State-of-the-art of screening methods for the rapid identification of chemicals in drinking water

ERNCIP thematic area
Chemical & Biological Risks in the Water Sector
Deliverable 1 - Task 6

Marta Llorca
Sara Rodríguez-Mozaz
Catalan Institute for Water Research (ICRA),
Girona, Spain

June 2013

The research leading to these results has received funding from the European Union as part of the European Reference Network for Critical Infrastructure Protection project.

Report EUR 26643 EN

Joint
Research
Centre

European Commission
Joint Research Centre
Institute for the Protection and Security of the Citizen

Contact information
Naouma Kourti
Address: Joint Research Centre, Via Enrico Fermi 2749, TP 721, 21027 Ispra (VA), Italy
E-mail: ern-cip@jrc.ec.europa.eu
Tel.: +39 0332 78 6045
Fax: +39 0332 78 5469

http://ipsc.jrc.ec.europa.eu/?id=688
http://www.jrc.ec.europa.eu/

This document replaces <State-of-the-art of screening methods for the rapid identification of chemicals in drinking water - Deliverable D1> with ISBN number <978-92-79-33132-9> and PUBSY request number <JRC83768>. The corrections made in the new document are <the term "Effect-Directed Analysis" replacing "Effect of Direct Analysis" at page 18 and 23>.

JRC89741
EUR 26643 EN
ISBN 978-92-79-38294-9
ISSN 1831-9424
doi:10.2788/80087

Luxembourg: Publications Office of the European Union, 2013

Printed in Italy

Task 6:

Review of screening techniques and methods for the rapid identification and quantification of unknown chemical contaminants.

Deliverable: State of the art of screening methods for the rapid identification of chemicals in drinking water

Authors: Marta Llorca, Sara Rodríguez-Mozaz

Responsible person: Sara Rodríguez-Mozaz

Catalan Institute for Water Research (ICRA), Girona, Spain

http://www.icra.cat

'The research leading to these results has received funding from the European Union as part of the European reference network for critical infrastructure protection (ERNCIP) project.'

Contents

1. Abstract

The contamination of drinking water is potentially harmful and poses a risk to public health. If any observation suggests a potential contamination of drinking water, such as consumer complaints about the alteration of the water's organoleptic properties, the appearance of health problems or an alarm triggered by sensors, a rapid identification of the hazard causing the problem is necessary. With regards to chemical contamination, EU Member States have several strategies to deal with the presence of unknown chemicals in water: there are screening methods as well as systematic approaches used for the analysis and identification of different groups of chemicals.

This report provides a brief overview of the existing methods for the non-targeted screening of organic compounds in water samples by means of mass spectrometry. This review is thus based on the studies and explorations that can be performed by different mass spectrometry approaches. In addition, the most relevant European institutions working on this topic and that are currently contributing to the development of the non-target screening of pollutants are presented.

2. Introduction

The main question regarding a water sample containing unknown substances is how to identify the contaminants that cause an environmental or health problem.

There are different analytical approaches and strategies to deal with this based on biological and chemical analyses. There are analytical tools that are currently in use for the identification of non-target compounds in a wide variety of matrices, including water. The applicability of a so-called non-target analysis depends strongly on current technical developments, which involves sample preparation as well as analyte detection, identification and confirmation (Hogenboom et al., 2009). In relation to this, some definitions of the key terms that will be used in this report are provided below.

— **Non-target compound**: this term refers to compounds detected in a sample that were not the target of the initial analysis (Schollée et al., 2012). This includes known and unknown compounds.

— **Known compound**: any analyte that has been previously identified by the analysers or that can be found in the bibliography or mass spectrometry libraries (Schollée et al., 2012).

— **Unknown compound**: the suspected compound has not been previously identified by the analysers and it cannot be found in the bibliography or mass spectrometry libraries (Schollée et al., 2012).

3. Analytical approaches for the identification of non-target compounds

3.1. Biological approach

There are different bioactivity-based screening tests. They are usually cost-effective, suited for high-throughput analysis and do not require specialised technicians or, in general, much sample preparation. However, activity-based assays, such as cell-based biosensors or growth inhibition assays, do not allow the unequivocal identification of an unknown compound (as the chemical analytical methods do) and, therefore, an additional chemical analysis is required after the bioactivity identification (Bovee et al., 2009). Nevertheless, although these techniques cannot compete with conventional analytical methods, they constitute a useful tool for rapid screening for both regulatory authorities and water facilities when only semi-quantitative data is needed to trigger an alarm. Positive samples can then be sent for detailed chemical analysis if needed, thus reducing the costs of monitoring programmes.

3.2. Chemical approach

Mass spectrometry (MS) based methodologies are widely used for non-target analysis since they allow the identification and the confirmation of non-target analytes (either known or unknown compounds). For organic compounds, liquid chromatography (LC) or gas chromatography (GC) are commonly used separation techniques and are usually coupled to MS. The identification of the detected compounds is thus based on chromatographic retention time combined with the mass spectrum.

