Glomerular Filtration Rate analysis by Iohexol (Omnipaque) clearance

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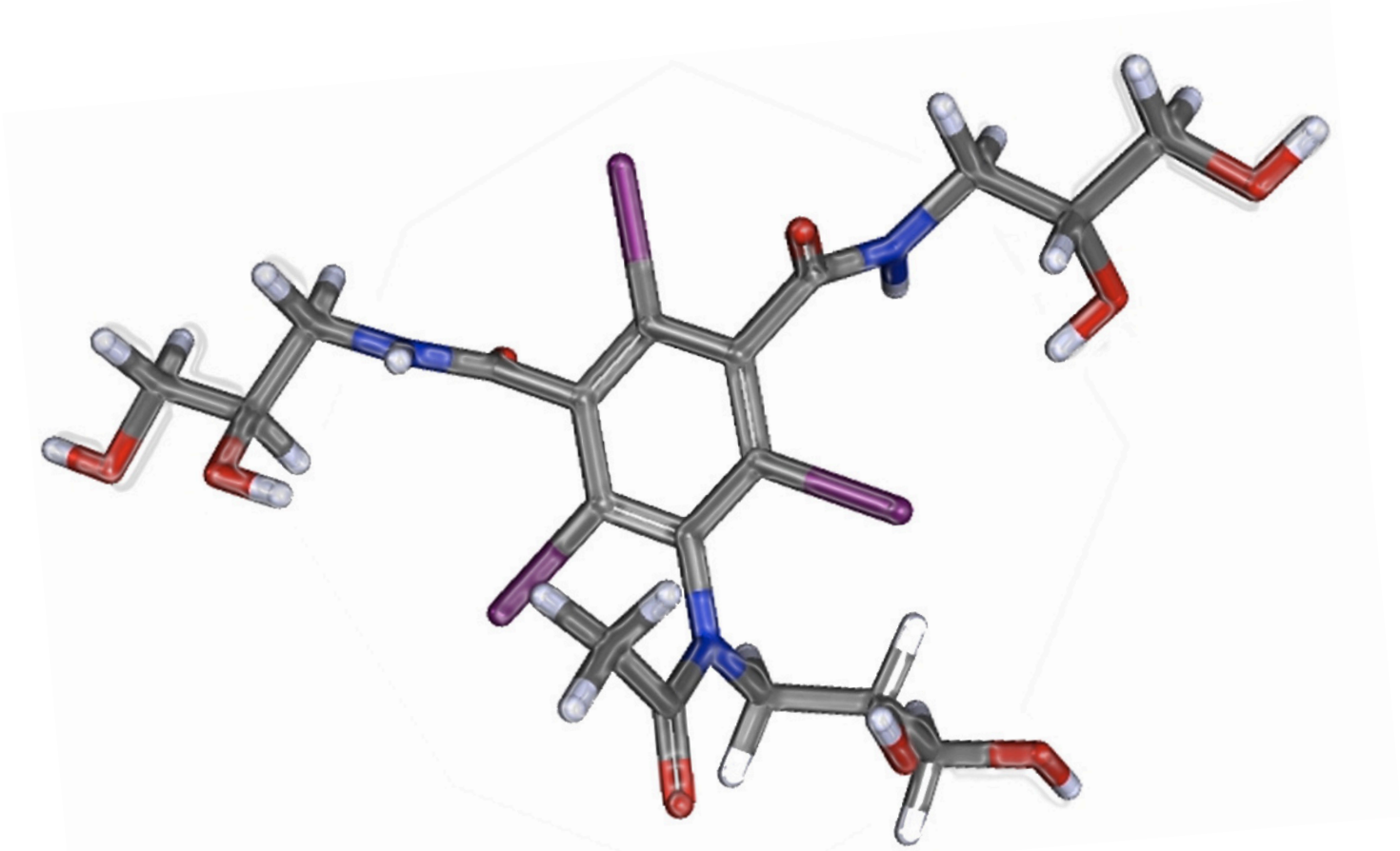

National Center for Biotechnology Information. PubChem Compound Database; CID=3730, https://pubchem.ncbi.nlm.nih.gov/compound/3730 0

Application Note:

ASSESSMENT OF GLOMERULAR FILTRATION RATE IN RATS BY MEASUREMENT OF IOHEXOL USING THE DELTADOT HIGH PERFORMANCE CAPILLARY ELECTROPHORESIS (HPCE) SYSTEM

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ABSTRACT

The chemical Iohexol can be used as a marker to rapidly indicate kidney function in small animals by measuring their glomerular filtration rate (GFR). Iohexol concentrations in rat plasma were measured using deltaDOT's High Performance Capillary Electrophoresis (HPCE) system with LFII® technology. Samples were analysed with no sample preparation other than the addition of an internal standard.

