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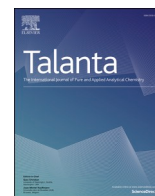
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## Simultaneous speciation of iodine-, gadolinium-, and platinum-based pharmaceuticals by HILIC-ICP-MS and its application to wastewaters

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### ABSTRACT

The presence of contaminants in drinking water resources is a topic of great interest, yet certain types of compounds as pharmaceutical residues are still under-documented. These contaminants include organometallic or halogenated complexes such as cancerostatic platinum compounds (CPC), gadolinium-based contrast agents (GBCA) and iodine contrast media (ICM). Because of their increasing use in recent years, their concentrations in effluents and surface waters can now reach  $\mu\text{g L}^{-1}$  for iodine compounds and  $\text{ng L}^{-1}$  for platinum- or gadolinium-based compounds.

This work aims to develop an analytical methodology for the simultaneous speciation of iodine-, platinum- and gadolinium-based complexes at ultratrace levels ( $\sim\text{ng L}^{-1}$ ). Our developments focused on hydrophilic interaction chromatography (HILIC) coupled with ICP-MS to separate and quantify ten drug residues, including three iodine-, three platinum- and four gadolinium-complexes. Three HILIC stationary phases (two zwitterionic, i.e. phosphorylcholine and ammonium-sulfonic acid phases, and one silica charged phase) were compared under different mobile phase compositions (organic solvent percentage, salt concentration and pH). Dipole moment computations using density functional theory (DFT) were performed to refine our understanding of observed compounds complex separation in HILIC. Phosphorylcholine stationary phase with an acetonitrile gradient from 75 % to 50 % in  $10\text{ mmol L}^{-1}$  ammonium formate aqueous solution was found best compromise to achieve the separation of the ten tested complexes. Achieved LODs for the various complexes were between  $1$  and  $3\text{ ng L}^{-1}$  Gd,  $3$  and  $17\text{ ng L}^{-1}$  Pt and  $46$  and  $63\text{ ng L}^{-1}$  I.

The developed method was applied to urban and hospital wastewaters. Two ICM (iohexol and iopamidol) and one GBCA (gadoterate) were quantified while no CPC were detected. Concentration of gadoterate was of the same order in the two types of wastewaters, i.e. between  $525$  and  $677\text{ ng L}^{-1}$  Gd. While iohexol was determined only in urban wastewaters at  $30\text{ }\mu\text{g L}^{-1}$  I, the concentration of iopamidol in urban wastewater was about 30 times lower than in hospital wastewater ( $134\text{ ng L}^{-1}$  I).

### 1. Introduction

The last few decades have been marked by the diversification and growing use of pharmaceutical products. Among them, organometallic or halogenated pharmaceuticals are worldwide and daily administered for diagnostic and therapeutic purposes [1]. They include complexes of different metals such as gadolinium-based contrast agents (GBCA) and cancerostatic platinum compounds (CPC) or, non-metal-based drugs like iodine contrast media (ICM). Between 2006 and 2022, computed tomography and magnetic resonance imaging examinations have been in an upward trend, respectively 5 % and 9 % per year in France [2,3], with a concomitant increase in the consumption of contrast agents such as

ICM or GBCA. In the same way, since 1990, growth rates up to 0.9 % per year [4] of new cancer cases resulted in a proportional increased use of antineoplastic drugs (CPC). Once administered and excreted from the body, these compounds are driven to wastewaters treatment plants where their remediation depends on the processes used and varies between  $\sim 20$  and  $\sim 60$  % for ICM [5] and, between 51 and 63 % for CPC [6,7]. The removal of GBCA varies from negligible (with activated sludges system) to 99.9 % with reverse osmosis treatment step that remains uncommon [8,9]. Most of the typically used wastewater treatments having little or no efficiency, the resulting contamination of water bodies requires to consider these compounds as contaminants of emerging and global concern.

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Concentrations of ICM compounds have been reported up to  $\mu\text{g L}^{-1}$  level in influents and effluents of wastewaters treatment plants in Germany, Australia, Spain and France [9–13] and up to few dozen of  $\text{ng L}^{-1}$  in drinking water in Germany [14,15]. Cancerostatic platinum compounds were determined at  $\mu\text{g L}^{-1}$  level in hospital wastewaters in Austria and United-Kingdom [16,17] and, between a few to several hundreds of  $\text{ng L}^{-1}$  in urban wastewater treatment plant influents and effluents in Slovenia and Iran [18,19]. Concentrations reported for GBCA reached  $\mu\text{g L}^{-1}$  level in hospital and urban wastewaters and,  $\text{ng L}^{-1}$  in surface and tap waters in Germany [20,21]. Monitoring their occurrence and behavior in the aquatic environment and further their potential impact toward exposed aquatic organisms, requires the development of sensitive analytical methods for the determination of these pharmaceuticals in water bodies.

The coupling between liquid chromatography (LC) and inductively coupled plasma mass spectrometry (ICP-MS) has been proven effective to separate and quantify these pharmaceuticals. An overview of published chromatographic procedures is given in Table S1. All these procedures have been specifically developed according to the single element (Gd, Pt or I) contained in drugs. Hydrophilic interaction liquid chromatography (HILIC) has been preferentially applied for GBCA speciation allowing to separate up to six compounds: gadobenate (Gd-BOPTA), gadodiamide (Gd-DTPA-BMA), gadoxetate (Gd-EOB-DTPA), gadobutrol (Gd-BT-DO3A), gadoterate (Gd-DOTA) and gadopentetic acid (Gd-DTPA) with detection limits (LOD) between 1 and  $157 \text{ ng L}^{-1}$  Gd [21–25]. In the case of ICM and CPC, reversed phase liquid chromatography (RP-LC) was mainly used before the “rediscovery” of HILIC in the 2000s. RP-LC allowed the separation of up to eight ICM (iopamidol, iothalamic acid, diatrizoate, iohexol, iomeprol, iopromide, ioxaglic acid and iodipamide) with LODs between 5 and  $970 \text{ ng L}^{-1}$  I [9–11] and, that of oxaliplatin, carboplatin and, cisplatin and its degradation products monoqua- and diaquacisplatin with detection limits between 9 and  $150 \text{ ng L}^{-1}$  Pt [16,19]. More recently, HILIC was also shown to be a convenient separation mechanism for iodine- and platinum-based pharmaceuticals with applications to environmental water samples giving LODs between 5 and  $1580 \text{ ng L}^{-1}$  I and, between 27 and  $575 \text{ ng L}^{-1}$  Pt [18].

The simultaneous and multi-elemental speciation of iodine-, gadolinium- and platinum-based pharmaceuticals is a challenging task because of the different physicochemical characteristics of complexes involving neutral, ionic or ionizable compounds of variable polarity. Target pharmaceutical complexes include polar compounds (e.g. Gd-BT-DO3A, Gd-HP-DO3A, iohexol), anionic complexes (e.g. Gd-BOPTA, diatrizoate) and low stability complexes which form(s) can vary according to matrix composition (e.g. cisplatin, oxaliplatin). Multimodal HILIC, combining different retention mechanisms stands as most appropriate chromatographic mode for their simultaneous separation. Indeed, the primary factors determining retention in HILIC, are polarity, hydrophilicity and ionicity [26]. Polarity is the central parameter to be considered since it controls the interaction between analytes and the water layer on the stationary phase. Hydrophilicity, which is in close connection with polarity, defines the partition of an analyte. Ionicity also might play an important role depending on the analyte, the stationary phase and other factors such as pH and salt concentration. At a given pH, strongly ionic analytes are naturally highly polar due to their charge, but their overall retention in HILIC derives from a combination of factors such as hydrophilic partitioning and electrostatic interactions with the stationary phase, which depend on structural features such as the presence of hydrophilic groups. Hydrophilicity is primarily promoted by the presence of polar functional groups, such as hydroxyl and amide, as well as charged groups like  $-\text{COO}^-$  and  $-\text{NH}_3^+$ . On the other hand, hydrophobicity arises from nonpolar structural features, such as alkyl chains, aromatic rings, or large nonpolar moieties like for instance bromine and iodine.

The mechanisms of retention in HILIC are thus dependent both on stationary phase surface and mobile phase composition which is

generally highly organic (>60 %). Changing the organic solvent percentage allows to modify the polarity of the mobile phase and therefore its eluting strength and hence the retention factor of the analytes. The addition of salt reduces electrostatic interactions on ionized functional groups, leading to increased retention of charged analytes undergoing repulsive interactions and decreased retention of charged analytes subject to attractive interactions. In the same way, the pH can also affect the charge state of both the stationary phase and ionizable analytes, the hydrophilicity of analytes and, therefore the processes of electrostatic interactions and hydrophilic partitioning.

Therefore, in this work a novel multi-elemental analysis method based on the coupling of HILIC with ICP-MS was developed for the speciation of iodine- (iohexol, iopamidol and diatrizoate), gadolinium- (gadobenate, gadoterate, gadoteridol and gadobutrol) and platinum- (cisplatin, oxaliplatin and carboplatin) containing pharmaceuticals in water bodies. Different stationary phases were compared for assessing their chromatographic efficiency for simultaneous quantitative speciation analysis of targeted pharmaceuticals. Observed separation was compared with polarity-based predictions assessed by computed dipole moments. The developed method was then successfully applied to GBCA, CPC and ICM analysis in hospital and urban wastewaters. To the best of our knowledge, this is the first HILIC-ICP-MS method proposed for simultaneous monitoring of iodine-, gadolinium- and platinum-containing pharmaceuticals in aquatic environment.

## 2. Material and methods

### 2.1. Reagents and standards

Mobile phase was prepared with ammonium formate ( $\geq 99.995$  % purity) purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France), acetonitrile (LC-MS grade) from Fisher Scientific (Illkirch, France) and ultrapure water obtained from a Milli-Q® IQ water system from Merck Millipore (Darmstadt, Germany). Mobile phase's pH was adjusted with formic acid ( $\geq 98$  % purity) from Sigma-Aldrich or ammonia solution (35 %) and was measured after calibration of the glass electrode system with conventional aqueous standards. As described by Alvarez-Segura et al. (2019), pH values of acetonitrile containing mobile phases are then provided in the  $\text{pH}_w$  scale [27].

