GHENT
UNIVERSITY

IRMS insights on EpiA-S, 6aOH-ADION and 19NA cases

Michael Polet – 22.09.2023

PART ONE: EPIA-S

PART TWO: 6AOH-ADION

PART THREE: 19NA

GHENT UNIVERSITY

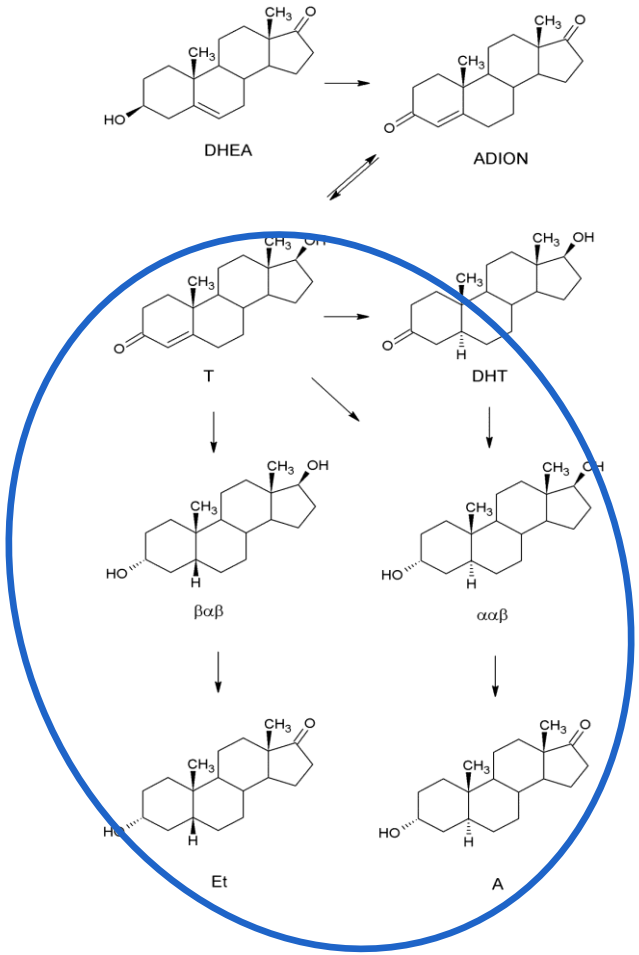
DoCoLab

PART ONE: EPIA-S

GHENT UNIVERSITY

DoCoLab

CONVENTIONAL IRMS TARGET COMPOUNDS



Conventional IRMS methodologies:

focus on glucuronides

GHENT UNIVERSITY

EPIA-S PROLONGS DETECTION TIME

Research article

Drug Testing and Analysis

Received: 14 July 2017 Revised: 10 August 2017 Accepted: 17 August 2017 Published online in Wiley Online Library: 27 October 2017

(www.drugtestinganalysis.com) DOI 10.1002/dta.2291

Epiandrosterone sulfate prolongs the detectability of testosterone, 4-androstenedione, and dihydrotestosterone misuse by means of carbon isotope ratio mass spectrometry

Thomas Piper,[a]* Marlen Putz,[a] Wilhelm Schänzer,[a] Valentin Pop,[b] Malcolm D. McLeod,[c] Dimanthi R. Uduwela,[c] Bradley J. Stevenson[c] and Mario Thevis[a,d]

EpiA-S prolongs IRMS detection time with factor 2 to 5 in comparison with the conventional TCs

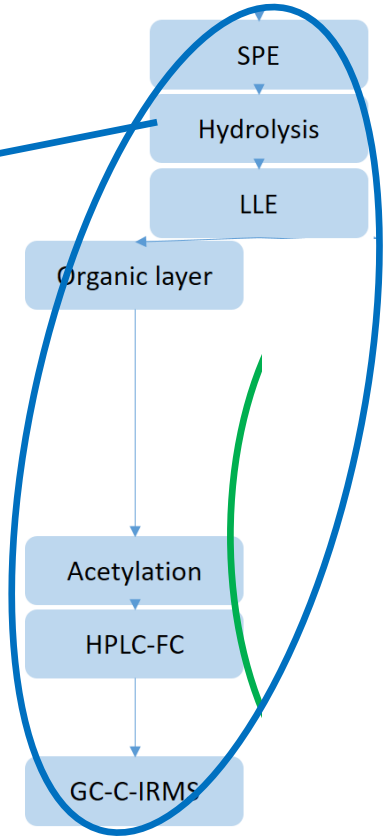
GHENT UNIVERSITY

SAMPLE PREP FOR GLUCURONIDES AND SULFATES

glucuronides

Glucuronidase

(*E. coli*)



