RIVM Letter Report 607013001/2007

Concentration of ‘forgotten’ substances using the XAD concentration method

Suitability of the method for hydrophilic chemicals

M.T. Collombon

Contact:
Wilko Verweij
RIVM/MEV/LER
wilko.verweij@rivm.nl

This investigation has been performed by order and for the account of Ministry of VROM,

Abstract

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In the nineties, RIVM developed a method to concentrate toxic substances on XAD (a synthetic resin). Using bioassays, the toxicity can be determined in the concentrate. 'Modern' toxic substances tend to be more polar then 'classic' ones. It was invetigated whether more polar substances are also concentrated using the applied method.

Key words: surface water, pollution, concentration, toxic substances

Rapport in het kort

Concentrering van 'vergeten' stoffen met gebruikmaking van de XAD-concentreringsmethode

In de jaren '90 is door het RIVM een methode ontwikkeld om toxische stoffen uit oppervlaktewater te concentreren op XAD (een chemische hars). In het concentraat kan de toxiciteit worden bepaald met behulp van bioassays. 'Moderne' probleemstoffen zijn in het algemeen meer polair dan 'klassieke' probleemstoffen. Onderzocht is of meer polaire stoffen ook worden geconcentreerd met de toegepaste methode.

Trefwoorden: oppervlaktewater, verontreiniging, concentrering, toxische stoffen

Contents

Summary

In the early ’90s RIVM developed a method to lower the detection levels for toxicity measurements in the aquatic environment, the XAD-concentration method. For this method, a combination of two synthetic resins is used to extract organic micropollutants from water; these micropollutants are then subsequently brought into a smaller volume of water (‘water concentrate’) that is suitable for toxicity testing. The method has proven to work well for ‘classic’ toxicants (hydrophobic substances with narcotic toxic mode of action, pesticides). Recently, questions were raised about the suitability of the method for new, ‘upcoming’ chemicals, chemicals that only in the last decade have been recognized as a potential environmental problem. These chemicals are of interest because of their occurrence in the aquatic environment and their (unknown) potential ecological impact. The question is, what substances or substance groups are these days regarded as ‘new’ or ‘forgotten’, and which of these substances are of interest for further validation of the XAD concentration procedure.

The purpose of this research was to collect information on the suitability of the XAD-concentration method for some selected ‘new’ or ‘forgotten’ chemicals that may not, or only partially, be concentrated using the XAD- concentration method.

Based on their occurrence in the environment, physical-chemical properties (log K_{ow} <2), specific toxic mode of action and chemical analysis possibilities, 7 substances were chosen for further validation of the method: Sulfamethoxazole, Caffeine, Metronidazole, Enalapril-maleate, Norfloxacin, Carbadox and Metformin. The substances were concentrated using the current XAD concentration method. Extraction recoveries varied from 1 to 58% of the measured initial amount, with an average of 30%. In acetone eluates, an average of 16% of the measured initial amount was recovered, and at the end of the concentration procedure 11% (avg.) of the measured initial amounts was measured in the water concentrates. All recoveries should be interpreted as indicative, as the analytical method, developed specifically for this research, was not validated yet.

In general, it was assumed that a low log K_{ow} may indicate a limited concentration efficiency when using the XAD-concentration method. No correlation was found between log K_{ow} and extraction recoveries for the selected substances. However, elution recoveries were usually correlated to log K_{ow}: the lower the log K_{ow}, the lower the elution efficiency.
Thus, low recoveries of low log K_{ow} substances are not only the result of a sub-optimal extraction, but also of a reduced elution from XAD. Next, chemical loss may occur during KD-distillation. In the experiments, this was likely to be caused by decomposition of the compounds rather than volatilization.

At this point, it is difficult to pronounce upon necessity to alter the concentration procedure to enhance recoveries of hydrophilic substances. New, wide spectrum extraction means have become commercially available. Whether or not to choose for a new extraction means should depend on the expected or maximum possible improvement of the concentration efficiency with regard to the inclusion of hydrophilic substances in water concentrates, while maintaining efficiency for hydrophobic substances (log K_{ow}>2), and without unintended introduction of toxicity by the method. Besides, one should consider the effect of improvement of the method on the toxicological recovery of the method: does improvement of the chemical recovery result in detection of (additional) toxicity when using non-selective short term bioassays?

Apart from a decision to change the extraction method, further optimization of the current method should also be considered. For instance, increasing the amount of XAD per water volume may enlarge the chemical recovery of the extraction. Although this may lead to a larger acetone eluate volume, and therefore to a somewhat prolonged distillation duration, the net return may be worth it.

1 Introduction

Surface water quality has been monitored since the 1970's. Chemical monitoring programmes were often based on routine measurement of chemicals that are regulated through maximum concentration levels in Environmental Quality Standards. Concentration levels of these chemicals have generally gone down as a result of measures that have been taken to meet these Environmental Quality Standards.

Chemical analysis techniques have improved in recent years. Nowadays techniques are available to detect low concentrations of many, including formerly undetectable, chemicals in the environment. Still, the majority of substances that are produced cannot be measured chemically, simply because no analytical technique is available (Kolpin *et al.*, 2002). Even if an analytical method is developed, chemical analysis is expensive and chemicals may be not routinely measured because there is no obligation to do so, due to lack of regulations (Barreveld *et al.*, 2001), or the concentration levels of chemicals may be too low to be detected. However, it is shown that only a minor part of the ecotoxicity that is observed in environmental samples can be explained by measured concentrations of known and identified compounds (Hendriks *et al*., 1994). Even if all concentrations of individual chemicals can be quantified, measured concentrations of chemicals alone do not give us sufficient information on a toxic effect that is caused by the combination of chemicals in a mixture.

For this reason, RIVM developed a method to monitor combined effects of (low concentrations of) substances in a mixture of unknown composition (Slooff *et al*., 1984, Struijs and Van Buren, 1995). This method comprises of a concentration procedure for organic micropollutants and subsequent toxicity testing. Organic micropollutants are extracted from water using a combination of two synthetic resins, XAD-4 and XAD-8. The micropollutants are then removed from the resins and brought into a 1000 fold smaller water volume ('water concentrate') that is suitable for toxicity testing.

