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Report of the First Confidence Building Exercise For Biomedical Sample Analysis

Armando alcaraz, Hugh Gregg

February 26, 2010

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This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

Report of the First Confidence Building Exercise for Biomedical Sample Analysis

Hugh R. Gregg, Armando Alcaraz
Physical and Life Sciences, LLNL

Summary

We participated in the first exercise to build confidence in the analysis of biomedical samples for trace levels of CW agents and/or their degradation, reaction or metabolites. The report is attached.

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ORGANISATION FOR THE PROHIBITION OF CHEMICAL WEAPONS

Report of the First Confidence Building Exercise For Biomedical Sample Analysis

Laboratory code: 22

Total number of pages: 49[1]

[1] Total number of pages including cover page and all attachments

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SUMMARY: PARTICIPATING LABORATORY

1. Participating laboratory

Laboratory code:	22
Name of the laboratory/institute participating in the test:	Lawrence Livermore National Laboratory
Contact person:	Mr. Armando Alcaraz
Address:	PO Box 808, M/S L-091 7000 East Avenue Livermore, CA 94551
Telephone number:	925-423-6889
Fax number:	925-423-9014
Email address:	Alcaraz1@llnl.gov

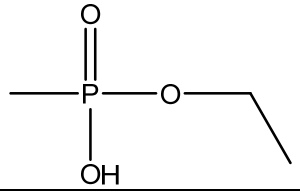
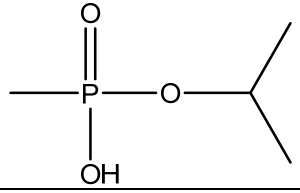
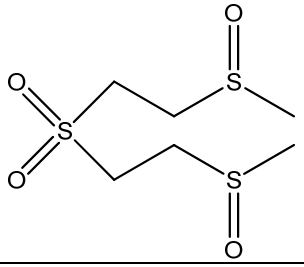
2. Analysts and authentication

	Name	Title	Date**	Signature**
1	Armando Alcaraz	Principal Investigator	2/23/10	Armando Alcaraz
2	Hugh Gregg	Co-PI, Senior Chemist	2/23/10	Hugh Gregg
3	Saphon Hok	Research Scientist	2/23/10	Saphon Hok
4	Heather Mulcahy	Scientist	2/23/10	Heather Mulcahy
5	Alex Vu	Scientist	2/23/10	Alex Vu
6				
7				
8				
9				
10				
11				

** Date and signature of the responsible analyst;

SUMMARY: CHEMICALS IDENTIFIED & METHODS

Laboratory code: 22

Sam. code	Chemical name	Chemical Abstract number	Chemical Structure	Molecular formula	Analytical Method	Derivative (if used)
2	Ethyl methylphosphonate	1382-53-7		$C_3H_9O_3P$	GC-MS (CI) LC-MS-MS	Pentafluoro-benzyl derivative
3 6	Isopropyl methylphosphonate	6838-93-3		$C_4H_{11}O_3P$	GC-MS (CI) LC-MS-MS	Pentafluoro-benzyl derivative
5 7	1,1'-Sulfonylbis[2-(methylsulfinyl)ethane]			$C_6H_{14}O_4S_3$	GC-GC-MS (EI) GC-MS (CI) LC-MS-MS	$TiCl_3$ reduction for GC methods

* Chemical number defined by the participating laboratory and used throughout the report for the reported chemical.

Note: There must be an unbroken chain of evidence linking each reported chemical to the original sample from which an aliquot was prepared and analyzed for the identification of this chemical.

OVERVIEW

LLNL received the following samples/reference standards for this exercise:

Tray/Box 1 contains: 7 vials with ~3.3mL each
Reference standard solutions provided to LLNL

Sample	Matrix	Spiked agent	Approximate concentration (µg/ml)
r1/22	Water	EMPA	10
r2/22	Water	IMPA	10
r3/22	Water	PMPA	10
r4/22	Water	MPA	10
r5/22	Water	TDG	10
r6/22	Water	SBMSE	10
r7/22	Water	MSMTESE	10

EMPA = O-ethyl methylphosphonic acid (*Ethyl methylphosphonate*)
IMPA = O-isopropyl methylphosphonic acid (*Isopropyl methylphosphonate*)
PMPA = O-pinacolyl methylphosphonic acid (*Pinacolyl methylphosphonate*)
MPA = methylphosphonic acid
TDG = thiodiglycol
SBMSE = 1,1'-Sulfonylbis[2-(methylsulfinyl)ethane]
MSMTESE = 1-Methylsulfinyl-2-[2-(methylthio)ethylsulfonyl]ethane

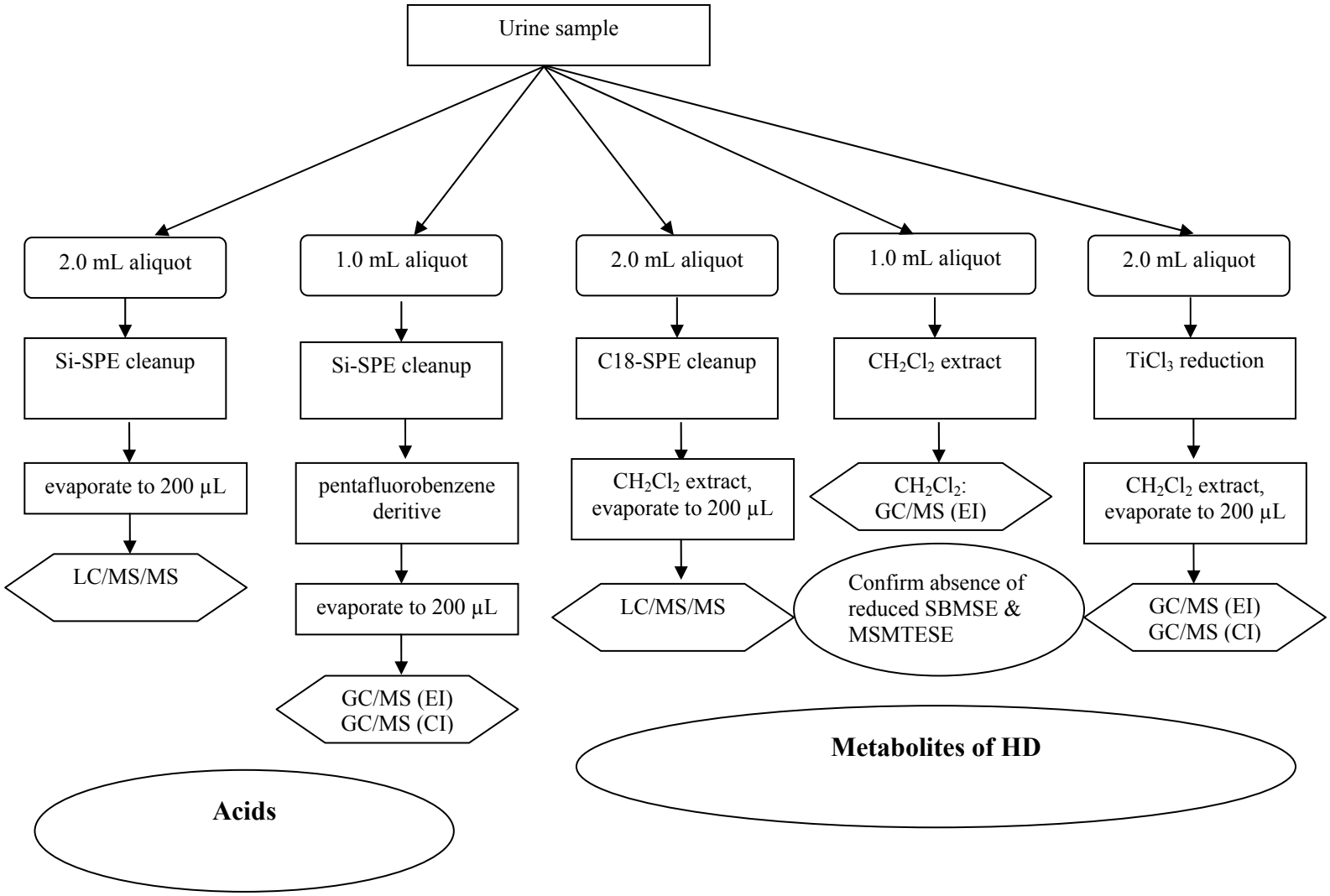
Tray/Box 2 contains: 24 vials with ~3.3mL each

Blanks	**Sample #2**	**Sample #3**	**Sample #4**	**Sample #5**	**Sample #6**	**Sample #7**
S1A/22	S2A/22	S3A/22	S4A/22	S5A/22	S6A/22	S7A/22
S1B/22	S2B/22	S3B/22	S4B/22	S5B/22	S6B/22	S7B/22
S1C/22	S2C/22	S3C/22	S4C/22	S5C/22	S6C/22	S7C/22
S1D/22						
S1E/22						
S1F/22						

The task is to identify the acids and urinary metabolites of mustard which were spiked into the samples in the at the 0, 10 or 100 ppb level.

Sample workup for all samples is the same, and follows the flowchart presented below. LC/MS and GC/MS were the analytical techniques employed.

Lab 22 currently does not have a method for the determination of low levels of thiodiglycol in urine.


Urine sample
2.0 mL aliquot
Si-SPE cleanup
evaporate to 200 µL
LC/MS/MS
1.0 mL aliquot
Si-SPE cleanup
pentafluorobenzene deritive
evaporate to 200 µL
GC/MS (EI)
GC/MS (CI)
2.0 mL aliquot
C18-SPE cleanup
CH_2Cl_2 extract, evaporate to 200 µL
LC/MS/MS
1.0 mL aliquot
CH_2Cl_2 extract
CH_2Cl_2: GC/MS (EI)
Confirm absence of reduced SBMSE & MSMTESE
2.0 mL aliquot
$TiCl_3$ reduction
CH_2Cl_2 extract, evaporate to 200 µL
GC/MS (EI)
GC/MS (CI)
Acids
Metabolites of HD

DETAILED SAMPLE PREPARATION

Acids:

Prep for GC/MS:

Silica SPE clean-up: This method similar to procedure used in Mawhinney(2007) and UK lab section 9.1. 1mL of sample was aliquoted for work-up in a clean 4mL sample vial. Sample was taken to dryness and the residue was extracted twice with 1.5mL of 5%H_2O in Acetonitrile. A Supelco Discovery-Silica column (500mg/3cc) was conditioned with 4.8mL of 25%H_2O in acetonitrile followed by 3mL of acetonitrile. Sample was taken through column and washed first with 2mL of acetonitrile and followed with 4mL of 10% H_2O in acetonitrile. Sample was eluted with 2.5mL of 25%H_2O in acetonitrile into clean 4mL vial.
Pentafluorobenzylation: Sample taken to dryness and 480µL of acetone was added to vial. Added to solution was 10µL of pentafluorobenzyl bromide and 10µL of diisopropylamine. Sample was placed on heat block and heated 75˚C for 1 hour. Sample allowed to cool and volume reduced to approximately 200µL and transferred to vial insert.
Samples: S1, S2, S3, S4, S5, S6, S7

Prep for LC/MS (for positive GC/MS samples only):

Silica SPE clean-up: This method similar to procedure used in Mawhinney(2007) and UK lab section 9.1. 1mL of sample was aliquoted for work-up in a clean 4mL sample vial. Sample was taken to dryness and the residue was extracted twice with 1.5mL of 5%H_2O in Acetonitrile. A Supelco Discovery-Silica column (500mg/3cc) was conditioned with 4.8mL of 25%H_2O in acetonitrile followed by 3mL of acetonitrile. Sample was taken through column and washed first with 2mL of acetonitrile and followed with 4mL of 10% H_2O in acetonitrile. Sample was eluted with 2.5mL of 25%H_2O in acetonitrile into clean 4mL vial. Sample volume was reduced to approximately 200µL.
Samples: S1, S2, S3, S6

HD metabolites:

Prep for GC/MS:

Titanium Chloride reduction: 2mL of sample was aliquoted for work-up in a clean 4mL sample vial. To this vial, 2mL of ca. 10% Titanium Chloride (in ~20-30% HCl) was added and vortexed for 5 seconds. Sample vial was placed on heating block and sample heated to 70˚C for 1 hour. Sample was then allowed to cool and liquid-liquid extraction performed using 3x 1mL of DCM solvent. DCM extract was volume reduced using gentle stream of nitrogen to approximately 200µL. Sample was transferred to vial insert.
Samples: S1, S2, S3, S4, S5, S6, S7

Prep for LC/MS (for positive GC/MS samples only):

C18 SPE clean-up: 2mL of sample was aliquoted for work-up. An Alltech C18 column (500mg/3cc) was conditioned with 3mL of methanol, followed by 3mL of HPLC water. 2mL sample was taken through column and washed with 3mL of water. Column was allowed to go to dryness and then proceeded to elute with 4mL of DCM into clean sample vial. Elutant was taken to dryness and taken back up in 200uL of HPLC H_2O. Sample was transferred to vial insert.
Samples: S1, S5, S7

DETAILED ANALYTICAL METHODS

GC-GC-MS (EI) parameters

TOF-GC/MS Method Name: OPCW 2D CW122809

Instrument name: LECO Pegasus IV GCxGC
Carrier gas type: Helium
Flow rate: 1.20 mL/min
[X] Corrected constant flow via pressure ramps
Inlet type: Split/Splitless
Injection mode: Pulsed-splitless
Inlet purge time: 35 sec
Inlet purge flow: 30 mL/min
Inlet pulse pressure: 40 psi
Inlet pulse time: 0.5 min
Inlet temperature: 250 °C
Primary Column type/phase: Agilent HP-5MS (5% diphenyl 95% dimethyl polysiloxane)
Primary Column Length x ID x Film thickness: 15 m x 0.25 mm x 0.25 μm
Secondary Column type/phase: Restek Rxi-17 (50% diphenyl 50% dimethyl polysiloxane)
Secondary Column Length x ID x Film thickness: 1 m x 0.1 mm x 0.1 μm
Oven equilibration time: 0.5 min
Primary Oven temperature program:
70 °C (0.5 min)
10 °C/min
280 °C (3 min)
Secondary Oven temperature program:
85 °C (0.5 min)
10 °C/min
295 °C (3 min)
Transfer line temperature: 295 °C

GCxGC parameters:
[X] Modulator enabled
Modulator temperature offset: 30 °C, relative to the GC oven temperature
Modulation period: 3 sec
Hot pulse time: 0.6 sec
Cool time between stages: 0.9 sec

MS parameters:
Acquisition delay: 90sec
Scan range: 30-600 m/z
Acquisition rate: 200 spectra/second
Detector voltage: 1650 V
Electron energy: 70 eV
Source temperature: 250 °C

GC-MS (CI) parameters

Parameter	Details
GC instrument	Agilent 6890
Column: type: dimensions (m x mm) film thickness (µm)	 Agilent HP5-MS 30m x 0.25 mm 0.25 µm
Guard column/retention gap	--
Carrier gas, flow rate (cm/s or psi)	He, 0.8 mL/min, 32 cm/sec
Temperature program	40°C/3 min, 8°C/min, 300°C/3 min
Injection type	Splitless
Injector temperature (°C)	250°C
Injection volume (µl)	1 µL
MS instrument (type)	Agilent 5973
Ionization type	CI – NH_3 for positive; CH_4 for negative
Emission current (µA)	242 µA
Electron energy (eV)	114 eV
Source temperature (°C)	150°C
Scan mode (MS full scan, SIM, MS-MS full scan, MRM)	*
Mass range (full scan) (m/z)	*
Extracted ions (full scan) (m/z)	*
Selected ions (SIM) (m/z), r.t. window[1]	*
Selected reactions (MRM) (m/z), r.t. window[1]	*
Resolution	0.7 u
Interface/transfer line temperature (°C)	280°C
Any additional parameters	

1. if appropriate

* Scan parameters are different for each analyte:

Analyte	Scan parameters
1,1'-Sulfonylbis[2-(methylthio)ethane]	Positive ion SIM: 232, 233, 234
Ethyl methylphosphonate	Negative ion SIM: 123
Isopropyl methylphosphonate	Negative ion SIM: 137

LC-MS-MS parameters

Parameter	**Details**	
	HD metabolites	Acids
LC instrument	LTQ XL-Orbitrap	
Column Type Dimensions (mm) Particle size (µm)	Waters Sunfire C8 2.1 x 150 mm 3.5 µm	Waters T3 Unbonded Silica 2.1 x 150 mm 3 µm
Oven temperature (°C)	30°C	
Mobile phase	Solvent A: Water w/0.1% formic acid Solvent B: ACN w/0.1% formic acid	
Gradient (or isocratic)	Gradient (%B/min: 5/0, 5/5, 80/15, 80/20, 5/23, 5/25)	
Injection type		
Injection volume (µl)	5 µL	
Loop volume (µl)		
MS instrument	Thermo LTQ Orbitrap	
Ionization, +ve / -ve	pos	Neg
Spray voltage (kV)	4 kV	
Source CID voltage (V)		
Source temperature (°C)	Capillary 250 °C	
Desolvation temperature (°C)[1]		
Desolvation gas, flow rate[1]	20 (sheath gas)	
Nebulizer gas, flow rate[1]	5 (sweep gas)	
Scan mode (MS full scan, SIM, MS-MS full scan, MRM)	*	
Scan range (full scan)	*	
Ions monitored (SIM), r.t. window[2]	*	
Reactions monitored (MRM), r.t. window[2]	*	
Collision energy	17	30

1. or other relevant source parameters
2. if appropriate

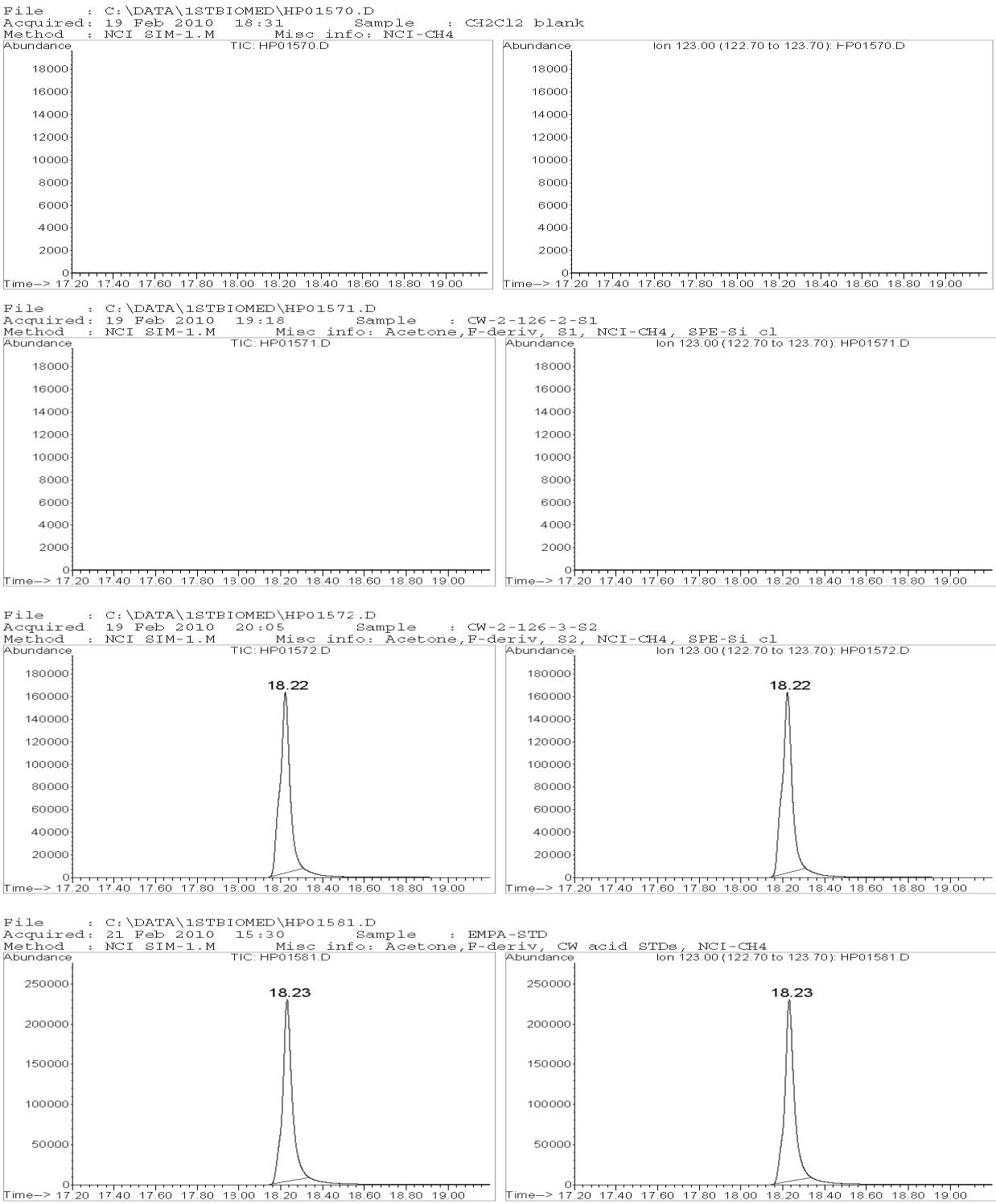
* Scan parameters are different for each analyte:

Analyte	Scan parameters
1-Methylsulfinyl-2-[2-(methylthio)ethylsulfonyl]ethane	MS/MS 253 → (70 to 280) Products of $[M+Na]^+$
1,1'-Sulfonylbis[2-(methylsulfinyl)ethane]	MS/MS 269 → (70 to 280) Products of $[M+Na]^+$
Ethyl methylphosphonate	MS/MS 123 → (50 to 210) Products of $[M-H]^-$
Isopropyl methylphosphonate	MS/MS 137 → (50 to 210) Products of $[M-H]^-$

RESULTS: URINE SAMPLE S2

β-Lyase metabolites of sulfur mustard were not found in this sample (after sample preparation that included $TiCl_3$ reduction and analysis by GC-GC-MS (EI)).

