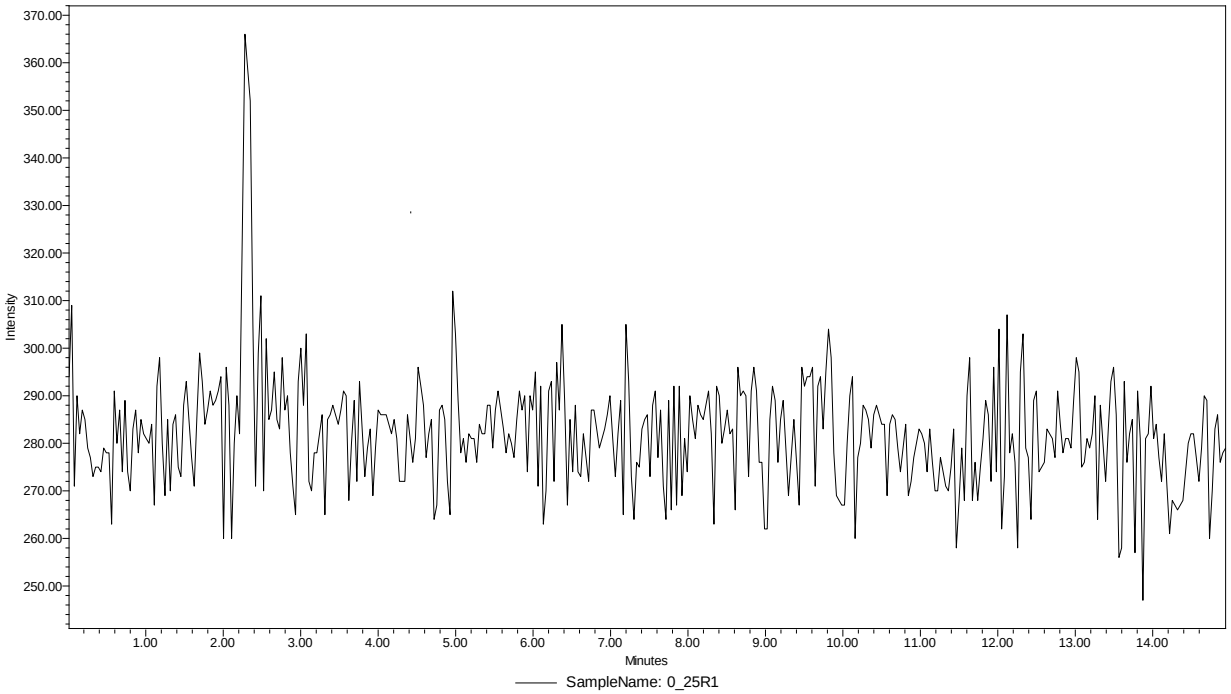

Fig. 25o product chromatographs for 0.025 ppm 5-FU 1st replicate.

0252

77

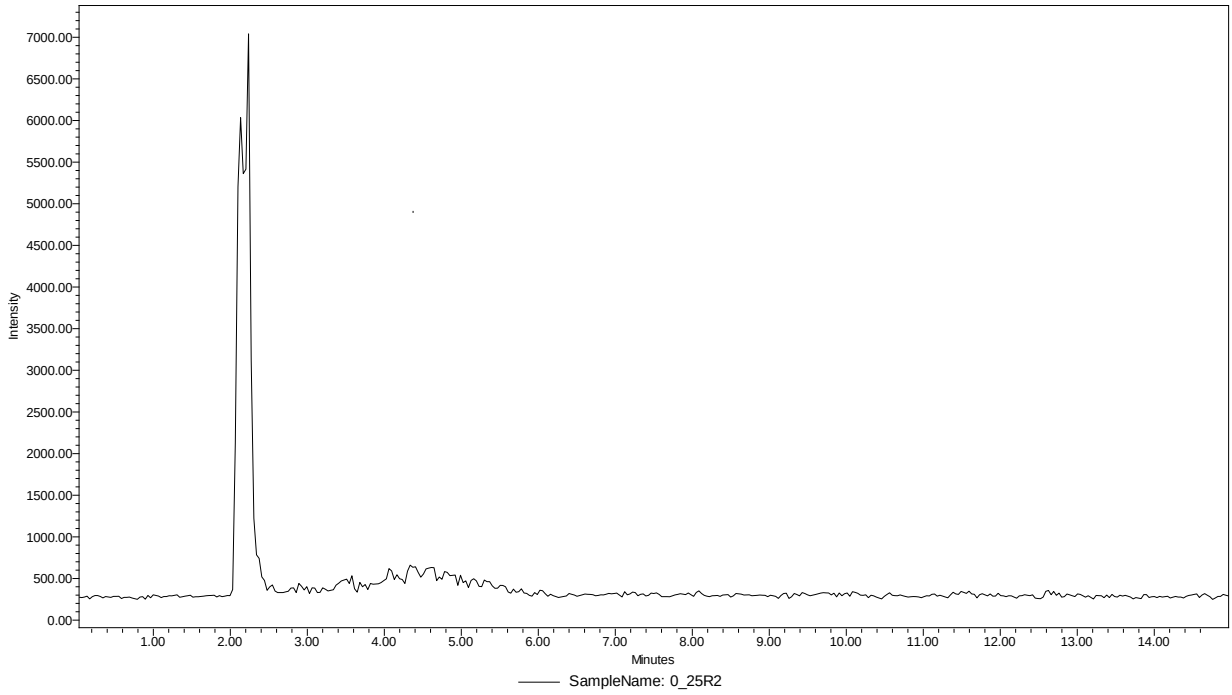

Fig. 25p product chromatographs for 0.025 ppm FAC 2nd replicate.

105

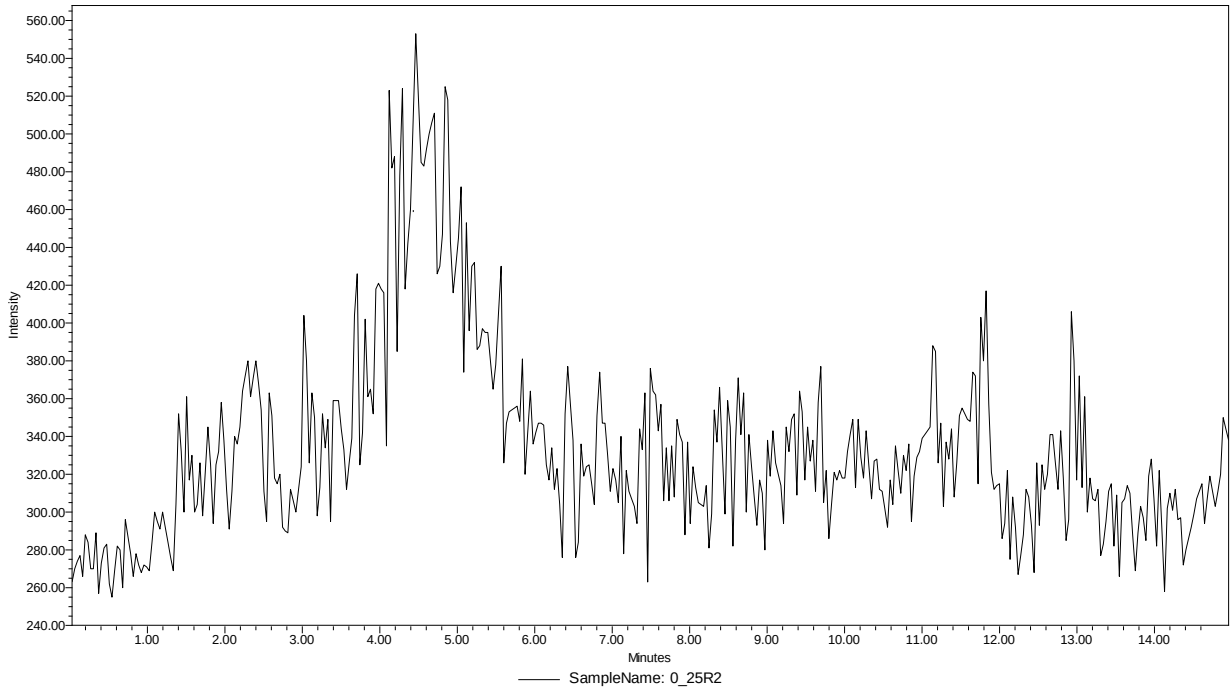

Fig. 25q product chromatographs for 0.025 ppm FMASld 2nd replicate.

106

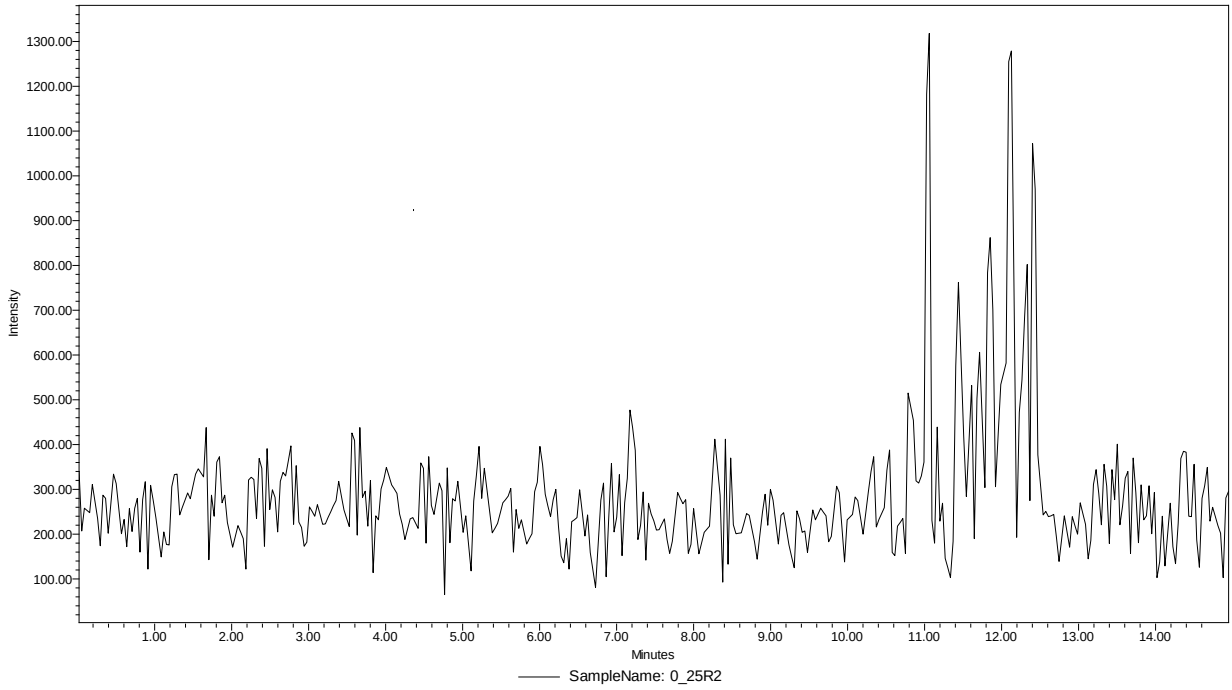

Fig. 25r product chromatographs for 0.025 ppm FBAL 2nd replicate.

149

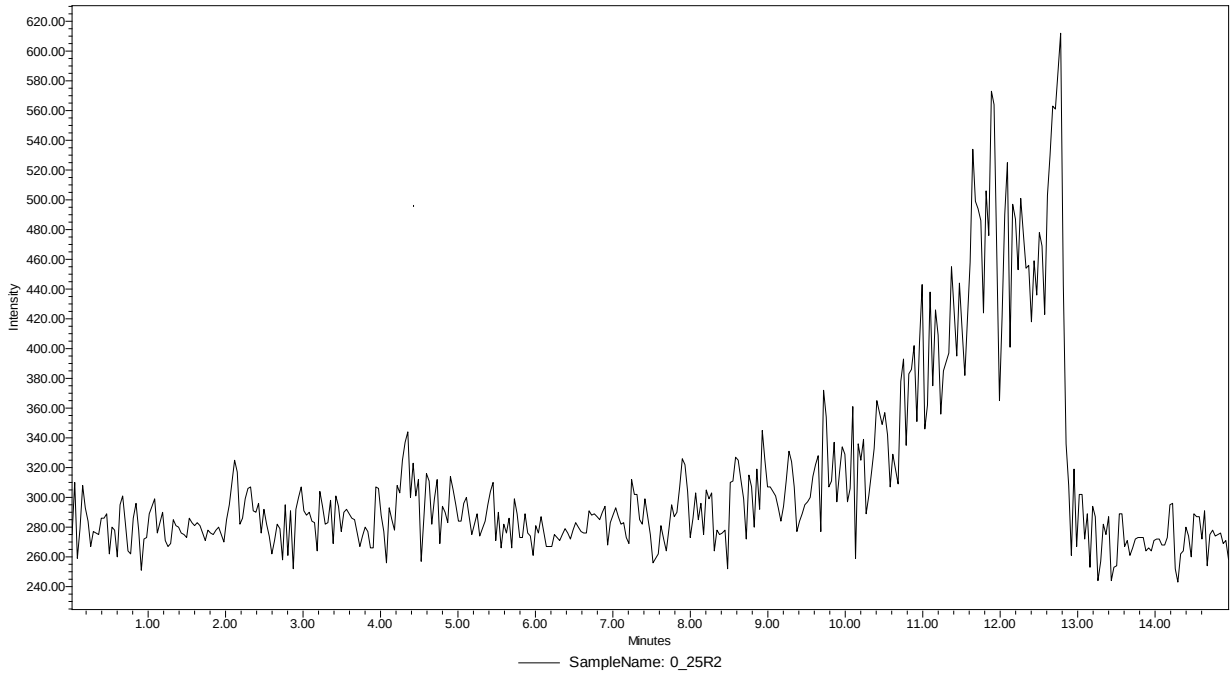

Fig. 25s product chromatographs for 0.025 ppm FUPA 2nd replicate.

129

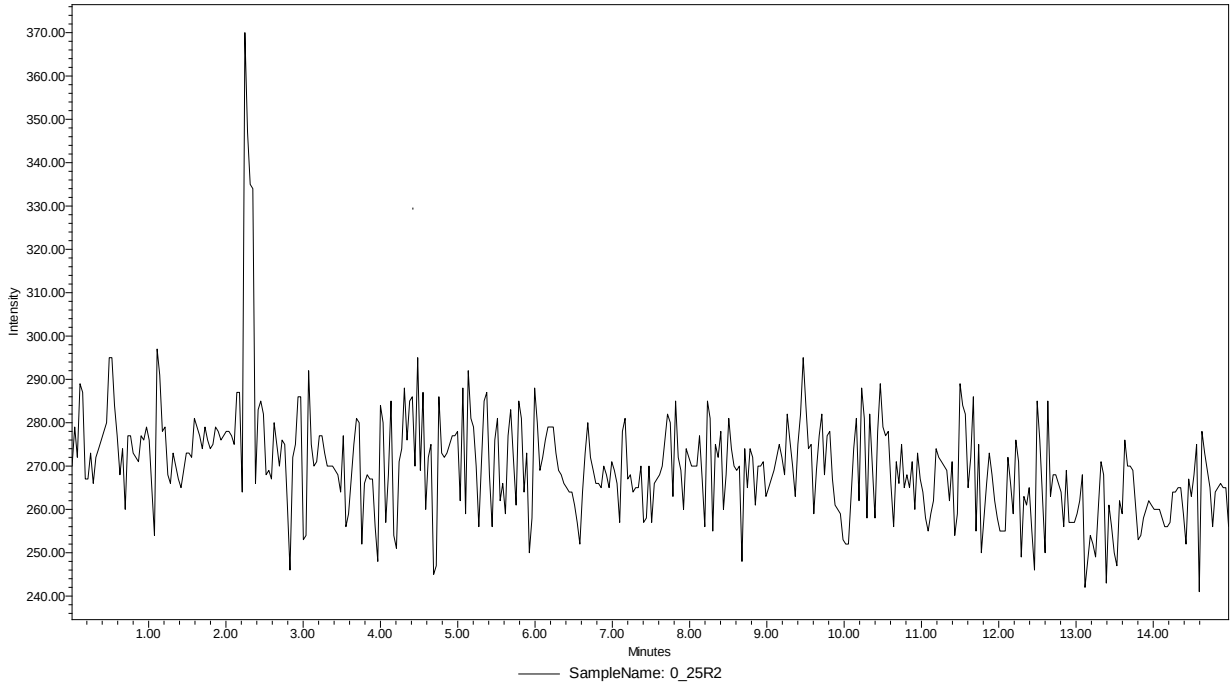

Fig. 25t product chromatographs for 0.025 ppm 5-FU 2nd replicate.

011

77

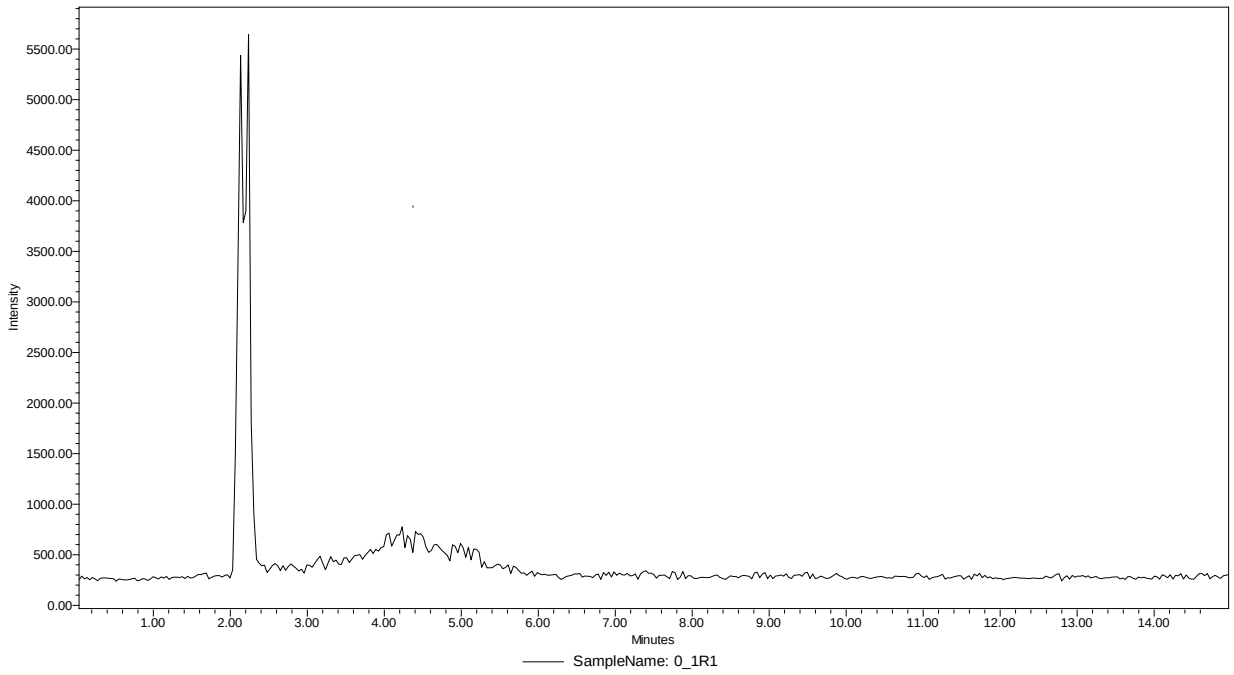

Fig. 25u product chromatographs for 0.01 ppm FAC 1st replicate.

105

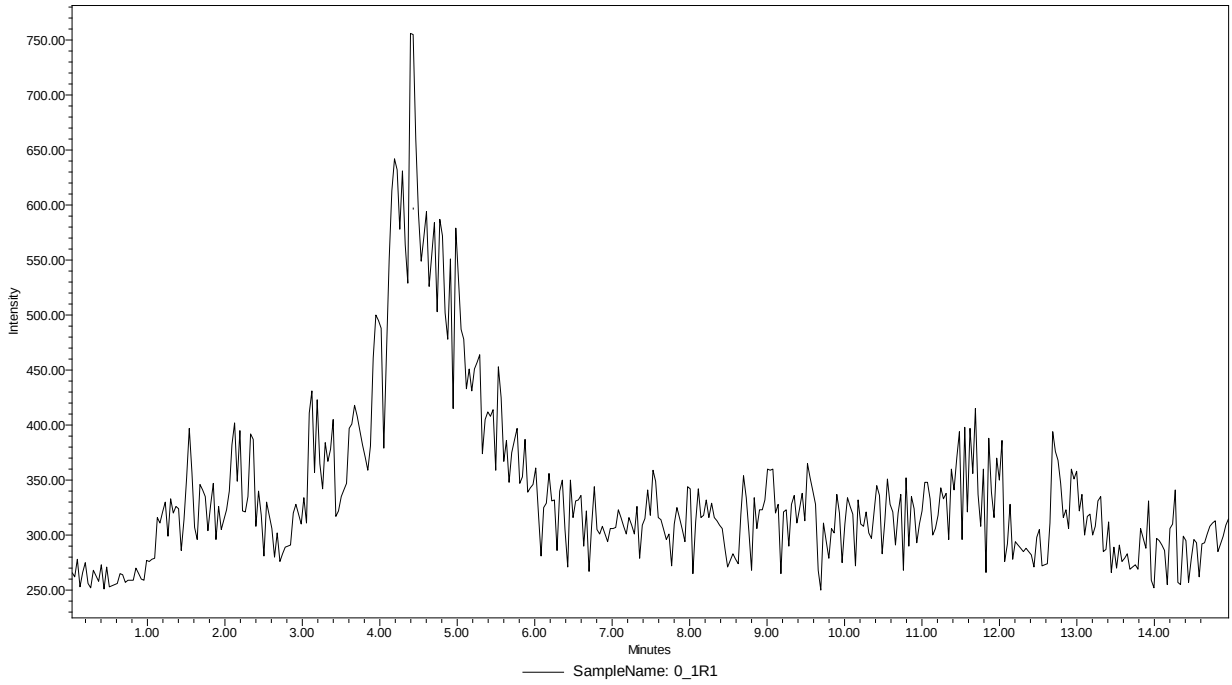

Fig. 25v product chromatographs for 0.01 ppm FMASld 1st replicate.

106

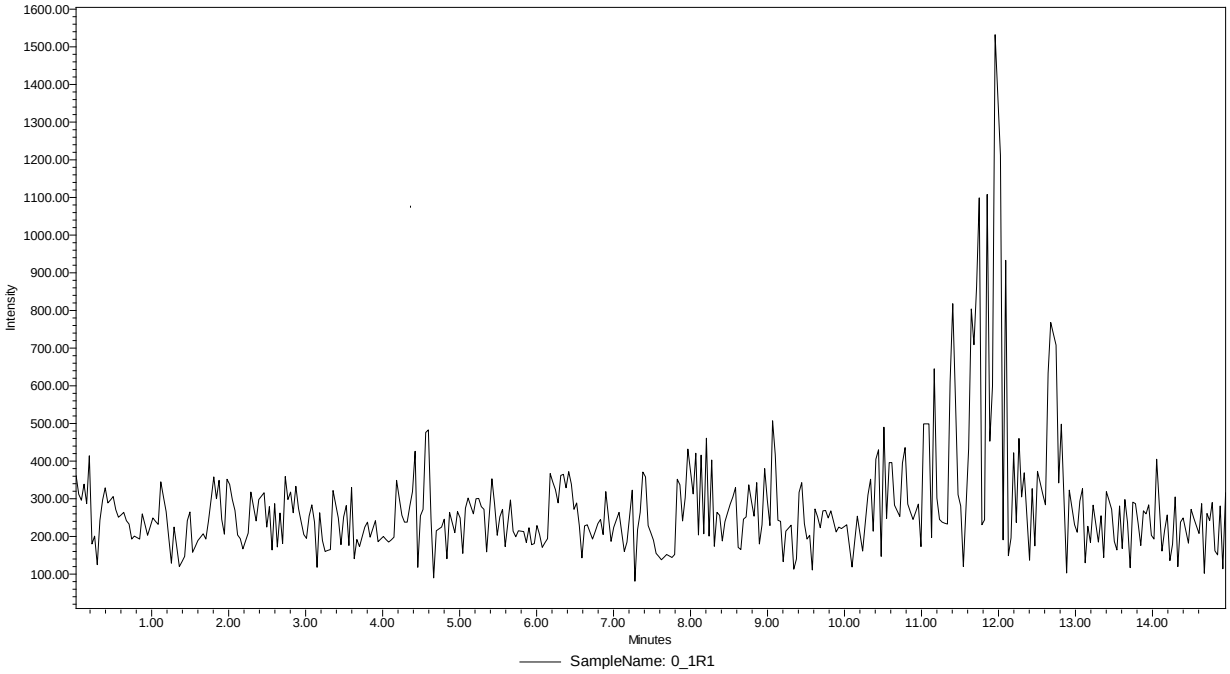

Fig. 25w product chromatographs for 0.01 ppm FBAL 1st replicate.

149

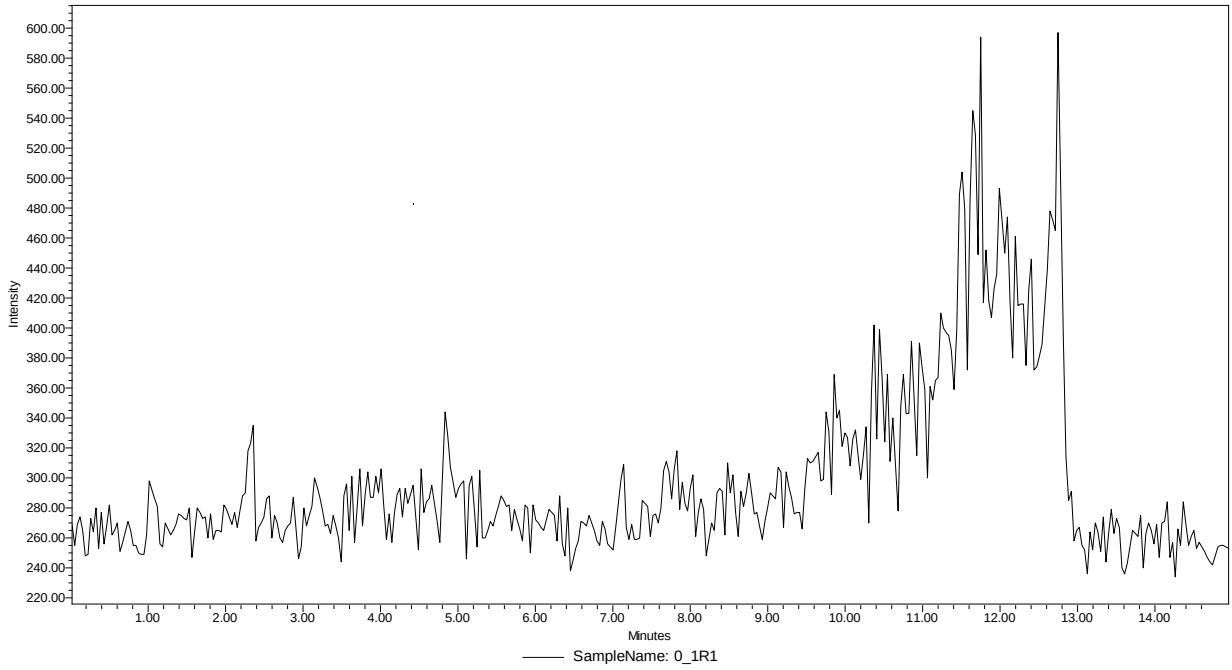

Fig. 25x product chromatographs for 0.01 ppm FUPA 1st replicate.

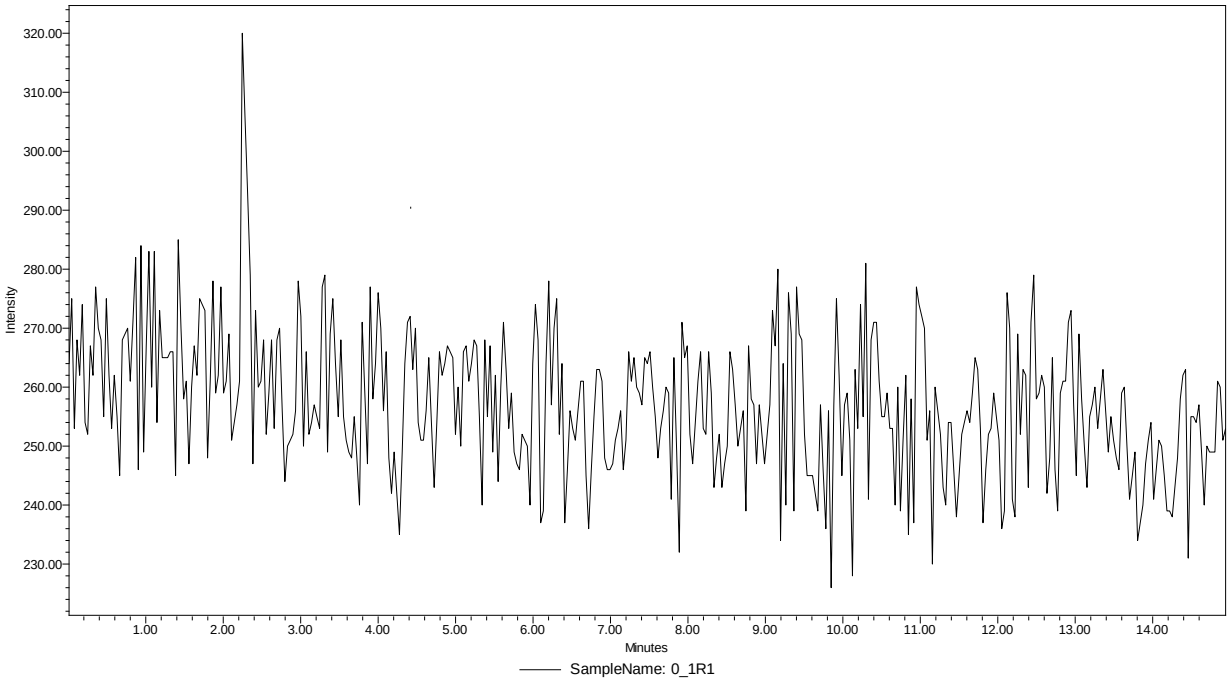

Fig. 25y product chromatographs for 0.01 ppm 5-FU 1st replicate.

012

77

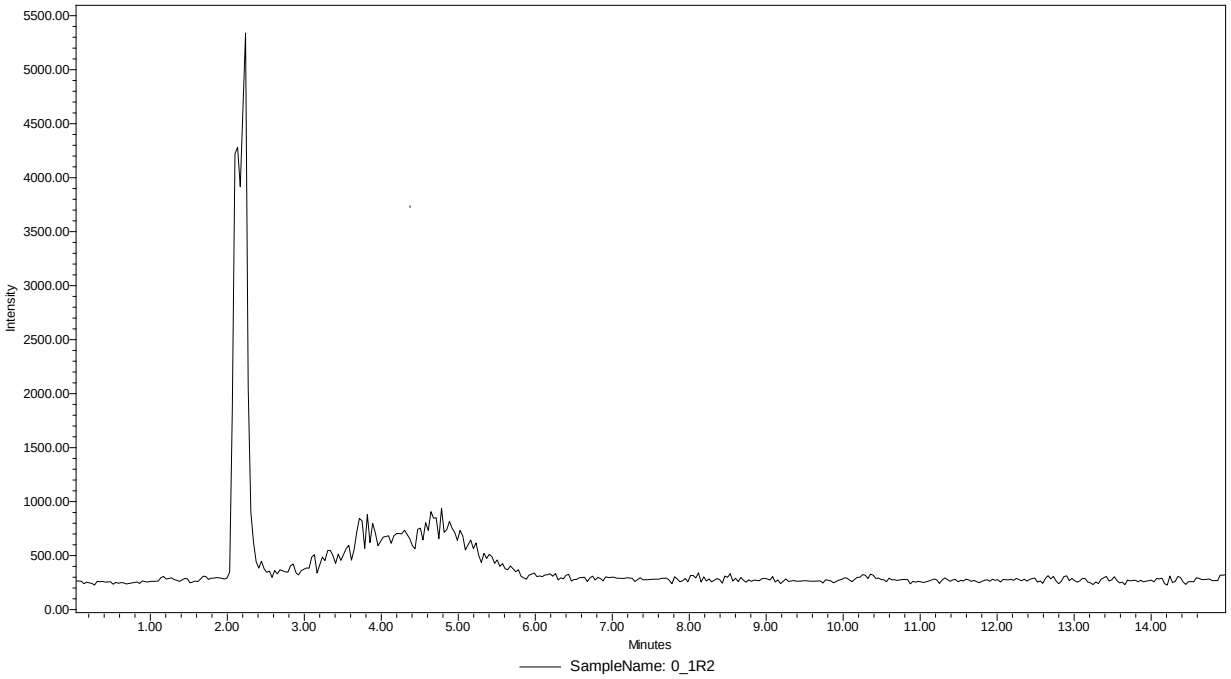

Fig. 25z product chromatographs for 0.01 ppm FAC 2nd replicate.

105

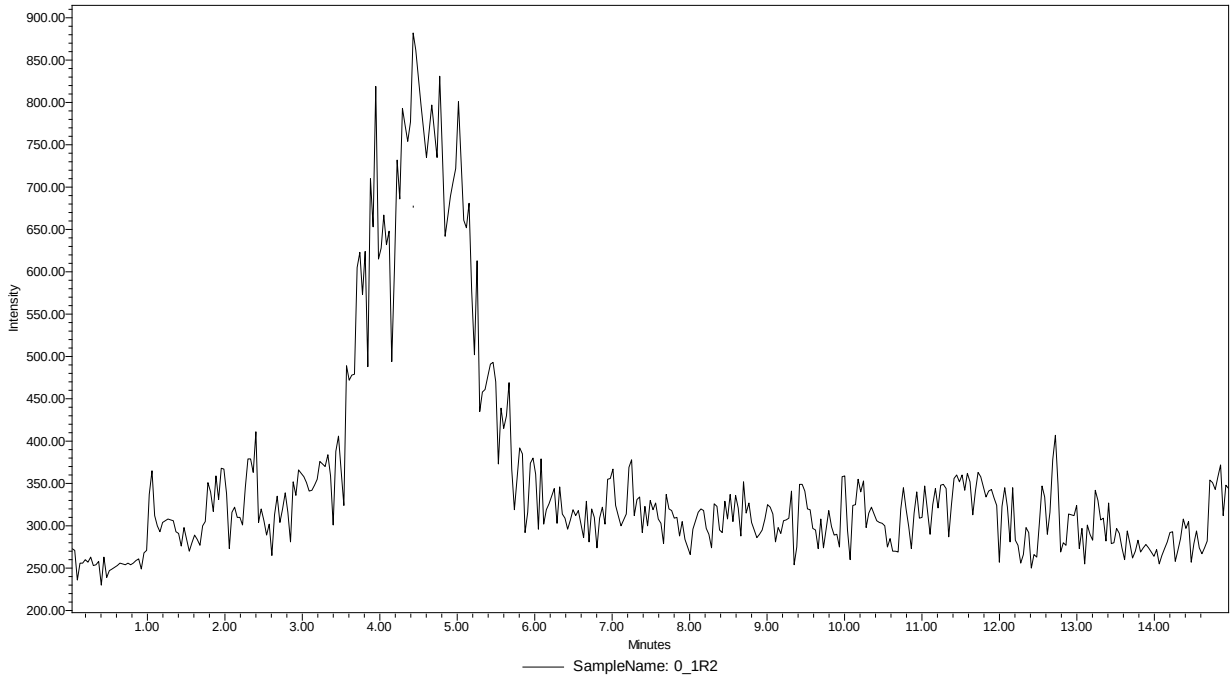

Fig. 25aa product chromatographs for 0.01 ppm FMASld 2nd replicate.

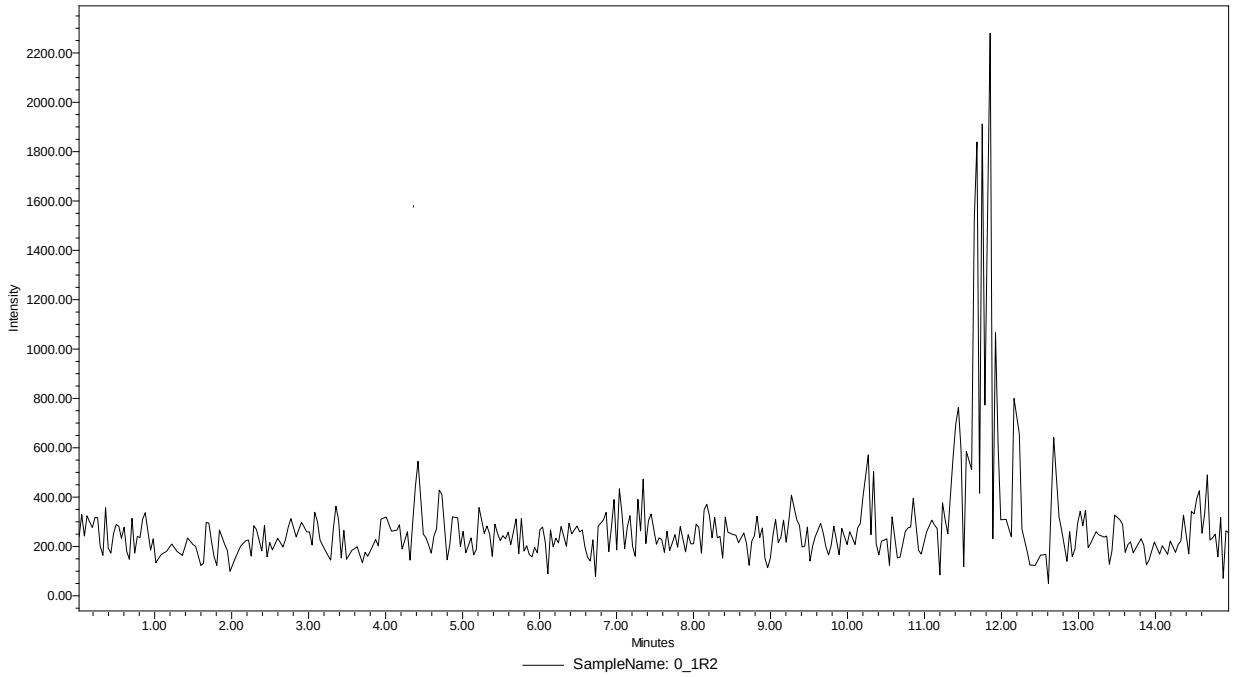

Fig. 25bb product chromatographs for 0.01 ppm FBAL 2nd replicate.

149

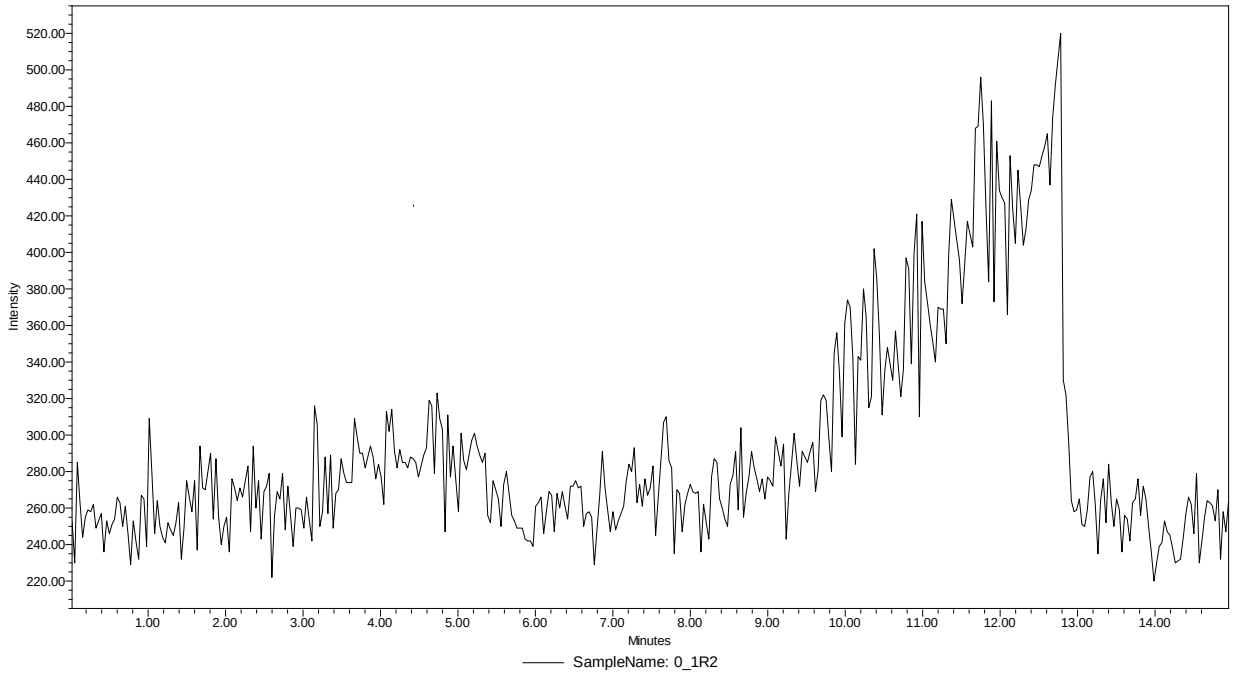

Fig. 25cc product chromatographs for 0.01 ppm FUPA 2nd replicate.

129

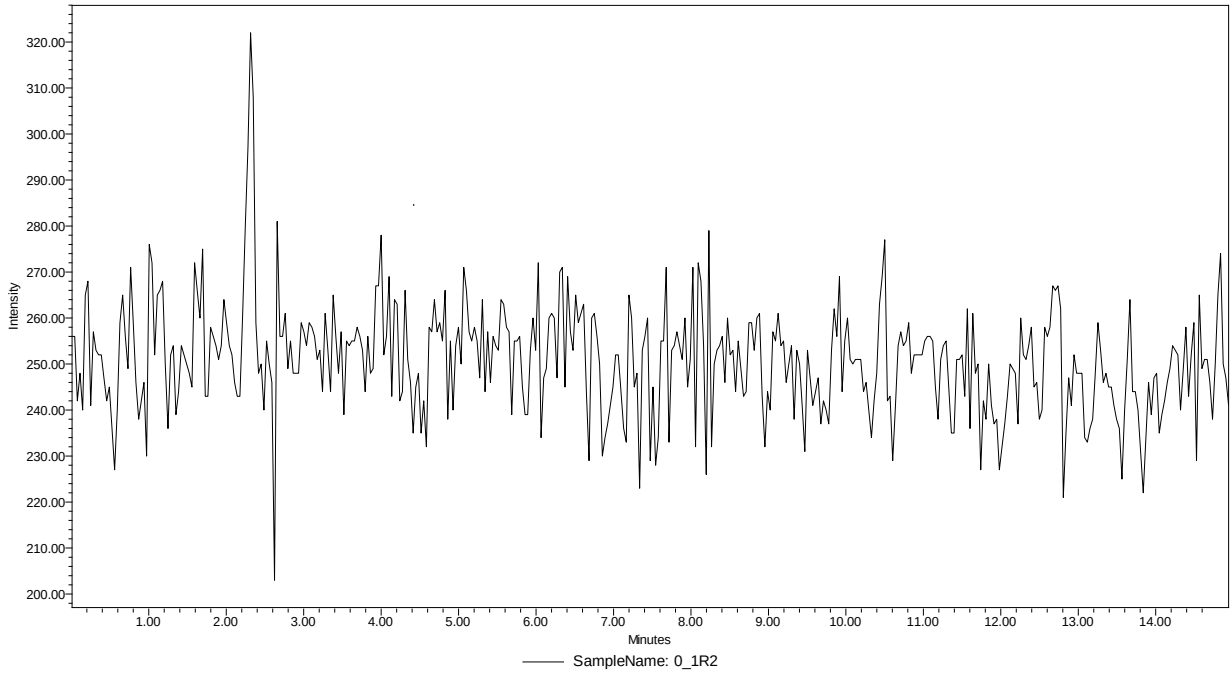

Fig. 25dd product chromatographs for 0.01 ppm 5-FU 2nd replicate.

051

77

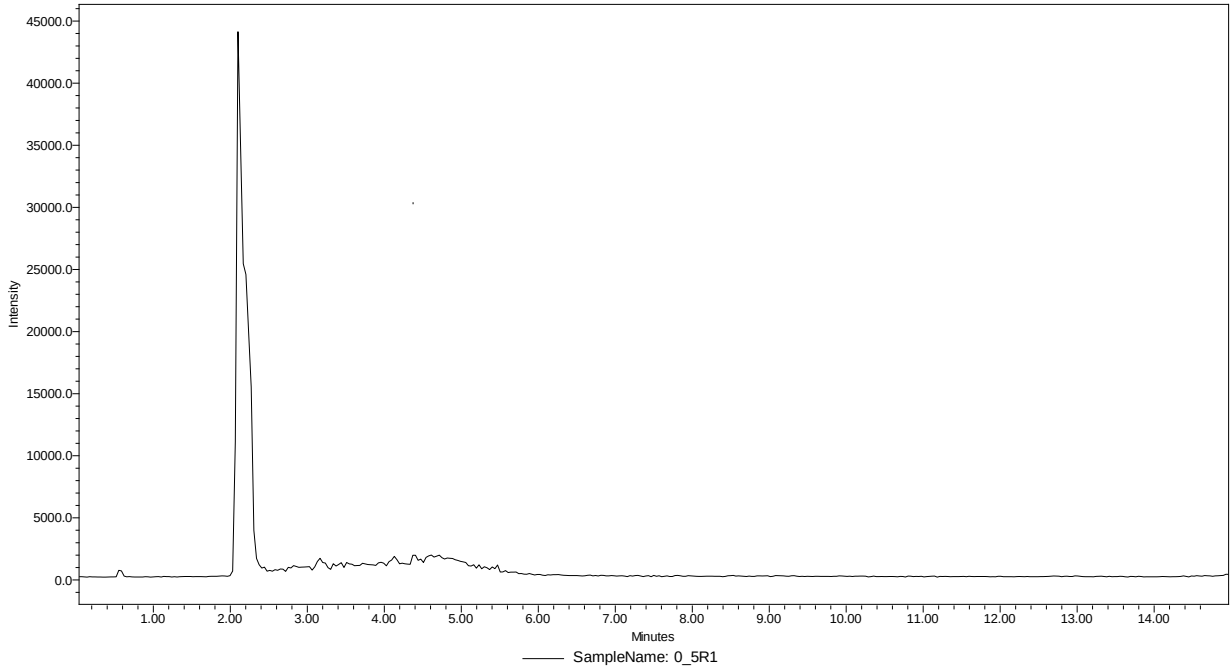

Fig. 25ee product chromatographs for 0.5 ppm FAC 1st replicate.

105

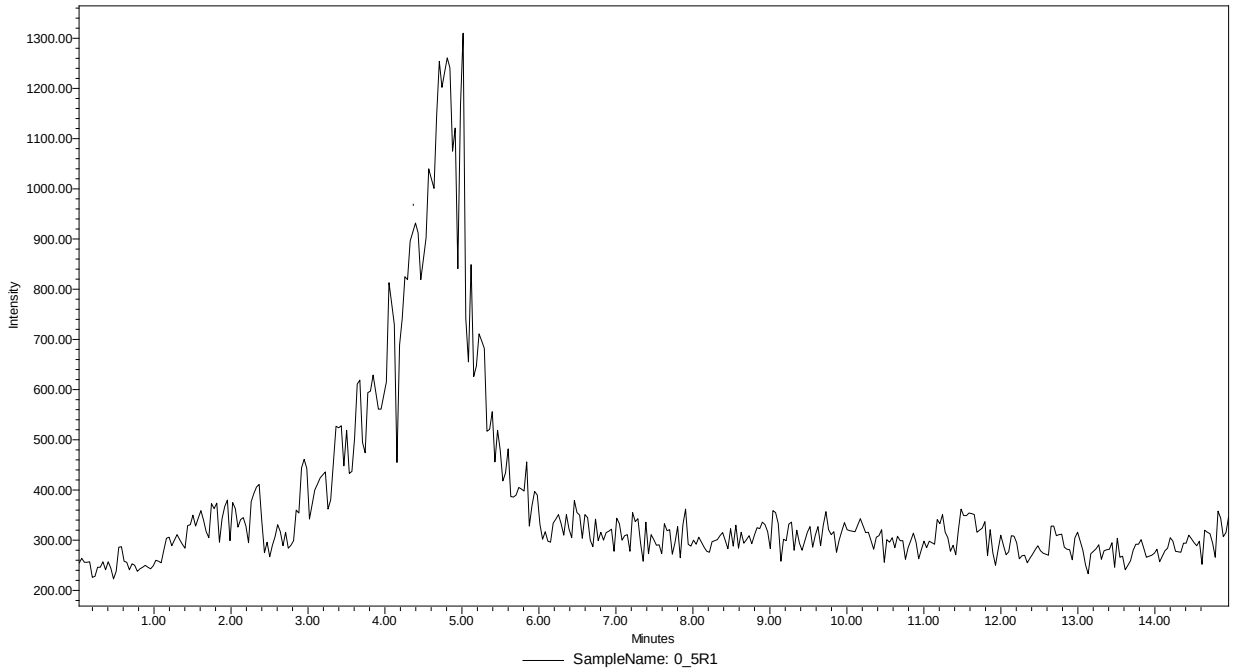

Fig. 25ff product chromatographs for 0.5 ppm FMASld 1st replicate.

106

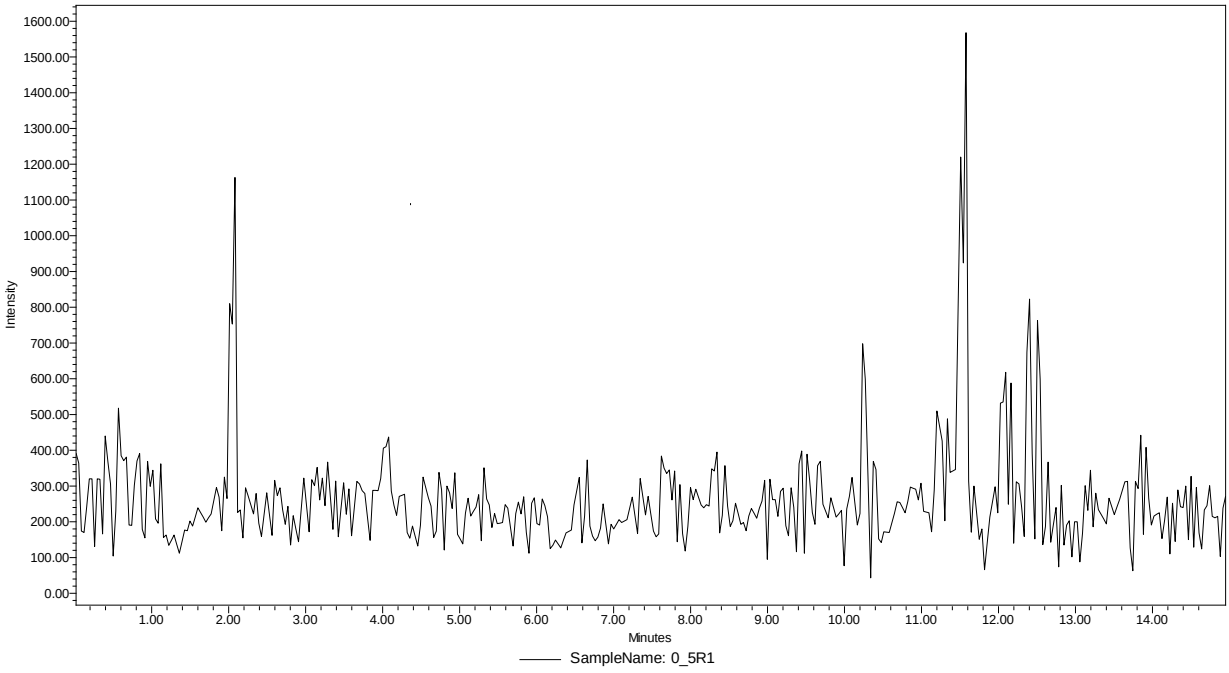

Fig. 25gg product chromatographs for 0.5 ppm FBAL 1st replicate.

149

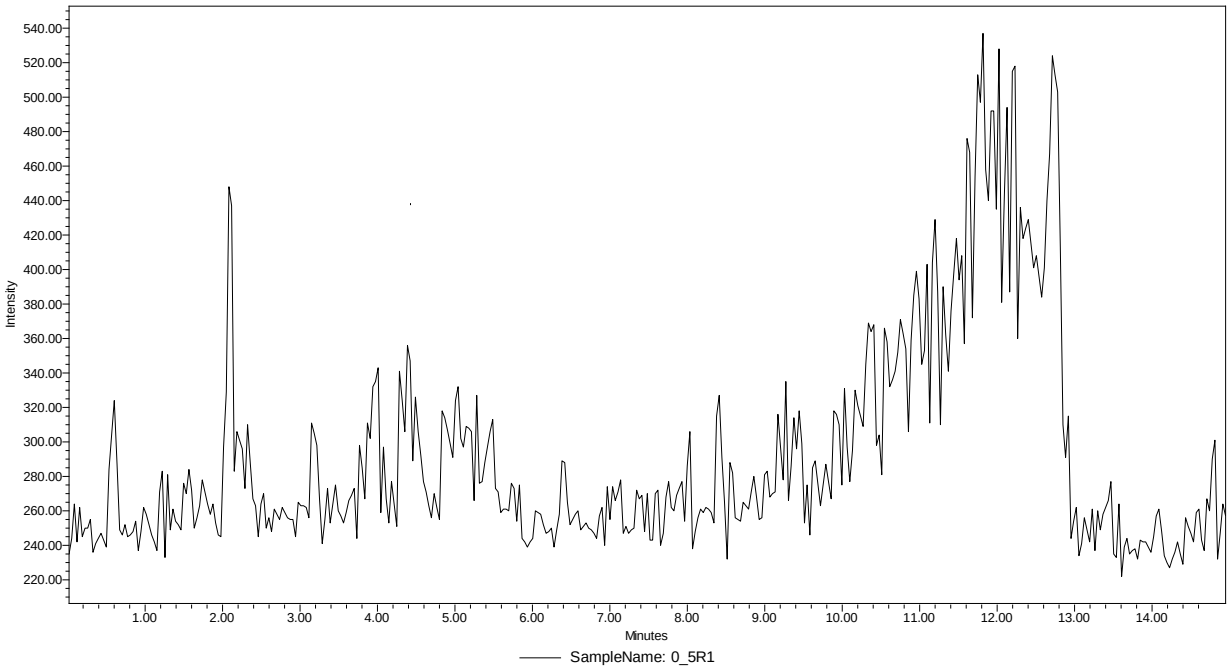

Fig. 25hh product chromatographs for 0.5 ppm FUPA 1st replicate.

129

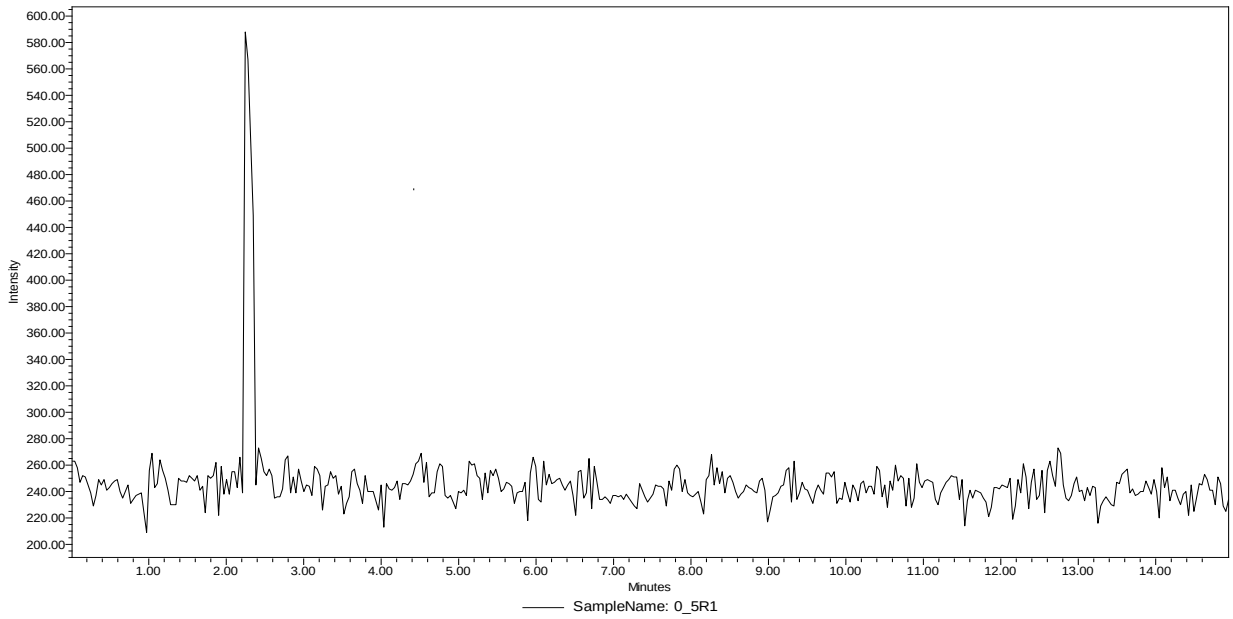

Fig. 25ii product chromatographs for 0.5 ppm 5-FU 1st replicate.

052

77

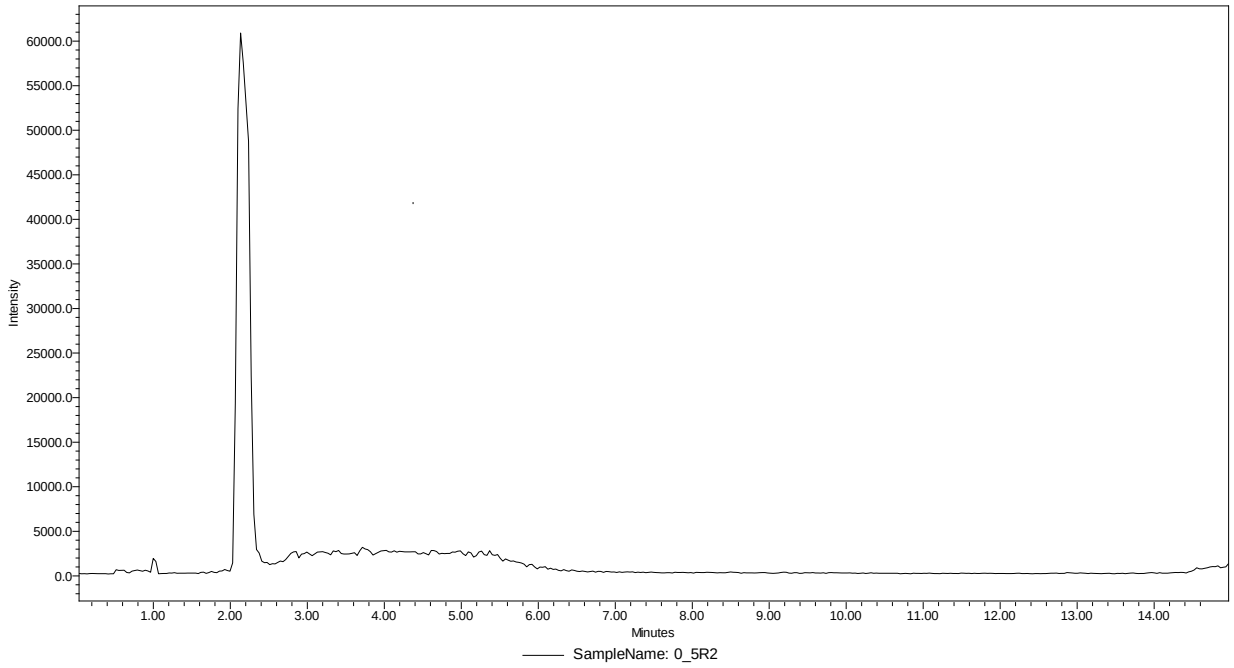

Fig. 25jj product chromatographs for 0.5 ppm FAC 2nd replicate.

105

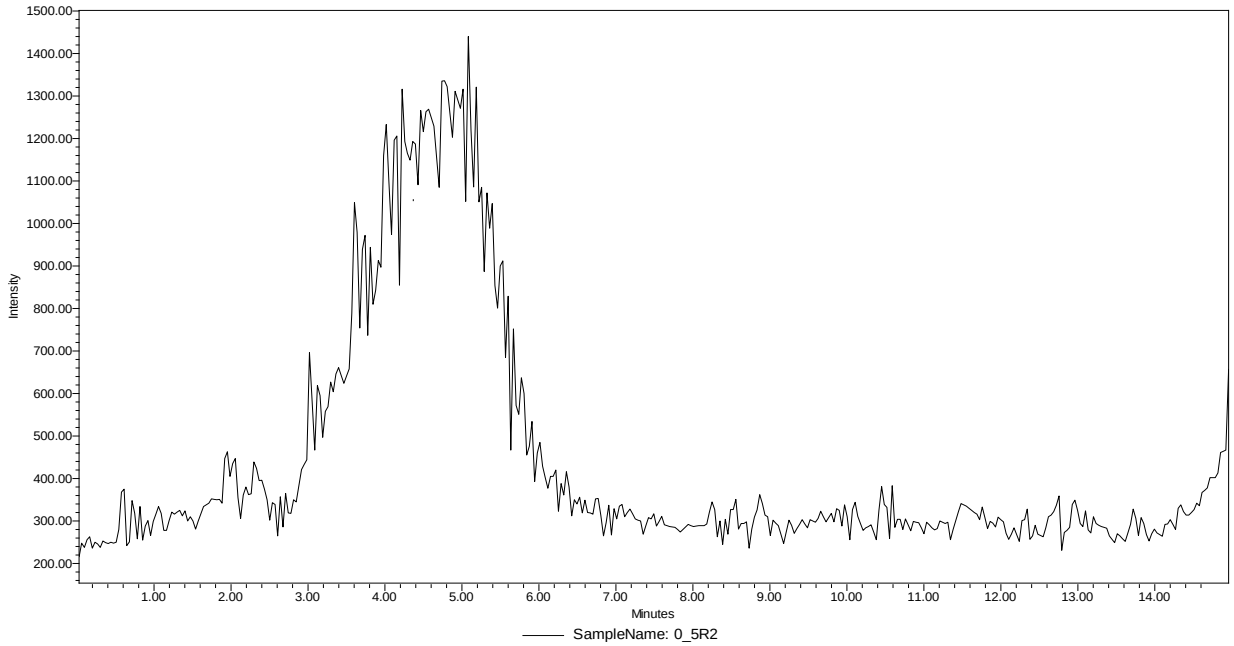

Fig. 25kk product chromatographs for 0.5 ppm FMASld 2nd replicate.

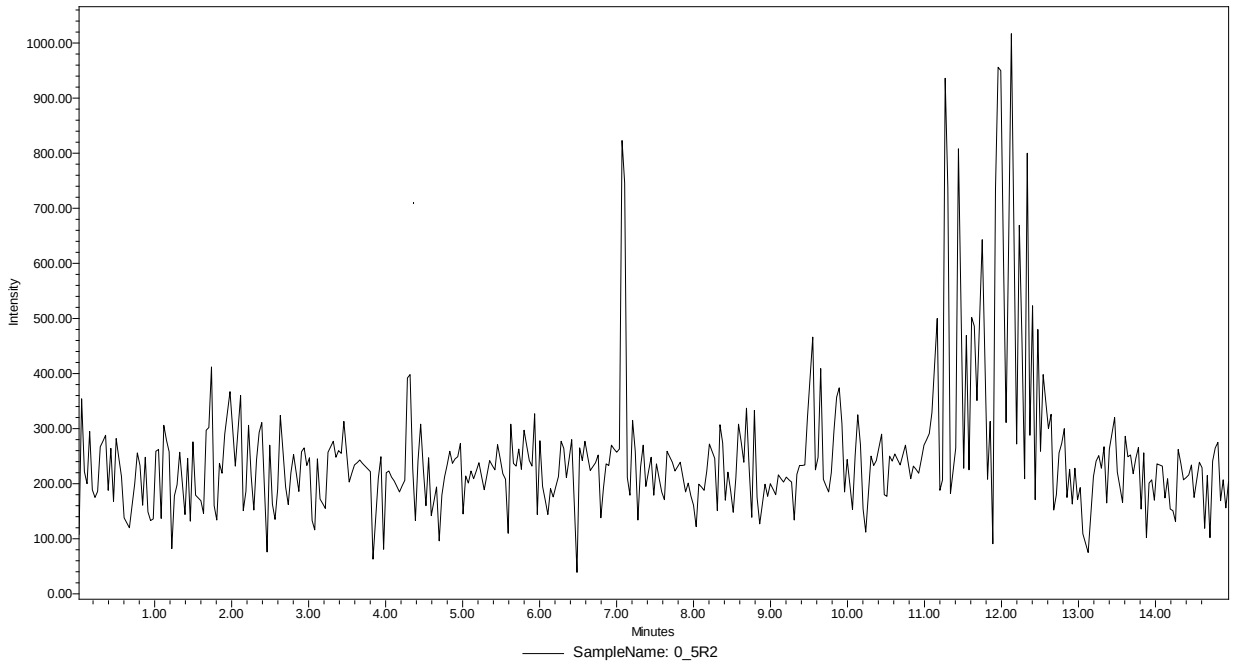

Fig. 25ll product chromatographs for 0.5 ppm FBAL 2nd replicate.

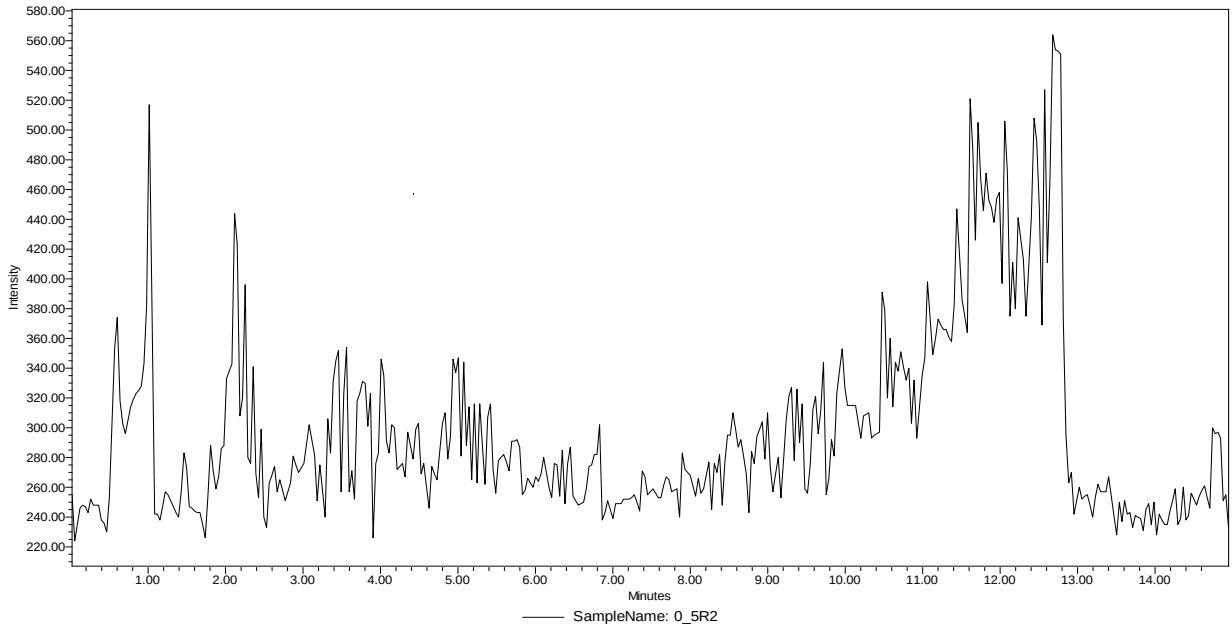

Fig. 25mm product chromatographs for 0.5 ppm FUPA 2nd replicate.

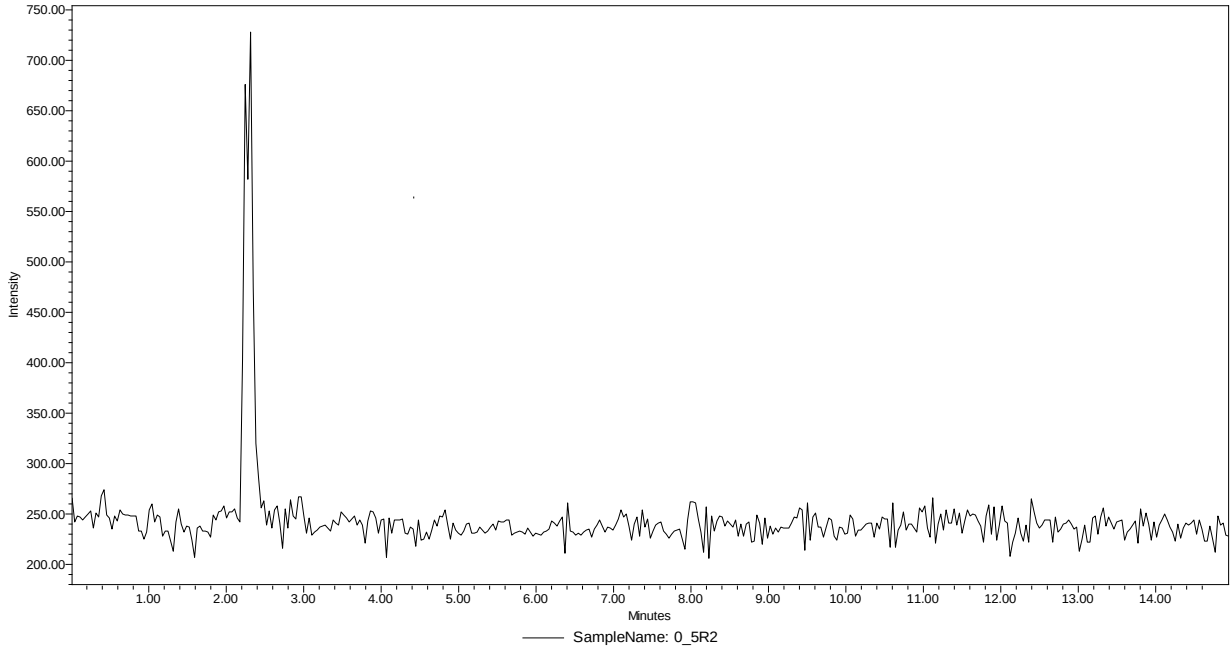

Fig. 25nn product chromatographs for 0.5 ppm 5-FU 2nd replicate.

11

77

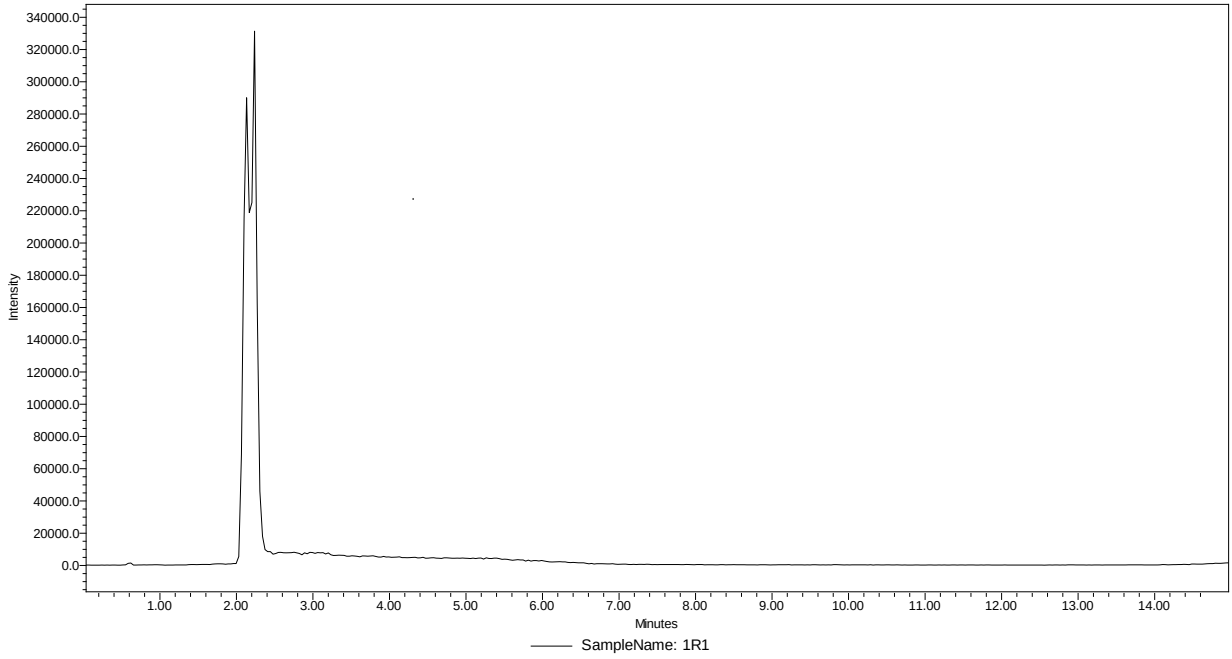

Fig. 25oo product chromatographs for 1.0 ppm FAC 1st replicate.

105

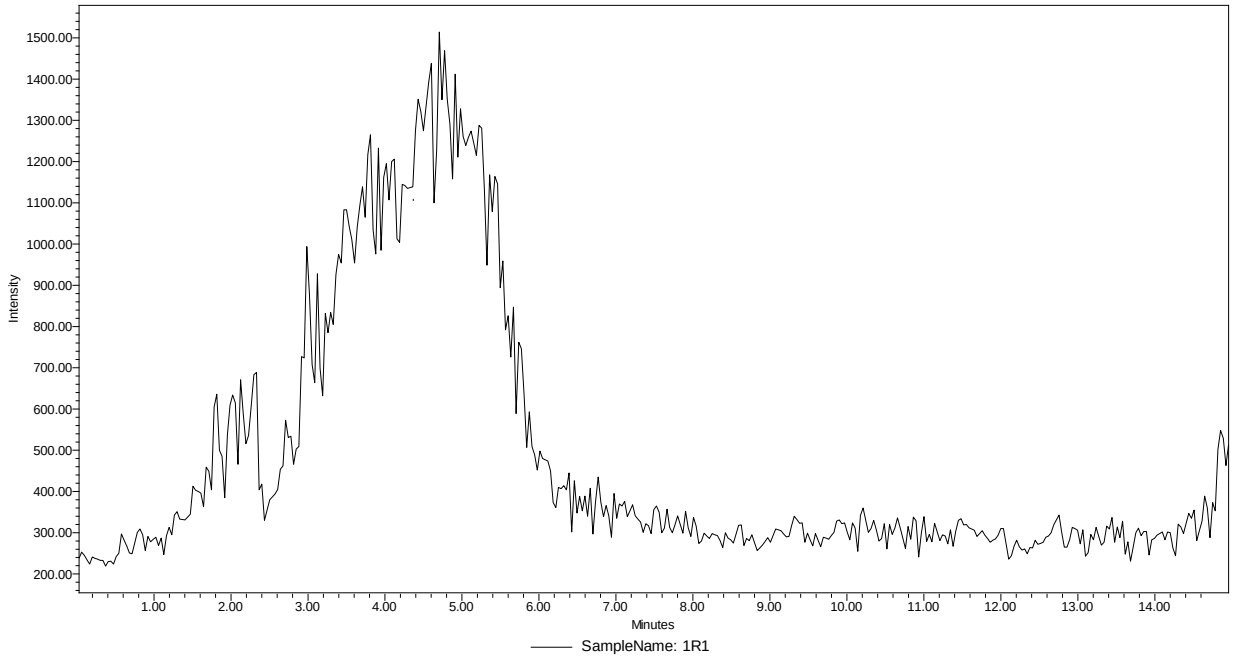

Fig. 25pp product chromatographs for 1.0 ppm FMASld 1st replicate.

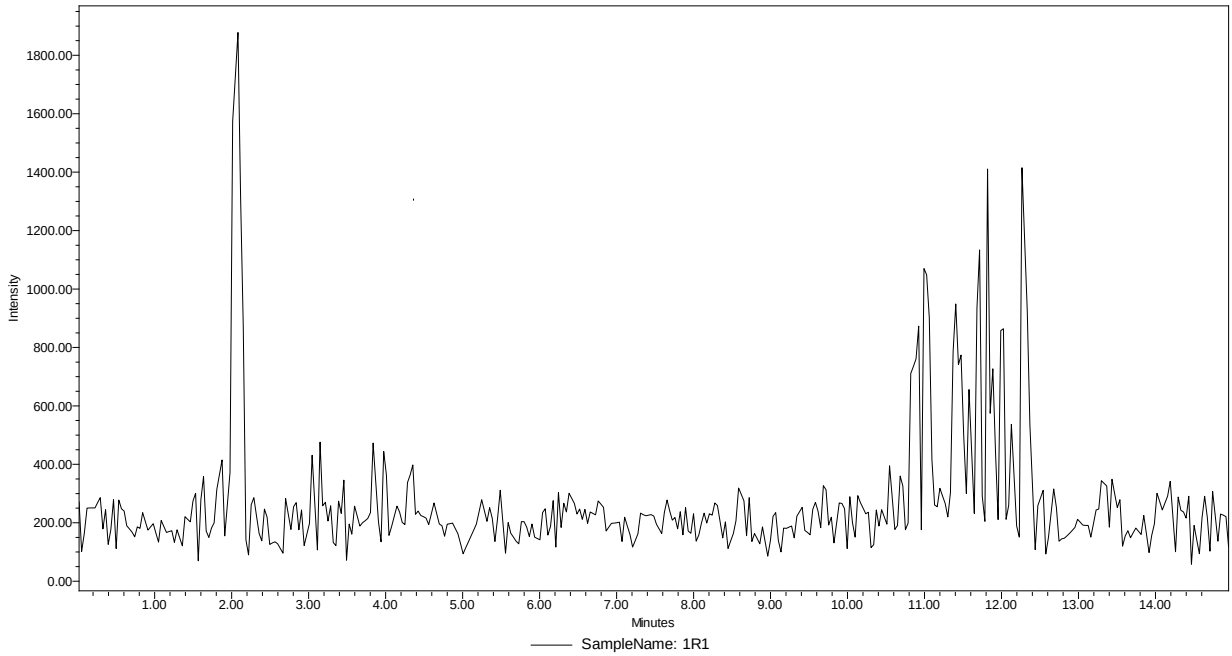

Fig. 25qq product chromatographs for 1.0 ppm FBAL 1st replicate.

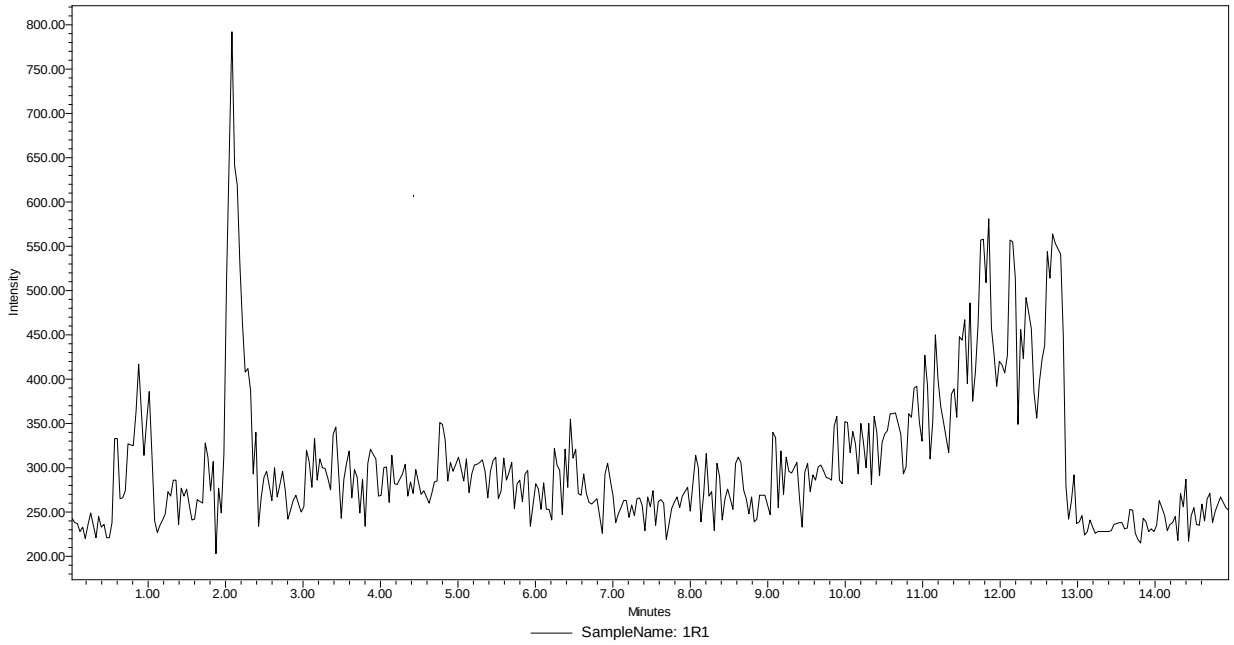

Fig. 25rr product chromatograph for 1.0 ppm FUPA 1st replicate.

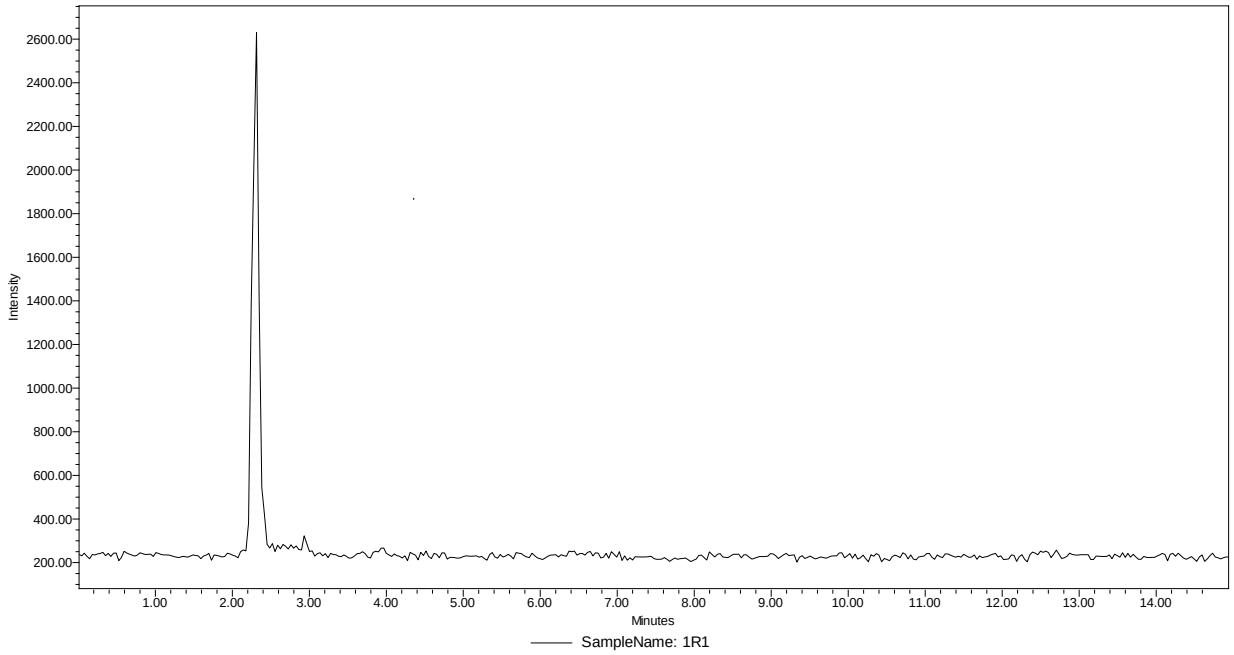

Fig. 25ss product chromatograph for 1.0 ppm 5-FU 1st replicate.

12

77

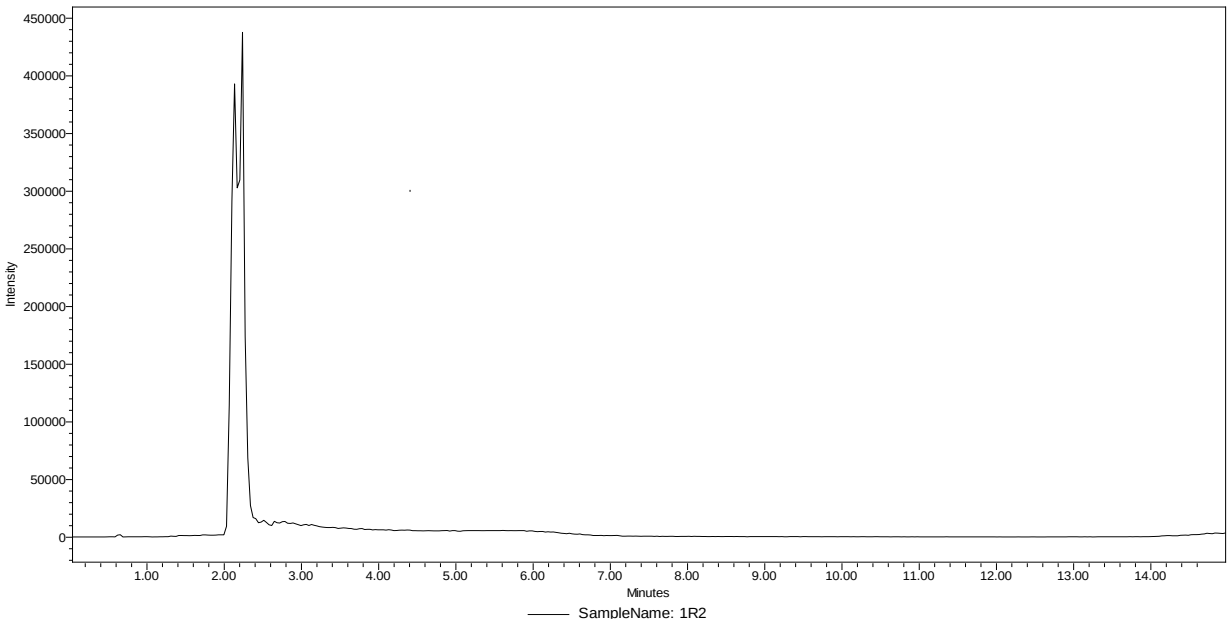

Fig. 25tt product chromatograph for 1.0 ppm FAC 2nd replicate.

105

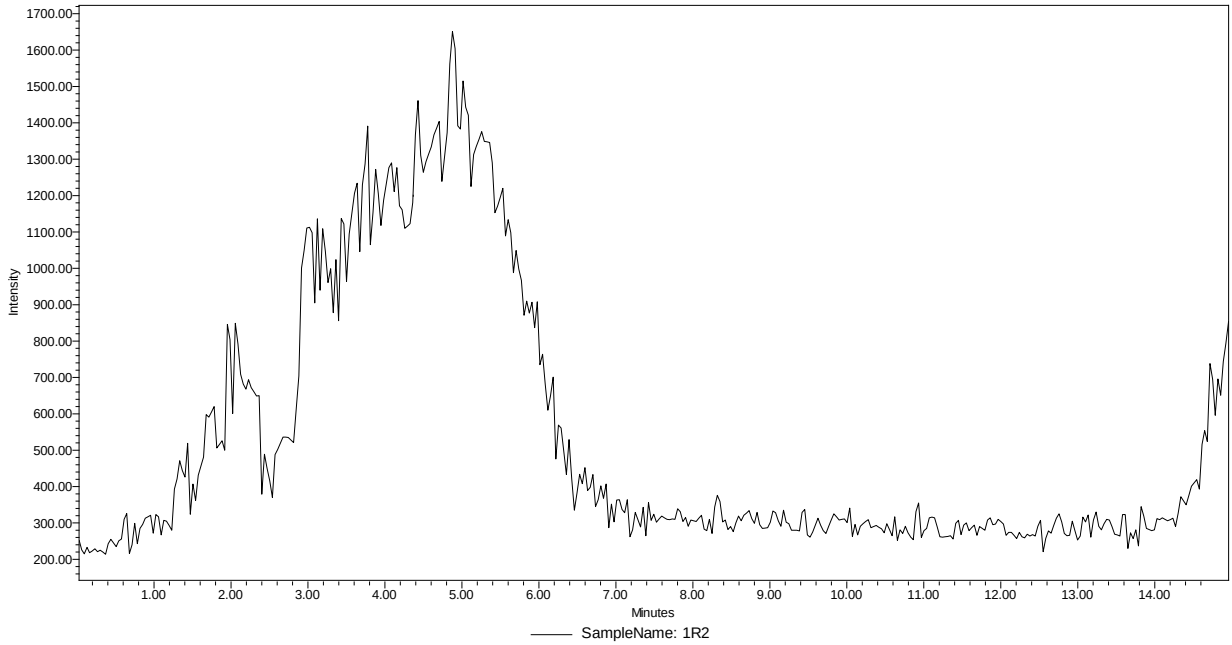

Fig. 25uu product chromatograph for 1.0 ppm FMASld 2nd replicate.

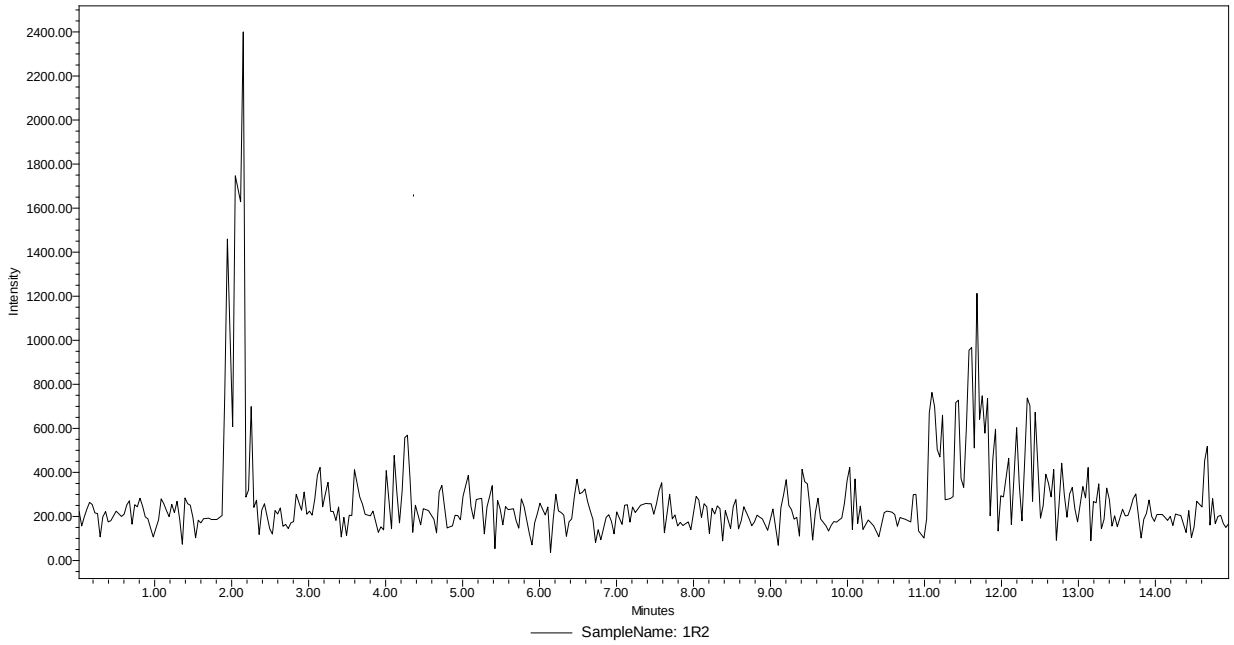

Fig. 25vv product chromatograph for 1.0 ppm FBAL 2nd replicate.

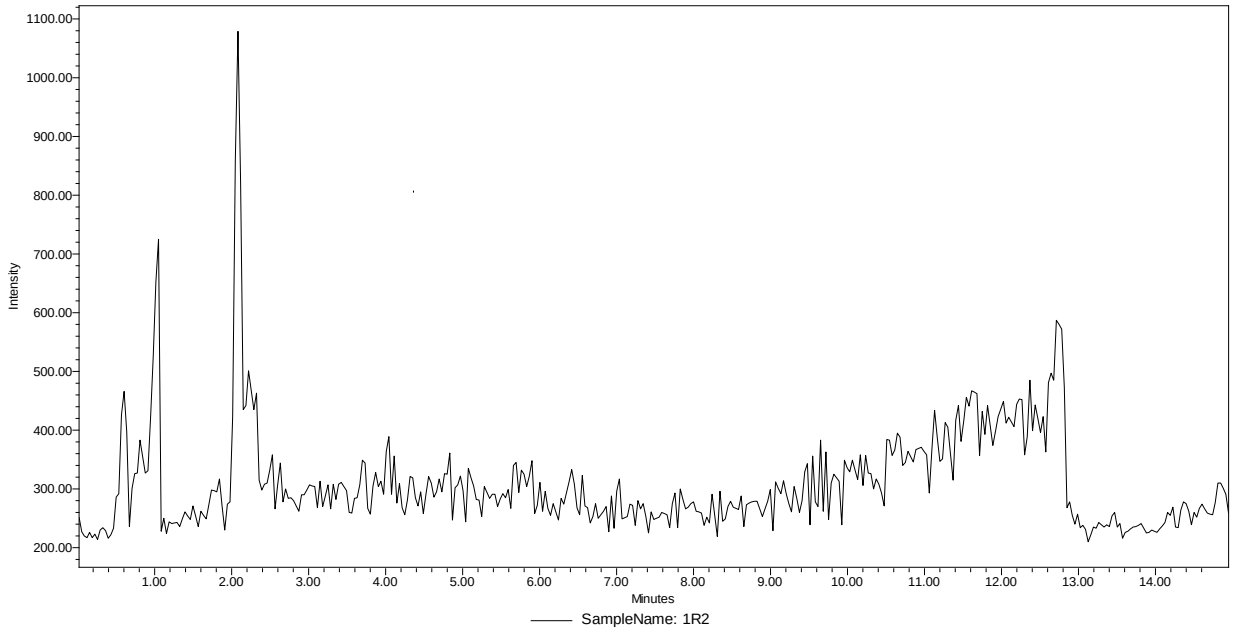

Fig. 25xx product chromatograph for 1.0 ppm FUPA 2nd replicate.

129

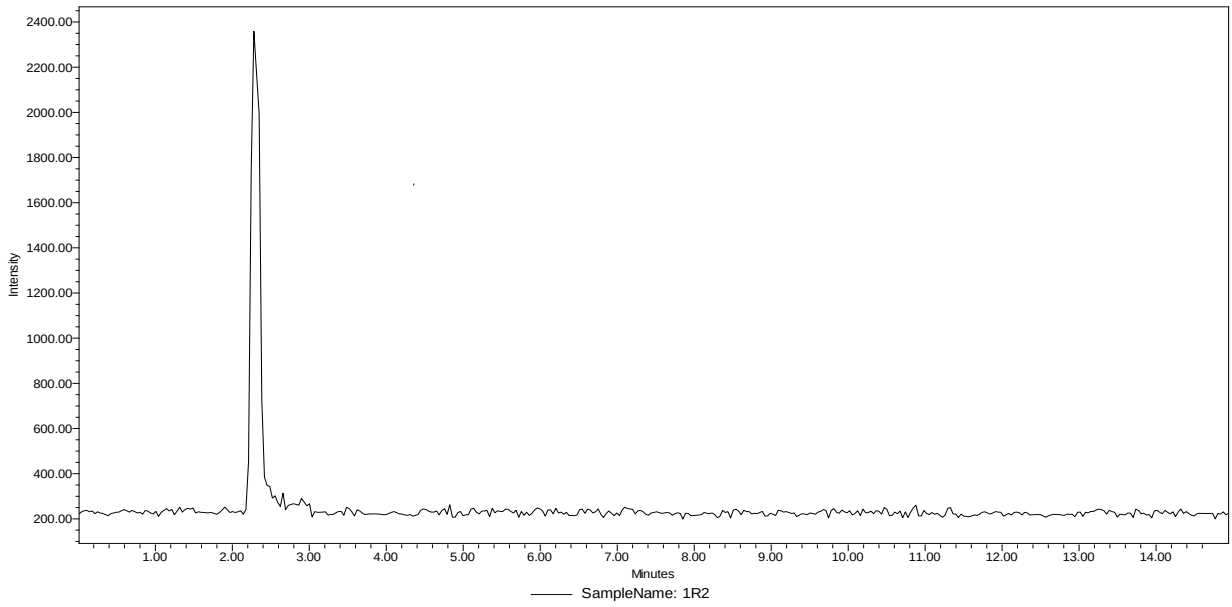

Fig. 25yy product chromatograph for 1.0 ppm 5-FU 2nd replicate.

51

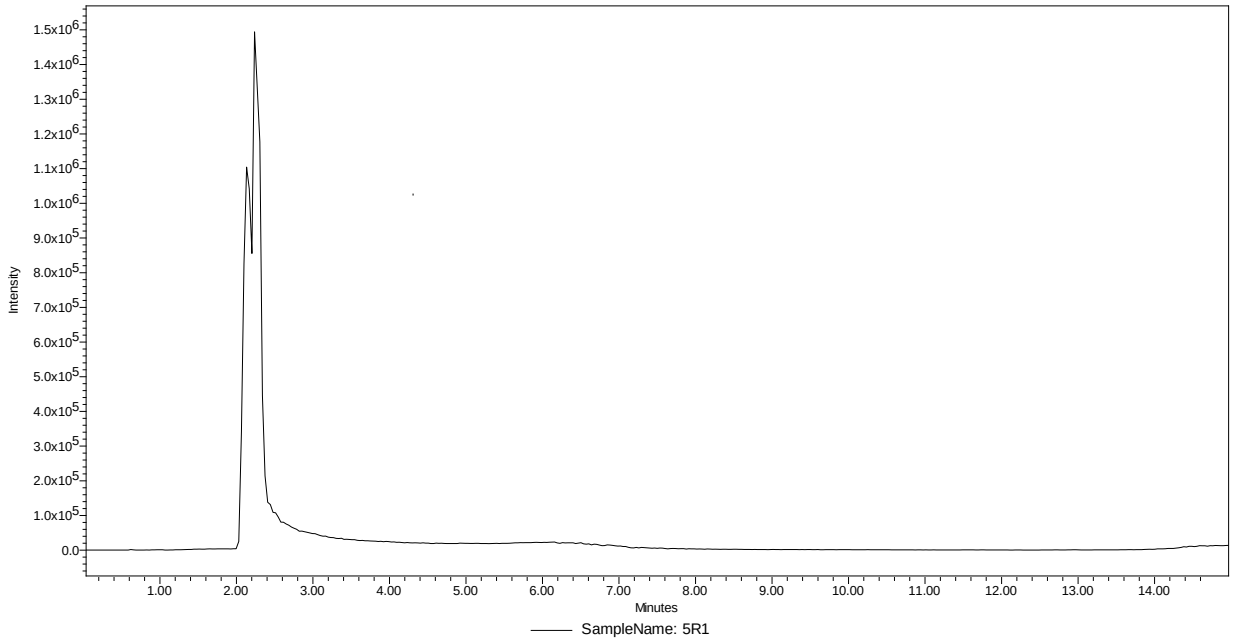

Fig. 25zz product chromatograph for 5.0 ppm FAC 1st replicate.

105

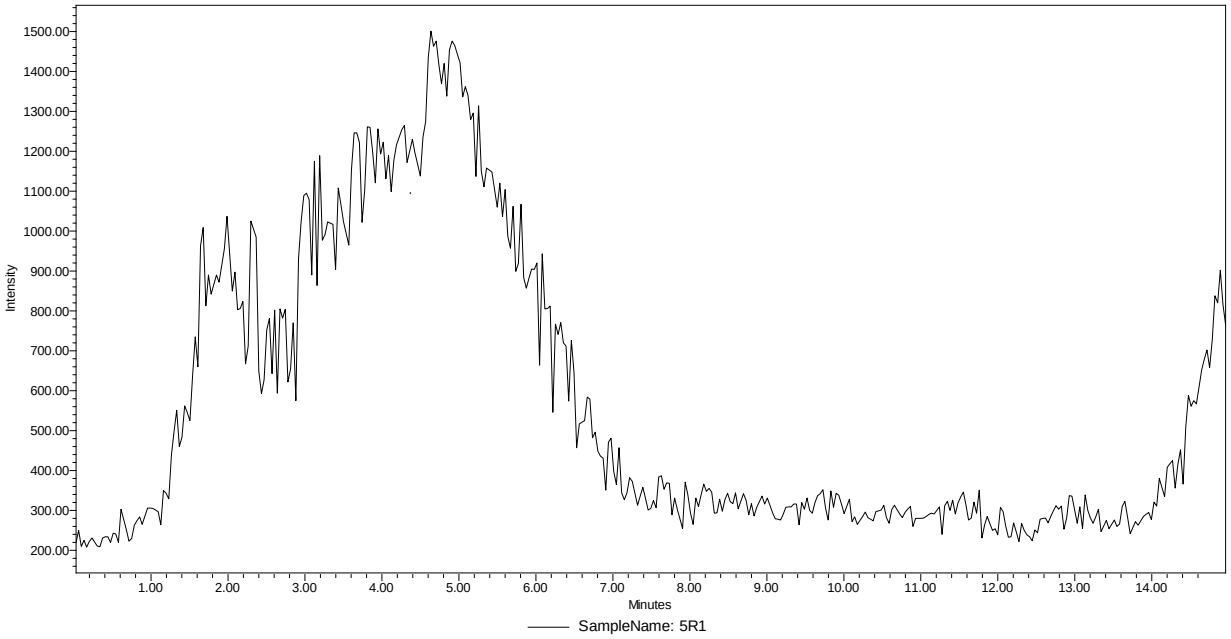

Fig. 25aaa product chromatograph for 5.0 ppm FMASld 1st replicate.

106

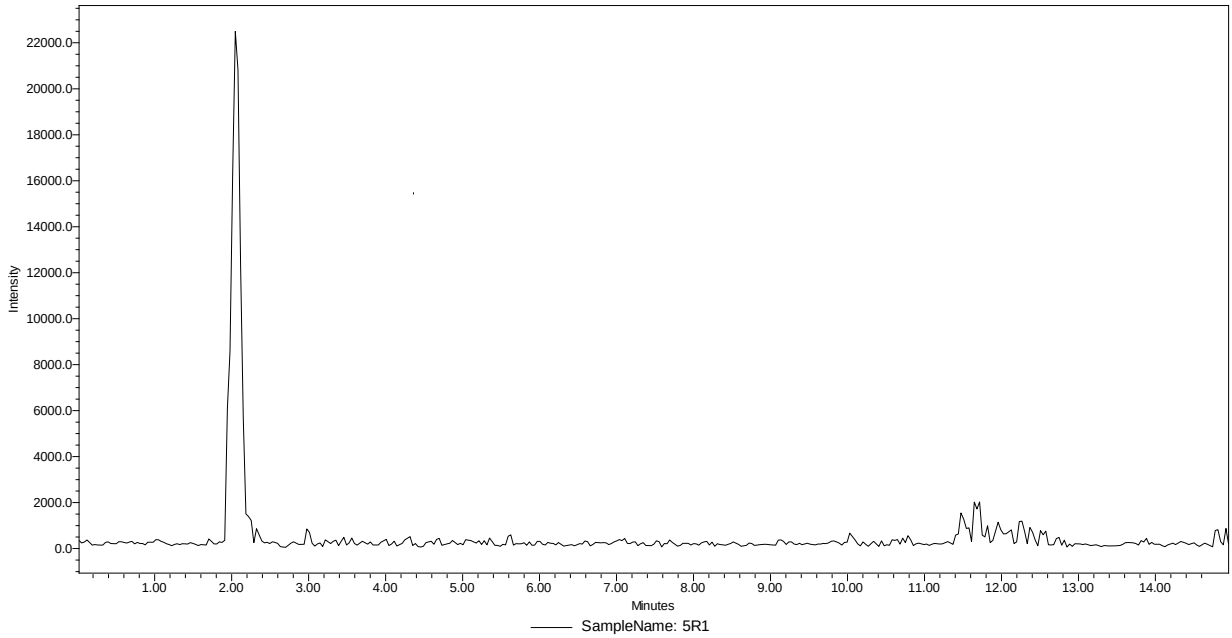

Fig. 25bbb product chromatograph for 5.0 ppm FBAL 1st replicate.

149

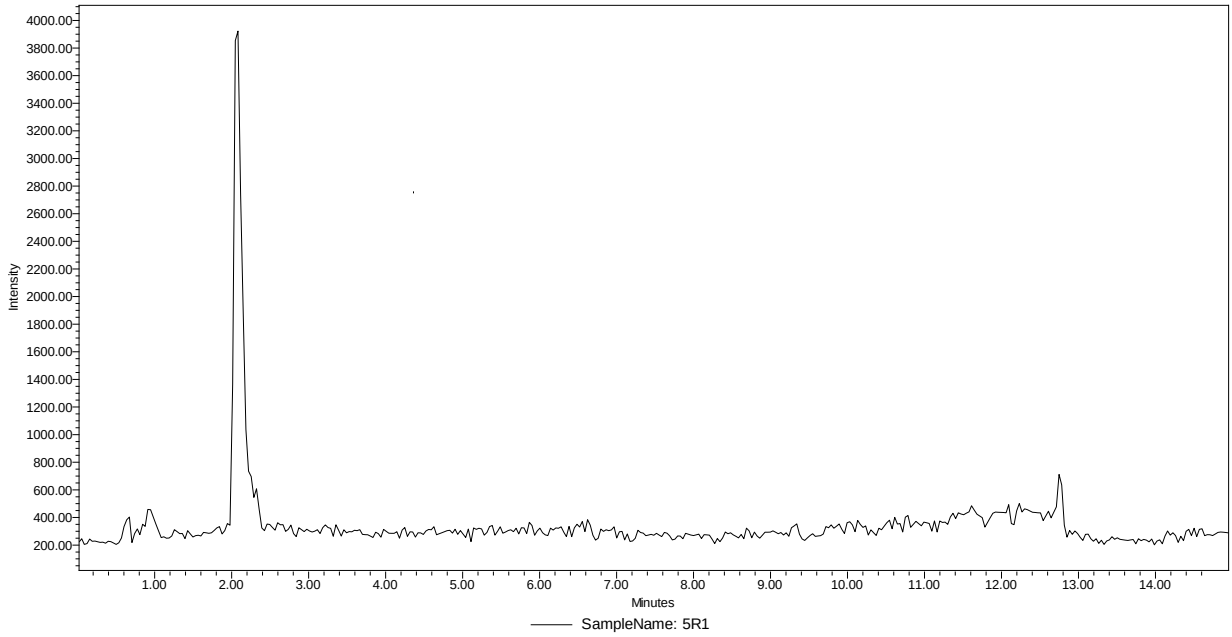

Fig. 25ccc product chromatograph for 5.0 ppm FUPA 1st replicate.

129

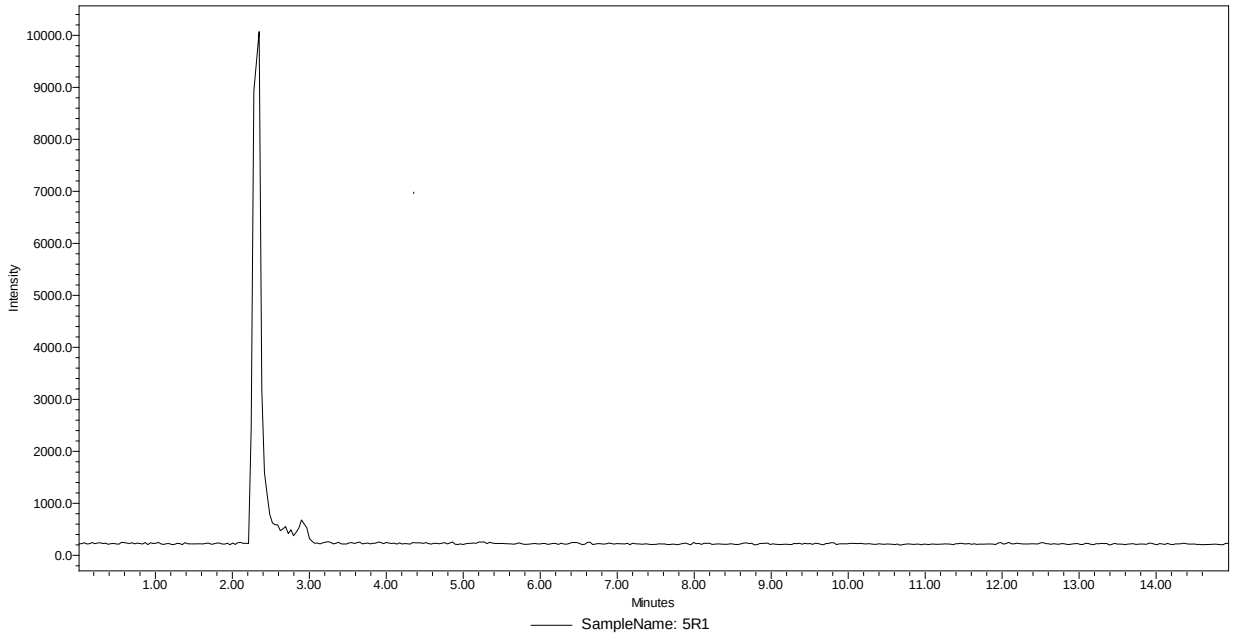

Fig. 25ddd product chromatograph for 5.0 ppm 5-FU 1st replicate.

52

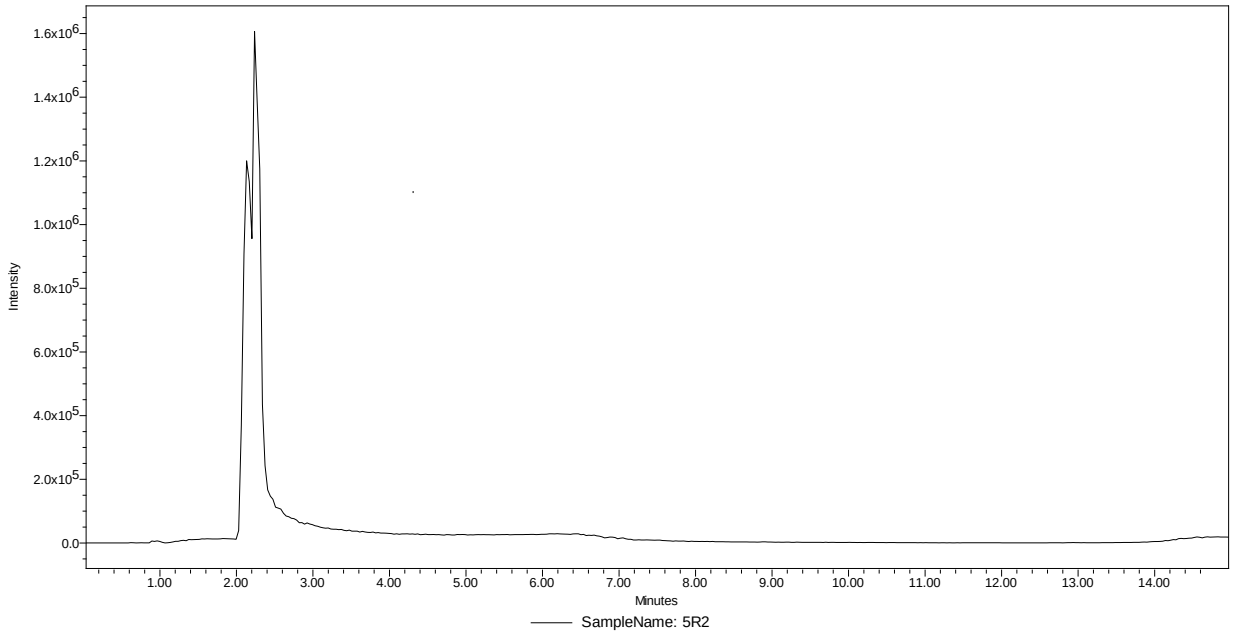

Fig. 25eee product chromatograph for 5.0 ppm FAC 2nd replicate.

105

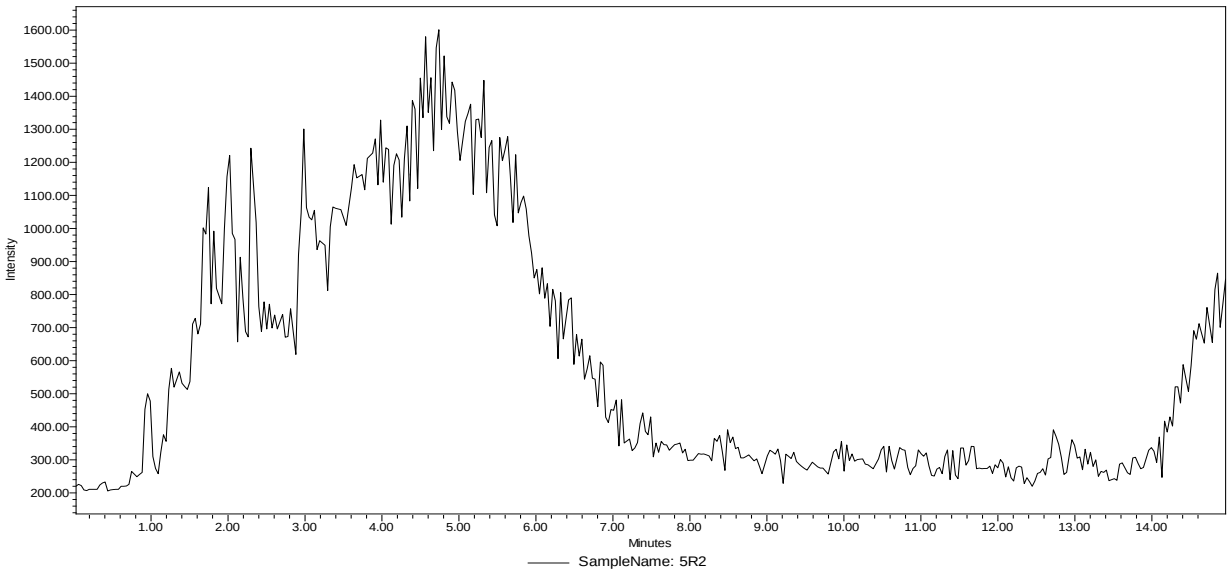

Fig. 25fff product chromatograph for 5.0 ppm FMASld 2nd replicate.

106

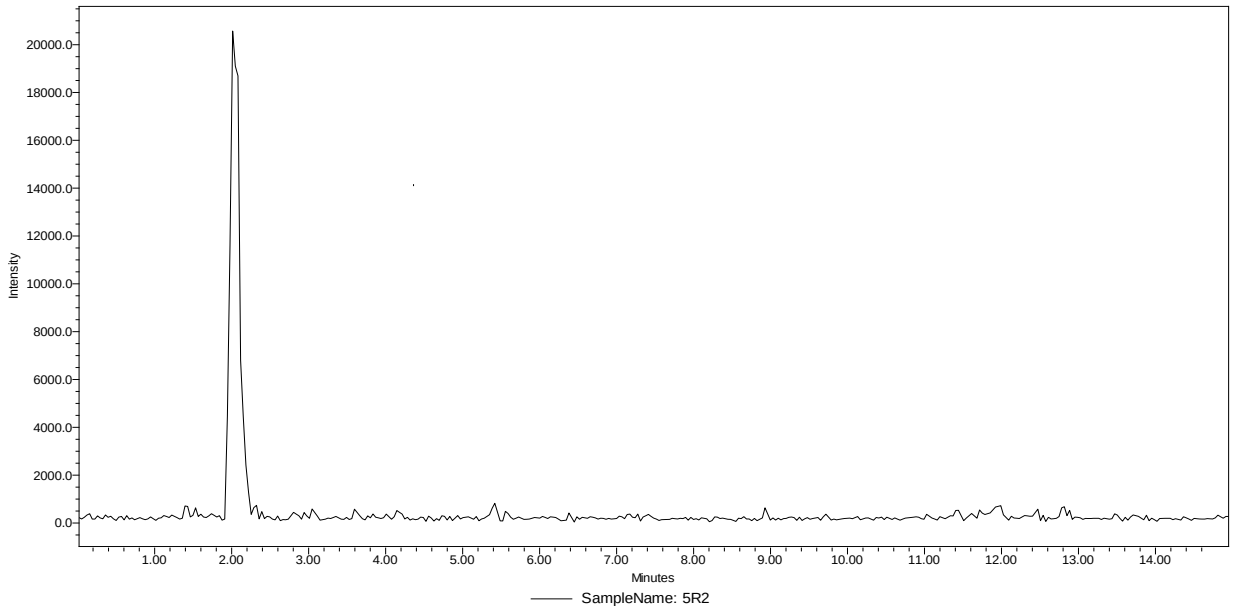

Fig. 25ggg product chromatograph for 5.0 ppm FBAL 2nd replicate.

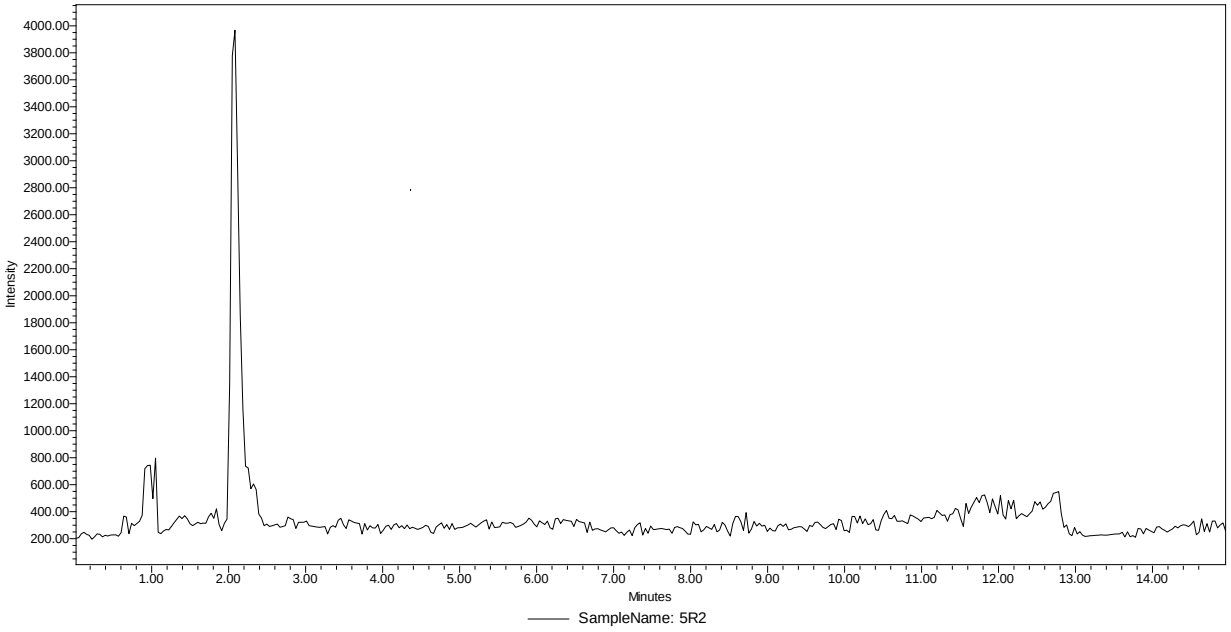

Fig. 25hhh product chromatograph for 5.0 ppm FUPA 2nd replicate.

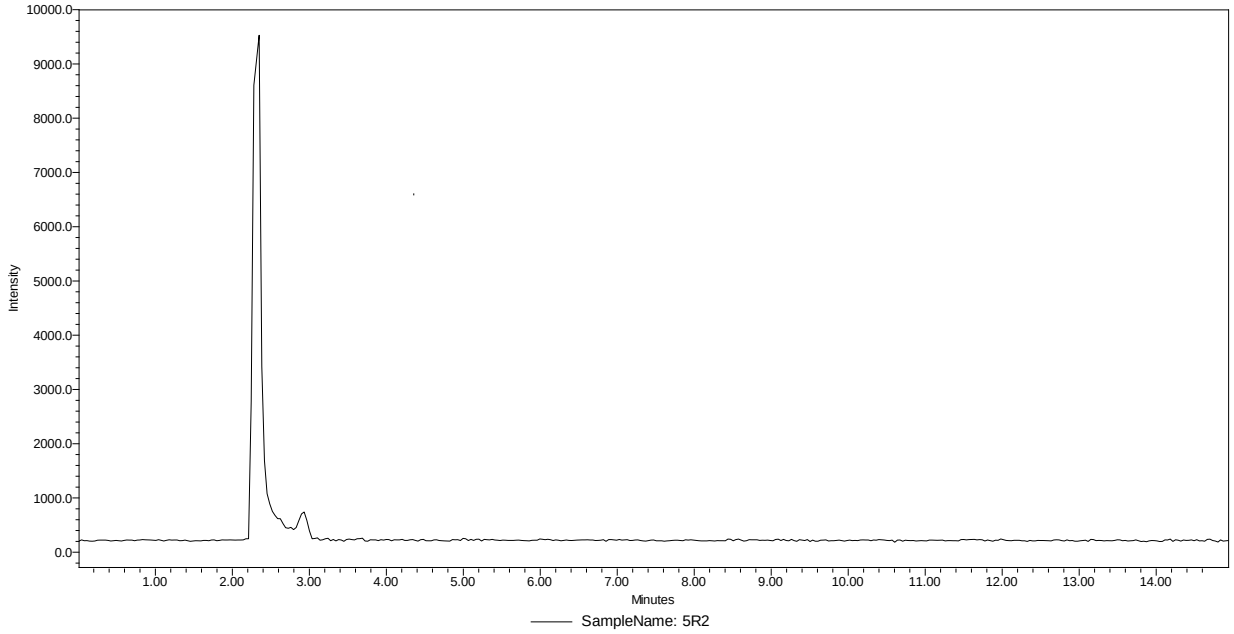

Fig. 25iii product chromatograph for 5.0 ppm 5-FU 2nd replicate.

A1r

77

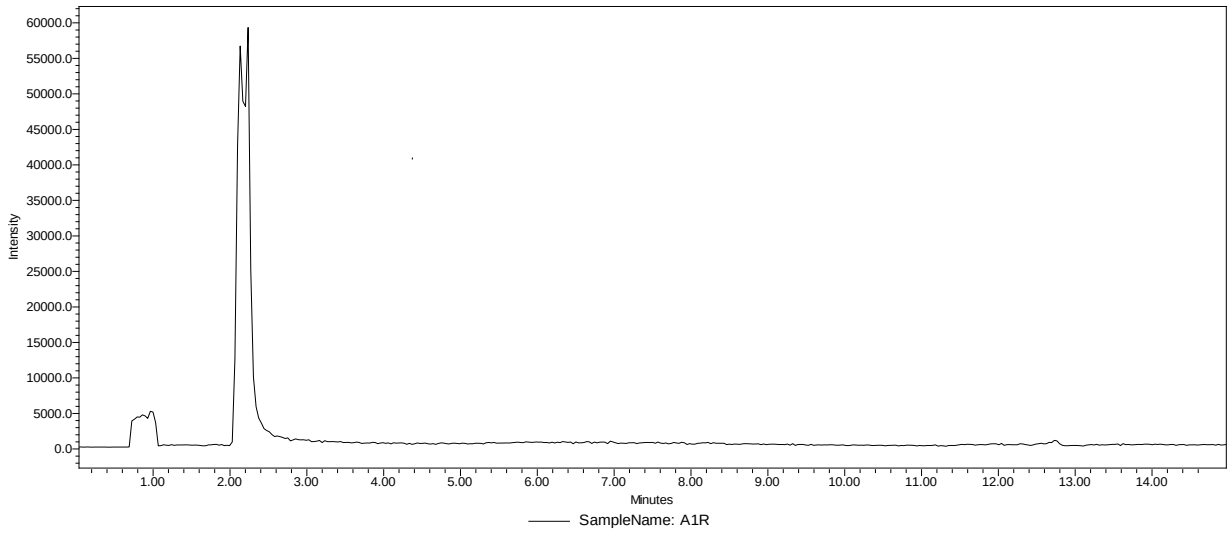

Fig. 25iii(1) product chromatograph for .05 ppm FAC 1st replicate.

105

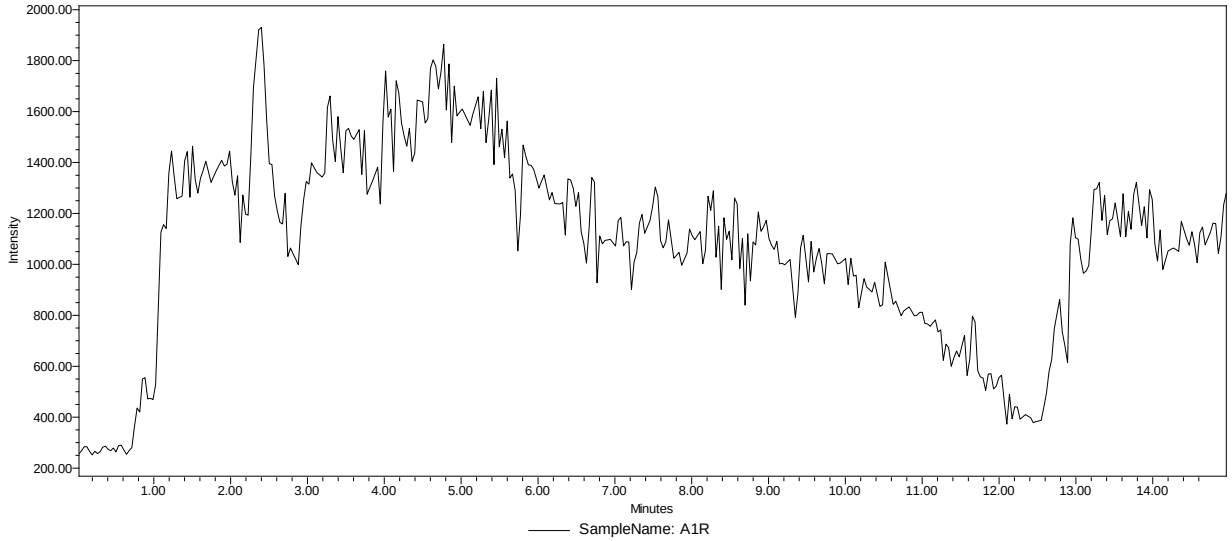

Fig. 25iii(2) product chromatograph for .05 ppm FMASld 1st replicate.

106

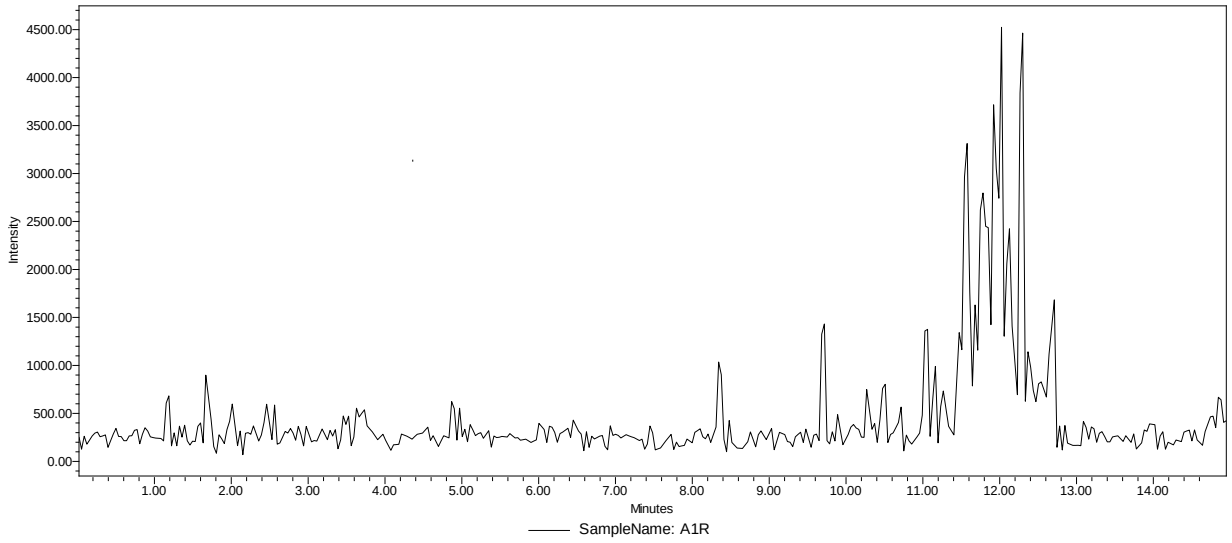

Fig. 25iii(3) product chromatograph for .05 ppm FBAL 1st

149

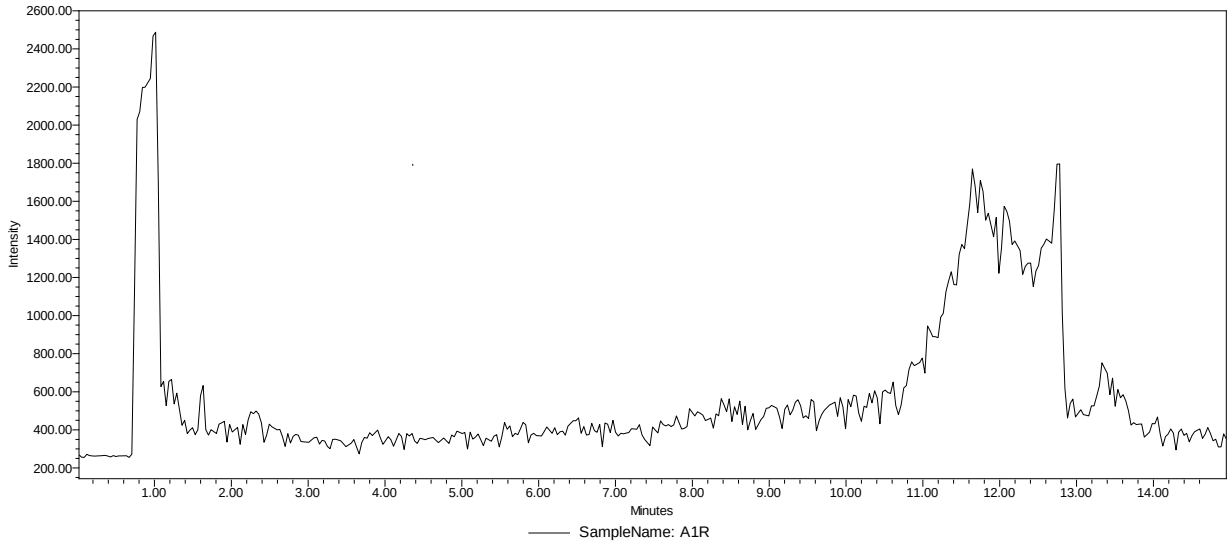

Fig. 25iii(4) product chromatograph for .05 ppm FUPA 1st

129

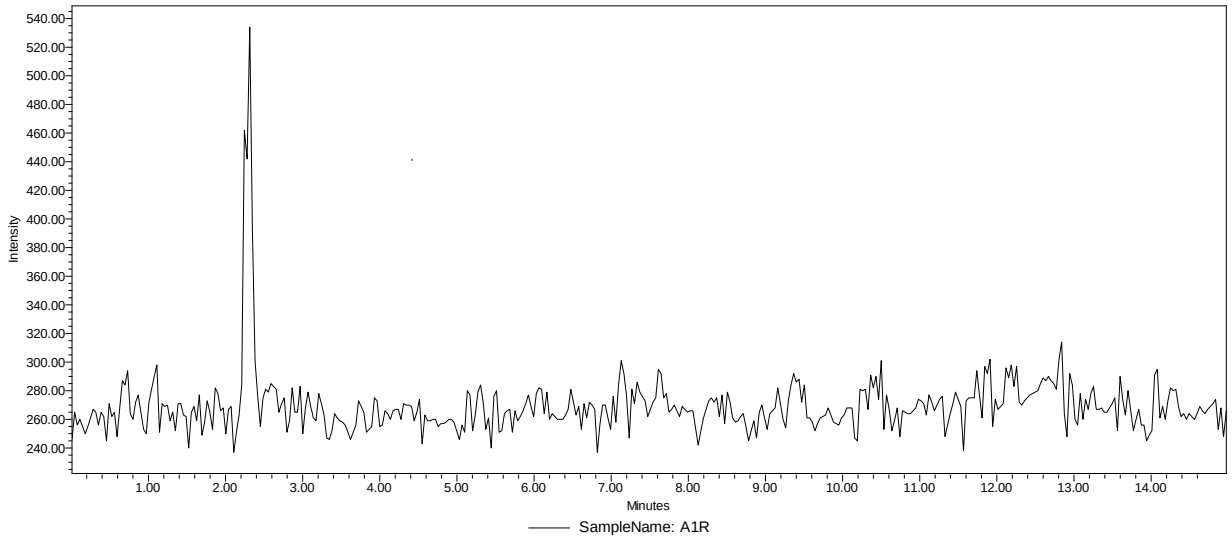

Fig. 25iii(5) product chromatograph for .05 ppm 5-FU 1st

A2

77

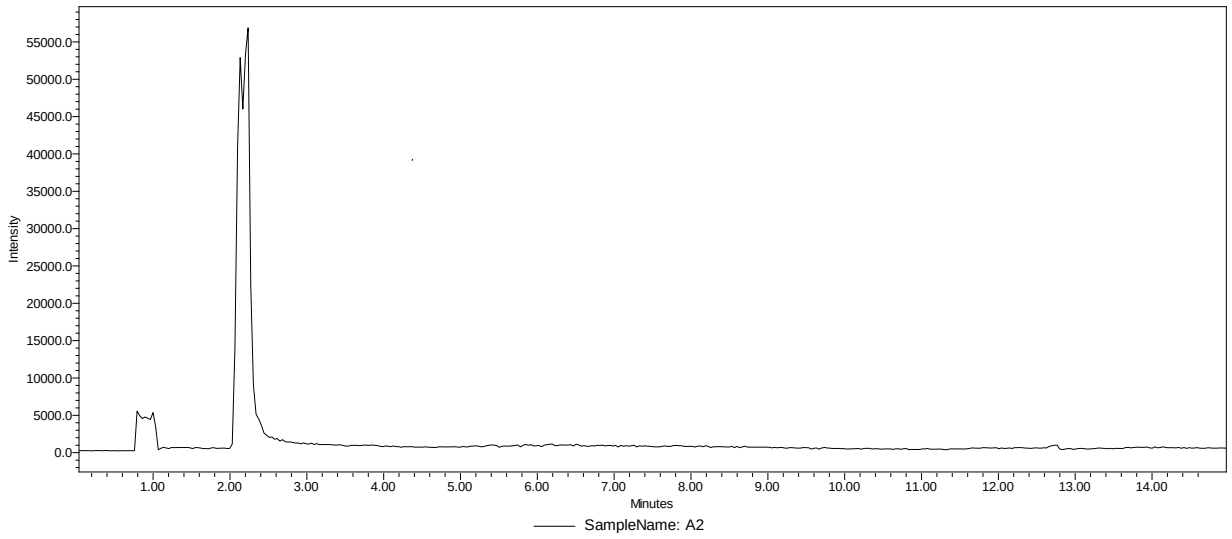

Fig. 25iii(6) product chromatograph for .05 ppm FAC 2nd

105

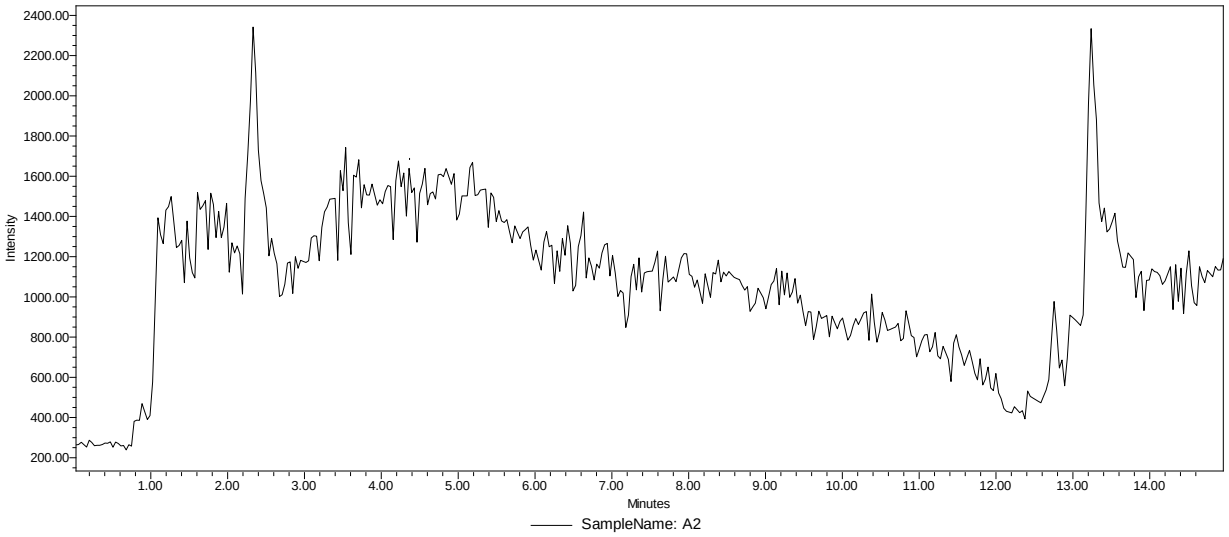

Fig. 25iii(7) product chromatograph for .05 ppm FMASld 2nd replicate.