sulfates

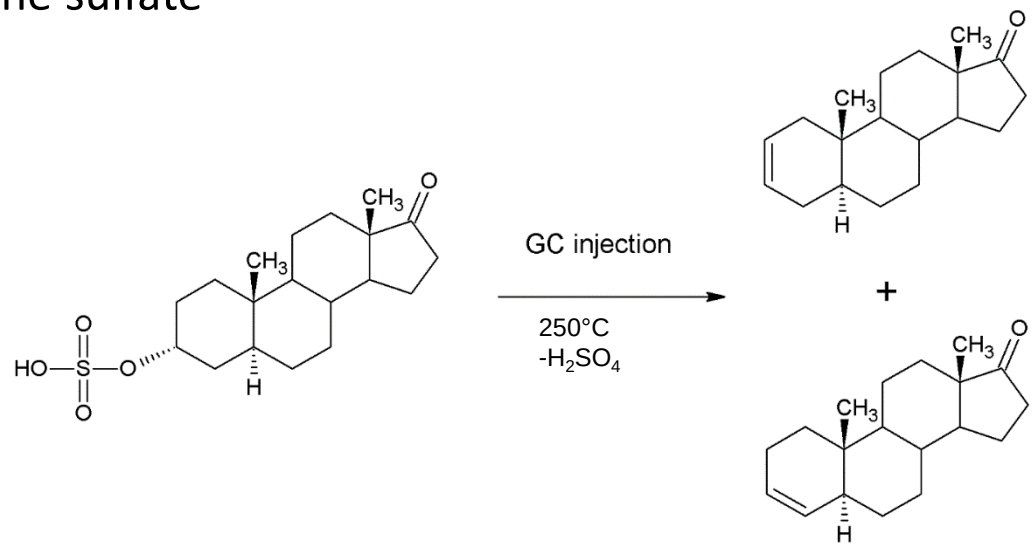
Chemical hydrolysis or

Sulfatase (e.g., *Helix pomatia*)

GHENT UNIVERSITY

NON-HYDROLYZED SULFATED STEROIDS ON GC

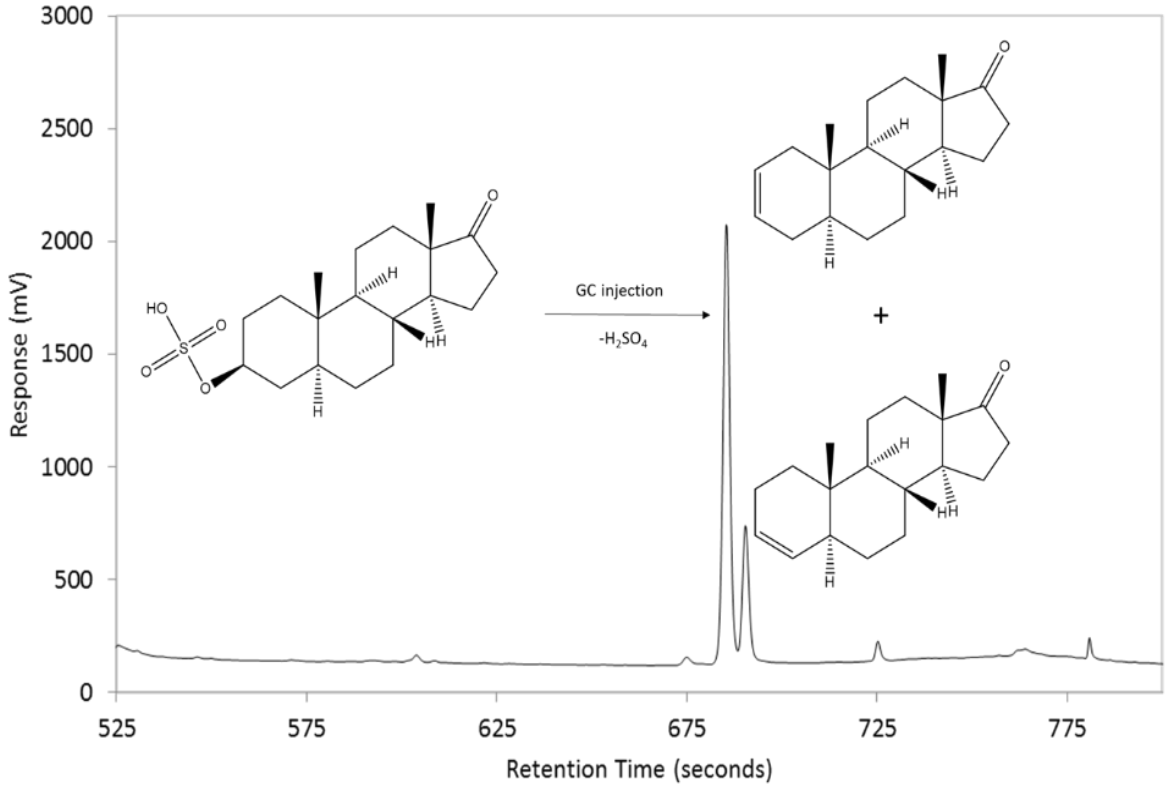
Androsterone sulfate



M. Polet, W. Van Gansbeke, A.D. Albertsdóttir, G. Coppieters, K. Deventer, P. Van Eenoo, Gas chromatography-mass spectrometry analysis of non-hydrolyzed sulfated steroids by degradation product formation. Drug testing and analysis. **2019**, 11, 1656.

