# Novel analytical methods for the identification of emerging contaminants in aquatic environments

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Academic Dissertation

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*“New directions in science are launched by new tools much more often than by new concepts. The effect of a concept-driven revolution is to explain new things in new ways. The effect of a tool-driven revolution is to discover new things that have to be explained.”*

*- Freeman Dyson, physicist and mathematician*

*“As crude a weapon as the cave man's club, the chemical barrage has been hurled against the fabric of life - a fabric on the one hand delicate and destructible, on the other miraculously tough and resilient, and capable of striking back in unexpected ways. These extraordinary capacities of life have been ignored by the practitioners of chemical control who have brought to their task no "high-minded orientation," no humility before the vast forces with which they tamper.”*

- *Rachel Carson, Silent Spring 1962*

*“Those who contemplate the beauty of the earth find reserves of strength that will endure as long as life lasts. There is symbolic as well as actual beauty in the migration of the birds, the ebb and flow of the tides, the folded bud ready for the spring. There is something infinitely healing in the repeated refrains of nature - the assurance that dawn comes after night, and spring after the winter.”*

*- Rachel Carson, Silent Spring 1962*

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## LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following four publications, which in the text are referred to by their Roman numerals:

I. Nurmi J. and Pellinen J. Multiresidue method for the analysis of emerging contaminants in wastewater by ultra performance liquid chromatography–time-of-flight mass spectrometry, J. Chromatogr. A 1218 (2011) 6712.

II. Nurmi J., Pellinen J. and Rantalainen A.-L. Critical evaluation of screening techniques for emerging environmental contaminants based on accurate mass measurements with time-of-flight mass spectrometry, J. Mass Spectrom. 47 (2012) 303.

III. Jernberg J., Pellinen J. and Rantalainen A.-L. Qualitative nontarget analysis of landfill leachate using gas chromatography time-of-flight mass spectrometry, Talanta 103 (2013) 384.

IV. Jernberg J., Pellinen J. and Rantalainen A.-L. Identification of organic xenobiotics in urban aquatic environments using time-of-flight mass spectrometry, Sci. Total Environ. 450-451 (2013) 1.



## THE AUTHOR'S CONTRIBUTION

Joonas Jernberg (formerly Nurmi) took the main responsibility for planning, carrying out the experimental laboratory work and processing the data produced in all four articles. Jernberg wrote the articles and is the corresponding author. Dr. Pellinen and Dr. Rantalainen participated in the planning of the studies and commented on the manuscripts. In articles III and IV, Rantalainen and Pellinen participated in the field sampling, respectively.

## ABSTRACT

Growth in economics and prosperity has been a global trend during recent decades and the use of chemicals has increased tremendously as a part of industrial production, agriculture and everyday life. The use of hazardous chemicals has been restricted by many intergovernmental treaties and legislation but new replacement chemicals are synthesized constantly and no decrease in the future production volumes of chemicals is soon expected. The term chemicalization is used to describe the increased use of chemicals and resultant environmental contamination. In the analysis of environmental samples, usually only some selected regulated compounds are measured. The problem with these target analyses is that other compounds remain undetected. Complementary techniques without any preselection of the analytes are thus required to identify new compounds.

The aim of this study was to develop novel instrumental techniques for the determination of organic compounds without any analyte preselection and to identify unknown anthropogenic contaminants in different water matrices. The methods developed were based on analytical separation, using gas and liquid chromatography combined with accurate mass measurement using time-of-flight mass spectrometry. The data produced were then processed with a deconvolution program to locate the chromatographic peaks and to extract their mass spectra. The measured accurate masses were then used to confirm the elemental compositions of the detected ions. The identification processes were validated, using spiked water samples, and finally the methods were applied to the identification of organic xenobiotics from wastewater effluent, stormwater, surface water and landfill leachate samples.

The results showed that analysis using time-of-flight mass spectrometry enables screening of large analyte groups without previous information on sample composition. The most comprehensive knowledge is yielded by analysing the sample with both gas and liquid chromatography. In many cases, tentative compound identification can be obtained if the deconvoluted spectra and accurate mass data are complemented with information, e.g. from spectral libraries and peak isotope patterns. This tentative identifications must, however, always be confirmed with a pure standard compound. The main limitations of the methods were related to insufficient features of the deconvolution program used. Most of the data-processing stages had to be performed manually or visually, which slows down the data processing and hinders their applicability, especially with large sample sets. Dozens of compounds were tentatively identified from water samples and several of them were also confirmed with a standard compound. The highest numbers of compounds were identified from wastewater effluent, stormwater and landfill leachate samples. The results confirmed the fact that anthropogenic waste streams are an important route for organic xenobiotics into the environment. Since the future volumes of chemicals will increase, the control and efficient treatment of these fluxes becomes evermore essential.

# TIIVISTELMÄ

Globaalin vaurastumisen myötä maapallon teollinen tuotanto, maatalous ja jokapäiväinen arkielämä perustuvat yhä enemmän erilaisten kemikaalien käyttöön. Tämän kehityksen haittapuoli on ympäristön kemikalisoituminen, jolla tarkoitetaan yhä lisääntyvää kemikaalien käyttöä ja sitä kautta tapahtuvaa ympäristön pilaantumista. Haitalliseksi tunnistettujen yhdisteiden käyttöä on rajoitettu useilla kansainvälisillä sopimuksilla ja kansallisella lainsäädännöllä, mutta uusia korvaavia yhdisteitä kehitetään ja otetaan käyttöön jatkuvasti, eikä kemikaalien käytön vähenemistä ole näköpiirissä. Pitkään jatkuneesta tutkimuksesta huolimatta vain harvojen kemiallisten yhdisteiden päästöt ja pitoisuudet ympäristössä tunnetaan. Tyypillisesti kemikaalien määritykset ympäristöstä rajoittuvat lainsäädännössä mainittuihin haitta-aineisiin muiden yhdisteiden jäädessä huomiotta. Ympäristönäytteiden kokonaisvaltaisempi analyysi edellyttää laboratoriolta uusia menetelmiä, jotka mahdollistavat myös etukäteen määrittelemättömien yhdisteiden tunnistamisen ja mittaamisen.

Tämän väitöskirjatutkimuksen tavoitteena on ollut kehittää uusia, ensisijaisesti kvalitatiivisia, instrumenttianalyyttisiä menetelmiä vesien sisältämien orgaanisten yhdisteiden määritykseen ilman etukäteisvalintaa sekä tunnistaa antropogeenisiä haitta-aineita erityyppisistä vesinäytteistä. Tutkimuksessa kehitetyt menetelmät perustuvat kaasu- ja nestekromatografiseen erotukseen ja analyyttien tarkan massan mittaukseen lentoaikamassaspektrometrillä sekä kerätyn aineiston käsittelyyn dekonvoluutio-ohjelmistolla. Analyysimenetelmiin liittyvät tunnistusprosessit validoitiin tutkimalla tunnettuja väkevöityjä näytteitä, jonka jälkeen menetelmiä sovellettiin Päijät-Hämeen alueelta kerättyihin jätevesi-, hulevesi-, pintavesi- ja kaatopaikan suotovesinäytteisiin.

Tutkimustulokset osoittivat, että analyysi lentoaikamassapektrometrillä mahdollistaa suurten yhdistejoukkojen seulonnan ilman ennakkotietoa näytteen koostumuksesta. Kattavin tieto näytteestä saavutetaan, kun näyte analysoidaan sekä kaasu- että nestekromatografisilla menetelmillä. Useissa tapauksissa mittauksilla voidaan saavuttaa alustava tunnistus yhdisteestä, mikäli tarkan massan mittauksiin yhdistetään tietoa esimerkiksi spektrikirjastoista ja ionien isotooppisuhteista. Lopullisen tunnistuksen tulee kuitenkin aina perustua malliaineen käyttöön. Tunnistusmenetelmien suurimmat rajoitteet johtuivat käytetyn dekonvoluutio-ohjelman puutteellisista ominaisuuksista, jonka vuoksi valtaosa työvaiheista jouduttiin suorittamaan manuaalisesti. Tulosten käsittelyn hitaus, erityisesti suurten näytemäärien yhteydessä, rajoittaakin vielä toistaiseksi menetelmien laaja-alaista sovellettavuutta. Tutkituista näytteistä tunnistettiin alustavasti kymmeniä yhdisteitä, joista osa varmistettiin myös malliaineilla. Suurimmat lukumäärät yhdisteitä tunnistettiin puhdistetusta jätevedestä, hulevedestä ja kaatopaikan suotovedestä. Tulokset osoittavat, että ihmisen toiminnasta syntyvät jätevirrat muodostavat tärkeän reitin orgaanisten yhdisteiden kulkeutumiselle ympäristöön. Kemikaalien käyttömäärien kasvaessa, näiden päästölähteiden kontrolloinnin ja asianmukaisen käsittelyn merkitys tulee entisestään korostumaan.

## ABBREVIATIONS

| | |
|---|---|
| ACN | acetonitrile |
| APCI | atmospheric pressure chemical ionization |
| APPI | atmospheric pressure photoionization |
| BRIICS | Brazil, Russia, India, Indonesia, China and South Africa |
| CI | chemical ionization |
| CID | collision-induced dissociation |
| EI | electron ionization |
| ESI | electrospray ionization |
| FWHM | full width at half maximum |
| FT-ICR | Fourier transform ion cyclotron resonance |
| GC | gas chromatography |
| HPLC | high-performance liquid chromatography |
| HR | high-resolution |
| ILD | instrumental limit of detection |
| ILQ | instrumental limit of quantification |
| IP | identification point |
| IT | ion trap |
| LC | liquid chromatography |
| LLE | liquid-liquid extraction |
| LOI | limit of identification |
| MDL | method detection limit |
| MeOH | methanol |
| MQL | method quantification limit |
| MRM | multiple reaction monitoring |
| MS | mass spectrometry / mass spectrometer |
| MS/MS | tandem mass spectrometry |
| m/z | mass-to-charge ratio |
| NIST | National Institute of Standards and Technology |
| nw-XIC | narrow-window extracted ion chromatogram |
| oa-TOF-MS | orthogonal acceleration time-of-flight mass spectrometry |
| OECD | Organization for Economic Cooperation and Development |
| PAH | polycyclic aromatic hydrocarbon |
| PBDE | polybrominated diphenyl ether |
| PCB | polychlorinated biphenyl |
| REACH | Registration, Evaluation, Authorization and Restriction of Chemicals |
| SBSE | stir bar sorptive extraction |
| SIM | selected ion monitoring |
| S/N | signal-to-noise ratio |
| SPE | solid phase extraction |
| SPME | solid phase microextraction |
| SRM | selected reaction monitoring |
| TDC | time-to-digital converter |

| | |
|---|---|
| TIC | total ion chromatogram |
| TOF | time-of-flight |
| UHPLC | ultra-high performance liquid chromatography |
| QqQ | triple-quadrupole |
| QTOF | quadrupole time-of-flight |
| $\Delta m$ | mass error |
| $\Delta t$ | variation in ion flight time |

# 1. INTRODUCTION

## 1.1 Chemicalization of the environment

Growth in economics and prosperity has been a global trend during recent decades. At the same time, the use of chemicals has increased tremendously as a part of industrial production, agriculture and everyday life. The Organization for Economic Cooperation and Development (OECD) predicted that global chemical production will increase about 3% per year and by year 2024 will be doubled from the level observed in year 2000 (OECD 2001). A very recent report (OECD 2012) stated that the same increasing trend will last at least until year 2050. Meanwhile, as production volumes are increasing, the chemical industry is also geographically relocating. The annual growth in the total production in Brazil, Russia, India, Indonesia, China and South Africa (BRIICS) will surpass the production of the OECD member countries after year 2030 (Fig. 1).

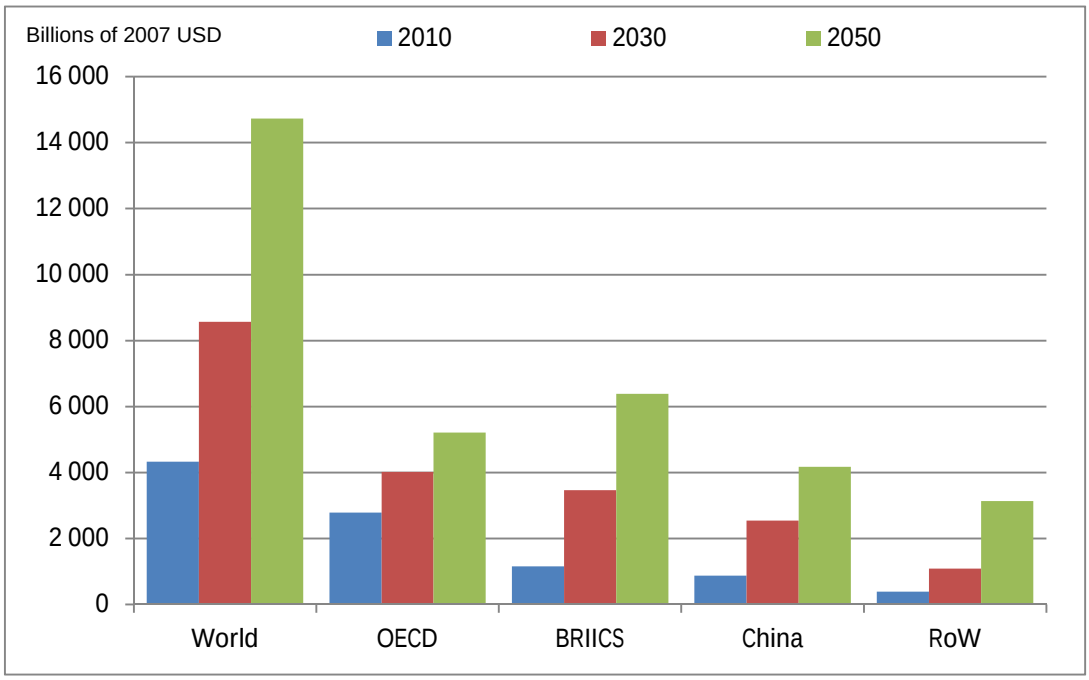



Fig. 1. Estimated development of chemical production volumes (OECD 2012). (RoW: rest of the World)

Since the 1960s, the health effects of chemicals have gained interest among researchers. Consequently, exposure to xenobiotics may adversely affect the development of humans (Barr et al. 2007, Grandjean et al. 2008), especially in the early stages of life (Main et al. 2006). In addition to their impacts on humans, chemicals may also harm the environment. This perspective was especially raised by Rachel Carson in her famous book *Silent Spring*, published in 1962 (Carson 1962). Hence, awareness of the dangers related to chemicals has increased, and the use of many toxic chemicals has been restricted in several countries. For example, the Stockholm Convention (European Parliament 2000) has since 2001 become ratified in 177 countries. This environmental treaty eliminates or restricts the use and production of several persistent organic pollutants.

Although the use of hazardous chemicals has continually been limited, the decrease in overall use of chemicals is not evident. Several undeniable benefits are gained from the use of chemicals, and despite the possible detriments, new chemicals are synthesized and introduced to the market all the time. According to Finnish Environmental Institute, more than 100 000 chemicals are currently used globally and nearly 30 000 products containing hazardous substances are imported or manufactured in Finland (Finnish Environmental Institute 2012). During or after use, most of these chemicals finally end up in the environment. In this thesis, the increased use of chemicals and resulting environmental contamination are defined by the term *chemicalization*. Intentional and negligent actions, e.g. dumping of chemical waste, are excluded from the definition. In the context of chemicalization, emissions of chemicals into the environment occur unintentionally and generally without people being aware of them.

