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# Quantification of triiodinated benzene derivatives and X-ray contrast media in water samples by liquid chromatography–electrospray tandem mass spectrometry

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## Abstract

A method for the analysis of iodinated X-ray contrast media and possible metabolites in environmental water samples is presented. The method consists of a sequential solid-phase extraction followed by high-performance liquid chromatography coupled with tandem mass spectrometry for detection. The recoveries for the analytes in tap water varies between 70 and 100%. For matrix poor samples the detection limit is in the lower ng/l range. In case of native, complex samples the recoveries are lower and the parallel analysis of a spiked sample is necessary to obtain reliable data. With the presented method iodinated X-ray contrast media could be detected and quantified in a sewage treatment plant effluent, in a receiving channel and lake. In the receiving lake the concentrations are still high with values between 0.5 and 4 µg/l. © 2001 Elsevier Science B.V. All rights reserved.

**Keywords:** Water analysis; Liquid chromatography–tandem mass spectrometry; X-Ray contrast media; Triiodinated benzene derivatives

## 1. Introduction

Already 10 years ago it has been recognised that some municipal treatment plant effluents exhibit high concentrations of adsorbable organo halogen (AOX) compounds. The differentiation of the sum parameter AOX into AOCl, AOBr and AOI revealed that the AOI contributes a large part to the AOX [1,2]. Based on the consumption of halogenated pharmaceuticals Gartiser et al. [3] showed that iodinated X-ray

contrast media are causing increased AOX concentrations of hospital waste water. Together with the fact that the weekly AOI concentration profile of a municipal treatment plant effluent, receiving hospital waste water, is characterized by low values over the weekend and high values during the week leads to the assumption that the iodinated contrast agents are responsible for the high AOI [2].

The worldwide consumption of the iodinated X-ray contrast media, which are all triiodinated benzene derivatives, is around 3500 tons per year. For one medical examination about 100 g of X-ray contrast media (ca. 30 g AOI) are applied [4]. Based on the required properties of the contrast agents they are very polar and persistent and are eliminated via

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urine or faeces unmetabolised after some hours of application. The very high water solubility and the persistency is a disadvantage from the environmental point of view. Municipal treatment plants are not able to remove the contrast agents [5,6]. Furthermore, iodinated compounds have been identified in all parts of the water cycle [7,8]. Iodinated compounds are present in the environment in high concentration, and therefore there is a big need to have reliable methods also for complex matrices. In this paper we will provide a detailed description for the analysis of the very polar iodinated benzene derivatives in water samples.

## 2. Experimental

### 2.1. Chemicals

Four iodinated contrast agents and four possible metabolites (purity >99%) were used to develop an analytical method. The structures and abbreviations used are given in Fig. 1. The iodinated contrast agents are from Schering (Berlin, Germany) and the possible metabolites are from Professor Sovak (University of California, San Diego, CA, USA). All organic solvents were gradient grade (LiChrosolv, Merck, Darmstadt, Germany, except methanol which

was from Baker). Trifluoroacetic acid (purity >98%) was purchased from Fluka (Deisenhofen, Germany) and nitric acid (suprapure, for pH adjustment), from Merck. Ultra pure water was produced out of deionised water by treatment in an Elga purification system (Ubstadt-Weiher, Germany).

### 2.2. Extraction

Sample extraction was performed on a Adsorbex sample preparation unit (Merck). Disposable 3-ml LiChrolut EN (200 mg; Merck) and Envi-Carb (250 mg; Supelco) cartridges were used for the sequential extraction. Before use the EN cartridges were first cleaned with ultra pure water and then activated with 9 ml methanol and 9 ml ultra pure water (pH 3.5). The Envi-Carb cartridges were treated similarly but with ultra pure water adjusted to pH 2. The samples, adjusted to pH 3.5, were first percolated with a flow-rate of 200 ml/h through the LiChrolut EN material and the filtrates were collected. After adjustment to pH 2 the filtrates were percolated (300 ml/h) through the Envi-Carb material. After extraction the cartridges were dried for ca. 1 min under vacuum. The compounds adsorbed on the EN material were eluted with 6 ml methanol. The Envi-Carb cartridges were eluted against extraction direction with a mixture (8 ml) of acetonitrile–ultra pure water (1:1,

