RESEARCH PAPER

Development, validation, and application of a novel LC-MS/MS trace analysis method for the simultaneous quantification of seven iodinated X-ray contrast media and three artificial sweeteners in surface, ground, and drinking water

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Abstract A new method for the simultaneous determination of iodated X-ray contrast media (ICM) and artificial sweeteners (AS) by liquid chromatography-tandem mass spectrometry (LC-MS/MS) operated in positive and negative ionization switching mode was developed. The method was validated for surface, ground, and drinking water samples. In order to gain higher sensitivities, a 10-fold sample enrichment step using a Genevac EZ-2 plus centrifugal vacuum evaporator that provided excellent recoveries (90±6 %) was selected for sample preparation. Limits of quantification below 10 ng/L were obtained for all compounds. Furthermore, sample preparation recoveries and matrix effects were investigated thoroughly for all matrix types. Considerable matrix effects were observed in surface water and could be compensated by the use of four stable isotope-labeled internal standards. Due to their persistence, fractions of diatrizoic acid, iopamidol, and acesulfame could pass the whole drinking water production process and were observed also in drinking water. To monitor the fate and occurrence of these compounds, the validated method was applied to samples from different stages of the drinking water production process of the Industrial Works of Basel (IWB). Diatrizoic acid was found as the most persistent compound which was eliminated by just 40 % during the whole drinking water treatment process, followed by iopamidol (80 % elimination) and acesulfame (85 % elimination). All other compounds were completely restrained and/or degraded by the soil and thus were not detected in groundwater. Additionally, a direct injection method without sample preparation achieving 3–20 ng/L limits of quantification was compared to the developed method.

Introduction

In the past, increasing attention has been given to the contamination of aquatic environment by pharmaceutical compounds and artificial sweeteners (AS). Iodinated X-ray contrast media compounds (ICM) (Table 1) are the most widely used pharmaceutical compounds for intravascular administration [1]. Very high doses up to 200 g ICM per patient are administered for medical X-ray imaging to increase the contrast between organs or vessels that could otherwise not be examined due to low X-ray absorption [2]. Artificial sweeteners (AS; Table 1) are used as sugar substitutes in the food and beverage industry, but also to give pharmaceuticals a sweet taste [3]. The large number of sweeteners available to the product developer enables the creation of sweet-tasting products without the calorie contribution of traditional sugars, as they are not decomposed as carbohydrates [4].

ICM and AS are used in large quantities throughout the world. Since they are metabolically stable, they are excreted mainly unchanged from the human body via urine or feces and reach the aquatic environment daily via sewage systems [3, 5]. These compounds have become chemicals of emerging concern to the public because of their high environmental concentrations and their potential to leak through wastewater-

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Table 1 Chemical structure, CAS number, and molecular weight of the investigated iodinated X-ray contrast media (ICM) and artificial sweeteners (AS)

Compound Chemical formula	CAS No.	Use/origin	MW (g/mol)	Chemical structure
Diatrizoic acid (DTZ) $C_{11}H_9I_3N_2O_4$	117-96-4	ICM (Ionic)	613.91	
Iothalamicacid (ITA) $C_{11}H_9I_3N_2O_4$	2276-90-6	ICM (Ionic)	613.91	
Ioxithalamic acid (IOX) $C_{12}H_{11}I_3N_2O_5$	28179-44-4	ICM (Ionic)	643.94	
Iopamidol (IOD) $C_{17}H_{22}I_3N_3O_8$	62883-00-5	ICM (Non-ionic)	777.08	
Iomeprol (IOM) $C_{17}H_{22}I_3N_3O_8$	78649-41-9	ICM (Non-ionic)	777.09	
Iohexol (IOH) $C_{19}H_{26}I_3N_3O_9$	66108-95-0	ICM (Non-ionic)	821.14	
Iopromide (IOP) $C_{18}H_{24}I_3N_3O_8$	73334-07-3	ICM (Non-ionic)	791.11	
Acesulfame (ACE) $C_4H_5NO_4S$	33665-90-6	AS	163.15	
Saccharin (SAC) $C_7H_5NO_3S$	81-07-2	AS	183.19	
Cyclamate (CYC) $C_6H_{13}NO_3S$	100-88-9	AS	179.24	

treatment plants (WWTP) and even reach drinking water [2, 6, 7]. Although ICMs and AS are believed not to be harmful, any subtle effects of mixtures, their metabolites, or other micropollutants are currently unknown and may pose ecological or human health issues. Given the very low biodegradability of some of these compounds, they also may accumulate in the environment [8].

Hirsch et al. [9] found iopamidol, diatrizoic acid, iomeprol, iopromide, ioxithalamic acid, and iothalamic acid at almost the same levels in influents and effluents of municipal WWTP

[2]. Whereas the AS saccharin and cyclamate are usually degraded by more than 90 % during wastewater treatment, acesulfame and sucralose pass through wastewater treatment plants mainly unchanged [6]. The elevated concentrations of ICM and AS in the aquatic environment can thus be explained by their persistence. Their polar character and stability against environmental degradation enable them to persist in the aquatic environment. These properties and their high concentrations in wastewater make these compounds ideal tracers for wastewater in surface and groundwater [6, 10].

