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Master Thesis

Active Bioaccumulation of Synthetic Micropollutants in Biofilms and Activated Sludge

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Abstract

Synthetic micropollutants are omnipresent in wastewaters and natural waters where they can have detrimental effects on ecosystems. Microbial communities such as biofilm and activated sludge interact with micropollutants in various ways, including accumulation: a term encompassing both passive adsorption and active bioaccumulation. In this master thesis, the QuEChERS extraction method was used in order to determine the amount of actively bioaccumulated compounds in biofilm and activated sludge. The firstly performed recovery experiment showed that out of 65 substances, 74% could be extracted with sufficient recoveries between 70% and 130% from both tested microbial communities. The following bioaccumulation experiment conducted with biofilm grown in river Ticino and activated sludge from wastewater treatment plant Airolo revealed that 20 of these substances accumulated to at least 10% of the initial amount in at least one of the two microbial communities (passive adsorption and/or active accumulation). Further, eleven of these substances showed active bioaccumulation to at least 5%: Four substances actively bioaccumulated only in biofilm, six substances only in activated sludge and one substance in both microbial communities. Several of the bioaccumulated compounds have already shown bioaccumulation in activated sludge and/or biofilm in other studies. Eight of the actively bioaccumulated compounds contain aliphatic amine moieties, which could facilitate active bioaccumulation.

keywords: micropollutants, biofilm, activated sludge, bioaccumulation, QuEChERS

Résumé

Les micropolluants de synthèse sont omniprésents dans les eaux polluées et les eaux de surface où ils peuvent avoir des effets néfastes sur les écosystèmes. Les communautés microbiennes comme les biofilms ou les boues activées interagissent avec les micropolluants de différentes manières, y compris en les accumulant. Le terme accumulation inclut la sorption passive ainsi que la bioaccumulation active. Dans ce projet de master, la méthode d'extraction "QuEChERS" a été mise en œuvre afin de déterminer la quantité de micropolluants bioaccumulés activement dans les biofilms et les boues activées. Les expériences de récupération ont montré que, parmi 65 substances, 74% ont pu être extraites des deux communautés microbiennes avec des taux de récupération allant de 70% à 130%. Ensuite, des expériences de bioaccumulation ont été effectuées avec du biofilm de la rivière du Tessin et avec de la boue activée de la station d'épuration d'Airolo. Les résultats ont montré que 20 substances ont été accumulées avec des taux d'au moins 10% de la quantité initiale dans au moins une des communautés microbiennes (sorption passive et/ou bioaccumulation active). De plus, onze de ces substances ont montré une bioaccumulation active d'au moins 5%: quatre substances ont été accumulées activement uniquement dans le biofilm, six substances ont été accumulées activement uniquement dans les boues activées et une substance a été accumulée activement dans les deux communautés microbiennes. Plusieurs de ces substances ont déjà montré une bioaccumulation dans d'autres études. Huit substances parmi les onze substances accumulées activement contiennent des groupes amines aliphatiques ce qui pourrait faciliter l'accumulation active.

mots clés: micropolluants, biofilm, boues activées, bioaccumulation, QuEChERS

Contents

1 Introduction

This master thesis is part of the SNF/DFG financed project "Unraveling the Molecular Mechanisms of Trace Contaminant Biotransformation from Wastewater to Natural Surface Water", which aims at deepening the understanding of biotransformation of micropollutants by two different types of microbial communities: activated sludge in wastewater treatment plants and biofilms in rivers. In recent studies, a so-called downstream effect has been observed, which means that some micropollutants are better transformed in biofilms grown downstream of wastewater treatment plant (WWTP) outfalls than upstream.[1,2] The SNF/DFG-project further investigates this downstream effect and tries to link it to the applied technology in the wastewater treatment plant, the genomic composition of the communities and the enzymes present.

This master thesis focuses on one part of this project: Active bioaccumulation of a defined set of micropollutants in biofilms and activated sludge. The next sections give some background information including recent study findings on micropollutants in biofilm and activated sludge, possible interactions between micropollutants and microbial communities from activated sludge and biofilms, and measurement methods.

1.1 Synthetic micropollutants

Synthetic micropollutants (MPs) are human-made substances which occur in µg/L to ng/L concentrations in wastewaters and natural waters.[1,3] The term "synthetic micropollutants" encompasses pesticides, pharmaceuticals, personal care products, additives, hydrocarbons, flame retardants, artificial sweeteners and other synthetic compounds present in low concentration.[3]

MPs enter the environment via different sources. An important point source are wastewater treatment plants (WWTPs). The concentration of pharmaceuticals, personal care products and artificial sweeteners is generally highest close to a WWTP outfall, especially when the WWTP has not implemented a micropollutant treatment.[4,5,6,7] These compounds get more and more diluted with increasing distance to the WWTP. The higher the proportion of wastewater in a stream, the higher is the concentration of MPs in the stream and the bigger is their impact on the environment.[7,8] Pesticides applied in agriculture, parks or private gardens can enter natural water bodies either directly by runoff from the field (with the load being highest after rain events)[6,7] or indirectly via the sewer system.[7]

It is important to study the fate of MPs as some MPs have known detrimental effects on the environment. For example, a study in a lake in Canada revealed that constant exposure to synthetic estrogen at a concentration of 5 to 6 ng/L led to the collapse of the fathead minnow (fish) population.[9] Effects of estrogenic endocrine disruptors were also observed downstream of wastewater treatment plants, e.g. feminization of fish and reproduction difficulties.[10,11] Studies have also shown the detrimental effects of non-steroidal anti-inflammatory drugs, such as diclofenac, on the environment.[12]

The effects of many other MPs are not yet fully understood but harmful effects not only for the environment but also for the human health are expected.[1,3,5,7,13] The vast number of different substances, for many of which no detailed ecotoxicological studies are available, makes it hard to assess the effect of MPs on ecosystems. In addition, studies have difficulties representing the mixture effect: Substances are not present in the environment as single, isolated compounds but in a mixture of hundreds or even thousands of different compounds. They might interact with each other, some substances can potentiate the effect of others or even new effects can occur when combining some substances. Therefore, assessing the true environmental impacts is very challenging.[3,7]

Nevertheless, for individual MPs there exists sufficient proof of the detrimental effects to call for action. Switzerland is one of the first countries that from 2013 on established concepts to reduce micropollutant concentration in natural waters by legal means. Wastewater treatment plants fulfilling certain criteria (such as size, receiving waters etc.) have to eliminate at least 80% of the micropollutant load and they must implement the additional treatment steps needed until 2040.[14,15,16,17] The removal of 80% is calculated based on a selection of approved substances.[17] MPs can partly be eliminated by biological transformation in the WWTP.[7] Studies have shown that MPs can also be transformed by other microbial communities such as biofilms.[1,18] It is interesting to study in more detail how these microbial communities interact with MPs in order to gain more insights on possible removal mechanisms.

In the rest of the report, biofilm and activated sludge will be referred to as *microbial communities*, well knowing that these two microbial communities are not the only ones existing.

1.2 Biofilms

Biofilms are one of the microbial communities whose interaction with MPs is studied in more detail in this project. The project focuses on periphytic river biofilms occurring in Swiss surface waters.

A river biofilm is a complex assemblage of microorganisms including bacteria, algae, archaea, unicellular eukaryotes, small invertebrates and funghi, which are enclosed in a matrix of extracellular polymeric substances (EPS).[8,19,18,20] This extracellular polymeric matrix (EPM) is composed of proteins, glycolipids, polysaccharides,

extracellular DNA and detritus and has elaborate structures including channels.[18,20] Channels are important for the biofilm organisms as they allow the exchange of nutrients and solutes with cells that are not at the surface of the biofilm. Biofilms generally present high biodiversity; their composition depends on environmental factors such as light availability, stream regime, pH etc. Thanks to their diversity, they play an important role in nutrient cycling in streams. The presence of different species, including phototrophs and heterotrophs leads to complementary resource partitioning and therefore allows optimised resource use.[20] Biofilms are at the lowest level of the foodweb and serve as important food source for diverse organisms such as many invertebrates and sometimes even fish.[18,20,21] This project examines periphytic biofilm: stream biofilms attached to a submerged substrate (often stones, rocks).[18,20]

1.2.1 MPs and biofilm

Biofilms are very diverse and as a result, the possible interaction mechanisms with synthetic MPs are numerous. The interaction mechanisms might even change from one biofilm to another as the biofilm composition depends on its geographic location and characteristics of the river water.[5,18] Therefore, if a specific MP interacts with a given biofilm in one way, it might not necessarily interact with other biofilms with a different composition in the same way.[2] Contaminants might passively adsorb to the biofilm, be transformed[1,2,18] or be actively bioaccumulated,[1,18] similarly as dissolved organic carbon (DOC) or nutrients are concentrated in biofilms.[20]

In this master thesis, the term accumulation is used to describe both active bioaccumulation as well as passive adsorption.

$$\text{accumulation} = \text{active bioaccumulation} + \text{passive adsorption} \tag{1}$$

The study of transformation capacity of biofilms towards MPs is covered in the SNF/DFG project but not in this master thesis.

Organic MPs with a high octanol-water partitioning coefficient (K_{ow}) often interact with rather apolar organic matrix, leading to passive adsorption to the biofilm.[5,18,22] Nevertheless, studies have shown that the amount of accumulated MPs is not significantly correlated with the K_{ow}, probably due to the diversity of the composition of the biofilms which allows many different interactions and not just hydrophobic interactions.[5,8,22]

Another possible interaction between a microbial community and MPs is active bioaccumulation of the MPs. An actively bioaccumulating substance is often actively transported inside the cell or kept in the cell through active mechanisms.[23] One such example is trapping in acidic vesicles (ion-trapping) as explained in detail in section 1.3.1. This active bioaccumulation mechanism is supposed to be especially important for eukaryotes such as protozoa and algae but it cannot be excluded for certain bacteria neither.[24] Certain MPs can actively bioaccumulate in biofilm because a corresponding molecular binding site is present.[5]

Accumulation of metals in biofilms has been shown[21,25] and recently, research is done to investigate the accumulation of other pollutants, including MPs in biofilms,[4,8,21,22] where only some studies clearly distinguish active bioaccumulation from passive adsorption.[1,18] To understand the fate of MPs in biofilm, it is important to distinguish between all different possible processes.

Due to the accumulative properties of biofilm, the amount of MPs stored in biofilms can give indications on the pollution of a river and the toxicological stress on its inhabitants.[5,8,21,22,25] Accumulated MPs in biofilms can still be present after longer periods of time even if the micropollutant concentration in the surface water has decreased[21,22] and biofilm can therefore be considered to act as passive sampler.[1,22] MPs might also be released back into natural waters from biofilms to establish a new equilibrium if the concentration in water decreases.[21]

As biofilms are an important food source, biomagnification of MPs can occur.[4,8,18] A study has shown biomagnification of TBEP, a flame retardant, which showed higher concentration in macroinvertebrates feeding on biofilm than in the biofilm itself.[4]

Not only do biofilms affect the fate of MPs by transforming or accumulating them[5,8] but also vice versa: Biofilms can be affected by (micro)pollutants. Most effects of MPs on biofilms are negative due to their toxicity.[6,8,22] However, also pollution induced community tolerance (PICT) has been observed meaning that exposed biofilms get tolerant to micropollutants.[5]

1.3 Activated sludge

Activated sludge is the second microbial community studied in this project that might interact with MPs. Activated sludge is an assembly of bacteria and other microorganisms responsible for the biological treatment in a WWTP. Wastewater is purified in several treatment units: First, mechanical processes remove solids and grease. Second, biological and/or chemical processes transform nutrients and organic matter and eventually, other physical processes follow for final treatment of the wastewater (e.g. treatment with activated carbon, filtration etc.).[26] The biological treatment can be performed by different methods but the worldwide most commonly applied biological process is treatment by activated sludge. It aims at removing organic compounds and nutrients by taking advantage of the metabolism of bacteria and other microorganisms.[26,27,28] Activated sludge undergoes

an internal recycling: After being mixed aerobically with the influent and treating the wastewater, it is separated from the water by sedimentation and partly recycled into the aeration tank where it can once more treat the influent.[13]

Activated sludge is composed of a diversity of microbial organisms (bacteria, protozoa etc.) that are able to degrade organic matter under aerobic conditions. Depending on the community composition, the residence time and the aeration, the microorganisms are also able to oxidize ammonia to nitrate, reduce nitrate to molecular nitrogen (under anoxic conditions) and remove phosphorus.[26,27,28] The exact composition of activated sludge depends on different factors such as geographic location, temperature, pH, characteristics of the inflow, exploitation of the treatment plant (residence and aeration time) etc.[26,29] Higher proportion of industrial wastewater - having different characteristics than municipal wastewater - results in a different community composition.[26,29] The community composition influences the performance of the WWTP. It is important to have well-equilibrated communities in order to reliably treat wastewater.[27] Still much research is going on trying to identify key organisms in wastewater treatment in order to further optimize biological treatment systems.[26,28]

1.3.1 MPs and activated sludge

Traditional WWTPs are designed to treat macropollutants such as organic matter and nutrients. Biological degradation in activated sludge is responsible for a big part of the removal of such macropollutants. However, MPs might also be affected and (partially) removed or transformed in conventional WWTPs through sorption to activated sludge, biological transformation by activated sludge, volatilization or abiotic reactions (photolysis, hydrolysis).[7] The removal pathway depends on the micropollutant and the environmental conditions. Generally, higher removal has been observed for more intense and longer-lasting biological treatment, indicating that activated sludge has an important role to play.[7,12]

Similarly as for biofilms, hydrophobic and/or positively charged compounds tend to adsorb to sludge.[7] Adsorption is the first step needed for further active internal bioaccumulation, however most studies do not differ whether the compounds are simply adsorbed or actively taken up into the cell of the organism.[12]

Kruglova et al. have shown that several synthetic MPs are actively taken up into the interior of bacterial cells in activated sludge. Some of the MPs (e.g. ibuprofen) were subsequently biologically degraded, other (e.g. diclofenac) remained unaltered in the bacterial cell or even moved back unaltered into the liquid phase again.[12] The release of the untransformed compound into the environment might occur due to an oversaturation in the cells or death of the microorganism.[12,24]

Gulde et al. have demonstrated that certain organic MPs actively bioaccumulate in protozoa present in activated sludge by a mechanism called ion trapping. This form of active bioaccumulation is possible for weak bases, which can be protonated / deprotonated at pHs around 7 to 10, mostly including amine-containing MPs. Ion trapping occurs when the pH outside the cell is higher than inside the cell. Due to the higher pH (e.g. pH 8), the neutral form of the compound can diffuse into the cell. From there, it can further diffuse into acidic vesicles where the pH is low, typically below 5. There, it gets protonated (positively charged) which renders a re-diffusion into the cytosol impossible. In this way, amine-containing substances actively accumulate in acidic vesicles. Ion trapping is possible in different kinds of eukaryotic cells and might not only occur in protozoa but also in algae. Most bacteria do not contain acidic vesicles and are thus not able to actively bioaccumulate MPs by ion-trapping. However, some bacteria have acidocalcisomes, a type of acidic vesicles, and might potentially be able to actively bioaccumulate MPs by ion-trapping too. One must pay attention to a possible saturation effect. Acidic vesicles are only able to accumulate substances up to a certain limit. Non-accumulation might be misinterpreted as incapacity to actively bioaccumulate when in fact the acidic vesicles are already oversaturated.[24]

The saturation effect has also been described for biofilms without referring to a specific accumulation mechanism.[18,30]

1.4 Determination of MPs in biofilms and activated sludge

1.4.1 Biofilms

Measuring the amount of contaminants accumulated in biofilms was for a long time challenging or even impossible.[18] Many different extraction methods were and are still used; some studies even renounced measuring the concentration in the biofilm and only used water measurements[18] or concentrations in sediment[22] to derive the accumulation in biofilm. However, it has been shown that the concentrations in water or sediment do not give reliable indications for the actual concentrations in biofilm.[5,22] In addition to the difficulty of measuring the accumulated amount, many studies do not differ the amount of passively adsorbed MPs from actively accumulated MPs.[4,8] In order to fully understand the processes taking place, it is important to know whether MPs are only adsorbed or actively bioaccumulated. An active bioaccumulation in the cell might influence the transformation pathway and can have impacts on the well-being of the organism that are more severe than the impacts of passive adsorption.

A possible promising method to determine the accumulated amount of MPs in biofilms is the QuEChERS extraction method. QuEChERS stands for Quick Easy Cheap Effective Rugged Safe. It is an extraction method which was developed in 2003 with the aim to have the characteristics that the name promises: Being an easy method which does not need a lot of time or expensive equipment, while being able to effectively extract substances using relatively safe extraction chemicals.[31] It has been used a lot in different forms to determine pesticide residues in food,[32,33] fish,[34] gammarids,[35] sediments[36] and many more matrices. Recently, it has also been used to extract MPs from biofilms.[1,5] Relative recoveries of 86 to 102% have been achieved for pesticides in food,[31] 70 to 130% for organic MPs in gammarids[35] and 70 to 130% for a majority (85%) of organic MPs in biofilm.[1]

1.4.2 Activated sludge

Similarly as in biofilms, the amount of organic MPs in activated sludge was hardly ever directly measured but only deduced from the incoming concentration and the concentration in the liquid phase which leads to many uncertainties.[12]

As a result, researchers tried to find suitable extraction methods. A typical extraction method is selective pressurised liquid extraction which is time-consuming and rather difficult to operate, particularly for small sample amounts.[37] Adapted QuEChERS extraction methods have been used to determine the amount of accumulated MPs on/in activated sludge.[37,38,39] Relative recoveries ranging from 62.6 to 130.5%[39] and 80 to 120%[38] have been achieved when applying an optimized protocol.

1.4.3 Extraction method for this master thesis

In order to simplify the laboratory procedure and to have comparable results for activated sludge and biofilm, the exact same QuEChERS-extraction protocol is used in this master thesis to determine the accumulated amount of MPs in both biofilm and activated sludge. The protocol used in this project differs from the ones used in other, above-cited studies by several points: It includes a fast preparation procedure that should destroy cells and free accumulated MPs from inside the cells. It uses other sorbents and partly other solvents, and it includes an additional cleaning step by doing a heptane extraction.[1,37,38,39] It is very close to the protocol used by Desiante et al.[1]

1.5 Goals

The goals of this master thesis are the following:

- Identify synthetic MPs which accumulate to a significant extent (>10%) in biofilms and/or activated sludge
- Interpret the accumulation, differ passive adsorption from active bioaccumulation and investigate whether certain MPs accumulate to a higher extent in activated sludge than in biofilms or vice versa.

In order to achieve these goals, the following methodological objectives are set and have to be achieved first:

- Confirm the suitability (acceptable relative recovery of 70% to 130%) of the QuEChERS extraction method as used by Desiante et al. for extracting MPs from biofilm.[1]
- Adapt the QuEChERS extraction method for MPs in activated sludge such as to achieve acceptable recovery rates (70% - 130 %).
- Develop and validate the standard addition method for substances without an internal standard such as to achieve acceptable recovery rates (70% - 130%) for those substances.

1.6 Hypotheses

Based on past studies, the hypotheses are as follows:

- The recovery rates using the QuEChERS extraction method are comparable for activated sludge and for biofilm.[1,38,39]
- Phenylurea herbicides are actively bioaccumulated to a significant amount.[1] This includes the compounds chlorotoluron, isoproturon, metoxuron and monuron.[40]
- Diclofenac is actively bioaccumulated.[12]

- Amine containing MPs are actively bioaccumulated. Especially for following amines, a bioaccumulation (or more specifically ion trapping) has been observed before and is expected to occur again: Levamisole, propranolol, fenfluramine, mexiletine, tramadol and venlafaxine.[24]
- Adsorbing compounds can be extracted by the QuEChERS method from the microbial communities both from the sorption control as well as from the biotic experiment. Actively bioaccumulating compounds can be found only or to a significantly higher extent in the biotic experiment than in the sorption control.[1]

2 Methods and Materials

2.1 Selection of micropollutants

The SNF/DGF project studies the fate of 187 MPs, composing of antibiotics, artificial sweeteners, fungicides, herbicides, insecticides, personal care products and pharmaceuticals. Due to the restricted time available for this master thesis, only a limited selection of those pollutants is investigated. The focus is on MPs for which no studies have been conducted before at Eawag or for which an accumulation was observed in earlier experiments in order to verify the results. This results in 78 substances, including one insecticide (icaridin), two artificial sweeteners (neohesperidin dc and neotame), four herbicides (chlorotoluron, isoproturon, metoxuron, monuron), one personal care product (benzophenone-3) and 70 pharmaceuticals. The full list of MPs can be found in the Annex A.1 in Table 4.

2.2 Preparation of stock solutions

The MPs were prepared in 10 mM stock solutions in DMSO (dimethyl sulfoxide). Lisinopril was dissolved in nanopure water because of its low solubility in DMSO. 14 submixes, each containing 12 to 14 compounds, were prepared in a concentration of 200 μM in ethanol. All submixes were further mixed together in a 10 μM final mix in ethanol. Three more mixes with concentrations of 1 μM, 100 nM and 10 nM were prepared by diluting the final mix in ethanol.

2.3 Sample collection

2.3.1 Samples for recovery experiment

The biofilm used for the recovery experiment was grown in artificial channels at Eawag fed by water from the Chriesbach River. The channels were equipped with glass slides on which biofilm was allowed to grow. The artificial channels underwent a light:dark cycle of 16:8 hours (dark from 21.00-5.00). The water temperature was around 14 - 15°C and the flow rate was set to approximately 45 L/s. After 4 to 7 weeks, the biofilm was scraped off the glass slides and suspended in filtered Chriesbach River water.

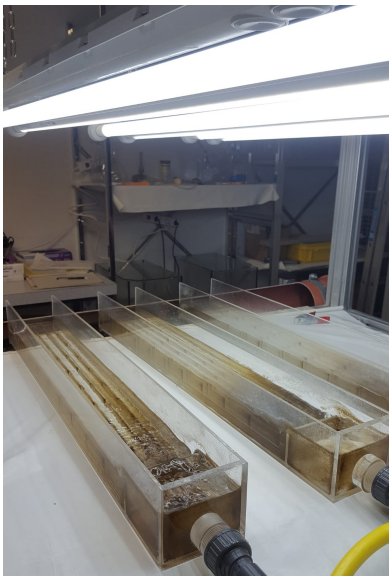


Figure 1: Artificial biofilm channels in Eawag facilities fed by water of the Chriesbach River

The activated sludge used for the recovery experiment was taken from ARA Neugut in Dübendorf, a wastewater treatment plant that treats the wastewater of 105'000 PE. The biological treatment eliminates organic matter, nitrifies, denitrifies and eliminates phosphorus.[41] Therefore, the sludge retention time is rather elevated with at least 8 - 10 days allowing for the development of diverse species.[7,42]

2.3.2 Biofilm samples for bioaccumulation experiment

For the bioaccumulation experiment, biofilm was grown in the river Ticino close to Airolo. Five sites were selected: Two sites upstream (ca. 150 m (Up2) and 2 km upstream (Up1)) of the WWTP in Airolo, one site immediately downstream of the WWTP (ca. 50 m downstream (Do1)) and two sites further downstream (ca.

700 m (Do2) and 1.5 km downstream (Do3)) of the WWTP outfall. These five sites were selected in order to fulfill requirements of the overall SNF/DFG study.

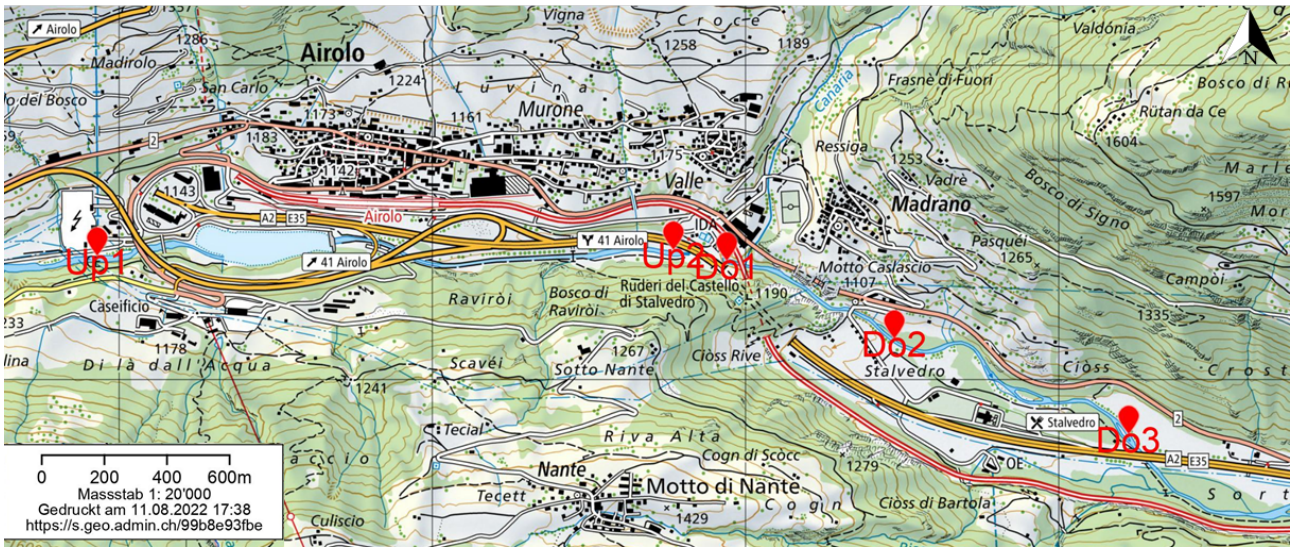


Figure 2: Map showing the five sites in river Ticino close to Airolo. IDA is Italian for WWTP. Two sites upstream the IDA/WWTP (Up1 and Up2) and three sites downstream the IDA/WWTP (Do1, Do2, Do3) were chosen. For this master thesis, the biofilms from all five sites were mixed. Map from map.geo.admin.ch.[43]

At each site, three boxes containing glass slides were installed in the river. One box at each site was used for the experiments of this master thesis. The glass slides in the boxes had to be submerged not lower than 50 cm and the flow rate had to be around 0.3 - 0.5 m/s. The boxes were left in the river for 6 weeks and then removed. To transport the biofilm grown on the glass slides back to Eawag, the glass slides were put in plastic bags filled with river water and put in boxes. At Eawag, the biofilm was scraped off the slides and suspended in Evian water. The biofilms from the five sites were mixed and suspended together in order to obtain an "average" biofilm of the five sites for this master thesis.

(a) Boxes containing glass slides for biofilm growth in river Ticino: The boxes had to be submerged and the flow rate had to be around 0.3 - 0.5 m/s.

(b) Glass slide overgrown with biofilm

Figure 3: Biofilm growth and harvesting in the field experiment.

2.3.3 Activated sludge samples for bioaccumulation experiment

Activated sludge from WWTP Airolo was taken for the bioaccumulation experiment. This WWTP is the first (farthest upstream) WWTP of the river Ticino. It was built in 1968 and since then improved several times. It only treats organic carbon; no nitrification or denitrification is implemented. Therefore it has a rather low sludge retention time and probably less diverse species and less protozoa than more advanced WWTPs.[7] It treats the wastewater of 6000 PE. The influent contains a significant amount of wastewater from dairy production and this WWTP thus faces challenges regarding foam production as well as grease and fat removal.[44]

2.4 QuEChERS extraction method

2.4.1 Sample preparation for QuEChERS

100 mL of biofilm or activated sludge suspension were centrifuged in two 50 mL Falcon tubes. The supernatant was discarded. The pellets were frozen at -20°C and then freeze-dried during 3 days. The pellets were crushed using a spatula until only a fine, homogeneous powder remained.

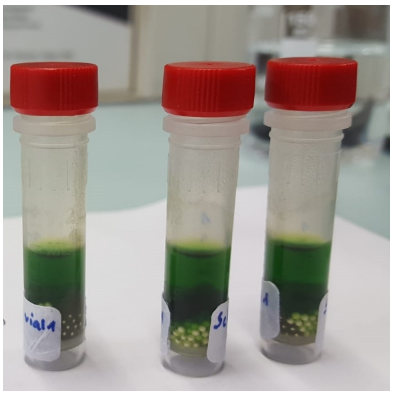
2.4.2 QuEChERS extraction

The QuEChERS extraction method was used to extract MPs from the microbial communities.

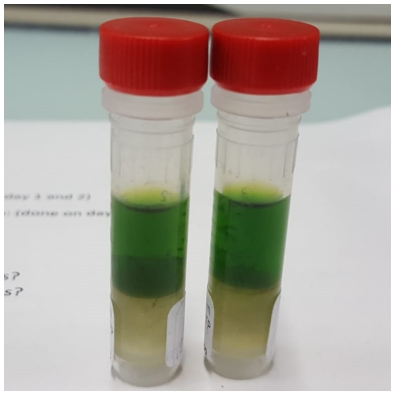
The freeze-dried powder was split in samples of 20 mg each and spiked with ISTD / standard solutions. The amount of ISTD and standard solutions spiked for the recovery experiment are displayed in Figures 6 and 7. For the bioaccumulation experiment, 24 μL ISTD (with the ISTD mix having a concentration of 100 ng/L) are spiked. The spiked standard solutions for the bioaccumulation experiment were: 0 nmol, 0.0024 nmol, 0.0024 nmol, 0.012 nmol, 0.024 nmol, 0.024 nmol. The solvent EtOH was let to evaporate overnight.

For every concentration and microbial community combination, three fast prep vials were prepared in a set A, B, C. For the extraction, 500 μL ACN, 500 μL nanopure water and 0.5 g of zirconium/glass pellets (diameter = 1mm, manufactured by Carl Roth GmbH+Co. KG) were added to the powderous biofilm or activated sludge in fast prep vial A. The samples were homogenized in a Fast Prep machine (FastPrep-24™ Classic bead beating grinder and lysis system, manufactured by MP Biomedicals) for 15 s at 6 m/s. Post, the samples were cooled in an ice-bath for 5 min. The fast prep procedure was repeated. After these two rounds of fast preparation, the samples were centrifuged for 6 min at 20'000 rcf at room temperature.

800 μL of the supernatant were transferred to vial B containing 300 mg QuEChERS salts (QuEChERS Final Polish EMR-Lipid; MgSO4/NaCl (4:1); manufactured by Agilent Technologies). Vial B was immediately shaken and vortexed for 1 min. The samples were centrifuged again as described above. With the addition of the QuEChERS salts, the nanopure and ACN phase were separated and MPs accumulated in the ACN phase.



(a) Fast preparation in vial A: The vial with the zirconium/glass beads was shaken vigorously in the fast preparation machine in order to free MPs from the biomass.



(b) Phase separation with QuEChERS salts in vial B.

Figure 4: Pictures illustrating the QuEChERS extraction method

Post, 200 μL of the upper, organic phase (ACN) were transferred into vial C. The extraction was repeated by adding 500 μL ACN to the homogenized sample in the vial A. The fast prep procedure was performed twice, including cooling between the two fast preparation steps. 600 μL of the second supernatant were transferred to the already used QuEChERS salt vial B. This vial was again shaken, vortexted and centrifuged. 600 μL of the supernatant was combined with the first extraction in vial C. The vials were stored at - 20°C overnight.

A heptane wash step was used to remove lipids from the extraction: 500 μL heptane were added to vial C, which was further shortly vortexed and centrifuged as described above. 400 μL of the upper heptane phase were transferred to a HPLC vial for further possible analysis. The sample was washed a second time with 500 μL of heptane, repeating the procedure. 500 μL of the upper heptane phase were transferred and combined with the first heptane washing in the same HPLC vial. Finally, 700 μL of the bottom phase (ACN) were transferred into a HPLC vial and stored at - 20°C until solvent exchange could be executed.

2.4.3 Solvent exchange

For the solvent exchange, the volume of ACN was reduced to approximately 50 μL in the turpovap machine (TurboVap® LV, manufactured by Biotage) under a nitrogen-stream of 0.8 L/s and in a water bath of 40°C. If needed (as in some recovery schemes), internal standards, standard solutions and substances were added. In recovery schemes where no internal standard / standard solutions / substances were added, pure ethanol was added in such a way that all samples from the different recovery schemes contained the same amount of solvent (ethanol). The vials were filled up with Evian water to a final volume of 1 mL. The vials were then frozen at -20°C until measuring.

2.4.4 Preparation for measurement

At the measuring day, the samples were thawed and centrifuged at 4000 rcf for 15 minutes. 800 μL of the supernatant were transferred into a new LC-MS vial.

2.5 Sample measurement with LC-MS

The chemical analysis was performed with a Thermo Scientific™ Q Exactive™ Hybrid Quadrupole-Orbitrap™ Mass Spectrometer coupled with Liquid Chromatography Thermo Scientific™ UltiMate™ 3000 UHPLC system by Thermo Fisher Scientific™. Phases were separated in an Atlantis T3 column (Waters HPLC column Atlantis 3 μm 3x150 mm, Part Number: 186003723, Column Serial No: 02073206814014). The mobile phase consisted of nanopure water (from generator at Eawag) and MeOH (OPTIMA®LC/MS Grade, Fisher Scientific) both containing 0.1% formic acid (98 - 100%, Merck KGaA, Darmstadt, Germany) and a total flow rate of 0.3 mL/min. The method can be seen in Figure 21 in the Annex A.4. The first minute, the ratio nanopure:MeOH was constantly 95:5. Then, MeOH linearly increased until the ratio nanopure:MeOH = 5:95 was reached after 17 minutes. This ratio was kept until 25 minutes after beginning of the run and then, in 0.1 minute, the ratio was set to the initial ratio of nanopure:MeOH = 95:5.

The first five minutes of the run were diverted into the waste and not analysed in the mass spectrometer. The samples were measured in positive / negative switching mode at a resolution of 70.000 at 200 m/z. Calibration standards were prepared in Evian water containing 5% acetonitrile (HPLC for gradient analysis, manufactured by Acros Organics) and ranged from 0.25 nM to 50 nM.

The software TraceFinder™ by Thermo Fisher Scientifics 5.1EFS (Thermo Scientific, Waltham, MA, USA, September 6, 2021) was used for the analysis of the HPLC-HRMS data. Further data analysis was conducted with the open source software R (version 4.2.1 (2022-06-23)). All codes are publicly available on GitHub: `https://github.com/AnnaB459/MT_ActiveBioaccumulationMicropollutants`

2.6 Quantification

2.6.1 Limit of quantification LOQ

The limit of quantification is the lowest concentration of the analyte where the concentration can be quantified. To determine the LOQ, the peaks in the calibration curve were checked. There needs to be a clear, Gaussian peak shape with at least five measurement sticks per peak in order for a concentration to be above the LOQ. The $\text{LOQ}_{calibration}$ was set to the lowest concentration for which these criteria are still fulfilled.

The LOQ needs to be adapted to the sample as there is a higher amount of matrix present in the samples than in the calibration samples, which will lead to a higher LOQ. With the following equation, the LOQ_{sample} can be determined:

$$\text{LOQ}_{sample}\ [\text{nM}] = \frac{\text{LOQ}_{calibration}\ [\text{nM}]}{\text{absolute recovery [-]}} \tag{2}$$

The calculation of the absolute recovery is explained in detail in section 2.7

Finally, the LOQ per weight of dry biomass (dwbiomass) can be determined:

$$\text{LOQ}_{sample,dwbiomass}\ [\text{nmol / g}] = \frac{\text{LOQ}_{sample}\ [\text{nmol / L}]}{\text{weight of biomass per sample volume [g / L]}} \tag{3}$$

The weight of the dry biomass per sample volume is equal to 20 mg per 1 mL for all experiments performed.

2.6.2 Internal standard calibration

For about a quarter of the tested substances, a corresponding internal standard (ISTD) is available to be used. An ISTD is a substance, which is very similar to the analyte and behaves ideally in exactly the same way as the analyte in the extraction and when measured but it can be clearly distinguished by analytical

methods. Typically, an isotope-labelled analyte is used (e.g. diclofenac-D4 for diclofenac), a so-called own internal standard. Own internal standards are good at correcting any effects and losses which might occur during preparation and measurement as their structure is identical to the one of the corresponding analyte.[45] An overview of the used substances with the associated internal standards can be found in the Annex A.1, Table 4.

A known amount of the internal standard was added before the sample was prepared. Thus, it underwent the same preparation steps as the analyte and experienced - due to its similarity with the analyte - the same losses and signal enhancement or diminution as the analyte. As the ratio $\frac{\text{analyte}}{\text{internal standard}}$ stayed constant throughout the whole procedure and the initial amount of internal standard was known, the true initial amount of analyte could be calculated.[45]

2.6.3 Standard addition

For the other tested substances, no corresponding own internal standard was available. Therefore, the standard addition calibration method was used. For this method, the sample was split in several sub-samples with equal weight and a different known amount of the analyte was added to each sub-sample. By plotting the signal (area of chromatogram) versus the known added amount of analyte, the true analyte concentration could be determined. Traditionally, the analyte additions are spaced equally and in the same order of magnitude as the expected analyte concentration.[45,46] However, precision has been shown to be much better when only extreme addition values are used and repeated several times.[46,47] In order to obtain correct results, the relationship between the signal (area) and concentration of analyte must be linear.[46] We are only interested in a limited concentration range and assume that the relationship is linear in this range for the tested substances.

Figure 5 illustrates the principle of determining the concentration by using standard addition with concentrations used in the recovery experiment. Six sub-samples spiked to concentrations of 0 nM (no spike), twice 2 nM, once 10 nM and twice 20 nM are plotted against the area obtained when measuring the samples. A linear regression line is fitted through these six points (in black).

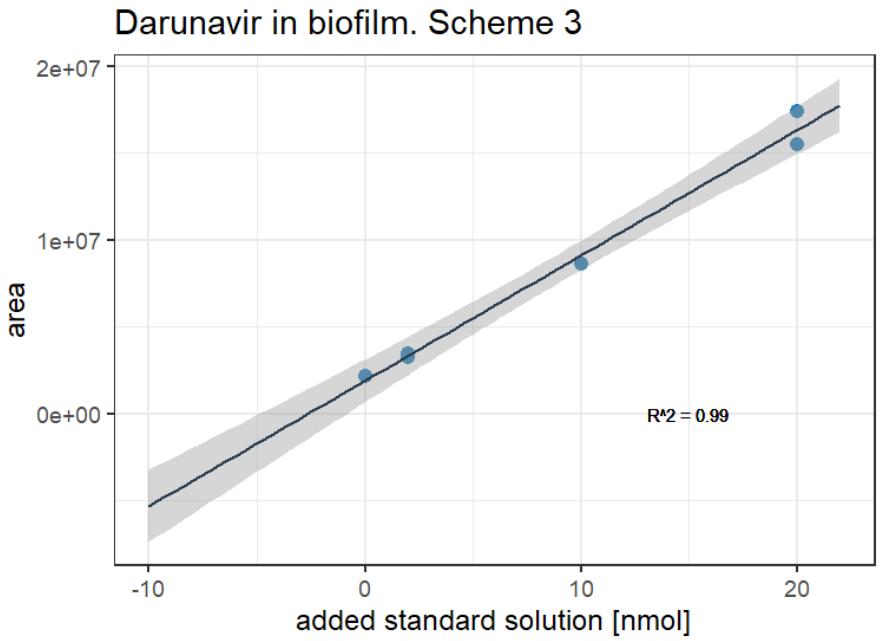


Figure 5: Illustration of standard addition for darunavir in a biofilm sample: Each blue point connects an added standard solution amount to its corresponding measured area in the chromatogram. A linear regression is fitted through all points. The true concentration is the absolute value of the intersection of the linear regression with the x-axis (here about 2.5 nmol). The error of the regression line is depicted with the shaded grey area (confidence interval = 95%). The goodness of fit of the linear regression is evaluated with the R^2-value (the better the fit, the closer the value to 1; here R^2=0.99).

Mathematically formulated:

$$\text{area(conc) [-]} = m[\text{nM}^{-1}] * \text{conc [nM]} + b \tag{4}$$

where m is the slope and b the intersection with the y-axis which are determined by the linear regression.

The actual, a priori unknown concentration of the analyte corresponds to the absolute x-value at the intersection of the regression line with the x-axis (where y = 0). Therefore:

$$\text{actual concentration [nM]} = |\frac{-b}{m\ [\text{nM}^{-1}]}| \tag{5}$$

If b is smaller than 0, the actual concentration is set to 0.

In Figure 5, the grey-shaded area depicts the confidence interval of 95%, meaning that the true linear regression is in this shaded area with a certainty of 95%. Both variables determined by the linear regression (slope m and intersection b) have a standard deviation. In order to know the exact impact of these standard deviations on the calculated concentration, an error propagation needs to be done.

To calculate the standard deviation of the actual concentration (which is calculated with equation 5), the following error propagation rule can be applied:[48]

$$\frac{\delta c_{act}}{c_{act}} = \sqrt{(\frac{\delta m}{m})^2 + (\frac{\delta b}{b})^2} \tag{6}$$

where c_{act} is the actual concentration and δ denotes the standard deviation.

2.7 Recovery experiment

The aim of the recovery experiment is to validate the method and to confirm that the concentration of the substances in biofilm or activated sludge can be reliably determined with the chosen QuEChERS extraction method. Different spiking schemes were used (see Figures 6 and 7) in order to calculate absolute and relative recoveries, the matrix factor and the extraction efficiency. Schemes 4 and 5 were used to determine background concentrations, schemes 1 to 3 allowed the quantification of different losses.

Each spiking scheme was run with six samples in order to allow for six standard additions. One sample consisted of 20 mg freeze-dried organic matter powder (either biofilm or activated sludge).

The concentrations of MPs used in the recovery experiment were chosen such as to be representative of the later accumulation experiment. The threshold of significant accumulation was set to 10%. Based on a maximum accumulation of about 80 ng citalopram in 300 mg freeze-dried biofilm (corresponding to approximately 0.001 nmol/mg freeze-dried biofilm) observed in a study done by Desiante et al.,[1] the upper limit of the added standard solutions was set to this maximal observed accumulation: 0.02 nmol on 20 mg freeze-dried biofilm. The lower limit of the added standard solutions was set to 0.002 nmol on 20 mg freeze-dried organic matter, corresponding to an accumulation of 10% of the maximal observed accumulation. In all samples, the amount of substances added corresponded to 20% of the maximal observed accumulation: 0.004 nmol on 20 mg freeze-dried biofilm.

At several steps of the QuEChERS extraction procedure only a fraction of the solvent containing the MPs was transferred and used in further steps. This resulted in a loss of MPs throughout the procedure; consequently the measured amount of MPs at the end did not correspond to the initial amount. First calculations showed a loss of 43% over the whole extraction procedure. Therefore, when spiking before the QuEChERS procedure, 1.75x more micropollutants / standard solutions / ISTD was spiked than when spiking after the QuEChERS procedure. This was mainly important for scheme 2 where the addition of the substances and the internal standards did not take place at the same moment. Figures 6 and 7 show the actual spiked amount.

Different spiking points and different amounts of spiked substances led to different amounts of solvent added. In order to exclude any effect of the solvent, ethanol was added to all samples, which were spiked with less than the maximal spiked amount of solvent. When spiking before the QuEChERS preparation procedure, ethanol was added to a final solvent amount of 155 μL in all samples. When spiking after the QuEChERS preparation procedure (before measuring), ethanol was added to a final solvent amount of 90 μL in all samples.

An overview of all different spiking amounts of substances, internal standards and standard solutions before the QuEChERS-extraction can be found in Table 6 in appendix A.3. Table 7 in appendix A.3 shows an overview of all different spiking amounts of substance, internal standards and standard solutions after the QuEChERS-extraction, directly before the measurement.

All the schemes underwent the same preparation procedure as described in section 2.4.2. The only differences from one scheme to another lied in the spiking time and amount, which are depicted in Figures 6 and 7.

2.7.1 Absolute recovery

The absolute recovery indicates how much of the analyte is lost during preparation and detection. It includes all possible effects; e.g. matrix effects, extraction efficiency, contamination of the instruments etc. A known amount of the substance is spiked into the sample; the sample is prepared and measured. In order to eliminate any background concentrations, the area from spiking scheme 4 is subtracted from the obtained area of scheme 3. This area is then compared to the area that the same amount of substance produces in the calibration curve.

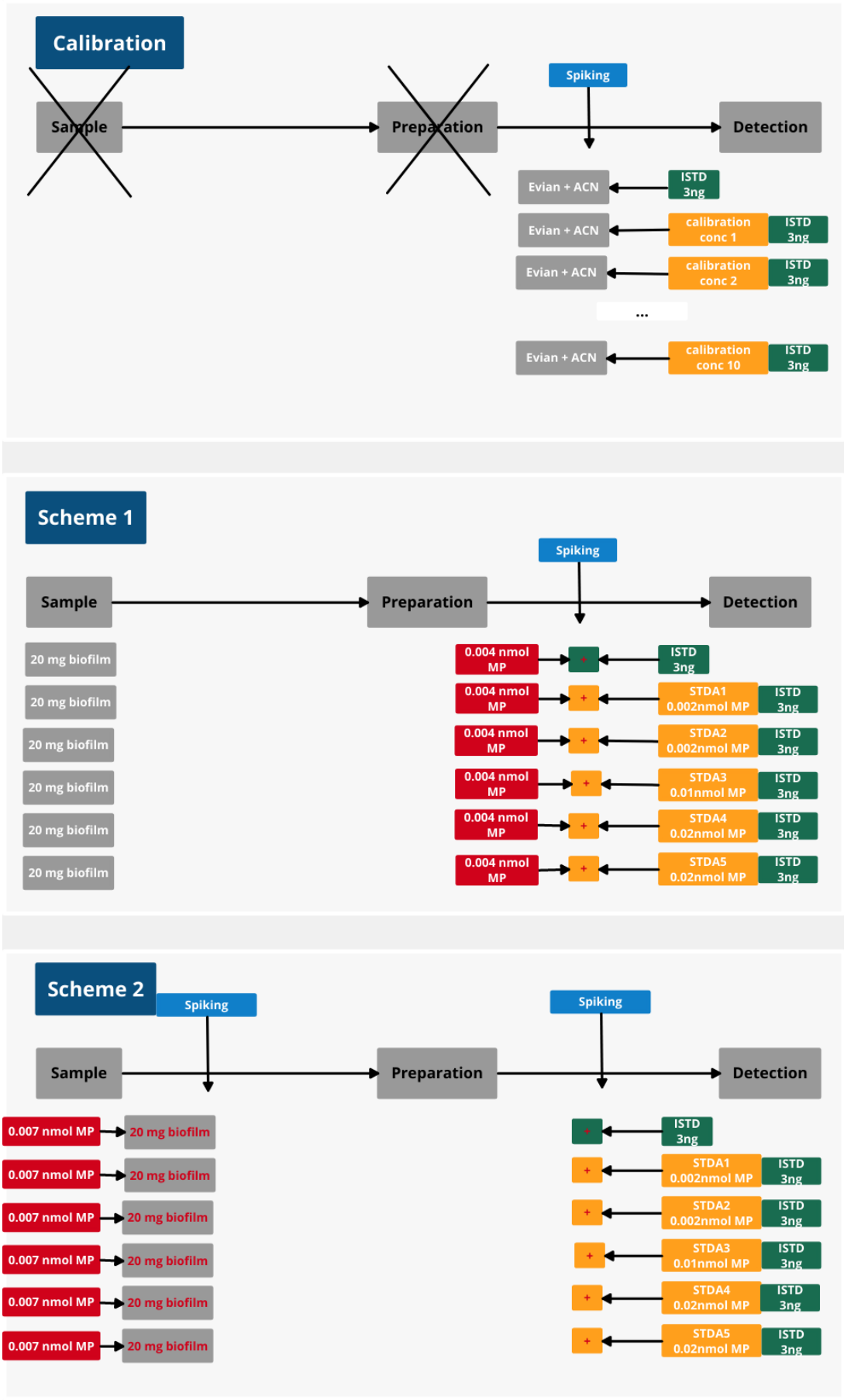


Figure 6: Calibration and Recovery Schemes 1 and 2 for biofilm. The principle is the same for activated sludge. The figure shows how much and when samples are spiked. MP stands for the micropollutant mixture of all tested substances, ISTD stands for internal standard, STDA stands for standard addition.

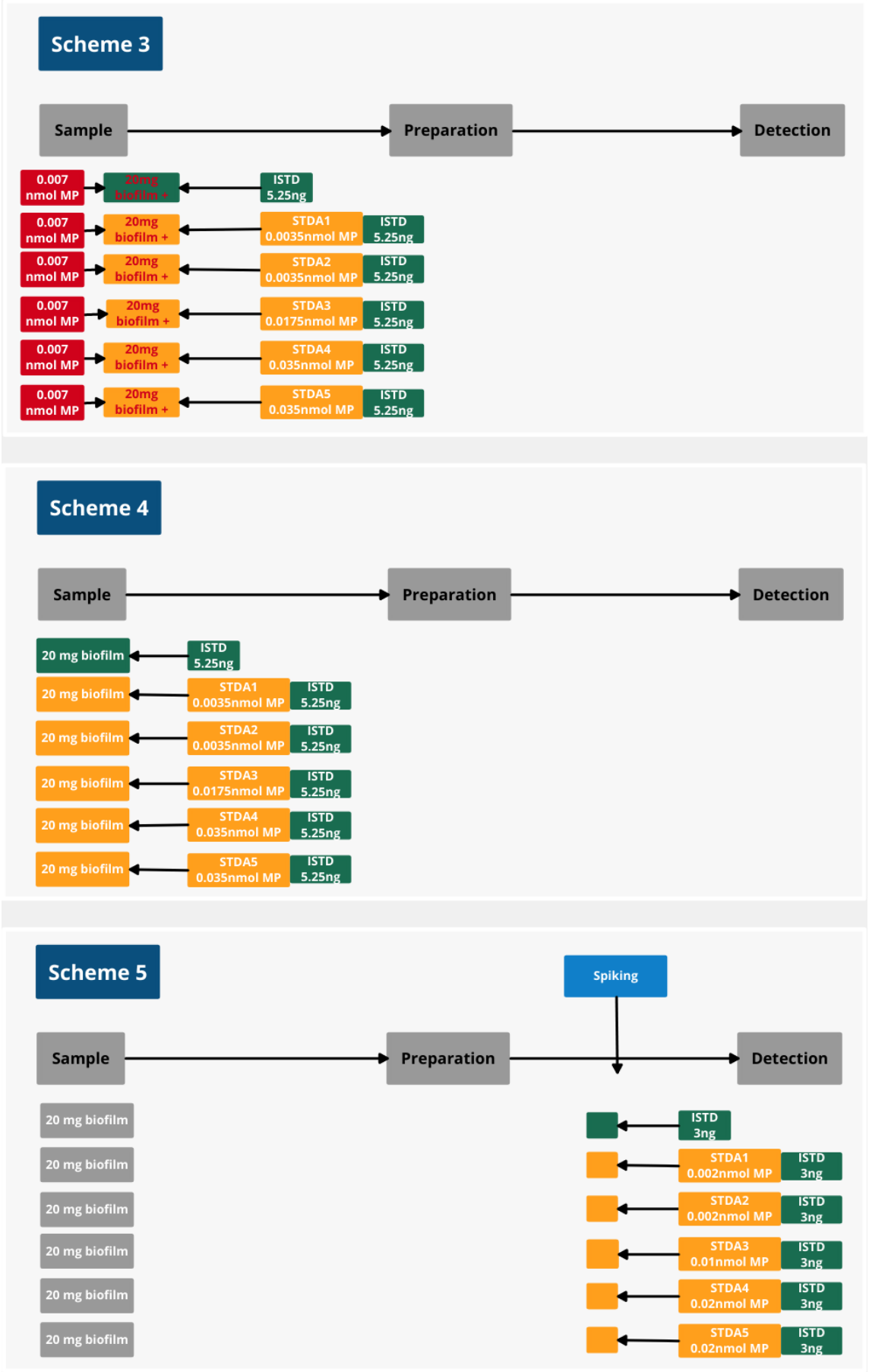


Figure 7: Recovery Schemes 3, 4 and 5 for biofilm. The principle is the same for activated sludge. The figure shows how much and when samples are spiked. MP stands for the micropollutant mixture of all tested substances, ISTD stands for internal standard, STDA stands for standard addition.

The subscripts in the following formulas refer to the recovery scheme.

$$\text{absolute recovery } [\%] = \frac{\text{Area}_3 - \text{Area}_4}{\text{Area}_{calibration}} * 100\% \tag{7}$$

2.7.2 Relative recovery

The relative recovery shows how much of the initial concentration can be reconstructed using corrections with internal standards or standard addition. The concentration in the formula is thus the concentration corrected with ISTD or standard addition. Ideally, relative recoveries should lie between 90% and 110%.[45] However, for experiments with biomass, relative recoveries between 80% and 120% can be considered as good and when they lie between 70% and 130%, they are still acceptable.[1,38]

$$\text{relative recovery } [\%] = \frac{\text{Concentration}_3 - \text{Concentration}_4}{\text{Concentration}_{theoretical}} * 100\% \tag{8}$$

The theoretical concentration is the concentration spiked in the beginning.

The concentrations of substances with an internal standard could be determined for each sample independently. As each sample was split in 6 sub-samples with different spiked amount, the relative recovery could be calculated for each of these sub-samples and the mean and the standard deviation of the recovery could be determined.

As the concentration of substances without an internal standard was calculated using the standard addition method, already those concentrations present a known standard deviation. This standard deviation was taken into account to calculate the standard deviation of the relative recovery by an error propagation.[48]

$$\delta\text{relative recovery}_{\text{stda method}} \ [\%] = \frac{\sqrt{(\delta\text{concentration}_3)^2 + (\delta\text{concentration}_4)^2}}{\text{concentration}_{theoretical}} \tag{9}$$

as δ stands for standard deviation.

2.7.3 Matrix factor

The matrix factor gives indications about ion suppression or enhancement in the matrix. A matrix factor close to 100% means that no ion suppression or enhancement occurs. If the matrix factor is higher than 100%, ion enhancement takes place. On the contrary, a matrix factor lower than 100% indicates ion suppression.

The matrix factor is calculated using different formulas depending on whether there is an internal standard of the substance available or not. For substances with an internal standard, it is calculated as follows:

$$\text{Matrix factor}_{\text{with ISTD}} \ [\%] = \frac{\text{Area ISTD}_1}{\text{Mean Area ISTD}_{calibration}} * 100\% \tag{10}$$

When no internal standard is available, the matrix factor is calculated with the following formula 11:

$$\text{Matrix factor}_{\text{no ISTD}} \ [\%] = \frac{\text{Area}_1 - \text{Area}_5}{\text{Area}_{calibration}} * 100\% \tag{11}$$

The area in the calibration corresponds to the area that is produced when the same amount of substance is spiked in pure solvent (Evian + 5% ACN) for the calibration curve. This means that areas for the same spiked amounts are compared.

2.7.4 Extraction efficiency

The extraction efficiency measures how well the extraction procedure performs. Extraction efficiency close to 100% indicates that the extraction procedure performs very well. It is calculated as follows:

$$\text{Extraction efficiency } [\%] = \frac{\text{Concentration}_2 - \text{Concentration}_4}{\text{Concentration}_1 - \text{Concentration}_4} * 100\% \tag{12}$$

2.7.5 Correction of calculations

During the course of the experiments, it became clear that the actual volume loss during the extraction was not 43% but only 17%, meaning that too much substance was spiked in the beginning, nominal $\frac{1.75}{1.2}$x too much. Therefore, the detected concentrations in the samples were corrected by a factor $\frac{1.2}{1.75}$ in order to compare them to the correct reference concentration. The areas were also corrected using the calibration which connects *substance area : IS area* with the concentration. As the IS area was known and the concentration had been corrected by a factor $\frac{1.2}{1.75}$, the corrected substance area could then easily be calculated. For the standard

addition method, the area was simply multiplied by $\frac{1.2}{1.75}$ as no calibration curve was calculated. As an ideal calibration curve would pass through the origin, meaning that a change of the area by a factor results in a change of the concentration by the same factor, this approximation is valid.

Furthermore, we realized that the whole idea of spiking more substance in the schemes before the extraction was an error of reasoning. In fact, the ratios in the calibration curve do not correspond to the ratios in the samples if not exactly the same amount of internal standard is spiked in the samples as in the calibration curve. The calibration is done by linking area ratios (*substance area : ISTD area*) to a concentration defined by the user in TraceFinder™. For example, in the calibration, an area ratio of 1:1 might be linked to 100 ng/L. However, as in the sample more substance and more internal standard were spiked, an area ratio of 1:1 corresponds to a higher concentration, e.g. 120 ng/L. This leads to an underestimation of the concentrations in the samples. In order to rectify this error, all concentrations of samples which were spiked too much (more than in the calibration curve) had to be corrected by a factor $\frac{\text{ISTD in sample}}{\text{ISTD in calibration}} = \frac{5.25}{4}$. The detailed calculations are shown in the corresponding R-code (recovery analysis with ISTD and recovery analysis with STDA) publicly available on GitHub:
`https://github.com/AnnaB459/MT_ActiveBioaccumulationMicropollutants`

2.7.6 Statistical tests

Statistical tests are used to compare two samples and to determine whether their mean or their median is equal.

The *unpaired two-sample t-test* is used to compare whether the means of two samples are statistically different. It can only be applied when both samples are normally distributed (check for normality with Shapiro-Wilks test) and when they have equal variance (check with F-test).[49,50,51] The null hypothesis is: Both samples have the same mean.

