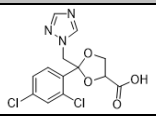
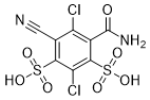
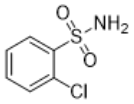
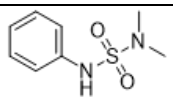
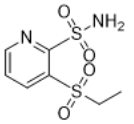
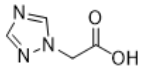
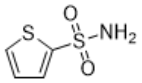
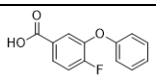
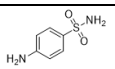
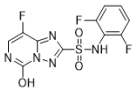
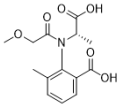
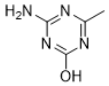
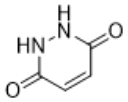
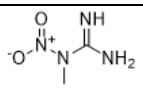
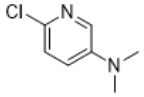
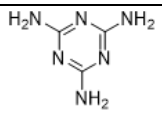
Determination of pesticide transformation products in drinking water by IC-HRMS/MS and LC-MS/MS

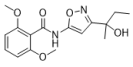
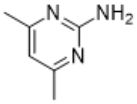
Contents

1. Scope

This document describes a standard analytical procedure of the determination of selected pesticide transformation products (Table 1) in drinking water samples by ion chromatography coupled to high resolution mass spectrometry and liquid chromatography coupled to mass spectrometry (IC-HRMS/MS and LC-MS/MS). The document also describes the validation of the quantitation method.

Table 1. List of targeted transformation products

No	*Compound name*	*Structure*	*Detection platform*	*Validated range µg/L*
1	2-(2,4-Dichlorophenyl)-2-(1*H*-1,2,4-triazol-ylmethyl)-1,3-dioxolane-4-carboxylic acid		IC(-)HRMS/MS	0.04 – 0.1
2	4-Carbamyl-2,5-dichloro-6cyano benzene-1,3-disulfonic acid		Not detected	
3	2-chlorobenzenesulfonamide		IC(-)HRMS/MS	0.04 – 0.1
4	N,N-Dimethyl-N'-phenylsulfamide		IC(-)HRMS/MS	0.04 – 0.1
5	3-(ethylsulfonyl)-2-pyridinesulfonamide		IC(-)HRMS/MS LC(+)-MS/MS	0.04 – 0.1
6	1,2,4-Triazole-1-acetic acid		LC(+)-MS/MS	not validated
7	thiophene sulphonamide		IC(-)HRMS/MS	0.04 – 0.1
8	4-fluoro-3-phenoxybenzoic acid		IC(-)HRMS/MS	not validated
9	4-aminobenzenesulphonamide		Not detected	
10	N-(2,6-difluorophenyl)-8-fluoro-5-hydroxy(1,2,4)triazolo(1,5-c)pyrimidine-2-sulfonamide		Not detected	
11	N-(2-carboxy-6-methylphenyl)-N-(methoxyacetyl)alanine		IC(-)HRMS/MS	0.04 – 0.1
12	4-amino-6-methyl-1,3,5-triazin-2-ol		LC(+)-MS/MS	not validated
13	1,2-dihydropyridazine-3,6-dione		IC(-)HRMS/MS	not validated
14	1-Methyl-3-nitroguanidine		IC(-)HRMS/MS	not validated
15	N-methyl(6-chloro-3-pyridyl)methylamine		IC(+)HRMS/MS LC(+)-MS/MS	0.04 – 0.1
16	Melamine		IC(+)HRMS/MS LC(+)-MS/MS	0.04 – 0.1

17	N-(3-(1-hydroxy-1-methyl-propyl)-5-isoxazolyl)-2,6,dimethoxybenzamide		Not delivered	
18	2-amino-4,6-dimethylpyrimidine		IC(+)HRMS/MS LC(+)-MS/MS	0.04-0.1

2. Reference

Development of sample preparation and analytical methods was based on the literature:

- Deeb, A.A., Schmidt, T.C., 2016. Tandem anion and cation exchange solid phase extraction for the enrichment of micropollutants and their transformation products from ozonation in a wastewater treatment plant. Anal. Bioanal. Chem. 408, 4219–4232. https://doi.org/10.1007/s00216-016-9523-y
- Scheurer, M., Brauch, H.J., Schmidt, C.K., Sacher, F., 2016. Occurrence and fate of nitrification and urease inhibitors in the aquatic environment. Environ. Sci. Process. Impacts 18, 999–1010. https://doi.org/10.1039/c6em00014b
- Herrero, P. et al. Comparison of triple quadrupole mass spectrometry and Orbitrap high-resolution mass spectrometry in ultrahigh performance liquid chromatography for the determination of veterinary drugs in sewage: Benefits and drawbacks. J. Mass Spectrom. 49, 585–596 (2014).

For method validation and calculation of results were used:

- B. Magnusson and U. Örnemark (eds.) Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics, (2nd ed. 2014). ISBN 978-91-87461-59-0. Available from www.eurachem.org
- Bekendtgørelse om kvalitetskrav til miljømålinger, BEK nr 1770 af 28/11/2020 (Gældende)
- HANDBOOK FOR CALCULATION OF MEASUREMENT UNCERTAINTY IN ENVIRONMENTAL LABORATORIES, NT TECHN REPORT 537, Approved 2003-05
- Evaluation of measurement data – Guide to the expression of uncertainty in measurement, JCGM 100:2008, (GUM 1995 with minor corrections)

Compounds recorded with high resolution were confirmed with in silico fragment-based identification:

- Ruttkies, C., Schymanski, E.L., Wolf, S. et al. MetFrag relaunched: incorporating strategies beyond in silico fragmentation. J Cheminform 8, 3 (2016). https://doi.org/10.1186/s13321-016-0115-9

Or with reference spectra from:

- Horai H, Arita M, Kanaya S, Nihei Y, Ikeda T, Suwa K, Ojima Y, Tanaka K, Tanaka S, Aoshima K, Oda Y, Kakazu Y, Kusano M, Tohge T, Matsuda F, Sawada Y, Hirai MY, Nakanishi H, Ikeda K, Akimoto N, Maoka T, Takahashi H, Ara T, Sakurai N, Suzuki H, Shibata D, Neumann S, Iida T, Tanaka K, Funatsu K, Matsuura F, Soga T, Taguchi R, Saito K, Nishioka T. MassBank: a public repository for sharing mass spectral data for life sciences. J Mass Spectrom. 2010 Jul;45(7):703-14. doi: 10.1002/jms.1777. PMID: 20623627.

3. Principle

Development process of analytical methods aimed for quantification of pesticide transformation products in drinking water samples is shown in the scheme below (Figure 1)

Drinking water sampled from the tap was used as matrix for development and validation purposes but methods are intended to be applicable for ground water samples, as well.

At first direct injection (DI) method, referred here in this report as Method 2, was tested on both systems IC(-)- HRMSMS and LC(+)-HRMSMS. The DI method was not used in the validation study because the estimated limit of detection (LD) for most of the analytes did not meet the acceptance criteria ≤ 0.01 µg/L. Time restrains of the project did not allow for further development of the DI method to account for the matrix effects and optimization of acquisition methods, which would be needed to obtain desired LDs.

Thus as an alternative was used solid phase extraction (SPE) method in tandem here referred to as Method 1 was used to extract analytes from drinking water. SPE method proved to be more suitable alternative than DI because estimated LD were meeting acceptance criteria and recoveries achieved for 10 of the analytes were > 80 %

Chemical analysis and validation was performed on replicate measurements of control sample at two different levels 0.04 µg/L and 0.1 µg/L.

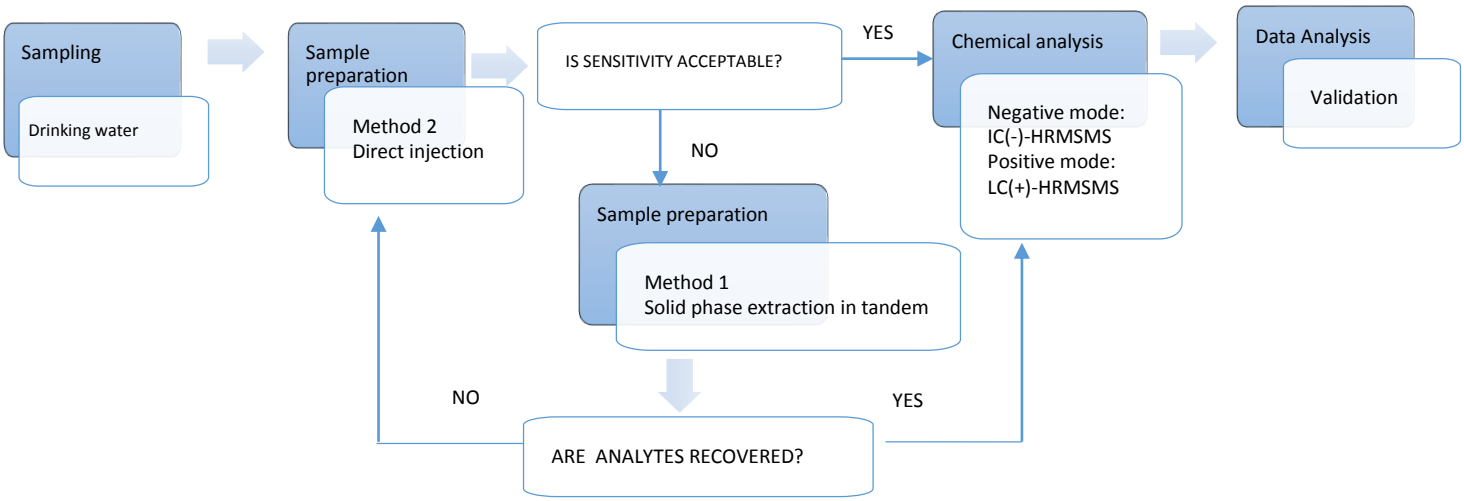


Figure 1 Scheme of the development process of analytical methods

4. Reagents and standards

Materials

For sample preparation two types solid phase extraction materials are used in tandem; Oasis® MAX cartridges, (Anion exchange, Waters, Denmark), 150 mg, 6mL Oasis® MCX cartridges, (Cation exchange, Waters, Denmark), 150 mg, 6 mL, SPE PTFE tubing and large volume adapters

Preparation of solutions for sample preparation

1. Wash solution A (water/ammonia solution, 95:5, v/v)

2. Wash solution B (water/formic acid, 98:2, v/v)
3. Elution solution A (methanol/ethyl acetate/formic acid mixture, 69:29:2, v/v/v)
4. Elution solution B (methanol/ethyl acetate/ammonium hydroxide mixture, 67.6:27.5:5, v/v/v)

All four SPE solutions are prepared in 100 mL volumetric flasks. Solutions must be prepared fresh due to instability of formic acid in methanol and water solutions[1]. At first, is volumetric flask filled with water (solutions 1-2) or methanol (solutions 3-4). Followed by the addition of acidic and basic reagents (formic acid and ammonia solution) using volumetric glass pipettes 2 mL for the formic acid solutions and 5 mL for ammonia solutions).

Preparation of calibration solutions

Matrix-matched calibration solutions for quantification are done with 7-point calibration solutions prepared in triplicates. Levels are listed in Table 2. The matrix-matched solution is a concentrated extract of a matrix (non-spiked tap water) that has been prepared following all the extraction and sample preparation steps of the analytical method.

Spiking calibration (STOCK) is prepared as mixture of single standard (Compound #1, vendor EEP) and 100x dilution of the original STOCK solutions (obtained from NEOCHEMA, AAR-022-10AN10 , 10 µg/mL) containing compounds (10 and 12) listed in (Table 3) and (NEOCHEMA, AAR-021-W10AN10, 10 µg/mL) containing remaining of the compounds in the table.

Table 2 Matrix-matched 7-point calibration solutions.

Name	*Stock addition*	*concentration [ng/mL]*	*Stock addition volume [µL]*	*Total volume [µL]*	*Addition of matrix*	***Final Concentration [ng/mL]***
STOCK		100				**1000**
Standard 7	Standard 7	100	100	1000	900	**100**
Standard 6	Standard 7	100	80	100	20	**80**
Standard 5	Standard 7	100	60	100	40	**60**
Standard 4	Standard 7	100	50	100	50	**50**
Standard 3	Standard 4	100	40	100	60	**40**
Standard 2	Standard 5	100	75	100	25	**30**
Standard 1	Standard 5	10	50	100	50	**20**

Table 3. List of standards of the investigated pesticide transformation products

Nr	*Compound*	*CAS*	*Purity%*
1	2-(2,4-Dichlorophenyl)-2-(1H-1,2,4-triazol-ylmethyl)-1,3-dioxolane-4-carboxylic acid	119725-91-6	96
2	4-Carbamyl-2,5-dichloro-6cyano benzene-1,3-disulfonic acid	not available	97.6

[1] Snoble, Karel et al. "Stability of Formic Acid in Methanol Solutions and the Implications for Use in LC-MS Gradient Elution Analysis." Lc Gc North America 26 (2008): 946-950.