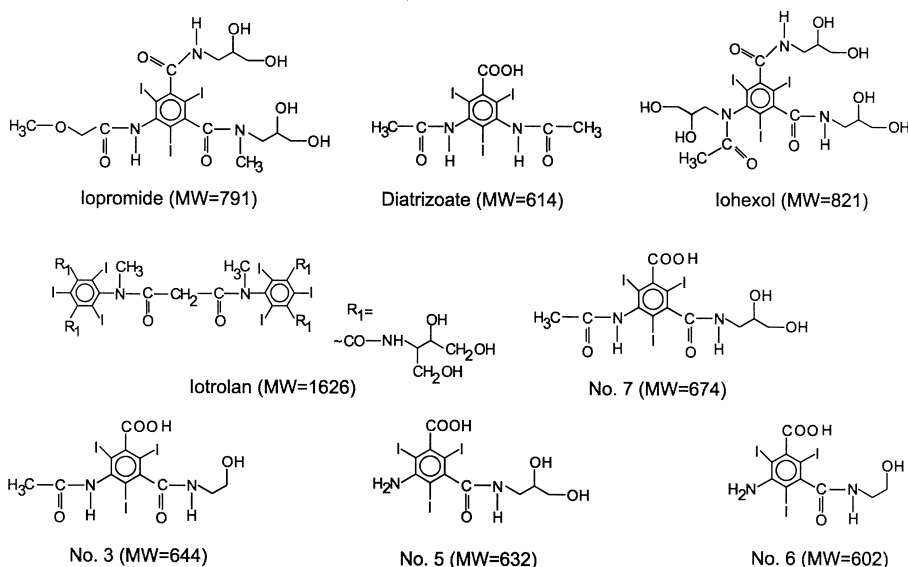


Fig. 1. Structures, abbreviations and molecular masses ( $M_r$ ) of iodinated contrast agents and possible metabolites.

v/v) and a trace ammonium acetate. For comparison one sample was extracted with the solid-phase Isolute ENV+ (IST, Separtis, Germany). The cartridge was conditioned with *n*-hexane, methanol and ultra pure water (pH 2.8). The sample adjusted to pH 2.8 was percolated through the ENV+ material. After extraction the solid phase was eluted with 4 ml methanol (for details see Ref. [8]).

The extracts were concentrated to a final volume of ca. 1 ml in an automatic SpeedVac concentrator (AS 160, Savant). The concentrated extracts were transferred to a 2.5-ml vial. In the case of native samples the LiChrolut EN and the Envi-Carb extracts were combined. Under a gentle nitrogen stream the solvent was evaporated. For analysis the native samples were dissolved in 0.5 ml of eluent A (see below) and all other extracts in 1 ml.

### 2.3. Recovery

Recoveries were evaluated by extraction of 500 ml tap water spiked with 2.5 and 0.5  $\mu\text{g}$  of each standard and by extraction of 1 l tap water with 10  $\mu\text{g}$  of each standard. The enrichment procedure was repeated three times for the lower concentrations (0.5 and 2.5  $\mu\text{g}$ ) and two times for the high concentration (10  $\mu\text{g}$ ). In blank experiments (without standard addition) none of the analytes could be detected.

### 2.4. Samples

A 24-h-mixed sample of a municipal waste water treatment plant effluent was kindly provided by the Berlin Waterworks (Berliner Wasserbetriebe). A grab sample of a channel and a surface water both influenced by the sewage effluent were sampled by ourselves. All samples were filtered through 0.45- $\mu\text{m}$  cellulose nitrate membranes (Satorius, Göttingen, Germany). If not analysed immediately, the samples were stored in the dark at 4°C.

