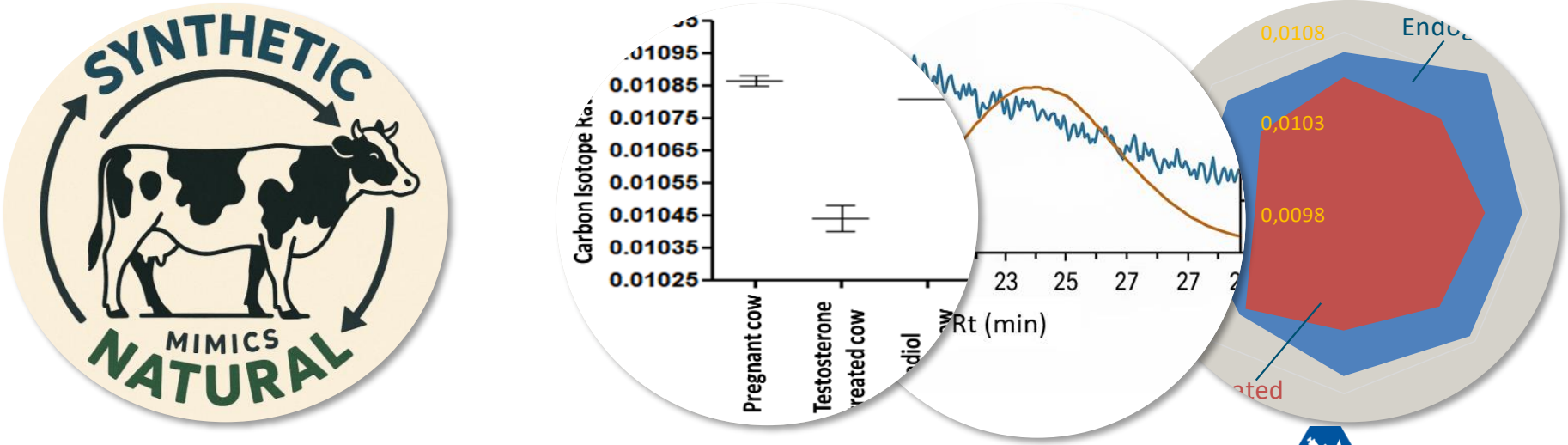
LC-ORBITRAP-IRMS FOR STABLE ISOTOPE RATIO MEASUREMENT?

Marco Blokland

Wageningen Food Safety Research



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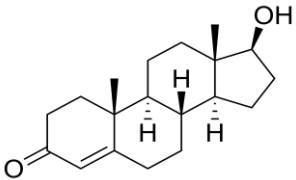
TRACES

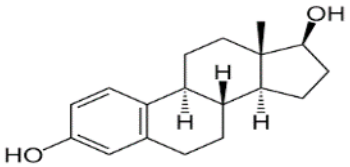
5 June 2025

Key challenges in residue analysis

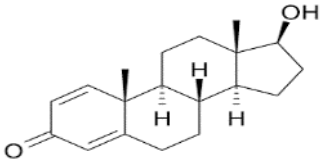
Distinguishing natural vs. synthetic origin



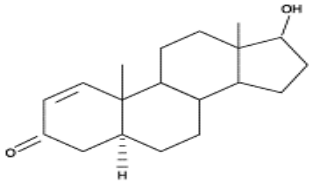
Testosterone



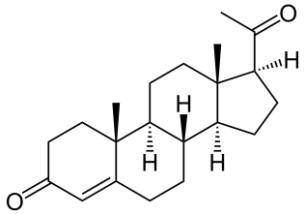
Estradiol



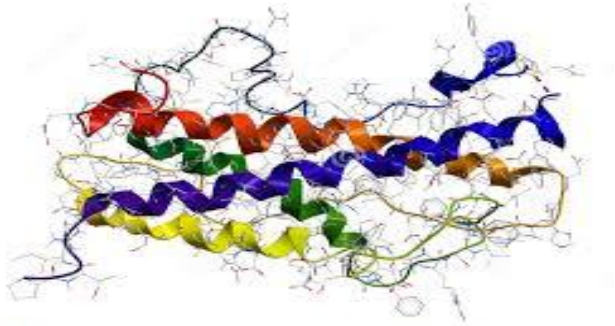
Boldenone

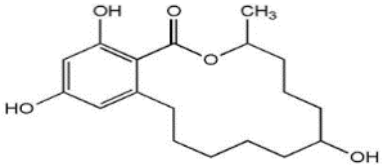


1-Testosterone

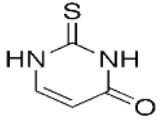


Progesterone

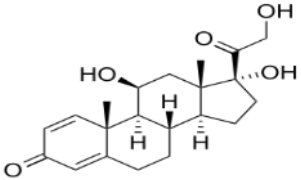

Somatotropin



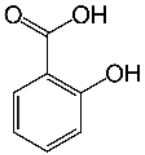
Zeranol



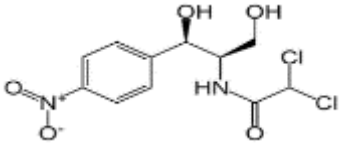
Thiouracil



Prednisolone



Salicylic Acid



CAP

Detecting synthetic analogs of natural compounds

Screening analysis:

- Detection of markers, for example 4-thiouracil together with 2-thiouracil
- Identify deviations from normal steroid profiles to flag anomalies

Confirmatory analysis

- Detect steroid-esters in diverse matrices: injection sites, hair, serum
- Isotope analysis using GC-c-IRMS

