



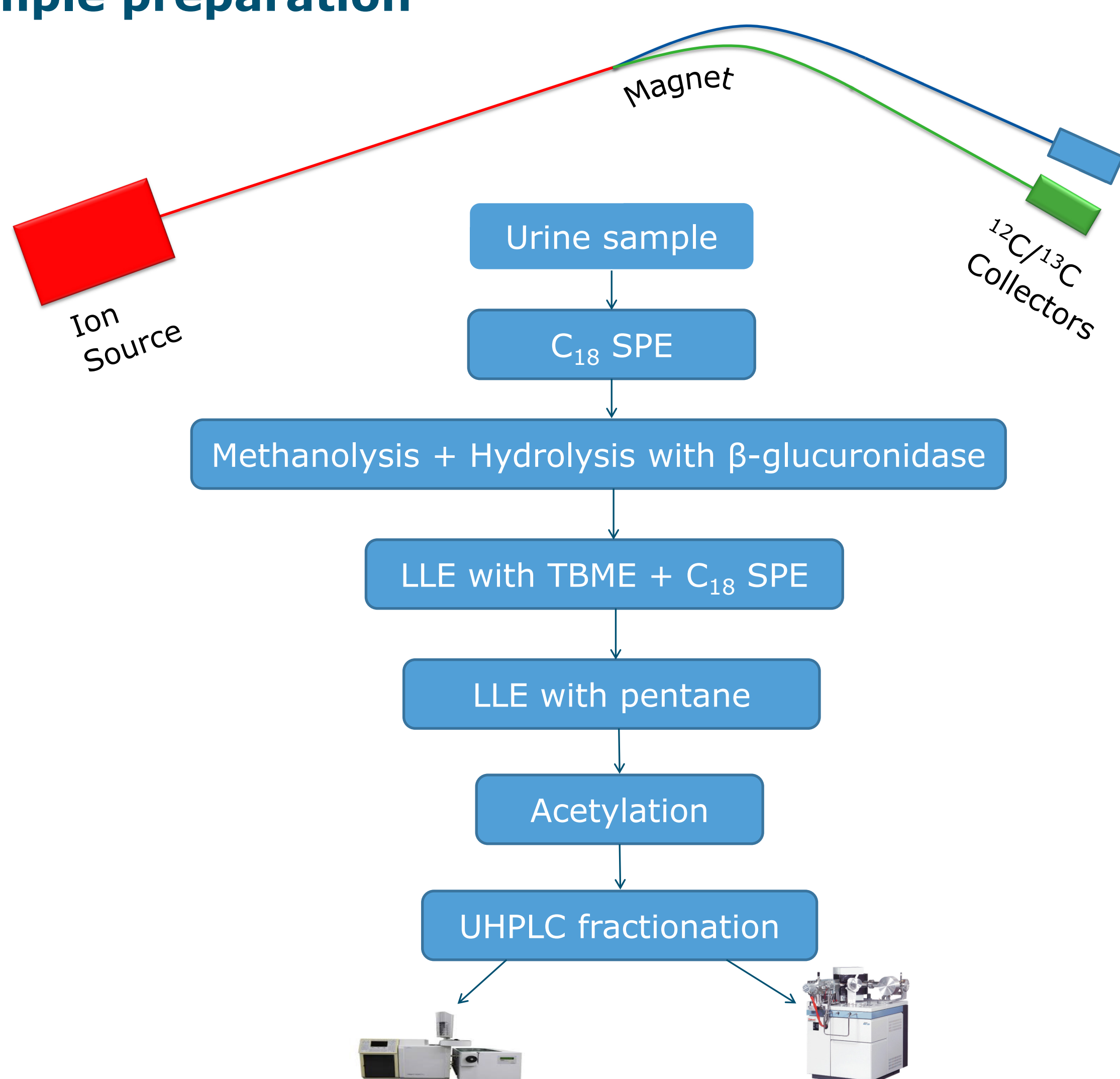
Application of GC-C-IRMS for the detection of natural hormone abuse in cattle

