Development of a solid phase extraction method for a wide group of pharmaceutical and industrial compounds in wastewater for the analysis with LC-MS/MS

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List of abbreviations

SPE= solid phase extraction

WWTP= wastewater treatment plant

SDS= sodium dodecyl sulfate

CBZ= carbamazepine

IBU= ibuprofen

NAP= naproxen

KET= ketoprofen

DIC= diclofenac

4CA= 4-chloroaniline

MET= metformin

NMZ= n-methylpiperazine

GUA= guanylurea

Index

Samenvatting 5
Introduction 6
Experimental 13
Results and discussion 20
Conclusion 39
Reference 40
Appendix 1. Parameters of mass spectrometry 43
Appendix 2. LC-MS/MS data of calibration standards 44
Appendix 3. LC-MS/MS data of the multi-component solutions 49
Appendix 4. LC-MS/MS data of calibration standards with internal standard 59
Appendix 5. LC-MS/MS data of wastewater samples 64

Samenvatting

Verscheidene studies hebben aangetoond dat de toegepaste waterzuiveringsinstallaties niet efficiënt genoeg zijn om geneesmiddelen te verwijderen [1,2]. Residuen van farmaceutische en industriële verbindingen en hun metabolieten zijn aangetroffen in afvalwater en oppervlaktewater in verschillende landen [2]. Vanwege deze kwestie is het belangrijk om een goede methode te hebben om veel verschillende farmaceutische of industriële verbindingen te extraheren. Vaste fase-extracties kunnen veel tijd in beslag nemen bij het extraheren van veel monsters, voornamelijk wanneer er meerdere extractiemethoden op de monsters worden toegepast. Daarom was het doel van dit onderzoek om één vaste fase-extractie methode te ontwikkelen voor een brede groep farmaceutische en industriële verbindingen. Voor de ontwikkeling van de methode werden er vijf parameters (kolom type, reagens, eluens volume, kolom grootte en pH) geselecteerd. De parameters zijn geoptimaliseerd in dezelfde volgorde als hiervoor benoemde. Elke parameter was individueel geoptimaliseerd. Om te bepalen of een parameter geoptimaliseerd was, werden water monsters gespiked met standaarden om vervolgens de recovery te bepalen. Voor de kolom type (stationaire fase) werden er drie kolommen (Oasis HLB, Oasis MCX en Sep-Pak C18) getest. De Oasis HLB kolom verkreeg de beste resultaten. Voor het reagens natriumdodecylsulfaat (SDS) werden er twee verschillende concentraties (2 mM en 6 mM) getest. De resultaten hadden aangetoond dat 6 mM SDS het meest geschikt concentratie was. Voor het eluens volume werden er twee verschillende hoeveelheden getest, 4 mL en 8 mL. De resultaten hadden aangetoond dat het eluent volume van 8 mL de meeste verbindingen had geëxtraheerd. Voor de optimalisatie van de kolom grootte werden er twee verschillende groottes, 60 mg en 150 mg, getest. De meest geschikte kolom grootte was 150 mg van Oasis HLB. Tot slot werd er gekeken of het toepassen van twee verschillende aanzuringsmethoden een verbetering in recovery resulteerde. Bij de eerste aanzuringsmethode werden alle werkoplossingen aangezuurd. Bij de tweede methode werd alleen het eluens aangezuurd. Beide aanzuringsmethoden waren niet geschikt voor deze methode. Met de geoptimaliseerde parameters werd voor alle componenten een recovery van >60% waargenomen. Dit concludeert dat er één vaste fase-extractiemethode is ontwikkeld voor een brede groep van farmaceutische en industriële verbindingen.

Introduction

This paper describes the development of one solid phase extraction method for a wide group of pharmaceutical and industrial compounds in wastewater that is analyzed with two LC-MS/MS methods.

At the moment there are many solid phase extraction methods for our compounds of interest but few methods are suitable for highly polar compounds. Also adding that solid phase extractions can be time consuming when extracting multiple samples. Applying multiple extraction methods to samples will even prolong the process. To save time one extraction method has been developed to extract all compounds of interest in one single step.

The following parameters have been optimized to obtain the main goal:

- Sorbent type (stationary phase)
- Addition of a reagent and in which concentration
- Eluent volume (to eluent the compounds)
- Sorbent size
- Addition of acids (to acidify the matrix)

Studies have indicated that applied wastewater treatment plants are not efficient enough to remove pharmaceuticals[1,2]. Despite being diluted and degraded, pharmaceuticals may have a toxic effect on the environment because they are continuously being released [5,6]. Residues of pharmaceutical and industrial compounds and their metabolites have been found in wastewater and surface water in a variety of countries[2]. With this matter it's important to have an good method to extract a lot of different pharmaceuticals or industrials compounds to monitor the compounds in wastewater. When this can be accomplished, quantification and identification can be performed. With this information it can be determined whether the current wastewater is contaminated and if so, how it can be removed through wastewater treatment plants.

Occurrence of pharmaceuticals in wastewater

Large amounts of pharmaceuticals are used worldwide. Pharmaceuticals is a large and diverse group of compounds designed to prevent, cure and treat disease and improve health. They have been used in significant quantities throughout the world. The production and consumption of pharmaceuticals increased over the years due to many reason for example; discoveries of new drugs and increase of population[1].Pharmaceutical compounds are bio-active and they have an effect on living beings. This could lead to a toxic effect on aquatic organisms[2]. Aquatic organisms come into contact with pharmaceuticals that end up in wastewater. Pharmaceuticals can enter the water supply in a variety of ways. For example after consumption, pharmaceutical compounds undergo metabolic processes in organism or pass through the body unaltered. Fractions of the parent compound are excreted in unmetabolized form or as metabolized (active or inactive) into sewage and wastewater treatment plants (WWTP). The metabolites that have been broken down from the pharmaceutical compounds can be more bio-active than the drug itself[1].Because of this matter research on pharmaceutical compounds in wastewater is needed.

Other sources that pharmaceuticals and their metabolites can be exposed to the wastewater aside from excretion of used pharmaceuticals by human or animals include production residues, improper disposal of expired medicines and unused drugs, hospitals, landfill leachates and accidental spillage during manufacturing and distribution[3,7]. See figure 1.

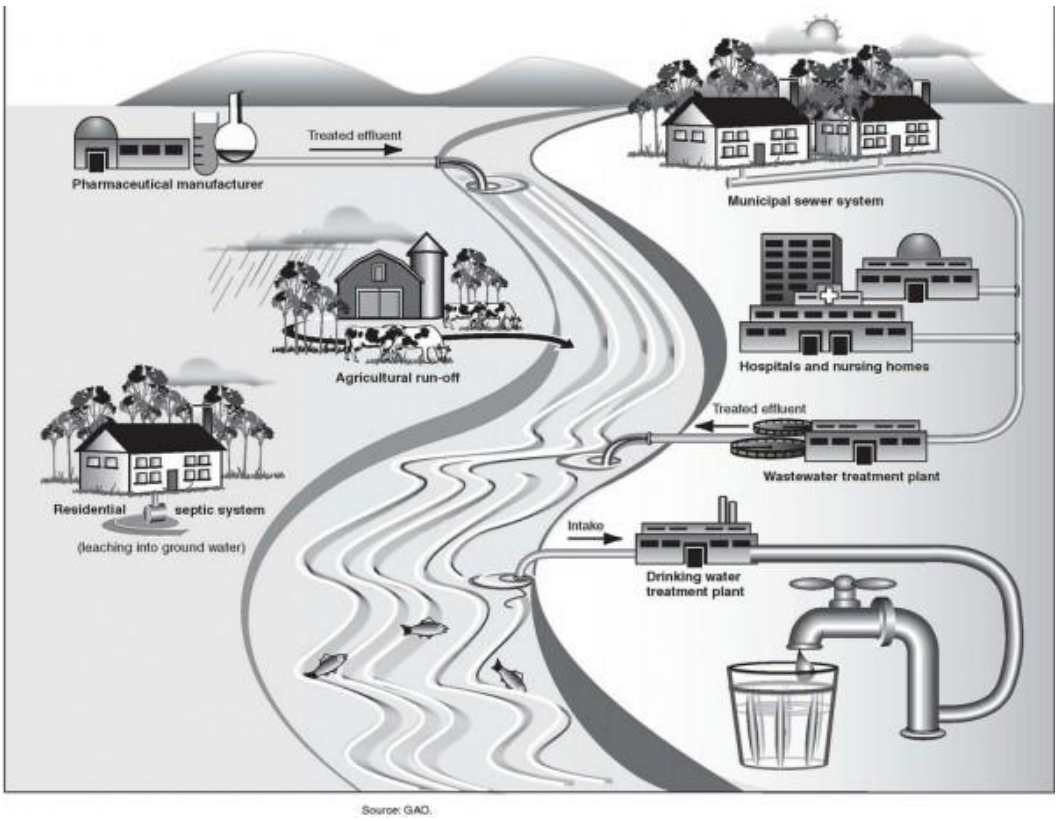

Figure1.Pathways of residue compounds in wastewater. Source GAO.

Wastewater treatment plants (WWTP) remove and purify contaminated wastewater. Physical or chemical and biological processes are used to clean up wastewater. However studies indicated that applied WWTP is not efficient enough to remove pharmaceuticals[1,2,3,6,8,17]. Residues of pharmaceutical compounds and their metabolites have been found in wastewater and surface water in a variety of countries[2]. A wide range of pharmaceuticals have been measured in wastewater and surface water with a range from ng/L to low µg/L[5,10,14].Exact reference values of the concentration of compounds that may be present in the water have not been found. Wastewater treatment plants (WWTPs) are generally not designed to remove complex pharmaceuticals, as they were built and upgraded with the aim of removing easily or moderately biodegradable carbon, nitrogen and phosphorus compounds and microorganisms[9].

High consumption and incomplete removal of pharmaceutical compounds can lead to reachable detectable concentrations of pharmaceuticals in wastewater. Despite being diluted and degraded, pharmaceuticals may have a toxic effect on the environment because they are continuously being released [5,6].Studies have also shown that combinations of pharmaceutical compounds have a much stronger toxic effect on the environment than the weak toxic effect of each compound individually[8,15].

With the developed solid phase extraction method the contamination of wastewater will be determined with 6 pharmaceutical compounds, 2 industrial compounds and 1 transformation compound. The selected pharmaceuticals belong to different classes; anti-inflammatory (ibuprofen, ketoprofen, naproxen, diclofenac) anti-epileptics (carbamazepine) and anti-diabetic (metformin). In Europe, usage of metformin has increased as it is among the top applied drugs for all stages of diabetic. Metformin is not metabolized in the human body and therefore passed through the body unaltered[11,16]. Compounds such as metformin are highly water soluble, which limits their removal during treatments of wastewater. In wastewater, metformin can have an affect on aquatic organisms and (or) undergo further biodegradation. Guanylurea, is the major transformation product of metformin[11].For this research two industrial compounds are chosen, 4-chloroaniline and n-methylpiperazine. 4-chloroaniline are used in the industrial production of pesticides, drugs, and dyestuffs. N-methylpiperazine is used for the production of plastics, resins, pesticides and brake fluid. See figure 2 for molecule structure of the compounds. Wastewater samples (influent and effluent) has been taken from 5 WWTP's across the Netherlands. Influent water samples are water samples that haven't been through a WWTP and are still contaminated. Effluent water sample are water sample that have been passed through the WWTP. In theory, influent wastewater contain higher concentration of pharmaceuticals in comparison to effluent wastewater.

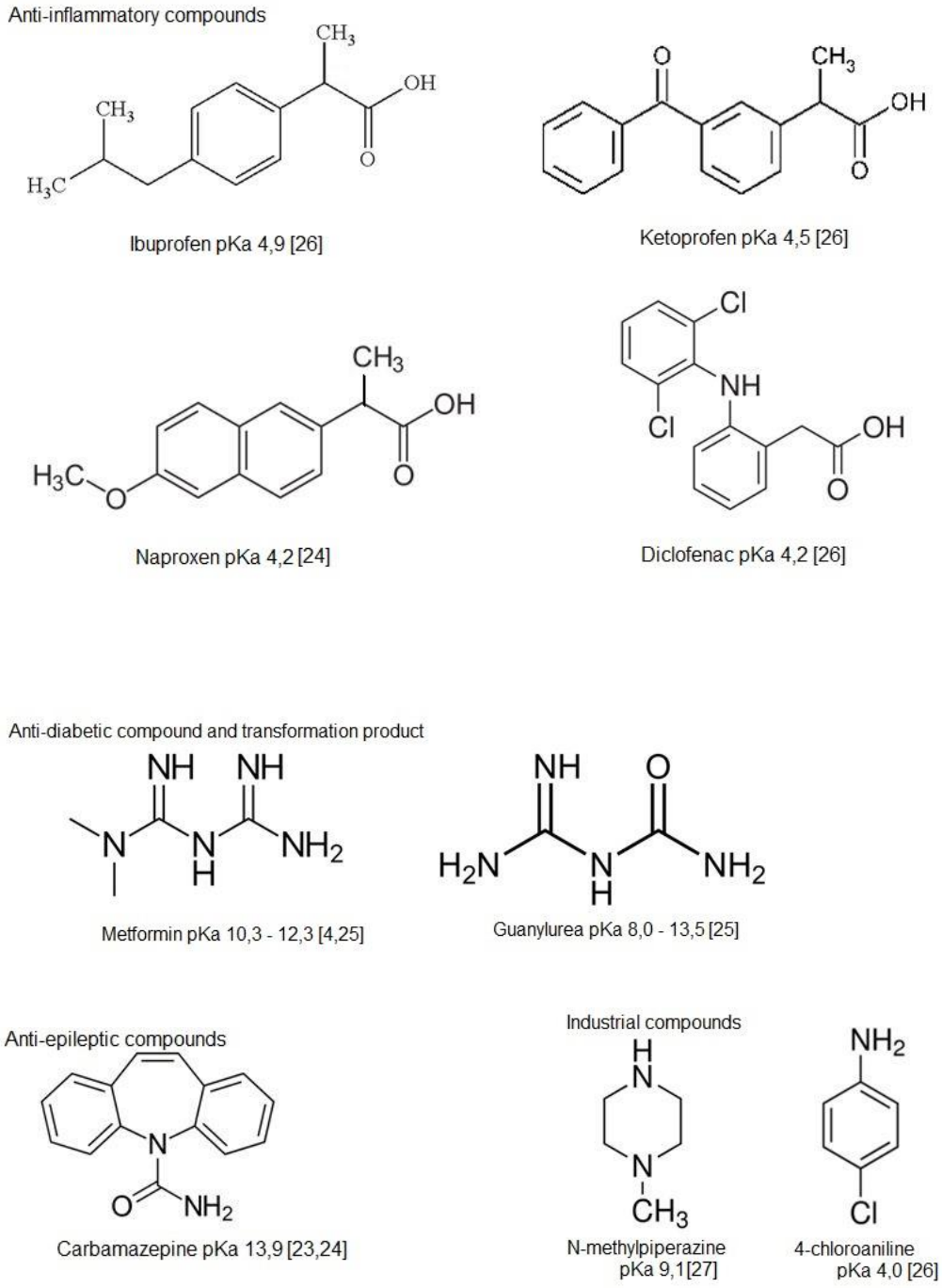

Figure 2.Molecule structure of the compounds with pKa.

For the analysis of pharmaceuticals in wastewater an extraction is involved for enrichment of the pharmaceuticals and clean up of matrix substances followed by instrumental analysis for separation and quantification. For water samples, solid phase extraction (SPE) is the most used method to extract pharmaceuticals of large volumes of water[1,2,3,4,6,7,8,12,13]. SPE is a sample preparation method to extract compounds that are dissolved in water and to separate them from other matrix compounds. SPE has different parameters that affect the efficiency of the extraction, for example the sorbent (functions as stationary phase that has interactions with the compounds to cause the compounds to have a retention time and 'stick' to the sorbent), elution solvents (functions as mobile phase), pH of the matrix and possible reagent. Therefore development of an extraction method was done with the aim of achieving a satisfying recovery for a wide group of compounds in a single extraction step.

For the optimization of the SPE the parameters: sorbents type,sorbent size,reagent, volume of solvent and acidification of the matrix were taken into account. In previous studies, the most used sorbents for extraction of pharmaceuticals were polymer sorbents Oasis HLB, Oasis MCX and silica-based Sep-Pak C18. Oasis HLB is an universal sorbent for acidic, neutral and basic compounds. Oasis HLB has a hydrophilic-lipophilic balance, water-wettable, reversed-phase sorbent. The hydrophilic-lipophilic balance is made from hydrophilic N-vinylpyrrolidone and lipophilic divinylbenzene. The lipophilic part provides reversed-phase capacity with a neutral polar 'hook' for enhanced retention of polor compounds[13]. See figure 3.

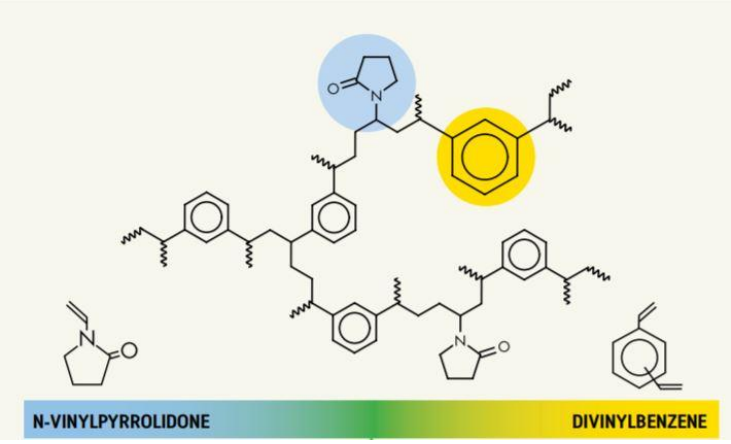

Figure 3.Oasis HLB has a hydrophilic N-vinylpyrrolidone and lipophilic divinylbenzene[13].

Oasis MCX is a strong mixed-mode cation exchange, water-wettable, polymeric sorbent. It is made from sulfonic groups to provide ion-exchange and reversed phase. Therefore MCX is suitable for neutral and cationic compounds[7,13]. See figure 4.

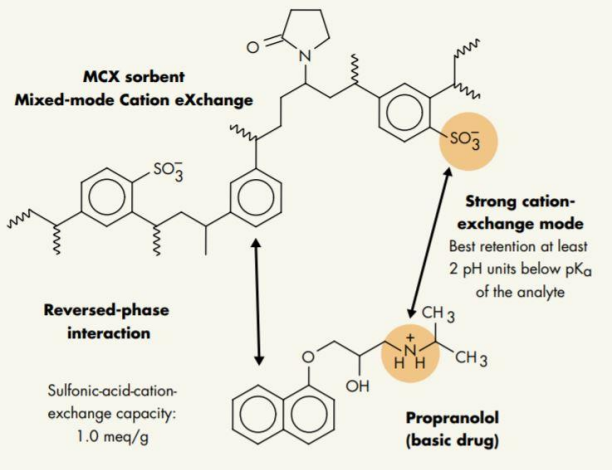

Figure 4.Oasis MCX with a strong mixed-mode cation exchange mode[13].

Sep-Pak C18 is a silica based sorbent that is non-polar. C18 has interactions with weak to high non-polar compounds from polar matrices, like water. Although it's a non-polar sorbent slightly polar compounds mind have a longer retention time with the sorbent than polar compounds that comes off immediately after eluation.

Currently the institute IBED is using a SPE method with Oasis HLB sorbent for drinking water with 'polar' compounds. The following method doesn't work for a wide group of compounds with a high polarity. Therefore it's interesting to see whether further optimization of the method includes compounds with a high polarity. It has been showed that adding an ion-pair reagent to an Oasis HLB method increases the recovery of highly polar compounds.[4]The ion-pair reagent that was used is sodium dodecyl sulfate (SDS). SDS is an anionic surfactant with a long non-polar and a polar sulfate group. See figure 5. The long non polar tail have a Van der waals interaction with the lipophilic part of the Oasis HLB sorbent. Where else the polar 'head' of the SDS has an ion exchange interaction with the compounds. Aside from the regular hydrophilic part of the Oasis HLB also have interaction with the compounds. This method is similar to the Oasis MCX sorbent, although with adding the ion-reagent separately the concentration of the ion-reagent can be varied to achieve to optimum interaction between the compounds and the sorbent. With the addition of SDS the compounds can have more interaction with the sorbent and have a longer retention time in the cartridge. This is good for the highly polar compounds.

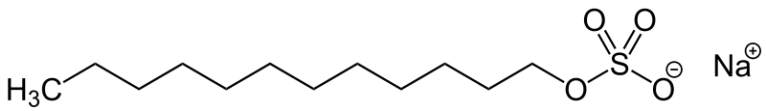

Figure 5. Sodium dodecyl sulfate (SDS)

In this research also will be tested whether acidification of the matrix improves the extractions of the compounds. Working with ion-exchange reagents (MCX and SDS) it is important that the compounds are ionized. By acidification of the compounds, the compounds are been protonated to have an interaction with the reagents. Furthermore the sorbent size of the cartridges will be tested to see whether the maximum of adsorbed compounds relates to the size of the sorbent material. Lastly to determine the optimal eluent volume to extract the maximum of adsorbed compounds, different volumes of eluent were tested.

To determine whether a method is optimized, a recovery percentage was determined with ultrapure water spiked with a known concentration of the tested compounds. The reproducibility of the method was determined by repeating the SPE method multiple times to see if the same recovery was achieved. For each SPE method a blank was included. The separation and quantification technique that is most used for the analysis of pharmaceuticals is liquid chromatography coupled with triple-quadrupole mass spectrometry (LC-MS/MS) due to the polarity of most of the compounds. LC-MS/MS is also high in selectivity, sensibility and robust detection.[6,7,8] Gas chromatography has been used in some cases, but most of the time it needs a time-consuming derivatization step, and is not compatible with thermolabile compounds.[3,8,12]

Multiple reaction monitoring (MSM) scan mode has been used for specific fragments for the quantification (and identification) of compounds in complex matrix. Two or three fragments were selected for identification, and the most intense fragment was used for quantification.[1,2,8] When using the LC-MS/MS, electrospray ionization (ESI) has been used as source because it allows rapid, accurate and sensitive analysis. ESI is a low-energy ionization that doesn't cause fragmentation of molecular ions and is therefore recommended for polar and thermally labile compounds such as the pharmaceuticals.[7]

In this research two different LC-columns are used to separate the compounds, a silica based C18 column and HILIC column. HILIC column is used for the highly polar compounds like metformin, guanylurea and n-methylpiperazine because on the C18 column the highly polar compounds are been flushed away with the water based mobile phase. HILIC is a combination of the characteristics of the 3 major methods in liquid chromatography: reversed phase, normal phase and ion chromatography.The stationary phase of HILIC is just like normal phase polar, while the mobile phase in HILIC is relatively polar and resembles reversed phase. HILIC uses eluent that consists of water and organic solvent just like reversed phase liquid chromatography. HILIC is suitable for aqueous matrices with (highly) polar compounds. As for normal phase liquid chromatography is not the case, it is not suitable for aqueous matrices. With the use of HILIC the highly polar compounds will have a better interaction with the stationary phase of the column that increases the retention time of the compounds in comparison with the C18 column.