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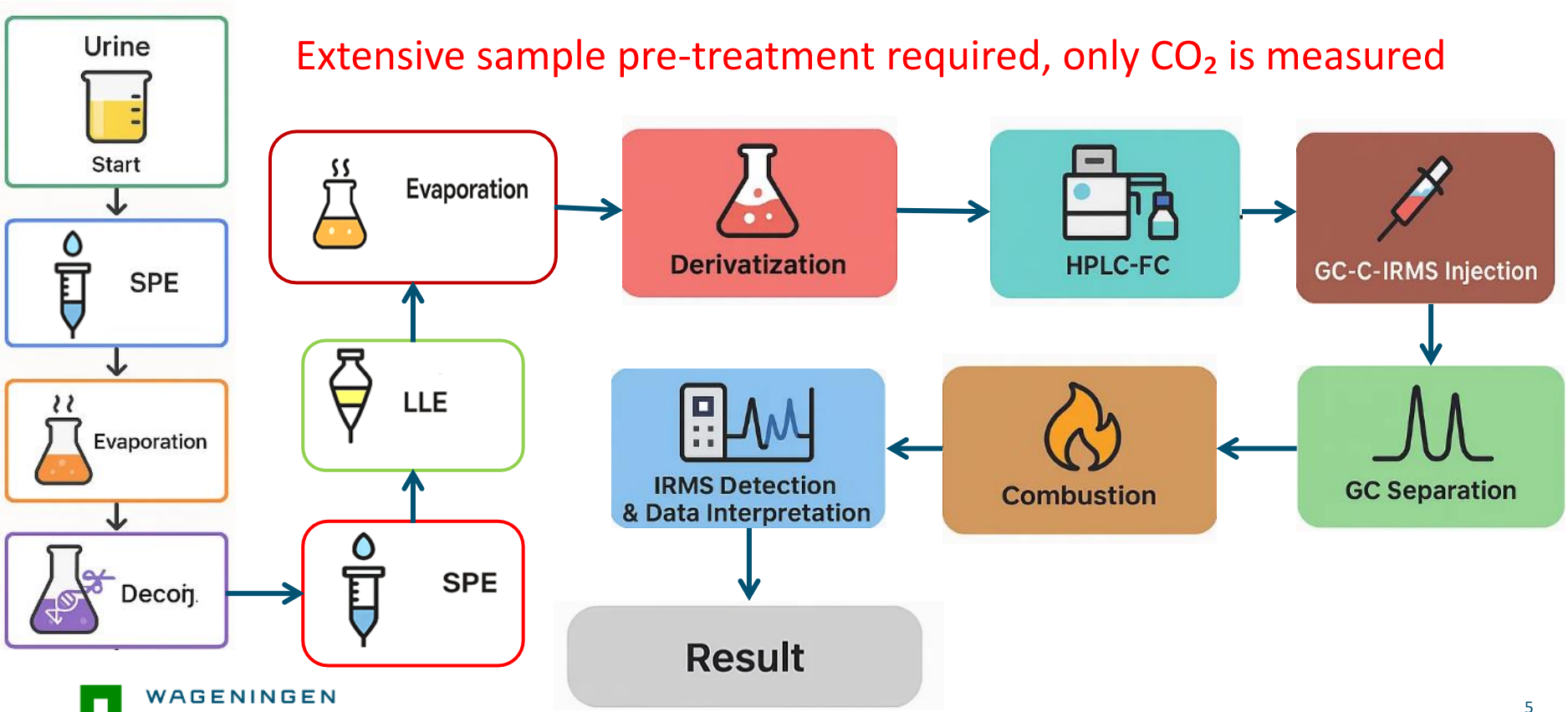
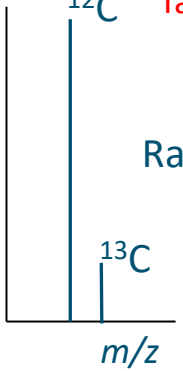
TOPICS FOR WHICH
GC-C-IRMS
CAN BE USED
OMPOUND-
SPECIFIC
POLLUTION
SOURCES
FOOD
AUTHENTICATION
DOPING
CONTROL

Sample analysis for GC-c-IRMS



Understanding IRMS language and criteria?

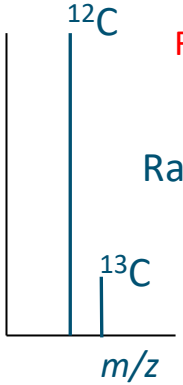
$R = \frac{^{13}C}{^{12}C}$ → IRMS outcome is denoted as $\delta^{13}C = \left(\frac{R\ Target\ Compound}{R\ Reference\ Standard} - 1\right) x1000 = xx\ ‰$



Target Compound

Ratio $\frac{^{13}C}{^{12}C}$ = 0.01080

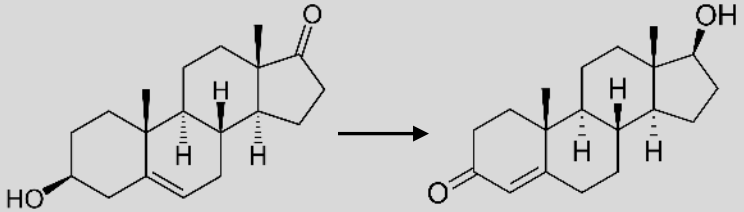
$\delta^{13}C = \left(\frac{0.01080}{0.01118} - 1\right) x1000 = -34\ ‰$



Reference Standard

Ratio $\frac{^{13}C}{^{12}C}$ = 0.01118

WADA criteria $\delta^{13}C$
(n ≥ 3) $\delta^{13}C$ = < 0.5 ‰



DHEA = ERC

Testosterone

$|\Delta\delta^{13}C| = |\delta^{13}C_{ERC} - \delta^{13}C_{TC}|$

Natural $\Delta\delta^{13}C| = |19 - 19| = 0$

After administration

$|\Delta\delta^{13}C| = |19 - 34| = 15$

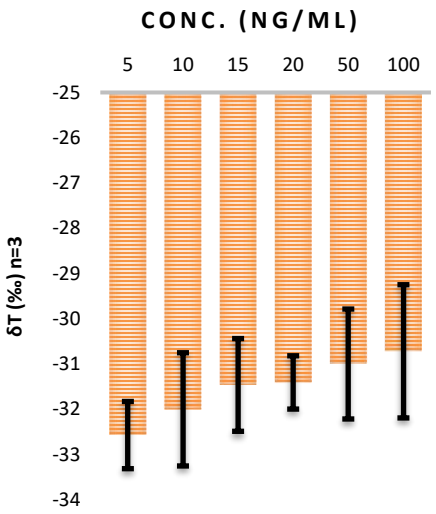
Non-Compliant

WADA criteria $\Delta\delta^{13}C > 3$ ‰

Examples of GC-c-IRMS results

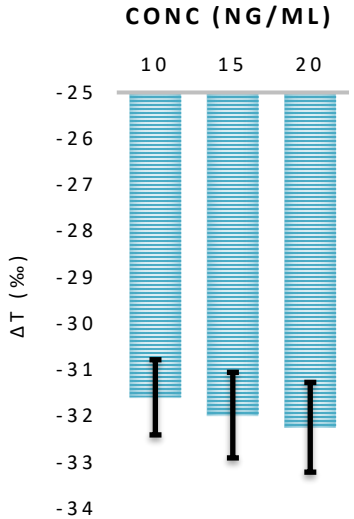
WADA criteria $\delta^{13}C$ ($n \geq 3$) $\delta^{13}C$ = < 0.5 ‰

