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LLNL-TR-635888

Report of the Third Biomedical Confidence Building Exercise

A. Alcaraz, A. Williams

April 30, 2013

Disclaimer

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 Third Biomedical Confidence Building Exercise

Laboratory code: 07

Total number of pages: 77

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SUMMARY: PARTICIPATING LABORATORY

1. Participating laboratory

Name of the laboratory/institute:	Lawrence Livermore National Laboratory
Contact person:	Mr. Armando Alcaraz
Address:	7000 East Ave, L-091 Livermore, CA 94550 USA
Telephone number:	1-925-423-6889
Email address:	alcaraz1@llnl.gov
Date of sample receipt:	February 5th, 2013
Date of report:	April 30th, 2013

2. Analysts and authentication

	Name	Title	Signature
1	Armando Alcaraz	Pricipal Investigator	
2	Deon Anex	Research Scientist	
3	Bradley Hart	FSC Director	
4	Saphon Hok	Research Scientist	
5	Carolyn Koester	Research Scientist	
6	Roald Leif	Research Scientist	
7	Brian Mayer	Research Scientist	
8	Tuijuana Mitchell-Hall	QA Manager	
9	Heather Mulcahy	Research Scientist	
10	Carlos Valdez	Research Scientist	
11	Alexander Vu	Research Scientist	
12	Audrey Williams	Research Scientist	

SUMMARY: QUALITY SYSTEM

Quality system:

☒ Described in a Quality Assurance Manual/Handbook. Quality system in accordance with:
☒ ISO/IEC 17025 ☐ Other/none: (describe below)

☒ Accreditation accepted; Year of accreditation: 2001
☐ Accreditation planned/pending; Year accreditation expected: _____

Accreditation body: A2LA
Scope of accreditation: CW analysis

Summary for laboratories with either "other" accreditation or no planned accreditation:

SAMPLE SUMMARY: A

Sample Code: P-301/07	**Laboratory Assigned Code:** CW-4-147-1
Description and condition of sample: Approximately 5 mL of plasma	

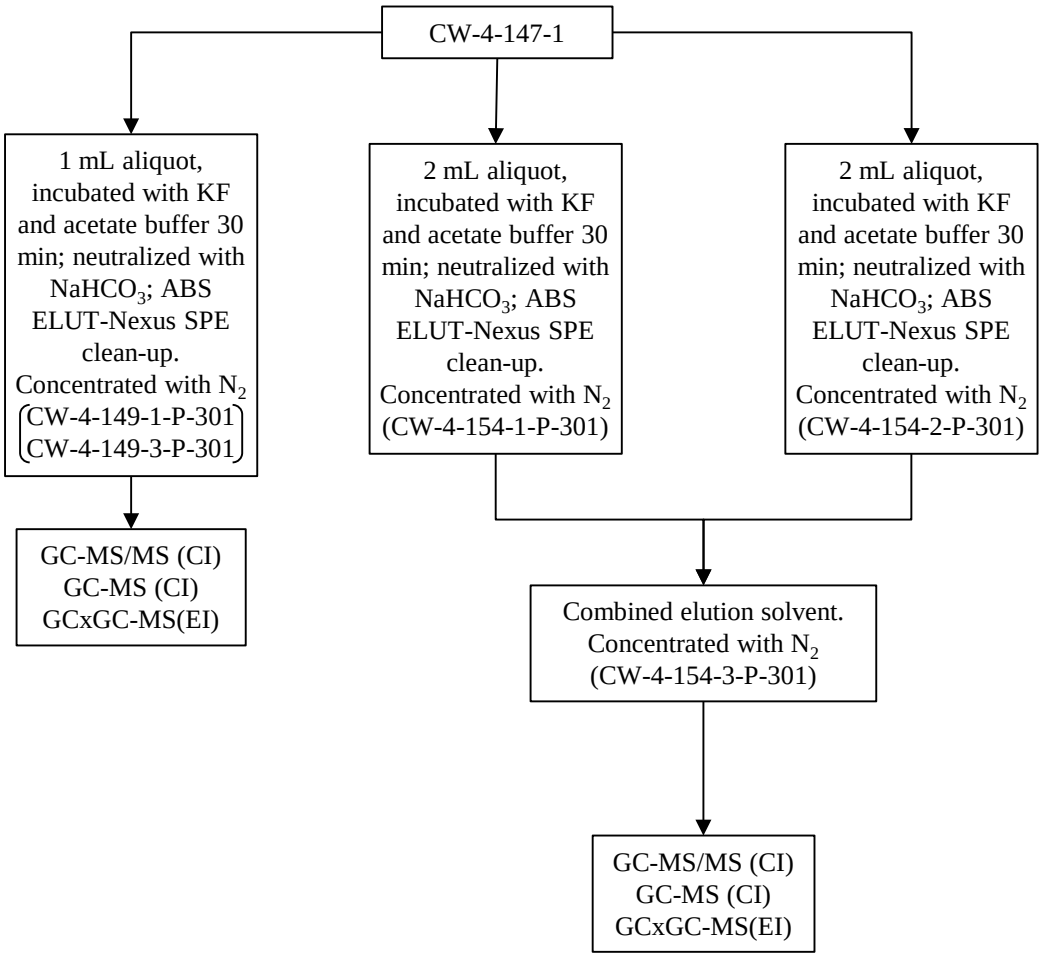
No chemicals relevant to the purpose of analysis were found.

SAMPLE PREPARATION DESCRIPTION: A

1. Sample preparation

Initial Aliquot Code	Type of Sample Preparation	Amount/ Volume	Sample/Blank Preparation Procedures	End Volume	Resulting Aliquot Code
CW-4-147-1	Fluoride Reactivation	1 mL	Added 3 mL of acetate buffer and 220 µL of 4.51 M KF. Incubated 30 minutes at room temperature. Neutralized with 0.8M Sodium Bicarbonate. ABS ELUT-Nexus cartridge cleanup. Eluted using 2 mL ethyl acetate and reduced sample volume to approximately 100 µL using nitrogen gas.	100 µL	CW-4-149-1-P-301
CW-4-149-1-P-301	Sample Split	50 µL	Split of sample CW-4-149-1-P-301.	50 µL	CW-4-149-3-P-301
CW-4-147-1	Fluoride Reactivation	2 mL	Added 3 mL of acetate buffer and 277 µL of 4.51 M KF. Incubated 30 minutes at room temperature. Neutralized with 0.8 M Sodium Bicarbonate. ABS ELUT-Nexus cartridge cleanup. Eluted using 2 mL ethyl acetate.	2 mL	CW-4-154-1-P-301
CW-4-147-1	Fluoride Reactivation	2 mL	Added 3mL of acetate buffer and 277 µL of 4.51 M KF. Incubated 30 minutes at room temperature. Neutralized with 0.8 M Sodium Bicarbonate. ABS ELUT-Nexus cartridge cleanup. Eluted using 2 mL ethyl acetate.	2 mL	CW-4-154-2-P-301
CW-4-154-1-P-301 CW-4-154-2-P-301	Concentrate combined samples	2 mLx2	Combined elution solvent from CW-4-154-1-P-301 and CW-4-154-2-P-301. Reduced sample volume to approximately 100 µL using nitrogen gas.	100 µL	CW-4-154-3-P-301

2. Additional information


CW-4-147-1
1 mL aliquot, incubated with KF and acetate buffer 30 min; neutralized with $NaHCO_3$; ABS ELUT-Nexus SPE clean-up. Concentrated with N_2 (CW-4-149-1-P-301 CW-4-149-3-P-301)
2 mL aliquot, incubated with KF and acetate buffer 30 min; neutralized with $NaHCO_3$; ABS ELUT-Nexus SPE clean-up. Concentrated with N_2 (CW-4-154-1-P-301)
2 mL aliquot, incubated with KF and acetate buffer 30 min; neutralized with $NaHCO_3$; ABS ELUT-Nexus SPE clean-up. Concentrated with N_2 (CW-4-154-2-P-301)
GC-MS/MS (CI) GC-MS (CI) GCxGC-MS(EI)
Combined elution solvent. Concentrated with N_2 (CW-4-154-3-P-301)
GC-MS/MS (CI) GC-MS (CI) GCxGC-MS(EI)

Description of sample preparation and analysis methods

Sample preparation and analysis methods were developed using an in-house standard made by incubating the nerve agent, Sarin, with commercially procured human blood plasma. Cholinesterase activities of the plasma sample were measured using method outlined in Ellman (1961) before and after incubation to ensure agent-adduction occurred. The resultant agent-adducted plasma was used for subsequent method development.

Adducted plasma samples were reactivated using the process outlined in Degenhardt *et al* (2004). 1 mL of plasma was added to 3 mL of acetate buffer solution [0.189 M acetic acid; 10.8 mM sodium acetate]. Appropriate amount of Potassium Fluoride [4.51M] was added to achieve a final concentration of 0.25 M KF in the unknown sample work-up. Sample was allowed to incubate at room temperature for 30 minutes. 0.5 mL of 0.8 M sodium bicarbonate was added to neutralize acid.

Several methods were attempted to isolate the reactivated sarin from the sample:

1. Direct dichloromethane extraction
2. SPE preparation #1
   Agilent ABS Elut-NEXUS (200 mg/ 6 mL)
   Conditioning: 2 mL N-hexane; 4 mL Ethyl Acetate; 4 mL H_2O
   Load 4.2 mL of reactivated sample.
   Elution: 2 mL Ethyl Acetate
3. SPE preparation #2
   Phenomenex Strata-X 33µm (30mg/ 3mL)
   Conditioning: 2 mL N-hexane; 4 mL Ethyl Acetate; 4 mL H_2O
   Load 4.2 mL of reactivated sample.
   Elution: 2 mL Ethyl Acetate

The following procedure describes, in detail, the method that was determined to be most successful and used for the sample (SPE preparation #1):
An Agilent ABS Elut-Nexus SPE cartridge was conditioned, by gravity, with 2 mL of *N*-hexane, 2x2 mL ethyl acetate, followed by 2x2 mL of H_2O. 1 mL reactivated plasma sample was then loaded into cartridge and allowed to pass through. Sample was eluted with 2 mL of ethyl acetate into a clean vial. Sample was reduced in volume to approximately 100 µL for analysis.

When the target compound was not detected in CW-4-147-1, a deviation was made to this method. CW-4-147-1 was reactivated by using two replicates of 2 mL instead of the 1 mL described above and the resulting eluted samples were combined for their respective samples to increase the analytical concentration of the target analyte, should it be present. The combined samples were then reduced in volume to approximately 100 µL for analysis.

References

G.L. Ellman. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharm 7: 88-95(1961).

C. E. A. M. Degenhardt, K. Pleijsier, M. J. van der Schans, J. P. Langenberg, K. E. Preston, M. I. Solano, V. L. Maggio, J. R. Barr. Improvements of the Fluoride Reactivation Method for the verification of nerve agent exposure. Journal of Analytical Toxicology. 28(5): 364-371 (2004).

SAMPLE SUMMARY: B

Sample Code: P-302/07	**Laboratory Assigned Code:** CW-4-147-2
Description and condition of sample: Approximately 5 mL of plasma	

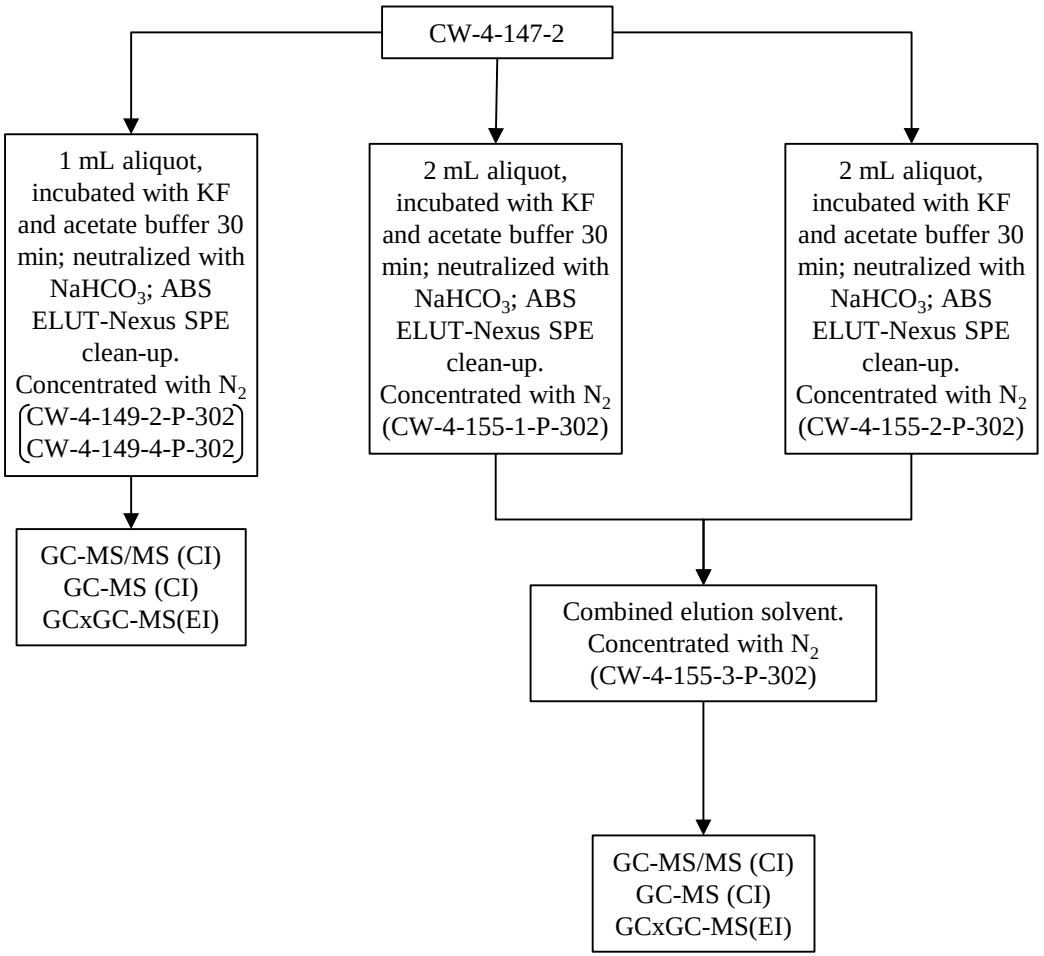
No chemicals relevant to the purpose of analysis were found.

SAMPLE PREPARATION DESCRIPTION: B

1. Sample preparation

Initial Aliquot Code	Type of Sample Preparation	Amount/ Volume	Sample/Blank Preparation Procedures	End Volume	Resulting Aliquot Code
CW-4-147-2	Fluoride Reactivation	1 mL	Added 3 mL of acetate buffer/0.25 M KF. Incubated 30 minutes at room temperature. Neutralized with 0.8M Sodium Bicarbonate. ABS ELUT-Nexus cartridge cleanup. Eluted using 2 mL ethyl acetate and reduced sample volume approximately 100 µL with nitrogen gas.	100 µL	CW-4-149-2-P-302
CW-4-149-2-P-302	Sample Split	100 µL	Split of sample CW-4-149-2-P-302.	50 µL	CW-4-149-4-P-302
CW-4-147-2	Fluoride Reactivation	2 mL	Added 3 mL of acetate buffer and 277 µL of 4.51 M KF. Incubated 30 minutes at room temperature. Neutralized with 0.8 M Sodium Bicarbonate. ABS ELUT-Nexus cartridge cleanup. Eluted using 2 mL ethyl acetate.	2 mL	CW-4-155-1-P-302
CW-4-147-2	Fluoride Reactivation	2 mL	Added 3 mL of acetate buffer and 277 µL of 4.51 M KF. Incubated 30 minutes at room temperature. Neutralized with 0.8 M Sodium Bicarbonate. ABS ELUT-Nexus cartridge cleanup. Eluted using 2 mL ethyl acetate.	2 mL	CW-4-155-2-P-302
CW-4-155-1-P-302 CW-4-155-2-P-302	Concentrate combined samples	2 mLx2	Combined elution solvent from CW-4-155-1-P-302 and CW-4-155-2-P-302. Reduced sample volume to approximately 100 µL using nitrogen gas.	100 µL	CW-4-155-3-P-302

2. Additional information


CW-4-147-2
1 mL aliquot, incubated with KF and acetate buffer 30 min; neutralized with $NaHCO_3$; ABS ELUT-Nexus SPE clean-up. Concentrated with N_2 (CW-4-149-2-P-302 CW-4-149-4-P-302
2 mL aliquot, incubated with KF and acetate buffer 30 min; neutralized with $NaHCO_3$; ABS ELUT-Nexus SPE clean-up. Concentrated with N_2 (CW-4-155-1-P-302)
2 mL aliquot, incubated with KF and acetate buffer 30 min; neutralized with $NaHCO_3$; ABS ELUT-Nexus SPE clean-up. Concentrated with N_2 (CW-4-155-2-P-302)
GC-MS/MS (CI) GC-MS (CI) GCxGC-MS(EI)
Combined elution solvent. Concentrated with N_2 (CW-4-155-3-P-302)
GC-MS/MS (CI) GC-MS (CI) GCxGC-MS(EI)

Description of sample preparation and analysis methods

Sample preparation and analysis methods were developed using an in-house standard made by incubating the nerve agent, Sarin, with commercially procured human blood plasma. Cholinesterase activities of the plasma sample were measured using method outlined in Ellman (1961) before and after incubation to ensure agent-adduction occurred. The resultant agent-adducted plasma was used for subsequent method development.

Adducted plasma samples were reactivated using the process outlined in Degenhardt *et al* (2004). 1 mL of plasma was added to 3 mL of acetate buffer solution [0.189 M acetic acid; 10.8 mM sodium acetate]. Appropriate amount of Potassium Fluoride [4.51M] was added to achieve a final concentration of 0.25 M KF in the unknown sample work-up. Sample was allowed to incubate at room temperature for 30 minutes. 0.5 mL of 0.8 M sodium bicarbonate was added to neutralize acid.

Several methods were attempted to isolate the reactivated sarin from the sample:

1. Direct dichloromethane extraction
2. SPE preparation #1
   Agilent ABS Elut-NEXUS (200 mg/ 6 mL)
   Conditioning: 2 mL N-hexane; 4 mL Ethyl Acetate; 4 mL H_2O
   Load 4.2 mL of reactivated sample.
   Elution: 2 mL Ethyl Acetate
3. SPE preparation #2
   Phenomenex Strata-X 33µm (30mg/ 3mL)
   Conditioning: 2 mL N-hexane; 4 mL Ethyl Acetate; 4 mL H_2O
   Load 4.2 mL of reactivated sample.
   Elution: 2 mL Ethyl Acetate

The following procedure describes, in detail, the method that was determined to be most successful and used for the sample (SPE preparation #1):
An Agilent ABS Elut-Nexus SPE cartridge was conditioned, by gravity, with 2 mL of *N*-hexane, 2x2 mL ethyl acetate, followed by 2x2 mL of H_2O. 1 mL reactivated plasma sample was then loaded into cartridge and allowed to pass through. Sample was eluted with 2 mL of ethyl acetate into a clean vial. Sample was reduced in volume to approximately 100 µL for analysis.

When the target compound was not detected in CW-4-147-2, a deviation was made to this method. CW-4-147-2 was reactivated by using two replicates of 2 mL instead of the 1 mL described above and the resulting eluted samples were combined for their respective samples to increase the analytical concentration of the target analyte, should it be present. The combined samples were then reduced in volume to approximately 100 µL for analysis.

References

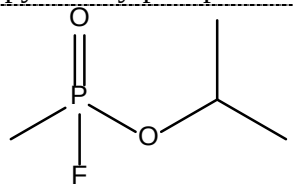
G.L. Ellman. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharm 7: 88-95(1961).

C. E. A. M. Degenhardt, K. Pleijsier, M. J. van der Schans, J. P. Langenberg, K. E. Preston, M. I. Solano, V. L. Maggio, J. R. Barr. Improvements of the Fluoride Reactivation Method for the verification of nerve agent exposure. Journal of Analytical Toxicology. 28(5): 364-371 (2004).

SAMPLE SUMMARY: C

Sample Code: P-303/07	**Laboratory Assigned Code:** CW-4-147-3
Description and condition of sample: Approximately 5 mL of plasma	

Chemical: C-1

Chemical name & Structure	**CAS #**	**Schedule**
O-Isopropyl methylphosphonofluoridate 	107-44-8	1.A.01

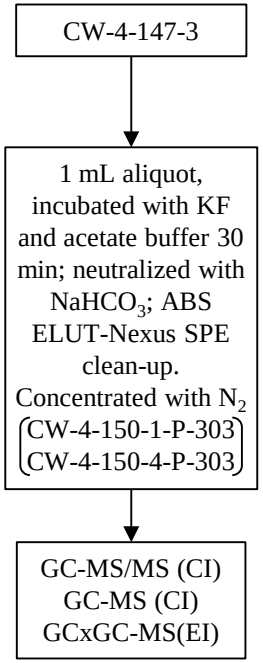
Aliquot(s)	**Original/derivative**	**Analysis technique**
CW-4-150-4-P-303	Original	GC-MS/MS (CI)
CW-4-150-4-P-303	Original	GC-MS (CI)
CW-4-150-1-P-303	Original	GCxGC-MS (EI)
Comments:		

SAMPLE PREPARATION DESCRIPTION: C

1. Sample preparation

Initial Aliquot Code	Type of Sample Preparation	Amount/ Volume	Sample/Blank Preparation Procedures	End Volume	Resulting Aliquot Code
CW-4-147-3	Fluoride Reactivation	1 mL	Added 3 mL of acetate buffer and 220 µL of 4.51 M KF. Incubated 30 minutes at room temperature. Neutralized with 0.8 M Sodium Bicarbonate. ABS ELUT-Nexus cartridge cleanup. Eluted using 2 mL ethyl acetate and reduced sample volume to approximately 100µL with nitrogen gas.	100 µL	CW-4-150-1-P-303
CW-4-150-1-P-303	Sample Split	50 µL	Split of sample CW-4-150-1-P-303.	50 µL	CW-4-150-4-P-303

2. Additional information


CW-4-147-3
1 mL aliquot, incubated with KF and acetate buffer 30 min; neutralized with $NaHCO_3$; ABS ELUT-Nexus SPE clean-up. Concentrated with N_2
(CW-4-150-1-P-303
CW-4-150-4-P-303)
GC-MS/MS (CI)
GC-MS (CI)
GCxGC-MS(EI)

Description of sample preparation and analysis methods

Sample preparation and analysis methods were developed using an in-house standard made by incubating the nerve agent, Sarin, with commercially procured human blood plasma. Cholinesterase activities of the plasma sample were measured using method outlined in Ellman (1961) before and after incubation to ensure agent-adduction occurred. The resultant agent-adducted plasma was used for subsequent method development.

Adducted plasma samples were reactivated using the process outlined in Degenhardt *et al* (2004). 1 mL of plasma was added to 3 mL of acetate buffer solution [0.189 M acetic acid; 10.8 mM sodium acetate]. Appropriate amount of Potassium Fluoride [4.51M] was added to achieve a final concentration of 0.25 M KF in the unknown sample work-up. Sample was allowed to incubate at room temperature for 30 minutes. 0.5 mL of 0.8 M sodium bicarbonate was added to neutralize acid.

Several methods were attempted to isolate the reactivated sarin from the sample:

1. Direct dichloromethane extraction
2. SPE preparation #1
   Agilent ABS Elut-NEXUS (200 mg/ 6 mL)
   Conditioning: 2 mL N-hexane; 4 mL Ethyl Acetate; 4 mL H_2O
   Load 4.2mL of reactivated sample.
   Elution: 2 mL Ethyl Acetate
3. SPE preparation #2
   Phenomenex Strata-X 33µm (30mg/ 3mL)
   Conditioning: 2 mL N-hexane; 4 mL Ethyl Acetate; 4 mL H_2O
   Load 4.2mL of reactivated sample.
   Elution: Ethyl Acetate

The following procedure describes, in detail, the method that was determined to be most successful and used for the sample (SPE preparation #1):
An Agilent ABS Elut-Nexus SPE cartridge was conditioned, by gravity, with 2 mL of *N*-hexane, 2x2 mL ethyl acetate, followed by 2x2 mL of H_2O. 1 mL reactivated plasma sample was then loaded into cartridge and allowed to pass through. Sample was eluted with 2 mL of ethyl acetate into a clean vial. Sample was reduced in volume to approximately 100 µL for analysis.

References

G.L. Ellman. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharm 7: 88-95(1961).

C. E. A. M. Degenhardt, K. Pleijsier, M. J. van der Schans, J. P. Langenberg, K. E. Preston, M. I. Solano, V. L. Maggio, J. R. Barr. Improvements of the Fluoride Reactivation Method for the verification of nerve agent exposure. Journal of Analytical Toxicology. 28(5): 364-371 (2004).

