Tracing gadolinium-based contrast agents from surface water to drinking water by means of speciation analysis

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A R T I C L E  I N F O

Article history:
Received 4 August 2015
Received in revised form 8 February 2016
Accepted 14 February 2016
Available online 17 February 2016

Keywords:
Contrast agents
Gadolinium
Speciation analysis
Drinking water
LC-ICP-MS

A B S T R A C T

In recent decades, a significant amount of anthropogenic gadolinium has been released into the environment as a result of the broad application of contrast agents for magnetic resonance imaging (MRI). Since this anthropogenic gadolinium anomaly has also been detected in drinking water, it has become necessary to investigate the possible effect of drinking water purification on these highly polar contaminants. Therefore, a novel highly sensitive method for speciation analysis of gadolinium is presented. For that purpose, the hyphenation of hydrophilic interaction liquid chromatography (HILIC) and inductively coupled plasma-mass spectrometry (ICP-MS) was employed. In order to enhance the detection power, sample introduction was carried out by ultrasonic nebulization. In combination with a novel HILIC method using a diol-based stationary phase, it was possible to achieve superior limits of detection for frequently applied gadolinium-based contrast agents below 20 pmol/L. With this method, the contrast agents Gd-DTPA, Gd-DOTA and Gd-BT-D03A were determined in concentrations up to 159 pmol/L in samples from several waterworks in a densely populated region of Germany alongside the river Ruhr as well as from a waterworks near a catchment lake. Thereby, the direct impact of anthropogenic gadolinium species being present in the surface water on the amount of anthropogenic gadolinium in drinking water was shown. There was no evidence for the degradation of contrast agents, the release of Gd\(^{3+}\) or the presence of further Gd species.

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1. Introduction

Contrast agents based on the rare earth element (REE) gadolinium (Gd) are frequently applied prior to medical examinations with magnetic resonance imaging (MRI) because of the paramagnetic properties of Gd\(^{3+}\) [1]. Those properties cause an increased longitudinal relaxation rate \(T_1\) of water protons during MRI scans, leading to an improved contrast of the resulting images. Due to the toxicity of Gd\(^{3+}\), it is delivered to the patients as chelate with polyaminocarboxylates, resulting in a fast and unmetabolized, mostly renal, excretion [2]. The first of these compounds to be commercially available was Gd-DTPA (gadopentetate), which has been introduced into the market in 1988. Since then, several other compounds from different pharmaceutical companies with different chelating agents were developed and commercialized. Fig. 1 shows the chemical structures of widely applied contrast agents, which were subject of this study [3]. These complexes can be separated in two groups: on the one hand those with linear ligands such as the above mentioned Gd-DTPA and Gd-BOPTA (gadobenate), and on the other hand those with macrocyclic ligands like Gd-DOTA (gadoterate) and Gd-BT-D03A (gadobutrol) [1]. The dosage per infusion of commercially available contrast agents is 0.05 mmol up to 0.3 mmol per kg body weight, meaning that about 1 g of gadolinium is being applied for one contrast agent enhanced MRI scan [3].

As a result of these high dosages and the unmetabolized excretion of the compounds, large amounts of anthropogenic gadolinium are released into the wastewater. In 1996, Bau and Dulski described an enrichment of gadolinium relatively to the other REEs in water samples from rivers and lakes as a result of this input [4]. This anthropogenic gadolinium anomaly has been investigated since then in a series of studies. It was shown that this phenomenon can be observed in rivers and lakes in highly populated regions with developed health care around the world [5–12]. Furthermore,
significant gadolinium anomalies were found even in the North Sea [13]. The emitted amount of anthropogenic gadolinium indicates that the contrast agents are not being removed during wastewater treatment. In 2010, investigations by Verplanck et al. revealed large gadolinium anomalies in treated wastewater in contrast to sewage sludge [14]. These results were confirmed by Telgmann et al., showing only a minor removal of approximately 10% of total gadolinium in batch experiments with a simulated aeration tank [15].

