Progress Report – September 2002

EPA Grant Number: R82941091-010
Title: Biomarkers for Air Pollutants: Development of Hemoglobin Adduct Methodology for Assessment of Exposure to Butadienes and Polycyclic Aromatic Hydrocarbons, SEER project of SIP: Experimental Program To Stimulate Competitive Research (EPSCoR) From The Commonwealth Of Kentucky
Investigators: Harrell E. Hurst and Steven R. Myers
Institution: University of Louisville
EPA Project Officer: Darrell Winner
Project Period: October 1, 2001 - September 30, 2003
Project Amount: $219,287 from EPA, $532,202 Total including cost sharing
Research Category: EPSCoR

Description:
We proposed to develop methodology that will measure systemic exposures to chloroprene (2-Cl-1,3-butadiene CAS-126-99-8) and selected polycyclic aromatic hydrocarbons (PAH: fluoranthene, CAS# 205-44-0; benzo(a)pyrene, CAS# 50-32-8). The methods will involve detection and measurement of covalent adducts to the abundant blood protein hemoglobin as biomarkers of exposure. The postulated adducts are presumed to be formed by electrophilic epoxide metabolites of these compounds. Analysis involves synthesis of derivatives through Edman cleavage of globin *N*-terminal valine adducts. Quantification will be accomplished by selected ion monitoring gas chromatography/mass spectrometry (SIM-GC/MS) using stable isotope internal standards. Adducts from PAH exposure will be analyzed after acid hydrolysis of labile PAH-Hb carboxylate adducts. Other potential sites, such as cysteine sulfhydryl and histidine imidazole groups, may be examined following proteolytic cleavage of Hb with trypsin. Analysis of cleaved peptides containing adducts will be accomplished by electrospray ionization (ESI) LC/MS and matrix assisted laser desorption ionization time of flight (MALDI-TOF) MS. Tandem MS studies using triple quadrupole or ion trap instruments will monitor selected ion decomposition pathways to provide additional analytical specificity and sensitivity.

Progress Summary: During the initial year of this grant, the contract was initiated, accounts were established, and initial experimental work was begun toward the proposed work. Equipment was ordered and received. Initial contact was made with industry scientists experienced in handling chloroprene, and safety protocols were developed in conjunction with the University of Louisville Department of Environmental Health and Safety for purification and handling of chloroprene and related compounds. A synthetic standard of the epoxide of chloroprene, 2-(1-chlorovinyl)oxirane or CVO, was provided by the International Institute of Synthetic Rubber Producers' Chloroprene Scientific Oversight Committee through Matthew Himmelstein of DuPont Haskell Laboratory. A postdoctoral fellow with expertise in mass spectrometry has been recruited from the University of New Orleans, and we are awaiting his arrival pending a visa extension from the Immigration and Naturalization Service. Initial in vitro experiments were begun. At this time positive chemical ionization GC/MS appears to offer best signal to noise for detection of hemoglobin adducts from the activated metabolite of chloroprene.

Preliminary Results: CVO, an electrophilic oxidative metabolite of chloroprene, reacts covalently with physiologic nucleophiles to produce adducts with various macromolecules. Presumably, this is a chemical mechanism involved in the toxicity of chloroprene, as this compound is analogous to 1,3-butadiene (BD) which exerts its carcinogenic effects through similar epoxide metabolites that form covalent adducts with DNA. Structures of chloroprene, butadiene, and CVO are shown in Figure 1.

The covalent reaction of CVO involves opening of an epoxide bond, which produces two potential isomeric adducts, as shown in Figure 2 where the macromolecule is a protein such as hemoglobin (Hb) with an N-terminal valine (Val).

Initial in vitro experiments have been conducted toward development of methods to detect these adducts. These involved reactions of CVO with racemic unlabeled valine, d_8-valine, valine-tyrosine-valine (Val-Tyr-Val or VYV) which is a tripeptide model for globin, and with hemoglobin solution from mouse red cells. Initial results did not easily reveal the presumed adduct derivatives from the opened epoxide using the Edman method followed by trimethylsilylation of the resultant hydroxyl group. Subsequent initial studies were conducted to discover reaction products using the model compounds valine, d_8-valine, and VYV. The methodology is detailed below, with experimental details in smaller font to distinguish methods from results and discussion. Following are figures that present structures and representative chromatographic and mass spectrometric data.

Preparation of Val-CVO, d_8-Val- CVO, and VYV-CVO adducts:
Model adducts were prepared and purified for potential use as potential standards for the Edman degradation and analysis by GC/MS.

Preparation of Val-CVO and d_8-Val-CVO: Dissolve valine (d, l racemic mixture from Sigma Chemical, 10mM, 1.17 mg/ml) in 0.2M phosphate buffer (pH 7.4). Mix 1.3 ml solution with 3.0 µl CVO (final concentration 25mM) in a 1.8 ml vial, incubate at 37°C.

Dissolve d_8-Val (10mM, 1.25 mg/ml) in 0.2M phosphate buffer (pH 7.4). Mix 1.22 ml solution with 2.8 µl CVO (final concentration 25mM) in a 1.8 ml vial, incubate at 37°C. Stop incubation after 50 hours of incubation. Keep the products in refrigerator before purification.

Purification of Val-CVO and d_8-Val-CVO: Wash column (Strata X column (30mg/1ml) from Phenomenex) with 1 ml 50% MeOH and 1 ml water, load 0.4 ml Val/CVO or d_8-Val/CVO incubation product, wash with 0.5 ml water, elute with 1.2 ml water. Repeat the process till the entire incubation product was processed. SPE fractions were analyzed by electrospray ionization ion trap mass spectrometry Finnigan LCQ Duo) after 1/10 dilution with 50% acetonitrile (ACN) containing 0.5% formic acid. Val-CVO and d_8-Val-CVO were found in 1.2 ml fractions. Small impurity peaks (m/z 118 and 176 in Val-CVO and m/z 126 and 184 in d_8-Val-CVO) were also detected in 1.2 ml fractions (Fig. 7 and 8). Val-CVO and d_8-Val-CVO are not well retained on Strata X column; a better purification method needs to be developed. Concentrate 1.2 ml fractions with Val-CVO and d_8-Val-CVO by Speedvac. Combine Val-CVO or d_8-Val-CVO fractions together and dry the samples after combination. This yielded some white residue in the vials but the amount was unknown.

Val-CVO and d_8-Val-CVO stock solutions: Dissolve residue of Val-CVO or d_8-Val-CVO fractions in 0.2 ml water.

Analysis of Val-CVO and d_8-Val-CVO by GC/MS:

Modified Edman degradation and trimethylsilylation were used to derivatize the adducted N-terminal amino acid (valine). Samples were analyzed by capillary gas chromatography/mass spectrometry using the HP5890/HP5973 GC/MS system.

