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A simple LC-MS method for the determination of iohexol and iothalamate in serum, using ioversol as an internal standard

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ABSTRACT

Background: Chronic kidney disease (CKD) is diagnosed and explored through the determination of the glomerular filtration rate (GFR). Our goal was to develop a simple LC-MS method for the determination in serum of 2 popular GFR markers, contrast agents iohexol and iothalamate, for routine use and comparison studies between the two markers. A similar contrast agent, ioversol, was used as an internal standard and the method underwent a rigorous validation protocol based on β-expectation tolerance intervals.
Methods: We adapted the HPLC-UV method from Cavalier et al. to our LC-MS system. Data treatment for the validation was performed using Multiquant 3.0 (Sciex, Framingham, MA, USA) and e.noval 3.0 software (Arlenda, Liège, Belgium).
Results: According to the validation results our method will give accurate and reliable results for concentrations ranging from 6.8 to 250 μg/ml for iohexol and 6.15 μg/ml to 250 μg/ml for iothalamate. In our practice these intervals are sufficient to determine both compounds in most patient samples. Samples with higher detected concentrations can always be diluted into range.
Conclusion: With its internal standard and extensive validation, our method is now ready for routine and clinical research use.

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1. Introduction

The determination of the glomerular filtration rate (GFR) is an important tool for the diagnosis and exploration of chronic kidney disease (CKD) [1–3].

It can be measured through the disappearance or appearance of a marker in blood or urine, respectively. The ideal marker is endogenous and present at a stable concentration in plasma, is freely filtered at the glomerulus (without tubular reabsorption or secretion), does not undergo extra-renal elimination and has low biding to proteins [1,3].

Inulin, a fructose polymer, meets all but one of these requirements, as it is exogenous. It is considered a safe compound [3]. Renal inulin clearance is considered a gold standard for the measurement of GFR, but the procedure to use is costly, time-consuming and impractical [3].

Serum creatinine, used with creatinine-based equations, is the most widespread method in daily clinical practice for GFR estimation, although it suffers many limitations, particularly in specific populations such as obese, anorectic and transplanted patients. Moreover, the equations used to calculate GFR from serum creatinine levels (Cockcroft-Gault, modification of diet in renal disease (MDRD), CKD-EPI, etc.) can introduce bias depending on factors such as age, presence and severity of CKD [4–9].

Other reference methods using different markers have been developed since as an alternative to inulin. Among them contrast agents like iohexol and iothalamate stand out as credible alternatives to inulin as markers, for plasmatic and/or renal clearance. Except for being exogenous, they possess all the features of a good GFR marker, and are readily available [10–16]. Regarding their safety, the risk of contrast-induced nephropathy (CIN) increases with the osmolarity of the agent employed, its concentration, the injected volume, as well as the presence and severity of CKD, among other risk factors. Nonetheless, this risk is low with the injection volume for GFR determination (5 ml in our case), especially if the patient receives proper hydration and care before the procedure [17–22].

In a previous study [23], our group developed a simple but accurate method for the quantification of iohexol in serum using protein precipitation, HPLC separation and UV detection. This method was thoroughly validated to ensure its reliability and has been used in our laboratory to this day.

Nonetheless, following current trends [24–26] we are gradually shifting our routine analyses to LC-MS/MS based methods, allowing us to gather them on a single device.

Therefore, our objective was to transfer the method to LC-MS/MS with selected reaction monitoring (SRM) to ensure the specificity of

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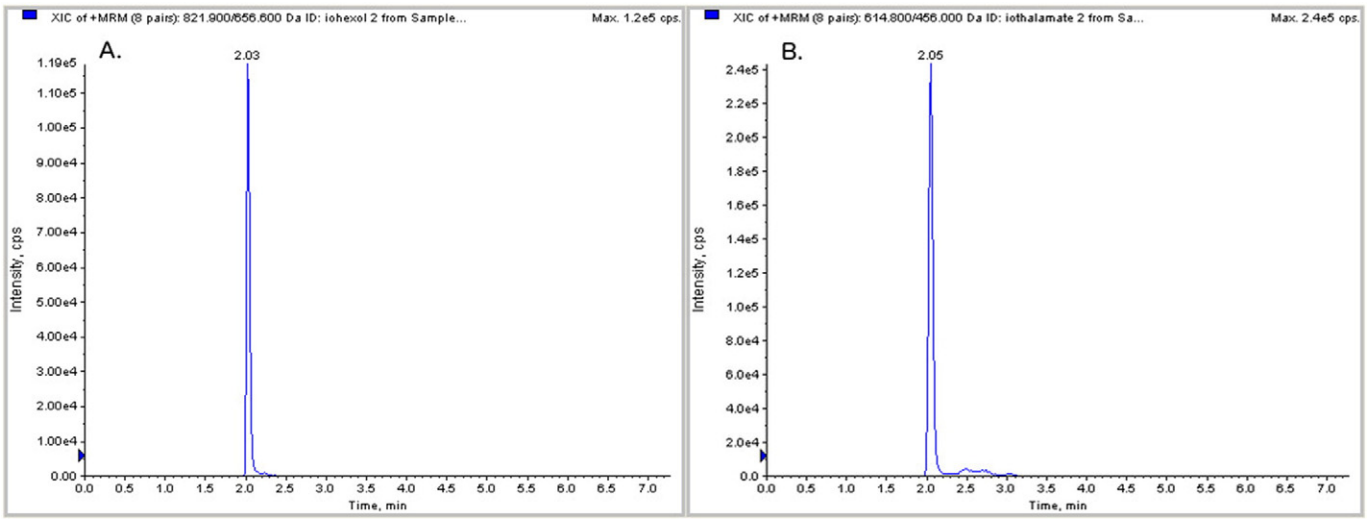