Recently, concentrations of some ICM and the AS acesulfame exceeded the International Association of Water Works in the Rhine Basin (IAWR) target values of 0.1 μg/L for ICM and 1 μg/L for AS in the Rhine river, particularly in the lower stretch of the Rhine downstream of Mainz [11, 12]. Especially non-ionic ICM such as iopamidol, iomeprol, and iopromide were present at concentrations in the higher nanogram per liter range in the Higher and Upper Rhine, since these non-ionic ICM are used preferentially in Switzerland. Acesulfame was the predominant AS with almost 10 times higher concentrations than the other AS in Rhine samples taken in Basel, Karlsruhe, Mainz, Cologne, and Düsseldorf [11, 12].

Due to their persistence, proportions of some of these compounds such as diatrizoic acid, iopamidol, and acesulfame could pass the whole drinking water purification stages and were detected in drinking water. The Industrial Works of Basel (IWB) provide about 50 % of Basel City's (northwestern part of Switzerland) drinking water for its 200,000 inhabitants. It applies a worldwide unique system for the drinking water production (Fig. 1) by artificial groundwater enrichment at the groundwater recharge plant "Lange Erlen". Here, forested recharge areas are flooded with surface water from the Rhine River. By seeping through the soil, the water is purified mechanically and biologically. To further purify the groundwater, deacidification, activated carbon filtration, and UV disinfection are applied before drinking water is provided to consumers [13].

Solid-phase extraction (SPE) combined with liquid chromatography (LC)-electrospray ionization (ESI)-tandem mass spectrometry (MS/MS) is the leading analytical methodology for the determination of ICM [9, 14–17] and AS [3, 18], achieving quantification limits in the order of tens of nanograms per liter. However, SPE methods can suffer from poor recovery [14, 19], are time consuming, and expensive, especially when sequential SPE columns are used [16, 20].

In their reviews, Perez et al. [2], Lange et al. [6], and Kokotou et al. [7] compare methods for the determination of ICM and AS; however, to date, no LC-MS/MS method has been reported for the simultaneous determination of ICM and AS. Combined analysis of ICM and AS remains difficult using a single mode of separation and extraction owing to their different physicochemical properties and polarities. Moreover, it requires positive and negative ionization switching modes, since ICM are usually measured in positive and AS in negative mode. Furthermore, to our knowledge, there is no method reported, neither for ICM nor for AS that reaches limits of quantification (LOQs) below 10 ng/L without using SPE enrichment.

The aim of this study was to develop a simple, sensitive, and robust method for the quantification of ICM and AS to regularly monitor the presence of these compounds at all stages of the drinking water production process. In this work, we describe a novel, sensitive, robust, and validated LC-MS/MS method for the simultaneous quantification of seven ICM and three important AS (Table 1) by applying a sample enrichment step through centrifugal vacuum evaporation. The method has been extensively validated on surface, ground, and drinking water and was applied to samples from different stages of the drinking water production process of the IWB. The developed method provides a less labor-intensive sample preparation and increased sample throughput while being sensitive and robust.

Materials and methods

Chemicals

All reference compounds (10 μg/mL, ICM in acetonitrile, AS in water) were purchased from Neochema GmbH & Co. KG (Bodenheim/Mainz, Germany). For additional quality control (QC), diatrizoic acid, iopamidol, iomeprol, iopromid, and iohexol were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany); iothalamic acid and ioxithalamic acid from a different batch as the reference compounds from Neochema GmbH & Co. KG (Bodenheim/Mainz, Germany); and acesulfame potassium, saccharin, and sodium cyclamate from Dr. Ehrenstorfer GmbH (Augsburg, Germany). All
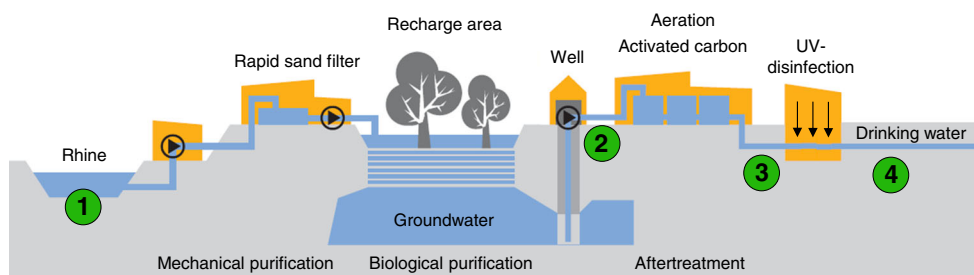

Fig. 1 Simplified scheme of the IWB drinking water production process in Basel (CH). The Rhine water (*1*) is first roughly purified by quartz sand filters (fast filtration system) before it is distributed over forested recharge areas in the "Langen Erlen". Several wells pump up groundwater from a depth of about 8–15 m. The collected groundwater (*2*) is then further purified by activated carbon filters in five basins (each with an activated carbon layer of about 2.5 m height). The activated carbon filter effluent (*3*) is then irradiated by UV light for disinfection what yields the ready drinking water (*4*). *1–4* Main sampling points in this work

QC compounds were of analytical grade (>92 %). Diatrizoic acid-d_6, iopamidol-d_8, acesulfame-d_4, and saccharin-$^{13}C_6$ were purchased from TRC Inc. (Toronto, Canada) and used as internal standards.