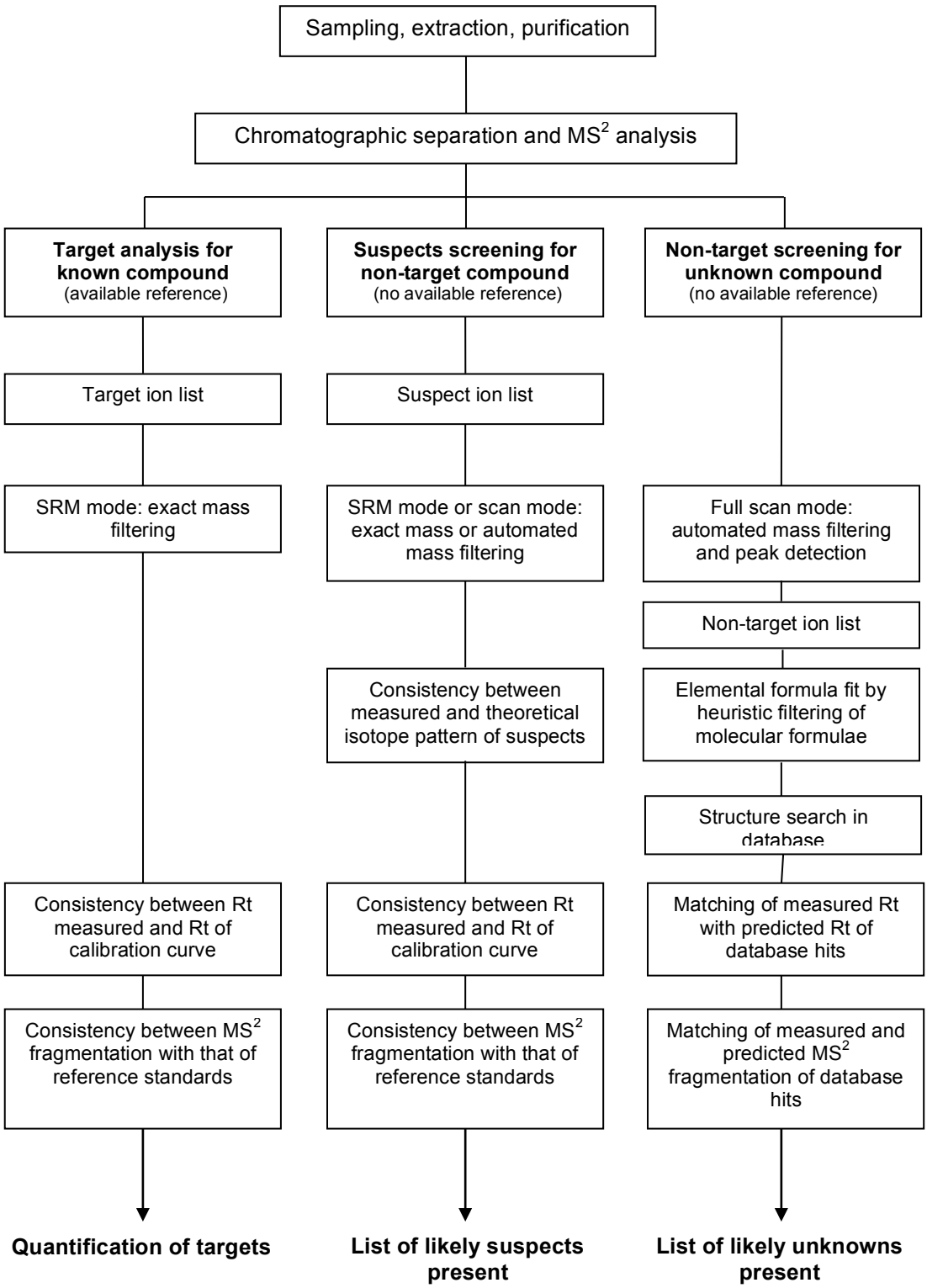
Different research projects have dealt, and are currently dealing, with this issue. An example is the NORMAN project, under the 6th framework programme, Priority 6.3 (Contract No 018486). The objective of the NORMAN project is to create a network of expert reference laboratories and related organisations in order to: (1) improve the exchange of information and data on emerging environmental contaminants between monitoring institutes, research centres and end-users (modelling experts, risk assessors and risk managers), and (2) to encourage the validation and harmonisation of common measurement methods and monitoring tools so that the demands of risk assessors and risk managers can be better met. Regarding the identification of unknown substances in the environment, sub-project 3 (SP3) of the NORMAN project deals with the access to and the evaluation of information about emerging

environmental substances. The main goal is to develop three web-based databases with particular attention to the parts of databases enabling the end-user to interpret the data and judge their representativeness, quality and comparability with other data sets. The three web-based databases are: (1) leading European experts, organisations (reference laboratories, research centres and monitoring institutes) and projects dealing with emerging pollutants; (2) geo-referenced monitoring data on target emerging substances; and (3) mass spectrometric information on provisionally identified and unknown substances (NORMAN-FP6).

In general, the workflow for the analysis of unidentified samples by LC, or GC, coupled to a mass spectrometer analyser, can be summarised as indicated below. This scheme, proposed by Schollée et al. (2012), will be considered in detail in the next sections.

1. Sample extraction or sample preparation, if necessary; in some cases water samples can be directly analysed.
2. Chromatographic separation coupled to mass spectrometry analysis including accurate mass and MS^2 fragmentation analysis.
3. Fiehn–Kind's seven golden rules.
4. Database searches.
5. Isotope patterns.
6. Structure–property relationships (including log K_{ow} approximations and chromatographic hydrophobicity index (CHI) approximations).
7. Computer-assisted analysis.

According to Krauss et al. (2010), three different analytical approaches can be distinguished depending on the objective of the study: (i) quantitative target analysis for known compounds (when reference standards are available); (ii) suspects screening for non-target compounds (when reference standards are not available); (iii) non-target screening for unknown compounds. Figure 1 shows a systematic workflow for the three approaches.



SRM: selected reaction monitoring; Rt: retention time.

Figure 1: Systematic workflow for the three screening approaches, adapted from Krauss et al. (2010)

3.2.1 Sampling process and sample extraction

The sampling procedure and sample shipment and preservation, as well as the sample preparation, are crucial steps prior to the proper analysis and should be optimised in order to ensure the accuracy of the final analytical results.

Prior sample extraction: the most common procedure for sampling is collecting water in glass bottles or bottles made of an inert material (such as polypropylene or polyethylene polymers). However, the use of glass or plastic bottles is sometimes questioned and discarded because of the possibility of the adsorption of organic analytes onto recipient walls and the extraction of the samples has to be performed *in situ*. Regarding the sample shipment, cold conditions are recommended. The preservation must be between 4 ºC and – 20 ºC, depending on the time that it will take to analyse the samples after sampling. The addition of some preservatives could be necessary in order to assure the traceability of the sample. For example, formaldehyde or sodium azide among others can be used for stopping biological processes and avoiding any biological degradation or transformation. These preservatives should be evaluated and considered carefully since they can interfere with the analysis as well as with the analytes in the sample.