Experimental

Materials and chemicals

The following pharmaceutical and industrial standards were used in this research. Ibuprofen (purity: 99%, cas: 15687271) was supplied by Acros. Diclofenac sodium salt (cas: 15307796), ketoprofen(purity: 98%, cas: 22071154), naproxen(purity: 99%, cas: 22204531), carbamazepine(cas: 298464), metformin hydrochloride(cas: 1115704), 4-chloroaniline (purity: 99%, cas: 106478), n-methylpiperazine (purity: 99%, cas: 109013), guanylurea sulfate salt hydrate (purity: 97%, cas: 207300865) were supplied by Sigma Aldrich.

For internal standard a mixture of isotope labelled compounds from 50 ppb were used (prepared by IBED). The mixture contained the following isotopes; metformin-D6, carbamazepine-C13, diclofenac-13C, ketoprofen-D3, naproxen-D3, guanylurea-15N4 and ibuprofen-D3. The ion-pair reagent that was used is sodium dodecyl sulfate (purity: 99%, code no. 17131301) was supplied by PlusOne.

The solvents, methanol absolute ULC/MS-CC/SFC (purity: 99,9%;cas: 67561), acetonitrile LC-MS (purity:99%, cas: 75058), formic acid ULC/MS-CC/SFC (purity:99%,cas: 64186), acetic acid glacial ULC/MS-CC/SFC (purity:99.9%,cas: 64197) were supplied by Biosolve and dichloromethane (purity: 99%, cas: 75092) was supplied by VWR.

The cartridges used for solid phase extraction were Oasis HLB(150 mg,60 µm), Oasis HLB (60mg, 60 µm), Sep-Pak VacC18 (500 mg,55-105 µm) and Oasis MCX(150 mg,60 µm) were supplied by Waters. The extraction manifold was supplied by Waters. The pump ME 1C was from Vacuubrand.

Other supplies that were used, roller mixer (SRT9D) from Stuart, centrifuge (Rotanta 460) from Hettich, ultrasonic cleaner USC-TH from VWR and water bath DWB-16 from LaboTech.

Wastewater sample(influent and effluent)of wastewater treatment plant from Amsterdam-West,Amstelveen,Bennekom,Eindhoven and Utrecht are stored at -20℃.

LC-MS/MS analysis

The analysis was performed on liquid chromatogram (UFLC-XR,Shimadzu) equipped with C18 column(1.6 µm, 50 x 2.0 mm, Shim-Pack, Shimadzu) with a securityGuard ultra-cartridge as guard column and HILIC column (1.7 µm, 50 x 2.1 mm, amide BEH, Waters).The liquid chromatogram was coupled to a mass spectrometry (MDS SCIEX 4000Qtrap) from Applied Biosystems equipped with an electrospray ionization source. The following parameters were used in positive and negative mode; collision gas: 6 L/h, curtain gas: 10 L/h, ion-spray voltage: 4000 C, temperature: 500 °C, ion source gas 1: 40 L/h and ion source gas 2: 50 L/h. Measurements were done in multiple reaction monitoring (MRM) mode, detailed parameters for MRM acquisition are presented in Appendix 1 table 1.

For the C18 column analyses, two chromatographic runs were performed in order to determine the compounds ibuprofen, diclofenac (in negative ionization mode) and ketoprofen, naproxen, carbamazepine and 4-chloroaniline (in positive ionization mode). Flow through the C18 column was maintained at 0,3 mL/min and the injection volume was 20 µL. The mobile phase for both ionization mode consisted of ultrapure water containing 0,1% (v/v) acetic acid (solvent A) and methanol (solvent B).The gradient elution program for both modes was started at 10% solvent B and increased to 100% at 1 min and hold at 100% to 2,5 min, afterwards solvent B was decreased to 10% to 4 min. Equilibration of the column was 3 min in both cases.

For the HILIC column analyses, the compounds metformin, n-methylpiperazine and guanylurea were determined in positive mode. Flow through the HILIC column was maintained at 0,4 mL/min and the injection volume was 20 µL. The mobile phase consisted of ultrapure water containing 5 mM ammonium format and 0,075% (v/v) formic acid (solvent A) and acetonitrile containing 5 mM ammonium format and 0,075% (v/v) formic acid (solvent B). The gradient eluation program started at 95% solvent B and decreased to 65% to 1,2 min then it was decreased to 50% to 1,4 min and hold to 3 min, afterwards solvent B was increased to 95% to 4 min. Equilibration of the column was 3 min.

Preparation stock solvents of the compounds

Of each compound a stock solution of 1000 ppm was prepared in methanol except for guanylurea. Guanylurea was prepared in ultrapure water. A multi-component solution for the development of the solid-phase extraction was obtained by diluting the stock solutions of the 9 compounds in ultrapure water to a final concentration of 1 ppm. All stock solutions and working solutions were stored in the fridge at 4˚C.

Preparation calibration standards

Calibration standards of each compound was prepared from the stock solutions of 1000 ppm. The following concentration 200 – 5 ppb was prepared by diluting the stock solutions of the compounds in methanol, except for guanylurea in ultrapure water, to a final concentration of 1 ppm. From this solution different volumes were pipetted in 10 mL vials with methanol, except for guanylurea, metformin and n-methylpiperazine they were diluted in ultrapure water, to obtain the final standard concentrations of 200 – 5 ppb. The standards were stored in the fridge at 4˚C. At the end the standards were diluted 10 times in 1 mL HPLC vials to a final concentration of 20 – 0,5 ppb to measure on the LC-MS/MS.The standards measured on the C18 column were diluted in ultrapure water and the standards measured on the HILIC column were diluted in acetonitrile. When measuring samples with internal standard, 100 µL of the internal standard of 50 ppb was added to the standards and diluted to 1 mL together with the standards to obtain 20 – 0,5ppb final concentration standards and 5 ppb internal standard.

Optimization sorbent

For the optimization of the sorbent type, three different sorbents were used; Oasis HLB, Sep-Pak C18 and Oasis MCX. For Oasis HLB, 150 mg cartridge was used, Sep-Pak C18 cartridge 500 mg and Oasis MCX 150 mg. For all methods a multi-component solution was used to pass through the cartridges as sample. For the method of Oasis HLB and Sep-Pak C18 no acid was added. For the method of Oasis MCX the multi-component solution contained 0,1% (v/v) formic acid[7]. Also all working solvents from the MCX method contained 0,1% (v/v) formic acid expect for the eluent methanol. The isolation solvent was obtained by adding 5% (v/v) of methanol in ultrapure water and 0.1% (v/v) formic acid.

For the Sep-Pak C18 method the eluent contained 3% acetic acid in methanol[18,19]. At each step the solvents were passed by the cartridges a slow flow rate. The methods used for the sorbents are shown in table 1. The eluates were collected in falcon tubes for evaporation and reconstitution.

Table1. Solid extraction methods of the sorbents Oasis HLB, Oasis MCX and Sep-Pak C18

Cartridge:	Oasis HLB	Sep-Pak C18	Oasis MCX
Condition:	*5 mL MeoH*	*5 mL MeOH*	*10 mL MeOH with 0,1% formic acid*
Pre-Cond:.	*5 mL H_2O*	*5 mL H_2O*	*5 ml H_2O with 0,1% formic acid*
Sample:	*10 mL*	*10 mL*	*8 mL containing 0,1% formic acid*
Wash:	*2 mL H_2O*	*2 mL H_2O*	*5 mL H_2O with 0,1% formic acid*
Dryness:	*15 min*	*15 min*	*10 min*
Isolation:	*-*	*-*	*1 mL 5% MeOH in H_2O with 0,1% formic acid*
Eluation:	*4 x 1,5 mL (6 mL) MeOH*	*5 mL MeOH + 3% HOAc*	*4 mL MeOH*

Optimization reagent

For the optimization of the reagent, sodium dodecyl sulfate (SDS), different concentrations were used. For the optimization of SDS, 2 mM and 6 mM SDS were used[4].The following method was used for each SDS concentration. Oasis HLB cartridges (150 mg) were conditioned using 10 mL of methanol. Pre-conditioning was performed by using 5 mL of ultrapure water and 5 mL SDS. After pre-conditioning 10 mL of the multi-component solution was used to pass through the cartridges as sample. Afterwards the cartridges were washed with 5 mL ultrapure water and dried for 10 min under vacuum. The isolation solvent was obtained by adding 5% (v/v) of methanol in ultrapure water and 1 mL of the solution was passed through the cartridges. The compounds were eluated with 4 mL methanol. At each step the solvents were passed by the cartridges a slow flow rate. The eluates were collected in falcon tubes for evaporation and reconstitution.

Optimization volume eluent

To determine the optimal eluent volume to extract the maximum of adsorbed compounds, different volumes (4 mL and 8 mL) of eluent were tested. Methanol was used as eluent. The following method was used for both extractions. Oasis HLB cartridges (150 mg) were conditioned using 10 mL of methanol. Pre-conditioning was performed by using 5 mL of ultrapure water and 5 mL 6 mM SDS. After pre-conditioning 10 mL of the multi-component solution was used to pass through the cartridges as sample. Afterwards the cartridges were washed with 5 mL ultrapure water and dried for 10 min under vacuum. The isolation solvent was obtained by adding 5% (v/v) of methanol in water and 1 mL of the solution was passed through the cartridges. The compounds were eluated with 4 mL and (8 mL for the other extraction) methanol. At each step the solvents were passed by the cartridges a slow flow rate. The eluates were collected in falcon tubes for evaporation and reconstitution.

Optimization sorbent size

For the optimization of the sorbent size, 2 different sizes were used from Oasis HLB, 150 mg and 60 mg cartridges. On both cartridges the same extraction method was performed. Both cartridges were conditioned using 10 mL of methanol. Pre-conditioning was performed by using 5 mL of ultrapure water and 5 mL 6 mM SDS. After pre-conditioning 10 mL of the multi-component solution was used to pass through the cartridges as sample. Afterwards the cartridges were washed with 5 mL ultrapure water and dried for 10 min under vacuum. The isolation solvent was obtained by adding 5% (v/v) of methanol in ultrapure water and 1 mL of the solution was passed through the cartridges. The compounds were eluated with 8 mL methanol. At each step the solvents were passed by the cartridges a slow flow rate. The eluates were collected in falcon tubes for evaporation and reconstitution.

Optimization pH matrix

To determine whether changing the pH increases the recovery, 2 types of acidifications were performed. The first acidification, 0,1% (v/v) formic acid was prepared for all working solvents including the multi-component solution and excluding the eluent. The second acidification, only the eluent was acidified with 0,1% (v/v) formic acid. For both cases the same method was used only in different pH, table 2. The cartridge used for this optimization was Oasis HLB (150 mg). The eluates were collected in falcon tubes for evaporation and reconstitution.

Table2.Solid extraction methods of 2 acidifications of solutions.

	Acidification method 1	**Acidification method 2**
Condition:	*10 mL MeOH with 0,1% formic acid*	*10 mL MeOH*
Pre-Cond:.	*5 ml H_2O with 0,1% formic acid,* *5 mL 6mM SDSwith 0,1% formic acid*	*5 mL H_2O, 5 mL 6 mM SDS*
Sample:	*8 mL with 0,1% formic acid*	*10 mL*
Wash:	*5 mL H_2O with 0,1% formic*	*5 mL H_2O*
Dryness:	*10 min*	*10 min*
Isolation:	*1 mL 5% MeOH in H_2O with 0,1% formic acid*	*1 mL 5% MeOH in H_2O*
Eluation:	*8 mL MeOH*	*8 mL MeOH with 0,1% formic acid*

Evaporation, reconstitution and dilution of the multi-component samples

The eluates collected in falcon tubes were evaporated at 60˚C with water bath to dryness by a gentle nitrogen stream. Then, the extracts were reconstituted in 1 mL of methanol. The reconstituted samples were further diluted to measure on the SCIEX.

Dilution for the C18 column: from the reconstituted samples 10 μL was pipetted and diluted with methanol to 1 mL. Then, 100 μL was pipetted from the previous dilution and diluted with water to 1 mL.

Dilution for the HILIC column: from the reconstituted samples 10 μL was pipetted and diluted with water to 1 mL. Then, 100 μL was pipetted from the previous dilution and diluted with acetonitrile to 1 mL.

Sampling from wastewater treatment plants

The wastewater samples (influent and effluent) have been taken from 5 wastewater treatment plants in Amsterdam-W, Amstelveen, Bennekom, Eindhoven and Utrecht and stored at – 20˚C.

Wastewater sample preparation

Wastewater samples were defrost to room temperature. Empty centrifuge tubes were weighed. In the centrifuge tubes 20 mL wastewater was added and the tubes with water were weighed again. Internal standard solution of 500 µL was added to the tubes with wastewater. Once again the tubes were weighed again. A blank and control sample were added to the batch of samples. A blank only contained ultrapure water and the control sample contained ultrapure water and 500 µL internal standard. The centrifuge tubes were homogenized on a roller shaker for 30 min. After homogenization the tubes were ultrasonicated for 10 min at room temperature and centrifuged for 5 min at 4000 rpm.

SPE method wastewater

Eventually the following method was used for the wastewater samples to extract the compounds from the matrix. Oasis HLB cartridges (150 mg) were conditioned using 10 mL of methanol. Pre-conditioning was performed by using 5 mL of ultrapure water and 5 mL 6 mM SDS. After pre-conditioning the upper layer of the samples in the centrifuge tubes were pipetted and passed through the cartridges. Afterwards the cartridges were washed with 10 mL ultrapure water and dried for 10 min under vacuum. The isolation solvent was obtained by adding 5% (v/v) of methanol in ultrapure water and 5 mL of the solution was passed through the cartridges. After the isolation solvent passed through the cartridges, 0,22 µm filters were placed between the cartridges and the crane. The compounds were eluated with 8 mL 0,1% formic acid (v/v) in methanol. At each step the solvents were passed by the cartridges a slow flow rate. The eluates were collected in falcon tubes for evaporation and reconstitution.

Concentration of the internal standard.

The internal standard (500 µL, 50 ppb) in the samples (20 mL) was diluted 41 times to a concentration of 1,22 ppb. From a beginning volume of 20,5 ml in total, the samples were passed through the cartridges and reconstituted in 0,5 mL methanol. After the reconstitution the internal standard was concentrated by 41 times to a concentration of 50 ppb. The samples were further diluted by 10 times to a concentration of 5 ppb of internal standard. This concentration of internal standard was prepared in the calibration standards.

As for the concentrations of the compounds in the water samples, the dilution with the internal standard and the amount of sample that had passed through were also taken into account.The final concentration measured on the mass spectrometry of the waste water samples were concentrated by 4 times.

Evaporation, reconstitution and dilution of the eluates from wastewater

The eluates collected in falcon tubes were evaporated at 60 ° C with water bath to dryness by a gentle nitrogen stream. Then, the extracts were reconstituted in 0,5 mL of methanol. The reconstituted samples were further diluted to measure on the SCIEX.

Dilution for the C18 column: from the reconstituted samples 100 µL was pipetted and diluted with water to 1 mL.

Dilution for the HILIC column: from the reconstituted samples 100 µL was pipetted and diluted with acetonitrile to 1 mL.

Results and discussion

To measure the concentration of the samples that were used to optimize the different parameters, standards were made for each compound. The standards were used to obtain calibration graphs to calculate the concentration of the samples.

The samples were analyzed with two types of LC-MS/MS methods. One LC-MS/MS method contained a C18 column and analyses were preformed in positive and negative mode. The second LC-MS/MS method contained a HILIC column and analyses were preformed in positive mode. In figure 6, 7 and 8 the chromatograms are given of the separation of the compounds from the multi-component solution passing through the C18 column in positive and negative mode and the HILIC column in positive mode. Since the liquid chromatogram is couple to the mass spectrometry, peaks of compounds that had the same retention time could still be distinguished from each other through their molecular mass and unique fragments pattern.

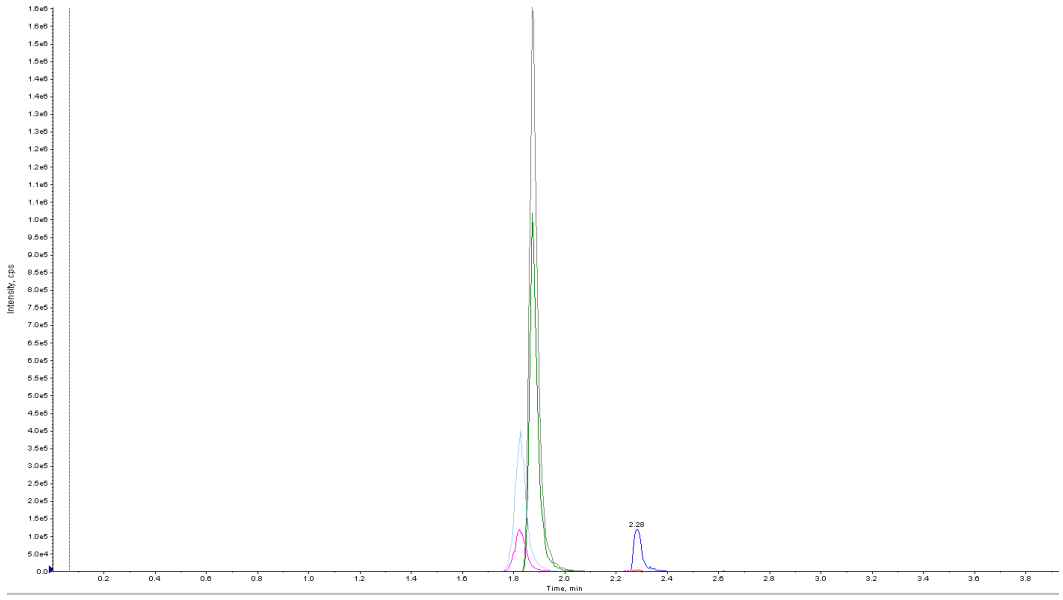

Figure 6. Chromatogram of the multi-component solution of the compounds guanylurea (1,80 min), metformin (1,88 min) and n-methylpiperazine (2,28 min) measured on the HILIC column in positive mode.

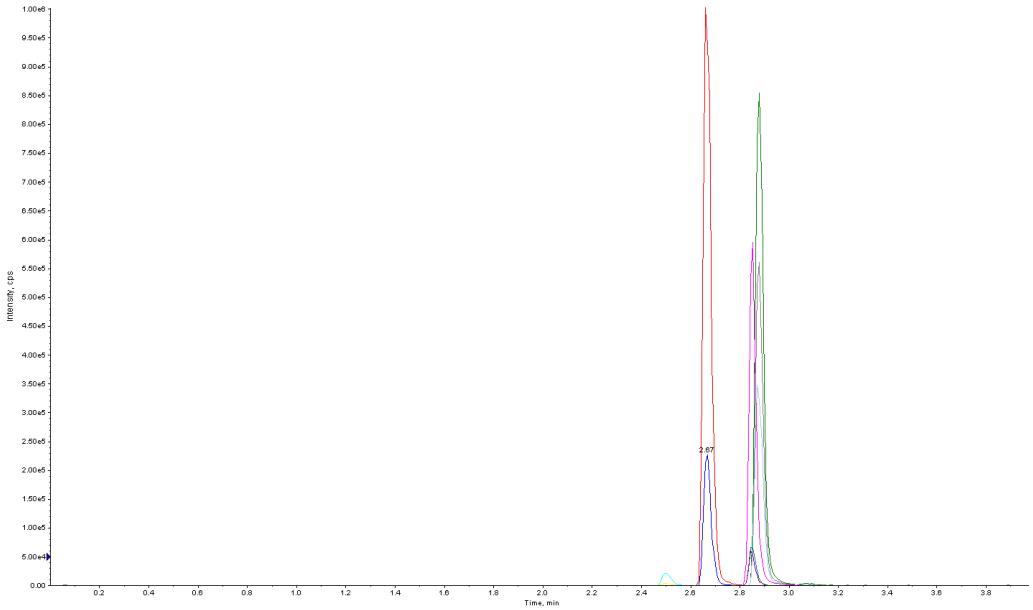

Figure 7. Chromatogram of the multi-component solution of the compounds 4-chloroaniline (2,50 min), carbamazepine (2,67 min) , ketoprofen(2,80 min) and naproxen (2,80 min) measured on the C18 column in positive mode.

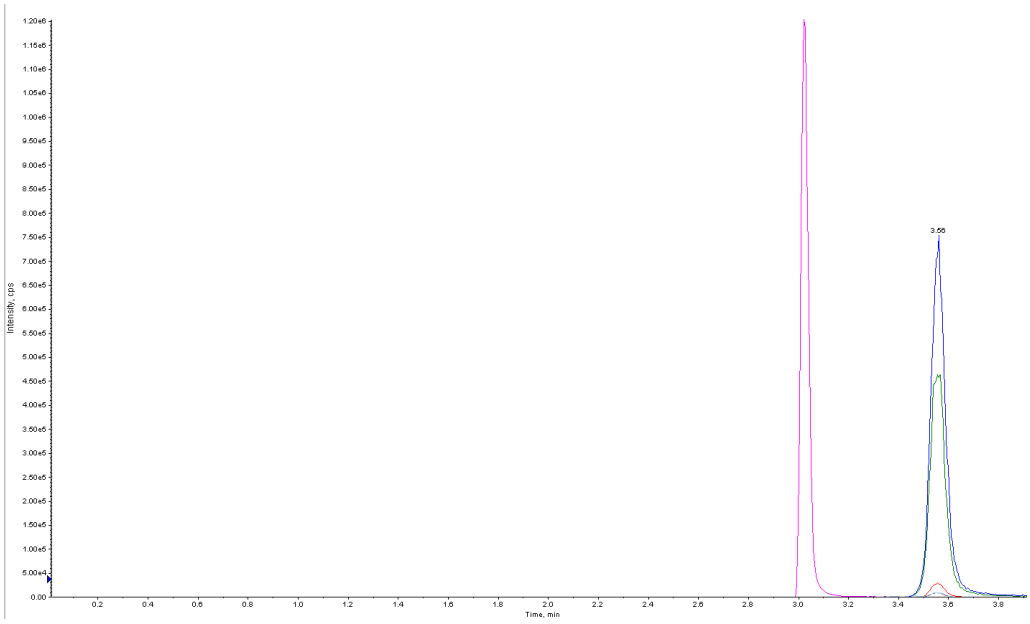

Figure 8. Chromatogram of the multi-component solution of the compounds ibuprofen (3,00 min) and diclofenac (3,59 min) measured on the C18 column in negative mode.