106

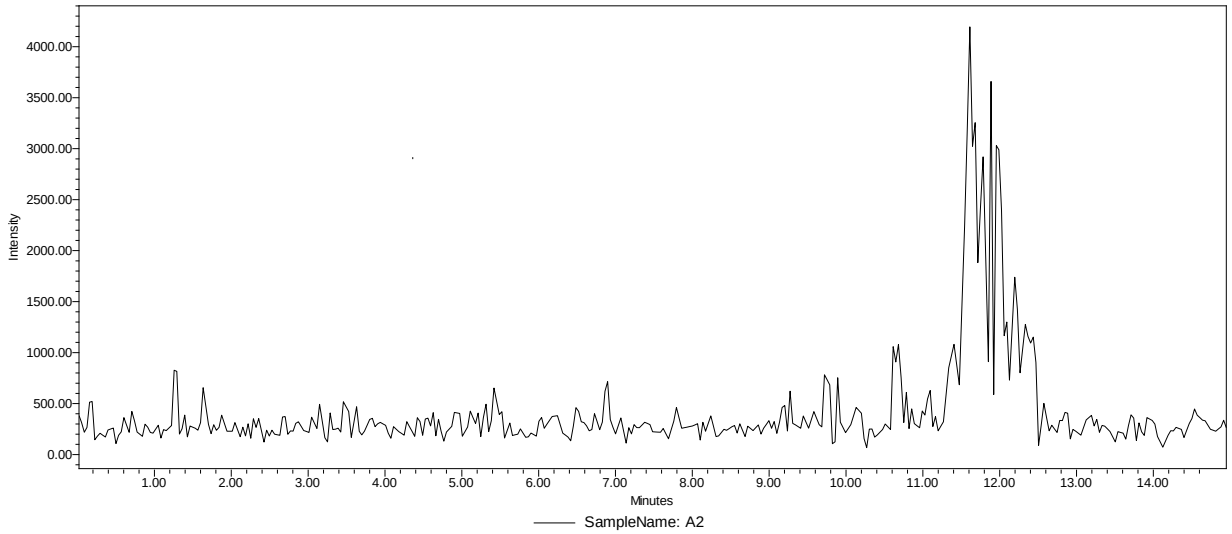

Fig. 25iii(8) product chromatograph for .05 ppm FBAL 2nd

149

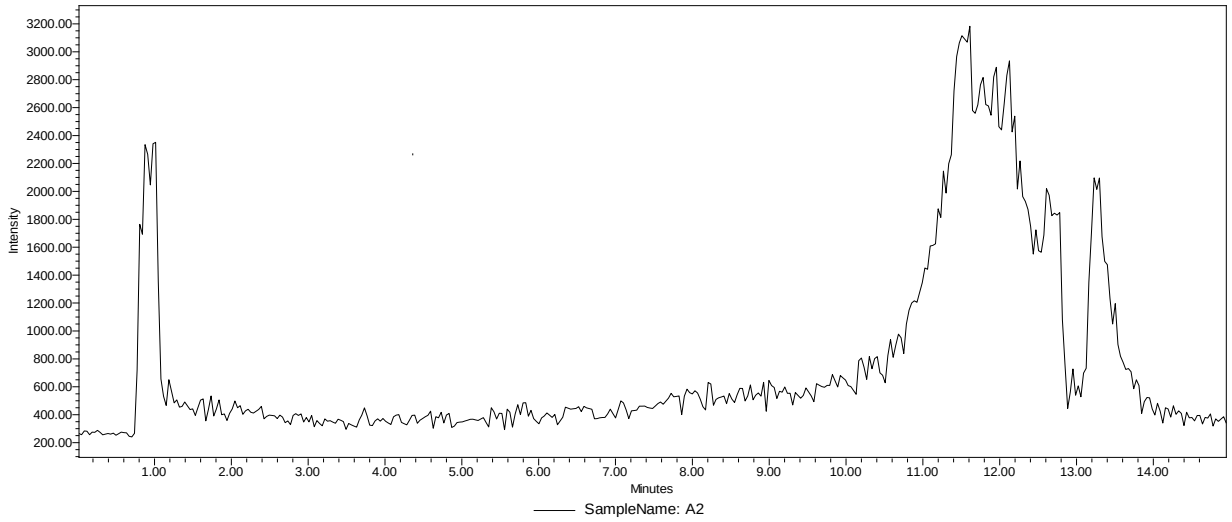

Fig. 25iii(9) product chromatograph for .05 ppm FUPA 2nd replicate.

129

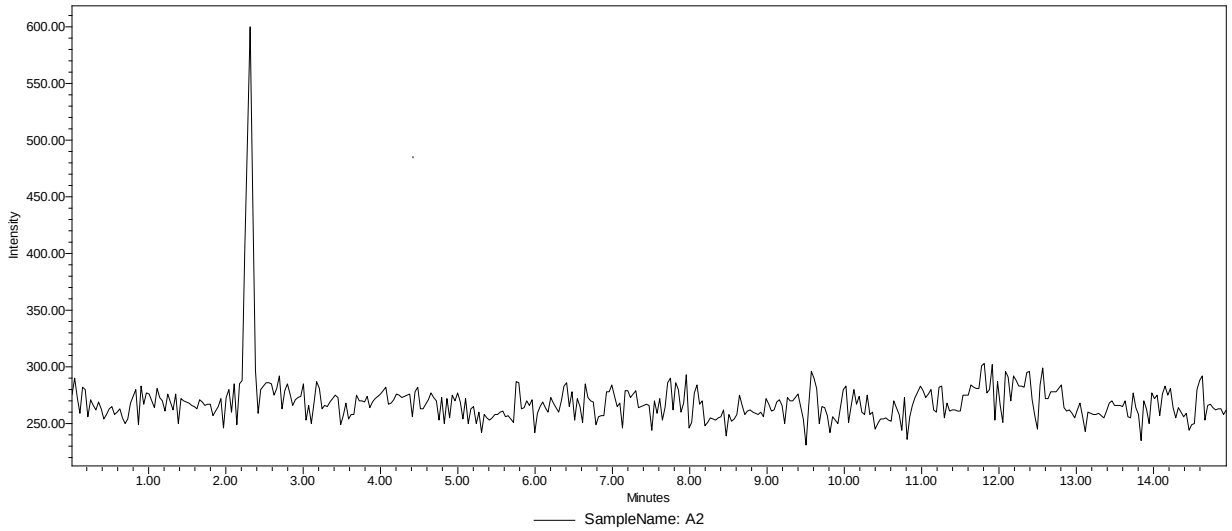

Fig. 25iii(10) product chromatograph for .05 ppm 5-FU 2nd replicate.

A3

77

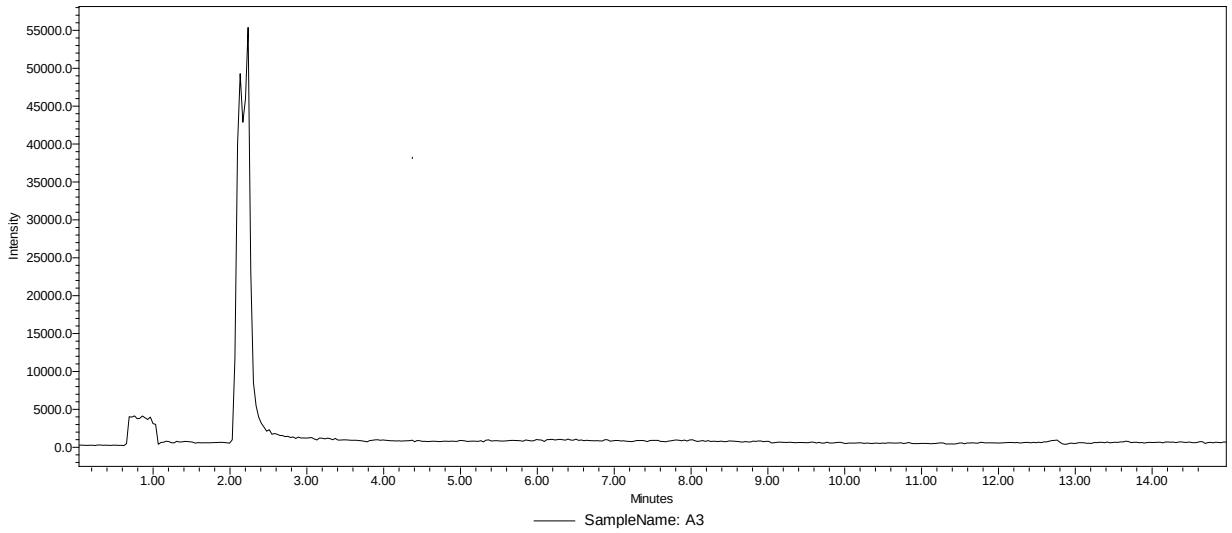

Fig. 25iii(11) product chromatograph for .05 ppm FAC 3rd replicate.

105

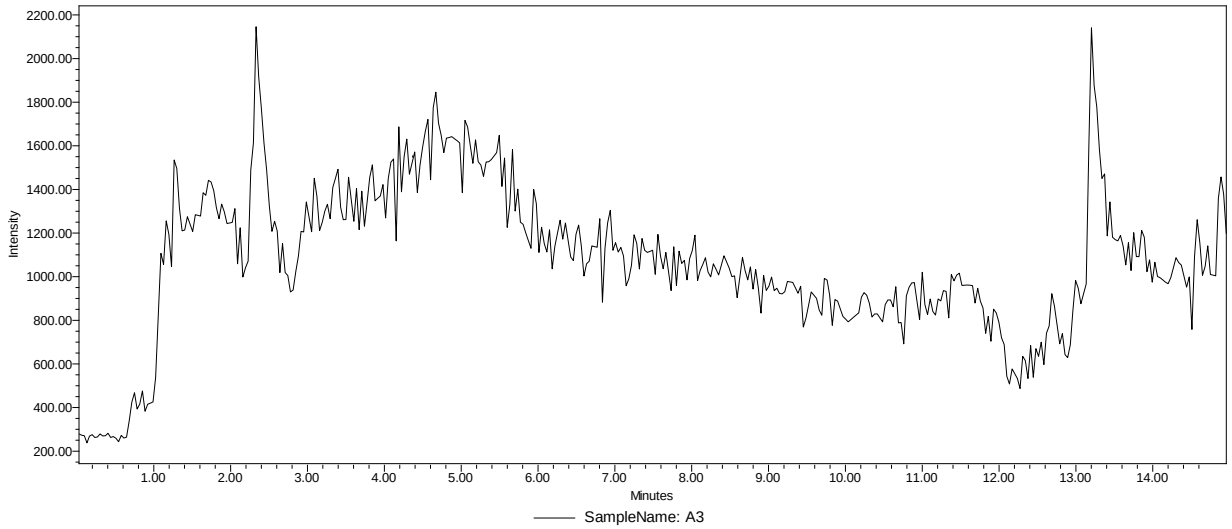

Fig. 25iii(12) product chromatograph for .05 ppm FMASld 3rd replicate.

106

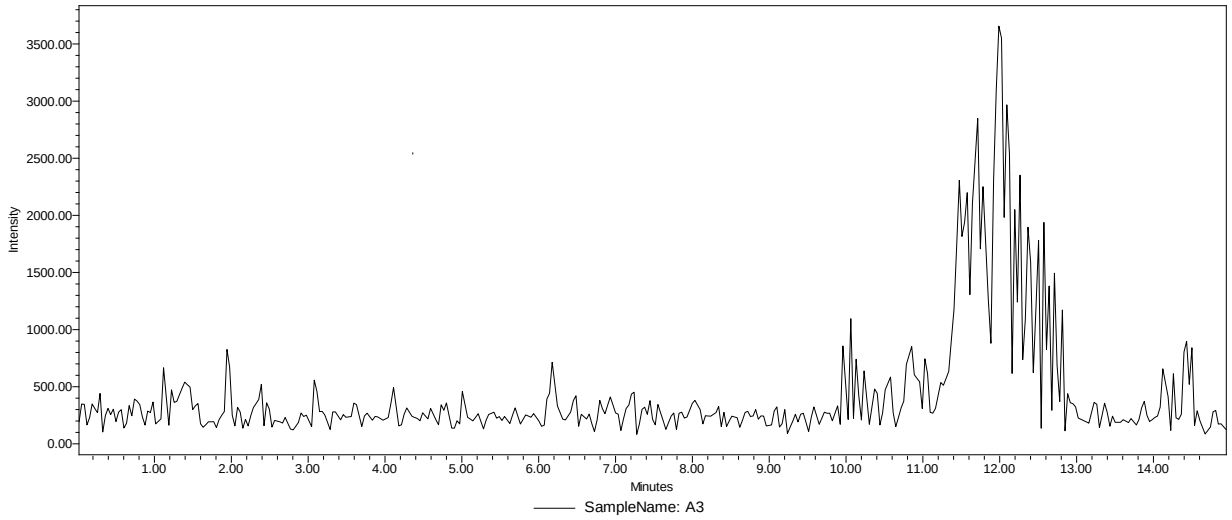

Fig. 25iii(13) product chromatograph for .05 ppm FBAL 3rd replicate.

149

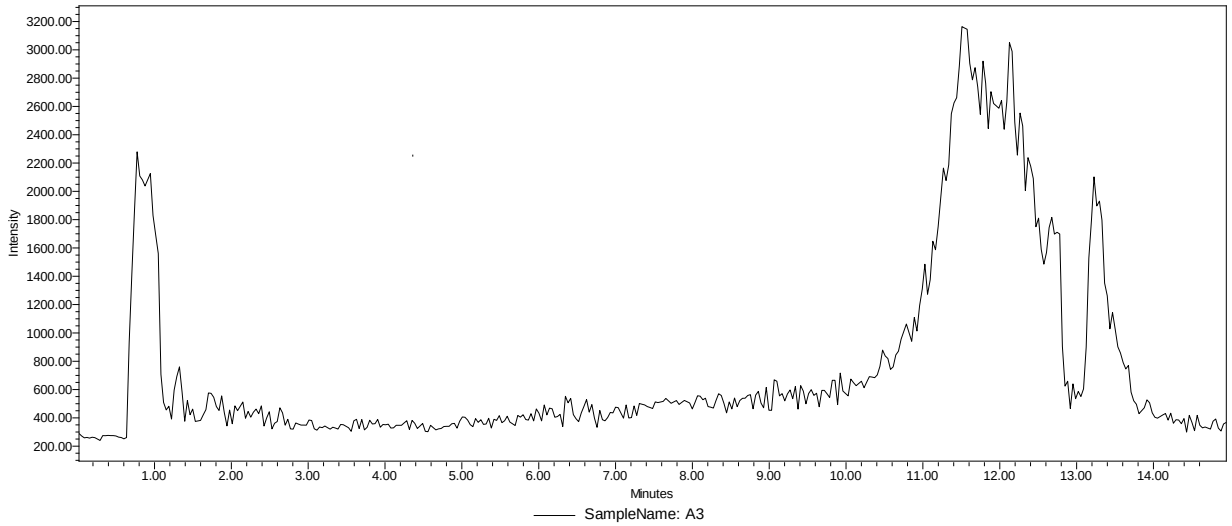

Fig. 25iii(14) product chromatograph for .05 ppm FUPA 3rd replicate.

129

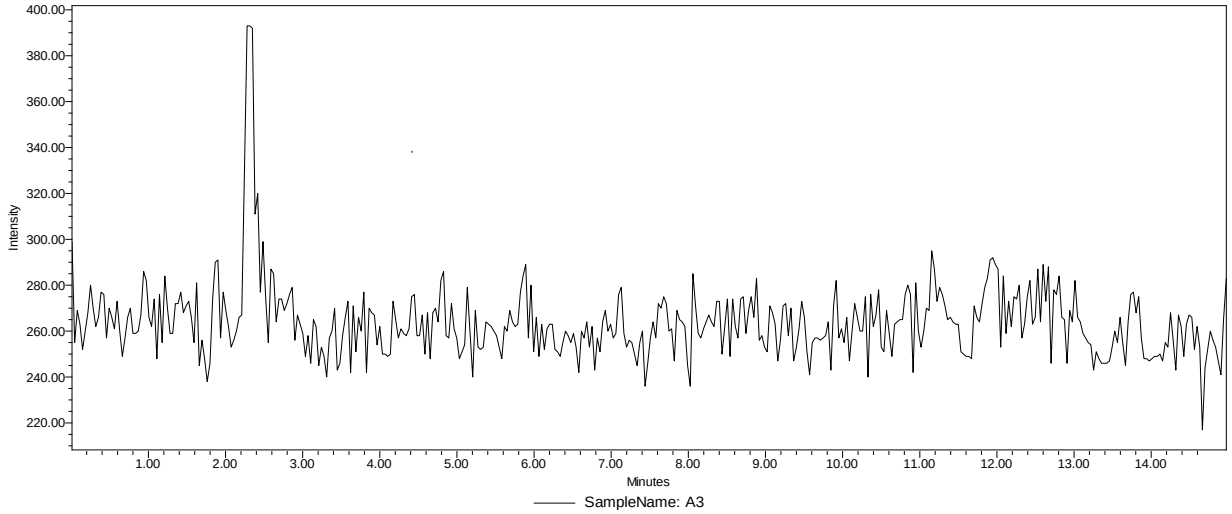

Fig. 25iii(15) product chromatograph for .05 ppm 5-FU 3rd replicate.

B1

77

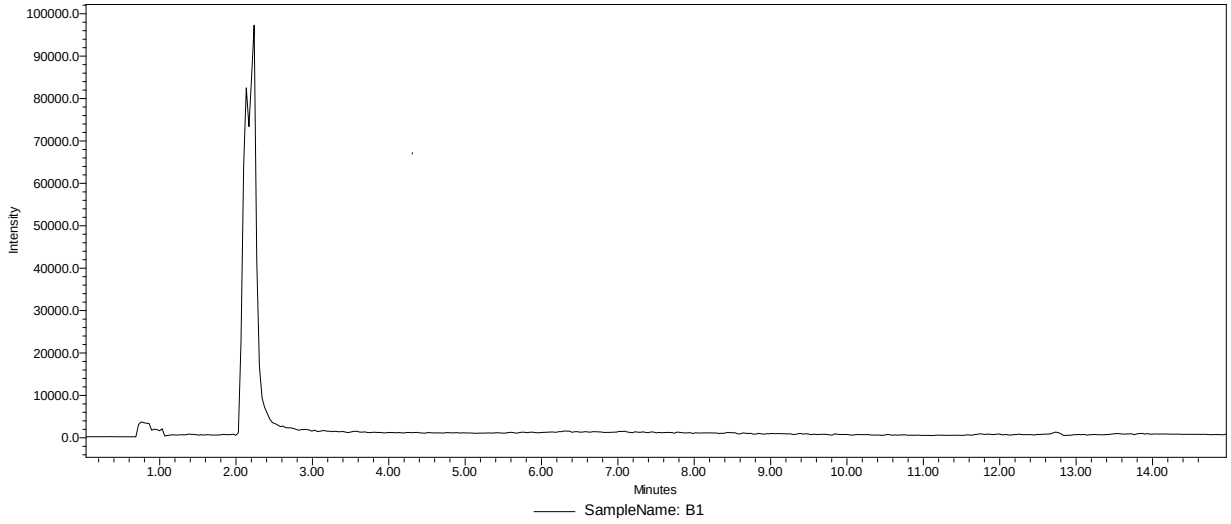

Fig. 25iii(16) product chromatograph for .100 ppm FAC 1st replicate.

105

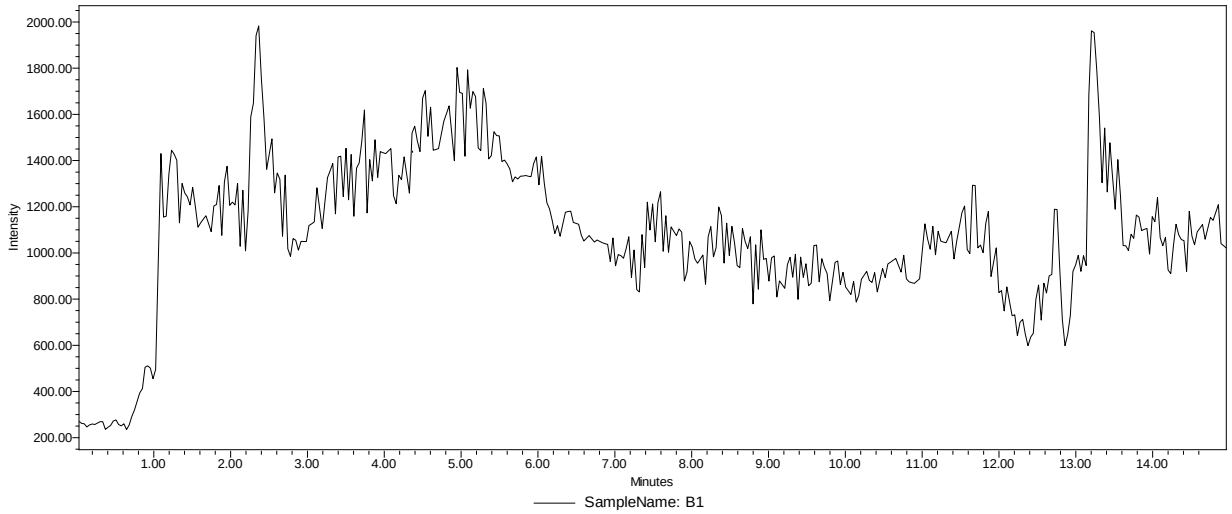

Fig. 25iii(17) product chromatograph for .100 ppm FMASld 1st replicate.

106

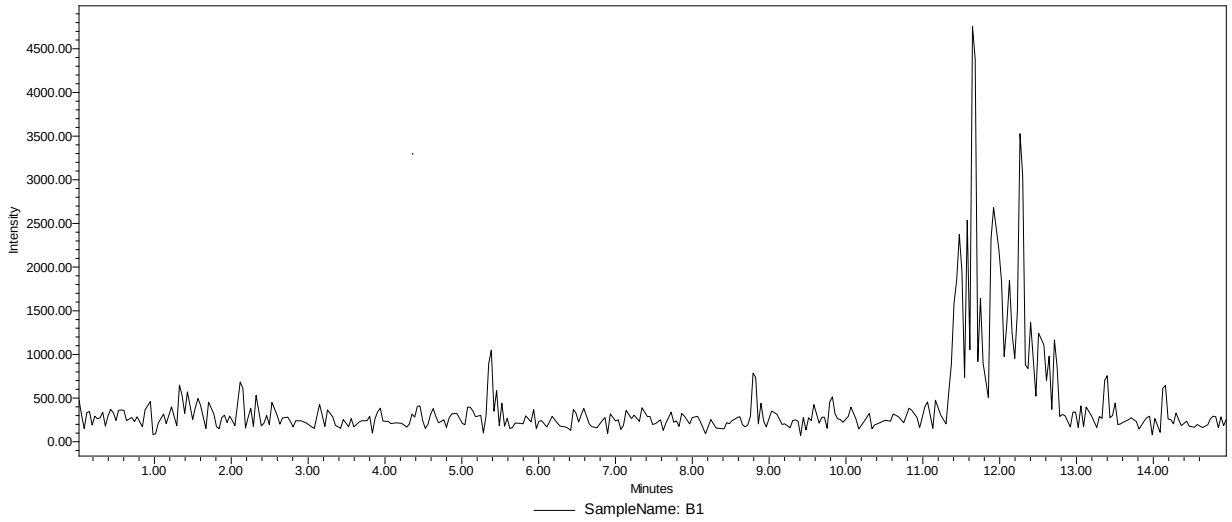

Fig. 25iii(18) product chromatograph for .100 ppm FBAL 1st replicate.

149

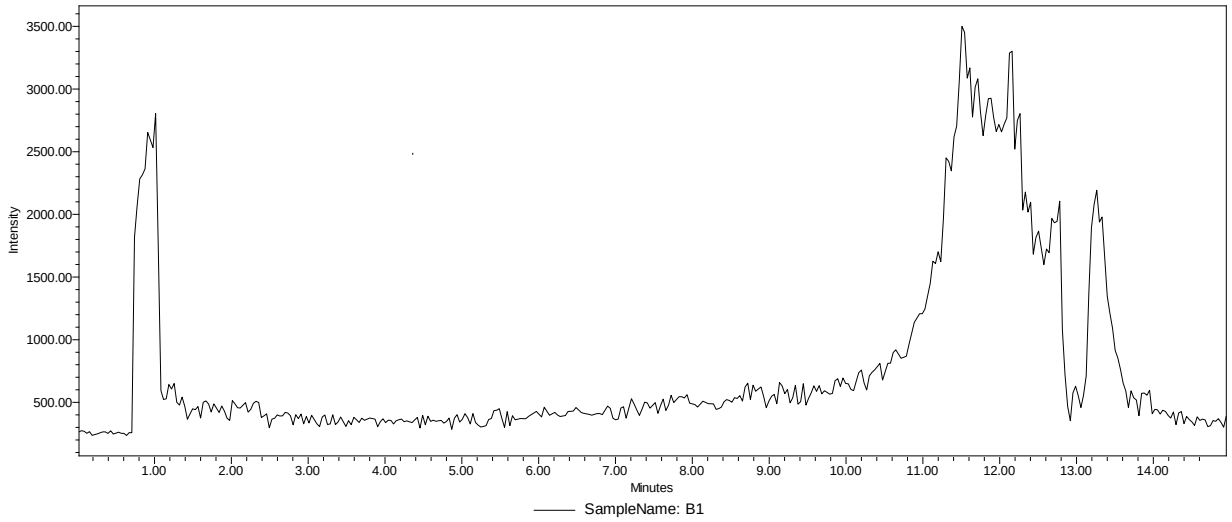

Fig. 25iii(19) product chromatograph for .100 ppm FUPA 1st replicate.

129

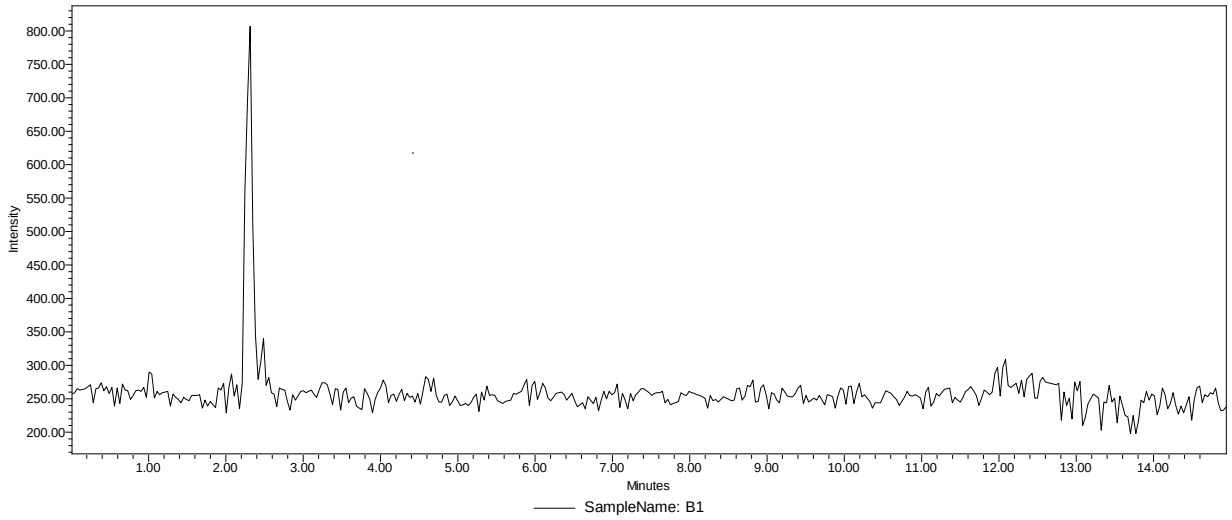

Fig. 25iii(20) product chromatograph for .100 ppm 5-FU 1st replicate.

B2

77

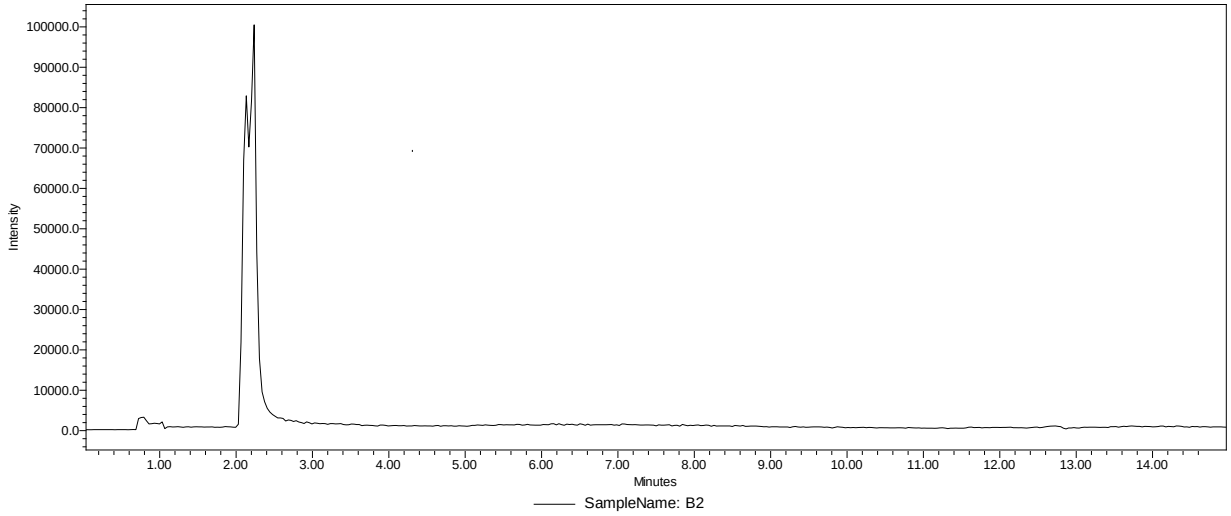

Fig. 25iii(21) product chromatograph for .100 ppm FAC 2nd replicate.

105

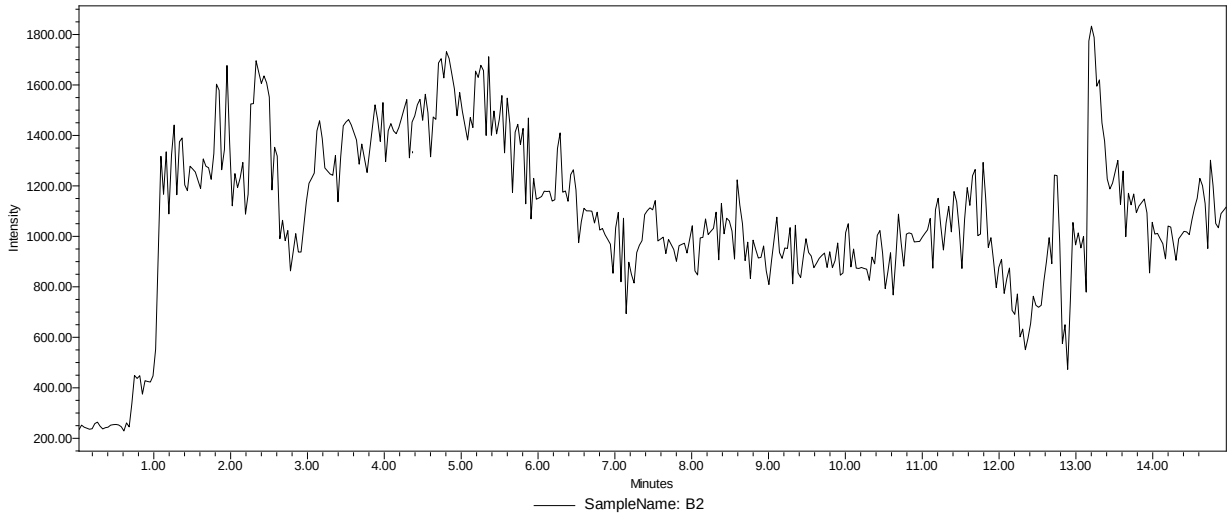

Fig. 25iii(22) product chromatograph for .100 ppm FMASld 2nd replicate.

106

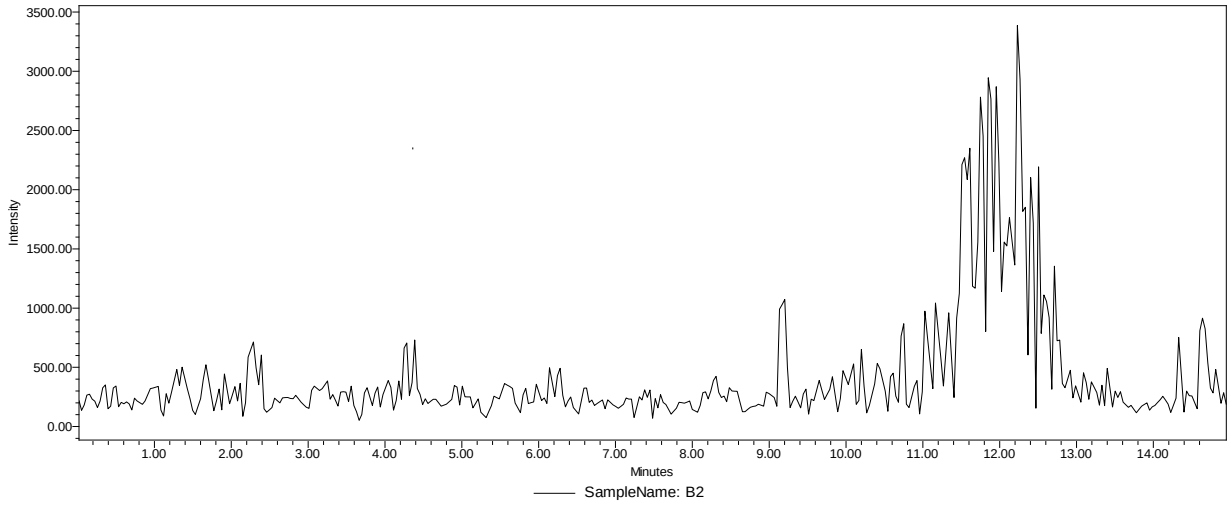

Fig. 25iii(23) product chromatograph for .100 ppm FBAL 2nd replicate.

149

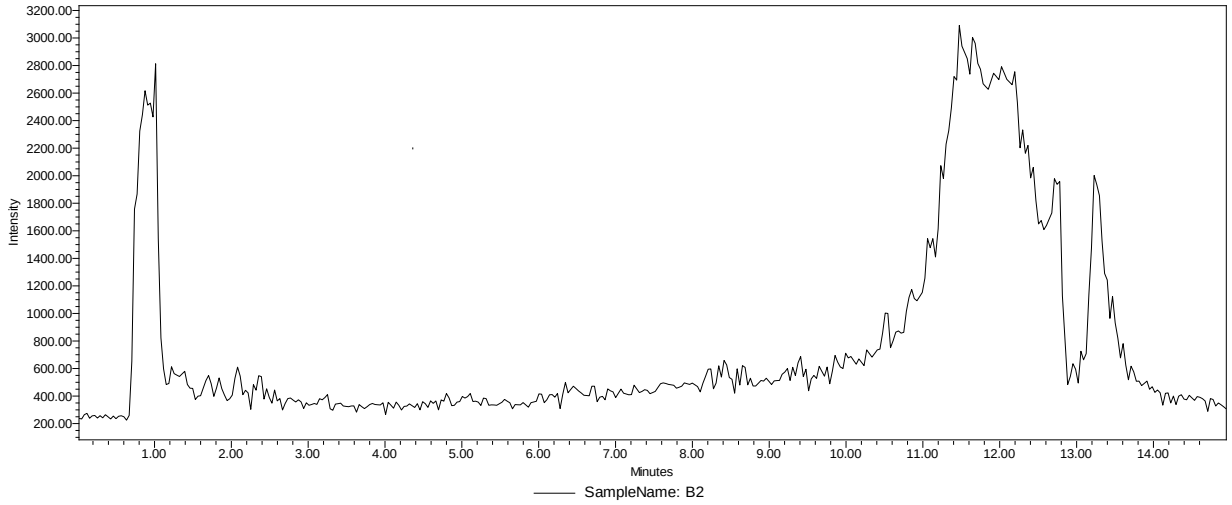

Fig. 25iii(24) product chromatograph for .100 ppm FUPA 2nd replicate.

129

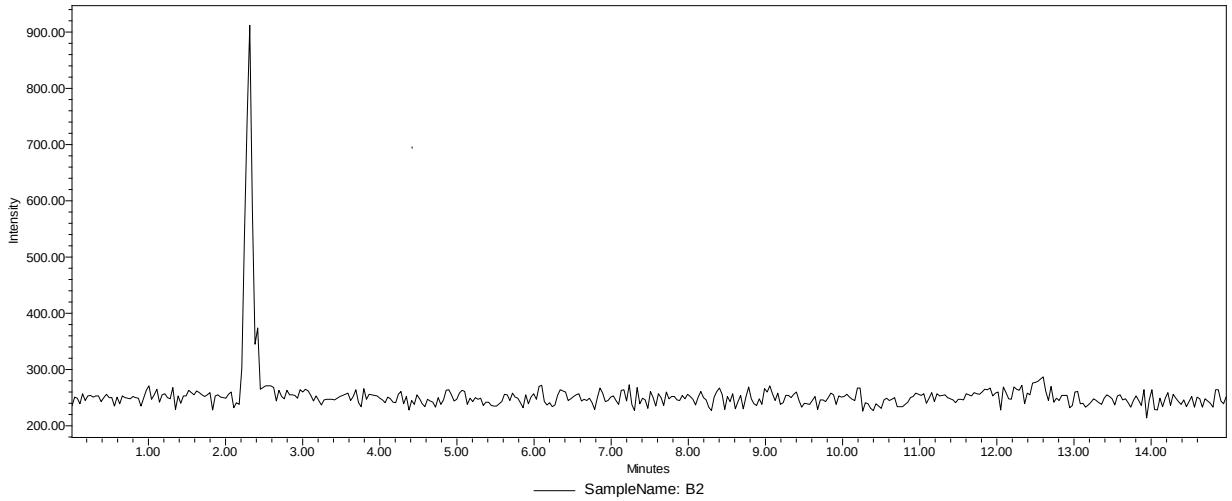

Fig. 25iii(25) product chromatograph for .100 ppm 5-FU 2nd replicate.

B3

77

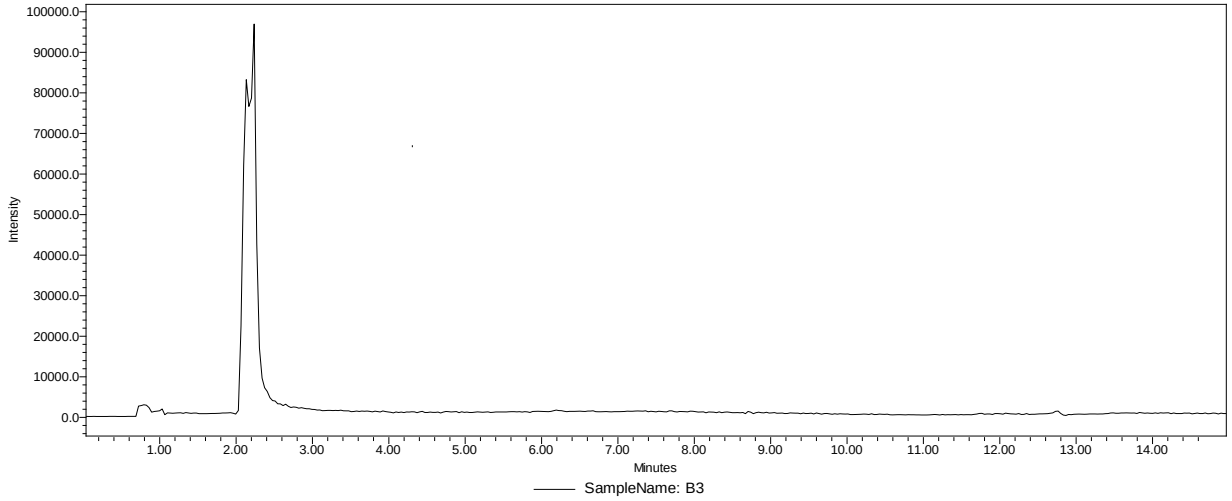

Fig. 25iii(26) product chromatograph for .100 ppm FAC 3rd replicate.

105

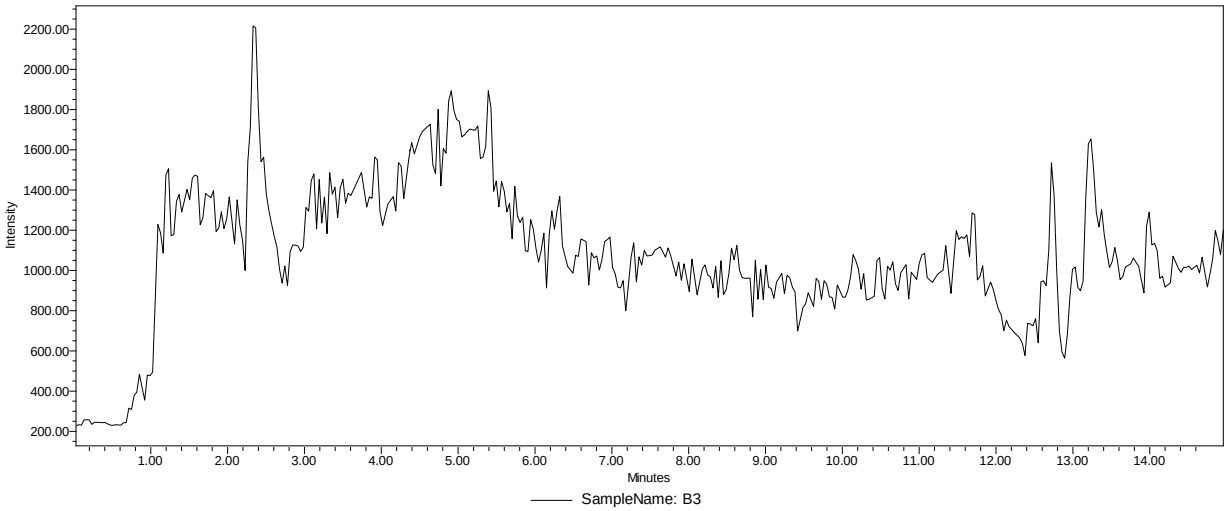

Fig. 25iii(27) product chromatograph for .100 ppm FMASld 3rd replicate.

106

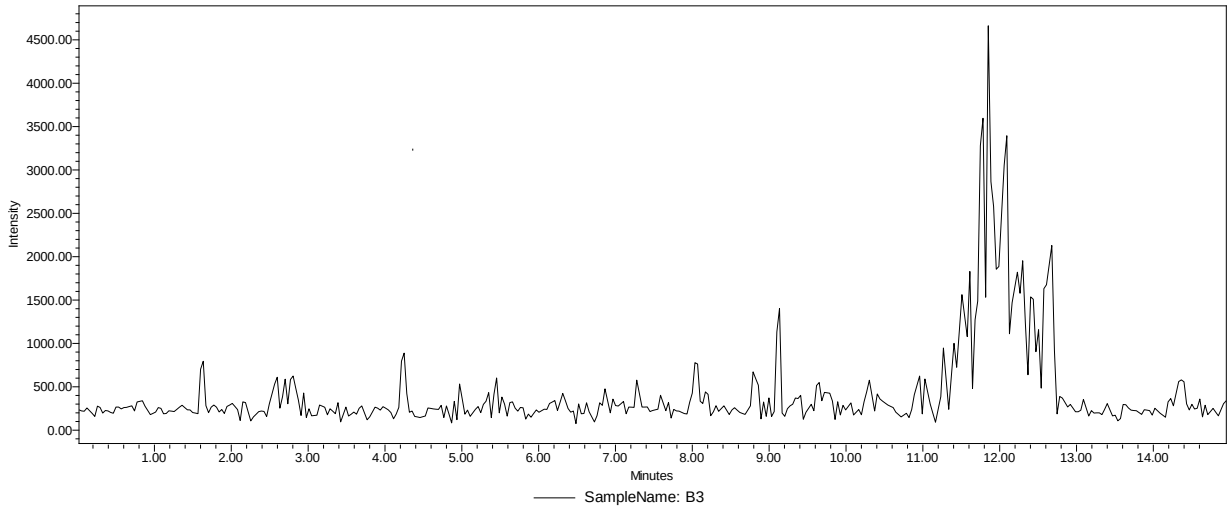

Fig. 25iii(28) product chromatograph for .100 ppm FBAL 3rd replicate.

149

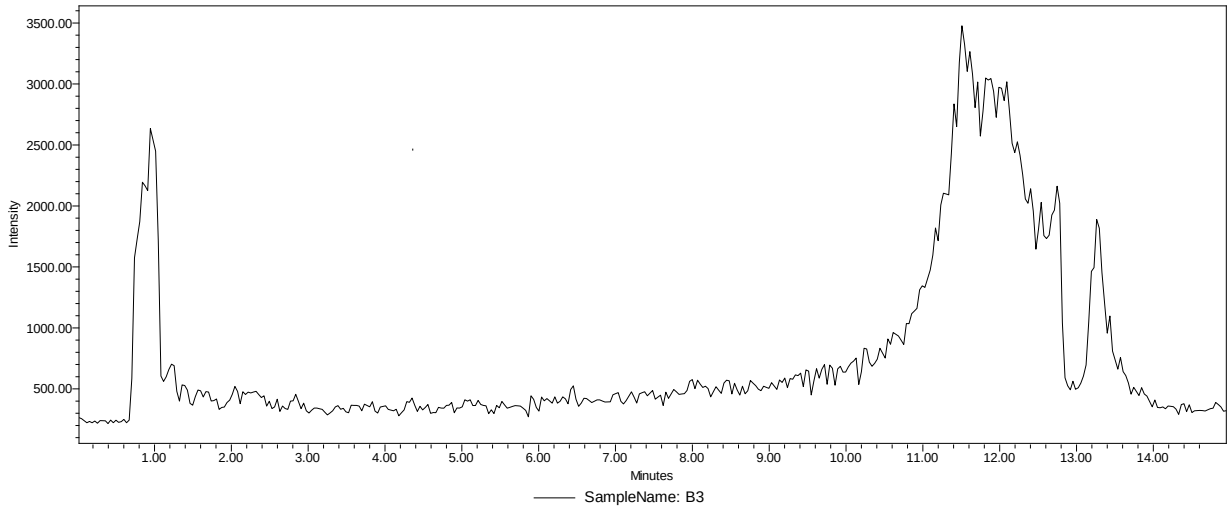

Fig. 25iii(29) product chromatograph for .100 ppm FUPA 3rd replicate.

129

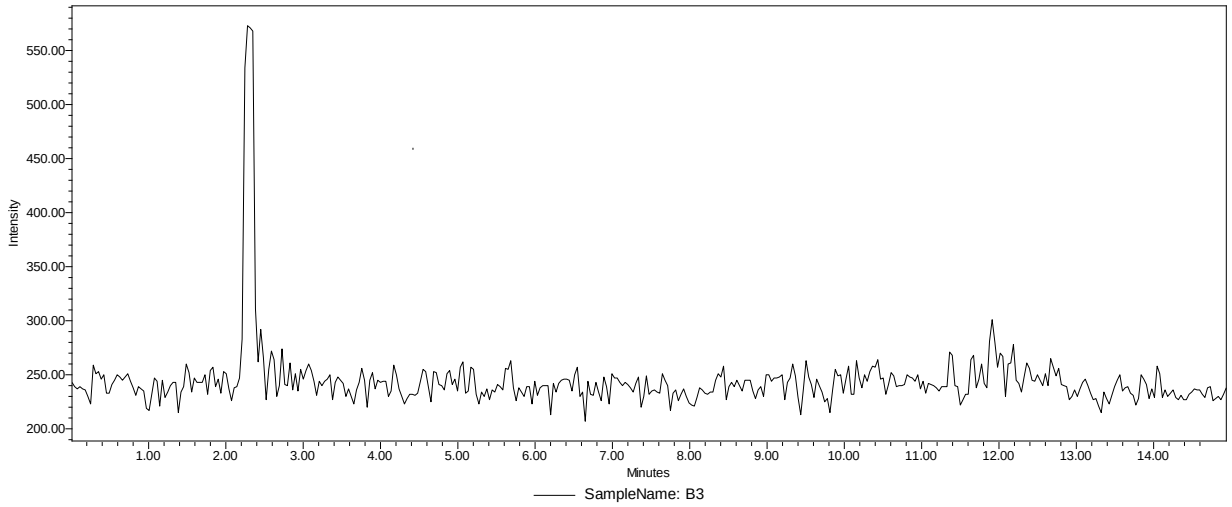

Fig. 25iii(30) product chromatograph for .100 ppm 5-FU 3rd replicate.

C1

77

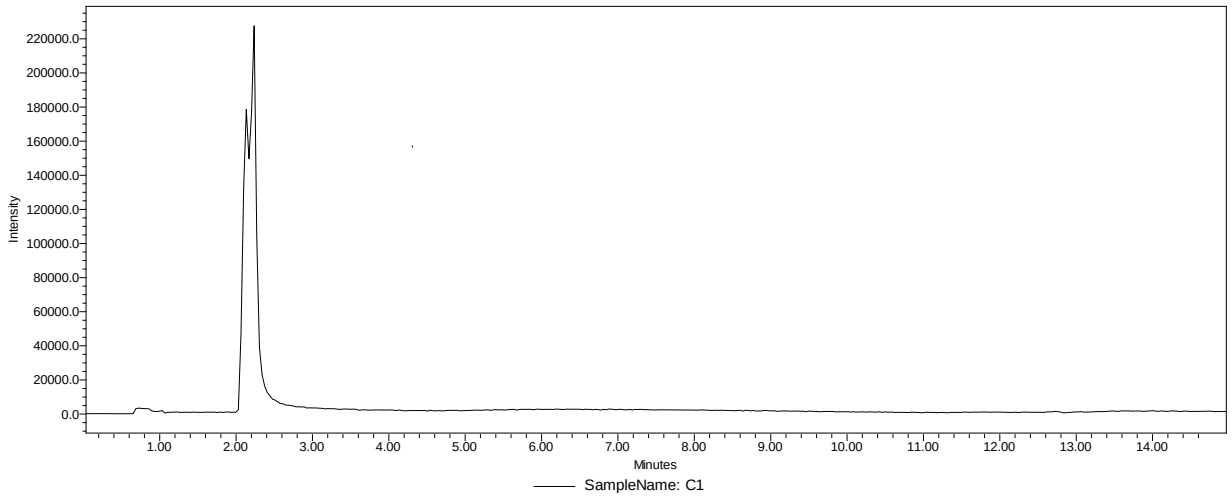

Fig. 25iii(31) product chromatograph for .250 ppm FAC 1st replicate.

105

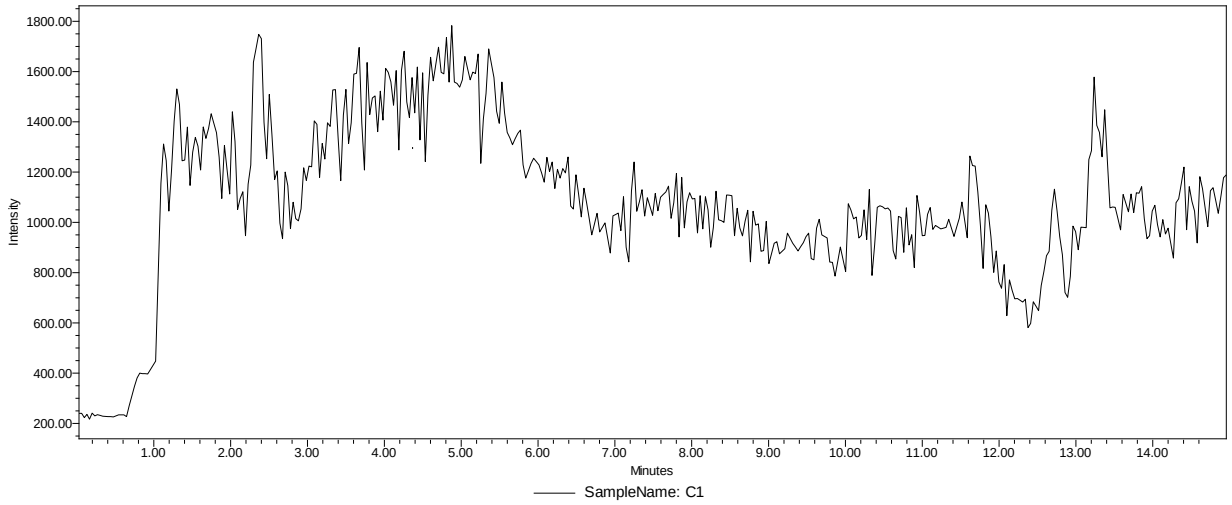

Fig. 25iii(32) product chromatograph for .250 ppm FMASld 1st replicate.

106

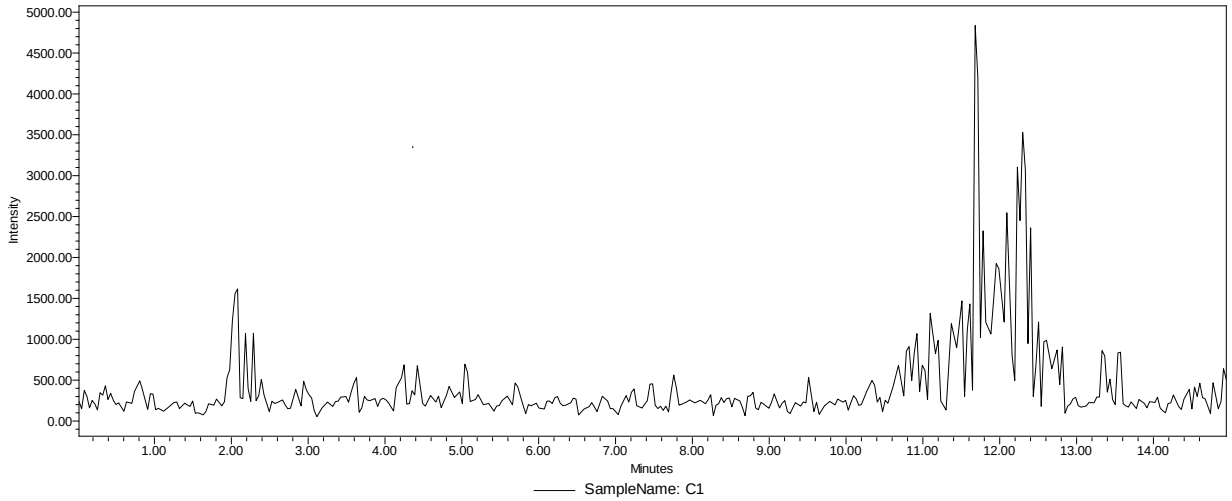

Fig. 25iii(33) product chromatograph for .250 ppm FBAL 1st replicate.

149

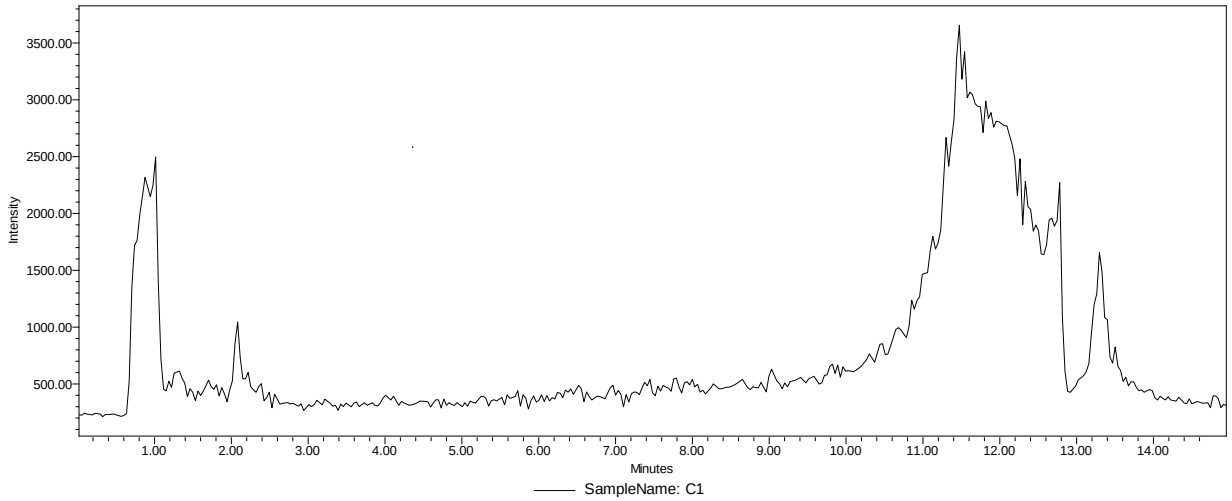

Fig. 25iii(34) product chromatograph for .250 ppm FUPA 1st replicate.

129

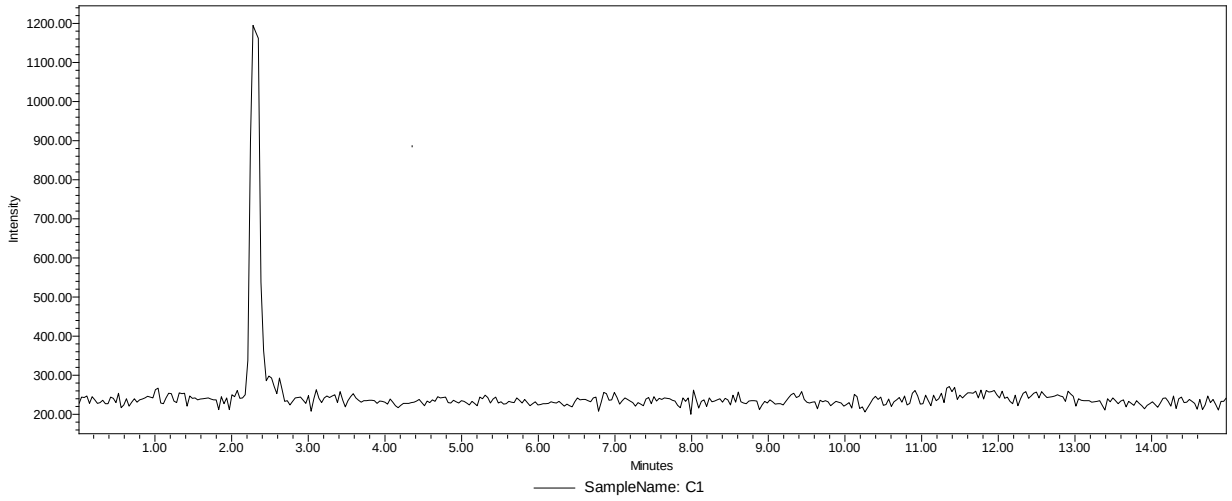

Fig. 25iii(35) product chromatograph for .250 ppm 5-FU 1st replicate.

C2

77

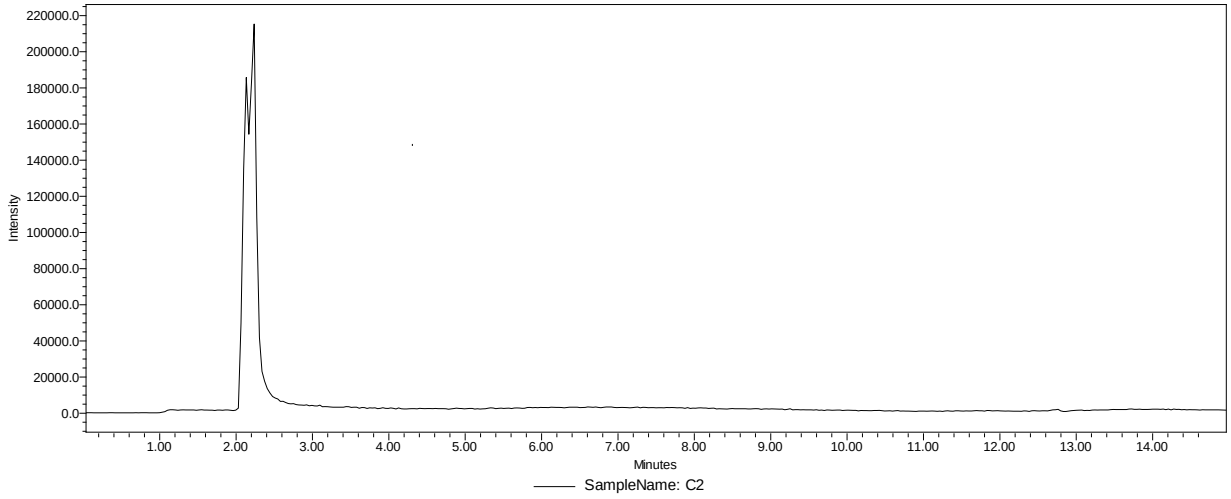

Fig. 25iii(36) product chromatograph for .250 ppm FAC 2nd replicate.

105

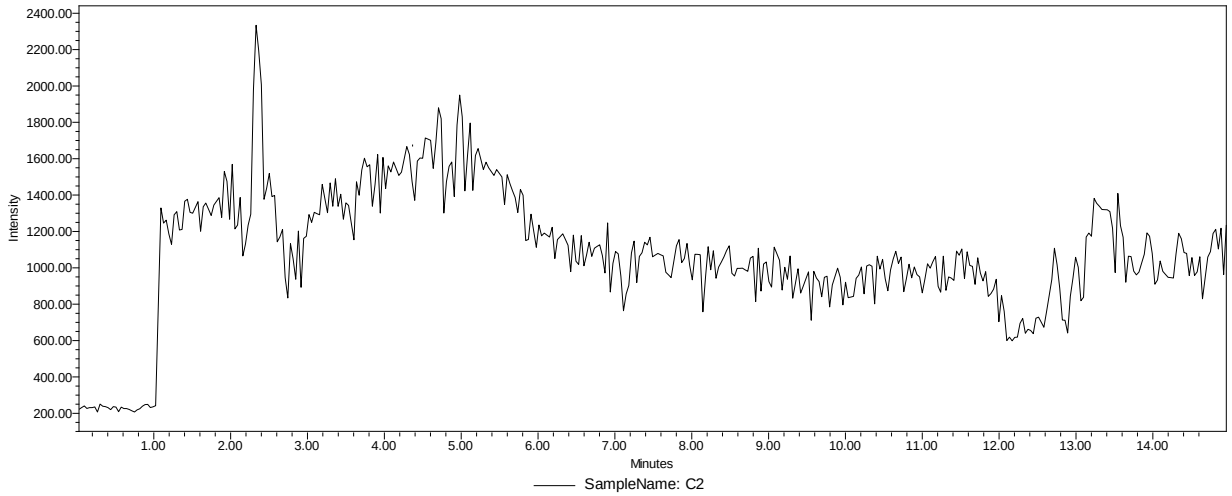

Fig. 25iii(37) product chromatograph for .250 ppm FMASld 2nd replicate.

106

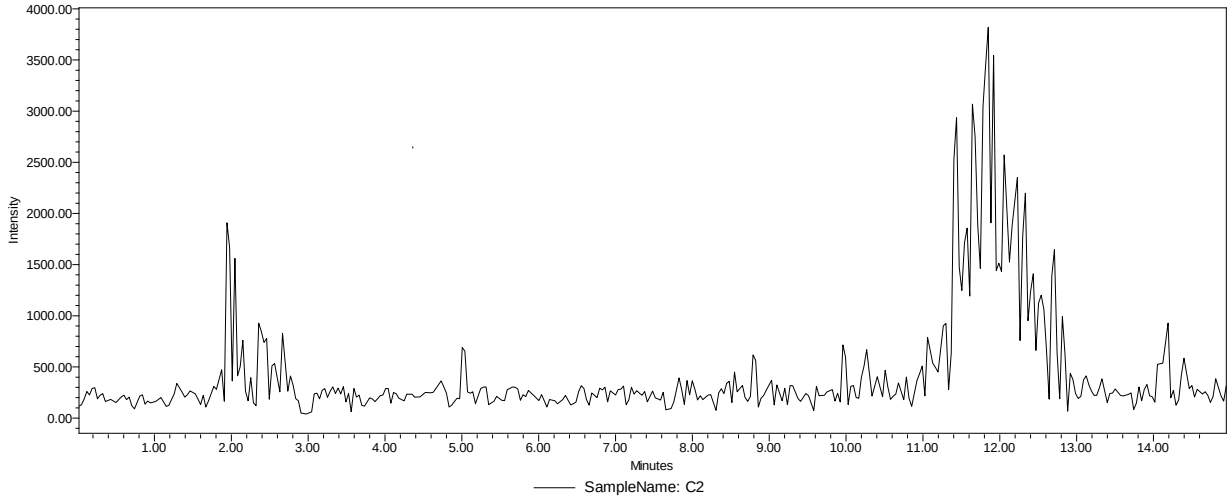

Fig. 25iii(38) product chromatograph for .250 ppm FBAL 2nd replicate.

149

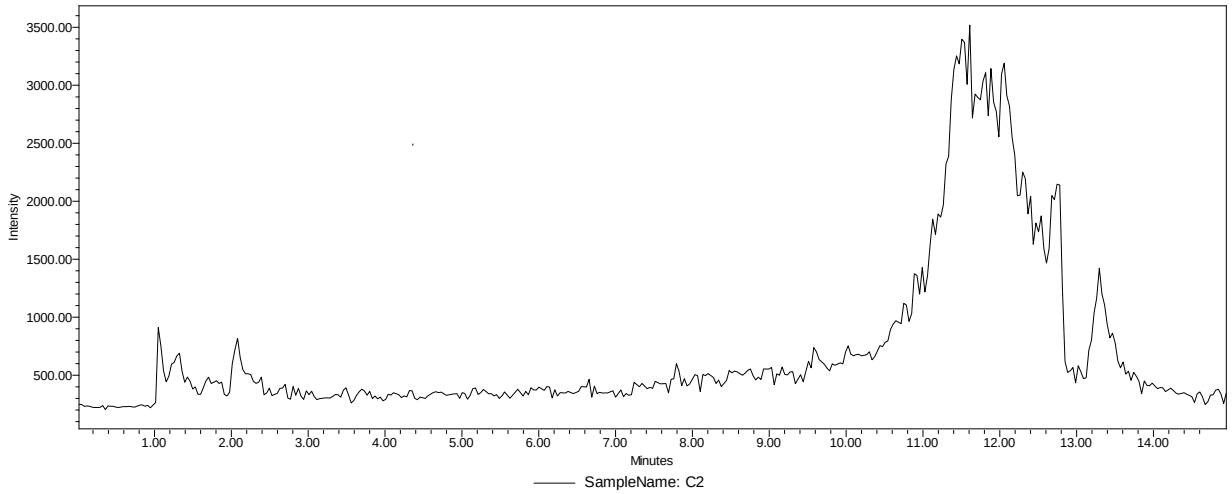

Fig. 25iii(39) product chromatograph for .250 ppm FUPA 2nd replicate.

129

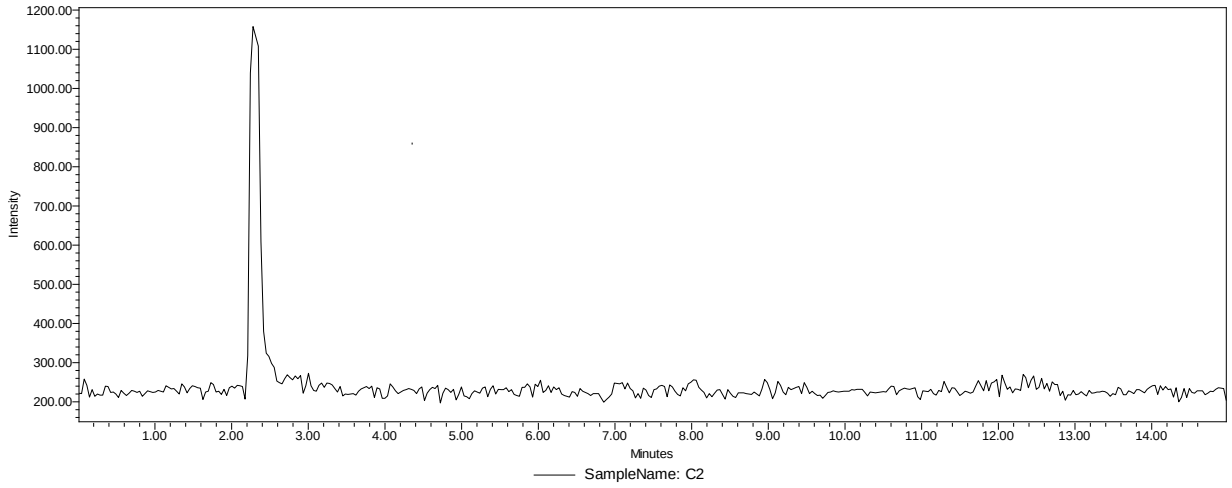

Fig. 25iii(40) product chromatograph for .250 ppm 5-FU 2nd replicate.

C3

77

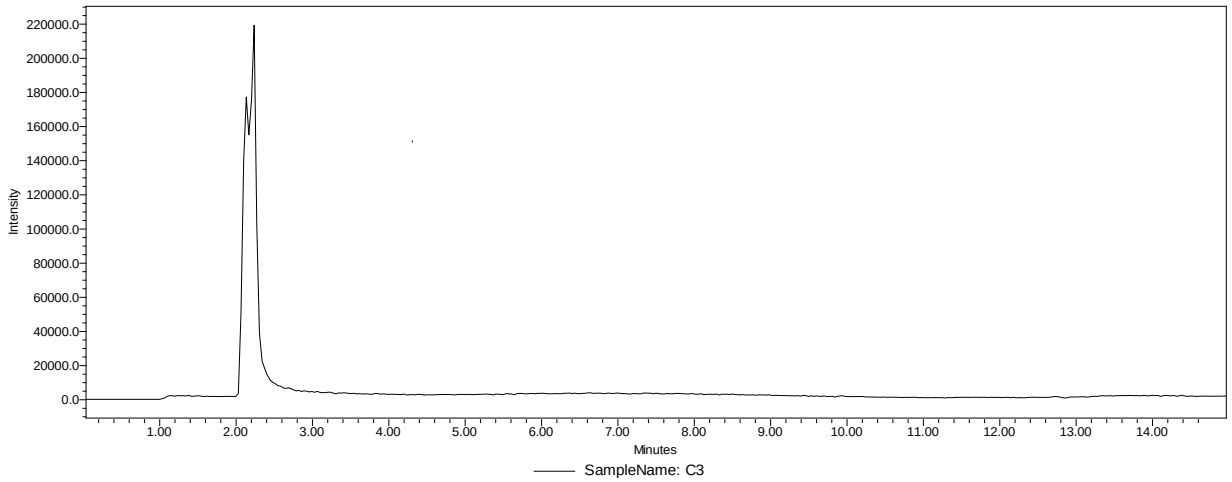

Fig. 25iii(41) product chromatograph for .250 ppm FAC 3rd replicate.

105

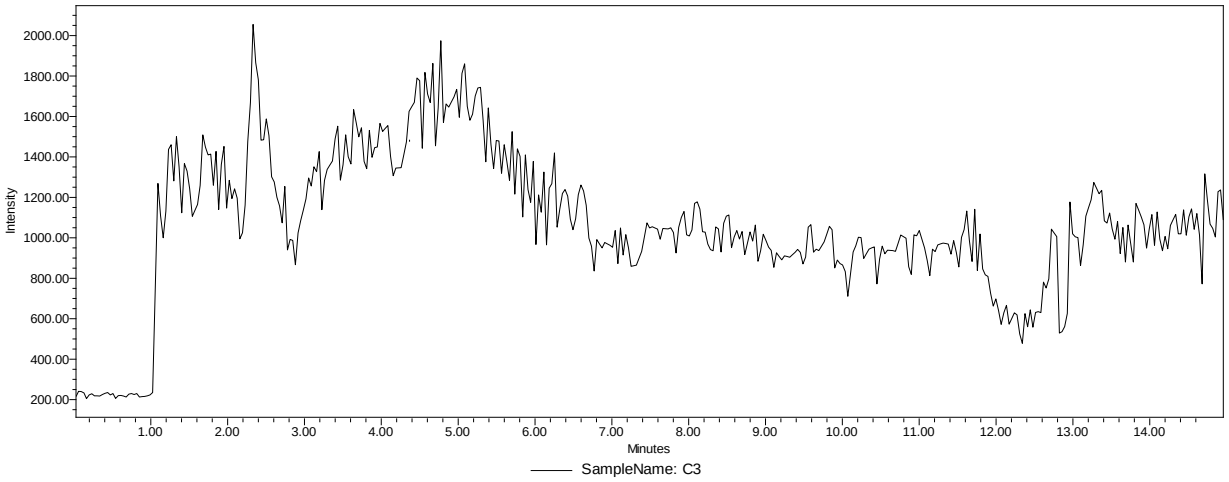

Fig. 25iii(42) product chromatograph for .250 ppm FMASId 3rd replicate.

106

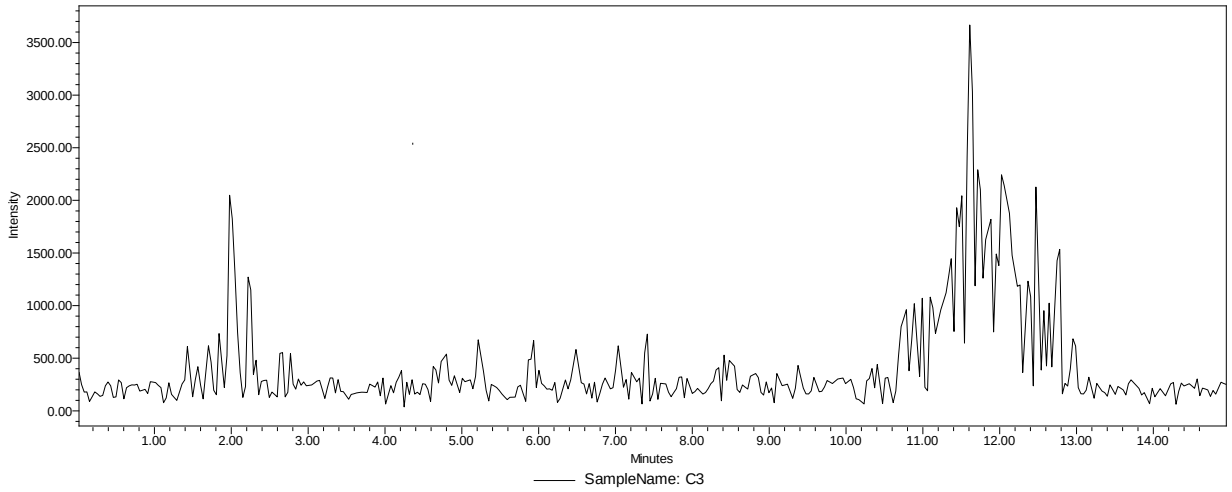

Fig. 25iii(43) product chromatograph for .250 ppm FBAL 3rd replicate.

149

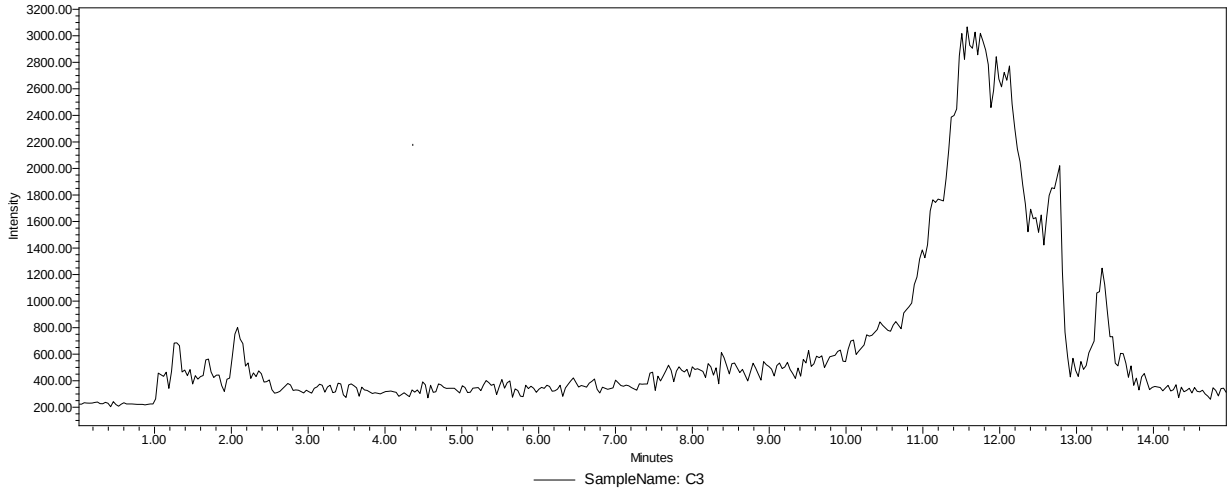

Fig. 25iii(44) product chromatograph for .250 ppm FUPA 3rd replicate.

129

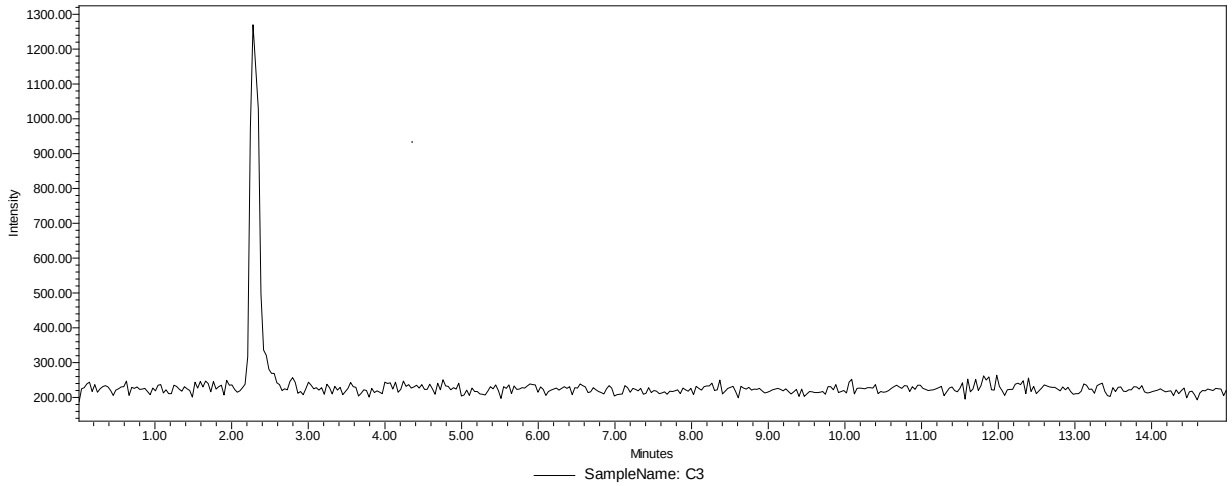

Fig. 25iii(45) product chromatograph for .250 ppm 5-FU 3rd replicate.

D1

77

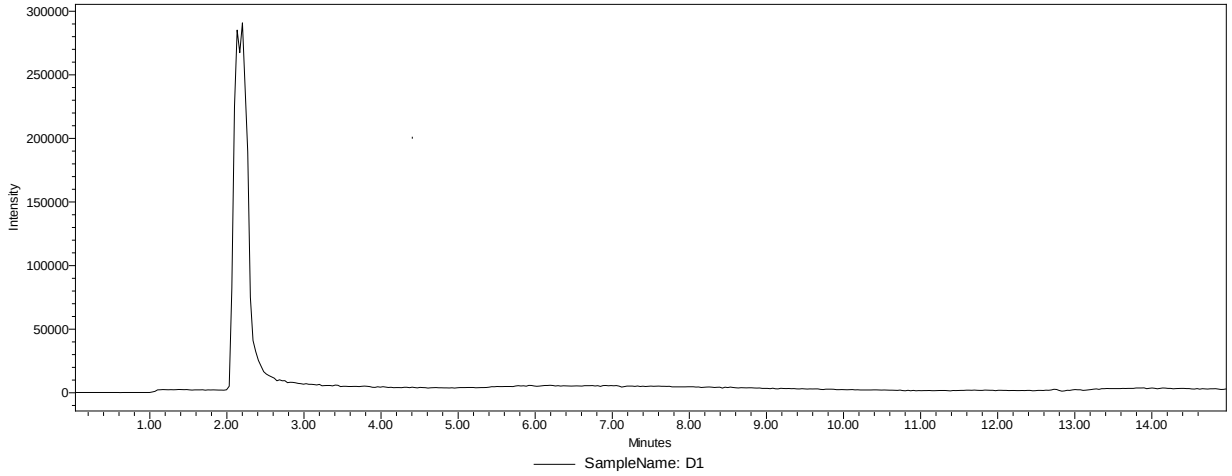

Fig. 25iii(46) product chromatograph for .50 ppm FAC 1st replicate.

105

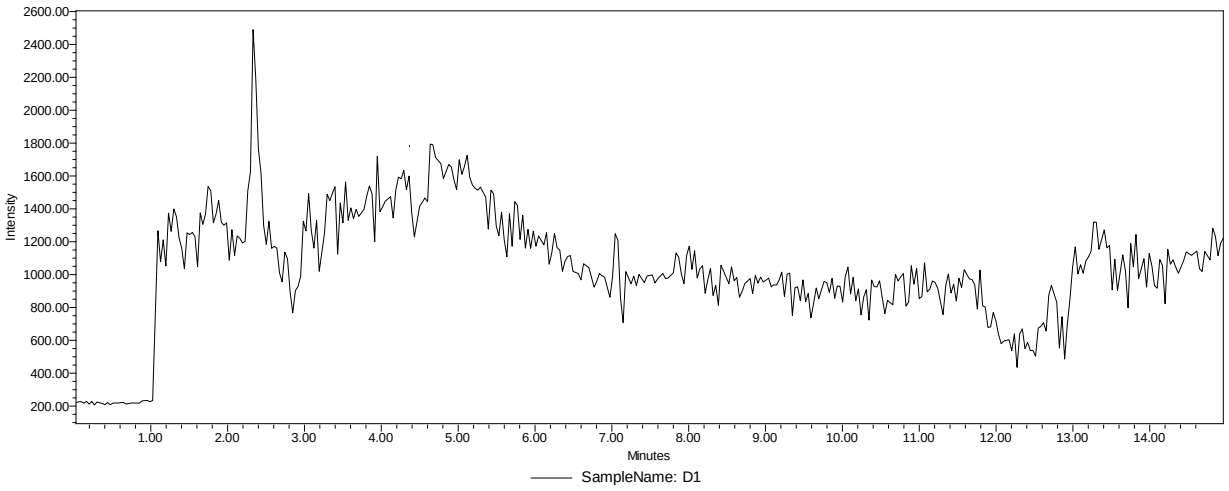

Fig. 25iii(47) product chromatograph for .50 ppm FMASld 1st replicate.

106

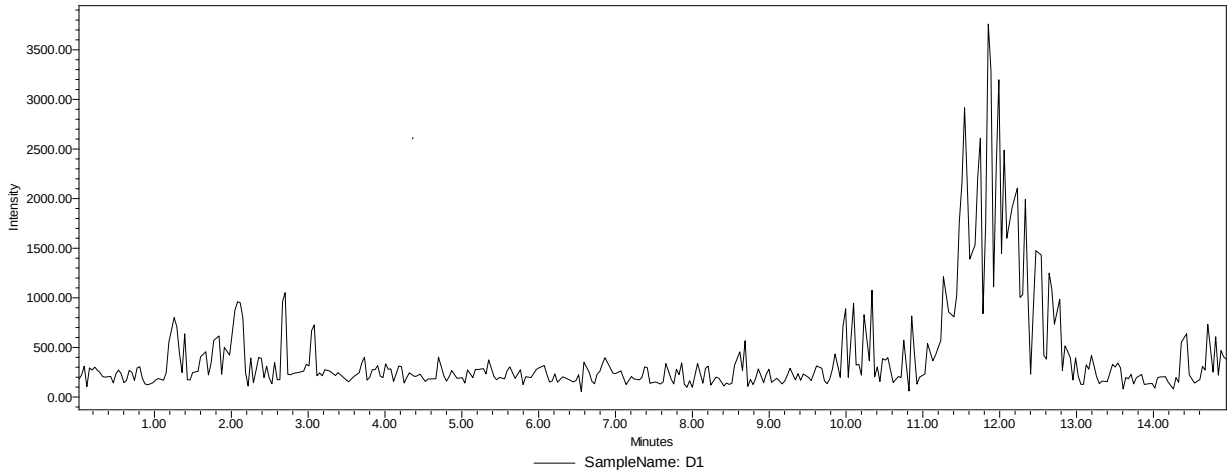

Fig. 25iii(48) product chromatograph for .50 ppm FBAL 1st replicate.

149

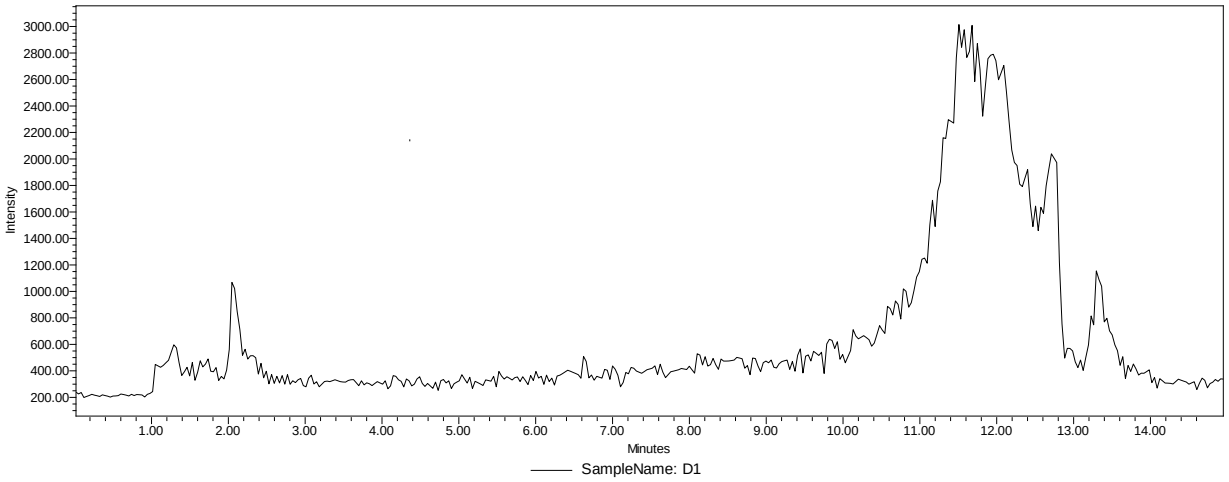

Fig. 25iii(49) product chromatograph for .50 ppm FUPA 1st replicate.

129

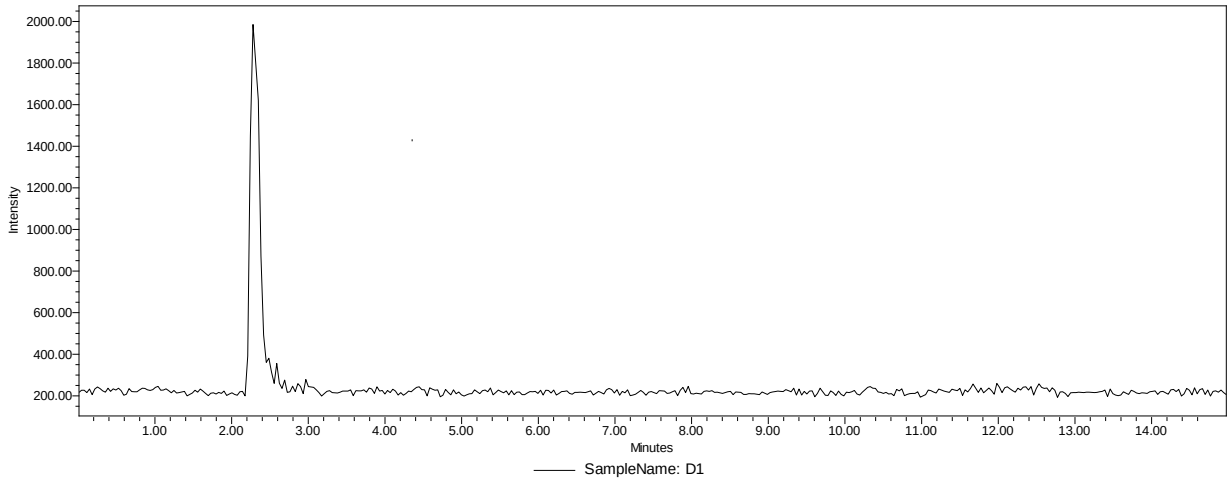

Fig. 25iii(50) product chromatograph for .50 ppm 5-FU 1st replicate.

D2

77

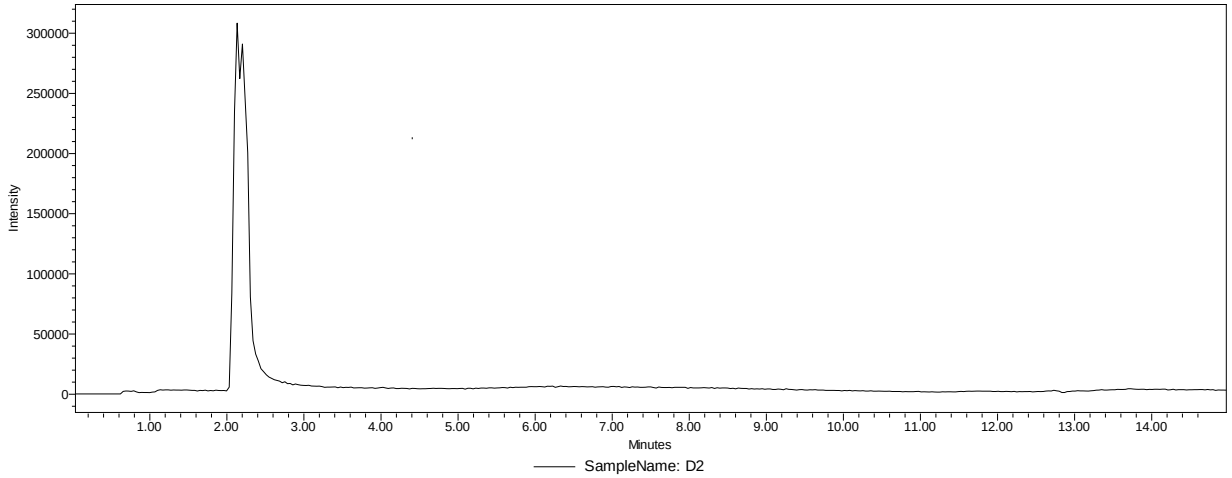

Fig. 25iii(51) product chromatograph for .50 ppm FAC 2nd replicate.

105

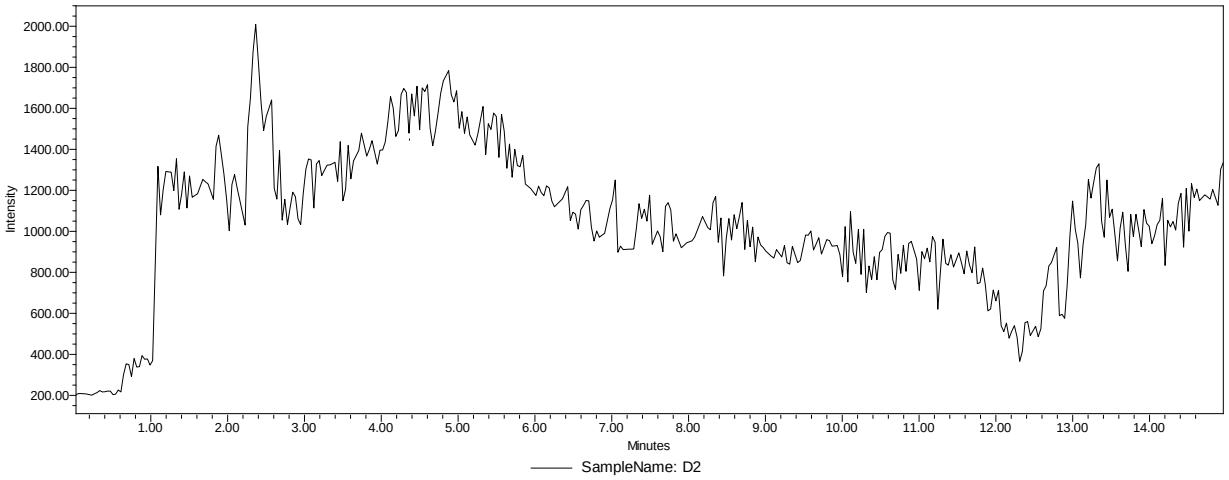

Fig. 25iii(52) product chromatograph for .50 ppm FMASld 2nd replicate.

106

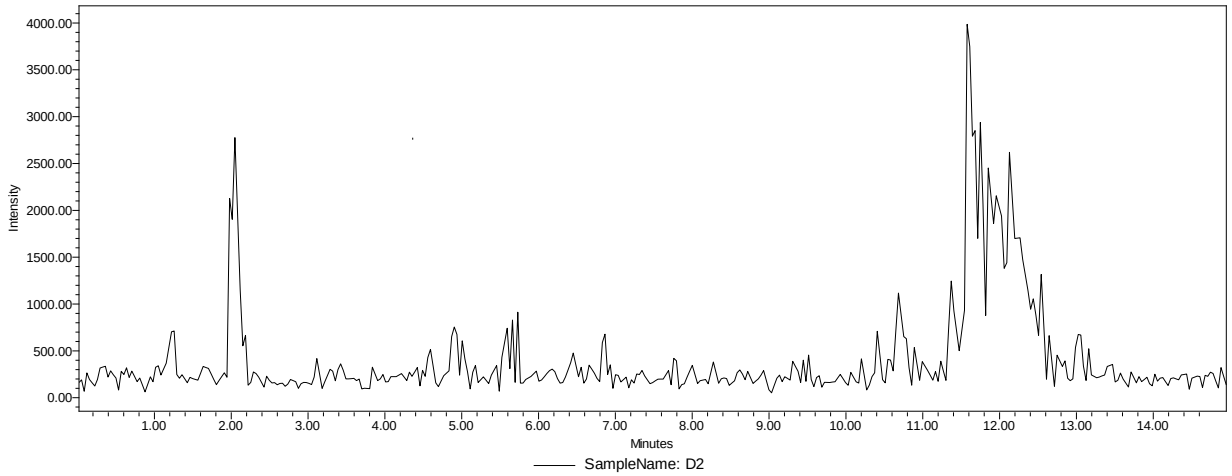

Fig. 25iii(53) product chromatograph for .50 ppm FBAL 2nd replicate.

149

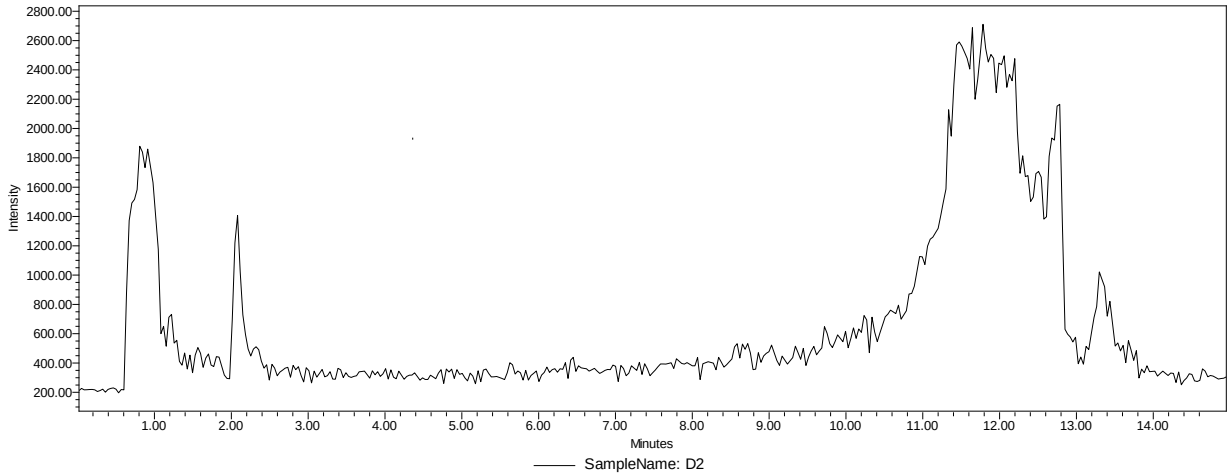

Fig. 25iii(54) product chromatograph for .50 ppm FUPA 2nd replicate.

129

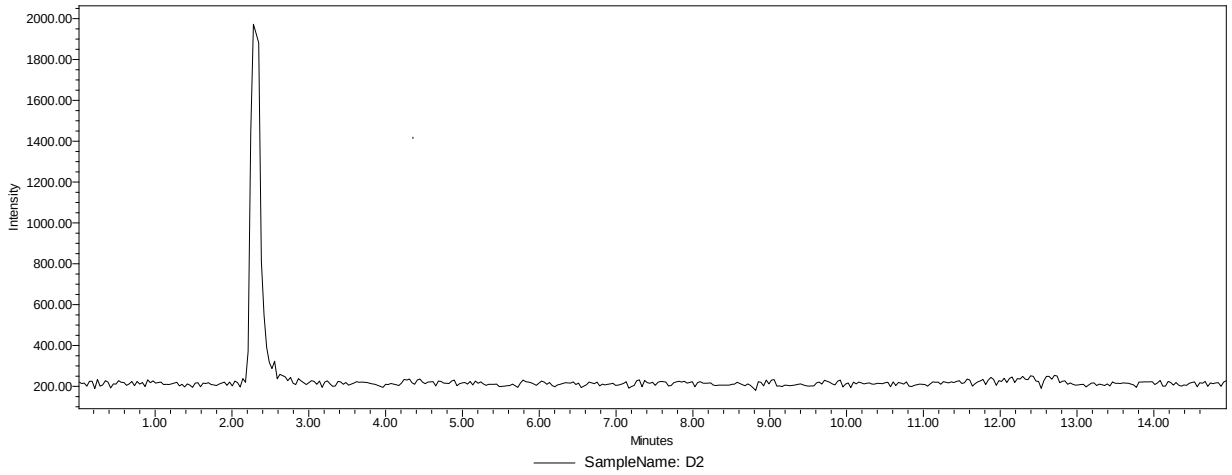

Fig. 25iii(55) product chromatograph for .50 ppm 5-FU 2nd replicate.

D3

77

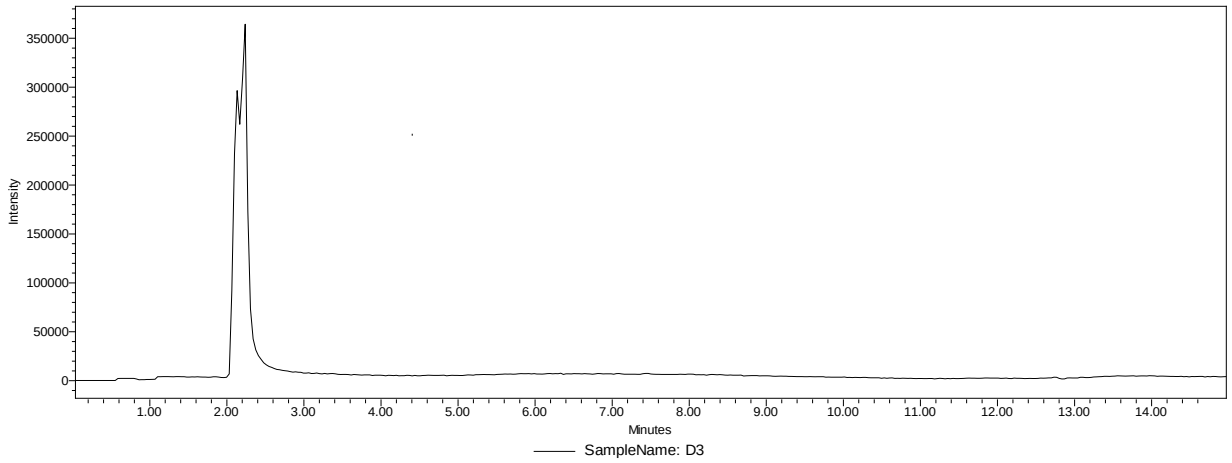

Fig. 25iii(56) product chromatograph for .50 ppm FAC 3rd replicate.

105

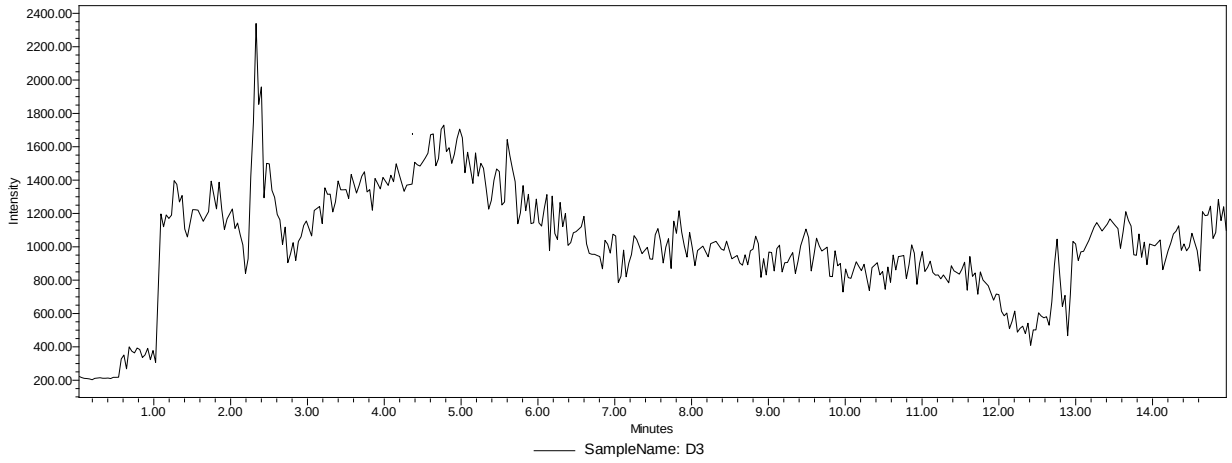

Fig. 25iii(57) product chromatograph for .50 ppm FMASld 3rd replicate.

106

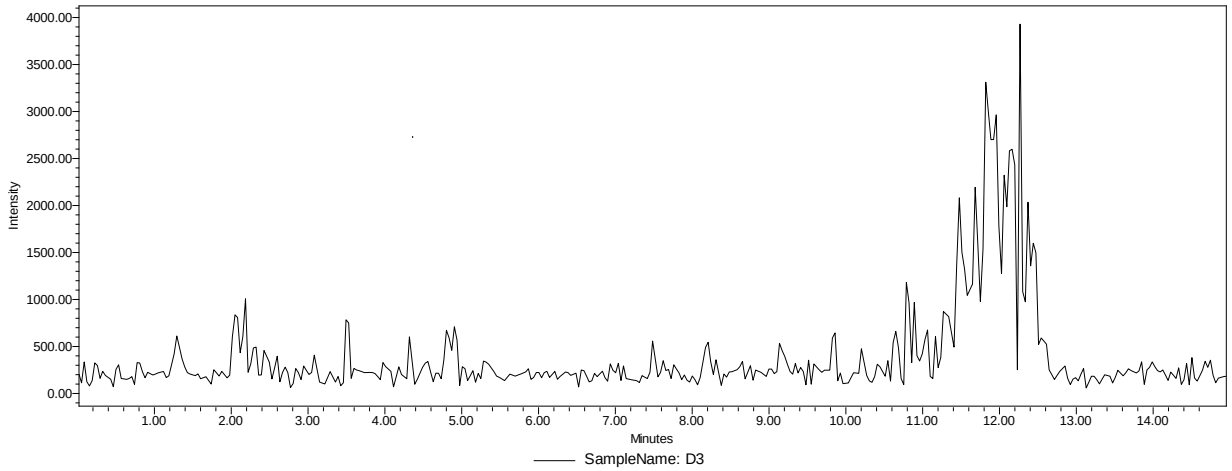

Fig. 25iii(58) product chromatograph for .50 ppm FBAL 3rd replicate.

149

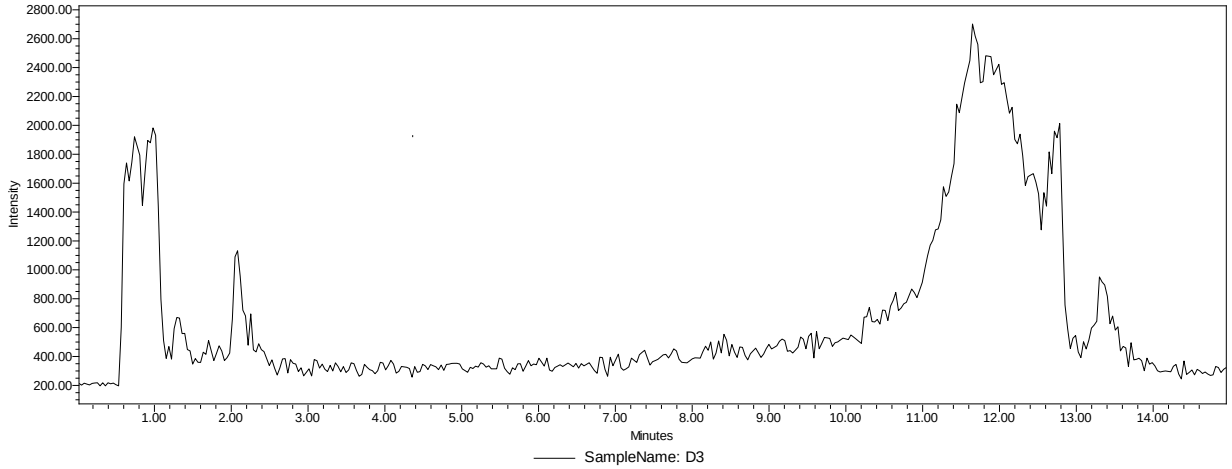

Fig. 25iii(59) product chromatograph for .50 ppm FUPA 3rd replicate.

129

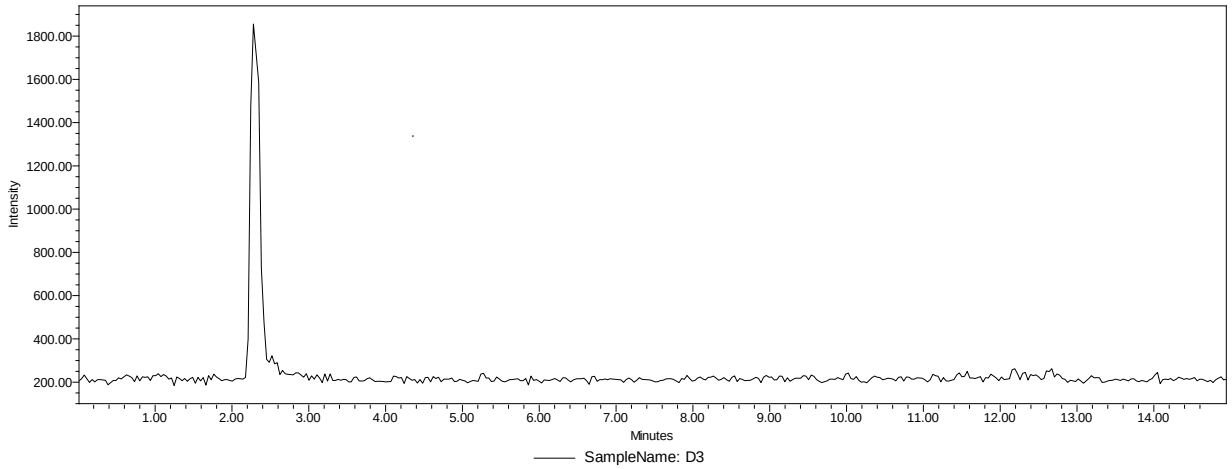

Fig. 25iii(60) product chromatograph for .50 ppm 5-FU 3rd replicate.

E1

77

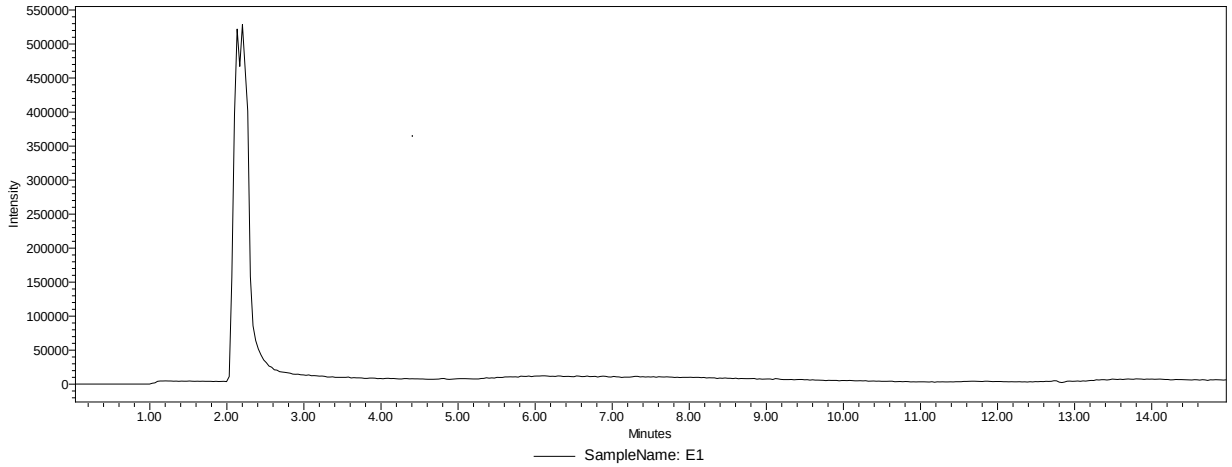

Fig. 25iii(61) product chromatograph for 1.0 ppm FAC 1st replicate.

105

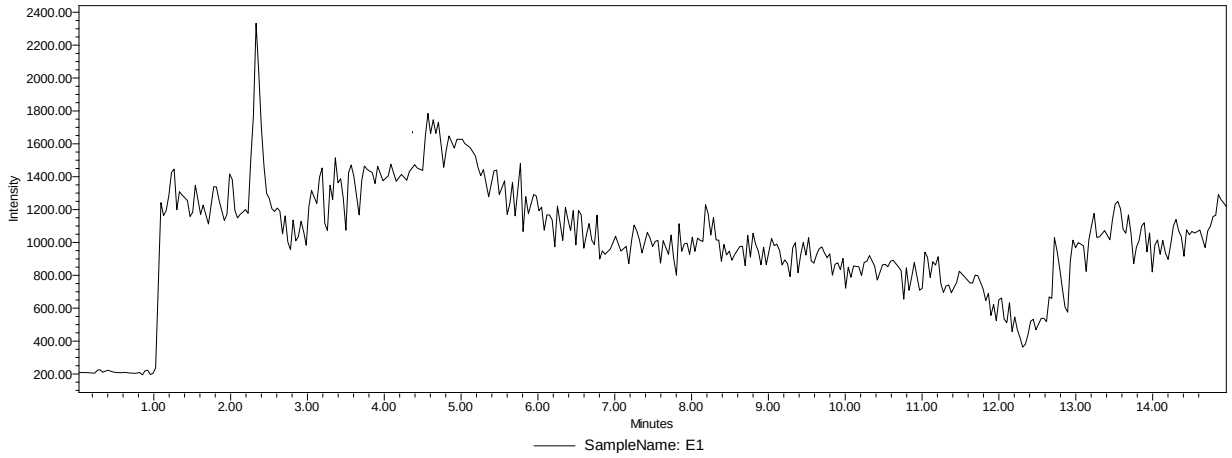

Fig. 25iii(62) product chromatograph for 1.0 ppm FMASld 1st replicate.

106

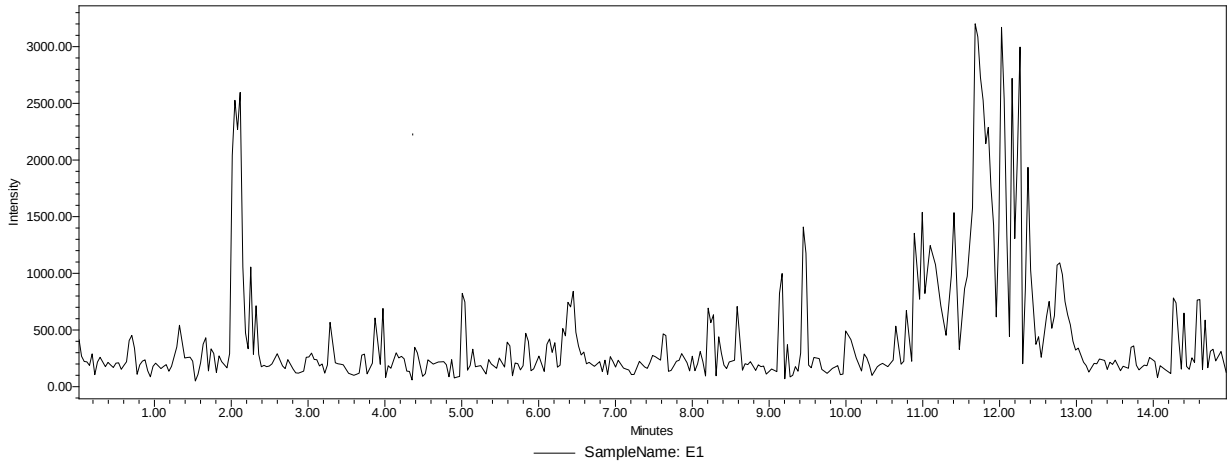

Fig. 25iii(63) product chromatograph for 1.0 ppm FBAL 1st replicate.

149

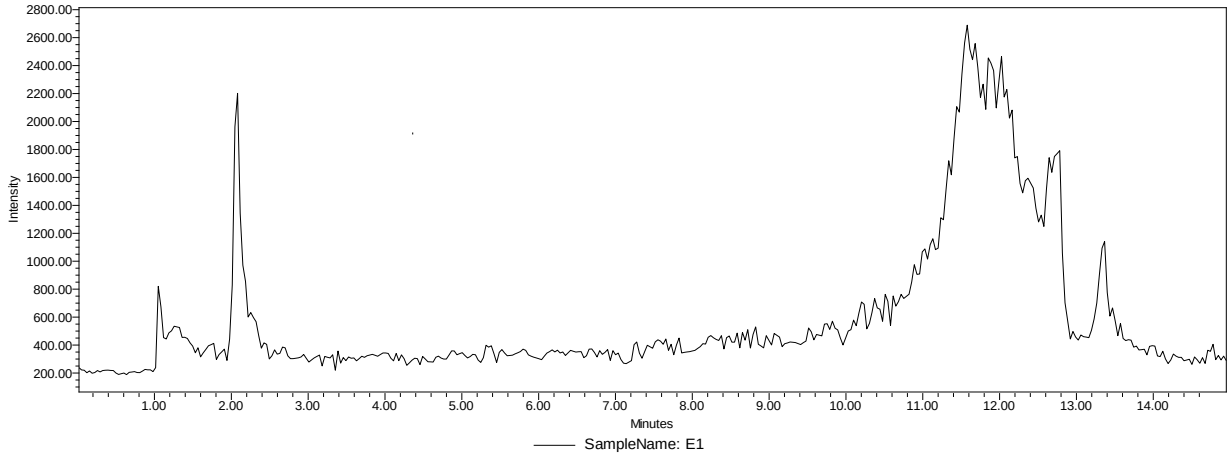

Fig. 25iii(64) product chromatograph for 1.0 ppm FUPA 1st replicate.

129

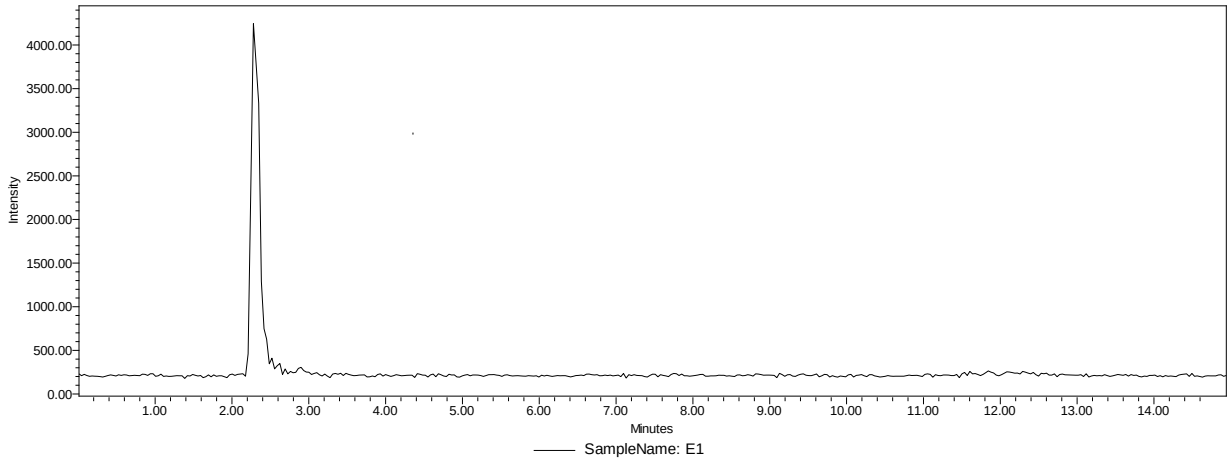

Fig. 25iii(65) product chromatograph for 1.0 ppm 5-FU 1st replicate.

E2

77

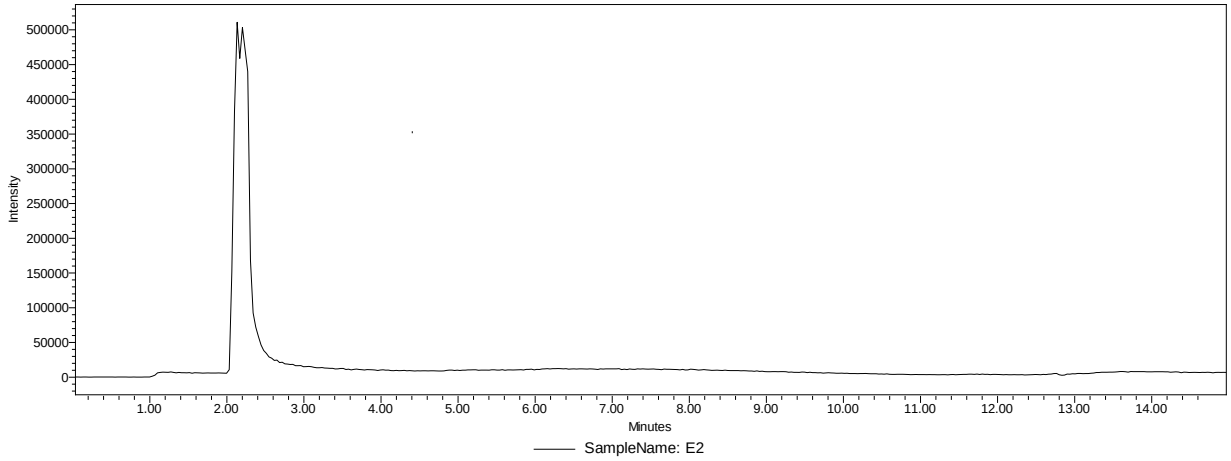

Fig. 25iii(66) product chromatograph for 1.0 ppm FAC 2nd replicate.

105

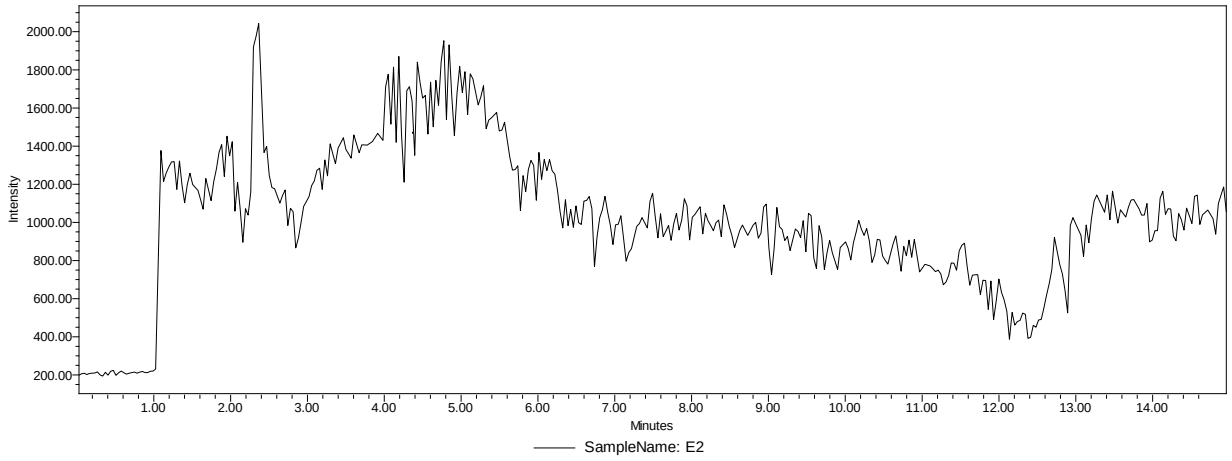

Fig. 25iii(67) product chromatograph for 1.0 ppm FMASld 2nd replicate.

106

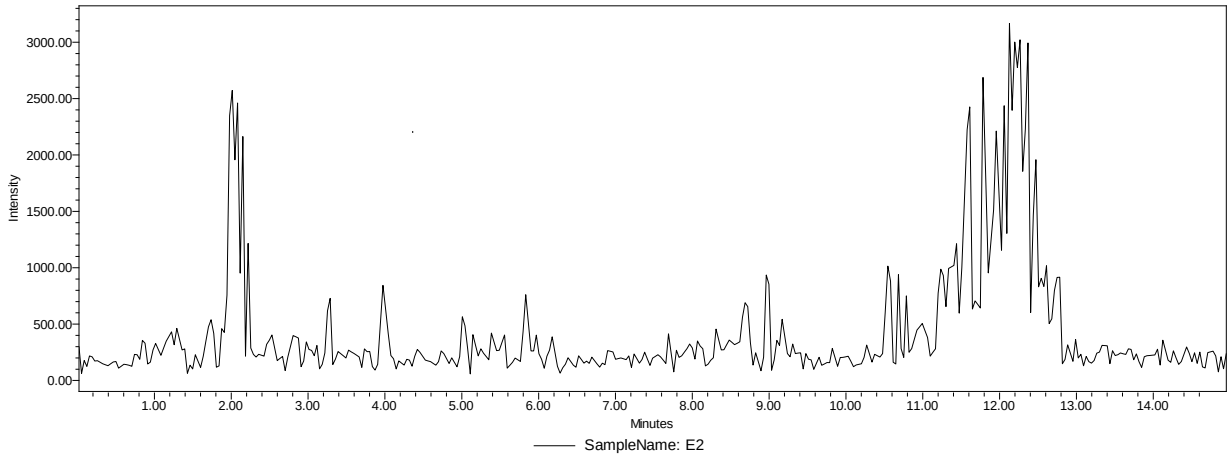

Fig. 25iii(68) product chromatograph for 1.0 ppm FBAL 2nd replicate.

149

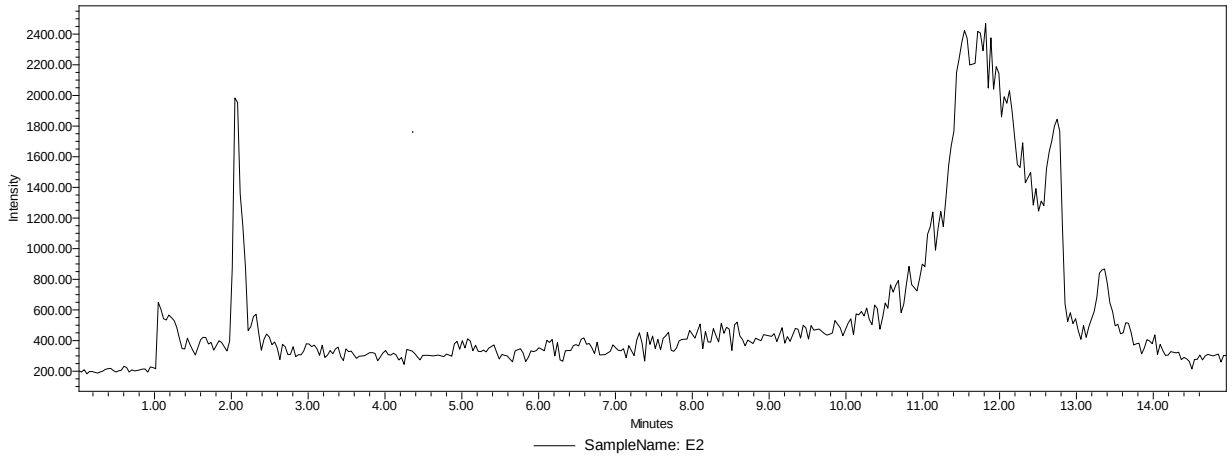

Fig. 25iii(69) product chromatograph for 1.0 ppm FUPA 2nd replicate.

129

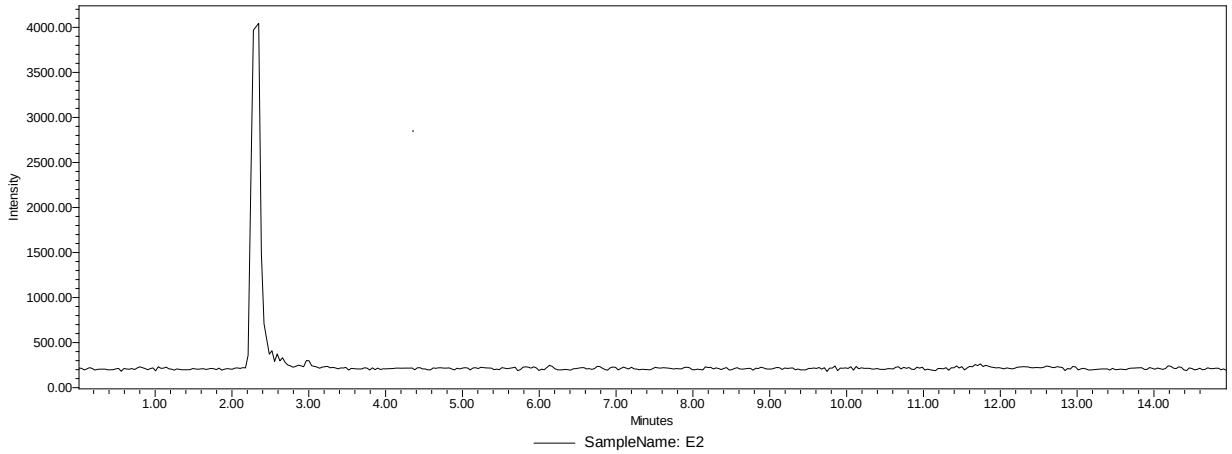

Fig. 25iii(70) product chromatograph for 1.0 ppm 5-FU 2nd replicate.

E3

77

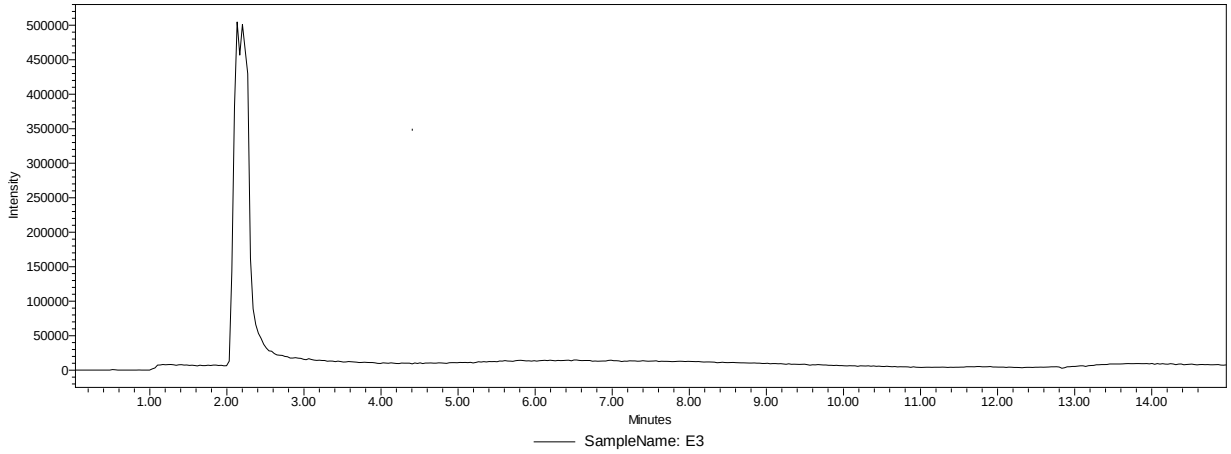

Fig. 25iii(71) product chromatograph for 1.0 ppm FAC 3rd replicate.

105

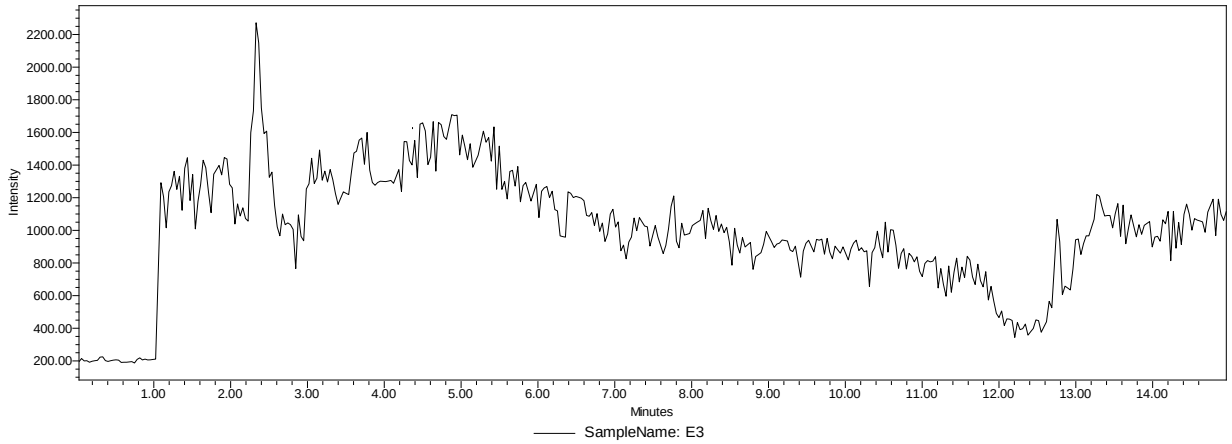

Fig. 25iii(72) product chromatograph for 1.0 ppm FMASld 3rd replicate.

106

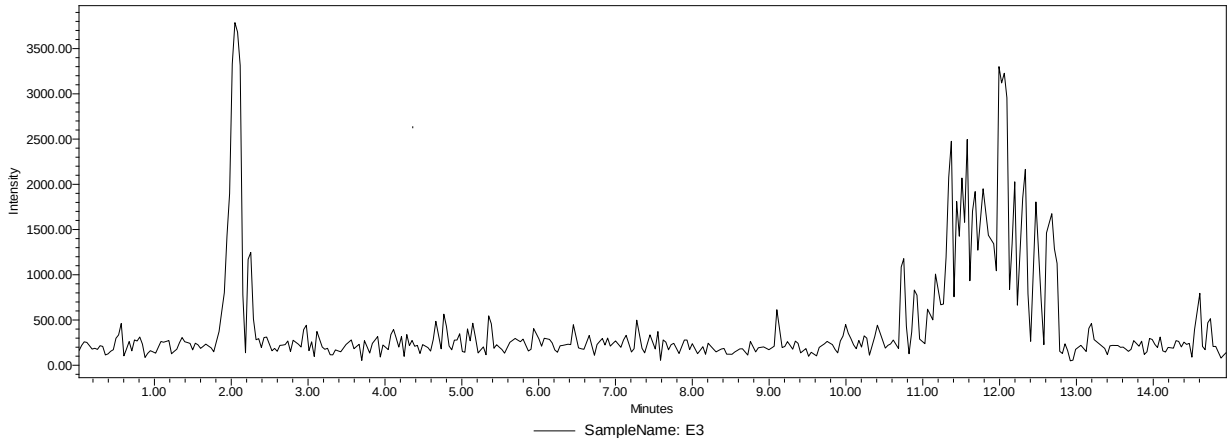

Fig. 25iii(73) product chromatograph for 1.0 ppm FBAL 3rd replicate.

149

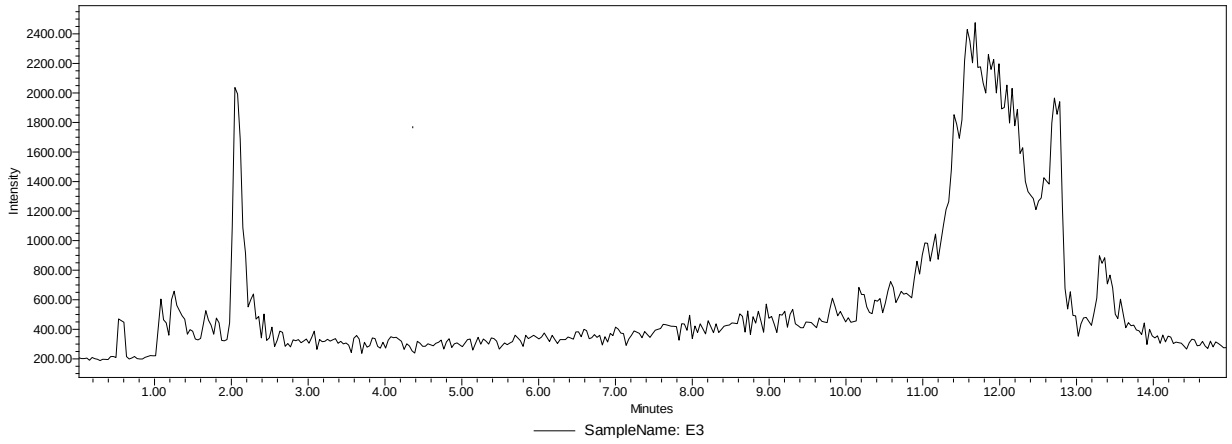

Fig. 25iii(74) product chromatograph for 1.0 ppm FUPA 3rd replicate.

129

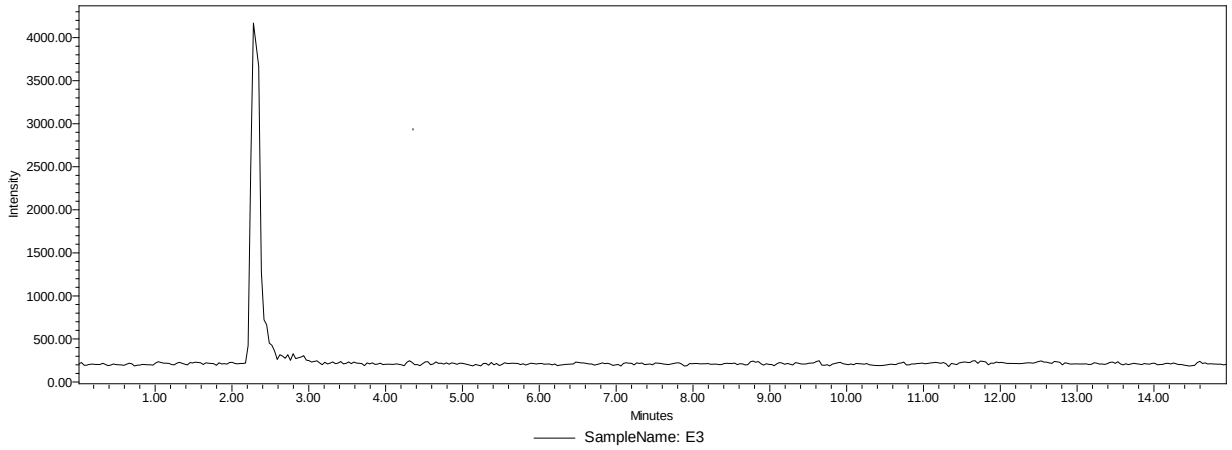

Fig. 25iii(75) product chromatograph for 1.0 ppm 5-FU 3rd replicate.

F1

77

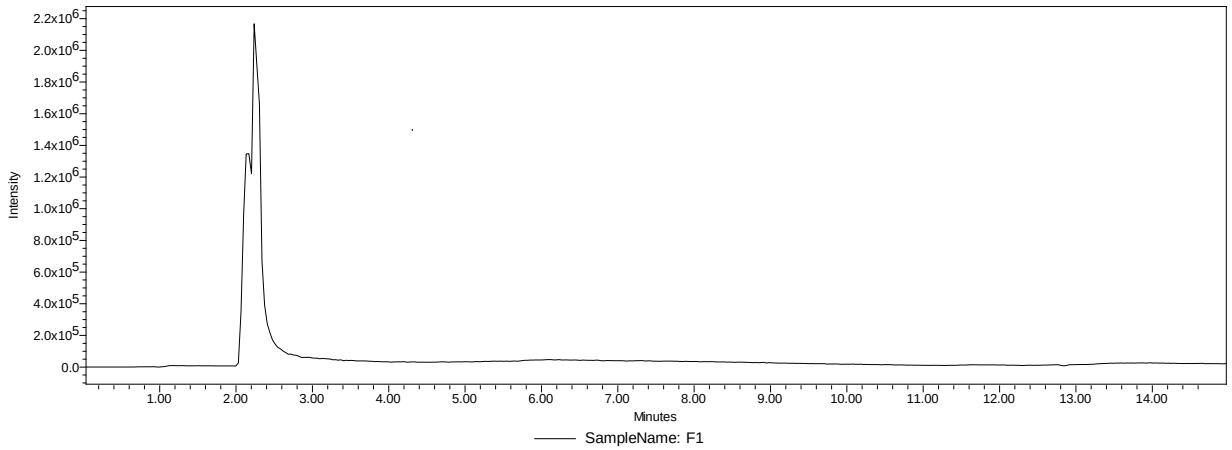

Fig. 25iii(76) product chromatograph for 5.0 ppm FAC 1st replicate.