Ethyl methyphosophonate was found. The following few pages present the GC-MS (CI) data for the derivative and the LC-MS-MS data for the un-derivitized acid.



CI chromatograms supporting identification; TIC on left; EIC (m/z **123**) on right.

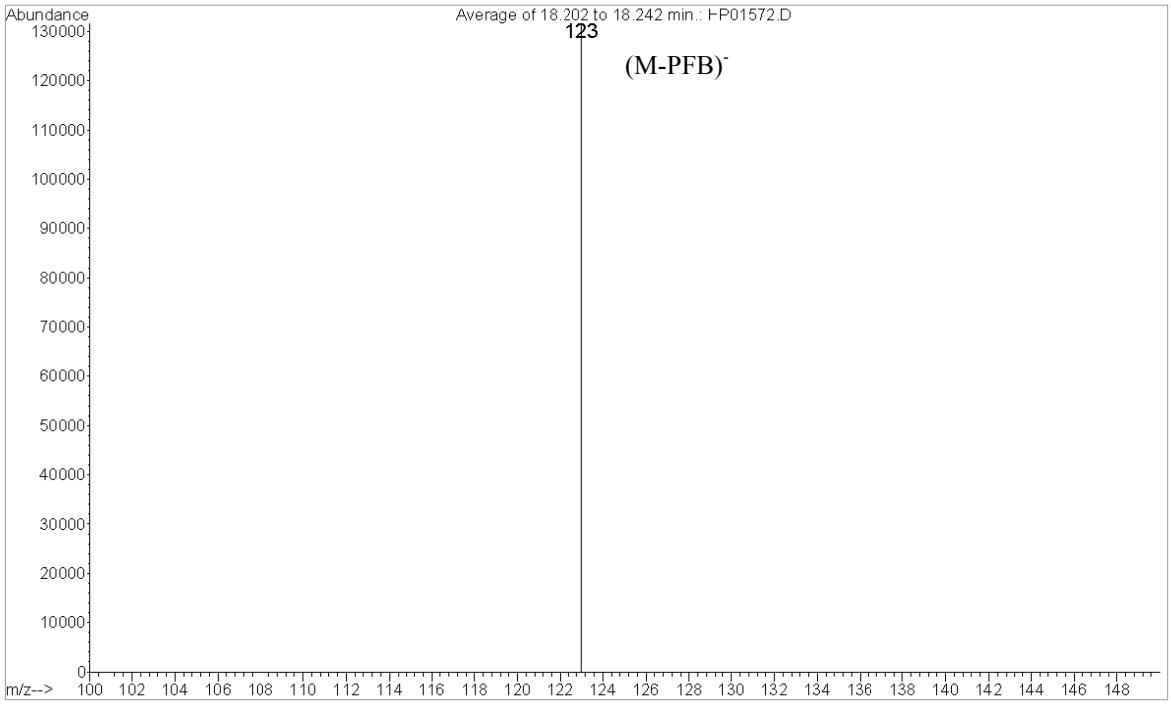
Top: Chromatograms of solvent blank.

Second: Chromatograms of urine blank S1.

Third: Chromatograms of Urine sample S2, retention time **18.22** min.

Bottom: Chromatograms of reference standard of PFB derivative of **Ethyl methylphosphonate** retention time **18.23** min.

```
File       :C:\DATA\1STBIOMED\HP01572.D
Acquired   : 19 Feb 2010  20:05     using AcqMethod NCI SIM-1.M
Sample Name: CW-2-126-3-S2
Misc Info  : Acetone,F-deriv, S2, NCI-CH4, SPE-Si cl
```

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File       :C:\DATA\1STBIOMED\HP01581.D
Acquired   : 21 Feb 2010  15:30     using AcqMethod NCI SIM-1.M
Sample Name: EMPA-STD
Misc Info  : Acetone,F-deriv, CW acid STDs, NCI-CH4
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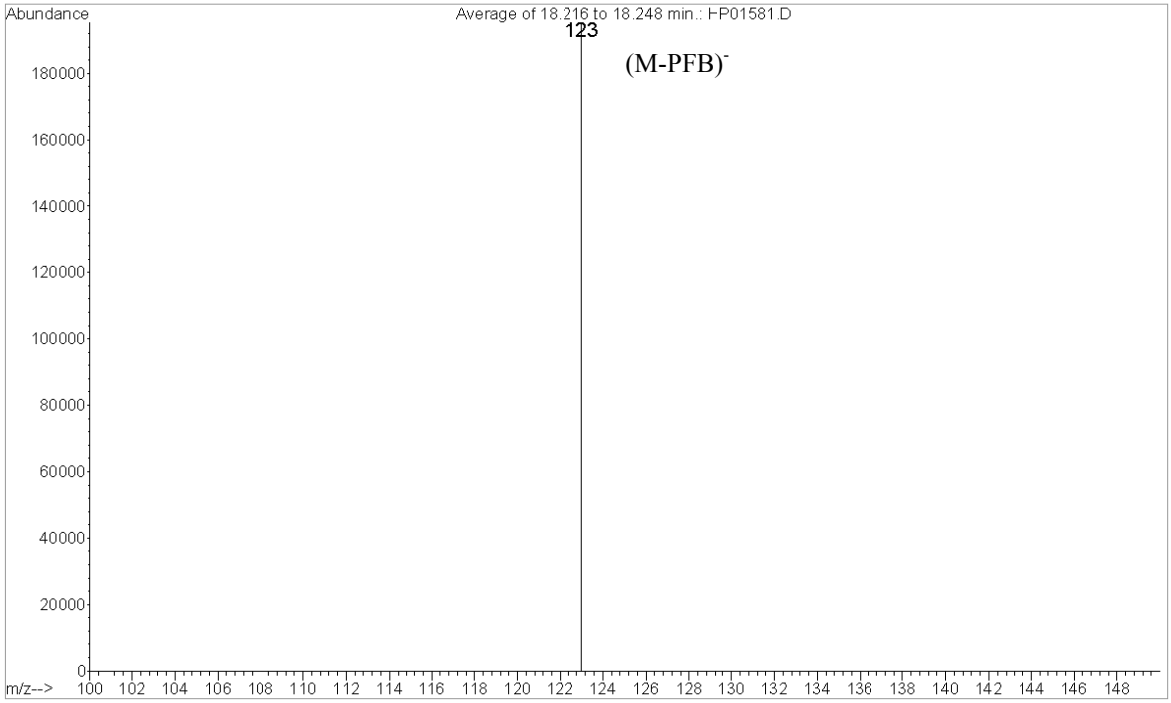
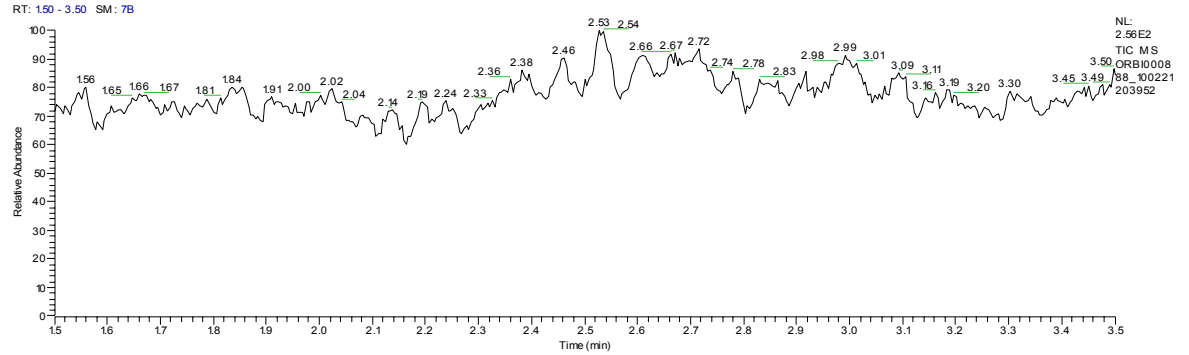
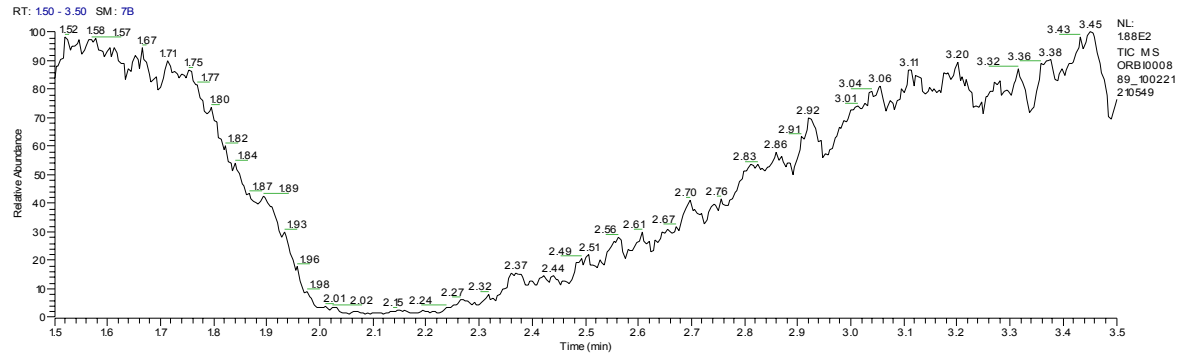


CI SIM mass spectrum (m/z 123) of:
Top: Spectrum of peak of interest from Urine sample S2.
Bottom: Reference standard of PFB derivative of **Ethyl methylphosphonate** (MW: **305**).

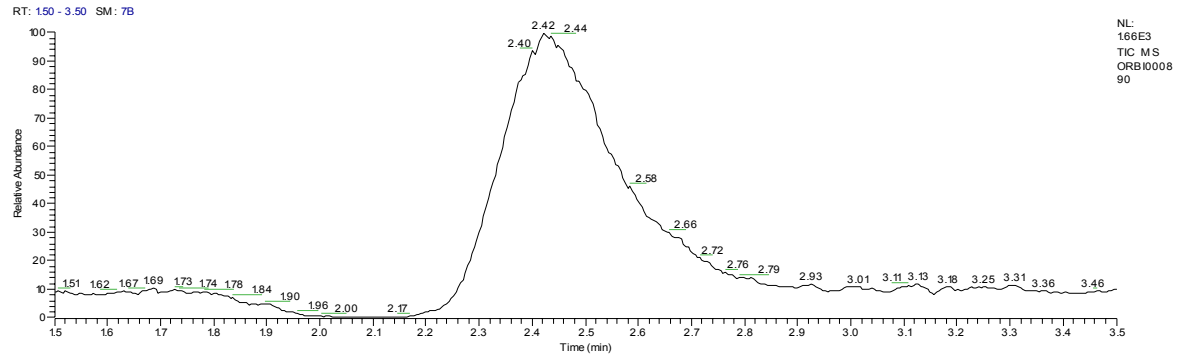
C:\Xcalibur\data\ORBI000888_100221203952 2/21/2010 8:39:52 PM Water blank
Neg, MSMS, T3 column, 200uL/min



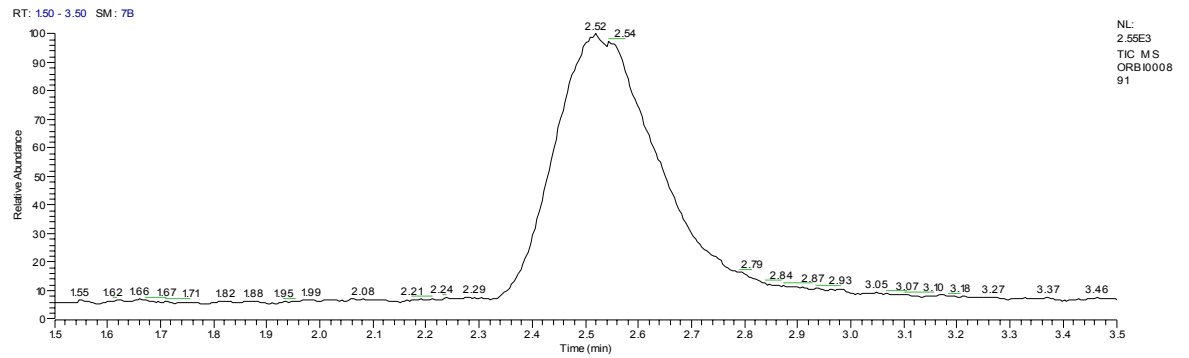
C:\Xcalibur\data\ORBI000889_100221210549 2/21/2010 9:05:49 PM Cw-2-127-6-S1
Neg, MSMS, T3 column, 200uL/min



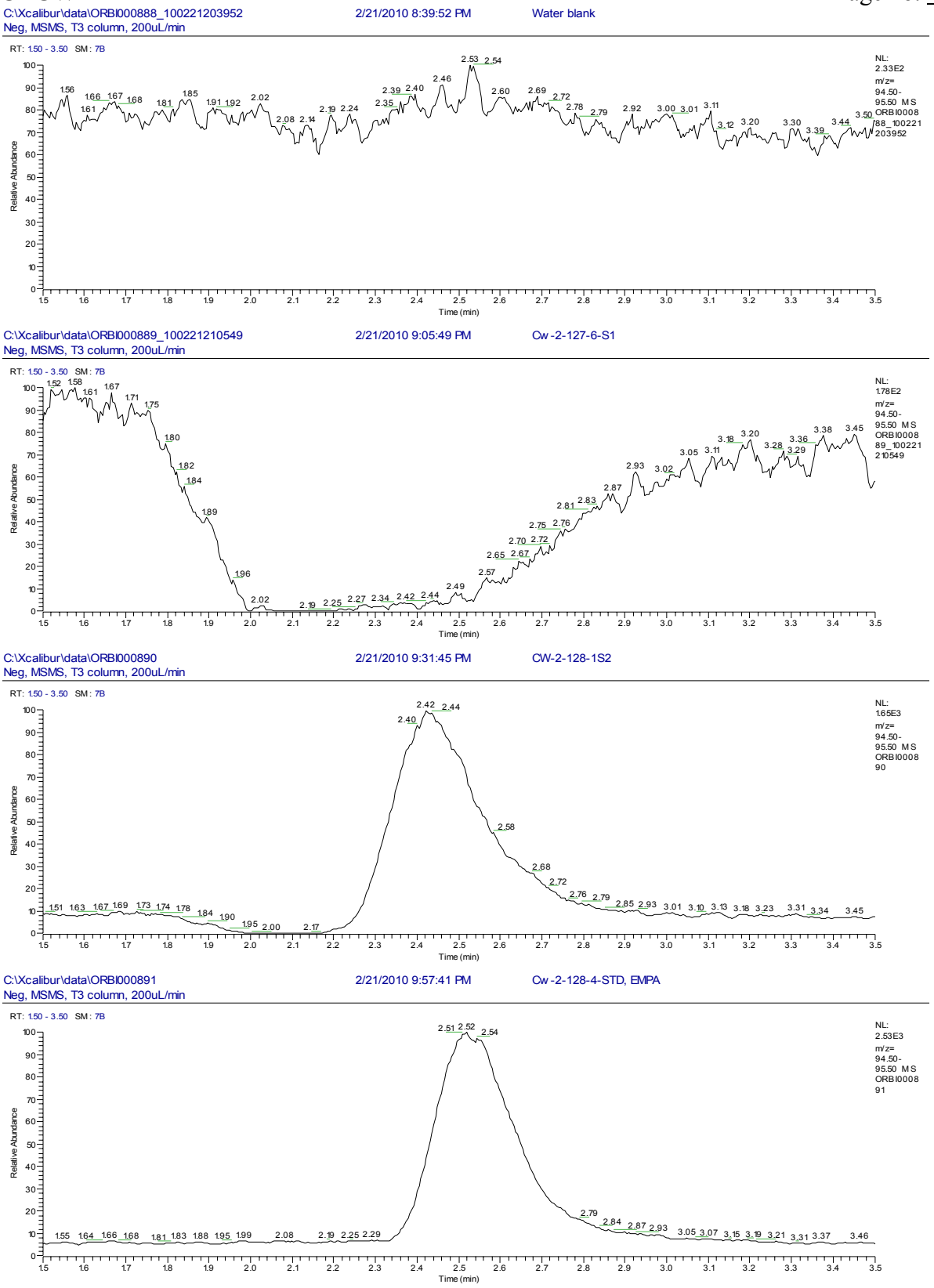
C:\Xcalibur\data\ORBI000890 2/21/2010 9:31:45 PM CW-2-128-1S2
Neg, MSMS, T3 column, 200uL/min



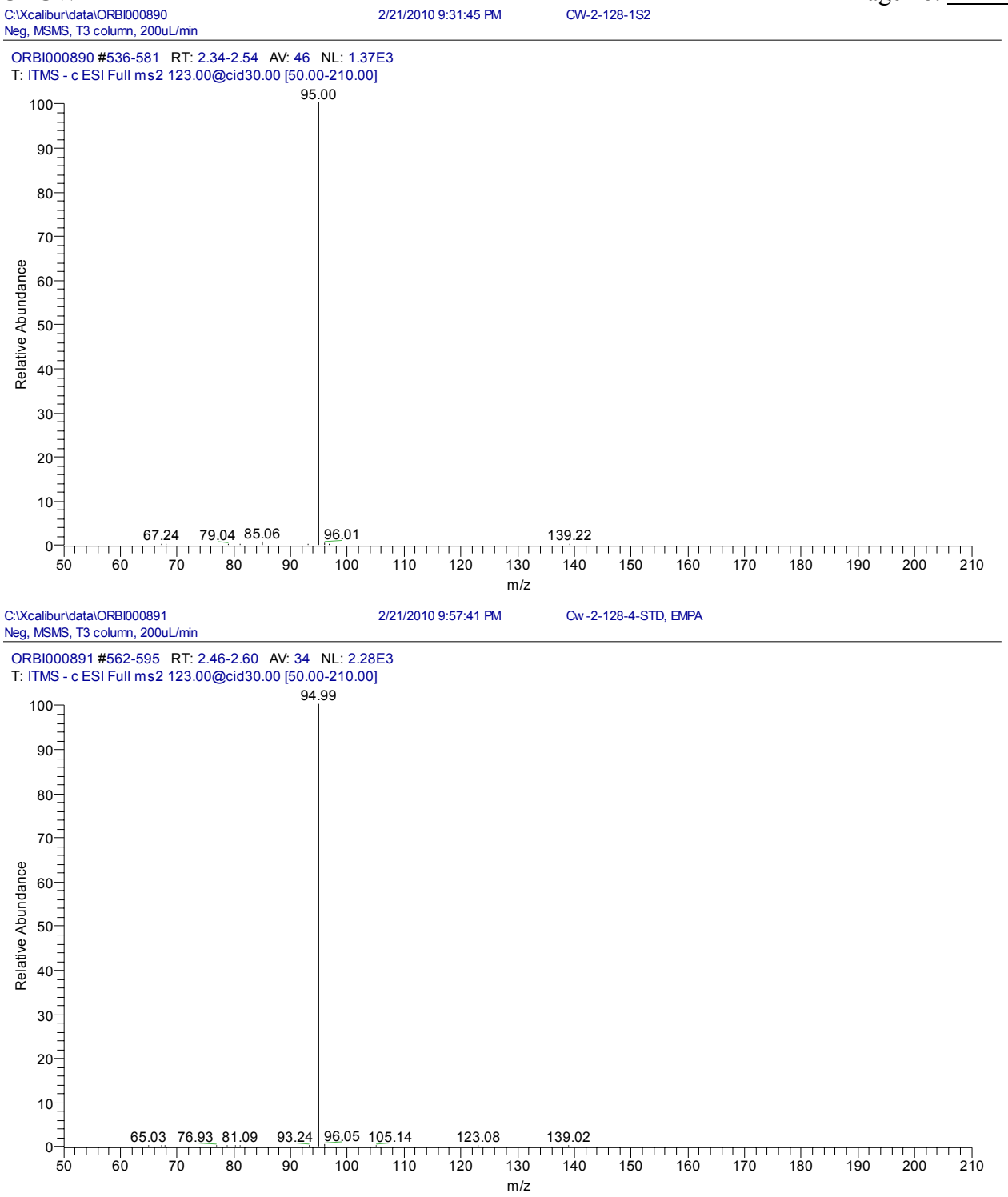
C:\Xcalibur\data\ORBI000891 2/21/2010 9:57:41 PM Cw-2-128-4-STD, EMPA
Neg, MSMS, T3 column, 200uL/min



LC/MS/MS chromatograms. Top: water blank. Next: Urine blank S1. Third: Urine sample S2. Bottom: reference standard of **Ethyl methylphosphonate**



LC/MS/MS EIC (m/z 95) chromatograms. Top: water blank. Next: Urine blank S1. Third: Urine sample S2. Bottom: reference standard of **Ethyl methylphosphonate**

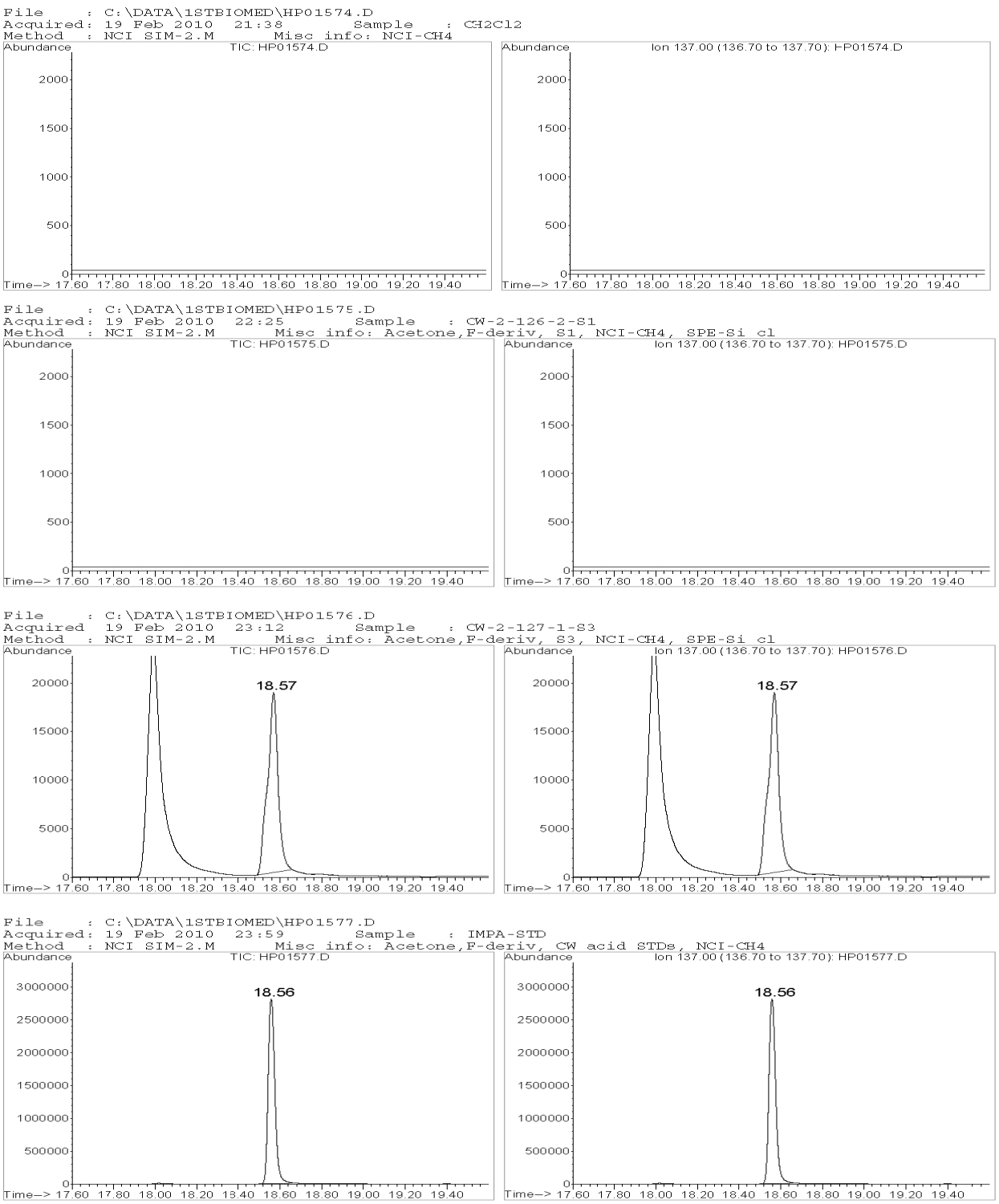


LC/MS/MS spectra – product spectra of parent $[M\text{-}H]^{+-}$ m/z 123. Top: Urine sample S2. Bottom: reference standard of **Ethyl methylphosphonate**

RESULTS: URINE SAMPLE S3

β-Lyase metabolites of sulfur mustard were not found in this sample (after sample preparation that included $TiCl_3$ reduction and analysis by GC-GC-MS (EI)).

