Use of non-target screening by means of LC-ESI-HRMS in water analysis

(Edition 1.0 2019)

Water Chemistry Society

Division of the Gesellschaft Deutscher Chemiker

Gesellschaft Deutscher Chemiker

Imprint

Edition 1.0 2019

Published in Mülheim an der Ruhr (Germany), December 2019

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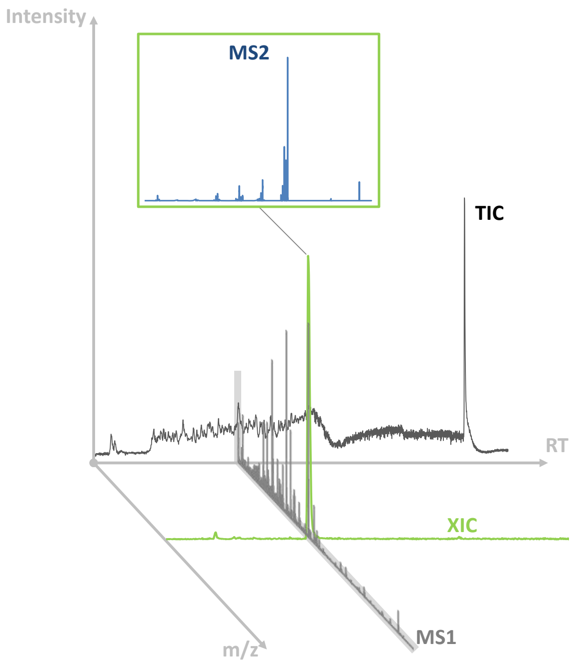
Guideline

Use of non-target screening by means of LC-ESI-HRMS in water analysis



Edition 1.0 2019

German Water Chemistry Society
'Non-Target Screening' Expert Committee

Non-Target Screening in water analysis

Guideline for the application of LC-ESI-HRMS for screening

Edition 1.0 2019

This guideline was developed by the members of the 'Non-Target Screening' expert committee of the German Water Chemistry Society.

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Citation recommendations

The guideline should be cited as follows: "W. Schulz, T. Lucke et al., Non-Target Screening in Water Analysis - Guideline for the application of LC-ESI-HRMS for screening (2019). Download at http://www.wasserchemische-gesellschaft.de"

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1 Introduction

The use of high performance liquid chromatography (HPLC) in combination with high resolution mass spectrometry (HRMS) enables the qualitative confirmation and quantification of organic trace substances. [1, 2, 3, 4, 5] In general, a differentiation is made between quantitative target analysis and qualitative Non-Target Screening (NTS). Target analysis uses predefined lists of substances that should be detected in a (water) sample, and whose concentrations are to be determined by reference substances. Non-Target Screening can detect both known substances and thus far not recorded or in many cases, entirely unknown substances. The retrospective data analysis of - for example - newly discovered or previously not considered substances is a particular advantage of HRMS compared with the use of low resolution mass spectrometers. [4]

This guideline defines the prerequisites and requirements for measurement technology, analysis and data interpretation.

Table 1.1 shows and explains examples of typical quantitative and qualitative tasks in water analysis (wastewater, groundwater, surface water or drinking water).

Table 1.1: Overview of typical tasks in water analysis[1]

Target analysis	Suspect-Target Screening	Non-Target Screening
• Monitoring of organic trace compounds to monitor thresholds • Monitoring of organic trace compounds to determine trends • Monitoring of organic trace compounds after contamination (accidents, fire, etc.) • Monitoring of individual process steps in wastewater and drinking water treatment (e. g.: breakthrough of an adsorption filter, removal efficiency of individual process steps)	• Search for known substances (e. g. pharmaceuticals, household and industrial chemicals, pesticides, transformation products, etc.) • Search for substances with specific structural properties (elements in the molecule, such as S, Cl, Br or functional groups such as COOH) • Comparison of positive findings from investigations by other laboratories or from literature data • Retrospective data analysis of archived HRMS data based on information on new substances • Rapid estimation of the presence of a compound at the investigated site • Decision-making basis to extend monitoring programs	• Search for additional compounds and their characterisation (beyond target monitoring) • Determination of differences (regarding organic trace compounds) between several samples (hydrogeology, time trends, processes regarding removal or formation of unknown substances) • Description of processes regarding behaviour of organic trace compounds • Detection and characterisation of transformation products (e. g. from known original compounds) • Detection / presence of compounds as a consequence of an event - determination of causes (toxicity – fish mortality, odour - taste, storm water, accident, fire, etc.) • Expansion / revision of monitoring programs (dynamic monitoring) • Identification of unknown substances with the aid of additional information (database comparison, comparison of MS/MS spectra from literature data or in-silico fragmentations) and measurements (reference substances, use of orthogonal techniques such as NMR or Raman spectroscopy)

[1]Revised from "Options in high resolution mass spectrometry (HRMS), use of Suspect and Non-Target analysis in monitoring practices of raw and drinking water" DVGW Information on Water No. 93

2 Scope

This guideline is intended to show fundamental aspects in the use of high performance liquid chromatography in combination with high resolution mass spectrometry. Aside from technical information pertaining to devices and potential contamination in sampling and measurements, this also includes data evaluation and quality assurance measures. The guideline is intended to assist the user in developing the method and interpreting the results.

3 Terms and abbreviations

The most important terms of mass spectrometry and high performance liquid chromatography with their definitions are compiled in the following Table 3.1.

Table 3.1: Compilation of abbreviations and terms of mass spectrometry and high performance liquid chromatography [6]

Accurate mass	The accurate mass of an ion is the mass experimentally determined (and recalibrated with a reference mass standard if applicable) in the mass spectrometer
APCI	***A****tmospheric* ***P****ressure* ***C****hemical* ***I****onization* Chemical ionisation at atmospheric pressure
Resolution	Least difference Δm of two *m/z* values in which two mass spectrometric peaks of the same intensity are deemed to be separated from each other (10% or 50% valley definition)
Resolving power R **(R = m/Δm)**	Quotient of the mass m determined in the mass spectrometer and the difference Δm of two *m/z* values that can be separated from each other [6]. The mass difference Δm of two *m/z* values can be measured from peak maximum to peak maximum at 5%, 10% or 50% of the peak height (full width at half maximum, FWHM) and should therefore be stated with the resolving power R.
CCS	***C****ollision* ***C****ross* ***S****ection* Molecular cross-section area calculated by ion mobility spectrometry as a measure of molecular size. Various mass spectrometers can be enhanced to include ion mobility spectrometry by modifying or adding an LC-MS system.
ESI	***E****lectro****s****pray* ***I****onisation*
Exact mass	The exact mass of an ion or molecule is the calculated mass for a given isotope composition (monoisotopic mass)
Feature	Features are peak shaped signals which are defined by their accurate mass (*m/z*) and retention time (RT) and fulfil the selection criteria for peak finding (e.g. intensity threshold).
FT-ICR-MS	***F****ourier* ***T****ransformation* ***I****on* ***C****yclotron* ***R****esonance* ***M****ass* ***S****pectrometer*

HILIC	***H**ydrophilic **I**nteraction **Li**quid **C**hromatography*, MS-compatible alternative to normal phase chromatography for separating strongly polar compounds consisting of a polar stationary phase (similar to normal phase chromatography; partly in combination with cation/ anion exchanger functions) using common RP eluents (water, methanol, acetonitrile)
HPLC	**H**igh **p**erformance **l**iquid **c**hromatography
IMS	**I**on **m**obility **s**pectrometry
Isotope pattern	The pattern that forms in the mass spectrum by the mass spectrometric separation of the various isotopes of the atoms in a molecule. The isotope pattern is dependent on the combination and frequency of the individual atoms in the molecule.
LC-HRMS	***L**iquid **C**hromatography - **H**igh **R**esolution **M**ass **S**pectrometry*
LIMS	***L**aboratory **I**nformation and **M**anagement **S**ystem*
MS	***M**ass **s**pectrum* Two-dimensional plot of the signal intensity of an ion (y axis) versus the *m/z* ratio (x axis)
m/z	Abbreviation for mass to charge ratio Mass divided by charge number (no dimensions)
Mass defect	The mass defect of an atom, molecule or ion is the difference between the nominal and the monoisotopic mass. Most organic molecules have a positive mass defect, since they are very often composed of atoms with nearly negligible negative (e.g. O, F) or small positive mass defects (e.g. H, N). Some elements such as chlorine and bromine have relatively large negative mass defects.
Mixed Mode	LC column material (stationary phase) with a combination of various functionalities to form hydrophobic and ionic (ion exchange) interactions
Monoisotopic mass	Exact mass of an ion or molecule calculated using the most commonly occurring natural isotopes of the elements. The monoisotopic mass of molecules or ions is also referred to as exact mass within this context.
MS^2:	Acquisition of product ion spectra (fragmentation spectra) by molecular fragmentation with various modes:
Targeted MS^2: MS^2 , MS/MS, ddMS	Specifically targeted (*Engl. **d**e**d**icated, also **D**ata **D**ependent*) fragmentation of individual ions to record fragmentation spectra that are as pure as possible
Automatically triggered MS^2: $MSMS^{all}$, AIF, DIA	Fragmentation of molecules in a selected mass range to record as many fragment ions as possible; this supplies overlaid fragment ion spectra *(**A**ll **I**on **F**ragmentation, **D**ata **I**ndependent **A**cquisition)*

Nominal mass	The nominal mass of an element is the integer number of the mass of its most common isotope, such as 12 u for carbon and 35 u for chlorine. To calculate the nominal mass of a molecule or ion, the nominal masses of the elements are multiplied by the number of atoms of each element in the molecule or ion.
NTS	***N****on-****T****arget* ***S****creening* Non-targeted analysis procedure without limitation to pre-selected substances. All substances that can be measured by chromatography and mass spectrometry by the applied analytical method are detected.
QA	**Q**uality **a**ssurance
RP	***R****eversed* ***P****hase in* *high performance liquid chromatography*
Sector-MS	*Sector field mass spectrometer*
TOF	**T**ime **o**f **F**light **m**ass spectrometer
u	Atomic mass unit defined as one twelfth of the mass of a carbon atom in its ground state: 1 u = 1.660 539 040 10^{-27} kg equal or equivalent to Da (Dalton)
UPLC	***U****ltra* ***P****erformance* ***L****iquid* ***C****hromatography*
UHPLC	***U****ltra* ***H****igh* ***P****erformance* ***L****iquid* ***C****hromatography,* High performance liquid chromatography with very high chromatographic separation performance on columns with small particle sizes (< 2 µm) and column pressures of up to 1500 bar

4 Basis of the procedure

The procedure is based on the use of high performance liquid chromatography coupled with high resolution mass spectrometry (LC-HRMS). [1] [7] This enables the detection of ions formed in the ion source at any time point in the selected mass range, and the determination of their accurate mass. Mass detection can be performed with a time-of-flight mass analyser (TOF), an Orbitrap or another high resolution mass spectrometer (FT-ICR, Sector-MS). The minimum requirement for high resolution mass spectrometers is a resolution of > 10,000 (10% valley definition overlap of the mass peaks to be separated [8]) or > 20,000 (FWHM definition based on the width at half maximum of height of the mass peak [9]) across the recorded mass range. The mass deviation between the measured (accurate) and theoretical (exact) masses should be < 5 ppm [10] at *m/z* 200 [9] and should be verified with regular calibrations. Compound identification requires the measurement of MS^2 spectra with accurate masses for individually selected precursors (MS/MS or $ddMS^2$) or if possible, simultaneously for all precursor ions (MS/MS^{all} or AIF or DIA). The evaluation of the obtained data is performed depending on the task and is structured into Suspect Target and Non-Target Screening (Table 1.1).

4.1 Non-Target Screening

In Non-Target Screening, LC-HRMS chromatograms are searched for so called features using suitable peak finding software (for a definition, see section 3). Due to isotope peaks and formation of various adduct ions of a molecule in the ion source and possible *in-source* fragmentation, it is necessary to perform componentisation. That is binning of all signals that originally come from one component (also see section 10.1.4). In order to remove false positive features, it is also necessary to perform a blank correction (also see section 10.1.3). Alignment furthermore makes sense when comparing different samples (also see section 10.1.2). This is generally followed by generating possible chemical formulas, using the accurate masses of the features, and if detected (concentration, sensitivity), the isotope patterns (also see section 10.1.5). In this context mass accuracy and resolving power play an important role in reducing the number of possible chemical formulas suggested. We furthermore refer to the "S*even Golden Rules" f*or reducing the number of chemical formulas which make sense from organic chemistry. [11] Various data bases and tools are available for identifying and interpreting features. The MS^2 information recorded for the features has proven essential to determine structures. Aside from matching in house and/or online substance databases (e. g. PubChem [12], ChemSpider [13], SUBSTANCE ID [14]) MS^2 data can also be used for querying analytical spectral data bases (e. g. Massbank [15], mzCloud [16]) and applying *in-silico* fragmentation tools (e. g. Metfrag [17]) (see also section 10.2.1.1). The number of possible structure suggestions for individual features herein drops successively as more information is incorporated into the queries. Since it is often not possible to unequivocally identify a feature, classification into different identification categories based on various matching criteria has proven helpful (also see section 10.2.1). Metadata, statistical methods and comparison of results from different samples (even without identification) can also provide significant assistance in solving analytical tasks (e.g. prioritising relevant features).

4.2 Suspect-Target Screening

Suspect-Target Screening uses a list of relevant substances or substance groups for the measurement task. The LC-HRMS chromatogram of the sample(s) is then evaluated only for the presence of these suspects using suitable software. Various strategies may be used here, such as using exact masses or chemical formulas. Confirmation of positive results (identification) generally requires an MS^2 spectrum of the sample and a reference compound or corresponding information from the literature.

5 Blanks

All types of blanks must be avoided or kept to a minimum. Sources of blanks can be assigned to different steps of the analytical procedure. The causes of blank values and how to avoid them in the individual work steps are explained below.

5.1 Sample blanks

Blanks due to sampling must be kept to a minimum. To avoid cross-contamination from sampling bottles or vials, they should only be used for sampling of one category like drinking, or surface or wastewater. This avoids the use of a glass bottle filled with wastewater for later drinking water sampling. All sampling bottles or glassware can be baked out in a heating furnace overnight at a temperature of at least 450 °C. Inert materials made from glass or stainless steel should be used as far as possible. If this is not possible, e.g. for technical reasons (composite samples from automatic samplers, temperature resistance), bottles made from plastisizer-free polymers or well washed (or old) plastic bottles should be used. Any handling of the sample, such as filling, pipetting or pre-concentration may cause contamination by organic trace compounds (also by laboratory personnel, e.g. due to skin protection or skin care products).

5.2 System blanks

Open handling (e.g. liquid transfer) should be avoided to reduce contamination. For the addition of charge carriers to the eluents to improve the ESI process (such as formic acid) ideally only baked-out glassware should be used (see section 8.1). The devices and analytical systems used should be regularly maintained and checked/tested for possible contamination, e.g. due to lubricants or additives of the used materials (tubes, seals, etc.).

5.3 Blank measurements

Regular blank measurements are used to check suitable conditions of sample bottles, vials and chemicals. For example, a sample blank and/or system blank can be used to perform a blank check. As sample blank an ultrapure laboratory water sample or synthetically buffered water sample can be used which was subjected to all analytical steps like sampling, sample storage, transport and preparation like the original sample. The system blank is obtained by measurement without a sample injection (zero injection). The resultant total ion chromatograms can be assessed by comparison of the signal intensities (see Appendix C.3). For blank assessment, an evaluation according to 10.1.1 has to be performed additionally. A blank check must be performed in each measurement sequence. When measuring samples with unknown contamination levels, a blank measurement is recommended between injections to avoid or detect carryover.

6 Sampling

6.1 General information

The sampling procedure for water samples is described different standard methods for a variety of parameters and parameter groups. [18] Controls for contamination or losses (e.g. by adsorption or instability of the sample during sample transport to the laboratory) can be performed for selected compounds; however, this is not the case in Non-Target Screening for the entire compound composition of the sample. Essential precautions must therefore be taken during sampling.

The required sample volume depends on sample preparation steps and injection volume. Stabilisation by adding acid or sodium azide (microbiology) may cause contamination and chemical reactions. It is recommended to immediately cool the sample to approx. 4 °C and perform the analysis as quickly as possible. If this is not possible, samples should be frozen at max. -18 °C until they are analysed. This also applies to retained samples. Loss due to freezing/thawing cycles is possible and must be taken into account.

6.2 Quality assurance in sampling

Performing quality assurance measures during sampling can avoid erroneous interpretation of measuring results. A suitable quality assurance measure must be verified for the task in question. The use of so called field blanks has proven useful for several measurement tasks, e.g. during pump sampling. Field blanks are clean water samples (e.g. ultrapure water) filled into bottles at the field site. This may reflect sample contamination during sampling or sample transport. In case of complex sample transport, a transport blank for each transport container (refrigerated box) is also useful.

6.3 Sample name / sample description

Sample names should be selected in a way that all data (raw data, evaluation) can be traced back to the sample unequivocally. The use of a unique laboratory number that is continuously used in all file names and documents is useful. The following Table 6.1 provides examples for background information of samples. For further information, we refer to the current standard documents for different sampling approaches. [18, 19, 20]

Additional information or specific characteristics (meta-information) during sampling must be included in the documents. This facilitates the interpretation of the screening data. For this the measurement objective to be clearly defined and known to the person who performs sampling.

