A validated capillary electrophoretic method for determination of iohexol in canine and feline plasma

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Introduction
Renal function can be monitored by estimation of the glomerular filtration rate (GFR) i.e. by measuring the clearance of iohexol. There is a lack of a validated capillary electrophoretic method that covers the concentration range for full curve clearance estimate of iohexol (i.e. 18-2900 mg/L). Renal function can be monitored by estimation of the glomerular filtration rate (GFR) i.e. by measuring the clearance of iohexol. There is a lack of a validated capillary electrophoretic method that covers the concentration range for full curve clearance estimate of iohexol (i.e. 18-2900 mg/L).

Method validation
The validation was performed in accordance with the guidelines [2] and included selectivity, accuracy, precision, recovery, range, linearity, stability of stock and working solutions as well as stability of clinical samples and BGE, sample carry-over, dilution of concentrations above the highest calibration point.

Conclusion
The proposed method covers the needed calibration range and is in good agreement with a previous LC method [3]. The analysis is simple to perform and has a low cost per sample (2-4 EUR in material cost), which makes it applicable for routine analysis of clinical samples.

References
[1] Schröder Schöler (www.schroder-schoeler.com) The values are predicted and in water

Experimental
Capillary electrophoresis:
Agilent G7100A
L=59.5 cm, 68 cm, 25°C, λmax 245 nm
Injection: 8 sec 35 mbar, +30 kV, EOF-marker: acrylamide
Sample preparation (protein precipitation):
100 µL plasma + 200 µL MeOH with 0.5 mM iopromide (I.S.)
Background electrolyte:
Borate buffer (since it is known to complexate with vicinal diols)

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Influence of pH
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Influence of ionic strength
The resolution between iohexol and the internal standard was decreased over 0.04 M, Figure 3. However, 0.06 M was chosen in the final method due to the higher buffer capacity and the longer time between the first migration peak and the electro-osmotic flow.

Method comparison
The method comparison was performed by analysis of 32 clinical samples (from dogs and cats administered with Omnipaque®) with CE and a validated LC-UV method [3]. The two methods showed excellent agreement, Figure 6.

Figure 1. The influence of pH on the resolution between iopromide and iohexol
BGE: sodium borate buffer pH 10.0-10.5 at I=0.04 M (grey) and 0.06 M (green). Sample: iohexol 82 mg/L and iopromide 40 mg/L (0.05 mM)

Figure 2. The influence of pH on the effective mobility
BGE: sodium borate buffer pH 10.0-10.5 at I=0.08 M (green marks) and 0.04 M (grey marks). Sample: iohexol 82 mg/L and iopromide 40 mg/L (0.05 mM)

Figure 3. Typical electropherograms of dog- and cat plasma
BGE: sodium borate buffer pH 10.0, 82 mg/L iohexol and 40 mg/L iopromide

Figure 4. The influence of the ionic strength on the effective mobility of iopromide and iohexol
BGE: sodium borate buffer pH 10.0. Sample: iohexol 82 mg/L and iopromide 40 mg/L (0.05 mM) ECF-marker: 1.0 mM acrylamide

Figure 5. Passing-Bablok analysis of the method comparison
A Passing-Bablok analysis between plasma samples analyzed with the CE in the LC method, y = 1.0021x + 0.0049. The solid line represents the Passing-Bablok regression line and the dashed line represent the 95 % confidence interval.

Figure 7. A typical plasma elimination curve of iohexol in a cat

Table 1. Inter- and intra-day accuracy and precision
The replicates (Six at each concentration level) are taken from six spiked plasma samples and are processed and analysed within three subsequent days.

Table 2. Influence of pH on the resolution between iohexol and the internal standard
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Table 3. Influence of the ionic strength on the resolution between iohexol and the internal standard
The ionic strength (M) of the BGE, Figure 2

Table 4. The influence of pH on the resolution between iopromide and iohexol
The influence of pH on the resolution between iopromide and iohexol was decreased over 0.04 M, Figure 3. However, 0.06 M was chosen in the final method due to the higher buffer capacity and the longer time between the first migration peak and the electro-osmotic flow.

Table 5. Influence of the ionic strength on the resolution between iopromide and iohexol
The ionic strength (M) of the BGE, Figure 2

Table 6. The influence of pH on the effective mobility of iopromide and iohexol
The effective mobility (cm²/Vs)*105 of iopromide and iohexol was decreased over 0.04 M, Figure 3. However, 0.06 M was chosen in the final method due to the higher buffer capacity and the longer time between the first migration peak and the electro-osmotic flow.

Table 7. Influence of the ionic strength on the effective mobility of iopromide and iohexol
The effective mobility (cm²/Vs)*105 of iopromide and iohexol was decreased over 0.04 M, Figure 3. However, 0.06 M was chosen in the final method due to the higher buffer capacity and the longer time between the first migration peak and the electro-osmotic flow.

Table 8. The influence of pH on the effective mobility of iopromide and iohexol
The effective mobility (cm²/Vs)*105 of iopromide and iohexol was decreased over 0.04 M, Figure 3. However, 0.06 M was chosen in the final method due to the higher buffer capacity and the longer time between the first migration peak and the electro-osmotic flow.

Table 9. Influence of the ionic strength on the effective mobility of iopromide and iohexol
The effective mobility (cm²/Vs)*105 of iopromide and iohexol was decreased over 0.04 M, Figure 3. However, 0.06 M was chosen in the final method due to the higher buffer capacity and the longer time between the first migration peak and the electro-osmotic flow.