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Target and Suspect Screening of Polar Organic Compounds in Raw and Potable Waters by Liquid Chromatography - High Resolution Accurate Mass Spectrometry

Target and Suspect Screening of Polar Organic Compounds in Raw and Potable Waters by Liquid Chromatography - High Resolution Accurate Mass Spectrometry

Methods for the Examination of Waters and Associated Materials

This booklet contains a method for the determination of unknown polar organic compounds in raw and potable waters by liquid chromatography – high resolution accurate mass spectrometry.

The method has not been validated and consequently details are included for information purposes only, as examples of the type of procedures that are available to analysts. Information on the use of this methods would be welcomed to assess its full capabilities.

Whilst this booklet may report details of the materials actually used, this does not constitute an endorsement of these products but serves only as an illustrative example. Equivalent products are available, and it should be understood that the performance characteristics of the method might differ when other materials are used. It is left to users to evaluate methods in their own laboratories.

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285. Target and Suspect Screening of Polar Organic Compounds in Raw and Potable Waters by Liquid Chromatography - High Resolution Accurate Mass Spectrometry.

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About this series

Introduction

This booklet is part of a series intended to provide authoritative guidance on recommended methods of sampling and analysis for determining the quality of drinking water, ground water, river water and sea water, wastewater and effluents as well as sewage sludges and biota.

In addition, short reviews of the most important analytical techniques of interest to the water and sewage industries are included.

Performance of methods

Ideally, all methods should be fully evaluated with results from performance tests. These methods should be capable of establishing, within specified or pre-determined and acceptable limits of deviation and detection, whether or not any sample contains concentrations of parameters above those of interest.

For a method to be considered fully evaluated, individual results from at least three laboratories should be reported. The specifications of performance generally relate to maximum tolerable values for total error (random and systematic errors) systematic error (bias) total standard deviation and limit of detection - often, full evaluation is not possible and only limited performance data may be available.

In addition, good laboratory practice and analytical quality control are essential if satisfactory results are to be achieved.

The preparation of booklets within the series “Methods for the Examination of Waters and Associated Materials” and their continuing revision is the responsibility of the Standing Committee of Analysts (SCA) - Established 1972 by the Department of the Environment.

At present, there are several working groups, each responsible for one section or aspect of water quality analysis:

1. General principles of sampling and accuracy of results
2. Microbiological methods
3. Inorganic and physical methods, metals and metalloids
4. Organic methods
5. Biological, biodegradability and inhibition methods
6. Radiochemistry methods

The actual methods and reviews are produced by smaller panels of experts in the appropriate field, in co-operation with the working group and main committee. The names of those members principally associated with these methods are listed at the back of this booklet.

Publication of new or revised methods will appear on our website – the library for which serves as a record of the bona fide methods developed and produced by the Standing Committee of Analysts.

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Every effort is made to avoid errors appearing in the published text. If, however, any are found, please notify the Secretary.

secretary@standingcommitteeofanalysts.co.uk

Users should ensure they are aware of the most recent version they seek.

Warning to users

The analytical procedures described in this booklet should only be carried out under the proper supervision of competent, trained analysts in properly equipped laboratories.

All possible safety precautions should be followed, and appropriate regulatory requirements complied with. This should include compliance with the Health and Safety at Work etc. Act 1974 and all regulations made under the Act, and the Control of Substances Hazardous to Health Regulations 2002 (SI 2002/2677). Where particular or exceptional hazards exist in carrying out the procedures described in this booklet, then specific attention is noted.

Numerous publications are available giving practical details on first aid and laboratory safety.

These should be consulted and be readily accessible to all analysts. Amongst such resources are:

HSE: [Information about health and safety at work](#)

RSC: [Laboratory best practices](#)

The Approved List of Biological Agents. (2023) Advisory Committee on Dangerous Pathogens of the Health and Safety Executive (HSE)

1 Introduction

“Target” or “Suspect screening” is an approach that is often used during investigations into suspected pollution events or when routine analysis fails to identify reasons for poor water quality. The approach consists of screening lists of multiple compounds of interest (the “suspects”) in complex samples using their molecular formula (and the resulting calculated exact mass) with or without reference standards. Although comprehensive, screening with large lists often results in many hits for the same exact mass. To eliminate false positives, data filtering using MS/MS fragments, retention time data and other metadata are employed. Appropriate selections of suspect lists associated with the environmental scenario in mind is also important to reduce the number of false positives and the significant data filtering work. Such defined suspect lists also allow to estimate whether the compounds can indeed be detected by the analytical method, thereby avoiding false negative results due to the wrong analytical method.

It should be noted that only qualitative or semi-quantitative data are obtained with the suspect screening workflows. It is possible to prioritize compounds on which to concentrate future target analysis when standards are available to purchase for identity confirmation and quantification.

As the varying levels of confidence associated with screening analysis, using high resolution accurate mass spectrometry (HRMS), are very difficult to convey concisely and accurately, Schymanski proposed a level system to ease the communication of identification and confidence of sample data. (Schymanski et al, 2014)

Targeted Screening

“Targets” are compounds of known chemical name and structure, for which quantitative methods may be available. Identification of targets is achieved by comparison with well characterised reference data (chromatographic retention time, MS, and MS/MS spectra) acquired from certified materials used to validate compound identity prior to analysis.

The level of confidence associated with target screening is 1. i.e., confirmed structure by reference standard. Method A contained in this booklet achieves a confidence level of 1.

Suspect screening

“Suspects” are known compounds in terms of chemical name and structure which are expected, or where there is a reasonable suspicion, to be present in a sample. To facilitate suspect screening, neutral monoisotopic masses and molecular formulae are included in large ‘suspect’ lists. In addition, several other predicted values (e.g., retention time, MS² fragment spectra) can also be included in the suspect list. The resulting suspect lists can be created as excel and comma separated values (CSV) formats allowing for easy import into various vendor software.

Sample data is analysed using software tools which compare experimental MS, MS² and isotope pattern data to calculated theoretical values. Experimental MS² spectra can be searched against various local and online spectral libraries and, where no MS² library data

exists, fragmentation modelling can be used for comparison. In most cases, analytical standards are not readily available and therefore, analytical methods are not always validated and compound identities and concentrations not definitive i.e., results are qualitative.

The level of confidence associated with suspect screening is Level 2-3. i.e., probable structure or tentative candidate structure.

Non-targeted Screening

Unknown compounds are those not contained within known compound list or spectral libraries, these include many metabolites and transformation products and in all likelihood account for the majority of signals in a data file. Generally, sample preparation and data acquisition are similar for suspect and non-targeted screening whereas data analysis/mining are different.

The level of confidence associated with non-targeted screening is Level 4-5. i.e. unequivocal molecular formula or exact mass of interest.

The procedures presented in this booklet are based on High Resolution Accurate Mass Spectrometry (HRMS) coupled with High Performance Liquid Chromatography. Method A by Time-of-Flight mass detection and Method B using an Orbitrap high resolution mass spectrometer. Identification of compounds requires MS² spectral data, generated by targeted or automatically triggered ion fragmentation. Evaluation using suitable software, is based on chromatographic retention time, mass accuracy, isotopic pattern and diagnostic MS/MS fragments by comparison with available chemical and mass spectrometry databases.

The techniques described in this booklet may be used in conjunction with other scan methods to provide a rapid response to Water Quality teams in the event of customer complaints or other potential incidents that could affect the quality of raw and potable waters.

A positive identification using any of the following methods could also help to pinpoint the source of a contaminant. A negative result however may not affect the actions taken by Water Quality Teams to remove the source of contamination, e.g. for a taste or odour complaint, mains-flushing or other appropriate techniques will still be carried out even if the analysis cannot identify the source.

The methods are designed only as screening tools to attempt to identify any polar or non-volatile compounds that may be present in the water samples. No quantification is performed on any identified compound. Method A contains examples of target compound calibrations that may be used to provide estimations of amounts of compounds present – the range may be adjusted accordingly to suit requirements.

It should be noted that there is overlap between this method and many other GC-MS based SVOC methods in terms of compounds identified – e.g. triazines, organophosphates and neonicotinoids.

This method therefore offers a complimentary technique for the confirmation of many compounds identified using low resolution GC-MS.

* Note: this book in no way endorses a particular instrument manufacturer or supplier, this is listed as a guide only to the configuration set up in the specific analytical sections to enhance understanding.

2 Acquisition options

There are various options of HRAM measurement technique that can be adopted dependant on requirements. This book considers both Time of Flight and Orbitrap mass analysers, others are available. For High Resolution Accurate mass analysers, the minimum resolution requirement is >20,000 FWHM (Fullwidth at half maximum) at m/z 100 and above. The minimum mass accuracy deviation limit (difference between measured and theoretical masses) should be <5ppm across the entire mass range. Measurement modes may be specifically targeted or automatically triggered molecular ion fragmentation to produce product ion (MS^2) details of which are outline in sections 2.1-2.2.

2.1 Data independent MS/MS

Data independent MS/MS methods using all-ion-fragmentation (AIF) techniques have recently gained attention in the analysis and identification of known and unknown pollutants. Various terminology is used by different manufacturers for the AIF technique including "AIF" (Thermo), 'All Ions MS/MS' (Agilent), broadband collision-induced dissociation or bbCID (Bruker) and MSE (Waters). Novel mass spectrometers with fast duty cycles and acquisition times up to 50 MS/MS scans per second over a wide mass range and at high resolution has allowed for the development of these techniques. Figure A1. illustrates the process of data independent MS/MS acquisition for a Q-TOF-MS.

The collision cell rapidly alternates between low and high collision energies and the result is a data file with a low energy channel (MS) that contains predominantly precursor ions and one or several high-energy channels that contain precursor and fragment ions (MS/MS). A ramped collision energy may also be used instead of using several high-energy channels forming composite mass spectra. Only the most stable compounds will have a precursor ion present at the highest collision energy setting. One advantage over data-dependent MS/MS is that very low intensity precursor ions are fragmented, even if there are co-eluting molecules with higher intensities that are usually triggered first in data-dependent MS/MS. Hence, in principle, all molecules in data-independent or AIF MS/MS will undergo fragmentation.

The obvious disadvantage for all-fragment-ion techniques analyses is that the direct link between a specific precursor ion and its corresponding product ions is broken, therefore, mixed product ion spectra are generated, that can originate from multiple precursor ions. Precursor ion determination when using the all-fragment-ion technique requires mass spectral deconvolution on the ms^2 level and retention time information. However, when working with suspect lists, where the compound's monoisotopic mass and potential adducts and fragment ions are known, then the task of identification is made considerably easier.

2.2 Data dependent ms²

In Data dependent MS/MS either full scan detection of a mass contained within a specified inclusion list, or detection of a signal above a specified threshold will trigger an MS/MS event where the detected precursor ion undergoes collision induced dissociation (CID).

Higher-energy collisional dissociation (HCD) is a CID technique specific to Thermo Fisher Scientific Orbitrap instruments in which fragmentation takes place outside of the orbitrap; the ions pass through the C-trap and into the HCD cell, where dissociation takes place. The ions are then returned to the C-trap before injection into the orbitrap for mass analysis. A schematic showing the referenced components of the Thermo Fisher Scientific Q-Exactive Orbitrap is available in appendix B.1.

A significant advantage of Data dependent MS/MS is the clear relationship between detected precursor and product ions and clean fragment scans produced, which allow for higher confidence in identification of detected components.

Significant disadvantages of Data dependent MS/MS are that some molecules will not undergo fragmentation, for example if the detected response is below the set trigger threshold, or if there are co-eluting molecules with higher intensities a MS/MS event may not trigger for the lower intensity precursor.

3 Sample Stability

Samples should be stored at 3 ± 2 °C prior to analysis. Drinking water samples should be preserved using sodium thiosulphate (80mg of sodium thiosulfate per litre of water).

In order to limit sample and compound degradation samples should be extracted as soon as possible after sampling or frozen as soon as possible if extraction is not to be performed.

4 References

- i. Water quality — Sampling Part 3: Preservation and handling of water samples (ISO 5667-3).
- ii. Standing Committee of Analysts. The Stability and Preservation of Drinking, Ground and Surface Water Samples 2018, November 2018.
- iii. Schymanski, E.L., Jeon, J., Gulde, R., Fenner, K., Ruff, M., Singer, H.P. and Hollender, J., 2014. Identifying small molecules via high resolution mass spectrometry: communicating confidence. DOI: 10.1021/es5002105.
- iv. International Organization for Standardization . (2019). Water quality — Multi-compound class methods — Part 1: Criteria for the identification of target compounds

by gas and liquid chromatography and mass spectrometry (ISO 21253-1:2019).

5 Glossary of Terms

ChemSpider	a free online chemical structure database that integrates and links many high-quality data sources. Contains approx. 100,000,000 structures. ChemSpider is owned by the Royal Society of Chemistry.
Exclusion List	List of masses that will not trigger a DDMS ² event if detected. For further details see instrument setup section B.8.3
FISh Score	Fragment Ion Search score is generated by comparison of observed ms ¹ and ms ² data with in-silico generated predicted fragmentation patterns of proposed structure.
Lock Masses	Permanent systematic masses which can be used to recalibrate mass alignment on each injection.
MDL	The method detection limit (MDL) is defined as the minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank results.
mSigma	a measure of isotopic fit obtained from Bruker TASQ software. Displays the spectral similarity score between the theoretical and the measured isotope pattern as a value between 0 and 1000 with zero being a perfect match.
MZCloud	Mass Spectral library curated by Thermo Scientific; Contains collections of ms ² spectra generated using various instruments and at a variety of fragmentation energies. Contains approx. 16,500 spectra for approx. 32,000 compounds.
MZCloud match score	Score generated by comparison of observed ms ¹ and ms ² data with ms ¹ and ms ² data contained within the MZCloud library for the proposed structure.
MZLogic score	Score generated by substructure similarity search of a proposed structure in the MZCloud library, followed by comparison of observed ms ¹ and ms ² data with MZCloud ms ¹ and ms ² data from similarity search result.
SFit score	Spectral Distance, measure of isotopic fit. Displays the spectral similarity score between the theoretical and the measured isotope pattern as a percentage.
SVOC	Semi Volatile Organic Compound.
TASQ	Target Analysis for Screening and Quantification, proprietary Bruker software used for processing data files obtained from Bruker mass spectrometers.

A Target Screening and Identification of Polar Organic Compounds in Raw and Potable Waters by high performance liquid chromatography high resolution quadrupole time of flight mass spectrometry (HPLC-HRMS-QTOF)

A1 Performance characteristics of the method

A1.1 Substances determined

Compounds included in databases curated with their masses, molecular adduct ions, retention times and characteristic fragment ions.

A1.2 Type of sample

Raw waters, drinking waters, process waters and passive sampling extracts.

A1.3 Basis of method

Samples are either analysed using direct aqueous injection or pre-concentrated using solid phase extraction prior to analysis using liquid chromatography with a time-of-flight mass spectrometric detection.

A1.4 Range of application

Typically, up to 10 µgL⁻¹. Where reference standards are available, semi-quantitative concentrations can be obtained from this method.

A1.5 Standard deviation

Data not yet available.

A1.6 Limit of detection

Typically, 0.01 - 0.10 µgL⁻¹, based on low level standard solutions. Lower detection limits can be obtained from sample pre-concentration.

A1.7 Bias

Data not yet available.

A2 Principle

Aqueous samples are either run by direct aqueous introduction (after the addition of mass labelled internal standards) or pre-concentrated using solid phase extraction disks. Solvent extracts obtained from disks are evaporated and reconstituted into water/methanol. Mass labelled internal standards are added prior to injection onto the analytical system. The resulting extracts are analysed by high performance liquid chromatography / high resolution quadrupole time of flight mass spectrometry (HPLC-HRMS-QTOF). Analytes are identified using Bruker TASQ software which scores compounds contained in the databases against

the following criteria: comparison to known chromatographic retention times, accurate mass, isotope patterns and fragment ions.

The level of confidence associated with this method is 1. i.e., confirmed structure by reference standard. (Schymanski et al, 2014).

A3 Interferences

Any compound that has a similar chromatographic retention time, similar mass, overlapping isotope masses and fragment ions to that of a target compound contained in the database may interfere. Co-eluting matrix components may cause signal suppression or enhancement. As data independent MS/MS mode is utilised with this method, the use of mass labelled standards which incorporate ¹³C, (and/or ¹⁵N and ¹⁸O) atoms within the backbone of the labelled compound is strongly recommended as they typically lead to mass fragments that differ in mass to those obtained from native compounds. (This should, however, be confirmed by running the mass labelled standards using the screening method). This prevents misidentification between the target compound and internal standard. An improved ability to compensate for ion suppression effects should also be observed when the ¹³C labelled internal standards are used.

