ENZYMATIC TRANSFORMATION OF CHIRAL PHARMACEUTICALS IN THE ENVIRONMENT AS REVEALED BY ENANTIOSELECTIVE CHROMATOGRAPHY

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Introduction

The average life-span of people is increasing continuously due to the demographic development. This goes in line with an increase in consumption of pharmaceuticals. In addition, a large number of compounds is also applied in veterinary medicine to the prevention and acute treatment of infectious diseases in intensive livestock farming. Furthermore, antibiotics are also used as growth promoters[1]. Compounds applied in human medicine as well as their metabolites are excreted with urine and faeces to sewer systems. Expired and surplus drugs are assumed to be disposed of via toilets to the sewage treatment plant (STP) by the consumer to an unknown extent. Elimination rates observed in the STP ranged from more than 80 % for acetylsalicylic acid, ibuprofen, bezafibrate, metoprolol and propranolol to less than 10 % for carbamazepine and x-ray contrast media[2,3]. In many cases, veterinary pharmaceuticals are directly released into the environment by their use in aquaculture, the dispersion of manure from treated livestock on fields or the therapeutic treatment of livestock on meadows. Although the aspect of pharmaceutical chemicals in the environment was occasionally mentioned in the late 1970s[4] and mid-80s[5,6], little attention had been paid to these substances as potential environmental pollutants until the early 1990s, when Stan and Linkerhägner[7] identified amazingly high concentrations of clofibric acid, metabolite of the lipid regulating agents clofibrate and etofibrate, in groundwater of the city of Berlin/Germany. Subsequently, investigations carried out by further research groups revealed the presence of a vast array of pharmaceutical residues in STP effluents and river water[2] in concentrations up to the µg/L-range. Among these were analgesics/antiphlogistics[8], β-blockers and β-sympathomimetics[9], antibiotics[10] and synthetic estrogens[11,12]. Some of them, especially clofibric acid, were even determined in drinking water[13] as well as in the North Sea[14], where this compound was found in concentrations similar to classical pollutants such as lindane (γ-HCH). As the widespread occurrence of pharmaceuticals demonstrates, they have to be regarded as a new class of priority environmental pollutants.

Knowledge about the fate of pharmaceuticals in the aquatic environment is limited. Therefore, in the present study enantioselective gas chromatography was applied to the investigation of enzymatic transformation processes occurring to chiral pharmaceuticals in the aquatic environment. Herein, special emphasis is placed on ibuprofen and its transformation products hydroxyibuprofen and carboxyibuprofen.

Materials and Methods

Ibuprofen was purchased from Synopharm (Barsbüttel, Germany), hydroxy-ibuprofen and carboxy-ibuprofen were synthesised according to Kurtz and Houser[15] and Tan et al.[16], respectively, and characterised by GC-MS and nuclear magnetic resonance spectroscopy (^{1}H-NMR). A detailed description of the sampling procedure, extraction, clean-up, fractionation and derivatisation steps is given in Weigel et al.[17], details concerning method validation is reported in Weigel et al.[18]. The chiral stationary phases include heptakis(6-*O*-*tert*-butyldimethylsilyl-2,3-di-*O*-methyl)-*β*-cyclodextrin (6-TBDMS-2,3-Me-*β*-CD)/50 % in OV-1701, 25 m × 250 µm × 0.1 µm or 12 m × 250 µm × 0.1 µm and 6-TBDMS-2,3-Me-*β*-CD/20 % in SE-52, 15 m × 250 µm × 0.1 µm (see the respective Figure captions).

Results and Discussion

Two water samples were taken at the inflow and outflow, respectively, of the STP of Hamburg, Germany, representing a state of the art plant including a biological treatment step. By contrast, sewage in Tromsø is collected in sewers and discharged either directly into the sea or after processing in one of the four sewage treatment plants. In the latter

case, sewage treatment consists of a mechanical filtration, but does not include any biological treatment. The two hospitals in the city discharge their waste water into the public sewer system without prior treatment. Sewage samples were taken at the main STP, receiving effluents from private households and commerce (relation about 5:1) of the major area of the inner city. The enantioselective separation of the ibuprofen enantiomers and that of its main metabolite hydroxyibuprofen determined in the respective water sample extracts are displayed in Figure 1, while the result for another transformation product, carboxyibuprofen, is shown in Figure 2.

