Quantification of the Contrast Dye Iohexol in Urine and Serum Matrices

Jesse C. Seegmiller, Ph.D.
AB SCIEX, Foster City, CA

Purpose

This application note describes a liquid chromatography tandem mass spectrometry (LC/MS/MS) method for the quantification of iohexol in urine and serum matrices. High performance liquid chromatography (HPLC) and capillary electrophoresis (CE) absorbance methods are susceptible to interferences from substances present in biological fluids. This method takes advantage of multiple reaction monitoring (MRM) on a tandem mass spectrometer to gain analytical selectivity and sensitivity.

Introduction

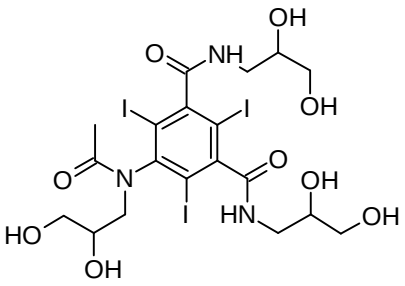
Iohexol has been used in clinical settings primarily for medical imaging purposes and for glomerular filtration rate (GFR) determinations[1,2] to assess kidney function.

This method was developed to demonstrate the superior sensitivity, specificity and robustness that can be achieved by using an AB SCIEX QTRAP® 5500 LC/MS/MS system for the analysis of iohexol in serum and urinary matrices.

Key Features of AB SCIEX™ Tandem Mass Spectrometry Systems

- Best-in-class triple quadrupole sensitivity allows quantification of low abundance analytes in complex matrices.
- Robust and reliable Turbo V™ ion source provides efficient ionization at LC flow rates up to 5 mL/min.
- Unique LINAC® collision cell technology permits greatly reduced dwell times for multi-target analyses, with no loss in sensitivity, and no false positives from cross-talk.
- Broad linear dynamic range allows accurate quantitation over a large range of analyte concentrations.
- Advanced MS/MS scan modes, including neutral loss and precursor ion scans, may be used in flexible combinations to achieve unprecedented selectivity.
- AB SCIEX software featuring full audit trail capability for regulatory compliance.
- Powerful and flexible report generation options, including export of results to LIMS or other software packages.

Figure 1. Chemical Structure of Iohexol.



Molecular Formula = $C_{19}H_{26}I_3N_3O_9$
Monoisotopic Mass = 820.8803
CAS# = 66108-95-0

Experimental Conditions

Calibration curves were constructed in water. Samples consisted of iohexol spiked into serum and urinary matrices. All samples were subjected to protein precipitation using acetonitrile containing internal standard (iothalamate), and centrifuged at 14000 x g. Supernatant was removed, further diluted with water, transferred to HPLC vials, and 5uL was injected on a QTRAP® 5500 LC/MS/MS system. HPLC conditions are described in Table 1.

Table 1. HPLC Conditions for the Quantitation of Iohexol on a QTRAP® 5500 LC/MS/MS System.

Time	%A	%B
0.1	97	3
0.3	97	3
2.3	35	65
2.8	35	65
2.9	5	95
3.4	5	95
3.5	97	3
6.3	97	3

Mobile Phase A: Water, 0.1% formic acid
Mobile Phase B: Acetonitrile, 0.1% formic acid
Column: Kinetex C18; 2.6µm 50mm x 2.1mm
Flow rate: 0.25 mL/min

Two MRM transitions were used for the analysis of iohexol – one for quantitation, and one for confirmatory purposes. A single MRM transition was used for a surrogate internal standard, iothalamate.

Results

Table 2 displays data demonstrating the method precision and robustness. The interassay imprecision was determined to be <4.0 %CV for the calibrators (N=15; five times per day over a three day period). Controls were also analyzed in both urine and serum matrices, and these displayed <4.2 %CV (N=15; five times per day over a three day period). These data provide strong evidence that this LC-MS/MS method affords excellent reproducibility in real sample matrices, and is suitable in clinical research settings.

Table 2. Calibrator and Control Reproducibility Data for Measurements of Iohexol

Calibrators			
	Mean	**s**	**%CV**
1 mg/L	0.964	0.023	2.43
10 mg/L	10.14	0.40	3.94
25 mg/L	25.23	0.68	2.68
50 mg/L	51.51	1.80	3.49
100 mg/L	98.16	3.06	3.12

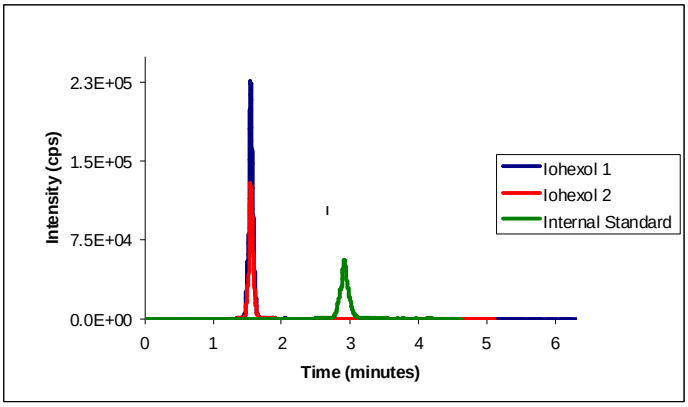
N=15, over 3 Days

Controls			
	Mean	**s**	**%CV**
Urine Low	13.04	0.42	3.20
Urine Medium	25.20	0.89	3.52
Urine High	51.91	2.16	4.15
Serum	4.71	0.13	2.81

N=15, over 3 Days

Figure 1 depicts an example chromatographic profile for the method, with a total run time of 6.3 minutes.

Figure 1. Representative Chromatogram for the Analysis of Iohexol.



Conclusions

An LC/MS/MS method was developed on an AB SCIEX QTRAP® 5500 system for the quantification of iohexol in urine and serum samples, using a structurally similar compound, iothalamate, as an internal standard. The method displayed excellent reproducibility for calibrators, QCs, and spiked serum and urine samples.

The reproducibility illustrated in this effort demonstrates that this method is appropriate for GFR determinations and could be used to measure GFR in renal transplant populations, as well as monitoring kidney function in clinical trials. The sensitivity, precision, robustness, and linear dynamic range of the AB SCIEX QTRAP® 5500 LC/MS/MS system makes this instrument an invaluable tool for the clinical research laboratory.

References

1. Schwartz GJ, Furth S, Cole SR, et al. Glomerular filtration rate via plasma iohexol disappearance: pilot study for chronic kidney disease in children. *Kidney Int* 2006; 69: 2070-2077.
2. Krutzen E, Back SE, Nilsson-Ehle I, et al. Plasma clearance of a new contrast agent, iohexol: a method for the assessment of glomerular filtration rate. *J Lab Clin Med* 1984; 104: 955-961.

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Headquarters
353 Hatch Drive | Foster City CA 94404 USA
Phone 650-638-5800
www.absciex.com

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