A4 Hazards

An appropriate risk assessment suitable for use must be undertaken prior to using the method. Staff engaged in the process must be suitably trained. Staff should also wear appropriate personal protection equipment / clothing. e.g. lab coat, safety spectacles. Solvent extractions should be undertaken in a fume hood. Skin contact or inhalation of all reagents and their solutions specified in this method should be avoided. Methanol and acetonitrile are toxic and flammable; these solvents should be handled away from sources of ignition. Formic acid is corrosive.

A5 Reagents

All reagents should be of sufficient purity that they do not give rise to interferences during the analysis and distilled, deionised or similar grade water should be used throughout.

All organic solvents should be of a very high purity (e.g. LCMS grade or chromatography grade).

All reagents should be tested for absence of target compounds by running appropriate procedural extraction and reagent blanks alongside samples.

- A5.1** Water, LCMS grade, or ultra-high purity (UHP) grade.
- A5.2** Acetonitrile, LCMS grade.
- A5.3** Methanol, LCMS grade.
- A5.4** Sodium hydroxide, pellets, ≥97.0% purity.
- A5.5** Hydrochloric acid, density 1.18 g/cm³ (Fisher Optima grade or equivalent)
- A5.6** Acetone, LCMS grade.
- A5.7** Propan-2-ol, LCMS grade.

- A5.8** Acetic acid, LCMS grade.
- A5.9** Formic acid, LCMS grade, $\geq 99\%$.
- A5.10** Ammonia solution, HPLC grade, 32%.
- A5.11** Ammonium formate, LCMS grade.
- A5.12** Ammonium acetate, LCMS grade.
- A5.13** Sodium formate, analytical grade, $>99\%$.
- A5.14** 5% v/v methanol in water

Add 50 mL of methanol to 950 mL of water in a 1000 mL glass volumetric flask and mix well. Smaller volumes can be used based on sample numbers. This solution is stable for a month.

A5.15 10% v/v methanol in water

Add 100 mL of methanol to 900 mL of water in a 1000 mL glass volumetric flask and mix well. Smaller volumes can be used based on sample numbers. This solution is stable for a month.

A5.16 (1:1 v/v) Acetonitrile:Methanol

Add 500 mL of acetonitrile to 500 mL of Methanol in a 1000 mL glass volumetric flask and mix well. Smaller volumes can be used based on sample numbers. This solution is stable for a month.

A5.17 2% Formic acid in (1:1 v/v) Acetonitrile:Methanol

Add 20 ml of formic acid to 980 mL of (1:1 v/v) Acetonitrile:Methanol in a 1000 mL glass volumetric flask and mix well. Smaller volumes can be used based on sample numbers. This solution is stable for a month.

A5.18 2% Ammonia solution in (1:1 v/v) Acetonitrile:Methanol

Add 20 mL of ammonia solution to 980 mL of (1:1 v/v) Acetonitrile:Methanol in a 1000 mL glass volumetric flask. Smaller volumes can be used based on sample numbers. This solution is stable for a month.

A5.19 LC Buffers

The required buffers can be weighed and measured for each eluent batch separately, but for easier eluent preparation and best reproducibility of retention times the following procedures can be used.

Buffer for Positive Ion Mode

Prepare the buffer concentrate using the following procedure to generate a concentrated buffer solution containing 2.5M ammonium formate and 5% formic acid. Avoid contact of the buffer concentrate with all plastic material to prevent

contamination with leachables (e.g. plasticizers, erucamide, non-ionic detergents etc)

- A5.19.1** Add 20 mL of methanol to a clean solvent rinsed 100mL glass volumetric flask followed by 5 mL of formic acid.
- A5.19.2** Add 15.771 g of ammonium formate (correct value for purity if necessary) to a clean solvent rinsed 100 mL glass beaker. Add 50 mL of water and gently agitate the beaker until the ammonium formate is completely dissolved. Transfer the solution to the 100mL volumetric flask carefully rinsing the glass beaker with small portions of water and transferring to the volumetric flask.
- A5.19.3** Stopper the volumetric flask and shake well to mix all components. Fill the volumetric flask with water to the 100 mL mark. Shake well.
- A5.19.4** For storage, transfer the buffer concentrate to a clean solvent rinsed screw cap 100 mL glass bottle. Store the buffer concentrate in a refrigerator at $3 \pm 2^{\circ}\text{C}$. This solution is stable for six months.

A5.20 Mobile Phase (Positive Ion Mode)

The following eluents are prepared using the buffer for positive ion mode:

A5.20.1 Eluent B

Methanol with 5mM ammonium formate and 0.01% formic acid

To 1 L (792 g) of methanol in a clean high purity borosilicate glass bottle add 2 x 1 mL of the buffer for positive ion mode. Close the bottle and swirl thoroughly to mix. This solution is stable for two weeks.

A5.20.2 Eluent A

Water/methanol (90:10) with 5mM ammonium formate and 0.01% formic acid.

To 900 ml (900 g) of water in a clean high purity borosilicate glass bottle add 2 x 900 μL (= 1.8 mL) buffer for positive ion mode. Add 100 mL (79.1 g) of Eluent B to the glass bottle. Close the bottle and swirl to mix thoroughly. This solution is stable for two weeks.

A5.21 Buffer for Negative Ion Mode

Prepare the buffer concentrate using the following procedure to generate a concentrated buffer solution containing 2.5M ammonium acetate. Avoid contact of the buffer concentrate with all plastic material to prevent contamination with leachables (e.g. plasticizers, erucamide, non-ionic detergents etc.)

- A5.21.1** Add 20 mL of methanol to a clean solvent rinsed 100 mL glass volumetric flask.
- A5.21.2** Weigh 19.270 g of ammonium acetate (correct value for purity if necessary) to a clean solvent rinsed 100 ml glass beaker. Add 50 ml of water and gently agitate the beaker until the ammonium acetate is completely dissolved. Transfer the solution to the 100 ml volumetric flask carefully rinsing the glass beaker with small portions of water and transferring to the volumetric flask.
- A5.21.3** Stopper the volumetric flask and shake well to mix all components. Fill the volumetric flask with water to the 100 mL mark. Shake well and check again that it's up to the mark.
- A5.21.4** For storage, transfer the buffer concentrate to a clean solvent rinsed screw cap 100 mL glass bottle. Store the buffer concentrate in a refrigerator at 3 ± 2 °C. This solution is stable for six months.

A5.22 Mobile Phase (Negative Ion Mode)

The following eluents are prepared using the buffer for negative ion mode:

A5.22.1 Eluent D

Methanol with 5 mM ammonium acetate

To 1 L (792 g) of methanol in a clean high purity borosilicate glass bottle add 2 x 1 mL of the buffer for negative ion mode. Close the bottle and swirl thoroughly to mix. This solution is stable for two weeks.

A.5.22.2 Eluent C

Water/methanol (90:10) with 5 mM ammonium acetate

To 900 ml (900 g) of water in a clean high purity borosilicate glass bottle add 2 x 900 μ L (= 1.8 mL) buffer for negative ion mode. Add 100 mL (79.1 g) of Eluent D to the glass bottle. Close the bottle and swirl to mix thoroughly. This solution is stable for two weeks.

A5.23 Mixed pesticide stock solutions, 50 mgL⁻¹

This standard is bought in as a mix (from Agilent Technologies) containing 316 compound pesticide reference material kit supplied in 14 glass ampoules. Compounds present in the kit are listed in Table 1, Appendix 1. See certificate of analysis for shelf life. Similar pesticide reference material mixes are available from other manufacturers.

PSM-105	Components:
PSM-105-A	GC Pesticide Standard no. 1 (1 x 1 mL)

PSM-105-B	GC Pesticide Standard no. 2 (1 x 1 mL)
PSM-105-C	LC Pesticide Standard no. 1 (1 x 1 mL)
PSM-105-D	LC Pesticide Standard no. 2 (1 x 1 mL)
PSM-105-E	LC Pesticide Standard no. 3 (1 x 1 mL)
PSM-105-F	LC Pesticide Standard no. 4 (1 x 1 mL)
PSM-105-G	LC Pesticide Standard no. 5 (1 x 1 mL)
PSM-105-H	LC Pesticide Standard no. 6 (1 x 1 mL)
PSM-105-I	LC Pesticide Standard no. 7 (1 x 1 mL)
PSM-105-J	LC Pesticide Standard no. 8 (1 x 1 mL)
PSM-105-K	LC Pesticide Standard no. 9 (1 x 1 mL)
PSM-105-L	LC/GC Pesticide Standard no. 1 (1 x 1 mL)
PSM-105-M	LC/GC Pesticide Standard no. 2 (1 x 1 mL)
PSM-105-N	GC/LC Pesticide Standard no. 1 (1 x 1 mL)

A5.24 Mixed pesticide intermediate solution, 50 µgL⁻¹

Add 25 µL of each standard stock solution into a 25 mL volumetric flask and make to volume with 10% v/v methanol in water. This solution may be stored at 3 ± 2 °C for up to 1 month.

A5.25 Mixed pharmaceutical stock solution, 100 mgL⁻¹

This standard is bought in as a custom mix (from Agilent Technologies) containing 24 compounds supplied in a single glass ampoule. List of compounds in Table 1, Appendix 1. See certificate of analysis for shelf life.

A5.26 Mixed pharmaceutical intermediate solution, 50 µgL⁻¹

Add 25 µL of each standard stock solution into a 50 mL volumetric flask and make to volume with 10% v/v methanol in water. This solution may be stored at 3 ± 2 °C for up to 1 month.

A5.27 Working standard solutions.

Prepare by adding the required amounts of intermediate calibration solution in the table below to the designated volumetric flasks with 10% v/v methanol in water. Transfer to 15 mL glass vials and label. These should be prepared with every batch of samples analysed.

Standard	Mixed intermediate solutions (A5.19 & A5.21 to add in µL)	Volumetric flask volume (mL)	Concentration (µgL ⁻¹)
Cal 1	1000	10	5.0
Cal 2	400	10	2.0
Cal 3	200	10	1.0
Cal 4	100	10	0.5
Cal 5	40	10	0.20
Cal 6	20	10	0.10

A5.28 Internal standard stock solution A

Fluconazole-13C3 and Caffeine-13C3 at 1000 mgL⁻¹ in methanol purchased from Cerilliant. The solution should be stored according to manufacturer's instructions. See certificate of analysis for shelf life.

A5.29 Internal standard stock solution B

Lamotrigine-13C, 15N4 at 500 mgL⁻¹ in methanol purchased from Cerilliant. The solution should be stored according to manufacturer's instructions. See certificate of analysis for shelf life.

A5.30 Internal standard stock solution C

Carbamazepine-13C6, Diazepam-D5, Acetaminophen- D4, at 100 mg L⁻¹ in methanol. Purchased from Cerilliant. The solution should be stored according to manufacturer's instructions. See certificate of analysis for shelf life.

A5.31 Internal standard intermediate solution, 1mgL⁻¹

Partially fill a 25 mL volumetric flask with methanol and add 25 µL of Internal standard stock solution A, 50 µL of Internal standard stock solution B and 250 µL of Internal standard stock solution C. Make up to the mark with methanol. This solution may be stored at 3 ± 2 °C for up to 6 months.

A5.32 Internal standard working solution, 200µgL⁻¹

Add 1000 µL of internal standard intermediate solution into a 5 mL volumetric flask and make to volume with methanol. This solution may be stored at 3 ± 2 °C for up to 1 month.

A5.33 Blank

A procedural blank is analysed with every batch of samples. 1000 mL of a suitable bottled or tap water is transferred to a labelled 1 Litre clear glass bottle. An aliquot of 500 mL is used for extraction. The blank should be prepared on the day of use.

A6 Apparatus

In addition to normal laboratory glassware and apparatus the following may be required.

A6.1 Glass sample bottles

1 L borosilicate glass bottle with PTFE-lined screw plastic cap. Glass bottles must be previously cleaned in a laboratory dishwasher with the standard wash cycle.

Clean bottles must then be heated in a muffle furnace at 400 °C for one hour prior to storage and use.

A6.2 Stainless steel vacuum manifold

The vacuum manifold can be obtained from UCT (UK distributors are Chromatography Direct) Similar vacuum manifolds are available from other manufacturers. Glass disk holders and reservoirs are required in addition to the steel manifold. The manifold is available as a 3 or 6 position system. For disk elution, Restek Diskcover-47 disk holders and reservoirs (or equivalent) are used.

A6.3 Vacuum pump and tubing

A6.4 Large (5 L) glass reservoir to hold waste solvents following disk conditioning and extracted water samples.

A6.5 Horizon Technologies HLB 47 mm Extraction disks (obtained from Biotage) or equivalent.

A6.6 Refrigerator and freezer, set 1-5 °C and less than -17 °C respectively with valid calibration status.

A6.7 Analytical Balance four decimal place for preparation of buffers and standards.

A6.8 Top Pan Balance, two decimal place for preparation of buffers.

A6.9 Measuring cylinders, various sizes.

A6.10 Micro-syringes, glass and/or micro pipettes with valid calibration status, various sizes.

A6.11 15 mL glass vials.

A6.12 40 mL clear glass vials and caps with PTFE lined septa for sample extraction.

A6.13 Flasks, volumetric, glass, various sizes.

A6.14 Pasteur pipettes, glass, disposable.

A6.15 Auto-sampler silanized vials, clear glass, 2 mL capacity screw capped.

These are used as silanization significantly reduces adsorption of compounds onto the surface of glass vials.

A6.16 Bruker Impact II Q-TOF Mass Spectrometer fitted with Bruker 'Apollo' electrospray source.

- A6.17** Dionex Ultimate 3000 UHPLC system consisting of a vacuum degasser, binary Pump, auto-sampler (25 µL loop and 10 µL mixer), and thermostated column compartment.
- A6.18** Dionex Acclaim RSLC 120 C18 analytical column (2.1 i.d. × 100 mm length, 2.2 µm particle size).
- A6.19** Waters VanGuard, Acquity UPLC BEH C18 1.7 µm, guard column.
- A6.20** Genevac 'Rocket' benchtop centrifugal vacuum rotary evaporator, with holders for 60 mL glass vials.

A7 Sample collection and preservation

Samples should be stored in a refrigerator at 3 ± 2 °C.

To limit sample and compound degradation samples should be extracted as soon as possible after sampling or frozen as soon as possible if extraction is not to be performed.

A8 Analytical procedure

A volume of 500 mL of river water sample is typically used for extraction. Check the pH of water samples and if outside the range 5.5-7.5 adjust to 7.0 with 1 M hydrochloric acid (HCl) or 1 M sodium hydroxide (NaOH) prior to SPE. If high levels of contaminants are expected (e.g. coloured sample, information from customer etc) then the sample can be analysed as is or diluted with UHP water.

- A8.1** Remove the sample from the cold room and invert to mix. If high levels of contaminants are expected (e.g. effluents), then transfer 1mL of sample or diluted sample to a silanized vial and cap and add 25 µL of IS to sample and shake to mix.
- A8.2** If the sample requires pre-concentration (e.g. clean freshwater) then the sample is extracted using solid phase extraction using the following procedure. Add 25 µL of IS to extract after pre-concentration and shake to mix.
- A8.3** Assemble the vacuum manifold and place a 47 mm Atlantic HLB-L extraction disk on the vacuum extraction assembly ready for conditioning.
- A8.4** Conditioning of the HLB extraction disk prior to sample extraction.
- A8.5** Open the valve on the vacuum manifold system and add 10 mL acetone to the reservoir. Apply vacuum until disk is visibly dry. Release vacuum.
- A8.6** Apply 10 mL isopropanol to the reservoir and apply vacuum until disk is visibly dry assuring no solvent remain on the disk. Release vacuum.