GHENT UNIVERSITY

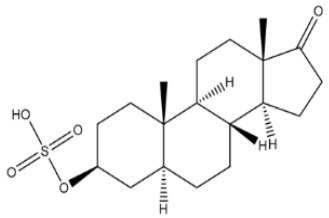
NON-HYDROLYZED EPIA-S ON GC



Column DB-17ms (30 m x 0,25 mm x 0,25 µm)

GHENT UNIVERSITY

NON-HYDROLYZED EPIA-S ON GC



EpiA-S standard

Helix pomatia hydrolysis

LLE

free EpiA on GC-C-IRMS

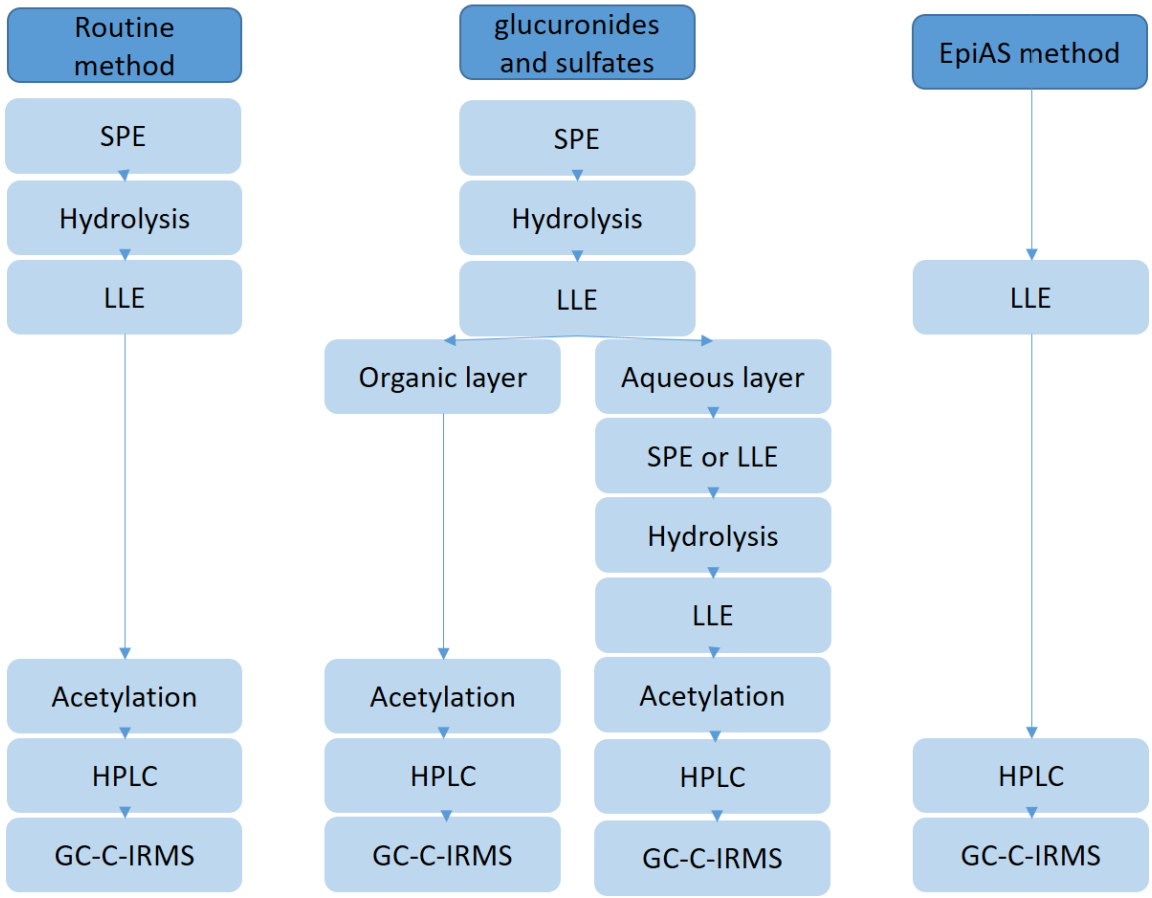
$\delta^{13}C$ = -30,58 ‰ (n = 10)
SD = 0,07 ‰