With regard to chemicalization, emerging contaminants have gained increasing interest in the field of environmental research in recent years (Al-Odaini et al. 2010, Bisceglia et al. 2010, Eggen et al. 2010, Nödler et al. 2010, Pedrouzo et al. 2009). Emerging contaminants are a structurally diverse and heterogeneous group of chemical compounds that are not currently covered by existing regulations or legislation, have not been studied widely and are believed to pose a threat to environmental ecosystems (Farré et al. 2008b). Generally, pharmaceuticals, hormones, personal care products, perfluorinated compounds, flame retardants, pesticides and their transformation products are included in this group (Richardson 2009, Richardson and Ternes 2011). Although a parent compound itself may not be harmful, various chemical reactions in the environment can change the chemical and physical properties and toxicity of the compound (Bedner and MacCrehan 2006). Despite previous research, knowledge of the occurrence of organic compounds in the environment is still limited. Reliable results of ecotoxicological studies are furthermore available for only a small fraction of the above-mentioned compound groups. Emerging contaminants originate from anthropogenic sources, which results in the increase in their concentrations, especially in urban areas (Diamond and Hodge 2007). Several emerging contaminants, such as flame retardants, are also used as ingredients in domestic products. The fate of these chemicals and the extent of their transport into the environment are dependent on their use and disposal. In households, products are commonly disposed with solid waste or with wastewater. Wastewater effluent, landfill leachate and stormwater runoff from urban areas are thus important routes of emerging contaminants into the environment.

## 1.2 Instrumental analysis of organic contaminants in the environment

The analyses of emerging organic contaminants in different environmental sample matrices are predominantly based on chromatographic separations and mass spectrometric detection (Wille et al. 2012). Before analysis, several sample pretreatment steps like filtration, pH adjustment, isolation from matrix and sample concentration are usually required. With aquatic samples, the concentration of analytes is usually performed along with extraction. Two most commonly used extraction techniques are liquid-liquid extraction (LLE) and solid phase extraction (SPE), but also other more novel techniques like solid phase microextraction (SPME) and stir bar sorptive extraction (SBSE) can be applied. For multiresidue methods, in which dozens or hundreds of compounds are determined in a single analysis, the extraction should not be too selective. Instead, rather generic technique is pursued to be able to extract as large variety of compounds as possible. LLE and SPE are well suited for this purpose and were thus used in this thesis. The use of SPME and SBSE is increasing but still limited due to requirements of specific instrument accessories. In this thesis, gas chromatography (GC) and liquid chromatography (LC) in combination with time-of-flight mass spectrometry (TOF-MS) were used. In the following three chapters, a brief review of these techniques and related analytical strategies are presented.

### *1.2.1 Gas chromatography and liquid chromatography*

Chromatography is a common name for separation techniques in which the separation is based on the interaction of an analyte between the stationary phase and the mobile phase. Analytes with differing tendencies to interactions with the phases travel through the chromatographic system over different lengths of time. GC and LC are the two most important techniques for the analysis of organic compounds in environmental samples. GC is best suited for volatile and semivolatile nonpolar compounds, while LC is used in the analysis of nonvolatile thermolabile compounds with wide ranges in polarity.

The introduction of chromatographic techniques dates back to 1906 (Tswett 1906a, Tswett 1906b) when the Russian botanist Mikhail Tswett first used the term *chromatography* when reporting his historical LC experiments. However, the active development of chromatography did not truly evolve until the 1940s. In 1941, Martin and Synge proposed the use of gas as a mobile phase (Martin and Synge 1941) and, as a consequence, the theory of gas-liquid partition chromatography, on which modern GC was founded, was presented in 1952 by the British scientists James and Martin (James and Martin 1952). The first commercial GC instruments were introduced in 1955 by the Burrell Corporation (Pittsburgh, PA, USA) and the PerkingElmer Corporation (Waltham, MA, USA). In modern GC, separations are mainly carried out in capillary columns in which a thin layer of the liquid stationary phase is bound on the inner wall of the capillary. Inert gas, usually helium or hydrogen, flowing through the capillary, is used as a mobile phase (carrier gas) to transport the vaporized analytes. The separation mechanism of GC

is based on gas-liquid partitioning and the analytes are separated according to their relative vapour pressures and solubility in the stationary phase. A wide variety of different stationary phases and dimensions for GC columns are currently available commercially and many priority pollutants are routinely measured with the existing well-established GC methods.

Until the 1960s, LC separations were carried out in packed glass cylinders and the movement of the liquid mobile phase was based on gravity. Introduction of the instrument capable of high-pressure pumping of liquid in 1969 started the occurrence in LC techniques. The most recent developments in LC technology have been seen in column chemistry, along with decreasing column dimensions and the particle size of the column packing material. Modern columns with sub-2-µm particle inner diameters provide superior chromatographic resolution and fast separation, but at the price of increased backpressure of the mobile phase flow. The new generation of LC instruments capable of operating at very high pressures (>1000 bar) have been termed as ultrahigh-performance liquid chromatography (UHPLC) devices to distinguish them from the traditional high-performance liquid chromatography (HPLC) instruments working at pressures <400 bar.

### *1.2.2 Time-of-flight mass spectrometry*

Mass spectrometers (MS) are instruments that determine the mass-to-charge ratio (m/z) and the abundance of gas-phase ions. MS instruments can detect only ions and thus the molecules analysed must first be ionized in an ion source before they can be separated and detected. The most common ionization techniques with GC are electron ionization (EI) and chemical ionization (CI). Electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI) and atmospheric pressure photoionization (APPI) are the predominant ionization techniques used with LC. After ionization, the ions with different m/z values are separated in an analyser. Finally, the abundance of the ions is measured by the detector. Various types of analysers are commercially available such as time-of-flight (TOF), magnetic sector, linear quadrupole, quadrupole ion trap (IT), Fourier transform ion cyclotron resonance (FT-ICR), orbitrap and several hybrid analysers (i.e. combinations of different analysers) and MS instruments are thus often classified according to the analyser (Gross 2004).

TOF analyser was first introduced by Stephens in 1946 at the American Physical Society (Stephens 1946). The schematic diagram of a modern TOF-MS is illustrated in Figure 2. In TOF analysers, ions of different m/z ratios are separated by simultaneously accelerating them to the same kinetic energy and channelling them into a field-free flight tube. During the acceleration, ions with different m/z ratios acquire different velocities, causing them to disperse in time during the flight and finally reach the detector at the end of the flight tube at different moments of time. The lighter ions arrive at the detector before the heavier ones. The flight time $t$ of an ion is proportional to the square

root of the m/z ratio according to Eq. 1, in which *s* is the travel distance, *e* the electron charge and *U* the acceleration voltage.

$$t = \frac{s}{\sqrt{2eU}}\sqrt{\frac{m}{z}} \qquad \text{(Eq. 1)}$$

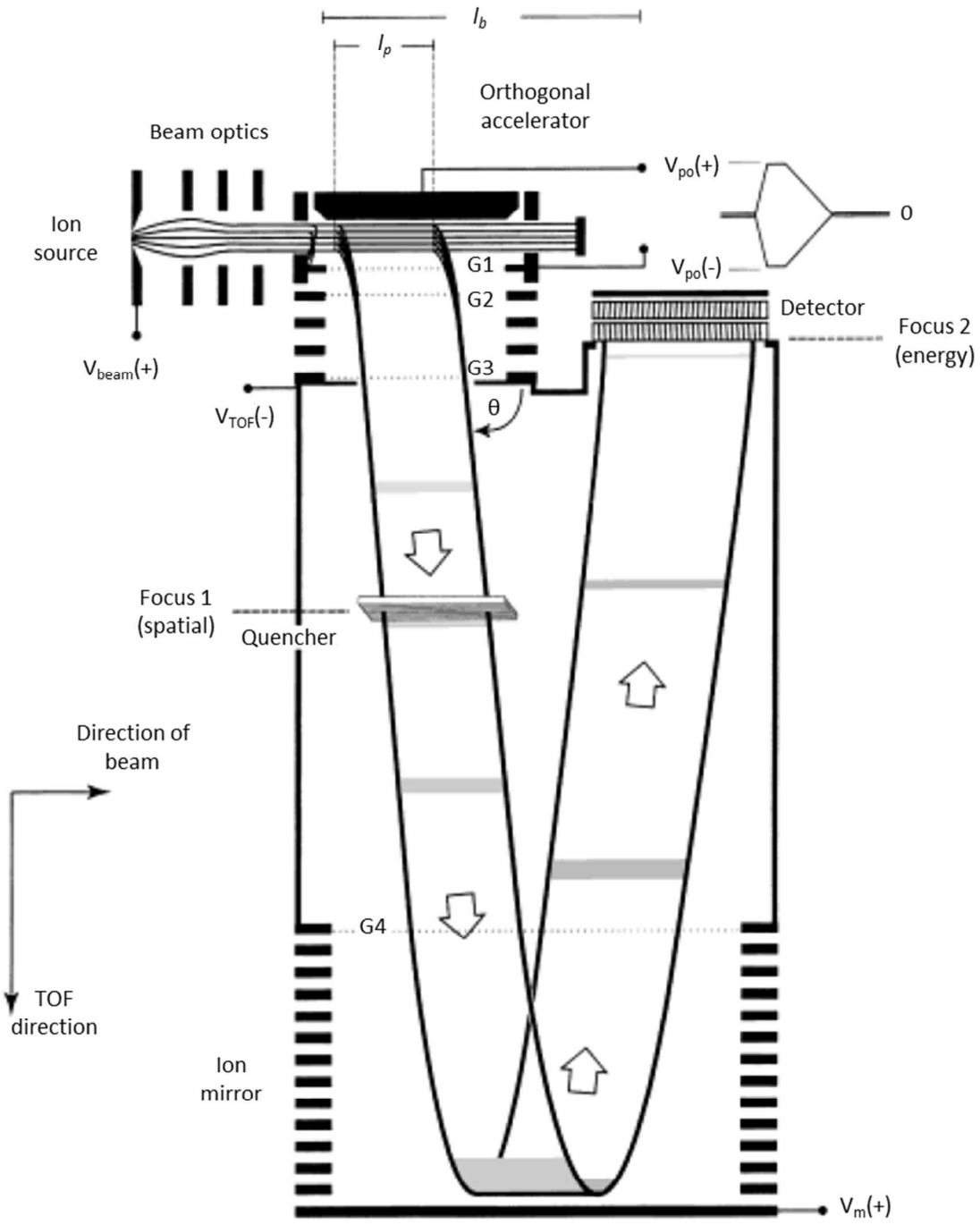



Fig. 2. Configuration of an oa-TOF system with single-stage reflectron. G1–G4: grids. Reproduced from (Guilhaus et al. 2000), with permission of John Wiley & Sons Ltd.

The term *resolution* describes the ability of an instrument to separate two ions with different m/z. In practice with TOF-MS, this means measurement of the variation in ion flight times ($\Delta t$) on the order of nanoseconds. The resolution ($R$) is defined in Eq. 2 as a ratio of the mass ($m$) to the difference in mass ($\Delta m$) measured as the width of the peak at a certain peak height (e.g. 5%, 10% or 50%). The use of full width at half maximum (FWHM) definition is widespread in connection with TOF-MS.

$$R = \frac{m}{\Delta m} \qquad \text{(Eq. 2)}$$

The two most important factors affecting the resolution of TOF analysers are the initial spatial and energy spread of the ions in the ion pulser at the beginning of the transient measurement cycle. These factors result in variation in the arrival time for a given mass and thus a decrease in resolution. Variation in the position (i.e. spatial spread) of an ion pulser is largely corrected, using Wiley-McLaren space focusing, in which a potential gradient is established across the pulser (Wiley and McLaren 1955). The other factor is related to the difference in energies. If two ions of the same mass have the same residual energy but in opposite directions, during the acceleration one of the ions will first move in the wrong direction for a brief moment before the repelling electric field reverses its direction. This so-called turnaround time will cause a significant input in $\Delta t$, reducing the resolution. The effect is generally reduced by minimizing the energy spread of the ions entering the ion pulser with beam-forming optics and slits. In addition, the turnaround time may be shortened by increasing the accelerating field.

The most important instrumental development used to overcome the effects of energy spread of TOF analysers was made in the late 1960s, when an instrumental part called the reflectron was introduced (Karataev et al. 1972, Mamyrin 2001). A reflectron is an ion mirror that consists of a series of ring electrodes at increasing potential. When the ions penetrate into the field of the reflectron, their kinetic energy is lost and their direction is reversed. The penetration depth of the ion is dependent on its kinetic energy, and those ions with higher energies travel longer distances. The reflectron thus corrects the variation in travel time by adjusting the travelling distance according to the kinetic energy.

Another important development related to coupling of TOF analysers with continuous ion sources (e.g. EI and ESI ion sources) was the introduction of orthogonal acceleration TOF-MS (oa-TOF-MS) (Coles and Guilhaus 1993, Dawson and Guilhaus 1989). In oa-TOF-MS, the direction of the TOF separation is orthogonally separated from the ion-beam axis of the ion source (see Fig. 2). The electrostatic force which accelerates the ions into the flight tube produces a component of the velocity that is independent of the axial velocity of the ion beam. The main advantages gained from this construction are (1) the ability to reduce the spread of the velocity component in the TOF direction (i.e. increase in resolution) and (2) the ability to control independently the ion-beam energy and drift energy (Guilhaus et al. 2000). The latter advantage maximizes the sampling

duty cycle by balancing the time required to fill the accelerator by the time required for the ions to drift to the detector, making them approximately the same. Commonly, the sampling duty cycle (i.e. ratio of the number of ions accelerated from accelerator to those entering to accelerator) of oa-TOF varies between 5% and 30% (Chernushevich et al. 2001). The overall mass analyser efficiency of the oa-TOF-MS can be derived by multiplying the duty cycle by the transmission of the analyser and the efficiency of the detector (Guilhaus et al. 2000).

Before the invention of the reflectron, TOF instruments were equipped with linear flight tubes on the order of 1–2 m long. These instruments suffered from low mass resolution (≤ 500 FWHM). Currently, modern oa-TOF-MS instruments with a built in reflectron provide R values >10 000 FWHM and are considered as high-resolution (HR) instruments. As a consequence, with continuous internal calibration techniques mass accuracies < 2 mDa are routinely obtained. The mass accuracy of TOF instruments is based on two factors: high mass resolution and a sufficient number of ions measured (Fjeldsted 2009). High resolving power minimizes the possibility of overlapping of two mass peaks by narrowing the peak width and the high number of ions reduces the uncertainty in establishing the mean value of the peak apex. The benefits of the accurate mass feature are especially highlighted in its ability to determine the elemental composition of a measured ion.

### *1.2.3 Analytical strategies and recent applications*

The analytical methods can generally be divided into *target analysis* and *nontarget analysis*, according to their purpose of use (Ibáñez et al. 2008). In target methods, only those compounds that have been selected for the analysis are determined and the method is validated solely for these compounds. The method is developed using pure standard compounds, and the maximum sensitivity for the target analytes is pursued. Single-quadrupole analysers in the selected ion monitoring (SIM) mode are very suitable for this purpose, but higher selectivity and sensitivity are generally obtained, using tandem MS (MS/MS) techniques. In MS/MS analysis, the analytes are detected via selected reaction monitoring (SRM), also called multiple reaction monitoring (MRM), by measuring product ions generated from precursor ions. Thus, the characteristic product ions must be known before analysis. Normally, detection of at least two SRM transitions is required to ensure proper identification of an analyte (Pozo et al. 2006).

The most frequently used analyser in target analyses is a triple-quadrupole (QqQ). The analyser provides high selectivity and sensitivity, which are usually required when working with complex environmental samples. Until recently, MS/MS target analyses with QqQs have mainly been associated with LC-MS instruments while the GC-MS analyses have mostly been carried out with single quadrupole analysers using SIM mode. However, statutory requirements for lower detection limits are increasing the use of QqQ-MS instruments also in GC analysis. The disadvantage of MS/MS techniques is that they lead up to biased information on samples, because only the user-defined data

obtained through SRMs or MRMs are saved. All the other sample data are discarded and sample compounds not specified beforehand remain unknown. Thus, target analysis using MS/MS is not a technique which allows for the determination of unknown compounds. Magnetic sector instruments are also still used to analyse organic pollutants (especially polychlorinated dibenzo-*p*-dioxins and dibenzofurans), but the high price and demanding operation of the instruments limit their wide use (Hernández et al. 2012).