- A8.7** Apply 10 mL methanol to the reservoir and allow approximately half of the methanol to percolate through the disk under gravity. Close the tap and allow the disk to soak for approximately one minute. Open the tap and allow the methanol to just enter the disk, leaving a thin layer of methanol on the disk.
- A8.8** Immediately apply 10 mL of ultra-high purity water and apply slight vacuum until a thin layer of water remains on the disk. Close the tap. Do not allow the disk to dry during disk conditioning.
- A8.9** Immediately add 500 mL of the sample and apply vacuum so that the flow rate through the disk is approximately 20 mLmin⁻¹ adjusting the vacuum as necessary during the extraction to maintain this flow rate. Draw the sample through the disk until the meniscus just enters the disk and then release vacuum.
- A8.10** Once the entire water sample has been extracted, the disk is washed with 3 mL of 5% methanol in water and a slight vacuum applied to pull the wash solvent slowly through the disk. The disk is then dried for about 60 min by pulling air through it at full vacuum.
- A8.11** Remove the filtration assembly from the filter flask or manifold, discard the water sample. If the disk has reached complete dryness place an appropriately sized sample collection tube (e.g. 40 mL) into the filter flask or manifold. If the disks have not reached complete dryness after 60 min then place disks on clean aluminium foil in a room free of dust and a low background concentration of target analytes. room for 16 hours (overnight) to air dry. A procedural blank can be used to determine if the room is suitable for drying the disks.
- A8.12** The disk is then eluted under gravity flow in the following order rinsing the sides of the reservoir in the process. A slight vacuum may be applied to aid the flow of the first elution solvent through the disk. Once flow is established the vacuum should be removed.
- A8.13** Elute twice with 3 mL of Acetonitrile:Methanol.
- A8.14** Elute twice with 3 mL mixture of Acetonitrile:Methanol (1:1 v/v) acidified with concentrated 2% formic acid.
- A8.15** Finally, elute twice with 3 mL of the mixture of Acetonitrile:Methanol (1:1 v/v) basified with 2% ammonia solution.
- A8.16** After elution the extracts are pooled, placed in a 60 mL glass tube and evaporated using a centrifugal vacuum rotary evaporator set to the low boiling point method (Genevac Rocket) to an end point of ~0.5 mL. Transfer the extract to a screw cap silanized vial and evaporate under a gentle flow of nitrogen gas until dryness is just achieved. Add 1000 µL of 10% methanol in water to the vial, cap, shake and allow to stand for 2 minutes. Store in laboratory freezer at less than -17 °C until analysis unless analysis is to commence within 24 hours when the sample can be stored refrigerated at 3 ± 2 °C.

A8.17 Prior to instrumental analysis completely thaw the sample extract and procedural blank extracts (if frozen) for approximately 1 hour and add 25 μL of internal standard working solution. Re-cap the vial and shake briefly for 10-20 seconds.

A8.18 Add 25 μL of internal standard working solution to 1000 μL of each extract in a screw cap silanized vial and mix well. Analyse the sample extracts and standards as described below.

A9 Analysis of extracts using LCMS Q-TOF

A9.1 Typical Operating Conditions for the Liquid Chromatograph

HPLC ACQUISITION PARAMETERS				
HPLC system	Dionex Ultimate 3000 UHPLC system consisting of a vacuum degasser, binary Pump, auto-sampler and thermostated column compartment			
Analytical Column	Dionex Acclaim RSLC 120 C ₁₈ analytical column (2.1 i.d. \times 100 mm length, 2.2 μm particle size)			
Guard Column	Waters VanGuard, Acquity UPLC BEH C ₁₈ 1.7 μm			
Column Temperature	30 $^{\circ}\text{C}$			
Injection volume	25 μL			
Mobile Phase A (positive ion)	Water/Methanol (90:10) with 5 mM ammonium formate and 0.01% formic acid			
Mobile Phase B (positive ion)	Methanol with 5 mM ammonium formate and 0.01% formic acid			
Mobile Phase A (negative ion)	Water/Methanol (90:10) with 5 mM ammonium acetate			
Mobile Phase B (negative ion)	Methanol with 5 mM ammonium acetate			
Gradient Program Timetable (positive and negative ion modes)				
	Time (minutes)	A (%)	B (%)	Flow rate (mL min ⁻¹)
Initial	0.00	99.0	1.0	0.200
1	3.0	61.0	39.0	0.200
2	14.0	0.10	99.9	0.400
3	16.0	0.10	99.9	0.480
4	16.1	99.0	1.0	0.480
5	19.0	99.0	1.0	0.480
6	19.1	99.0	1.0	0.200
7	20.0	99.0	1.0	0.200

A9.2 Typical Operating Conditions for the Bruker Mass Spectrometer

MS acquisition parameters (Positive Ion)	
Ionisation source	Electrospray
Drying and nebuliser gas	Nitrogen
Drying gas temperature	200 °C
Drying gas flow	8 L min ⁻¹
Nebulizer pressure	2 bar
Capillary voltage	2500 V (polarity automatically set by instrument)
End plate offset	500 V (polarity automatically set by instrument)
Mass range	30–1000 Da
Scan rate	2 Hz
Collision energy (low) MS	6 eV
Collision energy (high) MS/MS	30 eV (ramped 24-36 eV)

MS acquisition parameters (Negative Ion)	
Acquisition parameters	Electrospray
Drying and nebuliser gas	Nitrogen
Drying gas temperature	200 °C
Drying gas flow	8 L min ⁻¹
Nebulizer pressure	2 bar
Capillary voltage	2500 V (polarity automatically set by instrument)
End plate offset	500 V (polarity automatically set by instrument)
Mass range	30–1000 Da
Scan rate	2 Hz
Collision energy (low) MS	6 eV
Collision energy (high) MS/MS	30 eV (ramped 24-36 eV)

A9.3 Mass axis calibration

Prior to data acquisition, sodium formate calibrant, comprised of a mixture of 1 mM sodium formate in water/propan-2-ol/formic acid (1:1:0.01 v/v/v), is infused from a syringe pump at a flow rate of 180 µL/hr into the electrospray source.

After a stabilisation period of approx. 15 ± 5 min a manual mass calibration is performed. Calibration of the acquired sample data files is performed using the high-precision calibration (HPC) algorithm of the instrument. A standard deviation value of 0.5ppm or less (across the method mass range) indicates that the instrument is ready for data acquisition.

A SD value of >0.5 ppm indicates that the calibration failed and needs to be repeated. If it continues to fail, this may be due to contamination of the calibrant or the ion source which can lead to erroneous masses appearing in the mass spectrum resulting in poor calibration. Cleaning of the ion source or preparing a fresh calibrant solution will in most cases result in a successful mass axis calibration.

Calibrant is also introduced at the beginning and end of every sample chromatogram using the same conditions as mentioned above. This occurs between 0.1-0.8 minutes and 19.0-20

minutes when target analytes are not eluting. Mass calibration of the acquired sample data files is performed when processing data files via TASQ software.

A9.4 System suitability check

The working standard solution Cal 5 (0.2 µg/L) (A5.26), analysed with each batch of sample extracts, is used to perform a system suitability check. Individual compounds together with their acceptance criteria, are listed in the following tables.

(M+H+1) + or (M+H+2) + for halogenated compounds must be present in all compounds (including the mass labelled internal standards) listed in the following three tables.

Table 1A Pharmaceuticals

Compound	Maximum Mass Error (ppm)	Maximum mSigma value	Minimum Signal to Noise (S/N) ratio	Minimum no. of fragment ions to be identified in Cal 5 standard	Maximum RT deviation (+/- minute)
Amitriptyline	5	50	25	1	0.2
Benzoylecgonine	5	50	50	1	0.2
Citalopram	5	50	50	1	0.2
Sotalol	5	50	50	2	0.2
Trimethoprim	5	50	25	2	0.2

Table 2A Pesticides

Compound	Maximum Mass Error (ppm)	Maximum mSigma value	Minimum Signal to Noise (S/N) ratio	Minimum no. of fragment ions to be identified in Cal 5 standard	Maximum RT deviation (+/- minute)
Azoxystrobin	5	50	50	3	0.2
Diazinon	5	50	50	2	0.2
Ethion	5	50	50	2	0.2
Isopyrazam	5	50	75	2	0.2
Pirimiphos-methyl	5	50	50	2	0.2
Pyributicarb	5	50	50	3	0.2
Pyrifthalid	5	50	50	2	0.2
Tebupirimphos	5	50	50	2	0.2
Trifloxystrobin	5	50	50	1	0.2

Table 3A Mass labelled internal standards

Compound	Maximum Mass Error (ppm)	Maximum mSigma value	Minimum Signal to Noise (S/N) ratio	Maximum RT deviation (+/- minute)
Caffeine 13C3	5	50	200	0.2
Carbamazepine 13C6	5	50	500	0.2
Diazepam D5	5	50	500	0.2
Fluconazole 13C3	5	50	200	0.2
Lamotrigine 13C, 15N4	5	50	700	0.2
Paracetamol D4	5	50	200	0.2

One or more of the above compounds may occasionally fail the system suitability check. This does not necessarily indicate a problem with the mass spectrometer or liquid chromatograph. Non-volatile compounds from previously run samples may deposit on the ion source and result in a background containing ions of a similar or identical mass to those in the tables above. Cleaning of the ion source with a lint free cloth moistened with a 50/50

solution of isopropanol and water will in most cases remove the contamination and reduce the presence of background ions. If many compounds fail the system suitability check, or failures occur on a regular basis, then a deep clean of the entire system may be required which could include replacement of the guard and /or analytical column.

A10 Environmental conditions

The mass accuracy of the QTOF mass spectrometer may be affected by changes in room temperature, therefore temperature stability is very important. The room housing the Bruker Impact QTOF is maintained at 21 ± 2 °C.

A11 Performance data

Not yet available.

A12 Data Analysis - Operation of Bruker TASQ software

Target compounds (~ 2,500 substances) included in a combined database (Bruker pesticide Screener™ 2.1 and Tox Screener™ 2.1) are identified in solvent extracts based on their retention time, mass accuracy, isotopic pattern, and diagnostic MS/MS fragments.

NB: The databases, however, are not exhaustive of all the compounds that could be found in the sample extracts. No attempt is made to further identify such compounds manually using untargeted screening approaches.

The extracted ion chromatograms (EIC's) of all the compounds (i.e. protonated molecular adduct ions, plus those from sodium, ammonium and potassium if present at sufficient intensity) together with their associated fragment ions, are produced automatically using Bruker Target Analysis for Screening and Quantitation (TASQ® 2021b software). The sample EIC's produced are assessed against the following limits for all compounds in the two Bruker databases: ± 5 ppm for mass accuracy, isotopic fit < 250 (expressed as mSigma) and ± 0.3 min for the retention time tolerance.

Full details of the Bruker data analysis workflow employed in Method A is outlined in Appendix A. Other manufacturers of high-resolution accurate mass systems will employ their own data analysis software and workflows.

A12.1 Confirmation criteria for positive identification of analyte

The detection of the molecular adduct ion, its isotope(s) and at least one characteristic fragment ion for each precursor ion should be considered as mandatory for positive identification. If the isotope of the molecular adduct ion is absent, due to low intensity of the molecular adduct ion, then an additional characteristic fragment ion should be considered as mandatory. Manual evaluation of the data should be undertaken where necessary.

A12.2 Reporting of results for Method A.

It is recommended that when reporting results to a customer that a full list of compounds searched for (together with their respective CAS identifiers and semi-quant concentrations if

available) is included clearly identifying those that were present and absent. Typical formats for reporting include CSV, Excel or PDF, the former two being preferred for future search/mining of data for trend analysis.

B The Screening and Identification of target and suspect Polar Organic Compounds in Raw and Potable Waters by Liquid Chromatography Orbitrap HRAM Mass Spectrometry

B1 Performance characteristics of the method

B1.1 Substances determined

Compounds included in databases curated with their masses, molecular adduct ions, retention times and characteristic fragment ions.

B1.2 Type of sample

Surface waters, groundwaters, marine waters, surface waters and WWTW discharges.

B1.3 Basis of method

Samples are analysed using direct aqueous injection.

B1.4 Range of application

Typically, up to 500 ng L⁻¹. The range can be extended by sample dilution.

B1.5 Standard deviation

Data not yet available.

B1.6 Limit of detection

Typically, 5 ng L⁻¹-0.50 ng L⁻¹ Based on low level reference standards

B1.7 Bias

Data not yet available.

B1.8 System Suitability

To ensure adequate instrument performance, selected critical components are monitored from for minimum response and peak shape.

B2 Principle

Aqueous samples are analysed by direct aqueous introduction with in-line Solid Phase Extraction (SPE) coupled to high performance liquid chromatography / high resolution mass spectrometry (HPLC-HRMS). Analytes are identified using Thermo Tracefinder 5.1 and Compound Discoverer 3.3 software, which scores compounds contained in local and online databases against the following criteria: Comparison to chromatographic retention times if known, accurate mass, isotope patterns and fragment ions.

There are five levels of identification confidence based on the available data. These are described in Appendix C.

B3 Interferences

Isomers may be mis-identified, especially where no reference standard for retention time alignment is present and where m/z data cannot be differentiated.

Matrix interferences may cause signal suppression or enhancement. This is more relevant to quantified analysis rather than true compound identification.

B4 Hazards

Skin contact or inhalation of all reagents and their solutions specified in this method should be avoided. Methanol and acetonitrile are toxic and flammable; these solvents should be handled away from sources of ignition. Formic acid is corrosive.

B5 Reagents

All reagents should be of sufficient purity that they do not give rise to interferences during the analysis and distilled, deionised or similar grade water should be used throughout.

B5.1 Water, LCMS grade, or Ultra-high purity (UHP) grade.

B5.2 Acetonitrile, LCMS grade.

B5.3 Methanol, LCMS grade.

B5.4 Propan-2-ol, LCMS grade.

B5.5 Formic acid, LCMS grade, ~98-100% w/v.

B5.6 Ammonium formate, LCMS grade.

B5.7 Ammonium formate solution, 5M.

Dissolve 15.75 g \pm 0.1 g of ammonium formate in approximately 35 mL of water. Transfer to a 50 mL volumetric flask and make up to the mark with water. Mix thoroughly and transfer to an appropriately labelled 50 mL vial. This solution is stable for 6 months.

B5.8 Mobile phases.

B5.8.1 Mobile Phase A

5mM Ammonium Formate, 0.1% Formic Acid, 2% MeOH in Water.

Measure 400mL of water into a 500 mL measuring cylinder. Add 0.5 mL 5M ammonium formate solution and 0.5 mL formic acid. Add 10 mL methanol and make up to 500 mL with water. Transfer to HPLC mobile phase bottle “A” and mix thoroughly. This solution is stable for one month.

B5.8.2 Mobile Phase B

5mM Ammonium Formate, 0.1% Formic Acid, 2% Water in MeOH.

Measure 400mL of methanol into a 500 mL measuring cylinder. Add 0.5 mL 5M ammonium formate solution and 0.5 mL formic acid. Add 10 mL water and make up to 500 mL with methanol. Transfer to HPLC mobile phase bottle “B” and mix thoroughly. This solution is stable for one month.

B5.8.3 Mobile Phase C

0.1% Formic Acid in Water

Measure 400mL of water into a 500 mL measuring cylinder. Add 0.5 mL formic acid. Make up to 500 mL with water. Transfer to HPLC mobile phase bottle “C” and mix thoroughly. This solution is stable for one month.

B5.8.4 Mobile Phase D

0.1% Formic Acid in MeOH

Measure 400 mL of methanol into a 500 mL measuring cylinder. Add 0.5 mL formic acid. Make up to 500 mL with methanol. Transfer to HPLC mobile phase bottle “D” and mix thoroughly. This solution is stable for one month.

B5.9 Primary Internal Standards, 100 mg L⁻¹

These standards are sourced as individual and sub-composite pre-prepared solutions, supplied in glass ampoules or as solid material, and prepared in acetonitrile in the laboratory. The range of internal standards should be chosen to reflect any compound of potential interest.

Typically, for a range of pesticides and compounds of environmental concern isotopically labelled analogues of representative compounds would be selected such as: Diflubenzuron-¹³C₆, ciprofloxacin- D₈, carbamazepine- D₁₀, ranitidine- D₆, deltamethrin- D₅, isoproturon- D₆, diclofenac- D₆, dichlorvos- D₆, atrazine- D₅, simazine- D₁₀, diazinon- D₁₀, estrone- D₂, triclosan- D₃, dicamba- D₃, 2,4-D- D₃, bentazone- D₆, erythromycin-¹³C-D₃, triallate-¹³C-D₆ and mecoprop- D₆.

These solutions should be stored according to manufacturer’s instructions.

NB the isotopically labelled compounds listed above are a guide and may be expanded on or a different selection used.