Since the supply with drinking water also partly relies on surface water, it is possible to detect anthropogenic gadolinium in the tap water of urban agglomerations. Kulaksiz and Bau reported in 2011 large anthropogenic gadolinium anomalies in the western districts of Berlin (Germany) and only minor amounts in the eastern districts. This was a result of the drinking water supply in the western districts by means of bank filtration and thereby from surface water [16]. Samples from processing and purification of drinking water have not been analyzed so far. Furthermore, the formation of transformation products for example through ozonation or disinfection by UV irradiation has to be considered.

Therefore, it is necessary to develop highly sensitive specification analysis methods. Methods based on ion chromatography (IC), high performance liquid chromatography (HPLC), and size exclusion chromatography (SEC) have been developed for the chromatographic separation of gadolinium-based contrast agents [17–19]. In 2008, Künnemeyer et al. demonstrated the separation of commonly applied contrast agents using hydrophilic interaction liquid chromatography (HILIC) hyphenated to electrospray ionization mass spectrometry (ESI-MS) [20]. Since then, HILIC was applied in several studies for the analysis of biological and environmental samples using zwitterionic and unboned silica-based stationary phases. Especially the hyphenation of HILIC with inductively coupled plasma-mass spectrometry (ICP-MS) has proven to be a powerful method for the determination of gadolinium based contrast agents, mostly due to the uniquely high and element selective sensitivity of ICP-MS [21–24]. Nevertheless, the direct analysis of drinking water samples remains a difficult task with regard to the low concentration of the individual gadolinium species at the pmol/L level [25]. In general, the sample introduction into the plasma is a crucial parameter for sensitivity and therefore for the limit of detection using ICP-MS. Compared to conventional pneumatic nebulization, ultrasonic nebulization with subsequent desolvation offers a ten times increased efficiency of aerosol generation and an improved transport efficiency into the plasma [26]. The application of ultrasonic nebulization for HPLC-ICP-MS has been accomplished for the determination of several species of metals and metalloids such as mercury, platinum, arsenic and selenium [27–31].

This paper describes the development of a HILIC-ICP-MS method using ultrasonic nebulization for sample introduction. A new HILIC separation with a diol-based stationary phase was accomplished for the most commonly applied contrast agents. This method was then applied for the analysis of samples from different steps of drinking water purification in a series of waterworks. Additionally, total concentrations of gadolinium were determined by means of ICP-MS.

2. Materials and methods

2.1. Sampling and sample preparation

Water samples from six waterworks in Germany were analyzed during this study, covering different methods of drinking water purification from surface water. Those methods involve ground filtration, filtration through activated carbon as well as ozonation. Disinfection of the drinking water is carried out in those waterworks by addition of chlorine dioxide or UV irradiation. The location of the waterworks is shown in Fig. 2. One of those waterworks (A) is located near a large catchment lake, from which the raw water is obtained. The other five waterworks (B–F) are located in one of the most densely populated region of Germany alongside the river Ruhr. Therefore, it was assumed that significant amounts of anthropogenic gadolinium could be detected in the drinking water. In total, 21 Samples from different steps of the drinking water purification were taken with vessels of polypropylene (PP) at a single day in October 2014. The detailed sampling points in the respective waterworks are shown in the results section. Since the contrast agents Gd-DTPA and Gd-BOPHA show strong adsorption on glass surfaces, the use of glass vessels had to be avoided during sampling as well as during sample preparation [21]. The collected samples were cooled immediately below 10 °C for transport and subsequent analysis.

For determination of total gadolinium concentrations with ICP-MS, the samples were filtered through syringe filters with

![Fig. 1. Structures of the frequently applied gadolinium-based contrast agents for MRI examinations with respective trademarks, which were subject of this study.](image)
polytetrafluoroethylene (PTFE) membrane. 100 μL nitric acid (65%) and 1 mL rhodium solution as internal standard were added to 8 mL of the filtrate in a PP vessel and diluted to a volume of 10 mL. The final concentration of the internal standard was 1 μg/L. For speciation analysis with HILIC-ICP-MS, about 1.5 mL of the surface water samples were also filtered through syringe filters with PTFE membrane and filled into PP vials. The procedure of sample preparation was repeated three times for ICP-MS as well as for HILIC-ICP-MS measurements.