Standard
α–Testosterone

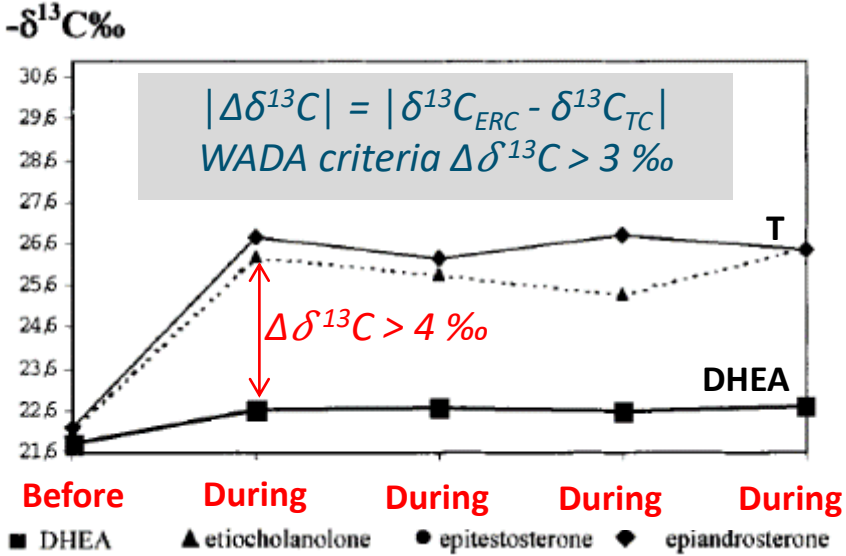


Average st.dev. $\delta^{13}C$=1.05 ‰

Spiked urine with
α–Testosterone



Average st.dev. $\delta^{13}C$=0.93 ‰



CV of $^{13}C/^{12}C$ = 0.08%

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Stéphanie Prévost, Tatiana Nicol, Fabrice Monteau, François André, Bruno Le Bizec
2001, Rapid Communications in Mass Spectrometry

Key Drawbacks of GC-c-IRMS

Highly Specialized Equipment:

Costly, niche IRMS instruments (high upfront/maintenance costs)

Molecular Information Lost:

Analytes converted to CO_2, destroys molecular structure/functional groups

GC-Dependent Specificity:

Relies on GC separation, co-elution risks mixed signals (mixed isotope ratios)

Lengthy Sample Prep:

Lengthy, expensive clean-up, rigorous purification required for accuracy

Expertise-Intensive:

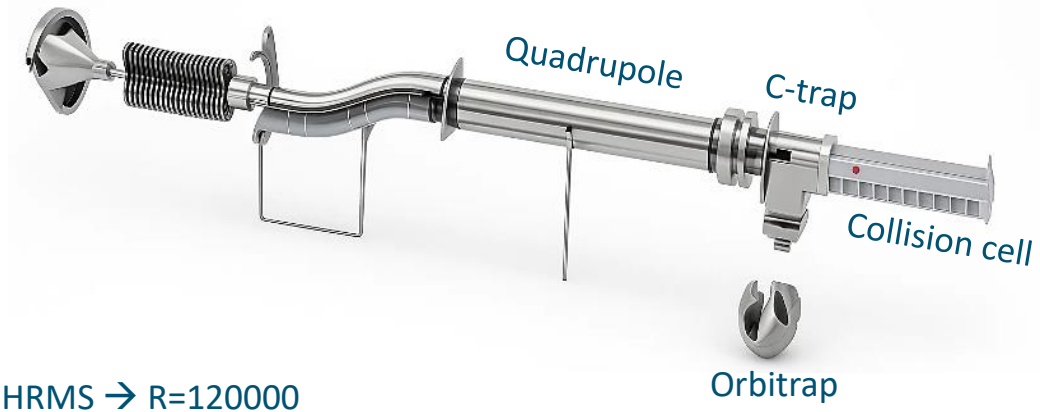
Requires expert operators, GC, IRMS, and data analysis skills

Nils Kuhlbusch (University of Münster & Thermo Fisher Scientific)

Presentation Topic:

Orbitrap Based Isotope Ratio Analysis of Caffeine in Complex Matrices

Electrospray



HRMS → R=120000

Pro's

- Compound-specific analysis without combustion retains molecular structure
- Simultaneous quantification of multiple isotopes (e.g. $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{2}H/^{1}H$) in a single run
- Expanded analytical scope with LC-HRMS