B5.9.2 Secondary Internal standards, 10 mg L⁻¹

Measure 1mL of each 100 mg L⁻¹ stock solution into 10mL volumetric flasks and make up to volume with acetonitrile. Mix thoroughly and transfer to a labelled bottle or vial. This solution is stored at 3 ± 2 °C and is stable for 6 months.

B5.9.3 Working Composite Internal Standard Spiking Solution, 10 µg L⁻¹

Measure 50 µL of each secondary internal standard into 50 mL volumetric flask. Make up to mark with acetonitrile. Mix thoroughly and transfer to a labelled vial. This solution is stored at 3 ± 2 °C and is stable for 3 months.

The use of internal standards in non-targeted screening is recommended to further enhance the integrity of data in terms of relative response, retention time reference and mass accuracy estimate.

It is advisable to analyse an Analytical Quality Control sample to demonstrate the ongoing performance of the method. A small selection of target compounds from key analytical groups may be selected for this purpose. AQC sample stock standard solutions should be obtained.

B5.10 Blanks

B5.10.1 Procedural Blank

A procedural blank is prepared on receipt of samples by filling a 50 ml polypropylene vial to the fill mark with water.

This blank is stored and analysed identically to a sample.

B5.10.2 Systematic Blank

A systematic blank is prepared when an exclusion list is required to be generated. This is prepared and stored as per the procedural blank with exception that internal standard is not added as per B8.1.3.

B6 Apparatus

In addition to normal laboratory glassware and apparatus the following may be required.

B6.1 Thermo Scientific Q-Exactive Focus high resolution accurate mass analyser.

B6.2 LC System: (Thermo EQUAN Max), Thermo Scientific ultimate 3000 LC system consisting of: LPG Pump, HPG (RS) Pump, column compartment and divert valve.

- B6.3** Centrifuge, typically Thermo Megafuge 16 or equivalent.
- B6.4** Centrifuge tubes.
- B6.5** Refrigerator and freezer, set 1-5 °C and less than -17 °C respectively with valid calibration status.
- B6.6** Analytical Balance four decimal place for preparation of buffers and standards.
- B6.7** Top Pan Balance, two decimal place for preparation of buffers.
- B6.8** Micro-syringes, glass and/or micro pipettes with valid calibration status, various sizes.
- B6.9** 15 mL glass vials.
- B6.10** 40 mL clear glass vials and caps with PTFE lined septa for sample extraction.
- B6.11** Flasks, volumetric, glass, various sizes.
- B6.12** Pasteur pipettes, glass, disposable.
- B6.13** Auto-sampler silanized vials, clear glass, 2 mL capacity screw capped.

B7 Sample collection and preservation

Samples are collected in 50 ml polypropylene vials. Samples should be stored at 3 ± 2 °C prior to analysis. To limit sample and compound degradation samples should be extracted as soon as possible after sampling or frozen as soon as possible if extraction is not to be performed.

B8 Analytical procedure

B8.1 Sample preparation.

- B8.1.1** Remove samples from the cold room or freezer and thaw. Invert to mix.
- B8.1.2** Transfer 9 mL of sample to a centrifuge tube.
- B8.1.3** Add 45 µL of internal standard working solution.
- B8.1.4** Add 950 µL of acetonitrile and cap the tube and mix thoroughly.
- B8.1.5** Centrifuge at 3000 rpm for 20 minutes.
- B8.1.6** Carefully transfer the supernatant liquid to an autosampler vial.
- B8.1.7** Prepare a procedural blank the same way using 9 mL of water and treat and analyse identically to a sample.
- B8.1.8** Prepare a systematic blank with 9 mL of water with only 950 µL acetonitrile treat and analyse identically to all samples as per 8.2.

B8.1.9 Analyse blank and sample extracts in both positive and negative polarity on the instrument using the typical operating conditions and parameters outlined in section B9.

B8.2 Sequence.

Analyse the sample extracts and standards in the following order: -

- Conditioning injections.
- Exclusion list from systematic Blank sample (see section B9.2.1)
- Exclusion list from systematic Blank sample opposite polarity
- Conditioning injections – set up exclusion list masses while this is running
- Blank
- AQC sample
- Samples

B9 Analysis using LCMS Orbitrap

B9.1 Typical Operating Conditions for the Liquid Chromatograph/ Mass Spectrometer

LC Conditions	
Instrument	Dionex Ultimate 3000 UHPLC system consisting of a vacuum degasser, high pressure binary LC pump, low pressure quaternary loading pump, auto-sampler/ on line and thermostated column compartment.
Trapping Column	Hypersil Gold aQ 20 mm x 2.1 mm x 12 µm
Analytical Column	Phenomenex Gemini C18 150 mm x 2 mm x 3 µm fitted with a 0.2 µm pre-column filter.
Column Oven Temperature	25 °C
Mobile Phases	<p>Mobile phase A: 98% Water (B5.1), 2% Methanol (B5.3), 5mM ammonium formate, 0.1% formic Acid (B5.5).</p> <p>Mobile phase B: 98% Methanol, 2% Water (B5.1), 5mM ammonium formate, 0.1% formic Acid.</p> <p>Mobile phase C: 0.1% formic acid (B5.5) in Water (B5.1)</p> <p>Mobile phase D: 0.1% formic acid (B5.5) in methanol (B5.3)</p>
Injection volume	2000 µL

TABLE 1B Loading Pump (Online SPE)

LPG Loading Pump			
Time (min)	Flow (mL min ⁻¹)	%C	%D
0	0.7	98	0

4	0.7	98	0
4.1	0.7	0	100
4.2	5	0	100
4.9	5	0	100
5.0	5	98	2
5.6	0.5	98	2
29.5	0.2	98	2
29.6	0.2	98	2

TABLE 2B Analytical Pump (UHPLC)

HPG Analytical Pump			
Time (min)	Flow (mL min ⁻¹)	%A	%B
0.0	0.3	98	2
5.0	0.3	98	2
10.0	0.3	30	70
17.5	0.3	10	90
18.2	0.3	0	100
24.5	0.3	0	100
26.5	0.3	0	100
26.6	0.3	98	2
29.6	Stop		

TABLE 3B Orbitrap Electrospray Conditions

HESI Source Parameters	
Sheath gas flow rate	40
Aux gas flow rate	10
Sweep gas flow rate	1
Spray voltage (kV)	3.5
Spray current (µA)	-
Capillary temp (°C)	320
S-lens RF level	50.0
Aux gas heater temp (°C)	320

TABLE 4B Optimised HRMS Conditions

Orbitrap Acquisition Settings		
Scan range (Positive and Negative)	Full MS, 100-1000 m/z	DDMS ² – Discovery
Resolution	70,000	17,500
Isolation window	NA	1.0 m/z
(N)CE / Stepped (N)CE	NA	Ce: 15, 30, 45

AGC Target	1e6	2e5
Max IT	200 ms	60 ms
Loop Count	NA	1
Microscans	1	NA
Spectrum data	Profile	Profile
Minimum AGC target	NA	6.00e ³
Dynamic exclusion	NA	10s
Exclude isotopes	NA	On

TABLE 5B Autosampler Settings

Autosampler Settings	
Syringe filling speed	50 $\mu\text{L s}^{-1}$
Filling speed –Wash 1	15 $\mu\text{L s}^{-1}$
Filling speed –Wash 2	10 $\mu\text{L s}^{-1}$
Inject to	LC Vlv 1
Injection Speed	100 $\mu\text{L s}^{-1}$
Pre injection delay	500 ms
Post injection delay	500 ms
Post clean with solvent 1	2
Post clean with solvent 2	2
Valve clean with solvent 1	1
Valve clean with solvent 2	1
Clean Vol	100%
Transfer time	240 s
Elution time	1000 s
Divert Valve Settings	
Position	Time (min)
1-2	0
1-6	2.9
1-2	5.5
1-6	27.0 - End

B9.2 Global Lists

B9.2.1 Exclusion List

Systematic Blank samples are run for each polarity and used to create an exclusion list. Once acquired the raw data file is transferred to Compound Discoverer software and filtered for signals where a DDMS² event has triggered in the blank sample.

This data is exported as a Tracefinder list and imported to the Instrument method as an exclusion list. Detection of listed masses in subsequent runs will not trigger an event. This prevents unnecessary triggering of DDMS² events from systematic sources.

The exclusion list must be generated and applied prior to commencing sample analysis. A new exclusion list must be generated monthly, or if changes have been made to the system e.g., change in solvent/reagent supplier, installation of a new column.

B9.3 Lock Masses

The following reference masses for both polarities are suggested but others may be added or removed. Interferences such as plasticizers, polysiloxanes and clusters from solvents are commonly used.

Polarity	Mass	Comment	Time (min)
Positive	445.12003	Polysiloxane	0-2
Positive	391.28420	Common plasticizer - diisooctyl phthalate	0-2
Negative	112.98550	Trifluoroacetic acid	0-2
Negative	226.97840	Trifluoroacetic acid dimer	0-2

B9.4 Mass alignment calibration

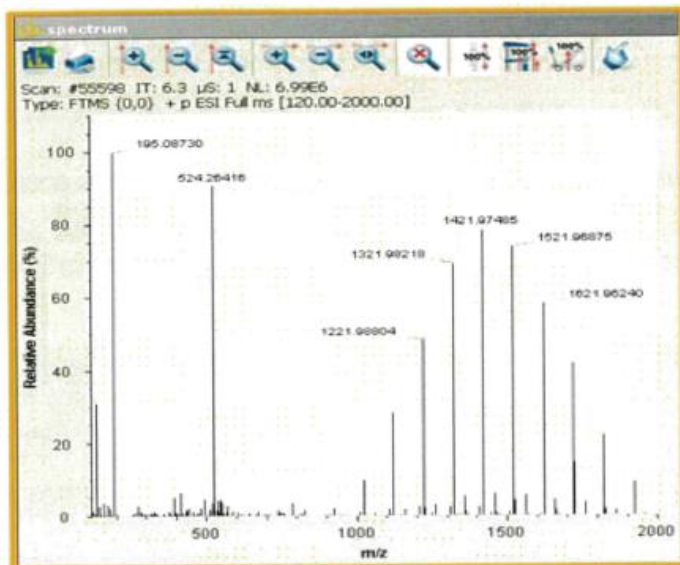
Immediately prior to analysis mass alignment calibrations are performed by infusion of both positive and negative calibration solutions. Orbitrap mass calibration is stable for 24-48 hours. The calibration mixture can be purchased ready to use or prepared in lab.

The compounds or stocks can be purchased from Thermo Fisher Scientific or Sigma.

Positive Mode	Negative mode
Caffeine	Sodium Dodecyl Sulfate
MRFA (L-methionyl-arginyl-phenylalanyl-alanine acetate)	Sodium Taurocholate
Ultramark® 1621	Ultramark® 1621

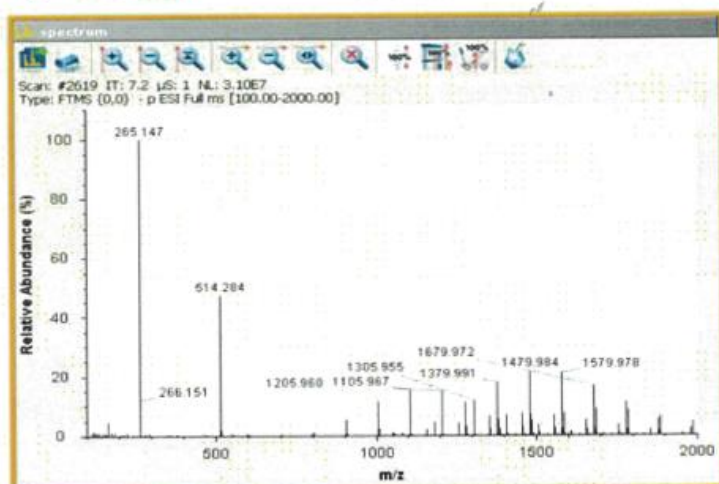
Further details can be found in the Exactive Operations manual.

Positive CalMix



#	m/z
1	138.066190
2	195.087652
3	524.264964
4	1121.997024
5	1221.990636
6	1321.984249
7	1421.977862
8	1521.971475
9	1621.965088
10	1721.958701

Negative CalMix



#	m/z
1	265.147903
2	514.284397
3	1279.997212
4	1379.990825
5	1479.984438
6	1579.978051
7	1679.971664
8	1779.965277

Figure 1B Mass Calibration Mix in both Positive and Negative Modes

B9.5 System Suitability criteria

The working standard solution (20 ng/L) containing 30 compounds spanning both polarities is analysed with each batch of samples. This is used to perform a system suitability check. Individual compounds together with their acceptance criteria, are listed in the following tables.

Table 6B System Suitability criteria +ve ion

Compound	Min Peak area	Mass accuracy (m/z delta)	Max RT deviation (RT delta)	Min isotopes match	Min Fragment ions
Erythromycin	>1,000,000	< ± 5 ppm	< ± 0.2 min	2	1
Omethoate	>300,000	< ± 5 ppm	< ± 0.2 min	1	n/a
Fenazaquin	>10,000,000	< ± 5 ppm	< ± 0.2 min	2	n/a

Table 7B System Suitability criteria -ve ion

Compound	Min Peak area	Mass accuracy (m/z delta)	Max RT deviation (RT delta)	Min isotopes match	Min Fragment ions
Bentazone	>1,000,000	< ± 5 ppm	< ± 0.2 min	2	1
Fipronil	>1,000,000	< ± 5 ppm	< ± 0.2 min	3	n/a

Table 8B System Suitability criteria Internal standard

Compound	Min Peak area	Mass accuracy (m/z delta)	Max RT deviation (RT delta)	Min isotopes match	Min Fragment ions
Erythromycin-13C,d3	>10,000,000	< ± 5 ppm	< ± 0.2 min	2	n/a
Triallate-13C,d6	>1,000,000	< ± 5 ppm	< ± 0.2 min	2	n/a
Mecoprop-d6	>1,000,000	< ± 5 ppm	< ± 0.2 min	2	n/a

B10 Environmental conditions

The room where the LC-HRAM is placed the temperature should be controlled at 21 ± 2 °C.

The autosampler has a temperature-controlled compartment for the storage of all vials, blanks, standards and samples that is controlled and preset within the control software.

B11 Performance data

It is possible to validate the procedure by carrying out a matrix spike recovery exercise looking at subsets of target compounds from different chemical groups. Quality assurance integrated within the procedure and instrumental detection capabilities may help negate reporting of false positives and false negatives.

Use of a quality control standard or internal standard can determine if an expected compound is reflected in the features detected, and the integrity of the spectral and chromatographic information is sufficient to identify at a higher level of confidence. Mass accuracy, low signal and matrix complexity can contribute to false positives.

False negatives can be mistaken for false positives if mass deviation is significant enough for the expected compounds features to be listed incorrectly. Incorrect identification may occur at low concentrations where spectral information may be insufficient to enable correct feature grouping, resulting in isotopic and adduct related features not related back to parent compound.

In complex matrices false negatives may occur where sample features cannot be distinguished from matrix and background by mass resolution. The use of second and third line studies can be of great use to further identify false positive and false negative results.

See Appendix B12 for example process.

B12 Data Analysis

Further details on instrument operation referenced in Appendix B.

B12.1 Target and Suspect screening Identification using Tracefinder Software

Targeted and suspect screening can be performed in Tracefinder 5.1. The application uses the mass-to-charge ratio and mass tolerance ranges to identify each compound from a compound database.

The area of the measured sample peak is compared against a pre-defined area threshold. Identification by searching within the specified retention time window and comparison of the measured m/z of the sample peak against the expected m/z of the target compound.

For confirmation the entire raw data file is searched and compares measured m/z against expected m/z within specified mass tolerance range. Fragment ion and isotopic pattern are also used for identification and confirmation with the latter an isotopic pattern score is generated for the best match between measured and expected isotopic patterns of the calculated elemental composition.

Library search can be used for identification and confirmation purposes, library entry with the highest score for the fragment ion spectrum in that library that matches the compound's ion spectrum.

A mass tolerance of 5 ppm, a retention time delta of ± 0.3 min, a minimum isotopic pattern score of 80% and where present a minimum of one fragment ion has been set for identification and confirmation criteria.

B12.2 Suspect and Non targeted screening using Compound Discoverer Software

Due to the clear relationship between precursor and product ions and the resulting improved identification confidence DDMS² is the preferred option for trace applications on orbitrap instrumentation with this capability, data independent acquisition options are more effective at higher levels.