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

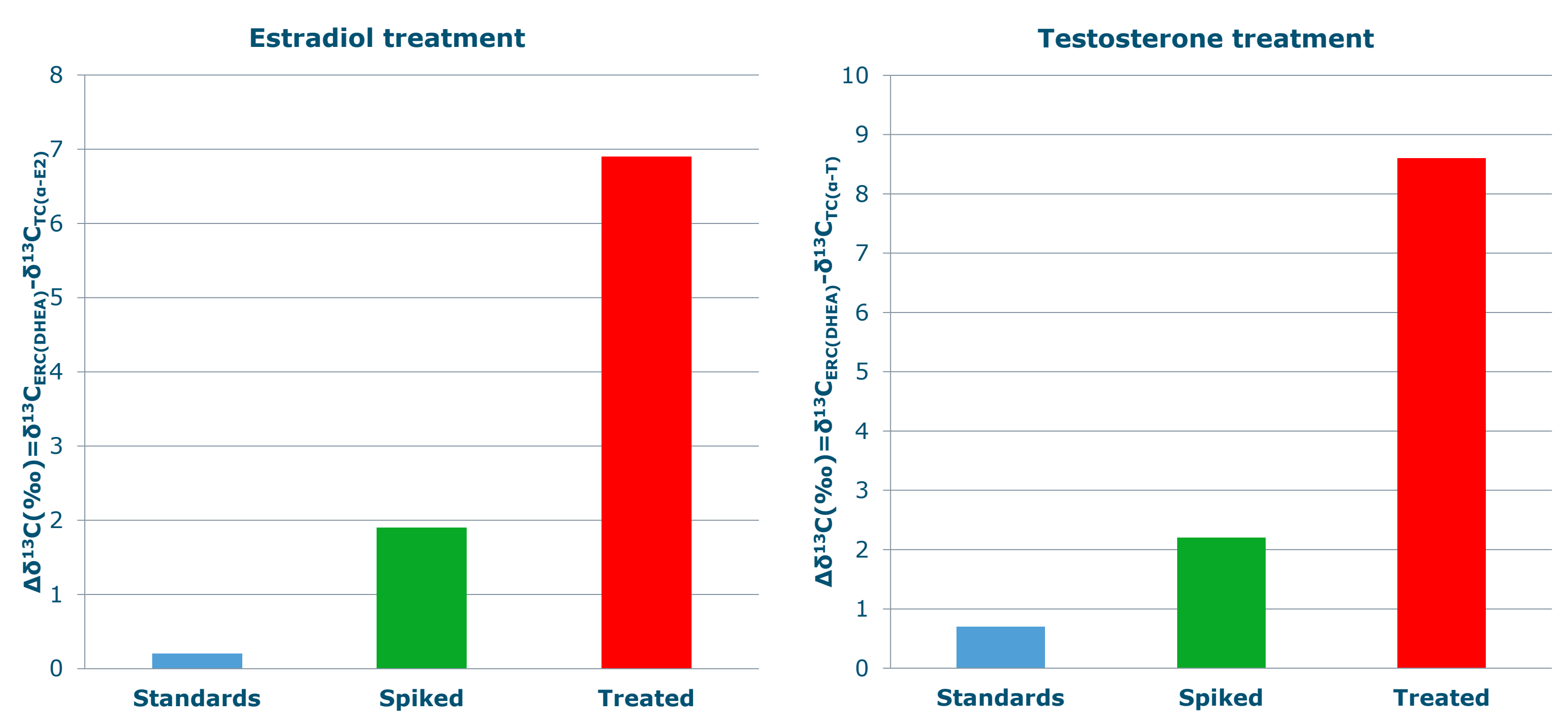
Background

The detection of natural steroid hormones originating from synthetic precursors has proven to be a challenge for the control of illegal use in livestock production. Endogenous steroid abuse can be confirmed by applying gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) that enables the accurate measurement of differences in the stable isotope ratio $^{13}\text{C}/^{12}\text{C}$. The $^{13}\text{C}/^{12}\text{C}$ isotope ratio of the main metabolites 17 α -estradiol and 17 α -testosterone were compared with dehydroepiandrosterone (DHEA) as an endogenous reference compound (ERC) to prove the exogenous and endogenous origin in cattle urine. The use of ERCs is necessary to compensate for variability of the $\delta^{13}\text{C}$ values mostly caused by differences in animal diet. Significant differences of $\delta^{13}\text{C}$ values between ERC and the target metabolite were observed, providing proof of administration. An effective clean-up procedure was developed based on UHPLC-fractionation of acetylated compounds. Large volume splitless injection was evaluated improving the overall sensitivity of the GC-C-IRMS technique.