Prior to every analysis, standard mixtures of ICM and AS with concentrations of 10 and 100 µg/L were prepared in order to build the calibration curve. Additional QC stock solutions with a concentration of 100 mg/L were prepared using methanol for ICM and methanol/water 50:50 for AS.

An internal standard stock solution was prepared by dissolving 1 mg of each stable isotope-labeled compound with methanol to a concentration of 10 mg/L. By diluting the stock solutions with nanopure water, an internal standard mixture with a final concentration of 50 µg/L was prepared, from which 10 µL was spiked to every sample prior to the enrichment. Of the aliquots of the IS mix, 800 µL were stored at −20 °C.

LC/MS-grade methanol, water, ammonium formate, ammonium acetate, formic acid, and acetic acid were supplied by Sigma-Aldrich (Steinheim, Germany). Evian water was purchased from H. Zysset (Basel, Switzerland). Nanopure water was prepared by a Thermo Scientific Barnstead Nanopure purification system (Thermo Fisher Scientific, New Hampshire, USA).

Sampling and sample preparation

Rhine water, collected groundwater before and after the active carbon filters, as well as drinking water samples were collected at different days over a period of 2 months, to follow concentration fluctuations of ICM and AS and examine the fate of ICM and AS in the drinking water production process.

All samples were collected in 250-mL amber glass bottles and stored in the dark at 4 °C until analysis within 2 weeks. Sample preservation was not necessary.

A 10-fold sample enrichment was performed in order to yield lower limits of quantification (<10 ng/L) than with direct injection. For quantification, all samples including calibration and control solutions were concentrated on a Genevac EZ-2 Plus vacuum centrifuge from Stepbios GmbH (Muttenz, Switzerland). Ten-milliliters of water samples were filled into 15 mL Pyrex test tubes (VWR, Dietikon, Switzerland) and IS was added. The complete water amount was then evaporated overnight at a maximum temperature of 54 °C. The precipitate was reconstituted in 1 mL of eluent A. After 10 min sonication and 10 min shaking, the samples were filtered with 0.2 µm nylon syringe filters from Infochroma (Zug, Switzerland) before submitting for LC-MS/MS analysis.

Liquid chromatography–mass spectrometry

All high-performance liquid chromatography (HPLC)-ESI-MS/MS measurements were performed using an Ultimate 3000 rapid separation LC-System from Dionex (Germering, Germany) equipped with a solvent degasser unit, a binary pump, an autosampler with three 40 vial plates and a temperature-controlled autosampler. The injection volume was 80 µL. A Zorbax Eclipse XDB C18 column (50× 4.6 mm, 1.8 µm) coupled to a Zorbax Eclipse XDB-C18 (12.5×4.6 mm, 5 µm) guard column from Agilent Technologies AG (Basel, Switzerland) was chosen for separation.

The gradient program was applied as follows: 0–6.4 min, 98–80 % A; 6.4–7 min, 80–5 % A; 7–9 min, 5 % A; 9–10 min, 5–98 % A; and 10–12 min, 98 % A. For elution, a constant flow rate of 0.5 mL/min was applied at a column temperature of 40 °C. To avoid potential carryover, before each injection, the needle of the injector was rinsed in the injection port with 200 µL methanol. To monitor instrumental and laboratory contaminations, regular instrument blanks were measured every five to 10 injections as well as blanks collected from certain sampling points.

The HPLC system was connected to an AB Sciex QTRAP 5500 mass spectrometer (Concord, Canada) fitted with an electrospray interface operated in positive and negative mode. Nitrogen provided by a nitrogen generator (Atlas Copco, Studen, Switzerland) was used as nebulizer, desolvation, as well as collision gas. Instrument control and data acquisition were performed with AB Sciex Analyst 1.6.2 linked to Dionex Chromatography MS Link 2.12.0. The ESI interface was operated for the analysis of ICM in positive mode with an ion spray voltage of +5.5 kV and an entrance potential of +10 V, whereas AS were analyzed in negative mode with an ion spray voltage of 4.5 kV and an entrance potential of −10 V. For both modes, a heater temperature of 600 °C, an ion source gas 1=60 and 2=50 psi, and a curtain gas of 25 psi were used. Both quadrupoles (Q1 and Q3) operated in unit mass resolution. In order to gain highest sensitivity, the analytes were measured in the scheduled MRM (sMRM) mode which allows monitoring each analyte only across its expected elution time.

To optimize MS and MS/MS parameter, single compound standard solutions with a concentration of 10 µg/mL were prepared in a 50/50 (*v:v*) mixture of eluents A and B and were directly infused into the MS. For each ICM compound, an automated compound optimization was performed in order to figure out the two most sensitive transitions and the optimal parameters for the declustering potential, collision energy, and cell exit potential. For all ICM except for iomeprol and iohexol, ammonium adducts were used as precursor ions. MS parameters for AS were adopted from Scheurer et al. [3]. Table 2 shows MRM transitions with the optimized MS parameters and the corresponding internal standard, used for quantification.