Target compounds are identified based on their retention time, mass accuracy, isotopic pattern and diagnostic MS/MS fragments. A chromatogram for each detected compound is produced automatically using Thermo Compound discoverer software. Each trace is a composite of its related adducts, and the peak area for a compound is the sum of the areas for its related adducts.

The software is used to identify detected compounds based on comparison of generated HRMS, ms^2 , and isotope data against the spectral libraries listed and where no library ms^2 is available, computer modelled expected fragmentation patterns.

- mzCloud (ms^2 data ~ 32,000 compounds)
- mzVault (ms^2 data ~ 16 000 compounds, likely to be mirrored in mzCloud)
- ChemSpider (ms^1 data ~ 100 000 000 compounds)

For positive identification matching algorithms are utilised to perform comparisons with reference ms^2 spectra or computer modelled expected fragmentation patterns. These comparisons produce match scores which must be within acceptable user defined limits.

Default mass tolerance for matching is ± 5 ppm.

Default isotope intensity tolerance = 30%

For relevant parameters e.g. observed isotope ratios, retention time or ms^2 fragmentation pattern, additional visual checks and manual sense checking should be performed.

Where ms^2 data is available and mzCloud/vault library ms^2 data exists, software uses matching algorithm to produce an mzCloud match score.

Where ms^2 data is available but no ms^2 library data exists, software searches mzCloud library for similar substructures and utilises precursor ion fingerprinting to perform comparisons with observed ms^2 spectra and produce a mzLogic score.

Spectral distance compares the observed isotopic pattern against the simulated pattern of a database candidate producing a SFit score. This is used to corroborate mzLogic matches (SFit >80) and prioritise candidates for FISh scoring. SFit is also used where no ms^2 data has been collected to prioritise likelihood of proposed structures. Alone it is insufficient to use for identification.

Where substructure similarity searching of library produces no mzLogic results, FISh scoring can be applied manually. FISh scoring compares observed fragments against in silico modelled ms^2 fragmentation data.

Level of confidence in identification of suspected compounds is based on either comparison with a reference standard or on the matched data source and mzCloud match/mzLogic/SFit/FISh scores obtained.

B12.3 Reporting

For Targeted and suspect compounds processed in Tracefinder, a report can be generated within the software. Select the required report format (may be customized), then select the format from CSV, Excel, PDF etc. For this example, method customer requirements are excel.

Reporting from Compound Discoverer – filter the results producing data that is not background and has been checked in the compounds table. Sort by mzCloud best match confidence and secondly by Area (max). Select create report and required template. The report will include assigned structure and any alternative structure proposals that have been assigned to a chromatographic signal, along with any comments made manually regarding confidence level and classification.

Members assisting with these methods.

Without the good will and support given by these individuals and their respective organisations SCA would not be able to continue and produce the highly valued and respected blue book methods.

Member		Organisation
Ian	Barnabas	Northumbrian Water
Richard	Brown	Independent
Kenny	Burnside	SEPA
Wayne	Civil	Environment Agency
Katherine	Clark	Subadra Consulting
Mark	Collins	Northern Ireland Water
Stuart	Collins	South West Water
Janine	Elliott	SEPA
David	Evans	ALS Environmental
Anna	Fee	Anglian Water
Leo	Firpo	South East Water
Marcus	Foster	ALS Environmental
Sarah	Gledhill	Thames Water
Anthony	Gravell	Cyfoeth Naturiol Cymru
Toni	Hall	Wessex Water
Beverley	Kerrigan	United Utilities
Leonard	McComb	ALS Environmental
Gavin	Mills	Severn Trent Water
Laura	Pinkney	United Utilities
Matthew	Rawlinson	Affinity Water
Sarah	Roberts	UKAS
Melanie	Schumacher	Cyfoeth Naturiol Cymru
Brian	Shields	Northumbrian Water
Kevin	Snaddon	Scottish Water
Catherine	Teehan	Dwr Cymru
Adrian	Thomas	Severn Trent Water
Lee	Thomas	Severn Trent Water
Sam	Towers	ALS Environmental
David	Turnbull	SES
Josie	Willott	South East Water
Shaun	Jones	mua Water

Correspondence

However well procedures may be tested, there is always the possibility of discovering hitherto unknown problems. Analysts with such information are requested to contact the Secretary of the Standing Committee of Analysts:

secretary@standingcommitteeofanalysts.co.uk

Amendment History

Target and Suspect Screening of Polar Organic Compounds in Raw and Potable Waters by Liquid Chromatography - High Resolution Accurate Mass Spectrometry is a new book. Therefore, there isn't an amendment history on this occasion.

Appendices

Appendix A

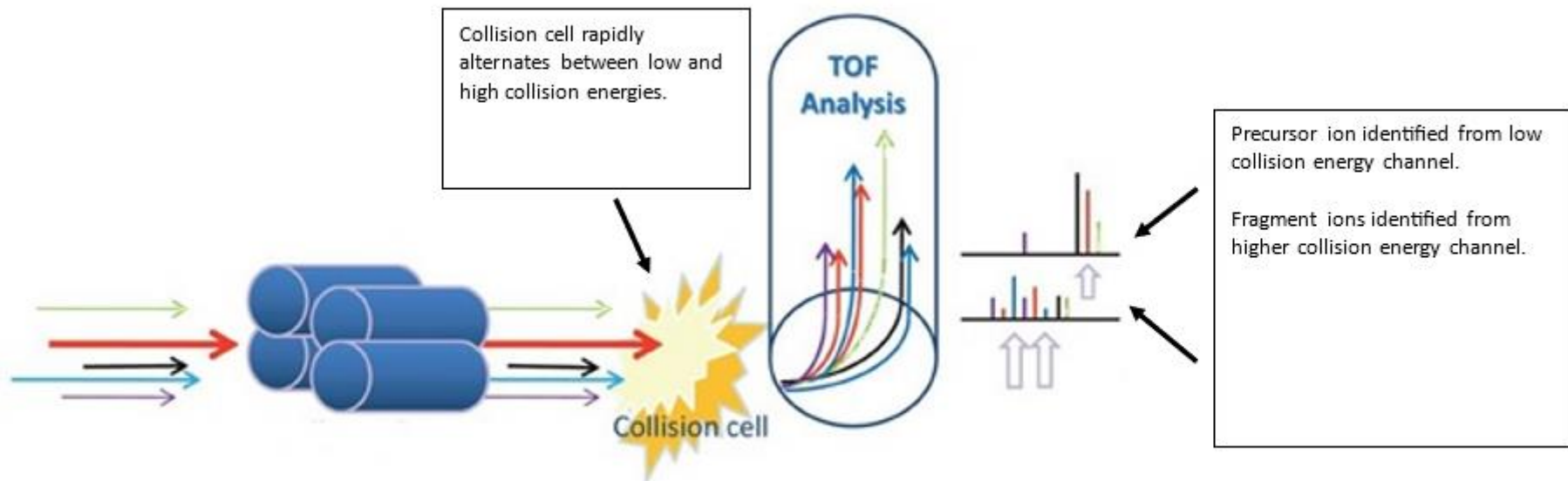


Figure A1 - Process of 'data independent MS/MS acquisition' for a Q-TOF-MS.

Diagram used with permission from Bruker UK Ltd.

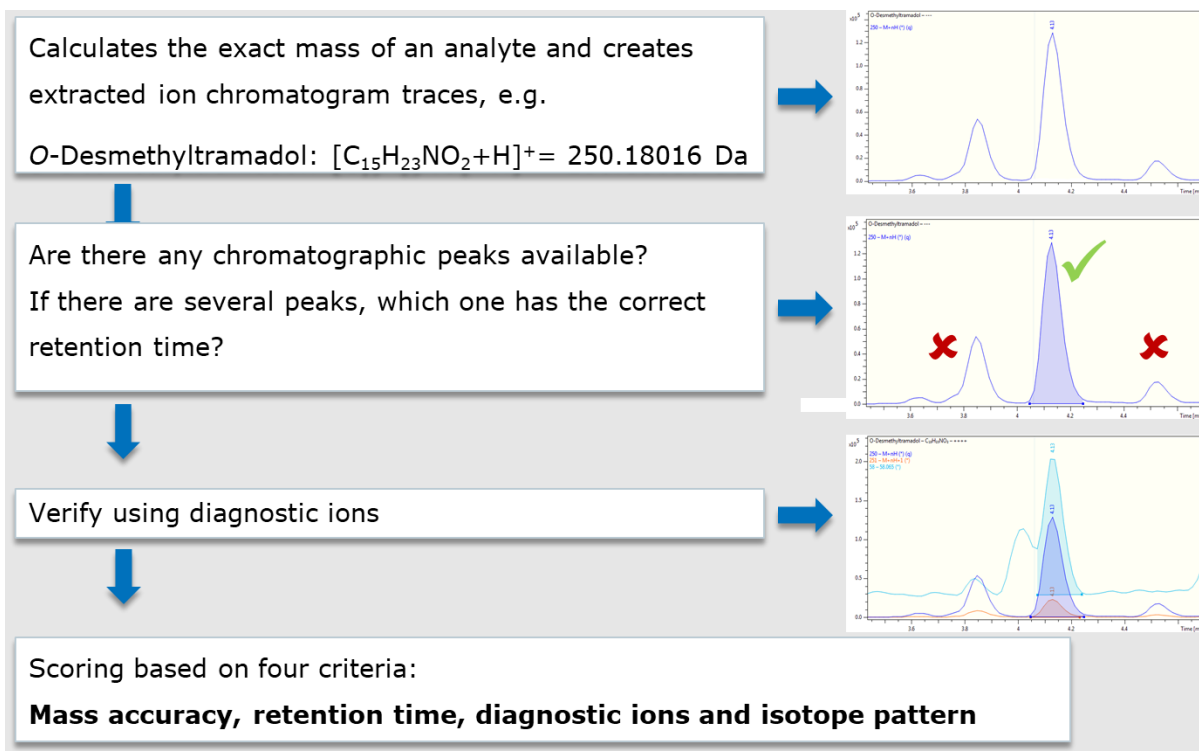


Figure A2 - HR-LC-MS/MS screening workflow – processing of data

Mass accuracy	→	Theoretical: 250.18016 Da (M+H) ⁺ Experimental: 250.18020 Da Δm/z: 0.04 mDa (0.16 ppm)	✓
Retention time	→	Theoretical: 4.06 min Experimental: 4.13 min ΔRT: 0.07 min	✓
Diagnostic ions	→	All diagnostic ions found?	✓
Isotope pattern	→	mSigma: 5.9	✓
Overall score	→	++++ O-Desmethyltramadol	✓

Figure A3 - HR-LC-MS/MS screening workflow – scoring of data

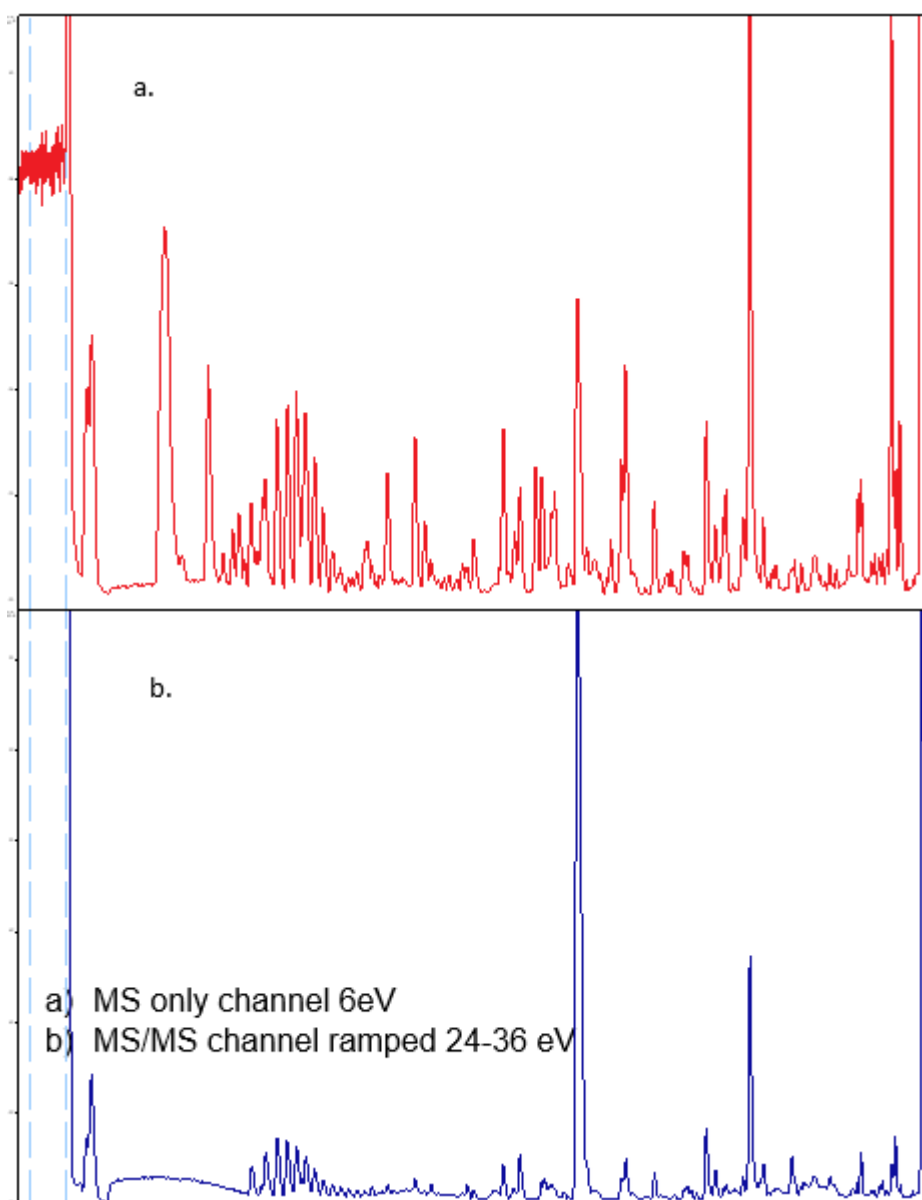


Figure A4 - Example sample chromatogram from BBCID analysis using Bruker Impact II Q-TOF.

Data Analysis Workflow for Bruker TASQ software (section A12 in Method A)

- A12.2** Open TASQ 2021b software and click on the **Batch Management** icon. All batches plus a full list of previously processed batches will appear under **Batch Navigator**.
- A12.3** Click on the **Import Batch** icon and select the directory (folder containing the data files) to be processed. The **specify batch name** will be auto filled with the folder name. Click **Next** and a new window **Select Data Sets for Import** opens allowing the analysts to choose which files are to be processed. Click finish.
- A12.4** Click on **Process Batch**. Load the TASQ data analysis method to process the data files. Click Finish to process the batch.
- A12.5** After processing, the results can be reviewed by clicking on the **Review Screening** icon on the main ribbon as shown in figure A5.
- A12.6** The **Analysis Results** view displays the results for a single analysis (sample) containing all analytes of the batch method. The analysis is selected via the **Select Data Set** list box in the Bruker ribbon as shown in figure A5. The **Analysis Results** table contains columns for mass accuracy, retention time, isotope pattern fit (mSigma) and qualifier ions. The individual EIC's are integrated during processing and peak areas displayed in the table.
- A12.7** The **Batch Results** view displays the results for a single analyte in all samples of a batch. See figure A6 (appendix A). The batch is selected via the **Batch navigator** or the context menu of batch management button in the **Bruker ribbon** at the top of the window. The analyte can be selected via the **Select Analyte** list box in the Bruker ribbon. See figure A6. The **Batch Results** table contains the same columns as the **Analysis Results** table (although the table can be amended to display only the info that the analyst requires) and results for mass accuracy, retention time, isotope pattern fit (mSigma) and qualifier ions are displayed, and the same colour coding used in the identification of the respective compound/analyte. An overall combined 'scoring' matrix displaying the confidence of the identification against the criteria set in the data analysis method is also shown in figure A6. The EIC's including the principal ion and qualifier ions, for each analyte are integrated during processing and peak areas displayed in the table.
- A12.8** An example and detailed EIC displaying the principal ion plus 8 other qualifier/confirmatory ion chromatograms for Atenolol in a standard is shown in figure A7.
- A12.9** An export of batch results for further data processing and reporting is possible and is shown in figure A8. Right click a batch or analysis node in the Batch Navigator and select Export Batch / Analysis to LIMS. Select a root folder by clicking the browse button or dropping a folder in the indicated field. From the drop-down list **select comma separated variable (.csv)** for the format. Check the single file for batch option. Click OK to export the batch data. The remaining options are left at the default

setting. **NB** The CSV file can be read by Microsoft Office Excel or similar spreadsheet applications.

