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Short communication

Identification and enantioselective gas chromatographic mass-spectrometric separation of *O*-desmethylnaproxen, the main metabolite of the drug naproxen, as a new environmental contaminant

Stephanie Selke[a], Markus Scheurell[a], Muhammad Raza Shah[b], Heinrich Hühnerfuss[a,*]

[a] University of Hamburg, Institute of Organic Chemistry, Martin-Luther-King-Platz 6, 20146 Hamburg, Germany
[b] HEJ Research Institute of Chemistry, University of Karachi, Karachi, Pakistan

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ABSTRACT

O-Desmethylnaproxen (2-(6-hydroxynaphthalen-2-yl)propanoic acid) was identified in 10 different water samples from Germany and Pakistan. In the Pakistan samples it was found in all samples, surface water and effluents, exhibiting estimated concentrations between 0.04 and 1.36 μg/L. In Germany it was only encountered in the STP-effluent with an average concentration of 0.23 μg/L. Furthermore, enantioselective GC analyses revealed differences in the enantiomeric ratios found in Germany and Pakistan. To the best of our knowledge this is the first report on the identification of *O*-desmethylnaproxen, the main metabolite of the drug naproxen, in environmental samples.

1. Introduction

Naproxen (2-(6-methoxynaphthalen-2-yl)propanoic acid) is one of the most frequently consumed pharmaceuticals worldwide in the treatment of pain and inflammation, belonging to the class of non-steroidal anti-inflammatory agents (NSAIDs). As a consequence, it has been detected in many environmental samples such as wastewater and surface water, and thus it belongs, like many other PPCPs, to the emerging pollutants [1–4]. It is known from other pharmaceutical drugs, for example ibuprofen or diclofenac, that their human metabolites are also present in the environment and have to be regarded as environmental pollutants too [5,6]. Their toxicity may be higher than that of the parent compound thus making their presence in the environment especially precarious [7,8]. Despite being such a well investigated pharmaceutical residue, there is no knowledge about the occurrence of the metabolites of naproxen in the environment, although approximately 30% of the given dose of naproxen is excreted in the form of its main metabolite *O*-desmethylnaproxen (2-(6-hydroxynaphthalen-2-yl)propanoic acid) [9,10].

An additional important aspect comprises the chirality of many pharmaceutically active compounds, because the stereoisomers may trigger different biological effects. For example, the chiral naproxen is administered in the pure *S*-form due to fact that the antipode shows toxic effects [11]. Newer results revealed that also the enantiomers of pharmaceuticals like propranolol or fluoxetine have different toxic effects [12,13]. In the recent years, emphasis was placed on the analysis of single enantiomers also in the environment in order to get a more detailed insight into the enantiomeric composition and the changes of chiral compounds. Thus it could be revealed that compounds like ibuprofen and naproxen undergo changes in the composition of their respective enantiomers during sewage treatment plant passage [14,15]. Furthermore, it could be shown that hydroxyibuprofen is enantioselectively formed from the mother compound ibuprofen in the aquatic environment [16].

The objective of the present study was to develop a method for the identification and the enantioselective analysis of acidic pharmaceuticals in different kind of water samples, with special emphasis on the transformation of naproxen in the aquatic environment. Within this work GC–MS-analysis with prior derivatization was used in order to avoid matrix effects typical for LC–MS–MS to improve sensitivity in matrix-rich environmental samples [17].

2. Materials and methods

2.1. Chemicals

O-Desmethylnaproxen was purchased from Aldrich (Steinheim, Germany), naproxen was synthesized according to [18],

* Corresponding author. Tel.: +49 40428384240; fax: +49 40428382893.
E-mail address: huehnerfuss@chemie.uni-hamburg.de (H. Hühnerfuss).

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Table 1
Origin of samples investigated herein.

