Determination of Iodinated Contrast Media in Aqueous Samples by Direct-Injection LC-MS/MS

Application Note

Environmental

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Abstract

Determination of iodinated contrast media in aqueous samples was performed by fast chromatography on an Agilent 1290 Infinity II LC that included an Agilent 1290 Infinity II Multisampler equipped with a 100 µL analytical head and loop cartridge. Detection was done with an Agilent 6495 Triple Quadrupole LC/MS. With this configuration, it was possible to detect the contrast agents at the low ng/L parts per trillion (ppt) level in water without any pretreatment except filtration. The method was developed for seven target contrast agents, and the performance and figures of merit were investigated on standard solutions as well as on various water samples.

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Introduction

Iodinated contrast media (ICMs) are globally used for medical X-ray imaging. These iodine-based chemicals are administered intravascularly, and absorb X-rays in vessels and tissues, enhancing the visibility of internal structures. Several ICMs are available. This class of pharmaceuticals is one of the most frequently used groups of chemicals applied in hospitals, and generally at relatively high doses[1].

Significant levels can be found in various aqueous matrices (hospital wastewater[1-3], surface water[2], ground water[4,5], and even drinking water[5]), and soil[6]. Studies have demonstrated that complete ICM removal in wastewater treatment plants is difficult or even impossible with state-of-the-art water treatment methods such as ozonation, UV irradiation, chlorination, microbial degradation, and so on[1,2]. Consequently, they are frequently detected at significant levels in wastewater treatment plant effluents. Moreover, potentially toxic iodine derivatives can be formed and released into the environment upon treatment[7].

The analysis of ICMs in the environment has gained considerable interest over the last decade. Earlier approaches measured the total absorbable organic iodine (AOI), whereas recent methods focus on the sensitive detection of individual ICMs and potential degradation products by LC-MS and LC-MS/MS[1-4,8]. These polar and sometimes acidic compounds can suffer from bad peak shapes in LC, and the chromatographic parameters need to be carefully chosen. To meet low ng/L (part per trillion, ppt) detection limits, an extraction step such as solid-phase extraction (SPE) is commonly performed[1,9].

This Application Note describes the use of the Agilent 6495 Triple Quadrupole LC/MS in combination with the Agilent 1290 Infinity II LC for the direct analysis of selected ICMs (Figure 1) in water samples at low ng/L (ppt) levels. Direct injection of water samples without any pretreatment except filtration was applied. Relatively large volumes need to be injected, requiring adequate control of the separation conditions. State-of-the-art mass spectrometry offers high sensitivity, allowing direct analysis of trace contaminants in water. This reduces time and cost per analysis, and minimizes errors that can be made during sample pretreatment.