105

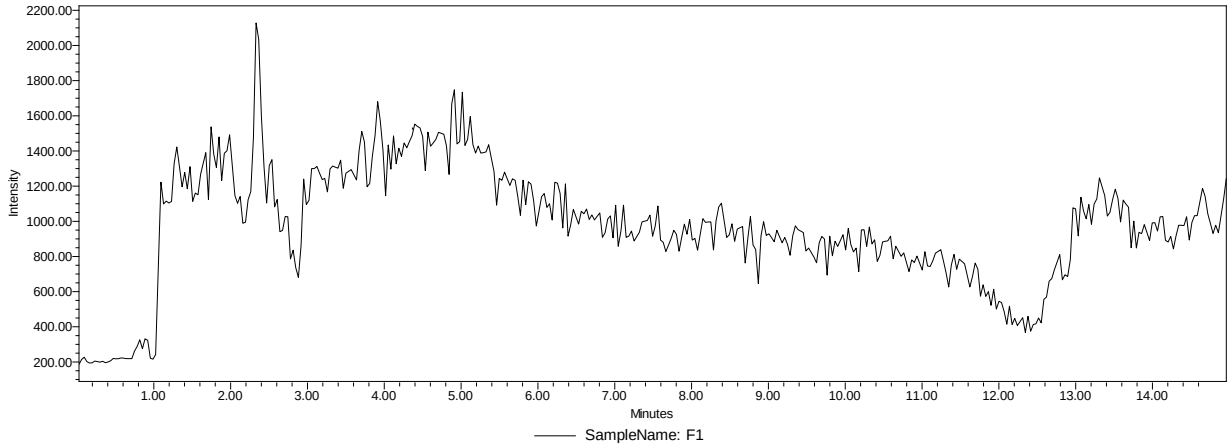

Fig. 25iii(77) product chromatograph for 5.0 ppm FMASld 1st replicate.

106

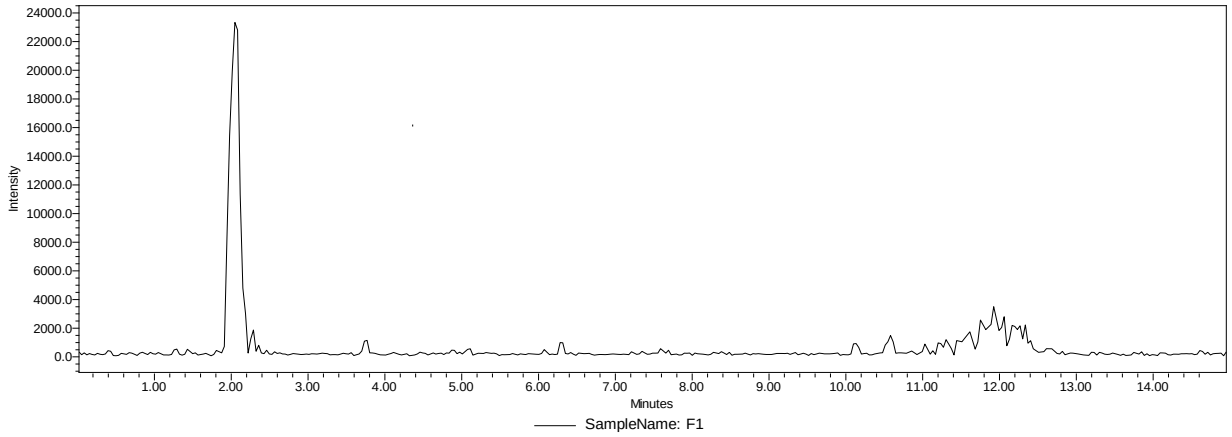

Fig. 25iii(78) product chromatograph for 5.0 ppm FBAL 1st replicate.

149

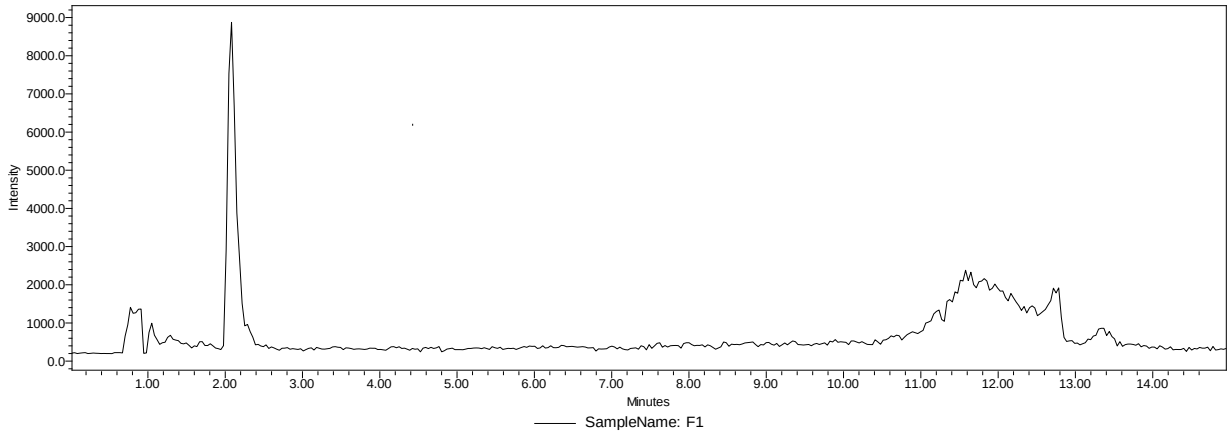

Fig. 25iii(79) product chromatograph for 5.0 ppm FUPA 1st replicate.

129

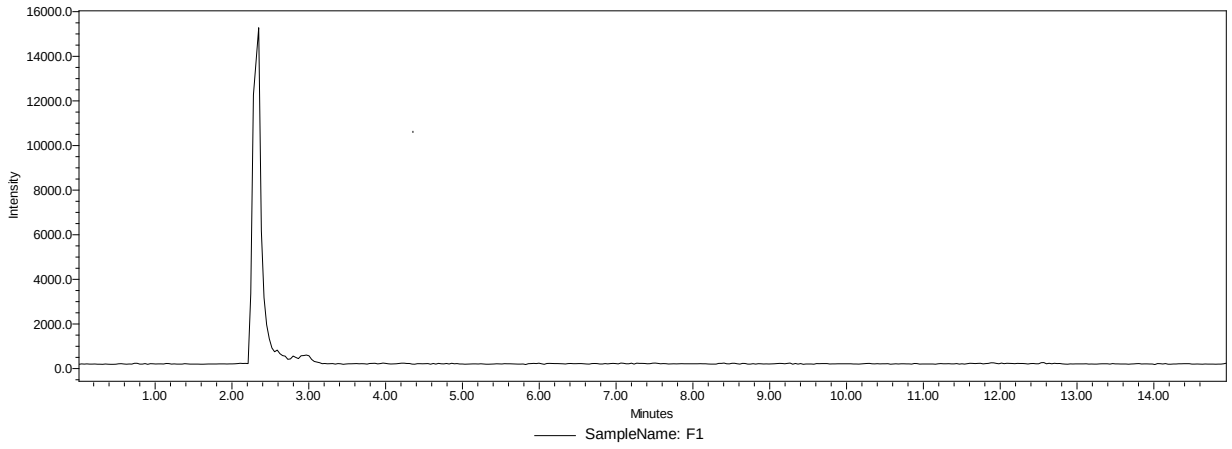

Fig. 25iii(80) product chromatograph for 5.0 ppm 5-FU 1st replicate.

F2

77

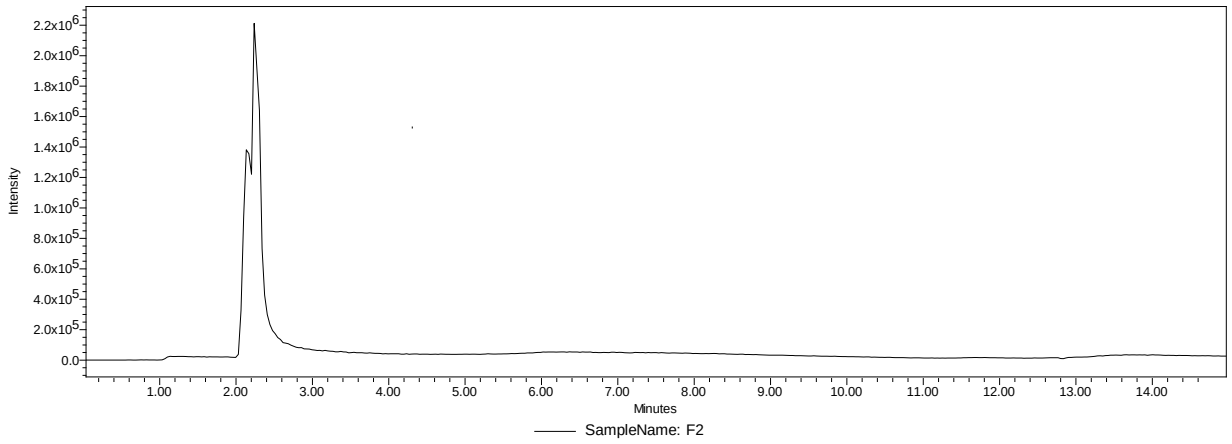

Fig. 25iii(81) product chromatograph for 5.0 ppm FAC 2nd replicate.

105

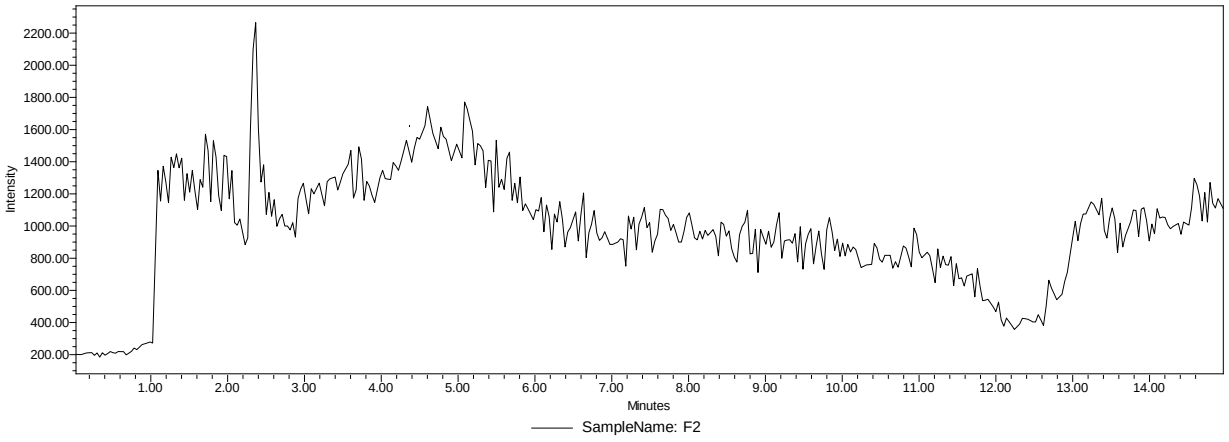

Fig. 25iii(82) product chromatograph for 5.0 ppm FMASld 2nd replicate.

106

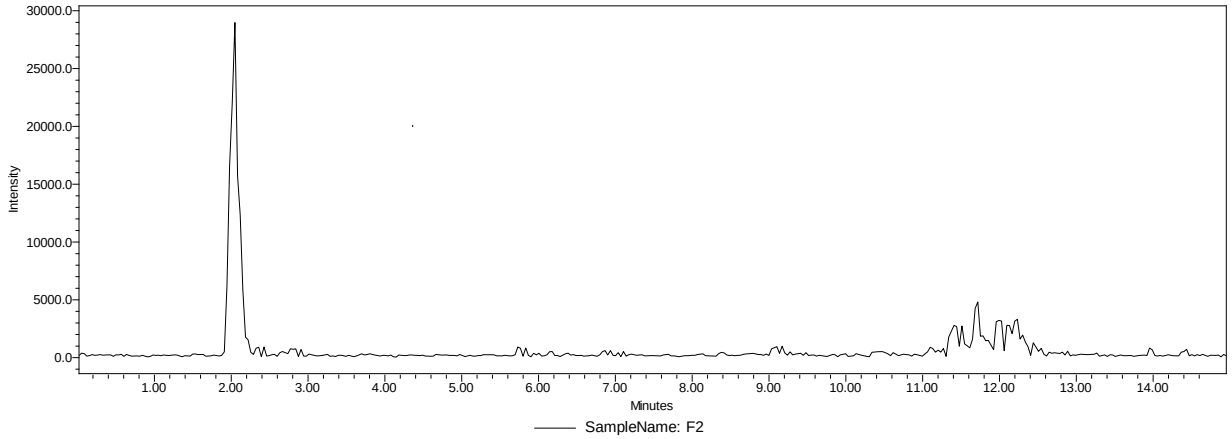

Fig. 25iii(83) product chromatograph for 5.0 ppm FBAL 2nd replicate.

149

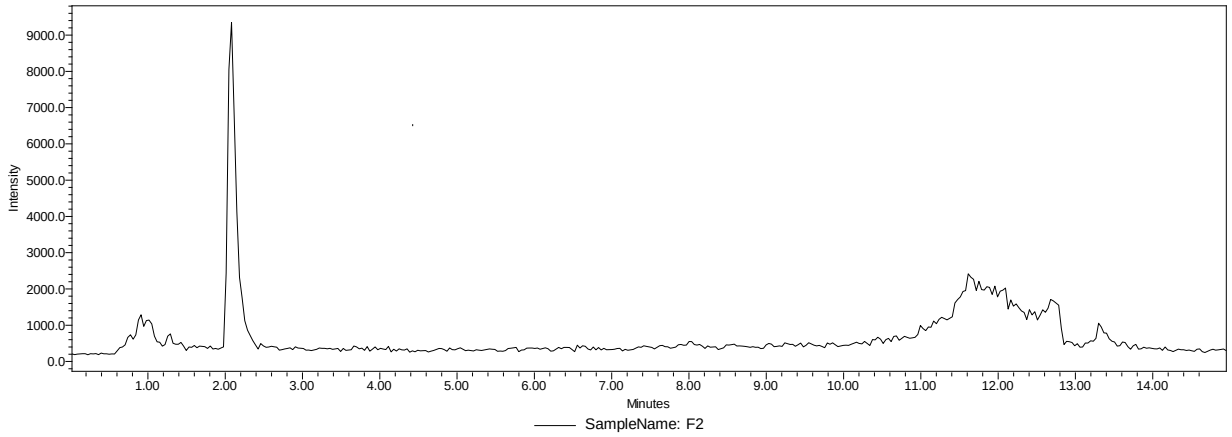

Fig. 25iii(84) product chromatograph for 5.0 ppm FUPA 2nd replicate.

129

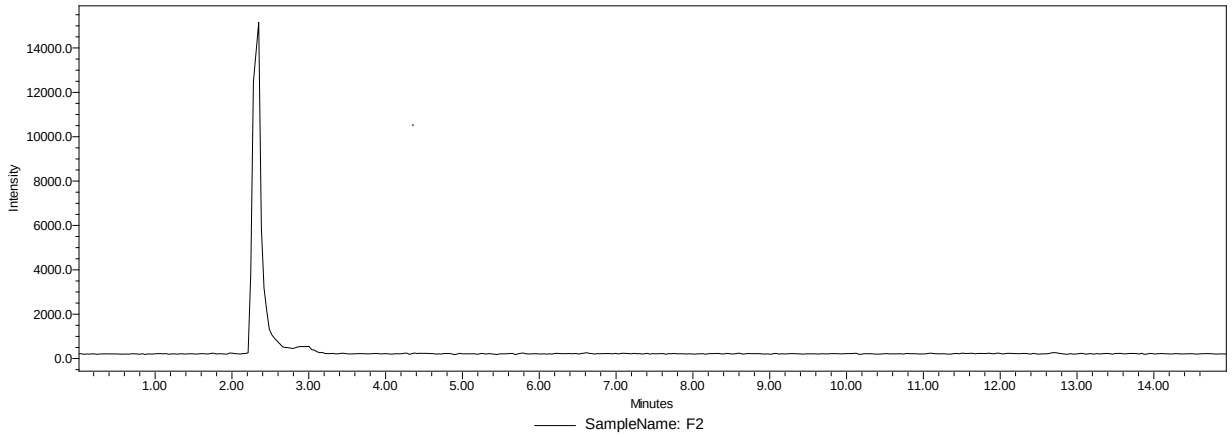

Fig. 25iii(85) product chromatograph for 5.0 ppm 5-FU 2nd replicate.

F3

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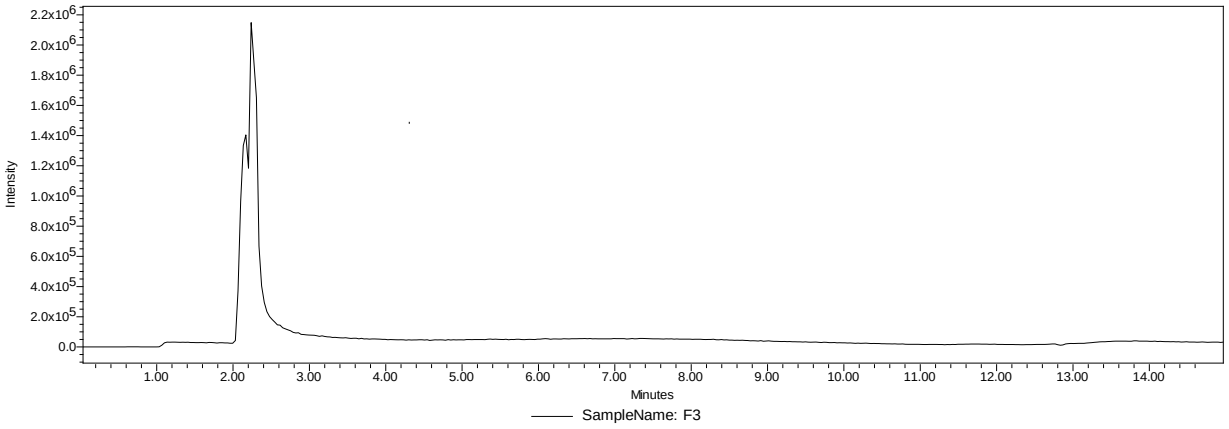

Fig. 25iii(86) product chromatograph for 5.0 ppm FAC 3rd replicate.

105

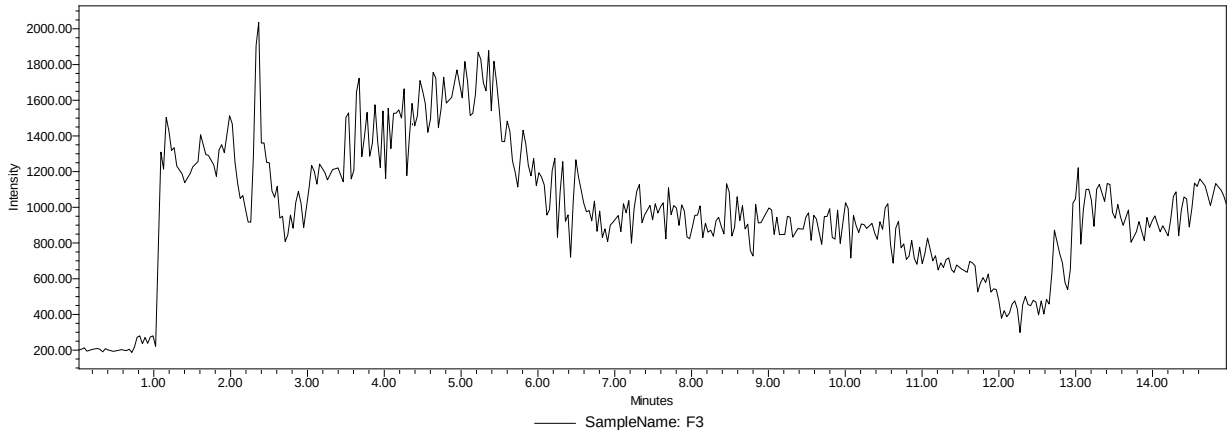

Fig. 25iii(87) product chromatograph for 5.0 ppm FMASld 3rd replicate.

106

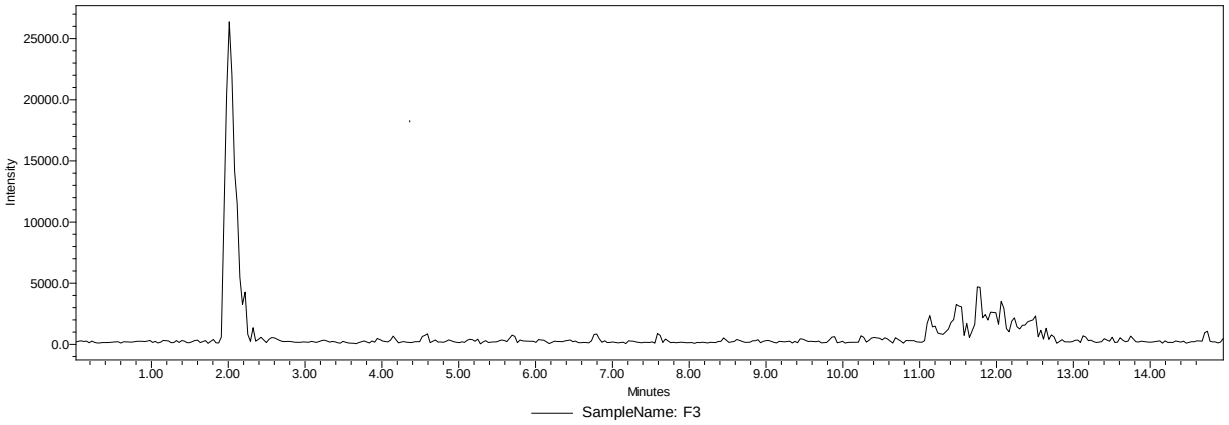

Fig. 25iii(88) product chromatograph for 5.0 ppm FBAL 3rd replicate.

149

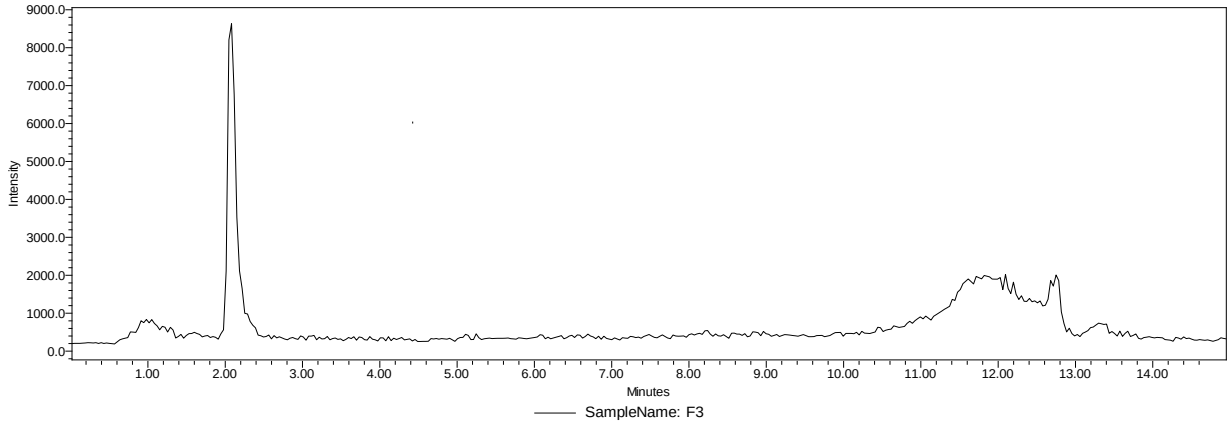

Fig. 25iii(89) product chromatograph for 5.0 ppm FUPA 3rd replicate.

129

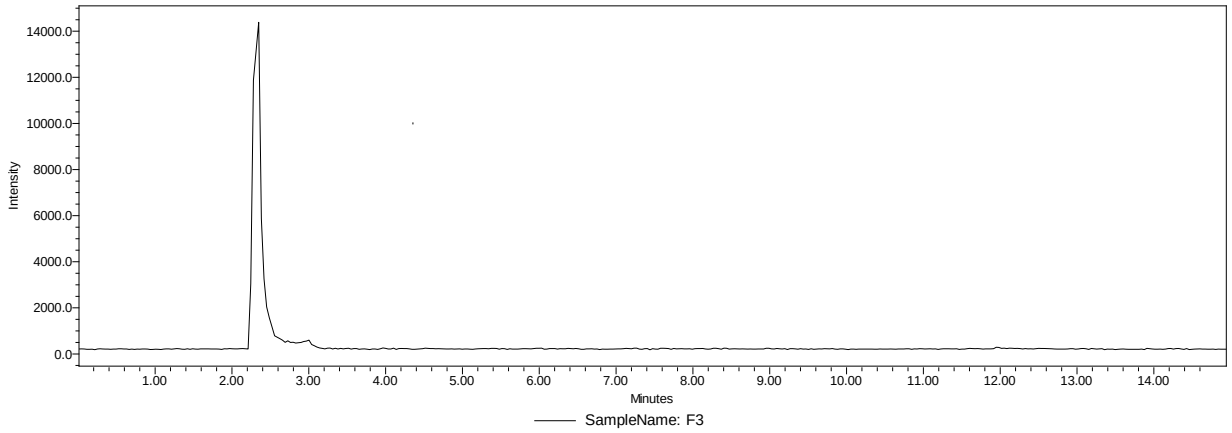

Fig. 25iii(90) product chromatograph for 5.0 ppm 5-FU 3rd replicate.

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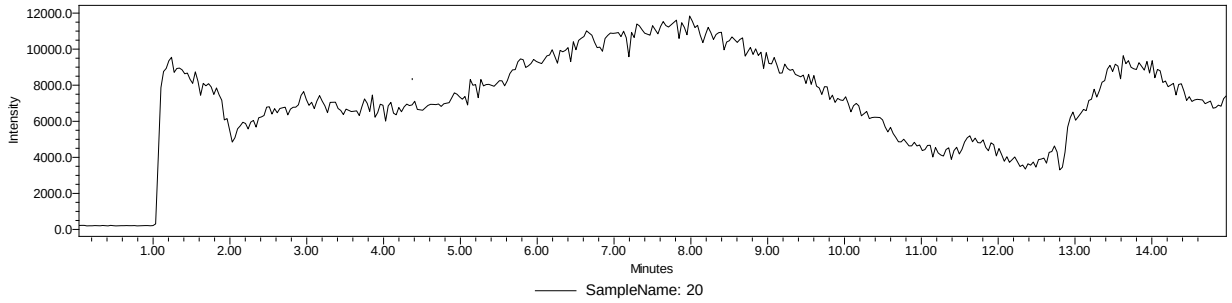

Fig. 26(1) product chromatograph for 5-FUr 1st replicate/screened for FAC.

105

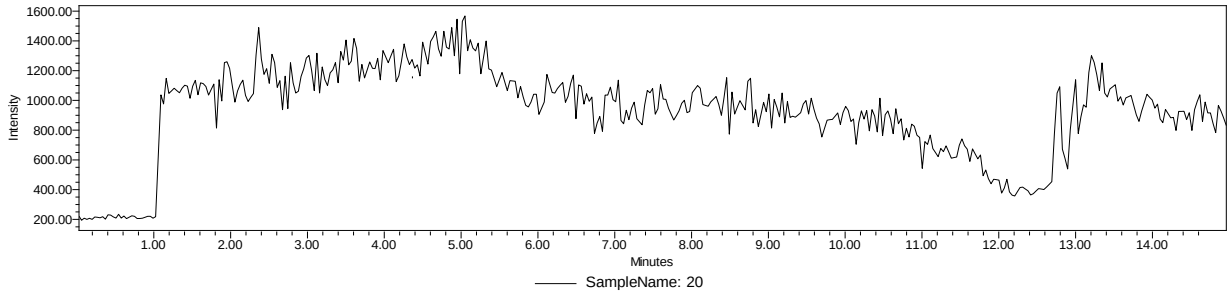

Fig. 26(2) product chromatograph for 5-FUr 1st replicate/screened for FMASld.

106

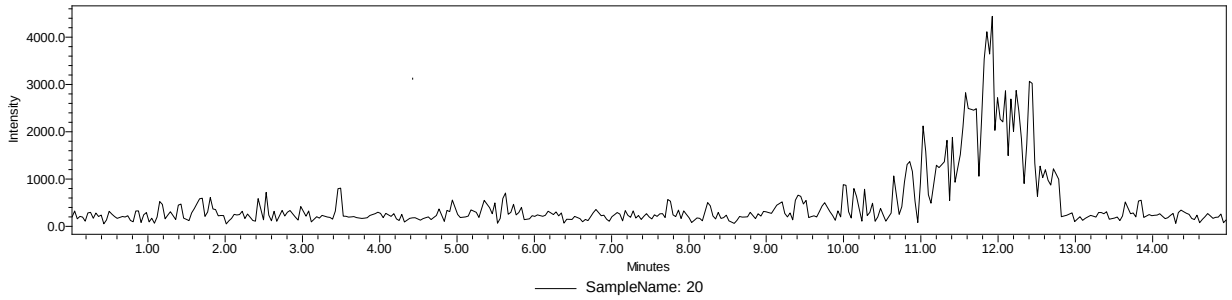

Fig. 26(3) product chromatograph for 5-FUr 1st replicate/screened for FBAL.

149

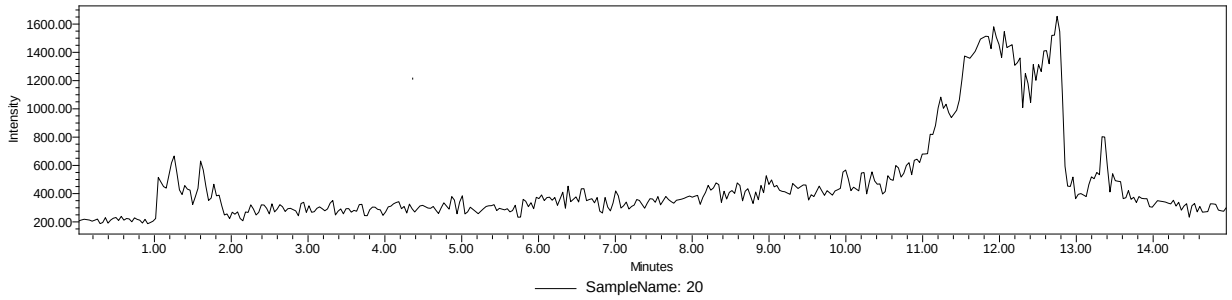

Fig. 26(4) product chromatograph for 5-FUr 1st replicate/screened for FUPA.

129

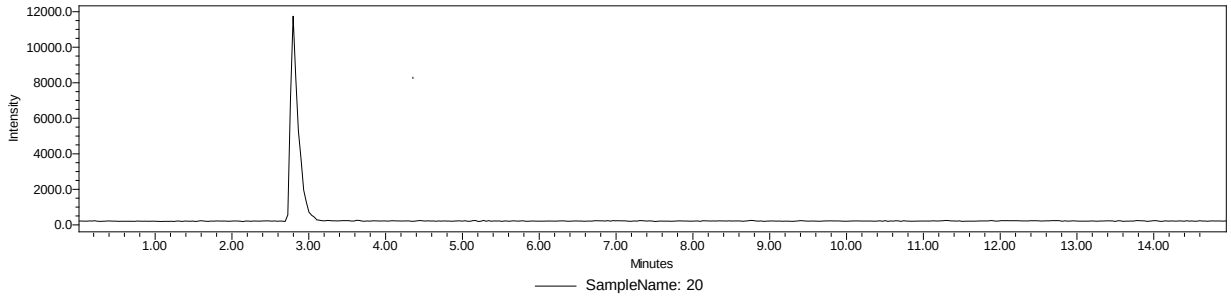

Fig. 26(5) product chromatograph for 5-FUr 1st replicate/screened for 5-FU.

20A

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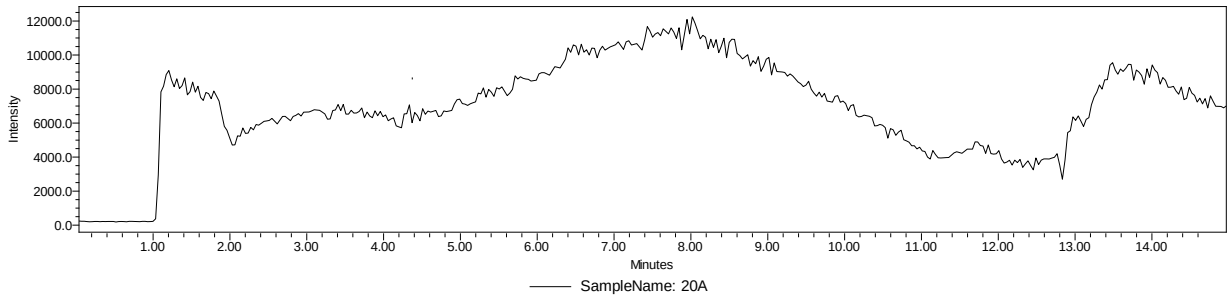

Fig. 26(6) product chromatograph for T/Alk$_0$ 1st replicate/screened for FAC.

105

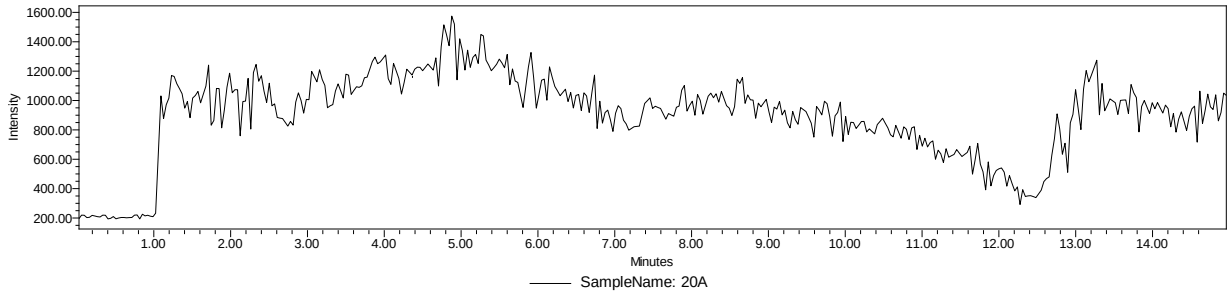

Fig. 26(7) product chromatograph for T/Alk$_0$ 1st replicate/screened for FMASld.

106

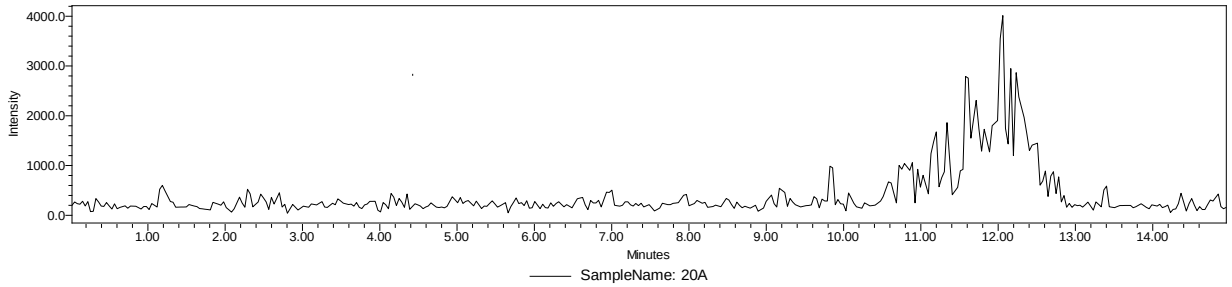

Fig. 26(8) product chromatograph for T/Alk$_0$ 1st replicate/screened for FBAL.

149

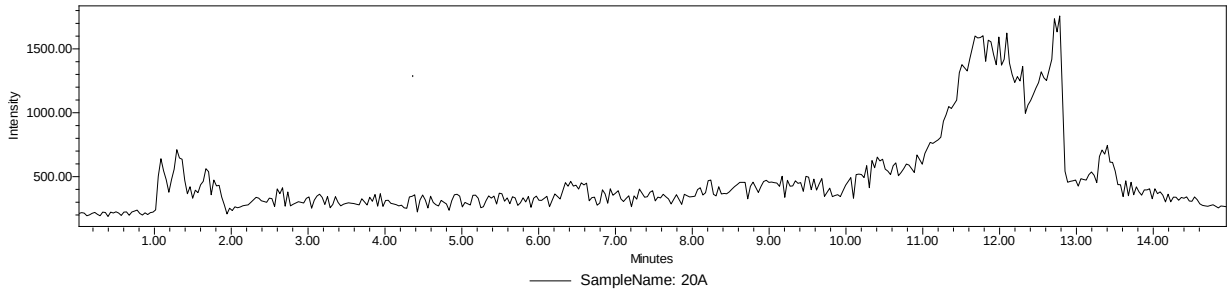

Fig. 26(9) product chromatograph for T/Alk$_0$ 1st replicate/screened for FUPA.

129

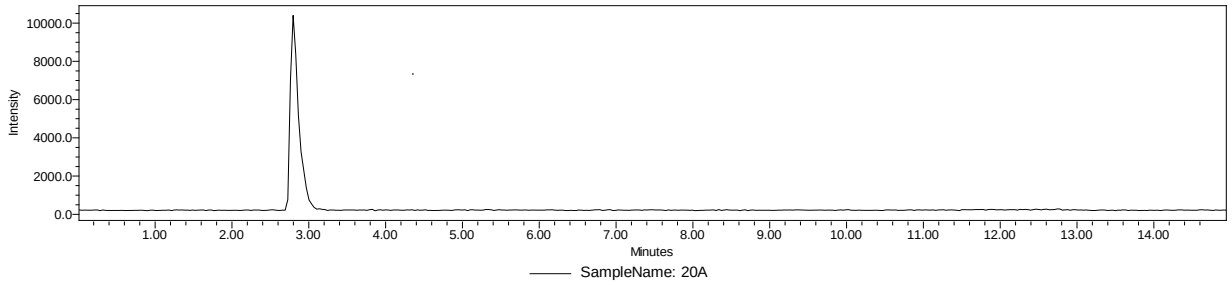

Fig. 26(10) product chromatograph for T/Alk$_0$ 1st replicate/screened for 5-FU.

21

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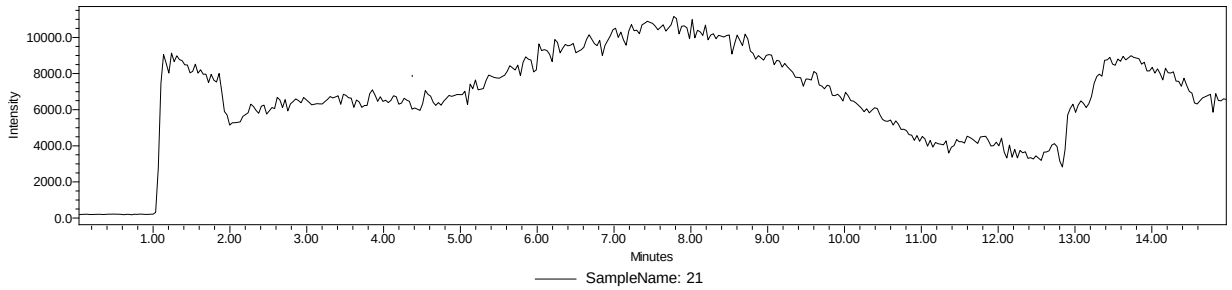

Fig. 26(11) Thermal/Alkali product chromatograph screening for FAC @ 1 day 1st replicate.

105

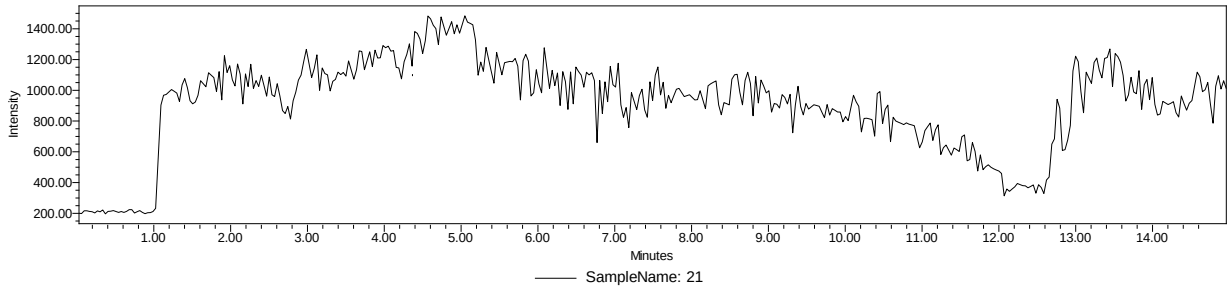

Fig. 26(12) Thermal/Alkali product chromatograph screening for FMASld @ 1 day 1st

106

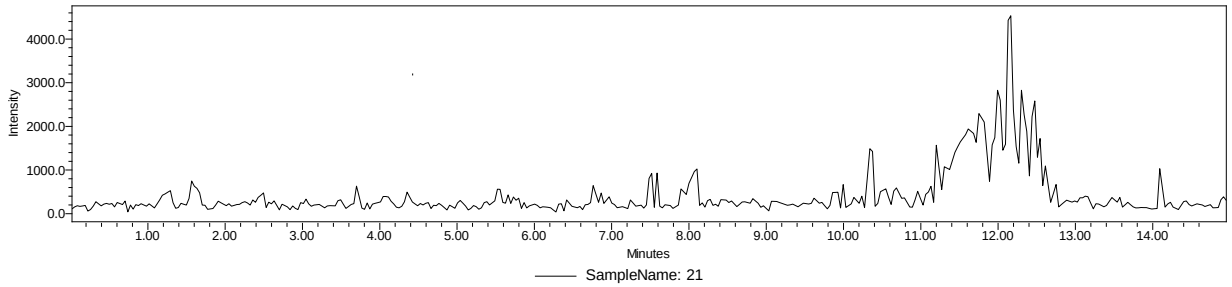

Fig. 26(13) Thermal/Alkali product chromatograph screening for FBAL @ 1 day 1st replicate.

149

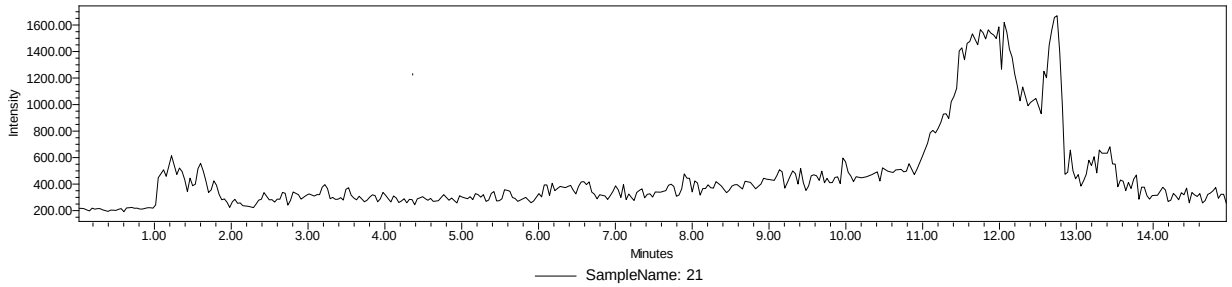

Fig. 26(14) Thermal/Alkali product chromatograph screening for FUPA @ 1 day 1st replicat

129

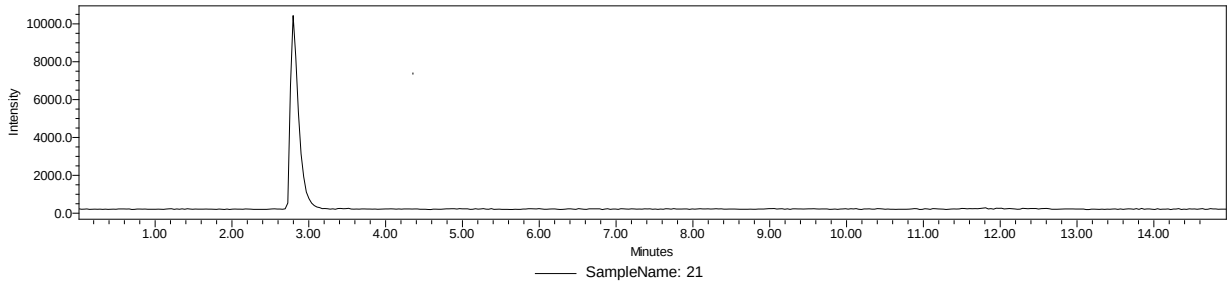

Fig. 26(15) Thermal/Alkali product chromatograph screening for 5-FU @ 1 day 1st replicate.

22

77

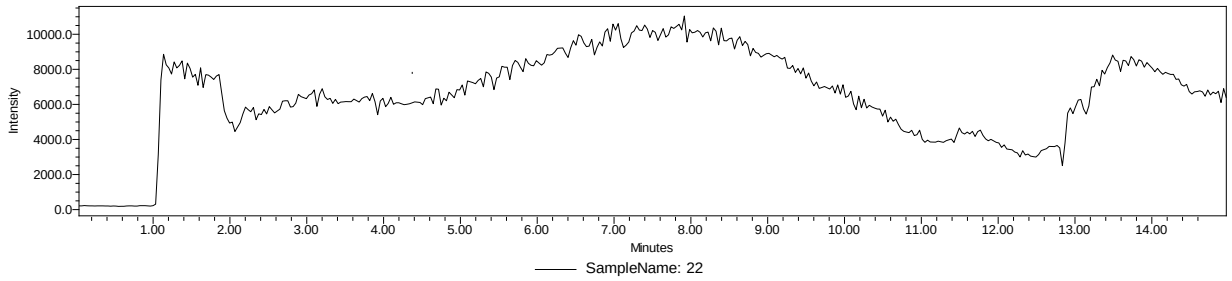

Fig. 26(16)Thermal/Alkali product chromatograph screening for FAC @ 1 day 2nd replicate.

105

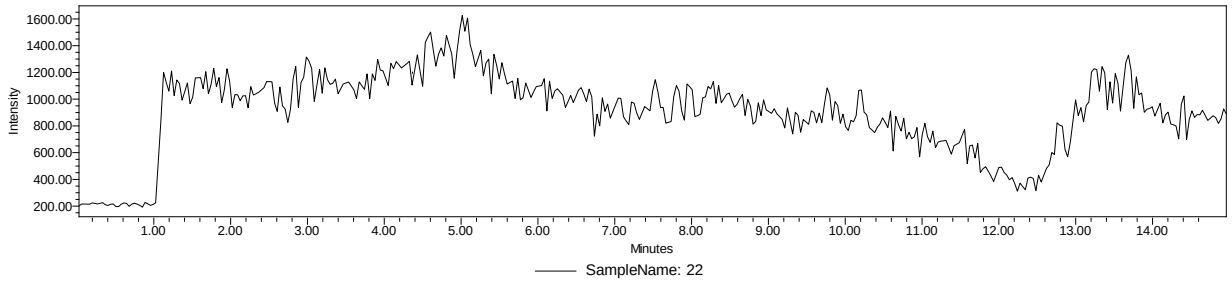

Fig. 26(17)Thermal/Alkali product chromatograph screening for FMASId @ 1 day 2nd replicate.

106

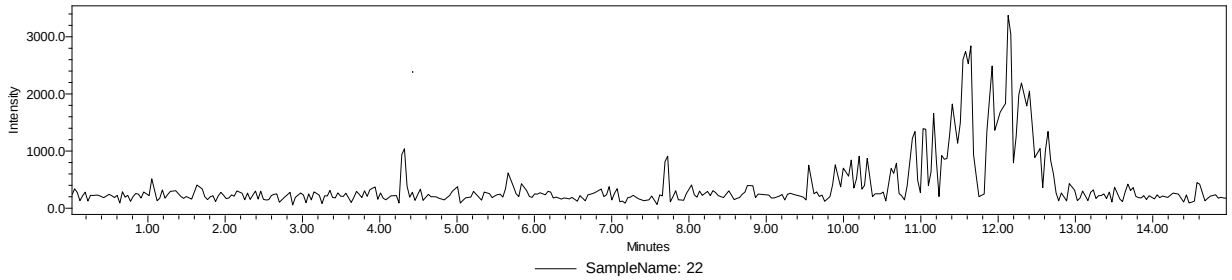

Fig. 26(18)Thermal/Alkali product chromatograph screening for FBAL @ 1 day 2nd replicate.

149

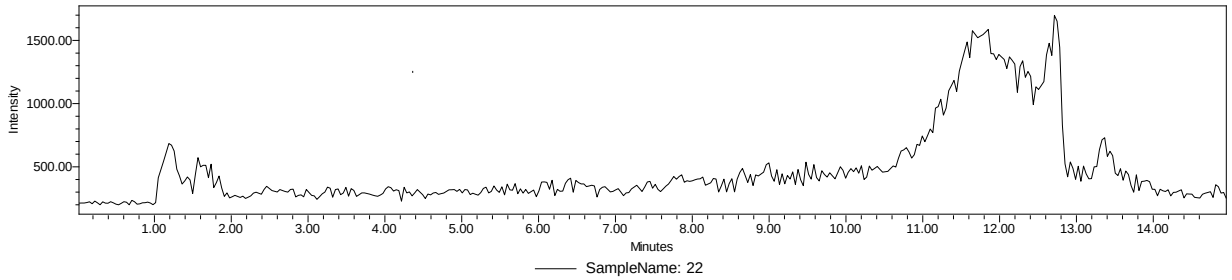

Fig. 26(19)Thermal/Alkali product chromatograph screening for FUPA @ 1 day 2nd replicate.

129

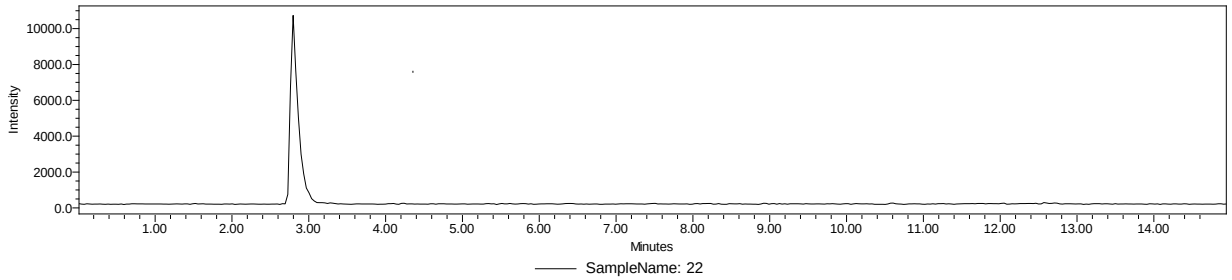

Fig. 26(20)Thermal/Alkali product chromatograph screening for 5-FU @ 1 day 2nd replicate.

23

77

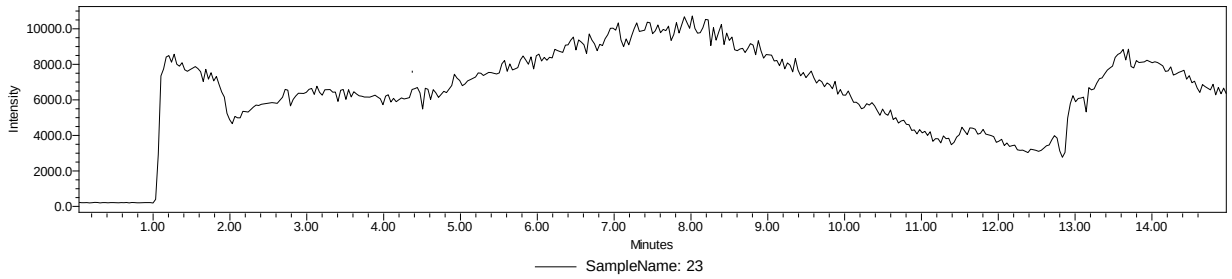

Fig. 26(21)Thermal/Alkali product chromatograph screening for FAC @ 1 day 3rd replicate.

105

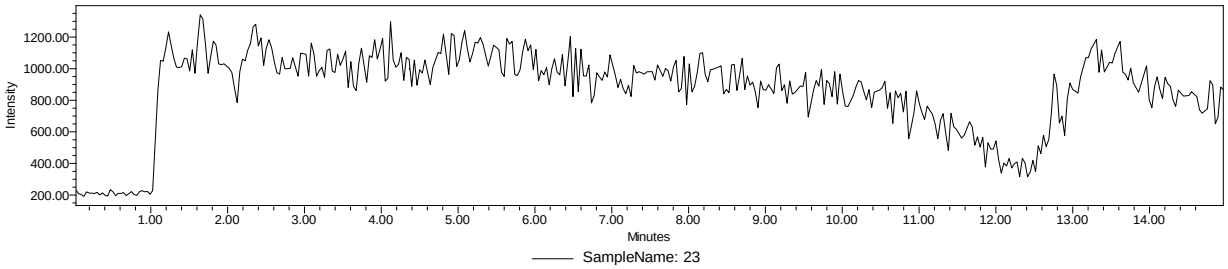

Fig. 26(22)Thermal/Alkali product chromatograph screening for FMASld @ 1 day 3rd replicate.

106

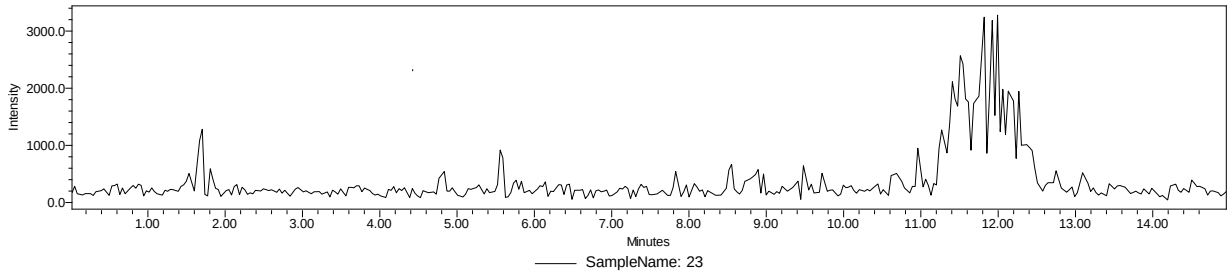

Fig. 26(23)Thermal/Alkali product chromatograph screening for FBAL @ 1 day 3rd replicate.

149

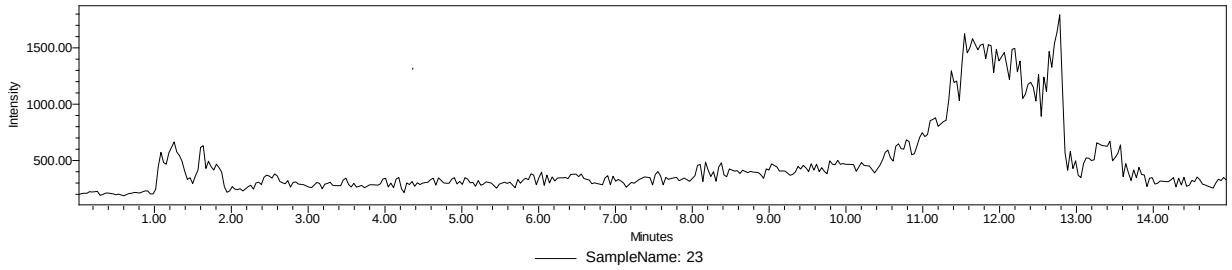

Fig. 26(24)Thermal/Alkali product chromatograph screening for FUPA @ 1 day 3rd replicate.

129

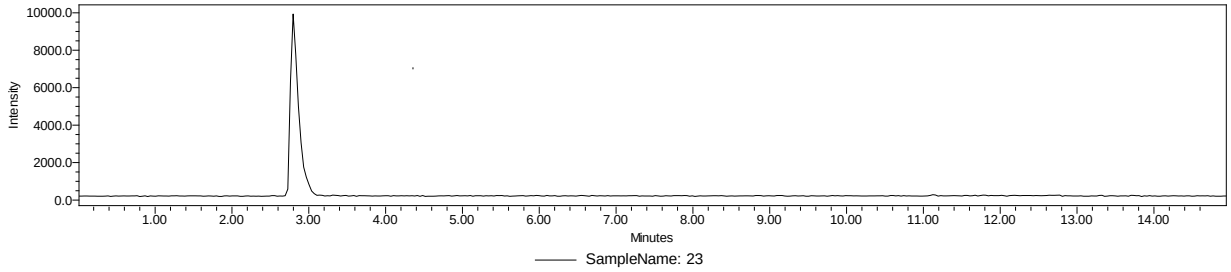

Fig. 26(25)Thermal/Alkali product chromatograph screening for 5-FU @ 1 day 3rd replicate.

24

77

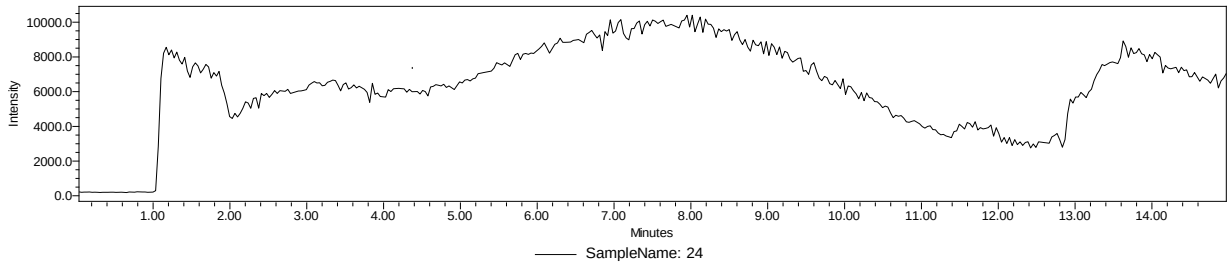

Fig. 26(26)Thermal/Alkali product chromatograph screening for FAC @ 2 day 1st replicate.

105

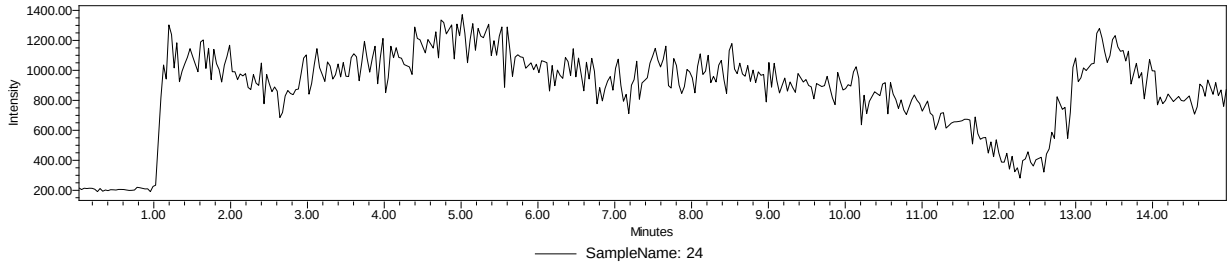

Fig. 26(27)Thermal/Alkali product chromatograph screening for FMASld @ 2 day 1st replicate.

106

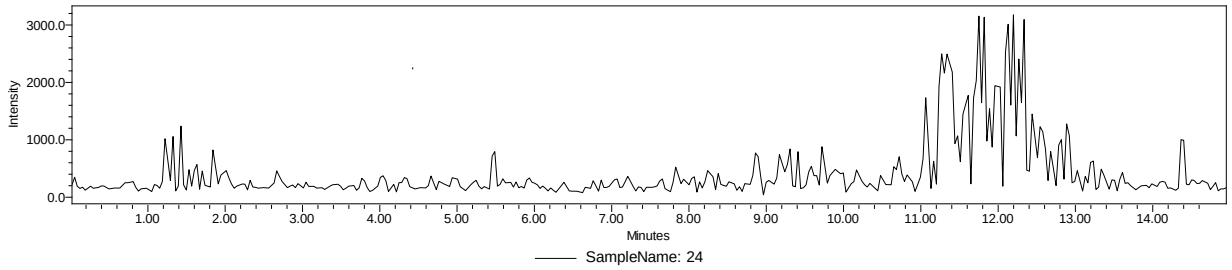

Fig. 26(28)Thermal/Alkali product chromatograph screening for FBAL @ 2 day 1st replicate.

149

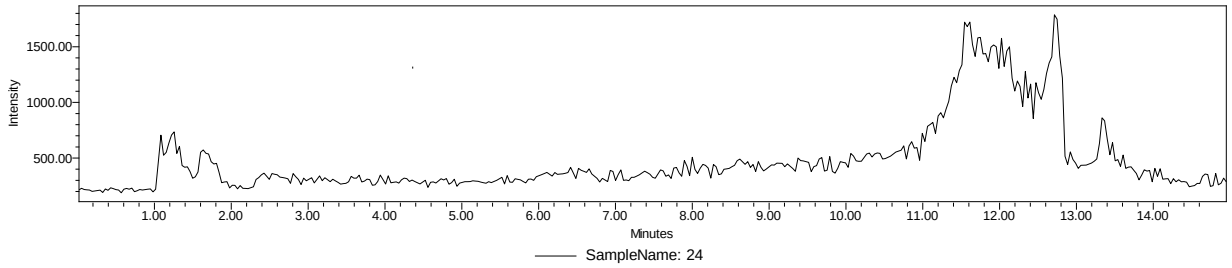

Fig. 26(29)Thermal/Alkali product chromatograph screening for FUPA @ 2 day 1st replicate.

129

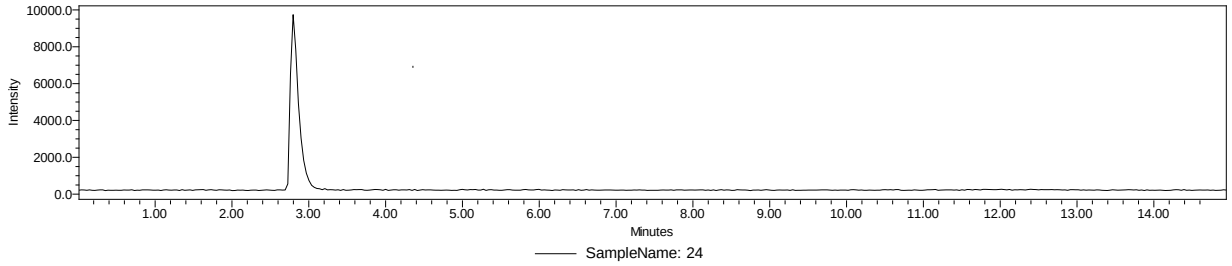

Fig. 26(30)Thermal/Alkali product chromatograph screening for 5-FU @ 2 day 1st replicate.

25

77

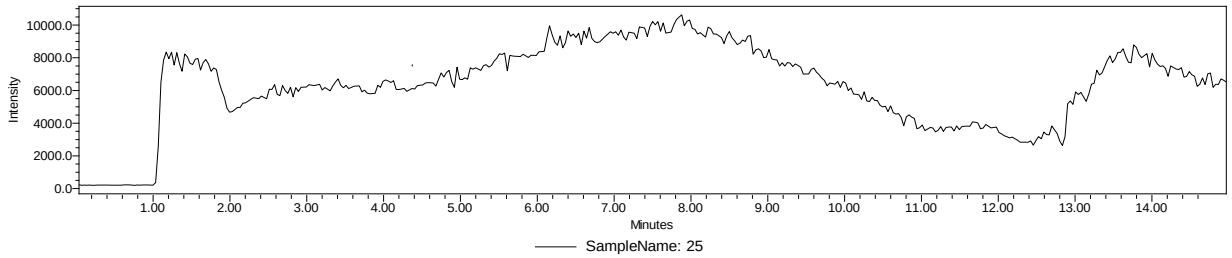

Fig. 26(31)Thermal/Alkali product chromatograph screening for FAC @ 2 day 2nd replicate.

105

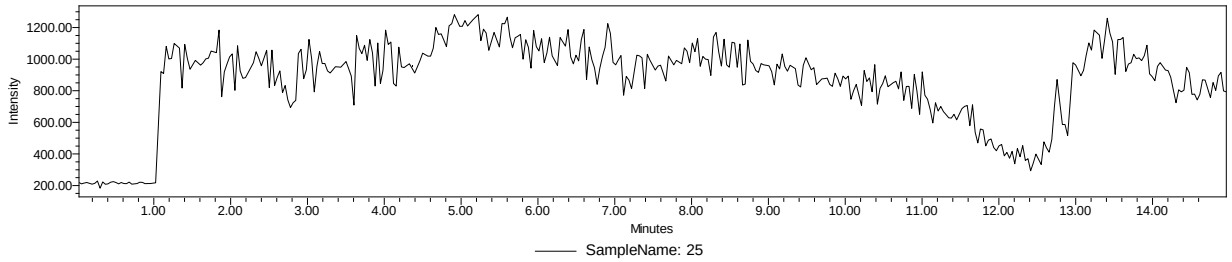

Fig. 26(32)Thermal/Alkali product chromatograph screening for FMASId @ 2 day 2nd replicate.

106

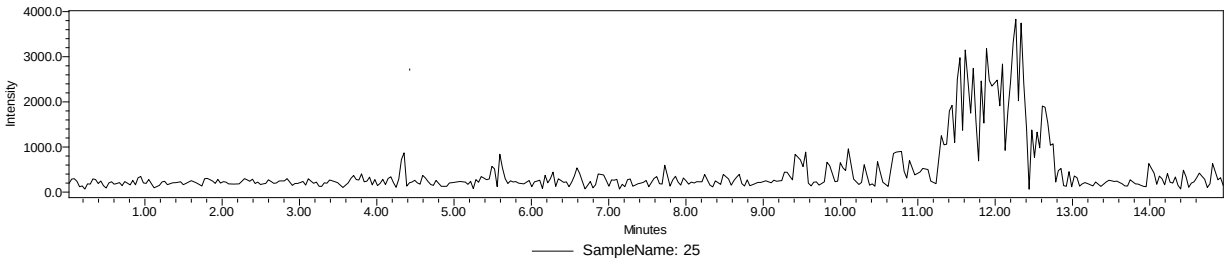

Fig. 26(33)Thermal/Alkali product chromatograph screening for FBAL @ 2 day 2nd replicate.

149

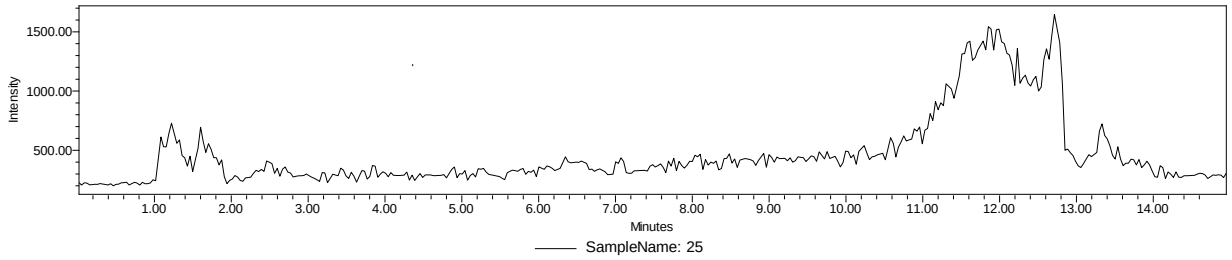

Fig. 26(34)Thermal/Alkali product chromatograph screening for FUPA @ 2 day 2nd replicate.

129

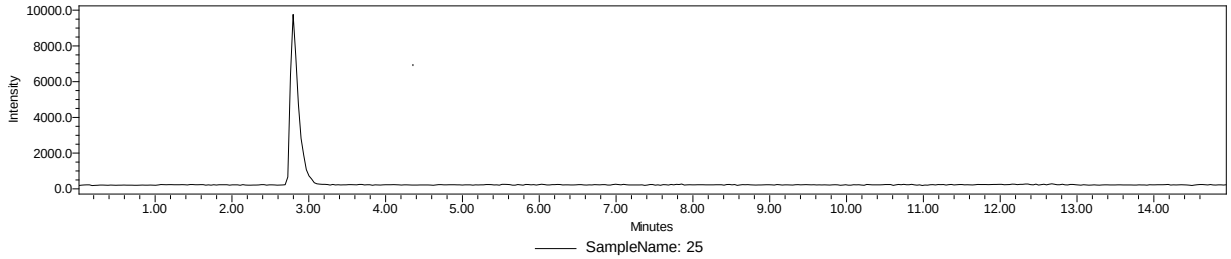

Fig. 26(35)Thermal/Alkali product chromatograph screening for 5-FU @ 2 day 2nd replicate.

26

77

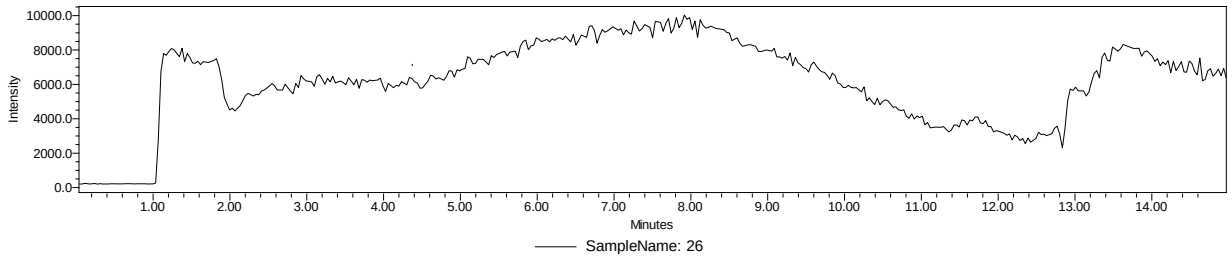

Fig. 26(36)Thermal/Alkali product chromatograph screening for FAC @ 2 day 3rd replicate.

105

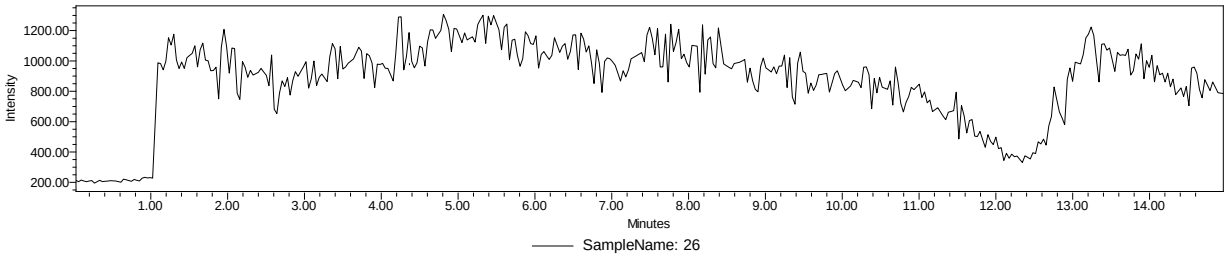

Fig. 26(37)Thermal/Alkali product chromatograph screening for FMASld @ 2 day 3rd replicate.

106

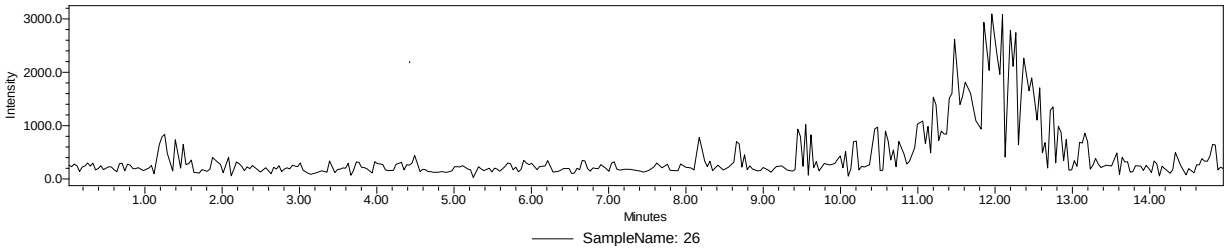

Fig. 26(38)Thermal/Alkali product chromatograph screening for FBAL @ 2 day 3rd replicate.

149

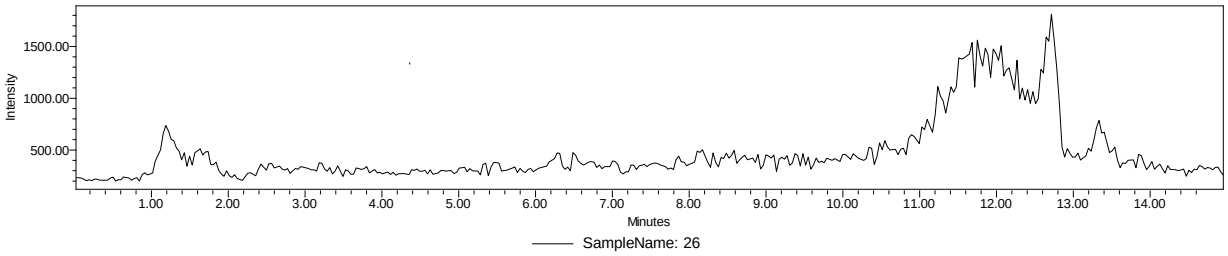

Fig. 26(39)Thermal/Alkali product chromatograph screening for FUPA @ 2 day 3rd replicate.

129

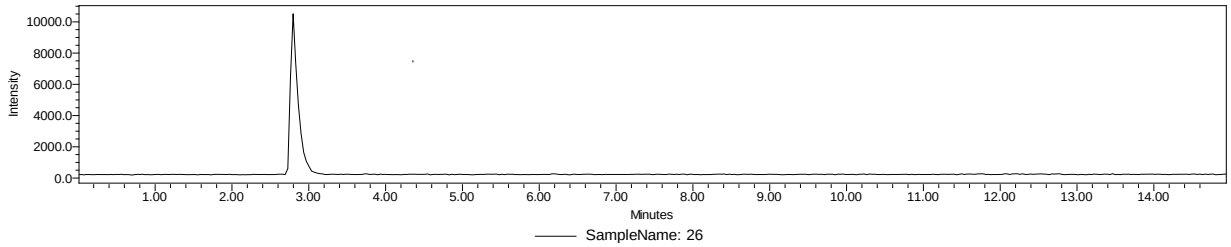

Fig. 26(40)Thermal/Alkali product chromatograph screening for 5-FU @ 2 day 3rd replicate.

27

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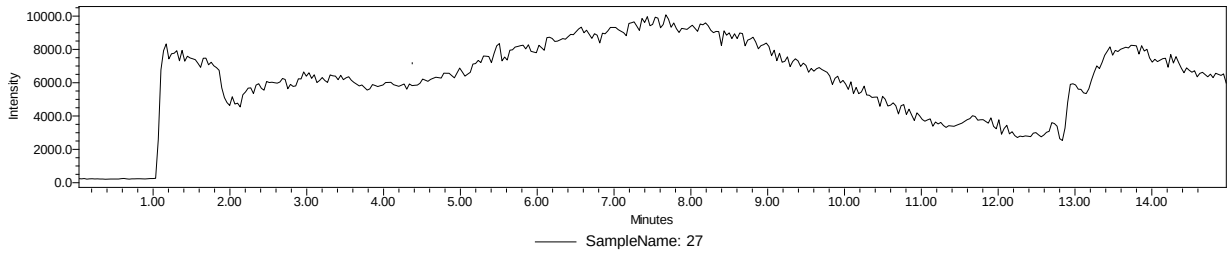

Fig. 26(41)Thermal/Alkali product chromatograph screening for FAC @ 3 day 1st replicate.

105

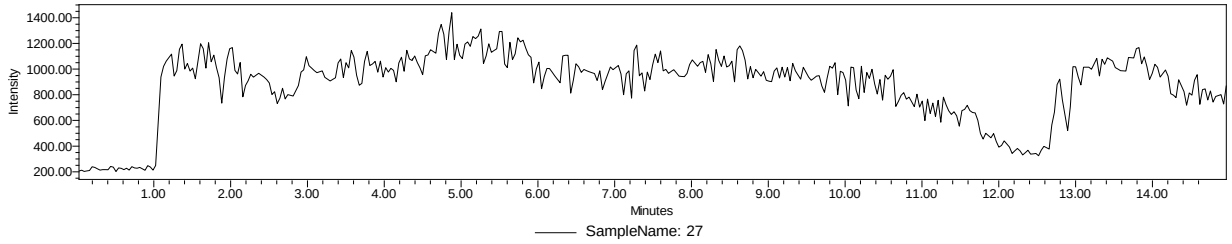

Fig. 26(42)Thermal/Alkali product chromatograph screening for FMASld @ 3 day 1st replicate.

106

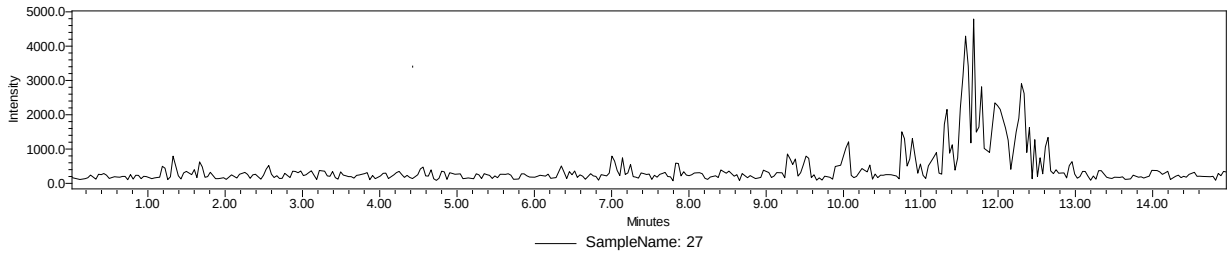

Fig. 26(43)Thermal/Alkali product chromatograph screening for FBAL @ 3 day 1st replicate.

149

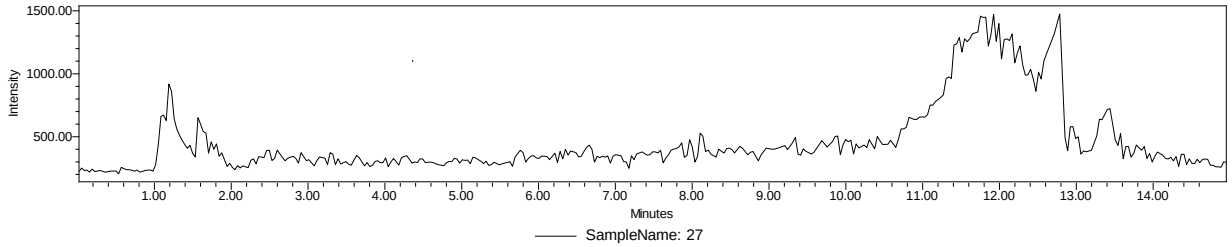

Fig. 26(44)Thermal/Alkali product chromatograph screening for FUPA @ 3 day 1st replicate.

129

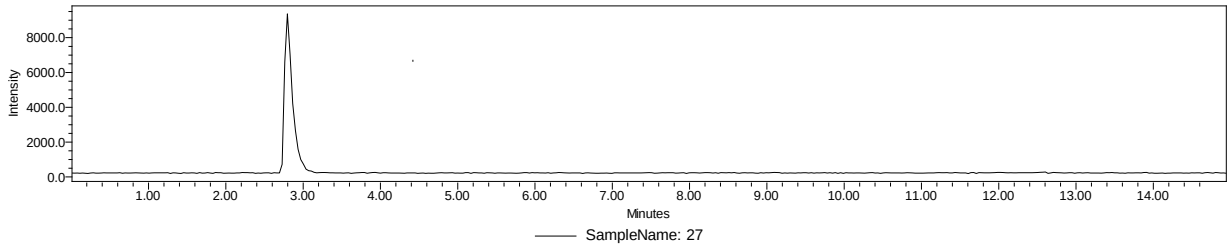

Fig. 26(45)Thermal/Alkali product chromatograph screening for 5-FU @ 3 day 1st replicate.

28

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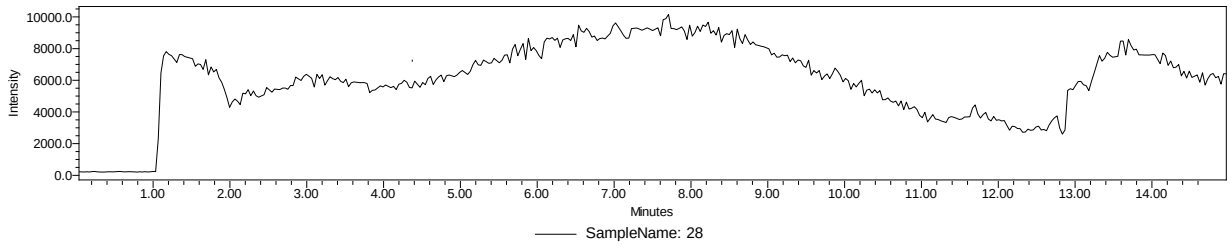

Fig. 26(46)Thermal/Alkali product chromatograph screening for FAC @ 3 day 2nd replicate.

105

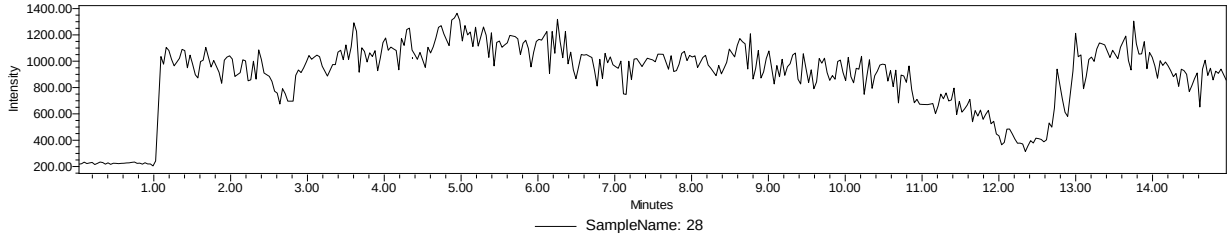

Fig. 26(47)Thermal/Alkali product chromatograph screening for FMASld @ 3 day 2nd replicate.

106

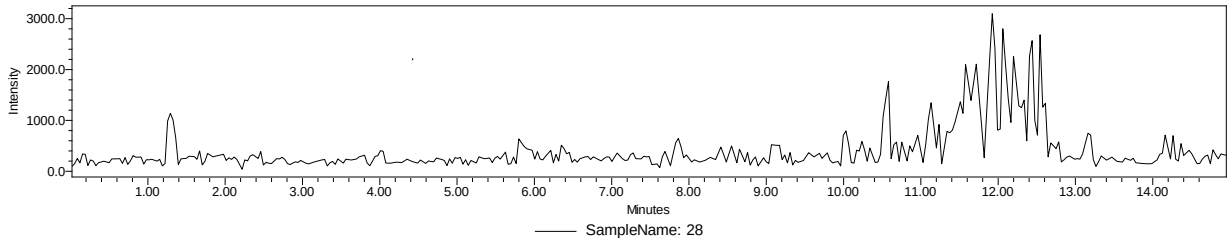

Fig. 26(48)Thermal/Alkali product chromatograph screening for FBAL @ 3 day 2nd replicate.

149

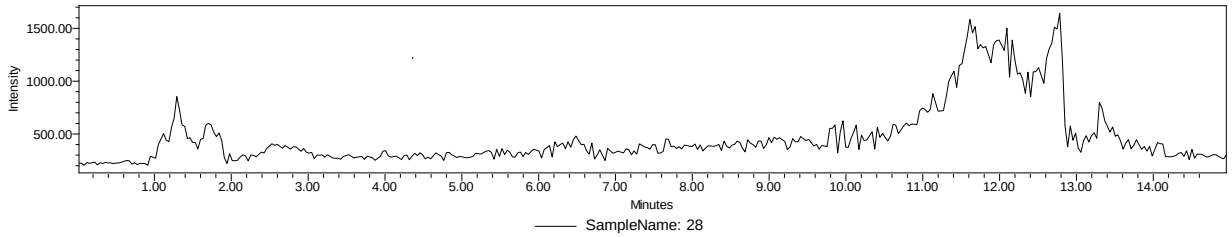

Fig. 26(49)Thermal/Alkali product chromatograph screening for FUPA @ 3 day 2nd replicate.

129

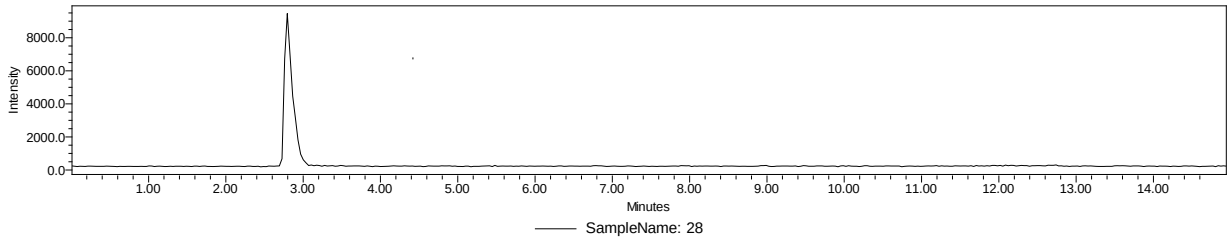

Fig. 26(50)Thermal/Alkali product chromatograph screening for 5-FU @ 3 day 2nd replicate.

29

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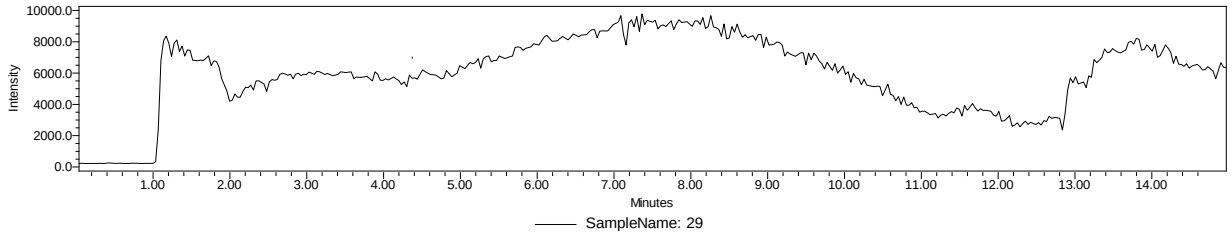

Fig. 26(51)Thermal/Alkali product chromatograph screening for FAC @ 3 day 3rd replicate.

105

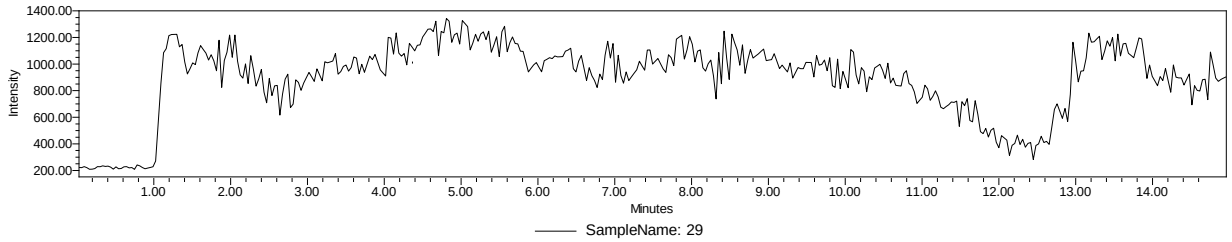

Fig. 26(52)Thermal/Alkali product chromatograph screening for FMASld @ 3 day 3rd replicate.

106

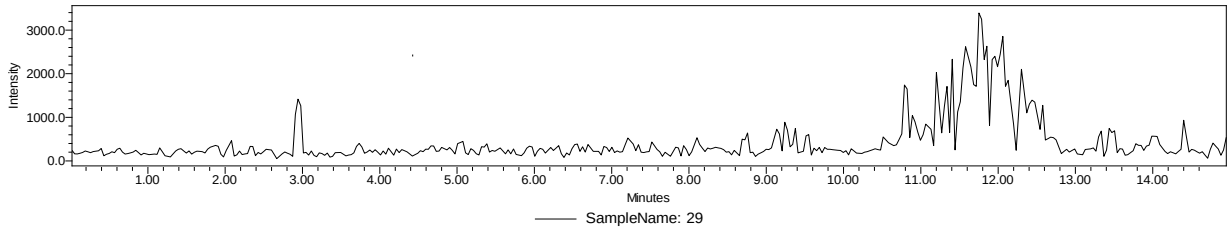

Fig. 26(53)Thermal/Alkali product chromatograph screening for FBAL @ 3 day 3rd replicate.

149

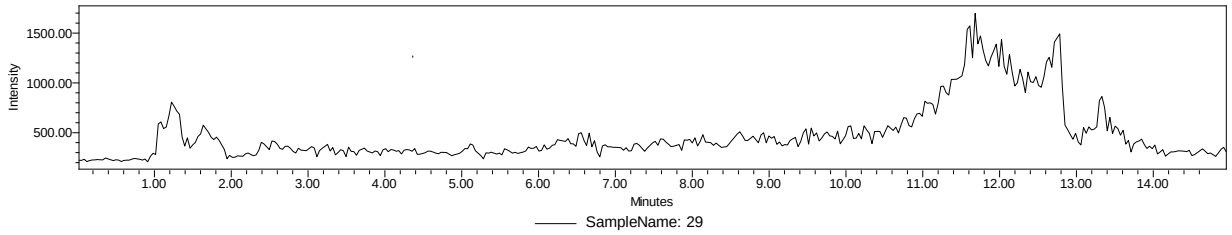

Fig. 26(54)Thermal/Alkali product chromatograph screening for FUPA @ 3 day 3rd replicate.

129

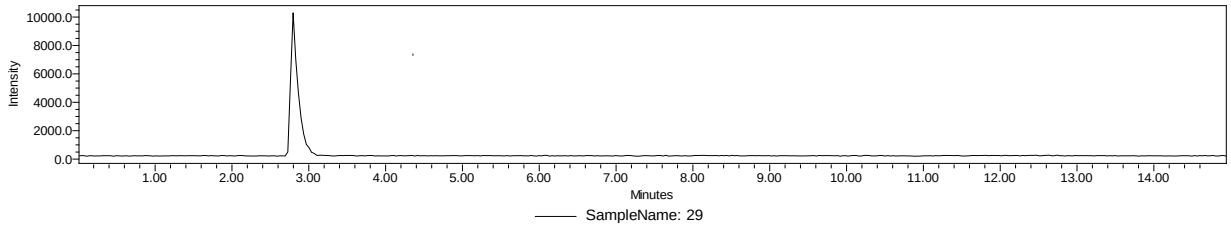

Fig. 26(55)Thermal/Alkali product chromatograph screening for 5-FU @ 3 day 3rd replicate.

30

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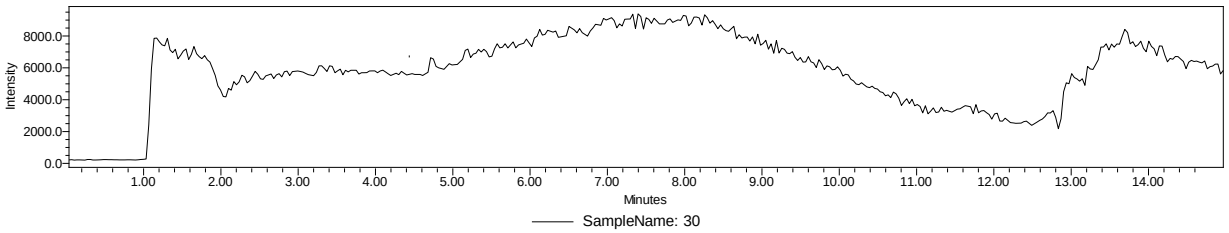

Fig. 26(56)Thermal/Alkali product chromatograph screening for FAC @ 4 day 1st replicate.

105

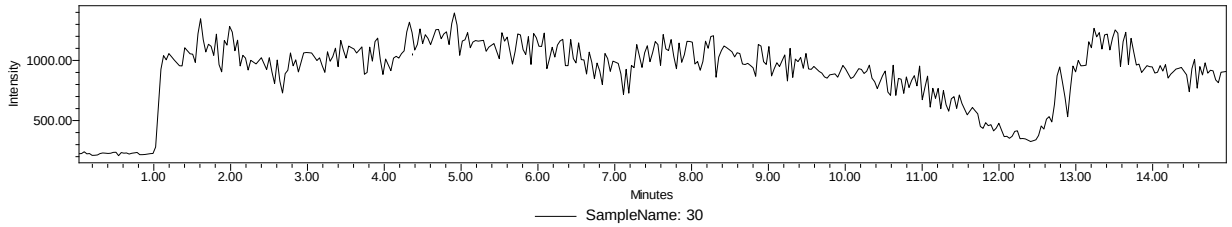

Fig. 26(57)Thermal/Alkali product chromatograph screening for FMASld @ 4 day 1st replica

106

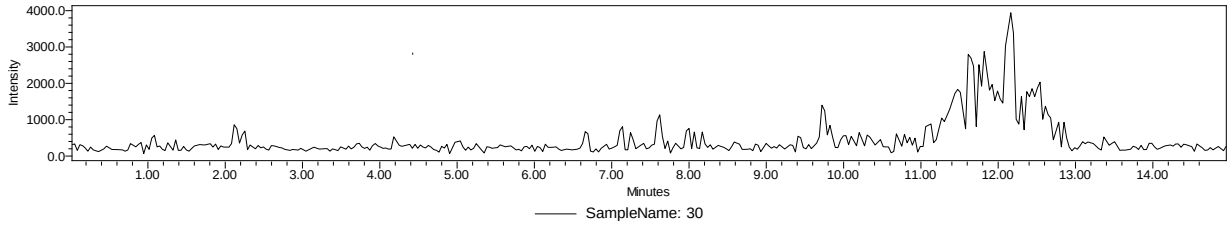

Fig. 26(58)Thermal/Alkali product chromatograph screening for FBAL @ 4 day 1st replicate.

149

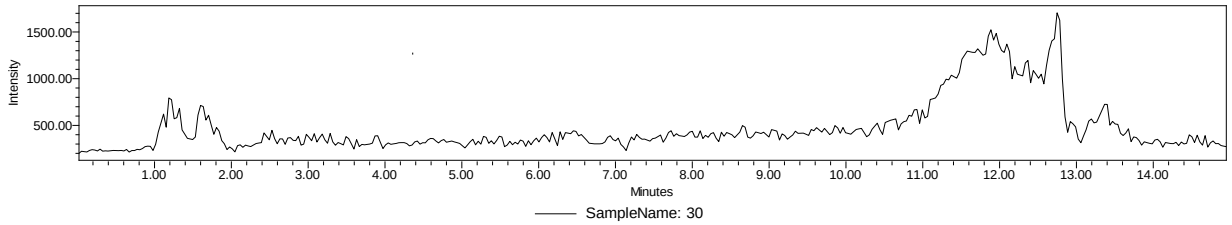

Fig. 26(59)Thermal/Alkali product chromatograph screening for FUPA @ 4 day 1st replicate.

129

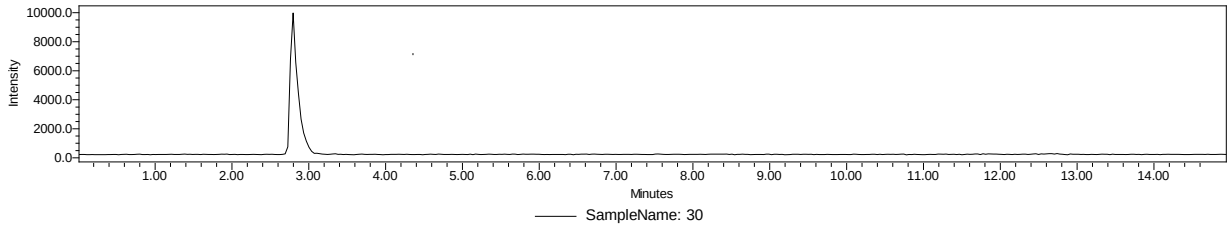

Fig. 26(60)Thermal/Alkali product chromatograph screening for 5-FU @ 4 day 1st replicate.

31

77

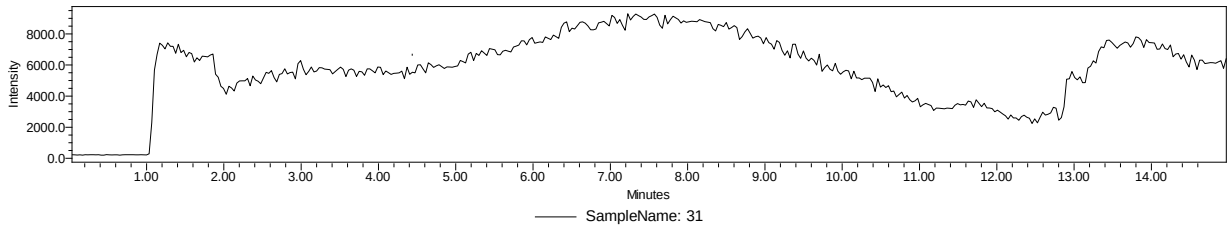

Fig. 26(61)Thermal/Alkali product chromatograph screening for FAC @ 4 day 2nd replicate.

105

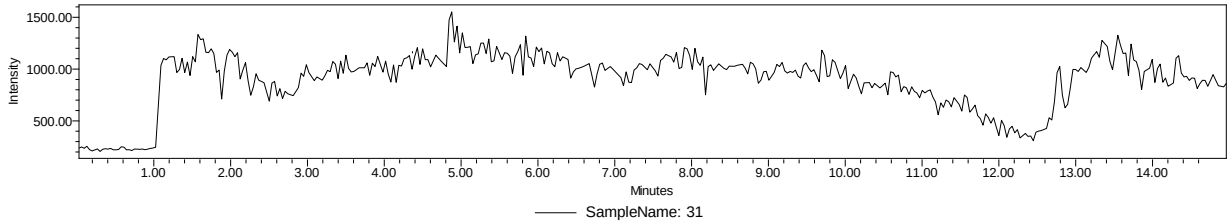

Fig. 26(62)Thermal/Alkali product chromatograph screening for FMASld @ 4 day 2nd replicate.

106

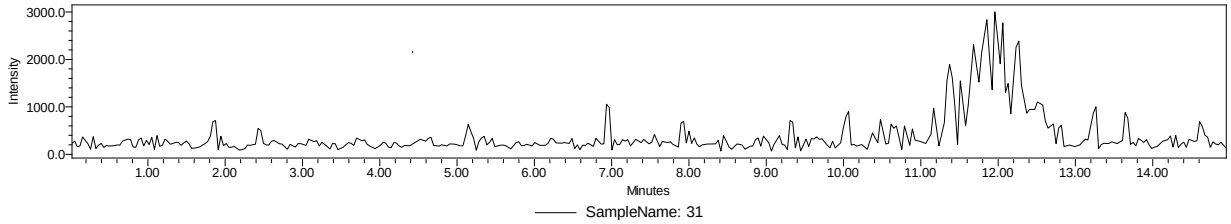

Fig. 26(63)Thermal/Alkali product chromatograph screening for FBAL @ 4 day 2nd replicate.

149

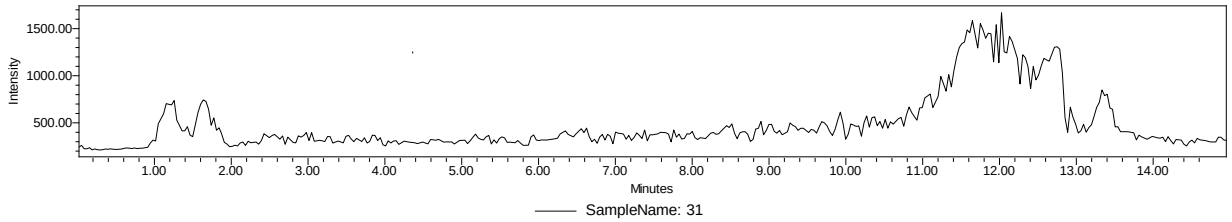

Fig. 26(64)Thermal/Alkali product chromatograph screening for FUPA @ 4 day 2nd replicate.

129

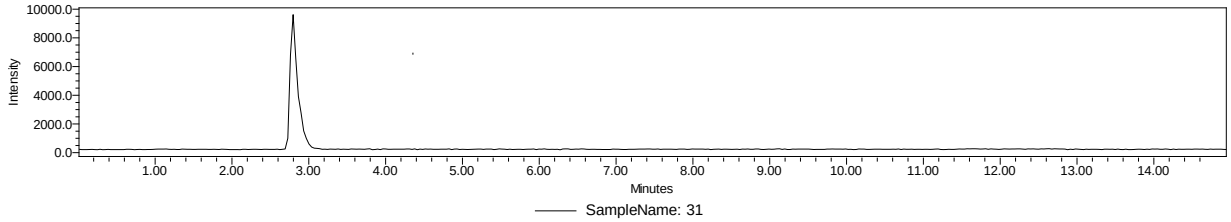

Fig. 26(65)Thermal/Alkali product chromatograph screening for 5-FU @ 4 day 2nd replicate.

32

77

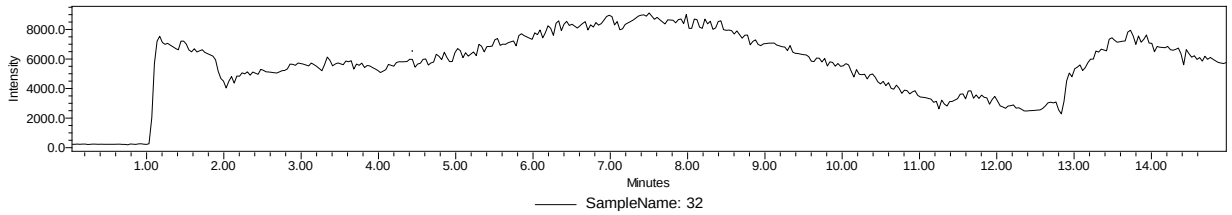

Fig. 26(66)Thermal/Alkali product chromatograph screening for FAC @ 4 day 3rd replicate.

105

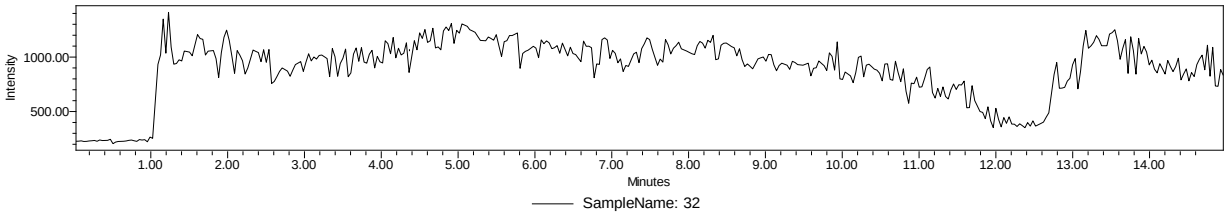

Fig. 26(67)Thermal/Alkali product chromatograph screening for FMASld @ 4 day 3rd replicate.

106

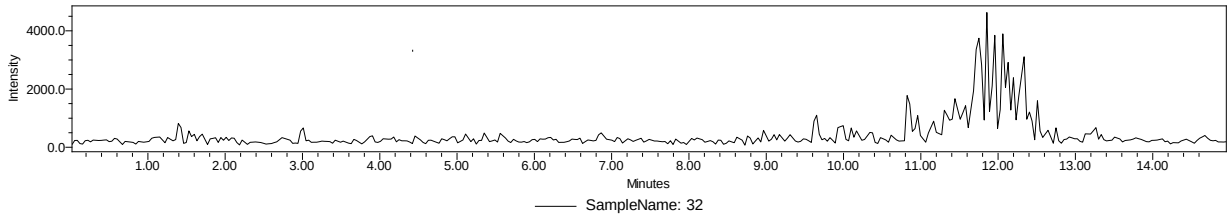

Fig. 26(68)Thermal/Alkali product chromatograph screening for FBAL @ 4 day 3rd replicate.

149

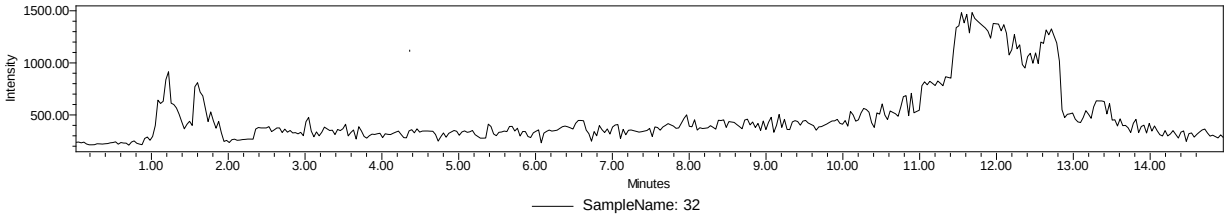

Fig. 26(69)Thermal/Alkali product chromatograph screening for FUPA @ 4 day 3rd replicate.

129

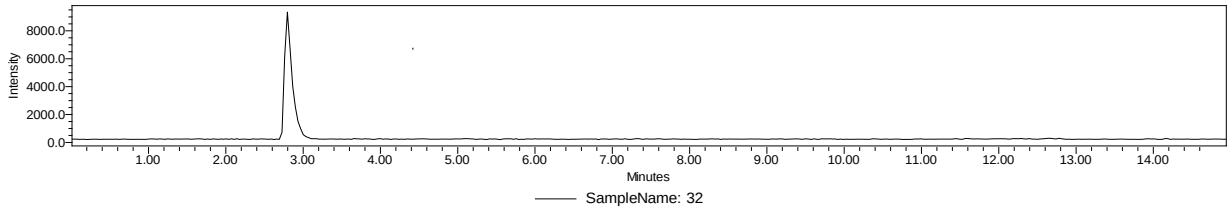

Fig. 26(70)Thermal/Alkali product chromatograph screening for 5-FU @ 4 day 3rd replicate.

33

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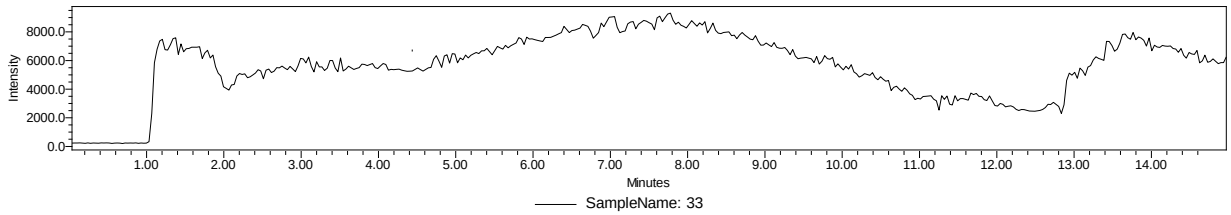

Fig. 26(71)Thermal/Alkali product chromatograph screening for FAC @ 5 day 1st replicate.

105

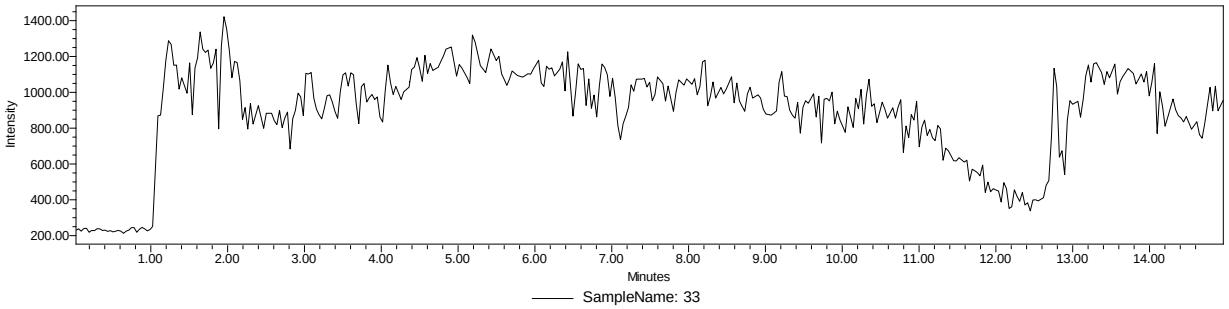

Fig. 26(72)Thermal/Alkali product chromatograph screening for FMASld @ 5 day 1st replicat

106

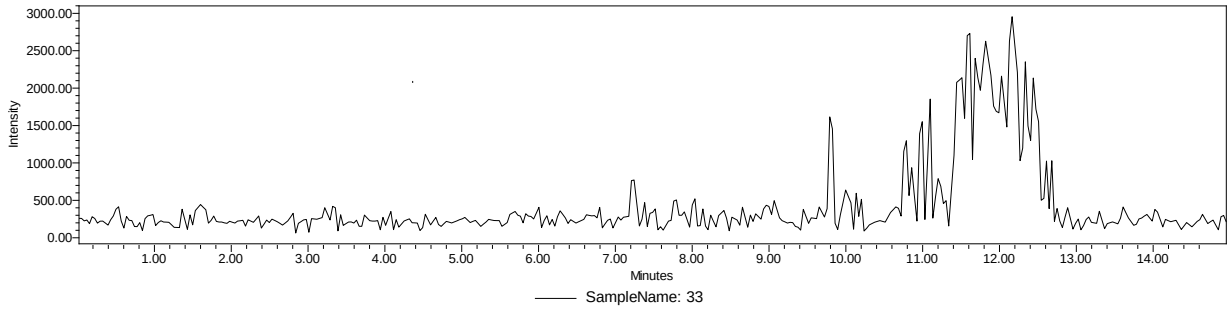

Fig. 26(73)Thermal/Alkali product chromatograph screening for FBAL @ 5 day 1st replicate.

149

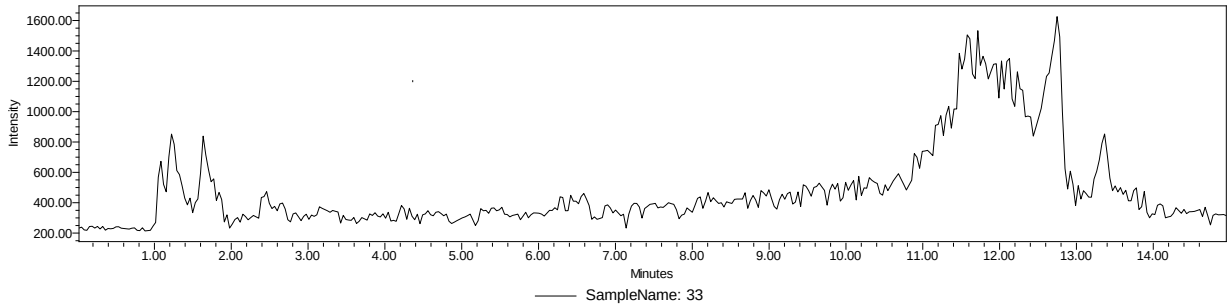

Fig. 26(74)Thermal/Alkali product chromatograph screening for FUPA @ 5 day 1st replicate.

129

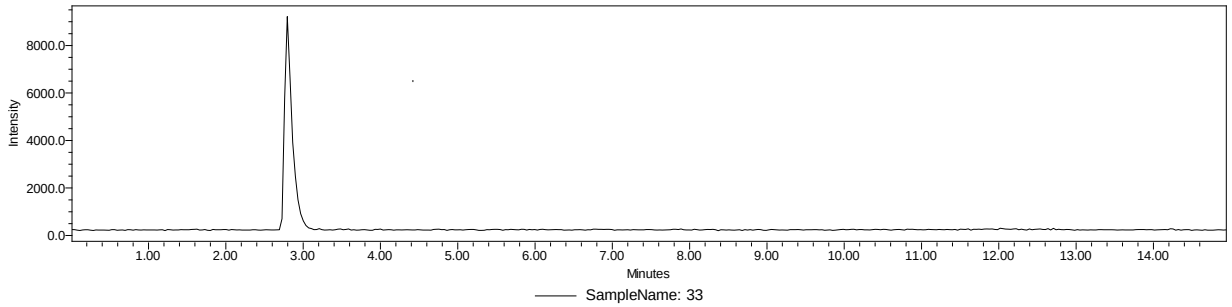

Fig. 26(75)Thermal/Alkali product chromatograph screening for 5-FU @ 5 day 1st replicate.

34

77

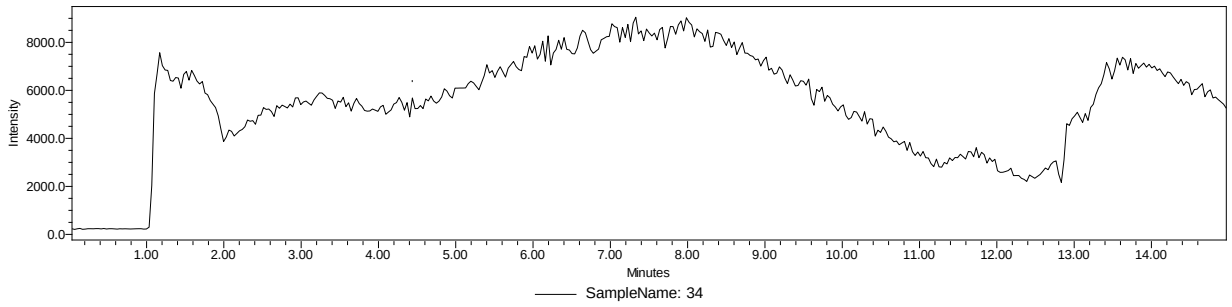

Fig. 26(76)Thermal/Alkali product chromatograph screening for FAC @ 5 day 2nd replicate.

105

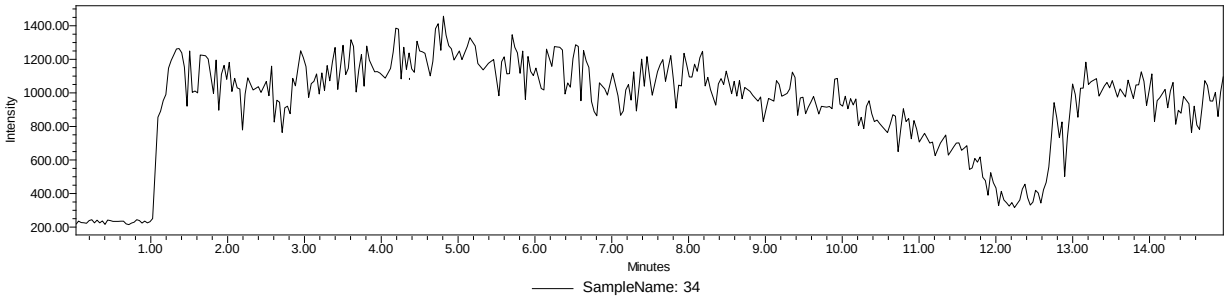

Fig. 26(77)Thermal/Alkali product chromatograph screening for FMASld @ 5 day 2nd replicate

106

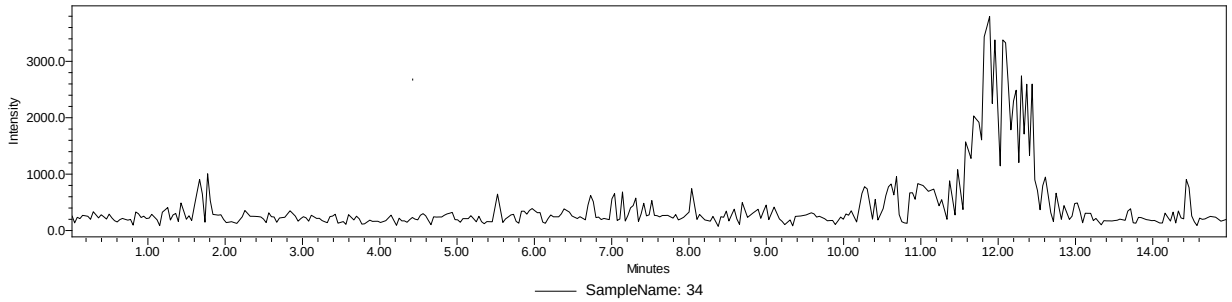

Fig. 26(78)Thermal/Alkali product chromatograph screening for FBAL @ 5 day 2nd replicate.

149

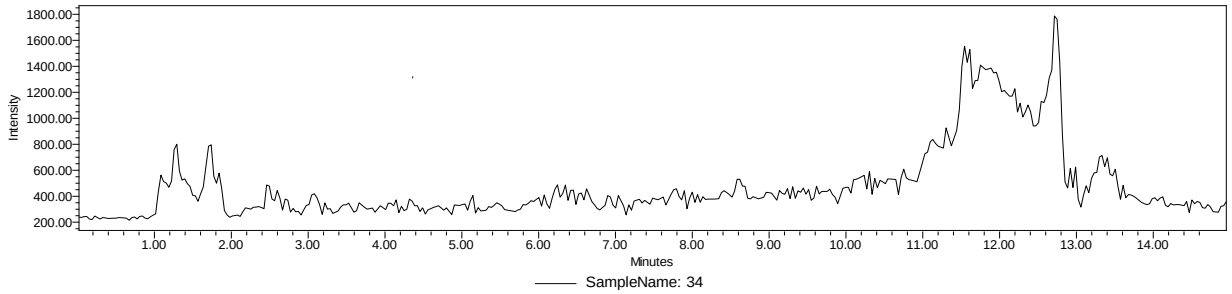

Fig. 26(79)Thermal/Alkali product chromatograph screening for FUPA @ 5 day 2nd replicate.

129

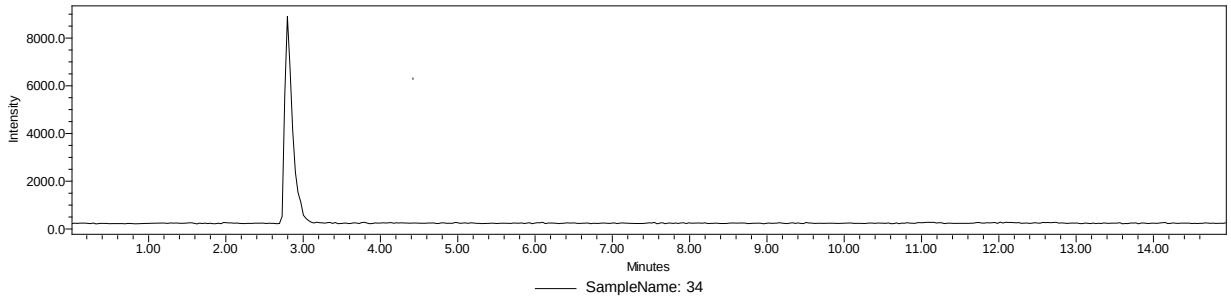

Fig. 26(80)Thermal/Alkali product chromatograph screening for 5-FU @ 5 day 2nd replicate.

35

77

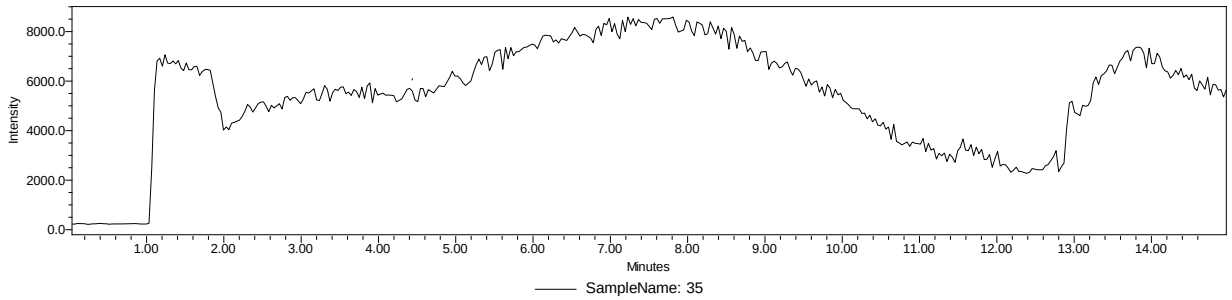

Fig. 26(81)Thermal/Alkali product chromatograph screening for FAC @ 5 day 3rd replicate.

105

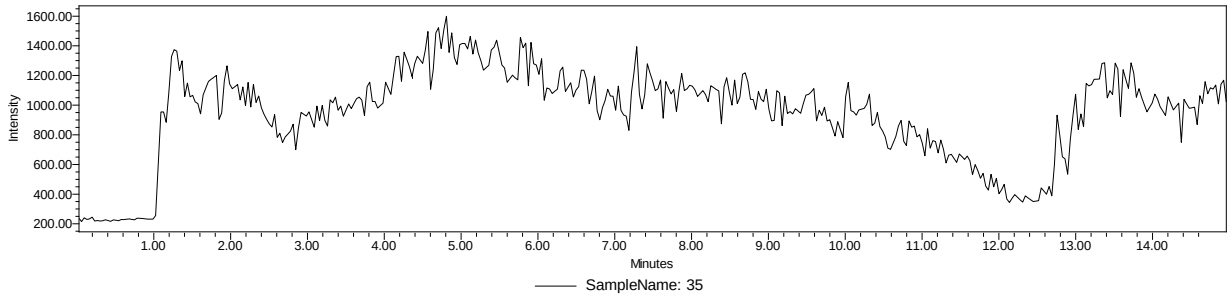

Fig. 26(82)Thermal/Alkali product chromatograph screening for FMASld @ 5 day 3rd replicate.

106

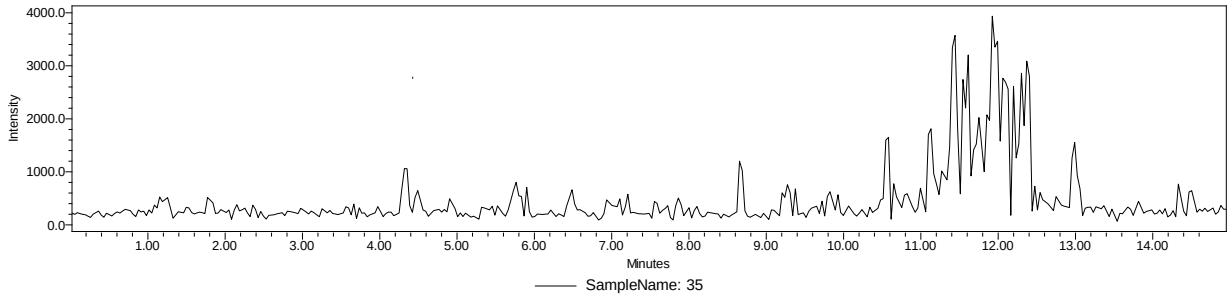

Fig. 26(83)Thermal/Alkali product chromatograph screening for FBAL @ 5 day 3rd replicate.

149

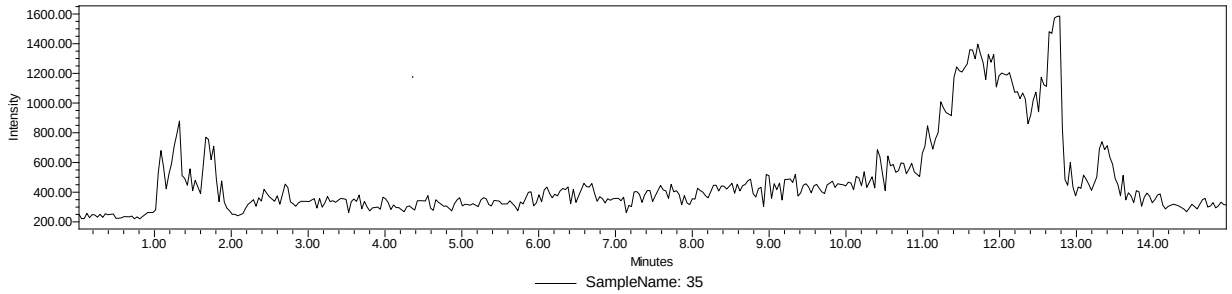

Fig. 26(84)Thermal/Alkali product chromatograph screening for FUPA @ 5 day 3rd replicate.

129

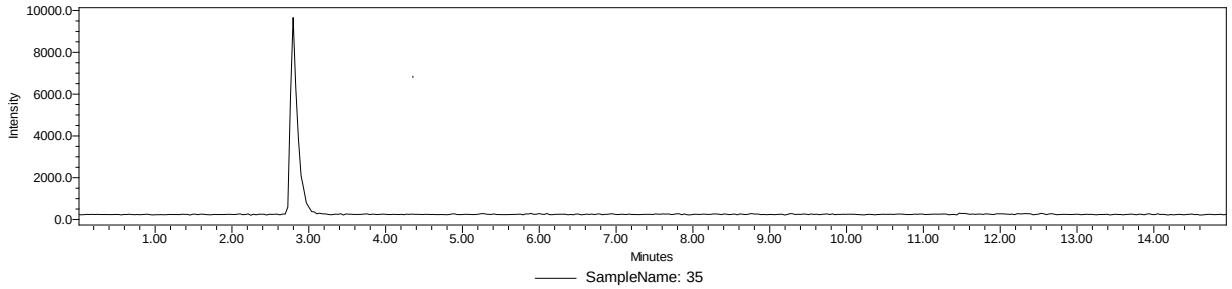

Fig. 26(85)Thermal/Alkali product chromatograph screening for 5-FU @ 5 day 3rd replicate.

36

77

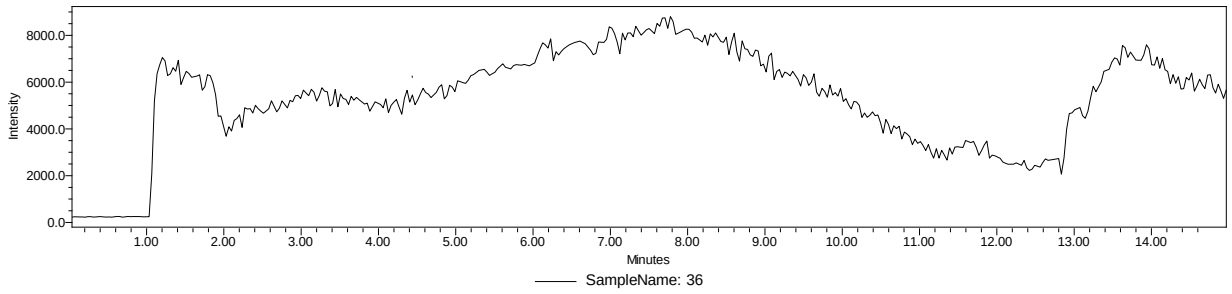

Fig. 26(86)Thermal/Alkali product chromatograph screening for FAC @ 6 day 1st replicate.

105

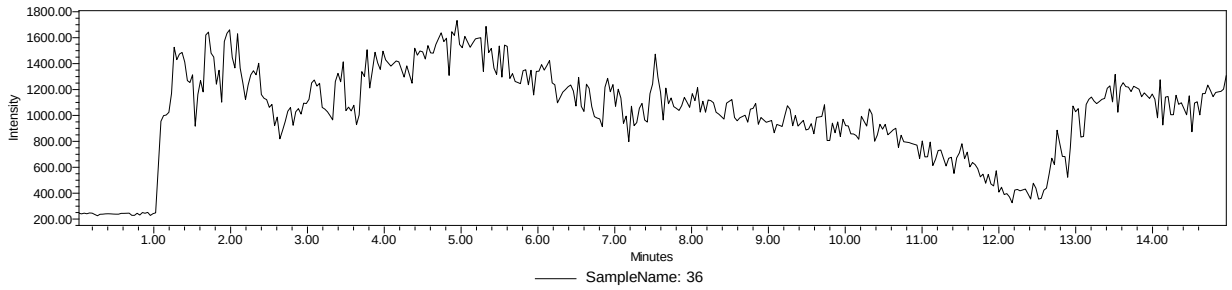

Fig. 26(87)Thermal/Alkali product chromatograph screening for FMASld @ 6 day 1st replicate.

106

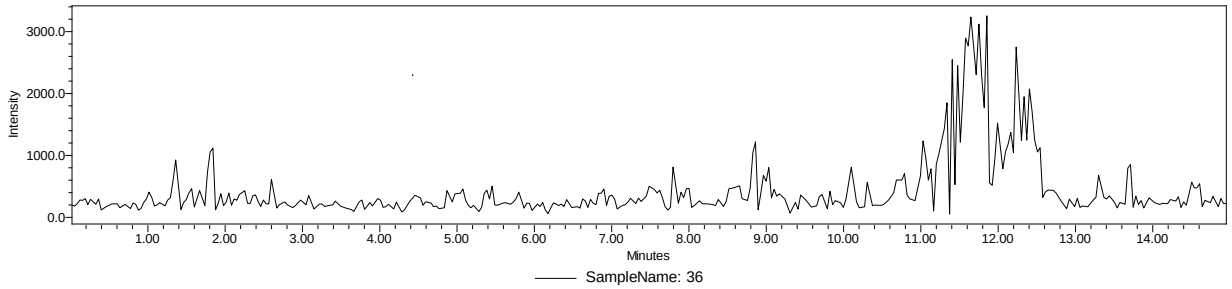

Fig. 26(88)Thermal/Alkali product chromatograph screening for FBAL @ 6 day 1st replicate.

149

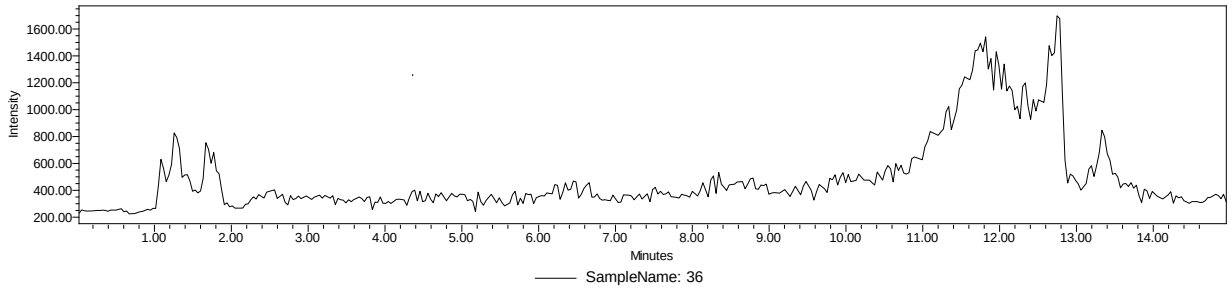

Fig. 26(89)Thermal/Alkali product chromatograph screening for FUPA @ 6 day 1st replicate

129

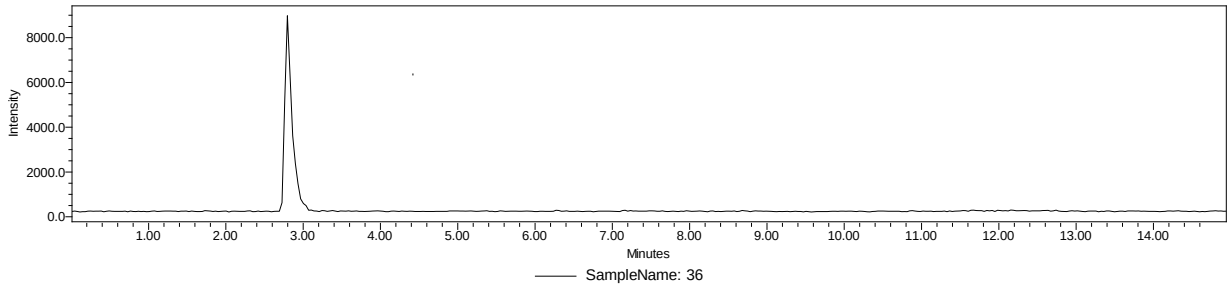

Fig. 26(90)Thermal/Alkali product chromatograph screening for 5-FU @ 6 day 1st replicate.

37

77

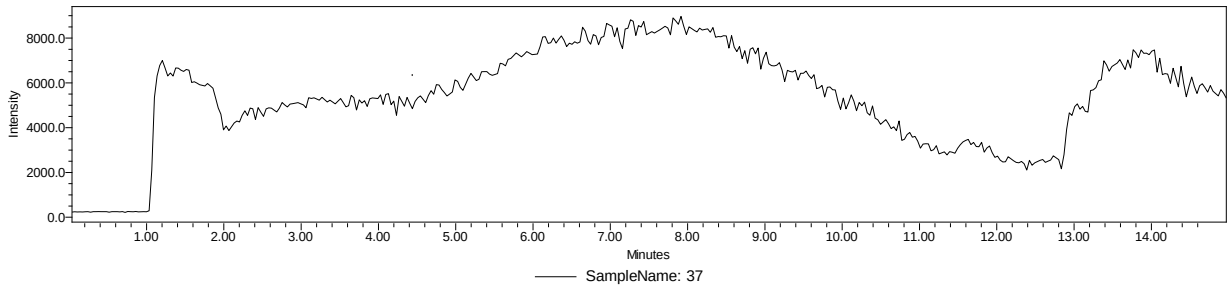

Fig. 26(91)Thermal/Alkali product chromatograph screening for FAC @ 6 day 2nd replicate.

105

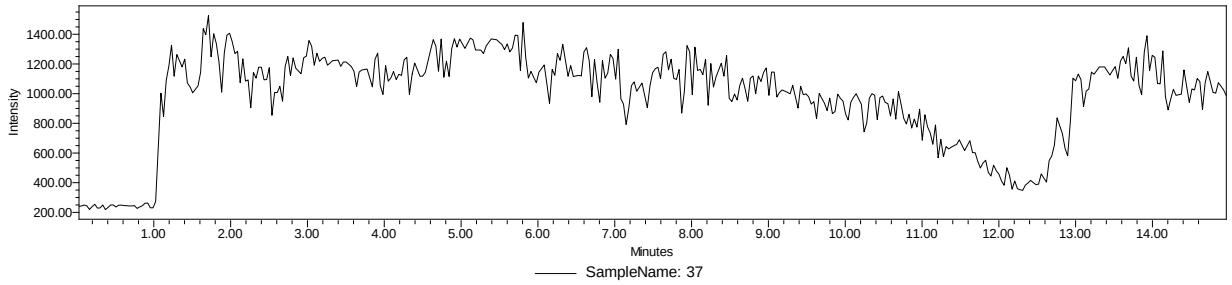

Fig. 26(92)Thermal/Alkali product chromatograph screening for FMASld @ 6 day 2nd replicate.

106

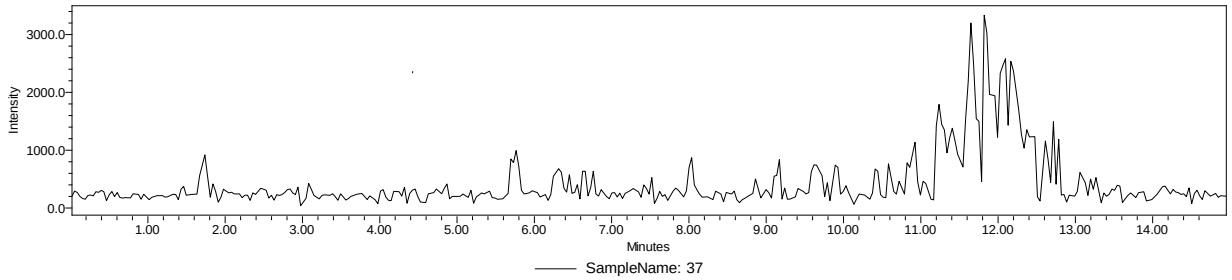

Fig. 26(93)Thermal/Alkali product chromatograph screening for FBAL @ 6 day 2nd replicate.

149

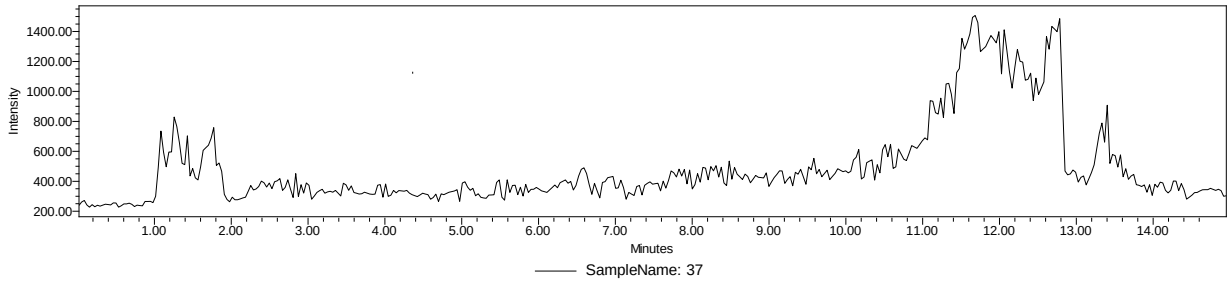

Fig. 26(94)Thermal/Alkali product chromatograph screening for FUPA @ 6 day 2nd replicate.