If the conditions for the unpaired two-sample t-test are not fulfilled (e.g. no normal distribution), an *unpaired two-sample Wilcoxon test* can be used to compare medians. The null hypothesis is: The two sample medians are equal.[52]

The *paired two-sample Wilcoxon test* is used to compare two samples pairwise (e.g. each substance in two different matrices). It is applied when the difference between the pairs is not normally distributed (if normal distribution, could use paired two-sample t-test). The null hypothesis is: The median of the differences "result in matrix A - result in matrix B" is not significantly different from zero.[52,53]

For all statistical tests, the significance level α is set to 5%, i.e. the null hypothesis is rejected whenever the obtained p-value is smaller than 0.05.

All statistical tests are performed with the built-in functions of R. The codes, including plotting of the results, are publicly available on GitHub (boxplot recoveries):
`https://github.com/AnnaB459/MT_ActiveBioaccumulationMicropollutants`

2.8 Bioaccumulation experiment

For the bioaccumulation experiment, the harvested biofilm from the glass slides of river Ticino was diluted in filtered river water such that a concentration of approximately 1 $g_{biomass} * L_{water}^{-1}$ was reached. This approximate concentration was determined by measuring the optical density OD at 685 nm, where an OD of 1 is expected to correspond to approximately 1 $g_{biomass} * L_{water}^{-1}$.

Activated sludge was diluted similarly in filtered effluent water such that a concentration of 1 $g_{biomass} * L_{water}^{-1}$ was reached.

The bioaccumulation experiment was performed in a temperature-controlled room at 17°C.

In order to determine which MPs are actively bioaccumulated and which MPs only passively adsorb, two reactors (biotic experiment and sorption control, see Figure 8) of both microbial communities (activated sludge and biofilm) containing each 600 mL biomass-water-suspension were prepared in 1L Schott bottles, which were constantly shaken with 160 min^{-1} throughout the course of the experiment. The biotic experiment reactor contained living biomass and underwent a light:dark cycle of 16:8 hours (dark from 21.00-5.00). In the biotic experiment reactor, all removal processes (biotransformation, abiotic transformation, passive adsorption, active bioaccumulation) could take place. The concentration in the solid phase of the biotic experiment reactor corresponded to the total accumulation. The biomass in the sorption control reactor was autoclaved (20 min at 121°C, 2 bar) and thus neither biotransformation nor any active bioaccumulation could take place. This reactor allowed determining the amount of passively adsorbed substances. Active bioaccumulation is the difference between the amount of substance detected in the solid phase of the biotic experiment minus the amount of substance detected in the solid phase of the sorption control. Biomass of all reactors was taken and extracted using QuEChERS. Additionally, samples from the supernatant were taken in order to make a mass balance in the end (described in detail in section 2.10).

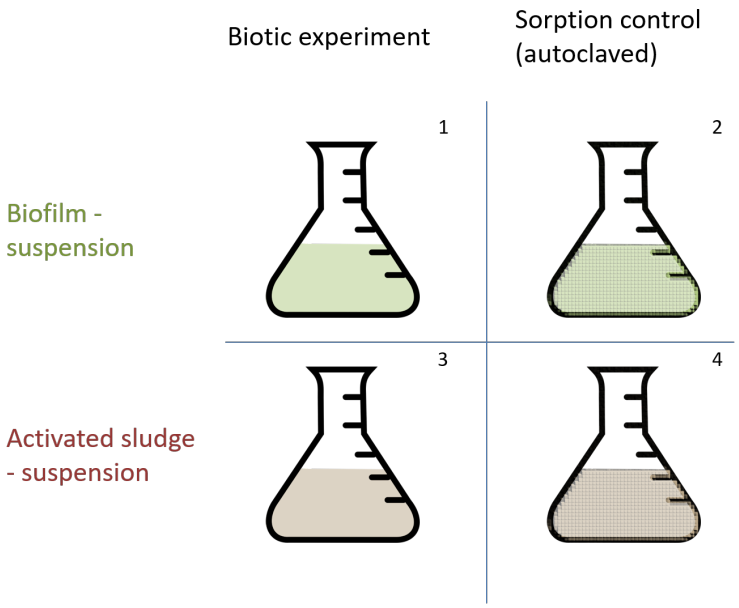


Figure 8: Overview of reactor scheme in bioaccumulation experiment. Reactor 1 was filled with 600 mL of the biofilm-riverwater suspension (testing accumulation in biofilm). Reactor 2 was filled with 600 mL of the biofilm-water mix and autoclaved (testing passive adsorption on biofilm). Reactor 3 was filled with 600 mL of the activated sludge - filtered effluent suspension (testing accumulation in activated sludge). Reactor 4 was filled with 600 mL of the activated sludge - filtered effluent suspension and autoclaved (testing passive adsorption on activated sludge).

A first solid sample was taken just before spiking (time point t0 for solid sample). The bottle was shaken in order to distribute the biomass homogeneously. 100 mL (2x 50 mL) were filled into Falcon tubes. The tubes were centrifuged at 4000 rcf for 20 minutes, the supernatant was discarded and the pellets were frozen at -20°C. Further, the reactors were spiked to a final concentration 10 nM for each substance. A first liquid sample was taken immediately after spiking (time point t0 for liquid sample): A sample of the well-shaken bottle (1 - 2 mL) was taken, centrifuged at 21300 rcf for 15min at 4°C. 1 mL of supernatant was transferred into a LC-MS-vial, spiked with internal standard (final ISTD-concentration = 2 μg/L) and stored at -20°C.

Two more samples were taken after 48 hours (time point t48) and after 96 hours (time point t96), liquid and solid samples were taken simultaneously, as described above. The biomass pellets were frozen at -20°C and then freeze-dried for 3 days.

The samples were then prepared and extracted as described in sections 2.4.1 to 2.4.4 and measured as explained in section 2.5.

2.9 Calculation of bioaccumulation

2.9.1 Accumulation

The accumulated amount is the amount of MPs extracted from the biotic experiment. It is normalized to the dry weight of the biomass (nmol substance per g biomass). It is calculated as follows for time points x (and x=48h, x=96h, respectively) with dwbiomass standing for freeze-dried weight of biomass in the sample, C for substance concentration in the sample and Cdw for substance concentration per dried weight biomass:

$$\Delta \mathrm{Cdw}_{t=x}\ [\mathrm{nmol}_{\mathrm{substance}}/\mathrm{g}_{\mathrm{biomass}}] = \frac{\mathrm{C}_{t=x}}{\mathrm{dwbiomass}_{t=x}} - \frac{\mathrm{C}_{t=0}}{\mathrm{dwbiomass}_{t=0}} \tag{13}$$

The adsorbed amount can be calculated with the same formula using the concentrations extracted from the sorption control reactors.

These calculations imply that at each time point the accumulation respective to time point 0 is calculated, corresponding to the actual accumulation observed in the experiment. This means that eventual accumulation which was already present in some samples before spiking (e.g. accumulation in the WWTP or in the river) is not taken into account.

For substances with an internal standard, this could be calculated up to 6 times per reactor and time point as for each reactor and time point up to six replicas were measured (due to the standard addition method). This multiple determination was used to calculate the mean and standard deviation of each time point and reactor. Further calculations were done with the obtained mean and standard deviation.

The concentration of the substances without an internal standard was calculated using the standard addition method. This means that only one concentration per time point and reactor was obtained but with a certain known standard deviation derived from the standard error of the linear regression.

All substances accumulating to at least 10% of their initial spiked amount at least at one time point are considered as accumulating substances.[1]

2.9.2 Active bioaccumulation

Active bioaccumulation is the amount of substances detected inside the living microbial communities that is not detected in the dead microbial communities, i.e. the sorption control. Therefore, the active bioaccumulation for time point t=x is calculated as follows with Cdw standing for substance per dry weight biomass, BE for the biotic experiment reactor, SC for the sorption control reactor.

$$\text{active bioacc}_{t=x}\ [\text{nmol}_{\text{substance}}/\text{g}_{\text{biomass}}] = \Delta\text{Cdw}_{BE,t=x} - \Delta\text{Cdw}_{SC,t=x} \tag{14}$$

In order to calculate what percentage of the initially present substance actively accumulated, equation 15 and 16 are used. In equation 15, the pure amount of substance accumulated (bioaccAmt) in the whole reactor is calculated. tdwbiomass stands for the total freeze-dried weight of the biomass, BE for the biotic experiment reactor, SC for the sorption control reactor, Cdw for substance per dry weight biomass. The tdwbiomass is the calculated based on measurements of 1/6 of the total biomass in each reactor. This is only an approximate measure. For comparison purposes, the mean tdwbiomass of both reactors containing the same microbial community is used for all calculations. The percentage accumulation is thus an approximate estimation.

$$\text{bioaccAmt}_{t=x}\ [\text{nmol}_{substance}] = \Delta\text{Cdw}_{BE,t=x} * \text{tdwbiomass}_{mean} - \Delta\text{Cdw}_{SC,t=x} * \text{tdwbiomass}_{mean} \tag{15}$$

Equation 16 calculates to what percentage this total accumulation corresponds.

$$\text{percentage accumulated}_{t=x}\ [\%] = \frac{\text{bioaccAmt}_{t=x}}{\text{initially spiked amount}} * 100\% \tag{16}$$

The initially spiked amount is equal to 5 nmol for all substances as described in section 2.8. All substances accumulating to at least 10% and actively bioaccumulating to at least 5% are considered as actively bioaccumulating substances. This limits are based on assumptions of Desiante et al., who performed very similar experiments.[1]

2.10 Mass balance

For some substances, a mass balance can be done in order to validate the method. The total amount of substances, which do not abiotically degrade, should stay constant in the sorption control reactor. The following equation should hold for time points x=48h and x=96h with C_{aq} standing for substance concentration in the water, $V_{aq,t=0}$ for the initial water volume, Cdw for substance per dry weight, SC for sorption control reactor, tdwbiomass for the total freeze-dried weight of the biomass.

$$\text{spiked amount substance [nmol]} = C_{aq,SC,t=x} * V_{aq,SC,t=0} + \ \Delta\text{Cdw}_{SC,t=x} * \text{tdwbiomass}_{SC} \tag{17}$$

This calculation can only be done for substances with an ISTD, as no standard additions were made on the aqueous samples. It is only done for compounds having a good relative recovery (80% - 120%).

It can also be calculated for the biotic experiment reactors; however, there the mass balance can probably not be closed as biotransformation can take place.

3 Results and Discussion

3.1 Exclusion of substances

Several of the initially 78 substances had to be excluded. Some substances are not clearly detectable when analysing them with LC-MS and are impossible to evaluate correctly in the calibration curve using the TraceFinder™ software.
Table 1 shows the excluded substances and the reason for their exclusion.

Excluded substance	**Reason for exclusion**
allopurinol	very broad peak, not clear if actually one or several peaks
benserazide	not detectable
mesalazine	too low retention time of around 3 minutes, not detectable with our measuring method
piracetam	very broad peak
simvastatin	only clearly detectable at very high concentrations (10 nM and more)
spironolactone	noise and peak not distinguishable, even at high concentrations
tenofovir	very broad peak with tailing

Table 1: Excluded substances that were not at all or not clearly detectable in the calibration curve.

In addition, the substances in Table 2 cannot be properly evaluated in samples with biomass, which is probably due to interaction with the biomass or disturbing noise from the matrix:

Excluded substance	**Reason for exclusion**
atorvastatin	very noisy, hard to distinguish
diphenhydramine	several peaks
hydroxychloroquine	no peak or no clearly identifiable peak
lamivudine	no clear peak
nintedanib	results inconsistent, same samples give different results
normorphine	no clear peak

Table 2: Excluded substances that were not at all or not clearly detectable in the biomass samples.

As it is not possible to determine the actual, true area and concentration of the above listed substances, any further evaluation or calculations are meaningless. Therefore, these substances are excluded from any further analysis, including the statistical testing and comparing. 65 substances are thus further analysed.

3.2 General comparison of internal standard method and standard addition method

On the following Table, the two used quantitation methods are compared qualitatively.

	Internal standard method	**Standard addition method with n standard additions**
sample amount	need only sample amount x	need sample amount n*x
preparation time	one preparation per sample	n preparations per sample
measurement time	one measurement per sample	n measurements per sample
analysis time	analyse 1 substance + 1 corresponding internal standard per sample	analyse each substance n times per sample
cost	internal standards very expensive	pure substances less expensive and already at hand
quality advantage	internal standard ideally behaves exactly like substance	less susceptible to individual spiking errors as multiple determination
quality disadvantage	spiking error might stay unnoticed	linear regression provokes more uncertainties especially when behaviour not 100% linear
requirements	need isotope-labelled internal standard	linear behaviour of substance within standard addition range

Table 3: Comparison of the internal standard method and the standard addition method with n standard additions.

3.3 Recovery results

There was a spiking error in sample n°5 of scheme 3, consequently, this sample is excluded from the analysis. The linear regression line to calculate the concentration for the standard addition method in scheme 3 is thus only based on five points and the mean concentration value of the samples with ISTD is the mean of five samples.

Due to the incorrect internal standard spiking amounts (see section 2.7.5), the extraction efficiency for substances with an internal standard is only calculated based on the concentrations in replicate 1 of all schemes (no standard addition).

Lisinopril cannot be detected in the samples that were spiked before the QuEChERS extraction procedure. The recovery for lisinopril is therefore 0. Lisinopril already showed different solubility behaviour when preparing stock solutions where it could not be dissolved in DMSO and was consequently dissolved in nanopure water. It is possible that the QuEChERS extraction method does not work for lisinopril due to its solubility properties.

The recovery for all other compounds is presented and discussed in the following sections.

Absolute and relative recovery, as well as the extraction efficiency and the matrix factor for each substance in activated sludge samples (AS) and biofilm samples (BF) are reported in tables in the Annex B.1.

3.3.1 Recoveries using the ISTD method

22 substances are evaluated with the internal standard method. The results are split in two tables: Table 8 in the Annex B.1 contains substances, which in at least one matrix have insufficient relative recoveries: below 0.7 or above 1.3. Table 9 in the Annex B.1 contains substances, which have good relative recoveries between 0.8 and 1.2 in both matrices. There are no substances which have relative recoveries between 0.7 and 0.8 or 1.2 and 1.3.

Only metoxuron and trimipramine show insufficient recoveries (Table 8 in the Annex B.1). Both metoxuron and trimipramine also have very low absolute recoveries, which shows that they are difficult to extract and/or analyse. Trimipramine also has low extraction efficiency indicating that the extraction is the main problematic step for this compound.

Table 9 in the Annex B.1 contains the remaining 20 substances evaluated with the ISTD method. They all have good relative recoveries between 0.8 and 1.2 in both matrices. Morphine presents good relative recovery even though in TraceFinder™ it was not clear whether there were two merged peaks of possibly two different substances or just one broader peak with mass and retention time of morphine. However, this good relative recovery might also be provoked by a systematic wrong integration in all samples. Therefore, special care needs to be taken when analysing the results of morphine. For most substances, absolute recoveries are about 0.3 to 0.4. Darunavir, losartan, morphine, pravastatin in biofilm and diclofenac in both matrices have low absolute recoveries with values below 0.2. Most of these substances present also low extraction efficiency in biofilm. The fact that substances show lower recovery in biofilm than in activated sludge will be discussed in more detail in section 3.3.5.

3.3.2 Recoveries using the standard addition method

The recovery results when using the standard addition method are split in three tables.

21 substances, displayed in table Table 10 in the Annex B.1, show insufficient recoveries (<0.7 or >1.3) when evaluated with the standard addition method. The R^2 of the linear regression is rather low for several of these substances (e.g. diosmin, sertraline), indicating that the standard addition method was not able to determine the concentrations reliably. Actually, all R^2 below 0.99 indicate difficulties with the linear regression. There are also some substances, which did not show Gaussian peak shapes when evaluating with TraceFinder™ (e.g. benzophenone 3, clomipramine, sertraline), which could lead to a bad relative recovery, eventually.

17 substances showed medium relative recovery between 0.7 and 0.8 or between 1.2 and 1.3 when evaluated with the standard addition method (Table 11 in the Annex B.1).

Finally, 26 substances showed good relative recovery between 0.8 and 1.2 when evaluated with the standard addition method (Table 12 in the Annex B.1). Similarly to the evaluation with ISTD, most absolute recoveries of substances showing good relative recovery lie in the range of 0.3 to 0.5. Only for abacavir, morphine and mycophenolic acid in biofilm and entacapone and valaciclovir in both matrices, they are below 0.2. For entacapone and valaciclovir, the extraction efficiencies are very low or even negative (an impossible result in principle), in addition the linear regression of valciclovir is rather low (0.97), indicating that special care needs to be taken when interpreting the results of these two substances.

3.3.3 Absolute recoveries

All absolute recoveries are depicted in the following boxplot (Figure 9).

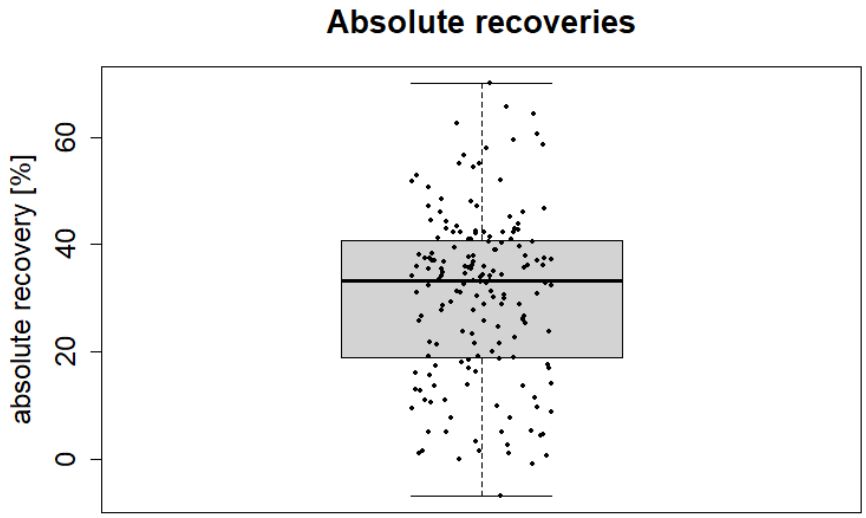


Figure 9: Boxplot of absolute recoveries obtained with both methods. It contains all 65 substances with 22 substances represented twice (once evaluated with the internal standard method, once evaluated with the standard addition method), each substance in both matrices, leading to a total of 174 points. The boxplot shows the median (bold black line), upper and lower quartiles (coloured box) and maximum/minimum variation outside the quartiles (dashed whisker lines) of all measurement points. The lower limit of the dashed whisker lines is defined as *max(min(all measurement points except outliers), lower quartile – 1.5 * inter-quartile range)*. The upper limit of the dashed whisker lines is defined as *min(max(all measurement points except outliers), upper quartile + 1.5 * inter-quartile range)*. All measurement points are displayed as black points, which are shifted randomly in horizontal direction in order to better visualize and distinguish different points. Empty points are outliers which are defined as being lower than *lower quartile - 1.5 * inter-quartile range* or higher than *upper quartile + 1.5 * inter-quartile range*. The median absolute recovery is 33%

The median absolute recovery is 33%. Negative absolute recoveries are in principle not possible and are produced by substances which are impossible to measure correctly with the applied method.

3.3.4 Comparison recoveries from the ISTD method with recoveries from the standard addition method

In a next step, the relative recovery obtained with both methods (internal standard vs. standard addition) is compared in Figure 10.

The relative recoveries for the standard addition method are more spread which translates into a higher inter-quartile range in Figure 10. However, there are also more substances evaluated with the standard addition method, which could naturally lead to such a higher spread. The median relative recovery obtained with the standard addition method is significantly higher with 107.65% compared to 98.75% obtained with the ISTD method.

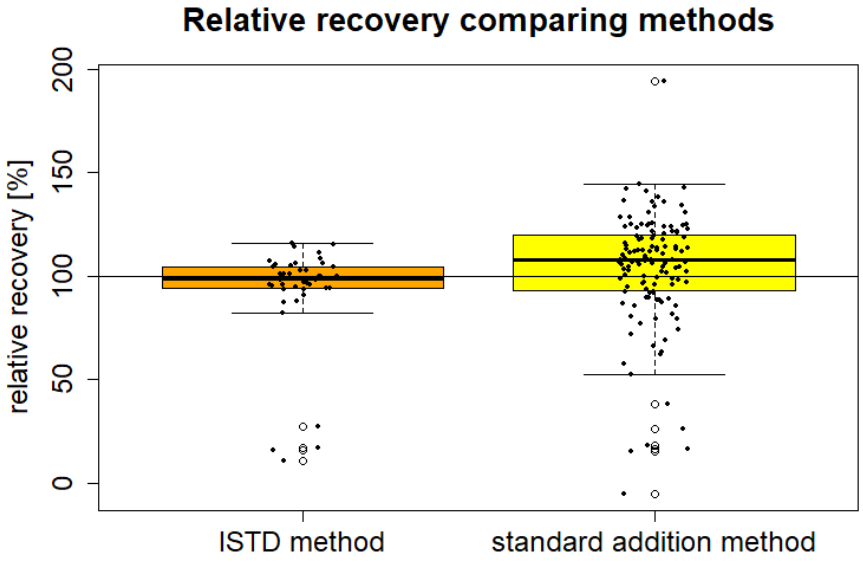


Figure 10: Boxplot of relative recoveries obtained with both methods. 22 substances are evaluated with the ISTD method. 65 substances are evaluated with the standard addition method, including the 22 substances which are also evaluated with the ISTD method. The boxplot shows the median (bold black line), upper and lower quartiles (coloured box) and maximum/minimum variation outside the quartiles (dashed whisker lines) of all other measurement points. The lower limit of the dashed whisker lines is defined as *max(min(all measurement points except outliers), lower quartile – 1.5 * inter-quartile range)*. The upper limit of the dashed whisker lines is defined as *min(max(all measurement points except outliers), upper quartile + 1.5 * inter-quartile range)*. All measurement points are displayed as black points, which are shifted randomly in horizontal direction in order to better visualize and distinguish different points. Empty points are outliers which are defined as being lower than *lower quartile - 1.5 * inter-quartile range* or higher than *upper quartile + 1.5 * inter-quartile range*. The median relative recovery obtained with the ISTD method is 98.75% and significantly lower than the median of 107.65% obtained with the standard addition method (unpaired two-sample Wilcoxon test, p-value=6e-04).

22 substances were evaluated with both methods and are compared one to one in Table 13 in the Annex B.1. Absolute recoveries are very close for both methods. In theory, they should be exactly the same as only areas are compared and no concentration calculation is performed. However, two different calibration curves were used for the standard addition method and the internal standard method (due to spiking errors as explained in section 2.7.5), which explain these slight variances. The fact that the absolute recoveries are very close even when using two different calibration curves shows the robustness of the measurement results.

A comparison of the relative recoveries of these 22 substances evaluated with both methods is also plotted in form of a boxplot in Figure 11.

Relative recoveries are higher when applying the standard addition method with a median of 108.85% compared to 98.75% obtained with the internal standard method. Relative recoveries of the standard addition method are more spread. The higher relative recovery when applying the standard addition method originates from a bias introduced by the standard addition method. There are several possible explanations for this bias. First, there might be a non-linear response of the detector at lower concentrations, meaning that the area at lower analyte concentrations decreases faster than at higher concentrations for a same concentration change.[45] Figure 12 illustrates the problem of applying a linear approximation to a dataset that is actually not linear.

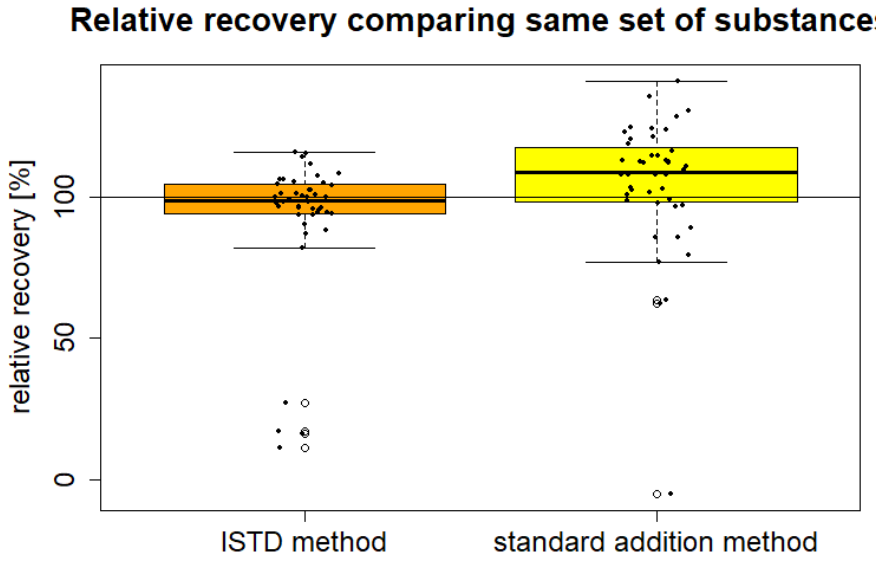


Figure 11: Boxplot of relative recoveries of 22 substances obtained with both methods. Comparison of the 22 substances that were evaluated with both methods. The boxplot shows the median (bold black line), upper and lower quartiles (coloured box) and maximum/minimum variation outside the quartiles (dashed whisker lines) of all other measurement points. The lower limit of the dashed whisker lines is defined as *max(min(all measurement points except outliers), lower quartile – 1.5 * inter-quartile range)*. The upper limit of the dashed whisker lines is defined as *min(max(all measurement points except outliers), upper quartile + 1.5 * inter-quartile range)*. All measurement points are displayed as black points, which are shifted randomly in horizontal direction in order to better visualize and distinguish different points. Empty points are outliers which are defined as being lower than *lower quartile - 1.5 * inter-quartile range* or higher than *upper quartile + 1.5 * inter-quartile range*. The median relative recovery obtained with the ISTD method is 98.75% and significantly lower than the median of 108.85% obtained with the standard addition method (paired two-sample Wilcoxon test, p-value=8e-03).

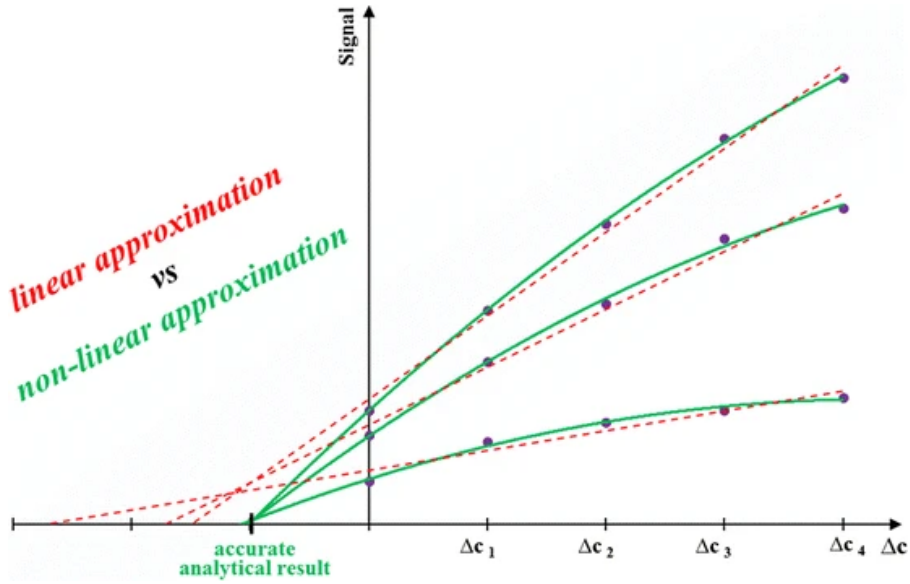
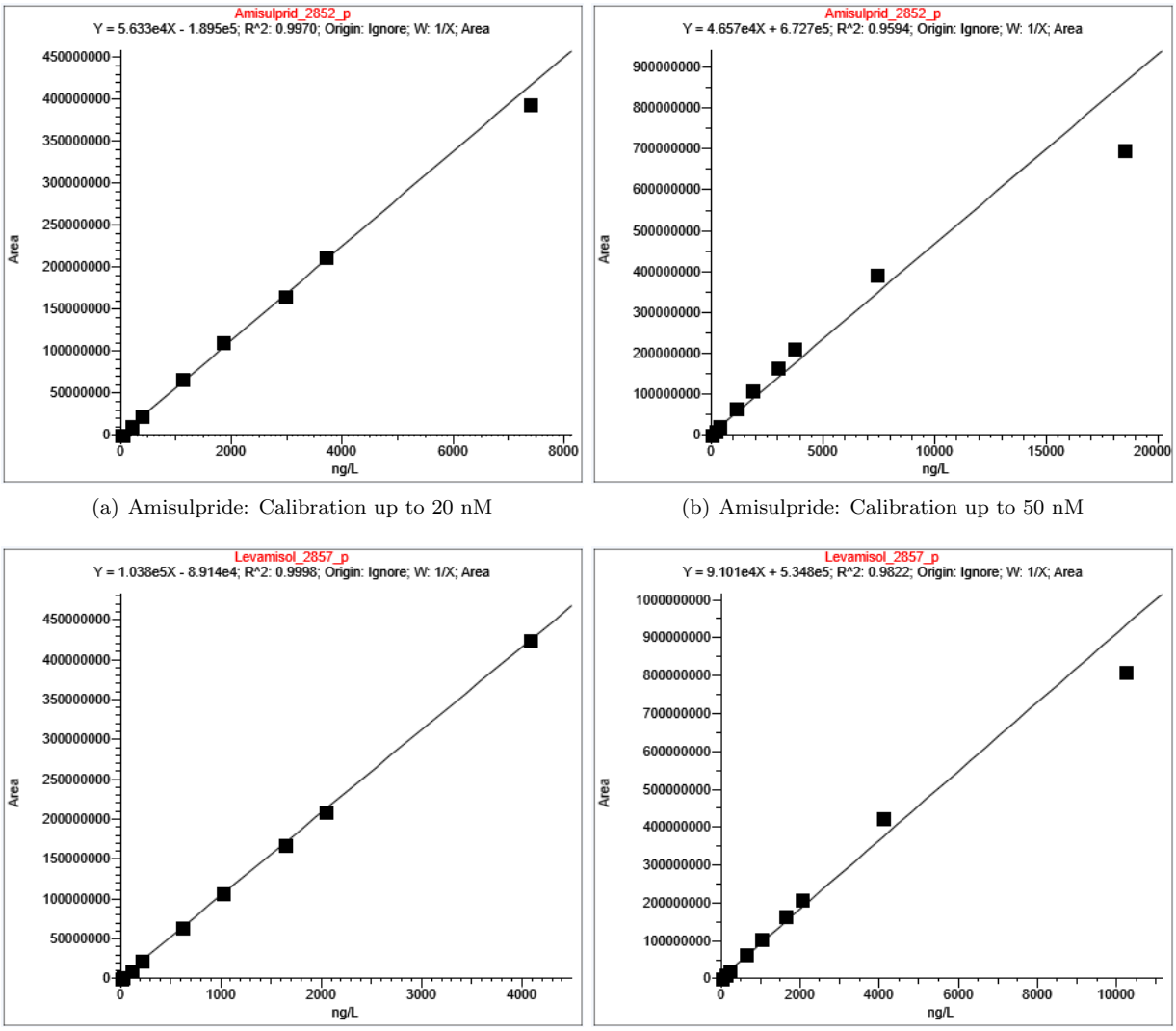


Figure 12: Illustration of the problem of linear regression when the actual response is not linear. Figure taken from Wieczorek et al.[54]

As can be seen in Figure 12, the intersection with the x-axis of the linear regression is further away from 0 than the accurate analytical result. Therefore, a nonlinear response leads to an overestimation of the actual concentration when applying the standard addition method. The overestimated concentration causes a higher relative recovery as the relative recovery is calculated by dividing the measured concentration by a fixed concen-

tration (consider formula 8). With our current data, we cannot further test this hypothesis. It is not possible to see in the calibration data when using an external calibration method whether there is a non-linear response of the detector and for most compounds, the R^2 of the linear regression is high (above 0.99). In order to confirm or reject this hypothesis, more measurements with more different concentrations would need to be taken and analysed.

Another bias could be introduced by ion suppression at higher concentration. This would lead to a relatively lower peak area of the highest standard addition points, manipulating the linear regression such that the slope is less steep, the intersection with the x-axis further from 0 and the concentration once more overestimated. Figure 13 illustrates this hypothesis.



(a) Amisulpride: Calibration up to 20 nM

(b) Amisulpride: Calibration up to 50 nM

(c) Levamisole: Calibration up to 20 nM

(d) Levamisole: Calibration up to 50 nM

Figure 13: Illustration of possible ion suppression effects. Comparison of the calibration curve when only the points up to 20 nM are used or when an additional point at 50 nM is used for the calibration curve. The Figure shows that the 50 nM measurement is below the calibration curve for both substances and thus undergoes ion suppression. This leads to a less steep slope when including the 50 nM point in the calibration curve. Pictures from Tracefinder™.

The highest calibration points in Subfigures 13(a) and 13(c) are at 20 nM (exclusion of highest calibration point at 50 nM, calibration curve only with all points below). In Subfigures 13(b) and 13(d), the calibration goes up to 50 nM. For both compounds, ion suppression is observed for the highest calibration point of 50 nM, which is below the linear regression line. If the highest calibration point is at 20 nM, the effect is less pronounced but still present for amisulpride. For levamisole, no effect can be seen at 20 nM.

During the recovery experiment, the highest standard addition is 0.02 nmol, which leads to a maximum concentration of 20 nM. In recovery schemes 1, 2 and 3, 0.004 nmol of substances are added, therefore, the final maximally measurable concentration is 24 nM: a concentration which could already undergo some ion suppression as shown by Figure 13. This might explain to a certain extent the higher relative recovery with the

standard addition method for amisulpride. For levamisole, the effect is not that clearly visible on the graphs and more data would be needed to make and confirm further hypotheses.

Extraction efficiencies are higher with the standard addition method, which could once more result from an overestimation of the concentration with the standard addition method. For most compounds, the extraction efficiency is around 0.7 to 0.8, for some higher, for some lower, indicating that generally the extraction is sufficient. Inefficient extraction is corrected when calculating the relative recovery.

Matrix factors are often slightly higher with the ISTD method. The use of a different formula for the calculation of the matrix factor depending on whether there is an internal standard available or not (see section 2.7.3) can explain this variation for some compounds. Though, even when using different formulas, the matrix factor of one method is most often in the range of the matrix factor +/- the standard deviation of the other method, indicating that the matrix factors obtained with the two methods are comparable. Many compounds have a matrix factor around 0.4 to 0.6, indicating that ion suppression takes place. No matrix factor above 1 is observed, therefore no ion enhancement takes place (see section 2.7.3 for more explanations).

3.3.5 Comparison recoveries in biofilm with recoveries in activated sludge

Finally, the absolute recovery obtained in both biological matrices is compared in Figure 14.

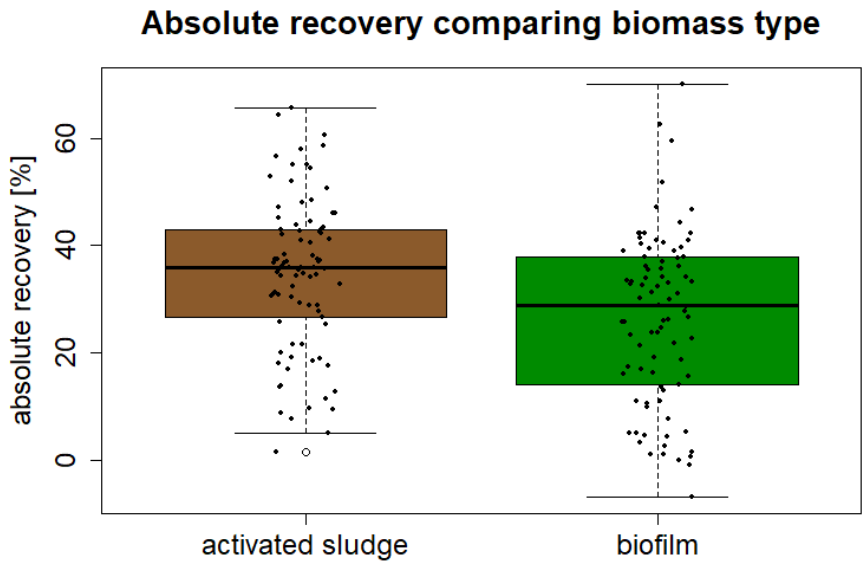


Figure 14: Boxplot of absolute recoveries obtained for biofilm and activated sludge samples. 65 substances are evaluated of which 22 substances are presented twice in each microbial community (once evaluated with the ISTD method, once evaluated with the stda method), leading to a total of 87 evaluated points per biomass type (biofilm or activated sludge). The boxplot shows the median (bold black line), upper and lower quartiles (coloured box) and maximum/minimum variation outside the quartiles (dashed whisker lines) of all measurement points. The lower limit of the dashed whisker lines is defined as *max(min(all measurement points except outliers), lower quartile – 1.5 * inter-quartile range)*. The upper limit of the dashed whisker lines is defined as *min(max(all measurement points except outliers), upper quartile + 1.5 * inter-quartile range)*. All measurement points are displayed as black points, which are shifted randomly in horizontal direction in order to better visualize and distinguish different points. Empty points are outliers which are defined as being lower than *lower quartile - 1.5 * inter-quartile range* or higher than *upper quartile + 1.5 * inter-quartile range*. The median absolute recovery is significantly higher in activated sludge with 35.95% compared to the median absolute recovery in biofilm which is 28.8% (paired two-sample Wilcoxon test, p-value=1.2e-09.)

Figure 14 shows and statistical tests confirm that absolute recovery in activated sludge is significantly higher. The lower absolute recovery of substances in biofilm could be due to the complexity of the biofilm matrix. Not only is the species diversity often very high in biofilms but they also present high amounts of extracellular polymeric substances (EPS). EPS limits the erosion of the cells under natural conditions[20] and could to some extent also protect the cells in the homogenization and extraction procedure of the recovery experiment. In addition, the high species diversity and EPS could both lead to more possible interactions with the analyte[18] and therefore loss in the extraction procedure.

3.3.6 Summary and implications of the recovery experiment

For 74% of all evaluated 65 substances, a sufficient relative recovery between 70% and 130% in both microbial communities is obtained. 58% of all substances have good relative recoveries between 80% and 120% in both microbial communities. Most compounds with insufficient recovery are compounds that were not used at Eawag before and that do not have an associated internal standard. Many of them exhibit a low R^2 when fitting the linear regression to determine the concentration using the standard addition method, which indicates difficulties in the extraction or measurement process.

The standard addition method results in higher relative recoveries than the ISTD method leading to a median above 100%. Moreover, relative recoveries obtained with the standard addition method are more scattered around the median. Possible explanations for this result are a non-linear response of the detector or ion suppression at higher concentrations.

Absolute recoveries are higher in activated sludge than in biofilm communities. Biofilm is supposed to be more diverse and contains more EPS. It is thus likely to interact and suppress MPs in more and more different ways.

The following substances will not be evaluated quantitatively in the accumulation experiment as they present insufficient recoveries:

- amlodipine, benzophenone 3, clomipramine, gliclazide, metoclopramide and valaciclovir in biofilm
- dextromethorphan, diosmin, levamisole, naloxone, pseudoephedrine amd trazodone in activated sludge
- bupropion, elvitegravir, metoxuron, sertraline and trimipramine in both matrices

Even though metoxuron and trimipramine can be recovered to a higher extent when applying the standard addition method, they are not evaluated quantitatively with the standard addition method as they have very low absolute recoveries respectively pose problems when evaluating with Tracefinder.

Lisinopril will not be analysed at all as it shows a recovery of 0.

3.4 LOQ

The Table with the LOQ of all substances can be found in the Annex B.2.

The LOQ in the sample is mostly below 1 nM. It can be high for some substances that typically also have low recoveries, such as diosmin, amlodipine, clomipramine, sertraline etc. (see section 3.3). It cannot be determined for lisinopril as it is not detectable in the QuEChERS-extracted samples. In addition, the absolute recoveries for valaciclovir and gliclazide in biofilm are negative, which is physically not possible. The absolute recoveries of these compounds are set to 0 and the LOQ cannot be determined.

3.5 Bioaccumulation experiment

The aim of the bioaccumulation experiment was to identify micropollutants that accumulate to a significant extent.

The obtained concentrations in the bioaccumulation experiment are not corrected with the recovery. Only substances with relative recoveries between 70 and 130% are evaluated quantitatively.

Results are given with standard deviations. For substances evaluated with the standard addition method, no standard deviation for time point 48h in the sorption control is available due to a loss of the activated sludge sorption control sample at time point 48h. Only two standard addition points are available at this point and this gives a perfect linear regression line through two points with an unknown and thus not plotted standard deviation.

In a first step, we determined which substances accumulated to more than 10% at least at one time point (t48 or t96) without differing in reactors (sorption control or biotic experiment), or microbial community (biofilm or activated sludge). 20 substances were identified to accumulate to at least 10% at at least one time point.

Next, the 20 substances were split in different accumulation patterns. Substances that only accumulated in the sorption control reactor or that accumulated to a higher extent in the sorption control reactor than in the biotic experiment reactor were not analysed further. For the remaining substances, the minimal active bioaccumulation was calculated as "accumulated amount in biotic experiment - accumulated amount in sorption control". The reasons for potentially more active bioaccumulation than this calculated minimal amount are discussed in detail in the discussion section (section 3.5.4). Substances that accumulated to a only slightly higher extent in the biotic experiment reactor than in the sorption control reactor ($< 5\%$ difference) and thus show some weak evidence for active bioaccumulation were grouped together but not further analysed in detail as it is not possible to determine whether this active bioaccumulation is significant. Finally, substances that actively bioaccumulated to a significant amount ($> 5\%$ of the initially spiked amount) were analysed in detail

and grouped according to the microbial community in which they actively bioaccumulated (activated sludge, biofilm or both). Desiante et al. assumed that less than 5% change was not significant,[1] therefore we chose this same significance level.

Figures of some substances are exemplarily shown for each group; for all other substances, figures can be found in the Annex B.3.2.

The full results of the bioaccumulation of all substances can be found in Table 15 in the Annex B.3.

3.5.1 Significantly adsorbing substances

Several substances only showed a significant accumulation in the sorption control reactor or a significantly higher accumulation in the sorption control reactor than in the biotic experiment reactor. This behaviour was observed for amlodipine, benzophenone-3 and diltiazem in activated sludge and for bisacodyl, entacapone and mirabegron in both microbial communities. Diltiazem is plotted as an example in Figure 15.

The results of all substances in activated sludge at 48h must be interpreted with care as part of the activated sludge sorption control sample of time point 48h was lost. There were only two points available for the linear regression of the standard addition method and therefore, the concentration at this time point could not be determined reliably (see also section 3.5.3 and figure 18, where the same phenomena occurred and is explained in more detail).

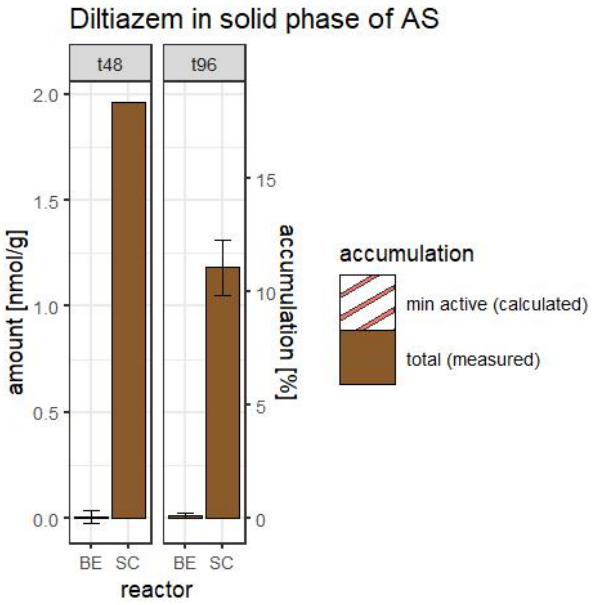


Figure 15: Adsorption of diltiazem. BE stands for biotic experiment, SC for sorption control, AS for activated sludge. For both time points, in brown the total accumulated amount in nmol substance / g freeze-dried biomass (left y-axis) and the corresponding percentage accumulation (right y-axis) in activated sludge are plotted. The minimal active bioaccumulation is calculated as difference between the accumulated amount in the biotic experiment and in the sorption control and is represented with red stripes. Diltiazem adsorbed to a high extent on the dead activated sludge (sorption control) but did not accumulate in the living activated sludge (biotic experiment), therefore no active bioaccumulation was observed.

Diltiazem was detected before in biofilm[4,8] and to very small extents in activated sludge[7] but without differing whether it adsorbed or actively bioaccumulated.

Adsorption processes are supposed to happen in both reactors: in the sorption control but also in the biotic experiment. However, no accumulated (and thus also no adsorbed) diltiazem was detected in the biomass from the biotic experiment. This could indicate rapid biotransformation: Once diltiazem has adsorbed to living activated sludge, it immediately gets biotransformed. This hypothesis can be confirmed once the results of the biotransformation experiment of the SNF/DFG-project are available. Similarly, all other substances, which adsorbed to a high extent in the sorption control but showed no or only little accumulation in the biotic experiment, are supposed to have undergone biotransformation in the biotic experiment reactor.

It is generally assumed that especially hydrophobic compounds tend to adsorb to biomass such as activated sludge or biofilm.[5,7,18,22] The log K_{ow} of the adsorbing substances in this experiment lie between 1 and 3.8, with most of them being around 2.5.[55] This means that these compounds are not very hydrophobic and that therefore, there is no clear relationship between the hydrophobicity and the tendency to adsorb for these

compounds. Other studies already stated that there was not always a clear relationship between log K_{ow} and adsorption and they assume that other interaction mechanisms might lead to adsorption.[5,8,22]

Entacapone contains a positively-charged N-atom and a negatively-charged O-Atom,[55] which might lead to an interaction with and adsorption to the organic matter.[18,5] However, absolute recoveries of entacapone were very low and the standard deviation of the detected accumulation was very high indicating that other extraction methods should be used as well in order to confirm adsorption of entacapone.

3.5.2 Slightly actively bioaccumulating substances

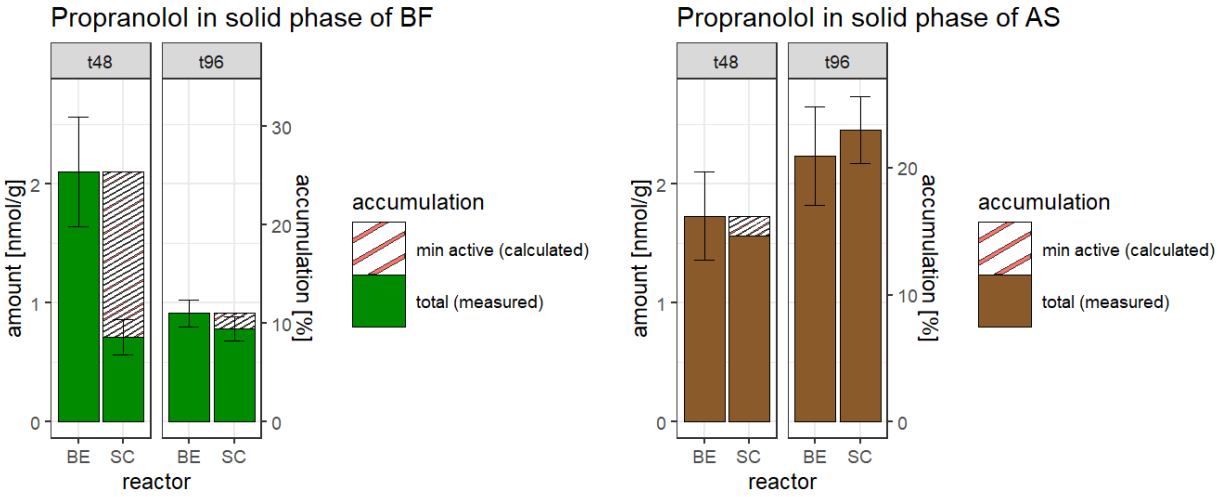
Some substances indicated active bioaccumulation, as the amount of accumulation was higher in the biotic experiment than in the sorption control. However, this difference is not seen as significant, as it is less than 5% of the initially spiked amount. This was observed for clomipramine, enzalutamide, metoclopramide and propranolol in activated sludge and for tolperison in biofilm. An example of not significant bioaccumulation is shown in Figure 16(b) where propranolol accumulates slightly in activated sludge after 48h but to less than 5%.

Clomipramine is a very hydrophobic compound with a log K_{ow} of 5.19[55] and about 30% of its initial amount adsorbed to activated sludge which is a typical behaviour for very hydrophobic compounds.[5,18,22] However, the other slightly actively bioaccumulating substances have lower log K_{ow} ranging from 2 to 4[55] and therefore no general relation between log K_{ow} and accumulation can be stated.

3.5.3 Significantly actively bioaccumulating substances

All substances that were detected to accumulate to more than 10% of their initially spiked amount at least at one time point and that showed active bioaccumulation of at least 5% at least at one time point, are considered as significantly actively bioaccumulating substances.

Four substances actively bioaccumulate only in biofilm to a significant extent (minimal active bioaccumulation in brackets), namely dextromethorphan (min. 10%), edoxaban (min. 8%), propranolol (min. 17%) and trazodone (min. 7%). These are all aliphatic amine containing compounds. The example of propranolol is shown in Figure 16.



(a) Propranolol: accumulation in biofilm.

(b) Propranolol: accumulation in activated sludge.

Figure 16: Accumulation of propranolol. BE stands for biotic experiment, SC for sorption control, BF for biofilm, AS for activated sludge. For both time points, in green/brown the total accumulated amount in nmol substance / g freeze-dried biomass (left y-axis) and the corresponding percentage accumulation (right y-axis) are plotted. The percentage accumulation is different for biofilm and activated sludge due to different biomass weights. The minimal active bioaccumulation is calculated as difference between the accumulated amount in the biotic experiment and in the sorption control and is represented with red stripes.

Desiante et al. observed adsorption of propranolol in biofilm but no active bioaccumulation.[1] Mastrángelo et al. detected propranolol in biofilm without differing adsorption from bioaccumulation.[56] Gulde et al. stated adsorption and active bioaccumulation of propranolol in activated sludge[24] and other studies detected propranolol to a high extent in activated sludge.[7] In this master thesis, active bioaccumulation of propranolol in activated sludge was only visible to about 2% after 48h and not at all after 96h. This might be due to different microbial community composition in our study compared to others. In fact, shorter sludge retention time (as

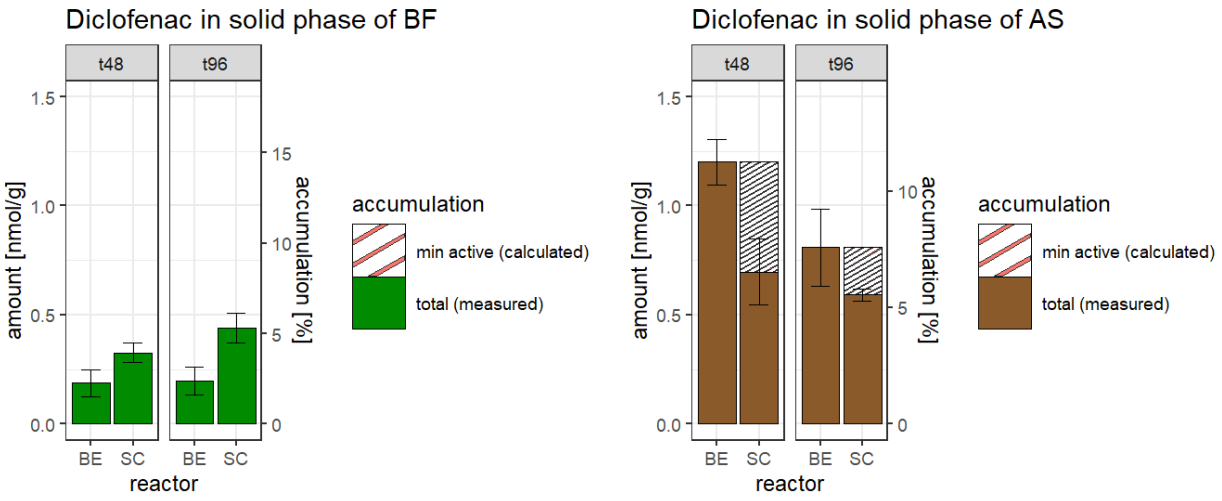
in WWTP Airolo) could lead to a less diverse activated sludge community composition containing less protozoa.[57,58] In biofilms of our study, propranolol actively bioaccumulates up to 17%. As propranolol contains an aliphatic amine-moiety with pK_a around 9.5,[55] ion trapping is possible and has actually been shown as being an accumulation mechanism of propranolol in activated sludge.[24] Therefore, this could also be the mechanism responsible for accumulation in biofilm. The accumulation of propranolol in biofilm is higher after 48h than after 96h. This effect could be due to biodegradation of propranolol. Once the results of the biotransformation experiment are available, they might enlighten the fate of propranolol in activated sludge and broaden the understanding of the fate of propranolol in biofilm.

Dextromethorphan (pK_a = 9.85[59]), edoxaban (pK_a = 6.33[59]) and trazodone (pK_a = 7.09[59]) all contain aliphatic amines with respective pK_a bigger than 5 (expected maximum pH inside acidic vesicles[24]). This means that a significant proportion is protonated at pH below 5 and could be trapped in acidic vesicles (mechanism of ion-trapping).[24]

Six substances actively bioaccumulated only in activated sludge to a significant extent (minimal active bioaccumulation in brackets), namely amisulpride (min. 5%), diclofenac (min. 5%), febuxostat (min. 7%), fenfluramine (min. 9%), ketamine (min. 9%) and tolperisone (min. 13%). Amisulpride, fenfluramine, ketamine and tolperisone contain aliphatic amine-moieties.

The initial concentration of diclofenac (approximately 0.3 nmol/g), febuxostat (approximately 0.1 nmol/g) and amisulpride (approximately 0.02 nmol/g) in activated sludge was already elevated indicating that these substances were accumulated in activated sludge in the wastewater treatment plant. This initial substance concentration was subtracted when calculating the accumulation after 48h and 96h in order to only evaluate the accumulation happening during our experiment.

The accumulation of diclofenac is shown in detail in Figure 17.



(a) Diclofenac: accumulation in biofilm.

(b) Diclofenac: accumulation in activated sludge.

Figure 17: Accumulation of diclofenac. BE stands for biotic experiment, SC for sorption control, BF for biofilm, AS for activated sludge. For both time points, in green/brown the total accumulated amount in nmol substance / g freeze-dried biomass (left y-axis) and the corresponding percentage accumulation (right y-axis) are plotted. The percentage accumulation is different for biofilm and activated sludge due to different biomass weights. The minimal active bioaccumulation is calculated as difference between the accumulated amount in the biotic experiment and in the sorption control and is represented with red stripes.

It has been shown by several studies that diclofenac adsorbs to or accumulates in activated sludge[7,12] and is present in biofilm.[4,5,8,18] There are also indications for active bioaccumulation in activated sludge[12] though most studies do not differ adsorption from active bioaccumulation. Active bioaccumulation in activated sludge can be confirmed in this master thesis. Active bioaccumulation in biofilm was not observed in our experiments. Adsorption of diclofenac to biofilm was detected, however the amount in the sorption control was always higher than in the biotransformation batch, indicating that in our experiments, diclofenac only adsorbed to biofilm (and this only to approximately 5%) and was not actively bioaccumulated. The bioaccumulation mechanism for diclofenac is unknown. Diclofenac has a low pK_a of about 4.0[59,55] and is thus not likely to undergo ion-trapping.[24]

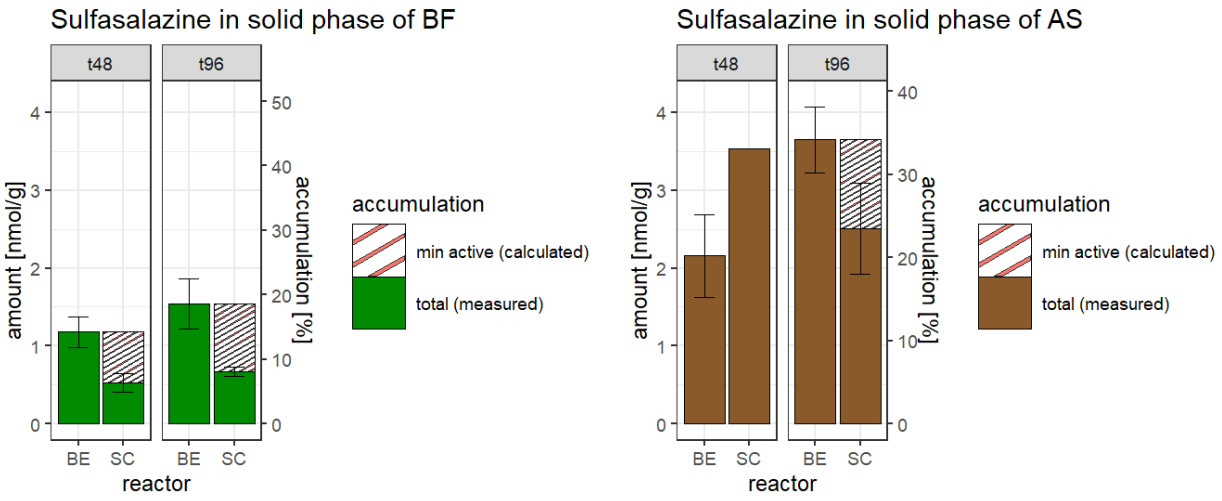
Four of the six compounds contain aliphatic amine-moieties and have high enough pK_a to undergo ion-trapping: Amisulpride with estimated pK_a between 7.05 and 9.3,[59,55] fenfluramine with estimated pK_a around 9.6 to 10.22,[59,55] ketamine with pK_a 7.16 - 7.5[59,55] and tolperisone with pK_a 8.78[59] might all be actively

bioaccumulated by the mechanism of ion-trapping as described by Gulde et al.[24] Gulde et al. already observed active bioaccumulation of fenfluramine in activated sludge protozoa,[24] which is confirmed in this study. Desiante et al. observed accumulation of amisulpride.[1]

Febuxostat has a pK_a of 3.59[59] and does not have an aliphatic amine-moiety, thus the active bioaccumulation is probably due to another mechanism than ion-trapping.