Sample extraction: solid phase extraction (SPE) is the most common procedure for extraction and/or preconcentration of contaminants. It is particularly well adapted to multi-residue analysis, including compounds with a wide range of chemical properties and polarities (Gómez et al., 2009), and can be automated. SPE usually requires a previous sample filtration, which in some cases can imply the loss of some important compounds in the filter: some substances may get attached to particulate matter in water or to the filter, and this aspect should be carefully considered before performing SPE. In those cases, liquid–liquid extraction is the technique of choice because it does not require pre-filtration of the sample. This extraction procedure allows the extraction of the analytes from the whole sample according to their affinity with hydrophilic and hydrophobic solvents, with protic and aprotic solvents, and with solvents with different polarities. In this sense, different fractions of the same sample can be analysed by different chromatographic methodologies (GC or LC). The analytes can be further separated by different chromatographic columns (reversed phase, normal phase (such as hydrophilic interaction liquid chromatography (HILIC)) and mobile phases according to the nature of the fraction.

Nonetheless, the simultaneous performance of different extraction strategies should be considered in order to cover a wide range of physico-chemical properties when target and non-target compound screening analyses have to be performed for an unidentified analyte. These extraction procedures could be standardised for cooperative purposes among different research laboratories for the detection of 'non-target' compounds including known and unknown analytes. However, when the presence of an untargeted compound is detected, the laboratories should be free to apply the most useful preparative method in order to identify the suspected chemical.

3.2.2 Chromatographic separation coupled to mass spectrometry analysis including accurate mass and MS^2 fragmentation analysis

The most widely used methodology for the detection of organic non-target compounds is based on MS analysis due to its versatility and the different available analysers. In general, the mass spectrometer is coupled to a chromatograph in order to separate the analytes beforehand according to their affinity with different solvents (LC), their volatilisation point (GC) and the stationary phases in the chromatographic column.

3.2.2.1 Chromatographic separation

Two techniques are described in this section: gas chromatography (GC) and liquid chromatography (LC).

Gas chromatography: gas chromatography–mass spectrometry (GC–MS) methods are most commonly used in the characterisation of (semi-)volatile and thermostable contaminants in water and, in general, for non-polar pollutants (Schollée et al., 2012). Examples of compounds usually analysed by GC–MS include organochlorine pesticides, polychlorinate biphenyls, polycyclic aromatic hydrocarbons, alkylphenols, dioxins and polybrominated diphenyl ethers (Hernández et al., 2011).

Liquid chromatography: liquid chromatography–mass spectrometry (LC–MS) methods have been used to extend the investigation of water contaminants to non-volatile, (highly) polar and thermally labile compounds such as pharmaceuticals, pesticides, endocrine-disrupting compounds and personal care products (Richardson 2007; Hogenboom et al., 2009; Hernández et al., 2011).

Both techniques should be used in order to cover a wide range of properties when there is no indication about the nature of the 'non-target' analyte.

3.2.2.2 Mass spectrometry analysis

There are several different types of mass analysers, classified according to ion movement and storage. The first is based on ion transport and includes electrostatic and magnetic sectors, quadrupoles (Q), time of flight (TOF) and hybrid combinations of these. The second type of analysers is based on ion storage such as ion traps (IT) and Fourier-transform ion cyclotron resonance (FT-ICR) (Llorca, 2012). The properties of mass analysers are evaluated according to mass range, mass resolution, ion transmission efficiency, mass accuracy, dynamic linear range, scan rate and sensitivity. Table 1 summarises some of these parameters as well as the main advantages and disadvantages of the analysers.

MS	Advantages	Disadvantages	Dynamic linear range	Mass accuracy	Mass resolution
Sector magnet	• High resolution • High sensitivity • Enough confirmation points	• Highly expensive	10 000	1–2 ppm	100 000
Q	• Low cost • High sensitivity	• Lack of confirmation points • Low resolution	10 000	100 000 ppm	4 000
QqQ	• Low cost • High sensitivity • SRM	• Low resolution	10 000	100 000 ppm	5 000
LIT	• Low cost • High efficiency in scan • MS^n experiments	• SRM with low sensitivity • Low resolution	10 000	50–200 ppm	1 000
QqLIT	• High efficiency in scan • SRM • High sensitivity • MS^n experiments	• Medium cost • Low resolution	1 000	100 000 ppm	7 000
TOF	• High resolution	• Lack of confirmation points • Medium cost	100	5 ppm (lock mass)	15 000
Q-TOF	• Medium resolution • SRM	• Medium cost • Low sensitivity		5 ppm (lock mass)	15 000
FT-ICR	• High resolution • Enough confirmation points • MS^n experiments	• Highly expensive	> 5 000	> 1 ppm	500 000
Orbitrap	• High resolution • Enough confirmation points • MS^n experiments	• Highly expensive	> 5 000	5 ppm 1–2 ppm (lock mass)	200 000

SRM: Selected reaction monitoring acquisition mode.

Table 1: **Main advantages and disadvantages of different mass spectrometers (Leonards et al., 2011; Llorca, 2012)**

Different acquisition modes can be operated depending on the analyser (see Table 1) in order to identify the analytes.

— **Full scan mode:** this acquisition scan mode allows the monitoring of a wide range of masses (mass to charge ratio), usually from 50 Da up to 2 000 Da, in a defined period of time, which is one of the major advantages of this acquisition mode. All the analysers can work in this mode, but the maximum potential of this technique is achieved when performing exact mass measurements. The exact mass allows an almost unequivocal identification of one ionised molecule. Only high-resolution mass spectrometers allow the exact mass measurement, working between 15 000 (for

medium–low resolution; i.e. TOF) and 150 000 of resolution (i.e. Orbitrap or Fourier-transform cyclotron). More detailed information can be seen in Table 1.