Ten commercially available pharmaceuticals were considered (Fig. 1). Three iodine contrast media (iohexol, iopamidol and diatrizoate) and three cancerostatic platinum compounds (cisplatin, oxaliplatin and carboplatin) were purchased from Sigma-Aldrich (European Pharmacopoeia Reference Standard, Saint-Quentin-Fallavier, France). Four commercially available gadolinium-based contrast agents including gadoterate (dotarem, Gd-DOTA,  $0.5 \text{ mol L}^{-1}$ ), gadoteridol (prohance, Gd-HP-DO3A,  $0.5 \text{ mol L}^{-1}$ ), gadobutrol (gadovist, Gd-BT-DO3A,  $1 \text{ mol L}^{-1}$ ) and gadobenate (multihance, Gd-BOPTA,  $0.5 \text{ mol L}^{-1}$ ) were purchased at a local pharmacy. Elemental concentration of drugs was verified by ICP-MS total analysis. Elemental calibration standard solutions of iodine ( $\text{KIO}_3$ ,  $1 \text{ g L}^{-1}$  as I), gadolinium ( $\text{Gd}_2\text{O}_3$ ,  $1 \text{ g L}^{-1}$  as Gd) and platinum ( $\text{H}_2\text{PtCl}_6$ ,  $1 \text{ g L}^{-1}$  as Pt) were bought from SCP Science (Courtaboeuf, France) as inorganic standards. Analytical developments were conducted with standard solutions containing the ten metallodrugs with or without the three inorganic forms, prepared in mobile phase or raw surface water as thereafter described.

### 2.2. HILIC-ICP-MS instrumentation

Analytical developments were carried out using three different HILIC columns: one silica based stationary phase (Accucore™,  $100 \times 2.1 \text{ mm}$ ,  $2.6 \mu\text{m}$  particle size, Thermo Fisher Scientific, Waltham, United States of America) and two zwitterionic stationary phases of cross-linked silica: with ammonium-sulfonic acid groups (NUCLEOSHELL®,  $100 \times 2 \text{ mm}$ ,  $2.7 \mu\text{m}$  particle size, Macherey-Nagel, Düren, Germany) and, with phosphorylcholine groups (PC HILIC,  $150 \times 4.6 \text{ mm}$ ,  $3 \mu\text{m}$  particle size,

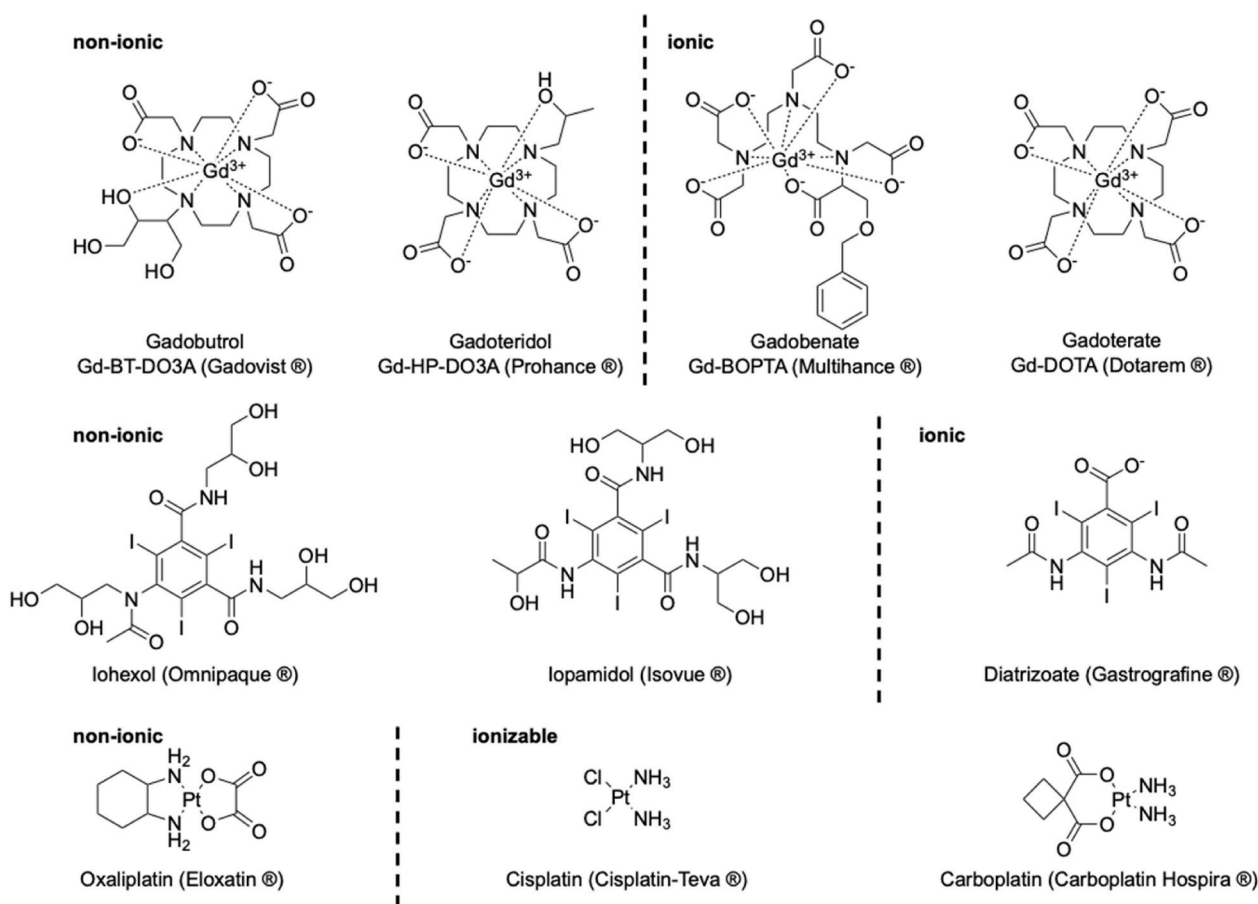


Fig. 1. Structures of considered pharmaceuticals according to drug formulations.

Osaka Soda, Osaka, Japan) (Table 1).

Chromatographic separations were conducted with an Agilent 1200 series HPLC system coupled to an Agilent 7900 ICP-MS (Agilent Technologies, Tokyo, Japan). ICP-MS was equipped with a MicroMist nebulizer, a Scott spray chamber, platinum cones and x-lenses. A 1 mm i.d. torch was used to lessen plasma loading and preserve its stability under organic solvent conditions. Samples were brought to the ICP through a polyetheretherketone (PEEK) tubing (0.125 mm i.d).

The sensitivity of the ICP-MS was daily optimized in aqueous mode

using a tuning solution containing  $1 \mu\text{g L}^{-1}$  Li, Y, Ce, and Tl in 2 %  $\text{HNO}_3$ . Optimization was refined by adjusting lenses settings under organic conditions using mobile phase (50–75 % acetonitrile + 25–50 % 10 mmol  $\text{L}^{-1}$  ammonium formate) spiked with  $1 \mu\text{g L}^{-1}$  I, Gd and Pt. Spray chamber temperature was set to  $-5^\circ\text{C}$  under organic solvent conditions in addition to  $\text{O}_2$  introduction (9 %) to avoid carbon deposition.  $^{127}\text{I}$ ,  $^{156}\text{Gd}$ ,  $^{157}\text{Gd}$ ,  $^{158}\text{Gd}$ ,  $^{194}\text{Pt}$ ,  $^{195}\text{Pt}$ , and  $^{196}\text{Pt}$  isotopes were monitored with integration time of 0.1 s.

Table 1

Characteristics of the stationary phases of analytical columns investigated [28,29].

Column name (stationary phase)	Structure of stationary phase	Hydrophilicity	Electrostatic interactions	Hydrogen bonds
Accucore™ (bare silica)		High	Negative, strong at pH > 4-5	Hydrogen donor and acceptor at pH < 4 Hydrogen acceptor at pH > 4-5
NUCLEOSHELL® (ammonium-sulfonic acid)		High	Negative, weak	Hydrogen acceptor
PC HILIC (phosphorylcholine)		High	Positive, weak	Hydrogen acceptor

### 2.3. Samples and standards preparation

Three types of water were collected: a surface water (43°05'21.6"N 0°15'02.4"W, Arthez d'Asson, France), on March 23, 2023, used as a negative control and as matrix for standard solutions during method development, a hospital wastewater (Dax, France), on November 20, 2023, and a daily representative urban wastewater from a treatment plant (WWTP) with a capacity of 20 000 inhabitant equivalents (Baudreix, France), on November 21, 2023.

All samples were collected into polypropylene containers previously washed with 5 % nitric acid (Atlantic Labo, Bruges, France), rinsed with ultrapure water and dried under laboratory hood. Before storage, samples were filtered at 0.45  $\mu\text{m}$  with acetate membrane filters (VWR, Rosny-sous-Bois, France) and then stored at  $-80\text{ }^{\circ}\text{C}$  for long term or at  $4\text{ }^{\circ}\text{C}$  just before analysis.

For total elements analysis, samples were diluted 100 and 10 times into ultrapure water for total iodine analysis, due to expected high level of iodine. External calibration (0.05–5  $\mu\text{g L}^{-1}$  range) was prepared in 2 %  $\text{HNO}_3$  for Gd and Pt and, in ultrapure water for I measurements. For speciation analysis, external calibration (in the ranges 0.001 to 0.5  $\mu\text{g L}^{-1}$ , 0.01 to 0.5  $\mu\text{g L}^{-1}$  and 0.05 to 30  $\mu\text{g L}^{-1}$  for Gd, Pt and I respectively) and standard additions quantification methods were compared. Because of the high levels of iodine and to lessen matrix effects, wastewater samples were diluted ten times into mobile phase of initial gradient conditions (75 % acetonitrile + 25 % 10  $\text{mmol L}^{-1}$  ammonium formate at  $\text{pH} = 6.8$ ). The injection volume in optimized gradient mode was 50  $\mu\text{L}$ , and external calibration solutions were also prepared in mobile phase solution of initial gradient conditions as described for samples.