The small volume of sample required means the method could be used to measure Iohexol concentrations in blood samples taken from rats given a bolus injection of Iohexol, in the form of the contrast agent Omnipaque 240. Measurement of the concentration at 30, 45 and 60 minutes enabled clearance of the Iohexol to be determined in approximately 20 minutes, and glomerular filtration rate to be calculated.

This application is directly applicable to the early diagnosis of chronic kidney disease (CKD) in companion animals such as cats and dogs as well as in human diagnostics.

INTRODUCTION

Kidney function is routinely assessed using metabolic biomarkers in blood, typically plasma creatinine concentrations. However, this method is unreliable, as creatinine may not change until more than 60 per cent of nephrons are already non-functional, and is also affected by factors such as muscle mass.

Glomerular filtration rate (GFR) on the other hand is directly correlated with functional renal mass, so that quantification of GFR is a direct measure of renal function. GFR is widely considered to be the 'gold standard' assay for kidney function.

GFR can be measured by the clearance of Iohexol, a contrast imaging agent which is not absorbed, metabolised or excreted, and is cleared solely by the kidney. To carry out the assessment, a bolus injection of Iohexol is given and then three serum samples are taken at specified times. An internal standard (Iopromide) is added and the samples are analysed to measure Iohexol concentrations, which together with the initial dose are used to calculate Iohexol clearance, and hence GFR.

MATERIALS AND METHODS

Serum samples were taken from four rats at 30, 45, 60, 90, 120, 180 and 240 minutes after injection with Omnipaque 240 at a dose of 1ml/kg.

The data presented in this report were collected on a deltaDOT HPCE system. All detection was performed in direct mode at 254 nm. Separation of the Iohexol and Iopromide was carried out in bare fused silica capillaries of total capillary length 41.5cm. The Peltier cooling system was set to 23°C.

Iopromide internal standard was prepared by dissolving in 25% Methanol/75% distilled water to a concentration of 10mg/mL. Iohexol calibrants were made up using Omnipaque 240 in canine serum after checking results did not differ from those made up in rat serum, due to a shortage of the latter matrix.

The sample was introduced using an electrokinetic injection and each sample was injected three times. Between injections, capillaries were flushed with 0.1M NaOH and then reconditioned using the runnng buffer (TBE + 3%(w/v) SDS).

RESULTS AND DISCUSSION

Analysis of separations was performed using deltaDOT's Generalised Separation Transform (GST) algorithm. GST is a method of combining the data from the 512 pixels in a natural way which preserves the peak shape information of the electropherograms while at the same time maximising the signal-to-noise ratio. Typically a 10-fold increase in signal-to-noise using GST is observed compared to the standard CE approach, where data is acquired from a single pixel.

An example electropherogram is shown in **Figure 1**, along with electropherograms from blank healthy and diseased rat plasma. Overlaid GST electropherograms from the entire calibration curve are shown in **Figure 2**. Migration time RSD for all replicates at all concentrations, in both healthy and diseased plasma was 0.822%.