Table 2 Retention times, MRM transitions (quantifier and qualifier), and corresponding internal standards used for quantification and optimized MS parameters

Compound	Retention time (min)	MRM transition (m/z)	Internal standard	DP (V)	CE (eV)	CXP (V)
Diatrizoic acid	4.2	*632/361*	638/367 (DTZ-d6)	50	35	10
		632/233			57	18
Iopamidol	3.7	*795/778*	803/786 (IOD-d8)	56	17	24
		778/559		186	31	16
Iomeprol	5.3	*778/405*	IOD-d8	176	57	12
		778/687			31	20
Iopromide	7.2	*809/792*	IOD-d8	176	35	16
		792/573		40	17	26
Iohexol	5.1	*822/804*	IOD-d8	171	29	24
		822/375			61	10
Iothalamic acid	4.5	*632/615*	DTZ-d6	51	17	18
		615/487			23	10
Ioxithalamic acid	3.6	*662/645*	DTZ-d6	61	17	20
		645/302		151	57	8
Acesulfame	3.0	*162/82*	166/86 (ACE-d4)	−35	−20	−11
		162/78			−42	−1
Saccharin	5.1	*182/42*	188/42 (SAC-$^{13}C_6$)	−75	−48	−3
		182/106			−26	−5
Cyclamate	6.7	*178/80*	SAC-$^{13}C_6$	−35	−38	−1
		178/96			−38	−1

Italicized mass transitions were used for quantification

DP declustering potential, *CE* collision energy, *CXP* collision cell exit potential, *DTZ* diatrizoic acid, *IOD* iopamidol, *ACE* acesulfame, *SAC* saccharin

Quantification and identification

Identification was accomplished via the chromatographic retention time and the ratio between the two most intense MRM transitions (quantifier and qualifier). For quantification a 7-point calibration including a 0 concentration was measured and a linear calibration curve (Analyte Conc./IS Conc. vs. Analyte Area/IS Area) was created. Due to the relatively large working range, a "$1/x$ weighting" was utilized. The calibration curve was built in Evian water since the matrix of Evian water is more similar to the sample matrices than the matrix of nanopure water in terms of salt and DOC concentrations.

Due to similarity of the chemical structure, diatrizoic acid-d$_6$ was used as internal standard for the quantification of diatrizoic acid, iothalamic acid and ioxithalamic acid, iopamidol-d$_8$ for iopamidol, iopromide, iomeprol and iohexol, acesulfame-d$_4$ for acesulfame, and saccharin-13C$_6$ for saccharin and cyclamate. All samples were spiked with a concentration of 50 ng/L of internal standard prior to the enrichment. Recoveries between 80 and 120 % for all compounds were achieved.

Method validation

For the determination of the linearity, a 10-point calibration curve was set up in Evian water ranging from 2.5 to 500 ng/L for ICM and 2.5 to 1600 ng/L for AS. Each calibration level was analyzed twice and the peak area ratios of the target analytes and their corresponding internal standard were calculated by the quantification function of Analyst 1.6.1, as well as the coefficients of determination (R^2). For the analysis, the working ranges were set at 10–500 ng/L for ICM and 10–1600 ng/L for AS.

The LOQs were calculated corresponding to the ICH guideline [21] as 10 times the S/N ratio by injecting an enriched 10 ng/L calibration solution. The noise levels of the single transitions of the calibration solution were compared to the ones in Rhine, ground, and drinking water, whereby no significant differences could be observed.

The repeatability was determined by measuring 10 replicates of the lower working range limit (10 ng/L) as well as 10 replicates of the upper working range limit (ICM, 500 ng/L; AS, 1,600 ng/L).

For investigation of the intermediate precision, the same Rhine, ground, and drinking water samples were spiked and measured in six series of measurements within 2 weeks. The concentrations were calculated via daily calibration curves.

Matrix effects and sample enrichment recoveries were investigated according to Ordonez et al. [22] by spiking samples either before or after the sample enrichment procedure with the same amount of analytes ($n=3$). All samples were spiked with 250 ng/L ICM and 500 ng/L AS. The response factor of the spiked samples was compared to the

response factor of a calibration curve, not undergoing the sample enrichment procedure. Thus, three response factors were obtained: calibration standard (R1), samples spiked before enrichment (R2), and samples spiked after enrichment (R3). Hence, matrix effects were calculated as $100 \times R3/R1$ and the recovery for the sample enrichment step as $100 \times R2/R3$.

To determine total recovery, the calibration samples were just as the samples submitted to the enrichment procedure to compensate the loss through sample preparation. In order to compensate matrix effects, the total recovery was calculated by taking internal standards into account and was investigated for all three matrices by five measurement series. Therefore, depending on the amount already present in the samples and the priority of the compounds, approximately the excepted amount already present in a sample was spiked in order to obtain meaningful values. Priority was given to iopamidol and acesulfame. Therefore, surface water was spiked with 250 ng/L ICM and 500 ng/L AS, groundwater with 50 ng/L ICM and 500 ng/L AS, and drinking water with 25 ng/L ICM and 50 ng/L AS. Five measurement series were performed within 5 weeks.

To ensure the quality of the reference compounds, additional quality control samples were measured. Therefore, a concentration of 25 ng/L ICM and 50 ng/L AS was prepared in Evian water and measured with every series of measurement in order to monitor the accuracy.