129

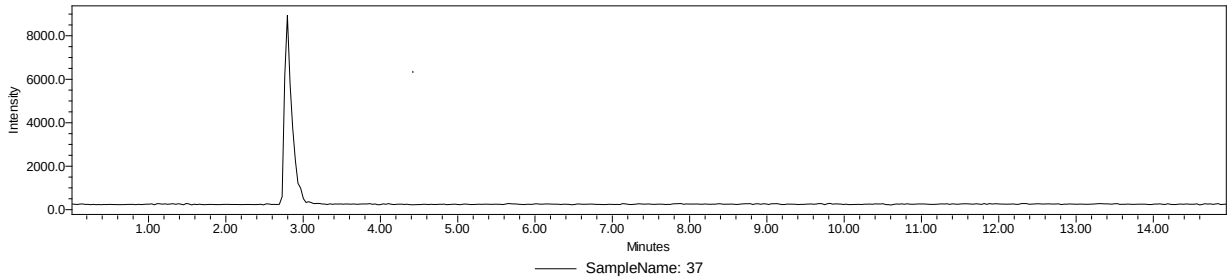

Fig. 26(95)Thermal/Alkali product chromatograph screening for 5-FU @ 6 day 2nd replicate.

38

77

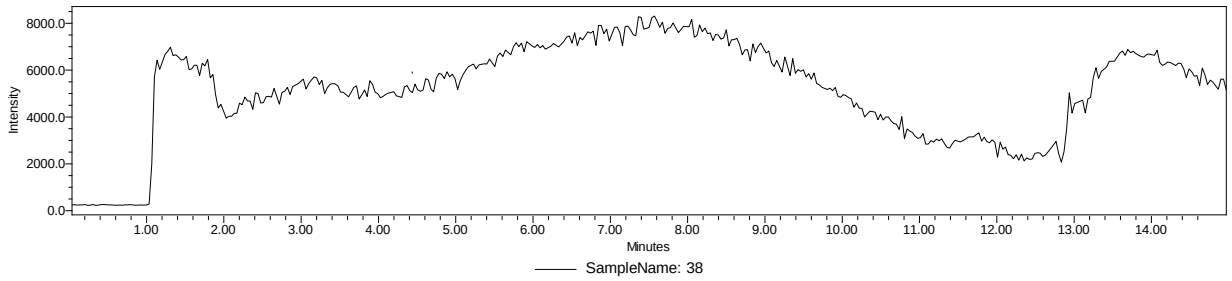

Fig. 26(96)Thermal/Alkali product chromatograph screening for FAC @ 6 day 3rd replicate.

105

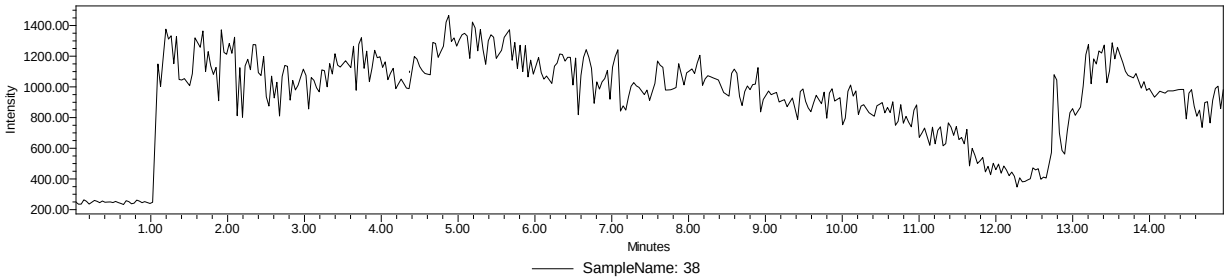

Fig. 26(97)Thermal/Alkali product chromatograph screening for FMASld @ 6 day 3rd replicate

106

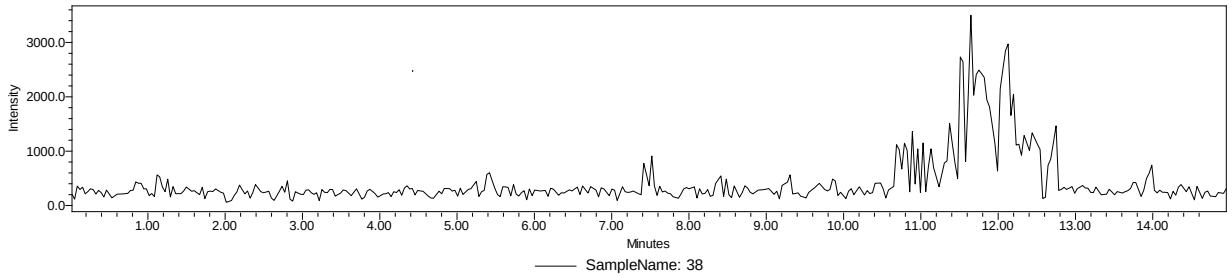

Fig. 26(98)Thermal/Alkali product chromatograph screening for FBAL @ 6 day 3rd replicate.

149

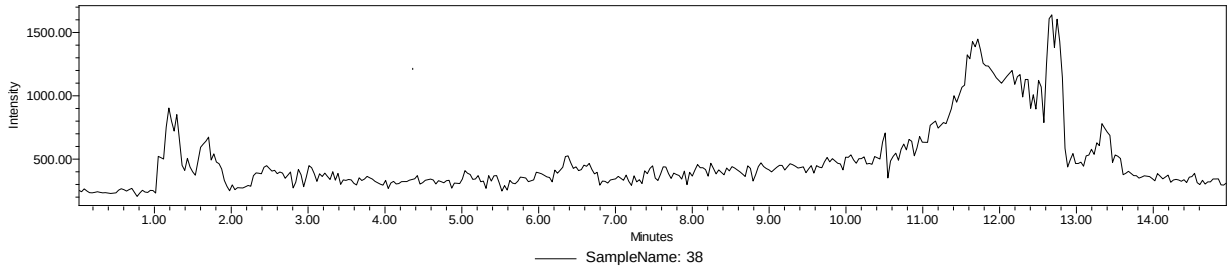

Fig. 26(99)Thermal/Alkali product chromatograph screening for FUPA @ 6 day 3rd replicate.

129

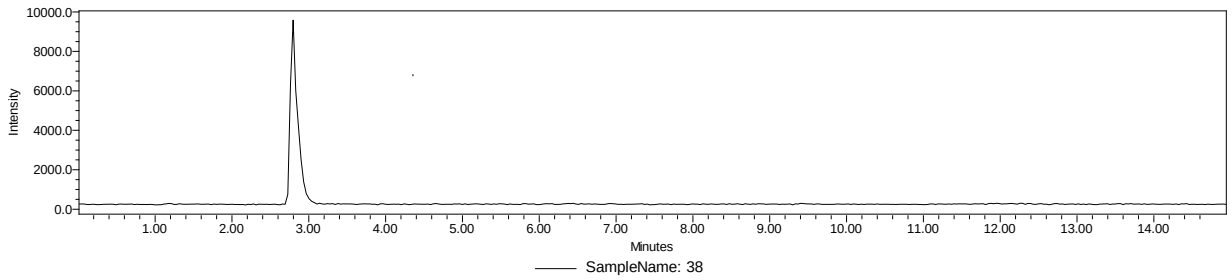

Fig. 26(100)Thermal/Alkali product chromatograph screening for 5-FU @ 6 day 3rd replicate.

39

77

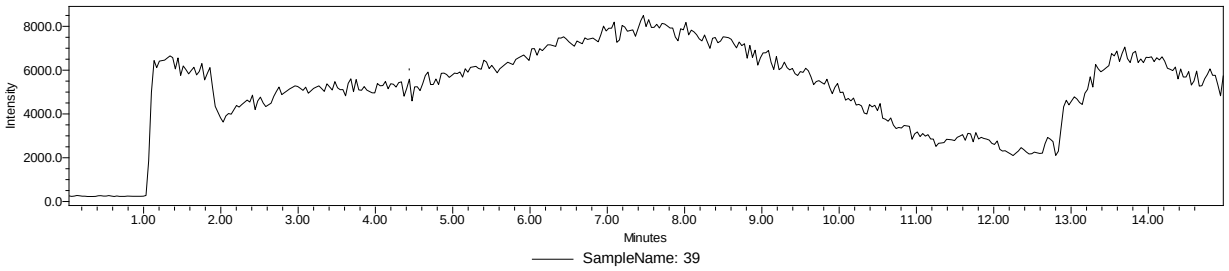

Fig. 26(101)Thermal/Alkali product chromatograph screening for FAC @ 7 day 1st replicate.

105

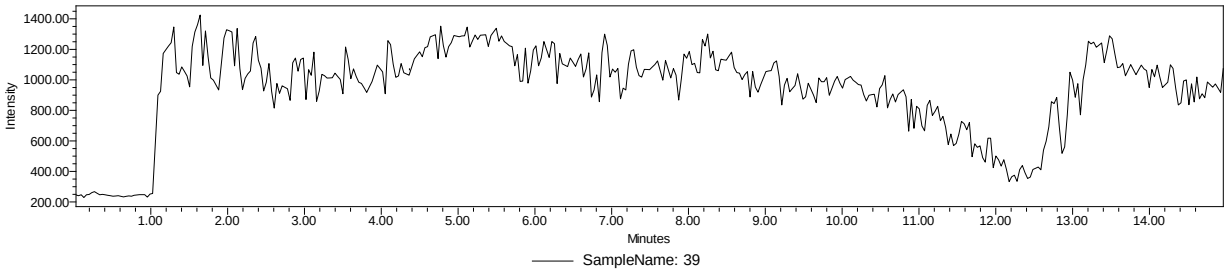

Fig. 26(102)Thermal/Alkali product chromatograph screening for FMASld @ 7 day 1st replicate.

106

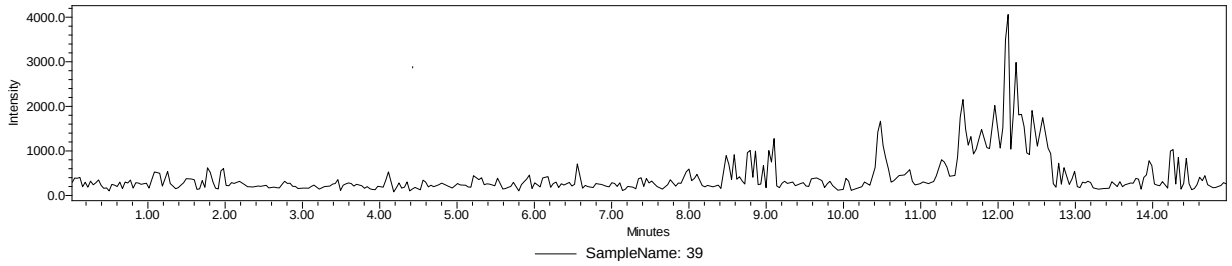

Fig. 26(103)Thermal/Alkali product chromatograph screening for FBAL @ 7 day 1st replicate.

149

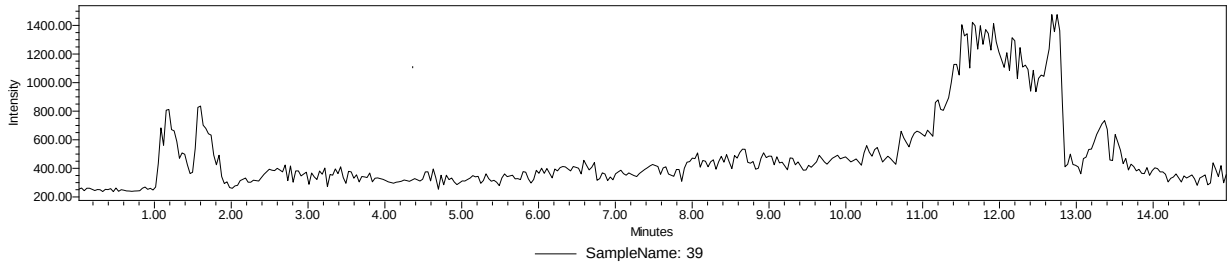

Fig. 26(104)Thermal/Alkali product chromatograph screening for FUPA @ 7 day 1st replicate.

129

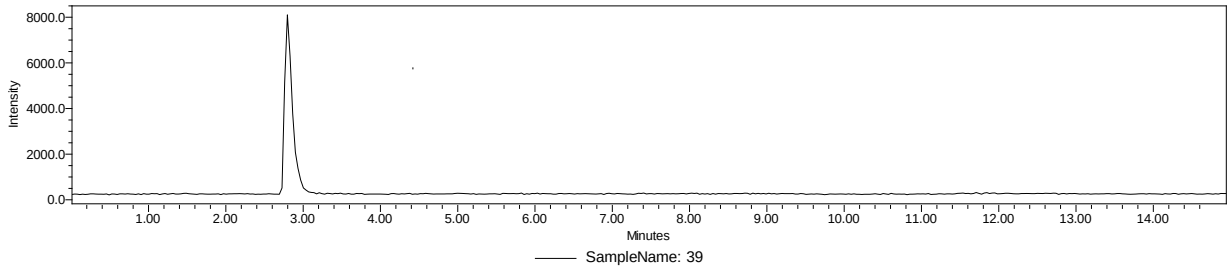

Fig. 26(105)Thermal/Alkali product chromatograph screening for 5-FU @ 7 day 1st replicate.

40

77

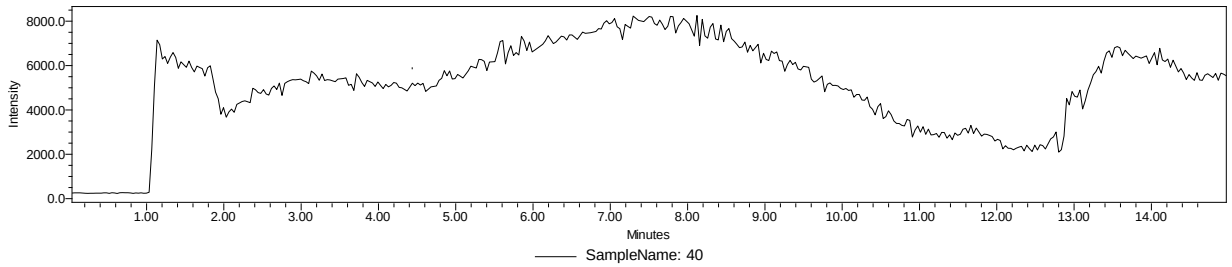

Fig. 26(106)Thermal/Alkali product chromatograph screening for FAC @ 7 day 2nd replicate.

105

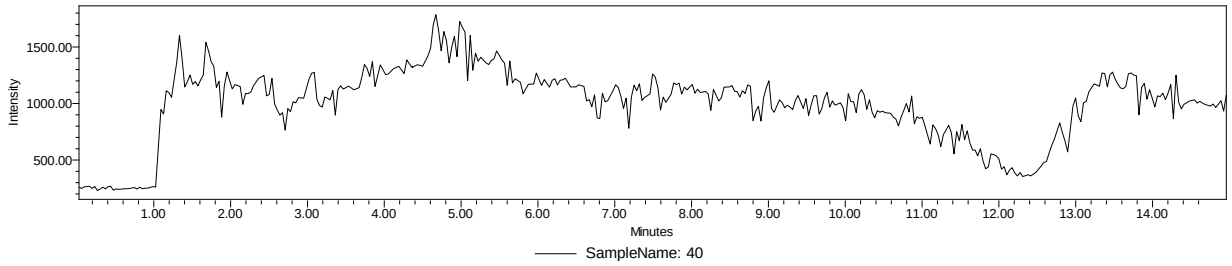

Fig. 26(107)Thermal/Alkali product chromatograph screening for FMASld @ 7 day 2nd replicate.

106

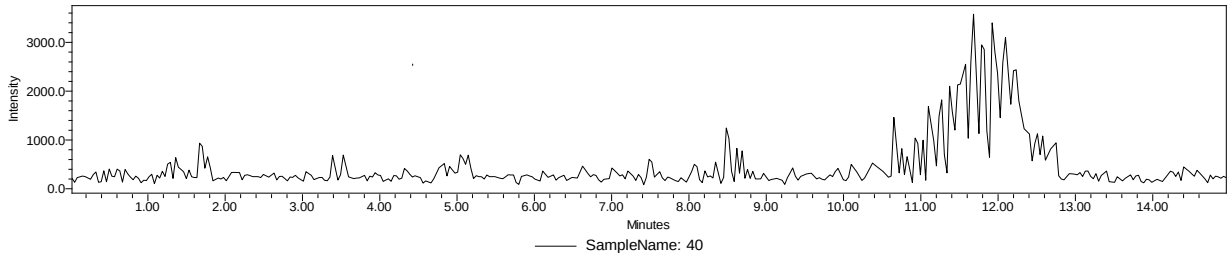

Fig. 26(108)Thermal/Alkali product chromatograph screening for FBAL @ 7 day 2nd replicate.

149

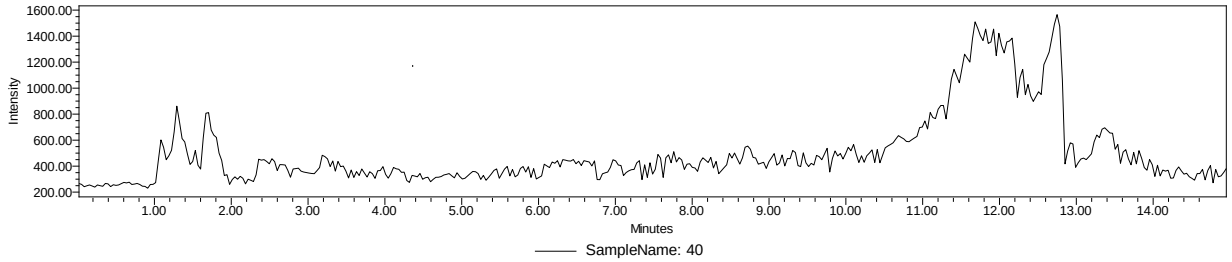

Fig. 26(109)Thermal/Alkali product chromatograph screening for FUPA @ 7 day 2nd replicate.

129

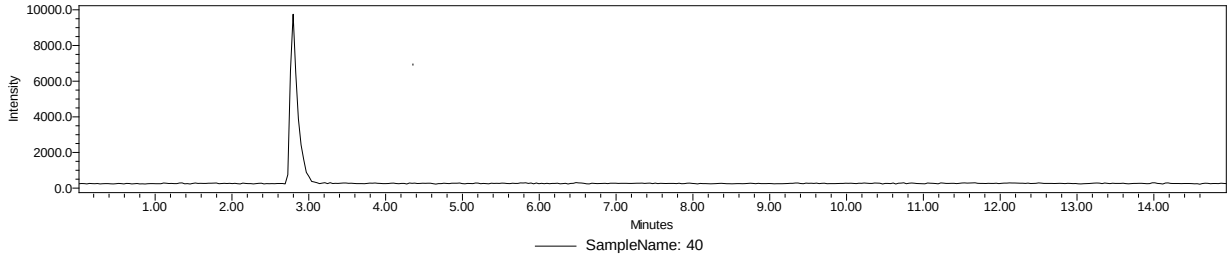

Fig. 26(110)Thermal/Alkali product chromatograph screening for 5-FU @ 7 day 2nd replicate.

41

77

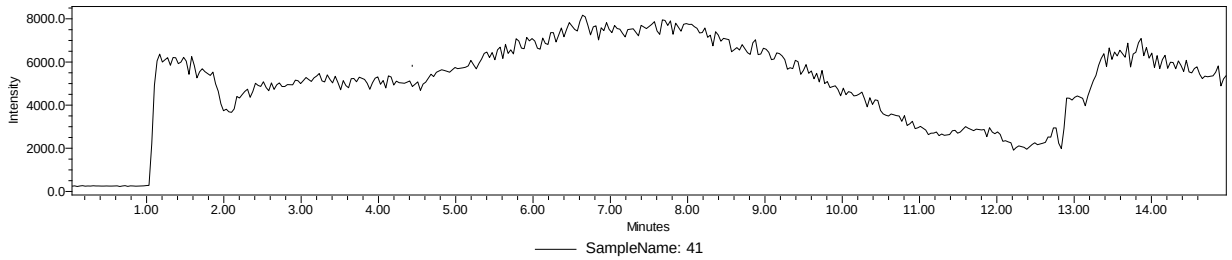

Fig. 26(111)Thermal/Alkali product chromatograph screening for FAC @ 7 day 3rd replicate.

105

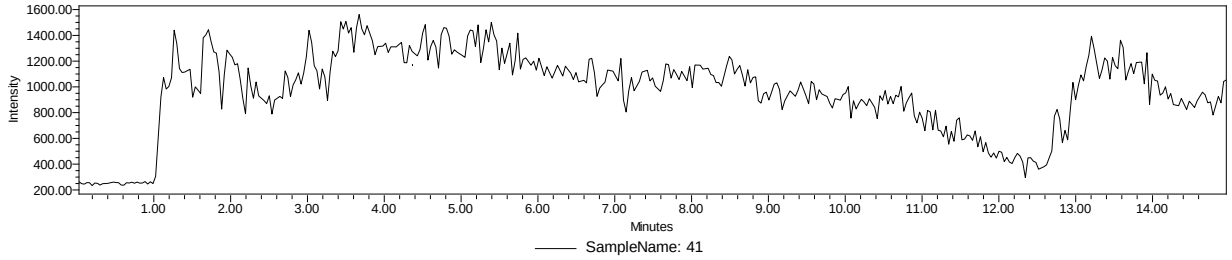

Fig. 26(112)Thermal/Alkali product chromatograph screening for FMASId @ 7 day 3rd replicate.

106

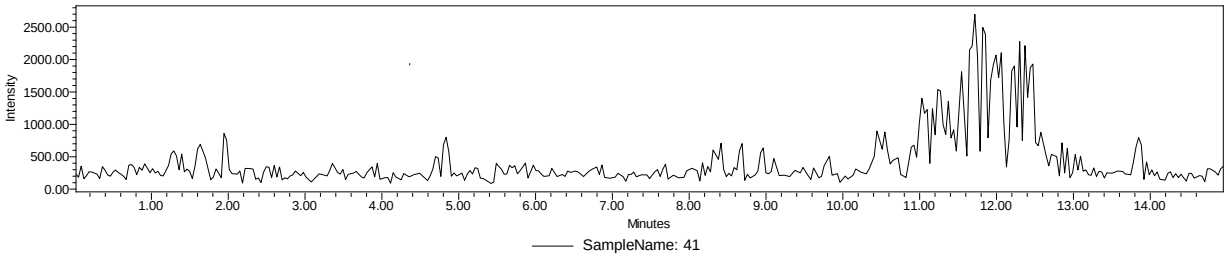

Fig. 26(113)Thermal/Alkali product chromatograph screening for FBAL @ 7 day 3rd replicate.

149

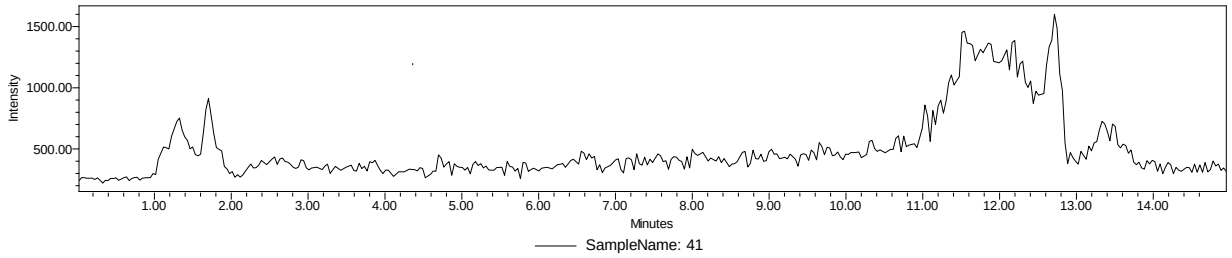

Fig. 26(114)Thermal/Alkali product chromatograph screening for FUPA @ 7 day 3rd replicate.

129

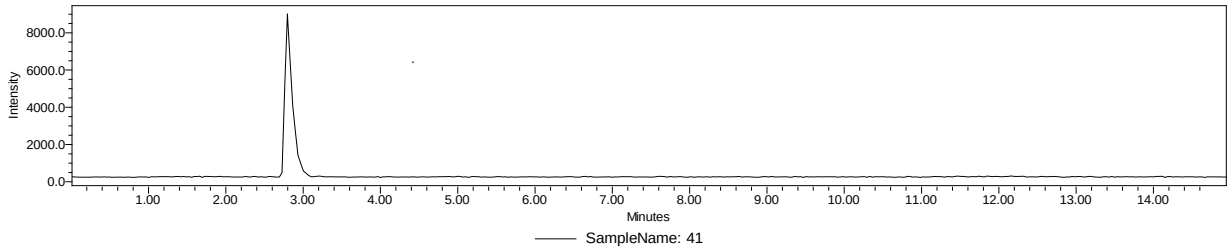

Fig. 26(115)Thermal/Alkali product chromatograph screening for 5-FU @ 7 day 3rd replicate.

1

77

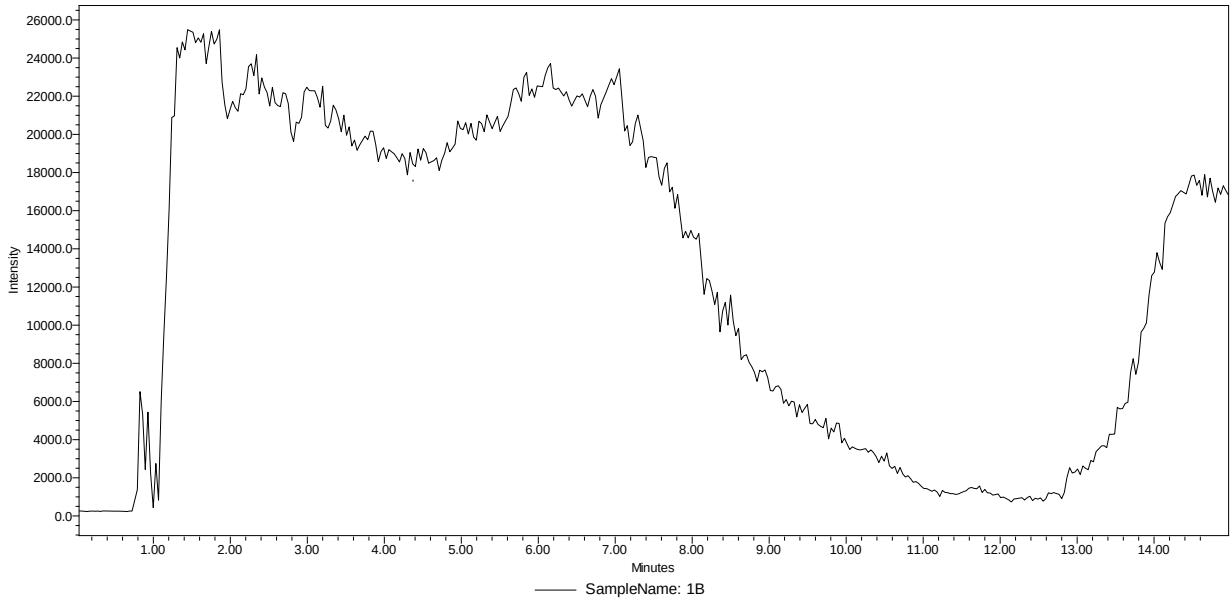

Fig. 27e Thermal/Acidic study's product chromatograph screening for FAC sample 1.

105

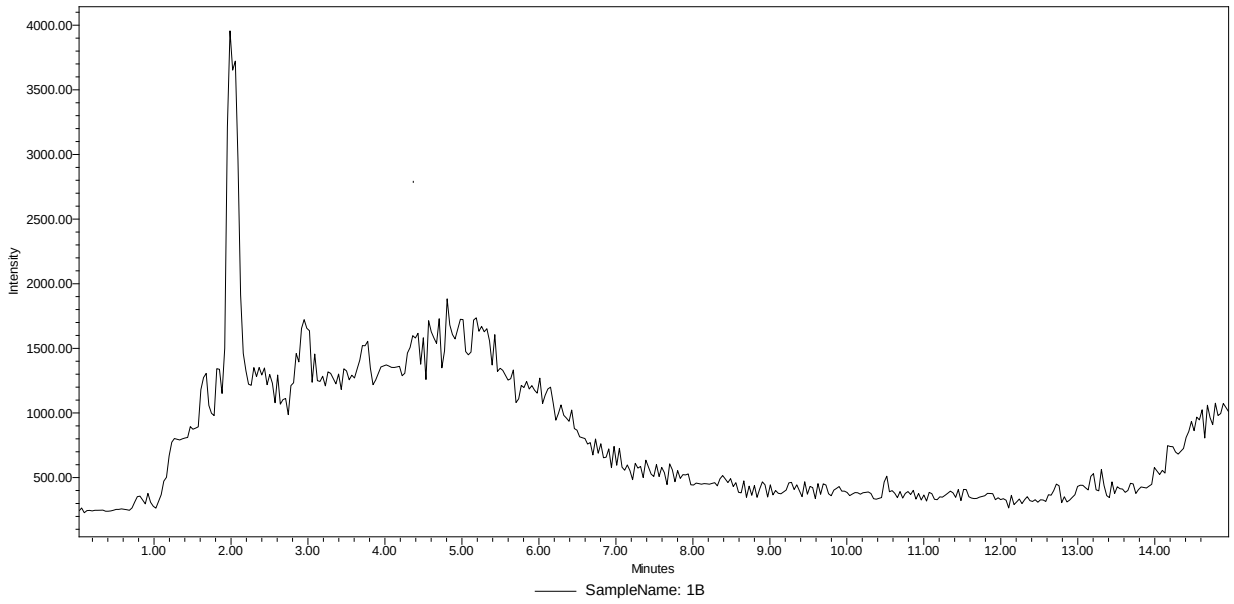

Fig. 27f Thermal/Acidic study's product chromatograph screening for FMASld sample 1.

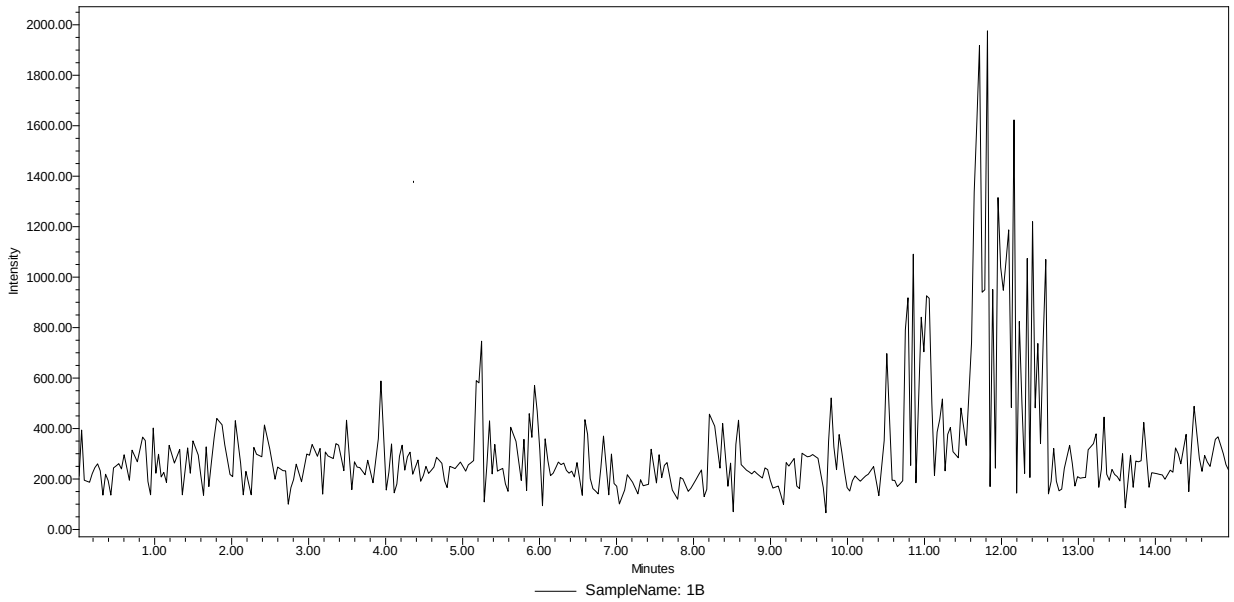

Fig. 27g Thermal/Acidic study's product chromatograph screening for FBAL sample 1.

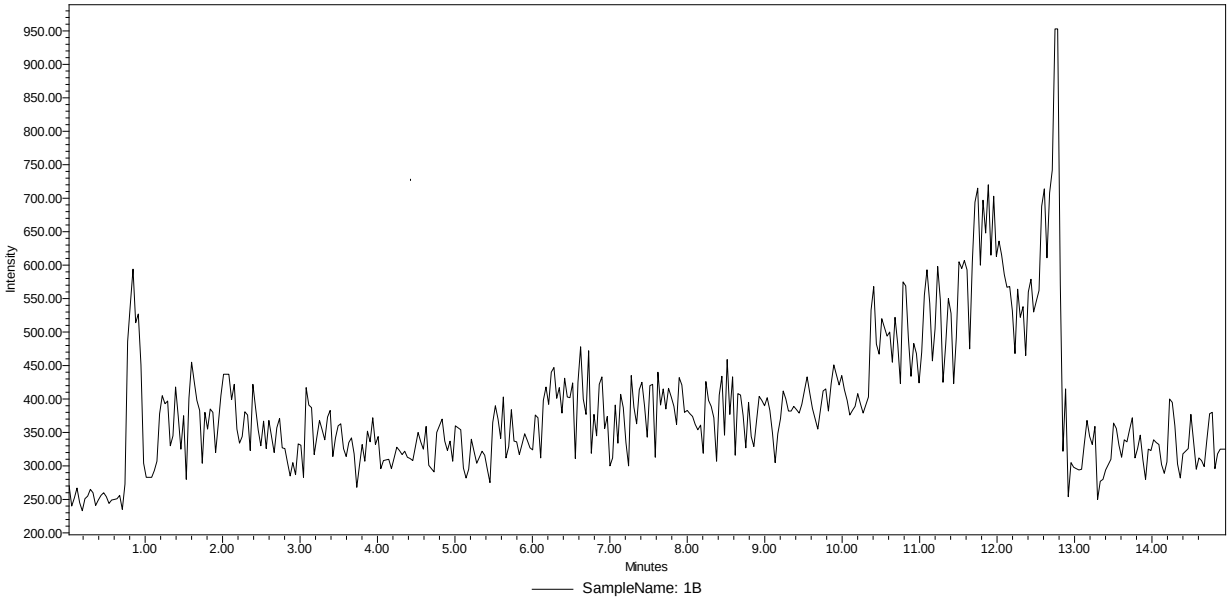

Fig. 27h Thermal/Acidic study's product chromatograph screening for FUPA sample 1.

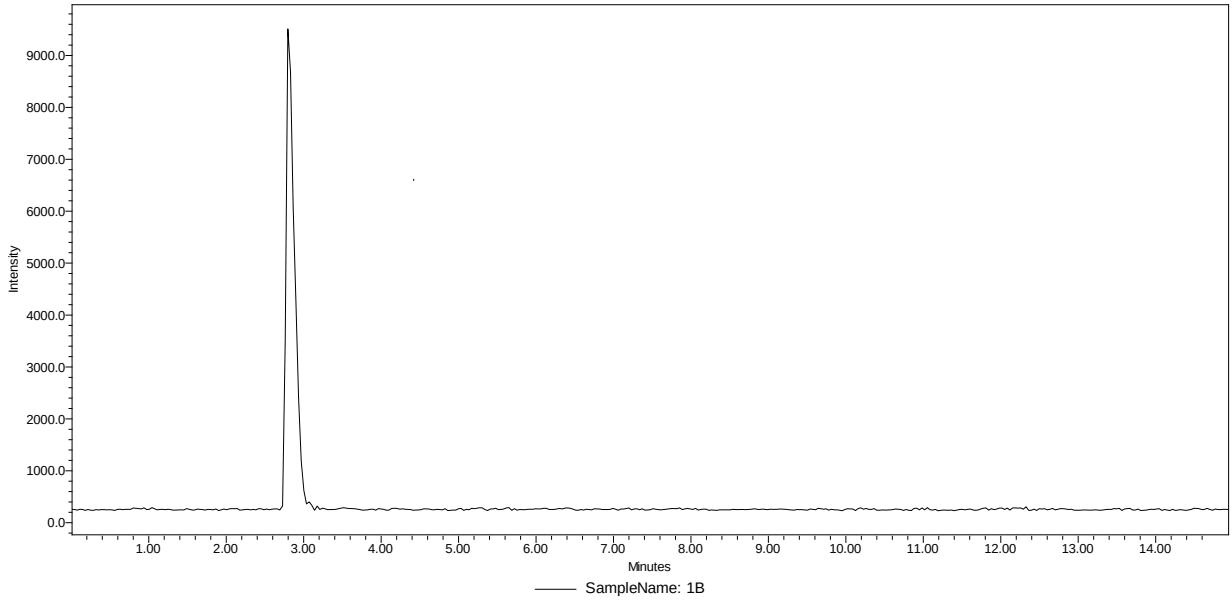

Fig. 27i Thermal/Acidic study's product chromatograph screening for 5-FU sample 1.

2

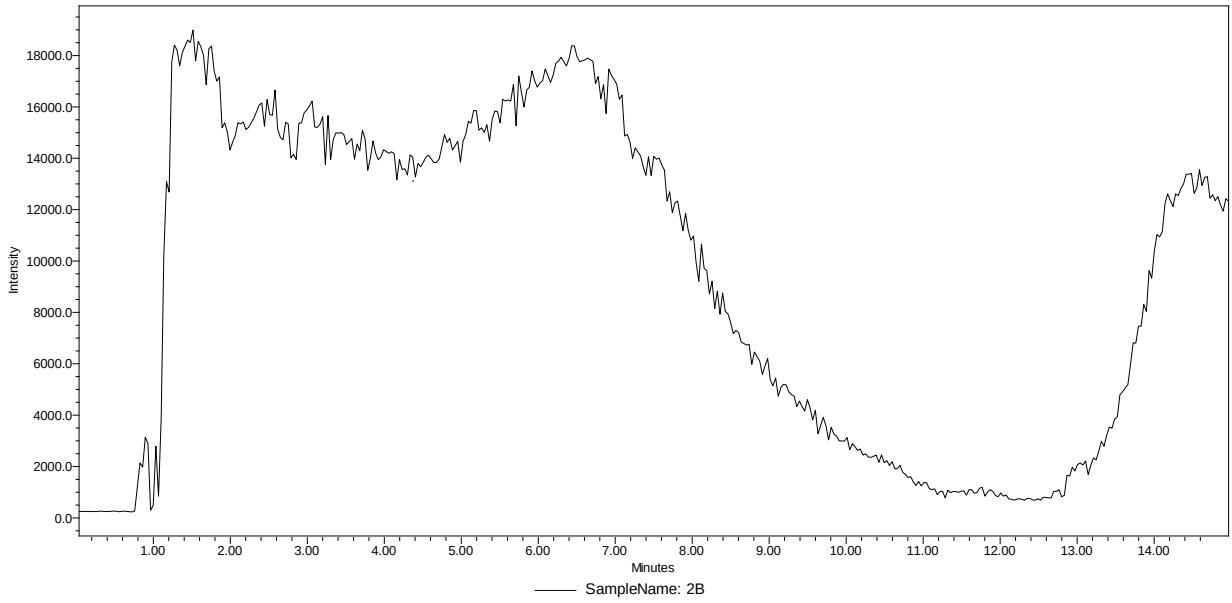

Fig. 27j Thermal/Acidic study's product chromatograph screening for FAC sample 2.

105

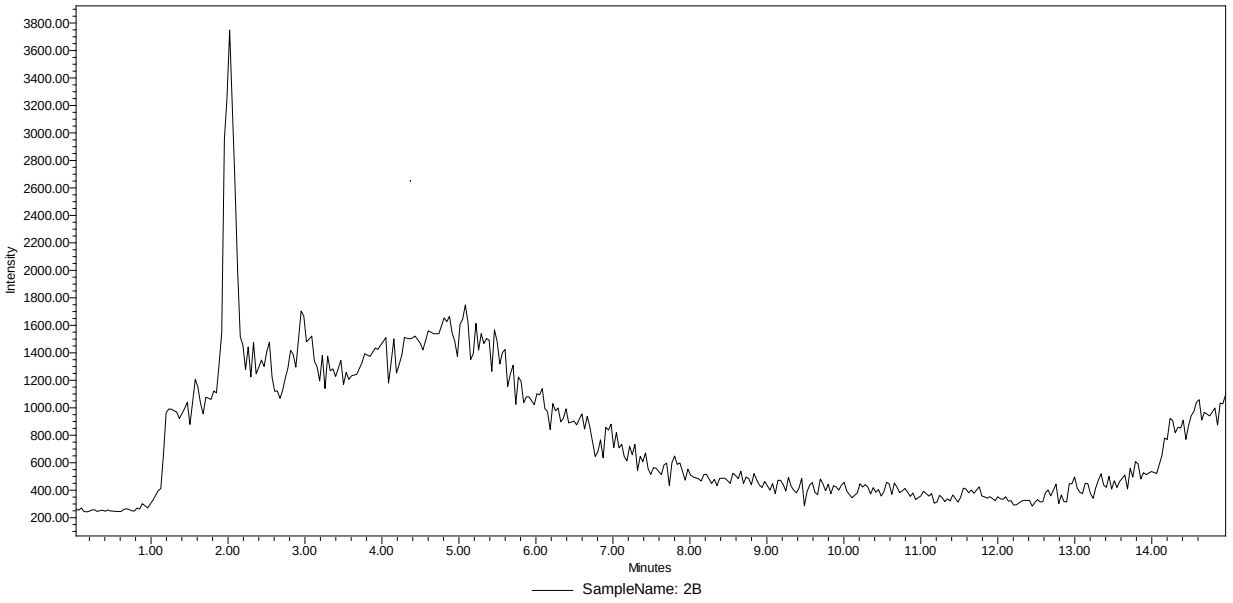

Fig. 27k Thermal/Acidic study's product chromatograph screening for FMASld sample 2.

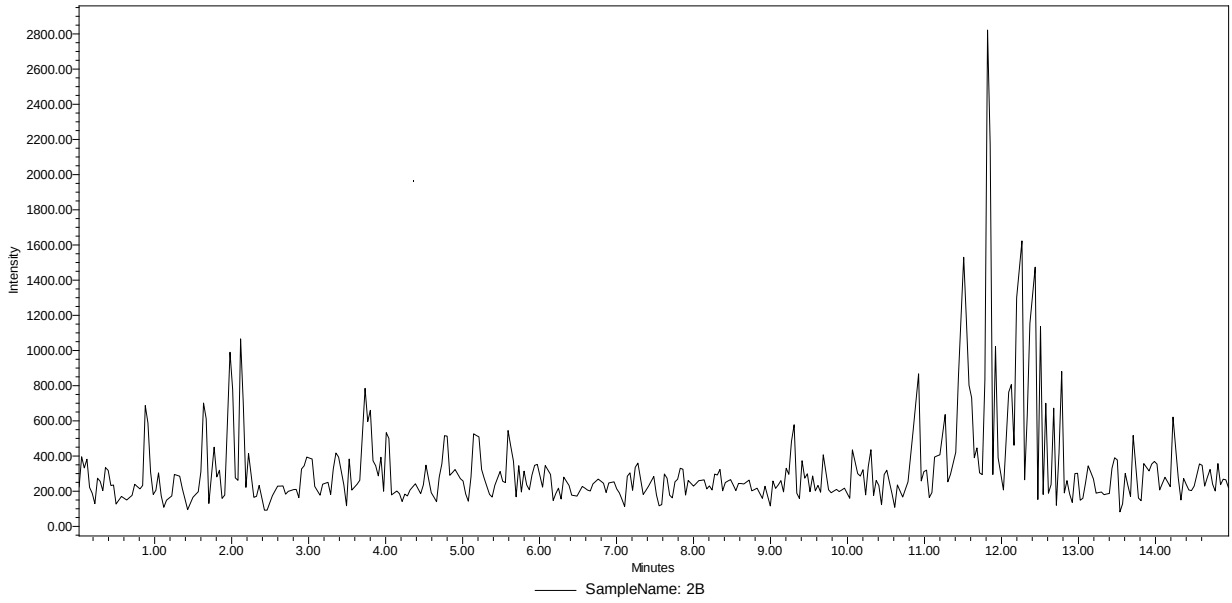

Fig. 27l Thermal/Acidic study's product chromatograph screening for FBAL sample 2.

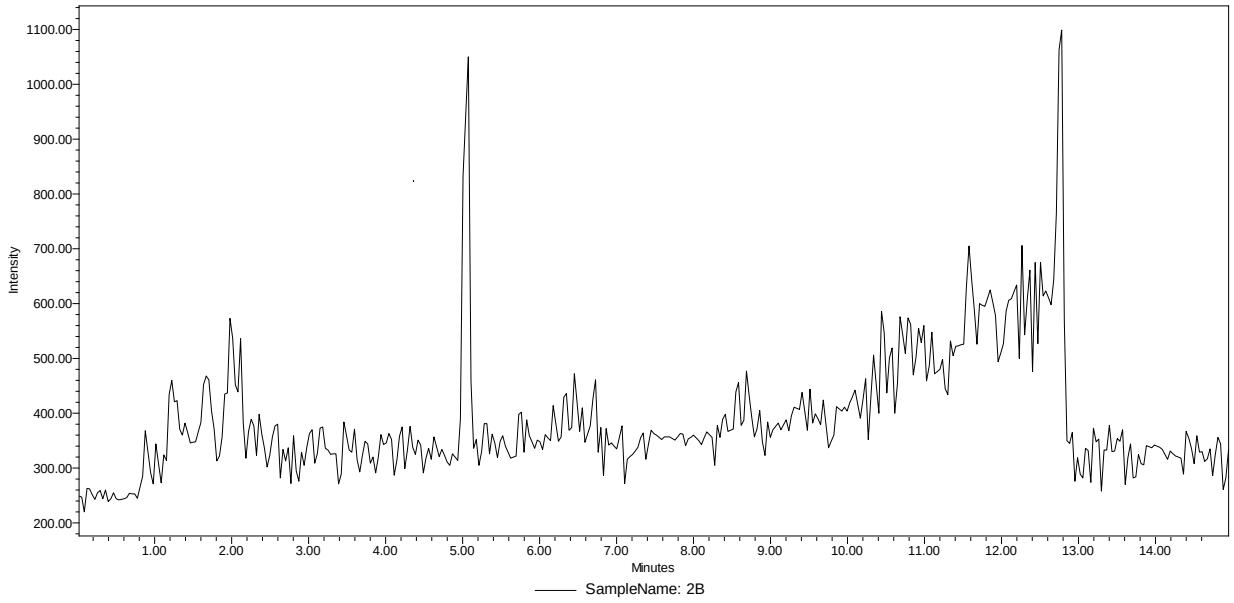

Fig. 27m Thermal/Acidic study's product chromatograph screening for FUPA sample 2.

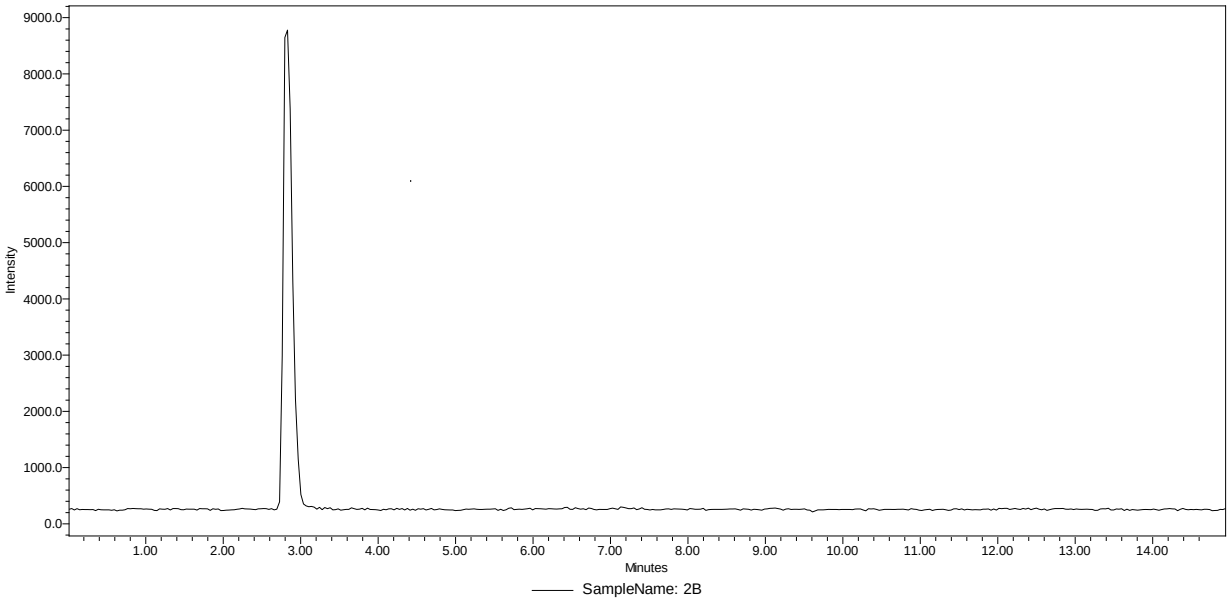

Fig. 27n Thermal/Acidic study's product chromatograph screening for 5-FU sample 2.

3

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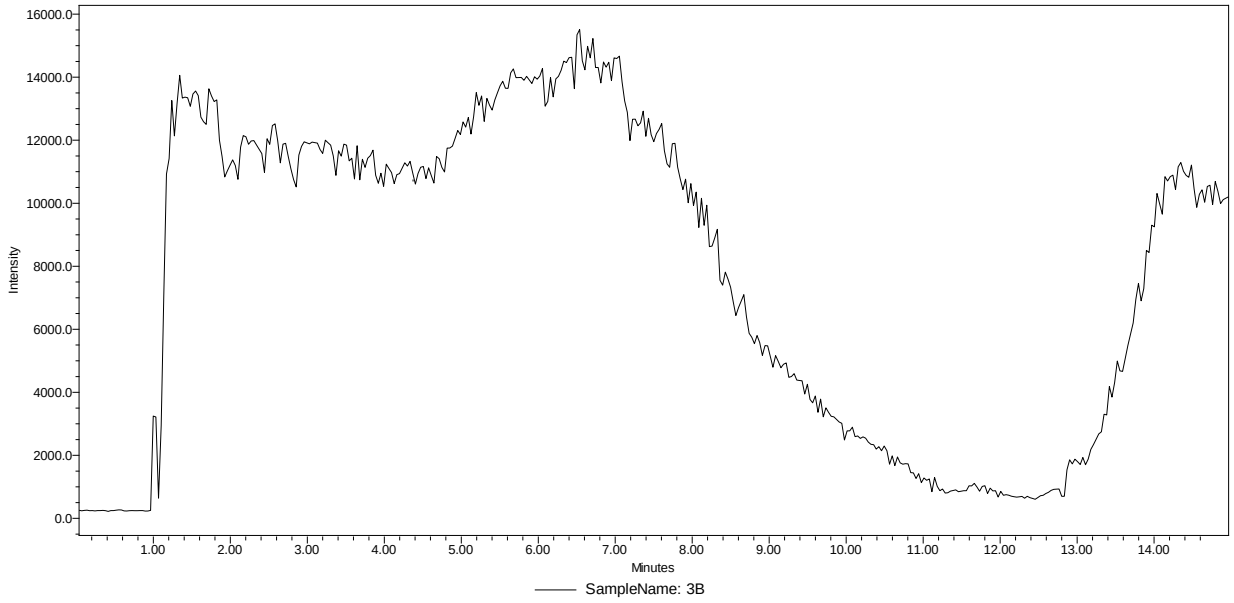

Fig. 27o Thermal/Acidic study's product chromatograph screening for FAC sample 3.

105

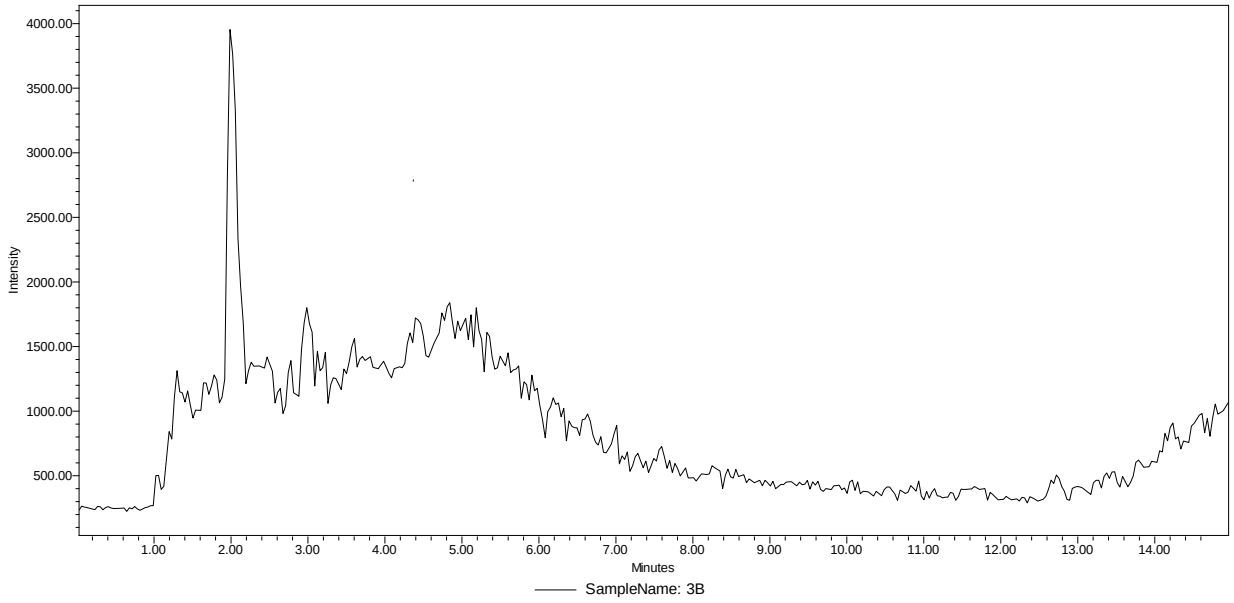

Fig. 27p Thermal/Acidic study's product chromatograph screening for FMASld sample 3.

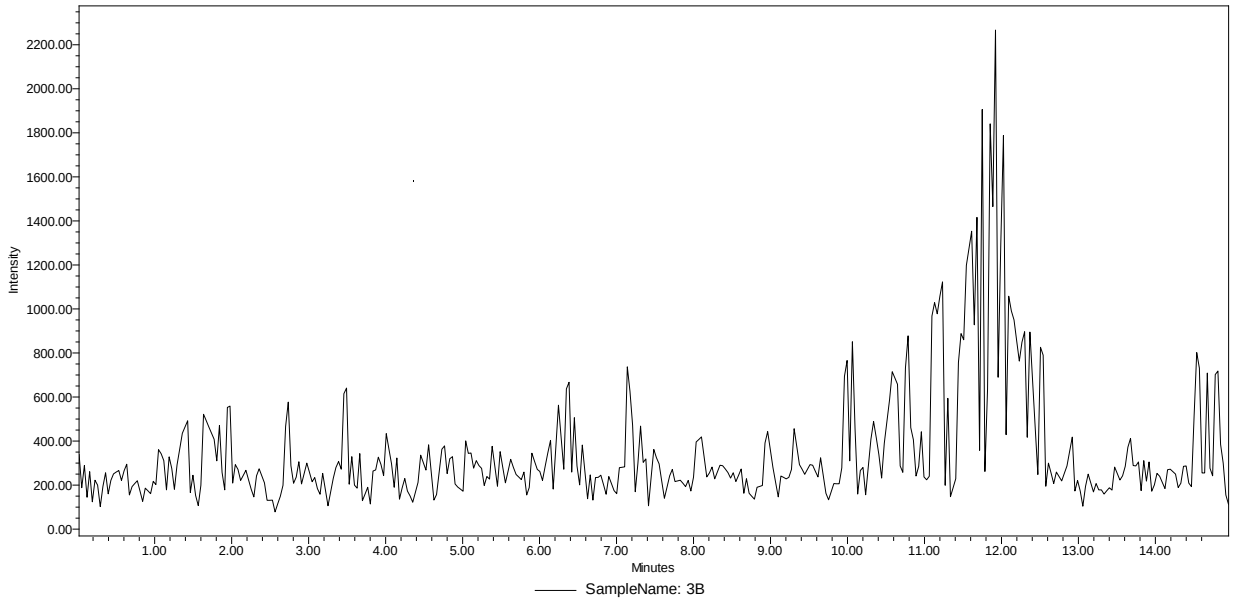

Fig. 27q Thermal/Acidic study's product chromatograph screening for FBAL sample 3.

149

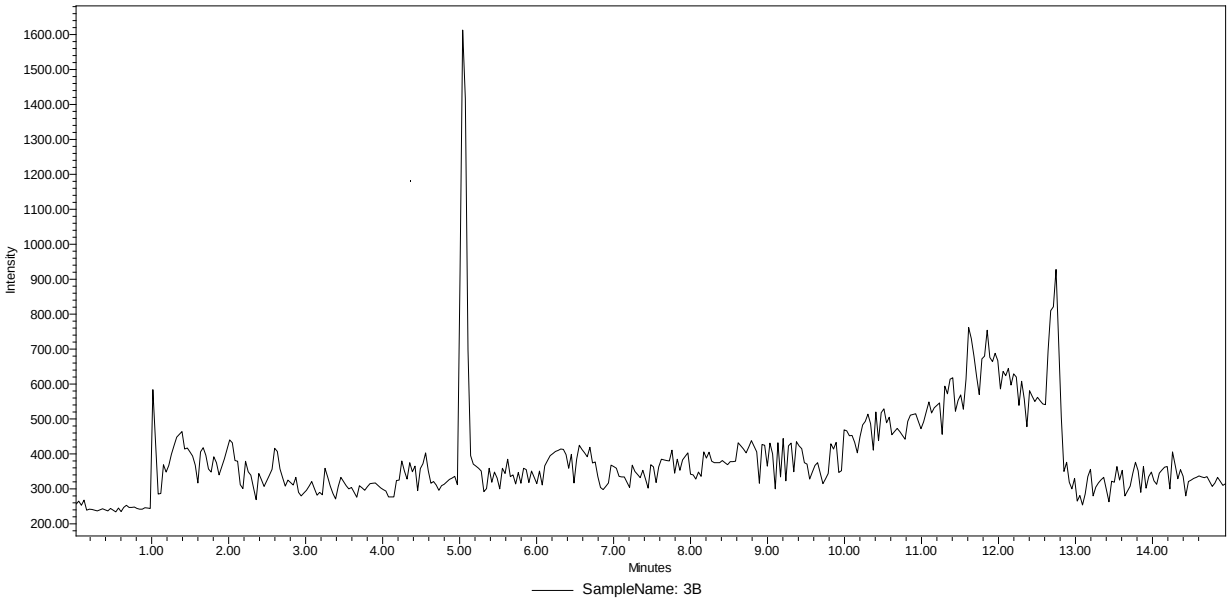

Fig. 27r Thermal/Acidic study's product chromatograph screening for FUPA sample 3.

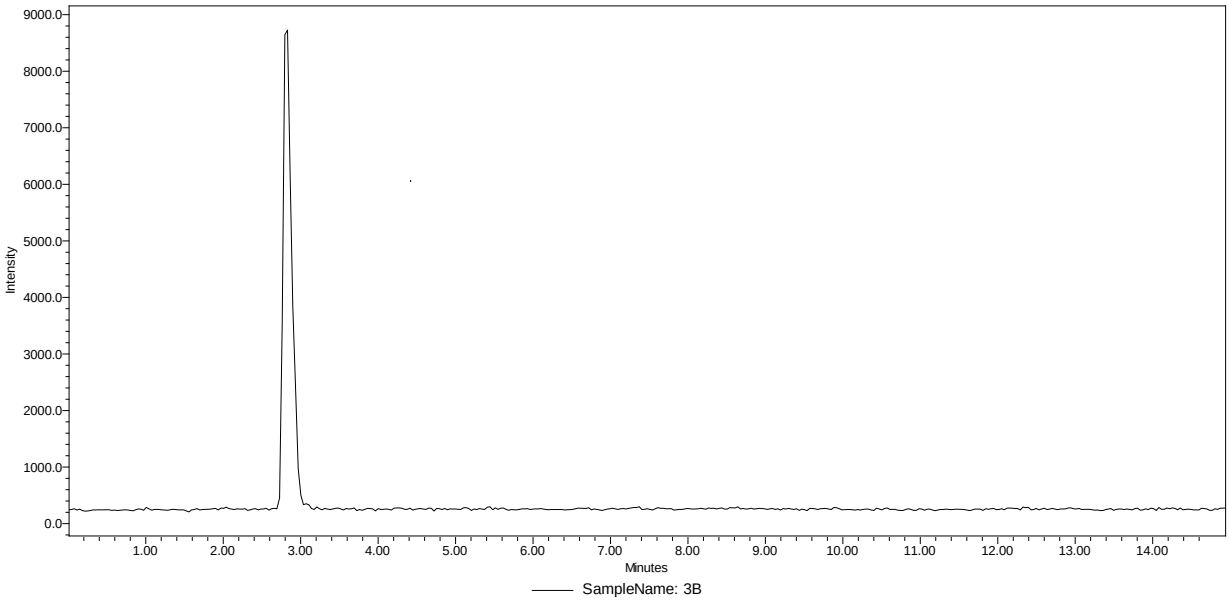

Fig. 27s Thermal/Acidic study's product chromatograph screening for 5-FU sample 3.

4

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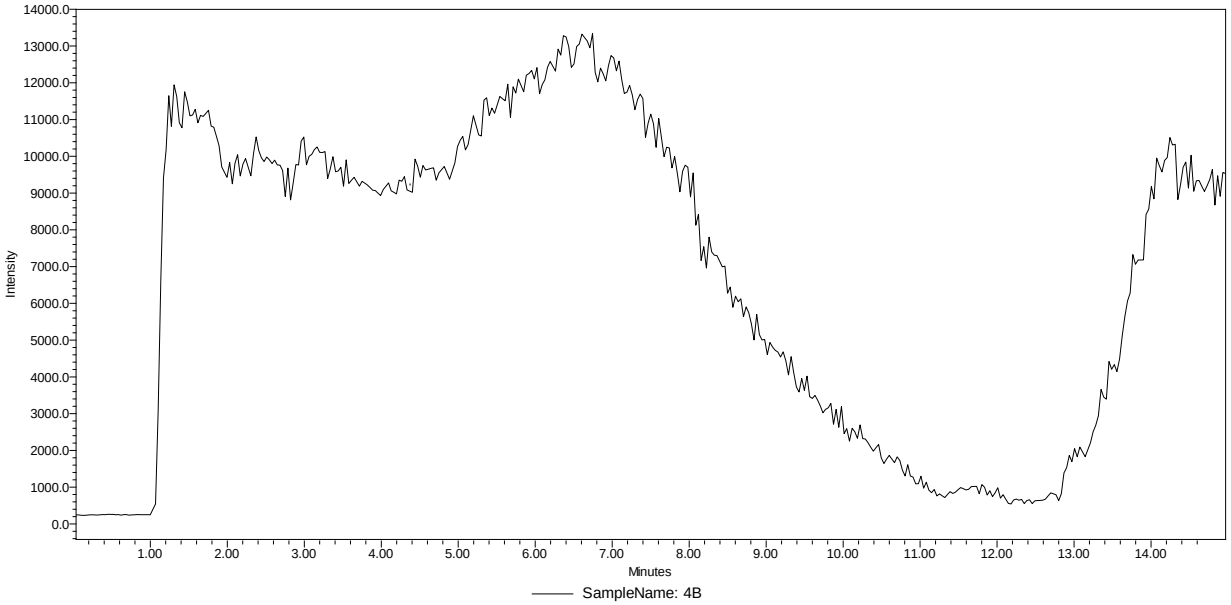

Fig. 27t Thermal/Acidic study's product chromatograph screening for FAC sample 4.

105

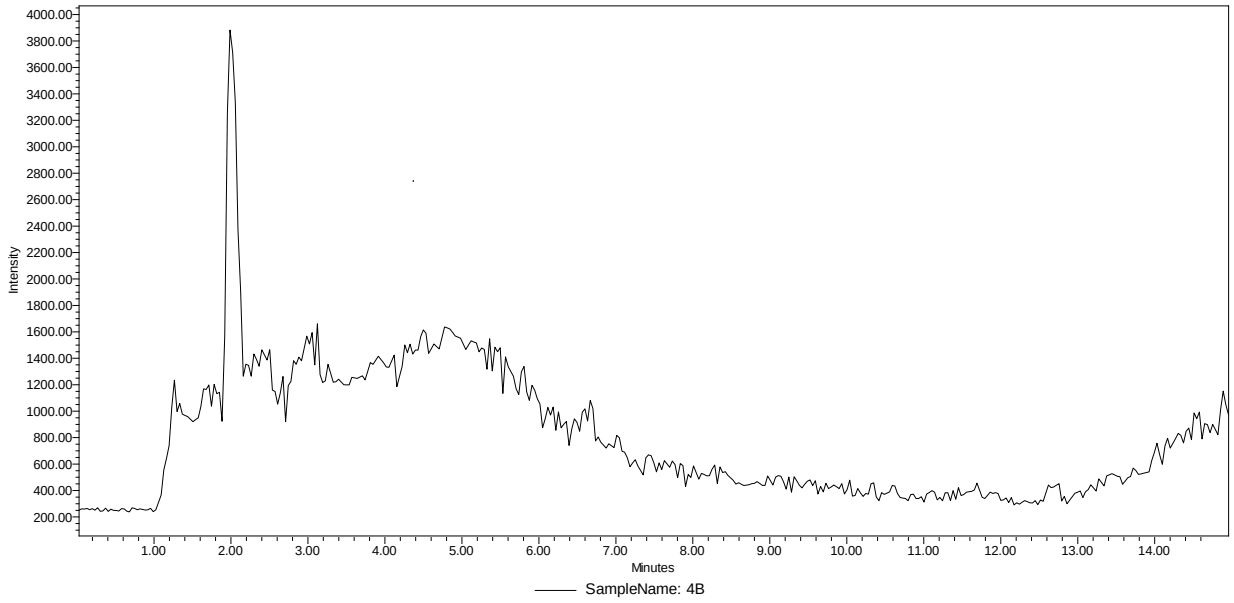

Fig. 27u Thermal/Acidic study's product chromatograph screening for FMASld sample 4.

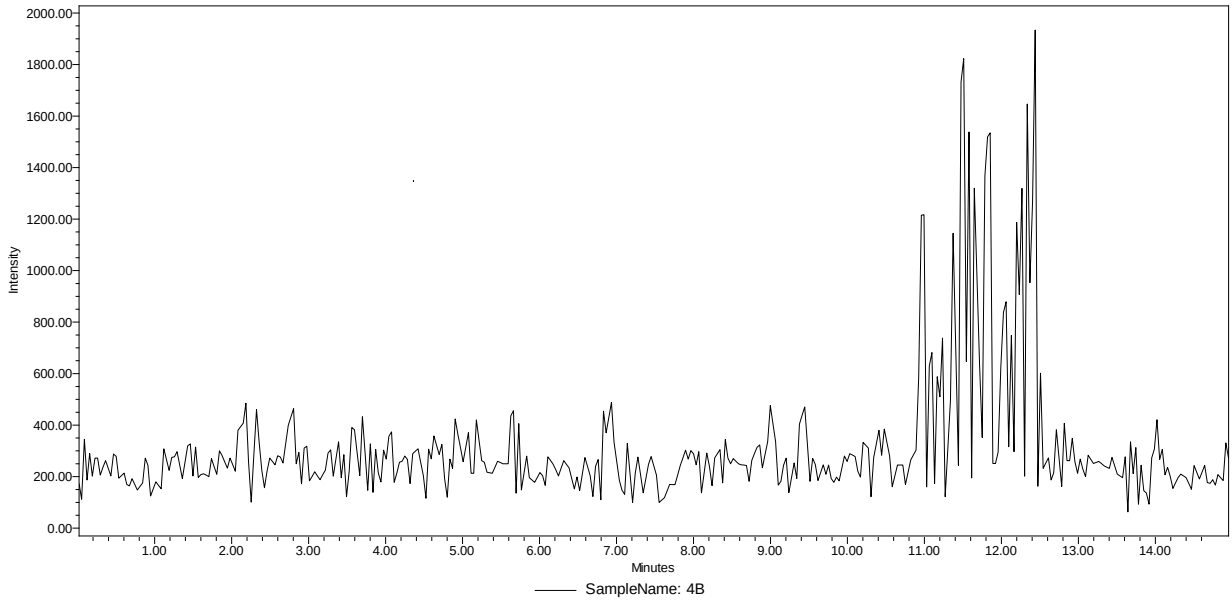

Fig. 27v Thermal/Acidic study's product chromatograph screening for FBAL sample 4.

149

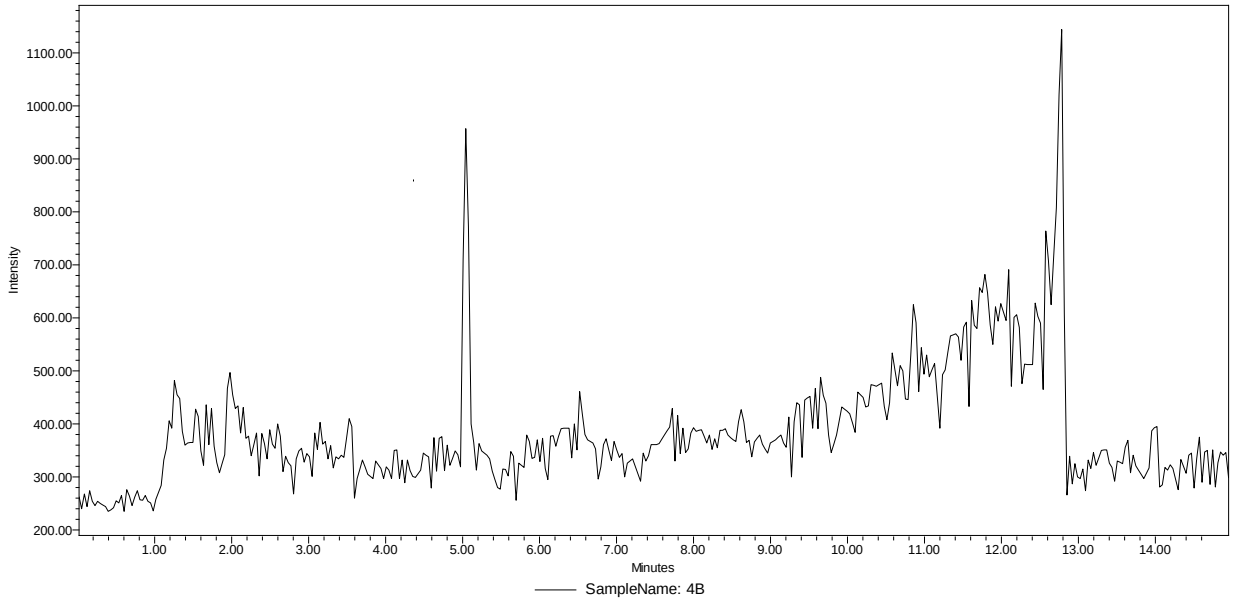

Fig. 27w Thermal/Acidic study's product chromatograph screening for FUPA sample 4.

129

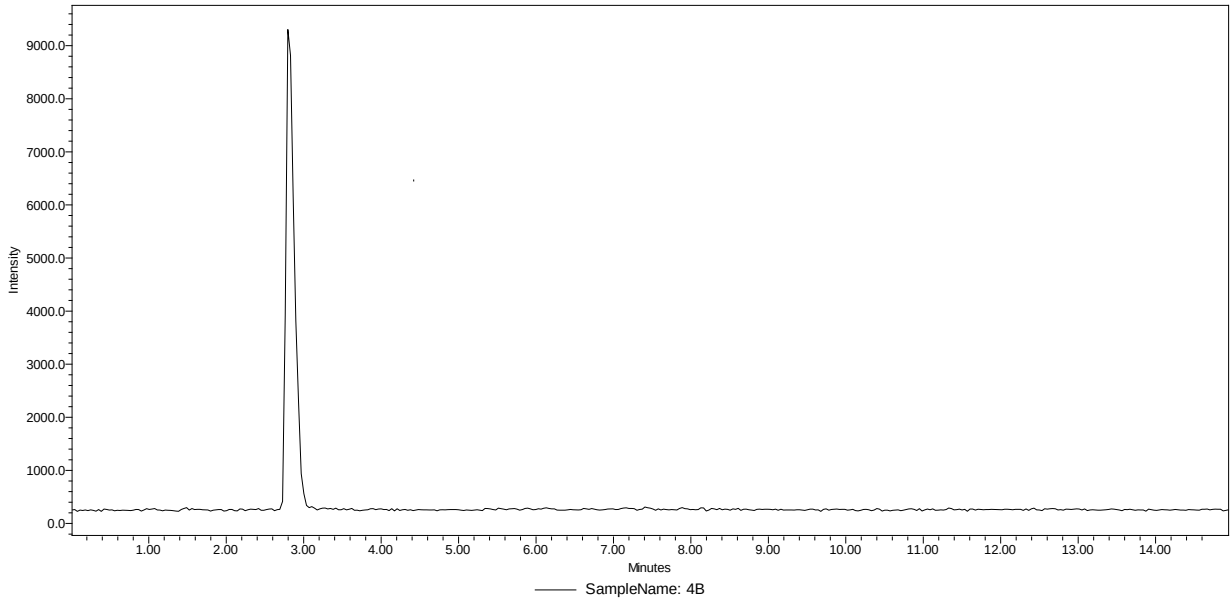

Fig. 27x Thermal/Acidic study's product chromatograph screening for 5-FU sample 4.

5

77

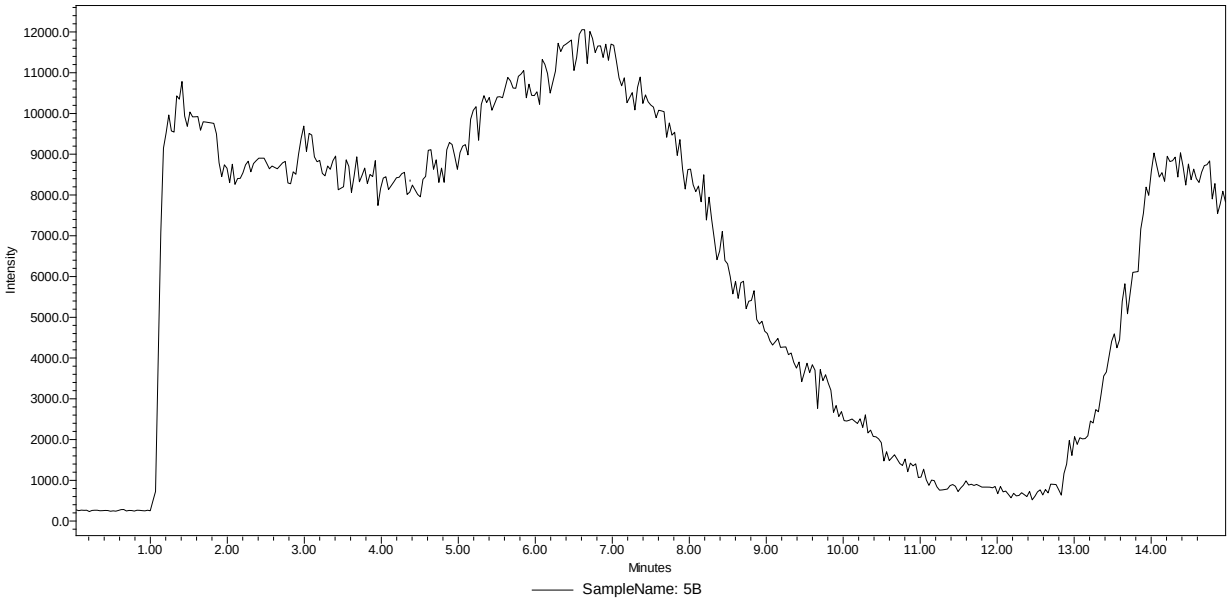

Fig. 27y Thermal/Acidic study's product chromatograph screening for FAC sample 5.

105

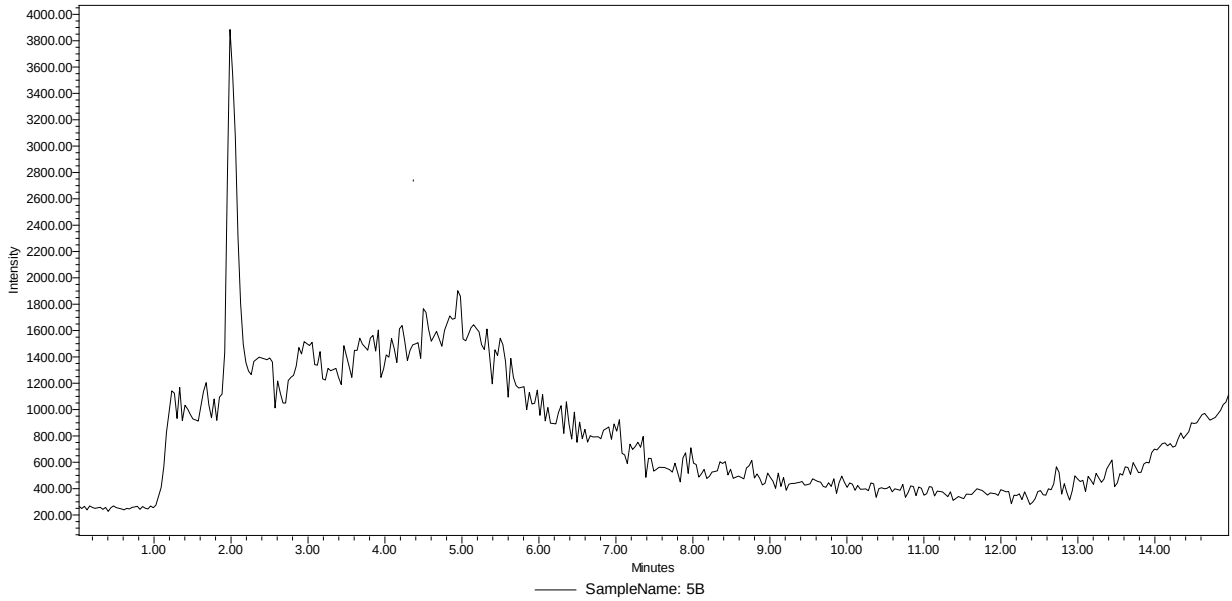

Fig. 27z Thermal/Acidic study's product chromatograph screening for FMASld sample 5.

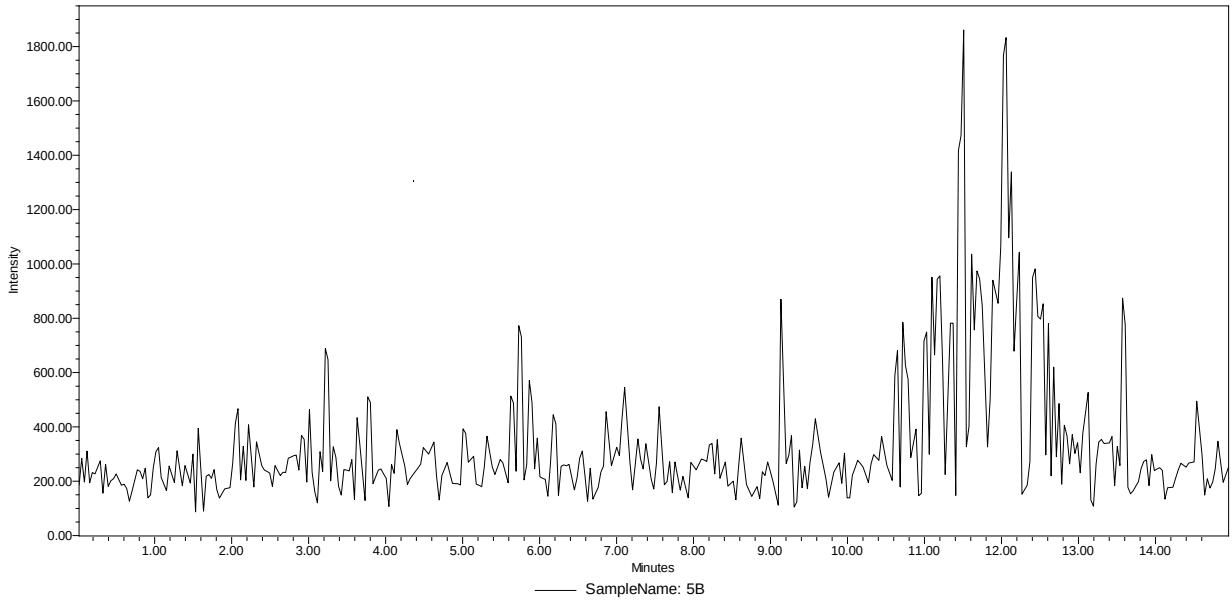

Fig. 27aa Thermal/Acidic study's product chromatograph screening for FBAL sample 5.

149

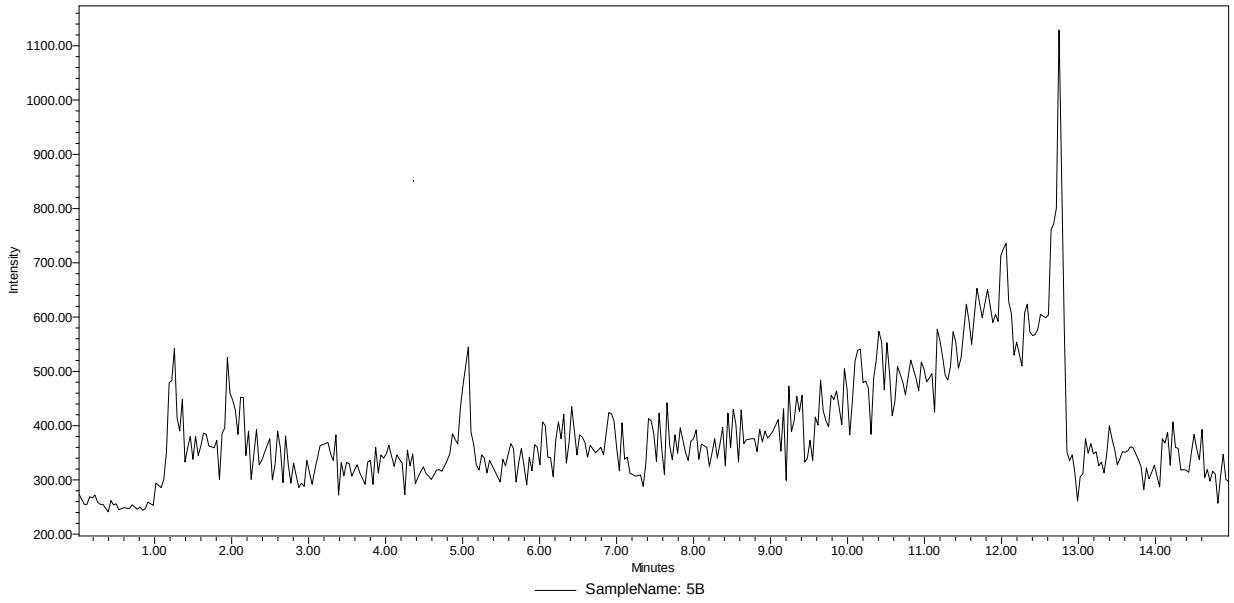

Fig. 27bb Thermal/Acidic study's product chromatograph screening for FUPA sample 5.

129

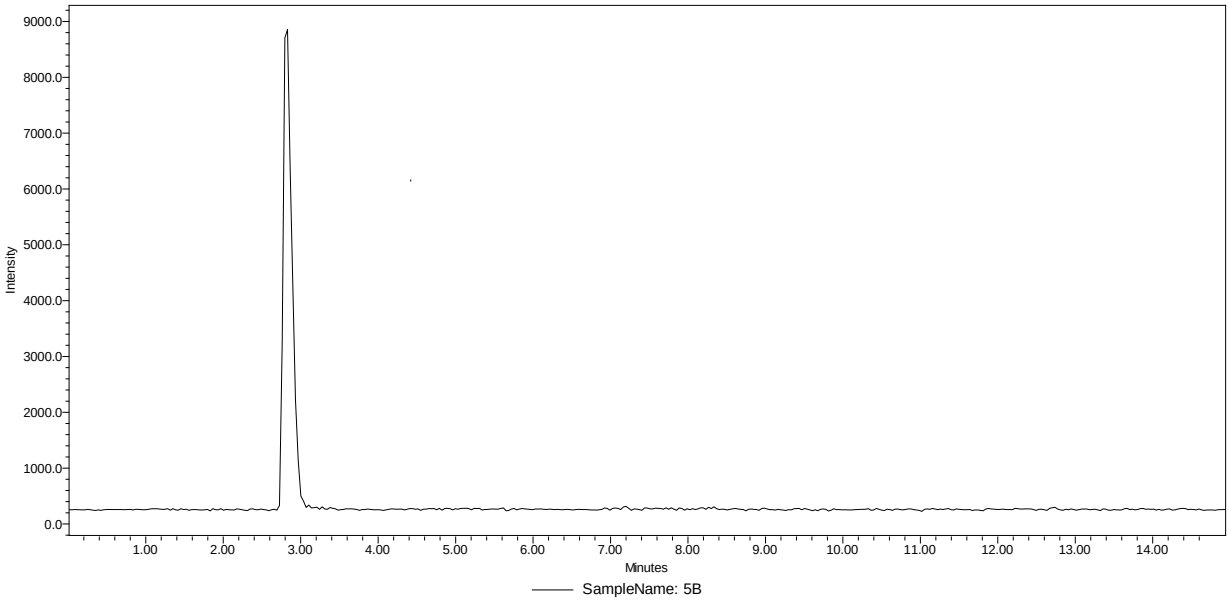

Fig. 27cc Thermal/Acidic study's product chromatograph screening for 5-FU sample 5.

6

77

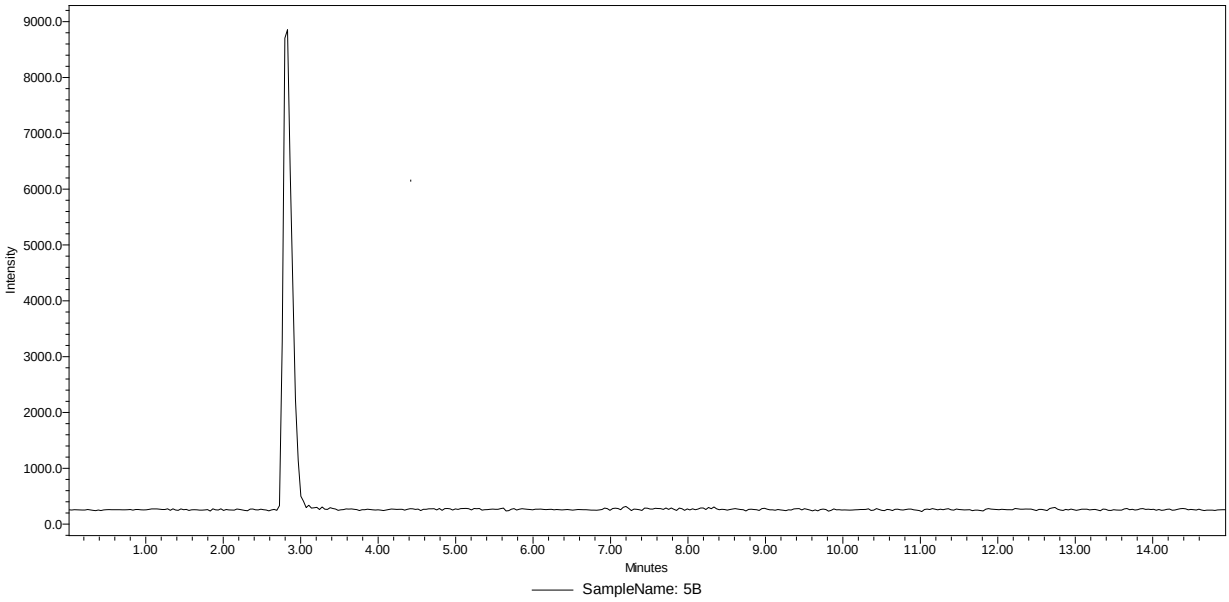

Fig. 27dd Thermal/Acidic study's product chromatograph screening for FAC sample 6.

105

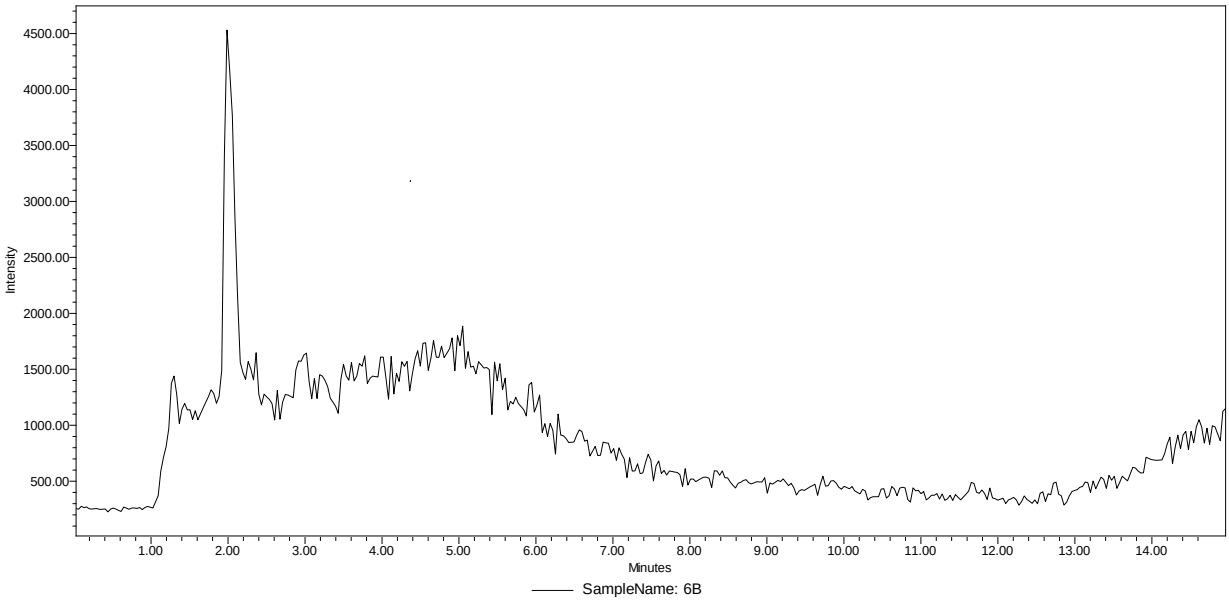

Fig. 27ee Thermal/Acidic study's product chromatograph screening for FMASld sample 6.

106

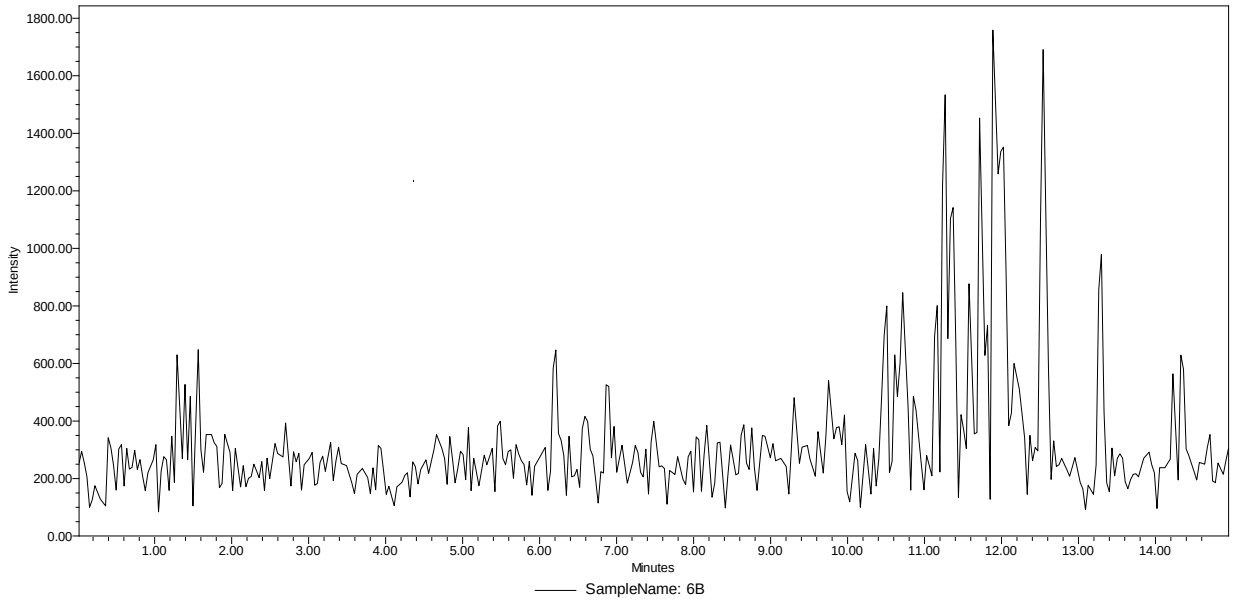

Fig. 27ff Thermal/Acidic study's product chromatograph screening for FBAL sample 6.

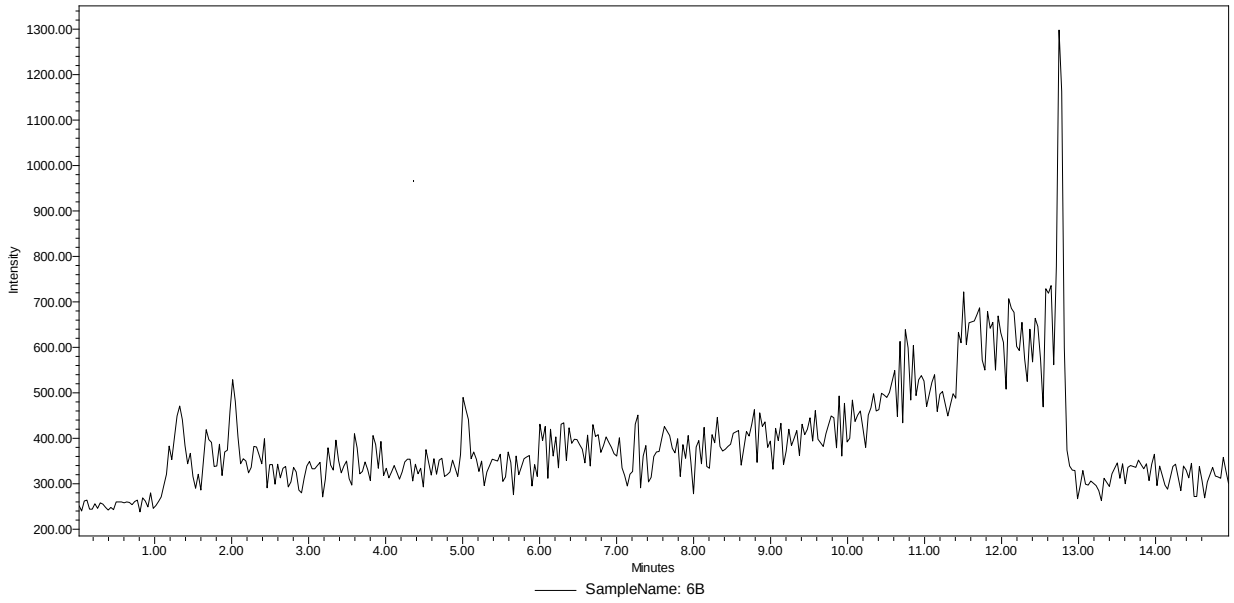

Fig. 27gg Thermal/Acidic study's product chromatograph screening for FUPA sample 6.

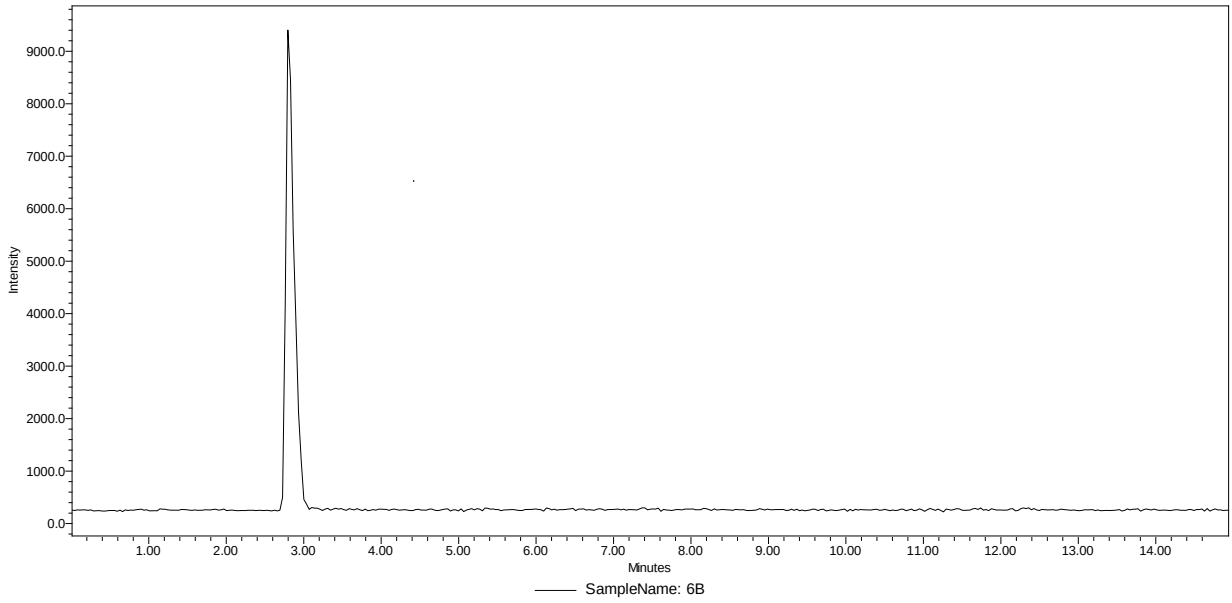

Fig. 27hh Thermal/Acidic study's product chromatograph screening for 5-FU sample 6.

7

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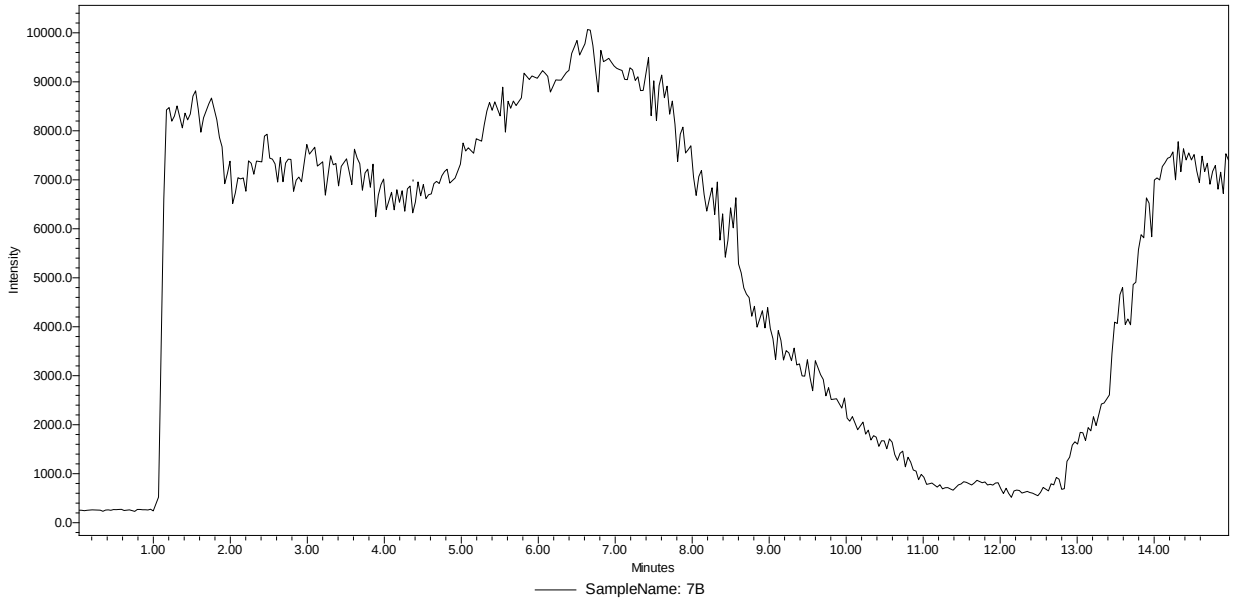

Fig. 27ii Thermal/Acidic study's product chromatograph screening for FAC sample 7

105

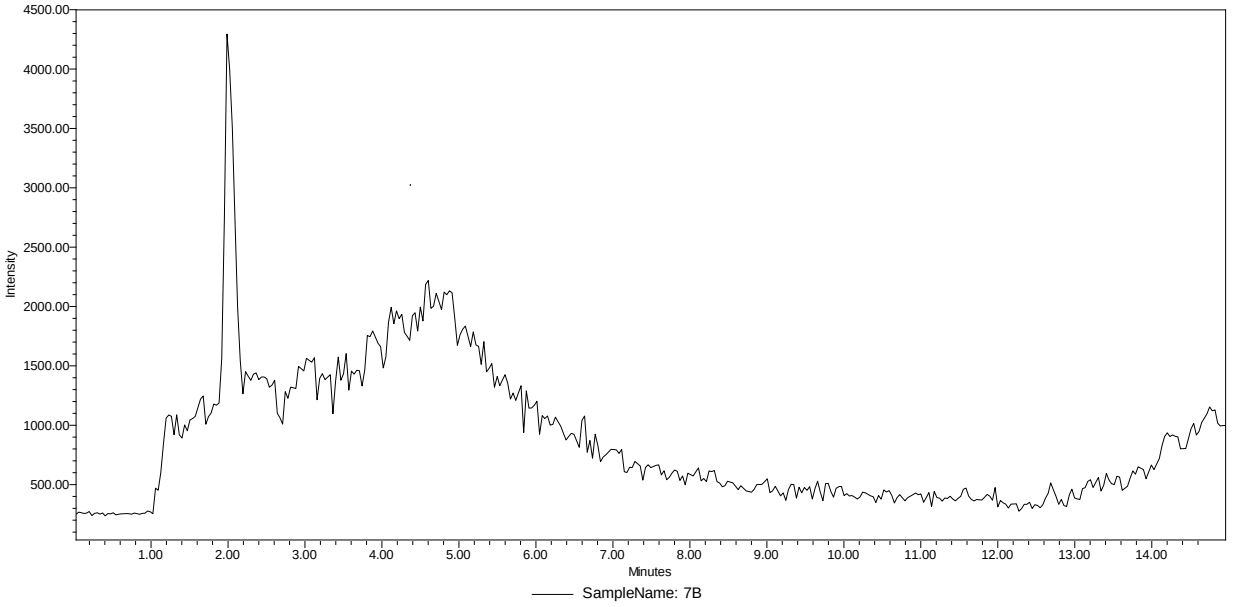

Fig. 27jj Thermal/Acidic study's product chromatograph screening for FMASld sam

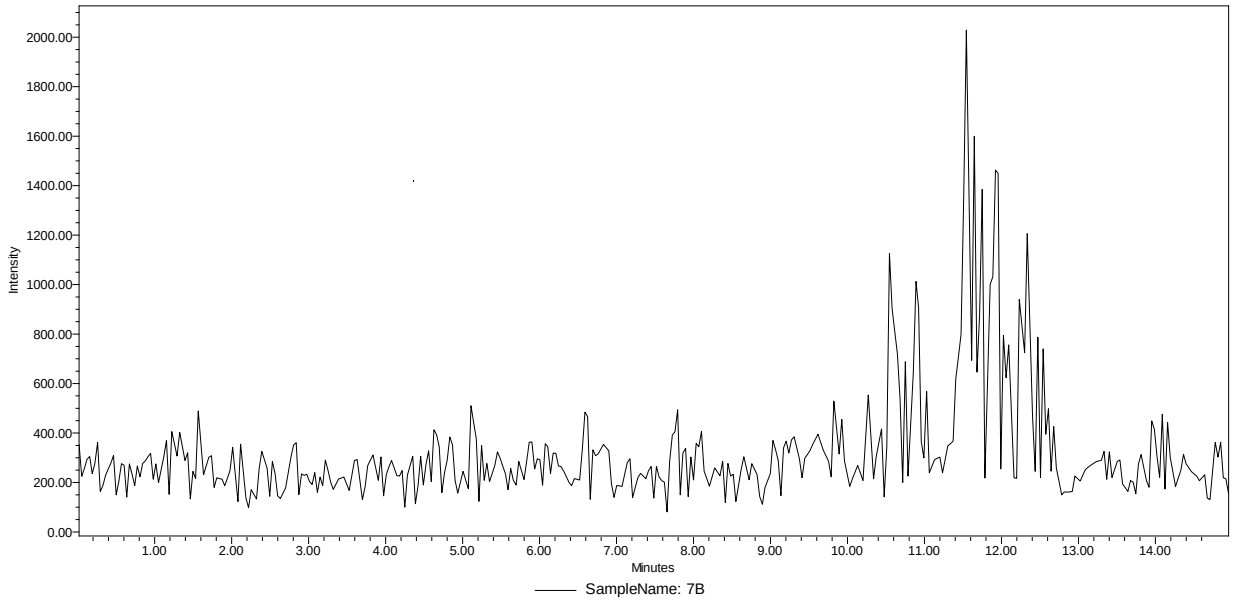

Fig. 27kk Thermal/Acidic study's product chromatograph screening for FBAL sample 7.

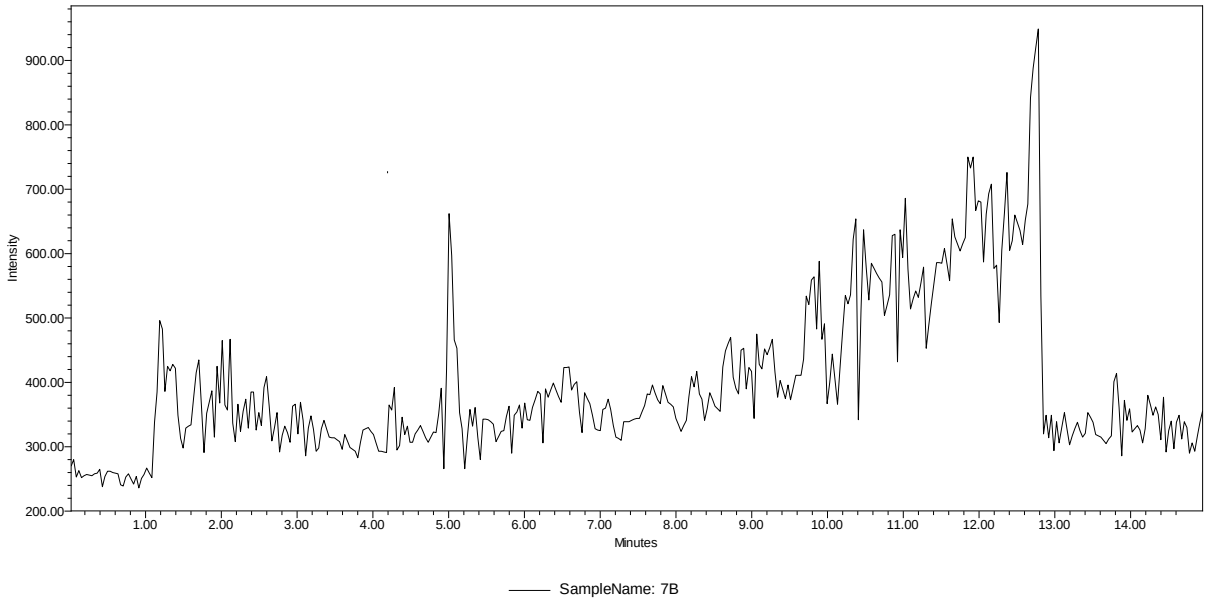

Fig. 27ll Thermal/Acidic study's product chromatograph screening for FUPA sample 7.

129

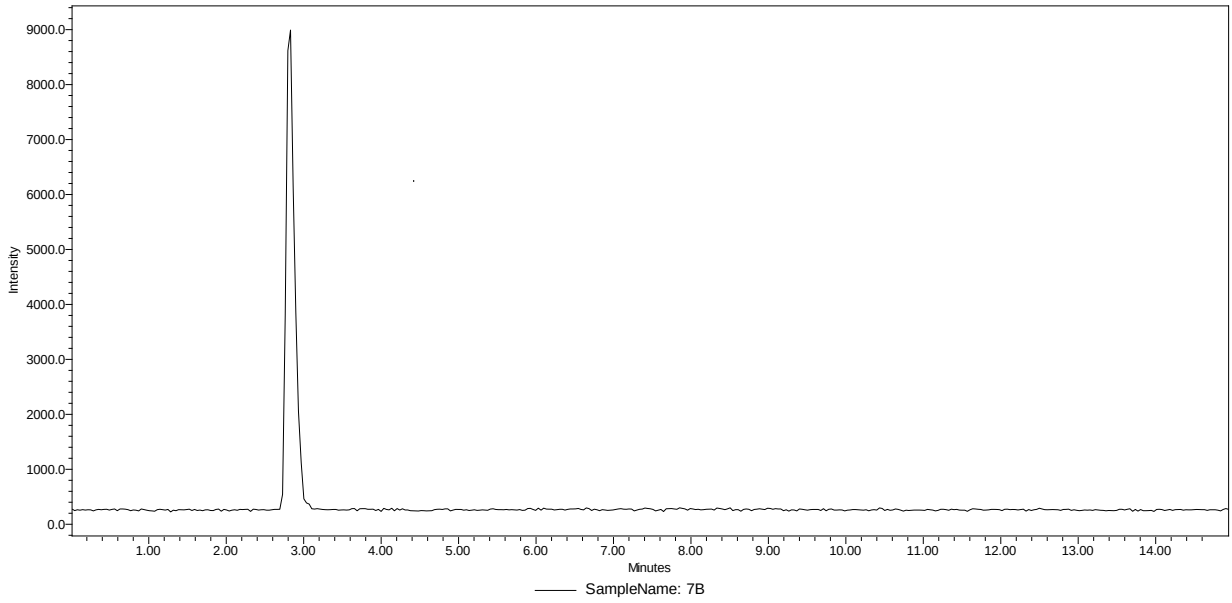

Fig. 27mm Thermal/Acidic study's product chromatograph screening for 5-FU sample 7.

8

77

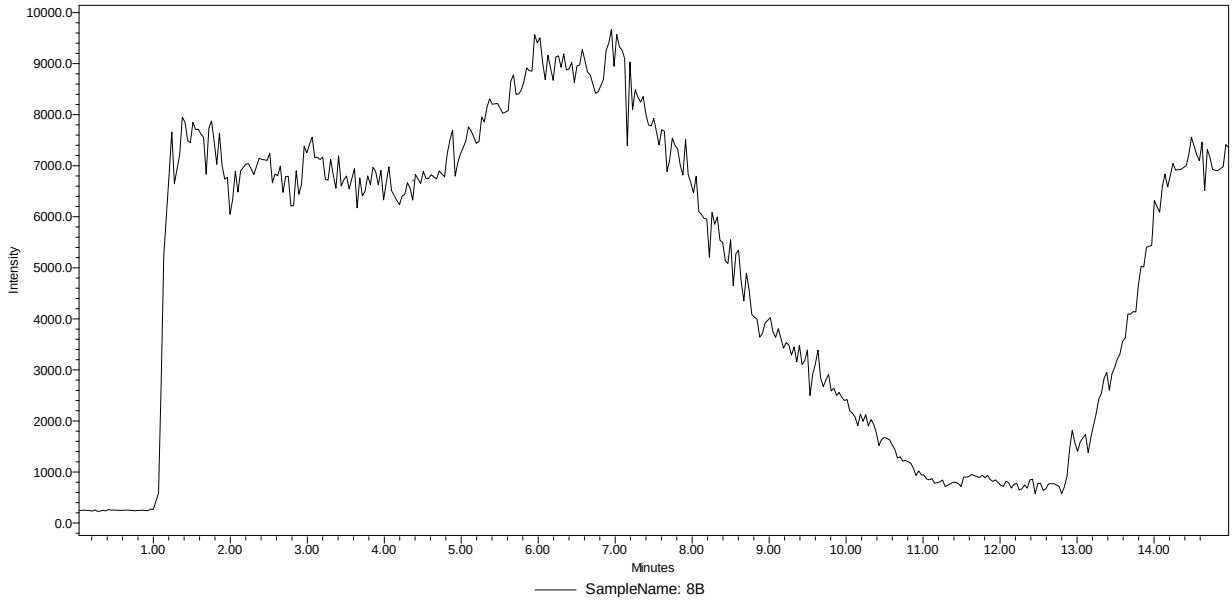

Fig. 27nn Thermal/Acidic study's product chromatograph screening for FAC sample 8.

105

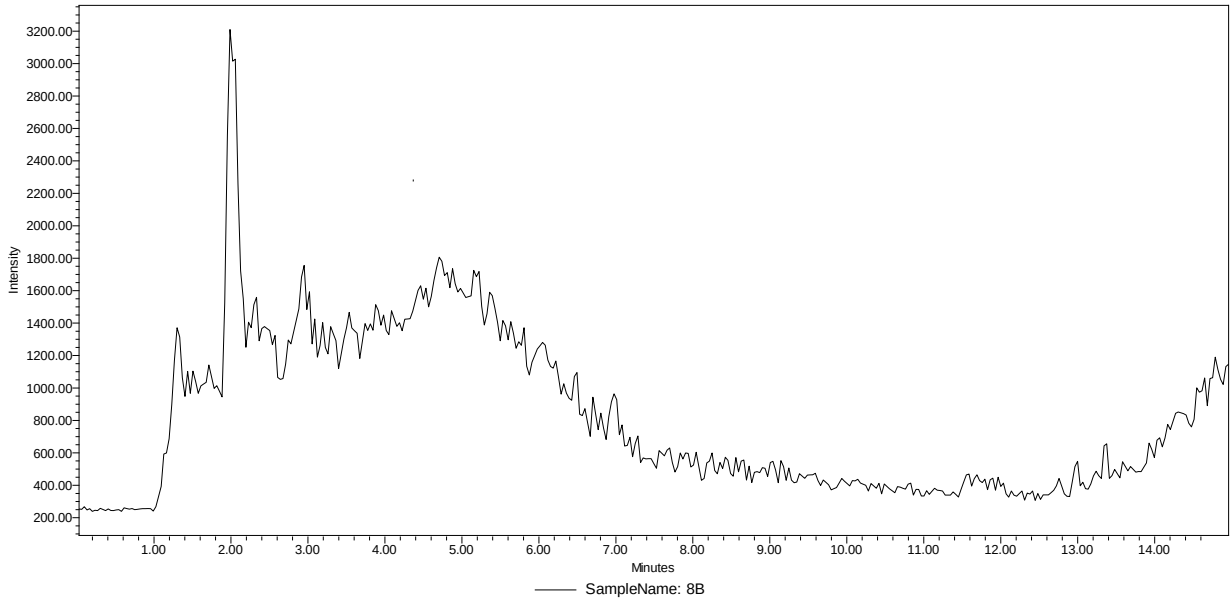

Fig. 27oo Thermal/Acidic study's product chromatograph screening for FMASld sample 8.

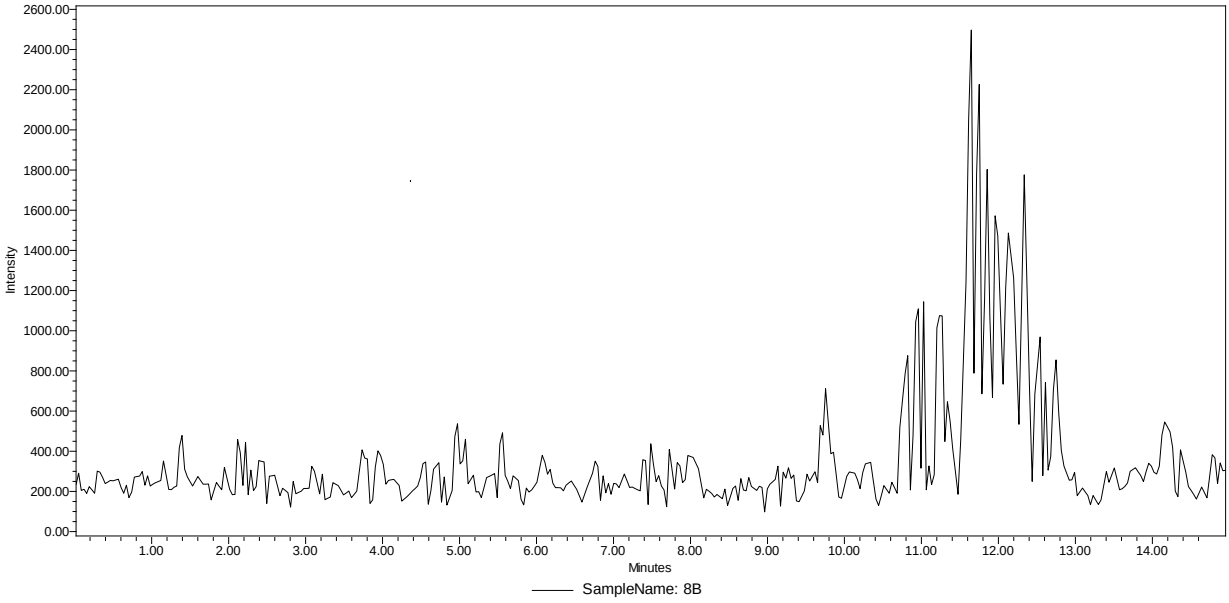

Fig. 27pp Thermal/Acidic study's product chromatograph screening for FBAL sample 8.

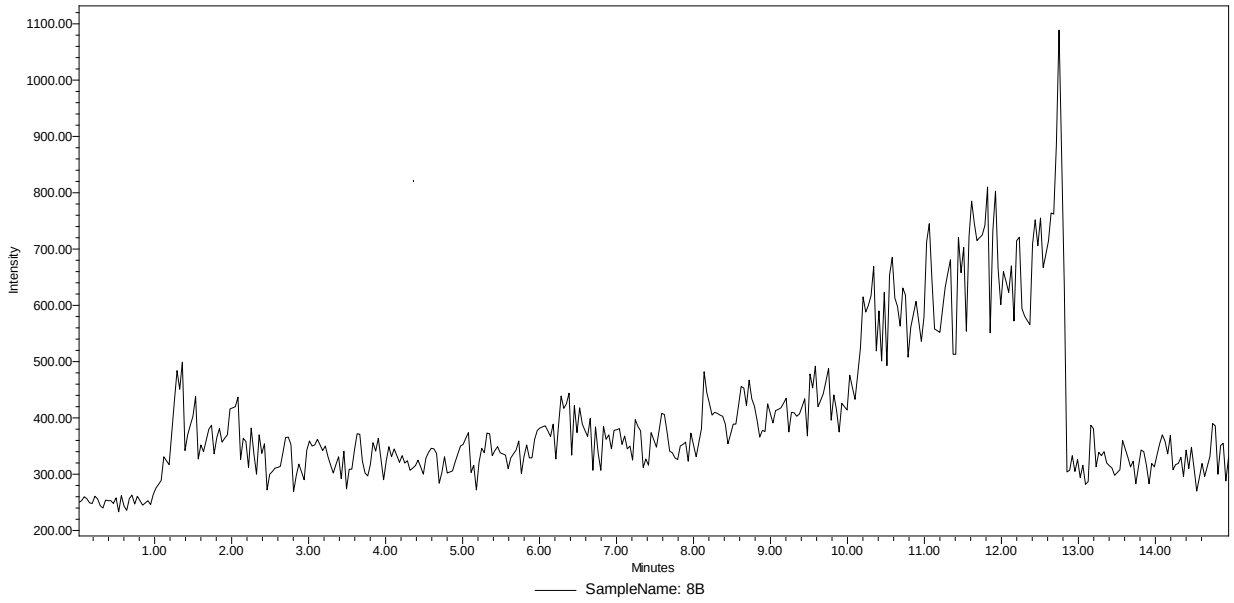

Fig. 27qq Thermal/Acidic study's product chromatograph screening for FUPA sample 8.

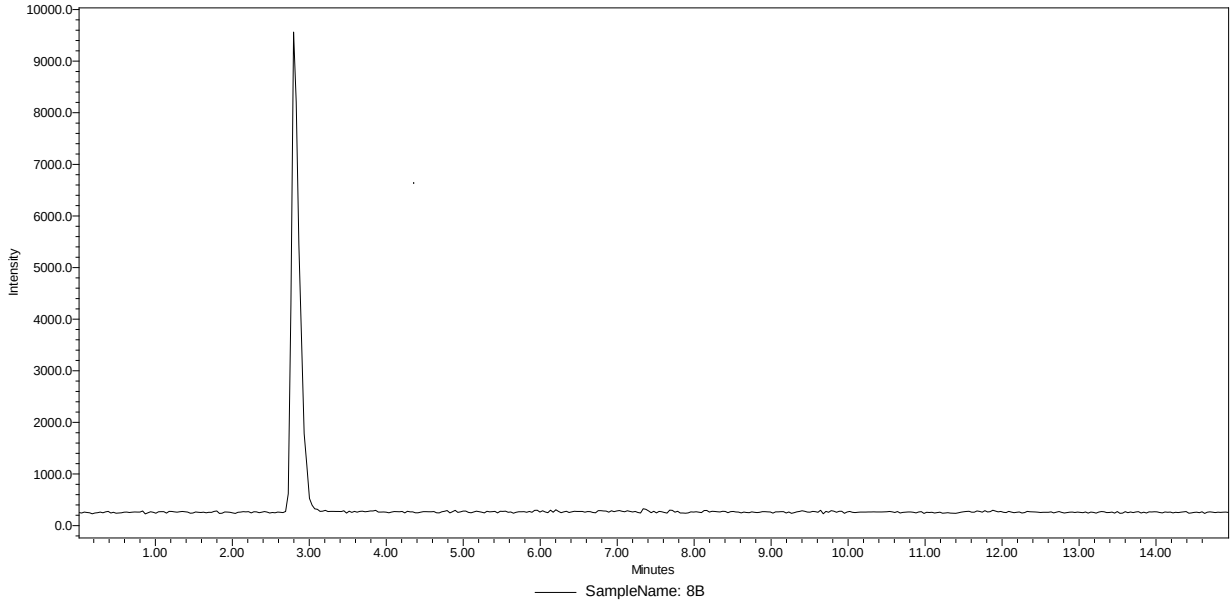

Fig. 27rr Thermal/Acidic study's product chromatograph screening for 5-FU sample 8.

9

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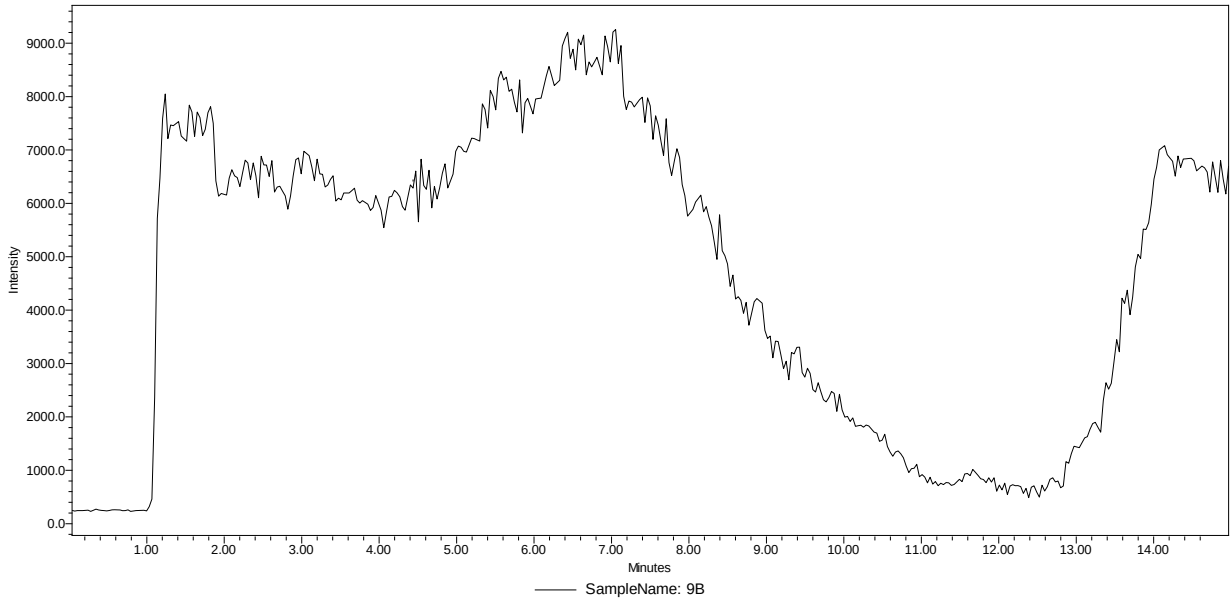

Fig. 27ss Thermal/Acidic study's product chromatograph screening for FAC sample 9.

105

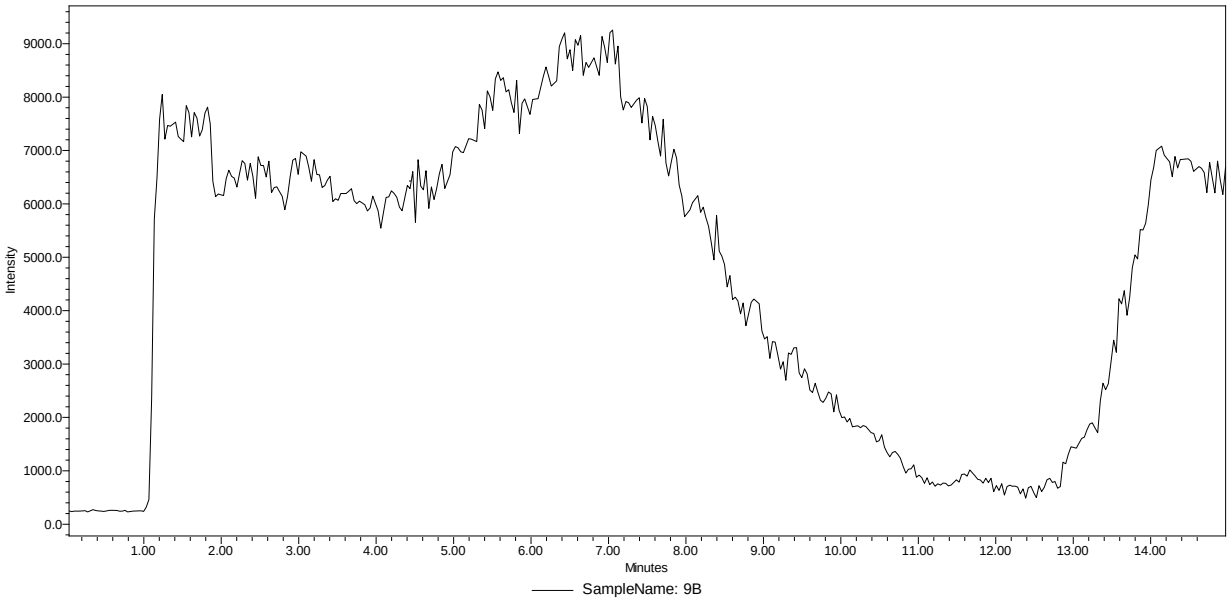

Fig. 27tt Thermal/Acidic study's product chromatograph screening for FMASld sample 9.

106

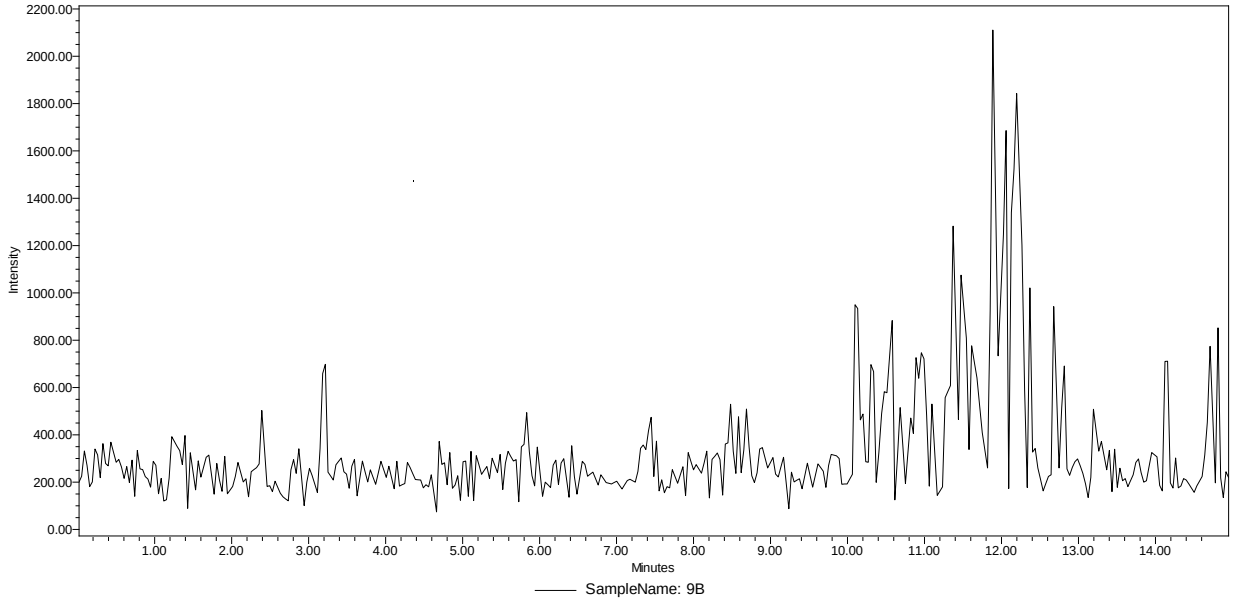

Fig. 27uu Thermal/Acidic study's product chromatograph screening for FBAL sample 9.

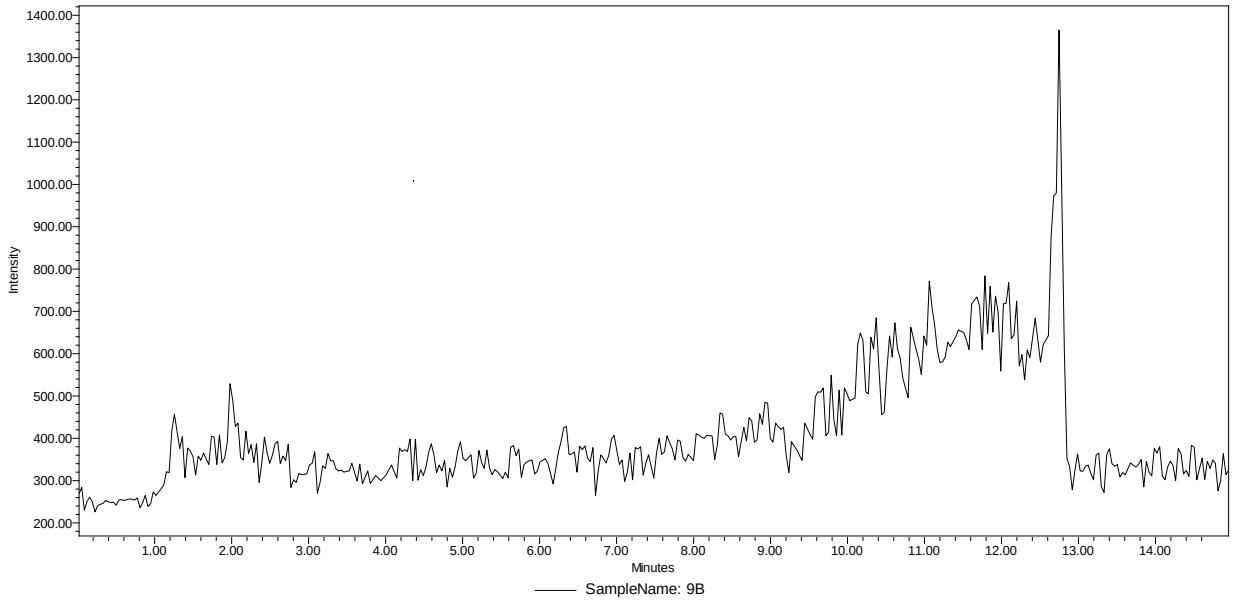

Fig. 27vv Thermal/Acidic study's product chromatograph screening for FUPA sample 9.

129

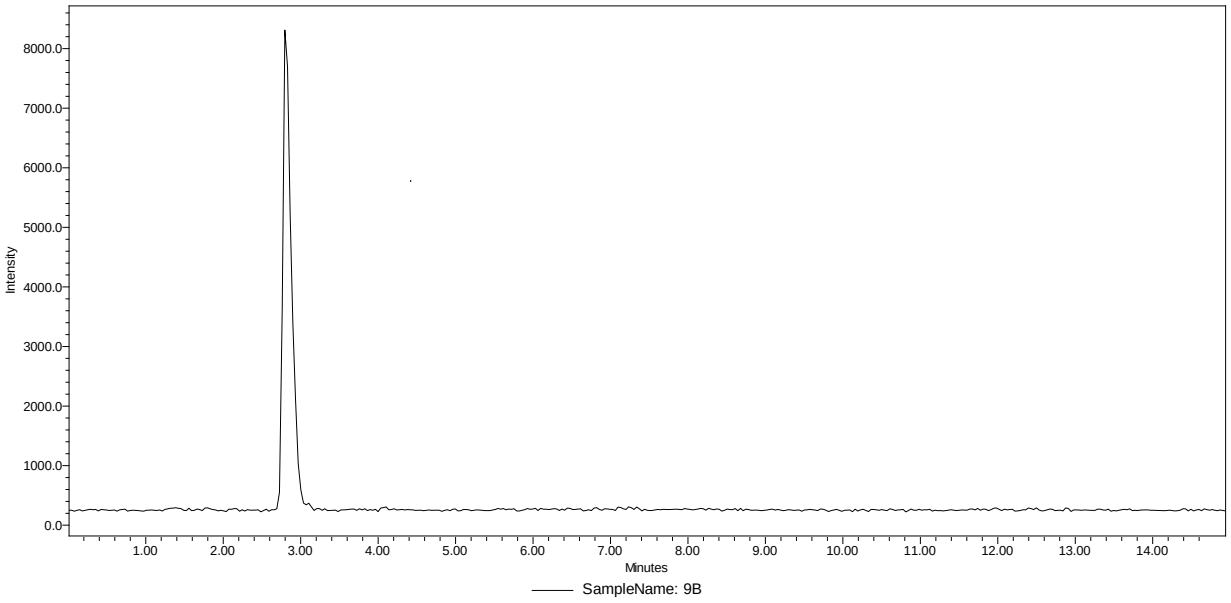

Fig. 27ww Thermal/Acidic study's product chromatograph screening for 5-FU sample 9.

10

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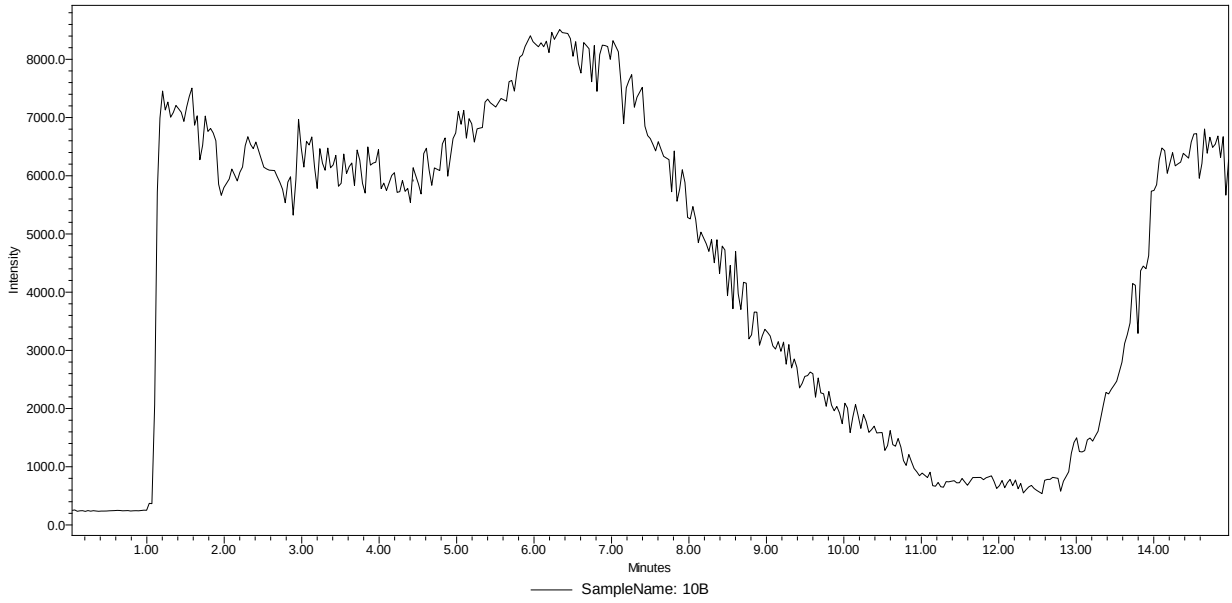

Fig. 27vv Thermal/Acidic study's product chromatograph screening for FAC sample 10.