Sample preparation



Results



For doping control purposes, WADA uses a threshold value of $|\Delta\delta^{13}\text{C}| > 3\text{‰}$ for non-compliant samples². By comparing urine samples from treated and untreated animals the resulting $|\Delta\delta^{13}\text{C}|$ values were 6.9‰ for 17 α -estradiol and 8.6‰ for 17 α -testosterone, clearly showing the capability of the method to detect positive samples.

Results

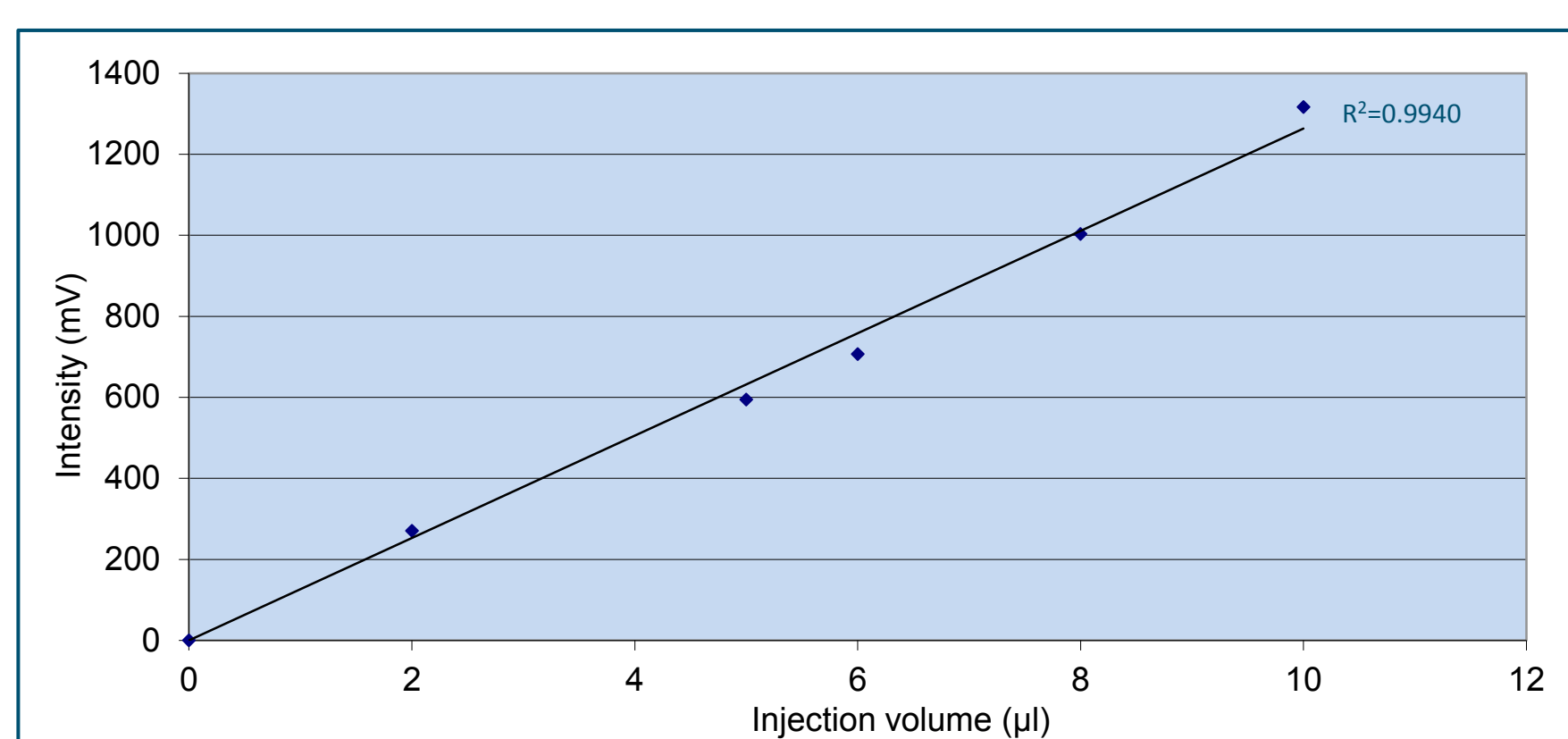


Figure 1. Linearity of large volume injection vs. intensity of a standard mixture.