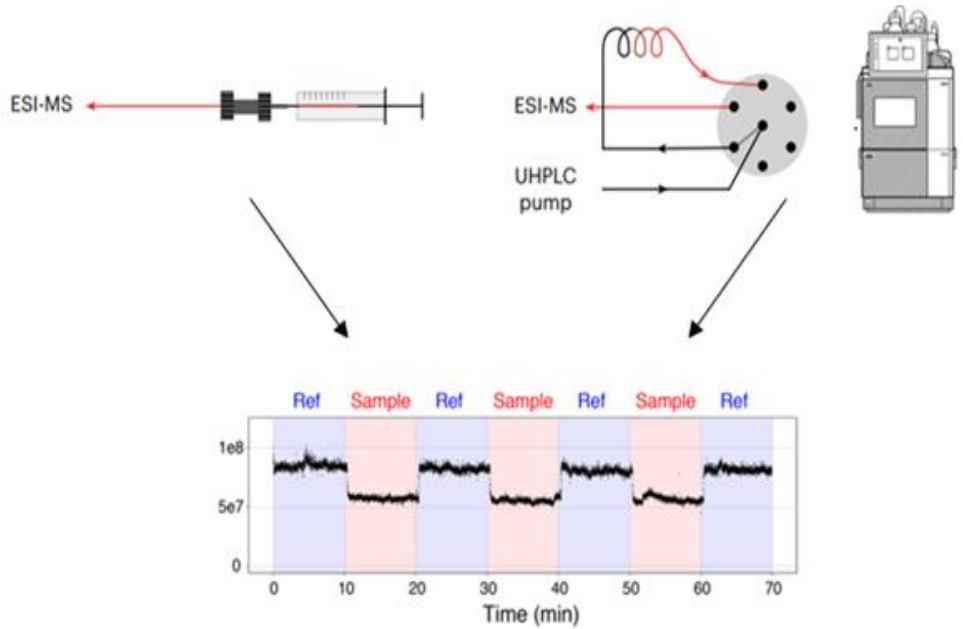
Con's

- Orbitrap-IRMS has a lower precision (±0.1–2‰) compared to GC-c-IRMS (±0.01–0.5‰)
- Requires careful Orbitrap optimization
- No experience on doping related questions using Orbitrap
- Long measurement times per compounds needed for stable isotope determination

Current state of the art LC-Orbitrap-IRMS

Direct Infusion

Flow Injection



- Need High Resolution & Mass Accuracy
- Optimized ESI Conditions, stable voltage, desolvation temperature, and solvent flow to minimize isotopic fractionation
- Avoid space-charge effects in the C-trap that distort isotope ratios
- Advanced algorithms for noise reduction and precise peak integration $N_{io} = \frac{S}{N} * \frac{C_N}{z} * \sqrt{\frac{R_N}{R}} * \sqrt{\mu}$
- Long measurement (many scans) for high-precision isotopic averaging

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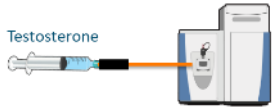
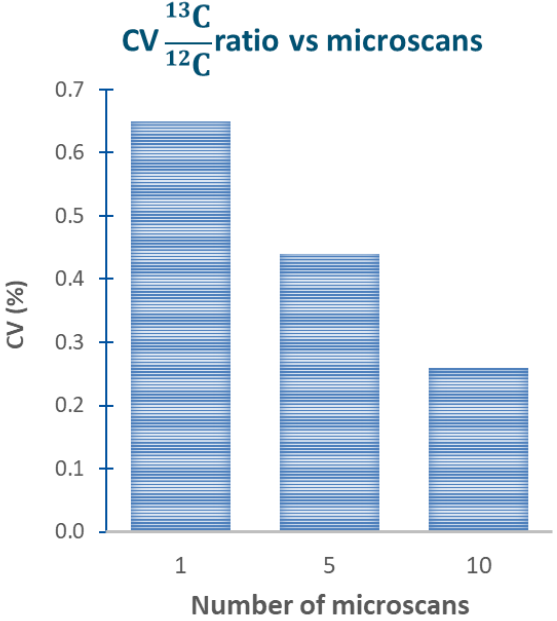
One and a half year research in a few slides



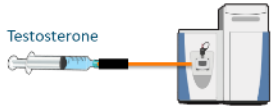
- Reproduce published results at our laboratory
- Infusion experiments of α–Testosterone on the LC-HRMS Exploris 120 (Q-Orbitrap)