Review Screening icon

Select Data Set list box

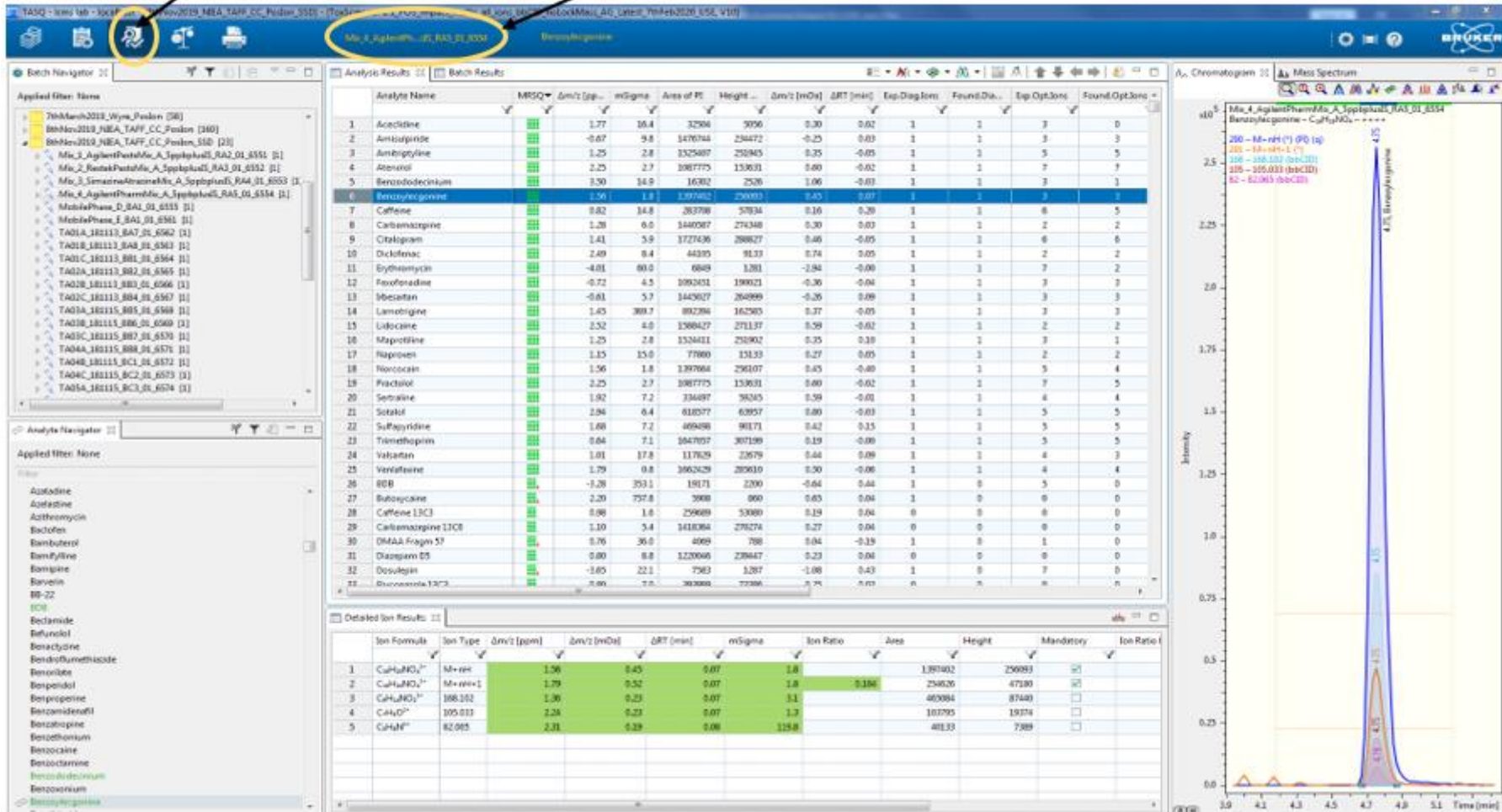


Figure A5 - Review Screening perspective in Analysis Result View

Select Analyte list box

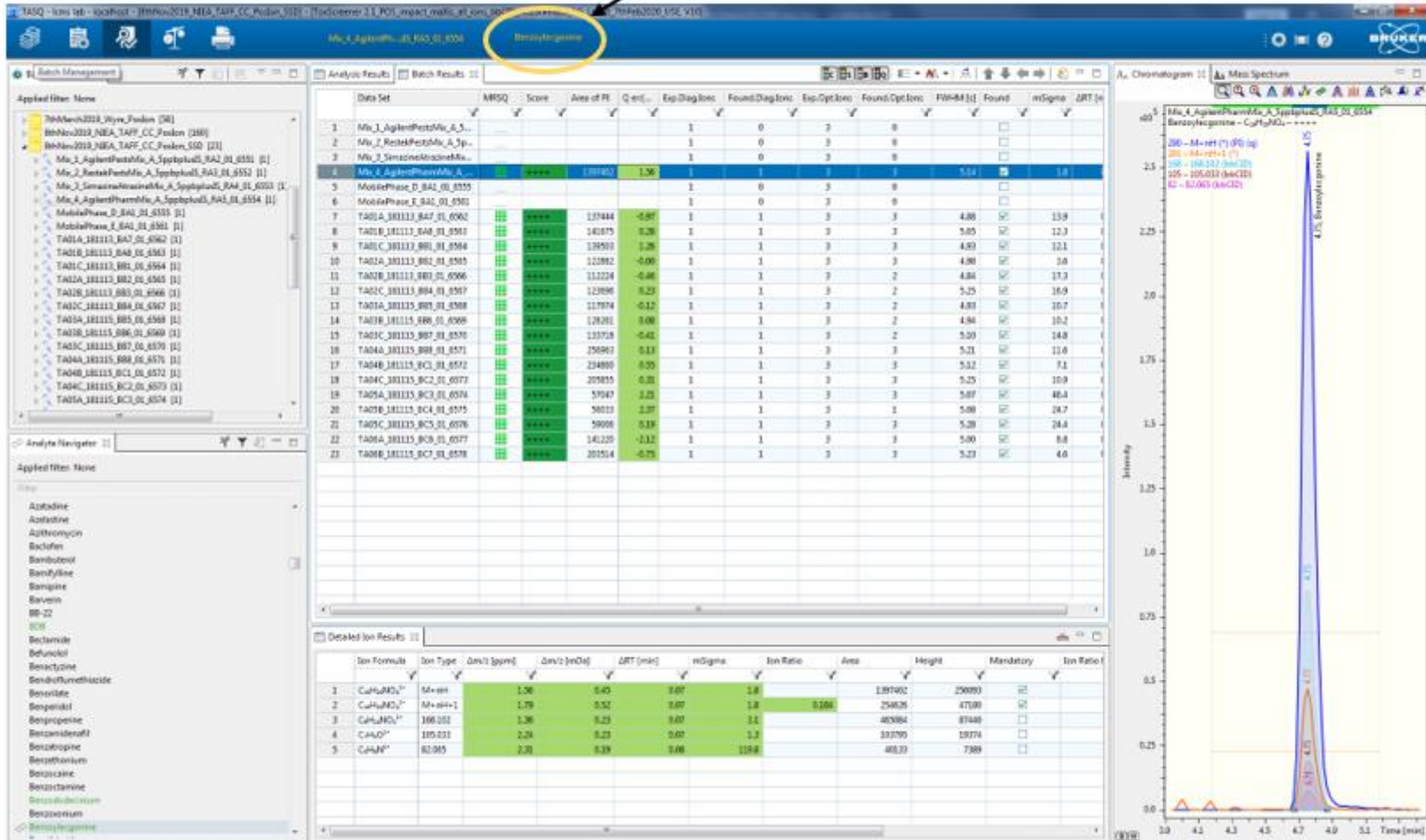


Figure A6 - Review Screening perspective in Batch Result View

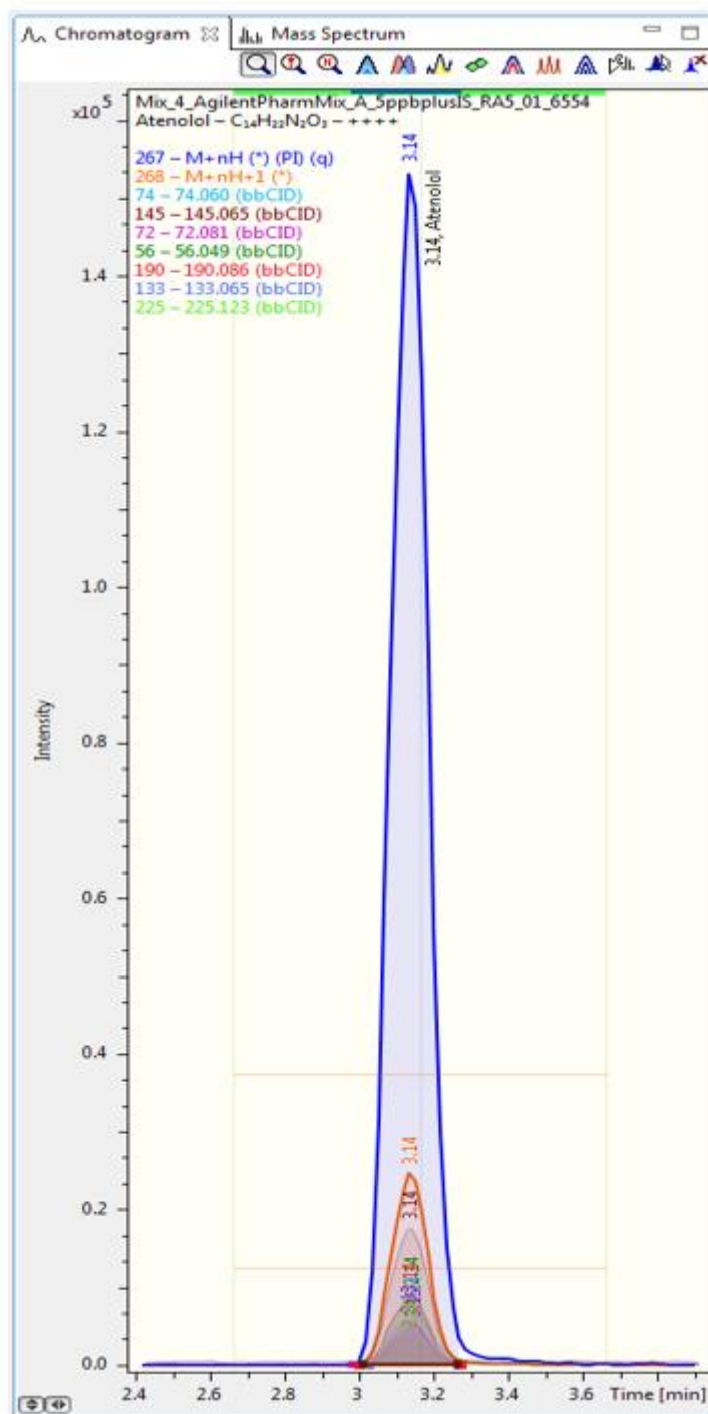


Figure A7 - Example extracted ion chromatograms (EIC) displaying the principal ion plus eight other confirmatory ion chromatograms for Atenolol in a standard.

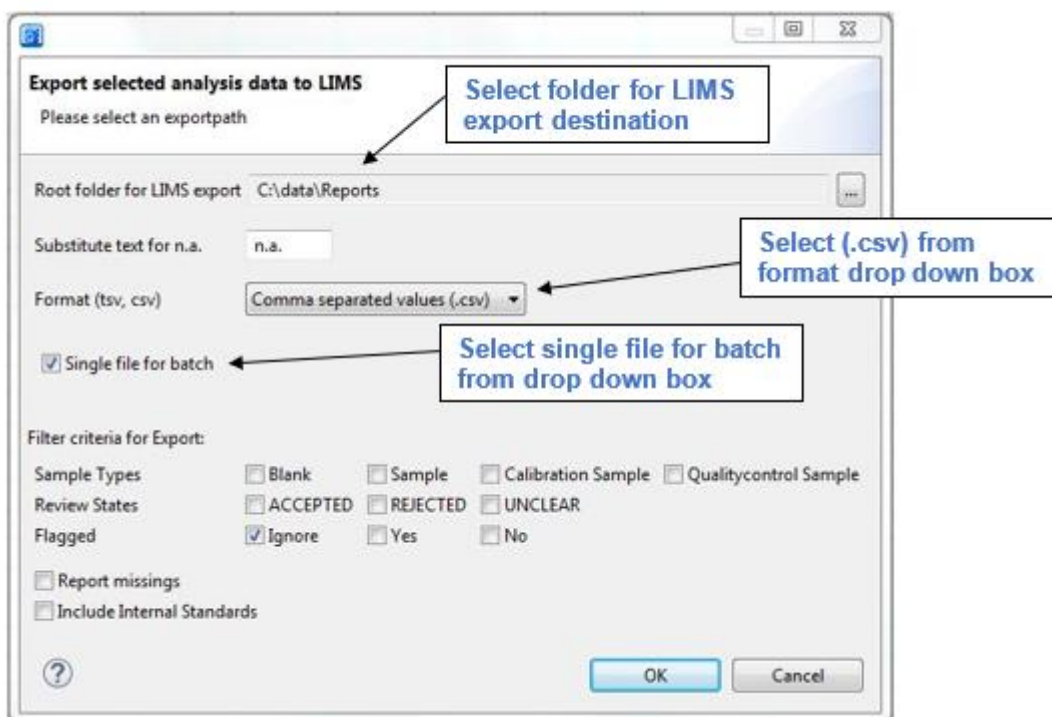


Figure A8 - Export batch data to LIMS dialogue box

Table A1 Compounds present in Agilent Mixes, PSM-105A through to PSM-105-N

PSM-105-A	PSM-105-B	PSM-105-C	PSM-105-D	PSM-105-E	PSM-105-F	PSM-105-G
Endrin	Chlorobenzilate	Myclobutanil	Propoxur	Metamifop	Pyrimisulfan	Ofurace
Bromobutide	Dieldrin	Cymoxanil	Methomyl	Dimethametrin	Thenylchlor	Methiocarb
Cyfluthrin	Bromopropylate	Dimethenamid	Propamocarb	Isopyrazam	Triflumizole	Triflumuron
BHC-Mix	Methoxychlor	Mandipropamid	Trifloxystrobin	Diafenthiuron	Tricyclazole	Monocrotophos
Fipronil	Aldrin	Fenoxaprop ethyl	Imidacloprid	Pyribenzoxim	Bifenazate	Fenobucarb
Disulfoton	Chinomethionate	Propaquizafop	Mepronil	Pyraclostrobin	Pyrimidifen	Omethoate
Fenvalerate	Tefluthrin	Molinate	Clofentezine	Pyraclofos	Tebufenozide	Phoxim
Chlorfenapyr	Procymidone	Bromacil	Metalaxyl	Benfuresate	Cyromazine	Thidiazuron
Captan	Fenitrothion	Fenpyroximate	Thiophanate methyl	Dithiopyr	Thiabendazole	Pencycuron
Indoxacarb	Diphenylamine	Clethodim	Tebufenpyrad	Esprocarb	Terbutylazine	Phosphamidon
Tetradifon	Dichloran	Bitertanol	Paclobutrazol	Pyrifalid	Kresoxim-methyl	Metolcarb
Deltamethrin	Cypermethrin	Buprofezin	Azoxystrobin	Pyributicarb	E-Pyriminobac-methyl	Triazophos
Thifluzamide	Iprodione	Hexythiazox	Oxadixyl	Gibberellic acid	Diphenamid	Profenophos