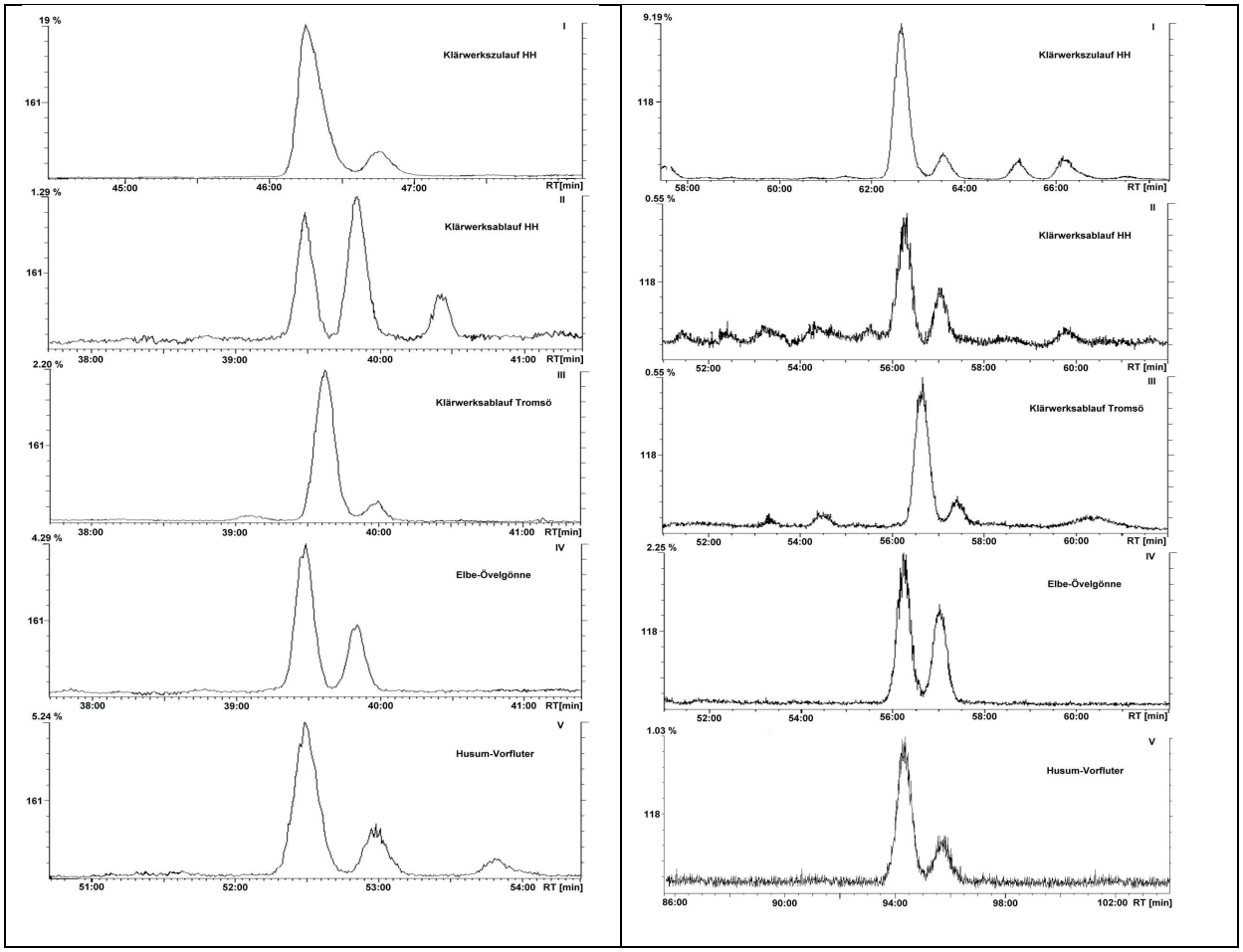

Figure 1, from top to bottom STP Hamburg influent, STP Hamburg effluent, STP Tromsø effluent, river Elbe Hamburg Övelgönne, Husum creek; **left hand side:** enantioselective separation of ibuprofen; stationary phase: I-IV) 6-TBDMS-2,3-Me-β-CD/50 % in OV-1701, V) 6-TBDMS-2,3-Me-β-CD/50 % in OV-1701 + VB-50; temperature programme: I) 233 K [60 °C] (5 min), 2.0 K/min until 423 K [150 °C] (75 min), II-IV) 233 K [60 °C] (5 min), 2.5 K/min until 423 K [150 °C] (25 min), V) 233 K [60 °C] (5 min), 2.5 K/min until 423 K [150 °C] (85 min)
right hand side: enantioselective separation of hydroxyibuprofen**;** stationary phase: Phase: I-IV) 6-TBDMS-2,3-Me-β-CD/50 % in OV-1701, V) 6-TBDMS-2,3-Me-β-CD/50 % in OV-1701 + VB-50; temperature programme: I) 233 K [60 °C] (5 min), 2.0 K/min until 423 K [150 °C] (75 min), II-IV) 233 K [60 °C] (5 min), 2.5 K/min until 423 K [150 °C] (25 min), V) 233 K [60 °C] (5min), 2.5 K/min until 423 K [150 °C] (85 min)

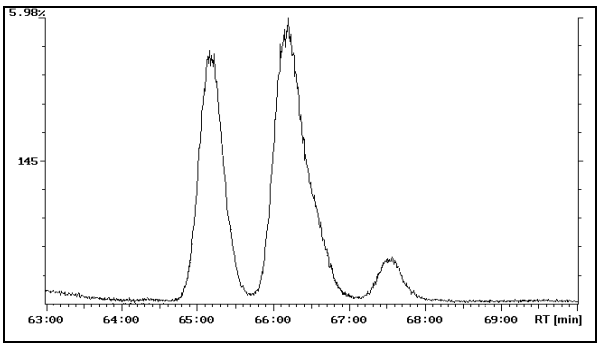

Figure 2: Enantioselective separation of carboxy-ibuprofen in the STP Hamburg influent; stationary phase: 6-TBDMS-2,3-Me-β-CD/50 % in OV-1701; temperature programme: 343 K [70 °C] (2 min), 10 K/min, 403 K [130 °C] (45 min), 5 K/min, 423 K [150 °C] (15 min)

