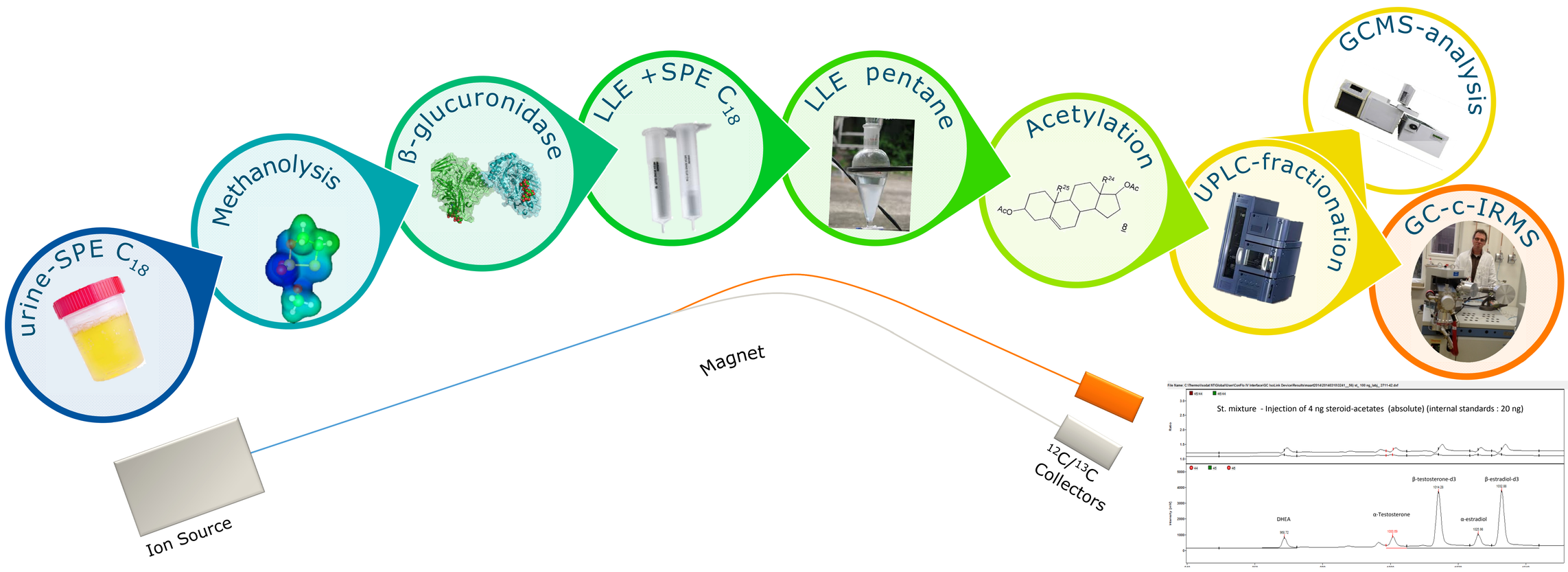
RIKILT
WAGENINGEN UR

GC-c-IRMS for analysis of natural hormone abuse in urines of cattle

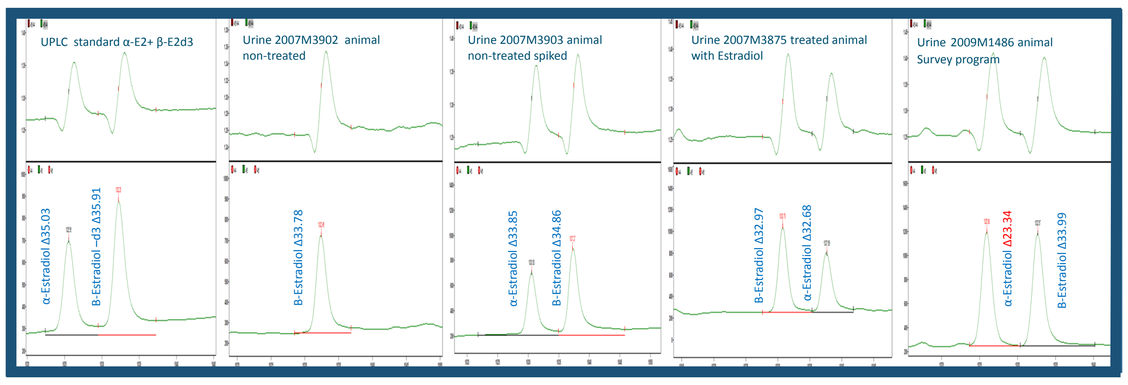
Paul Zoontjes, Hennie van Rossum, Marco Blokland, Leen van Ginkel, Saskia Sterk