GC-MS/MS (CI) TECHNIQUE METHOD AND ANALYSIS DESCRIPTION

Identification

Chemical: C-1
Aliquot code: CW-4-150-4-P-303
Datafile name: TSQ0328

Compound identified as: Original Compound
Compound reference: Reference Chemical (OPCW)
Match algorithm and match factor: NIST, 980/999

Analysis Method

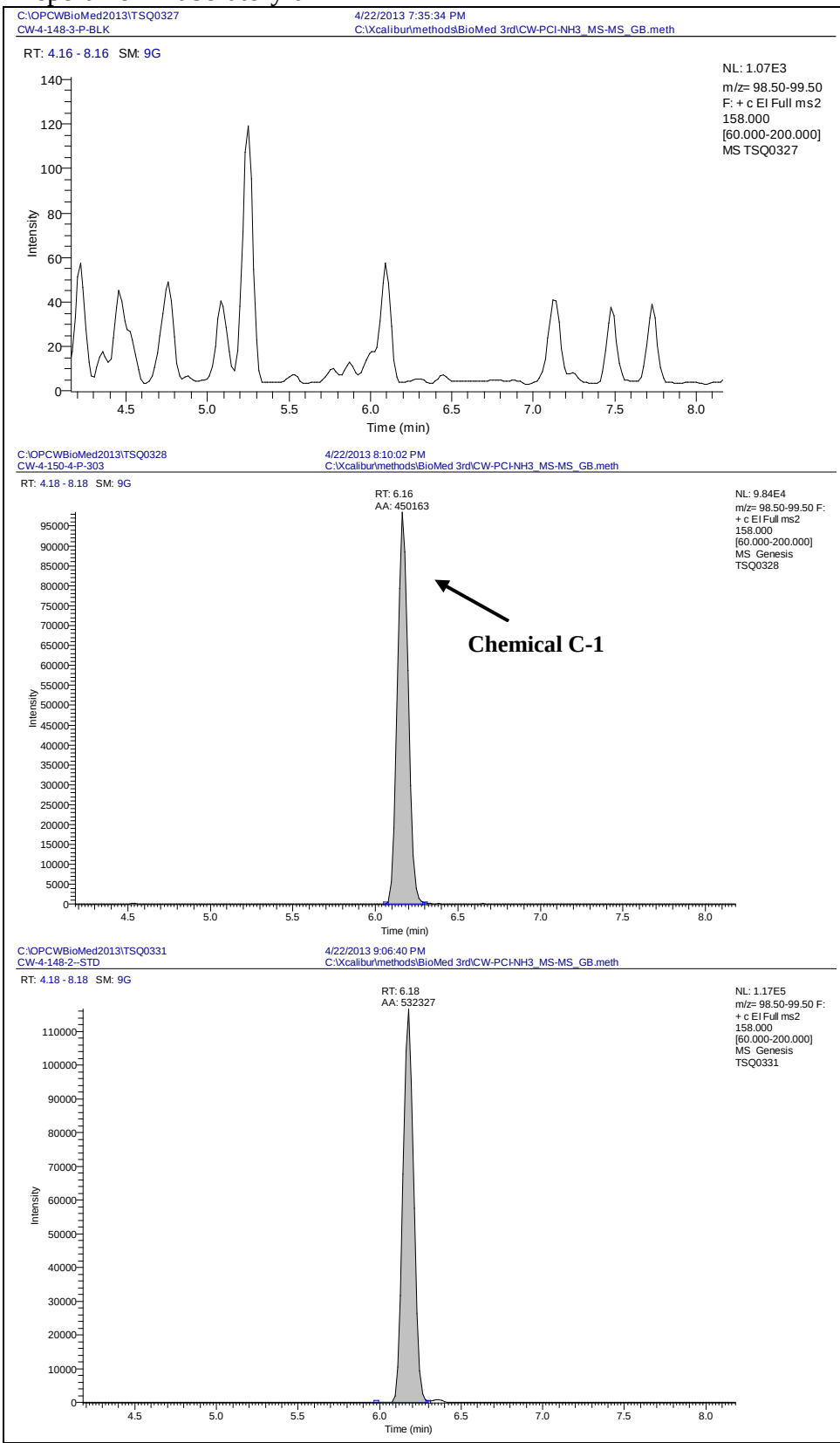
GC Instrument manufacturer and type: Thermo Fisher Trace GC Ultra
Carrier gas: Helium
Flow control/rate: Constant flow, 1 mL/min
Injection mode: Splitless, 0.60 min
Injector temperature: 230 ºC
Column brand/phase: Agilent HP-5MS: (5%-Phenyl)-methylpolysiloxane
Column Length x ID x Film thickness: 30 m x 0.25 mm x 0.25 µm
GC temperature programme: 40 ºC (3 min), 8 ºC/min to 90 ºC, 20 ºC/min to 300 ºC (1 min)

MS Instrument manufacturer and type: Thermo Fisher TSQ Quantum
Solvent delay time: 3 min
Electron energy: 100 eV
Reaction gas: Ammonia
Ionisation polarity: Positive
Scan range/time: 60-200 m/z in 1 second
Mass resolution: 0.7
Type of MS/MS scan: Product ion scan
Precursor ion(s): m/z 158
Collision gas: Argon
Collision Energy: 10

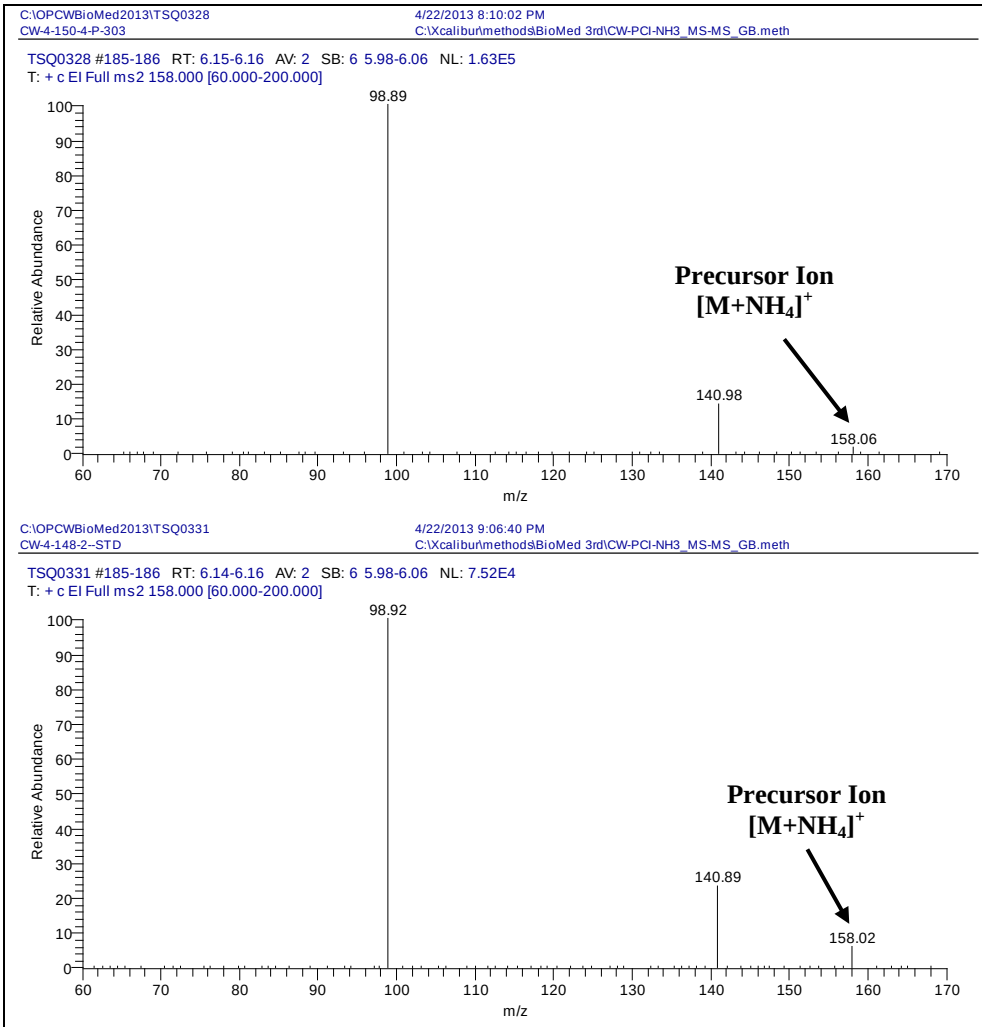
Ions/transitions	Sample		Standard		Criteria	Result
	Area*	Ion Ratio	Area*	Ion Ratio	Tolerance#	
99	450163	1.0	532327	1.0	N/A	N/A
141	62037	0.138	57494	0.108	±30%	27.6%

*****Peak area of the ion, % intensity compared to the most abundant ion
Compare with the relative ion intensity of the standard: >50%: ±20%; >20% to 50%: ±25%; >10% to 20%: ±30%; ≤10%: ±50%

Remarks



GC-MS/MS (CI) chromatograms supporting identification of **Chemical C-1;** EIC
Top: Chromatogram of the blank.
Center: Chromatogram of the sample (aliquot code listed in header).
Bottom: Chromatogram of reference chemical of **O-Isopropyl methylphosphonofluoridate.**



CI product mass spectra of:
Top: **Chemical C-1** (aliquot code listed in header).
Bottom: Reference chemical of **O-Isopropyl methylphosphonofluoridate.**

GC-MS(CI) TECHNIQUE METHOD AND ANALYSIS DESCRIPTION

Identification

Chemical: C-1
Aliquot code: CW000626.D
Datafile name: CW-4-150-4-P-303

Compound identified as: Original Compound
Compound reference: Reference Chemical (OPCW)

Analysis Method

GC Instrument manufacturer and type: Agilent 6890
Carrier gas: Helium
Flow control/rate: Constant Flow, 32 cm/sec
Injection mode: Pulsed Splitless, 0.75 min
Injector temperature: 250 ºC
Column brand/phase: Agilent HP-5MS: (5%-Phenyl)-methylpolysiloxane
Column Length x ID x Film thickness: 30 m x 0.25 mm x 0.25 µm
GC temperature programme: 40 ºC (3 min), 8 ºC/min to 100 ºC, 25 ºC/min to 300 ºC (3 min)

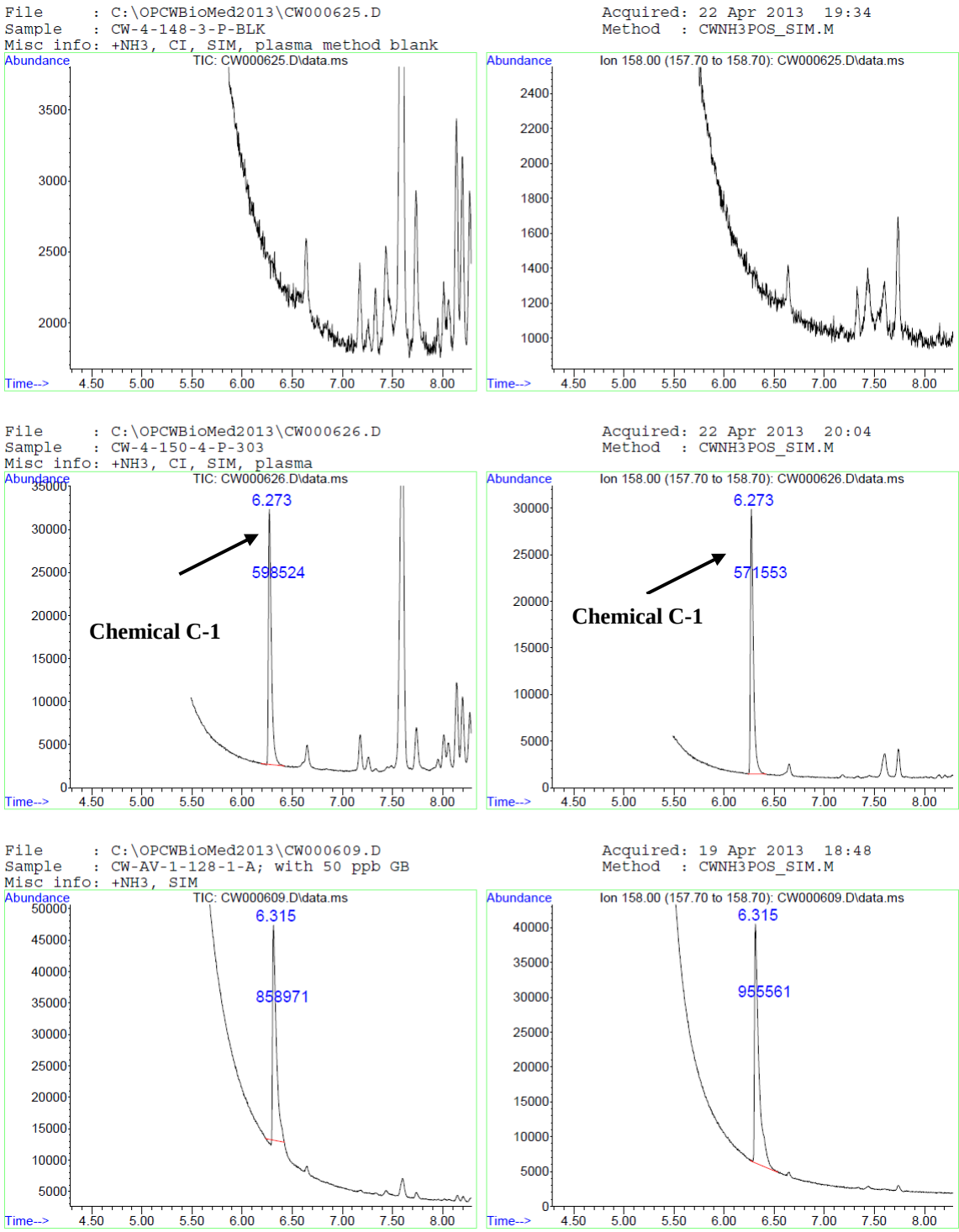
MS Instrument manufacturer and type: Agilent 5975 MSD
Solvent delay time: 5.4 min
Electron energy: 82 eV
Scan range/time: SIM, m/z 141, 158, 100 µs dwell time
Source temperature: 230 ºC
Reaction gas: Ammonia **and flow:** 21%
Ionisation polarity: Positive

Ions/transitions	Sample		Standard		Criteria	Result
	Area*	Ion Ratio	Area*	Ion Ratio	Tolerance[#]	
141	21504	0.038	28172	0.029	±50%	27.6%
158	571553	1.0	955561	1.0	N/A	N/A

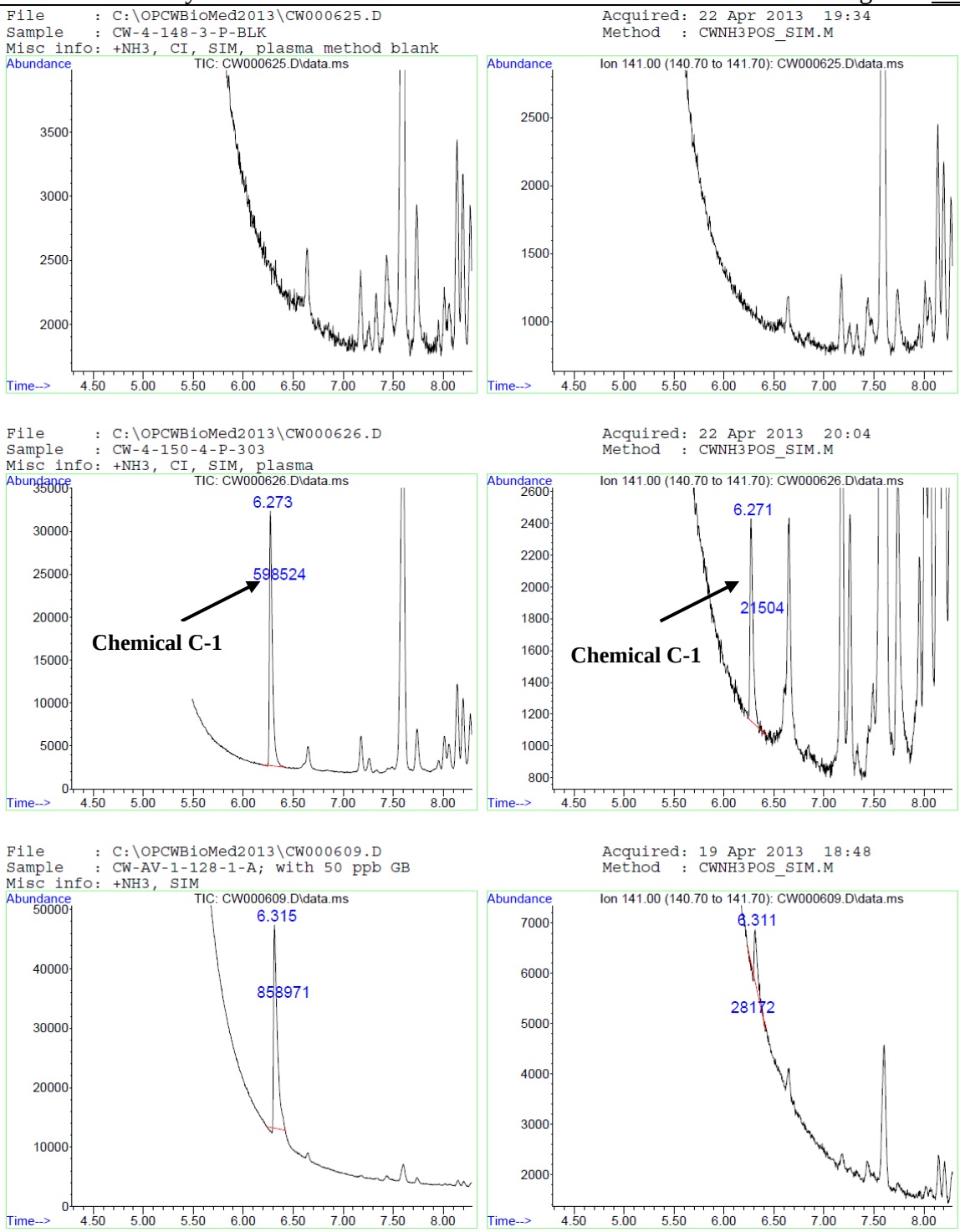
*****Peak area of the ion, % intensity compared to the most abundant ion
[#] Compare with the relative ion intensity of the standard: >50%: ±20%; >20% to 50%: ±25%; >10% to 20%: ±30%; ≤10%: ±50%

Remarks

Due to the solvent delay of 5.4 min, the chromatogram could not be presented with a window of the retention time of the compound minus 2 min.



EI chromatograms supporting identification of **Chemical C-1;**
TIC on left, EIC (m/z 158) on right.
Top: Chromatogram of the blank.
Center: Chromatogram of the sample (aliquot code listed in header).
Bottom: Chromatogram of reference chemical of **O-Isopropyl methylphosphonofluoridate.**



EI chromatograms supporting identification of **Chemical C-1;**
TIC on left, EIC (m/z 141) on right.
Top: Chromatogram of the blank.
Center: Chromatogram of the sample (aliquot code listed in header).
Bottom: Chromatogram of reference chemical of **O-Isopropyl methylphosphonofluoridate.**

GCXGC-MS(EI) TECHNIQUE METHOD AND ANALYSIS DESCRIPTION

Identification

Chemical: C-1
Aliquot code: CW-4-150-1-P-303
Datafile name: TOF1335

Chemical identified as: Original Compound
Chemical reference: Reference Chemical
Match algorithm and match factor: 914/999

Analysis Method

GC Instrument manufacturer and type: Agilent 6890 GC
Carrier gas: Helium
Flow rate: 1.10 mL/min
[X] Corrected constant flow via pressure ramps
Injection mode: Splitless, 0.17 min
Injector temperature: 250^{o} C
Primary Column:
Brand/phase: Agilent HP-5 MS UI: (5% diphenyl 95% dimethyl polysiloxane)
Column Length x ID x Film thickness: 30 m x 0.25 mm x 0.25 µm
GC temperature programme: 40^{o}C (3 min), 8^{o}C/min, 300^{o}C (3 min)
Secondary Column:
Brand/phase: Restek Rxi-17: (50%-Phenyl)-methylpolysiloxane
Column Length x ID x Film thickness: 1.5 m x 0.18 mm x 0.18 µm
GC temperature programme: 50^{o}C (3 min), 8^{o}C/min, 310^{o}C (3 min)
[X] Modulator enabled
Modulator temperature offset: 20 °C, relative to the GC secondary oven temperature
Modulation period: 3.5 sec
Hot pulse time: 0.6 sec
Cool time between stages: 1.15 sec

MS Instrument manufacturer and type: LECO Pegasus 4D
Solvent delay time: 3 minutes
Detector voltage: 1750 V
Electron energy: 70 eV
Mass resolution: 0.6 u
Scan range: 29-600 m/z
Scan rate: 200 spectra/sec
Source temperature: 250 ^{o}C

Ions/transitions	Sample		Standard		Criteria	Result
	Area*	Ion Ratio	Area*	Ion Ratio	Tolerance[#]	
39	3989.4	0.162	27388	0.152	±30%	6.6%
81	3972.7	0.162	27547	0.153	±30%	5.5%
99	24559	1.00	179664	1.00	N/A	N/A
125	4239.4	0.173	36495	0.203	±25%	15.0%

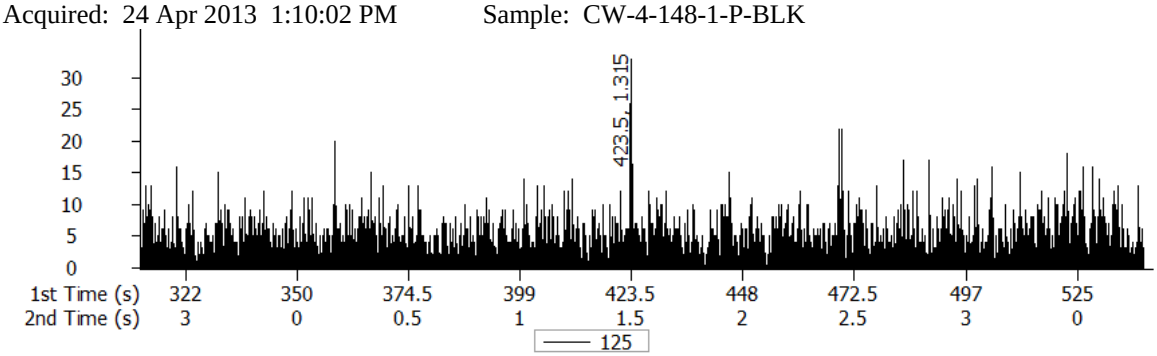
*****Peak area of the ion, % intensity compared to the most abundant ion

[#] Compare with the relative ion intensity of the standard: >50%: ±20%; >20% to 50%: ±25%; >10% to 20%: ±30%; ≤10%: ±50%

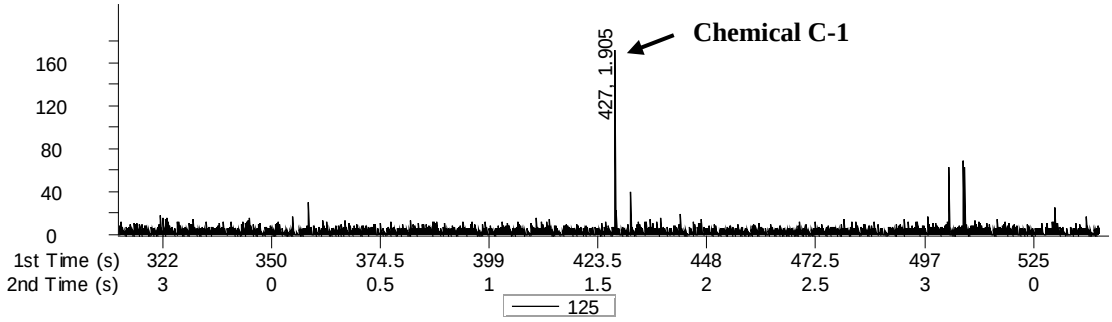
Remarks

A small peak is present in the blank within 0.1 min of the target chemical. This peak has been tentatively identified as hexamethylcyclotrisiloxane through library matching (NIST, 960/999). The mass spectrum of this compound and the library spectrum are included.