Non-hydrolyzed EpiA-S on IRMS

$\delta^{13}C$ = -30,56 ‰ (n = 56)
SD = 0,22 ‰

GHENT UNIVERSITY

SAMPLE PREPARATION



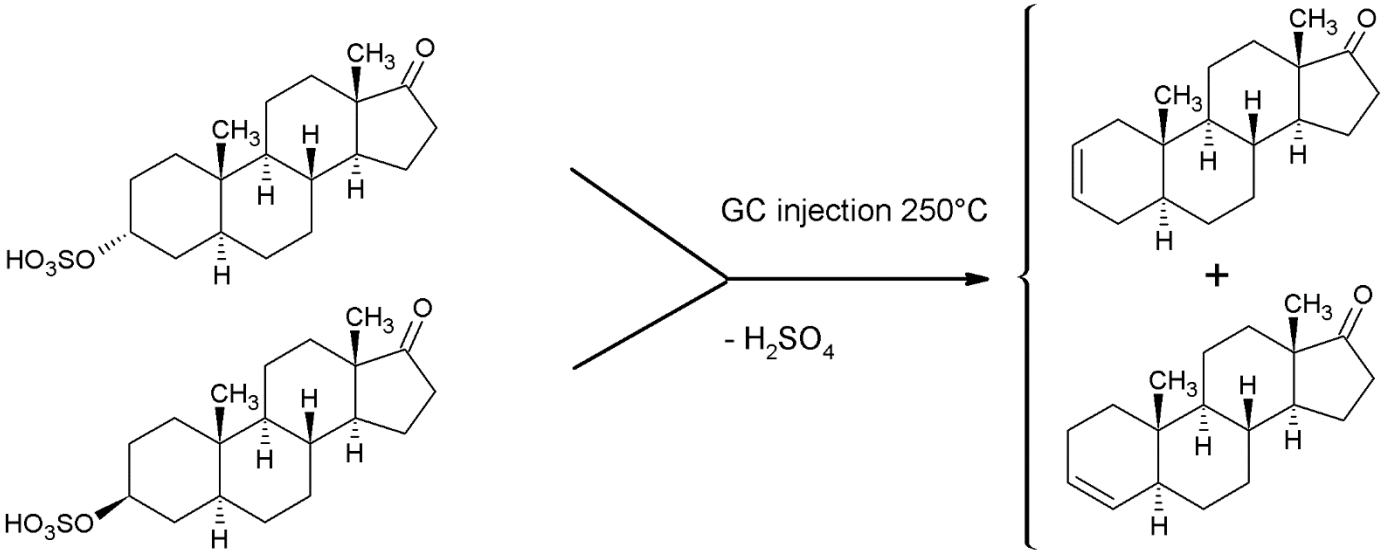
GHENT UNIVERSITY

HPLC-FC

A-S and EpiA-S need to be separated on HPLC-FC

A-S

EpiA-S

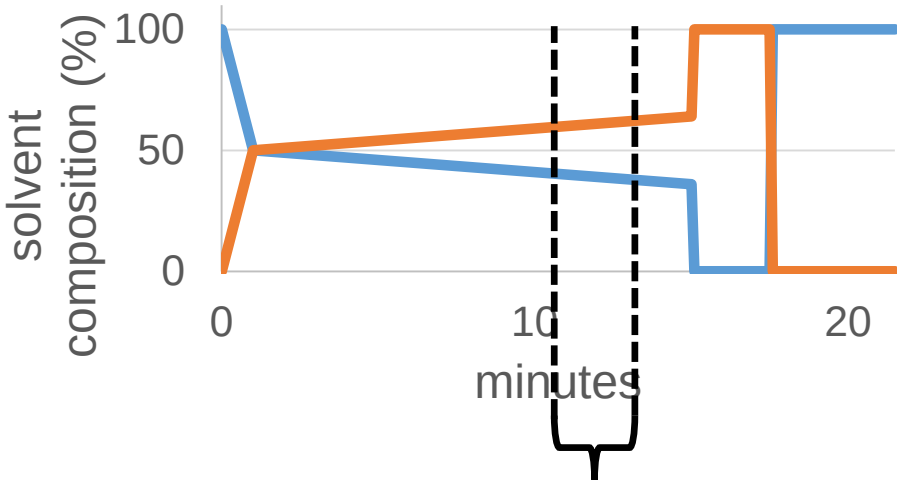


GHENT UNIVERSITY

HPLC-FC

Hypersil Gold C18 column 150 x 4,6 mm x 5 µm

A) H_2O (0,01 % formic acid; 20 mM NH_4OOCH)
B) MeOH (0,01 % formic acid; 20 mM NH_4OOCH)

