Development and validation of an LC-MS/MS method for simultaneous determination of iohexol and creatinine for GFR estimation in cats using VAMS samples

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Background

Chronic Kidney Disease (CKD) is the most common metabolic disease in cats. Unfortunately, unravelling the cause of the disease remains a challenge. As early diagnosed cats are prognostically in favor, early detection of CKD is crucial. Currently, the gold standard to assess renal function is measurement of glomerular filtration rate (GFR). However, this assessment still has important practical limitations. To overcome some of these drawbacks, microsampling can be used. Application of volumetric absorptive microsampling (VAMS) in feline nephrology would be of tremendous value, aligning with animal welfare and improving practical feasibility of GFR estimations.

Methods

In this context, we developed and validated an LC-MS/MS method for the simultaneous determination of iohexol and creatinine in plasma, blood and VAMS samples. Special attention was paid to matrix compatibility, as human blood is used to quantify cat samples. Furthermore, a clinical study was conducted in which 20 cats were enrolled to collect both conventional venous blood, plasma and VAMS samples in parallel at 8 different time points for GFR measurement. Clinical method validation was performed using the validated methods, including comparative evaluation of plasma, liquid and dried blood (VAMS samples).

Results

The LC-MS/MS methods were developed and validated based on international guidelines, also taking into account DBS-specific parameters. The methods fulfilled all pre-set validation acceptance criteria. For VAMS, reproducible (CV<15%) IS-compensated relative recovery values were obtained, showing no hematocrit-dependence (compared to a hct of 0.35 L/L). The analysis of the clinical study samples is currently ongoing. Results from this will be presented at the conference.

Conclusions

In conclusion, a microsampling protocol was established for validation of the methods to minimize the use of cat blood. The framework of this study provides a reference for future studies, facilitating implementation of the 3Rs principle in bio-analytical method validation. Application of the methods on real GFR samples will point out the suitability of this VAMS technology as an alternative for conventional quantification of creatinine and iohexol in plasma.

Key Words

Volumetric absorptive microsampling (VAMS), glomerular filtration rate (GFR), chronic kidney disease (CKD)