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A Label-Free High Performance Capillary Electrophoresis (HPCE) method for the determination of Glomerular Filtration Rate (GFR) values in feline subjects administered with the contrast agent Iohexol (Omnipaque™) is described. This capillary electrophoresis technique was found to give quicker results, with minimal sample preparation and lower costs compared to traditional HPLC techniques.

Materials and Methods

Residual serum samples from 14 healthy adult cats, which had been administered Iohexol for GFR assessment by a traditional High Performance Liquid Chromatography technique with ultraviolet detection (HPLC-UV), were obtained. Each of the 14 cats had had blood samples taken at 120, 180 and 240 min time-points following intravenous administration of a bolus dose of 647mg/kg of Iohexol. Serum was separated from whole blood and stored at -80°C. An Iohexol standard calibration curve was prepared in feline plasma and used to determine the concentration of Iohexol remaining in each of the feline plasma samples at each of the 3 time-points. GFR was calculated using the method described by Finch *et al.* (2011)¹. The correction formula used is given below. A Bland-Altman plot was used to assess differences between GFR values obtained using the current capillary electrophoresis method and traditional HPLC-UV.

$$CI_{(corrected)} = (1.036 \times CI_{(uncorrected)}) - (0.062 \times CI_{(uncorrected)}^2)$$

where CI is clearance

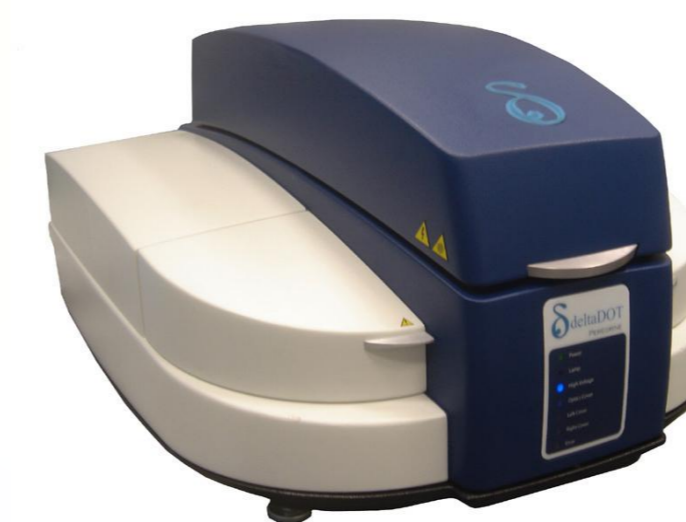
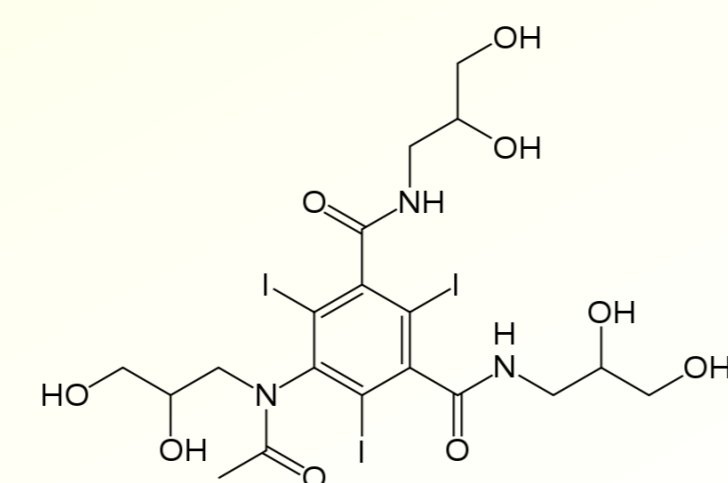
Iohexol concentrations in each of the serum samples were measured using deltaDOT's Label-Free HPCE system. Data were analysed using deltaDOT's Generalised Separation Transform (GST) and Equiphase Vertexing Algorithm (EVA).

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. A factor of 10 increase in signal-to-noise using GST as compared to single electropherograms is typically observed.

EVA is a technique developed by deltaDOT which uses a distance-time constraint to greatly increase the signal-to-noise, improve the resolving power of the system and to allow associations of a given band in the electrophoretic window with a given injection.

