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The hunt for highly polar substances

KWR Watercycle Research Institute

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The hunt for highly polar chemicals

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Watercycle Research Institute

BTO *Managementsamenvatting*

Zoeken naar zeer polaire stoffen

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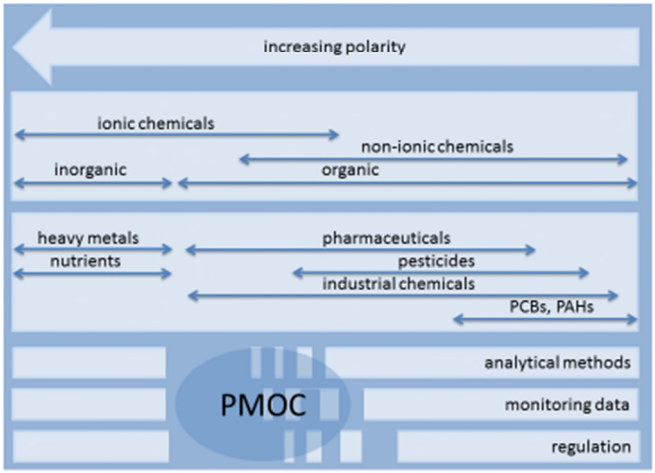
De verspreiding van zeer polaire stoffen in het milieu is groot, omdat zij goed uitspoelen naar grondwater en slecht worden verwijderd in zuiveringsinstallaties van afval- en drinkwater. Een goede methode om de aanwezigheid en het gedrag van zeer polaire stoffen in de waterketen te monitoren ontbreekt nog. Er is een HILIC non-target analyse methode ontwikkeld en gevalideerd voor 38 zeer polaire stoffen in oppervlaktewater en drinkwater. De methode biedt handvatten om de zeer polaire fractie van de “overige antropogene stoffen” uit het Drinkwaterbesluit te meten op een niveau van 1 µg/L. De methode is in te zetten voor de screening naar onbekende zeer polaire stoffen.



Kennishiaten van persistente zeer polaire organische stoffen (PMOC=Persistent Mobile Organic Compounds, (Figure adapted from [1])

Belang: meer kennis nodig over (aanwezigheid van) zeer polaire stoffen in de waterketen

Momenteel is nog te weinig bekend over de zeer polaire stoffen die in de waterketen (kunnen) voorkomen. Deze stoffen zijn relevant, omdat ze mobiel zijn en moeilijk uit water kunnen worden verwijderd. Er is behoefte aan meer kennis over de (i) modellering, (ii) analyse, (iii) monitoring, (iv) en het gedrag in het milieu en in zuiveringsprocessen van deze stoffen. Omdat er nieuwe analytische methoden beschikbaar zijn om zeer polaire organische verbindingen in water te bestuderen, is het tijd om een monitoringmethode te vinden die werkt voor dergelijke zeer polaire verbindingen in de waterketen.

Aanpak: bronnenonderzoek en ontwikkeling monitoringmethode met massaspectrometerie

Op basis van literatuur en andere bronnen is een classificatieschema ontwikkeld voor zeer polaire stoffen en zijn relevante kandidaatstoffen geselecteerd (hoofdstuk 2). Daarna is geïnventariseerd welke analytische methoden voorhanden zijn om deze stoffen te scheiden en is per techniek bepaald hoe geschikt die is voor inzet in non-target screening (hoofdstuk 3). Vervolgens

is een non-target screeningsmethode ontwikkeld die is gebaseerd op hydrofiele interactie-vloeistofchromatografie (HILIC), gekoppeld aan hoge resolutie massaspectrometrie. Deze methode is gevalideerd met een set van 38 kandidaatstoffen die voorkomen in drink- en oppervlaktewater (hoofdstuk 4).

Resultaten: non-target screening nu ook voor zeer polaire stoffen

Er is een overzicht gemaakt van zeer polaire stoffen / stofgroepen die relevant kunnen zijn voor de drinkwatersector, geselecteerd op basis van (beperkte) meetgegevens in de waterketen, gegevens over productie en toepassing en literatuurgegevens. Deze stoffen zijn geclassificeerd op hydrofobiciteit (vetminnendheid) en oplosbaarheid. Er zijn technieken geselecteerd die geschikt zijn om zeer polaire stoffen te scheiden: capillaire elektroforese, ionchromatografie, HILIC, mixed-mode chromatografie en superkritische vloeistofchromatografie. Om deze technieken te kunnen toepassen bij non-target screening moeten ze: (i) een zo'n breed mogelijk pakket van zeer polaire stoffen tegelijkertijd kunnen scheiden en (ii) te koppelen zijn met detectie met hoge resolutie-massaspectrometrie. Met HILIC is een screeningsmethode voor zeer polaire stoffen uitgewerkt.

De ontwikkelde HILIC screening is gebaseerd op de screening beschreven in BTO.2015.076. Er zijn 16 stoffen toegevoegd aan de methode, waardoor nu 38 stoffen worden geanalyseerd. Daarnaast is er een monstervoorbewerking ontwikkeld, op basis van verdampen van het water, om de gevoeligheid te verbeteren. De Orbitrap Fusion wordt ingezet voor de detectie van de stoffen. Deze ultrahoge resolutie massaspectrometer biedt mogelijkheden voor screening naar – en structuuropheldering van onbekende zeer polaire stoffen. Testen voor de validatie van de methode laten goede prestatiekenmerken zien in oppervlakte - en drinkwater. Een selectie van 38 representatieve stoffen kan worden gemeten op een niveau van 1 µg/L of lager. Dat betekent dat de methode geschikt is voor monitoring van het zeer polaire spectrum van de categorie "overige antropogene stoffen" uit het Drinkwaterbesluit. Dankzij de combinatie van deze nieuwe methode met de reeds bestaande non-target methoden is het nu mogelijk een zeer breed spectrum van organische microverontreinigingen te meten en een completer beeld van de chemische waterkwaliteit te krijgen.

De ontwikkelde HILIC non-target methode is inmiddels toegepast in het BTO project *Meten is weten - zeer polaire stoffen in bronnen van drinkwater* en in het DPWE-project '*Screening op (kleine) polaire stoffen door de zuiveringen heen in koude en warme periode'* (BTO 2018.023). Zeer polaire bekende en onbekende stoffen zijn met de HILIC screening-methode gesignaleerd in (bronnen van) drinkwater in Nederland en Vlaanderen.

Implementatie: methode inzetten bij monitoring en evaluatie en verder ontwikkelen

Omdat nog veel informatie rondom zeer polaire stoffen in de waterketen ontbreekt, is het belangrijk de ontwikkelde methode in te zetten om inzicht te krijgen in de aanwezigheid en het gedrag van zeer polaire stoffen in de waterketen en tijdens de zuivering. Gegevens uit deze monitoring kunnen vervolgens worden gebruikt om stoffen te selecteren voor onderzoek naar hun gezondheidskundige relevantie.

Daarnaast is het belangrijk de methode ook verder te ontwikkelen zodat deze geschikt is voor (sterk) zure verbindingen. Met de huidige deze methode zijn dergelijke stoffen nog niet goed of gevoelig genoeg te bepalen.

Rapport

Dit onderzoek is beschreven in rapport BTO2018.022 *The hunt for highly polar substances*. Aanvullende informatie over de meetmethode is te vinden in de rapportage BTO2015.076 "HILIC screening - analyse van zeer polaire stoffen in water" en de ontwikkelde methode is ontwikkelde methode is al toegepast, zie BTO2018.023 *Meten is weten - zeer polaire stoffen in bronnen van drinkwater*.

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Summary

Highly polar chemicals are mobile in an aqueous environment. They are poorly removed from wastewater and by drinking water treatment. Consequently, these chemicals can be expected in wastewater effluent, surface water, groundwater and even in drinking water. Analytical methods for the analysis of trace levels of these compounds in water are largely lacking. Within this study a non-target screening was developed for analysis of highly polar chemicals, based on sample evaporation and reconstituting in organic solvent, followed by HILIC coupled to high resolution mass spectrometry. The method was validated with 38 highly polar chemicals in surface water and drinking water. Despite the fact that the method does not cover all highly polar chemicals – especially strong acidic substances appear difficult to be separated and detected - the developed method allows to detect a wide array of highly polar chemicals at a level of 1 µg/L or lower. The method can therefore be applied to analyze "other anthropogenic substances" that are not yet specified in "*Het Drinkwaterbesluit*" can be applied to screen of new and yet unidentified highly polar substances. The method is complementary to currently applied target and non-target methods for less polar substances. Thereby, it can be applied for a better (drinking) water quality assessment.

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1 Introduction

1.1 Background

Micro contaminants are a key issue for the drinking water companies as they can pose a human health risk [2]. Even if they are not expected to have any human health effects [3], their mere presence indicate contamination routes of anthropogenic micro contaminants in the water cycle and along treatment trains, illustrating the vulnerability of drinking water (sources) and can generate public concern [4, 5].

Historically, the focus of environmental chemistry and toxicology in the (urban) water cycle was on two classes of chemicals 1) metals, in their dissolved form and (2) (persistent) hydrophobic organic chemicals. This focus is driven by increasing emissions, by detection of (eco) toxicological effects and especially by the availability of sensitive analytical tools.

Heavy metals occur naturally, but intensive mining in the nineteenth and twentieth centuries lead to environmental pollution and the observation of effects in the environment. Dissolved ionic species of certain metals can be very toxic to living organisms including humans. Development of techniques such as Atomic Absorption Spectrometry (AAS) where the absorbance of specific wavelengths of light by metals is used enabled (environmental) analysis of metals in aqueous samples. More recently Inductively Coupled Plasma Mass Spectrometry (ICPMS) is used to detect metallic species in aqueous samples based on their specific mass.

Most persistent hydrophobic organic chemicals are man-made. The research and legislative focus on hydrophobic organic chemicals was driven by the combination of increasing production after the second world war [6], observation of effects [7], and availability of analytical tools to detect these substances at low concentrations in environmental matrices. Hydrophobic persistent chemicals are toxic since they tend to accumulate in fatty tissues and membranes of living organisms due to their hydrophobic nature (bioaccumulation). The presence of these substances in cell membranes disturbs normal functioning of the membrane, thereby affecting the organism. The toxicological effect caused by these substances is called narcosis, as (sub)lethal effects of these substances show signs of sedation [8]. The hydrophobic and persistent nature of the substances allows them to be accumulated through food webs, this effect is called biomagnification [9].

The environmental analysis of these chemicals was enabled by techniques based on gas chromatographic separation followed by detection using several types of detectors such as Flame Ionisation Detector, Thermal Conductivity Detector, Electron Capture Detector Nitrogen Phosphorous Detector and Mass Spectrometer. Since the eighties of the twentieth century, the introduction and development of liquid chromatography in combination with various detectors has drawn attention towards more polar, water soluble and less volatile chemicals. This technique enabled, for example, the analysis of many non-volatile pharmaceuticals in environmental waters [10].

Highly polar organic chemicals that are very mobile in aqueous matrices are currently largely omitted from environmental monitoring. Even novel techniques such as non-target screening based on reversed phase chromatography [11] and assessment of effects using bioassays

misses these chemicals, since they are poorly extracted by the commonly applied solid phase extraction techniques.