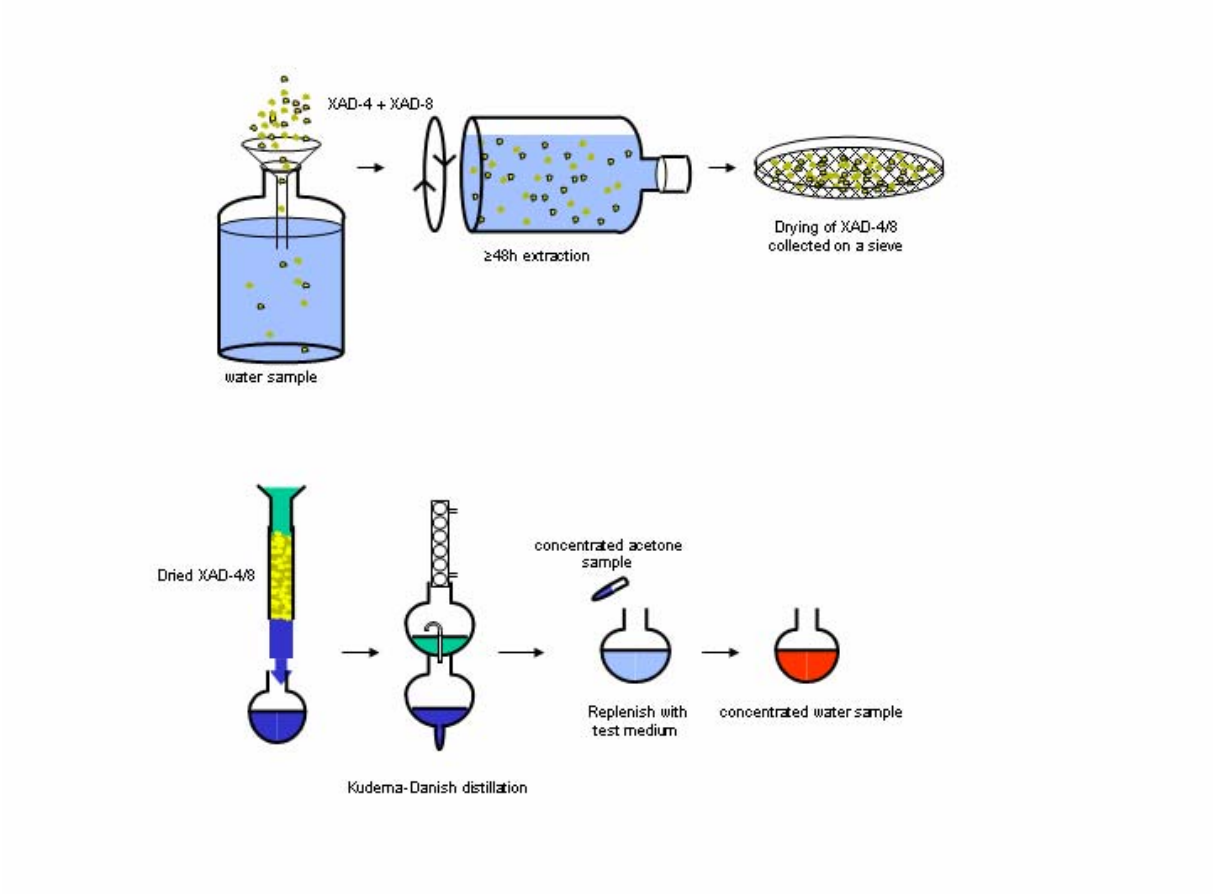

Figure 1 The XAD-concentration method

This water concentrate is used in short term bioassays to evaluate the toxic effect of the organic micropollutant mixture.

The concentration method has proven to be suitable for many, mainly 'classic'[1] chemicals, ranging from hydrophobic chemicals and volatile chemicals to some detergents (Van Stee *et al.*, 2002, Struijs and Van de Kamp, 2001). Of the chemicals that were used in spike experiments, an average of ca. 60% of the nominal initial quantities was measured in the water concentrates (Struijs and Van de Kamp, 2001).

Only recently, attention was paid to substances that were 'new' or 'forgotten' (Barreveld et al, 2001, Roig *et al.*, 2005). 'New' or 'forgotten' substances are substances that are possibly posing a risk to the environment, but are not under regulatory attention and detection in the (aquatic) environment does not automatically lead to an alarm (Van Wezel and Kalf. 2000, Barreveld *et al*, 2001).

Questions were raised about the suitability of the method for new, 'upcoming' chemicals, chemicals that only in the last decade have been recognized as a potential environmental problem. These chemicals are of interest because of their occurrence in the aquatic environment and their (unknown) potential ecological impact. These new chemicals may have properties different from the 'classic' contaminants known to have adverse effects on ecosystems, and may therefore exhibit different chemical and toxicological behaviour. The question is what substances or substance groups may be regarded as 'new' or 'forgotten', and which of these substances are of interest for further validation of the XAD concentration procedure.

The purpose of this research was to collect information on the suitability of the concentration method for some selected 'new' or 'forgotten' chemicals that may not, or only partially, be concentrated using the XAD- concentration method. Based on the results of the study, it will be discussed whether or not the XAD concentration method should be adjusted.

[1] 'Classic' substances are hydrophobic substances that are known to cause adverse effects in the environment, due to their presence, persistence and toxicity, *e.g.* PAH's, PCB's, dioxins, chlorobenzenes, pesticides etc.

2 Material and Methods

2.1 Selection of substances

The selection of chemicals was focused on chemicals that may not, or only partially, be concentrated using the XAD-concentration method. This means in the first place that the chemicals are not efficiently extracted from water using the combination of XAD-4/8. Next, it must be considered that a substance may be lost during acetone elution or Kuderna-Danish distillation, or at both steps in the procedure.

This information was connected to lists of chemicals that are already under policy attention (WFD, 2000, Laane *et al.*, 2001), and lists with 'new', 'emerging' of 'forgotten' substances (Roig *et al*., 2005, Barreveld *et al.*, 2001). Attention was paid also to other possible substances of interest. Information was gathered on physical-chemical characteristics, type of application, production volumes and potential toxicity.

2.2 Selection criteria

In the past, substances may not have been recognized as toxicants because of

- their specific effects at extremely low concentrations due to specific (anti-)estrogenic mode of action (*e.g.* flame retardants, some pesticides, synthetic estradiol), their low concentrations in the environment, but high acute toxicity (*e.g.* fragrances);
- low concentrations in the environment combined with low acute toxicity, but possibly long term effects at low concentrations due to a specific toxic mode of action (*e.g.* antibiotics).

Many of these chemicals could not be analyzed before due to a lack of suitable chemical analysis methods and were therefore 'forgotten'.

For selection of substances from these substance 'groups', two principally different type of selection criteria must be considered: selection criteria with regard to methodological limitations of the XAD-concentration method and criteria with regard to toxicity measurement of substances.

2.2.1 Selection criteria with regard to XAD-concentration method limitations

Chemicals are not concentrated to a sufficient extent when they fail in one of the steps: XAD extraction, acetone elution and KD-distillation. However, chemicals that are not extracted in the first place are lost for the remaining steps in the procedure. As a result, the first focus should be on the extraction step. Next, acetone solubility, volatility and temperature stability of a substance should be assessed.

Substances (not) adsorbing onto XAD

To find selection criteria for substances not adsorbing to XAD, it is necessary to understand what kind of substances do adsorb when present in water.

The adsorption process of organic molecules to XAD is defined by the interaction between the XAD and organic compounds. In order to achieve extraction from water, the properties of compounds must meet the following criteria (AWWA-KIWA, 1988):
- the molecules must be hydrophobic, or partly hydrophilic and partly hydrophobic;
- the affinity of the hydrophilic part of the molecule for water must be lower than the affinity of the hydrophobic part of the molecule for XAD.

Only organic molecules adsorb onto XAD-4 and XAD-8, inorganic substances, including heavy metal ions, are excluded from adsorption. Also, molecules larger than the pore size of XAD (pore size XAD-8: 225 Å, XAD-4: 50Å) may not be adsorbed effectively. For large molecules, XAD's adsorption capacity is limited to the outside surface of the XAD spheres.

Adsorption efficiency of a partly hydrophilic and partly hydrophobic organic substance is determined by the relative size of the hydrophilic and the hydrophobic parts of the molecule. A molecule containing, compared to a hydrophilic group, a relatively large hydrophobic group will be adsorbed more easily than a molecule that has a small hydrophobic part compared to the hydrophilic group.

Polarity or hydrophobicity
For convenience's sake, 'new', (relatively) water soluble organic micropollutants are often referred to as 'polar' substances.Whilst molecules can be described as "polar" or "non-polar" or "semi-polar" it must be noted that this is often a relative term, with one molecule simply being *more polar* or *more non-polar* than another. As such, there are no ultimate properties which can be ascribed to polar or non-polar molecules.
It should be realized that a hydrophilic substance indeed must be polar, but a polar substance is not necessarily hydrophilic. A polar organic molecule may consist of one or more polar and non-polar parts. Size and positioning of the polar and non-polar parts in a molecule will determine hydrophilicity of a molecule. There is no 'hard' measure for polarity, polarity of a molecule is therefore no criterion for selection of substances.

Log K_{ow} as selection criterion
From literature research and results of former research, hydrophilicity or hydrophobicity, expressed as log K_{ow} possibly is a criterion for selection. Struijs and Van Buren (1995) reported that (rather) hydrophobic chemicals (log K_{ow} >2) are probably effectively extracted from water using XAD. Hydrophilic substances (log K_{ow} <2) may be not efficiently extracted.