Isopropyl methyphosophonate was found. The following few pages present the GC-MS (CI) data for the derivative and the LC-MS-MS data for the un-derivitized acid.



CI chromatograms supporting identification; TIC on left; EIC (m/z **137**) on right.

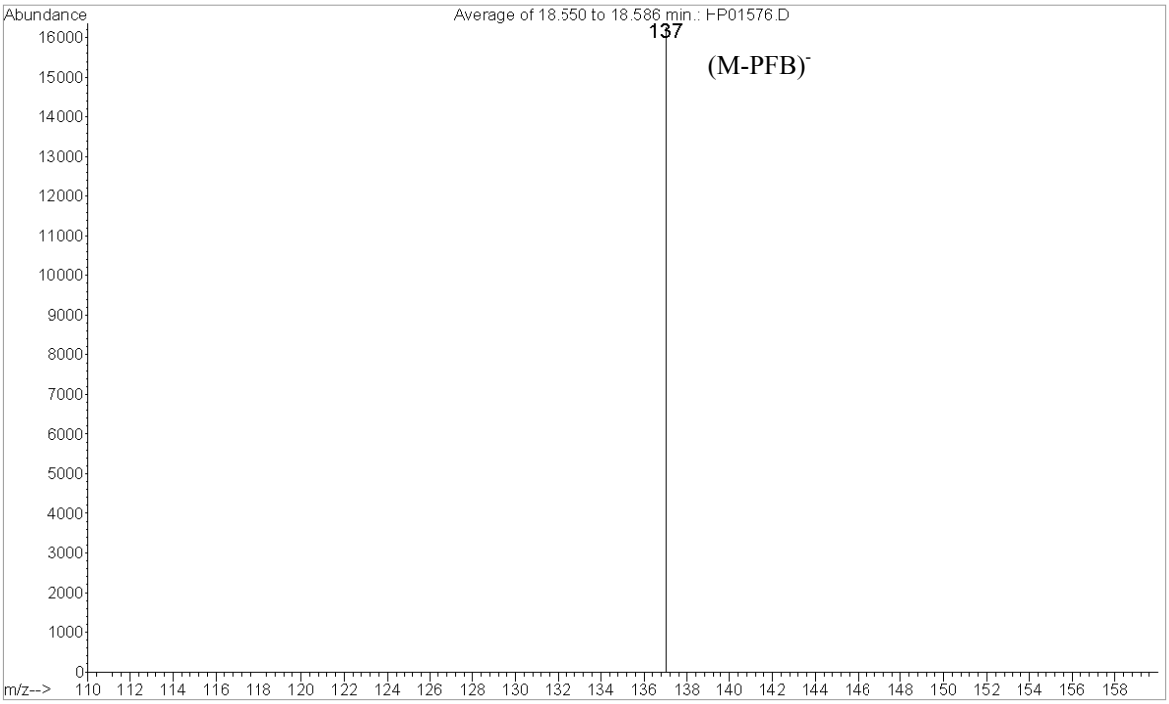
Top: Chromatograms of solvent blank.

Second: Chromatograms of urine blank S1.

Third: Chromatograms of Urine sample S3, retention time **18.57** min.

Bottom: Chromatograms of reference standard of PFB derivative of **Isopropyl methylphosphonate** retention time **18.56** min.

```
File       :C:\DATA\1STBIOMED\HP01576.D
Acquired   : 19 Feb 2010  23:12     using AcqMethod NCI SIM-2.M
Sample Name: CW-2-127-1-S3
Misc Info  : Acetone,F-deriv, S3, NCI-CH4, SPE-Si cl
```

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Sample Name: IMPA-STD
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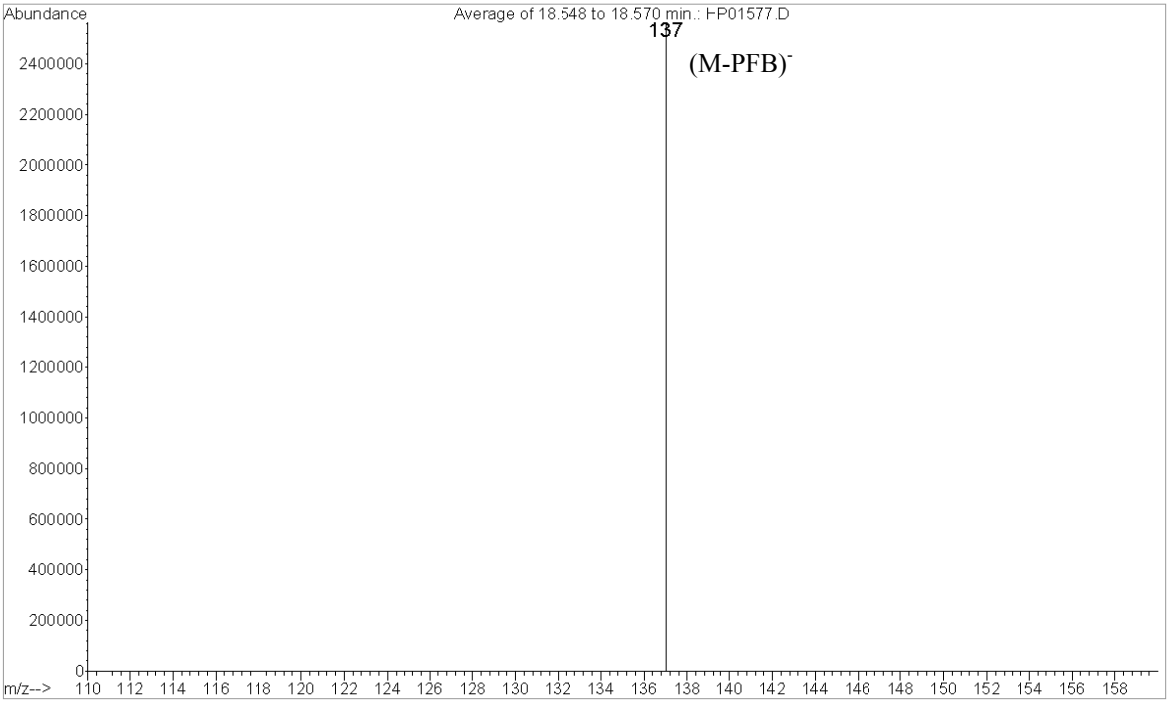
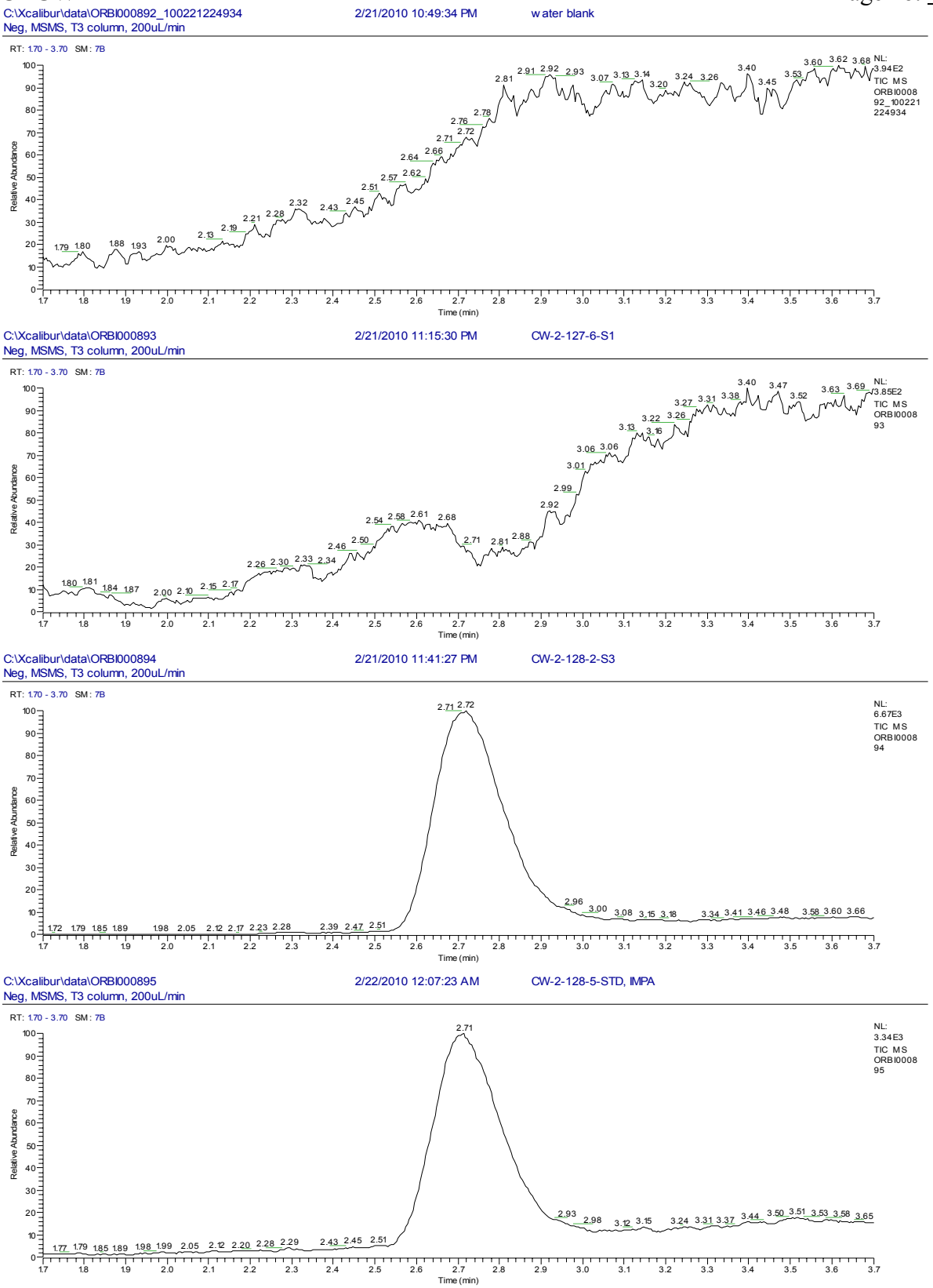
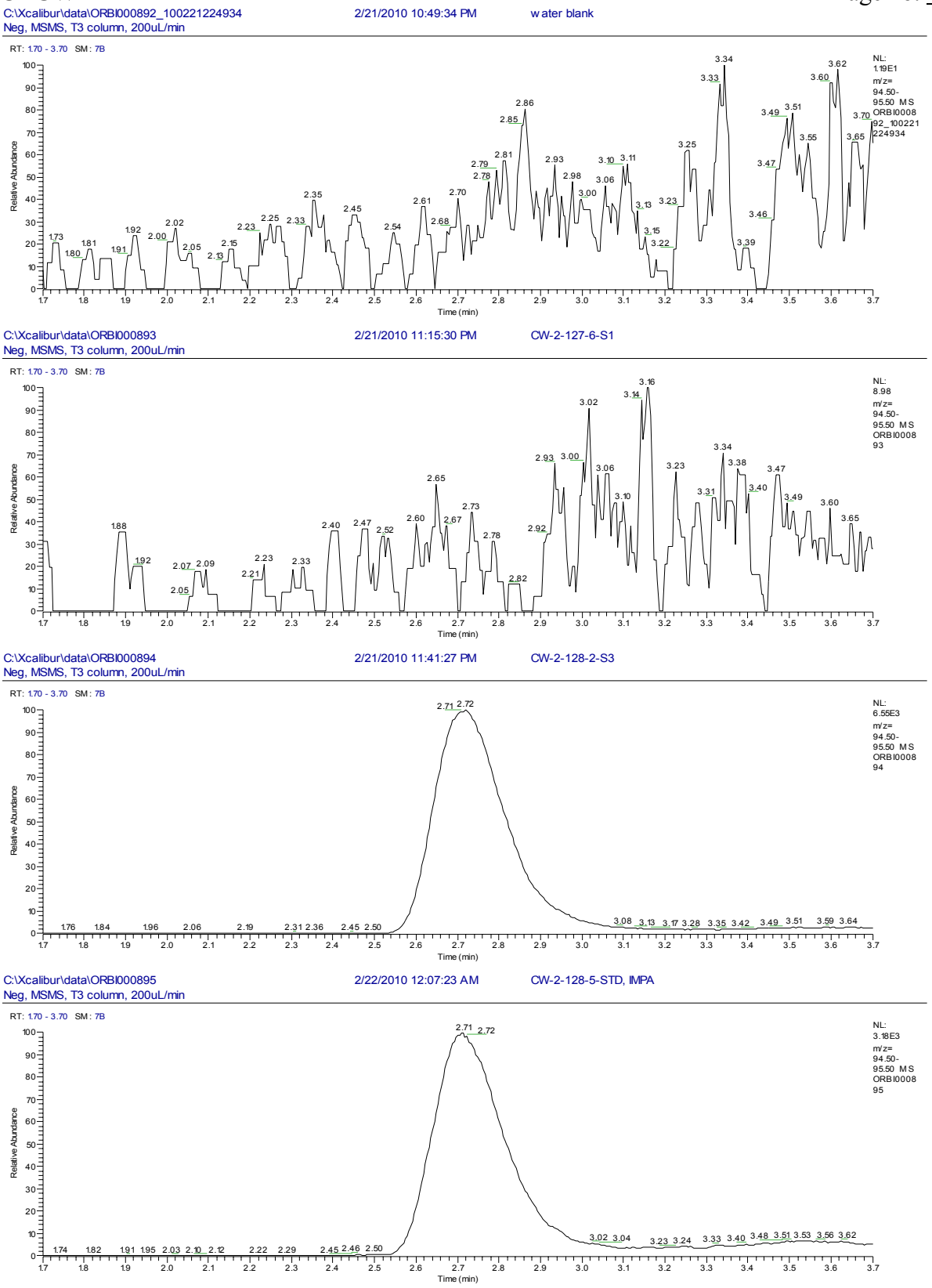


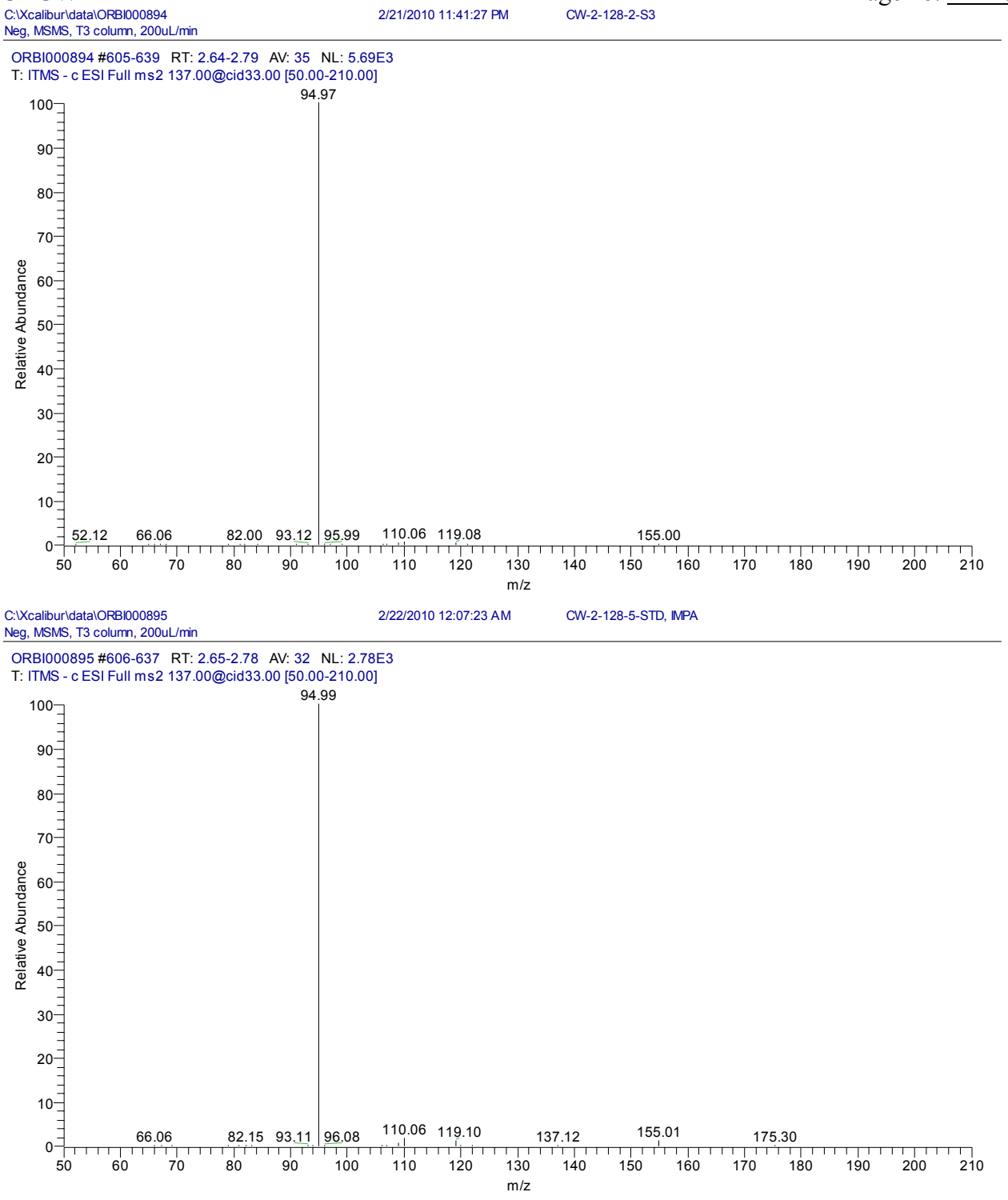
CI SIM mass spectrum (m/z 137) of:
Top: Spectrum of peak of interest from Urine sample S3.
Bottom: Reference standard of PFB derivative of **Isopropyl methylphosphonate** (MW: **319**).



LC/MS/MS chromatograms. Top: water blank. Next: Urine blank S1. Third: Urine sample S3. Bottom: reference standard of **Isopropyl methylphosphonate**



LC/MS/MS EIC (m/z 95) chromatograms. Top: water blank. Next: Urine blank S1. Third: Urine sample S3. Bottom: reference standard of **Isopropyl methylphosphonate**



LC/MS/MS spectra – product spectra of parent $[M-H]^-$ m/z 137. Top: Urine sample S3. Bottom: reference standard of **Isopropyl methylphosphonate**

RESULTS: URINE SAMPLE S4

β-Lyase metabolites of sulfur mustard were not found in this sample (after sample preparation that included $TiCl_3$ reduction and analysis by GC-GC-MS (EI)).

None of the phosphonic acids were found.