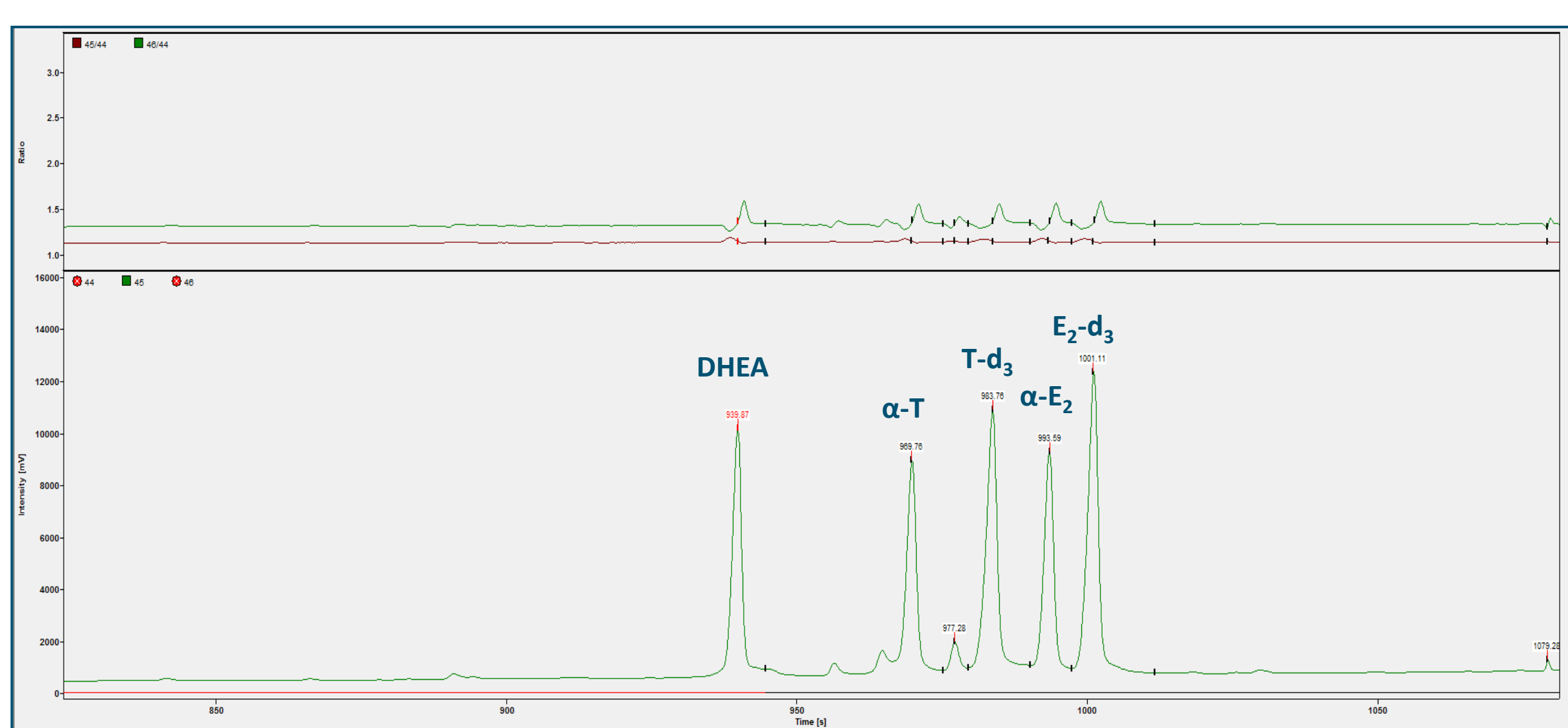


Figure 2. GC-C-IRMS chromatogram of a standard mixture (lower trace is ^{13}C and upper trace is isotopic swing).

Conclusions

- The GC-C-IRMS method proved its capability for the detection of steroid abuse.
- By the use of $\Delta\delta^{13}\text{C}$ values we successfully differentiated between treated and untreated samples for 17 α -testosterone and 17 α -estradiol.
- UHPLC-fractionation of the acetylated derivatives was of key importance providing clean final extracts and reducing the total time of the sample clean-up.
- Large volume splitless injection up to 10 μl improved the overall sensitivity of the method by a factor of 5 compared to 2 μl injection offering the possibility of confirmatory analysis at a detection limit of 5 ng mL⁻¹.

References

- 1) Steroids. 2012;77(11):1050-1060.

Acknowledgements

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