Recently, it was argued that mobile (polar) persistent chemicals are of relevance since their polar nature makes them very mobile in the aqueous environment and their persistence enables them to remain in the (aqueous) environment for a long time [1, 12]. What holds for natural systems also holds for techniques applied in water treatment systems. This makes Persistent Mobile Organic Chemicals (PMOC) also a challenge for organisations responsible for wastewater treatment and drinking water production. The environmentally relevant hydrophobic organic chemicals are generally less relevant for wastewater treatment and especially drinking water treatment, since their hydrophobic nature prevents them from passing multiple treatment barriers in wastewater treatment and the production of drinking water from surface water [13]. Furthermore the hydrophobic nature also prevents them from passage of soil or sediment, so it is unlikely that relevant concentrations of very hydrophobic substances end up in groundwater or river bank filtrate. Unlike hydrophobic substances, removing PMOC from wastewater and water used for the production of drinking water is difficult, which makes them relevant for the water sector.

1.2 Objectives

The objectives of this study are to (1) classify and select highly polar chemicals relevant for drinking water [12] (2) define analytical challenges and blind spots for highly polar chemicals, (3) evaluating innovative tools to for chemical and biological characterization of highly polar chemicals, (4) prepare these innovative tools for environmental monitoring [14] to gain insight in their concentration and relevance by applying the developed method on environmental samples (this is described within the related report BTO2018-023). Finally (5) this research forms the basis for a roadmap for the development and/or application of monitoring tools and human health risk assessment.

As stated earlier, highly polar substances are scarcely monitored in the water cycle. There is limited knowledge on occurrence of these substances in the water cycle. Consequently, the determination of relevant highly polar substances is difficult. Therefore non-target screening methods for this class of substances are required to screen for relevant polar substances and direct further (target) analyses, fate studies an toxicological evaluations. The core of the present study is the development of a non-target screening method for polar substances that is validated with an array of polar an highly polar substances with different charges, an sizes. This training set enables one to evaluate the non-target screening method and define analytical gaps. The non-target method for (very) polar substances is developed to track down highly polar substances in the urban water cycle.

Chapter 2 provides a set of criteria to define and classify polar substances and proposes a list of candidate substances for method development and validation. This classification is of importance since the term polar organic substances is used for substances with a wide array of properties in scientific literature. Chapter 3 gives a literature overview of analytical methods an approaches to detect (very) polar substances, and evaluates them for the purpose of environmental monitoring in aqueous matrices. Chapter 4 describes and evaluates a novel analytical method for non-target screening of (very) polar substances, while Chapter 5 evaluates the relevance and value of developed analytical technique for future monitoring of highly polar substances.

2 Classification of polar and mobile chemicals

This chapter illustrates classification schemes for chemical properties and in particular substance polarity and mobility. Furthermore a selection of polar chemical (classes) is made that will be used in the experimental section.

2.1 Framing polarity and mobility

Polar substances can be defined in many ways. Technically a highly polar substance means that the overall charge, determined by the electrons present in the molecule, is unevenly distributed over the surface of the molecule, however without an unbalance between the number of electrons and protons within a molecule (such an unbalance would result in a charged, or ionic species). The heterogeneous distribution generates (slightly) negatively and (slightly) positively charged sites on the molecular surface and is indicated by the net charge deviation from homogenous distribution of charge over the molecule (∂-sign). In extreme cases the distribution of electrons over a molecule can be so heterogeneously that one site of a molecule is negatively charged (there are one or more electrons more than protons at an active group of a molecule) while another site is positively charged (there are more protons than electrons at the active group). Theoretically this would mean that zwitter-ions (an ion that holds both an negatively charged site and a positively charged site, rendering a net neutrally charged chemical) would be the most polar chemical possible. However, within this study we define the polarity of a chemical by the difficulty to separate the substance from water. The ionic properties of zwitterions allow ionic interactions with dissolved ionic species and charged surfaces of sorbents [15-17]. This makes them less mobile in the environment and potentially less soluble in water.

Water itself is also a polar substance and its polarity enables hydrogen bonding which in turn determines its extraordinary properties. So where we use the term (very) polar substances within this report we use it from an operational perspective of separation and detection from an aqueous phase. We do not necessarily mean the substances with the highest heterogeneity of charge on their molecular surface, but substances that easily form hydrogen bonds with water and are highly soluble in water, because this makes them difficult so separate from water. Actually these substances are very much alike water. Such substances have the following properties. Active groups like alcohols, ethers, aldehydes, ketones, esthers, carboxylic acids etc. possess the ability to form hydrogen bonds with water molecules. An active group that is able to form hydrogen bonds improves the solubility in water. The energy that comes from the formation of this new hydrogen bond between the molecule and water largely balances out the energy that is required to dissolve in water by breaking existing hydrogen bonds between the water molecules [18]. This means that the hydrogen bond forming sites in relation to the substance volume or surface strongly correlates with its solubility. Thereby, it inversely correlates with adsorption on and partitioning in sorbents with different properties than water. So in practice this means that such molecules are difficult to isolate from water by sorbents for analysis or treatment.

2.2 Classifying and selecting (persistent) polar substances

As was discussed shortly in the introduction, highly polar organic substances or mobile organic compounds are much alike water. Their properties and behaviour have hardly been

studied, and they have not been included in monitoring programs nor in regulation. This is due to (1) the absence or limited application of suitable analytical techniques for aqueous environmental samples, (2) the fact that they are not likely to bioaccumulate or biomagnify and (3) the limited attention in regulatory frameworks. Highly polar substances are of particular interest when they persist in the environment or treatment systems. Due to limited monitoring data the selection of environmental or drinking water-relevant highly polar substances is a challenge, as was clearly pointed out in the paper of Reemtsma *et al.* [1] who metaphorically used "mind the gap" for the missing information on this underexposed class of PMOC. These chemicals are mobile (in water) because of their polar –water alike- nature. Figure 2-1 taken from Arp et al. illustrates a classification scheme for persistent and mobile chemicals in aqueous matrices. Mobile chemicals are defined by their polarity which is measured by the distribution coefficient between water and organic matter, normalized for the fraction organic carbon (in soil or sediment; log K_{OC} *of* D_{OC}*)* or the aqueous solubility (in mass per liter). The persistence is defined by the half-life of a substance in aqueous matrices. As stated by the authors, these substances can be found by an analytical approach, in which you combine the most suitable analytical techniques with non-target screening techniques to track them in environmental samples, or to screen for presumably polar (and persistent) substances within lists of produced or applied chemicals [19, 20]. Within the current study the primary focus is on highly polar (mobile) substances.

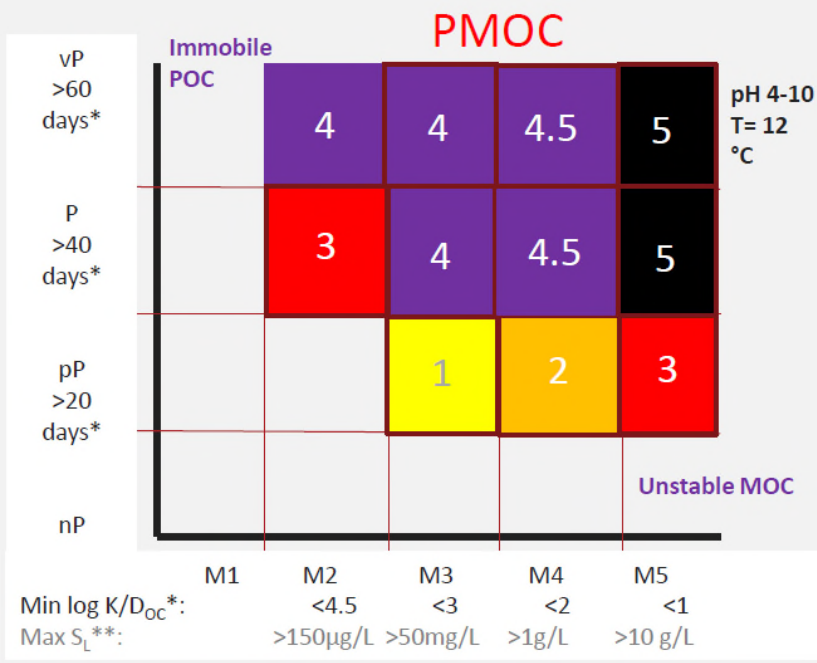

FIGURE 2-1: CLASSIFICATION OF PERSISTENT MOBILE ORGANIC CHEMICALS. VP = VERY PERSISTENT, P = PERSISTENT, PP PARTIALLY PERSISTENT, NP NOT PERSISTENT ; M1-M5 ARE MOBILITY CLASSES RANGING FROM NOT MOBILE M1 TO VERY MOBILE M5. THESE CLASSES CAN BE DEFINED BY EITHER SOLUBILITY OR DISTRIBUTION COEFFICIENTS BETWEEN WATER AND ORGANIC MATERIAL, NORMALISED FOR THE CARBON CONTENT. FIGURE OBTAINED FROM ARP ET AL. [21].

Detecting mobile substances in the environment requires suitable techniques and specific knowledge on the domain of application of these techniques [20, 21]. Selecting relevant mobile (persistent) substances without environmental monitoring data therefore requires other types of information such as information on production application or use (emission) in combination with their persistence and mobility in the environment [12].

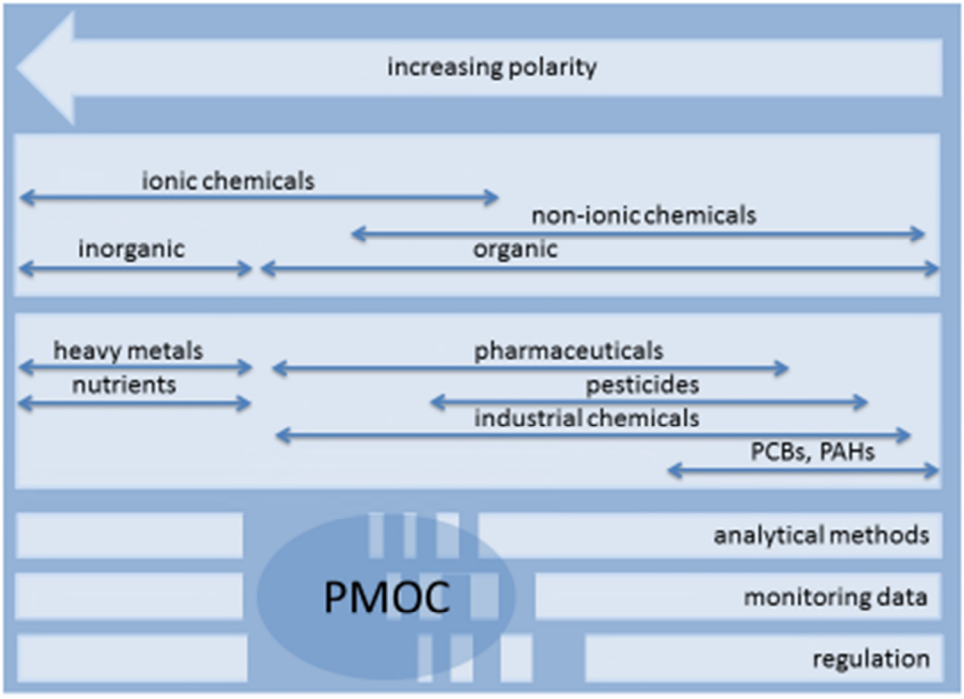

FIGURE 2-2: SCALING PERSITENT MOBILE CHEMICALS (PMOC) AND DEFINING THE KNOWLEDGE GAP (FIGURE ADAPTED FROM [1])

Various sources have been used to generate an overview of polar substances that can be of relevance for the drinking water sector. Table 2-1 gives an overview of classes of chemicals that are or cover soluble hydrophilic chemicals. The generic criterion that was used was a log K_{ow} < 1 and solubility < 10 mg/L-level. In this initial selection their persistence is not evaluated.