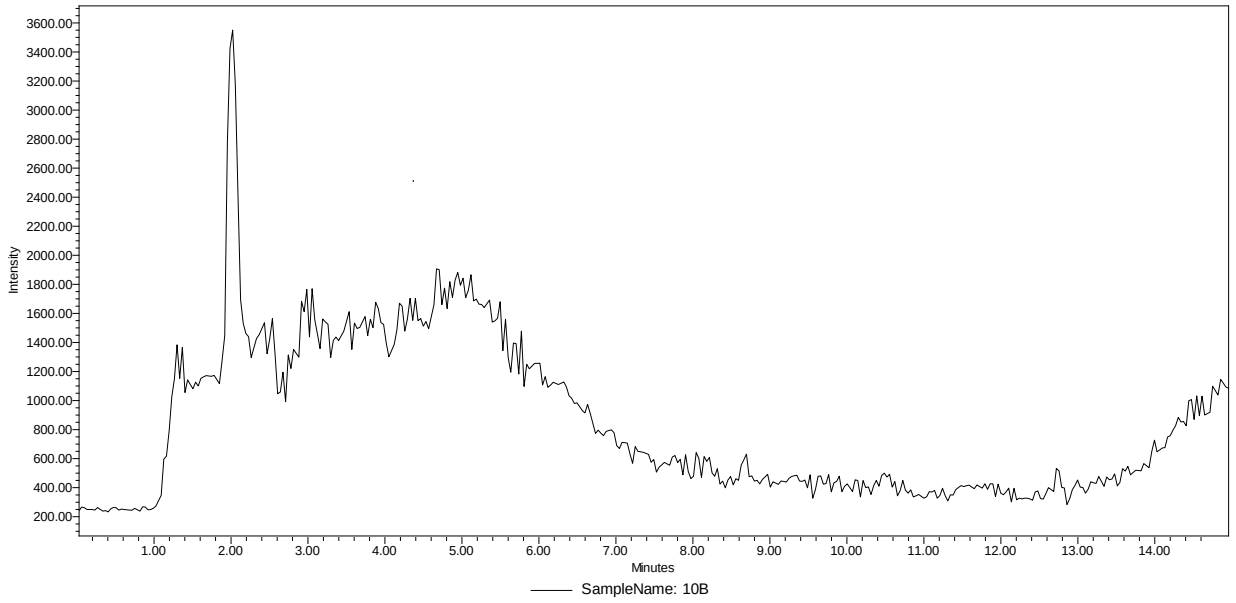

Fig. 27xx Thermal/Acidic study's product chromatograph screening for FMASld sample 10.

106

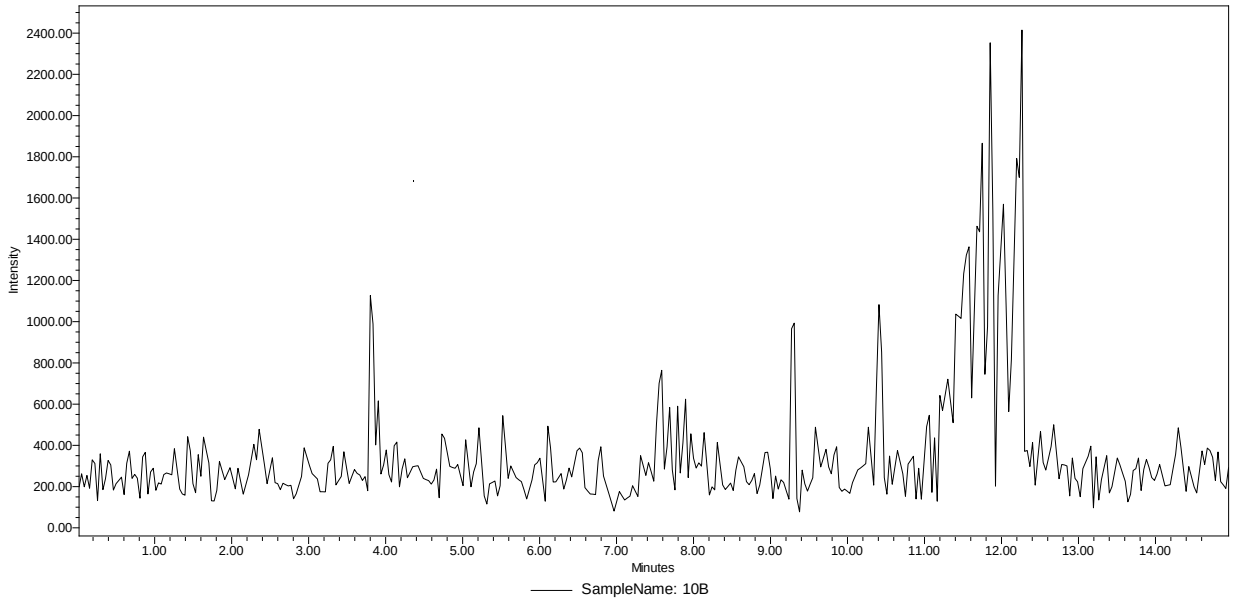

Fig. 27yy Thermal/Acidic study's product chromatograph screening for FBAL sample 10.

149

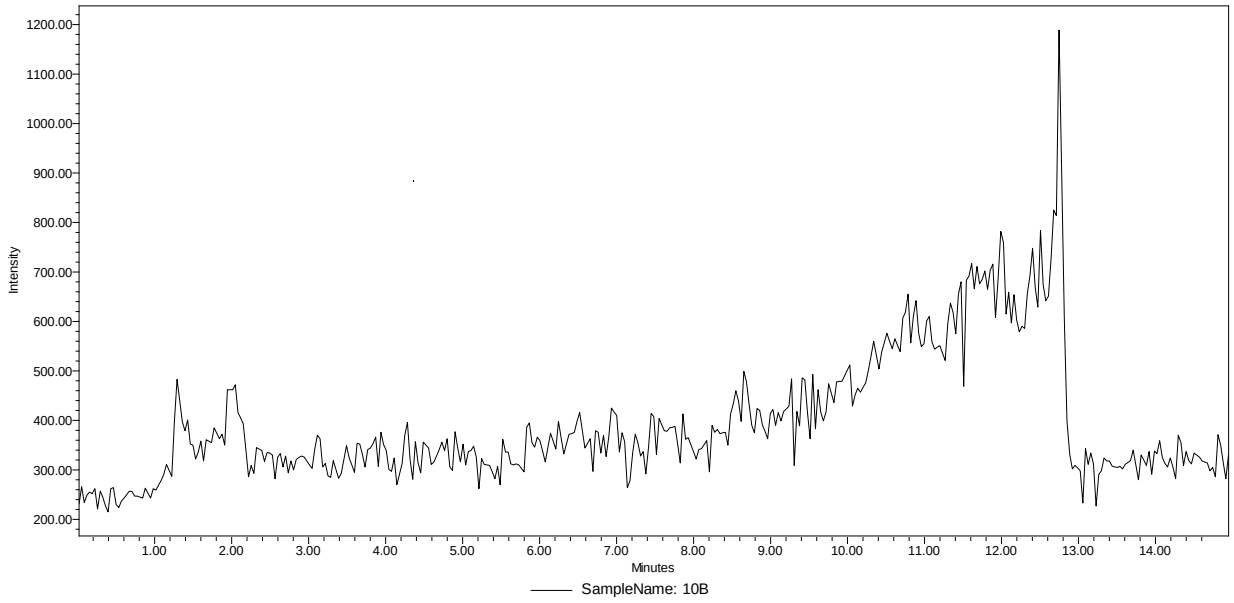

Fig. 27zz Thermal/Acidic study's product chromatograph screening for FUPA sample 10.

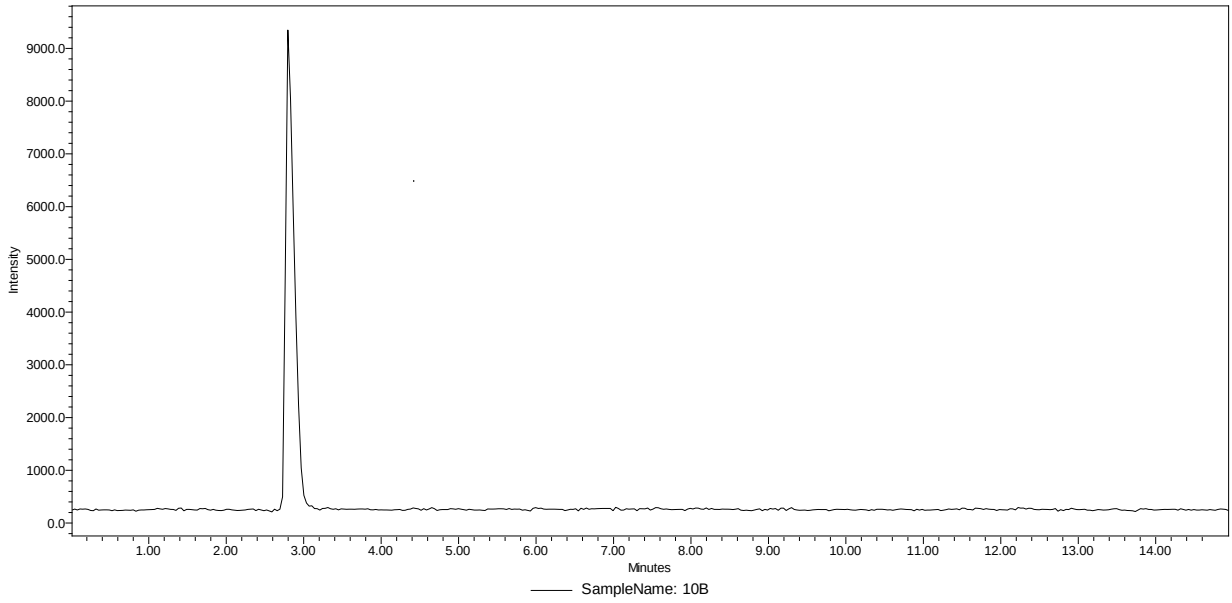

Fig. 27aa Thermal/Acidic study's product chromatograph screening for 5-FU sample 10.

11

77

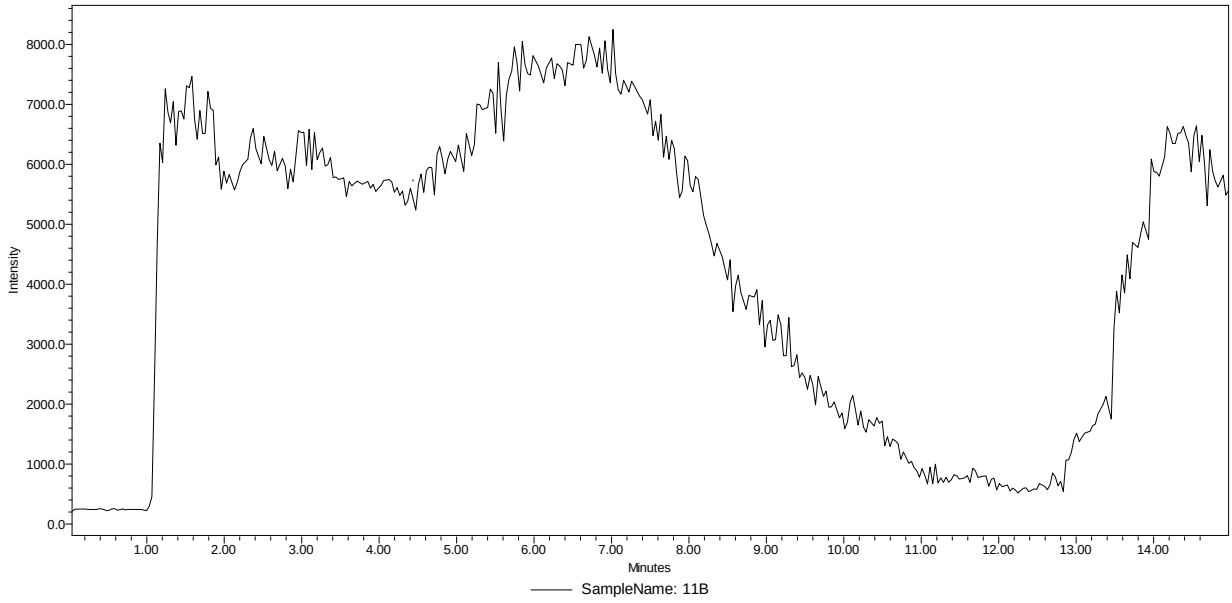

Fig. 27bb Thermal/Acidic study's product chromatograph screening for FAC sample 11.

105

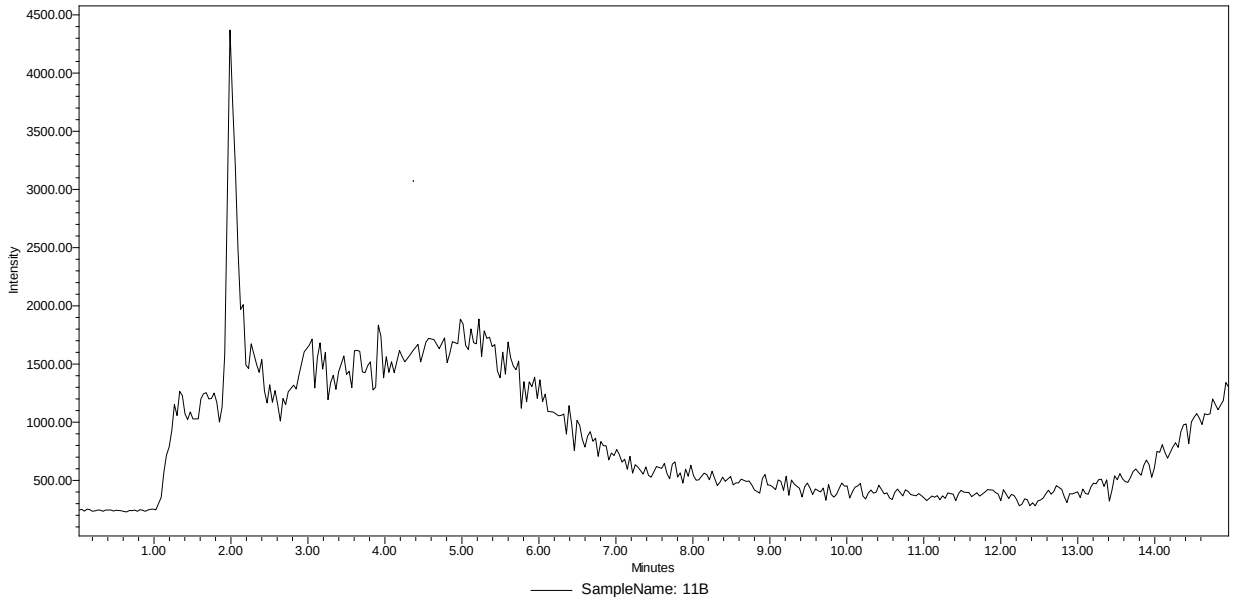

Fig. 27cc Thermal/Acidic study's product chromatograph screening for FMASld sample 11.

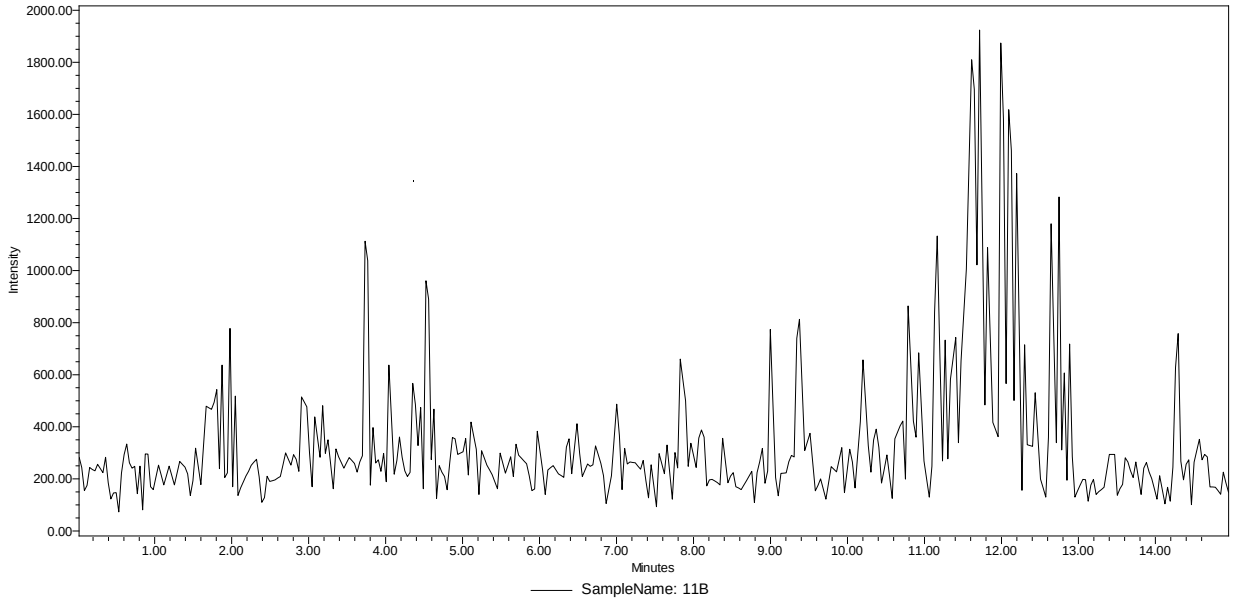

Fig. 27dd Thermal/Acidic study's product chromatograph screening for FBAL sample 11.

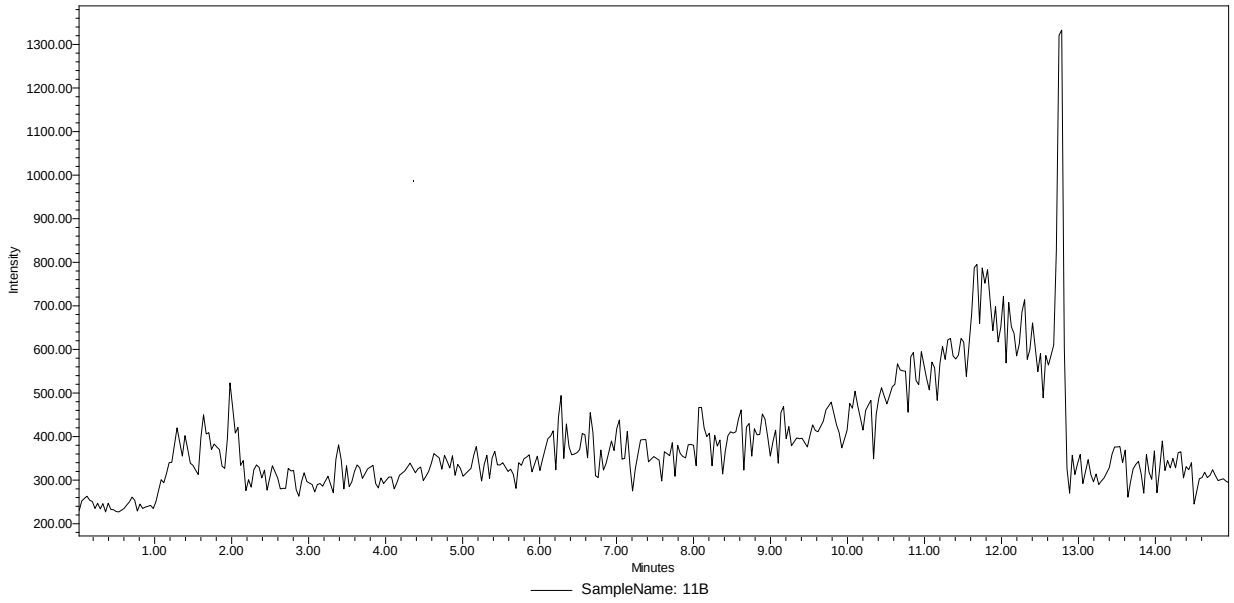

Fig. 27ee Thermal/Acidic study's product chromatograph screening for FUPA sample 11.

129

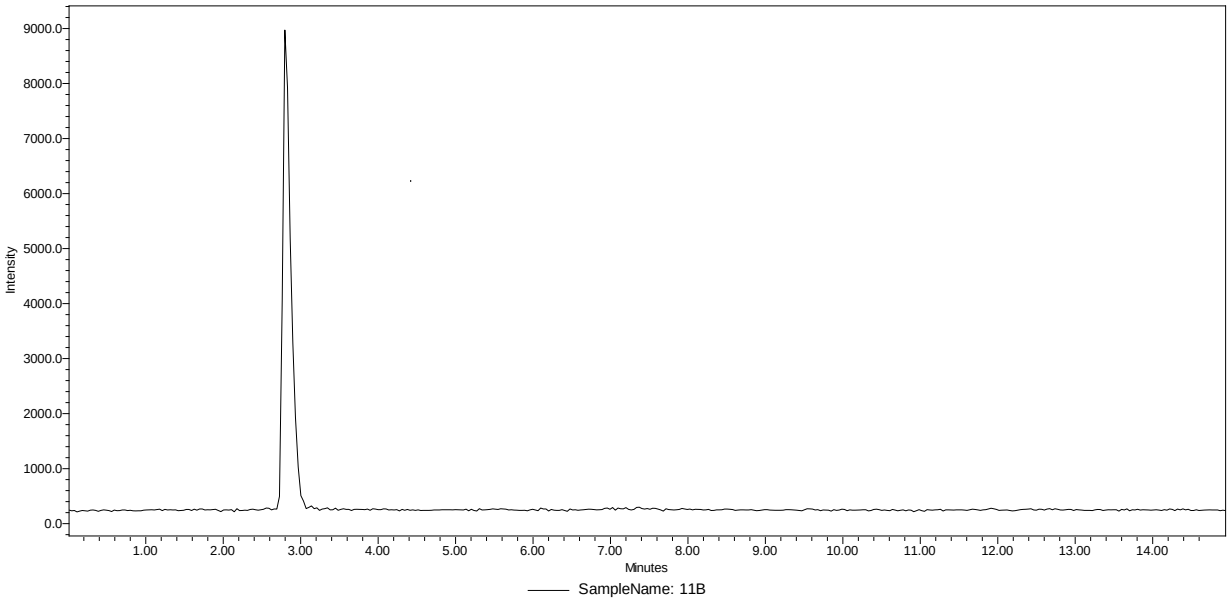

Fig. 27ff Thermal/Acidic study's product chromatograph screening for 5-FU sample 11.

12

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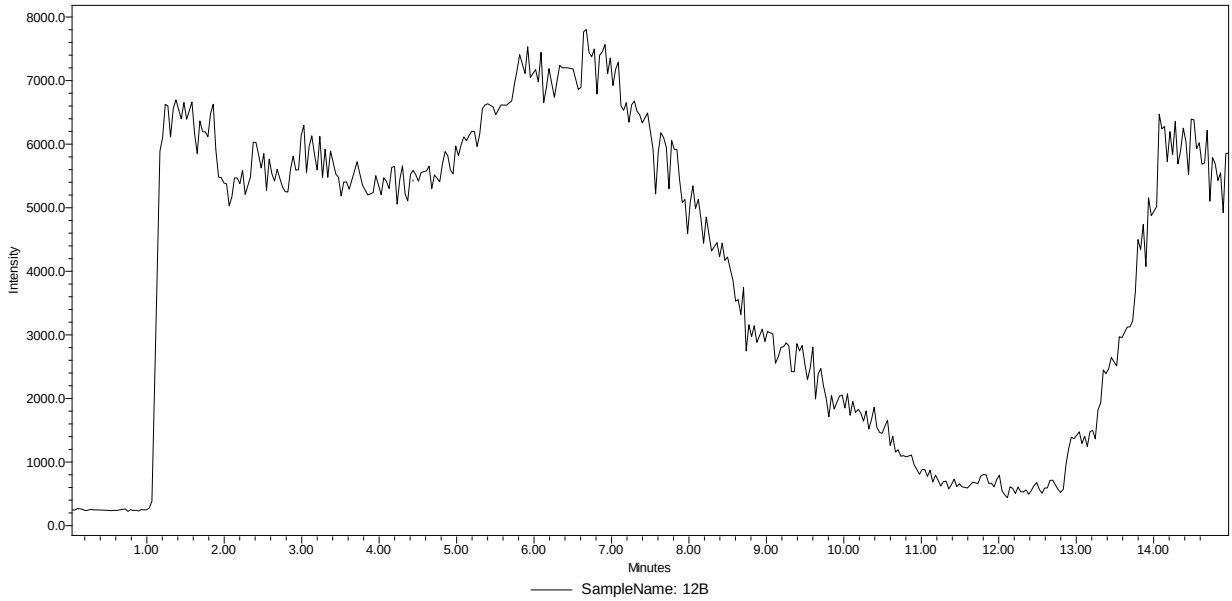

Fig. 27gg Thermal/Acidic study's product chromatograph screening for FAC sample 12.

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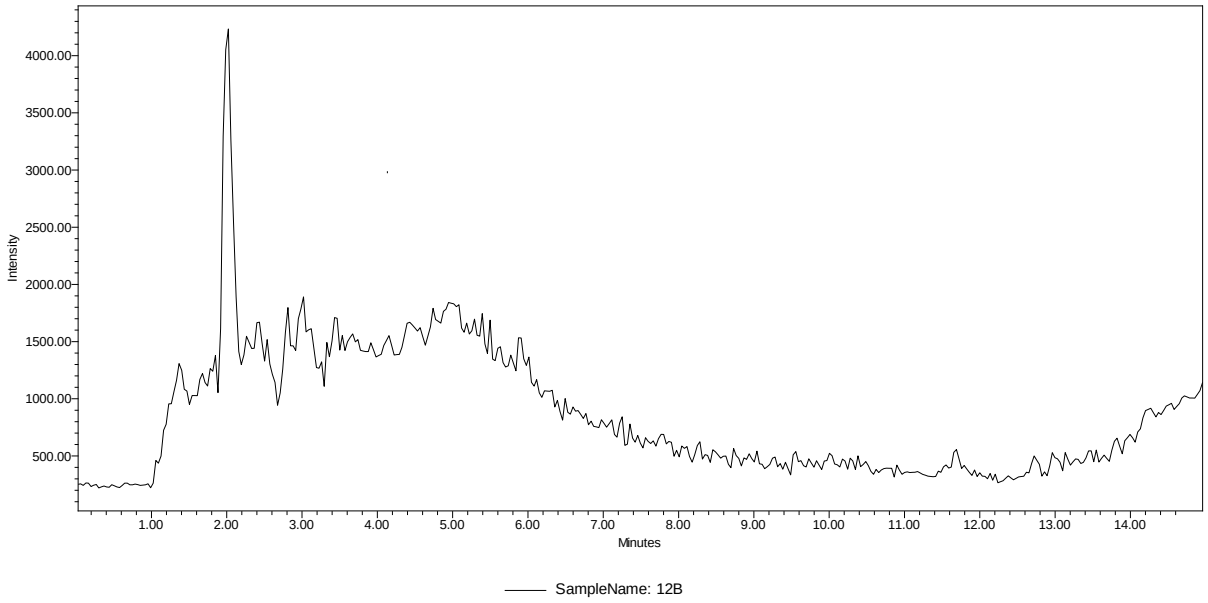

Fig. 27hh Thermal/Acidic study's product chromatograph screening for FMASld sample 12.

106

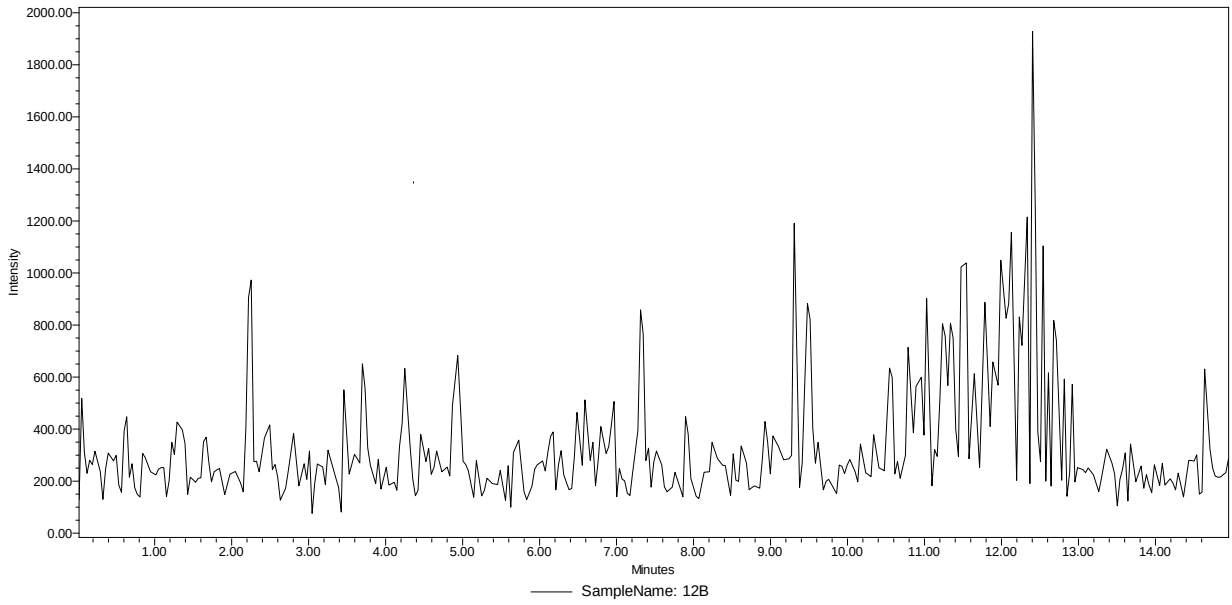

Fig. 27ii Thermal/Acidic study's product chromatograph screening for FBAL sample 12.

149

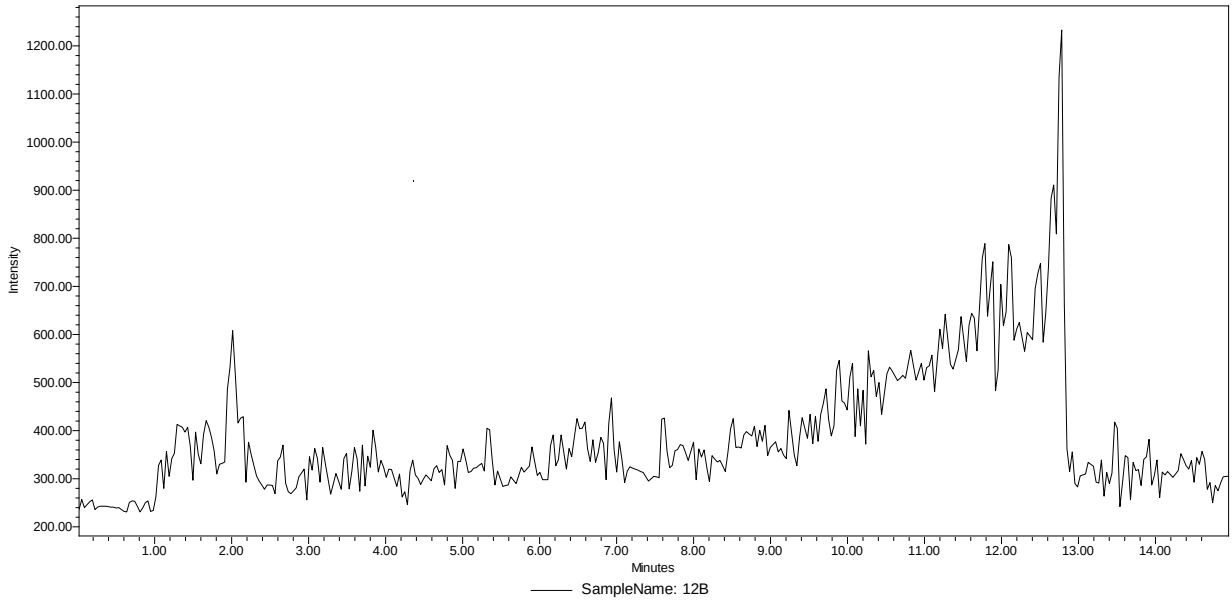

Fig. 27jj Thermal/Acidic study's product chromatograph screening for FUPA sample 12.

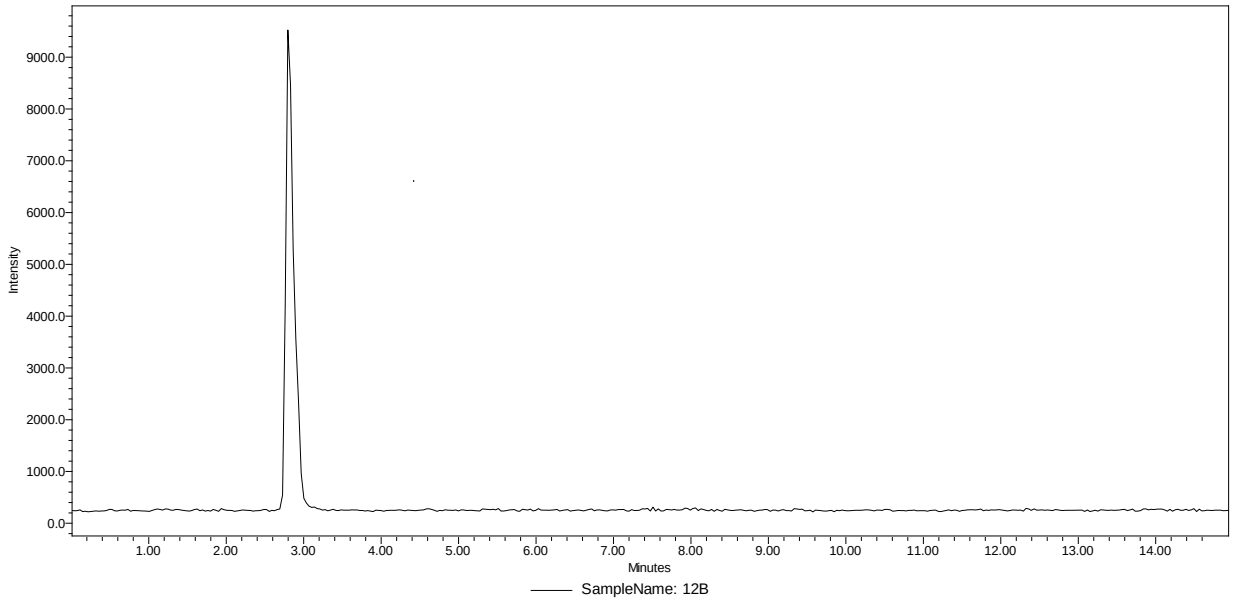

Fig. 27kk Thermal/Acidic study's product chromatograph screening for 5-FU sample 12.

13

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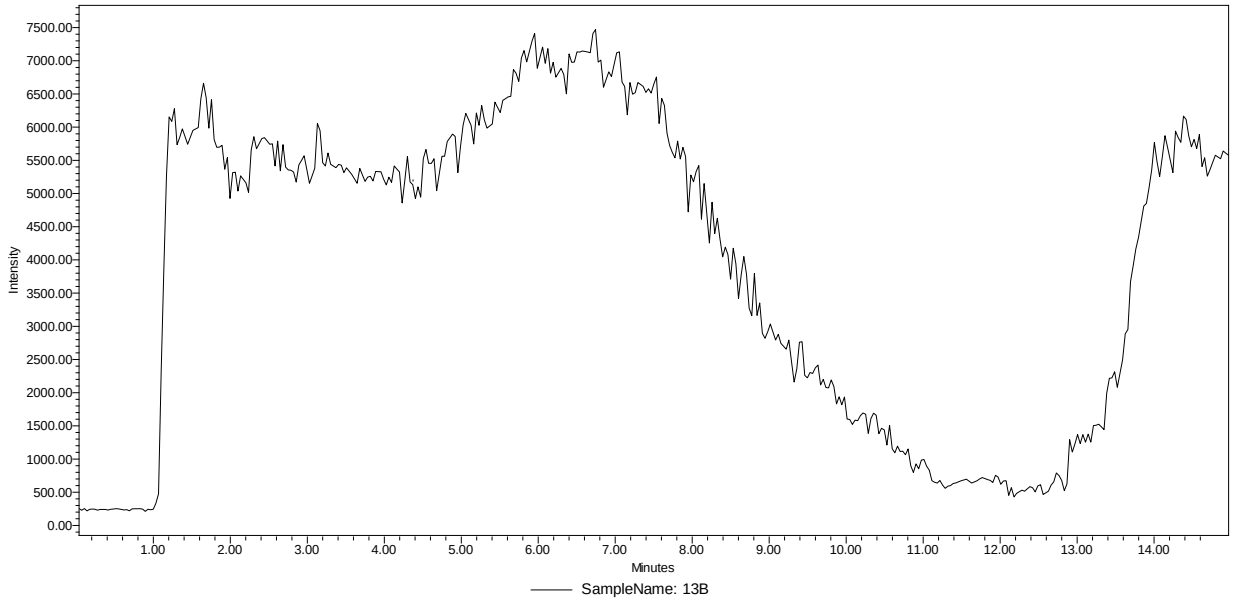

Fig. 27ll Thermal/Acidic study's product chromatograph screening for FAC sample 13.

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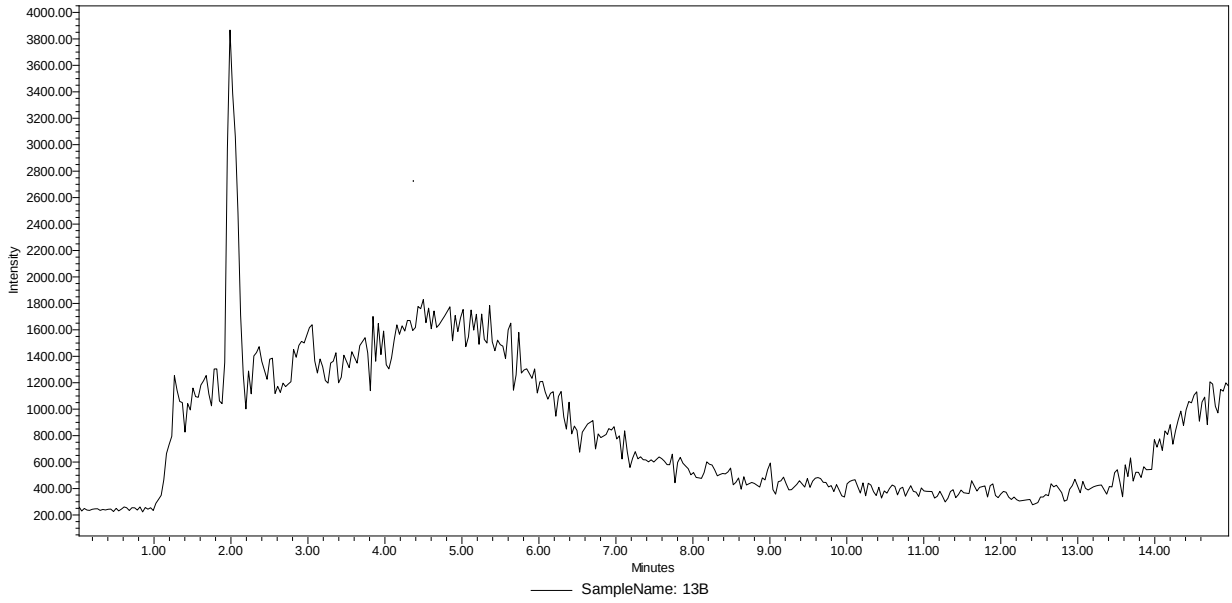

Fig. 27mm Thermal/Acidic study's product chromatograph screening for FMASld sample 13.

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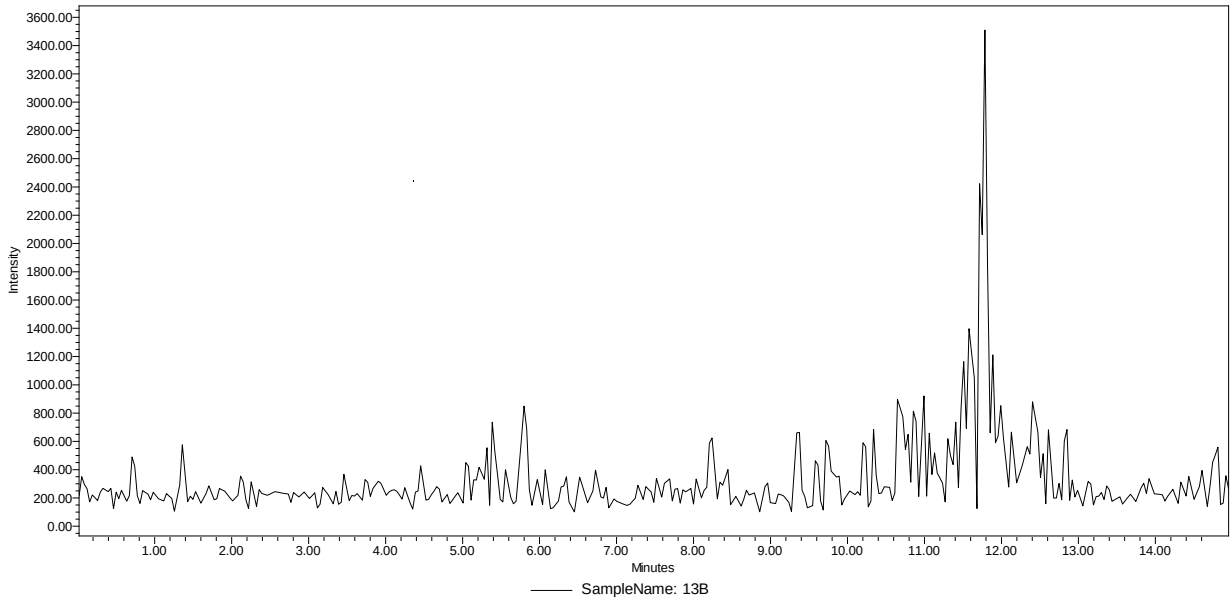

Fig. 27nn Thermal/Acidic study's product chromatograph screening for FBAL sample 13.

149

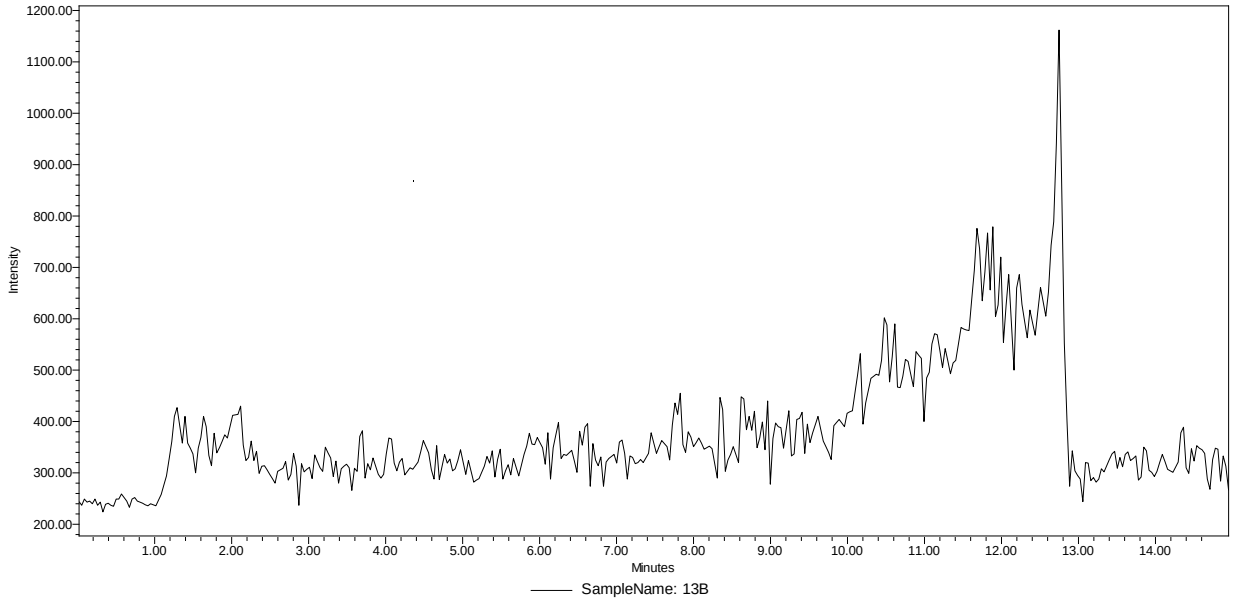

Fig. 27oo Thermal/Acidic study's product chromatograph screening for FUPA sample 13.

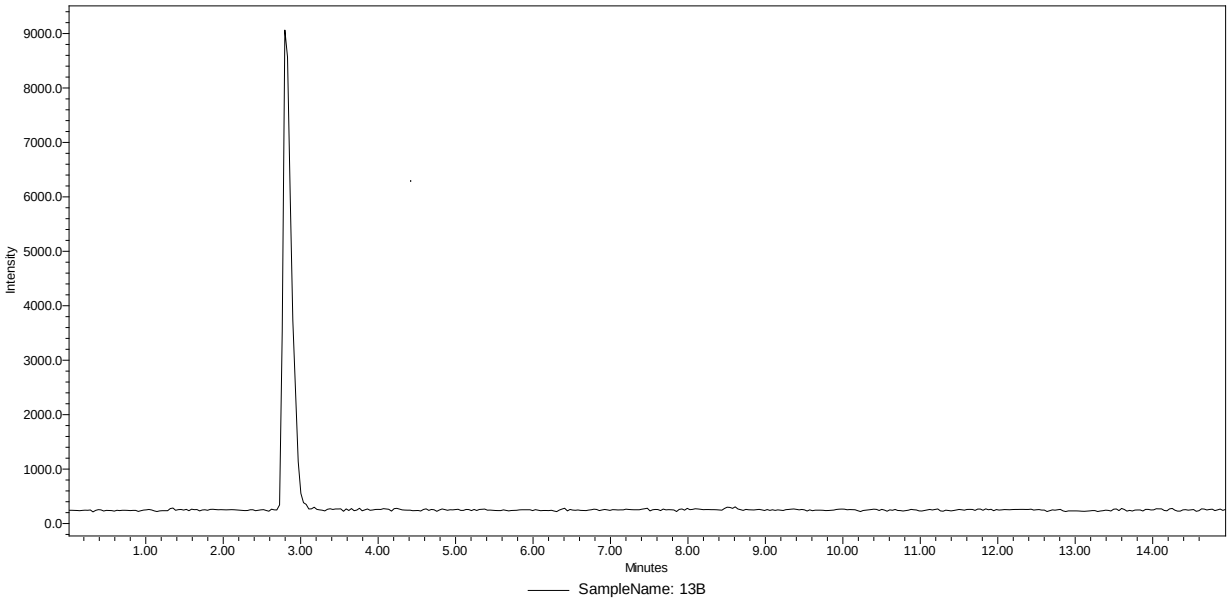

Fig. 27pp Thermal/Acidic study's product chromatograph screening for 5-FU sample 13.

14

77

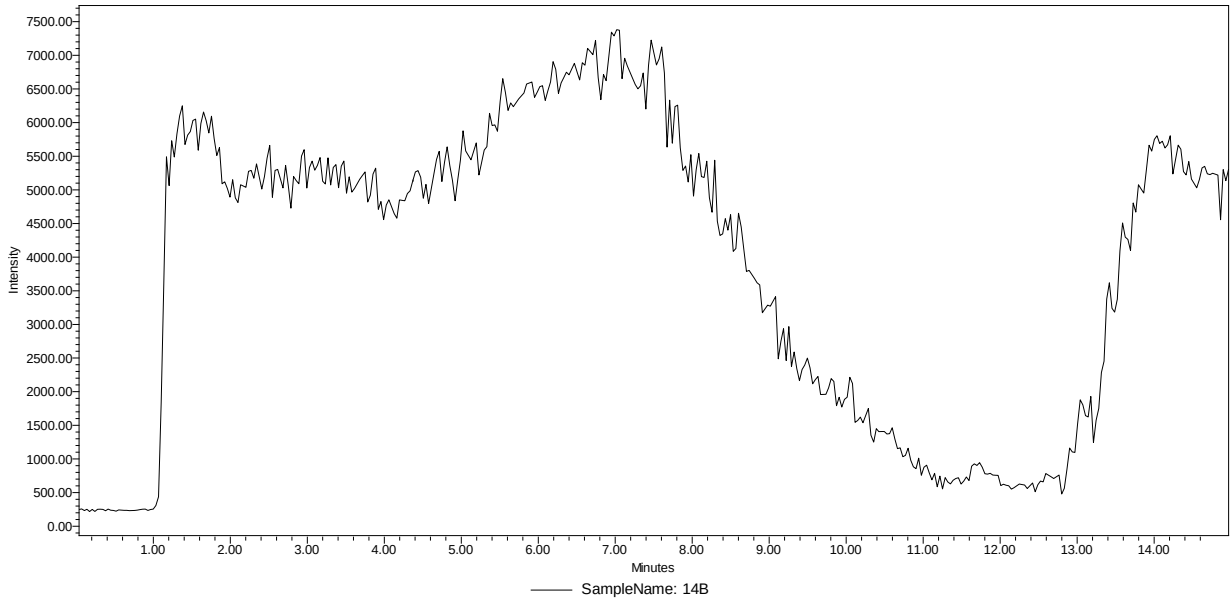

Fig. 27qq Thermal/Acidic study's product chromatograph screening for FAC sample 14.

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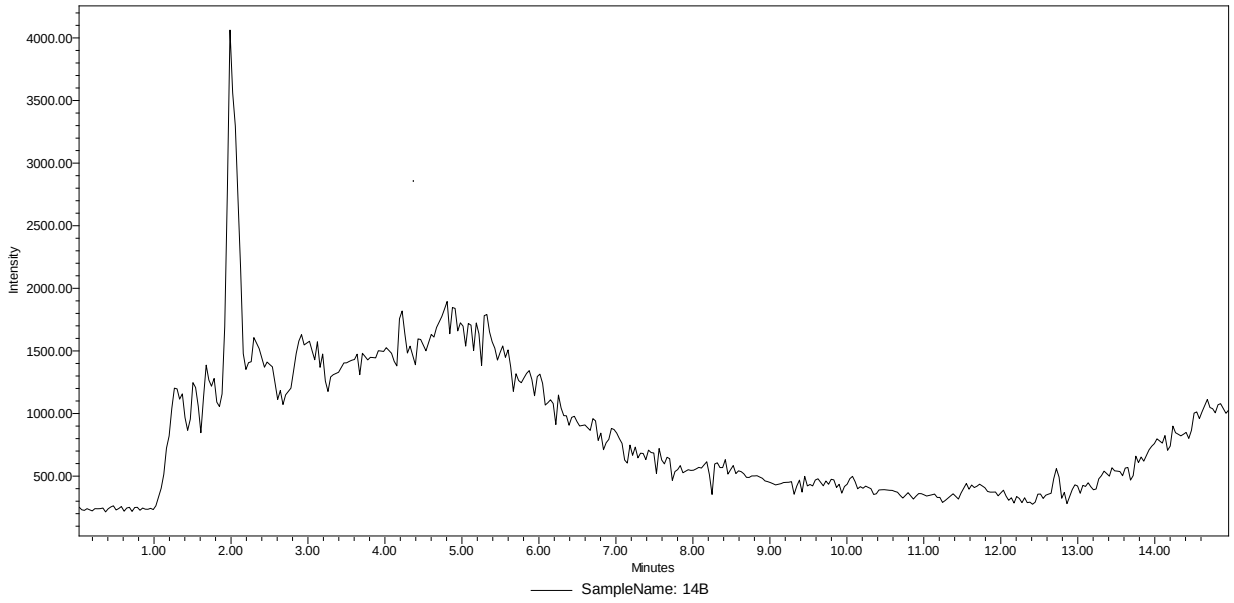

Fig. 27rr Thermal/Acidic study's product chromatograph screening for FMASld sample 14.

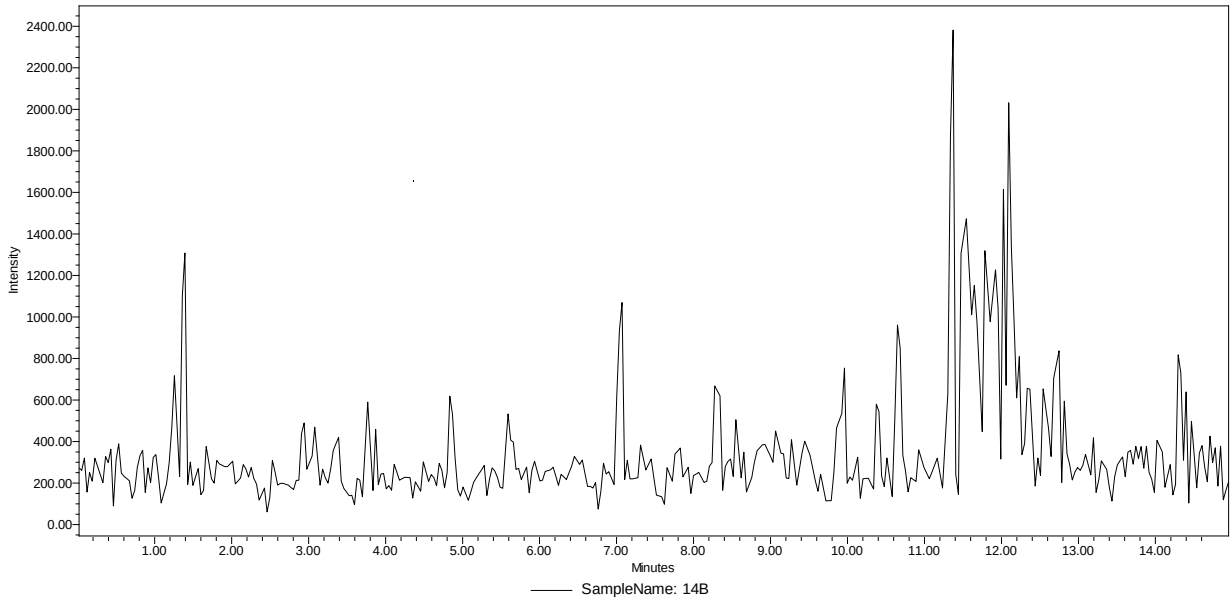

Fig. 27ss Thermal/Acidic study's product chromatograph screening for FBAL sample 14.

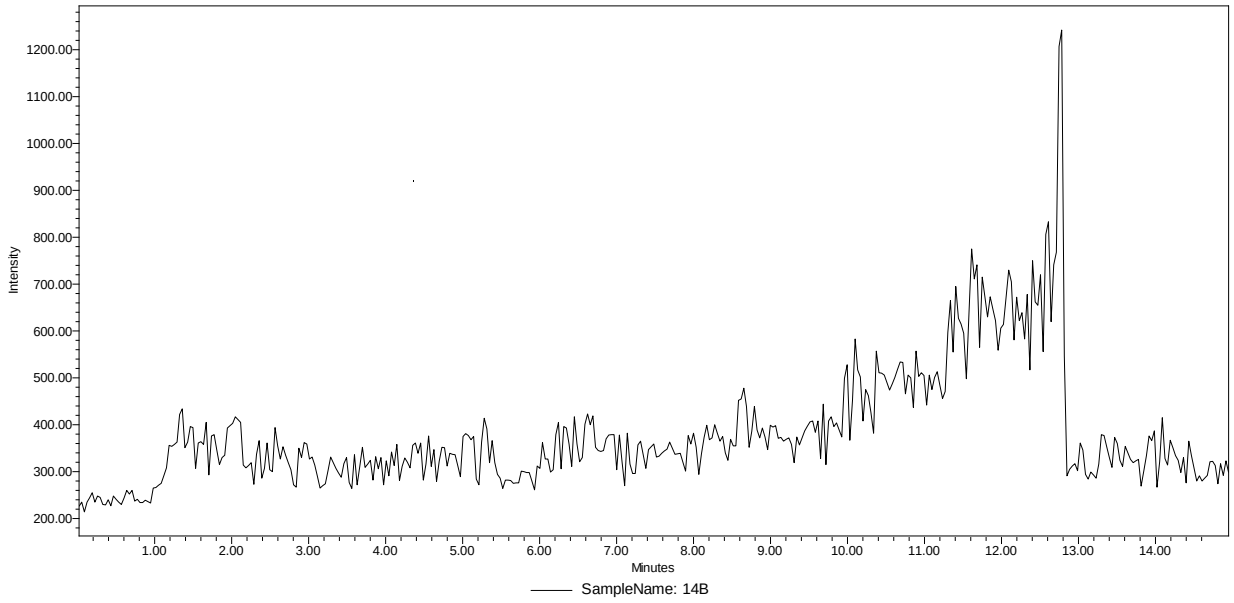

Fig. 27tt Thermal/Acidic study's product chromatograph screening for FUPA sample 14.

129

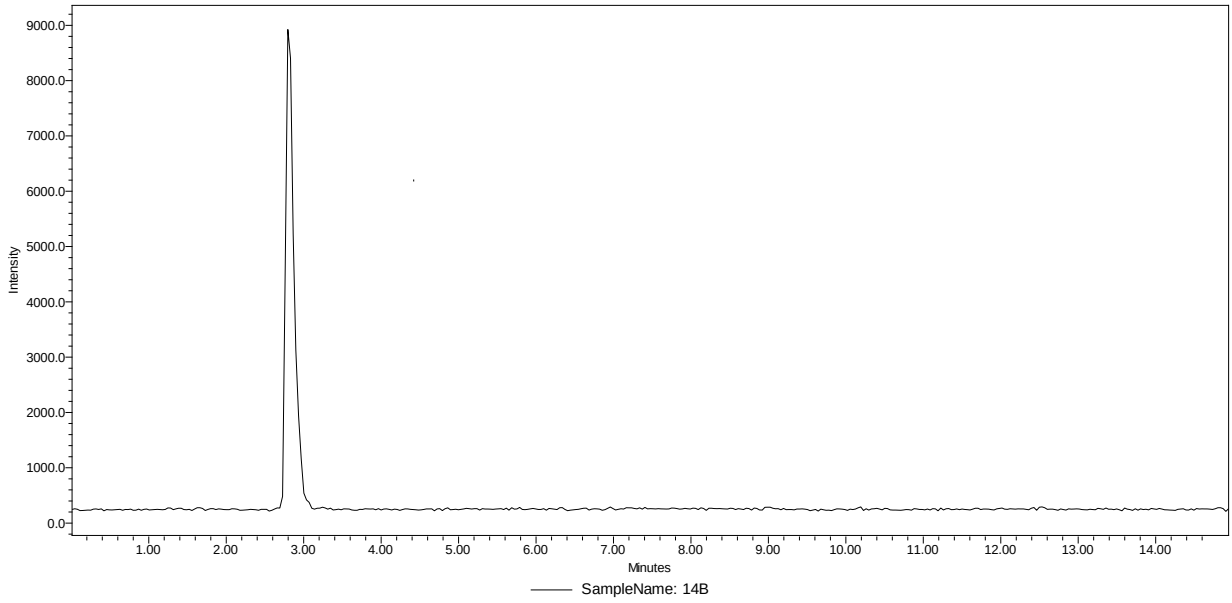

Fig. 27uu Thermal/Acidic study's product chromatograph screening for 5-FU sample 14.

15

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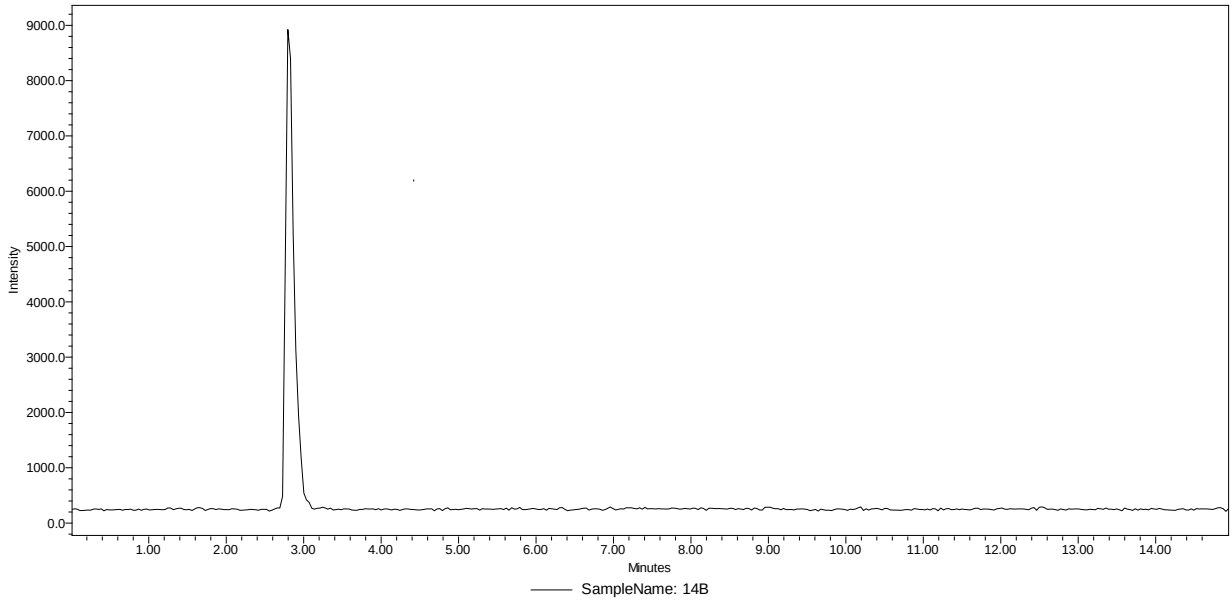

Fig. 27vv Thermal/Acidic study's product chromatograph screening for FAC sample 15.

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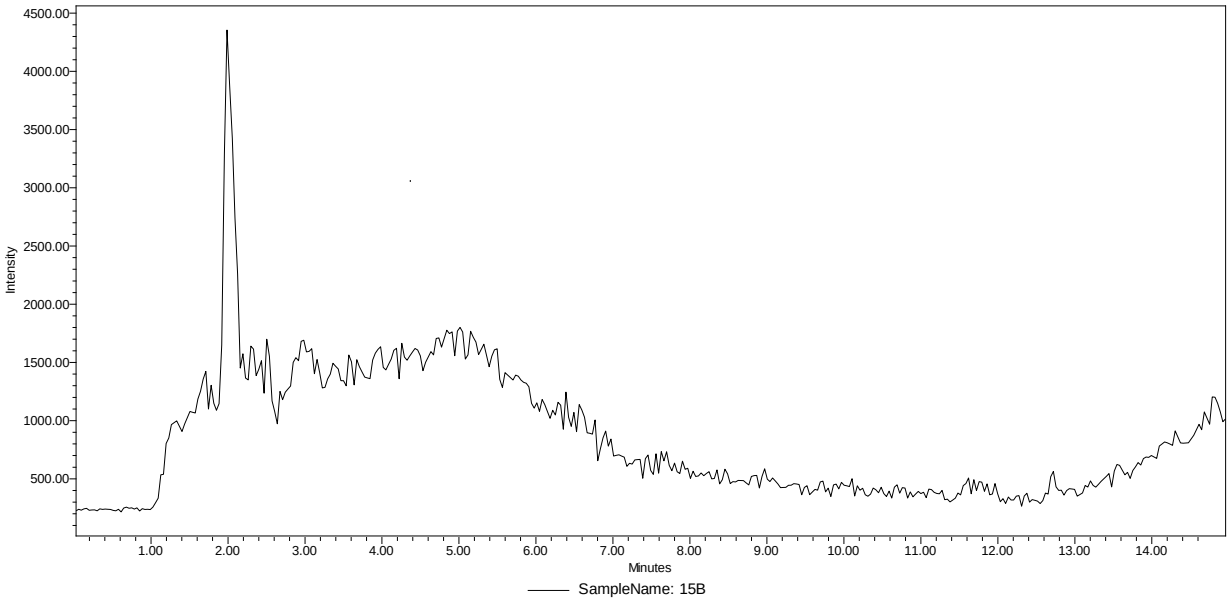

Fig. 27ww Thermal/Acidic study's product chromatograph screening for FMASld sample 15.

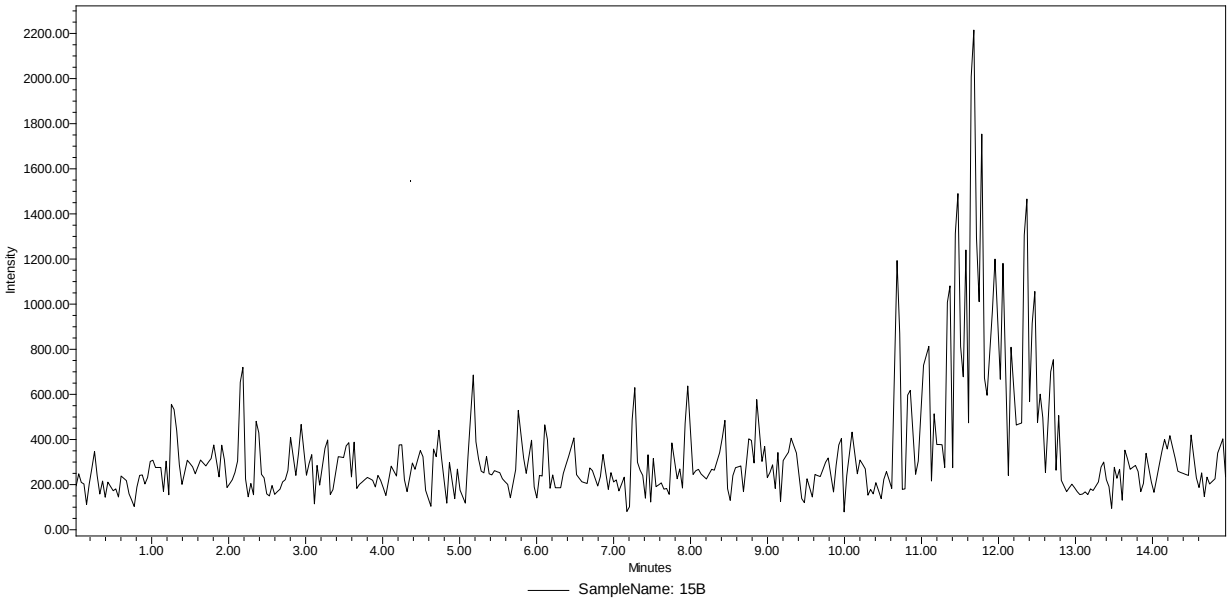

Fig. 27xx Thermal/Acidic study's product chromatograph screening for FBAL sample 15.

149

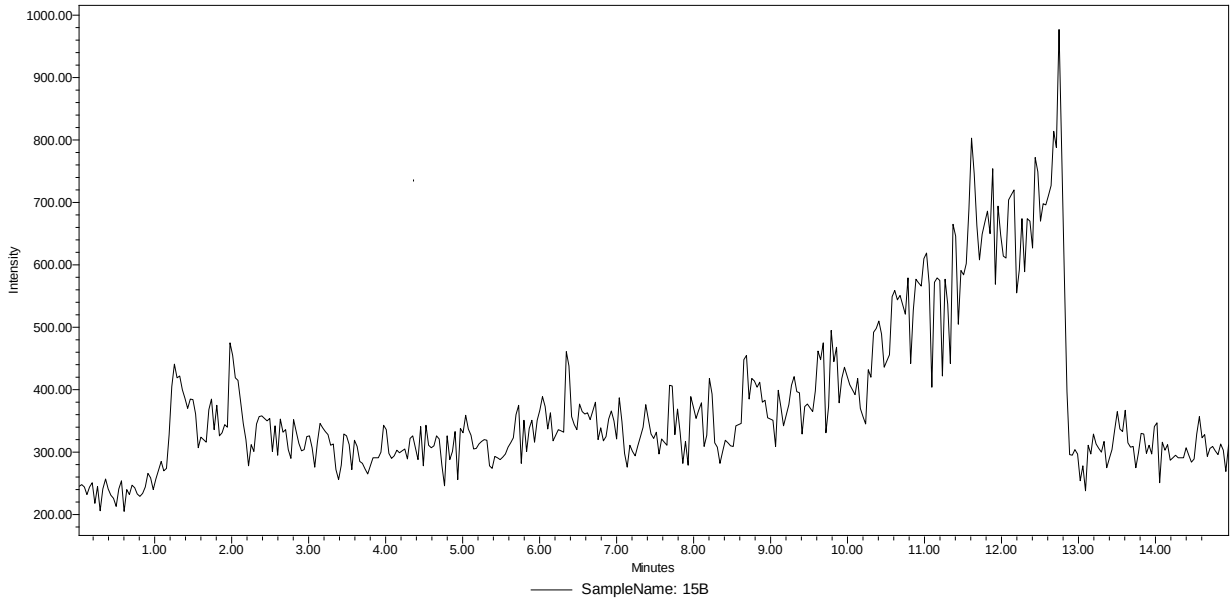

Fig. 27yy Thermal/Acidic study's product chromatograph screening for FUPA sample 15.

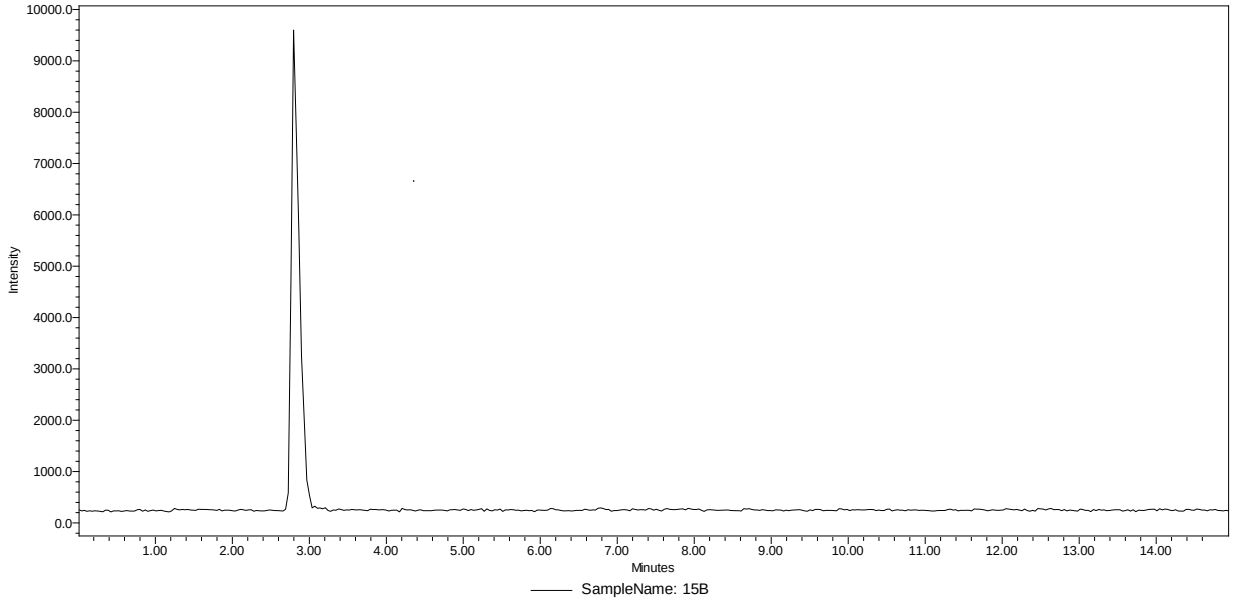

Fig. 27zz Thermal/Acidic study's product chromatograph screening for 5-FU sample 15.

16

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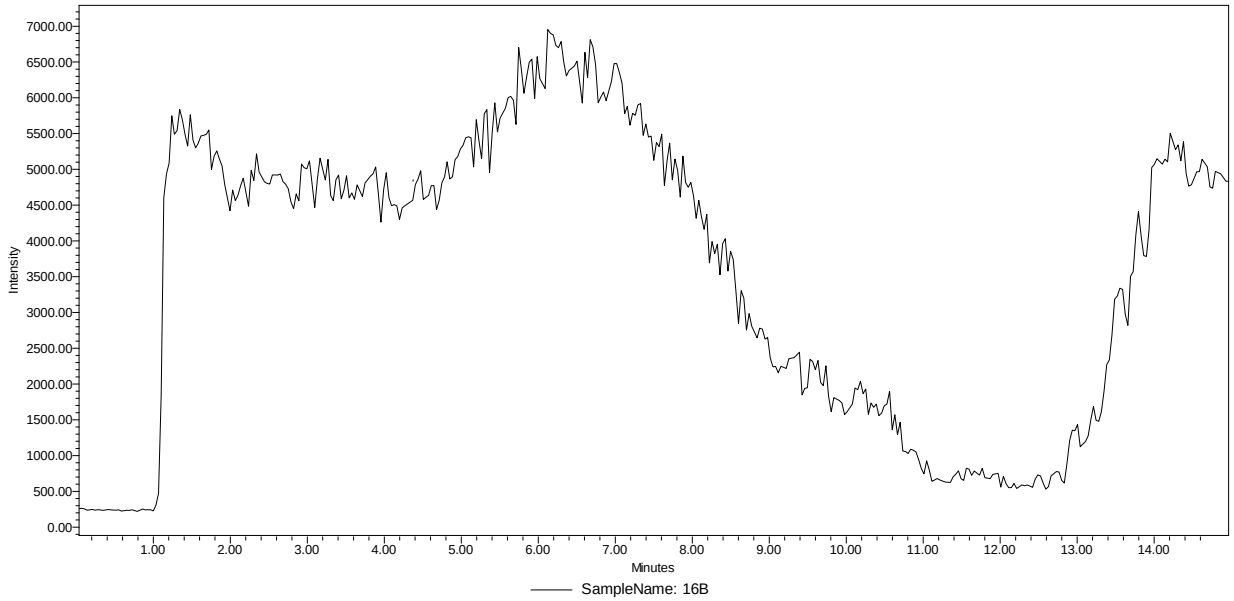

Fig. 27aaa Thermal/Acidic study's product chromatograph screening for FAC sample 16.

105

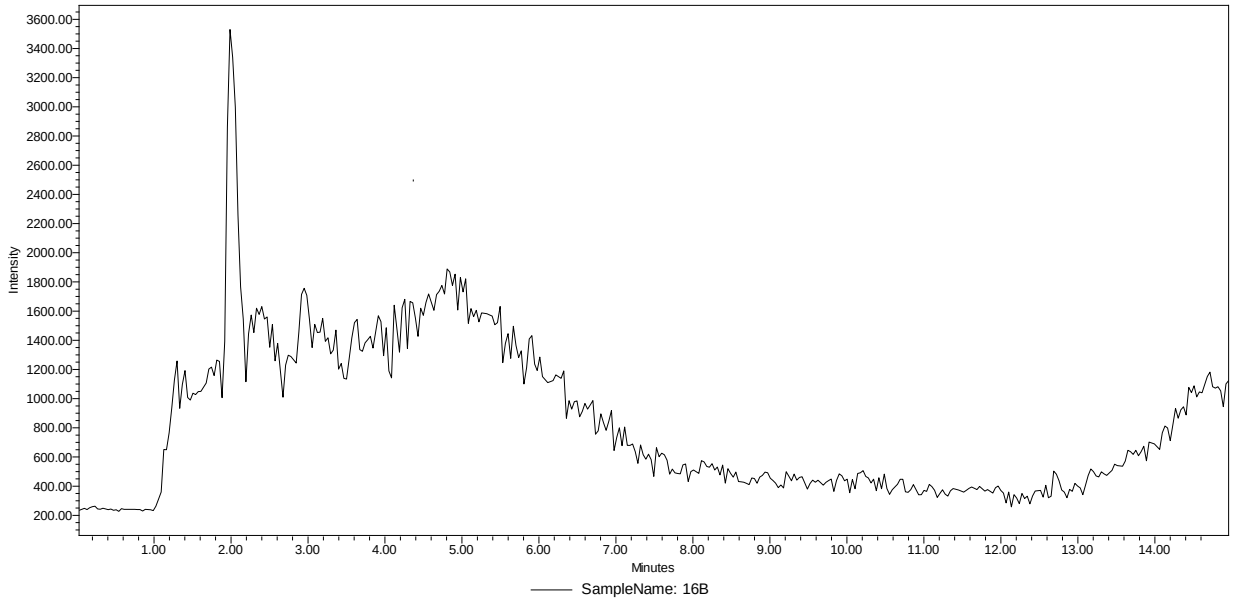

Fig. 27bbb Thermal/Acidic study's product chromatograph screening for FMASld sample 16.

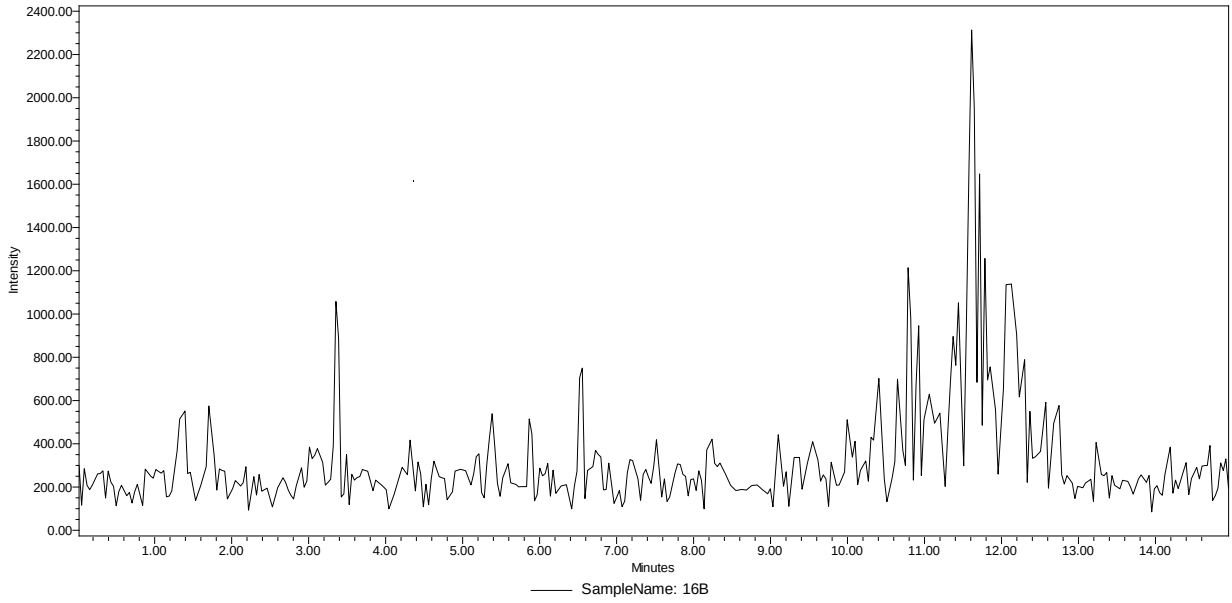

Fig. 27ccc Thermal/Acidic study's product chromatograph screening for FBAL sample 16.

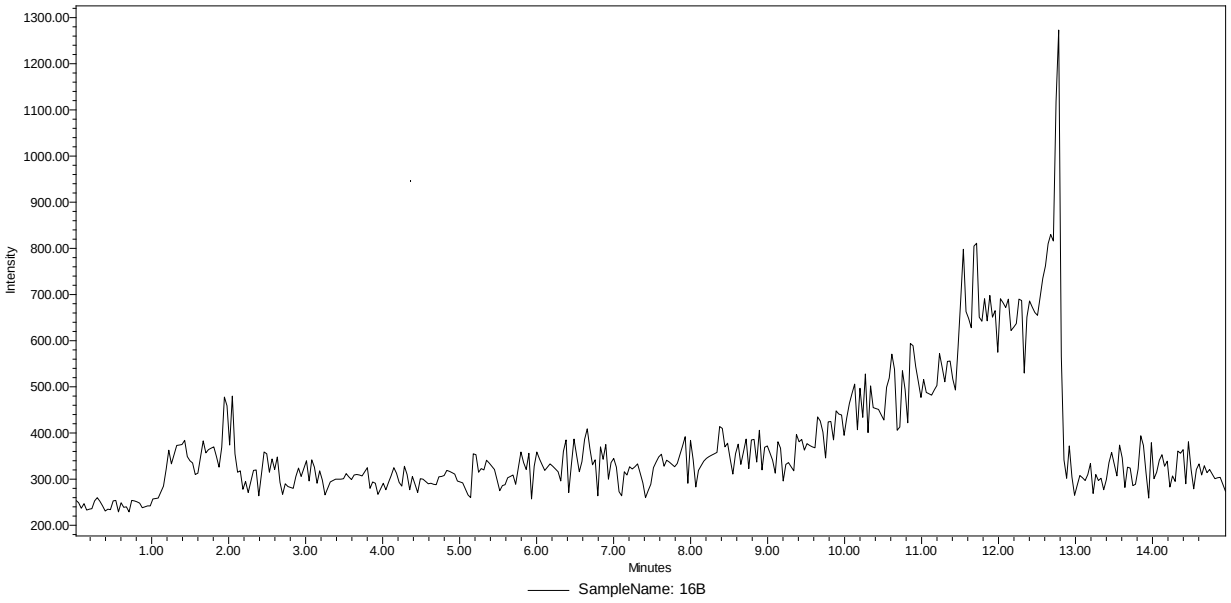

Fig. 27ddd Thermal/Acidic study's product chromatograph screening for FUPA sample 16.

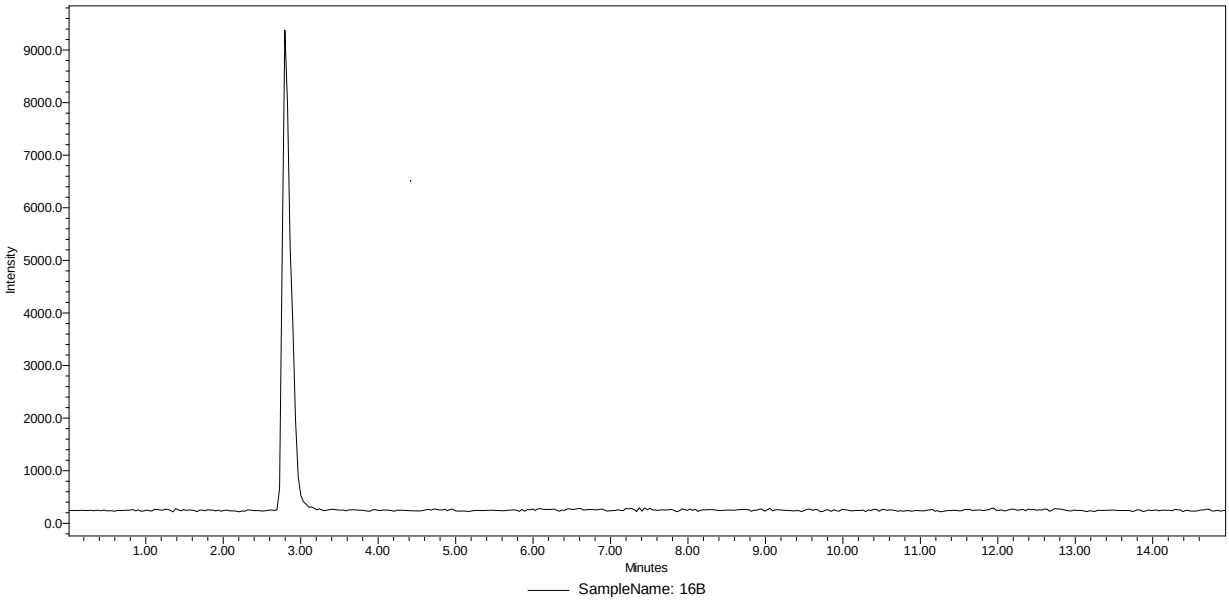

Fig. 27eee Thermal/Acidic study's product chromatograph screening for 5-FU sample 16.

17

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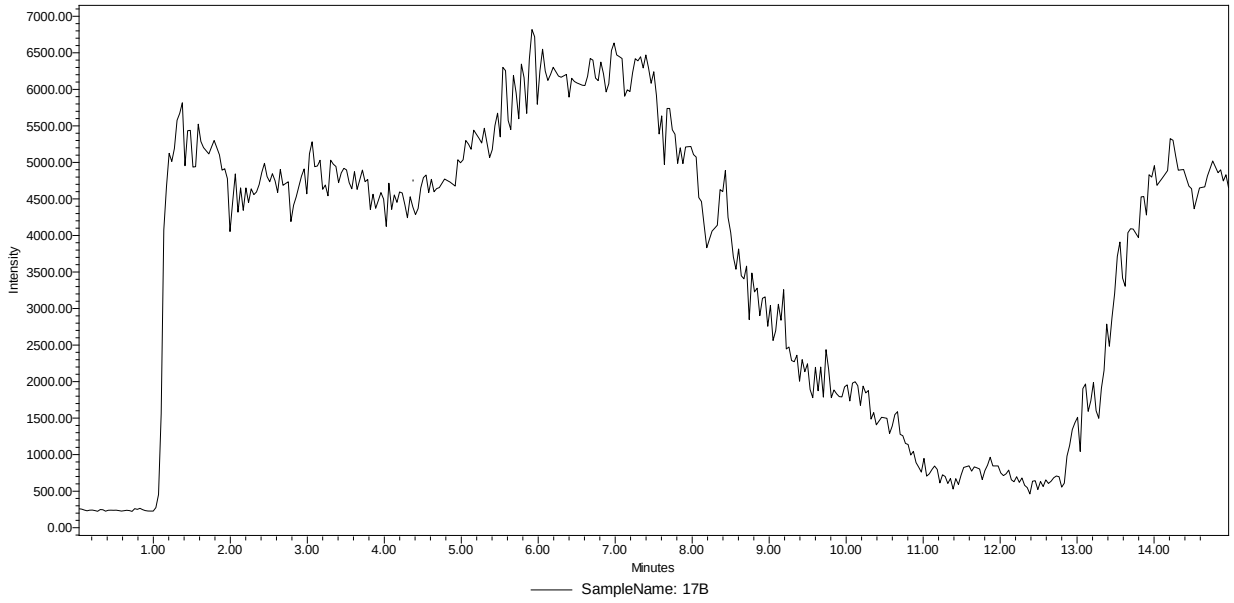

Fig. 27fff Thermal/Acidic study's product chromatograph screening for FAC sample 17.

105

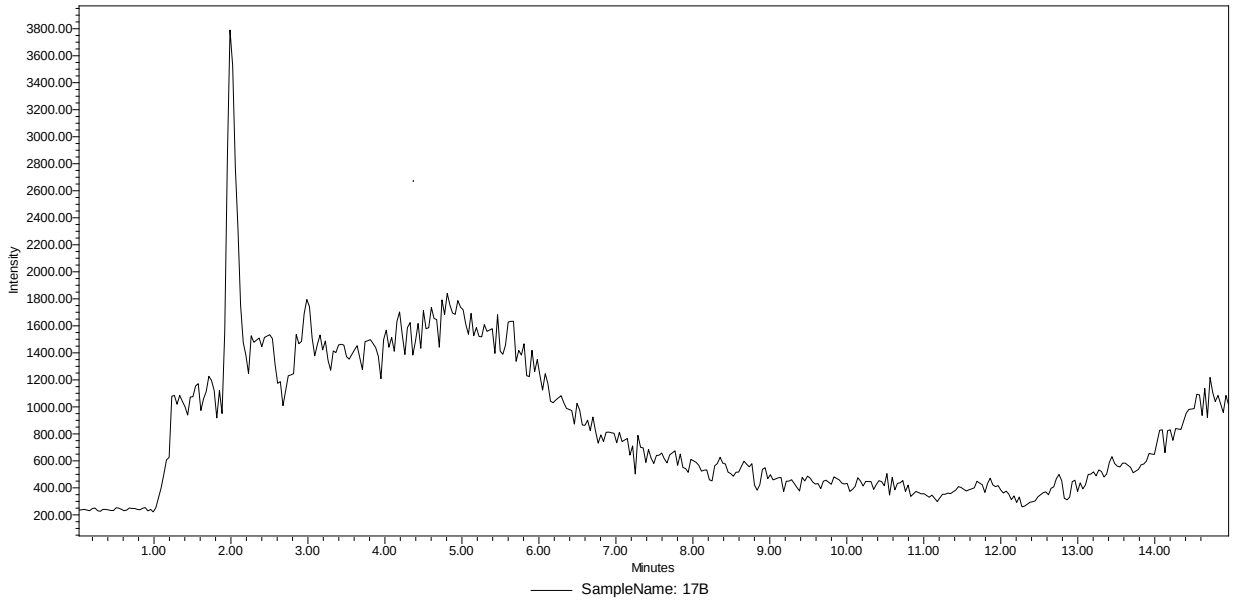

Fig. 27ggg Thermal/Acidic study's product chromatograph screening for FMASld sample 17.

106

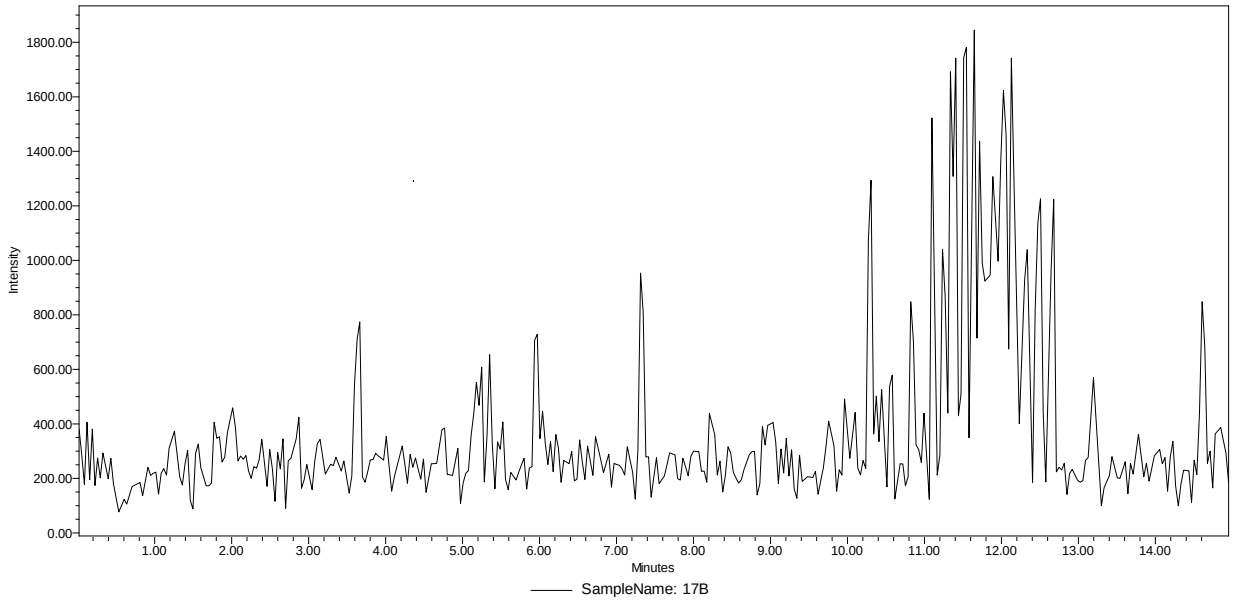

Fig. 27hhh Thermal/Acidic study's product chromatograph screening for FBAL sample 17.

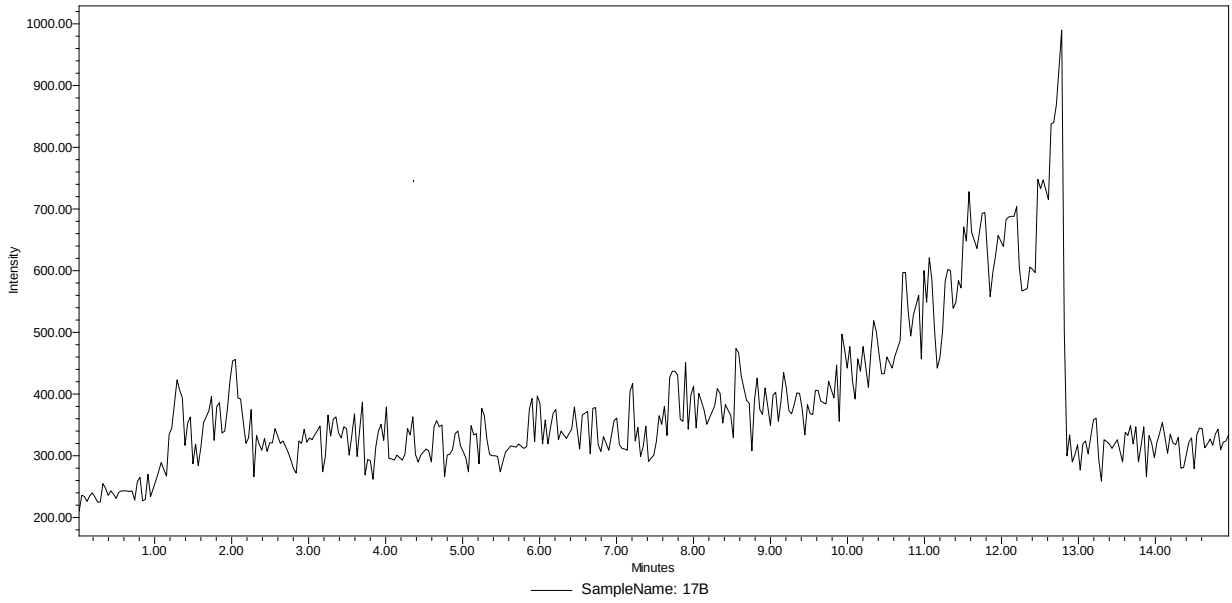

Fig. 27iii Thermal/Acidic study's product chromatograph screening for FUPA sample 17.

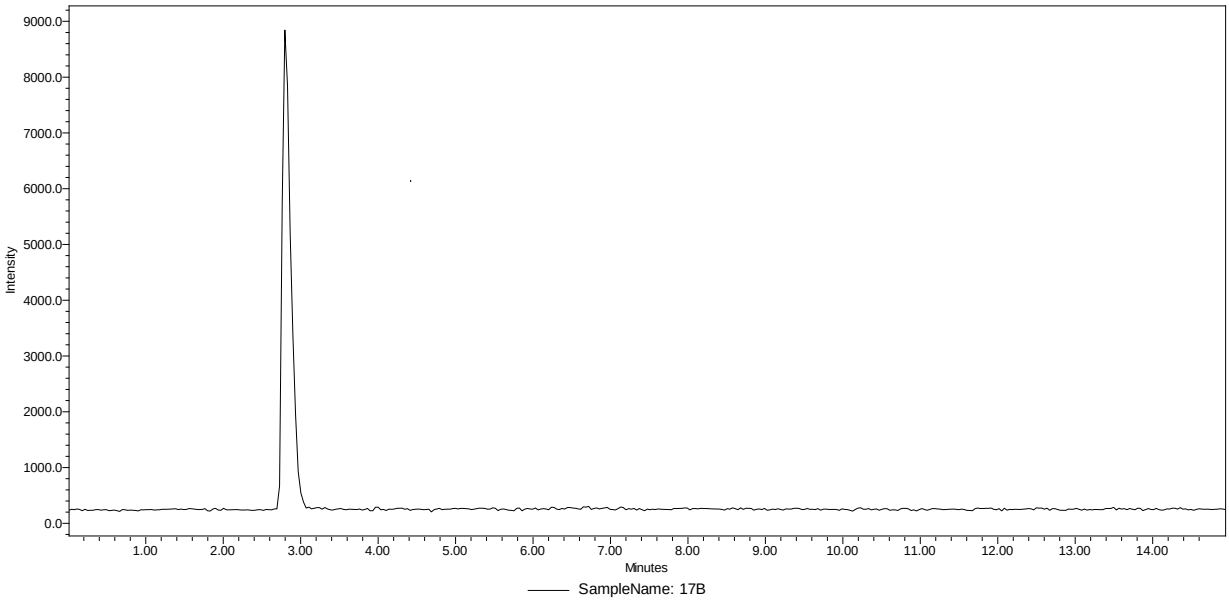

Fig. 27jjj Thermal/Acidic study's product chromatograph screening for 5-FU sample 17.

18

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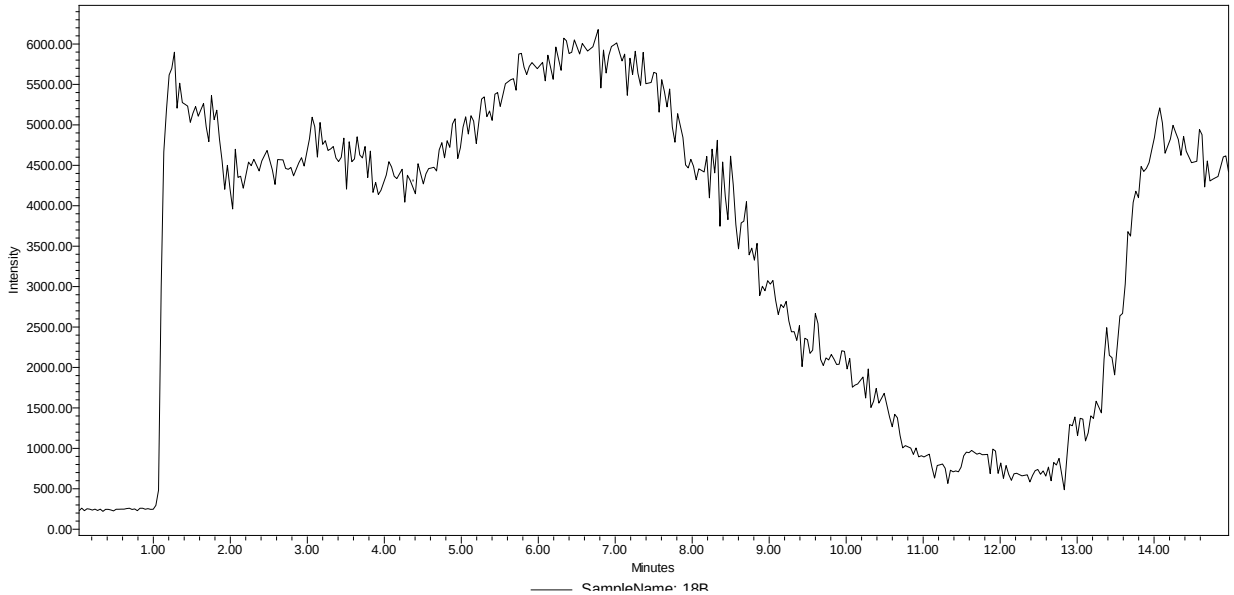

Fig. 27iii Thermal/Acidic study's product chromatograph screening for FAC sample 18.

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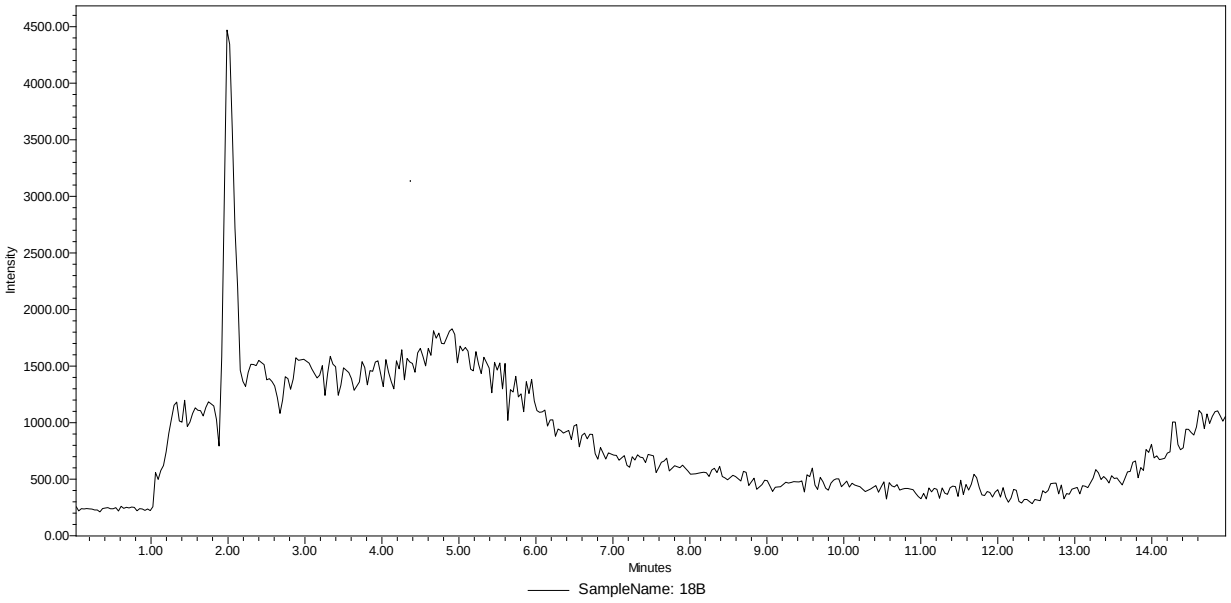

Fig. 27jjj Thermal/Acidic study's product chromatograph screening for FMASld sample 18.

106

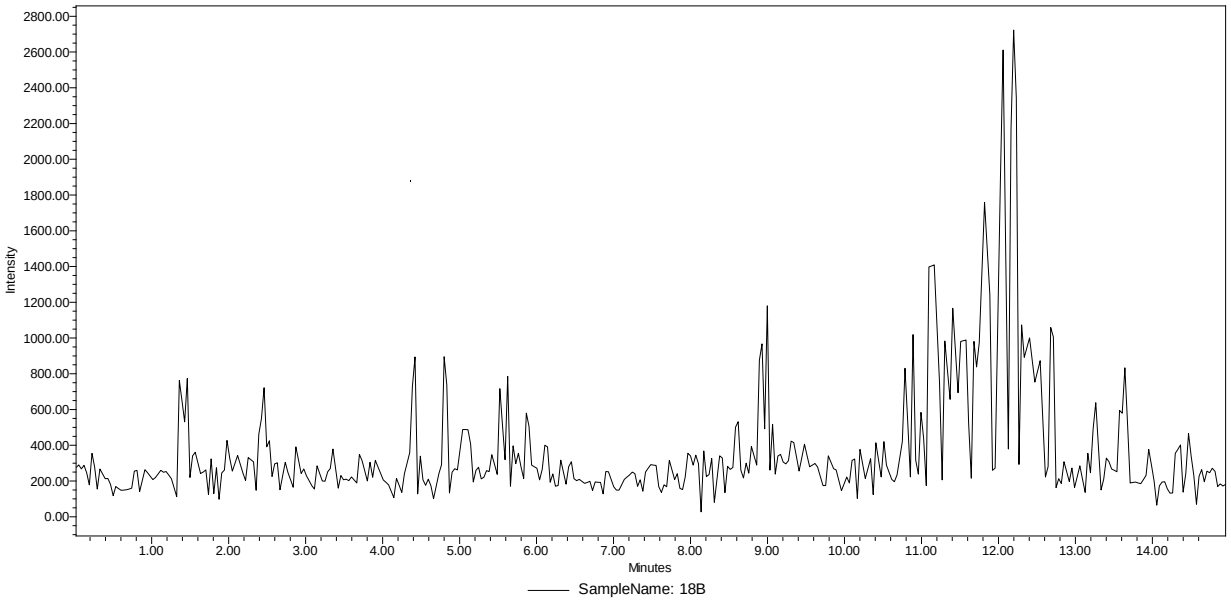

Fig. 27kkk Thermal/Acidic study's product chromatograph screening for FBAL sample 18.

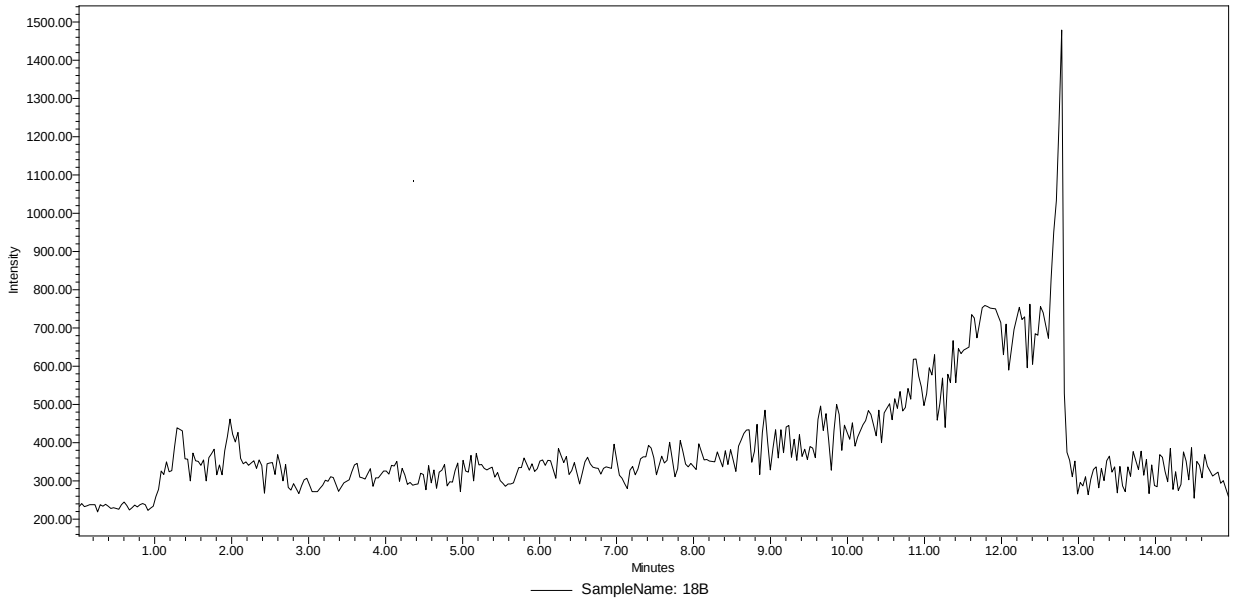

Fig. 27lll Thermal/Acidic study's product chromatograph screening for FUPA sample 18.

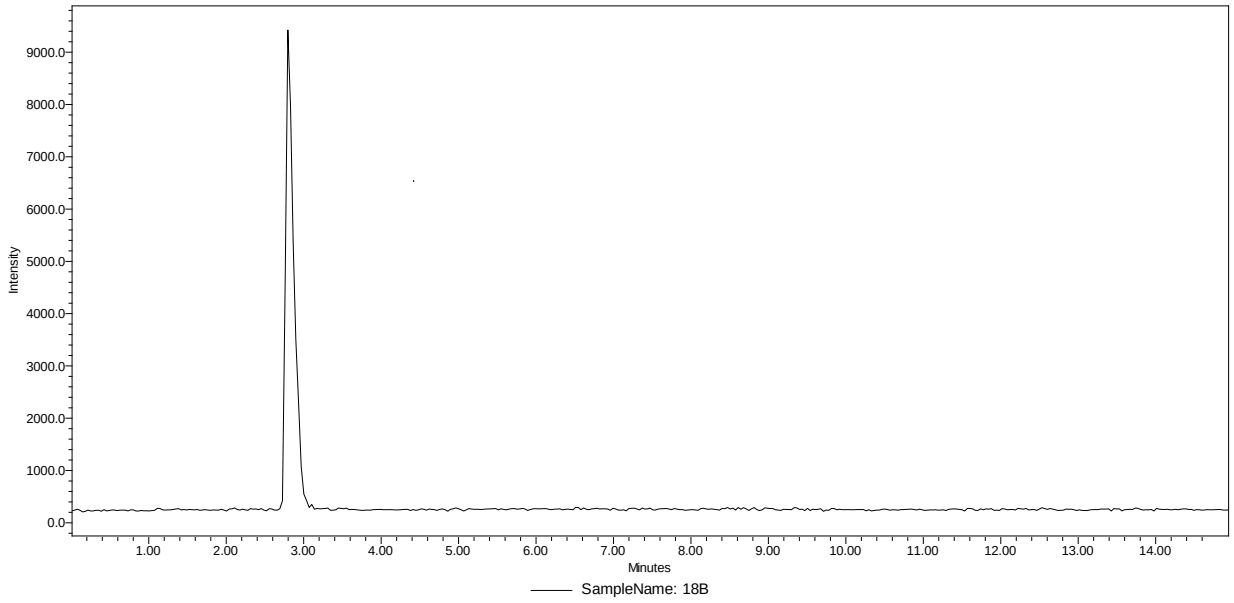

Fig. 27mmm Thermal/Acidic study's product chromatograph screening for 5-FU sample 18.

19

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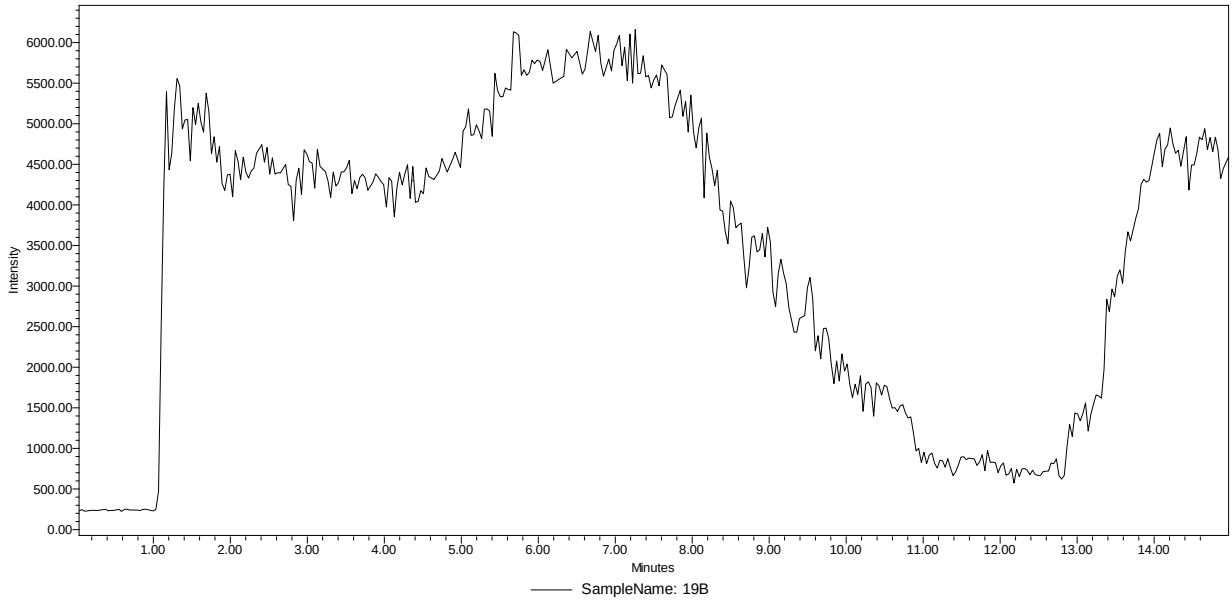

Fig. 27nnn Thermal/Acidic study's product chromatograph screening for FAC sample 19.

105

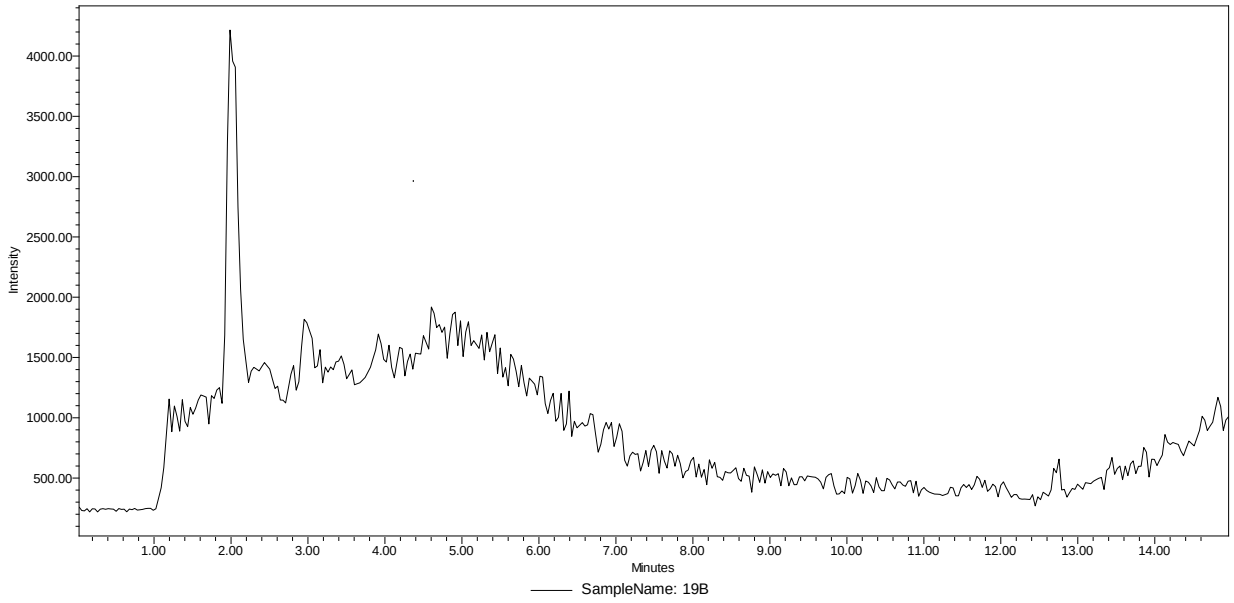

Fig. 27ooo Thermal/Acidic study's product chromatograph screening for FMASld sample 19.

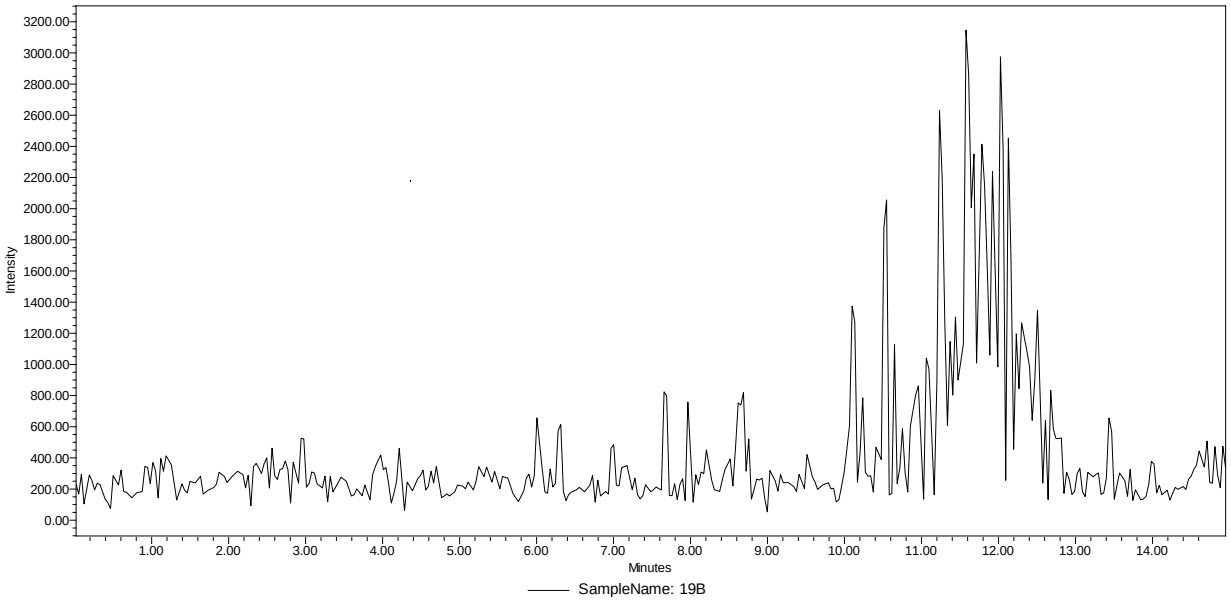

Fig. 27ppp Thermal/Acidic study's product chromatograph screening for FBAL sample 19.

149

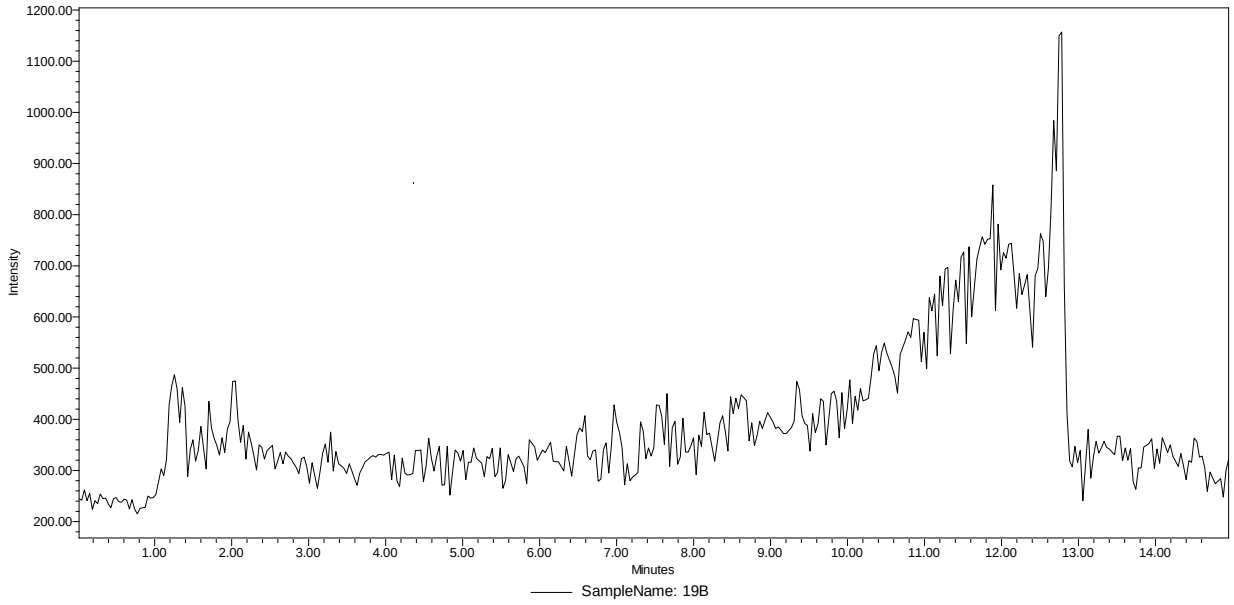

Fig. 27qqq Thermal/Acidic study's product chromatograph screening for FUPA sample 19.

129

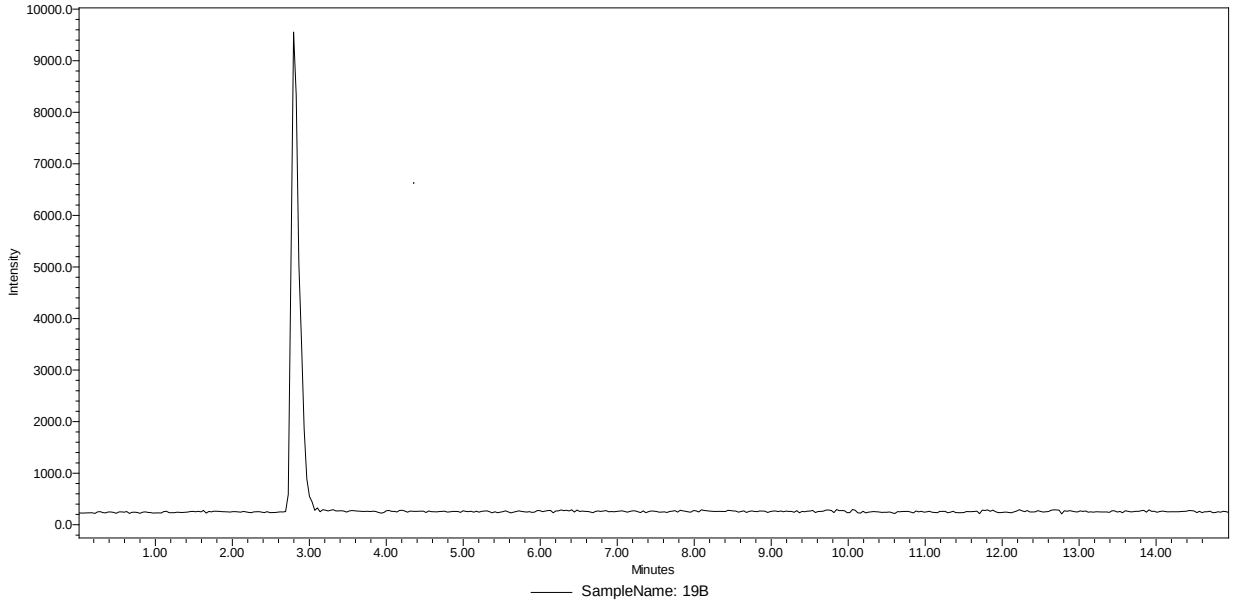

Fig. 27rrr Thermal/Acidic study's product chromatograph screening for 5-FU sample 19.

1

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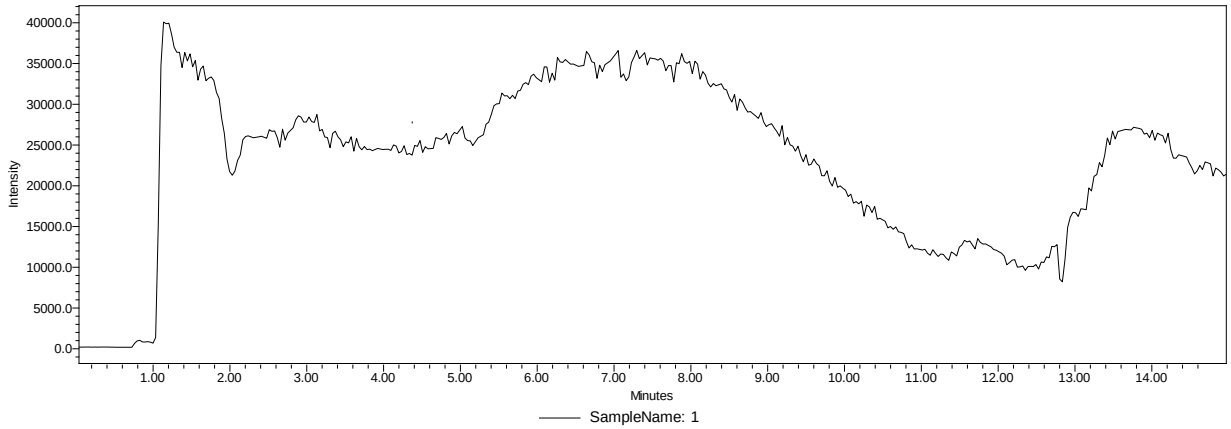

Fig. 28(1) product chromatograph for 5-FUr 1st replicate/screen for FAC.

105

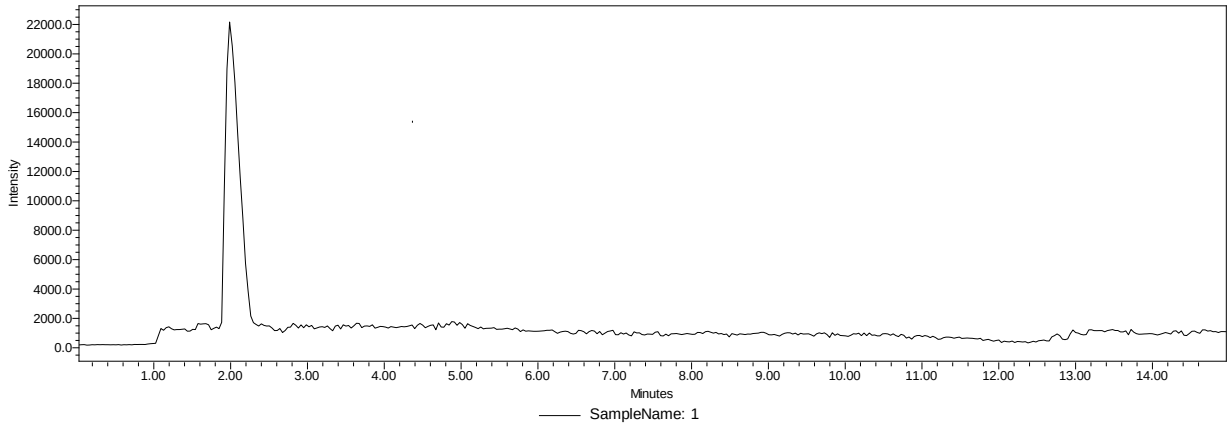

Fig. 28(2) product chromatograph for 5-FUr 1st replicate/screen for FMASld.

106

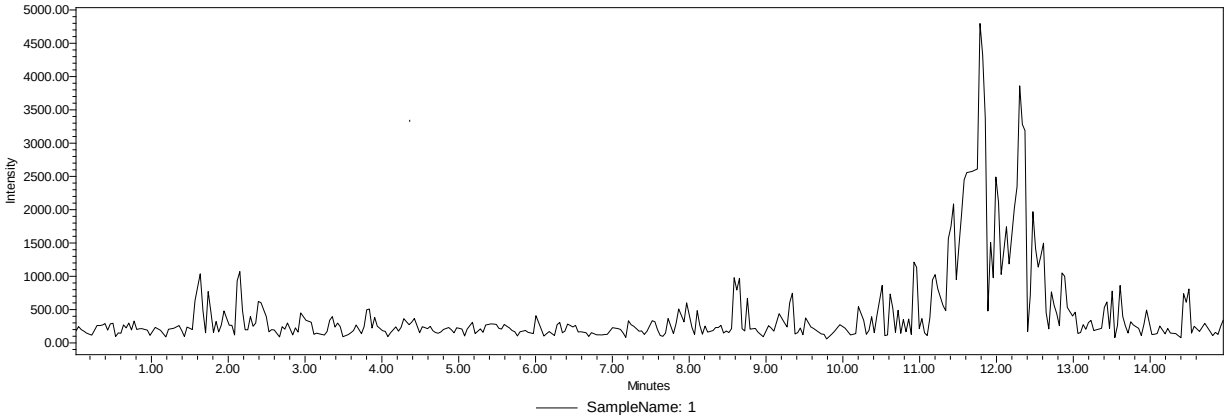

Fig. 28(3) product chromatograph for 5-FUr 1st replicate/screen for FBAL.

149

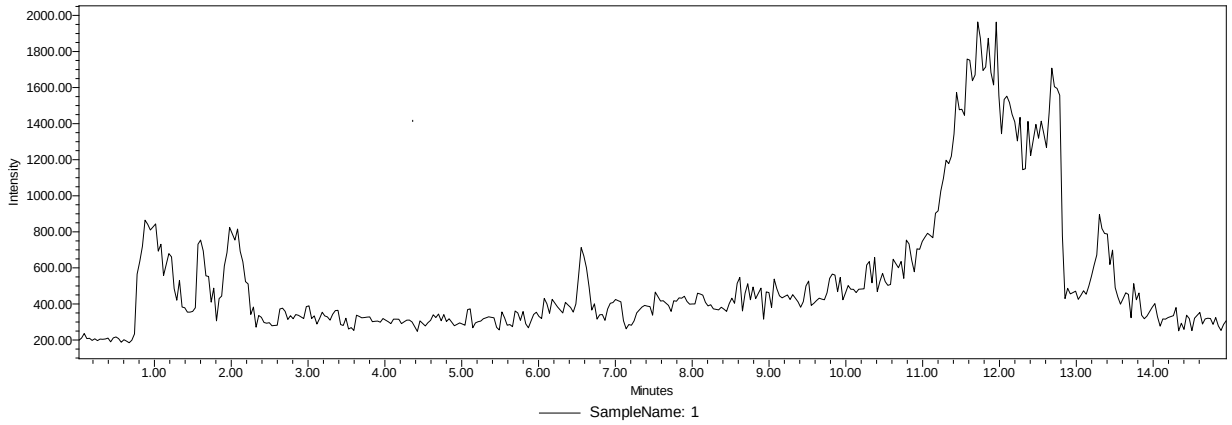

Fig. 28(4) product chromatograph for 5-FUr 1st replicate/screen for FUPA.

129

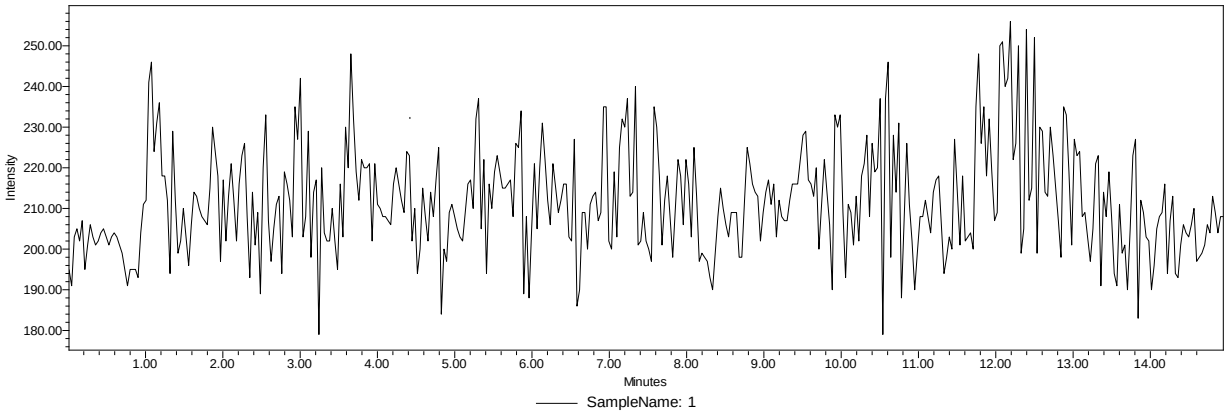

Fig. 28(5) product chromatograph for 5-FUr 1st replicate/screen for 5-FU.

1A

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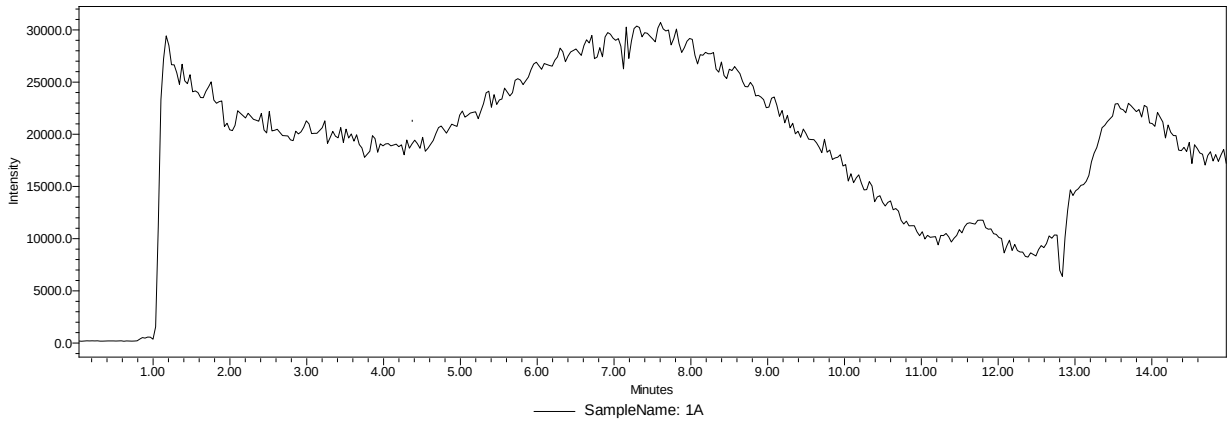

Fig. 28(6) product chromatograph for ts_0 1st replicate/screen for FAC.

105

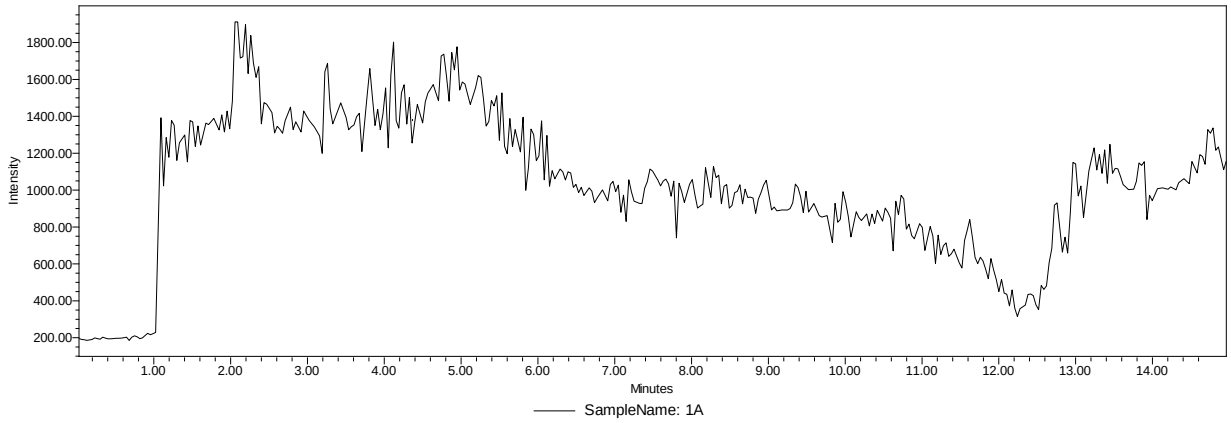

Fig. 28(7) product chromatograph for ts_0 1st replicate/screen for FMASld.

106

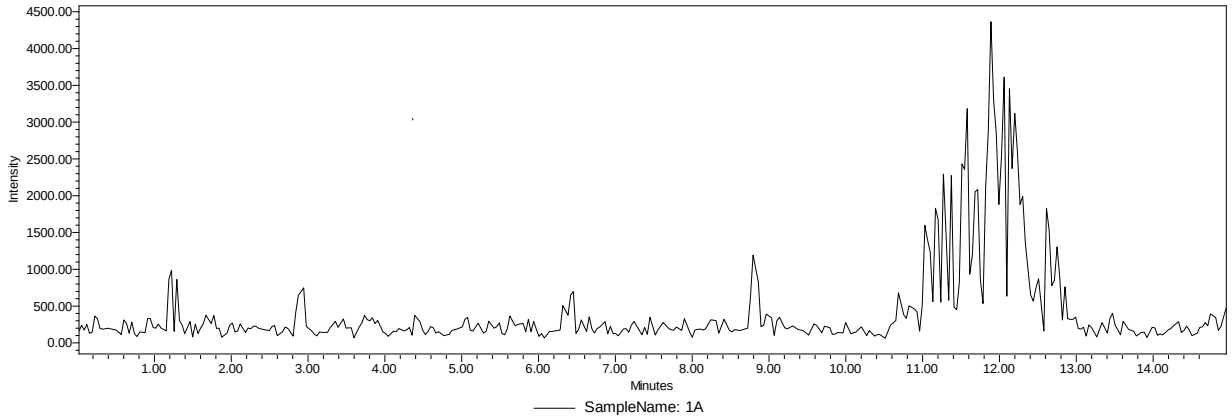

Fig. 28(8) product chromatograph for ts_0 1st replicate/screen for FBAL.

149

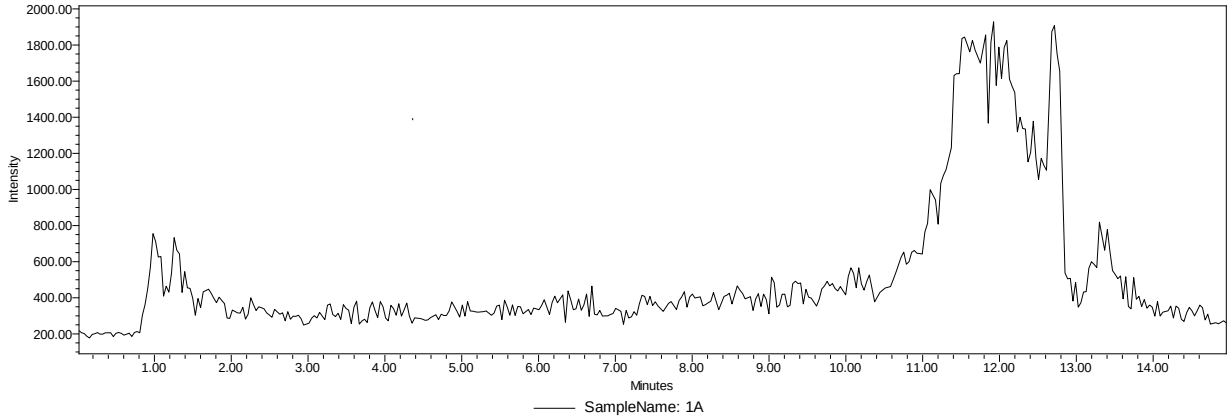

Fig. 28(9) product chromatograph for ts_0 1st replicate/screen for FUPA.

129

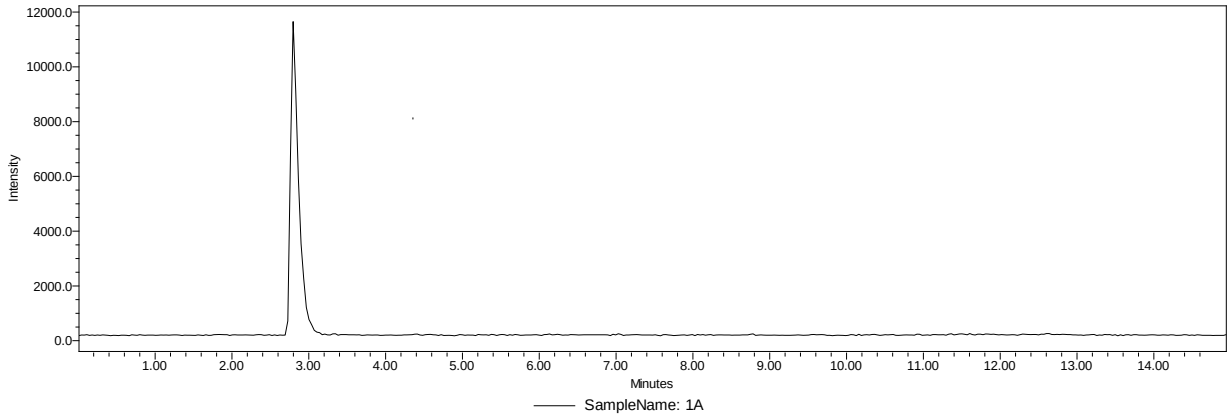

Fig. 28(10) product chromatograph for ts_0 1st replicate/screen for 5-FU.