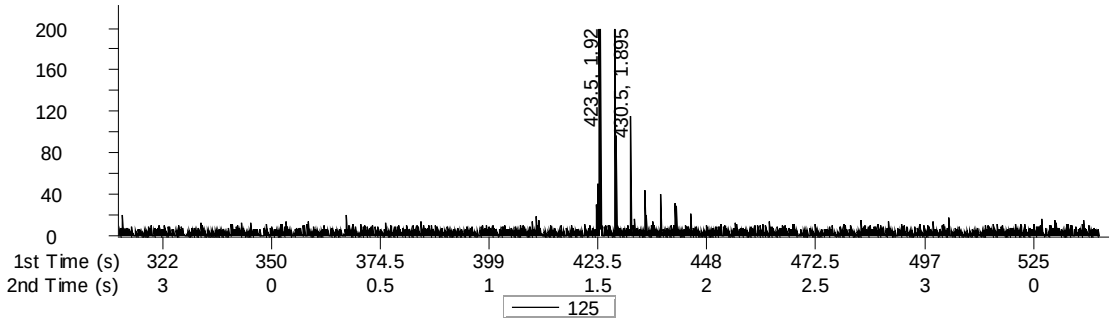
File : C:\LLNL\TOF1334
Method: 2D OPCW020613
Acquired: 24 Apr 2013 1:10:02 PM Sample: CW-4-148-1-P-BLK



File : C:\LLNL\TOF1335
Method: 2D OPCW020613
Acquired: 24 Apr 2013 1:52:49 PM Sample: CW-4-150-1-P-303

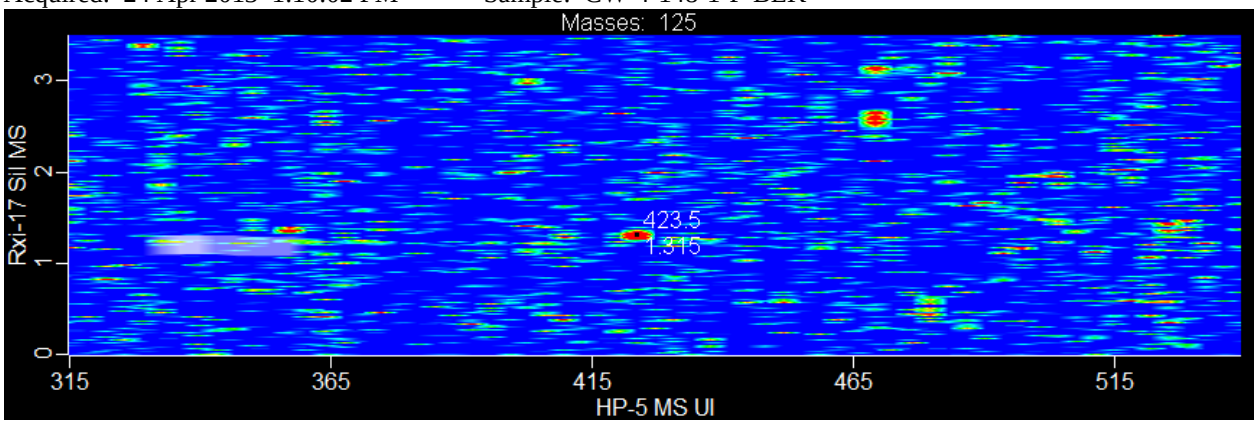


File : C:\LLNL\TOF1336
Method: 2D OPCW020613
Acquired: 24 Apr 2013 4:34:02 PM Sample: CW-4-150-3-SARINSTD

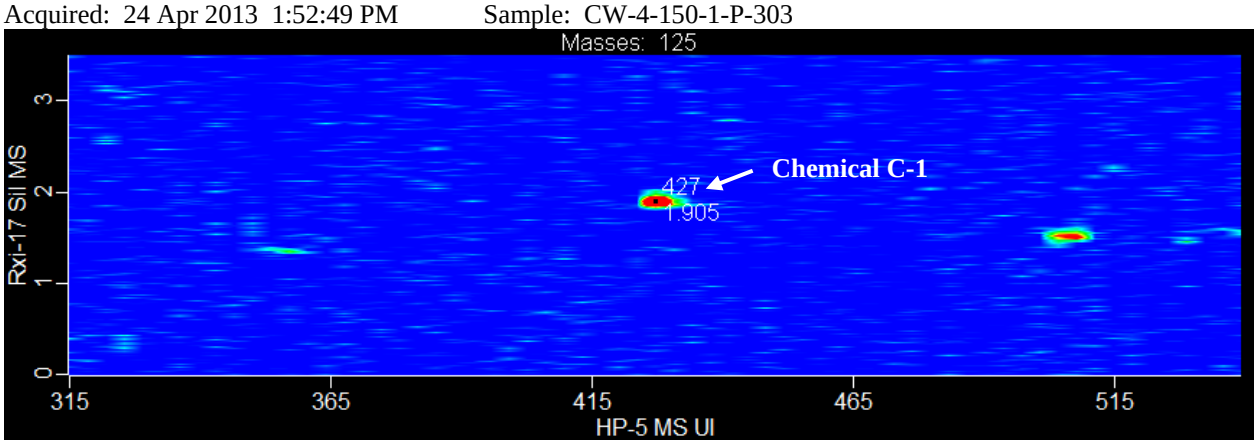


EI chromatograms supporting identification of **Chemical C-1**; EIC
Top: Chromatogram of the blank.
Center: Chromatogram of the sample (aliquot code listed in header).
Bottom: Chromatogram of reference chemical of **O-Isopropyl methylphosphonofluoridate.**

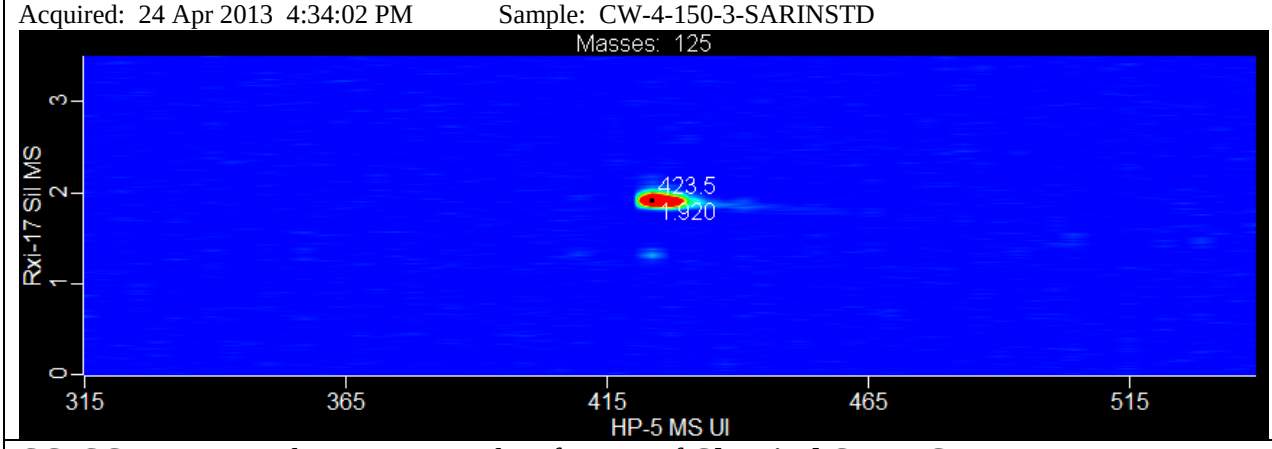
File : C:\LLNL\TOF1334
Method: 2D OPCW020613
Acquired: 24 Apr 2013 1:10:02 PM Sample: CW-4-148-1-P-BLK



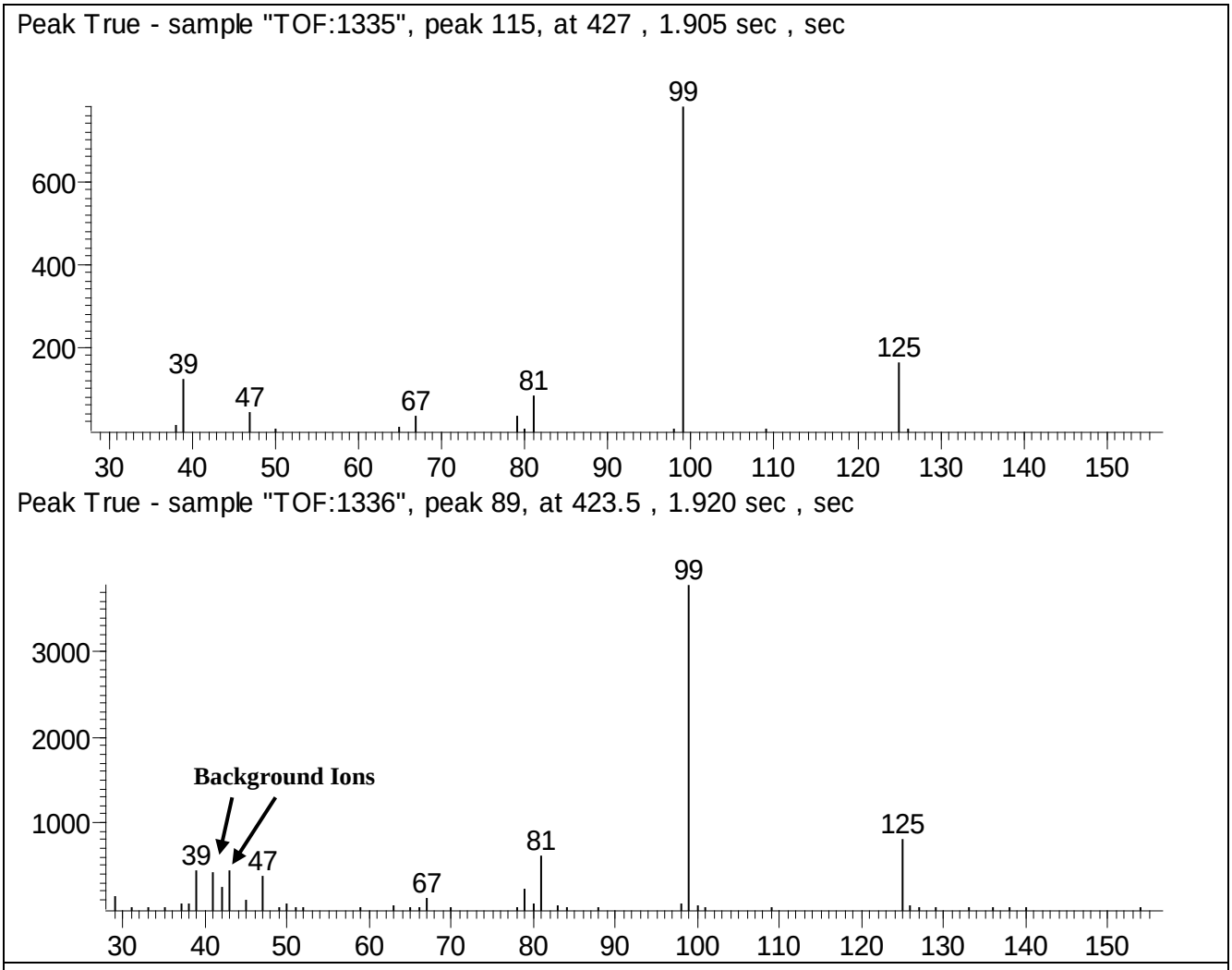
File : C:\LLNL\TOF1335
Method: 2D OPCW020613
Acquired: 24 Apr 2013 1:52:49 PM Sample: CW-4-150-1-P-303



File : C:\LLNL\TOF1336
Method: 2D OPCW020613
Acquired: 24 Apr 2013 4:34:02 PM Sample: CW-4-150-3-SARINSTD

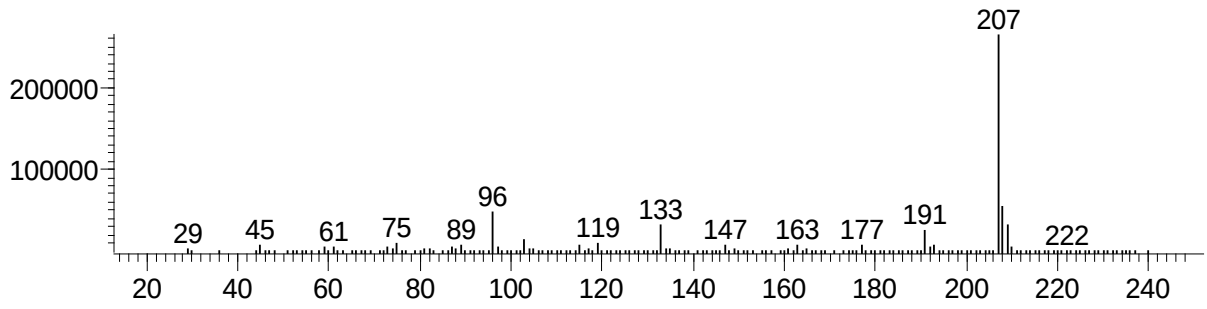


GCxGC EI contour plot supporting identification of **Chemical C-1**; EIC
Top: Contour plot of the blank.
Center: Contour plot of the sample (aliquot code listed in header).
Bottom: Contour plot of reference chemical of **O-Isopropyl methylphosphonofluoridate.**

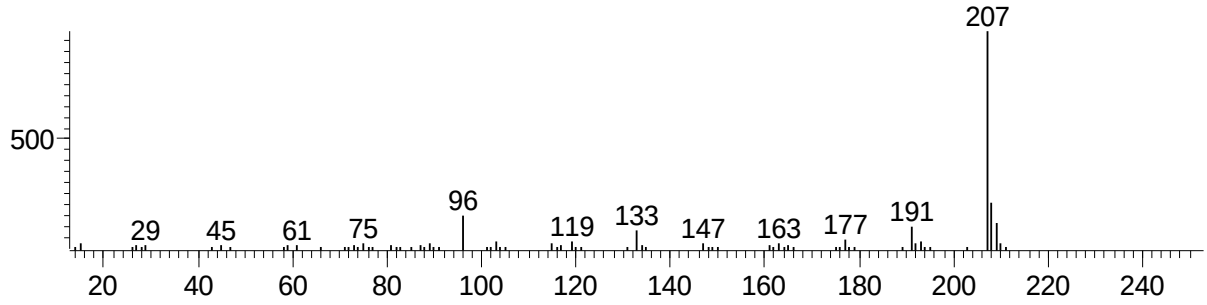


EI mass spectra of:
Top: **Chemical C-1** (file name listed in header).
Bottom: Reference chemical of **O-Isopropyl methylphosphonofluoridate.**

Peak True - sample "TOF:1334", peak 102, at 423.5 , 1.315 sec , sec



Library Hit - similarity 960, "Cyclotrisiloxane, hexamethyl-"

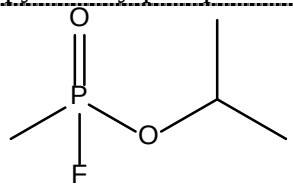


EI mass spectra of:
Top: Background contaminant found in blank within ± 0.1 min of reported chemical (file name listed in header).
Bottom: Library mass spectrum of hexamethylcyclotrisiloxane.

SAMPLE SUMMARY: D

Sample Code: P-304/07	**Laboratory Assigned Code:** CW-4-147-4
Description and condition of sample: Approximately 5 mL of plasma	

Chemical: D-1

Chemical name & Structure	**CAS #**	**Schedule**
O-Isopropyl methylphosphonofluoridate 	107-44-8	1.A.01

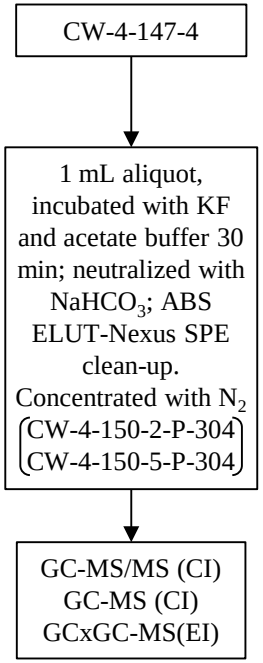
Aliquot(s)	**Original/derivative**	**Analysis technique**
CW-4-150-5-P-304	Original	GC-MS/MS (CI)
CW-4-150-5-P-304	Original	GC-MS (CI)
Comments:		

SAMPLE PREPARATION DESCRIPTION: D

1. Sample preparation

Initial Aliquot Code	Type of Sample Preparation	Amount/ Volume	Sample/Blank Preparation Procedures	End Volume	Resulting Aliquot Code
CW-4-147-4	Fluoride Reactivation	1 mL	Added 3 mL of acetate buffer and 220 µL of 4.51 M KF. Incubated 30 minutes at room temperature. Neutralized with 0.8 M Sodium Bicarbonate. ABS ELUT-Nexus cartridge cleanup. Eluted using 2 mL ethyl acetate and reduced sample volume approximately 100 µL.	100 µL	CW-4-150-2-P-304
CW-4-150-2-P-304	Sample Split	50 µL	Split of sample CW-4-150-2-P-304.	50 µL	CW-4-150-5-P-304

2. Additional information


CW-4-147-4
1 mL aliquot, incubated with KF and acetate buffer 30 min; neutralized with $NaHCO_3$; ABS ELUT-Nexus SPE clean-up. Concentrated with N_2
(CW-4-150-2-P-304
CW-4-150-5-P-304)
GC-MS/MS (CI)
GC-MS (CI)
GCxGC-MS(EI)

Description of sample preparation and analysis methods

Sample preparation and analysis methods were developed using an in-house standard made by incubating the nerve agent, Sarin, with commercially procured human blood plasma. Cholinesterase activities of the plasma sample were measured using method outline in Ellman (1961) before and after incubation to ensure agent-adduction occurred. The resultant agent-adducted plasma was used for subsequent method development.

Adducted plasma samples were reactivated using the process outlined in Degenhardt *et al* (2004). 1 mL of plasma was added to 3 mL of acetate buffer solution [0.189 M acetic acid; 10.8 mM sodium acetate]. Appropriate amount of Potassium Fluoride [4.51M] was added to achieve a final concentration of 0.25 M KF in the unknown sample work-up. Sample was allowed to incubate at room temperature for 30 minutes. 0.5 mL of 0.8 M sodium bicarbonate was added to neutralize acid.

Several methods were attempted to isolate the reactivated sarin from the sample:

4. Direct dichloromethane extraction
5. SPE preparation #1
   Agilent ABS Elut-NEXUS (200 mg/ 6 mL)
   Conditioning: 2 mL N-hexane; 4 mL Ethyl Acetate; 4 mL H_2O
   Load 4.2 mL of reactivated sample.
   Elution: 2 mL Ethyl Acetate
6. SPE preparation #2
   Phenomenex Strata-X 33µm (30mg/ 3mL)
   Conditioning: 2 mL N-hexane; 4 mL Ethyl Acetate; 4 mL H_2O
   Load 4.2 mL of reactivated sample.
   Elution: 2 mL Ethyl Acetate

The following procedure describes, in detail, the method that was determined to be most successful and used for the sample (SPE preparation #1):
An Agilent ABS Elut-Nexus SPE cartridge was conditioned, by gravity, with 2 mL of *N*-hexane, 2x2 mL ethyl acetate, followed by 2x2 mL of H_2O. 1 mL reactivated plasma sample was then loaded into cartridge and allowed to pass through. Sample was eluted with 2 mL of ethyl acetate into a clean vial. Sample was reduced in volume to approximately 100 µL for analysis.

References

G.L. Ellman. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharm 7: 88-95(1961).

C. E. A. M. Degenhardt, K. Pleijsier, M. J. van der Schans, J. P. Langenberg, K. E. Preston, M. I. Solano, V. L. Maggio, J. R. Barr. Improvements of the Fluoride Reactivation Method for the verification of nerve agent exposure. Journal of Analytical Toxicology. 28(5): 364-371 (2004).

GC-MS/MS (CI) TECHNIQUE METHOD AND ANALYSIS DESCRIPTION

Identification

Chemical: D-1
Aliquot code: CW-4-150-5-P-304
Datafile name: TSQ0334

Compound identified as: Original Compound
Compound reference: Reference Chemical (OPCW)
Match algorithm and match factor: NIST 948/999

Analysis Method

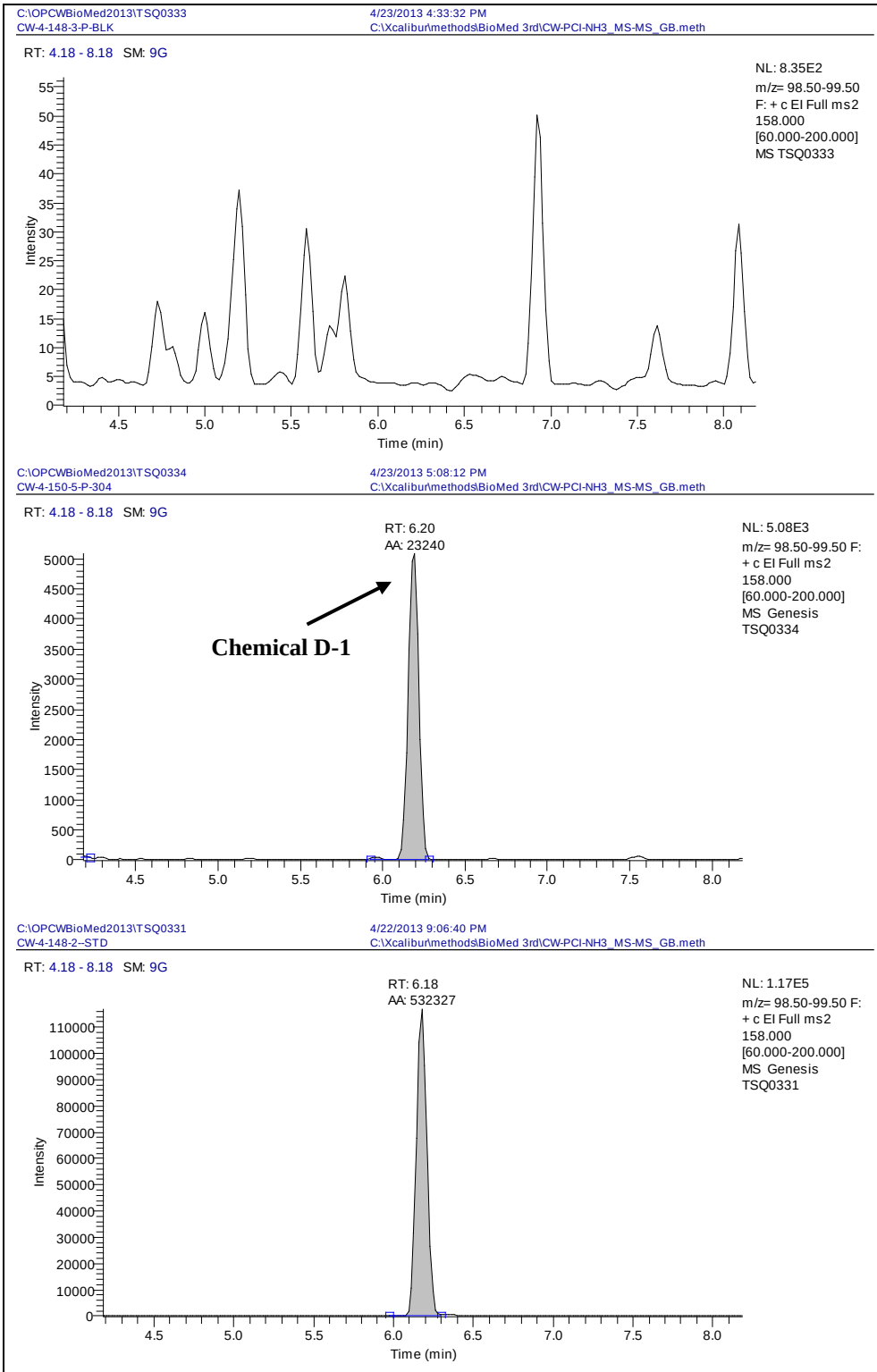
GC Instrument manufacturer and type: Thermo Fisher Trace GC Ultra
Carrier gas: Helium
Flow control/rate: Constant flow, 1 mL/min
Injection mode: Splitless, 0.60 min
Injector temperature: 230 ºC
Column brand/phase: Agilent HP-5MS: (5%-Phenyl)-methylpolysiloxane
Column Length x ID x Film thickness: 30 m x 0.25 mm x 0.25 µm
GC temperature programme: 40 ºC (3 min), 8 ºC/min to 90 ºC, 20 ºC/min to 300 ºC (1 min)

MS Instrument manufacturer and type: Thermo Fisher TSQ Quantum
Solvent delay time: 3 min
Electron energy: 100 eV
Reaction gas: Ammonia
Ionisation polarity: Positive
Scan range/time: 60-200 m/z in 1 second
Mass resolution: 0.7
Type of MS/MS scan: Product ion scan
Precursor ion(s): m/z 158
Collision gas: Argon
Collision Energy: 10

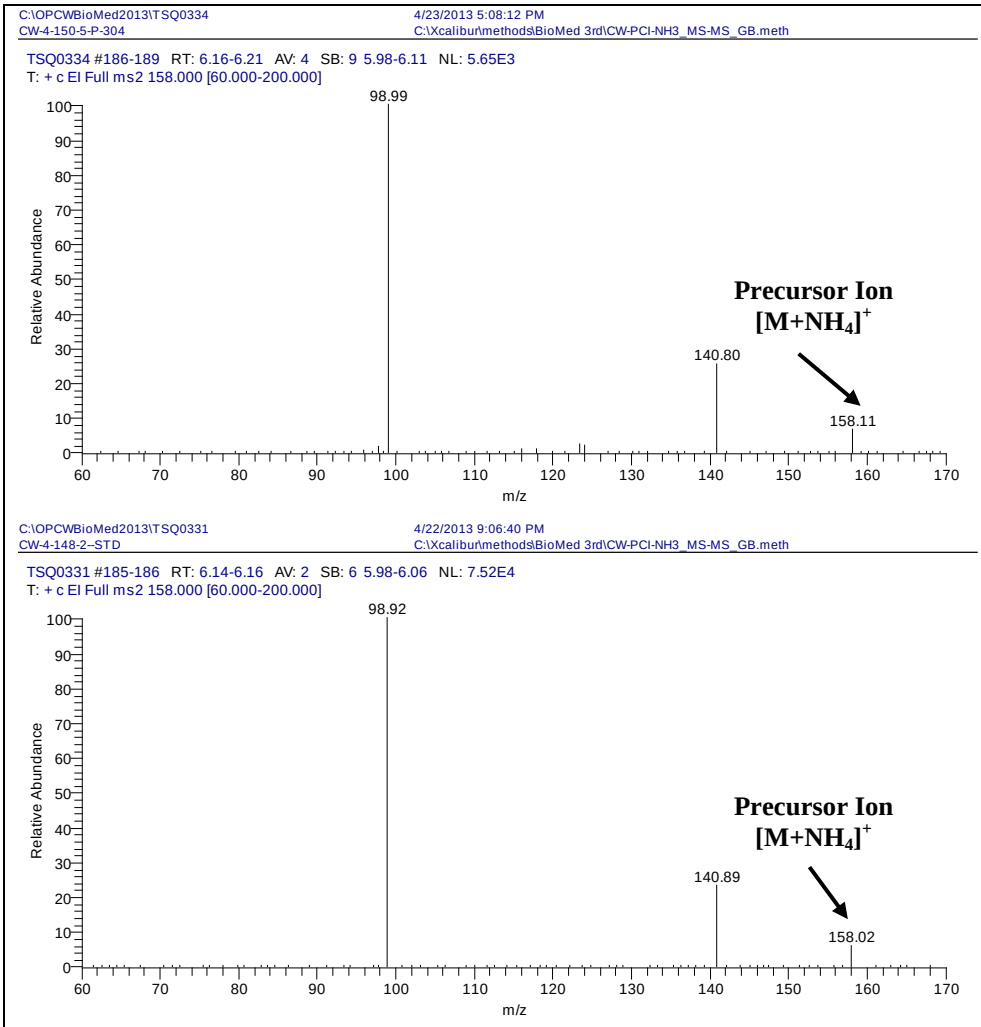
Ions/transitions	Sample		Standard		Criteria	Result
	Area*	Ion Ratio	Area*	Ion Ratio	Tolerance#	
99	23240	1.0	532327	1.0	N/A	N/A
141	3087	0.133	57494	0.108	±30%	23.0%