The aim of nontarget analysis is to carry out search for as many compounds in a sample as possible with the focus on compounds not previously known to be present (Krauss et al. 2010). During the nontarget analysis, all m/z within the defined mass range generated from the compounds eluting from the column by ionization are recorded throughout the analysis, i.e. full-spectrum data are saved. It is evident that with nontarget methods it is not possible to identify all the compounds present in the sample, which may result in false-negative results. This is due to the inherent nature of the analytical procedure, as the chosen sample preparation technique, mobile phase, ionization process etc., of always excluding some of the compounds. An important feature of nontarget methods is that the full-spectrum dataset enables the retrospective analysis of the sample even years after the data acquisition.

The prerequisite for nontarget analysis is an instrument that is capable of recording the full spectrum rapidly, so that sufficient numbers of data points are obtained during the elution of chromatographic peak. Modern TOF-MS instruments fulfil these demands. The instruments provide high mass resolution combined with high full-spectrum sensitivity and speed. In addition to plain TOF analyser, different hybrid TOF analysers are also available. The most common analyser combination is a quadrupole TOF (QTOF), which has become a standard analyser in commercial modern TOF instruments. Another somewhat less usual hybrid analyser is an IT-TOF. The additional advantageous feature of these hybrid TOF analysers compared to plain TOF is the possibility to perform MS/MS analysis. With QTOF instruments the fragmentation is limited to $MS^2$ while with IT-TOF $MS^n$ fragmentation is enabled. In nontarget analysis, analyte identification is usually based on the use of accurate mass. The accurate mass ion chromatograms from the location of chromatographic peak are extracted and combined with a special deconvolution program to form a spectrum of the unknown compound. Then, the elemental composition of the ions in a spectrum is deduced from the accurate mass of the ions. Often the number of candidate elemental compositions needs to be reduced with the aid of information obtained, e.g. from isotopic patterns, spectral libraries, elemental filters, retention time and chemical databases.

Another possible accurate mass-based approach, using full-spectrum data is *posttarget analysis* (Hernández et al. 2005). In this technique, the exact masses of the analytes of interest are extracted from the total ion chromatogram (TIC) after the analysis. The resulting narrow-window extracted ion chromatogram (nw-XIC) quickly reveals whether the analyte of interest might be present in the sample. If several peaks representing the

same mass appear, the validity of the candidates needs to be assessed, using spectral libraries, isotope peak patterns, presence of fragment ion peaks or retention times.

Target analysis has so far been the primary technique used in environmental analytical chemistry. The problem with this method is it is not able to detect unknown compounds. Current lists of target compounds additionally become outdated rapidly. The increased use of TOF-MS instruments can be seen as a new trend in the field of environmental research to address this problem (Ferrer et al. 2006, Ferrer and Thurman 2010b). The applicability of GC-TOF-MS for the nontarget analysis of complex sample matrices, such as human breast adipose tissue (Hernández et al. 2009) and honeybee samples (Portolés et al. 2009), has recently been reported. Further information on the analytical strategies using high-resolution mass spectrometry (HRMS) and GC-TOF-MS can be found e.g. in the extensive reviews of Hernández et al. (Hernández et al. 2012, Hernández et al. 2011b). The use of LC-TOF-MS, including some applications of nontarget analysis, is presented in a monograph by Ferrer and Thurman (Ferrer and Thurman 2009). Recent applications of LC-TOF-MS and GC-TOF-MS for the analysis of anthropogenic organic compounds in aquatic matrices are presented in Tables 1 and 2, respectively. The tables show that TOF-MS instruments are the most commonly used in identification of an analyte of interest. The identification of transformation products is an especially important application area. TOF-MS devices have also been used in quantitative analysis, but to a minor extent. This is mainly due to the rather narrow dynamic ranges of TOF analysers and the saturation of the detectors at high sample concentrations. The demanding selectivity requirements of quantitative analysis (European Commission 2002) are generally more easily fulfilled, using QqQ instruments and MS/MS techniques.

The identification of emerging contaminants requires new techniques and methods that are capable of determining large numbers of analytes in a single analysis by producing full-spectrum datasets. Although some environmental TOF-MS applications can be found in the literature, publications in which nontarget analysis was used to identify unknown compounds are still scarce. TOF-MS is still mostly used in a posttarget manner, and in most publications nontarget analysis was only briefly demonstrated but not widely applied. In this thesis, the uncovered potential of TOF-MS for the identification of organic compounds is explored by the analysis of aquatic samples.

Table 1. Recent LC-TOF-MS applications in the analysis of anthropogenic organic compounds in aquatic matrices.

| Analytes of interest | Number of analytes | Sample matrix[a] | Instrumentation[b] | Analyte identification[c] | Quantitative analysis[d] | Reference |
|---|---|---|---|---|---|---|
| Artificial sweeteners | <20 | GW, SW, WW | HPLC-TOF-MS | x | x | (Ferrer and Thurman 2010b) |
| Transformation products of bisphenol A | <20 | LW, WW | HPLC-TOF-MS | x | - | (Mezcua et al. 2006) |
| Transformation products of diclofenac | <20 | LW | HPLC-TOF-MS | x | - | (Agüera et al. 2005) |
| Transformation products of enalapril | <20 | LW | UHPLC-QTOF-MS | x | - | (Pérez et al. 2007) |
| Identification of unknowns | 20-100 | GW, SW, WW, LL | HPLC-QTOF-MS | x | - | (Ibáñez et al. 2005) |
| Pesticides | 20-100 | SW | HPLC-TOF-MS | - | - | (Sasaki et al. 2006) |
| Pesticides | <20 | SW | Capillary-LC-TOF-MS | - | - | (Holm et al. 2003) |
| Pesticide + transformation products | >100 | SW | HPLC-TOF-MS | x | x | (Ferrer and Thurman 2007) |
| Pesticide + transformation products | 1-20 | DW | UHPLC-QTOF-MS | x | - | (Brix et al. 2009) |
| Pharmaceuticals | 1-20 | DW, GW, SW | HPLC-QTOF-MS | x | - | (Stolker et al. 2004) |
| Pharmaceuticals | 20-100 | SW, WW | UHPLC-QTOF-MS | - | x | (Farré et al. 2008a) |
| Pharmaceuticals | 20-100 | WW | HPLC-TOF-MS | x | x | (Gómez et al. 2007) |
| Pharmaceuticals | 20-100 | SW, WW | UHPLC-QTOF-MS | x | x | (Petrovic et al. 2006) |
| Pharmaceuticals + transformation products | <20 | DW | UHPLC-IT-TOF-MS | x | - | (Melton and Brown 2012) |
| Pharmaceuticals + transformation products | <20 | DW, GW, SW, WW | HPLC-QTOF-MS | x | x | (Ferrer and Thurman 2010a) |
| Pharmaceuticals + transformation products | >100 | WW | UHPLC-QTOF-MS | x | - | (Hernández et al. 2011a) |
| Pharmaceuticals + transformation products | >100 | WW | HPLC-QTOF-MS | x | - | (Ferrer and Thurman 2012) |
| Pharmaceuticals and pesticides | >100 | WW | UHPLC-QTOF-MS | x | - | (Pitarch et al. 2010) |
| Pharmaceuticals and pesticides | >100 | WW | HPLC-QTOF-MS | x | - | (Gómez-Ramos et al. 2011) |
| Pharmaceuticals and pesticides | 20-100 | WW | HPLC-TOF-MS | x | - | (Martínez Bueno et al. 2007) |
| Pharmaceuticals and pesticides | >100 | SW, WW | HPLC-QTOF-MS | x | x | (Gómez et al. 2010) |
| Surfactants | 20-100 | SW, WW | HPLC-TOF-MS | x | x | (Lara-Martín et al. 2011) |

[a] DW: drinking water, GW: groundwater, SW: surface water, WW: wastewater, LW: laboratory water, LL: landfill leachate
[b] HPLC: high-performance liquid chromatography, UHPLC: ultrahigh-performance liquid chromatography, IT: ion trap
[c] In the application, the TOF analyser is used in the identification of the compounds.
[d] In the application, the TOF analyser is used to quantitate the analytes of interest.

Table 2. Recent GC-TOF-MS applications in the analysis of anthropogenic organic compounds in aquatic matrices.

| Analytes of interest[a] | Number of analytes | Sample matrix[b] | Instrumentation | Analyte identification[c] | Quantitative analysis[d] | Reference |
|---|---|---|---|---|---|---|
| Identification of unknowns | 20-100 | SW | GC-(EI)-TOF-MS | x | - | (Serrano et al. 2011) |
| Organophosphate triesters | <20 | GW, SW | GC-(EI)-TOF-MS | x | - | (Nácher-Mestre et al. 2011) |
| Pesticides | <20 | GW | GC-(EI)/(CI)-TOF-MS | x | - | (Portolés et al. 2011b) |
| Pesticides, PAHs | 20-100 | AW, SW | GC-(EI)-TOF-MS | - | x | (Amelin et al. 2011) |
| Pesticides, PAHs, octyl/nonyl phenols | 20-100 | GW, SW, WW, LL | GC-(EI)-TOF-MS | x | x | (Hernández et al. 2007) |
| Pesticides, PCBs, PBDEs | 20-100 | WW | GC-(EI)-TOF-MS | x | x | (Pitarch et al. 2010) |
| Pesticides, PCBs, PBDEs, PAHs | 20-100 | GW, SW, WW | GC-(EI)-TOF-MS | x | - | (Portolés et al. 2007) |
| Pesticides, PCBs, PBDEs, PAHs | >100 | GW, SW, WW | GC-(EI)-TOF-MS | x | - | (Portolés et al. 2011a) |
| Pesticides, PCBs, PBDEs, PAHs | >100 | SW | GC-(EI)-TOF-MS | x | x | (Serrano et al. 2012) |
| Pesticides, personal care products | <20 | SW, WW | GC-(EI)-TOF-MS | x | x | (Gómez et al. 2009) |

[a] PCB: polychlorinated biphenyl, PBDE: polybrominated diphenyl ether, PAH: polycyclic aromatic hydrocarbon

[b] AW: artesian water, GW: groundwater, SW: surface water, WW: wastewater, LL: landfill leachate

[c] In the application, the TOF analyser is used in the identification of the compounds.

[d] In the application, the TOF analyser is used to quantitate the analytes of interest.

## 2. OBJECTIVES OF THE STUDY

The main objective of this study was to develop novel analytical methods for the identification of organic contaminants in aquatic environments using LC-TOF-MS and GC-TOF-MS. The ultimate aim was to assess the accurate mass features of TOF-MS and to apply them to qualitative posttarget and nontarget analysis. To fulfil these aims, several environmental water samples representing different matrices were collected and analysed. The data produced were processed, using posttarget and nontarget analysis.

More specifically, the aims of the study were the following:

- To develop a general instrumental multiresidue method for LC-TOF-MS for posttarget and nontarget analyses of different types of water samples and for compounds with variable properties (I)
- To develop a systematic procedure to process the data from LC-TOF-MS in qualitative posttarget and nontarget analyses of emerging contaminants in water samples (II)
- To develop a systematic procedure to process the data from GC-TOF-MS in qualitative nontarget analysis of emerging contaminants in complex environmental water samples (III)
- To estimate the feasibility of TOF-MS for posttarget and nontarget techniques for the identification of organic contaminants in environmental water samples (II–IV)
- To assess the identification reliability and to explore the possible limitations of posttarget and nontarget analysis (II–IV).

# 3. MATERIALS AND METHODS

A short summary of the used analytical methods and instrumentation is given in this chapter. A more detailed description of the experimental work can be found in the original publications.

## 3.1 Samples and sample preparation

Water samples representing various matrices were collected in solvent-rinsed glass bottles as grab samples from the City of Lahti, southern Finland, and its surroundings. The sample types were wastewater effluent (I, II, IV), landfill leachate (III), lake and river water (IV) and stormwater (IV) samples. The sample volumes varied between 100 and 1000 mL. The samples were stored in the dark at 4 °C and were prior to extraction filtered on fibreglass filters. The organic compounds in the samples were isolated before analysis, using either SPE (I, II, IV) or LLE (III). Ultrapure laboratory water samples were always processed in parallel with the environmental water samples.

In publications I and II, the water samples were acidified to pH 2.0 with 37% HCl and then passed through the SPE cartridges. Oasis MCX 150 mg/6 mL from Waters Corp. (Milford, MA, USA) and a Strata-X 200 mg/6 mL from Phenomenex Inc. (Torrance, CA, USA) were used in series. The analytes were eluted with a solution containing 5% $NH_3$ (aq) in methanol (MeOH) (v/v). In publication IV, the water samples were acidified to pH 3.0 with 1 M HCl and then extracted with Oasis HLB 200 mg/5 mL glass cartridges from Waters. The analytes were eluted with dichloromethane and ethyl acetate. Using LLE, an aliquot of leachate sample was first extracted with *n*-hexane and then with dichloromethane (III). The extracts were concentrated with a rotary evaporator and transferred into glass tubes. The samples for GC-TOF-MS analyses were dried after extraction, using granular anhydrous sodium sulphate. Finally, all samples were concentrated under a gentle nitrogen flow to the final sample volume and transferred into sample vials.

## 3.2 Liquid chromatography-time-of-flight mass spectrometry

The LC-TOF-MS analyses were performed in publications I and II. The LC separations were carried out with the ACQUITY UPLC™ (Waters). Chromatographic separations of compounds were carried out, using an ACQUITY UPLC BEH C18 column (100 mm x 2.1 mm, 1.7 µm) with an ACQUITY BEH C18 VanGuard™ precolumn (5 mm x 2.1 mm, 1.7 µm). The temperature of the column chamber was set to 35 °C. In positive ESI (ESI(+)), the mobile phase was composed of solvent A (5 mM ammonium bicarbonate, pH 9.5, pH adjusted with $NH_3$ (aq)) and B (100% MeOH). In negative ESI (ESI(-)), the mobile phase was composed of solvents A (0.05% acetic acid in water (v/v)) and B (0.05% acetic acid in MeOH (v/v)). Gradient elution with a flow rate of 0.45 mL/min was used.

The analytes were detected, using an oa-TOF-MS Micromass LCT Premier XE (Micromass® MS Technologies, Manchester, UK) in the W optics mode with ESI(+) and ESI(-). The resolution of the instrument in the W mode is > 11 000 FWHM. The capillary voltages were 3000 V and 2800 V for ESI(+) and ESI(-), respectively. The desolvation gas flow of nitrogen was 800 L/h and temperature 350 °C. The source temperature was 120 °C. The acquisition rate was 0.15 s per scan, with interscan delay of 0.01 s between scans. The measured mass range (m/z) was 100–1000 in the centroid mode.

To ensure accuracy of the mass measurements, all analyses were performed with lock mass ion m/z of 557.2802 for ESI(+) and m/z of 555.2645 for ESI(-) correction by continuously injecting a leucine-enkephaline solution (300 pg/µL, 1:1 acetonitrile (ACN):$H_2O$) at a flow rate of 25 µL/min via a reference sprayer. In measuring, the lock mass ion software automatically corrects for any possible drift in the mass axis. The lock mass ion was measured every 50 scans.

## 3.3 Gas chromatography-time-of-flight mass spectrometry

The GC-TOF-MS analyses were performed in publications III and IV. The analyses were carried out, using an oa-TOF-MS GCT Premier instrument from Micromass® MS Technologies. Chromatographic separations of the sample compounds were performed, using a ZB-5MSi column (30 m x 0.25 mm x 0.25 µm) with a deactivated guard column (2 m x 0.25 mm) from Phenomenex. Helium was used as the carrier gas in a constant flow mode of 1.0 mL/min. The samples were injected, using a GC Pal injection system (CTC Analytics, Zwingen, Switzerland) and splitless injection technique.