Background

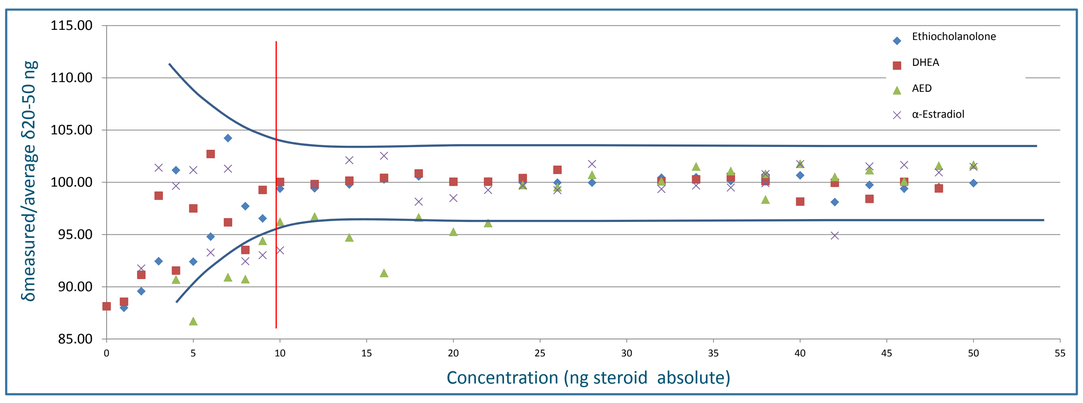
The use of hormones as growth promoters for fattening purposes in cattle has been banned in the European Union since the early eighties. Control of the illegal use of natural steroid hormones in cattle is still a challenge since no conclusive method and non-ambiguous analytical criteria are available. The ability of gas chromatography / combustion / isotope ratio mass spectrometry (GC-c-IRMS) to confirm the administration of estradiol and testosterone to cows has been investigated. This was done by comparison of the $^{13}C/^{12}C$ isotopic ratio of the main urinary metabolites, i.e. 17α-estradiol and 17α-testosterone, with endogenous reference compounds (ERCs) to differentiate the endogenous or exogenous origin. For this purpose sample clean-up methods were developed based on humane urine analysis protocols[1] which were used to determine whether an athlete has been abusing an exogenous natural hormone. Preliminary results obtained with the developed clean-up method (LC-fractionation using a UPLC column resulting in small (<0.3 min) selective fractions) and GC-c-IRMS analysis are presented.



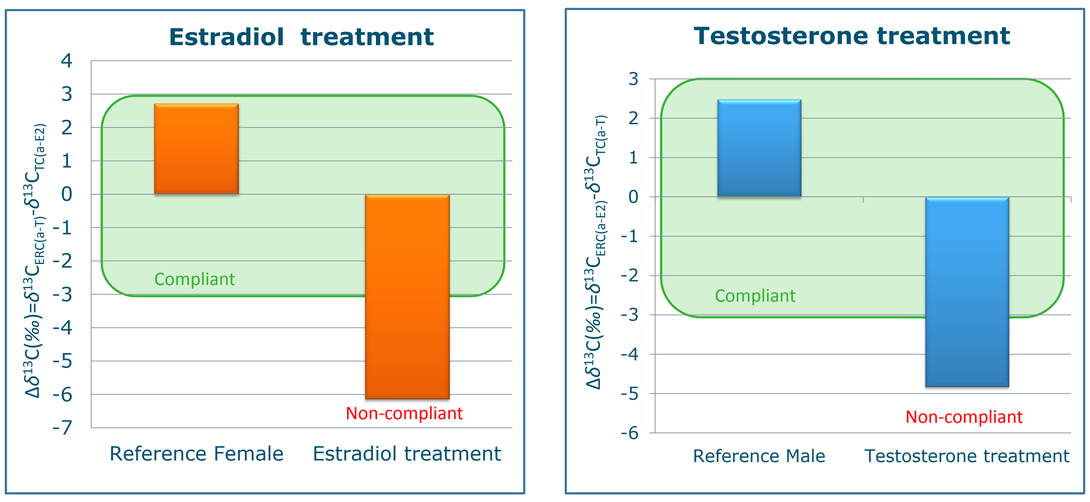
The figure below shows GC-c-IRMS chromatograms of a standard, urine of a non-treated animal, a non-treated-spiked urine, an urine of an estradiol treated animal and a suspected sample of urine (lower trace is ^{13}C, upper trace isotopic swing).



To determine the stability of the delta ($\delta^{13}C$) value of the GC-c-IRMS system, standards of different concentration (1-50 ng absolute) containing different steroids were analysed. Stable delta values were obtained > 10 ng steroids absolute injection.



Samples of urine from a bovine population with and without treatment of testosterone or estradiol were analysed and their corresponding $\Delta\delta^{13}C$ values were compared. A difference of more than 3 $\Delta\delta^{13}C$ value is considered proof of abuse[2]. See figure below for the average $\Delta\delta^{13}C$ values of these measurements.



Conclusions

- Delta values start deviating below 10 ng absolute steroids
- Sensitivity can possibly be improved by use of large volume injection
- High-resolution UPLC-fractionation gives clean extracts
- First experiments demonstrate that applied method gives a pronounced discrimination between exogenous and endogenous natural steroids

RIKILT Wageningen UR
P.O. Box 230, 6700 AE Wageningen
Contact: paul.zoontjes@wur.nl
T + 31 (0)317 48 04 37

Scan for PDF

Acknowledgment: This project was financially supported by the Dutch Ministry of Economic Affairs and the EC - DG SANCO

References
1) Steroids. 2012;77(11):1050-60
2) J Agric Food Chem. 2013;61(30):7242-9

European Union Reference Laboratory