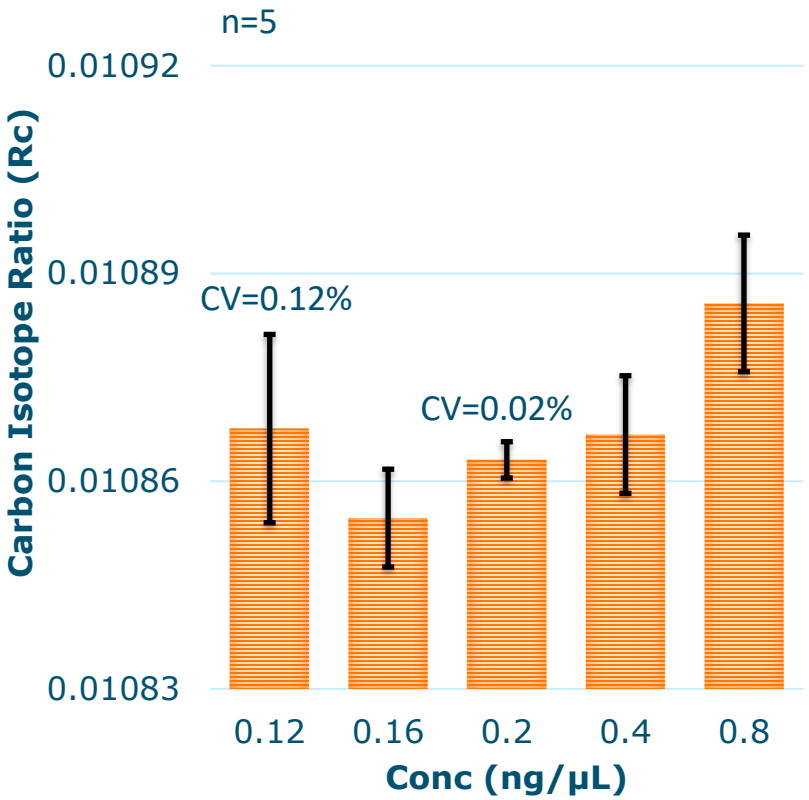


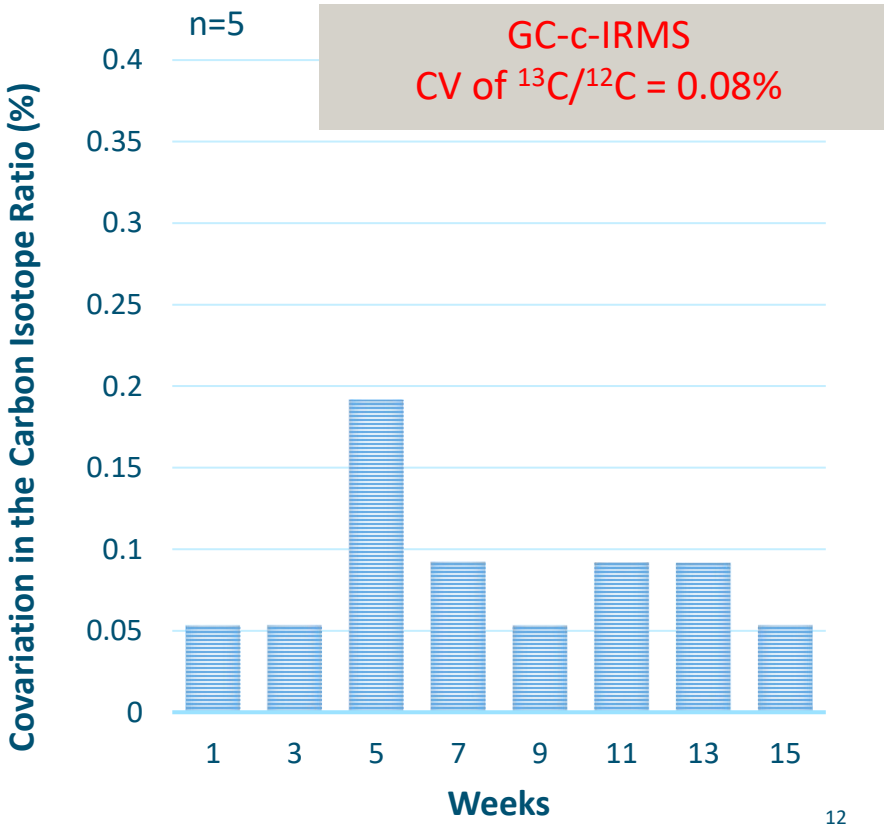
Lots of experiments were performed to obtain the lowest possible variability between measurements

- Determine the effect of the resolution on the CV
- Optimized the AGC to reduce space charging effects in the C-trap
- Effect of the scan-range on the CV
- Optimise the S-lens
- Effect of number of microscans on the measurement
- Etc.

Concentration and day-to-day stability





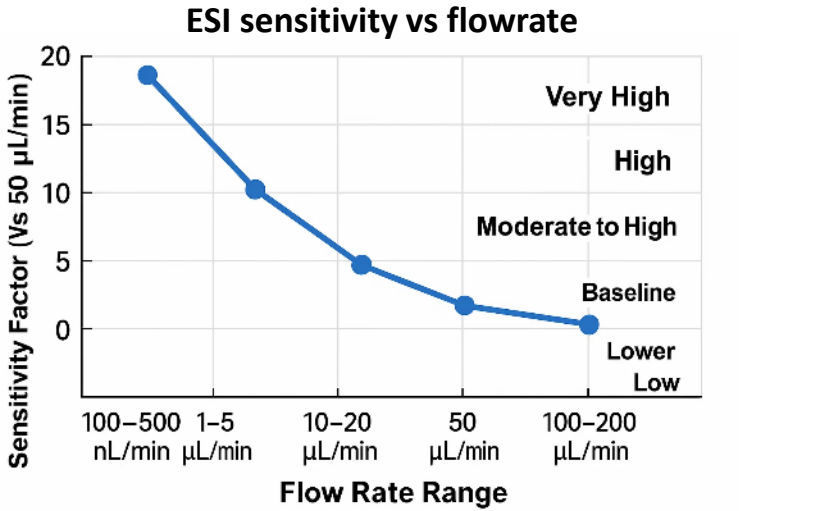


“Peak broadening” for high-precision isotope ratios

Prolonged peak measurement (minutes) with maximized chromatographic resolution and sensitivity

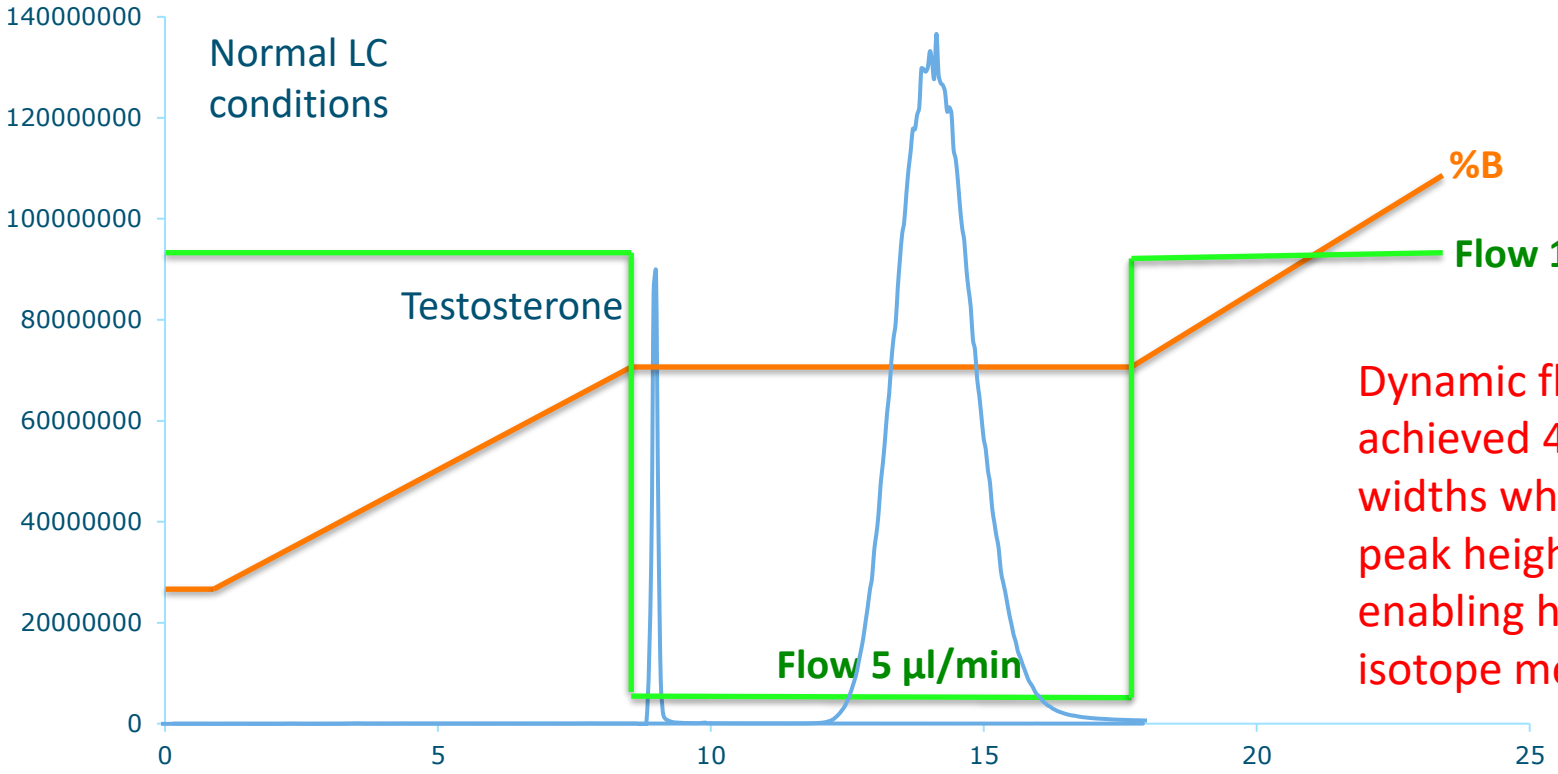
"Controlled" peak broadening
- Wider-bore columns
- Larger particles
- Longer columns