The TOF-MS was operated in the EI mode at 70 eV. The resolution of the instrument was > 7000 FWHM. The acquisition rate was 0.09 s per scan with an interscan delay of 0.01 s between scans. The measured mass range (m/z) was 50–550 in the centroid mode. The lock mass ion m/z of 218.9856 was produced by continuously injecting a lock mass compound (heptacosafluorotributylamine) into the ion source from an internal reservoir.

## 3.4 Data processing

The LC-TOF-MS and GC-TOF-MS instruments were controlled, using the MassLynx program from Waters. The analysed data were processed with TargetLynx and ChromaLynx XS, two application managers of the MassLynx. The TargetLynx is a program for target analysis and quantitative working, while the ChromaLynx XS program is a peak detection and spectral deconvolution tool with automatic library searching, screening and comparison features. The ChromaLynx was used to process the data in all post-target and nontarget analyses performed. The National Institute of Standards and Technology (NIST) mass spectral library 2008 was used for compound identification with GC-TOF-MS analysis.

# 4. RESULTS AND DISCUSSION

The ionization techniques used in GC-MS and LC-MS instruments differ from each other considerably. Especially the different analyte fragmentation is a factor that affects the appearance of the related mass spectra and therefore also the identification of unknown compounds. The developed methods must thus contain separate elements that are not interchangeable between the GC and LC techniques. The presentation of the results is therefore divided into two parts. Chapter 4.1 summarises the results from the LC-TOF-MS analyses and chapter 4.2 the results from the GC-TOF-MS analyses.

## 4.1 Liquid chromatography-time-of-flight mass spectrometry

The LC-TOF-MS experiments were performed in publications I and II. First, a multiresidue method for use in posttarget and nontarget analysis was developed and validated (I). The sample extraction procedure and the mobile phase of the chromatographic separation were optimized to obtain maximum recovery and ionization efficiency in ESI, respectively. The instrumental method developed was then applied to posttarget and nontarget analysis and validated, using spiked wastewater effluent samples (II). Finally, the method was applied to analysis of unspiked wastewater samples.

### *4.1.1 Development and optimization of the multiresidue method*

In posttarget and nontarget analysis, the sample pretreatment and the chromatographic method must be generic to enable the analysis of large numbers of compounds with varying chemical properties. In addition, the selected mobile phase composition greatly affects the ionization efficiency of the analytes in LC-MS techniques (Kostiainen and Kauppila 2009). A C18 reverse-phase chromatography column (100 mm x 2.1 mm 1.7 µm) was chosen and MeOH was used as an organic solvent in the eluent. The additives for the organic and aqueous mobile phases were optimized during method development (I). The tested mobile phase compositions are presented in Table 3.

Table 3. Mobile phase compositions tested.

| | Eluent A | Eluent B |
|---|---|---|
| 1 | 0.1% formic acid in water | 0.1% formic acid in MeOH |
| 2 | 0.1% acetic acid in water | 0.1% acetic acid in MeOH |
| 3 | 5 mM ammonium acetate in water | 5 mM ammonium acetate in MeOH |
| 4 | 0.05% ammonia (aq) in water | 0.05% ammonia (aq) in MeOH |
| 5 | 5 mM ammonium bicarbonate in water (pH 9.5) | MeOH |

For ESI(+), a basic mobile phase with $NH_3$ (aq) resulted in the highest ionization efficiency, but appeared to be unstable in terms of pH, causing drifting of the retention times for some analytes. Ammonium bicarbonate with higher buffering capacity was therefore chosen as an additive for the mobile phase. For ESI(-), acidic solutions generally resulted in better peak shape and provided higher retention factors than basic or

neutral mobile phases. Acetic acid, with a slightly higher ionization efficiency than formic acid, was chosen for use as the mobile phase.

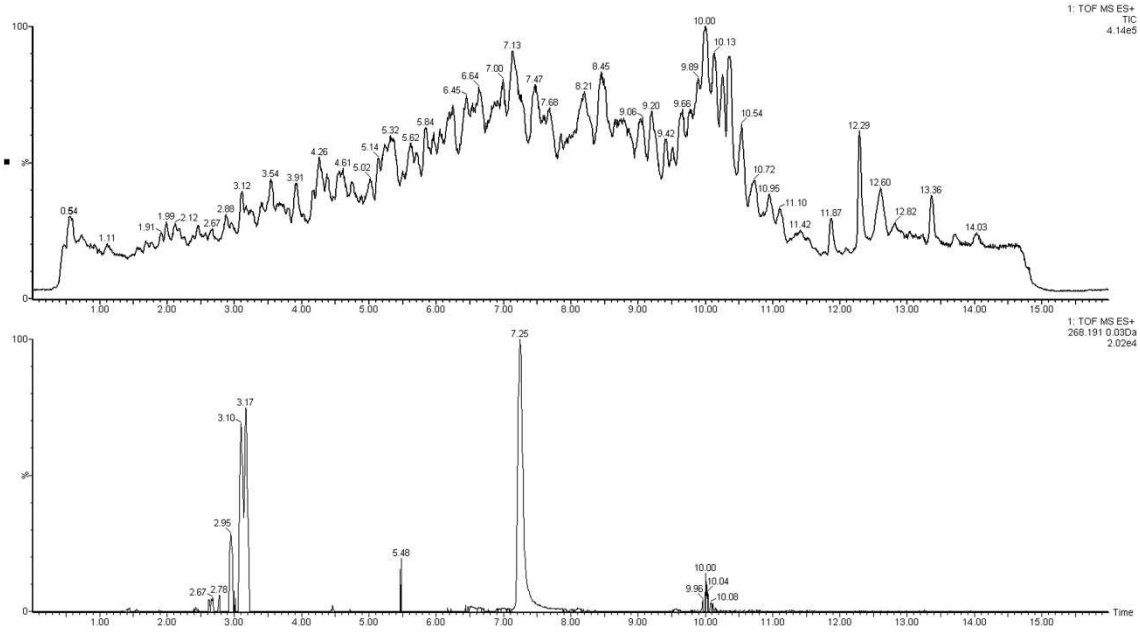



Fig 3. TIC (ESI(+)) of wastewater effluent sample (upper) and nw-XIC of m/z 268.1912 corresponding to metoprolol (retention time 7.25 min) (lower).

The recoveries of the analytes in the various SPE sorbents were estimated by comparing four commercial sorbent materials (Table 4) under neutral and acidic conditions (I). The sorbents were evaluated by spiking wastewater effluent with a mixture of 83 compounds (pesticides and pharmaceuticals) to a final concentration of 100 ng/L and comparing the peak areas of the spiked analytes. The results of the experiments showed that selection of a single sorbent leads to loss of some compounds. Thus, a combination of the two best cartridges (Oasis MCX and Strata-X), using acidic sample pH, was chosen. The cartridges were assembled in series and the same sample first passed through the Oasis MCX and then the Strata-X. By using two different sorbent types (reverse phase and cation exchange), neutral, acidic and basic analytes could be extracted simultaneously.

The median values for recovery were 95% and 105% for low (75 ng/L) and high (500 ng/L) level measurement, respectively. Only for five compounds were the recoveries less than 70%. The disadvantage of the use of two cartridges is increased matrix suppression in ionization, which in turn decreases the sensitivity of the method. Figure 3 illustrates a typical wastewater sample chromatogram measured at ESI(+) and nw-XIC of a detected analyte (metoprolol). The median values of matrix suppression for the low and high concentration levels were 33% and 26% for ESI(+) and 38% and 37% for ESI(-). The suppression observed in this study was more extensive than that previously reported for wastewater (Marín et al. 2009). On the other hand, the capability of TOF-MS instruments for ion extraction with very narrow mass windows increases the selec-

tivity and sensitivity of the method (Fig. 3), so that the resulting overall sensitivity was considered to be high enough for the analytical purposes.

Table 4. SPE sorbent materials and conditions tested.

| Sorbent | Size | Sorbent type[a] | Sample pH(1) | Sample pH(2) | Manufacturer |
|---|---|---|---|---|---|
| Strata-X | 500 mg/6 mL | RP | 3.0 | 6.5 | Phenomenex |
| Oasis HLB | 500 mg/6 mL | RP | 3.0 | 6.5 | Waters |
| Strata-X-C | 500 mg/6 mL | CEX | 3.0 | - | Phenomenex |
| Oasis MCX | 500 mg/6 mL | CEX | 3.0 | - | Waters |

[a] RP: reverse phase, CEX: cation exchange

### *4.1.2 Target analysis*

Although the primary aims of this study concerned qualitative methods, the quantitative performance of the LC-TOF-MS method developed was also examined with target analysis of pesticides and pharmaceuticals (I). The sensitivity of the LC-TOF-MS instrument was assessed by measuring the instrumental limit of detection (ILD) and instrumental limit of quantification (ILQ) values for all 84 compounds used in method development. For ESI(+), the median value of ILD was 7.5 pg and for ILQ 19 pg. For ESI(-), the values were 20 pg and 46 pg. The sensitivity of the method was evaluated with method detection limit (MDL) and method quantification limit (MQL) values based on the signal-to-noise ratio (S/N) value. For MDL and MQL calculation S/N values of 3 and 10 were used, respectively. The median MDL and MQL values for positive polarity were 15 and 28 ng/L and for negative polarity 26 and 49 ng/L, which are comparable to or somewhat higher than those in other multiresidue methods (Petrovic et al. 2006).

Table 5. Concentrations[a,b] (ng/L) of quantified compounds in wastewater effluent.

| **ESI(+)-** | Replicate 1 | Replicate 2 |
|---|---|---|
| Diuron | < MQL[c] | < MQL |
| Atenolol | 330 ± 2.9 | 300 ± 7.2 |
| Cyclophosphamide | < MQL | < MQL |
| Metoprolol | 1100 ± 59 | 830 ± 52 |
| Trimethoprim | 780 ± 22 | 650 ± 17 |
| Warfarin | < MQL | < MQL |
| **ESI(-)** | Replicate 1 | Replicate 2 |
| Diclorprop | 61 ± 0.3 | 61 ± 0.2 |
| MCPA | 50 ± 0.1 | 49 ± 0.3 |
| Mecoprop | 70 ± 0.3 | 70 ± 0.6 |
| Bezafibrate | 39 ± 0.4 | 40 ± 0.3 |
| Diclofenac | 420 ± 1.1 | 410 ± 0.3 |
| Furosemide | 2200 ± 180 | 2100 ± 110 |
| Hydrochlorothiazide | 1800 ± 23 | 1500 ± 52 |
| Ketoprofen | 250 ± 2.8 | 250 ± 5.5 |

[a] Not corrected with recovery.

[b] Standard deviation of three replicate injections.

[c] MQL: method quantification limit

The linear range of the method varied between two and three orders of magnitude. Knowledge of the linear range of the analyte is essential because the microchannel plate detector used in TOF-MS may saturate even at low μg/L levels. The target analytes were quantified in the wastewater effluent, using the standard addition method. Three pesticides and eight pharmaceuticals were found in concentrations up to ~2200 ng/L (Table 5). The results showed that the LC-TOF-MS instrument can also be used for quantitative work when the method is adequately validated.

### *4.1.3 Posttarget analysis*

Posttarget analysis of the LC-TOF-MS data were first validated, using a wastewater effluent sample spiked with a mixture of 88 standard compounds (pesticides and pharmaceuticals) at concentrations of 75 and 500 ng/L. 67 of these compounds were detected at ESI(+) and 21 at ESI(-). Data with and without retention time information on the spiked analytes were used to assess the identification performance of posttarget analysis. The outlines of posttarget and nontarget analysis, using LC-TOF-MS experiments are illustrated in Figure 4.

Posttarget analyses were first performed with retention time data information (Fig. 4, experiment 1). In this experiment, nw-XICs of ± 30 mDa corresponding to the exact protonated and deprotonated masses of posttarget analytes were extracted from the TIC and screened within the defined retention time window of ± 0.2 min. The classification of the identification results in MassLynx is based on the mass error value (Δm), which is the difference between the exact and measured mass. A user-defined cutoff-Δm (mDa) value was used in defining whether the identification was stated as correct (Δm < cutoff-Δm) or tentative (cutoff-Δm < Δm < 30 mDa) by the software. The results showed that with the cutoff-Δm value of 5 mDa, more than 90% of the compounds were correctly identified and therefore, 5 mDa was used as the cutoff-Δm value throughout the study. The proper cutoff-Δm value is naturally dependent on the mass accuracy of the MS instrument and should thus be separately verified for each instrument.

Posttarget analysis with retention time information is an efficient technique for screening the presence of analytes of interest in the sample. However, if the retention time is unknown, as is often the case with emerging contaminants, the task is more challenging. This was studied in experiment 2 of Figure 4, in which nw-XICs of ± 30 mDa corresponding to the exact protonated and deprotonated masses of posttarget analytes were extracted from the TIC without retention time information. Now, all components with Δm less than the cutoff-Δm value were labelled as possible positive identifications, of which only one (or possibly none) could be the correct one. A four-stage identification process was developed to diminish the number of these candidates (Fig. 4, experiment 2).

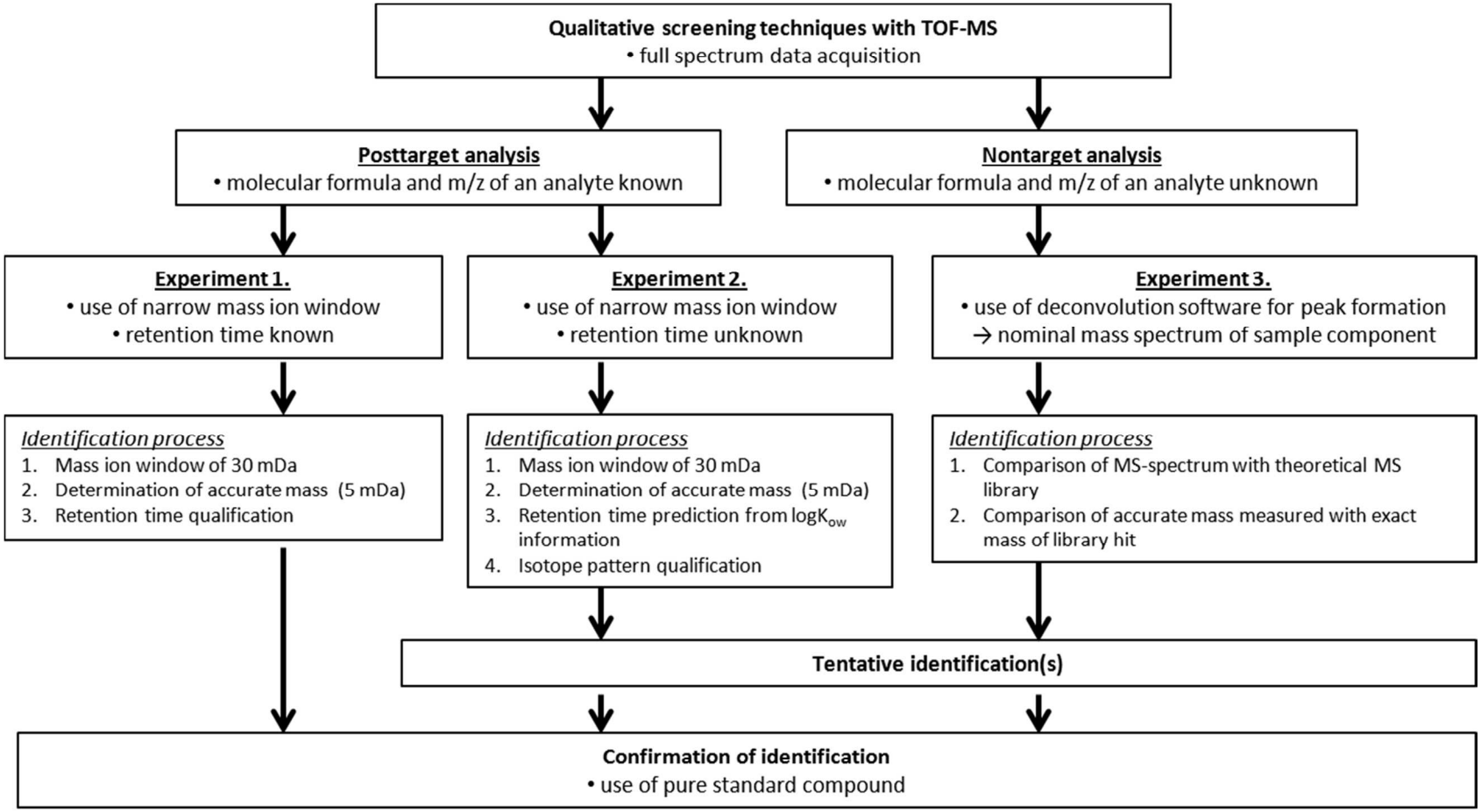



Fig. 4. Outlines of LC-TOF-MS posttarget and nontarget experiments performed.