Lambda-cyhalothrin	Carbophenothion	Etofenprox	Flutolanil	Quinmerac	Mefenacet	Bendiocarb
Endosulfan sulfate	Chlorpyriphos-methyl	Thiamethoxam	Aldicarb	Pentoxazone	Oxadiazon	Methabenzthiazuron
Parathion (ethyl)	Chlorfenvinphos	Pyriproxyfen	Fenazaquin	Dimepiperate	Isoprothiolane	Iprovalicarb
Flucythrinate	Terbufos	Cyazofamid	Pymetrozine	Carpropamid	Pyroquilon	Pirimiphos-methyl
Butachlor	Trifluralin	Benthiavalicarb-isopropyl	Flusilazole	Forchlorfenuron (CPPU)	Metrafenone	Quinalphos
Indanofan	Etridiazole	Methoxyfenozide	Mepanipyrim	Fentrazamide	Furathiocarb	Linuron
Ethalfuralin	Dicofol	Propanil	Flufenacet	Fenoxanil	Napropamide	Phosalone
Permethrins (isomer mix)	Chlorothalonil	Carbaryl	Triadimefon	Daimuron	Flumioxazin	Benfuracarb
DDT-Mix	Ethion	Clomazone	Oxamyl	Cyclosulfamuron	Chromafenozide	Flufenoxuron
Pyridalyl	Diclofop methyl	Ethoxyquin	Carboxin	Fluopicolide	Acibenzolar-s-methyl	Quizalofop-ethyl
Bifenthrin	Isofenphos	Famoxadone	Prochloraz	Cafenstrole	Abamectin	Thiodicarb
Fenpropathrin	Heptachlor	Dimethomorph	Carfentrazone-ethyl		Benzoximate	Malathion
Chlordane (tech)	Zoxamide	Spirodiclofen	Penconazole		Hexazinone	Isoprocab

Anilofos	Triadimenol	Iprobenfos	Pirimicarb		Nuarimol	Vamidothion
Methyl parathion	Oxyfluorfen		Clothianidin		Fenarimol	Piperophos
Fthalide	Tokuthion		Pyrimethanil		Pyridaben	Diethofencarb
Halfenprox	Bifenox				Cyhalofop-butyl	Phenthoate
Tralomethrin	Vinclozolin					Diuron
	Chlorpropham					Thiobencarb
	Mecarbam					Pyrazophos
	Pirimiphos-ethyl					Ethiofencarb
	Pentachloronitrobenzene					

PSM-105-H	PSM-105-I	PSM-105-J	PSM-105-K	PSM-105-L	PSM-105-M	PSM-105-N
Metconazole	Uniconazole	Oxaziclomefone	Metazosulfuron	Picoxystrobin	Pendimethalin	Chlorfluazuron
Etrimfos	Sulfoxaflor	Penoxsulam	Flucetosulfuron	Penthiopyrad	Prometryn	Imazalil
Diniconazole	Pyrazolynate	Tiadinil	Halosulfuron-methyl	Methidathion	Mevinphos	Dichlofluanid
Thiacloprid	Sethoxydim	Fluacrypyrim	Thifensulfuron (methyl)	Simeconazole	Metobromuron	Folpet
EthoproP	Pyrifluquinazon	Fosthiazate	Azimsulfuron	Cyflufenamid	Fenamiphos	EPN

Haloxyfop (free acid)	Dinotefuran	Imibenconazole	Chlorsulfuron	Pyridaphenthion	Simetryn	Lufenuron
Cadusafos	Flonicamid	Inabenfide	Ethoxysulfuron	Fenothiocarb	Phorate	Silafluofen
Diazinon	Amisulbrom	Saflufenacil	Nicosulfuron	Fluopyram	Propiconazole	Fludioxonil
Fluquinconazole	Imicyafos	Demeton-s-methyl	Imazosulfuron	Metolachlor	Terbutryn(e)	Probenazole
Teflubenzuron	Benzobicyclon	Dimethylvinphos	Chlorantraniliprol e	Spiromesifen	Tolclofos-methyl	Tolyfluanid
Dichlorvos	Ethaboxam	Spinetoram	Bensulfuron- methyl	Thiazopyr	Tebupirimphos	Alachlor
Diflubenzuron	Etoxazol	Ametoctradin		Fenamidone	Carbofuran	
Fenhexamid				Cyprodinil	Dimethoate	
Fenthion				Metribuzin		
Cyproconazole				Quinoclamine		
Tetraconazole				Ferimzone		
Tebuconazole				Novaluron		

Appendix B
Orbitrap

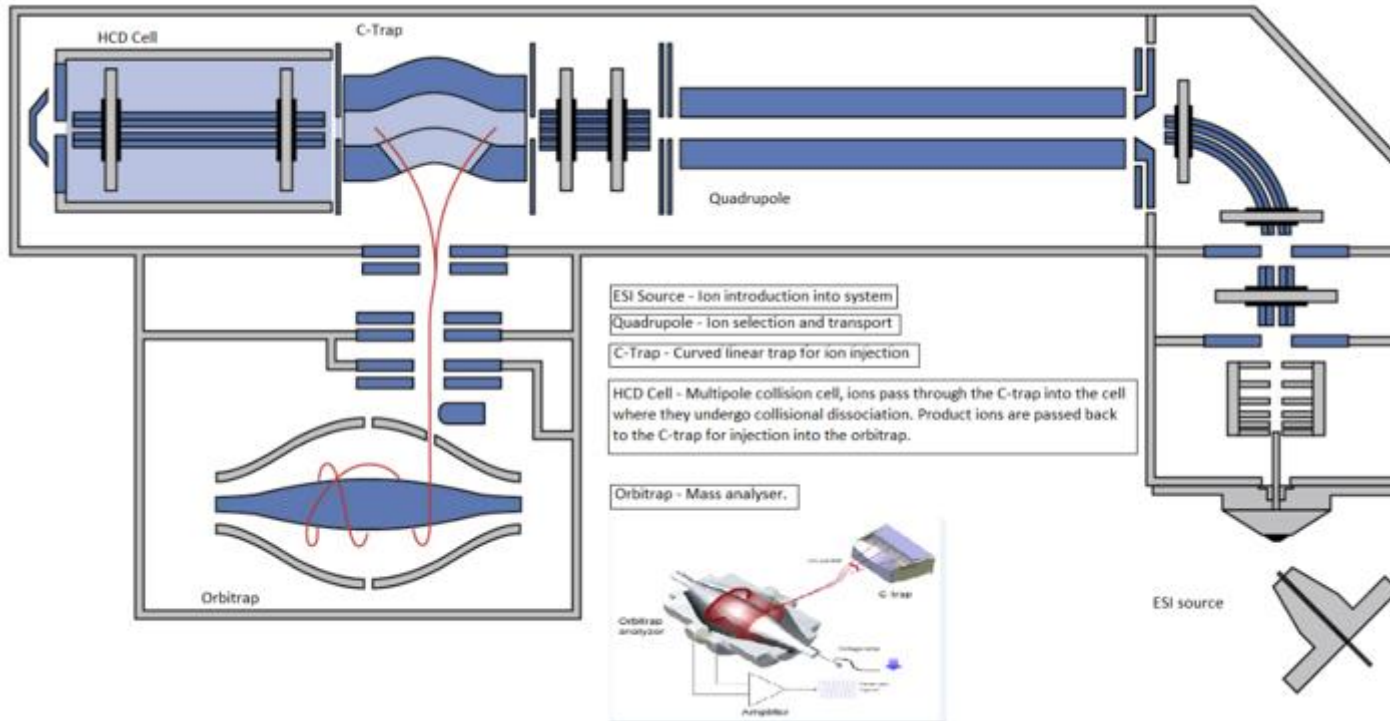


Figure B1 - Schematic of Thermo Scientific Q-Exacte Orbitrap.
 Images used with permission from Thermo Fisher Scientific UK.

B2 Compound Discover Example Workflow

- B12.2.1** Open the compound discoverer application and select “new study and analysis” to open the wizard and name the study.
- B12.2.2** Select the appropriate workflow (in this example “Workflow Templates\Environmental\Environmental w Stats Unknown ID x Online and Local Database searches”)
- B12.2.3** Select the files to be included in the study and set the process blank sample type to “blank”. Where required add relevant study factors to aid interpretation of results produced e.g. Categorical or numerical study factors. NB for routine screening analysis factors are generally not required but may be useful to aid interpretation in some situations e.g. investigative or incident response samples.
- B12.2.4** Where required define ratio to calculate based on study factors e.g. Bulk ratio to define ratios within sample groups that will be useful when interpreting results (upstream vs downstream or inlet vs outlet).
- B12.2.5** Open the workflow tab to edit how data analysis is performed. Most settings should be left at default values with the exception of the following:
- Detect compounds node – default min Peak intensity 500,000/100,00(+/-) may be altered dependant on purpose of analysis or other relevant factors
 - Search ChemSpider – database selection may be altered to either cover additional references or reduce analysis time. Database choice may be influenced by sample type, monitoring purpose, suspected contaminant to name a few. Typical selctions from options Massbank, ACRaR, Cayman, Drugbank, FDA UNII-NCM, EPA TOXcast, EPA DSSTox, EAWAG Biocatalysis Biodegradation.

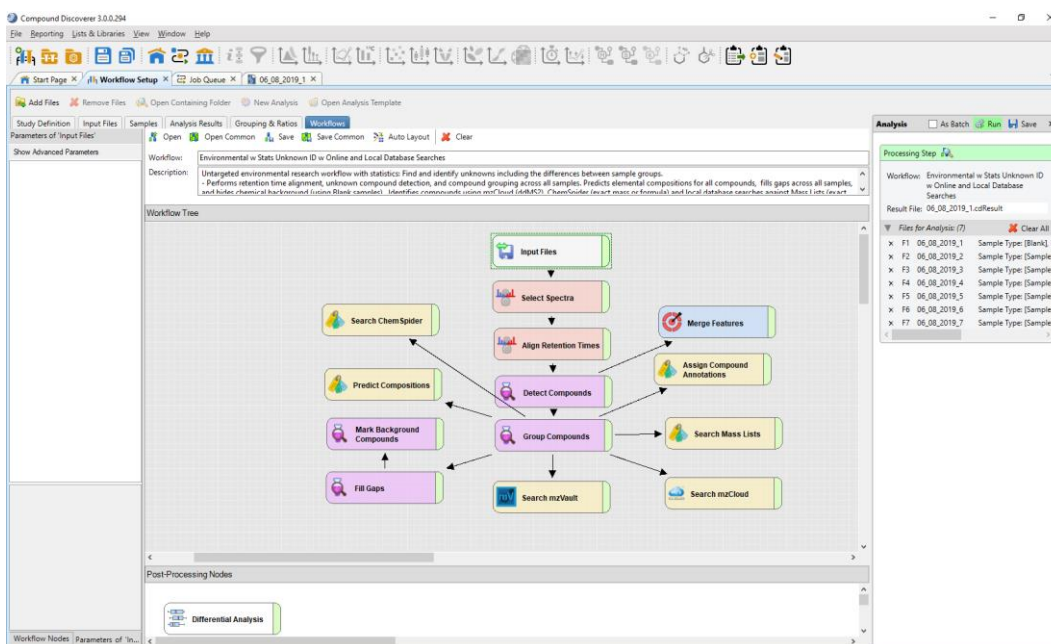


Figure B2 - Example of workflow tab

B12.2.6 Remove everything except the blank and the first file from the right-hand analysis pane. Check the output file name corresponds to the sample and check the run button. Repeat for all the samples in the batch. Processing of a full batch may take several hours depending on sample composition, workflow settings and number of files to process, but individual completed sample results can be opened and assessed will subsequent files are processed.

B12.2.7 Results open as a new tab that shows a list of all the detected peaks, a chromatogram and spectrum for the selected line in the compounds table.

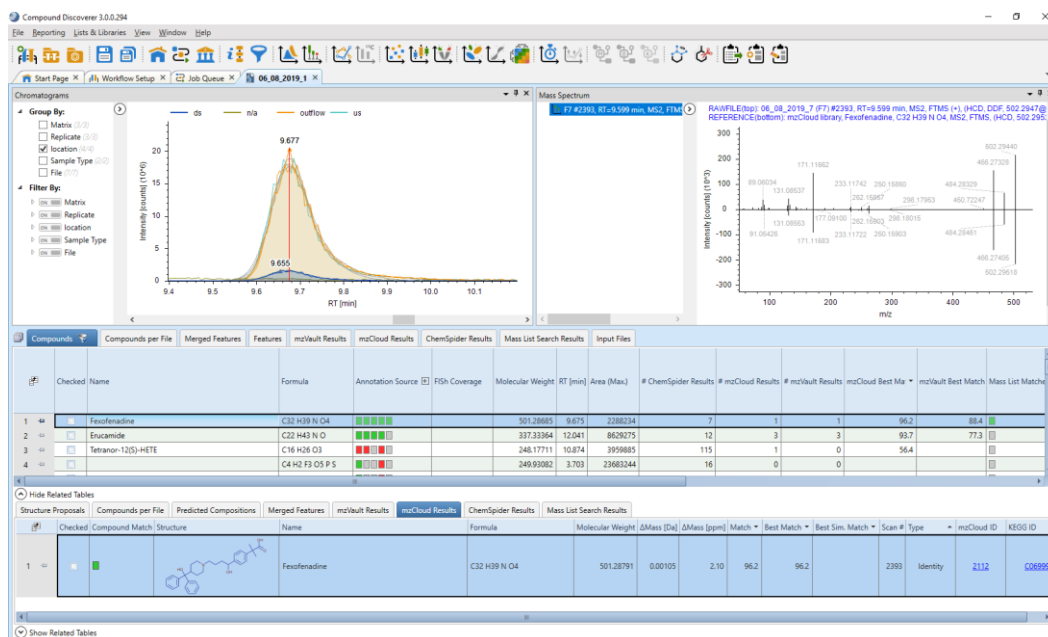


Figure B3 - Example of results tab

B12.2.8 Assess m/z Cloud matching.

B12.2.8.1 Use the filter tool to filter results to display only those which have a m/z cloud best match confidence greater than 60. A good match gives a high level of confidence in identification as it has been confirmed by comparison with library ms^2 spectrum.

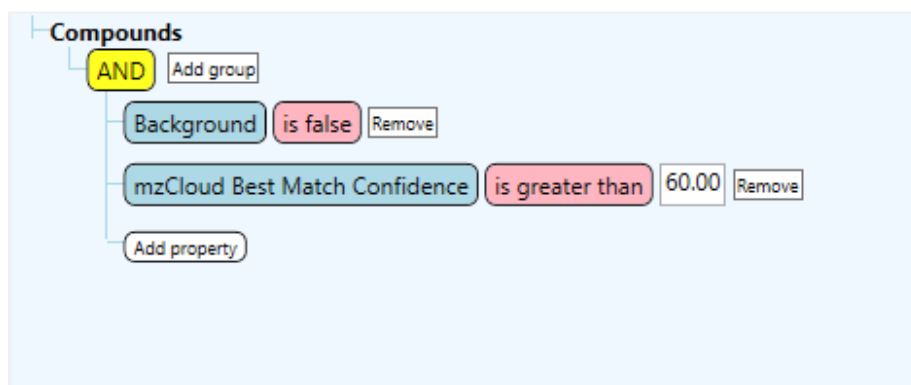


Figure B4 - Filter for mzCloud best match Confidence

B12.2.8.2 Sort by m/z cloud best match confidence and click on the top result line in compounds table, then click show related tables.

B12.2.8.3 Open m/z cloud results tab to show all m/z cloud hits for the selected line. Select the result with the highest match score. Assuming other assessments are acceptable a match of >90 is required to include as a confirmed structure, a score between 60 and 90 is acceptable to include as a tentative proposal.

B12.2.8.4 Assess the likelihood of proposed compound in sample with respect to matrix, location and any supporting information.

B12.2.8.5 Assess the proposed structure with respect to observed retention time, ms^2 spectrum against m/z cloud reference spectrum and look for corroborating isotope patterns. Look for corroborating matches in ChemSpider and mass lists for improved confidence of identification.

B12.2.8.6 If assessments are acceptable, right click on entry and select add to structure proposals and click on the checked box. Then move on to the next highest m/z cloud match if score is similar as they may also be a possibility.

B12.2.9 Assess mass lists, predicted composition

B12.2.9.1 Filter out data that has already been checked and look for those which have mass list match, predicted composition match and related ms^2 data.