Gradient/flow optimization
- Shallow gradients or isocratic holds
- Low flow rates



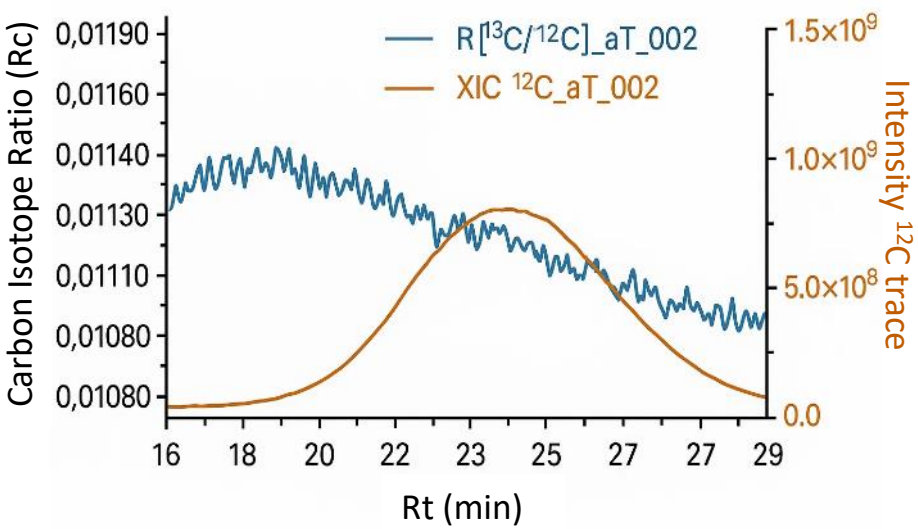
Advanced Tactics
- Peak Parking: trap analyte in a loop → slowly elute (5–10 µL/min) to Orbitrap.
- An idea was to use dynamic flow reduction: Lower flow during elution peak, no literature available

Dynamic flow reduction example

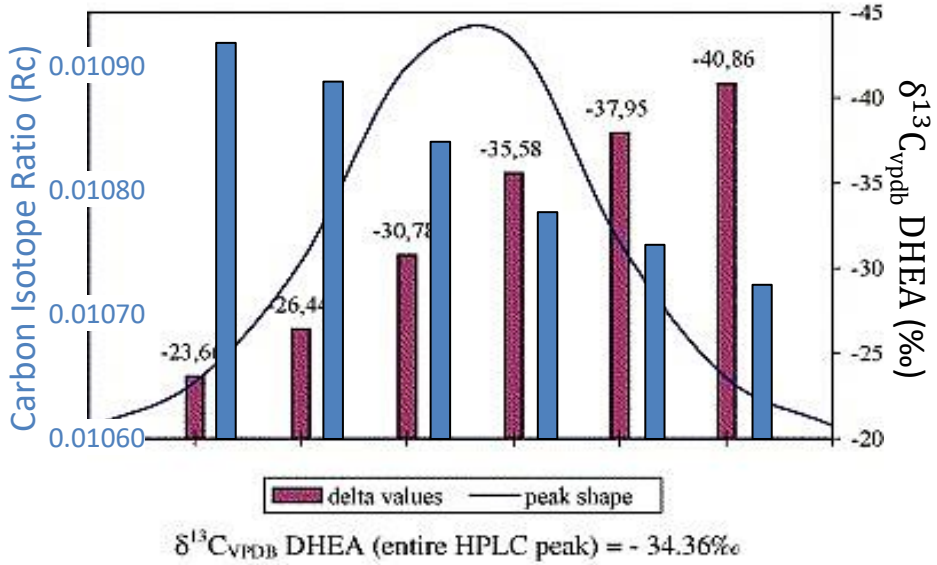


Dynamic flow reduction achieved 4-5 minute peak widths while maintaining peak height intensity, enabling high-precision isotope measurements