The first and second stages of the process were based on the accurate mass. At the first stage, candidates within an nw-XIC of 30 mDa were extracted from the TIC and at the second stage, only candidates with a Δm less than 5 mDa were qualified. The results showed that after these two stages there were still too many possible candidates within that accurate mass window. Logarithm of octanol-water partition coefficient ($logK_{ow}$) as a measure of the hydrophobicity can be used to determine whether the retention time of a candidate compound is similar to that of compounds with similar $logK_{ow}$ values. This feature of $logK_{ow}$ values has previously been used to reduce the number of candidates in the analysis of transformation products of organic contaminants (Kern et al. 2009) and was here utilized in the third stage. To be able to calculate the expected retention time window, the retention times and $logK_{ow}$ values of the standard compounds were plotted and a linear regression line with 95% prediction intervals was calculated. As a result, a retention time window of approximately 5 min was obtained for compounds with a certain $logK_{ow}$ value and all the candidates with retention times outside of this window were excluded. The fourth stage was based on examination of the isotope peak pattern and was applied to all compounds containing chlorine or bromine atom(s). All candidates not showing the proper isotope pattern were rejected.

At the concentration of 500 ng/L with ESI(+) for 31 of 67 compounds spiked to sample (altogether 88 compounds) there was only one (correctly identified) candidate left after the fourth identification stage. For 20 of 67 compounds, two candidates were left and for 10 of 67 compounds more than two candidates were left after the fourth stage of the identification process. For 6 of 67 compounds, the process gave false results. With ESI(-), for 19 of 21 compounds only one (correctly identified) candidate was already left after the third stage. Two compounds showed false-negative results. Results from the validation experiments of posttarget analysis showed that LC-TOF-MS can provide tentative identification of an analyte when the accurate mass is accompanied by the analyte's structure-related steps. The final identification must, however, always rely on the use of a pure standard compound.

The posttarget analysis without retention time data was finally applied for unspiked wastewater effluent samples. The presence of 147 pharmaceuticals and 54 metabolites was screened by posttarget analysis, using positive and negative ionization modes. All the compounds having more than one candidate at the final stage were considered as unidentified. In all, 36 compounds were tentatively identified. The presence of the six candidates (metronidazole, paroxetine, trimethoprim, diclofenac, furosemide and ibuprofen) could be confirmed by the analysis of pure standards and retention time comparisons. The compounds nordiazepam, oxazepam and temazepam were tentatively identified at the fourth stage, but their presence could not be confirmed with standard compounds. Results showed that posttarget analysis, using LC-TOF-MS, is feasible method and can be applied for identification of compounds in environmental samples. Indication of the presence of a compound in a sample can be obtained, even before the corresponding standard compound is available. However, there is a risk of occasional false-negative and false-positive identifications and the results must be examined with care.

Further effort should be made to speed up the development of more intelligent software for screening unknown compounds. So far, knowledge on the content of a sample can be obtained with thorough manual inspection, but larger-scale automated data treatment is still hindered by the lack of advanced features in the software.

### *4.1.4 Nontarget analysis*

The performance of nontarget analysis was estimated by spiking wastewater effluent with six pharmaceuticals (bezafibrate, diclofenac, furosemide, hydrochlorothiazide, ibuprofen and ketoprofen) representing potential aquatic contaminants at a concentration of 500 ng/L (Fig. 4, experiment 3). To simplify the experiment, only compounds ionizing at ESI(-) were selected. The sample was then analysed, using LC-TOF-MS with ESI(-) and processed as an unknown. The sample components were deconvoluted from the TIC, using ChromaLynx with nw-XICs of 20 mDa and mass range of 100–500 Da. Only one m/z was included in the deconvoluted spectra because in-source collision-induced dissociation (CID) was not used and the compounds were not expected to undergo extensive fragmentation. A library containing the theoretical mass spectra of the six spiked pharmaceuticals was used for component identification.

The correspondence between the library spectrum and the deconvoluted spectrum is expressed as the forward fit parameter, with possible values varying between 0 (no similarities) and 1000 (complete similarity). The number of deconvoluted components is reduced by setting a cutoff value for the forward fit parameter. The identification was assessed using two forward fit cutoff values: 600 and 850. The results of these experiments are presented in Table 6. Four of the six spiked compounds were correctly identified, using the lower forward fit cutoff value (600), but with the higher cutoff value (850) only one compound was correctly identified.

Table 6. Results of nontarget analysis of spiked wastewater sample.

| | Stage 1. id. criteria: forward fit > 600[a] | | Stage 2. id. criteria: forward fit > 850 | |
|---|---|---|---|---|
| Compound | true analyte found | number of false-positives[b] | true analyte found | number of false-positives |
| Bezafibrate | yes | 6 | no | 6 |
| Diclofenac | no | 15 | no | 14 |
| Furosemide | yes | 16 | no | 3 |
| Hydrochlorothiazide | yes | 13 | no | 13 |
| Ibuprofen | yes | 3 | yes | 3 |
| Ketoprofen | no | 12 | no | 8 |

[a] User-defined identification criteria used in the software.

[b] Spectral comparison based on nominal mass.

Table 6 also shows the numbers of components that were falsely identified as spiked compounds. These numbers were generally too high for practical nontarget analysis. For example, the program found 16 deconvoluted components at different retention times, for which it proposed furosemide as compound identification based on the library search. The search is based only on spectra with nominal masses, but the results can

also be scored by fitness of accurate mass. It was found that among the group of components having the same identification, only the true spiked compound usually fulfilled the requirement for maximum Δm of 5 mDa.

The main problems with nontarget analysis were related to the deconvolution program and its library search features. In the program, the deconvoluted spectrum is composed by selecting the ions in decreasing order of intensities, which leads to very improbable spectra, especially in a system, such as LC-TOF-MS, that is not inherently producing fragment ions. One example of a deconvoluted component spectrum is illustrated in Figure 5 (lower) containing two ions: m/z 299 and 425. Instead of intensity-based selection, the deconvolution of isotope pattern peaks or adduct peaks would provide more relevant component spectra. The library search proposed diclofenac as a hit for the unknown component in Figure 5. The identification was based on the minor isotope peak m/z 299 and was clearly incorrect. The emphasis of the library search should be on the presence of the theoretical base peak of the analyte, not on the minor isotope peaks.

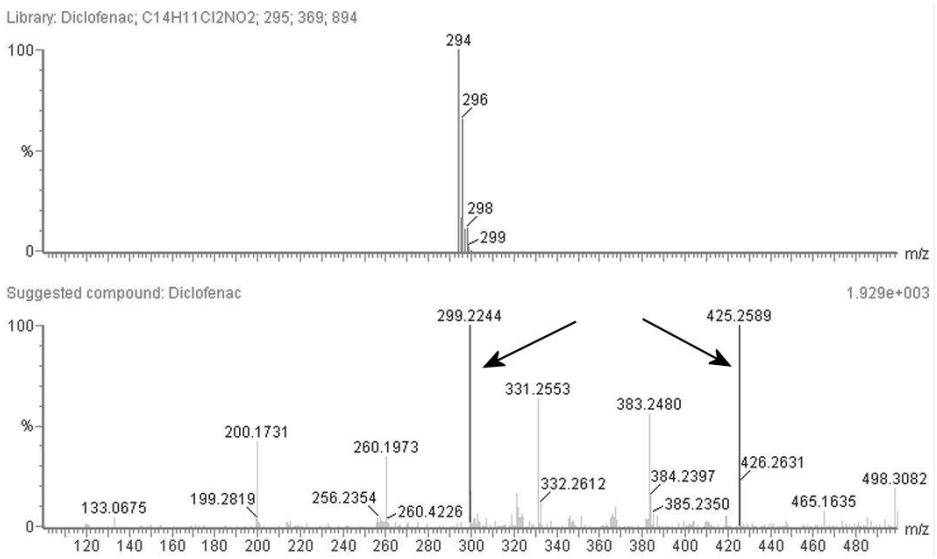



Fig. 5. Example of false library identification. Library spectrum of diclofenac (upper) and the experimental component spectrum (lower). Two ions selected by the program for the deconvoluted component spectrum are indicated with arrows. The compound detected could not be identified.

Nontarget analysis using the ChromaLynx deconvolution program for LC-TOF-MS data proved unfeasible, due to the lack of sufficient tools for effective data processing. High numbers of false identifications, even with a very limited library, and increased amounts of insignificant data made processing of the results time-consuming and ineffective. Additional tools for nontarget software are required before an automated and large-scale screening of complex samples can efficiently be performed with libraries containing hundreds of compounds.

## 4.2 Gas chromatography-time-of-flight mass spectrometry

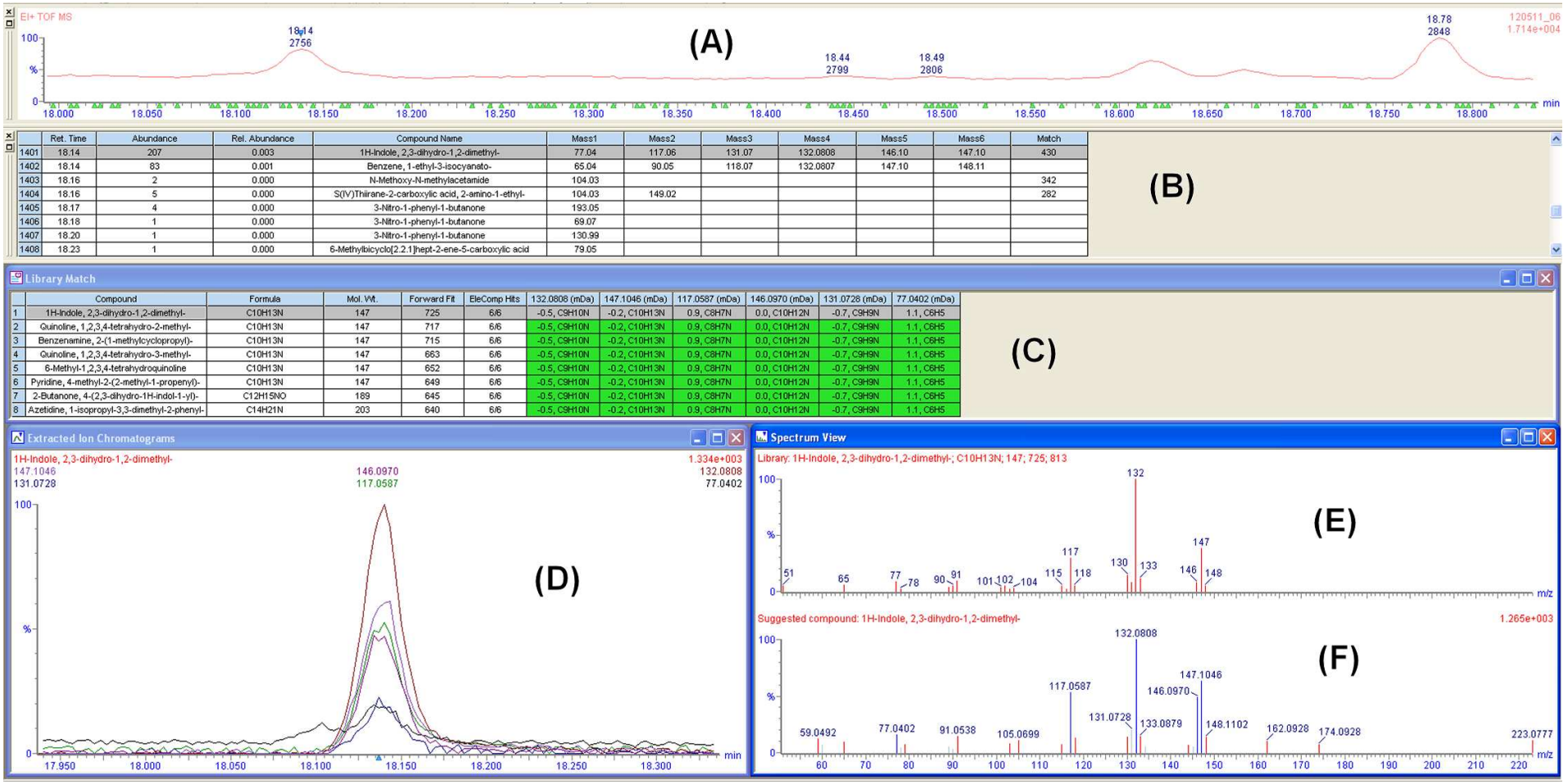
This chapter summarizes the results from the experiments, using GC-TOF-MS (III, IV). The nontarget method for GC-TOF-MS was optimized and validated, using a landfill leachate sample as the complex sample matrix (III). The nontarget method developed was then applied to the analysis of various urban and suburban water samples (IV). In this study, nontarget analysis was additionally complemented with posttarget analysis.

### *4.2.1 Nontarget analysis*

Nontarget analysis using GC-(EI)-TOF-MS data and a deconvolution program, was performed in publications III and IV. A six-stage identification process for nontarget analysis was developed by spiking a raw landfill leachate sample with a standard mixture containing 11 different semivolatile compounds (EPA method 526 mixture) at concentrations of 100, 500 and 2500 ng/L and processing the data obtained with ChromaLynx (III). The general layout of ChromaLynx in the nontarget analysis mode (using EI data) is illustrated in Figure 6.

The identification process was based on accurate mass measurements and comparison of the deconvoluted spectra with a NIST spectral library. The workflow of the developed process is shown in Figure 7. First, all components with a forward fit parameter value of less than 700 were rejected. The selection of a value (numerical value between 0 and 1000 indicating the similarity of experimental MS spectrum and library MS spectrum) of 700 was based on the results obtained in method development, using spiked samples. The decrease in identification reliability was noted at lower forward fit values. In the second stage, components containing less than three ions deconvoluted in a spectrum were rejected. Those components consisting of only one or two ions generally originated from noise or lock mass ions, and were therefore rejected. In the third stage, components with an ion abundance value of less than 10 were omitted.