*****Peak area of the ion, % intensity compared to the most abundant ion
Compare with the relative ion intensity of the standard: >50%: ±20%; >20% to 50%: ±25%; >10% to 20%: ±30%; ≤10%: ±50%

Remarks



GC-MS/MS (CI) chromatograms supporting identification of **Chemical D-1;** EIC
Top: Chromatogram of the blank.
Center: Chromatogram of the sample (aliquot code listed in header).
Bottom: Chromatogram of reference chemical of **O-Isopropyl methylphosphonofluoridate.**



CI product mass spectra of:
Top: **Chemical D-1** (aliquot code listed in header).
Bottom: Reference chemical of **O-Isopropyl methylphosphonofluoridate.**

GC-MS(CI) TECHNIQUE
METHOD AND ANALYSIS DESCRIPTION

Identification

Chemical: D-1
Aliquot code: CW-4-150-5-P-304
Datafile name: CW000630.D

Compound identified as: Original Compound
Compound reference: Reference Chemical (OPCW)

Analysis Method

GC Instrument manufacturer and type: Agilent 6890
Carrier gas: Helium
Flow control/rate: Constant Flow, 32 cm/sec
Injection mode: Pulsed Splitless, 0.75 min
Injector temperature: 250 ºC
Column brand/phase: Agilent HP-5MS: (5%-Phenyl)-methylpolysiloxane
Column Length x ID x Film thickness: 30 m x 0.25 mm x 0.25 µm
GC temperature programme: 40 ºC (3 min), 8 ºC/min to 100 ºC, 25 ºC/min to 300 ºC (3 min)

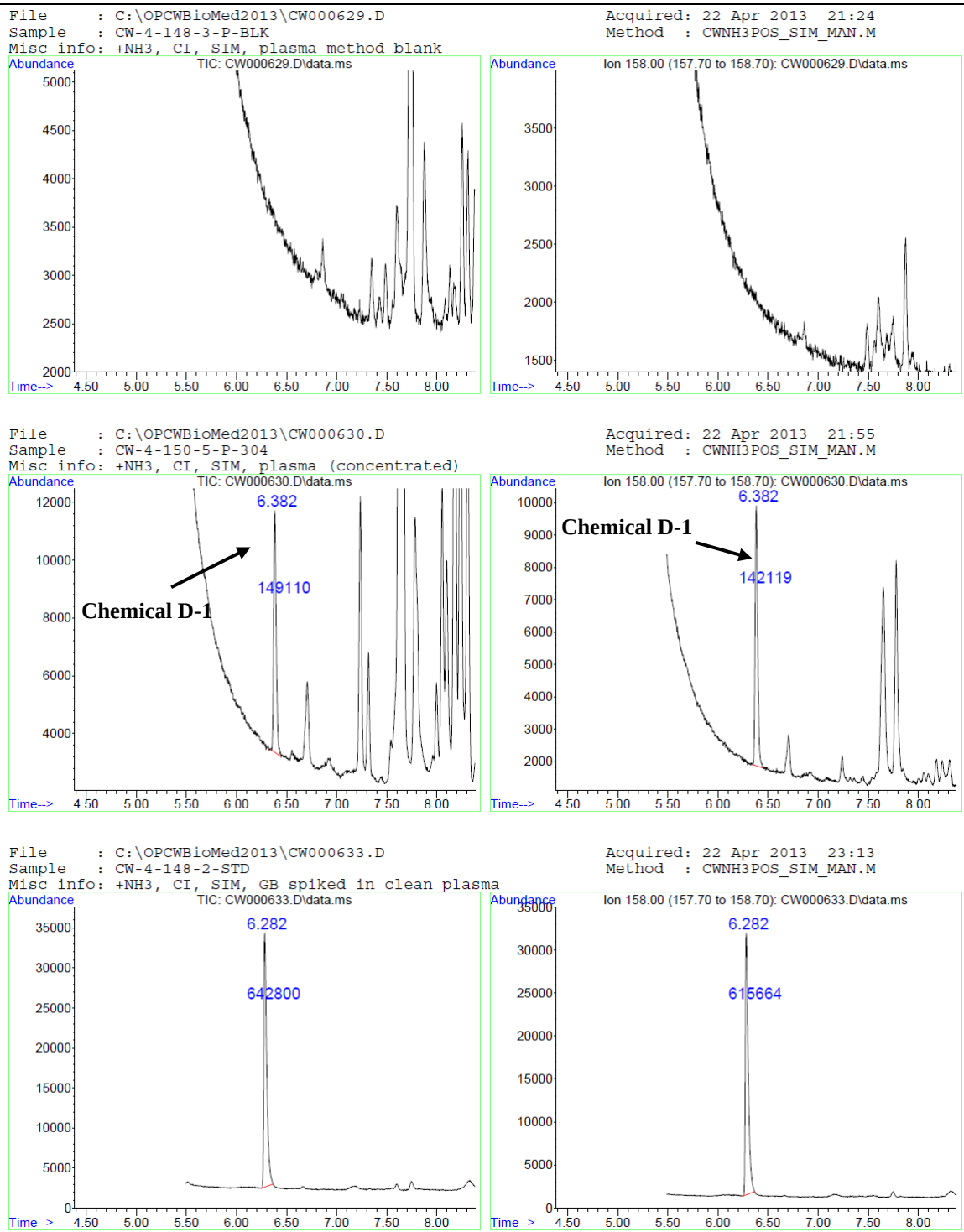
MS Instrument manufacturer and type: Agilent 5975 MSD
Solvent delay time: 5.4 min
Electron energy: 82 eV
Scan range/time: SIM, m/z 141, 158, 100 µs dwell time
Source temperature: 230 ºC
Reaction gas: Ammonia **and flow:** 21%
Ionisation polarity: Positive

Ions/transitions	Sample		Standard		Criteria	Result
	Area*	Ion Ratio	Area*	Ion Ratio	Tolerance#	
141	6306	0.044	28724	0.047	±50%	4.9%
158	142119	1.000	615664	1.000	N/A	N/A

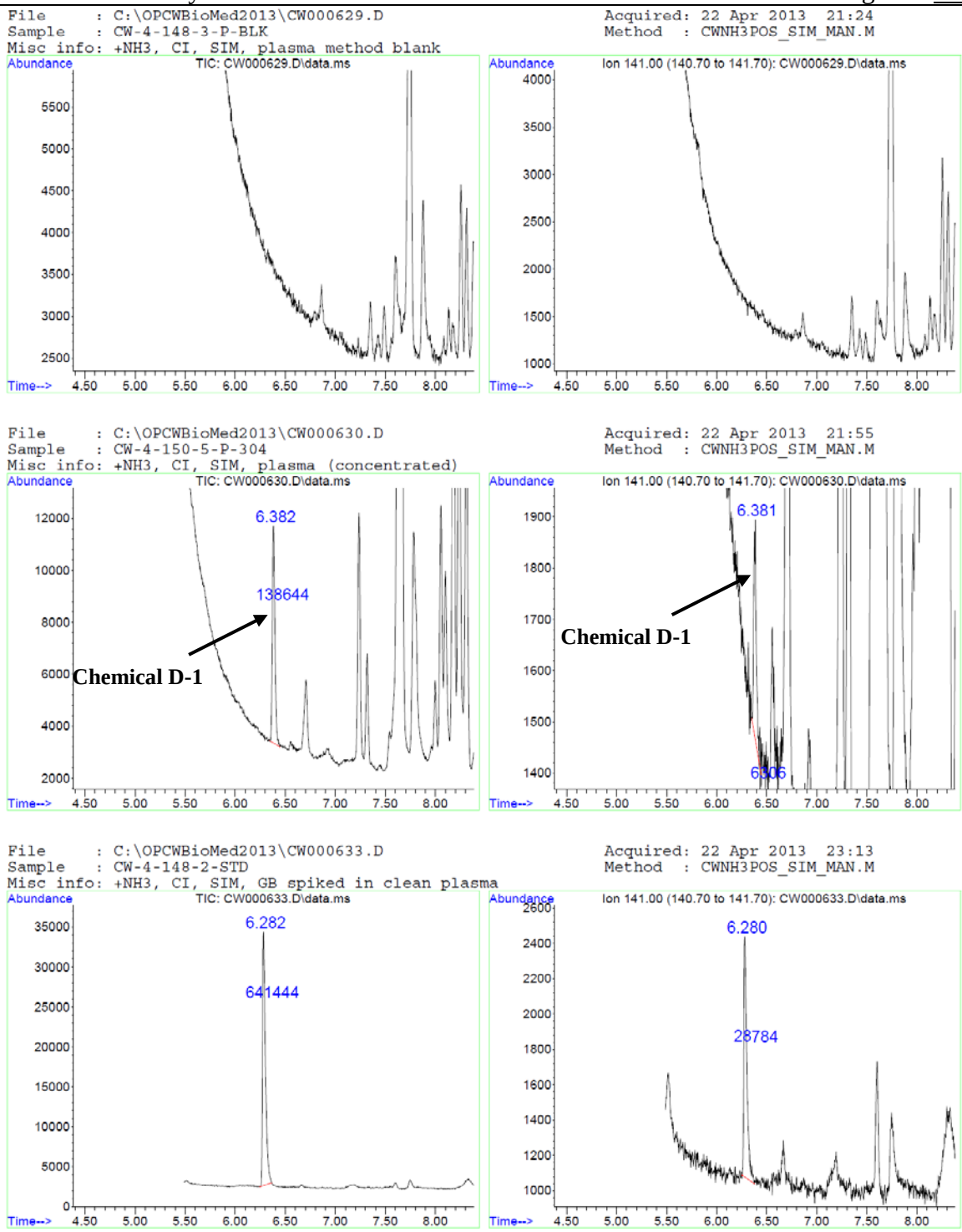
*****Peak area of the ion, % intensity compared to the most abundant ion
Compare with the relative ion intensity of the standard: >50%: ±20%; >20% to 50%: ±25%; >10% to 20%: ±30%; ≤10%: ±50%

Remarks

Due to the solvent delay of 5.4 min, the chromatogram could not be presented with a window of the retention time of the compound minus 2 min.



EI chromatograms supporting identification of **Chemical D-1;**
TIC on left, EIC (m/z 158) on right.
Top: Chromatogram of the blank.
Center: Chromatogram of the sample (aliquot code listed in header).
Bottom: Chromatogram of reference chemical of **O-Isopropyl methylphosphonofluoridate.**

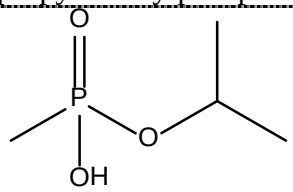


EI chromatograms supporting identification of **Chemical D-1;**
TIC on left, EIC (m/z 141) on right.
Top: Chromatogram of the blank.
Center: Chromatogram of the sample (aliquot code listed in header).
Bottom: Chromatogram of reference chemical of **O-Isopropyl methylphosphonofluoridate.**

SAMPLE SUMMARY: E

Sample Code: U-305/07	**Laboratory Assigned Code:** CW-4-147-5
Description and condition of sample: Approximately 10 mL of urine	

Chemical: E-1

Chemical name & Structure	CAS #	Schedule
Isopropyl methylphosphonate 	1832-54-8	2.B.04

Aliquot(s)	Original/derivative	Analysis technique
CW-4-151-2-U-305	Pentafluorobenzyl derivative	GC-MS/MS(CI)
CW-4-151-2-U-305	Pentafluorobenzyl derivative	GC-MS/MS(CI)
Comments:		

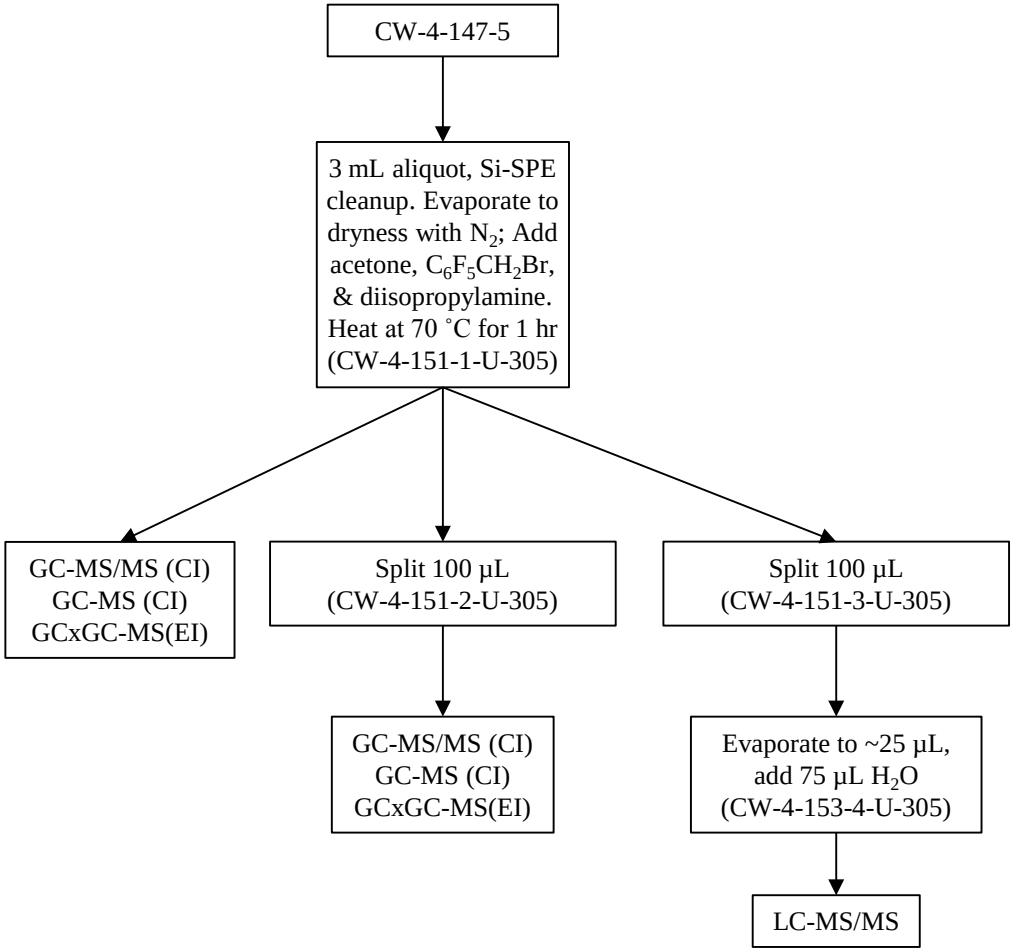
SAMPLE PREPARATION DESCRIPTION: E

1. Sample preparation

Initial Aliquot Code	Type of Sample Preparation	Amount/ Volume	Sample/Blank Preparation Procedures	End Volume	Resulting Aliquot Code
CW-4-147-5	Urine Clean-Up and Pentafluorobenzyl Derivatization	3 mL	Silica SPE cartridge cleanup. Eluted using 3 mL 25% H_2O in acetonitrile. Reduced sample volume to complete dryness with nitrogen gas. Added 300 µL of acetone, 5 µL pentafluorobenzyl bromide and 5 µL diisopropylamine. Sample heated at 70˚C for one hour.	310 µL	CW-4-151-1-U-305
CW-4-151-1-U-305	Sample Split	100 µL	Split of sample CW-4-151-1-U-305.	100 µL	CW-4-151-2-U-305
CW-4-151-1-U-305	Sample Split	100 µL	Split of sample CW-4-151-1-U-305.	100 µL	CW-4-151-3-U-305
CW-4-151-3-U-305	Preparation for LC-MS Analysis	100 µL	Reduced volume to ~25 µL with nitrogen gas. Added 75 µL of H_2O.	100 µL	CW-4-153-4-U-305

2. Additional information

SPE=solid phase extraction


CW-4-147-5
3 mL aliquot, Si-SPE cleanup. Evaporate to dryness with N_2; Add acetone, $C_6F_5CH_2Br$, & diisopropylamine. Heat at 70 ˚C for 1 hr (CW-4-151-1-U-305)
GC-MS/MS (CI) GC-MS (CI) GCxGC-MS(EI)
Split 100 µL (CW-4-151-2-U-305)
Split 100 µL (CW-4-151-3-U-305)
GC-MS/MS (CI) GC-MS (CI) GCxGC-MS(EI)
Evaporate to ~25 µL, add 75 µL H_2O (CW-4-153-4-U-305)
LC-MS/MS

Description of sample preparation and analysis methods

Sample preparation and analysis methods were developed using an in-house standard made by spiking a mixture of methylphosphonic acid, ethyl methylphosphonic acid, isopropyl methylphosphonic acid, and pinacolyl methylphosphonic acid into commercially procured human urine. The resultant acid-containing urine was used for subsequent method development.

Several SPE methods were attempted to isolate the phosphonic acids from the urine sample:

1. Alltech Silica (500 mg/4 mL)
   Conditioning: 25%H_2O in acetonitrile; 3 mL of acetonitrile
   Load 3x1 mL of urine sample.
   Wash: 2mL acetonitrile; 2 mL 10% H_2O in acetonitrile.
   Elution: 25%H_2O (in acetonitrile)
2. Agilent ABS Elut-NEXUS (200 mg/ 6 mL)
   Conditioning: N-hexane; Ethyl Acetate; H_2O
   Load 3x1 mL of urine sample
   Elution: Ethyl Acetate
3. Phenomenex Strata-X 33µm (30 mg/ 3 mL)
   Sample pretreatment: Dilute sample 1:1 with Acetate Buffer (pH ~3.5)
   Conditioning: Methanol; Acetate Buffer (pH~3.5)
   Load: 1 mL of urine sample
   Wash: Acetate Buffer (pH ~3.5); methanol
   Elution: 5% ammonium hydroxide in methanol

The following procedure describes, in detail, the method that was determined to be most successful and used for the sample (SPE preparation #1):
Spiked urine samples were processed following cleanup conditions found in Mawhinney (2007). An Alltech silica SPE was conditioned with 4mL 25% water in acetonitrile, followed by 3 mL of acetonitrile. Sample, 3x1 mL, was loaded onto cartridge and washed with 2 mL acetonitrile and 2 mL of 10% water in acetonitrile. Samples were eluted with using 25% water in acetonitrile.

Mawhinney took urine sample to dryness and reconstituted before introduction to SPE cartridge; however, in a side by side comparison, we found loading urine directly onto a conditioned cartridge resulted in the best recovery. Additionally, success has been reported with using polymeric SPE for cleanup and although we found success with one polymeric SPE, our best recovery was using silica SPE.

The urine sample eluted from the SPE cartridge was derivatized before analysis. The eluted sample was taken to dryness. Following the process outlined in Palit (2004), the dried samples were derivatized by adding 5 µL pentafluorobenzyl bromide, 5 µL diisopropylamine and 300 µL acetone and heating to 70°C for 1 hour.

References

Mawhinney, D.B., Hameli, E.I., Fraser, R., Silva, S.S., Pavlopoulos, A.J. Kobelski, R.J. J.; The determination of organophosphate nerve agent metabolites in human urine by hydrophilic interaction liquid chromatography tandem mass spectrometry. J. Chromatogr. B. 852 (2007) 235-243.

Palit, M., Gupta, A.K., Jain, R. and Raza, S.K.; Determination of pentafluorobenzyl derivatives of phosphonic and phosphonothionic acids by gas chromatography-mass spectrometry. J Chromatogr. A, 1043 (2004) 275-284.

GC-MS/MS (CI) TECHNIQUE METHOD AND ANALYSIS DESCRIPTION

Identification

Chemical: E-1
Aliquot code: CW-4-151-2-U-305
Datafile name: TSQ0358
Compound identified as: Pentafluorobenzyl derivative
Compound reference: Reference Chemical (own synthesis)
Match algorithm and match factor: NIST, 981/999

Analysis Method

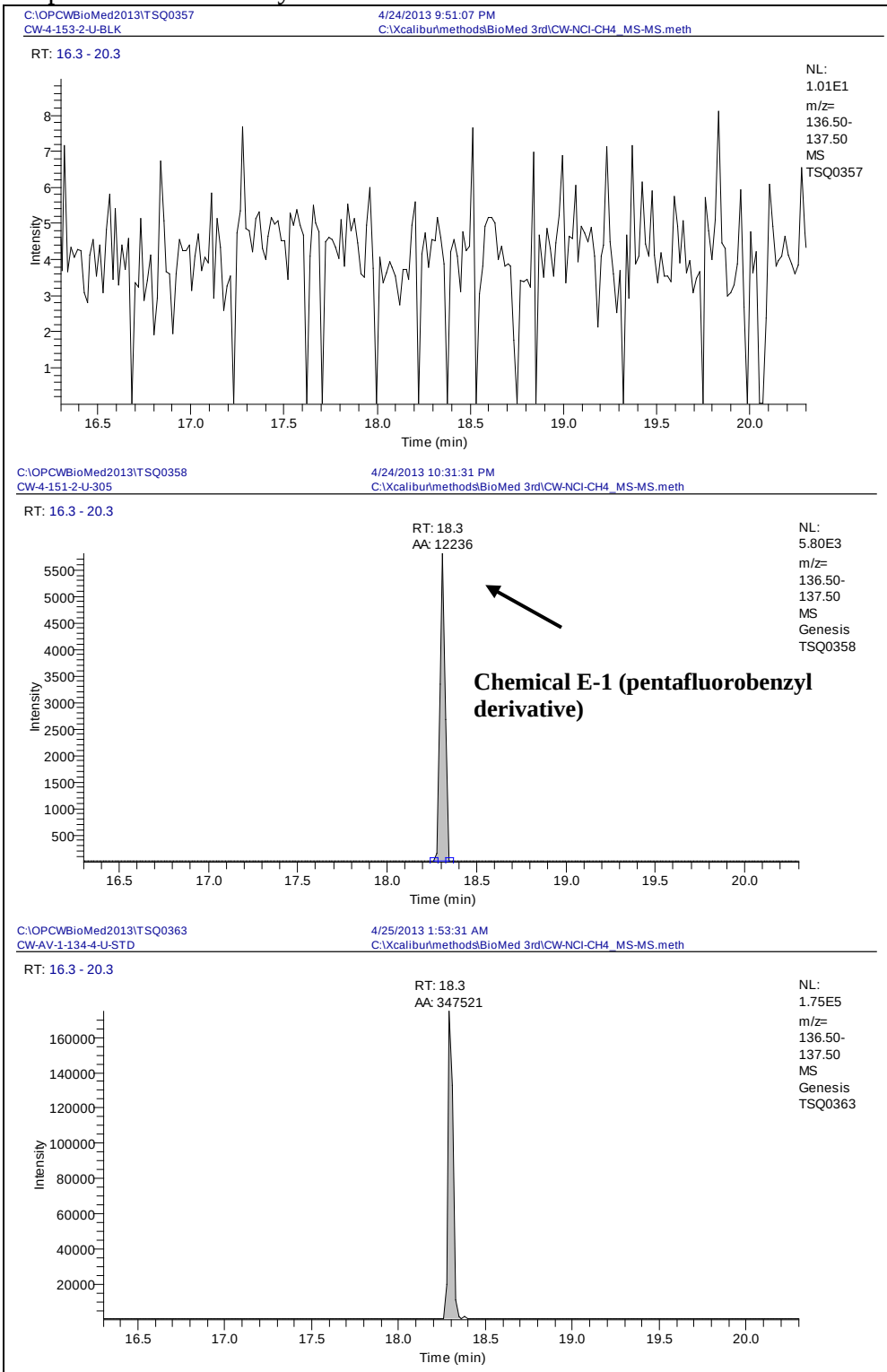
GC Instrument manufacturer and type: Thermo Fisher Trace GC Ultra
Carrier gas: Helium
Flow control/rate: Constant flow, 1 mL/min
Injection mode: Splitless, 0.60 min
Injector temperature: 230 ºC
Column brand/phase: Agilent HP-5MS: (5%-Phenyl)-methylpolysiloxane
Column Length x ID x Film thickness: 30 m x 0.25 mm x 0.25 µm
GC temperature programme: 40 ºC (3 min), 8 ºC/min to 215 ºC, 20 ºC/min to 300 ºC (1 min)

MS Instrument manufacturer and type: Thermo Fisher TSQ Quantum
Solvent delay time: 3 min
Electron energy: 100 eV
Reaction gas: Methane
Ionisation polarity: Negative
Scan range/time: 50-500 m/z in 1 second
Mass resolution: 0.7
Type of MS/MS scan: Product ion scan
Precursor ion(s): m/z 137
Collision gas: Argon
Collision Energy: 10