3	2-chlorobenzenesulfonamide	6961-82-6	98.0
4	N,N-Dimethyl-N'-phenylsulfamide	4710-17-2	99.3
5	3-(ethylsulfonyl)-2-pyridinesulfonamide	117671-01-9	97.0
6	1H-1,2,4-triazol-1-ylacetic acid	28711-29-7	94.5
7	thiophene sulphonamide	6339-87-3	99.9
8	4-fluoro-3-phenoxybenzoic acid	77279-89-1	98.2
9	4-aminobenzenesulphonamide	63-74-1	99.9
10	N-(2,6-difluorophenyl)-8-fluoro-5-hydroxy(1,2,4)triazolo(1,5-c)pyrimidine-2-sulfonamide	292085-54-2	95.5
11	N-(2-carboxy-6-methylphenyl)-N-(methoxyacetyl)alanine	104390-56-9	99.0
12	4-amino-6-methyl-1,3,5-triazin-2-ol	16352-06-0	95.0
13	1,2-dihydropyridazine-3,6-dione	123-33-1	99.0
14	N-methyl-N-nitroguanidine	62409-38-5	94.9
15	N-methyl(6-chloro-3-pyridyl)methylamine	41288-91-9	97.1
16	Melamine	108-78-1	99.0
17	N-(3-(1-hydroxy-1-methyl-propyl)-5-isoxazolyl)-2,6,dimethoxybenzamide	127842-34-6	
18	2-amino-4,6-dimethylpyrimidine	767-15-7	99.9

*, substance 17 is currently under production/synthesis.

5. Apparatus

IC-HRMS/MS analysis

Mass spectrometric analysis is performed using a Orbitrap Q Exactive HF tandem mass spectrometer equipped with a heated electrospray ionization interface (HESI-II) nitrogen filled higher-energy C-trap dissociation collision cell (HCD, Thermo Fisher Scientific). The chromatographic separation was done with a Dionex IonPac™ AS11-4µm, Analytical column (2 x 250 mm) using an ion chromatograph ICS-6000 Dionex with cooled autosampler (8 °C, Thermo Fisher Scientific).

Analytical Determination

Ion chromatographic separation is achieved in negative mode with gradient elution using potassium hydroxide (KOH) in water. Gradient steps are described in Table 4. Column temperature is set at 35°C. The Orbitrap mass spectrometer is operating with heated electrospray in negative ionization mode (ESI-) using the following parameters: spray voltage, 4 kV; sheath gas (N2, >95%), 40; auxiliary gas (N2, >95%), 10; tube lens voltage, 50 V; heater temperature, 260 °C; and capillary temperature, 425 °C. The Orbitrap tandem mass spectrometer was operated in negative ionization parallel reaction monitoring mode (PRM). An isolation window of 1.5 Da from a substance inclusion list (with the substances listed in Table 3) was fragmented in the collision cell (HCD) at 15 and 50 normalized collision energy. An MS2 full spectrum of ion fragments were recorded at 60,000 in resolution with an automatic gain control target of 5e4 and maximum

C-trap injection time of 250 ms. From pure analytical standards MS2-fragments were verified and two MS2 fragments were extracted (XIC) at a 5 ppm tolerance (Table 5).

Table 4. Ion chromatography conditions.

<table>
<tr><td>Column</td><td colspan="4">Dionex IonPac™ AS11-HC-4µm, Analytical (2 x 250 mm), AG11-HC-4µm (2 x 50 mm)</td></tr>
<tr><td>KOH gradient:</td><td>No</td><td>Time</td><td>Concentration KOH (mM)</td><td>Curve</td></tr>
<tr><td></td><td>1</td><td>0.000</td><td>6.00</td><td></td></tr>
<tr><td></td><td>2</td><td>5.000</td><td>10.00</td><td>5</td></tr>
<tr><td></td><td>3</td><td>11.000</td><td>60.00</td><td>5</td></tr>
<tr><td></td><td>4</td><td>13.000</td><td>60.00</td><td>5</td></tr>
<tr><td></td><td>5</td><td>13.100</td><td>6.00</td><td>5</td></tr>
<tr><td></td><td>6</td><td>20.000</td><td>6.00</td><td></td></tr>
<tr><td>Eluent source</td><td colspan="4">Dionex EGC 500 KOH eluent
Cartridge, Dionex CR-ATC 600 trap
column and high pressure degas
module</td></tr>
<tr><td>Flow rate</td><td colspan="4">0.45 mL/min</td></tr>
<tr><td>Injection volume</td><td colspan="4">12 µL (limited sample injection) using a 25 µL sample loop</td></tr>
<tr><td>Column temperature</td><td colspan="4">40 °C</td></tr>
<tr><td>Detection/suppressor compartment:</td><td colspan="4">35 °C</td></tr>
<tr><td>Detection 1:</td><td colspan="4">Suppressed conductivity,
Dionex AERS™ 600, 2mm, 4.2 V, external water mode (delivered by a Dionex AXP-MS
pump at 0.45 mL/min)</td></tr>
</table>

In the *Table 5* are presented parent, quantification and confirmation ions and their respective retention time. Please note that retention times presented in table are for analyte recorded in matrix. If analytes are recorded in pure solvent retention time shift is be observed. Ion chromatography is very sensitive to pH differences in between different matrices as the separation is based on increasing pH gradient. Fragments

that contain superscript in table were confirmed with in silico prediction and with reference spectra (see chapter 2, Reference). The obtained HRMS/MS fragment information may be applied to traditional MS/MS systems, e.g. triple-quadrupole platforms in MRM or SRM mode (Herrero et al., 2014).

Table 5. Parent ion, quantification ion, confirmation ion and matrix-matched retention time used in IC-HRMS/MS method

Compound number	*Parent ion (m/z)*	*Quantification ion (m/z)*	*Confirmation ion (m/z)*	*RT (min)*
1	342.0054	253.9895	108.0204	8.6
3	189.9736	77.9654[b]	126.0115[b]	8.4
4	199.0547	91.0427[b]	155.0047[b]	8.8
5	249.0009	157.0076[a]	93.0458	9.7
7	161.9689	82.9961[a]	78.0015[a]	10.5
8	231.0463	93.0345[a]	167.0502[a]	20.3
11	294.0983	204.0666[b]	89.0244[b]	10.7
13	111.0200	82.0061	83.0138	6.7
14	117.0418	61.0043[a]	55.0301	2.9

[a)] confirmed with MetFrag (see references); [b)] confirmed with reference spectra (see references)

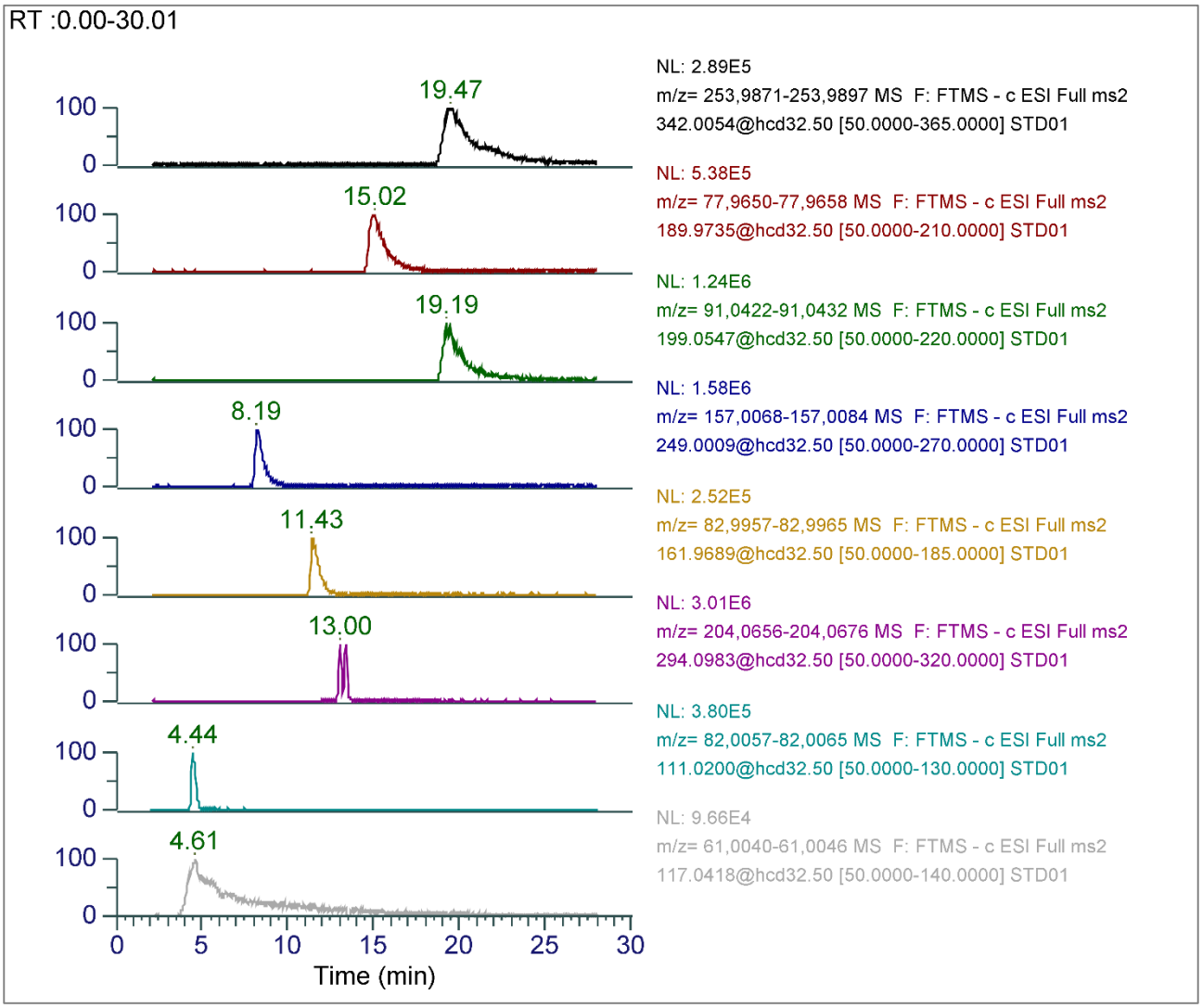


Figure 2. Displayed are quantification ions of targeted analytes in standard mixture. Analytes from top to bottom are: 1, 3, 4, 5, 7, 11, 13 and 14. Note that compound 5 could be measured in both positive and negative mode (Figure 4) and compound 8 is missing on the picture because analytical IC column was changed from AS19-4µm to AS11-HC-4µm and analyte could no longer be detected with this column.

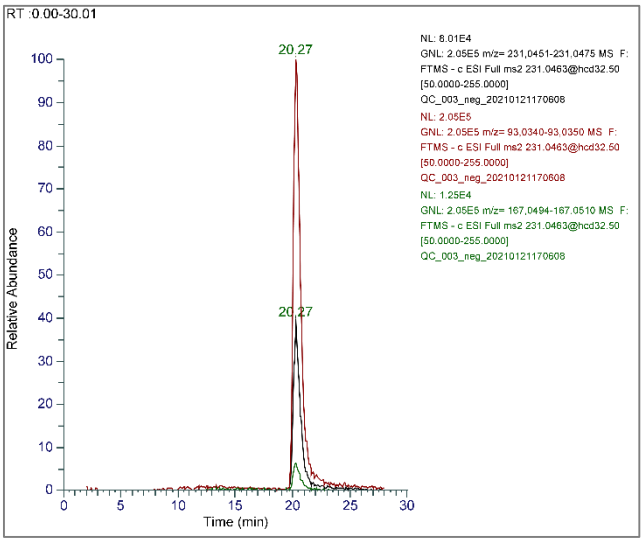


Figure 3 Displayed is parent, quantification and confirmation ions of compound #8 eluted on an analytical IC column AS19-4µm. It was not possible to detect this compound on the AS11-HC-4µm IC column.