This hypothesis may be supported by the work that was done by Van Stee *et al.* (2002). To get information on unknown substances in surface water (indicated as 'forgotten' substances), they isolated and concentrated organic micropollutants from water using the combination of XAD-4/8. The isolated substances were analyzed using gas chromatography combined with mass spectrometry (GC-MS). Of the encountered substances, 78% exhibited log K_{ow}>2, leaving 22% of the encountered substances with log K_{ow} <2. Only 4% of the substances exhibited a log K_{ow} <0.
Of most organic pollutants that were analyzed using this method (*i.e.* concentration *plus* chemical analysis), chemical recovery is >80%. Of the non-volatile chemicals with recovery below 40%, most had log K_{ow} <2. The hypothesis that a low log K_{ow} can be a selection criterion for our study seems to be supported by these data.

However, it should be noted that the chemical analysis method GC-MS is selective for hydrophobic substances. The fact that only a limited number of low log K_{ow}-substances were encountered may have been caused by either limited extraction by XAD-4/8, by selective chemical analysis, or both. This means that it is possible that relatively hydrophilic substances may also be concentrated using XAD, but they simply are not discovered due to the limitations of the chemical analysis methods that are used.

With regard to limitations to the XAD-concentration method, selected substances should be organic, exhibit a low log K_{ow}, (log K_{ow} <2) and the molecule should be not too large.

2.2.2 Selection criteria with regard to toxicity

Besides methodological limitations, mainly substances that may pose a risk to the environment are of interest for linking the presence of chemicals to any toxic effect.

Substances that are generally regarded as a problem for ecosystem health are expected to be present in the environment. A substance must be produced, used in sufficiently large quantities and be persistent enough to be encountered. Next, to pose a risk for organisms, the substance should be biologically active. In order to be biologically active, the substance must be bioavailable, *i.e.* the substance must be able to pass cell membranes to reach the point of action in the cells of an organism, where the

toxicant can cause its effects. For toxicity, both concentration levels causing effects and toxic mode of action are important. When considering modification of the concentration method to make it suitable for new or forgotten substances, these properties also have to be taken into account.

Substances on priority lists in general are known to be toxic with bioaccumulative potential, organic micropollutants with log K_{ow} >2, or they are heavy metals and are therefore not in the scope of this work. Van Wezel and Kalf (2000) evaluated existing chemicals with regard to production volumes, use, persistence, bioaccumulative potential and toxicity, with the aim to pay attention to 'forgotten' substance groups that may pose a risk to the environment. They concluded that substance groups that can be considered as 'substances deserving more policy attention' are:

- Pharmaceuticals
- Disinfectants
- (Anti-) estrogenic compounds
- Biotransformation products of pesticides
- Fluorescent whitening agents
- Flame retardants
- Fragrances

Most substance groups regarded as 'deserving more policy attention', do not exhibit low log K_{ow} properties. (Anti-) estrogen substances, fluorescent whiteners, flame retardants, fragrances are assumed (Struijs and Van Buren, 1995) and demonstrated (Barreveld *et al.*, 2001) to be extracted efficiently using XAD, probably due to their log K_{ow} >2. Only *part* of the pharmaceuticals, *part* of the disinfectants, and *part* of the transformation products of pesticides exhibit a log K_{ow}< 2. From these groups, the low log K_{ow} chemicals were selected in order to match them with other criteria.

2.2.3 Selection criteria with regard to chemical analysis

As stated earlier, for many substances a chemical analysis method is not readily available. Chemical analysis is therefore the final selection criterion. For most low log Kow substances a chemical analysis method had to be developed. Measurement of the selected substances should be possible in water, acetone and a mixture of acetone and water; in water measurement should be possible at both low and high concentration levels, and in acetone at high concentration levels. Preferably the substances were to be analyzed using a single method. Furthermore, if available, information should be assessed on acetone solubility, volatility, temperature stability, persistence, bioaccumulative potential and toxicity of the substances.

2.3 Experimental setup and sampling

The selected substances were spiked into 10 l of mineral water (Spa blauw) and 2.5 ml XAD-4/-8 was added. Extraction was performed for 48 hours in the dark at room temperature (ca. 20°C). After extraction, the XAD was collected on a sieve (50 µm) and the XAD was dried overnight (ca. 18 hours) under a hood, at room temperature. The XAD then was eluted with ca. 4.25 ml acetone (exact elution volume determined) and the acetone was evaporated by Kuderna-Danish distillation. The distillation residue was then replenished with mineral water to 10 ml. The experiment was performed on three replicates. The concentration procedure was performed according SOP LER 303/03 (2003) and SOP LER 313/02 (2003).

Test substance concentrations were 100 -500 µg/l at the start of the experiment (nominal initial concentrations), maximum expected concentrations in water concentrates were 100 – 500 mg/l. Samples were collected from stock solutions, spiked mineral water , waste water after extraction , acetone and water concentrates.

Table 1 Nominal initial concentrations of the selected substances

	Nominal initial concentration			No. of samples collected
	Sulfamethoxazole	Other substances	Unit	
Stock solution	1*	0.02**	mg/ml	1+1
Spiked mineral water	100	500	µg/l	3
Waste water after extraction	0-100	0-500	µg/l	3
Acetone eluate	235	1176	µg/ml	3
Water concentrate	100	500	mg/l	3

* Stock solution of single substance in acetone

** Stock solution of mixed substances in water

2.4 Chemical analysis

Samples were analyzed by LC-MS, by the NEN-EN-ISO/IEC 17025 certified RIVM Laboratory for Food- and Residue Analyses (RIVM-ARO). For the analysis of the selected substances, a new operating procedure had to be developed.

3 Results

3.1 Selected substances

Candidates for selection are listed in Annex A. Combining low log K_{ow}'s, and chemical analysis possibilities, a list of 7 substances was composed (Table 2). All selected chemicals are non-volatile. As acetone solubility and temperature stability were unknown before selection, these properties were not taken into account in the selection. All selected substances have been designed for a specific application and have been detected in the environment in recent years (Derksen *et al.*, 2001, Derksen *et al.*, 2004, Kolpin *et al.*, 2002, Rijs *et al.*, 2003)

The chemicals were combined in a test mixture to further validate the developed concentration method using XAD-4/8. More detailed information on substance properties, purities and suppliers is listed in Annex B.

Table 2 Selected low log K_{ow} substances

Substance name	Application	Cas No.	Log K_{ow}	Molecular formula	Molecular weight
Sulfamethoxazole	Antibiotic	723-46-6	0.89	$C_{10}H_{11}N_3O_3S$	253.3
Caffeine	Stimulant	58-08-2	-0.07	$C_8H_{10}N_4O_2$	194.19
Metronidazole	Antibiotic	443-48-1	-0.1	$C_6H_9N_3O_3$	171.16
Enalapril-maleate	Antihypertensive	76095-16-4	-0.74	$C_{24}H_{32}N_2O_9$	492.53
Norfloxacin	Antibiotic	70458-96-7	-1	$C_{16}H_{18}FN_3O_3$	319.34
Carbadox	Antibiotic, growth promotor	5-7-6804	-1.37	$C_{11}H_{10}N_4O_4$	262.23
Metformin	Antidiabetic	657-24-9	-2.64	$C_4H_{11}N_5$	129.17

3.2 Experimental results

3.2.1 Extraction

Extraction efficiency (or recovery) was calculated from spiked concentrations and waste water concentrations, assuming that no other process than adsorption onto XAD had taken place. The amount that disappeared from water relative to the quantity measured in spiked water (measured initial quantity) is assumed to be adsorbed onto XAD.

$$\text{Extraction recovery\%} = \frac{m_{spike} - m_{waste\,water}}{m_{spike}} * 100\%$$

Average extraction recovery for the 7 substances was 30% of the measured initial quantities. Extraction recovery was plotted against log K_{ow} (Figure 2). Four substances were extracted at a rate between 39 and 58%, 2 at ca. 10% and only 1 substance was extracted almost not at all (metronidazole, 1±1%). There is no correlation between extraction efficiency and log K_{ow}.