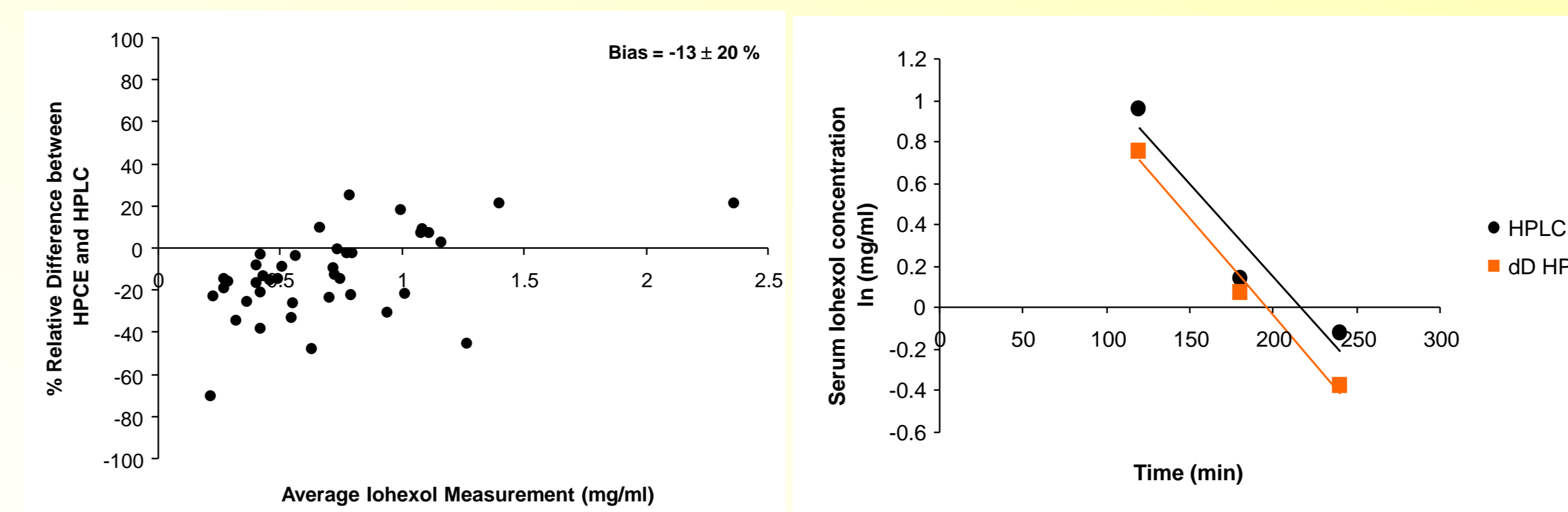
Samples were prepared and run using deltaDOT's proprietary protocols.

Structure of Iohexol



GFR Calculations

Absolute Iohexol concentrations obtained using the two methods were compared using a Bland Altman plot, from which GFR values were calculated. Although absolute Iohexol values obtained using deltaDOT's HPCE were slightly lower than those obtained with HPLC, calculating the rate of Iohexol loss (i.e GFR) from each of these values gave excellent agreement. The average bias in GFR measurements for the two methods was calculated at -0.12% ± 15%.