— **Selected reaction monitoring (SRM) mode:** this type of acquisition mode scan is performed using two mass spectrometers in tandem. In the first analyser, the selected ion or ions from the molecule are isolated **(selected ion monitoring (SIM))**, fragmented and finally the product ions from the fragmentation of the molecule are isolated in the second analyser. The high selectivity of this technique allows the identification as well as the quantification of the compound. Analysers that can work in this mode are triple quadrupoles (QqQ); ion traps (IT); hybrid analysers such as quadrupoles-linear ion traps (QqLIT) or quadrupoles-time of flight (QqTOF); and the hybrid high-resolution mass spectrometer quadrupole-Orbitrap (Q-Exactive). More detailed information can be seen in Table 1.

— **Combination of SRM and full scan:** different operating modes can combine both acquisition modes. As an example, the **information/data-dependent acquisition (IDA** or **DDA)** or **collision-induced dissociation (CID)** automatically run experiments based on results obtained from previous experiments. A full scan screening is performed in the first quadrupole (Q) and followed by a full scan in the second Q, the LIT, the TOF or the Orbitrap. This second scan is dependent on the IDA/DDA parameters. Another example is the **enhanced product ion (EPI)**. This mode consists of a SIM analysis in the first analyser and scan in the second one. This operating mode is typical for QqLITs. The major advantage for these hybrid analysers is the combination of either selectivity and efficiency (Q-LIT) or selectivity and resolving power (Q-TOF and Q- Orbitrap).

The acquisition modes are operated depending on the screening purposes. In the context of this document, two different screenings are differentiated: (i) target screening and (ii) non-target screening. In terms of MS, **target screening** implies searching for thousands of known compounds that can be present in a sample (Hernández et al., 2011). This type of search is carried out, in general, by SRM mode although it can be performed in full scan mode too. This screening is performed for the study of **known compounds** (or **known-knowns**) (Godula et al., 2011). On the other hand, the **non-target screening** consists of a first component detection step working in a full scan acquisition mode, followed by a search for the detected components in home-made

spectral libraries, public libraries and/or in the bibliography (Diaz et al., 2012). This screening is performed for **non-target compounds** including **known** and **unknown analytes** (**unknown-knowns** and **unknown-unknowns**, respectively) (Godula et al., 2011). The search for unknown-unknowns is considered as the true untargeted analysis (looking for differences from the norm through sample profiling to spot the unexpected, then characterise and identify) (Godula et al., 2011). More detailed information about both screening types combined with the capabilities of working with **low-** and **high-resolution mass spectrometers** (**LRMS** and **HRMS**, respectively) is given in the next section.

i. Target screening for target compounds (known-knowns)

Different analysers can be employed in order to identify target compounds. The most common acquisition mode is by SRM in a QqQ or a hybrid QqLIT for LRMS analysers. Both instruments will work with a target screening method for the characterisation of the molecules by MS^2 experiments. Target screenings carried out by HRMS are usually performed in order to confirm the identity of a suspected target compound that is difficult to identify by LRMS.

ii. Non-target screening for non-target compounds with suspected analytes (unknown-knowns)

In terms of non-target screening, the meaning of suspect screening and unknown screening should be differentiated. However, in both cases the procedure by MS and MS^2 analysis is exactly the same, i.e. working in full scan mode. Regarding the non-target screening for suspects (unknown-knowns), once an unknown compound is suspected (known), its identification can be performed following the different steps explained in the previous section (target screening for target compounds).

The most used technique for the identification of non-target analytes is based on accurate mass with high-resolution mass spectrometers such as time of flight (medium–high resolution), magnetic sector, Orbitrap and Fourier-transform ion cyclotron resonance (Krauss et al., 2010; Gómez-Ramos et al., 2011; Schollée et al., 2012). The use of high resolution also allows structural studies differentiating between isobaric compounds (compounds with the same nominal mass but different accurate mass) eluted at the same retention time (Kind et al., 2007). However, LRMS can be used in order to rule out any unknown-known compounds when a non-target analyte is

suspected. The most commonly used instruments are the QqQ and the hybrid QqLIT. The possibility to perform MS^n experiments with some analysers working at LRMS is another important tool for identification purposes. This type of experiment can be performed in instruments such as the hybrid QqLIT or the IT. The isolation of the generated ions during the MS^1, MS^2 and the consequent fragmentation experiments allows the generation of MS^3 ions. This is a useful technique for structural elucidation studies during the identification of the suspected non-target compound.

In the case of HRMS, the theoretical exact masses of analytes are typically extracted from the full-spectrum acquisition data using a narrow-mass window extracted ion chromatogram (nw-XIC) with a small mass window (i.e. 0.01–0.02 Da) (Hernández et al., 2011). The full scans are often performed between 100 and 1 000 Da. However, this mass range only works for molecules smaller than 1 000 Da. In the case of macromolecules, such as microcystines or polymers, this detection mode would eventually provide information only related to multicharged ions and to some monocharged fragments or, in the case that the polymers can be easily fragmented, the detection is based on monocharged monomers. Nonetheless, the target screenings by SRM and IDA/DDA modes are the techniques of choice. The fragmentation obtained in MS^2 is highly useful for elucidating the structures of unknown compounds as well as for the isotopic pattern information that can help during the identification of the suspected compound (Hernández et al., 2011).