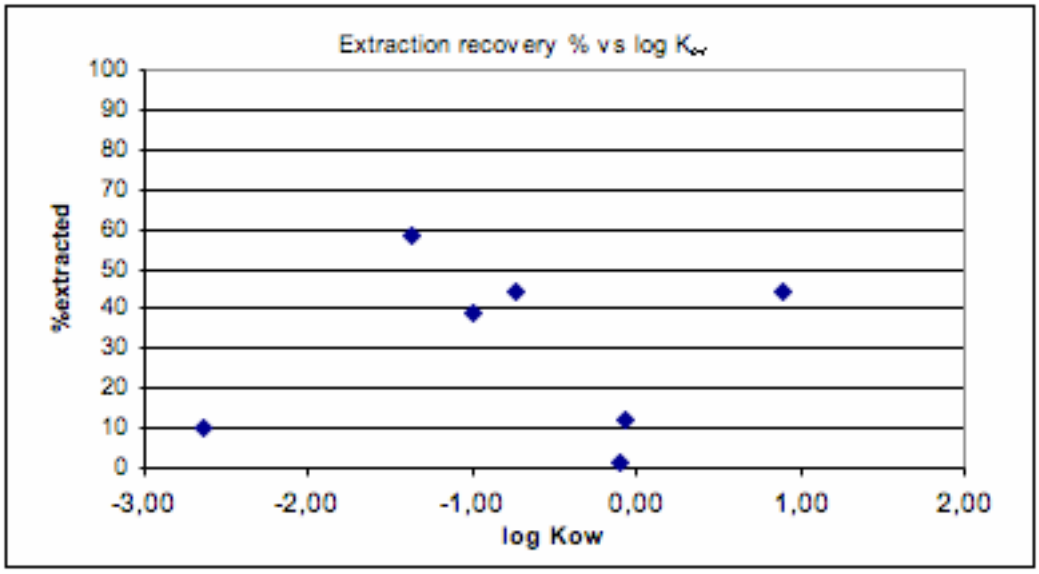

Figure 2 Extraction recovery% vs. log K_{ow} for 7 selected substances

3.2.2 Elution

The elution efficiency was calculated relative to the measured initial quantity (m_{spike}), resulting in the combined recovery of extraction, drying, and elution.

$$\text{Elution recovery (1)} = \frac{m_{acetone}(\mu g)}{m_{spike}(\mu g)} * 100\%$$

From extraction recovery (avg. 30%) to elution recovery (avg. 16%), an average of 14% of the measured initial quantities was lost. Losses at this stage vary: sulfamethoxazole was not lost at all (44% recovery both after extraction and after extraction plus elution), and metformin was completely lost (10% recovery after extraction, 0% recovery after extraction plus elution). Although metronidazole was almost not extracted from water, the same quantity of the substance that was extracted from water was recovered in the acetone eluate.

The elution recovery was also calculated relative to the quantity of a substance that was assumed to be adsorbed onto XAD:

$$\text{Elution recovery (2)} = \frac{m_{acetone}(\mu g)}{m_{spike}(\mu g) - m_{waste\,water}(\mu g)} * 100\%$$

The elution recovery (2) of a substance was plotted against its log K_{ow} (Figure 3). Of the eluted chemicals, 4 were recovered at levels above 80% of the adsorbed quantity; norfloxacin was eluted from XAD at 47%, and Carbadox at 17%.

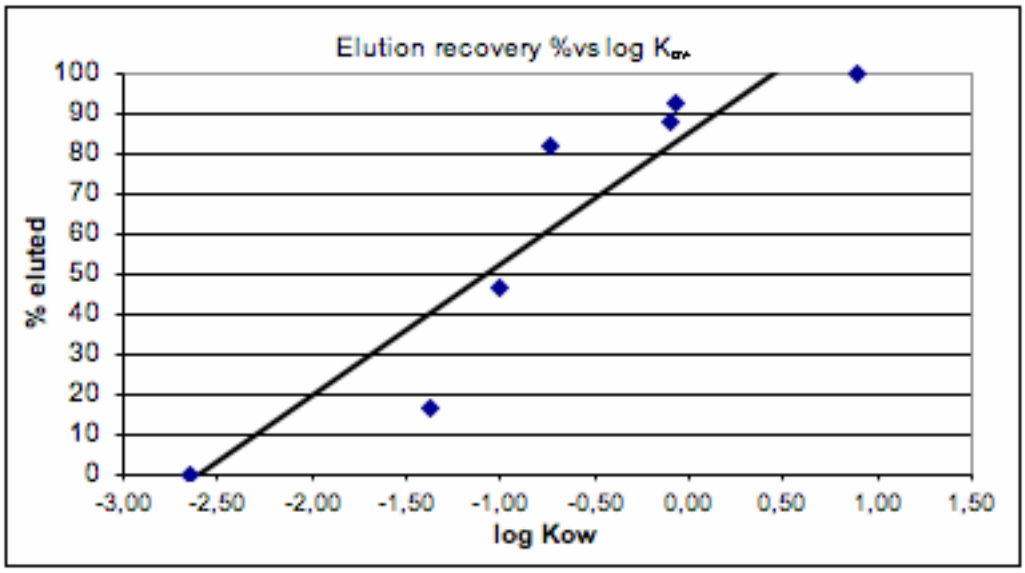

Figure 3 Elution recovery% (calculated from adsorbed amounts) vs. log K_{ow}'s for 7 substances. R^2 = 0.86.

When considering just acetone elution from XAD, the correlation between elution recovery% and log K_{ow} is linear for these 7 substances: the lower the log K_{ow}, the lower the elution recovery.

3.2.3 Kuderna Danish Distillation

After distillation and replenishing of the residues, the average overall recovery% in the water concentrates was 11%. Relative to the quantities in acetone eluates, 69% was recovered in the water concentrates, and thus 31% was lost. As expected, there is no correlation between log K_{ow} and KD-distillation recovery.

Table 3 Percentage of substance quantity recovered in water concentrates from acetone eluate

Substance	Water concentrate
Sulfamethoxazole	66
Caffeine	91
Metronidazole	50
Enalapril-maleate	76
Norfloxacin	61
Carbadox	50
Metformin	0
Average	69

3.2.4 Overall results

Chemical recovery of each step in the procedure was calculated relative to the quantity spiked in water (measured initial quantity or m_{spike}) and presented as % of m_{spike} (Figure 4). The extraction efficiency is presented in blue, the recovery after acetone elution in yellow and the recovery after KD distillation (overall-recovery) in red. The substances are arranged in order of decreasing log K_{ow}, the substance with the highest log K_{ow} is on the left side of the figure (sulfamethoxazole, log K_{ow} = 0.89).