No	Sample	Position	Location characteristics
Hamburg			
1	Sewage treatment plant effluent	53°31′12.61″N 09°55′10.43″E	Municipal sewage treatment facility with mechanical, biological and chemical treatment
2	River	53°31′31.67″N 09°56′15.20″E	Elbe—Koehlbrand
3	River	53°32′37.52″N 09°54′51.17″E	Elbe—main river, stream downwards of 2
4	River	53°33′34.27″N 09°59′49.23″E	Outer Alster—an artificial dammed region of the tributary of the Elbe
5	River	53°35′33.97″N 09°59′40.05″E	Alster river, stream upwards of 4
Pakistan			
1	River	24°51′03.38″N 67°05′39.33″E	Malir River—major river in Karachi
2	Communal effluent	24°47′56.02″N 67°02′00.12″E	Clifton effluent—across the beach, untreated into the Arabian sea
3	Harbor lagoon	24°50′29.10″N 66°59′21.02″E	Mangrove lagoon—part of Karachi harbor
4	Drainage	24°48′51.96″N 67°07′10.17″E	Landhi—(Korangi drain) stream downwards of 5
5	Drainage	24°50′04.07″N 67°07′11.66″E	Landhi—(Korangi drain) stream downwards of 6
6	Drainage	24°50′54.27″N 67°11′24.25″E	Landhi—low-income residential area, open drainage (Korangi drain): canals receiving communal and industrial effluents, wash-off and rain
7	River	24°53′18.42″N 67°01′34.80″E	Lyari River—major river in Karachi
8	Drainage	24°48′23.49″N 67°06′10.02″E	Landhi—(Korangi drain) end of pipe, flowing untreated into Gizri creek
9	River	24°55′47.05″N 67°07′10.12″E	Lyari River—stream upwards of 7

mecoprop methylester D_3 was obtained from Dr. Ehrenstorfer (Augsburg, Germany). Methylchloromethanoate (MCM) and all solvents (SupraSolv grade, except water LiChroSolv grade and pyridine p.a. grade) were bought from Merck (Darmstadt, Germany). All gases of 5.0 grade were purchased from Linde (Germany).

2.2. Water samples

Water samples from the effluent of a sewage treatment plant (STP) and from the receiving river were taken between 2004 and 2009 in different sampling campaigns in the region of Hamburg, Germany (samples 1–3). The surface water samples were collected close to the point where the cleaned sewage water is released into a River Elbe tributary as well as at a point situated 3 km further downstream in the main River Elbe. Further samples were taken at the Elbe tributary Alster (samples 4 and 5; Table 1).

In Pakistan, surface water samples were collected during two campaigns in Karachi, in December 2006 and April 2007 (Fig. 1 and Table 1). Sample 1 was taken from the Malir River, while samples 7 and 9 stem from the Lyari River, the two major rivers flowing through Karachi. Sample 3 was taken in the mangrove lagoon, which is part of Karachi harbor receiving effluents from the center parts of the city. Samples 4, 5, 6 and 8 were taken from an open drainage canal system (Korangi drain) receiving untreated residential and industrial effluents as well as wash-off and rain water from the Landhi residential district. Sample 2 was taken from the end of a pipe eluting wastewaters from the district of Clifton across the beach into the Arabian Sea.

2.3. Water sampling and analytical procedures

All samples were taken in 2.5 L amber glass bottles with a sampling device designed in the working group and filtered through a glass fibre filter (GF-A, Whatman), a sample volume of 2 L of which was extracted over 1 g of Oasis HLB (Waters, Germany). After extraction of the sample the solid phase was dried with nitrogen, before being eluted with 40 mL of methanol. The resulting methanol eluates were then evaporated to dryness and derivatized with MCM, according to [19], to form the methyl esters (COOH-functions) or carbonate diesters (OH-functions), respectively. The resulting *n*-hexane phase of the derivatization reaction was spiked with 100 μL of an internal standard (mecoprop methylester [1 μg/mL]) and evaporated under a gentle stream of nitrogen to a final volume of 100 μL. GC–MS analysis was performed on a Magnum ITD 40 (Finnigan MAT, Bremen, Germany) ion trap mass spectrometer with the following conditions: EI at 70 eV, manifold temperature 473 K, emission current 10 μA, dwell time 100 μs, and scan range 40–500 *m*/*z* (full scan mode). It was coupled to a Varian 3400 GC system (Sunnyvale, CA, USA), separation was performed on a VF-5MS column, analogue to DB-5 (Varian, Sunnyvale, CA, USA), length 30 m, ID 0.2 mm, film thickness 0.33 μm, carrier gas Helium 5.0, transfer line 523 K run with an A 200 SE autosampler (CTC Analytics, Zwingen, Switzerland), injected volume 2 μL. The column was temperature programmed as follows: 333 K (2.5 min) with 6 K/min to 523 K (kept for 15 min). For the enantioselective separation a modified β-cyclodextrin was used (2.3-Di-*O*-methyl-6-*tert*butyldimethylsilyl-β-cyclodextrin [Hydrodex-β-6TBDM] from Macherey-Nagel, Germany, length 25 m, ID 0.25 mm, film thickness 0.1 μm). The column was temperature programmed as follows: 343 K (15 min) with 2 K/min to 493 K (kept for 30 min) with a carrier gas pressure of 12 PSI.