All compounds except amisulpride are rather hydrophobic with log K_{ow} around 3.5[55] which might facilitate the adsorption to biomass[5,18,22] and the consecutive active bioaccumulation.

One substance showed significant active bioaccumulation in both microbial communities: Sulfasalazine with active bioaccumulation of at least 11% in both microbial communities. The results for sulfasalazine are shown in Figure 18.



(a) Sulfasalazine: accumulation in biofilm.

(b) Sulfasalazine: accumulation in activated sludge.

Figure 18: Accumulation of sulfasalazine. BE stands for biotic experiment, SC for sorption control, BF for biofilm, AS for activated sludge. For both time points, in green/brown the total accumulated amount in nmol substance / g freeze-dried biomass (left y-axis) and the corresponding percentage accumulation (right y-axis) are plotted. The percentage accumulation is different for biofilm and activated sludge due to different biomass weights. The minimal active bioaccumulation is calculated as difference between the accumulated amount in the biotic experiment and in the sorption control and is represented with red stripes.

Sulfasalazine does not contain aliphatic amine-moieties but it contains a sulfonamide with estimated pK_a between 6.5[59,55] and 9.7.[60] However, sulfasalazine is anionic at higher pH and only neutral (not positively charged) at pH 5, therefore ion trapping is unlikely. Gulde et al. have shown that sulfathiazole, an antibiotic containing also a sulfonamide with a similar pK_a (6.9) is not ion-trapped.[24] The active bioaccumulation mechanism of sulfasalazine in our experiments remains thus unknown.

Sulfasalazine shows inconsistent behaviour in activated sludge: At time point 48h, the amount in the sorption control is significantly higher (indicating possibly biotransformation). At time point 96h, the amount in the biotic experiment is significantly higher (indicating active bioaccumulation). This same behaviour was observed for apixaban, clomipramine, dexlansoprazole, dextromethorphan, diosmin, elvitegravir, mexiletine and sertraline in activated sludge though only clomipramine accumulated to a significant extent. This pattern was not observed in biofilm. Also, amlodipine, benzophenone-3, bisacodyl, diltiazem, entacapone and mirabegron showed higher adsorption after 48h than after 96h in the activated sludge samples of the sorption control. All substances showing this pattern are substances evaluated without an internal standard and their concentration at time point 48h is only determined by a linear regression through two measurement points due to a loss of the corresponding sample (standard addition). Therefore, the concentration at time point 48h is not reliably determined and might be incorrect. The more reliable results of time point 96h indicate that apixaban, clomipramine, dexlansoprazole, dextromethorphan, diosmin, elvitegravir, mexiletine and sertraline actively bioaccumulate in activated sludge to some (not significant) extent. Recoveries for dextromethorphan, diosmin, elvitegravir and sertraline are insufficient, thus their results cannot be interpreted quantitatively. In order to have stronger evidence for active bioaccumulation of all these compounds, the experiments should be repeated.

3.5.4 Discussion

It needs to be noted that the calculated amount of active bioaccumulation corresponds to a minimal value. In the biotic experiment reactor, substances can also be biotransformed, which can influence the amount of adsorption. We assumed that the amount adsorbed in the biotic experiment was equal to the amount adsorbed in the sorption control. However, the amount adsorbed in the biotic experiment might get (partially) biotransformed (as assumed for substances in section 3.5.1) or actively bioaccumulated. This means that the proportion of active bioaccumulation to adsorption would be higher than calculated. Adsorption in the biotic experiment reactor could also be lower than in the sorption control due to less total substance mass present in the system (because of biotransformation). Assuming that there is an equilibrium between the dissolved amount and the adsorbed amount of a substance, the total adsorbed amount would decrease with increasing biotransformation in the biotic reactor and not correspond to the observed adsorbed amount in the sorption control reactor. This would once more lead to an underestimation of active bioaccumulation. In addition, the adsorption characteristics of autoclaved biomass could differ from those of living biomass, which would also influence the amount of adsorbed substances.

Eight compounds out of eleven showing active bioaccumulation to a significant extent contain aliphatic amine-moieties. It is thus very likely that one of the main mechanisms for active bioaccumulation is ion-trapping (see section 1.3.1 for detailed description of the mechanism). Some aliphatic amine containing substances did not show active bioaccumulation to a significant extent even though it was observed in previous studies, especially levamisole, mexiletine, tramadol and venlafaxine did not actively bioaccumulate to a significant extent in our study even though they showed ion-trapping before,[24] respectively could not be evaluated quantitatively due to insufficient recoveries. However, they all actively bioaccumulated to some extent, which was lower than the significance level chosen for our study. This might be due to a different community composition. For this master thesis, activated sludge of an only C-eliminating WWTP (Airolo) was used, whereas Gulde et al. used activated sludge of an also N-eliminating WWTP (Neugut, Dübendorf), which possibly contains more diverse species and protozoa.[24,57,58]

As expected,[12] diclofenac accumulated to a significant extent and also actively bioaccumulated to at least 5% in activated sludge. The mechanisms for this accumulation are still unknown.

Contrarily to our hypothesis, phenylurea herbicides did not accumulate to a significant amount. The total accumulation of all four phenylurea herbicides (chlorotoluron, isoproturon, metoxuron, monuron) is below 10% and their active bioaccumulation is below 5%; in fact, it is mostly just slightly above 0%. Desiante et al. observed active bioaccumulation of isoproturon in biofilm up to 30%,[1] which could not be confirmed in this study. Also in further studies, isoproturon was detected in the solid phase of biofilm[5,61] and of activated sludge[7] without differing between adsorption and active bioaccumulation. Other studies detected phenylurea herbicides such as diuron in biofilm[62,63] or activated sludge[7] without differing between adsorption and active bioaccumulation. Carles et al. did not detect any bioconcentration (substance amount in biofilm / substance amount in water = 0) of chlorotoluron in biofilm[5] and therefore no accumulation. Gulde et al. could not observe any ion-trapping of isoproturon or chlorotoluron in activated sludge.[24] Overall, when combining the results of this study and other studies on the fate of phenylurea herbicides in microbial communities, they are not totally consistent and further studies are thus needed to clarify.

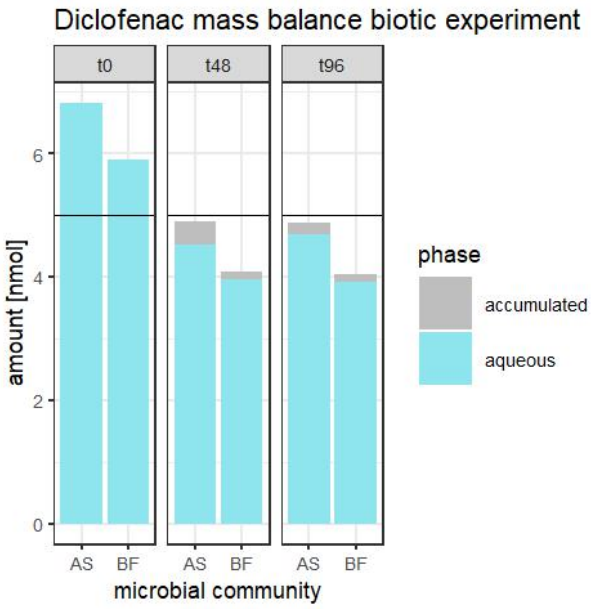
The results for benzophenone-3 and clomipramine in biofilm, dextromethorphan, diosmin, levamisole and trazodone in activated sludge and bupropion, elvitegravir, sertraline and trimipramine in both microbial communities indicate that these substances might accumulate. However, as the recoveries are insufficient, it is not possible to determine whether a significant accumulation or an active bioaccumulation has taken place. Other extraction methods such as selective pressurised liquid extraction could possibly lead to a better recovery for these compounds and could be used to confirm or reject their accumulation.

3.5.5 Mass balance

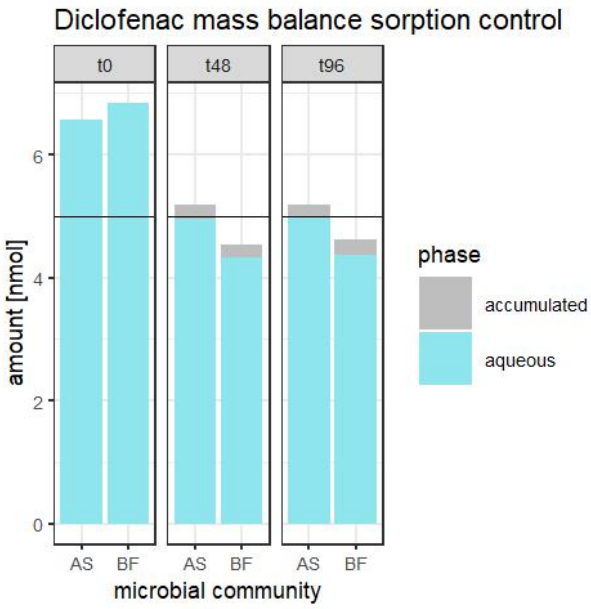
Two of the actively bioaccumulated compounds were measured with their own internal standard and hence, their aqueous concentration could be determined readily. Therefore, a mass balance for these compounds could be calculated. It is shown in the following Figures 19 and 20. Mass balances of the other compounds with an internal standard can be found as plots in the Annex B.3.2 and in Table 16 in the Annex B.3.1. Mass balances for metoclopramide and trimipramine are not calculated due to the insufficient recoveries of these compounds.

As can be seen in Figure 19, the amount of diclofenac in biofilm is lower in the biotic experiment (about 20% loss) than in the sorption control experiment (about 10% loss) after 48h and after 96h. More data could confirm whether this decrease is significant and whether this is due to biotransformation. The amount of diclofenac in the activated sludge reactor does not decrease significantly below 5 nM, indicating no biotransformation. Kruglova et al. found low biological removal rates of diclofenac in activated sludge.[12] Typical removal values of diclofenac in WWTPs lie around 20%.[7] The results of the biotransformation experiments performed for the SNF/DFG-study will enlighten whether diclofenac was really not removed in our activated sludge community

and whether there was significant removal in biofilm communities. As our results indicate that diclofenac was not biotransformed in activated sludge, the calculated minimal active bioaccumulation might correspond to the real active bioaccumulation.

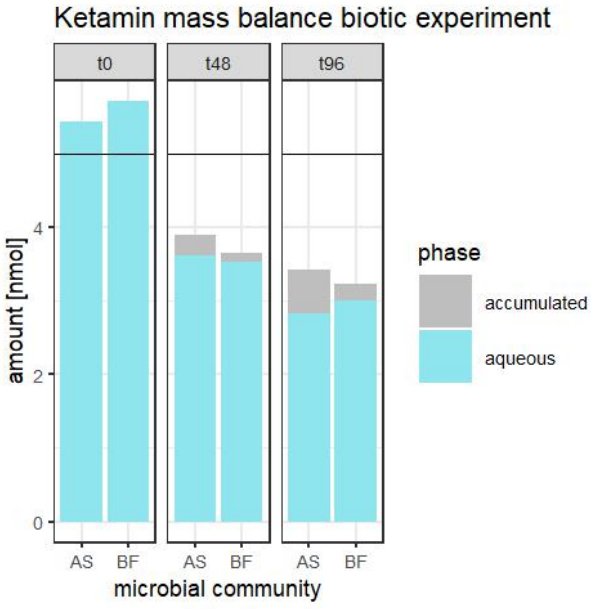


(a) Diclofenac: mass balance in biotic experiment.

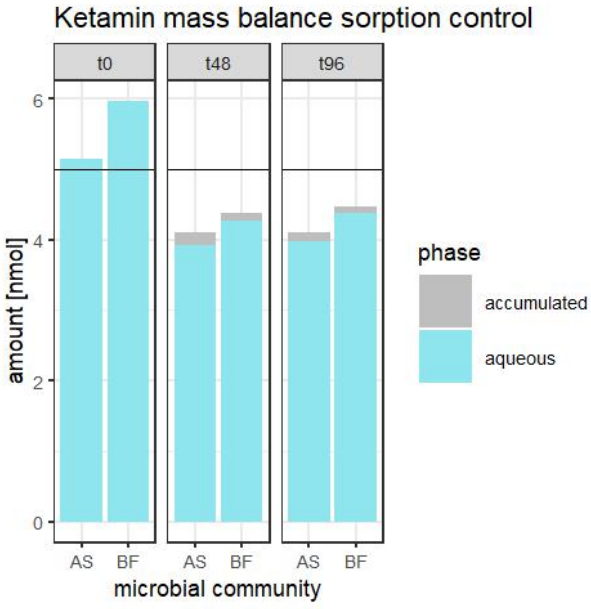


(b) Diclofenac: mass balance in sorption control.

Figure 19: Mass balance of diclofenac. AS stands for activated sludge, BF for biofilm. The initially spiked amount was 5 nmol and is represented with a horizontal black line. In blue, the amount of substances in the aqueous sample is represented; in grey the amount of substances in the solid sample (accumulated) is represented.



(a) Ketamine: mass balance in biotic experiment.



(b) Ketamine: mass balance in sorption control.

Figure 20: Mass balance of ketamine. AS stands for activated sludge, BF for biofilm. The initially spiked amount was 5 nmol and is represented with a horizontal black line. In blue, the amount of substances in the aqueous sample is represented; in grey the amount of substances in the solid sample (accumulated) is represented.

As shown in Figure 20, the total amount of ketamine in the biotic experiment decreases significantly over time (more than 30% loss compared to spiked amount of 5 nmol in biofilm and activated sludge), which indicates

biotransformation of ketamine. The results of the biotransformation experiment will give more information on the biotransformation rate and the order of the degradation reaction.

Assuming that the degradation of ketamine led to a new adsorption-dissolution-equilibrium, the actual active bioaccumulation in activated sludge was probably higher than the calculated 9% (see section 3.5.4).

Ideally, the total amount in the sorption control should be 5 nmol at all three time points as no removal processes (except abiotic) can take place. However, the total initial amount is higher than 5 nmol for both substances shown above and also for many other substances. The total amount of substance is around 5 nmol or lower after 48h and 96h but similar at these two time points in the sorption control.

Several reasons could lead to this unclosed sorption control mass balance: underestimation of the amount in the solid phase at t48 and t96, underestimation of the amount in the liquid phase at t48 and t96 or overestimation of the amount in the liquid phase at t0.

Underestimation of the amount in the solid phase can be excluded as the substance amount extracted with QuEChERS after 48h and 96h should be about 5 - 10 times higher in order to obtain the same total amount as initially. This means that the actual relative recovery would only be 10 - 20%. Even though the conditions of the recovery experiment (spiking on freeze-dried biomass) are not exactly the same as the conditions of the bioaccumulation experiment (spiking in reactor with suspended, autoclaved biomass), the actual relative recovery should still be in the same order of magnitude as in the recovery experiment (around 100%) and not so much smaller.

The lower total amount after 48h and 96h could also be due to an abiotic degradation. However, as the same pattern is observable for almost all substances and the total amount after 48h and after 96h is very similar, this explanation seems unlikely. Also, substances that were studied by Desiante et al.[1] and are partly overlapping with substances of this study did not show an abiotic degradation.

Insufficient mixing of the batch reactor when taking the aqueous sample at t0 could lead to an overestimation of the substances initially present. In fact, the total amount of substance at t0 is very often around 5.5 nmol or even above 6 nmol in the biomass suspension. Yet, the initial total amount spiked was only 5 nmol. In order to have the true initial concentration and exclude any fast adsorption effects, the first liquid sample was taken very quickly after spiking and only after short mixing, which seems to have been too fast and led to an overestimation of the initial concentration. The systematic higher measured concentration of substances in biofilm in the sorption control reactor at time point 0h compared to activated sludge could even confirm this handling error: The biofilm in the sorption control reactor was even worse mixed than the activated sludge in the sorption control reactor after spiking in the beginning.

Still, even when assuming that the total detected amount after 48h and 96h is true, it is in average about 4 to 4.5 nmol, meaning that about 10% - 20% of the substance is missing in the mass balance. This can be observed for most substances indicating that possible handling errors could have led to this lower amount. The samples were taken by three different persons on the three different sampling days and possible effects as changing *sample volume : ISTD volume* cannot be excluded. Desiante et al. considered the mass balance as closed if it had no more than 25% deviation of the initial spiked amount (100%).[1] Applying this principle, the mass balance can be closed in the sorption control for 17 of 20 evaluated compounds. Only for pantoprazole, neotame and morphine, less than 75% of the initially spiked amount was found. Morphine did not show a nice Gaussian peak in Tracefinder™ but rather two merged peaks, therefore measurement problems of morphine cannot be excluded. Desiante et al. did not study these three compounds,[1] therefore the results of the SNF/DFG-biotransformation experiment are needed in order to know whether abiotic degradation takes place. Neotame degrades readily in the biotic experiment reactor (almost complete degradation after 48h) and some degradation mechanisms could possibly also occur in the sorption control. All three compounds show good relative recovery between 80 and 120% in the QuEChERS extraction, meaning that the loss probably does not occur in the QuEChERS extraction.

4 Conclusion and Outlook

In order to better understand the fate of MPs in contact with microbial communities, bioaccumulation experiments with activated sludge and biofilm were performed.

An appropriate method to determine the accumulated amount of micropollutants in biomass was successfully developed. The QuEChERS method applied in this master thesis allowed determining the accumulated amount of 48 (74%) of 65 evaluated substances in activated sludge and in biofilm with sufficient recoveries (relative recovery between 70% and 130%). The absolute recoveries of the substances in activated sludge are mostly higher than those of the substances in biofilm, possibly due to the higher complexity of the biofilm matrix.

The developed method allowed identifying eleven MPs that actively bioaccumulated to more than 5% in at least one microbial community. Dextromethorphan, edoxaban, propranolol and trazodone actively bioaccumulated in biofilm. Amisulpride, diclofenac, febuxostat, fenfluramine, ketamine and tolperisone actively bioaccumulated in activated sludge. Sulfasalazine actively bioaccumulated in both microbial communities. Eight of these substances contain aliphatic amine-moieties with pKa's above 5 (mostly in range 7 to 10) and are thus susceptible to undergo ion-trapping in protozoa as it has already been shown for two of these compounds (propranolol and fenfluramine in activated sludge).[24] Ion-trapping might therefore be an important active bioaccumulation process. The active bioaccumulation mechanism of diclofenac, febuxostat and sulfasalazine remains unknown. Several of the eleven actively bioaccumulated substances have shown active bioaccumulation in experiments performed in other studies even though the exact percentage active bioaccumulation varied from one study to another.[1,12,24]

Contrarily to our expectations, phenylurea herbicides[1] did not accumulate significantly. Results from different studies are not well aligned,[1,5,7,24,61,62,63] therefore additional experiments are needed in order to better understand their removal fate.

Diclofenac actively bioaccumulated as hypothesized.[12]

Finally, a mass balance was calculated for 20 compounds in order to further validate the experiments and gain additional insights. The mass balance could be closed in the sorption control reactor for 17 of the 20 compounds, i.e. 100% +/- 25% of the initially spiked amount could be detected after 48h and 96h. This is considered as sufficient to validate the method as we were able to close the mass balance for a higher proportion of compounds than Desiante et al.[1] who applied the same method. The mass balance for three compounds could not be closed. They showed a loss of more than 25% of the initial amount after 48h and 96h. It should be investigated whether these three compounds degrade abiotically.

This master study has some limitations and room for improvement:

First, the performed experiments have some weaknesses and could be improved as follows:

In order for the standard addition method to perform correctly, the signal that a compound produces should be linearly connected to its concentration. We never tested linearity of the included substances due to time constraints. In order to be sure that the relationship is linear, more concentrations that are different should be measured and statistically evaluated. Such an evaluation could also enlighten reasons for higher recovery with the standard addition method.

Several calculation and reasoning errors occurred during the planning of the recovery experiments. Even though they could be corrected by calculations, the recovery experiment should be repeated to have truly reliable results.

The calculated percentage accumulation is only an approximation. In a future study, the complete microbial community should be extracted, freeze-dried and weighted after termination of the experiment. This would lead to a more accurate result for time point 96h. The total amount of biomass can change throughout the experiment; therefore it remains challenging to calculate the exact percentage accumulation for the other time points.

The activated sludge sample loss at time point 48h led to uncertainties in the result and inconsistency whether certain substances without an associated ISTD accumulate or not. Even though a tendency for accumulation and often also for active bioaccumulation can be stated thanks to the result of the time point 96h, experiments with activated sludge should be repeated in order to reliably determine the bioaccumulation of several compounds (see section 3.5.3).

Second, the overall setup of this study has some limitations:

The presence of almost 200 substances in concentration of 10 nM each leads to an overall substance concentration of 2 μM. This is an artificial situation and does not reflect real exposure in the environment. Studies indicate that when testing such high concentrations in biofilm, there could be a saturation effect, i.e. the biofilm is saturated with accumulated micropollutants or other pollutants (such as metals) and cannot accumulate more MPs or other types of pollutants. In nature however, this other MPs could potentially also accumulate as the biofilm is not saturated and there still is capacity for uptake.[18,30] The same effects are possibly present in activated sludge too but less pronounced as activated sludge is exposed to higher substance concentrations in WWTPs than biofilm in rivers. A saturation effect could also occur due to the limitations of ion trapping.

Only a limited amount of substances can be trapped[24] and the acidic vesicles in activated sludge and/or biofilm communities could be saturated in the experiments performed for this study and therefore not accumulate the same substances and/or not to the same extent as in natural environments. It would be especially interesting to test single substances in lower concentration which showed a low tendency to bioaccumulate (order of magnitude of a few percent) to see whether they would accumulate to a higher extent in the absence of competition, especially compounds, which are fairly alike (for example phenylurea herbicides).

In addition, the adsorbed amount in the biotic experiment reactor could be lower than in the sorption control reactor, which could also lead to an underestimation of the actual active bioaccumulation (see also section 3.5.4).

Further, one must keep in mind that the dwbiomass includes also sediments, which in biofilms of this master thesis present up to 50% of the total mass. Part of the MPs could also sorb on this inert material. Active bioaccumulation can only take place in organic biomass, therefore the actively bioaccumulated substances per real weight organic biomass would actually be higher.

The results of this master study form part of a bigger picture of the SNF/DFG-project "Unraveling the Molecular Mechanisms of Trace Contaminant Biotransformation from Wastewater to Natural Surface Water". The biotransformation results will further enlighten the fate of accumulated MPs in this study. For example, several MPs showed higher bioaccumulation after 48h than after 96h. With the biotransformation data, it will become clear whether this phenomenon could really be due to biotransformation. In addition, the biotransformation data might indicate why more accumulation of certain substances was observed in one microbial community than in the other. For some substances, lower accumulation in one microbial community might simply be due to faster biotransformation in this microbial community. The genomic data and the genetic microbial community composition might give more indications why certain substances tend to bioaccumulate more in one microbial community than in the other. Data from this master study will be used to distinguish biotransformation from bioaccumulation.

It would be interesting to perform ecotoxicological studies with the bioaccumulating substances. This would allow seeing whether their effect on the organism is more severe because of their accumulation or if the organisms get more tolerant as shown by first studies done by Carles et al.[5] The ecotoxicological effect could be evaluated by measuring primary and secondary production of biofilm / activated sludge, or measuring the photosynthetic efficiency of biofilm when exposed to a strongly accumulating substance compared to exposure to a similar but non-bioaccumulating substance. In order to evaluate the tolerance, microbial communities should be exposed to constant low MP concentrations. Ecotoxicological tests should be performed before and after this exposure.[5] Ecotoxicological evaluation of in biofilm accumulating compounds is particularly relevant as biofilm is an important food source and the accumulated compounds might biomagnify throughout the trophic food chain.[4,8,18]

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Finally, many thanks to my boyfriend, my friends and family, who supported me during the last 6 months and who were able to take my mind off my master thesis sometimes in order to relax and recharge my forces.

6 Nomenclature

List of Abbreviations

The following abbrevations are used in this report:

ACN	acetonitrile
AS	activated sludge
BE	biotic experiment
BF	biofilm
bioacc	bioaccumulation
DFG	Deutsche Forschungsgemeinschaft (German Research Foundation)
DOC	dissolved organic carbon
EPM	extracellular polymeric matrix
EPS	extracellular polymeric substances
fdw	freeze-dried weight
ISTD	internal standard
LC-MS	Liquid Chromatography - Mass Spectrometry
LOQ	limit of quantification
MP	micropollutant
OD	optical density
PE	Person Equivalent
QuEChERS	Quick Easy Cheap Effective Rugged Safe
RT	Retention Time
SC	sorption control
SNF	Schweizerischer Nationalfonds (Swiss National Science Foundation)
STDA	standard addition
TBEP	Tris(2-butoxyethyl) phosphate

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A Additional information

A.1 Full list of micropollutants

Substance	Associated ISTD	Substance class
Abacavir	-	Pharmaceutical
Acemetacin	-	Pharmaceutical
Albuterol	Albuterol-D4	Pharmaceutical
Allopurinol	Allopurinol-13C,15N2	Pharmaceutical
Amisulpride	Amisulpride-D5	Pharmaceutical
Amlodipine	-	Pharmaceutical
Apixaban	-	Pharmaceutical
Apremilast	-	Pharmaceutical
Atorvastatin	Atorvastatin-D5	Pharmaceutical
Benzophenone-3	-	Personal care
Bisacodyl	-	Pharmaceutical
Bupropion	-	Pharmaceutical
Chlorotoluron	Chlorotoluron-D6	Herbicide
Clomipramine	-	Pharmaceutical
Codeine	Codeine-D6	Pharmaceutical
Cyclophosphamide	Cyclophosphamide-D4	Pharmaceutical
Darunavir	Darunavir-D4	Pharmaceutical
Dexlansoprazole	-	Pharmaceutical
Dextromethorphan	-	Pharmaceutical
Diclofenac	Diclofenac-D4	Pharmaceutical
Diltiazem	-	Pharmaceutical
Diosmin	-	Pharmaceutical
Diphenhydramine	-	Pharmaceutical
Edoxaban	-	Pharmaceutical
Elvitegravir	-	Pharmaceutical
Emtricitabine	Emtricitabine-13C,15N2	Pharmaceutical
Entacapone	-	Pharmaceutical
Enzalutamide	-	Pharmaceutical
Etodolac	Etodolac-D3	Pharmaceutical
Febuxostat	-	Pharmaceutical
Fenfluramine	-	Pharmaceutical
Gliclazide	-	Pharmaceutical
Hydroxychloroquine	-	Pharmaceutical
Icaridin	-	Insecticide
Ifosfamide	-	Pharmaceutical
Isoproturon	Isoproturon-D6	Herbicide
Ketamine	Ketamine-D3	Pharmaceutical
Lamivudine	-	Pharmaceutical
Levamisole	-	Pharmaceutical
Lisinopril	-	Pharmaceutical
Losartan	Losartan-D4	Pharmaceutical
Mesalazine	-	Pharmaceutical
Metoclopramid	-	Pharmaceutical
Metoxuron	Metoxuron-D6	Herbicide
Mexiletine	-	Pharmaceutical
Mirabegron	-	Pharmaceutical
Moclobemide	-	Pharmaceutical
Monuron	-	Herbicide
Morphine	Morphine-D3	Pharmaceutical
Mycophenolic acid	-	Pharmaceutical
Naloxone	-	Pharmaceutical
Neohesperidin dihydrochalcone	-	Artificial sweetener
Neotame	Neotame-D3	Artificial sweetener

Substance	Associated ISTD	Substance class
Nintedanib	-	Pharmaceutical
Normorphine	-	Pharmaceutical
Olmesartan	-	Pharmaceutical
Pantoprazole	Pantoprazole-D3	Pharmaceutical
Piracetam	-	Pharmaceutical
Pirfenidone	-	Pharmaceutical
Pravastatin	Pravastatin-D3	Pharmaceutical
Primidone	Primidone-D5	Pharmaceutical
Propranolol	-	Pharmaceutical
Pseudoephedrine	-	Pharmaceutical
Rivaroxaban	-	Pharmaceutical
Sacubitril	-	Pharmaceutical
Sertraline	-	Pharmaceutical
Simvastatin	-	Pharmaceutical
Spironolactone	-	Pharmaceutical
Sulfasalazine	-	Pharmaceutical
Tenofovir	Tenofovir-D6	Pharmaceutical
Tolperison	-	Pharmaceutical
Torsemide	Torsemide-D7	Pharmaceutical
Tramadol	Tramadol-D6	Pharmaceutical
Trazodone	-	Pharmaceutical
Trimipramine	Trimipramine-D3	Pharmaceutical
Valaciclovir	-	Pharmaceutical
Venlafaxine	Venlafaxine-D6	Pharmaceutical

Table 4: All micropollutants included in this master project

A.2 Chemicals used

Name	Supplier
Heptane for HPLC >99%	Sigma-Aldrich
Acetonitrile, HPLC for gradient analysis	Acros Organics
Nanopure Water	From generator at Eawag
QuEChERS Final Polish EMR-Lipid (Sodium Chloride : Magnesium Sulfate 1:4)	Agilent Technologies
Accessories for BeadBeater ® zirconium/glass pellets, 1.0mm	Carl Roth GmbH+Co. KG
Methanol (OPTIMA ® LC/MS Grade)	Fisher Scientific
Ethanol absolute for analysis EMSURE ® ACS,ISO,Reag. Ph Eur	Sigma-Aldrich

Table 5: Material used

A.3 Exact spiking amounts

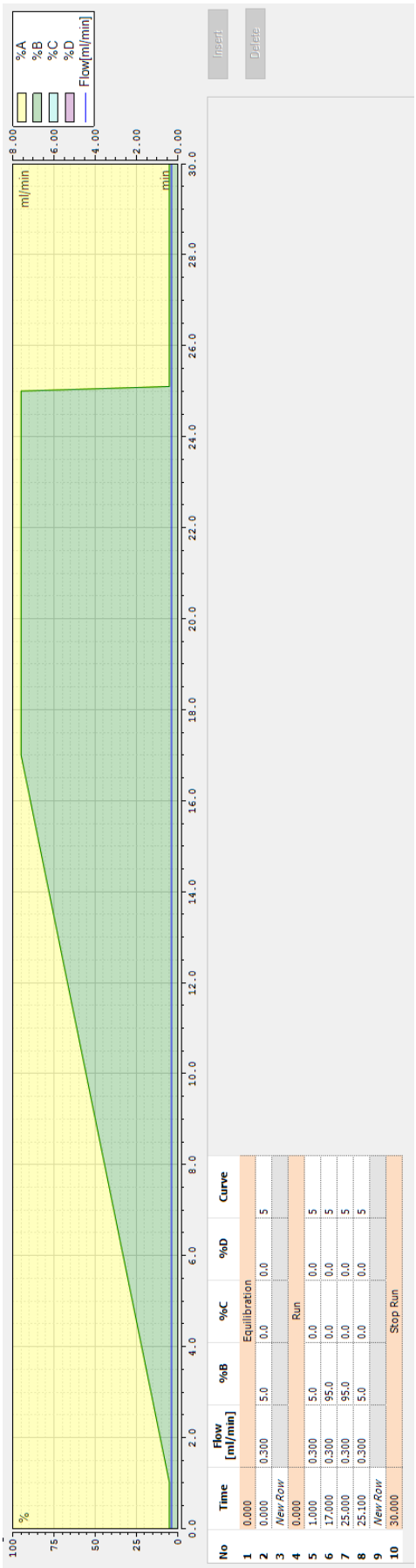
	added amount of STD before preparation	**added amount of ISTD before preparation**	**added amount of STDA before preparation**
Scheme 1-1	0	0	0
Scheme 1-2	0	0	0
Scheme 1-3	0	0	0
Scheme 1-4	0	0	0
Scheme 1-5	0	0	0
Scheme 1-6	0	0	0
Scheme 2-1	70µL of 100nM-solution = 0.007 nmol	0	0
Scheme 2-2	70µL of 100nM-solution = 0.007 nmol	0	0
Scheme 2-3	70µL of 100nM-solution = 0.007 nmol	0	0
Scheme 2-4	70µL of 100nM-solution = 0.007 nmol	0	0
Scheme 2-5	70µL of 100nM-solution = 0.007 nmol	0	0
Scheme 2-6	70µL of 100nM-solution = 0.007 nmol	0	0
Scheme 3-1	70µL of 100nM-solution = 0.007 nmol	52.5µL of 100µg/L-solution = 5.25 ng	0
Scheme 3-2	70µL of 100nM-solution = 0.007 nmol	52.5µL of 100µg/L-solution = 5.25 ng	35µL of 100nM-solution = 0.0035nmol
Scheme 3-3	70µL of 100nM-solution = 0.007 nmol	52.5µL of 100µg/L-solution = 5.25 ng	35µL of 100nM-solution = 0.0035nmol
Scheme 3-4	70µL of 100nM-solution = 0.007 nmol	52.5µL of 100µg/L-solution = 5.25 ng	17.5µL of 1µM-solution = 0.0175nmol
Scheme 3-5	70µL of 100nM-solution = 0.007 nmol	52.5µL of 100µg/L-solution = 5.25 ng	35µL of 1µM-solution = 0.035nmol
Scheme 3-6	70µL of 100nM-solution = 0.007 nmol	52.5µL of 100µg/L-solution = 5.25 ng	35µL of 1µM-solution = 0.035nmol
Scheme 4-1	0	52.5µL of 100µg/L-solution = 5.25 ng	0
Scheme 4-2	0	52.5µL of 100µg/L-solution = 5.25 ng	35µL of 100nM-solution = 0.0035nmol
Scheme 4-3	0	52.5µL of 100µg/L-solution = 5.25 ng	35µL of 100nM-solution = 0.0035nmol
Scheme 4-4	0	52.5µL of 100µg/L-solution = 5.25 ng	17.5µL of 1µM-solution = 0.0175nmol
Scheme 4-5	0	52.5µL of 100µg/L-solution = 5.25 ng	35µL of 1µM-solution = 0.035nmol
Scheme 4-6	0	52.5µL of 100µg/L-solution = 5.25 ng	35µL of 1µM-solution = 0.035nmol
Scheme 5-1	0	0	0
Scheme 5-2	0	0	0
Scheme 5-3	0	0	0
Scheme 5-4	0	0	0
Scheme 5-5	0	0	0
Scheme 5-6	0	0	0

Table 6: Amounts spiked before doing the QuEChERS extraction

	added amount of STD after preparation	**added amount of ISTD after preparation**	**added amount of STDA after preparation**
Scheme 1-1	40µL of 100nM-solution = 0.004 nmol	30µL of 100µg/L-solution = 3ng	0
Scheme 1-2	40µL of 100nM-solution = 0.004 nmol	30µL of 100µg/L-solution = 3ng	20µL of 100nM-solution = 0.002nmol
Scheme 1-3	40µL of 100nM-solution = 0.004 nmol	30µL of 100µg/L-solution = 3ng	20µL of 100nM-solution = 0.002nmol
Scheme 1-4	40µL of 100nM-solution = 0.004 nmol	30µL of 100µg/L-solution = 3ng	10µL of 1µM-solution = 0.01nmol
Scheme 1-5	40µL of 100nM-solution = 0.004 nmol	30µL of 100µg/L-solution = 3ng	20µL of 1µM-solution = 0.02nmol
Scheme 1-6	40µL of 100nM-solution = 0.004 nmol	30µL of 100µg/L-solution = 3ng	20µL of 1µM-solution = 0.02nmol
Scheme 2-1	0	30µL of 100µg/L-solution = 3ng	0
Scheme 2-2	0	30µL of 100µg/L-solution = 3ng	20µL of 100nM-solution = 0.002nmol
Scheme 2-3	0	30µL of 100µg/L-solution = 3ng	20µL of 100nM-solution = 0.002nmol
Scheme 2-4	0	30µL of 100µg/L-solution = 3ng	10µL of 1µM-solution = 0.01nmol
Scheme 2-5	0	30µL of 100µg/L-solution = 3ng	20µL of 1µM-solution = 0.02nmol
Scheme 2-6	0	30µL of 100µg/L-solution = 3ng	20µL of 1µM-solution = 0.02nmol
Scheme 3-1	0	0	0
Scheme 3-2	0	0	0
Scheme 3-3	0	0	0
Scheme 3-4	0	0	0
Scheme 3-5	0	0	0
Scheme 3-6	0	0	0
Scheme 4-1	0	0	0
Scheme 4-2	0	0	0
Scheme 4-3	0	0	0
Scheme 4-4	0	0	0
Scheme 4-5	0	0	0
Scheme 4-6	0	0	0
Scheme 5-1	0	30µL of 100µg/L-solution = 3ng	0
Scheme 5-2	0	30µL of 100µg/L-solution = 3ng	20µL of 100nM-solution = 0.002nmol
Scheme 5-3	0	30µL of 100µg/L-solution = 3ng	20µL of 100nM-solution = 0.002nmol
Scheme 5-4	0	30µL of 100µg/L-solution = 3ng	10µL of 1µM-solution = 0.01nmol
Scheme 5-5	0	30µL of 100µg/L-solution = 3ng	20µL of 1µM-solution = 0.02nmol
Scheme 5-6	0	30µL of 100µg/L-solution = 3ng	20µL of 1µM-solution = 0.02nmol

Table 7: Amounts spiked after the preparation, just before detection

A.4 LC-MS method



No	Time	Flow [ml/min]	%B	%C	%D	Curve
1	0.000			Equilibration		
2	0.000	0.300	5.0	0.0	0.0	5
3	New Row					
4	0.000			Run		
5	1.000	0.300	5.0	0.0	0.0	5
6	17.000	0.300	95.0	0.0	0.0	5
7	25.000	0.300	95.0	0.0	0.0	5
8	25.100	0.300	5.0	0.0	0.0	5
9	New Row					
10	30.000			Stop Run		

Figure 21: Composition of flow for the measurement with LC-MS. Eluent A is nanopure water with 0.1% formic acid. Eluent B is MeOH with 0.1% formic acid.

B Full results

B.1 Recovery experiment results

	mean absolute recovery +/- stdv	mean relative recovery +/- stdv	extraction efficiency	matrix factor +/- stdv	TraceFinder™
Metoxuron AS	0.094 +/- 0.306	0.273 +/- 0.916	0.897	0.439 +/- 0.029	double peak
Metoxuron BF	0.050 +/- 0.298	0.161 +/- 0.762	0.892	0.481 +/- 0.023	double peak
Trimipramine AS	0.177 +/- 0.039	0.111 +/- 0.008	0.388	0.444 +/- 0.071	good
Trimipramine BF	0.016 +/- 0.011	0.170 +/- 0.121	0.088	0.048 +/- 0.013	good

Table 8: Substances evaluated with insufficient relative recoveries (below 0.7 or higher than 1.3) in at least one matrix evaluated with the internal standard method. AS stand for activated sludge matrix. BF stands for biofilm matrix. The column Tracefinder indicates how well the substance could be evaluated with Tracefinder (good Gaussian peak shape etc.).

	mean absolute recovery +/- stdv	mean relative recovery +/- stdv	extraction efficiency	matrix factor +/- stdv	TraceFinder™
Albuterol AS	0.372 +/- 0.023	0.982 +/- 0.040	0.534	0.618 +/- 0.048	medium
Albuterol BF	0.362 +/- 0.098	1.025 +/- 0.076	0.649	0.661 +/- 0.070	medium
Amisulpride AS	0.430 +/- 0.081	0.940 +/- 0.062	0.774	0.639 +/- 0.070	good
Amisulpride BF	0.471 +/- 0.048	1.025 +/- 0.054	0.749	0.635 +/- 0.055	good
Chlorotoluron AS	0.354 +/- 0.025	0.999 +/- 0.105	0.814	0.474 +/- 0.011	good
Chlorotoluron BF	0.333 +/- 0.036	0.957 +/- 0.079	0.813	0.436 +/- 0.025	good
Codeine AS	0.461 +/- 0.064	1.082 +/- 0.203	0.67	0.612 +/- 0.069	good
Codeine BF	0.342 +/- 0.113	0.945 +/- 0.134	0.683	0.624 +/- 0.067	good
Cyclophosphamid AS	0.350 +/- 0.047	0.968 +/- 0.090	1.057	0.503 +/- 0.034	good
Cyclophosphamid BF	0.422 +/- 0.057	1.010 +/- 0.153	0.847	0.564 +/- 0.022	good
Darunavir AS	0.307 +/- 0.052	0.985 +/- 0.169	0.811	0.429 +/- 0.022	good
Darunavir BF	0.046 +/- 0.079	0.871 +/- 0.222	0.291	0.452 +/- 0.040	good
Diclofenac AS	0.140 +/- 0.080	0.881 +/- 0.243	-1.107	0.218 +/- 0.019	medium
Diclofenac BF	0.137 +/- 0.039	0.955 +/- 0.265	0.852	0.210 +/- 0.024	medium
Emtricitabine AS	0.371 +/- 0.054	1.054 +/- 0.136	0.542	0.737 +/- 0.030	good

	mean absolute recovery +/- stdv	mean relative recovery +/- stdv	extraction efficiency	matrix factor +/- stdv	TraceFinder™
Emtricitabine BF	0.260 +/- 0.157	1.043 +/- 0.042	0.519	0.751 +/- 0.025	good
Etodolac AS	0.644 +/- 0.093	0.999 +/- 0.116	0.736	0.822 +/- 0.034	good
Etodolac BF	0.626 +/- 0.075	1.076 +/- 0.035	0.779	0.898 +/- 0.049	good
Isoproturon AS	0.288 +/- 0.089	0.980 +/- 0.182	0.955	0.581 +/- 0.022	good
Isoproturon BF	0.313 +/- 0.090	1.143 +/- 0.065	0.901	0.505 +/- 0.018	good
Ketamin AS	0.405 +/- 0.048	0.905 +/- 0.039	0.792	0.561 +/- 0.036	good
Ketamin BF	0.409 +/- 0.106	0.965 +/- 0.114	0.755	0.553 +/- 0.037	good
Losartan AS	0.551 +/- 0.196	1.011 +/- 0.068	0.681	0.629 +/- 0.020	good
Losartan BF	0.111 +/- 0.048	0.820 +/- 0.251	0.277	0.603 +/- 0.023	good
Morphine AS	0.278 +/- 0.073	1.159 +/- 0.085	0.535	0.513 +/- 0.061	bad
Morphine BF	0.187 +/- 0.105	1.001 +/- 0.108	0.518	0.530 +/- 0.070	bad
Neotame AS	0.429 +/- 0.059	1.062 +/- 0.069	0.748	0.632 +/- 0.032	good
Neotame BF	0.424 +/- 0.095	1.046 +/- 0.071	0.782	0.645 +/- 0.029	good
Pantoprazol AS	0.565 +/- 0.026	1.051 +/- 0.106	0.807	0.726 +/- 0.058	good
Pantoprazol BF	0.414 +/- 0.215	0.960 +/- 0.163	0.783	0.661 +/- 0.007	good
Pravastatin AS	0.217 +/- 0.061	0.935 +/- 0.178	0.493	0.496 +/- 0.008	good
Pravastatin BF	0.130 +/- 0.130	1.115 +/- 0.090	0.461	0.493 +/- 0.012	good
Primidone AS	0.294 +/- 0.068	1.155 +/- 0.220	0.766	0.367 +/- 0.029	good
Primidone BF	0.333 +/- 0.041	0.961 +/- 0.264	0.831	0.442 +/- 0.023	good
Torasemid AS	0.484 +/- 0.076	0.990 +/- 0.221	0.793	0.629 +/- 0.025	good
Torasemid BF	0.380 +/- 0.053	1.061 +/- 0.048	0.689	0.651 +/- 0.011	good
Tramadol AS	0.374 +/- 0.063	0.944 +/- 0.092	0.561	0.535 +/- 0.027	medium
Tramadol BF	0.395 +/- 0.026	0.935 +/- 0.053	0.788	0.583 +/- 0.032	medium
Venlafaxin AS	0.367 +/- 0.056	1.011 +/- 0.072	0.683	0.594 +/- 0.026	good
Venlafaxin BF	0.389 +/- 0.054	1.003 +/- 0.075	0.699	0.628 +/- 0.036	good

Table 9: Substances with good relative recoveries: in both matrices between 0.8 and 1.2, evaluated with the ISTD method. AS stands for activated sludge matrix. BF stands for biofilm matrix. The column Tracefinder indicates how well the substance could be evaluated with Tracefinder (good Gaussian peak shape etc.).

	mean absolute recovery +/- stdv	mean relative recovery +/- stdv	extraction efficiency	matrix factor +/- stdv	TraceFinder™	R^2 in linear regression
Amlodipine AS	0.188 +/- 0.035	1.074 +/- 0.161	1.013	0.214 +/- 0.138	good	0.970 +/- 0.036
Amlodipine BF	0.012 +/- 0.013	0.166 +/- 0.249	0	0.030 +/- 0.062	medium	0.970 +/- 0.073
Benzophenone 3 AS	0.088 +/- 0.008	0.885 +/- 0.281	0.748	0.060 +/- 0.110	good	0.978 +/- 0.016
Benzophenone 3 BF	0.051 +/- 0.028	0.382 +/- 0.186	0	0.035 +/- 0.036	low conc bad	0.978 +/- 0.027
Bisacodyl AS	0.015 +/- 0.016	0.154 +/- 0.277	-0.011	0.712 +/- 0.109	good	0.990 +/- 0.013
Bisacodyl BF	0.278 +/- 0.087	1.442 +/- 0.280	0.385	0.602 +/- 0.154	good	0.990 +/- 0.010
Bupropion AS	0.130 +/- 0.017	1.426 +/- 0.252	0.147	0.443 +/- 0.080	good	0.995 +/- 0.006
Bupropion BF	0.033 +/- 0.017	1.362 +/- 0.274	0.077	0.397 +/- 0.164	good	0.995 +/- 0.006
Clomipramine AS	0.171 +/- 0.047	1.253 +/- 0.260	0.763	0.239 +/- 0.143	good	0.962 +/- 0.042
Clomipramine BF	0.006 +/- 0.013	0.528 +/- 0.225	0.427	0.022 +/- 0.010	medium	0.962 +/- 0.052
Cyclophosphamide AS	0.344 +/- 0.045	1.024 +/- 0.067	1.236	0.381 +/- 0.080	good	0.996 +/- 0.007
Cyclophosphamide BF	0.342 +/- 0.189	1.305 +/- 0.129	1.422	0.382 +/- 0.197	good	0.996 +/- 0.001
Darunavir AS	0.323 +/- 0.054	1.032 +/- 0.069	1.023	0.380 +/- 0.035	good	0.999 +/- 0.000
Darunavir BF	0.044 +/- 0.075	0.624 +/- 0.204	0.63	0.348 +/- 0.135	good	0.999 +/- 0.004
Dextromethorphan AS	0.342 +/- 0.102	1.308 +/- 0.318	1.098	0.430 +/- 0.029	good	0.989 +/- 0.013
Dextromethorphan BF	0.111 +/- 0.025	0.886 +/- 0.202	0.869	0.155 +/- 0.060	good	0.989 +/- 0.015
Diclofenac AS	0.136 +/- 0.078	-0.049 +/- 0.273	0.811	0.162 +/- 0.050	medium	0.985 +/- 0.010
Diclofenac BF	0.106 +/- 0.059	0.890 +/- 0.164	1.602	0.230 +/- 0.123	medium	0.985 +/- 0.008
Diosmin AS	0.191 +/- 0.225	0.664 +/- 4.120	1.185	0.248 +/- 0.254	good	0.844 +/- 0.214
Diosmin BF	0.027 +/- 0.058	1.143 +/- 0.324	0.158	0.340 +/- 0.148	good	0.844 +/- 0.054
Elvitegravir AS	0.097 +/- 0.066	1.940 +/- 0.933	0.37	0.200 +/- 0.062	good	0.938 +/- 0.045
Elvitegravir BF	0.078 +/- 0.114	0.576 +/- 0.289	0.216	0.114 +/- 0.109	good	0.938 +/- 0.037
Gliclazide AS	0.427 +/- 0.060	0.969 +/- 0.081	0.956	0.419 +/- 0.131	good	0.999 +/- 0.001
Gliclazide BF	-0.067 +/- 0.125	0.693 +/- 0.130	0.197	0.482 +/- 0.184	good	0.999 +/- 0.002
Levamisole AS	0.360 +/- 0.140	1.380 +/- 0.060	1.063	0.551 +/- 0.133	good	0.997 +/- 0.004
Levamisole BF	0.262 +/- 0.127	1.179 +/- 0.206	0.944	0.408 +/- 0.188	good	0.997 +/- 0.005
Losartan AS	0.545 +/- 0.194	0.965 +/- 0.149	1.027	0.571 +/- 0.128	good	0.996 +/- 0.002
Losartan BF	0.100 +/- 0.049	0.636 +/- 0.224	0.439	0.476 +/- 0.140	good	0.996 +/- 0.006
Metoclopramide AS	0.375 +/- 0.088	1.253 +/- 0.107	0.912	0.505 +/- 0.173	good	0.996 +/- 0.005
Metoclopramide BF	0.266 +/- 0.130	1.420 +/- 0.156	0.884	0.398 +/- 0.187	good	0.996 +/- 0.007
Metoxuron AS	0.368 +/- 0.071	1.079 +/- 0.165	1.089	0.359 +/- 0.080	double peak	0.997 +/- 0.004
Metoxuron BF	0.358 +/- 0.139	1.357 +/- 0.178	1.457	0.421 +/- 0.194	double peak	0.997 +/- 0.006
Naloxone AS	0.591 +/- 0.077	1.342 +/- 0.197	1.043	0.528 +/- 0.210	good	0.994 +/- 0.006
Naloxone BF	0.521 +/- 0.085	1.183 +/- 0.118	1.047	0.508 +/- 0.267	good	0.994 +/- 0.007
Pseudoephedrine AS	0.311 +/- 0.145	1.357 +/- 0.122	1.031	0.508 +/- 0.152	good	0.996 +/- 0.003
Pseudoephedrine BF	0.265 +/- 0.058	1.020 +/- 0.093	0.968	0.398 +/- 0.148	good	0.996 +/- 0.004
Sertraline AS	0.077 +/- 0.035	0.264 +/- 0.873	1.359	0.109 +/- 0.087	medium	0.846 +/- 0.142
Sertraline BF	0.001 +/- 0.013	0.184 +/- 0.307	0	0.017 +/- 0.009	low conc bad	0.846 +/- 0.050
Tramadol AS	0.346 +/- 0.069	1.409 +/- 0.152	1.308	0.363 +/- 0.110	medium	0.994 +/- 0.005
Tramadol BF	0.354 +/- 0.067	1.120 +/- 0.110	1.035	0.411 +/- 0.130	medium	0.994 +/- 0.003

	mean absolute recovery +/- stdv	mean relative recovery +/- stdv	extraction efficiency	matrix factor +/- stdv	TraceFinder™	R^2 in linear regression
Trazodone AS	0.437 +/- 0.074	1.337 +/- 0.200	1.104	0.475 +/- 0.139	good	0.996 +/- 0.003
Trazodone BF	0.333 +/- 0.062	1.239 +/- 0.175	0.613	0.145 +/- 0.348	good	0.996 +/- 0.032

Table 10: Substances with insufficient relative recoveries: in at least one matrix (activated sludge AS or biofilm BF) relative recovery <0.7 or >1.3 which is indicated in red. Evaluated with the standard addition method (stda). R^2 evaluates the goodness of the linear regression fit with a value closer to 1 indicating a better fit. The column Tracefinder indicates how well the substance could be evaluated with Tracefinder (good Gaussian peak shape etc.).

	mean absolute recovery +/- stdv	mean relative recovery +/- stdv	extraction efficiency	matrix factor +/- stdv	TraceFinder™	R^2 in linear regression
Acemetacin AS	0.194 +/- 0.033	0.894 +/- 0.221	1.668	0.264 +/- 0.039	good	0.990 +/- 0.007
Acemetacin BF	0.172 +/- 0.030	0.741 +/- 0.170	1.047	0.219 +/- 0.067	good	0.990 +/- 0.006
Amisulpride AS	0.411 +/- 0.078	1.119 +/- 0.141	1.205	0.458 +/- 0.156	good	0.994 +/- 0.005
Amisulpride BF	0.424 +/- 0.078	1.244 +/- 0.183	1.143	0.396 +/- 0.262	good	0.994 +/- 0.006
Apixaban AS	0.430 +/- 0.074	1.082 +/- 0.160	1.168	0.428 +/- 0.068	good	0.994 +/- 0.009
Apixaban BF	0.471 +/- 0.113	1.232 +/- 0.146	1.416	0.558 +/- 0.163	good	0.994 +/- 0.002
Apremilast AS	0.613 +/- 0.344	1.249 +/- 0.015	1.211	0.786 +/- 0.171	good	0.997 +/- 0.004
Apremilast BF	0.709 +/- 0.111	1.282 +/- 0.245	1.242	0.689 +/- 0.273	good	0.997 +/- 0.007
Diltiazem AS	0.439 +/- 0.073	1.210 +/- 0.178	1.153	0.499 +/- 0.091	good	0.995 +/- 0.004
Diltiazem BF	0.218 +/- 0.055	0.935 +/- 0.205	0.692	0.144 +/- 0.181	good	0.995 +/- 0.020
Emtricitabine AS	0.359 +/- 0.055	0.795 +/- 0.094	0.686	0.692 +/- 0.058	good	0.996 +/- 0.003
Emtricitabine BF	0.258 +/- 0.138	0.856 +/- 0.059	0.638	0.517 +/- 0.270	good	0.996 +/- 0.003
Enzalutamide AS	0.261 +/- 0.031	1.238 +/- 0.148	1.212	0.207 +/- 0.143	good	0.993 +/- 0.005
Enzalutamide BF	0.241 +/- 0.040	0.870 +/- 0.194	0.914	0.188 +/- 0.160	good	0.993 +/- 0.028
Febuxostat AS	0.269 +/- 0.022	1.073 +/- 0.159	1.744	0.344 +/- 0.190	good	0.990 +/- 0.005
Febuxostat BF	0.193 +/- 0.048	0.717 +/- 0.146	1.224	0.254 +/- 0.047	good	0.990 +/- 0.005
Icaridin AS	0.317 +/- 0.073	1.214 +/- 0.122	1.097	0.414 +/- 0.080	good	0.995 +/- 0.004
Icaridin BF	0.303 +/- 0.044	0.949 +/- 0.098	1.095	0.336 +/- 0.159	good	0.995 +/- 0.008
Isoproturon AS	0.310 +/- 0.093	1.189 +/- 0.116	1.167	0.345 +/- 0.102	good	0.996 +/- 0.002
Isoproturon BF	0.310 +/- 0.081	1.214 +/- 0.250	2.534	0.344 +/- 0.078	good	0.996 +/- 0.101

	mean absolute recovery +/- stdv	mean relative recovery +/- stdv	extraction efficiency	matrix factor +/- stdv	TraceFinder™	R^2 in linear regression
Mirabegron AS	0.586 +/- 0.174	0.921 +/- 0.075	0.898	0.715 +/- 0.153	good	0.996 +/- 0.004
Mirabegron BF	0.235 +/- 0.045	0.791 +/- 0.138	0.53	0.388 +/- 0.243	good	0.996 +/- 0.004
Pravastatin AS	0.216 +/- 0.061	0.771 +/- 0.154	0.652	0.410 +/- 0.049	good	0.990 +/- 0.010
Pravastatin BF	0.157 +/- 0.123	1.286 +/- 0.200	0.606	0.428 +/- 0.231	good	0.990 +/- 0.009
Primidone AS	0.304 +/- 0.064	1.246 +/- 0.137	1.237	0.332 +/- 0.082	good	0.993 +/- 0.010
Primidone BF	0.361 +/- 0.042	1.080 +/- 0.127	1.304	0.409 +/- 0.154	good	0.993 +/- 0.014
Propranolol AS	0.414 +/- 0.067	1.257 +/- 0.106	0.99	0.501 +/- 0.083	good	0.996 +/- 0.003
Propranolol BF	0.241 +/- 0.056	1.074 +/- 0.152	0.836	0.198 +/- 0.117	good	0.996 +/- 0.012
Tolperison AS	0.385 +/- 0.015	1.216 +/- 0.066	0.8	0.573 +/- 0.093	good	0.993 +/- 0.007
Tolperison BF	0.144 +/- 0.033	1.253 +/- 0.162	0.573	0.258 +/- 0.058	good	0.993 +/- 0.006
Trimipramine AS	0.181 +/- 0.044	1.230 +/- 0.206	0.555	0.290 +/- 0.136	good	0.986 +/- 0.013
Trimipramine BF	0.012 +/- 0.004	0.856 +/- 0.195	0.43	0.055 +/- 0.033	good	0.986 +/- 0.034
Venlafaxine AS	0.383 +/- 0.058	1.206 +/- 0.145	1.126	0.500 +/- 0.014	good	0.996 +/- 0.002
Venlafaxine BF	0.391 +/- 0.061	1.238 +/- 0.109	0.976	0.436 +/- 0.293	good	0.996 +/- 0.017