TABLE 2-1 CLASSES OF POLAR/MOBILE CHEMICALS THAT CAN BE RELEVANT FOR THE DRINKING WATER SECTOR

Group of compounds	**Reference**	**Examples**
Pesticides		
Organophosphate pesticides	[22, 23]	acephate, methamidophos, omethoate
Quaternary ammonium chemicals	[1, 24, 25]	paraquat, diquat, chlormequat, mepiquat
Other polar pesticides	[1, 26]	glyphosate, AMPA, bentazone, cyromazine, maleïnehydrazide
Transformation products of pesticides	[27, 28]	AMPA, desphenyl-chloridazon, N,N-dimethylsulfamide
Pharmaceuticals		
Antibiotics	[29, 30]	amprolium, gentamycin, neomycin, paromycin, streptomycin
Anti-cancer drugs	[25, 31, 32]	5-fluoracil, cytarabine, gemtitabine

Group of compounds	**Reference**	**Examples**
Drugs of Abuse	[33-35]	GHB
Other pharmaceuticals	[1, 3, 36-40]	caffeine, metformin, gabapentin
Transformation products of pharmaceuticals	[41]	dihydroxycarbamazepine, guanylurea
Contrast media		
Diagnostic Contrast Media	[1, 39, 42, 43]	diatrizoic acid, iomeprol, iopromide, iopamidol, iohexol
Consumer products		
Artifical sweeteners	[44, 45]	acesulfame, saccharin, cyclamate, and sucralose
Amino acids		phenylalanine, tyrosine, tryptophan
Estrogen conjugates		
Estrogen conjugates	[46]	estrone, estradiol, and estriol
Industrial products		
Benzatriazoles	[47]	1H-benzo-1,2,3-triazole (BTri) and its methylated analogues (tolyltriazole, TTri)
Aromatic sulphonic acids	[48]	naphthalene-1,5-disulphonic acid, nafthalene-1,3,6-trisulphonic acid
Complexing agents	[1, 49]	ethylenediamino tetraacetate (EDTA) and diethylenetriamino pentaacetate (DTPA)
Halogenated methanesulfonic acids	[50]	trifluoromethanesulfonic acid, chloromethanesulfonic acid, bromomethanesulfonic acid
Organophosphates	[1]	fosethyl ammonium, TCEP
Perfluorinated chemicals (PFAAs)	[1]	perfluoric acid, Gen X
Amino-phenols	[51]	diethanolamine, triethanolamine
Solvents	[1]	ETBE, MTBE, n-methyldiethanolamine
Triazines	[52]	ammeline, ammelide, cyanuric acid, melamine, melam, melem
Other industrial by-products	[53-55]	acrylamide, acrylic acid, pyrazole, urotropine
Disinfection byproducts	[1, 52, 56-58]	dichloroacetic acid, NDMA (formed from DMS)

2.3 Selecting polar/mobile substances for analysis

Within this study we focus on the most mobile (polar) substances. Very polar substances are poorly extracted from water and separated by the most commonly applied analytical techniques for monitoring in the water sector (reversed phase HPLC). We therefore select substances that are difficult or impossible to analyse with commonly applied reversed phase HPLC separation. Table 2-2 gives the pre-selected chemicals. The ultimate selection of a substance was based also on pragmatic issues such as the availability and affordability of standards.

TABLE 2-2: PRE-SELECTED POLAR/MOBILE CHEMICALS FOR ANALYSIS WITH THEIR LOG - OCTANOL-WATER PARTITION COEFFICIENT (LOG K_{ow}) AND AQUEOUS SOLUBILIYU AT 25˚C

Chemical	**Log K_{ow} EpiSuite[1]**	**Aqueous solubility EpiSuite[1] (mg/L 25˚C)**	**Remark**
Maleic hydrazide	-0.84	4510	Polar pesticides, growth regulator
Cyanuric acid	1.95	2000	Triazine
Ammeline	-3.65	75	Triazine, transformation

Chemical	**Log K_{ow} EpiSuite**[1]	**Aqueous solubility EpiSuite**[1] **(mg/L 25°C)**	**Remark**
			products melamine
Ammelide	-1.22	76.9	Triazine
Melam	0.38	13620	Triazine, condensation product of melamine
Melem	-1.22	390400	Heptazine, flame retardant
Trifluoromethanesulfonic acid (TFSA)	-0.49	197500	Sulphonic acids
HFPO-DA (GenX)	3.36[a]	27.2	Perfluorinated chemicals (PFAAs)
Fosetyl-aluminum	-2.4	111000	Phosphonates
GHB	-0.4	1000000	Psychoactive drug
Metformin	-2.64	1000000	Pharmaceuticals, anti-diabetic
Urotropine	-4.15	449000	Industrial by-products
Guanylurea	-1.22	62020	Pharmaceuticals, transformation product of metformin
Iopromide	-2.05	23.75	Diagnostic contrast media
Iomeprol	-2.79	126.4	Diagnostic contrast media
Iohexol	-3.05	106.5	Diagnostic contrast media
Paraquat	-4.5[2]	620000	Quaternary ammonium chemicals
Diquat	-2.82[2]	442600	Quaternary ammonium chemicals
Glyphosate	-3.4	1050000	Polar pesticides, herbicide, phosphonate
AMPA	-2.47	1000000	Polar pesticides, transformation product
Tetrapropylammonium (TPA)	1.45	2409	Industrial compound
Desphenyl-chloridazon	-1.59	1000000	Polar pesticides, transformation products
N,N-Dimethylsulfamide	-1.11	42370	Polar pesticides, transformation products
Acesulfame K	-2.67	1000000	Artifical sweeteners
Acrylamide	-0.67	390000	Industrial compound
Acrylic acid	0.35	1000000	Industrial compound
Niacin	0.36	18000	Vitamin B3 complex
Diatrizoic acid	1.37	8.885	Diagnostic contrast media
Cyromazin	0.96	13000	Polar pesticides, insecticide, triazine
Phenylalanine	-1.38	28200	Amino acid
Tyrosine	-2.26	400	Amino acid
Tryptophane	-1.06	13400	Amino Acid
5-Fluorouracil	-0.89	11100	Pharmaceutical, cytostatica
Cytarabine	-2.51	175700	Pharmaceutical, cytostatica
Gemcitabine	-2.01	51390	Pharmaceutical, cytostatica

Chemical	**Log K_{ow} EpiSuite**[1]	**Aqueous solubility EpiSuite**[1] **(mg/L 25°C)**	**Remark**
Acephate	-0.85	818000	Polar pesticides, insecticide, phospates
Methamidophos	-0.8	1000000	Polar Pesticides, insecticide, phospates
Omethoate	-1.49	1000000	Polar pesticides, insecticide, phospates
Dichloroacetic acid	0.92	1000000	Chlorination by-product
Melamine	-1.37	3230	Triazine
Cytosine	-1.73	8000	DNA base
Naphtalene-1,5-disulphonic acid	-0.94	1000000	Sulphonic acid
Naphtalene-1,3,6-trisulphonic acid	-1.88	194700	Sulphonic acid
Gabapentine	-1.1	4491	Pharmaceutical, anti-epileptica
Amprolium	-2.5	1000000	Veterinary pharmaceutical, antiprotozoal
Diethanolamine	-1.43	1000000	Intermediate pesticides synthesis
Triethanolamine	-1	1000000	Additive
N-Methyldiethanolamine	-1.5	1000000	Solvent
Streptomycin	-7.53	1000000	Antibiotic
Gentamycin	-1.88	961800	Antibiotic
Paromomycin	-6.92	1000000	Antibiotic
Neomycin	-9.41	1000000	Antibiotic
Chlormequat	-3.44	1000000	Quaternary ammonium chemicals, growth regulator
Mepiquat	-2.82	1000000	Quaternary ammonium chemicals

[1] *Experimental database matches were preferred over modelled values*

[2] *Determination of octanol water partition coefficients of surfactant-like substances is not possible, therefore this parameter is not suitable to determine their mobility [59]*

Substances with very mobile properties (and according to the PMOC criteria: persistent) are most challenging in water treatment as well as for chemical analysis. The solubility is determined by various molecular properties such as charge (i.e. neutral, positively charged, negatively charged and zwitterionic substances, charge distribution (e.g. surfactant like substances, hydrogen bonding). Within this study we aim to develop an analytical method that covers a broad array of polar substances, but inevitably, such a method will not be optimal for all highly polar soluble substances. Chapter 3 discusses the analytical possibilities an challenges in more detail.

3 Analytical tools for highly polar chemicals in water

This chapter gives an overview of available analytical techniques to determine trace levels of for highly polar chemicals in aqueous matrices.

3.1 Analytical methods for polar substances

The development and application of various analytical tools lead to increased awareness of a broad array of anthropogenic chemicals and their metabolites in the aqueous environment and drinking water. Polar and mobile chemicals are less well studied in aqueous samples since their isolation from water and subsequent separation is difficult. Nowadays most chemical water quality monitoring methods of micro contaminants make use of reversed phase (RP) liquid chromatography (LC) using hydrophobic C18 or similar columns in combination with a mass spectrometer.This technique is used to separate the chemicals present in the sample extract thus enabling identification and quantification. In RP-LC the analytes are dissolved in a polar mobile phase (predominantly water), which is conducted over a stationary a-polar phase. Due to interaction of the analytes with the stationary phase, these analytes will be separated and will leave the HPLC column at different times. Highly polar chemicals are poorly retained on C18 columns and elute in the “void volume” together with polar matrix components present in water samples, resulting in ineffective ionisation in the LC-MS interface [1]. Some novel advances in RP technology have come to the market, such as columns stable within an extended pH range and columns tolerating a completely aqueous mobile phase which allow separation of more polar compounds. Despite these developments an analytical gap still exist for the analysis of PMOCs.

There are various separation techniques available for the analysis of highly polar compounds, i.e. capillary electrophoresis, ion chromatography, hydrophilic interaction chromatography (HILIC), mixed-mode chromatography and super critical fluid chromatography. These techniques are described in more detail in this chapter. It is desired to have a method available that can separate the total chemical space from the highly polar compounds (i.e. strong acids, neutral, strong bases, zwitterionic, and permanently charged compounds). Moreover the possibilities of coupling these different separation techniques to high resolution mass spectrometry to perform suspect and/or non-target screening are evaluated.

3.2 Capillary electrophoresis

Electrophoresis is the separation of charged polar chemicals in aqueous solution under the influence of an electric field [60]. Positively charge chemicals move towards the negatively charged cathode and negatively charged chemicals towards the anode. How fast these ions can travel is determined by their charge and size and by the viscosity of the liquid. All three parameters have a profound effect on the separation of the ions. The following equation describes the mobility of the ions:

$$\mu = \frac{v}{E} = \frac{q}{6\pi r\eta}$$

μ = mobility, v = velocity, q = charge, r = radius, η = viscosity

The equation reveals that the mobility increases with an increasing charge, but is reduced when the size of the ion gets larger. The more viscous the liquid in the capillary, the slower the ions move through the capillary.

The most common type of electrophoresis for inorganic and organic chemicals is capillary electrophoresis (CE) [61]. CE coupled to a mass spectrometer has shown considerable potential for profiling ionogenic chemicals. In pressure assisted CE flow rates are very low and are typically in the range of 20 – 100 nL/min. The result of this low flow-rate is the formation of small droplets in the ionisation chamber. This is advantageous for ESI as the transfer of ions into the mass spectrometer is improved. Furthermore, ion suppression is reduced.