2.4. Semi-quantitative estimation

Since the method used in this study was originally developed for the parent compound naproxen and not for its metabolite *O*-desmethylnaproxen, no exact linearity range, LOD, LOQ or recovery rate can be given for the latter compound. Therefore,

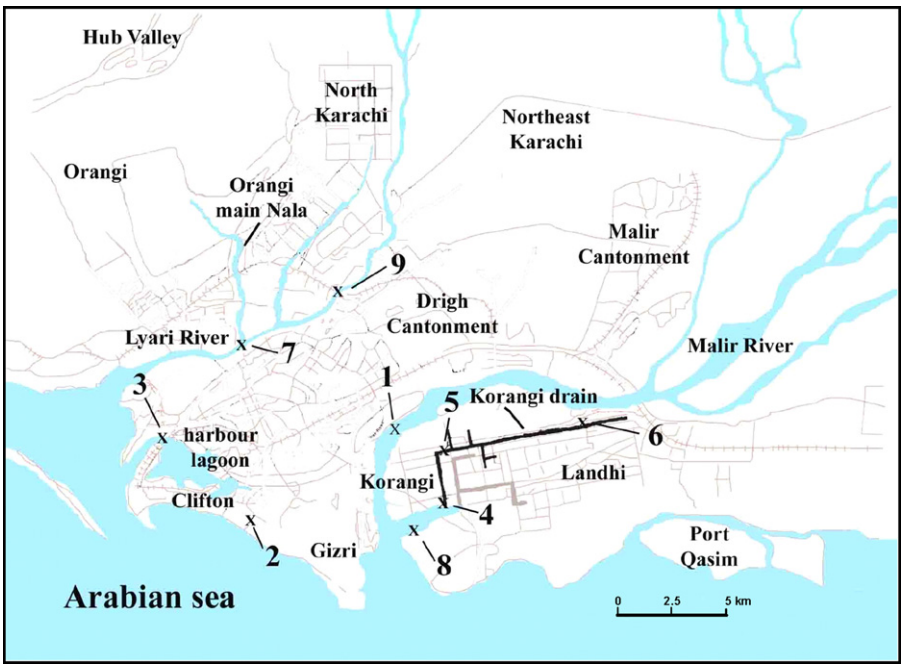

Fig. 1. Map of Karachi showing the rivers and the draining system, sampling locations are marked (X).

a so-called semi-quantitative concentration estimation of *O*-desmethylnaproxen was possible only using a procedure as follows: peak area ratios of *O*-desmethylnaproxen/internal standard were compared to the peak area ratios of the external standard/internal standard. The resulting concentrations were corrected by the recovery rate of naproxen (70 ± 7%), for which all crucial quality assurance parameters were determined during the validation of the present method for a linearity range of 4–1830 ng/L. Furthermore, a great number of blank control measurements were performed, in particular, in the course of the sampling campaign many field blanks were processed as well as procedural blanks after every four samples. *O*-Desmethylnaproxen was not found in any of them.

3. Results and discussion

3.1. Identification

O-Desmethylnaproxen was identified by comparing mass spectra of an external standard and the respective spectra obtained for water sample extracts (Fig. 2). In Germany, it was found in the STP-effluent sample extracts taken in different years, but in none of the surface water samples. In the former case, the concentrations comprised the μg/L-range [average value 0.23 μg/L]. In contrast, the mother compound naproxen was present in all analyzed samples from the Hamburg area. The concentrations were thereby in the STP-effluent comparable to those of the metabolite, in the surface water samples from the Elbe river they were in the lower ng/L-range. The concentrations of naproxen and *O*-desmethylnaproxen found in the samples are given in Table 2.

With regard to the samples taken in Karachi, *O*-desmethylnaproxen was found in all nine water sample extracts presented herein, i.e., in the methylated fractions of the methanol eluate. Two of the surface water samples (samples 1 and 7), taken from the two major rivers in Karachi, Malir and Lyari River, exhibited the highest concentrations, 1.34 and 1.36 μg/L, respectively. This may be due to the fact that no sewage treatment process exists in Karachi, all wastewaters, independent of their origin, mainly flow through open canals into the rivers and finally into the open sea. This may conceivably explain that in sample 9, stream upwards of 7, the concentration found is approximately half as much [0.66 μg/L], since two additional canals (nalas) drain into the Lyari River until reaching sample point 7. Furthermore, a great number of companies produce generic pharmaceuticals. For example, in the case of the Malir River regime, to the best of our knowledge at least eight companies can be specified, none of which treats their effluents before they enter into the Malir River. *O*-Desmethylnaproxen was also detected in the samples taken from the drainage system (Korangi drain) of Landhi town, a low-income residential area in Karachi. In this case the highest concentration was found at the beginning of the canal system (sample 6; 1.09 μg/L). Further down stream

Table 2
Approximate concentrations of *O*-desmethylnaproxen (assignment of samples see Table 1).