2.2. Chemicals and consumables

Nitric acid (65%, Suprapur), gadolinium standard for ICP-MS (1000 mg/L), and acetonitrile were purchased from Merck KGaA (Darmstadt, Germany). Rhodium solution (1000 mg/L) from SCP Science (Baie D’Urfé, Canada) was used as internal standard for ICP-MS. Formic acid and ammonium formate were obtained from Fluka Chemie GmbH (Buchs, Switzerland). The contrast agent standards were purchased as infusion solutions from the respective pharmaceutical companies: Dotarem (Gd-DOTA, 0.5 mol/L) by Guerbet (Sulzbach, Germany), Magnevist (Gd-DTPA, 0.5 mol/L) and Gadovist (Gd-BT-DO3A, 1.0 mol/L) by Bayer-Schering Pharma AG (Berlin, Germany), and Multihance (Gd-BOPTA, 0.5 mol/L) by Nycomed (Konstanz, Germany). All chemicals were used in the highest quality available. Water was purified by an Aquatron Water Still purification system model A4000D (Barloworld Scientific, Nemours Cedex, France). Syringe filters with PTFE membranes (0.2 μm pore size) were purchased from VWR International (Darmstadt, Germany).

2.3. Stock and standard solutions

The determination of total gadolinium concentrations with ICP-MS was carried out by external calibration in a range from 5 to 500 ng/L. Rhodium solution was added as internal standard prior to final dilution of the sample solutions as well as to the calibration solutions with a final concentration of 1 μg/L.

Solutions of the contrast agents Gd-BOPTA, Gd-BT-DO3A, Gd-DOTA and Gd-DTPA were prepared in a concentration of 1 nmol/L each by dilution of the infusion solutions for determination of the retention times. For quantification of single contrast agents by means of HILIC-ICP-MS, a mixed stock solution with a concentration of 50 nmol/L for Gd-BOPTA, Gd-BT-DO3A, Gd-DOTA and Gd-DTPA...
was prepared. External calibration was carried out in a range from 10 to 500 pmol/L.

2.4. Instrumentation and experimental parameters

2.4.1. ICP-MS

The total gadolinium concentration in the water samples was determined using a quadrupole-based iCAP Qc ICP-MS (Thermo Fisher Scientific, Bremen, Germany) that was controlled by the Qtegra software version 2.4 (Thermo Fisher Scientific). The instrument was equipped with a PFA MicroFlow nebulizer (Elemental Scientific, Omaha, NE, USA), a Peltier-cooled cyclonic spray chamber (Thermo Fisher Scientific), a quartz injector pipe with an inner diameter of 3.5 mm and a SC-4-S autosampler (Elemental Scientific). The interface consisted of a nickel sampler and a nickel skimmer. Measurements were carried out in the standard mode (STD) without pressurizing the optional collision/reaction cell of the ICP-MS in order to achieve highest sensitivity. The following parameters were used: RF power 1550 W, cool gas flow 16 L/min, auxiliary gas flow 0.8 L/min and nebulizer gas flow 0.6 L/min and oxygen flow 0.04 L/min. The oxygen flow was added after the ultrasonic nebulizer into the sample aerosol in order to prevent carbon buildup in the interface. The isotopes $^{156}$Gd, $^{157}$Gd, $^{158}$Gd and $^{160}$Gd were monitored with a sample time of 0.01 s each. 100 samples per peak were measured in a mass window of 10% for each isotope. The software Origin version 9.1.0 (OriginLab Corporation, Northampton, USA) was employed for data analysis. The HILIC-ICP-MS chromatograms based on the sum of intensities of the isotopes mentioned above were smoothed with the Savitzky–Golay algorithm using a second order polynomial regression over 90 data points.