By means of the total recovery and the accuracy from the QC samples, the measurement uncertainty was estimated according to ISO 17025 by a series of four measurements. Thereby, the random deviation was determined by measuring four times the recovery of identically spiked samples and the systematic deviation by measuring four identically prepared control standards.

Samples of all three matrix types were stored at 4 °C in brown glass bottles and measured regularly over a time period of 2 weeks. This experiment was repeated three times.

Results and discussion

LC-MS/MS method development

To find out HPLC eluent composition that yields the best MS sensitivity, solutions of the two most important ICMs (diatrizoic acid and iopamidol) were prepared. Different additives with various combinations of ammonium acetate (NH_4Ac) and ammonium formate (NH_4Fo) as buffers and formic acid (FA) and acetic acid (AA) as pH modifiers were tested by direct infusion into the MS. The combination of 5 mmol/L ammonium formate and 0.1 % formic acid yielded the highest intensities and was therefore chosen for the HPLC method.

The requirements for the HPLC column were the ability to manage large sample loadings and provide a satisfying separation within a reasonable time frame. After screening various C18 and C8 columns, best results were achieved with a Phenomenex Synergi Fusion-RP C18 (3×100 mm, 2.5 μm) and an Agilent Zorbax Eclipse-XDB C18 (50×4.6 mm, 1.8 μm). Baseline separation was achieved with the Synergi column for most compounds except for iohexol and iomeprol. However, lower LOQs due to sharper peaks and shorter run times were achieved with the Agilent Zorbax Eclipse-XDB column. This column benefits from smaller particles and reduced column length compared to the Phenomenex Synergi Fusion. The chromatograms obtained with this column in positive and negative ionization switching modes are shown in Figure 2.

Although no baseline separation could be achieved for some compounds with the Zorbax Eclipse XDB column, all peaks except for diatrizoic acid and iothalamic acid (Fig. 2a) could be isolated through their specific MS/MS transitions (see Table 2). Diatrizoic acid and iothalamic acid have partially the same transitions, since the molecules are very similar and have the same molecular mass. Nevertheless, the achieved HPLC separation was sufficient for further analysis. All compounds were unequivocally confirmed on basis of retention time and a compound specific qualifier ion.

Typically, acidic solutions favor positive ion mode analysis while basic solutions favor negative ion mode analysis. That is also the reason why Scheurer et al. [3] applied a post column addition of a strong base in order to facilitate the ionization of some weekly acidic sweeteners. However, in the case of acesulfame, saccharin, and cyclamate, which all have pK_a values around 2, acidic LC conditions (pH=4) were appropriate for ESI ionization in negative mode.

Since switching between negative and positive ionization mode could lead to a significant loss of sensitivity, a crucial point for low LOQs was that the two important AS, acesulfame and saccharin, were well separated from the two important ICM, iopamidol and diatrizoic acid (Fig. 2a, b). Where the detection windows of acesulfame and iopamidol overlapped, a noise increase could be observed.

Method validation

Initially, it was intended to develop a direct injection method without sample preconcentration. However, the obtained LOQs were slightly too high for our requirements, but still very impressive for such a straightforward method. Thus, a brief discussion of some results of the direct injection method is given. In order to test whether a direct injection would be possible, it was decided to investigate ICM and AS in two separate methods. For the same reason, instead of 80 μL, 100 μL were injected. By injecting a solution of 10 ng/L and calculating the S/N ratio of 10/1, LOQs ranging between
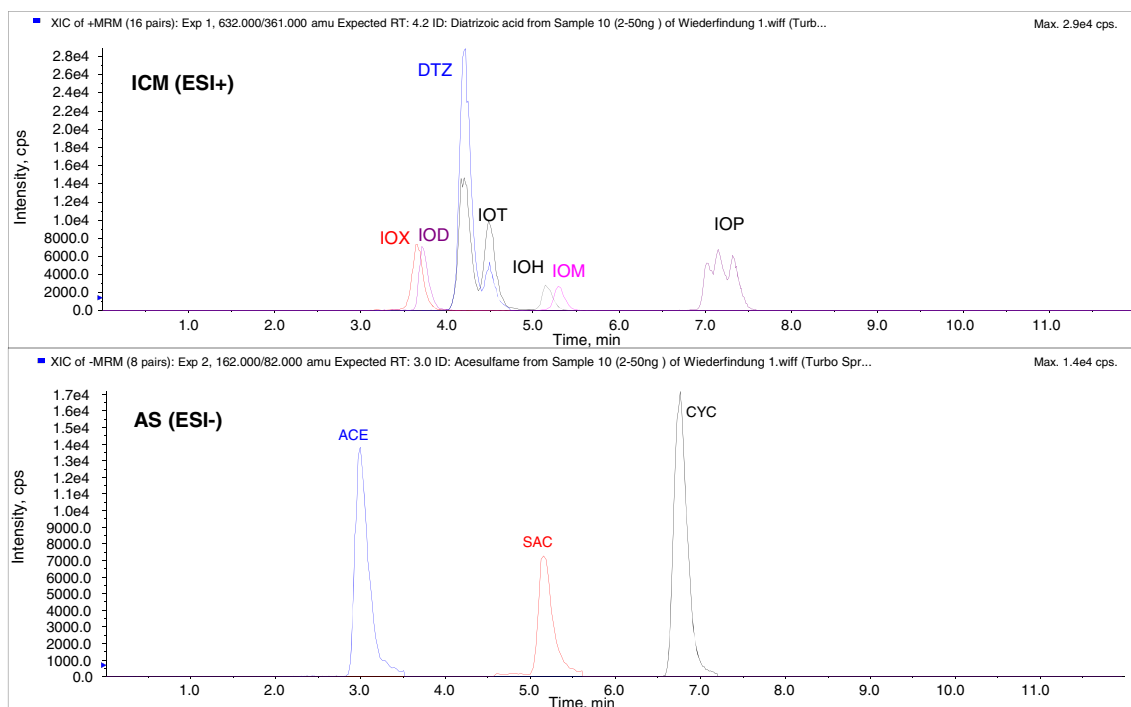
Fig. 2 Extracted ion chromatograms of seven ICM measured in positive ESI mode and three AS measured in negative ESI mode at a concentration of 50 ng/L