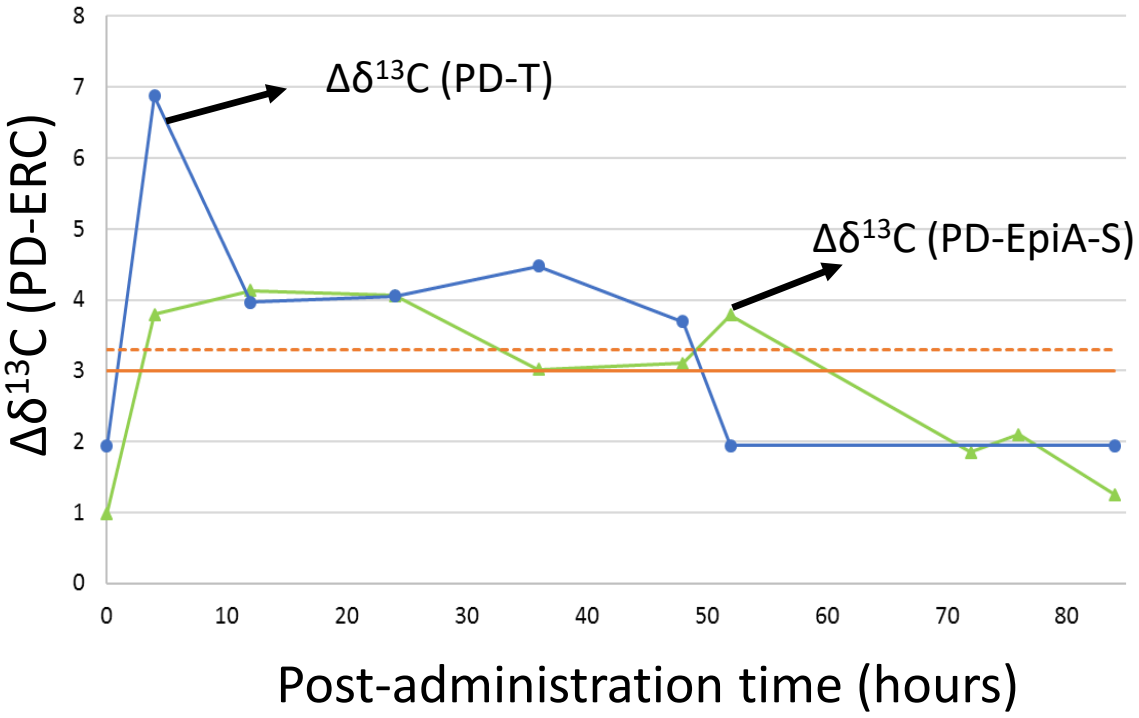


GHENT UNIVERSITY

EpiA-S

ADMINISTRATION STUDY: DHEA

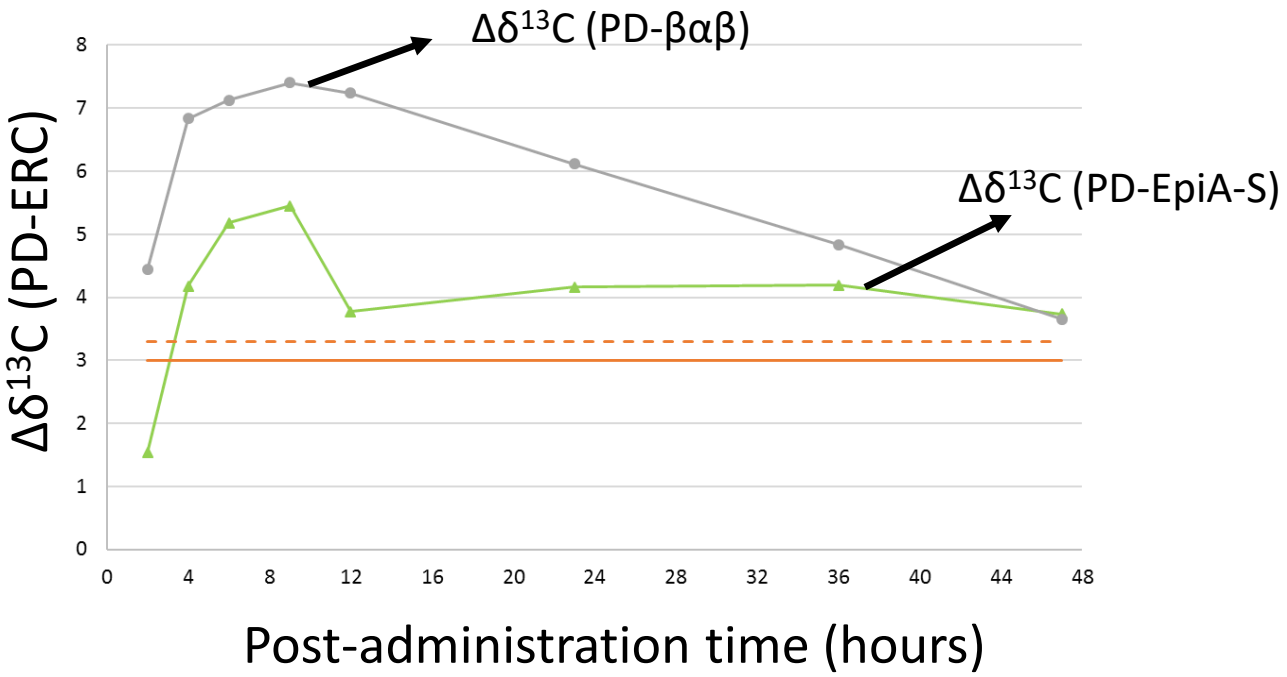
Single dose 50 mg DHEA (oral)