Table 6.1: Exemplary compilation of sample accompanying information

Information	Description / example
Sampling site	Precise description E.g. flow kilometre, name of groundwater measuring site, geographic coordinates
Sampling type	Pumped sample, grab sample, tap sample, combined sample, qualified randomised sample
Special features of sampling	Use of a power generator, environmental factors (e.g. adjacent fertilisation at the time of sampling...)
Sample vessel	Glass, lids, caps, pre-treatment of sample bottle, materials in contact with the sample during sampling?
Weather	Sun, precipitation
Blank samples	Field blank, transport blank
Meta-information	Characterization of sampling sites
Analytical task should be known	special features such as discharges, production plants, agricultural activities, flooding

7 Reagents

7.1 General information

Specific requirements for purity must be considered for all reagents used. The contribution of impurities to the blank has to be minimized or should be as low as possible or negligible in relation to the analyte signals relevant for the analytical task. This must be checked regularly (see section 5).

7.2 Eluents

Solvents (e.g. methanol, acetonitrile) and water must be suitable for HPLC and mass spectrometry. Special qualities are commercially available. If the bottles used for this purpose cannot be baked out (see section 8.1), they should be easy to clean and reserved for use in screening.

7.3 Operating gases for mass spectrometers

The operating gases for the mass spectrometer have to fulfill the minimum requirements of the manufacturer. This also includes the gas line materials.

7.4 Reference substances

Reference substances are necessary for confirmation of the identification of compounds (see section 10.2.1). They should have a purity of at least 95% if possible. Solutions of several reference substances (multicomponent standard) can also be used to monitor the stability of the LC-HRMS system (see Appendix E).

7.5 Internal standard substances (IS)

Isotope labelled compounds should be used (see Appendix B.1). They are used in each sample to check measurement stability and may provide indication of matrix effects. For example, the IS can be automatically added with the autosampler by co-injection of each sample (e.g. 95 µL sample + 5 µL IS).

7.6 Preparation of solutions

During preparation of solutions each step must be checked for potential contamination. Contact with plastic materials should be avoided as far as possible. Use of glass syringes has proven beneficial in practice.

7.6.1 Stock solution (reference substances)

Stock solutions should be stored at max. -18 °C, protected against light and evaporation. A shelf life of at least one year is generally expected under these conditions.

7.6.2 Spiking solutions (IS)

The concentrations of spiking solutions should be adjusted to the detection sensitivity of the compound. This guarantees sufficient signal intensity for detection of internal standards while avoiding overdoses. Overdoses of IS may cause signal suppression during ionisation of compounds present in the sample.

7.6.3 QA standard (control standard)

A multicomponent standard with compounds should be used which cover both the mass and the retention time range of the LC-HRMS method as comprehensively as possible. A multicomponent standard spiked to a sample matrix should be used particularly when checking the generic peak finding process. In best case the reference matrix should be an aliquot of a representative environmental sample that is available in sufficient quantities (spiked if required). This also expands the compound pattern by unknowns at a variety of concentration levels. This allows to monitor also the peak finding parameters which are intensity dependent (see section 10.1.1) to avoid false positive and false negative results.

8 Devices

8.1 General information

Devices or device parts that come into contact with the water sample must be free of residues that may cause blanks. Glassware should be used if possible, since it can be cleaned well by baking out, e.g. at 450 °C for 4 h (see also section 5).

8.2 Sample glass vials

Use crimp capped vials with septa and a nominal volume of 1.5 mL, suitable for the injector system Baking out glass vials at 450 °C for at least four hours. The cleaned sample vials must be stored protected against contamination until use. This also applies to sampling bottles. Since it is not possible to bake out crimp caps and septa, septa materials providing low blanks should be used. For example, PTFE-coated septa should be given preference over rubber septa.

8.3 High performance liquid chromatography

8.3.1 General information

HPLC systems that are used for screening together with mass spectrometers generally consist of degassing systems, low-pulsation analytical pump systems (suitable for binary gradient elution), sample loading system (optimally cooled for preserving sample storage until measurement) and a column oven.

8.3.2 HPLC column

HPLC columns that have sufficient retention should be used when MS-compatible eluents (organic solvents and volatile buffers) are applied based on the analytical task, the analyte spectrum and blank requirements for detection (data quality).

In addition to reversed phase materials (RP) - typically C18 or polar modified C18 materials - columns with other separating mechanisms such as HILIC or mixed mode materials can be used. The necessary requirements for eluents and ionisation additives must be fulfilled for the HRMS (e.g. for the ion source) and data quality. Examples of measurement methods are listed in Appendix C.1.

Reference materials (or IS) that cover the entire separation range should be regularly measured to verify robustness. Reference substances can also be used for standardisation, that is the retention time index RTI (Table B.3) which enables a comparison of retention times between laboratories (Table B.4)

8.4 Mass spectrometers

8.4.1 General information

The HRMS mass analysers most commonly used today in routine laboratory work include time-of-flight mass spectrometers ((Q-)TOF) and Orbitrap systems. Both are used for Non-Target screening, typically in the Tandem-MS mode with automated recording of fragmentation spectra. The measurements are normally performed in a specific acquisition mode (e.g. in positive or negative mode), so that two runs are required to completely record all ion species. Diagrams and explanations for QTOF and Orbitrap systems are shown in Appendix D. Examples for used MS methods are shown in Appendix C.2.

Minimum requirements are given to perform screening measurements using LC-HRMS:

- The mass ***resolving power*** should be at least 10,000 [8, 9] (10% valley definition). This is approximately equal to 20,000 (FWHM definition).
- The ***mass range*** should be selected according to the analytical task. In environmental analysis, most molecules of interest are in a range between *m/z* 100 and no more than *m/z* 1200.
- ***Mass accuracy*** should be at least within 5 ppm [9, 10] at *m/z* 200 to limit the number of possible chemical formulas.
- Various recording modes described in Table 9.3 for ***fragmentation spectra (MS²)*** are possible. The basic requirements for HRMS should also be fulfilled for MS^2 spectra (R = at least 10,000 [8, 9] and mass deviation of no more than 5 ppm [10])
- The required ***sensitivity*** depends on the task and applied chromatography (injection volume) and should permit detection of analytes in the range of approx. 10 pg on column. For water samples detection limits in the lower ng/L range are required to consider threshold values.
- ***System stability*** must always be ensured with respect to sensitivity and mass accuracy (see Figure E.3 – control charts to check MS performance by mass accuracy, resolution and sensitivity).

8.4.2 Ion source

The selection of the ion source depends on the analytical task. Thus far, electrospray ionisation (ESI) has best proven itself due to its universal and robust applications. Other ionisation techniques (such as APCI) can be used analogously depending on the task or the analytes to be detected.

9 Implementation

9.1 Sample preparation

Sample preparation depends on the task, the type of water sample (e.g. seepage, wastewater, surface water, groundwater, drinking water) and the sensitivity of the available LC-HRMS system. In order to avoid blanks due to impurities (see section 5), the final goal of sample preparation should be to perform only absolutely necessary steps and be aware of all contamination sources in this process. [21] Table 9.1 shows examples of various sample preparation and sample injection methods.

Table 9.1: Benefits and disadvantages of individual steps in sample preparation and sample loading

Description	Procedure (example)	Benefits	Disadvantages
<u>**Sample preparation**</u>			
Filtration	Pre-filter with membrane filter made of regenerated cellulose, cellulose acetate, PTFE or glass fibre	Homogeneous sample	Contamination, sorption, requires a lot of work and material, becomes clogged
Preservation	Refrigeration (4 °C, -18 °C), stabiliser	Acts differently on various analytes	
Solid phase extraction (SPE)	Sorbent material and quantity, pH, solvent	Potentially high accumulation factor, matrix separation	Contamination, sorption, specific to compound groups, requires a lot of work and material
Centrifugation	at least 2500 x g, 10 min	simple and rapid implementation	Risk of breakthrough, contamination and sorption during any liquid handling
<u>**Sample injection**</u>			
Direct injection, without SPE	no more than 100 µL	unchanged sample, low sample volume required	Sample storage of large retained sample quantities
Co-injection of internal standard (IS)	95 µL sample and 5 µL IS	saves time, highly reproducible	Cannot be performed with all autosamplers
Online SPE	Sorbent material and quantity, pH, solvent	Complete automation is possible	Contamination, sorption, specific for substance groups, requires a lot of material

9.2 Chromatography

Chromatographic separation must not be disregarded despite the selective HRMS. Retention time (RT) is an important criterion for identifying a compound and reflects physical/chemical properties (such as polarity). The type of separation to be used depends on the task. If the separation performance of a classic C18-HPLC column is not sufficient, column materials with smaller particle diameters (such as UHPLC columns) can be used. The applied phase must be selected based on the polarity range of the compounds to be to be separated (log D). Aside from C18 materials, polarity enhanced chromatography may also be necessary.

Additional requirements may apply for an efficient chromatographic separation depending on the task. MS-compatible, volatile buffers and ionisation additives must be used for separation. The reproducibility and stability of the separation are very important so that comparison within and between different datasets are possible. The comparison of chromatograms, such as a time series over months, requires high long-term stability (see Appendix E and E.2). An RT tolerance of 0.15 minutes (analogously [10]) can be defined as the minimum requirement for RT stability. Retention times can be confirmed with chromatographic reference materials. On the one hand, this makes it possible to record robustness of the separation, on the other hand, it also enables the standardisation of the covered separation range (with regard to polarity). This retention time standardisation over an RT index (RTI) system can ensure the transferability of results between laboratories with different LC methods in screening approaches (see Table B.3 for an example of an RT standard).

9.3 Mass spectrometry

The HRMS mass analysers most commonly used today in routine laboratory work are Q-TOF and Orbitrap (see Appendix D).

9.3.1 Ion source / ionisation technique

The use of an electrospray ionisation source has been shown to be the preferred ionisation technique for the use of Non-Target Screening in water analysis. Non-Target Screening requires an ion source that covers a wide polarity range of analytes with sufficient sensitivity. It is important that the source parameters (such as temperature, gas flows, voltages) for ionisation are selected in a way that fragmentation reactions (*in-source* fragmentation) or adduct formations in the source are minimised. Despite the generally soft ionisation mode of ESI, fragment formation in the source cannot generally be avoided. Alternatively, depending on the task or samples, other ionisation techniques such as APCI may be useful. Table 9.2 shows a list of typical adducts and fragments that may form in electrospray ionisation. For a detailed list of typical adducts and fragments, including substance examples, we refer to Appendix G.

Table 9.2: Adduct and fragment formation in the source in electrospray ionisation

	ESI+	**ESI-**
Substance properties	Sufficient alkaline compounds that attract protons or other cations	Sufficient acidic compounds that dissociate a proton (in the gas phase)
Ionisation	Addition of cations e. g. H^+, Na^+, NH_4^+, K^+	Dissociation of a proton or attraction of an anion, e. g. $-H^+$, $+Cl^-$, $+HCOO^-$
Typical adducts	$[M+H]^+$, $[M+Na]^+$, $[M+NH_4]^+$, $[M+nH]^{n+}$	$[M-H]^-$, $[M+HCOO]^-$, $[M+Cl]^-$, dimers
Fragmentation	Gentle ionisation and thereby relatively few fragments (*in-source* fragmentation not always readily detectable), fragmentation by MS/MS collision energy	
Typical fragments	$[M+H-H_2O]^+$, $[M+H-CO_2]^+$, $[M+H-C_2H_6O]^+$	$[M-H-CO_2]^-$, $[M-F]^-$

9.3.2 Measuring technique

The goal in Suspect Target and Non-Target Screening is to obtain as much analytical information as possible about the sample during LC-HRMS measurement. Various measurement modes can be used, depending on the task. The measurement techniques are summarised in Table 9.3. In addition to the acquisition of high resolution mass spectra, depending on the scan speed of the MS, the MS^2 spectra can be recorded by specific or automatically triggered precursors (see Figure 9.1). MS data acquisition (one full scan spectrum per cycle, including MS^2 spectra) has to be selected in a way that sufficient data points to reconstruct the chromatographic peaks are always guaranteed. Therefore, the full cycle time must be adjusted to the chromatographic method. Peaks should be represented by at least 12 data points across the peak for robust data evaluation. [10] To acquire more information in qualitative screening, a lower scan rate can be accepted. However at least 6 to 8 data points are required here as well, since an increase in measurement deviations would otherwise render a reproducible evaluation difficult or impossible.

Table 9.3: Compilation of the different MS measuring techniques with brief descriptions

Measuring technique	**Description**
HRMS or FS	*HRMS: High Resolution Mass Spectrometry* *FS: Full-Scan* Detection of accurate masses of all ions formed in the ion source within a specified mass spectrum over the entire chromatographic run time.
MS/HRMS	Selection and fragmentation of an ion (precursor) and detection of accurate masses of formed fragments. The precursor ion is selected according to various criteria:
MS/MS Target	Selection of specific precursor masses of which an MS/MS is measured.
Data dependent acquisition (DDA)	Selection of several MRM/SRM experiments in one measurement. The device scans for precursor ions across the entire cycle time and MS/MS fragmentation is triggered if a threshold for signal intensity is exceeded. (example in Appendix D)
Data independent acquisition (DIA) and analogous measuring modes (MS^E, MS/MS^{all} or AIF)	Permanent/alternating fragmentation of all molecule ions Option for rapidly scanning selected mass range windows consecutively (MS^E, SWATH®) are available from some manufacturers. Significantly more complex data evaluation! (example in Appendix D)

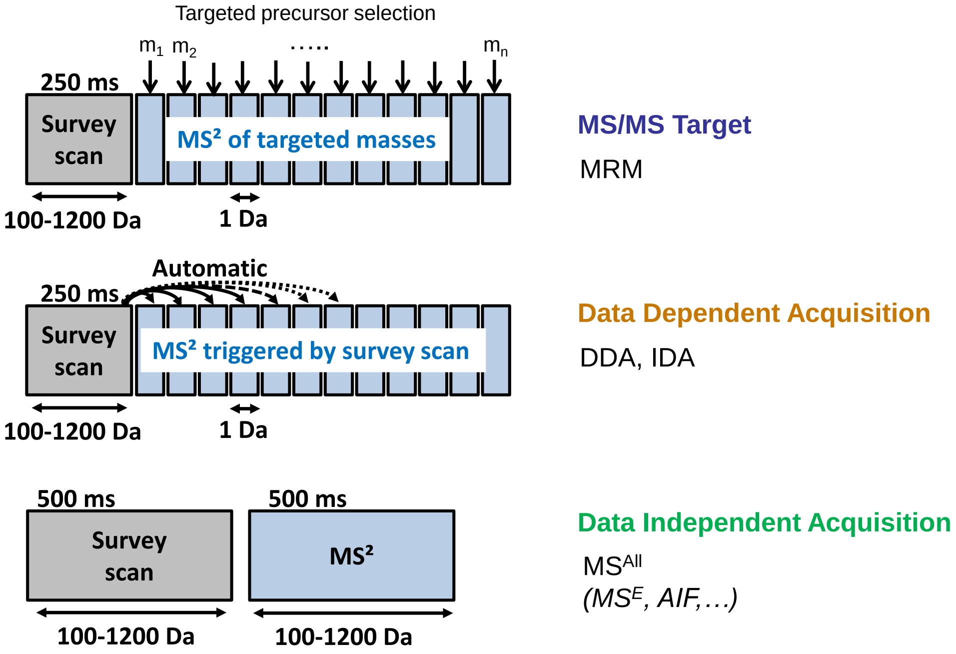


Figure 9.1: Schematic diagram of various possible MS² measuring modes

9.3.3 Mass calibration and mass accuracy

Depending on the measurement system, it is necessary to perform and/or check mass calibration at regular intervals and document the results. Calibrate all measurement (MS and MS²) and ionisation modes (ESI positive and negative) according to the manufacturer's instructions. Use the specified calibration solutions or standards. The mass calibration can be performed internally and/or externally and must cover the relevant mass range.

9.3.4 QA of LC-HRMS measurement

The use of isotope labelled standards (see section 7.5) as internal standards covering the retention time and mass range is required to verify system stability regarding retention time, mass accuracy, sensitivity and matrix effects.

10 Evaluation

10.1 Measurement data

The manufacturer's software is generally used to evaluate LC-HRMS data. This may be complemented or replaced by software from other manufacturers or proprietary developments for specific tasks and problems. Additionally, numerous *open-source* algorithms have been developed, and may also have advantages compared to individual approaches. Free availability and comparability across different instrument platforms are the advantages of open-source algorithms. With it different data formats from different platforms can be processed using the same workflow after converting the original acquisition data into free formats, such as *.mzML or *.mz(X)ML.

The first steps of data processing are decisive for the results of Non-Target Screening [22] and will be explained individually in further detail below.

10.1.1 Peak finding

The determination of features is the first crucial step. All further steps of data evaluation depend won the results of peak finding. Peak finding may be performed manually depending on the task, e.g. based on a Suspect Target List. In Non-Target Screening, this is done by a specific peak finding algorithm. There are various strategies, three of which are listed here as examples:

- The first strategy considers the two coordinates of RT and *m/z* independently. The variation of mass is examined by the *m/z* axis and the course of intensity is examined by the retention time axis. Hereby the definition of an intensity threshold is a decisive criterion for feature detection.
- The second strategy consists of the analysis of extracted ion chromatograms within a narrow *m/z* range. The resulting ion chromatograms can then be examined for chromatographic peaks independently of each other, using a suitable filter (e.g. a second-order Gauss filter). In this strategy, the search for peaks in the complete *m/z* range is avoided.
- The third strategy consists of a model fit to the raw data. For example, a model may consist of a three-dimensional fit of an isotope pattern starting with the peak of highest adundance and subsequent subtraction. This process is iteratively applied until only white noise is left.

For further details refer to [23] .

For optimisation of all peak finding parameters the problem of false positive or false negative results should be taken into account. Excessively strict parameters lead to false negative findings, that is, real signals are no more detected automatically. On the other hand, excessively generous parameters increase the false positive rate by recording noise, which is erroneously detected as a peak. This contradictory behaviour of false positive and false negative findings makes it more difficult to optimise peak finding and requires compromises. Here, it is advisable to minimise the number of false negative findings and initially accept an increased false positive rate. This can be reduced by filter criteria afterwards (after the actual peak finding process). The so called intensity threshold which defines the minimum signal height of features has a major impact on the result. This value should be selected in a way that the majority of known features within the relevant concentration range can still be detected.