3 and 20 ng/L (diatrizoic acid, 5 ng/L; iopamidol, 10 ng/L; acesulfame, 3 ng/L) for all 10 compounds were obtained. LOQs for all compounds were not higher than 10 ng/L, except for iohexol with an LOQ of 20 ng/L. In addition, the precision was determined by injecting six times a concentration of 10 ng/L. The CV for all compounds was ≤12 %. Furthermore, matrix effects were estimated by comparing standard calibration solutions in Evian water with spiked samples of surface, ground, and drinking water. Except for acesulfame (50–60 % recovery), no significant matrix effects were observed for all matrices. Total recoveries were obtained by taking internal standards into account and ranged between 83 and 110 %.

However, since the working range should cover 10 to 500 ng/L for ICM and 10 to 1600 ng/L for AS, respectively, LOQs ≤10 ng/L for all compounds are needed. Hence, the direct injection method did not yield sufficient sensitivity. Therefore, a sample enrichment step was incorporated.

The 10-point calibration curve was found to be linear over a working range of 10–500 ng/L for ICM and 10–1600 ng/L for AS. The coefficients of determination were higher than 0.999 for all compounds (Table 3), what points out the high accuracy of the method.

Based on a sample volume of 10 mL, which was 10-fold concentrated by evaporation, LOQs below 10 ng/L could be achieved for all compounds (see Table 3).

The precision of the 10 replicates was excellent with relative standard deviation values ranging between 3 and 6 % for

the lower working range limit and 1 and 3 % for the upper working range limit.

The intermediate precision for surface, ground and drinking water ranged between 2 and 10 % with an average precision for the 10 compounds of 5 % for surface and drinking water and 4 % for groundwater.

Sample preparation recoveries and matrix effects were investigated for all three matrix types: surface, ground, and drinking water. As shown in Fig. 3a, sample preparation recovery was almost similar for all compounds with an excellent average recovery of 90±4 % and an average RSD of 2 % for triplicate injections.

Table 3 Linear regression determined by a 10-point calibration curve ($n=2$) and LOQs calculated with a S/N ratio of 10

Compound	R^2	LOQ (ng/L)
Diatrizoic acid	0.9997	1
Iopamidol	0.9997	5
Iomeprol	0.9994	5
Iopromide	0.9995	5
Iothalamicacid	0.9994	3
Ioxitalamic acid	0.9996	4
Iohexol	0.9997	8
Acesulfame	0.9999	2
Saccharin	0.9992	7
Cyclamate	0.9995	1