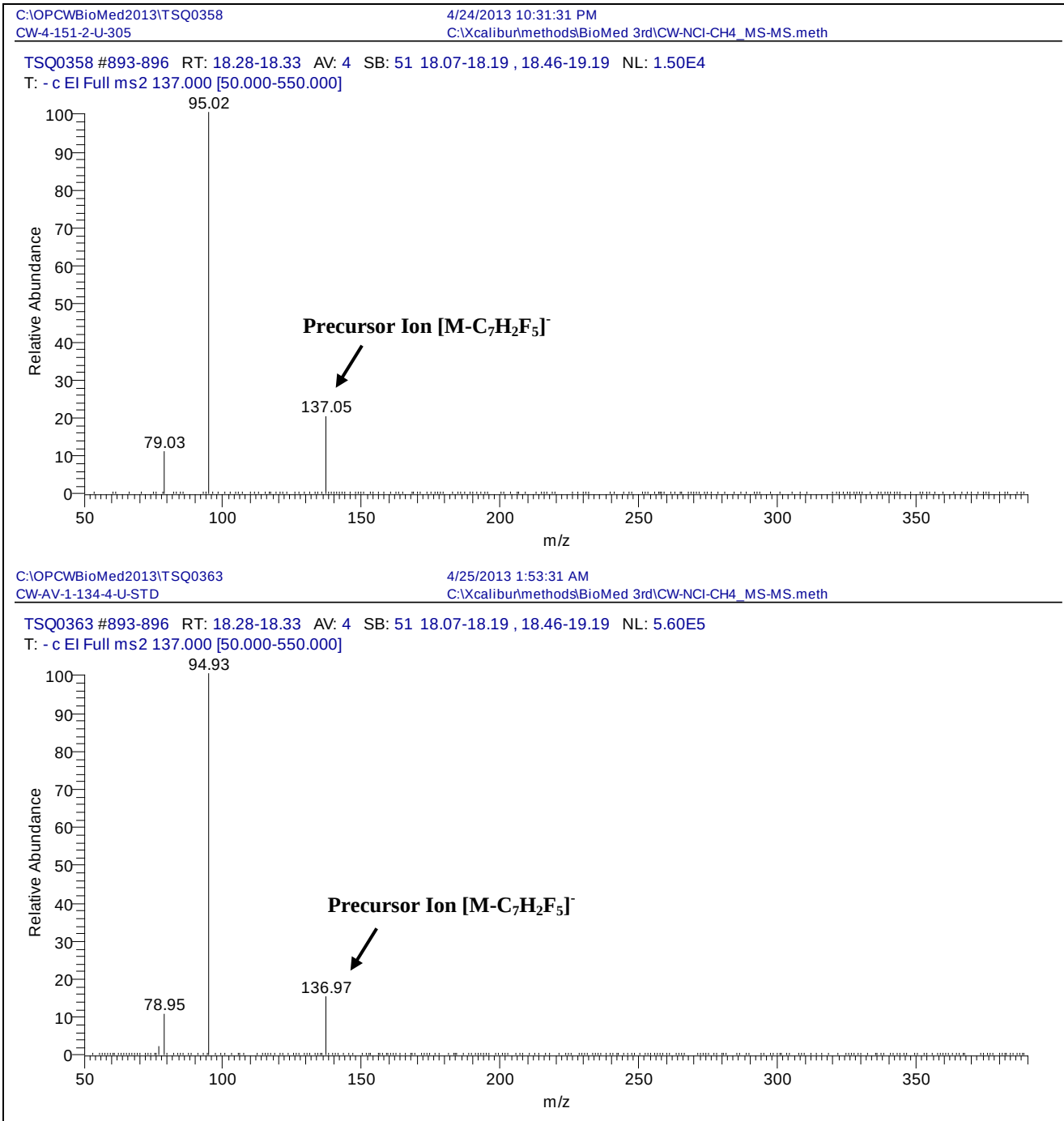
Ions/transitions	Sample		Standard		Criteria	Result
	Area*	Ion Ratio	Area*	Ion Ratio	Tolerance#	
79	6684	0.104	243159	0.109	±30%	4.4%
95	61457	1.0	2338092	1.0	N/A	N/A
137	12236	0.149	347521	0.199	±30%	33.0%

*****Peak area of the ion, % intensity compared to the most abundant ion
Compare with the relative ion intensity of the standard: >50%: ±20%; >20% to 50%: ±25%; >10% to 20%: ±30%; ≤10%: ±50%

Remarks



GC-MS/MS (CI) chromatograms supporting identification of **Chemical E-1 (pentafluorobenzyl derivative)**; TIC
Top: Chromatogram of the blank.
Center: Chromatogram of the sample (aliquot code listed in header).
Bottom: Chromatogram of reference chemical of **Pentafluorobenzyl derivative of isopropyl methylphosphonate.**



CI product mass spectra of:
Top: **Chemical E-1 (pentafluorobenzyl derivative)** (aliquot code listed in header).
Bottom: Reference chemical of **Pentafluorobenzyl derivative of isopropyl methylphosphonate.**

GC-MS/MS (CI) TECHNIQUE METHOD AND ANALYSIS DESCRIPTION

Identification

Chemical: E-1
Aliquot code: CW-4-151-2-U-305
Datafile name: TSQ0379
Compound identified as: Pentafluorobenzyl derivative
Compound reference: Reference Chemical (own synthesis)
Match algorithm and match factor: NIST, 848/999

Analysis Method

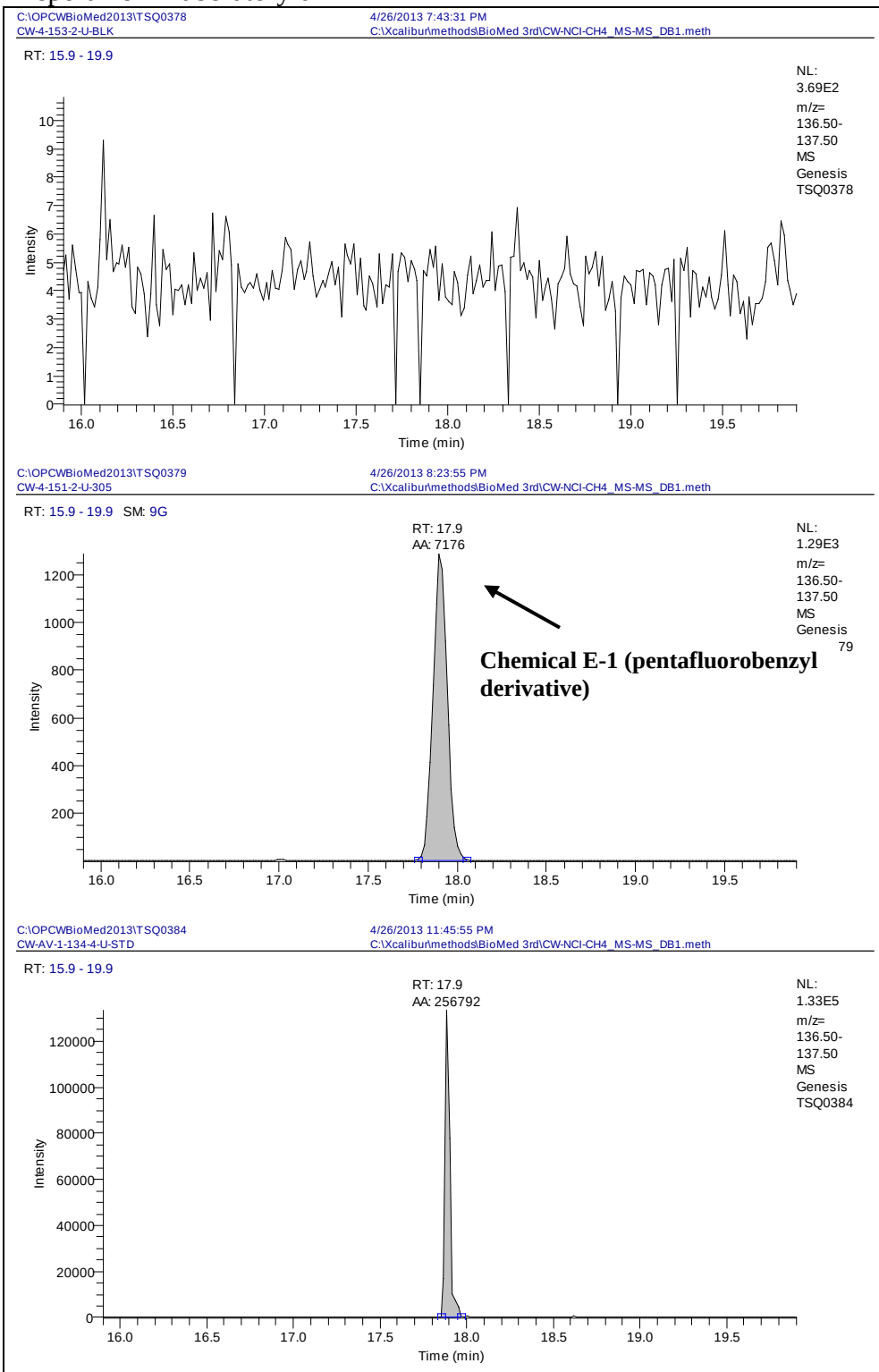
GC Instrument manufacturer and type: Thermo Fisher Trace GC Ultra
Carrier gas: Helium
Flow control/rate: Constant flow, 1 mL/min
Injection mode: Splitless, 0.60 min
Injector temperature: 230 ºC
Column brand/phase: Agilent DB-1: 100% Dimethylpolysiloxane
Column Length x ID x Film thickness: 30 m x 0.25 mm x 0.25 µm
GC temperature programme: 40 ºC (3 min), 8 ºC/min to 215 ºC, 20 ºC/min to 300 ºC (1 min)

MS Instrument manufacturer and type: Thermo Fisher TSQ Quantum
Solvent delay time: 3 min
Electron energy: 100 eV
Reaction gas: Methane
Ionisation polarity: Negative
Scan range/time: 50-500 m/z in 1 second
Mass resolution: 0.7
Type of MS/MS scan: Product ion scan
Precursor ion(s): m/z 137
Collision gas: Argon
Collision Energy: 10

Ions/transitions	Sample		Standard		Criteria	Result
	Area*	Ion Ratio	Area*	Ion Ratio	Tolerance#	
79	2700	0.022	211094	0.113	±30%	78.1%
95	120425	1.000	1862500	1.000	N/A	N/A
137	7176	0.109	256792	0.138	±30%	21.0%

*****Peak area of the ion, % intensity compared to the most abundant ion
Compare with the relative ion intensity of the standard: >50%: ±20%; >20% to 50%: ±25%; >10% to 20%: ±30%; ≤10%: ±50%

Remarks

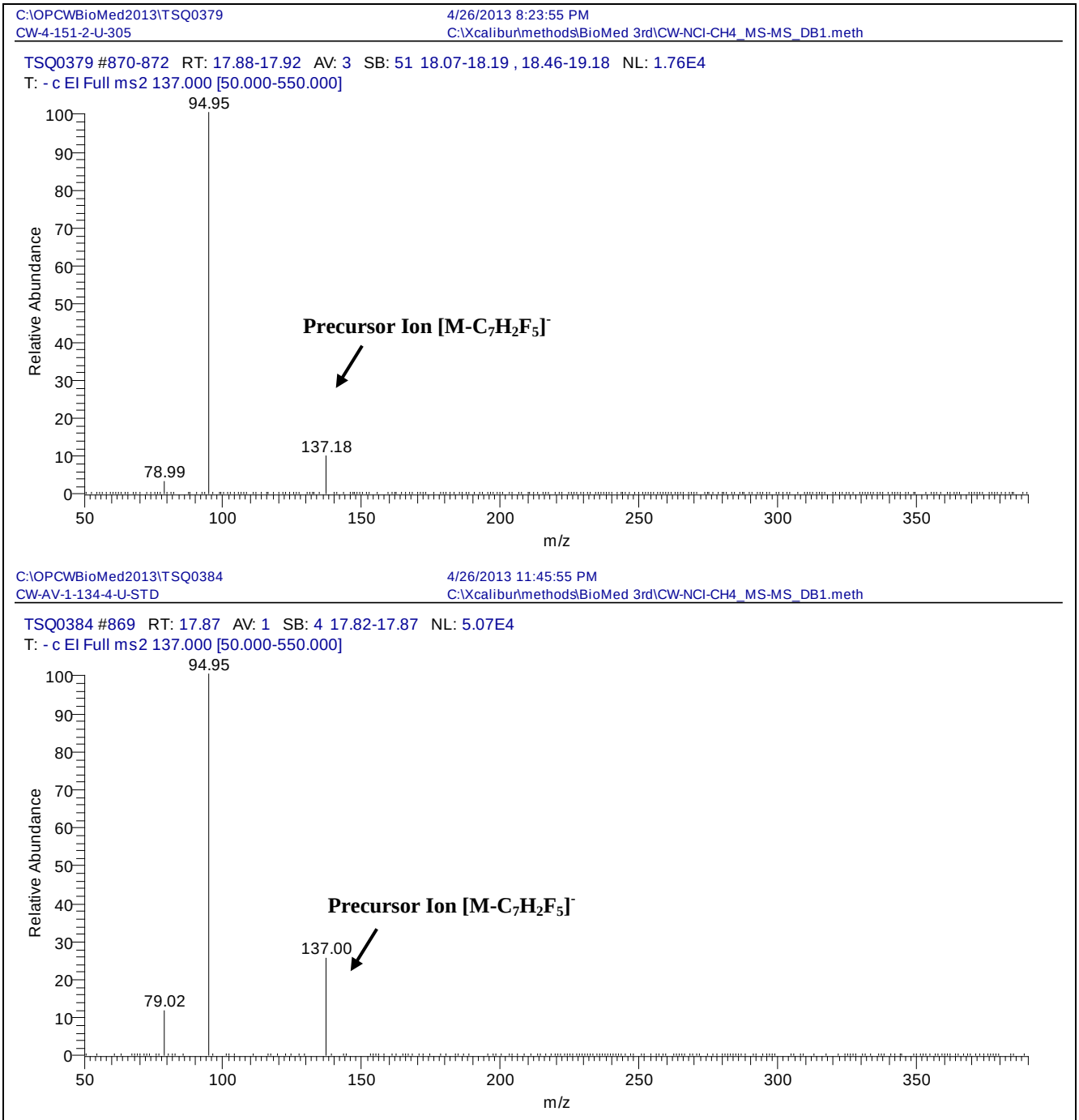


GC-MS/MS (CI) chromatograms supporting identification of **Chemical E-1 (pentafluorobenzyl derivative)**; TIC

Top: Chromatogram of the blank.

Center: Chromatogram of the sample (aliquot code listed in header).

Bottom: Chromatogram of reference chemical of **Pentafluorobenzyl derivative of isopropyl methylphosphonate.**

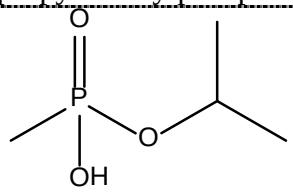


CI product mass spectra of:
Top: **Chemical E-1 (pentafluorobenzyl derivative)** (aliquot code listed in header).
Bottom: Reference chemical of **Pentafluorobenzyl derivative of isopropyl methylphosphonate.**

SAMPLE SUMMARY: F

Sample Code: U-306/07	**Laboratory Assigned Code:** CW-4-147-6
Description and condition of sample: Approximately 10 mL of urine	

Chemical: F-1

Chemical name & Structure	CAS #	Schedule
Isopropyl methylphosphonate 	1832-54-8	2.B.04

Aliquot(s)	Original/derivative	Analysis technique
CW-4-152-2-U-306	Pentafluorobenzyl derivative	GC-MS/MS(CI)
CW-4-152-2-U-306	Pentafluorobenzyl derivative	GC-MS/MS(CI)
Comments:		

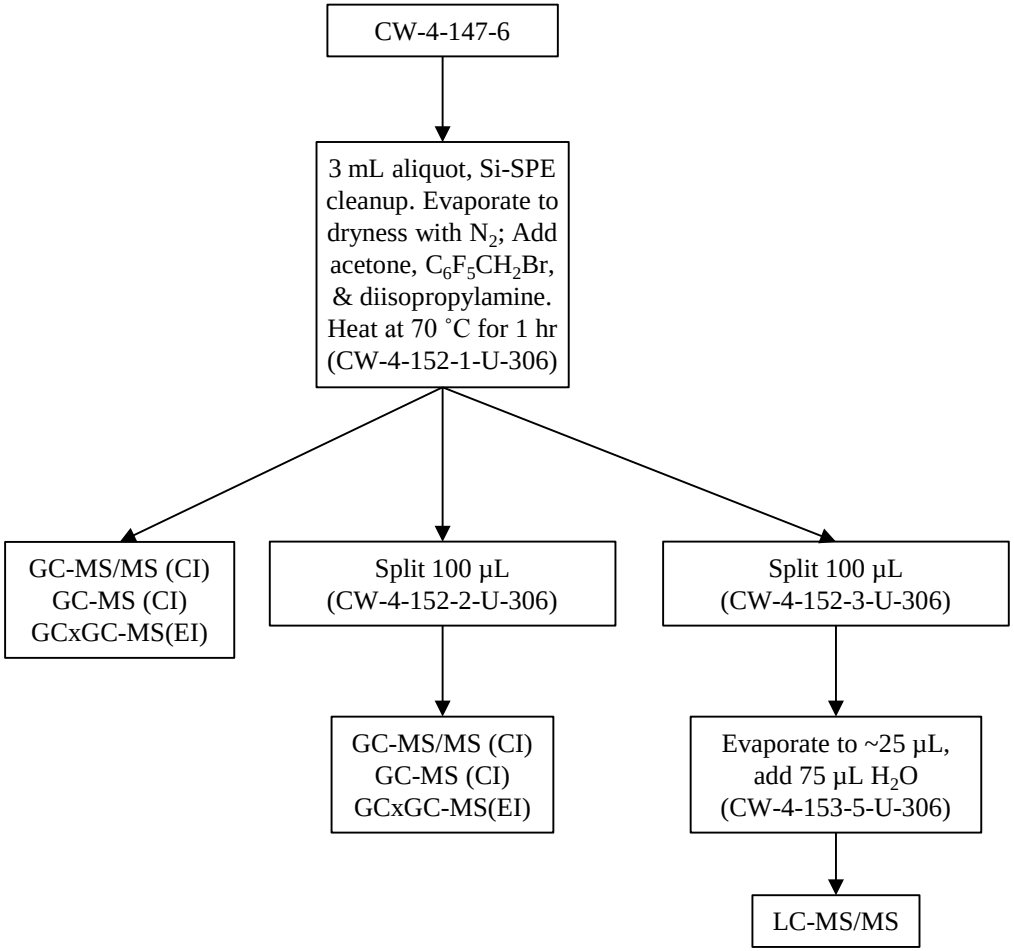
SAMPLE PREPARATION DESCRIPTION: F

1. Sample preparation

Initial Aliquot Code	Type of Sample Preparation	Amount/ Volume	Sample/Blank Preparation Procedures	End Volume	Resulting Aliquot Code
CW-4-147-6	Urine Clean-Up and Pentafluorobenzyl Derivatization	3 mL	Silica SPE cartridge cleanup. Eluted using 3 mL 25% H_2O in acetonitrile. Reduced sample volume to complete dryness with nitrogen gas. Added 300 µL of acetone, 5 µL pentafluorobenzyl bromide and 5 µL diisopropylamine. Sample heated at 70˚C for one hour.	310 µL	CW-4-152-1-U-306
CW-4-152-1-U-306	Sample Split	100 µL	Split of sample CW-4-152-1-U-306.	100 µL	CW-4-152-2-U-306
CW-4-152-1-U-306	Sample Split	100 µL	Split of sample CW-4-152-1-U-306.	100 µL	CW-4-152-3-U-306
CW-4-152-3-U-306	Preparation for LC-MS Analysis	100 µL	Reduced volume to ~25 µL with nitrogen gas. Added 75 µL of H_2O.	100 µL	CW-4-153-5-U-306

2. Additional information

SPE=solid phase extraction


CW-4-147-6
3 mL aliquot, Si-SPE cleanup. Evaporate to dryness with N_2; Add acetone, $C_6F_5CH_2Br$, & diisopropylamine. Heat at 70 ˚C for 1 hr (CW-4-152-1-U-306)
GC-MS/MS (CI) GC-MS (CI) GCxGC-MS(EI)
Split 100 µL (CW-4-152-2-U-306)
Split 100 µL (CW-4-152-3-U-306)
GC-MS/MS (CI) GC-MS (CI) GCxGC-MS(EI)
Evaporate to ~25 µL, add 75 µL H_2O (CW-4-153-5-U-306)
LC-MS/MS

Description of sample preparation and analysis methods

Sample preparation and analysis methods were developed using an in-house standard made by spiking a mixture of methylphosphonic acid, ethyl methylphosphonic acid, isopropyl methylphosphonic acid, and pinacolyl methylphosphonic acid into commercially procured human urine. The resultant acid-containing urine was used for subsequent method development.

Several SPE methods were attempted to isolate the phosphonic acids from the urine sample:

1. Alltech Silica (500 mg/4 mL)
   Conditioning: 25%H_2O in acetonitrile; 3 mL of acetonitrile
   Load 3x1 mL of urine sample.
   Wash: 2mL acetonitrile; 2 mL 10% H_2O in acetonitrile.
   Elution: 25%H_2O (in acetonitrile)
2. Agilent ABS Elut-NEXUS (200 mg/ 6 mL)
   Conditioning: N-hexane; Ethyl Acetate; H_2O
   Load 3x1 mL of urine sample
   Elution: Ethyl Acetate
3. Phenomenex Strata-X 33µm (30 mg/ 3 mL)
   Sample pretreatment: Dilute sample 1:1 with Acetate Buffer (pH ~3.5)
   Conditioning: Methanol; Acetate Buffer (pH~3.5)
   Load: 1 mL of urine sample
   Wash: Acetate Buffer (pH ~3.5); methanol
   Elution: 5% ammonium hydroxide in methanol

The following procedure describes, in detail, the method that was determined to be most successful and used for the sample (SPE preparation #1):
Spiked urine samples were processed following cleanup conditions found in Mawhinney (2007). An Alltech silica SPE was conditioned with 4mL 25% water in acetonitrile, followed by 3 mL of acetonitrile. Sample, 3x1 mL, was loaded onto cartridge and washed with 2 mL acetonitrile and 2 mL of 10% water in acetonitrile. Samples were eluted with using 25% water in acetonitrile.

Mawhinney took urine sample to dryness and reconstituted before introduction to SPE cartridge; however, in a side by side comparison, we found loading urine directly onto a conditioned cartridge resulted in the best recovery. Additionally, success has been reported with using polymeric SPE for cleanup and although we found success with one polymeric SPE, our best recovery was using silica SPE.

The urine sample eluted from the SPE cartridge was derivatized before analysis. The eluted sample was taken to dryness. Following the process outlined in Palit (2004), the dried samples were derivatized by adding 5 µL pentafluorobenzyl bromide, 5 µL diisopropylamine and 300 µL acetone and heating to 70°C for 1 hour.

References

Mawhinney, D.B., Hameli, E.I., Fraser, R., Silva, S.S., Pavlopoulos, A.J. Kobelski, R.J. J.; The determination of organophosphate nerve agent metabolites in human urine by hydrophilic interaction liquid chromatography tandem mass spectrometry. J. Chromatogr. B. 852 (2007) 235-243.

Palit, M., Gupta, A.K., Jain, R. and Raza, S.K.; Determination of pentafluorobenzyl derivatives of phosphonic and phosphonothionic acids by gas chromatography-mass spectrometry. J Chromatogr. A, 1043 (2004) 275-284.