Modified Edman degradation with trimethylsilylation for GC/MS: Mix 10 µl Val-CVO or d_8-Val-CVO stock solutions, or 10 µl VYV-CVO stock solution, with 0.5 ml 1-propanol, 1.0 ml 0.5M $KHCO_3$, and 7 µl neat pentafluorophenylisothiocyanate (PFPITC, Fluka Chemical Co.), set samples on rotator overnight, incubate samples at 50°C for 2 hours, extract twice with 2 ml hexane, dry hexane phase under nitrogen, dissolve residue in 1 ml toluene, wash twice with 2 ml 0.1N Na_2CO_3, dry toluene phase under nitrogen, incubate with 25 µl ACN and 25 µl *N,O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) at 60°C for 1 hour, and inject 1 µl into GC/MS (Hewlett Packard HP5890/HP5973 with 70eV electron impact ionization). Compound separation was achieved using a capillary column (DB-5MS, 15 m × 0.25 mm I.D. × 0.25 µm film thickness) from J&W Scientific (Folsom, CA). The GC injection port and GC/MS interface temperature were set to 280°C, with constant helium carrier gas pressure at 5 psig. Injections were made in splitless mode with the inlet port purged after 1 min following injection. The GC oven temperature was held initially at 100°C for 1 min, then increased at a rate of 10°C min^{-1} to 300°C, which was held for 5 min. Components eluting from GC were detected by mass spectrometry with electron ionization (70eV) in full scan mode.

Val-CVO derivatives, in the expected forms as 1-(3-chloro-2-trimethylsilyloxy-but-3-enyl)-5-isopropyl-3-(pentafluorophenyl)-2-thioxoimidazolidin-4-one or 1-[2-chloro-1-(trimethylsilyloxymethyl)-prop-2-enyl]-5-isopropyl-3-(pentafluorophenyl)-2-thioxoimidazolidin-4-one, were not detected following Edman degradation and trimethylsilylation. Such reactions would produce derivatives of nominal mass m/z 500. Instead many large unknown peaks were detected by GC/MS as evident in Figure 3 (Val-CVO), and in Figure 4 (d_8-Val-CVO). These peaks are likely from reagents because identical chromatograms were obtained from blank analyses containing reagent only.

Valine and d_8-valine were used as models for amino acid adducts, and these provided insights into the chemistry of the reactions involved in these analyses. Use of both isotopic valine structures allowed examination of the data for pairs of ions to distinguish spectra of interest from other spectra derived from background and other components of the injected mixtures. Likewise the naturally occurring isotopes of chlorine, ^{35}Cl and ^{37}Cl in a 3:1 ratio, provide indicators of the presence of ions containing adducts from CVO. Detailed inspection of mass spectra from full scan analyses indicated a single chromatographic peak with these characteristics. That corresponded to an ion resulting from loss of HOTMS from the expected derivative (see structures, Figure 5). It is not clear whether the loss of HOTMS occurred during derivatization, in the GC/MS injection port, or during ionization, although subsequent chemical ionization studies make the latter least likely. This product was detected at retention time (Rt) 12.66 min (noted by arrows in Figure 3 and with d_8-valine in Figure 4, and was not detected in blank sample analyses. The intensity of the peak was very low. Based on its mass (m/z 410 for Val-CVO derivative, Figure 3; and m/z 417 for d_8-Val-CVO derivative) this adduct derivative is postulated to be homologous with addition of chlorobutadiene to valine, as shown in Figure 5. From the mass spectra in Figure 4, it is evident that hydrogen exchange occurs with replacement of one deuterium atom during the Edman reaction, as the molecular ion of the detected d_8-valine derivative was m/z 417, a net mass increase of 7 over m/z 410 for the unlabeled valine analog in which hydrogen exchange is not evident. The apparent

yields of the reactions are low in the current reaction data. It is not clear which of the two structures corresponds to the detected peaks, or whether separate peaks exist for the isomeric structures.

Analysis of VYV-CVO by Ion Trap Mass Spectrometry:
To provide standard reference material for the Edman degradation and to address uncertainties inherent in these results with valine, studies were conducted with the model tripeptide VYV. This material reacted with CVO in solution, as shown in Figure 6. About 50% and 70% of VYV was converted to VYV-CVO after 24 and 50 hours of incubation, respectively, as estimated from ESI/MS peak intensities of VYV and VYV-CVO following HPLC analysis.

Preparation of VYV-CVO: Dissolve VYV (Sigma Chemical Co., 10mM, 3.80 mg/ml) in 0.2M phosphate buffer (pH 7.4). Mix 1.1 ml VYV solution with 2.5 µl CVO (final concentration 25mM) in a 1.8 ml vial, incubate at 37°C. Check VYV-CVO appearance in the reaction mixture at various times during incubation.

The products were purified by solid phase extraction (SPE) and examined by electrospray (ESI) ion trap mass spectrometry (ITMS) following infusion into the instrument (Figure 7). Unmodified VYV and VYV-$(CVO)_2$ were not detected from the sample. However a small impurity peak containing chlorine was detected with nominal mass at m/z 367. This possibly is an adduct of an impurity, m/z 263, in VYV.

Purification of VYV-CVO by SPE: Wash column (Strata X column (30mg/1ml) with 1 ml 50% ACN with 0.5% formic acid and 1 ml 0.5% formic acid, load 0.25 to 0.3 ml unpurified VYV-CVO, wash with 0.5% formic acid (1.5, 1.5, and 1.5 ml), elute with 5% ACN (1.3, 1.5, and 1.5ml), 7.5% ACN (1.3, 1.5ml), 10% ACN (1.3, 1.5ml), and 50% ACN (1.5ml). (All ACN solvents have 0.5% formic acid). Repeat till all VYV/CVO reaction mixture is processed. Check fractions by ESI/MS (Quattro LC) after 1/50 dilution with 50% ACN containing 0.5% formic acid. VYV was found in 0.5% formic acid fractions and 1st 5% ACN fraction, VYV-CVO was found in 5% ACN fractions and small amount in 1st 7.5% ACN fraction, VYV-$(CVO)_2$ was found in 7.5% ACN and 10% ACN fractions. Second and third 5% ACN fractions and 1st 7.5% ACN fraction were concentrated by Speedvac, combined together, and dried by Speedvac.

More than 1 mg purified VYV-CVO was obtained.

Check of purity of VYV-CVO stock solution by ESI/MS: Dissolve 0.55 mg purified VYV-CVO in 1.10 ml 0.25% formic acid (0.5 mg/ml, 1.035 nmol/µl) to make stock solution. Check purity of VYV-CVO stock solution by ESI/MS after 1/100 dilution with 50% ACN containing 0.5% formic acid and infusion into the ITMS.

To further examine the products of the reaction and to examine the relative amounts of the isomers resulting from opening of either of the epoxide bonds, the purified material was examined by HPLC with on-line ESI ITMS.

Confirmation of VYV-CVO: VYV-CVO stock solution was diluted 1/10 with 0.5% formic acid and 10 µl was injected to a Spectra System 4000 HPLC with Spectra System AS3000 auto sampler. The separation column was Waters Spherisorb 5 µm ODS2 (4.6×250mm). A gradient started from 0% Solvent B (95% ACN with 0.05% TFA) and increased to 50% Solvent B in 15 minutes and then Solvent B was maintained at 50% for 10 minutes (Solvent A was 5% ACN with 0.05% TFA). The flow rate was set at 0.5 ml/min. Under these conditions, two peaks at Rt 20.4 and 20.7 min were detected (Fig. 2).

Two eluted modified peptides were detected by ion trap MS (Finnigan LCQ Duo) in ESI mode. The M+H ions of both of the peaks were m/z 484, as seen in Figure 8. These peaks, almost equal in abundance, are consistent with proposed isomeric adduct structures shown in Figure 6. Little difference was evident in the mass spectra of these isomers. Identities of the isomers' adduct positions could not be assigned from the MS data.