At the inflow of the STP Hamburg the enantiomeric separations (Figure 1, top left) reflect the characteristics of the transformation of ibuprofen in the human body: after having been consumed as a racemate, (*R*,*S*)-ibuprofen is largely being transformed into the active *S*-enantiomer, 15 % of which are leaving the human body preferentially as *S*-enantiomer (first peak in Fig. 1) together with minor amounts of the *R*-enantiomer (second peak, Fig.1)[19]. This result is in line with observations of Buser et al.[20] who reported enantiomeric ratios of ER = *S*/*R* = 5.5 – 8. The enantiomeric excess of the main metabolite (45 % of the parent compound) at the inflow implies a preferential formation of the (2 *S*)-hydroxyibuprofen (first eluting peak, Figure 1, top right) in humans. The same characteristics were found at the inflow of the STP Tromsø and, as in the latter case no biological treatment step is included, at the outflow of the STP Tromsø as well (Figure 1, third row from top). The enantioselective separation of carboxy-ibuprofen stereoisomers thus far achieved for STP Hamburg influent sample extracts is shown in Figure 2. It has to be noted that a maximum of four peaks can be expected because two stereogenic centres are present. Presently, it must be left open as to whether co-elution of two of the stereoisomers or the presence of three stereoisomers explains the prevalence of three peaks only in the chromatograms. Basically, 26 % of the parent compound is assumed to be metabolised to (2´*RS*, 2 *S*)-carboxyibuprofen[19].