GC-MS/MS (CI) TECHNIQUE METHOD AND ANALYSIS DESCRIPTION

Identification

Chemical: F-1
Aliquot code: CW-4-152-2-U-306
Datafile name: TSQ0360

Compound identified as: Pentafluorobenzyl derivative
Compound reference: Reference Chemical (own synthesis)
Match algorithm and match factor: NIST, 920/999

Analysis Method

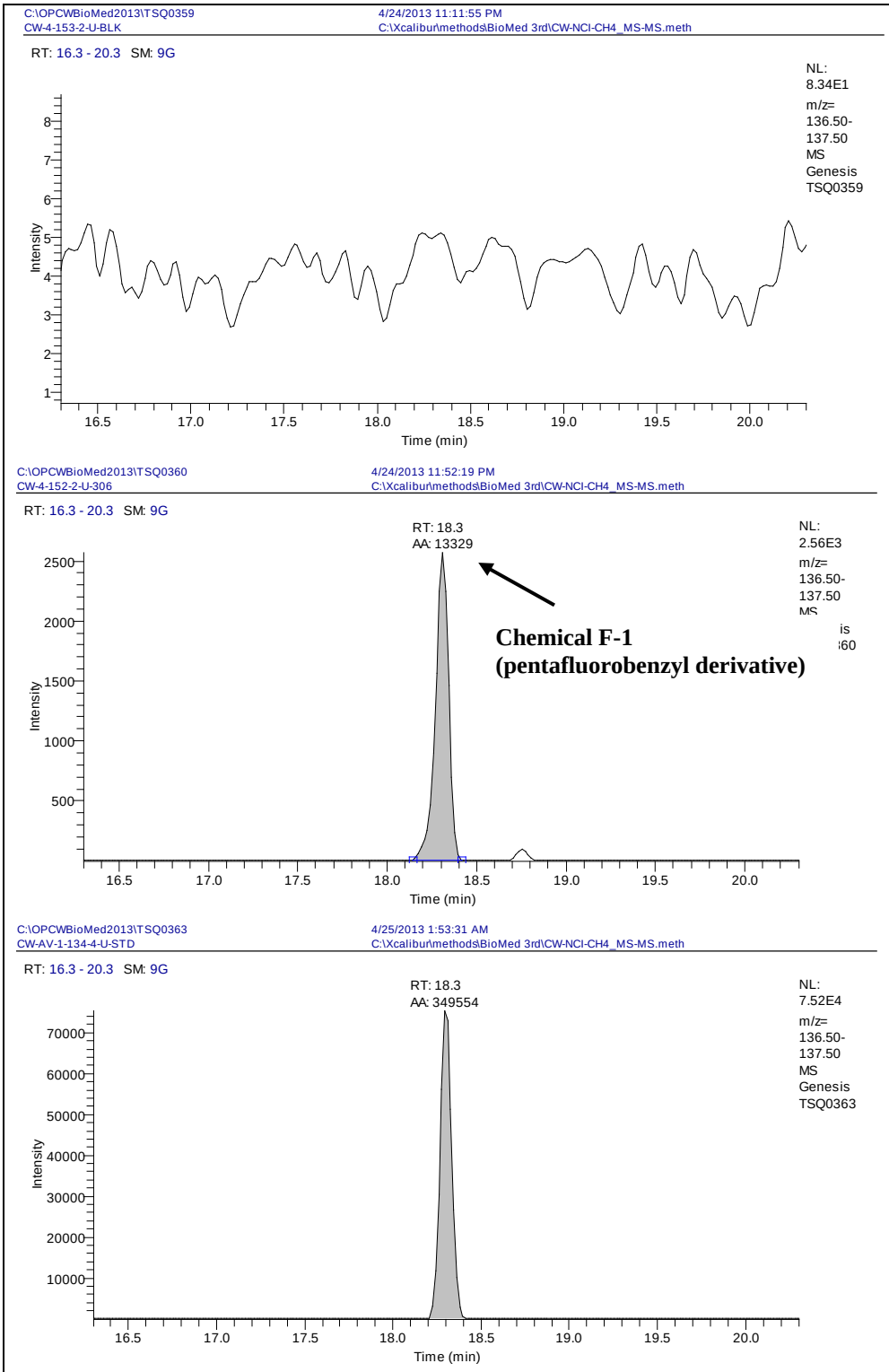
GC Instrument manufacturer and type: Thermo Fisher Trace GC Ultra
Carrier gas: Helium
Flow control/rate: Constant flow, 1 mL/min
Injection mode: Splitless, 0.60 min
Injector temperature: 230 ºC
Column brand/phase: Agilent HP-5MS: (5%-Phenyl)-methylpolysiloxane
Column Length x ID x Film thickness: 30 m x 0.25 mm x 0.25 µm
GC temperature programme: 40 ºC (3 min), 8 ºC/min to 215 ºC, 20 ºC/min to 300 ºC (1 min)

MS Instrument manufacturer and type: Thermo Fisher TSQ Quantum
Solvent delay time: 3 min
Electron energy: 100 eV
Reaction gas: Methane
Ionisation polarity: Negative
Scan range/time: 50-500 m/z in 1 second
Mass resolution: 0.7
Type of MS/MS scan: Product ion scan
Precursor ion(s): m/z 137
Collision gas: Argon
Collision Energy: 10

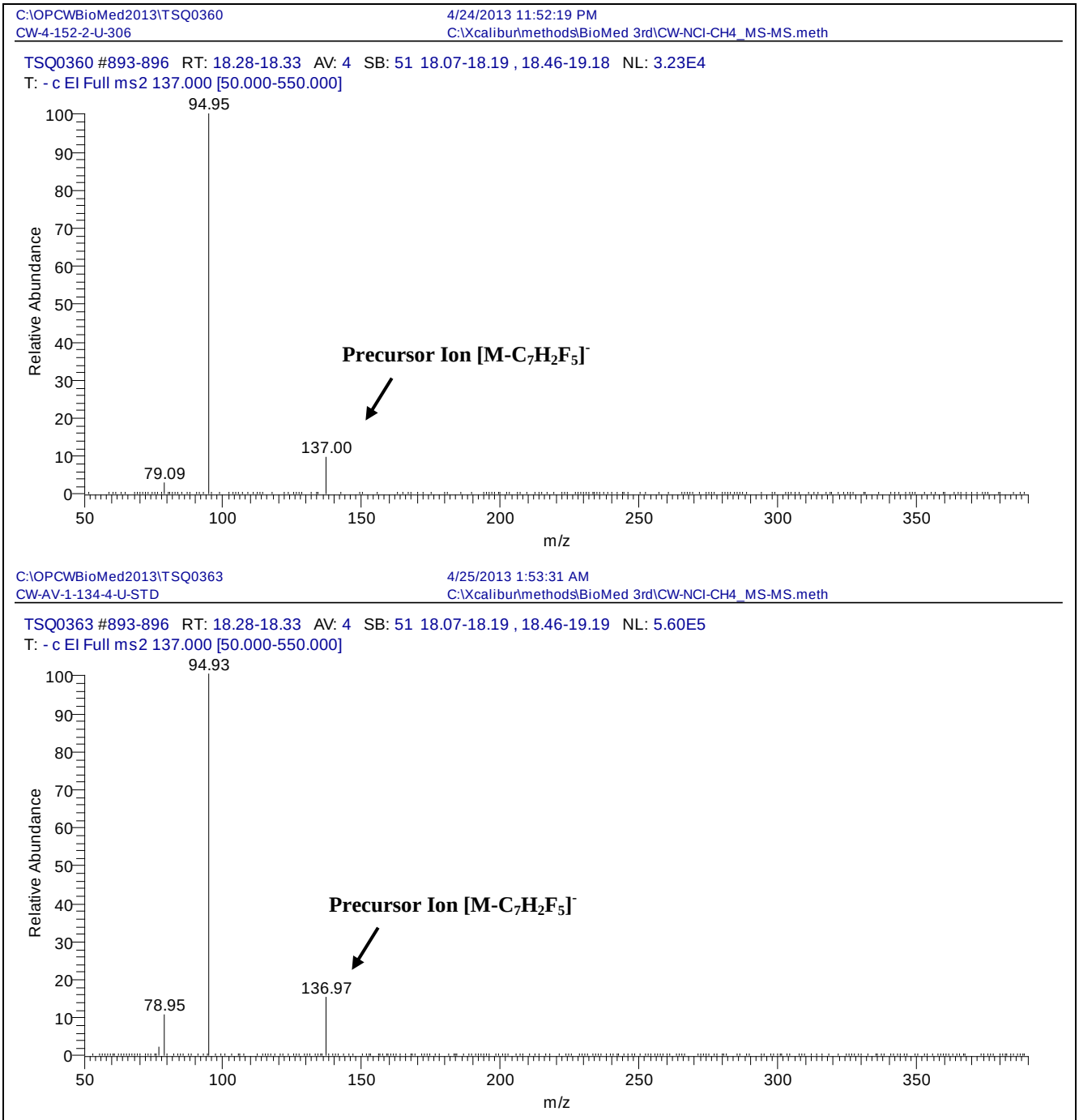
Ions/transitions	Sample		Standard		Criteria	Result
	Area*	Ion Ratio	Area*	Ion Ratio	Tolerance#	
79	3424	0.025	243296	0.104	±30%	73.2%
95	139394	1	2338092	1.0	N/A	N/A
137	13329	0.096	347521	0.199	±30%	27.2%

*****Peak area of the ion, % intensity compared to the most abundant ion
Compare with the relative ion intensity of the standard: >50%: ±20%; >20% to 50%: ±25%; >10% to 20%: ±30%; ≤10%: ±50%

Remarks



GC-MS/MS (CI) chromatograms supporting identification of **Chemical F-1 (pentafluorobenzyl derivative)**; TIC
Top: Chromatogram of the blank.
Center: Chromatogram of the sample (aliquot code listed in header).
Bottom: Chromatogram of reference chemical of **Pentafluorobenzyl derivative of isopropyl methylphosphonate.**



CI product mass spectra of:
Top: **Chemical F-1(pentafluorobenzyl derivative)** (aliquot code listed in header).
Bottom: Reference chemical of **Pentafluorobenzyl derivative of isopropyl methylphosphonate.**

GC-MS/MS (CI) TECHNIQUE METHOD AND ANALYSIS DESCRIPTION

Identification

Chemical: F-1
Aliquot code: CW-4-152-2-U-306
Datafile name: TSQ0381

Compound identified as: Pentafluorobenzyl derivative
Compound reference: Reference Chemical (own synthesis)
Match algorithm and match factor: NIST, 820/999

Analysis Method

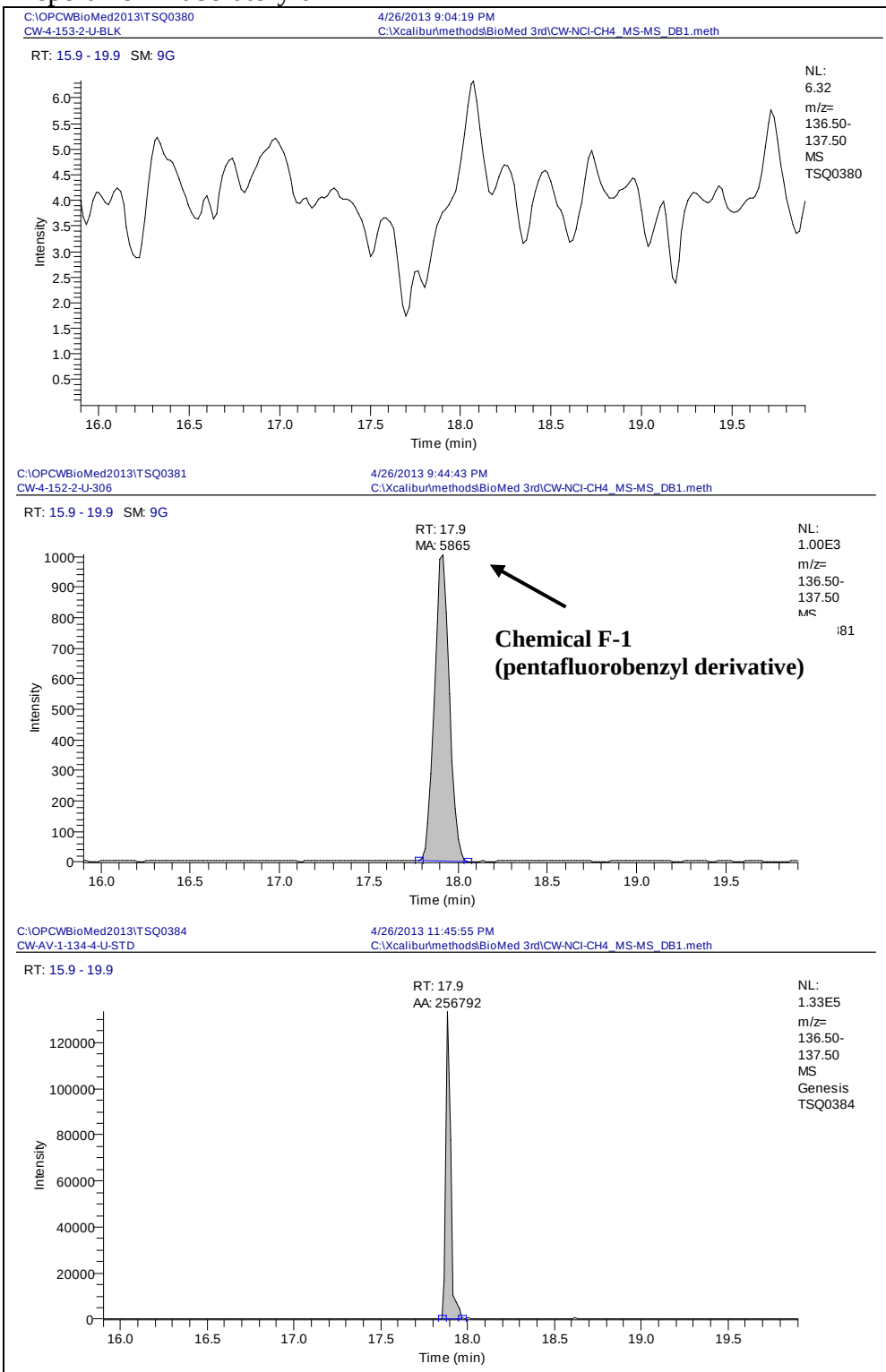
GC Instrument manufacturer and type: Thermo Fisher Trace GC Ultra
Carrier gas: Helium
Flow control/rate: Constant flow, 1 mL/min
Injection mode: Splitless, 0.60 min
Injector temperature: 230 ºC
Column brand/phase: Agilent DB-1: 100% Dimethylpolysiloxane
Column Length x ID x Film thickness: 30 m x 0.25 mm x 0.25 µm
GC temperature programme: 40 ºC (3 min), 8 ºC/min to 215 ºC, 20 ºC/min to 300 ºC (1 min)

MS Instrument manufacturer and type: Thermo Fisher TSQ Quantum
Solvent delay time: 3 min
Electron energy: 100 eV
Reaction gas: Methane
Ionisation polarity: Negative
Scan range/time: 50-500 m/z in 1 second
Mass resolution: 0.7
Type of MS/MS scan: Product ion scan
Precursor ion(s): m/z 137
Collision gas: Argon
Collision Energy: 10

Ions/transitions	Sample		Standard		Criteria	Result
	Area*	Ion Ratio	Area*	Ion Ratio	Tolerance[#]	
79	2700	0.022	211094	0.113	±30%	80.2%
95	120425	1.000	1862500	1.000	N/A	N/A
137	5865	0.049	256792	0.138	±30%	64.7%

*****Peak area of the ion, % intensity compared to the most abundant ion
[#] Compare with the relative ion intensity of the standard: >50%: ±20%; >20% to 50%: ±25%; >10% to 20%: ±30%; ≤10%: ±50%

Remarks

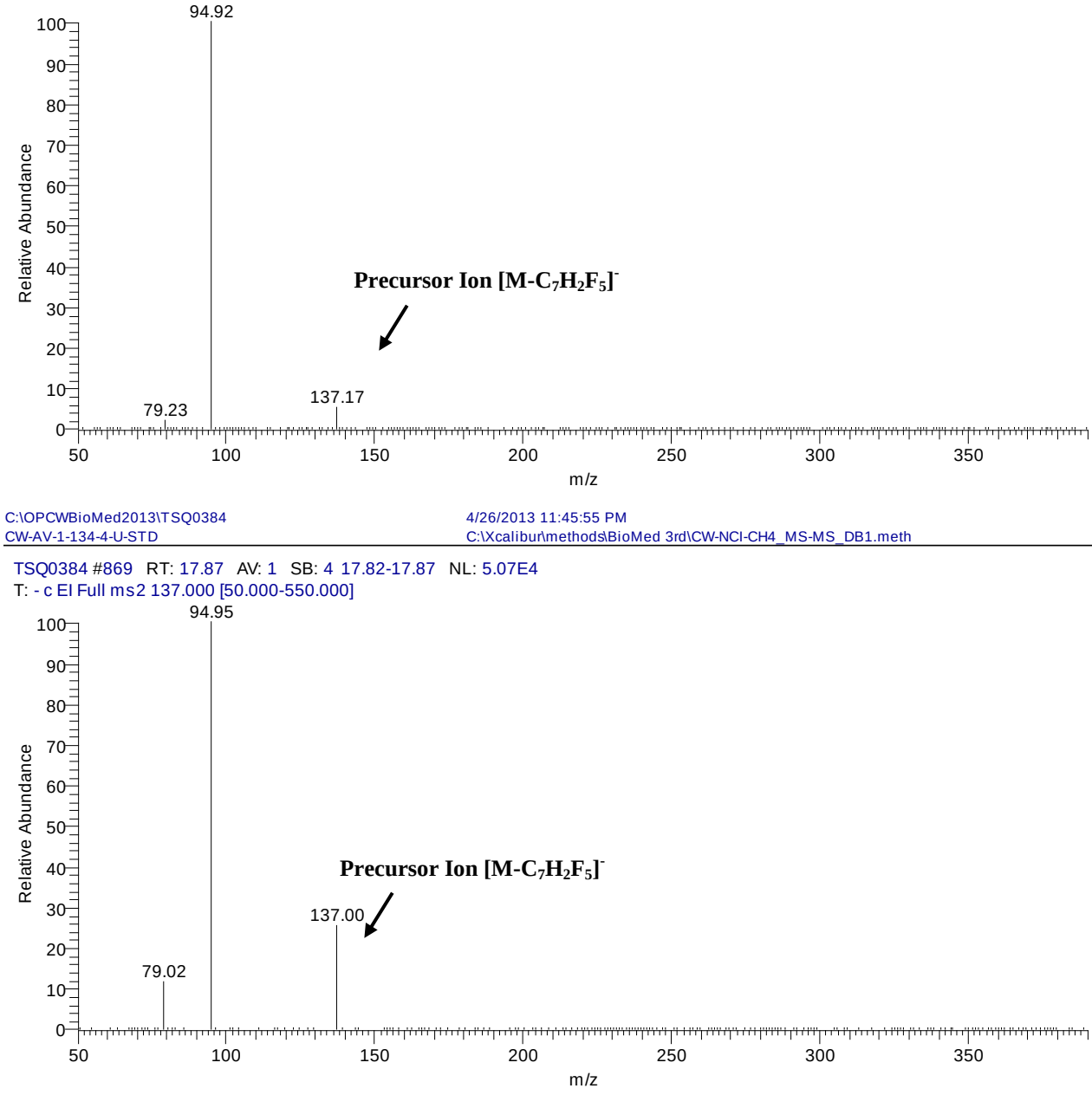


GC-MS/MS (CI) chromatograms supporting identification of **Chemical F-1 (pentafluorobenzyl derivative)**; TIC

Top: Chromatogram of the blank.

Center: Chromatogram of the sample (aliquot code listed in header).

Bottom: Chromatogram of reference chemical of **Pentafluorobenzyl derivative of isopropyl methylphosphonate.**

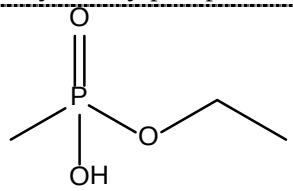


CI product mass spectra of:
Top: **Chemical F-1(pentafluorobenzyl derivative)** (aliquot code listed in header).
Bottom: Reference chemical of **Pentafluorobenzyl derivative of isopropyl methylphosphonate.**

SAMPLE SUMMARY: G

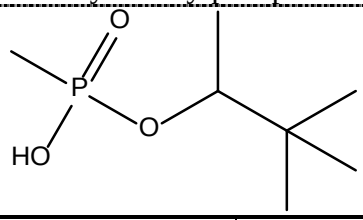
Sample Code: U-307/07	**Laboratory Assigned Code:** CW-4-147-7
Description and condition of sample: Approximately 10 mL of urine	

Chemical: G-1

Chemical name & Structure	**CAS #**	**Schedule**
Ethyl methylphosphonate 	1832-53-7	2.B.04

Aliquot(s)	**Original/derivative**	**Analysis technique**
CW-4-152-5-U-307	Pentafluorobenzyl derivative	GC-MS/MS(CI)
CW-4-152-5-U-307	Pentafluorobenzyl derivative	GC-MS/MS(CI)
Comments:		

Chemical: G-2

Chemical name & Structure	**CAS #**	**Schedule**
Pinacolyl methylphosphonate 	616-52-4	2.B.04

Aliquot(s)	**Original/derivative**	**Analysis technique**
CW-4-152-5-U-307	Pentafluorobenzyl derivative	GC-MS/MS(CI)
CW-4-152-5-U-307	Pentafluorobenzyl derivative	GC-MS/MS(CI)
CW-4-153-6-U-307	Pentafluorobenzyl derivative	LC-MS/MS
Comments:		

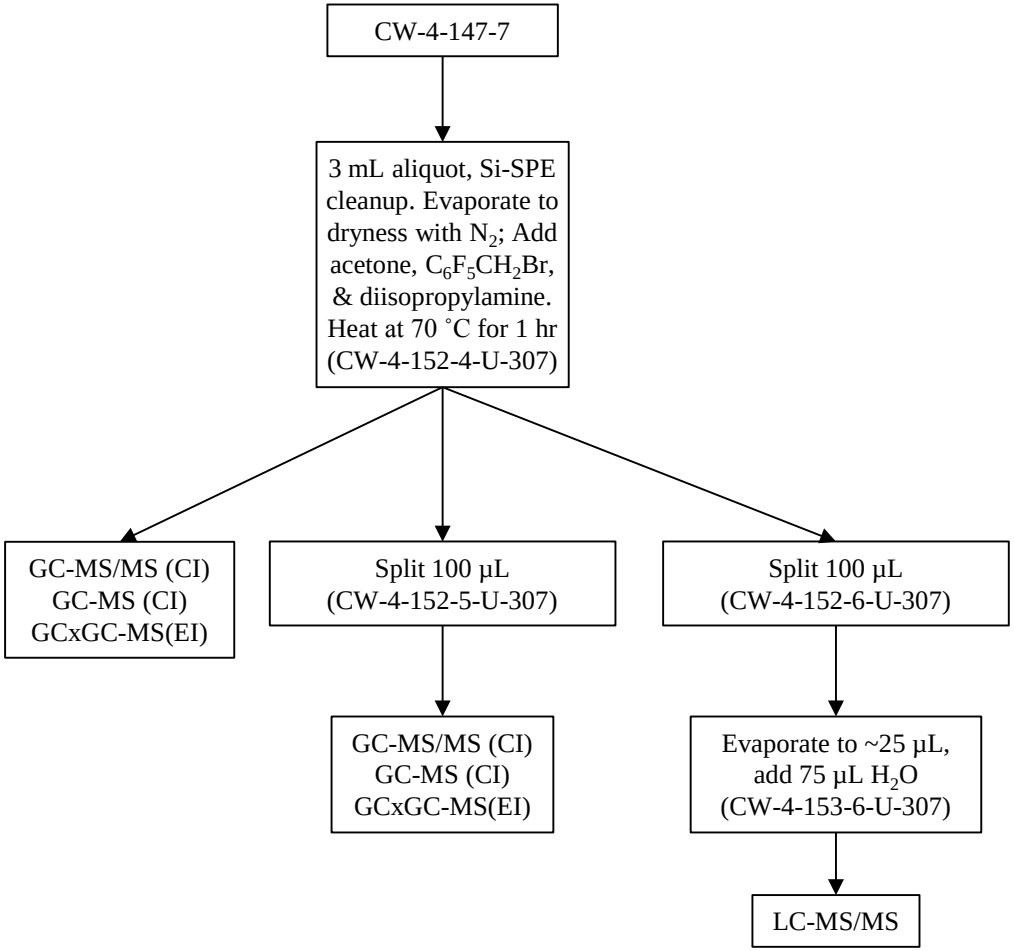
SAMPLE PREPARATION DESCRIPTION: G

1. Sample preparation

Initial Aliquot Code	Type of Sample Preparation	Amount/ Volume	Sample/Blank Preparation Procedures	End Volume	Resulting Aliquot Code
CW-4-147-7	Urine Clean-Up and Pentafluorobenzyl Derivatization	3 mL	Silica SPE cartridge cleanup. Eluted using 3 mL 25% H_2O in acetonitrile. Reduced sample volume to complete dryness with nitrogen gas. Added 300 µL of acetone, 5 µL pentafluorobenzyl bromide and 5 µL diisopropylamine. Sample heated at 70˚C for one hour.	310 µL	CW-4-152-4-U-307
CW-4-152-4-U-307	Sample Split	100 µL	Split of sample CW-4-152-4-U-307.	100 µL	CW-4-152-5-U-307
CW-4-152-4-U-307	Sample Split	100 µL	Split of sample CW-4-152-4-U-307.	100 µL	CW-4-152-6-U-307
CW-4-152-6-U-307	Preparation for LC-MS Analysis	100 µL	Reduced volume to ~25 µL with nitrogen gas. Added 75 µL of H_2O.	100 µL	CW-4-153-6-U-307

2. Additional information

SPE=solid phase extraction


CW-4-147-7
3 mL aliquot, Si-SPE cleanup. Evaporate to dryness with N_2; Add acetone, $C_6F_5CH_2Br$, & diisopropylamine. Heat at 70 ˚C for 1 hr (CW-4-152-4-U-307)
GC-MS/MS (CI) GC-MS (CI) GCxGC-MS(EI)
Split 100 µL (CW-4-152-5-U-307)
Split 100 µL (CW-4-152-6-U-307)
GC-MS/MS (CI) GC-MS (CI) GCxGC-MS(EI)
Evaporate to ~25 µL, add 75 µL H_2O (CW-4-153-6-U-307)
LC-MS/MS

Description of sample preparation and analysis methods

Sample preparation and analysis methods were developed using an in-house standard made by spiking a mixture of methylphosphonic acid, ethyl methylphosphonic acid, isopropyl methylphosphonic acid, and pinacolyl methylphosphonic acid into commercially procured human urine. The resultant acid-containing urine was used for subsequent method development.

Several SPE methods were attempted to isolate the phosphonic acids from the urine sample:

1. Alltech Silica (500 mg/4 mL)
   Conditioning: 25%H_2O in acetonitrile; 3 mL of acetonitrile
   Load 3x1 mL of urine sample.
   Wash: 2mL acetonitrile; 2 mL 10% H_2O in acetonitrile.
   Elution: 25%H_2O (in acetonitrile)
2. Agilent ABS Elut-NEXUS (200 mg/ 6 mL)
   Conditioning: N-hexane; Ethyl Acetate; H_2O
   Load 3x1 mL of urine sample
   Elution: Ethyl Acetate
3. Phenomenex Strata-X 33µm (30 mg/ 3 mL)
   Sample pretreatment: Dilute sample 1:1 with Acetate Buffer (pH ~3.5)
   Conditioning: Methanol; Acetate Buffer (pH~3.5)
   Load: 1 mL of urine sample
   Wash: Acetate Buffer (pH ~3.5); methanol
   Elution: 5% ammonium hydroxide in methanol

The following procedure describes, in detail, the method that was determined to be most successful and used for the sample (SPE preparation #1):
Spiked urine samples were processed following cleanup conditions found in Mawhinney (2007). An Alltech silica SPE was conditioned with 4mL 25% water in acetonitrile, followed by 3 mL of acetonitrile. Sample, 3x1 mL, was loaded onto cartridge and washed with 2 mL acetonitrile and 2 mL of 10% water in acetonitrile. Samples were eluted with using 25% water in acetonitrile.

Mawhinney took urine sample to dryness and reconstituted before introduction to SPE cartridge; however, in a side by side comparison, we found loading urine directly onto a conditioned cartridge resulted in the best recovery. Additionally, success has been reported with using polymeric SPE for cleanup and although we found success with one polymeric SPE, our best recovery was using silica SPE.

The urine sample eluted from the SPE cartridge was derivatized before analysis. The eluted sample was taken to dryness. Following the process outlined in Palit (2004), the dried samples were derivatized by adding 5 µL pentafluorobenzyl bromide, 5 µL diisopropylamine and 300 µL acetone and heating to 70°C for 1 hour.

References

Mawhinney, D.B., Hameli, E.I., Fraser, R., Silva, S.S., Pavlopoulos, A.J. Kobelski, R.J. J.; The determination of organophosphate nerve agent metabolites in human urine by hydrophilic interaction liquid chromatography tandem mass spectrometry. J. Chromatogr. B. 852 (2007) 235-243.