From the calibration graphs, linear regression (R^2:>0,9999) were obtained of each compound and used to quantify the extracted compounds. The fragmentations (Q1/Q3) from the compounds with the highest intensity were used for quantification. From the calculated concentrations of the samples the recovery was determined. The measured blanks that were added to the calibration standards and the extraction blanks obtained only background noise.

As an example, in figure 9 an calibration graph is given from one of the compounds (carbamazepine) to illustrate the linearity of the graph with the obtained equation of y= (263249,2 ± 770,9)x - 1038,91 ± 8230,4; BI95%; n=12; $s_{y/x}$= 7819,58; R^2 0,9999. In Appendix 2 the data of calibration standards of all compounds are given in tables 1-9. In table 3 the equations are given that were obtained of the measured standards.

Table3. Equations obtained from the measured calibration standards in Appendix 2.

Compound	Equation
Ketoprofen	y=(201319,9 ± 599,5)x + 2900,4 ± 6889,3; BI95%; n=12; $s_{y/x}$= 6902,9; R^2 0,9999
Naproxen	y=(136564,4 ± 343,0)x - 338,3 ± 3686,3; BI95%; n=12; $s_{y/x}$= 3592,0; R^2 0,9999
4-chloroaniline	y=(175373,7 ± 452,2)x + 780,5 ± 5188,1; BI95%; n=12; $s_{y/x}$ = 5142,1; R^2 0,9999
Diclofenac	y=(327842,2 ± 808,8)x + 49192,8 ± 9570,7; BI95%; n=12; $s_{y/x}$ = 9371,6; R^2 0,9999
Ibuprofen	y=(271412,7 ± 488,2)x + 29205,5 ± 5910,8; BI95%; n=12; $s_{y/x}$ = 5818,0; R^2 0,9999
N-methylpiperazine	y=(36832,6 ± 75,1)x + 2005 ± 798,8; BI95%; n=12; $s_{y/x}$ = 788,7; R^2 0,9999
Metformin	y=(489798,3 ± 638,3)x + 50041,8 ± 5464,1; BI95%; n=12; $s_{y/x}$ = 5233,3; R^2 0,9999
Guanylurea	y=(143903,5 ± 378,6)x + 24405,7 ± 3653,7; BI95%; n=12; $s_{y/x}$ = 3424,9; R^2 0,9999

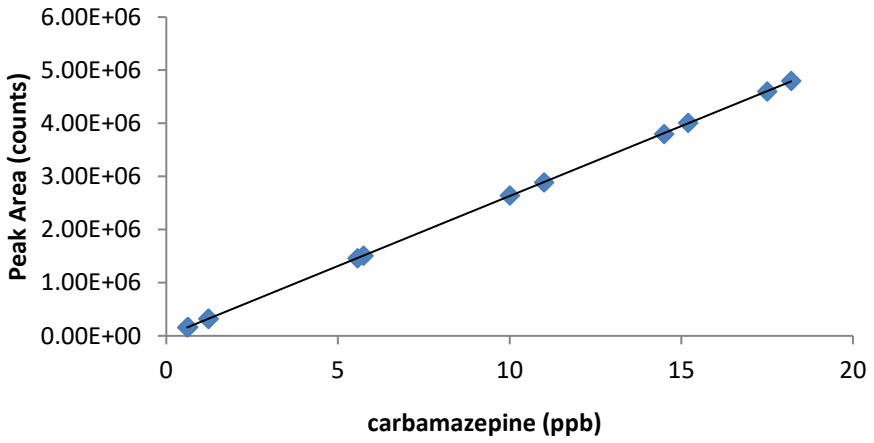

Figure 9. Calibration graph of carbamazepine measured on C18 column in positive mode. y= (263249,2 ± 770,9)x - 1038,91 ± 8230,4; BI95%; n=12; sy/x= 7819,58; R^2 0,9999

Theoretical concentration of carbamazepine in solution

For the preparation of the multi-component solution, stock solutions (1000 ppm) of the 9 compounds were diluted in ultrapure water to obtain the concentration of 1 ppm. As example, carbamazepine was diluted to a concentration of 0,9279 ppm. When 10 mL of the multi-component solution was taken to pass through the cartridges and afterwards reconstituted in 1 mL methanol, the concentration of carbamazepine was concentrated by 10 times to a concentration of 9,279 ppm. The sample then was diluted 1000 times to a concentration of 9,279 ppb. This was the theoretical concentration of carbamazepine in the sample.

Measured concentration of carbamazepine in solution

After the extractions the multi-component samples were measured on the LC-MS/MS. In Appendix 3 the data of the multi-component solution samples are given in tables 1-9. As example, in table 4 the samples extracted with the Oasis HLB method are given with the analyzed compound carbamazepine. In order determine the concentration of carbamazepine of the samples the following calculation was performed.

Table 4. Carbamazepine data of the multi-component samples extracted with Oasis HLB method. The concentration is shown in ppb, the peak area in counts and the recovery in percentage %.

Sample	Analyte	Peak area (counts)	Calculated concentration (ppb)	Calculated recovery (%)
1	Carbamazepine	2,19E+06	8,33	89,77
2	Carbamazepine	2,44E+06	9,27	99,90
3	Carbamazepine	2,41E+06	9,18	98,93

Calculations:

Example of concentration calculation

To calculate the concentration of carbamazepine in the samples, the equation obtained from the calibration graph was used. y = 263249,2 x - 1038,9

The measured peak area was y and the concentration was x.

$$x = \frac{y + 1038{,}9}{263249{,}2}$$

$$x = \frac{2.19 \times 10^6 + 1038{,}9}{263249{,}2} = 8{,}33\ ppb$$

Example of recovery calculation

The recovery was calculated with the measured concentration carbamazepine in the sample and the theoretical concentration that was added to the samples. The theoretical concentration of carbamazepine in the sample was 9,279 ppb.

$$Recovery\ \% = \frac{measured\ concentration\ (ppb) \times 100}{9{,}279}$$

$$Recovery\ \% = \frac{8{,}33 \times 100}{9{,}279} = 89{,}77\ \%$$

Example of standard deviation calculation

The average recovery of carbamazepine of the samples was 96,20 %
The standard deviation was calculated with the formula indicated below.

$$s = \sqrt{\frac{\sum(x - \bar{x})^2}{n - 1}}$$

$x = calculated\ recovery\ of\ the\ sample.$
$\bar{x} = average\ recovery\ of\ the\ samples.$ $n =$ $number\ of\ measured\ samples.$

$$s = \sqrt{\frac{62{,}49}{3 - 1}} = 5{,}59\ \%$$

Optimization sorbent

For the optimization of the sorbent type, three different sorbents were used; Oasis HLB, Sep-Pak C18 and Oasis MCX. For Oasis HLB, 150 mg cartridge was used, Sep-Pak C18 500 mg and Oasis MCX 150 mg cartridges. For all methods a multi-component solution was used to pass through the cartridges as sample. As a correction for possible contamination a blank was used. To measure reproducibility, the samples were extracted in triplicates. From the known concentrations of the compounds in the multi-component solution, the recovery was calculated from the compounds that had passed through the cartridges. In figure 10, the recovery of each compound from all cartridges are given.

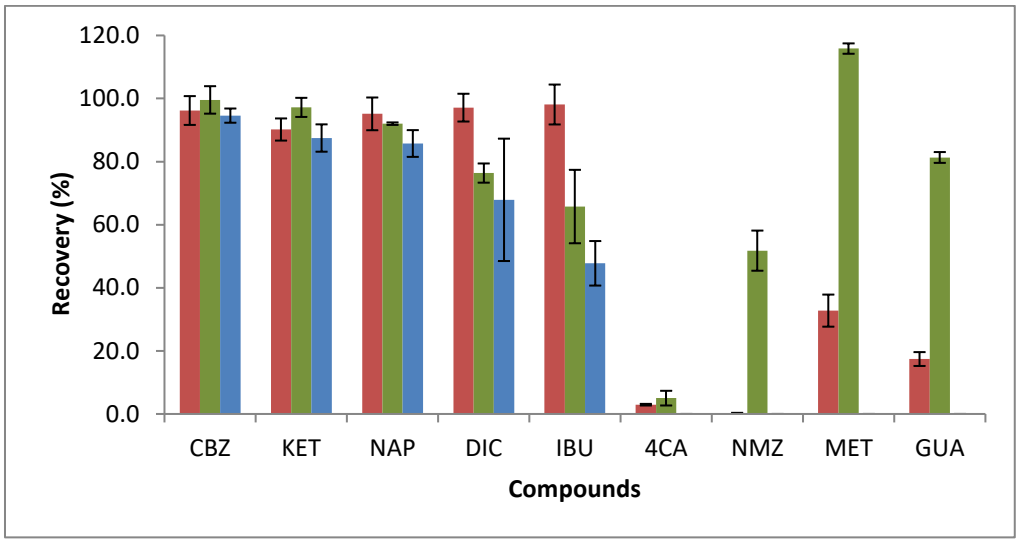

Figure 10.Recovery of each compound from 3 types of cartridges(red: Oasis HLB, blue: Oasis MCX and green: Sep-Pak C18). (CBZ: carbamazepine, KET: ketoprofen, NAP: naproxen, DIC: diclofenac, IBU: ibuprofen, 4CA: 4-chloroaniline, NMZ: n-methylpiperazine, MET: metformin and GUA: guanylurea); n: 3.

In figure 10, it can be seen that the Oasis MCX is the least suitable sorbent compared to Oasis HLB and Sep-Pak C18. Only the compounds carbamazepine, ketoprofen and naproxen obtained a recovery of >80%. Oasis MCX is a strong mixed-mode cation exchange polymeric sorbent. The compounds with low recovery probably had a strong interaction that could not be broken with the eluent. It is not recommended to change the level of acidification, because more acidification have a negative impact to most compounds and acidifying less will not protonate the compounds enough. If there is a strong interaction with the compounds and sorbent an eluent with high basic pH is recommended. The pKa of the compounds metformin, guanylurea and n-methylpiperazine are high therefore an addition of sodium hydroxide with 1 or 2 pKa level higher than the compounds is recommended to wash away the compounds from the sorbent. The most suitable sorbent is Oasis HLB. The compounds carbamazepine, ketoprofen, naproxen, diclofenac and ibuprofen obtained a recovery of >90%. Oasis HLB is an universal sorbent for acidic, neutral and basic compounds. Oasis HLB has a hydrophilic-lipophilic balance that provides reversed-phase capacity with a neutral polar ‘hook’ for enhanced retention of polor

compounds. Oasis HLB provides more possibilities, as it contained two sources of interaction, for optimization to obtain better recovery for the compounds 4-chloroaniline, n-methylpiperazine, metformin and guanylurea. These compounds are high in polarity.

Sep-Pak C18 sorbent obtained also good recovery >80% for the compounds carbamazepine, ketoprofen, naproxen and metformin. In comparison with Oasis HLB, Sep-Pak C18 obtained better recovery for the highly polar compounds 4-chloroaniline, n-methylpiperazine, metformin and guanylurea. From this results, Oasis HLB is chosen as sorbent because it provides both reversed-phase capacity and polar interaction.

Optimization reagent

For the optimization of the reagent, sodium dodecyl sulfate (SDS), different concentration were used. For the optimization of SDS, 2 mM and 6 mM SDS were used. Other than the reagent concentration, both extractions were in the same conditions. For this optimization Oasis HLB 150 mg cartridges were used, as result from the optimization of sorbent type. As a correction for possible contamination a blank was used. To measure reproducibility, the samples were extracted in triplicates. From the known concentrations of the compounds in the multi-component solution, the recovery was calculated from the compounds that had passed through the cartridges. In figure 11, the recovery of each compound from different reagent concentrations are given.

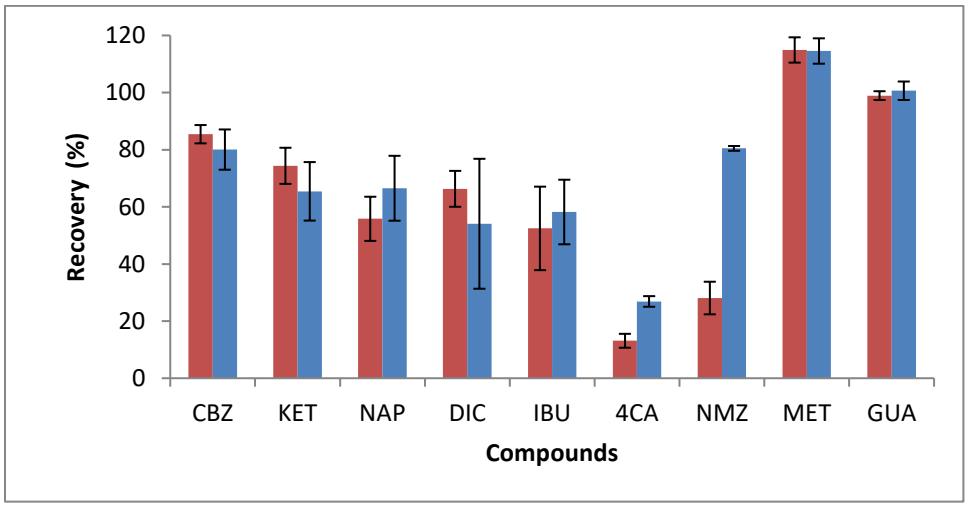

Figure 11.Recovery of each compound from different reagent concentrations, 2 mM SDS (red) and 6 mM SDS (blue) (CBZ: carbamazepine, KET: ketoprofen, NAP: naproxen, DIC: diclofenac, IBU: ibuprofen, 4CA: 4-chloroaniline, NMZ: n-methylpiperazine, MET: metformin and GUA: guanylurea); n: 3.

In figure 11, Oasis HLB 150 mg cartridges were used, as result from the optimization of sorbent type. Although the sorbent was not suitable for all compounds. Studies have showed by adding an ion-pair reagent to an Oasis HLB method increases the recovery of highly polar compounds[4].

The ion-pair reagent that was used is sodium dodecyl sulfate (SDS). SDS is an anionic surfactant with a long non-polar and a polar sulfate group. By using this ion-pair reagent the highly polar compounds have more retention time in the cartridges. The compounds can have three possible interactions with the column, Van Der Waals, polar and ion exchange interaction. The results in figure 11 showed that ion-pair reagent improved the recovery of 4-chloroaniline, n-methylpiperazine, metformin and guanylurea. In comparison with figure 10, the compounds carbamazepine, ketoprofen, naproxen, diclofenac and ibuprofen obtained a decrease of recovery. There is a possibility that by extending retention time with SDS the compounds carbamazepine, ketoprofen, naproxen, diclofenac and ibuprofen are not completely extracted from the cartridges. By adding more eluent this problem can be solved or by adding a solution that can break the interaction of the compounds in the cartridges. Figure 11 also showed that using a higher concentration of SDS increases the recovery of the compounds naproxen, ibuprofen, 4-chloroaniline, n-methylpiperazine, metformin and guanylurea. From this results, 6 mM SDS is chosen as concentration because it obtained an increase of recovery.

Optimization volume eluent

To determine the optimal eluent volume to extract the maximum of adsorbed compounds, different volumes (4 mL and 8 mL) of eluent were tested. Oasis HLB 150 mg cartridges and 6 mM SDS reagent were used, as result from the optimization of sorbent type and reagent concentration. As a correction for possible contamination a blank was used. To measure reproducibility, the samples were extracted in triplicates for the method with 4 mL methanol as eluent and eight times for the method with 8 mL methanol as eluent. From the known concentrations of the compounds in the multi-component solution, the recovery was calculated from the compounds that had passed through the cartridges. In figure 12, the recovery of each compound from different volumes (4 mL and 8 mL) of eluent are given.

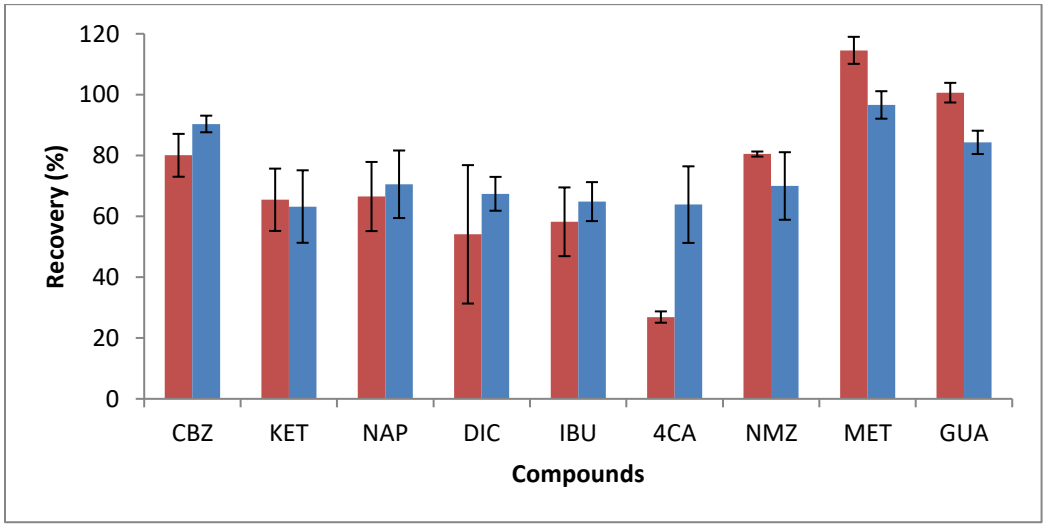

Figure 12.Recovery of each compound from different volumes of eluent, 4 mL methanol (red) and 8 mL eluent (blue).(CBZ: carbamazepine, KET: ketoprofen, NAP: naproxen, DIC: diclofenac, IBU: ibuprofen, 4CA: 4-chloroaniline, NMZ: n-methylpiperazine, MET: metformin and GUA: guanylurea); n: 3 (red); n: 8 (blue).

Results of figure 11 showed that using an ion reagent decreases the recovery of the compounds carbamazepine, ketoprofen, naproxen, diclofenac and ibuprofen. There is a possibility that by extending retention time with the ion reagent the compounds are not completely extracted from the cartridges. By changing the volume of the eluent this problem can be solve. In figure 12, two extractions are shown. In blue, 8 mL methanol was used to extract the compounds. In red, previous method of 4 mL methanol was used to extract. The results showed that by increasing the eluent volume the compounds carbamazepine, naproxen, diclofenac and ibuprofen obtained an increase of recovery. Although it caused a decrease of recovery for the compounds n-methylpiperazine, metformin and guanylurea. A compromise between the compounds must been made.

Optimization sorbent size

For the optimization of the sorbent size, two different sizes were used from Oasis HLB, 150 mg and 60 mg cartridges. On both cartridges the same extraction method was performed. For the optimization Oasis HLB cartridges, 6mM SDS reagent and 8 mL methanol as eluent were used, as result from the optimization of sorbent type and size, reagent concentration and volume amount of the eluent. To measure reproducibility, measurements were performed four times for the method with 60 mg of Oasis HLB cartridges and eight times for the method with 150 mg of Oasis HLB cartridges. From the known concentrations of the compounds in the multi-component solution, the recovery was calculated from the compounds that had passed through the cartridges. In figure 13, the recovery of each compound from two different sorbent sizes (60 mg and 150 mg) are given.

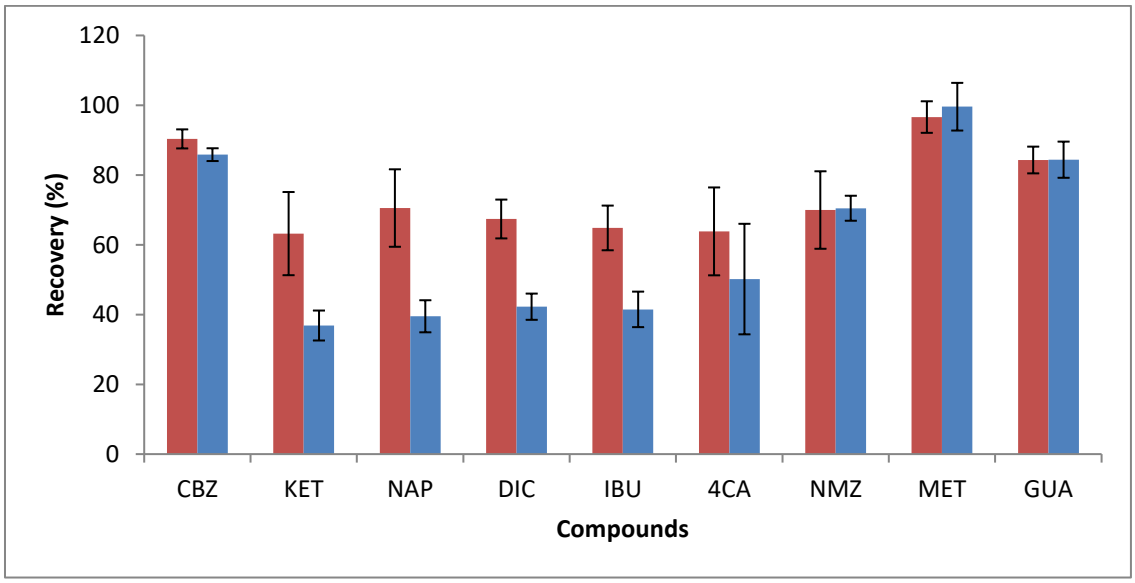

Figure 13. Recovery of each compound from different sorbent size, Oasis HLB 60 mg (blue) and Oasis HLB 150 mg (red).(CBZ: carbamazepine, KET: ketoprofen, NAP: naproxen, DIC: diclofenac, IBU: ibuprofen, 4CA: 4-chloroaniline, NMZ: n-methylpiperazine, MET: metformin and GUA: guanylurea); n: 8 (red); n: 4 (blue).

This optimization was used to see if a smaller sorbent obtained the same recovery than the previously used Oasis HLB sorbent of 150 mg. This optimization was done because the 60 mg sorbent is cheaper in comparison to the 150 mg sorbent. If the same recovery was obtained on the 60 mg sorbent, the cheapest alternative would be used to perform more extraction in the future. In figure 13, two extractions are given in the same optimized conditions except for the sorbent size. In blue, 60 mg Oasis HLB sorbent was used to extract the compounds. In red, previous 150 mg Oasis HLB sorbent was used to extract. The results showed that by using 60 mg Oasis HLB sorbent a decrease of recovery was obtained from most of the compounds.