To confirm VYV-CVO structure, MS/MS spectra of m/z 484 were acquired with ITMS collision energy set at 40%, and are displayed in Figure 9. The chlorine-containing fragment ion at m/z 367, which represents loss of $C_5H_{11}NO_2$ (derived from carboxy-terminal valine) from the protonated molecular ion (m/z 484), confirmed the modification of the amino group by CVO. Changes in collision energy to 30 and 50% did not cause significant difference in MS/MS spectra.

Detection of Val-CVO from VYV-CVO by GC/MS:

The modified Edman degradation was used to cleave and derivatize the adducted N-terminal valine in this tripeptide. Derivatized samples were analyzed using the HP5890/HP5973 GC/MS system.

Modified Edman degradation with trimethylsilylation for GC/MS: Process 10 µl VYV-CVO stock solution as described for derivatization and GC/MS analysis of Val-CVO above.

The intact Val-CVO derivative was not detected; rather many large unknown peaks were detected that likely originate from reagents as noted before. This early analysis will be reviewed in light of the discovery of formation of the chlorobutadiene derivative noted above with Edman degradation and trimethylsilylation.

Mouse Globin Modification by CVO:

An initial attempt was made to use the preliminary assay methods to detect N-terminal valine adducts following reaction of CVO with globin solution from mouse red blood cells, as this is the protein of interest as a biomarker of exposure to chloroprene. These studies were conducted in vitro using packed red blood cells from an animal supplier.

Mouse Hb solution: Transfer 1.2 ml of packed mouse (C57 from Harlan) red blood cells to 2 vials, centrifuge and remove plasma, wash twice with 2 ml BSA (1 ml each vial), lyse the red blood cells with 2 ml water, centrifuge, and collect Hb solution (about 2.5 ml).

Preparation of CVO modified globin: Incubate 1.0 ml mouse Hb (above) with 1.8 µl neat CVO (final concentration 20 mM) at 37ºC. Found precipitate was formed from mouse Hb after 24 hours incubation. Dilute Hb solution with 2 ml water, precipitate globin with 12 ml cold acidified acetone, wash precipitate twice with 12 ml acetone, and dry globin under nitrogen.

The prepared globin appeared normal, but the ESI spectrum from the globin was very noisy, possibly due to breakdown of globin during incubation with the high concentration of CVO. Future experiments with red cells will use headspace vapor phase exposure.

Detection of Val-CVO in CVO modified globin by GC/MS:

Preparations from modified mouse globin were analyzed by GC/MS.

Edman degradation of modified mouse globin: Dissolve 4.22 mg globin in 0.5 ml 1-propanol, 1.0 ml 0.5M $KHCO_3$, and 7 µl PFPITC, set samples on rotator overnight, incubate samples at 50ºC for 2 hours, extract twice with 2 ml hexane, dry hexane phase under nitrogen, dissolve residue in 1 ml toluene, wash twice with 2 ml 0.1N Na_2CO_3, dry toluene phase under nitrogen, incubate with 25 µl ACN and 25 µl BSTFA at 60ºC for 1 hour, and analyze the sample by GC/MS in SIM mode (m/z 368, 410, and 412 were monitored).

Val-CVO was detected from the sample of mouse globin exposed to CVO, but many impurity peaks were evident and the signal to noise for the chlorobutadiene analog (m/z 410) was not good. It was decided to attempt development of a cleanup method for better result.

Trial cleanup procedure with C18 disk: Dissolve 4.93 mg modified globin in 0.5 ml 1-propanol, 1.0 ml 0.5M $KHCO_3$, and 7 µl PFPITC, set samples on rotator overnight, incubate samples at 50ºC for 2 hours, extract twice with 2 ml hexane, dry hexane phase under nitrogen, add 1 ml water to one of the globin samples, vortex and centrifuge (there was some insoluble material). Load the aqueous phase on a SPEC PLUS C18 disk (washed with 1 ml MeOH and 1 ml water), wash with 2 ml water, elute with 1 ml MeOH, dry elute under nitrogen, add 25 µl ACN and 25 ul BSTFA, incubate at 50ºC for 1 hour, and inject 1 µl to GC/MS.

Ion chromatograms were cleaner than samples processed without solid phase extraction, but most of the derivative appeared to be lost in sample processing.

Detection of Val-CVO in exposed mouse globin using isobutane positive chemical ionization (PCI) and selected ion monitoring:

Under PCI conditions (with isobutane gas flow set at 20), the MS spectrum of the chlorobutadiene derivative from Val-CVO was dominated by the M+H ion (m/z 411) from the resultant chlorobutadiene adduct, as illustrated in Figure 10. This procedure was applied to the sample of modified (CVO exposed) mouse globin.

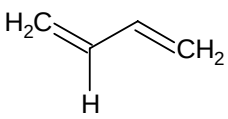
Selected ion monitoring was examined as a potential means to enhance sensitivity in analysis of valine-CVO adducts. This included electron impact (EI) and positive chemical ionization (PCI) techniques, as shown in Figure 11. Here molecular ions corresponding to the ^{35}Cl and ^{37}C isotopes are designated with arrows at Rt of about 12.6 min. During these initial studies, the PCI mode offered the most promising signal to noise of these techniques.

Under PCI SIM GC/MS conditions much better intensity of the peaks and much lower baseline were obtained, as illustrated in Figure 11 in comparison with the EI mode of ionization. The PCI method presently offers the best signal to noise of those examined. However, none of the methods examined can yet be considered satisfactory as the high sensitivity analysis methodology needed for use with this biomarker of trace exposure to chloroprene.

Future Activities: Efforts will continue toward refinement of GC/MS and other MS techniques to detect chemical modification of mouse globin following exposure to CVO. The primary objective will be to find a technique that offers greatest promise for detection of trace amounts of the globin adducts that result from exposure to CVO. Additional studies will focus on globin exposed to CVO in headspace to determine if the conditions of exposure adversely influence detection of adducts. Experiments detailed here will be repeated in hope of finding an ion mass offering additional sensitivity. Experiments next planned will examine the abundance of the corresponding ions following negative chemical ionization. Other studies will examine in greater detail use of HPLC MS/MS and product ions (illustrated in Figure 9) in hope of finding a more sensitive means of detecting the globin adducts. These will combine the use of the Edman degradation with MS/MS product ion detection (so-called multiple reaction monitoring). Following resolution of the problem of detection and additional refinement of an assay for these adducts, mice will be treated with chloroprene to examine suitability of the mass spectrometric assay(s) as a biomarker of chloroprene exposure.

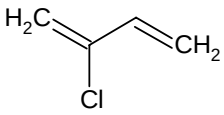
Publications and Presentations: No publication has occurred as yet from these efforts.

Supplemental Keywords: exposure, ambient air, carcinogen, chloroprene, epoxides, hemoglobin adducts, VOC, analytical, monitoring, mammalian, metabolism, KY, Kentucky



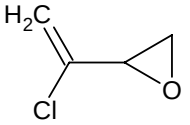
buta-1,3-diene

BD



2-chlorobuta-1,3-diene

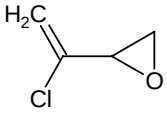
chloroprene



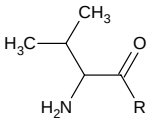
2-(1-chlorovinyl)oxirane

CVO

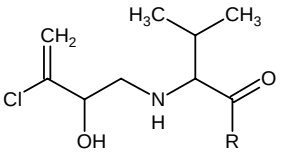
Figure 1.