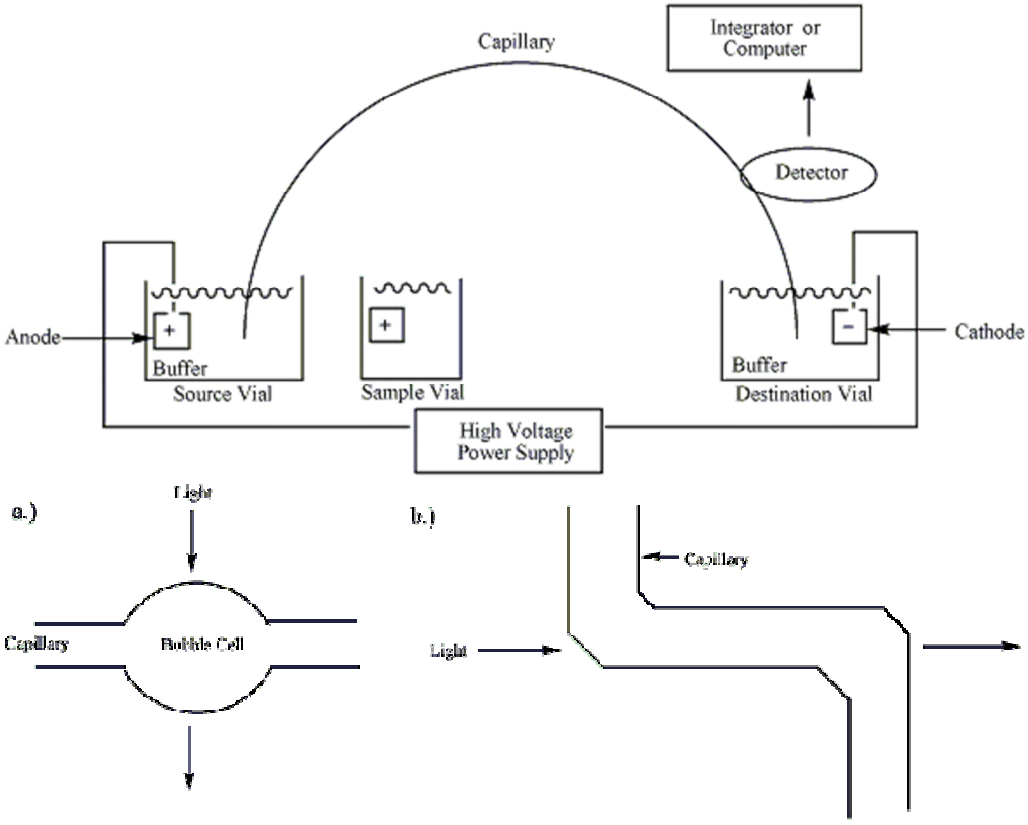

FIGURE 3-1: SCHEMATIC DIAGRAM OF A CLASSICAL CE- SET-UP (TOP). TWO POSSIBLE WAYS TO DETECT CHEMICALS IN A CAPILLARY (BOTTOM)

In a classical CE setup a closed electric circuit exists between the two ends of the capillary. This is usually achieved by placing both ends of the capillary in vials with a buffer solution. Between these two solutions a voltage difference exists (see Figure3-1). The charged chemicals can then travel from one side of the capillary to the other. They are detected e.g. in the capillary using florescence or UV detection. When an mass spectrometer needs to be used as detector to reveal the identity of the chemicals, this kind of setup is not possible. An interface that connects the outlet of the capillary to the ionisation chamber (mostly ESI so far) must close the electric circuit by delivering the buffer solution (Figure 3-2). So far, such an interface with a so-called sheath liquid had a flow rate of 5 – 10 µL/min. This was a concern for applying this technique to detect low concentrations in samples, as the sheath liquid dilutes the sample coming from the CE. Consequently, the sensitivity is compromised. Recent advantages in the field of CE, however, have made it possible to couple CE to the MS

without the necessity to use sheath liquids. The result is a significantly improved sensitivity (nanomolar level) while using small sample injection volumes (20 nL).

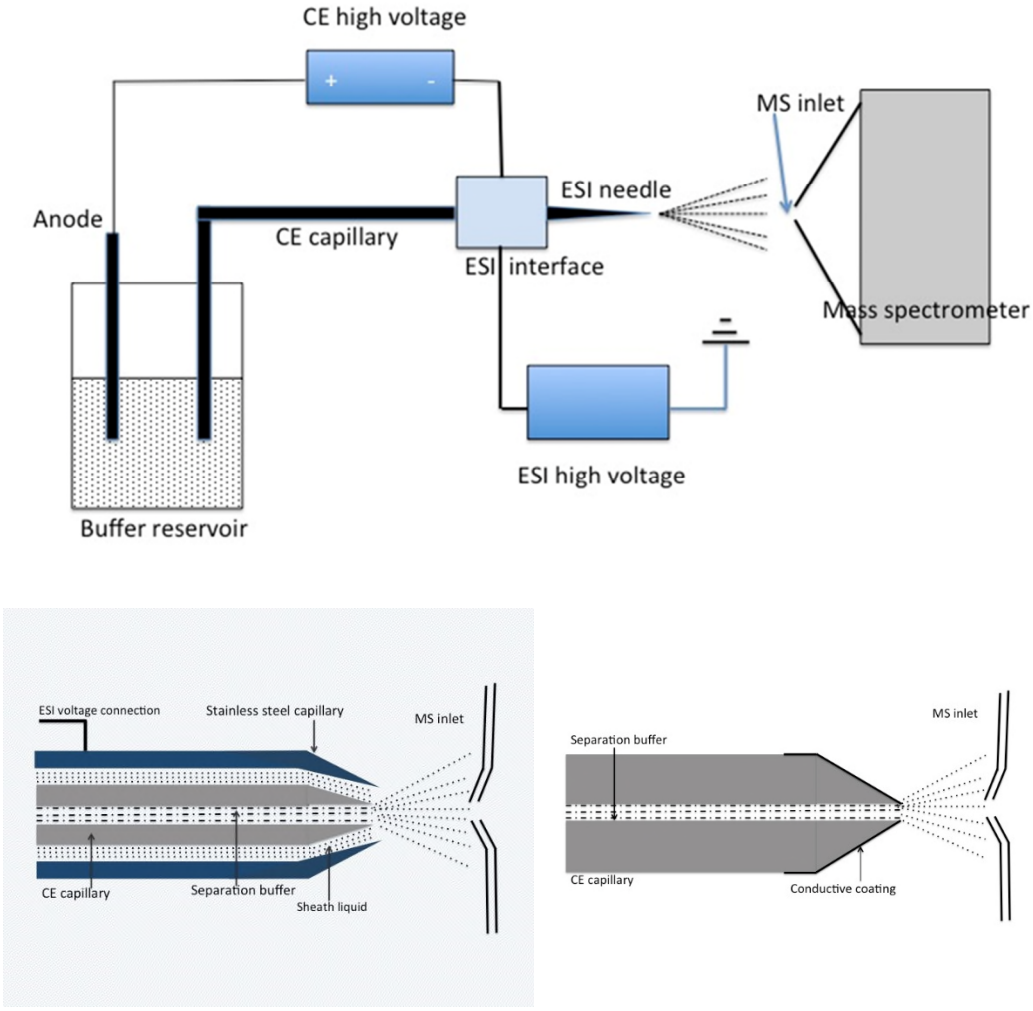

FIGURE 3-2: SCHEMATIC DIAGRAM OF CE-ESI/MS (TOP). SHEATH-LIQUID INTERFACE (BOTTOM LEFT). SHEATHLESS INTERFACE (BOTTOM RIGHT).

As mentioned before, separation by CE is achieved by applying an electrical field between two ends. In the classical set-up this meant that only like-charged ions could be separated. Oppositely charged ions were not separated. To separate them, the polarity has to be reversed. To separate only ions of the same charge, the sample is brought into the capillary by dipping it into the sample vial (Figure 3-1). Next the capillary is moved to the source vial. From then on the chemicals are subjected to an electric field, which triggers the movement towards the appropriate electrode and the detector. A classical system only allows for either cations or anions to be detected in the same run. However, evidently it is possible to inject the same sample again and run the method with opposite electrical field. This type of separation can be accelerated by increasing the flow of the solution in the capillary. Under these conditions one speaks of pressure driven separation, otherwise of electroosmotic flow (EOF)-driven separation.

To separate both types of ions a different set-up is necessary. The scheme in Figure 3-3 shows the representation of two set-ups that allow the separation of anions and cations at

the same time [61]. The left figure shows that if the EOF is high enough, that cations and anions can be separated simultaneously. The mechanism behind this rather contradictory effect is explained by JW Jorgenson and K DeArman Lukacs [62].

In another method the sample is introduced into the capillary twice (Figure 3-3 right) [61]. The capillary is dipped into the vial with the sample, pressure is applied and the first sample rich liquid is allowed to flow towards the other end of the capillary. Then the capillary is dipped into the sample vial a second time and the sample is introduced a second time into the capillary. This sample, however, remains at the entrance of the capillary. The detector is placed in the middle of the capillary. If now an electrical field is applied both cation and anions are moving towards the detector. A prerequisite for this method to work with an MS is, that the detector can switch polarity quickly, meaning it can measure in negative and positive mode in one run.

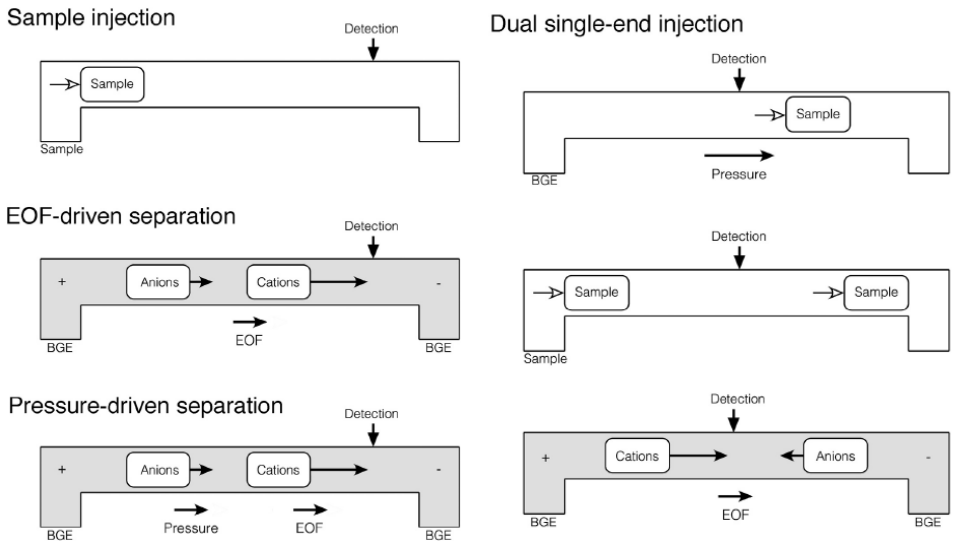

FIGURE 31-3: SINGLE SAMPLE (LEFT) AND DUAL SAMPLE (RIGHT) SEPARATION MODE. BGE = BACK GROUND ELECTROLYTE. EOF = ELECTRO OSMOTIC FLOW. ADOPTED FROM J SÁIZ, IJ KOENKA, TD MAI, PC HAUSER AND C GARCÍA-RUIZ [61].

Alternatively, a set-up using two detectors can be used (Figure 3-4). In this case the sample is introduced into the capillary and moves to the middle. Next, the voltage gradient is provided. The ions move towards the two ends of the capillary and are detected. This is, however, a more expensive approach, certainly when two mass spectrometers have to be installed. Therefore, the one detector solution is desirable.

Apart from these separation methods, there are several other approaches, especially for dual mode detection [61].