Dynamic flow reduction and isotope fractionation

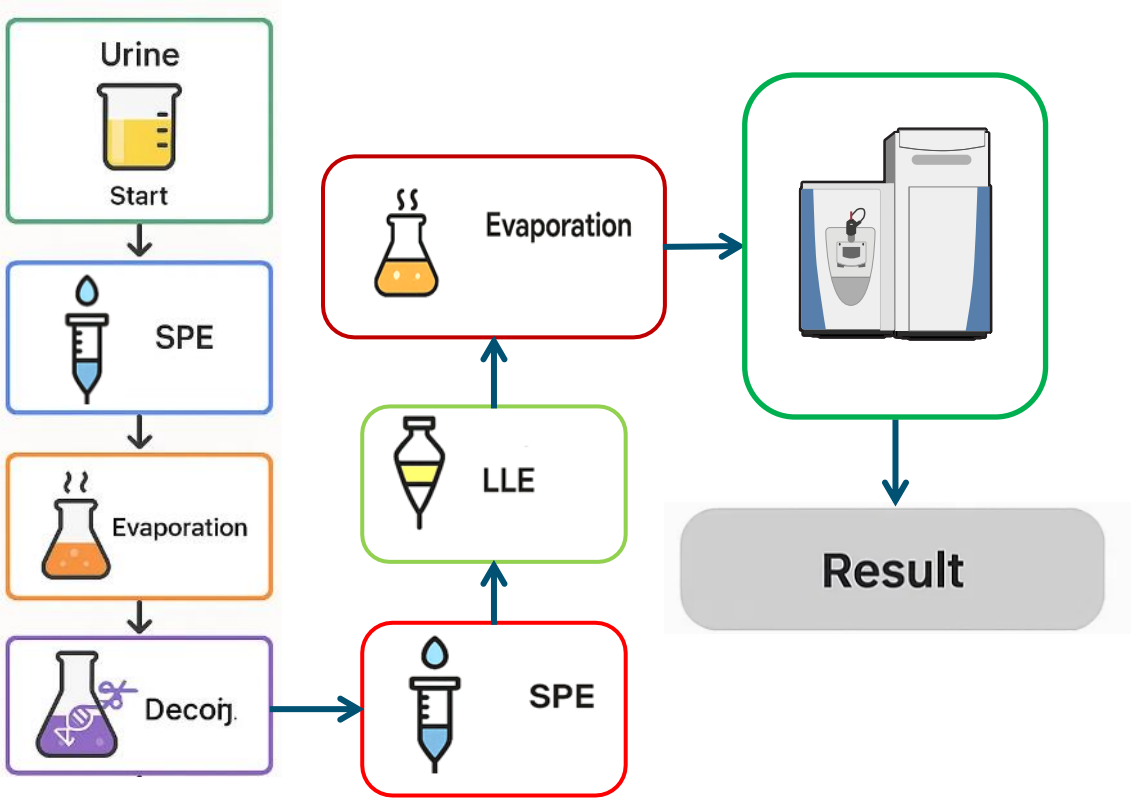


DHEA measured on a C_{18} LC column



C. Buisson, M. Hebestreit, A. Preiss Weigert, K. Heinrich, H. Fry, U. Flenker, S. Banneke, S. Prevost, F. Andre, W. Schaenzer, E. Houghton, B. Le Bizec
Journal of Chromatography A, 1093, 2005, 69-80

Urine sample analysis on the Orbitrap-IRMS



Same clean-up as GC-c-IRMS

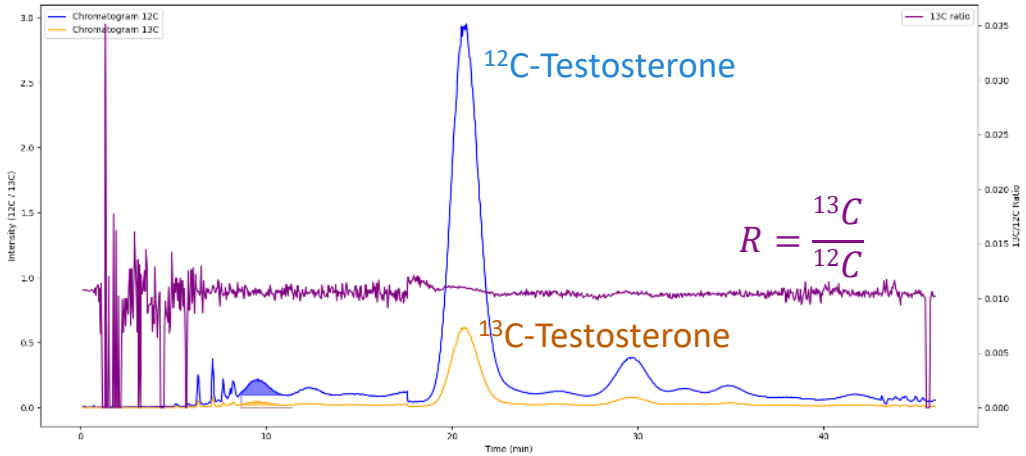
Inter Laboratory Comparison samples

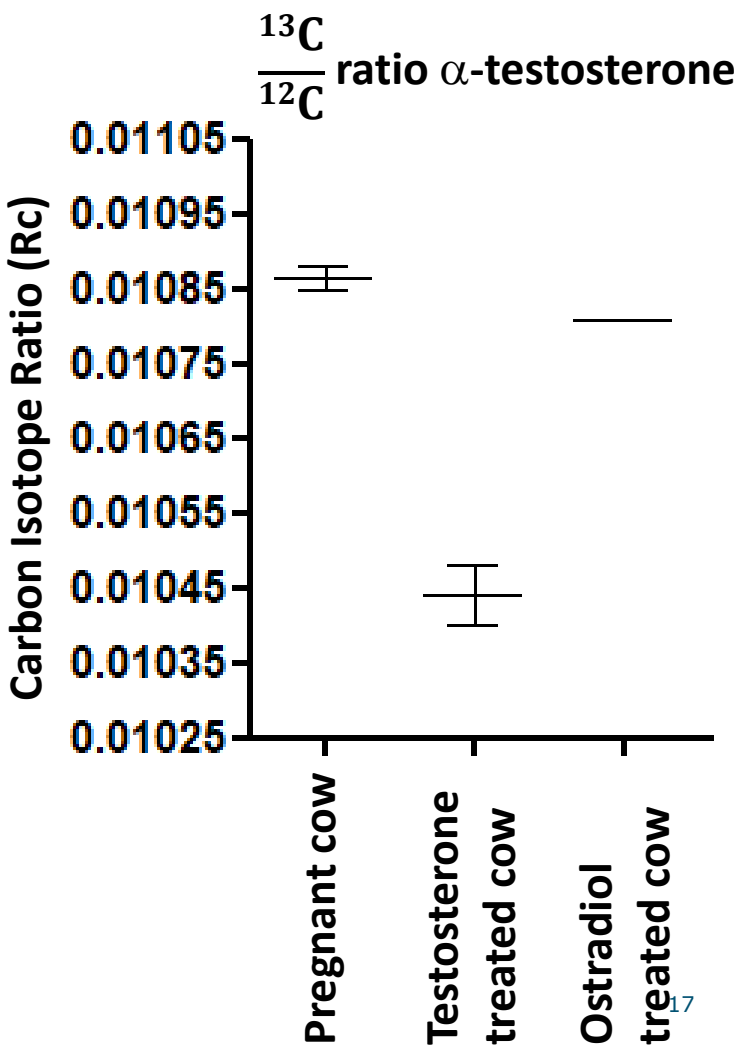
Animal study samples

Inter Laboratory Comparison samples

Sample type	Origin α-testosterone
Pregnant cow	Endogenous
Testosterone treated cow	Synthetic
Oestradiol treated cow	Endogenous