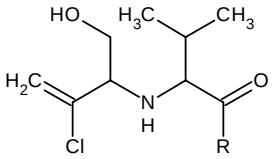
2-(1-chlorovinyl)oxirane



N-terminal valine in Hb



3-(3-chloro-2-hydroxybut-3-enyl) adduct



3-(2-chloro-1-(hydroxymethyl)prop-2-enyl) adduct

Figure 2.

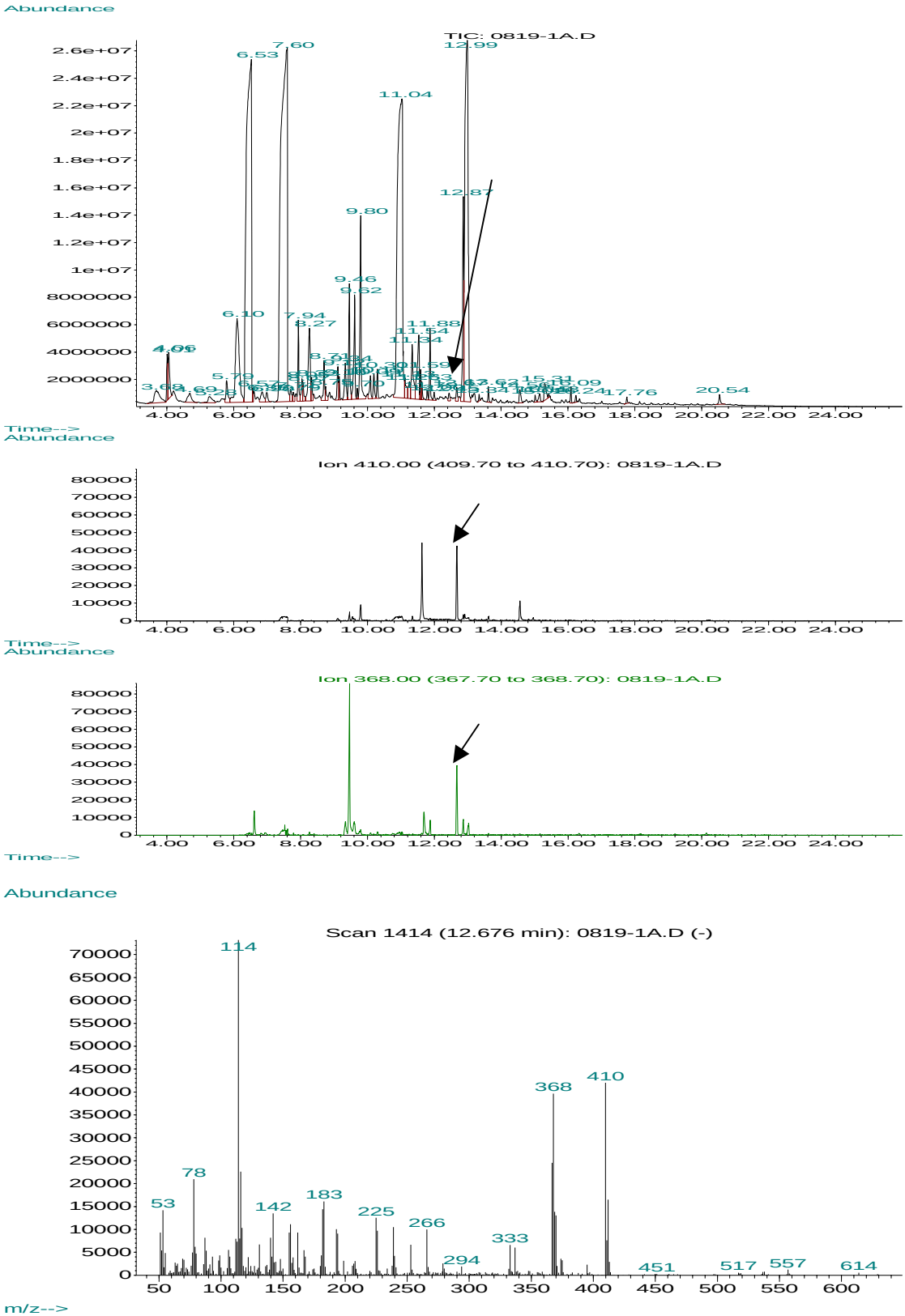

Abundance
TIC: 0819-1A.D
Time-->
Abundance
Ion 410.00 (409.70 to 410.70): 0819-1A.D
Time-->
Abundance
Ion 368.00 (367.70 to 368.70): 0819-1A.D
Time-->
Abundance
Scan 1414 (12.676 min): 0819-1A.D (-)
m/z-->

Figure 3.