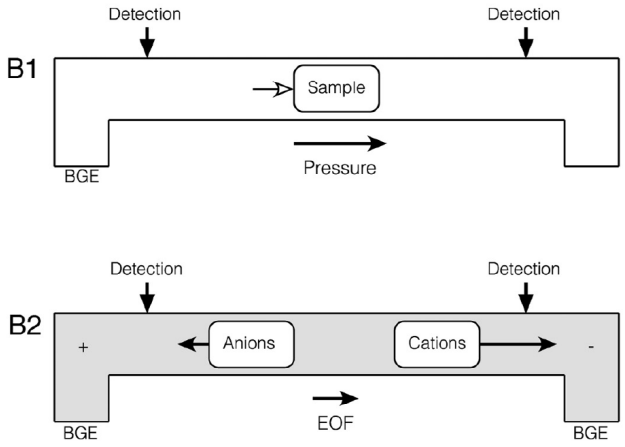

FIGURE 3-4: DUAL ION SEPARATION USING TWO DETECTORS. BGE = BACK GROUND ELECTROLYTE. EOF = ELECTRO OSMOTIC FLOW. ADOPTED FROM J SÁIZ, IJ KOENKA, TD MAI, PC HAUSER AND C GARCÍA-RUIZ [61].

CE allows the separation and detection of charged chemicals. Both anions and cations can be separated and detected simultaneously. Injections volumes can be very low (double-digit nano litres) while maintaining a high sensitivity (nano molar). The disadvantage of this technique is that during a run no pH adjustments can be made. Therefore, chemicals with different pK_as have to be analysed with different methods.

Electrophoresis can be applied *e.g.* in metabolomics [63], peptide analysis [64], arsenic quantification [65], inorganic salts determination [66] and organic compound detection [60].

3.3 Ion chromatography

Ion chromatography is a process that allows the separation of ions and polar molecules based on their charge [67, 68]. It can be used for most charged molecules including micropollutants, large proteins, small nucleotides and amino acids.

Ion-exchange chromatography retains analyte molecules on the column based on coulombic (ionic) interactions. The stationary phase surface displays ionic functional groups that interact with analyte ions of opposite charge. This type of chromatography is further subdivided into cation - and anion exchange chromatography. The basis of the separation is the varying attraction of different ions in a solution to the oppositely charged sites on the stationary phase (the ion exchanger). Aqueous solutions with different salts (e.g. sodium carbonate) and pH changes are used for elution in ion chromatography. This can be done either isocratic or with a gradient. In contrast to RP, NP and HILIC chromatography no organic solvents are needed [68]. The advantage of this method is that charged molecules can easily be separated from water soluble non-charged chemicals. By adjusting the pH of the mobile phase, also chemicals with different pK_a values can be separated. On the other hand, exclusively charged chemicals can be separated.

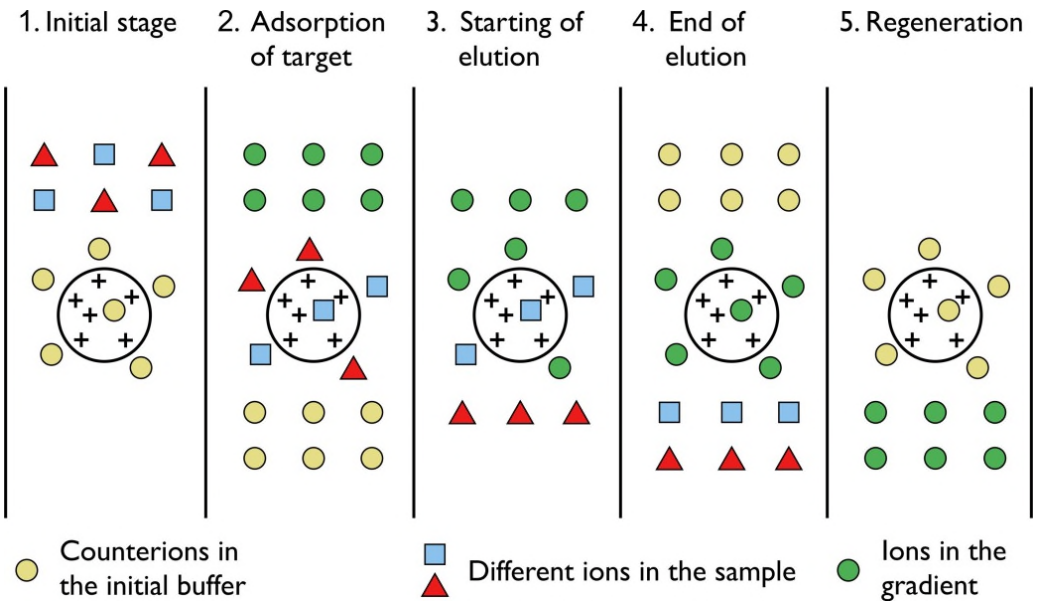

FIGURE 3-5: SEPARATION MECHANISM OF ION CHROMATOGRPAHY.

There are in principle three possibilities to elute the analyte from the column: i) by changing the pH of the mobile phase and neutralising the analyte, by ii) changing the pH of the solution and neutralising the surface charge of the stationary phase and iii) by displacement of the analyte by a different counter ion.

Like CE ion chromatography can only separate charged chemicals. However, the possibility to run a pH-gradient allows to separate chemicals with different pK_as. In case of CE this is not possible, because the pH of the solution cannot be changed during separation.

3.4 HILIC

HILIC is a method that was developed to separate carbohydrates in 1975 [69]. Since then it's field of application has been extended to all kinds of polar organic chemicals [70-72]. The setup of a HILIC system is comparable to RP-chromatography and NP-chromatography, however there are significant differences, which are shown in Table 3-1.

TABLE 3-1: CHARACTERISTICS OF DIFFERENT CHROMATOGRAPHIC SEPARATIONS

Chromatographic method	Stationary phase	Mobile phase	Solvents	Interactions
Reversed phase	Apolar	Polar	H_20/MeCN	Apolar interactions
Normal phase	Polar	Apolar	$CHCl_3$, hexane	Hydrogen bonding
HILIC	Polar	Apolar-polar	H_20, MeCN, MeOH	Partitioning, coulomb forces, hydrogen-bonding, dipole-dipole interaction, van der Waals

Also in case of HILIC a mobile phase is conducted over a solid phase, which is a hydrophilic material and the mobile phase is a mixture of a water-miscible organic solvent and small

amounts of water [72]. In HILIC the analytes partition between two liquid layers, the mobile phase and a stationary aqueous layer close to the polar solid phase. The affinity (partition coefficient) of the analyte for the aqueous phase controls the retention. Apart from partitioning, interaction between the analytes and the solid phase can also play a role once they are in the aqueous layer. Interactions include coulomb forces, hydrogen-bonding, dipole-dipole interaction, and van der Waals interactions. In HILIC chromatography apolar chemicals have hence the tendency to elute first followed by the more polar ones. For the water layer to be formed, the mobile phase needs to contain at least 2-3 % of water. Therefore, HILIC chromatography cannot be performed without water.

3.4.1 Stationary phases

Several types of stationary phases exist and they have a significant influence on the separation quality. Therefore, choosing the correct column is very important for HILIC chromatography. All column materials have in common that they have a polar surface, that allows the formation of a stationary water-layer. These materials can be subdivided into several classes: neutral, charged and zwitterionic.

Amides, diols, and silica belong to the group of neutral phases. Amide columns are predominately employed for the separation of oligosaccharides and peptides. Diol phases are useful for the analysis of proteins, polar metabolites and vitamins. Amines and silica phases (pH > 4-5) are two of the most common charged phases for HILIC chromatography. Amine columns are used in the analysis of proteins and metabolites. Silica is used to separate positively charged chemicals due to the negative charges on its surface at pH >4. Zwitterionic phases contain equal amounts of positively and negatively charged groups on the surface. They can bind water extremely well to the surface and therefore the partitioning between the water-phase and the mobile organic phase is the predominant retention mechanism. They work very well for acids, bases and zwitterionic chemicals.

3.4.2 Mobile phase

The mobile phase consists mainly of a water-miscible organic solvent and at least 2-3% of water. This amount of water is necessary to trigger the formation of the stationary aqueous layer on the surface of the column material. If this layer is absent, direct interaction between analytes and the surface takes place. The consequence is a completely different separation pattern, if separation can be achieved at all. Another prerequisite is that the organic solvent does not show any hydrogen acceptor or donor abilities or at least keeps it at a minimum, because these organic solvents would compete with the water molecules for the stationary phase but also with the analytes. As a consequence chemicals elute faster. The elution strength for some frequently used HILIC solvents is as follows:

acetone < acetonitrile < tetrahydrofuran < isopropanol < ethanol < methanol < water

Effects of salts

The salts (e.g. ammonium acetate or bicarbonate) are added to control electrostatic interactions between charged analytes and the stationary phase. An increase in salt concentration results in a decreased interaction between charged analytes and charged columns. In case of electrostatic attraction this will in turn reduce retention time, in case of electrostatic repulsion this will increase retention time.

pH of the mobile phase

A change of pH will affect the analytes in the mobile phase. The pH will control the charge of these chemicals. Therefore, the pH is adjusted to bring chemicals of interest in their charged state. The column is in most cases not effected by the pH, with the exception of silica

columns. At a low pH (< 4) they lose their negative charge. This will alter the separation mechanism significantly and hence retention times.

3.5 Mixed-mode liquid chromatography

In mixed-mode liquid chromatography (MMLC) more than one interaction takes place with analytes and the stationary phase, which allows the determination of analytes with different physicochemical properties in one run (e.g. ionic, basic, acid and neutral chemicals). The secondary interaction should substantially contribute to the retention of the analytes, and should not be too weak. Often MMLC combines polar interactions (e.g. via RP or HILIC) with ion exchange mechanisms. Currently, mixed-mode columns can be purchased from different manufactures, either with anionic or cationic exchange capabilities, or, state-of-the-art columns in which both types of ion exchanges mechanisms are included [73, 74].

Vughs *et al.* report a target method metformin and melamine using the ODS-CX15 mixed-mode column, comprising of RP and cation-exchange, but this method was not applicable for screening purposes [14]. Montes *et al.* successfully applied a trifunctional mixed-mode column, combining RP, anion and cation exchange, coupled to high resolution mass spectrometry for a suspect screening of polar compounds in real water samples. 22 compounds in concentrations from 6 ng/L to 540 µg/L were identified in the water samples [75].

3.6 Super critical fluid chromatography

Supercritical fluid chromatography (SCF) with normal phase columns and a supercritical fluid such as carbon dioxide as the mobile phase has recently gained attention due to its potential to analyse polar compounds. Its suitability remains to be proven. Bieber *et al.* successfully applied SCF combined with mass spectrometry for screening of polar compounds in wastewater treatment plant effluent samples [76]. They showed that this technique was able to extend the polarity range compared with the analysis of the same sample in reversed phase chromatography.

3.7 Conclusions

To fill the analytical gap in PMOC analysis, we aimed to develop a method that is able to (i) separate as much as possible from the chemical space from the highly polar compounds (i.e. strong acids, neutral, strong basic, zwitter ionic, and. ionogenic compounds) and (ii) to hyphenate the separation technique to high resolution mass spectrometry to be able to perform suspect and/or non-target screening. Therefore the techniques described in this chapter are evaluated for both purposes in Table 3-2.