To optimize the peak finding step for each new measurement campaign, spiking of known (isotope labelled) compounds in the relevant concentration range (0.1 µg/L) to sample matrix is recommended (QA control sample; see section 7.6.3). Since the peak finding step is strongly dependent on signal abundance, a sufficient long-term stability of the MS sensitivity is required (see Appendix E.2). Intensity-dependent parameters (such as threshold value for white noise ("noise threshold")) are particularly decisive in generic peak extraction and define the number of features found by the algorithm. This limits false positive results and avoids false negatives. For technical reasons (e.g. adjustment of detector voltage, replacement of detector or ESI needle), the base sensitivity of a MS may deviate between two measuring series. Therefore, the intensity-dependent parameters of the peak finding algorithm has to be adjusted in such cases; an example of such a strategy is shown in Appendix F. This is also the case if an existing data evaluation method is transferred to a new MS machine.

Validation based on the QA control sample can be used to assess and optimize the "performance" of the data evaluation method. Common figures of merit such as the false positive rate, recall or precision allow a comprehensive evaluation of this step. The quality of all subsequent steps and therefore the final results are significantly affected by this step which emphasises its importance.

10.1.2 Alignment

Alignment consists of binning the same features within an individual sample or between various samples. The detected features are compared by retention time and mass domains. The result is a data matrix consisting of features (lines) and samples (columns) with the peak height or peak area as the matrix input. In order to improve the binning between the samples, a retention time correction and mass recalibration can be performed, e.g. using internal standards (see Appendix B.1).

10.1.3 Blank correction

The consideration of the blank must be particularly emphasised in data processing. It is primarily used to minimise false positive findings. The blank must be selected in a way that the samples are suitably matched. If the incorrect blank is included in the data evaluation, there is a risk of eliminating real features (generation of false negative findings). A system, field or transport blank is recommended in direct sample measurements. For processed samples such as SPE extracts, false positive findings are kept to a minimum by selecting an extraction blank. Further explanations on possible blanks and their consideration are provided in section 5.

10.1.4 Componentisation

A compound can generate various adducts during ionisation (see Appendix G). There is also an isotope pattern for each of these adducts. The ion source may also produce fragmentations that generate further features for a molecule. Numerous features may therefore be assigned to one compound under certain conditions. Componentisation detects these features and merges them into one compound. Terms used for these binned compounds vary depending on the software package and manufacturer (e.g. Molecular Feature (Agilent), Bucket (Bruker), Feature (Sciex), Merged Feature (ThermoFisher)).

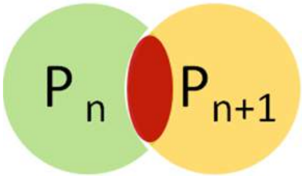
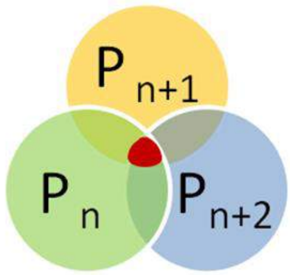
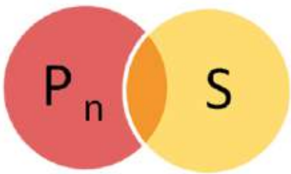
10.1.5 Generation of chemical formula

Possible chemical formulas can be suggested based on accurate mass and isotope pattern. The "Seven Golden Rules" for determining chemical formulas from measurement data are described in [11]. The more precise the accurate mass, the fewer options for possible chemical formulas will result. The nature and extent of suggestions for chemical formula also depends on the selected elements used to calculate the chemical formulas. An unequivocal chemical formula is only rarely obtained from the measurement data. [11]

10.2 Interpretation

Validated data from evaluation (see section Table 1.1) are a prerequisite for solving the analytical tasks (10.1). The results can be shown for example in a mass retention time plot (scatter plot). The determined scatter plots can be considered as quantities P_n (in a mathematical sense). The elements of the quantities are the features (components), characterised by the accurate mass and retention time. Intensities can be similarly evaluated according to the task. Some tasks for temporal resolved sample series are compiled in Table 10.1 using the notation of set theory.

Table 10.1: Schematic diagram of scatter plot comparison using set theory

Task	Symbolic depiction	Quantity theoretical description
Feature is contained in two consecutive samples		$P_n \cap P_{n+1}$
Feature is contained in three consecutive samples		$P_n \cap P_{n+1} \cap P_{n+2}$
Feature is contained in all 14 samples of the series	$S = P_n \cap P_{n+1} \cap \ldots P_{14}$	
Feature is contained in only one sample of the series		$P_n \setminus S$

10.2.1 Identification

Depending on the available information and degree of confidence, compound identification can be subdivided into categories or levels of confidence. [24] Uniform categorisation is a prerequisite for comparing results from different laboratories. For communication of the results from Non-Target Screening generally two groups of recipients can be distinguished. One group includes recipients without detailed knowledge on measurement technology and data evaluation, while the other group possesses this detailed knowledge. The purpose of differentiating the communication of results in this way is to focus on the information that is significant to the recipient. Table 10.2 shows the classification with the corresponding levels of confidence.

The categorisation is based on the information generated with LC-HRMS, namely the retention time, accurate mass and measured MS^2 spectra. Other measurement data such as CCS values (Collision Cross Section) from ion mobility measurements may further contribute to delimiting database hits and confirm substance identification. [25]

Table 10.2: Classification of the identification of features from HRMS screening

Presentation of results and processing of features (signals) from HRMS screening

Customer log		Processor log		Reference data				
Signal*	Statement	Signal*	Statement	Accurate mass	RT (RTI)	MS2 database	MS2 reference	MS2 Insilico
Cat. 1	Identified substance	Category 1	Confirmed substance / structure	✓	✓	✓	✓	!
Cat. 2	Probable substance	Category 2***	Probable substance/ structure	✓	!	✓	✗	!
Cat. 3	Suggested compound from chemical formula	Category 3a	Possible structure, information of metadata	✓	!	✗	✗	✓
		Category 3b	Possible compound	✓	✓	✗	✗	✗
Cat. 4	Signal of a compound	Category 4a**	Chemical formula	✓	✗	✗	✗	✗
		Category 4b	Feature (signal)	✗	✗	✗	✗	✗

* A signal is characterised by the accurate mass, the retention time and the abundance.

** A sum formula can be stated when at least two isotopes and/or adducts can be identified in the signals.

*** Confirmation by a reference standard is required.

Legend:
- ✗ not present
- ! can be present
- ✓ must be present

10.2.1.1 Databases

The use of databases can be a rapid and effective method to support the identification of features. Success is dependent on search criteria and the extents of database entries. A variety of databases are available on the internet. For general chemical databases with several million entries such as PubChem [12], ChemSpider [13], there may be hundreds of hits for a queried mass or chemical formula. Some databases permit prioritisation of multiple hits by meta-information. For example, a retention time estimate using quantitative structure retention models may help to prioritise suggested structures that match the measured retention. [26] Other metadata that can be used to prioritise hits includes e.g. the mumber of literature references, toxicity data or intended uses and quantities of a compound. The working platform FOR-IDENT [27] with the database STOFF-IDENT [substance identification] [14] and other environmentally relevant compound databases such as Chemistry Dashboard [28] and Norman Network Databases [29] provide support specifically for identifying substances relevant for water. Databases are queried not only for accurate mass or chemical formulas, but also for further information (for metadata, see 10.2.1.2). In order to prioritise an individual compound suggestion from multiple hits for a queried mass or chemical formula, the FOR-IDENT platform uses the standardised retention time, chemical formula and/or MS-MS spectra (matching with *in-silico* fragmentation spectra).

10.2.1.2 Metadata

Further information on the analysed sample is helpful for identifying features or compounds. Such metadata may include e.g. properties of substances, where they have been found, application areas, production volumes, possible transformation products or by-products from production or usage.

10.2.2 Statistical methods

Depending on the high quantity and complexity of data obtained in Non-Target Screening, multivariate statistical methods like the principal component analysis (PCA) are helpful in data evaluation. [30] Various software tools offer a variety of options for different statistical approaches. [22]

11 Reporting of results

A documentation of the used workflow is mandatory to obtain comparable analytical results from LC-HRMS measurements as far as possible. Particularly when using databases, it is possible to obtain comparable results by careful selection documentation of the parameters used for the query. The parameterisations of data processing and database queries must be documented as comprehensively as possible to ensure traceability.

A uniform description of the confidence of the identification of unknown features (categorisation) is a further prerequisite for comparable LC-HRMS screening results (see 10.2.1).

12 Collaborative trial

12.1 Participants

Name	Institution / Company
Brüggen, Susanne	Landesamt für Natur, Umwelt und Verbraucherschutz NRW D - 47051 Duisburg
Dünnbier, Uwe	Labor der Berliner Wasserbetriebe (BWB) D - 13629 Berlin
Fink, Angelika Götz, Sven	Hessenwasser GmbH & Co. KG D - 64293 Darmstadt
Geiß, Sabine	Thüringer Landesanstalt für Umwelt und Geologie Environmental analysis / environmental radioactivity D-07745 Jena
Letzel, Thomas Grosse, Sylvia	Technical University of Munich (TUM) D - 80333 Munich
Petri, Michael	ZV Bodensee-Wasserversorgung D - 78354 Sipplingen
Scheurer, Marco	DVGW-Technologiezentrum Wasser (German Water Centre) D - 76139 Karlsruhe
Schlüsener, Michael Kunkel, Uwe	German Federal Institute of Hydrology D - 56068 Koblenz
Schulz, Wolfgang Lucke, Thomas	Zweckverband Landeswasserversorgung (LW) D - 89129 Langenau
Singer, Heinz	Eawag CH - 8600 Dübendorf
Stötzer, Sebastian	Bachema AG CH - 8952 Schlieren
Schlett, Claus	Westfälische Wasser- and Umweltanalytik GmbH D - 45891 Gelsenkirchen
Seiwert, Bettina	HelmholtzCentre for Environmental Research GmbH - UFZ Analytical Department D - 04318 Leipzig
Sengl, Manfred	Bavarian Environment Agency D - 86179 Augsburg
Türk, Jochen	Institut für Lebensmittel- and Umweltforschung e.V. (IUTA) D - 47229 Duisburg
Zwiener, Christian	University of Tübingen Environmental Analytical Chemistry D - 72074 Tübingen

12.2 Implementation

Within the scope of the "Non-Target Screening" expert committee of the Wasserchemischen Gesellschaft (see 12.1), two collaborative trials have been performed.

12.2.1 Collaborative trial A

- Participants:
    - Sent to 18 participants (returned 15 datasets)
    - MS manufacturers: Agilent, SCIEX, ThermoFisher, Waters
- Sample set:
    - Blanks and methanolic reference standards (10 mg/L) for dilution by the participant
    - 5 substances for positive and negative electrospray ionisation, respectively
    - 2 additional substances that can be ionised in both ESI modes
- Specifications:
    - Fixed injection volume of 10 µL (for comparative evaluation of MS sensitivity)
    - Literature spectra of known compounds
- Analysis:
    - (Suspect) Target Screening for known compounds using the LC-HRMS methods established among the participants
- Task:
    - Dilution of the standard solution in decade steps
    - Single measurement of dilutions to determine detection limits (detection of at least two fragment ions)
    - Comparison of MS-MS spectra with literature spectra
    - Triplicate measurements at the detection limit
- Recorded data:
    - Applied method
    - Precursor masses
    - Detection limits

12.2.2 Collaborative trial B

- Participants:
    - 21 participants (returned 18 datasets)
    - MS manufacturers: Agilent, SCIEX, Bruker, Thermo, Waters
- Sample set:
    - 4 randomised spiked water samples from the river Danube, S Germany (unspiked, 0.025, 0.10 and 0.50 µg/L)
    - 24 spiked compounds (not known to the participant, but included in suspect list)
- Specifications:
    - Suspect/Non-Target Screening (established workflows)
    - Suspect list (approx. 200 substances)
    - RTI-Standard. (TUM) – data return and evaluation TUM
- Analysis:
    - Established screening workflows (Suspect or Non-Target)
- Task:
    - Identification of spiked compounds
    - Verification of chemcial formulas (isotopes)
    - Type of identification (database, reference standard)
    - Identification and categorisation (according to 10.2.1)

12.3 Results

12.3.1 Methods used

All participants used LC separation with reversed phase chromatography with methanol or acetonitrile and acid additives to improve ESI ionisation. All participants used electrospray ionisation in both the positive and negative mode. Automated detection of MS/MS spectra in the same run was dependent on the scan speed of the mass spectrometers. If automatic recording was not possible, MS/MS spectra were obtained in separate runs and used for evaluation.

12.3.2 Sensitivity

System sensitivity was evaluated by dilution of the methanolic solutions of 10 mg/L per substance in decadic increments with water. The dilution at which two of the reported fragment ions could be barely detected at an injection volume of 10 µL was defined as the detection limit (Figure 12.1).

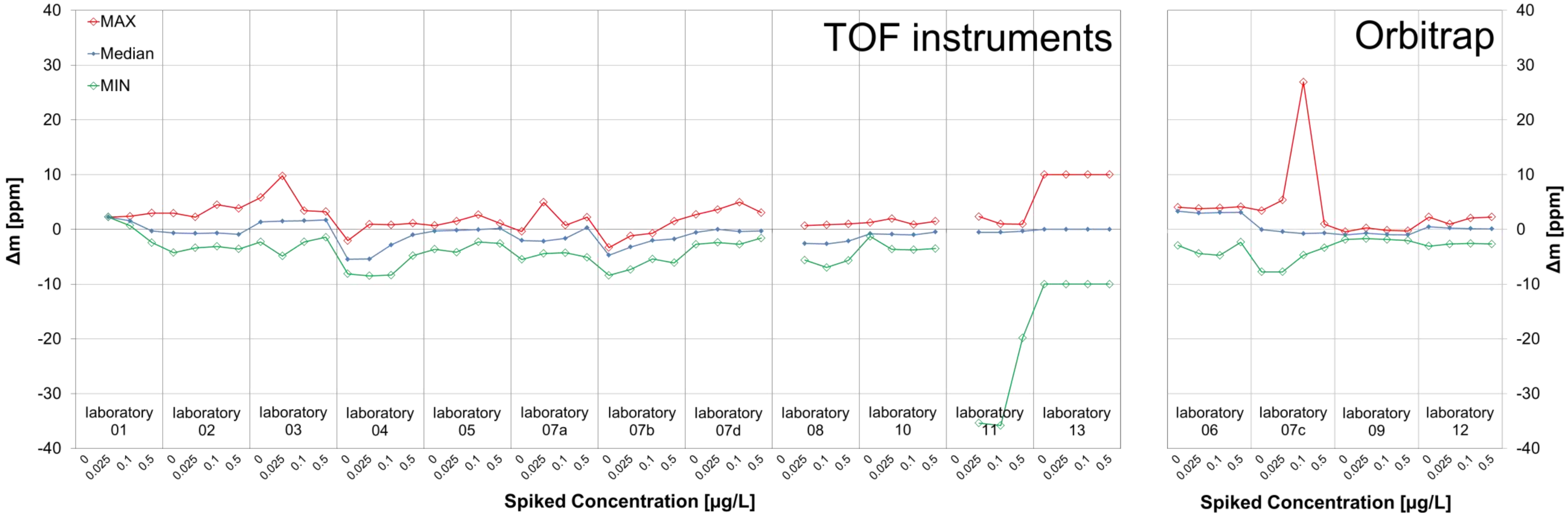
Detection Limits in µg/L												
		No. 1	No. 6	No. 7	No. 8	No. 10	No. 11	No. 13	No. 2	No. 3	No. 4	No. 12
ESI pos	Alachlor	1	100	1	1	100	10	0.1	0.01	100	0.1	0.01
	Atrazine	0.1	10	0.1	0.1	10	0.1	0.1	0.1	0.0001	0.01	0.01
	Clarithromycin	0.1	1000	1	1	10	1	0.1	0.1	0.001	0.1	0.01
	Gabapentin	1	100	0.1	1	10	1	0.1	n.n	0.0001	0.01	1
	Quinoxyfen	0.1	1000	0.1	0.1	10	0.1	0.1	0.01	0.0001	0.1	0.01
	Valsartan	0.1	n.n.	0.1	1	100	1	0.1	0.01	0.0001	0.01	1
	Candesartan	0.001	100	0.1	0.1	1	1	0.1	0.1	0.0001	0.01	1
ESI neg	PFNA	n.n.	1000	0.1	1	100	10	n.a.	1	0.0001	0.1	0.1
	HCT	1	1000	1	1	10	10	n.a.	0.1	0.0001	0.1	1
	Mecoprop	1	1000	1	1	10	10	n.a.	0.1	0.0001	0.1	1
	Ioxynil	0.01	1000	0.1	0.1	1	1	n.a.	0.01	0.0001	0.1	0.1
	Dinoseb	0.01	100	0.1	0.1	0.1	0.1	n.a.	0.01	0.0001	0.1	0.01
	Valsartan	0.01	n.n.	0.1	1	10	10	n.a.	0.1	0.0001	0.1	1
	Candesartan	0.001	100	0.1	1	10	10	n.a.	0.1	0.001	0.1	1
not measured / analysed		TOF instruments							Orbitrap			

Figure 12.1: Comparison of detection limits with two detectable fragment ions (laboratories 6 and 3 outliers) PFNA: Perfluorononanoic acid; HCT: Hydrochlorothiazide

12.3.3 Mass accuracy

The overall median of all mass deviations of molecular ions of the spiked compounds was below 5 ppm. There were no differences found in the mass precisions between the TOF and Orbitrap mass spectrometers. The mass deviations were furthermore independent of the spiked concentrations (Figure 12.2).