3. Results and discussion

3.1. Method development

The determination of gadolinium species in drinking water is a challenging task especially considering the low concentrations to be expected. Therefore, a novel HILIC-ICP-MS method was developed combining a diol HILIC separation and improved ICP-MS detection. The latter was achieved by ultrasonic nebulization and subsequent desolvation for sample introduction. Since the nebulization efficiency directly affects the transport efficiency of the analytes into the plasma, this has to be considered to be a crucial parameter regarding the sensitivity of a method. The efficiency of ultrasonic nebulization and the resulting signal stability are strongly depending on the flow rate of the introduced solution. It could be observed that it was possible to achieve a sufficiently stable signal under HILIC conditions with a flow rate of 800 μL/min. Additionally, the employed double focusing sector field ICP-MS generally provides a high sensitivity if used in low resolution, because of the maximized ion transmission. The relatively high accelerating voltage also improves sensitivity compared to most quadrupole-based systems.

The employed diol HILIC method combines this high flow rate with an isotropic separation of the most commonly applied contrast agents, which is shown in Fig. 3. As can be seen, it was possible
to separate and to detect the contrast agents Gd-BOPTA, Gd-BT-DOTA and Gd-DTPA in a concentration range from 10 to 500 pmol/L. The relative standard deviation (RSD) of the baseline signal from a blank analysis was 5.4%. Thus, the sensitivity and the signal to noise ratio of this HILIC-ICP-MS method were sufficient to perform speciation analysis of gadolinium in drinking water.

### 3.2. Figures of merit

The LODs were determined by means of signal to noise ratio of three (S/N = 3), whereas the LOQs were determined by means of a signal to noise ratio of ten (S/N = 10). For total gadolinium the LOD was 0.6 pmol/L and the LOQ was 2.1 pmol/L. The LOD for Gd-BT-DOTA was as low as 8 pmol/L, for Gd-DOTA 11 pmol/L and for Gd-DTPA 14 pmol/L. Thus, the respective LOQ was 26 pmol/L for Gd-BT-DOTA, 37 pmol/L for Gd-DOTA and 46 pmol/L for Gd-DTPA. As shown in Table 1, this is a significant improvement compared to earlier studies involving the determination of contrast agents in environmental samples and drinking water by means of HILIC-ICP-MS. Linearity in the relevant concentration range was verified based on the triplicate measurement of the calibration solutions. The resulting regression coefficients were 0.999 for total Gd, 0.999 for Gd-BT-DOTA, 0.999 for Gd-DOTA and 0.997 for Gd-DTPA. The average RSD of retention times was 0.2% for Gd-BT-DOTA, 0.6% for Gd-DOTA and 0.6% for Gd-DTPA. The column recovery for speciation analysis was found to be 93% for Gd-BT-DOTA, 95% for Gd-DOTA and 101% for Gd-DTPA.

### 3.3. Analysis of water samples

The above described HILIC-ICP-MS method was employed for the analysis of samples from different waterworks. Additionally, total gadolinium concentrations were determined with ICP-MS for evaluation of the mass balance. The HILIC-ICP-MS chromatograms of the analyzed samples from waterworks F and a 500 pmol/L standard solution are displayed in Fig. 4.

The chromatograms show that the contrast agents Gd-BT-DOTA, Gd-DOTA and Gd-DTPA could be detected in samples from different steps of the drinking water purification, while Gd-BOPTA was not detectable in any sample. The direct comparison of the samples with the standard indicates that the concentration of the individual species could be expected to be found in the lower pmol/L range. The signal intensity of Gd-BT-DOTA and Gd-DOTA is slightly decreased after ozonation, while it is constant for Gd-DTPA. Therefore, the HILIC-ICP-MS measurements demonstrate that the above mentioned contrast agents in the drinking water can be traced back through the whole purification process.