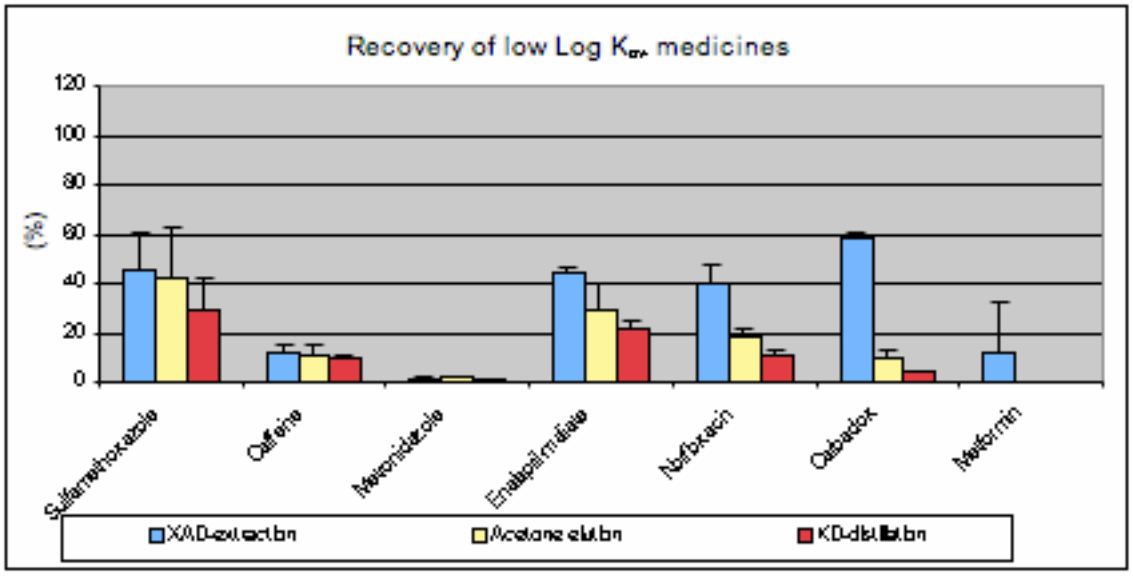

Figure 4 Chemical recovery of selected substances, recoveries relative to measured initial quantities

All chemicals were extracted from water to some extent, and all substances but one (metformin) were recovered in the water concentrates, at an average of 11% (Table 4). Metronidazole was recovered in waste water at 99% of the measured initial concentration.

Table 4 Substance concentrations in spiked water, quantities in waste water, acetone and water concentrates relative to the measured initial quantities

	Spiked mineral water		Waste water		Extracted from water		Acetone		Water concentrate	
	Nominal initial concentrations	Measured initial quantity	measured quantity		measured quantity		measured quantity		measured quantity	
Substance	(µg/l)	%	avg.% ± sd		avg.% ± sd		avg.% ± sd		avg.% ± sd	
Sulfamethoxazole	100	100	56	15	44	15	44	21	29	13
Caffeine	500	100	88	3	12	3	11	4	10	1
Metronidazole	500	100	99	1	1	1	2	1	1	0
Enalapril-maleate	500	100	55	2	45	2	29	10	22	3
Norfloxacin	500	100	61	8	39	8	18	3	11	2
Carbadox	500	100	42	3	58	3	10	4	5	0
Metformin	500	100	90	21	10	21	0	0	0	0
Average		100	**70**	22	**30**	22	**16**	16	**11**	11

Details on chemical analyses are presented in Annex C.

4 Discussion

4.1 Extraction efficiency

In figure 4, substances are arranged in order of decreasing log $K_{ow.}$ There is no apparent connection between log K_{ow} and extraction efficiency, *e.g.* the recovery of carbadox (log K_{ow} = -1,37) is substantially higher than the recovery of caffeine (log K_{ow} = -0.07). However, all substances show substantial loss at the extraction stage. A log K_{ow}<2 may be considered as indicative for reduced recovery in the concentration procedure.

4.2 Elution efficiency

Elution recoveries seem to be connected to log K_{ow} via acetone solubility of a substance, as elution recovery decreases with decreasing acetone solubility and decreasing log K_{ow} (Table 5).

Table 5 Acetone solubilities as determined roughly in the RIVM laboratory

	log K_{ow}	Acetone solubility	% eluted from XAD
Sulfamethoxazole	0.89	9600	100
Caffeine	-0.07	3050	93
Metronidazole	-0.10	2700	88
Enalapril-maleate	-0.74	2100	82
Norfloxacin	-1.00	1960	47
Carbadox	-1.37	<700	17
Metformin	-2.64	<1400	0

Although acetone solubilities were determined roughly with the aim only to get an indication on solubility of the substances, it is clear that acetone solubility decreases with decreasing log K_{ow}. This is reflected in the elution efficiencies of the substances.

4.3 Kuderna Danish distillation

In general, it takes about 20-30 min. to reduce an acetone eluate to 0.2 - 0.3 ml. In this experiment, all three samples kept cooking for a long time without volume reduction, only after ca 2 – 2.5 hours of boiling at 67°C, volume reduction started to take place. The final distillation residues were 0.6 – 3 ml. The average loss of chemicals from the acetone eluate to the water concentrate was 32%. This loss is probably partly due to the duration of the distillation. As the chemicals are rather non-volatile and the end concentrations in the water concentrates were well below their water solubility, volatility and limited water solubility were probably not the cause of chemical loss. The substances may (partially) have been decomposed during distillation.

Loss of chemicals could have been less if the distillation duration could have been limited to a shorter period. However, volume reduction of the sample is necessary to avoid a too high acetone concentration in the water concentrate to avoid unintended toxic effects in toxicity studies. Due to this, the distillation had to be prolonged, possibly resulting in the extra chemical loss.

4.4 Chemical recovery of the overall concentration procedure

When looking at the results of the experiment with 7 substances, an average recovery of 11% was obtained, ranging from 0% to 29%. No direct link between low log K_{ow} and overall recovery can be found (Figure 3). However, none of the substances showed extraction recoveries above 60%, and the average recovery of the 7 chemicals in the concentration procedure is substantially lower than 93%

average extraction recovery of hydrophobic substances and pesticides in former research (Struijs and Van de Kamp, 2001).
In analytical chemistry, an overall recovery of 11% average for a substance is usually not regarded as acceptable. However, it should be noted that even with an overall recovery as low as 10%, the substance amounts in the water concentrate are 100-fold of the substance concentrations in the original water sample when applying the XAD concentration procedure.

For the existing concentration method, the aim always has been to concentrate as many (organic) micropollutants from water as effective as possible, in order to reach low detection levels for toxicity and to get an impression of overall toxicity caused by 'all' micropollutants. Principally it is impossible to achieve 100% chemical recovery when concentrating 'the' micropollutants from (surface) water. For many organic micropollutants, particularly the substances with log $K_{ow} > 2$, the XAD concentration procedure may work quite well (Barreveld *et al.* (2001), Struijs and Van de Kamp (2001), Van Stee *et al.*, (2002)), provided that the acetone solubility is sufficiently high, the volatility sufficiently low and the substance is temperature resistant up to 70°C: chemical recoveries are often >80%.

The results of the experiment with the 7 selected substances however indicate that hydrophilic organic micropollutants are not '100% effectively' concentrated using the existing concentration method. When the aim is to monitor toxicity of *all* chemicals in water as stress factor in ecosystems, the concentration method should be more effective for a wider spectrum of chemicals, including hydrophilic substances.

4.5 What is regarded as a good recovery?

Principally, the ambition is to maximize chemical recovery of micropollutants and 100% recovery is the ultimate goal. However, chemical loss during the concentration procedure has to be accepted. A balance between effort to maximize chemical recovery and the maximum possible yield (in terms of toxicological effects to be measured) needs to be found.

Whether 10% chemical recovery is considered as 'good' or 'bad' is based on the goal to be reached and expectations on the possible maximum concentration of a substance. For the substances that were selected for this research, recoveries were expected to be lower than the recoveries of the 'classic' chemicals, without having a clue in which direction to think with regard to this.

The question is: to what extent do the hydrophilic substances contribute to the toxicity of the micropollutant mixture that is measured when using the water concentrates. When a substance in surface water generally is found at the ng/l level (like many antibiotics (Derksen et al., 2001, Kolpin et al, 2002, Schrap *et al.*, 2003)), the maximum concentration level in a 1000 fold concentrate will be at the µg/l level. If the substance exposes acute toxicity at the mg/l level, does the addition to a test mixture result in substantial addition of toxicity to the water concentrate, or is the addition of toxicity too small to detect when using acute bioassays? From what concentration level of a hydrophilic substance do or can we expect addition to the acute toxicity of a test mixture?