Figure 12.2: Mass deviations in the MS mode (laboratories 8 and 11: unspiked samples not measured)



12.3.4 Mass accuracy of fragment masses (MS/MS)

Qualitative differences in the fragmentation spectra were mainly due to different collision energies. The mass accuracy of the fragment ions differed between the TOF and Orbitrap MS. Time-of-flight mass spectrometers (Figure 12.3) show a slightly greater mass deviation in MS/MS experiments compared to Orbitrap devices (Figure 12.4). The deviations are usually in the range below 5 mDa for TOF MS, corresponding to a relative deviation of 5 to 50 ppm. For Orbitrap MS the absolute mass deviations are usually below 2 mDa, corresponding to a relative deviation of 2 to 40 ppm (mass range *m/z* 50 - 1000).

Figure 12.3: *Mass deviations of MS/MS fragments of spiked compounds (TOF insruments); sorted by fragment mass and separated by ionisation mode*

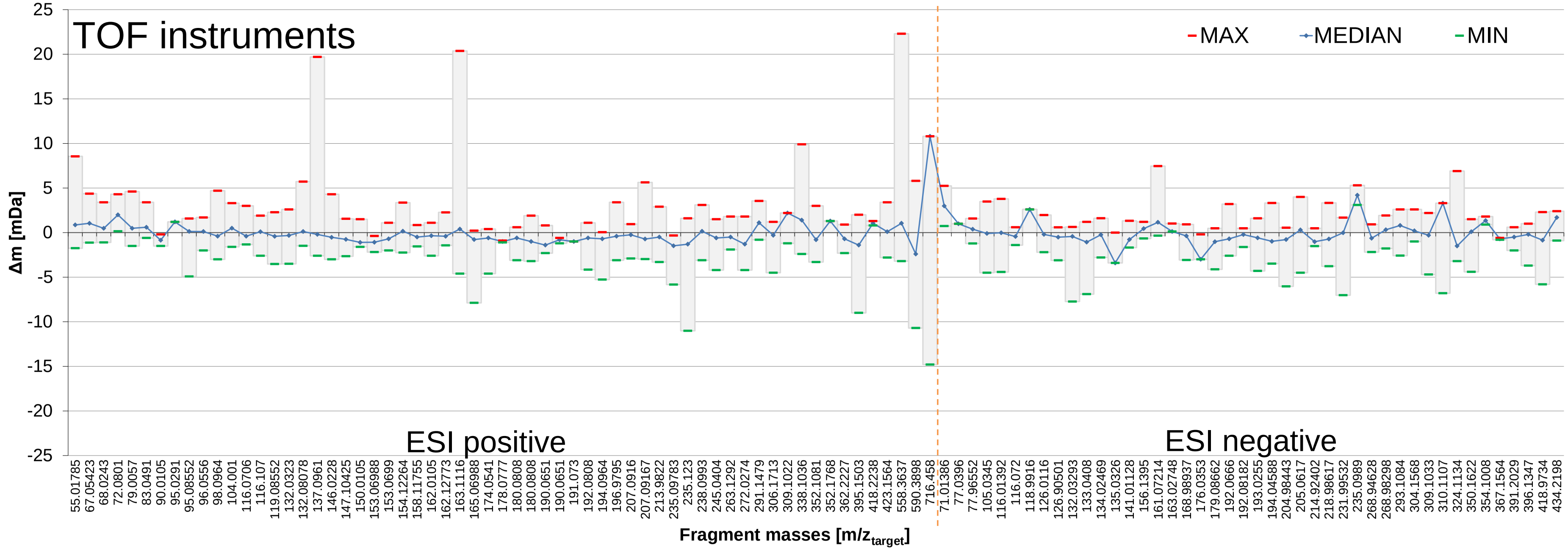
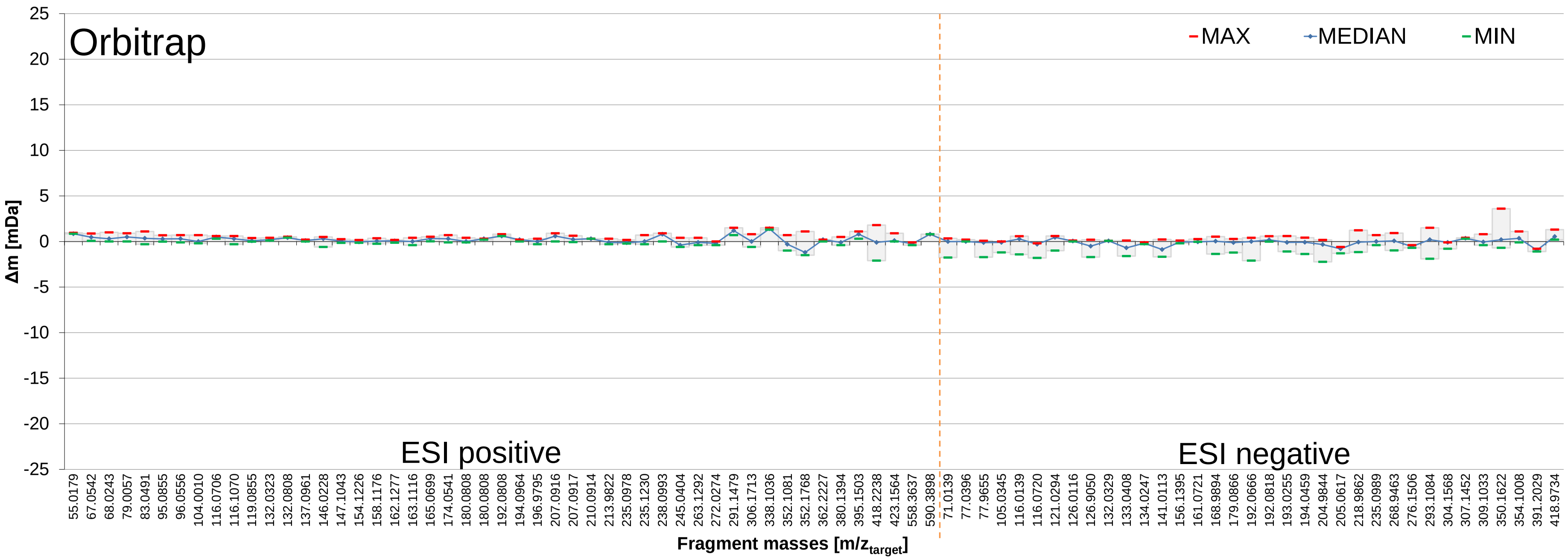


Figure 12.4: *Mass deviations of MS/MS fragments of spiked compounds (Orbitrap); sorted by fragment mass and separated by ionisation mode*

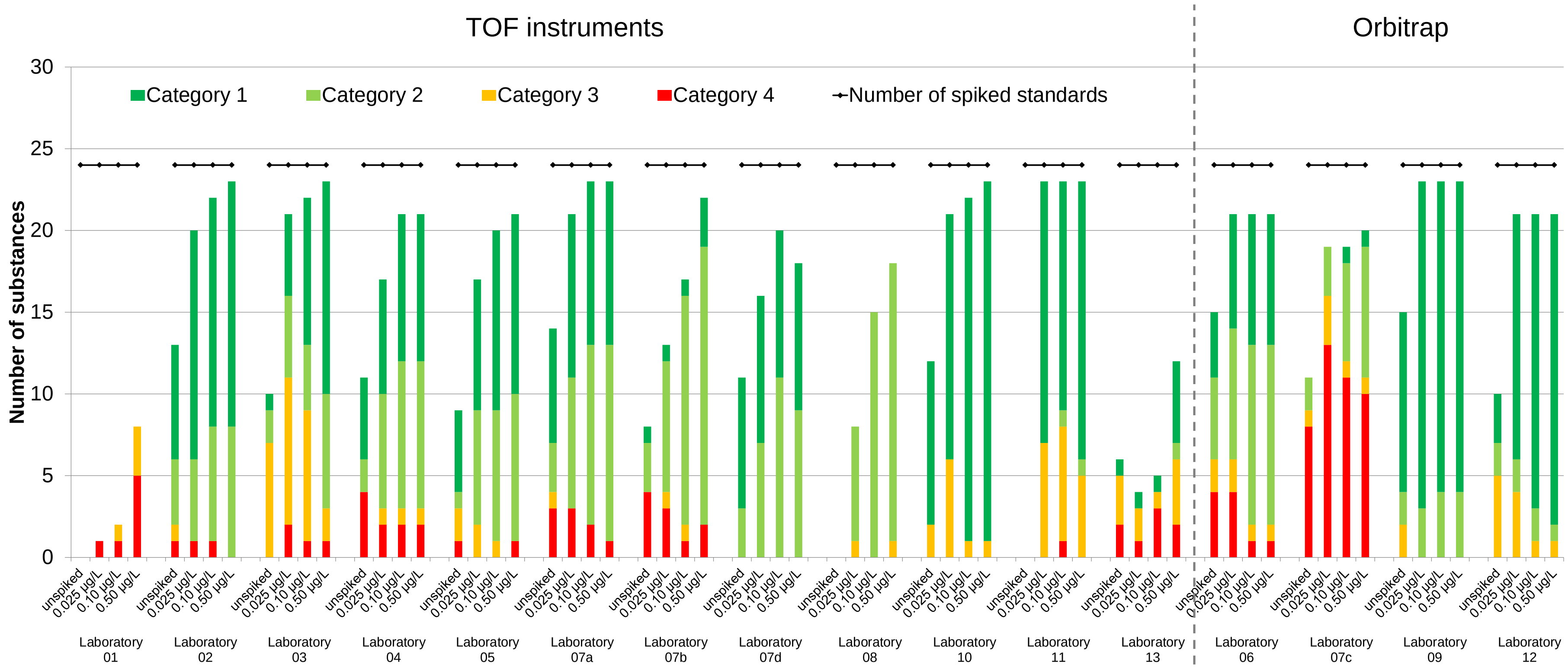


12.3.5 Data evaluation and substance identification

Figure 12.5 shows the numbers of the correctly identified standard substances of the participating laboratories. Compound identifications were categorised according to the criteria shown in section 10.2.1. The increase in the fraction of identifications in categories 1 (confirmed compound identification) and 2 (probable identification) with increasing spiking levels is clearly visible. This is generally due to the increased ability to detect a clean and meaningful MS/MS spectrum.

Results from laboratory 7 are a special case. The participation of a laboratory with altogether four LC-HRMS systems (7a to 7d operated by another person) reveals that the applied MS (particularly the software options) and the available database (measured reference standards and MS^2 spectra) have a major impact on the number of confirmed identifications. Significantly fewer substances were correctly identified and confirmed in particular from laboratory 7c. The number of qualitative detections was similar to the other platforms. This may be due to a low number of available reference spectra or a more complex software for the identification step. Last but not least, the experience of the user and the time put into the data evaluation also play a decisive role.

Figure 12.5: Comparison of identified compounds of participating laboratories according to identification categories 1 to 4



12.3.6 Comparison of Workflows

In addition to the collaborative trial, one of the datasets of the second trial was evaluated using three different workflows to determine the influence of the approach on the number of correctly identified compounds (Figure 12.6).

The three applied workflows were structured as follows:

1. Suspect screening for the entire suspect list (200 compounds) and manual inspection of the identification by matching of MS^2 spectra libraries
2. Non-Target approach with peak finding by the *open-source*-tool envipy [31] and subsequent manual inspection of identification based on reference spectra
3. Non-Target approach (internally at the laboratory) with data evaluation and subsequent FOR-IDENT query to prioritise suggested hits. Identification using a MS^2 spectra database.

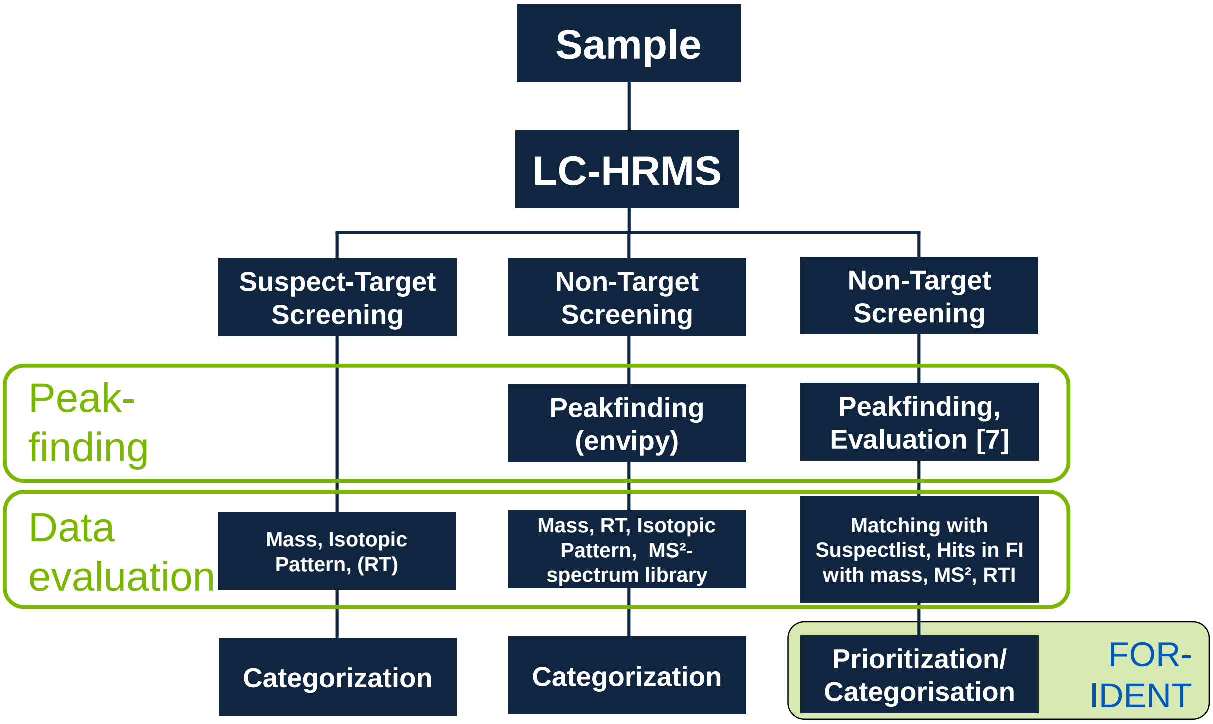


Figure 12.6: Structure of three different workflows for detection and identification of substances

The comparison of the results of the three workflows (Figure 12.7) demonstrates good detectability of the spiked compounds. For workflow 2 (Figure 12.7, middle), the number of detected compounds (categories 1 to 4) is slightly below the other two workflows. This might be due to insufficient optimisation of the peak finding parameters. The peak finding in the third workflow was developed on the LC-HRMS system used for the measurement and is therefore surely best suited to this system. This is reflected by the high detection numbers. The preconditions for the identification (MS^2 spectra, databases) were the same in all cases. The barely different number of compounds found in categories 1 and 2 reveals that. The benefits of automation are therefore best demonstrated in terms of the required time. The detection of compounds was only scarcely affected by the choice of workflows.

The first workflow (Suspect-Target Screening) required the most time, since processing and manual inspection of the hits of 200 substances for identification was necessary in this case. Furthermore, reference spectra had to be searched in databases available on the internet for all compounds not already included in the available spectra library.

However, the extent of manual steps in the workflow drop considerably from 1) to 3). This is due to automated peak finding in cases 2) and 3), but also specifically due to automated prioritisation of suggested hits by FOR-IDENT in case 3). As expected, with increasing concentration levels the number of detected compounds also increased.

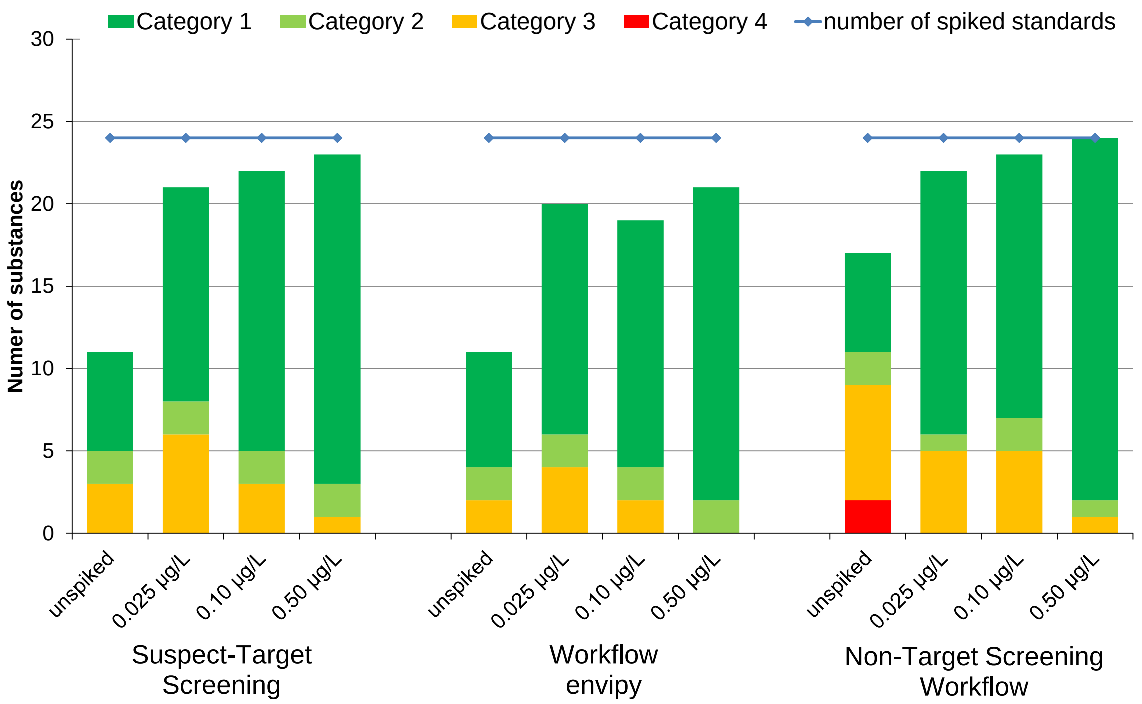


Figure 12.7: Comparison of identification results of a dataset with three different evaluation workflows

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Appendix A. "Non-Target Screening" expert committee

A.1 Background and tasks

In 2009, the Water Chemistry Society (a divison of the Gesellschaft Deutscher Chemiker e.V) founded the Non-Target Screening expert committee. The idea was to provide support in the identification of trace organic compounds in LC-MS analysis by providing a suitable compound database (also applicable for data from low-resolution MS). The development of high resolution mass spectrometers for routine use has shifted the tasks in the direction of target analysis, Suspect Target and Non-Target Screening. The tasks include: Developing strategies for Non-Target Screening, comparability of results based on various analytical platforms, standardisation of Suspect Target Screening, and quality assurance.

