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Short communication

Formation of an artifact of diclofenac during acidic extraction of environmental water samples

K. Reddersen, Th. Heberer*

Institute of Food Chemistry, Technical University Berlin, Gustav-Meyer-Allee 25, Sekr. TIB 4/3-1, 13355 Berlin, Germany

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Abstract

Solid-phase extraction at an acidic pH is used as a common sample preparation method for analyzing residues of the analgesic drug diclofenac (2-[(2,6-dichlorophenyl)amino] benzeneacetic acid) in environmental water samples. This paper describes the matrix-dependent formation of an artifact of diclofenac during sample preparation resulting in an up to 40% underestimation of diclofenac concentrations especially in matrix-prone samples such as sewage effluents or surface water. The artifact most likely being formed during acidification of the sample was unequivocally identified as 1-(2,6-dichlorophenyl)indolin-2-one by capillary gas chromatography–mass spectrometry. To avoid an underestimation of the analytical results quantification of both diclofenac and its artifact is recommended.

Keywords: Environmental analysis; Water analysis; Diclofenac

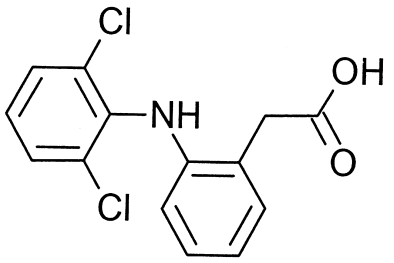

Fig. 1. Structure of diclofenac (2-[(2,6-dichlorophenyl)amino] benzeneacetic acid).

1. Introduction

Diclofenac (2-[(2,6-dichlorophenyl)amino] benzeneacetic acid shown in Fig. 1) is one of the most frequently administered non-steroidal drugs with analgesic, antipyretic, and anti-inflammatory properties mainly used for the treatment of rheumatic diseases or to relieve other pain. Diclofenac is applied orally, rectally or by injection but to a large degree also dermally as an ointment. The annual consumption in Germany has been estimated at about $75 \cdot 10^3$ kg [1]. In recent years, diclofenac was also found as an environmental contaminant in sewage, surface, ground, and drinking water samples [1–9]. In long-term monitoring investigations of sewage and surface water samples in Berlin, Germany [2], it was identified as one of the environmentally most important pharmaceutically active compounds

*Corresponding author. Tel.: +49-30-3147-2796; fax: +49-30-3147-2823.
E-mail address: thomas.heberer@tu-berlin.de (Th. Heberer).

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(PhACs) present in the water cycle at concentrations up to the μg/l level. No significant removal of diclofenac is observed during municipal sewage treatment [2,3]. Additional to dilution a pronounced and continuous decrease of diclofenac concentrations has been observed in contaminated surface waters, especially in summer [2,3]. Buser et al. [3] explained this phenomenon by photochemical degradation processes, an assumption which has recently also been confirmed by other authors [10,11]. In soil-column experiments and in investigations at bank-filtration sites, diclofenac was identified as a contaminant that is adsorbed and removed at considerable rates [12–16]. Nevertheless, it can also turn up into water-supply wells or drinking water at low-ng/l concentrations [1,4,12,15,16].

Some of the analytical methods described in recent literature apply solid-phase extraction (SPE), chemical derivatization, and gas chromatography–mass spectrometry (GC–MS) for the analysis of diclofenac in environmental water samples [3,6,8,17–19]. At neutral pH the carboxylic acid diclofenac is almost completely present in water samples in its dissociated form. Thus, it is important to lower the pH value to less then 2 to guarantee the protonation of the acidic moiety making it much better extractable from the aqueous matrix. Especially when using SPE with reversed-phase (RP) adsorbents (e.g., RP-C_{18}), adjusting the samples to a low pH value is essential to improve recovery rates to an acceptable and almost complete level. The acidification of the water samples may, however, also lead to undesirable by-products as will be described in this paper. Additionally, it will be demonstrated that the formation of different amounts of an artifact of diclofenac during acidic SPE also depends on the amount and composition of the matrix in the water samples.

2. Experimental

2.1. Chemicals and reagents

Diclofenac was obtained from Sigma–Aldrich (Steinheim, Germany), 1-(2,6-dichlorophenyl)indolin-2-one from Mikromol (Luckenwalde, Germany). Pentafluorobenzyl bromide (PFBBr) was purchased from Sigma–Aldrich and triethylamine from Merck (Darmstadt, Germany). Solvents used for sample preparation were of ‘‘ultra-residue purity’’ obtained from Merck.