The results of the quantification of individual species by HILIC-ICP-MS and total gadolinium by ICP-MS are shown in Table 2, Figs. 5 and 6. It can be seen that individual contrast agents could be detected and quantified in the surface water, after all purification steps and in the disinfected drinking water. The comparison of summed up concentrations of individual contrast agents and the total Gd concentrations in the surface water and drinking water samples shows a gap in the mass balance. This gap is increased in the drinking water mostly due to the extremely low concentrations of Gd-BT-DOTA, which is found below the LOQ in most samples. In case of the surface water sample from the catchment lake at waterworks A, the contrast agents represent 71% of the total Gd concentration. In the respective drinking water sample, this amount was decreased to 63%. The contrast agents in the samples from the Ruhr represent 87% up to 108% of the total gadolinium. There is also a larger gap in the mass balance of the drinking water samples with an amount of 66–81%. Nevertheless, this cannot be considered as evidence for the release of Gd³⁺ or a significant degradation of individual species, because Gd-BT-DOTA was quantified slightly above the LOQ or was found below the LOQ in most samples.

### Table 2

Sampling points in the respective waterworks and quantification results. Gadolinium-based contrast agents were determined by means of HILIC-ICP-MS and total gadolinium was determined by means of ICP-MS.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Gd-based contrast agents</th>
<th>Total Gd (pmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gd-BT-DOTA (pmol/L)</td>
<td>Gd-DOTA (pmol/L)</td>
</tr>
<tr>
<td>Waterworks A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface water</td>
<td>&lt;LOQ</td>
<td>55 (±20)</td>
</tr>
<tr>
<td>After filtration</td>
<td>&lt;LOQ</td>
<td>49 (±15)</td>
</tr>
<tr>
<td>Drinking water</td>
<td>–</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>Waterworks B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface water</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>Ground filtrate</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>Drinking water after disinfection (UV irradiation)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Waterworks C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface water</td>
<td>40 (±3)</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>Ground filtrate</td>
<td>30 (±4)</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>Drinking water after disinfection (chlorine dioxide)</td>
<td>&lt;LOQ</td>
<td>–</td>
</tr>
<tr>
<td>Waterworks D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface water</td>
<td>30 (±5)</td>
<td>56 (±20)</td>
</tr>
<tr>
<td>Ground filtrate</td>
<td>&lt;LOQ</td>
<td>48 (±9)</td>
</tr>
<tr>
<td>After ozonation</td>
<td>&lt;LOQ</td>
<td>&lt;LOQ</td>
</tr>
<tr>
<td>After filtration</td>
<td>&lt;LOQ</td>
<td>40 (±9)</td>
</tr>
<tr>
<td>Drinking water after disinfection (UV irradiation)</td>
<td>&lt;LOQ</td>
<td>45 (±4)</td>
</tr>
<tr>
<td>Waterworks E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface water</td>
<td>49 (±14)</td>
<td>61 (±2)</td>
</tr>
<tr>
<td>Ground filtrate</td>
<td>26 (±6)</td>
<td>81 (±5)</td>
</tr>
<tr>
<td>Drinking water after disinfection (chlorine dioxide)</td>
<td>&lt;LOQ</td>
<td>74 (±24)</td>
</tr>
<tr>
<td>Waterworks F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface water</td>
<td>38 (±20)</td>
<td>85 (±12)</td>
</tr>
<tr>
<td>After ozonation</td>
<td>&lt;LOQ</td>
<td>46 (±3)</td>
</tr>
<tr>
<td>Ground filtrate</td>
<td>&lt;LOQ</td>
<td>37 (±18)</td>
</tr>
<tr>
<td>Drinking water after disinfection (chlorine dioxide)</td>
<td>&lt;LOQ</td>
<td>44 (±3)</td>
</tr>
</tbody>
</table>
As mentioned above, the raw water from waterworks A is obtained from a catchment lake. In this case, the contrast agents Gd-DOTA and Gd-DTPA were quantified in the surface water, while Gd-BT-DOTA was found to be below the LOQ. In the drinking water, the concentration of Gd-DTPA was 167 pmol/L and Gd-DOTA was below the LOQ. Total gadolinium concentrations were determined in a range of 254 pmol/L in the drinking water to 307 pmol/L in the surface water.