Table 11: Substances with medium relative recovery: in at least one matrix (activated sludge AS or biofilm BF) relative recovery in range [0.7, 0.8] or [1.2, 1.3], evaluation with the standard addition (stda) method. Relative recovery in the other matrix is either better or also in one of these two ranges. R^2 evaluates the goodness of the linear regression fit with a value closer to 1 indicating a better fit. The column Tracefinder indicates how well the substance could be evaluated with Tracefinder (good Gaussian peak shape etc.).

	mean absolute recovery +/- stdv	mean relative recovery +/- stdv	extraction efficiency	matrix factor +/- stdv	TraceFinder™	R^2 in linear regression
Abacavir AS	0.456 +/- 0.052	1.174 +/- 0.158	1.111	0.400 +/- 0.211	good	0.996 +/- 0.003
Abacavir BF	0.162 +/- 0.208	1.118 +/- 0.189	1.096	0.319 +/- 0.348	good	0.996 +/- 0.004
Albuterol AS	0.362 +/- 0.021	1.161 +/- 0.154	0.979	0.489 +/- 0.122	medium	0.993 +/- 0.007
Albuterol BF	0.340 +/- 0.094	1.124 +/- 0.100	0.84	0.399 +/- 0.320	medium	0.993 +/- 0.008

	mean absolute recovery +/- stdv	mean relative recovery +/- stdv	extraction efficiency	matrix factor +/- stdv	TraceFinder™	R^2 in linear regression
Chlorotoluron AS	0.329 +/- 0.023	0.990 +/- 0.024	1.19	0.444 +/- 0.131	good	0.997 +/- 0.005
Chlorotoluron BF	0.302 +/- 0.039	1.079 +/- 0.178	1.216	0.284 +/- 0.169	good	0.997 +/- 0.004
Codeine AS	0.460 +/- 0.064	1.127 +/- 0.162	0.942	0.402 +/- 0.204	good	0.993 +/- 0.007
Codeine BF	0.324 +/- 0.110	1.128 +/- 0.099	0.942	0.437 +/- 0.232	good	0.993 +/- 0.010
Dexlansoprazole AS	0.524 +/- 0.076	1.091 +/- 0.102	0.933	0.480 +/- 0.208	good	0.997 +/- 0.004
Dexlansoprazole BF	0.381 +/- 0.046	0.925 +/- 0.032	0.709	0.235 +/- 0.312	good	0.997 +/- 0.009
Edoxaban AS	0.507 +/- 0.033	1.102 +/- 0.112	1.167	0.510 +/- 0.063	good	0.998 +/- 0.002
Edoxaban BF	0.442 +/- 0.063	1.069 +/- 0.039	0.821	0.346 +/- 0.367	good	0.998 +/- 0.014
Etodolac AS	0.656 +/- 0.095	0.971 +/- 0.171	1.014	0.737 +/- 0.195	good	0.993 +/- 0.005
Etodolac BF	0.595 +/- 0.125	1.007 +/- 0.102	0.983	0.557 +/- 0.631	good	0.993 +/- 0.012
Entacapone AS	0.114 +/- 0.028	0.992 +/- 0.236	-0.93	0.792 +/- 0.719	good	0.993 +/- 0.005
Entacapone BF	0.053 +/- 0.009	0.895 +/- 0.101	0.029	0.149 +/- 0.811	good	0.993 +/- 0.010
Fenfluramine AS	0.374 +/- 0.092	1.177 +/- 0.127	1.091	0.453 +/- 0.096	good	0.996 +/- 0.005
Fenfluramine BF	0.290 +/- 0.041	1.057 +/- 0.079	0.914	0.294 +/- 0.111	good	0.996 +/- 0.004
Ifosfamid AS	0.354 +/- 0.042	1.138 +/- 0.141	1.451	0.397 +/- 0.084	good	0.994 +/- 0.004
Ifosfamid BF	0.410 +/- 0.051	1.196 +/- 0.160	1.27	0.345 +/- 0.202	good	0.994 +/- 0.007
Ketamine AS	0.420 +/- 0.052	0.988 +/- 0.088	0.986	0.497 +/- 0.057	good	0.995 +/- 0.005
Ketamine BF	0.405 +/- 0.107	0.980 +/- 0.189	1.025	0.439 +/- 0.178	good	0.995 +/- 0.009
Mexiletine AS	0.379 +/- 0.058	1.122 +/- 0.123	1.277	0.492 +/- 0.049	good	0.996 +/- 0.005
Mexiletine BF	0.292 +/- 0.063	0.875 +/- 0.175	0.787	0.376 +/- 0.137	good	0.996 +/- 0.005
Moclobemid AS	0.485 +/- 0.048	1.138 +/- 0.060	1.092	0.538 +/- 0.089	good	0.996 +/- 0.004
Moclobemid BF	0.407 +/- 0.091	1.115 +/- 0.145	0.843	0.349 +/- 0.259	good	0.996 +/- 0.012
Monuron AS	0.343 +/- 0.079	1.070 +/- 0.147	1.076	0.313 +/- 0.116	good	0.996 +/- 0.004
Monuron BF	0.336 +/- 0.057	1.139 +/- 0.128	1.049	0.353 +/- 0.124	good	0.996 +/- 0.006
Morphine AS	0.288 +/- 0.076	1.148 +/- 0.282	0.677	0.353 +/- 0.202	bad	0.986 +/- 0.005
Morphine BF	0.175 +/- 0.110	1.144 +/- 0.141	0.767	0.347 +/- 0.208	bad	0.986 +/- 0.005
Mycophenolic acid AS	0.203 +/- 0.025	1.039 +/- 0.076	0.793	0.312 +/- 0.055	good	0.993 +/- 0.011
Mycophenolic acid BF	0.165 +/- 0.052	1.191 +/- 0.225	0.706	0.283 +/- 0.157	good	0.993 +/- 0.006
Neohesperidin dc AS	0.360 +/- 0.096	1.060 +/- 0.106	0.734	0.487 +/- 0.121	good	0.994 +/- 0.007
Neohesperidin dc BF	0.227 +/- 0.053	1.000 +/- 0.170	0.67	0.445 +/- 0.276	good	0.994 +/- 0.007
Neotame AS	0.428 +/- 0.058	1.015 +/- 0.158	1.06	0.546 +/- 0.116	good	0.993 +/- 0.010
Neotame BF	0.397 +/- 0.105	1.107 +/- 0.082	1.026	0.499 +/- 0.246	good	0.993 +/- 0.006
Olmesartan AS	0.535 +/- 0.191	0.961 +/- 0.197	0.897	0.624 +/- 0.126	good	0.995 +/- 0.005
Olmesartan BF	0.250 +/- 0.051	0.921 +/- 0.117	0.637	0.527 +/- 0.308	good	0.995 +/- 0.006
Pantoprazol AS	0.550 +/- 0.025	1.097 +/- 0.120	1.003	0.568 +/- 0.155	good	0.996 +/- 0.003
Pantoprazol BF	0.379 +/- 0.197	1.130 +/- 0.061	1.202	0.504 +/- 0.125	good	0.996 +/- 0.004
Pirfenidone AS	0.451 +/- 0.118	1.054 +/- 0.118	1.022	0.415 +/- 0.130	good	0.997 +/- 0.002
Pirfenidone BF	0.427 +/- 0.042	1.063 +/- 0.155	1.292	0.450 +/- 0.171	good	0.997 +/- 0.005
Rivaroxaban AS	0.364 +/- 0.018	1.068 +/- 0.165	1.305	0.390 +/- 0.032	good	0.994 +/- 0.009
Rivaroxaban BF	0.374 +/- 0.066	1.047 +/- 0.106	1.298	0.420 +/- 0.128	good	0.994 +/- 0.004
Sacubitril AS	0.352 +/- 0.051	1.047 +/- 0.104	0.921	0.507 +/- 0.165	good	0.993 +/- 0.006
Sacubitril BF	0.331 +/- 0.052	1.045 +/- 0.114	1.423	0.435 +/- 0.285	good	0.993 +/- 0.018
Sulfasalazine AS	0.255 +/- 0.044	0.958 +/- 0.126	0.984	0.369 +/- 0.053	good	0.997 +/- 0.002
Sulfasalazine BF	0.216 +/- 0.026	0.805 +/- 0.141	0.888	0.346 +/- 0.094	good	0.997 +/- 0.006

	mean absolute recovery +/- stdv	mean relative recovery +/- stdv	extraction efficiency	matrix factor +/- stdv	TraceFinder™	R^2 in linear regression
Torasemid AS	0.471 +/- 0.072	1.080 +/- 0.025	1.267	0.578 +/- 0.051	good	0.997 +/- 0.002
Torasemid BF	0.329 +/- 0.115	1.030 +/- 0.066	0.922	0.451 +/- 0.234	good	0.997 +/- 0.007
Valaciclovir AS	0.050 +/- 0.014	0.814 +/- 0.215	0.152	0.456 +/- 0.225	good	0.971 +/- 0.027
Valaciclovir BF	-0.008 +/- 0.076	0.927 +/- 0.125	0.212	0.437 +/- 0.259	good	0.971 +/- 0.023

Table 12: Substances with good relative recovery in range 0.8 to 1.2 in both matrices (AS for activated sludge and BF for biofilm), evaluated with the standard addition (stda) method. R^2 evaluates the goodness of the linear regression fit with a value closer to 1 indicating a better fit. The column Tracefinder indicates how well the substance could be evaluated with Tracefinder (good Gaussian peak shape etc.).

substance	mean absolute recovery +/- stdv	mean relative recovery +/- stdv	extraction efficiency	matrix factor +/- stdv	R^2 in linear regression
Albuterol AS ISTD	0.372 +/- 0.023	0.982 +/- 0.040	0.534	0.618 +/- 0.048	
Albuterol AS STDA	0.362 +/- 0.021	1.161 +/- 0.154	0.979	0.489 +/- 0.122	0.993 +/- 0.007
Albuterol BF ISTD	0.362 +/- 0.098	1.025 +/- 0.076	0.649	0.661 +/- 0.070	
Albuterol BF STDA	0.340 +/- 0.094	1.124 +/- 0.100	0.84	0.399 +/- 0.320	0.993 +/- 0.008
Amisulprid AS ISTD	0.430 +/- 0.081	0.940 +/- 0.062	0.774	0.639 +/- 0.070	
Amisulprid AS STDA	0.411 +/- 0.078	1.119 +/- 0.141	1.205	0.458 +/- 0.156	0.994 +/- 0.005
Amisulprid BF ISTD	0.471 +/- 0.048	1.025 +/- 0.054	0.749	0.635 +/- 0.055	
Amisulprid BF STDA	0.424 +/- 0.078	1.244 +/- 0.183	1.143	0.396 +/- 0.262	0.994 +/- 0.006
Chlorotoluron AS ISTD	0.354 +/- 0.025	0.999 +/- 0.105	0.814	0.474 +/- 0.011	
Chlorotoluron AS STDA	0.329 +/- 0.023	0.990 +/- 0.024	1.19	0.444 +/- 0.131	0.997 +/- 0.005
Chlorotoluron BF ISTD	0.333 +/- 0.036	0.957 +/- 0.079	0.813	0.436 +/- 0.025	
Chlorotoluron BF STDA	0.302 +/- 0.039	1.079 +/- 0.178	1.216	0.284 +/- 0.169	0.997 +/- 0.004
Codein AS ISTD	0.461 +/- 0.064	1.082 +/- 0.203	0.67	0.612 +/- 0.069	
Codein AS STDA	0.460 +/- 0.064	1.127 +/- 0.162	0.942	0.402 +/- 0.204	0.993 +/- 0.007
Codein BF ISTD	0.342 +/- 0.113	0.945 +/- 0.134	0.683	0.624 +/- 0.067	
Codein BF STDA	0.324 +/- 0.110	1.128 +/- 0.099	0.942	0.437 +/- 0.232	0.993 +/- 0.010
Cyclophosphamid AS ISTD	0.350 +/- 0.047	0.968 +/- 0.090	1.057	0.503 +/- 0.034	
Cyclophosphamid AS STDA	0.344 +/- 0.045	1.024 +/- 0.067	1.236	0.381 +/- 0.080	0.996 +/- 0.007
Cyclophosphamid BF ISTD	0.422 +/- 0.057	1.010 +/- 0.153	0.847	0.564 +/- 0.022	

substance	mean absolute recovery +/- stdv	mean relative recovery +/- stdv	extraction efficiency	matrix factor +/- stdv	R^2 in linear regression
Cyclophosphamid BF STDA	0.342 +/- 0.189	1.305 +/- 0.129	1.422	0.382 +/- 0.197	0.996 +/- 0.001
Darunavir AS ISTD	0.307 +/- 0.052	0.985 +/- 0.169	0.811	0.429 +/- 0.022	
Darunavir AS STDA	0.323 +/- 0.054	1.032 +/- 0.069	1.023	0.380 +/- 0.035	0.999 +/- 0.000
Darunavir BF ISTD	0.046 +/- 0.079	0.871 +/- 0.222	0.291	0.452 +/- 0.040	
Darunavir BF STDA	0.044 +/- 0.075	0.624 +/- 0.204	0.63	0.348 +/- 0.135	0.999 +/- 0.004
Diclofenac AS ISTD	0.140 +/- 0.080	0.881 +/- 0.243	-1.107	0.218 +/- 0.019	
Diclofenac AS STDA	0.136 +/- 0.078	-0.049 +/- 0.273	0.811	0.162 +/- 0.050	0.985 +/- 0.010
Diclofenac BF ISTD	0.137 +/- 0.039	0.955 +/- 0.265	0.852	0.210 +/- 0.024	
Diclofenac BF STDA	0.106 +/- 0.059	0.890 +/- 0.164	1.602	0.230 +/- 0.123	0.985 +/- 0.008
Emtricitabine AS ISTD	0.371 +/- 0.054	1.054 +/- 0.136	0.542	0.737 +/- 0.030	
Emtricitabine AS STDA	0.359 +/- 0.055	0.795 +/- 0.094	0.686	0.692 +/- 0.058	0.996 +/- 0.003
Emtricitabine BF ISTD	0.260 +/- 0.157	1.043 +/- 0.042	0.519	0.751 +/- 0.025	
Emtricitabine BF STDA	0.258 +/- 0.138	0.856 +/- 0.059	0.638	0.517 +/- 0.270	0.996 +/- 0.003
Etodolac AS ISTD	0.644 +/- 0.093	0.999 +/- 0.116	0.736	0.822 +/- 0.034	
Etodolac AS STDA	0.656 +/- 0.095	0.971 +/- 0.171	1.014	0.737 +/- 0.195	0.993 +/- 0.005
Etodolac BF ISTD	0.626 +/- 0.075	1.076 +/- 0.035	0.779	0.898 +/- 0.049	
Etodolac BF STDA	0.595 +/- 0.125	1.007 +/- 0.102	0.983	0.557 +/- 0.631	0.993 +/- 0.012
Isoproturon AS ISTD	0.288 +/- 0.089	0.980 +/- 0.182	0.955	0.581 +/- 0.022	
Isoproturon AS STDA	0.310 +/- 0.093	1.189 +/- 0.116	1.167	0.345 +/- 0.102	0.996 +/- 0.002
Isoproturon BF ISTD	0.313 +/- 0.090	1.143 +/- 0.065	0.901	0.505 +/- 0.018	
Isoproturon BF STDA	0.310 +/- 0.081	1.214 +/- 0.250	2.534	0.344 +/- 0.078	0.996 +/- 0.101
Ketamin AS ISTD	0.405 +/- 0.048	0.905 +/- 0.039	0.792	0.561 +/- 0.036	
Ketamin AS STDA	0.420 +/- 0.052	0.988 +/- 0.088	0.986	0.497 +/- 0.057	0.995 +/- 0.005
Ketamin BF ISTD	0.409 +/- 0.106	0.965 +/- 0.114	0.755	0.553 +/- 0.037	
Ketamin BF STDA	0.405 +/- 0.107	0.980 +/- 0.189	1.025	0.439 +/- 0.178	0.995 +/- 0.009
Losartan AS ISTD	0.551 +/- 0.196	1.011 +/- 0.068	0.681	0.629 +/- 0.020	
Losartan AS STDA	0.545 +/- 0.194	0.965 +/- 0.149	1.027	0.571 +/- 0.128	0.996 +/- 0.002
Losartan BF ISTD	0.111 +/- 0.048	0.820 +/- 0.251	0.277	0.603 +/- 0.023	
Losartan BF STDA	0.100 +/- 0.049	0.636 +/- 0.224	0.439	0.476 +/- 0.140	0.996 +/- 0.006
Metoxuron AS ISTD	0.094 +/- 0.306	0.273 +/- 0.916	0.897	0.439 +/- 0.029	
Metoxuron AS STDA	0.368 +/- 0.071	1.079 +/- 0.165	1.089	0.359 +/- 0.080	0.997 +/- 0.004
Metoxuron BF ISTD	0.050 +/- 0.298	0.161 +/- 0.762	0.892	0.481 +/- 0.023	
Metoxuron BF STDA	0.358 +/- 0.139	1.357 +/- 0.178	1.457	0.421 +/- 0.194	0.997 +/- 0.006
Morphin AS ISTD	0.278 +/- 0.073	1.159 +/- 0.085	0.535	0.513 +/- 0.061	
Morphin AS STDA	0.288 +/- 0.076	1.148 +/- 0.282	0.677	0.353 +/- 0.202	0.986 +/- 0.005
Morphin BF ISTD	0.187 +/- 0.105	1.001 +/- 0.108	0.518	0.530 +/- 0.070	
Morphin BF STDA	0.175 +/- 0.110	1.144 +/- 0.141	0.767	0.347 +/- 0.208	0.986 +/- 0.005
Neotam AS ISTD	0.429 +/- 0.059	1.062 +/- 0.069	0.748	0.632 +/- 0.032	
Neotam AS STDA	0.428 +/- 0.058	1.015 +/- 0.158	1.06	0.546 +/- 0.116	0.993 +/- 0.010
Neotam BF ISTD	0.424 +/- 0.095	1.046 +/- 0.071	0.782	0.645 +/- 0.029	
Neotam BF STDA	0.397 +/- 0.105	1.107 +/- 0.082	1.026	0.499 +/- 0.246	0.993 +/- 0.006
Pantoprazol AS ISTD	0.565 +/- 0.026	1.051 +/- 0.106	0.807	0.726 +/- 0.058	
Pantoprazol AS STDA	0.550 +/- 0.025	1.097 +/- 0.120	1.003	0.568 +/- 0.155	0.996 +/- 0.003
Pantoprazol BF ISTD	0.414 +/- 0.215	0.960 +/- 0.163	0.783	0.661 +/- 0.007	

substance	mean absolute recovery +/- stdv	mean relative recovery +/- stdv	extraction efficiency	matrix factor +/- stdv	R^2 in linear regression
Pantoprazol BF STDA	0.379 +/- 0.197	1.130 +/- 0.061	1.202	0.504 +/- 0.125	0.996 +/- 0.004
Pravastatin AS ISTD	0.217 +/- 0.061	0.935 +/- 0.178	0.493	0.496 +/- 0.008	
Pravastatin AS STDA	0.216 +/- 0.061	0.771 +/- 0.154	0.652	0.410 +/- 0.049	0.990 +/- 0.010
Pravastatin BF ISTD	0.130 +/- 0.130	1.115 +/- 0.090	0.461	0.493 +/- 0.012	
Pravastatin BF STDA	0.157 +/- 0.123	1.286 +/- 0.200	0.606	0.428 +/- 0.231	0.990 +/- 0.009
Primidon AS ISTD	0.294 +/- 0.068	1.155 +/- 0.220	0.766	0.367 +/- 0.029	
Primidon AS STDA	0.304 +/- 0.064	1.246 +/- 0.137	1.237	0.332 +/- 0.082	0.993 +/- 0.010
Primidon BF ISTD	0.333 +/- 0.041	0.961 +/- 0.264	0.831	0.442 +/- 0.023	
Primidon BF STDA	0.361 +/- 0.042	1.080 +/- 0.127	1.304	0.409 +/- 0.154	0.993 +/- 0.014
Torasemid AS ISTD	0.484 +/- 0.076	0.990 +/- 0.221	0.793	0.629 +/- 0.025	
Torasemid AS STDA	0.471 +/- 0.072	1.080 +/- 0.025	1.267	0.578 +/- 0.051	0.997 +/- 0.002
Torasemid BF ISTD	0.380 +/- 0.053	1.061 +/- 0.048	0.689	0.651 +/- 0.011	
Torasemid BF STDA	0.329 +/- 0.115	1.030 +/- 0.066	0.922	0.451 +/- 0.234	0.997 +/- 0.007
Tramadol AS ISTD	0.374 +/- 0.063	0.944 +/- 0.092	0.561	0.535 +/- 0.027	
Tramadol AS STDA	0.346 +/- 0.069	1.409 +/- 0.152	1.308	0.363 +/- 0.110	0.994 +/- 0.005
Tramadol BF ISTD	0.395 +/- 0.026	0.935 +/- 0.053	0.788	0.583 +/- 0.032	
Tramadol BF STDA	0.354 +/- 0.067	1.120 +/- 0.110	1.035	0.411 +/- 0.130	0.994 +/- 0.003
Trimipramin AS ISTD	0.177 +/- 0.039	0.111 +/- 0.008	0.388	0.444 +/- 0.071	
Trimipramin AS STDA	0.181 +/- 0.044	1.230 +/- 0.206	0.555	0.290 +/- 0.136	0.986 +/- 0.013
Trimipramin BF ISTD	0.016 +/- 0.011	0.170 +/- 0.121	0.088	0.048 +/- 0.013	
Trimipramin BF STDA	0.012 +/- 0.004	0.856 +/- 0.195	0.43	0.055 +/- 0.033	0.986 +/- 0.034
Venlafaxin AS ISTD	0.367 +/- 0.056	1.011 +/- 0.072	0.683	0.594 +/- 0.026	
Venlafaxin AS STDA	0.383 +/- 0.058	1.206 +/- 0.145	1.126	0.500 +/- 0.014	0.996 +/- 0.002
Venlafaxin BF ISTD	0.389 +/- 0.054	1.003 +/- 0.075	0.699	0.628 +/- 0.036	
Venlafaxin BF STDA	0.391 +/- 0.061	1.238 +/- 0.109	0.976	0.436 +/- 0.293	0.996 +/- 0.017

Table 13: Comparison of recoveries obtained with the ISTD (internal standard) method and obtained with the STDA (standard addition) method for 22 compounds. AS stands for activated sludge matrix, BF stands for biofilm matrix.

The negative relative recovery of diclofenac in activated sludge when applying the standard addition method is striking. Further analysis shows that the background concentration of diclofenac in activated sludge from ARA Neugut was already very high (about 13.5 nmol) compared to the spiked amount in scheme 3 (4 nmol). This could explain the incapability of the standard addition method (standard additions between 0 and 20 nmol) to reach a sufficient relative recovery.

B.2 LOQ

Compound	LOQ calibration [nM]	LOQ sample [nM]	LOQ sample per biomass [nmol / g biomass]
Abacavir_AS	0.05	0.11	0.0055
Abacavir_BF	0.05	0.31	0.0154
Acemetacin_AS	0.25	1.29	0.0644
Acemetacin_BF	0.25	1.45	0.0727
Albuterol_AS	0.05	0.13	0.0067
Albuterol_BF	0.05	0.14	0.0069
Amisulpride_AS	0.1	0.24	0.0120
Amisulpride_BF	0.1	0.23	0.0117
Amlodipine_AS	0.25	1.33	0.0665
Amlodipine_BF	0.25	20.83	1.0417
Apixaban_AS	0.25	0.58	0.0291
Apixaban_BF	0.25	0.53	0.0265
Apremilast_AS	1	1.63	0.0816
Apremilast_BF	1	1.41	0.0705
Benzophenone 3_AS	0.05	0.57	0.0284
Benzophenone 3_BF	0.05	0.98	0.0490
Bisacodyl_AS	0.05	3.33	0.1667
Bisacodyl_BF	0.05	0.18	0.0089
Bupropion_AS	0.1	0.77	0.0385
Bupropion_BF	0.1	3.03	0.1515
Chlorotoluron_AS	0.05	1.00	0.0500
Chlorotoluron_BF	0.05	1.00	0.0500
Clomipramine_AS	0.05	0.29	0.0146
Clomipramine_BF	0.05	8.33	0.4167
Codeine_AS	0.01	0.02	0.0011
Codeine_BF	0.01	0.03	0.0015
Cyclophosphamid_AS	0.05	0.14	0.0071
Cyclophosphamid_BF	0.05	0.12	0.0059
Darunavir_AS	0.01	0.03	0.0016
Darunavir_BF	0.01	0.23	0.0114
Dexlansoprazole_AS	0.25	0.48	0.0239
Dexlansoprazole_BF	0.25	0.66	0.0328
Dextromethorphan_AS	0.05	0.15	0.0073
Dextromethorphan_BF	0.05	0.45	0.0225
Diclofenac_AS	0.25	1.79	0.0893
Diclofenac_BF	0.25	1.82	0.0912
Diltiazem_AS	0.05	0.12	0.0059
Diltiazem_BF	0.05	0.25	0.0126
Diosmin_AS	1	5.24	0.2618
Diosmin_BF	1	37.04	1.8519
Edoxaban_As	0.25	0.49	0.0247
Edoxaban_BF	0.25	0.57	0.0283
Elvitegravir_AS	0.1	1.03	0.0515
Elvitegravir_BF	0.1	1.28	0.0641
Emtricitabine_AS	0.5	1.35	0.0674

Compound	LOQ calibration [nM]	LOQ sample [nM]	LOQ sample per biomass [nmol / g biomass]
Emtricitabine_BF	0.5	1.92	0.0962
Entacapone_AS	0.05	0.44	0.0219
Entacapone_BF	0.05	0.94	0.0472
Enzalutamide_AS	0.25	0.96	0.0479
Enzalutamide_BF	0.25	1.04	0.0519
Etodolac_AS	0.05	0.08	0.0039
Etodolac_BF	0.05	0.08	0.0039
Febuxostat_AS	0.05	0.08	0.0040
Febuxostat_BF	0.05	0.26	0.0130
Fenfluramine_AS	0.01	0.03	0.0013
Fenfluramine_BF	0.01	0.03	0.0017
Gliclazide_AS	0.05	0.12	0.0059
Gliclazide_BF	0.05	n.a.	n.a.
Icaridin_AS	0.05	0.16	0.0079
Icaridin_BF	0.05	0.17	0.0083
Ifosfamid_AS	0.25	0.71	0.0353
Ifosfamid_BF	0.25	0.61	0.0305
Isoproturon_AS	0.05	0.17	0.0087
Isoproturon_BF	0.05	0.16	0.0080
Ketamin_AS	0.1	0.25	0.0123
Ketamin_BF	0.1	0.24	0.0122
Levamisole_AS	0.05	0.15	0.0076
Levamisole_BF	0.05	0.19	0.0095
Lisinopril_AS	0.5	n.a.	n.a.
Lisinopril_BF	0.5	n.a.	n.a.
Losartan_AS	0.1	0.18	0.0091
Losartan_BF	0.1	0.90	0.0450
Metoclopramide_AS	0.1	0.27	0.0136
Metoclopramide_BF	0.1	0.33	0.0167
Metoxuron_AS	0.1	1.06	0.0532
Metoxuron_BF	0.1	2.00	0.1000
Mexiletine_AS	0.05	0.13	0.0066
Mexiletine_BF	0.05	0.17	0.0086
Mirabegron_AS	1	1.71	0.0853
Mirabegron_BF	1	4.26	0.2128
Moclobemid_AS	0.05	0.10	0.0052
Moclobemid_BF	0.05	0.12	0.0061
Monuron_AS	0.05	0.14	0.0068
Monuron_BF	0.05	0.14	0.0070
Morphin_AS	0.1	0.36	0.0180
Morphin_BF	0.1	0.53	0.0267
Mycophenolsaeure_AS	0.1	0.49	0.0246
Mycophenolsaeure_BF	0.1	0.61	0.0303
Naloxone_AS	0.1	0.17	0.0085
Naloxone_BF	0.1	0.19	0.0096
Neohesperidin dihydrochalcon_AS	0.5	1.50	0.0749
Neohesperidin dihydrochalcon_BF	0.5	2.44	0.1220
Neotame_AS	0.05	0.12	0.0058

Compound	LOQ calibration [nM]	LOQ sample [nM]	LOQ sample per biomass [nmol / g biomass]
Neotame_BF	0.05	0.12	0.0059
Olmesartan_AS	0.1	0.19	0.0093
Olmesartan_BF	0.1	0.40	0.0200
Pantoprazol_AS	0.05	0.09	0.0044
Pantoprazol_BF	0.05	0.12	0.0060
Pirfenidone_AS	0.05	0.11	0.0055
Pirfenidone_BF	0.05	0.12	0.0059
Pravastatin_AS	0.05	0.23	0.0115
Pravastatin_BF	0.05	0.38	0.0192
Primidone_AS	0.5	1.70	0.0850
Primidone_BF	0.5	1.50	0.0751
Propranolol_AS	0.05	0.13	0.0063
Propranolol_BF	0.05	0.23	0.0113
Pseudoephedrine_AS	0.1	0.32	0.0161
Pseudoephedrine_BF	0.1	0.38	0.0189
Rivaroxaban_AS	0.5	1.37	0.0687
Rivaroxaban_BF	0.5	1.34	0.0668
Sacubitril_AS	0.05	0.14	0.0071
Sacubitril_BF	0.05	0.15	0.0076
Sertraline_AS	0.05	0.65	0.0325
Sertraline_BF	0.05	50.00	2.5000
Sulfasalazine_AS	0.05	0.20	0.0098
Sulfasalazine_BF	0.05	0.23	0.0116
Tolperison_AS	1	2.60	0.1299
Tolperison_BF	1	6.94	0.3472
Torasemid_AS	0.05	0.11	0.0053
Torasemid_BF	0.05	0.15	0.0075
Tramadol_AS	0.1	0.27	0.0134
Tramadol_BF	0.1	0.25	0.0127
Trazodone_AS	0.1	0.23	0.0114
Trazodone_BF	0.1	0.30	0.0150
Trimipramin_AS	0.05	0.28	0.0141
Trimipramin_BF	0.05	3.13	0.1563
Valaciclovir_AS	0.25	5.00	0.2500
Valaciclovir_BF	0.25	n.a.	n.a.
Venlafaxin_AS	0.01	0.03	0.0014
Venlafaxin_BF	0.01	0.03	0.0013

Table 14: LOQ of all compounds. LOQ calibration is the lowest concentration which can reliably be determined in the calibration curve. LOQ sample is the lowest concentration which can reliably be determined in the sample (taking into account absolute recoveries). LOQ sample per biomass is normalised to the amount of biomass present. AS stands for activated sludge, BF stands for biofilm.

B.3 Full bioaccumulation results

type	C0 [nmol/g biomass]	mean C48 [nmol/g biomass]	corresponds to accumulated %	stdv C48 [nmol/g biomass]	mean C96 [nmol/g biomass]	corresponds to accumulated %	stdv C96 [nmol/g biomass] %
BF_btq Abacavir	0.00	0.12	1.43	0.02	0.04	0.54	0.03
BF_scq Abacavir	0.00	0.16	1.96	0.07	0.13	1.57	0.04
AS_btq Abacavir	0.00	0.01	0.07	0.05	0.02	0.20	0.01
AS_scq Abacavir	0.01	0.48	4.50	NA	0.29	2.72	0.01
active_bioacc_BF Abacavir	0.00	-0.04	-0.53	0.07	-0.09	-1.03	0.05
active_bioacc_AS Abacavir	-0.01	-0.47	-4.43	NA	-0.27	-2.52	0.02
BF_btq Acemetacin	0.00	0.06	0.67	0.01	0.00	0.00	0.00
BF_scq Acemetacin	0.00	0.41	4.90	0.08	0.43	5.23	0.04
AS_btq Acemetacin	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AS_scq Acemetacin	0.00	0.91	8.54	NA	0.72	6.77	0.06
active_bioacc_BF Acemetacin	0.00	-0.35	-4.23	0.08	-0.43	-5.23	0.04
active_bioacc_AS Acemetacin	0.00	-0.91	-8.54	NA	-0.72	-6.77	0.06
BF_btq Albuterol	0.00	0.12	1.46	0.05	0.11	1.37	0.11
BF_scq Albuterol	0.00	0.15	1.81	0.06	0.13	1.54	0.13
AS_btq Albuterol	0.00	0.20	1.84	0.07	0.11	1.00	0.15
AS_scq Albuterol	0.00	0.33	3.06	0.09	0.28	2.62	0.03
active_bioacc_BF Albuterol	0.00	-0.03	-0.35	0.08	-0.01	-0.18	0.17
active_bioacc_AS Albuterol	0.00	-0.13	-1.22	0.11	-0.17	-1.61	0.16
BF_btq Amisulprid	0.00	0.58	7.02	0.06	0.58	7.02	0.02
BF_scq Amisulprid	0.00	0.51	6.17	0.05	0.53	6.40	0.04
AS_btq Amisulprid	0.02	0.97	9.07	0.08	1.13	10.61	0.08
AS_scq Amisulprid	0.01	0.70	6.52	0.07	0.64	5.99	0.01
active_bioacc_BF Amisulprid	0.00	0.07	0.85	0.08	0.05	0.62	0.05
active_bioacc_AS Amisulprid	0.01	0.27	2.55	0.11	0.49	4.62	0.08
BF_btq Amlodipine	0.00	0.43	5.15	0.15	0.05	0.56	0.09
BF_scq Amlodipine	0.00	0.09	1.09	0.08	0.03	0.37	0.04
AS_btq Amlodipine	0.04	0.27	2.53	0.06	0.14	1.30	0.03
AS_scq Amlodipine	0.00	442.39	4140.81	NA	1.64	15.36	0.29
active_bioacc_BF Amlodipine	0.00	0.34	4.05	0.17	0.02	0.19	0.10
active_bioacc_AS Amlodipine	0.04	-442.12	-4138.28	NA	-1.50	-14.06	0.29
BF_btq Apixaban	0.00	0.59	7.09	0.04	0.49	5.87	0.03
BF_scq Apixaban	0.00	0.55	6.67	0.10	0.55	6.70	0.03
AS_btq Apixaban	0.01	0.67	6.23	0.07	0.65	6.07	0.07
AS_scq Apixaban	0.00	0.92	8.60	NA	0.56	5.21	0.03
active_bioacc_BF Apixaban	0.00	0.04	0.42	0.11	-0.07	-0.83	0.04
active_bioacc_AS Apixaban	0.00	-0.25	-2.37	NA	0.09	0.86	0.07
BF_btq Apremilast	0.00	0.14	1.72	0.04	0.02	0.28	0.03
BF_scq Apremilast	0.00	0.06	0.75	0.06	0.03	0.37	0.06
AS_btq Apremilast	0.00	0.52	4.83	0.07	0.32	3.01	0.04
AS_scq Apremilast	0.00	0.47	4.40	NA	0.28	2.60	0.01
active_bioacc_BF Apremilast	0.00	0.08	0.98	0.07	-0.01	-0.09	0.06
active_bioacc_AS Apremilast	0.00	0.05	0.43	NA	0.04	0.41	0.04
BF_btq Benzophenon 3	0.00	0.93	11.19	0.20	0.74	8.98	0.12
BF_scq Benzophenon 3	0.00	1.64	19.76	0.41	1.79	21.65	0.46
AS_btq Benzophenon 3	0.00	0.44	4.09	0.10	0.03	0.25	0.06

type	C0 [nmol/g biomass]	mean C48 [nmol/g biomass]	corresponds to accumulated %	stdv C48 [nmol/g biomass]	mean C96 [nmol/g biomass]	corresponds to accumulated %	stdv C96 [nmol/g biomass] %
AS_scq Benzophenon 3	0.00	12.77	119.57	NA	3.62	33.87	2.56
active_bioacc_BF Benzophenon 3	0.00	-0.71	-8.58	0.46	-1.05	-12.67	0.48
active_bioacc_AS Benzophenon 3	0.00	-12.34	-115.48	NA	-3.59	-33.62	2.56
BF_btq Bisacodyl	0.00	0.03	0.32	0.02	0.00	0.05	0.04
BF_scq Bisacodyl	0.00	2.80	33.83	1.24	1.11	13.42	0.31
AS_btq Bisacodyl	0.02	0.05	0.45	0.21	0.13	1.21	0.39
AS_scq Bisacodyl	0.00	2.32	21.73	NA	1.53	14.29	0.08
active_bioacc_BF Bisacodyl	0.00	-2.78	-33.51	1.24	-1.11	-13.37	0.32
active_bioacc_AS Bisacodyl	0.02	-2.27	-21.27	NA	-1.40	-13.09	0.40
BF_btq Bupropion	0.08	0.91	10.97	0.09	1.00	12.03	0.10
BF_scq Bupropion	0.03	0.47	5.64	0.16	0.21	2.48	0.05
AS_btq Bupropion	0.00	0.63	5.93	0.11	1.54	14.39	0.21
AS_scq Bupropion	0.00	0.54	5.09	NA	0.38	3.60	0.08
active_bioacc_BF Bupropion	0.05	0.44	5.33	0.19	0.79	9.55	0.11
active_bioacc_AS Bupropion	0.00	0.09	0.84	NA	1.15	10.79	0.23
BF_btq Chlorotoluron	0.00	0.27	3.24	0.09	0.29	3.47	0.06
BF_scq Chlorotoluron	0.00	0.26	3.15	0.02	0.21	2.57	0.04
AS_btq Chlorotoluron	0.00	0.65	6.07	0.13	0.58	5.42	0.14
AS_scq Chlorotoluron	0.00	0.49	4.60	0.14	0.45	4.22	0.03
active_bioacc_BF Chlorotoluron	0.00	0.01	0.09	0.09	0.07	0.90	0.07
active_bioacc_AS Chlorotoluron	0.00	0.16	1.47	0.19	0.13	1.19	0.14
BF_btq Clomipramine	0.00	1.40	16.94	0.73	0.29	3.47	0.08
BF_scq Clomipramine	0.00	0.53	6.42	0.17	0.25	3.00	0.07
AS_btq Clomipramine	0.02	2.02	18.88	0.28	2.94	27.52	1.02
AS_scq Clomipramine	0.00	4.09	38.24	NA	2.77	25.93	1.50
active_bioacc_BF Clomipramine	0.00	0.87	10.51	0.75	0.04	0.47	0.11
active_bioacc_AS Clomipramine	0.02	-2.07	-19.36	NA	0.17	1.59	1.81
BF_btq Codein	0.00	0.25	3.08	0.07	0.27	3.25	0.05
BF_scq Codein	0.00	0.23	2.73	0.05	0.23	2.73	0.06
AS_btq Codein	0.01	0.17	1.60	0.05	0.07	0.70	0.07
AS_scq Codein	0.00	0.54	5.03	0.10	0.36	3.37	0.00
active_bioacc_BF Codein	0.00	0.03	0.34	0.09	0.04	0.52	0.07
active_bioacc_AS Codein	0.01	-0.37	-3.42	0.11	-0.29	-2.67	0.07
BF_btq Cyclophosphamid	0.00	0.07	0.82	0.07	0.04	0.53	0.07
BF_scq Cyclophosphamid	0.00	0.12	1.40	0.03	0.07	0.85	0.03
AS_btq Cyclophosphamid	0.00	0.32	2.97	0.08	0.23	2.12	0.10
AS_scq Cyclophosphamid	0.00	0.19	1.82	0.11	0.13	1.26	0.05
active_bioacc_BF Cyclophosphamid	0.00	-0.05	-0.58	0.07	-0.03	-0.31	0.08
active_bioacc_AS Cyclophosphamid	0.00	0.12	1.15	0.13	0.09	0.86	0.11
BF_btq Darunavir	0.00	0.34	4.05	0.07	0.37	4.46	0.22
BF_scq Darunavir	0.00	0.44	5.34	0.23	0.42	5.07	0.11
AS_btq Darunavir	0.00	0.75	7.00	0.07	0.64	5.97	0.08
AS_scq Darunavir	0.00	0.62	5.78	0.07	0.55	5.18	0.00
active_bioacc_BF Darunavir	0.00	-0.11	-1.29	0.24	-0.05	-0.60	0.24
active_bioacc_AS Darunavir	0.00	0.13	1.22	0.10	0.08	0.79	0.08
BF_btq Dexlansoprazole	0.00	0.19	2.25	0.01	0.09	1.06	0.02
BF_scq Dexlansoprazole	0.00	0.25	2.98	0.10	0.15	1.82	0.02

type	C0 [nmol/g biomass]	mean C48 [nmol/g biomass]	corresponds to accumulated %	stdv C48 [nmol/g biomass]	mean C96 [nmol/g biomass]	corresponds to accumulated %	stdv C96 [nmol/g biomass] %
AS_btq Dexlansoprazole	0.00	0.62	5.84	0.09	0.53	4.96	0.03
AS_scq Dexlansoprazole	0.00	0.75	7.06	NA	0.37	3.47	0.05
active_bioacc_BF Dexlansoprazole	0.00	-0.06	-0.73	0.10	-0.06	-0.76	0.03
active_bioacc_AS Dexlansoprazole	0.00	-0.13	-1.23	NA	0.16	1.49	0.06
BF_btq Dextromethorphan	0.00	1.53	18.53	0.43	0.86	10.40	0.18
BF_scq Dextromethorphan	0.00	0.71	8.52	0.15	0.58	6.95	0.07
AS_btq Dextromethorphan	0.00	1.91	17.86	0.29	3.12	29.24	0.72
AS_scq Dextromethorphan	0.00	2.07	19.41	NA	1.48	13.87	0.20
active_bioacc_BF Dextromethorphan	0.00	0.83	10.01	0.46	0.29	3.45	0.20
active_bioacc_AS Dextromethorphan	0.00	-0.17	-1.55	NA	1.64	15.37	0.74
BF_btq Diclofenac	0.00	0.19	2.27	0.06	0.20	2.40	0.06
BF_scq Diclofenac	0.00	0.33	3.93	0.05	0.44	5.31	0.07
AS_btq Diclofenac	0.38	1.20	11.24	0.10	0.81	7.57	0.18
AS_scq Diclofenac	0.19	0.70	6.52	0.15	0.59	5.53	0.03
active_bioacc_BF Diclofenac	0.00	-0.14	-1.66	0.08	-0.24	-2.91	0.09
active_bioacc_AS Diclofenac	0.19	0.50	4.72	0.18	0.22	2.03	0.18
BF_btq Diltiazem	0.00	0.71	8.58	0.10	0.36	4.30	0.05
BF_scq Diltiazem	0.00	0.44	5.29	0.10	0.42	5.06	0.05
AS_btq Diltiazem	0.00	0.01	0.06	0.03	0.01	0.13	0.01
AS_scq Diltiazem	0.00	1.96	18.36	NA	1.18	11.07	0.13
active_bioacc_BF Diltiazem	0.00	0.27	3.29	0.14	-0.06	-0.76	0.07
active_bioacc_AS Diltiazem	0.00	-1.96	-18.31	NA	-1.17	-10.94	0.13
BF_btq Diosmin	0.30	0.12	1.43	0.09	0.13	1.53	0.18
BF_scq Diosmin	0.02	0.26	3.20	0.24	0.17	2.07	0.17
AS_btq Diosmin	1.40	1.55	14.46	0.72	1.81	16.96	1.54
AS_scq Diosmin	1.24	2.57	24.06	NA	0.81	7.54	0.50
active_bioacc_BF Diosmin	0.28	-0.15	-1.77	0.26	-0.05	-0.55	0.25
active_bioacc_AS Diosmin	0.16	-1.03	-9.60	NA	1.01	9.42	1.62
BF_btq Edoxaban	0.00	0.92	11.11	0.09	0.58	7.01	0.04
BF_scq Edoxaban	0.00	0.24	2.92	0.09	0.38	4.63	0.05
AS_btq Edoxaban	0.00	0.78	7.31	0.13	0.70	6.53	0.05
AS_scq Edoxaban	0.00	0.71	6.67	NA	0.42	3.93	0.03
active_bioacc_BF Edoxaban	0.00	0.68	8.19	0.13	0.20	2.38	0.06
active_bioacc_AS Edoxaban	0.00	0.07	0.64	NA	0.28	2.60	0.06
BF_btq Elvitegravir	0.00	2.58	31.11	1.05	1.18	14.22	0.47
BF_scq Elvitegravir	0.00	4.73	57.13	4.32	1.60	19.26	0.33
AS_btq Elvitegravir	0.51	1.28	12.01	0.26	5.53	51.78	2.90
AS_scq Elvitegravir	0.40	6.27	58.66	NA	1.15	10.78	0.16
active_bioacc_BF Elvitegravir	0.00	-2.15	-26.01	4.45	-0.42	-5.04	0.57
active_bioacc_AS Elvitegravir	0.10	-4.98	-46.65	NA	4.38	41.00	2.90
BF_btq Emtricitabine	0.00	0.10	1.22	0.05	0.06	0.68	0.03
BF_scq Emtricitabine	0.00	0.13	1.54	0.02	0.09	1.11	0.04
AS_btq Emtricitabine	0.00	0.23	2.11	0.07	0.03	0.27	0.09
AS_scq Emtricitabine	0.00	0.24	2.21	0.10	0.16	1.52	0.02
active_bioacc_BF Emtricitabine	0.00	-0.03	-0.32	0.05	-0.04	-0.43	0.05
active_bioacc_AS Emtricitabine	0.00	-0.01	-0.10	0.12	-0.13	-1.26	0.09
BF_btq Entacapone	0.00	0.33	4.03	0.06	0.11	1.28	0.04

type	C0 [nmol/g biomass]	mean C48 [nmol/g biomass]	corresponds to accumulated %	stdv C48 [nmol/g biomass]	mean C96 [nmol/g biomass]	corresponds to accumulated %	stdv C96 [nmol/g biomass] %
BF_scq Entacapone	0.00	3.30	39.82	1.80	3.29	39.74	3.25
AS_btq Entacapone	0.00	0.71	6.68	0.14	0.81	7.58	0.08
AS_scq Entacapone	0.00	3.07	28.78	NA	1.41	13.21	0.38
active_bioacc_BF Entacapone	0.00	-2.96	-35.79	1.81	-3.18	-38.45	3.26
active_bioacc_AS Entacapone	0.00	-2.36	-22.10	NA	-0.60	-5.63	0.39
BF_btq Enzalutamide	0.00	0.44	5.33	0.04	0.37	4.52	0.05
BF_scq Enzalutamide	0.00	0.62	7.49	0.15	0.54	6.51	0.03
AS_btq Enzalutamide	0.00	1.72	16.10	0.25	1.66	15.53	0.14
AS_scq Enzalutamide	0.00	1.61	15.06	NA	1.80	16.89	0.41
active_bioacc_BF Enzalutamide	0.00	-0.18	-2.16	0.15	-0.16	-1.99	0.06
active_bioacc_AS Enzalutamide	0.00	0.11	1.04	NA	-0.15	-1.36	0.44
BF_btq Etodolac	0.00	0.08	0.91	0.06	0.05	0.59	0.02
BF_scq Etodolac	0.00	0.18	2.18	0.04	0.15	1.85	0.04
AS_btq Etodolac	0.00	0.57	5.31	0.07	0.35	3.30	0.07
AS_scq Etodolac	0.00	0.36	3.38	0.10	0.26	2.47	0.03
active_bioacc_BF Etodolac	0.00	-0.10	-1.27	0.07	-0.10	-1.26	0.04
active_bioacc_AS Etodolac	0.00	0.21	1.94	0.12	0.09	0.82	0.07
BF_btq Febuxostat	0.00	0.36	4.34	0.03	0.37	4.42	0.05
BF_scq Febuxostat	0.00	0.50	6.08	0.11	0.70	8.41	0.06
AS_btq Febuxostat	0.12	1.94	18.20	0.31	1.35	12.63	0.12
AS_scq Febuxostat	0.01	1.23	11.53	NA	1.20	11.24	0.15
active_bioacc_BF Febuxostat	0.00	-0.14	-1.74	0.12	-0.33	-4.00	0.08
active_bioacc_AS Febuxostat	0.11	0.71	6.67	NA	0.15	1.39	0.20
BF_btq Fenfluramine	0.00	0.63	7.61	0.08	0.74	8.88	0.03
BF_scq Fenfluramine	0.00	0.28	3.41	0.14	0.21	2.49	0.01
AS_btq Fenfluramine	0.00	1.04	9.75	0.16	1.67	15.65	0.26
AS_scq Fenfluramine	0.00	0.92	8.66	NA	0.70	6.57	0.15
active_bioacc_BF Fenfluramine	0.00	0.35	4.20	0.16	0.53	6.39	0.03
active_bioacc_AS Fenfluramine	0.00	0.12	1.10	NA	0.97	9.08	0.30
BF_btq Gliclazide	0.00	0.04	0.50	0.03	0.01	0.07	0.04
BF_scq Gliclazide	0.00	0.08	1.01	0.06	0.05	0.58	0.02
AS_btq Gliclazide	0.00	0.34	3.21	0.06	0.23	2.14	0.02
AS_scq Gliclazide	0.00	0.38	3.55	NA	0.24	2.28	0.05
active_bioacc_BF Gliclazide	0.00	-0.04	-0.51	0.07	-0.04	-0.51	0.04
active_bioacc_AS Gliclazide	0.00	-0.04	-0.33	NA	-0.02	-0.15	0.05
BF_btq Icaridin	0.00	0.11	1.27	0.02	0.06	0.69	0.03
BF_scq Icaridin	0.00	0.19	2.24	0.09	0.06	0.78	0.02
AS_btq Icaridin	0.00	0.01	0.13	0.03	0.02	0.17	0.02
AS_scq Icaridin	0.04	0.50	4.71	NA	0.21	1.97	0.02
active_bioacc_BF Icaridin	0.00	-0.08	-0.97	0.09	-0.01	-0.09	0.04
active_bioacc_AS Icaridin	-0.04	-0.49	-4.58	NA	-0.19	-1.80	0.03
BF_btq Ifosfamid	0.01	0.09	1.13	0.02	0.07	0.79	0.03
BF_scq Ifosfamid	0.00	0.14	1.72	0.05	0.07	0.87	0.04
AS_btq Ifosfamid	0.00	0.26	2.48	0.04	0.18	1.72	0.03
AS_scq Ifosfamid	0.00	0.48	4.52	NA	0.19	1.80	0.02
active_bioacc_BF Ifosfamid	0.01	-0.05	-0.59	0.05	-0.01	-0.08	0.05
active_bioacc_AS Ifosfamid	0.00	-0.22	-2.05	NA	-0.01	-0.08	0.04

type	C0 [nmol/g biomass]	mean C48 [nmol/g biomass]	corresponds to accumulated %	stdv C48 [nmol/g biomass]	mean C96 [nmol/g biomass]	corresponds to accumulated %	stdv C96 [nmol/g biomass] %
BF_btq Isoproturon	0.00	0.27	3.20	0.07	0.29	3.54	0.02
BF_scq Isoproturon	0.00	0.20	2.42	0.02	0.15	1.78	0.02
AS_btq Isoproturon	0.00	0.51	4.81	0.08	0.37	3.44	0.13
AS_scq Isoproturon	0.00	0.44	4.13	0.09	0.34	3.23	0.03
active_bioacc_BF Isoproturon	0.00	0.06	0.78	0.08	0.15	1.76	0.03
active_bioacc_AS Isoproturon	0.00	0.07	0.68	0.12	0.02	0.21	0.13
BF_btq Ketamin	0.00	0.21	2.57	0.07	0.38	4.55	0.09
BF_scq Ketamin	0.00	0.19	2.30	0.05	0.14	1.71	0.06
AS_btq Ketamin	0.00	0.61	5.67	0.06	1.27	11.85	0.08
AS_scq Ketamin	0.00	0.38	3.51	0.06	0.30	2.80	0.01
active_bioacc_BF Ketamin	0.00	0.02	0.27	0.09	0.23	2.83	0.11
active_bioacc_AS Ketamin	0.00	0.23	2.15	0.09	0.97	9.05	0.08
BF_btq Levamisol	0.00	0.56	6.71	0.08	0.74	8.89	0.03
BF_scq Levamisol	0.00	0.53	6.36	0.10	0.46	5.56	0.04
AS_btq Levamisol	0.00	1.15	10.77	0.17	2.29	21.42	0.33
AS_scq Levamisol	0.00	1.10	10.32	NA	0.64	5.96	0.07
active_bioacc_BF Levamisol	0.00	0.03	0.34	0.12	0.28	3.33	0.05
active_bioacc_AS Levamisol	0.00	0.05	0.45	NA	1.65	15.46	0.34
BF_btq Lisinopril	NA	NA	NA	NA	NA	NA	NA
BF_scq Lisinopril	NA	NA	NA	NA	NA	NA	NA
AS_btq Lisinopril	NA	NA	NA	NA	NA	NA	NA
AS_scq Lisinopril	NA	NA	NA	NA	NA	NA	NA
active_bioacc_BF Lisinopril	NA	NA	NA	NA	NA	NA	NA
active_bioacc_AS Lisinopril	NA	NA	NA	NA	NA	NA	NA
BF_btq Losartan	0.00	0.18	2.18	0.04	0.15	1.87	0.11
BF_scq Losartan	0.00	0.31	3.73	0.06	0.33	3.98	0.10
AS_btq Losartan	0.14	0.86	8.09	0.10	0.66	6.16	0.07
AS_scq Losartan	0.04	0.60	5.59	0.09	0.50	4.66	0.01
active_bioacc_BF Losartan	0.00	-0.13	-1.55	0.07	-0.17	-2.11	0.15
active_bioacc_AS Losartan	0.10	0.27	2.50	0.13	0.16	1.50	0.07
BF_btq Metoclopramid	0.00	0.61	7.34	0.08	0.55	6.69	0.09
BF_scq Metoclopramid	0.00	0.49	5.94	0.16	0.57	6.87	0.10
AS_btq Metoclopramid	0.00	1.05	9.80	0.11	1.35	12.64	0.20
AS_scq Metoclopramid	0.00	1.52	14.25	NA	1.17	10.95	0.18
active_bioacc_BF Metoclopramid	0.00	0.12	1.39	0.18	-0.01	-0.18	0.13
active_bioacc_AS Metoclopramid	0.00	-0.48	-4.45	NA	0.18	1.69	0.27
BF_btq Metoxuron	0.01	0.17	2.04	0.03	0.23	2.73	0.10
BF_scq Metoxuron	0.00	0.17	2.11	0.02	0.14	1.68	0.05
AS_btq Metoxuron	0.00	0.42	3.94	0.10	0.31	2.93	0.10
AS_scq Metoxuron	0.00	0.38	3.57	0.10	0.27	2.49	0.03
active_bioacc_BF Metoxuron	0.01	-0.01	-0.07	0.04	0.09	1.05	0.11
active_bioacc_AS Metoxuron	0.00	0.04	0.38	0.14	0.05	0.44	0.10
BF_btq Mexiletine	0.00	0.42	5.02	0.07	0.38	4.56	0.04
BF_scq Mexiletine	0.00	0.39	4.67	0.07	0.25	2.98	0.02
AS_btq Mexiletine	0.00	0.53	4.94	0.08	0.77	7.19	0.09
AS_scq Mexiletine	0.00	0.80	7.51	NA	0.64	5.96	0.10
active_bioacc_BF Mexiletine	0.00	0.03	0.35	0.10	0.13	1.57	0.04