RESULTS: URINE SAMPLE S5

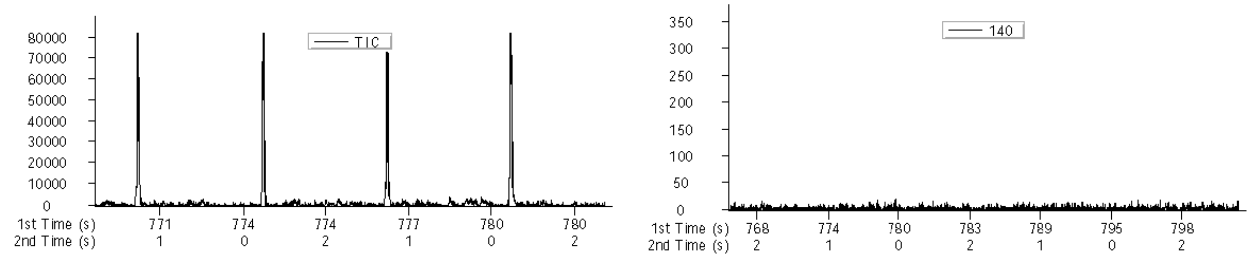
A β-Lyase metabolite of sulfur mustard was found in this sample (after sample preparation that included $TiCl_3$ reduction and analysis by GC-GC-MS (EI)) and confirmed with GC-MS (CI) and LC-MS-MS.

Present was 1,1'-Sulfonylbis[2-(methylsulfinyl)ethane] (SBMSE), but no evidence of 1-Methylsulfinyl-2-[2-(methylthio)ethylsulfonyl]ethane (MSMTESE) was observed.

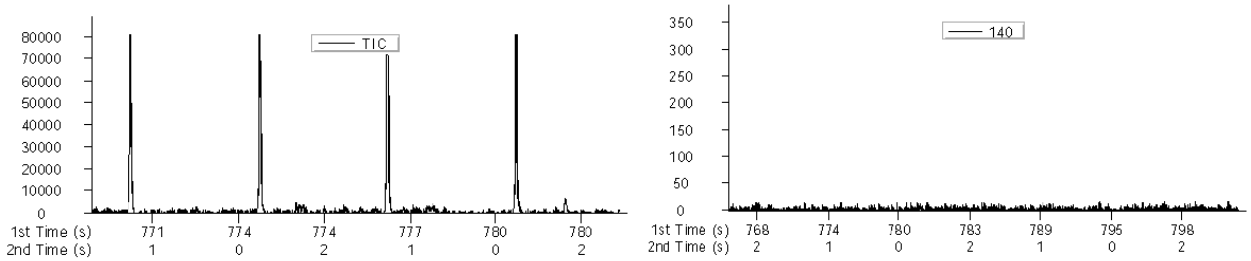
None of the phosphonic acids were found.

The following few pages present the GC-GC-MS (EI) and GC-MS (CI) data for the derivative and the LC-MS-MS data for the un-derivitized metabolite.

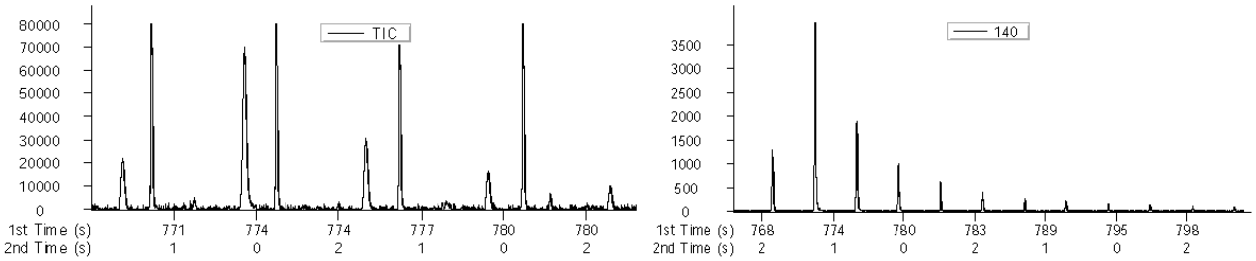
File : C:\LLNL2010\TOF71
Acquired: 14 Jan 2009 2:35:45 PM Sample: CH2Cl2 Blank Lot# 7284M



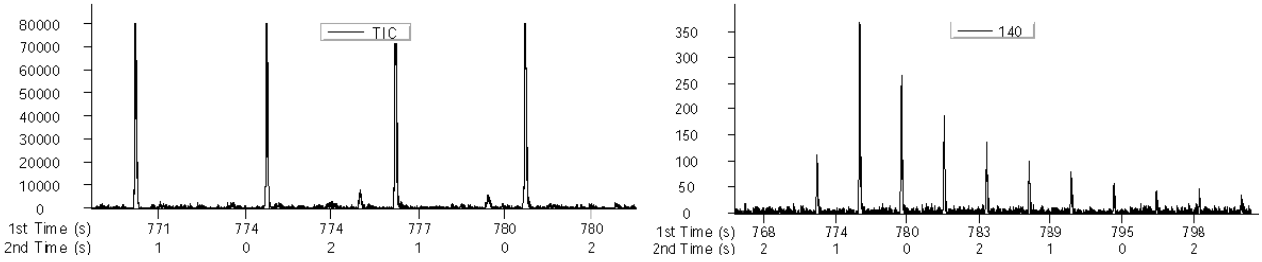
File : C:\LLNL2010\TOF72
Acquired: 14 Jan 2009 3:02:50 PM Sample: CW-2-122-3-S1



File : C:\LLNL2010\TOF73
Acquired: 14 Jan 2009 3:39:35 PM Sample: CW-2-123-1-S5

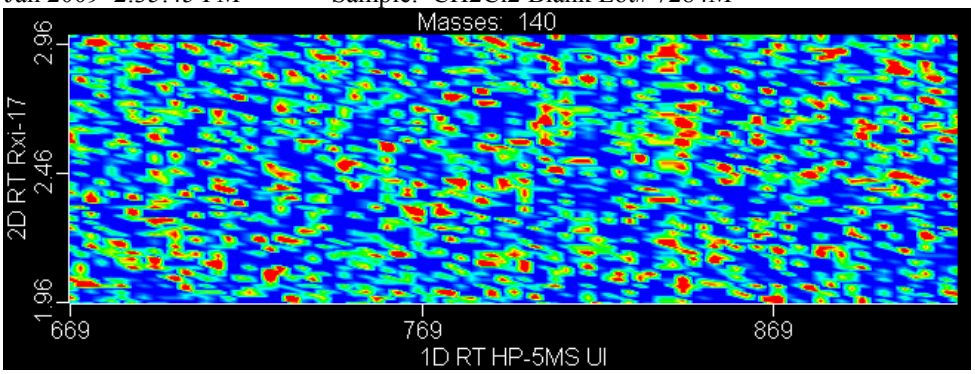


File : C:\LLNL2010\TOF74
Acquired: 14 Jan 2009 4:16:16 PM Sample: 0.5 ppm SBMTE

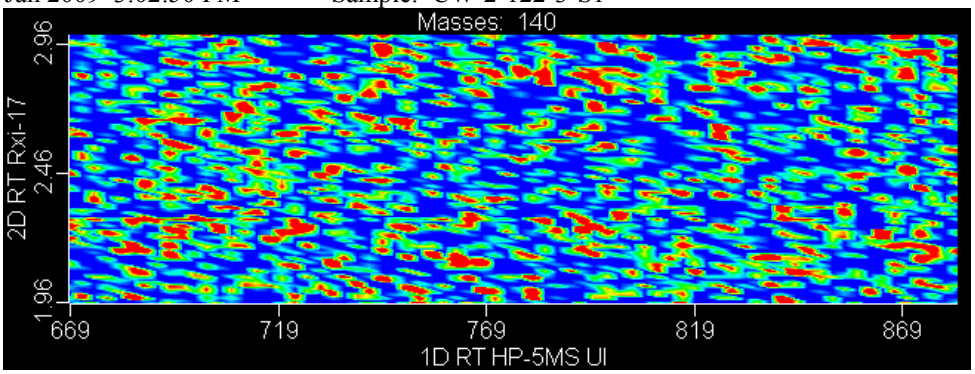


EI chromatograms supporting identification; TIC on left; EIC (m/z **140**) on right
Top: Chromatograms of solvent blank.
Second: Chromatograms of urine blank S1.
Third: Chromatograms of urine sample S5, retention time 771, 2.710 sec
Bottom: Chromatograms of reference standard of **1,1'-Sulfonylbis[2-9methylthio)ethane]**, retention time 774, 2.660 sec

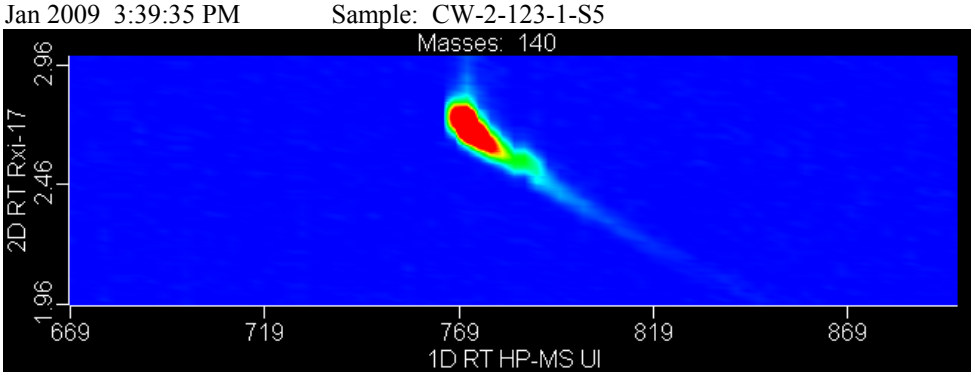
File : C:\LLNL2010\TOF71
Acquired: 14 Jan 2009 2:35:45 PM Sample: CH2Cl2 Blank Lot# 7284M



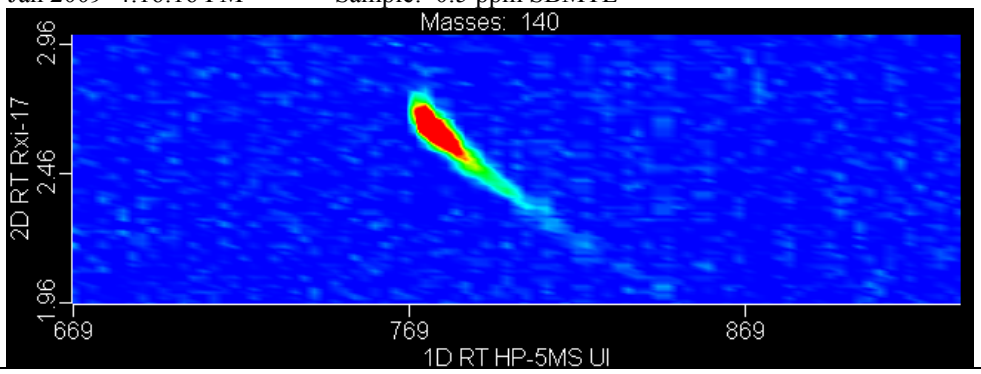
File : C:\LLNL2010\TOF72
Acquired: 14 Jan 2009 3:02:50 PM Sample: CW-2-122-3-S1



File : C:\LLNL2010\TOF73
Acquired: 14 Jan 2009 3:39:35 PM Sample: CW-2-123-1-S5



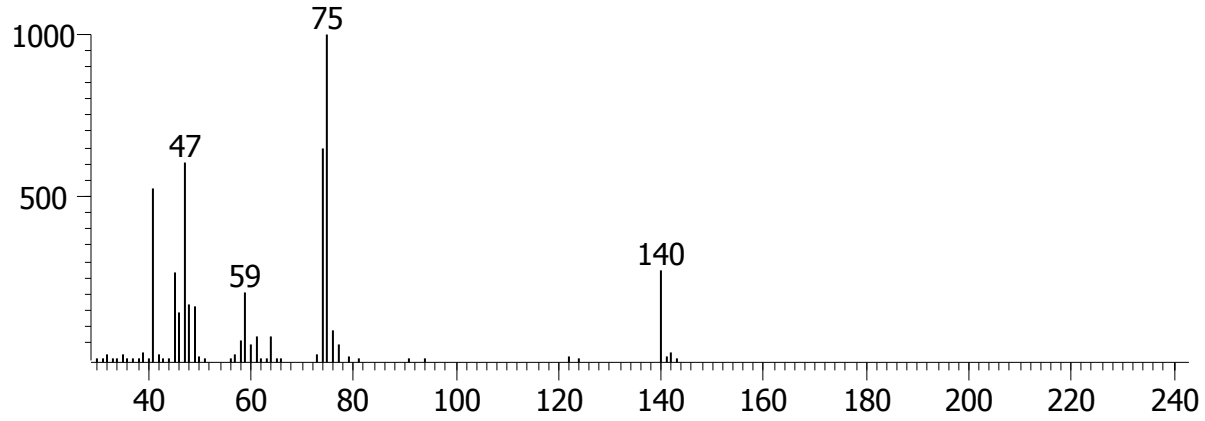
File : C:\LLNL2010\TOF74
Acquired: 14 Jan 2009 4:16:16 PM Sample: 0.5 ppm SBMTE



2D Contour plot supporting identification; EIC (m/z **140**)
Top: Contour plot of solvent blank; Second: Contour plot of urine blank S1; Third: Contour plot of urine sample S5, retention time 771, 2.710 sec; Bottom:Contour plot of reference standard of **1,1'-Sulfonylbis[2-9methylthio)ethane]**, retention time 774, 2.660 sec

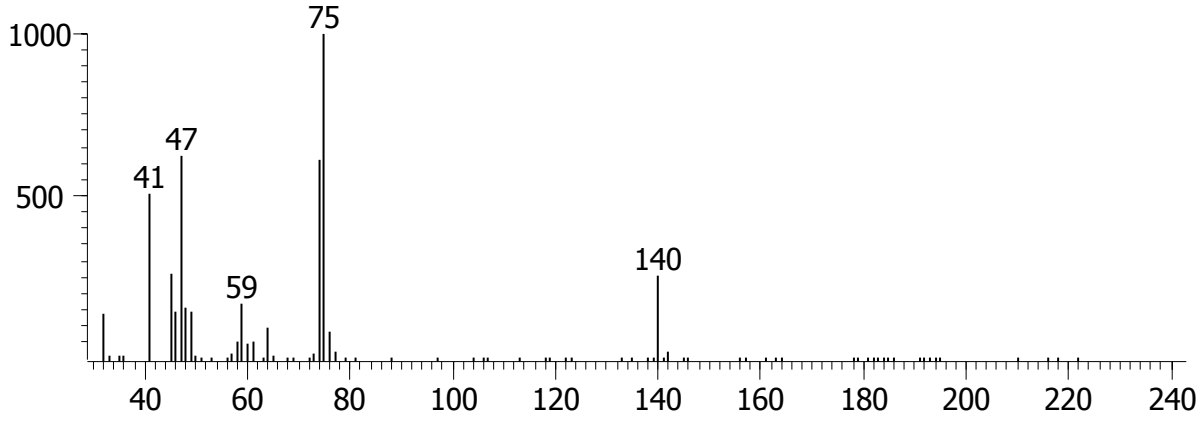
File : C:\LLNL2010\TOF73
Acquired: 14 Jan 2009 3:39:35 PM Sample: CW-2-123-1-S5

Peak True - sample "TOF:73", peak 185, at 771 , 2.710 sec , sec

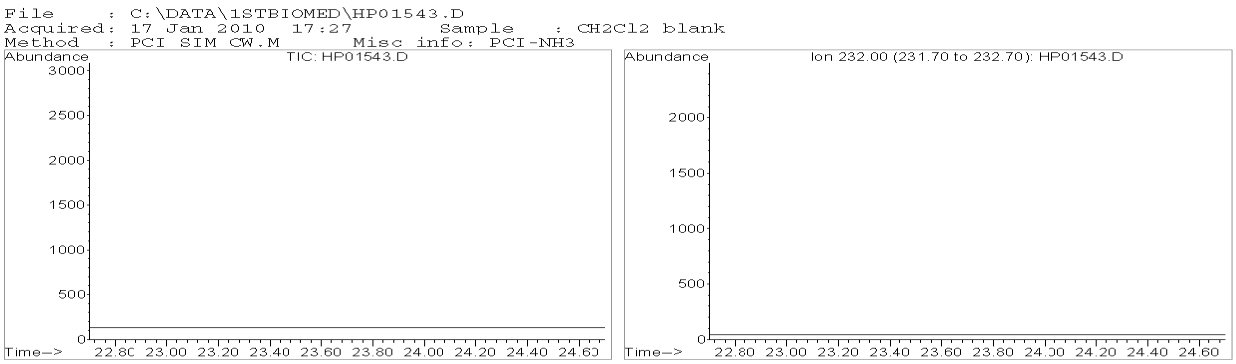


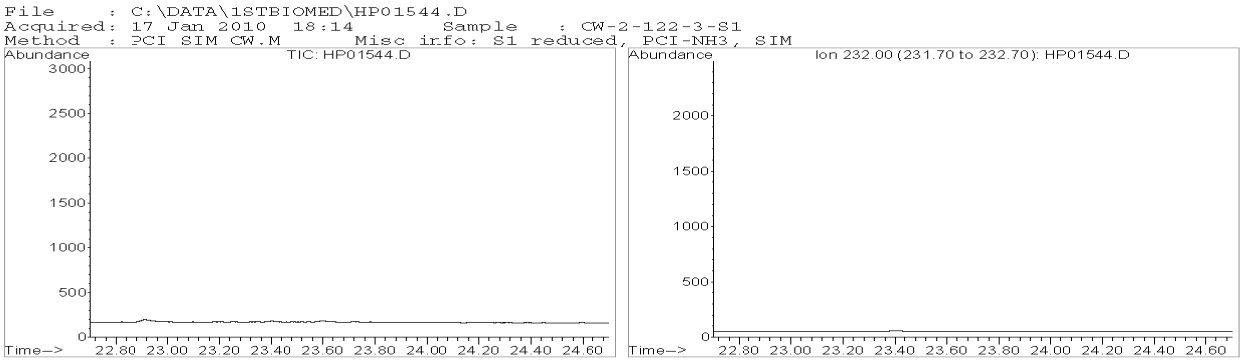
File : C:\LLNL2010\TOF74
Acquired: 14 Jan 2009 4:16:16 PM Sample: 0.5 ppm SBMTE

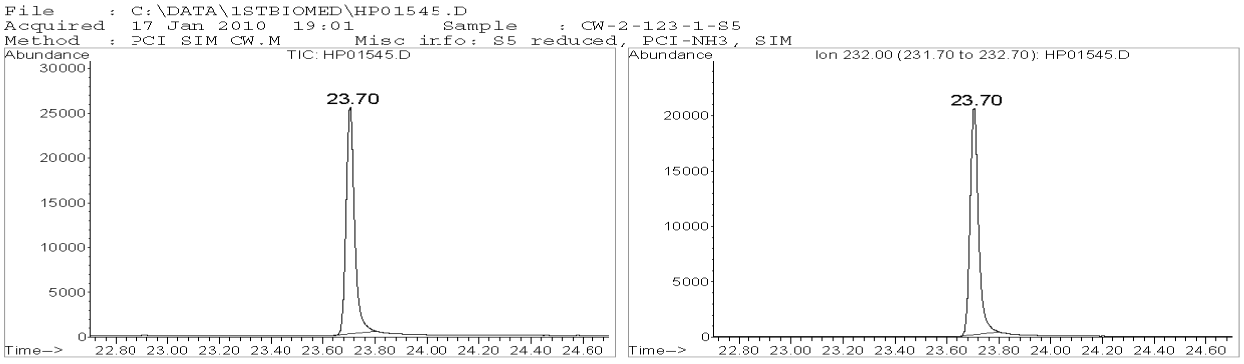
Peak True - sample "TOF:74", peak 116, at 774 , 2.660 sec , sec

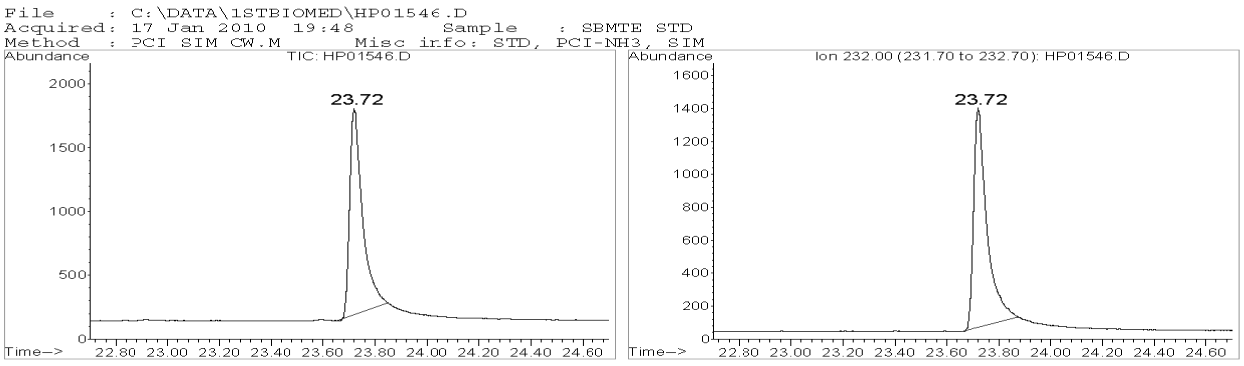


EI mass spectrum (m/z 75, 140) of:
Top: Spectrum of urine sample S5, retention time 771, 2.710 sec
Bottom: Reference standard of **1,1'-Sulfonylbis[2-9methylthio)ethane]. (MW: 232).**









CI chromatograms supporting identification; TIC on left; EIC (m/z **232**) on right.