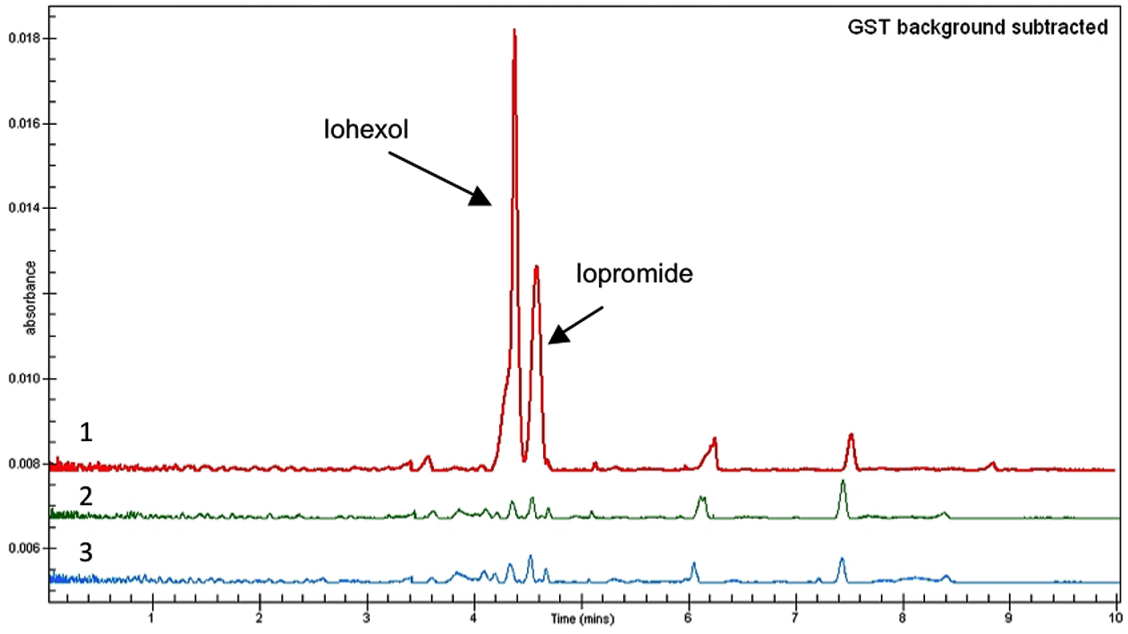

Figure 1: *GST electropherograms of 1. rat plasma spiked at 269.6 µg/mL Iohexol with 150 µg/mL Iopromide IS; 2. Blank healthy rat plasma; 3. Blank diseased rat plasma*

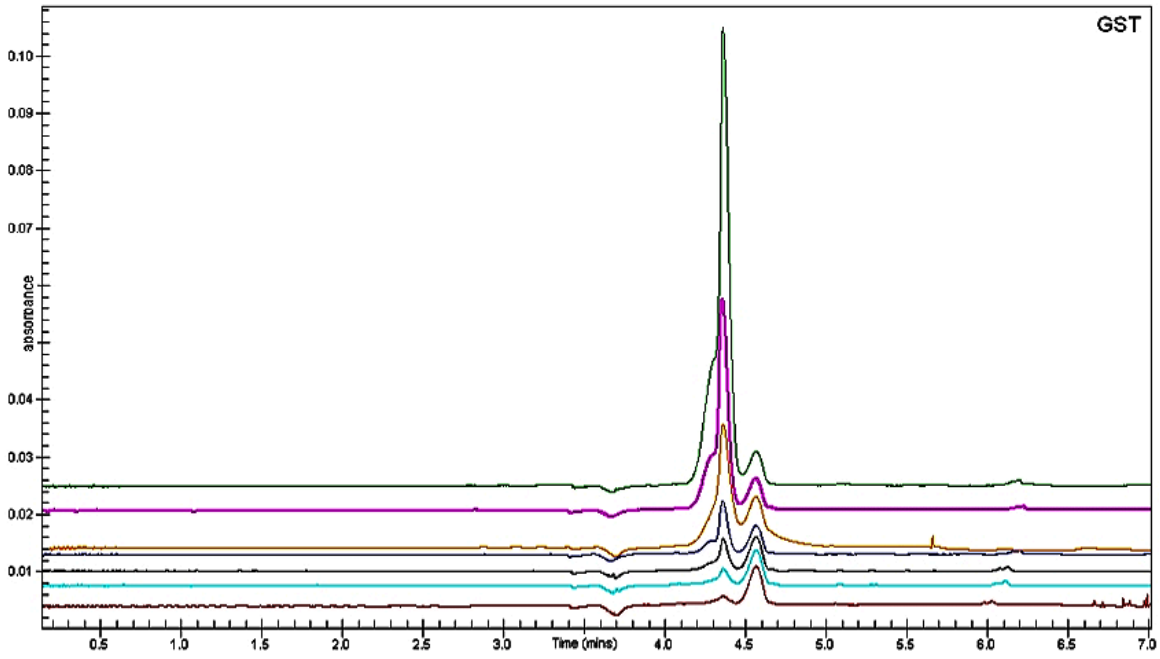

Figure 2. *GST electropherograms of rat plasma spiked from 33.7 to 2156.67 µg/mL Iohexol (15.625 to 1000 µg/mL iodine).*

Comparison of the calibration using rat plasma to that from canine serum (see **Figure 3**) demonstrated that canine serum is an acceptable alternative (<2% difference in mean accuracy). Calibration against canine serum was used to generate the rest of the results in this report, due to a lack of availability of rat plasma.