Many hydrophilic substances are pharmaceuticals. They are designed for specific action and should not cause severe acutely toxic effects in an organism at relatively high concentrations. This is reflected in the low acute toxicity of these substances (Derksen *et al.*, 2001) However, unintended toxic effects, possibly related to their specific mode of action, may occur at low concentration levels in the aquatic environment (Schrap *et al*, 2003, Derksen *et al.*, 2004). These specific effects will probably not be detected when using acute bioassays only.

If the contribution of this kind of substances to the non-specific, acute toxicity of a mixture is negligible and therefore undetectable, and the specific effects of these substances are undetectable too, why then putting so much effort in adjusting the concentration procedure to increase the recovery of hydrophilic substances?

Nevertheless, due to regulation of substance use (and the setting of maximum permissible concentrations (MPC)), concentration levels of ‘classic’ hydrophobic chemicals in surface water are decreasing. A tendency towards production of chemicals with less accumulative potential (=less hydrophobic) has already been noticed. As a result, more hydrophilic chemicals will appear in the environment. Little is known about long term effects of low concentrations of chemicals that exhibit specific chronic toxicity like pharmaceuticals.

4.6 Chemical analysis

Chemical analysis was performed using a new chemical analysis method that had not been validated yet. Due to this, the chemical recovery of the substances was not corrected for systematic errors. In most cases, the results of chemical analysis were consistent. However, some discrepancies were encountered. In some cases, no explanation could be found for a deviation in measured concentration (*e.g.*, the concentrations in one of the acetone eluates was half of the concentrations in the other two eluates for all chemicals). No explanation could be found in the actual chemical analysis of the acetone samples. The concentrations in the KD-residues of the three samples were rather consistent. Due to this, the deviating concentrations in acetone eluates were not used for calculation of the average concentrations in acetone.

Due to concentration and dilution, ‘noise’ is introduced in the samples, which may complicate chemical analysis. Mass balance and a validated method are necessary in order to be able to detect errors. With few measurements it may be difficult to differentiate between variation in chemical analysis and variation as a result of the sample treatment.

However, the chemical analysis was performed in a NEN-EN-ISO/IEC 17025 certified laboratory, with one LC-MS apparatus that was operated by 1 technician who measured the samples in a short period of time. Therefore, recoveries for the subsequent steps in the concentration procedure are reported relative to the measured initial amounts (100%). The results should be interpreted as indicative.

5 Conclusions

For the 7 selected substances, chemical recovery in the water concentrates at the end of the concentration procedure was 11%. This is substantially lower than the recovery for the hydrophobic substances of the narcotic and pesticide test mixtures in former research.

A log K_{ow} <2 may indicate that a substance may show a reduced XAD extraction efficiency. However, there is no correlation between low log K_{ow} and extraction recovery. For the selected substances, there was a correlation between log K_{ow} and elution recovery: the lower the log K_{ow}, the lower the acetone solubility and the lower the elution efficiency.

Low recovery of the selected substances in the overall concentration procedure is determined mainly by low extraction yields (average loss: 70%), but also by low elution efficiencies (average loss: 47% of the substances that were adsorbed onto XAD) and during distillation (average loss of substances from the acetone eluate: 32%). Thus - beside extraction - elution and KD-distillation efficiency *also* play an important role in the effectivity of the concentration method. When looking for improvement of the concentration method, all steps in the procedure should be taken into account.

Many of the chemicals that are potentially troublesome for the environment are (relatively) hydrophobic and will probably be extracted from water using XAD, the XAD-concentration method seems to be suitable. Even relatively water soluble substances are concentrated to some extent. However, no information is available on the suitability of the method for substances that are not known to be harmful to the environment. Still, harmful effects of chemicals that cannot be analyzed or measured can be missed, especially when these effects are subtle.

6 Recommendations

At this point, it is difficult to pronounce upon necessity to alter the concentration procedure. Whether or not to choose for a new extraction means should depend on the expected or maximum possible improvement of the concentration efficiency with regard to the inclusion of hydrophilic substances in water concentrates, while maintaining efficiency for hydrophobic substances (log K_{ow}>2), and without unintended introduction of toxicity by the method. Besides, one should consider the effect of improvement of the method on the toxicological recovery of the method: does improvement of the chemical recovery result in detection of additional toxicity when using non-selective short term bioassays?

The method using XAD water concentrates in short term toxicity tests was developed to be able to measure low concentrations of non-specific general toxicants (narcotic substances) and toxicity caused by highly toxic substances (pesticides). If one is after specific effects of low concentrations of a substance (group), it should be realized that the other micropollutants - that will be concentrated along with the substance of interest - may interfere with any toxic effects caused by the substance of interest. When one is interested in effects of chemicals with low acute toxicity and specific toxicity at relatively low concentration levels, focus probably should be on specific extraction of that substance (group). Focusing on a specific group of substances however is not within the scope of the method that was developed to evaluate toxicity of a toxicant mixture of unknown composition.

If it is decided to invest in the concentration method (anyway), the concentration efficiency may be improved by the use of new extraction means, *e.g.* new adsorption products that are available for extraction of a wide spectrum of chemicals from water (Annex D). These products may be able to improve extraction efficiencies including ‘new’ or ‘forgotten’ chemicals. Adjustment of the extraction method would then be a way to broaden the spectrum of chemicals to be concentrated, which in turn may lead to lower toxicity detection levels. Suitability of this kind of products for concentrating chemicals for toxicity testing could be evaluated with regard to chemical *and* toxicological recovery.

For the reasons mentioned above, it may not be worth the investment to alter the current concentration procedure to catch more of the hydrophilic substances. On the other hand, as the composition of the organic micropollutant mixture is shifting towards less bioaccumulative, more hydrophilic substances, toxic effects of this kind of substances should be monitored.

The current concentration method may be optimized for hydrophilic substances. Some suggestions for improvements are:

1. Enlarging of the XAD : water volume ratio

The amount of XAD that is used per water volume was set to 2.5 ml XAD-4/8 per 10l water. This amount was determined using hydrophobic chemicals, substances with high affinity for XAD. Results from experiments with some hydrophilic substances (bentazone, metoxuron), including experiments with humic acids added to the water samples, indicate that this amount may be not sufficient. Enlarging the amount of XAD per water volume (e.g. to ca 5 ml/10l water) increases the adsorption surface, which may result in a more effective extraction of hydrophilic substances.
A larger XAD:water volume ratio will result in a larger acetone eluate volume, which in turn may lead to extended distillation duration. Effects on chemical recovery, *e.g.* extra chemical loss due to extended distillation duration, should be examined. However, these losses may be smaller than the gains due to extra extraction recovery.

2. Prolongation of the extraction period

If XAD is not saturated after 48h of extraction, prolonged extraction may also result in better recoveries. If extended extraction does not result in higher recoveries, and degradation of the substance during the extraction period can be excluded, the amount of XAD may have been too small.

3. additional elution of XAD with another solvent
Elution with a solvent with properties different from acetone may result in better recoveries of substances that do not dissolve in acetone. Both elution before and after drying of XAD can be considered. The solvent should be completely removable and the extra step should not introduce unintended toxicity.

Acknowledgements

Special thanks to Klaas Wubs and Peter Kootstra (RIVM-ARO) for advice on and execution of the chemical analyses.