2.2. Sample preparation

Details of the analytical procedure are described elsewhere [17,20]. Briefly, SPE is carried out at pH 2 using RP-C_{18} material (Bakerbond Polar Plus from Mallinckrodt-Baker, Griesheim, Germany). After elution with methanol, diclofenac is derivatized by adding 100 μl of a PFBBr solution (2%, v/v, in toluene), 4 μl triethylamine and by heating the sample for 1 h at 100 °C.

2.3. Instrumentation

GC–MS analysis was carried out using a HP 6890 gas chromatograph and a HP 5973 quadrupole mass spectrometer from Agilent Technologies (Waldbronn, Germany). For chromatographic separation a (5% phenyl)methylpolysiloxane column (HP5MS, 30 m×0.25 mm I.D., 0.25 μm film thickness) from Agilent Technologies was used. The carrier gas was Helium 5.0 (99.999% purity). The flow velocity was set to 37 cm/s (1 ml/min) in constant flow mode, injection volume was 2 μl. The temperature program was started at 100 °C (held for 1 min), set at a rate of 30 °C/min up to 150 °C (held for 2 min), at a rate of 3 °C/min up to 205 °C (held for 1 min), at a rate of 10 °C/min up to 260 °C (held for 5 min), and finally at a rate of 10 °C/min up to 280 °C (held for 14 min).

3. Results and discussion

When analyzing sewage effluents and surface water samples with GC–MS in the full scan mode, a second analyte peak was detected in some of the chromatograms. As shown in Fig. 2, this formerly unknown analyte peak was present with a considerable intensity in the initially residue-free surface water sample fortified with diclofenac at a concentration of 200 ng/l. The original sample collected from a non-contaminated surface water sampling site did not contain any diclofenac residues which was also confirmed in a preceding analysis of the same