LC-MS/MS analysis

Mass spectrometric analysis is performed using a QTrap 5500 QqLIT (quadrupole-linear ion trap, capable of MS3 using nitrogen as collision gas) equipped with a TurboIon electrospray source (AB Sciex). The chromatographic separation was done using a Hypercarb column (2.1 x 100 mm, ThermoFisher Scientific) on an Agilent 1200 Series UHPLC system with an autosampler operated at ambient temperature (Agilent, Palo Alto, CA, USA).

Analytical Determination

Optimized method is based on the method from (Scheurer et al., 2016). Mass spectrometric analysis is performed using a QTrap 5500 equipped with a TurboIon source (AB Sciex). The source settings are: Ion Spray Voltage (IS) +5.5 kV; Source temperature 600 ºC; Curtain gas, 20; Gas 1, 60; Gas 2, 70; Collision gas low. The chromatographic separation parameters are listed in *Table 6*. Mass spectrometric analysis is performed in multiple reaction monitoring (MRM). Parent and product ions of analysed and their optimized voltages are listed in *Table 7*.

Table 6 Liquid chromatography conditions.

Column	Hypercarb column (2.1 x 100 mm, 3 µm, Thermo Fisher Scientific)		
Gradient:	*Time*	*Solvent A*	*Solvent B*
	0	95	5
	14	5	95
	18	5	95
	20	95	5
	36	95	5

Mobile phase composition	Solvent A: 0.1 % formic acid in water Solvent B: 0.1 % formic acid in Methanol
Flow rate	0.15 mL/min
Injection volume	10 µL Method 1 100 µL Method 2 (direct injection)
Column temperature	40 °C

Table 7. List of parent ions, product ions, declustering potentials, collision energies and matrix-matched retention times for selected analytes used in LC-MS/MS method.

Compound number	*Parent ion (m/z)*	*Product ion (m/z)*	*Declustering Potential DP [eV]*	*Collision Energy CE [eV]*	*RT*
5	251	106	80	20	23.3
5	251	78	80	50	23.3
6	128	73	80	50	22.4
6	128	69	80	20	22.4
12	127	69	100	30	20.0
12	127	86	100	30	20.0
15	157	126	80	20	16.9
15	159*	128	80	20	16.9
16	127	85	100	20	20.9
16	127	68	100	40	20.9
18	124	67	180	40	19.2
18	124	42	180	50	19.2

*compound contains Cl atom therefore second parent ion is 159 m/z for ^{37}Cl-isotope.

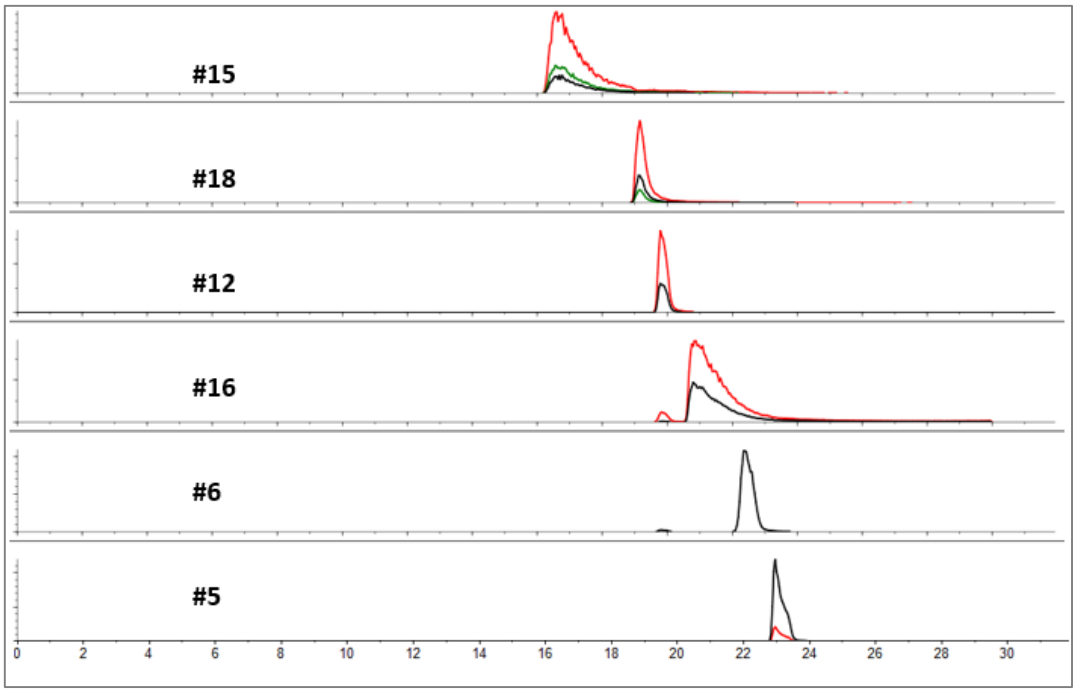


Figure 4. MRM transitions of 7 analytes in the control sample at concentration c= 0.1 µg/mL. Chromatograms of compounds #16 and #6 contain isobaric interference at retention time 20 min from compound #12. Further trimming and steepness optimization of gradient is recommended.

6. Procedure

Sample Storage

Samples are stored in freezer (-20 °C) until analysis.

Sample Preparation

Solid phase extraction in tandem (method 1)

Solid phase extraction (SPE) in tandem described by (Deeb and Schmidt, 2016) was selected as suitable sample preparation technique.

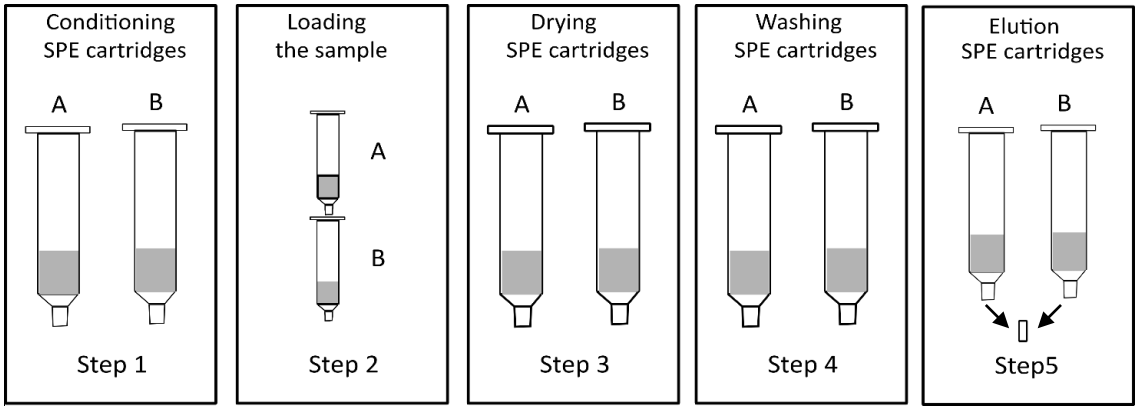


Figure 5 Scheme of 5 steps in tandem SPE. SPE cartridge A is MAX material, while SPE B is MCX material.

Procedure:

A. Anion exchange cartridge to capture anions (MAX)

B. Cation exchange cartridge to capture cations (MCX)

1. Sample: 1000 mL drinking water or groundwater
3. Conditioning of the cartridges A (MAX) and B (MCX) is done separately with 3 x 3 mL methanol
4. Equilibration of the cartridges (separately) with 3 x 5 mL of water
5. Loading of the spiked sample with 1-2 drops/s on the cartridges (in tandem)
6. Cartridges are dried with slight vacuum and separated before washing and elution steps.
7. Washing (Washing is done individually for both cartridges)
   → Washing of MAX-cartridge is done with 2 mL water/ammonia solution (95:5, v/v), pH =11.2
   → Washing of MCX-cartridge is done with 2 mL water/formic acid (98:2, v/v), pH=2.4
8. Elution (Elution is done individually for both cartridges)
   → Elution of MAX-cartridge was done with 6 mL of methanol/ethyl acetate/formic acid mixture (69:29:2, v/v/v), pH = 2.6
   → Elution of MCX-cartridge was done with 6 mL methanol/ethyl acetate/ammonium hydroxide mixture (67.6:27.5:5, v/v/v), pH = 10.5
9. Eluates are combined and reconstituted in 1 mL of milli-Q-water. Reconstitution is done in two steps: At first, to combined eluates is added 0.5 mL of milli-Q-water and mixture is evaporated under nitrogen stream at 30 °C to <2 mL . After that is added again 0.5 mL of milli-Q-water and mixture is evaporated to <1 mL . Finally is mixture adjusted to 1 mL by weighing.
10. Combined extracts were reconstituted in 1 mL of milli-Q-water.
11. Extracts are stored in the freezer (-20 °C) until analysis.

Direct injection method (method 2)

As method 2 was selected direct injection method to cover analytes with low recoveries on SPE. 1 mL of sample is filtered through syringe filters (KX Sprøjtefilter (syringe filter) RC-regenerated cellulose, 4mm 0,22µm) and directly injected.

Quantification

$$C_{Sample} = C_{added} \times \frac{S_{sample}}{S_{sample\ plus\ added} - S_{sample}}$$

7. Calculation of results

Recovery

$$\%RE = \frac{C_{pre-spiked\ sample}}{C_{Theoretical\ amount}} \times 100$$

Limit of Detection (LD)

Calculation of limit of detection (LD) is based on (Executive Order on quality requirements for environmental measurements) [2]

$$LD = 3 \cdot s_w$$

$$s_w^2 = (d_1^2 + d_2^2 + d_3^2 + ... + d_n^2)/2n$$

where d_1, d_2, d_3, … D_n is the difference between the results of the individual duplicate determinations of a total of n duplicate determinations of control samples.

Expanded measurement uncertainty U_{rel} and U_{abs}

Calculation of expanded measurement uncertainty is based on Nordtest report[3]

Measurement uncertainty U is expanded Measurement Uncertainty, estimated from control sample results, , using a coverage factor of 2 to reach approximately 95% confidence level

U_{abs} – Expanded combined uncertainty defined in absolute units (µg/L) and is based on replicate analysis of control samples spiked at low level (0.04 µg/L)

U_{rel} – Expanded combined uncertainty expressed as percentage (%) and calculated from replicate analysis of control samples spiked at high level (0.1 µg/L)

Calculation:

$$U_{abs} = u_{abs}(c) * 2$$

$$U_{rel} = u_{rel}(c) * 2$$

Where 2 is coverage factor and small $u_{abs}(c)$ and $u_{rel}(c)$ are combined uncertainties (containing variance of intermediate precision and bias component) in absolute and relative values, respectively.

$$u_{abs}(c) = \sqrt{u_{bias(abs)}^2 + s_T^2 + u_{ref}^2}$$

Where $u_{bias(abs)}^2$ is bias calculated as

$$u_{bias(abs)}^2 = (100 - \text{Recovery}) /100*0.04$$

s_T^2 is total standard deviation within laboratory determined on the basis of replicate analysis of control samples (reference executive order)

And u_{ref}^2 is uncertainty on the "true" value of the control sample. It is calculated by the GUM method[4] and include the preparation of standards, control samples and the purity of the standards

$$U_{rel}(c) = \sqrt{u_{bias(rel)}^2 + CV_T^2 + u_{ref}^2}$$

[2] Bekendtgørelse om kvalitetskrav til miljømålinger, BEK nr 1770 af 28/11/2020 (Gældende)

[3] HANDBOOK FOR CALCULATION OF MEASUREMENT UNCERTAINTY IN ENVIRONMENTAL LABORATORIES, NT TECHN REPORT 537, Approved 2003-05

[4] Evaluation of measurement data – Guide to the expression of uncertainty in measurement, JCGM 100:2008, (GUM 1995 with minor corrections)

Where $u^2_{bias(abs)}$ is bias in relative values calculated as

$$u^2_{bias(abs)} = (100 - \text{Recovery}) /100$$

And CV_T^2 is relative standard deviation.

8. Problematic compounds

This chapter contains discussion on chemical properties and the reasons why some of the compounds here referred to as: "problematic compounds" were not detected or extraction recovery of these compounds are very low.