This result was expected, because there was less material that the compounds could interact with. Therefore, 150 mg Oasis HLB sorbent was used for extraction. In previous studies the most common Oasis HLB sorbent that was used for extraction was 500 mg sorbent[6,20,21]. This may increase the recovery of the compounds, as it has the possibility to have more interaction with the sorbent which increases the retention time of the compounds. Although aside from the sorbent size, the particle size also determines the recovery of the extraction. By decreasing the particle size the samples have a longer interaction with the sorbent. The used particle size in these extractions were 60 μm. It is recommended to use 30 μm particle size to increase the retention time. Unfortunately, in this study there was no remaining time to test this.

Optimization pH matrix

To determine whether changing the pH increases the recovery, two types of acidifications were performed. The first acidification, 0,1% (v/v) formic acid was prepared for all working solvents including the multi-component solution and excluding the eluent. For the second acidification, only the eluent was acidified with 0,1% (v/v) formic acid. In both cases, the same method was used, only the pH conditions were different. Oasis HLB 150 mg cartridges, 6 mM SDS reagent and 8 mL methanol eluent were used. From the known concentrations of the compounds in the multi-component solution, the recovery was calculated from the compounds that had passed through the cartridges. In figure 14, the recovery of the compound are given from the first acidification method. In figure 15, the recovery of the compounds are given from the second acidification method.

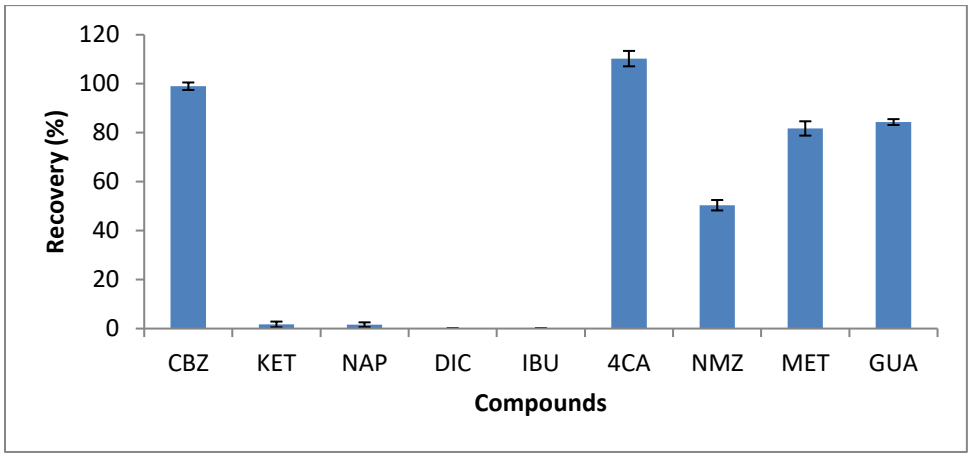

Figure 14. Recovery of each compound from the first acidification method. CBZ: carbamazepine, KET: ketoprofen, NAP: naproxen, DIC: diclofenac, IBU: ibuprofen, 4CA: 4-chloroaniline, NMZ: n-methylpiperazine, MET: metformin and GUA: guanylurea); n: 3.

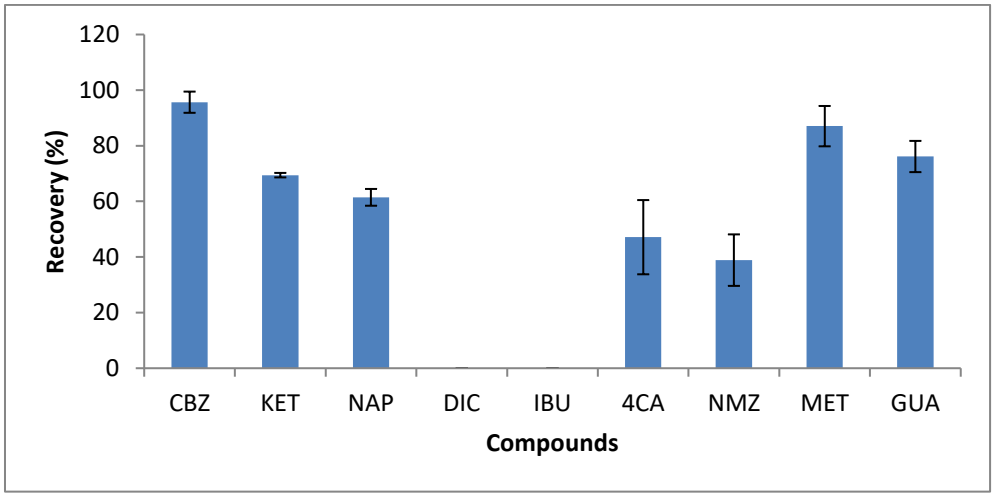

Figure 15. Recovery of each compound from the second acidification method.(CBZ: carbamazepine, KET: ketoprofen, NAP: naproxen, DIC: diclofenac, IBU: ibuprofen, 4CA: 4-chloroaniline, NMZ: n-methylpiperazine, MET: metformin and GUA: guanylurea); n: 3.

In figure 14 and 15, both extractions did not give a recovery for ibuprofen and diclofenac. A decrease of recovery is given for the compounds 4-chloroaniline and n-methylpiperazine in comparison with the previous extractions. In both extractions, figure 14 and 15, carbamazepine maintained a recovery of >95%. In the previous extraction in figure 13, the working solvents were not acidified which gave 4-chloroaniline a recovery of 64 ± 13%. In figure 14, all working solutions were acidified with 0,1% (v/v) formic acid except for the eluent solvent of methanol which gave 4-chloroaniline a recovery of 110 ± 3%. In previous extractions this amount of recovery of 4-chloroaniline was not obtained before. The results showed that by acidifying the working solutions, no recovery to a decrease of recovery was obtained for a couple of compounds. Only an improvement of recovery was shown for 4-chloroaniline by using the method from figure 14. This may be that the 4-chloroaniline compound was been protonated by the first acidification method and therefore had a better interaction with the column. Further optimization of pH should be done with different pH values or different acid or basic compounds.

Optimal method

The optimized parameters showed that the following method is the most suitable to extract all compounds of interest in one single step. In figure 16 the recovery of the compounds are given. All compounds obtained a descant amount of recovery with one single method.

Cartridge: Oasis HLB (150 mg)
Condition: 10 mL methanol
Pre-condition: 5 mL H2O and 5 mL 6 mM SDS
Sample: 10 mL
Wash: 5 mL H2O
Vacuum: 10 mins vacuumdrying
Isolation: 1 mL 5 % (v/v) of methanol in water
Eluation: 8 mL methanol

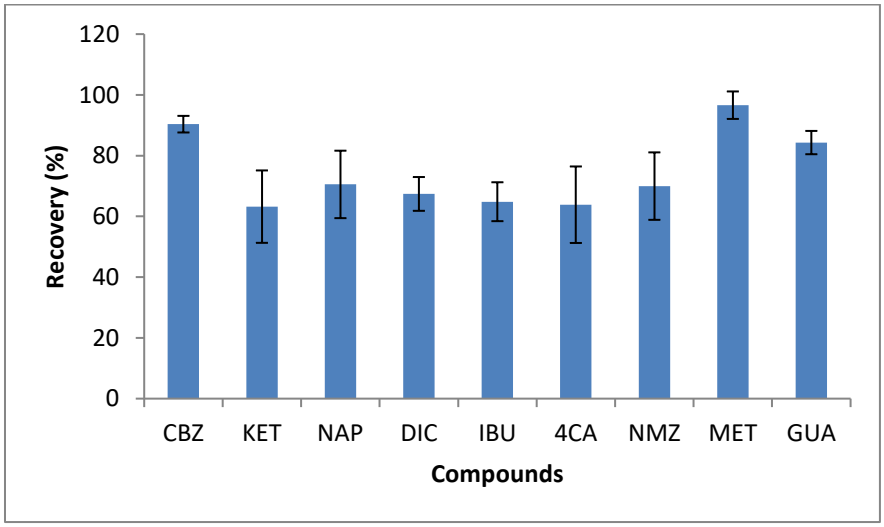

Figure 16. Recovery of each compound with the optimal method. (CBZ: carbamazepine, KET: ketoprofen, NAP: naproxen, DIC: diclofenac, IBU: ibuprofen, 4CA: 4-chloroaniline, NMZ: n-methylpiperazine, MET: metformin and GUA: guanylurea); n: 8.

As for the wastewater sample more solvents were used for the washing (10 mL) and isolation (5 mL) step because the wastewater samples were more contaminated then the multi-component solutions. It was also observed that the wastewater samples were not thoroughly clean after the extractions therefore a filtration was needed afterwards.

Wastewater sample from wastewater treatment plants

Wastewater samples (influent and effluent) have been taken from 5 wastewater treatment plants across the Netherlands. After sample preparation the upper layer of the samples were used for extraction. By the weighed of the remained solution, the exact amount of solutions that had passed through the cartridges were been calculated. The addition of internal standard aims to correct for any loss of compounds throughout the process. The internal standard were also measured in MRM mode on the mass spectrometry. The fragmentation (Q1/Q3) from the compounds with the highest intensity was used for quantification. Aside from adding a blank to the batch of samples, a control sample was added that contained ultrapure water and the same amount of internal standard like the water samples. The purpose of the control sample was to see how the isotopes in ultrapure water matrix would react throughout the process and to see if the amount of internal standard added in the tubes were found back in the measurement on the mass spectrometry.

Subsequently, an incorrect elution step of 0,1% (v/v) formic acid in methanol was performed. Due to time-constraint the wastewater samples were extracted before the measurements of figure 15 had occurred. It was assumed that the method would work. It was not known that the method used in figure 15 was not optimal for this research. Therefore the measurements of the wastewater were not reliable for the compounds ibuprofen and diclofenac.

The blanks that were added to the calibration standards and the extraction blanks both obtained only background noise. In figure 17 and 18 the chromatograms are given of the control samples measured on the C18 column in positive mode and the HILIC column in positive mode. The control sample measured on the C18 column in negative mode did not obtain peaks for the internal standards ibuprofen-D3 and diclofenac-C13 because the extraction method that was used did not work for the compounds ibuprofen and diclofenac. In figure 17 the internal standards carbamazepine-C13 (2,54 min) and ketoprofen-D3 (2,63 min) were seen clearly. In figure 18 the internal standard metformin-D6 (2,00 min) was seen.

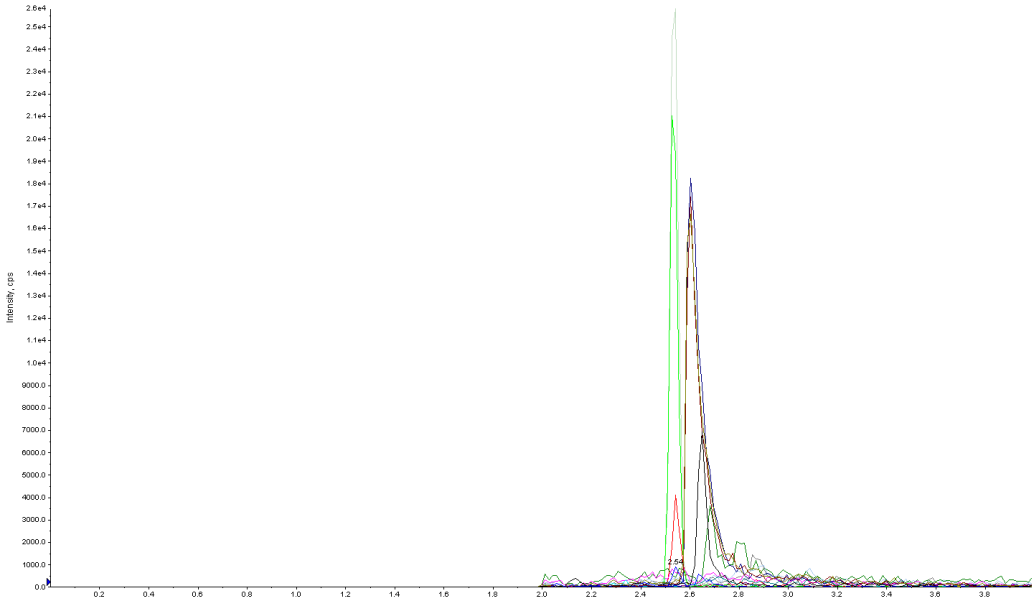

Figure 17. Chromatogram of the control sample carbamazepine-C13(2,54 min) and ketoprofen-D3 (2,63 min)measured on the C18 column in positive mode

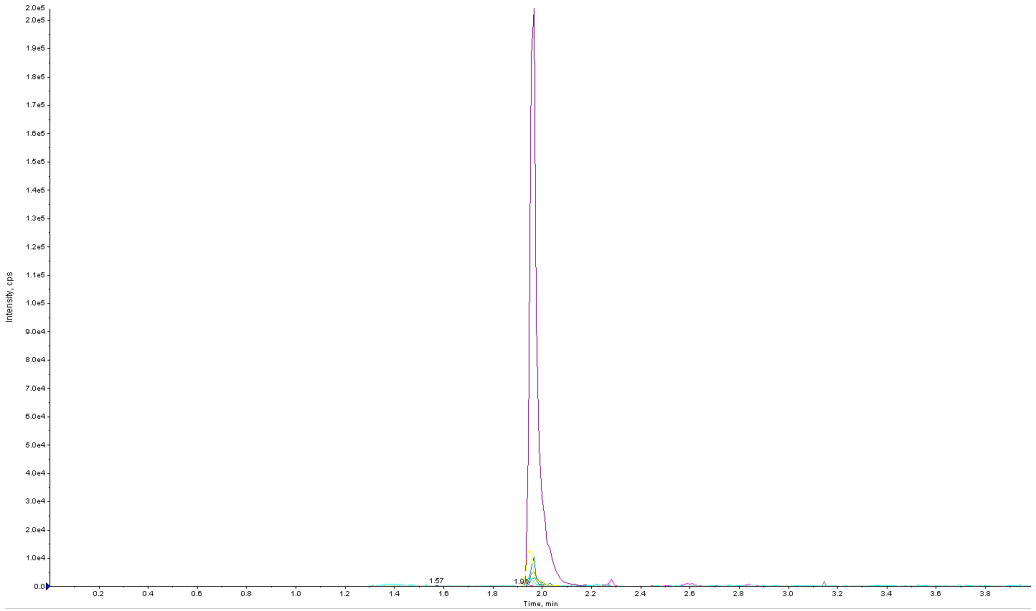

Figure 18. Chromatogram of the control sample metformin-D3 (2,00 min) measured on the HILIC column in positive mode.

As an example, in figure 19 and 20 the chromatograms are given of the Utrecht inflow 2 sample measured on the C18 column in positive mode and the HILIC column in positive mode. On the C18 column negative mode chromatogram no peaks were obtained. In Appendix 5 the data of the wastewater samples are given in tables 1-9.

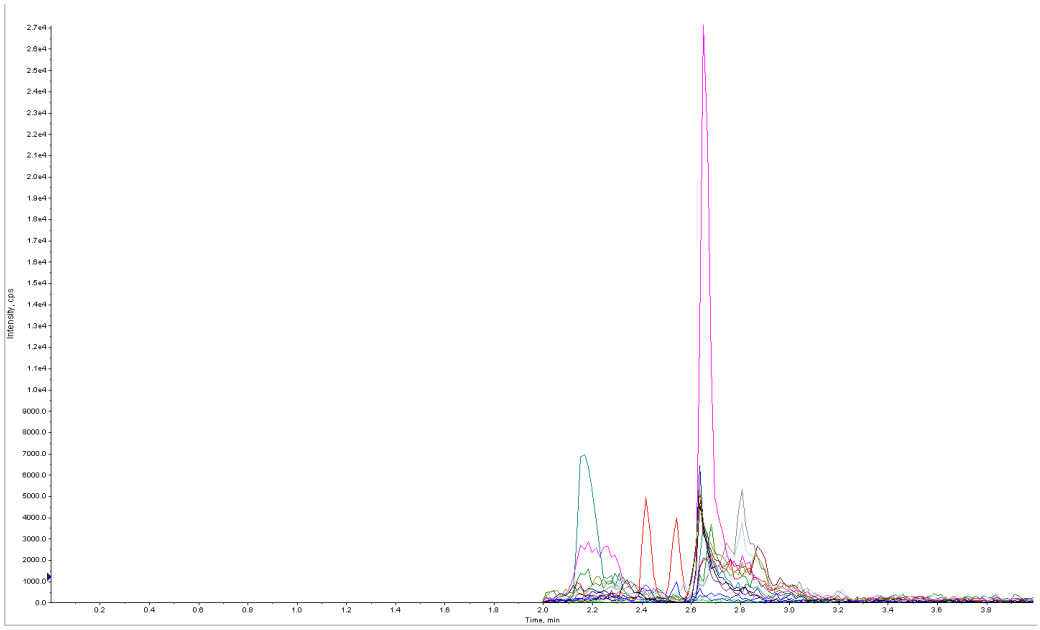

Figure 19. Chromatogram of Utrecht inflow 2 sample naproxen (2,65 min) measured on the C18 column in positive mode

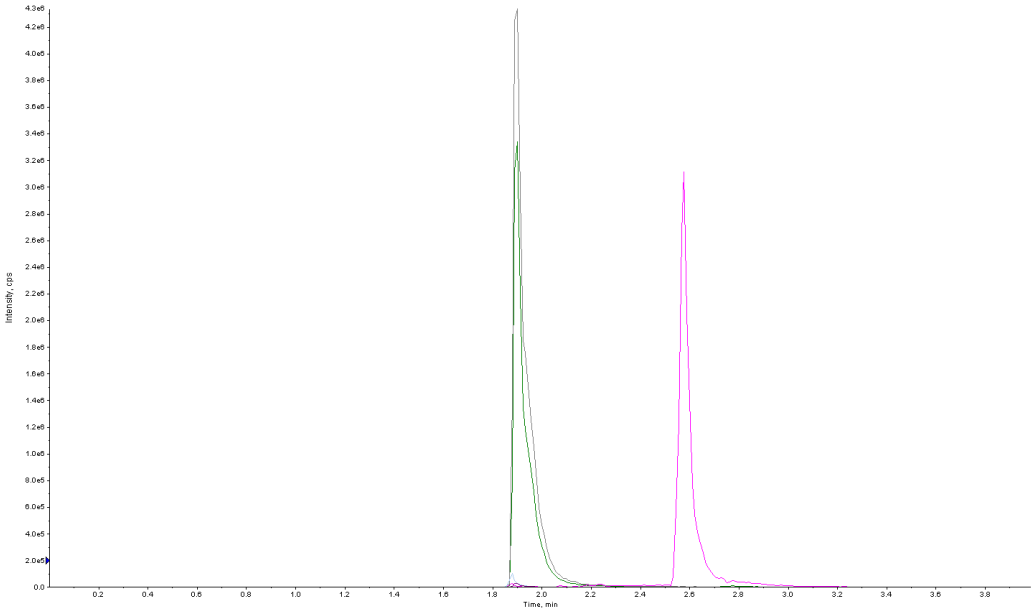

Figure 20. Chromatogram of Utrecht inflow 2 sample metformin (1,89 min) and guanylurea (2,57 min) measured on the HILIC column in positive mode.

To calculate the concentration of the compounds in the wastewater samples, calibration standards were made that contained 5 ppb internal standards. The wastewater samples that were prepared for the LC-MS/MS measurements also contained 5 ppb internal standard theoretically. The samples were corrected when a loss in internal standard was measured in the samples. In Appendix 4 the data of the calibration standards (with internal standards) of all compounds are given in tables 1-9. In table 5 the equations are given that were obtained of the measured standards in Appendix 4.

Table 5. Equations obtained from the measured calibration standards in Appendix 4.

Compound	Equation
Carbamazepine	y=(363590,8 ± 27594,1)x + 111001,1 ± 296270,9; BI95%; n=12; sy/x= 290743,1; R^2 0,9885
Ketoprofen	y=(325089,4 ± 6753)x + 26890,9 ± 78061,8; BI95%; n=12; sy/x= 76422,4; R^2 0,9991
Naproxen	y=(158891,3 ± 8892,5)x + 46028,4 ± 96267,6; BI95%; n=12; sy/x= 96098,2; R^2 0,9937
4-chloroaniline	y=(122954 ± 162,3)x - 1145,3 ± 1881; BI95%; n=12; sy/x= 1895,1 ; R^2 0,9999
Diclofenac	y=(377718,4 ± 4812,7)x + 28867,3 ± 57466,8; BI95%; n=12; sy/x= 57407,7; R^2 0,9998
Ibuprofen	y=(300840,1 ± 1852,4)x + 24114 ± 22623,3; BI95%; n=12; sy/x= 22431; R^2 0,9999
N-methylpiperazine	y=(21440,3 ± 51,6)x + 864,1 ± 553,7; BI95%; n=12; sy/x= 551,2; R^2 0,9999
Metformin	y=(363278 ± 42831,3)x + 208235,4 ± 368147,9; BI95%; n=12; sy/x= 371810; R^2 0,9728
Guanylurea	y=(44147,8 ± 1491,1)x + 4183,4 ± 14364,8 ; BI95%; n=12; sy/x= 14408,4; R^2 0,9977

Although the software corrected the samples with internal standard, the following calculations can be performed to determine the concentration of the samples. The calculations will deviate from the software as it works with exact data's.

As an example, the concentration of the compound metformin in the Utrecht inflow 2 sample is calculated. In table 6 the data of the calibration standards of metformin and the Utrecht inflow 2 sample are given with the internal standard of metformin-D6.

Table 6. LC-MS/MS calibration data of metformin. The concentration is shown in ppb, the peak area in counts and peak height in cps. The internal standard used is metformin-D6.