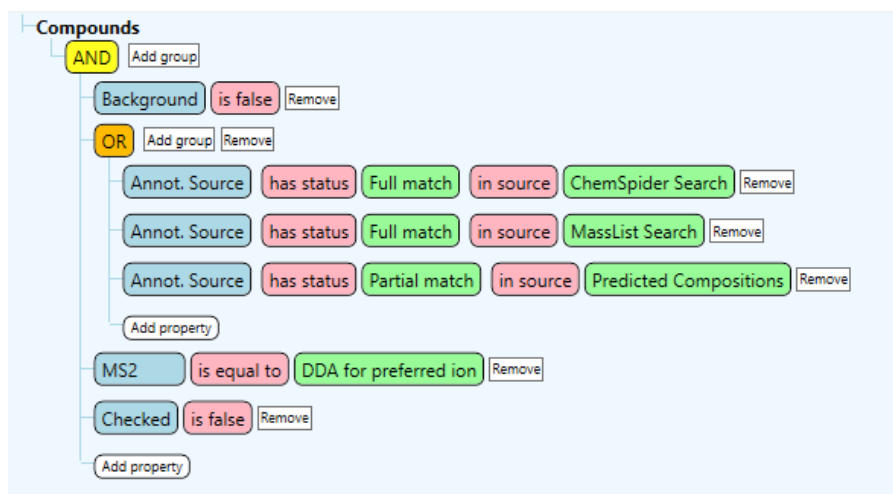


Figure B5 - Filter for ChemSpider predicted composition match

- B12.2.9.2** Sort by max area, click on top results in compound table, then check mass list tab to show all hits for selected line. Ensure prioritised by mz logic score. SFit score is used if no mz logic result available
- B12.2.9.3** Assess likelihood of presence of top listed compound with respect to matrix, location and any supporting information.
- B12.2.9.4** Assess proposed structure with respect to observed retention time. Assess observed ms spectrum against expected spectrum.
- B12.2.9.5** Where available use m/z logic score to assess confidence of identification, m/z logic score >90 is acceptable to include as confirmed structure, a score between 60- 90 is acceptable to include as a tentative structure proposal.

Where m/z logic score does not exceed 60, or no m/z logic data available, SFit and Fish scoring can be used to prioritize results. (SFit scoring alone cannot confirm a structure definitely but can help to confirm unequivocal chemical formula. SFit score must be >80 for a proposed compound to be considered for reporting.

Fish scoring can be used to assess SFit possibilities if more than one present. Right click on the entry in the structure proposals and select apply Fish scoring. This will generate a score based on comparison of observed ms² data, with in-silico generated theoretical fragments from the proposed structure. Fish scoring can provide some structural information giving increased level of confidence. A minimum score of 60 is required for inclusion as a tentative candidate.

It is not always possible to differentiate between compounds with very similar structures, which produce similar SFit, m/z logic and Fish scores. In this instance send the top 3 possibilities to structure proposals and include comment that identification is not definitive.

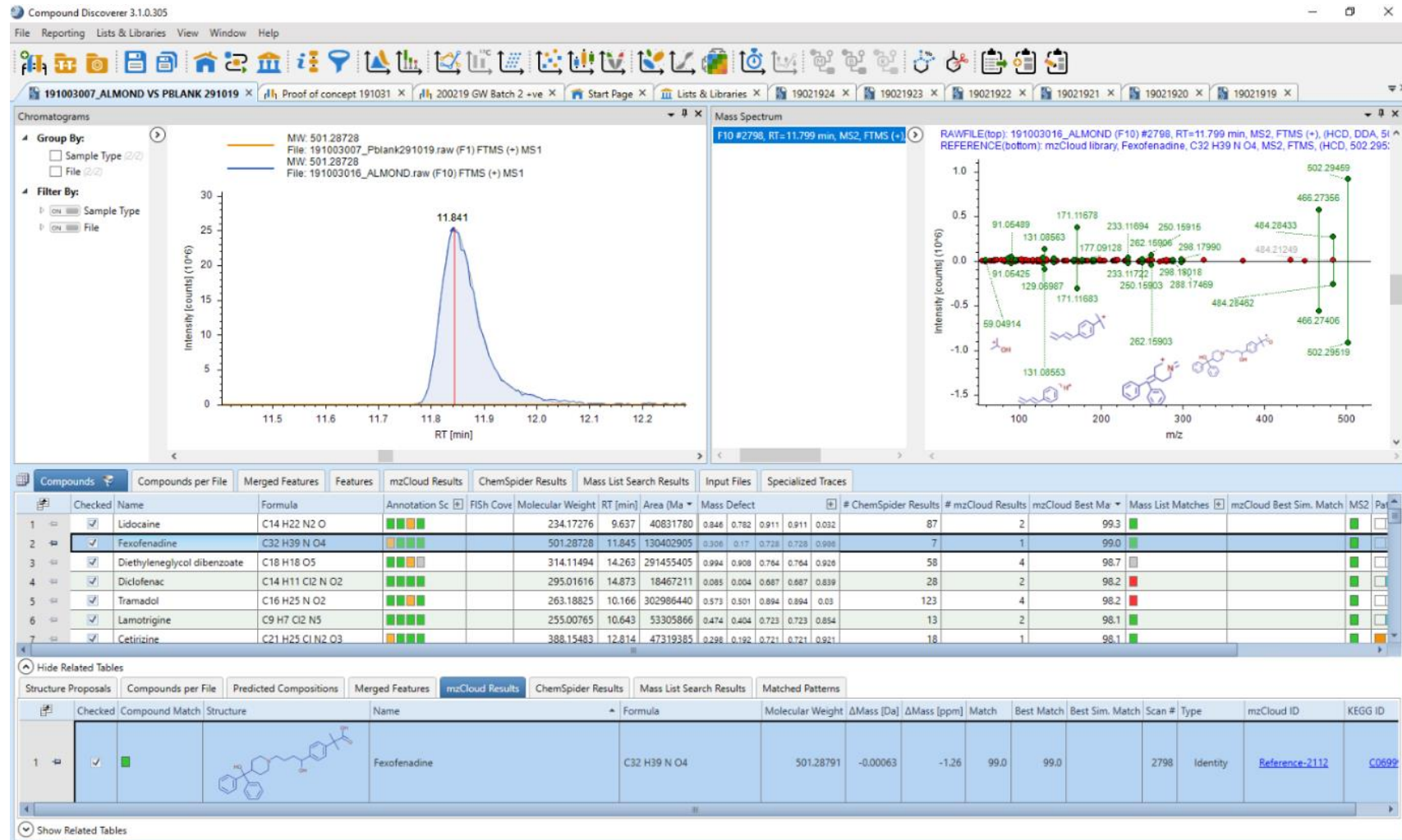


Figure B6 - Compound Discoverer output window

Compound Discoverer Primary Results fields relating to figure B2

- Name – where a name has been assigned, it is displayed here
- Formula – proposed elemental composition.
- Annotation source – traffic light system shows which of the selected annotation sources show a positive identification for the assigned name.
 - Green represents a full match against the selected library
 - Amber represents a partial match or is not the top hit against the selected library
 - Red represents no match against selected library due to spectral anomaly such as an invalid mass.
 - Grey indicates that there was no entry in the selected library.
- FISh coverage – In silico evaluation of how well a proposed structure explains the observed spectral features. Can only be performed on compounds with proposed structures.
- Molecular weight – Shows the monoisotopic molecular weights for assigned components.
- RT – shows observed Retention Time for detected components.
- Area (Max) – shows the maximum area observed at specified mass and retention time in any sample included in the analysis.
- #ChempSpider results – number of possible chemspider matches for specified mass and retention time. This could include several hits for the same compound or a selection of isomers.
- #MZCloud results – number of possible, MZCloud matches for specified mass and retention time
- #MZVault results – number of possible, MZVault matches for specified mass and retention time
- MZCloud best match – displays confidence level of best match found in MZCloud Database
- MZVault best match – displays confidence level of best match found in MZVault Database
- MZCloud best sim match – best m/z similarity match for specified mass and retention time, Similarity match assessed in m/z Logic workflow node when no MZCloud hit is found.
- ms² – Green if ms² data available for specified mass and retention time, Red if no data available

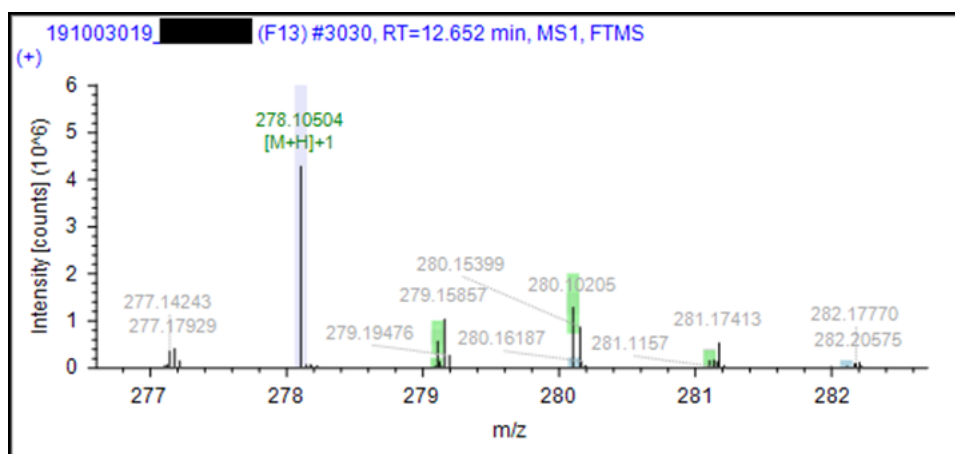


Figure B7 - Isotope Assessment

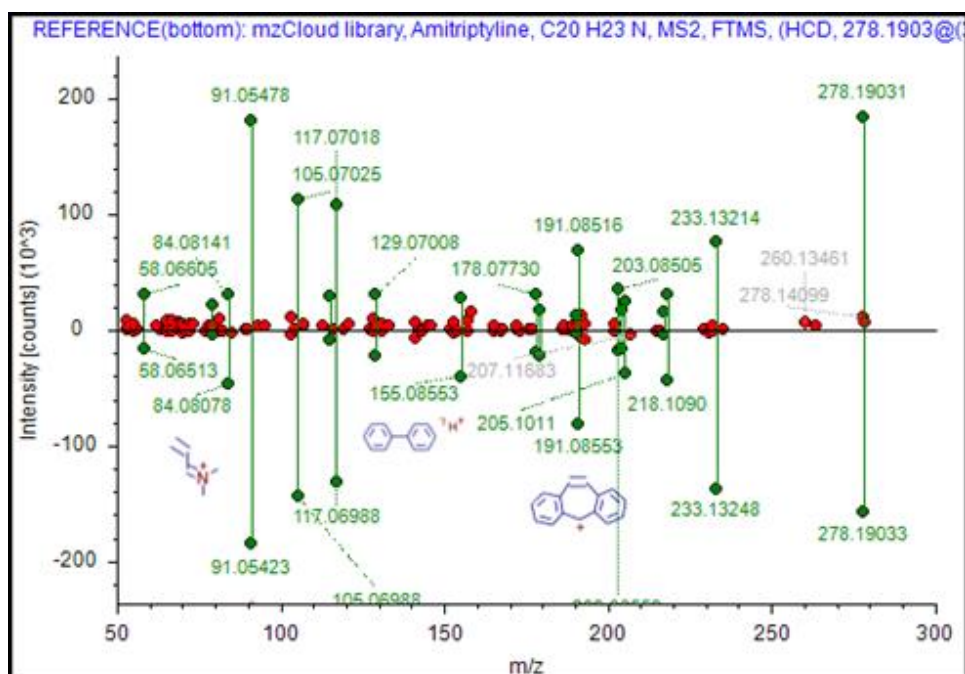
Isotope assessment is done using the top right panel of the Compound Discoverer output window.

Isotope assessment

When a line in the compounds table is selected this panel will show the ms Spectrum for the selected entry.

The panel will show the observed spectrum along with expected ions and abundances, shown as range bars superimposed on the spectrum. These are colour coded to indicate how well the observed spectrum matches the expected.

- A grey/violet bar indicates the base ion upon which the expected abundances are based
- A green bar indicates the presence of an expected ion with abundance within the tolerance range.
- A red bar indicates expected ion either out with tolerance range or not detected.



The expected fragments from the library are shown on the lower plot with experimental data on the upper. Matching fragments are shown in green while missing or unexpected fragments are shown in red.

It is usual to see some low intensity red points on the plot which relate to instrument noise, these are of little consequence.

If significant unexpected masses appear or expected high abundance masses are missing, consider alternative identification possibilities.

Appendix C

Confidence levels

Level 1 – Confirmed structure

For full unequivocal confirmation a reference standard for the suspected compound must be obtained and analysed. Confirmation of component identity is accomplished via comparison of retention time, High Resolution Mass spectrum, isotopic ratios, and where an ms^2 scan has triggered the presence of confirmatory ms^2 fragment(s) with the laboratory standard material

- Retention time must match reference standard within 10 seconds.
- Mass must be within 5 ppm of compound database value.
- Isotopic pattern match must exceed 80%.

Due to the nature of DDMS² acquisition there may be occasions where no ms^2 event triggers for a component; where ms^2 data is available it must meet the following criteria.

- Most abundant expected fragment must be observed.

This combination is suitable as an absolute identifier of the presence of the compound.

Level 2 – Probable structure

Result ms^1 and ms^2 data has been confirmed against library ms^1 and ms^2 data

- Compounds identified with a best match >90 in MZCloud or MZVault libraries
- Compounds identified by chemspider with MZLogic score >90

Level 3 Tentative candidate(s).

Result ms^1 and ms^2 data compared against library ms^1 and computer-generated expected fragmentation ms^2 data.

- Compounds identified with a best match between 60 and 90 in MZCloud or MZVault libraries.
- Compounds identified by chemspider/mass list with MZLogic score >60
- Compounds identified by chemspider with SFit score >80 and FISh score >60

Level 4 unequivocal chemical formula

- Formula identified by predicted composition or chemspider with SFit only and no generated ms^2 data.
-

Level 5 – Retention Time and Exact mass of interest

- Compounds detected but not identified. No attempt is made to further identify such compounds manually.

Figure C1 below outlines an identification confidence level system proposed by Schymanski et al, 2014 for high resolution mass spectrometric analysis. MS² is intended to also represent any form of MS fragmentation (e.g., MS^e, MSⁿ).

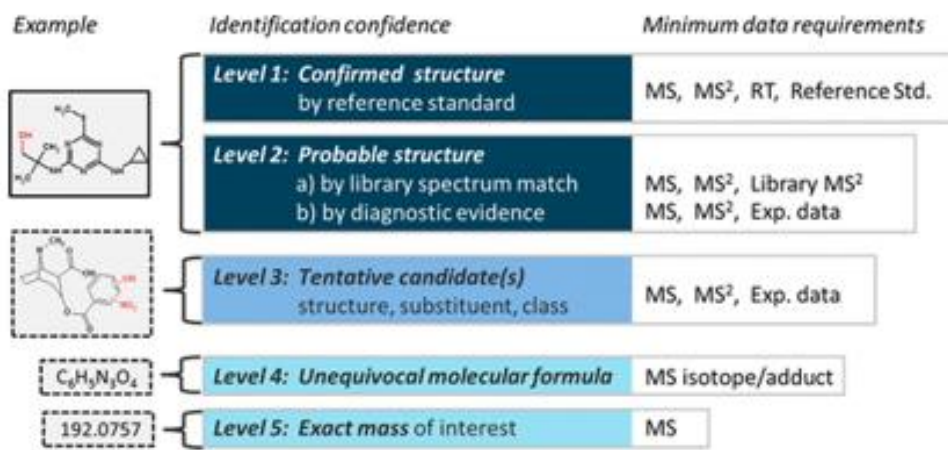


Figure C1 - Proposed identification confidence levels in high resolution mass spectrometric analysis.

Appendix D

Proficiency testing schemes for target, suspect and non-target LCMS screening.

D1.1 VIO XX-39b wide screening, semi-quantitative using a LC-MS in drinking, surface ground and waste-water.

[EPTIS – VIO XX-39b wide screening, semi-quantitative using a LC-MS in drinking, surface, ground and wastewater \(EPTIS-ID 232901\) \(bam.de\)](#)

D1.2 PTS Food Pesticides: 66 - Multiresidue screening of pesticides

[EPTIS – PTS Food Pesticides: 66 - Multiresidue screening of pesticides \(EPTIS-ID 156465\) \(bam.de\)](#)

[D2.3](#) The Bipea scheme is 37m – Fresh waters – Multi-pesticides –

<https://www.bipea.org/water/#div2>

D3.4 Chemical Contamination Incident in Drinking Water Proficiency Test

[Fapas Proficiency testing from Fera | Fapas Proficiency Testing from Fera](#)

Appendix E

Example report from method A

(Includes all compounds screened for using method A)

Results can be filtered to display only those compounds present in the sample, this is achieved by applying the filter to column D and choosing box 'P' only. Fifty-four compounds are displayed.



Example Report -
Method A.xlsx

sca standing
committee of
analysts