type	C0 [nmol/g biomass]	mean C48 [nmol/g biomass]	corresponds to accumulated %	stdv C48 [nmol/g biomass]	mean C96 [nmol/g biomass]	corresponds to accumulated %	stdv C96 [nmol/g biomass] %
active_bioacc_AS Mexiletine	0.00	-0.27	-2.57	NA	0.13	1.22	0.13
BF_btq Mirabegron	0.00	0.72	8.71	0.08	0.22	2.64	0.04
BF_scq Mirabegron	0.00	0.98	11.79	0.27	1.37	16.55	0.24
AS_btq Mirabegron	0.00	0.06	0.60	0.08	0.01	0.11	0.03
AS_scq Mirabegron	0.00	2.69	25.21	NA	1.62	15.12	0.40
active_bioacc_BF Mirabegron	0.00	-0.26	-3.08	0.28	-1.15	-13.90	0.25
active_bioacc_AS Mirabegron	0.00	-2.63	-24.61	NA	-1.60	-15.01	0.40
BF_btq Moclobemid	0.00	0.18	2.18	0.01	0.17	2.11	0.05
BF_scq Moclobemid	0.00	0.24	2.87	0.09	0.17	2.04	0.01
AS_btq Moclobemid	0.00	0.62	5.78	0.08	0.48	4.49	0.03
AS_scq Moclobemid	0.00	0.43	4.02	NA	0.31	2.90	0.03
active_bioacc_BF Moclobemid	0.00	-0.06	-0.69	0.09	0.01	0.07	0.05
active_bioacc_AS Moclobemid	0.00	0.19	1.76	NA	0.17	1.59	0.04
BF_btq Monuron	0.00	0.13	1.63	0.01	0.11	1.29	0.02
BF_scq Monuron	0.00	0.15	1.78	0.10	0.11	1.27	0.03
AS_btq Monuron	0.00	0.42	3.96	0.05	0.33	3.07	0.02
AS_scq Monuron	0.00	0.64	5.99	NA	0.24	2.22	0.02
active_bioacc_BF Monuron	0.00	-0.01	-0.15	0.10	0.00	0.02	0.04
active_bioacc_AS Monuron	0.00	-0.22	-2.04	NA	0.09	0.85	0.03
BF_btq Morphin	0.00	0.06	0.71	0.08	0.02	0.19	0.04
BF_scq Morphin	0.00	0.22	2.60	0.06	0.20	2.44	0.09
AS_btq Morphin	0.00	0.06	0.53	0.09	0.04	0.34	0.11
AS_scq Morphin	0.00	0.49	4.56	0.08	0.42	3.92	0.01
active_bioacc_BF Morphin	0.00	-0.16	-1.90	0.10	-0.19	-2.25	0.10
active_bioacc_AS Morphin	0.00	-0.43	-4.03	0.12	-0.38	-3.58	0.11
BF_btq Mycophenolsaeure	0.00	0.06	0.70	0.02	0.01	0.13	0.04
BF_scq Mycophenolsaeure	0.02	0.11	1.39	0.04	0.09	1.07	0.05
AS_btq Mycophenolsaeure	0.00	0.00	0.00	0.00	0.01	0.09	0.02
AS_scq Mycophenolsaeure	0.00	0.48	4.47	NA	0.17	1.63	0.09
active_bioacc_BF Mycophenolsaeure	-0.02	-0.06	-0.69	0.05	-0.08	-0.95	0.06
active_bioacc_AS Mycophenolsaeure	0.00	-0.48	-4.47	NA	-0.16	-1.54	0.09
BF_btq Naloxone	0.00	0.15	1.82	0.04	0.05	0.65	0.01
BF_scq Naloxone	0.00	0.23	2.78	0.07	0.19	2.30	0.02
AS_btq Naloxone	0.00	0.20	1.84	0.05	0.05	0.48	0.01
AS_scq Naloxone	0.00	0.49	4.58	NA	0.38	3.54	0.03
active_bioacc_BF Naloxone	0.00	-0.08	-0.95	0.08	-0.14	-1.66	0.02
active_bioacc_AS Naloxone	0.00	-0.29	-2.74	NA	-0.33	-3.07	0.04
BF_btq Neohesperidin dc	0.00	0.00	0.05	0.02	0.00	0.00	0.00
BF_scq Neohesperidin dc	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AS_btq Neohesperidin dc	0.00	0.00	0.00	0.00	0.00	0.01	0.01
AS_scq Neohesperidin dc	0.00	0.57	5.30	NA	0.39	3.68	0.03
active_bioacc_BF Neohesperidin dc	0.00	0.00	0.05	0.02	0.00	0.00	0.00
active_bioacc_AS Neohesperidin dc	0.00	-0.57	-5.30	NA	-0.39	-3.67	0.03
BF_btq Neotam	0.00	0.00	0.00	0.07	0.00	0.00	0.05
BF_scq Neotam	0.00	0.12	1.43	0.01	0.05	0.64	0.03
AS_btq Neotam	0.00	0.00	0.00	0.09	0.00	0.00	0.09
AS_scq Neotam	0.00	0.23	2.15	0.10	0.16	1.45	0.02

type	C0 [nmol/g biomass]	mean C48 [nmol/g biomass]	corresponds to accumulated %	stdv C48 [nmol/g biomass]	mean C96 [nmol/g biomass]	corresponds to accumulated %	stdv C96 [nmol/g biomass] %
active_bioacc_BF Neotam	0.00	-0.12	-1.43	0.07	-0.05	-0.64	0.06
active_bioacc_AS Neotam	0.00	-0.23	-2.15	0.13	-0.16	-1.45	0.10
BF_btq Olmesartan	0.00	0.08	0.92	0.02	0.02	0.22	0.05
BF_scq Olmesartan	0.00	0.12	1.48	0.04	0.11	1.35	0.06
AS_btq Olmesartan	0.00	0.28	2.65	0.07	0.20	1.83	0.03
AS_scq Olmesartan	0.00	0.44	4.08	NA	0.15	1.40	0.03
active_bioacc_BF Olmesartan	0.00	-0.05	-0.57	0.05	-0.09	-1.13	0.08
active_bioacc_AS Olmesartan	0.00	-0.15	-1.43	NA	0.05	0.43	0.04
BF_btq Pantoprazol	0.00	0.17	2.11	0.05	0.16	1.89	0.05
BF_scq Pantoprazol	0.00	0.22	2.70	0.02	0.21	2.52	0.05
AS_btq Pantoprazol	0.03	0.60	5.64	0.09	0.55	5.17	0.08
AS_scq Pantoprazol	0.00	0.50	4.66	0.10	0.42	3.92	0.01
active_bioacc_BF Pantoprazol	0.00	-0.05	-0.58	0.06	-0.05	-0.63	0.07
active_bioacc_AS Pantoprazol	0.03	0.10	0.98	0.13	0.13	1.25	0.08
BF_btq Pirfenidone	0.00	0.08	0.91	0.02	0.05	0.55	0.03
BF_scq Pirfenidone	0.00	0.15	1.87	0.06	0.16	1.91	0.06
AS_btq Pirfenidone	0.00	0.32	2.99	0.02	0.21	1.98	0.02
AS_scq Pirfenidone	0.00	0.44	4.15	NA	0.28	2.63	0.06
active_bioacc_BF Pirfenidone	0.00	-0.08	-0.96	0.06	-0.11	-1.36	0.07
active_bioacc_AS Pirfenidone	0.00	-0.12	-1.15	NA	-0.07	-0.65	0.07
BF_btq Pravastatin	0.00	0.07	0.84	0.05	0.04	0.45	0.04
BF_scq Pravastatin	0.00	0.15	1.85	0.03	0.11	1.37	0.04
AS_btq Pravastatin	0.00	0.02	0.20	0.11	0.04	0.42	0.10
AS_scq Pravastatin	0.00	0.25	2.37	0.11	0.17	1.55	0.04
active_bioacc_BF Pravastatin	0.00	-0.08	-1.00	0.06	-0.08	-0.93	0.06
active_bioacc_AS Pravastatin	0.00	-0.23	-2.17	0.16	-0.12	-1.13	0.11
BF_btq Primidon	0.00	0.06	0.75	0.06	0.03	0.32	0.07
BF_scq Primidon	0.00	0.12	1.50	0.07	0.08	0.93	0.06
AS_btq Primidon	0.00	0.39	3.66	0.08	0.30	2.82	0.09
AS_scq Primidon	0.00	0.30	2.85	0.10	0.19	1.77	0.03
active_bioacc_BF Primidon	0.00	-0.06	-0.75	0.09	-0.05	-0.61	0.09
active_bioacc_AS Primidon	0.00	0.09	0.82	0.12	0.11	1.05	0.09
BF_btq Propranolol	0.00	2.10	25.40	0.46	0.91	11.02	0.12
BF_scq Propranolol	0.00	0.71	8.63	0.15	0.78	9.47	0.10
AS_btq Propranolol	0.04	1.73	16.20	0.37	2.24	20.94	0.41
AS_scq Propranolol	0.05	1.57	14.66	NA	2.46	22.99	0.28
active_bioacc_BF Propranolol	0.00	1.39	16.77	0.48	0.13	1.55	0.16
active_bioacc_AS Propranolol	-0.01	0.16	1.54	NA	-0.22	-2.05	0.50
BF_btq Pseudoephedrine	0.02	0.23	2.80	0.06	0.24	2.95	0.03
BF_scq Pseudoephedrine	0.00	0.31	3.72	0.08	0.15	1.76	0.01
AS_btq Pseudoephedrine	0.00	0.82	7.70	0.10	0.19	1.76	0.02
AS_scq Pseudoephedrine	0.00	0.59	5.54	NA	0.30	2.83	0.05
active_bioacc_BF Pseudoephedrine	0.02	-0.08	-0.92	0.10	0.10	1.20	0.04
active_bioacc_AS Pseudoephedrine	0.00	0.23	2.16	NA	-0.11	-1.07	0.05
BF_btq Rivaroxaban	0.00	0.35	4.20	0.03	0.25	2.97	0.02
BF_scq Rivaroxaban	0.00	0.36	4.38	0.09	0.35	4.17	0.04
AS_btq Rivaroxaban	0.00	0.57	5.31	0.08	0.41	3.85	0.03

type	C0 [nmol/g biomass]	mean C48 [nmol/g biomass]	corresponds to accumulated %	stdv C48 [nmol/g biomass]	mean C96 [nmol/g biomass]	corresponds to accumulated %	stdv C96 [nmol/g biomass] %
AS_scq Rivaroxaban	0.00	1.02	9.59	NA	0.67	6.32	0.01
active_bioacc_BF Rivaroxaban	0.00	-0.01	-0.18	0.10	-0.10	-1.20	0.05
active_bioacc_AS Rivaroxaban	0.00	-0.46	-4.28	NA	-0.26	-2.47	0.04
BF_btq Sacubitril	0.00	0.12	1.44	0.03	0.09	1.13	0.06
BF_scq Sacubitril	0.00	0.18	2.22	0.08	0.14	1.73	0.04
AS_btq Sacubitril	0.00	0.00	0.00	0.00	0.00	0.00	0.01
AS_scq Sacubitril	0.00	0.52	4.87	NA	0.29	2.70	0.01
active_bioacc_BF Sacubitril	0.00	-0.06	-0.78	0.08	-0.05	-0.60	0.07
active_bioacc_AS Sacubitril	0.00	-0.52	-4.87	NA	-0.29	-2.70	0.01
BF_btq Sertraline	0.00	1.18	14.20	0.44	0.31	3.80	0.13
BF_scq Sertraline	0.00	0.36	4.37	0.09	0.17	2.10	0.03
AS_btq Sertraline	0.92	1.03	9.64	0.24	4.39	41.08	3.38
AS_scq Sertraline	0.58	4.51	42.25	NA	1.80	16.86	0.61
active_bioacc_BF Sertraline	0.00	0.81	9.83	0.45	0.14	1.70	0.14
active_bioacc_AS Sertraline	0.35	-3.48	-32.61	NA	2.59	24.22	3.44
BF_btq Sulfasalazine	0.00	1.18	14.25	0.20	1.54	18.63	0.32
BF_scq Sulfasalazine	0.00	0.53	6.34	0.12	0.67	8.10	0.06
AS_btq Sulfasalazine	0.00	2.16	20.18	0.53	3.65	34.16	0.43
AS_scq Sulfasalazine	0.00	3.53	33.04	NA	2.50	23.42	0.58
active_bioacc_BF Sulfasalazine	0.00	0.65	7.91	0.23	0.87	10.53	0.33
active_bioacc_AS Sulfasalazine	0.00	-1.37	-12.86	NA	1.15	10.73	0.72
BF_btq Tolperison	0.00	0.85	10.26	0.15	0.48	5.76	0.03
BF_scq Tolperison	0.00	0.61	7.32	0.14	0.45	5.40	0.04
AS_btq Tolperison	0.00	1.18	11.01	0.28	2.07	19.34	0.41
AS_scq Tolperison	0.00	1.13	10.59	NA	0.72	6.76	0.07
active_bioacc_BF Tolperison	0.00	0.24	2.94	0.21	0.03	0.36	0.05
active_bioacc_AS Tolperison	0.00	0.05	0.43	NA	1.34	12.58	0.41
BF_btq Torasemid	0.00	0.14	1.65	0.04	0.09	1.04	0.01
BF_scq Torasemid	0.00	0.19	2.28	0.03	0.13	1.58	0.05
AS_btq Torasemid	0.00	0.42	3.90	0.05	0.28	2.61	0.13
AS_scq Torasemid	0.00	0.30	2.81	0.10	0.20	1.86	0.04
active_bioacc_BF Torasemid	0.00	-0.05	-0.63	0.05	-0.05	-0.54	0.05
active_bioacc_AS Torasemid	0.00	0.12	1.09	0.11	0.08	0.76	0.13
BF_btq Tramadol	0.00	0.22	2.63	0.05	0.28	3.36	0.07
BF_scq Tramadol	0.00	0.22	2.61	0.03	0.20	2.42	0.07
AS_btq Tramadol	0.06	0.57	5.38	0.12	0.86	8.05	0.07
AS_scq Tramadol	0.03	0.41	3.82	0.11	0.30	2.83	0.04
active_bioacc_BF Tramadol	0.00	0.00	0.02	0.06	0.08	0.94	0.10
active_bioacc_AS Tramadol	0.04	0.17	1.55	0.16	0.56	5.22	0.08
BF_btq Trazodone	0.00	1.08	13.00	0.25	0.64	7.69	0.11
BF_scq Trazodone	0.00	0.49	5.87	0.13	0.83	10.02	0.14
AS_btq Trazodone	0.13	1.74	16.26	0.43	1.35	12.60	0.17
AS_scq Trazodone	0.05	2.01	18.85	NA	1.79	16.75	0.59
active_bioacc_BF Trazodone	0.00	0.59	7.13	0.28	-0.19	-2.33	0.18
active_bioacc_AS Trazodone	0.08	-0.28	-2.58	NA	-0.44	-4.15	0.61
BF_btq Trimipramin	0.00	2.37	28.60	0.17	2.16	26.04	0.14
BF_scq Trimipramin	0.00	1.24	15.02	0.07	1.03	12.40	0.08

type	C0 [nmol/g biomass]	mean C48 [nmol/g biomass]	corresponds to accumulated %	stdv C48 [nmol/g biomass]	mean C96 [nmol/g biomass]	corresponds to accumulated %	stdv C96 [nmol/g biomass] %
AS_btq Trimipramin	0.00	3.70	34.61	0.14	3.51	32.87	0.21
AS_scq Trimipramin	0.00	5.08	47.56	0.19	5.29	49.56	0.03
active_bioacc_BF Trimipramin	0.00	1.12	13.58	0.18	1.13	13.64	0.16
active_bioacc_AS Trimipramin	0.00	-1.38	-12.95	0.23	-1.78	-16.69	0.21
BF_btq Valaciclovir	0.00	0.00	0.00	0.00	0.00	0.00	0.00
BF_scq Valaciclovir	0.00	0.02	0.30	0.11	0.02	0.27	0.04
AS_btq Valaciclovir	0.00	0.00	0.00	0.00	0.00	0.00	0.00
AS_scq Valaciclovir	0.00	0.35	3.32	NA	0.04	0.33	0.07
active_bioacc_BF Valaciclovir	0.00	-0.02	-0.30	0.11	-0.02	-0.27	0.04
active_bioacc_AS Valaciclovir	0.00	-0.35	-3.32	NA	-0.04	-0.33	0.07
BF_btq Venlafaxin	0.00	0.78	9.37	0.07	0.81	9.76	0.05
BF_scq Venlafaxin	0.00	0.58	7.02	0.02	0.57	6.94	0.04
AS_btq Venlafaxin	0.02	0.70	6.53	0.09	1.00	9.38	0.12
AS_scq Venlafaxin	0.01	0.46	4.33	0.14	0.42	3.96	0.01
active_bioacc_BF Venlafaxin	0.00	0.19	2.35	0.07	0.23	2.82	0.06
active_bioacc_AS Venlafaxin	0.01	0.23	2.20	0.16	0.58	5.42	0.12

Table 15: Full bioaccumulation results. btq stands for biotransformation-QuEChERS and shows the accumulated amount of substance found in the biotic experiment reactor. scq stands for sorption control-QuEChERS and shows the amount of substance found in the sorption control reactor sorbed to the cell. AS stands for activated sludge. BF stands for biofilm. active_bioacc is the amount of actively bioaccumulated substance in biofilm resp. activated sludge.

B.3.1 Mass balance full results

Substance	**Reactor and phase**	**t0 [nmol]**	**t48 [nmol]**	**t96 [nmol]**
Albuterol	SC biofilm aq	5.9	4.4	4.5
Albuterol	SC biofilm qq	0.0	0.1	0.1
Albuterol	SC biofilm total	5.9	4.5	4.6
Albuterol	SC activated sludge aq	5.3	4.1	4.2
Albuterol	SC activated sludge qq	0.0	0.2	0.1
Albuterol	SC activated sludge total	5.3	4.3	4.3
Albuterol	BE biofilm aq	6.1	3.8	3.8
Albuterol	BE biofilm qq	0.0	0.1	0.1
Albuterol	BE biofilm total	6.1	3.9	3.9
Albuterol	BE activated sludge aq	6.6	2.2	1.1
Albuterol	BE activated sludge qq	0.0	0.1	0.1
Albuterol	BE activated sludge total	6.6	2.3	1.1
Amisulprid	SC biofilm aq	6.1	4.0	4.2
Amisulprid	SC biofilm qq	0.0	0.3	0.3
Amisulprid	SC biofilm total	6.1	4.3	4.5
Amisulprid	SC activated sludge aq	5.0	3.8	4.1
Amisulprid	SC activated sludge qq	0.0	0.3	0.3
Amisulprid	SC activated sludge total	5.0	4.1	4.4
Amisulprid	BE biofilm aq	5.6	3.5	3.8
Amisulprid	BE biofilm qq	0.0	0.4	0.4
Amisulprid	BE biofilm total	5.6	3.9	4.1

Substance	Reactor and phase	t0 [nmol]	t48 [nmol]	t96 [nmol]
Amisulprid	BE activated sludge aq	5.1	3.7	3.7
Amisulprid	BE activated sludge qq	0.0	0.4	0.5
Amisulprid	BE activated sludge total	5.1	4.1	4.2
Chlorotoluron	SC biofilm aq	6.5	4.2	4.6
Chlorotoluron	SC biofilm qq	0.0	0.2	0.1
Chlorotoluron	SC biofilm total	6.5	4.4	4.7
Chlorotoluron	SC activated sludge aq	5.6	3.9	3.8
Chlorotoluron	SC activated sludge qq	0.0	0.2	0.2
Chlorotoluron	SC activated sludge total	5.6	4.2	4.0
Chlorotoluron	BE biofilm aq	5.8	4.1	4.4
Chlorotoluron	BE biofilm qq	0.0	0.2	0.2
Chlorotoluron	BE biofilm total	5.8	4.3	4.6
Chlorotoluron	BE activated sludge aq	5.0	4.0	3.9
Chlorotoluron	BE activated sludge qq	0.0	0.3	0.3
Chlorotoluron	BE activated sludge total	5.0	4.3	4.2
Codein	SC biofilm aq	7.0	4.3	4.9
Codein	SC biofilm qq	0.0	0.1	0.1
Codein	SC biofilm total	7.0	4.5	5.1
Codein	SC activated sludge aq	5.9	4.4	4.4
Codein	SC activated sludge qq	0.0	0.3	0.2
Codein	SC activated sludge total	5.9	4.6	4.5
Codein	BE biofilm aq	5.8	3.9	4.0
Codein	BE biofilm qq	0.0	0.2	0.2
Codein	BE biofilm total	5.8	4.0	4.2
Codein	BE activated sludge aq	5.7	0.7	0.1
Codein	BE activated sludge qq	0.0	0.1	0.0
Codein	BE activated sludge total	5.7	0.7	0.2
Cyclophosphamid	SC biofilm aq	6.4	4.5	4.5
Cyclophosphamid	SC biofilm qq	0.0	0.1	0.0
Cyclophosphamid	SC biofilm total	6.4	4.6	4.6
Cyclophosphamid	SC activated sludge aq	5.2	4.0	4.1
Cyclophosphamid	SC activated sludge qq	0.0	0.1	0.1
Cyclophosphamid	SC activated sludge total	5.2	4.1	4.2
Cyclophosphamid	BE biofilm aq	6.4	4.4	4.5
Cyclophosphamid	BE biofilm qq	0.0	0.0	0.0
Cyclophosphamid	BE biofilm total	6.4	4.4	4.6
Cyclophosphamid	BE activated sludge aq	5.9	4.5	4.2
Cyclophosphamid	BE activated sludge qq	0.0	0.1	0.1
Cyclophosphamid	BE activated sludge total	5.9	4.7	4.3
Darunavir	SC biofilm aq	6.0	3.7	3.5
Darunavir	SC biofilm qq	0.0	0.3	0.3
Darunavir	SC biofilm total	6.0	4.0	3.8
Darunavir	SC activated sludge aq	5.0	3.8	3.9
Darunavir	SC activated sludge qq	0.0	0.3	0.3
Darunavir	SC activated sludge total	5.0	4.1	4.2
Darunavir	BE biofilm aq	5.6	3.1	3.1
Darunavir	BE biofilm qq	0.0	0.2	0.2
Darunavir	BE biofilm total	5.6	3.3	3.3
Darunavir	BE activated sludge aq	4.4	2.4	2.8
Darunavir	BE activated sludge qq	0.0	0.3	0.3
Darunavir	BE activated sludge total	4.4	2.7	3.1
Diclofenac	SC biofilm aq	6.8	4.3	4.4
Diclofenac	SC biofilm qq	0.0	0.2	0.3
Diclofenac	SC biofilm total	6.8	4.5	4.6
Diclofenac	SC activated sludge aq	6.6	4.9	5.0
Diclofenac	SC activated sludge qq	0.0	0.2	0.2
Diclofenac	SC activated sludge total	6.6	5.2	5.2

Substance	Reactor and phase	t0 [nmol]	t48 [nmol]	t96 [nmol]
Diclofenac	BE biofilm aq	5.9	4.0	3.9
Diclofenac	BE biofilm qq	0.0	0.1	0.1
Diclofenac	BE biofilm total	5.9	4.1	4.0
Diclofenac	BE activated sludge aq	6.8	4.5	4.7
Diclofenac	BE activated sludge qq	0.0	0.4	0.2
Diclofenac	BE activated sludge total	6.8	4.9	4.9
Emtricitabine	SC biofilm aq	6.1	4.3	4.5
Emtricitabine	SC biofilm qq	0.0	0.1	0.1
Emtricitabine	SC biofilm total	6.1	4.4	4.6
Emtricitabine	SC activated sludge aq	4.8	4.4	3.6
Emtricitabine	SC activated sludge qq	0.0	0.1	0.1
Emtricitabine	SC activated sludge total	4.8	4.5	3.7
Emtricitabine	BE biofilm aq	5.8	3.9	4.1
Emtricitabine	BE biofilm qq	0.0	0.1	0.0
Emtricitabine	BE biofilm total	5.8	4.0	4.1
Emtricitabine	BE activated sludge aq	5.5	2.9	0.0
Emtricitabine	BE activated sludge qq	0.0	0.1	0.0
Emtricitabine	BE activated sludge total	5.5	3.0	0.0
Etodolac	SC biofilm aq	6.2	4.4	4.5
Etodolac	SC biofilm qq	0.0	0.1	0.1
Etodolac	SC biofilm total	6.2	4.5	4.6
Etodolac	SC activated sludge aq	5.1	4.0	4.0
Etodolac	SC activated sludge qq	0.0	0.2	0.1
Etodolac	SC activated sludge total	5.1	4.1	4.1
Etodolac	BE biofilm aq	5.9	2.6	2.4
Etodolac	BE biofilm qq	0.0	0.0	0.0
Etodolac	BE biofilm total	5.9	2.7	2.4
Etodolac	BE activated sludge aq	5.4	3.4	3.3
Etodolac	BE activated sludge qq	0.0	0.3	0.2
Etodolac	BE activated sludge total	5.4	3.7	3.4
Isoproturon	SC biofilm aq	5.7	4.4	4.6
Isoproturon	SC biofilm qq	0.0	0.1	0.1
Isoproturon	SC biofilm total	5.7	4.5	4.7
Isoproturon	SC activated sludge aq	5.3	4.3	4.1
Isoproturon	SC activated sludge qq	0.0	0.2	0.2
Isoproturon	SC activated sludge total	5.3	4.5	4.3
Isoproturon	BE biofilm aq	5.5	3.7	4.4
Isoproturon	BE biofilm qq	0.0	0.2	0.2
Isoproturon	BE biofilm total	5.5	3.9	4.5
Isoproturon	BE activated sludge aq	5.5	3.8	3.5
Isoproturon	BE activated sludge qq	0.0	0.2	0.2
Isoproturon	BE activated sludge total	5.5	4.1	3.7
Ketamin	SC biofilm aq	6.0	4.3	4.4
Ketamin	SC biofilm qq	0.0	0.1	0.1
Ketamin	SC biofilm total	6.0	4.4	4.5
Ketamin	SC activated sludge aq	5.1	3.9	4.0
Ketamin	SC activated sludge qq	0.0	0.2	0.1
Ketamin	SC activated sludge total	5.1	4.1	4.1
Ketamin	BE biofilm aq	5.7	3.5	3.0
Ketamin	BE biofilm qq	0.0	0.1	0.2
Ketamin	BE biofilm total	5.7	3.7	3.2
Ketamin	BE activated sludge aq	5.4	3.6	2.8
Ketamin	BE activated sludge qq	0.0	0.3	0.6
Ketamin	BE activated sludge total	5.4	3.9	3.4
Losartan	SC biofilm aq	6.3	4.2	4.5
Losartan	SC biofilm qq	0.0	0.2	0.2
Losartan	SC biofilm total	6.3	4.3	4.6

Substance	Reactor and phase	t0 [nmol]	t48 [nmol]	t96 [nmol]
Losartan	SC activated sludge aq	5.6	4.2	4.3
Losartan	SC activated sludge qq	0.0	0.3	0.2
Losartan	SC activated sludge total	5.6	4.5	4.5
Losartan	BE biofilm aq	5.8	3.4	3.4
Losartan	BE biofilm qq	0.0	0.1	0.1
Losartan	BE biofilm total	5.8	3.5	3.5
Losartan	BE activated sludge aq	5.4	3.5	3.3
Losartan	BE activated sludge qq	0.0	0.3	0.2
Losartan	BE activated sludge total	5.4	3.8	3.6
Morphin	SC biofilm aq	5.7	3.4	3.3
Morphin	SC biofilm qq	0.0	0.1	0.1
Morphin	SC biofilm total	5.7	3.5	3.4
Morphin	SC activated sludge aq	4.6	3.1	3.2
Morphin	SC activated sludge qq	0.0	0.2	0.2
Morphin	SC activated sludge total	4.6	3.4	3.4
Morphin	BE biofilm aq	5.6	1.2	0.3
Morphin	BE biofilm qq	0.0	0.0	0.0
Morphin	BE biofilm total	5.6	1.2	0.4
Morphin	BE activated sludge aq	5.2	0.1	0.0
Morphin	BE activated sludge qq	0.0	0.0	0.0
Morphin	BE activated sludge total	5.2	0.1	0.0
Neotam	SC biofilm aq	5.9	3.5	3.1
Neotam	SC biofilm qq	0.0	0.1	0.0
Neotam	SC biofilm total	5.9	3.6	3.1
Neotam	SC activated sludge aq	5.2	3.8	3.7
Neotam	SC activated sludge qq	0.0	0.1	0.1
Neotam	SC activated sludge total	5.2	3.9	3.7
Neotam	BE biofilm aq	4.2	0.1	0.0
Neotam	BE biofilm qq	0.0	0.0	0.0
Neotam	BE biofilm total	4.2	0.1	0.0
Neotam	BE activated sludge aq	4.6	0.0	0.0
Neotam	BE activated sludge qq	0.0	0.0	0.0
Neotam	BE activated sludge total	4.6	0.0	0.0
Pantoprazol	SC biofilm aq	6.0	3.7	3.7
Pantoprazol	SC biofilm qq	0.0	0.1	0.1
Pantoprazol	SC biofilm total	6.0	3.9	3.8
Pantoprazol	SC activated sludge aq	4.9	3.5	3.4
Pantoprazol	SC activated sludge qq	0.0	0.2	0.2
Pantoprazol	SC activated sludge total	4.9	3.7	3.6
Pantoprazol	BE biofilm aq	5.5	3.5	3.3
Pantoprazol	BE biofilm qq	0.0	0.1	0.1
Pantoprazol	BE biofilm total	5.5	3.6	3.4
Pantoprazol	BE activated sludge aq	4.8	2.8	2.5
Pantoprazol	BE activated sludge qq	0.0	0.3	0.2
Pantoprazol	BE activated sludge total	4.8	3.0	2.7
Pravastatin	SC biofilm aq	6.1	4.6	4.9
Pravastatin	SC biofilm qq	0.0	0.1	0.1
Pravastatin	SC biofilm total	6.1	4.7	5.0
Pravastatin	SC activated sludge aq	5.3	4.2	4.2
Pravastatin	SC activated sludge qq	0.0	0.1	0.1
Pravastatin	SC activated sludge total	5.3	4.4	4.2
Pravastatin	BE biofilm aq	5.9	4.2	4.1
Pravastatin	BE biofilm qq	0.0	0.0	0.0
Pravastatin	BE biofilm total	5.9	4.2	4.2
Pravastatin	BE activated sludge aq	5.5	0.1	0.0
Pravastatin	BE activated sludge qq	0.0	0.0	0.0
Pravastatin	BE activated sludge total	5.5	0.1	0.0

Substance	Reactor and phase	t0 [nmol]	t48 [nmol]	t96 [nmol]
Primidon	SC biofilm aq	6.3	4.7	4.8
Primidon	SC biofilm qq	0.0	0.1	0.0
Primidon	SC biofilm total	6.3	4.8	4.9
Primidon	SC activated sludge aq	5.8	4.4	4.5
Primidon	SC activated sludge qq	0.0	0.1	0.1
Primidon	SC activated sludge total	5.8	4.6	4.6
Primidon	BE biofilm aq	6.0	4.1	4.3
Primidon	BE biofilm qq	0.0	0.0	0.0
Primidon	BE biofilm total	6.0	4.2	4.3
Primidon	BE activated sludge aq	5.5	4.5	4.4
Primidon	BE activated sludge qq	0.0	0.2	0.1
Primidon	BE activated sludge total	5.5	4.7	4.6
Torasemid	SC biofilm aq	6.2	4.1	4.5
Torasemid	SC biofilm qq	0.0	0.1	0.1
Torasemid	SC biofilm total	6.2	4.2	4.6
Torasemid	SC activated sludge aq	5.3	4.1	4.1
Torasemid	SC activated sludge qq	0.0	0.1	0.1
Torasemid	SC activated sludge total	5.3	4.3	4.2
Torasemid	BE biofilm aq	6.1	4.1	4.3
Torasemid	BE biofilm qq	0.0	0.1	0.1
Torasemid	BE biofilm total	6.1	4.2	4.3
Torasemid	BE activated sludge aq	5.4	4.3	4.3
Torasemid	BE activated sludge qq	0.0	0.2	0.1
Torasemid	BE activated sludge total	5.4	4.4	4.4
Tramadol	SC biofilm aq	6.0	4.3	4.9
Tramadol	SC biofilm qq	0.0	0.1	0.1
Tramadol	SC biofilm total	6.0	4.4	5.0
Tramadol	SC activated sludge aq	5.9	4.8	4.7
Tramadol	SC activated sludge qq	0.0	0.2	0.1
Tramadol	SC activated sludge total	5.9	4.9	4.9
Tramadol	BE biofilm aq	5.8	3.9	4.0
Tramadol	BE biofilm qq	0.0	0.1	0.2
Tramadol	BE biofilm total	5.8	4.0	4.1
Tramadol	BE activated sludge aq	6.1	4.4	3.6
Tramadol	BE activated sludge qq	0.0	0.2	0.4
Tramadol	BE activated sludge total	6.1	4.7	3.9
Venlafaxin	SC biofilm aq	6.1	4.2	4.3
Venlafaxin	SC biofilm qq	0.0	0.4	0.3
Venlafaxin	SC biofilm total	6.1	4.6	4.6
Venlafaxin	SC activated sludge aq	5.2	4.2	4.2
Venlafaxin	SC activated sludge qq	0.0	0.2	0.2
Venlafaxin	SC activated sludge total	5.2	4.4	4.4
Venlafaxin	BE biofilm aq	5.6	3.3	3.1
Venlafaxin	BE biofilm qq	0.0	0.5	0.5
Venlafaxin	BE biofilm total	5.6	3.7	3.6
Venlafaxin	BE activated sludge aq	5.4	4.2	3.6
Venlafaxin	BE activated sludge qq	0.0	0.3	0.5
Venlafaxin	BE activated sludge total	5.4	4.5	4.0

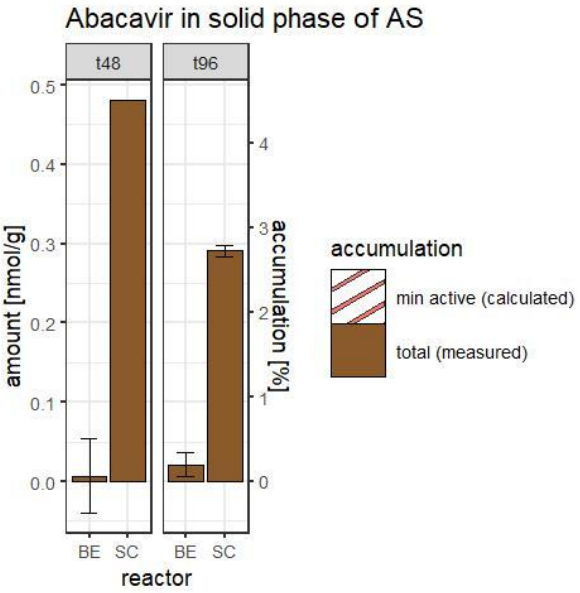
Table 16: Mass balance results of all compounds with an ISTD. SC stands for the sorption control reactor, BE for the biotic experiment reactor. aq stands for the amount in the aqueous sample, qq stands for the amount in the solid sample extracted with QuEChERS. total is the sum of both phases and should ideally add up to 5 nmol in the sorption control reactors.

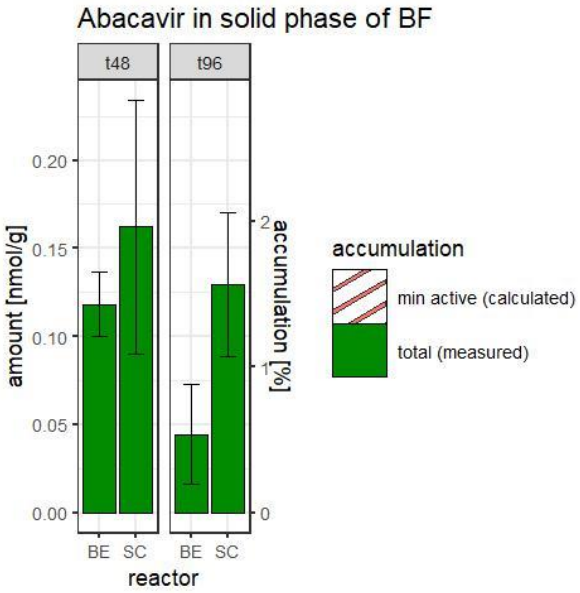
B.3.2 Plots of accumulation and mass balance

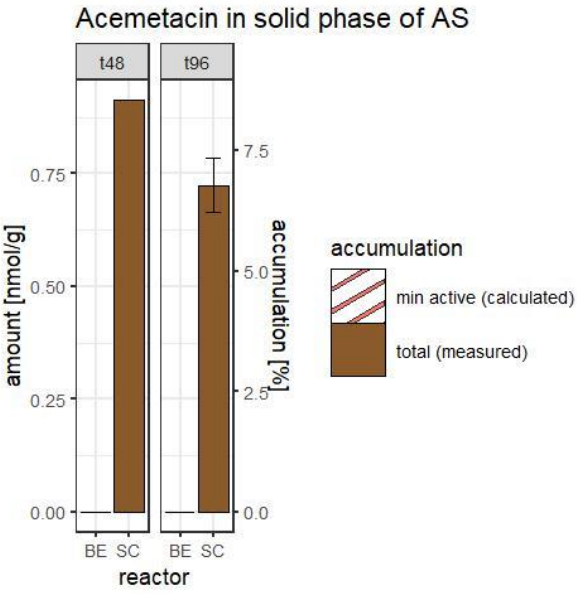
On the following pages, the plots of accumulation of all compounds are presented in alphabetical order. BE stands for biotic experiment, SC for sorption control, BF for biofilm, AS for activated sludge. For both time points, in green/brown the total accumulated amount in nmol substance / g freeze-dried biomass (left y-axis) and the corresponding percentage accumulation (right y-axis) are plotted. The percentage accumulation is different for biofilm and activated sludge due to different biomass weights. The minimal active bioaccumulation is calculated as difference between the accumulated amount in the biotic experiment and in the sorption control and is represented with red stripes.

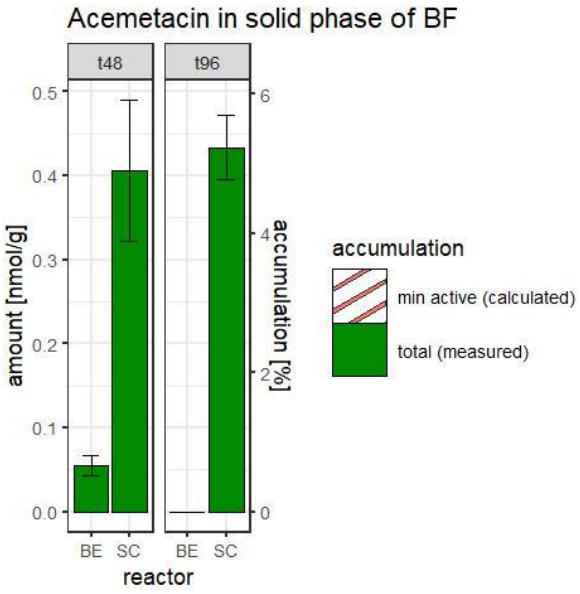
They are followed by plots of the mass balance of compounds with an internal standard in alphabetical order. AS stands for activated sludge, BF for biofilm. The initially spiked amount was 5 nmol and is represented with a horizontal black line. In blue, the amount of substances in the aqueous sample is represented; in grey the amount of substances in the solid sample (accumulated) is represented.

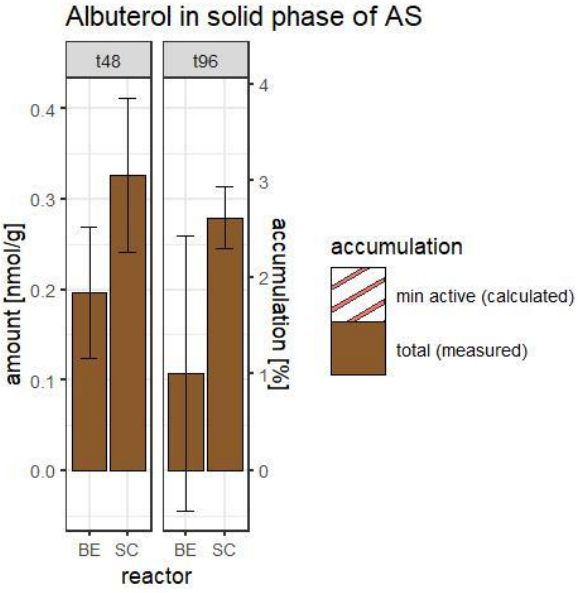
Accumulation plots

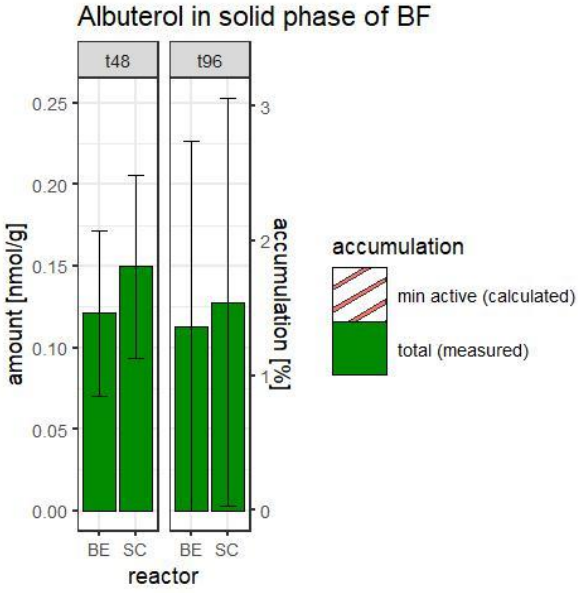


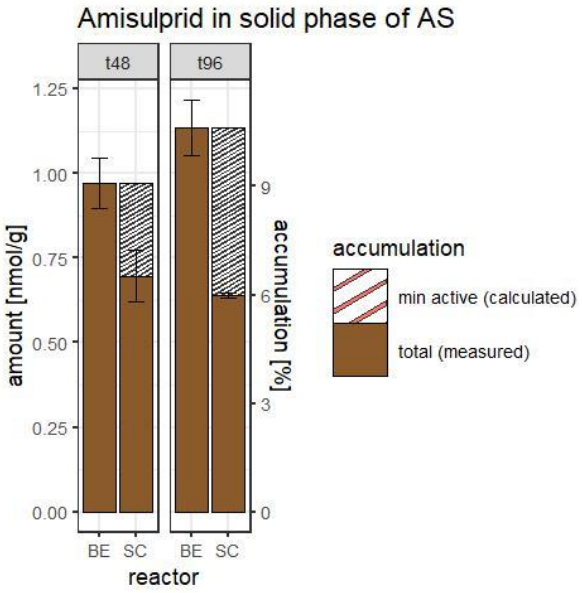


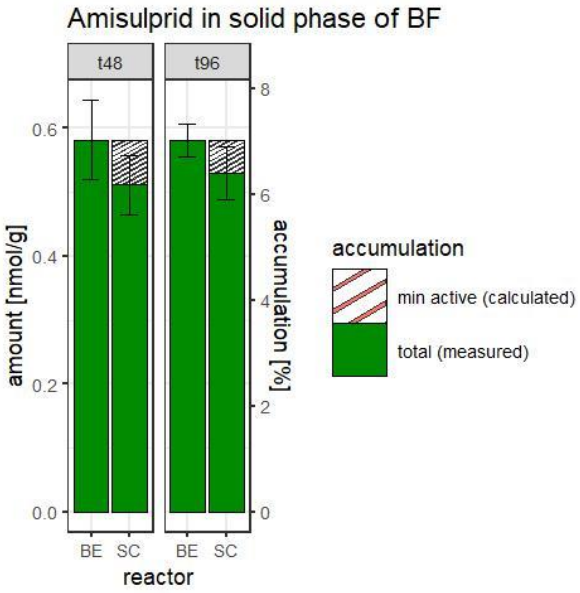


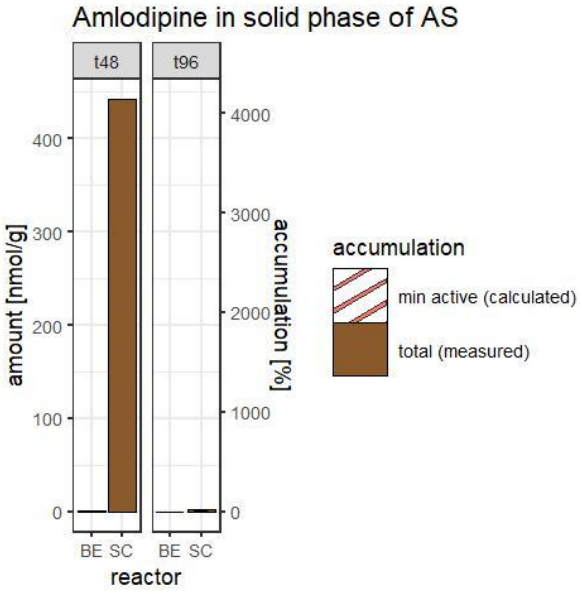


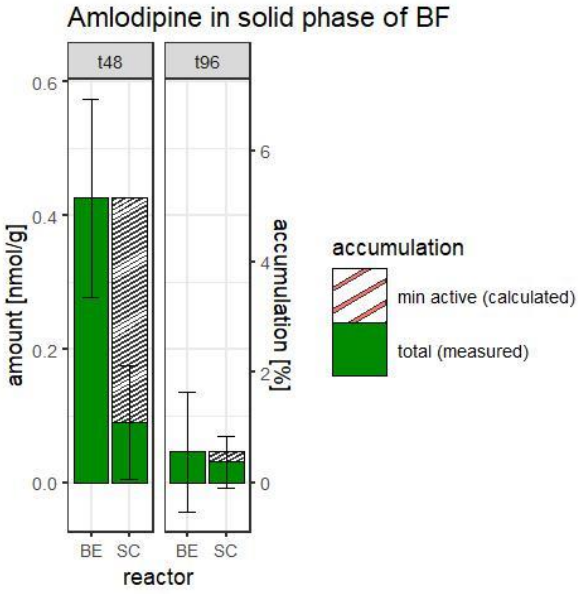


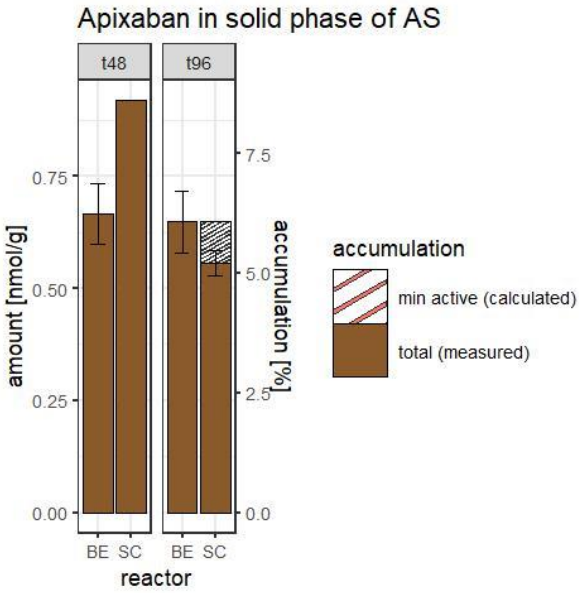


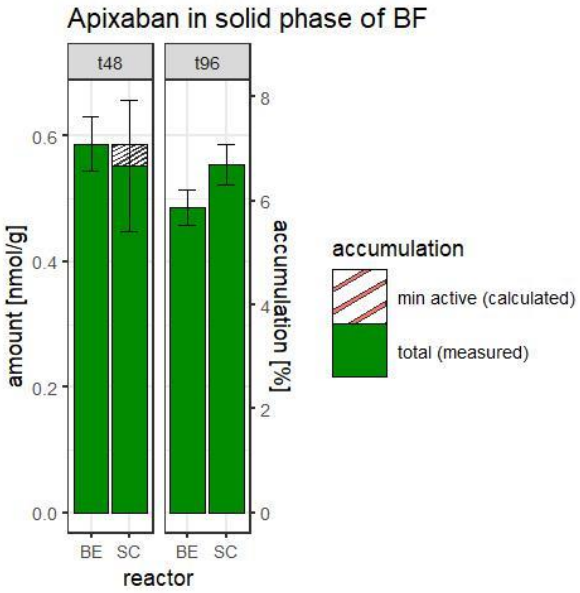

Amisulprid in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

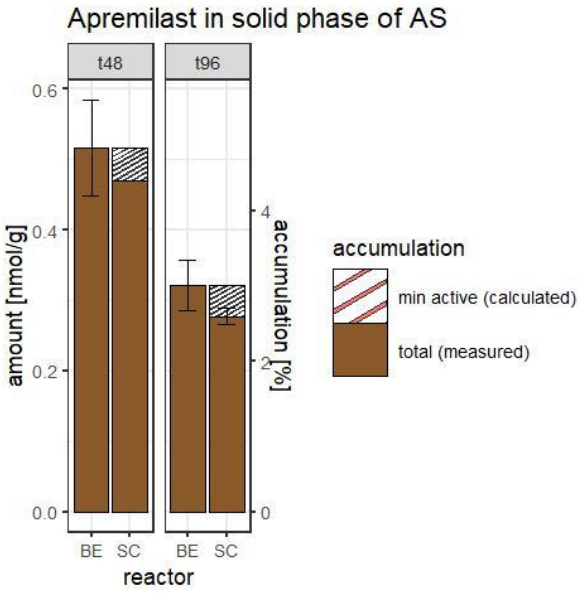

Amisulprid in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

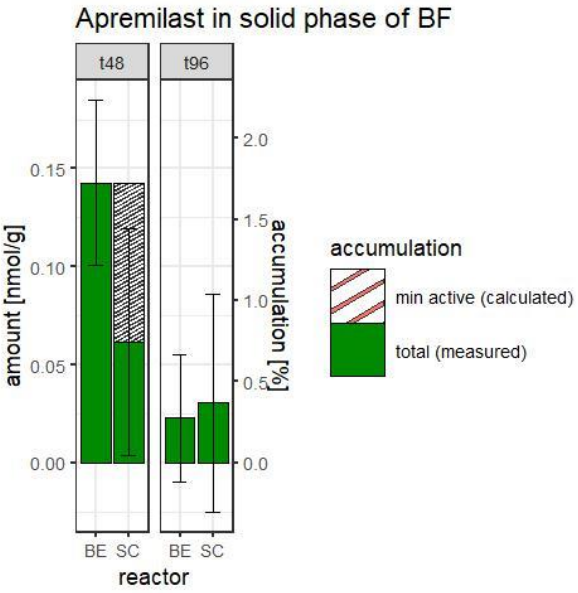

Amlodipine in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

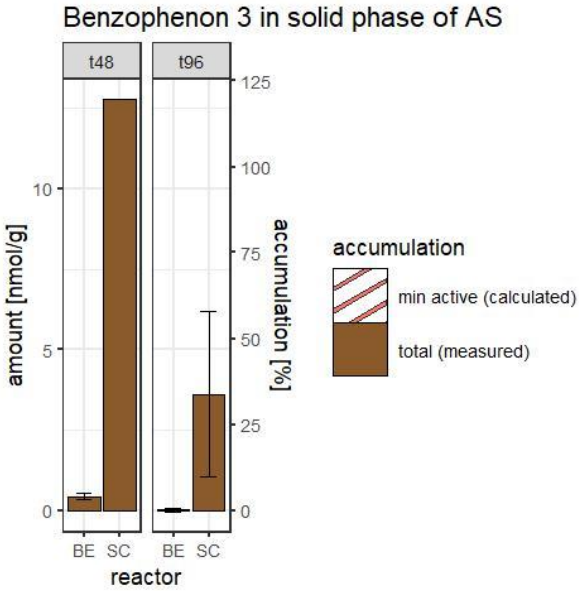

Amlodipine in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

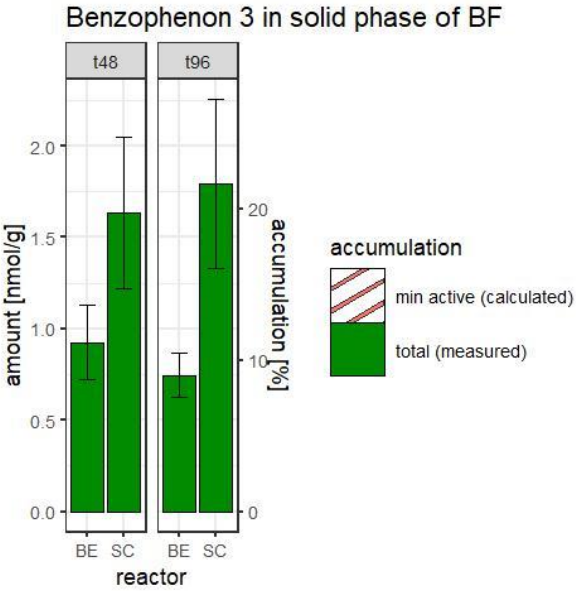

Apixaban in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

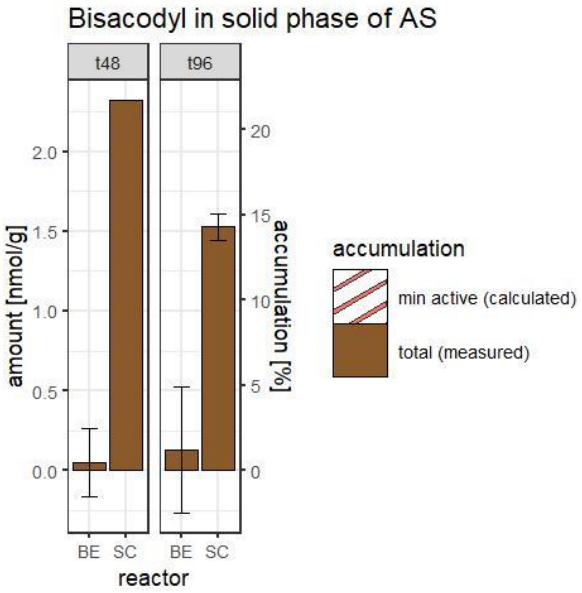

Apixaban in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

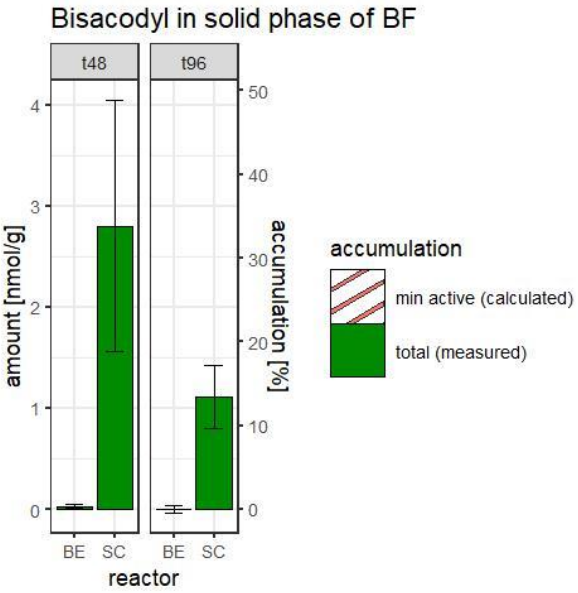

Apremilast in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
reactor
BE
SC
accumulation
min active (calculated)
total (measured)

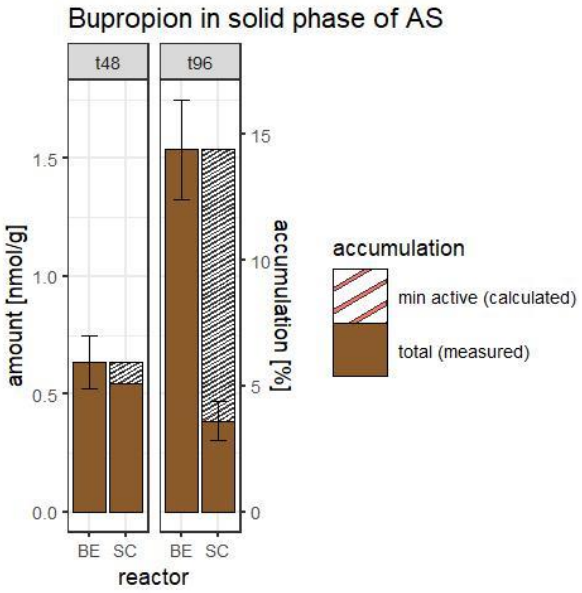

Apremilast in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
reactor
BE
SC
accumulation
min active (calculated)
total (measured)

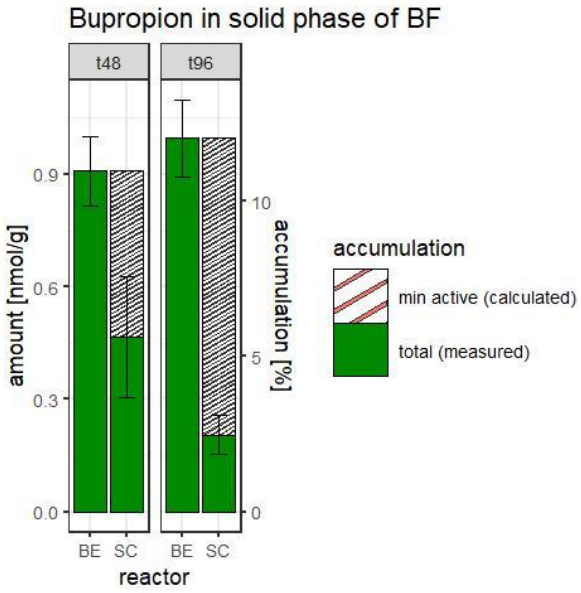

Benzophenon 3 in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
reactor
BE
SC
accumulation
min active (calculated)
total (measured)

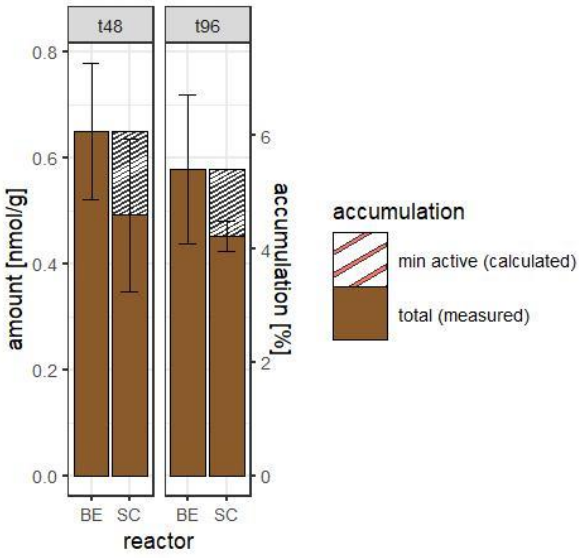

Benzophenon 3 in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
reactor
BE
SC
accumulation
min active (calculated)
total (measured)

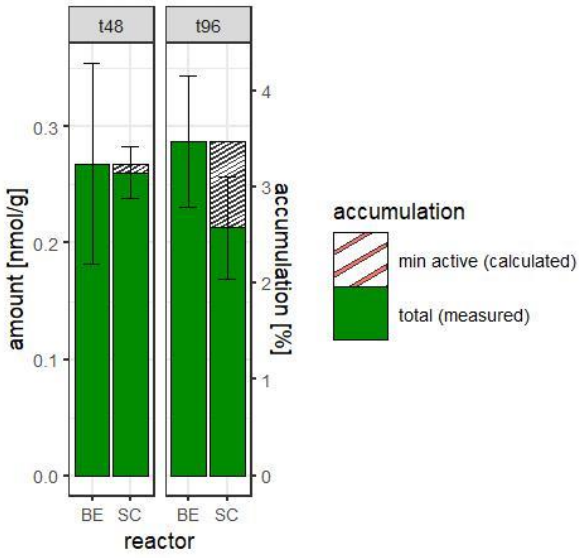

Bisacodyl in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
reactor
BE
SC
accumulation
min active (calculated)
total (measured)