### 2.4. Molecular polarity computations

Based on the molecular structure of the analytes of interest, Gd-DOTA, Gd-HP-DO3A, and Gd-BT-DO3A are expected to be the most hydrophilic due to their multiple hydroxyl and carboxyl groups. Gd-BOPTA should exhibit lower hydrophilicity, because the presence of a phenyl moiety introduces hydrophobic character. Iopamidol and iohexol are expected to have a more balanced polarity, where hydroxyl and amide groups reduce the hydrophobicity of their iodine-substituted aromatic rings. Finally, diatrizoate is expected to be the least hydrophilic due to the presence of fewer polar groups. However, although qualitative assessments based on functional groups provide an initial understanding, they cannot precisely quantify the extent of polarity differences or predict slight variations in retention behavior. Therefore, in this work we assessed the polarity of the analyzed compounds through dipole moment computations using density functional theory (DFT). Dipole moment is a measure of the overall polarity of a molecule, caused by differences in electronegativity between atoms and the shape of the molecule. To model the compounds of interest we relied on crystallographic data for the Gd- complexes to build reference models while the iodine-containing derivatives were manually constructed. Molecular geometries were optimized using the PBE0 functional [30] and the Def2-SVP basis set [31], which includes pseudopotentials for heavy elements such as gadolinium and iodine. To ensure a more precise evaluation of electronic properties, dipole moments were computed with the higher-accuracy Def2-TZVPP basis set [31]. Solvation effects were modeled using the CPCM [32] implicit solvation model with water and acetonitrile as solvents, separately. Since HILIC involves mostly localized solvent environments rather than a uniform mixture, separate calculations were performed using pure water (to model the structured water layer near the stationary phase) and pure acetonitrile (to approximate the bulk mobile phase). Frequency calculations confirmed that the optimized geometries correspond to true minima on the potential energy surface (see SI for more details). It is important to note that in solution, some of the studied compounds exist predominantly in their charged forms, which makes their dipole moments dependent on

the choice of the geometrical origin. Therefore, to obtain relative comparisons we computed dipole moments using a uniform computational protocol centering each molecule in its center of mass. For CPC, as the degradation of cisplatin and oxaliplatin was observed, no computation was performed.

### 2.5. Chromatography optimization

In order to identify most appropriate separation conditions for individual HILIC column, the influence of the main mobile phase parameters (acetonitrile percentage, ammonium formate concentration and pH) was evaluated in isocratic elution mode (Table S2). The variation range of these parameters was selected from previously reported conditions [22,24,33] and column tolerances defined by suppliers. Surface waters spiked with each drug alone and with the mixture of the ten drugs were injected in triplicate to identify and determine compounds retention time. Flow rate was maintained at  $0.8\text{ mL min}^{-1}$  and injection volume was set to 5  $\mu\text{L}$ . For each parameter variation, retention factor ( $k'$ ) and resolution ( $R_s$ ) were calculated to determine its influence on pharmaceutical's retention and identify optimum column and conditions.

Then optimization of the chromatographic separation was refined for the previously selected PC HILIC column, with an elution gradient of acetonitrile (A) and 10  $\text{mmol L}^{-1}$  ammonium formate aqueous solution at  $\text{pH} = 6.1$  (B). Gradient and post-gradient equilibration time were carefully optimized leading to following conditions: 0–0.5 min: 25 % B; 0.5 to 1.5 min: 25 %–50 % B; 1.5 to 9 min: 50 % B; 9 to 10 min: 50–25 % B; 10 to 30 min: 25 % B. Flow rate was maintained at  $0.8\text{ mL min}^{-1}$  and injection volume was set to 50  $\mu\text{L}$  for all experiments (from optimization with standards to sample analysis).

For pharmaceutical quantification and determination of analytical figures of merit, when compounds were split into several peaks, the sum of all peak areas of each compound was used.

### 2.6. Column recovery determination

The recovery rate of the PC HILIC column for each pharmaceutical was calculated as the ratio of its peak areas resulting from chromatographic and flow injection analyses (FIA). The FIA of every standard was obtained in isocratic mode, using two mobile phase compositions (75 % and 50 % acetonitrile) to match initial and final conditions of acetonitrile gradient. The recovery rate was determined using FIA peak area obtained in the mobile phase conditions closest to those of chromatographic analysis regarding retention time of each compound.

## 3. Results and discussion

A panel of four different chromatographic columns was available for analytical developments, i.e., the three HILIC columns presented above (§ 2.2) and a fourth HILIC column with bridged ethylene-silica stationary phase (Xbridge BEH,  $50 \times 2.1\text{ mm}$ , 2.5  $\mu\text{m}$  particle size, Waters, Milford, United States of America). First tests with different mobile phase compositions indicated that the Xbridge BEH column did not allow the separation of any of the drugs of interest, therefore, this column was ruled out for further developments.

### 3.1. Development of HILIC-ICP-MS method

#### 3.1.1. Preliminary tests

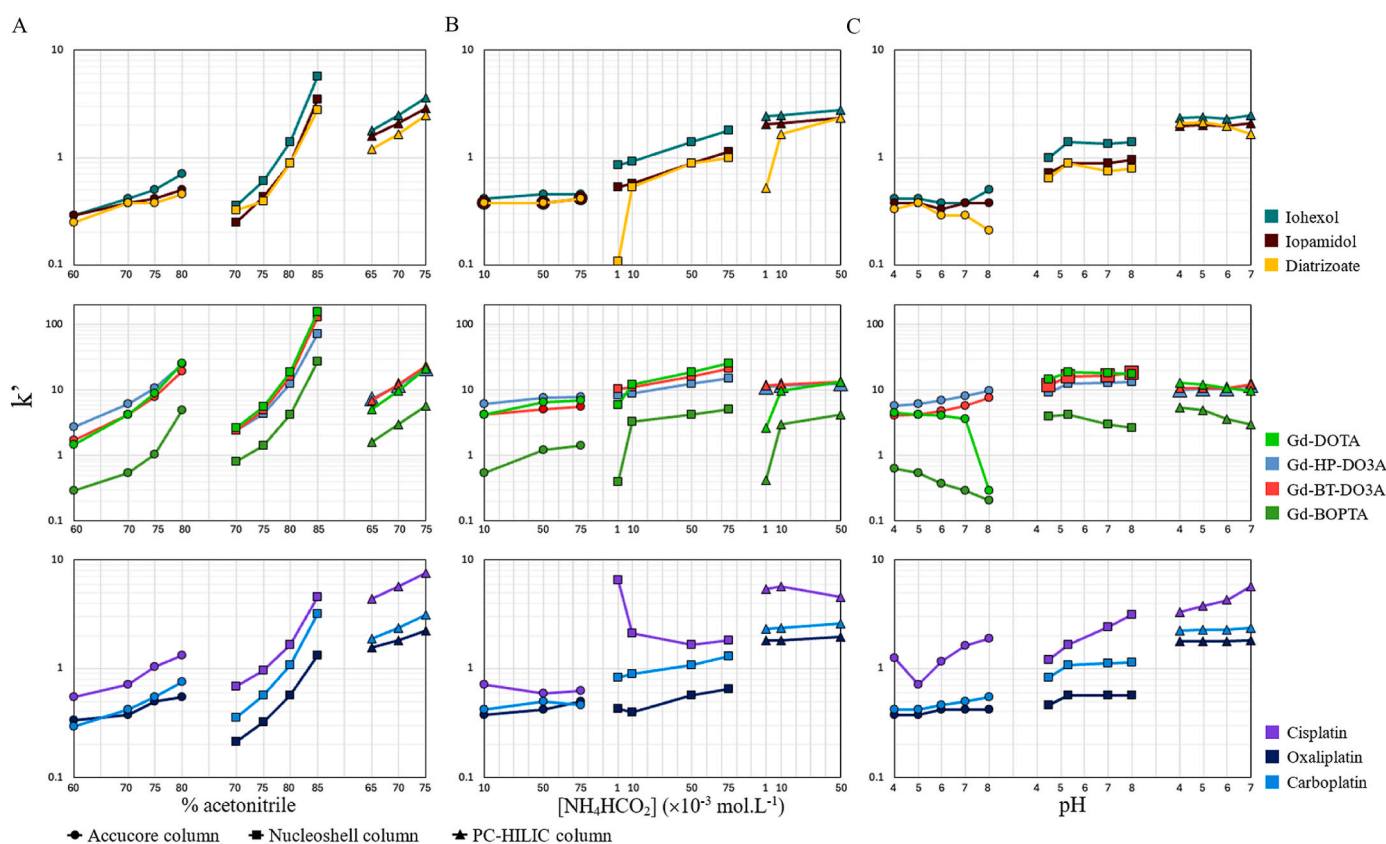
The three HILIC columns (Accucore™; NUCLEOSHELL®; PC HILIC) investigated in this study were selected according to previous studies dedicated to GBCA speciation analysis [22,24,25,33]. Considering mobile phase composition, acetonitrile is traditionally used in HILIC chromatography as it is neither acceptor nor donor of protons avoiding to compete with water molecules [29]. Some preliminary tests corroborated that the use of methanol resulted in poor analyte retention. The

use of a salt-free mobile phase resulted in decreased retention times and a poor reproducibility and was also discarded. Addition of ammonium acetate instead of ammonium formate in the mobile phase was evaluated. As no difference was observed, ammonium formate was chosen due to the higher purity of the available stock. Thus, a mix between acetonitrile and aqueous ammonium formate solution was used. In the case of PC HILIC column, the use of pure water mobile phase has been described for the separation of six GBCA (Gd-DTPA, Gd-EOB-DTPA, Gd-DOTA, Gd-DTPA-BMA, Gd-BT-DO3A and Gd-HP-DO3A) [25]. Using pure water mobile phase, we first similarly observed the separation of the ten pharmaceuticals with an unprecedented sensitivity of detection. However, after few days of analyses and despite column storage under recommended organic conditions between each session of analysis, irreversible degradation of the stationary phase occurred. As a result, pure water mobile phase was ruled out.