Compound #2 (4-Carbamyl-2,5-dichloro-6cyano benzene-1,3-disulfonic acid)

Based on theoretical calculation, compound #2 is preferably ionised into a double charged adduct by losing two hydrogens from sulfonic groups [M-2H] (blue line on the picture below, Figure 6). The reason why we have previously reported this compound as non-detected is that our hypothesis on formation of parent ion was incorrect and we were looking for wrong parent ion. Compound was monitored as parent ion [M-2OH] with m/z of 194.9399, instead m/z of 185.9346 which corresponds to [M-2H] adduct should be used. As a confirmation ion should be used fragment with a mass of 79.9574 m/z (picture below), this fragment is confirmed with theoretical in silico prediction (metfrag). As data recorded for this compound is incorrect and could not be used for calculation of validation parameters but above-mentioned parent and quantification ions should be used in the future instead.

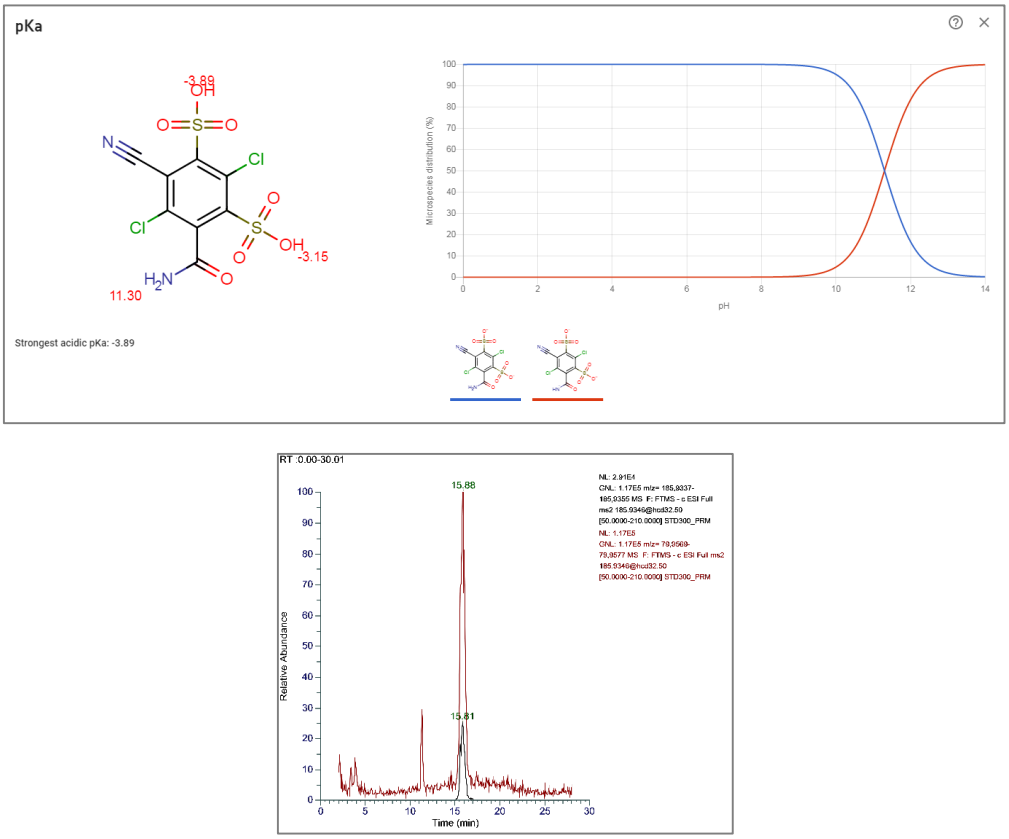


Figure 6 (top) Dissociation curve of compound #2, taken from https://chemicalize.com/app/calculation; (bottom) parent ion and fragment of compound #2

Compound #9 (4-aminobenzenesulphonamide)

Based on theoretical calculations compound #9 can exist as both positive and negative species with strongest acid pkA of 10.99 and most basic pkA of 2.27 (calculated with web tool chemicalize[5]). The compound was also detected experimentally in reference spectra and in both forms in both positive and negative mode under similar conditions (composition of mobile phase etc.) (massbank). Based on this knowledge we hypothesize that our current methods are suitable for detection, however more testing is needed.

Compound #10 (N-(2,6-difluorophenyl)-8-fluoro-5-hydroxy(1,2,4)triazolo(1,5-c)pyrimidine-2-sulfonamide)

Compound #10 could be observed in the data and spectra of this compound contain peaks that are confirmed theoretically but peak is observed in all injections including water blanks. Therefore, this compound was classified as not detected and identity of observed peaks was not investigated any further. According to prediction is compound #10 preferably ionised as negatively charged adduct $[M-H]^-$ with mass to charge ratio of 344.0071 m/z. Fragments of this compound are ions with m/z of 153.0218 and 296.9888 are observed in our experimental data (picture below). Parent ion and fragments are confirmed on theoretical basis by web tool MetFrag (see references)

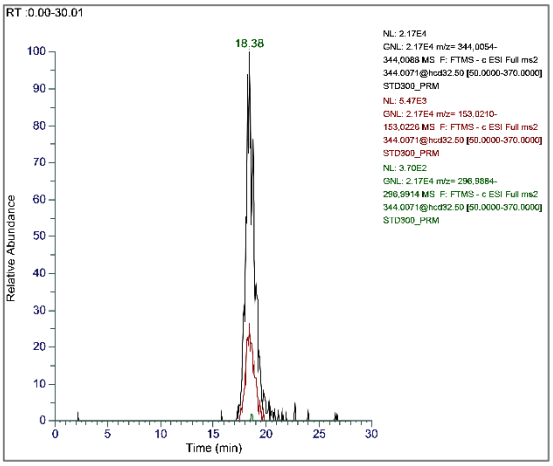


Figure 7 Plotted are parent ion and two fragments of compound #10

Compounds #13 and #14

Compounds are detected with IC-HRMS/MS and identity of the compounds is confirmed with in silico prediction (see appendix B). Validation results for these compounds could not be calculated due to low recovery of this compound with reported SPE protocol. Further investigation and optimization of SPE method is necessary to achieve good recovery for this compound with SPE.

Alternatively, direct injection was tested but results were not satisfactory as compound could be detected only at spiking level of 100 µg/L in the presence of matrix. Analytes were not observed with lower spiking levels. Large limit of detection could be explained by suppression of the signal by matrix i.e, background ions saturated analytical column and signal of the analyte was suppressed. Recommended is use of guard filters

[5] https://chemicalize.com/app/calculation;

before running samples with ion chromatography. As an example, could be SPE filters that can remove transition metals and neutralize the sample matrix before injection. This was not tested and more work is need.

9. Analysis quality

Quality of the analysis was based on measurement of 8 control samples at high and low levels (0.04 and 0.1 µg/L). Here are presented validation results for 9 analytes shown in Table 8 (note that validation parameters were obtained for analytes without any superscript in the table). Acceptable accuracy and precision with expanded measurement uncertainty (U_{rel}) lower than 30 % were confirmed for compounds 1, 3, 4 and 11. All 9 validated analytes had previously shown good extraction recovery and acceptable accuracy in preliminary studies but were however not confirmed for the compounds 7, 15 and 16 in this validation study. Low recovery of these compounds at higher validation level (0.1 µg/L) and increased sample volume are the reason measurement uncertainty being higher than 50 %. Further optimization of sample preparation protocol is necessary for these compounds in order to achieve acceptable measurement precision and accuracy. Presented are also estimated detection limits (LD) and extraction recoveries for compounds 6, 8 and 12. Results are not validated for these compounds.

Table 8 Validation parameters recovery estimated at two levels (0.04 and 0.1 µg/L), limit of detection (LD), Relative expanded measurement uncertainty (U_{rel}) and absolute expanded measurement uncertainty (U_{abs})

No	Method	Recovery Low level (%)	Recovery High level (%)	LD (µg/L)	U_{rel} (%)	U_{abs} (µg/L)
1	SPE-IC-HRMS/MS	122	96	0.010	8	0.019
3	SPE-IC-HRMS/MS	112	84	0.004	32	0.014
4	SPE-IC-HRMS/MS	113	89	0.010	21	0.014
5	SPE-IC-HRMS/MS	103	78	0.016	45	0.009
	SPE- LC-MS/MS	94	65	0.013	70	0.018
6[a]	DI-LC-MS/MS			0.010[a]		
7	SPE-IC-HRMS/MS	136	22	0.017	141	0.078
8[b]	SPE-IC-HRMS/MS	87 ± 40[b]		0.02[b]		
11	SPE-IC-HRMS/MS	151	88	0.021	25	0.046
12[a]	DI-LC-MS/MS			0.05[a]		
13[c]	DI-IC-HRMS/MS			100		
14[c]	DI-IC-HRMS/MS			100		
15	SPE- LC-MS/MS	107	74	0.009	55	0.013
16	SPE- LC-MS/MS	95	35	0.006	133	0.029
18	SPE- LC-MS/MS	101	82	0.007	39	0.010

[a)] results obtained with direct injection method with LC-MS/MS, results are not validated; [b)] result obtained by using different ion chromatography column (AS19-4µm), not validated; [c)] direct injection with IC-HRMS/MS was tested but results could not be evaluated due to high concentration of background ions, further optimization is needed.

10. Conclusion

In summary, above presented methods were aimed for 18 compounds that are summarized in Table 1.

Compound #17 was under production/synthesis and therefore could not be included in method development.

Qualitative results were obtained for only for 14 compounds which were detected with LC-MSMS and/or IC-HRMSMS platforms remaining compounds #2, #9 and #10 were not detected.

Quantitative results were obtained only for 12 compounds as compounds #13 and #14 could not be extracted with SPE and direct injection method was not working due to large ion suppression effect.

For compounds #6, #12 was direct injection suitable option but results were not validated due to time limitations of the project.

Compound #8 was not validated as compound was detected with different IC column and this compound is not retained with column material used in validation study.

Quantitative results were further validated for 9 compounds (#1, #3, #4, #5, #7, #11, #15, #16 and #18) and results are presented in Table 8.

Methods require further optimization in order to achieve more effective methods with better extraction recovery and thereby lower measurement uncertainty. Moreover, the addition of internal standards should be pursued further.

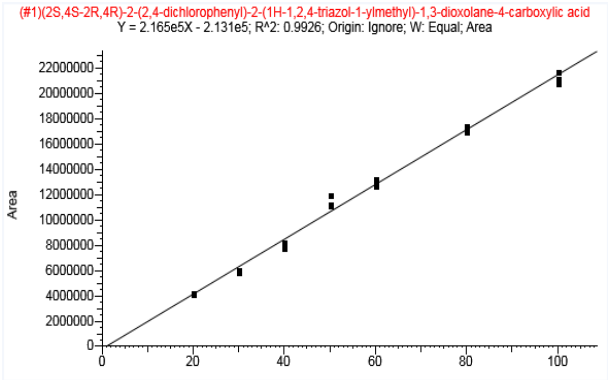
Solution for compounds that have low recoveries is optimization of solid phase extraction protocol to recover more analytes and or optimization of analytical methods to achieve lower detection limits with direct injection method.

It is possible to apply the obtained HRMS/MS-fragment substance information (i.e. IC-HRMS/MS) for SRM methods on triple-quadrupole or other MS/MS systems.

