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Analysis of the Contrast Dye Iohexol in Human Urine/Serum using PaperSpray Technology

Yu Zhu, Katherine Walker, Neloni R. Wijeratne, Mary Blackburn, Thermo Fisher Scientific, San Jose, CA

ABSTRACT

Purpose: To eliminate the time-consuming sample preparation step with a faster and reliable analytical method of iohexol using paper spray technology.

Methods: A new high-throughput and automated paper spray system was used to analyze iohexol in different human matrices. Traditional multi-step sample preparation is replaced with a one step spike-and-spot method.

Results: LOQ of iohexol is 1 ug/mL and 5 ug/mL in urine and serum, respectively. Quantification of iohexol produced linear, precise, and accurate results with minimal sample preparation.

INTRODUCTION

PaperSpray technology is a rapid analysis technology specifically suitable for clinical samples. Quick sample turnaround times of 2 minutes or less make it very competitive compared to traditional LC/MS-based techniques. Minimal sample preparation is required for analysis of dried urine or blood spots from a piece of triangular shaped paper. The new Thermo Scientific™ VeriSpray™ system uses PaperSpray technology to make clinical workflows faster and more efficient by combining ease-of-use and increased automation with the speed that PaperSpray technology provides. The VeriSpray system consists of the VeriSpray ion source and the VeriSpray plate loader (Figure 1, left). The VeriSpray plate loader magazine holds up to 10 VeriSpray sample plates (Figure 1, right). Each VeriSpray sample plate contains 24 single use paper strips (12 on each side, A and B). Through Thermo Scientific™ Xcalibur™ software sequence setup, the full magazine can be run in an automated way.

Figure 1. VeriSpray ion source with plate loader (left) and VeriSpray sample plate (right).

Here we demonstrate a VeriSpray PaperSpray method for the analysis of iohexol from human urine and serum. Glomerular filtration rate (GFR) is the best overall indicator of kidney function. The standard method for measuring GFR is renal clearance of inulin, which is invasive, expensive and time consuming. Hence, there exists a need for an easy and safe method for GFR measurement. Use of iodinated dyes such as iohexol is the current widely used method for evaluation of GFR, as it has shown to be safe and readily available. Using PaperSpray technology, matrix samples spiked with iohexol can be spotted on a piece of paper, and analyzed within 1 minutes.

MATERIALS AND METHODS

Sample Preparation

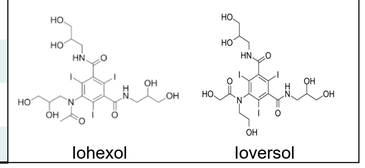
Iohexol was spiked into urine and serum at concentrations ranging from 1-1500 ug/mL and 5 – 1500 ug/mL respectively. QC samples were prepared at 3 additional concentration points (20 ug/mL, 250 ug/mL and 1200 ug/mL). Ioversol was used as an internal standard due to the structural similarity and numerous reported applications in literature. An Internal standard (IS) mixture was prepared by dissolving ioversol in methanol at a concentration of 22.2 ug/mL. 50 uL of the serum sample was crashed with 450 uL IS mixture. Sample was vortexed for 30 seconds and centrifuged at 14,000 g for 3 min. 10 uL supernatant was loaded on a PaperSpray plate.

Test Method(s)

The rewet/spray solvent used was 95% acetonitrile, 5% water, and 0.1 % formic acid. Data were acquired for 1 minute per analyte, and each concentration level of calibrators and QCs was measured 5 times separately for quantification. Compounds were analyzed on a Thermo Scientific™ TSQ Altis™ mass spectrometer. Three transitions were monitored for iohexol, and one transition for internal standard ioversol. The spray voltage was 3.5 kV, applied from 0.1 to 0.9 min, the inlet capillary temperature was set to 350 ˚C, and the distance of paper tip to inlet was approximately 5.3 mm. Thermo Scientific™ TraceFinder™ software, version 4.1 was used for data analysis.

Table 1. Compound structure and SRM transitions.