In the fourth stage, at least three of the deconvoluted ions had to pass the Δm limit of 5 mDa. The calculation of identification points (IP) was additionally added to the process to enhance the plausibility of the identification. The implementation of IPs is presented in the European Commission Guideline for identification and quantification of organic residues (European Commission 2002). The accumulation of IPs is based on the number of ions measured. In addition to IPs, the qualification of identification requires fulfilment of certain criteria of ion ratios. However, the use of accurate mass measurements in terms of IPs is not included in the statute. Complementary criteria for accurate mass measurements have been proposed in the literature (Hernández et al. 2004, Nielen et al. 2007) and the scoring used was modified from that of Hernández et al. (Hernández et al. 2007). A Δm less than 2.0 mDa gained two IPs per ion and a Δm from 2.0 to 5.0 mDa gained one IP. At least four IPs were required to proceed to the next stage.




| | Ret. Time | Abundance | Rel. Abundance | Compound Name | Mass1 | Mass2 | Mass3 | Mass4 | Mass5 | Mass6 | Match |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1401 | 18.14 | 207 | 0.003 | 1H-Indole, 2,3-dihydro-1,2-dimethyl- | 77.04 | 117.06 | 131.07 | 132.0808 | 146.10 | 147.10 | 430 |
| 1402 | 18.14 | 83 | 0.001 | Benzene, 1-ethyl-3-isocyanato- | 65.04 | 90.05 | 118.07 | 132.0807 | 147.10 | 148.11 | |
| 1403 | 18.16 | 2 | 0.000 | N-Methoxy-N-methylacetamide | 104.03 | | | | | | 342 |
| 1404 | 18.16 | 5 | 0.000 | S(IV)Thiirane-2-carboxylic acid, 2-amino-1-ethyl- | 104.03 | 149.02 | | | | | 282 |
| 1405 | 18.17 | 4 | 0.000 | 3-Nitro-1-phenyl-1-butanone | 193.05 | | | | | | |
| 1406 | 18.18 | 1 | 0.000 | 3-Nitro-1-phenyl-1-butanone | 69.07 | | | | | | |
| 1407 | 18.20 | 1 | 0.000 | 3-Nitro-1-phenyl-1-butanone | 130.99 | | | | | | |
| 1408 | 18.23 | 1 | 0.000 | 6-Methylbicyclo[2.2.1]hept-2-ene-5-carboxylic acid | 79.05 | | | | | | |

| | Compound | Formula | Mol. Wt. | Forward Fit | EleComp Hits | 132.0808 (mDa) | 147.1046 (mDa) | 117.0587 (mDa) | 146.0970 (mDa) | 131.0728 (mDa) | 77.0402 (mDa) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1H-Indole, 2,3-dihydro-1,2-dimethyl- | C10H13N | 147 | 725 | 6/6 | -0.5, C9H10N | -0.2, C10H13N | 0.9, C8H7N | 0.0, C10H12N | -0.7, C9H9N | 1.1, C6H5 |
| 2 | Quinoline, 1,2,3,4-tetrahydro-2-methyl- | C10H13N | 147 | 717 | 6/6 | -0.5, C9H10N | -0.2, C10H13N | 0.9, C8H7N | 0.0, C10H12N | -0.7, C9H9N | 1.1, C6H5 |
| 3 | Benzenamine, 2-(1-methylcyclopropyl)- | C10H13N | 147 | 715 | 6/6 | -0.5, C9H10N | -0.2, C10H13N | 0.9, C8H7N | 0.0, C10H12N | -0.7, C9H9N | 1.1, C6H5 |
| 4 | Quinoline, 1,2,3,4-tetrahydro-3-methyl- | C10H13N | 147 | 663 | 6/6 | -0.5, C9H10N | -0.2, C10H13N | 0.9, C8H7N | 0.0, C10H12N | -0.7, C9H9N | 1.1, C6H5 |
| 5 | 6-Methyl-1,2,3,4-tetrahydroquinoline | C10H13N | 147 | 652 | 6/6 | -0.5, C9H10N | -0.2, C10H13N | 0.9, C8H7N | 0.0, C10H12N | -0.7, C9H9N | 1.1, C6H5 |
| 6 | Pyridine, 4-methyl-2-(2-methyl-1-propenyl)- | C10H13N | 147 | 649 | 6/6 | -0.5, C9H10N | -0.2, C10H13N | 0.9, C8H7N | 0.0, C10H12N | -0.7, C9H9N | 1.1, C6H5 |
| 7 | 2-Butanone, 4-(2,3-dihydro-1H-indol-1-yl)- | C12H15NO | 189 | 645 | 6/6 | -0.5, C9H10N | -0.2, C10H13N | 0.9, C8H7N | 0.0, C10H12N | -0.7, C9H9N | 1.1, C6H5 |
| 8 | Azetidine, 1-isopropyl-3,3-dimethyl-2-phenyl- | C14H21N | 203 | 640 | 6/6 | -0.5, C9H10N | -0.2, C10H13N | 0.9, C8H7N | 0.0, C10H12N | -0.7, C9H9N | 1.1, C6H5 |

Fig. 6. Layout of nontarget screening results in ChromaLynx software, using GC-TOF-MS. (A) Sample chromatogram, (B) components/ions deconvoluted, (C) accurate mass-scoring table, (D) extracted ion chromatograms of the component selected, (E) library spectrum of the library hit selected and (F) deconvoluted sample spectrum.

In the fifth stage, two alternative cases emerged. If the difference in forward fit values between the best and second-best library match (Δ(forward fit)) was more than 100, the component was considered tentatively identified (stage 5a). If the Δ(forward fit) was less than 100 (i.e. components having similar EI spectra) and all components qualified had the same elemental formula, the identity of the best library match was reported to illustrate one possible component structure (stage 5b). The final (sixth) stage of the process was confirmation of the tentative identification with a pure standard material, if available.

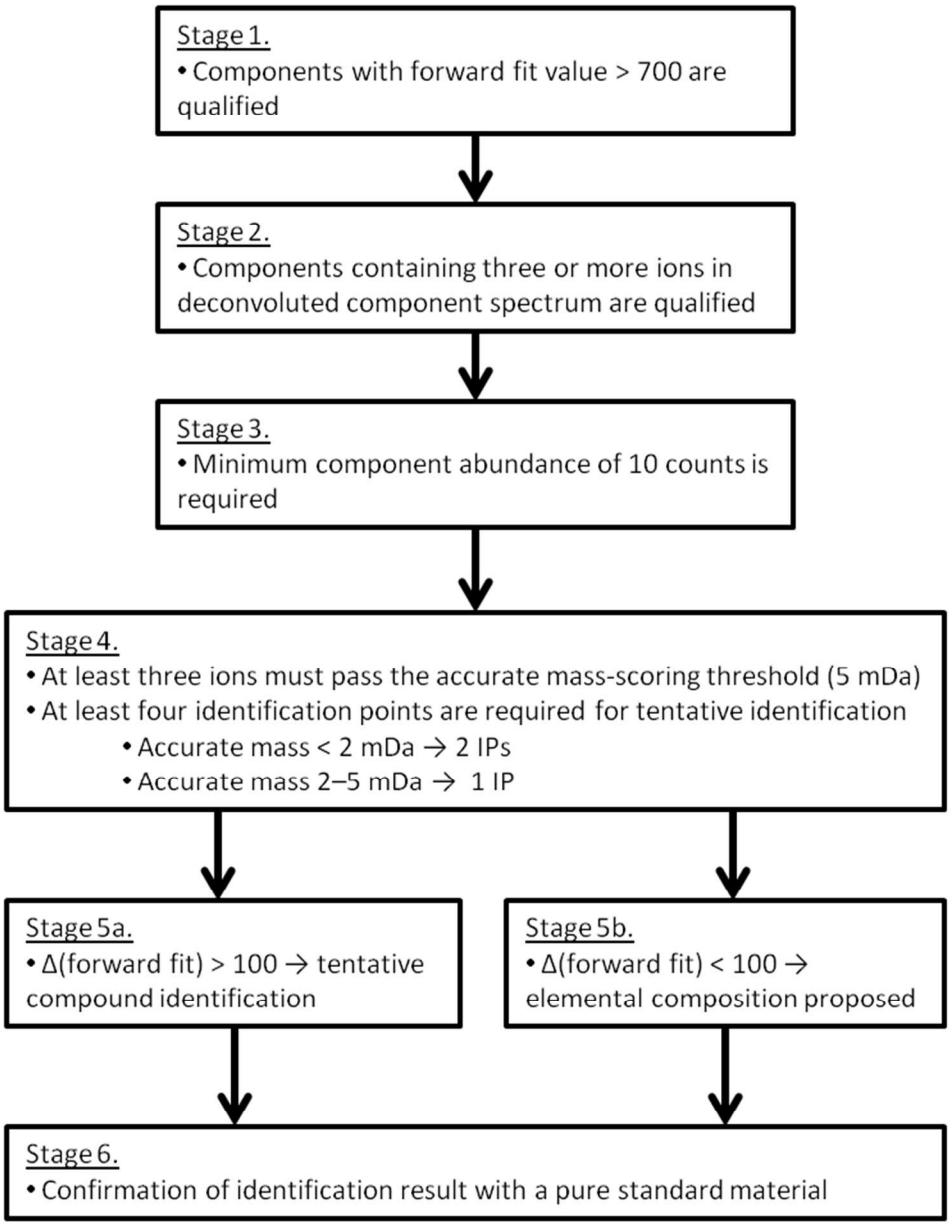



Fig. 7. Workflow of the nontarget identification process.

The lowest concentration tested at which a compound was detected and identified following the six-stage process was defined as the limit of identification (LOI) of the com-

pound. The LOI was 2500 ng/L for 4 out of 11 compounds, 500 ng/L for 3 out of 11 compounds and 100 ng/L for 1 out of 11 compounds. Three compounds were not detected in any of the spiked samples. The results showed that the intensity of the ion peaks formed in EI of an analyte is a critical parameter that affects the success of nontarget detection. The best LOIs (i.e. highest sensitivity) were obtained with compounds having only a few intensive fragment ions in their EI spectra. In contrast, heavy fragmentation led to poor LOIs. The forward fit parameter value also decreased when the concentration of an analyte approached the LOI value. Coelution with a matrix compound may additionally interfere with the detection of an analyte, since the combined EI spectrum of two different compounds does not yield relevant library matches. The identification can also be hampered if the accuracy of the mass measurement is lost. With TOF-MS, this is mainly due to the saturation of the time-to-digital converter (TDC) due to high analyte concentrations.

The nontarget analysis was applied to the analysis of different water samples. For landfill leachate samples, the sample preparation was based on a very generic LLE with two organic solvents (*n*-hexane and dichloromethane) (III) and for other water samples, SPE using polymeric reverse-phase sorbent material was used (IV). The numbers of tentatively identified compounds are presented in Table 7. As can be seen, the highest numbers of compounds were identified from landfill leachate, wastewater effluent and urban stormwater. The identity of five compounds was afterwards confirmed with standard compounds available in the laboratory. The chemical structures of these compounds are presented in Figure 8.

Table 7. Numbers of tentatively identified compounds in different sample matrices using nontarget analysis.

| Sample matrix | Sample preparation technique | Number of tentatively identified compounds |
|---|---|---|
| Landfill leachate (*n*-hexane extract) | LLE | 24 |
| Landfill leachate (dichloromethane extract) | LLE | 20 |
| Wastewater effluent | SPE | 21 |
| Receiving water (river)[a] | SPE | 6 |
| Stormwater, urban | SPE | 15 |
| Stormwater, rural | SPE | 1 |
| Surface water | SPE | 4 |

[a] Receiving water for the wastewater effluent studied.

The results showed that GC-TOF-MS can produce valuable information on the sample composition, and can reveal the presence of compounds that would not have been found using traditional target analysis techniques. Nontarget screening may be used as a parallel technique with target analysis to obtain more information on the sample. Compared with nontarget analysis using LC-MS data, the existence of spectral libraries eases tentative identification. If the compound spectrum is not stored in the library, identification without any other configuration of mass spectrometers becomes very challenging, even with GC-TOF-MS. Functioning of the program used in nontarget analysis again played the most significant role. The program must be capable of extracting essential information from the immense amount of full-spectrum data. Different thresholds and set-

tings are additionally required to efficiently filter the data, since many false components originating from noise were also deconvoluted. All identification stages subsequent to the first had to be performed manually, since the software did not offer such filtering options. When more than a few samples are processed, this current approach is very time-consuming and tedious. Consequently, the limitations of the program applied formed a bottleneck for this nontarget screening method, as in the case of nontarget analysis using LC-TOF-MS. Additional tools for the nontarget screening software are thus required to obtain a more automated method, making extensive screening studies feasible.

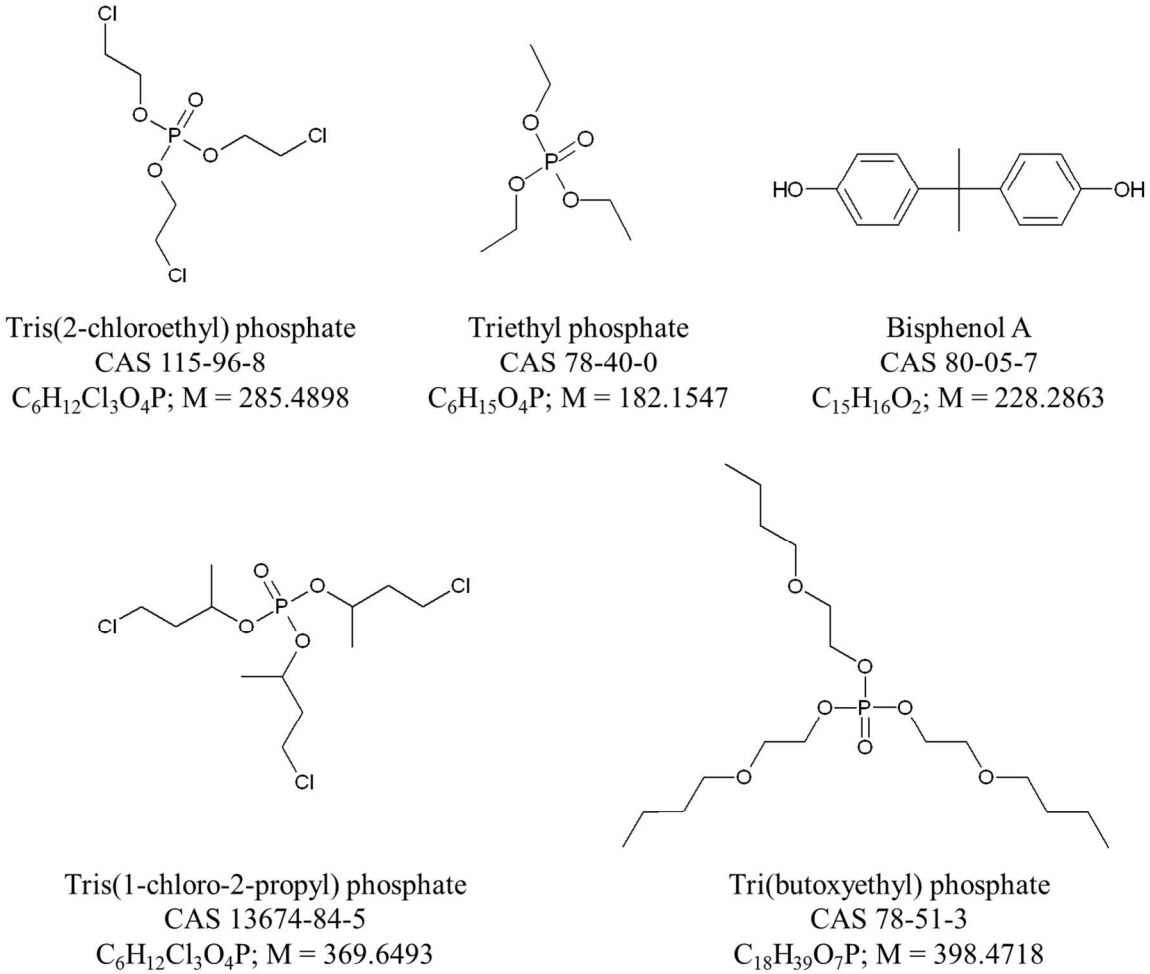



Fig. 8. Structures, CAS numbers, molecular formulas and exact masses of compounds identified in nontarget analysis of wastewater and stormwater.