11.Appendix A – calibration curves

CALIBRATION:
Matrix matched calibration curve
- low level

#1



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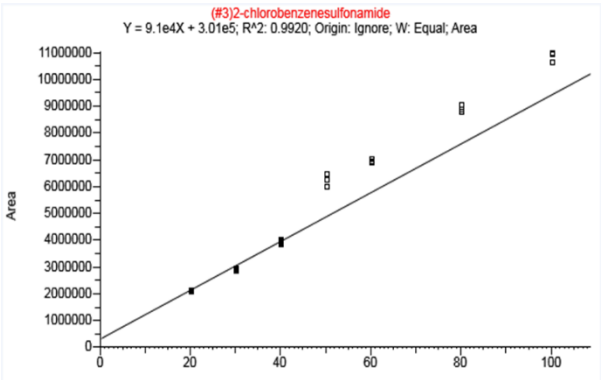
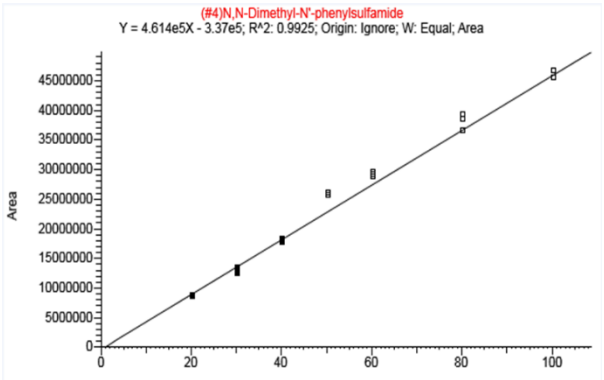
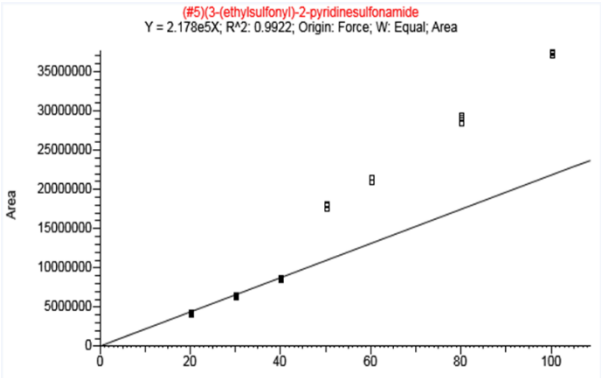


#4



#5



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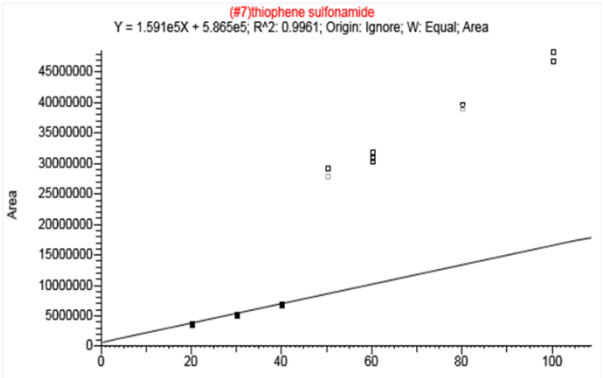
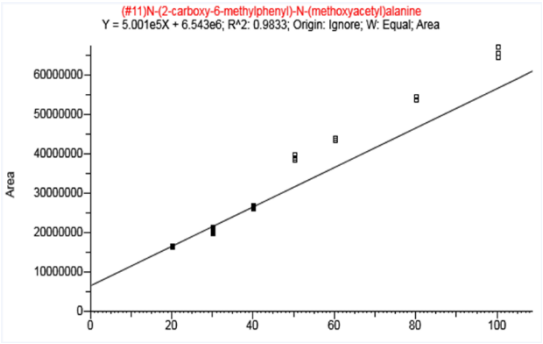
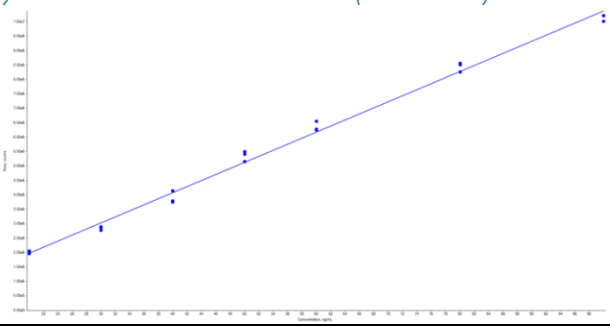


#11



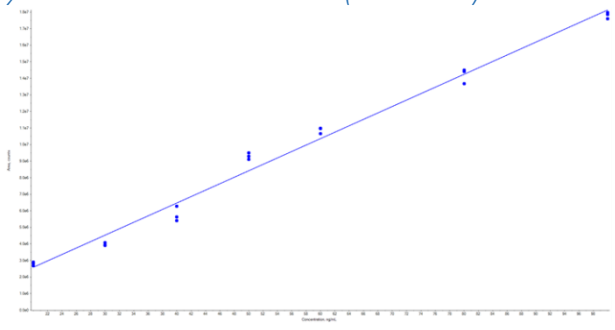
#18

y = 1.05e+005 x + -1.21e+005 (r = 0.9968)



#15

y = 1.94e+005 x + -1.3e+006 (r = 0.9935)



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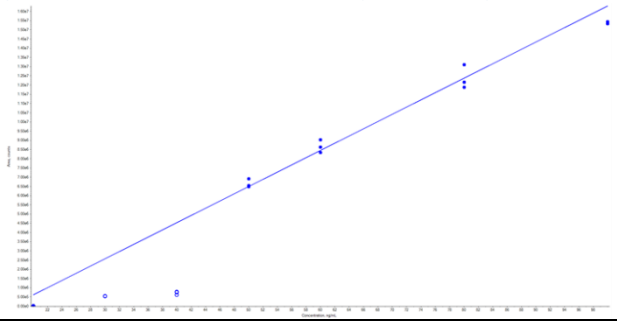
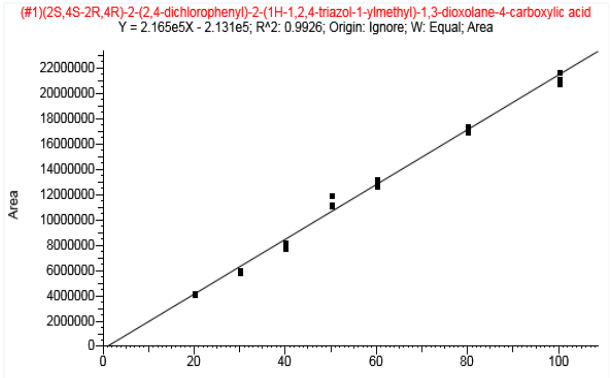
y = 1.96e+005 x + -3.31e+006 (r = 0.9905)

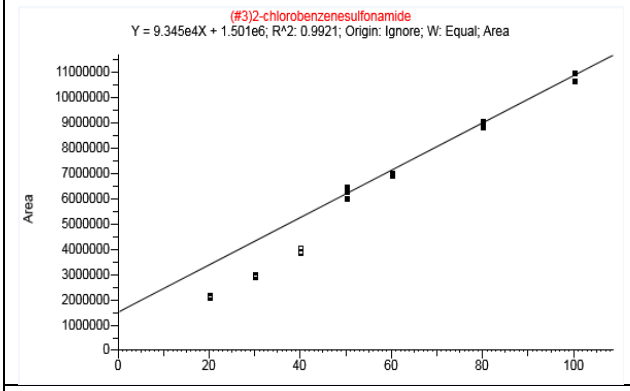


High level

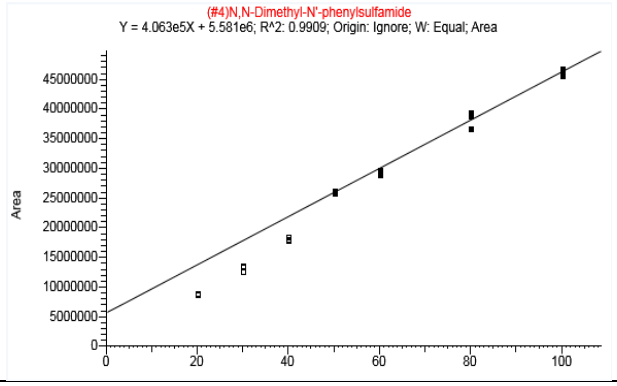
#1



#3



#4



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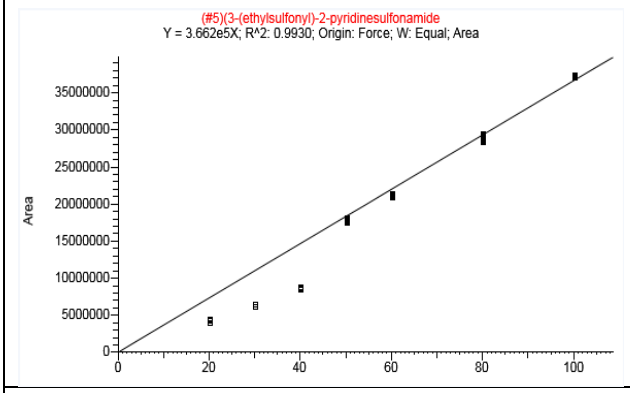

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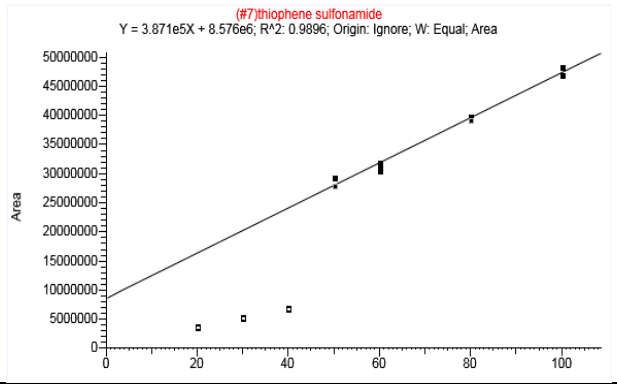

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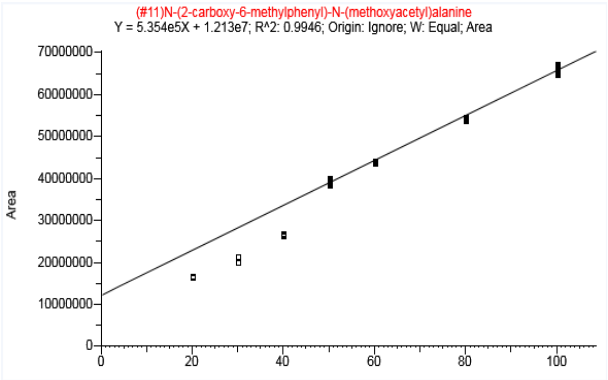
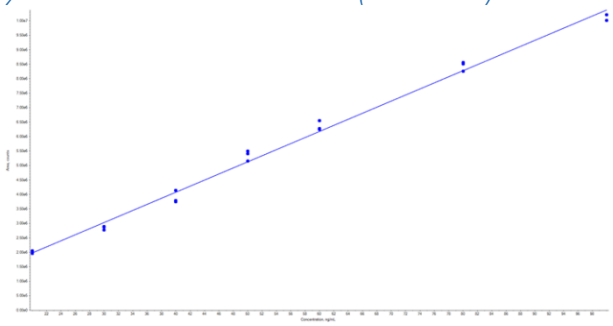


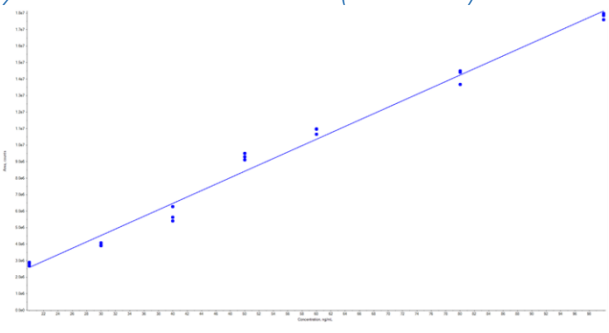
#18

y = 1.05e+005 x + -1.21e+005 (r = 0.9968)



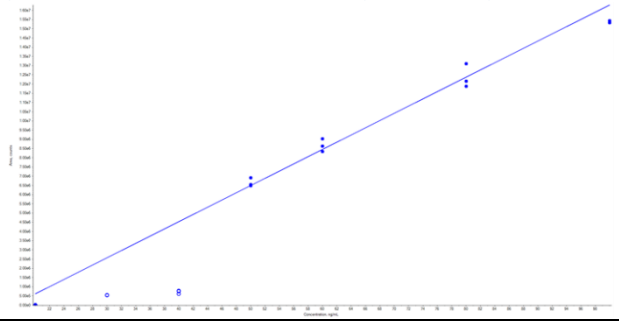
#15

y = 1.94e+005 x + -1.3e+006 (r = 0.9935)