GHENT UNIVERSITY

ADMINISTRATION STUDY: ADION

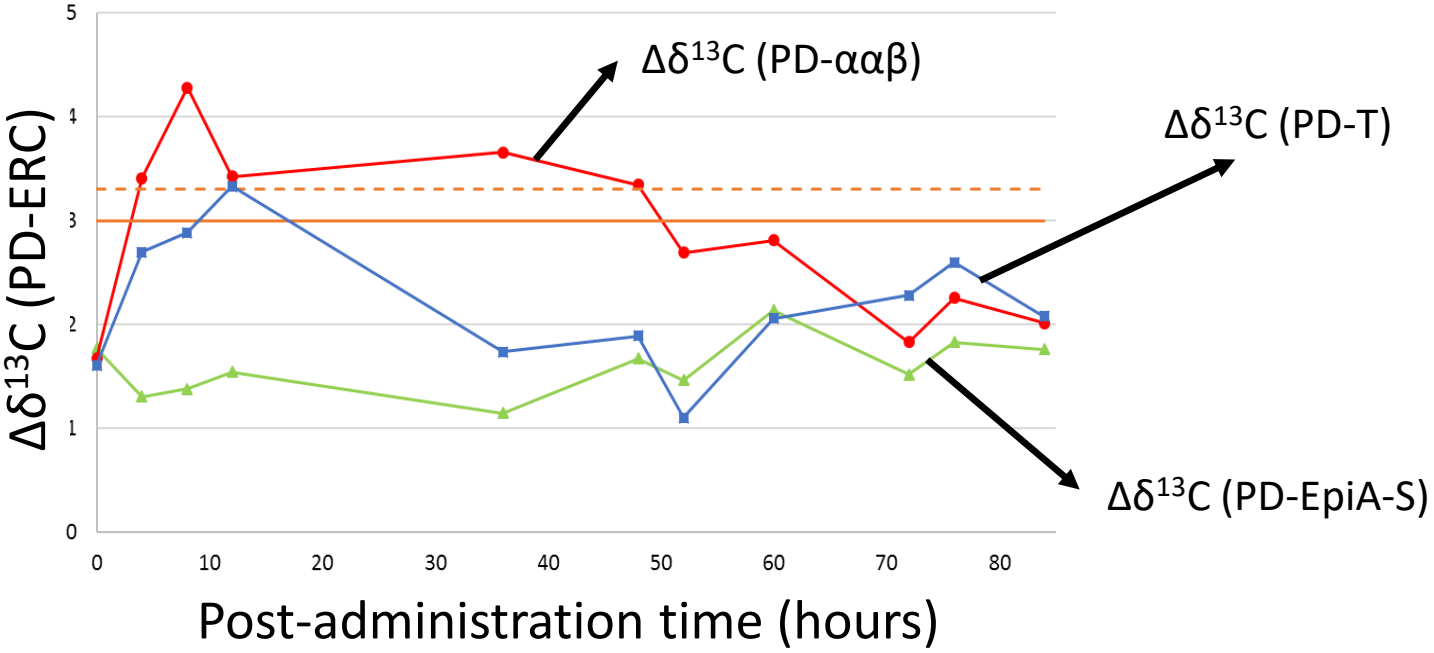
Single dose 50 mg ADION (oral)