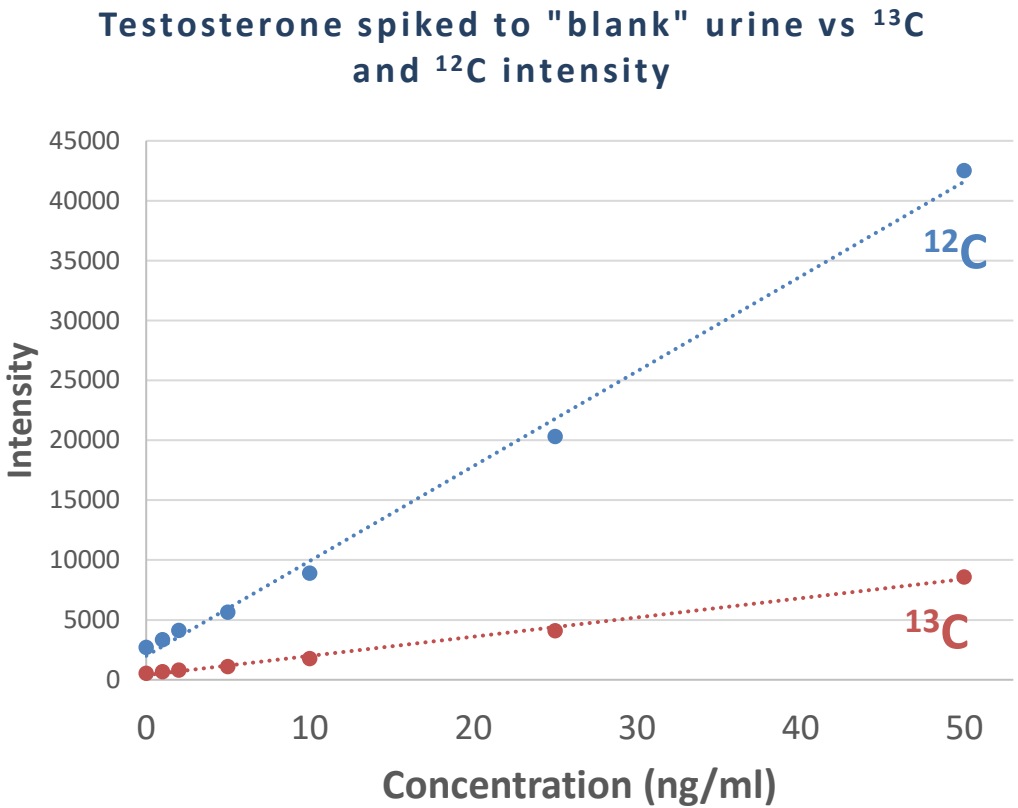
Samples were measured in duplicate

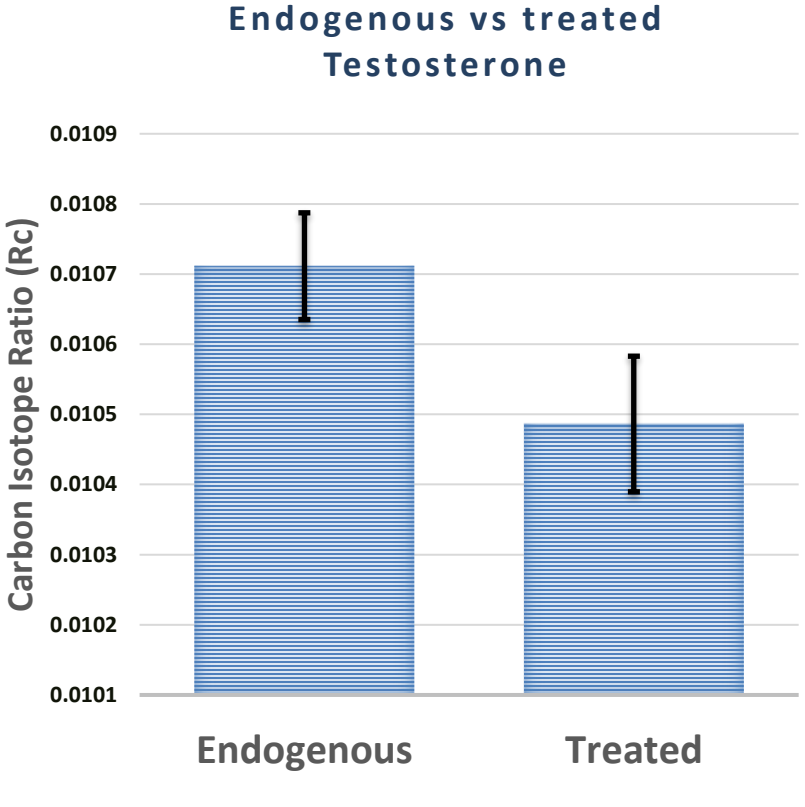




Animal study samples

Eight samples before treatment and 8 after treatment of a cow with Testosterone





Conclusion

- Extensive optimization of source/MS settings is a must for precision
- LC-Orbitrap-IRMS can measure very reproducible $^{13}C/^{12}C$ values when optimised
- Dynamic flow reduction (5 µL/min) enables 4–5 min peak widths without sensitivity loss
- Automated data extraction tools essential for processing broad peaks
- Absolute $^{13}C/^{12}C$ ratios show high promise based on animal studies

Future Perspectives

- Incorporate Certified Standards to correct for instrument drift
- Include Endogenous Reference Compounds (ERCs) to correct for biological variability
- Explore MS/hrMS Fragmentation to determine fragment-specific isotope ratios for unambiguous compound verification
- Full validation of Orbitrap-IRMS for steroids
- Hopefully, discussion with other interested laboratories for this techniques

Thank the team

Gregg Roelofs

Krystian Janiszewski

Josha Jager

Pim Pinto

Saskia Sterk

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