Optimization of SPE clean up and validation of quantitative determination of corticosteroids in urine by LC-MS/MS

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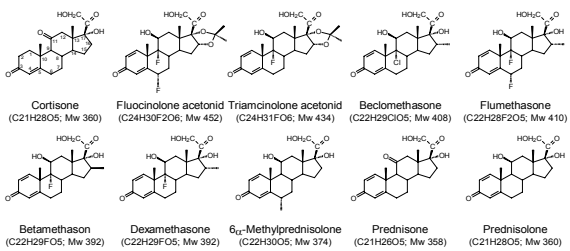
Introduction

A solid phase extraction (SPE) method for extraction and clean up of nine synthetic corticosteroids from porcine and bovine urine has been optimised for quantification by reversed-phase high-performance liquid chromatography negative electrospray ionisation mass spectrometry (LC-MS/MS).

The final method has been validated for urine according to EU regulations[1] for determination of residues of veterinarian drugs in products of animal origin.

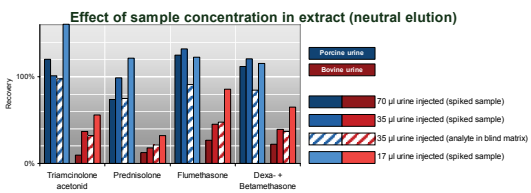
Cortisone and corticosteroids in the method



Experiments with clean up

Wash: Water. Elution: Methanol:

Initially, acceptable recoveries with negligible matrix interference for five synthetic corticosteroids (triamcinolone acetonid, flumethasone, dexamethasone, betamethasone and prednisolone) were produced from porcine urine (absolute recoveries: 74-125%) with a simple clean up procedure based on application of urine on a mixed mode polymeric strong anion exchange SPE column (Oasis MAX), washing with water and elution with methanol. From bovine urine, however, recoveries were low (10-27%).

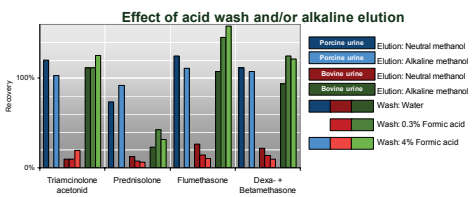


Dilution experiments showed that the low recovery from bovine urine was caused by suppression of the signal during the measuring process and not due to losses during clean up.

Washing with acid or alkaline solutions in combination with elution with neutral methanol showed little effect towards reducing the suppression.

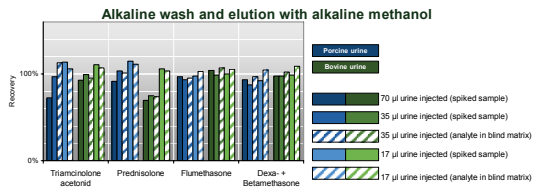
Wash: Neutral or acid water. Elution: Alkaline methanol:

By elution with alkaline methanol (0.1M ammonia in methanol), suppression was effectively eliminated for all analytes except prednisolone.



Wash: Alkaline water. Elution: Alkaline methanol:

By combining an alkaline washing procedure (0.1M ammonia in water) with elution with alkaline methanol (0.1M ammonia in methanol), suppression also decreased for prednisolone, and by reducing the amount of cleaned up sample injected to the chromatographic system from the equivalent of 70 µl urine to approximately 20 µl, suppression of prednisolon was minimized (absolute recoveries from porcine urine: 92-115%, from bovine urine: 99-111%).



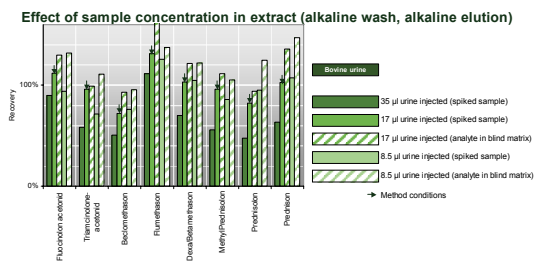
Final method

Hydrolysis:

To include conjugated corticosteroids in the analysis, the sample was hydrolysed with Helix Pomatia ß-glucuronidase/aryl sulfatase. This process showed some effects on the recoveries, so for the final method, which also included fluocinolone acetonid, 6α-methylprednisolone, beclomethasone and prednisone, a quantification based on spiked samples put through the entire analytical procedure was used. For quantification of triamcinolone acetonid an internal standard (triamcinolone acetonid-D6) was used.

Clean up:

3 ml of centrifuged urine was brought to pH 5.1 with acetate buffer, hydrolysed over night at 37°C with Helix Pomatia β-glucuronidase/aryl sulfatase and centrifuged. A SPE-column (Oasis MAX, 150 mg) was conditioned with methanol, water, and 1 ml 0.33M acetate buffer. 3 ml of the hydrolysed urine was loaded onto the column. After washing with water and 6 ml 0.1M ammonia in water, corticosteroids were eluted with 2 ml 0.1M ammonia in methanol. After evaporation (40°C, nitrogen) and dissolution with 300µl acetonitril and 900µl 0.1% formic acid, corticosteroids were quantified by LC/MS-MS.



LC/MS-MS:

LC-system: Agilent 1100; 10µl injected on a Zorbax Eclipse XDB (2.1x100 mm, 1.7µm), 40°C; eluent: acetonitril/0.1% formic acid (3+7) at 0.22 ml/min.
MS: Waters Micromass Ultima (ESI-); two MS-MS daughter ions for each compound.

Validation

During validation, the method showed average *relative recoveries* from 96 to 103% with the exception of beclomethason (113%).

Average *absolute recoveries* were 81-99%.

Internal reproducibility, determined by triplicates from spiking at three different levels in six analytical series was 7-19% (at 2-4 µg/l) except for prednison and prednisolon (26-27% at 3-6 µg/l).

Quantification limits (decision limits[2], CCα at 1%) were demonstrated (by spiking 8-21 samples at CCα) to be not higher than 1 µg/l (3 µg/l for prednison and prednisolon).

Detection capabilities[2] (CCβ at 5%) were demonstrated (by spiking 11-21 samples at CCβ) to be not higher than 1,5 µg/l (4.5 µg/l for prednison and prednisolon).

[1] European Commission Decision 2002/657/EC, Off.J.Eur.Commun.L 221, (2002) 8.

[2] Capability of detection – Part 1. ISO 11843-1:1997, Geneva 1997.