The general identification of the suspected compound is made by the comparison with the reference standard (the chromatographic retention time, the fragmentation pattern and the isotopic pattern). However, two different strategies are applied when this standard reference is not available (Krauss et al., 2010; Hernández et al., 2011): (1) comparison of the main MS^2 fragments with the MS^2 product ions reported in the literature, and/or; (2) by justifying the accurate mass fragments manually or using specialised software for structural elucidation (e.g. MassFragment (Waters) and MetFrag (MetFrag)). Both strategies are efficient in the identification and confirmation processes of suspected analytes (Hernández et al., 2011). In this context, hybrid instruments such as QqTOF, Q-Exactive and LTQ-Orbitrap (linear IT in tandem with an Orbitrap) allow to perform the fragmentation elucidation, MS^2 (QqTOF and Q-Exactive) – MS^n (LTQ-Orbitrap) experiments, working at HRMS (with the exception of QqTOF that works at medium resolution). The main goal of this instrumentation is the undeniable identification by exact mass of the suspected compound.

iii. Non-target screening for non-target compounds with unknown analytes (unknown-unknowns)

Unknown screening for unknown studies (unknown-unknowns) and, in a strict sense, non-target screening, starts without any a priori information on the compounds to be detected (Krauss et al., 2010; Hernández et al., 2011; Schollée et al., 2012). In terms of environmental analysis, it represents a high difficulty level. As Krauss et al. (2010) remarked, the 'unconstrained boundary conditions and a structure proposition for a peak detected by high-resolution MS and MS^2 spectra involves several work-intensive data and expert-processing steps'.

The following steps are the most commonly used in non-target workflows for unknown studies: (i) an automated peak detection by exact mass filtering from the chromatographic run; (ii) an assignment of an elemental formula to the exact mass of interest; and (iii) a database search of plausible structures for the determined elemental formula (Hogenboom et al., 2009; Krauss et al., 2010; Hernández et al., 2011).

For identification purposes, and within the European framework of the Commission Decision 2002/657/EC guidelines, the HRMS precursor and product ions (working with resolution R > 20 000) earn 2 and 2.5 identification points (Krauss et al., 2010). These identification points are sufficient according to the Commission guidelines for the unequivocal identification of an unknown compound. However, more analyses are recommended in order to avoid the detection of false positives such as proton or carbon nuclear magnetic resonance (NMR) (Schollée et al., 2012).

Nonetheless, LRMS can be used in order to get some structural information (see previous section), but HRMS is required to achieve a high degree of certainty for the identification of unknowns (Gros et al., 2012).

3.2.3 Fiehn–Kind's seven golden rules

During the structural elucidation steps, different structures are proposed. However, some of them are unfeasible. In order to rule out these structures, Kind et al. (2007) developed an algorithm for filtering molecular formulae based on seven heuristic rules making an automatic exclusion of non-correct formulae. Different researchers have used at least six of these principles to identify non-target analytes (Schollée et al.,

2012): (1) restrictions for element numbers, (2) Lewis and Senior check, (3) isotopic pattern filter, (4) hydrogen/carbon element ratio check, (5) heteroatom ratio check, and (6) element probability check.

Most of the available software used for structural elucidation operates on the basis of these seven golden rules.

3.2.4 Database searches

This search is performed after the tentative elemental assignment, based on HRMS data, when the standard of the presumed identified compound is not available to be compared with or when the synthesis of the proposed compound is not feasible.

Different computational prediction fragmentation software tools are available (see Table 2). These software tools are useful for fragmentation assignments as well as for unknown-knowns' mass spectra identification.

Database and software		Confirm accurate mass	Mass spectra library	Fragment-ation prediction	Fragment-ation mecha-nism	Biological, toxicological, physico-chemical properties	Available online
Mass Frontier (Thermo Scientific)	Software	Yes	Yes	Yes	Yes	No	No
ChromaLynx XS (Waters)	Software	Yes	Yes	No	No	No	No
Data Explorer (Applied Biosystems)	Software	Yes	Yes	No	No	No	No
National Institute of Standards and Technology mass spectra library (NIST)	Database	Yes	Yes	No	No	No	No
MetFrag (MetFrag)	Database	Yes	Yes	Yes	No	No	Yes
PubChem database (PubChem)	Database	Yes	No	No	No	Yes	Yes
ChemSpider (ChemSpider)	Database	Yes	No	No	No	Yes	Yes
Merck Index Online (Merck Index)	Database	Yes	No	No	No	Yes	Yes
NORMAN MassBank (NORMAN-FP6)	Database	Yes	Yes	Yes	No	No	Yes
MetFusion (IPB Halle)	Database	Yes	Yes	Yes	No	No	Yes

Table 2: **Databases**

3.2.5 Isotope patterns

The identification of isotope patterns is of high relevance in the case of molecules containing heteroatoms, especially in the presence of halogens such as chloride or bromide. Isotope pattern is usually included in the fragmentation prediction software tools and can help during the elucidation of the chemical formula of the suspected compound.

3.2.6 Structure–property relationships (including log K_{ow} approximations and chromatographic hydrophobicity index approximations)

After the tentative structural elucidation of an unknown compound, other complementary identification strategies can be performed for confirmatory purposes. In general, the most common procedure is the study of the different structure–property relationships of the proposed compound. The physico-chemical properties of a compound allow the prediction of the retention time in LC or GC techniques, making possible the confirmation of the suspected compound by a comparison of the empirical with the theoretical retention time. For example, the octanol–water constant or chromatographic hydrophobicity index (CHI) index are calculated theoretically by the use of different available software such as the PubChem database or Virtual Computational Chemistry Laboratory, among others.

However, the unequivocal confirmation can only be performed by comparison to a reference standard (Schollée et al., 2012).

3.2.7 Computer-assisted analysis

A computer-assisted analysis is employed to differentiate any suspected analyte from the matrix. It is usual to compare a matrix blank with the sample containing the non-target analyte. There are different software tools that allow deconvolution for peak picking and the removal of background noise (Krauss et al., 2010; Schollée et al., 2012) and that allow deconvolution of the spectral peaks by comparing different chromatograms, for example SIEVE (Thermo_Scientific), MarkerLynx (Waters), MarkerView (ABSciex) or Mass Profiler Professional (Agilent). Other similar open-source softwares are XCMS (xcmsonline) and MZmine (Pluskal et al.) for the EnviMass Excel tool (Eawag).

3.3. Biological and chemical analysis

The combination of toxicological and chemical analyses is currently gaining in importance in order to better understand any possible risks associated to a suspected sample. The toxicity identification evaluation (TIE), or the Effect-Directed Analysis (EDA), approach can also help in identifying emerging contaminants in the environment including non-target compounds.