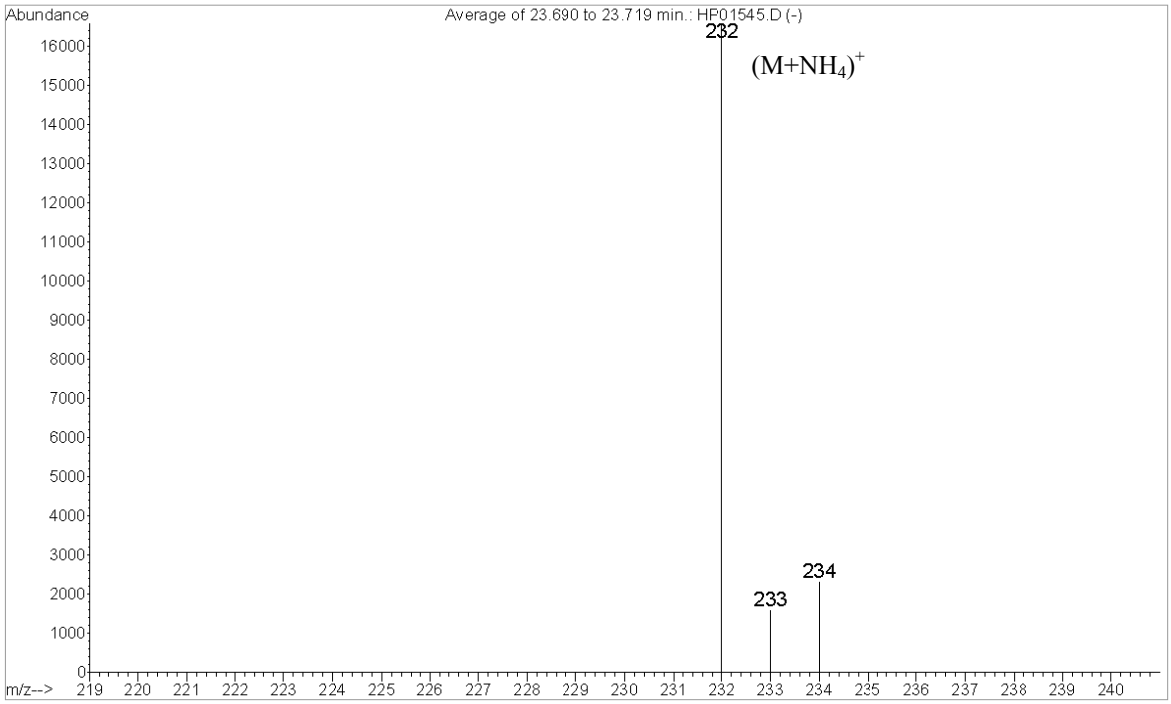
Top: Chromatograms of solvent blank.

Second: Chromatograms of urine blank S1.

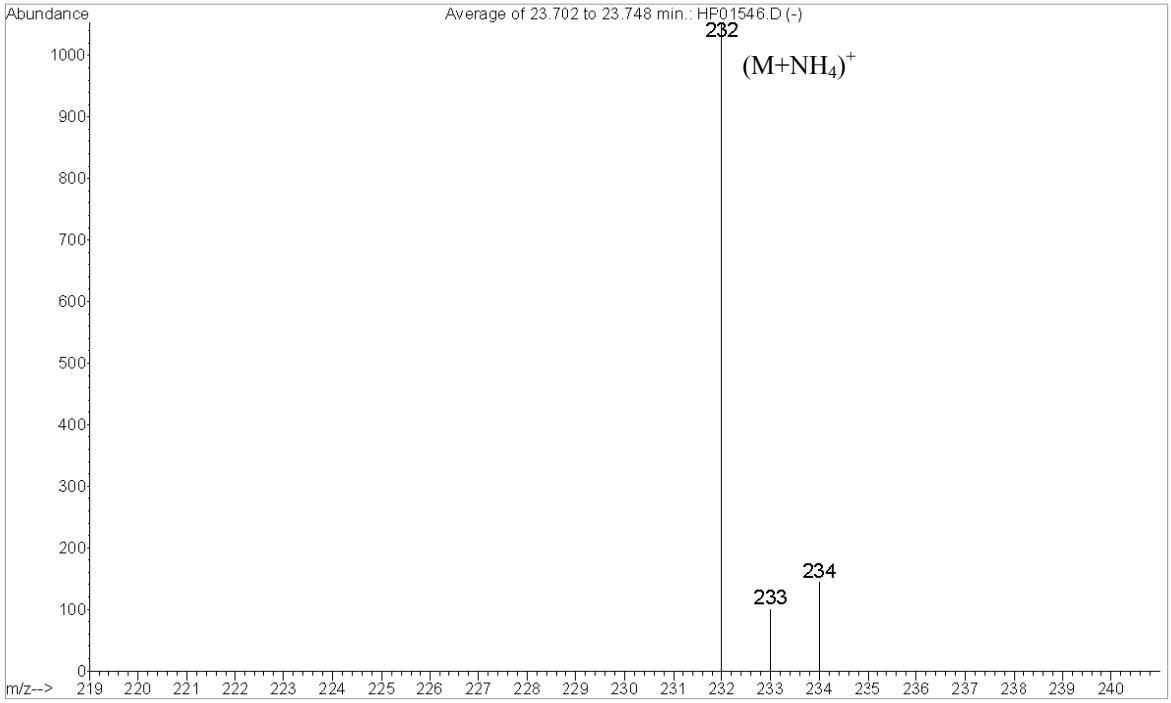
Third: Chromatograms of Urine sample S5, retention time **23.70** min.

Bottom: Chromatograms of reference standard of **1,1'-Sulfonylbis[2-(methylthio)ethane]**, retention time **23.72** min.

```
File       :C:\DATA\1STBIOMED\HP01545.D
Acquired   : 17 Jan 2010  19:01     using AcqMethod PCI SIM CW.M
Sample Name: CW-2-123-1-S5
Misc Info  : S5 reduced, PCI-NH3, SIM
```

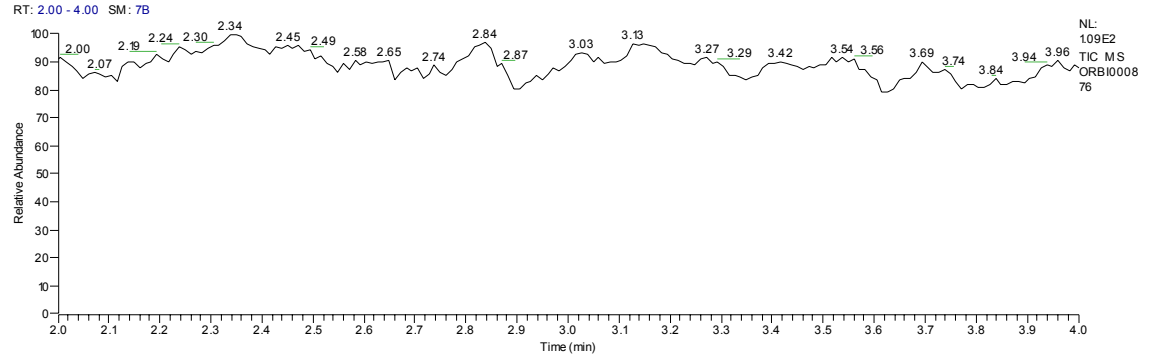



```
File       :C:\DATA\1STBIOMED\HP01546.D
Acquired   : 17 Jan 2010  19:48     using AcqMethod PCI SIM CW.M
Sample Name: SBMTE STD
Misc Info  : STD, PCI-NH3, SIM
```

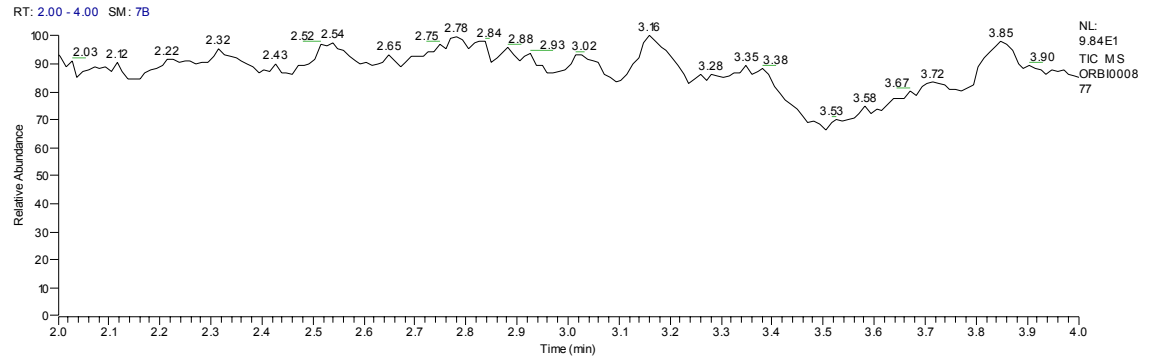


CI SIM mass spectrum (m/z 232, 233, 234) of:
Top: Spectrum of peak of interest from Urine sample S5.
Bottom: Reference standard of **1,1'-Sulfonylbis[2-(methylthio)ethane]**. (MW: **214**).

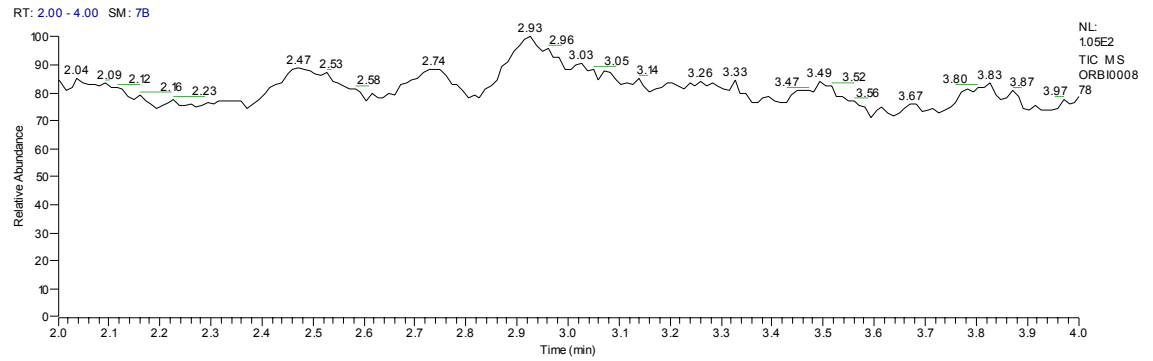
C:\Xcalibur\data\ORBI000876 1/17/2010 3:12:22 PM Water blank
Pos, MSMS, Sun column, 200uL/min



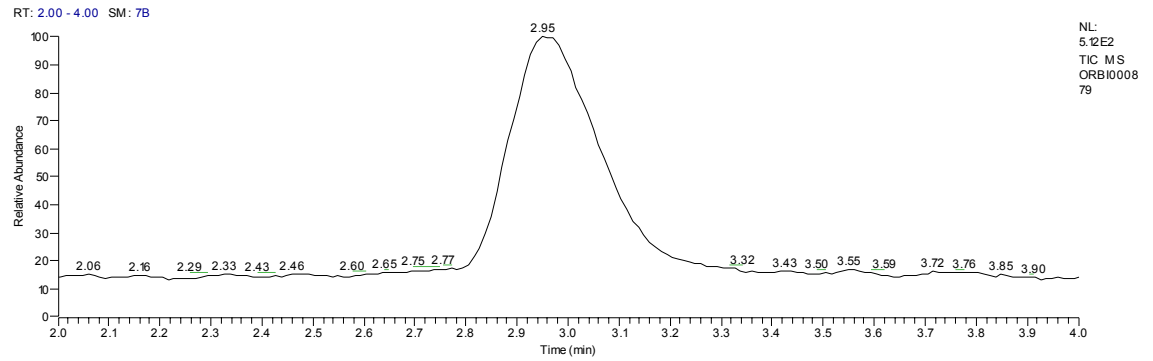
C:\Xcalibur\data\ORBI000877 1/17/2010 3:43:19 PM CW-2-124-4-S1
Pos, MSMS, Sun column, 200uL/min



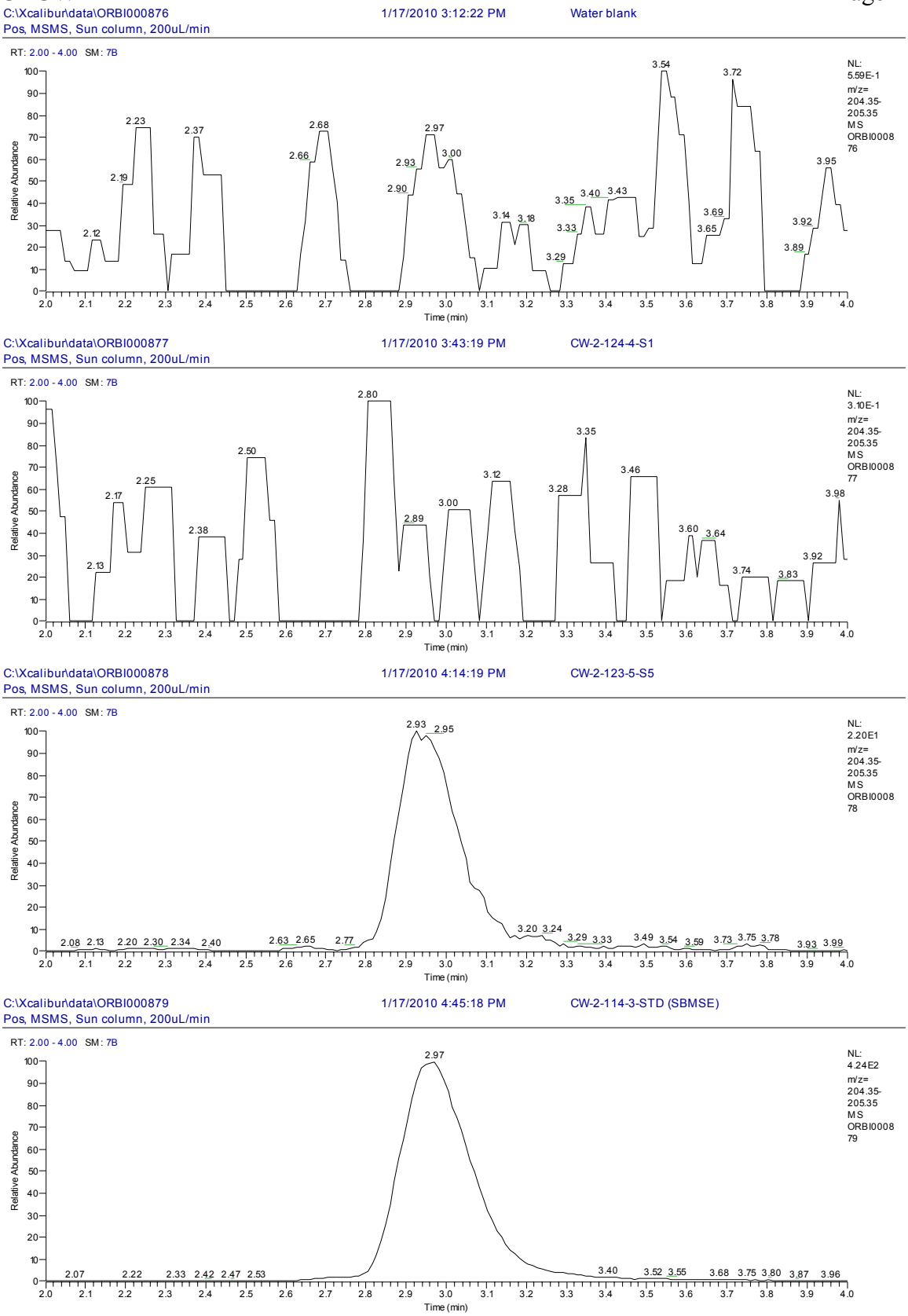
C:\Xcalibur\data\ORBI000878 1/17/2010 4:14:19 PM CW-2-123-5-S5
Pos, MSMS, Sun column, 200uL/min



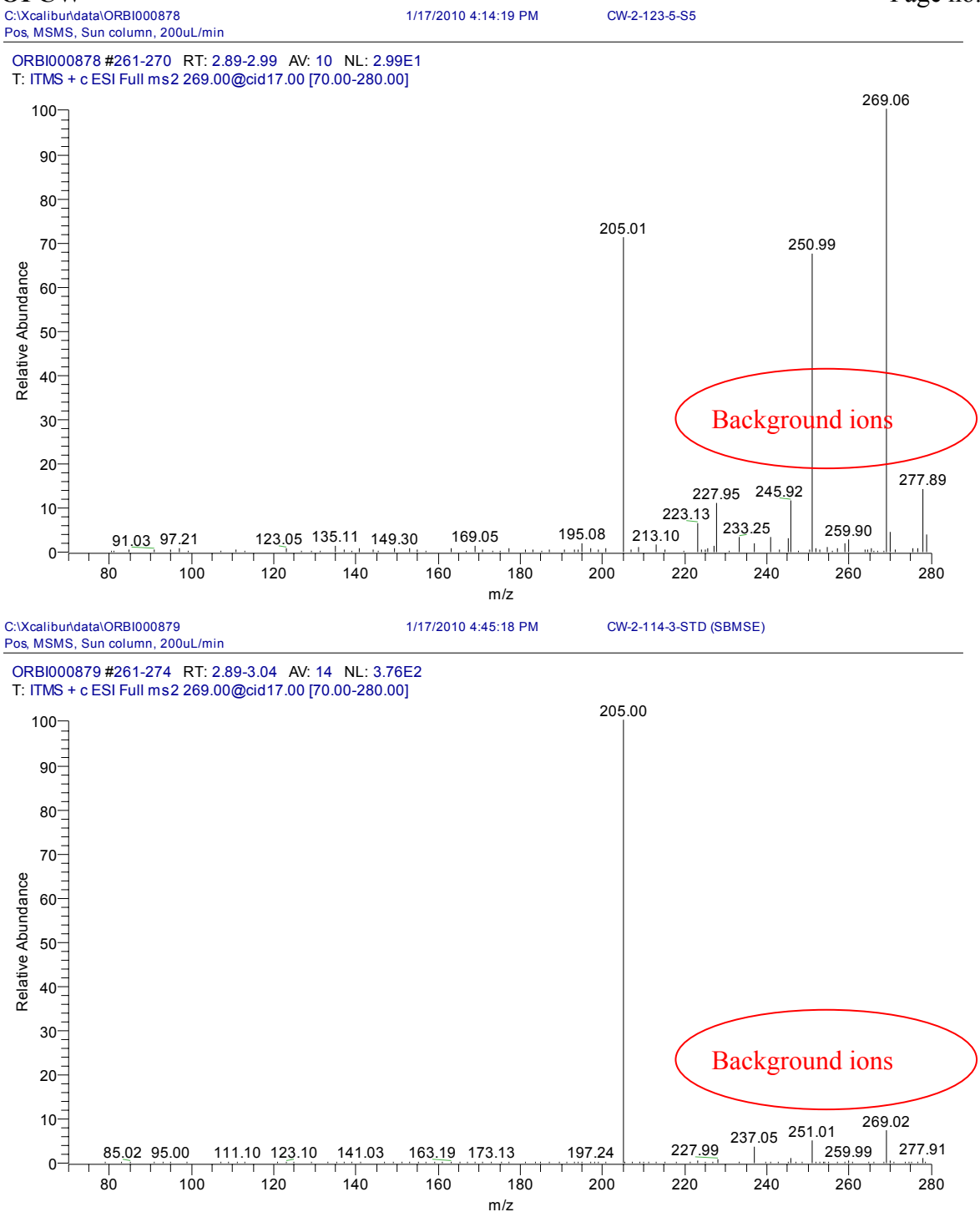
C:\Xcalibur\data\ORBI000879 1/17/2010 4:45:18 PM CW-2-114-3-STD (SBMSE)
Pos, MSMS, Sun column, 200uL/min



LC/MS/MS chromatograms. Top: water blank. Next: Urine blank S1. Third: Urine sample S5. Bottom: reference standard of 1,1'-Sulfonylbis[2-(methylsulfinyl)ethane].



LC/MS/MS EIC (m/z 205) chromatograms. Top: water blank. Next: Urine blank S1. Third: Urine sample S5. Bottom: reference standard of 1,1'-Sulfonylbis[2-(methylsulfinyl)ethane].

