Iohexol quantitation and possible degradation kinetics in human urine using mass spectrometry coupled to liquid chromatography (LC)

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Introduction

- Iohexol is an iodine contrast media. Its clearance is used to evaluate glomerular flitration rate (GFR), and indicator of kidney function (1,2)

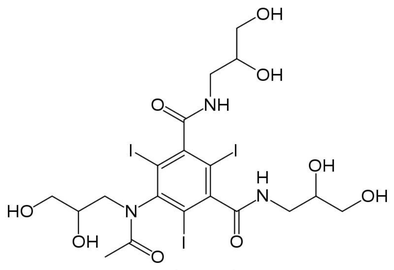

Figure 1: Iohexol structure

- This clearance is calculated using LC-MS/MS approaches on human plasma and urines. In these approaches, urines or plasma are taken from patients who were administered Iohexol at different timepoints and Iohexol is quantified at each time using one MRM approach. Once those value obtained, kinetics can be performed and GFR is calculated.
- **Some discrepancies can occur between urine and plasma results from the same patient and no study clearly explained this**.

Objectives of the study

- **Assessing molecule profile variations occurring over time with patients that took Iohexol using LC coupled with high resolution mass spectrometry.**

Material and Methods

- For urine samples: samples are first centrifuged, and the supernatant is diluted 100 times with LC-MS grade water before injection in a NanoACQUITY UPLC system coupled with a SYNAPT XS instrument operating in positive ion mode.
- 8 urine samples coming from 8 different patients are considered.
- For each sample, 4 timepoints are taken: T120 minutes, T180 minutes, T240 minutes and T300 minutes.
- Gradient conditions:
  * Flow: 9µl/min
  * Injection volume: 5 µl
  * Mobile phases:
  A: H_2O, 0.1% HCOOH
  B: Acetonitrile, 0.1% HCOOH

Time (min)	%A	%B
0	0	100
0.1	0	100
1	100	0
3	100	0
5	0	100
7	0	100

- Technical triplicates are performed.
- Standard samples (commercial Iohexol drug: “Omnipaque 240”) are also analyzed as quality control. Blank urines are also taken as reference.

Previous results

Table 1: Iohexol plasmatic and urinary clearances for all considered samples calculated using LC-MS/MS approach.

Sample	Iohexol plasmatic clearance (ml/min)	Iohexol urinary clearance (ml/min)	Discrepancies (%)
1	86	78	10.2
2	**148**	**126**	**17.5**
3	115	/	/
4	98	97	1
5	72	67	7.5
6	97	98	1
7	118	106	11.3
8	77	76	1.3

Previous results

- Values presented on table 1 have been calculated using classical MRM (LC-MS/MS) approaches. Sample 2 clearly shows the biggest discrepancy between urine and plasma.

Results on standard samples

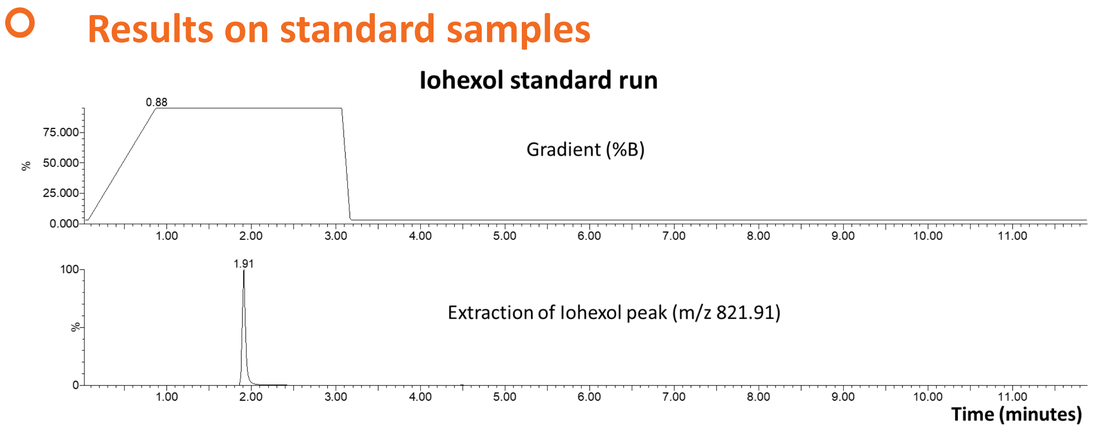

Figure 2: Above: gradient B %; Bellow: extraction of Iohexol peak (m/z 821.91). Injection concentration: 2µg/ml

- Figure 2 depicts the peak shape of m/z 821.91. The elution occurs in the middle of 100% B plateau.