### 2.5. High-performance liquid chromatography–tandem mass spectrometry (HPLC–MS–MS)

HPLC analysis was carried out using a HP system (Hewlett-Packard series 1100, Waldbronn, Germany) comprising a vacuum solvent degassing unit, a binary high-pressure gradient pump, an automatic sample injector and a photodiode array absorbance

(PDA) detection system. The HPLC system was coupled with a orthogonal Z-spray-electrospray interface to a mass spectrometer (Quattro-LC; Micromass, Manchester, UK). Drying gas and nebulizing gas was nitrogen generated from pressurized air (Whatman, Haverhill, USA). Electrospray ionisation was used in the positive ion (PI) mode. The compounds were detected in the selected-reaction monitoring (SRM) mode. Argon 5.0 (Messer Griesheim, Germany) was used as collision gas. Infusion experiments were performed using a Modell 11 syringe pump (Harvard, Holliston, USA).

For separation a reversed-phase column [Phenomenex: Luna 3  $\mu\text{C}_{18}(2)$ ; 150 $\times$ 2 mm] was used. The linear gradient elution programme was as follows, where A=ultra pure water+0.05% (w/v) trifluoroacetic acid (TFA) and B=methanol+0.05% TFA (w/v): from 0% B to 5% B in 10 min, to 25% B after 25 min; to 0% B after 27 min, which than was hold for 5 min. The flow-rate was 0.25 ml/min. The column oven was set to 45°C and the injection volume was 10  $\mu\text{l}$ .

### 2.6. Quantification

For UV and MS detection external calibration was applied. Stock solutions of standards dissolved in ultra pure water were diluted using eluent A. All real samples were analysed with and without standard addition (1  $\mu\text{g}$  of each compound). The standards were added before extraction. The concentration of each analyte in the non spiked and spiked sample were calculated. The recovery for each compound was determined by subtraction of the two data sets (spiked–non spiked sample). The concentrations of the native samples were than corrected based on the recoveries of the native, spiked samples thus, losses during the enrichment procedure and a possible decreased ionisation yield, caused by the matrix of samples, are compensated.

## 3. Results and discussion

### 3.1. HPLC–UV

With the HPLC conditions described in the Experimental section all eight available standards can be separated (Fig. 2). The two peaks for Iopromide

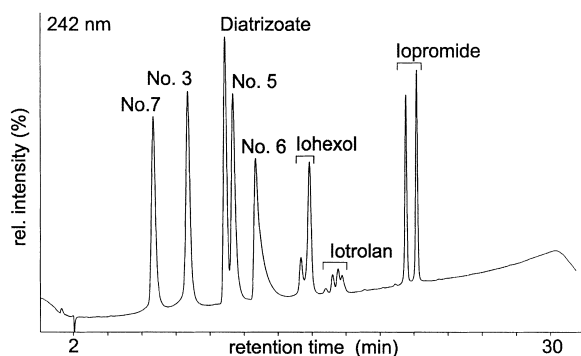


Fig. 2. UV chromatogram of standards diluted in eluent A. For structures see Fig. 1.

and Iohexol and the four peaks for Iotrolan are caused by stereoisomerism [9,10]. A separation of all compounds is not possible using a similar column (RP-C<sub>18</sub>) with a higher particle size (5 μm). With ion-pair chromatography (tetrabutylammonium bromide, pH 7.5) a poor separation is achieved. Ion-pair chromatography cannot be recommended if MS detection is required, because the ionisation yield is very low. Furthermore, Hirsch et al. [8] observed a decreasing sensitivity when using ion pair chromatography, due to pollution of the MS source by the ion pair reagents. The compounds can be detected using UV absorption at 242 nm. In case of standard solutions 1 ng absolute of each compound can be detected, except Iotrolan which detection limit is around 5 ng absolute. Linear calibration curves of standards in eluent A with concentrations of 0.1, 0.5, 1, 2.5 and 5 μg/ml were obtained. Regression coefficients were above  $r^2=0.999$ . The areas of the isomers for Iopromide and Iohexol were added for quantification.