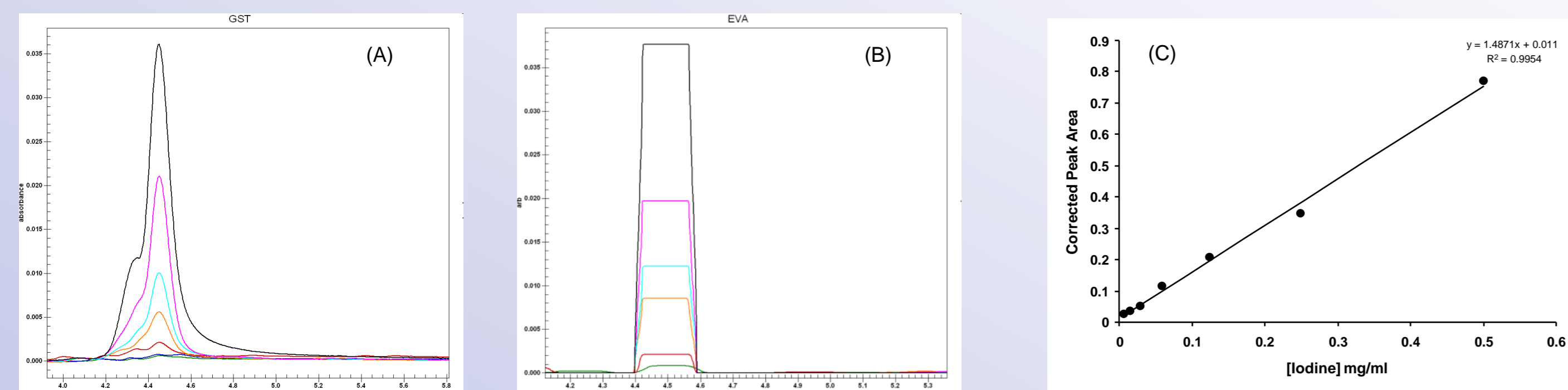


| Cat | HPLC - GFR values | deltaDOT HPCE - GFR values | Mean | Difference | % Relative Difference |
|-----|-------------------|----------------------------|------|------------|-----------------------|
| 1 | 1.35 | 1.59 | 1.47 | -0.24 | -16.36 |
| 2 | 0.82 | 0.92 | 0.87 | -0.10 | -11.44 |
| 3 | 1.68 | 1.18 | 1.43 | 0.50 | 34.59 |
| 4 | 1.64 | 1.54 | 1.59 | 0.09 | 5.87 |
| 5 | 2.54 | 2.27 | 2.40 | 0.27 | 11.35 |
| 6 | 1.13 | 1.42 | 1.27 | -0.29 | -22.83 |
| 7 | 1.78 | 1.62 | 1.70 | 0.16 | 9.42 |
| 8 | 1.50 | 1.71 | 1.61 | -0.21 | -13.13 |
| 9 | 3.11 | 2.79 | 2.95 | 0.32 | 11.02 |
| 10 | 2.25 | 2.23 | 2.24 | 0.02 | 0.98 |
| 11 | 2.12 | 2.44 | 2.28 | -0.32 | -14.07 |
| 12 | 2.51 | 2.33 | 2.42 | 0.18 | 7.43 |
| 14 | 2.76 | 2.88 | 2.82 | -0.13 | -4.45 |

(A) Bland-Altman plot of % Relative Iohexol concentration difference plotted against mean Iohexol concentration; (B) Comparison between observed Iohexol values for the HPLC method and deltaDOT's HPCE method for a particular cat; (C) Table depicting GFR values obtained using HPLC vs. deltaDOT's HPCE, which gives an average bias of -0.12 ± 15%.

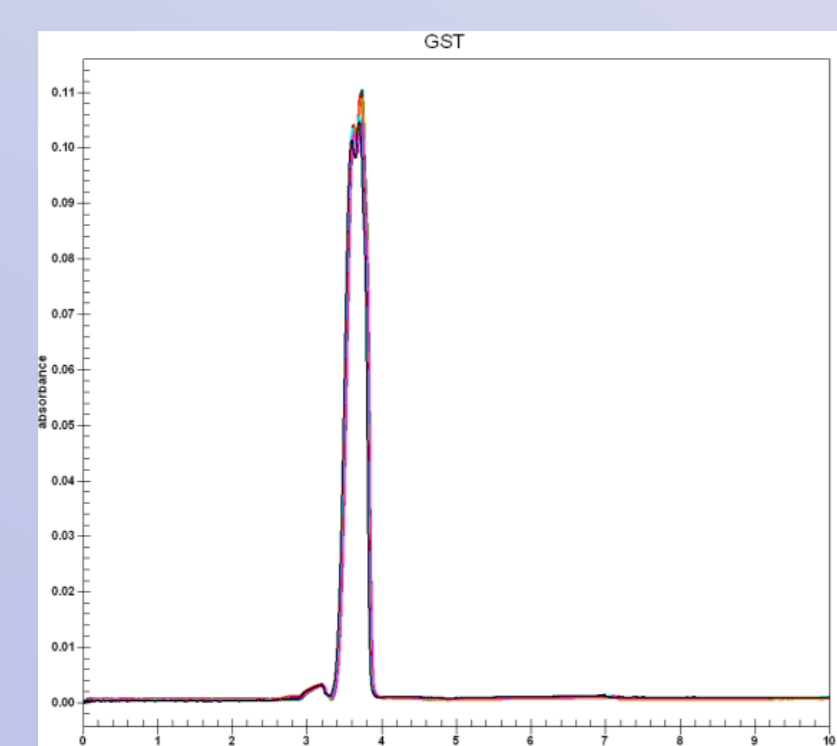
Calibration, Repeatability and Accuracy

No difference between samples stored in heparin tubes and samples stored in EDTA tubes was observed during the calibration process.



GST electropherogram (A) and EVA trace (B) depicting the separation of Iohexol spiked into feline serum. A calibration curve was generated from this data (C). No interference from the feline serum was observed.

Repeatability was assessed by calculating the relative standard deviation (RSD). % RSD values of 1.4% in terms of Iohexol concentration were observed demonstrating excellent repeatability of the system. Accuracy of measurements was determined by measuring the concentration of a 125µg/ml Iohexol solution and was calculated at 104%.



GST electropherogram depicting 6 consecutive injections of a 1mg/ml solution of Iohexol.

Summary

- High Performance Capillary Electrophoresis is a rapid and cost-effective method for assessing GFR in feline subjects.
- Minimal sample preparation is required prior to analysis, thereby decreasing the likelihood of any experimental bias.
- Excellent repeatabilities are achieved.
- This validation study has proven that GFR values obtained with this novel HPCE technique are comparable to traditional HPLC techniques, allowing for easy transfer from the standard protocols.
- A commercially available HPCE assay will allow easy next-day estimation of feline GFR in veterinary practice using only 3 samples taken after injection of an Iohexol bolus.
- We are currently working towards using this HPCE assay for monitoring GFR values using a single Iohexol measurement.

[1] Finch NC, Syme HM, Elliott J, Peters AM, Gerritsen R, Croubels S and Heiene R (2011) Glomerular filtration rate estimation by use of a correction formula for slope-intercept plasma Iohexol clearance in cats. *Am J Vet Res* 72:1652-1659.