Palit, M., Gupta, A.K., Jain, R. and Raza, S.K.; Determination of pentafluorobenzyl derivatives of phosphonic and phosphonothionic acids by gas chromatography-mass spectrometry. J Chromatogr. A, 1043 (2004) 275-284.

GC-MS/MS (CI) TECHNIQUE METHOD AND ANALYSIS DESCRIPTION

Identification

Chemical: G-1
Aliquot code: CW-4-152-5-U-307
Datafile name: TSQ0362

Compound identified as: Pentafluorobenzyl derivative
Compound reference: Reference Chemical (own synthesis)
Match algorithm and match factor: NIST, 804/999

Analysis Method

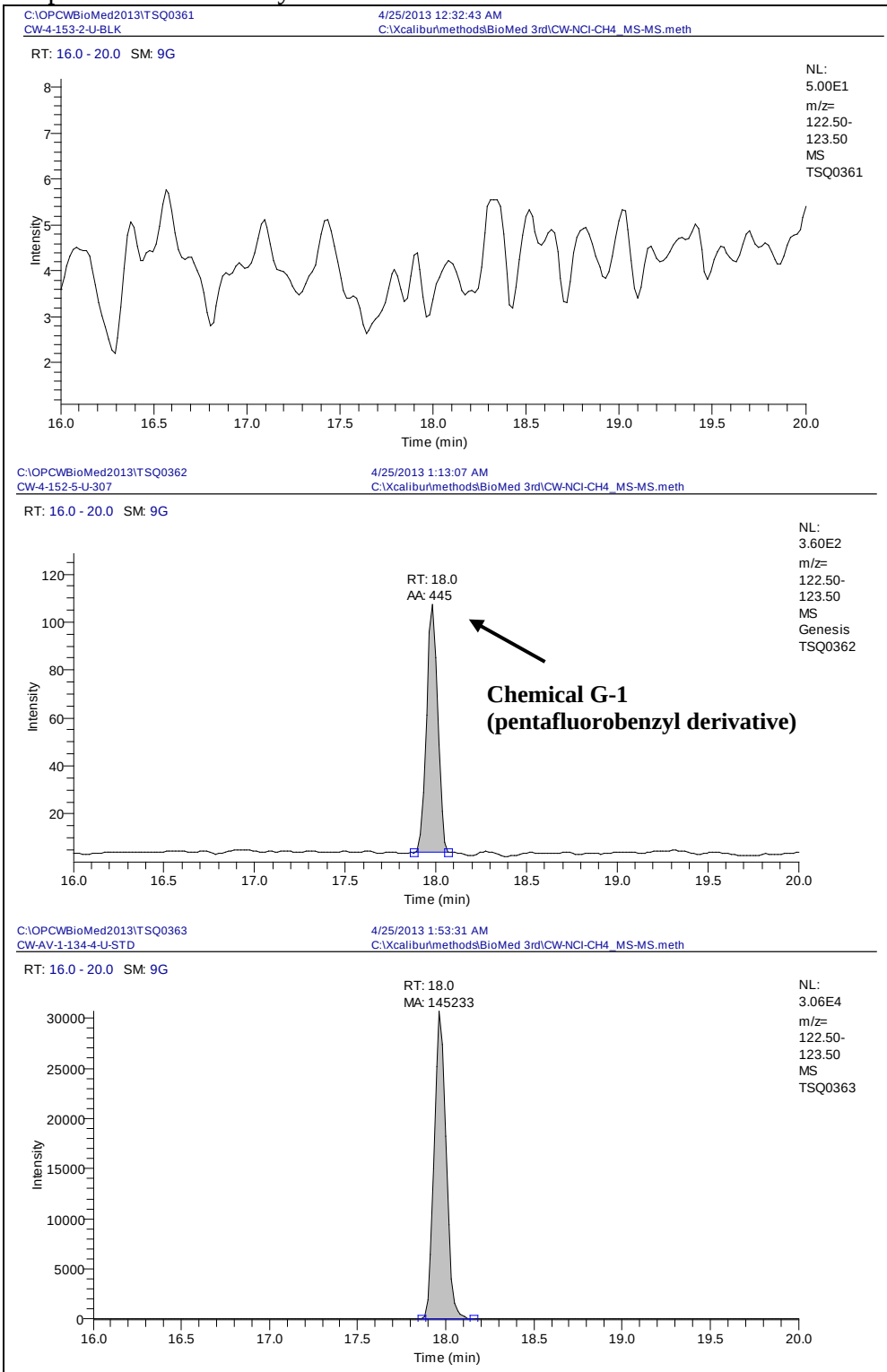
GC Instrument manufacturer and type: Thermo Fisher Trace GC Ultra
Carrier gas: Helium
Flow control/rate: Constant flow, 1 mL/min
Injection mode: Splitless, 0.60 min
Injector temperature: 230 ºC
Column brand/phase: Agilent HP-5MS: (5%-Phenyl)-methylpolysiloxane
Column Length x ID x Film thickness: 30 m x 0.25 mm x 0.25 µm
GC temperature programme: 40 ºC (3 min), 8 ºC/min to 215 ºC, 20 ºC/min to 300 ºC (1 min)

MS Instrument manufacturer and type: Thermo Fisher TSQ Quantum
Solvent delay time: 3 min
Electron energy: 100 eV
Reaction gas: Methane
Ionisation polarity: Negative
Scan range/time: 50-500 m/z in 1 second
Mass resolution: 0.7
Type of MS/MS scan: Product ion scan
Precursor ion(s): m/z 123
Collision gas: Argon
Collision Energy: 10

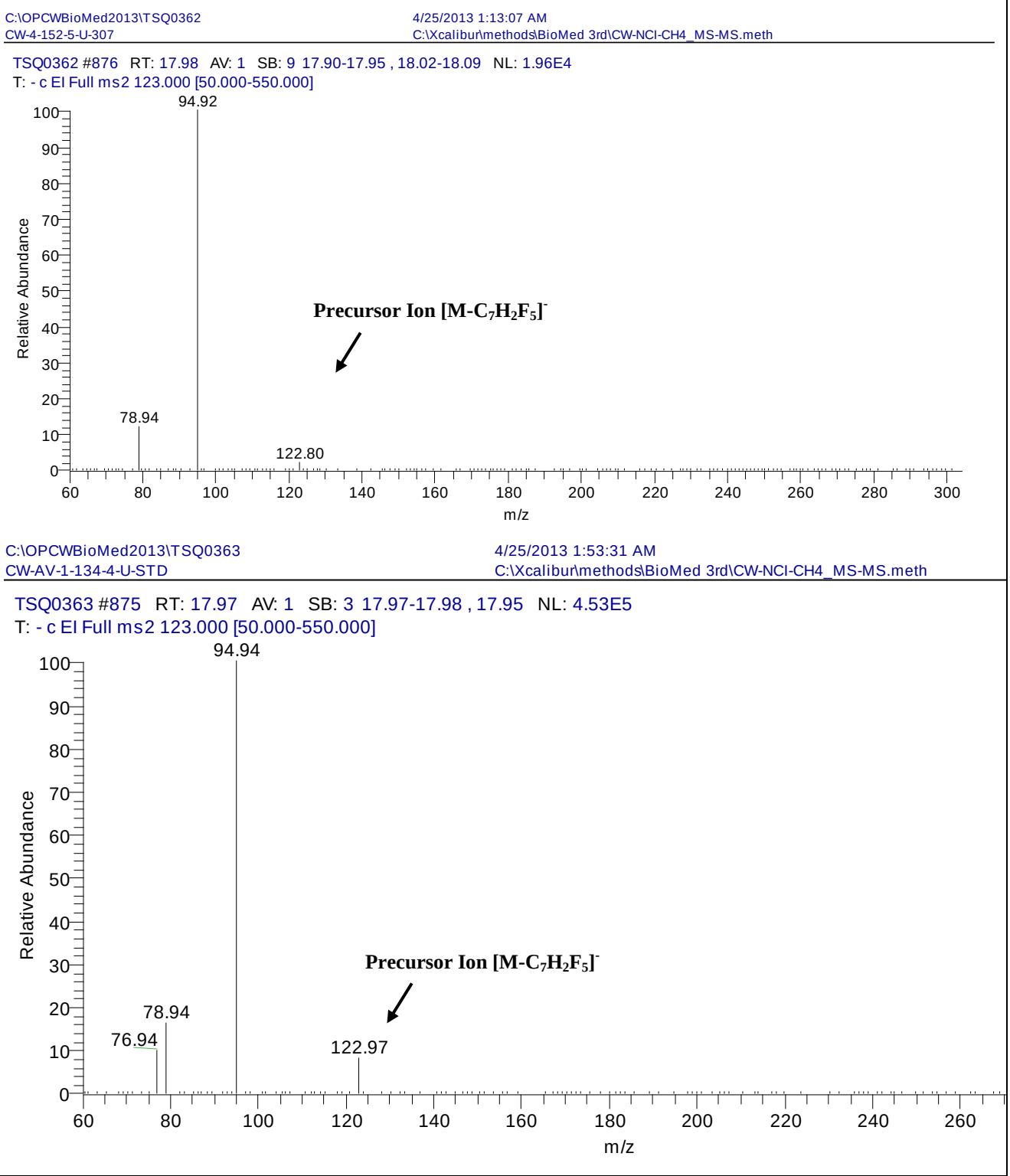
Ions/transitions	Sample		Standard		Criteria	Result
	Area*	Ion Ratio	Area*	Ion Ratio	Tolerance#	
79	2358	0.049	307914	0.178	±30%	72.4%
95	48048	1	1731474	1	N/A	
123	445	0.009	145233	0.084	±50%	89.0%

*Peak area of the ion, % intensity compared to the most abundant ion
Compare with the relative ion intensity of the standard: >50%: ±20%; >20% to 50%: ±25%; >10% to 20%: ±30%; ≤10%: ±50%

Remarks



GC-MS/MS (CI) chromatograms supporting identification of **Chemical G-1 (pentafluorobenzyl derivative)**; TIC
Top: Chromatogram of the blank.
Center: Chromatogram of the sample (aliquot code listed in header).
Bottom: Chromatogram of reference chemical of **Pentafluorobenzyl derivative of ethyl methylphosphonate.**



CI product mass spectra of:
Top: **Chemical G-1(pentafluorobenzyl derivative)** (aliquot code listed in header).
Bottom: Reference chemical of **Pentafluorobenzyl derivative of ethyl methylphosphonate.**

GC-MS/MS (CI) TECHNIQUE METHOD AND ANALYSIS DESCRIPTION

Identification

Chemical: G-1
Aliquot code: CW-4-152-5-U-307
Datafile name: TSQ0383

Compound identified as: Pentafluorobenzyl derivative
Compound reference: Reference Chemical (own synthesis)
Match algorithm and match factor: NIST, 851/999

Analysis Method

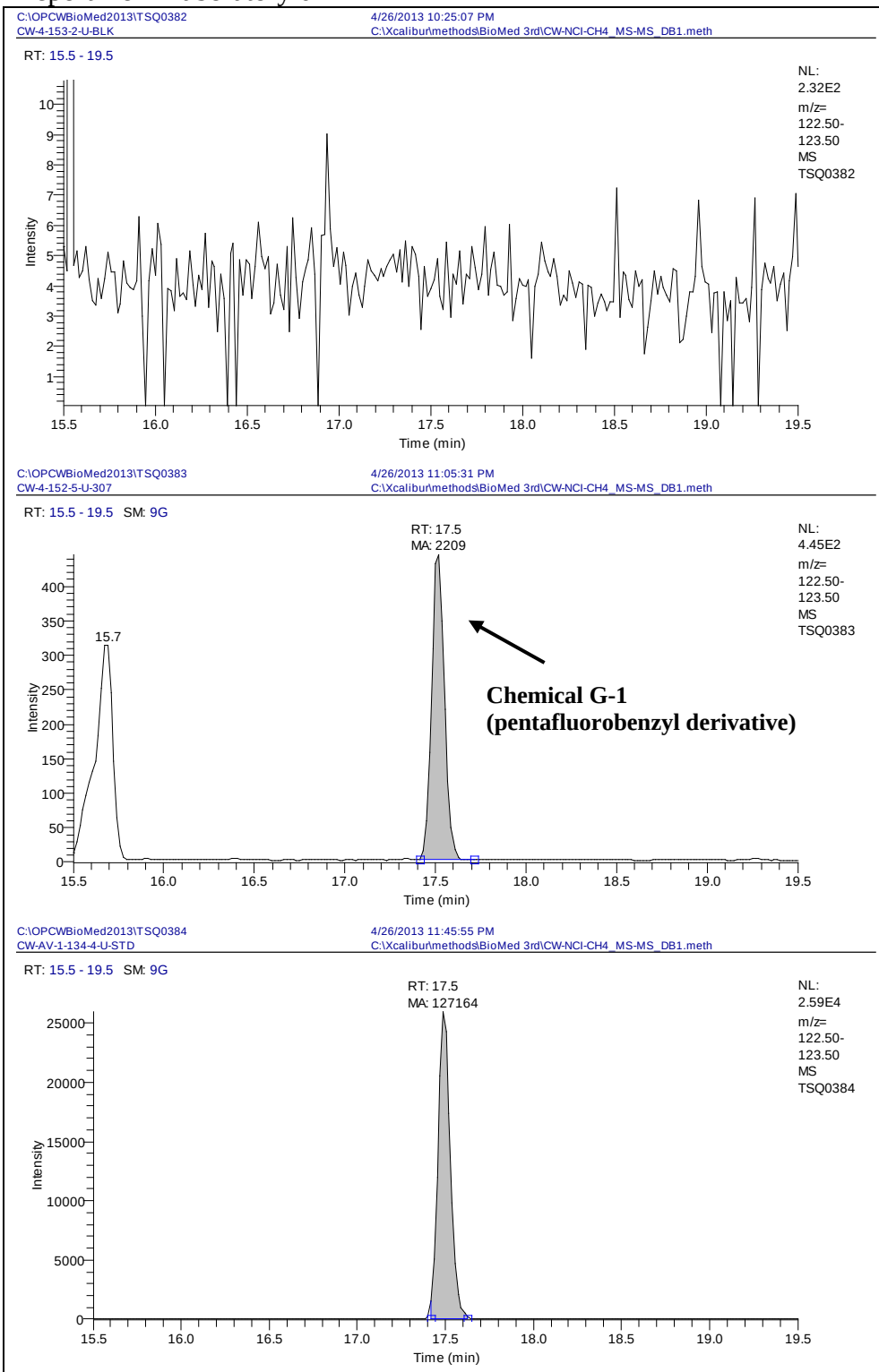
GC Instrument manufacturer and type: Thermo Fisher Trace GC Ultra
Carrier gas: Helium
Flow control/rate: Constant flow, 1 mL/min
Injection mode: Splitless, 0.60 min
Injector temperature: 230 ºC
Column brand/phase: Agilent DB-1: 100% Dimethylpolysiloxane
Column Length x ID x Film thickness: 30 m x 0.25 mm x 0.25 µm
GC temperature programme: 40 ºC (3 min), 8 ºC/min to 215 ºC, 20 ºC/min to 300 ºC (1 min)

MS Instrument manufacturer and type: Thermo Fisher TSQ Quantum
Solvent delay time: 3 min
Electron energy: 100 eV
Reaction gas: Methane
Ionisation polarity: Negative
Scan range/time: 50-500 m/z in 1 second
Mass resolution: 0.7
Type of MS/MS scan: Product ion scan
Precursor ion(s): m/z 123
Collision gas: Argon
Collision Energy: 10

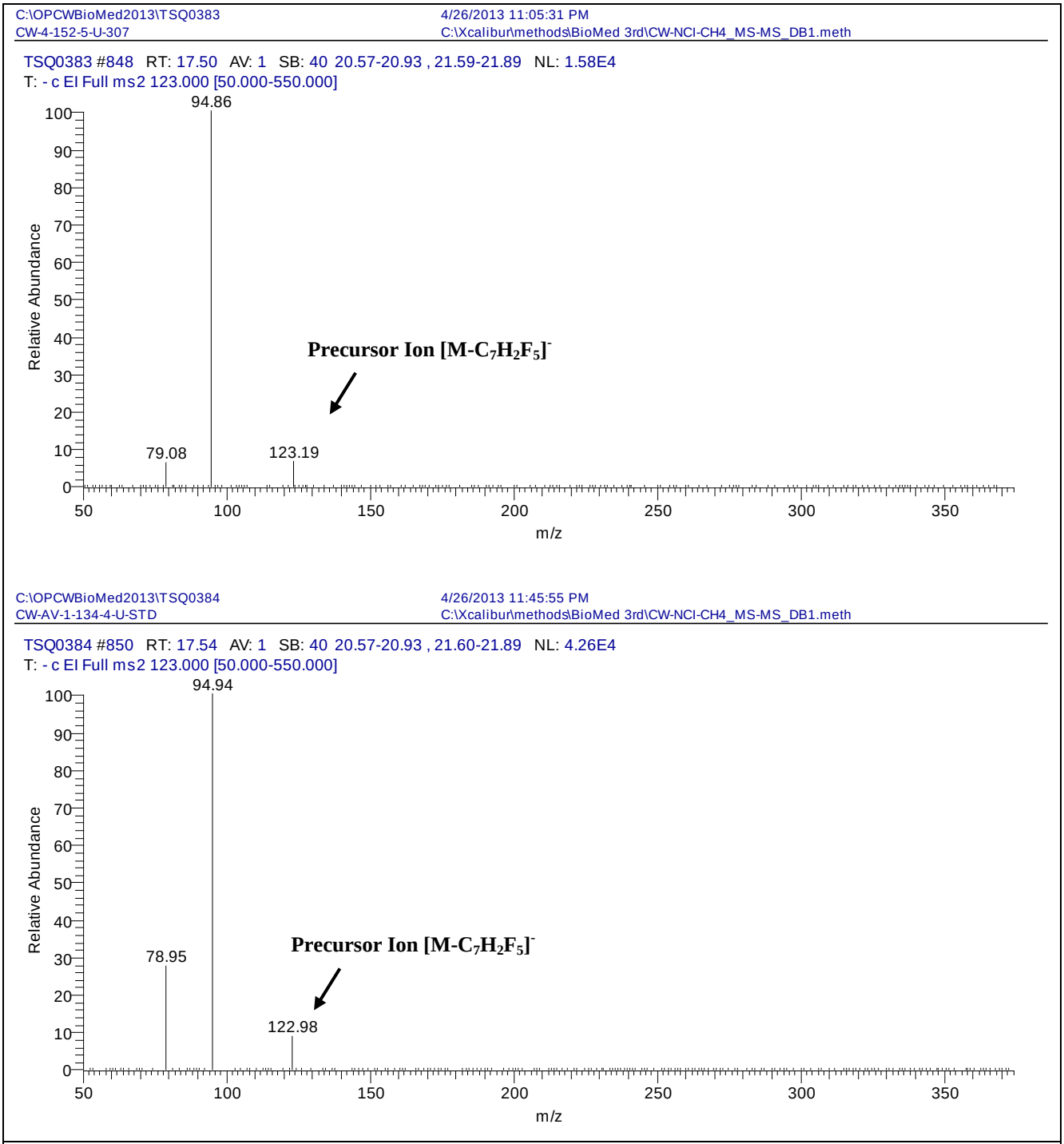
Ions/transitions	Sample		Standard		Criteria	Result
	Area*	Ion Ratio	Area*	Ion Ratio	Tolerance[#]	
79	2141	0.038	293708	0.182	±30%	79.4%
95	57029	1.000	1610485	1.000	N/A	N/A
123	2209	0.039	127164	0.079	±50%	40.9%

*****Peak area of the ion, % intensity compared to the most abundant ion
[#] Compare with the relative ion intensity of the standard: >50%: ±20%; >20% to 50%: ±25%; >10% to 20%: ±30%; ≤10%: ±50%

Remarks



GC-MS/MS (CI) chromatograms supporting identification of **Chemical G-1 (pentafluorobenzyl derivative)**; TIC
Top: Chromatogram of the blank.
Center: Chromatogram of the sample (aliquot code listed in header).
Bottom: Chromatogram of reference chemical of **Pentafluorobenzyl derivative of ethyl methylphosphonate.**



CI product mass spectra of:
Top: **Chemical G-1(pentafluorobenzyl derivative)** (aliquot code listed in header).
Bottom: Reference chemical of **Pentafluorobenzyl derivative of ethyl methylphosphonate.**

GC-MS/MS (CI) TECHNIQUE METHOD AND ANALYSIS DESCRIPTION

Identification

Chemical: G-2
Aliquot code: CW-4-152-5-U-307
Datafile name: TSQ0362

Compound identified as: Pentafluorobenzyl derivative
Compound reference: Reference Chemical (own synthesis)
Match algorithm and match factor: NIST, 815/999

Analysis Method

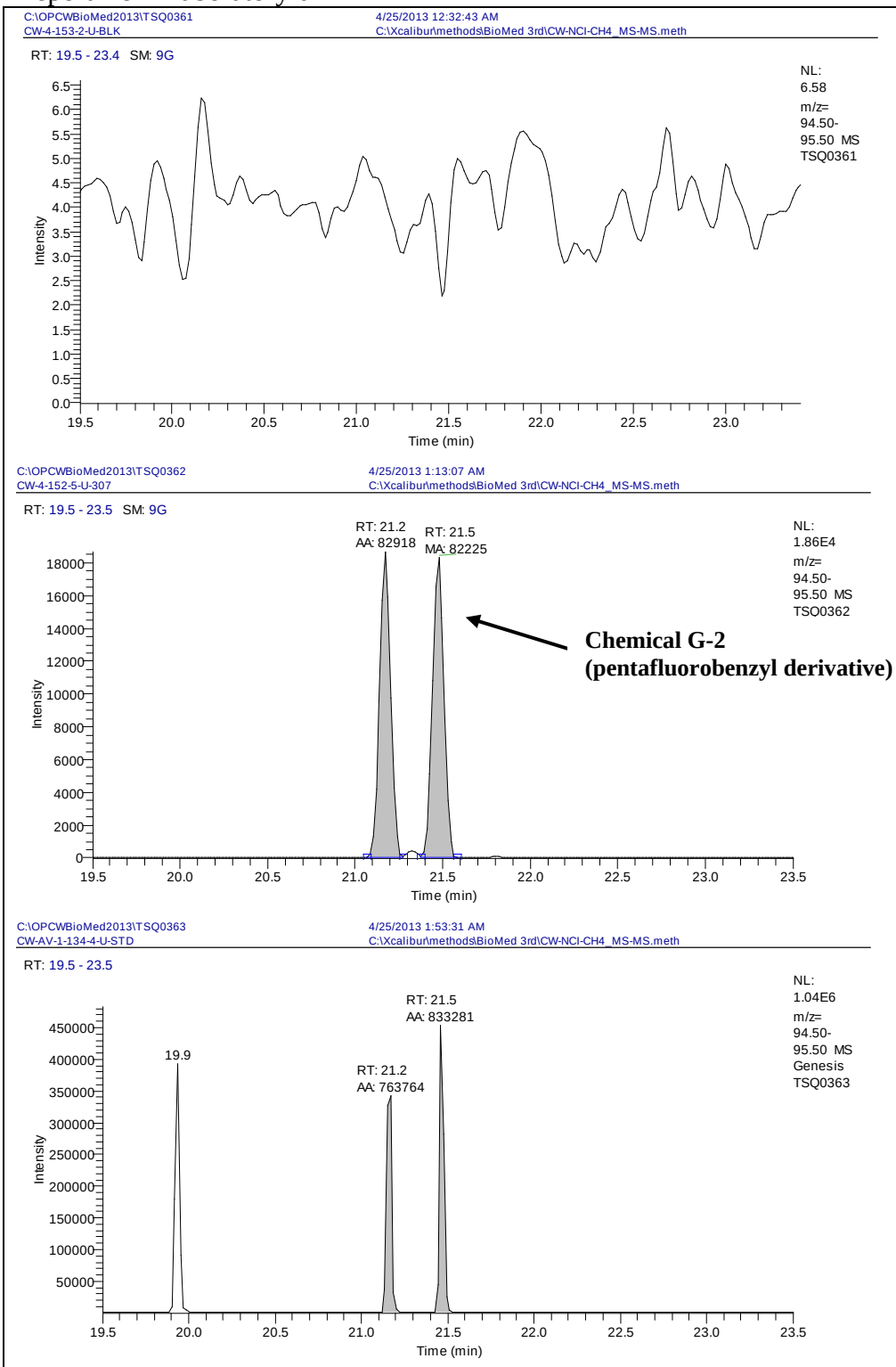
GC Instrument manufacturer and type: Thermo Fisher Trace GC Ultra
Carrier gas: Helium
Flow control/rate: Constant flow, 1 mL/min
Injection mode: Splitless, 0.60 min
Injector temperature: 230 ºC
Column brand/phase: Agilent HP-5MS: (5%-Phenyl)-methylpolysiloxane
Column Length x ID x Film thickness: 30 m x 0.25 mm x 0.25 µm
GC temperature programme: 40 ºC (3 min), 8 ºC/min to 215 ºC, 20 ºC/min to 300 ºC (1 min)

MS Instrument manufacturer and type: Thermo Fisher TSQ Quantum
Solvent delay time: 3 min
Electron energy: 100 eV
Reaction gas: Methane
Ionisation polarity: Negative
Scan range/time: 50-500 m/z in 1 second
Mass resolution: 0.7
Type of MS/MS scan: Product ion scan
Precursor ion(s): m/z 179
Collision gas: Argon
Collision Energy: 10

Ions/transitions	Sample		Standard		Criteria	Result
	Area*	Ion Ratio	Area*	Ion Ratio	Tolerance[#]	
79	14388	0.175	128937	0.155	±30%	13.1%
95	82225	1.0	833281	1.0	N/A	N/A
179	5910	0.067	60141	0.072	±50%	7.0%