Compound	Concentrations [μg/L]								
Karachi samples	1	2	3	4	5	6	7	8	9
Naproxen	32.0	23.1	0.15	14.0	0.69	24.3	24.6	15.5	11.4
O-Desmethylnaproxen	1.34	0.56	0.04	0.28	0.08	1.09	1.36	0.52	0.66

Compound	Concentrations [μg/L]				
Hamburg samples	1	2	3	4	5
Naproxen	0.24	0.01	<0.01	<0.01	n.d.
O-Desmethylnaproxen	0.23	n.d.	n.d.	n.d.	n.d.

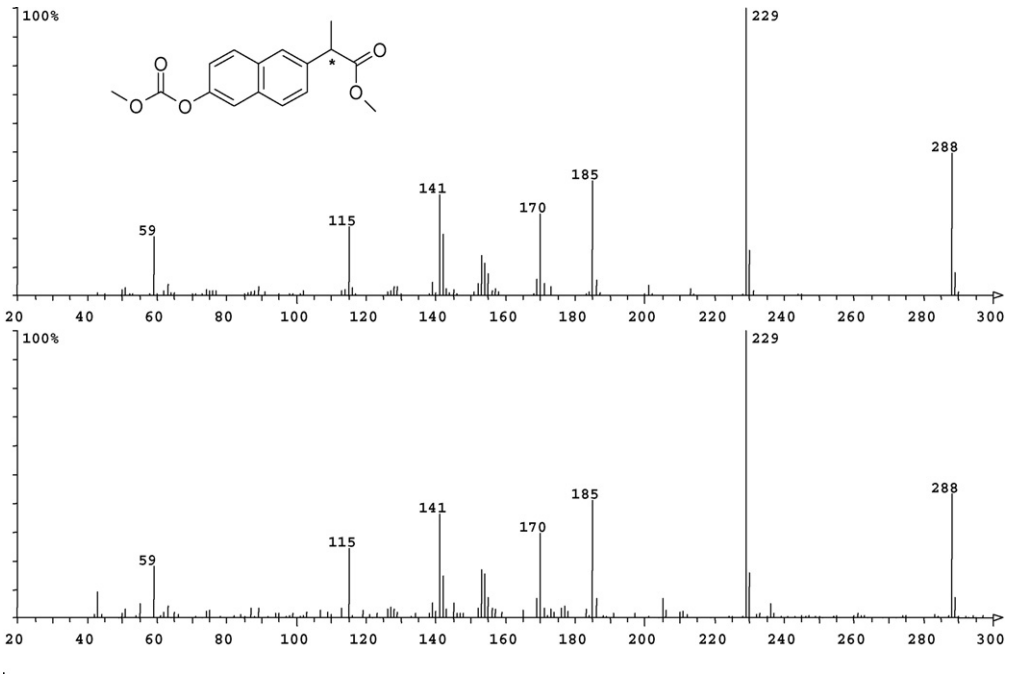

Fig. 2. Mass spectra of *O*-desmethylnaproxen as MCM derivative; above: external standard; below: sample 2 extract.

it is being diluted to concentrations of 0.08 μg/L in sample 5, rising again until reaching Gizri creek (samples 4 and 8; 0.28 and 0.52 μg/L, respectively). At the outflow of the harbor lagoon (sample 3) only a low concentration of *O*-desmethylnaproxen could be detected [0.04 μg/L]. Another relatively high concentration was found in the Clifton effluent [0.56 μg/L], a subterranean drainage system, receiving wastewater mainly from offices and high-income households. The mother compound naproxen was also present in all analyzed Karachi water samples (Table 2).

3.2. Enantioselective analysis

For the enantioselective analysis of *O*-desmethylnaproxen the same type of chiral selector and the same chromatographic conditions as for the mother compound naproxen were used. The enantiomers of the latter one are thus separated by 95% (Fig. 3a). Matrix effects resulting from different kind of water samples like wastewater or surface water had almost no influence on the separation of the enantiomers. Since *O*-desmethylnaproxen was available only in the form of the *S*-enantiomer, the quality of the enantioselective resolution could be checked on the basis of real environmental samples only (Fig. 3c).