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Annex A Candidate substances for selection

Compound name	CAS nr.	Category	Sub-category	Log K_{ow}	Solubility (mg/L)		log(1/H)*	measured recovery	P/E[c]
					in water	in acetone			
Glyphosate	1071-83-6	Pesticides	Herbicide	-2.8	12000[1]	practically insoluble	10.1	-	
Metformin	657-24-9	Prescription drugs	Ant diabetic	-2.6[1]	1000000[1]		15.1	-	E
Olaquindox	23696288	Prescription drugs	Antibiotic	-2.3	436000[1]		26.9	-	
Methotrexate	59-05-2	Prescription drugs	Cytostatic	-1.9[1]	2600[1]		30.8	-	
Carbadox	6804-07-5	Prescription drugs	Antibiotic	-1.4[1]	15000[1]		22.3	-	E
Oxytetracycline	79-57-2	Prescription drugs	Antibiotic, (Tetracyclines)	-1.2	313[1]		24.8	-	E
Tetracycline	64-75-5	Prescription drugs	Antibiotic, (Tetracyclines)	-1.2	249000[1]		30.9	-	E
Tri-ethyleneglycol-Monobutylether	143-24-8	Solvent	Glycols	-1.0[1]	254000[1]		10.3	35[a]	
Norfloxacin	70458-96-7	Prescription drugs	Antibiotic (Quinolines)	-1.0[1]	178000[1]		18.1	-	E
Tri-ethyleneglycol-dimethylether	112-49-2	Solvent	glycols	-0.8[1]	249000[1]		8.5	30 [a]	
Enalaprilat	76420-72-9	Prescription drugs	Antihypertensive	-0.7[1]	25000	?	18.1	-	E
Chlortetracycline	57-62-5	Prescription drugs	Antibiotic (Tetracyclines)	-0.6	630[1]		23.5	-	E
Bentazone	25058-89-0	Pesticides	Herbicide	-0.5	570		3.7	30-35; 1.4[b]	
Diglyme = Diethyleenglycol methylether	111-96-6	Solvent		-0.4[1]	1000000[1]		6.3	5 [a]	
Dipropyleneglycol-monomethylether	34590-94-8	Solvent		-0.4[1]	1000000[1]		7.0	<5 [a]	
Sulfadiazine	68-35-9	Prescription drugs	Antibiotic, (Sulfonamides)	-0.1[1]	77[1]		9.8	-	
Caffeine	58-08-2	nonPrescription drugs	Stimulant	-0.07	21000[1]	20000	18.7	5 [a]	
Metronidazole	443-48-1/ 69198-10-3	Prescription drugs	Antibiotic, antiprotozoa	-0.1[1]	9500[1]		10.8	-	
Sulfathiazole	72-14-0	Prescription drugs	Antibiotic, Sulfonamides	0.1				-	E
Cotinine, metabolite caffeine	486-56-6	nonPrescription drugs	caffeine metabolite	0.1	999000[1]			-	E
Mevinphos	7786-34-7	Pesticides	Organophosphorus pesticide	0.1	600000		2.4	60[b]	

Compound name	CAS nr.	Category	Sub-category	Log K_{ow}	Solubility (mg/L)		log(1/H)*	measured recovery	P/E[c]
					in water	in acetone			
Sulfamerazine	127-58-2	Prescription drugs	Antibiotic, Sulfonamides	0.2				-	E
Ranitidine	66357-35-5	Prescription drugs	Antacid	0.3	24700[1]			-	E
Ciprofloxacin	85721-33-1	Prescription drugs	Antibiotic, (fluoro)quinolines	0.3[1]	30000		18.3	-	E
Sulfachloropyridazine	80-32-0	Prescription drugs	Antibiotic, Sulfonamides	0.3	7000[1]			-	E
Cimetidine	51481-61-9	Prescription drugs	Antacid	0.4	9380[1]			-	E
Sodiumdodecanebenzene-sulfonate (LAS)	25155-30-0	Detergent		0.5	800			38[b]	
Paracetamol = acetaminophen	103-90-2	NonPrescription drugs	analgesic	0.5	14000[1]			-	E
Sulfamethizole	144-82-1	Prescription drugs	Antibiotic (Sulfonamides)	0.5	1050[1]			-	E
2-butoxy-ethanol	111-76-2	Solvent	Aliphatic compounds	0,57 - 0,83				10[a]	
Propylene glycol methylether-acetate	108-65-6	Solvent	Glycols	0.6[1]	198000[1]		5.5	25[a]	
Lincomycin	154-21-2	Prescription drugs	Antibiotic (Macrolides)	0.6	927[1]			-	E
N,N-dimethylbenzamide	611-74-5			0.6	22600[1]			>80[a]	
Albuterol	18559-94-9	Prescription drugs	beta-agonist, asthma medicine	0.6	14100			-	E
Caprolactam	105-60-2		Production of nylon and poly-urethanes, partly originating from XAD-4	0.7[1]	772000[1]		7.6	5[a]	
Enrofloxacin	93106-60-6	Prescription drugs	Antibiotic, (fluoro)quinolines	0.7[1]	3400[1]		17.8	-	E
Cyclohexanon	108-94-1	Solvent	Aliphatic compounds	0.8[1]	25000[1]		5.0	15[a]	
3-methyl-2-butanon	563-80-4	Solvent	Aliphatic compounds	0.8[1]	60800[1]		4.0	10[a]	
Amoxicillin	26787780	Prescription drugs	Antibiotic, (Penicillines)	0.9					
Sulfamethoxazole	723-46-6	Prescription drugs	Antibiotic (oxazoles)	0.9	610[1]	good	12.2	53	E
2-pentanon	107-87-9	Solvent, fragrance	Aliphatic compounds	0.91	430001		4.1	10a	
Trimethoprim	738-70-5	Prescription drugs	Antibiotic	0.9					E
Oxolinic acid	14698294	Prescription drugs	Antibiotic	0.9	3.2[1]				

Compound name	CAS nr.	Category	Sub-category	Log K_{ow}	Solubility (mg/L)		log(1/H)*	measured recovery	P/E[c]
					in water	in acetone			
Sarafloxacin	98105-99-8	Prescription drugs	Antibiotic (Quinolines)	1.1[1]	1140[1]		18.7		E
Digoxigenin	1672-46-4	Prescription drugs	Cardiac stimulant metabolite	1.1	403[1]			-	E
Triethyl phosphate	78-40-0	(chloro) alkylphosphates	plasticizer and flame retardant in cellulose plastics	1.1	>115250		2.4	50[a]	
Acetyl salicylic acid	50-78-2	Non-Prescription drugs	analgesic	1.2	4600[1]				
Codeine	76-57-3	Prescription drugs	analgesic	1.2	9000[1]			-	E
Dichloromethane	75-09-2	Aliphatic compounds	Halogenated alkanes	1.3[1]	13000[1]		3.9		P
Digoxin	20830-75-5	Prescription drugs	Cardiac stimulant	1.3	64.8			-	E
4-methyl-2-pentanon	108-10-1	Solvent	Aliphatic compounds,	1.3[1]	19000[1]		3.9	45[a]	
Isoforone	78-59-1	Solvent	for varnish, fragrance	1.3			5.2	75[a]	
2,2-dimethoxypropane	77-76-9	Aliphatic compounds	synthesis	1.41	75201		4.1	5a	
Phenol	108-95-2	Phenols	Disinfectant, synthesis	1.5	80000		6.4	5[a]	E
1,2-dichloroethane	107-06-2	Aliphatic compounds	Halogenated alkanes	1.5[1]	8600[1]		2.9		P
Acetophenone	98-86-2	Fragrance, solvent	anti-corrosion, rubber additive, dye additive,	1.6			5.0	92[a]	E
Metoxuron	19937-59-8	Pesticides	Herbicide	1.6	678		2.8	63[b]	
5-methyl-1h-benzotriazole	136-85-6		Anticorrosive	1.7[1]	3070[1]		6.8		
Flumequine	42835256	Prescription drugs	Antibiotic , fluoroquinolones	1.6[1]	2190[1]		12.6		
Phthalic anhydride	85-44-9		Plastic manufacturing	1.6[1]					
2-hexanol	626-93-7	Solvent	plasticizer, pharmacy, leather working, fragrant	1.8	13700[1]				
Nitrobenzene	98-95-3	Solvent	nitrobenzene shoe polish, synthesis	1.9[1]	2090[1]		4.6		
Sulfamethoxine	155-92-3	Prescription drugs	Antibiotic, Sulfonamides	1.9					
Penicillin G.	61-33-6/ 69-57-8	Prescription drugs	Antibiotic, penicillin	1.9					
Benzoic acid	65-85-0			1.9[1]					
Tri(2-chloroethyl) phosphate	115-96-8	Flame retardant	(chloro) alkylphosphates	0,54-1,78	>5000		4.4		
Prozac = fluoxetine	56296-78-7, 54910-89-3	Prescription drugs	Antidepressant	1-1,8-2,6					

[*]H: Henry's Law constant
[a]Recovery measured in samples concentrated with XAD-4 only
[b] Recovery measured in samples that were concentrated using XAD4 and XAD-8
[c] P: priority substance acoording to WFD; E: Emerging substance (SWIFT)
1 Data from Syracuse phys-chem database http://www.syrres.com/esc/physdemo.htm

Annex B Selected substances in order of decreasing Log K_{ow}.