GHENT UNIVERSITY

ADMINISTRATION STUDY: T GEL

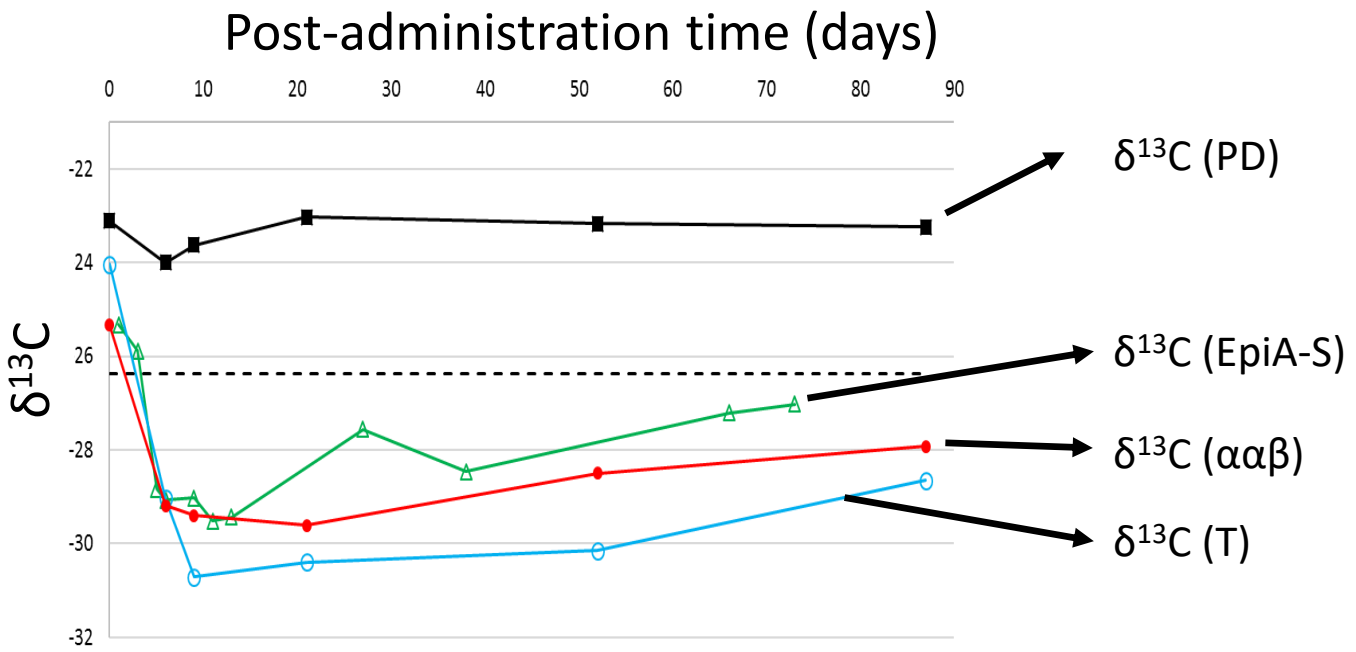
Single dose 50 mg T gel (transdermal)



GHENT UNIVERSITY

ADMINISTRATION STUDY: NEBIDO

Single dose 1 g Nebido (intramuscularly)

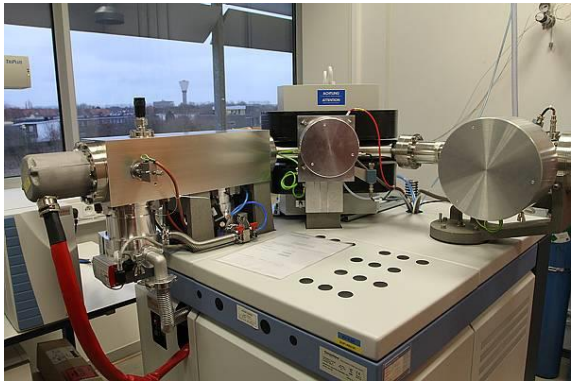


GHENT UNIVERSITY

CONCLUSIONS

1. Fast method (performed in addition to the conventional method)

2. Prolongation of detection window is very limited



GHENT UNIVERSITY

RESEARCH ARTICLE

WILEY

Development and validation of a fast gas chromatography combustion isotope ratio mass spectrometry method for the detection of epiandrosterone sulfate in urine

Laurie De Wilde | Pieter Van Renterghem | Peter Van Eenoo | Michaël Polet

SPECIAL ISSUE - RESEARCH ARTICLE

WILEY

Evaluation of epiandrosterone as a long-term marker of testosterone use

Vinod S. Nair[1] | Christine E. Doman[1] | Matthew S. Morrison[1] | Geoffrey D. Miller[1] | Jacob Husk[1] | Peter van Eenoo[2] | Andre K. Crouch[1] | Daniel Eichner[1]

1. SMRTL publication also showed very limited added value
2. Analysis of EpiA-S no longer in our scope

GHENT UNIVERSITY

PART TWO: 6AOH-ADION

GHENT UNIVERSITY

DoCoLab

STEROID PROFILE

Administration of endogenous anabolic steroids (T, ADION, DHEA, DHT,...)

$\Rightarrow$ Elevation of one or more steroid profile parameters

$\Rightarrow$ IRMS confirmation

Classic steroid profile parameters:

T, EpiT, DHEA, DHT, ADION

main metabolites: A, Et, aab-diol, bab-diol

T/E

etc.