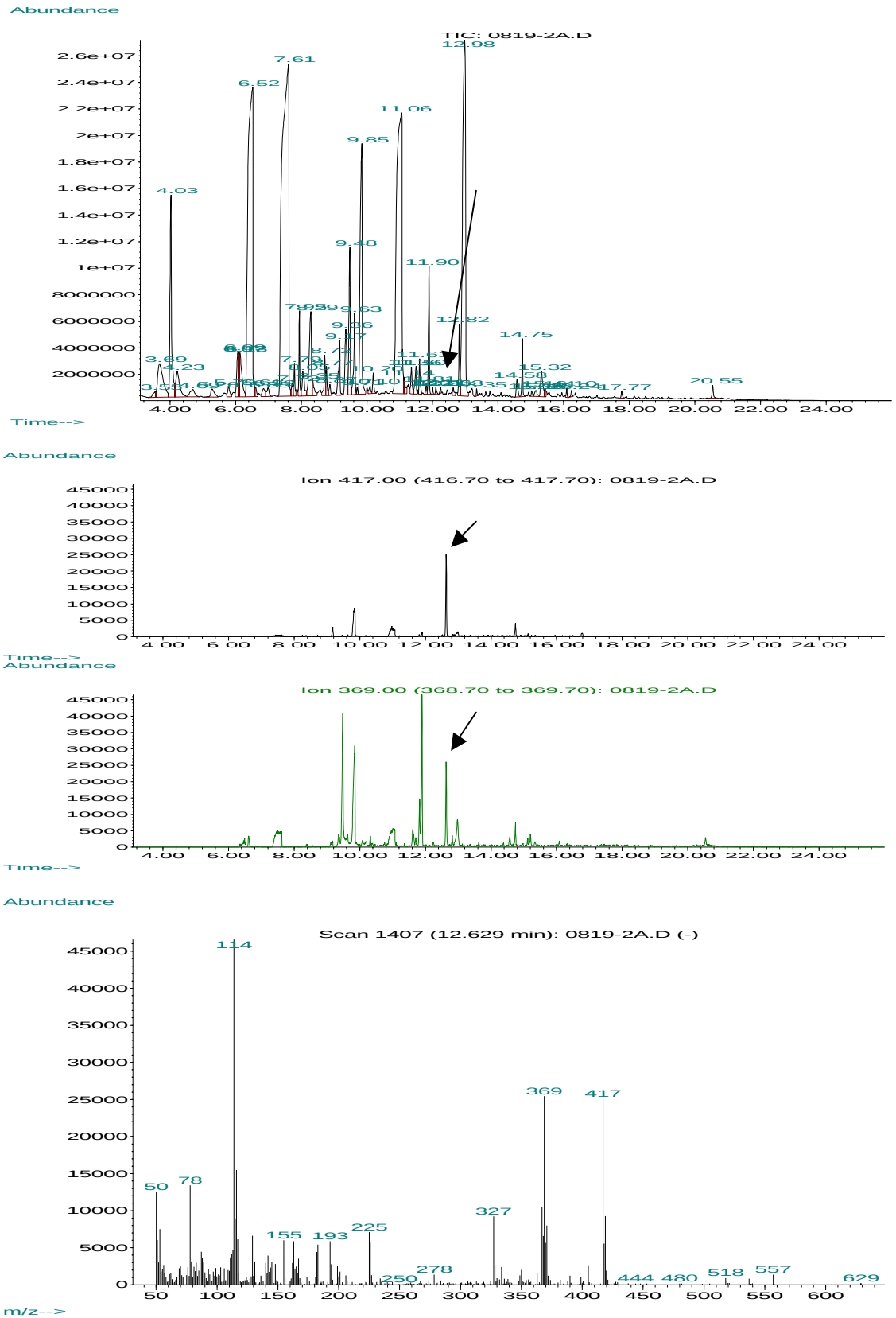

Abundance
TIC: 0819-2A.D
12.98
7.61
6.52
11.06
9.85
4.03
9.48
11.90
9.63
12.82
14.75
15.32
20.55
Time-->
Abundance
Ion 417.00 (416.70 to 417.70): 0819-2A.D
Time-->
Abundance
Ion 369.00 (368.70 to 369.70): 0819-2A.D
Time-->
Abundance
Scan 1407 (12.629 min): 0819-2A.D (-)
114
369
417
50
78
327
155
193
225
278
444
480
518
557
629
m/z-->

Figure 4.

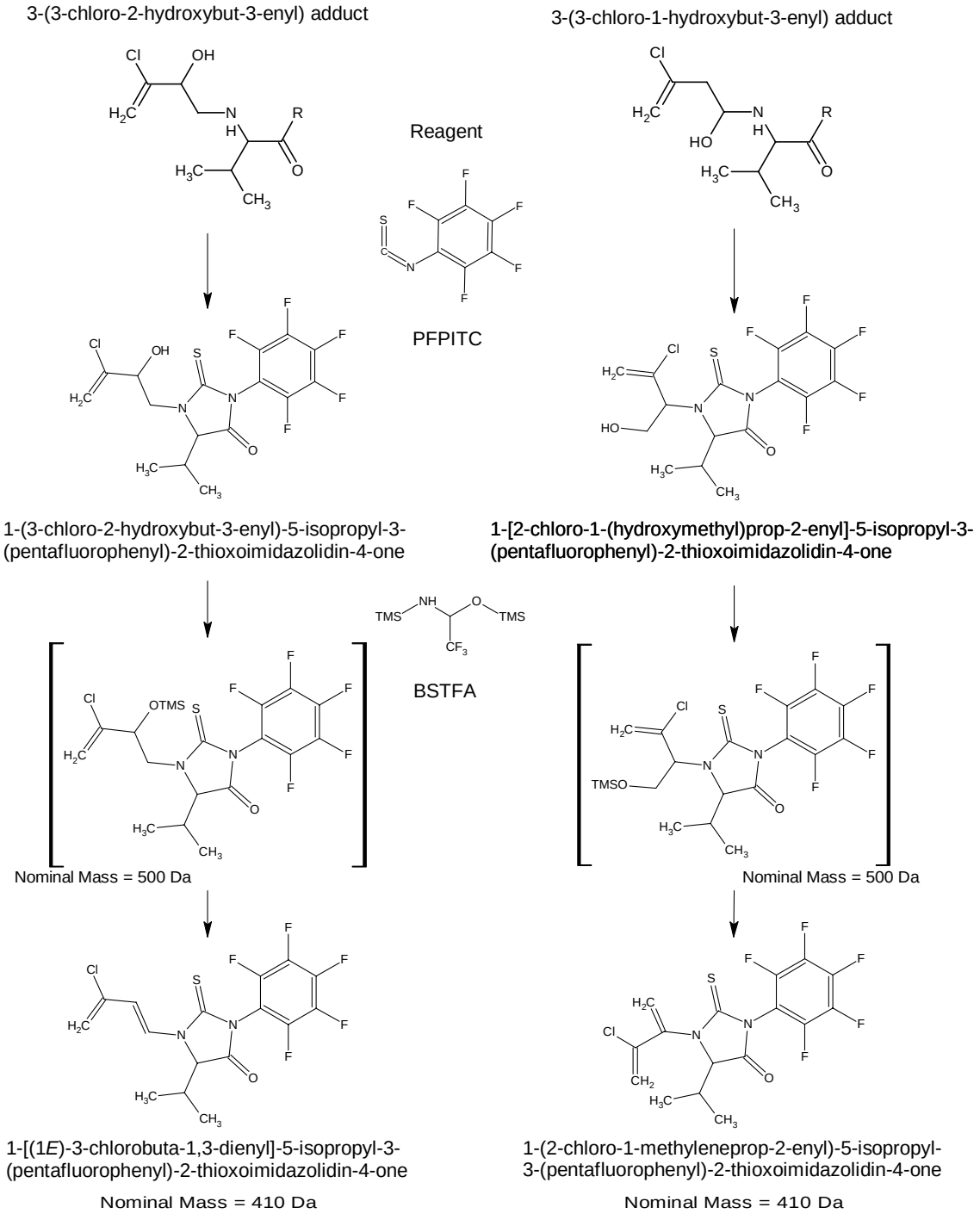
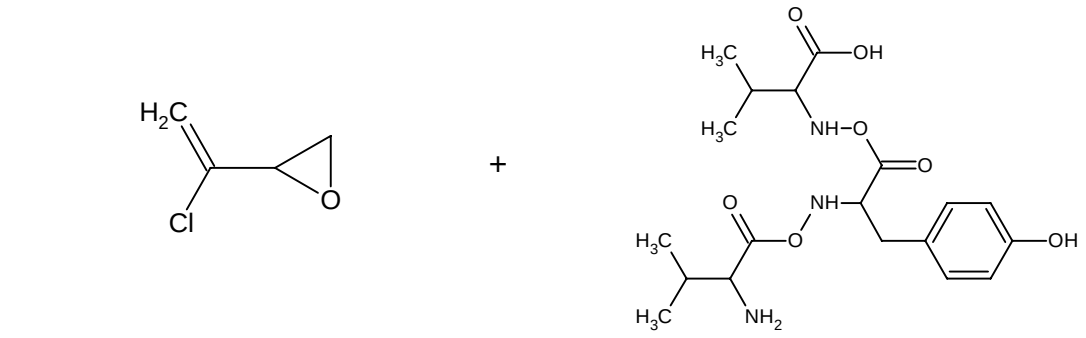
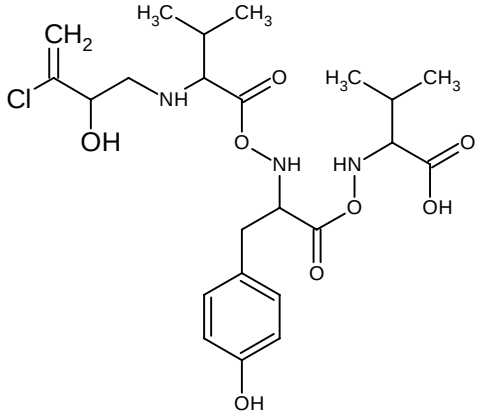