Based on these initial tests, stationary phase characteristics and previous studies, starting mobile phase compositions for columns evaluation were set to: 70 % acetonitrile + 30 % 10 mmol L<sup>-1</sup> ammonium formate at  $s_w$  pH = 5 for Accucore™ column [24,33], 80 % acetonitrile + 20 % 50 mmol L<sup>-1</sup> ammonium formate at  $s_w$  pH = 5.3 for Nucleoshell® column [22] and, 70 % acetonitrile + 30 % 10 mmol L<sup>-1</sup> ammonium formate at  $s_w$  pH = 7 for PC HILIC column. Each parameter was then successively modified from those initial conditions (Table S2), to evaluate its influence on the retention and separation of targeted pharmaceuticals in isocratic conditions (Fig. 2 and Tables S3 and S4). Whatever mobile phase composition, iodinated contrast agents and platinum drugs eluted first, while gadolinium complexes exhibited intermediate (Gd-BOPTA) to last retention times (Gd-HP-DO3A, Gd-BT-DO3A, Gd-DOTA).

### 3.1.2. Effect of acetonitrile percentage

Increasing acetonitrile percentage led to a common trend observed resulting in increased retention of the analytes, due to stronger partitioning effect (Fig. 2A). Independently of the column, this effect was particularly marked for those Gd complexes which have the greatest polarities as determined by the computed molecular dipole moments (Table S5). For Gd-DOTA, Gd-HP-DO3A and, Gd-BT-DO3A with highest dipole moments (>25 Debye), retention factors increased of ca. (average) 11-fold for Accucore™ column between 60 and 80 % acetonitrile, of 44-fold for NUCLEOSHELL® column between 70 and 85 % acetonitrile, and of 4-fold for PC HILIC column between 65 and 75 % acetonitrile. Gd-DOTA, having high dipole moment and the most deprotonated carboxylic moiety, was the compound whose retention was most impacted by acetonitrile percentage until changing its elution order from second to fourth (Accucore™ column) or third (PC HILIC column) position. This change was not observed for NUCLEOSHELL® column with which Gd-DOTA eluted last whatever acetonitrile percentage, most likely due to the highest salt content used with this column. The increase of acetonitrile percentage resulted in unchanged elution order for ICM and CPC but provided higher values of their retention factors with zwitterionic stationary phases (Table S3) together with better resolution (Table S4). Iohexol, as the most polar ICM compound analyzed, remained the last ICM to be eluted regardless of the acetonitrile percentage. Moreover, whatever the column, a general broadening of peaks, even more important for compounds of higher polarity (GBCA), was observed with highest acetonitrile percentage conditions. This resulted in an average loss of 50 % of peak height and resolution for compounds with close retention times (e.g. Gd-HP-DO3A and Gd-BT-DO3A, or Gd-DOTA and Gd-BT-DO3A).



**Fig. 2.** Evolution of retention factors,  $k'$ , for pharmaceutical complexes according to varying mobile phase composition with A) acetonitrile percentage, B) salt concentration and C) pH mobile phase for Accucore™ (circle), NUCLEOSHELL® (square) and PC HILIC (triangle) columns. The influence of one parameter is evaluated while the two other remained constant at their corresponding value of starting mobile phase compositions (respectively for Accucore™, NUCLEOSHELL® and PC HILIC, constant values are 70, 80 and, 70 % acetonitrile; 10, 50 and 10 mmol L<sup>-1</sup> ammonium formate; = 5, 5.3 and 7, as detailed in Table S2).

### 3.1.3. Effect of salt concentration

Regardless of the column, a similar increased retention was observed for all the compounds, except cisplatin, with increasing ammonium formate concentration (Fig. 2B). From the lowest salt concentration, this effect is all the more marked for compounds with anionic character (e.g. diatrizoate, Gd-BOPTA and Gd-DOTA). Indeed, among ICM, the retention of the first eluting compound, i.e. diatrizoate, increased significantly (c.a 5 to 9-fold), compared with iohexol and iopamidol retention which was almost unchanged in the range of ammonium formate concentrations experimented. The same behavior was observed for Gd-BOPTA, the single linear dianionic Gd-complex, whose retention had the highest increase among GBCA, with increasing concentration of ammonium formate. In the case of Gd-DOTA, macrocyclic and anionic Gd-complex, its elution order changed from second to third or last with ammonium formate concentration increase. Furthermore, electrostatic interactions between negatively charged analytes and the stationary phases may be attractive in the case of phosphorylcholine phase or repulsive in the case of silica and sulfobetaine phases (Table 1). Our results suggested that such interactions were not prevalent with phosphorylcholine stationary phase as the retention of negatively charged analytes was raised in the presence of increased salt concentrations. Silica and sulfobetaine negatively charged phases induce electrostatic repulsions which reduction with increased salt concentrations may contribute to increase the retention of negatively charged analytes. A closer look at retention factors values for ammonium formate concentration  $>10 \text{ mmol L}^{-1}$  (Table S3) showed a continuous increase for anionic as well as uncharged ICM and GBCA, that may be explained by hydrogen-bonding interactions promoted by salt concentration in the mobile phase [34].

Among CPC, oxaliplatin and carboplatin remained poorly retained on silica and sulfobetaine negatively charged stationary phases whatever salt concentration. Only cisplatin retention was influenced and lowered by the reduction of electrostatic attractions on sulfobetaine phase when ammonium formate concentration was increased. The retention of CPC on the phosphorylcholine phase of PC-HILIC column was almost unchanged in the presence or absence of salt suggesting weak electrostatic interactions between CPC and this stationary phase.

### 3.1.4. Effect of pH mobile phase

Changes in  $^s_{\text{w}}\text{pH}$  mobile phase in the range 4 to 8, resulted in unchanged retention of the non-ionic compounds iopamidol, iohexol and oxaliplatin while the retention of non-ionic Gd-HP-DO3A and Gd-BT-DO3A was increased (in greater or lesser degree depending on the column) (Fig. 2C). Such different behavior between non-ionic compounds may be attributed to the presence of hydrophobic moieties in the first group (cyclic or aromatic hydrocarbons) while alcoholic functional groups of Gd-HP-DO3A and Gd-BT-DO3A may promote hydrogen bonding to hydrogen acceptor stationary phases. The retention of diatrizoate, Gd-BOPTA and Gd-DOTA that may be partially protonated at pH around 4–5 was generally decreased with pH increase. In the case of Accucore<sup>TM</sup> column, for  $^s_{\text{w}}\text{pH} > 5$ , silanol groups of its stationary phase are deprotonated resulting in a lower retention of negatively charged compounds. In the case of both zwitterionic stationary phases, the retention of Gd-BOPTA decreased with increasing pH. Diatrizoate and Gd-DOTA behaved in a similar way (and in much less extent) on PC HILIC column while their retention was slightly increased on NUCLEOSHELL<sup>®</sup> column. Cisplatin and, to a lesser extent, carboplatin were more retained on both zwitterionic columns with increasing pH.

### 3.1.5. Comparison of observed retention and computed dipole moments

The retention ordering of the studied analytes in HILIC can first be interpreted in terms of hydrophilicity, as analytes with greater hydrophilic character tend to interact more strongly with the stationary phase. The computed dipole moments of all gadolinium-based contrast agents were found significantly higher ( $>24.4 \text{ D}$ ) than that of the iodinated contrast agents ( $<6.9 \text{ D}$ ) (Table S5). The higher polarity of Gd-

complexes stems from their structural composition, including multiple highly polar functional groups, and their ionic nature. In contrast, the reduced polarity of the iodinated compounds arises from their neutral net charges and weak localized electronic effects of their amide and hydroxyl groups, incorporated to enhance water solubility but do not significantly increase overall polarity.

Among the iodinated contrast agents, iohexol has the highest dipole moment, followed by iopamidol and diatrizoate. This trend is consistent with their retention times observed in PC HILIC column that enables their separation, where iohexol elutes last among the three (279 s), while iopamidol (249 s) and diatrizoate (214 s) elute earlier.

Regarding Gd-complexes their retention times do not strictly follow polarity-based predictions. Despite having the highest dipole moment, ionic Gd-DOTA elutes earlier than both Gd-HP-DO3A and Gd-BT-DO3A in Accucore<sup>TM</sup> and PC HILIC columns. This deviation suggests that additional retention mechanisms beyond polarity, such as solvation effects or hydrogen bonding with the stationary phase, influence their retention behavior. For instance, from a structural point of view, Gd-BT-DO3A and Gd-HP-DO3A differ in the nature of their side groups, i.e. Gd-BT-DO3A features three hydroxyl groups in its side groups, while Gd-HP-DO3A bears only one. The additional hydroxyls of Gd-BT-DO3A increases its potential for hydrogen bonding with the hydrophilic stationary phase relative to Gd-DOTA most likely leading to longer retention times. For Gd-BOPTA, computations were performed on its fully deprotonated form, with a net charge of  $-2$ , which should predominate at pH 7 [35]. This feature alone would suggest, with a dipole moment at  $26.7 (26.5) \text{ D}$ , a strong interaction with the stationary phase. However, this prediction contradicts experimentally observed early elution that was close to iodinated contrast agents. The observed behavior can be attributed to the presence of the hydrophobic phenyl ring, which as in the case of the iodine-based agents, appears to reduce its hydrophilic character and thus its affinity for the water-rich stationary phase. The distinct behavior of Gd-BOPTA compared to the other Gd complexes has also been reported by Laurent et al. [36], who observed a significant interaction between Gd-BOPTA and human serum albumin, in contrast to the other Gd-complexes. Laurent et al. [36] attributed this preference to the benzyloxymethyl side group which, carrying the hydrophobic aromatic ring, plays a key role in Gd-BOPTA's solvation behavior. This observation supports the hypothesis that the phenyl ring of benzyloxymethyl side group should play an important role in retention behavior by reducing its effective hydrophilicity and in extension its retention times.