### 3.2. LC–MS–MS

UV detection of the compounds in the enriched complex mixture is not possible, thus the analytes were detected by SRM, after collision induced dissociation. Infusion experiments were performed to determine intensive product ions. The precursor ions, the detected product ions and the collision energies are listed in Table 1. The double charged ion of Iotrolan is produced in higher yield than the single charged ion [7]. It was not possible to receive intensive product ions of Iotrolan by collision-induced dissociation, thus Iotrolan was not analysed by SRM.

The mass spectrometer was calibrated over a concentration range from 0.5 to 50 ng absolute of each compound when real samples were analysed. Linear regression coefficients higher than  $r^2=0.999$  were obtained for Iopromide, Iohexol, No. 3 and No. 6. For the other compounds  $r^2$  was  $>0.996$ . For the determination of recoveries of standards in tap water the MS was calibrated over the expected concentration range. For these samples the calibration was just done over 1 decade ( $r^2>0.999$ , for all compounds).

Fig. 3 shows the total ion chromatogram of SRM fragments of a standard mixture diluted with eluent A. The absolute amount of each compound is 0.5 ng. The sensitivity of the selected compounds is different (see Fig. 3) and 0.25 ng of Iopromide, Iohexol and No. 6 are still detectable. If 0.5 ng absolute of each compound is used as detection limit for SRM, then the limit of detection (LOD) for the hole method is 50 ng/l (enrichment: 1000). In case of matrix poor samples the LOD can be increased by increasing the

Table 1  
Precursor ions, product ions and collision energies (CEs) used for LC–MS–MS analysis

Compound	Precursor ion ( $m/z$ )	Product ion ( $m/z$ )	CE (eV)	Loss of
Iopromide	792	573	24	HI; C <sub>3</sub> H <sub>8</sub> NO <sub>2</sub>
Iohexol	822	803	20	H <sub>2</sub> O
Diatrizoate	615	361	20	2I
Iotrolan	814	n.d.	n.d.	
No. 7	675	428	22	HI; C <sub>4</sub> H <sub>8</sub> NO <sub>3</sub>
No. 3	645	584	18	C <sub>2</sub> H <sub>6</sub> NO
No. 5	633	541	22	C <sub>3</sub> H <sub>8</sub> NO <sub>2</sub>
No. 6	603	541	20	C <sub>2</sub> H <sub>6</sub> NO

n.d., Not detectable.

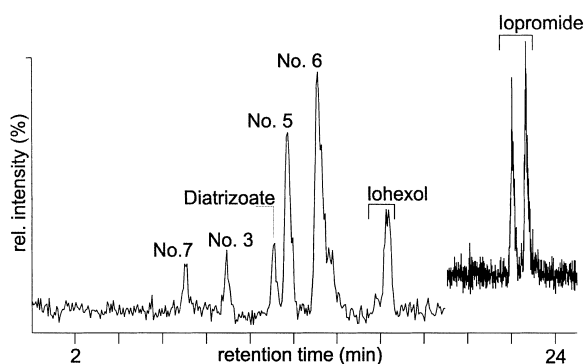


Fig. 3. Total ion chromatogram of SRM fragments of standards diluted in eluent A. Absolute amount injected: 0.5 ng of each compound.

enrichment and the injection volume (e.g., enrichment 2000, injection volume 20  $\mu\text{l}$ ).

The sensitivity of the MS detection can be reduced by TFA [11] and using, instead, e.g., formic acid might well have improved the LODs. However, this aspect was not studied for the triiodinated compounds. A separation of all compounds is probably not possible when using a different acid, and co-elution of matrix compounds could reduce the ionisation yield too. Admittedly, the detection and quantification of co-eluting compounds is possible when using SRM [8], but we preferred to have a baseline separation for the analysis of the complex samples.

