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Liquid Chromatography-Isotope Ratio Mass Spectrometry in Food Residue Control

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Background

Residues that can also have an endogenous origin, need confirmative testing by carbon isotope ratio analysis. For this, gas chromatography-combustion-isotope ratio mass spectrometry (GC-c-IRMS) was traditionally used within Wageningen Food Safety Research (WFSR). However, GC-c-IRMS is often not applicable for non-volatile's or require derivatisation. To overcome this limitation for non-volatile's, liquid chromatography-isotope ratio mass spectrometry (LC-IRMS) can be used. This is a challenge, as the interface design between LC and IRMS requires the need for carbon-free eluents, thereby limiting usable LC separation techniques. Here we present preliminary results using LC-IRMS as a confirmatory technique for endogenous and/or exogenous steroids, antipyretics and thyreostats.

Detection limits of LC-IRMS

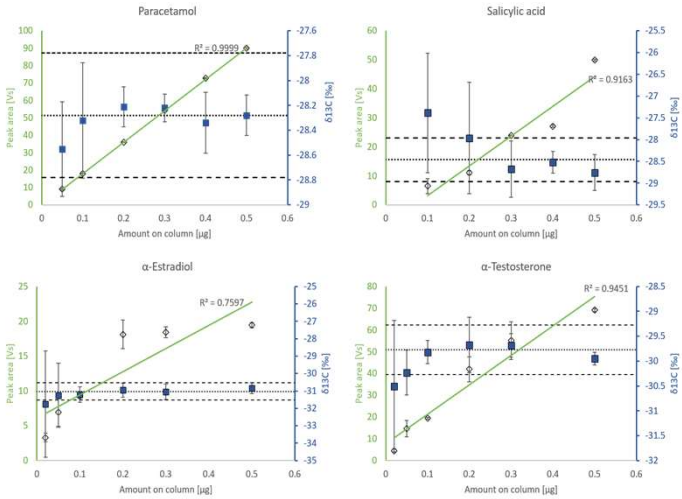

Figure 1. Determination of the detection limit for paracetamol, salicylic acid, α-estradiol and α-testosterone in LC-IRMS analysis using pure water as eluens. The squares represent the $\delta^{13}C$, diamonds the peak area. The linear curve fit of the peak area of $^{44}CO2$ and the correlation coefficient for plotting peak area vs. absolute amount are shown. Error bars indicate the SD of 5-fold measurements. The dotted line indicates the calculated mean value of $\delta^{13}C$. The horizontal dashed lines represent the interval of mean value ±0.5‰.

LC-IRMS chromatograms of steroids in bovine urine

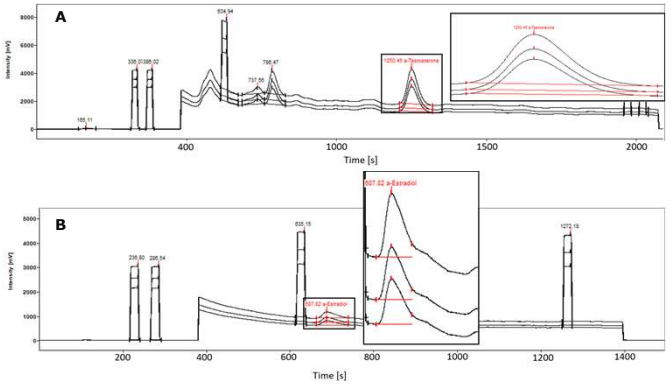

Figure 2. Chromatograms obtained by LC-IRMS of a bovine urine sample of A) α-testosterone (absolute 100 ng) spiked in bovine urine, and B) an estradiol treated animal. Chromatography performed using 10 mM phosphate buffer (pH 3.0) with a flow of 0.4 mL/min and a temperature gradient of 80 – 140 °C

δ13C differences natural and synthetic α-estradiol

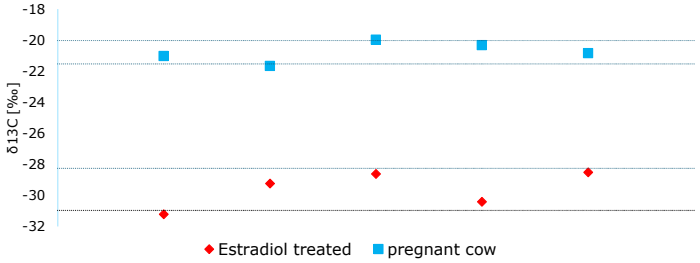

Figure 3. α-estradiol $\delta^{13}C$ difference between a blank urine batch from a pregnant animal and a blank urine batch from an estradiol treated animal. The dashed lines represents the 99% confidence intervals

Differences in $\delta^{13}C$ values between blank urine of a pregnant animal and an estradiol treated animal were established. Indicating the potential in differentiating between natural and synthetic homologues in animal husbandry and broadening the scope of IRMS as confirmatory analysis to polar non-volatile compounds.

Potential of high-temperature LC separation

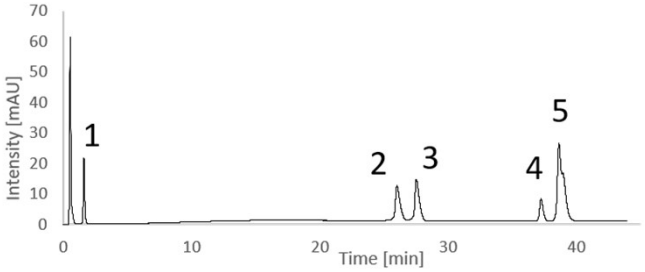

Figure 4. Chromatogram obtained by high-temperature LC photodiode array detection of 1) 2-thiouracil, 2) prednisolone, 3) prednisone, 4) α-zearanol, and 5) zearalenone. Chromatography performed using 10 mM phosphate buffer (pH 3.0) with a flow of 0.5 mL/min and a temperature gradient of 40 - 140 °C

Outlook

For LC-IRMS, samples need extensive clean-up and purification compared to LC-MS/MS confirmatory methods. But combined with carbon-free eluents, the present data demonstrates that LC-IRMS has potential to distinguish between the exo- or endogenous origin of substances in animal husbandry. Further research will be conducted to develop reliable LC-IRMS confirmatory methods for these substances.

Acknowledgements

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