3-(3-chloro-2-hydroxybut-3-enyl) adduct
3-(3-chloro-1-hydroxybut-3-enyl) adduct
Reagent
PFPITC
1-(3-chloro-2-hydroxybut-3-enyl)-5-isopropyl-3-(pentafluorophenyl)-2-thioxoimidazolidin-4-one
1-[2-chloro-1-(hydroxymethyl)prop-2-enyl]-5-isopropyl-3-(pentafluorophenyl)-2-thioxoimidazolidin-4-one
BSTFA
Nominal Mass = 500 Da
Nominal Mass = 500 Da
1-[(1E)-3-chlorobuta-1,3-dienyl]-5-isopropyl-3-(pentafluorophenyl)-2-thioxoimidazolidin-4-one
Nominal Mass = 410 Da
1-(2-chloro-1-methyleneprop-2-enyl)-5-isopropyl-3-(pentafluorophenyl)-2-thioxoimidazolidin-4-one
Nominal Mass = 410 Da

Figure 5.

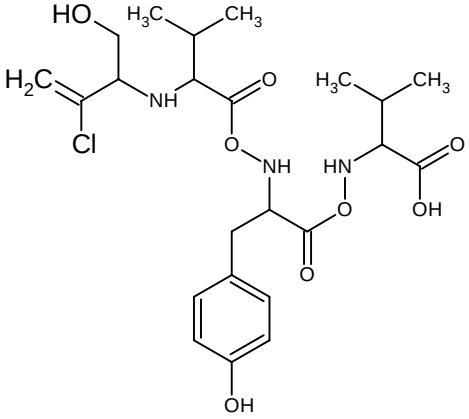


2-(1-chlorovinyl)oxirane <**CVO**>

valine-tyrosine-valine <**VYV**>



N-(3-chloro-2-hydroxybut-3-enyl)-valine-tyrosine-valine <**C2HB-VYV**>



N-(2-chloro-1-(hydroxymethyl)prop-2-enyl)-valine-tyrosine-valine <**C1HB-VYV**>

Figure 6.

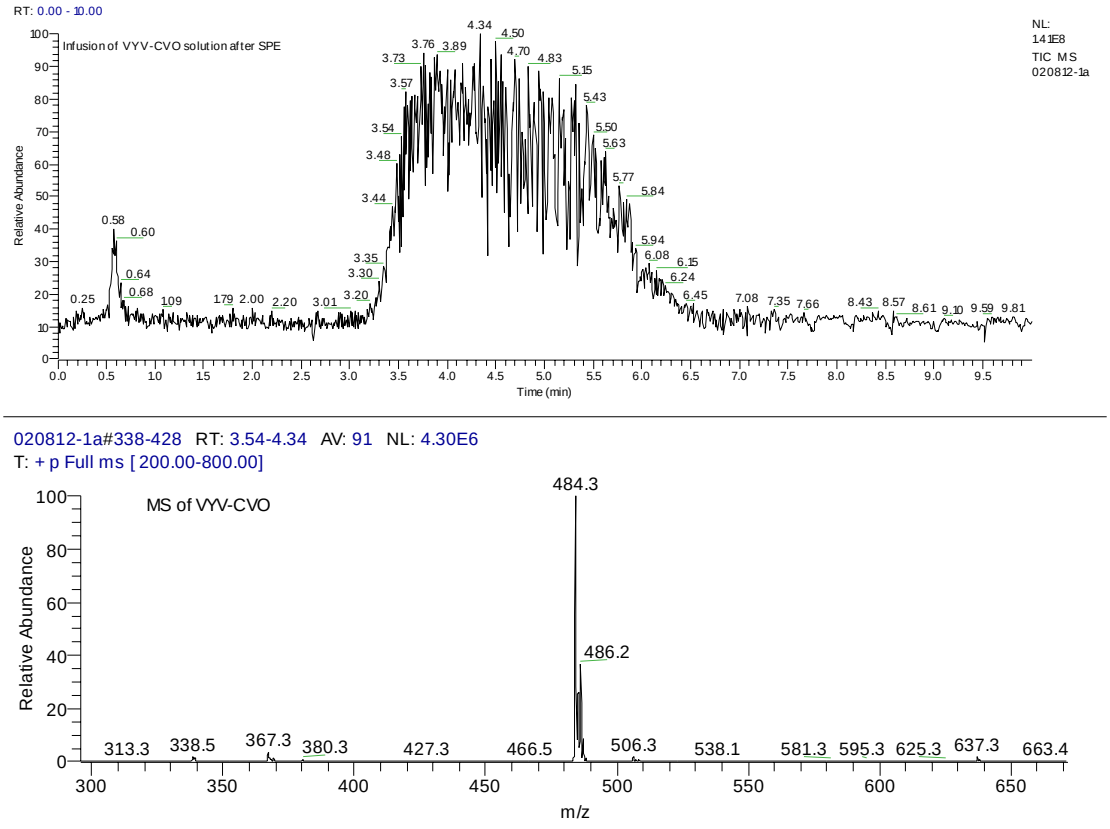

RT: 0.00 - 10.00
Infusion of VYV-CVO solution after SPE
NL:
141E8
TIC MS
020812-1a
Relative Abundance
Time (min)
020812-1a#338-428 RT: 3.54-4.34 AV: 91 NL: 4.30E6
T: + p Full ms [200.00-800.00]
MS of VYV-CVO
484.3
486.2
313.3
338.5
367.3
380.3
427.3
466.5
506.3
538.1
581.3
595.3
625.3
637.3
663.4
Relative Abundance
m/z

Figure 7.