Fig. 3 Recoveries (*n*=3) for the sample enrichment step (**a**), matrix effects investigated by spiking 250 ng/L ICM and 500 ng/L AS (**b**), and total recoveries (**c**) for Rhine, ground, and drinking water
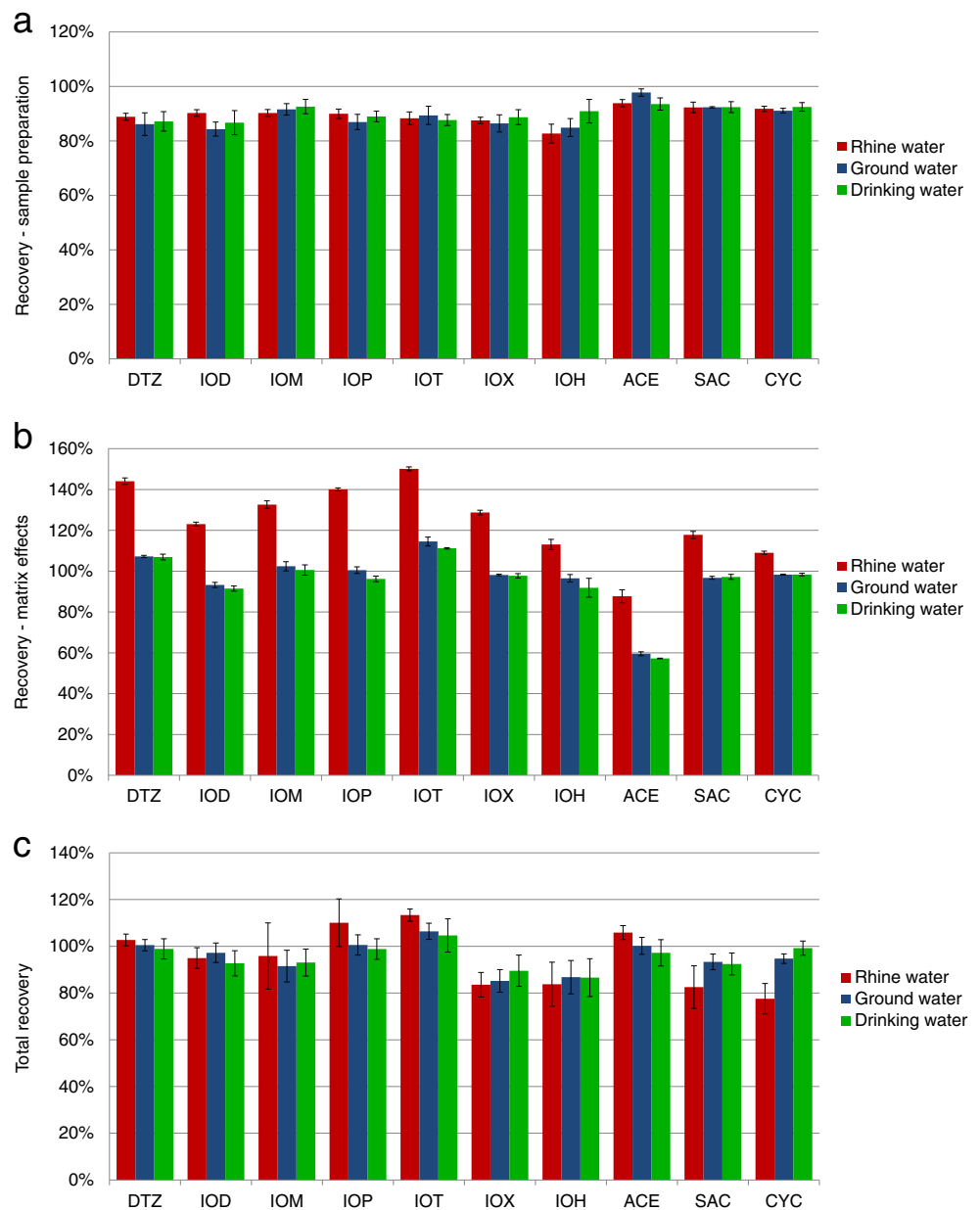
As shown in Fig. 3b, matrix effects occurred especially in Rhine water. For all ICM, ion enhancement was observed in this matrix type. However, in ground and drinking water, no significant matrix effects could be observed for all compounds except acesulfame. For this, ion suppression led to recoveries of about 60 %.

Most likely, the enhancement effect can be attributed to the high salt concentration in Evian water, which leads to an ion suppression in the calibration solution. On the other hand, ground and drinking water have higher salt concentrations than Rhine water and are hence more similar to Evian water. Thus, it could be expected that relative to the salt rich Evian water, the less salt containing Rhine water exerts less ion suppression. However, the focus of our investigations is on

ground and drinking water. Therefore, Evian water is a good reference to investigate possible matrix effects.

Matrix effects for compounds like the ionic ICM diatrizoic acid, ioxithalamic acid, and iothalamic acid are very similar. Therefore, diatrizoic acid-d_6 can be used as internal standard for these three compounds. For the same reason, iopamidol-d_8 was used for iopamidol, iomeprol, iopromide, and iohexol and saccharin-$^{13}C_6$ for saccharin and cyclamate.

To investigate the total recovery, calibration samples were also submitted to sample enrichment. In addition, internal standards were taken into account for the recovery calculation.

As presented in Fig. 3c, good results were obtained with mean recoveries from five independent measurement series between 78 and 113 %, covering all three matrices. Especially

Table 4 Concentrations (ng/L) of ICM and AS in Rhine, collected ground and drinking water from the drinking water production process measured between March and April 2013

Compound	Rhine water ($n=16$)				Groundwater ($n=8$)				Drinking water ($n=18$)			
	Mean	RSD (%)	Max	Min	Mean	RSD (%)	Max	Min	Mean	RSD (%)	Max	Min
Diatrizoic acid	32	25	47	20	27	11	32	24	19	9	22	16
Iopamidol	146	49	286	47	63	28	94	36	28	20	43	17
Iomeprol	153	53	385	66	–	–	–	–	–	–	–	–
Iopromide	144	36	240	69	–	–	–	–	–	–	–	–
Iothalamic acid	–	–	–	–	–	–	–	–	–	–	–	–
Ioxithalamic acid	41	23	57	24	–	–	–	–	–	–	–	–
Iohexol	32	36	56	15	–	–	–	–	–	–	–	–
Acesulfame	764	11	916	619	415	25	524	180	122	11	144	92
Saccharin	50	21	76	37	–	–	–	–	–	–	–	–
Cyclamate	33	22	51	25	–	–	–	–	–	–	–	–

for the three most important compounds, diatrizoic acid, iopamidol, and acesulfame recoveries were found almost 100 % with relative standard deviations ≤5 % for all three matrices.

The measurement uncertainties were estimated by four measurement series. For the most relevant compounds, measurement uncertainties were determined as 18 % for diatrizoic acid, 27 % for iopamidol, and 11 % for acesulfame.