EDA or TIE experiments are based on the study of different biological effects in different organisms. If these analyses conclude that the sample represents a biological threat, then the chemical analysis is performed to identify the corresponding toxicant. In these experiments, it is common practice to fractionate the sample in order to better isolate and identify the possible toxicant by biological tests as well as to identify the toxicant through chemical studies (Hogenboom et al., 2009; Brack et al., 2011). An example of a workflow is shown in Figure 2.

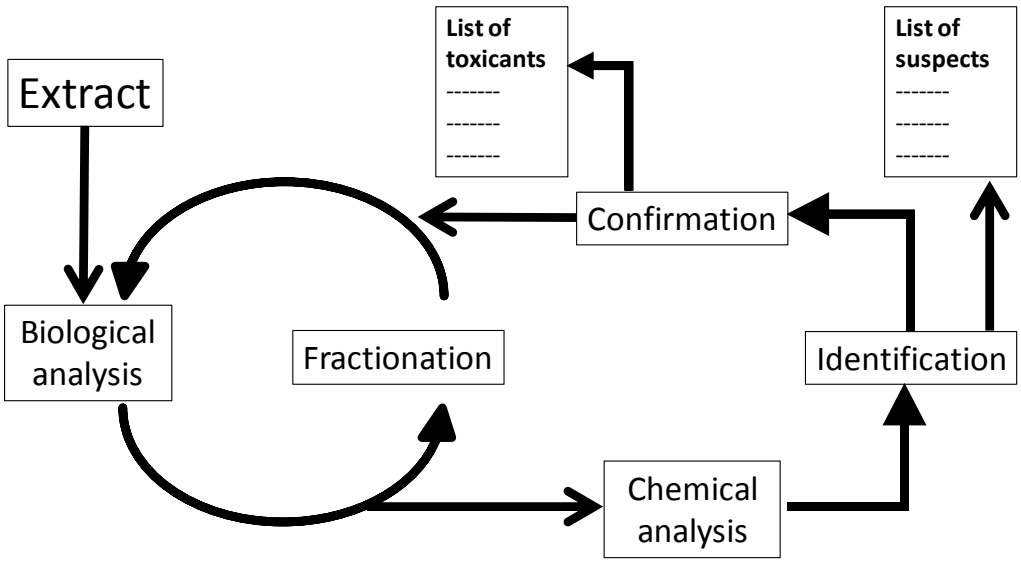


Figure 2: Workflow for EDA analyses, adapted from Leonards et al. (2011)

These types of studies were started in the early 1980s and are nowadays applied for non-target screenings studies (Brack et al., 2011). However, the time required to perform these integrated analyses, the specificity and the difficult interpretation of the results make these methods unsuitable for routine studies.

3.4. European expert laboratories in the analysis of non-target compounds in water

Several European research groups are currently developing different strategies for the screening of non-target compounds in environmental samples (including water samples). Their protocols are based on cutting-edge instrumentation, using accurate mass and fragmentation analysis. An overview of the most remarkable groups performing this kind of research is shown in Table 3.

Institution	Department	Led by	Address	Country
Faculty of Bioscience Engineering, Ghent University	Analysis and technology of organic minor components — Research Group Environmental Organic Chemistry and Technology, Department of Sustainable Organic Chemistry and Technology	Prof. Dr Kristof Demeestere	Coupure Links 653, 9000 Ghent	Belgium
Ruđer Bošković Institute	Laboratory for Analytical Chemistry and Biogeochemistry of Organic Compounds, Division for Marine and Environmental Research	Dr Marijan Ahel	Bijenička cesta 54, HR-10000 Zagreb	Croatia
Helmholtz Centre for Environmental Research (UFZ)	Department of Effect-Directed Analysis	Dr Werner Brack	Permoserstraße 15, 04318 Leipzig	Germany
Hochschule Fresenius Idstein	Chemistry and Biology	Prof. Dr Thomas P. Knepper	Limburger Straße 2, 65510 Idstein	Germany
Landeswasser-versorgung	Non-Target Screening, Committee II on Water Composition and Water Quality	Dr Wolfgang Schluz	Am Spitzigen Berg 1, 89129 Langenau	Germany
Leibniz Institute of Plant Biochemistry (IPB)	Bioinformatics and Mass Spectrometry; Stress and Developmental Biology	Dr Steffen Neumann	Weinberg 3, 06120 Halle (Saale)	Germany
Institute of Environmental Assessment and Water Research (IDAEA-CSIC)	Service of Mass Spectrometry	Prof. Dr Josep Caixach	Jordi Girona, 18–26 08034 Barcelona	Spain
Institute of Environmental Assessment and Water Research (IDAEA-CSIC)	Department of Environmental Chemistry	Prof. Dr Damià Barceló	Jordi Girona, 18–26 08034 Barcelona	Spain
University Jaume I	Research Institute of Pesticides and Water	Dr Félix Hernández	University Jaume I, 12071 Castellón	Spain
University of Almeria	Pesticide Residue Research Group, Department of Hydrogeology and Analytical Chemistry	Prof. Dr Amadeo Fernández-Alda	La Cañada de San Urbano, 04120 Almería	Spain
Official Food Control Authority of the Canton of Zurich	—	Dr Anton Kauffmann	Kantonales Labor Zürich, Fehrenstrasse 15, 8032 Zürich	Switzerland
Swiss Federal Institute of Aquatic Science and Technology	Department of Environmental Chemistry	Dr Julianne Hollender	Ueberlandstrasse 133, 8600 Dübendorf	Switzerland
KWR Watercycle Research Institute	Chemical Water Quality and Health	Prof. Dr Pim de Voogt	Groningenhaven 7, 3433 PE Nieuwegein	The Netherlands
University of Amsterdam	Institute of Biodiversity and Ecosystem Dynamics	Prof. Dr Pim de Voogt	Nieuwe Achtergracht 166, 1018 WV Amsterdam	The Netherlands
Wageningen University and Research Centre	RIKILT	Dr Linda Stolker	Akkermaalsbos 2, 6708 WB Wageningen	The Netherlands

Table 3: European research groups specialised in the identification of target and non-target compounds

4. Future perspectives

Based on the reported literature and the findings of recent projects, the main limitation is the difficulty associated with data processing in order to get the information from one sample analysis. The detection of unknowns can be relatively easy, but the identification of these compounds represents a highly difficult step and qualified expertise is needed. However, the main research groups focused on the study of non-target compounds in the environment are currently working on the generation of different user-friendly software processors for analyte identification.