*****Peak area of the ion, % intensity compared to the most abundant ion
[#] Compare with the relative ion intensity of the standard: >50%: ±20%; >20% to 50%: ±25%; >10% to 20%: ±30%; ≤10%: ±50%

Remarks

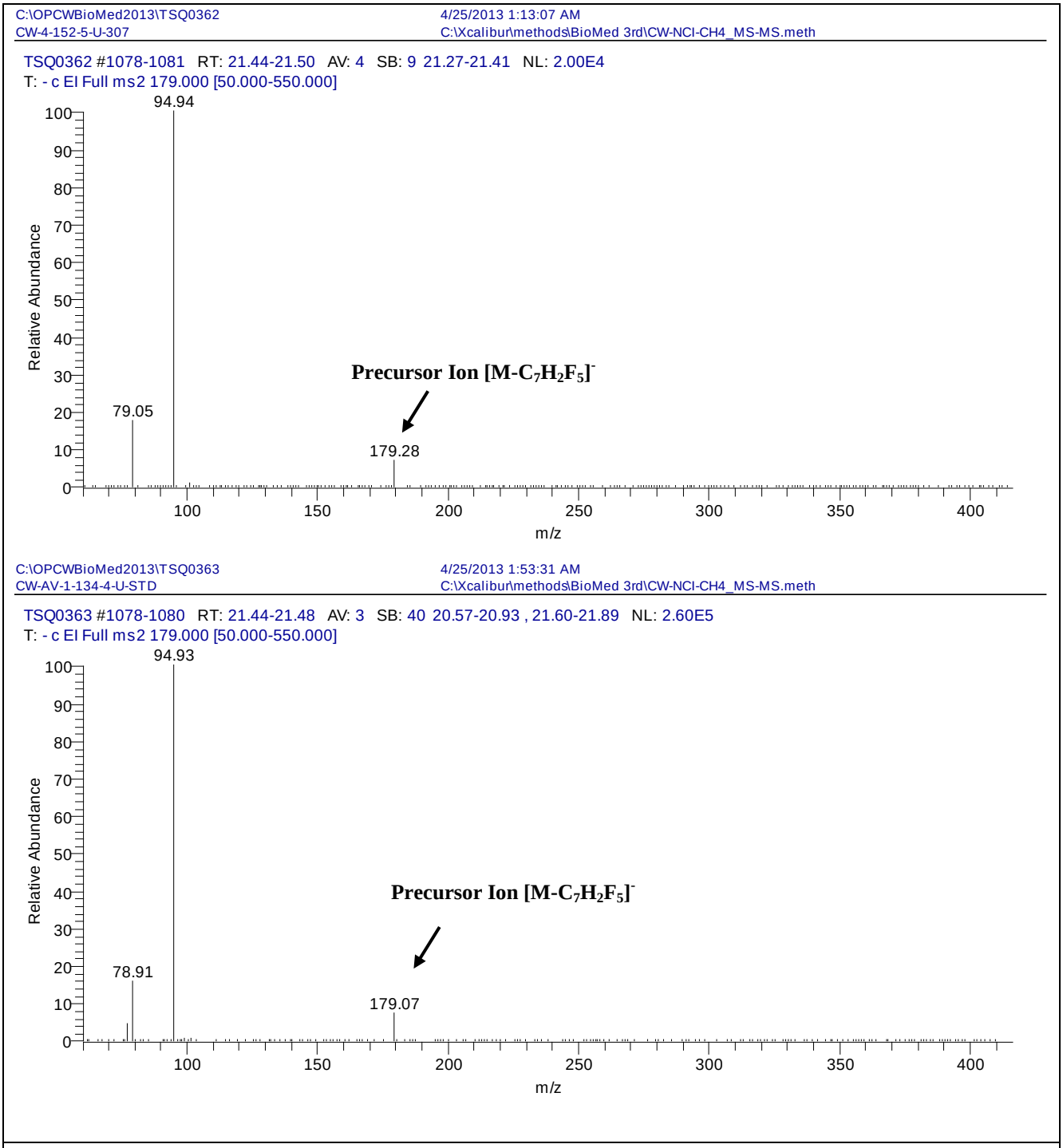


GC-MS/MS (CI) chromatograms supporting identification of **Chemical G-2 (pentafluorobenzyl derivative)**; TIC

Top: Chromatogram of the blank.

Center: Chromatogram of the sample (aliquot code listed in header).

Bottom: Chromatogram of reference chemical of **Pentafluorobenzyl derivative of pinacolyl methylphosphonate.**



CI product mass spectra of:
Top: **Chemical G-2(pentafluorobenzyl derivative)** (aliquot code listed in header).
Bottom: Reference chemical of **Pentafluorobenzyl derivative of pinacolyl methylphosphonate.**

GC-MS/MS (CI) TECHNIQUE METHOD AND ANALYSIS DESCRIPTION

Identification

Chemical: G-2
Aliquot code: CW-4-152-5-U-307
Datafile name: TSQ0383

Compound identified as: Pentafluorobenzyl derivative
Compound reference: Reference Chemical (own synthesis)
Match algorithm and match factor: NIST, 931/999

Analysis Method

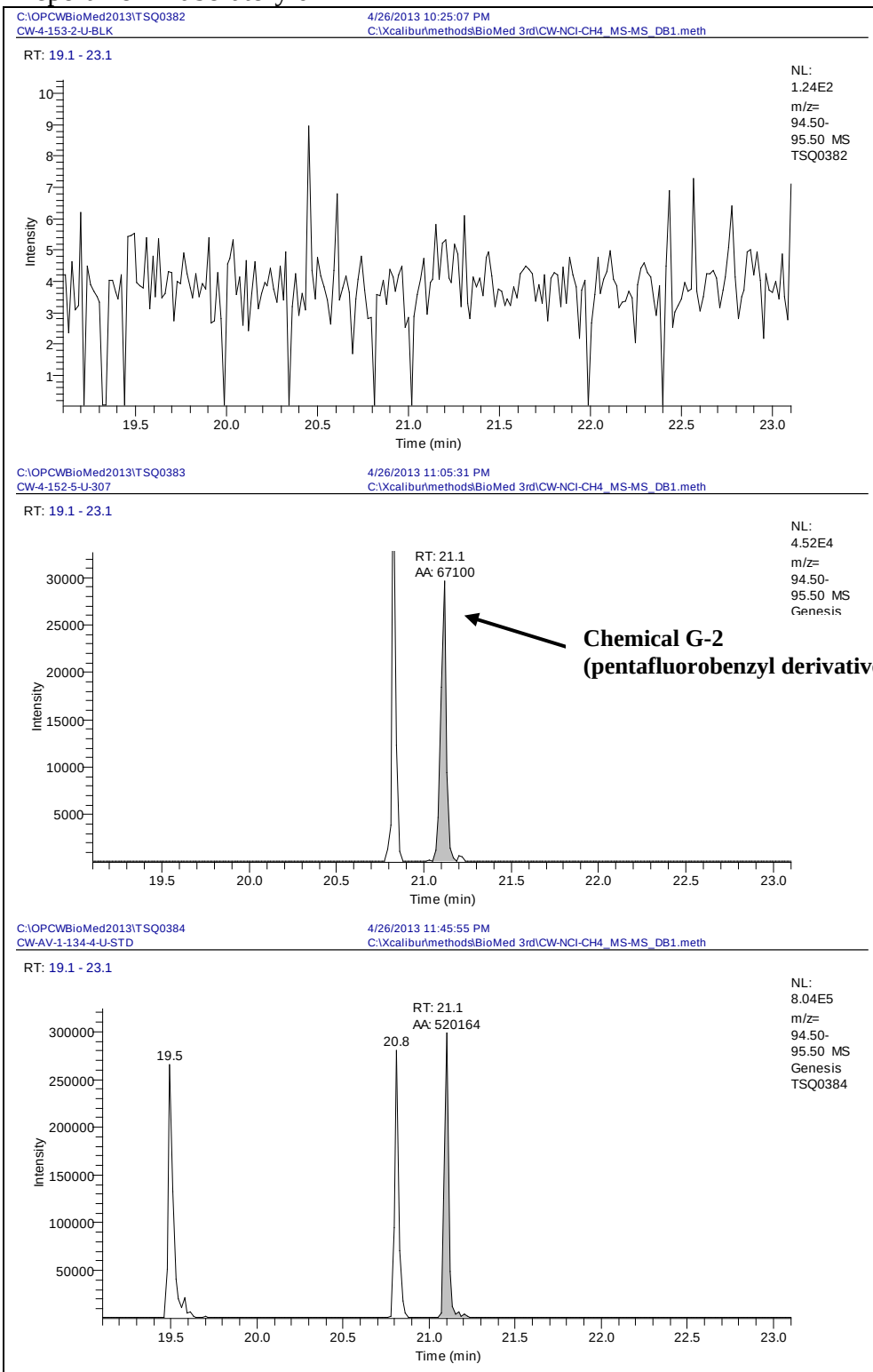
GC Instrument manufacturer and type: Thermo Fisher Trace GC Ultra
Carrier gas: Helium
Flow control/rate: Constant flow, 1 mL/min
Injection mode: Splitless, 0.60 min
Injector temperature: 230 ºC
Column brand/phase: Agilent DB-1: 100% Dimethylpolysiloxane
Column Length x ID x Film thickness: 30 m x 0.25 mm x 0.25 µm
GC temperature programme: 40 ºC (3 min), 8 ºC/min to 215 ºC, 20 ºC/min to 300 ºC (1 min)

MS Instrument manufacturer and type: Thermo Fisher TSQ Quantum
Solvent delay time: 3 min
Electron energy: 100 eV
Reaction gas: Methane
Ionisation polarity: Negative
Scan range/time: 50-500 m/z in 1 second
Mass resolution: 0.7
Type of MS/MS scan: Product ion scan
Precursor ion(s): m/z 179
Collision gas: Argon
Collision Energy: 10

Ions/transitions	Sample		Standard		Criteria	Result
	Area*	Ion Ratio	Area*	Ion Ratio	Tolerance[#]	
79	6607	0.098	79743	0.153	±30	35.8%
95	67100	1.000	520164	1.000	N/A	N/A
179	3430	0.051	37110	0.071	±50%	28.3%

*****Peak area of the ion, % intensity compared to the most abundant ion
[#] Compare with the relative ion intensity of the standard: >50%: ±20%; >20% to 50%: ±25%; >10% to 20%: ±30%; ≤10%: ±50%

Remarks

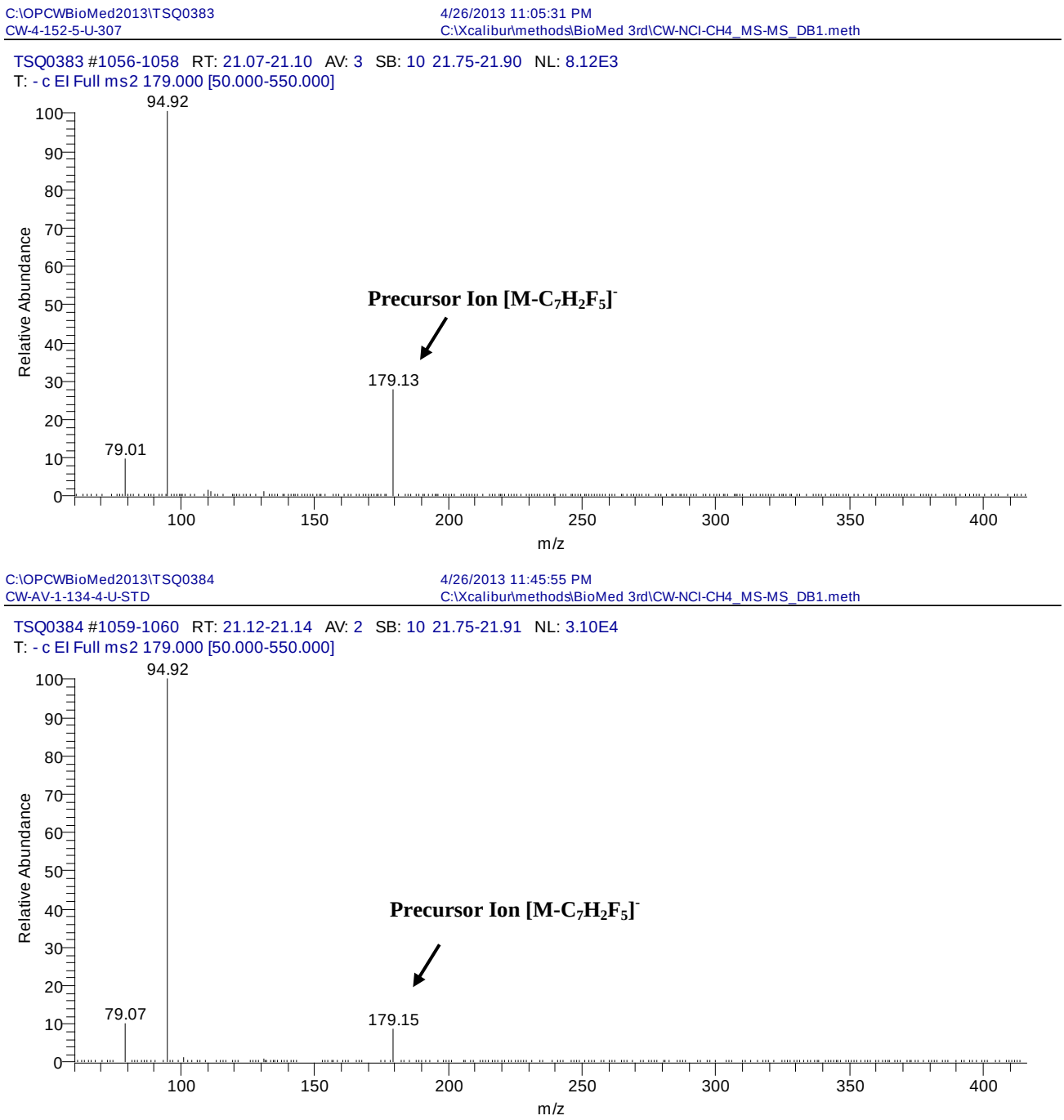


GC-MS/MS (CI) chromatograms supporting identification of **Chemical G-2 (pentafluorobenzyl derivative)**; EIC

Top: Chromatogram of the blank.

Center: Chromatogram of the sample (aliquot code listed in header).

Bottom: Chromatogram of reference chemical of **Pentafluorobenzyl derivative of pinacolyl methylphosphonate.**



CI product mass spectra of:
Top: **Chemical G-2(pentafluorobenzyl derivative)** (aliquot code listed in header).
Bottom: Reference chemical of **Pentafluorobenzyl derivative of pinacolyl methylphosphonate.**

LC-API-MS/MS TECHNIQUE METHOD AND ANALYSIS DESCRIPTION

Identification

Chemical: G-2
Aliquot code: CW-4-153-6-U-307
Datafile name: ORBI004531

Compound identified as: Pentafluorobenzyl Derivative
Compound reference: Reference Chemical (own synthesis)

Analysis Method

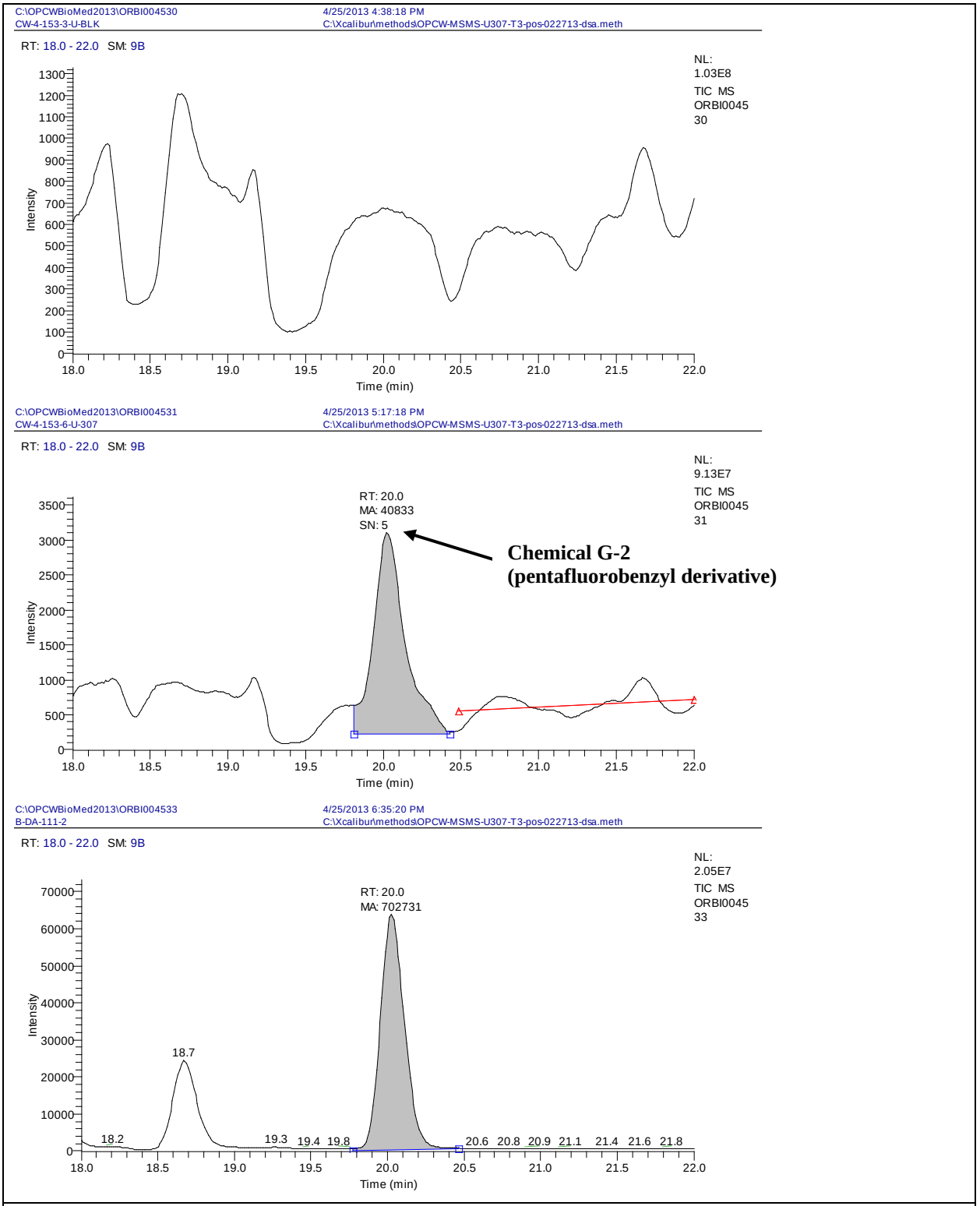
LC Instrument manufacturer and type: Thermo Surveyor Plus
Injection volume: 10 µL
Eluent composition: A = H_2O with 0.1% formic acid
B = acetonitrile with 0.1% formic acid
Elution programme: 95% A for 5 min, linear gradient to 20% A in 10 min, hold at 20% A for 10 min, regenerate column by returning to 95% A in 3 min, hold at 95% A for 10 min.
Flow rate: 200 µL/min
Column brand/phase: Waters Atlantis T3/C18
Column Length x ID x Particle size: 150 mm x 2.1 mm x 3 µm
Column temperature: 30 °C

MS Instrument manufacturer and type: Thermo LTQ XL
Ionization type: Electrospray
Ionisation polarity: Positive
Electrospray/APCI voltage: 4.0 kV
Type of MS/MS scan: Product ion scan
Scan range: m/z 75-750 u
Scan time: 60 microsec/u
Collision energy: 13 eV
Collision gas: Helium
Mass resolution: Unit
Precursor ion(s): m/z 277

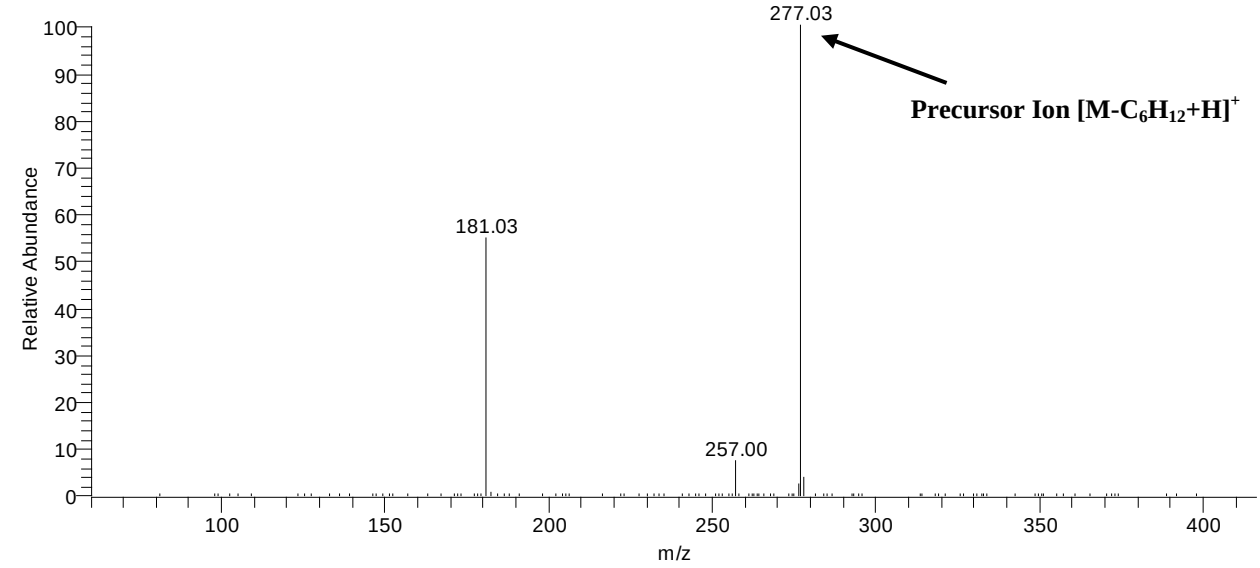
Ions/transitions	Sample		Standard		Criteria	Result
	Area*	Ion Ratio	Area*	Ion Ratio	Tolerance#	
181	9489	0.449	128565	0.313	±25%	43.7%
257	1179	0.056	14673	0.036	±50%	56.5%
277	21115	1.000	411161	1.000	N/A	N/A

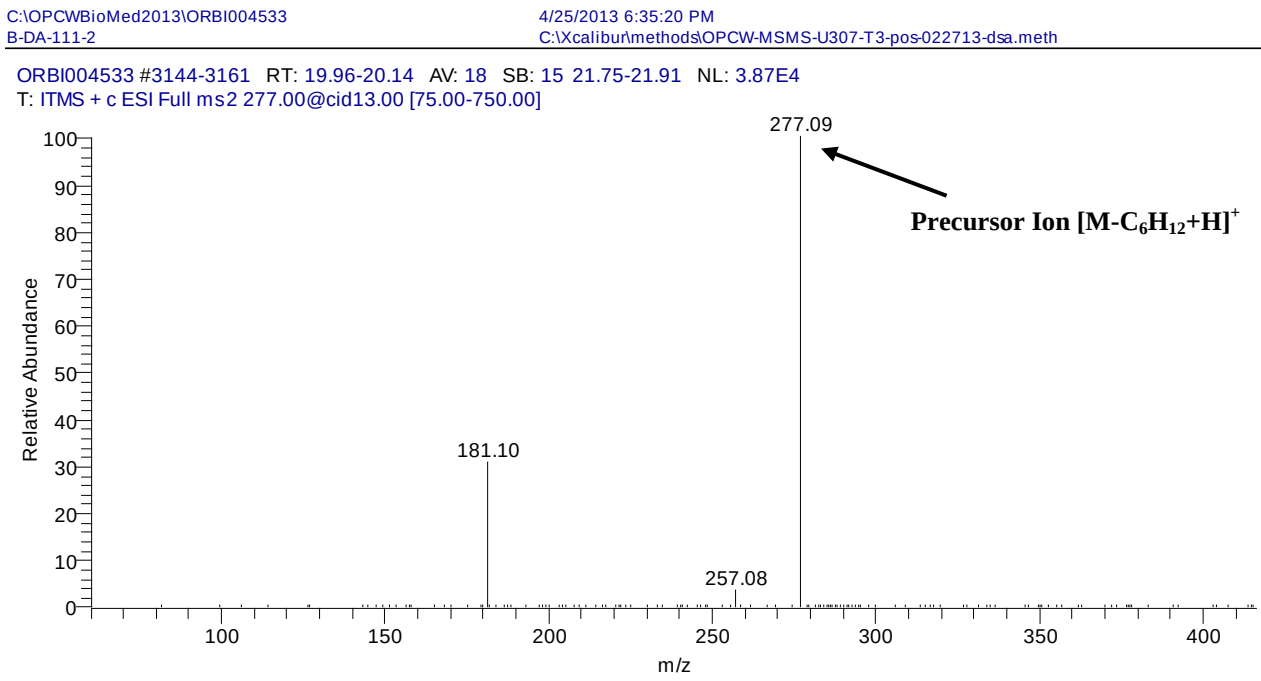
*Peak area of the ion, % intensity compared to the most abundant ion
Compare with the relative ion intensity of the standard: >50%: ±20%; >20% to 50%: ±25%; >10% to 20%: ±30%; ≤10%: ±50%

Remarks



LC-MS/MS chromatograms supporting identification of **Chemical G-2 (Pentafluorobenzyl derivative)**; TIC.
Top: Chromatogram of the blank.
Center: Chromatogram of the sample (aliquot code listed in header).
Bottom: Chromatogram of reference chemical of **Pentafluorobenzyl derivative of pinacolyl methylphosphonate.**





ESI product mass spectra of:
Top: **Chemical G-2 (Pentafluorobenzyl derivative)** (aliquot code listed in header).
Bottom: Reference chemical of **Pentafluorobenzyl derivative of pinacolyl methylphosphonate.**