In contrast to waterworks A, the further samples were collected from waterworks alongside the river Ruhr. Only the linear contrast agent Gd-DTPA could be quantified in the samples from waterworks B with a concentration of 82 pmol/L in the drinking water, which was disinfected by UV irradiation. The results of waterworks C, some kilometers downstream the river Ruhr, confirm this finding. In addition to Gd-DTPA, the macrocyclic contrast agent Gd-BT-DOTA was quantified with a concentration of 40 pmol/L in the surface water and 30 pmol/L in the ground filtrate.

Waterworks D is located in more densely populated region compared to waterworks B and C. As a result of that, there is an increased total gadolinium concentration as well as increased concentrations of individual species in the surface water, exposing a significant anthropogenic input. Again, this amount of contrast agents and thereby the anthropogenic input was also found in the drinking water. In the surface water, the concentration was 30 pmol/L for Gd-BT-DOTA, 56 pmol/L for Gd-DOTA and 116 pmol/L for Gd-DTPA. Nearly similar concentrations were found in the drinking water after disinfection by means of UV irradiation for Gd-DOTA and Gd-DTPA, while Gd-BT-DOTA was below the LOQ. The total gadolinium concentration was 188 pmol/L in the drinking water.

The analysis of the samples from waterworks E also shows high concentrations of total gadolinium and contrast agents compared to the waterworks B and C upstream the Ruhr. A total gadolinium concentration of 242 pmol/L was determined in the surface water, whereas it was 274 pmol/L in the ground filtrate and 298 pmol/L in the drinking water after addition of chlorine dioxide. The concentration in the surface water was 100 pmol/L for Gd-DTPA, 61 pmol/L for Gd-DOTA and 49 pmol/L for Gd-BTDO3A. In the drinking water after disinfection by chlorine dioxide, the concentration of Gd-BT-DOTA was below the LOQ, while Gd-DTPA and Gd-DOTA were quantified in a concentration of 110 pmol/L and 45 pmol/L, respectively.

Since Waterworks F is located in close vicinity of waterworks E, the initial amount of anthropogenic gadolinium in the surface water was also increased compared to the waterworks upstream. The resulting concentration of the most abundant contrast agent Gd-DTPA was 103 pmol/L in the drinking water. Therefore,
analysis of samples from the waterworks E and F substantiates the results from waterworks D, indicating an increased anthropogenic input of gadolinium downstream the Ruhr. The concentrations of contrast agents were also clearly increased in the drinking water produced from the related waterworks.

4. Conclusions

In this work, a new highly sensitive HILIC-ICP-MS method for speciation analysis of gadolinium was presented, involving a diol HILIC column and sample introduction by ultrasonic nebulization. Because of the resulting superior LODs and LOQs, the frequently applied contrast agents Gd-DTPA, Gd-DOTA and Gd-BT-DOD3A could be detected and quantified in samples from the process of drinking water purification. Furthermore, the linear contrast agent Gd-DTPA was by far the most abundant species to be detected in all samples. There was no evidence for additional Gd species or transformation products being present in the analyzed samples. Since only single samples were taken and considering the standard deviations as well, an interpretation of the data regarding a possible removal of contrast agents is not possible.

It was possible to demonstrate the direct impact of anthropogenic gadolinium species in the surface water on the amount of anthropogenic gadolinium in the drinking water in a densely populated region of Germany at the river Ruhr. In the waterworks further downstream the river, the analysis of the produced drinking water revealed higher concentrations of individual contrast agents as well as total gadolinium compared to the waterworks upstream. This finding directly correlates with the respective surface water samples. In case of the samples from a catchment lake and the drinking water from this lake, there was a similar correlation. Nevertheless, it is necessary to conduct further investigations on a larger scale, especially regarding the time dependency of sampling. The presented methods provide the capability to perform such studies and serve as a basis for further method development.

Acknowledgements

The authors would like to thank Dr. Claus Schlett from the Westfälische Wasser- und Umweltanalytik GmbH as well as the GELENWASSER AG, the Wasserwerke Westfalen GmbH and the Wassergewinnung Essen GmbH for collecting and providing samples.

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