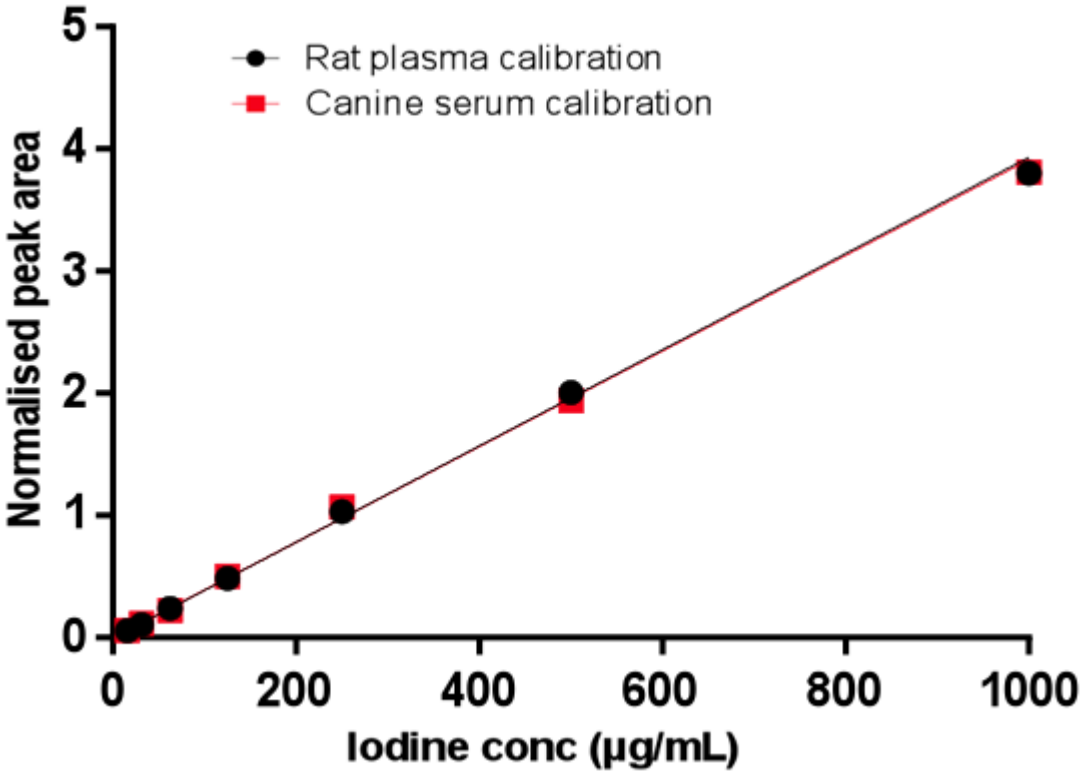

Figure 3. *Overlaid calibration curves from canine serum and rat plasma*

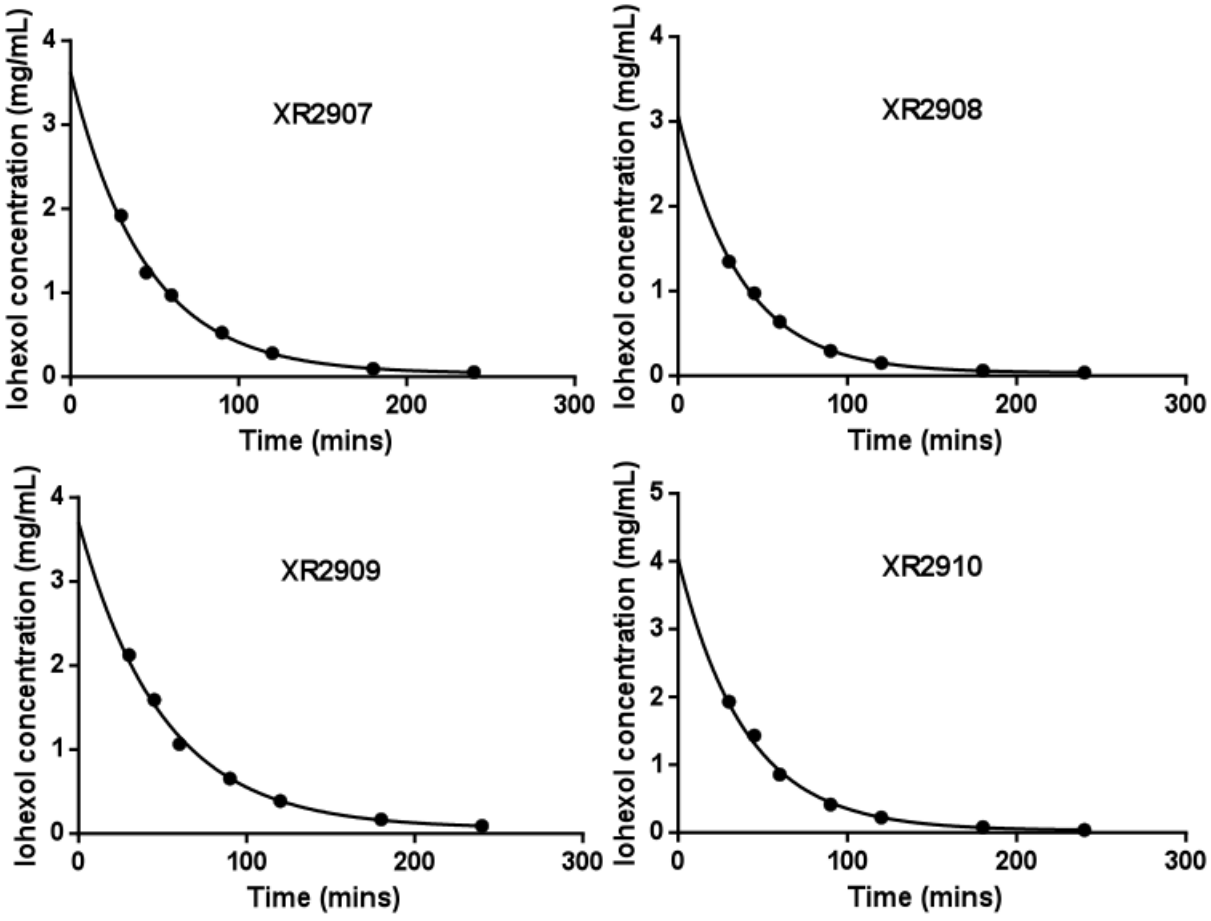

Figure 4. *Data from timed rat samples plotted with best fit monoexponential decay curves. The time scale is minutes post-injection, the assay time is 20 minutes*

RAT	AUC (7 points) (A7)	AUC (3 points)	Adjusted AUC (3 points) (AA3)	A7/AA3
XR2907	91.070	89.094	92.614	0.983
XR2908	63.165	61.701	64.139	0.985
XR2909	107.377	99.586	103.520	1.037
XR2910	90.802	87.662	91.125	0.996

***Table 1**: Comparison of the AUC (Area Under the Curve) generated using all seven time points and just the first three time points*

The fitting of the curves shown in **Figure 4** allows the area under the curve (AUC) to be generated. This allows the GFR to be calculated as the clearence of Iohexhol by the kidney is inversely proportional to the area under the curve. It can be seen that there is a strong correlation between the adjusted AUC and the 'normal AUC(7) showing that only 3 data points are needed meaning the animal is subjected to less stress during the procedure.

CONCLUSION

The analysis of Iohexol (Omnipaque) on the deltaDOT HPCE platform provides a new, reliable tool for the analysis of kidney function by glomerular filtration rate in small animals.

This data suggests that an accurate measure of AUC, and thereby clearance of iohexol and GFR can be calculated from three samples taken from rats at 30, 45 and 60 minutes after a bolus injection of Omnipaque 240.

This provides a sensitive and accurate assessment of kidney function in rats without the need to sacrifice the animal, allowing repeated measurements of kidney function over a period of time to be carried out and small changes in kidney function to be detected.