A significantly different enzymatic transformation of ibuprofen and formation of hydroxyibuprofen, respectively, can be inferred from the results obtained for the outflow of the STP Hamburg (Figure 1, second row from top). In the case of the parent compound, obviously the 2 *S*-enantiomer is preferentially transformed giving rise to an inversion of the enantiomeric excess in comparison with the inflow characteristics. Regarding the hydroxyibuprofen, a similar inversion tendency was observed.

In the river Elbe (Figure 1, second row from bottom), the enzymatic microbial transformation appears to show a transformation preference similar to that encountered in the human body. As a consequence, the enantiomeric excesses at the outflow of the STP Hamburg are inverted again. In order to verify this conclusion with regard to the principally different enzymatic transformation of ibuprofen within the STP Hamburg and the river water, a model experiment was carried out. A water sample of a small creek the water of which is flowing into the German Bight was taken, including the microbial community of this water body. The water was given into a self-contained water reservoir that was kept in the river, however, without any exchange between river water and reservoir water. The latter was spiked with ibuprofen and kept at this position for about one month in July and August. The enantioselective separation of ibuprofen is displayed in Figure 3 at the beginning of the experiment (top left) and after a transformation period of one month (bottom left). While no hydroxyibuprofen was present at the beginning of the experiment, an enantioselective microbial formation of this main metabolite can be inferred from the results obtained after one month (Figure 3, bottom right).

In conclusion, different enzymatic metabolism of ibuprofen and formation of the metabolites hydroxyibuprofen and carboxyibuprofen encountered in the human body, in a sewage treatment plant with biological treatment step and in rivers can be verified by enantioselective chromatographic analyses of water sample extracts.

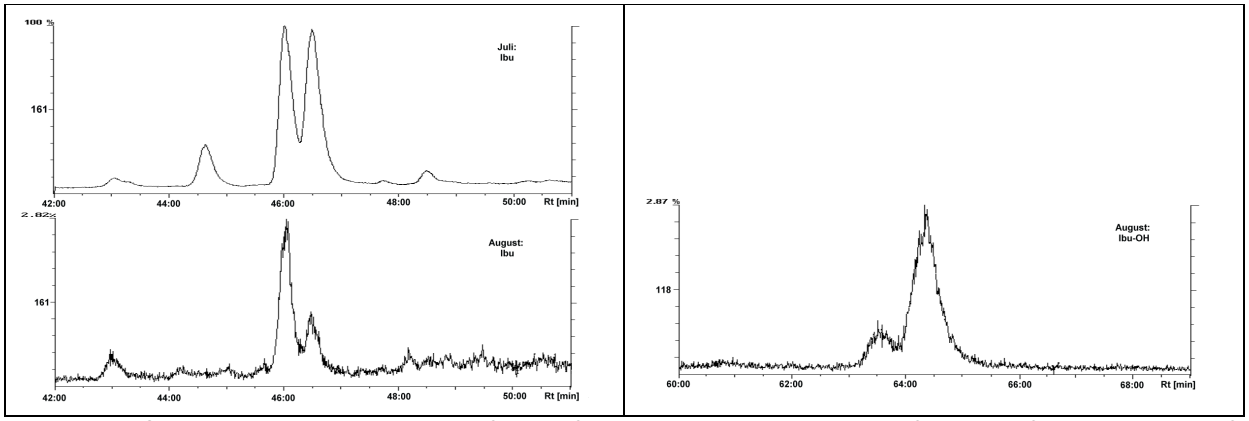

Figure 3, left: Enantioselective separation of ibuprofen at the beginning (top) and after a transformation period of one month carried out by a microbial community taken from a creek in Northern Germany; **right:** Enantioselective separation of hydroxyibuprofen formed from the spiked ibuprofen after a transformation period of one month (see above); in both cases stationary phase**:** 6-TBDMS-2,3-Me-β-CD/50 % in OV-1701 and temperature programme: 233 K [60 °C] (5 min), 2.0 K/min until 423 K [150 °C] (65 min)

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