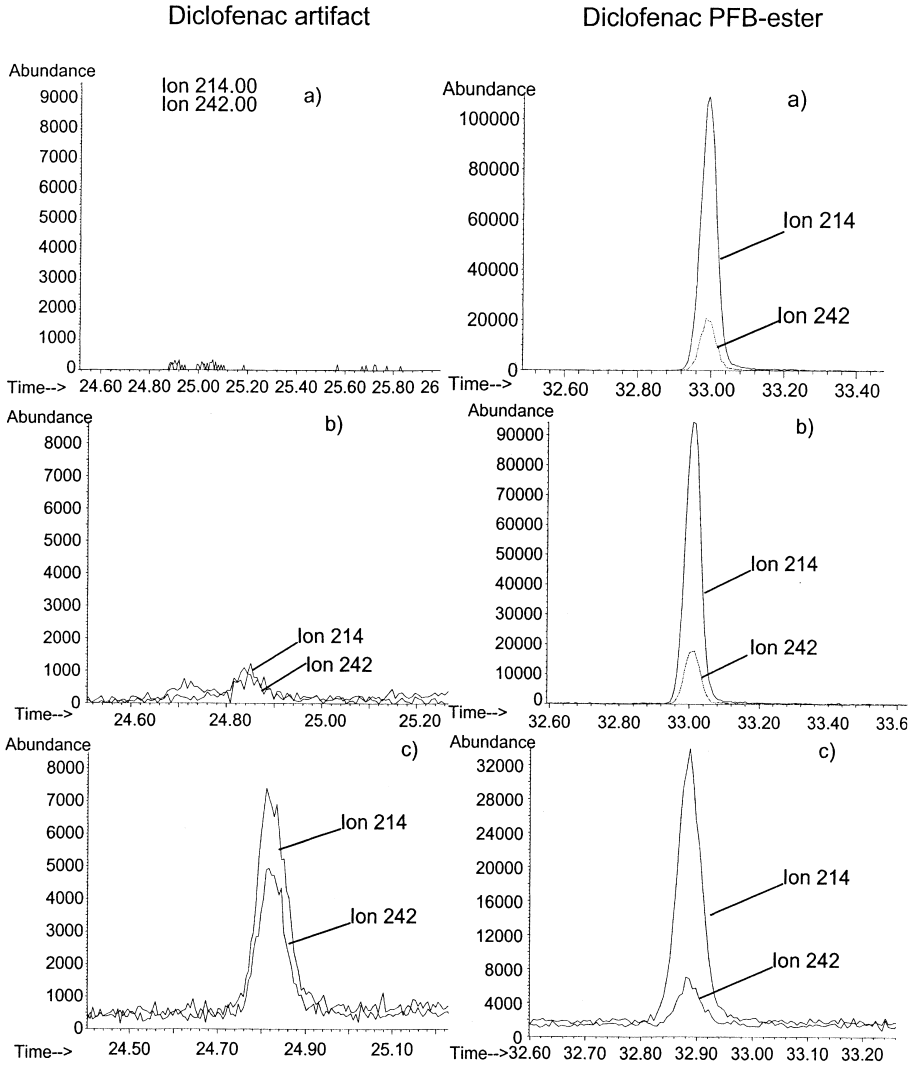

Fig. 2. Reconstructed ion chromatograms (RICs) recorded with GC–MS in the full-scan mode of (a) a derivatized standard of diclofenac (100 ng), (b) a derivatized extract of a distilled water sample spiked with diclofenac at 200 ng/l (extracted volume: 500 ml), and (c) a derivatized extract of a fortified surface water sample containing 200 ng/l of diclofenac residues (extracted volume: 500 ml).

sample. On the other hand, the unknown compound peak was not or only found at low intensities in the reconstructed ion chromatograms (RICs) recorded for the derivatized standard compound and the derivatized extract of a distilled water sample that was also spiked with 200 ng/l of diclofenac prior to sample extraction (Fig. 2).

The mass spectra of the unknown peak and of diclofenac derivatized with PFBBr are shown in Figs. 3 and 4. Several fragment ions occurring in the mass spectrum of the unknown compound are similar to those recorded in the mass spectrum of diclofenac PFB ester. But the molecular chlorine-cluster ions of the diclofenac PFB ester at m/z 475, 477, and 479 are missing in the mass spectrum of the unknown analyte as well as the fragment ion at m/z 181 originating from the PFB moiety. This suggests that in contrast to diclofenac, the unknown analyte does not possess a carboxylic group that can and would have been derivatized with PFBBr. On the other

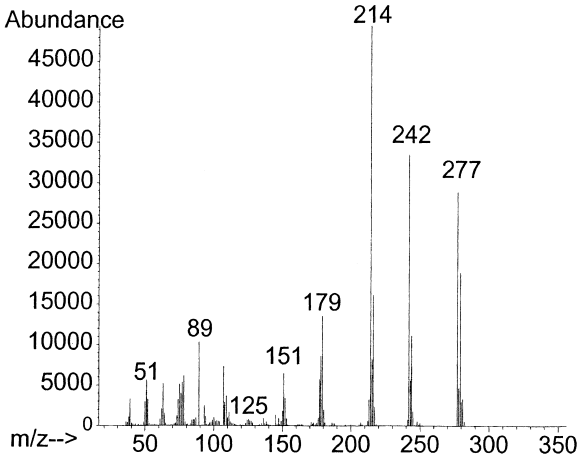

Fig. 3. Mass spectrum of the diclofenac artifact recorded with GC–MS in the full scan mode using electron impact ionization at 70 eV.

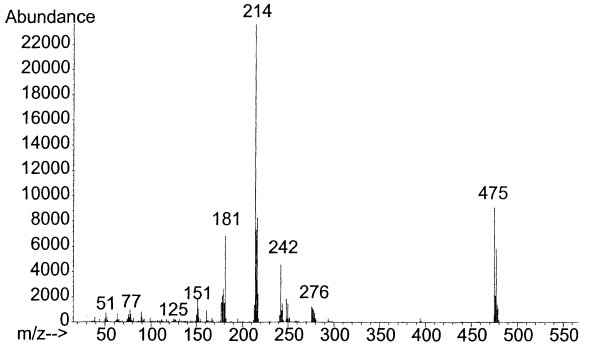

Fig. 4. Mass spectrum of diclofenac derivatized with PFBBr recorded with GC–MS in the full scan mode using electron impact ionization at 70 eV.

hand, the unknown compound must structurally be very similar to diclofenac as it almost matches with the lower mass ions found in the mass spectrum of diclofenac PFB ester.

Fig. 5 shows the chemical structures of mass fragments with m/z 214/216 and 242/244 appearing in the electron impact ionization (EI) mass spectrum of diclofenac PFB ester. Proposing the same identity for these mass fragments in the mass spectra of diclofenac PFB ester and the unknown analyte, the only plausible identity of this compound was 1-(2,6-dichlorphenyl)indolin-2-one, a commercially available standard reference compound. The structure of this artifact is given in Fig. 6. It results from the condensation of the carboxylic- and the amino-group in the molecule of diclofenac forming an additional nitrogen-containing ring in the molecule. This structure also explains why this compound can not be derivatized. The molecular ion cluster in the mass spectrum of this compound at m/z 277, 279, and 281 (Fig. 3) represents a characteristic isotopic cluster of a dichloro compound. Assuming the ions at m/z 277, 279, and 281 to be the molecular ions of this compound, the odd values suggest a compound

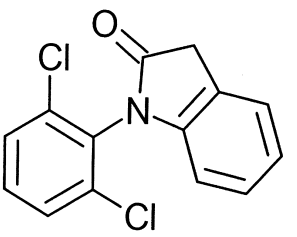

Fig. 6. Structure of 1-(2,6-dichlorophenyl)indolin-2-one (diclofenac artifact).