Sample Name	Sample Type	Analyte Peak Area (counts)	Analyte Peak Height (cps)	IS Peak Area (counts)	IS Peak Height (cps)	Calculated Concentration (ppb)
Blank	Blank	0,00E+00	0,00E+00	0,00E+00	0,00E+00	N/A
Blank	Blank	0,00E+00	0,00E+00	0,00E+00	0,00E+00	N/A
MET1	Standard	1,82E+05	4,28E+04	2,79E+04	6,27E+03	0,413
MET1	Standard	1,76E+05	4,09E+04	3,12E+04	7,30E+03	0,357
MET2	Standard	3,47E+05	7,97E+04	2,49E+04	5,66E+03	0,871
MET2	Standard	3,31E+05	7,58E+04	3,37E+04	8,15E+03	0,616
MET3	Standard	1,64E+06	3,71E+05	2,83E+04	6,85E+03	3,59
MET3	Standard	1,66E+06	3,77E+05	2,88E+04	6,97E+03	3,58
MET4	Standard	3,11E+06	7,11E+05	2,80E+04	6,44E+03	6,86
MET4	Standard	3,13E+06	7,14E+05	2,08E+04	4,40E+03	9,33
MET5	Standard	4,70E+06	1,06E+06	2,62E+04	6,21E+03	11,1
MET5	Standard	4,35E+06	9,87E+05	2,56E+04	6,11E+03	10,5
MET6	Standard	5,78E+06	1,30E+06	2,08E+04	4,72E+03	17,2
MET6	Standard	5,58E+06	1,22E+06	2,47E+04	5,38E+03	14
Utrecht inflow 2	Sample	1,59E+07	2,79E+06	1,17E+03	3,51E+02	839

Calculations:

Example of concentration calculation

To calculate the concentration of metformin in the wastewater samples, the following equation obtained from the calibration graph was used. y= 363278 x + 208235,4

The measured peak area was y and the concentration was x.

$$x = \frac{y - 208235{,}4}{363278}$$

$$x = \frac{1{,}59 \times 10^7 - 208235{,}4}{363278} = 43{,}19\ ppb$$

The average IS peak area of the standards was 2,67 * 10^4 counts. The IS peak area of the sample was 1,17 * 10^3 counts. The calculated concentration was corrected with the internal standard.

$$Corrected\ conc.(ppb) = \frac{Average\ IS\ peak\ area\ (counts)}{IS\ peak\ area\ of\ sample\ (counts)} \times calculated\ conc.(ppb)$$

$$Corrected\ conc.(ppb) = \frac{2{,}67\ \times\ 10^4}{1{,}17\ \times\ 10^3} \times 43{,}19 = 987\ ppb.$$

The final concentrations measured on LC-MS/MS were concentrated by 4 times, further corrections were done and corrections for minor sample dilutions with the internal standard from sample preparations and amount of sample that had passed through the cartridges were included.

$$Final\ conc.(ppb) = \frac{corrected\ conc.(ppb)}{4} \times dilutions\ correction \times cartridges\ correction$$

$$Final\ conc.(ppb) = \frac{987}{4} \times 1{,}025 \times 1{,}009 = 255.2\ ppb$$

The standard deviation was calculated the same way as for the multi-component solution samples.

Table 7 shows the final concentrations of the compounds found in influent and effluent wastewater from different locations.

Table 7.Concentrations of the compounds in influent and effluent wastewater.

	Carbamazepine (µg/L)	Ketoprofen (µg/L)	Naproxen (µg/L)	Diclofenac (µg/L)	Ibuprofen (µg/L)	Metformin (µg/L)	N-methylpiperazine (µg/L)	Guanylurea (µg/L)
Amsterdam-West infl.	-	-	-	0,236 ± 0,284	9,380 ± 8,826	35,189 ± 14,697	0,052 ± 0,005	14,424 ± 4,319
Amsterdam-Westeffl.	0,648 ± 0,076	-	-	-	-	0,256 ± 0,115	-	39,256 ± 8,114
Amstelveen infl.	-	0,297 ± 0,266	3,727 ± 1,159	-	-	107,992 ± 18,030	0,010 ± 0,001	1,701 ± 0,084
Amstelveen effl.	-	-	1,267 ± 0,221	-	-	5,243 ± 0,841	-	75,050 ± 5,751
Bennekom infl.	-	0,718 ± 0,184	5,126 ± 0,831	-	-	220,109 ± 61,989	0,035 ± 0,004	4,011 ± 0,488
Eindhoven infl.	-	-	8,265 ± 1,346	-	-	124,620 ± 17,563	0,011 ± 0,011	32,614 ± 5,962
Eindhoven effl.	-	0,213 ± 0,204	0,356 ± 0,171	-	-	2,002 ± 0,079	-	26,647 ± 0,308
Utrecht infl.	-	-	3,610 ± 0,024	-	-	167,977 ± 60,599	-	14,506 ± 1,011
Utrecht effl.	-	0,047 ± 0,082	1,079 ± 0,185	-	-	2,658 ± 0,122	-	22,124 ± 1,151

In table 7, the corrected concentrations of the compounds in the wastewater samples are given. The concentrations were corrected for internal standards, dilutions and exact amount of sample that had passed through the cartridges. From the results of the control samples, no internal standard were found for the compounds ibuprofen-D3, diclofenac-C13 and naproxen-D3. For ibuprofen-D3 and diclofenac-C13 this can be explained by the fact that in the extraction method a wrong eluation step was carried out that did not provided any recovery for the compounds ibuprofen and diclofenac. This has also been the case for the isotopes that have the same chemical structure. For 4-chloroaniline, none of the sample contained the compound. Low concentrations were observed for carbamazepine, ketoprofen, naproxen, diclofenac and n-methylpiperazine. High concentrations were observed for metformin and guanylurea, since guanylurea is a transformation product of metformin. In addition, it was easy to see that influent wastewater samples observed high concentrations of metformin and after treatment (wastewater treatment plants) the concentration of metformin has decreased. This was clearly visible in the effluent wastewater samples. Also was observed that the influent water samples have low concentration of guanylurea before the treatment than after the treatment, because after the treatment metformin in the effluent water biodegradates into its transformation product guanylurea which increases the concentration guanylurea in the effluent water[16,22]. This can also explain the low concentration metformin in the effluent water.

Conclusion

The aim of this research was to develop one solid phase extraction method for a wide group of pharmaceutical and industrial compounds. For the development of a solid phase extraction method, five parameters (sorbent type, reagent, eluent, sorbent size and pH) were optimized to obtain the main goal. For the sorbent type (stationary phase) three sorbents (Oasis HLB, Oasis MCX and Sep-Pak C18) were tested. The Oasis HLB sorbent obtained the best results. For the parameter reagent, the addition of the ion-exchange reagent sodium dodecyl sulfate (SDS) was tested with two different concentrations (2 mM and 6 mM). The results showed that 6 mM SDS was the most suitable concentration. For the eluent volume two different volumes were tested, 4 mL and 8 mL. The results showed that the 8 mL eluent volume extracted the most compounds. For the optimization of the sorbent size two different sizes, 60 mg and 150 mg, were tested. The most suitable sorbent size was 150 mg Oasis HLB. And for the parameter of addition of acids, two different acidification methods were tested. Both methods were not suitable for this method.

With the optimized parameters a recovery of >60% was observed. This concludes that a solid phase extraction method has been developed for a wide group of pharmaceutical and industrial compounds. Further optimizations are needed to improve the recovery of the compounds. In the future it is recommended to optimize the pH value. As it improved the compounds ibuprofen and 4-chloroaniline. Different pH values of acids should be used to test the optimum. Furthermore it is recommended for the extractions with MCX to add sodium hydroxide to the eluent to obtain 1 or 2 pKa value higher than the basic compounds. This is to break the strong interaction of the compounds and sorbent. Also the particle size of the sorbent should be optimized. By decreasing the particle size the samples will have a longer interaction with the sorbent because the load of the sample is smaller. It was also observed that the wastewater sample was not thoroughly clean after the extractions therefore a filtration was needed afterwards.

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Appendix 1. Parameters of mass spectrometry

Tabel1. Parameters of the mass spectrometry used of the compounds in this research.

	Q1	Q3	DP	CE	CXP	Retention time (min)
N-methylpiperazine Tr.1	101	58	116	27	8	2,28
N-methylpiperazine Tr.2	101	44	116	33	6	2,28
Metformin Tr.1	130,2	70,9	47	32	4	1,88
Metformin Tr.2	130,2	60	47	19	4	1,88
Guanylurea Tr.1	103	60	47	16	10	1,80
Guanylurea Tr.2	103	86	47	13	13	1,80
Metformin-D6 Tr.1	136,1	76,9	120	52	4	1,88
Metformin-D6 Tr.2	136,1	60	63	21	2	1,88
Guanylurea-15N4 Tr.1	107	89	74	14	10	1,80
Guanylurea-15N4 Tr.2	107	63	74	17	10	1,80
Guanylurea-15N4 Tr.3	107	45	74	38	10	1,80
Carbamazepine Tr.1	237	192	91	29	12	2,67
Carbamazepine Tr.2	237	194	91	29	12	2,67
Ketoprofen Tr.1	255	209	90	18	10	2,80
Ketoprofen Tr.2	255	105	90	35	4	2,80
Ketoprofen Tr.3	255	77	90	63	2	2,80
Naproxen Tr.1	231	185	63	17	9	2,80
Naproxen Tr.2	231	115	63	82	4	2,80
Naproxen Tr3	231	141	63	65	8	2,80
4-chloroaniline Tr.1	128	93	60	27	7	2,50
4-chloroaniline Tr.2	128	75	60	48	3	2,50
Carbamazepine-C13 Tr.1	243	200	120	52	4	2,67
Carbamazepine-C13 Tr.2	243	198	120	52	4	2,67
Ketprofen-D3 Tr.1	258,3	211,7	75	17	12	2,80
Ketprofen-D3 Tr.2	258,3	105	75	32	12	2,80
Ketprofen-D3 Tr.3	258,3	77	75	60	12	2,80
Naproxen-D3 Tr.1	234	188,4	60	19	10	2,80
Naproxen-D3 Tr.2	234	115	60	80	10	2,80
Diclofenac Tr.1	294	250	-47	-18	-15	3,59
Diclofenac Tr.2	294	214	-47	-30	-13	3,59
Ibuprofen Tr.1	205	161	-33	-11	-6	3,00
Ibuprofen-D3 Tr.1	208	164	-43	-10	-8	3,00
Diclofenac-C13 Tr.1	300	256	-55	-14	-5	3,59
Diclofenac-C13 Tr.2	302	258	-55	-14	-5	3,59

Appendix 2. LC-MS/MS data of calibration standards

Table 1. LC-MS/MS calibration data of carbamazepine. The concentration is shown in ppb, the peak area in counts and peak height in cps.

Sample Name	Sample Type	Analyte Peak Area (counts)	Analyte Peak Height (cps)	Calculated Concentration (ppb)
Blank	blank	0,00E+00	0,00E+00	N/A
Blank	blank	0,00E+00	0,00E+00	N/A
CBZ1	standard	1,67E+05	4,92E+04	0,634
CBZ1	standard	1,59E+05	4,70E+04	0,606
CBZ2	standard	3,23E+05	9,60E+04	1,23
CBZ2	standard	3,22E+05	9,54E+04	1,22
CBZ3	standard	1,51E+06	4,40E+05	5,74
CBZ3	standard	1,46E+06	4,20E+05	5,57
CBZ4	standard	2,89E+06	8,41E+05	11
CBZ4	standard	2,64E+06	7,55E+05	10
CBZ5	standard	3,80E+06	1,09E+06	14,5
CBZ5	standard	4,01E+06	1,15E+06	15,2
CBZ6	standard	4,60E+06	1,32E+06	17,5
CBZ6	standard	4,80E+06	1,38E+06	18,2

Table 2. LC-MS/MS calibration data of ketoprofen. The concentration is shown in ppb, the peak area in counts and peak height in cps.

Sample Name	Sample Type	Analyte Peak Area (counts)	Analyte Peak Height (cps)	Calculated Concentration (ppb)
Blank	blank	0,00E+00	0,00E+00	N/A
Blank	blank	0,00E+00	0,00E+00	N/A
KET1	standard	1,22E+05	3,45E+04	0,605
KET1	standard	1,19E+05	3,43E+04	0,591
KET2	standard	1,10E+04	3,03E+03	0,0546
KET2	standard	1,08E+04	3,00E+03	0,0535
KET3	standard	1,13E+06	3,30E+05	5,6
KET3	standard	1,12E+06	3,24E+05	5,54
KET4	standard	2,19E+06	6,27E+05	10,8
KET4	standard	2,21E+06	6,39E+05	10,9
KET5	standard	3,20E+06	9,22E+05	15,9
KET5	standard	3,18E+06	9,10E+05	15,8
KET6	standard	4,00E+06	1,15E+06	19,9
KET6	standard	3,97E+06	1,14E+06	19,7

Table 3. LC-MS/MS calibration data of naproxen. The concentration is shown in ppb, the peak area in counts and peak height in cps.

Sample Name	Sample Type	Analyte Peak Area (counts)	Analyte Peak Height (cps)	Calculated Concentration (ppb)
Blank	blank	0,00E+00	0,00E+00	N/A
Blank	blank	0,00E+00	0,00E+00	N/A
NAP1	standard	6,63E+04	1,99E+04	0,486
NAP1	standard	6,69E+04	1,99E+04	0,49
NAP2	standard	1,33E+05	3,88E+04	0,976
NAP2	standard	1,44E+05	4,30E+04	1,06
NAP3	standard	7,26E+05	2,14E+05	5,32
NAP3	standard	7,02E+05	2,08E+05	5,15
NAP4	standard	1,40E+06	4,19E+05	10,3
NAP4	standard	1,37E+06	4,03E+05	10
NAP5	standard	2,05E+06	6,02E+05	15
NAP5	standard	2,08E+06	6,06E+05	15,2
NAP6	standard	2,46E+06	7,13E+05	18
NAP6	standard	2,52E+06	7,31E+05	18,5

Table4. LC-MS/MS calibration data of 4-chloroaniline. The concentration is shown in ppb, the peak area in counts and peak height in cps.

Sample Name	Sample Type	Analyte Peak Area (counts)	Analyte Peak Height (cps)	Calculated Concentration (ppb)
Blank	blank	0,00E+00	0,00E+00	N/A
Blank	blank	9,11E+02	2,08E+02	N/A
4CA1	standard	8,84E+04	2,35E+04	0,504
4CA1	standard	8,71E+04	2,30E+04	0,496
4CA2	standard	1,92E+05	5,14E+04	1,09
4CA2	standard	1,88E+05	4,91E+04	1,07
4CA3	standard	9,24E+05	2,44E+05	5,27
4CA3	standard	9,00E+05	2,37E+05	5,13
4CA4	standard	1,87E+06	4,87E+05	10,7
4CA4	standard	1,80E+06	4,61E+05	10,2
4CA5	standard	2,74E+06	7,07E+05	15,6
4CA5	standard	2,72E+06	6,99E+05	15,5
4CA6	standard	3,53E+06	9,00E+05	20,1
4CA6	standard	3,57E+06	9,12E+05	20,4

Table5. LC-MS/MS calibration data of diclofenac. The concentration is shown in ppb, the peak area in counts and peak height in cps.

Sample Name	Sample Type	Analyte Peak Area (counts)	Analyte Peak Height (cps)	Calculated Concentration (ppb)
Blank	blank	0,00E+00	0,00E+00	N/A
Blank	blank	1,60E+04	2,96E+03	N/A
DIC1	standard	2,06E+05	4,48E+04	0,477
DIC1	standard	2,06E+05	4,41E+04	0,479
DIC2	standard	3,93E+05	8,41E+04	1,05
DIC2	standard	4,05E+05	8,74E+04	1,08
DIC3	standard	1,93E+06	4,10E+05	5,74
DIC3	standard	1,94E+06	4,15E+05	5,77
DIC4	standard	3,76E+06	7,69E+05	11,3
DIC4	standard	3,67E+06	7,45E+05	11,1
DIC5	standard	5,41E+06	1,09E+06	16,3
DIC5	standard	5,37E+06	1,09E+06	16,2
DIC6	standard	6,76E+06	1,37E+06	20,5
DIC6	standard	6,70E+06	1,36E+06	20,3

Table6. LC-MS/MS calibration data of ibuprofen. The concentration is shown in ppb, the peak area in counts and peak height in cps.

Sample Name	Sample Type	Analyte Peak Area (counts)	Analyte Peak Height (cps)	Calculated Concentration (ppb)
Blank	blank	0,00E+00	0,00E+00	N/A
Blank	blank	9,56E+03	3,49E+03	N/A
IBU1	standard	1,65E+05	6,28E+04	0,498
IBU1	standard	1,68E+05	6,45E+04	0,51
IBU2	standard	3,27E+05	1,22E+05	1,1
IBU2	standard	3,43E+05	1,31E+05	1,15
IBU3	standard	1,54E+06	5,67E+05	5,55
IBU3	standard	1,53E+06	5,67E+05	5,55
IBU4	standard	3,14E+06	1,17E+06	11,5
IBU4	standard	3,24E+06	1,23E+06	11,8
IBU5	standard	4,42E+06	1,61E+06	16,2
IBU5	standard	4,46E+06	1,63E+06	16,3
IBU6	standard	5,78E+06	2,09E+06	21,2
IBU6	standard	5,76E+06	2,12E+06	21,1

Table7 . LC-MS/MS calibration data of n-methylpiperazine. The concentration is shown in ppb, the peak area in counts and peak height in cps.

Sample Name	Sample Type	Analyte Peak Area (counts)	Analyte Peak Height (cps)	Calculated Concentration (ppb)
Blank	blank	1,68E+03	4,61E+02	N/A
Blank	blank	1,13E+03	4,25E+02	N/A
NMZ1	standard	1,45E+04	4,98E+03	0,346
NMZ1	standard	1,46E+04	5,40E+03	0,35
NMZ2	standard	3,38E+04	1,16E+04	0,869
NMZ2	standard	3,11E+04	1,05E+04	0,796
NMZ3	standard	1,84E+05	6,37E+04	4,93
NMZ3	standard	1,92E+05	6,56E+04	5,17
NMZ4	standard	3,79E+05	1,31E+05	10,2
NMZ4	standard	4,05E+05	1,40E+05	10,9
NMZ5	standard	5,39E+05	1,73E+05	14,6
NMZ5	standard	4,88E+05	1,58E+05	13,2
NMZ6	standard	6,64E+05	2,15E+05	18
NMZ6	standard	7,13E+05	2,18E+05	19,3

Table8. LC-MS/MS calibration data of metformin. The concentration is shown in ppb, the peak area in counts and peak height in cps.

Sample Name	Sample Type	Analyte Peak Area (counts)	Analyte Peak Height (cps)	Calculated Concentration (ppb)
Blank	blank	2,77E+02	8,71E+01	N/A
Blank	blank	3,71E+02	1,15E+02	N/A
MET1	standard	2,33E+05	8,30E+04	0,376
MET1	standard	2,52E+05	9,09E+04	0,414
MET2	standard	4,89E+05	1,75E+05	0,898
MET2	standard	5,00E+05	1,81E+05	0,92
MET3	standard	2,33E+06	7,99E+05	4,64
MET3	standard	2,33E+06	8,11E+05	4,65
MET4	standard	4,05E+06	1,36E+06	8,17
MET4	standard	4,19E+06	1,43E+06	8,45
MET5	standard	5,72E+06	1,92E+06	11,6
MET5	standard	5,68E+06	1,91E+06	11,5
MET6	standard	7,30E+06	2,38E+06	14,8
MET6	standard	7,16E+06	2,32E+06	14,5

Table 9. LC-MS/MS calibration data of guanylurea. The concentration is shown in ppb, the peak area in counts and peak height in cps.

Sample Name	Sample Type	Analyte Peak Area (counts)	Analyte Peak Height (cps)	Calculated Concentration (ppb)
Blank	blank	9,58E+01	5,34E+01	N/A
Blank	blank	2,38E+02	8,71E+01	N/A
GUA1	standard	1,02E+05	3,01E+04	0,54
GUA1	standard	9,77E+04	2,74E+04	0,511
GUA2	standard	2,05E+05	6,19E+04	1,25
GUA2	standard	1,89E+05	5,44E+04	1,15
GUA3	standard	8,08E+05	2,28E+05	5,45
GUA3	standard	8,38E+05	2,31E+05	5,65
GUA4	standard	1,43E+06	3,88E+05	9,79
GUA4	standard	1,36E+06	3,60E+05	9,26
GUA5	standard	1,92E+06	5,11E+05	13,2
GUA5	standard	1,99E+06	5,07E+05	13,6
GUA6	standard	2,34E+06	6,21E+05	16,1
GUA6	standard	2,31E+06	6,02E+05	15,9

Appendix 3. LC-MS/MS data of the multi-component solutions

Table 1. LC-MS/MS data of each compound of the Oasis HLB method. The concentration is shown in ppb, the peak area in counts and peak height in cps.

Sample	Analyte	Analyte Peak Area (counts)	Analyte Peak Height (cps)	Calculated Concentration (ppb)
1	N-methylpiperazine	1,70E+03	4,15E+02	< 0
2	N-methylpiperazine	1,24E+03	3,56E+02	< 0
3	N-methylpiperazine	3,40E+03	9,10E+02	0,0446
4	N-methylpiperazine	2,47E+03	6,08E+02	0,0193
blank	N-methylpiperazine	5,80E+02	1,53E+02	< 0
1	Metformin	1,04E+06	3,67E+05	2,02
2	Metformin	8,68E+05	3,06E+05	1,67
3	Metformin	1,17E+06	4,18E+05	2,28
4	Metformin	1,31E+06	4,65E+05	2,57
blank	Metformin	9,96E+02	3,25E+02	< 0
1	Guanylurea	2,24E+05	6,34E+04	1,39
2	Guanylurea	2,08E+05	6,21E+04	1,28
3	Guanylurea	2,51E+05	7,46E+04	1,58
4	Guanylurea	2,80E+05	8,20E+04	1,78
blank	Guanylurea	4,50E+02	1,57E+02	< 0
1	Carbamazepine	2,19E+06	6,19E+05	8,33
2	Carbamazepine	2,44E+06	6,98E+05	9,27
3	Carbamazepine	2,41E+06	6,89E+05	9,18
1	Ketoprofen	1,76E+06	5,08E+05	8,75
2	Ketoprofen	1,90E+06	5,42E+05	9,4
3	Ketoprofen	1,93E+06	5,56E+05	9,59
1	Naproxen	1,12E+06	3,30E+05	8,21
2	Naproxen	1,27E+06	3,73E+05	9,33
3	Naproxen	1,24E+06	3,64E+05	9,11
1	4-chloroaniline	4,68E+04	1,23E+04	0,267
2	4-chloroaniline	5,31E+04	1,42E+04	0,303
3	4-chloroaniline	5,82E+04	1,53E+04	0,332
1	Diclofenac	3,13E+06	6,71E+05	9,39
2	Diclofenac	3,42E+06	7,24E+05	10,3
3	Diclofenac	3,47E+06	7,50E+05	10,4
1	Ibuprofen	2,55E+06	9,56E+05	9,28
2	Ibuprofen	2,87E+06	1,08E+06	10,5
3	Ibuprofen	2,96E+06	1,11E+06	10,8

Table 2. LC-MS/MS data of each compound of the C18 method. The concentration is shown in ppb, the peak area in counts and peak height in cps.