Overall, our computational study at the molecular level shows that, while retention behavior may depend on the combined influence of factors such as solvation, hydrogen bonding, and hydrophobic substituents, descriptors such as molecular polarity can serve as a useful starting point for understanding retention, particularly when experimental physicochemical data are lacking.

### 3.1.6. Column selection

Optimum conditions for each column were determined according to the calculated values of retention factor ( $k' > 1$ , Table S3) and resolution ( $R_s > 0.6$ , Table S4). With Accucore<sup>TM</sup> column, satisfactory separation conditions were obtained only for GBCA using 70 % acetonitrile + 30 %  $10 \text{ mmol L}^{-1}$  ammonium formate and  $^s_{\text{w}}\text{pH} = 7$  (Cf. Fig. S6a, Table S4). Under these conditions, the four Gd-complexes were detected in a total 250 s run, two peaks were observed for Gd-DOTA suggesting the separation of its isomers which one coeluted with Gd-BOPTA. Birka et al. (2013) reported similar observation of significant shoulder of the Gd-BOPTA peak using this column and mobile phase at pH value of 3.75 [33]. However, under all experimented conditions with Accucore<sup>TM</sup> column, the separation of ICM as well as CPC was not achieved (the three ICM, oxaliplatin and carboplatin eluting in the void volume).

Only the zwitterionic columns allowed an improvement of multi-elemental compounds separation and initial conditions proved to be the most effective in terms of separation.

The NUCLEOSHELL® column allowed the separation of the three CPC in addition to the four GBCA with mobile phase of 80 % acetonitrile + 20 % 50 mmol L<sup>-1</sup> ammonium formate and  $s_w$ pH = 5.3, within 600 s run (Cf. Fig. S6b, Table S4). With PC HILIC column and a mobile phase of 70 % acetonitrile + 30 % 10 mmol L<sup>-1</sup> ammonium formate and  $s_w$ pH = 7, the separation of all the complexes of the three elements was obtained within 1100 s although without baseline separation between Gd-HP-DO3A and Gd-BT-DO3A (Cf. Fig. S6c, Table S4). However, Gd-BOPTA was eluted in two peaks suggesting, as indicated above, the separation of different isomers of this compound. Under all tested conditions for PC HILIC column, iohexol was also eluted in two peaks, the second one corresponding to 20 % of total area as previously reported by Echeverría et al. (2013) [11]. This can be explained by the center of asymmetry and sites of restricted rotation that induce multiple isomeric forms for this compound [37]. Finally, the signal intensity was strongly compound dependent for CPC: cisplatin was almost non-detected and oxaliplatin signal was only 30 % of carboplatin one, suggesting a poor recovery rate for these compounds as discussed below.

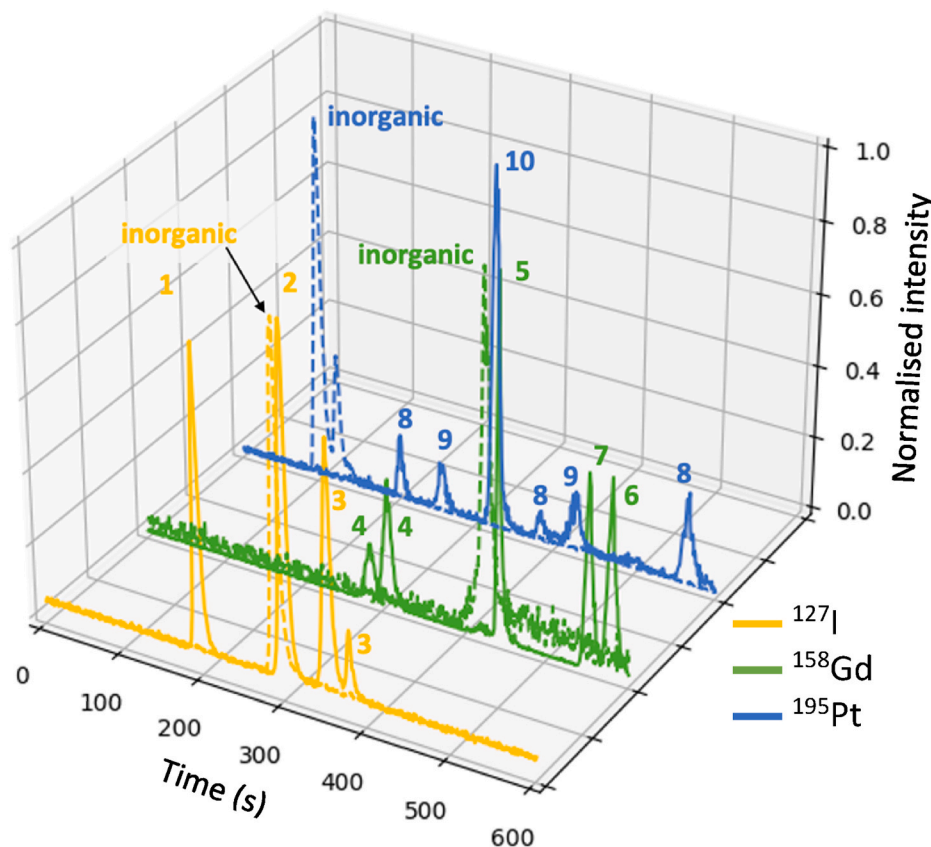
An estimation of detectable concentrations was done for the resolved pharmaceuticals and the three columns in their corresponding optimal isocratic elution conditions (Table S6). In general, these values reach several hundreds of ng L<sup>-1</sup> for ICM and between tens to few hundreds of ng L<sup>-1</sup> for GBCA and CPC remaining higher than the ones achieved in literature (Table S1). In view of quantifying pharmaceuticals at ng L<sup>-1</sup> level in environmental water samples, regardless of the column used, sensitivity of the method needs to be improved of ca. 50–100 factor for ICM and factor 10 for GBCA and CPC. PC HILIC column was selected for further optimization as it proved possible to achieve satisfactory overall

separation of all the targeted compounds.

### 3.1.7. PC HILIC separation improvement with an elution gradient

The use of mobile phase gradient was investigated next with the dual objective of improving sensitivity and chromatographic resolution between Gd-BT-DO3A and Gd-HP-DO3A which peaks were not fully resolved in isocratic elution.

Based on previously obtained results with PC HILIC indicating higher retention of all pharmaceuticals at high acetonitrile content (Fig. 2, Table S3), starting acetonitrile percentage was set at 75 % ( $s_w$ pH = 6.8) to first focus on ICM separation. Then, the gradient profile was adjusted to decrease acetonitrile content to 50 % ( $s_w$ pH = 6.5) in order to speed up elution of all the compounds and increase the global sensitivity while maintaining the minimal recommended acetonitrile percentage for this column. In these conditions and with an injection volume of 50  $\mu$ L, the ten pharmaceuticals were eluted within 10 min. As HILIC requires long re-equilibration times [29,38], 20 min, *i.e.* 6 column volumes, were necessary between runs to re-establish initial conditions, leading to 30 min run time per sample. Although more than 12 column volumes are usually recommended for HILIC re-equilibration in gradient elution, reproducible chromatographic runs were obtained with 6 column volumes equilibration as highlighted by RSD values of standards retention times (0.2 to 2.2 %,  $n = 10$ , Table S7). As illustrated in Fig. 3, run gradient separation with injection volume to 50  $\mu$ L, allowed to improve the separation between Gd-HP-DO3A and Gd-BT-DO3A whose resolution factor ( $R_s$ ) increased from 0.7 in isocratic mode to 1.1 with gradient mode. The low stability of cisplatin and oxaliplatin standard solutions was difficult to prevent and resulted in compounds elution in a plurality



**Fig. 3.** Typical chromatogram of the ten pharmaceutical standards mixture (full line) and inorganic standards (dotted line). Gradient elution: 0–0.5 min: 25 % B; 0.5 to 1.5 min: 25 %–50 %; 1.5 to 9 min: 50 % B; 9 to 10 min: 50–25 % B; 10 to 30 min: 25 % B. (A) = acetonitrile, (B) = 10 mmol L<sup>-1</sup> ammonium formate aqueous solution.  $V_{\text{INJECTION}} = 50 \mu\text{L}$ ; flow rate = 0.8 mL min<sup>-1</sup>. ICM (yellow trace): 1 = diatrizoate, 2 = iopamidol, 3 = iohexol; GBCA (green trace): 4 = Gd-BOPTA, 5 = Gd-DOTA, 6 = Gd-BT-DO3A, 7 = Gd-HP-DO3A; CPC (blue trace): 8 = cisplatin, 9 = oxaliplatin, 10 = carboplatin. Standard concentrations were 5  $\mu\text{g L}^{-1}$  I; 100 ng L<sup>-1</sup> Gd and 250 ng L<sup>-1</sup> Pt for drugs and 5  $\mu\text{g L}^{-1}$  I; 1000 ng L<sup>-1</sup> Gd and 1000 ng L<sup>-1</sup> Pt for inorganic forms. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

of chromatographic peaks. Freshly prepared standards solution showed 35 % and 45 % loss of signal intensity after 72 h. This can be explained by some speciation modification by exchange of  $\text{Cl}^-$  with  $\text{OH}^-$  and/or  $\text{H}_2\text{O}$  for cisplatin [39] and exchange of oxalate moiety with  $\text{H}_2\text{O}$  for oxaliplatin [40] leading to the formation of mono- and di-aqua intermediates. Aquation of cisplatin would be prevented at chloride concentrations higher than  $\sim 5.5 \text{ g L}^{-1}$  [16,41], concentration not suitable with ICP-MS detection.

The chromatographic behavior of inorganic forms of target elements was also checked to verify eventual co-elution with corresponding complexed forms (Fig. 3). Although eluting as two peaks at  $96 \pm 2$  and  $118 \pm 3 \text{ s}$ , inorganic platinum standard ( $\text{PtCl}_6^{2-}$ ) was clearly separated from CPC. Although iodate and gadolinium (III) eluted (at  $284 \pm 3 \text{ s}$  and  $425 \pm 4 \text{ s}$ , respectively) close to iopamidol and Gd-DOTA respectively, the differentiation between inorganic and complexed forms was still allowed although without baseline separation.