2

77

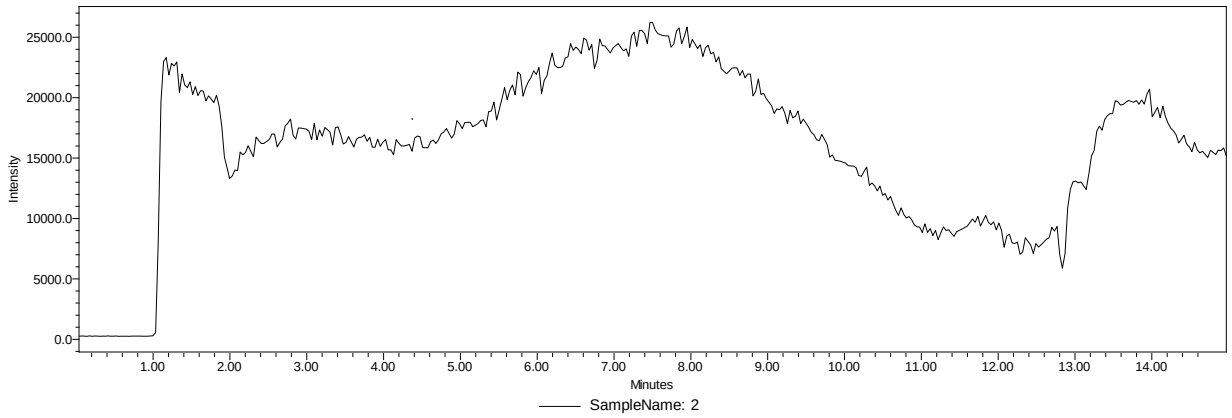

Fig. 28(11) Thermal/Saline product chromatograph screening for FAC @ .04 day 1st replicate.

105

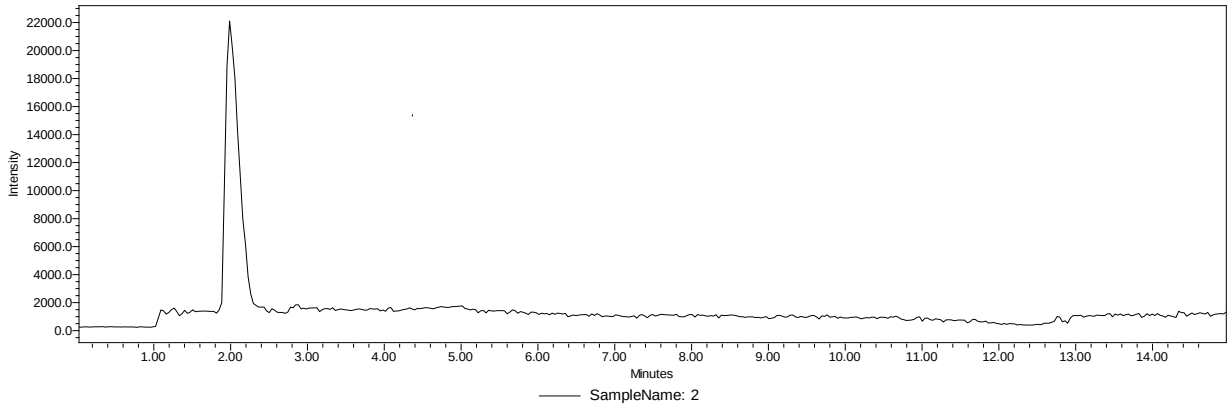

Fig. 28(12) Thermal/Saline product chromatograph screening for FMASld @ .04 day 1st replicate.

106

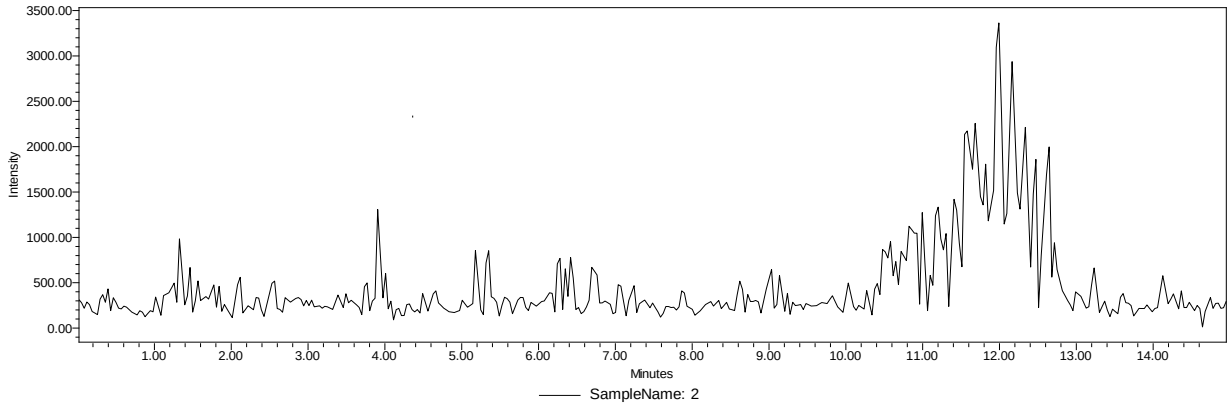

Fig. 28(13) Thermal/Saline product chromatograph screening for FBAL @ .04 day 1st replicate.

149

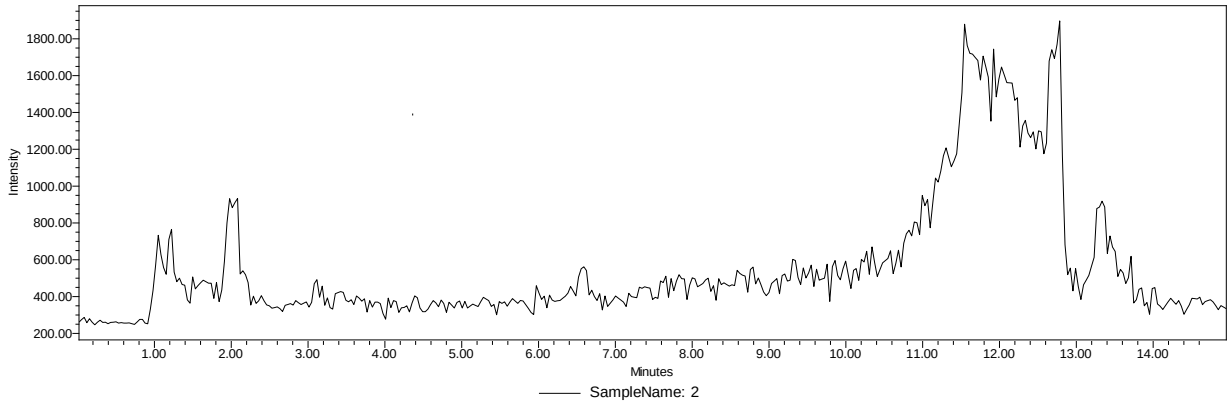

Fig. 28(14) Thermal/Saline product chromatograph screening for FUPA @ .04 day 1st replicate.

129

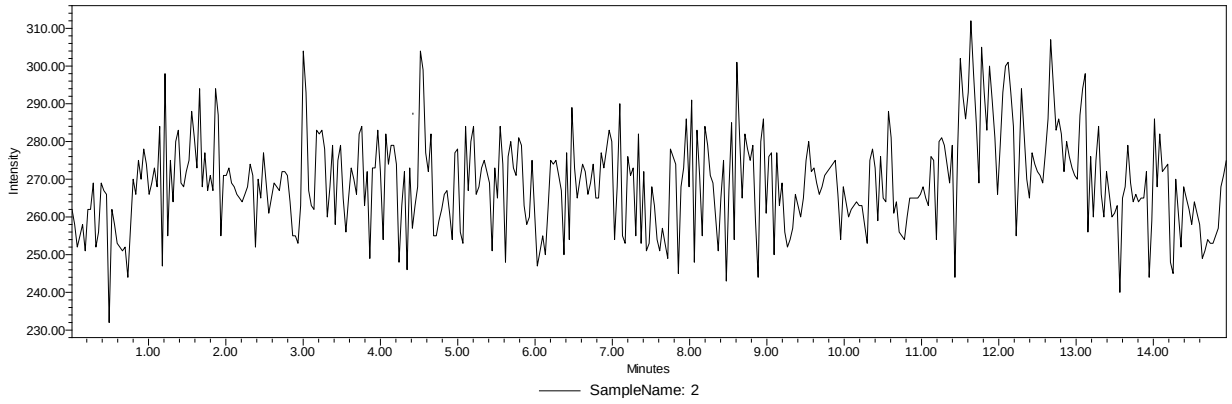

Fig. 28(15) Thermal/Saline product chromatograph screening for 5-FU @ .04 day 1st replicate.

3

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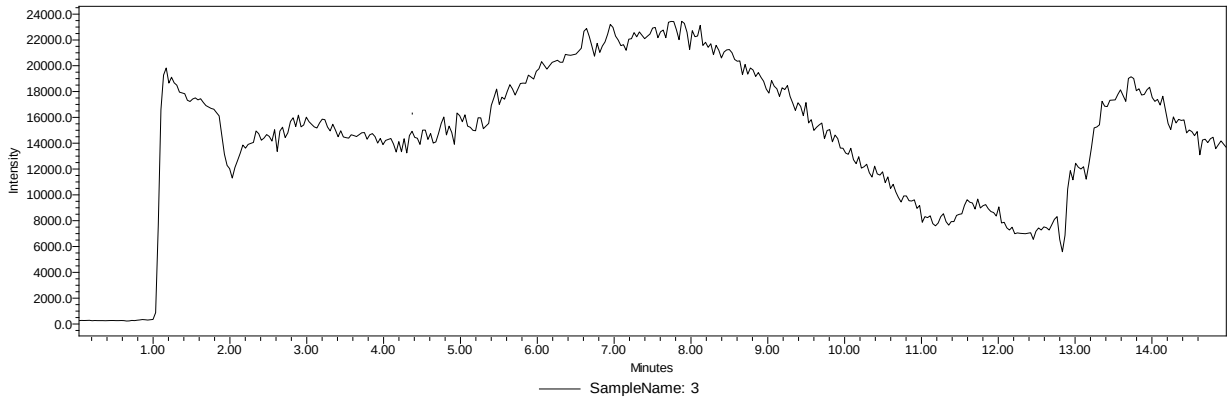

Fig. 28(16)Thermal/Saline product chromatograph screening for FAC @ .04 day 2nd replicate.

105

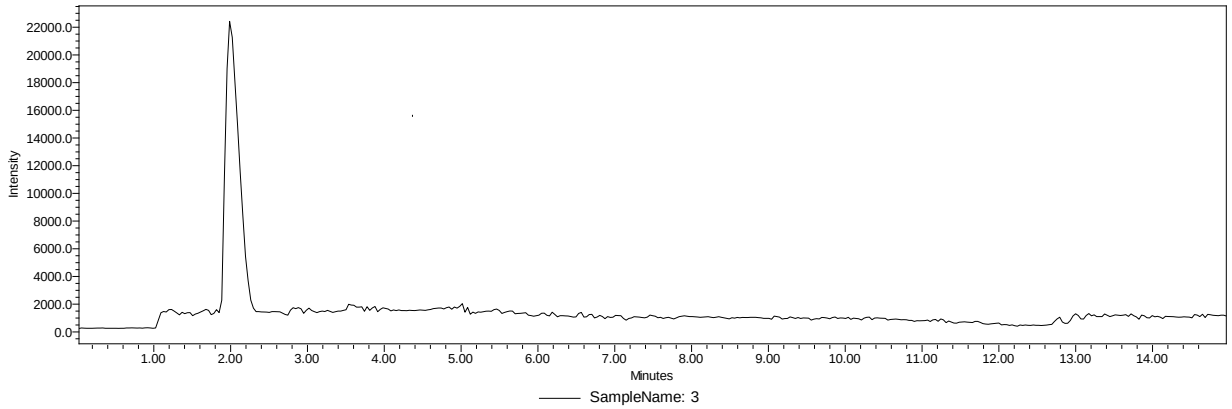

Fig. 28(17)Thermal/Saline product chromatograph screening for FMASld @ .04 day 2nd replic

106

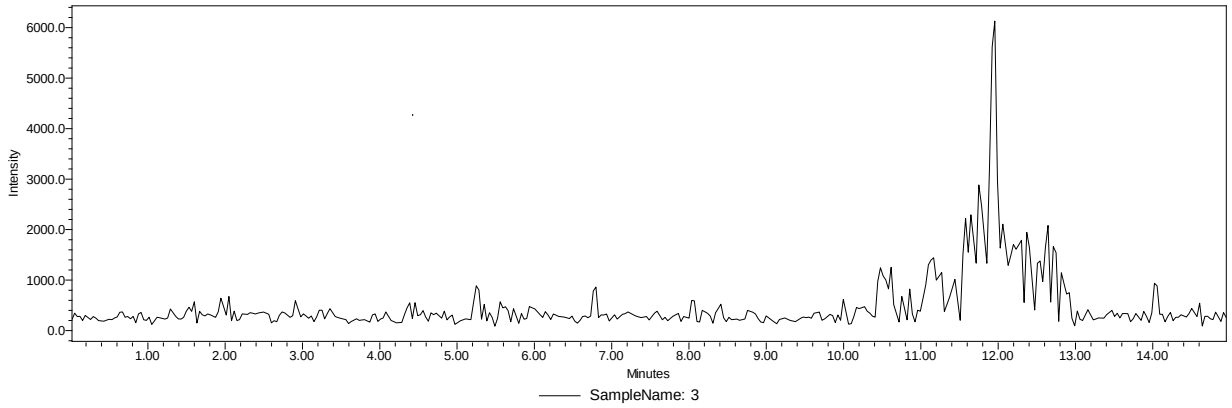

Fig. 28(18)Thermal/Saline product chromatograph screening for FBAL @ .04 day 2nd replicate.

149

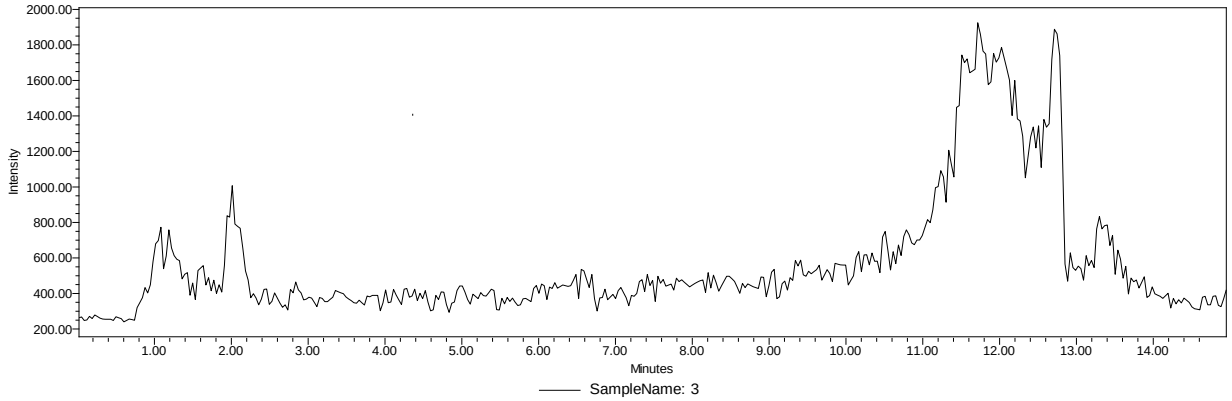

Fig. 28(19)Thermal/Saline product chromatograph screening for FUPA @ .04 day 2nd replicate.

129

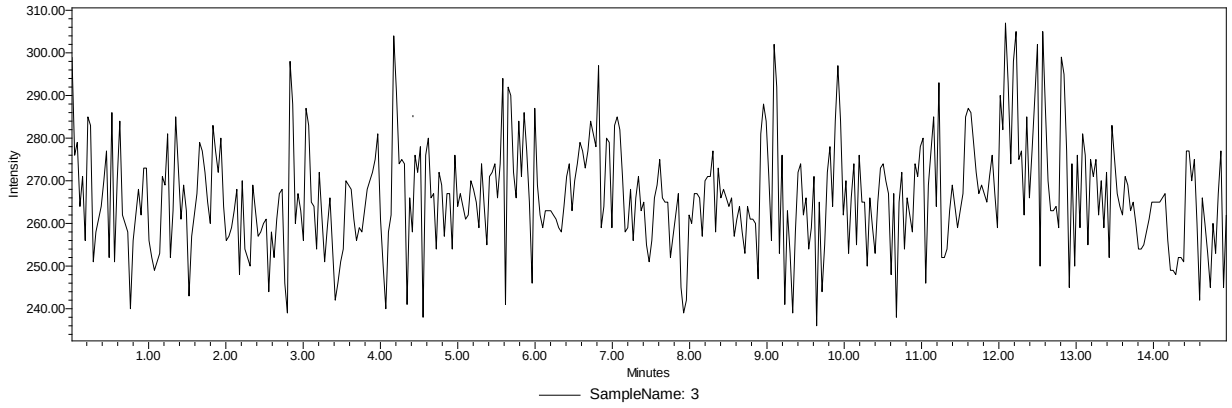

Fig. 28(20)Thermal/Saline product chromatograph screening for 5-FU @ .04 day 2nd replicate.

4

77

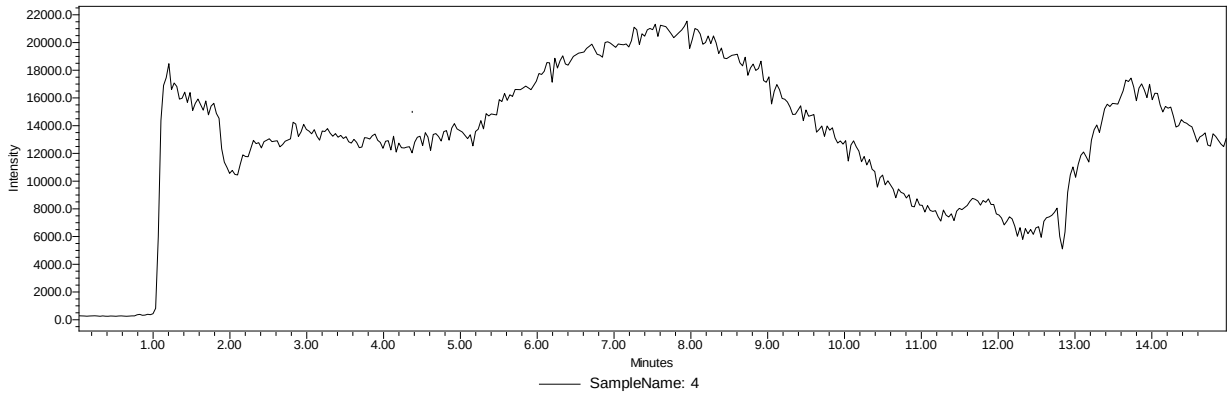

Fig. 28(21)Thermal/Saline product chromatograph screening for FAC @ .04 day 3rd replicate.

105

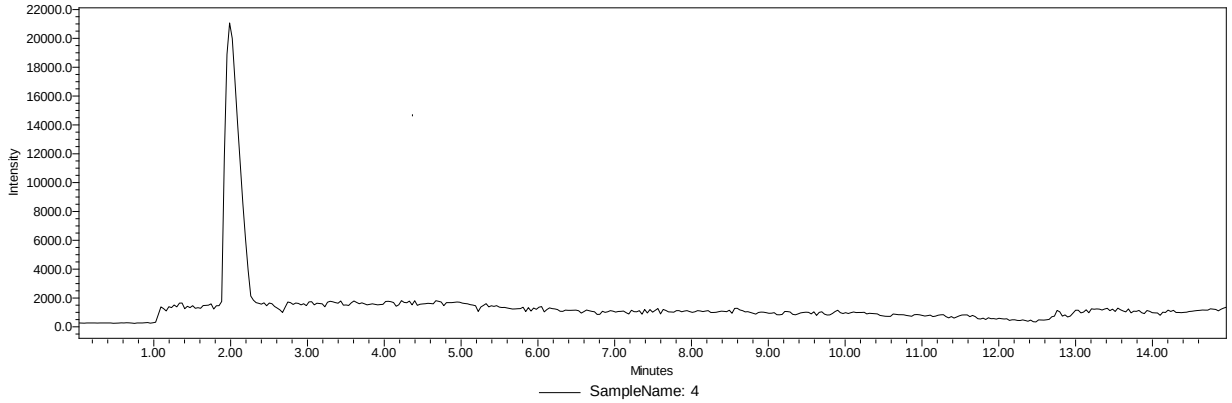

Fig. 28(22)Thermal/Saline product chromatograph screening for FMASId @ .04 day 3rd replicate.

106

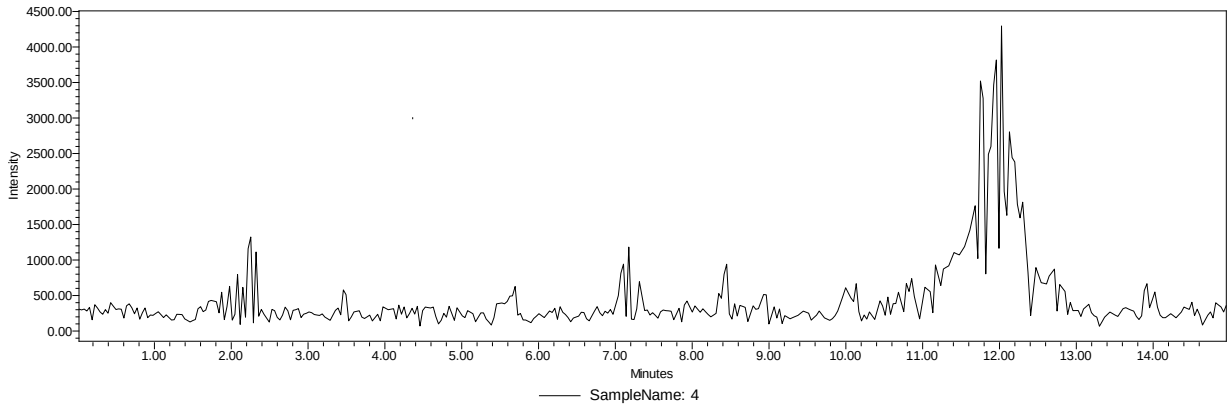

Fig. 28(23)Thermal/Saline product chromatograph screening for FBAL @ .04 day 3rd replicate.

149

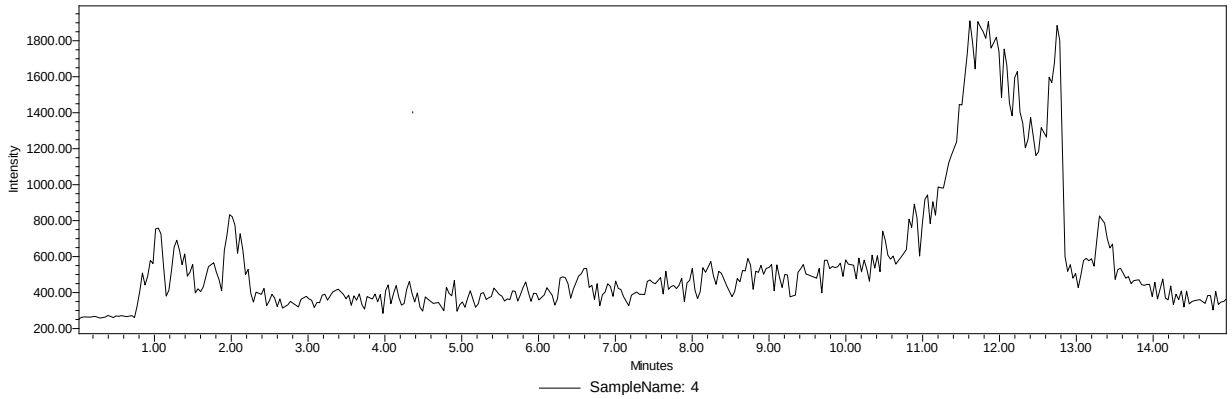

Fig. 28(24)Thermal/Saline product chromatograph screening for FUPA @ .04 day 3rd replicate.

129

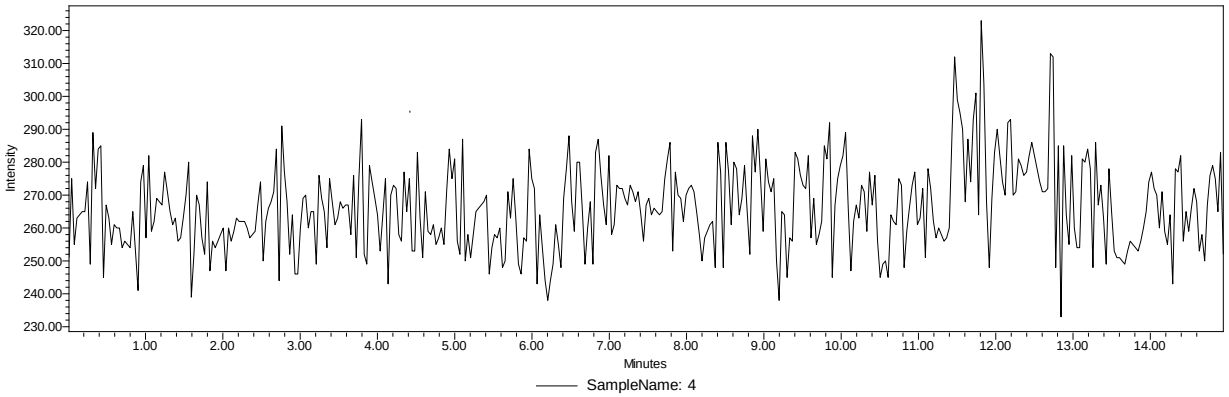

Fig. 28(25)Thermal/Saline product chromatograph screening for 5-FU @ .04 day 3rd replicate.

5

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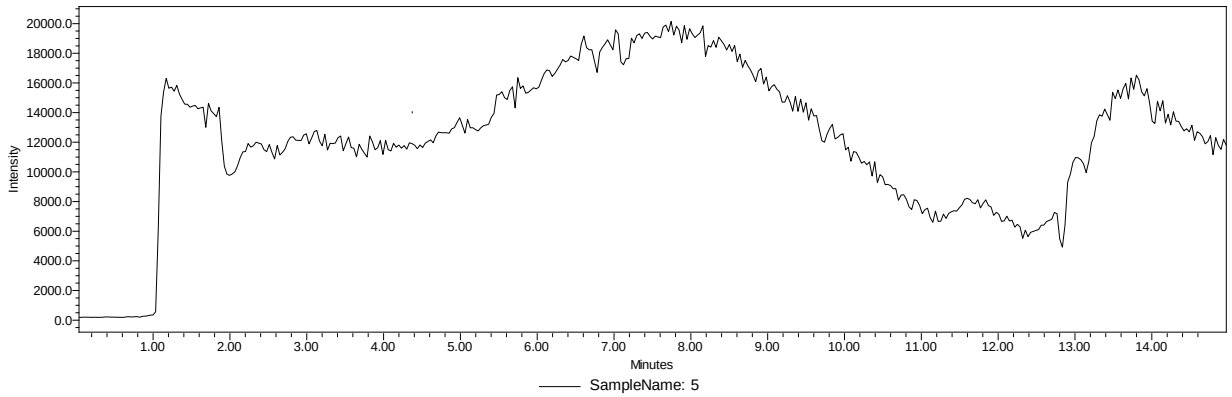

Fig. 28(26)Thermal/Saline product chromatograph screening for FAC @ .08 day 1st replicate.

105

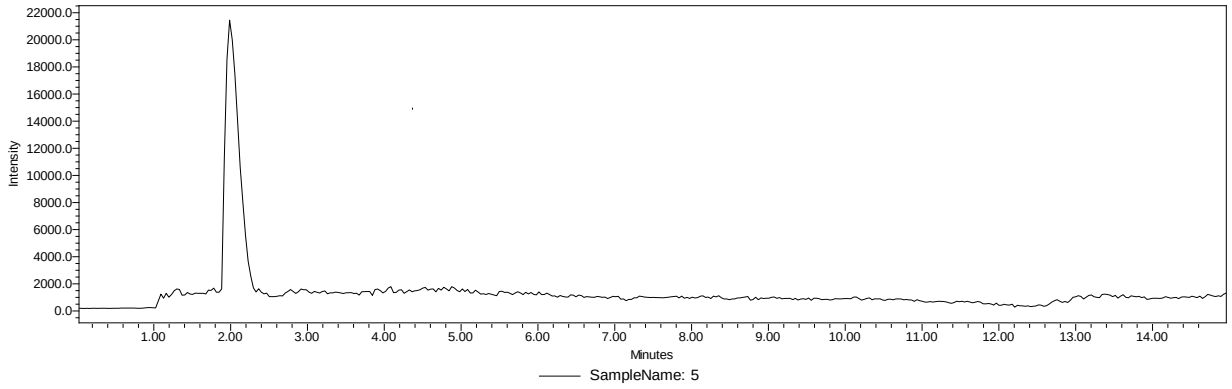

Fig. 28(27)Thermal/Saline product chromatograph screening for FMASld @ .08 day 1st replicate.

106

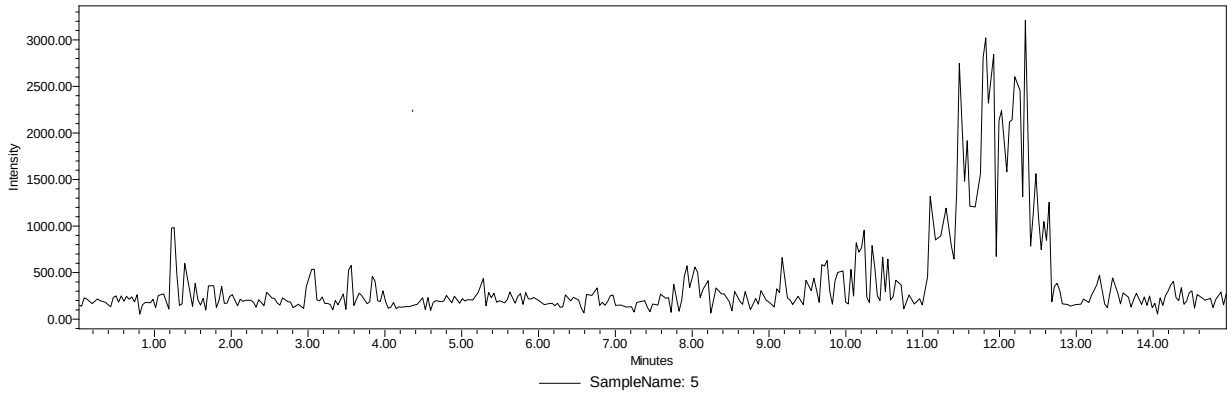

Fig. 28(28)Thermal/Saline product chromatograph screening for FBAL @ .08 day 1st replicate.

149

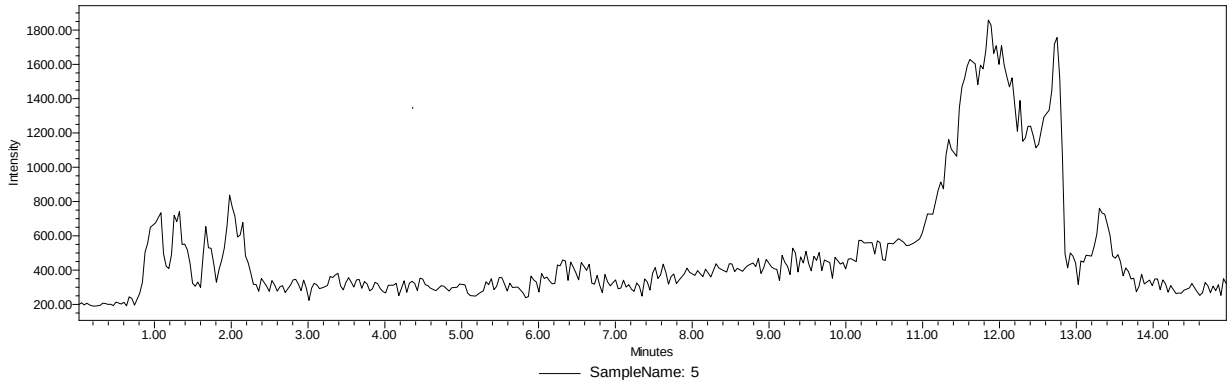

Fig. 28(29)Thermal/Saline product chromatograph screening for FUPA @ .08 day 1st replicate.

129

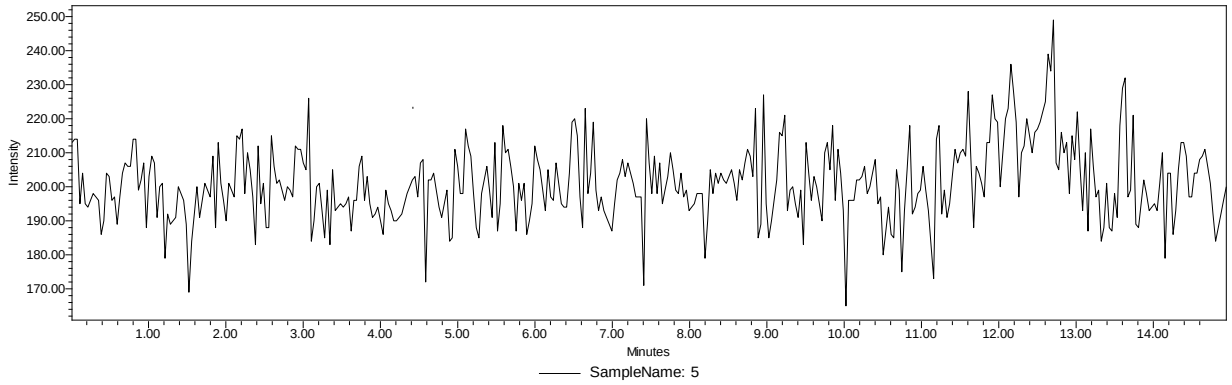

Fig. 28(30)Thermal/Saline product chromatograph screening for 5-FU @ .08 day 1st replicate.

6

77

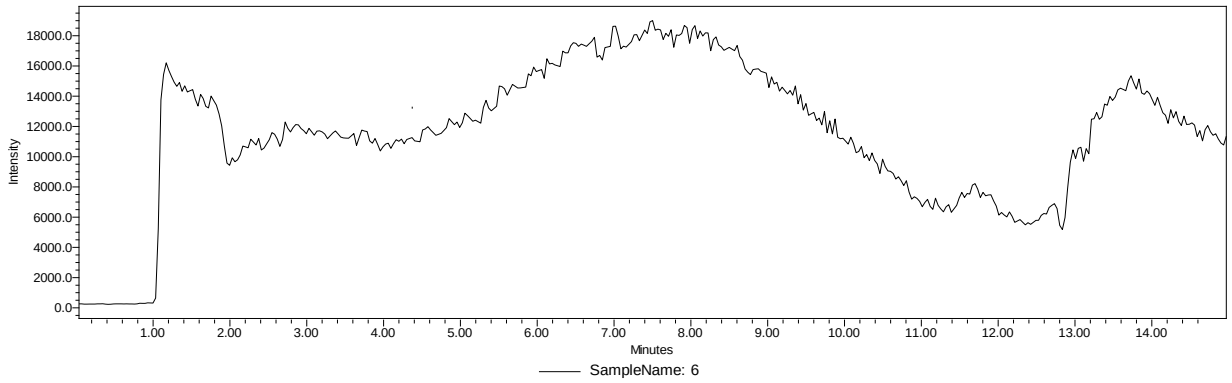

Fig. 28(31)Thermal/Saline product chromatograph screening for FAC @ .08 day 2nd replicate.

105

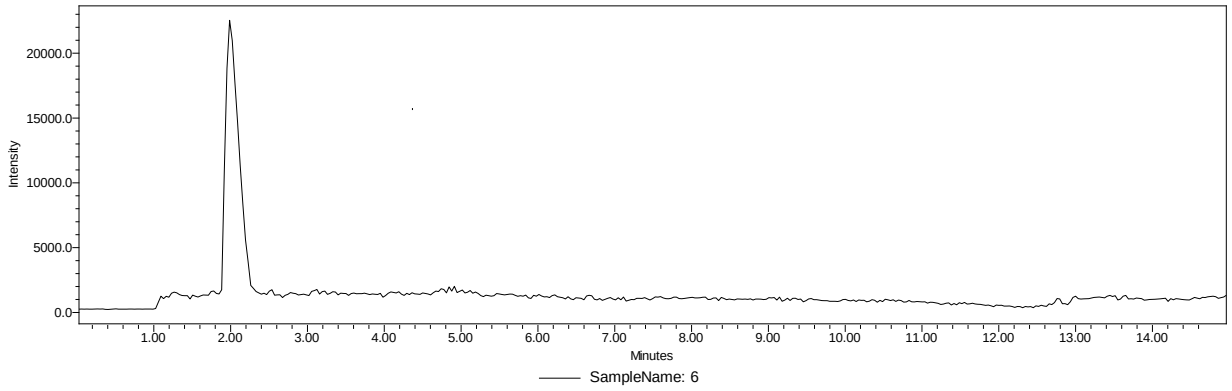

Fig. 28(32)Thermal/Saline product chromatograph screening for FMASld @ .08 day 2nd

106

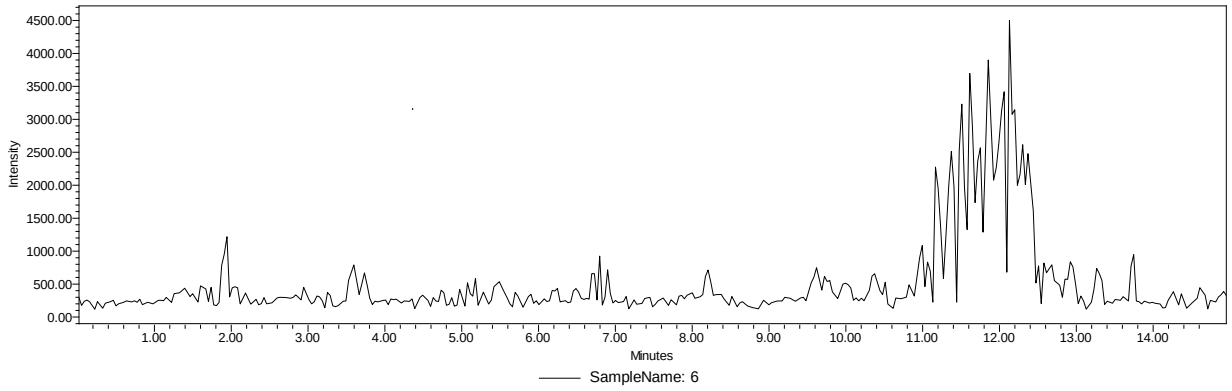

Fig. 28(33)Thermal/Saline product chromatograph screening for FBAL @ .08 day 2nd replicate.

149

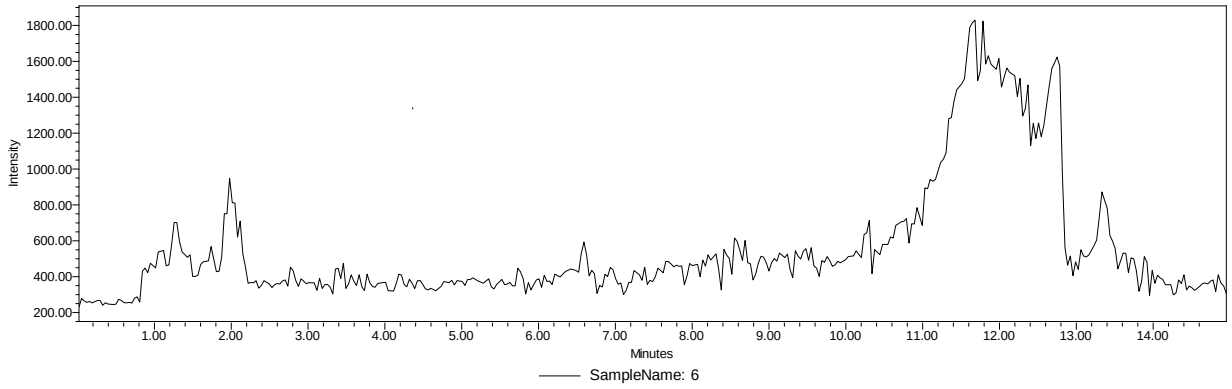

Fig. 28(34)Thermal/Saline product chromatograph screening for FUPA @ .08 day 2nd replicate.

129

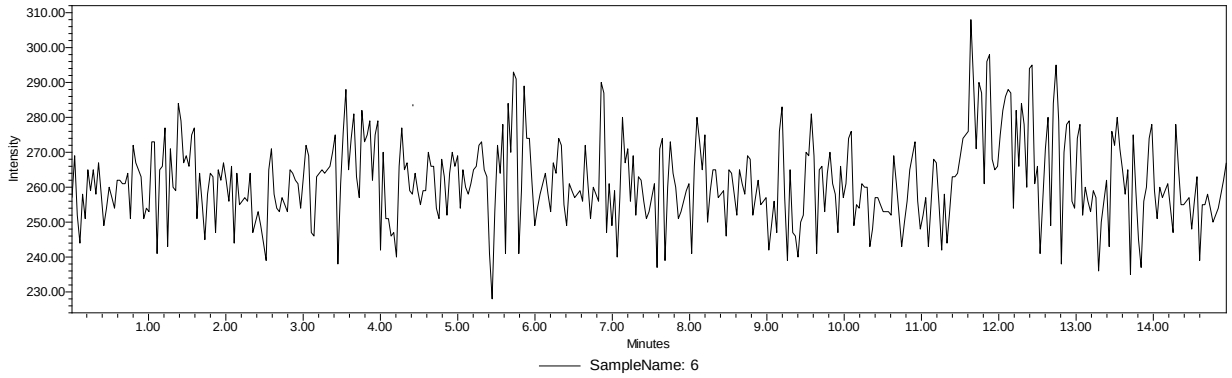

Fig. 28(35)Thermal/Saline product chromatograph screening for 5-FU @ .08 day 2nd replicate.

7

77

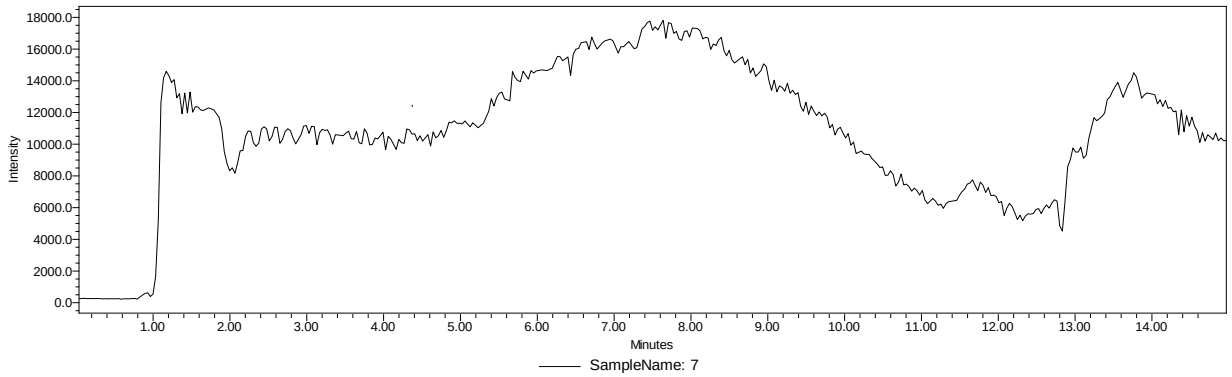

Fig. 28(36)Thermal/Saline product chromatograph screening for FAC @ .08 day 3rd replicate.

105

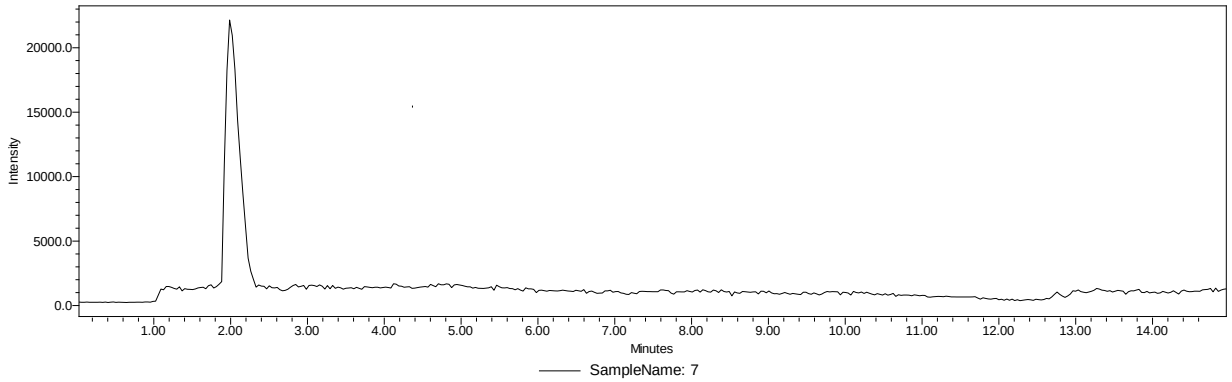

Fig. 28(37)Thermal/Saline product chromatograph screening for FMASld @ .08 day 3rd replicate.

106

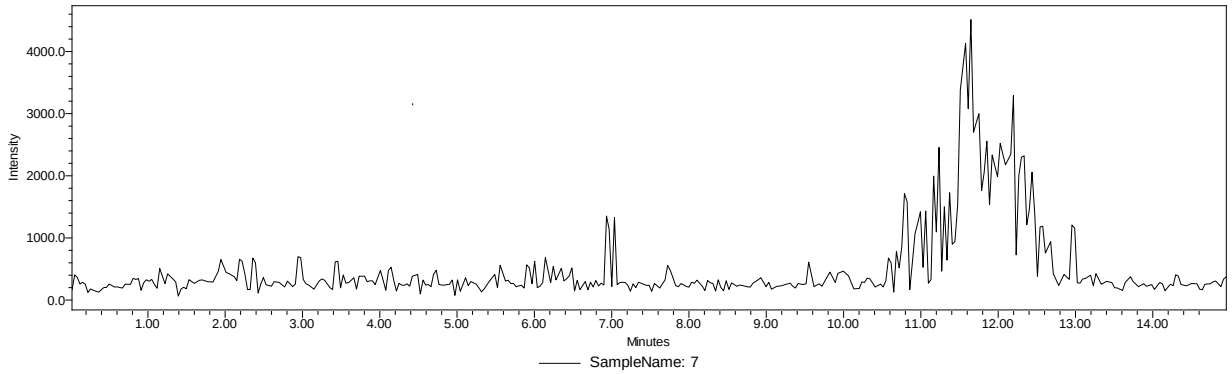

Fig. 28(38)Thermal/Saline product chromatograph screening for FBAL @ .08 day 3rd replicate.

149

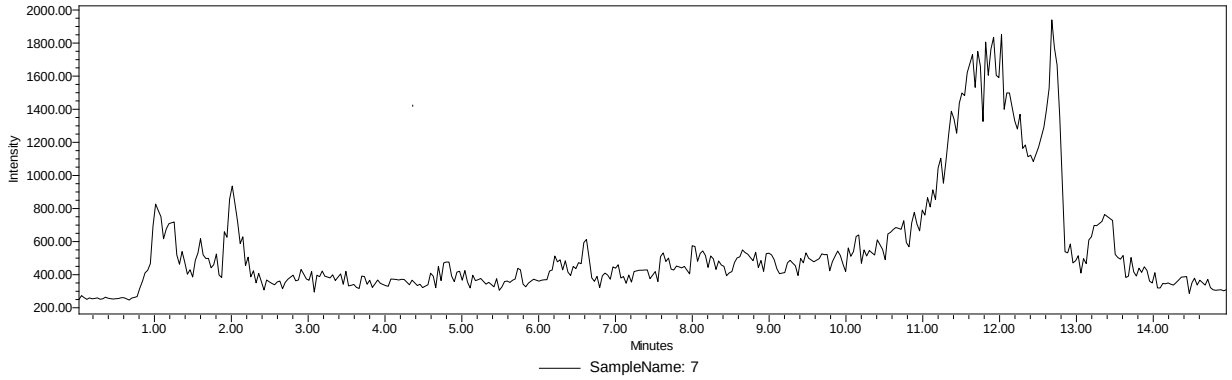

Fig. 28(39)Thermal/Saline product chromatograph screening for FUPA @ .08 day 3rd replicate.

129

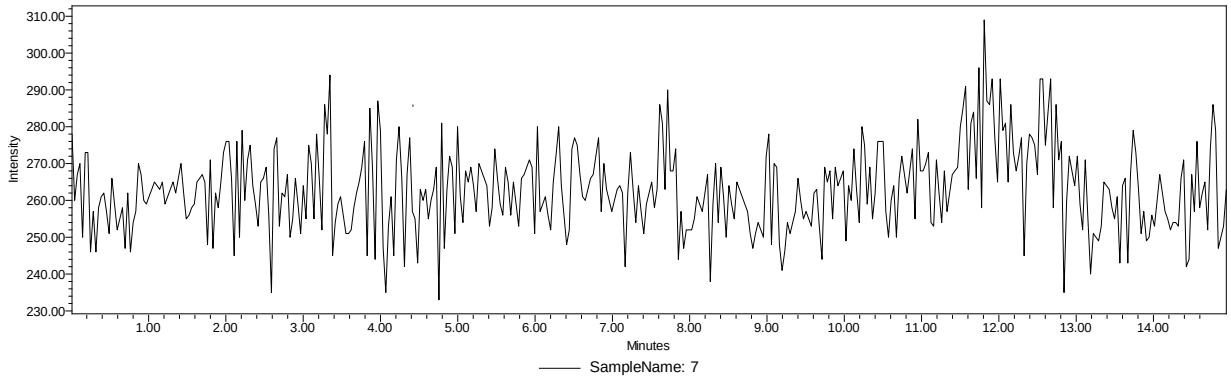

Fig. 28(40)Thermal/Saline product chromatograph screening for 5-FU @ .08 day 3rd replicate.

8

77

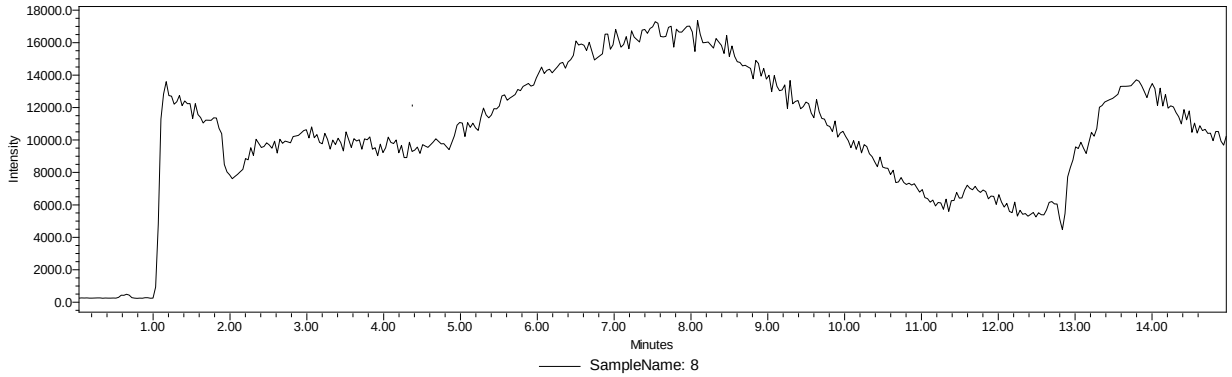

Fig. 28(41)Thermal/Saline product chromatograph screening for FAC @ .13 day 1st replicate.

105

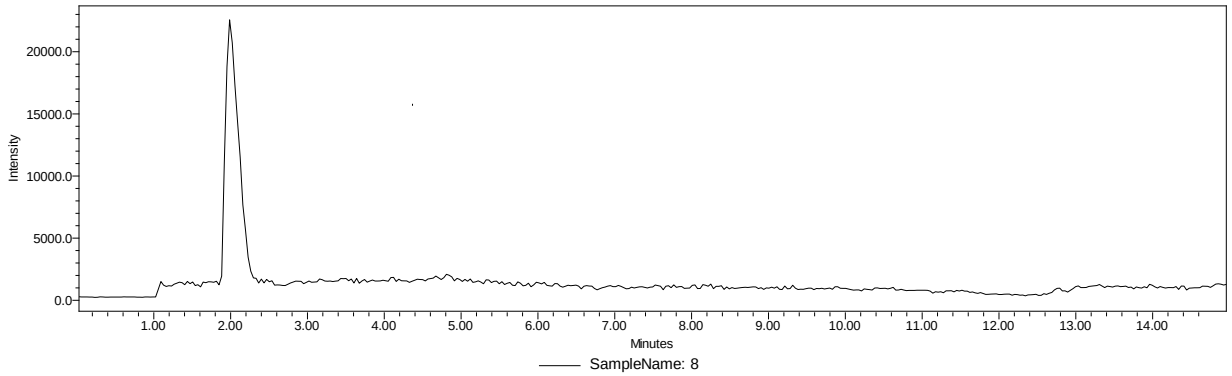

Fig. 28(42)Thermal/Saline product chromatograph screening for FMASld @ .13 day 1st replicate.

106

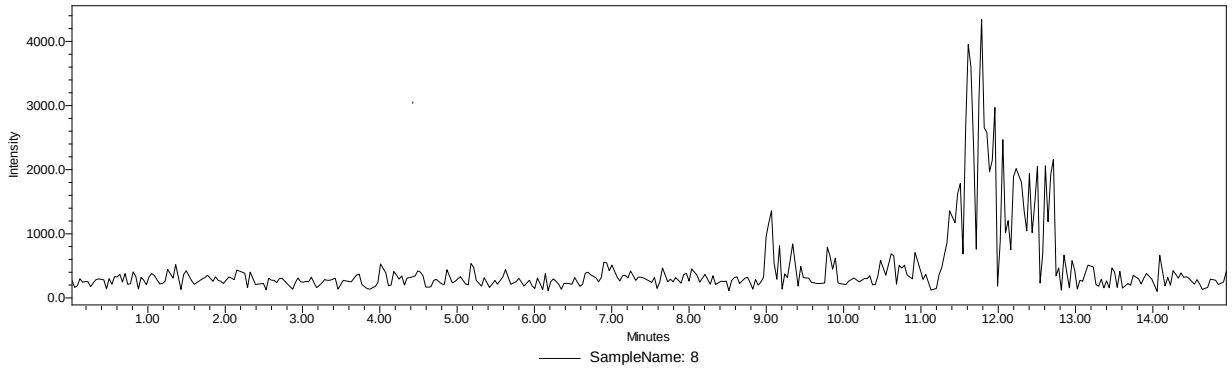

Fig. 28(43)Thermal/Saline product chromatograph screening for FBAL @ .13 day 1st replicate.

149

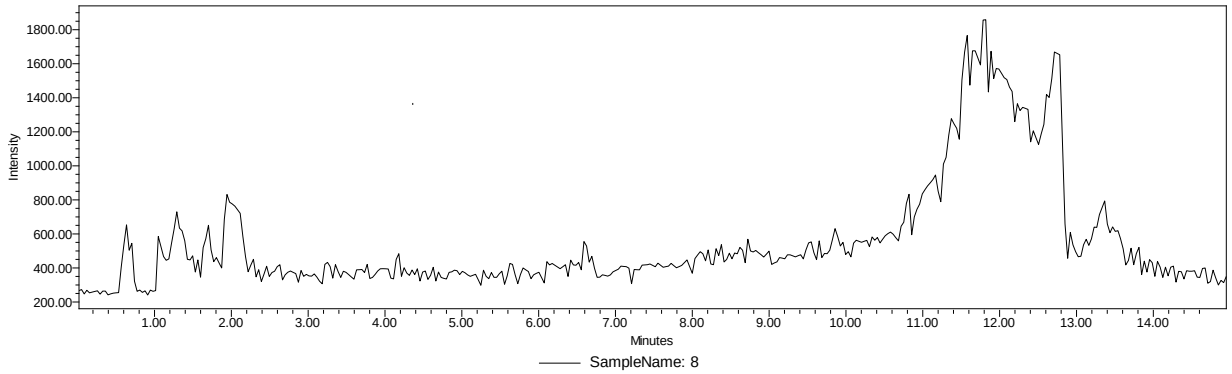

Fig. 28(44)Thermal/Saline product chromatograph screening for FUPA @ .13 day 1st replicate.

129

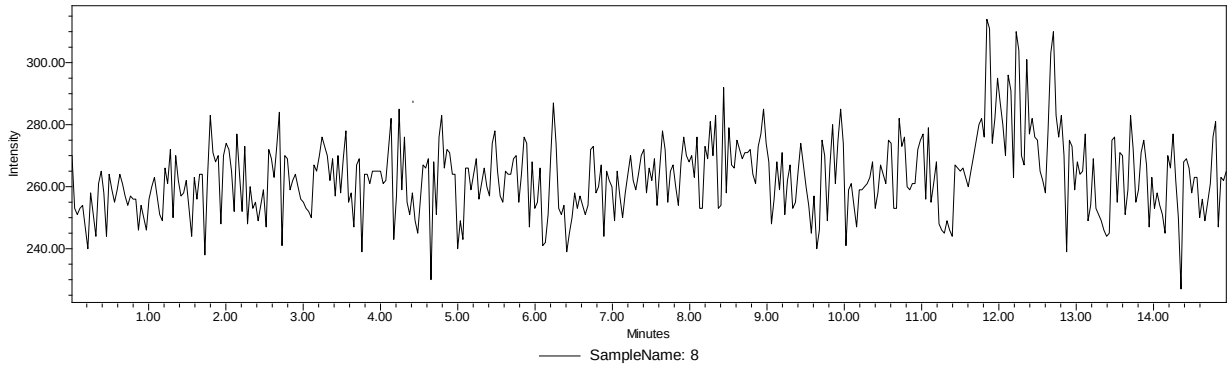

Fig. 28(45)Thermal/Saline product chromatograph screening for 5-FU @ .13 day 1st replicate.

9

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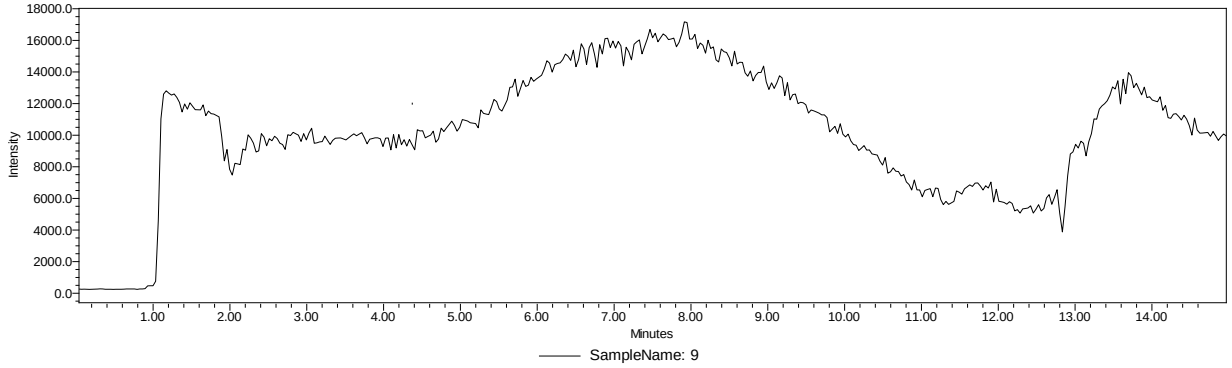

Fig. 28(46)Thermal/Saline product chromatograph screening for FAC @ .13 day 2nd replicate.

105

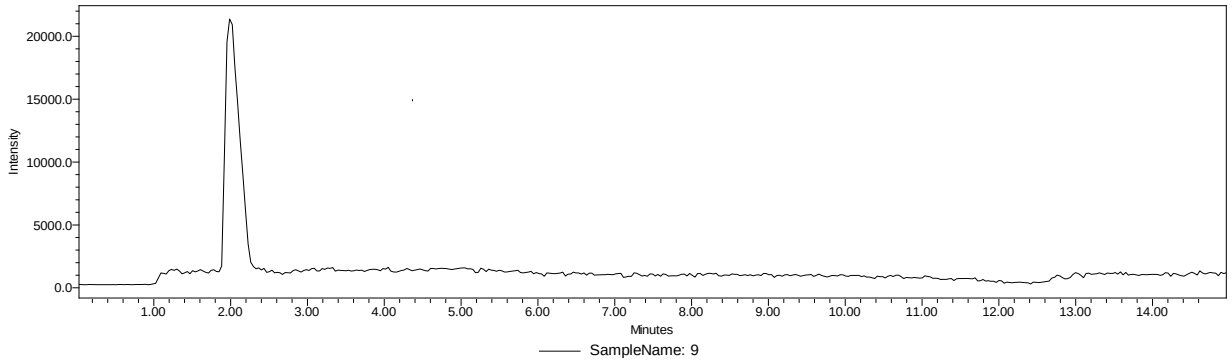

Fig. 28(47)Thermal/Saline product chromatograph screening for FMASld @ .13 day 2nd replicate.

106

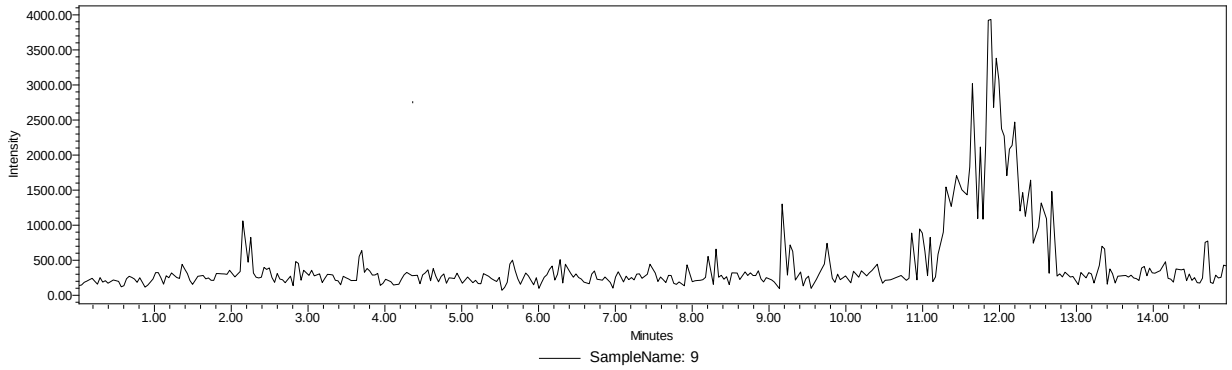

Fig. 28(48)Thermal/Saline product chromatograph screening for FBAL @ .13 day 2nd replicate.

149

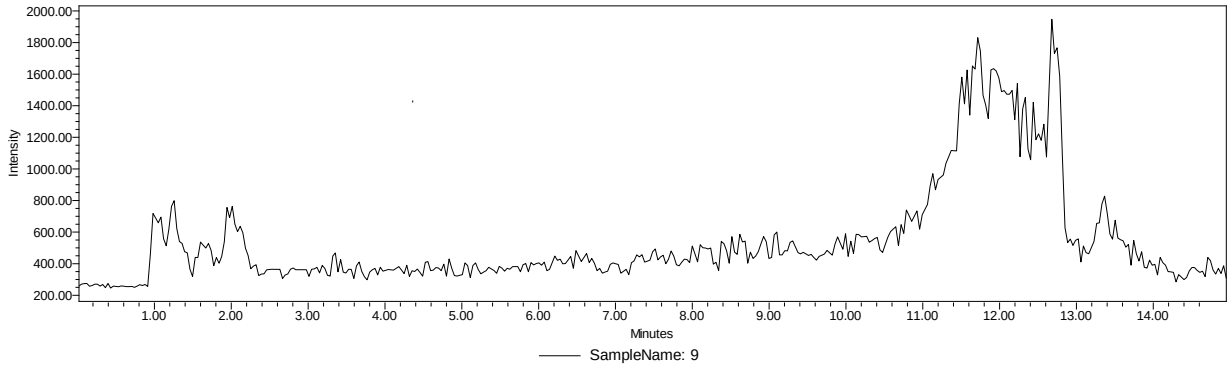

Fig. 28(49)Thermal/Saline product chromatograph screening for FUPA @ .13 day 2nd replicate.

129

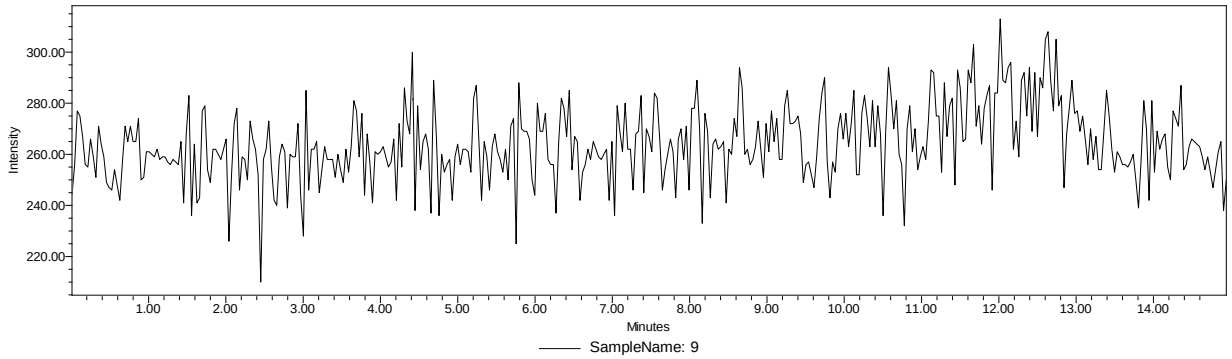

Fig. 28(50)Thermal/Saline product chromatograph screening for 5-FU @ .13 day 2nd replicate.

10

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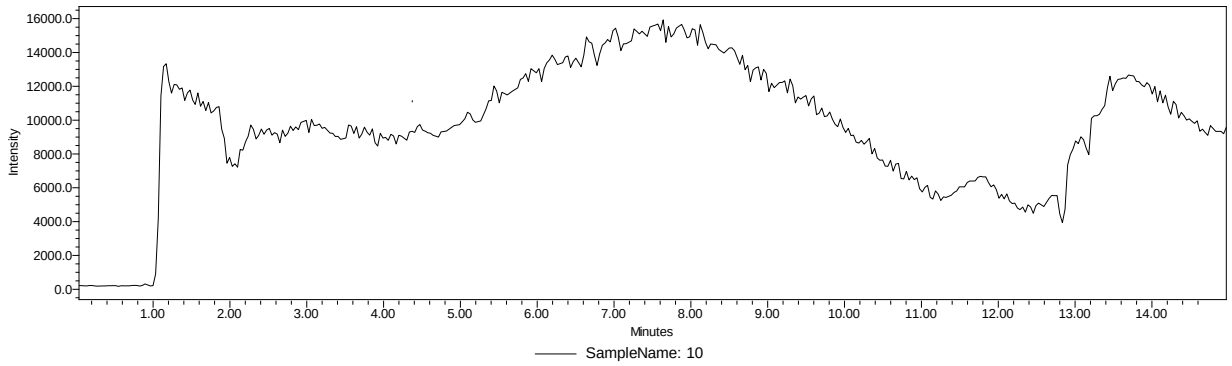

Fig. 28(51)Thermal/Saline product chromatograph screening for FAC @ .13 day 3rd replicate.

105

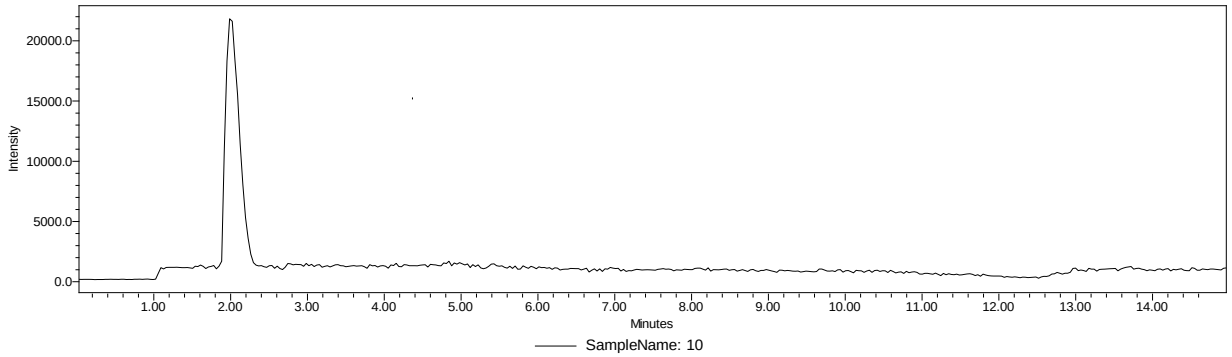

Fig. 28(52)Thermal/Saline product chromatograph screening for FMASld @ .13 day 3rd replicate.

106

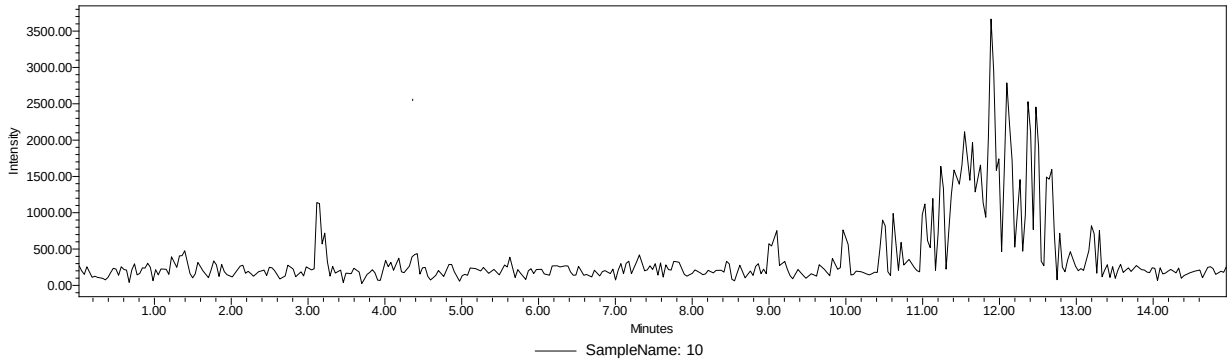

Fig. 28(53)Thermal/Saline product chromatograph screening for FBAL @ .13 day 3rd replicate.

149

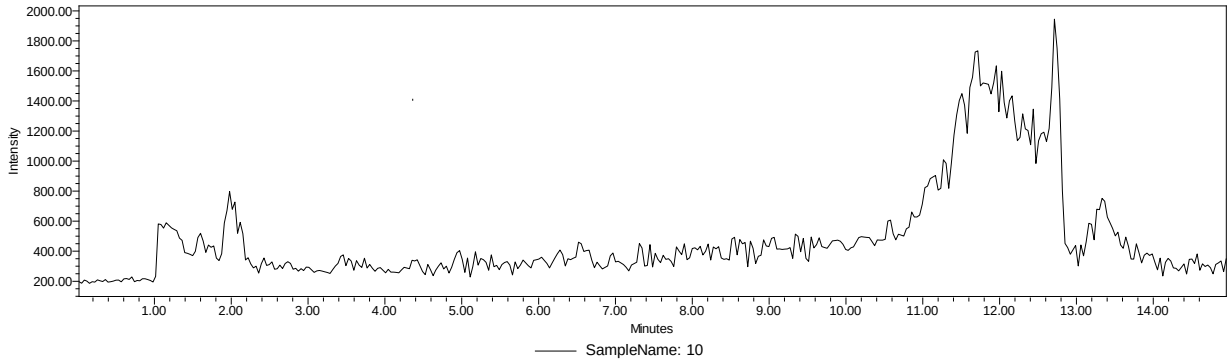

Fig. 28(54)Thermal/Saline product chromatograph screening for FUPA @ .13 day 3rd replicate.

129

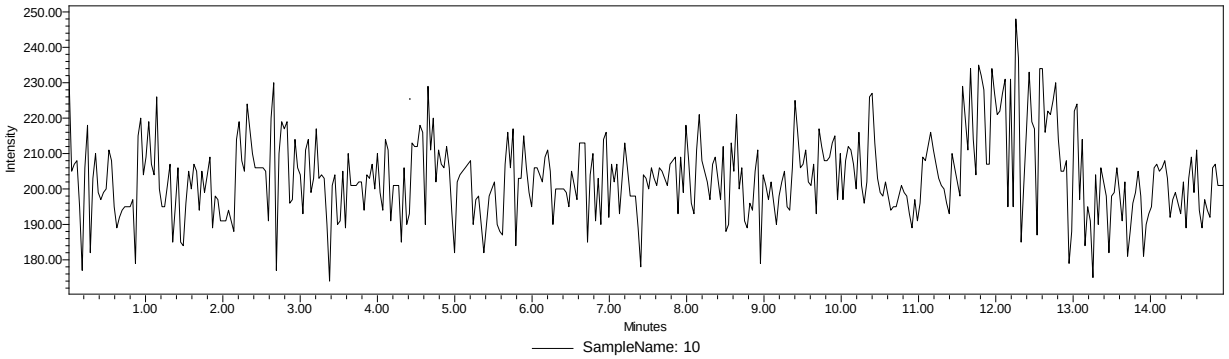

Fig. 28(55)Thermal/Saline product chromatograph screening for 5-FU @ .13 day 3rd replicate.

11

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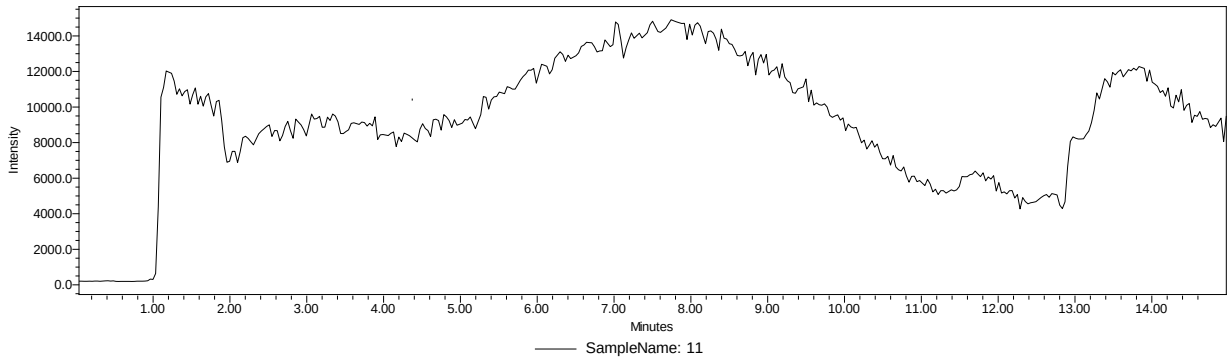

Fig. 28(56)Thermal/Saline product chromatograph screening for FAC @ .17 day 1st replicate.

105

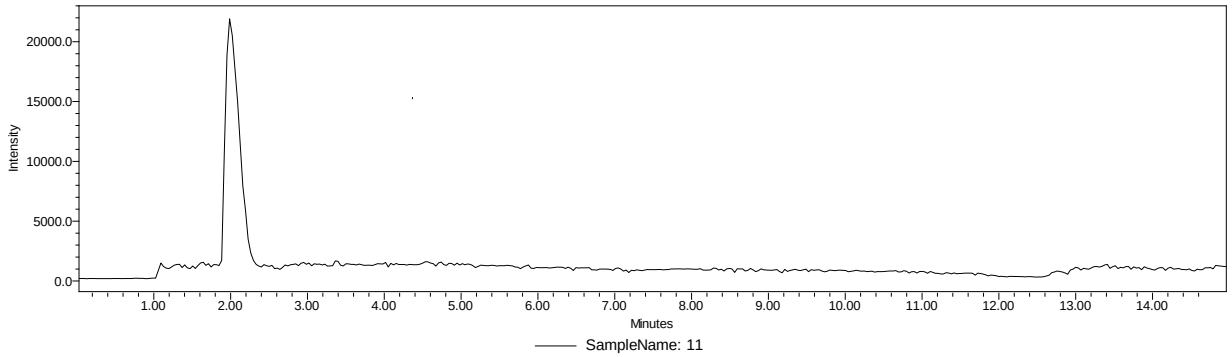

Fig. 28(57)Thermal/Saline product chromatograph screening for FMASld @ .17 day 1st replicate.

106

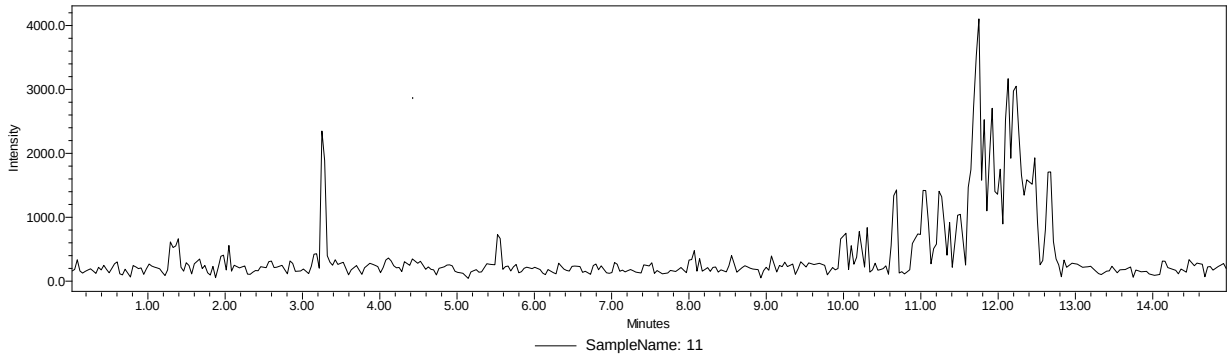

Fig. 28(58)Thermal/Saline product chromatograph screening for FBAL @ .17 day 1st replicate.

149

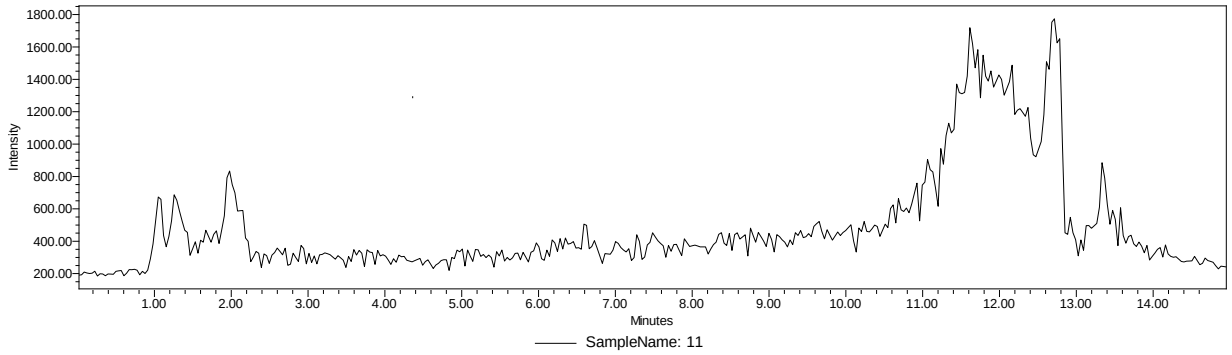

Fig. 28(59)Thermal/Saline product chromatograph screening for FUPA @ .17 day 1st replicate.

129

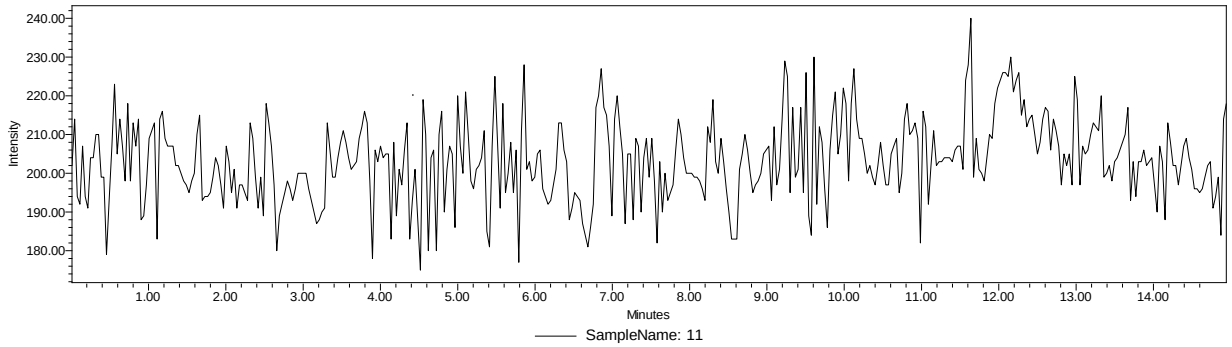

Fig. 28(60)Thermal/Saline product chromatograph screening for 5-FU @ .17 day 1st replicate.

12

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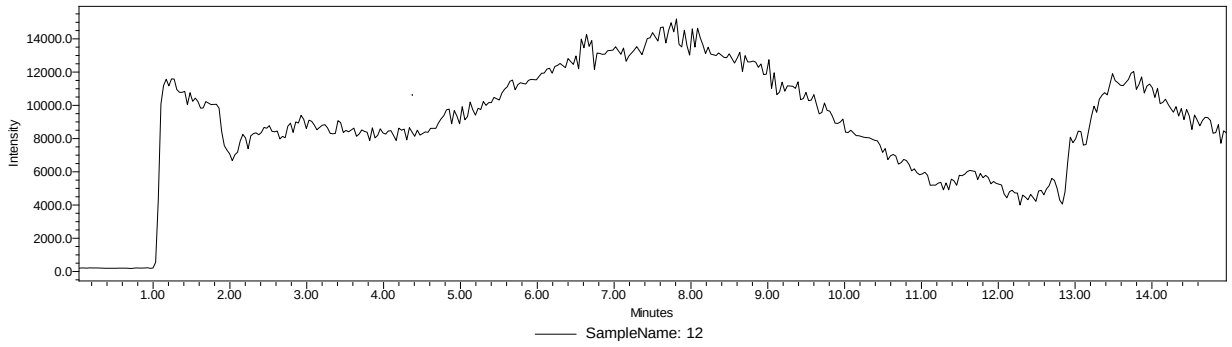

Fig. 28(61)Thermal/Saline product chromatograph screening for FAC @ .17 day 2nd replicate.

105

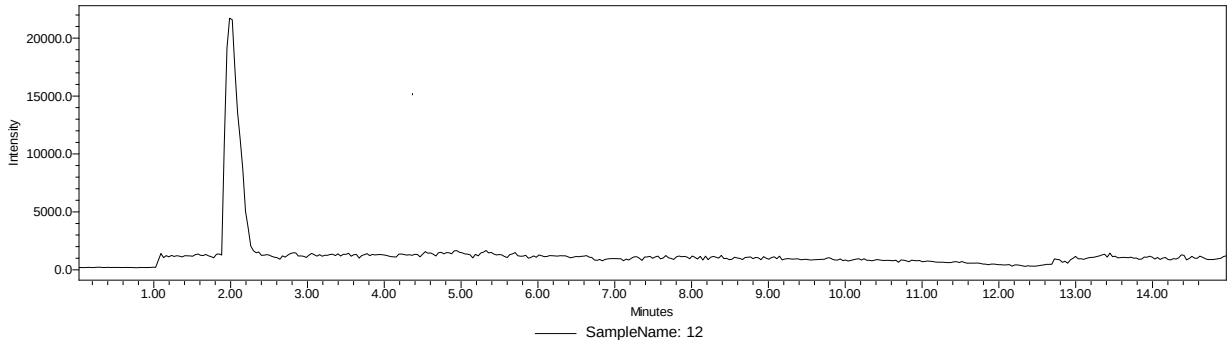

Fig. 28(62)Thermal/Saline product chromatograph screening for FMASld @ .17 day 2nd replicate.

106

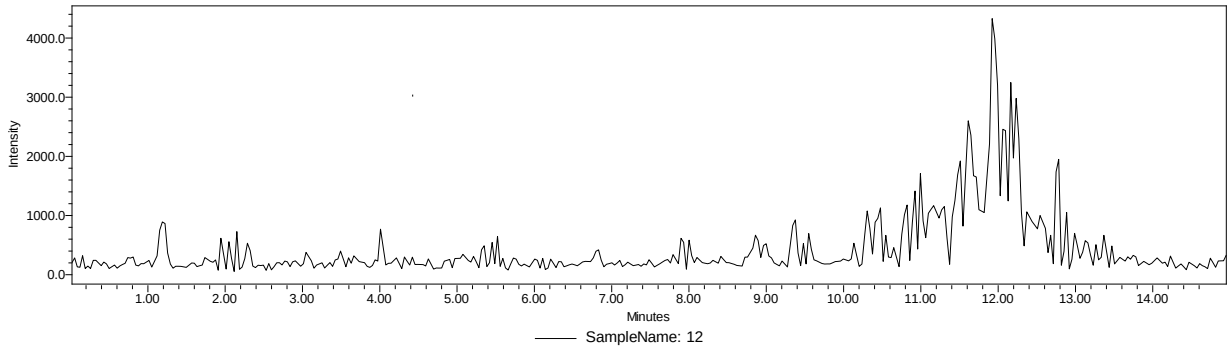

Fig. 28(63)Thermal/Saline product chromatograph screening for FBAL @ .17 day 2nd replicate.

149

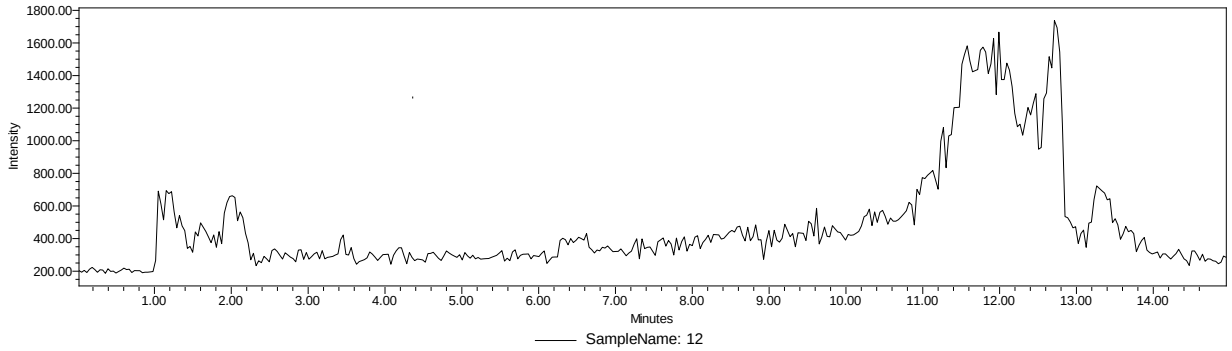

Fig. 28(64)Thermal/Saline product chromatograph screening for FUPA @ .17 day 2nd replicate.

129

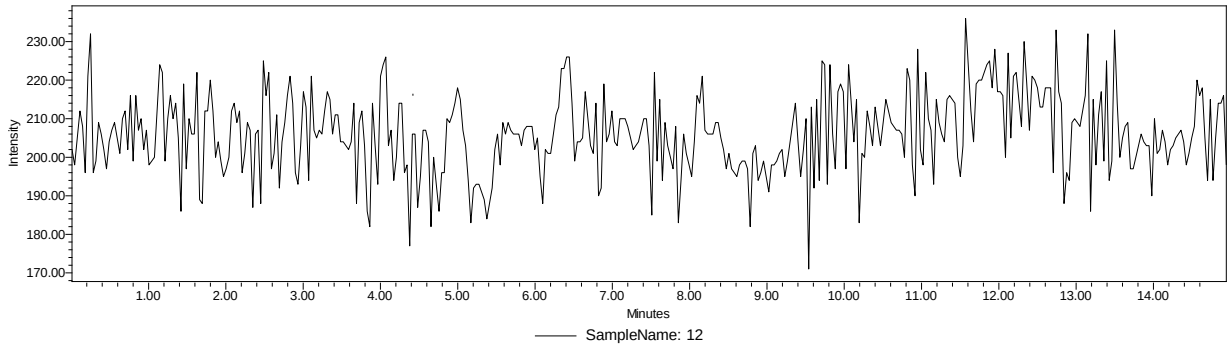

Fig. 28(65)Thermal/Saline product chromatograph screening for 5-FU @ .17 day 2nd replicate.

13

77

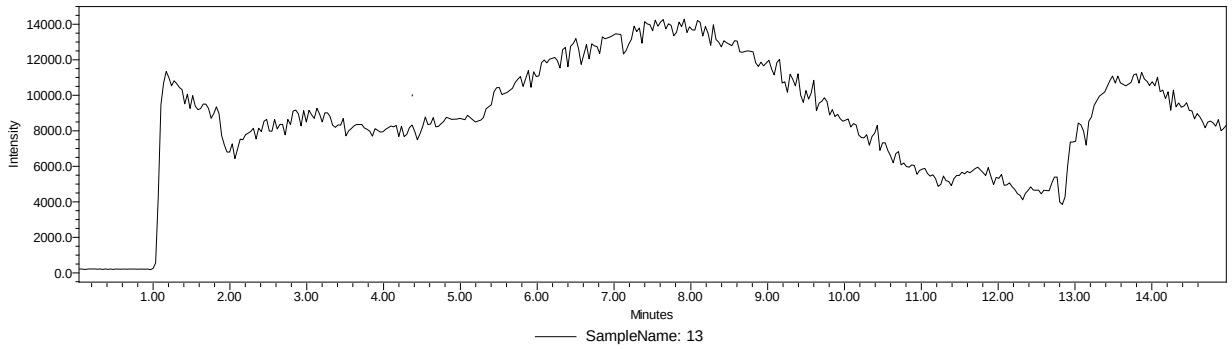

Fig. 28(66)Thermal/Saline product chromatograph screening for FAC @ .17 day 3rd replicate.

105

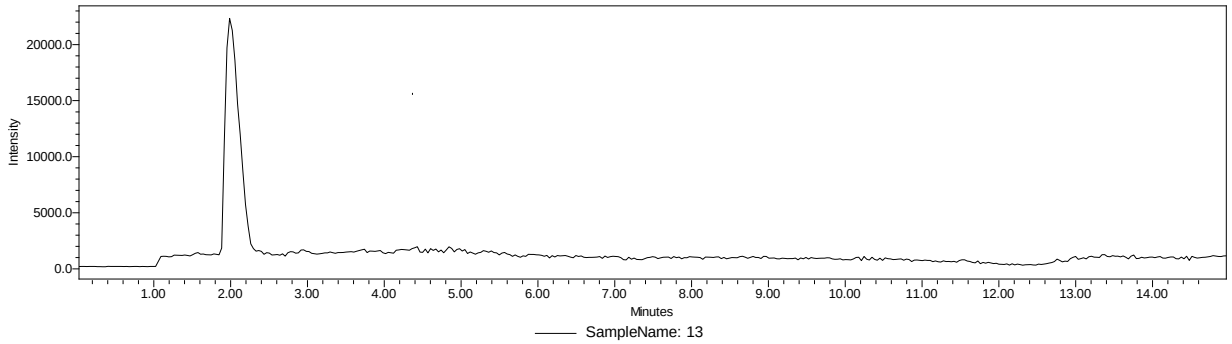

Fig. 28(67)Thermal/Saline product chromatograph screening for FMASld @ .17 day 3rd replicate.

106

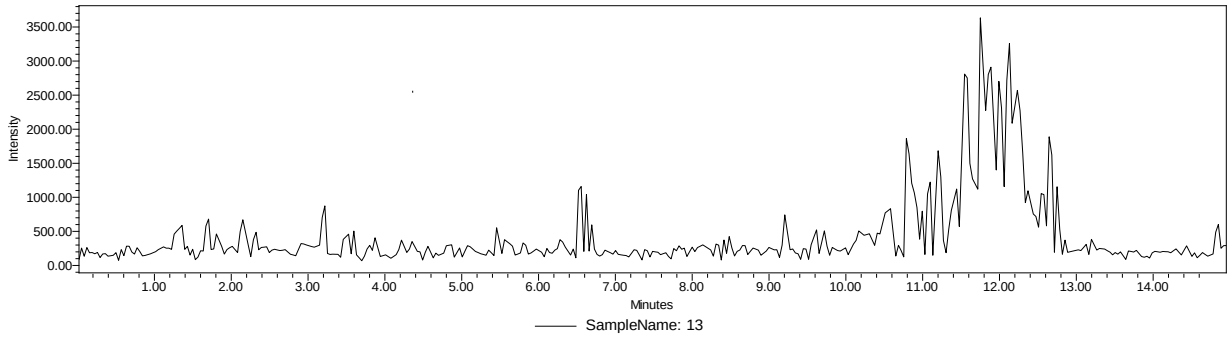

Fig. 28(68)Thermal/Saline product chromatograph screening for FBAL @ .17 day 3rd replicate.

149

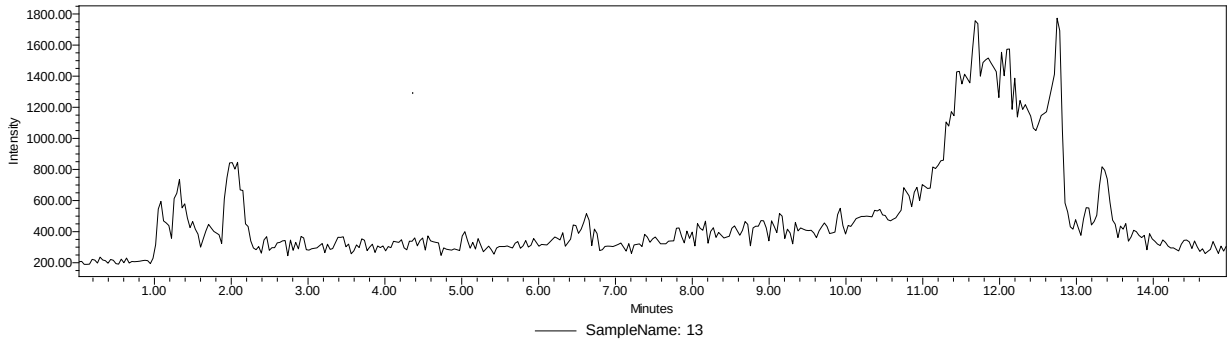

Fig. 28(69)Thermal/Saline product chromatograph screening for FUPA @ .17 day 3rd replicate.

129

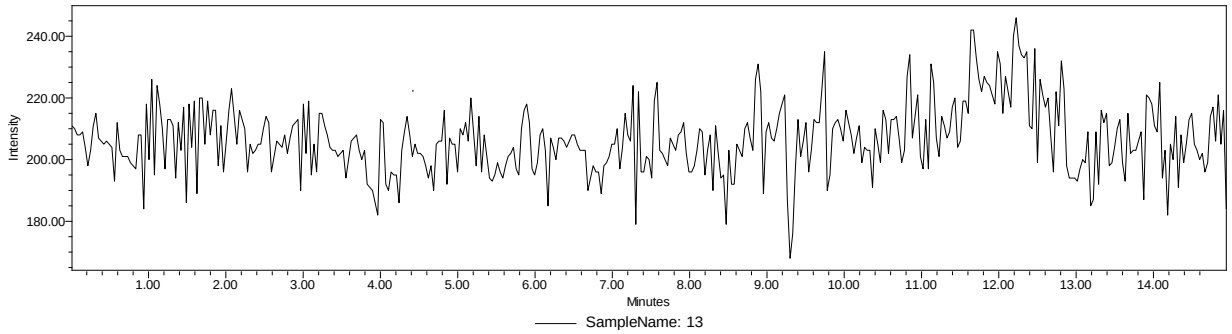

Fig. 28(70)Thermal/Saline product chromatograph screening for 5-FU @ .17 day 3rd replicate.

14

77

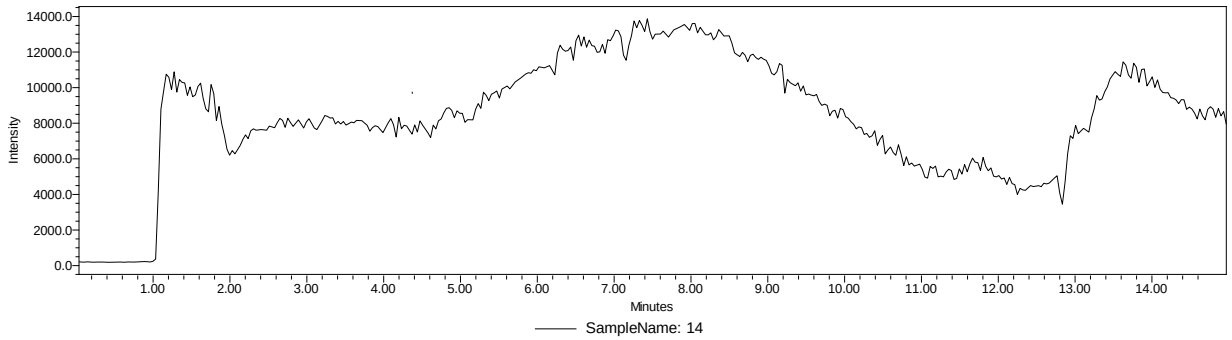

Fig. 28(71)Thermal/Saline product chromatograph screening for FAC @ .21 day 1st replicate.

105

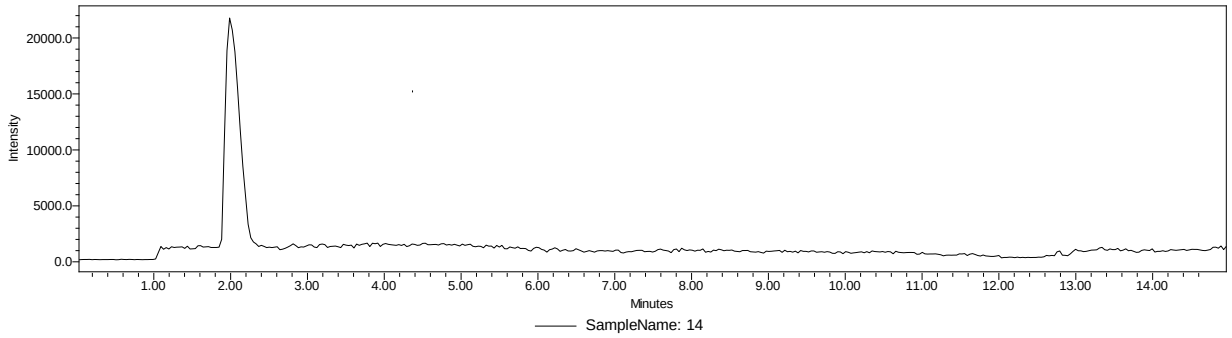

Fig. 28(72)Thermal/Saline product chromatograph screening for FMASld @ .21 day 1st replicate.

106

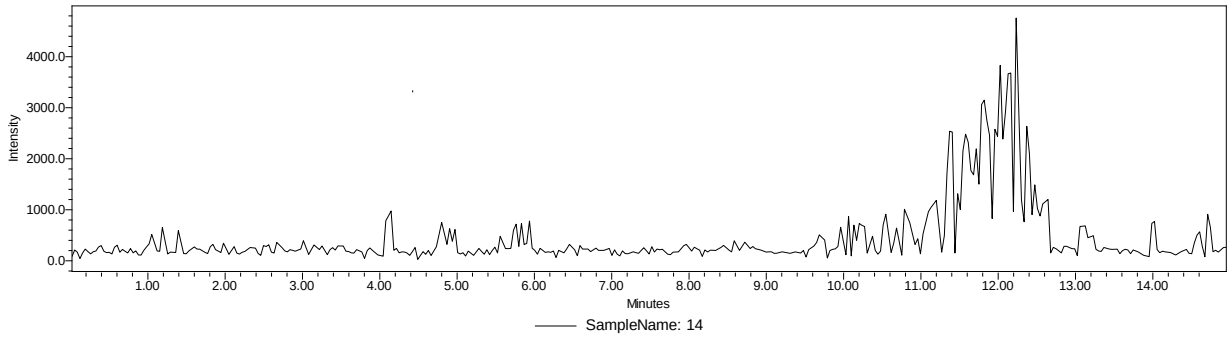

Fig. 28(73)Thermal/Saline product chromatograph screening for FBAL @ .21 day 1st replicate.

149

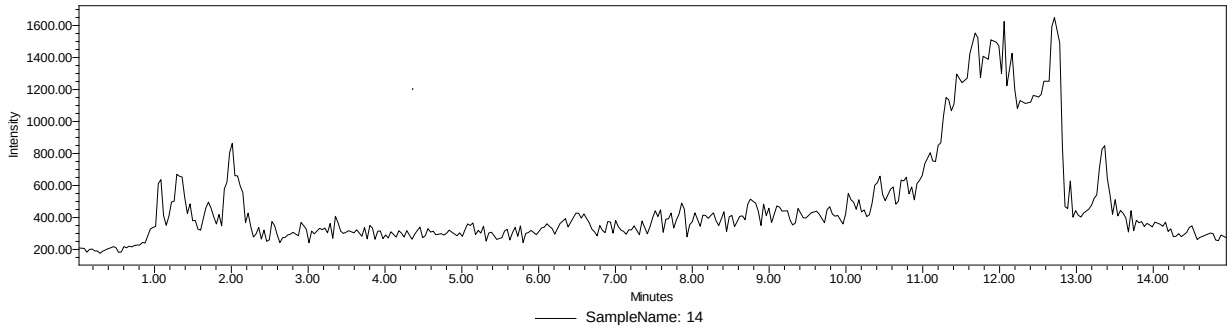

Fig. 28(74)Thermal/Saline product chromatograph screening for FUPA @ .21 day 1st replicate.

129

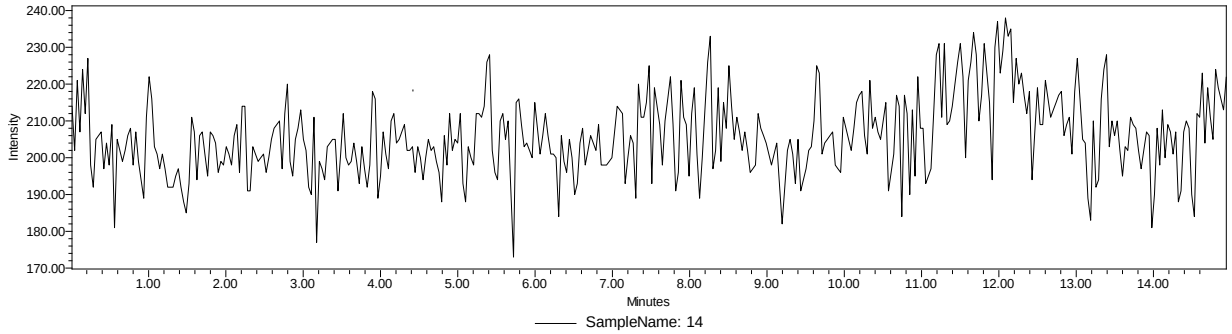

Fig. 28(75)Thermal/Saline product chromatograph screening for 5-FU @ .21 day 1st replicate.

15

77

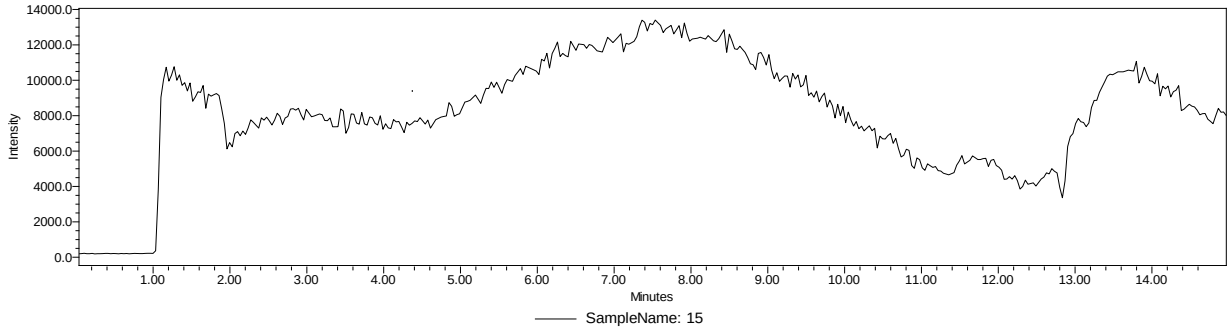

Fig. 28(76)Thermal/Saline product chromatograph screening for FAC @ .21 day 2nd replicate.

105

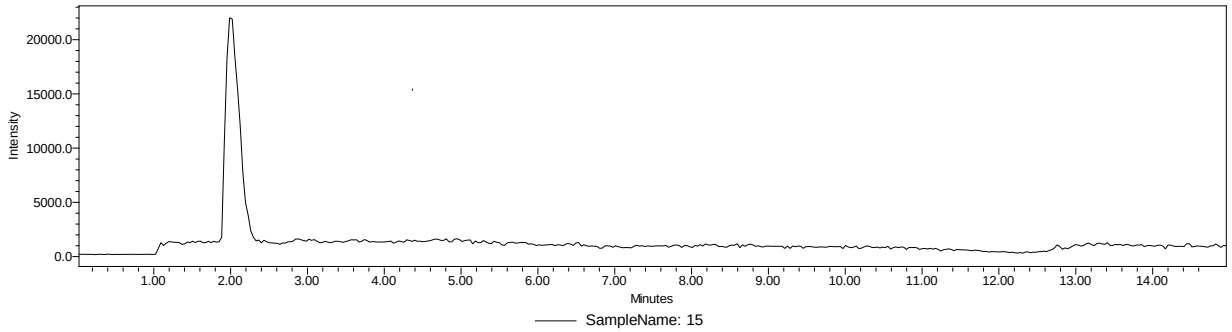

Fig. 28(77)Thermal/Saline product chromatograph screening for FMASld @ .21 day 2nd replicate.

106

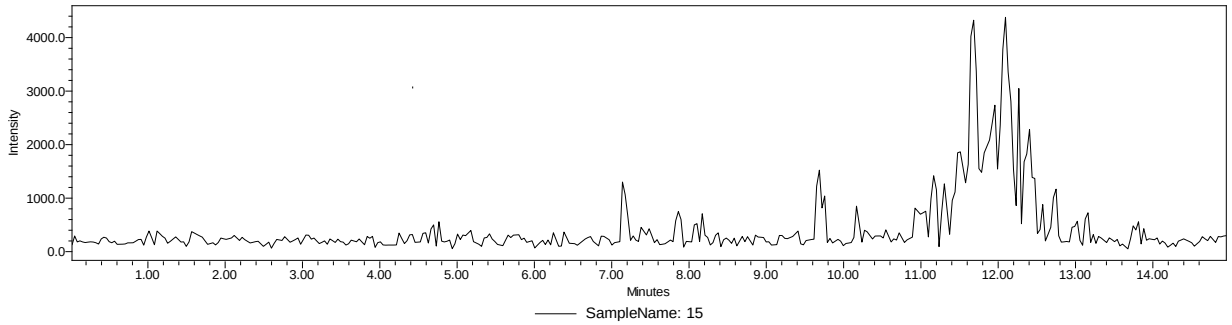

Fig. 28(78)Thermal/Saline product chromatograph screening for FBAL @ .21 day 2nd replicate.

149

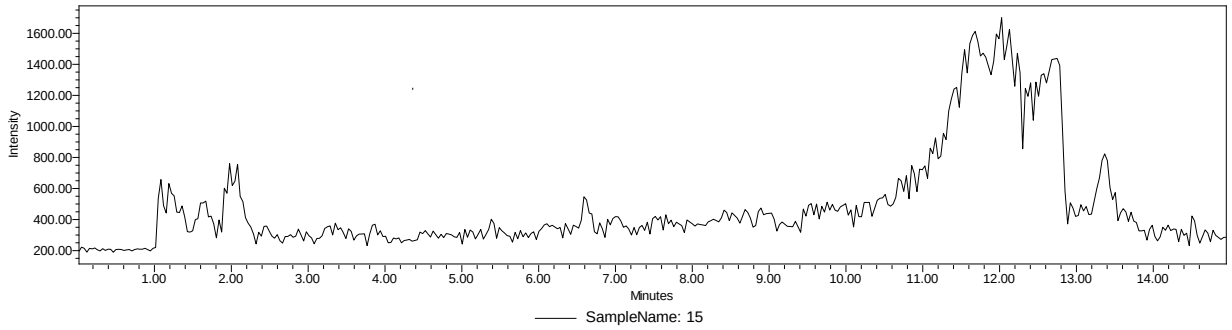

Fig. 28(79)Thermal/Saline product chromatograph screening for FUPA @ .21 day 2nd replicate.

129

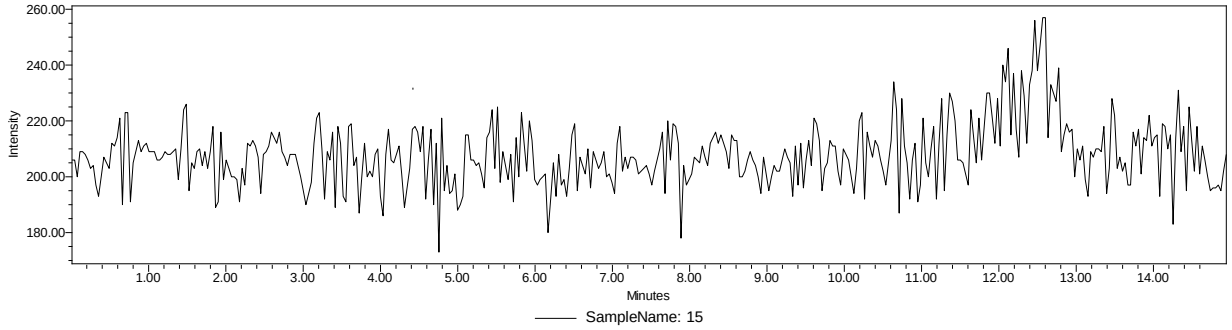

Fig. 28(80)Thermal/Saline product chromatograph screening for 5-FU @ .21 day 2nd replicate.

16

77

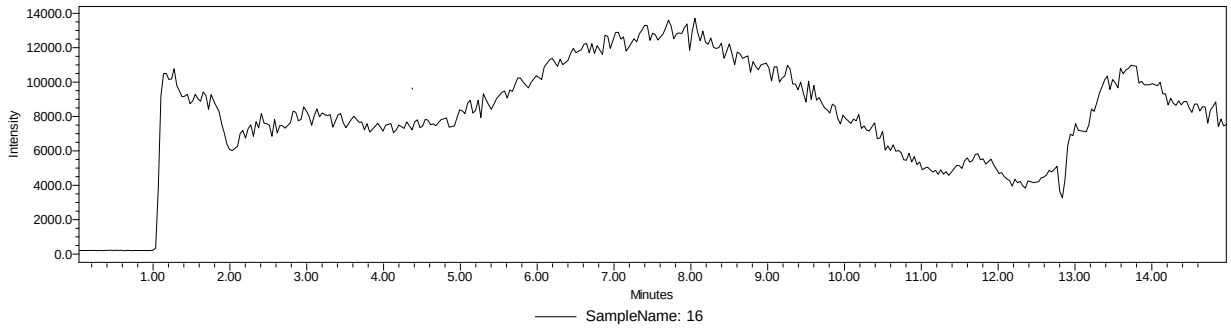

Fig. 28(81)Thermal/Saline product chromatograph screening for FAC @ .21 day 3rd replicate.

105

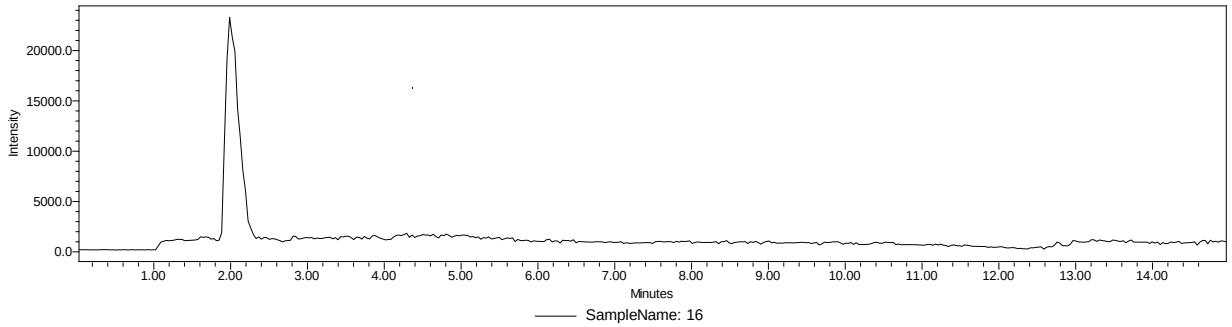

Fig. 28(82)Thermal/Saline product chromatograph screening for FMASld @ .21 day 3rd replicate.

106

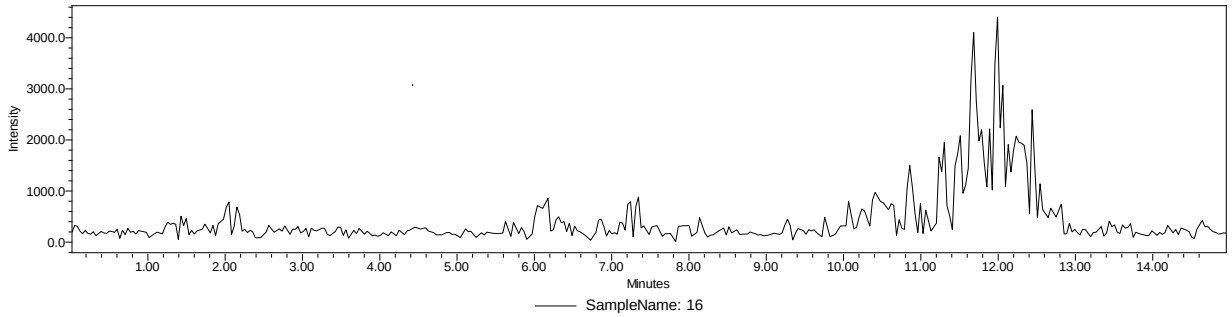

Fig. 28(83)Thermal/Saline product chromatograph screening for FBAL @ .21 day 3rd replicate.

149

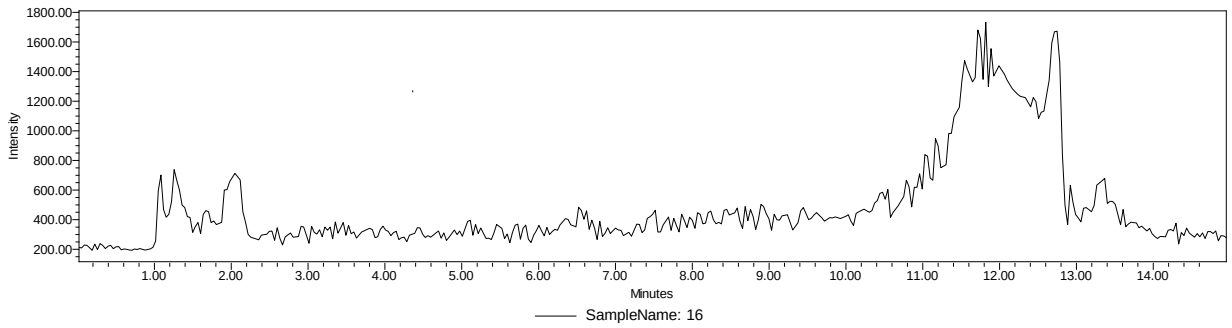

Fig. 28(84)Thermal/Saline product chromatograph screening for FUPA @ .21 day 3rd replicate.

129

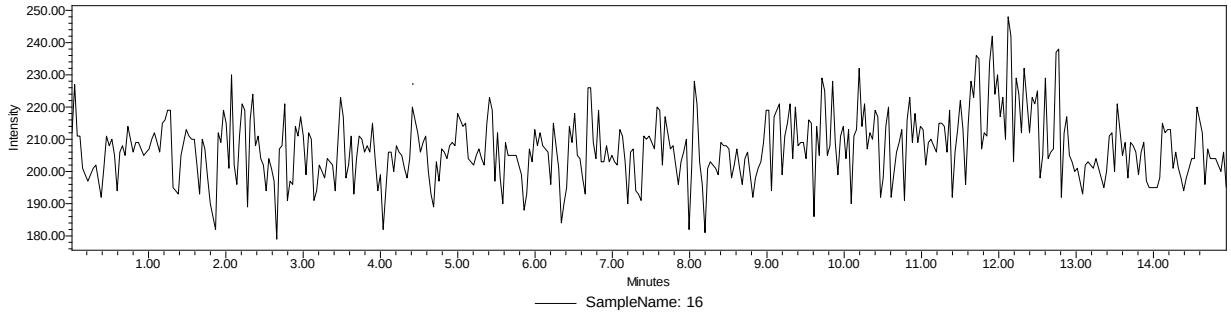

Fig. 28(85)Thermal/Saline product chromatograph screening for 5-FU @ .21 day 3rd replicate.

17

77

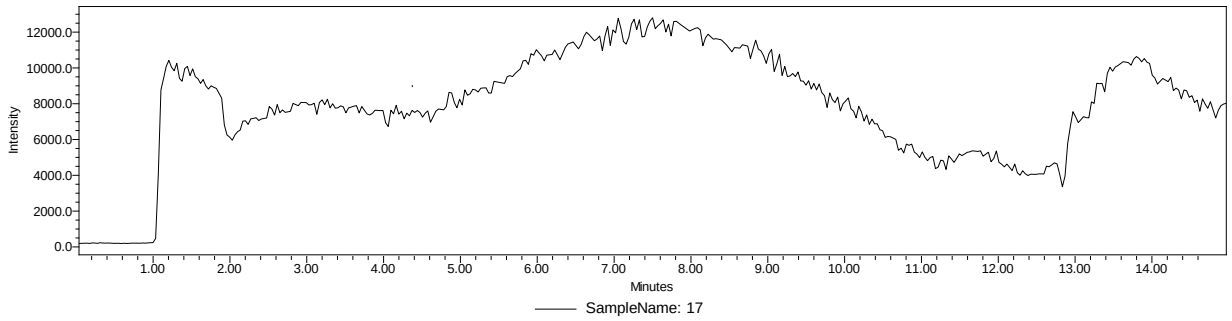

Fig. 28(86)Thermal/Saline product chromatograph screening for FAC @ .38 day 1st replicate.

105

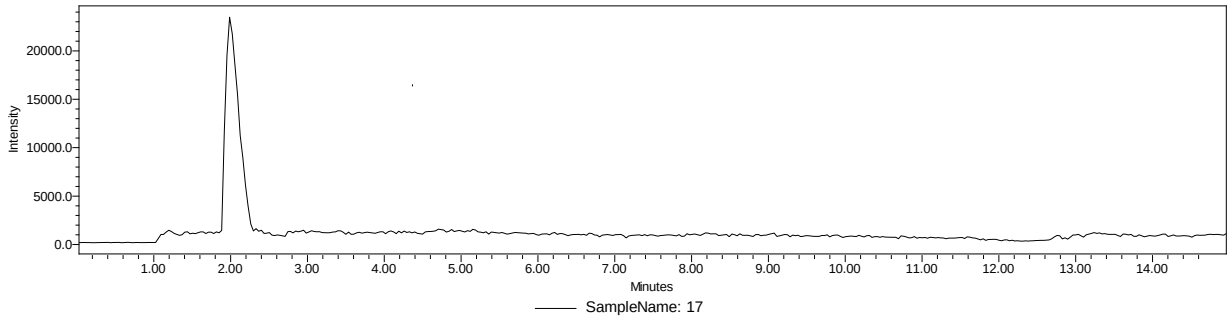

Fig. 28(87)Thermal/Saline product chromatograph screening for FMASld @ .38 day 1st replicate.

106

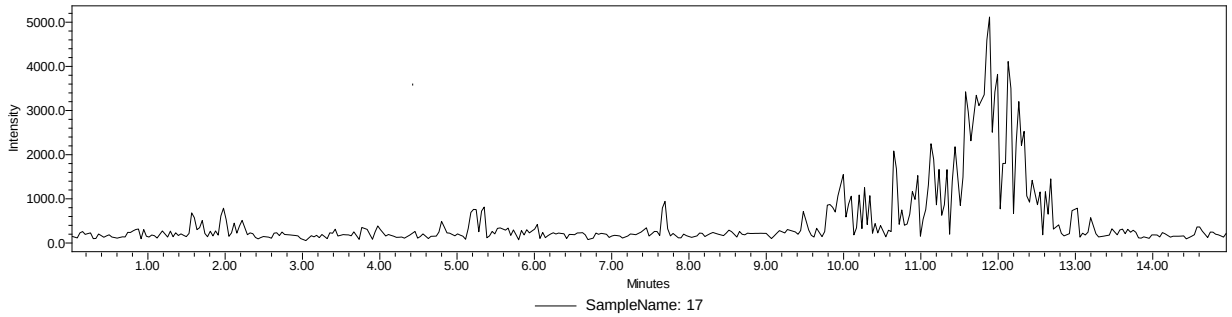

Fig. 28(88)Thermal/Saline product chromatograph screening for FBAL @ .38 day 1st replicate.

149

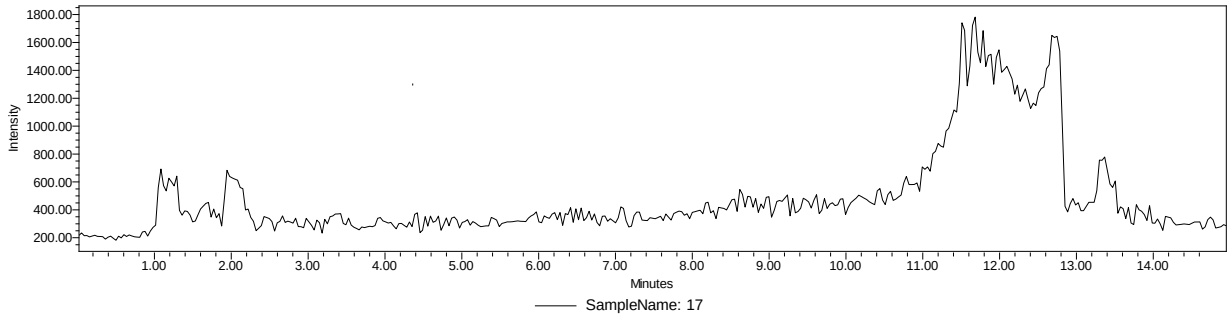

Fig. 28(89)Thermal/Saline product chromatograph screening for FUPA @ .38 day 1st replicate.

129

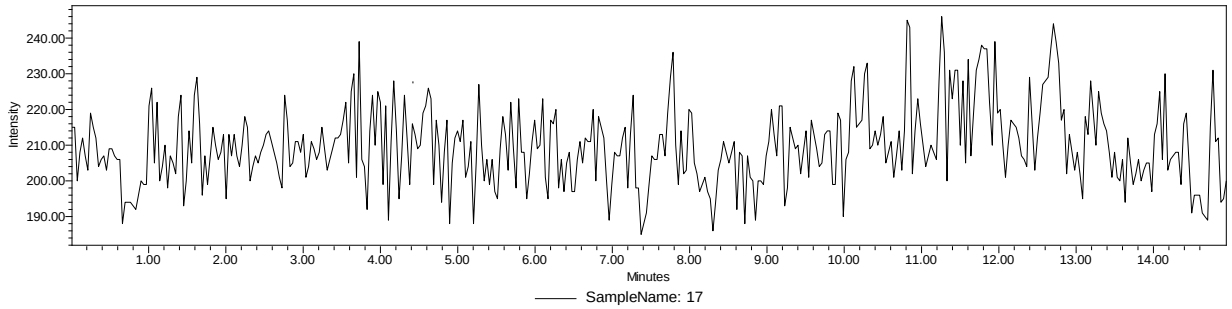

Fig. 28(90)Thermal/Saline product chromatograph screening for 5-FU @ .38 day 1st replicate.

18

77

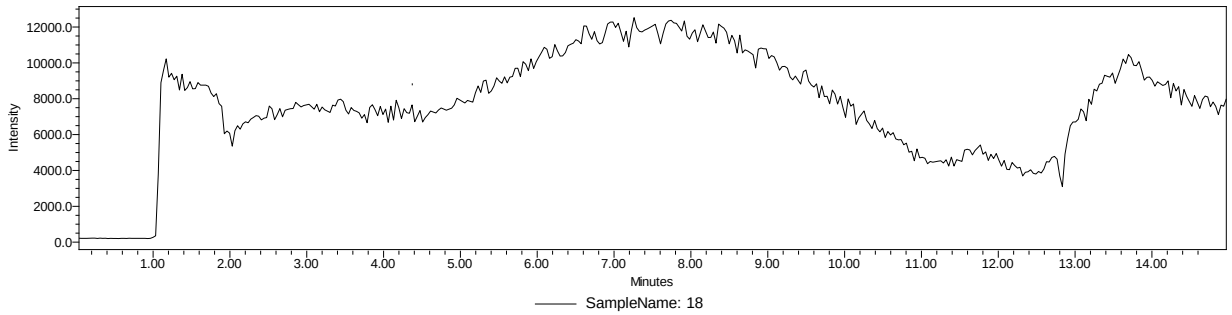

Fig. 28(91)Thermal/Saline product chromatograph screening for FAC @ .38 day 2nd replicate.

105

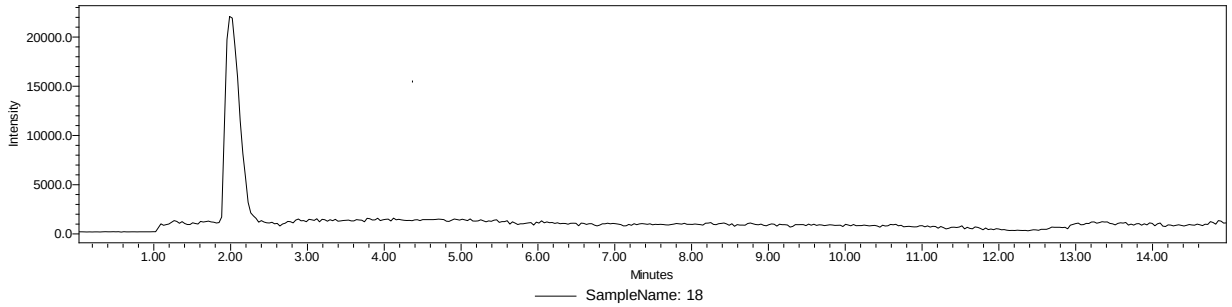

Fig. 28(92)Thermal/Saline product chromatograph screening for FMASld @ .38 day 2nd replicate.

106

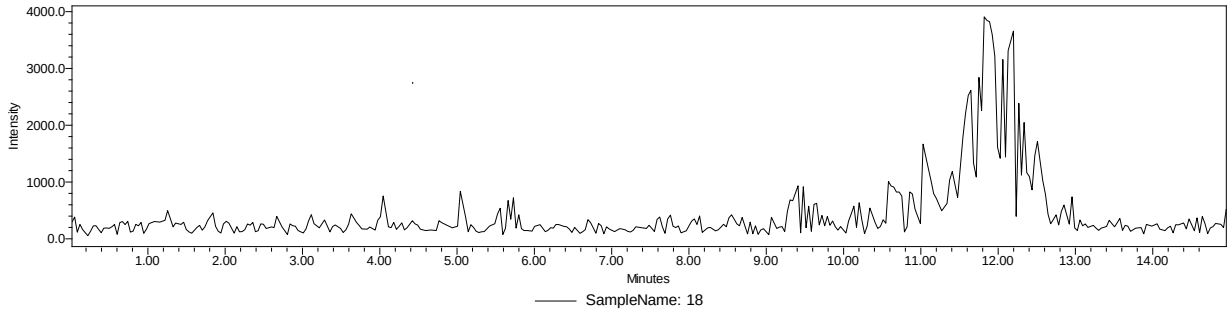

Fig. 28(93)Thermal/Saline product chromatograph screening for FBAL @ .38 day 2nd replicate.

149

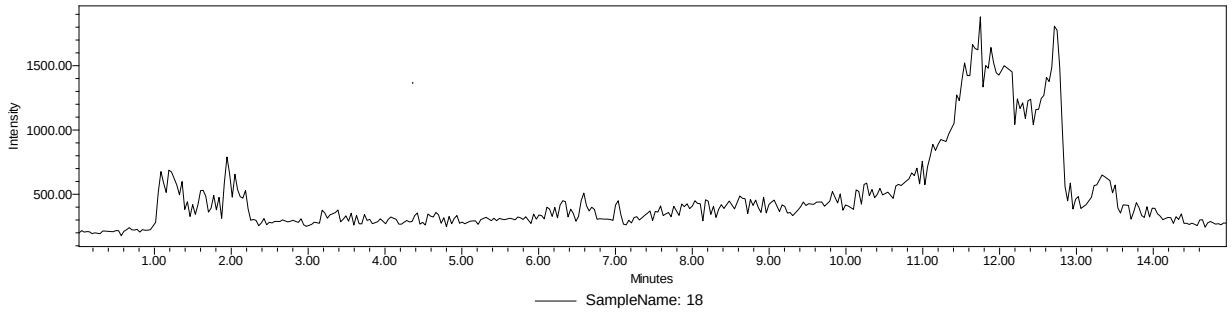

Fig. 28(94)Thermal/Saline product chromatograph screening for FUPA @ .38 day 2nd replicate.

129

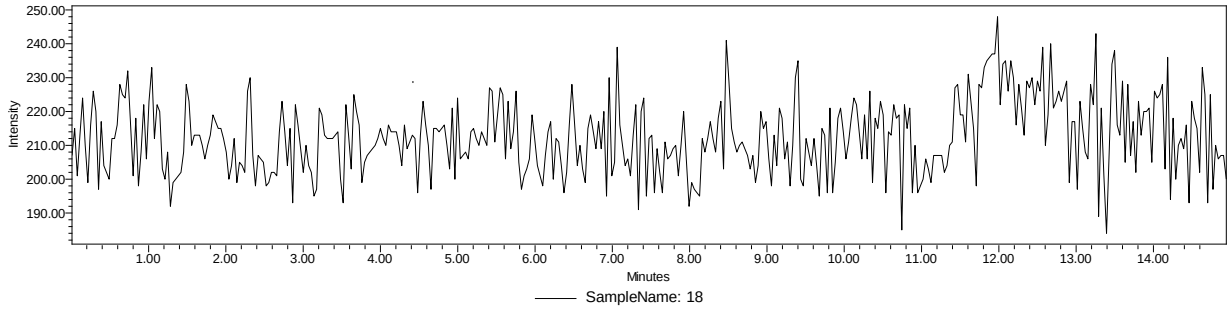

Fig. 28(95)Thermal/Saline product chromatograph screening for 5-FU @ .38 day 2nd replicate.

19

77

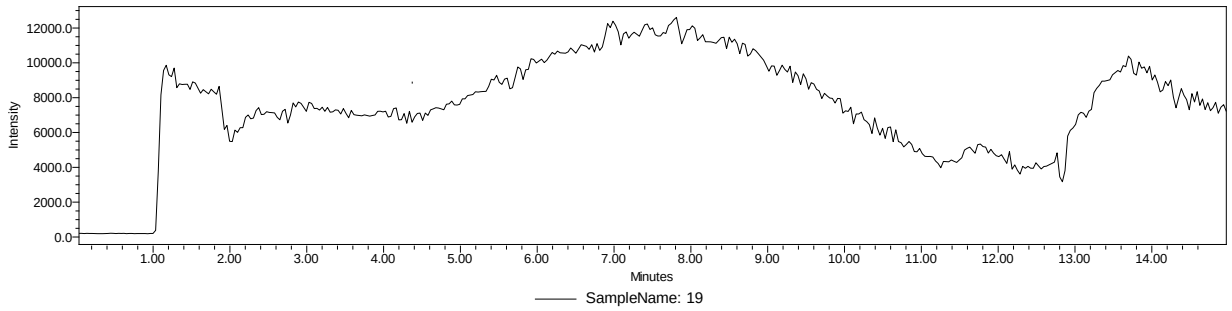

Fig. 28(96)Thermal/Saline product chromatograph screening for FAC @ .38 day 3rd replicate.

105

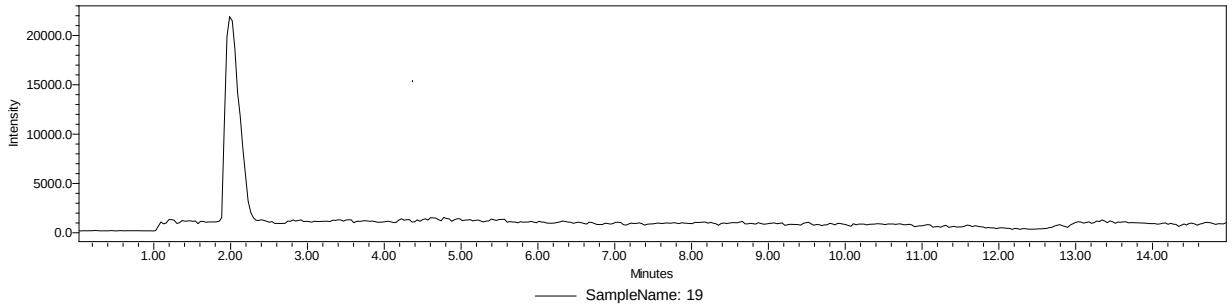

Fig. 28(97)Thermal/Saline product chromatograph screening for FMASld @ .38 day 3rd replicate.

106

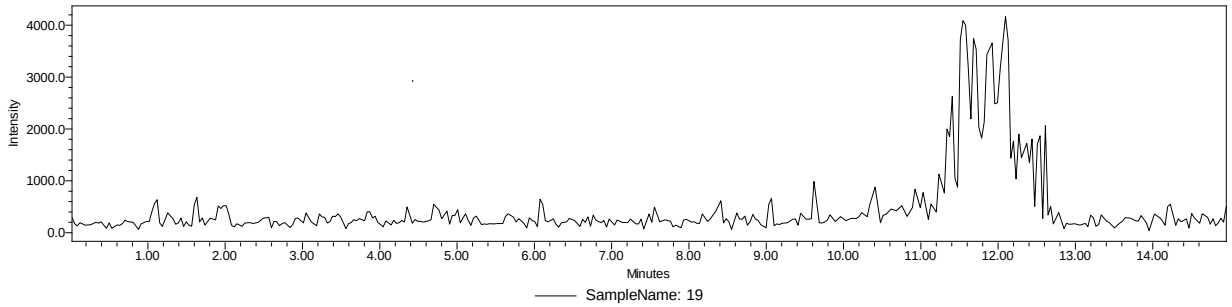

Fig. 28(98)Thermal/Saline product chromatograph screening for FBAL @ .38 day 3rd replicate.

149

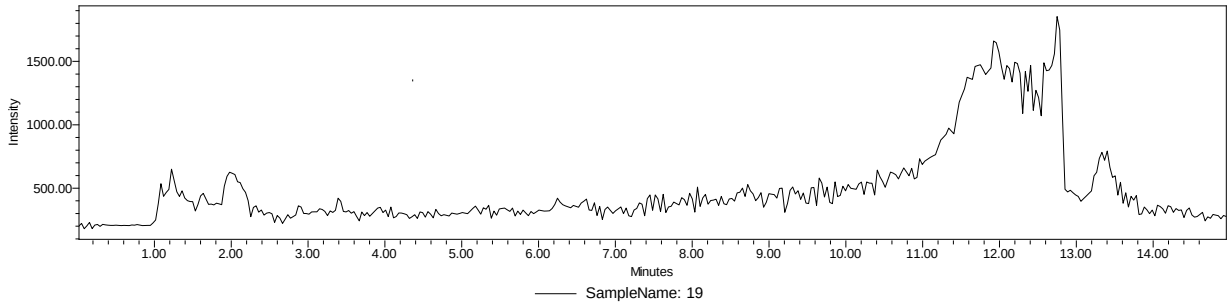

Fig. 28(99)Thermal/Saline product chromatograph screening for FUPA @ .38 day 3rd replicate.

129

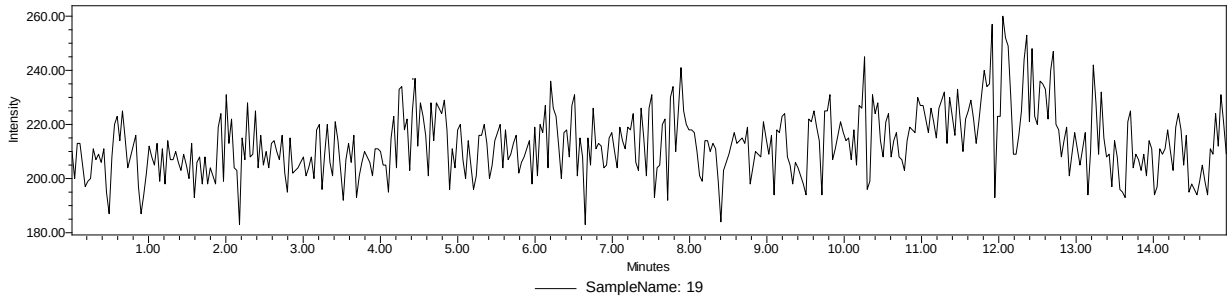

Fig. 28(100)Thermal/Saline product chromatograph screening for 5-FU @ .38 day 3rd replicate.