### 3.3. Solid-phase extraction/recovery

The detection and quantification of trace compounds requires enrichment. The quality of the described sequential solid-phase extraction (SPE) has been controlled by the recoveries, determined for different initial concentrations of analytes in tap water. The enrichment for three initial concentrations has been performed several times to check the repeatability. The recovery as the sum of the two extracts is in general high and varies between 80 and 100%, except for Iotrolan which was only recovered by 55% and, at the lowest initial concentration the recovery for Nos. 7 and 6 was 70% (Table 2). The recoveries obtained by UV and MS detection are slightly different. Sodium adducts which are formed during MS analysis are most probably the reason for the different recoveries. Under consideration of the standard deviation, the sequential extraction procedure is reproducible for the enrichment of standards in tap water. It is not possible to achieve good recoveries for all compounds by just one extraction step (Fig. 4). With the first extraction more than 40% of Nos. 5, 6, Iohexol and Iopromide can be extracted, whereas the yield for the other compounds is poor. The second extraction step is necessary to obtain good overall recoveries.

### 3.4. Analysis of native samples

To evaluate the method a sewage treatment plant

Table 2  
Recovery (sum of both extracts) of standards in tap water

Compound	Recovery (%) and standard deviation				
	10 $\mu\text{g}/\text{l}^{\text{a}}$ ( $n=2$ )	5 $\mu\text{g}/\text{l}^{\text{a}}$ ( $n=3$ )	1 $\mu\text{g}/\text{l}^{\text{a}}$ ( $n=3$ )	5 $\mu\text{g}/\text{l}^{\text{b}}$ ( $n=3$ )	1 $\mu\text{g}/\text{l}^{\text{b}}$ ( $n=3$ )
Iopromide	90 $\pm$ 10	80 $\pm$ 5	80 $\pm$ 5	90 $\pm$ 5	100 $\pm$ 5
Iohexol	80 $\pm$ 15	90 $\pm$ 5	90 $\pm$ 5	90 $\pm$ 5	90 $\pm$ 5
Iotrolan	n.a.	n.a.	n.a.	55 $\pm$ 5	n.d.
Diatrizoate	90 $\pm$ 15	100 $\pm$ 10	100 $\pm$ 10	85 $\pm$ 5	100 $\pm$ 5
No. 7	100 $\pm$ 10	100 $\pm$ 5	80 $\pm$ 10	80 $\pm$ 5	70 $\pm$ 10
No. 3	90 $\pm$ 10	100 $\pm$ 10	100 $\pm$ 10	90 $\pm$ 5	85 $\pm$ 5
No. 5	n.a.	85 $\pm$ 5	90 $\pm$ 5	90 $\pm$ 5	85 $\pm$ 5
No. 6	100 $\pm$ 5	80 $\pm$ 5	80 $\pm$ 5	90 $\pm$ 5	70 $\pm$ 10

n.d., Not detectable; n.a.: not analysed.  $n$ =Number of experiments.

<sup>a</sup> Quantified using SRM detection.

<sup>b</sup> Quantified using UV detection.