In addition, the lack of sample processing and analysing agreements has also been identified as a problem. For this reason, the unification of efforts and analytical steps through the implementation of different standardised protocols as well as the performance of inter-laboratory studies is important (May 2011). These protocols must include the sampling process, sample pre-treatment (if necessary) and, finally, sample analysis (NORMAN-FP6, May 2011). In view of this objective and within the NORMAN-FP6 project, the organisation of a collaborative trial on non-target screening of selected water samples from the Danube river with the GC-MS and LC-HR-MS(MS) methodologies available in participating laboratories will be carried out this year (2013). This activity will be pursued in 2014 with the treatment of the results, drafting of the report and organisation of a workshop for discussion and dissemination of the results (NORMAN-FP6 2013). Another approach is the so-called 'water security initiative' launched by the United States Environmental Protection Agency (EPA) in order to 'develop robust, comprehensive, and fully coordinated surveillance and monitoring systems, including international information, for … water quality that provides early detection and awareness of disease, pest, or poisonous agents' (Homeland Security Presidential Directive 9 (2004)), and whose methodology has been recently published (EPA 2013a; EPA 2013b).

5. List of acronyms

CHI: Chromatographic hydrophobicity index

CID: Collision-induced dissociation

DDA: Data-dependent acquisition

EDA: Effect-Directed Analysis

EPI: Enhanced product ion

FT-ICR: Fourier-transform ion cyclotron resonance

GC: Gas chromatography

GC–MS: Gas chromatography coupled to mass spectrometry

HILIC: Hydrophilic interaction liquid chromatography

HRMS: High-resolution mass spectrometer

IDA: Information-dependent acquisition

IT: Ion trap analyser

K_{ow}: Octanol–water distribution constant

LC: Liquid chromatography

LC–MS: Liquid chromatography coupled to mass spectrometry

LRMS: Low-resolution mass spectrometer

MS: Mass spectrometry

MS^2: Mass spectrometry experiments in tandem

MS^n: n Mass spectrometry experiments in tandem

NMR: Nuclear magnetic resonance

Q: Quadrupole analyser

QqLIT: Hybrid quadrupole-linear ion trap analyser

QqQ: Triple quadrupole analyser

Q-TOF: Hybrid quadrupole time of flight analyser

R: Resolution or resolving power

Rt: Retention time

SIM: Selected ion monitoring

SPE: Solid phase extraction

SRM: Selected reaction monitoring

TIE: Toxicity identification evaluation

TOF: Time of flight analyser

6. References

'ALOGPS 2.1 program', Virtual Computational Chemistry Laboratory, http://www.vcclab.org/lab/alogps/start.html.

Applied Biosystems, Matrix Science: AB Sciex Data Explorer, http://www.matrixscience.com/help/instruments_data_explorer.html.

Bovee, T.F.H. and Pikkemaat, M.G., 'Bioactivity-based screening of antibiotics and hormones', *Journal of Chromatography A,* Vol. 1216, Issue 46, Elsevier, November 2009, pp. 8035–8050.

Brack, W. and Burgess, R., 'Considerations for incorporating bioavailability in effect-directed analysis and toxicity identification evaluation', *Effect-directed analysis of complex environmental contamination,* Springer Berlin Heidelberg, April 2011, pp. 41–68.

'ChemSpider — The free chemical database', http://www.chemspider.com/.

'ChromaLynx', Waters, http://www.waters.com/waters/nav.htm?cid=513759&locale=es_ES.

Commission Decision 2002/657/EC of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results, OJ L 221, 17.8.2002, p. 8.

Diaz, R., Ibanez, M., Sancho, J.V. and Hernandez, F., 'Target and non-target screening strategies for organic contaminants, residues and illicit substances in food, environmental and human biological samples by UHPLC-QTOF-MS', *Analytical Methods,* Vol. 4, Issue 1, Royal Society of Chemistry, 2012, pp. 196–209.

Eawag — Department of Environmental Chemistry, http://www.eawag.ch/forschung/uchem/index_EN.

'EnviMass', Swiss Federal Institute of Aquatic Science and Technology (Eawag), http://www.eawag.ch/forschung/uchem/software/enviMass1.

Godula, M., Charlton, A. and Mittendorf, K., 'Non-targeted searching for food contaminants using Orbitrap high-resolution mass spectrometry', 2011, http://www.aoaceurope.com/2011pr/file %2021.pdf.

Gómez-Ramos Mdel, M., Pérez-Parada, A., García-Reyes, J.F., Fernández-Alba, A.R. and Agüera, A., 'Use of an accurate-mass database for the systematic identification of transformation products of organic contaminants in wastewater effluents', *Journal of Chromatography A*, Vol. 1218, Issue 44, Elsevier, November 2011, pp. 8002–8012.

Gómez, M. J., Gómez-Ramos, M.M., Agüera, A., Mezcua, M., Herrera, S. and Fernández-Alba, A. R., 'A new gas chromatography/mass spectrometry method for the simultaneous analysis of target and non-target organic contaminants in waters', *Journal of Chromatography A*, Vol. 1216, Issue 18, Elsevier, May 2009, pp. 4071–4082.

Gros, M., Rodríguez-Mozaz, S. and Barceló, D., 'Use of enhanced product ion scans in quantitative and qualitative analysis of water pollutants', AB SCIEX Webinar, 2012.