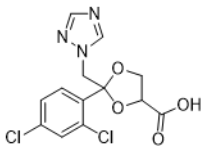
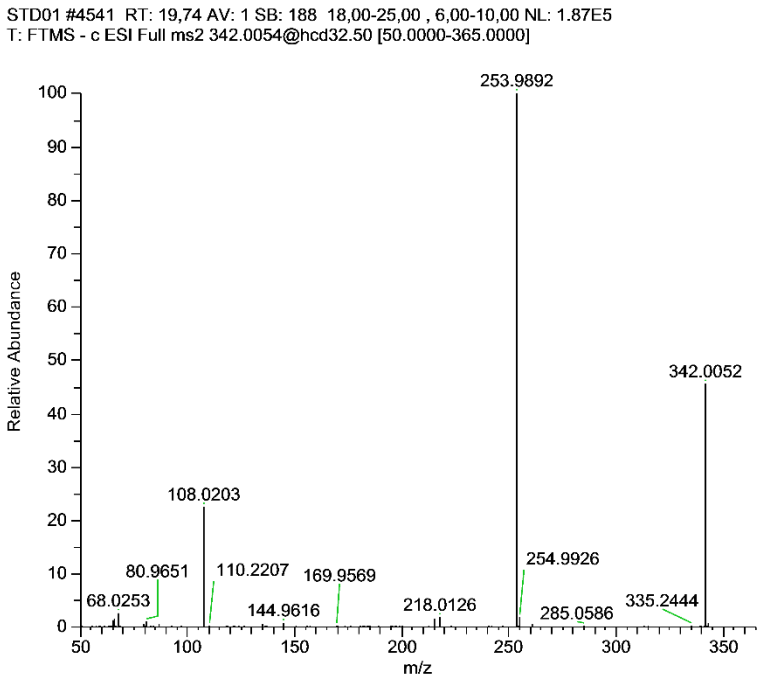
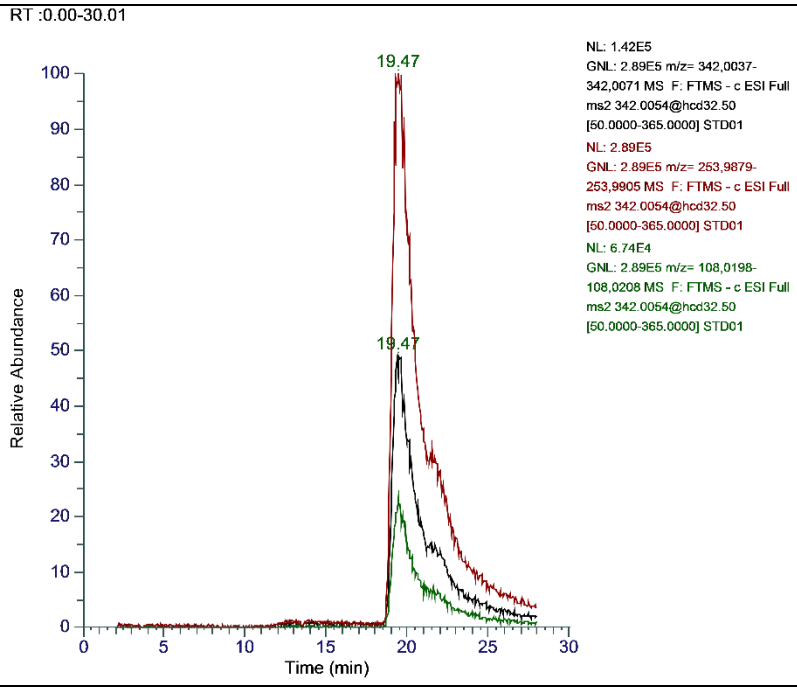


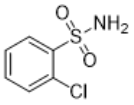
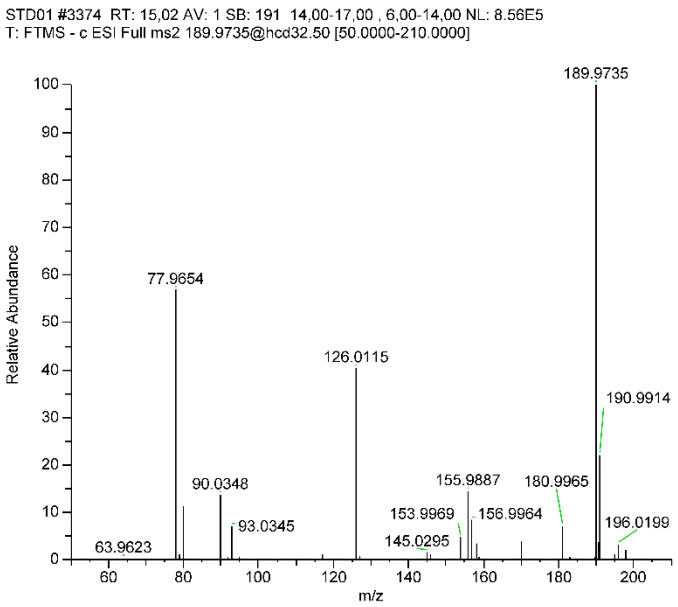
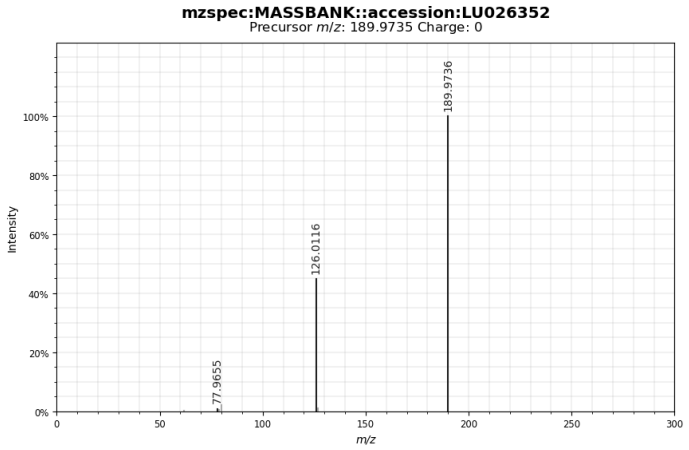
#16

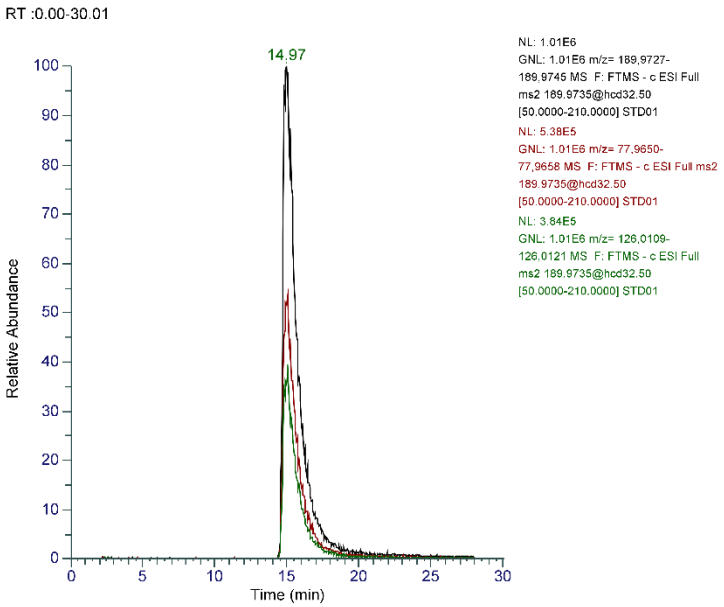
y = 1.96e+005 x + -3.31e+006 (r = 0.9905)

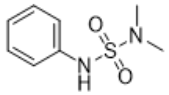
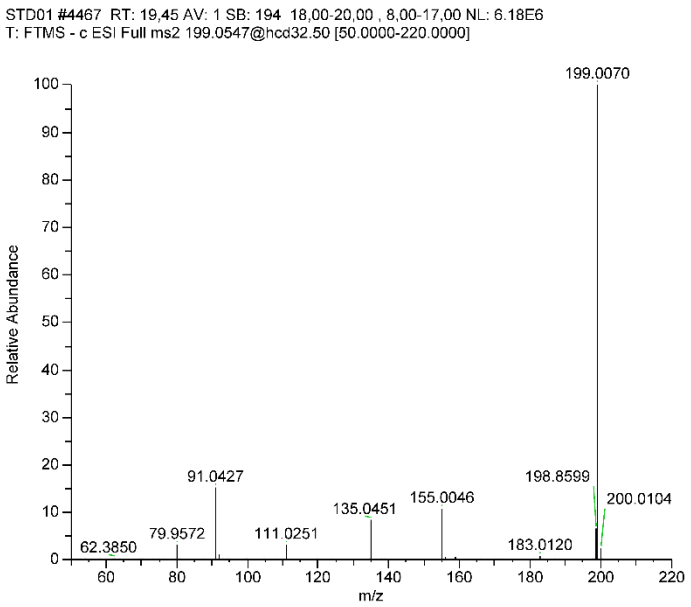
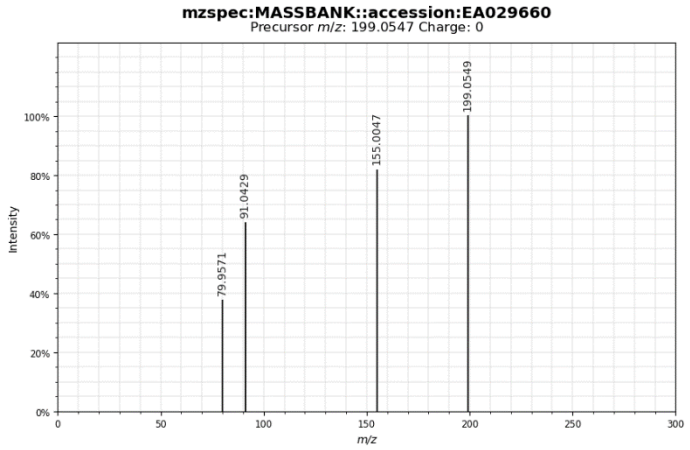


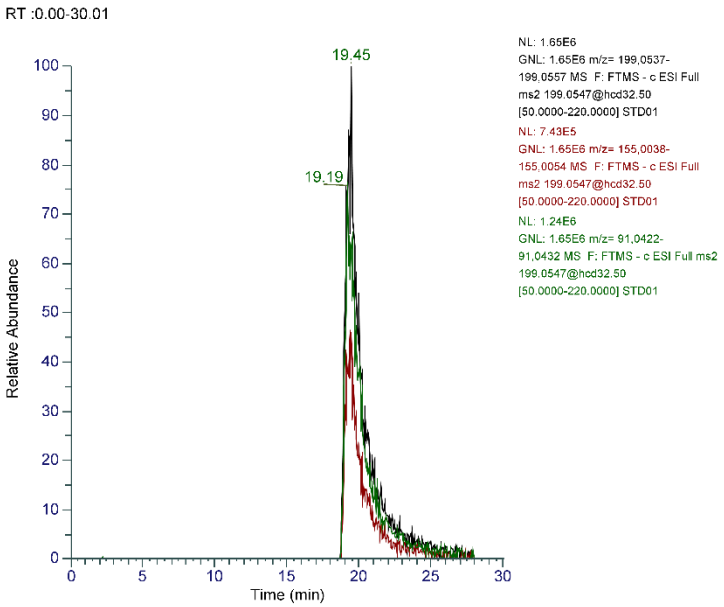
12. Appendix B – Substance chromatograms and mass spectrums

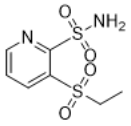
Compound number: Compound name: Chemical formula: InChIKey: Structure:	Compound #1 2-(2,4-Dichlorophenyl)-2-(1H-1,2,4-triazol-ylmethyl)-1,3-dioxolane-4-carboxylic acid C13H11Cl2N3O4 Not available 
Experimental MS2 spectra: Parent ion: 342.0053 [M-H] Fragments: 253.9893 (QI) 108.0203(CI)	
Overlaid parent ion, quantification (QI) ion and confirmation ion (CI)	