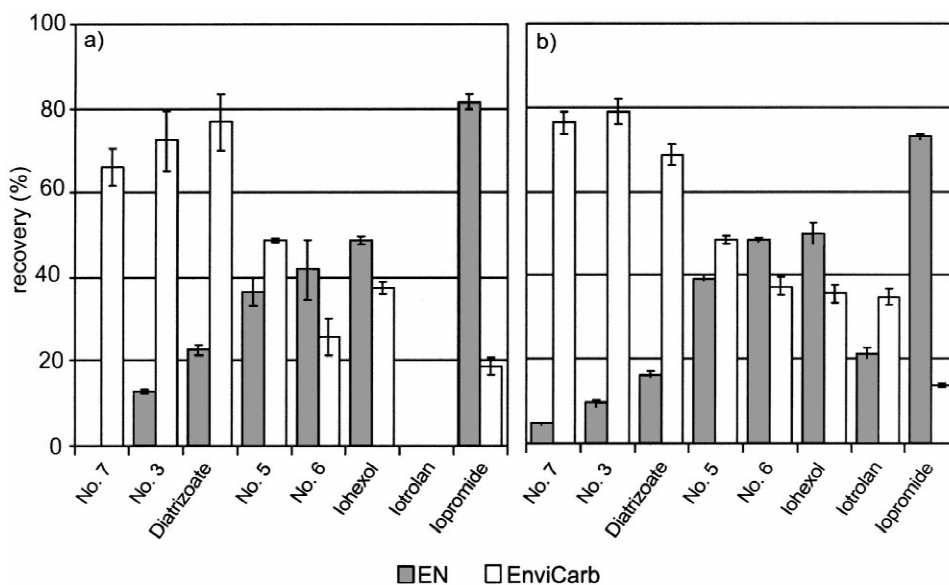


Fig. 4. Recovery of the triiodinated benzene derivatives in the EN and EnviCarb eluate. Initial concentration of each compound in tap water: (a) 1 µg/l and (b) 5 µg/l.  $n$ =Number of experiments.

effluent, a channel and a lake, both receiving the effluent were analysed. A 500-ml volume of lake water, 50 ml of the channel water and the effluent were extracted with and without standard addition. No. 5 was not analysed in the real samples, because from literature it is known that No. 5 does not occur in environmental water samples [6,7].

In all samples Iopromide, Iohexol and Diatrizoate are detectable (Table 3). In the sewage treatment plant effluent the concentration of X-ray contrast media is very high with values up to 20 µg/l for Iopromide, 13 µg/l for Diatrizoate and 7 µg/l for Iohexol. In the receiving channel the concentrations are reduced by ca. 2, due to dilution. Even in the receiving lake the concentration of X-ray contrast media are high with values varying between 4 and 0.5 µg/l. A possible metabolite (No. 3) has been detected in the sewage treatment plant effluent and in the channel water (Fig. 5). The occurrence of No. 3 as a metabolite has to be controlled and confirmed by analysis of more samples.

The recoveries for the triiodinated benzene derivatives in the lake water vary between 65 and 110%. The recoveries are roughly comparable with those obtained for the enrichment of standards in tap water, but day-to-day changes are obvious, especially

for the ionic compounds (Diatrizoate, Nos. 7, 6, and 3). The recovery for the compounds in the more complex samples varies between 30 and 65% (sequential SPE). For these samples the recoveries are reduced up to 40% for the non ionic compounds (Iopromide, Iohexol) and up to 65% for the ionic compounds, compared with the recoveries in tap water. The reduced recovery is caused by the matrix of the complex samples. The capacity of the extraction material can be reduced for the analytes of interest. A reduced ionisation yield caused by the matrix is not responsible for the low recoveries. The concentrations of the non spiked samples were corrected based on the recoveries obtained by analysing samples spiked with all analytes of interest, thus the influence of the matrix is compensated.

To check the quality of the results, the original complex samples (acidified to pH 2) were just concentrated fivefold with the automated SpeedVac concentrator. Losses caused by this procedure were determined to be maximal 8% by using 6 ml water, spiked with 0.5 and 2.5 µg of each standard. The spiked water was treated like the eluates (see Experimental section) and the concentration of the standards were determined in a final volume of 1 ml by HPLC–UV analysis. Additionally, the channel

**Table 3**  
Concentration of triiodinated benzene derivatives, standard deviation (SD) of a triplicate determination and recovery (in parentheses)