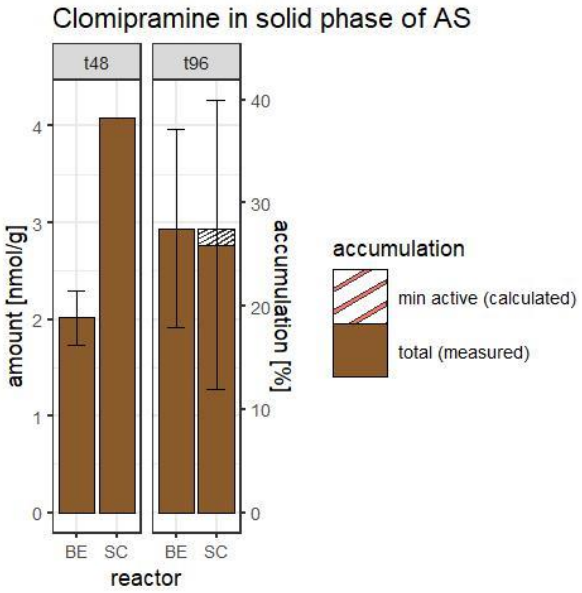

Bisacodyl in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
reactor
BE
SC
accumulation
min active (calculated)
total (measured)

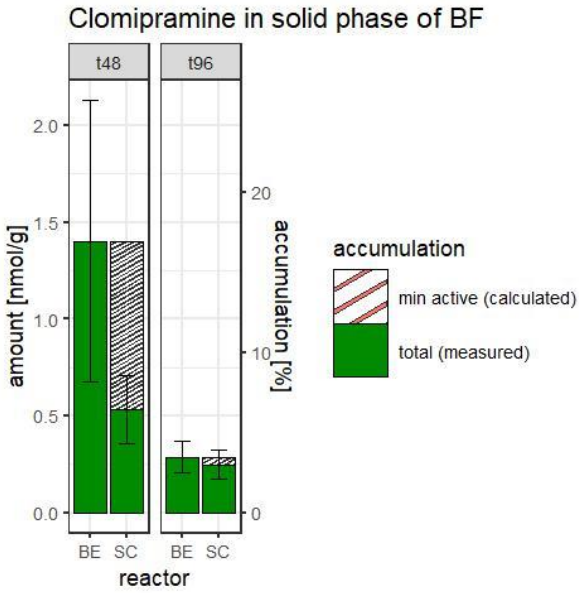

Bupropion in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

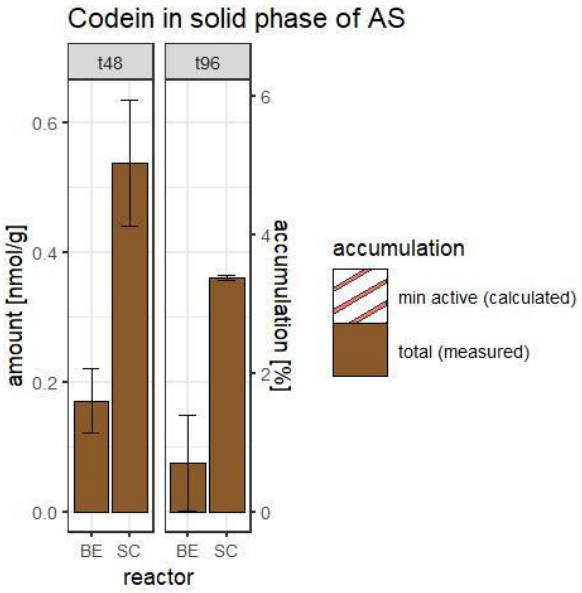

Bupropion in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

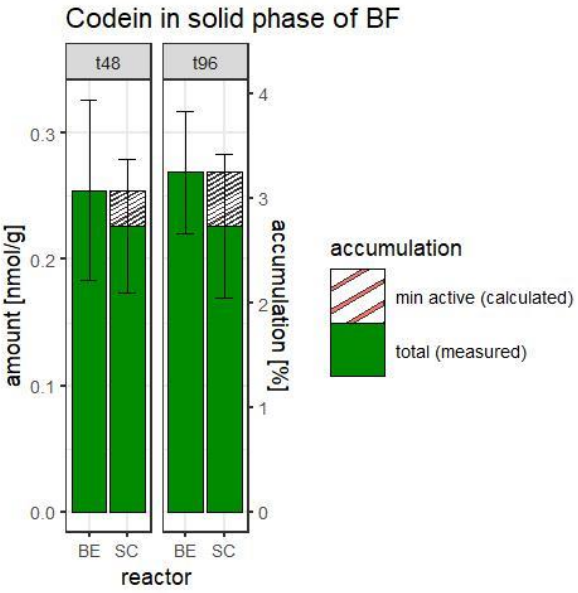

Chlorotoluron in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

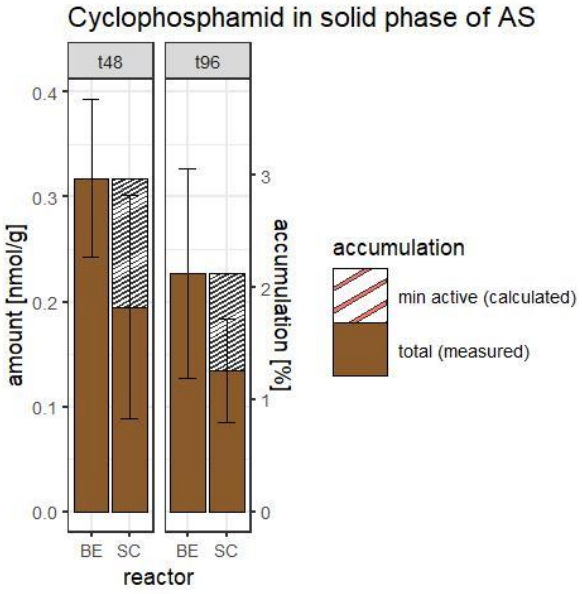

Chlorotoluron in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

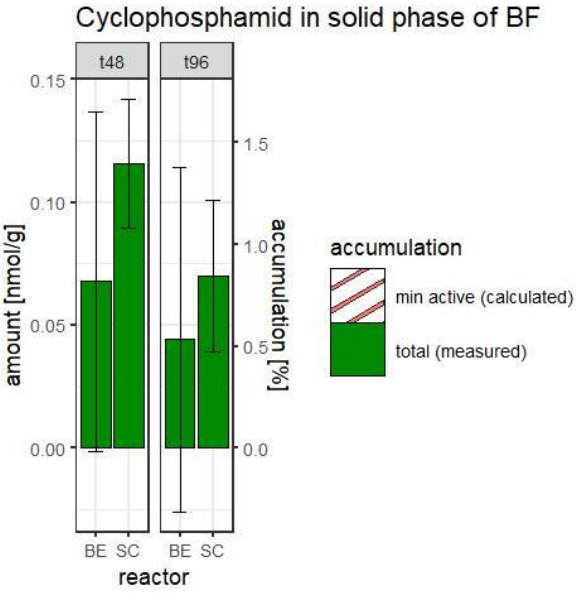

Clomipramine in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

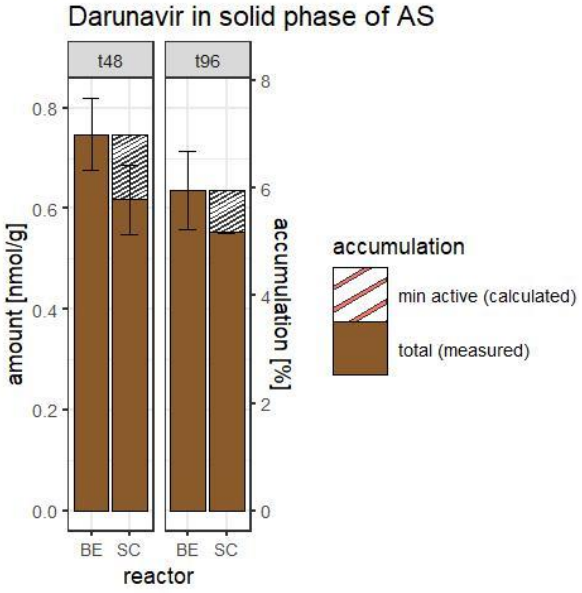

Clomipramine in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

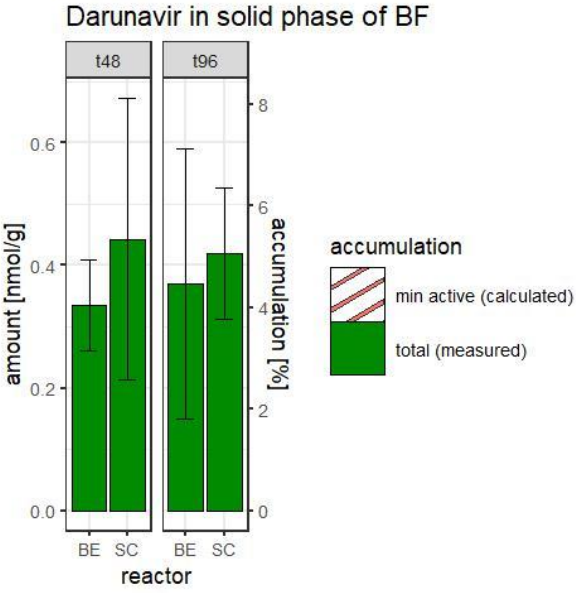

Codein in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE
SC
reactor

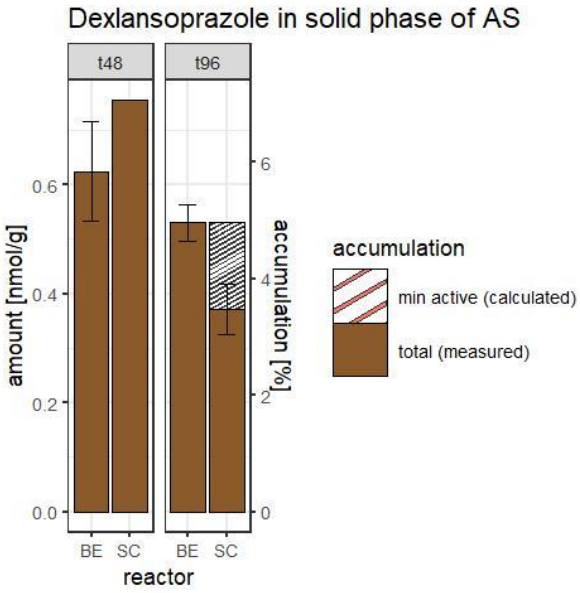

Codein in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE
SC
reactor

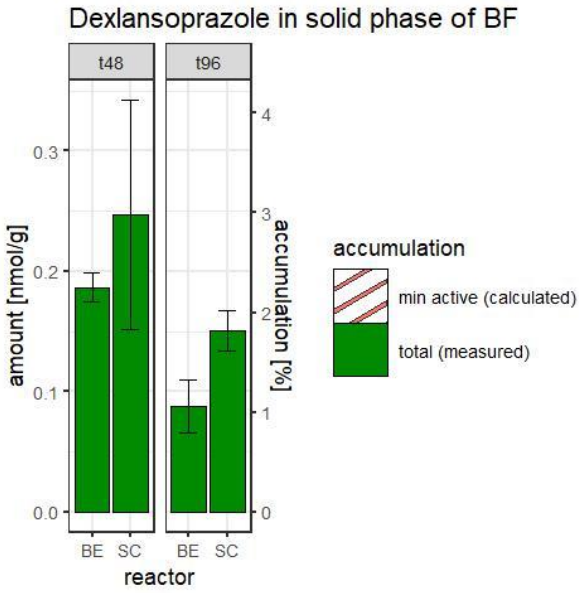

Cyclophosphamid in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE
SC
reactor

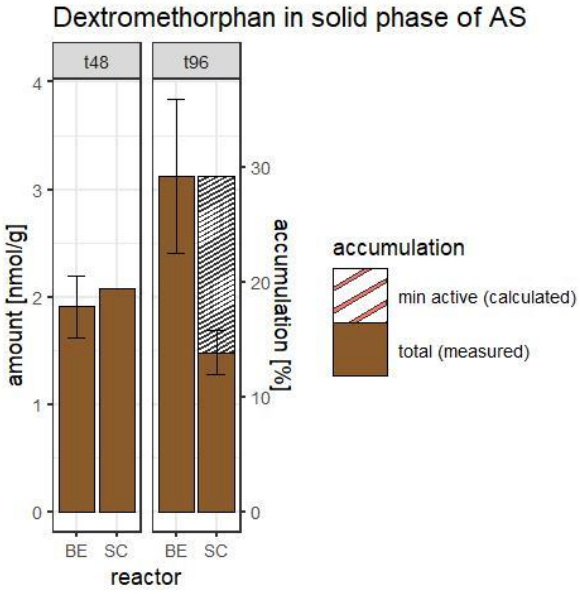

Cyclophosphamid in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE
SC
reactor