1

77

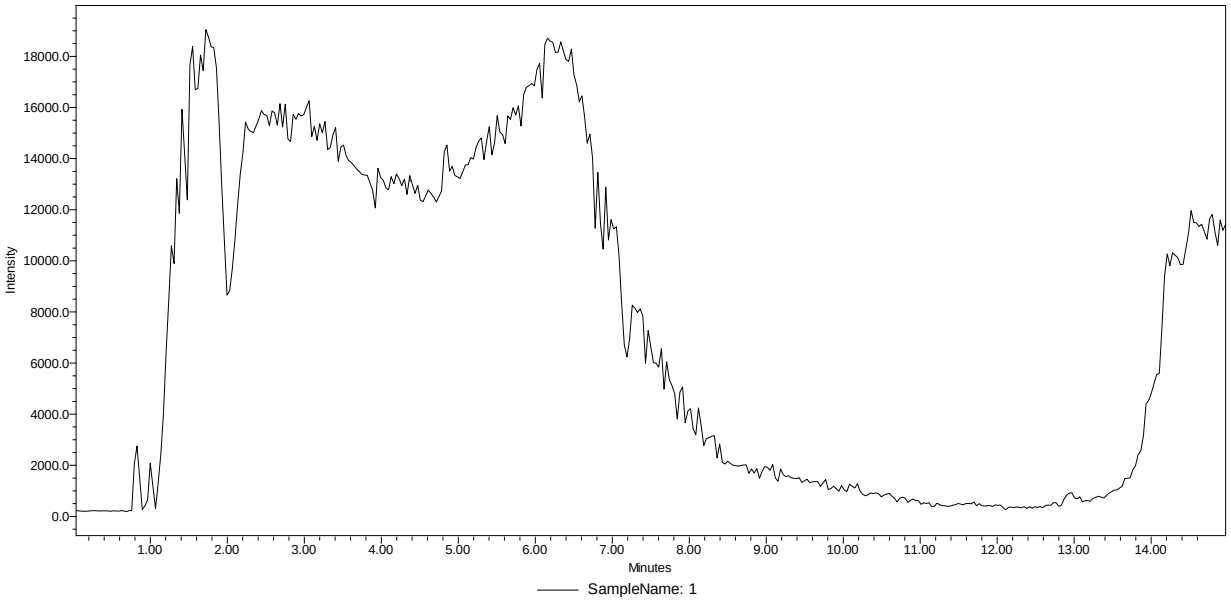

Fig. 29e Photolytic/Alkaline product chromatographs for FAC/sample 1.

105

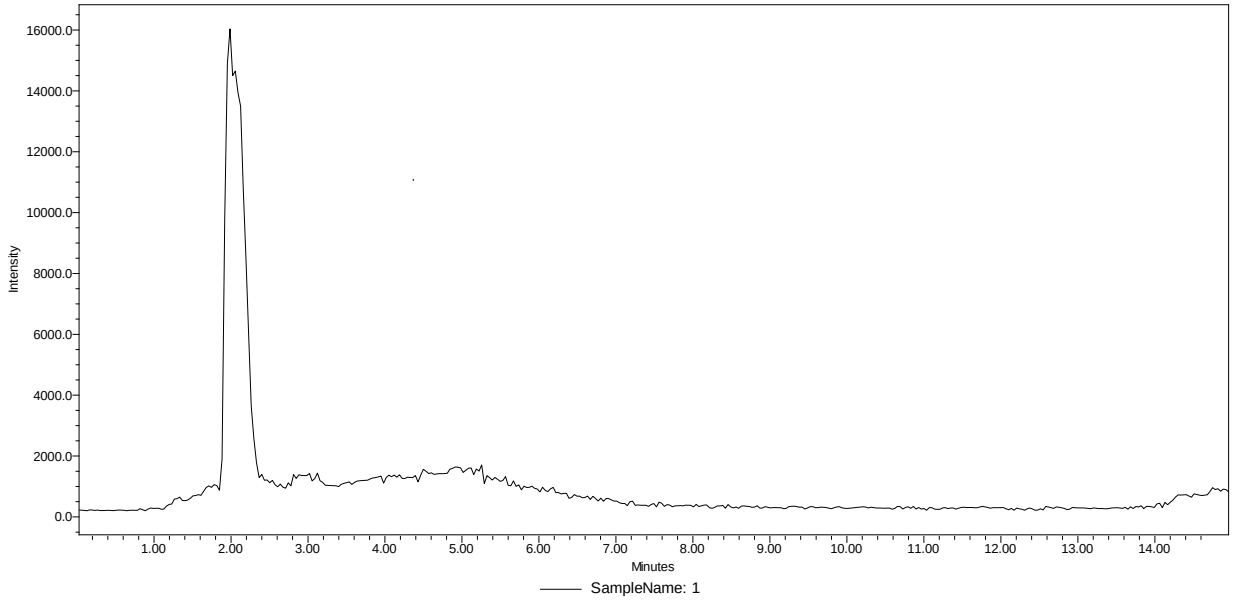

Fig. 29f Photolytic/Alkaline product chromatographs for FMASld/sample 1.

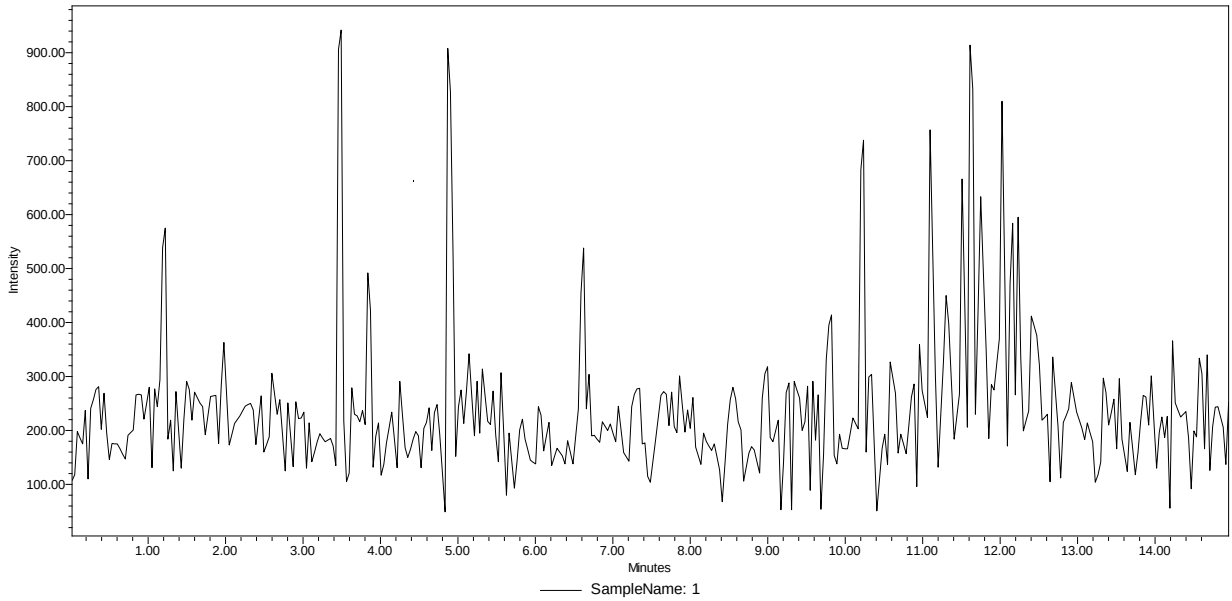

Fig. 29g Photolytic/Alkaline product chromatographs for FBAL/sample 1.

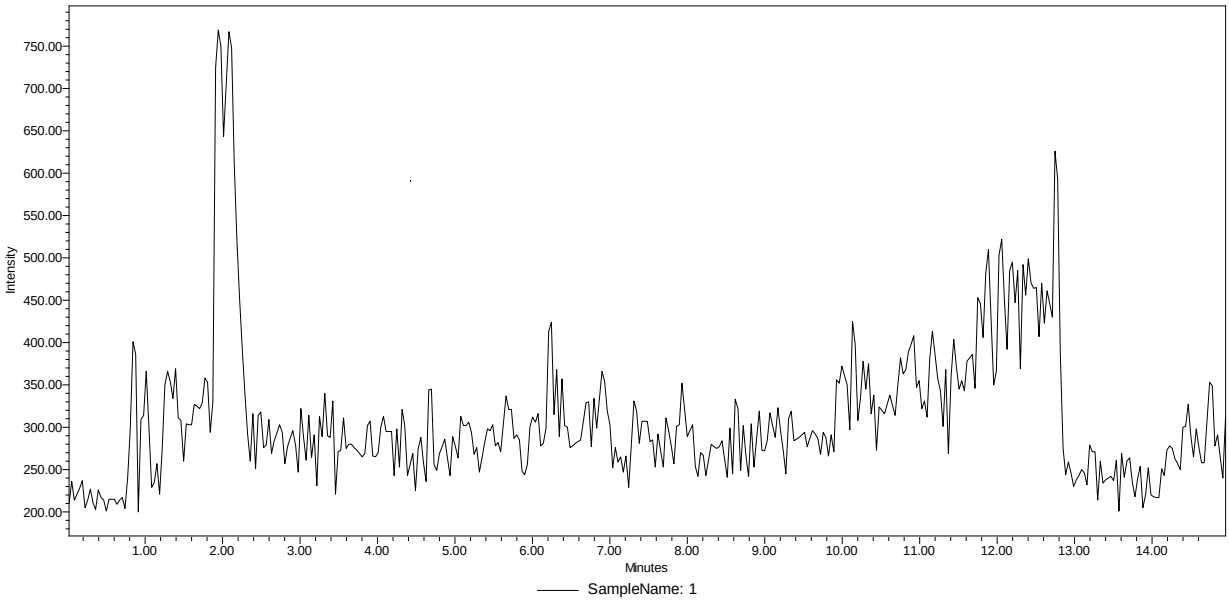

Fig. 29h Photolytic/Alkaline product chromatographs for FUPA/sample 1.

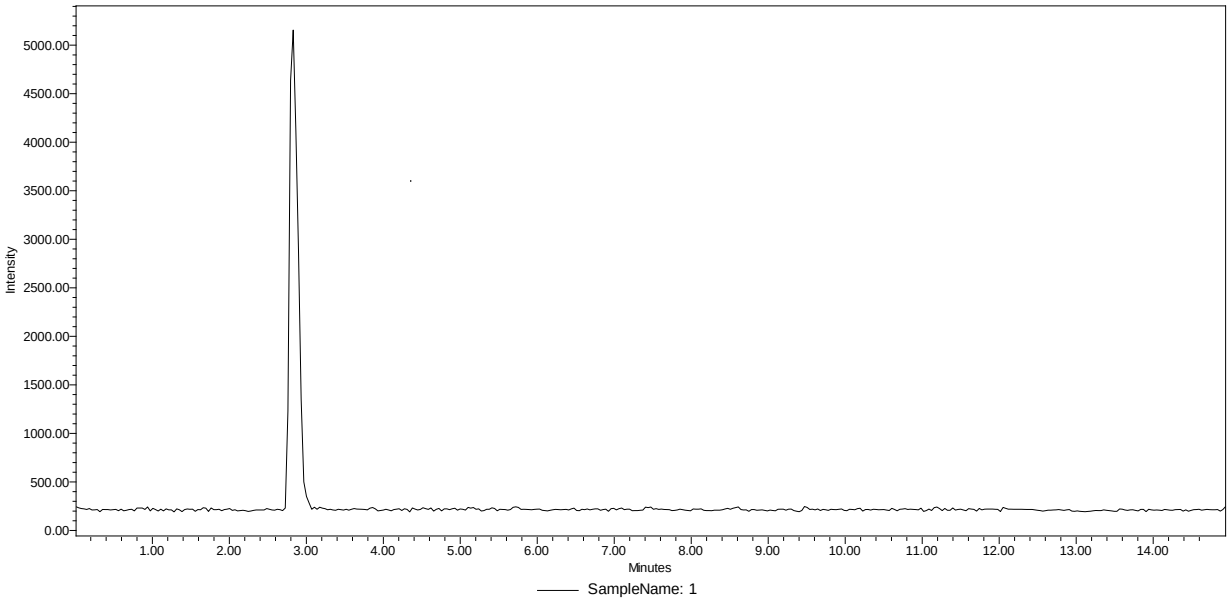

Fig. 29i Photolytic/Alkaline product chromatographs for 5-FU/sample 1.

2

77

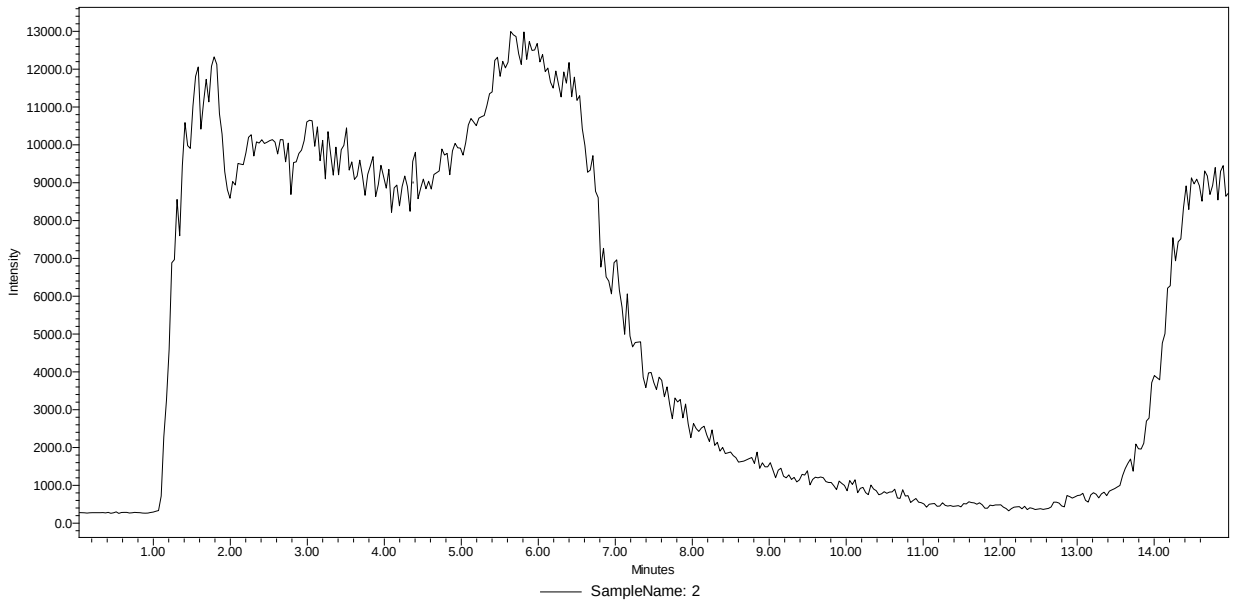

Fig. 29j Photolytic/Alkaline product chromatographs for FAC/sample 2.

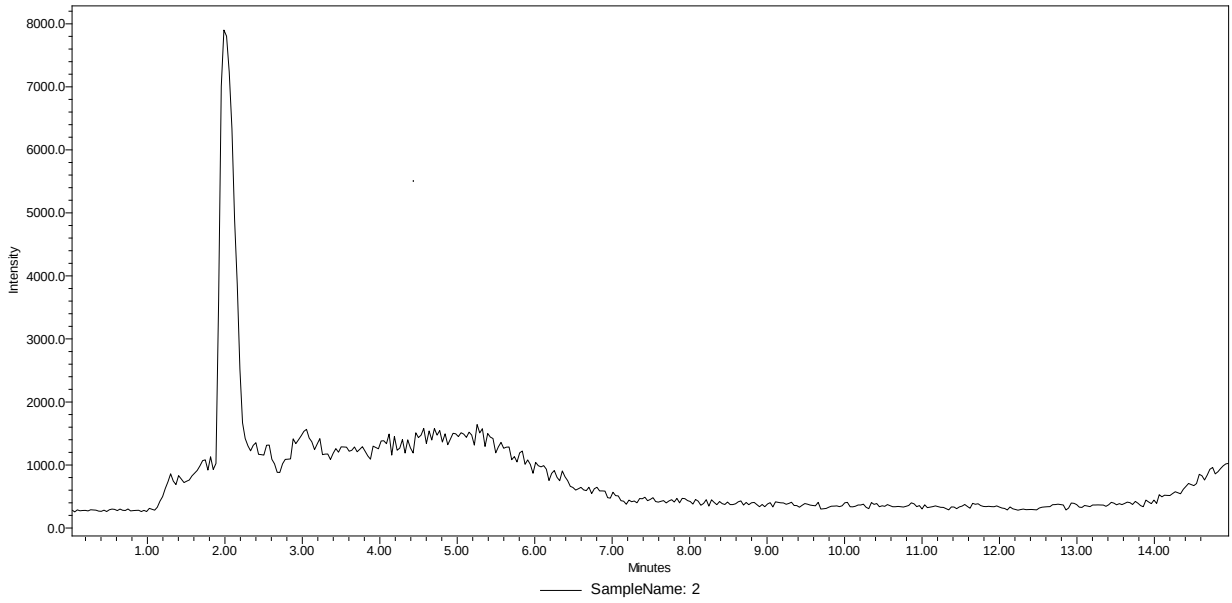

Fig. 29k Photolytic/Alkaline product chromatographs for FMASld/sample 2.

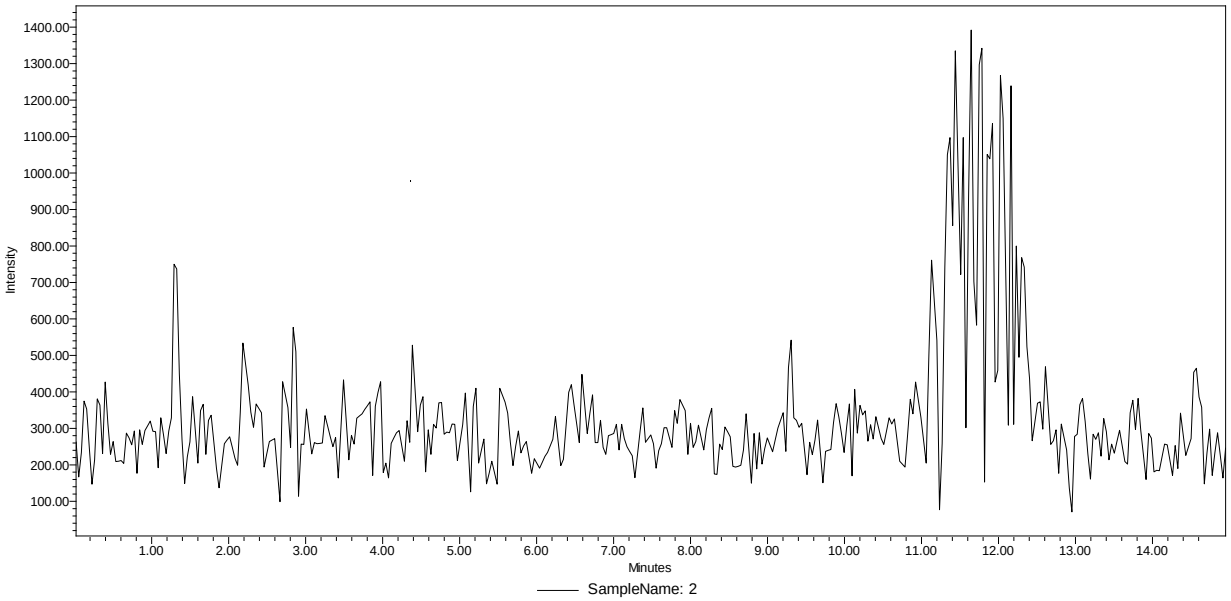

Fig. 29l Photolytic/Alkaline product chromatographs for FBAL/sample 2.

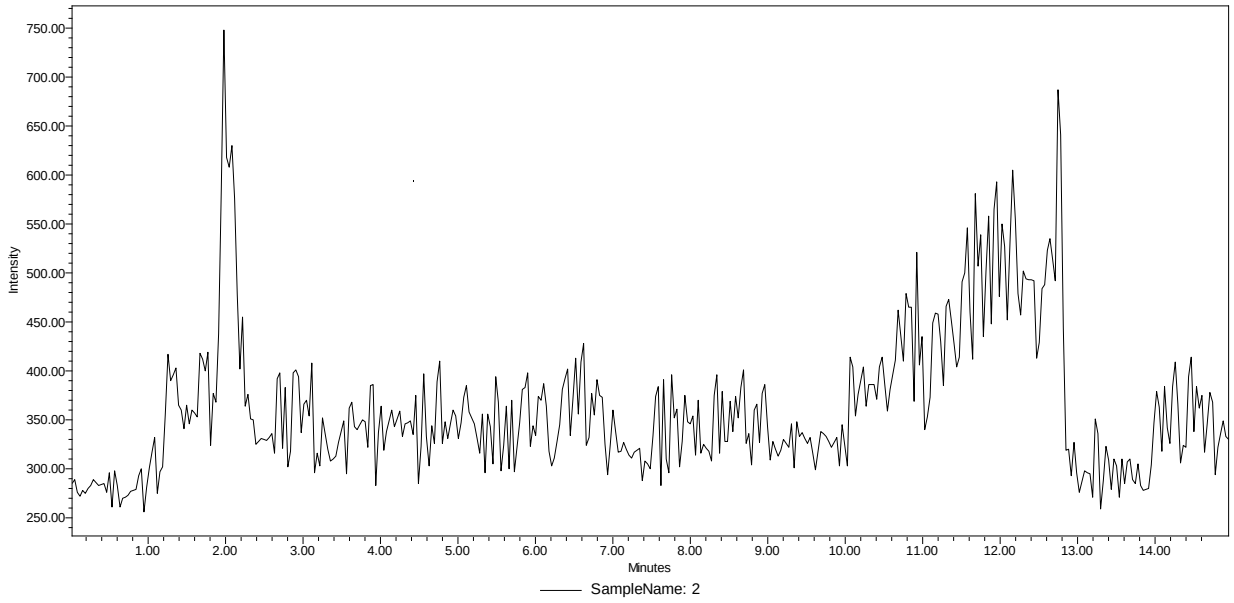

Fig. 29m Photolytic/Alkaline product chromatographs for FUPA/sample 2.

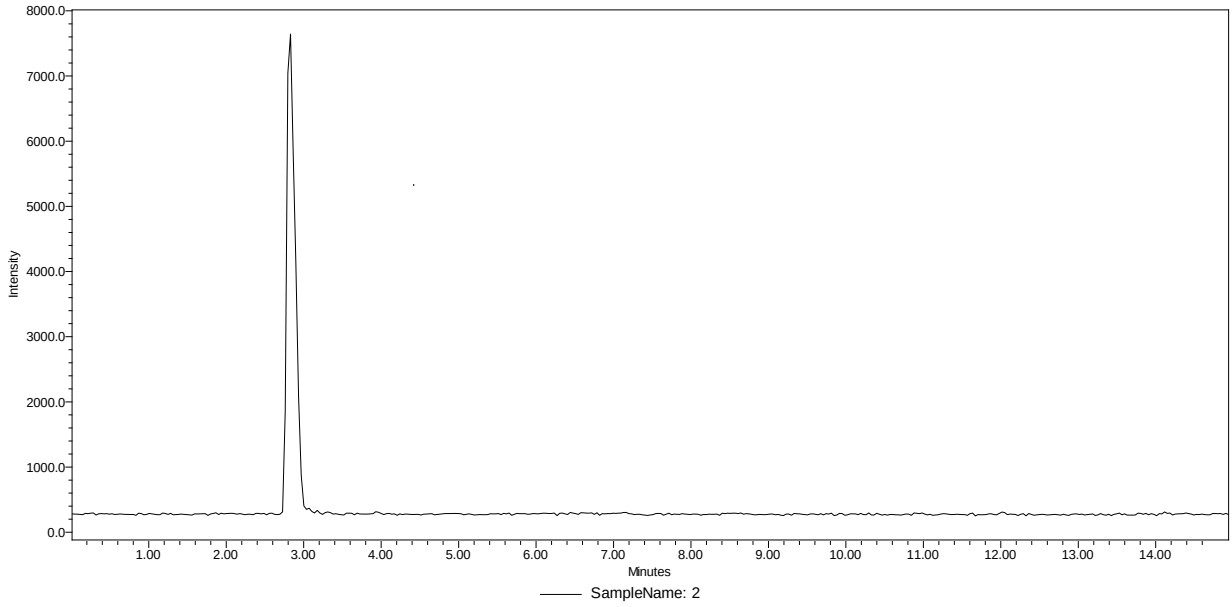

Fig. 29n Photolytic/Alkaline product chromatographs for 5-FU/sample 2.

3

77

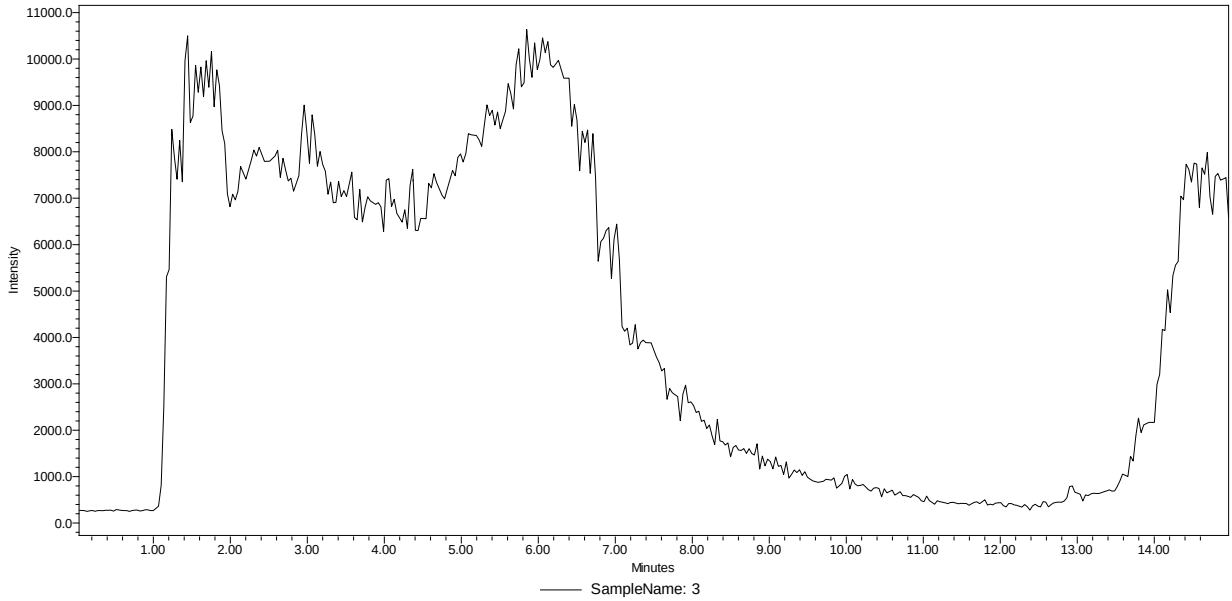

Fig. 29o Photolytic/Alkaline product chromatographs for FAC/sample 3.

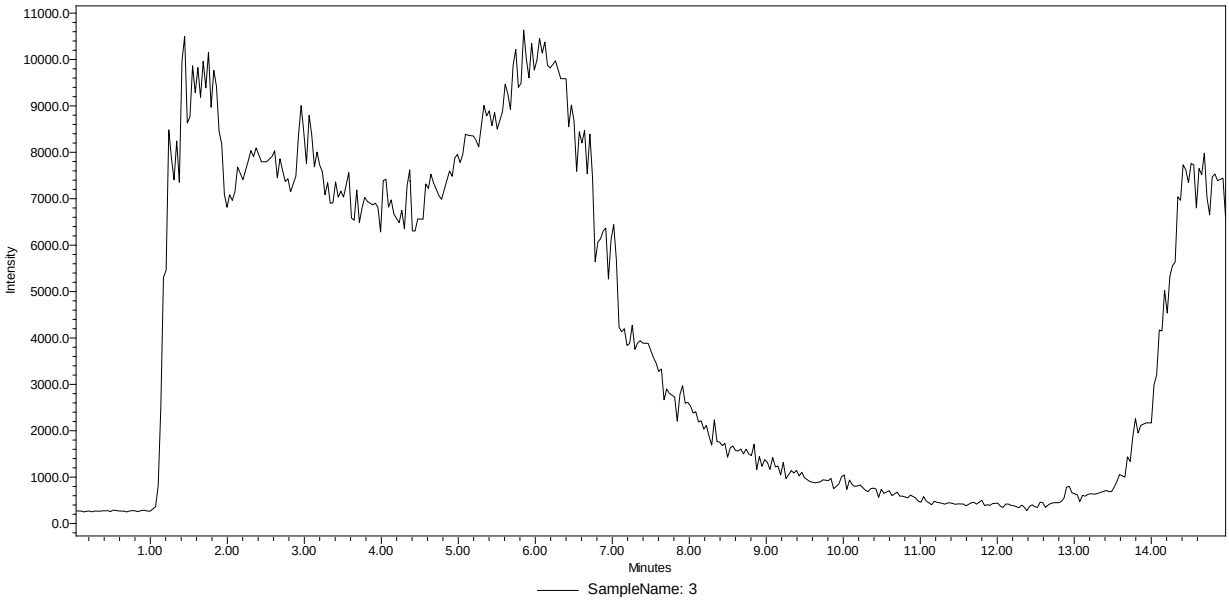

Fig. 29p Photolytic/Alkaline product chromatographs for FMASld/sample 3.

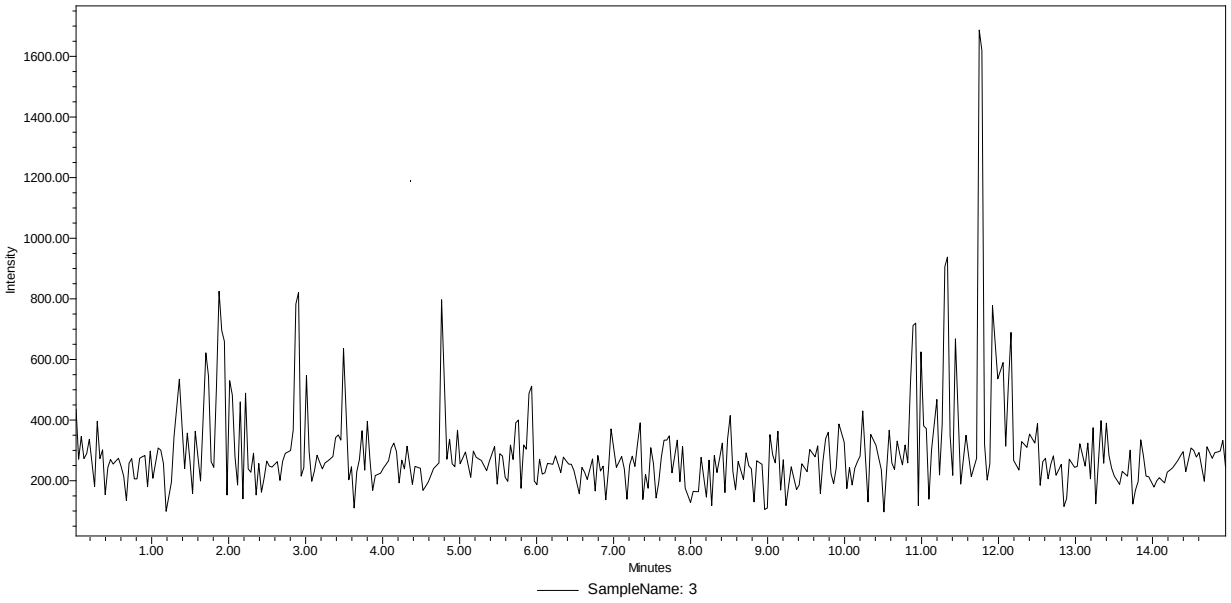

Fig. 29q Photolytic/Alkaline product chromatographs for FBAL/sample 3.

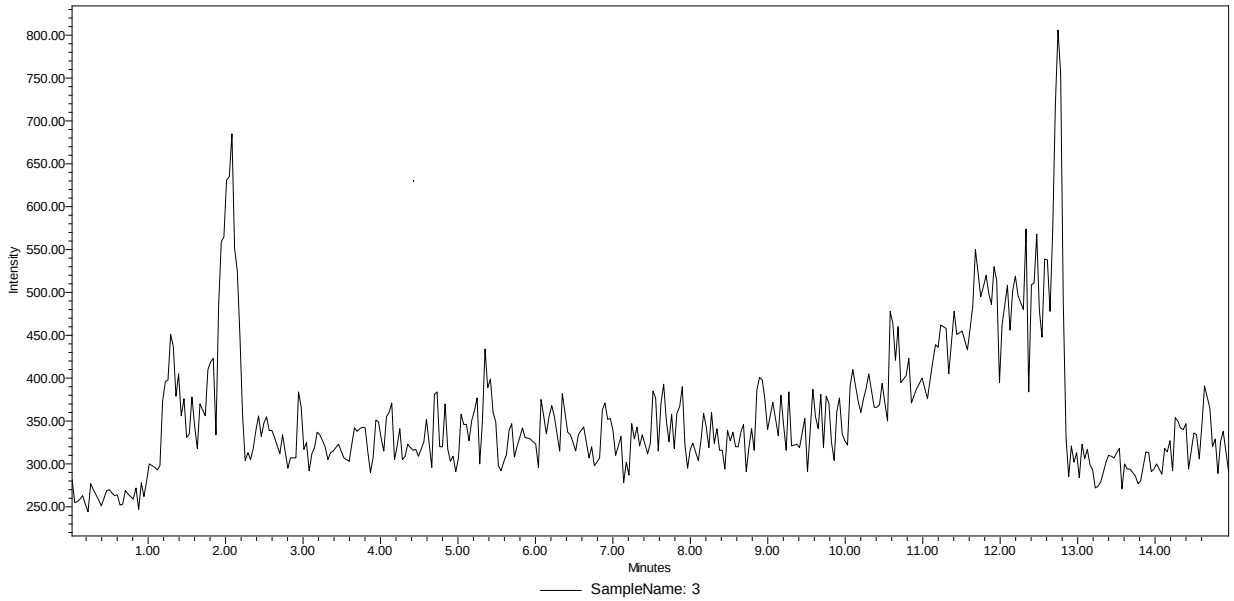

Fig. 29r Photolytic/Alkaline product chromatographs for FUPA/sample 3.

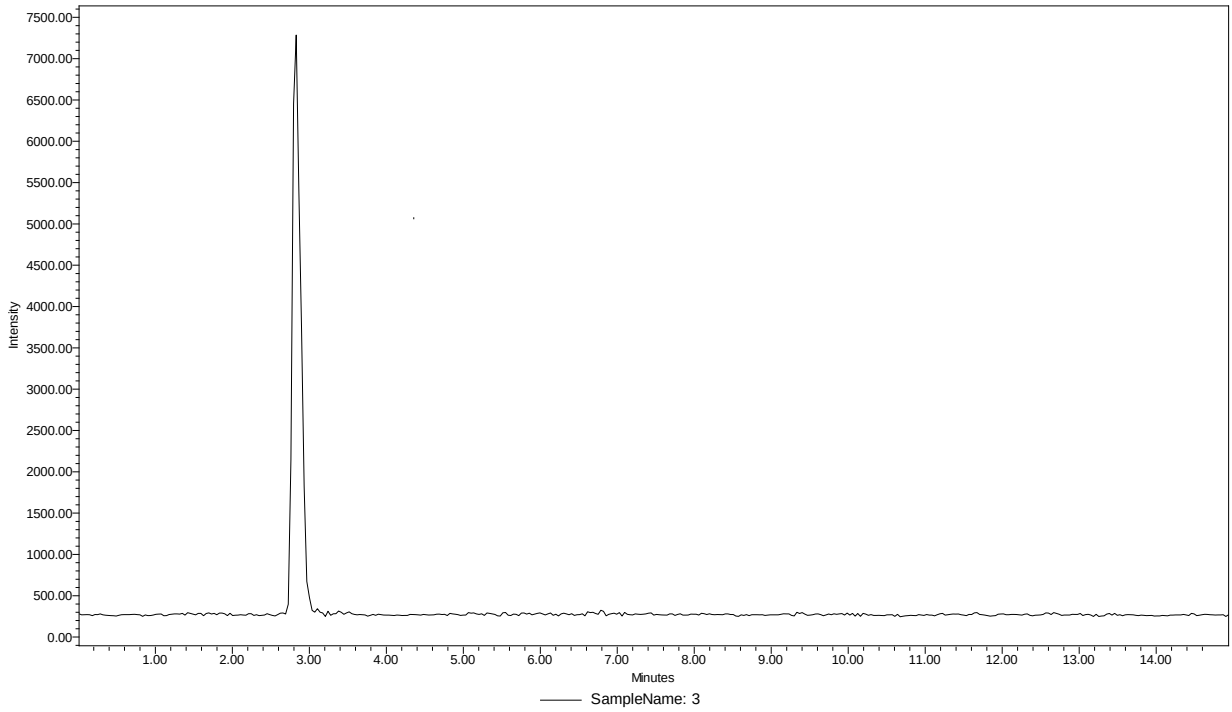

Fig. 29s Photolytic/Alkaline product chromatographs for 5-FU/sample 3.

4

77

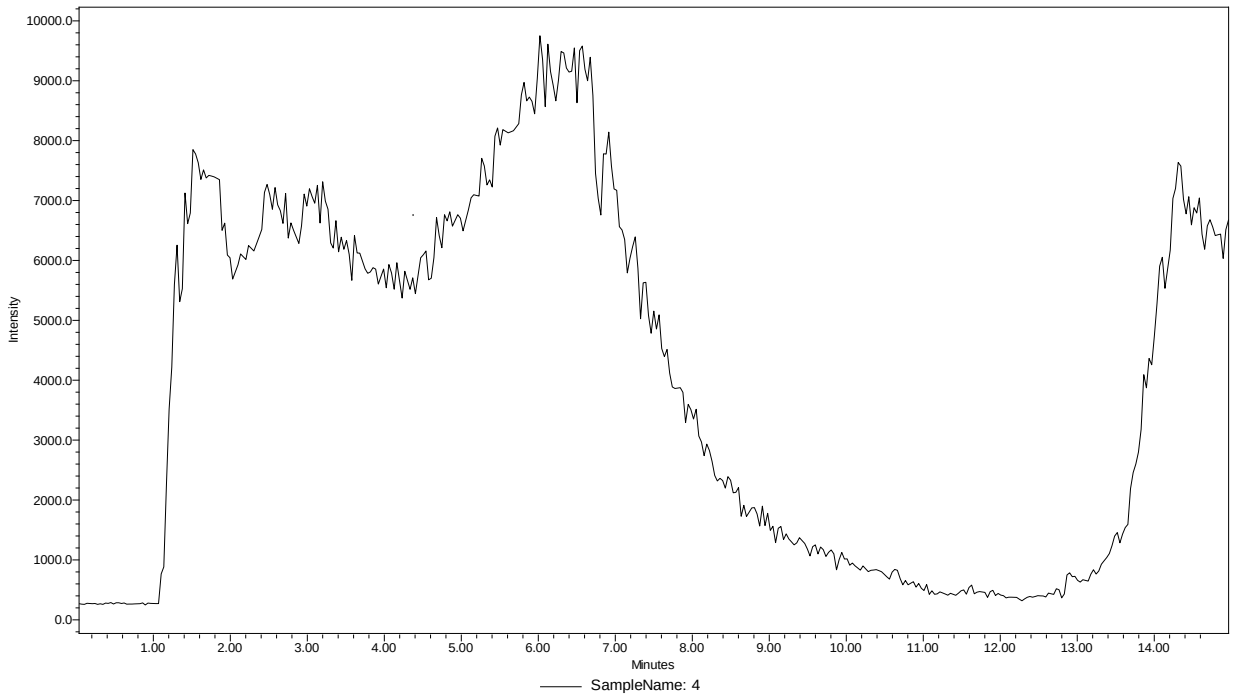

Fig. 29t Photolytic/Alkaline product chromatograph for FAC/sample 4.

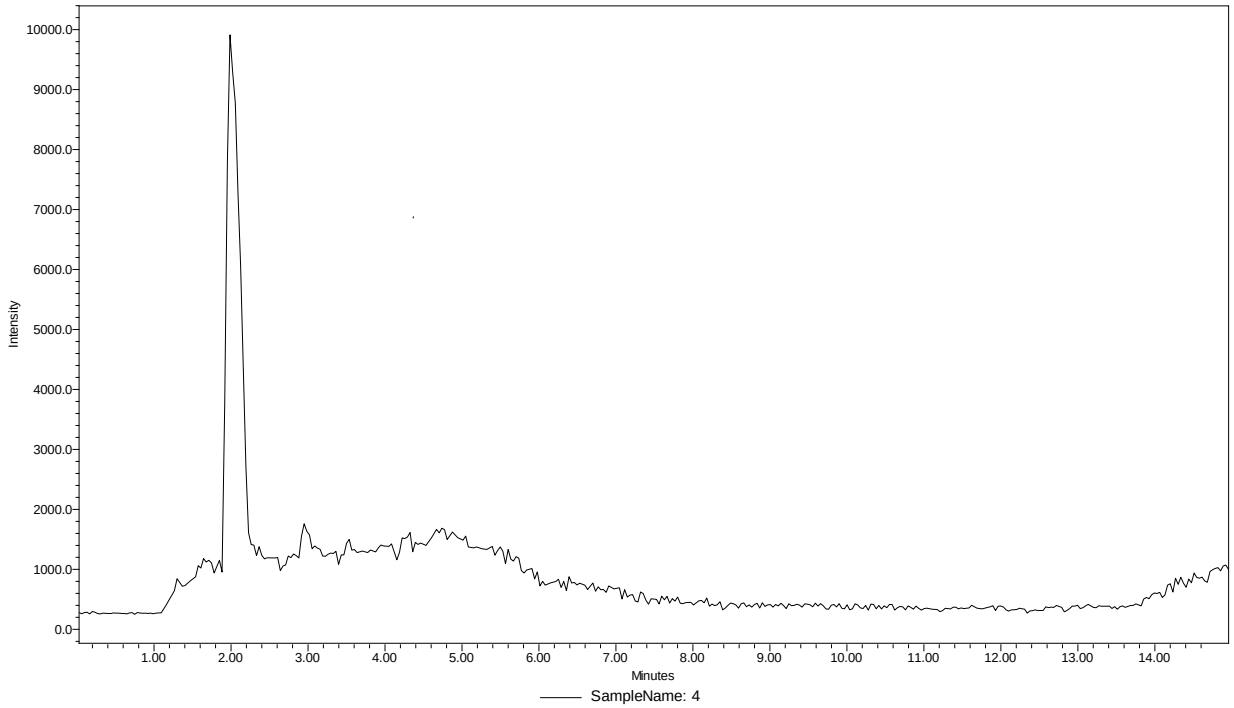

Fig. 29u Photolytic/Alkaline product chromatograph for FMASld/sample 4.

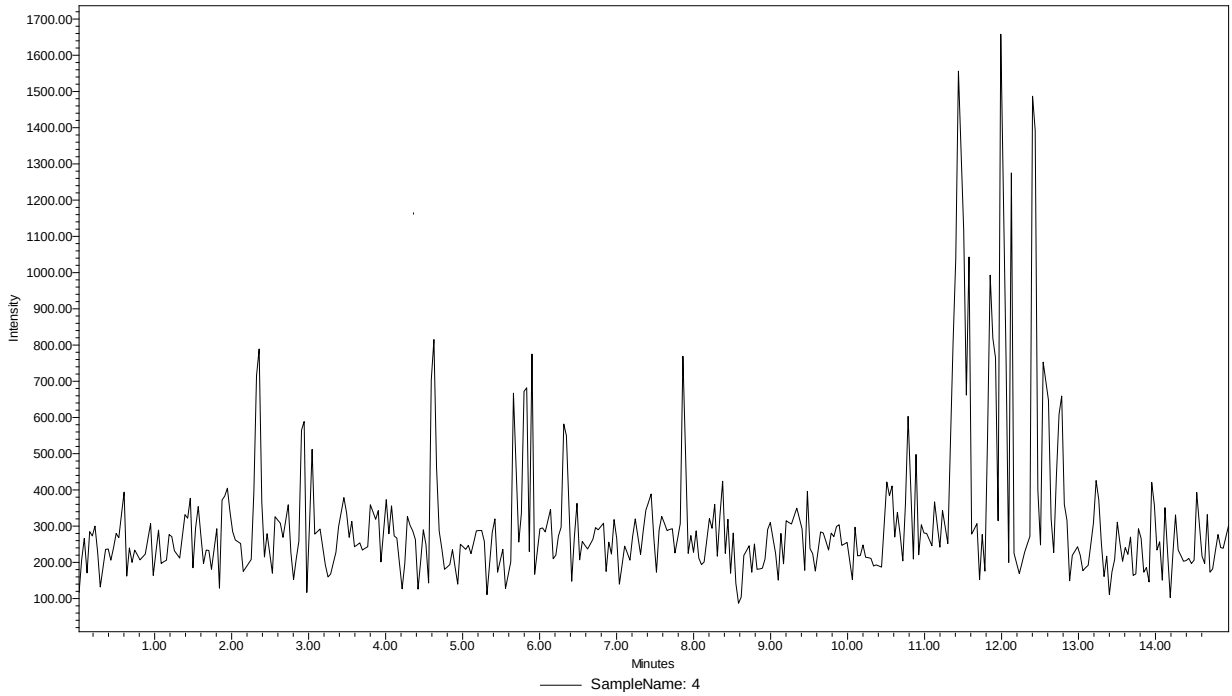

Fig. 29v Photolytic/Alkaline product chromatograph for FBAL/sample 4.

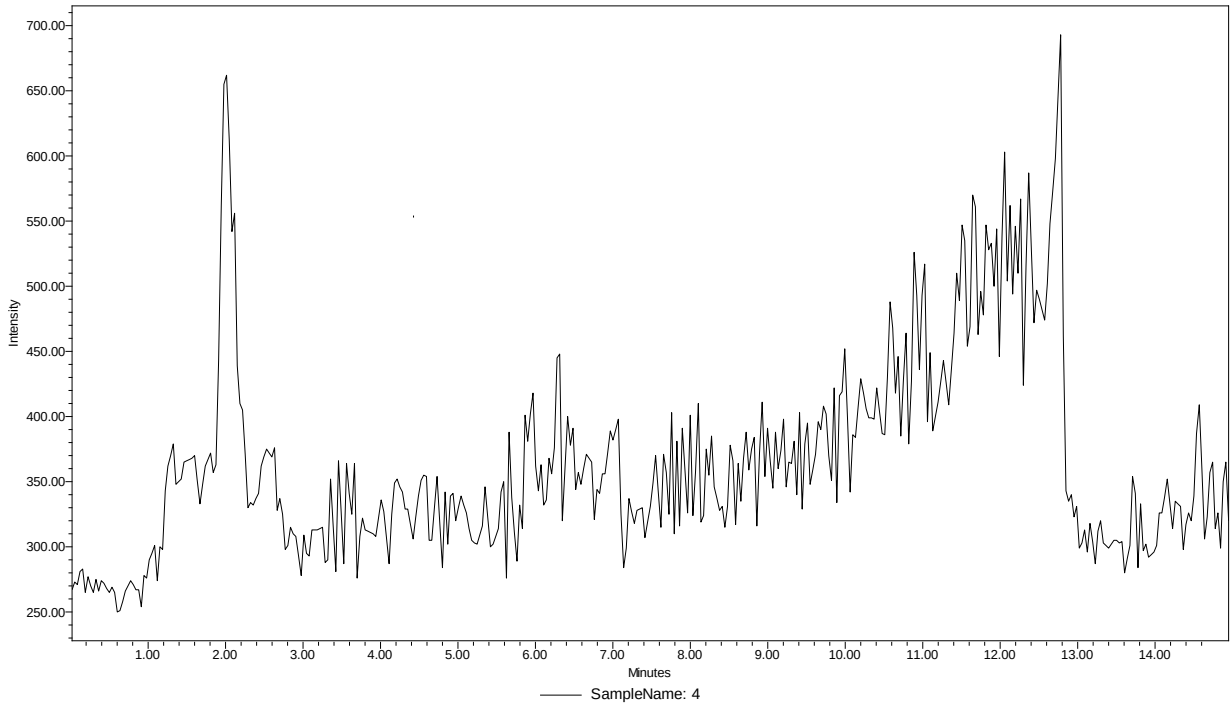

Fig. 29w Photolytic/Alkaline product chromatograph for FUPA/sample 4.

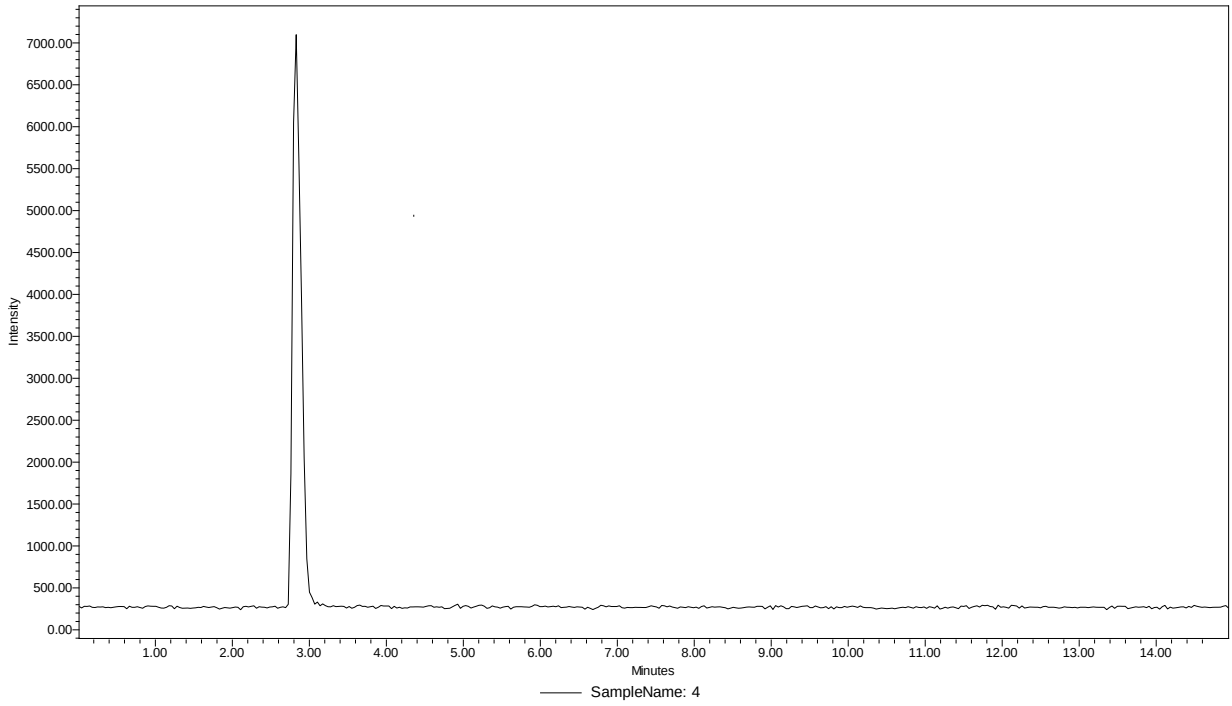

Fig. 29x Photolytic/Alkaline product chromatograph for 5-FU/sample 4.

5

77

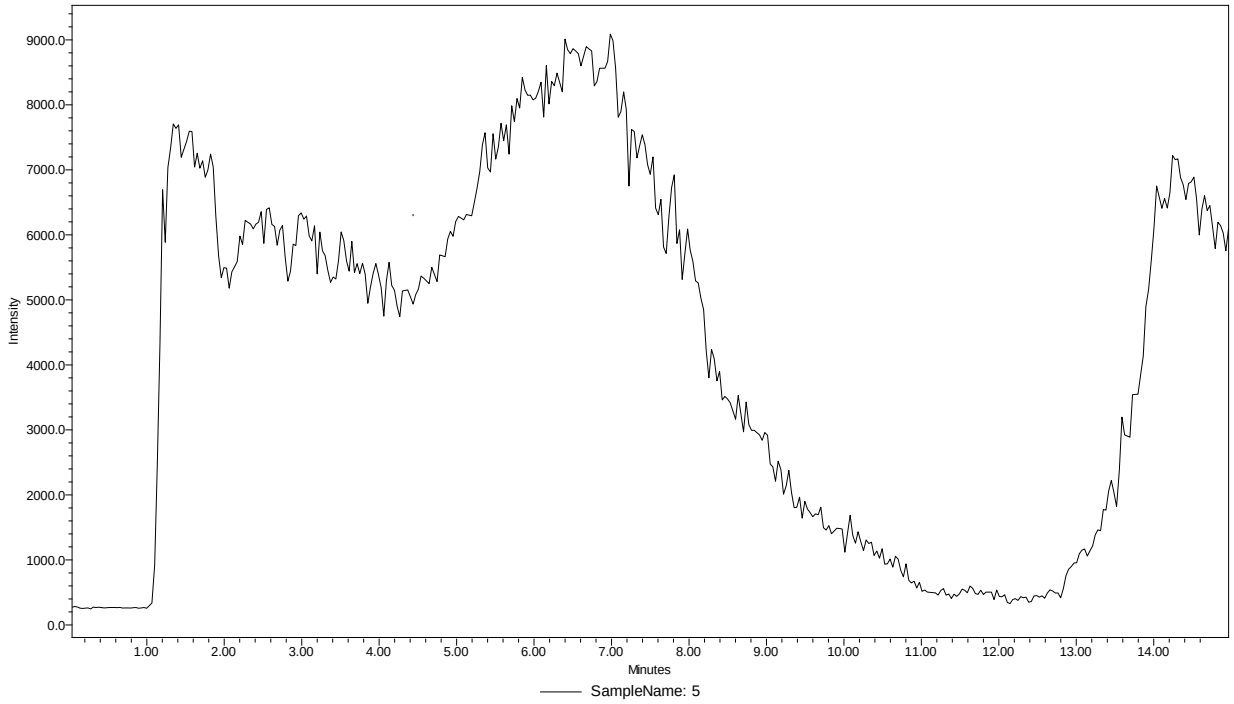

Fig. 29y Photolytic/Alkaline product chromatograph for FAC/sample 5.

105

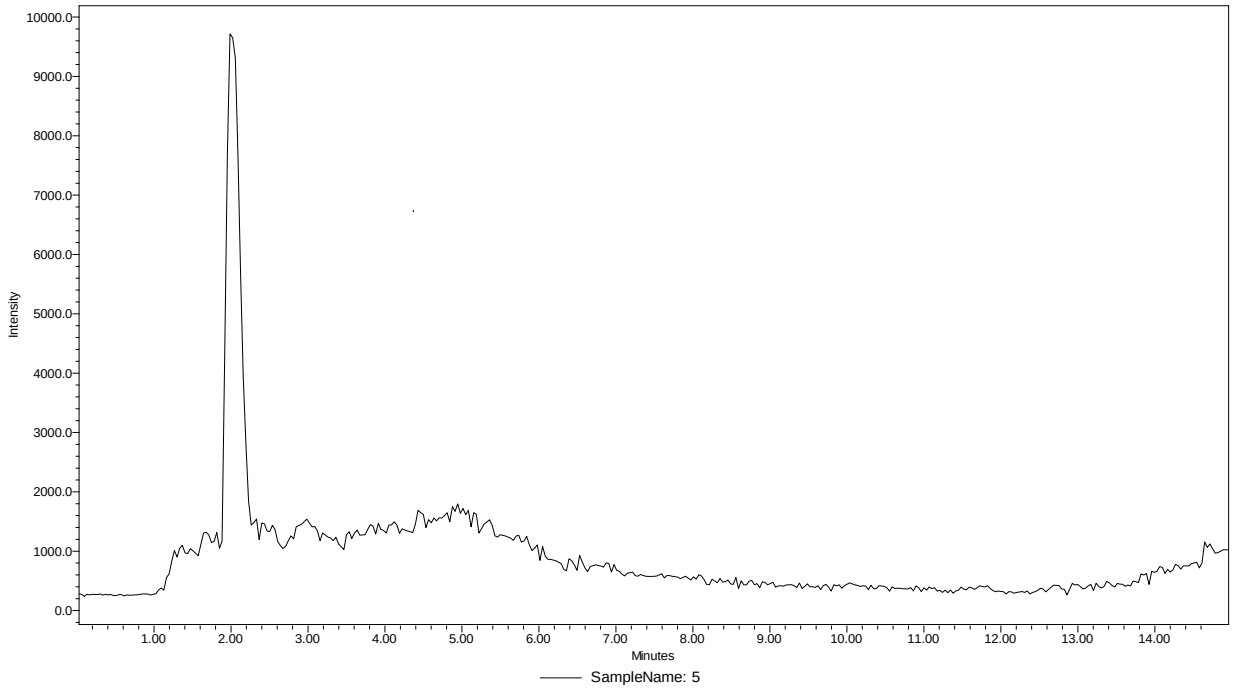

Fig. 29z Photolytic/Alkaline product chromatograph for FMASld/sample 5.

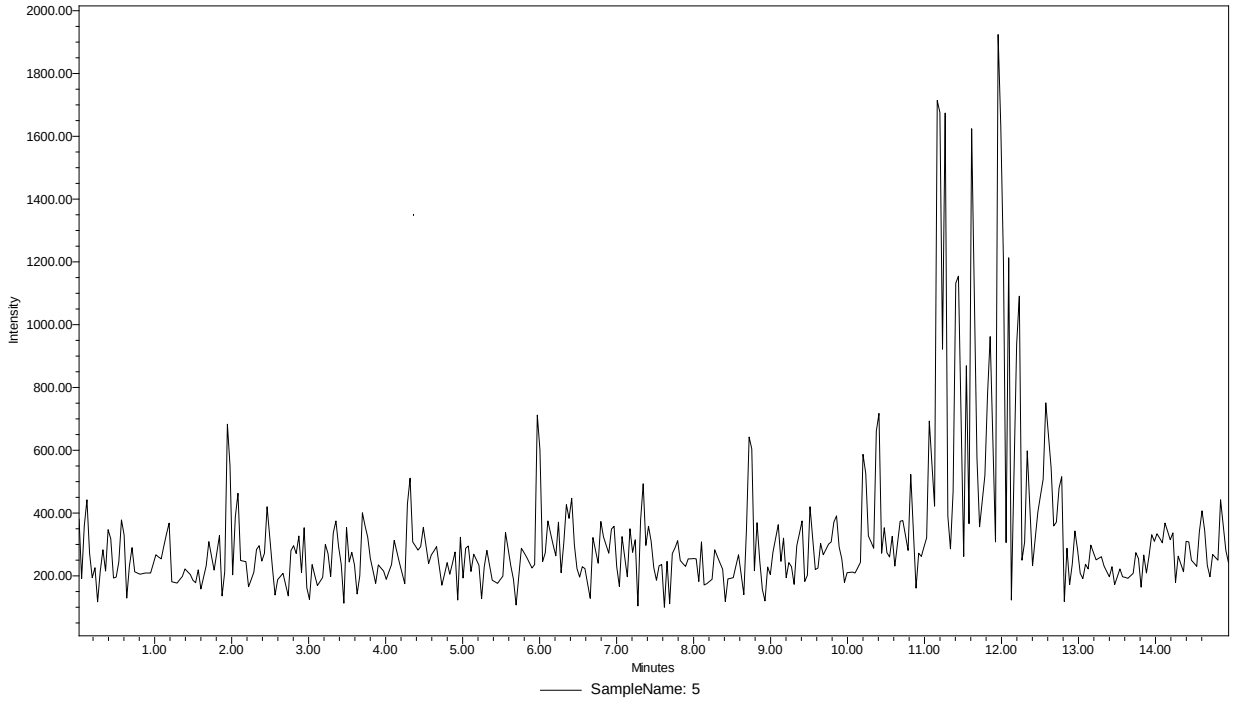

Fig. 29aa Photolytic/Alkaline product chromatograph for FBAL/sample 5.

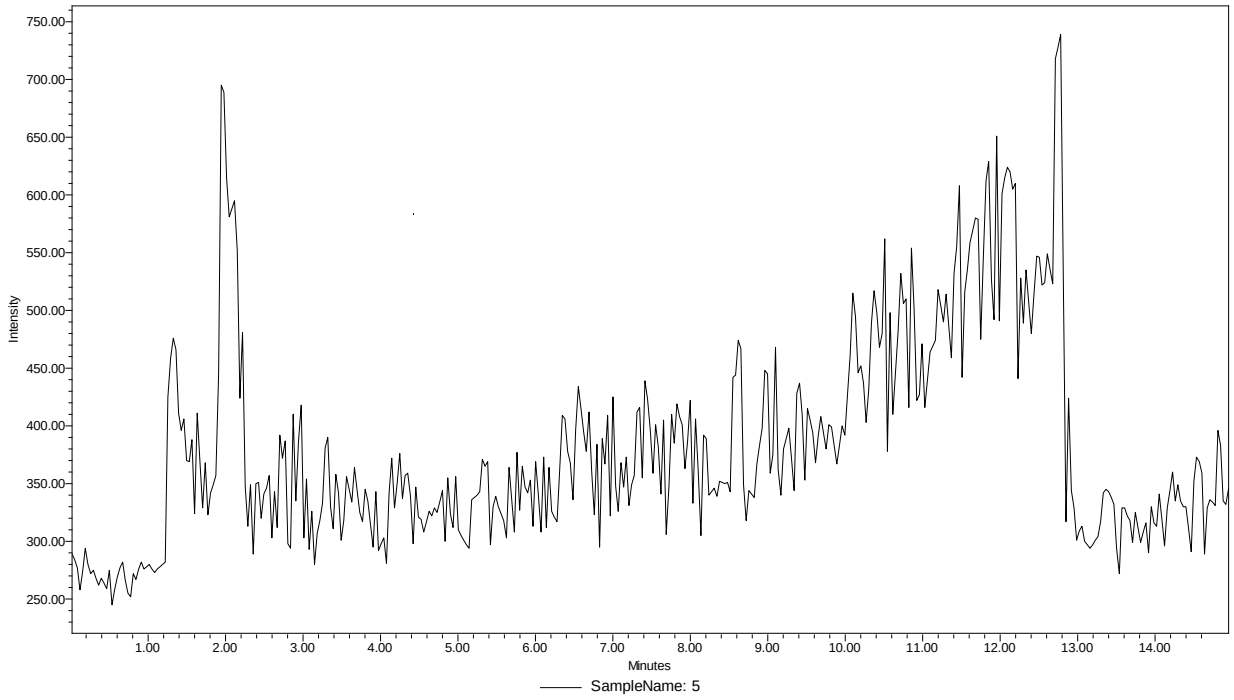

Fig. 29bb Photolytic/Alkaline product chromatograph for FUPA/sample 5.

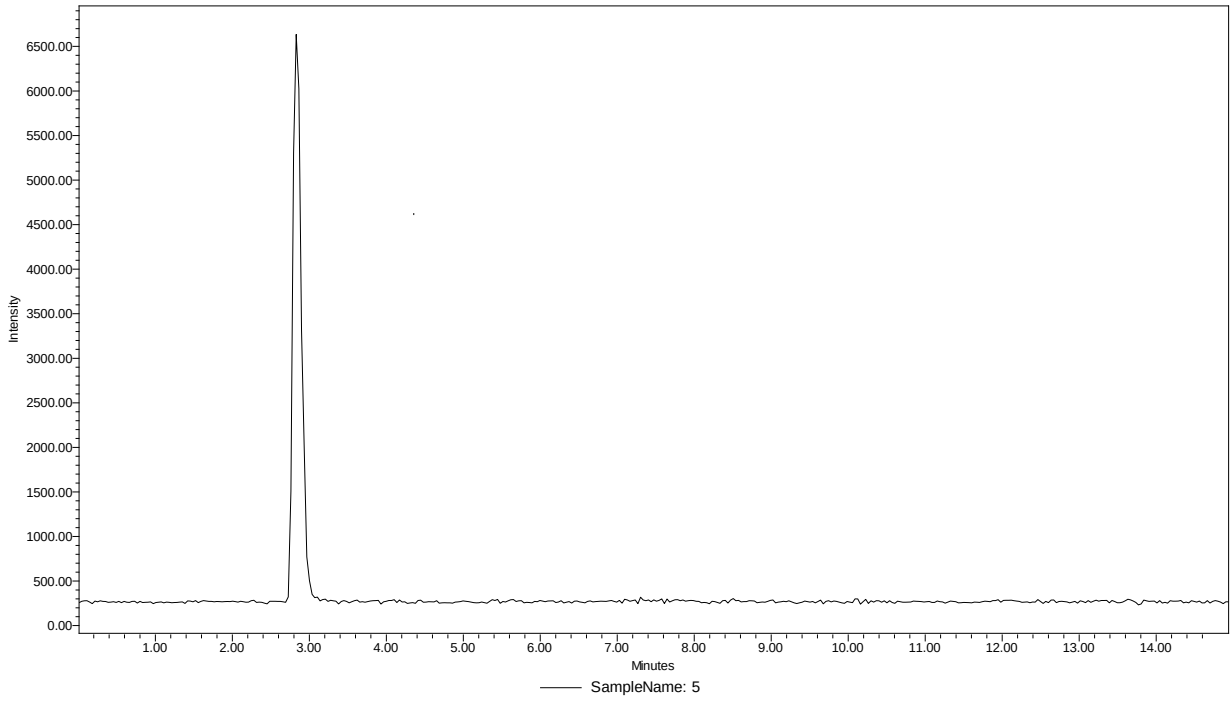

Fig. 29cc Photolytic/Alkaline product chromatograph for 5-FU/sample 5.

6

77

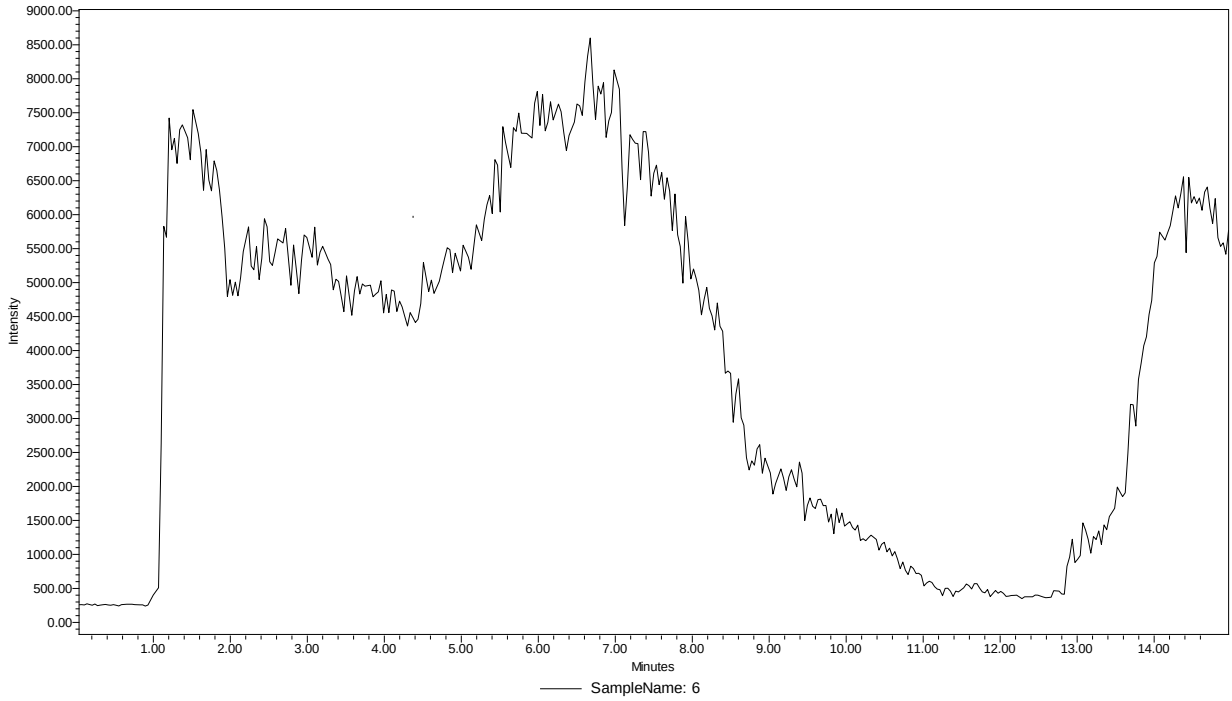

Fig. 29dd Photolytic/Alkaline product chromatograph for FAC/sample 6.

105

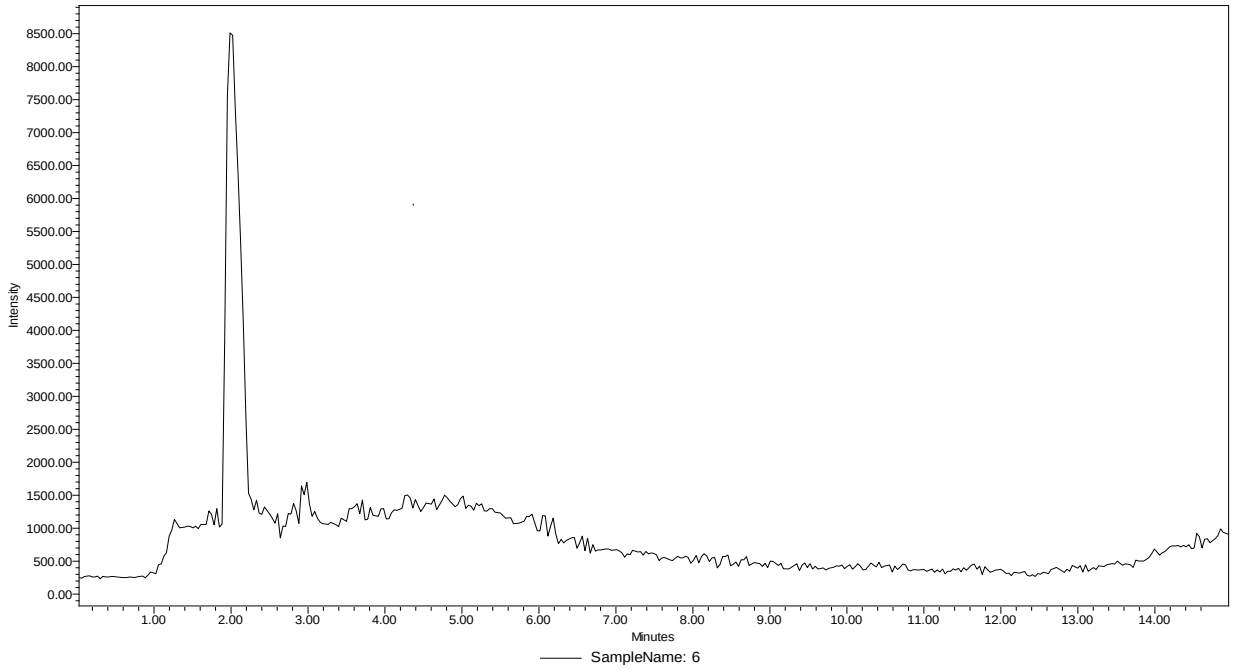

Fig. 29ee Photolytic/Alkaline product chromatograph for FMASld/sample 6.

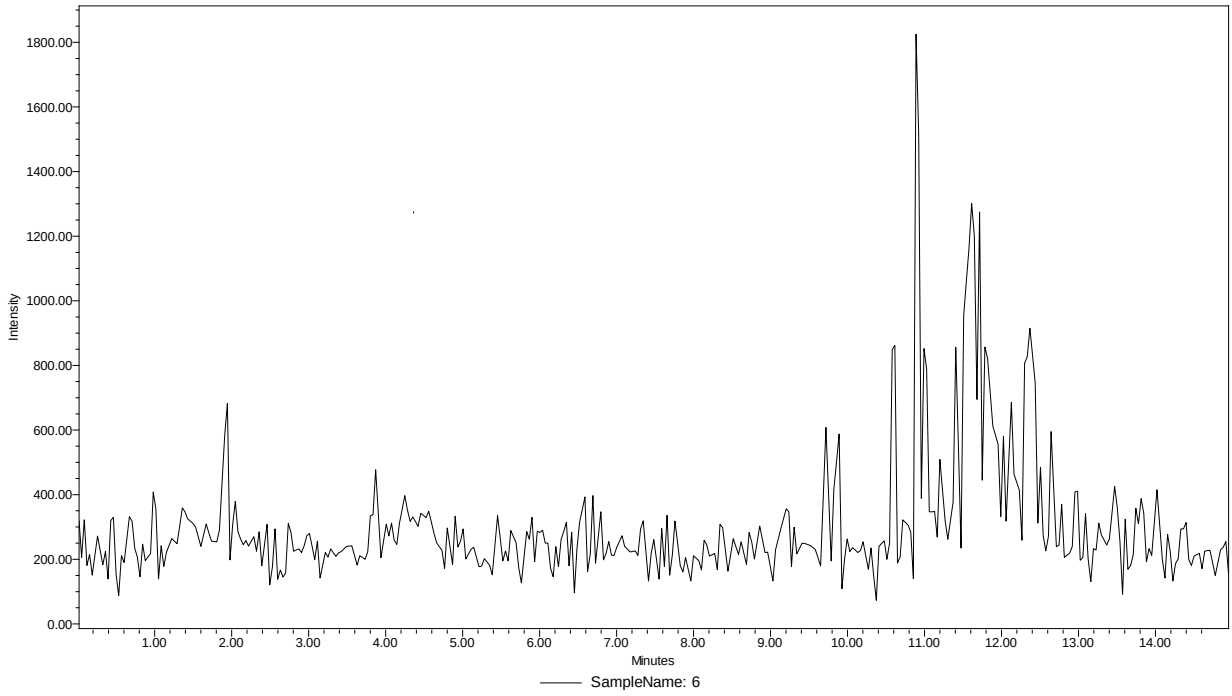

Fig. 29ff Photolytic/Alkaline product chromatograph for FBAL/sample 6.

149

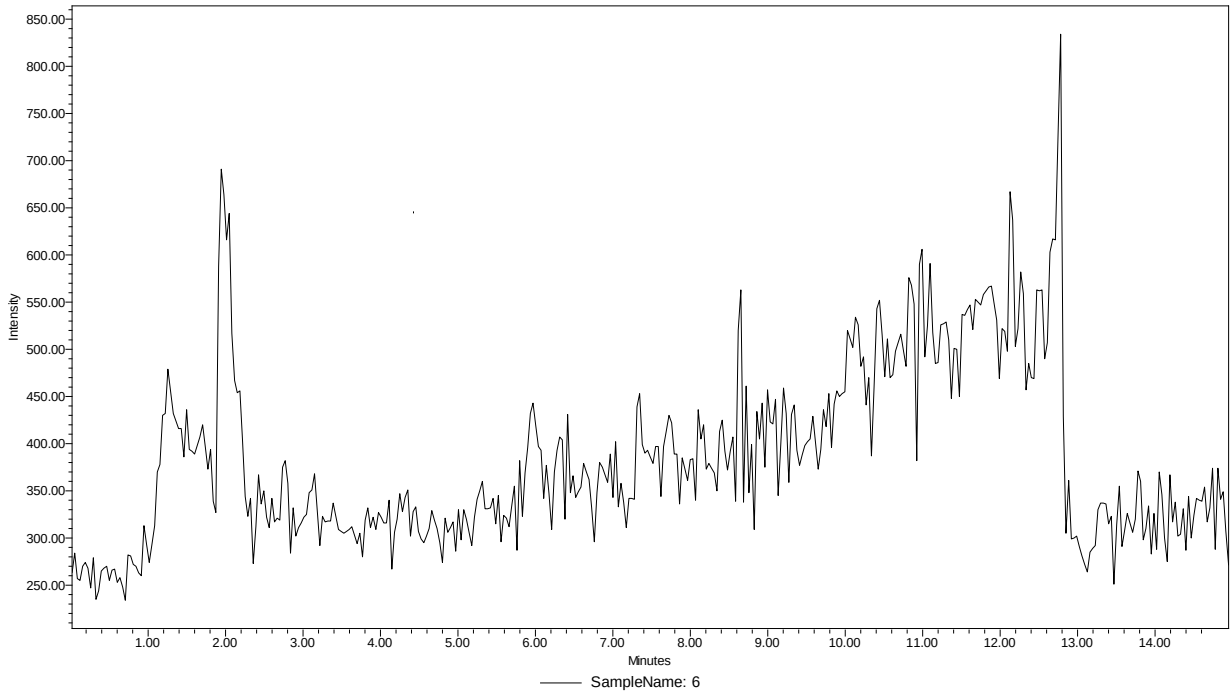

Fig. 29gg Photolytic/Alkaline product chromatograph for FUPA/sample 6.

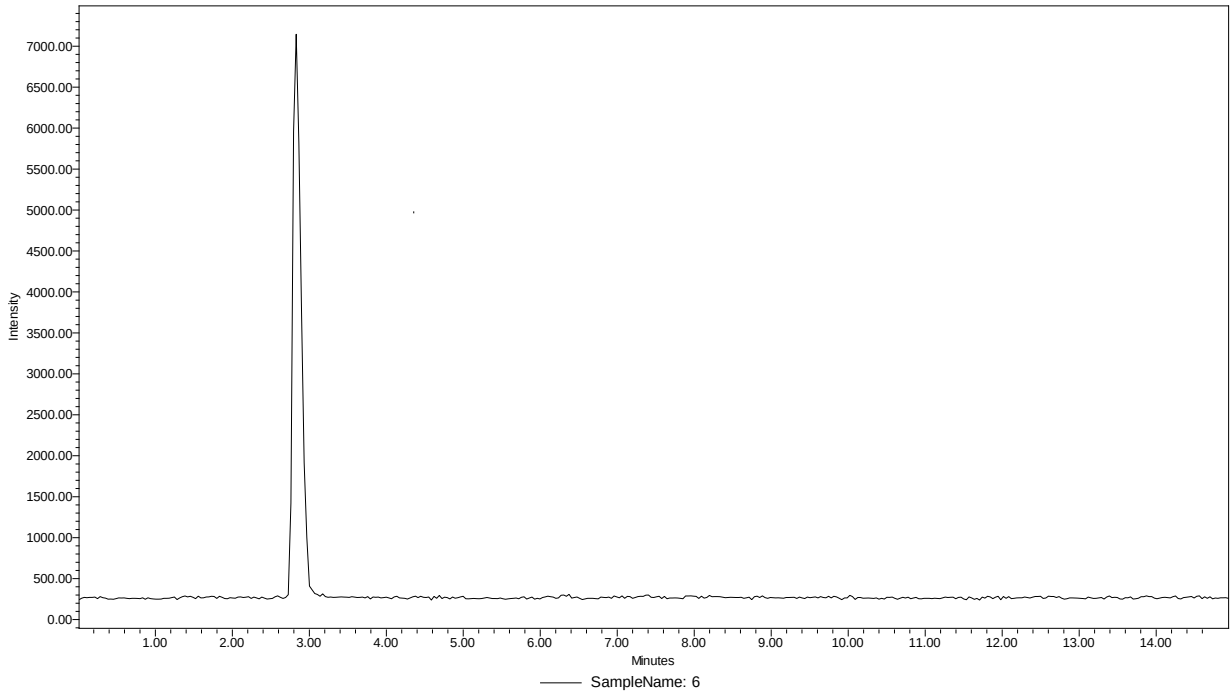

Fig. 29hh Photolytic/Alkaline product chromatograph for 5-FU/sample 6.

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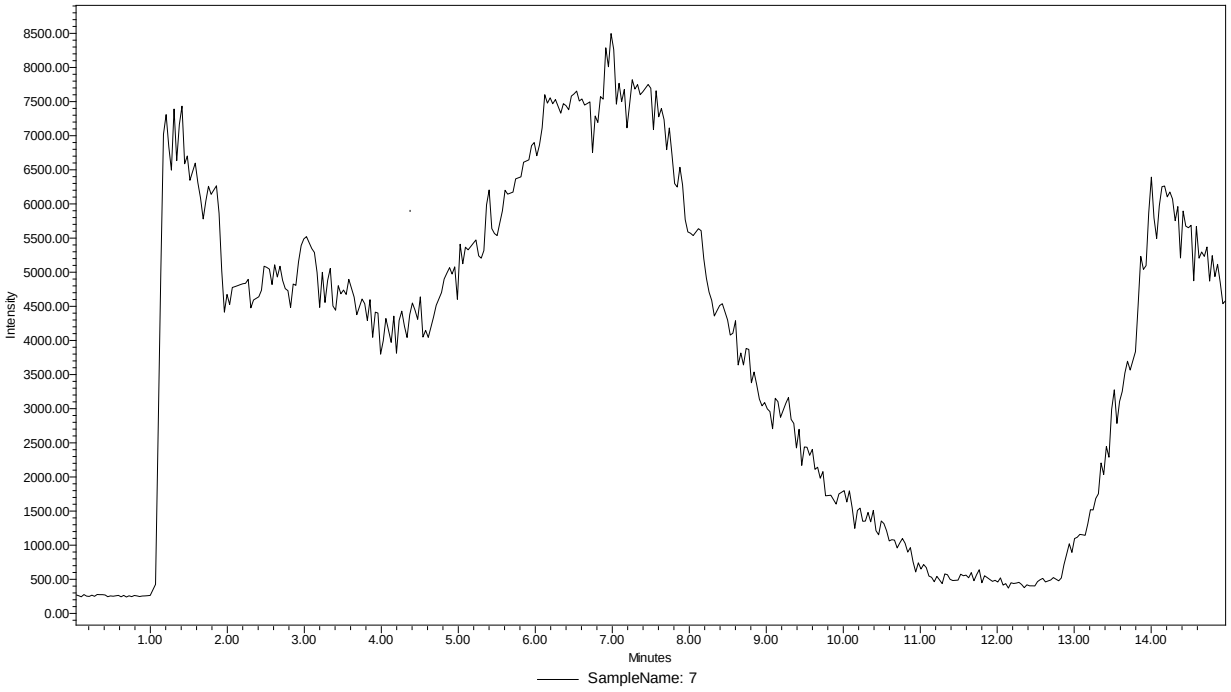

Fig. 29ii Photolytic/Alkaline product chromatograph for FAC/sample 7.

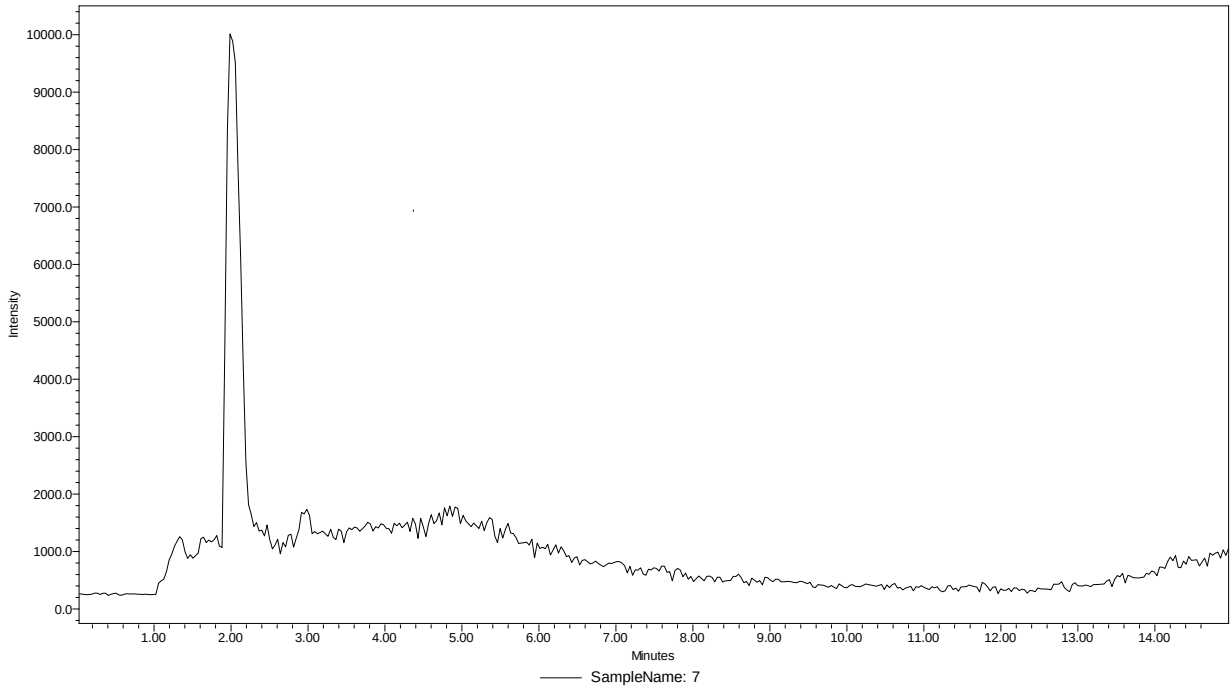

Fig. 29jj Photolytic/Alkaline product chromatograph for FMASld/sample 7.

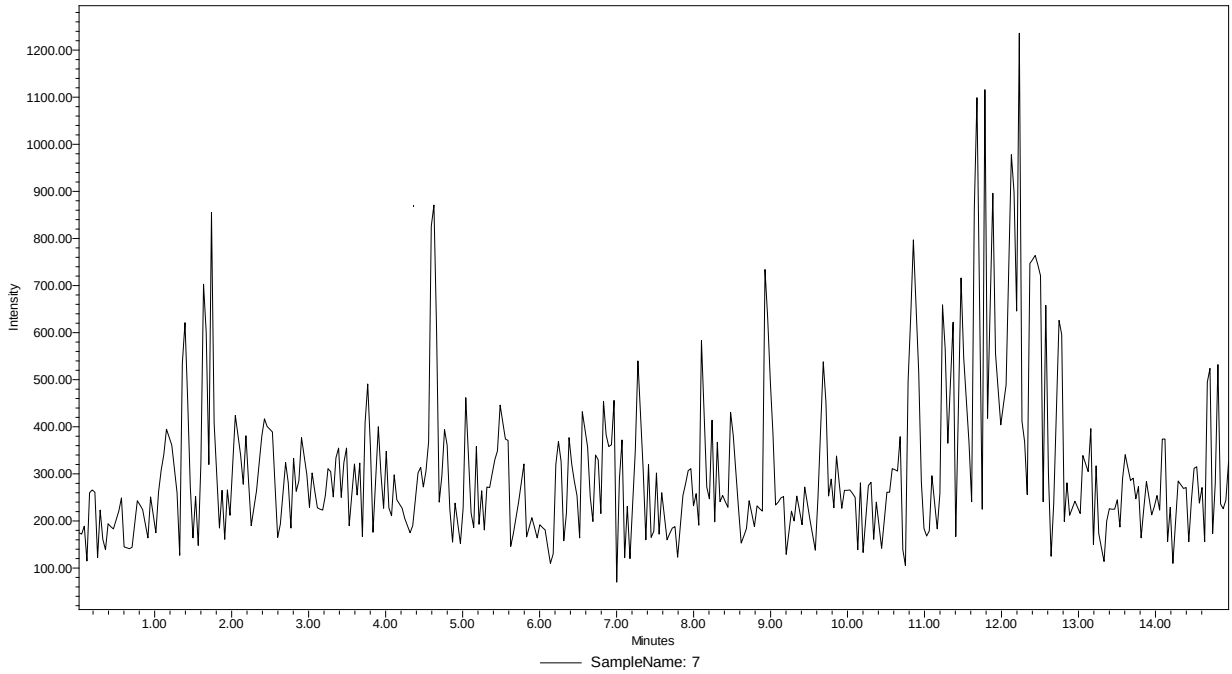

Fig. 29kk Photolytic/Alkaline product chromatograph for FBAL/sample 7.

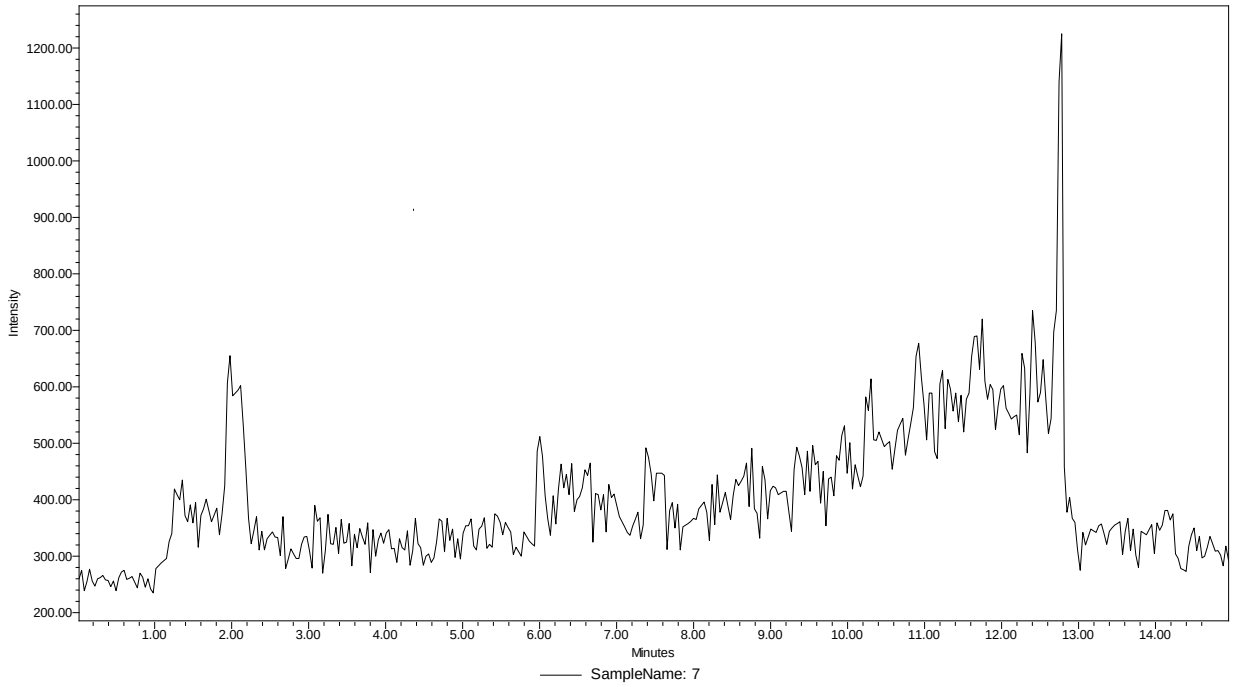

Fig. 29ll Photolytic/Alkaline product chromatograph for FUPA/sample 7.

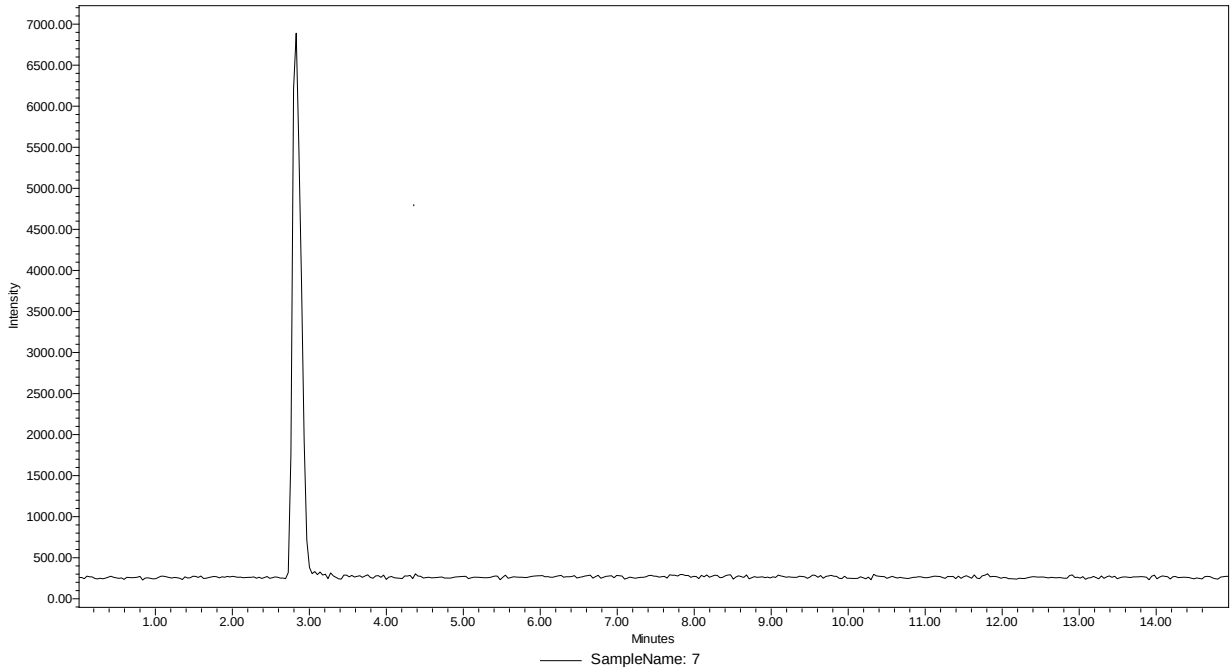

Fig. 29mm Photolytic/Alkaline product chromatograph for 5-FU/sample 7.

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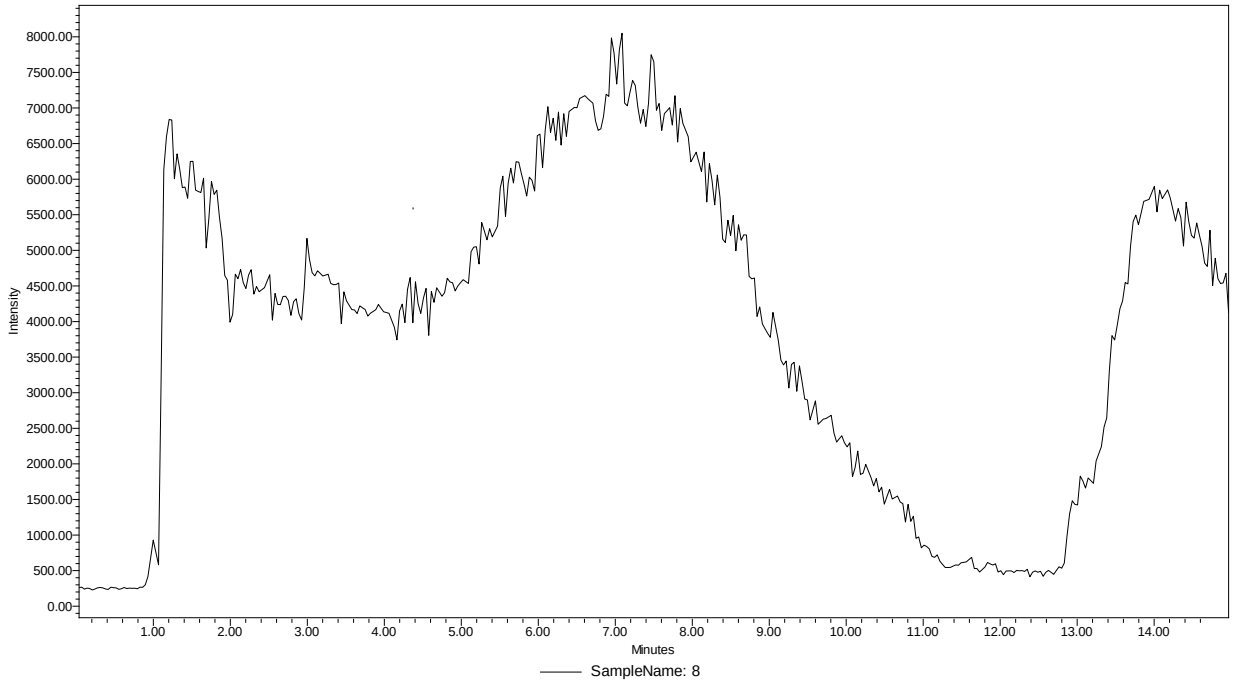

Fig. 29nn Photolytic/Alkaline product chromatograph for FAC/sample 8.

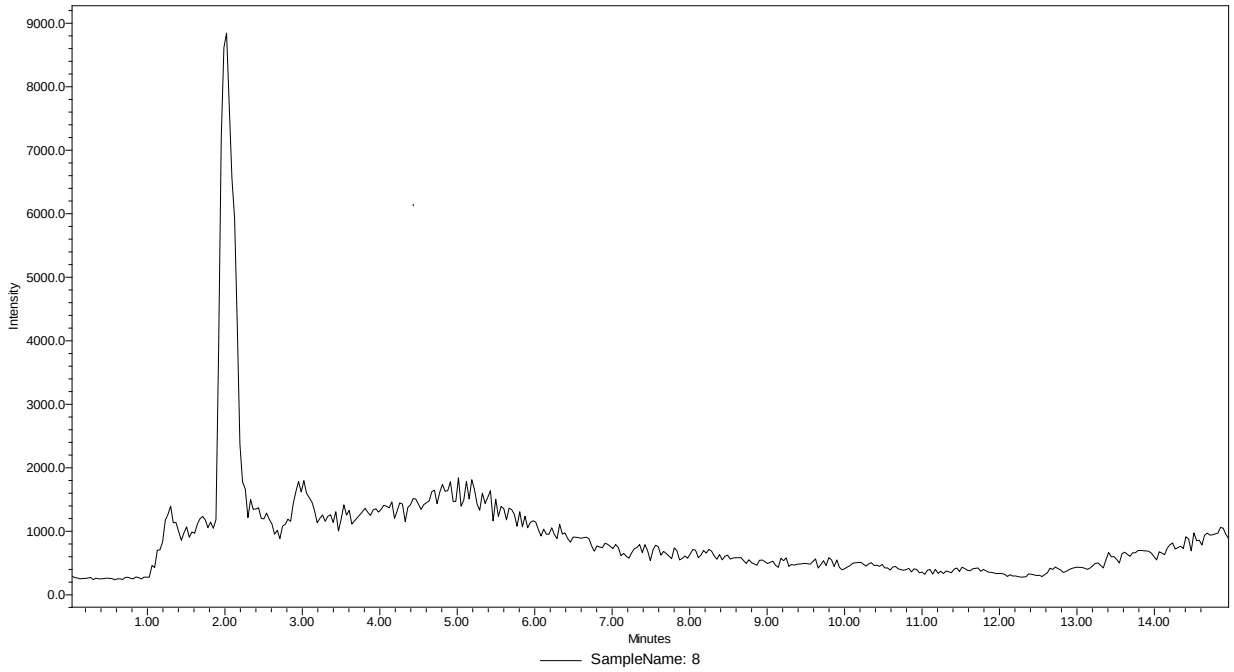

Fig. 29oo Photolytic/Alkaline product chromatograph for FMASld/sample 8.

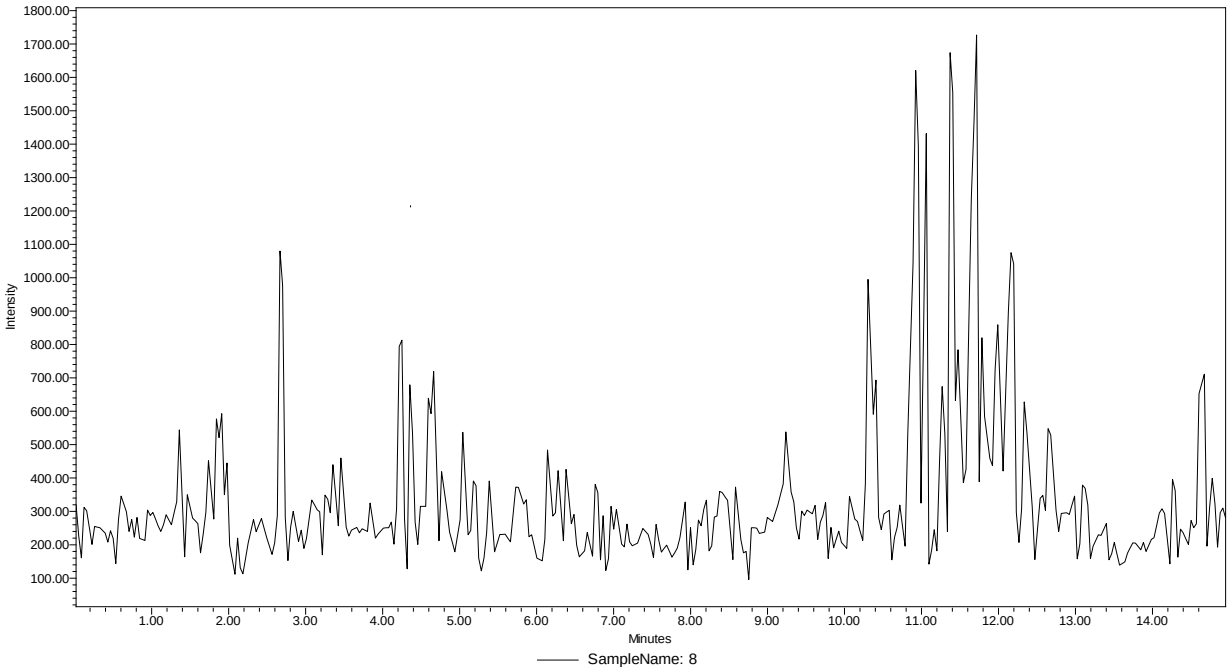

Fig. 29pp Photolytic/Alkaline product chromatograph for FBAL/sample 8.

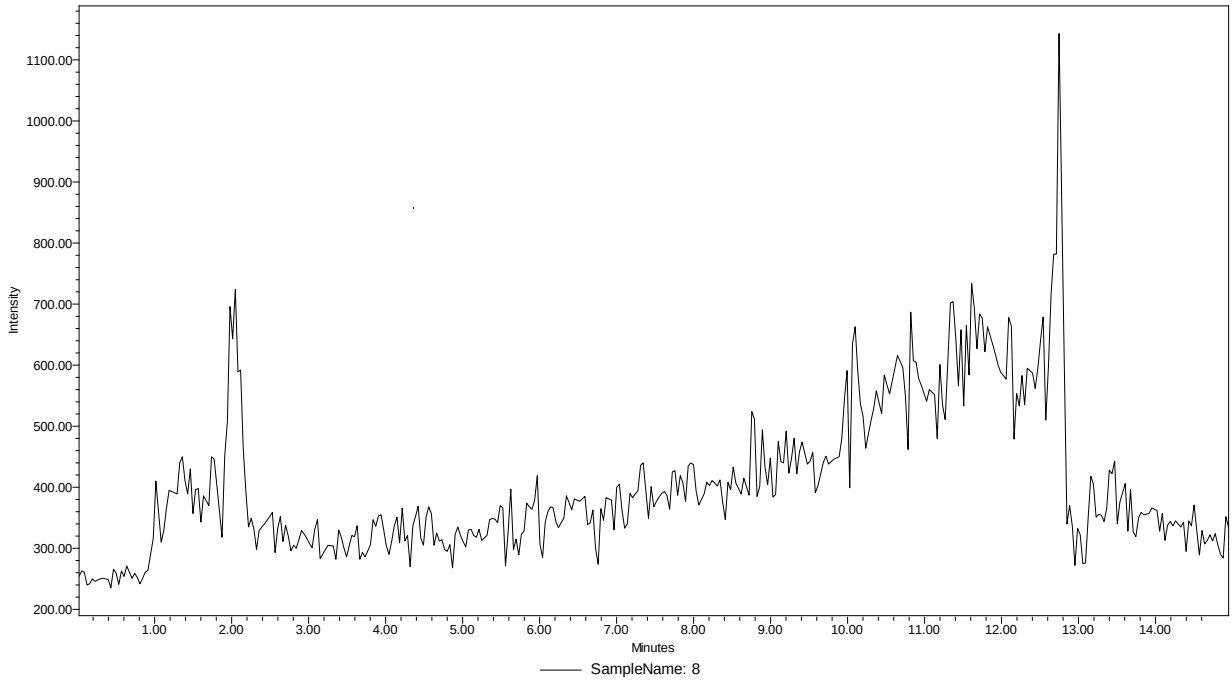

Fig. 29qq Photolytic/Alkaline product chromatograph for FUPA/sample 8.

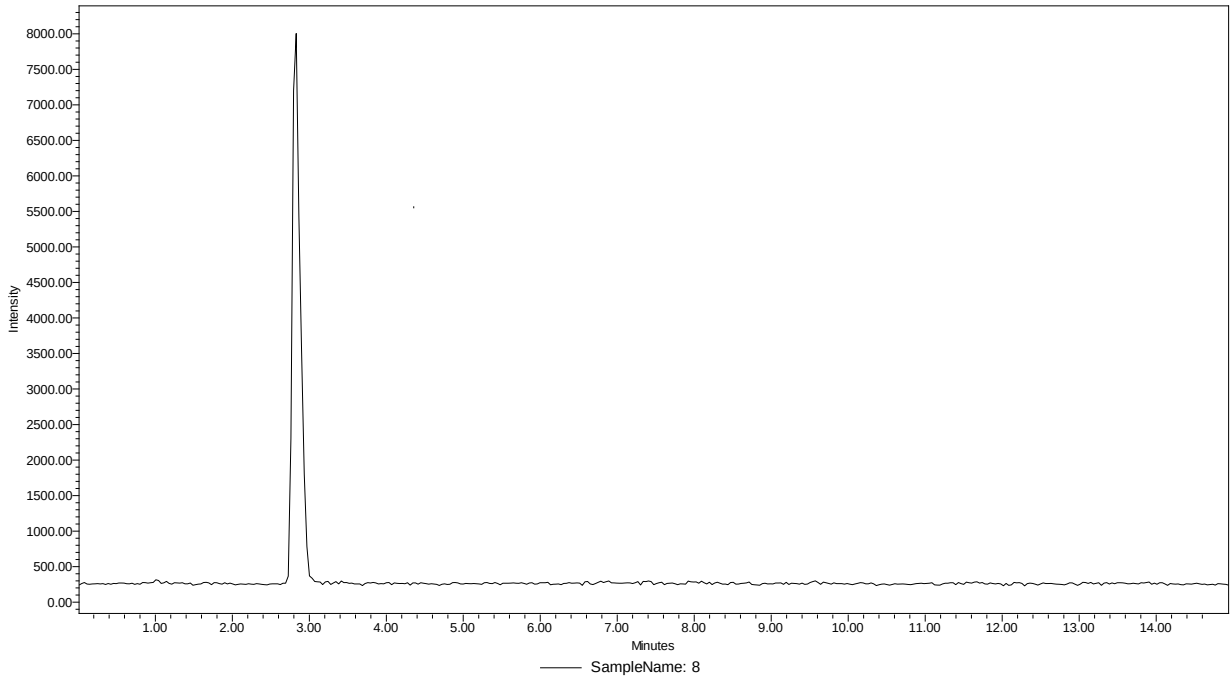

Fig. 29rr Photolytic/Alkaline product chromatograph for 5-FU/sample 8.

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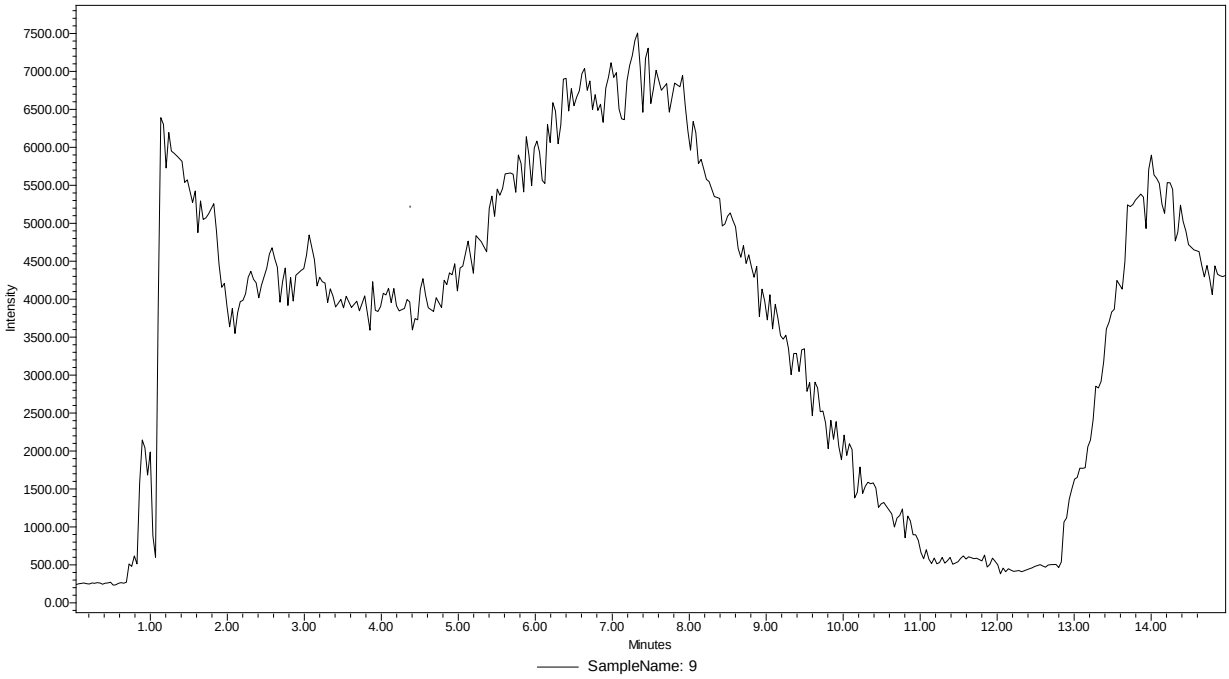

Fig. 29ss Photolytic/Alkaline product chromatograph for FAC/sample 9.

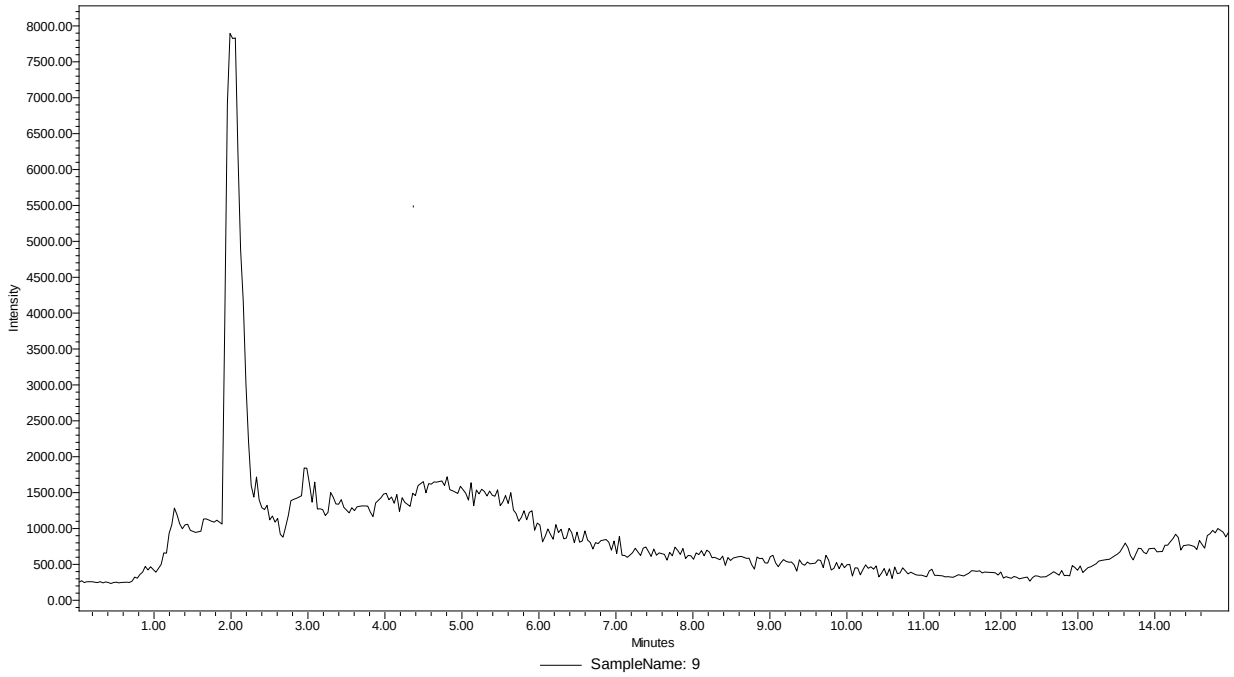

Fig. 29tt Photolytic/Alkaline product chromatograph for FMASld/sample 9.

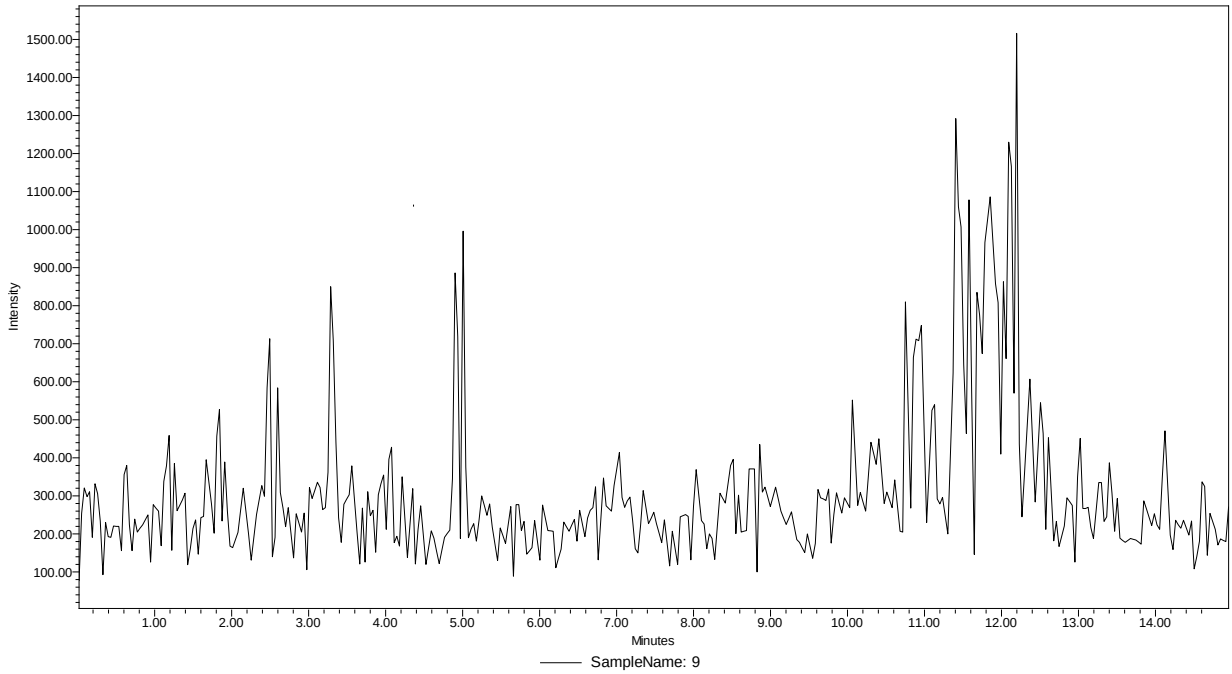

Fig. 29uu Photolytic/Alkaline product chromatograph for FBAL/sample 9.

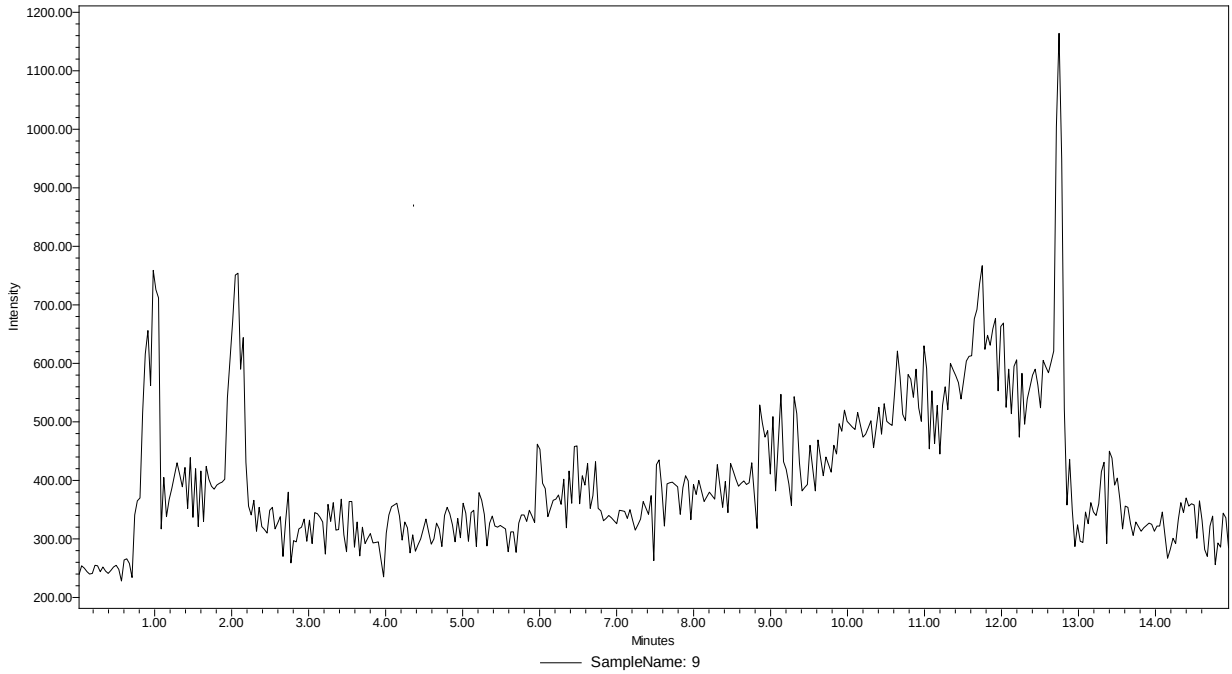

Fig. 29vv Photolytic/Alkaline product chromatograph for FUPA/sample 9.

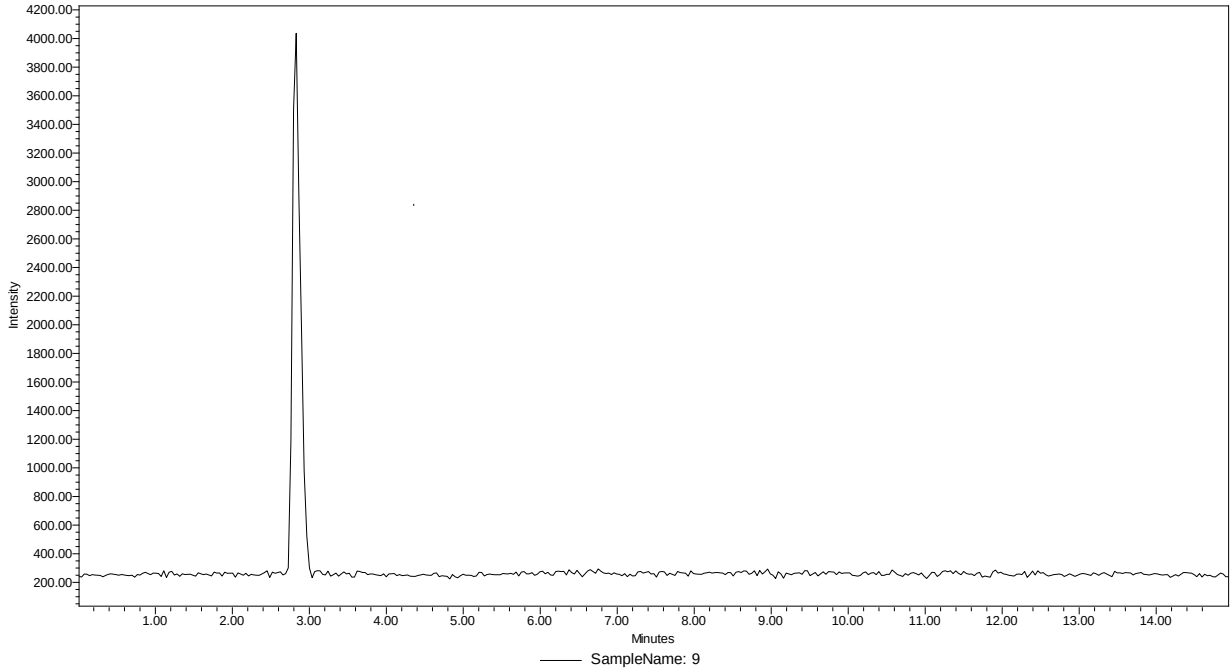

Fig. 29ww Photolytic/Alkaline product chromatograph for 5-FU/sample 9.

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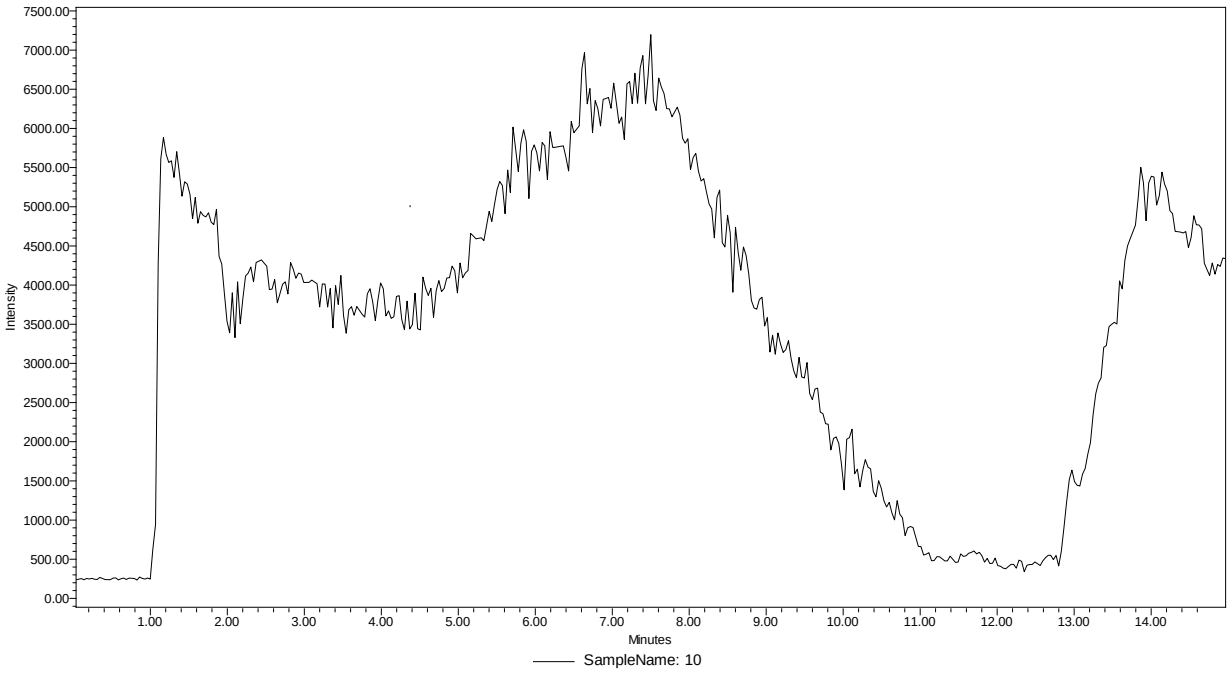

Fig. 29xx Photolytic/Alkaline product chromatograph for FAC/sample 10.

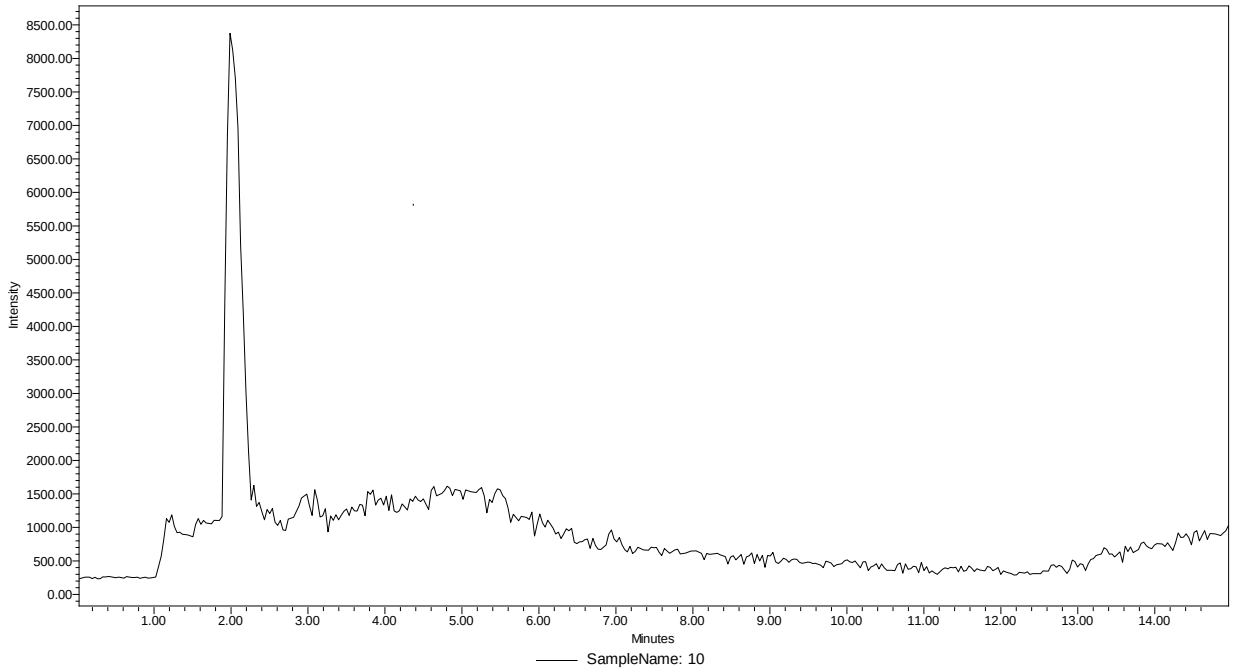

Fig. 29yy Photolytic/Alkaline product chromatograph for FMASld/sample 10.

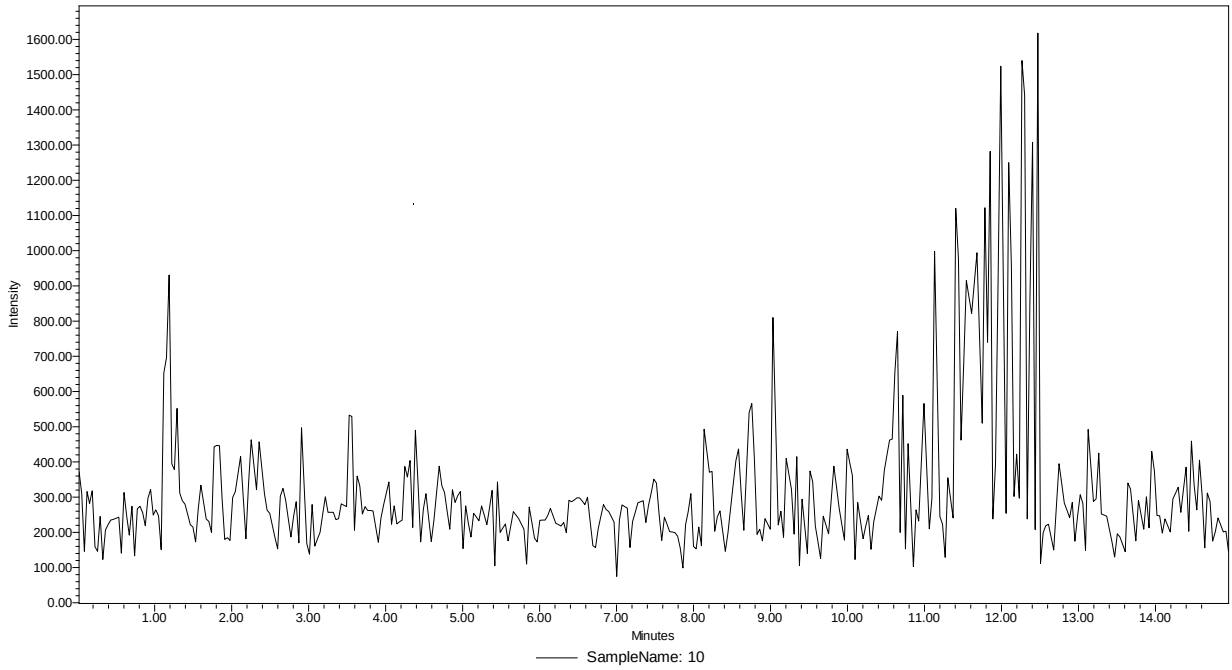

Fig. 29zz Photolytic/Alkaline product chromatograph for FBAL/sample 10.

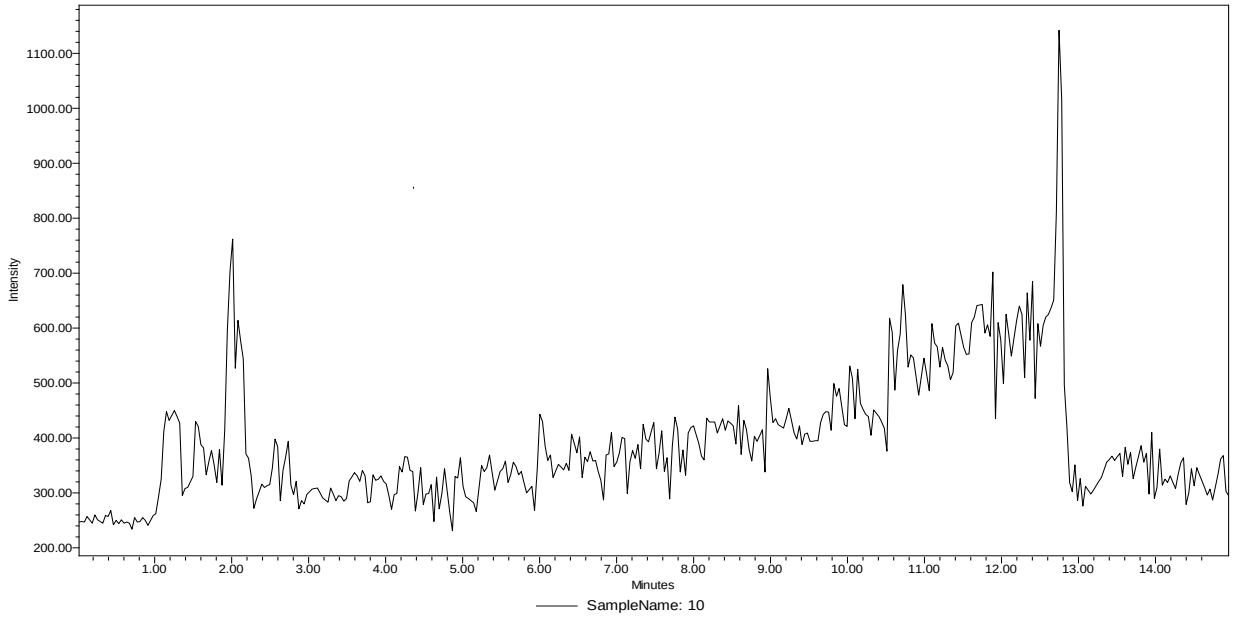

Fig. 29aaa Photolytic/Alkaline product chromatograph for FUPA/sample 10.

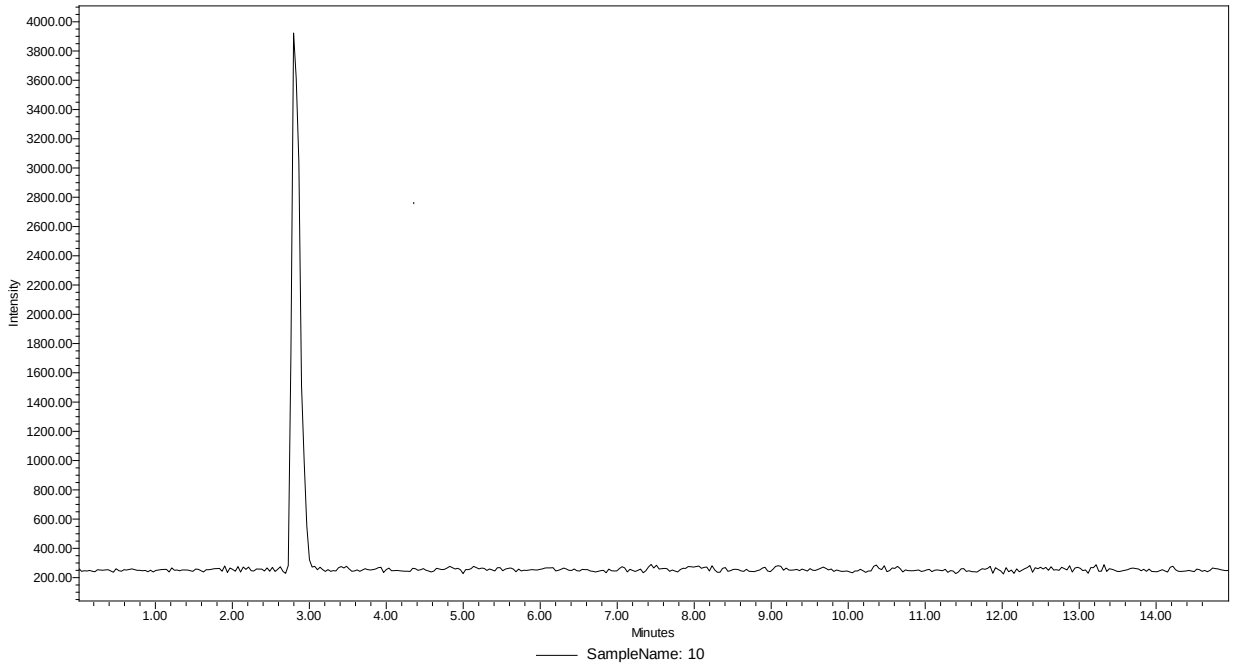

Fig. 29bbb Photolytic/Alkaline product chromatograph for 5-FU/sample 10.

11

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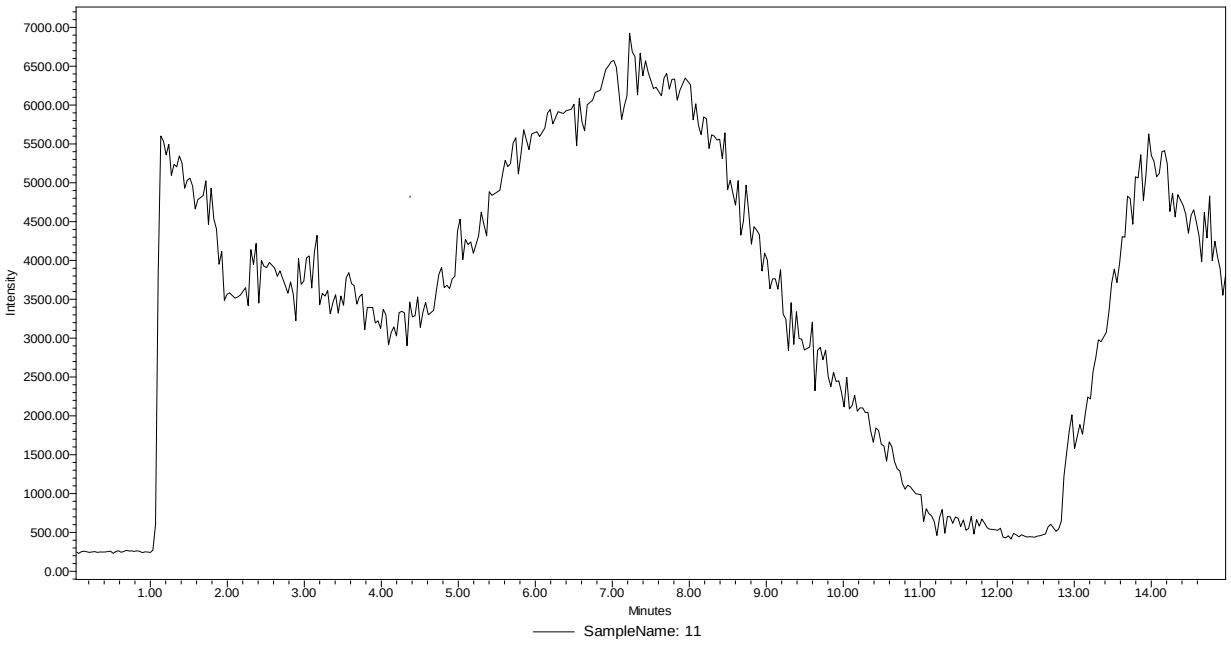

Fig. 29ccc Photolytic/Alkaline product chromatograph for FAC/sample 11.