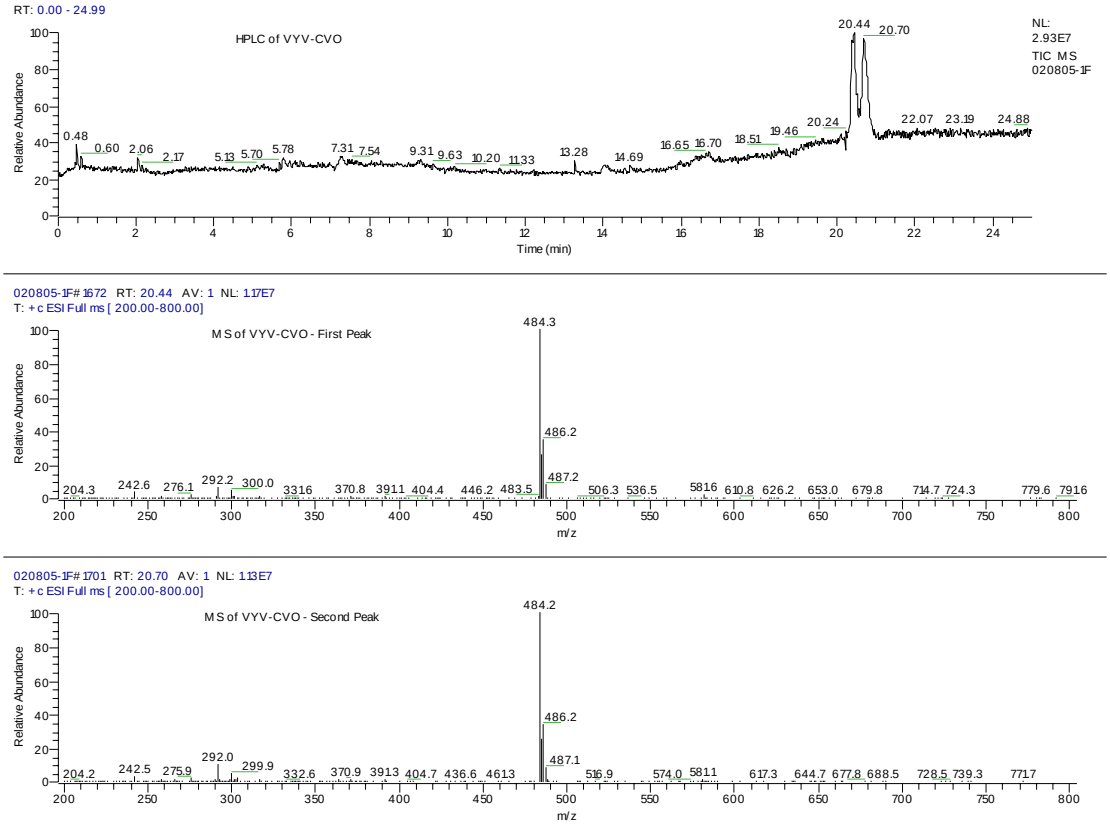

RT: 0.00 - 24.99
HPLC of VYV-CVO
NL:
2.93E7
TIC MS
020805-1F
Relative Abundance
Time (min)
020805-1F# 1672 RT: 20.44 AV: 1 NL: 1.17E7
T: + c ESI Full ms [200.00-800.00]
MS of VYV-CVO - First Peak
m/z
020805-1F# 1701 RT: 20.70 AV: 1 NL: 1.13E7
T: + c ESI Full ms [200.00-800.00]
MS of VYV-CVO - Second Peak
m/z

Figure 8.

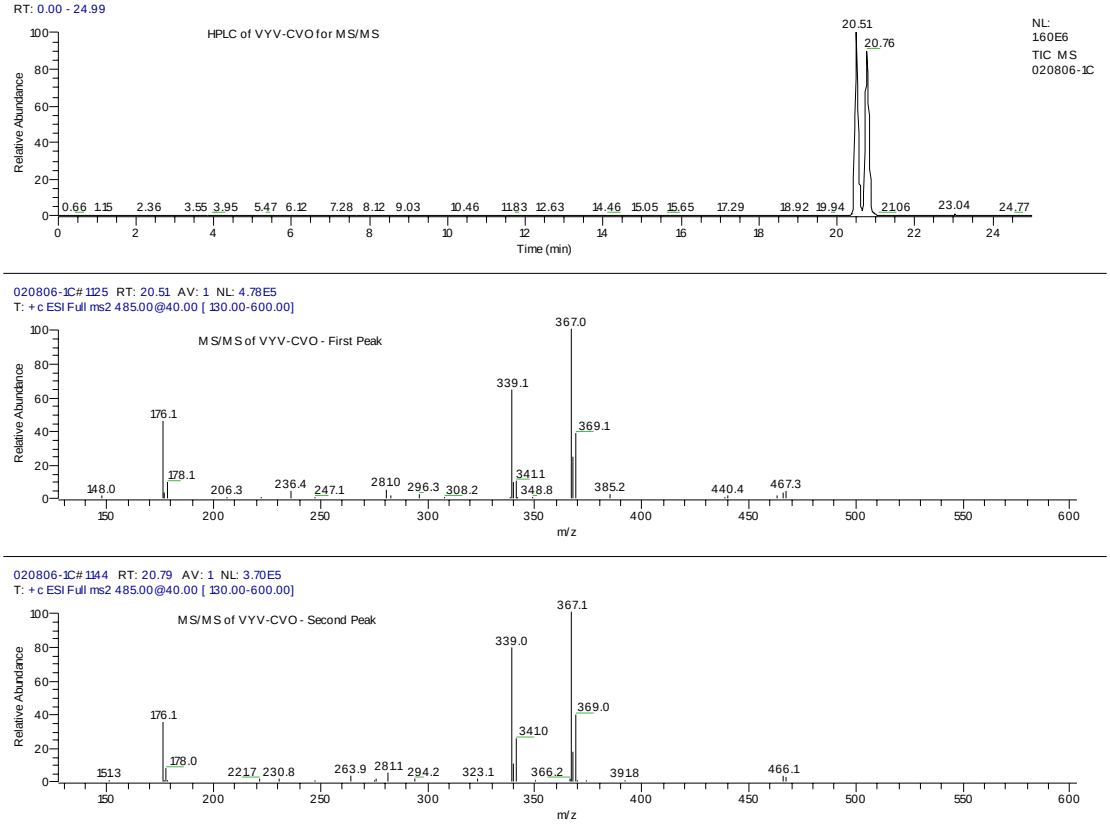

RT: 0.00 - 24.99
HPLC of VYV-CVO for MS/MS
Relative Abundance
Time (min)
NL:
160E6
TIC MS
020806-1C
20.51
20.76
020806-1C# 1125 RT: 20.51 AV: 1 NL: 4.78E5
T: + c ESI Full ms2 485.00@40.00 [130.00-600.00]
MS/MS of VYV-CVO - First Peak
367.0
339.1
176.1
369.1
178.1
341.1
m/z
020806-1C# 1144 RT: 20.79 AV: 1 NL: 3.70E5
T: + c ESI Full ms2 485.00@40.00 [130.00-600.00]
MS/MS of VYV-CVO - Second Peak
367.1
339.0
176.1
369.0
341.0
178.0
m/z

Figure 9.