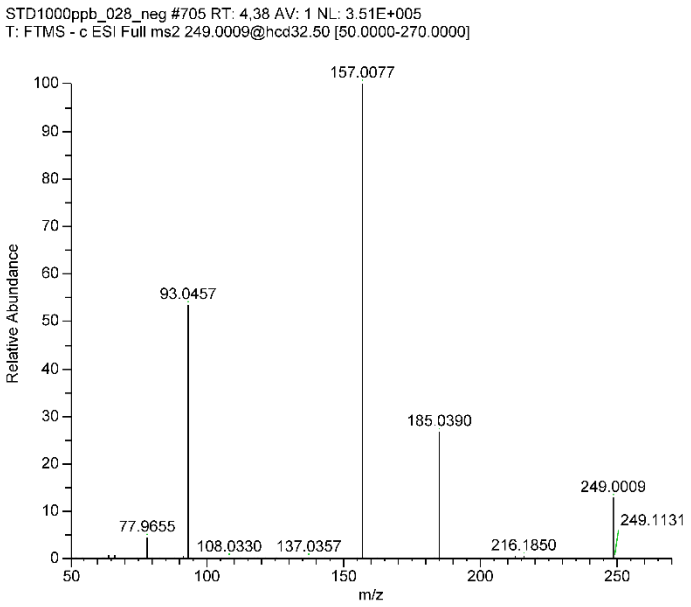
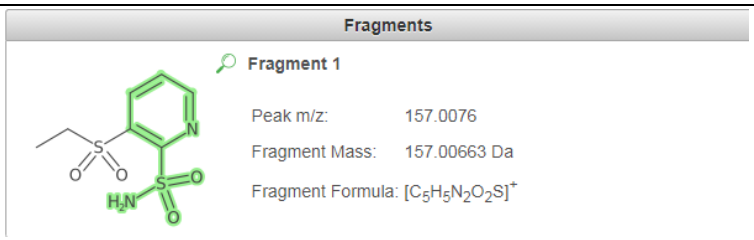
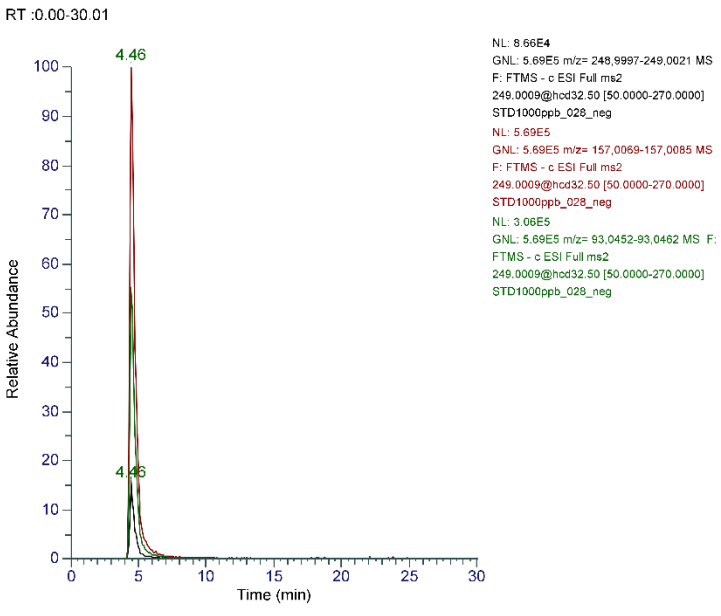
Compound number: Compound name: Chemical formula: InChIKey: Structure:	Compound #3 2-chlorobenzenesulfonamide C6H6ClNO2S JCCBZCMSYUSCFM-UHFFFAOYSA-N 
Experimental spectra (background substracted) MS2 spectra	
Reference spectra (see matching fragments)	 Kondić, T.;Singh, R.;Elapavalore, A.;Schymanski, E.

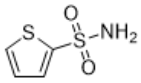
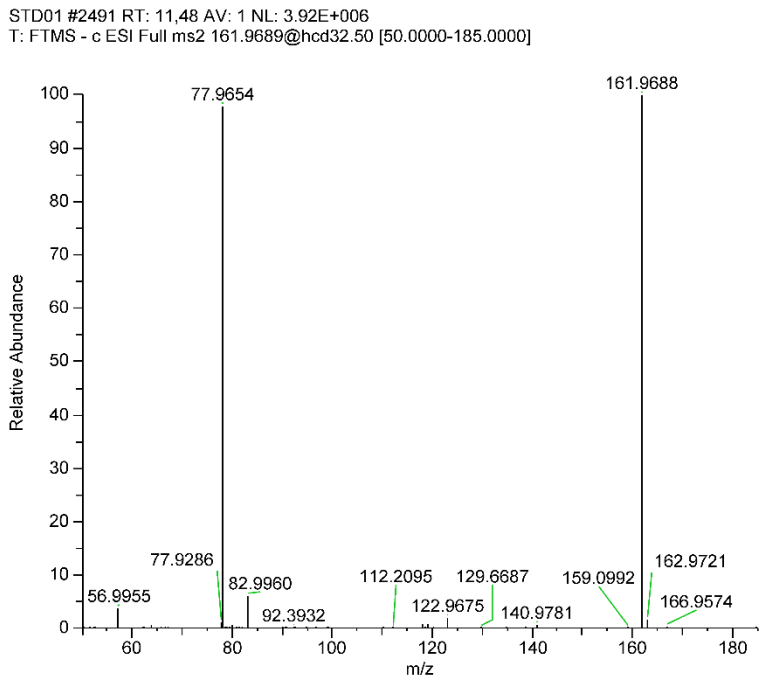
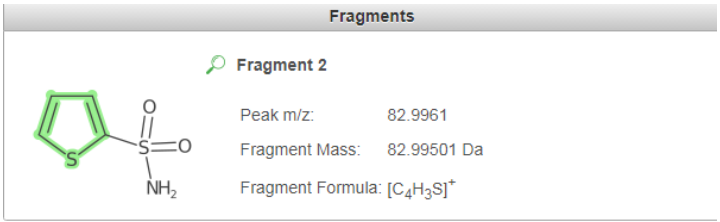
Overlaid parent ion, quantification (QI) ion and confirmation ion (CI)	

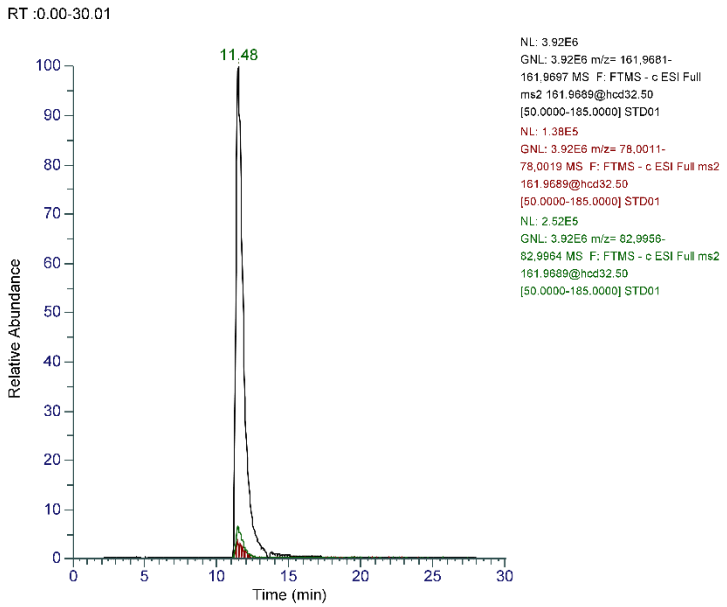
Compound number: Compound name: Chemical formula: InChIKey: Structure:	Compound #4 N,N-Dimethyl-N'-phenylsulfamide C8H12N2O2S QCDQDISRALTLNQ-UHFFFAOYSA-N 
Experimental spectra (background substracted) MS2 spectra	
Reference spectra (see matching fragments)	 Kondić, T.;Singh, R.;Elapavalore, A.;Schymanski, E.

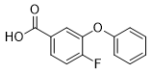
Overlaid parent ion, quantification (QI) ion and confirmation ion (CI)	

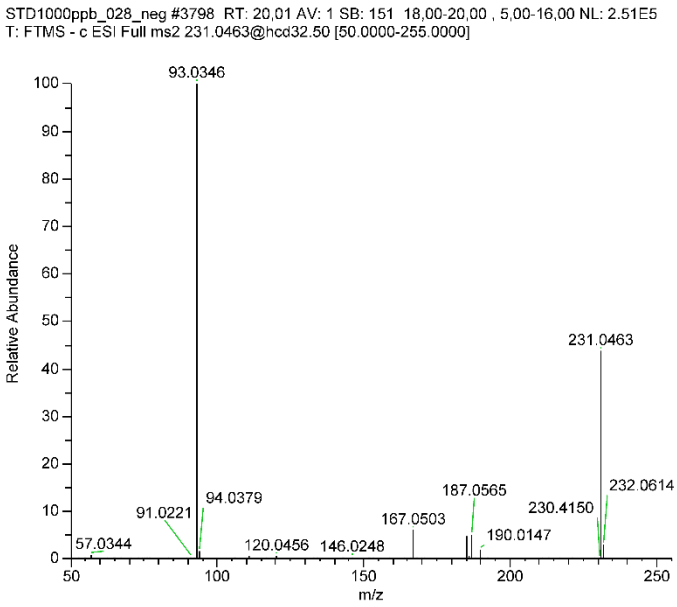
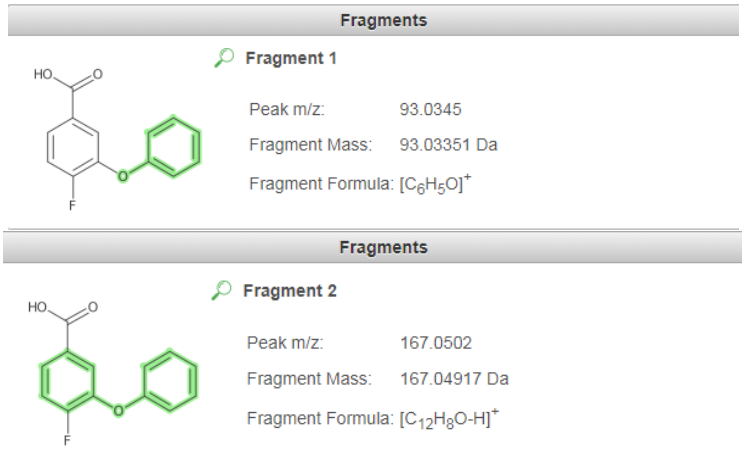
Compound number: Compound name: Chemical formula: InChIKey: Structure:	Compound #5 3-(ethylsulfonyl)-2-pyridinesulfonamide C7H10N2O4S2 ZVAJJLYQUHJURI-UHFFFAOYSA-N 

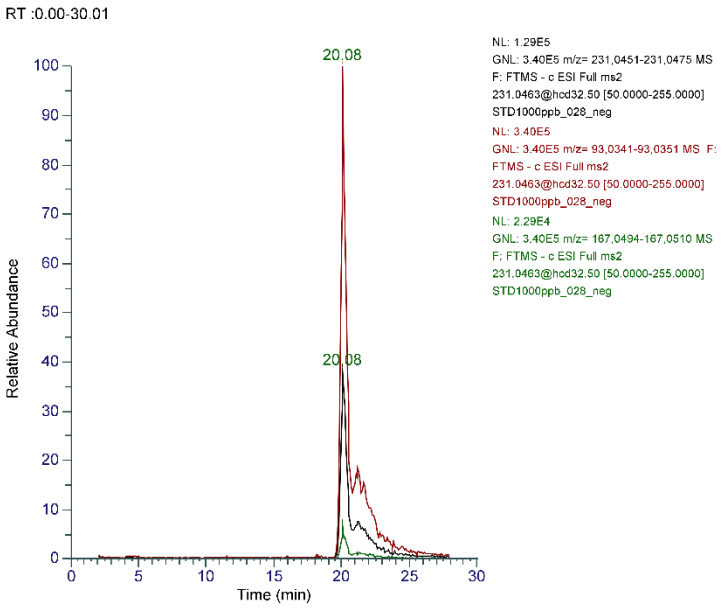
Experimental spectra (background substracted) MS2 spectra	
Metfrag (Fragment 157.0077 was confirmed with in silico prediction, see attached screenshot)	
Overlaid parent ion, quantification (QI) ion and confirmation ion (CI)	

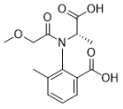
Compound number: Compound name: Chemical formula: InChIKey: Structure:	Compound #7 thiophene sulphonamide C4H5NO2S2 KTFDYVNEGTXQCV-UHFFFAOYSA-N 
Experimental spectra (background substracted) MS2 spectra	
MetFrag (Fragments 82.9960 was confirmed with in silico prediction, see attached screenshot)	