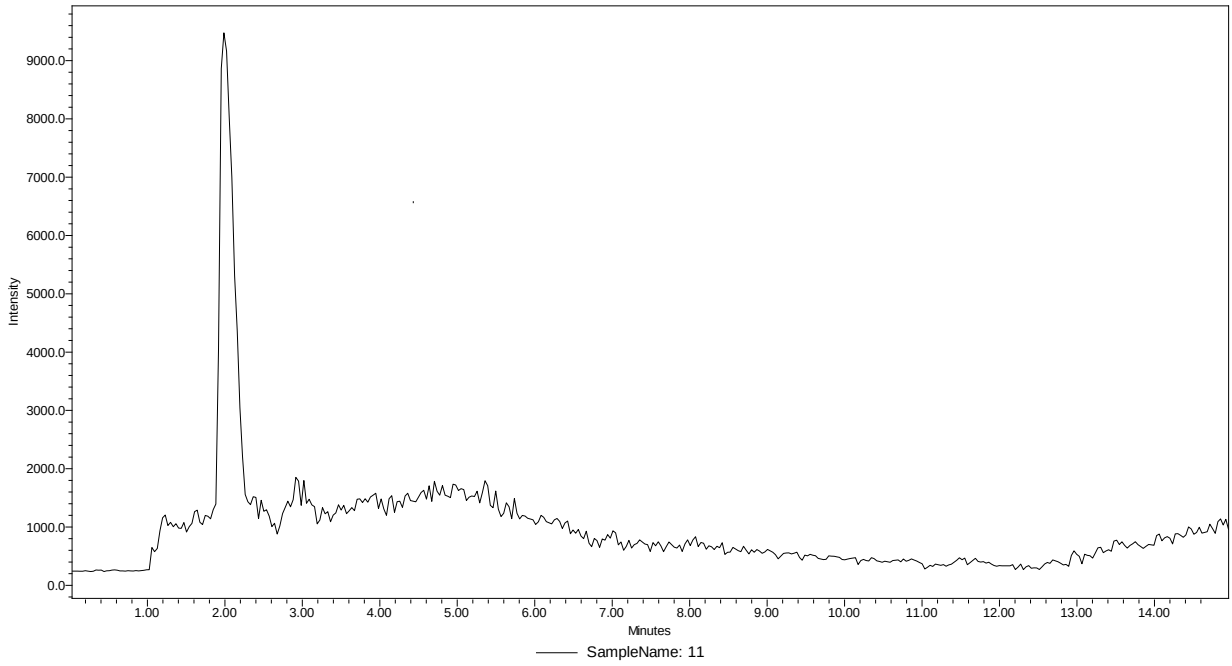

Fig. 29ddd Photolytic/Alkaline product chromatograph for FMASld/sample 11.

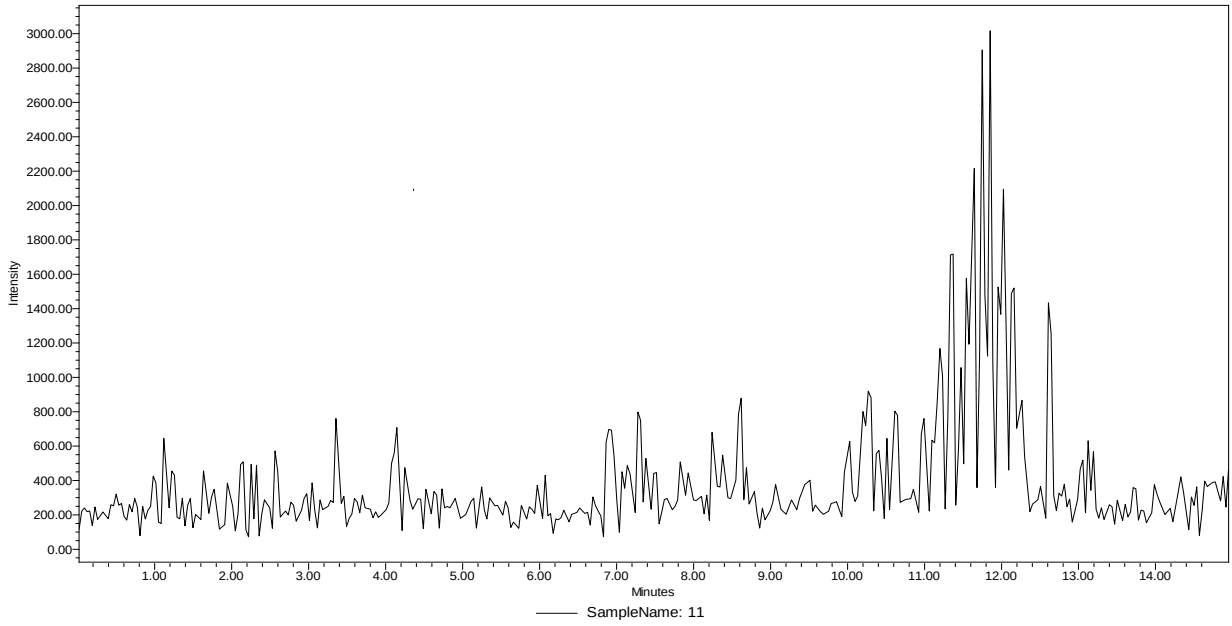

Fig. 29eee Photolytic/Alkaline product chromatograph for FBAL/sample 11.

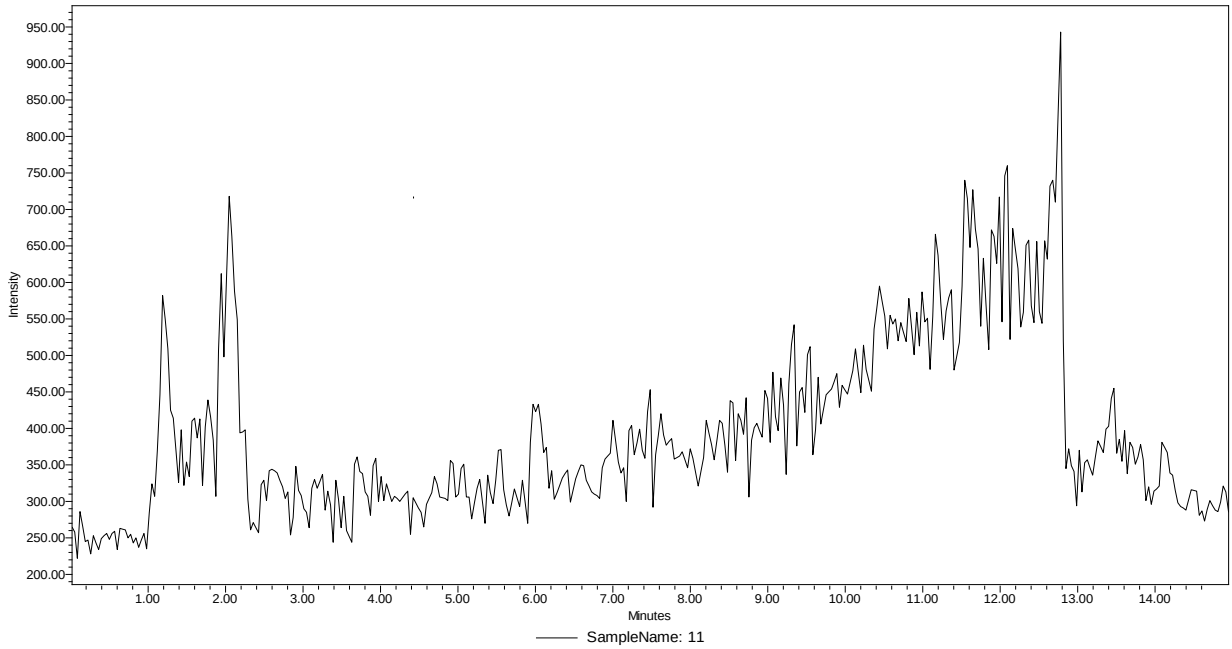

Fig. 29fff Photolytic/Alkaline product chromatograph for FUPA/sample 11.

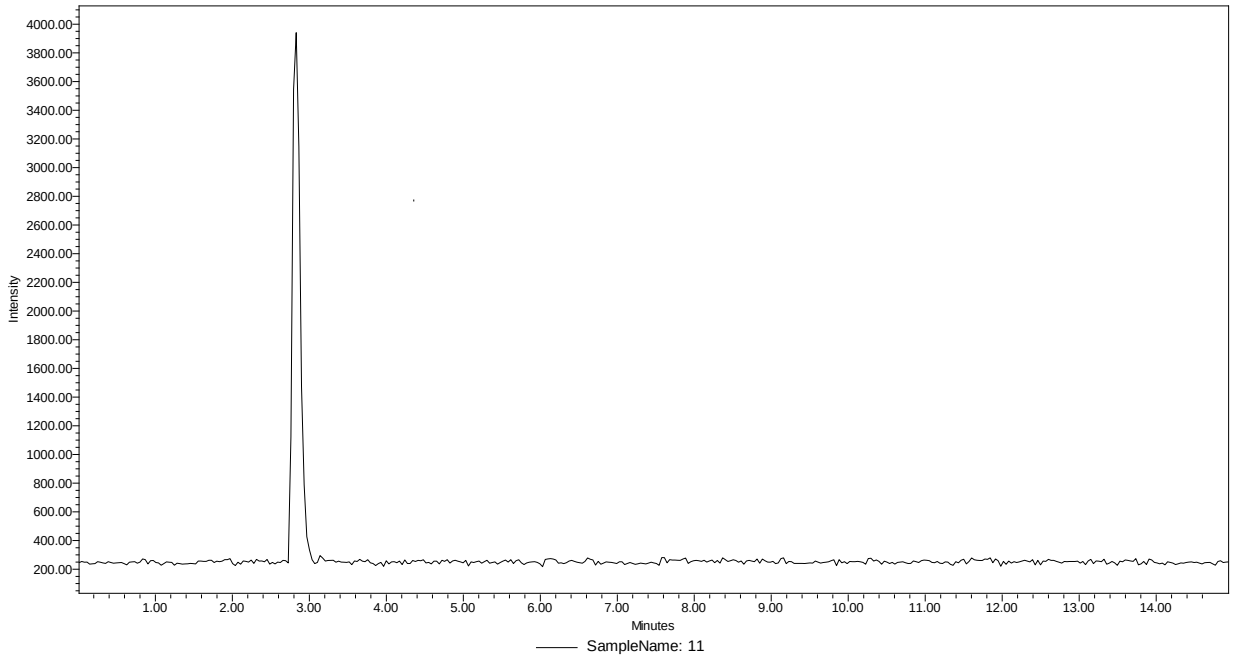

Fig. 29ggg Photolytic/Alkaline product chromatograph for 5-FU/sample 11.

12

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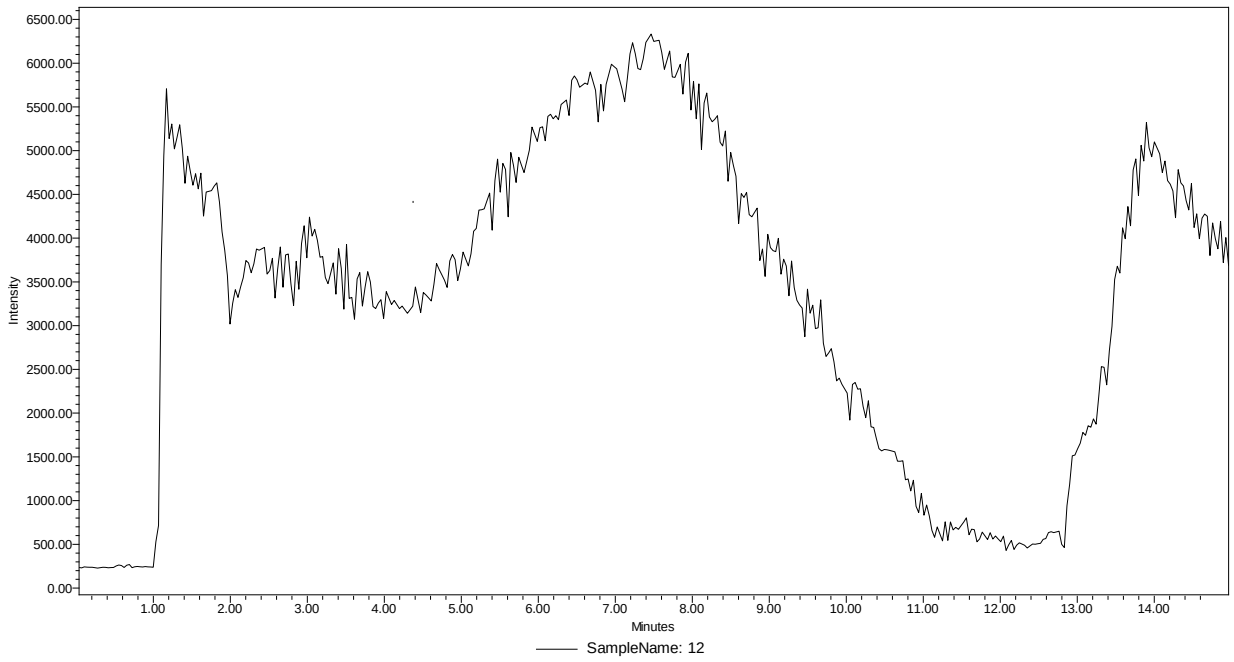

Fig. 29hhh Photolytic/Alkaline product chromatograph for FAC/sample 12.

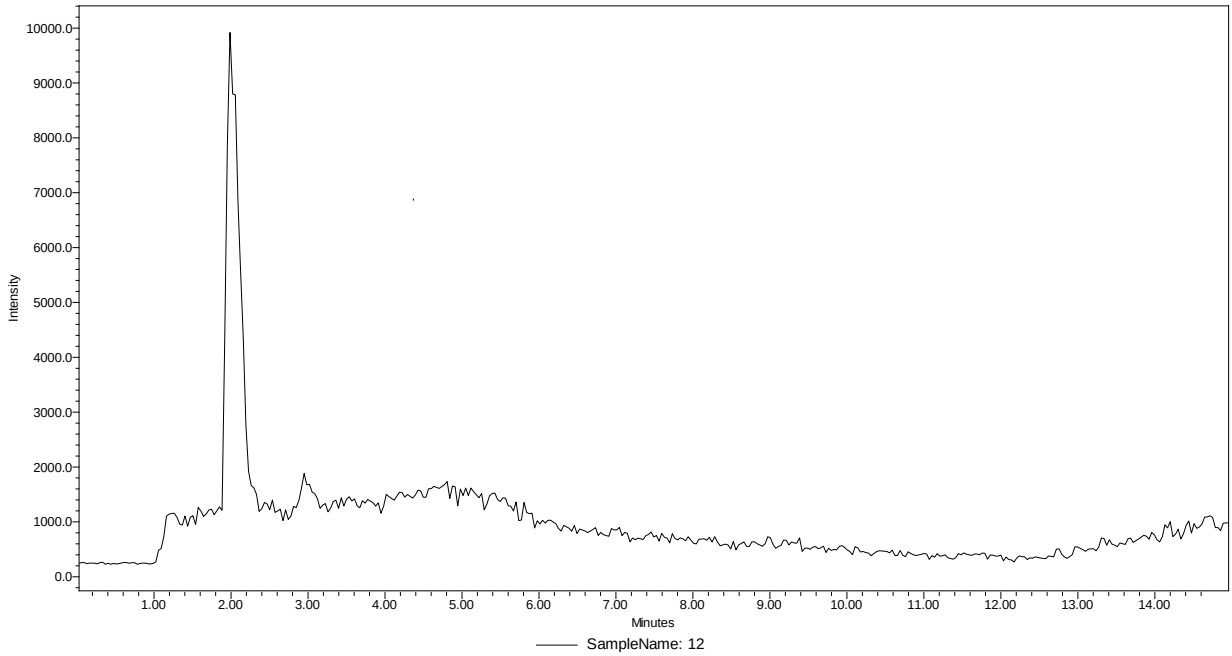

Fig. 29iii Photolytic/Alkaline product chromatograph for FMASld/sample 12.

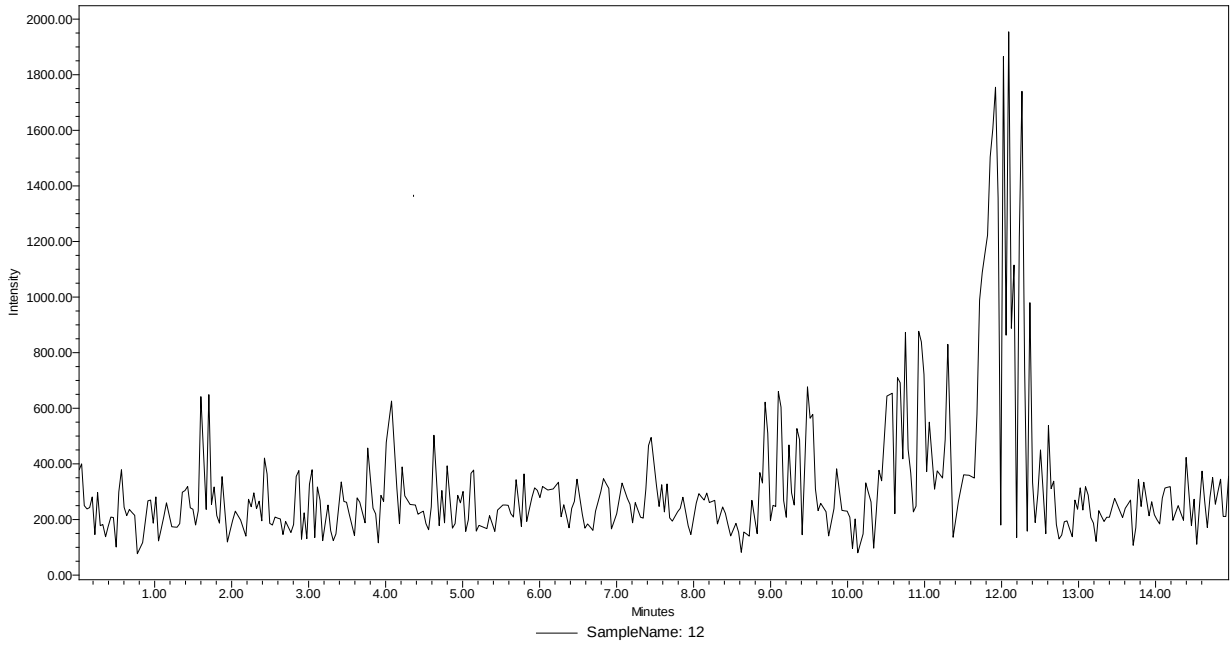

Fig. 29jjj Photolytic/Alkaline product chromatograph for FBAL/sample 12.

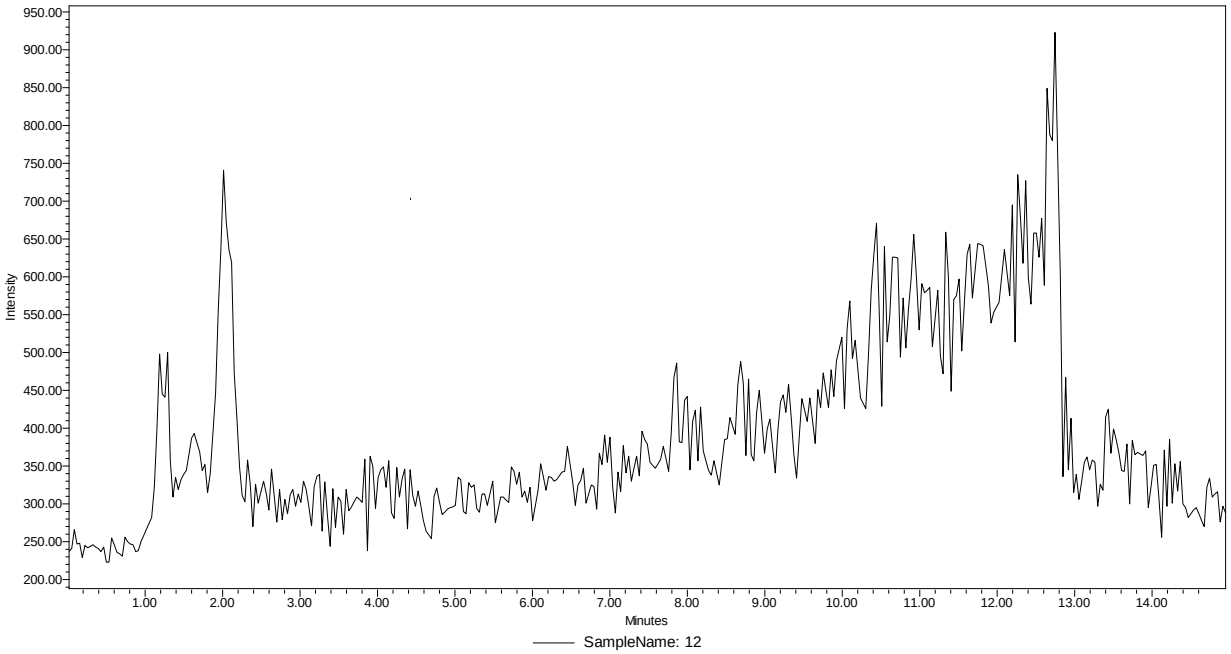

Fig. 29kkk Photolytic/Alkaline product chromatograph for FUPA/sample 12.

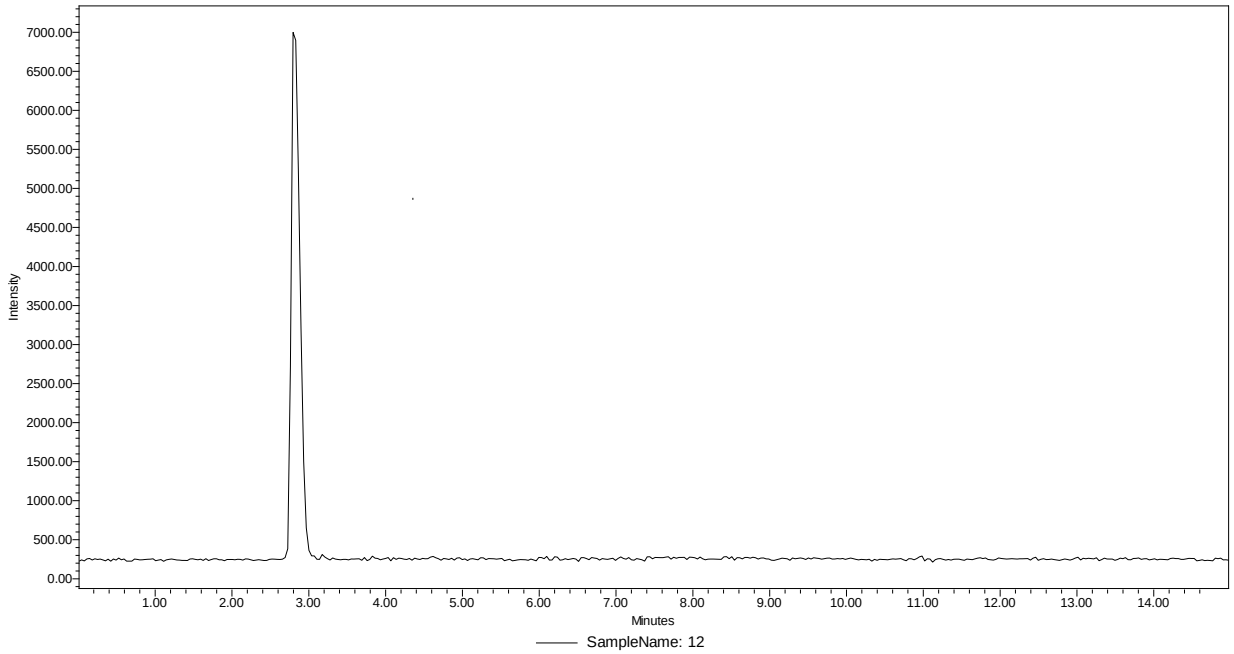

Fig. 29lll Photolytic/Alkaline product chromatograph for 5-FU/sample 12.

13

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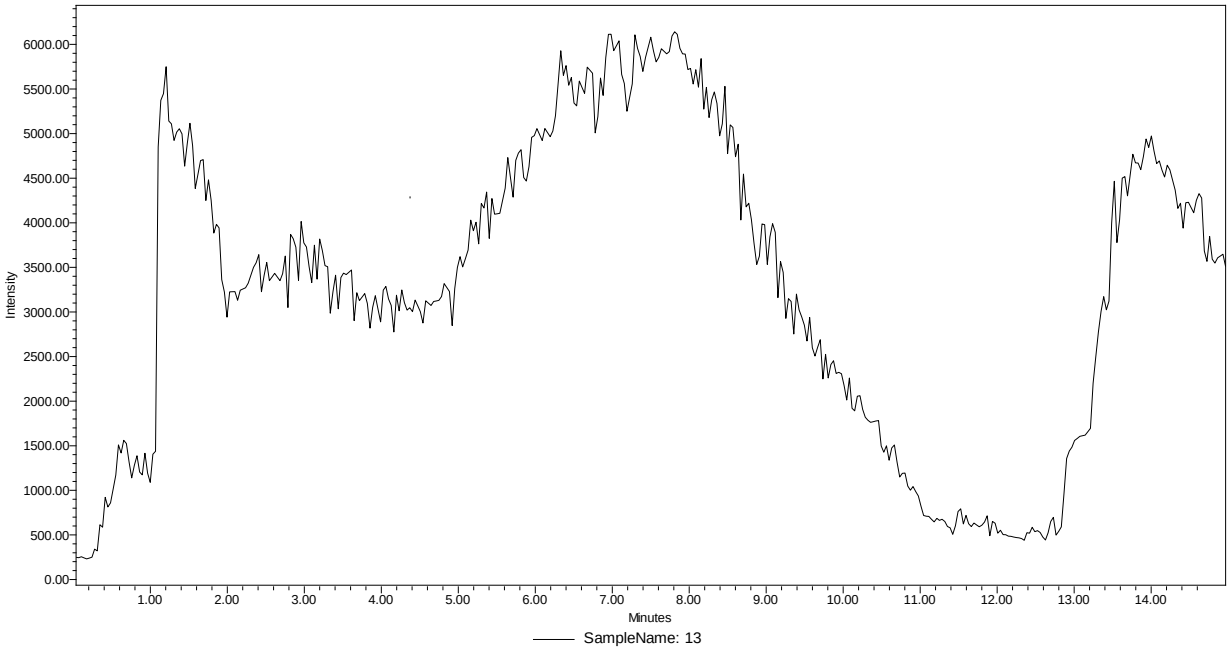

Fig. 29mmm Photolytic/Alkaline product chromatograph for FAC/sample 13.

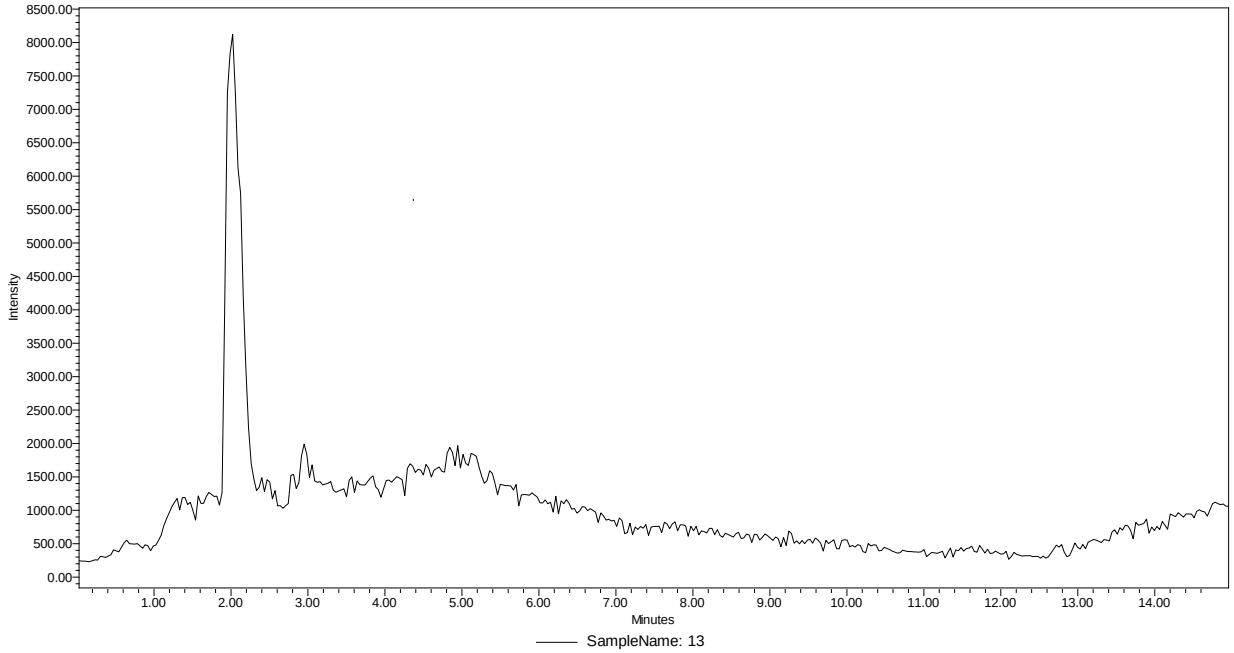

Fig. 29nnn Photolytic/Alkaline product chromatograph for FMASld/sample 13.

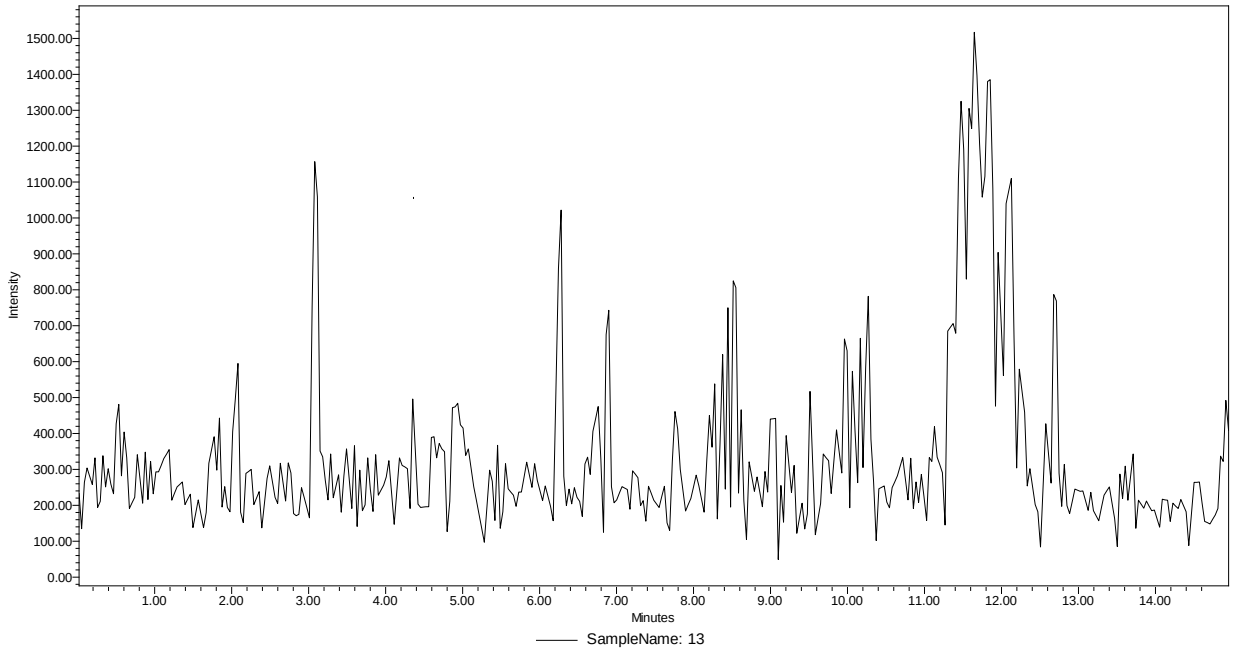

Fig. 29ooo Photolytic/Alkaline product chromatograph for FBAL/sample 13.

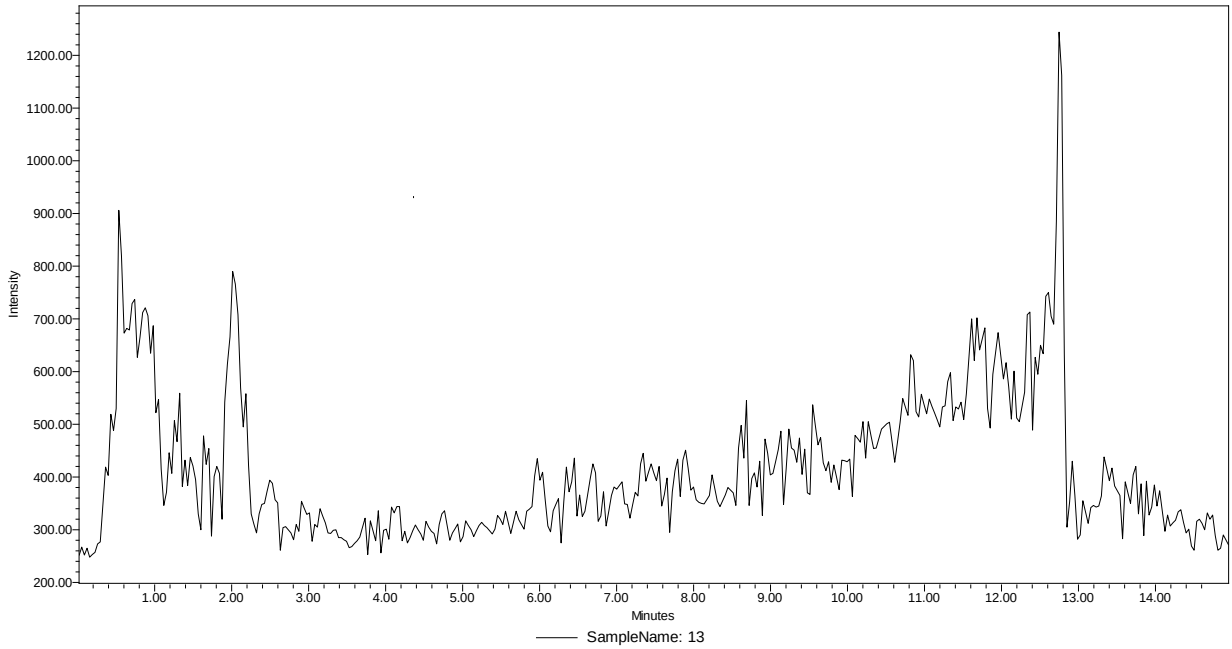

Fig. 29ppp Photolytic/Alkaline product chromatograph for FUPA/sample 13.

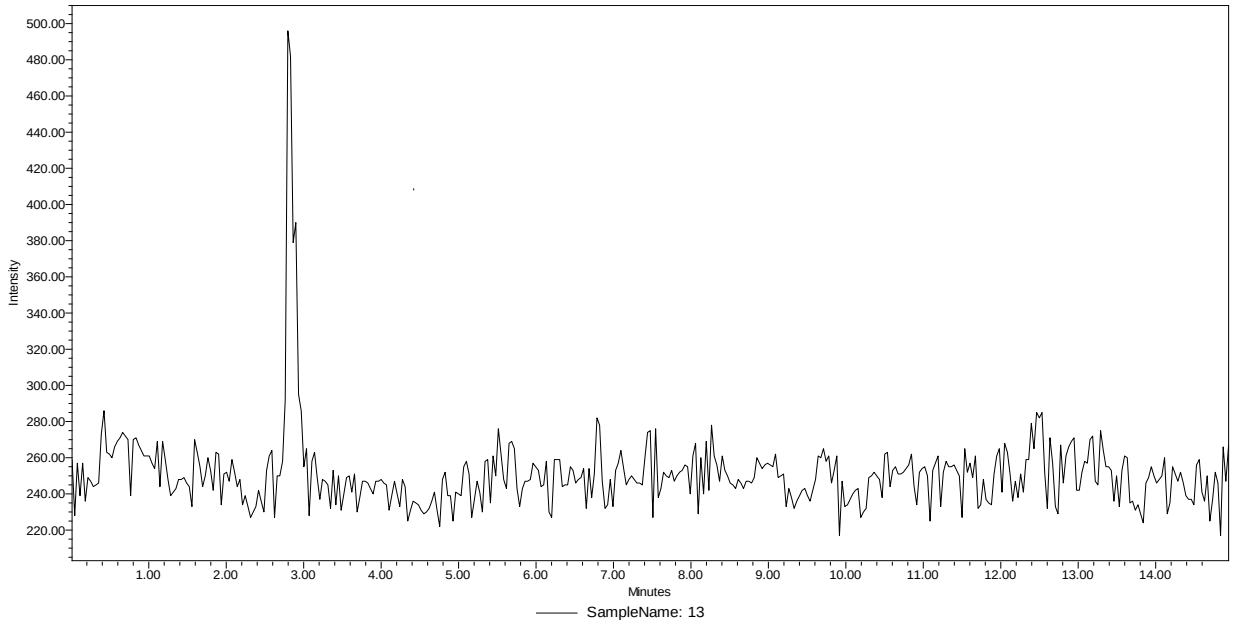

Fig. 29qqq Photolytic/Alkaline product chromatograph for 5-FU/sample 13.

14

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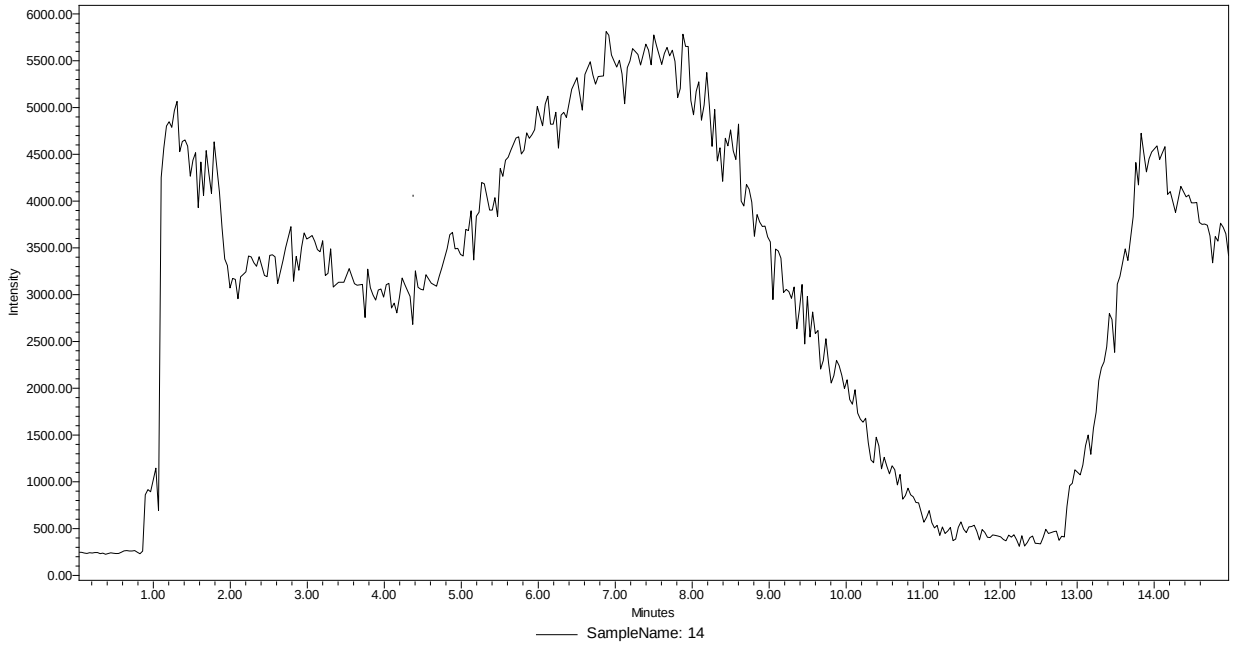

Fig. 29rrr Photolytic/Alkaline product chromatograph for FAC/sample 14.

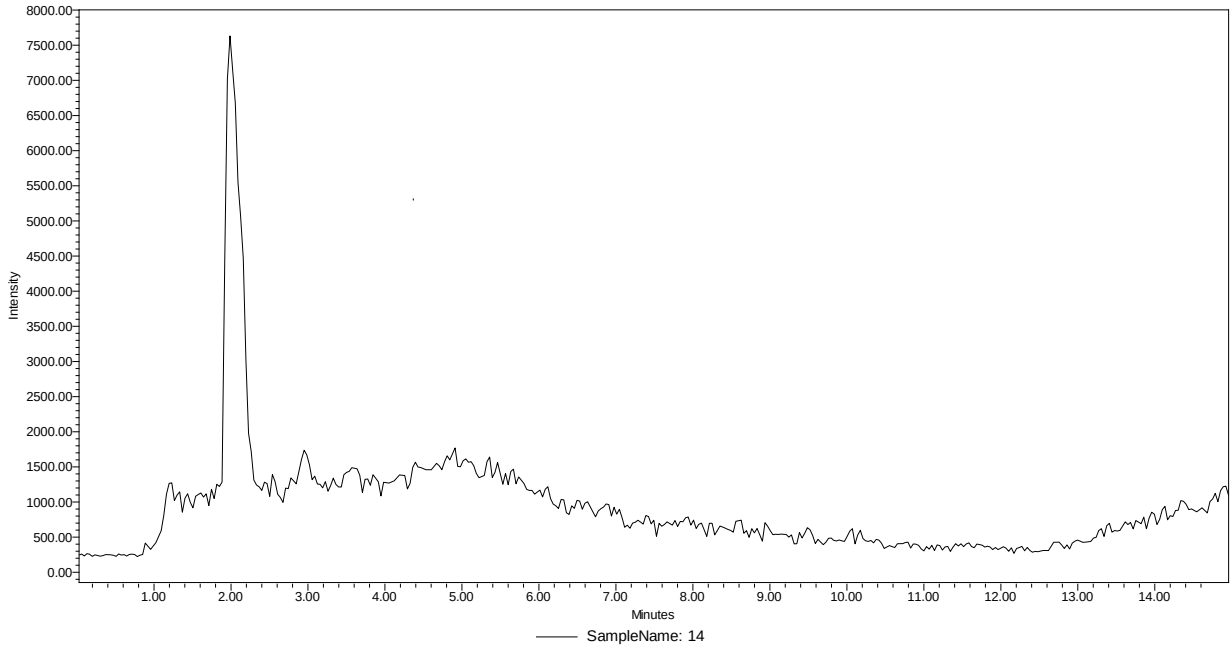

Fig. 29sss Photolytic/Alkaline product chromatograph for FMASId/sample 14.

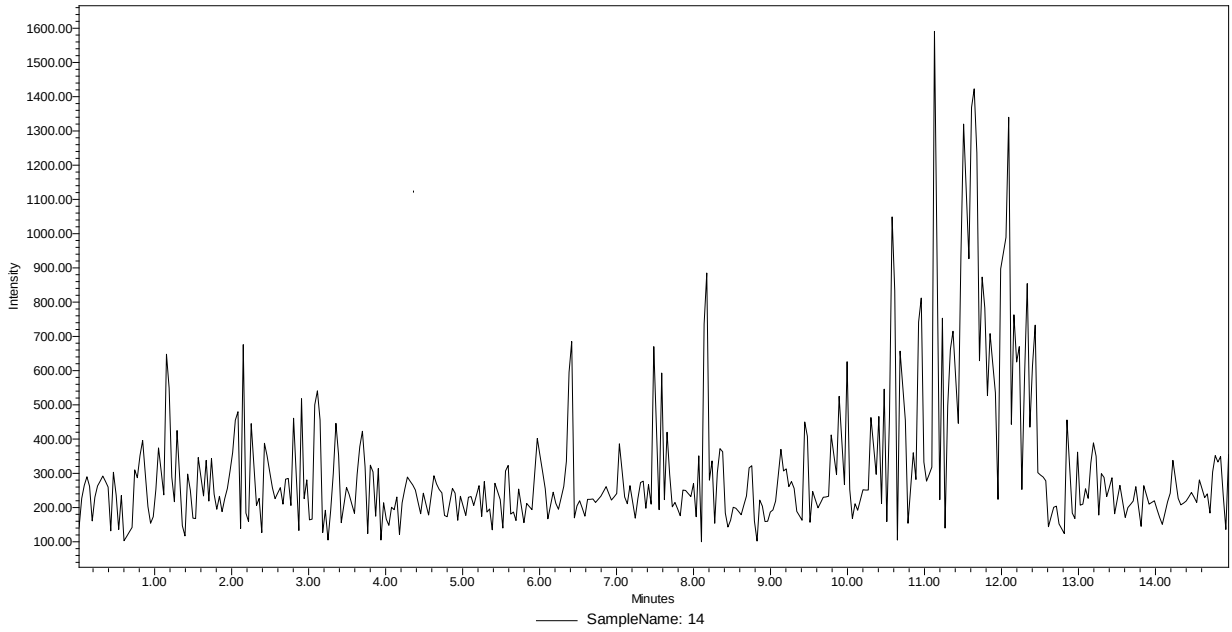

Fig. 29ttt Photolytic/Alkaline product chromatograph for FBAL/sample 14.

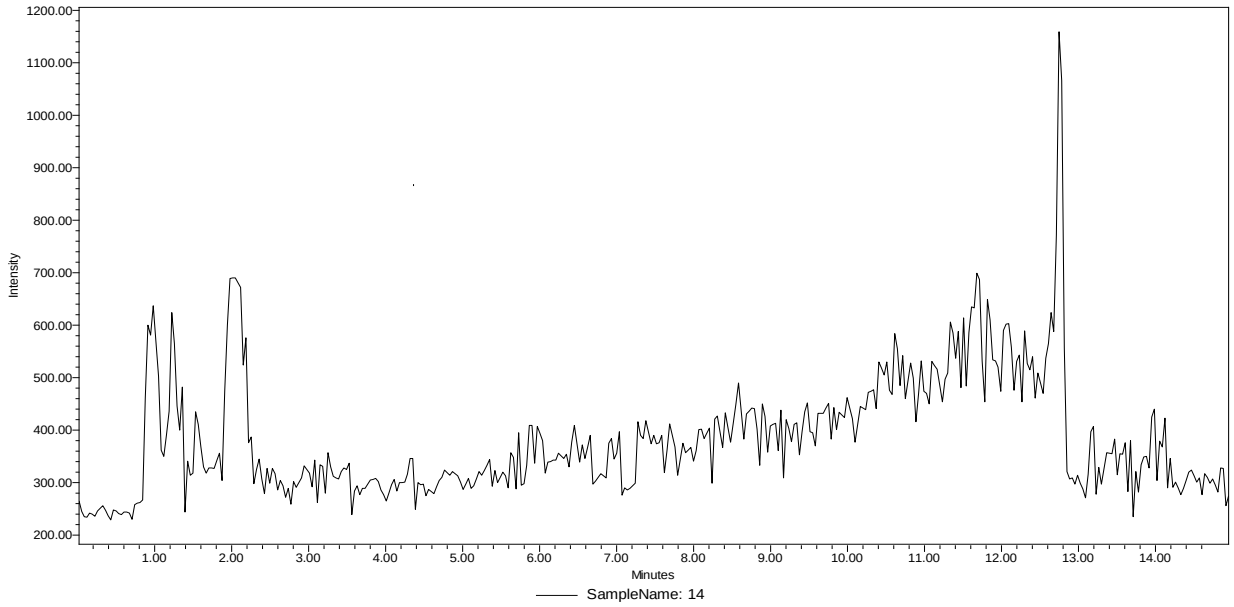

Fig. 29uuu Photolytic/Alkaline product chromatograph for FUPA/sample 14.

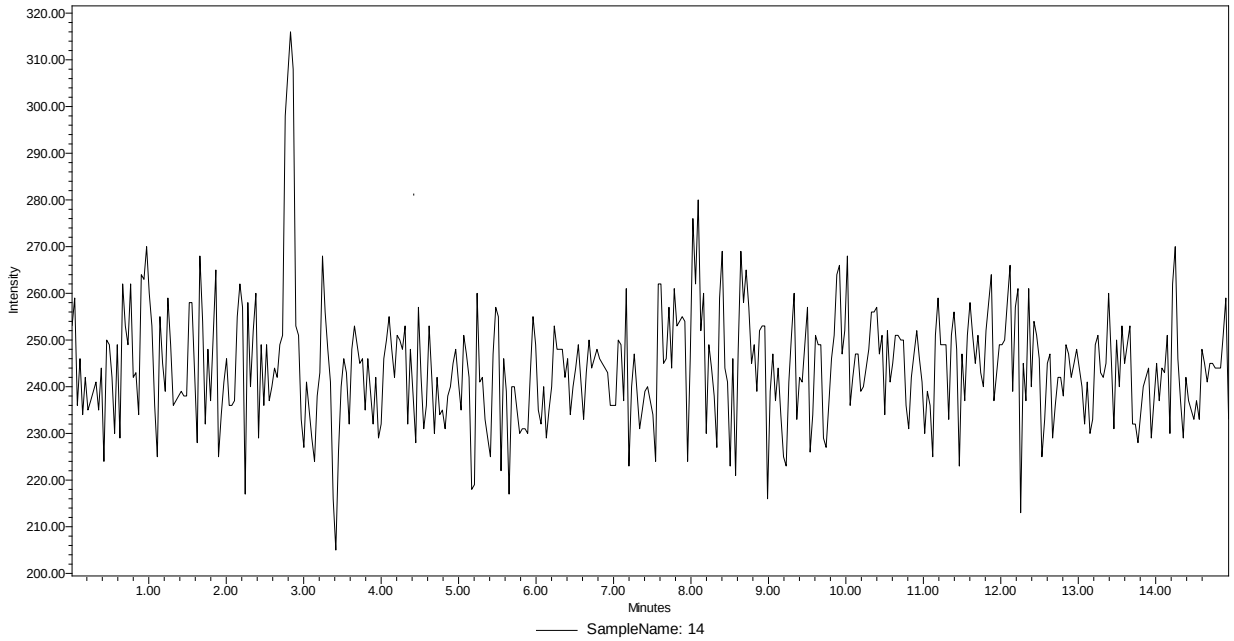

Fig. 29vvv Photolytic/Alkaline product chromatograph for 5-FU/sample 14.

15

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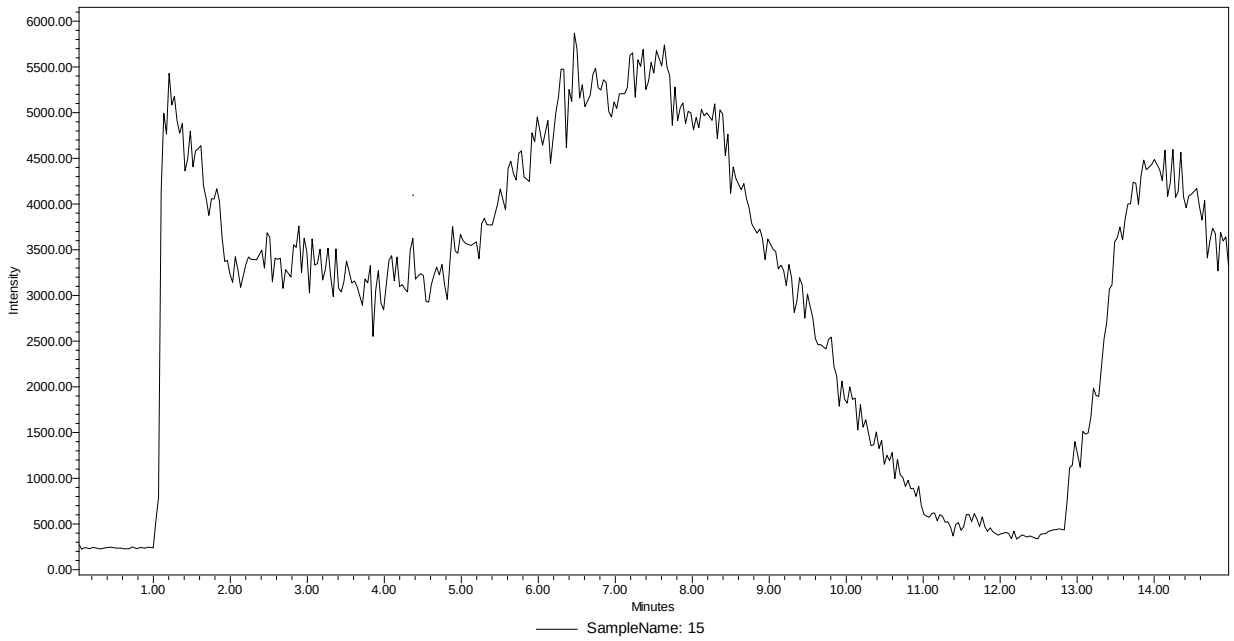

Fig. 29www Photolytic/Alkaline product chromatograph for FAC/sample 15.

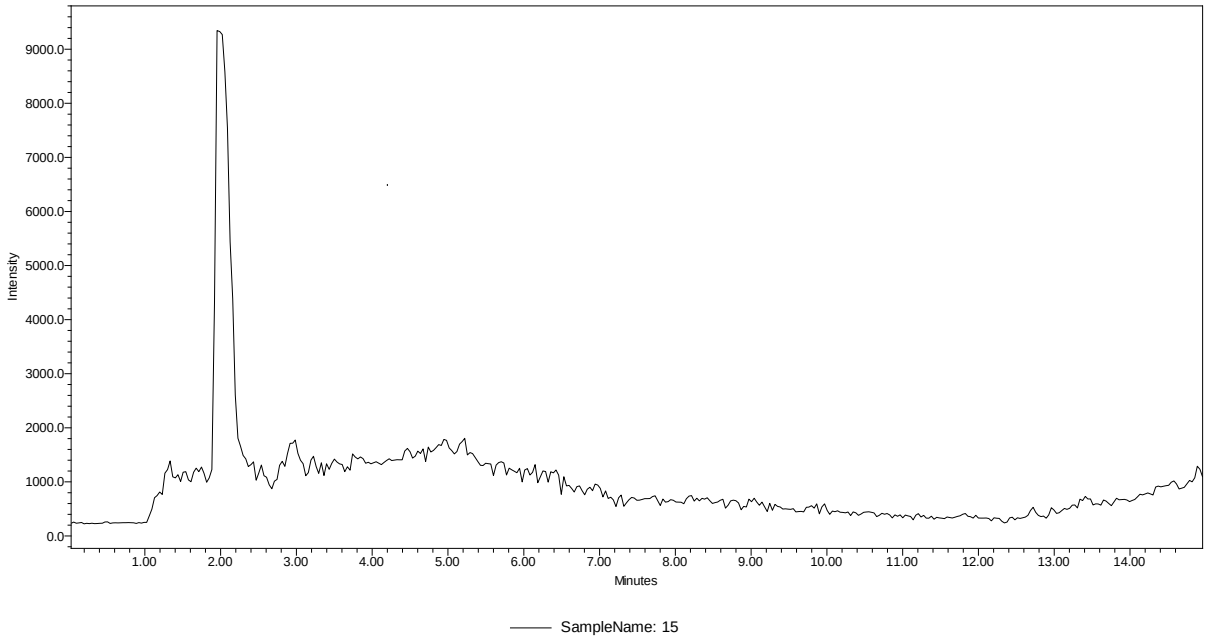

Fig. 29xxx Photolytic/Alkaline product chromatograph for FMASld/sample 15.

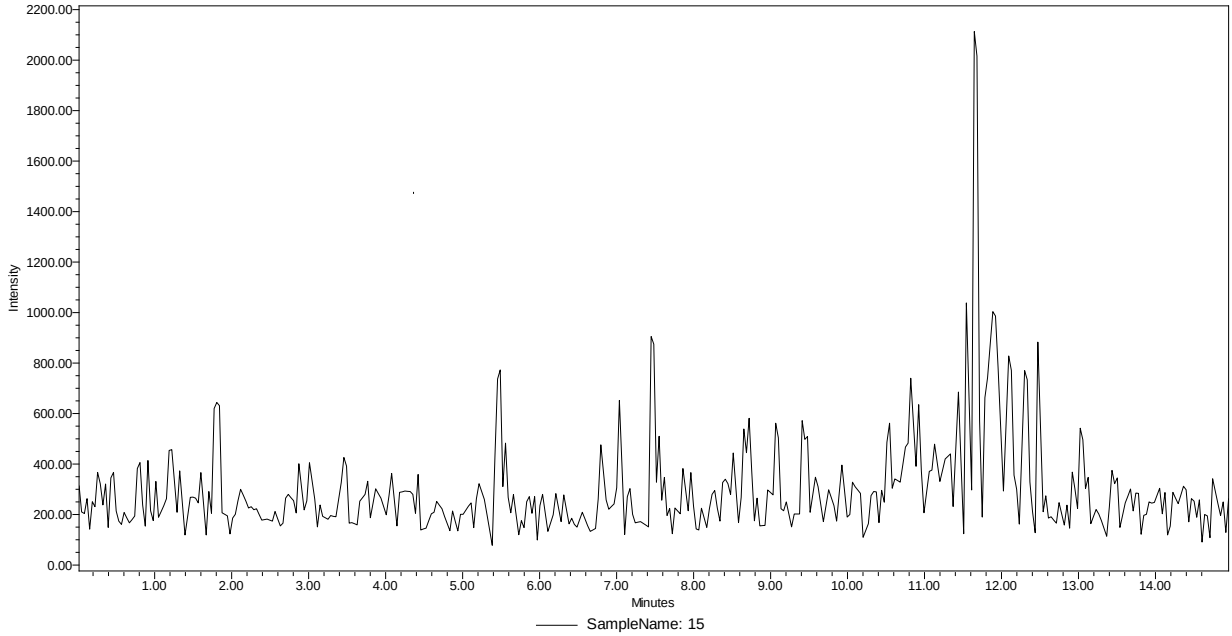

Fig. 29yyy Photolytic/Alkaline product chromatograph for FBAL/sample 15.

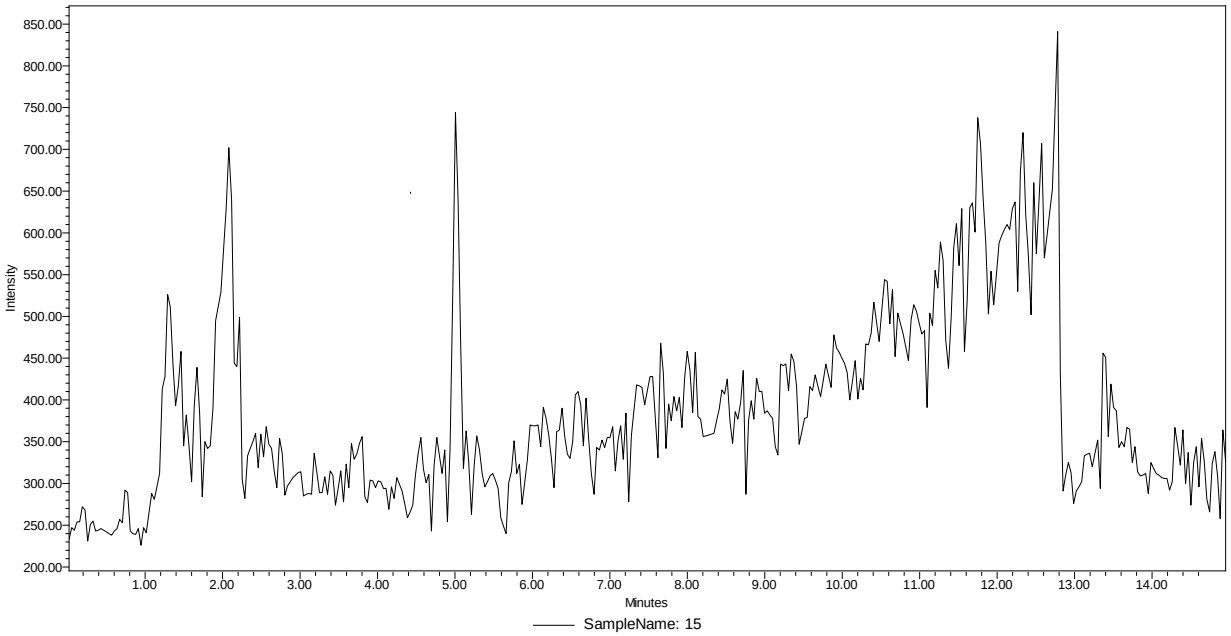

Fig. 29zzz Photolytic/Alkaline product chromatograph for FUPA/sample 15.

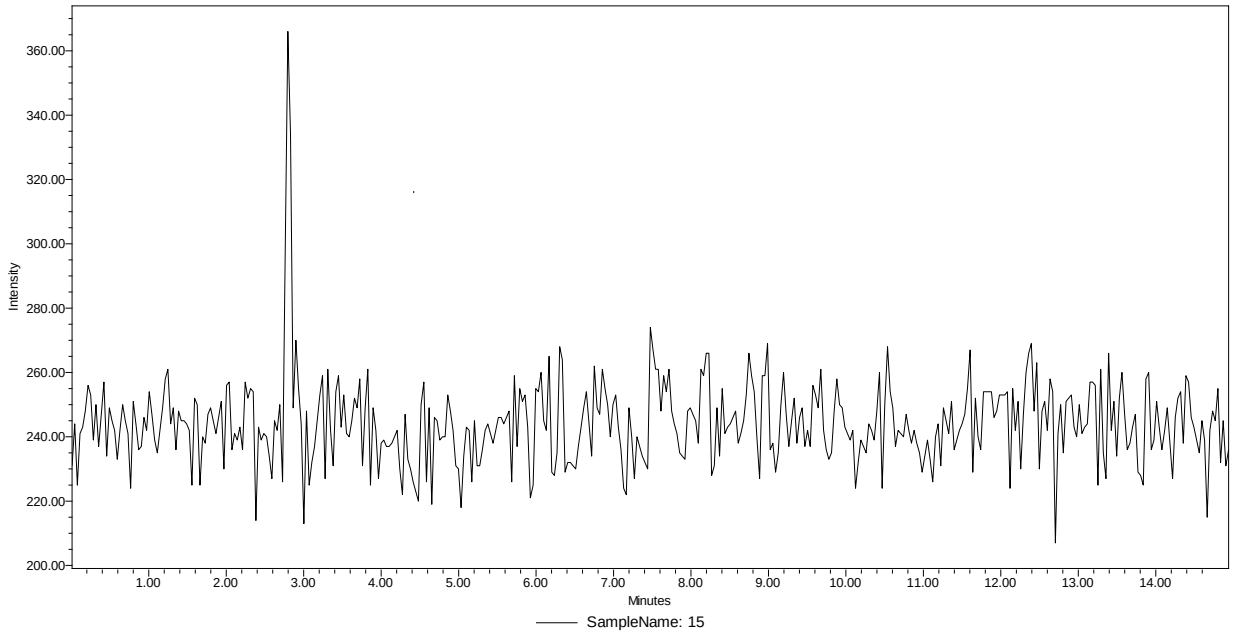

Fig. 29aaaa Photolytic/Alkaline product chromatograph for 5-FU/sample 15.

16

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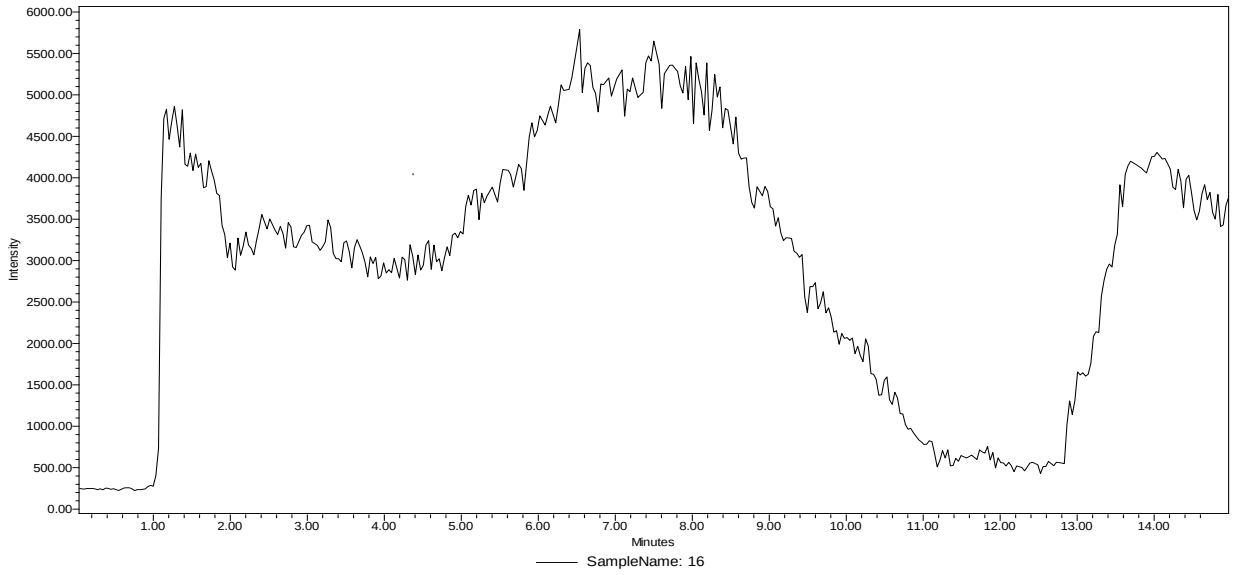

Fig. 29bbbb Photolytic/Alkaline product chromatograph for FAC/sample 16.

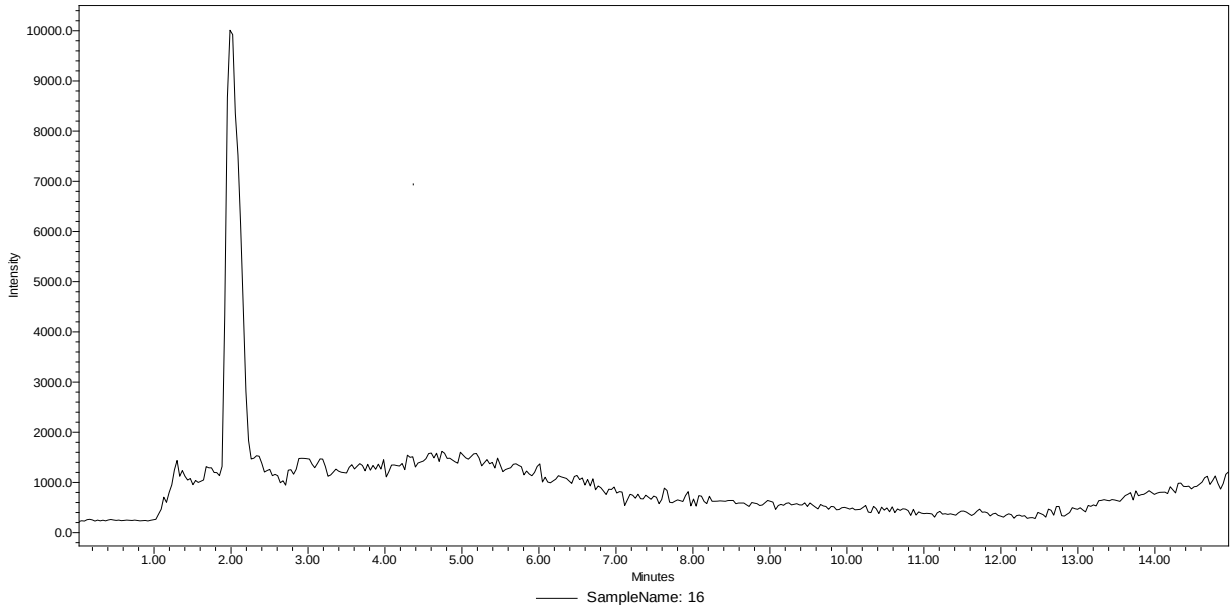

Fig. 29cccc Photolytic/Alkaline product chromatograph for FMASId/sample 16.

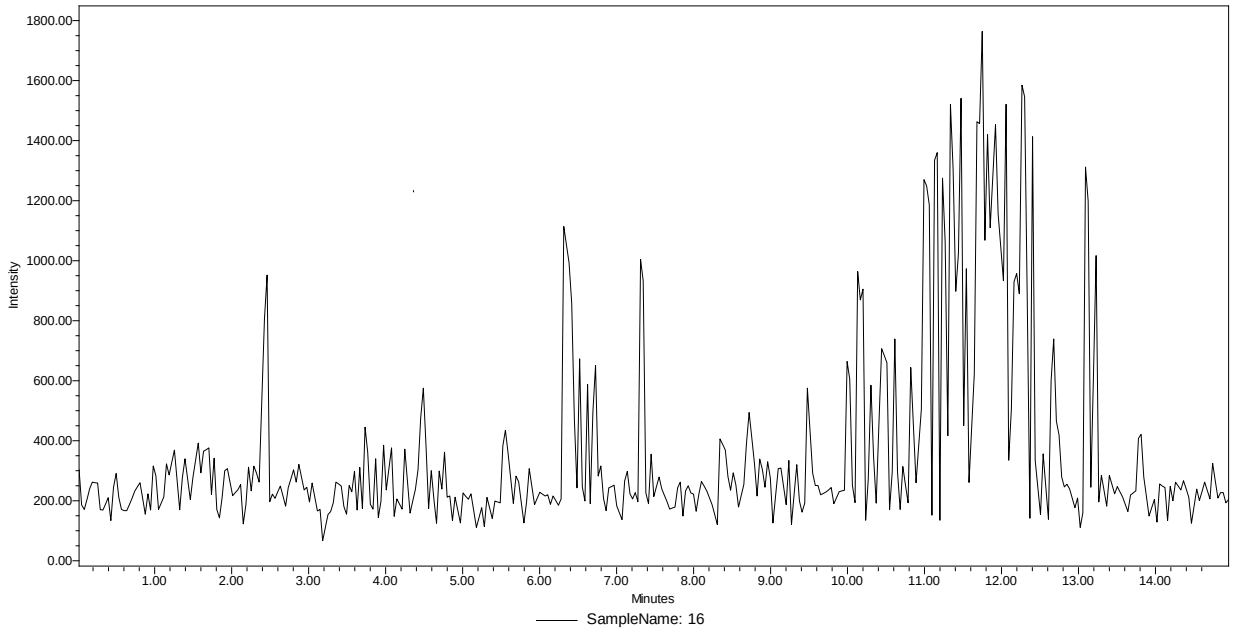

Fig. 29dddd Photolytic/Alkaline product chromatograph for FBAL/sample 16.

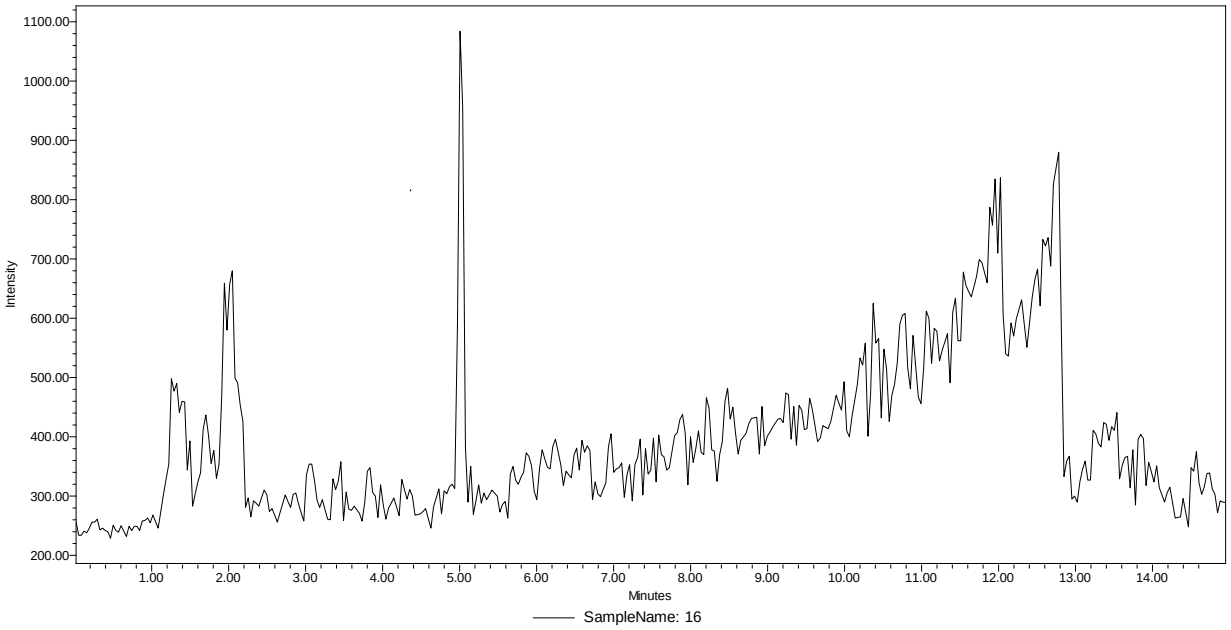

Fig. 29eeee Photolytic/Alkaline product chromatograph for FUPA/sample 16.

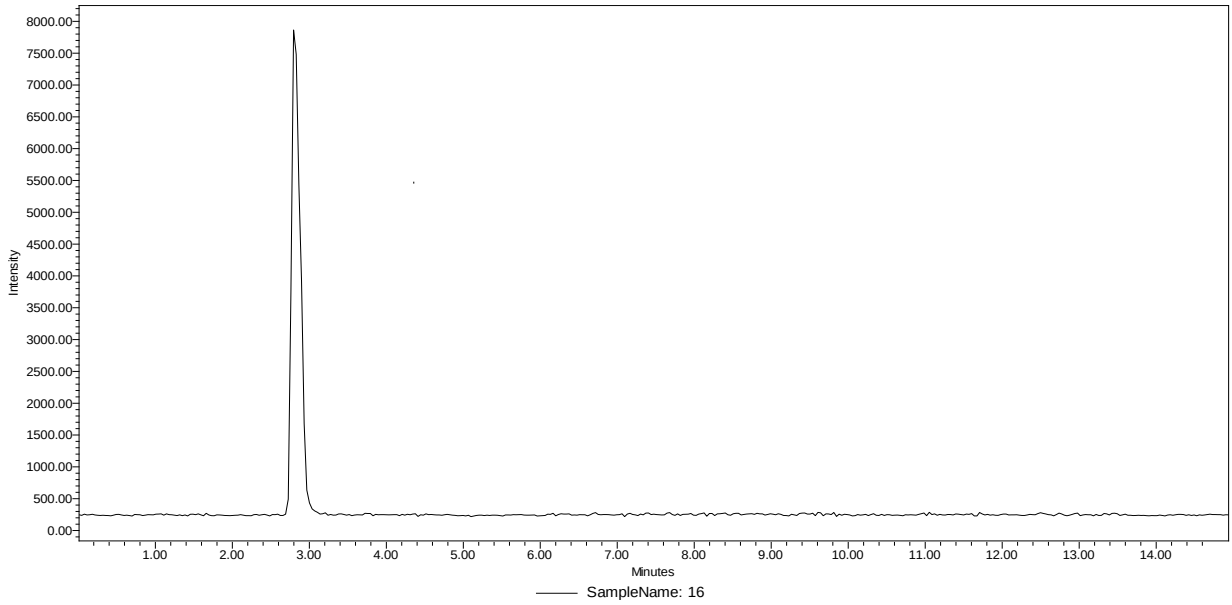

Fig. 29ffff Photolytic/Alkaline product chromatograph for 5-FU/sample 16.

17

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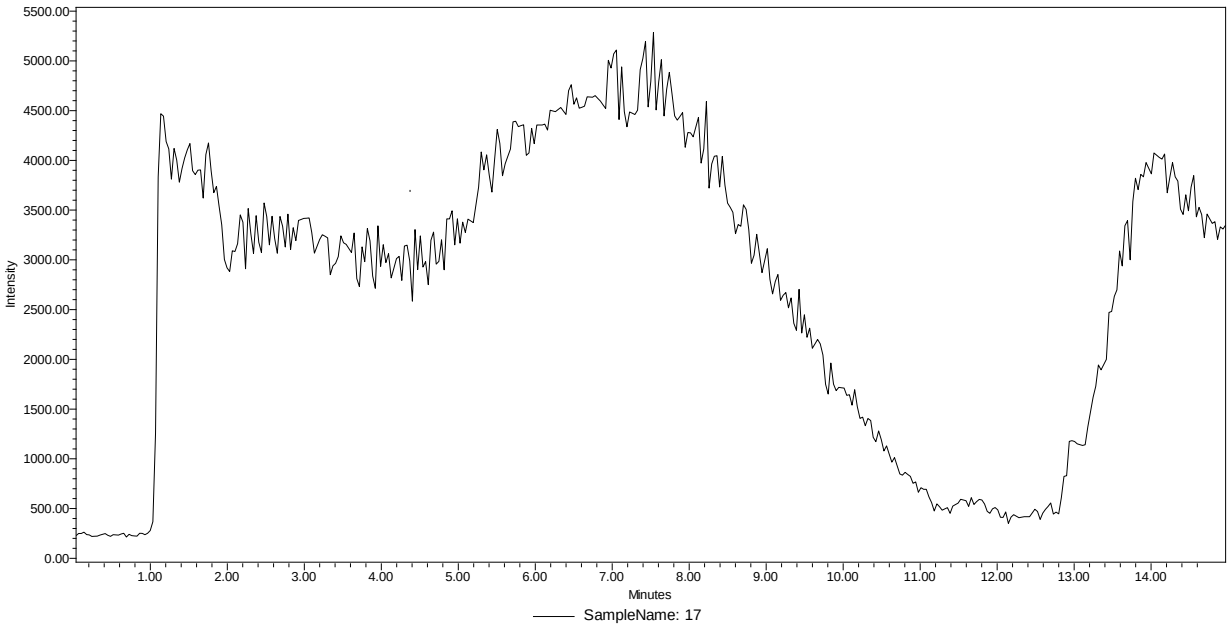

Fig. 29gggg Photolytic/Alkaline product chromatograph for FAC/sample 17.

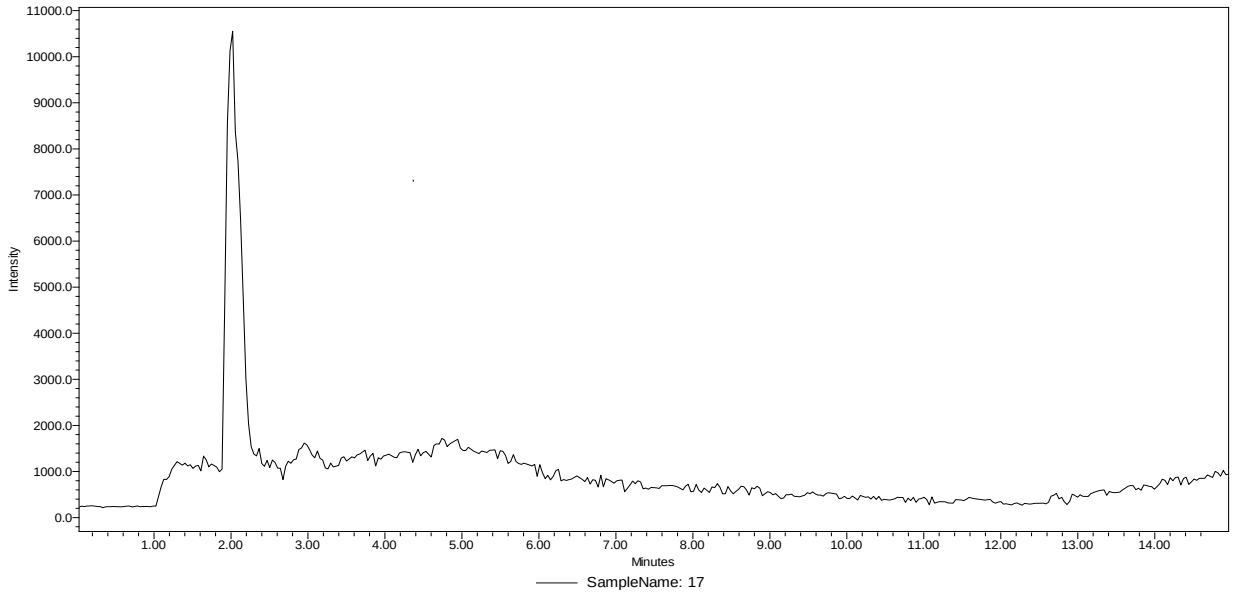

Fig. 29hhhh Photolytic/Alkaline product chromatograph for FMASld/sample 17.

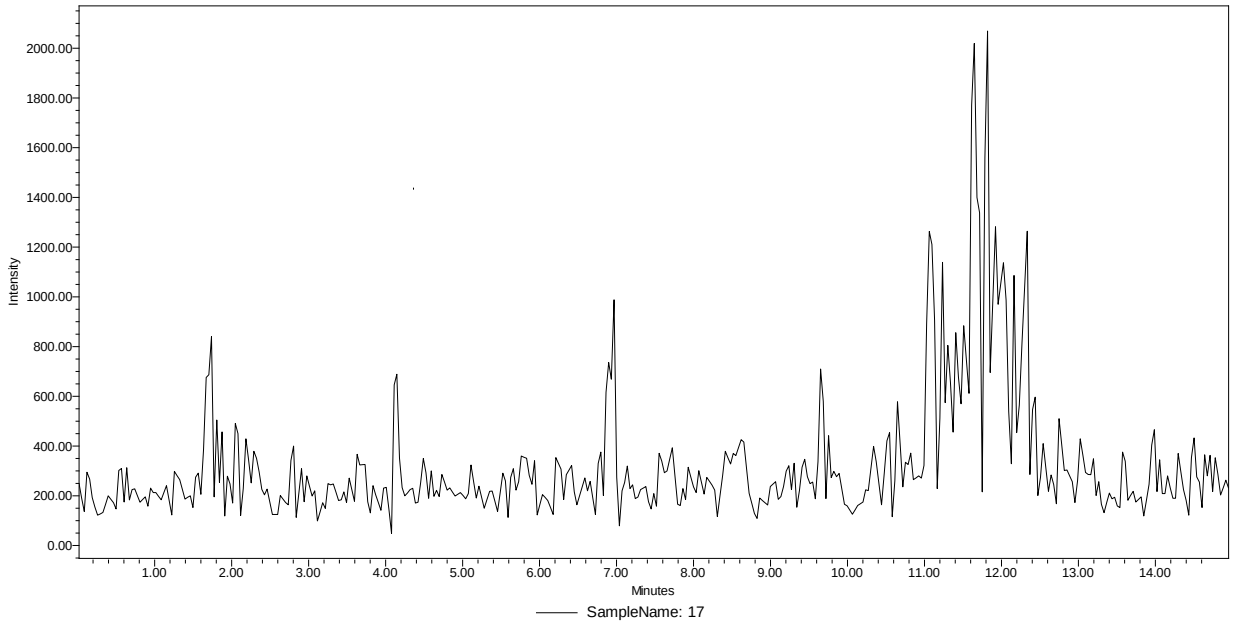

Fig. 29iiii Photolytic/Alkaline product chromatograph for FBAL/sample 17.

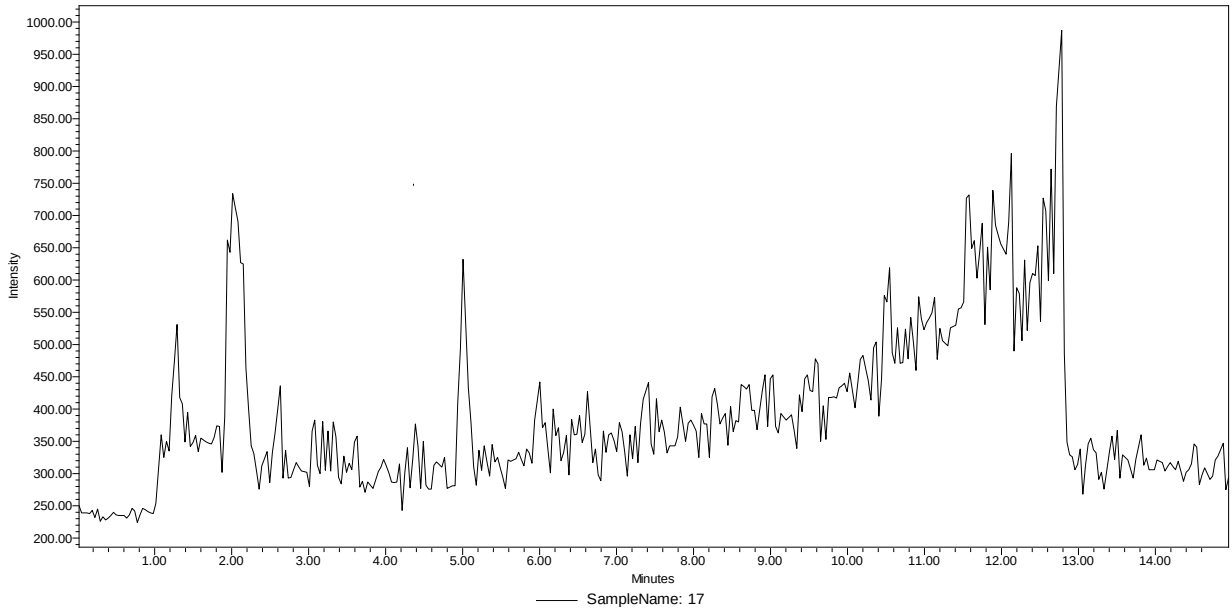

Fig. 29jjjj Photolytic/Alkaline product chromatograph for FUPA/sample 17.

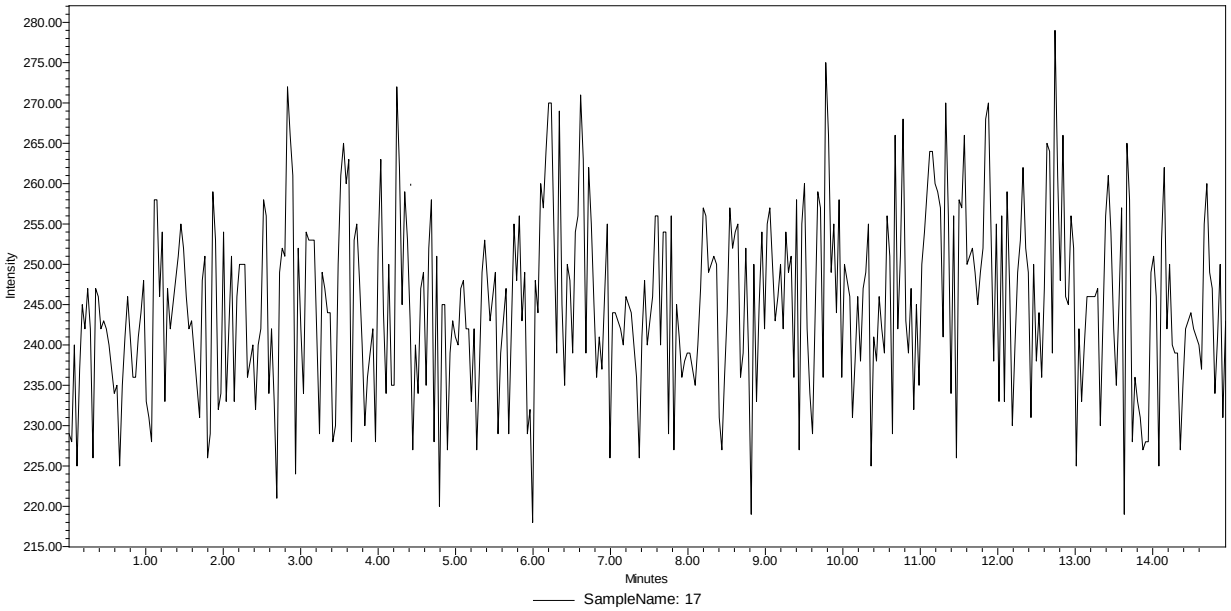

Fig. 29kkkk Photolytic/Alkaline product chromatograph for 5-FU/sample 17.

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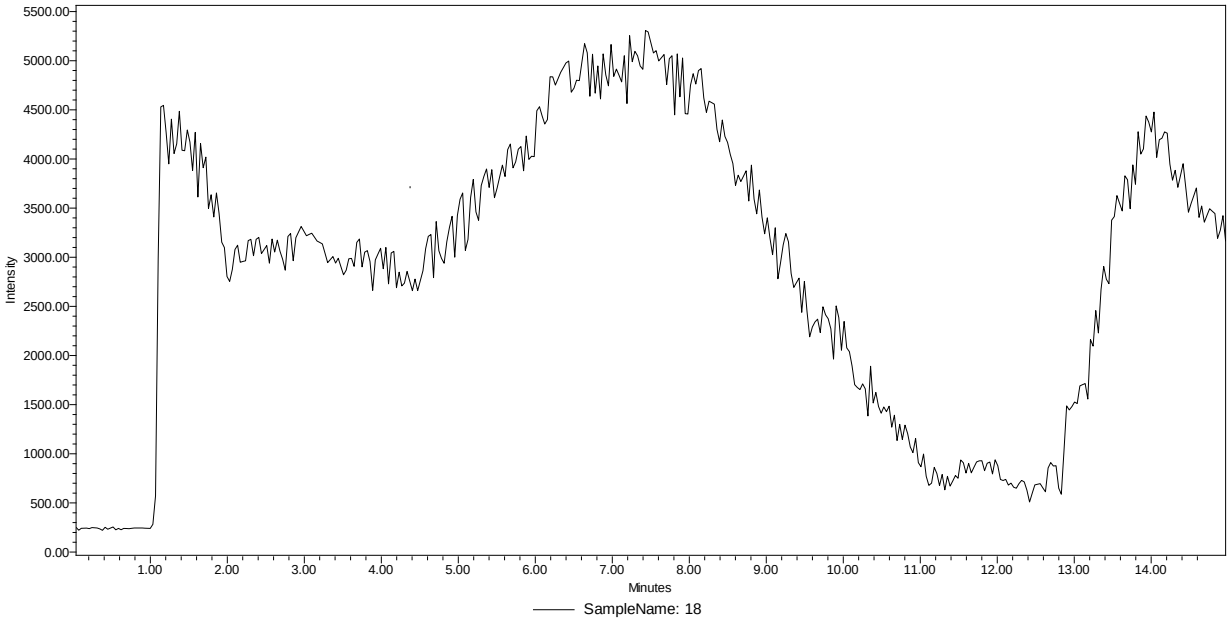

Fig. 29llll Photolytic/Alkaline product chromatograph for FAC/sample 18.

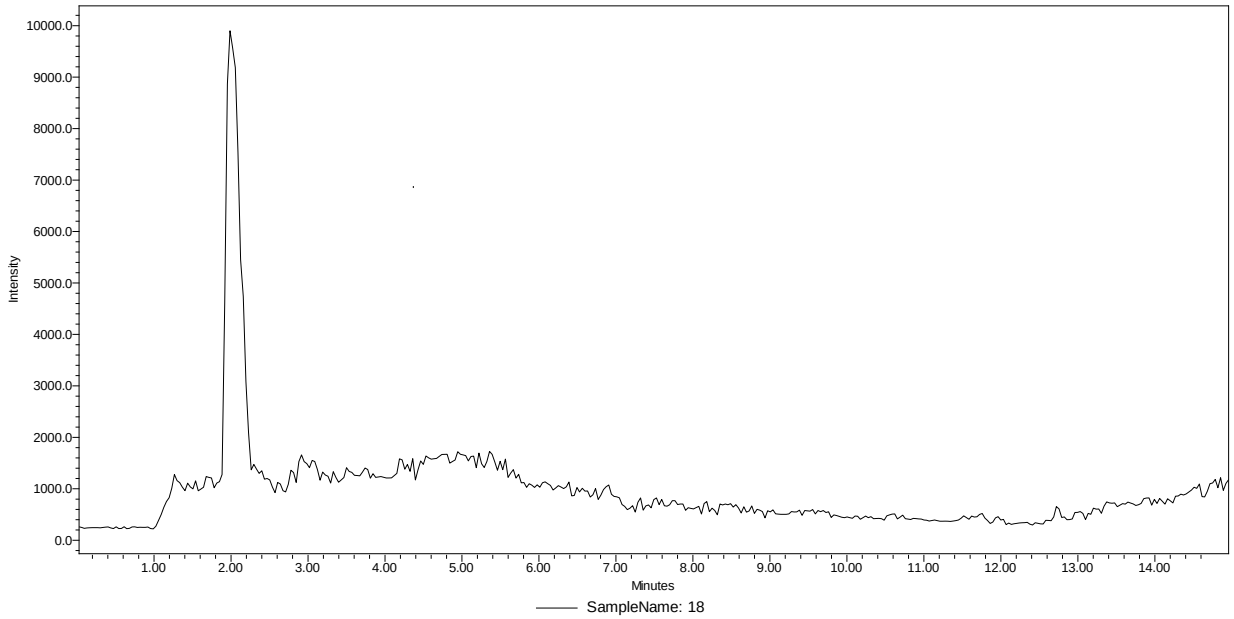

Fig. 29mmmm Photolytic/Alkaline product chromatograph for FMASld/sample 18.

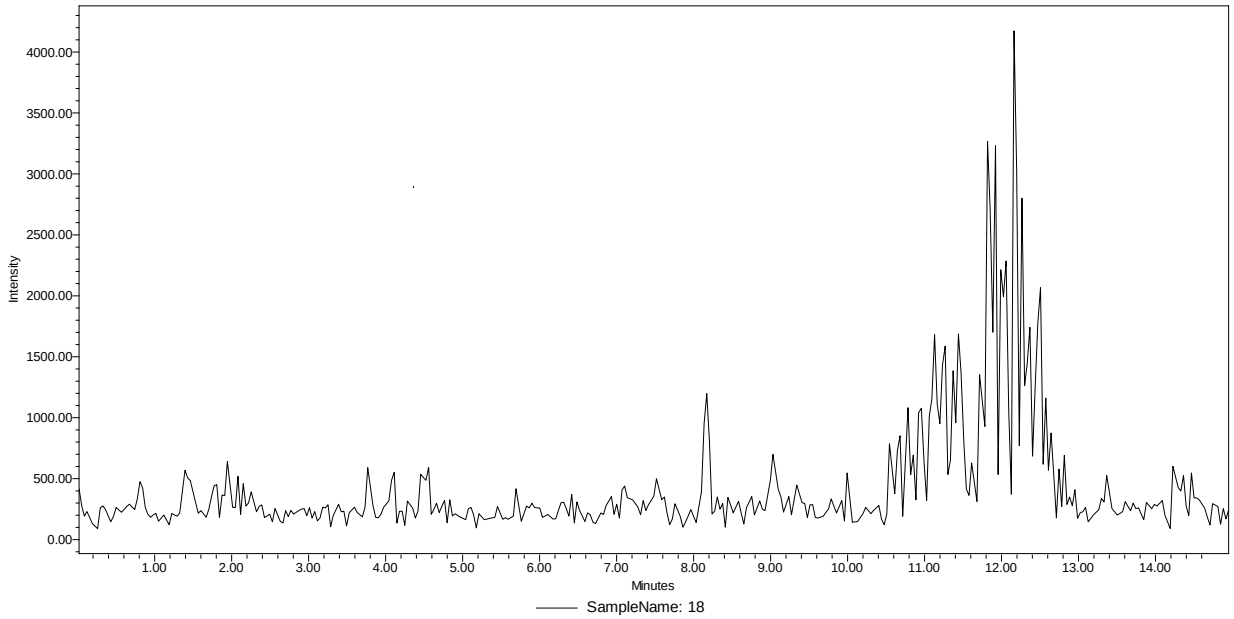

Fig. 29nnnn Photolytic/Alkaline product chromatograph for FBAL/sample 18.

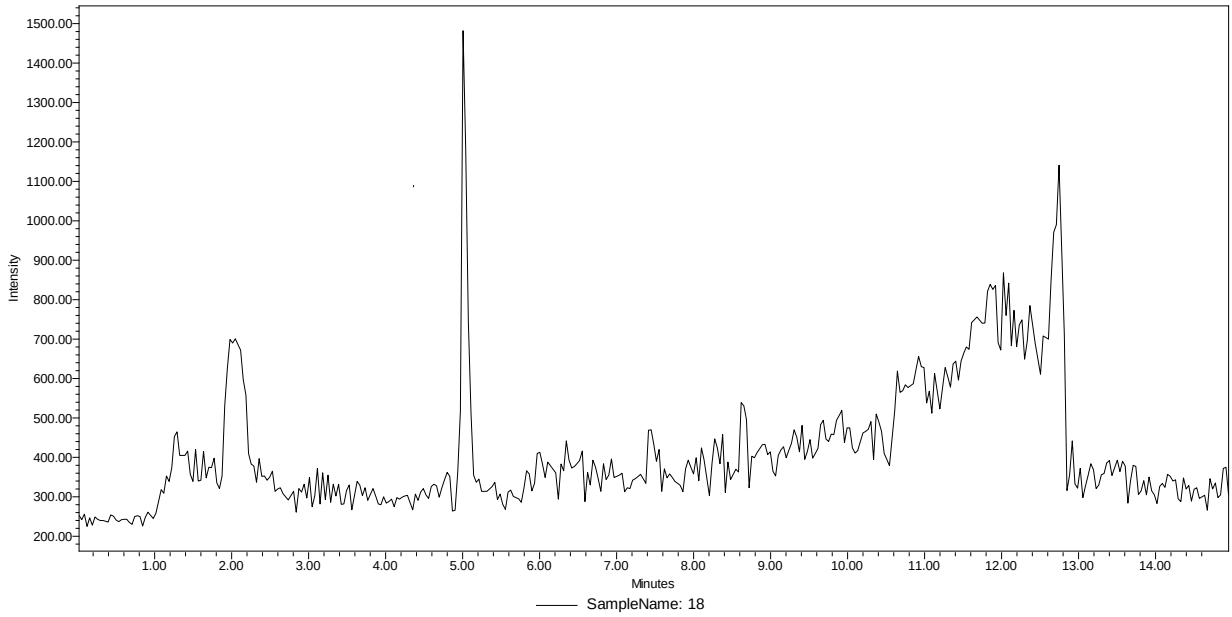

Fig. 29oooo Photolytic/Alkaline product chromatograph for FUPA/sample 18.

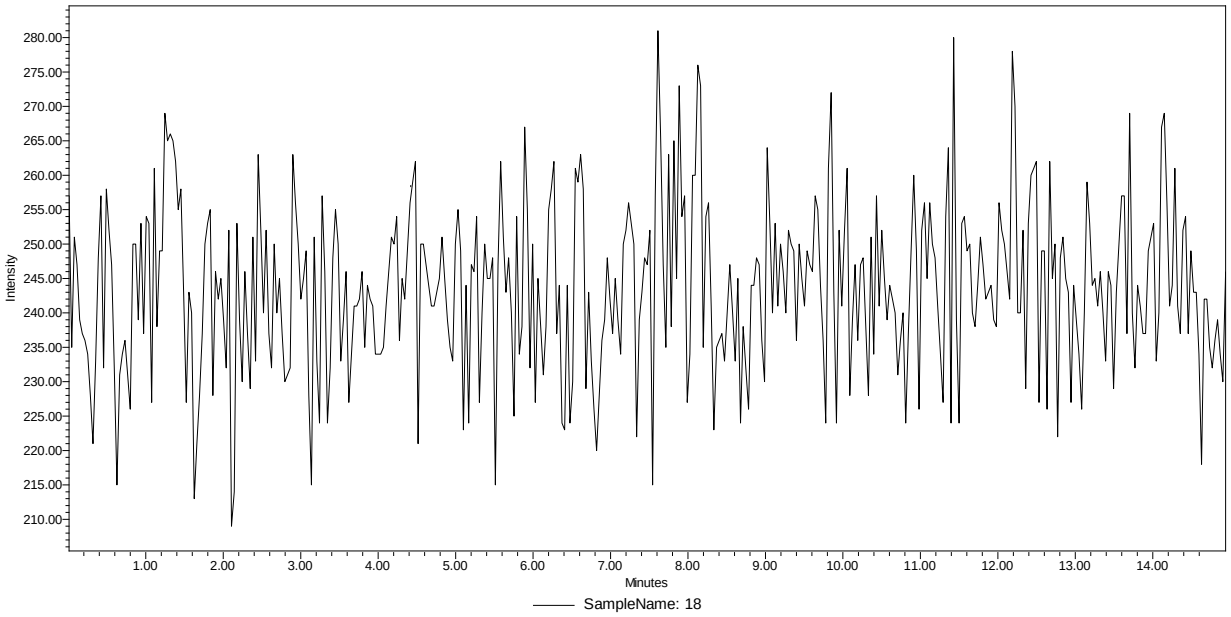

Fig. 29pppp Photolytic/Alkaline product chromatograph for 5-FU/sample 18.

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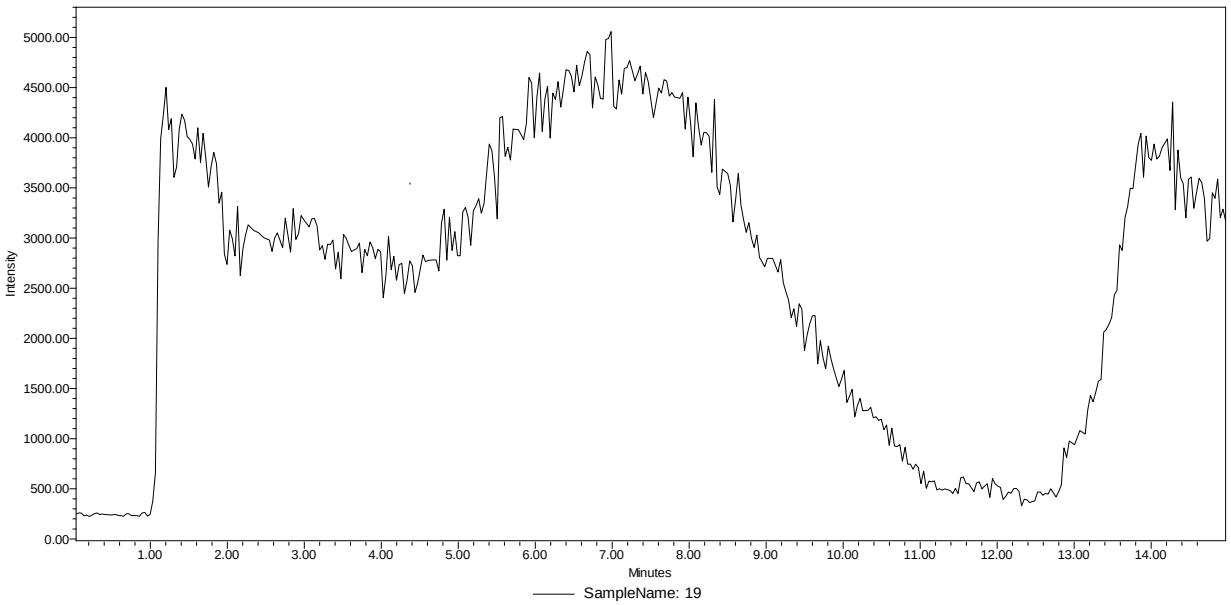

Fig. 29qqqq Photolytic/Alkaline product chromatograph for FAC/sample 19.

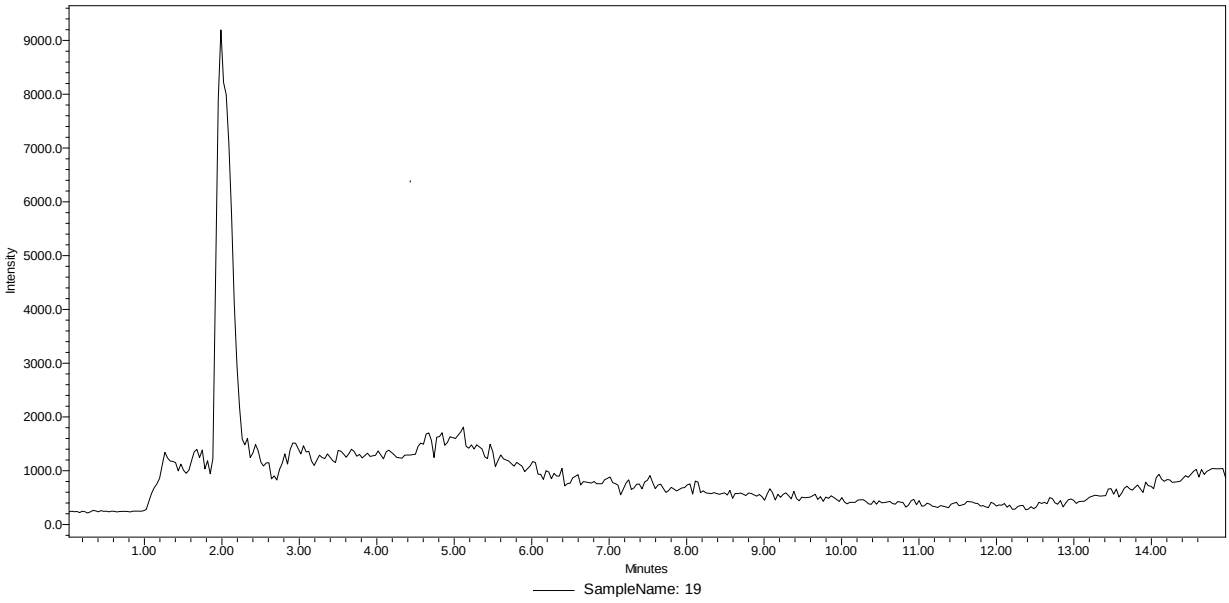

Fig. 29rrrr Photolytic/Alkaline product chromatograph for FMASld/sample 19.

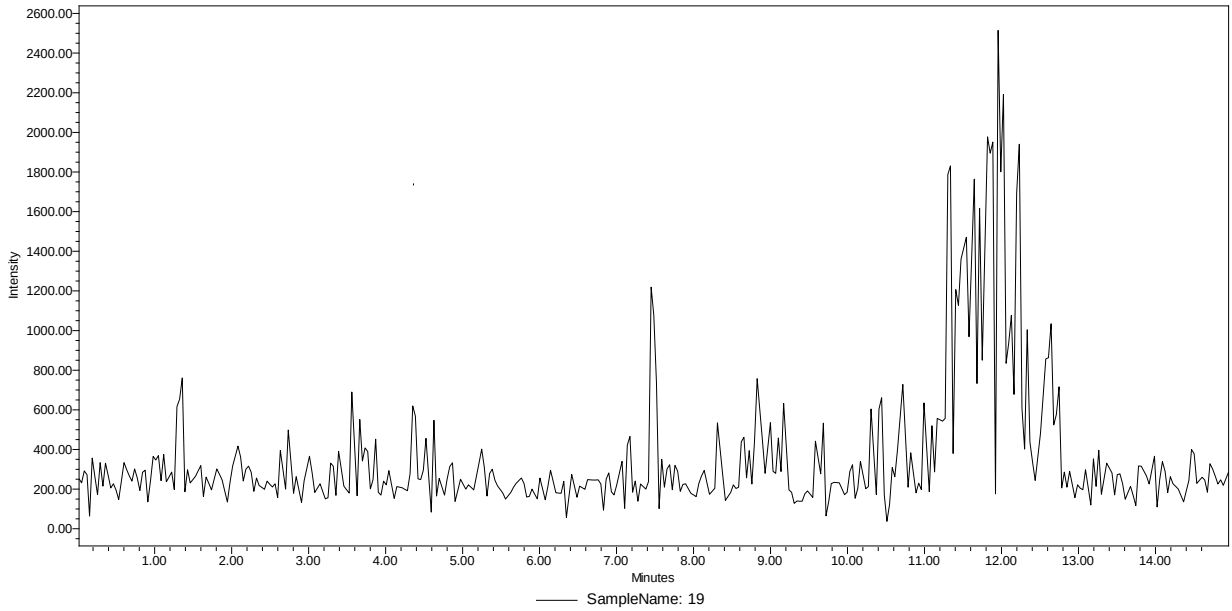

Fig. 29ssss Photolytic/Alkaline product chromatograph for FBAL/sample 19.

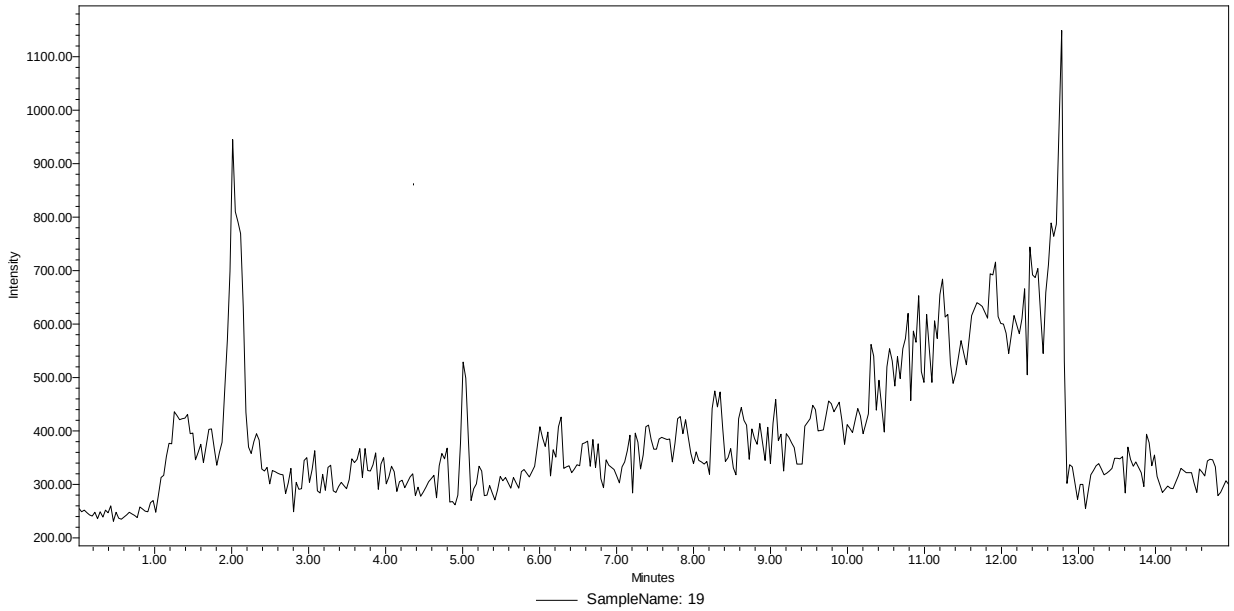

Fig. 29tttt Photolytic/Alkaline product chromatograph for 5-FU/sample 19.

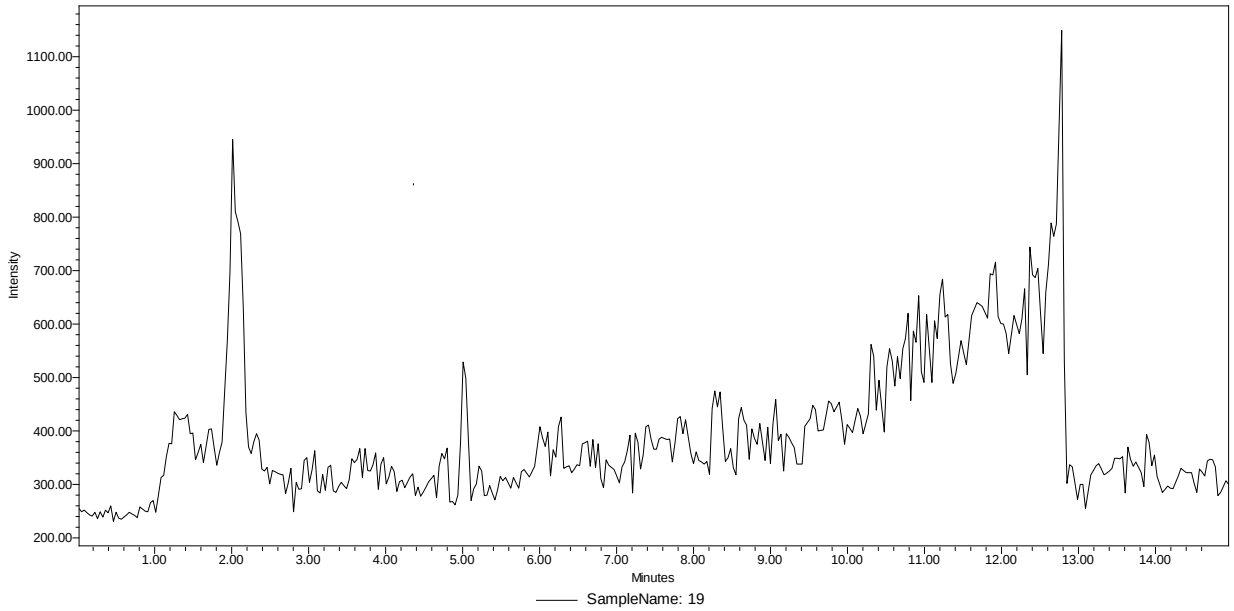

Fig. 29uuuu Photolytic/Alkaline product chromatograph for 5-FU/sample 19.

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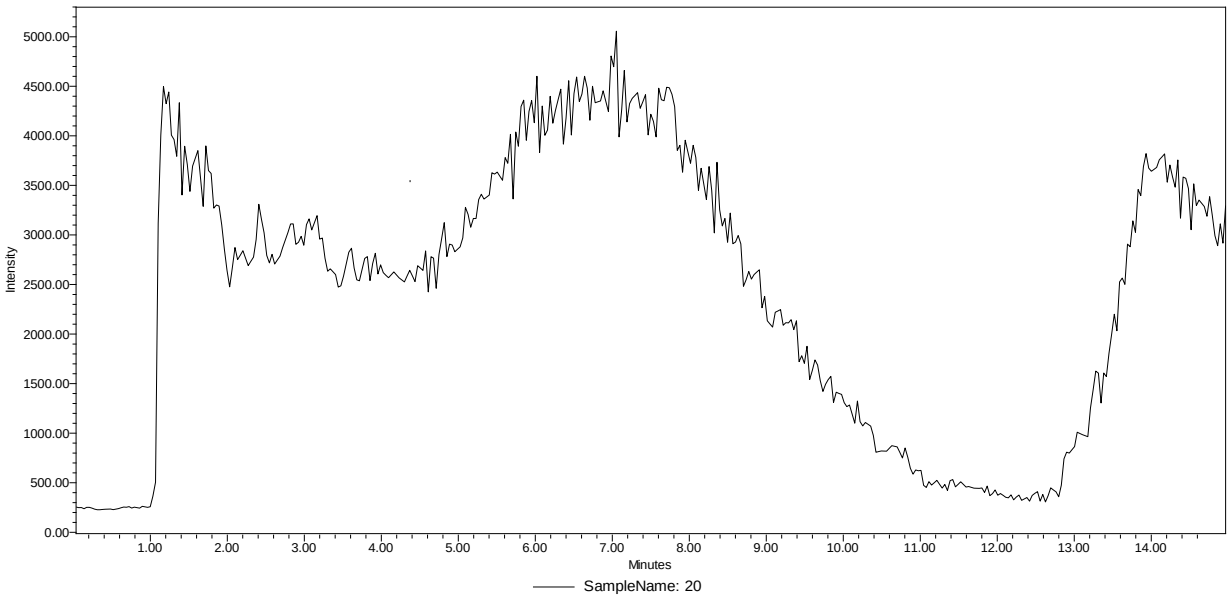

Fig. 29vvvv Photolytic/Alkaline product chromatograph for FAC/sample 20.

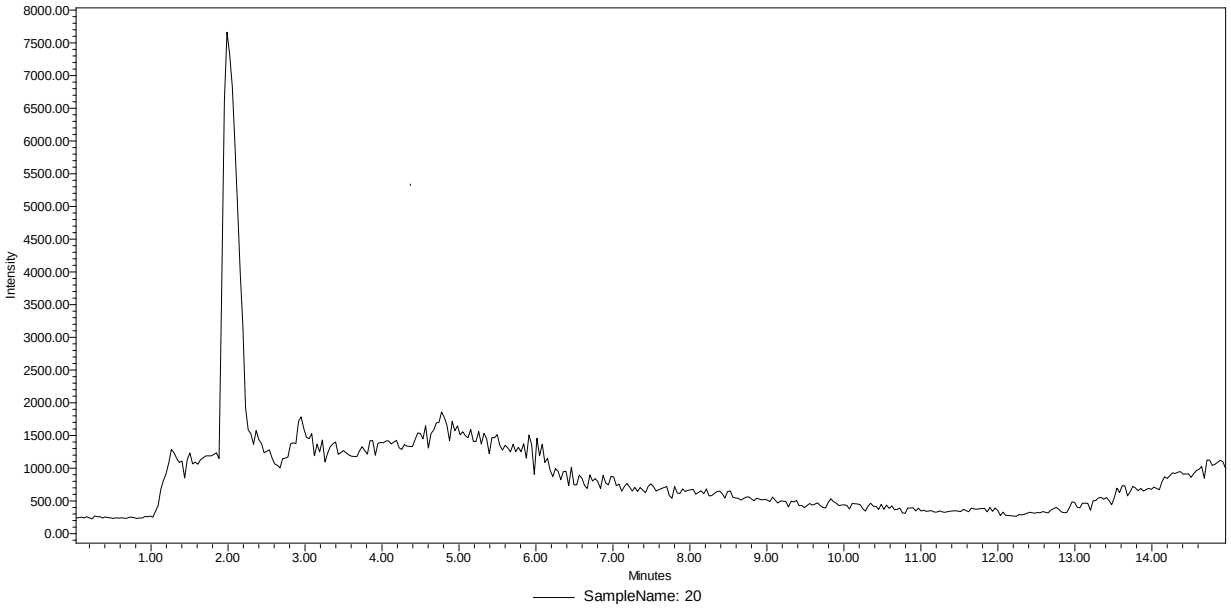

Fig. 29wwww Photolytic/Alkaline product chromatograph for FMASld/sample 20.

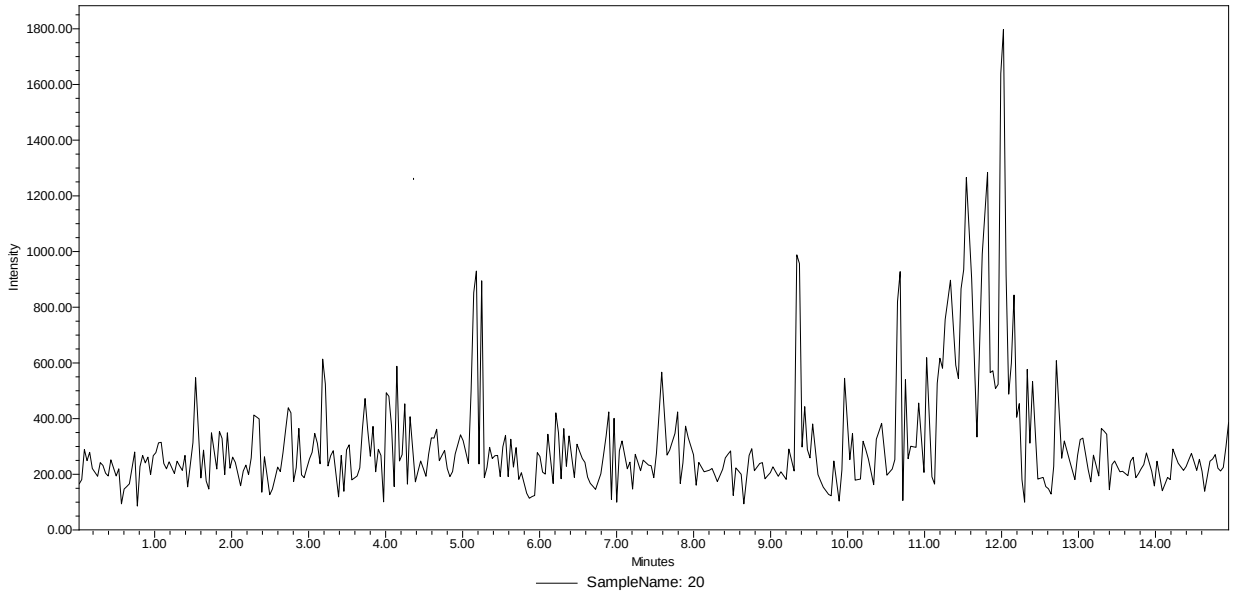

Fig. 29xxxx Photolytic/Alkaline product chromatograph for FBAL/sample 20.

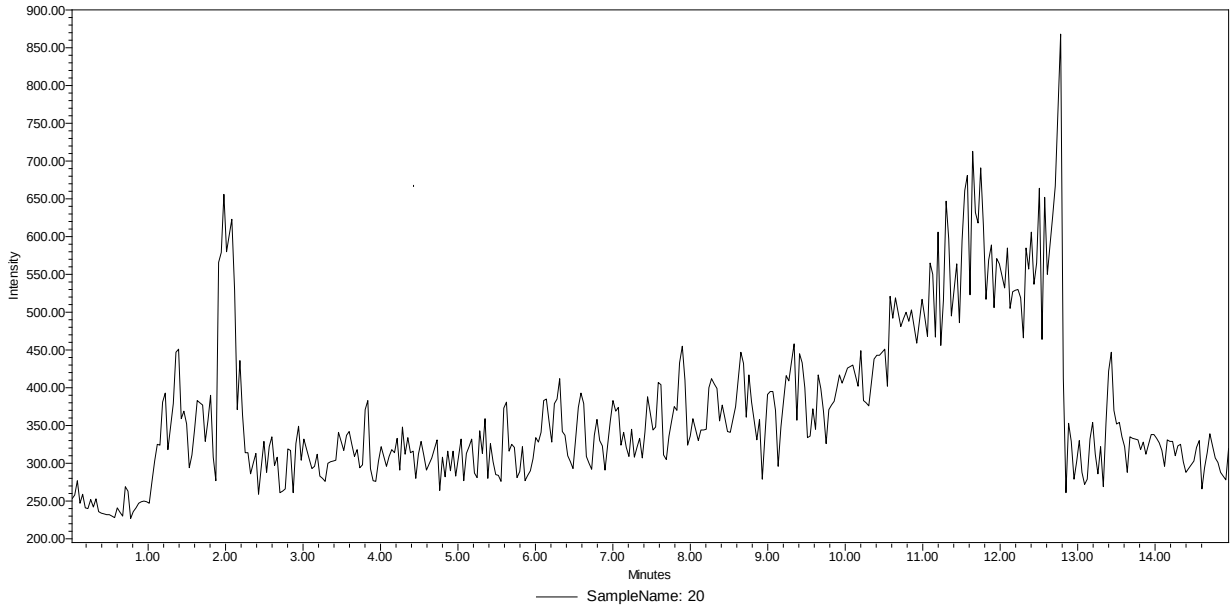

Fig. 29yyyy Photolytic/Alkaline product chromatograph for FUPA/sample 20.

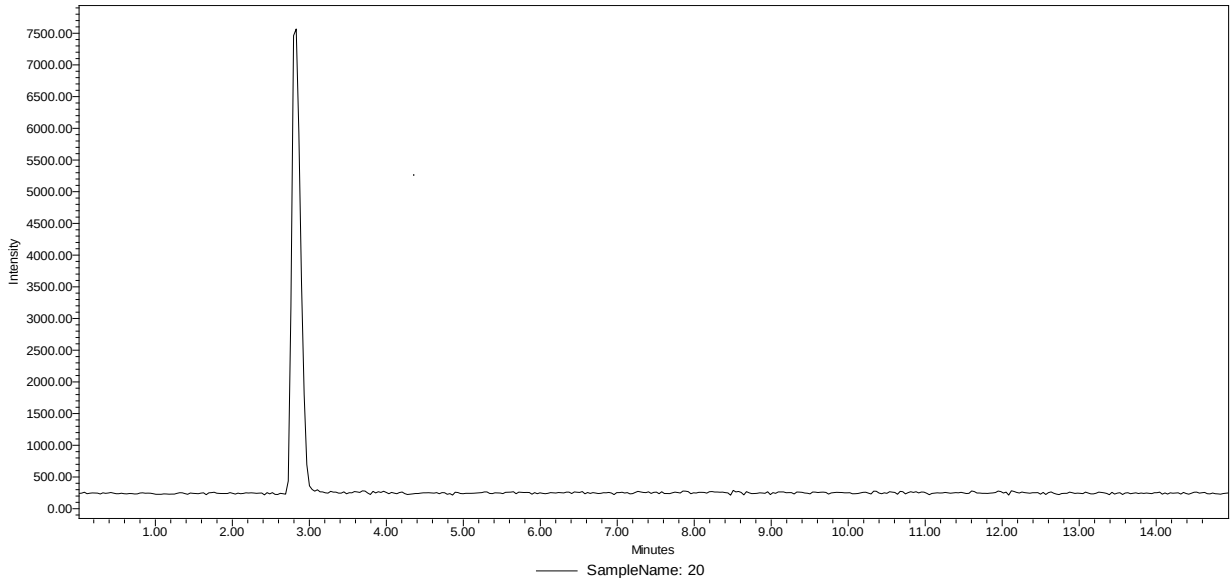

Fig. 29zzzz Photolytic/Alkaline product chromatograph for 5-FU/sample 20.

21

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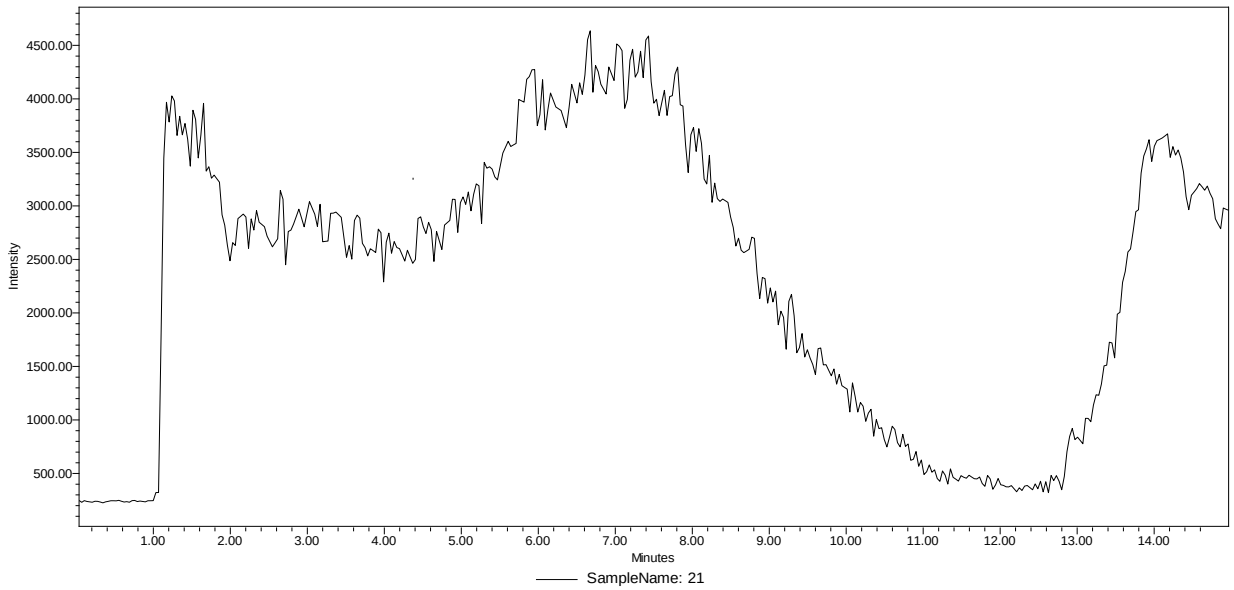

Fig. 29aaaaa Photolytic/Alkaline product chromatograph for FAC/sample 21.

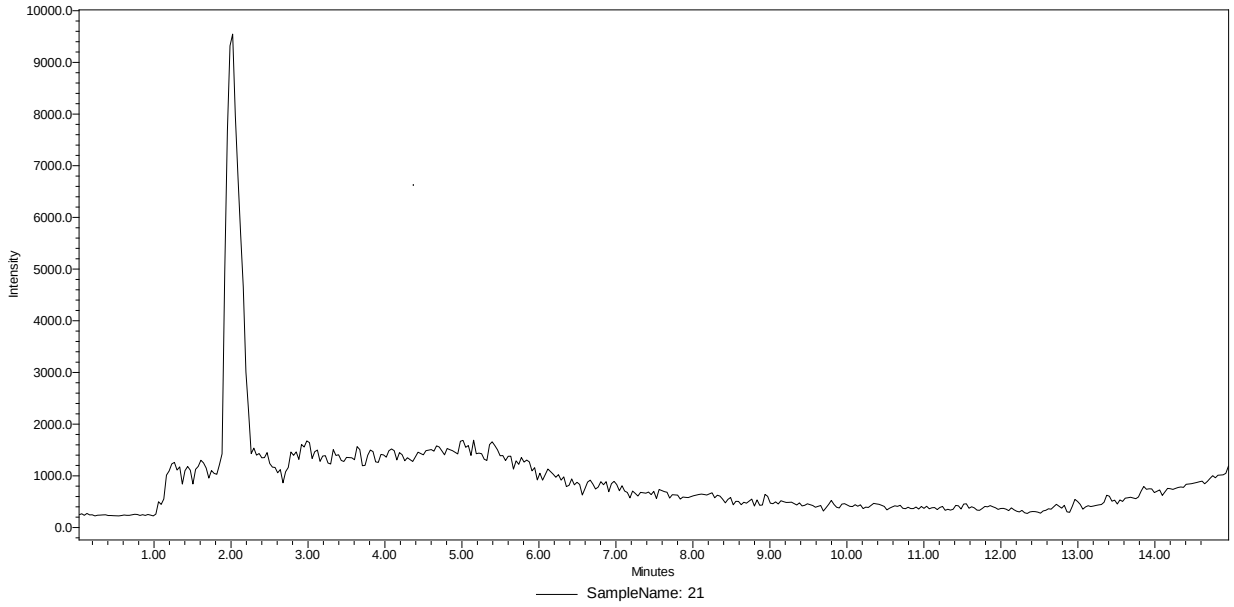

Fig. 29bbbbb Photolytic/Alkaline product chromatograph for FMASld/sample 21.

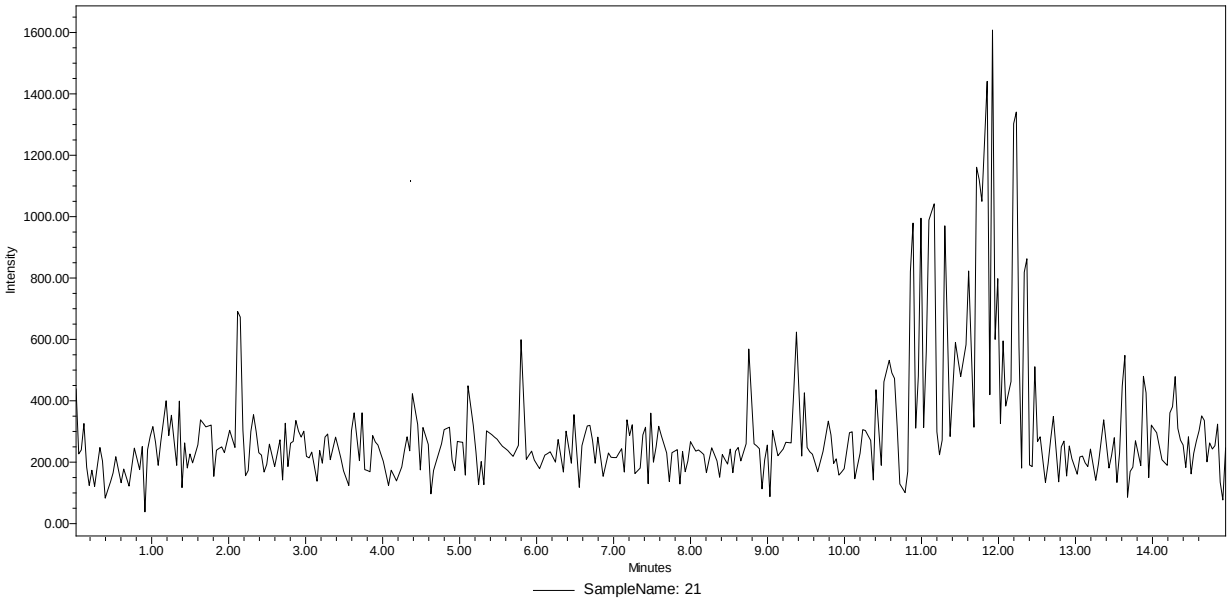

Fig. 29ccccc Photolytic/Alkaline product chromatograph for FBAL/sample 21.

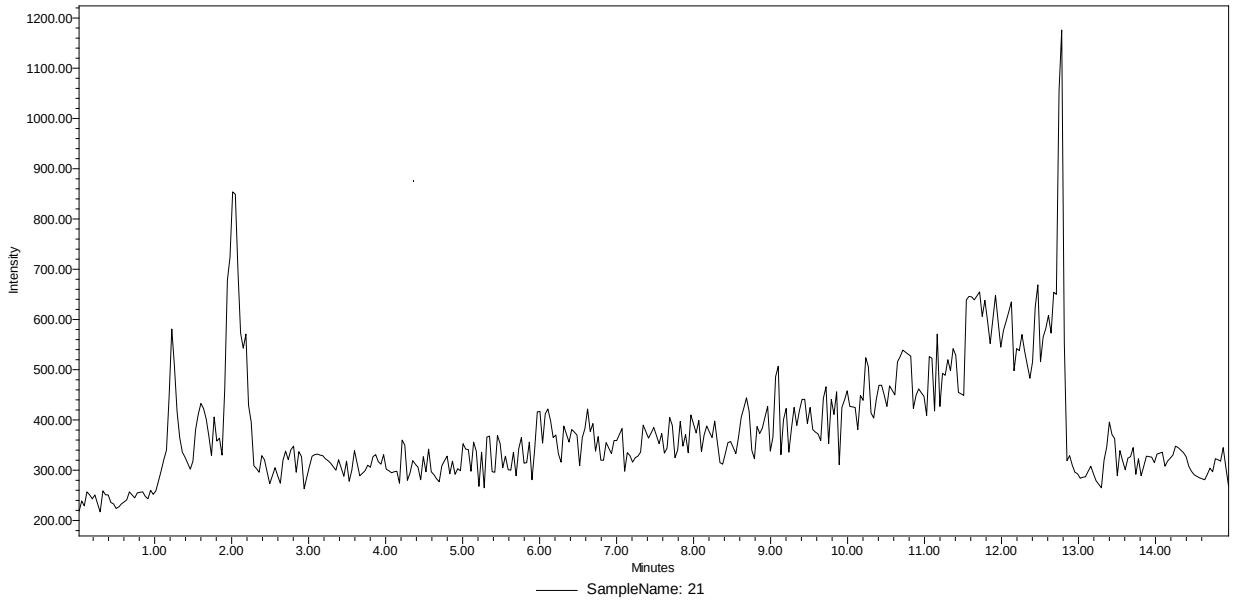

Fig. 29ddddd Photolytic/Alkaline product chromatograph for FUPA/sample 21.

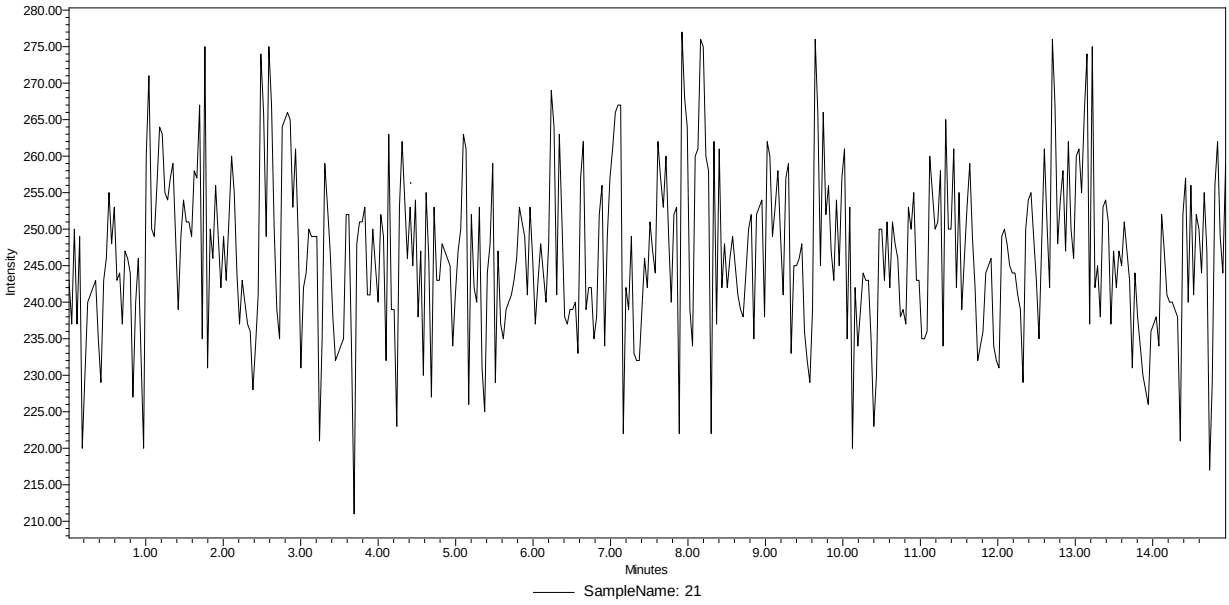

Fig. 29eeeee Photolytic/Alkaline product chromatograph for 5-FU/sample 21.

VITA

Eddie LaReece Pittman was born in Tarboro, North Carolina which is where he grew up and graduated from Tarboro High School. He then entered the U.S. Army Reserves and received several athletic physical testing honors. Soon thereafter he commenced his college career at the North Carolina State University and was recognized as an Honors student after his first year. He then transferred his studies down to Florida and eventually earned his Bachelor of Science in chemistry from the University of South Florida in Tampa. During that time, he worked at an environmental analytical lab called GTEL and it was there where he developed strong interests in environmental related issues. Years later, he pursued and completed his Master of Science degree in chemistry at the University of North Carolina Wilmington under the guidance of Dr. Ned Martin. This achievement helped him get several PhD program offers from which he chose the University of Missouri to pursue under guidance of Dr. Renee JiJi with intent on having his research agenda correlated with environmental issues. Dr. Chu Lin played a significant role in this correlation process. Finally, with the completion of the PhD agenda here at Mizzou, he anticipates on publishing at least 6 research articles and is on track in eventually purchasing full ownership of his own environmental

analytical environmental lab in Canada in which both may be seen as rare accomplishments.