Stabilization is of special importance for nonpersistent AS such as saccharin and cyclamate, especially when dealing with surface or wastewater samples [6]. Van Stempvoord et al. [23] did some stability tests in waste and groundwater and found that the concentration of cyclamate declined within 3 weeks in refrigerated samples, whereas acesulfame and saccharin were stable during this period.

Our investigations showed that for all three matrices, no significant concentration changes (<15 %) occurred during 2 weeks of storage at 4 °C.

Application to Rhine, ground, and drinking water samples

The developed and validated analytical method was applied to the determination of ICM and AS in different water samples including surface, ground, and drinking water from the IWB drinking water production process. Therefore, Rhine water, collected groundwater from 13 wells and drinking water samples were measured. Table 4 shows the calculated mean concentrations, the relative standard deviation, and the measured max and min values for each compound. Except for iothalamic acid, all compounds were detected in Rhine water in Basel. Highest concentrations were found for the non-ionic ICM iopamidol (146 ng/L), iomeprol (153 ng/L), and iopromide (144 ng/L) and for acesulfame (764 ng/L).

Iomeprol, iopromide, ioxithalamic acid, iohexol, saccharin, and cyclamate were neither detected in ground nor in drinking water samples (below LOQ).

The attenuation of the concentrations is a result of different processes during percolation through the soil (e.g., biodegradation, dissolution, and sorption) and depends on the residence time in the soil. However, it can be assumed that degradation plays a major role, since the results are in accordance with the reported behavior of iomeprol, iopromide, and iohexol during bank filtration [24, 20, 25]. The removal of saccharin and cyclamate through soil also fits with the findings of Scheurer et al. [26]. They confirmed their degradation in several tested waterworks using biologically active treatment units like river bank filtration (RBF) or artificial groundwater recharge.

Diatrizoic acid, iopamidol, and acesulfame, however, were detected in all ground (27, 63, and 415 ng/L) and drinking water (19, 28, and 122 ng/L) samples (Table 4). Figure 4 presents the reduction of concentrations of these compounds during the drinking water production process, created with normalized mean concentrations from Table 4.
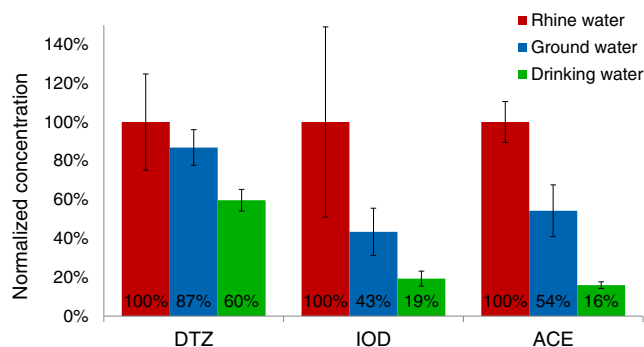
Fig. 4 Decrease of relative concentrations during soil passage (groundwater) and by activated carbon filtration/UV irradiation (drinking water)

Diatrizoic acid was found to be the most persistent compound. About 60 % of its initial concentration in Rhine water could still be detected in drinking water, whereas about 15–20 % of iopamidol and acesulfame persisted throughout the purification process (see Fig. 4).

In order to estimate the removal by the five activated carbon filters in use (see Fig. 1) and to investigate the influence of UV irradiation on the concentration, individual measurements were performed with samples of the activated carbon filter influent and effluent ($n=3$, respectively) as well as samples directly before and after UV irradiation ($n=3$, respectively). These measurements showed that diatrizoic acid was removed to about 14 ± 8 %, iopamidol to 52 ± 6 %, and acesulfame to 60 ± 1 % through activated carbon filtration (average for all five activated carbon basins) whereas samples measured after UV irradiation showed consistently lower concentrations than samples measured prior to it. Thereby, diatrizoic acid concentration decreased by 25 ± 5 %, iopamidol by 19 ± 8 %, and acesulfame by 30 ± 5 %.

Measurements of individual effluents of the five activated carbon basins showed that the adsorption through activated carbon filters depends strongly on the quality of the activated carbon material. The five activated carbon filters had an age about 3–15 months during this work. The newest filter (3 months; load, ~20 m^3/kg) could adsorb up to 100 % of acesulfame, 20 % of diatrizoic acid, and 65 % of iopamidol of the influent concentration.

Conclusion

A sensitive LC-MS/MS method was developed which allows the simultaneous quantification of seven ICM and three AS in surface, ground, and drinking water. The method combines the analysis of the two compound classes in a single chromatographic run. Instead of using labor-intensive SPE, an easy sample preparation was performed by applying a sample enrichment step through centrifugal vacuum evaporation in order to achieve LOQs below 10 ng/L. The developed method supports increased sample throughput and is less labor intensive and cheaper than comparable methods using SPE enrichment. Validation confirmed the high linear working range, high sensitivity, and robustness of the method. The use of four isotope-labeled internal standards enabled compensation of matrix effects and facilitated accurate quantification. The method was successfully applied to Rhine, ground, and drinking water samples. Statements about the occurrence and fate of these compounds in the drinking water production process of the IWB could be made.

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