A.2 Members of the expert committee

Table A.1: Members of the "Non-Target Screening" expert committee

Name	Institution / Address
Head: **Schulz, Wolfgang**[1]	Zweckverband Landeswasserversorgung Laboratory of operation control and research Am Spitzigen Berg 1 D-89129 Langenau
Achten, Christine **Oberleitner, Daniela**	University of Münster Institute of Geology and Palaeontology Applied Geology Correnstr. 24 D-48149 Münster
Balsaa, Peter **Hinnenkamp, Vanessa**	IWW Water Centre Moritzstr. 8 D-45476 Mülheim a.d.R.
Brüggen, Susanne	Landesamt für Natur, Umwelt und Verbraucherschutz NRW Wuhanstraße 6 D-47051 Duisburg
Dünnbier, Uwe[1] **Liebmann, Diana**	Labor der Berliner Wasserbetriebe (BWB) Motardstr. 35 D-13629 Berlin
Fink, Angelika **Götz, Sven**	Hessenwasser GmbH & Co. KG Gräfenhäuser Straße 118 D-64293 Darmstadt
Geiß, Sabine	Thüringer Landesanstalt für Umwelt and Geologie Environmental Analysis / Environmental Radioactivity Göschwitzer Str. 41 D-07745 Jena
Hohrenk Lotta	University of Duisburg-Essen Instrumental Analytical Chemistry (IAC) Universitätsstr. 5 D-45141 Essen
Härtel, Christoph	Ruhrverband Kronprinzenstr. 37 D-45128 Essen
Letzel, Thomas[1]	Technical University of Munich (TUM) Am Coulombwall 3 D-85748 Garching
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Name	Institution / Address
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Petri, Michael	ZV Bodensee-Wasserversorgung Laboratory of operation control and research Süßenmühle 1 D-78354 Sipplingen
Sawal, George	Federal Environment Agency FG II 2.5 Laboratory for Water Analysis Bismarckplatz 1 D-14193 Berlin
Scheurer, Marco **Nürenberg, Gudrun**	DVGW-Technologiezentrum Wasser (German Water Centre) Karlsruher Str. 84 D-76139 Karlsruhe
Schlüsener, Michael	German Federal Institute of Hydrology Am Mainzer Tor 1 D-56068 Koblenz
Seiwert, Bettina	Helmholtz-Centre for Environmental Research GmbH – UFZ Analytical Department Permoserstraße 15 D-04318 Leipzig
Sengl, Manfred[1] **Kunkel, Uwe**	Bavarian Environment Agency Bürgermeister-Ulrich-Str. 160 D-86179 Augsburg
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Türk, Jochen	Institut für Lebensmittel- and Umweltforschung e.V. (IUTA) Bliersheimer Str. 58-60 D-47229 Duisburg
Zwiener, Christian	University of Tübingen Environmental Analytical Chemistry at the Center for Applied Geoscience Hölderlinstr. 121 D-72074 Tübingen

[1] Project Partner in the BMBF Research Project FOR-IDENT (funding ID 02WRS1354D)

Appendix B. Mass and RT Testing

B.1 Isotopic labelled Internal Standards

Table B.1: List of isotope labelled internal standards, Eawag ($N_{ESI+} = 123$, $N_{ESI-} = 56$)[1]

No.	Name	Chemical formula	Retention time [min]
1	2,4-D-d3 $^{(-)}$	$C_8H_3{}^2H_3Cl_2O_3$	9.7
2	2,6-dichlorobenzamide-3,4,5-d3 $^{(+)}$	$C_7H_2{}^2H_3Cl_2NO$	5.8
3	5-methylbenzotriazole-d6	$C_7H{}^2H_6N_3$	6.5
4	Acetyl-sulfamethoxazole-d5	$C_{12}H_8{}^2H_5N_3O_4S$	7.0
5	Alachlor-d13 $^{(+)}$	$C_{14}H_7{}^2H_{13}ClNO_2$	12.8
6	Amisulpride-d5	$C_{17}H_{22}{}^2H_5N_3O_4S$	5.1
7	Atazanavir-d5	$C_{38}H_{47}{}^2H_5N_6O_7$	10.2
8	Atenolol acid-d5	$C_{14}H_{16}{}^2H_5NO_4$	4.8
9	Atenolol-d7 $^{(+)}$	$C_{14}H_{15}{}^2H_7N_2O_3$	4.5
10	Atomoxetine-d3 $^{(+)}$	$C_{17}H_{18}{}^2H_3NO$	7.7
11	Atorvastatin-d5	$C_{33}H_{30}{}^2H_5FN_2O_5$	11.8
12	Atrazine-d5 $^{(+)}$	$C_8H_9{}^2H_5ClN_5$	9.7
13	Atrazine-2-hydroxy-d5	$C_8H_{10}{}^2H_5N_5O$	4.9
14	Atrazine-desisopropyl-d5 $^{(+)}$	$C_5H_3{}^2H_5ClN_5$	5.5
15	Azithromycin-d3 $^{(+)}$	$C_{38}H_{69}{}^2H_3N_2O_{12}$	5.8
16	Azoxystrobin-d4 $^{(+)}$	$C_{22}H_{13}{}^2H_4N_3O_5$	11.8
17	Bentazon-d6	$C_{10}H_6{}^2H_6N_2O_3S$	9.4
18	Benzotriazole-d4	$C_6H{}^2H_4N_3$	5.5
19	Bezafibrate-d4	$C_{19}H_{16}{}^2H_4ClNO_4$	10.4
20	Bicalutamide-d4	$C_{18}H_{10}{}^2H_4F_4N_2O_4S$	11.0
21	Caffeine-d9 $^{(+)}$	$C_8H{}^2H_9N_4O_2$	5.0
22	Candesartan-d5	$C_{24}H_{15}{}^2H_5N_6O_3$	9.3
23	Carbamazepine-d8 $^{(+)}$	$C_{15}H_4{}^2H_8N_2O$	8.4
24	Carbamazepine-10,11-epoxide-13C,d2 $^{(+)}$	$C_{14}{}^{13}CH_{10}{}^2H_2N_2O_2$	7.2
25	Carbendazim-d4 $^{(+)}$	$C_9H_5{}^2H_4N_3O_2$	4.8
26	Cetirizine-d8	$C_{21}H_{17}{}^2H_8ClN_2O_3$	8.3
27	Chloridazon-d5	$C_{10}H_3{}^2H_5ClN_3O$	6.4
28	Chloridazon-methyl-desphenyl-d3	$C_5H_3{}^2H_3ClN_3O$	4.5
29	Chlorotoluron-d6 $^{(+)}$	$C_{10}H_7{}^2H_6ClN_2O$	9.3
30	Chlorpyrifos-d10 $^{(+)}$	$C_9H{}^2H_{10}Cl_3NO_3PS$	15.9
31	Chlorpyrifos-methyl-d6 $^{(+)}$	$C_7H{}^2H_6Cl_3NO_3PS$	14.4
32	Citalopram-d6 $^{(+)}$	$C_{20}H_{15}{}^2H_6FN_2O$	7.3
33	Clarithromycin-N-methyl-d3 $^{(+)}$	$C_{38}H_{66}{}^2H_3NO_{13}$	8.4
34	Climbazole-d4	$C_{15}H_{13}{}^2H_4ClN_2O_2$	8.4
35	Clofibric acid-d4 $^{(-)}$	$C_{10}H_7{}^2H_4ClO_3$	10.2
36	Clopidogrel carboxylic acid-d4 $^{(+)}$	$C_{15}H_{10}{}^2H_4ClNO_2S$	6.1
37	Clothianidin-d3	$C_6H_5{}^2H_3ClN_5O_2S$	6.3
38	Clotrimazole-d5 $^{(+)}$	$C_{22}H_{12}{}^2H_5ClN_2$	8.7
39	Clozapine-d8 $^{(+)}$	$C_{18}H_{11}{}^2H_8ClN_4$	6.5
40	Codeine-13C,d3 $^{(+)}$	$C_{17}{}^{13}CH_{18}{}^2H_3NO_3$	4.7
41	Cyclophosphamide-d4 $^{(+)}$	$C_7H_{11}{}^2H_4Cl_2N_2O_2P$	7.0
42	Cyprodinil-d5 $^{(+)}$	$C_{14}{}^2H_5H_{10}N_3$	10.7
43	Darunavir-d9	$C_{27}H_{28}{}^2H_9N_3O_7S$	10.4
44	Desethylatrazine-15N3 $^{(+)}$	$C_6H_{10}ClN_2{}^{15}N_3$	6.5
45	Desphenyl Chloridazon-15N2 $^{(+)}$	$C_4H_4ClN{}^{15}N_2O$	2.9
46	Diazepam-d5 $^{(+)}$	$C_{16}H_8{}^2H_5N_2OCl$	10.7
47	Diazinon-d10 $^{(+)}$	$C_{12}H_{11}{}^2H_{10}N_2O_3PS$	14.1
48	Dichlorprop-d6 $^{(-)}$	$C_9H_2{}^2H_6Cl_2O_3$	10.7
49	Diclofenac-d4	$C_{14}H_7{}^2H_4Cl_2NO_2$	12.1
50	Diflufenican-d3	$C_{19}H_8{}^2H_3F_5N_2O_2$	14.7
51	Dimethenamid-d3 $^{(+)}$	$C_{12}H_{15}{}^2H_3ClNO_2S$	11.7
52	Dimethoate-d6 $^{(+)}$	$C_5H_6{}^2H_6NO_3PS_2$	6.7
53	Diuron-d6	$C_9H_4{}^2H_6Cl_2N_2O$	9.8
54	Emtricitabine-13C,15N2 $^{(+)}$	$C_7{}^{13}CH_{10}FN{}^{15}N_2O_3S$	4.5
55	Epoxiconazole-d4 $^{(+)}$	$C_{17}H_9{}^2H_4ClFN_3O$	11.9
56	Eprosartan-d3	$C_{23}H_{21}{}^2H_3N_2O_4S$	6.6

[1] Eawag - Environmental Chemistry

No.	Name	Chemical formula	Retention time [min]
57	Erythromycin-13C2 (+)	$C_{35}{}^{13}C_2H_{67}NO_{13}$	7.4
58	Fenofibrate-d6 (+)	$C_{20}H_{15}{}^2H_6ClO_4$	15.9
59	Fipronil-13C2,15N2	$C_{10}{}^{13}C_2H_4Cl_2F_6N_2{}^{15}N_2OS$	13.4
60	Fluconazole-d4	$C_{13}H_8{}^2H_4F_2N_6O$	5.9
61	Fluoxetine-d5 (+)	$C_{17}H_{13}{}^2H_5F_3NO$	8.4
62	Furosemide-d5 (-)	$C_{12}H_6{}^2H_5ClN_2O_5S$	8.3
63	Gabapentin-d4	$C_9H_{13}{}^2H_4NO_2$	4.7
64	Hydrochlorothiazide-13C,d2	$C_6{}^{13}CH_6{}^2H_2ClN_3O_4S_2$	5.1
65	Ibuprofen-d3 (+)	$C_{13}H_{15}{}^2H_3O_2$	12.4
66	Imidacloprid-d4	$C_9H_6{}^2H_4ClN_5O_2$	6.5
67	Indomethacin-d4	$C_{19}H_{12}{}^2H_4ClNO_4$	12.1
68	Irbesartan-d3	$C_{25}H_{25}{}^2H_3N_6O$	8.8
69	Irgarol-d9 (+)	$C_{11}H_{10}{}^2H_9N_5S$	9.8
70	Isoproturon-d6 (+)	$C_{12}H_{12}{}^2H_6N_2O$	9.7
71	Lamotrigine-13C3,d3 (+)	$C_6{}^{13}C_3H_4{}^2H_3Cl_2N_5$	5.4
72	Levetiracetam-d3 (+)	$C_8H_{11}{}^2H_3N_2O_2$	4.8
73	Lidocaine-d10 (+)	$C_{14}H_{12}{}^2H_{10}N_2O$	5.3
74	Linuron-d6	$C_9H_4{}^2H_6Cl_2N_2O_2$	11.4
75	MCPA-d3 (-)	$C_9H_6{}^2H_3ClO_3$	9.8
76	Mecoprop-d6 (-)	$C_{10}H_5{}^2H_6ClO_3$	10.6
77	Mefenamic acid-d3	$C_{15}H_{12}{}^2H_3NO_2$	13.2
78	Mesotrione-d3	$C_{14}H_{10}{}^2H_3NO_7S$	8.8
79	Metalaxyl-d6 (+)	$C_{15}H_{15}{}^2H_6NO_4$	9.8
80	Methiocarb-d3 (+)	$C_{11}H_{12}{}^2H_3NO_2S$	11.2
81	Methylprednisolone-d3 (+)	$C_{22}H_{27}{}^2H_3O_5$	8.4
82	Metolachlor-d6 (+)	$C_{15}H_{16}{}^2H_6ClNO_2$	12.8
83	Metolachlor-ESA-d11	$C_{15}H_{12}{}^2H_{11}NO_5S$	7.2
84	Metoprolol-d7 (+)	$C_{15}H_{18}{}^2H_7NO_3$	5.6
85	Metronidazole-d4 (+)	$C_6H_5{}^2H_4N_3O_3$	4.7
86	Metsulfuron-methyl-d3	$C_{14}H_{12}{}^2H_3N_5O_6S$	8.8
87	Morphine-d3 (+)	$C_{17}H_{16}{}^2H_3NO_3$	4.3
88	N,N-diethyl-3-methylbenzamide-d10 (+)	$C_{12}H_7{}^2H_{10}NO$	9.8
89	N,O-didesmethyl venlafaxine-d3 (+)	$C_{15}H_{20}{}^2H_3NO_2$	5.1
90	N4-Acetyl-sulfathiazole-d4	$C_{11}H_7{}^2H_4N_3O_3S_2$	5.4
91	Naproxen-d3 (+)	$C_{14}H_{11}{}^2H_3O_3$	10.3
92	Nelfinavir-d3	$C_{32}H_{42}{}^2H_3N_3O_4S$	8.9
93	Nicosulfuron-d6	$C_{15}H_{12}{}^2H_6N_6O_6S$	7.8
94	Octhilinone-d17 (+)	$C_{11}H_2{}^2H_{17}NOS$	11.5
95	O-Desmethylvenlafaxine-d6 (+)	$C_{16}H_{19}{}^2H_6NO_2$	5.2
96	Oxazepam-d5	$C_{15}H_6{}^2H_5ClN_2O_2$	8.8
97	Oxcarbazepine-d4 (+)	$C_{15}H_8{}^2H_4N_2O_2$	7.5
98	Paracetamol-d4 (+)	$C_8H_5{}^2H_4NO_2$	4.7
99	Phenazone-d3 (+)	$C_{11}H_9{}^2H_3N_2O$	5.8
100	Pirimicarb-d6 (+)	$C_{11}H_{12}{}^2H_6N_4O_2$	5.9
101	Pravastatin-d3 (-)	$C_{23}H_{33}{}^2H_3O_7$	8.1
102	Primidone-d5 (+)	$C_{12}H_9{}^2H_5N_2O_2$	5.8
103	Prochloraz-d7 (+)	$C_{15}H_9{}^2H_7Cl_3N_3O_2$	11.0
104	Propamocarb free base-d7 (+)	$C_9H_{13}{}^2H_7N_2O_2$	4.6
105	Propazine-d6 (+)	$C_9H_{10}{}^2H_6ClN_5$	11.0
106	Propiconazole-d5 (+)	$C_{15}H_{12}{}^2H_5Cl_2N_3O_2$	13.0
107	Propranolol-d7 (+)	$C_{16}H_{14}{}^2H_7NO_2$	6.7
108	Pyrimethanil-d5 (+)	$C_{12}H_8{}^2H_5N_3$	9.1
109	Ranitidine-d6	$C_{13}H_{16}{}^2H_6N_4O_3S$	4.5
110	Ritalinic acid-d10 (+)	$C_{13}H_7{}^2H_{10}NO_2$	5.2
111	Ritonavir-d6 (+)	$C_{37}H_{42}{}^2H_6N_6O_5S_2$	12.4
112	Simazine-d5 (+)	$C_7H_7{}^2H_5ClN_5$	8.3
113	Sotalol-d6	$C_{12}H_{14}{}^2H_6N_2O_3S$	4.5
114	Sulcotrione-d3	$C_{14}H_{10}{}^2H_3ClO_5S$	9.0
115	Sulfadiazine-d4	$C_{10}H_6{}^2H_4N_4O_2S$	5.1
116	Sulfadimethoxine-d4	$C_{12}H_{10}{}^2H_4N_4O_4S$	7.7
117	Sulfamethazine-13C6	$C_6{}^{13}C_6H_{14}N_4O_2S$	5.9
118	Sulfamethoxazole-d4	$C_{10}H_7{}^2H_4N_3O_3S$	6.8
119	Sulfapyridine-d4	$C_{11}H_7{}^2H_4N_3O_2S$	5.3
120	Sulfathiazole-d4	$C_9H_5{}^2H_4N_3O_2S_2$	5.1
121	Tebuconazole-d6 (+)	$C_{16}H_{16}{}^2H_6ClN_3O$	12.2
122	Terbuthylazine-d5 (+)	$C_9H_{11}{}^2H_5ClN_5$	11.3