TABLE 3-2: TECHNIQUES FOR THE ANALYSIS OF HIGHLY POLAR SUBSTANCES

Analytical technique	Polarity range		Possibility hyphenation to mass spectrometry	Non-target screening possibility for the whole PMOC range	Availability at KWR
	charged	neutral			
CE	+	-	+/-	-	-
IC	+	-	+/-	-	-
HILIC	+	+	+	+	+
MMLC	+	+	+	+	+
SFC	+	+	+	?	-

+:possible, -: not possible, +/-: possible but not straight forward, ?: remains to be proven

We wanted to cover an as large as possible polarity range in one method. Therefore, capillary electrophoresis and ion chromatography were discarded as solely charged compounds can be separated, making them less attractive for a generic screening method for highly polar substances. SCF has only recently been applied for highly polar substances and its suitability needs to be proven. The technique is not available at KWR, ruling out this possibility.

For this research project we chose to focus on HILIC for the development of a generic approach to screen for highly polar compounds in water, because of prior experience with this technique and the good hyphenation possibilities, i.e. the high organic solvent content in the mobile phase facilitates ionisation. Chapter 4 describes the developed HILIC screening method.

4 Development of a non-target screening method for highly polar substances

In this chapter, a HILIC-MS method, combining target analysis and suspect and non-target screening, for highly polar substances in water samples is described. In addition, the performance characteristics of the method in surface water and in drinking water are depicted.

4.1 Rationale behind method improvement

The method is based on the HILIC non-target screening described in the BTO report 2015.076 [14]. Here it was recommended to further improve the method to obtain lower detection limits for the highly polar substances, and to get more insight in which part of the highly polar substances can be analysed with this screening method. The rationale behind reaching lower detection limits was to determine (at least) a level of 1 µg/L in order to be able to meet the level stated in the Drinking water Directive for "other anthropogenic substances" (in Dutch "overige antropogene stoffen") [77].

The improvement of the HILIC screening was realized in the current project by:

- increasing the number of target compounds in the method from 22 to 38,
- using a more sensitive high resolution mass spectrometer, i.e. an Orbitrap Fusion mass spectrometer instead of a QToF mass spectrometer for increased screening capabilities, elucidation of unknowns and increased sensitivity,
- development of a pre-treatment approach for surface and drinking water samples to improve sensitivity.

4.2 Materials and Methods

4.2.1 Chemicals

All solvents used were of analytical grade quality. Acetonitrile and methanol (ultra-gradient HPLC grade) were obtained from Avantor Performance Materials B.V. (Deventer, the Netherlands). Formic acid (HPLC quality) was purchased from Sigma-Aldrich (Steinheim, Germany). Ultrapure water was obtained by purifying demineralized water in an Elga Purelab Chorus ultrapure water system. (High Wycombe, United Kingdom). Reference standards were obtained from Toronto Research Chemicals (Toronto, Canada) and Sigma Aldrich (Zwijndrecht, the Netherlands)

4.2.2 Reference standard solutions

In Table 4-1, the compound name, the CAS number, formula, accurate mass of the protonated molecule ($[M+H]^+$) or deprotonated molecule ($[M-H]^-$), are shown for the reference standards. Stock solutions of the HILIC reference standards and internal standards (chlormequat-d9 and sotalol-d7) were prepared at a concentration of ~100 mg/L in acetonitrile. The internal standards were used (i) to check the injection of the sample and (ii) to study matrix effects for the target analysis and for semi- quantification (internal standard equivalents/L) in case of an suspect and unknown screening.

TABLE 4-1: OVERVIEW OF LC-MS RELATED PROPERTIES OF THE REFERENCE COMPOUNDS

Compound name	Ionisation mode	Accurate mass	Retention time (min)	Molecular ion
5-Fluorocystosine	pos	130.04112	5.13	$[M+H]^+$
Acephate	pos	184.01918	2.10	$[M+H]^+$
Ammelide	pos	129.04070	3.66	$[M+H]^+$
Ammeline	pos	128.05669	7.01	$[M+H]^+$
Amprolium	pos	243.16042	10.70	$[M]^+$
Chlormequat	pos	122.07310	8.80	$[M]^+$
Cotinine	pos	177.10224	4.32	$[M+H]^+$
Cyromazin	pos	167.10397	5.71	$[M+H]^+$
Cytarabine	pos	244.09280	6.56	$[M+H]^+$
Cytosine	pos	112.05054	6.94	$[M+H]^+$
Diatrizoic acid	pos	614.77690	7.10	$[M+H]^+$
Gabapentine	pos	172.13321	8.81	$[M+H]^+$
Gemcitabine	pos	264.07904	3.08	$[M+H]^+$
Guanylurea	pos	103.06144	7.30	$[M+H]^+$
Iohexol	pos	821.88761	7.10	$[M+H]^+$
Iopamidol	pos	777.86140	4.70	$[M+H]^+$
Iopromide	pos	791.87705	4.75	$[M+H]^+$
Maleic hydrazide	pos	113.03455	2.66	$[M+H]^+$
Melam	pos	236.11152	8.19	$[M+H]^+$
Melamine	pos	127.07267	6.38	$[M+H]^+$
Melem	pos	219.08497	6.16	$[M+H]^+$
Mepiquat	pos	114.12773	10.05	[M]+
Metformin	pos	130.10872	8.60	$[M+H]^+$
Methamidophos	pos	142.00862	2.13	$[M+H]^+$
Niacin	pos	124.03930	5.67	$[M+H]^+$
N-Methyldiethanolamine	pos	120.10191	9.50	$[M+H]^+$
Omethoate	pos	214.02974	2.17	$[M+H]^+$
Phenylalanine	pos	166.08626	8.60	$[M+H]^+$
Tetrapropylammonium (TPA)	pos	186.22218	7.19	$[M+H]^+$
Tryptophane	pos	205.09715	8.36	$[M+H]^+$
Tyrosine	pos	182.08117	8.87	$[M+H]^+$
Urotropine	pos	141.11347	11.30	$[M+H]^+$
5-Fluorouracil	neg	129.01058	1.81	$[M-H]^-$
Cyanuric acid	neg	128.01016	1.77	$[M-H]^-$
Dichloroacetic acid	neg	126.93591	2.10	$[M-H]^-$
Ethyl sulphate	neg	124.99085	1.37	$[M-H]^-$
Naphthalene-1,5-disulfonic acid	neg	286.96896	1.69	$[M-H]^-$
Trifluoromethanesulfonic acid	neg	148.95257	1.37	$[M-H]^-$
Internal standards				
Chlormequat-d9	pos	131.12960		$[M+H]^+$
Sotalol-d8	pos	279.16240		$[M+H]^+$
	neg	278.15612		$[M-H]^-$
Did not make in in the final method				
Diethanolamine	pos	106.08626		$[M+H]^+$
Triethanolamine	pos	150.11247		$[M+H]^+$
Diquat	pos	184.09950		$[M]^+$
Paraquat	pos	186.11515		$[M]^+$
Perchlorate	neg	98.94851		$[M]^+$

Sometimes it was necessary to add water to the stock solutions to improve the solubility. Working solutions were prepared in ultrapure water and acetonitrile (5:95%; v/v). Stock solutions were stored at a temperature of -20 ˚C. Working solutions were stored at 7˚C for a maximum of 1 week.

4.2.3 Water samples

Tap water was obtained from the town of Nieuwegein (The Netherlands). Surface water samples were taken from the Lekkanaal at Nieuwegein, which is connected to the River Rhine, in a stainless steel container that had previously been thoroughly washed and rinsed. These surface water samples were stored at 4 ˚C in the dark for a maximum of 1 week. For non-target screening a blank sample, consisting of 1 L of ultra-pure water in the sample bottle, was processed as using the same protocol as the water sample from the sampling campaign.

4.2.4 Sample preparation

Five mL water was transferred to a glass tube. The sample was evaporated to 250 µL at a temperature of 300 ˚C using an automated blow-down apparatus (Barkey, Germany). Next, 50 µL internal standard solution, containing 100 µg/L chlormequat-d9 and sotalol-d7, and 4.7 mL acetonitrile were added the sample, resulting in a final concentration of internal standards of 1 µg/L in 95/5 (v/v) acetonitrile/water. Samples were filtered using a 0.2 µm filter (Phenomenex) and transferred to an autosampler file prior to LC-MS analysis.

4.2.5 LC-MS conditions

A Tribrid Orbitrap Fusion mass spectrometer (ThermoFisher Scientific, Bremen, Germany) provided with an electrospray ionisation source was interfaced to a Vanquish HPLC system (ThermoFisher Scientific, Bremen, Germany). With every batch run mass calibration was performed using Pierce ESI positive and negative ion calibration solution. The vaporizer and capillary temperature were maintained at 350 and 300 ˚C, respectively. Sheath, auxiliary and sweep gases were set to arbitrary units of 45, 5 and 5. The source voltage was set to 3.0 kV in the positive mode, and -2.5kV the negative mode respectively. The RF lens was set to 50 %. Full scan high accuracy mass spectra was acquired in the range of 80 –1300 m/z with the resolution set at 120,000 FWHM and quadruple isolation was used for acquisition with a 5 ppm mass window. Data dependent acquisition was performed using a High Collision Dissociation (HCD) energy at 35% and an FT resolution of 15,000 FWHM.

For the chromatographic separation an Agilent Zorbax Hilic plus (150 mm × 2.1 mm I.D., particle size 1.8 µm) (Agilent) preceded by a krudkatcher ULTRA HPLC In-line Filter, 0.5 µm was used. The column temperature was maintained at 25 ˚C. Eluent A consisted of 95% ultra-pure water and 5% acetonitrile (v/v) with 5 mM ammonium formate at a pH of 3. Eluent B consisted of 95% acetonitrile and 5% ultra-pure water (v/v) with 5 mM ammonium formate at a pH of 3. The gradient started linear from 100% B to 90% B in 4 min. Next, from 90% B to 20% B in 11 minutes, and the %B stayed at 20% for 6 minutes. The %B was increased to 100 in 1 minute, and the column was equilibrated at 100% B for 8 min. The flow rate was 0.3 mL/min and 100 µL of sample was injection onto the LC column.

4.2.6 Data analysis

Target analysis

Data processing for target analysis was performed using Xcalibur version 2.2. Identification of the compounds was performed by comparing the accurate mass of the of the molecular ion (see Table 4-1), two accurate MS2 fragment ions, and the retention time of the signals of a target compound in the matrix to those obtained by the standard reference solutions.

Suspect and non-target screening

Data analysis for suspect and non-target screening was performed using Compound Discoverer 2.1 (Thermo Fisher) for peak picking, componentization, chlorine pattern scoring, suspect screening (using the target list of 38 target compounds, and Chemspider) and automatic MS2 fragment searches in mzCloud. An overview of the Compound Discoverer workflow and the data processing parameters is provided in Annex I and II, respectively.

4.2.7 Validation

The LOD of the whole method was determined by spiking reference standards in drinking water and in surface water at concentrations of 0.01, 0.05, 0.2, 1 and 5 µg/L. This was done four times per matrix, and samples were analysed in duplicate (eight measurements). The LOD is defined by using the standard deviation of the repeatability for the lowest concentration that was detected, and a taking into account a confidence interval of 99% with one-side probability. The limit of quantitation (LOQ) for each compound was then determined by using the LOD multiplied by 3. Repeatability and recovery were determined at a spiked concentration of 1 µg/L or 5 µg/L.