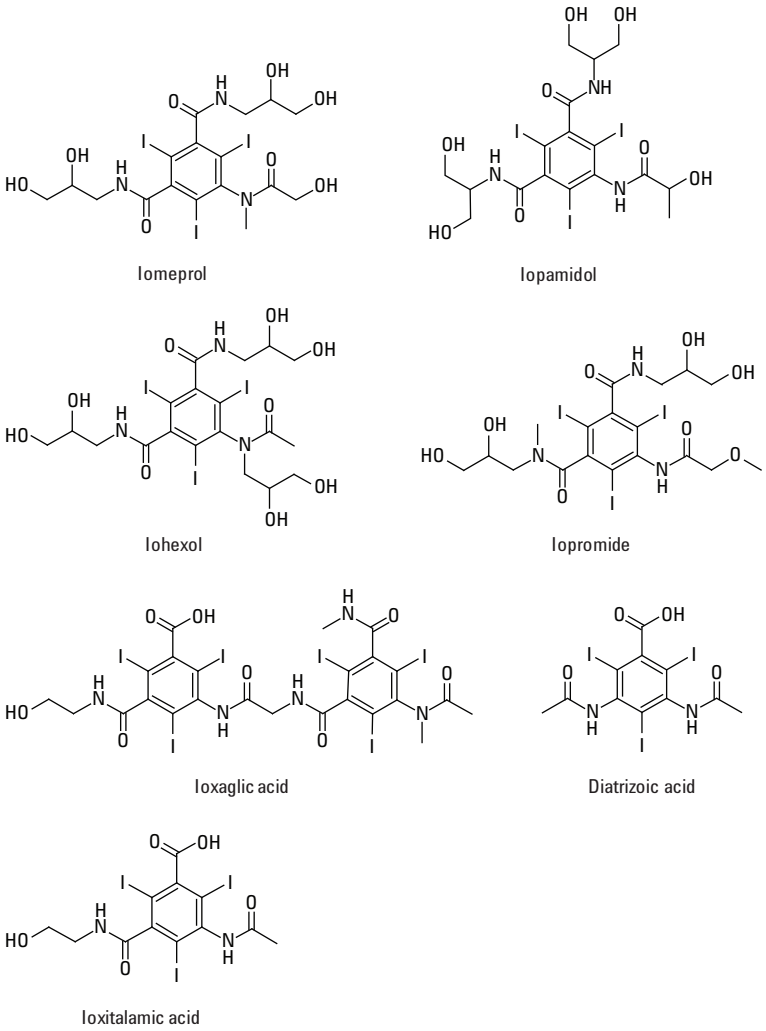

Figure 1. Structures of the studied ICMs.

Experimental

Instrumentation

An Agilent 1290 Infinity II LC and an Agilent 6495 Triple Quadrupole MS with an Agilent Jet Stream technology source were used. The 1290 Infinity II LC was configured as follows:

- Agilent 1290 Infinity II High Speed Pump (G7120A) with a 35 µL Jet Weaver mixer
- Agilent 1290 Infinity II Multisampler (G7167B) with analytical head 100 µL (G4267-60043) and sample loop-flex 100 µL (G4267-60500)
- Agilent 1290 Infinity II Multicolumn Thermostat (G7116B) with an Agilent InfinityLab Quick Connect heat exchanger assembly for standard flow (G7116-60015), InfinityLab Quick Connect fittings 0.12 × 105 mm (5067-5957), and an Agilent ZORBAX Eclipse Plus C18 RRHD, 2.1 × 150 mm, 1.8 µm column (959759-902)

Method parameters

Parameter	Description
Mobile phase	A) 0.1 % (v/v) acetic acid in water B) Acetonitrile
Flow rate	0.4 mL/min
Gradient	0 to 1 minutes – 0 %B 1 to 5 minutes – 0 to 73 %B 5 to 5.5 minutes – 73 to 95 %B 5.5 to 7 minutes – 95 %B
Post time	3 minutes at 0 %B
Temperature	15 °C
Injection	100 µL, with needle wash (flush port, 5 seconds, water/methanol 1/1)

Detection parameters

Parameter	Description
Mode	MS/MS
Ionization	Electrospray, positive ionization
Drying gas temperature	220 °C
Drying gas flow	13 L/min
Nebulizer pressure	40 psig
Sheath gas temperature	390 °C
Sheath gas flow	11 L/min
Capillary voltage	3,500 V
Nozzle voltage	1,600 V
Dynamic MRM	See Table 1
Delta EMV	200
Cycle time	200 ms 0 to 3 minutes – Waste 3 to 5 minutes – MS 5 minutes to end – Waste

Table 1 shows the acquisition parameters for the selected ICMs.

Solutions and samples

The ICMs were purchased from LGC Standards SARL (Molsheim, France), and deuterated internal standards were provided by an Agilent customer, but can be ordered, for example, from Santa Cruz Biotechnology, Inc., Dallas, Texas.

Separate stock solutions of the standards and internal standards were prepared in methanol/water 50/50 v/v at 5 and 1 mg/L (ppm), respectively. These solutions were diluted to 100 µg/L (ppb) working solutions in methanol/water 10/90 v/v. All subsequent dilutions were made in deionized HPLC water.