Fig. 1. Chromatograms for a 5 μl injection of a 25 μg/ml iohexol/iothalamate calibration standard.

our determination method. Moreover, we wanted to incorporate an internal standard to reduce bias in our results. Another contrast agent, ioversol, has been chosen for this purpose, as it shares most of its structure with iohexol and should exhibit similar behavior through the analytical process. Finally, we also wanted to incorporate iothalamate in our method, as its use for GFR measurement is common in the U.S. and comparison studies between iohexol and iothalamate for this application are necessary [27,28].

The method would then undergo an exhaustive analytical validation process to be fit for routine use. To our knowledge, we are the first to publish a method with this set of features.

2. Materials and methods

2.1. Terminology

First of all, here is a brief reminder of the meaning of key statistical terms and concepts underlying the validation method.

- Trueness is "the closeness of agreement between the average value obtained from a large series of test results and an accepted reference value". It refers to the systematic measurement error [29–31].
- Precision is "the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions". It refers to the random measurement error [29–38].
- Accuracy is "the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found" [29–38]. It refers to the total measurement error, a combination of systematic and random measurement errors [20–29].
- Uncertainty of measurement is "a parameter associated with the result of a measurement, that characterizes the dispersion of the values that could reasonably be attributed to the measurand" [31,35,36].
- β-Expectation tolerance intervals are used to describe populations. They contain a proportion β of the future individual values of a population within their limits. In our validation, they estimate the accuracy of quantification for each validation point. With $\beta = 0.95$, 95% of future results corresponding to a validation point will lie in the interval determined during validation [37,38].

2.2. Chemicals

Iohexol (5-[acetyl(2,3-dihydroxypropyl)amino]-1-*N*,3-*N*-bis(2,3-dihydroxypropyl)-2,4,6-triiodobenzene-1,3-dicarboxamide, brand name Omnipaque™ 240 mg I/ml) was purchased from GE Healthcare

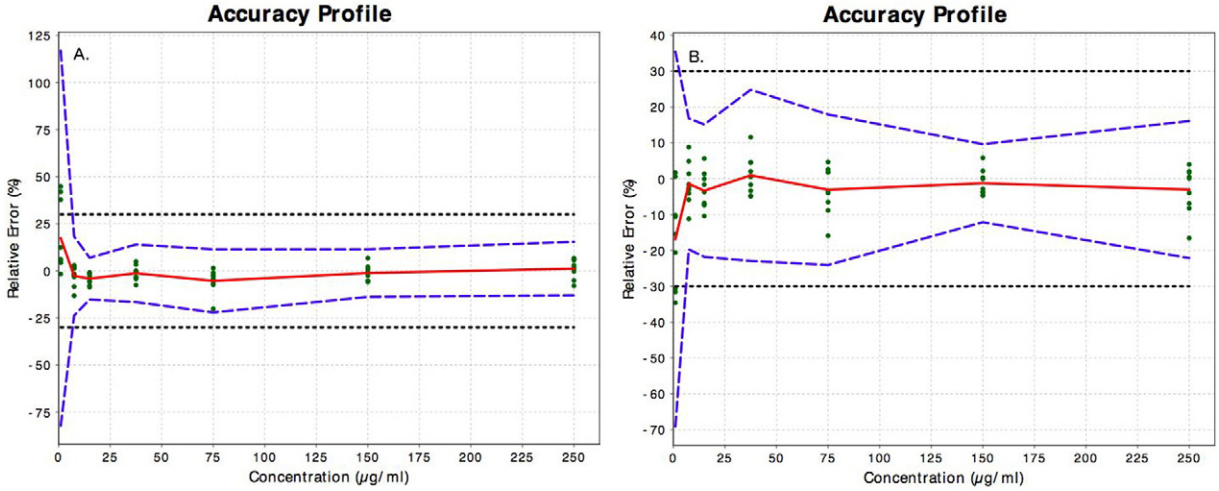

Fig. 2. Accuracy profiles for iohexol (A) and iothalamate (B).

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