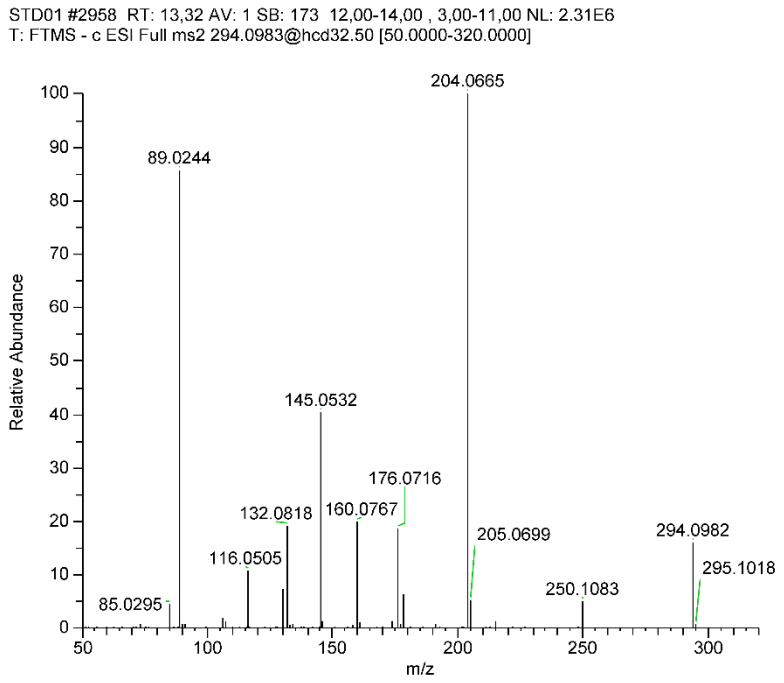
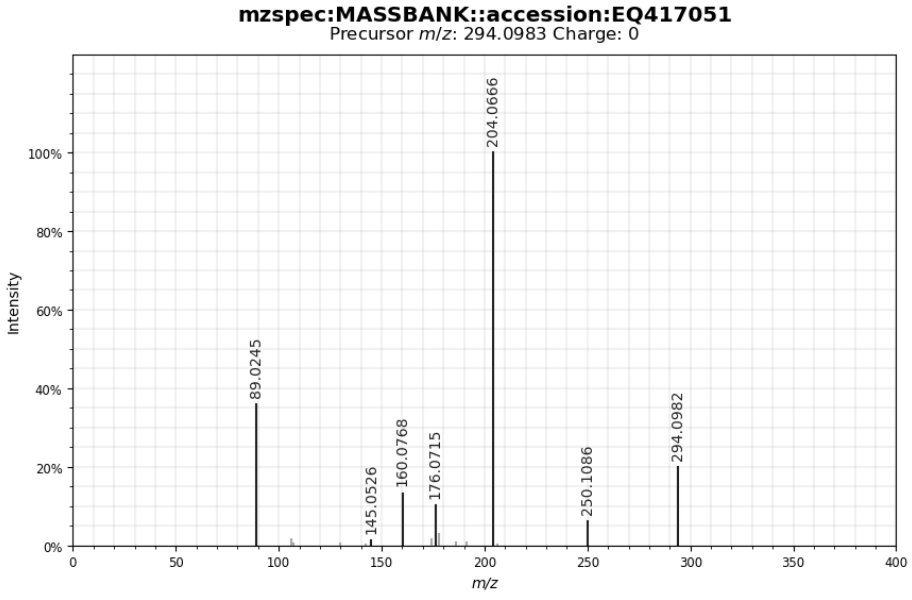
Overlaid parent ion, quantification (QI) ion and confirmation ion (CI) Comment: compound fragments very little	

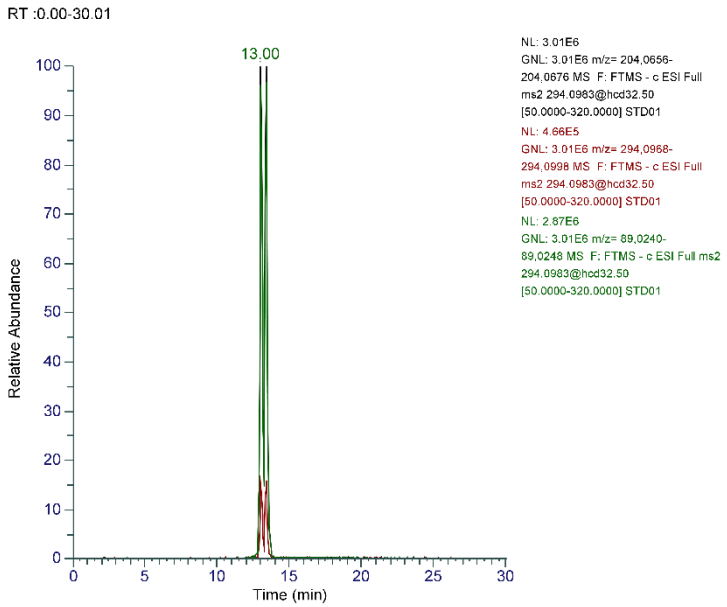
Compound number: Compound name: Chemical formula: InChIKey: Structure:	Compound #8 4-fluoro-3-phenoxybenzoic acid C13H9FO3 VLXNXMTVRWIUJZ-UHFFFAOYSA-N 

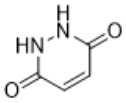
Experimental spectra (background substracted) MS2 spectra	
MetFrag (Fragments 93.0346 and 167.0503 were confirmed with in silico prediction, see attached screenshot)	

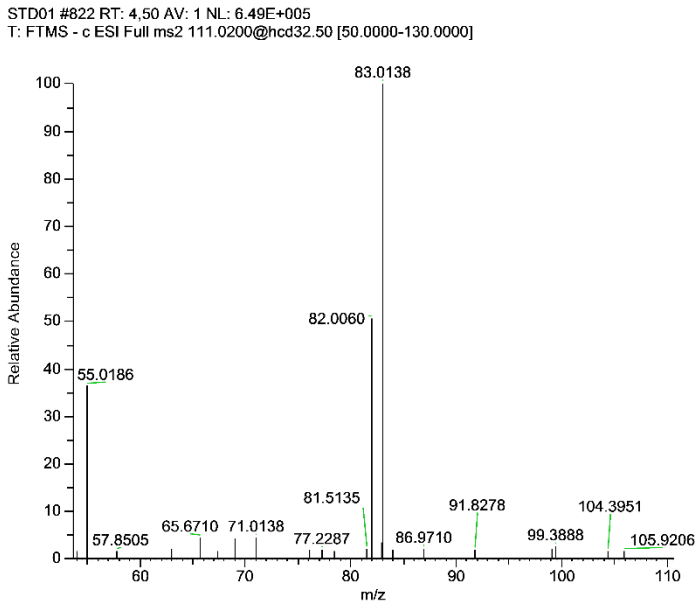
Overlaid parent ion, quantification (QI) ion and confirmation ion (CI) Comment: compound was detected with IC AS19-4µm column	

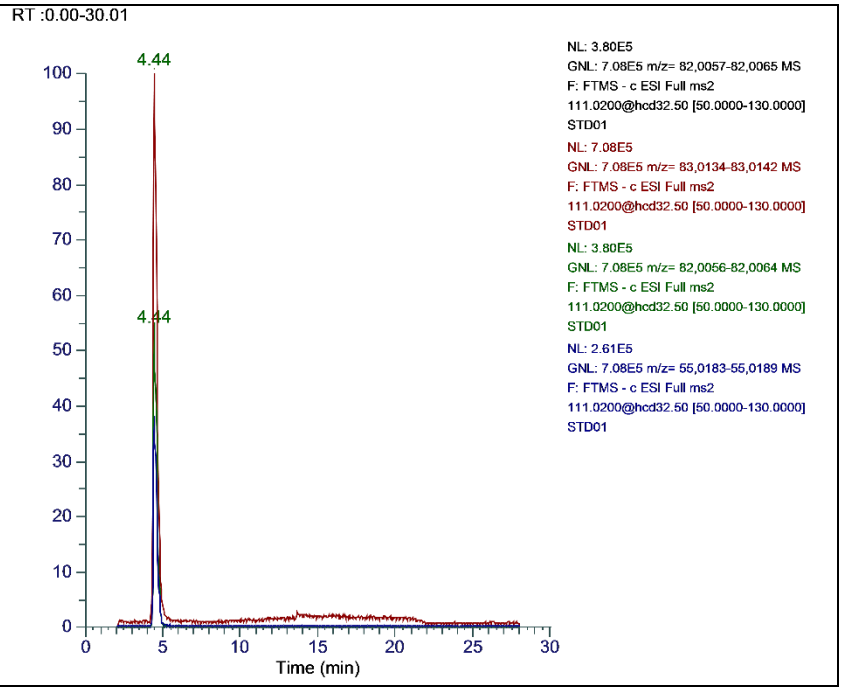
Compound number: Compound name: Chemical formula: InChIKey: Structure:	Compound #11 N-(2-carboxy-6-methylphenyl)-N-(methoxyacetyl)alanine C14H17NO6 WFTHOCDLKYPFJX-UHFFFAOYSA-N 

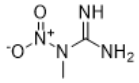
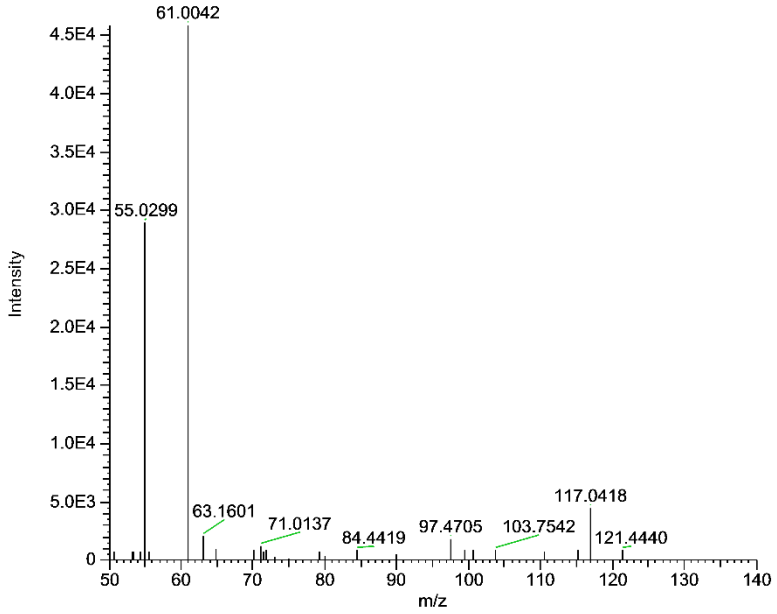
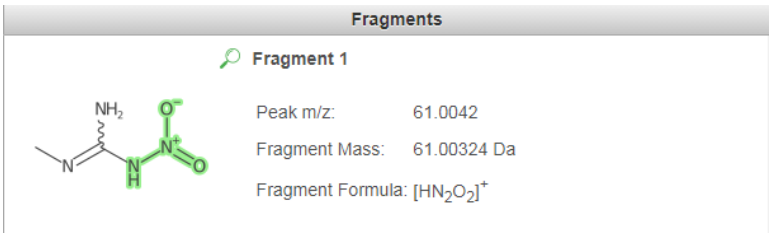
Experimental spectra (background substracted) MS2 spectra	
Reference spectra (see matching fragments)	

Overlaid parent ion, quantification (QI) ion and confirmation ion (CI) Comment: Compound is present in two forms	

Compound number: Compound name: Chemical formula: InChIKey: Structure:	Compound #13 1,2-dihydropyridazine-3,6-dione C4H4N2O2 BGRDGMRNKXEXQD-UHFFFAOYSA-N 

Experimental spectra (background substracted) MS2 spectra	
MetFrag (Fragments 55.018682.006 and 83.0136 were confirmed with in silico prediction, see screnshots on right)	**Fragments** **Fragment 1** Peak m/z: 55.0186 Fragment Mass: 55.01785 Da Fragment Formula: $[C_3H_2O]+H^+$ **Fragments** **Fragment 2** Peak m/z: 82.006 Fragment Mass: 82.00493 Da Fragment Formula: $[C_4H_2O_2]^+$ **Fragments** **Fragment 3** Peak m/z: 83.0138 Fragment Mass: 83.01276 Da Fragment Formula: $[C_4H_2O_2]+H^+$

Overlaid parent ion, quantification (QI) ion and confirmation ion (CI)	

Compound number: Compound name: Chemical formula: InChIKey: Structure:	Compound #14 N-methyl-N-nitroguanidine C2H6N4O2 XCXKNNGWSDYMMS-UHFFFAOYSA-N 
Experimental spectra (background substracted) MS2 spectra	STD01 #879 RT: 4,72 AV: 1 SB: 118 4,00-10,00 , 2,00-3,00 NL: 4.58E4 T: FTMS - c ESI Full ms2 117.0418@hcd32.50 [50.0000-140.0000]  post_high #1005 RT: 5,22 AV: 1 SB: 260 4,00-11,00 , 11,00-19,00 NL: 1.39E4 T: FTMS - c ESI Full ms2 117.0418@hcd32.50 [50.0000-140.0000]
MetFrag	

Overlaid parent ion, quantification ion and confirmation ion