Water samples were stored at 4 °C. After removal from the refrigerator, they were allowed to reach room temperature, then agitated. An aliquot was taken, and an internal standard was added. For the spiking experiments, a stock solution of the ICMs was added. A portion of the water sample was filtered through a syringe filter (Captiva Premium Syringe Filter Regenerated Cellulose, 25 mm, 0.45 µm, Agilent Technologies, p/n 5190-5111) into an autosampler vial.

Table 1. Dynamic MRM acquisition parameters for the compounds under investigation. The first transition is the quantifier transition.

Compound	Precursor ion (*m/z*)	MS1 Resolution	Product ion (*m/z*)	MS2 Resolution	RT (min)	RT Window (min)	Collision energy (V)	Cell accelerator voltage
Iopamidol	777.9	Unit	541.9	Wide	3.73	1	32	3.5
	777.9	Unit	558.9	Wide	3.73	1	24	3.5
	777.9	Unit	386.9	Wide	3.73	1	42	1
Ioxitalamic acid	662.0	Unit	645.0	Wide	3.76	1	5	3
	662.0	Unit	429.0	Wide	3.76	1	33	3
	662.0	Unit	302.0	Wide	3.76	1	50	3
Iohexol	821.9	Unit	803.9	Wide	3.80	1	24	1.5
	821.9	Unit	656.9	Wide	3.80	1	30	1
	821.9	Unit	375.0	Wide	3.80	1	52	6
Iomeprol	777.8	Unit	404.8	Wide	3.73	1	44	3.5
	777.8	Unit	558.7	Wide	3.73	1	28	3.5
	777.8	Unit	532.0	Wide	3.73	1	22	3.5
Diatrizoic acid	614.8	Unit	360.4	Wide	3.88	1	25	1
	614.9	Unit	233.0	Wide	3.88	1	47	3
Iopromide	792.0	Unit	573.0	Wide	4.04	1	28	2
	792.0	Unit	558.9	Wide	4.04	1	28	2
Ioxaglic acid	1269.6	Wide	610.8	Wide	4.51	1	40	2
	1269.6	Wide	455.9	Wide	4.51	1	60	5.5
Internal standards								
Iopamidol-D8	786.0	Unit	391.0	Wide	3.73	1	42	1
	786.0	Unit	546.0	Wide	3.73	1	32	3.5
Iohexol-D5	826.9	Unit	809.0	Wide	3.80	1	20	1.5
Iomeprol-D3	781.0	Unit	562.0	Wide	3.86	1	22	3.5
	781.0	Unit	535.0	Wide	3.86	1	22	3.5
Iopromide-D3	795.1	Unit	576.0	Wide	4.03	1	28	2
	795.1	Unit	299.9	Wide	4.03	1	28	2

Results and Discussion

The selected ICMs are highly polar, and the analytical conditions had to be carefully chosen to obtain adequate retention, acceptable peak shape, and good efficiency. Moreover, care had to be taken so that the sensitivity of the mass spectrometer was not affected by the choice of the mobile phase. All LC parameters were carefully optimized, and reversed-phase LC with a mobile phase of acetic acid in water and acetonitrile proved to give the best overall results in terms of chromatography and detection. The column was cooled to 15 °C to increase retention. Figure 2 shows the results for the quantifier transitions for a 100 µL injection of a standard solution containing 200 ng/L (A) and 20 ng/L (B) of ICMs. Note that the isomers iopamidol and iomeprol are nicely separated. The total analysis time including reconditioning was 10 minutes.

Method precision and linearity were evaluated with injections of standard solutions in deionized water. Five replicate injections of 20 and 200 ppt standard solutions were carried out to determine the repeatability of injection. Single injections of standard solutions between 1 to 1,000 ppt were done to assess linearity and sensitivity. Calibration was performed on each ICM between the limit of detection (LOD) and 1,000 ppt (9 to 12 levels for each compound). Table 2 summarizes the results.