Enantioselective analysis revealed that in the Hamburg STP-effluent sample 1 the first eluting enantiomer, which is the *S*-enantiomer of *O*-desmethylnaproxen, was the dominating one (Fig. 3c). The *R*-enantiomer was present at approximately 20%. The resolution of the *O*-desmethylnaproxen enantiomers is comparable to that of naproxen, where no statement on the influence of matrix can be given. It is worth noting that the enantiomeric ratio of *O*-desmethylnaproxen in the STP-effluent showed the same ratio as that of the mother compound (data not shown) [15]. In contrast, in all Karachi samples analyzed in this work exclusively the *S*-enantiomer was present (Fig. 3d and e). Thus, again the same

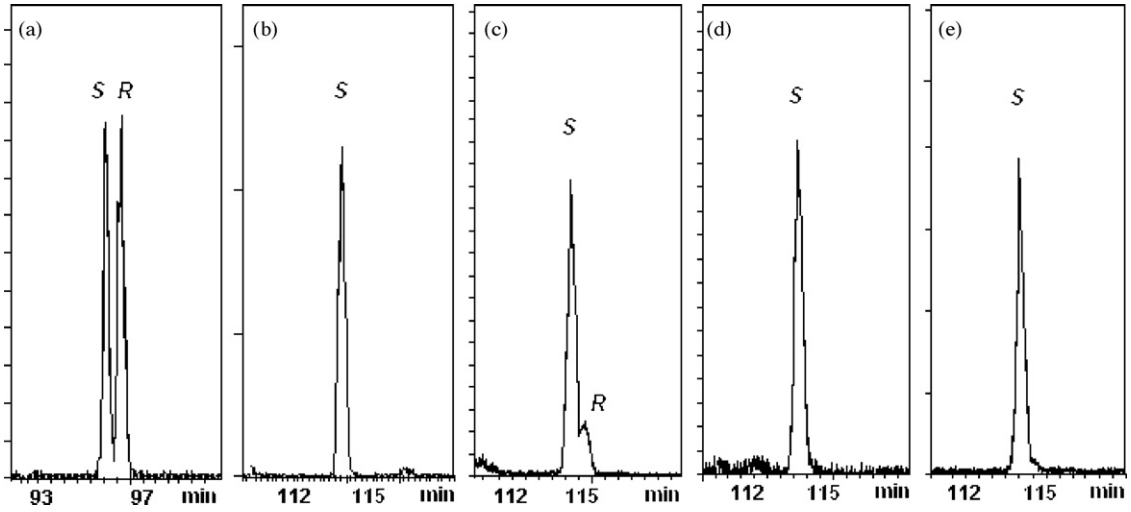

Fig. 3. Enantioselective GC–MS-analysis. (a) and (b) Standard solutions of naproxen and *O*-desmethylnaproxen, *O*-desmethylnaproxen in (c) STP-effluent (Hamburg, sample 1), (d) surface water (Pakistan sample 1), (e) surface water (Pakistan sample 7)

enantiomeric composition as for naproxen was found in these samples. Due to the fact that no sewage treatment process exists in Karachi and that the parent compound is administered in the pure *S*-form, it can be conceivably assumed that the sewage treatment process is responsible for the presence of the *R*-form of both compounds. Ecotoxicologically, the presence of the *R*-form in the STP-effluent could be of importance since the *R*-form of the mother compound is far more toxic than its antipode [11]. The outcome of this study gives further rise to the discussion on the importance of transformation products of environmental contaminants. Furthermore, it can be stated that newer environmental contaminants, such as PPCPs are not a problem solely for the industrialized countries but also in the developing world, especially in their fast growing mega-cities.

4. Conclusion

The naproxen metabolite *O*-desmethylnaproxen was detected in different types of water samples stemming from Germany and Pakistan. In Germany it was found in the effluent of a STP only, while in Pakistan it was encountered in effluents as well as in surface waters. The analysis of the single enantiomers showed that in Pakistan the *S*-form was present only, in contrast to the German STP-effluent where also the antipode was present. In both cases, the enantiomeric ratios reflect those of the mother compound also detected in the samples (data not shown). This implies that *O*-desmethylnaproxen is likely formed non-stereoselectively from the mother compound not only during sewage treatment process [20] and human metabolism, but also under environmental conditions. This is indicated by the relatively high amounts found in the surface water samples from Pakistan [0.66–1.36 μg/L] and the ratios compared to the mother compound, especially in sample 3. To the best of our knowledge this is the first report on the occurrence of *O*-desmethylnaproxen in environmental samples.

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