No.	Name	Chemical formula	Retention time [min]
123	Terbutryn-d5 (+)	$C_{10}H_{14}{}^{2}H_5N_5S$	9.4
124	Thiamethoxam-d3 (+)	$C_8H_7{}^{2}H_3ClN_5O_3S$	5.7
125	Tramadol-d6 (+)	$C_{16}H_{19}{}^{2}H_6NO_2$	5.6
126	Trimethoprim-d9 (+)	$C_{14}H_9{}^{2}H_9N_4O_3$	4.9
127	Valsartan-13C5,15N	$C_{19}{}^{13}C_5H_{29}N_4{}^{15}NO_3$	10.8
128	Valsartan acid-d4	$C_{14}H_6{}^{2}H_4N_4O_2$	7.3
129	Venlafaxine-d6 (+)	$C_{17}H_{21}{}^{2}H_6NO_2$	6.3
130	Verapamil-d6 (+)	$C_{27}H_{32}{}^{2}H_6N_2O_4$	8.1

(+): ESI positive mode

(-): ESI negative mode

Table B.2: List of isotope labelled internal standards, LW[1]

Name	Chemical formula	Retention time [min]
Benzotriazole-d4 (+/-)	$C_6HN_3{}^{2}H_4$	5.4
Chloridazon-d5 (+/-)	$C_{10}H_3ClN_3O^{2}H_5$	6.3
Propazine-d6 (+)	$C_9H_{10}ClN_5{}^{2}H_6$	10.7
Diuron-d6 (+/-)	$C_9H_4Cl_2N_2O^{2}H_6$	9.6
Lidocaine-d10 (+)	$C_{14}H_{12}N_2O^{2}H_{10}$	5.2
Sotalol-d6 (+/-)	$C_{12}H_{14}N_2O_3S^{2}H_6$	4.4
Hydrochlorothiazide-13C,d2 (-)	$C_6H_6ClN_3O_4S_2{}^{13}C^{2}H_2$	5.1
Diazinon-d10 (+)	$C_{12}H_{11}N_2O_3PS^{2}H_{10}$	13.8
Sulfadimethoxine-d6 (+/-)	$C_{12}H_8N_4O_4S^{2}H_6$	7.5
Azoxystrobin-d4 (+)	$C_{22}H_{13}N_3O_5{}^{2}H_4$	11.5
Irbesartan-d4 (+/-)	$C_{25}H_{24}N_6O^{2}H_4$	8.6
Bicalutamide-d4 (+/-)	$C_{18}H_{10}F_4N_2O_4S^{2}H_4$	10.7
Darunavir-d9 (+/-)	$C_{27}H_{28}N_3O_7S^{2}H_9$	10.1
Fipronil-13C2,15N2 (+/-)	$C_{10}H_4Cl_2F_6N_2OS^{13}C_2{}^{15}N_2$	13.1

(+): ESI positive mode

(-): ESI negative mode

B.2 Standard for retention time standardisation and use

Table B.3: List of possible reference standards for RT monitoring and standardisation (distribution across the polarity range that can be covered with RP-LC)

Name	Chemical formula	logP (log K_{OW})
Metformin	$C_4H_{11}N_5$	-1.36
Chloridazon	$C_{10}H_8ClN_3O$	1.11
Carbetamide	$C_{12}H_{16}N_2O_3$	1.65
Monuron	$C_9H_{11}ClN_2O$	1.93
Metobromuron	$C_9H_{11}BrN_2O_2$	2.24
Chlorbromuron	$C_9H_{10}BrClN_2O_2$	2.85
Metconazole	$C_{17}H_{22}ClN_3O$	3.59
Diazinon	$C_{12}H_{21}N_2O_3PS$	4.19
Quinoxyfen	$C_{15}H_8Cl_2FNO$	4.98
Fenofibrate	$C_{20}H_{21}ClO_4$	5.28

[1] List of the Zweckverband Landeswasserversorgung

Table B.4: List of substances found in collaborative trial B with the number of RTI detections from 6 laboratories with the mean logD deviations and standard deviations

Name	CAS No.	Sum formula	logD (pH 3)	ESI mode	N_{RTI} (out of a total of 6 laboratories)	$\bar{x}$ Δ logD	s Δ logD
Gabapentin	*60142-96-3*	$C_9H_{17}NO_2$	*-2.00*	pos	18	1.4	0.61
				neg	12	1.5	0.73
Metoprolol acid	*56392-14-4*	$C_{14}H_{21}NO_4$	*-1.69*	pos	15	1.1	0.62
				neg	4	1.0	0.01
Propranolol	525-66-6	$C_{16}H_{21}NO_2$	-0.66	pos	15	1.1	0.31
				neg	-	-	-
Hydrochlorothiazide	58-93-5	$C_7H_8ClN_3O_4S_2$	-0.58	pos	10	-0.5	0.18
				neg	14	-0.3	0.27
Caffeine	58-08-2	$C_8H_{10}N_4O_2$	-0.55	pos	17	0.0	0.24
				neg	-	-	-
Clarithromycin	*81103-11-9*	$C_{38}H_{69}NO_{13}$	*-0.26*	pos	16	1.6	0.45
				neg	4	2.1	0.45
Atrazine-2-hydroxy	*2163-68-0*	$C_8H_{15}N_5O$	*0.00*	pos	14	-0.4	0.41
				neg	10	-0.6	0.08
Metamitron	41394-05-2	$C_{10}H_{10}N_4O$	0.24	pos	14	-0.3	0.14
				neg	7	-0.2	0.02
Sulfathiazole	72-14-0	$C_9H_9N_3O_2S_2$	0.93	pos	13	-0.7	0.24
				neg	9	-0.8	0.12
Desethylatrazine	6190-65-4	$C_6H_{10}ClN_5$	1.02	pos	15	-0.8	0.08
				neg	-	-	-
1,2,3-benzotriazole	*95-14-7*	$C_6H_5N_3$	*1.30*	pos	15	-0.6	0.06
				neg	11	-0.6	0.07
2,4-dinitrophenol	*51-28-5*	$C_6H_4N_2O_5$	*1.53*	pos	15	-0.2	0.55
				neg	18	-0.1	0.55
4-methyl-1H-benzotriazole	*29878-31-7*	$C_7H_7N_3$	*1.78*	pos	13	-0.5	0.09
				neg	6	-0.6	0.10
5-methyl-1H-benzotriazole	*136-85-6*	$C_7H_7N_3$	*1.81*	pos	16	-0.6	0.11
				neg	11	-0.6	0.11
4-chlor-benzoic acid	74-11-3	$C_7H_5ClO_2$	2.20	pos	3	-0.5	0.66
				neg	6	-0.3	0.47
N,N-diethyltoluamide	134-62-3	$C_{12}H_{17}NO$	2.50	pos	15	-0.6	0.86
				neg	-	-	-
Isoproturon	34123-59-6	$C_{12}H_{18}N_2O$	2.57	pos	14	-0.3	0.11
				neg	-	-	-
Mecoprop	7085-19-0	$C_{10}H_{11}ClO_3$	2.85	pos	13	-0.2	0.35
				neg	13	-0.2	0.35
Dimethenamid	87674-68-8	$C_{12}H_{18}ClNO_2S$	2.92	pos	14	-0.1	0.07
				neg	-	-	-
Dinoterb	*1420-07-1*	$C_{10}H_{12}N_2O_5$	*3.09*	pos	12	0.0	0.53
				neg	15	0.5	0.59
Valsartan acid	164265-78-5	$C_{14}H_{10}N_4O_2$	3.14	pos	18	-1.5	0.44
				neg	18	-1.5	0.44
Metolachlor	51218-45-2	$C_{15}H_{22}ClNO_2$	3.45	pos	16	0.0	0.16
				neg	-	-	-
Bezafibrate	41859-67-0	$C_{19}H_{20}ClNO_4$	3.93	pos	16	-1.4	0.28
				neg	16	-1.4	0.28
Gemfibrozil	*25812-30-0*	$C_{15}H_{22}O_3$	*4.37*	pos	4	0.1	0.57
				neg	5	0.2	0.52

Appendix C. Methodology

C.1 Examples of LC methods

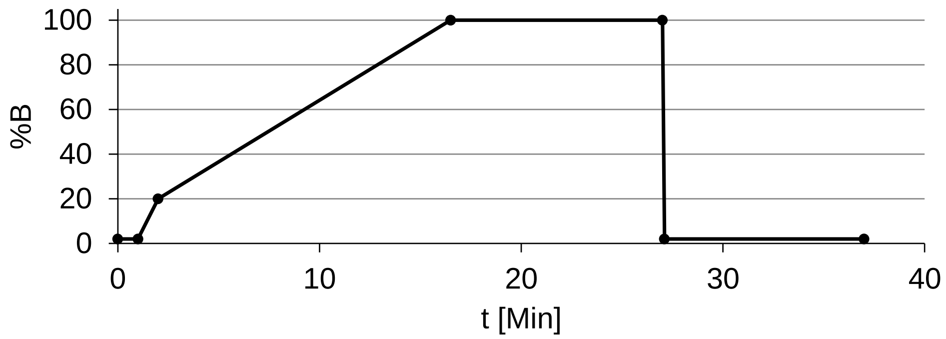
In the following two exemplary LC methods for chromatographic separation are shown.

Method A:

Eluents			
	A: MilliQ + 0.1% v/v formic acid		
	B: Acetonitrile + 0.1% v/v formic acid		
Injection volume	95 µL sample + 5 µL isotope marked standard mix		
Column temperature	40°C		
Flow rate	0.3 mL/min		
Column	Agilent Zorbax Eclipse Plus C18		
	Narrow Bore RR	2.1x150 mm	3.5 µm
	PN:	959763-902	
Pre-column	Phenomenex Cartridge Holder		
	C18 4x2.0 mm ID		
	PN:	AJO-4286	

Gradient

%B	2	2	20	100	100	2	2
t [min]	0	1	2	16.5	27	27.1	37



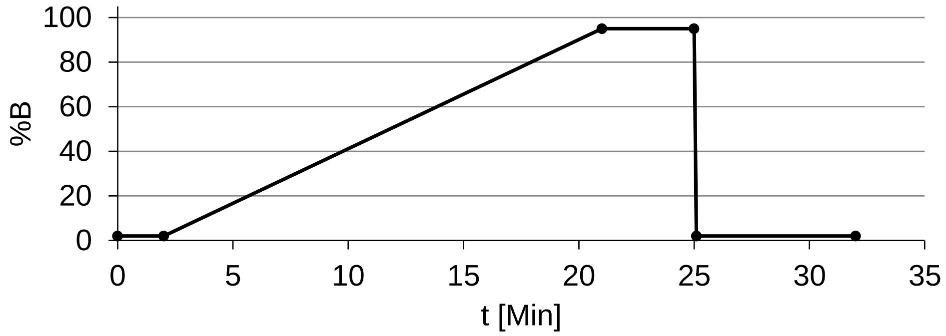
Method B:

Eluents		
	A:	MilliQ + 0.1% v/v formic acid
	B:	Acetonitrile + 0.1% v/v formic acid

Injection volume	95 µL sample + 5 µL isotope marked standard mix	
Column temperature	40°C	
Flow rate	0.6 mL/min	
Column	Restek Ultra Aqueous C18	
	250 x 4.6 mm	5 µm
	Cat:	9178575
Pre-column	Restek Ultra AQ C18	
	10 x 4 mm	
	Cat:	917850210

Gradient

%B	2	2	95	95	2	2
t [min]	0	2	21	25	25.1	32



C.2 Examples of MS methods

In the following two exemplary MS methods are given for a Time-of-Flight and a Orbitrap mass spectrometer.

Table C.1: exemplary MS method for a time-of-flight mass spectrometer

Ion Source	
Gas Flows	Gas 1: 35 psi Gas 2: 45 psi Curtain Gas: 40 psi Collision Gas: 6/medium
Temperature	550 °C
ISVF	5500 V (+) -4500 V (-)
Declustering Potential	60 V (+) -100 (-)
TOF-MS Scan	
Mass Range	MS: 100 – 1200 Da TOF-MS: 250 ms
MS²	
Mass Range	30 – 1200 Da
Collision Energy	40 eV (+) -40 eV (-)
Collision Energy Spread	20 eV
MS² Acquisition in IDA or SWATH mode	
IDA Triggering	
Accumulation Time	65 ms
Max number of MS² per cycle	12
Minimum intensity	100 cps
Exclude Isotopes	Within 4 Da
Mass Tolerance	5 ppm
Include/Exclude List	None
Dynamic Background subtract	On
SWATH	
Accumulation Time	50 ms
Mass range	100 – 1200 Da
Number of SWATH windows	16

Table C.2: exemplary MS method for an Orbitrap mass spectrometer

Ion Source	
Gas Flows	Sheat Gas: 40 Aux gas flow: 15 Sweep Gas: 50
Temperature	Capillary:350 °C Aux Gas: 400 °C
Spray Voltage	3500 V
MS Scan	
Mass Range	Full MS: 120 – 1200 m/z
Resolution	30,000
Microscans	1
Maximum inject time	50 ms
Full MS / dd-MS² (TopN)	
Full MS	
Resolution	120,000
AGC Target	$3e^6$
Maximum IT	100 ms
Scan Range	120 – 1200 m/z
dd-MS²	
Resolution	15,000
AGC Target	$1e^5$
Maximum IT	50 ms
Loop count	5
Isolation window	1.3 m/z
Fixed firsr mass	50.0 m/z
(N)CE / stepped N(CE)	Nce: 80
dd Settings	
Minimum AGC target	$8.00e^3$
Apex trigger	3 to 10 s
Charge Exclusion	-
Peptide Match	Preferred
Exclude isotopes	On
Dynamic exclusion	15.0 s

C.3 Blank measurements

The following shows the total ion chromatograms for the two LC methods A and B after electrospray ionisation in positive and negative mode. The intensity axis has the same scale in all chromatograms.

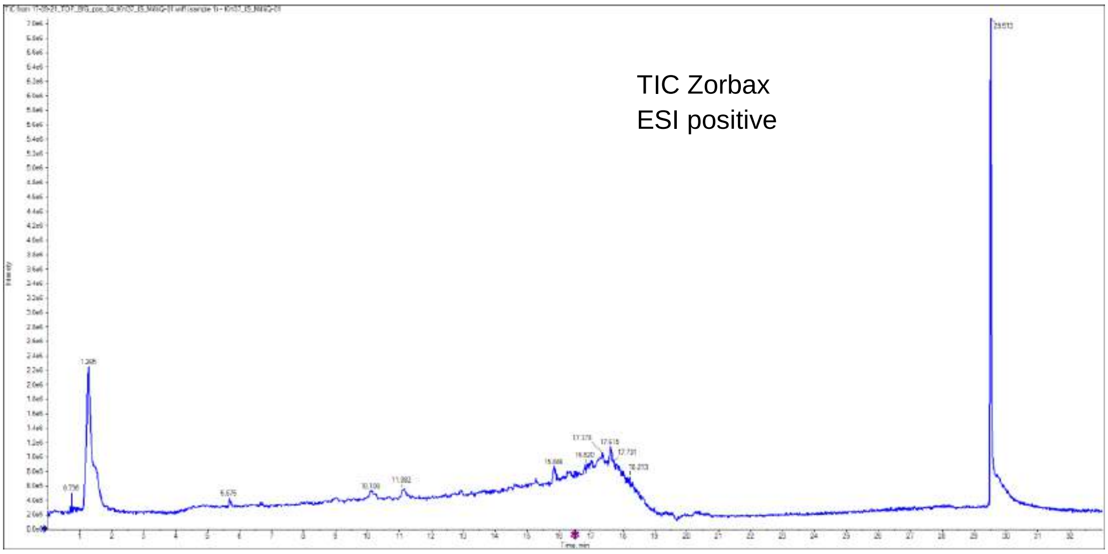


Figure C.1: Total ion chromatogram for LC method A; positive electrospray.

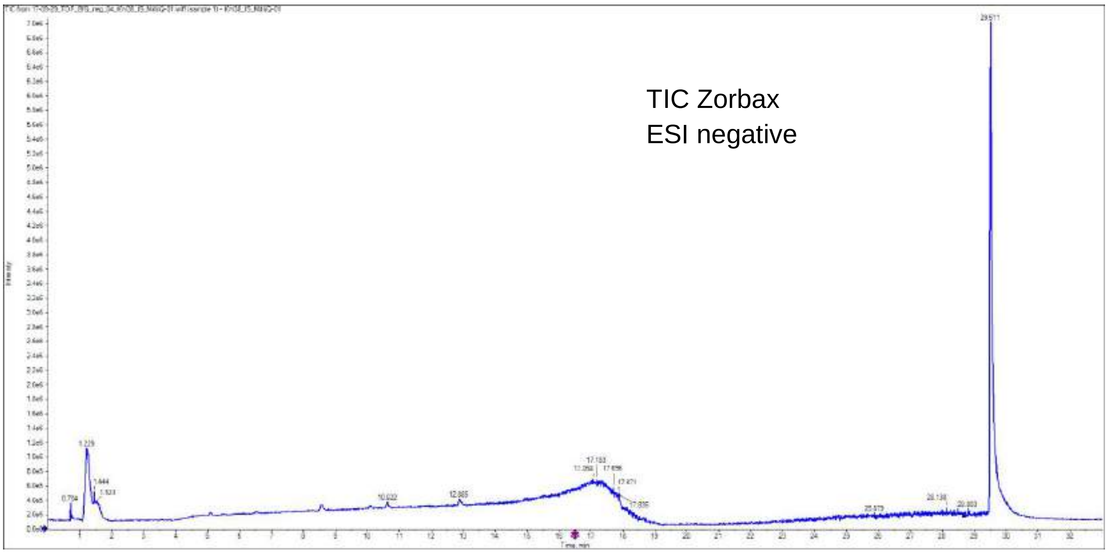


Figure C.2: Total ion chromatogram for LC method A; negative electrospray.