Hernández, F., Sancho, J.V., Ibáñez, M. and Portolés, T., 'Time-of-flight and quadrupole time-of-flight mass spectrometry for identifying unknown contaminants and degradation products in the environment', *Encyclopedia of Analytical Chemistry,* John Wiley & Sons, Ltd, 2011.

Hogenboom, A.C., van Leerdam, J.A. and de Voogt, P., 'Accurate mass screening and identification of emerging contaminants in environmental samples by liquid chromatography-hybrid linear ion trap Orbitrap mass spectrometry', *Journal of Chromatography A*, Vol. 1216, Issue 3, Elsevier, January 2009, pp. 510–519.

'In silico fragmentation for computer assisted identification of metabolite mass spectra', MetFrag, http://msbi.ipb-halle.de/MetFrag/.

Kind, T. and Fiehn, O. (2007), 'Seven golden rules for heuristic filtering of molecular formulae obtained by accurate mass spectrometry', *BMC Bioinformatics,* Vol. 8, Issue 1, 2007, p. 105.

Krauss, M., Singer, H. and Hollender, J., 'LC-high resolution MS in environmental analysis: from target screening to the identification of unknowns', *Analytical and Bioanalytical Chemistry*, Vol. 397, Issue 3, March 2010, pp. 943–951.

Leonards, P.E.G., Brix, R., Barceló, D. and Lamoree, M., 'Advanced GC–MS and LC–MS tools for structure elucidation in effect-directed analysis', *Effect-directed analysis of complex environmental contamination*, Vol. 15, Springer, 2011, pp. 143–165.

'List of scientific activities organised by the NORMAN network in 2013', NORMAN-FP6, 2013, http://www.norman-network.net/public_docs/about_us/list_scientific_activities_norman_2013_to_members_8feb2013.pdf.

Llorca, M. 'Analysis of Perfluoroalkyl Substances in food and environmental matrices', PhD thesis, IDAEA-CSIC, Barcelona, 2012.

'MassFragment', Waters, http://www.waters.com/waters/nav.htm?cid=1000943.

'Mass Frontier 7.0 Spectral Interpretation Software', Thermo Scientific, http://www.thermoscientific.com/ecomm/servlet/productsdetail_11152_L10877_80588_11961841_-1.

May, B., 'Potable water contamination emergency: The analytical challenge', *Water contamination emergencies: Monitoring, understanding and acting*, Royal Society of Chemistry, 2011, pp. 110–116.

'MetFusion', IPB_Halle, http://msbi.ipb-halle.de/MetFusion/.

'National Institute of Standards and Technology mass spectra library', NIST, http://www.sisweb.com/software/ms/nist.htm.

'Network of reference laboratories for monitoring of emerging environmental pollutants', NORMAN-FP6, http://www.norman-network.net/index_php.php?menu2=public/about_us/about_us&module=public/about_us/home.

'NORMAN MassBank', NORMAN-FP6, http://massbank.normandata.eu/MassBank/.

Pluskal, T., Castillo, S., Villar-Briones, A. and Orešič, M., 'MZmine 2', http://mzmine.sourceforge.net/.

'PubChem database', PubChem, http://pubchem.ncbi.nlm.nih.gov/.

Richardson, S.D., 'Water analysis: Emerging contaminants and current issues', *Analytical Chemistry*, Vol. 79, Issue 12, ACS Publications, June 2007, pp. 4295–4324.

Schollée, J. and de Voogt, P. 'State of the art: Structure elucidation and formula finding of emerging contaminants in water', Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, 2012.

'SIEVE™ software for differential expression', Thermo Scientific, http://www.thermoscientific.com/ecomm/servlet/productsdetail_11152___11962143_-1.

'The Merck index database', http://themerckindex.cambridgesoft.com/.

'Water: Legislation and directives — Water security initiative', United States Environmental Protection Agency, http://water.epa.gov/infrastructure/watersecurity/lawsregs/initiative.cfm.

'Water quality event detection system challenge: Methodology and findings', United States Environmental Protection Agency, 2013a.

'Water security initiative: Guidance for building laboratory capabilities to respond to drinking water contamination', Environmental Protection Agency, 2013b.

'XCMS Online — Scrips Centre for Metabolomics', http://metlin.scripps.edu/xcms/.

European Commission
EUR 26643 EN – Joint Research Centre – Institute for the Protection and Security of the Citizen (IPSC)

Title: State-of-the-art of screening methods for the rapid identification of chemicals in drinking water
Authors: Marta Llorca, Sara Rodríguez-Mozaz, Catalan Institute for Water Research (ICRA), Girona, Spain

Luxembourg: Publications Office of the European Union

2013 – 29 pp. – 21.0 x 29.7 cm

EUR – Scientific and Technical Research series – ISSN 1831-9424

ISBN 978-92-79-38294-9

doi:10.2788/80087

Abstract

The contamination of drinking water is potentially harmful and poses a risk to public health. If any observation suggests a potential contamination of drinking water, such as consumer complaints about the alteration of the water's organoleptic properties, the appearance of health problems or an alarm triggered by sensors, a rapid identification of the hazard causing the problem is necessary. With regards to chemical contamination, EU Member States have several strategies to deal with the presence of unknown chemicals in water: there are screening methods as well as systematic approaches used for the analysis and identification of different groups of chemicals.

This report provides a brief overview of the existing methods for the non-targeted screening of organic compounds in water samples by means of mass spectrometry. This review is thus based on the studies and explorations that can be performed by different mass spectrometry approaches. In addition, the most relevant European institutions working on this topic and that are currently contributing to the developmentof the non-target screening of pollutants are presented.

LB-NA-26643-EN-N

As the Commission's in-house science service, the Joint Research Centre's mission is to provide EU policies with independent, evidence-based scientific and technical support throughout the whole policy cycle. Working in close cooperation with policy Directorates-General, the JRC addresses key societal challenges while stimulating innovation through developing new methods, tools and standards, and sharing its know-how with the Member States, the scientific community and international partners.

Publications Office