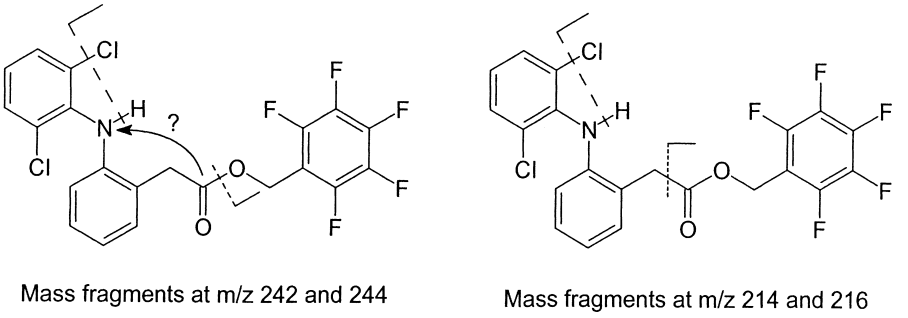

Fig. 5. Chemical structures of mass fragments with m/z 214 and 242 appearing in the EI mass spectrum of diclofenac PFB ester.

containing an odd number of nitrogen atoms. The mono-chlorine ion cluster containing the mass fragments at m/z 242 and 244 is obtained after cleavage of one chlorine atom from the molecular ion. The mass fragments at m/z 214 and 216 result from the further cleavage of the CO moiety. Finally, the identity of the unknown compound was confirmed by comparing the mass spectrum and retention time of the unknown peak with the commercially available reference standard of 1-(2,6-dichlorophenyl)indolin-2-one.

The formation of this artifact during analysis of environmental samples has not yet been described. But in a paper published in 1975, Geiger et al. [21] used the formation of this artifact in acidic solution for the detection of diclofenac in biological matrices.

Our investigations have also shown a strong matrix dependency of the amount of diclofenac being transformed into the artifact during sample preparation. Quantitation of the artifact was performed by external calibration. When spiking purified water with diclofenac the formation of the artifact was negligible (Fig. 2b). When analyzing native surface water samples the amount of the artifact was between 10 and 60% compared to the amount of the parent compound (diclofenac was found at concentrations of up to 1.03 μg/l in Berlin) and in native municipal sewage effluents it was between 10 and 40% (mean sewage effluent concentration of diclofenac in Berlin: 2.64 μg/l). The artifact was also detected at considerable rates in diclofenac-residue containing bank-filtered water collected downstream from municipal sewers. Several spiking experiments with residue-free surface water samples collected upstream from municipal sewers identified the acidification step as the source most likely being responsible for the formation of the artifact (Fig. 2c). On the other hand, the derivatization step could almost be excluded as a possible source for the formation of the artifact (see Fig. 2a). This was also confirmed by spiking sample extracts of residue-free surface water samples prior to derivatization and by alternatively applying *N*-(*tert.*-butyldimethylsilyl)-*N*-methyl-trifluoracetamide (MTBSTFA) [20] for the derivatization of sample extracts fortified with diclofenac prior to SPE. It can, however, not be excluded that the artifact is additionally also formed in the environment by sunlight radiation. This may also explain the higher shares of the artifact detected in native surface and bank-filtered water samples (up to more than 60% of the parent compound values) compared to municipal sewage effluent samples (up to 40%).

Thus, neglecting the formation of this artifact during acidic extraction may lead to an underestimation of the results for diclofenac strongly depending on the amount and the composition of the matrix in the water samples. An underestimation of up to 30 and 40% in sewage effluent and surface water samples, respectively, was observed in our field studies when only the derivatized amount of diclofenac was used for quantitation. This problem can, however, be overcome by additional quantification of the artifact and by including this result into the calculation of the total amount of diclofenac in the sample.

4. Conclusion

This paper describes the formation of an artifact of the drug diclofenac during acidic extraction of environmental water samples. The artifact most likely formed in the acidification step of the sample preparation was identified as 1-(2,6-dichlorophenyl)indolin-2-one. To avoid an underestimation of the results when analyzing diclofenac in sewage, surface, and bank-filtered water samples using acidic extraction, the additional quantification of this artifact and the addition of the respective result to the total amount of diclofenac is recommended.

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