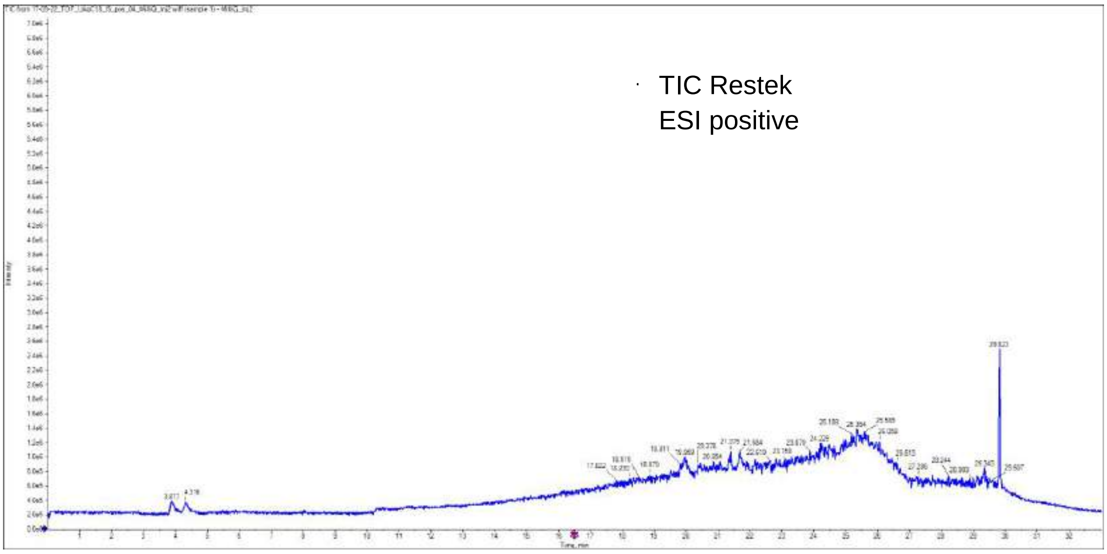


Figure C.3: Total ion chromatogram for LC method B; positive electrospray.

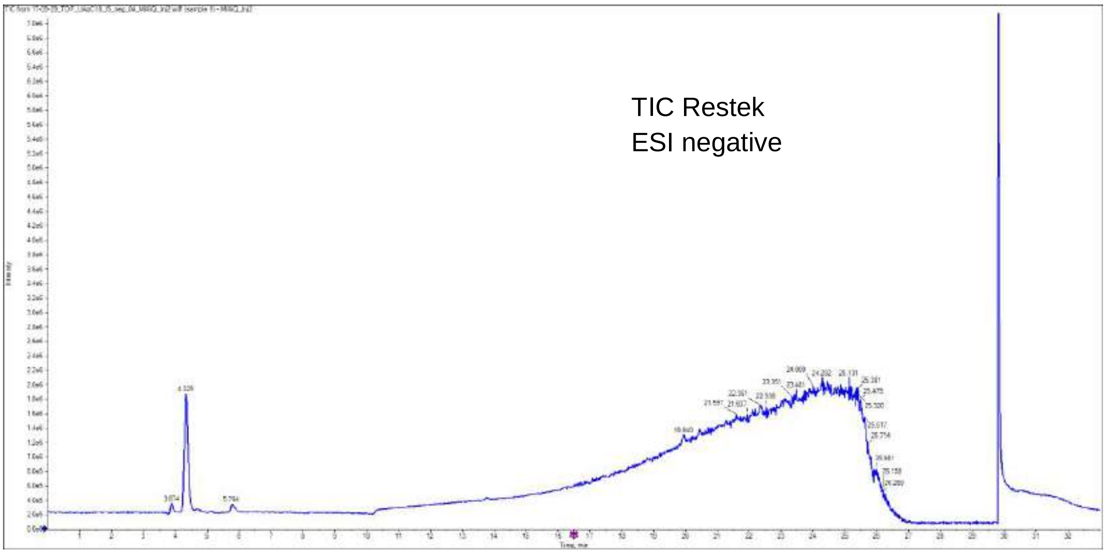


Figure C.4: Total ion chromatogram for LC method B; negative electrospray.

C.4 Retention time mass plot of blanks

The features detected in blanks are compared by scatter plots (mass vs. retention for ESI+ and ESI-). The red dots represent the isotope labelled internal standards. The internal standards should be evenly distributed over the mass and retention time range (polarity range) as much as possible.

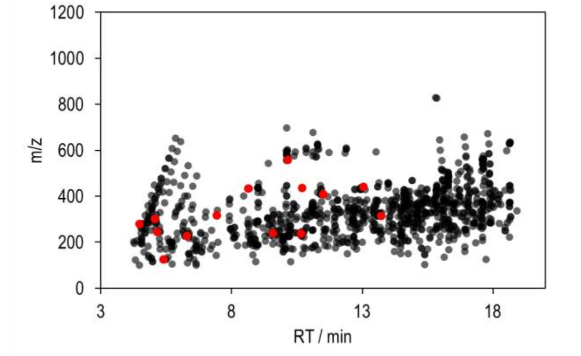
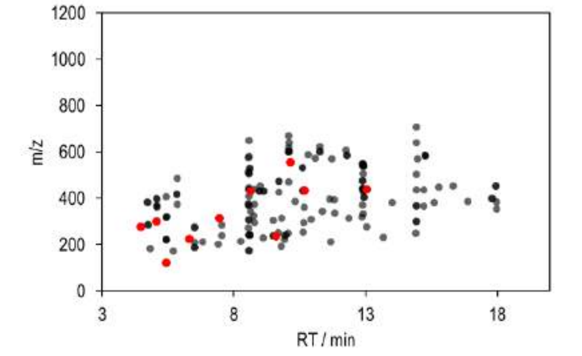
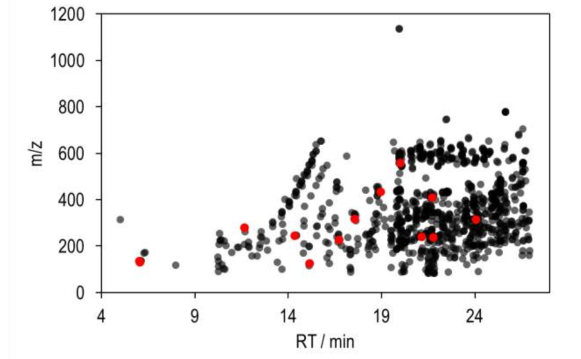
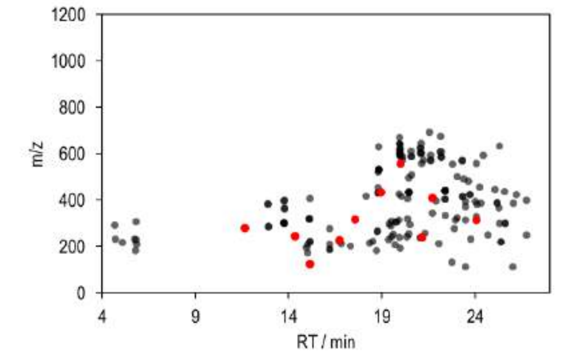
Method A, ESI pos	Method A, ESI neg
	
Method B, ESI pos	**Method B, ESI neg**
	

Figure C.5: Scatter plots ("point clouds") mass vs. RT for the two separation methods A and B, for positive and negative ESI mode

The mass vs. retention time plots in Figure C.5 show clear differences for methods A and B, largely due to the different stationary phases of the separation columns.

Appendix D. Measurement technique

D.1 HRMS mass spectrometer

The Orbitrap is the most recent development in ion trap mass spectrometers. The ion trap contains a central, spindle-shaped electrode. The ions are introduced into the Orbitrap radially to the central electrode and move in orbits around the central electrode due to electrostatic attraction. Since the ions are not introduced into the centre of the chamber, but decentralised, they simultaneously oscillate along the axis of the central electrode. The frequency of these oscillations generates signals in detector plates that are converted into the corresponding *m/z* ratios by means of Fourier transformation.

A time-of-flight mass spectrometer (TOF-MS) consists of a tube under vacuum with a very rapid detector at its end. In principle, TOF technique is based on the principle that ions accelerated to the same kinetic energy have different velocities depending on their mass. Lighter ions are faster than heavier ions and therefore reach the detector earlier during their flight through a field free region (flight tube). In practice, TOF instruments with ion reflectors or reflectrons in which ions are reflected by an additional electrical field at the end of the flight tube. In this way the flight distance is doubled and the energy dispersion of the ions is focused. This minimises the speed dispersion of ions of the same mass, which started from slightly different positions and had already different initial velocities during acceleration (Doppler effect). The length of the flight distance is decisive for the resolving power of the mass spectrometer.

Orbitrap

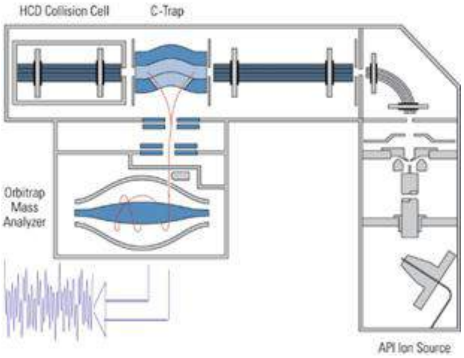


Image source:
Thermo Fischer Scientific

Time-of-flight mass spectrometer (TOF)

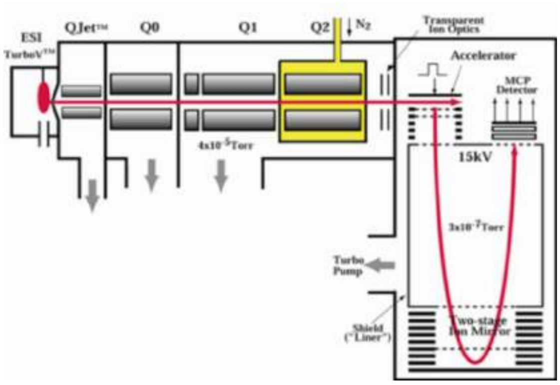
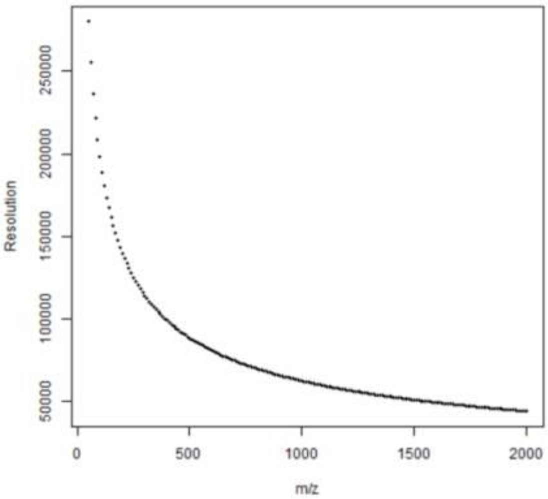


Image source: Sciex®



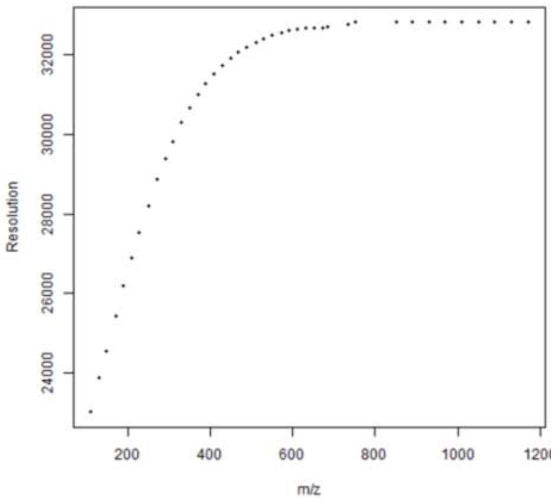


Figure D.1: *Set-up of the mass spectrometers Orbitrap (left) and time-of-flight mass spectrometer (right) and their mass resolving power (resolution) depending on the mass range (bottom) [32]*

Appendix E. System stability

E.1 Chromatography

Reproducibility of retention time

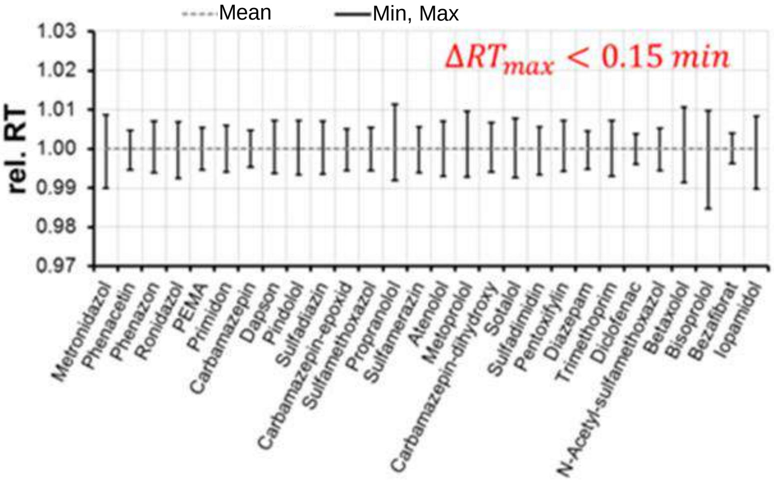


Figure E.1: Retention time stability over a period of 10 months (N = 134 measurements)

E.2 Mass spectrometry

Long term stability of sensitivity

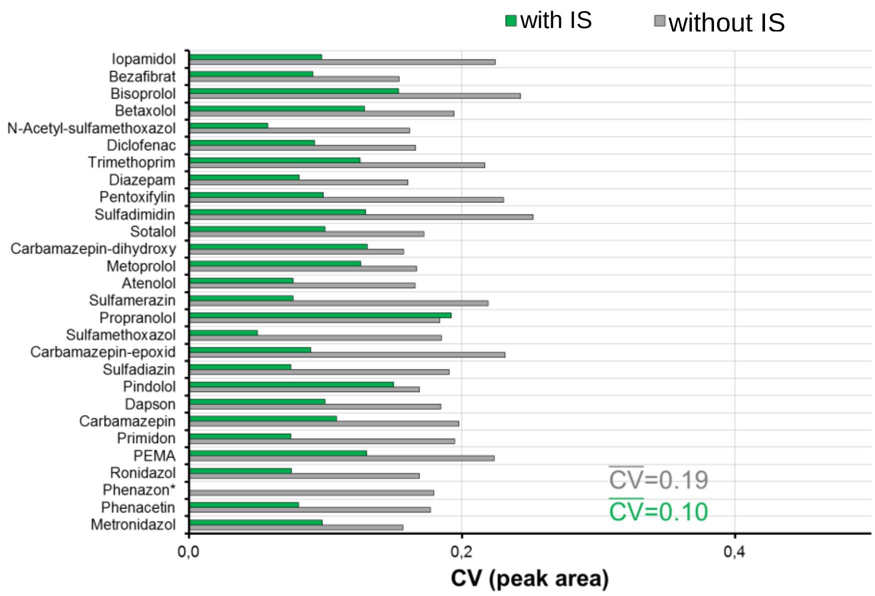


*Figure E.2: Stability of device sensitivity over a period of 10 months (N=134) without (grey) and with (green) internal standardisation (*phenazone as IS)*

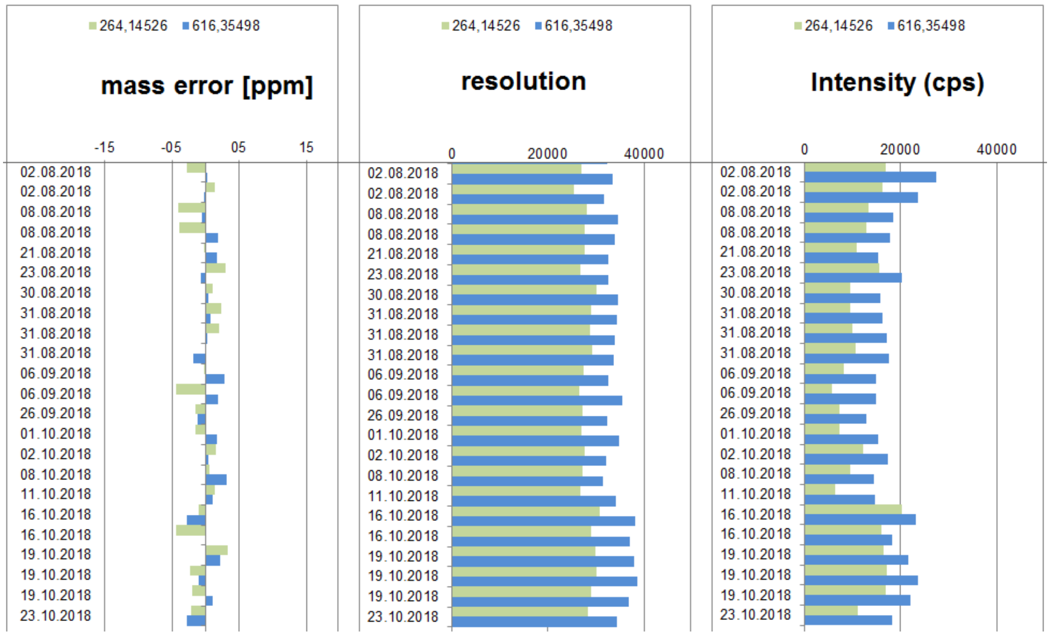


Figure E.3: *Control charts to check MS performance via mass accuracy, resolving power (resolution) and sensitivity*

Appendix F. Data analysis

F.1 Adjustment of intensity dependent parameters for peak finding using the example of the "noise threshold" of the MarkerViewTM software (SCIEX)

Replicate measurements of an aliquot of a wastewater treatment plant effluent spiked with 64 compounds (QA control sample) from various sampling times over one year showed a variation of the sensitivity levels of LC-HRMS instrument. The previously optimised values for the "noise threshold" at 100 (positive ion mode) or 75 (negative ion mode) didn´t give any satisfactory results for peak finding algorithm (Figure F.2). Higher signal intensities for true features improved the overall sensitivity but also increased the noise level. In order to adjust the "noise threshold", the mean "noise" (median) across all spiked compounds was determined from the control sample for each measurement. Using the optimisation measurements, a "noise threshold" was calculated from each of these values. The "noise" plotted vs. "noise threshold" resulted in a linear correlation which ose formula can be used for further adjustments (Figure F.1).