Compound	Effluent STP <sup>a*</sup> ( $\mu\text{g}/1\pm\text{SD}$ )	Effluent STP <sup>b*</sup> ( $\mu\text{g}/1\pm\text{SD}$ )	Receiving channel <sup>a</sup> ( $\mu\text{g}/1\pm\text{SD}$ )	Receiving channel <sup>b</sup> ( $\mu\text{g}/1\pm\text{SD}$ )	Receiving channel <sup>c</sup> ( $\mu\text{g}/1\pm\text{SD}$ )	Receiving lake <sup>a</sup>		
						February ( $\mu\text{g}/1\pm\text{SD}$ )	March ( $\mu\text{g}/1\pm\text{SD}$ )	June ( $\mu\text{g}/1\pm\text{SD}$ )
Iopromid	20 $\pm$ 2 (60%)	21 $\pm$ 2	11 $\pm$ 0.5 (50%)	8.5 $\pm$ 1	8.5 $\pm$ 0.5 (55%)	4 $\pm$ 1 (100%)	3 $\pm$ 0.5 (110%)	2 $\pm$ 0.5 (90%)
Iohexol	7 $\pm$ 1 (60%)	7 $\pm$ 1	2 $\pm$ 0.5 (65%)	2 $\pm$ 0.5	1 $\pm$ 0.5 (50%)	n.a.	n.a.	0.5 $\pm$ 0.1 (95%)
Iotrolan	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Diatrizoate	13 $\pm$ 1 (35%)	15 $\pm$ 2	6 $\pm$ 0.5 (30%)	8 $\pm$ 1	7 $\pm$ 0.5 (65%)	2.5 $\pm$ 0.5 (80%)	1.5 $\pm$ 0.5 (80%)	3.0 $\pm$ 0.5 (70%)
No.7	n.a.	n.a.	n.a.	n.a.	n.d. (58%)	n.d. (55%)	n.d. (80%)	n.a.
No. 3	1 $\pm$ 0.5 (30%)	n.d.	1 $\pm$ 0.5 (50%)	n.d.	1 $\pm$ 0.5 (55%)	n.d. (65%)	n.d. (80%)	n.d. (85%)
No. 5	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
No. 6	n.d. (50%)	n.d.	n.d. (60%)	n.d.	n.d. (70%)	n.a.	n.a.	n.d. (65%)

\*Sewage treatment plant 24 h mixed sample (sum of both extracts). n.a., Not analysed; n.d., not detected.

<sup>a</sup> Sequential SPE.

<sup>b</sup> Concentrated sample.

<sup>c</sup> SPE with ENV+.

water was extracted with ENV+ cartridges, according to the method published by Hirsch et al. [8]. The concentrations of the triiodinated benzene derivatives are comparable as determined with the sequential SPE (Table 3). Thus, reliable concentrations can be achieved by a correction based on recoveries, which requires the analysis of samples spiked with all compounds of interest and, is thus time intensive.

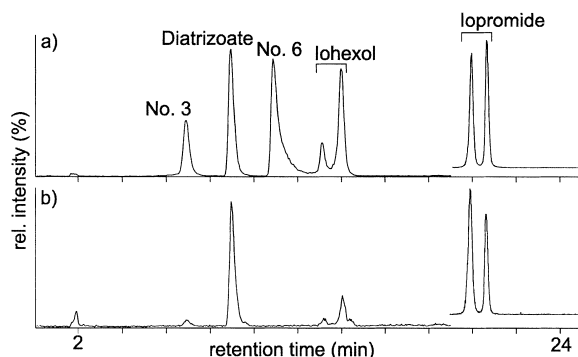


Fig. 5. Chromatogram (SRM fragments) of the enriched channel water. (a) Spiked sample, (b) non spiked sample.

#### 4. Conclusion

The developed analytical procedure allows one to detect and quantify triiodinated benzene derivatives in water samples. With the sequential extraction method the very polar and partly ionic compounds can be enriched. Possible metabolites of the iodinated contrast media can be ionic, like some of the original compounds. Especially for the enrichment of the ionic compounds the sequential extraction procedure is necessary. The recoveries are good for matrix poor samples and the LOD is in the lower ng/l range. For very complex samples like waste water treatment plant effluents the recoveries and the LOD are reduced. The capacity of the ENV+ material is higher and should be used instead of the EN material, at least for complex samples. For matrix-rich samples reliable concentrations can be obtained if a spiked sample is analysed in parallel.

The occurrence of iodinated contrast agents in waste water treatment plant effluents as well as in the receiving waters shows the importance of the compounds as organic pollutants. In a running project the degradation of contrast media under environmental conditions is under investigation.

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