### *4.2.2 Posttarget analysis*

Assessment of nontarget analysis revealed that its ability to identify compounds decreases at low concentrations. Nontarget analysis of the water sample set was thus complemented with posttarget analysis in which 101 emerging contaminants were screened from the samples (IV). The list was compiled from the NORMAN Network database

(NORMAN Network 2011). The exact mass of one selected ion in the EI spectrum of a particular compound (preferably a molecular ion) was extracted from the TIC. During data procession, nw-XICs of 30 mDa were extracted from the raw data, and each peak containing the defined ion within the defined mass range was considered a possible identifier. A predetermined Δm value of ± 5 mDa was additionally demanded for the components to qualify for further investigation. The deconvoluted candidate spectra were then visually compared with the NIST library spectra and, if in addition to the target ion at least two of the most intense library spectrum ions were present, the compound was considered tentatively identified. In this visual inspection, isotope patterns of molecular and fragment ions originating from chlorine and bromine were especially emphasized. $^{13}C$ and $^{34}S$ also produce isotopic peaks into spectrum, but their natural abundances are only 1.1% and 4.3%, respectively. Because of the low intensity of these isotope peaks compared to those of e.g. $^{37}Cl$ (24.2%) and $^{81}Br$ (49.3%), the tentative identification would be very prone to false-positive, especially with low analyte concentration and noisy background. Thus, only chlorine and bromine with more intense and easily recognizable isotope patterns were taken into consideration. The tentative identifications were confirmed with a pure standard compound, if available. Tentative identifications from wastewater effluent sample, wastewater receiving water sample (river) and two stormwater samples, using posttarget analysis, are presented in Table 8. In all, 18 compounds were tentatively identified, using posttarget analysis, and 10 of these were confirmed with a standard compound.

Table 8. Tentatively identified compounds, using posttarget analysis.

| | | | Sample[b] | | | |
|---|---|---|---|---|---|---|
| Compound | Use of compound | ID[a] | WW | RW | ST1 | ST2 |
| Tetraacetylethylenediamine | Bleaching agent | - | x | x | - | - |
| Tris(2-chloroethyl)phosphate | Flame retardant | x | x | x | - | x |
| Tris(2-chloroisopropyl)phosphate | Flame retardant | x | x | x | - | x |
| Tris(1,3-dichloroisopropyl)phosphate | Flame retardant | x | x | x | - | - |
| Ethanol, 2-butoxy-, 1,1',1''-phosphate | Flame retardant | x | x | x | x | - |
| Triphenylphosphate | Flame retardant | x | x | x | x | - |
| 4-Oxoisophorone | Personal care product | - | x | x | x | x |
| N,N-Diethyltoluamide | Personal care product | - | x | x | - | - |
| Galaxolide | Personal care product | x | x | x | - | - |
| Triethylphosphate | Plasticizer | x | x | x | x | x |
| Tri-isobutylphosphate | Plasticizer | x | x | x | x | x |
| Tri-*n*-butylphosphate | Plasticizer | x | x | x | x | x |
| Triethylcitrate | Plasticizer | - | x | x | x | - |
| N-butyl-benzenesulphoamide | Plasticizer | - | x | x | x | x |
| Bisphenol A | Plasticizer | x | x | x | x | x |
| Tributylacetylcitrate | Plasticizer | - | - | - | - | x |
| 2-(Methylthio)benzothiazole | Rubber component | - | x | x | x | x |
| 2(3H)-Benzothiazolone | Rubber component | - | x | - | x | x |

[a] Identification confirmed with a pure standard compound.

[b] WW: wastewater effluent, RW: receiving water, ST: stormwater

Posttarget analysis can be used to detect analytes at lower concentration levels than in nontarget analysis. For example, triethylphosphate in stormwater and bisphenol A in wastewater effluent could only be detected using posttarget analysis. The usefulness of posttarget analysis is, however, strongly user-dependent, because the program only locates ions preselected by the analyst. The spectra of the analytes studied must be available for the selection of the target ion and the validity assessment of the candidates found. The program used for posttarget analysis proved to be a bottleneck of data processing. The ChromaLynx program lacks the library comparison feature in the posttarget mode and therefore the tentative identification had to be performed visually. The method is thus best suited for small sample sets. In addition, the candidate search in the program was limited to the use of only one ion. The number of candidate compounds reported could easily be further limited, using more than one ion during the screening. The most urgent needs for improvements in nontarget and posttarget analysis are related to the programs used to process the HRMS data produced.

The results showed that the highest number of compounds was tentatively identified, using a combination of nontarget and posttarget analysis. Posttarget and nontarget analyses both have some faults, and if the data are treated carelessly, there is clearly a risk of false-positive identification. The analysis of blank and replicate samples is additionally necessary to reveal possible contamination during sample pretreatment. The identification principles and criteria should therefore always be clearly stated. The identification criteria should be kept rigid, although this equates to a lower number of identified compounds. The policy throughout this thesis has been that final confirmation on compound identity always required the use of an authentic standard compound.

## 5. CONCLUSIONS

The increasing use of chemicals and resulting chemicalization of the environment is a global environmental problem that poses new challenges for analytical laboratories. In addition to the usual results with known contaminants, information about new potentially harmful emerging contaminants is increasingly becoming desired. This forces laboratories to widen their methodological repertoire to analyse novel contaminants from various sample matrices. Traditional target techniques may not be sufficient and complementary approaches are needed. Nontarget and posttarget identification methods using the GC-TOF-MS and the LC-TOF-MS developed in this thesis address this issue.

All the methods developed were based on accurate mass measurements, which proved to be a useful tool in tentative identification. Accurate mass determination is an efficient way to decrease the number of candidates of tentatively identified compounds. Despite this advantageous feature, nontarget analysis of an unknown sample is never a simple task. The mass resolution of TOF-MS is simply not sufficient for the unquestioning determination of the elemental composition of an ion. Instruments with higher mass-resolving power, e.g. the Orbitrap MS or FT-ICR MS would be required for this purpose. However, the mass accuracy provided by TOF-MS is suitable for judging whether

the elemental composition of some proposed compound matches the mass of the ion(s) recorded (i.e. posttarget analysis). However, accurate mass alone is only seldom sufficient for reliable identification, and complementary information, e.g. from isotope patterns, spectral libraries and structure-related properties is usually required. In nontarget analysis with TOF-MS, the deconvolution program must first be used to detect the compound and its ions. Spectral libraries, determined accurate mass and other data can then be used to tentatively identify the component detected. Identification using this approach, however, requires knowledge of the ions of the particular candidate compound (e.g. spectral library or standard compound). If no spectral comparison can be performed, the premises for identification are rather poor. All results from TOF-MS data using posttarget and nontarget analysis should be treated as tentative identifications until the final confirmation with a pure standard compound has been performed.

The utilization of two different separation techniques (i.e. GC and LC) enabled the analysis of compounds with varying physicochemical properties and thus provided expanded knowledge of the sample composition (e.g. of the wastewater effluent sample). The MS data produced in GC-MS and LC-MS are, however, quite different compared to each other due to the inherent operational principles of the associated ionization techniques and the mechanism of chromatographic separation. Generally, LC-MS data are more challenging for identification purposes due to the low number of ions formed in the ion source. The fragmentation can be enhanced using quadrupole TOF-MS instruments, but then special programs are usually required to predict the fragmentation pathways, especially if the standard compound is not available, since the extensive spectral libraries for ESI mass spectra are not available. In this context, GC-TOF-MS was more feasible for nontarget analysis than LC-TOF-MS. For posttarget analysis, both techniques were well suited and were applicable for tentative compound identification. The main limitations of nontarget and posttarget analyses with both analytical techniques were related to insufficient features of the deconvolution program used. An excessive amount of data processing had to be performed manually or visually, which becomes unfeasible with large sample sets. In my opinion, the most urgent needs for improvements related to posttarget and nontarget analysis with TOF-MS currently lie in the development of efficient data-processing software. Tremendous advances in the development of TOF-MS instruments have already been seen during the last decade and devices with superior analytical performance have been introduced into the market. This trend will most probably continue and, in this regard, corresponding progress in related software can reasonably be expected.

The posttarget and nontarget methods developed were used to identify organic contaminants in different aquatic matrices. Dozens of compounds were tentatively identified and several of them were also confirmed with standard compounds. Anthropogenic sources such as wastewater effluent, landfill leachate and urban stormwater contained the highest numbers of different compounds that could be identified. It was further found that several organic xenobiotics originate from urban areas and drift into the environment. Further research is, however, required to fully understand the ecotoxicological

significance of this chemical burden. In this thesis, the usability of nontarget and posttarget techniques was demonstrated with water samples, but with modified sample pretreatment procedures, the same analytical approaches may also be applied to other environmental matrices, such as soil or air samples. I believe that TOF-MS combined with nontarget analysis could provide valuable information, e.g. on the transformation products of emerging contaminants in various matrices and transport of chemicals in the environment. Application areas for posttarget and nontarget analysis can also be found in other branches of organic analytical chemistry such as forensic and petrochemical science.

The need in the field of environmental analysis for methods capable of analysing samples without any preselection is indisputable. The methods presented in this thesis provide some tools to address this challenge. The interest in posttarget and nontarget analysis is apparent, since these techniques can be used to reveal the presence of hitherto unknown contaminants in the environment. Several serious examples of global pollution with organic contaminants, e.g. chlorinated pesticides, brominated flame retardants and perfluorinated compounds, are known from history. If local environmental contamination of some novel compounds could be detected at an early stage, the global spread of the contaminants could be avoided with efficient instant remedies. Although the importance of posttarget and nontarget analysis has been highlighted throughout the thesis, it should not be interpreted as the disparagement of target analysis. Anyway, new sensitive quantitative target methods are still needed for the assessment of the occurrence of organic contaminants in the environment.

Emerging contaminants mainly originate from different branches of the chemical industry and usually the manufacturing companies have the exact chemical compositions and properties of their products. Unfortunately, this information is mainly not available in the literature. Protection of the environment is one important motivating factor among scientists working with analytical chemistry and environmental pollution. The fact that important information does already exist, but is still not available, not only breeds frustration among environmental scientists, but also wastes research resources. If environmentally relevant scientific data were open and accessible, research on the fate and effects of chemicals would also be much faster and more effective.

Chemicals have become a part of our daily life during the last century. Generally, their use ameliorates our quality of life and supports matters that are considered as truisms in the Western welfare state. The flip side of this phenomenon has been shown in several scientific studies, since trace concentrations of organic xenobiotics have been found in the environment throughout the world. Occasional large-scale chemical accidents additionally highlight the disquieting risks related to the use of chemicals, if treated carelessly. These problems do not arise solely from the developing countries but can also occur in countries of high technology. The regulation of chemicals has become tighter in Europe with the Registration, Evaluation, Authorization and Restriction of Chemicals

(REACH) regulation, but wider intergovernmental cooperation is still required to effectively control the risks and to protect the environment.

Fifty years have now passed since the publication of Rachel Carson's book Silent Spring. The world has changed greatly, but the message of the book is still topical. The scientific focus has turned from DDT and other pesticides to emerging contaminants, while analytical techniques have developed enormously. The production volumes have, however, simultaneously increased and the related environmental risks have not disappeared anywhere. Despite all the progress, I'm afraid that mankind has not yet fully learned the lesson from history. Chemicals can and should be used when truly needed, but only in a responsible way and with minimal possible volumes. The purpose of use must always be justified, and environmentally friendly alternatives should be favoured. Requisite safety precautions must be taken into account throughout the life cycle of the chemical product, ending up in disposal trough use of appropriate waste treatment processes. Improvements in waste management, especially in highly urbanized and industrialized areas, remain a key issue in the prevention of future environmental contamination.

# 6. ACKNOWLEDGEMENTS

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# 7. REFERENCES


Agüera, A., Pérez Estrada, L.A., Ferrer, I., Thurman, E.M., Malato, S. & Fernández-Alba, A.R. 2005: Application of time-of-flight mass spectrometry to the analysis of phototransformation products of diclofenac in water under natural sunlight. - *J. Mass Spectrom.* 40: 908-915.

Al-Odaini, N.A., Zakaria, M.P., Yaziz, M.I. & Surif, S. 2010: Multi-residue analytical method for human pharmaceuticals and synthetic hormones in river water and sewage effluents by solid-phase extraction and liquid chromatography–tandem mass spectrometry. - *J. Chromatogr. A* 1217: 6791-6806.

Amelin, V., Nikeshina, T. & Tret'yakov, A. 2011: Identification and quantification of pesticides and polycyclic aromatic hydrocarbons in water and food by gas chromatography-mass spectrometry. - *J. Anal. Chem.* 66: 918-923.

Barr, D.B., Bishop, A. & Needham, L.L. 2007: Concentrations of xenobiotic chemicals in the maternal-fetal unit. - *Reprod. Toxicol.* 23: 260-266.

Bedner, M. & MacCrehan, W.A. 2006: Transformation of acetaminophen by chlorination produces the toxicants 1,4-benzoquinone and N-acetyl-p-benzoquinone imine. - *Environ. Sci. Technol.* 40: 516-522.

Bisceglia, K.J., Yu, J.T., Coelhan, M., Bouwer, E.J. & Roberts, A.L. 2010: Trace determination of pharmaceuticals and other wastewater-derived micropollutants by solid phase extraction and gas chromatography/mass spectrometry. - *J. Chromatogr. A* 1217: 558-564.

Brix, R., Bahi, N., Lopez de Alda, M.J., Farré, M., Fernandez, J. & Barceló, D. 2009: Identification of disinfection by-products of selected triazines in drinking water by LC-Q-ToF-MS/MS and evaluation of their toxicity. - *J. Mass Spectrom.* 44: 330-337.

Carson, R. *Silent Spring.* Houghton Mifflin Company: Boston (MA), 1962; pp. 368.

Chernushevich, I.V., Loboda, A.V. & Thomson, B.A. 2001: An introduction to quadrupole-time-of-flight mass spectrometry. - *J. Mass Spectrom.* 36: 849-865.

Coles, J. & Guilhaus, M. 1993: Orthogonal acceleration — a new direction for time-of-flight mass spectrometry: Fast, sensitive mass analysis for continuous ion sources. - *TrAC Trends Anal. Chem.* 12: 203-213.

Dawson, J.H.J. & Guilhaus, M. 1989: Orthogonal-acceleration time-of-flight mass spectrometer. - *Rapid Commun. Mass Sp.* 3: 155-159.

Diamond, M.L. & Hodge, E. 2007: Urban contaminant dynamics: from source to effect. - *Environ. Sci. Technol.* 41: 3796-3800.

Eggen, T., Moeder, M. & Arukwe, A. 2010: Municipal landfill leachates: A significant source for new and emerging pollutants. - *Sci. Total Environ.* 408: 5147-5157.

European Commission 2002: European Commission Decision 2002/657/EC, implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. - *Off. J. Eur. Commun.* L221: 8.

European Parliament 2000: Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 Establishing a Framework for Community Action in the Field of Water Policy. - *Off. J. Eur. Commun.* L327: 1.

Farré, M., Gros, M., Hernández, B., Petrovic, M., Hancock, P. & Barceló, D. 2008a: Analysis of biologically active compounds in water by ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry. - *Rapid Commun. Mass Sp.* 22: 41-51.

Farré, M., Pérez, S., Kantiani, L. & Barceló, D. 2008b: Fate and toxicity of emerging pollutants, their metabolites and transformation products in the aquatic environment. - *TrAC Trends Anal. Chem.* 27: 991-1007.

Ferrer, I. & Thurman, E.M. 2012: Analysis of 100 pharmaceuticals and their degradates in water samples by liquid chromatography/quadrupole time-of-flight mass spectrometry. - *J. Chromatogr. A* 1259: 148-157.

Ferrer, I. & Thurman, E.M. 2010a: Identification of a new antidepressant and its glucuronide metabolite in water samples using liquid chromatography/quadrupole time-of-flight mass spectrometry. - *Anal. Chem.* 82: 8161-8168.

Ferrer, I. & Thurman, E.M. 2010b: Analysis of sucralose and other sweeteners in water and beverage samples by liquid chromatography/time-of-flight mass spectrometry. - *J. Chromatogr. A* 1217: 4127-4134.

Ferrer, I. & Thurman, E.M. 2009: Liquid chromatography time-of-flight mass spectrometry. Principles, tools, and applications for accurate mass analysis. - *Chemical Analysis. A series of monographs on analytical chemistry and its applications* Vol. 173: 261.