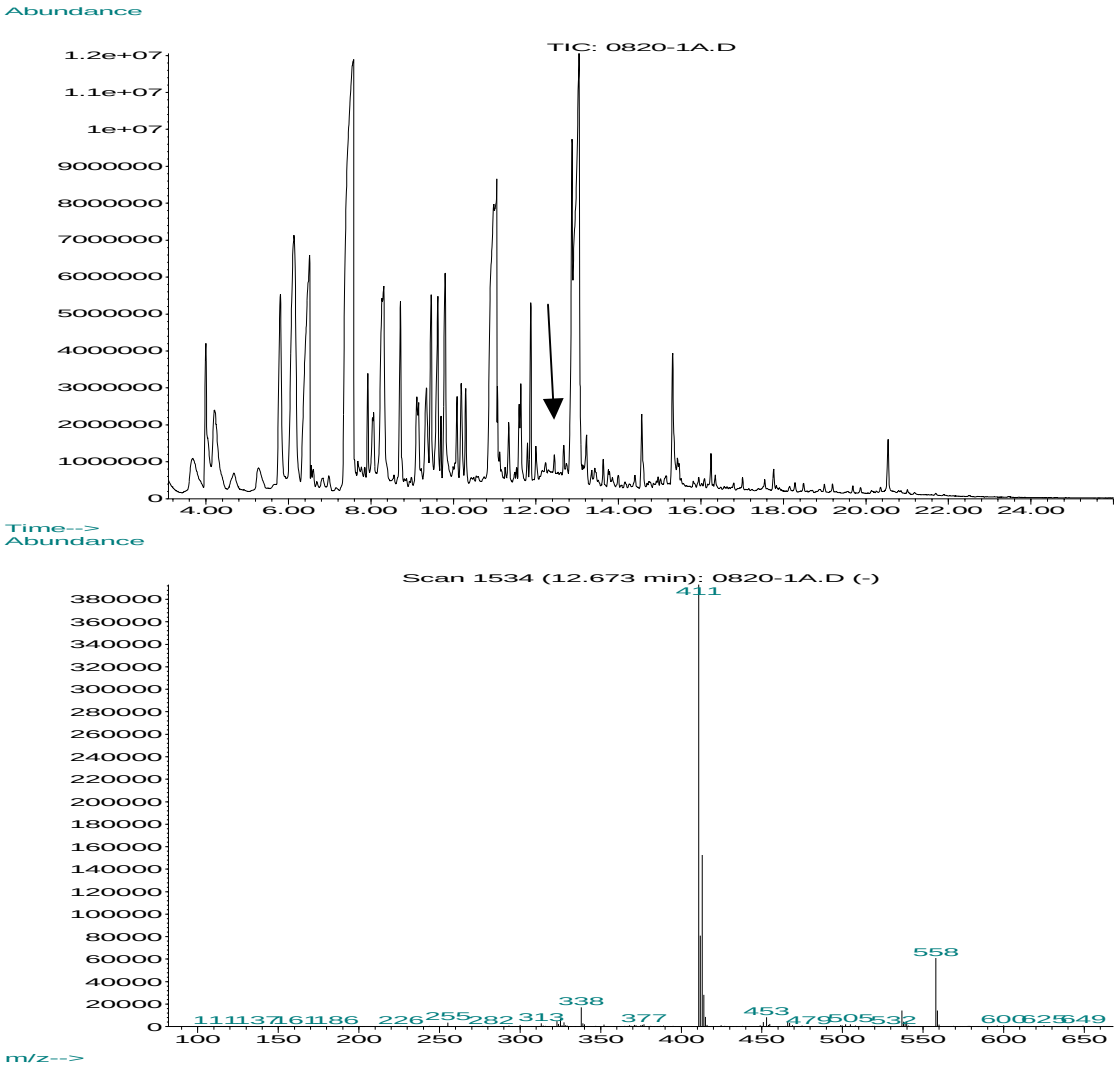
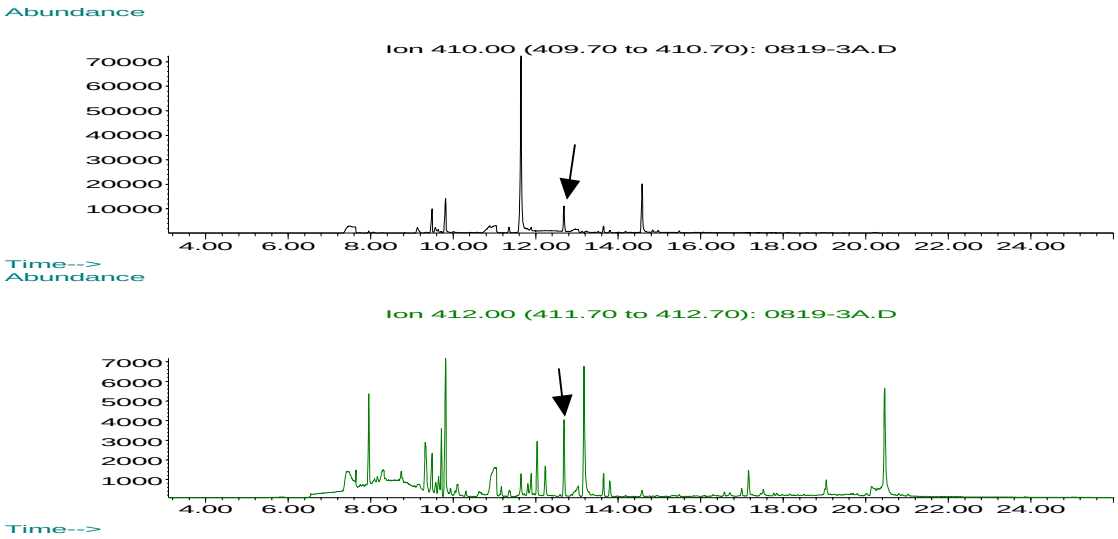

Abundance
TIC: 0820-1A.D
1.2e+07
1.1e+07
1e+07
9000000
8000000
7000000
6000000
5000000
4000000
3000000
2000000
1000000
0
4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00
Time-->
Abundance
Scan 1534 (12.673 min): 0820-1A.D (-)
411
558
338
453
380000
360000
340000
320000
300000
280000
260000
240000
220000
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
111 137 161 186 226 255 282 313 377 479 505 532 600 625 649
100 150 200 250 300 350 400 450 500 550 600 650
m/z-->

Figure 10.

EI Mode



PCI Mode

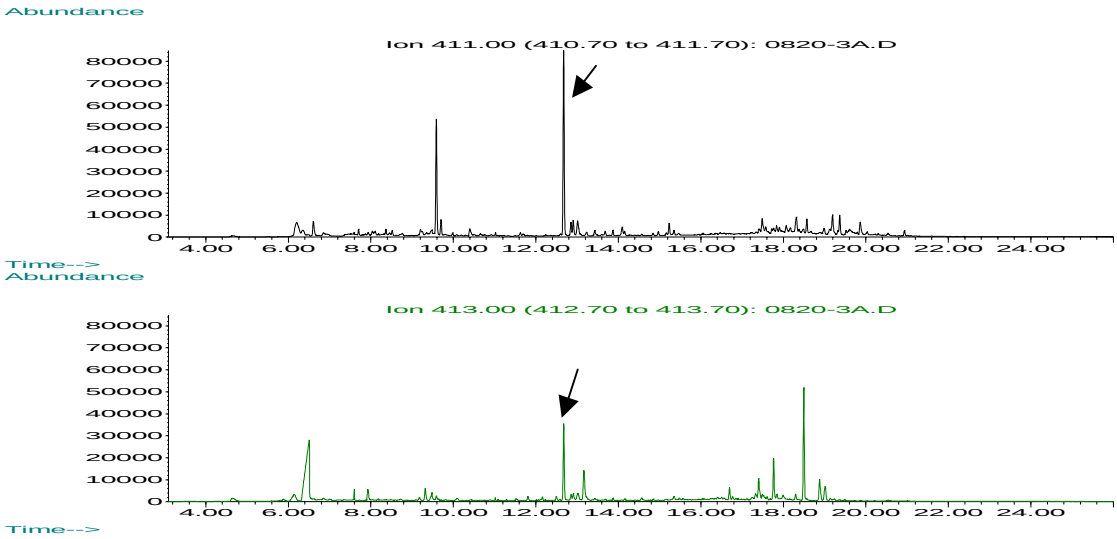


Figure 11.