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Time-saving sample treatments for the screening of steroid esters in bovine hair

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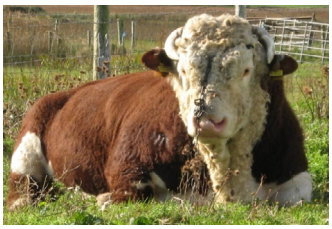
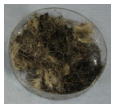
Introduction

In the EU the use of growth promoting agents is prohibited.
To monitor illegal use, hair of animals can be used as a matrix.
Hair can easily be collected, transported, stored and extracted.
The collection method is non-invasive.
Following the administration of veterinary drugs, the detection of these compounds in hair offers a retrospective detection (e.g. compared with urine, liver, kidney or fat).



The entire method of analysis currently used is very time-consuming. The collected hair is washed with water, dried, cut into pieces of < 1cm and pulverized in a ball mill. Furthermore there is a time-consuming solid-phase extraction (SPE) clean-up step.
In this study simplified extraction protocols were developed aiming at a reduced analysis time.

Sample treatment



Incurred bovine hair →
Estradiol benzoate ('EBe'; 5 & 300µg/kg)
Testosterone cypionate ('TCy'; 20, 820µg/kg)
Testosterone decanoate ('TDe';20, 530µg/kg)

Currently (screening & confirmation)	New, time saving (screening)
±3g. hair	200mg. unwashed & uncut hair
← Wash with water	← Add 10ml. DCM
← Dry 24h. at 45°C	← 10min. ultrasonification
Cutting & grinding ± 1g.	
200mg. ground hair	
← Digest 1h. with 25mM TCEP	
← Add MeOH	
← Centrifuge 5min. 1700G	
← Add water	
Polym. C18 SPE column	
← Activate with ACN, MeOH & H_2O	
← Apply hairmixture	
← Wash with MeOH/water (60:40, v/v)	
← Elute with ACN & ethylacetate	
Evaporate to dryness with N_2	Evaporate to dryness with N_2
← Reconstitute in 200µl. mob. fase	← Reconstitute in 200µl. mob. fase
	← Filter the extract
LC (UPLC™)-(ESI+)MS/MS	LC (UPLC™)-(ESI+)MS/MS

Results

By using several extraction techniques (solid-phase extraction (SPE), pressurised liquid extraction (PLE), PLE with diatomaceous earth and an ultrasonic water bath) (figure 1), with several extraction solvents (aceton, DCM, MeOH), DCM gives by far the best results.

Figure 1:

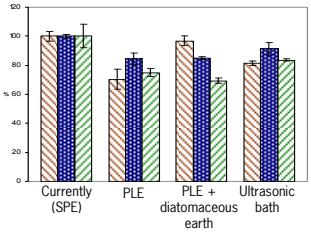


Figure 2:

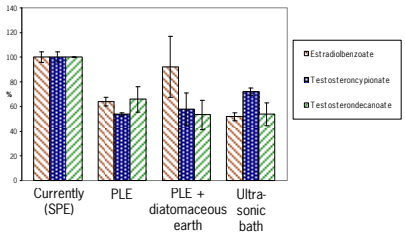


Figure 3:

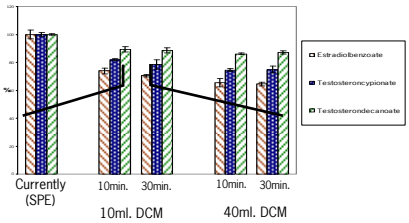
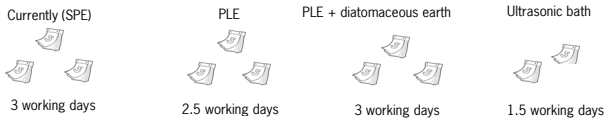


Figure 1: Comparison of several extraction techniques with the current (normalized) LC-MS/MS method in yield% for steroid esters (after correction for the internal standards) with corresponding standard deviations. High concentrations of steroid esters. Extraction solvent is DCM.
Figure 2: Same as figure 1, only with low concentrations of steroid esters
Figure 3: Comparison of several extractions in an ultrasonic bath with the current (normalized) LC-MS/MS method in yield% for steroid esters (after correction for the internal standards) with corresponding standard deviations. High concentrations of steroid esters. Extraction solvent is DCM.

To handle ten samples of hair (including standard addition, MMS-series, starting the LC-MS/MS, handling the data etc. etc.) the total analysis time it takes is approximately:



Conclusion

For screening purposes of representative steroid esters in hair of bovine calves, ultrasonification in dichloromethane is a useful approach: time-saving is about 50% compared to the currently used method. To further shorten the LC-time additional research can be done on a DESI-MS.

References

Stolker A.A.M., et all. Detectability of testosterone esters and estradiol benzoate in bovine hair and plasma following pour-on treatment. Analytical and Bioanalytical Chemistry, 395 (2009) 1075-1087.

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