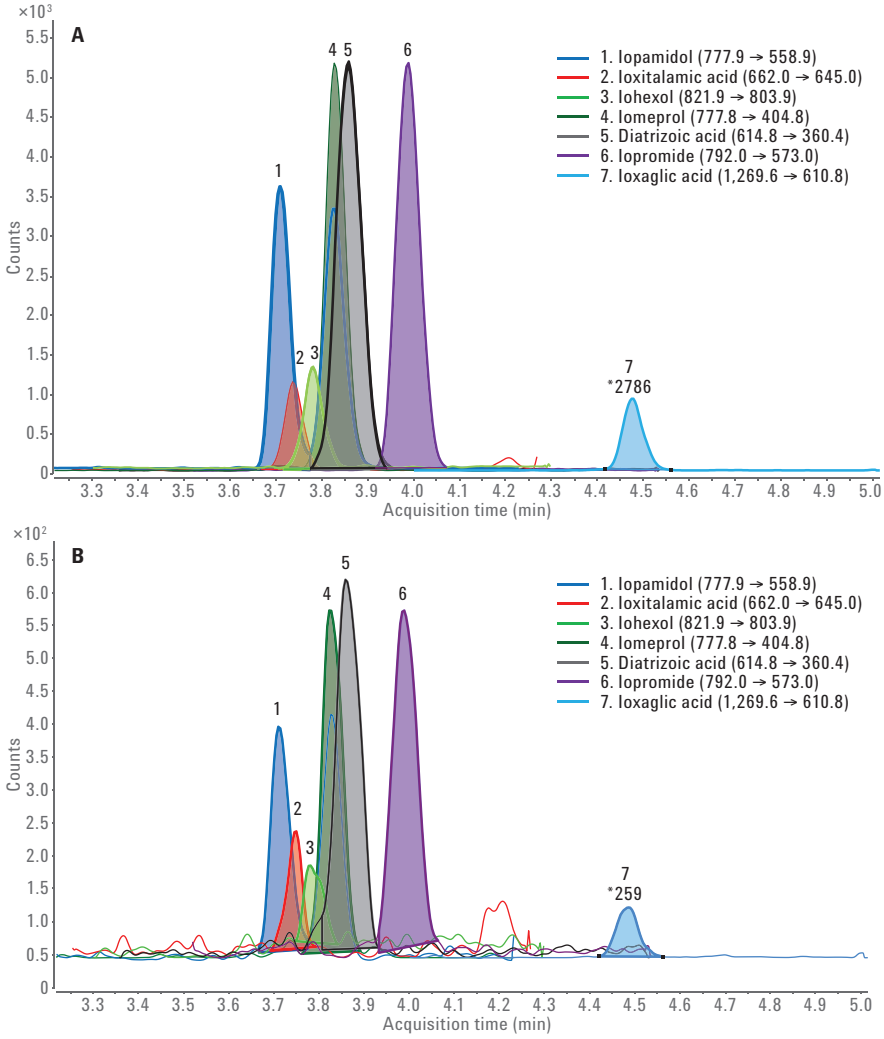

Figure 2. Data obtained for a 100 µL direct injection of a 200 (A) and 20 (B) ng/L (ppt) standard solution in water. EIC of the quantifier transitions of the nonlabeled ICMs.

Table 2. Method performance parameters.

			20 ppt		200 ppt			
Compound	ISTD	RT	Conc (ppt)	RSD % (n = 5)	Conc (ppt)	RSD % (n = 5)	R^2 (10–1,000 ppt)*	LOD (ppt)
Iopamidol	Iopamidol-D8	3.70	19.69	8.72	196.61	6.26	0.9986	2
Ioxitalamic acid	Iopamidol-D8	3.73	19.32	8.10	192.21	3.02	0.9962	10
Iohexol	Iohexol-D5	3.77	20.11	6.74	199.41	7.97	0.9983	5
Iomeprol	Iomeprol-D3	3.82	19.06	4.43	190.34	1.43	0.9995	1
Diatrizoic acid	Iomeprol-D3	3.85	18.86	5.35	190.56	5.73	0.9963	2
Iopromide	Iopromide-D3	3.98	20.80	4.34	189.91	5.78	0.9968	2
Ioxaglic acid	Iopromide-D3	4.47	17.47	9.12	184.59	7.25	0.9970	2

*Calibration curve type: linear, ignore origin, weighting 1/x

A selection of water samples (tap water, surface water, and wastewater treatment plant effluent) was analyzed to test the usefulness of the developed method on different matrices. The result for the various samples are shown in Figure 3 (tap water), Figure 4 (surface water), and Figure 5 (wastewater treatment plant effluent).