4.3 Results and discussion

4.3.1 Optimization of non-target screening method for highly polar compounds

The HILIC non-target screening method described in the BTO report 2015.076 [14] was used as basis. First, the scope of the method was enlarged by adding polar components to the method, in total 38 compounds can now be analysed in comparison with 22 in the previous method. For compounds which are ionised in positive ionisation mode, retention times (see table 4-1) are distributed evenly throughout the LC gradient, i.e. ranging from 1.81 till 11.3 min. The retention for compounds measured in negative ionisation mode on this HILIC column is less pronounced. They all elute at very early, i.e. between 1.37 min and 2.10 min. The high resolution of the mass spectrometer makes it possible to distinguish highly polar compounds within this tight time window, but it is not ideal for screening purposes. Second, the Orbitrap Fusion mass spectrometer was applied, instead of a QToF mass spectrometer, which allows ultra-high resolution mass spectrometry, i.e. a maximum of 450,000 (FWHM) at m/z 200, that can be used for an improved screening and structure elucidation of unknown compounds. Third, a pre-treatment approach for surface and drinking water samples was developed, in order to inject more sample onto the HILIC column, and subsequently improve sensitivity. The pre-treatment method comprised of evaporation of the water sample, and subsequently reconstitution of the sample in a solution containing high organic solvent concentration. The water sample is not concentrated by this procedure, but the composition of the sample changes from 100% water, to a high concentration organic solvent, i.e. 95% acetonitril and 5% water (v/v). In total 100 µl of this sample is injected onto the column, i.e. 100 µl of the original sample. This volume is 10 times higher compared with our previous HILC non-target screening method as described in BTO report 2015.076 [14], in which 10 µl of water sample could be injected. It is a time consuming step, but one that shows promising results. Other non-discriminating methods, like freeze drying [75] and or two-stage SPE procedures [76] have been described for the sample preparation for determination of highly polar compounds and are also worth exploring.

4.3.2 Method performance

The method performance of the whole analytical method was determined in drinking and surface water. The limit of detection (LOD), limit of quantification (LOQ), repeatability (RSD) and recovery were determined for 38 compounds in both matrices. The validation results are shown in Table 4-2 for drinking water and Table 4-3 for surface water.

The validation results for the HILIC method for highly polar chemicals in drinking and surface water are satisfactory. The LOQs of these 38 compounds range from 0.01-2.0 µg/L with an average of 0.2 µg/L for drinking water. For surface water the values are slightly higher, i.e. the LOQs vary from 0.01-5.0 µg/L with an average of 0.6 µg/L. The reproducibility for all compounds, except for maleic hydrazide in drinking water, are lower than 20%. The recoveries are on average 99.4% and 89.7% for drinking and surface water, respectively. Three compounds in drinking water and seven in surface water fall outside the recovery range of 75-125%, which is a generally accepted range for recovery.

TABLE 4-2: METHOD PERFORMANCE CHARACTERISTICS FOR HILIC SCREENING METHOD IN DRINKING WATER

Compound	LOD (µg/L)	LOQ (µg/L)	Repeatability @ 1 µg/L (%) (n=8)	Recovery @ 1 µg/L (%) (n=8)
5-Fluorocystosine	0.003	0.01	7.2	102.9
Acephate	0.197	0.5	6.8	93.1
Ammelide	0.028	0.1	14.1	58.7
Ammeline	0.012	0.05	7.7	97.1
Amprolium	0.018	0.05	11.8	85.3
Chlormequat	0.005	0.02	8.5	102.7
Cotinine	0.003	0.01	7.0	109.4
Cyromazin	0.004	0.01	3.2	107.5
Cytarabine	0.024	0.05	9.4	93.0
Cytosine	0.01	0.05	7.4	105.9
Diatrizoic acid	0.087	0.2	13.8	114.5
Gabapentine	0.01	0.05	6.0	99.4
Gemcitabine	0.003	0.01	7.1	108.7
Guanylurea	0.022	0.05	6.0	85.4
Iohexol	0.243	0.5	7.7	104.5
Iopamidol	0.082	0.2	7.0	88.5
Iopromide	0.046	0.2	9.4	102.2
Maleic hydrazide	0.134	0.5	27.0	16.2
Melam	0.002	0.01	13.1	101.6
Melamine	0.191	0.5	4.7	118.5
Melem	0.023	0.05	10.2	97.4
Mepiquat	0.006	0.02	9.6	116.8
Metformin	0.004	0.05	8.4	104.0
Methamidophos	0.072	0.2	7.5	114.2
Niacin	0.009	0.03	6.5	101.8
N-Methyldiethanolamine	0.027	0.05	9.7	95.2
Omethoate	0.013	0.05	8.4	91.9
Phenylalanine	0.047	0.2	8.7	94.8
Tetrapropylammonium (TPA)	0.006	0.02	10.1	100.9
Tryptophane	0.179	0.5	5.4	101.2
Tyrosine	0.458	1	15.4	98.2
Urotropine	0.04	0.1	11.0	116.8
5-Fluorouracil	0.008	0.05	4.0	110.7
Cyanuric acid	0.051	0.2	12.8	109.7
Dichloroacetic acid	0.028	0.1	5.4	84.7
Ethyl sulphate	0.017	0.05	6.8	137.9
Naphthalene-1,5-disulfonic acid	0.138	0.2	16.9	104.1
Triflic acid	0.527	2	5.0*	101.4*

**: Repeatability and recovery for this compound were determined at a concentration of 5 µg/L.*

TABLE 4-3: METHOD PERFORMANCE CHARACTERISTICS FOR HILIC SCREENING METHOD IN SURFACE WATER

Compound	LOD (µg/L)	LOQ (µg/L)	Repeatability @ 1 µg/L (%)	Recovery @ 1 µg/L (%)
5-Fluorocystosine	0.002	0.01	3.8	84.5
Acephate	0.158	0.50	8.2	62.1
Ammelide	0.009	0.10	11.0	14.5
Ammeline	0.019	0.05	5.3	114.6
Amprolium	0.084	0.20	3.9	89.0
Chlormequat	0.005	0.02	9.7	105.1
Cotinine	0.003	0.01	10.9	77.1
Cyromazin	0.002	0.01	3.2	107.8
Cytarabine	0.088	0.20	5.3	107.7
Cytosine	0.020	0.05	3.6	100.2
Diatrizoic acid	0.093	0.20	8.9	99.7
Gabapentine	0.087	0.20	7.8	97.2
Gemcitabine	0.007	0.10	5.6	22.1
Guanylurea	0.116	0.50	2.9	112.0
Iohexol	0.204	0.50	6.3	101.2
Iopamidol	0.110	0.20	9.4	92.6
Iopromide	0.110	0.20	8.8	88.1
Maleic hydrazide	0.071	0.50	6.0	38.0
Melam	0.003	0.01	8.4	90.6
Melamine	0.187	0.50	3.0	96.4
Melem	0.020	0.05	15.6	97.8
Mepiquat	0.007	0.02	7.3	92.5
Metformin	0.237	0.50	7.5	92.8
Methamidophos	0.098	0.20	8.0	99.0
Niacin	0.006	0.03	6.9	104.2
N-Methyldiethanolamine	0.020	0.05	8.0	97.9
Omethoate	0.010	0.05	8.3	101.4
Phenylalanine	0.885	5.0	4.9*	108.0*
Tetrapropylammonium (TPA)	0.008	0.02	2.2	107.4
Tryptophane	1.295	5.0	9.9*	79.7*
Tyrosine	1.607	5.0	9.8*	88.2*
Urotropine	0.449	1.0	3.4	141.8
5-Fluorouracil	0.024	0.05	6.3	108.4
Cyanuric acid	0.014	0.05	7.6	94.9
Dichloroacetic acid	0.093	0.20	9.8	86.9
Ethyl sulphate	0.036	0.10	4.4	70.0
Naphthalene-1,5-disulfonic acid	0.037	0.20	6.1	37.9
Triflic acid	0.376	2.0	18.6*	97.4*

**: Repeatability and recovery for this compound were determined at a concentration of 5 µg/L.*

4.3.3 Comparison 2015 and 2018 method

Table 4-4 shows a comparison of the HILIC non-target method developed in 2015 and the one developed and evaluated in the present study. The limits of quantification (LOQ) of both methods are depicted in the table, as well as the change in sensitivity. There is an overlap of 20 compounds in both methods. For 16 compounds the LOQ changed (factor 1.25 till 100), for 3 compounds the LOQ did not change, and for 1 compound, namely phenylalanine, the

sensitivity was reduced by a factor of 5 in the method developed within this study(2018.022). Overall, the sensitivity of the method has improved.

TABLE 4-4: COMPARISION OF LIMIT OF QUANTIFICATION (SURFACE WATER) BETWEEN THE HILIC SCREENING FROM 2015 AND 2018.

Compound name	Ionisation mode	LOQ (µg/L) 2015.076	LOQ (µg/L) 2018.022	Change in sensitivity* (2018/2015)
5-Fluorocystosine	pos		0.01	
Acephate	pos	0.5	0.50	1
Ammelide	pos		0.10	
Ammeline	pos		0.05	
Amprolium	pos	5	0.20	25
Chlormequat	pos	0.25	0.02	12.5
Cotinine	pos		0.01	
Cyromazin	pos		0.01	
Cytarabine	pos	0.25	0.20	1.25
Cytosine	pos	0.1	0.05	2
Diatrizoic acid	pos		0.20	
Diethanolamine	pos	1.0		
Gabapentine	pos	0.5	0.20	2.5
Gemcitabine	pos	0.1	0.10	1
Guanylurea	pos	1	0.50	2
Iohexol	pos	1	0.50	2
Iopamidol	pos	0.5	0.20	2.5
Iopromide	pos		0.20	
Maleic hydrazide	pos		0.50	
Melam	pos		0.01	
Melamine	pos		0.50	
Melem	pos		0.05	
Mepiquat	pos	0.25	0.02	12.5
Metformin	pos	1	0.50	2
Methamidophos	pos	0.5	0.20	2.5
Niacin	pos		0.03	
N-Methyldiethanolamine	pos	0.25	0.05	5
Omethoate	pos	0.1	0.05	2
Phenylalanine	pos	1.0	5.0	0.2
Tetrapropylammonium (TPA)	pos		0.02	
Triethanolamine	pos	1.0		
Tryptophane	pos#	10	5.0	2
Tyrosine	pos#	10	5.0	2
Urotropine	pos	1	1.0	1
5-Fluorouracil	neg	5	0.05	100
Cyanuric acid	neg		0.05	
Dichloroacetic acid	neg		0.20	
Ethyl sulphate	neg		0.10	
Naphthalene-1,5-disulfonic acid	neg		0.20	
Triflic acid	neg		2.0	

**: Change in sensitivity, >1 means sensitivity has improved in the 2018 method, <1means sensitivity has declined in the 2018 method, =1, sensitivity stayed the same*

#: this compound was detected in negative ionisation mode in the 2015 method

4.4 Conclusion

The validation results show that the newly developed method for the highly polar compounds in drinking - and surface water, based on sample evaporation and reconstituting in organic solvent, followed by HILIC coupled to high resolution mass spectrometry, has significantly better performance characteristics compared to the 2015 method. The non-target screening method allows to screen for highly polar compounds at a level below 1 µg/L for in drinking water and in sources for drinking water.