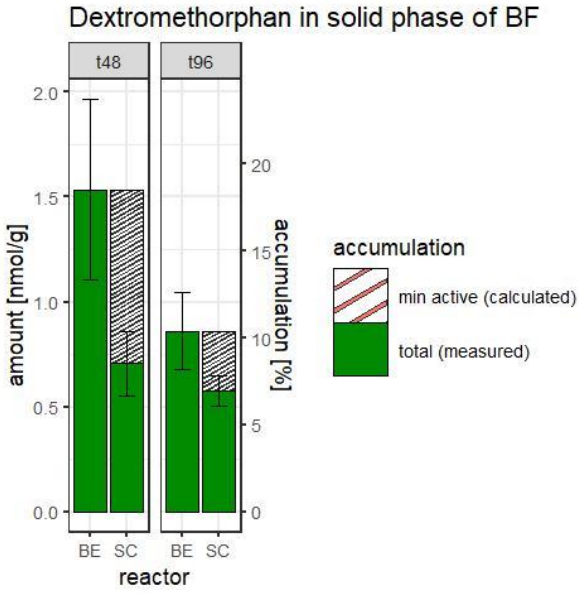

Darunavir in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE
SC
reactor

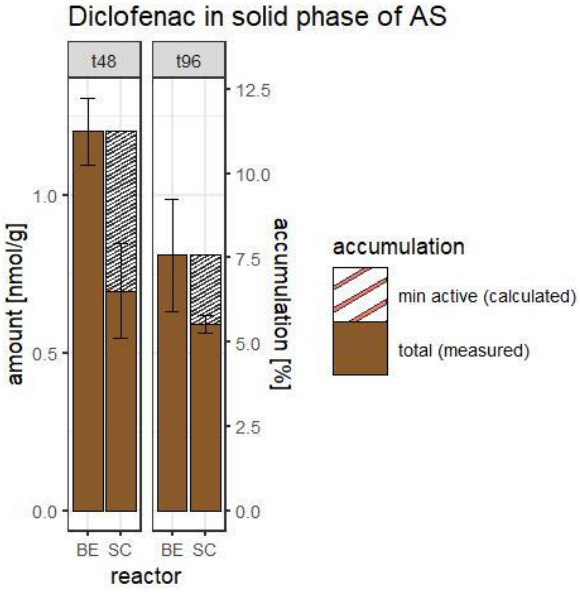

Darunavir in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE
SC
reactor

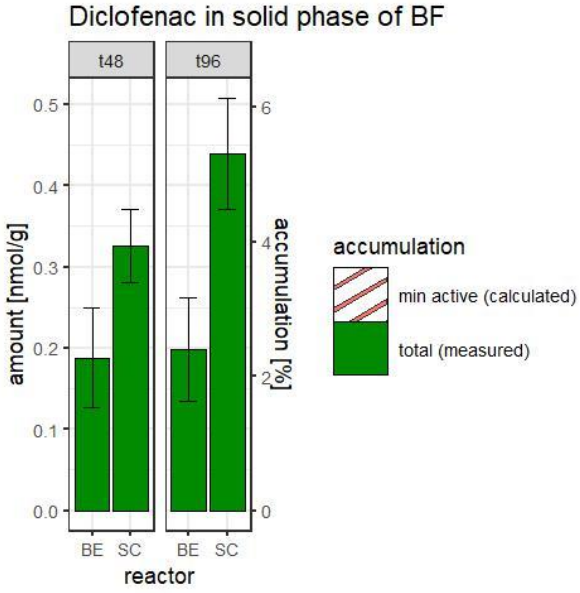

Dexlansoprazole in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

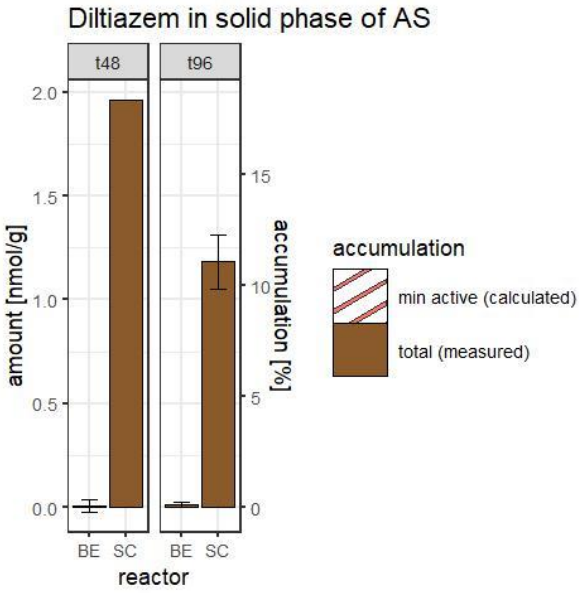

Dexlansoprazole in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

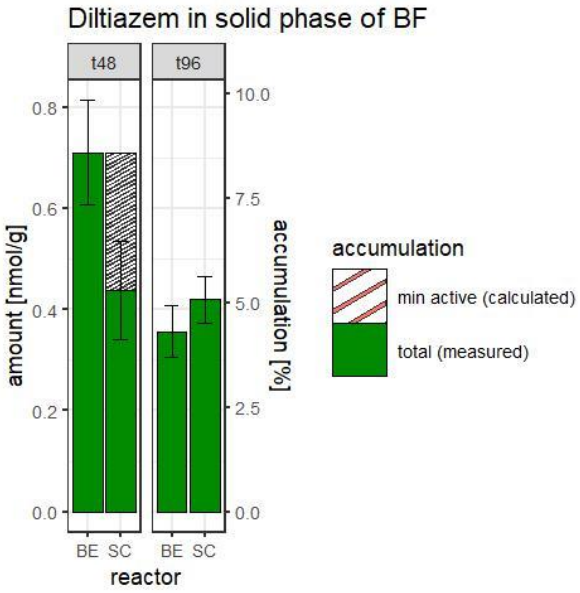

Dextromethorphan in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

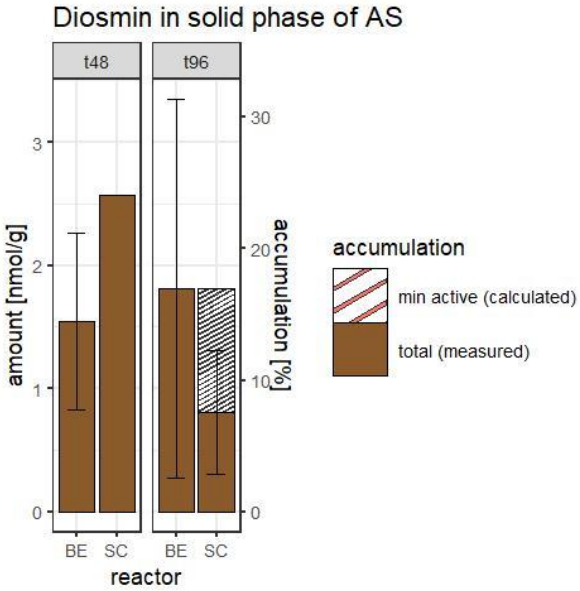

Dextromethorphan in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

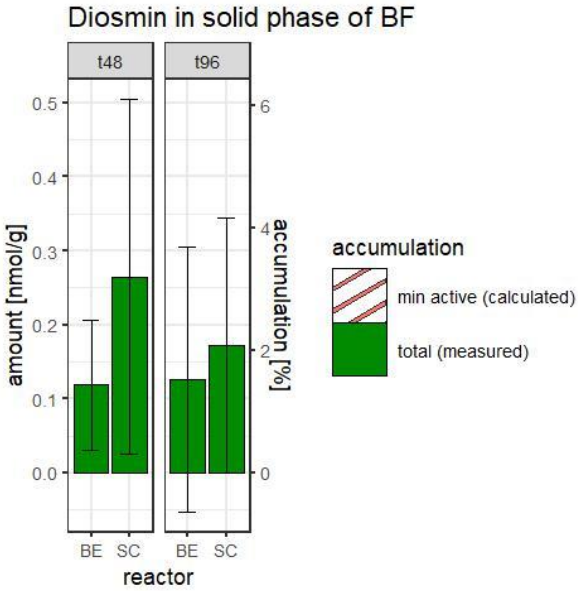

Diclofenac in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

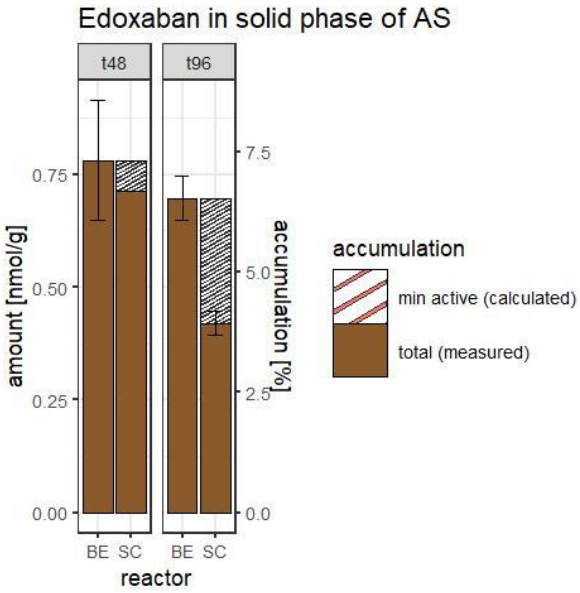

Diclofenac in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

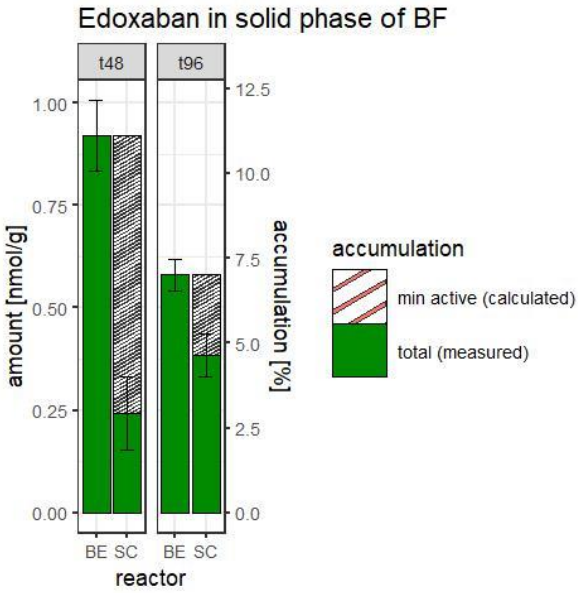

Diltiazem in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

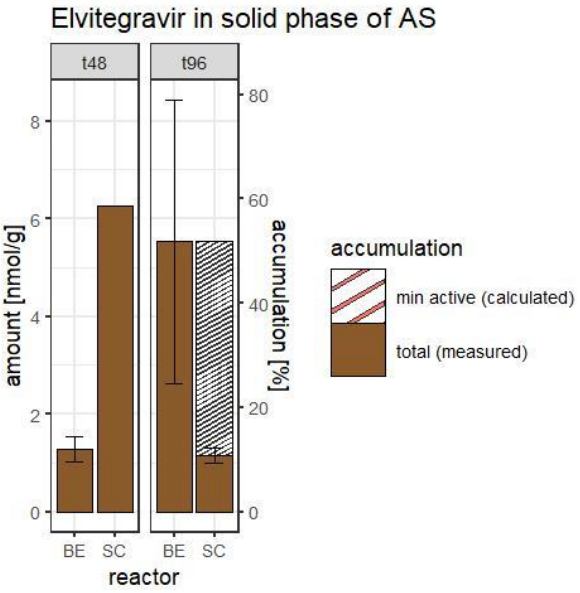

Diltiazem in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

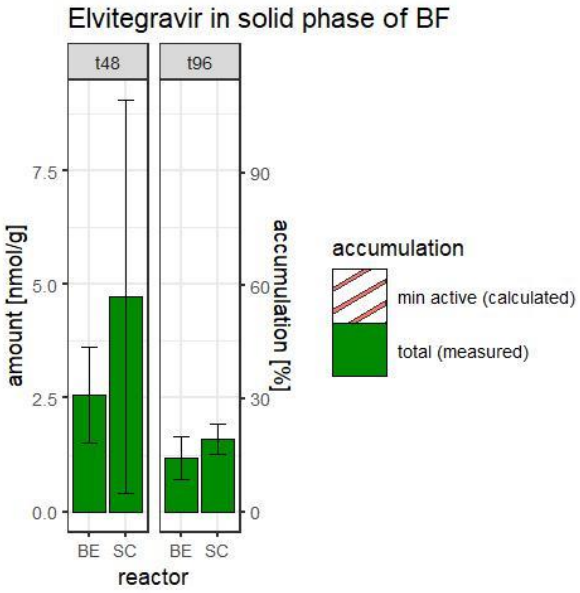

Diosmin in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

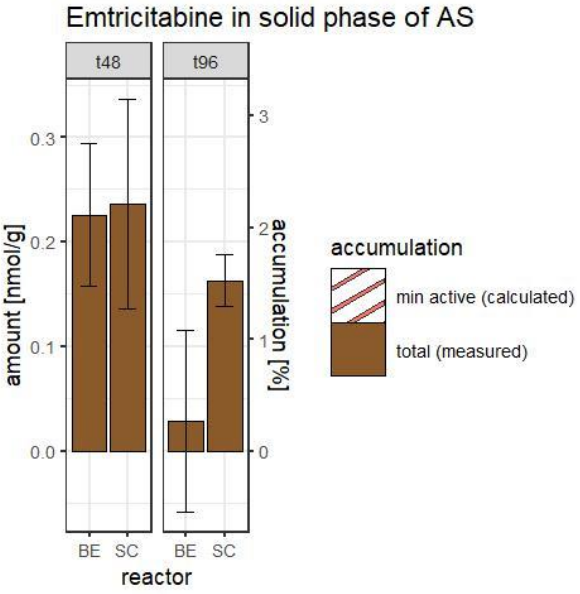

Diosmin in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

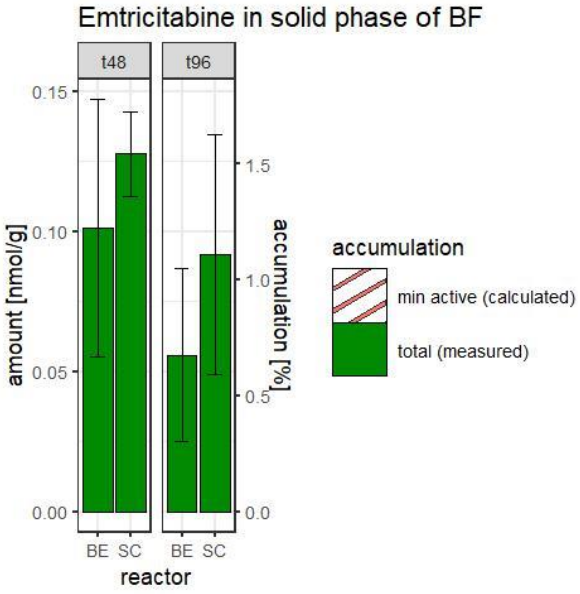

Edoxaban in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

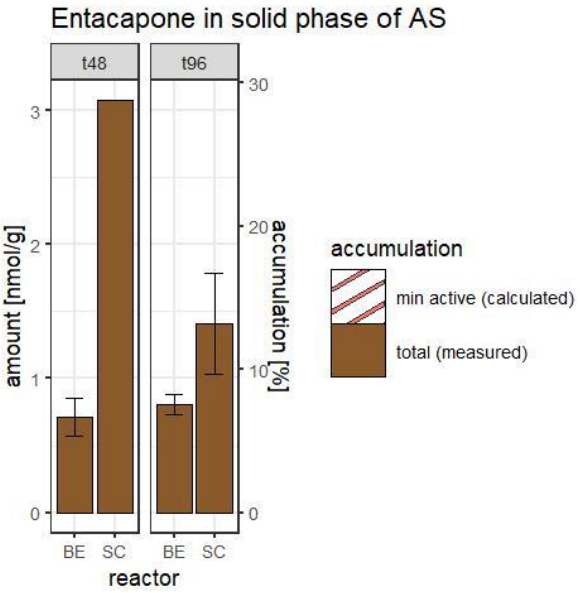

Edoxaban in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

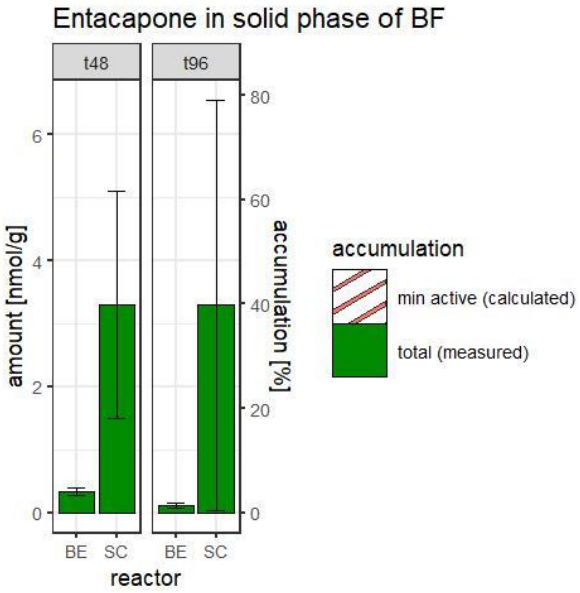

Elvitegravir in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

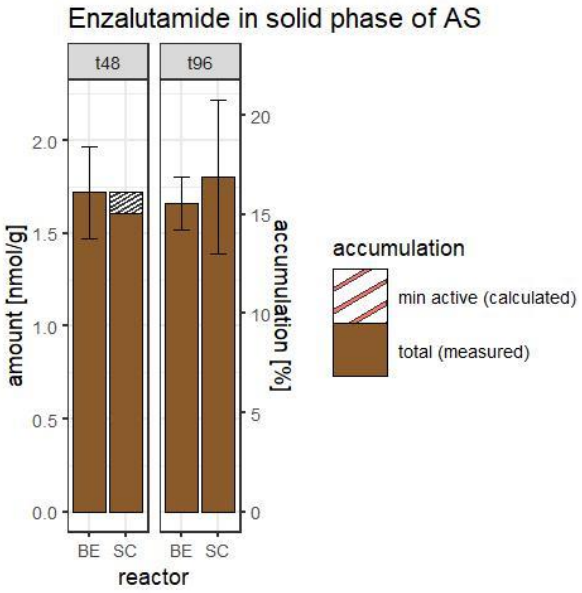

Elvitegravir in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

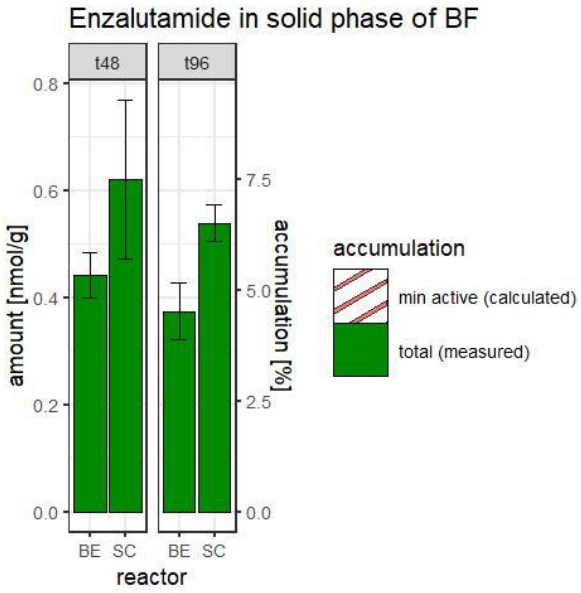

Emtricitabine in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

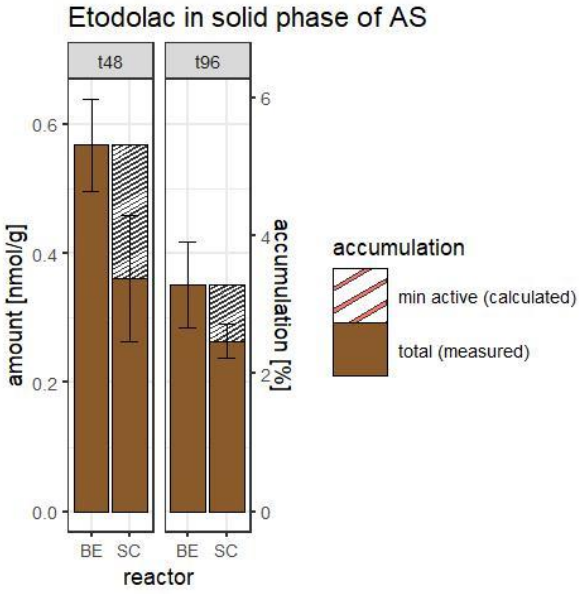

Emtricitabine in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

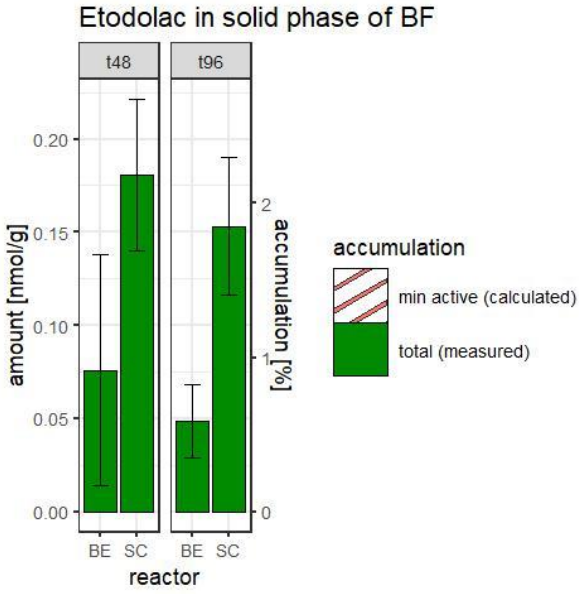

Entacapone in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

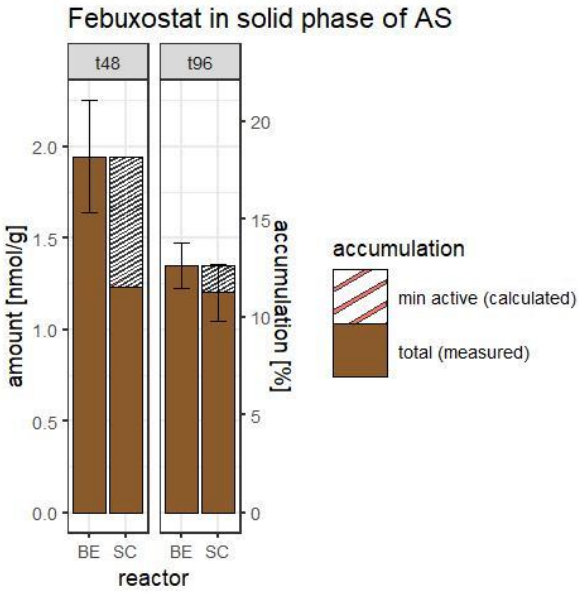

Entacapone in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

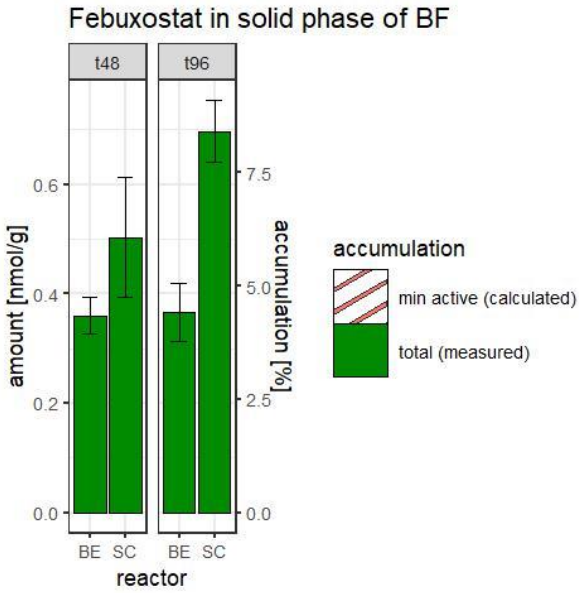

Enzalutamide in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

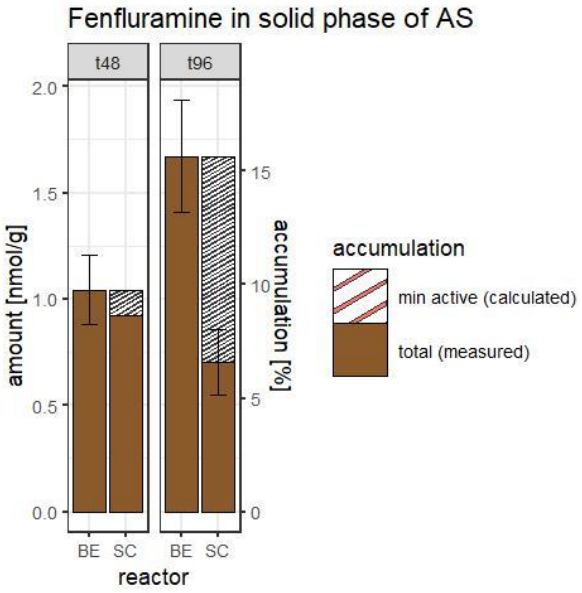

Enzalutamide in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

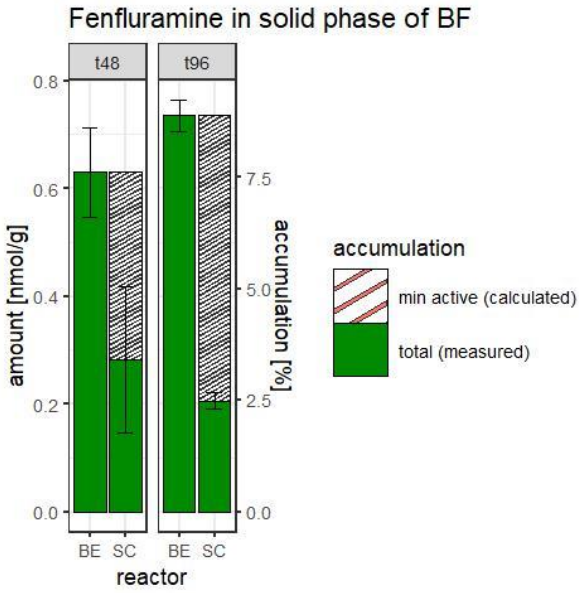

Etodolac in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

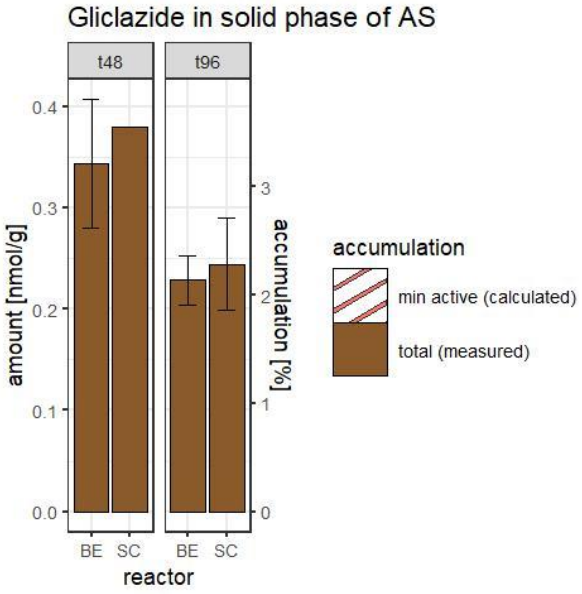

Etodolac in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

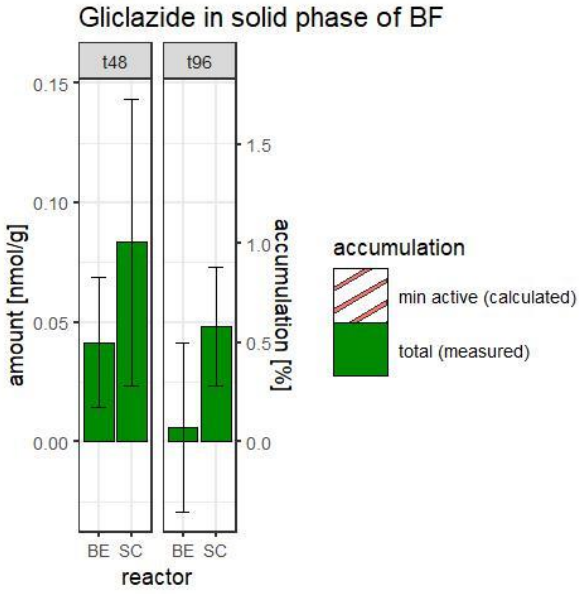

Febuxostat in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

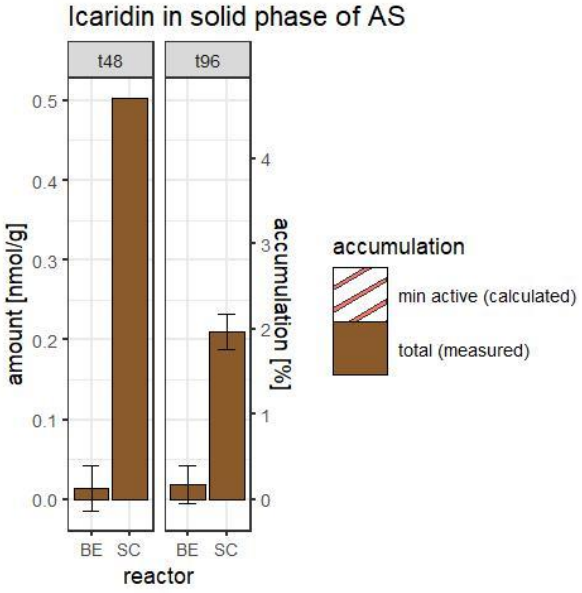

Febuxostat in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

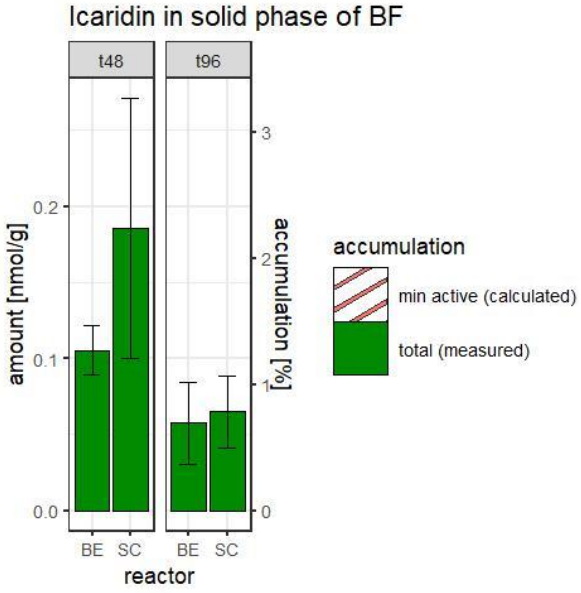

Fenfluramine in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

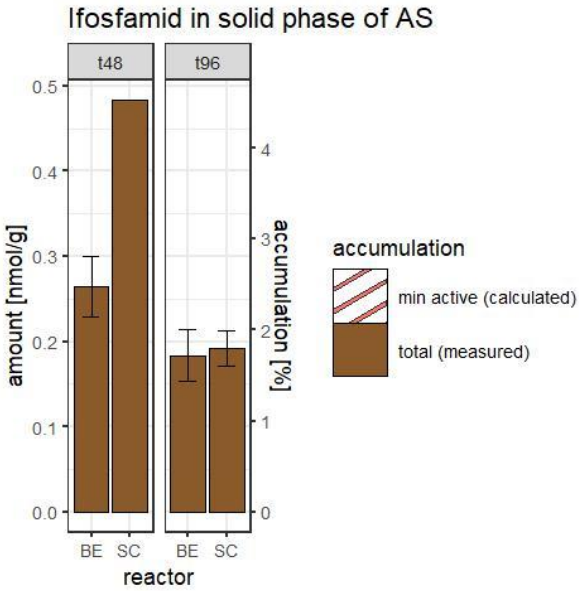

Fenfluramine in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

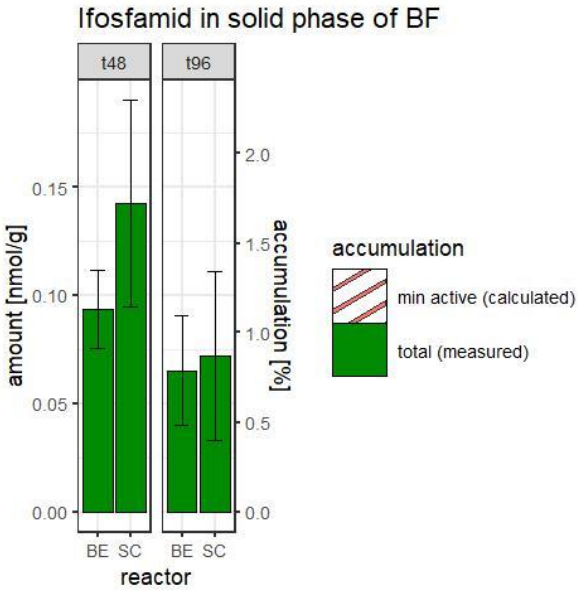

Gliclazide in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

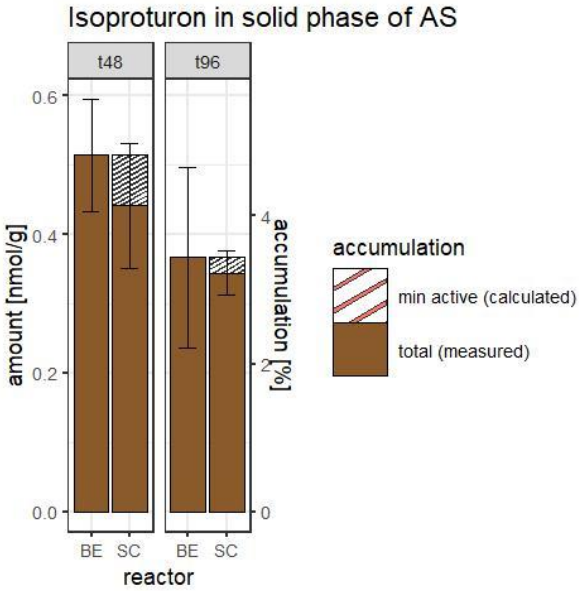

Gliclazide in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

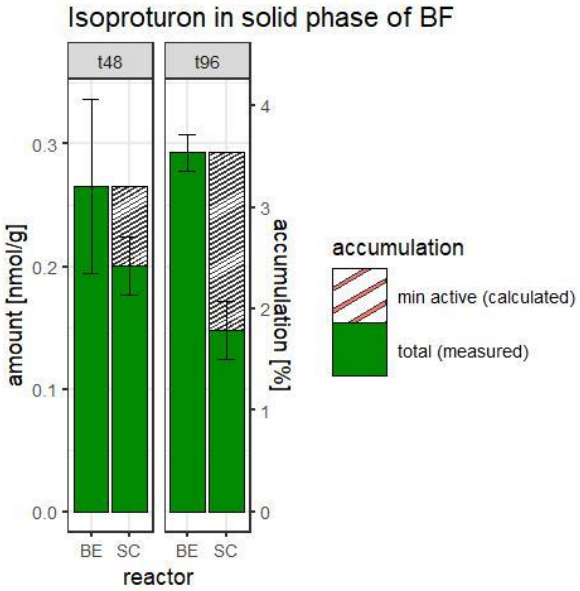

Icaridin in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

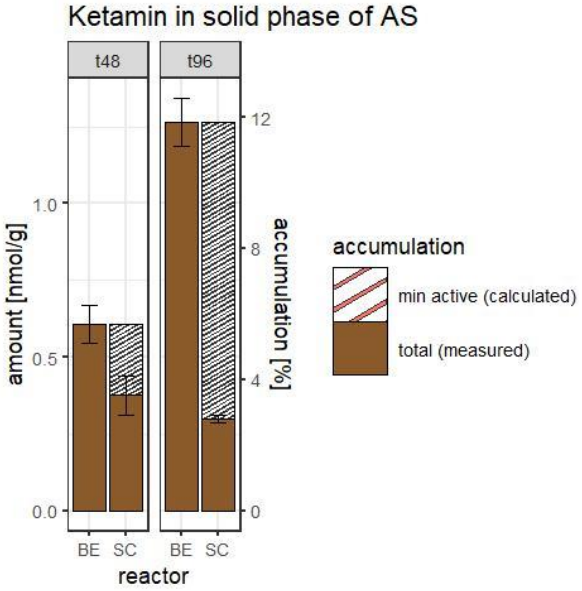

Icaridin in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

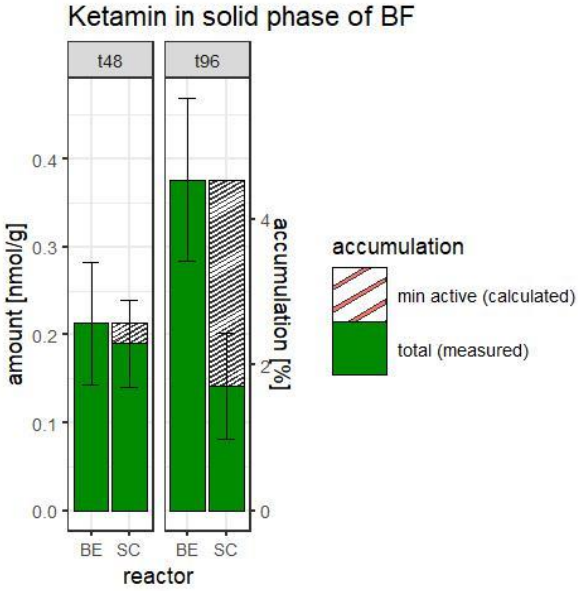

Ifosfamid in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

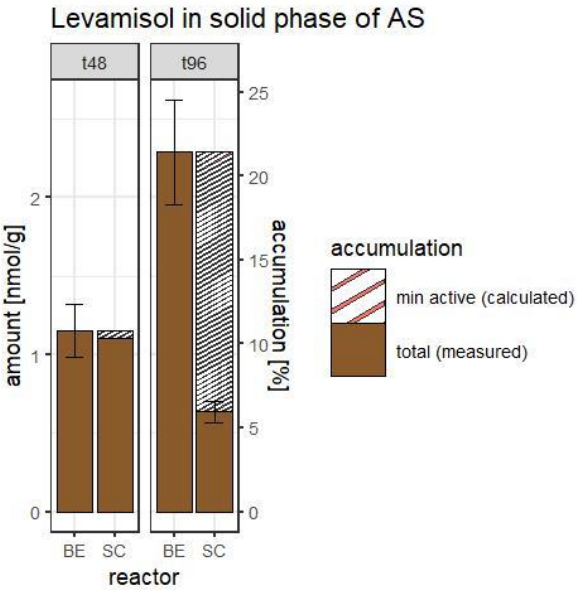

Ifosfamid in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

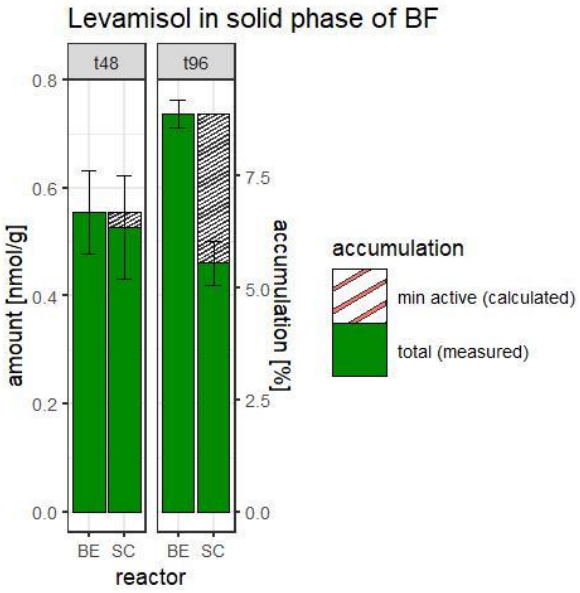

Isoproturon in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

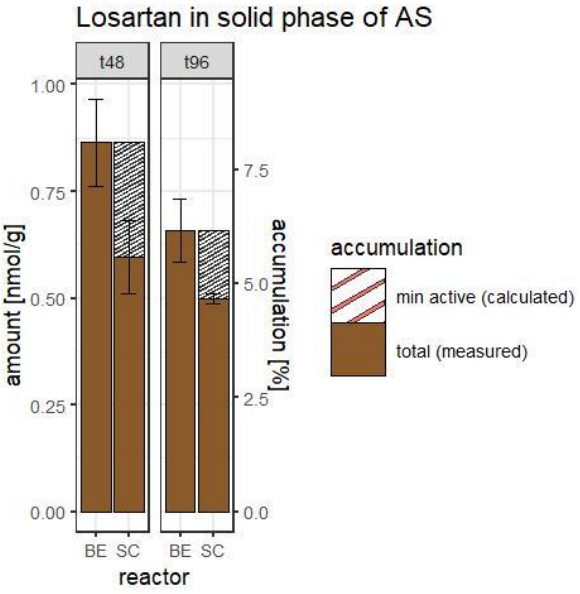

Isoproturon in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

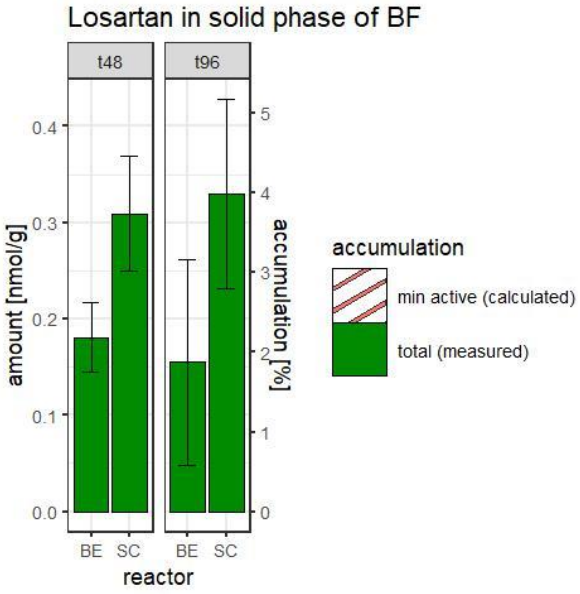

Ketamin in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

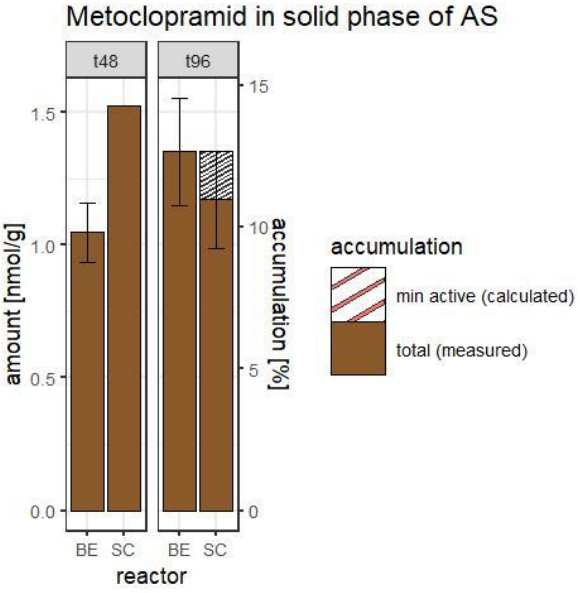

Ketamin in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

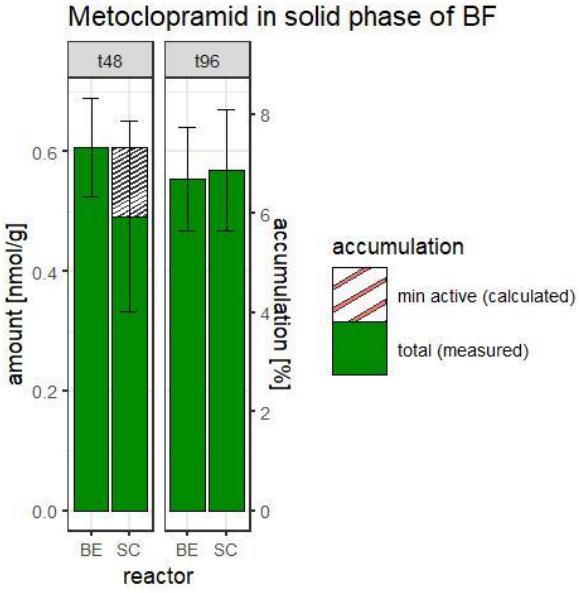

Levamisol in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

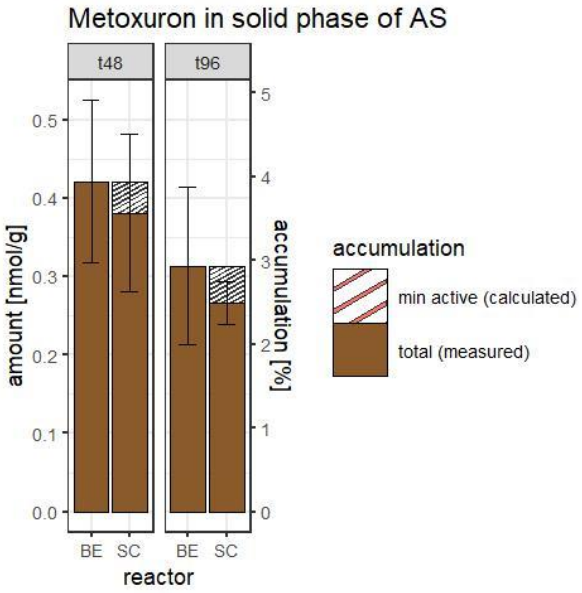

Levamisol in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

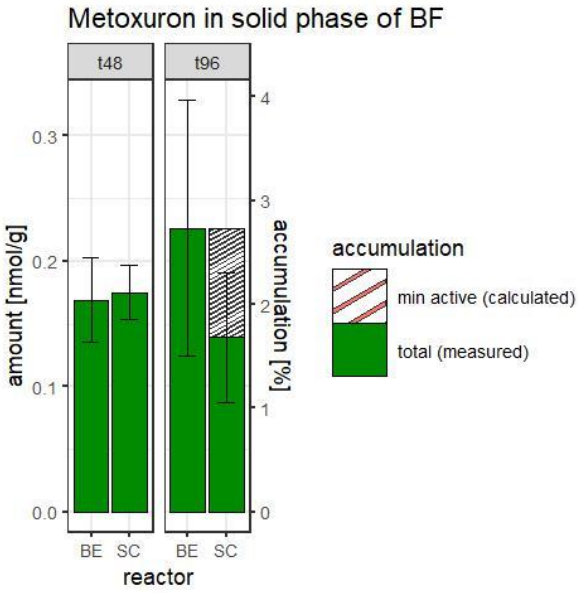

Losartan in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

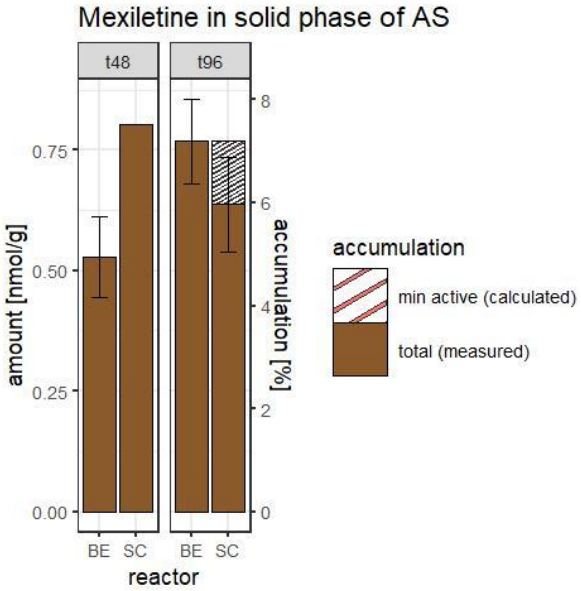

Losartan in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

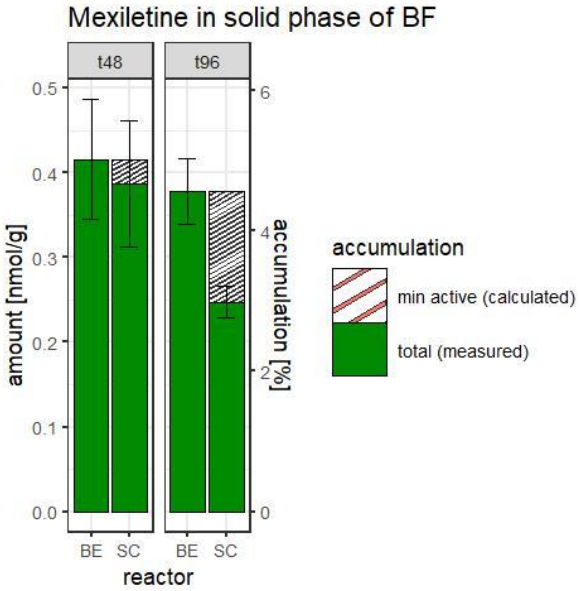

Metoclopramid in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

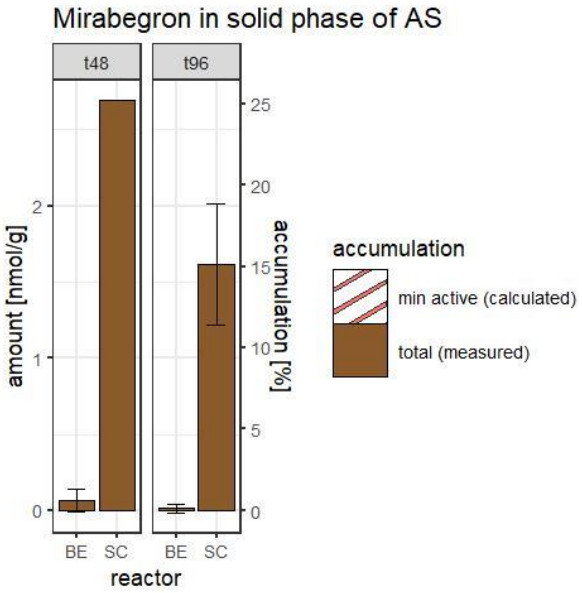

Metoclopramid in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

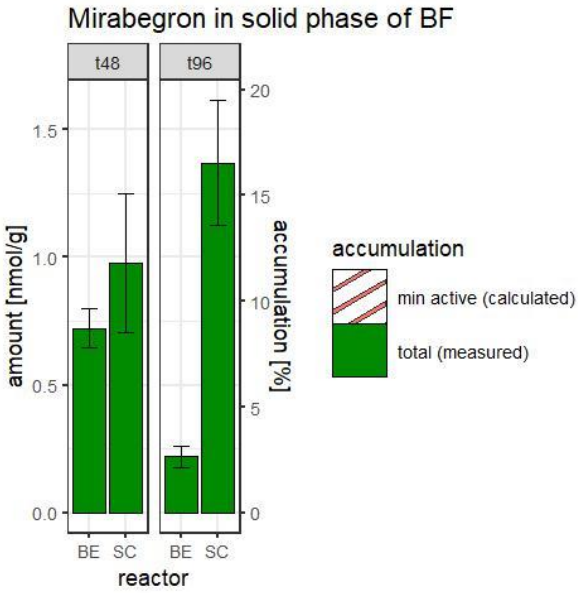

Metoxuron in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE
SC
reactor

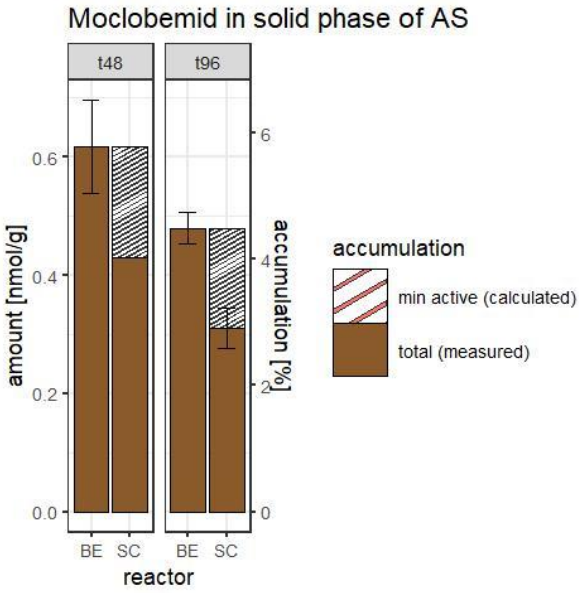

Metoxuron in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE
SC
reactor

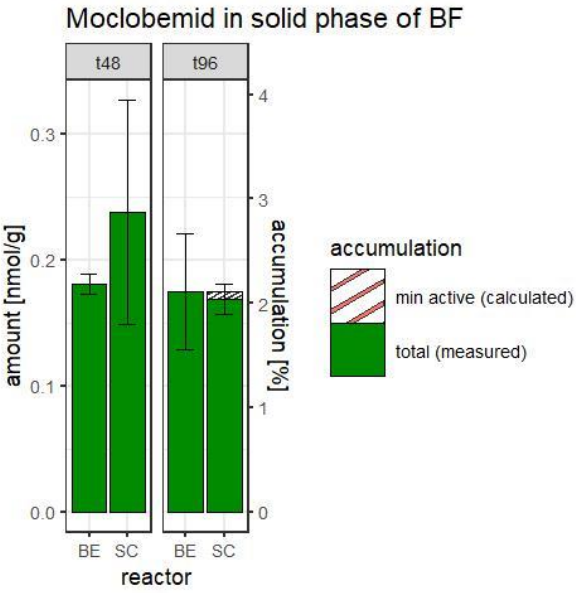

Mexiletine in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE
SC
reactor

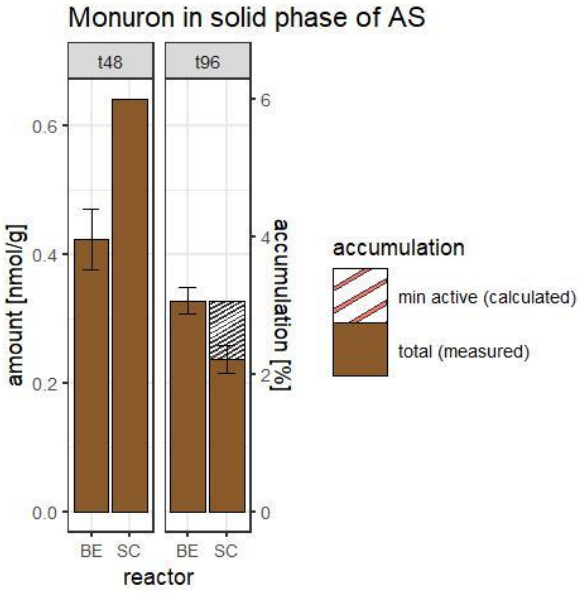

Mexiletine in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE
SC
reactor

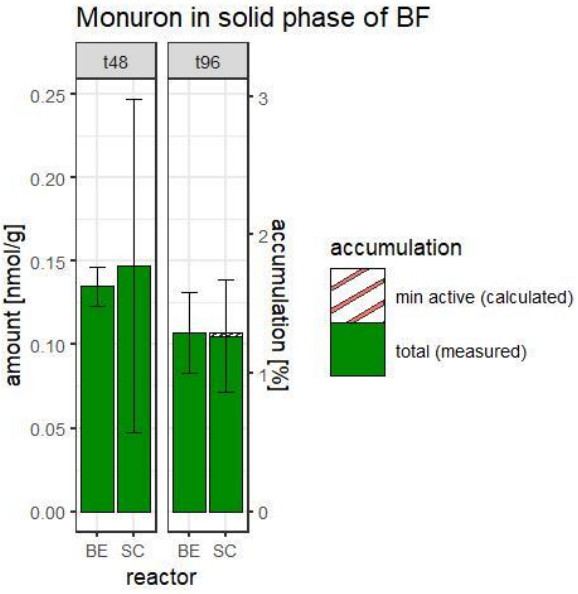

Mirabegron in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE
SC
reactor

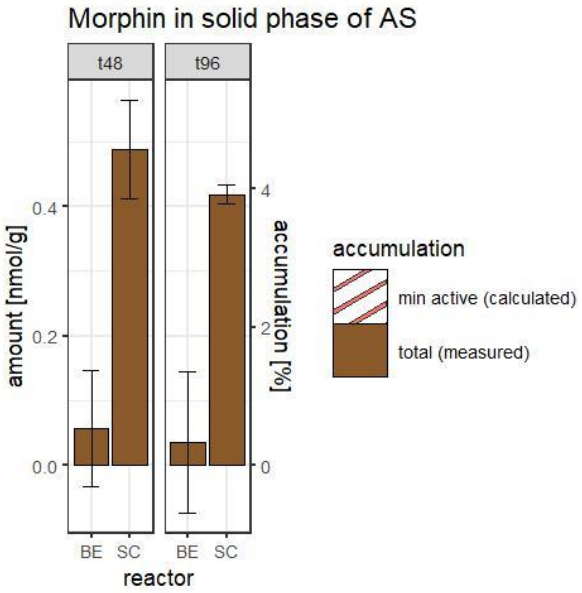

Mirabegron in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE
SC
reactor

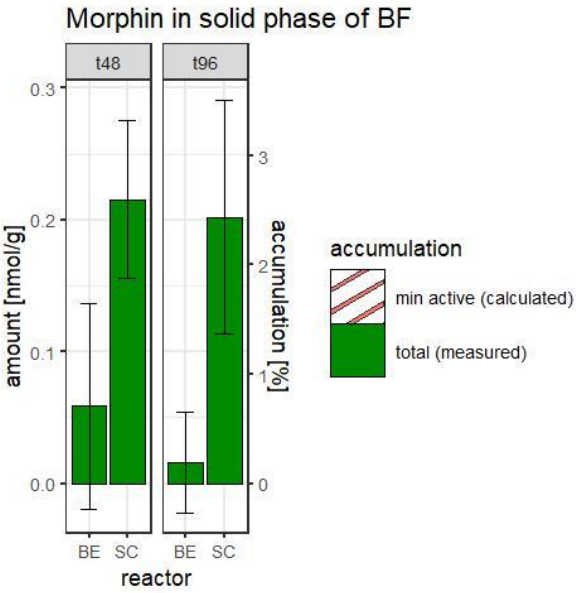

Moclobemid in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE
SC
reactor
accumulation
min active (calculated)
total (measured)

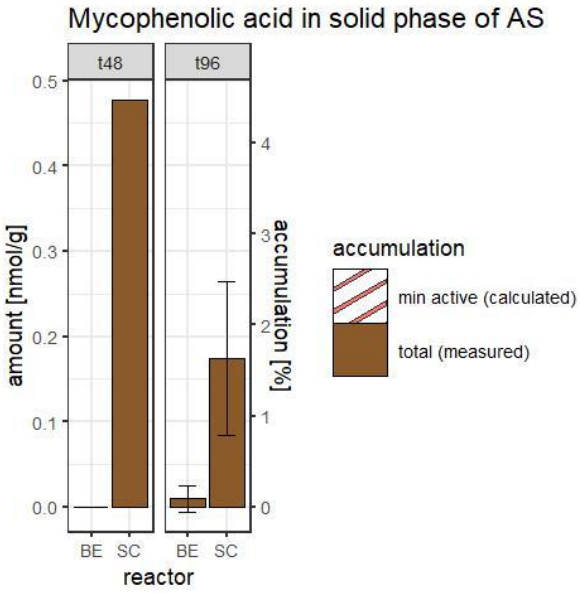

Moclobemid in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE
SC
reactor
accumulation
min active (calculated)
total (measured)

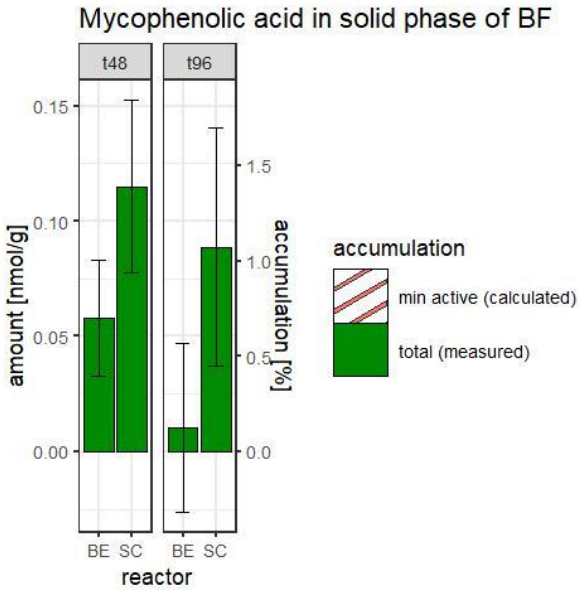

Monuron in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE
SC
reactor
accumulation
min active (calculated)
total (measured)

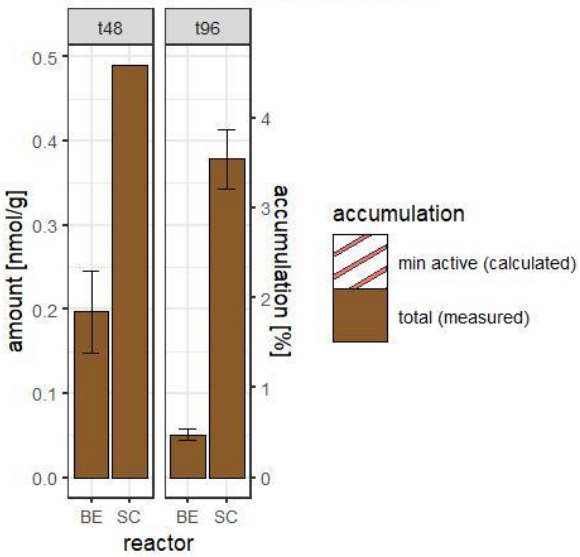

Monuron in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE
SC
reactor
accumulation
min active (calculated)
total (measured)

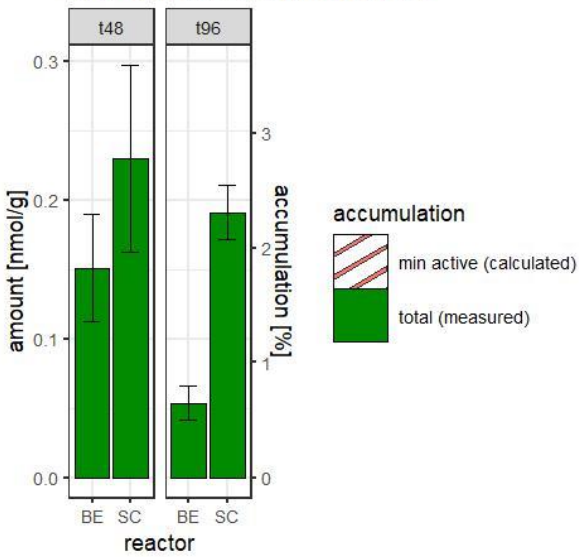

Morphin in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE
SC
reactor
accumulation
min active (calculated)
total (measured)

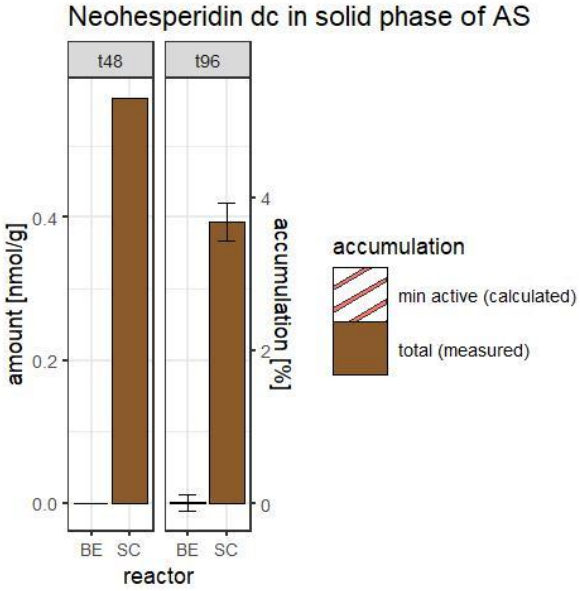

Morphin in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE
SC
reactor
accumulation
min active (calculated)
total (measured)

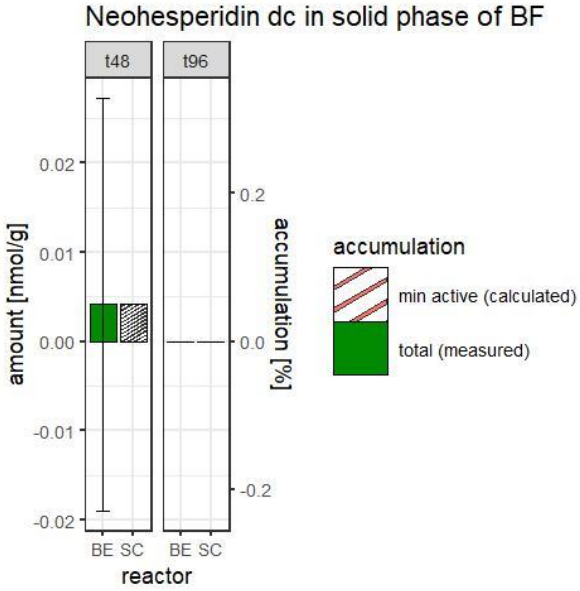

Mycophenolic acid in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

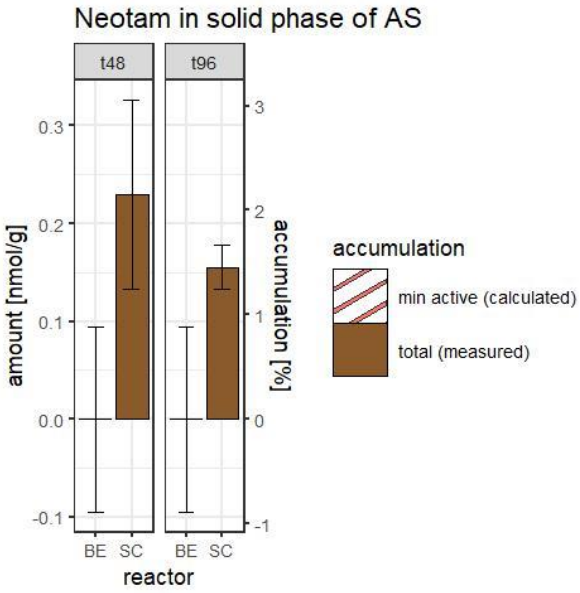

Mycophenolic acid in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

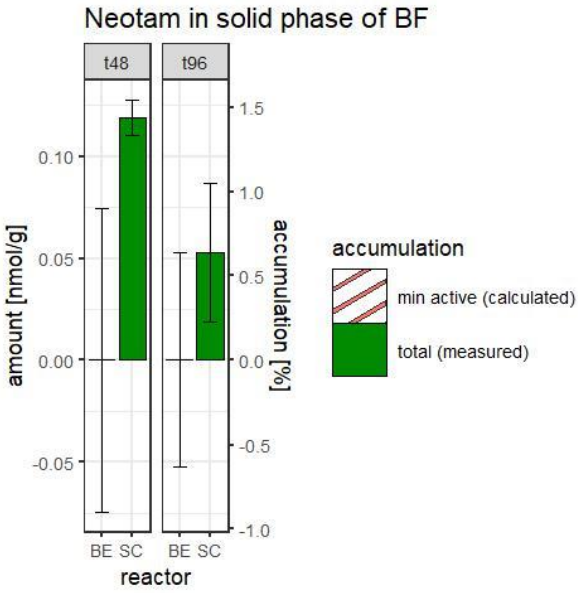

Naloxone in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

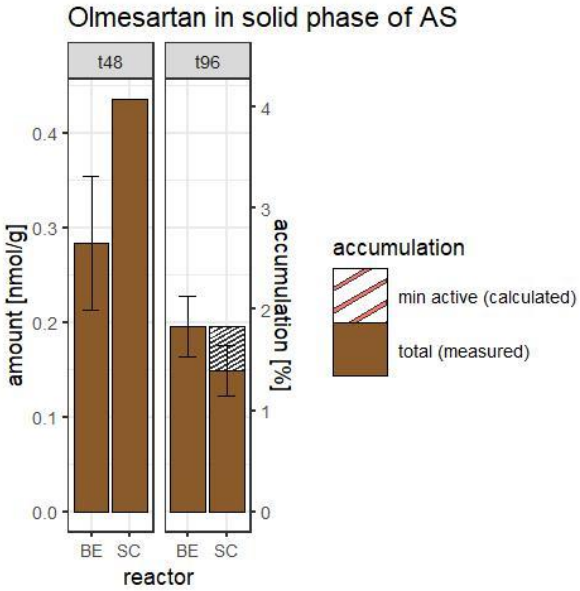

Naloxone in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

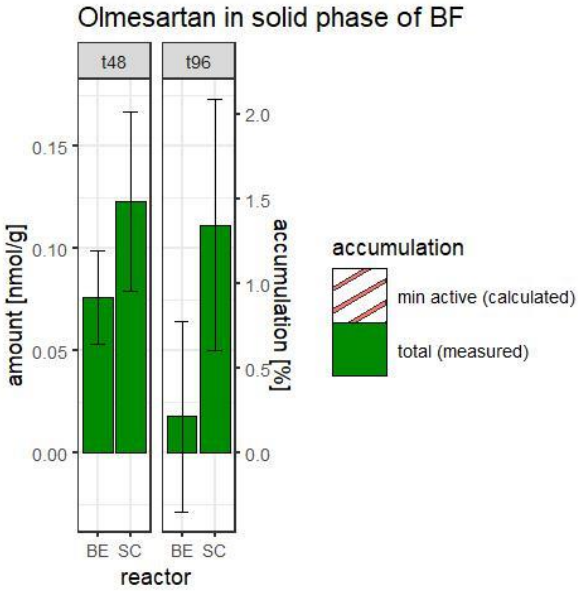

Neohesperidin dc in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

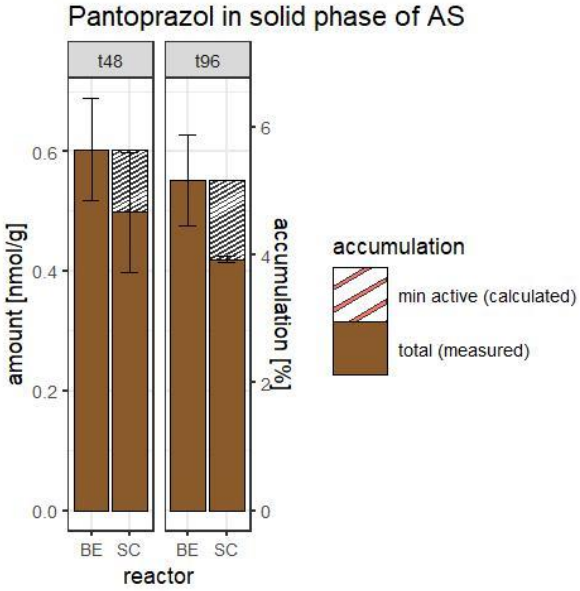

Neohesperidin dc in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

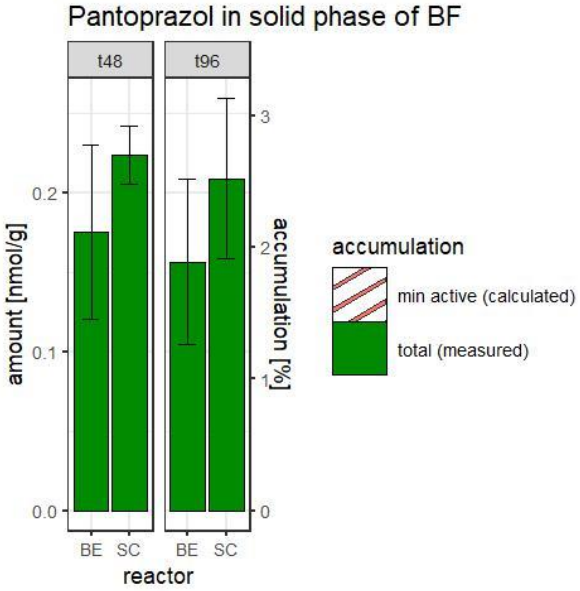

Neotam in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

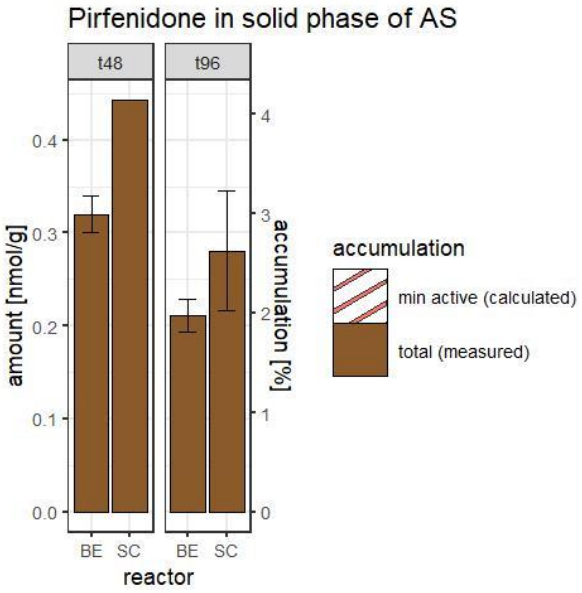

Neotam in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

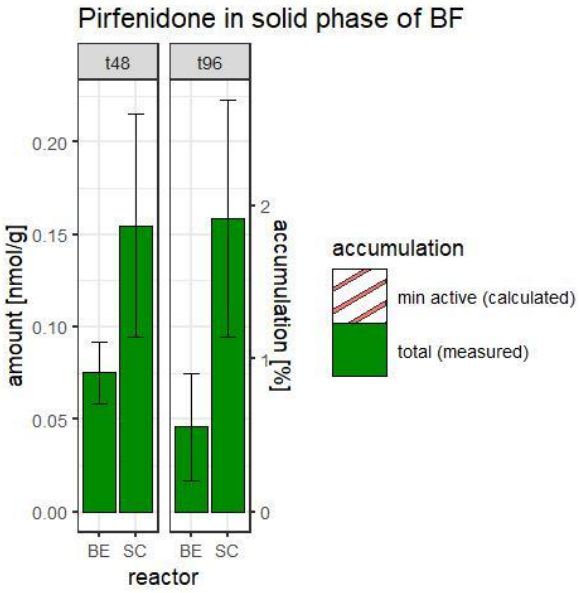

Olmesartan in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

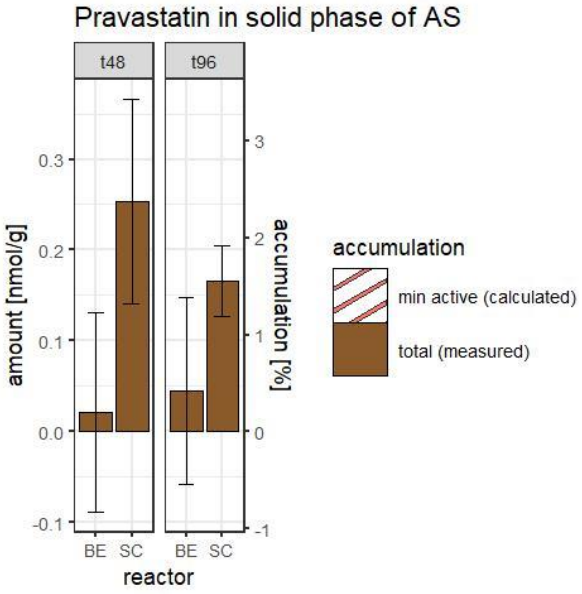

Olmesartan in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

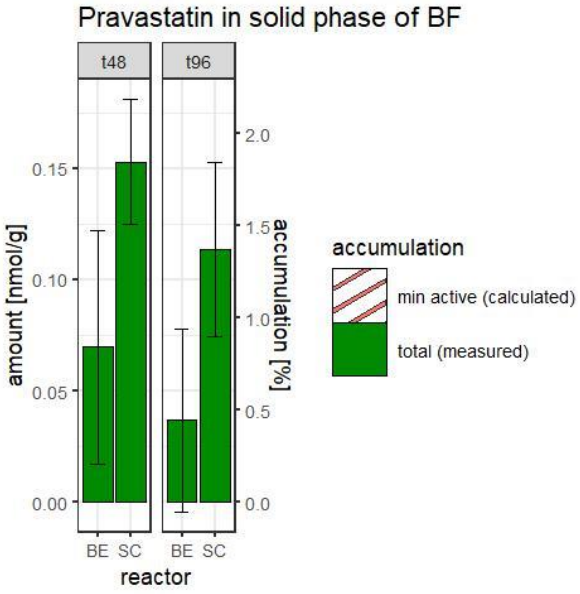

Pantoprazol in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

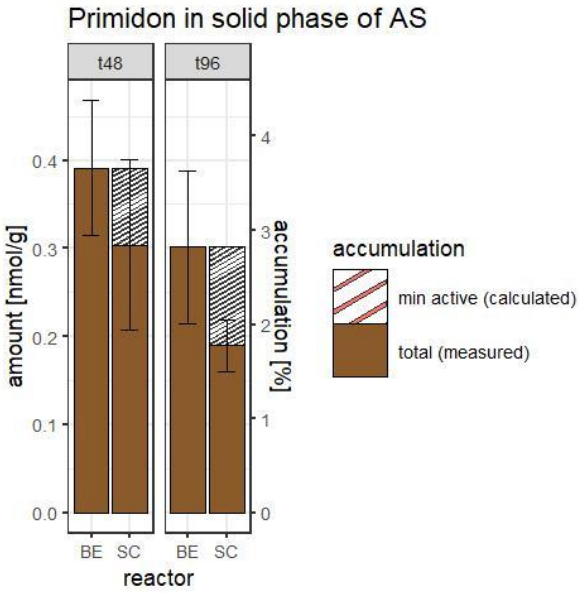

Pantoprazol in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

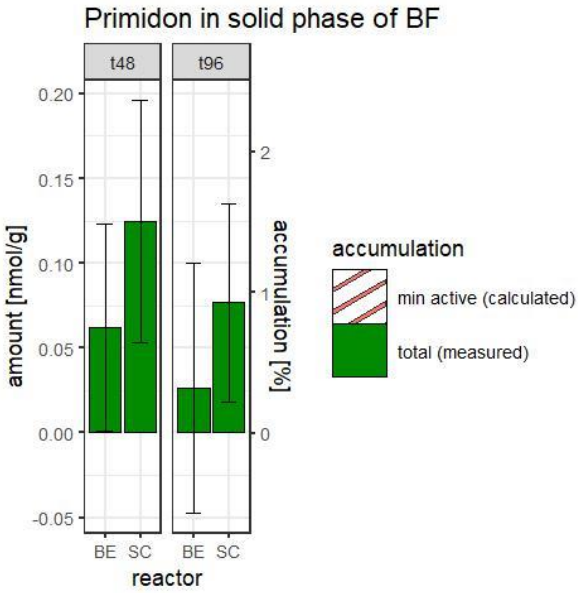

Pirfenidone in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

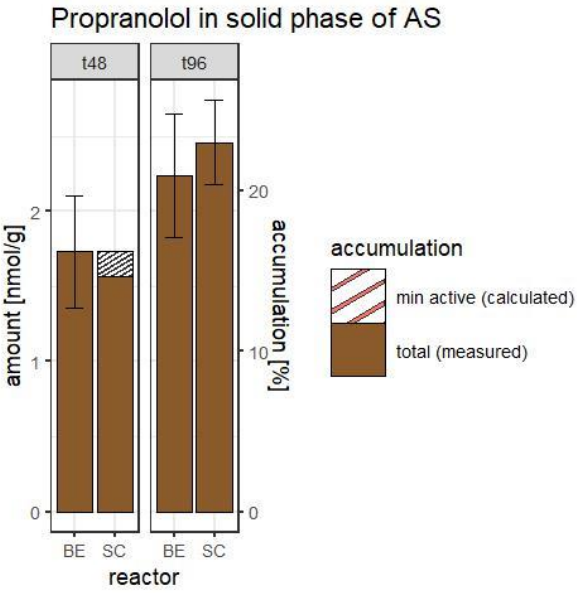

Pirfenidone in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

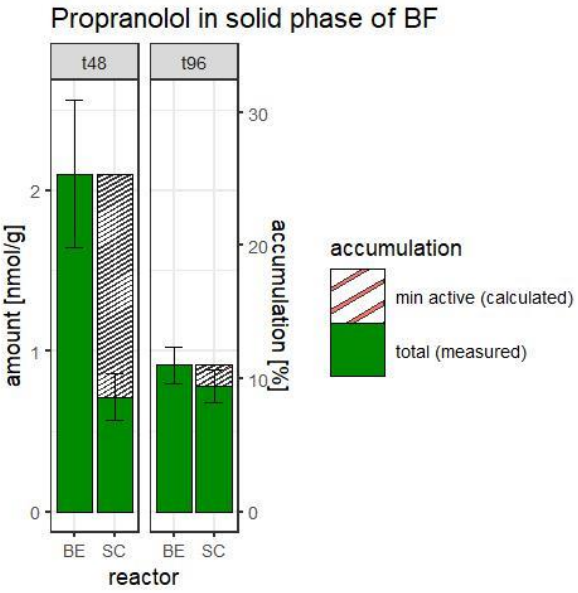

Pravastatin in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

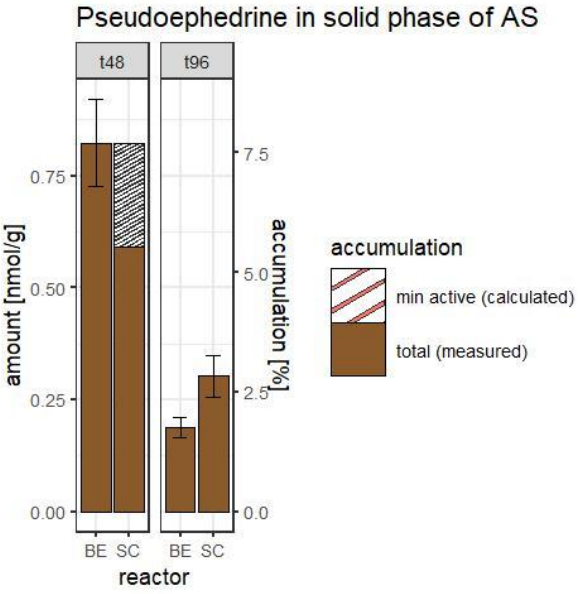

Pravastatin in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

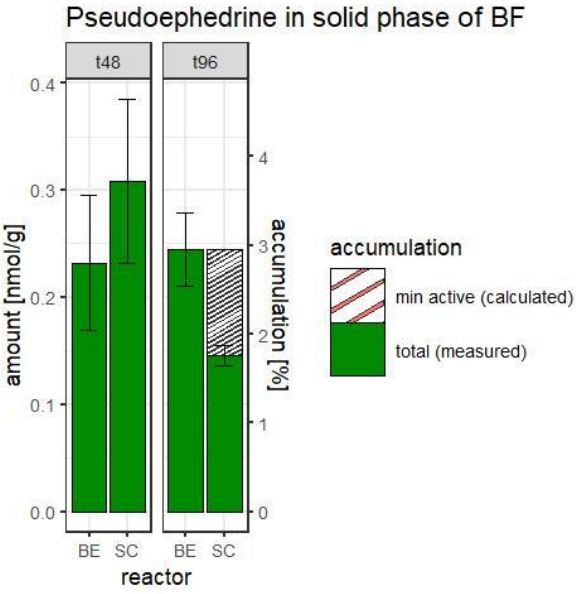

Primidon in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

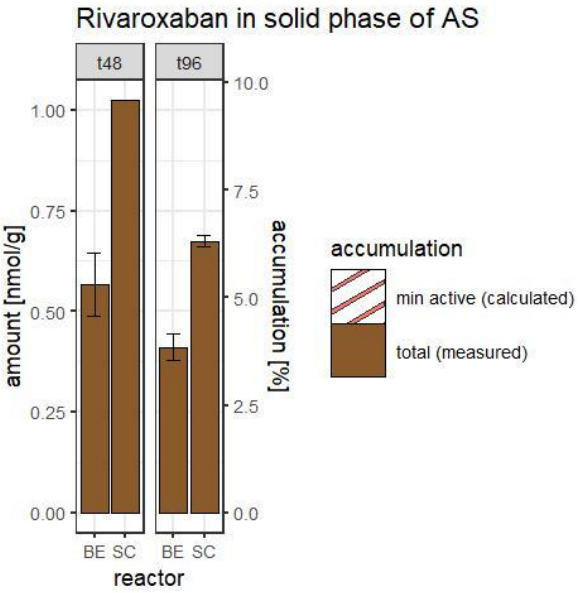

Primidon in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

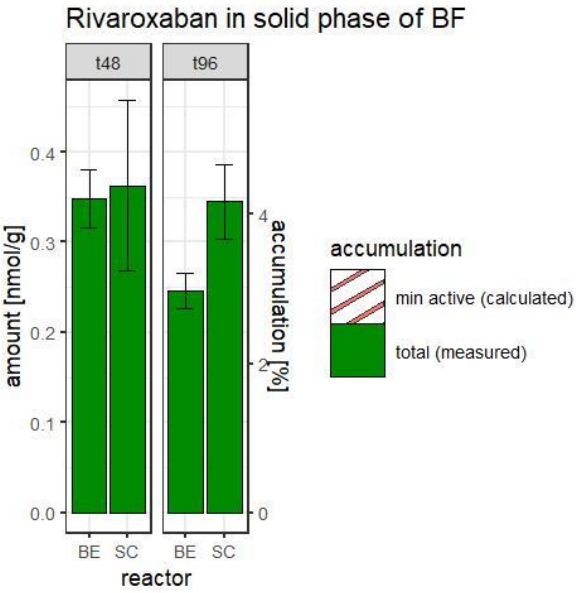

Propranolol in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

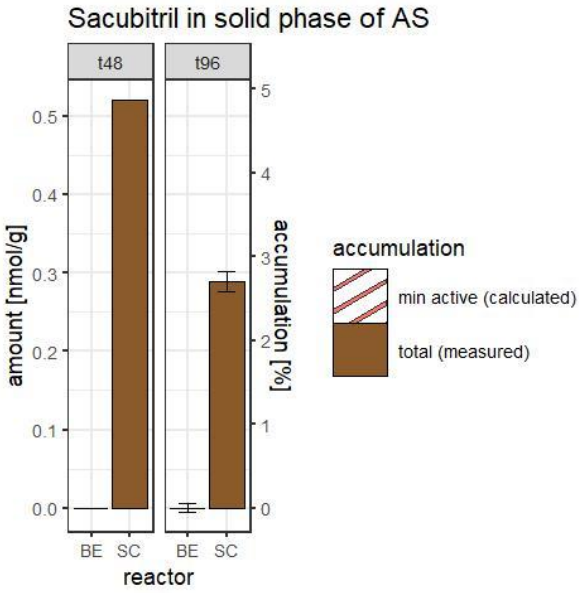

Propranolol in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

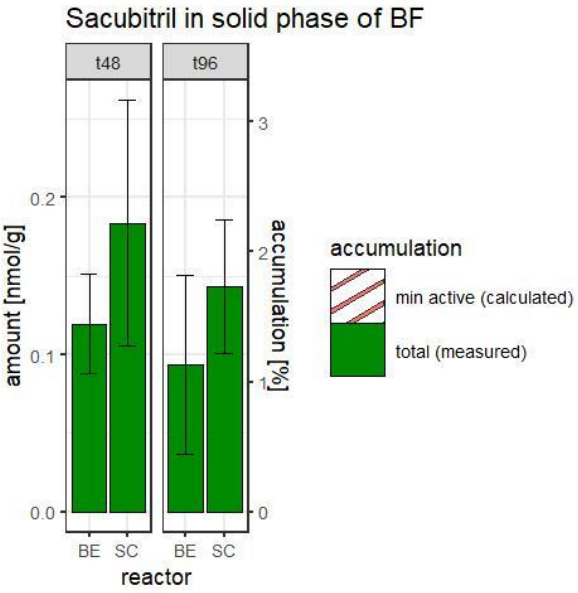

Pseudoephedrine in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

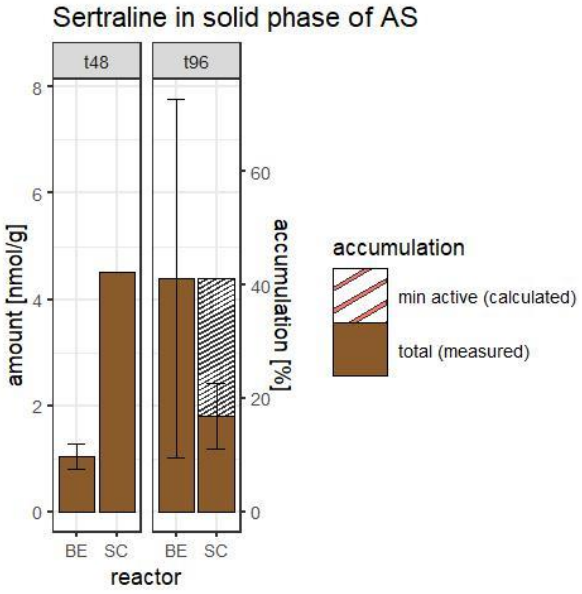

Pseudoephedrine in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

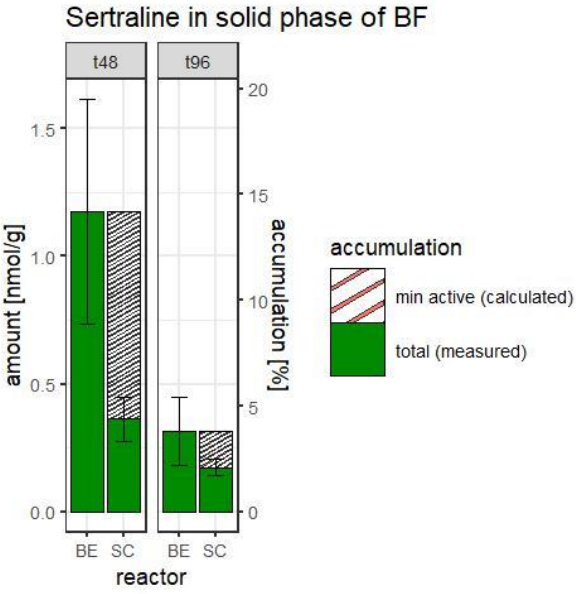

Rivaroxaban in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

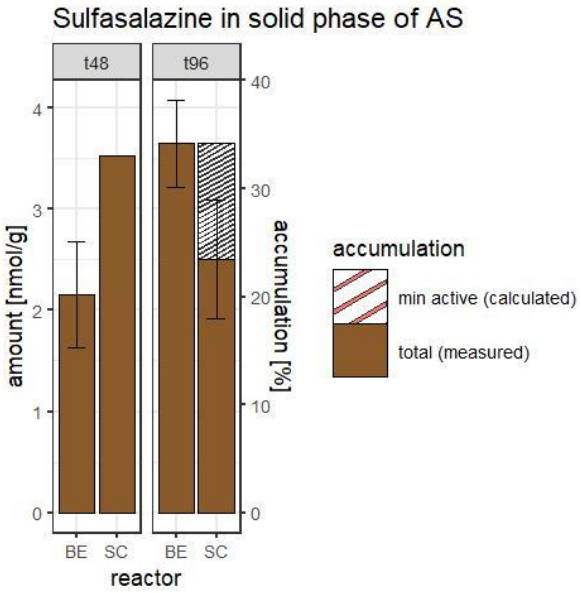

Rivaroxaban in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

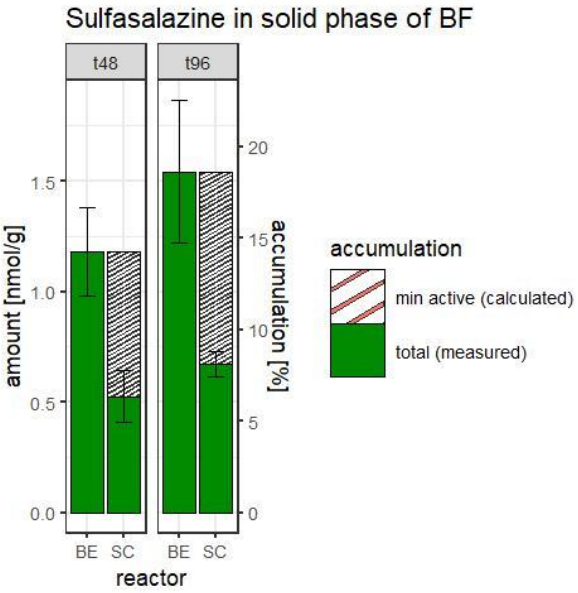

Sacubitril in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE
SC
reactor
accumulation
min active (calculated)
total (measured)

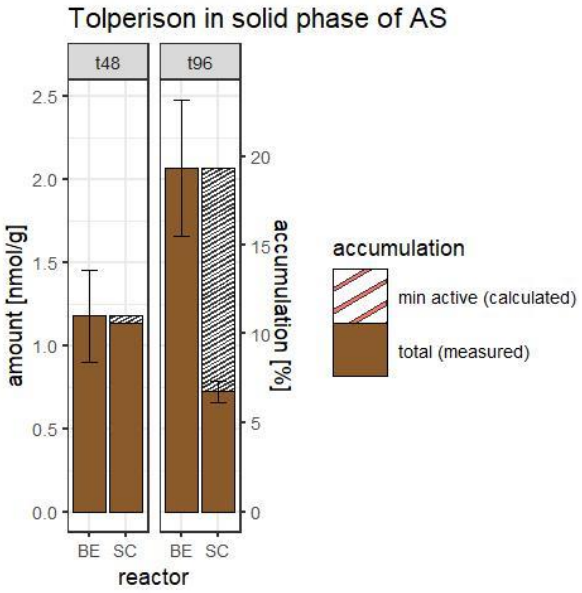

Sacubitril in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE
SC
reactor
accumulation
min active (calculated)
total (measured)

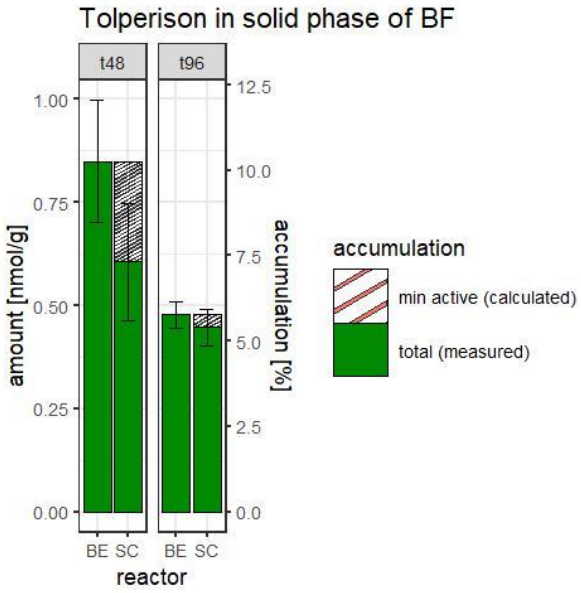

Sertraline in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE
SC
reactor
accumulation
min active (calculated)
total (measured)

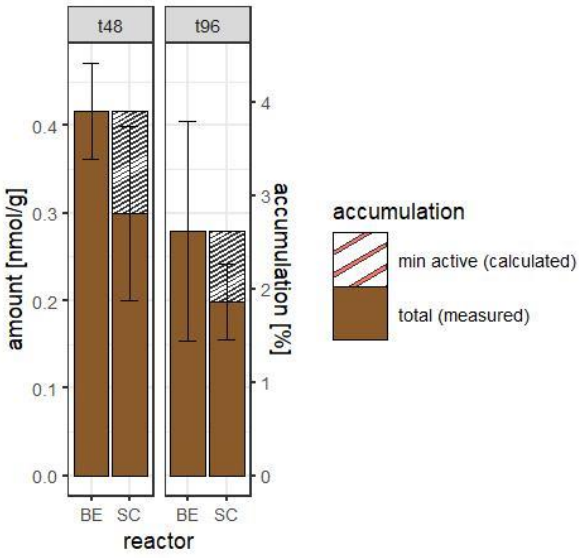

Sertraline in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE
SC
reactor
accumulation
min active (calculated)
total (measured)

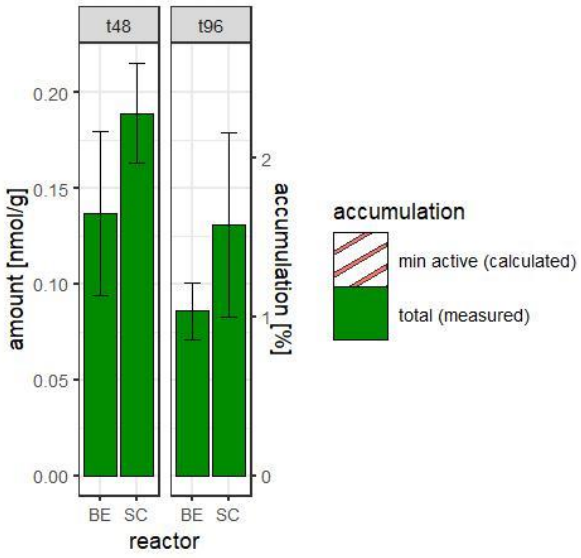

Sulfasalazine in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE
SC
reactor
accumulation
min active (calculated)
total (measured)

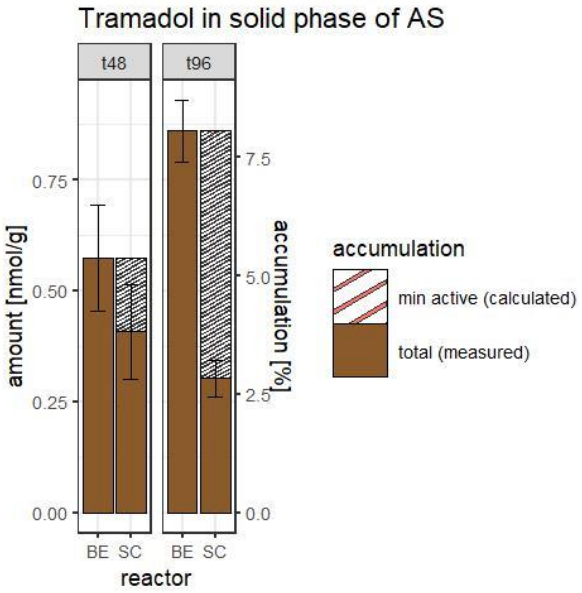

Sulfasalazine in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE
SC
reactor
accumulation
min active (calculated)
total (measured)