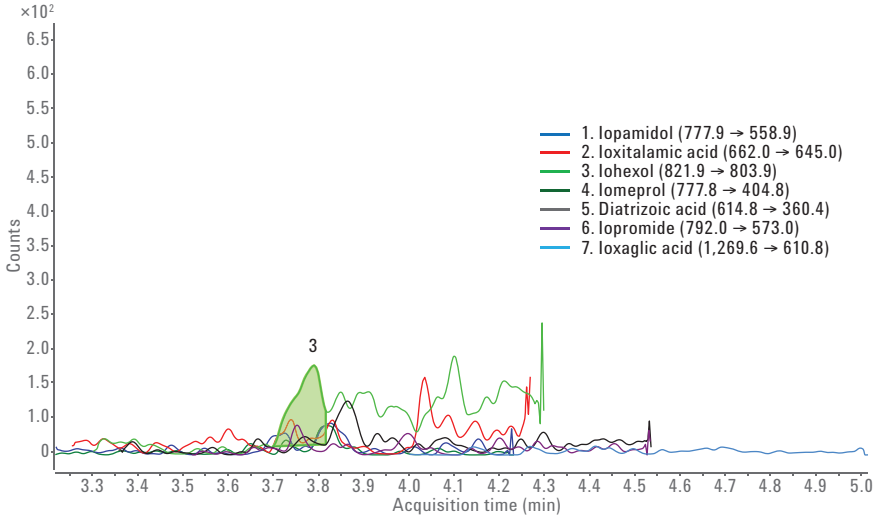

Figure 3. Result for a 100-µL injection of tap water. EIC of only the quantifier transitions of the nonlabeled ICMs.

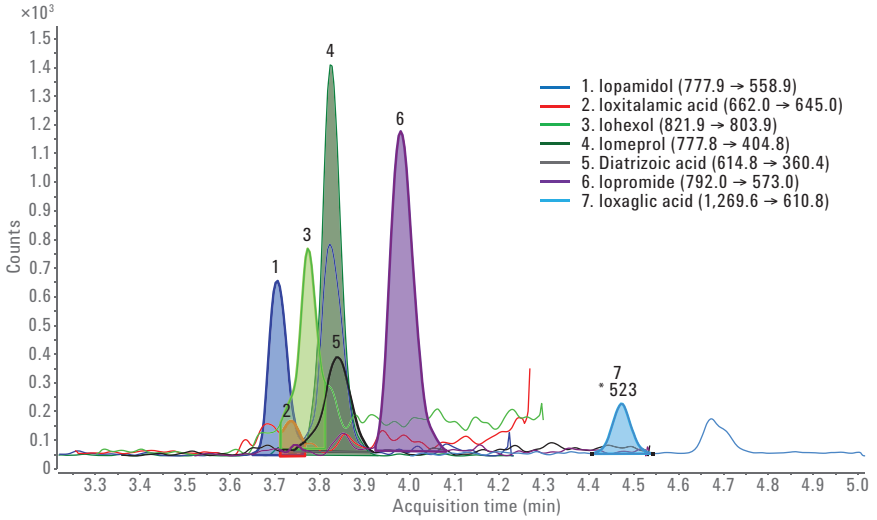

Figure 4. Result for a 100-µL injection of surface water. EIC of only the quantifier transitions of the nonlabeled ICMs.

The samples were analyzed before, and after addition of 200 ng/L (ppt) of the test compounds. Quantitation was carried out on spiked and unspiked samples to determine the recovery (Table 3). Isotopically labeled internal standards were available only for some of the compounds under investigation. The internal standard correction for ICMs, for which an internal standard was not available, was done with the internal standard having a retention time (RT) close to the ICM RT. Table 1 gives the selected internal standard for each ICM.

