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WIDE-RANGE SCREENING OF BANNED SUBSTANCES IN BOVINE URINE

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Introduction

Since 1988, in the European Union the monitoring plans to detect the illicit use of growth promoters in farm such as steroids, stilbenes or thyreostats play an important role to protect the public health. Most of controls targeting group A substances (banned) are carried out on urine, that is the matrix of election, since it is relatively easy to collect and samples can be taken before slaughter avoiding contaminated meat reaches the market. The strategy commonly used for their determination in the official control laboratories is the application, at first, of a screening test followed by a confirmatory method in case of suspect sample(s). The routine activities are generally carried out using several single-class methods to cover all the required classes with high times of analysis and costs. In addition, each method requires a preliminary intensive work to be developed, validated and to obtain its accreditation. A way to improve the cost-effectiveness of these controls can be the development of a wide-range multi-class screening method in urine. In fact, in the last years this strategy has been successfully realized for other groups of residues (veterinary drugs, pesticides and mycotoxins), mainly thanks to the tremendous improvement of chromatographic and mass spectrometric equipment.
This work describes the development and validation of a targeted screening procedure for 52 banned compounds in bovine urine: lactones of the resorcylic acid (RALs), nitroimidazoles, steroids, stilbenes, dapsone and chloramphenicol. In addition, two classes belonging to group B, such as corticosteroids (B2f) and sedatives (B2d), were included, too. The method was developed using liquid chromatography coupled to hybrid high-resolution mass spectrometry (LC-Q Exactive).

Experimental

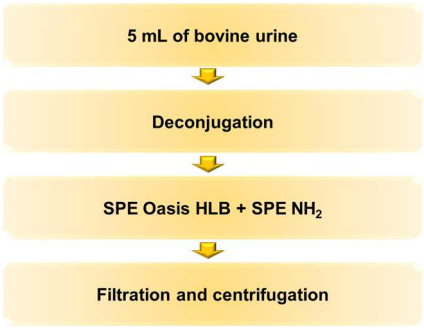

Figure 1 - *Flow diagram of sample preparation*

MASS SPECTROMETER: **Q-Exactive (quadrupole-Orbitrap) - Thermo Scientific**			
LC SYSTEM: **Ultimate 3000TM - Thermo Scientific**			
Column	Kinetex XB - C18 100A (100 x 3.0 mm, 2.6 µm, Phenomenex)		
Flow	0.4 mL/min	**Injection volume**	10 µL
Mobile phase	CH_3CN with 0.1 % Acetic Acid / H_2O with 0.1 % Acetic Acid (HESI+)		**BETA-AGONISTS, CORTICOSTROIDS, DAPSONE, NITROIMIDAZOLES, SEDATIVES STEROIDS**
	CH_3CN / H_2O (HESI-)		**CHLORAMFENICOL, RALs, STILBENES**

The validation study was carried out following Commission Decision 2002/657/EC (2002). Bovine urines from different animals were spiked at three levels: 1, 2 and 5 µg/L. At the same time, the same urines were analyzed without any spiking for a total of 126 analyses (63 urine samples). The percentage of false negative (FN) results was assessed to estimate the detection capability (CCβ) of each analyte.

Results and Discussion

Because of the several interfering substances, the Parallel Reaction Monitoring (PRM) was adopted as acquisition mode, since the use of MS full scan did not permit to achieve the required concentrations (CRL Guidance Paper, 2007). After the method development, the validation data were evaluated to check the percentage of false compliant results at each validation level. The procedure was not suitable for beta-nortestosterone, diethylstilbestrol and stanozolol. For other six compounds further validation experiments are necessary since the tested levels (1, 2 and 5 µg/L) did not enable any definitive conclusion.
The main difficulty experienced during the method development was the dramatic difference among each individual sample (urine-to-urine). In Figure 2 an "in-house dirty urine" (unspiked and spiked at 2 µg/L) and the test material analyzed within the FAPAS Proficiency Test PT 02246 "Synthetic Hormones in Bovine Urine" (2014) are presented. The final report assigned the following values: 3.95 for methyltestosterone (MT), 2.65 for zeranol (ZER) and 7.58 µg/L for taleranol (TAL). In the chromatogram c), the MT signal is dramatically suppressed, whereas in the PT urine (chromatogram b) its peak is well detectable (3.95 µg/L). In the chromatograms e) and f) similar concentrations of ZER were present (2.65 *vs* 2 µg/L, respectively), but very different amount of signal: the ZER peak (16.43 min) in chromatogram f) is very close to limit of detection, whereas in chromatogram e) is about fifty-fold higher.

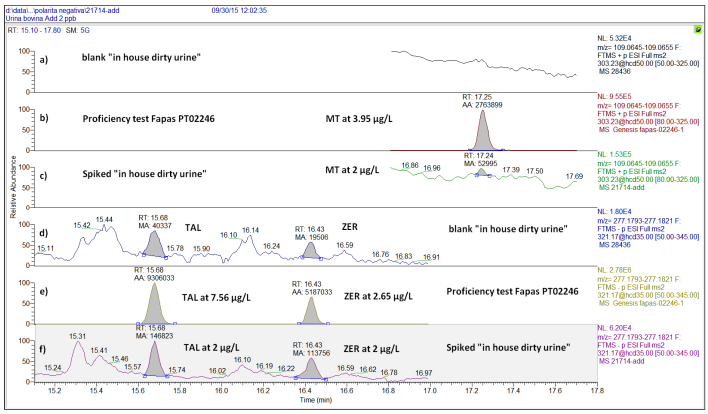

Figure 2 – *Comparison between an in house dirty urine and the urine of PT 02246 (FAPAS)*

Conclusions

The method here developed is suitable to detect 43 out of 52 investigated compounds. The MS acquisition mode chosen was PRM, which provided high selectivity because the MS/MS data were acquired in high resolution mode that could separate co-isolated background ions from the target analyte ions. Joining MS/MS experiments with high mass accuracy, suitable detection capabilities (CCβs) were obtained for most of the investigated substances. The price to pay was the loss of capacity to perform untargeted analysis and, consequently, to find unexpected contaminants, that is one of the most celebrated characteristics of HR analysers. The undoubtable gain was the high sample throughput. Increasing the number of substances simultaneously checked and decreasing the screening methods to apply in order to control the same set of compounds, a great improvement of the cost-effectiveness of the official controls could be obtained.

References

- Commission Decision 2002/657/EC of 12 August 2002. *Off. J. Eur. Communities* 2002; L22, 8-36.
- CRL Guidance Paper of 7th December 2007. CRL view on state of the art analytical methods for national residue control plan.

Acknowledgments
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