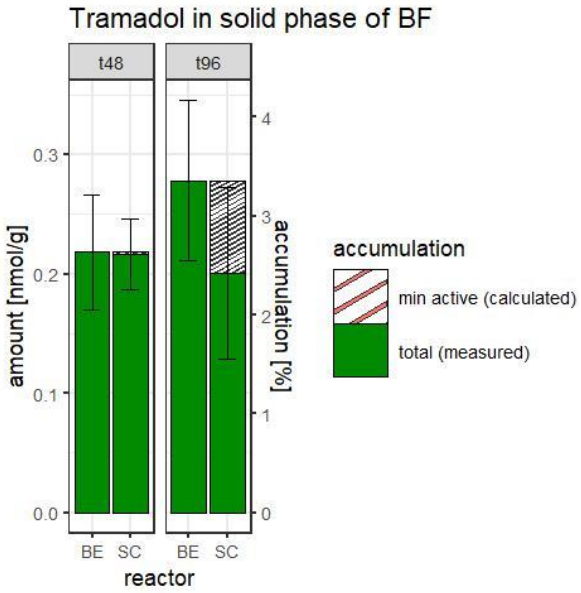

Tolperison in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

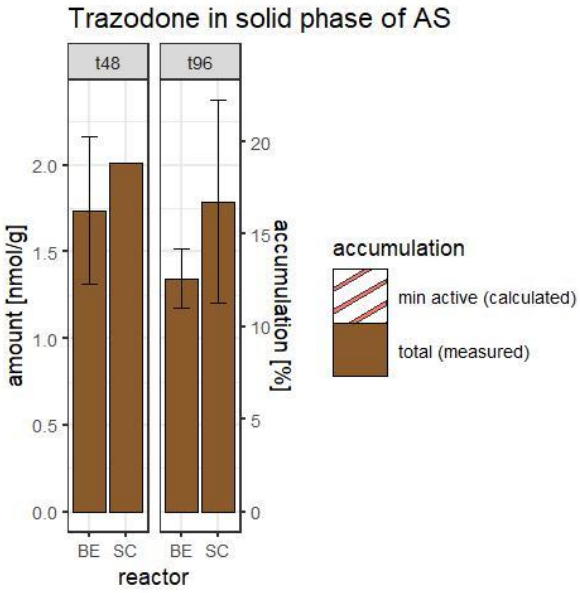

Tolperison in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

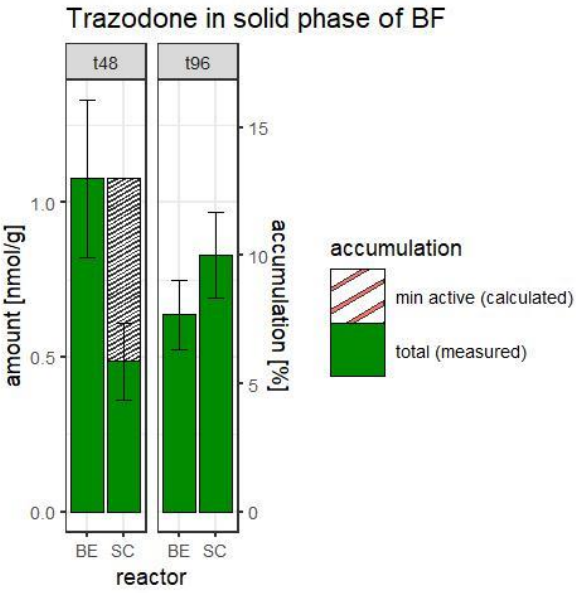

Torasemid in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

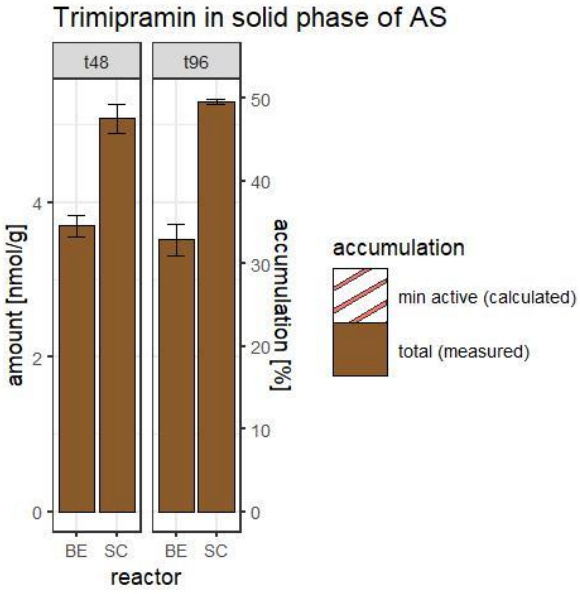

Torasemid in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

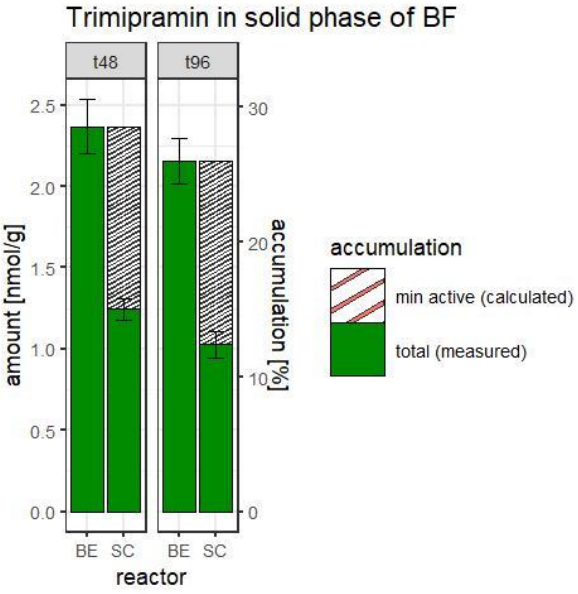

Tramadol in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

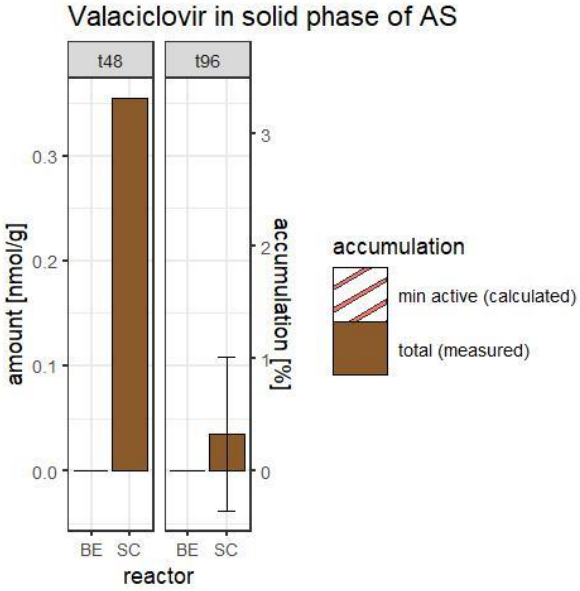

Tramadol in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
accumulation
min active (calculated)
total (measured)
BE SC BE SC
reactor

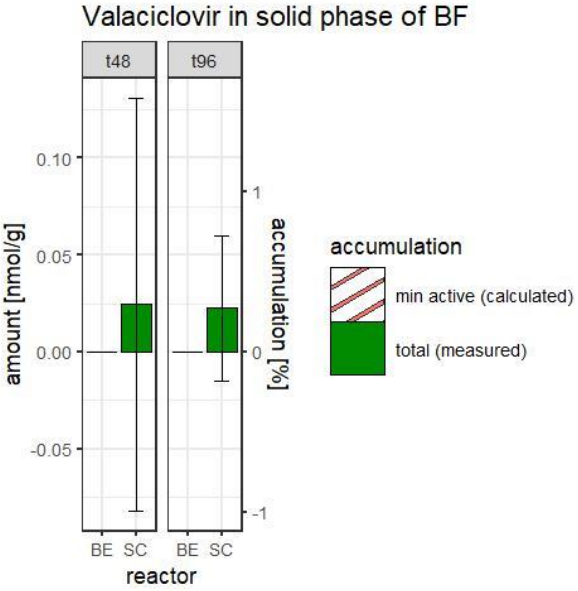

Trazodone in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

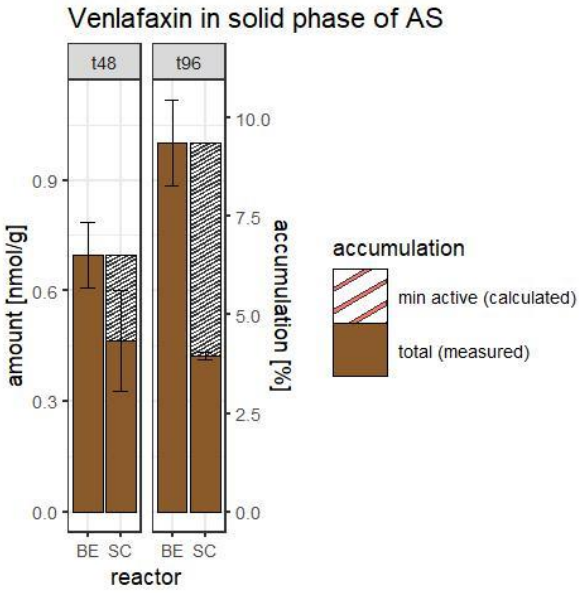

Trazodone in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

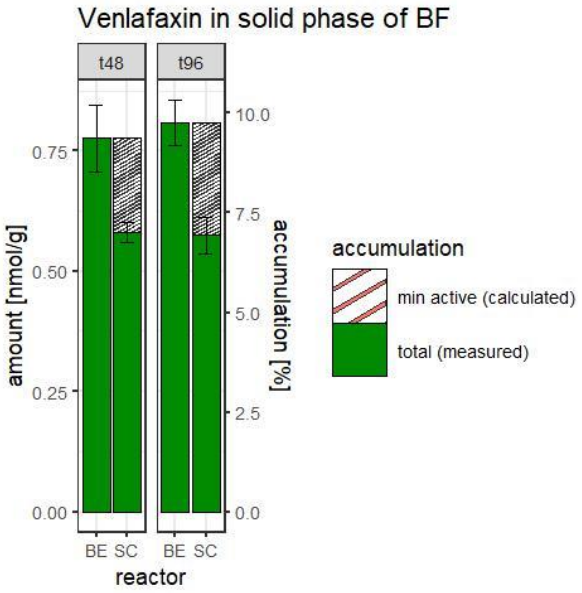

Trimipramin in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)


Trimipramin in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

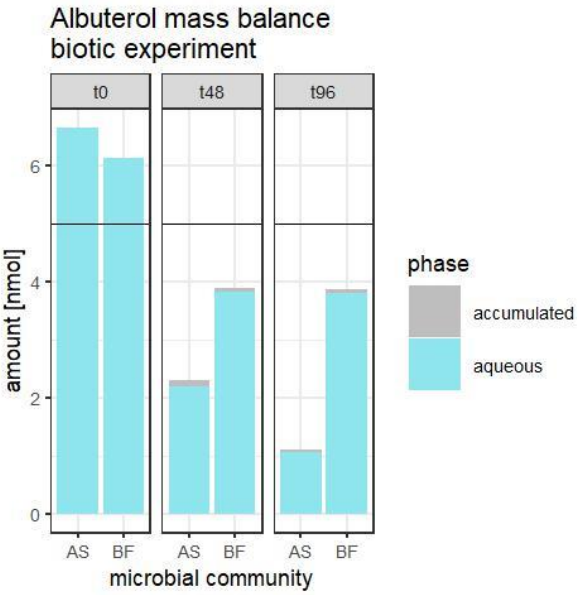

Valaciclovir in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

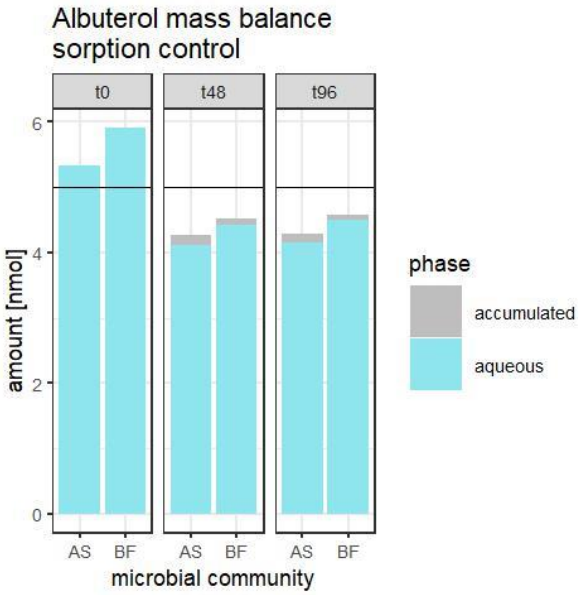

Valaciclovir in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

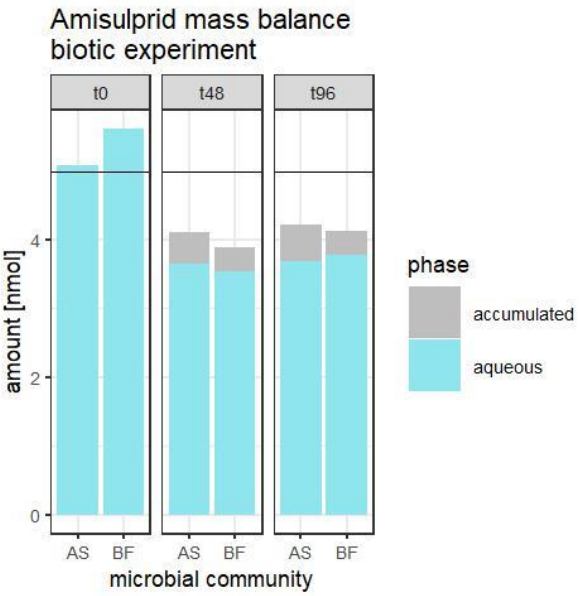

Venlafaxin in solid phase of AS
t48
t96
amount [nmol/g]
accumulation [%]
0.0
0.3
0.6
0.9
2.5
5.0
7.5
10.0
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

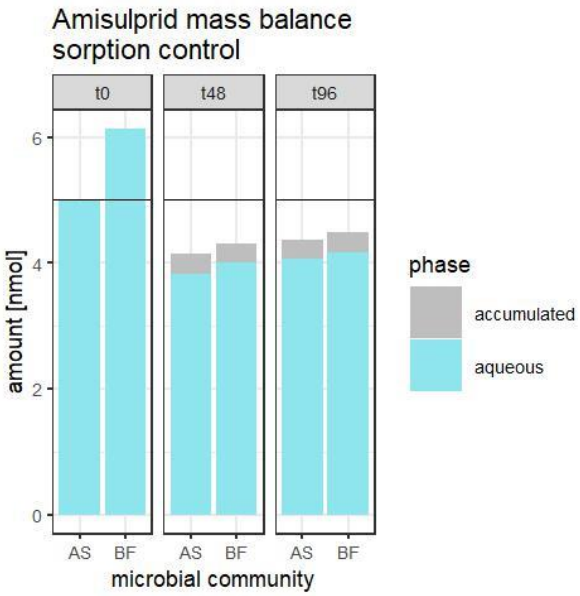

Venlafaxin in solid phase of BF
t48
t96
amount [nmol/g]
accumulation [%]
0.00
0.25
0.50
0.75
0.0
2.5
5.0
7.5
10.0
BE SC BE SC
reactor
accumulation
min active (calculated)
total (measured)

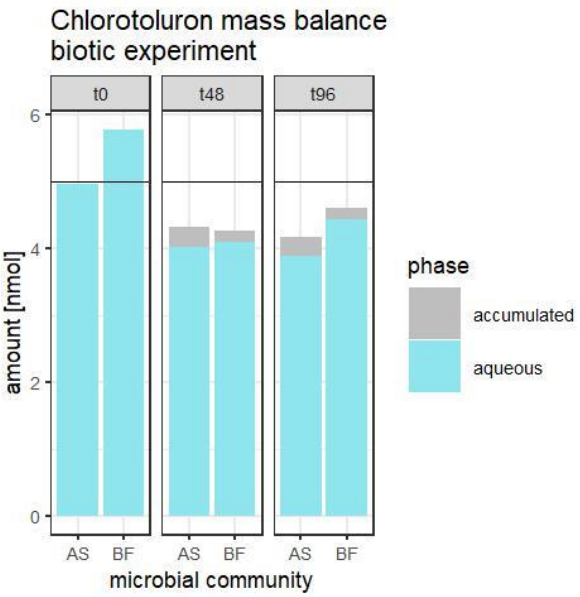
Mass balance plots

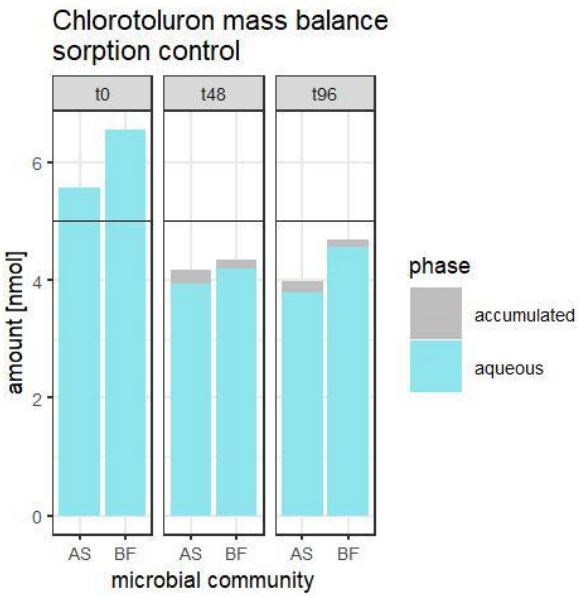


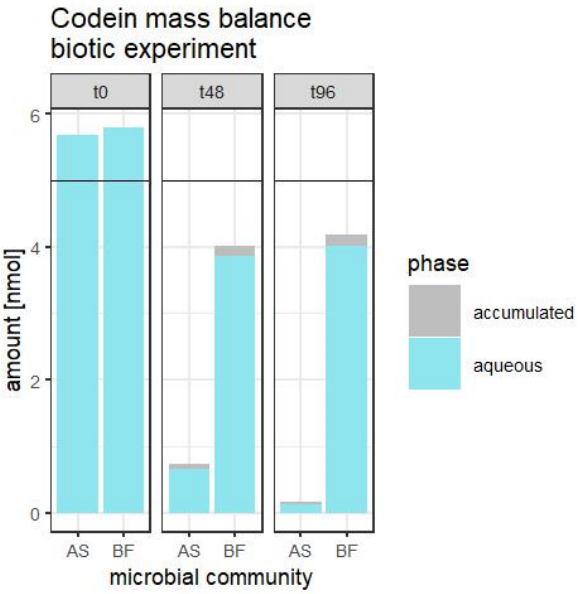


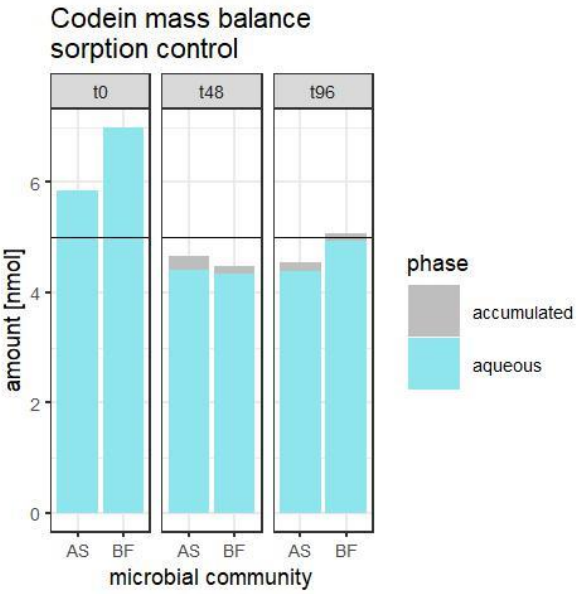


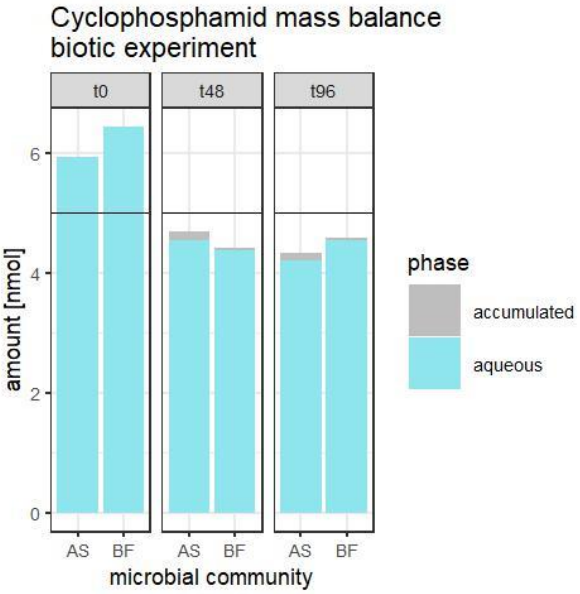


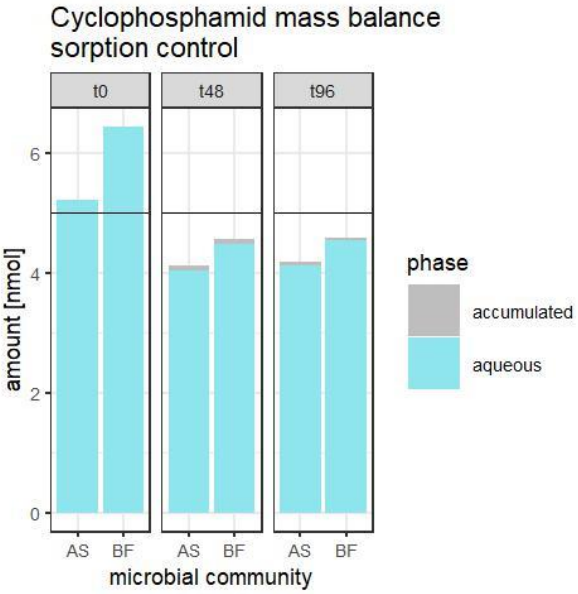


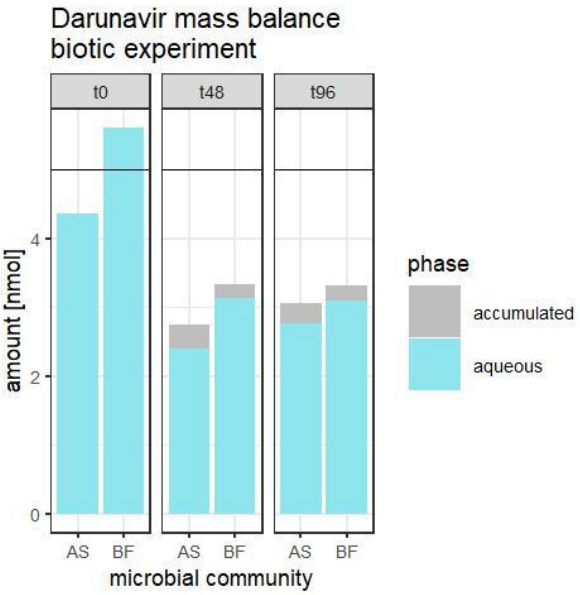


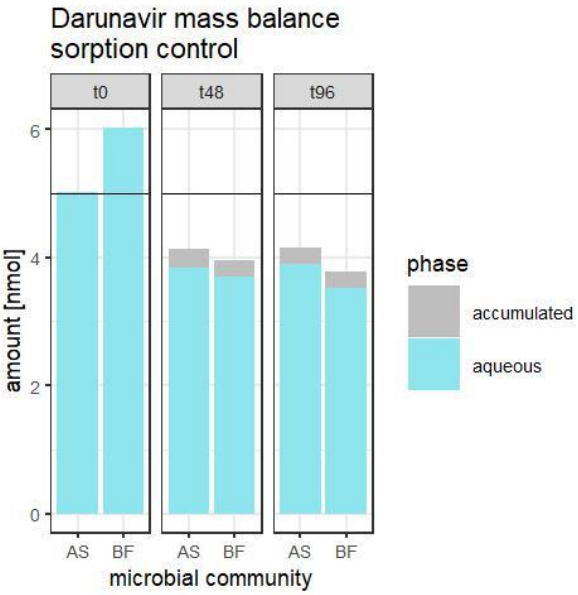

Codein mass balance
biotic experiment
t0
t48
t96
amount [nmol]
0
2
4
6
AS
BF
microbial community
phase
accumulated
aqueous

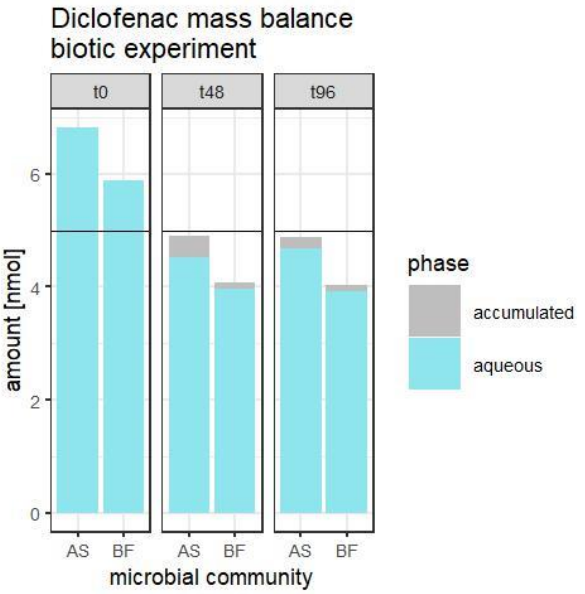

Codein mass balance
sorption control
t0
t48
t96
amount [nmol]
0
2
4
6
AS
BF
microbial community
phase
accumulated
aqueous

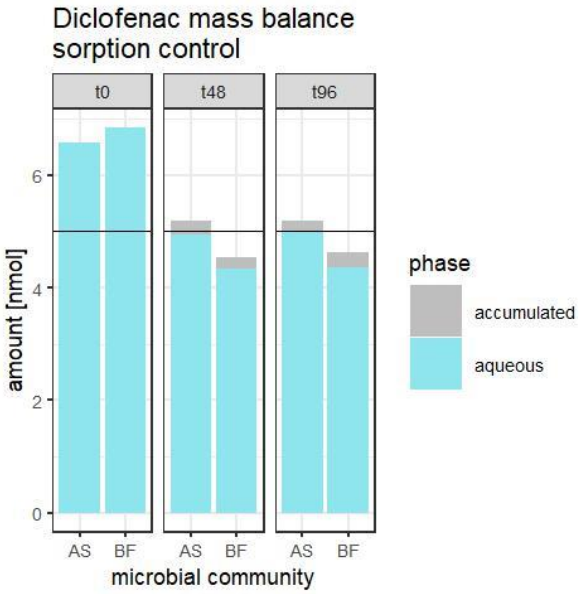

Cyclophosphamid mass balance
biotic experiment
t0
t48
t96
amount [nmol]
0
2
4
6
AS
BF
microbial community
phase
accumulated
aqueous

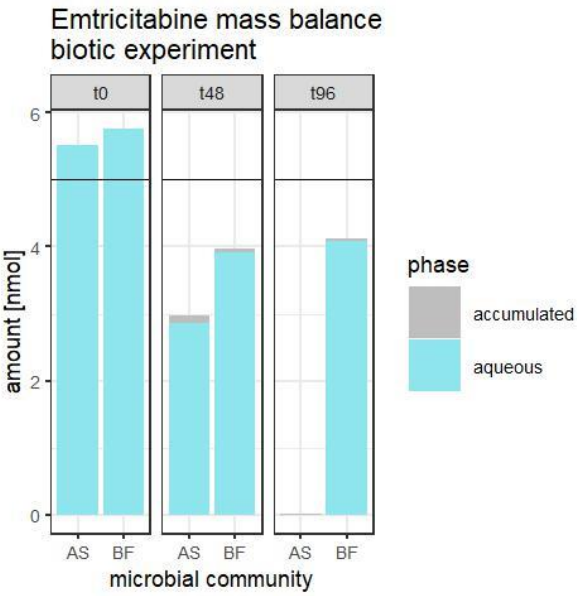

Cyclophosphamid mass balance
sorption control
t0
t48
t96
amount [nmol]
0
2
4
6
AS
BF
microbial community
phase
accumulated
aqueous

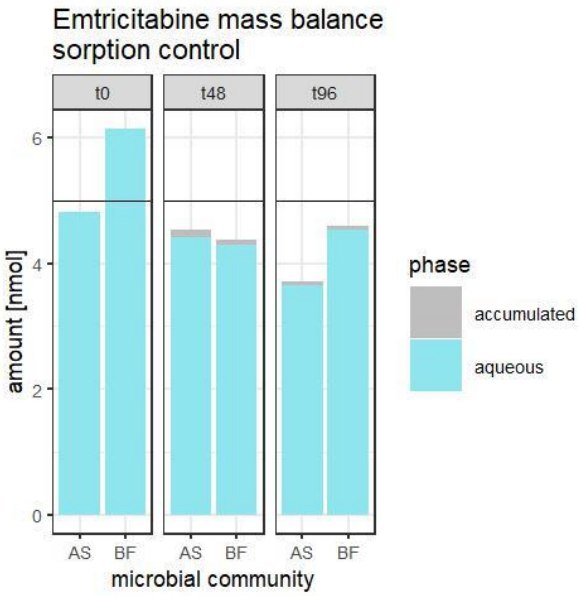

Darunavir mass balance
biotic experiment
t0
t48
t96
amount [nmol]
0
2
4
AS
BF
microbial community
phase
accumulated
aqueous

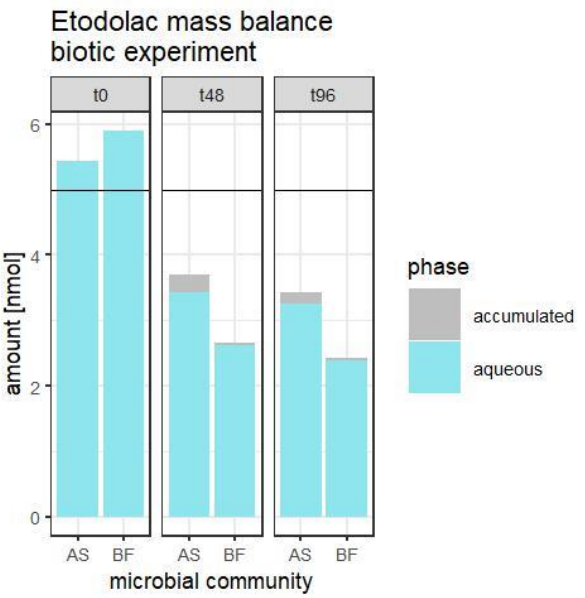

Darunavir mass balance
sorption control
t0
t48
t96
amount [nmol]
0
2
4
6
AS
BF
microbial community
phase
accumulated
aqueous

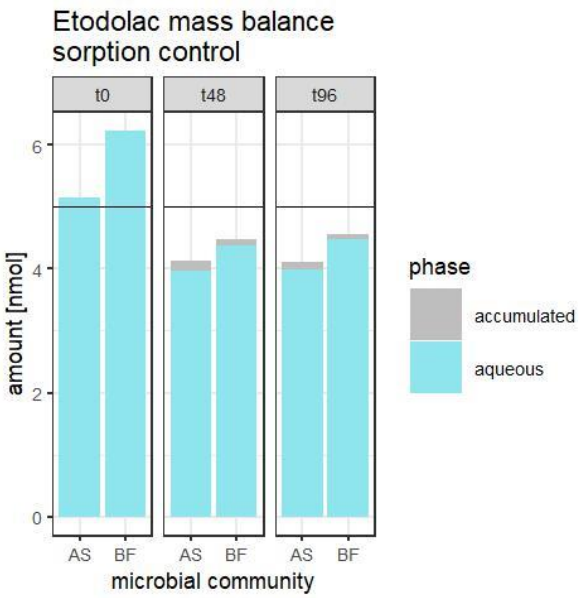

Diclofenac mass balance
biotic experiment
t0
t48
t96
amount [nmol]
0
2
4
6
AS
BF
microbial community
phase
accumulated
aqueous

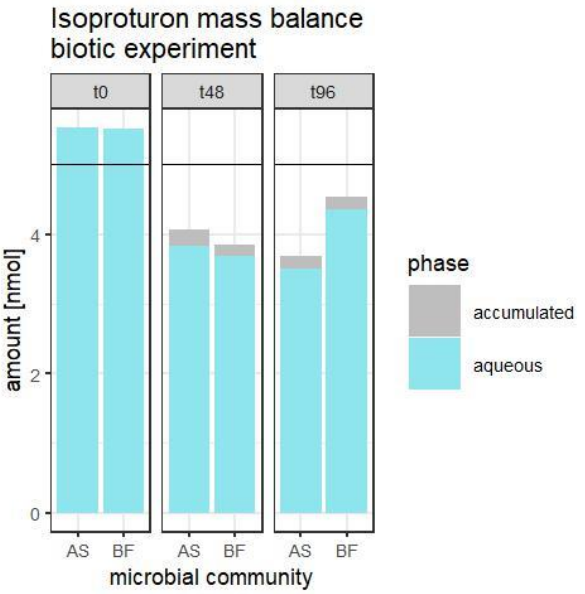

Diclofenac mass balance
sorption control
t0
t48
t96
amount [nmol]
0
2
4
6
AS
BF
microbial community
phase
accumulated
aqueous

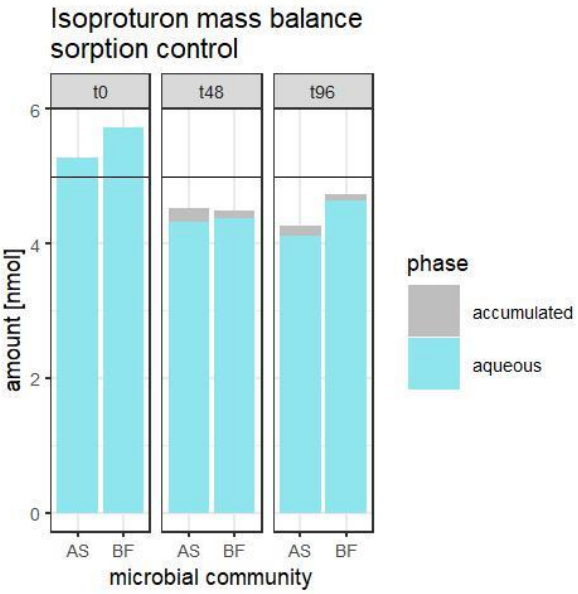

Emtricitabine mass balance
biotic experiment
t0
t48
t96
amount [nmol]
0
2
4
6
AS
BF
microbial community
phase
accumulated
aqueous

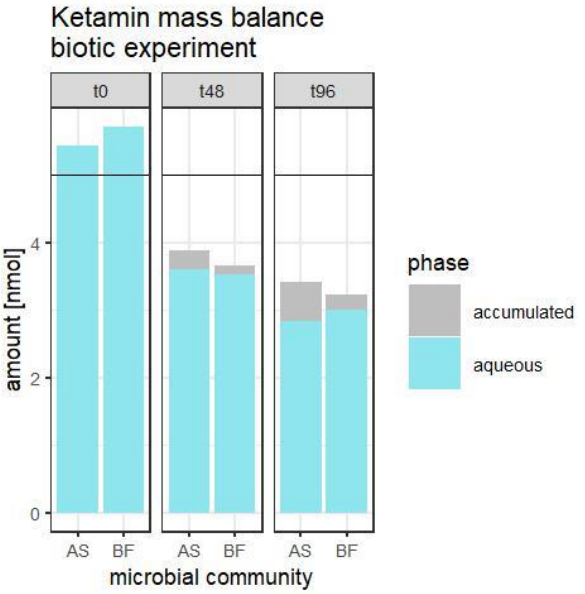

Emtricitabine mass balance
sorption control
t0
t48
t96
amount [nmol]
0
2
4
6
AS
BF
microbial community
phase
accumulated
aqueous

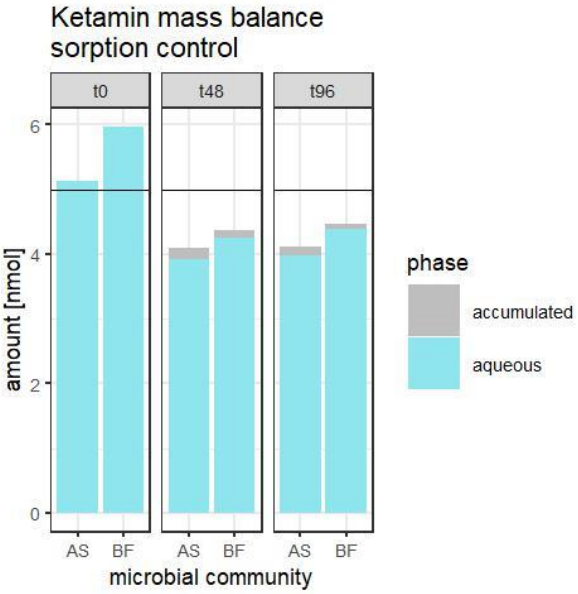

Etodolac mass balance
biotic experiment
t0
t48
t96
amount [nmol]
0
2
4
6
AS
BF
microbial community
phase
accumulated
aqueous

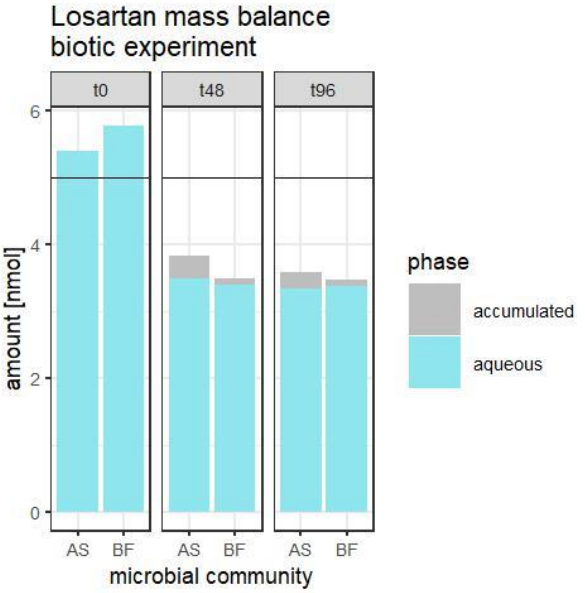

Etodolac mass balance
sorption control
t0
t48
t96
amount [nmol]
0
2
4
6
AS
BF
microbial community
phase
accumulated
aqueous

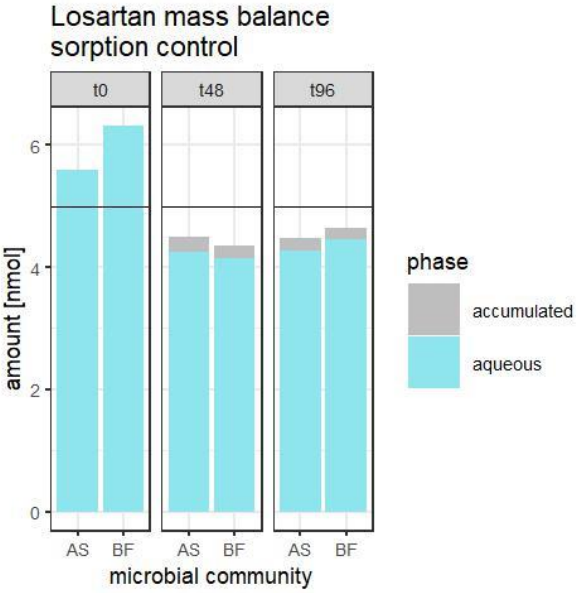

Isoproturon mass balance
biotic experiment
t0
t48
t96
amount [nmol]
0
2
4
AS
BF
microbial community
phase
accumulated
aqueous

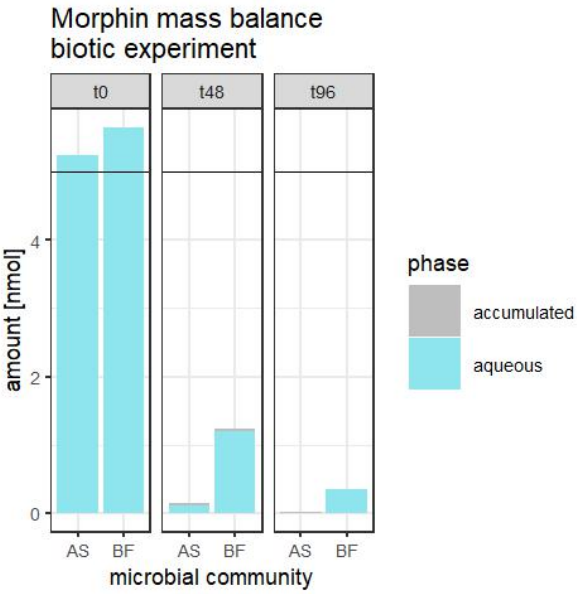

Isoproturon mass balance
sorption control
t0
t48
t96
amount [nmol]
0
2
4
6
AS
BF
microbial community
phase
accumulated
aqueous

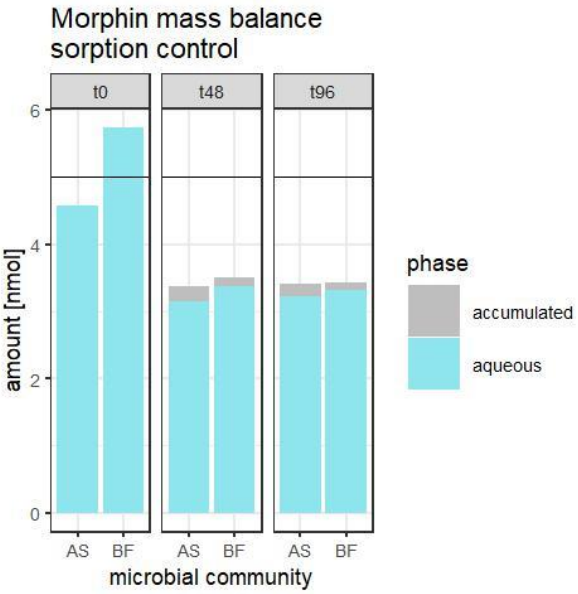

Ketamin mass balance
biotic experiment
t0
t48
t96
amount [nmol]
0
2
4
AS
BF
microbial community
phase
accumulated
aqueous


Ketamin mass balance
sorption control
t0
t48
t96
amount [nmol]
0
2
4
6
AS
BF
microbial community
phase
accumulated
aqueous


Losartan mass balance
biotic experiment
t0
t48
t96
amount [nmol]
0
2
4
6
AS
BF
microbial community
phase
accumulated
aqueous


Losartan mass balance
sorption control
t0
t48
t96
amount [nmol]
0
2
4
6
AS
BF
microbial community
phase
accumulated
aqueous


Morphin mass balance
biotic experiment
t0
t48
t96
amount [nmol]
0
2
4
AS
BF
microbial community
phase
accumulated
aqueous


Morphin mass balance
sorption control
t0
t48
t96
amount [nmol]
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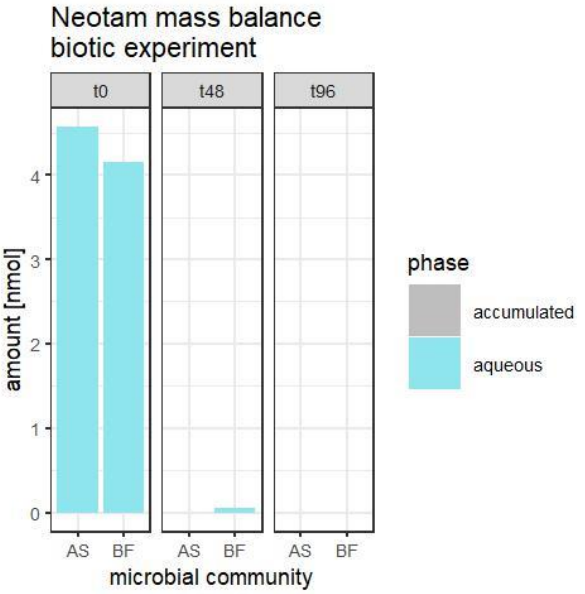
2
4
6
AS
BF
microbial community
phase
accumulated
aqueous


Neotam mass balance
biotic experiment
t0
t48
t96
amount [nmol]
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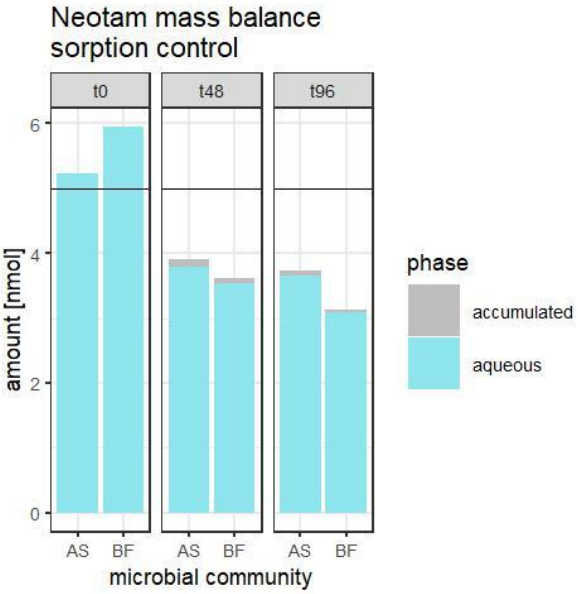
1
2
3
4
AS
BF
microbial community
phase
accumulated
aqueous


Neotam mass balance
sorption control
t0
t48
t96
amount [nmol]
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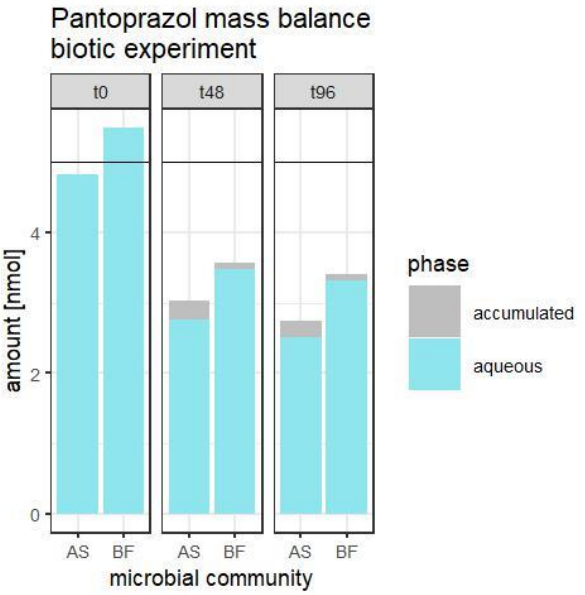
2
4
6
AS
BF
microbial community
phase
accumulated
aqueous


Pantoprazol mass balance
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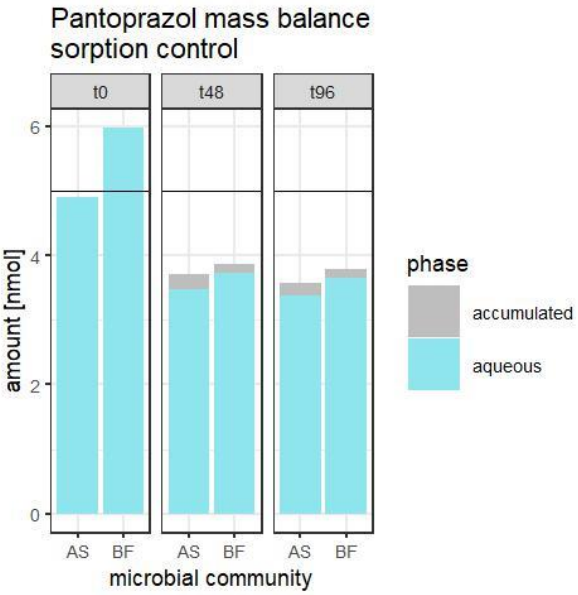
t48
t96
amount [nmol]
0
2
4
AS
BF
microbial community
phase
accumulated
aqueous


Pantoprazol mass balance
sorption control
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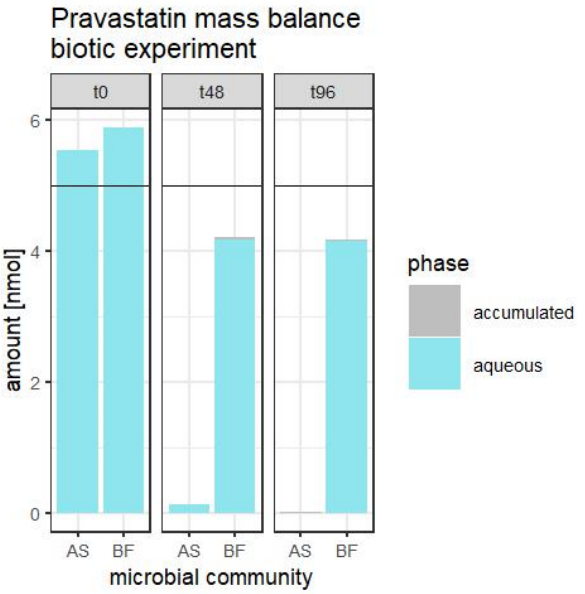
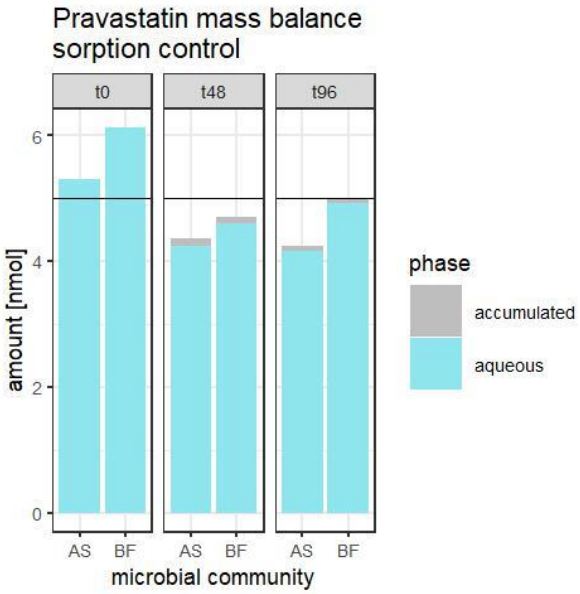
t48
t96
amount [nmol]
0
2
4
6
AS
BF
microbial community
phase
accumulated
aqueous


Pravastatin mass balance
biotic experiment
t0
t48
t96
amount [nmol]
0
2
4
6
AS
BF
microbial community
phase
accumulated
aqueous


Pravastatin mass balance
sorption control
t0
t48
t96
amount [nmol]
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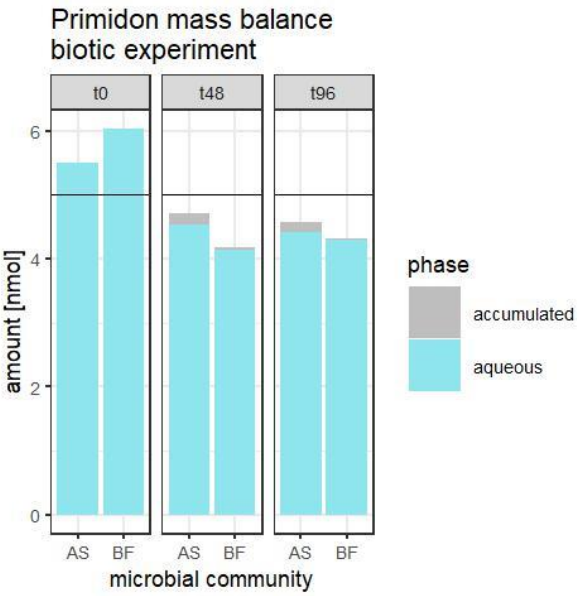
2
4
6
AS
BF
microbial community
phase
accumulated
aqueous


Primidon mass balance
biotic experiment
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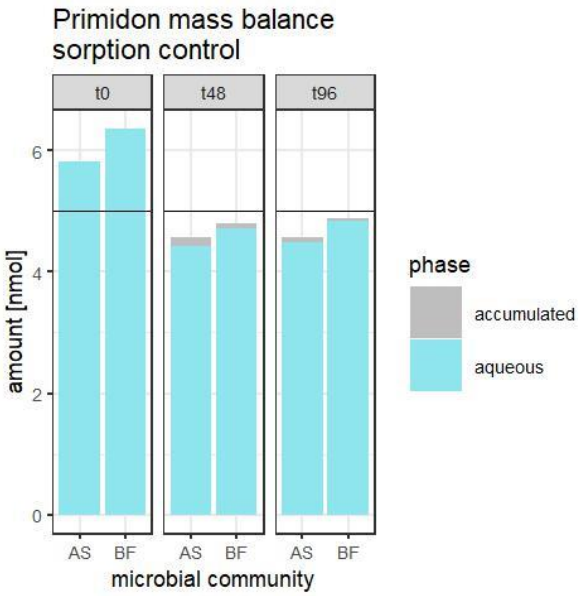
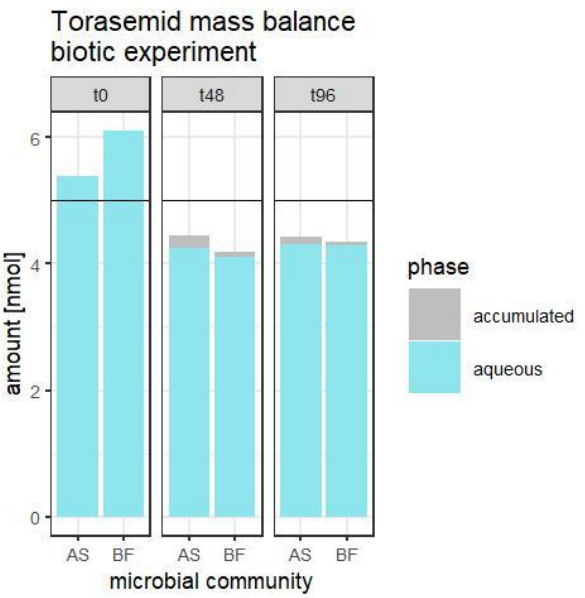
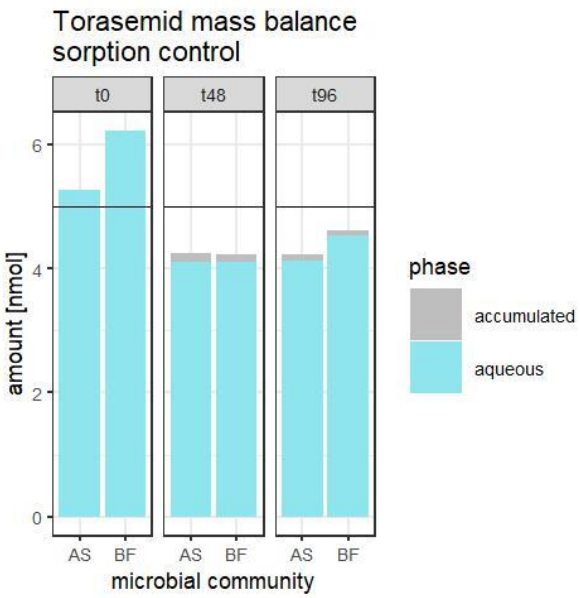
t48
t96
amount [nmol]
0
2
4
6
AS
BF
microbial community
phase
accumulated
aqueous


Primidon mass balance
sorption control
t0
t48
t96
amount [nmol]
0
2
4
6
AS
BF
microbial community
phase
accumulated
aqueous


Torasemid mass balance
biotic experiment
t0
t48
t96
amount [nmol]
0
2
4
6
AS
BF
microbial community
phase
accumulated
aqueous


Torasemid mass balance
sorption control
t0
t48
t96
amount [nmol]
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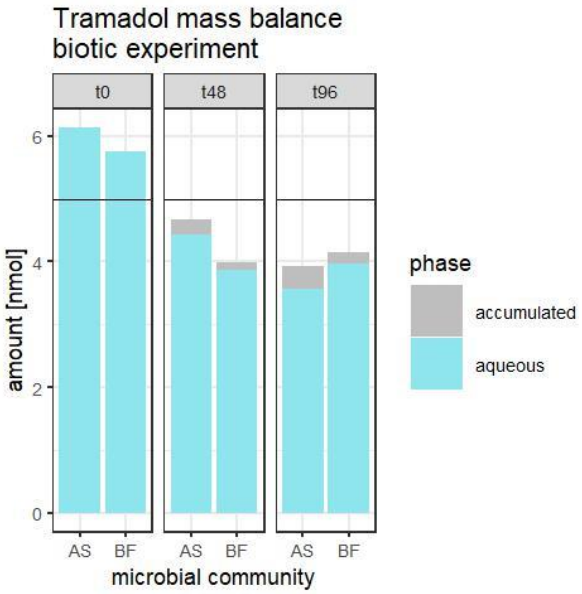
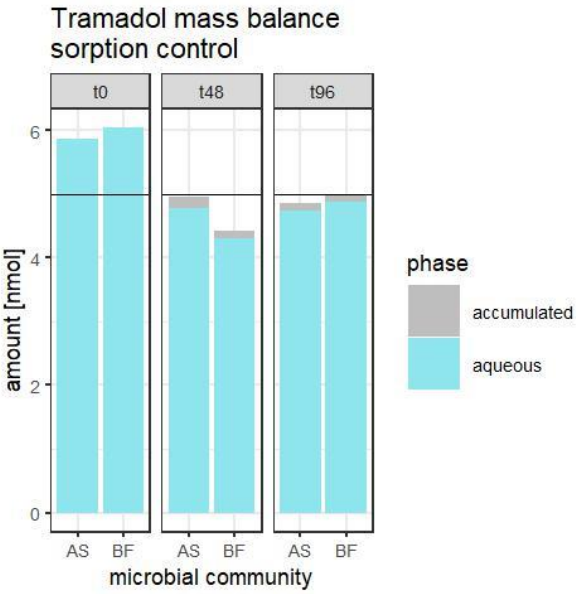
2
4
6
AS
BF
microbial community
phase
accumulated
aqueous


Tramadol mass balance
biotic experiment
t0
t48
t96
amount [nmol]
0
2
4
6
AS
BF
microbial community
phase
accumulated
aqueous


Tramadol mass balance
sorption control
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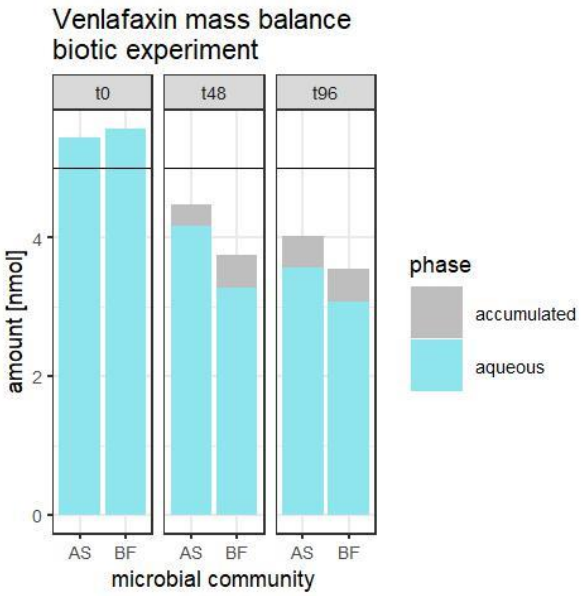
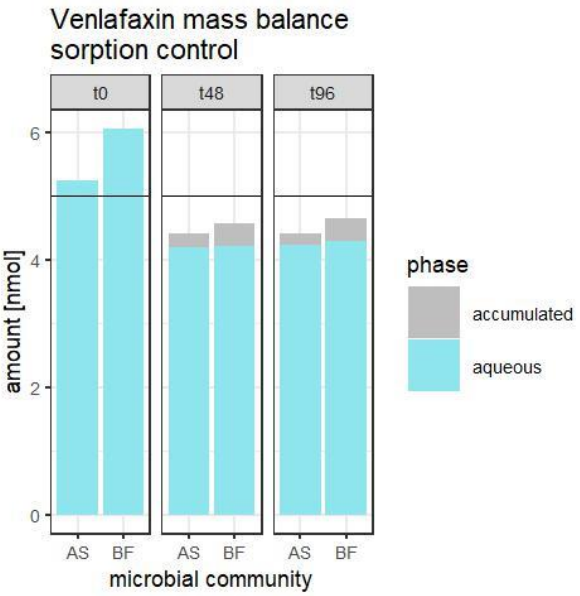
t48
t96
amount [nmol]
0
2
4
6
AS
BF
microbial community
phase
accumulated
aqueous


Venlafaxin mass balance
biotic experiment
t0
t48
t96
amount [nmol]
0
2
4
AS
BF
microbial community
phase
accumulated
aqueous


Venlafaxin mass balance
sorption control
t0
t48
t96
amount [nmol]
0
2
4
6
AS
BF
microbial community
phase
accumulated
aqueous