Ferrer, I. & Thurman, E.M. 2007: Multi-residue method for the analysis of 101 pesticides and their degradates in food and water samples by liquid chromatography/time-of-flight mass spectrometry. - *J. Chromatogr. A* 1175: 24-37.

Ferrer, I., Fernandez-Alba, A., Zweigenbaum, J.A. & Thurman, E.M. 2006: Exact-mass library for pesticides using a molecular-feature database. - *Rapid Commun. Mass Sp.* 20: 3659-3668.

Finnish Environmental Institute 2012: http://www.ymparisto.fi/default.asp?contentid=406546&lan=en&clan=fi. Accessed 19.11.2012:

Fjeldsted, J.C. Accurate mass measurements with orthogonal axis time-of-flight mass spectrometry, In *Liquid chromatography time-of-flight mass spectrometry: principles, tools and applications for accurate mass analysis*, Ferrer, I. and Thurman, E.M., Eds.; John Wiley & Sons, Inc.: Hoboken, New Jersey, 2009; Vol.173 pp. 3-15.

Gómez, M.J., Gómez-Ramos, M.M., Malato, O., Mezcua, M. & Férnandez-Alba, A.R. 2010: Rapid automated screening, identification and quantification of organic micro-contaminants and their main transformation products in wastewater and river waters using liquid chromatography–quadrupole-time-of-flight mass spectrometry with an accurate-mass database. - *J. Chromatogr. A* 1217: 7038-7054.

Gómez, M.J., Gómez-Ramos, M.M., Agüera, A., Mezcua, M., Herrera, S. & Fernández-Alba, A.R. 2009: A new gas chromatography/mass spectrometry method for the simultaneous analysis of target and non-target organic contaminants in waters. - *J. Chromatogr. A* 1216: 4071-4082.

Gómez, M.J., Malato, O., Ferrer, I., Aguera, A. & Fernandez-Alba, A. 2007: Solid-phase extraction followed by liquid chromatography-time-of-flight-mass spectrometry to evaluate pharmaceuticals in effluents. A pilot monitoring study. - *J. Environ. Monit.* 9: 718-729.

Gómez-Ramos, M.d.M., Pérez-Parada, A., García-Reyes, J.F., Fernández-Alba, A.R. & Agüera, A. 2011: Use of an accurate-mass database for the systematic identification of transformation products of organic contaminants in wastewater effluents. - *J. Chromatogr. A* 1218: 8002-8012.

Grandjean, P., Bellinger, D., Bergman, Å., Cordier, S., Davey-Smith, G., Eskenazi, B., Gee, D., Gray, K., Hanson, M., Van Den Hazel, P., Heindel, J.J., Heinzow, B., Hertz-Picciotto, I., Hu, H., Huang, T.T., Jensen, T.K., Landrigan, P.J., McMillen, I.C., Murata, K., Ritz, B., Schoeters, G., Skakkebæk, N.E., Skerfving, S. & Weihe, P. 2008: The Faroes statement: human health effects of developmental exposure to chemicals in our environment. - *Basic Clin. Pharm. Toxicol.* 102: 73-75.

Gross, J.H. *Mass Spectrometry. A textbook.* Springer-Verlag: Berlin, 2004; pp. 518.

Guilhaus, M., Selby, D. & Mlynski, V. 2000: Orthogonal acceleration time-of-flight mass spectrometry. - *Mass Spectrom. Rev.* 19: 65-107.

Hernández, F., Sancho, J.V., Ibáñez, M., Abad, E., Portolés, T. & Mattioli, L. 2012: Current use of high-resolution mass spectrometry in the environmental sciences. - *Anal. Bioanal. Chem.* 403: 1251-1264.

Hernández, F., Portolés, T., Pitarch, E. & López, F.J. 2007: Target and nontarget screening of organic micropollutants in water by solid-phase microextraction combined with gas chromatography/high-resolution time-of-flight mass spectrometry. - *Anal. Chem.* 79: 9494-9504.

Hernández, F., Ibáñez, M., Sancho, J.V. & Pozo, Ó.J. 2004: Comparison of different mass spectrometric techniques combined with liquid chromatography for confirmation of pesticides in environmental water based on the use of identification points. - *Anal. Chem.* 76: 4349-4357.

Hernández, F., Ibáñez, M., Gracia-Lor, E. & Sancho, J.V. 2011a: Retrospective LC-QTOF-MS analysis searching for pharmaceutical metabolites in urban wastewater. - *J. Sep. Sci.* 34: 3517-3526.

Hernández, F., Portolés, T., Pitarch, E. & López, F.J. 2011b: Gas chromatography coupled to high-resolution time-of-flight mass spectrometry to analyze trace-level organic compounds in the environment, food safety and toxicology. - *TrAC Trends Anal. Chem.* 30: 388-400.

Hernández, F., Portolés, T., Pitarch, E. & López, F.J. 2009: Searching for anthropogenic contaminants in human breast adipose tissues using gas chromatography-time-of-flight mass spectrometry. - *J. Mass Spectrom.* 44: 1-11.

Hernández, F., Pozo, Ó.J., Sancho, J.V., López, F.J., Marín, J.M. & Ibáñez, M. 2005: Strategies for quantification and confirmation of multi-class polar pesticides and transformation products in water by LC–$MS^2$ using triple quadrupole and hybrid quadrupole time-of-flight analyzers. - *TrAC Trends Anal. Chem.* 24: 596-612.

Holm, A., Molander, P., Lundanes, E. & Greibrokk, T. 2003: Determination of rotenone in river water utilizing packed capillary column switching liquid chromatography with UV and time-of-flight mass spectrometric detection. - *J. Chromatogr. A* 983: 43-50.

Ibáñez, M., Sancho, J.V., Hernández, F., McMillan, D. & Rao, R. 2008: Rapid non-target screening of organic pollutants in water by ultraperformance liquid chromatography coupled to time-of-light mass spectrometry. - *TrAC Trends Anal. Chem.* 27: 481-489.

Ibáñez, M., Sancho, J.V., Pozo, Ó.J., Niessen, W. & Hernández, F. 2005: Use of quadrupole time-of-flight mass spectrometry in the elucidation of unknown compounds present in environmental water. - *Rapid Commun. Mass Sp.* 19: 169-178.

James, A.T. & Martin, A.J.P. 1952: Gas-liquid partition chromatography: the separation and micro-estimation of volatile fatty acids from formic acid to dodecanoic acid. - *Biochem. J.* 50: 679-690.

Karataev, V.I., Shmikk, D.V. & Mamyrin, B.A. 1972: New method for focusing ion bunches in time-of-flight mass spectrometers. - *J. Tech. Phys.* 16: 1177-1189.

Kern, S., Fenner, K., Singer, H.P., Schwarzenbach, R.P. & Hollender, J. 2009: Identification of transformation products of organic contaminants in natural waters by computer-aided prediction and high-resolution mass spectrometry. - *Environ. Sci. Technol.* 43: 7039-7046.

Kostiainen, R. & Kauppila, T.J. 2009: Effect of eluent on the ionization process in liquid chromatography–mass spectrometry. - *J. Chromatogr. A* 1216: 685-699.

Krauss, M., Singer, H. & Hollender, J. 2010: LC-high resolution MS in environmental analysis: from target screening to the identification of unknowns. - *Anal. Bioanal. Chem.* 397: 943-951.

Lara-Martín, P.A., González-Mazo, E. & Brownawell, B.J. 2011: Multi-residue method for the analysis of synthetic surfactants and their degradation metabolites in aquatic systems by liquid chromatography–time-of-flight-mass spectrometry. - *J. Chromatogr. A* 1218: 4799-4807.

Main, K.M., Mortensen, G.K., Kaleva, M.M., Boisen, K.A., Damgaard, I.N., Chellakooty, M., Schmidt, I.M., Suomi, A., Virtanen, H.E., Petersen, J.H., Andersson, A., Toppari, J. & Skakkebæk, N.E. 2006: Human breast milk contamination with phthalates and alterations of endogenous reproductive hormones in infants three months of age. - *Environ. Health Perspect.* 114: 270-276.

Mamyrin, B.A. 2001: Time-of-flight mass spectrometry (concepts, achievements, and prospects). - *Int. J. Mass Spectrom.* 206: 251-266.

Marín, J.M., Gracia-Lor, E., Sancho, J.V., López, F.J. & Hernández, F. 2009: Application of ultra-high-pressure liquid chromatography–tandem mass spectrometry to the determination of multi-class pesticides in environmental and wastewater samples: Study of matrix effects. - *J. Chromatogr. A* 1216: 1410-1420.

Martin, A.J.P. & Synge, R.L.M. 1941: A new form of chromatogram employing two liquid phases. - *Biochem. J.* 35: 1358-1368.

Martínez Bueno, M.J., Agüera, A., Gómez, M.J., Hernando, M.D., García-Reyes, J.F. & Fernández-Alba, A.R. 2007: Application of liquid chromatography/quadrupole-linear ion trap mass spectrometry and time-of-flight mass spectrometry to the determination of pharmaceuticals and related contaminants in wastewater. - *Anal. Chem.* 79: 9372-9384.

Melton, T.C. & Brown, S.D. 2012: The fate of sulfamethazine in sodium-hypochlorite-treated drinking water: monitoring by LC-MSn-IT-TOF. - *Int. J. Med. Chem.* vol. 2012 Article ID 693903: 1-6.

Mezcua, M., Ferrer, I., Hernando, M.D. & Fernández-Alba, A.R. 2006: Photolysis and photocatalysis of bisphenol A: Identification of degradation products by liquid chromatography with electrospray ionization/time-of-flight/mass spectrometry (LC/ESI/ToF/MS). - *Food Addit. Contam.* 23: 1242-1251.

Nácher-Mestre, J., Serrano, R., Portolés, T. & Hernández, F. 2011: Investigation of organophosphate esters in fresh water, salt and brine samples by GC-TOF MS. - *Anal. Methods* 3: 1779-1785.

Nielen, M.W.F., van Engelen, M.C., Zuiderent, R. & Ramaker, R. 2007: Screening and confirmation criteria for hormone residue analysis using liquid chromatography accurate mass time-of-flight,

Fourier transform ion cyclotron resonance and orbitrap mass spectrometry techniques. - *Anal. Chim. Acta* 586: 122-129.

Nödler, K., Licha, T., Bester, K. & Sauter, M. 2010: Development of a multi-residue analytical method, based on liquid chromatography–tandem mass spectrometry, for the simultaneous determination of 46 micro-contaminants in aqueous samples. - *J. Chromatogr. A* 1217: 6511-6521.

NORMAN Network 2011: http://www.norman-network.net. Accessed 13.1.2012:

OECD Organisation for Economic Co-operation and Development 2012: Environmental outlook to 2050.

OECD Organisation for Economic Co-operation and Development 2001: Environmental outlook for the Chemicals Industry.

Pedrouzo, M., Borrull, F., Marcé, R.M. & Pocurull, E. 2009: Ultra-high-performance liquid chromatography–tandem mass spectrometry for determining the presence of eleven personal care products in surface and wastewaters. - *J. Chromatogr. A* 1216: 6994-7000.

Pérez, S., Eichhorn, P. & Barceló, D. 2007: Structural characterization of photodegradation products of enalapril and its metabolite enalaprilat obtained under simulated environmental conditions by hybrid quadrupole-linear ion trap-MS and quadrupole-time-of-flight-MS. - *Anal. Chem.* 79: 8293-8300.

Petrovic, M., Gros, M. & Barcelo, D. 2006: Multi-residue analysis of pharmaceuticals in wastewater by ultra-performance liquid chromatography–quadrupole–time-of-flight mass spectrometry. - *J. Chromatogr. A* 1124: 68-81.

Pitarch, E., Portolés, T., Marín, J., Ibáñez, M., Albarrán, F. & Hernández, F. 2010: Analytical strategy based on the use of liquid chromatography and gas chromatography with triple-quadrupole and time-of-flight MS analyzers for investigating organic contaminants in wastewater. - *Anal. Bioanal. Chem.* 397: 2763-2776.

Portolés, T., Ibáñez, M., Sancho, J.V., Ló pez, F.J. & Hernández, F. 2009: Combined Use of GC-TOF MS and UHPLC-(Q)TOF MS To Investigate the Presence of Nontarget Pollutants and Their Metabolites in a Case of Honeybee Poisoning. - *J. Agric. Food Chem.* 57: 4079-4090.

Portolés, T., Pitarch, E., López, F.J. & Hernández, F. 2011a: Development and validation of a rapid and wide-scope qualitative screening method for detection and identification of organic pollutants in natural water and wastewater by gas chromatography time-of-flight mass spectrometry. - *J. Chromatogr. A* 1218: 303-315.

Portolés, T., Pitarch, E., López, F.J., Hernández, F. & Niessen, W.M.A. 2011b: Use of soft and hard ionization techniques for elucidation of unknown compounds by gas chromatography/time-of-flight mass spectrometry. - *Rapid Commun. Mass Sp.* 25: 1589-1599.

Portolés, T., Pitarch, E., López, F.J., Sancho, J.V. & Hernández, F. 2007: Methodical approach for the use of GC-TOF MS for screening and confirmation of organic pollutants in environmental water. - *J. Mass Spectrom.* 42: 1175-1185.

Pozo, Ó.J., Sancho, J.V., Ibáñez, M., Hernández, F. & Niessen, W.M.A. 2006: Confirmation of organic micropollutants detected in environmental samples by liquid chromatography tandem mass spectrometry: Achievements and pitfalls. - *TrAC Trends Anal. Chem.* 25: 1030-1042.

Richardson, S.D. & Ternes, T.A. 2011: Water analysis: emerging contaminants and current issues. - *Anal. Chem.* 83: 4614-4648.

Richardson, S.D. 2009: Water analysis: emerging contaminants and current issues. - *Anal. Chem.* 81: 8654-8654.

Sasaki, H., Yonekubo, J. & Hayakawa, K. 2006: A new on-line sample preparation system for the liquid chromatography/time-of-flight mass spectrometry simultaneous analysis of pesticides in river water. - *Anal. Sci.* 22: 835-840.

Serrano, R., Portolés, T., Blanes, M.A., Hernández, F., Navarro, J.C., Varó, I. & Amat, F. 2012: Characterization of the organic contamination pattern of a hyper-saline ecosystem by rapid screening using gas chromatography coupled to high-resolution time-of-flight mass spectrometry. - *Sci. Total Environ.* 433: 161-168.

Serrano, R., Nácher-Mestre, J., Portolés, T., Amat, F. & Hernández, F. 2011: Non-target screening of organic contaminants in marine salts by gas chromatography coupled to high-resolution time-of-flight mass spectrometry. - *Talanta* 85: 877-884.

Stephens, W.E. 1946: A pulsed mass spectrometer with time dispersion. - *Phys. Rev.* 69: 691-691.

Stolker, A.M., Niesing, W., Hogendoorn, E., Versteegh, J.M., Fuchs, R. & Brinkman, U.T. 2004: Liquid chromatography with triple-quadrupole or quadrupole-time of flight mass spectrometry for screening and confirmation of residues of pharmaceuticals in water. - *Anal. Bioanal. Chem.* 378: 955-963.

Tswett, M. 1906a: Physikalisch-Chemische Studien über das Chlorophyll. Die Adsorption. - *Ber. Dent. Botan. Ges.* 24: 316-326.

Tswett, M. 1906b: Adsorptionanalyse und chromatographische Methode. Anwendung auf die Chemie des Chlorophylls. - *Ber. Dent. Botan. Ges.* 24: 384-393.

Wiley, W.C. & McLaren, I.H. 1955: Time-of-flight mass spectrometer with improved resolution. - *Rev. Sci. Instrum.* 26: 1150-1157.

Wille, K., De Brabander, H.F., Vanhaecke, L., De Wulf, E., Van Caeter, P. & Janssen, C.R. 2012: Coupled chromatographic and mass-spectrometric techniques for the analysis of emerging pollutants in the aquatic environment. - *TrAC Trends Anal. Chem.* 35: 87-108.