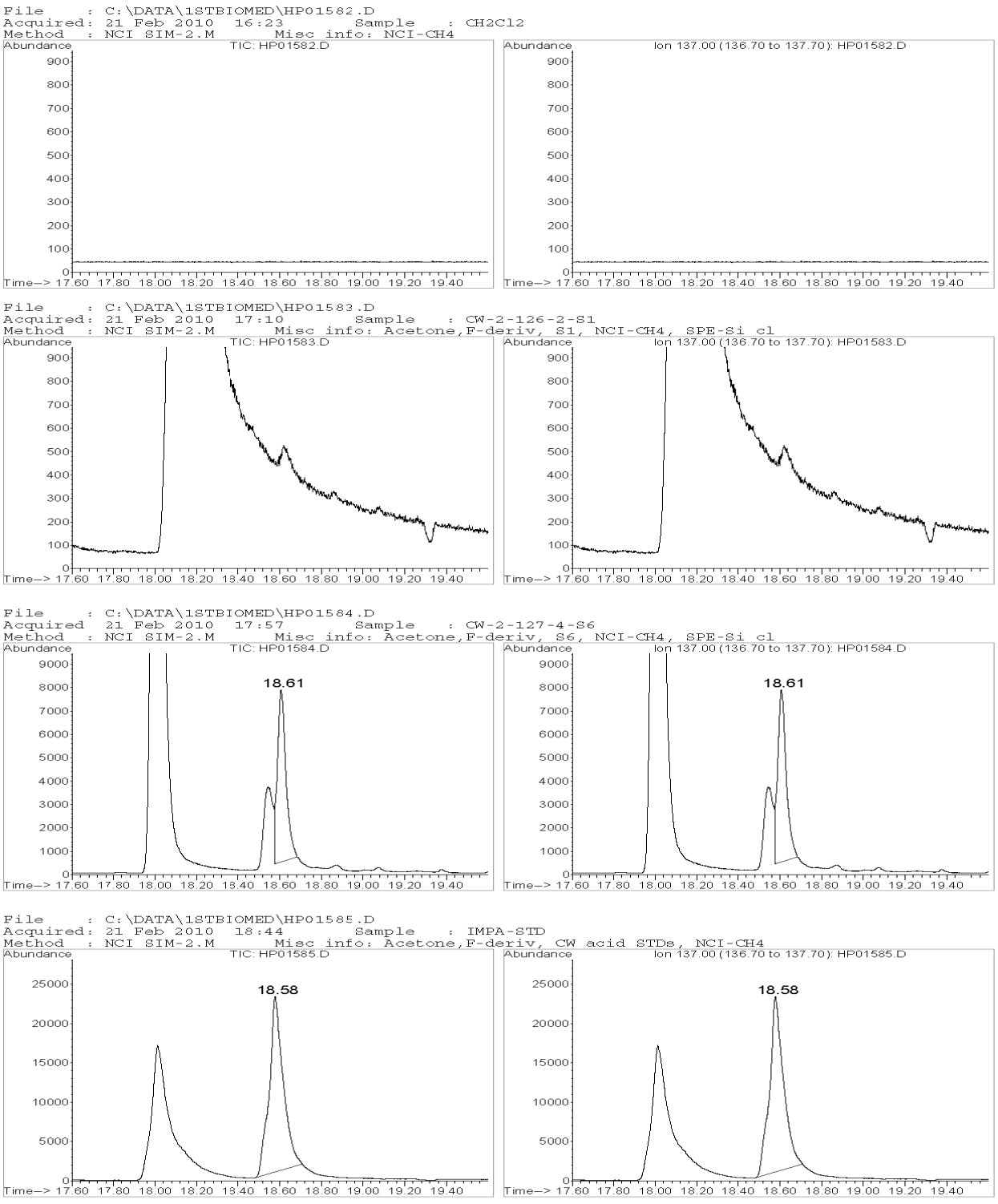


LC/MS/MS spectra – product spectra of parent $[M+Na]^+$ m/z 269. Top: Urine sample S5. Bottom: reference standard of 1,1'-Sulfonylbis[2-(methylsulfinyl)ethane].

RESULTS: URINE SAMPLE S6

β-Lyase metabolites of sulfur mustard were not found in this sample (after sample preparation that included $TiCl_3$ reduction and analysis by GC-GC-MS (EI)).

Isopropyl methyphosophonate was found. The following few pages present the GC-MS (CI) data for the derivative and the LC-MS-MS data for the un-derivitized acid.



CI chromatograms supporting identification; TIC on left; EIC (m/z **137**) on right.

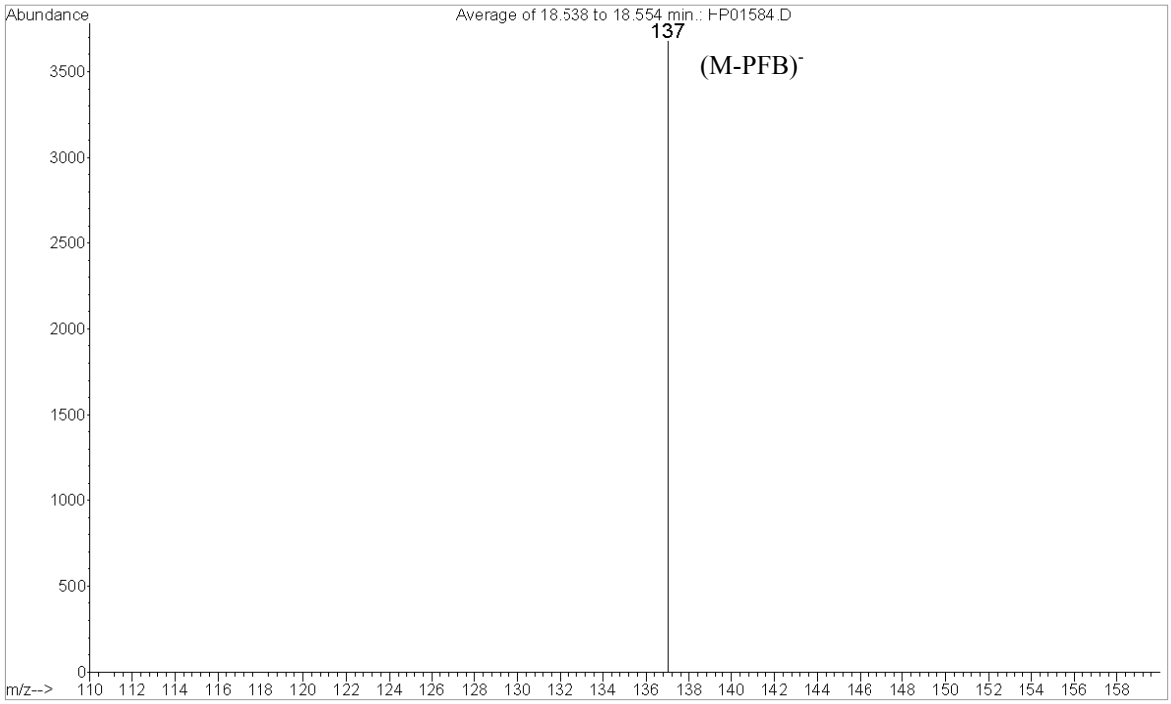
Top: Chromatograms of solvent blank.

Second: Chromatograms of urine blank S1.

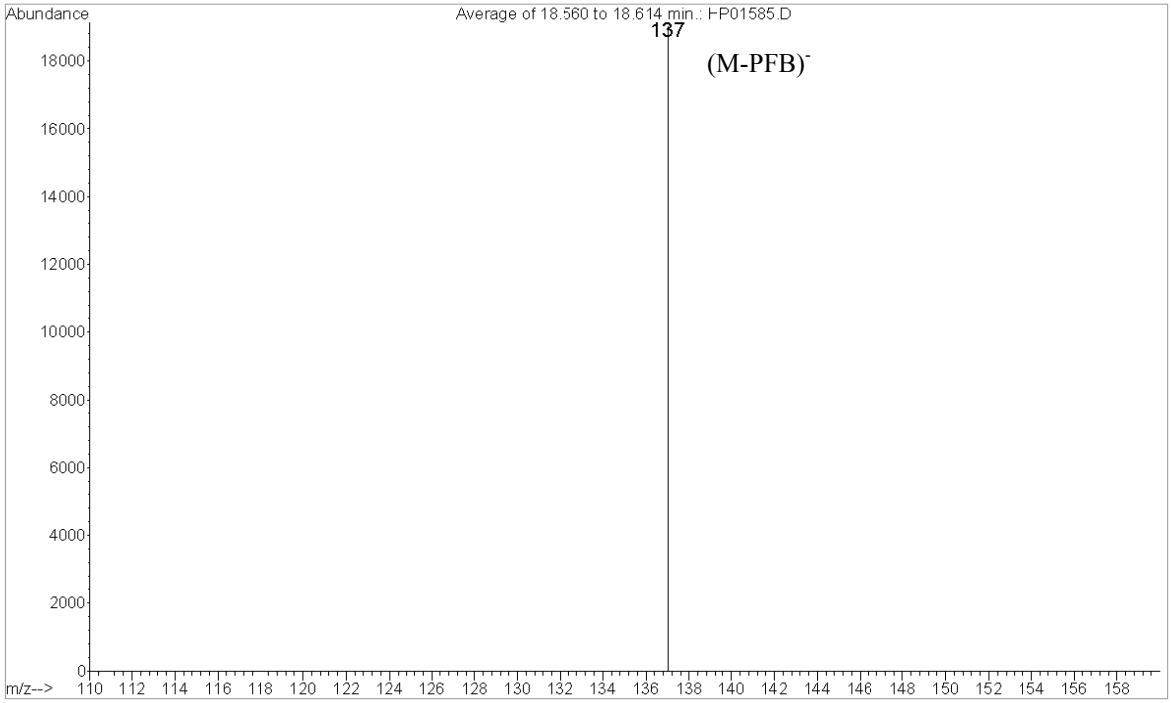
Third: Chromatograms of Urine sample S6, retention time **18.61** min.

Bottom: Chromatograms of reference standard of PFB derivative of **Isopropyl methylphosphonate** retention time **18.58** min.

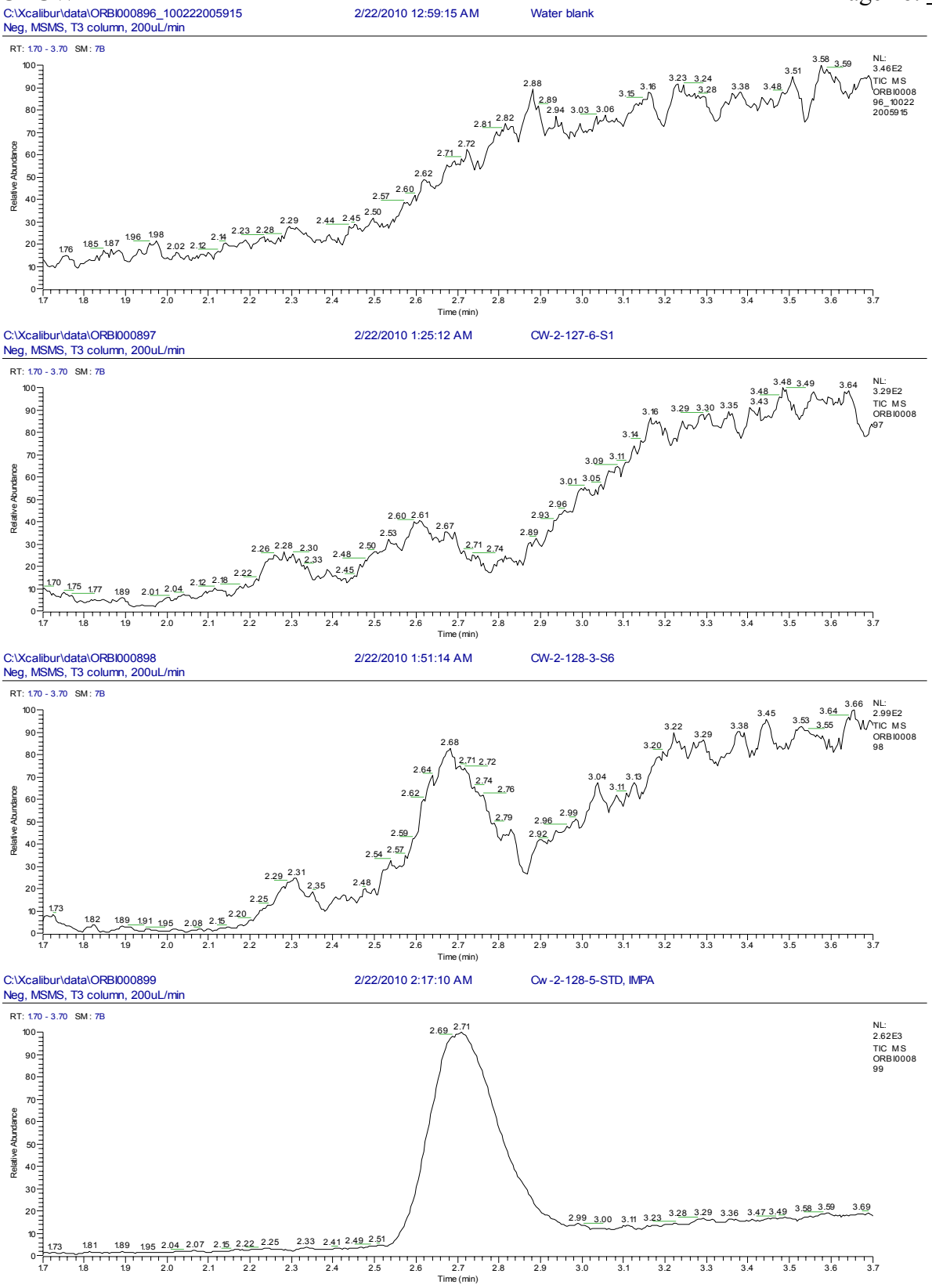
```
File       :C:\DATA\1STBIOMED\HP01584.D
Acquired   : 21 Feb 2010  17:57     using AcqMethod NCI SIM-2.M
Sample Name: CW-2-127-4-S6
Misc Info  : Acetone,F-deriv, S6, NCI-CH4, SPE-Si cl
```




```
File       :C:\DATA\1STBIOMED\HP01585.D
Acquired   : 21 Feb 2010  18:44     using AcqMethod NCI SIM-2.M
Sample Name: IMPA-STD
Misc Info  : Acetone,F-deriv, CW acid STDs, NCI-CH4
```

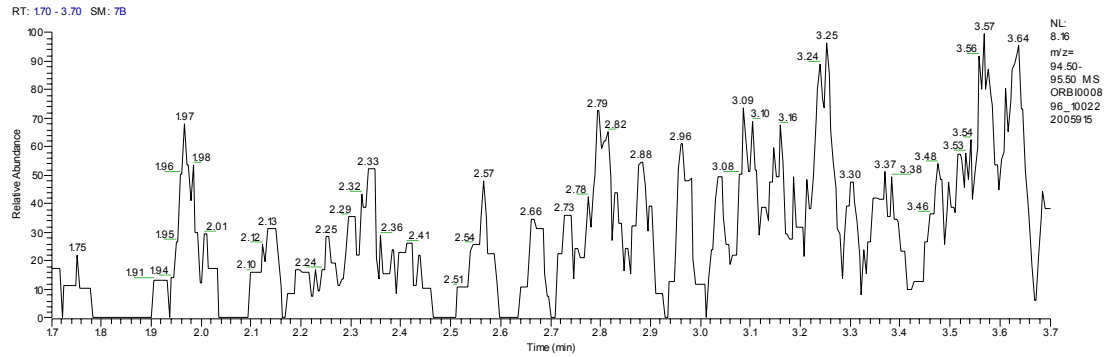


CI SIM mass spectrum (m/z 137) of:
Top: Spectrum of peak of interest from Urine sample S6.
Bottom: Reference standard of PFB derivative of **Isopropyl methylphosphonate** (MW: **319**).

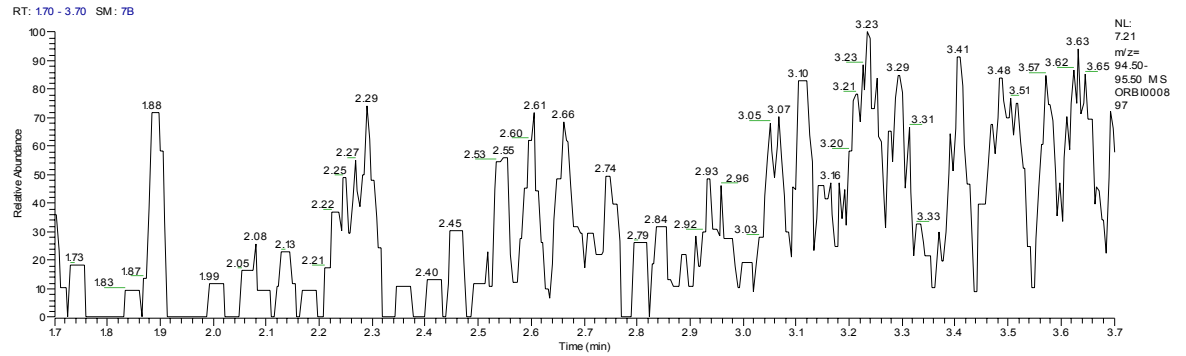


LC/MS/MS chromatograms. Top: water blank. Next: Urine blank S1. Third: Urine sample S6. Bottom: reference standard of **Isopropyl methylphosphonate**

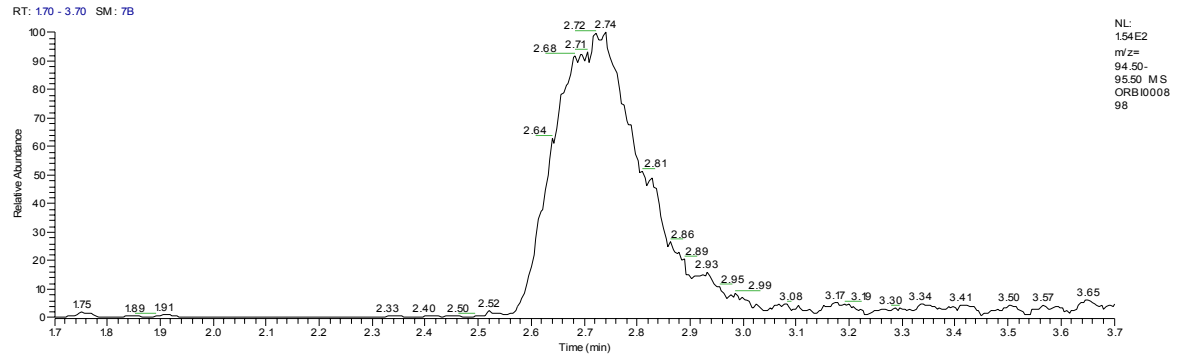
C:\Xcalibur\data\ORBI000896_100222005915 2/22/2010 12:59:15 AM Water blank
Neg, MSMS, T3 column, 200uL/min



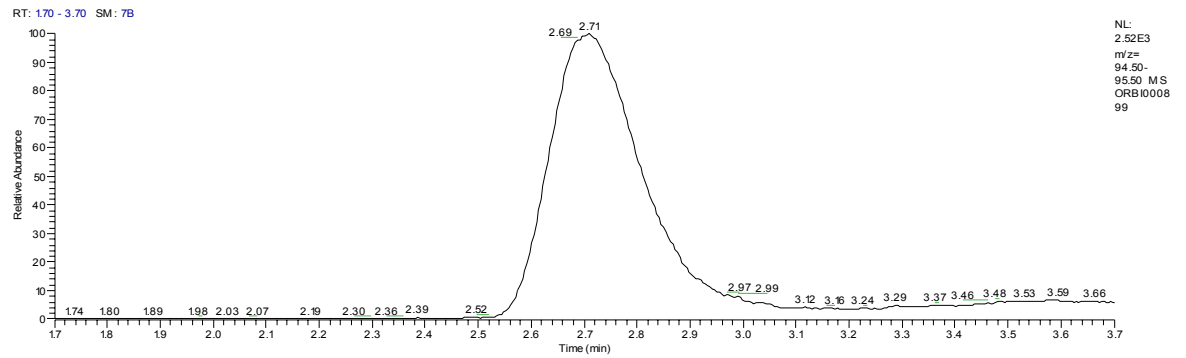
C:\Xcalibur\data\ORBI000897 2/22/2010 1:25:12 AM CW-2-127-6-S1
Neg, MSMS, T3 column, 200uL/min



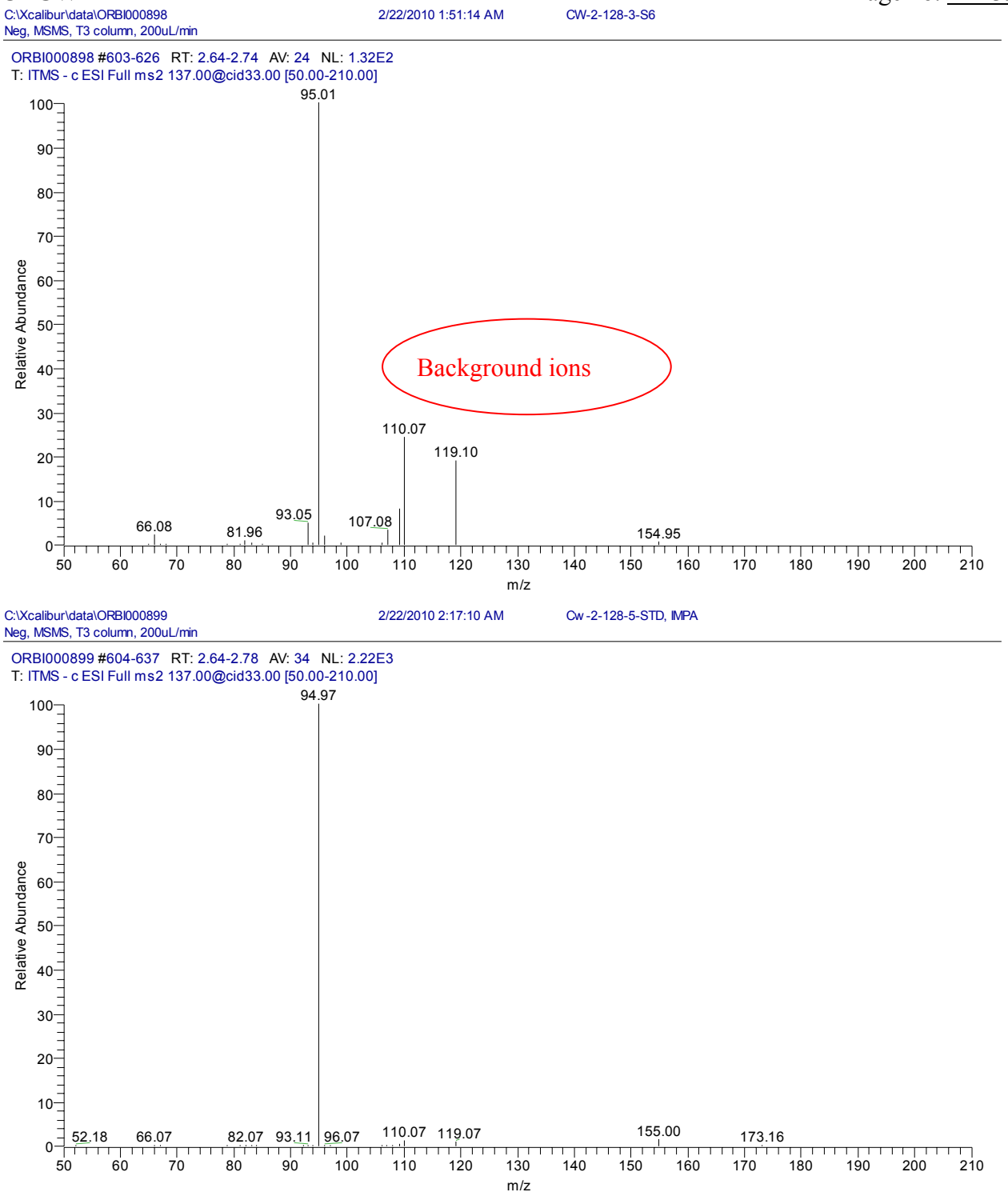
C:\Xcalibur\data\ORBI000898 2/22/2010 1:51:14 AM CW-2-128-3-S6
Neg, MSMS, T3 column, 200uL/min



C:\Xcalibur\data\ORBI000899 2/22/2010 2:17:10 AM Cw-2-128-5-STD, IMPA
Neg, MSMS, T3 column, 200uL/min



LC/MS/MS EIC (m/z 95) chromatograms. Top: water blank. Next: Urine blank S1. Third: Urine sample S6. Bottom: reference standard of **Isopropyl methylphosphonate**



LC/MS/MS spectra – product spectra of parent $[M-H]^-$ m/z 137. Top: Urine sample S6. Bottom: reference standard of **Isopropyl methylphosphonate**

RESULTS: URINE SAMPLE S7

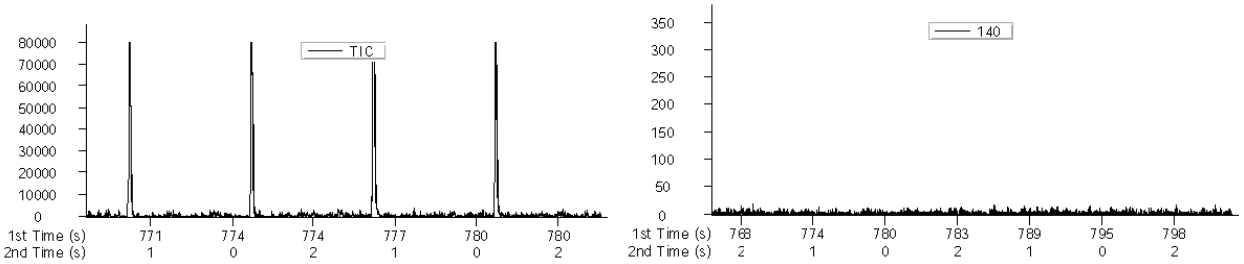
A β-Lyase metabolite of sulfur mustard was found in this sample (after sample preparation that included $TiCl_3$ reduction and analysis by GC-GC-MS (EI)) and confirmed with GC-MS (CI) and LC-MS-MS.