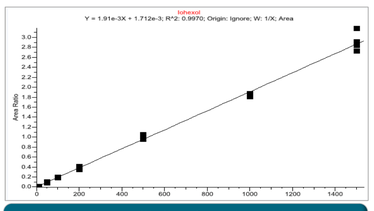
Compound	Precursor (m/z)	Product (m/z)	Collision Energy (V)	Chemical Structure
Iohexol 1	821.887	374.613	46.79	
Iohexol 2	821.887	528.863	43.5	
Iohexol 3	821.887	501.0	48.68	
Ioversol	807.9	589.1	42	



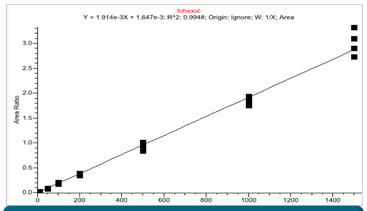
RESULTS

This study provided a method for the analysis of iohexol directly from urine and serum. Different dilution ratios and IS solvent compositions were tested and evaluated based on absolute area counts and precision of response ratios. A final dilution of 1:9 (serum: IS mixture) was chosen as the optimized condition. Calibration curves for iohexol were constructed by integrating the resulting chronograms (n=5). The calibration curves are linear over the tested concentration range: 1-1500 ug/mL in urine; 5-1500 ug/mL in serum (Figure 2).

Figure 2. Calibration curve for iohexol in urine (left) and serum (right).



Range of quantitation for Iohexol in Urine: 1 – 1500 ug/mL, N=5



Range of quantitation for Iohexol in Serum: 5 – 1500 ug/mL, N=5

The precision was under 10% for all urine calibrator levels (Table 2) and under 15% for all serum calibrator levels (Table 3). Calibrator accuracy was under 20% for all levels in both matrices. QC samples were placed at concentration points in between the calibrator levels and met precision (<10%) and accuracy (<10%) criteria.

Table 2. Iohexol cal accuracy and %CV in urine.

Cal Level	Conc. (ug/mL)	Accuracy	%CV	S/N
1	1	15.02	7.85	5
2	5	16.21	6.66	14
3	10	-2.11	2.76	26
4	50	-8.04	8.73	132
5	100	-1.92	3.59	242
6	200	-6.76	7.19	551
7	500	0.26	4.72	1746
8	1000	-4.21	1.32	3584
9	1500	-0.76	5.74	5309

Table 3. Iohexol cal accuracy and %CV in serum.

Cal Level	Conc. (ug/mL)	Accuracy	%CV	S/N
1	5	2.89	5.80	8.7
2	10	-3.43	13.42	16
3	50	-13.18	3.70	77
4	100	-7.41	7.12	161
5	200	-10.48	4.80	240
6	500	-8.50	7.52	617
7	1000	-6.46	4.38	1760
8	1500	-4.72	7.40	2431

Three QC levels (low, medium, high) were placed at concentration points in between the calibrator levels and met precision (<10%) and accuracy (<10%) criteria in both matrices. Result are shown in Table 4 (urine matrix) and Table 5 (serum matrix).

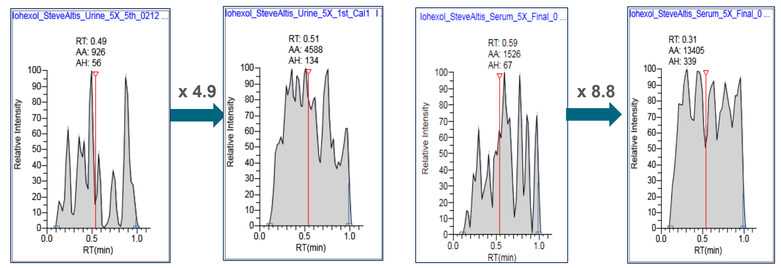
Table 4. Urine QC accuracy and precision.

QC	Theoretical Conc.	Mean	Accuracy %	CV %
Urine Low	20	21.8424	9.2	1.42
Urine Medium	250	227.2214	-9.1	2.51
Urine High	1200	1155.9064	-3.6	2.27

Table 5. Serum QC accuracy and precision.

QC	Theoretical Conc.	Mean	Accuracy %	CV %
Serum Low	20	18.5986	-7.007	0.95
Serum Medium	250	255.1682	2.06728	4.88
Serum High	1200	1255.6368	4.6364	9.45

Figure 3. Chronograms of iohexol at LOQ level in urine (Left) and serum (right).



Urine Matrix Blank | Urine LOQ = 1 ug/mL %CV = 7.85% | Serum Matrix Blank | Serum LOQ = 5 ug/mL %CV = 5.8%

LOQ level of iohexol is 1 ug/mL in urine and 5 ug/mL in serum, LOQ acceptance criteria is defined as follows: R^2 ≥ 0.98, Accuracy ≤ 20%, %CV ≤ 15% and S/N ≥ 4.

CONCLUSIONS

- PaperSpray technology is well suited for fast and reliable clinical analysis, because of its accuracy, sensitivity, ease of sample preparation and short analysis time within a 1 minute window.
- Quantification of iohexol is easily achievable in the required measurement range.
- The new VeriSpray ion source enables PaperSpray analysis as an easy, fast, and more automated choice than other traditional platforms.

REFERENCES

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