Sample	Analyte	Analyte Peak Area (counts)	Analyte Peak Height (cps)	Calculated Concentration (ppb)
1	N-methylpiperazine	1,67E+05	5,69E+04	4,48
2	N-methylpiperazine	2,18E+05	7,47E+04	5,86
3	N-methylpiperazine	1,73E+05	6,26E+04	4,65
blank	N-methylpiperazine	4,25E+02	1,55E+02	< 0
1	Metformin	3,82E+06	1,34E+06	7,69
2	Metformin	3,74E+06	1,26E+06	7,53
3	Metformin	3,69E+06	1,27E+06	7,43
blank	Metformin	3,18E+03	8,58E+02	< 0
1	Guanylurea	1,02E+06	2,80E+05	6,93
2	Guanylurea	1,02E+06	2,92E+05	6,94
3	Guanylurea	1,07E+06	2,92E+05	7,25
blank	Guanylurea	6,30E+02	1,45E+02	< 0
1	Carbamazepine	3,16E+06	5,72E+05	8,17
2	Carbamazepine	3,05E+06	5,41E+05	7,88
3	Carbamazepine	3,38E+06	6,07E+05	8,75
1	Ketoprofen	2,98E+06	5,10E+05	9,1
2	Ketoprofen	2,85E+06	4,88E+05	8,71
3	Ketoprofen	3,08E+06	5,23E+05	9,4
1	Naproxen	1,26E+06	2,25E+05	7,83
2	Naproxen	1,28E+06	2,28E+05	7,91
3	Naproxen	1,27E+06	2,29E+05	7,89
1	4-chloroaniline	5,51E+04	1,31E+04	0,314
2	4-chloroaniline	1,30E+05	3,04E+04	0,74
3	4-chloroaniline	5,06E+04	1,20E+04	0,288
blank	4-chloroaniline	0,00E+00	0,00E+00	No Peak
1	Diclofenac	2,69E+06	4,79E+05	7,16
2	Diclofenac	2,57E+06	4,55E+05	6,85
3	Diclofenac	2,83E+06	5,04E+05	7,55
1	Ibuprofen	2,41E+06	6,74E+05	8,03
2	Ibuprofen	1,61E+06	4,41E+05	5,34
3	Ibuprofen	1,81E+06	4,95E+05	6,01

Table 3. LC-MS/MS data of each compound of the MCX method. The concentration is shown in ppb, the peak area in counts and peak height in cps.

Sample	Analyte	Analyte Peak Area (counts)	Analyte Peak Height (cps)	Calculated Concentration (ppb)
1	N-methylpiperazine	1,56E+03	3,02E+02	< 0
2	N-methylpiperazine	1,13E+03	3,28E+02	< 0
3	N-methylpiperazine	9,29E+02	3,17E+02	< 0
blank	N-methylpiperazine	1,18E+03	3,31E+02	< 0
1	Metformin	0,00E+00	0,00E+00	No Peak
2	Metformin	0,00E+00	0,00E+00	No Peak
3	Metformin	0,00E+00	0,00E+00	No Peak
blank	Metformin	0,00E+00	0,00E+00	No Peak
1	Guanylurea	6,11E+02	1,86E+02	< 0
2	Guanylurea	4,02E+02	9,90E+01	< 0
3	Guanylurea	2,33E+02	9,46E+01	< 0
blank	Guanylurea	4,27E+02	1,26E+02	< 0
1	Carbamazepine	4,57E+06	1,07E+06	6,92
2	Carbamazepine	4,68E+06	1,09E+06	7,09
3	Carbamazepine	4,41E+06	1,06E+06	6,69
blank	Carbamazepine	1,52E+04	3,47E+03	0,0231
1	Ketoprofen	4,52E+06	1,01E+06	7,38
2	Ketoprofen	4,62E+06	1,05E+06	7,54
3	Ketoprofen	4,12E+06	9,53E+05	6,72
blank	Ketoprofen	3,09E+04	5,55E+03	0,0504
1	Naproxen	1,79E+06	4,22E+05	6,1
2	Naproxen	1,80E+06	4,27E+05	6,13
3	Naproxen	1,61E+06	3,97E+05	5,5
blank	Naproxen	1,36E+04	2,50E+03	0,0464
1	4-chloroaniline	0,00E+00	0,00E+00	No Peak
2	4-chloroaniline	0,00E+00	0,00E+00	No Peak
3	4-chloroaniline	0,00E+00	0,00E+00	No Peak
blank	4-chloroaniline	0,00E+00	0,00E+00	No Peak
1	Diclofenac	3,58E+06	7,09E+05	6,73
2	Diclofenac	3,64E+06	7,24E+05	6,86
3	Diclofenac	1,89E+06	3,95E+05	3,37
blank	Diclofenac	9,66E+03	1,94E+03	< 0
1	Ibuprofen	1,88E+06	5,36E+05	4,15
2	Ibuprofen	2,11E+06	5,81E+05	4,71
3	Ibuprofen	1,51E+06	4,22E+05	3,26
blank	Ibuprofen	3,51E+03	1,23E+03	< 0

Table 4. LC-MS/MS data of each compound of the Oasis HLB with 2 mM SDS method. The concentration is shown in ppb, the peak area in counts and peak height in cps.

Sample	Analyte	Analyte Peak Area (counts)	Analyte Peak Height (cps)	Calculated Concentration (ppb)
1	N-methylpiperazine	7,97E+04	2,84E+04	2,11
2	N-methylpiperazine	9,69E+04	3,41E+04	2,58
3	N-methylpiperazine	1,28E+05	4,54E+04	3,44
blank	N-methylpiperazine	5,25E+02	1,79E+02	< 0
1	Metformin	3,55E+06	1,20E+06	7,14
2	Metformin	3,80E+06	1,29E+06	7,65
3	Metformin	3,88E+06	1,33E+06	7,82
blank	Metformin	7,11E+04	2,47E+04	0,0462
1	Guanylurea	1,23E+06	3,35E+05	8,39
2	Guanylurea	1,28E+06	3,42E+05	8,71
3	Guanylurea	1,26E+06	3,47E+05	8,6
blank	Guanylurea	1,25E+03	3,27E+02	< 0
1	Carbamazepine	2,60E+06	4,66E+05	6,73
2	Carbamazepine	2,78E+06	5,05E+05	7,19
3	Carbamazepine	2,84E+06	5,09E+05	7,36
1	Ketoprofen	1,66E+06	2,88E+05	5,07
2	Ketoprofen	1,74E+06	2,98E+05	5,3
3	Ketoprofen	2,10E+06	3,57E+05	6,42
1	Naproxen	6,61E+05	1,19E+05	4,09
2	Naproxen	7,38E+05	1,32E+05	4,57
3	Naproxen	9,14E+05	1,65E+05	5,67
1	4-chloroaniline	2,23E+05	5,19E+04	1,27
2	4-chloroaniline	1,52E+05	3,50E+04	0,868
3	4-chloroaniline	2,40E+05	5,66E+04	1,37
blanc	4-chloroaniline	0,00E+00	0,00E+00	No Peak
1	Diclofenac	2,10E+06	3,78E+05	5,59
2	Diclofenac	2,29E+06	4,24E+05	6,11
3	Diclofenac	2,64E+06	4,65E+05	7,02
1	Ibuprofen	9,79E+05	2,69E+05	3,23
2	Ibuprofen	1,67E+06	4,68E+05	5,55
3	Ibuprofen	2,01E+06	5,46E+05	6,68

Table 5. LC-MS/MS data of each compound of the Oasis HLB with 6 mM SDS method. The concentration is shown in ppb, the peak area in counts and peak height in cps.

Sample	Analyte	Analyte Peak Area (counts)	Analyte Peak Height (cps)	Calculated Concentration (ppb)
1	N-methylpiperazine	2,86E+05	9,89E+04	7,7
2	N-methylpiperazine	2,92E+05	1,02E+05	7,88
3	N-methylpiperazine	2,86E+05	9,22E+04	7,72
blank	N-methylpiperazine	3,79E+02	1,17E+02	< 0
1	Metformin	3,68E+06	1,26E+06	7,4
2	Metformin	3,90E+06	1,34E+06	7,85
3	Metformin	3,56E+06	1,16E+06	7,15
blank	Metformin	2,55E+03	6,42E+02	< 0
1	Guanylurea	1,24E+06	3,36E+05	8,42
2	Guanylurea	1,33E+06	3,59E+05	9,09
3	Guanylurea	1,27E+06	3,46E+05	8,63
blank	Guanylurea	1,93E+03	3,42E+02	< 0
1	Carbamazepine	4,43E+06	9,78E+05	7,87
2	Carbamazepine	4,54E+06	1,06E+06	8,06
3	Carbamazepine	3,70E+06	8,80E+05	6,58
blank	Carbamazepine	1,87E+04	4,26E+03	0,0331
1	Ketoprofen	3,85E+06	8,64E+05	7,65
2	Ketoprofen	3,48E+06	8,12E+05	6,92
3	Ketoprofen	2,61E+06	6,17E+05	5,2
blank	Ketoprofen	1,42E+04	2,57E+03	0,0281
1	Naproxen	1,90E+06	4,51E+05	7,54
2	Naproxen	1,61E+06	3,78E+05	6,39
3	Naproxen	1,24E+06	3,02E+05	4,93
blank	Naproxen	9,10E+03	1,66E+03	0,0361
1	4-chloroaniline	3,96E+05	9,26E+04	2,26
2	4-chloroaniline	4,04E+05	9,29E+04	2,3
3	4-chloroaniline	4,62E+05	1,07E+05	2,63
blank	4-chloroaniline	0,00E+00	0,00E+00	No Peak
1	Diclofenac	1,32E+06	2,32E+05	2,5
2	Diclofenac	3,20E+06	5,65E+05	6,45
3	Diclofenac	4,08E+06	6,77E+05	8,3
blank	Diclofenac	1,84E+04	2,25E+03	< 0
1	Ibuprofen	2,02E+06	5,67E+05	4,71
2	Ibuprofen	2,82E+06	8,32E+05	6,71
3	Ibuprofen	3,22E+06	9,13E+05	7,68
blank	Ibuprofen	1,39E+04	3,18E+03	< 0

Table6. LC-MS/MS data of each compound of the Oasis HLB with 6 mM SDS method with increased eluent volume of 8 mL and a 150 mg cartridge. The concentration is shown in ppb, the peak area in counts and peak height in cps.

Sample	Analyte	Analyte Peak Area (counts)	Analyte Peak Height (cps)	Calculated Concentration (ppb)
1	N-methylpiperazine	1,54E+05	3,65E+04	6,69
2	N-methylpiperazine	1,35E+05	3,35E+04	5,84
3	N-methylpiperazine	1,56E+05	3,80E+04	6,78
4	N-methylpiperazine	1,78E+05	4,50E+04	7,7
5	N-methylpiperazine	1,77E+05	4,35E+04	7,68
6	N-methylpiperazine	1,18E+05	2,58E+04	5,13
7	N-methylpiperazine	1,93E+05	4,77E+04	8,38
8	N-methylpiperazine	1,31E+05	3,12E+04	5,69
blank	N-methylpiperazine	2,31E+02	7,32E+01	0,0527
1	Metformin	3,27E+06	8,47E+05	7,5
2	Metformin	3,03E+06	7,87E+05	6,94
3	Metformin	2,79E+06	7,13E+05	6,41
4	Metformin	3,29E+06	8,45E+05	7,55
5	Metformin	3,17E+06	8,09E+05	7,27
6	Metformin	3,12E+06	8,01E+05	7,16
7	Metformin	3,15E+06	8,10E+05	7,23
8	Metformin	3,15E+06	8,03E+05	7,22
blank	Metformin	0,00E+00	0,00E+00	No Peak
1	Guanylurea	4,36E+05	1,08E+05	7,89
2	Guanylurea	4,10E+05	1,00E+05	7,42
3	Guanylurea	3,83E+05	9,39E+04	6,94
4	Guanylurea	4,18E+05	1,02E+05	7,57
5	Guanylurea	4,48E+05	1,09E+05	8,11
6	Guanylurea	4,05E+05	9,90E+04	7,34
7	Guanylurea	4,19E+05	1,04E+05	7,6
8	Guanylurea	4,01E+05	9,84E+04	7,27
blank	Guanylurea	1,87E+03	4,10E+02	< 0
1	Carbamazepine	4,78E+06	1,11E+06	8,36
2	Carbamazepine	4,83E+06	1,10E+06	8,44
3	Carbamazepine	5,06E+06	1,21E+06	8,86
4	Carbamazepine	4,63E+06	1,08E+06	8,09
5	Carbamazepine	4,73E+06	1,13E+06	8,28
6	Carbamazepine	4,64E+06	1,09E+06	8,12
7	Carbamazepine	4,58E+06	1,05E+06	8
8	Carbamazepine	4,75E+06	1,09E+06	8,31
blank	Carbamazepine	3,59E+03	9,86E+02	0,00627
1	Ketoprofen	3,56E+06	8,09E+05	6,46
2	Ketoprofen	4,70E+06	1,06E+06	8,52
3	Ketoprofen	3,60E+06	8,16E+05	6,53
4	Ketoprofen	3,78E+06	8,51E+05	6,85
5	Ketoprofen	4,25E+06	9,68E+05	7,7
6	Ketoprofen	2,94E+06	6,60E+05	5,32
7	Ketoprofen	3,11E+06	6,90E+05	5,64
8	Ketoprofen	2,49E+06	5,49E+05	4,51
blank	Ketoprofen	1,38E+04	2,95E+03	0,025

1	Naproxen	1,48E+06	3,55E+05	6,74
2	Naproxen	1,76E+06	4,07E+05	8,01
3	Naproxen	1,45E+06	3,51E+05	6,6
4	Naproxen	1,54E+06	3,64E+05	7
5	Naproxen	1,68E+06	3,94E+05	7,62
6	Naproxen	1,20E+06	2,87E+05	5,48
7	Naproxen	1,27E+06	2,93E+05	5,77
8	Naproxen	1,05E+06	2,43E+05	4,79
blank	Naproxen	2,49E+03	6,59E+02	0,0113
1	4-chloroaniline	2,77E+05	6,86E+04	5,59
2	4-chloroaniline	2,29E+05	5,61E+04	4,62
3	4-chloroaniline	3,31E+05	8,19E+04	6,68
4	4-chloroaniline	3,32E+05	8,27E+04	6,7
5	4-chloroaniline	3,20E+05	7,83E+04	6,45
6	4-chloroaniline	4,48E+05	1,09E+05	9,02
7	4-chloroaniline	2,72E+05	6,65E+04	5,49
8	4-chloroaniline	3,83E+05	9,24E+04	7,72
blank	4-chloroaniline	0,00E+00	0,00E+00	No Peak
1	Diclofenac	3,33E+06	5,66E+05	6,79
2	Diclofenac	3,92E+06	6,76E+05	8,03
3	Diclofenac	3,62E+06	6,75E+05	7,4
4	Diclofenac	3,20E+06	5,69E+05	6,51
5	Diclofenac	3,16E+06	5,54E+05	6,43
6	Diclofenac	3,43E+06	5,84E+05	7
7	Diclofenac	3,62E+06	6,44E+05	7,39
8	Diclofenac	3,04E+06	5,48E+05	6,18
blank	Diclofenac	1,51E+04	2,93E+03	< 0
1	Ibuprofen	2,47E+06	6,46E+05	6,53
2	Ibuprofen	3,00E+06	7,89E+05	7,99
3	Ibuprofen	2,65E+06	7,42E+05	7,02
4	Ibuprofen	2,58E+06	7,31E+05	6,84
5	Ibuprofen	2,74E+06	7,58E+05	7,26
6	Ibuprofen	2,36E+06	6,66E+05	6,24
7	Ibuprofen	2,53E+06	7,33E+05	6,71
8	Ibuprofen	2,12E+06	6,04E+05	5,57
blank	Ibuprofen	3,72E+03	1,27E+03	< 0

Table 7. LC-MS/MS data of each compound of the Oasis HLB with 6 mM SDS method with increased eluent volume of 8 mL and a 60 mg cartridge. The concentration is shown in ppb, the peak area in counts and peak height in cps.

Sample	Analyte	Analyte Peak Area (counts)	Analyte Peak Height (cps)	Calculated Concentration (ppb)
1	N-methylpiperazine	1,45E+05	3,66E+04	6,27
2	N-methylpiperazine	1,53E+05	3,86E+04	6,63
3	N-methylpiperazine	1,57E+05	3,73E+04	6,81
4	N-methylpiperazine	1,66E+05	4,22E+04	7,22
blank	N-methylpiperazine	0,00E+00	0,00E+00	No Peak
1	Metformin	2,93E+06	7,53E+05	6,72
2	Metformin	3,36E+06	8,72E+05	7,72
3	Metformin	3,08E+06	7,97E+05	7,08
4	Metformin	3,48E+06	8,85E+05	8
blank	Metformin	0,00E+00	0,00E+00	No Peak
1	Guanylurea	3,90E+05	9,71E+04	7,07
2	Guanylurea	4,44E+05	1,10E+05	8,05
3	Guanylurea	3,90E+05	9,50E+04	7,06
4	Guanylurea	4,37E+05	1,06E+05	7,92
blank	Guanylurea	0,00E+00	0,00E+00	No Peak
1	Carbamazepine	4,51E+06	1,06E+06	7,89
2	Carbamazepine	4,37E+06	9,98E+05	7,64
3	Carbamazepine	4,63E+06	1,09E+06	8,11
4	Carbamazepine	4,54E+06	1,09E+06	7,94
blank	Carbamazepine	5,18E+03	1,37E+03	0,00907
1	Ketoprofen	2,10E+06	4,67E+05	3,8
2	Ketoprofen	2,15E+06	4,69E+05	3,89
3	Ketoprofen	1,71E+06	3,89E+05	3,09
4	Ketoprofen	2,37E+06	5,68E+05	4,3
blank	Ketoprofen	1,55E+04	2,88E+03	0,0282
1	Naproxen	8,27E+05	1,91E+05	3,76
2	Naproxen	8,35E+05	1,90E+05	3,79
3	Naproxen	6,49E+05	1,57E+05	2,95
4	Naproxen	8,99E+05	2,21E+05	4,09
blank	Naproxen	2,56E+03	6,63E+02	0,0116
1	4-chloroaniline	1,49E+05	3,66E+04	3,01
2	4-chloroaniline	2,62E+05	6,33E+04	5,29
3	4-chloroaniline	2,33E+05	5,67E+04	4,7
4	4-chloroaniline	3,74E+05	9,37E+04	7,54
blank	4-chloroaniline	0,00E+00	0,00E+00	No Peak
1	Diclofenac	2,03E+06	3,51E+05	4,03
2	Diclofenac	2,28E+06	4,13E+05	4,56
3	Diclofenac	2,00E+06	3,51E+05	3,97
4	Diclofenac	2,45E+06	4,73E+05	4,91
blank	Diclofenac	1,23E+04	2,07E+03	< 0
1	Ibuprofen	1,52E+06	4,24E+05	3,93
2	Ibuprofen	1,70E+06	4,80E+05	4,43
3	Ibuprofen	1,48E+06	4,10E+05	3,81
4	Ibuprofen	1,97E+06	5,94E+05	5,16
blank	Ibuprofen	3,71E+03	1,24E+03	< 0

Table 8. LC-MS/MS data of each compound of the Oasis HLB with 6 mM SDS method and addition of 0,1% formic acid in working solution. The concentration is shown in ppb, the peak area in counts and peak height in cps.

Sample	Analyte	Analyte Peak Area (counts)	Analyte Peak Height (cps)	Calculated Concentration (ppb)
1	N-methylpiperazine	1,31E+05	4,68E+04	4,01
2	N-methylpiperazine	1,19E+05	3,94E+04	3,64
3	N-methylpiperazine	1,30E+05	4,65E+04	3,96
blank	N-methylpiperazine	9,15E+02	2,11E+02	< 0
1	Metformin	1,74E+06	6,07E+05	5,24
2	Metformin	1,60E+06	5,58E+05	4,8
3	Metformin	1,67E+06	5,78E+05	5,03
blank	Metformin	0,00E+00	0,00E+00	No Peak
1	Guanylurea	2,09E+05	6,52E+04	6,09
2	Guanylurea	2,03E+05	6,50E+04	5,89
3	Guanylurea	2,04E+05	6,29E+04	5,94
blank	Guanylurea	4,09E+02	7,60E+01	< 0
1	Carbamazepine	4,69E+06	1,11E+06	7,11
2	Carbamazepine	4,73E+06	1,10E+06	7,17
3	Carbamazepine	4,86E+06	1,15E+06	7,37
blank	Carbamazepine	1,30E+04	2,52E+03	0,0196
1	Ketoprofen	3,24E+04	6,92E+03	0,0529
2	Ketoprofen	8,21E+04	1,92E+04	0,134
3	Ketoprofen	1,73E+05	4,12E+04	0,282
blank	Ketoprofen	1,05E+04	2,17E+03	0,0171
1	Naproxen	1,37E+04	2,44E+03	0,0468
2	Naproxen	2,92E+04	6,39E+03	0,0996
3	Naproxen	6,05E+04	1,45E+04	0,206
blank	Naproxen	2,96E+03	4,05E+02	0,0101
1	4-chloroaniline	9,25E+05	2,19E+05	8,85
2	4-chloroaniline	9,86E+05	2,43E+05	9,44
3	4-chloroaniline	9,34E+05	2,22E+05	8,94
blank	4-chloroaniline	0,00E+00	0,00E+00	No Peak
1	Diclofenac	2,57E+04	5,21E+03	< 0
2	Diclofenac	5,30E+04	1,09E+04	< 0
3	Diclofenac	5,36E+04	1,09E+04	< 0
blank	Diclofenac	6,72E+03	1,45E+03	< 0
1	Ibuprofen	1,47E+04	4,06E+03	< 0
2	Ibuprofen	4,15E+04	1,23E+04	< 0
3	Ibuprofen	9,66E+04	2,86E+04	< 0
blank	Ibuprofen	0,00E+00	0,00E+00	No Peak

Table 9. LC-MS/MS data of each compound of the Oasis HLB with 6 mM SDS method and addition of 0,1% formic acid in eluant solution. The concentration is shown in ppb, the peak area in counts and peak height in cps.