Present was 1,1'-Sulfonylbis[2-(methylsulfinyl)ethane] (SBMSE), but no evidence of 1-Methylsulfinyl-2-[2-(methylthio)ethylsulfonyl]ethane (MSMTESE) was observed.

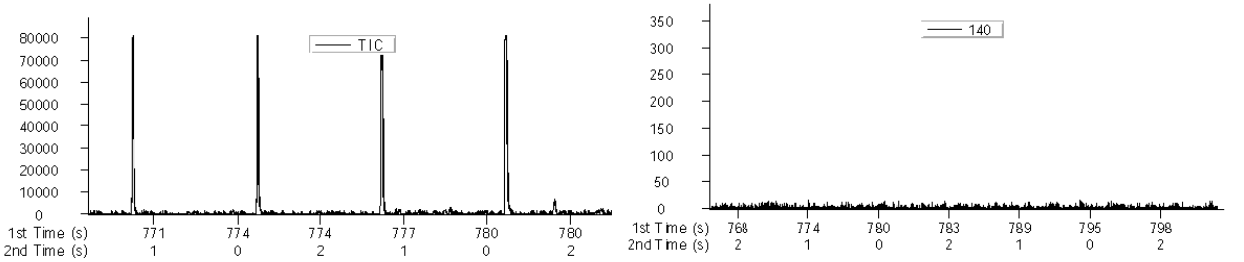
None of the phosphonic acids were found.

The following few pages present the GC-GC-MS (EI) and GC-MS (CI) data for the derivative and the LC-MS-MS data for the un-derivitized metabolite.

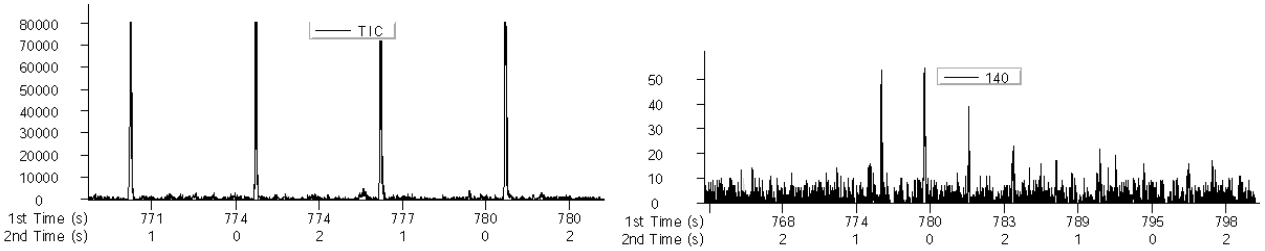
File : C:\LLNL2010\TOF75
Acquired: 14 Jan 2009 4:52:51 PM Sample: CH2Cl2 Blank Lot# 7284M



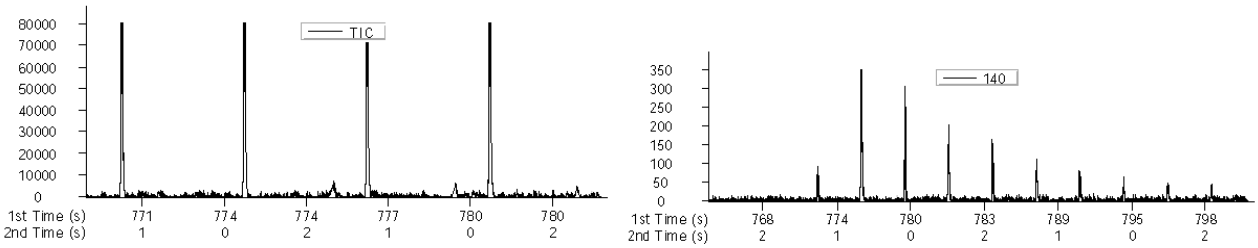
File : C:\LLNL2010\TOF76
Acquired: 14 Jan 2009 5:29:26 PM Sample: CW-2-122-3-S1



File : C:\LLNL2010\TOF77
Acquired: 14 Jan 2009 6:05:59 PM Sample: CW-2-123-3-S7

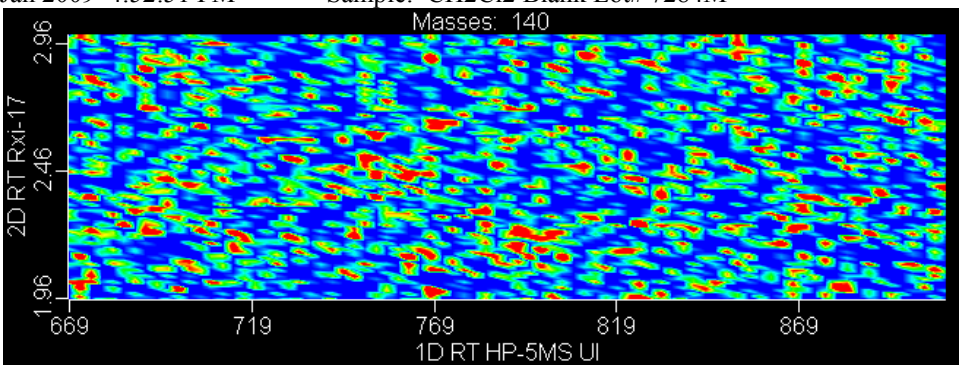


File : C:\LLNL2010\TOF78
Acquired: 14 Jan 2009 4:16:16 PM Sample: 0.5 ppm SBMTE

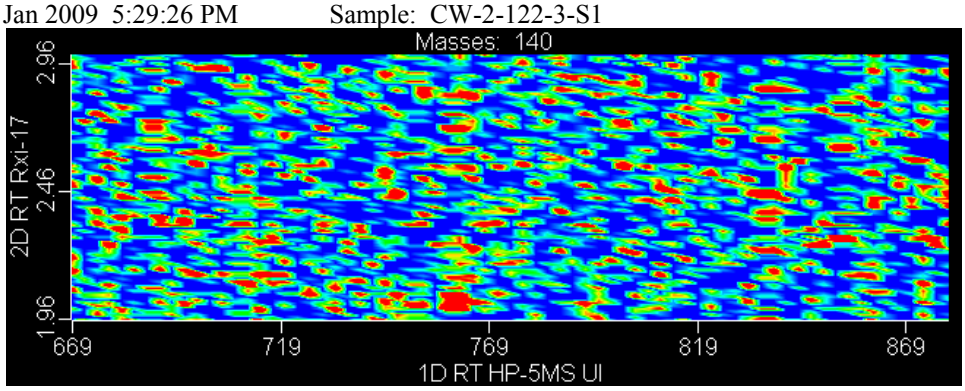


EI chromatograms supporting identification; TIC on left; EIC (m/z **140**) on right
Top: Chromatograms of solvent blank.
Second: Chromatograms of urine blank S1.
Third: Chromatograms or urine sample S7, retention time 774, 2.658 sec
Bottom: Chromatograms of reference standard of **1,1'-Sulfonylbis[2-9methylthio)ethane]**, retention time 774, 2.655 sec

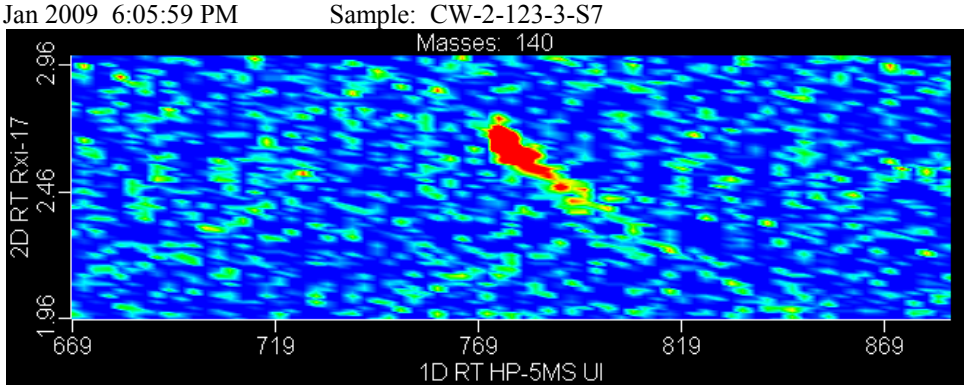
File : C:\LLNL2010\TOF75
Acquired: 14 Jan 2009 4:52:51 PM Sample: CH2Cl2 Blank Lot# 7284M



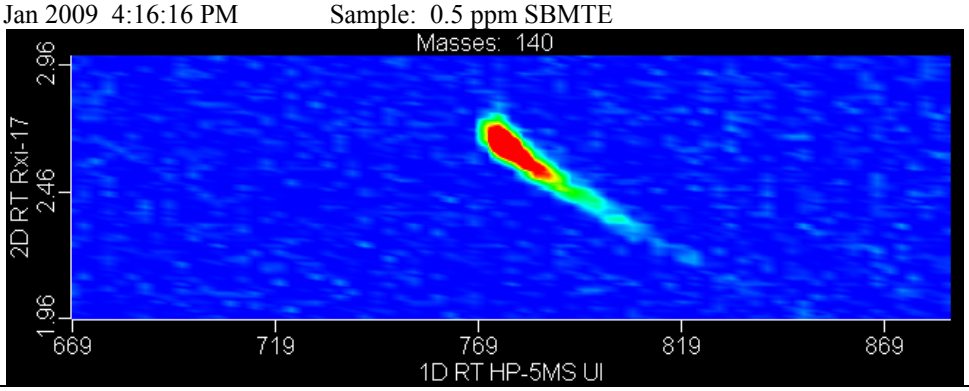
File : C:\LLNL2010\TOF76
Acquired: 14 Jan 2009 5:29:26 PM Sample: CW-2-122-3-S1



File : C:\LLNL2010\TOF77
Acquired: 14 Jan 2009 6:05:59 PM Sample: CW-2-123-3-S7



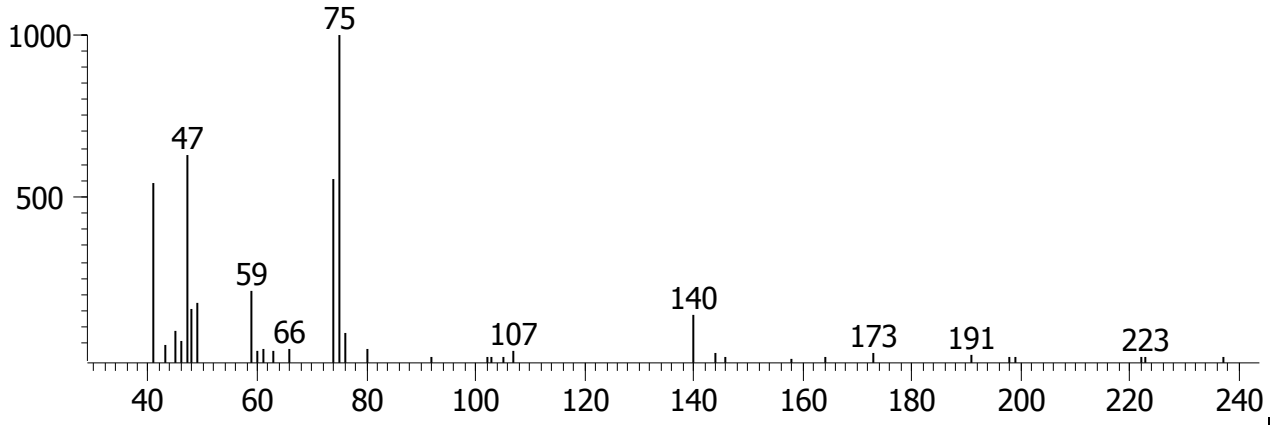
File : C:\LLNL2010\TOF78
Acquired: 14 Jan 2009 4:16:16 PM Sample: 0.5 ppm SBMTE



2D Contour plot supporting identification; EIC (m/z **140**)
Top: Contour plot of solvent blank; Second: Contour plot of urine blank S1; Third: Contour plot of urine sample S7, retention time 774, 2.658 sec; Bottom: Contour plot of reference standard of **1,1'-Sulfonylbis[2-9methylthio)ethane]**, retention time 774, 2.655 sec

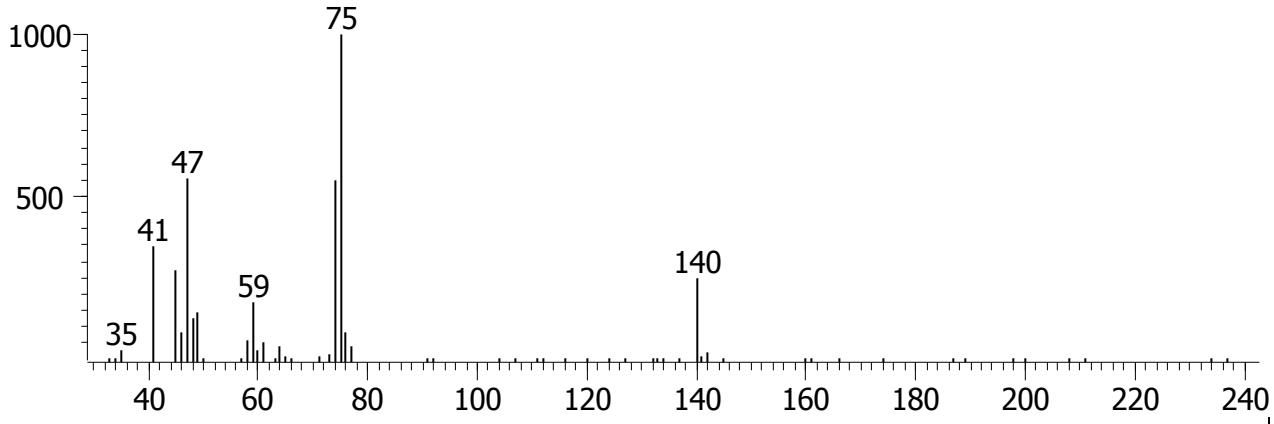
File : C:\LLNL2010\TOF77
Acquired: 14 Jan 2009 6:05:59 PM Sample: CW-2-123-3-S7

Peak True - sample "TOF:77", peak 150, at 774 , 2.658 sec , sec

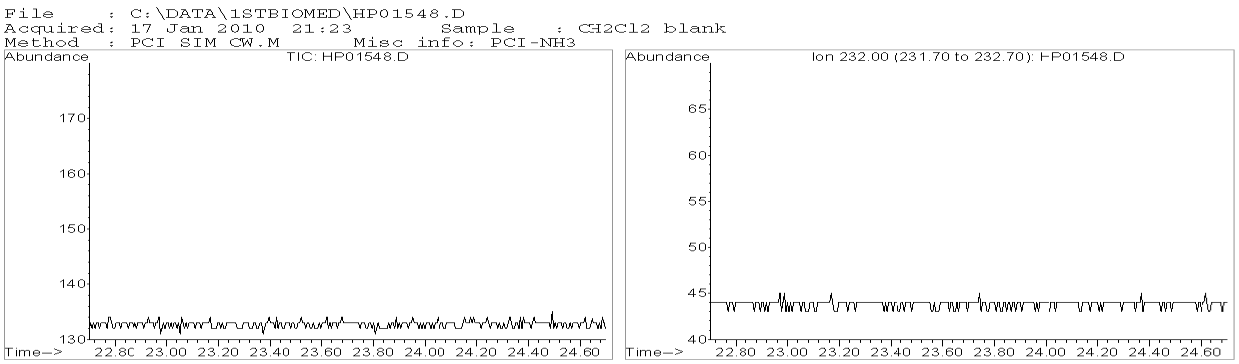


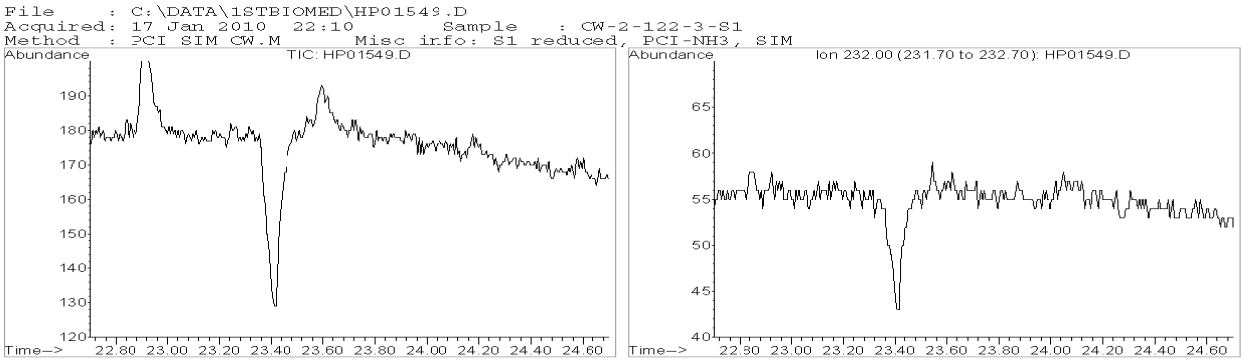
File : C:\LLNL2010\TOF78
Acquired: 14 Jan 2009 4:16:16 PM Sample: 0.5 ppm SBMTE

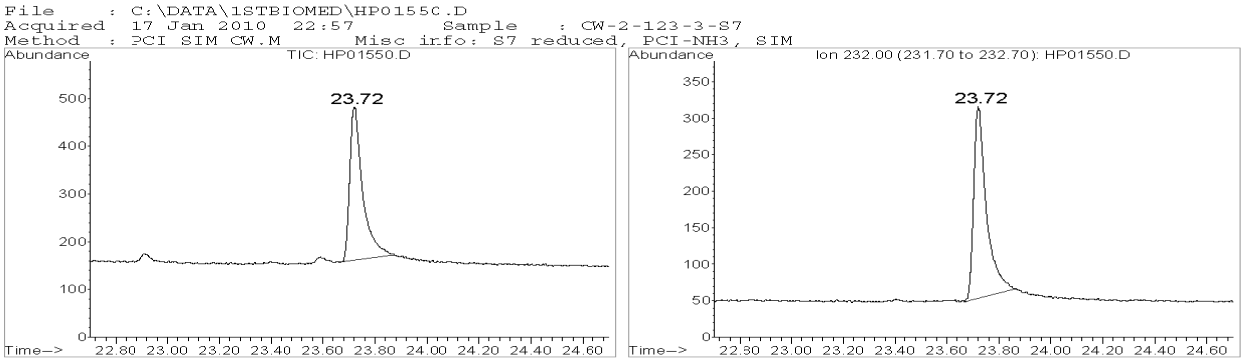
Peak True - sample "TOF:78", peak 120, at 774 , 2.655 sec , sec

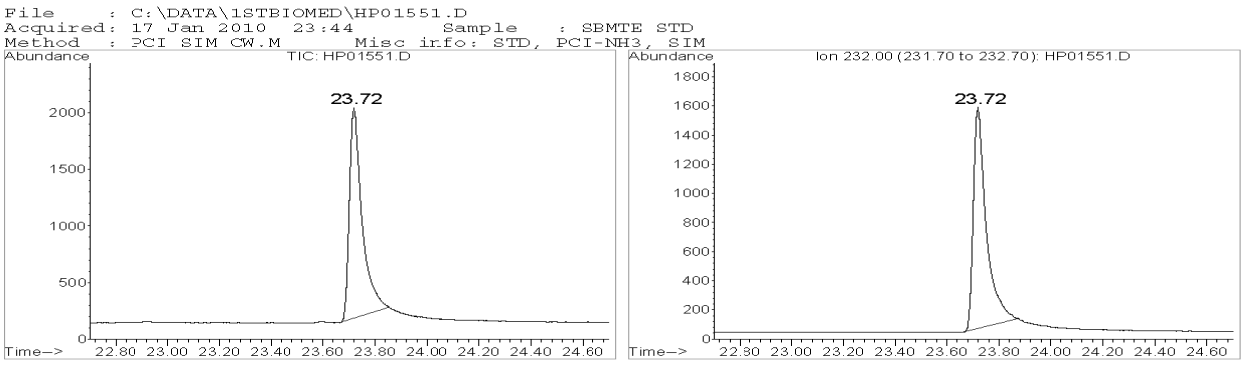


EI mass spectrum (m/z 75, 140) of:
Top: Spectrum of urine sample S7, retention time 774, 2.658 sec
Bottom: Reference standard of **1,1'-Sulfonylbis[2-9methylthio)ethane]. (MW: 232).**









CI chromatograms supporting identification; TIC on left; EIC (m/z **232**) on right.