The advantages offered by the deltaDOT HPCE instrument in the analysis of Iohexol are simplicity of operation and speed of separation The technique is being applied in clinical and industrial setting for animal health and food formulation studies.

REFERENCES

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Iohexol (Omnipaque) analysis for Chronic Kidney Disease diagnosis by glomerular filtration rate (GFR) analysis.

The chemical Iohexol can be used as a marker to rapidly indicate kidney function in small animals by measuring their glomerular filtration rate (GFR). Iohexol concentration in rat plasma was measured using deltaDOT's High Performance Capillary Electrophoresis system and proprietary LFII® technology.

Samples were analysed with no sample preparation other than the addition of an internal standard. The small volume of sample required means the method could be used to measure Iohexol concentrations in blood samples taken from rats given a bolus injection of Iohexol, in the form of the contrast agent Omnipaque 240.

Measurement of the concentration at 30, 45 and 60 minutes enabled clearance of the Iohexol to be determined, and glomerular filtration rate to be calculated.

This technique is directly applicable to the diagnosis of chronic kidney disease (CKD) in companion animals such as cats and dog and is also in human diagnostics.

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deltaDOT has developed and is commercialising innovative capillary electrophoresis technologies and products in the bioscience sector. The company has a strong proprietary position and extensive expertise in instrumentation automation, computing and analysis which will contribute to improvements in knowledge, profitability and process time throughout drug discovery and general life sciences research.