### 3.2. Analytical figures of merits

The limits of detection ( $\text{LOD} = 3\sigma/a$ ) and quantification ( $\text{LOQ} = 10\sigma/a$ ) reported in Table 2 were calculated with standard deviation ( $\sigma$ ) over the entire run (30 min) of mobile phase injections ( $n = 3$ ) and slopes ( $a$ ) of the external calibrations (with correlation coefficient  $R^2$  greater than 0.999). In these optimal conditions, LOD were between 46 and  $63 \text{ ng L}^{-1}$  for ICM, between 1 and  $3 \text{ ng L}^{-1}$  for GBCA and, between 3 and  $17 \text{ ng L}^{-1}$  for CPC (Table 2). These values are in the same order of magnitude as the ones previously reported for ICM [10–13], CPC [16,18,19] and, similar to those previously reported for GBCA [20,21,24,25,42–44]. The absolute LOD ( $\text{LOD}^A$ ), also reported in Table 2, were calculated by considering the injection volume of each method. Except for CPC, the  $\text{LOD}^A$  values obtained in this work remain in the average of values obtained in literature.

Column recovery rates were above 93 % (Table 2) for all pharmaceuticals except cisplatin and oxaliplatin for which recoveries were 64 % and 46 % respectively. These lower recovery rates can be explained by cisplatin and oxaliplatin aquation as discussed above. Calculated recoveries were above 95 % for inorganic I and Pt while inorganic Gd was strongly retained on the stationary phase (26 % recovery).

The developed method allowed thus for the first time the multi-

**Table 2**

Quantification (LOQ) and detection limits (LOD) given as I, Gd and Pt for the simultaneous analysis of I-, Gd- and Pt-based pharmaceuticals by PC HILIC-ICP-MS under gradient elution.

	Column recovery (%)	LOQ ( $\text{ng L}^{-1}$ )	LOD ( $\text{ng L}^{-1}$ ) ( $\text{LOD}^A$ (pg))		
			This study	Literature	Ref
Diatrizoate	$94 \pm 4$	191	57 (3)	3–242 (0.02–24)	[10–13]
Iopamidol	$96 \pm 1$	153	46 (2)	8–113 (0.04–11)	
Iohexol	$99 \pm 3$	211	63 (3)	19–733 (0.23–13)	
Gd-BOPTA	$100 \pm 1$	10	3	3–63 (0.15)	[20,42–44]
Gd-DOTA	$93 \pm 3$	3	1	2–48 (0.05)	[20,21,24,25,42–44]
Gd-BT-DO3A	$94 \pm 2$	7	2	1–62 (0.10)	
Gd-HP-DO3A	$93 \pm 1$	6	2	2–22 (0.10)	[25,42]
Cisplatin	$64 \pm 3$	47	14	11–58 (0.70)	[16,18,19]
Oxaliplatin	$46 \pm 5$	56	17	4–74 (0.85)	
Carboplatin	$93 \pm 3$	11	3	7–53 (0.15)	

elemental pharmaceuticals analysis with comparable sensitivity to that of previous methods focused on the separation of complexes of a single element.

### 3.3. Application to wastewaters analysis

In a first step, total elemental analysis was carried out for hospital and domestic wastewaters. Results indicated high total concentration of iodine (about  $400 \mu\text{g L}^{-1}$ ) in hospital effluent whereas it was *ca.* 10 times lower in domestic wastewater (Table 3). Fairly similar levels (*ca.*  $600 \text{ ng L}^{-1}$ ) of Gd were found in both wastewaters. Platinum total concentrations were at the low  $\text{ng L}^{-1}$  level in domestic effluent and *ca.* 5 times higher in hospital effluent.

For speciation analysis, the chromatography reproducibility was checked in spiked diluted wastewaters resulting in retention times similar to those obtained with standard solutions (Table S7). Representative chromatograms are shown in Fig. 4 for both wastewater samples. No CPC could be detected in both matrices which may be explained by the low total Pt concentrations determined and the poorly developed oncology department of this hospital. Two matching peaks were identified by standard additions in hospital effluent (Fig. 4a) corresponding to iopamidol and Gd-DOTA. A third additional matching signal corresponding to iohexol was identified in domestic wastewater (Fig. 4b). In both samples two other iodine-containing peaks were detected at the same retention times of  $\sim 113 \text{ s}$  and  $\sim 315 \text{ s}$  but didn't match with any of the three available ICM in our laboratory or with inorganic I standards. This may suggest the presence of other ICM or biotic/abiotic degradation products of ICM or other iodine containing compounds.

Quantification of pharmaceuticals was performed by both external calibration and standard additions. For unknown iodine-containing peaks, concentrations were estimated using the slopes obtained for iodate as these compounds did not match any of the available organic ICM. Determined concentrations are presented in Table 3. Both quantification methods gave similar results for detected compounds as the difference of their response slopes with external calibration and standard additions was 15 % or less (Table S7). A larger difference (20–23 %) between external calibration and standard additions in hospital wastewater slopes was however observed for Gd-HP-DO3A and Gd-BT-DO3A (not detected in samples) (Table S7). This indicates hospital wastewater matrix effects persisting even after ten-times dilution that could lead to underestimation of Gd-HP-DO3A and Gd-BT-DO3A if left unchecked. These matrix effects were not observed in the case of diluted urban wastewater.

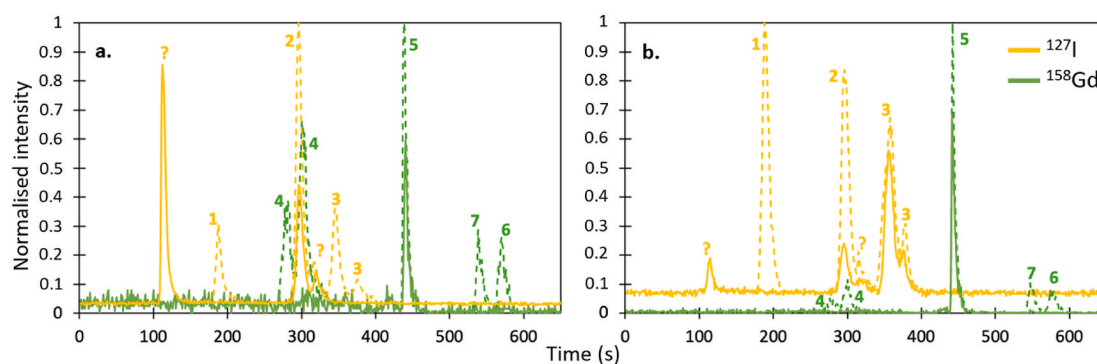
In hospital wastewater, measured concentration of iopamidol of  $134 \pm 5 \mu\text{g L}^{-1}$  I was between 2 and 10 times lower than the maximum concentrations reported for this compound in Europe [45,46]. Indeed, a high variability of ICM concentrations including iopamidol, up to a factor 100, is observed according to the daily number of consultations requiring the use of the compound [12,45]. The type of ICM used also influences these results. For example, in France, Sordet et al. (2018) did not detect iopamidol in hospital wastewater but iohexol at concentrations up to  $1766 \mu\text{g L}^{-1}$  I [12]. In our study, unknown iodine compounds accounted for more than 60 % of the total iodine content in hospital wastewater. Given their major proportion and previously cited literature results, it is reasonable to assume that the unknown iodine compounds probably derived from ICMs other than those targeted in this work or from degradation products. Only few studies have quantified GBCA in hospital wastewater. Künnemeyer et al. (2009) did not detect Gd-DOTA but reported a concentration of Gd-BT-DO3A of  $3.8 \mu\text{g L}^{-1}$  Gd, *i.e.* 7 times higher than the one of Gd-DOTA measured in this work [22]. As with ICM, it remains difficult to compare these results due to the variable daily use of the different complexes.

In urban wastewater, iopamidol and iohexol were measured at concentrations of  $4.5 \pm 0.3$  and  $27.5 \pm 0.5 \mu\text{g L}^{-1}$  I respectively. In the literature, various studies on ICM were carried out in WWTP with

**Table 3**

Total elemental and detected pharmaceuticals concentrations (in  $\mu\text{g L}^{-1}$  I and  $\text{ng L}^{-1}$  Gd/Pt) in hospital and urban wastewaters. Species quantification with external calibration and standard additions (shaded grey line).

Wastewater	[I] <sub>total</sub>	Iohexol	Iopamidol	Unknown-I	% $\Sigma\text{ICM}/I_{\text{tot}}$
Hospital	399 ± 38	< LOD	134 ± 5	267 ± 6	34 ± 3
		< LOD	153 ± 9		38 ± 4
Urban	45 ± 5	27.5 ± 0.5	4.5 ± 0.3	6.4 ± 0.5	78 ± 8
		32 ± 2	7.6 ± 0.5		87 ± 10
	[Gd] <sub>total</sub>	Gd-DOTA	% $\Sigma\text{GBCA}/Gd_{\text{tot}}$		
Hospital	665 ± 37	525 ± 36	79 ± 7		
		589 ± 43	89 ± 8		
Urban	578 ± 32	677 ± 20	117 ± 7		
		658 ± 21	114 ± 7		
	[Pt] <sub>total</sub>				
Hospital	33 ± 7				
Urban	6 ± 3				



**Fig. 4.** PC HILIC-ICPMS chromatograms (under optimized gradient elution) of hospital (a.) and domestic (b.) wastewaters. Dotted lines correspond to standard additions of respective drugs: ICM: 1 = diatrizoate, 2 = iopamidol, 3 = iohexol; GBGA: 4 = Gd-BOPTA, 5 = Gd-DOTA, 6 = Gd-BT-DO3A, 7 = Gd-HP-DO3A.

capacities 10 to 20 times higher than the one studied in this work [11, 15,47–49]. These compounds were found at similar concentrations, ranging from 0.7 to 10.3  $\mu\text{g L}^{-1}$ . Unknown iodine compounds accounted for almost 14 % of the total iodine concentration. While this is rarely described or observed in most studies, Kormos et al., were able to identify and quantify up to six biotransformation products of iopromide, iomeprol and iohexol in urban wastewaters, representing between 1 and 11 % of total iodine concentration [15]. Among the GBGA, Gd-DOTA was quantified at  $677 \pm 20 \text{ ng L}^{-1}$  Gd, i.e. almost equally as in hospital wastewater. Some of the reported concentrations in the literature for this compound are in the same range (214–456  $\text{ng L}^{-1}$  Gd [42,43]), while mostly measured levels are 10 to 100 times lower (7–100  $\text{ng L}^{-1}$  Gd [20–22,24,25,33,43,44]). Regarding the studied WWTP capacity, categorized as a large-scale facility, and knowing that it is not connected to any health system, our results underline the contribution of at home individual emissions as diffuse sources of contrast agents. Due to high rainfalls during the sampling period, the WWTP flow rate was twice the usual level (3200  $\text{m}^3 \text{ day}^{-1}$  instead of 1500  $\text{m}^3 \text{ day}^{-1}$ ) that may have probably contributed to a dilution of expected concentrations at usual capacity.

