Poster

DEVELOPMENT AND PRELIMINARY VALIDATION OF A MULTICLASS METHOD FOR THE DETERMINATION OF BANNED SUBSTANCES AND CORTICOSTEROIDS IN ANIMAL URINE BY LC-MS/MS

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Scope. In the European Union, monitoring prohibited substances and authorized veterinary drugs in livestock is essential to protect public health and ensure food safety. Traditionally, official control is performed with several different analytical methods to detect all the required classes/compounds. The aim of this work was the development and validation of a multiclass method for the simultaneous determination of 34 substances belonging to 5 different classes in animal urine: 13 β-agonists, 9 corticosteroids, 3 resorcylic acid lactones (RALs), 6 sedatives, and 3 stilbenes.

Methods. The deconjugation step was carried out adding 0.8 mL of acetate buffer 0.2 M (pH 5.2) to 1 mL of centrifuged urine and adjusting the pH at 5.2 with acetic acid 1 M. Then 20 µL of beta-glucuronidase from Helix pomatia were added and the sample was placed at 55 °C for two hours. After cooling, the pH was adjusted to 10 ± 0.2. Sample cleanup was performed by Simplified Liquid Extraction (SLE) using Novum (12 cc) column (Phenomenex). Analytes were eluted with 10 mL of tert-butyl methyl ether, evaporated at 40 °C, and the dry residue was redissolved in 200 µL of a water/methanol (80:20, v/v) mixture containing 0.1 % acetic acid. Instrumental analysis was carried out using an Exion LC™ system coupled to a triple quadrupole/linear ion trap mass spectrometer QTRAP 6500+ (SCIEX) operating in both positive and negative electrospray ionization modes. Chromatographic separation was optimized using two targeted injections (Kinetex XB-C18 100 Å, 100 x 3 mm, 2.6 µm, Phenomenex): a multi-analyte run with acidified mobile phases (0.1% acetic acid in both water and acetonitrile) for 13 β-agonists, 9 corticosteroids, 3 RALs, and 6 sedatives, and a dedicated run with non-acidified mobile phases specifically tailored for 3 stilbenes to ensure optimal ionization. Preliminary validation experiments were carried out according approach reported on the guidelines of Regulation (EU) 2021/808. Selectivity, linearity, matrix effect, trueness (recovery), intra-laboratory reproducibility, decision limit (CCα), were evaluated across three independent validation series. Lowest Calibration Levels (LCL) are set at 0.1, 0.45, or 0.9 µg/L based on the specific Minimum Method Performance Requirements (MMPRs).

Results. The marked differences in the physicochemical properties of the analytes made selective clean-up particularly challenging. After the development, preliminary results obtained in intra-laboratory reproducibility conditions (7 replicates in 3 different days) demonstrated recovery > 50 % for all analytes and precision expressed as coefficients of variation < 20 % for the majority of the analytes. Moreover, good selectivity and linearity were observed.

Conclusions. Developing this method was highly challenging, due to the matrix complexity and the extremely low detection limits of the analytes (i.e., sub-parts-per-billion). The preliminary results demonstrate that the developed method has the potential to be fit-for-purpose as qualitative confirmation method according to Commission Implementing Regulation (EU) 2021/808. Once completely validated, the implemented analytical approach could offer a significant reduction in time and cost per sample compared to single-class determinations.