Results on urine patient samples

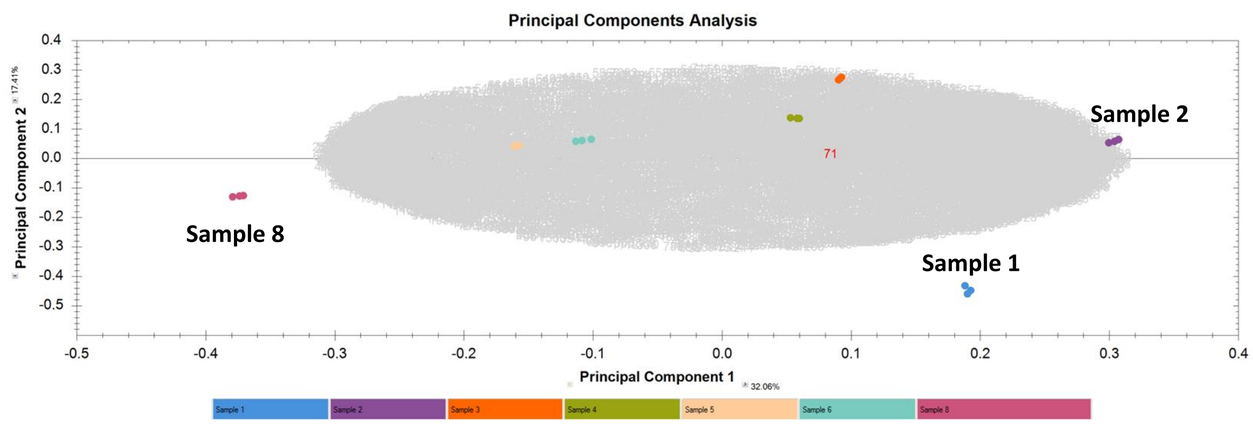

Figure 3: Unsupervised principal components analysis plot of timepoint T180. PC1: 32.06% and PC2: 17.41%

- Unsupervised PCA results performed on T180 timepoint is depicted on figure 3. Sample 1 and sample 2 are clearly more associated with higher values of PC1.
- These observation can be correlated with values in table 1.
- All triplicates are also well clustered indicating the reproducibility of the method.

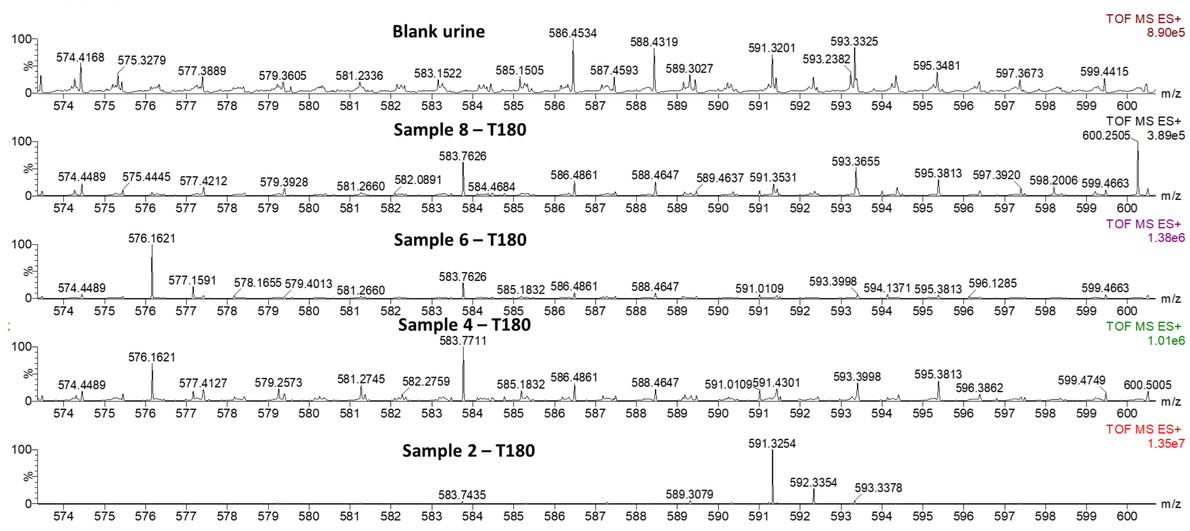

Figure 4: Comparison of total mass spectra obtained at same time point (T + 180 minutes) with different patients. Sample 2 clearly show high abundance of m/z 591.3254

- Total mass spectra coming from various samples at the same timepoint (T + 180 minutes) are compared on figure 4.
- m/z 591.3254 seems specific from sample 2.
- Sample 2 is also the sample with the biggest discrepancy between urinary and plasmatic clearance (see table 1).

Work in progress

- Comparison between plasma and urine samples.
- Extension of the study to more patients.
- Other statistical analysis tool will be considered.
- Evaluation of sample preparation effect: addition of one protein precipitation step in progress.

Conclusions and prospects:

1) m/z 591.3254 looks specific to sample with high discrepancies between urinary and plasmatic.
2) MS/MS is in progress to identify m/z 591.3254
3) More patient samples will be analyzed.
4) Corresponding plasma samples will be analyzed

References

(1) P. Delanaye, T. Melsom, N. Ebert, S. E. Bäck, C. Mariat, E. Cavalier, J. Björk, A. Christensson, U. Nyman, E. Porrini, G. Remuzzi, P. Ruggenenti, E. Schaeffner, I. Soveri, G. Sterner, B. O. Eriksen and F. Gaspari, *Clin. Kidney J.*, 2016, **9**, 700–704.

(2) P. Delanaye, N. Ebert, T. Melsom, F. Gaspari, C. Mariat, E. Cavalier, J. Björk, A. Christensson, U. Nyman, E. Porrini, G. Remuzzi, P. Ruggenenti, E. Schaeffner, I. Soveri, G. Sterner, B. O. Eriksen and S. E. Bäck, *Clin. Kidney J.*, 2016, **9**, 682–699.