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An LC–MS/MS method for the determination of budesonide and 16α-hydroxyprednisolone in dog plasma

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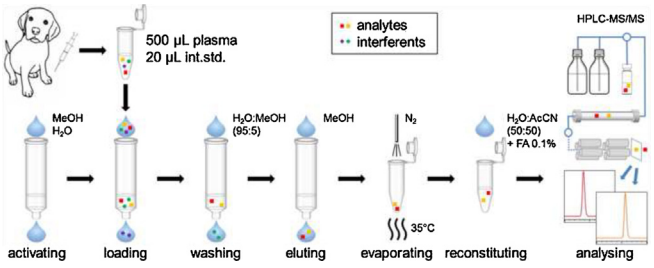
GRAPHICAL ABSTRACT



ABSTRACT

Although budesonide is frequently used in veterinary medicine for the treatment of canine respiratory and bowel inflammatory diseases, knowledge is lacking regarding its kinetics in this species. We developed and validated a liquid chromatography–tandem mass spectrometry method for the determination of budesonide and its metabolite 16α-hydroxyprednisolone in dog plasma. The analytes were extracted by solid phase extraction and analysis was performed by high performance liquid chromatography–tandem mass spectrometry, with positive electrospray ionization.

- This method allows budesonide and one of its main metabolites to be simultaneously quantified in dog plasma at fairly low concentrations.
- The proposed protocol is very easy and fast to execute, without compromising analytical performances.

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- A small amount (0.5 mL) of plasma is required, making this approach suitable for pharmacokinetic studies also in small sized dogs.

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Method details

An LC–MS/MS method for the quantitative determination of budesonide (BUD) and one of its main metabolite, 16α-hydroxyprednisolone (16α-HP), in dog plasma was developed and validated. The simultaneous quantification of the two molecules, the rapidity and simplicity of the procedure, and the low volume of sample required make the proposed technique suitable for pharmacokinetic studies involving also small-sized dogs. In addition, the developed protocol was employed in a clinical trial on dogs of different breeds, orally treated with BUD once a day for 30 days, demonstrating to be suitable for the purpose [1].

Materials

BUD and betamethasone (BET) standards were purchased from Sigma–Aldrich (Milan, Italy); 16α-HP was purchased from Du-Hope International Group (Nanjing, China). The internal standard solution was prepared diluting BET in water:acetonitrile (1:1) at a concentration of 50 ng/mL.

Sample preparation

1. Add 20 μL of BET internal standard solution to 0.5 mL of plasma.
2. Activate the Strata-X cartridge (1 mL, 30 mg) (Phenomenex, Torrance, USA) with 1 mL of methanol and wash with 1 mL of water.
3. Load the sample on the cartridge, at a flow speed of 2 mL/min.
4. Wash the cartridge with 3 mL of a solution of 5% methanol in water.
5. Dry the cartridge under low vacuum conditions for 30 s.
6. Elute the analytes with 1.5 mL of methanol at a flow speed of 2 mL/min, and completely remove the remaining solvent through vacuum.
7. Evaporate the eluate to dryness under nitrogen stream and heating at 35 °C.
8. Dissolve the sample in 100 μL of a mixture of water: acetonitrile (1:1) containing 1% formic acid and vortex for 30 s.
9. Transfer the reconstituted extract to autosampler vial for LC–MS/MS analysis.

LC–MS/MS conditions

The liquid chromatograph was an Alliance 2695 system consisting of a quaternary pump, solvent degasser, auto sampler and column heater (Waters Corporation, Milford, USA). Chromatographic separation was achieved on a Waters XBridge MS C18 column (3.5 μm, 2.1 mm × 150 mm) in combination with a protecting guard column (3.5 μm, 2.1 mm × 5 mm) of the same type (Waters Corporation, Milford, USA). The mobile phase was water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B).

The following gradient program, time (% A − % B), was applied: 0 min (90–10), 5 min (10–90), 8.5 min (10–90), 9.5 min (90–10), 13.0 min (90–10). The flow rate was 0.3 mL/min and the column temperature was maintained at 35 °C. An example of the obtained chromatograms is shown in Fig. 1.