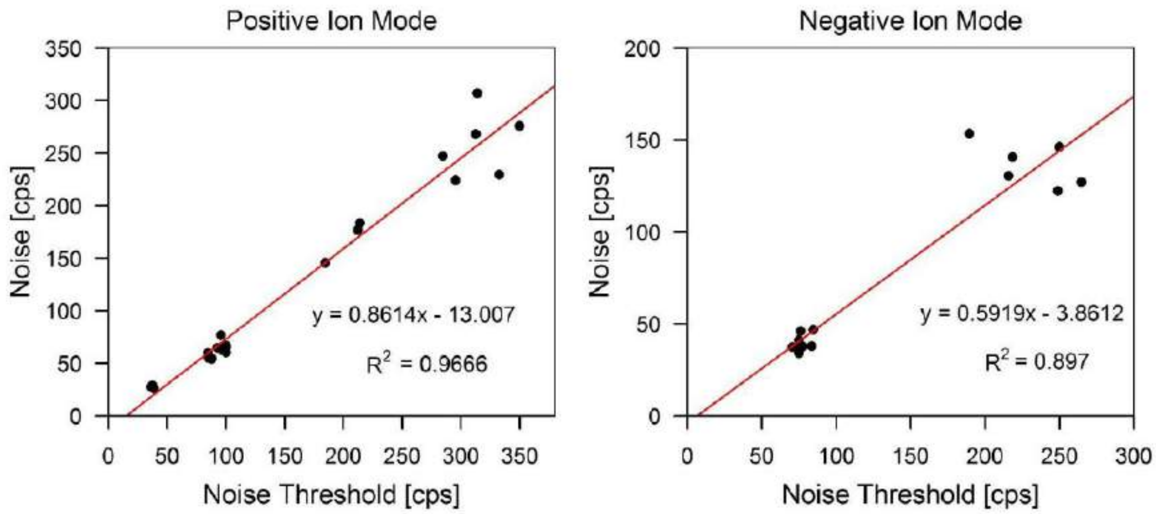


Figure F.1: Correlation between "noise" and the calculated "noise threshold"

The use of these adjusted values for the "noise threshold" showed that the share fraction of false positive results (FPs) of the features again matched that of the original optimisation (Figure F.2). Adjustment based on the median of white noise therefore works very well. However, the total number of features varied if the "noise threshold" changed. At a higher instrument sensitivity, further features with low signal intensity can also be detected which are not detectable at lower instrument sensitivity. Therefore, results based on the number of features, are only comparable if the differences of instrument sensitivities are not too high.

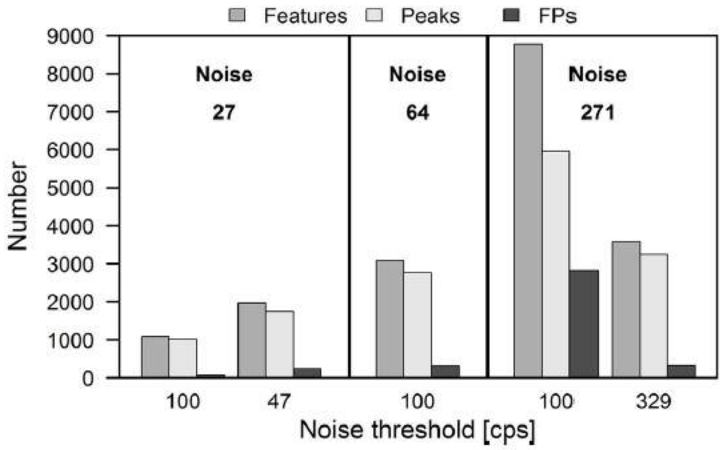


Figure F.2: *Change in the number of features, true peaks and false positive results (FPs) based on the "noise threshold" (100 cps and calculated value from the linear adjustment function) for the measurements ("positive ion mode") of a spiked wastewater treatment plant effluent for three different levels of instrument sensitivity. Left: LC-HRMS with low sensitivity, centre: LC-HRMS during optimisation, right: LC-HRMS with higher sensitivity. See the following for further details [2]*

Appendix G. Adduct formation when using an ESI source

G.1 Adducts and in-source fragments

Table G.1: Examples of detected adducts and in-source fragments of known substances

Type	Name (split/added elements)	Polarity	Description	Mass difference in comparison to $[M+H]^+$ or $[M-H]^-$	Exemplary compounds
Adduct	+O	both	Addition of an oxygen	15.99491	2-mercaptobenzoxazole, 2-mercaptobenzothiazole
Adduct	$+NH_4$	positive	Addition of ammonium	17.02654	Diatrizoate, ethofumesate, iopromide
Adduct	+Na	both	Addition of sodium	21.98194	pos: Carbamazepine, metolachlor / neg: Valsartan, olmesartan
Adduct	+HCl	negative	Addition of HCl	35.97667	Ethidimuron, dimefuron, methoxyfenozide
Adduct	+K	positive	Addition of potassium	37.95588	Azoxystrobin, dimoxystrobin, praziquantel
Adduct	$+C_2H_8N$	positive	Addition of ethylamine	45.05784	Dimethoate, tetraglyme, dimefuron, metalaxyl
Adduct	$+CH_2O_2$	negative	Addition of formic acid	46.00548	Flecainide, aliskiren, fluconazole
Adduct	$+C_2H_4O_2$	negative	Addition of acetic acid/ sodium cluster	60.02113	-
Adduct	$+HNO_3$	negative	Addition of nitrate	62.99564	Clothianidin, fluconazole
Adduct	$+NaCH_2O_2$	negative	Addition of formic acid/ sodium cluster	67.98743	Penoxsulam, diphenylphosphinic acid, haloxyfop,
Adduct	$+NaC_2H_4O_2$	negative	Addition of acetic acid/ sodium cluster	83.0109	-
Adduct	$+NaNO_3$	negative	Addition of nitrate/ sodium cluster	84.97814	Bromacil, chlorothanonil R611965
Fragment	$-C_7H_8N_2O_4S$	positive		-216.02103	Metazachlor metabolite BH 479 9
Fragment	$-C_{10}H_{14}O_4$	positive		-198.0905	Kresoxim-methyl
Fragment	$-C_5H_6O_4N_2S$	positive		-190.00483	Metazachlor metabolite 479M008
Fragment	$-C_9H_{11}O_4$	positive		-183.06554	Kresoxim-methyl
Fragment	$-C_6H_8O_2N_2S$	positive		-172.0312	Metazachlor metabolite BH 479 11
Fragment	$-C_8H_8O_3$	positive		-152.04789	Dimoxystrobin metabolites 505M08 and 505M09
Fragment	$-C_6H_8O_3$	positive		-152.0472	Kresoxim-methyl
Fragment	$-C_5H_4O_3N_2$	positive		-140.02274	Metazachlor metabolite NOA409045
Fragment	$-C_4H_8O_5$	positive		-136.03772	Metalaxyl metabolite CGA 108906
Fragment	$-C_2O_2F_9$	negative		-127.00069	ADONA
Fragment	$-C_7H_5ON$	negative		-119.03711	Carbetamide
Fragment	$-C_3H_2O_5$	positive		-117.99077	Metolachlor metabolite CGA 357704

Type	Name (split/added elements)	Polarity	Description	Mass difference in comparison to $[M+H]^+$ or $[M-H]^-$	Exemplary compounds
Fragment	$-C_7H_8O$	positive		-108.05737	Kresoxim-methyl
Fragment	$-C_3H_9O_3N$	positive		-107.05879	Dimoxystrobin metabolites 505M08 and 505M09
Fragment	$-C_2H_2O_3S$	negative		-105.97301	Dimethenamid metabolite M31, Metazachlor metabolite CGA 368208
Fragment	$-C_3H_4O_4$	negative		-104.01151	Dimethenamid metabolite M23
Fragment	$-C_3H_8O_3$	positive		-92.04721	Kresoxim-methyl
Fragment	$-C_2H_6O_3$	negative		-90.03224	Metalaxyl metabolite CGA 108906
Fragment	$-C_5H_{11}ON$	positive		-89.08406	Diphenhydramine
Fragment	$-C_5H_{12}O$	positive		-88.08882	Pendimethalin
Fragment	$-C_3H_5O_2$	positive		-88.05298	Metolachlor metabolite CGA 50267
Fragment	$-C_2O_4$	negative		-87.98021	Quinmerac metabolite BH 518-2
Fragment	$-C_2H_2O_2N_2$	negative		-86.01218	Thiacloprid metabolite M30
Fragment	$-C_2H_3ON_3$	negative		-85.02816	Tritosulfuron metabolite M635H003
Fragment	$-SO_3$	positive	Splitting of SO_3	-79.95682	Sitagliptin-N-sulphate
Fragment	$-C_2H_4O_3$	positive		-76.01596	Kresoxim-methyl, metolachlor metabolite CGA 37735
Fragment	$-C_3H_5O_2$	positive		-73.0295	Metolachlor metabolite CGA 50267
Fragment	$-C_3H_4O_2$	negative		-72.02058	Mecoprop, fenoprop, fluziprop
Fragment	$-C_2O_3$	negative		-71.98419	Dimethenamid metabolite M23
Fragment	$-C_5H_{10}$	positive		-70.07825	Pendimethalin
Fragment	$-C_3H_4N_2$	positive		-68.03745	Prochloraz, metazachlor metabolite 479M004, Metazachlor metabolite 479M008
Fragment	$-C_5H_6$	positive		-66.04641	Propyzamide
Fragment	$-CH_4O_3$	positive		-64.01605	2-OH-ibuprofen
Fragment	$-C_2H_4O_2$	positive		-60.02168	Metalaxyl metabolite CGA 108906
Fragment	$-C_2H_2O_2$	both		-58.00493	Kresoxim-methyl, metolachlor metabolite CGA 37735
Fragment	$-C_2H_3ON$	both		-57.02146	DCPMU, carbofuran, carbaryl
Fragment	$-C_4H_8$	positive		-56.0626	Bromacil, terbuthylazine, bupropion, methoxyfenozide
Fragment	$-C_3H_4O$	negative		-56.0256	Ketoprofen
Fragment	$-3*H_2O$	positive	3-fold water splitting	-54.03168	Prednisolone
Fragment	$-CH_6O_2$	positive		-50.03733	Dimethachlor metabolite SYN 530561
Fragment	$-CH_5ON$	positive		-47.03711	Kresoxim-methyl
Fragment	$-C_2H_6O$	positive		-46.04241	Mefenpyr-diethyl, fenoxycarb, ethofumesate, pethoxamid
Fragment	$-CH_4ON$	positive		-46.02929	Levetiracetam
Fragment	$-CH_2O_2$	both		-46.00548	Naproxen, ibuprofen
Fragment	$-CO_2$	negative		-43.98986	Diatrizoate, N-methyl-pregabalin
Fragment	-CHON	negative		-43.00581	DCPU, tritosulfuron metabolite M635H001
Fragment	$-C_3H_6$	positive		-42.0475	Flufenacet metabolite AZ14777

Type	Name (split/added elements)	Polarity	Description	Mass difference in comparison to $[M+H]^+$ or $[M-H]^-$	Exemplary compounds
Fragment	$-2*H_2O$	positive	2-fold water splitting	-36.02112	Prednisolone
Fragment	-Cl	positive	Splitting of chloride	-34.9683	3,4-dichloraniline
Fragment	$-CH_4O$	both		-32.02622	Dimethenamid, metolachlor, oxfendazole
Fragment	$-CH_5N$	positive		-31.04219	Sertraline
Fragment	$-CH_2O$	positive		-30.01111	Topramezone metabolite M670H05
Fragment	-HF	negative	Splitting of fluoride	-20.00623	Diflubenzuron
Fragment	$-H_2O$	both	Water splitting	-18.01056	pos: 10,11-dihydroxy-10,11-dihydrocarbamazepine, gabapentin / neg: Diclofenac, PFBA, diatrizoate
Fragment	$-NH_4$	positive		-17.02654	Levetiracetam, amoxicillin
Fragment	$-CH_4$	positive		-16.0313	1,2-dihydro-2,2,4-trimethylquinoline
Fragment	-O	positive	Splitting of an oxygen	-15.99491	Ranitidine-N-oxide, 5-chloro-2-mercaptobenzoxazole

Other adducts, *in-source* fragments or typical blank values and impurities in the LC-(HR)MS are described in the literature. [32]

Appendix H. Workflow

H.1 Example of a typical screening workflow

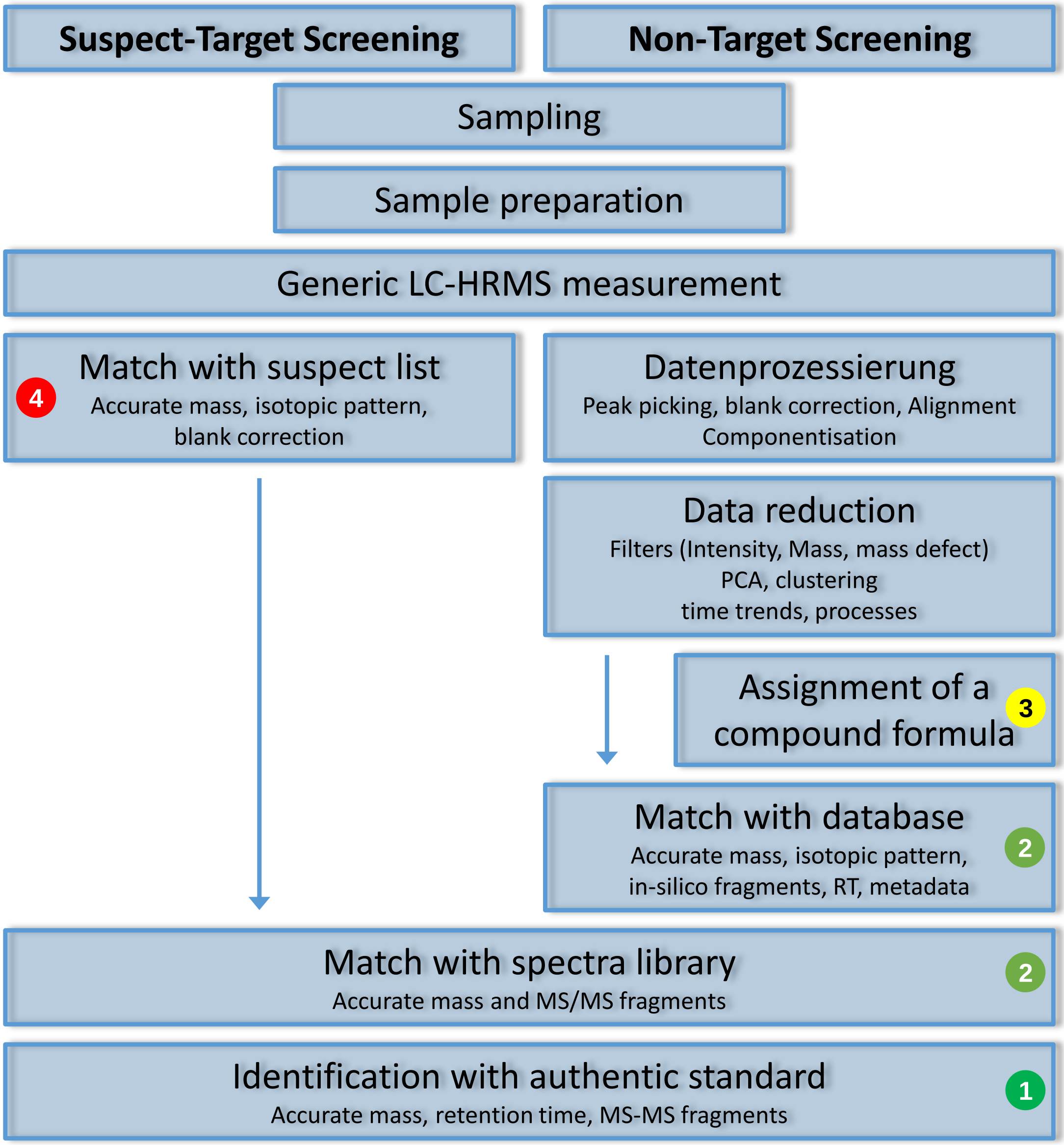


Figure H.1: *Exemplary workflow for suspect and non-target screening, including categorisation of the compound identification (see also 10.2.1)*

Other exemplary workflows are found in the literature. [1]