Substance name	Application	Casnr	Molecular formula	Molecular weight	Log K_{ow}	Solubility in water* (mg/l)	Solubility in acetone* (mg/l)	Henry's law constant (atm.m^3.mol^{-1})	Vapour pressure (Pa)	Batch-no.	Purity
Sulfamethoxazole	Antibiotic	723-46-6	$C_{10}H_{11}N_3O_3S$	253.3	0.89	370-610	9600	$6.42*10^{-13}$	$9.24*10^{-06}$	064K1257	100%
Caffeine	Stimulant	58-08-2	$C_8H_{10}N_4O_2$	194.19	-0.07	21000	3050	$1.9*10^{-19}$	$9.771*10^{-07}$	014K0036	100%
Metronidazole	Antibiotic	443-48-1	$C_6H_9N_3O_3$	171.16	-0.1	9500	2700	$1.6*10^{-11}$	$4.07*10^{-05}$	033K1473	100%
Enalapril-maleate	Anti-hyper-tensive	76095-16-4	$C_{24}H_{32}N_2O_9$	492.53	-0.74	8.94	2100	$7.85*10^{-19}$	$2.93*10^{-17}$	055K1446	>99%
Norfloxacin	Antibiotic	70458-96-7	$C_{16}H_{18}FN_3O_3$	319.34	-1	178000	1960	$8.7*10^{-19}$	$9.02*10^{-11}$	083H0921	>98%
Carbadox	Antibiotic, growth promotor	5-7-6804	$C_{11}H_{10}N_4O_4$	262.23	-1.37	15000	<700	$4.5*10^{-23}$	$8.26*10^{-08}$	030H0349	99%
Metformin	Anti-diabetic	657-24-9	$C_4H_{11}N_5$	129.17	-2.64	1000000	<1400	$7.64*10^{-16}$	$4.52*10^{-01}$	085K0205	>99%

*Acetone solubilities as determined in the RIVM lab to get an indication

Supplier is Sigma-Aldrich.

Annex C Data on chemical analysis: Substance Concentrations

Table C1 Stock solutions. Nominal concentrations, actual concentrations based on weight and actual concentrations based on chemical analysis

Stock solution	nominal concentration (mg/ml)	actual concentration based on mass (mg/ml)	Actual concentration, based on LC-MS analysis(mg/ml)	Ratio analysis:mass (%)
Sulfamethoxazole	1*	1.00	0.9314	93.1
Caffeine	0.02**	0.0201	0.0202	100.5
Metronidazole	0.02**	0.0198	0.0216	109.1
Enalapril-maleate	0.0198**	0.0199	0.0155	78.2
Norfloxacin	0.0196**	0.0194	0.0241	123.8
Carbadox	0.0198**	0.0208	0.0130	62.8
Metformin	0.0198**	0.0203	0.0199	97.9

*: stock solutions in acetone
**: stock solution in water

Table C2 Spiked mineral water, nominal concentrations in mineral water, concentrations based on weight and based chemical analysis.

Spiked mineral water	Nominal concentrations	Measured initial concentrations				
		based on weight	chemical analysis			average ± sd (n=3)
	(µg/l)	(µg/l)	(µg/l)	(µg/l)	(µg/l)	(µg/l)
Sulfamethoxazole	100	100	77	102	95	91±13
Caffeine	500	502	485	493	505	494±10
Metronidazole	500	494	556	570	571	566±8
Enalapril-maleate	500	497	403	398	402	401±3
Norfloxacin	500	486	623	476	685	595±107
Carbadox	500	519	334	374	385	364±27
Metformin	500	507	466	410	406	427±34

Table C3 Waste water concentrations

Waste water	expected concentration range	Measured concentrations			
		chemical analysis			average ± sd (n=3)
	(µg/l)	(µg/l)	(µg/l)	(µg/l)	(µg/l)
Sulfamethoxazole	0-100	56	44	50	50±6
Caffeine	0-500	433	447	424	435±11
Metronidazole	0-500	540	565	570	558±16
Enalapril-maleate	0-500	235	214	219	223±11
Norfloxacin	0-500	381	323	360	355±30
Carbadox	0-500	129	163	164	152±20
Metformin	0-500	303	415	416	378±65

Table C4 Amounts of substances recovered in acetone eluates

Acetone eluates	expected amount range	Measured amounts			
		chemical analysis			average ± sd (n=3)
	(µg)	(µg)	(µg)	(µg)	(µg)
Sulfamethoxazole	0-1000	477	211	460	382±149
Caffeine	0-5000	666	336	643	549±185
Metronidazole	0-5000	114	55	124	98±37
Enalapril-maleate	0-5000	1409	695	1449	1184±424
Norfloxacin	0-5000	1189	713	1433	1112±366
Carbadox	0-5000	452	243	382	359±106
Metformin	0-5000	0	0	0	0

Table C5 Concentrations in water concentrates: expected concentration ranges and measured concentrations

Water concentrates	expected concentration range	Measured concentrations			
		chemical analysis			average ± sd (n=3)
	(mg/l)	(mg/l)	(mg/l)	(mg/l)	(mg/l)
Sulfamethoxazole	0-100	19	44	18	27±15
Caffeine	0-500	48	55	46	50±4
Metronidazole	0-500	7	7	5	7±1
Enalapril-maleate	0-500	93	94	75	87±11
Norfloxacin	0-500	65	63	65	64±1
Carbadox	0-500	15	17	18	17±2
Metformin	0-500	0	0	0	0

Annex D **Availability new sorbents for wide spectrum extraction from water.**

New sorbents that are commercially available. They are applied in chromatography. Details on application and use have to be collected.

Supplier	Sorbent name	Details
Waters	OASIS-HLB	Alfred Middendorp, waters_nederland@waters.com
Mallinckrodt Baker	Bakerbond SDB-SC	Jan.Jacobs@emea.tycohealthcare.com
J.T. Baker	Bakerbond-Speedisk	
	Bakerbond SDB 1	
	active carbon	
Supelco/Sigma Aldrich	Discovery DPA-6S	particle size 50µm < size < 180 µm
Varian	Hayesep B	
	Hayesep T	
Argonaut UK (Biotage)	Insolute env+	Claire.Desbrow@eu.biotage.com
	Insolute C18	

Potential use is dependent on extraction environmental conditions, do they have to be adapted (pH), and ease to use. A sorbent should preferably have a not too small particle size, so that it can be added to a water sample (analogous to the use of XAD). After extraction, it must be easy to collect. Sorbents that have extremely small particle sizes and therefore must be used in a chromatography column may be unpractical because of clogging of the column when using it. Alternative way is to find a way to adsorb chemicals in an ‘inverted tea bag’ (adsorption from water into the tea bad instead of releasing chemicals from the teabag into water).

OASIS-HLB is used by Heike Schmitt (Utrecht University) for extraction of Sulfamethoxazole, adjustment of pH.