5 Discussion, conclusion and recommendations

5.1 Discussion and conclusion

Terminology for mobile and polar substances is often used without a clear definition. Environmental analytical chemistry historically focuses on (very) a-polar or hydrophobic substances, so substances that are less hydrophobic are easily considered polar. The challenge for environmental analysis of (highly) polar substances is determined by the incapability of common gas chromatographic and reversed phase liquid chromatographic techniques to enable a proper separation-and therefore detection of these substances. This is why the term highly polar substances was used throughout this report. In order to provide a clear classification framework, the classification made by Reemtsma e*t al.* was adopted [1], and methods were developed for substances that are poorly detected by commonly applied gas chromatographic and reversed phase liquid chromatographic separation techniques. These are generally substances with high solubility (in gram per litre range or higher) and low log K_{OW} (below ~2, and especially negative log K_{OW} values). A list of relevant substance classes with polar groups was made and from that list candidates were selected for method development and validation. Substances with an array of chemical structures were selected in order to provide a sound basis for method development and validation, as the method developed is intended to be used for non-target screening purposes. Non-target screening requires a generic method that is able to detect a wide array of substances with various active groups and properties. Thereby the method developed enables to cover a wide array of highly polar substances and complements existing non-target screening methods developed for less polar substances using reversed phase liquid chromatography [78]. Together these techniques are able to cover a wide range of polarities of substances. This allows better characterization of the chemical water quality considering organic contaminants, making the non-target screening method for highly polar substances developed in this study combined with non-target screening with currently applied non-target screening methods for lesser polar substances a valuable combination of tools in water quality assessment.

A HILIC non-target screening approach including a pre-treatment was developed and validated for the determination of highly polar compounds in surface water and in drinking water. 38 compounds can be determined with this method. An Orbitrap Fusion mass spectrometer was applied as detector, allowing ultra-high resolution mass spectrometry, i.e. a maximum of 450,000 (FWHM) at m/z 200, which can be used for an improved non-target screening and structure elucidation of unknown compounds. The non-target screening method developed allows to screen for highly polar compounds at a level below 1 µg/L for in drinking water and in sources for drinking water.

It is very challenging to develop a single method that covers the whole chemical space of highly polar compounds, from strong acidic, to neutral and strong basic compound and also including amphoteric and ionic compounds (e.g. quaternary amines). For compounds measured in negative ionisation mode, e.g. strong acids like cyanuric acid, naphthalene-1,5-disulfonic acid and triflic acid, the HILIC non-target screening method is not optimal because those compounds show limited retention on the column used. For those compounds it is therefore strongly advised to explore other separation options, for example other HILIC

columns, different separation conditions, mixed-mode chromatography columns and WAX columns (weak anion exchange). Also it is advised to explore more possibilities for sample pre-treatment/concentration for highly polar compounds. It can be envisioned that two methods are needed to cover the whole space of highly polar organic chemicals.

The HILIC non-target screening method developed is complementary to other non-target screening methods, like GC-MS screening, and LC MS screening with reverse phase columns. The limit of quantification for most compounds was far below 1 µg/L, making the method suitable to analyse the parameter "*overige antropogene stoffen"* as named in het Drinkwaterbesluit (2011)". The method developed was successfully applied in a monitoring study applied to samples from (sources of) drinking water of the Dutch drinking water companies and the Flemish de Watergroep. The results of the monitoring study are described in the BTO report 2018.023 [79].

5.2 Recommendations

We recommend to extend "*the hunt*" for polar compounds by:

- applying this screening method to determine (and identify) "novel" highly polar compounds in the water cycle,
- applying this screening method in the water cycle to obtain insight in the presence and quantity of highly polar compounds in various compartments of the water cycle
- applying the method to study the behaviour of highly polar substances in the environment and during water treatment
- developing a non-target screening method for compounds that are analysed in negative ionisation mode, i.e. acidic compound (e.g. TFA, F3-MSA, halogenated sulphonic acids),
- getting more knowledge on the toxicological effects of highly polar compounds and on mixture toxicity by combining screening with effect-based tests such as bioassays

6 Literature

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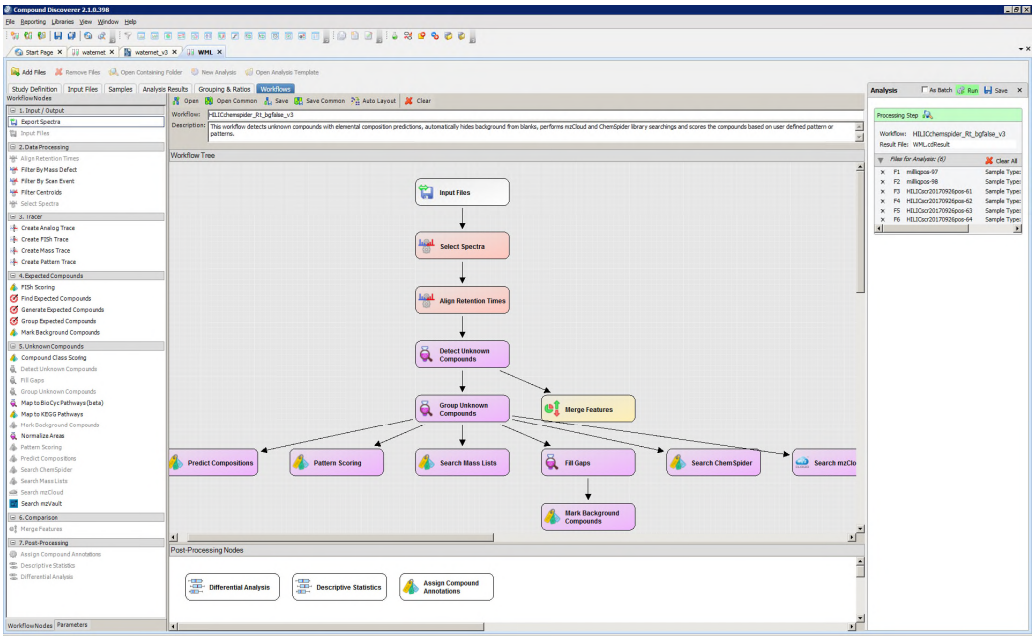
Annex 1 Compound Discoverer 2.1 workflow overview



Annex 2 Summary of data processing parameters and Compound Discoverer 2.1 settings

Processing node 1: Select Spectra

---

1. General Settings:
- Precursor Selection: Use MS(n - 1) Precursor
- Use New Precursor Reevaluation: True
- Use Isotope Pattern in Precursor Reevaluation: True
- Store Chromatograms: False

2. Spectrum Properties Filter:
- Lower RT Limit: 2.5
- Upper RT Limit: 0
- First Scan: 0
- Last Scan: 0
- Ignore Specified Scans: (not specified)
- Lowest Charge State: 0
- Highest Charge State: 0
- Min. Precursor Mass: 80 Da
- Max. Precursor Mass: 5000 Da
- Total Intensity Threshold: 0
- Minimum Peak Count: 1

3. Scan Event Filters:
- Mass Analyzer: (not specified)
- MS Order: Any
- Activation Type: (not specified)
- Min. Collision Energy: 0
- Max. Collision Energy: 1000
- Scan Type: Any
- Polarity Mode: (not specified)

4. Peak Filters:
- S/N Threshold (FT-only): 1.5

5. Replacements for Unrecognized Properties:
- Unrecognized Charge Replacements: 1
- Unrecognized Mass Analyzer Replacements: ITMS
- Unrecognized MS Order Replacements: MS2
- Unrecognized Activation Type Replacements: CID
- Unrecognized Polarity Replacements: +
- Unrecognized MS Resolution@200 Replacements: 60000
- Unrecognized MSn Resolution@200 Replacements: 30000

Processing node 2: Align Retention Times

---

1. General Settings:
- Alignment Model: Adaptive curve
- Alignment Fallback: Use Linear Model
- Maximum Shift [min]: 0.5
- Shift Reference File: True
- Mass Tolerance: 5 ppm
- Remove Outlier: True

Processing node 3: Detect Unknown Compounds
---
1. General Settings:
- Mass Tolerance [ppm]: 5 ppm
- Intensity Tolerance [%]: 30
- S/N Threshold: 10
- Min. Peak Intensity: 1000000
- Ions:
    [2M+H]+1
    [M+2H]+2
    [M+ACN+H]+1
    [M+H]+1
    [M+H-H2O]+1
    [M+K]+1
    [M+Na]+1
    [M+NH4]+1
- Base Ions: [M+H]+1; [M-H]-1
- Min. Element Counts: C H
- Max. Element Counts: C90 H190 Br3 Cl4 K2 N10 Na2 O15 P2 S5

2. Peak Detection:
- Filter Peaks: True
- Max. Peak Width [min]: 0.8
- Remove Singlets: True
- Min. # Scans per Peak: 5
- Min. # Isotopes: 1

Processing node 4: Group Unknown Compounds
---
1. Compound Consolidation:
- Mass Tolerance: 5 ppm
- RT Tolerance [min]: 0.1

2. Fragment Data Selection:
- Preferred Ions: [M+H]+1; [M-H]-1

Processing node 7: Fill Gaps
---
1. General Settings:
- Mass Tolerance: 5 ppm
- S/N Threshold: 1.5
- Use Real Peak Detection: True

Processing node 5: Mark Background Compounds
---
1. General Settings:
- Max. Sample/Blank: 5
- Max. Blank/Sample: 0
- Hide Background: False

Processing node 20: Search mzCloud
---
1. Search Settings:
- Compound Classes: All
- Match Ion Activation Type: True
- Match Ion Activation Energy: Match with Tolerance
- Ion Activation Energy Tolerance: 20
- Apply Intensity Threshold: True
- Precursor Mass Tolerance: 10 ppm
- FT Fragment Mass Tolerance: 0.0025 Da
- IT Fragment Mass Tolerance: 0.4 Da
- Identity Search: HighChem HighRes
- Similarity Search: None
- Library: Reference
- Post Processing: Recalibrated
- Match Factor Threshold: 20
- Max. # Results: 10

Processing node 21: Pattern Scoring
---
1. General Settings:
- Isotope Patterns: Cl; Cl2; Br; Br2; Cl3
- Mass Tolerance: 5 ppm
- Intensity Tolerance [%]: 30
- SN Threshold: 10
- Min. Spectral Fit [%]: 0

Processing node 6: Predict Compositions
---
1. Prediction Settings:
- Mass Tolerance: 5 ppm
- Min. Element Counts: C H
- Max. Element Counts: C90 H190 Br3 Cl4 N10 O15 P2 S5
- Min. RDBE: -1
- Max. RDBE: 40
- Min. H/C: 0.1
- Max. H/C: 3
- Max. # Candidates: 10
- Max. # Internal Candidates: 200

2. Pattern Matching:
- Intensity Tolerance [%]: 30
- Intensity Threshold [%]: 0.1
- S/N Threshold: 3
- Min. Spectral Fit [%]: 10

- Min. Pattern Cov. [%]: 90
- Use Dynamic Recalibration: True

3. Fragments Matching:
- Use Fragments Matching: True
- Mass Tolerance: 5 ppm
- S/N Threshold: 3

Processing node 23: Search Mass Lists
---
1. Search Settings:
- Input file(s): hilic.csv;extracompc18.csv
- Mass Tolerance: 5 ppm
- Show extra Fields as Columns: False
- Consider Retention Time: True
- RT Tolerance : 0.05

Processing node 26: Search ChemSpider
---
1. Search Settings:
- Mass Tolerance: 5 ppm
- Database(s): KEGG
- Max. # of results per compound: 100
- Max. # of Predicted Compositions to be searched per Compound: 3
- Result Order (for Max. # of results per compound): Order By Reference Count (DESC)

2. Predicted Composition Annotation:
- Check All Predicted Compositions: False

Processing node 14: Merge Features
---
1. Peak Consolidation:
- Mass Tolerance: 5 ppm
- RT Tolerance [min]: 0.1

Processing node 15: Differential Analysis
---
1. General Settings:
- Log10 Transform Values: True

Processing node 24: Descriptive Statistics

No parameters

Processing node 25: Assign Compound Annotations
---
1. General Settings:
- Mass Tolerance: 5 ppm

2. Data Sources:
- Data Source #1: mzCloud Search
- Data Source #2: Predicted Compositions
- Data Source #3: MassList Match
- Data Source #4: ChemSpider Search