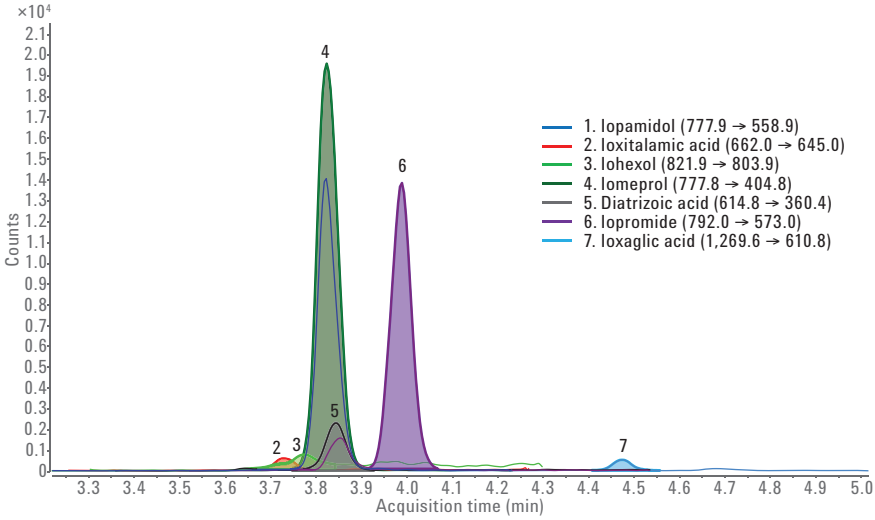

Figure 5. Result for a 100-µL injection of water treatment effluent sample. EIC of only the quantifier transitions of the nonlabeled ICMs.

Table 3. Quantitative results for the selected samples. Samples were analyzed before, and after spiking with 200 ng/L (ppt).

	Tap water			Surface water			Water treatment effluent		
	Unspiked (ppt)	Spiked (ppt)	Recovery %	Unspiked (ppt)	Spiked (ppt)	Recovery %	Unspiked (ppt)	Spiked (ppt)	Recovery %
Iopamidol	0.0	260.4	130.2	56.3	254.9	99.3	0.0	202.9	101.5
Ioxitalamic acid	0.0	237.7	118.8	17.7	192.5	87.4	351.5	306.1	-22.7
Iohexol	20.3	266.1	122.9	178.0	425.9	124.0	369.9	596.9	113.5
Iomeprol	0.0	250.6	125.3	97.0	298.0	100.5	2,154.7	2,427.5	136.4
Diatrizoic acid	0.0	228.2	114.1	23.2	223.0	99.9	194.3	403.1	104.4
Iopromide	0.0	235.9	117.9	60.9	224.1	81.6	1,035.0	1,203.2	84.1
Ioxaglic acid	0.0	214.0	107.0	49.3	208.8	79.7	243.9	435.8	95.9

In tap water, traces of iohexol were detected, illustrating that the purification process did not completely remove this iodinated X-ray contrast medium. The two other samples contained all compounds under investigation. In surface water, the concentrations ranged from 17 to 178 ng/L. The wastewater treatment effluent contained amounts of iomeprol and iopromide outside the calibrated range. The spiking experiments resulted in recoveries between 75 and 125 % for most of the compounds and samples. Outliers are caused by matrix effects (for example, iopamidol in tap water or ioxithalamic acid in wastewater treatment effluent), or values that are out of the calibration range (for example, iomeprol in water treatment effluent). The value of –22.7 for ioxitalamic acid is most probably due to ion suppression/enhancement effects. Since no internal standard was available for this compound, matrix effects can influence the result. The latter sample was also injected after dilution with deionized water (1/10), and results were, in terms of recovery, similar to the surface water sample.

Conclusion

Direct injection of water samples was carried out for the analysis of low ng/L (ppt) levels of iodinated contract media. The Agilent 1290 Infinity II Multisampler equipped with a 100 µL loop and analytical head allowed efficient loading of 100 µL of sample with no detectable carryover. High sensitivity was achieved by using the Agilent 6495 Triple Quadrupole MS with Agilent Jet Stream Technology.

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