The comparison of total element concentrations with the sum of pharmaceutical concentrations (Table 3) showed good agreement, between 79 and 117 %, for Gd for which only Gd-DOTA was detected confirming that the method is accurate enough to quantify this pharmaceutical in such complex matrices. The lower recovery of 79 % obtained in hospital wastewater is similar to the one reported by

Künnemeyer et al. (2009) of 81 %. It suggests that detected Gd-DOTA did not represent total Gd concentration, the presence of free gadolinium retained on the column (with poor recovery rate) may not be excluded.

#### 4. Conclusion

This study presents the development of an analytical method for the simultaneous speciation of iodine-, platinum- and gadolinium-based pharmaceuticals at ultratrace levels in environmental waters. For the first time the separation of ten complexes of these elements was achieved with HILIC chromatography and their detection was obtained using ICP-MS. Zwitterionic columns experimented proved their chromatographic efficiency, the phosphorylcholine based stationary phase allowing the separation of the ten pharmaceuticals. Furthermore, DFT computations were performed to examine the correlation between permanent dipole moments, used as a measure of molecular polarity, and observed retention times. The results showed that, in general, higher dipole moments correlate with stronger interactions with the hydrophilic stationary phase. However, structural features such as hydrophobic groups can further influence retention independently. The use of an elution gradient was necessary to find the best compromise between separation and sensitivity. The limits of detection were between 46 and 63  $\text{ng L}^{-1}$  I for ICM, 1 and 3  $\text{ng L}^{-1}$  Gd for GBGA and, 3 and 17  $\text{ng L}^{-1}$  Pt for CPC. The developed method was applied to complex aqueous samples of hospital and urban wastewaters. Two pharmaceuticals were

identified into hospital effluent, iopamidol at over a hundred  $\mu\text{g L}^{-1}$  I and Gd-DOTA at several hundred  $\text{ng L}^{-1}$  Gd. Few studies have measured the concentration of GBCA in hospital wastewater, and overall, comparison of the results for these samples remains difficult due to the high variability of ICM and GBCA concentrations observed in these waters, directly related to the daily patient load requiring the use of these compounds. In urban wastewater, two ICM were identified, iopamidol and iohexol, at lower levels than into hospital wastewater, as expected from literature results quantifying these compounds from a few  $\mu\text{g L}^{-1}$  I to more than  $10 \mu\text{g L}^{-1}$  I. As in hospital wastewater, Gd-DOTA is the only GBCA identified and quantified in urban wastewater, at a similar level of over  $600 \text{ ng L}^{-1}$  Gd as reported by various studies and revealing the importance of the contribution of individual emissions through the global flow of these compounds. Two additional iodine-containing peaks corresponding to any available pharmaceuticals or other iodine compounds were detected into both water matrices. No CPC were detected in any of these wastewaters due to the low total platinum concentration, the analysis of wastewater from a hospital with a more important oncology department should allow to detect some of these compounds. Finally, time series monitoring of the concentrations of these compounds in the various wastewaters and sites appears to be the next step and is of particular interest in order to gain a better understanding of their respective concentration levels, as well as to potentially identify new compounds.

#### CRedit authorship contribution statement

**Joseph Côme:** Writing – original draft, Methodology, Investigation, Formal analysis. **Maïté Bueno:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Panaghiotis Karamanis:** Writing – review & editing, Methodology, Investigation. **Florence Pannier:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Sandra Mounicou:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.talanta.2025.128974>.

#### Data availability

Data will be made available on request.

#### References

- [1] K.D. Mjos, C. Orvig, Metalloids in medicinal inorganic chemistry, *Chem. Rev.* 114 (2014) 4540–4563, <https://doi.org/10.1021/cr400460s>.
- [2] Health Care Use - Computed Tomography (CT) Exams - OECD Data, theOECD (n. d.). <http://data.oecd.org/healthcare/computed-tomography-ct-exams.htm> (accessed May 13, 2024).
- [3] Health care use - magnetic resonance imaging (MRI) exams - OECD data, theOECD (n. d.). <http://data.oecd.org/healthcare/magnetic-resonance-imaging-mri-exams.htm> (accessed May 13, 2024).
- [4] Incidence des principaux cancers en France métropolitaine en 2023 et tendances depuis 1990, *Bulletin Épidémiologique Hebdomadaire*, 2023, pp. 188–204.
- [5] J. Margot, C. Kienle, A. Magnet, M. Weil, L. Rossi, L.F. de Alencastro, C. Abegglen, D. Thonney, N. Chèvre, M. Schärer, D.A. Barry, Treatment of micropollutants in municipal wastewater: ozone or powdered activated carbon? *Sci. Total Environ.* 461–462 (2013) 480–498, <https://doi.org/10.1016/j.scitotenv.2013.05.034>.
- [6] J. Zhang, V.W.C. Chang, A. Giannis, J.-Y. Wang, Removal of cytostatic drugs from aquatic environment: a review, *Sci. Total Environ.* 445–446 (2013) 281–298, <https://doi.org/10.1016/j.scitotenv.2012.12.061>.
- [7] Y. Roque-Diaz, M. Sanadar, D. Han, M. López-Mesas, M. Valiente, M. Tolazzi, A. Melchior, D. Veciani, The dark side of platinum based cytostatic drugs: from detection to removal, *Processes* 9 (2021) 1873, <https://doi.org/10.3390/pr9111873>.
- [8] M.G. Lawrence, J. Keller, Y. Poussade, Removal of magnetic resonance imaging contrast agents through advanced water treatment plants, *Water Sci. Technol.* 61 (2010) 685–692, <https://doi.org/10.2166/wst.2010.885>.
- [9] S. Pérez, D. Barceló, Fate and occurrence of X-ray contrast media in the environment, *Anal. Bioanal. Chem.* 387 (2007) 1235–1246, <https://doi.org/10.1007/s00216-006-0953-9>.
- [10] F. Busetti, K.L. Linge, J.W. Blythe, A. Heitz, Rapid analysis of iodinated X-ray contrast media in secondary and tertiary treated wastewater by direct injection liquid chromatography-tandem mass spectrometry, *J. Chromatogr. A* 1213 (2008) 200–208, <https://doi.org/10.1016/j.chroma.2008.10.021>.
- [11] S. Echeverría, F. Borrull, N. Fontanals, E. Pocurull, Determination of iodinated X-ray contrast media in sewage by solid-phase extraction and liquid chromatography tandem mass spectrometry, *Talanta* 116 (2013) 931–936, <https://doi.org/10.1016/j.talanta.2013.07.080>.
- [12] M. Sordet, A. Buleté, E. Vuilliet, A rapid and easy method based on hydrophilic interaction chromatography coupled with tandem mass spectrometry (HILIC-MS/MS) to quantify iodinated X-ray contrast in wastewaters, *Talanta* 190 (2018) 480–486, <https://doi.org/10.1016/j.talanta.2018.08.006>.
- [13] K. Ribbers, L. Breuer, R.-A. Düring, Detection of artificial sweeteners and iodinated X-ray contrast media in wastewater via LC-MS/MS and their potential use as anthropogenic tracers in flowing waters, *Chemosphere* 218 (2019) 189–196, <https://doi.org/10.1016/j.chemosphere.2018.10.193>.
- [14] R. Hirsch, T.A. Ternes, A. Lindart, K. Haberer, R.-D. Wilken, A sensitive method for the determination of iodine containing diagnostic agents in aqueous matrices using LC-electrospray-tandem-MS detection, *Fresenius J. Anal. Chem.* 366 (2000) 835–841, <https://doi.org/10.1007/s002160051581>.
- [15] J.L. Kormos, M. Schulz, T.A. Ternes, Occurrence of iodinated X-ray contrast media and their biotransformation products in the urban water cycle, *Environ. Sci. Technol.* 45 (2011) 8723–8732, <https://doi.org/10.1021/es2018187>.
- [16] S. Hann, Zs Stefánka, K. Lenz, G. Stingeder, Novel separation method for highly sensitive speciation of cancerostatic platinum compounds by HPLC/ICP-MS, *Anal. Bioanal. Chem.* 381 (2005) 405–412, <https://doi.org/10.1007/s00216-004-2839-z>.
- [17] N. Vyas, A. Turner, G. Sewell, Platinum-based anticancer drugs in waste waters of a major UK hospital and predicted concentrations in recipient surface waters, *Sci. Total Environ.* 493 (2014) 324–329, <https://doi.org/10.1016/j.scitotenv.2014.05.127>.
- [18] J. Vidmar, A. Martincić, R. Milačić, J. Ščančar, Speciation of cisplatin in environmental water samples by hydrophilic interaction liquid chromatography coupled to inductively coupled plasma mass spectrometry, *Talanta* 138 (2015) 1–7, <https://doi.org/10.1016/j.talanta.2015.02.008>.
- [19] Y. Ghafari, M. Yunesian, R. Nabizadeh, A. Mesdaghinia, M.H. Dehghani, M. Alimohammadi, Platinum cytotoxic drugs in the municipal wastewater and drinking water, a validation method and health risk assessment, *Hum. Ecol. Risk Assess.* 24 (2017) 1–13, <https://doi.org/10.1080/10807039.2017.1400372>.
- [20] C.S.K. Raju, A. Cossmer, H. Scharf, U. Panne, D. Lück, Speciation of gadolinium based MRI contrast agents in environmental water samples using hydrophilic interaction chromatography hyphenated with inductively coupled plasma mass spectrometry, *J. Anal. At. Spectrom.* 25 (2009) 55–61, <https://doi.org/10.1039/B919959D>.
- [21] M. Birka, C.A. Wehe, O. Hachmöller, M. Sperling, U. Karst, Tracing gadolinium-based contrast agents from surface water to drinking water by means of speciation analysis, *J. Chromatogr. A* 1440 (2016) 105–111, <https://doi.org/10.1016/j.chroma.2016.02.050>.
- [22] J. Künnemeyer, L. Terborg, B. Meermann, C. Brauckmann, I. Möller, A. Scheffer, U. Karst, Speciation analysis of gadolinium chelates in hospital effluents and wastewater treatment plant sewage by a novel HILIC/ICP-MS method, *Environ. Sci. Technol.* 43 (2009) 2884–2890, <https://doi.org/10.1021/es803278n>.
- [23] L. Telgmann, C.A. Wehe, M. Birka, J. Künnemeyer, S. Nowak, M. Sperling, U. Karst, Speciation and isotope dilution analysis of gadolinium-based contrast agents in wastewater, *Environ. Sci. Technol.* 46 (2012) 11929–11936, <https://doi.org/10.1021/es301981z>.
- [24] M. Horstmann, R. Gonzalez de Vega, David P. Bishop, U. Karst, P.A. Doble, D. Clases, Determination of gadolinium MRI contrast agents in fresh and oceanic waters of Australia employing micro-solid phase extraction, HILIC-ICP-MS and bandpass mass filtering, *J. Anal. At. Spectrom.* 36 (2021) 767–775, <https://doi.org/10.1039/D0JA00493F>.
- [25] S. Okabayashi, L. Kawane, N.Y. Mrabawani, T. Iwai, T. Narukawa, M. Tsuboi, K. Chiba, Speciation analysis of Gadolinium-based contrast agents using aqueous eluent-hydrophilic interaction liquid chromatography hyphenated with inductively