Sample	Analyte	Analyte Peak Area (counts)	Analyte Peak Height (cps)	Calculated Concentration (ppb)
1	N-methylpiperazine	5,86E+04	1,39E+04	2,57
2	N-methylpiperazine	1,08E+05	2,65E+04	4,71
3	N-methylpiperazine	8,54E+04	2,14E+04	3,73
blank	N-methylpiperazine	0,00E+00	0,00E+00	No Peak
1	Metformin	2,54E+06	6,56E+05	5,82
2	Metformin	3,11E+06	7,97E+05	7,13
3	Metformin	2,78E+06	7,21E+05	6,38
blank	Metformin	0,00E+00	0,00E+00	No Peak
1	Guanylurea	3,32E+05	8,19E+04	6
2	Guanylurea	3,97E+05	9,71E+04	7,19
3	Guanylurea	3,64E+05	8,97E+04	6,58
blank	Guanylurea	6,17E+02	1,53E+02	< 0
1	Carbamazepine	3,68E+06	6,77E+05	8,27
2	Carbamazepine	4,02E+06	7,07E+05	9,03
3	Carbamazepine	3,72E+06	6,78E+05	8,35
blank	Carbamazepine	0,00E+00	0,00E+00	No Peak
1	Ketoprofen	2,20E+06	3,76E+05	6,77
2	Ketoprofen	2,26E+06	3,81E+05	6,96
3	Ketoprofen	2,25E+06	3,81E+05	6,9
blank	Ketoprofen	1,30E+04	2,59E+03	0,0401
1	Naproxen	9,38E+05	1,67E+05	5,58
2	Naproxen	1,02E+06	1,78E+05	6,1
3	Naproxen	9,17E+05	1,61E+05	5,46
blank	Naproxen	9,44E+03	1,32E+03	0,0562
1	4-chloroaniline	4,69E+05	8,42E+04	4,64
2	4-chloroaniline	6,21E+05	1,12E+05	6,15
3	4-chloroaniline	3,00E+05	5,39E+04	2,97
blank	4-chloroaniline	0,00E+00	0,00E+00	No Peak
1	Diclofenac	1,17E+04	2,52E+03	< 0
2	Diclofenac	1,52E+04	3,09E+03	< 0
3	Diclofenac	2,22E+04	4,42E+03	< 0
blank	Diclofenac	5,80E+01	1,86E+01	< 0
1	Ibuprofen	7,68E+03	2,38E+03	< 0
2	Ibuprofen	1,12E+04	3,50E+03	< 0
3	Ibuprofen	2,00E+04	6,10E+03	< 0
blank	Ibuprofen	0,00E+00	0,00E+00	No Peak

Appendix 4. LC-MS/MS data of calibration standards with internal standard

Table 1. LC-MS/MS calibration data of carbamazepine. The concentration is shown in ppb, the peak area in counts and peak height in cps. The internal standard used is carbamazepine-C13.

Sample Name	Sample Type	Analyte Peak Area (counts)	Analyte Peak Height (cps)	IS Peak Area (counts)	IS Peak Height (cps)	Calculated Concentration (ppb)
Blank	Blank	0,00E+00	0,00E+00	0,00E+00	0,00E+00	N/A
Blank	Blank	0,00E+00	0,00E+00	0,00E+00	0,00E+00	N/A
CBZ1	Standard	2,00E+05	8,14E+04	7,41E+04	3,03E+04	0,474
CBZ1	Standard	1,98E+05	7,93E+04	7,91E+04	3,35E+04	0,437
CBZ2	Standard	4,31E+05	1,64E+05	6,91E+04	2,56E+04	1,15
CBZ2	Standard	4,17E+05	1,63E+05	7,34E+04	2,74E+04	1,04
CBZ3	Standard	1,97E+06	7,30E+05	7,01E+04	2,56E+04	5,34
CBZ3	Standard	2,06E+06	7,72E+05	7,26E+04	2,59E+04	5,39
CBZ4	Standard	3,91E+06	1,45E+06	8,33E+04	3,30E+04	8,94
CBZ4	Standard	4,01E+06	1,44E+06	7,91E+04	3,06E+04	9,66
CBZ5	Standard	5,64E+06	1,97E+06	7,08E+04	2,48E+04	15,2
CBZ5	Standard	5,30E+06	1,73E+06	6,38E+04	2,50E+04	15,9
CBZ6	Standard	7,30E+06	2,45E+06	7,15E+04	2,59E+04	19,5
CBZ6	Standard	6,23E+06	1,86E+06	7,06E+04	2,55E+04	16,9

Table 2. LC-MS/MS calibration data of ketoprofen. The concentration is shown in ppb, the peak area in counts and peak height in cps. The internal standard used is ketoprofen-D3.

Sample Name	Sample Type	Analyte Peak Area (counts)	Analyte Peak Height (cps)	IS Peak Area (counts)	IS Peak Height (cps)	Calculated Concentration (ppb)
Blank	Blank	0,00E+00	0,00E+00	0,00E+00	0,00E+00	N/A
Blank	Blank	0,00E+00	0,00E+00	0,00E+00	0,00E+00	N/A
KET1	Standard	1,74E+05	5,96E+04	9,67E+05	3,32E+05	0,503
KET1	Standard	1,78E+05	5,92E+04	9,48E+05	3,21E+05	0,526
KET2	Standard	3,71E+05	1,32E+05	1,00E+06	3,58E+05	1,08
KET2	Standard	4,01E+05	1,33E+05	1,06E+06	3,60E+05	1,1
KET3	Standard	1,86E+06	6,23E+05	1,01E+06	3,36E+05	5,59
KET3	Standard	1,85E+06	6,57E+05	1,05E+06	4,00E+05	5,36
KET4	Standard	3,59E+06	1,25E+06	9,80E+05	3,40E+05	11,1
KET4	Standard	3,60E+06	1,22E+06	9,80E+05	3,46E+05	11,2
KET5	Standard	5,17E+06	1,62E+06	1,01E+06	3,38E+05	15,6
KET5	Standard	5,00E+06	1,49E+06	9,82E+05	3,15E+05	15,5
KET6	Standard	6,58E+06	2,00E+06	9,75E+05	3,20E+05	20,5
KET6	Standard	6,58E+06	2,09E+06	1,02E+06	3,56E+05	19,7

Table 3. LC-MS/MS calibration data of naproxen. The concentration is shown in ppb, the peak area in counts and peak height in cps. The internal standard used is naproxen-D3.

Sample Name	Sample Type	Analyte Peak Area (counts)	Analyte Peak Height (cps)	IS Peak Area (counts)	IS Peak Height (cps)	Calculated Concentration (ppb)
Blank	Blank	0,00E+00	0,00E+00	0,00E+00	0,00E+00	N/A
Blank	Blank	0,00E+00	0,00E+00	0,00E+00	0,00E+00	N/A
NAP1	Standard	8,74E+04	2,77E+04	6,36E+04	2,31E+04	0,461
NAP1	Standard	8,14E+04	2,65E+04	5,91E+04	2,12E+04	0,462
NAP2	Standard	1,42E+05	5,30E+04	5,59E+04	1,99E+04	0,859
NAP2	Standard	1,65E+05	5,67E+04	6,10E+04	2,42E+04	0,911
NAP3	Standard	8,77E+05	3,15E+05	6,07E+04	2,32E+04	4,91
NAP3	Standard	8,74E+05	3,32E+05	5,97E+04	2,12E+04	4,98
NAP4	Standard	1,66E+06	6,10E+05	5,25E+04	2,20E+04	10,8
NAP4	Standard	1,65E+06	6,40E+05	6,09E+04	2,45E+04	9,21
NAP5	Standard	2,33E+06	8,72E+05	5,95E+04	2,17E+04	13,3
NAP5	Standard	2,30E+06	7,96E+05	5,28E+04	2,02E+04	14,8
NAP6	Standard	3,16E+06	1,07E+06	5,30E+04	1,85E+04	20,3
NAP6	Standard	3,05E+06	1,07E+06	5,58E+04	2,08E+04	18,6

Table 4. LC-MS/MS calibration data of 4-chloroaniline. The concentration is shown in ppb, the peak area in counts and peak height in cps.

Sample Name	Sample Type	Analyte Peak Area (counts)	Analyte Peak Height (cps)	IS Peak Area (counts)	IS Peak Height (cps)	Calculated Concentration (ppb)
Blank	Blank	0,00E+00	0,00E+00	N/A	N/A	N/A
Blank	Blank	0,00E+00	0,00E+00	N/A	N/A	N/A
4CA1	Standard	5,73E+04	2,25E+04	N/A	N/A	0,474
4CA1	Standard	6,37E+04	2,35E+04	N/A	N/A	0,526
4CA2	Standard	1,25E+05	4,83E+04	N/A	N/A	1,02
4CA2	Standard	1,26E+05	4,76E+04	N/A	N/A	1,03
4CA3	Standard	5,81E+05	2,21E+05	N/A	N/A	4,74
4CA3	Standard	6,39E+05	2,37E+05	N/A	N/A	5,21
4CA4	Standard	1,24E+06	4,56E+05	N/A	N/A	10,1
4CA4	Standard	1,16E+06	4,68E+05	N/A	N/A	9,44
4CA5	Standard	1,98E+06	7,42E+05	N/A	N/A	16,1
4CA5	Standard	1,95E+06	7,23E+05	N/A	N/A	15,9
4CA6	Standard	2,53E+06	9,12E+05	N/A	N/A	20,6
4CA6	Standard	2,56E+06	9,38E+05	N/A	N/A	20,8

Table 5. LC-MS/MS calibration data of diclofenac. The concentration is shown in ppb, the peak area in counts and peak height in cps. The internal standard used is diclofenac-C13.

Sample Name	Sample Type	Analyte Peak Area (counts)	Analyte Peak Height (cps)	IS Peak Area (counts)	IS Peak Height (cps)	Calculated Concentration (ppb)
Blank	Blank	0,00E+00	0,00E+00	0,00E+00	0,00E+00	N/A
Blank	Blank	0,00E+00	0,00E+00	0,00E+00	0,00E+00	N/A
DIC1	Standard	2,18E+05	4,28E+04	1,08E+06	1,81E+05	0,514
DIC1	Standard	1,79E+05	3,73E+04	9,40E+05	1,62E+05	0,486
DIC2	Standard	3,87E+05	7,64E+04	1,01E+06	1,80E+05	0,973
DIC2	Standard	3,84E+05	7,41E+04	1,00E+06	1,77E+05	0,972
DIC3	Standard	2,06E+06	3,61E+05	9,63E+05	1,63E+05	5,42
DIC3	Standard	2,06E+06	3,77E+05	9,81E+05	1,72E+05	5,33
DIC4	Standard	4,12E+06	7,01E+05	9,82E+05	1,67E+05	10,6
DIC4	Standard	4,11E+06	6,99E+05	9,60E+05	1,65E+05	10,9
DIC5	Standard	5,97E+06	1,00E+06	9,65E+05	1,70E+05	15,7
DIC5	Standard	6,30E+06	1,05E+06	9,78E+05	1,62E+05	16,3
DIC6	Standard	7,97E+06	1,28E+06	9,55E+05	1,57E+05	21,1
DIC6	Standard	8,06E+06	1,35E+06	9,50E+05	1,62E+05	21,5

Table 6. LC-MS/MS calibration data of ibuprofen. The concentration is shown in ppb, the peak area in counts and peak height in cps. The internal standard used is ibuprofen-D3.

Sample Name	Sample Type	Analyte Peak Area (counts)	Analyte Peak Height (cps)	IS Peak Area (counts)	IS Peak Height (cps)	Calculated Concentration (ppb)
Blank	Blank	0,00E+00	0,00E+00	0,00E+00	0,00E+00	N/A
Blank	Blank	0,00E+00	0,00E+00	0,00E+00	0,00E+00	N/A
IBU1	Standard	1,70E+05	4,73E+04	1,67E+06	4,36E+05	0,516
IBU1	Standard	1,75E+05	4,75E+04	1,65E+06	4,34E+05	0,54
IBU2	Standard	3,50E+05	9,41E+04	1,63E+06	4,33E+05	1,13
IBU2	Standard	3,45E+05	9,40E+04	1,65E+06	4,46E+05	1,1
IBU3	Standard	1,70E+06	4,47E+05	1,64E+06	4,31E+05	5,6
IBU3	Standard	1,71E+06	4,55E+05	1,68E+06	4,47E+05	5,5
IBU4	Standard	3,40E+06	8,52E+05	1,65E+06	4,15E+05	11,2
IBU4	Standard	3,44E+06	8,34E+05	1,67E+06	4,15E+05	11,2
IBU5	Standard	5,01E+06	1,27E+06	1,64E+06	4,23E+05	16,6
IBU5	Standard	4,94E+06	1,26E+06	1,64E+06	4,26E+05	16,3
IBU6	Standard	6,46E+06	1,54E+06	1,63E+06	4,05E+05	21,5
IBU6	Standard	6,55E+06	1,59E+06	1,64E+06	4,19E+05	21,7

Table 7. LC-MS/MS calibration data of n-methylpiperazine. The concentration is shown in ppb, the peak area in counts and peak height in cps.

Sample Name	Sample Type	Analyte Peak Area (counts)	Analyte Peak Height (cps)	IS Peak Area (counts)	IS Peak Height (cps)	Calculated Concentration (ppb)
Blank	Blank	0,00E+00	0,00E+00	N/A	N/A	N/A
Blank	Blank	0,00E+00	0,00E+00	N/A	N/A	N/A
NMZ1	Standard	7,01E+03	1,83E+03	N/A	N/A	0,296
NMZ1	Standard	6,82E+03	1,60E+03	N/A	N/A	0,287
NMZ2	Standard	1,46E+04	3,50E+03	N/A	N/A	0,65
NMZ2	Standard	1,32E+04	3,00E+03	N/A	N/A	0,585
NMZ3	Standard	1,16E+05	2,64E+04	N/A	N/A	5,36
NMZ3	Standard	1,16E+05	2,65E+04	N/A	N/A	5,36
NMZ4	Standard	2,32E+05	5,32E+04	N/A	N/A	10,8
NMZ4	Standard	2,18E+05	5,06E+04	N/A	N/A	10,1
NMZ5	Standard	2,57E+05	5,45E+04	N/A	N/A	11,9
NMZ5	Standard	3,53E+05	7,97E+04	N/A	N/A	16,4
NMZ6	Standard	3,73E+05	7,61E+04	N/A	N/A	17,4
NMZ6	Standard	4,25E+05	9,35E+04	N/A	N/A	19,8

Table 8. LC-MS/MS calibration data of metformin. The concentration is shown in ppb, the peak area in counts and peak height in cps. The internal standard used is metformin-D6.

Sample Name	Sample Type	Analyte Peak Area (counts)	Analyte Peak Height (cps)	IS Peak Area (counts)	IS Peak Height (cps)	Calculated Concentration (ppb)
Blank	Blank	0,00E+00	0,00E+00	0,00E+00	0,00E+00	N/A
Blank	Blank	0,00E+00	0,00E+00	0,00E+00	0,00E+00	N/A
MET1	Standard	1,82E+05	4,28E+04	2,79E+04	6,27E+03	0,413
MET1	Standard	1,76E+05	4,09E+04	3,12E+04	7,30E+03	0,357
MET2	Standard	3,47E+05	7,97E+04	2,49E+04	5,66E+03	0,871
MET2	Standard	3,31E+05	7,58E+04	3,37E+04	8,15E+03	0,616
MET3	Standard	1,64E+06	3,71E+05	2,83E+04	6,85E+03	3,59
MET3	Standard	1,66E+06	3,77E+05	2,88E+04	6,97E+03	3,58
MET4	Standard	3,11E+06	7,11E+05	2,80E+04	6,44E+03	6,86
MET4	Standard	3,13E+06	7,14E+05	2,08E+04	4,40E+03	9,33
MET5	Standard	4,70E+06	1,06E+06	2,62E+04	6,21E+03	11,1
MET5	Standard	4,35E+06	9,87E+05	2,56E+04	6,11E+03	10,5
MET6	Standard	5,78E+06	1,30E+06	2,08E+04	4,72E+03	17,2
MET6	Standard	5,58E+06	1,22E+06	2,47E+04	5,38E+03	14

Table 9. LC-MS/MS calibration data of guanylurea. The concentration is shown in ppb, the peak area in counts and peak height in cps. The internal standard used is guanylurea-15N4.

Sample Name	Sample Type	Analyte Peak Area (counts)	Analyte Peak Height (cps)	IS Peak Area (counts)	IS Peak Height (cps)	Calculated Concentration (ppb)
Blank	Blank	0,00E+00	0,00E+00	0,00E+00	0,00E+00	N/A
Blank	Blank	0,00E+00	0,00E+00	0,00E+00	0,00E+00	N/A
GUA1	Standard	1,95E+04	4,51E+03	4,67E+04	1,07E+04	0,39
GUA1	Standard	1,98E+04	4,49E+03	4,05E+04	8,64E+03	0,455
GUA2	Standard	3,73E+04	8,09E+03	4,24E+04	9,34E+03	0,81
GUA2	Standard	3,80E+04	8,29E+03	4,42E+04	9,41E+03	0,791
GUA3	Standard	2,05E+05	4,26E+04	4,04E+04	8,64E+03	4,62
GUA3	Standard	1,90E+05	3,99E+04	4,26E+04	9,22E+03	4,06
GUA4	Standard	3,98E+05	8,20E+04	4,09E+04	8,50E+03	8,86
GUA4	Standard	3,86E+05	8,01E+04	4,07E+04	8,61E+03	8,64
GUA5	Standard	5,41E+05	1,13E+05	3,96E+04	8,59E+03	12,4
GUA5	Standard	5,58E+05	1,19E+05	4,23E+04	9,11E+03	12
GUA6	Standard	7,69E+05	1,62E+05	3,88E+04	8,27E+03	18
GUA6	Standard	7,88E+05	1,64E+05	4,15E+04	8,89E+03	17,3

Appendix 5. LC-MS/MS data of wastewater samples

Table 1. LC-MS/MS n-methylpiperazine data of wastewater sample from 5 locations. The concentration is shown in ppb, the peak area in counts and peak height in cps.

Sample	Analyte	Analyte Peak Area(counts)	Analyte Peak Height(cps)	IS Peak Area (counts)	IS Peak Height(cps)	Calculated Concentration (ppb)
SPE blank	NMZ	0,00E+00	0,00E+00	N/A	N/A	No Peak
SPE control	NMZ	0,00E+00	0,00E+00	N/A	N/A	No Peak
Utrecht Inflow 1	NMZ	1,38E+03	3,94E+02	N/A	N/A	0,0335
Utrecht Inflow 2	NMZ	1,11E+03	3,54E+02	N/A	N/A	0,0208
Utrecht Inflow 3	NMZ	7,19E+02	2,45E+02	N/A	N/A	0,00255
Utrecht Outflow 1	NMZ	0,00E+00	0,00E+00	N/A	N/A	No Peak
Utrecht Outflow 2	NMZ	0,00E+00	0,00E+00	N/A	N/A	No Peak
Utrecht Outflow 3	NMZ	0,00E+00	0,00E+00	N/A	N/A	No Peak
Eindh. Inflow 1	NMZ	2,42E+03	5,24E+02	N/A	N/A	0,082
Eindh. Inflow 2	NMZ	5,85E+02	2,14E+02	N/A	N/A	< 0
Eindh. Inflow 3	NMZ	1,54E+03	4,45E+02	N/A	N/A	0,0409
Eindh. Outflow 1	NMZ	0,00E+00	0,00E+00	N/A	N/A	No Peak
Eindh. Outflow 2	NMZ	0,00E+00	0,00E+00	N/A	N/A	No Peak
Eindh. Outflow 3	NMZ	0,00E+00	0,00E+00	N/A	N/A	No Peak
Amstelv. Inflow 1	NMZ	1,42E+03	3,91E+02	N/A	N/A	0,0354
Amstelv. Inflow 2	NMZ	1,60E+03	4,27E+02	N/A	N/A	0,0436
Amstelv. Inflow 3	NMZ	1,55E+03	4,28E+02	N/A	N/A	0,0412
Amstelv. Outflow 1	NMZ	0,00E+00	0,00E+00	N/A	N/A	No Peak
Amstelv. Outflow 2	NMZ	0,00E+00	0,00E+00	N/A	N/A	No Peak
Amstelv. Outflow 3	NMZ	0,00E+00	0,00E+00	N/A	N/A	No Peak
Bennekom Inflow 1	NMZ	3,97E+03	1,00E+03	N/A	N/A	0,154
Bennekom Inflow 2	NMZ	3,55E+03	8,45E+02	N/A	N/A	0,135

Bennekom Inflow 3	NMZ	3,28E+03	8,61E+02	N/A	N/A	0,122
Amsterdam Inflow 1	NMZ	2,06E+03	5,26E+02	N/A	N/A	0,186
Amsterdam Inflow 2	NMZ	2,11E+03	5,63E+02	N/A	N/A	0,188
Amsterdam Inflow 3	NMZ	2,57E+03	6,91E+02	N/A	N/A	0,21
Amsterdam Outflow 2	NMZ	0,00E+00	0,00E+00	N/A	N/A	No Peak
Amsterdam Outflow 3	NMZ	0,00E+00	0,00E+00	N/A	N/A	No Peak

Table 2. LC-MS/MS metformin data of wastewater sample from 5 locations. The concentration is shown in ppb, the peak area in counts and peak height in cps. The internal standard used is metformin-D6.

Sample	Analyte	Analyte Peak Area(counts)	Analyte Peak Height (cps)	IS Peak Area (counts)	IS Peak Height (cps)	Calculated Concentration (ppb)
SPE blank	MET	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
SPE control	MET	0,00E+00	0,00E+00	1,85E+04	4,40E+03	No Peak
Utrecht Inflow 1	MET	1,44E+07	2,57E+06	1,22E+03	3,75E+02	728
Utrecht Inflow 2	MET	1,59E+07	2,79E+06	1,17E+03	3,51E+02	839
Utrecht Inflow 3	MET	1,60E+07	2,86E+06	2,54E+03	6,01E+02	387
Utrecht Outflow 1	MET	2,11E+06	4,98E+05	1,32E+04	3,25E+03	9,89
Utrecht Outflow 2	MET	1,88E+06	4,52E+05	1,10E+04	2,91E+03	10,6
Utrecht Outflow 3	MET	1,80E+06	4,30E+05	1,03E+04	2,80E+03	10,8
Eindh. Inflow 1	MET	1,43E+07	2,52E+06	1,63E+03	4,03E+02	545
Eindh. Inflow 2	MET	1,45E+07	2,53E+06	2,16E+03	4,90E+02	416
Eindh. Inflow 3	MET	1,49E+07	2,55E+06	2,04E+03	4,47E+02	449
Eindh. Outflow 1	MET	1,58E+06	3,78E+05	1,29E+04	3,11E+03	7,59
Eindh. Outflow 2	MET	1,64E+06	3,89E+05	1,28E+04	3,06E+03	7,92
Eindh. Outflow 3	MET	1,57E+06	3,72E+05	1,26E+04	2,97E+03	7,7
Amstelv. Inflow 1	MET	1,59E+07	2,91E+06	2,22E+03	5,47E+02	442
Amstelv.	MET	1,55E+07	2,89E+06	2,82E+03	6,37E+02	341

Inflow 2						
Amstelv. Inflow 3	MET	1,57E+07	2,85E+06	2,02E+03	4,98E+02	478
Amstelv. Outflow 1	MET	3,22E+06	7,60E+05	9,23E+03	2,42E+03	21,5
Amstelv. Outflow 2	MET	3,56E+06	8,40E+05	1,31E+04	3,16E+03	16,8
Amstelv. Outflow 3	MET	3,96E+06	9,33E+05	1,06E+04	2,64E+03	23,1
Bennekom Inflow 1	MET	1,07E+07	1,55E+06	5,93E+02	1,84E+02	1120
Bennekom Inflow 2	MET	1,15E+07	1,69E+06	8,55E+02	2,41E+02	832
Bennekom Inflow 3	MET	1,20E+07	1,69E+06	1,17E+03	3,71E+02	633
Amsterdam Inflow 1	MET	1,58E+07	2,85E+06	6,83E+02	1,65E+02	121
Amsterdam Inflow 2	MET	1,63E+07	2,98E+06	4,33E+02	1,33E+02	197
Amsterdam Inflow 3	MET	1,63E+07	2,95E+06	1,04E+03	2,79E+02	82,5
Amsterdam Outflow 2	MET	4,59E+05	1,13E+05	1,89E+03	4,84E+02	1,33
Amsterdam Outflow 3	MET	4,26E+05	1,06E+05	3,59E+03	6,80E+02	0,687

Table 3. LC-MS/MS guanylurea data of wastewater sample from 5 locations. The concentration is shown in ppb, the peak area in counts and peak height in cps. The internal standard used is guanylurea-15N4.