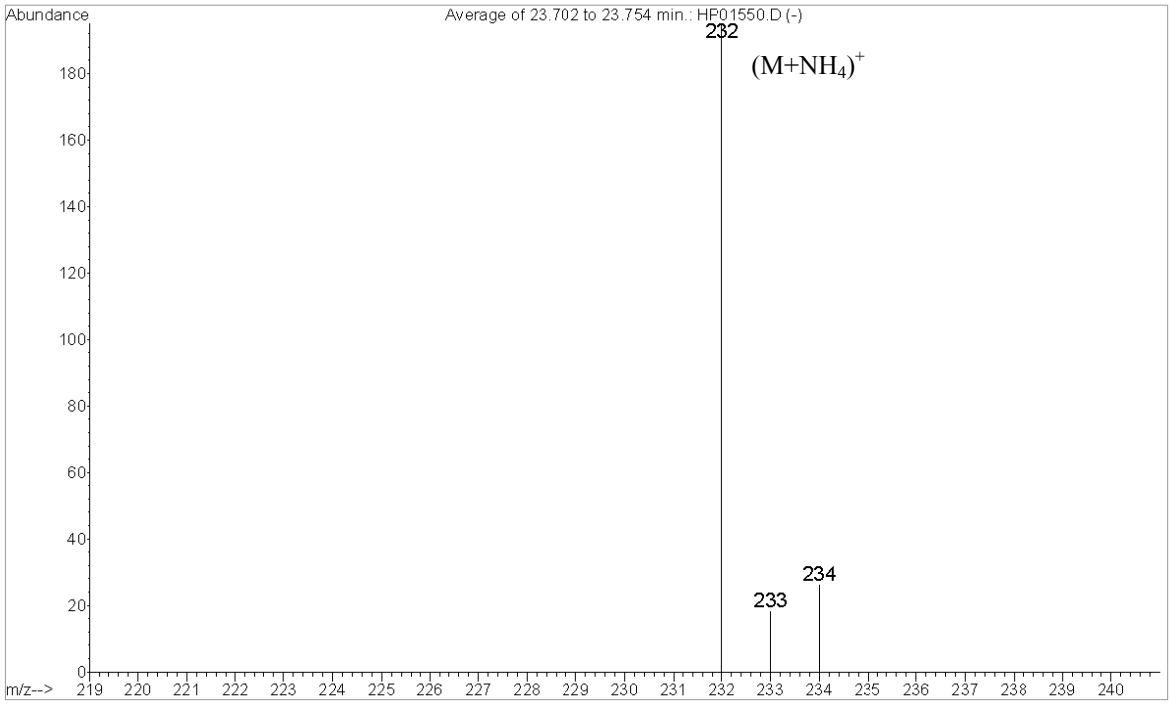
Top: Chromatograms of solvent blank.

Second: Chromatograms of urine blank S1.

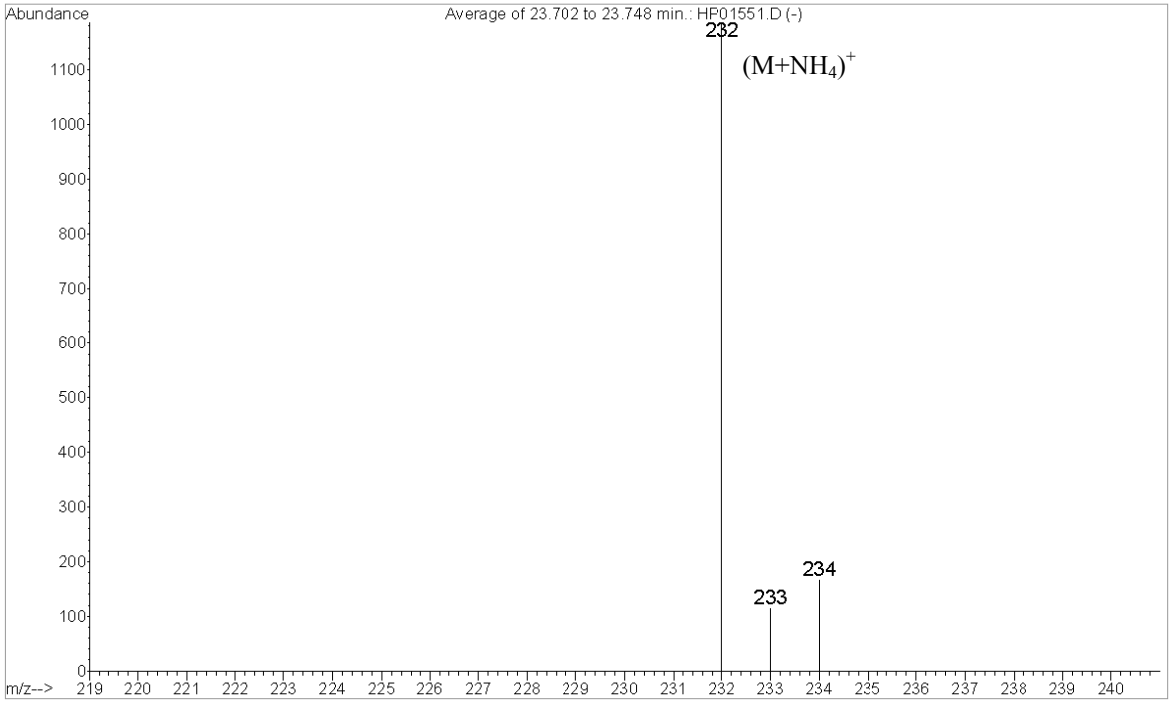
Third: Chromatograms of Urine sample S7, retention time **23.72** min.

Bottom: Chromatograms of reference standard of **1,1'-Sulfonylbis[2-(methylthio)ethane]**, retention time **23.72** min.

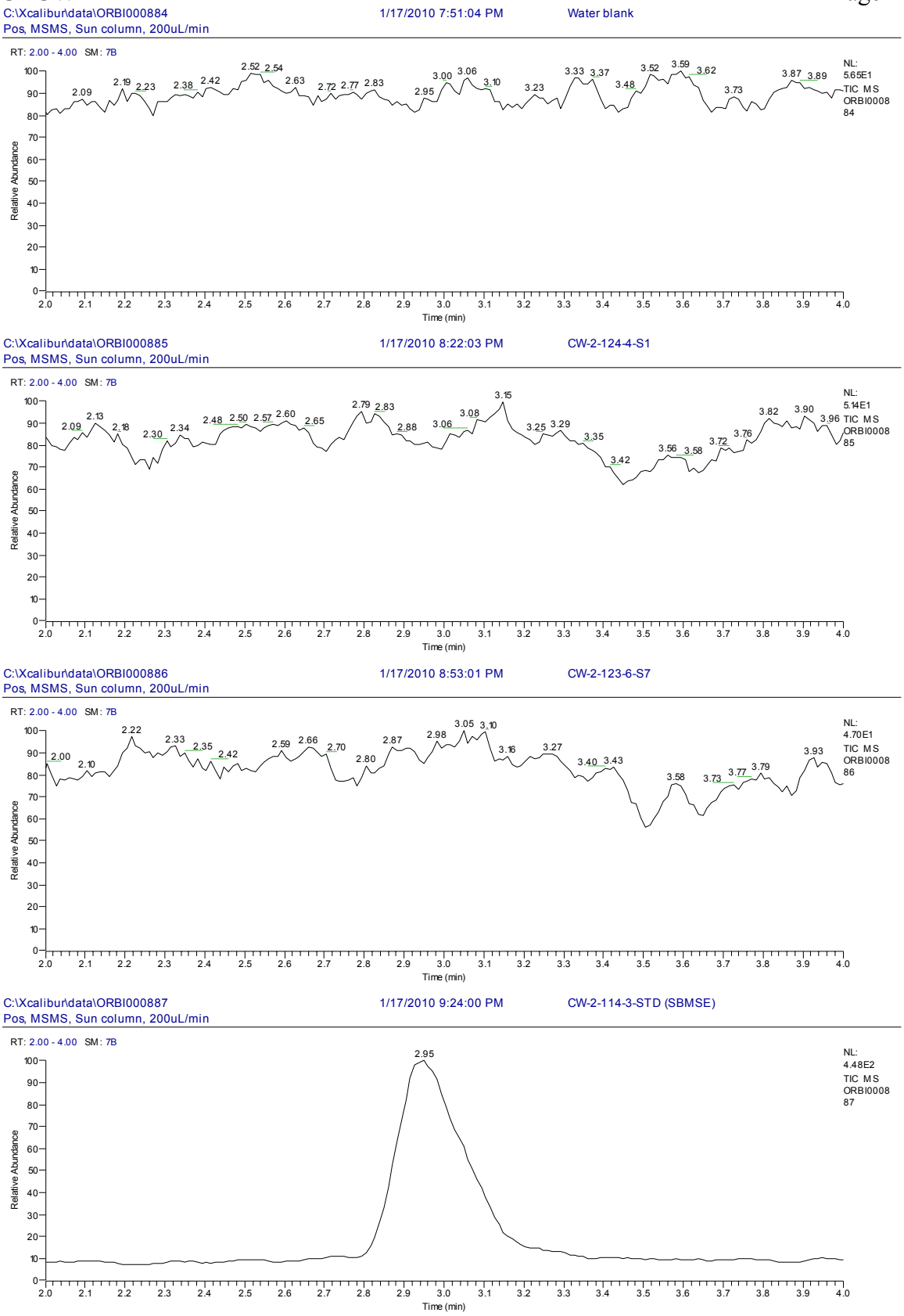
```
File       :C:\DATA\1STBIOMED\HP01550.D
Acquired   : 17 Jan 2010  22:57     using AcqMethod PCI SIM CW.M
Sample Name: CW-2-123-3-S7
Misc Info  : S7 reduced, PCI-NH3, SIM
```




```
File       :C:\DATA\1STBIOMED\HP01551.D
Acquired   : 17 Jan 2010  23:44     using AcqMethod PCI SIM CW.M
Sample Name: SBMTE STD
Misc Info  : STD, PCI-NH3, SIM
```

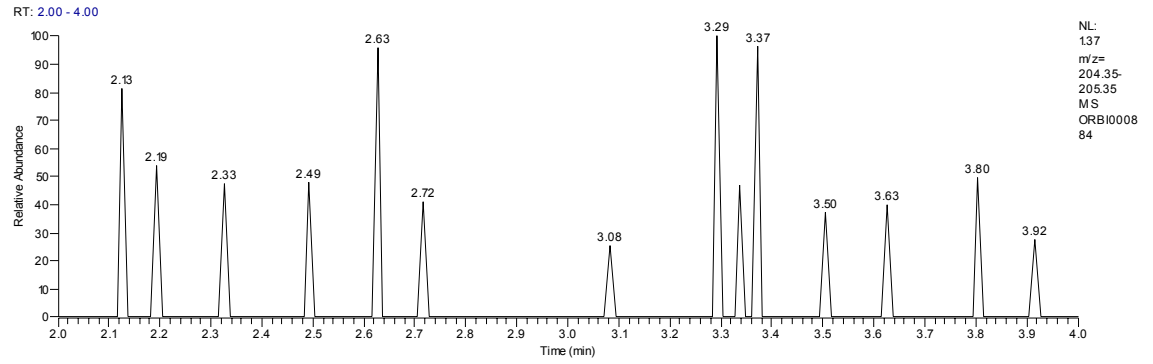


CI SIM mass spectrum (m/z 232, 233, 234) of:
Top: Spectrum of peak of interest from Urine sample S7.
Bottom: Reference standard of **1,1'-Sulfonylbis[2-(methylthio)ethane]**. (MW: **214**).

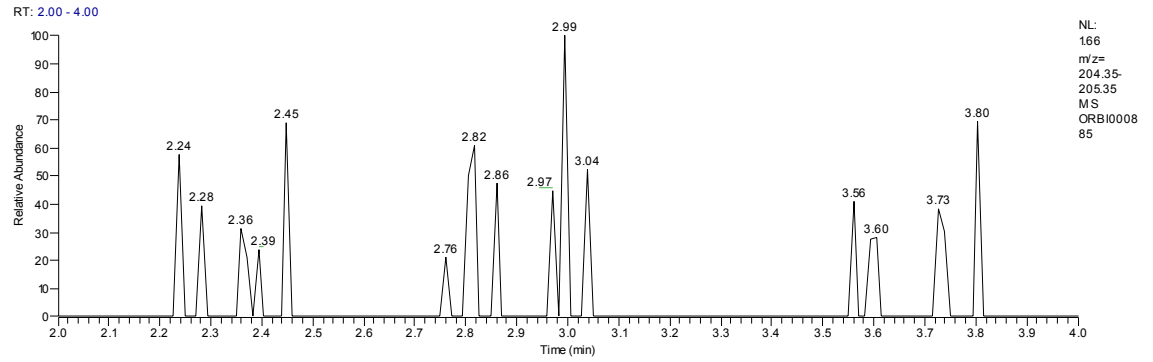


LC/MS/MS chromatograms. Top: water blank. Next: Urine blank S1. Third: Urine sample S7. Bottom: reference standard of 1,1'-Sulfonylbis[2-(methylsulfinyl)ethane].

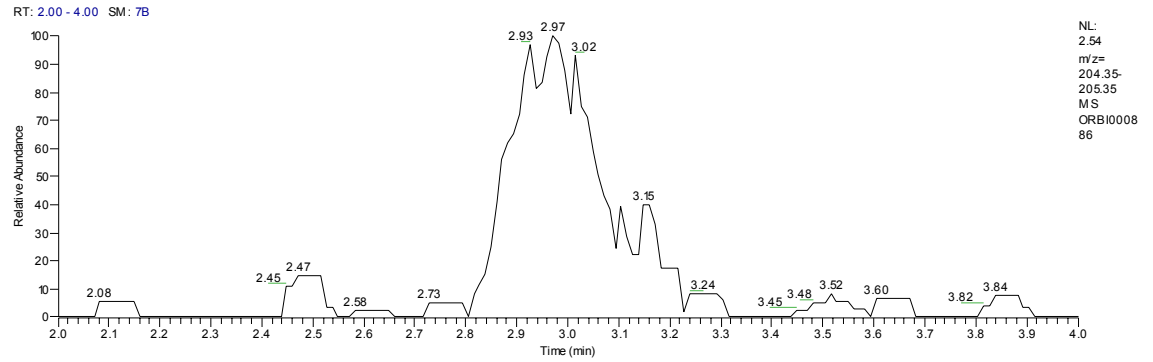
C:\Xcalibur\data\ORBI000884 1/17/2010 7:51:04 PM Water blank
Pos, MSMS, Sun column, 200uL/min



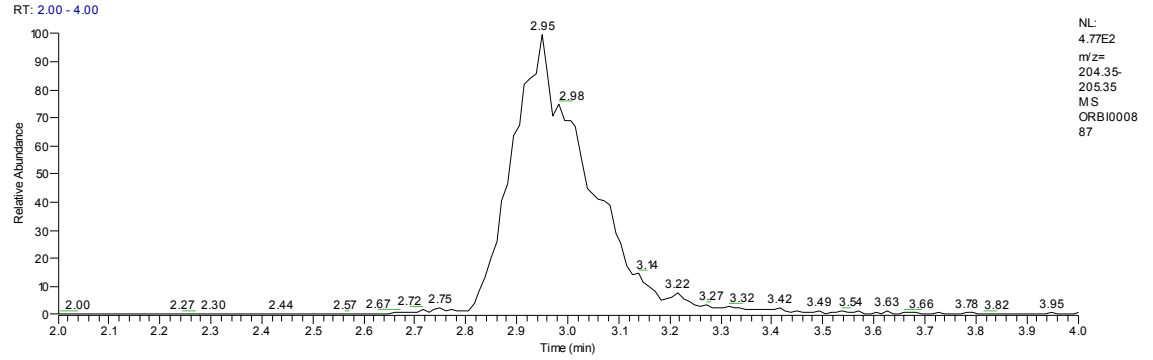
C:\Xcalibur\data\ORBI000885 1/17/2010 8:22:03 PM CW-2-124-4-S1
Pos, MSMS, Sun column, 200uL/min



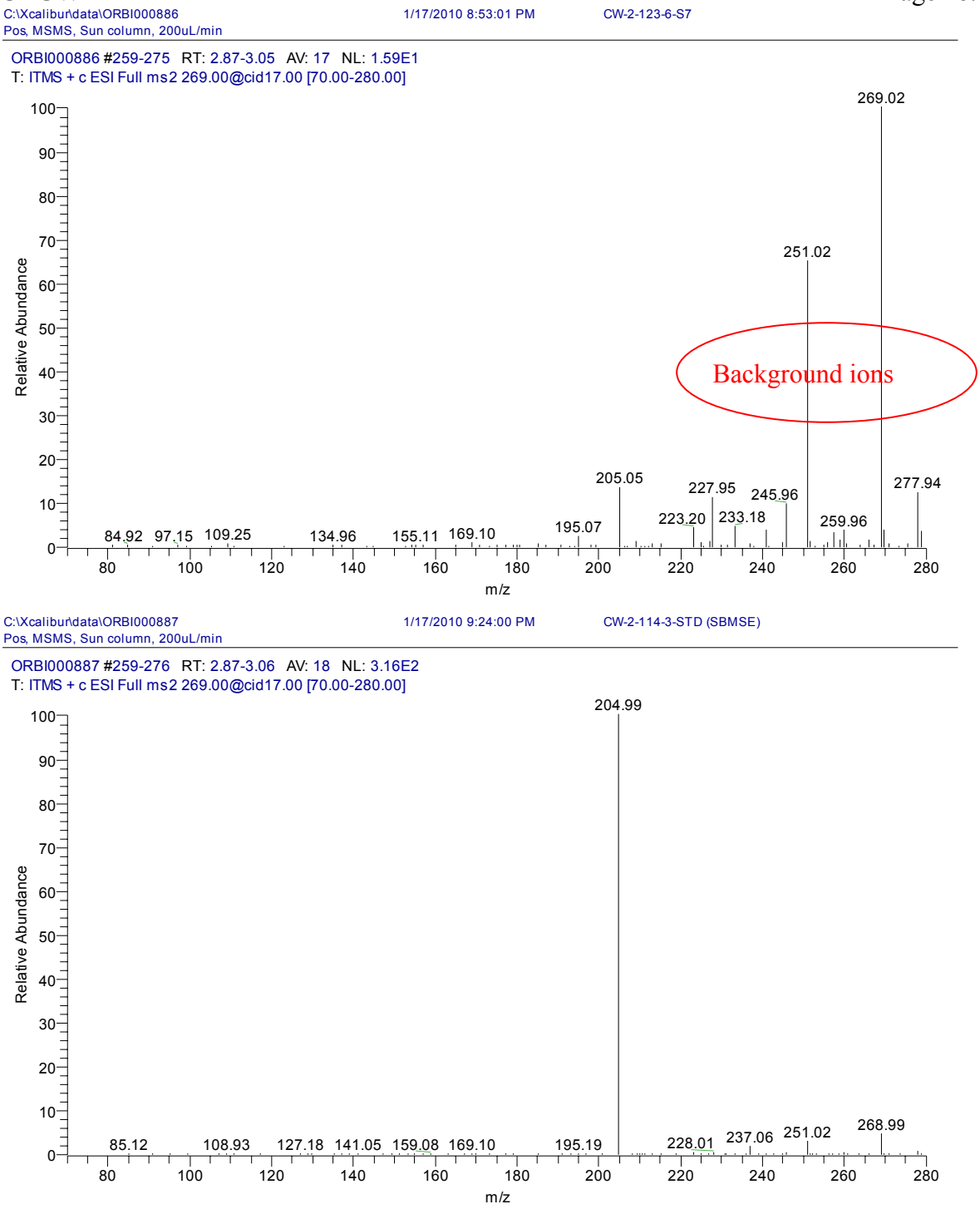
C:\Xcalibur\data\ORBI000886 1/17/2010 8:53:01 PM CW-2-123-6-S7
Pos, MSMS, Sun column, 200uL/min



C:\Xcalibur\data\ORBI000887 1/17/2010 9:24:00 PM CW-2-114-3-STD (SBMSE)
Pos, MSMS, Sun column, 200uL/min



LC/MS/MS EIC (m/z 205) chromatograms. Top: water blank. Next: Urine blank S1. Third: Urine sample S5. Bottom: reference standard of 1,1'-Sulfonylbis[2-(methylsulfinyl)ethane].



LC/MS/MS spectra – product spectra of parent $[M+Na]^+$ m/z 269. Top: Urine sample S5. Bottom: reference standard of 1,1'-Sulfonylbis[2-(methylsulfinyl)ethane].

COMMENTS

1. General

Samples were screened with GC/MS techniques, then confirmed with LC/MS.

2. Sample preparation

3. Analysis

No chemicals relevant to this scenario were present in sample 4.

4. Statement on the absence of derivatized chemicals(s) in original sample(s) and/or ambiguity of Lewisite(s) identification