- coupled plasma-mass spectrometry, *Talanta* 222 (2021) 121531, <https://doi.org/10.1016/j.talanta.2020.121531>.
- [26] B. Buszewski, S. Noga, Hydrophilic interaction liquid chromatography (HILIC)—A powerful separation technique, *Anal. Bioanal. Chem.* 402 (2012) 231–247, <https://doi.org/10.1007/s00216-011-5308-5>.
- [27] T. Alvarez-Segura, X. Subirats, M. Rosés, Retention-pH profiles of acids and bases in hydrophilic interaction liquid chromatography, *Anal. Chim. Acta* 1050 (2019) 176–184, <https://doi.org/10.1016/j.aca.2018.11.021>.
- [28] P. Jandera, Stationary and mobile phases in hydrophilic interaction chromatography: a review, *Anal. Chim. Acta* 692 (2011) 1–25, <https://doi.org/10.1016/j.aca.2011.02.047>.
- [29] G. Greco, T. Letzel, Main interactions and influences of the chromatographic parameters in HILIC separations, *J. Chromatogr. Sci.* 51 (2013) 684–693, <https://doi.org/10.1093/chromsci/bmt015>.
- [30] C. Adamo, V. Barone, Toward reliable density functional methods without adjustable parameters: the PBE0 model, *J. Chem. Phys.* 110 (1999) 6158–6170, <https://doi.org/10.1063/1.478522>.
- [31] F. Weigend, R. Ahlrichs, Balanced basis sets of split valence, triple zeta valence and quadruple zeta valence quality for H to Rn: design and assessment of accuracy, *Phys. Chem. Chem. Phys.* 7 (2005) 3297–3305, <https://doi.org/10.1039/B508541A>.
- [32] V. Barone, M. Cossi, Quantum calculation of molecular energies and energy gradients in solution by a conductor solvent model, *J. Phys. Chem. A* 102 (1998) 1995–2001, <https://doi.org/10.1021/jp9716997>.
- [33] M. Birka, C.A. Wehe, L. Telgmann, M. Sperling, U. Karst, Sensitive quantification of gadolinium-based magnetic resonance imaging contrast agents in surface waters using hydrophilic interaction liquid chromatography and inductively coupled plasma sector field mass spectrometry, *J. Chromatogr. A* 1308 (2013) 125–131, <https://doi.org/10.1016/j.chroma.2013.08.017>.
- [34] C. West, E. Auroux, Deconvoluting the effects of buffer salt concentration in hydrophilic interaction chromatography on a zwitterionic stationary phase, *J. Chromatogr. A* 1461 (2016) 92–97, <https://doi.org/10.1016/j.chroma.2016.07.059>.
- [35] F. Uggeri, S. Aime, P.L. Anelli, M. Botta, M. Brocchetta, C. de Haeen, G. Ermondi, M. Grandi, P. Paoli, Novel contrast agents for magnetic resonance imaging. Synthesis and characterization of the ligand BOPTA and its Ln(III) complexes (Ln = Gd, La, Lu). X-ray structure of disodium (TPS-9-145337286-C-S)-[4-Carboxy-5,8,11-tris(carboxymethyl)-1-phenyl-2-oxa-5,8,11-triazatridecan-13-oato(5-)] gadolinate(2-) in a mixture with its enantiomer, *Inorg. Chem.* 34 (1995) 633–643, <https://doi.org/10.1021/ic00107a017>.
- [36] S. Laurent, C. Henoumont, D. Stanicki, S. Boutry, E. Lipani, S. Belaid, R.N. Muller, L. Vander Elst, Paramagnetic gadolinium complexes, in: *MRI Contrast Agents*, Springer, Singapore, 2017, pp. 23–53, [https://doi.org/10.1007/978-981-10-2529-7\\_4](https://doi.org/10.1007/978-981-10-2529-7_4).
- [37] J. Dunn, Y. Lin, D. Miller, M. Rogic, W. Neumann, S. Woulfe, D. White, Stereoisomerism in contrast media isoversol, *Investig. Radiol.* 25 (1990) S102.
- [38] D.V. McCalley, Managing the column equilibration time in hydrophilic interaction chromatography, *J. Chromatogr. A* 1612 (2020) 460655, <https://doi.org/10.1016/j.chroma.2019.460655>.
- [39] O.B. Belskaya, Transformations of a metal complex precursor of the active component in the preparation of Pt/Al<sub>2</sub>O<sub>3</sub> catalysts, *Chem. Sustain. Dev.* 19 (2011) 33–43.
- [40] D.M. Kweekel, H. Gelderblom, H.-J. Guchelaar, Pharmacology of oxaliplatin and the use of pharmacogenomics to individualize therapy, *Cancer Treat. Rev.* 31 (2005) 90–105, <https://doi.org/10.1016/j.ctrv.2004.12.006>.
- [41] R.F. Greene, D.C. Chatterji, P.K. Hiranaka, J.F. Gallelli, Stability of cisplatin in aqueous solution, *Am. J. Hosp. Pharm.* 36 (1979) 38–43, <https://doi.org/10.1093/ajhp/36.1.38>.
- [42] M. Macke, C.D. Quarles, M. Sperling, U. Karst, Fast and automated monitoring of gadolinium-based contrast agents in surface waters, *Water Res.* 207 (2021) 117836, <https://doi.org/10.1016/j.watres.2021.117836>.
- [43] U. Lindner, J. Lingott, S. Richter, N. Jakubowski, U. Panne, Speciation of gadolinium in surface water samples and plants by hydrophilic interaction chromatography hyphenated with inductively coupled plasma mass spectrometry, *Anal. Bioanal. Chem.* 405 (2013) 1865–1873, <https://doi.org/10.1007/s00216-012-6643-x>.
- [44] U. Lindner, J. Lingott, S. Richter, W. Jiang, N. Jakubowski, U. Panne, Analysis of Gadolinium-based contrast agents in tap water with a new hydrophilic interaction chromatography (ZIC-cHILIC) hyphenated with inductively coupled plasma mass spectrometry, *Anal. Bioanal. Chem.* 407 (2015) 2415–2422, <https://doi.org/10.1007/s00216-014-8368-5>.
- [45] D. Weissbrodt, L. Kovalova, C. Ort, V. Pazhepurackel, R. Moser, J. Hollender, H. Siegrist, C.S. McArdell, Mass flows of X-ray contrast media and cytostatics in hospital wastewater, *Environ. Sci. Technol.* 43 (2009) 4810–4817, <https://doi.org/10.1021/es8036725>.
- [46] L. Kovalova, H. Siegrist, H. Singer, A. Wittmer, C.S. McArdell, Hospital wastewater treatment by membrane bioreactor: performance and efficiency for organic micropollutant elimination, *Environ. Sci. Technol.* 46 (2012) 1536–1545, <https://doi.org/10.1021/es203495d>.
- [47] T.A. Ternes, R. Hirsch, Occurrence and behavior of X-ray contrast media in sewage facilities and the aquatic environment, *Environ. Sci. Technol.* 34 (2000) 2741–2748, <https://doi.org/10.1021/es991118m>.
- [48] T.A. Ternes, M. Bonerz, N. Herrmann, B. Teiser, H.R. Andersen, Irrigation of treated wastewater in Braunschweig, Germany: an option to remove pharmaceuticals and musk fragrances, *Chemosphere* 66 (2007) 894–904, <https://doi.org/10.1016/j.chemosphere.2006.06.035>.
- [49] W. Seitz, R. Winzenbacher, A survey on trace organic chemicals in a German water protection area and the proposal of relevant indicators for anthropogenic influences, *Environ. Monit. Assess.* 189 (2017), <https://doi.org/10.1007/s10661-017-5953-z>.