Sample	Analyte	Analyte Peak Area (counts)	Analyte Peak Height (cps)	IS Peak Area (counts)	IS Peak Height (cps)	Calculated Concentration (ppb)
SPE blank	GUA	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
SPE control	GUA	0,00E+00	0,00E+00	2,86E+04	6,89E+03	No Peak
Utrecht Inflow 1	GUA	1,63E+05	3,62E+04	2,77E+03	7,06E+02	53,7
Utrecht Inflow 2	GUA	2,09E+05	4,71E+04	3,10E+03	8,51E+02	61,2
Utrecht Inflow 3	GUA	2,08E+05	4,72E+04	3,50E+03	8,02E+02	53,9
Utrecht Outflow 1	GUA	9,48E+05	2,21E+05	9,36E+03	2,19E+03	92
Utrecht Outflow 2	GUA	9,16E+05	2,18E+05	1,00E+04	2,48E+03	83,2
Utrecht Outflow 3	GUA	8,27E+05	1,92E+05	8,81E+03	2,14E+03	85,3
Eindh. Inflow 1	GUA	3,11E+05	7,18E+04	1,90E+03	5,24E+02	148
Eindh. Inflow 2	GUA	3,35E+05	7,70E+04	2,60E+03	6,96E+02	117

Eindh. Inflow 3	GUA	3,07E+05	6,92E+04	2,67E+03	7,21E+02	104
Eindh. Outflow 1	GUA	1,36E+06	3,11E+05	1,19E+04	2,82E+03	104
Eindh. Outflow 2	GUA	1,48E+06	3,40E+05	1,32E+04	3,15E+03	102
Eindh. Outflow 3	GUA	1,36E+06	3,13E+05	1,21E+04	2,90E+03	103
Amstelv. Inflow 1	GUA	3,45E+04	8,31E+03	4,79E+03	1,25E+03	6,56
Amstelv. Inflow 2	GUA	3,40E+04	8,32E+03	4,90E+03	1,25E+03	6,32
Amstelv. Inflow 3	GUA	3,14E+04	7,49E+03	4,09E+03	1,05E+03	6,98
Amstelv. Outflow 1	GUA	2,48E+06	5,70E+05	7,60E+03	1,83E+03	296
Amstelv. Outflow 2	GUA	2,88E+06	6,66E+05	8,35E+03	2,01E+03	314
Amstelv. Outflow 3	GUA	3,18E+06	7,01E+05	1,07E+04	2,50E+03	269
Bennekom Inflow 1	GUA	2,59E+04	5,92E+03	1,32E+03	3,86E+02	17,9
Bennekom Inflow 2	GUA	3,20E+04	7,75E+03	2,07E+03	5,48E+02	14,1
Bennekom Inflow 3	GUA	3,10E+04	6,96E+03	1,87E+03	5,31E+02	15,1
Amsterdam Inflow 1	GUA	1,12E+05	2,61E+04	2,06E+02	6,37E+01	45,5
Amsterdam Inflow 2	GUA	1,25E+05	2,95E+04	1,42E+02	4,68E+01	74,4
Amsterdam Inflow 3	GUA	1,16E+05	2,71E+04	2,24E+02	7,48E+01	43,7
Amsterdam Outflow 2	GUA	1,98E+06	4,29E+05	1,26E+03	3,02E+02	132
Amsterdam Outflow 3	GUA	1,90E+06	4,16E+05	9,06E+02	2,34E+02	177

Table 4. LC-MS/MS carbamazepine data of wastewater sample from 5 locations. The concentration is shown in ppb, the peak area in counts and peak height in cps. The internal standard used is carbamazepine-C13.

Sample	Analyte	Analyte Peak Area(counts)	Analyte Peak Height (cps)	IS Peak Area (counts)	IS Peak Height(cps)	Calculated Concentration (ppb)
SPE blank	CBZ	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
SPE control	CBZ	0,00E+00	0,00E+00	6,33E+04	2,70E+04	No Peak
Utrecht Inflow 1	CBZ	0,00E+00	0,00E+00	1,09E+03	4,93E+02	No Peak
Utrecht Inflow 2	CBZ	0,00E+00	0,00E+00	8,05E+02	2,93E+02	No Peak
Utrecht Inflow 3	CBZ	0,00E+00	0,00E+00	9,73E+02	3,24E+02	No Peak
Utrecht Outflow 1	CBZ	0,00E+00	0,00E+00	1,12E+03	4,68E+02	No Peak
Utrecht Outflow 2	CBZ	0,00E+00	0,00E+00	1,18E+03	4,92E+02	No Peak
Utrecht Outflow 3	CBZ	0,00E+00	0,00E+00	2,23E+03	5,35E+02	No Peak
Eindh. Inflow 1	CBZ	0,00E+00	0,00E+00	9,17E+02	3,20E+02	No Peak
Eindh. Inflow 2	CBZ	0,00E+00	0,00E+00	8,61E+02	2,81E+02	No Peak
Eindh. Inflow 3	CBZ	0,00E+00	0,00E+00	8,98E+02	3,31E+02	No Peak
Eindh. Outflow 1	CBZ	0,00E+00	0,00E+00	1,03E+03	3,63E+02	No Peak
Eindh. Outflow 2	CBZ	0,00E+00	0,00E+00	1,10E+03	5,33E+02	No Peak
Eindh. Outflow 3	CBZ	0,00E+00	0,00E+00	9,17E+02	3,50E+02	No Peak
Amstelv. Inflow 1	CBZ	0,00E+00	0,00E+00	1,47E+03	4,09E+02	No Peak
Amstelv. Inflow 2	CBZ	0,00E+00	0,00E+00	6,92E+02	2,21E+02	No Peak
Amstelv. Inflow 3	CBZ	0,00E+00	0,00E+00	9,35E+02	5,55E+02	No Peak
Amstelv. Outflow 1	CBZ	0,00E+00	0,00E+00	8,42E+02	3,27E+02	No Peak
Amstelv. Outflow 2	CBZ	0,00E+00	0,00E+00	1,16E+03	6,61E+02	No Peak
Amstelv. Outflow 3	CBZ	0,00E+00	0,00E+00	1,25E+03	5,58E+02	No Peak
Bennekom Inflow 1	CBZ	0,00E+00	0,00E+00	7,66E+02	2,59E+02	No Peak
Bennekom Inflow 2	CBZ	0,00E+00	0,00E+00	4,11E+02	3,35E+02	No Peak
Bennekom Inflow 3	CBZ	0,00E+00	0,00E+00	5,03E+02	2,40E+02	No Peak
Amsterdam	CBZ	1,83E+04	3,03E+03	0,00E+00	0,00E+00	#DEEL/0!

Inflow 1						
Amsterdam Inflow 2	CBZ	1,12E+04	2,15E+03	0,00E+00	0,00E+00	#DEEL/0!
Amsterdam Inflow 3	CBZ	6,55E+03	1,36E+03	0,00E+00	0,00E+00	#DEEL/0!
Amsterdam Outflow 2	CBZ	3,49E+05	5,69E+04	2,28E+03	4,50E+02	2,34
Amsterdam Outflow 3	CBZ	3,71E+05	5,70E+04	2,06E+03	4,19E+02	2,76

Table 5. LC-MS/MS ketoprofen data of wastewater sample from 5 locations. The concentration is shown in ppb, the peak area in counts and peak height in cps. The internal standard used is ketoprofen-D3.

Sample	Analyte	Analyte Peak Area(counts)	Analyte Peak Height (cps)	IS Peak Area (counts)	IS Peak Height (cps)	Calculated Concentration (ppb)
SPE blank	KET	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
SPE control	KET	0,00E+00	0,00E+00	7,07E+04	1,77E+04	No Peak
Utrecht Inflow 1	KET	0,00E+00	0,00E+00	1,28E+04	3,78E+03	No Peak
Utrecht Inflow 2	KET	0,00E+00	0,00E+00	1,48E+04	5,96E+03	No Peak
Utrecht Inflow 3	KET	0,00E+00	0,00E+00	1,56E+04	7,43E+03	No Peak
Utrecht Outflow 1	KET	0,00E+00	0,00E+00	5,87E+04	2,90E+04	No Peak
Utrecht Outflow 2	KET	1,79E+04	5,17E+03	9,09E+04	3,42E+04	0,556
Utrecht Outflow 3	KET	0,00E+00	0,00E+00	8,69E+04	3,55E+04	No Peak
Eindh. Inflow 1	KET	0,00E+00	0,00E+00	1,80E+04	4,69E+03	No Peak
Eindh. Inflow 2	KET	0,00E+00	0,00E+00	1,41E+04	4,87E+03	No Peak
Eindh. Inflow 3	KET	0,00E+00	0,00E+00	1,44E+04	5,76E+03	No Peak
Eindh. Outflow 1	KET	0,00E+00	0,00E+00	2,17E+04	1,17E+04	No Peak
Eindh. Outflow 2	KET	1,18E+04	4,34E+03	3,87E+04	1,87E+04	0,883
Eindh. Outflow 3	KET	2,08E+04	4,86E+03	3,89E+04	1,71E+04	1,59
Amstelv. Inflow 1	KET	1,87E+04	6,39E+03	2,79E+04	1,20E+04	2
Amstelv. Inflow 2	KET	0,00E+00	0,00E+00	3,14E+04	1,58E+04	No Peak
Amstelv. Inflow 3	KET	1,77E+04	6,49E+03	3,57E+04	1,34E+04	1,47
Amstelv. Outflow 1	KET	0,00E+00	0,00E+00	9,42E+04	3,67E+04	No Peak

Amstelv. Outflow 2	KET	0,00E+00	0,00E+00	8,63E+04	4,01E+04	No Peak
Amstelv. Outflow 3	KET	0,00E+00	0,00E+00	9,06E+04	3,28E+04	No Peak
Bennekom Inflow 1	KET	1,82E+04	5,57E+03	1,50E+04	7,24E+03	3,65
Bennekom Inflow 2	KET	1,29E+04	5,18E+03	1,68E+04	6,55E+03	2,3
Bennekom Inflow 3	KET	1,12E+04	4,27E+03	1,35E+04	6,45E+03	2,48
Amsterdam Inflow 1	KET	2,61E+04	3,14E+03	0,00E+00	0,00E+00	#DEEL/0!
Amsterdam Inflow 2	KET	8,55E+04	5,14E+03	0,00E+00	0,00E+00	#DEEL/0!
Amsterdam Inflow 3	KET	3,77E+04	3,12E+03	0,00E+00	0,00E+00	#DEEL/0!
Amsterdam Outflow 2	KET	7,88E+04	6,69E+03	0,00E+00	0,00E+00	#DEEL/0!
Amsterdam Outflow 3	KET	6,79E+04	5,78E+03	0,00E+00	0,00E+00	#DEEL/0!

Table 6. LC-MS/MS naproxen data of wastewater sample from 5 locations. The concentration is shown in ppb, the peak area in counts and peak height in cps. The internal standard used is naproxen-D3.

Sample	Analyte	Analyte Peak Area (counts)	Analyte Peak Height (cps)	IS Peak Area (counts)	IS Peak Height (cps)	Calculated Concentration (ppb)
SPE blank	NAP	0,00E+00	0,00E+00	1,01E+03	4,09E+02	No Peak
SPE control	NAP	0,00E+00	0,00E+00	1,46E+03	5,33E+02	No Peak
Utrecht Inflow 1	NAP	5,14E+04	1,34E+04	1,26E+03	2,34E+02	13,9
Utrecht Inflow 2	NAP	8,86E+04	2,73E+04	2,16E+03	6,79E+02	14
Utrecht Inflow 3	NAP	6,82E+04	2,39E+04	1,65E+03	7,11E+02	14,1
Utrecht Outflow 1	NAP	4,50E+04	1,26E+04	4,49E+03	1,22E+03	3,41
Utrecht Outflow 2	NAP	6,43E+04	1,72E+04	4,58E+03	1,64E+03	4,77
Utrecht Outflow 3	NAP	4,59E+04	1,38E+04	3,46E+03	1,37E+03	4,52
Eindh. Inflow 1	NAP	9,49E+04	2,22E+04	9,75E+02	2,80E+02	33,2
Eindh. Inflow 2	NAP	8,87E+04	2,77E+04	8,67E+02	5,20E+02	34,9
Eindh. Inflow 3	NAP	1,08E+05	3,52E+04	1,45E+03	4,03E+02	25,4
Eindh. Outflow 1	NAP	9,24E+03	2,55E+03	2,51E+03	8,32E+02	1,25
Eindh.	NAP	1,78E+04	3,79E+03	2,94E+03	1,07E+03	2,05

Outflow 2						
Eindh. Outflow 3	NAP	7,80E+03	2,68E+03	3,29E+03	9,35E+02	0,8
Amstelv. Inflow 1	NAP	1,13E+05	3,60E+04	2,47E+03	8,20E+02	15,6
Amstelv. Inflow 2	NAP	9,06E+04	3,47E+04	3,24E+03	9,32E+02	9,53
Amstelv. Inflow 3	NAP	1,21E+05	3,44E+04	2,24E+03	8,81E+02	18,4
Amstelv. Outflow 1	NAP	6,42E+04	2,59E+04	4,02E+03	1,57E+03	5,43
Amstelv. Outflow 2	NAP	6,62E+04	2,38E+04	5,71E+03	1,49E+03	3,95
Amstelv. Outflow 3	NAP	6,33E+04	2,22E+04	3,95E+03	1,44E+03	5,46
Bennekom Inflow 1	NAP	1,04E+05	3,13E+04	1,55E+03	3,22E+02	22,9
Bennekom Inflow 2	NAP	1,14E+05	4,09E+04	2,36E+03	6,42E+02	16,4
Bennekom Inflow 3	NAP	1,12E+05	4,07E+04	1,83E+03	5,67E+02	20,9
Amsterdam Inflow 1	NAP	1,30E+05	2,34E+04	0,00E+00	0,00E+00	#DEEL/0!
Amsterdam Inflow 2	NAP	1,99E+05	3,68E+04	0,00E+00	0,00E+00	#DEEL/0!
Amsterdam Inflow 3	NAP	1,48E+05	2,87E+04	0,00E+00	0,00E+00	#DEEL/0!
Amsterdam Outflow 2	NAP	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Amsterdam Outflow 3	NAP	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!

Table 7. LC-MS/MS 4-chloroaniline data of wastewater sample from 5 locations. The concentration is shown in ppb, the peak area in counts and peak height in cps.

Sample	Analyte	Analyte Peak Area (counts)	Analyte Peak Height (cps)	IS Peak Area (counts)	IS Peak Height (cps)	Calculated Concentration (ppb)
SPE blank	4CA	0,00E+00	0,00E+00	N/A	N/A	No Peak
SPE control	4CA	0,00E+00	0,00E+00	N/A	N/A	No Peak
Utrecht Inflow 1	4CA	0,00E+00	0,00E+00	N/A	N/A	No Peak
Utrecht Inflow 2	4CA	0,00E+00	0,00E+00	N/A	N/A	No Peak
Utrecht Inflow 3	4CA	0,00E+00	0,00E+00	N/A	N/A	No Peak
Utrecht Outflow 1	4CA	0,00E+00	0,00E+00	N/A	N/A	No Peak
Utrecht Outflow 2	4CA	0,00E+00	0,00E+00	N/A	N/A	No Peak
Utrecht Outflow 3	4CA	0,00E+00	0,00E+00	N/A	N/A	No Peak
Eindh. Inflow 1	4CA	0,00E+00	0,00E+00	N/A	N/A	No Peak
Eindh. Inflow 2	4CA	0,00E+00	0,00E+00	N/A	N/A	No Peak
Eindh. Inflow 3	4CA	0,00E+00	0,00E+00	N/A	N/A	No Peak
Eindh. Outflow 1	4CA	0,00E+00	0,00E+00	N/A	N/A	No Peak
Eindh. Outflow 2	4CA	0,00E+00	0,00E+00	N/A	N/A	No Peak
Eindh. Outflow 3	4CA	0,00E+00	0,00E+00	N/A	N/A	No Peak
Amstelv. Inflow 1	4CA	0,00E+00	0,00E+00	N/A	N/A	No Peak
Amstelv. Inflow 2	4CA	0,00E+00	0,00E+00	N/A	N/A	No Peak
Amstelv. Inflow 3	4CA	0,00E+00	0,00E+00	N/A	N/A	No Peak
Amstelv. Outflow 1	4CA	0,00E+00	0,00E+00	N/A	N/A	No Peak
Amstelv. Outflow 2	4CA	0,00E+00	0,00E+00	N/A	N/A	No Peak
Amstelv. Outflow 3	4CA	0,00E+00	0,00E+00	N/A	N/A	No Peak
Bennekom Inflow 1	4CA	0,00E+00	0,00E+00	N/A	N/A	No Peak
Bennekom Inflow 2	4CA	0,00E+00	0,00E+00	N/A	N/A	No Peak
Bennekom Inflow 3	4CA	0,00E+00	0,00E+00	N/A	N/A	No Peak
Amsterdam	4CA	7,58E+03	1,46E+03	N/A	N/A	0,0883

Inflow 1						
Amsterdam Inflow 2	4CA	0,00E+00	0,00E+00	N/A	N/A	No Peak
Amsterdam Inflow 3	4CA	0,00E+00	0,00E+00	N/A	N/A	No Peak
Amsterdam Outflow 2	4CA	0,00E+00	0,00E+00	N/A	N/A	No Peak
Amsterdam Outflow 3	4CA	0,00E+00	0,00E+00	N/A	N/A	No Peak

Table 8. LC-MS/MS ibuprofen data of wastewater sample from 5 locations. The concentration is shown in ppb, the peak area in counts and peak height in cps. The internal standard used is ibuprofen-D3.

Sample	Analyte	Analyte Peak Area (counts)	Analyte Peak Height (cps)	IS Peak Area (counts)	IS Peak Height (cps)	Calculated Concentration (ppb)
SPE blank	IBU	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
SPE control	IBU	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Utrecht Inflow 1	IBU	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Utrecht Inflow 2	IBU	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Utrecht Inflow 3	IBU	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Utrecht Outflow 1	IBU	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Utrecht Outflow 2	IBU	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Utrecht Outflow 3	IBU	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Eindh. Inflow 1	IBU	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Eindh. Inflow 2	IBU	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Eindh. Inflow 3	IBU	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Eindh. Outflow 1	IBU	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Eindh. Outflow 2	IBU	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Eindh. Outflow 3	IBU	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Amstelv. Inflow 1	IBU	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Amstelv. Inflow 2	IBU	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Amstelv. Inflow 3	IBU	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Amstelv. Outflow 1	IBU	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!

Amstelv. Outflow 2	IBU	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Amstelv. Outflow 3	IBU	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Bennekom Inflow 1	IBU	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Bennekom Inflow 2	IBU	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Bennekom Inflow 3	IBU	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Amsterdam Inflow 1	IBU	5,20E+04	1,34E+04	1,93E+04	5,68E+03	0,602
Amsterdam Inflow 2	IBU	8,65E+04	1,68E+04	9,60E+03	1,77E+03	2,11
Amsterdam Inflow 3	IBU	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Amsterdam Outflow 2	IBU	1,08E+03	3,46E+02	0,00E+00	0,00E+00	#DEEL/0!
Amsterdam Outflow 3	IBU	7,26E+02	2,29E+02	0,00E+00	0,00E+00	#DEEL/0!

Table 9. LC-MS/MS diclofenac data of wastewater sample from 5 locations. The concentration is shown in ppb, the peak area in counts and peak height in cps. The internal standard used is diclofenac-C13.

Sample	Analyte	Analyte Peak Area (counts)	Analyte Peak Height (cps)	IS Peak Area (counts)	IS Peak Height (cps)	Calculated Concentration (ppb)
SPE blank	DIC	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
SPE control	DIC	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Utrecht Inflow 1	DIC	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Utrecht Inflow 2	DIC	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Utrecht Inflow 3	DIC	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Utrecht Outflow 1	DIC	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Utrecht Outflow 2	DIC	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Utrecht Outflow 3	DIC	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Eindh. Inflow 1	DIC	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Eindh. Inflow 2	DIC	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Eindh. Inflow 3	DIC	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Eindh. Outflow 1	DIC	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Eindh.	DIC	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!

Outflow 2						
Eindh. Outflow 3	DIC	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Amstelv. Inflow 1	DIC	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Amstelv. Inflow 2	DIC	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Amstelv. Inflow 3	DIC	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Amstelv. Outflow 1	DIC	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Amstelv. Outflow 2	DIC	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Amstelv. Outflow 3	DIC	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Bennekom Inflow 1	DIC	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Bennekom Inflow 2	DIC	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Bennekom Inflow 3	DIC	0,00E+00	0,00E+00	0,00E+00	0,00E+00	#DEEL/0!
Amsterdam Inflow 1	DIC	7,45E+05	1,93E+05	5,51E+03	2,23E+03	67,4
Amsterdam Inflow 2	DIC	1,60E+06	4,15E+05	1,95E+04	5,94E+03	40,7
Amsterdam Inflow 3	DIC	8,84E+03	2,38E+03	0,00E+00	0,00E+00	#DEEL/0!
Amsterdam Outflow 2	DIC	5,45E+02	1,57E+02	0,00E+00	0,00E+00	#DEEL/0!
Amsterdam Outflow 3	DIC	3,97E+02	1,31E+02	0,00E+00	0,00E+00	#DEEL/0!