GHENT UNIVERSITY

STEROID PROFILE

Minor metabolites:

4OH-ADION (formestane)

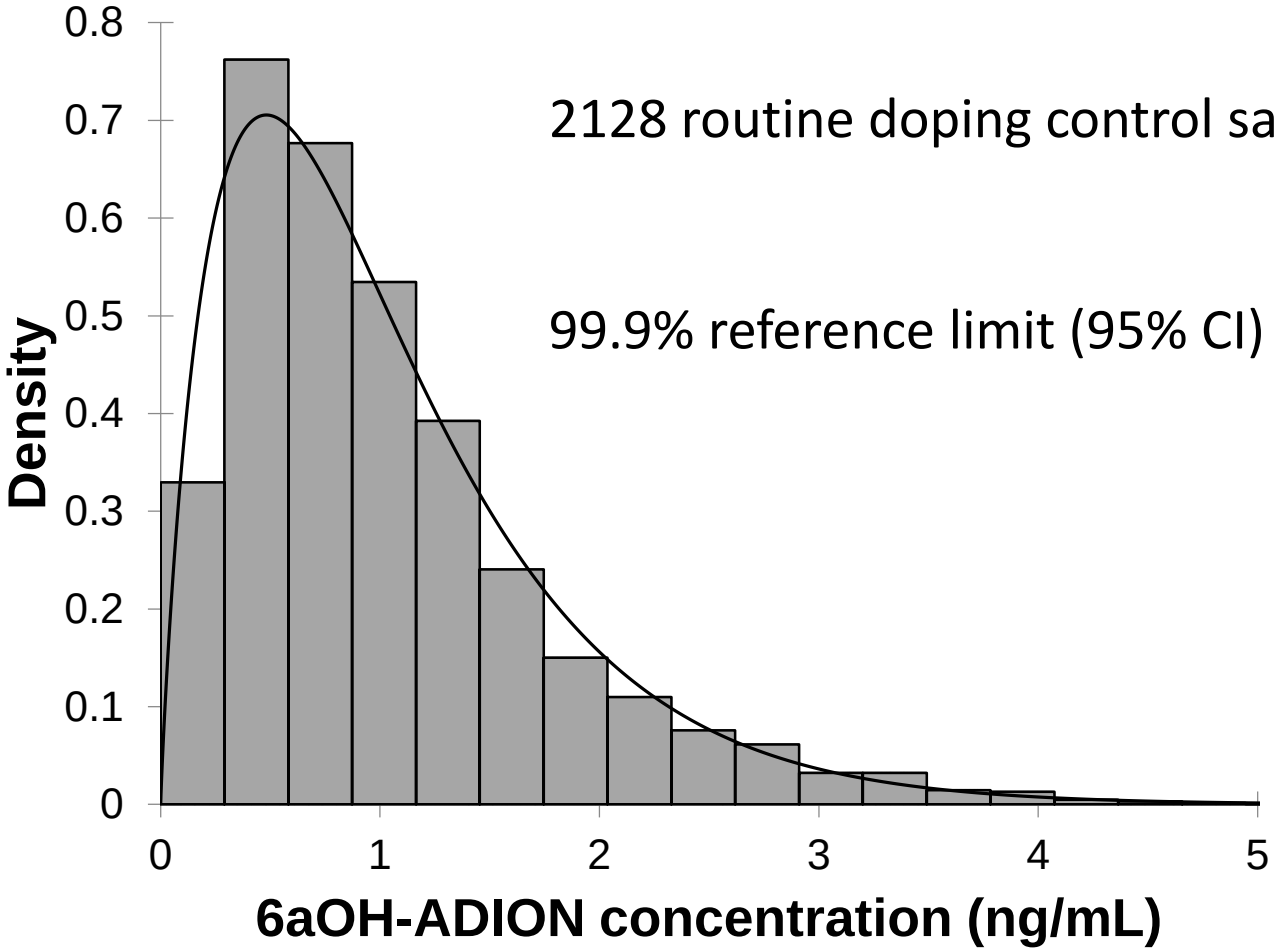
7bOH-DHEA

6aOH-ADION

$\Rightarrow$ Important parameters as well [1-5)]

[1)]Cawley, A. T., et al. *Forensic Science International*, **143**, 103 (2004).
[2)]Cawley, A. T., et al. *Rapid Communications in Mass Spectrometry*, **22**, 4147 (2008).
[3)]Van Renterghem, P., et al. *Journal of Chromatography B*, **876**, 225 (2008).
[4)]Van Renterghem, P., et al. *Steroids*, **75**, 154 (2010).
[5)]Van Renterghem, P., et al. *Steroids*, **75**, 1047 (2010).

REFERENCE POPULATION

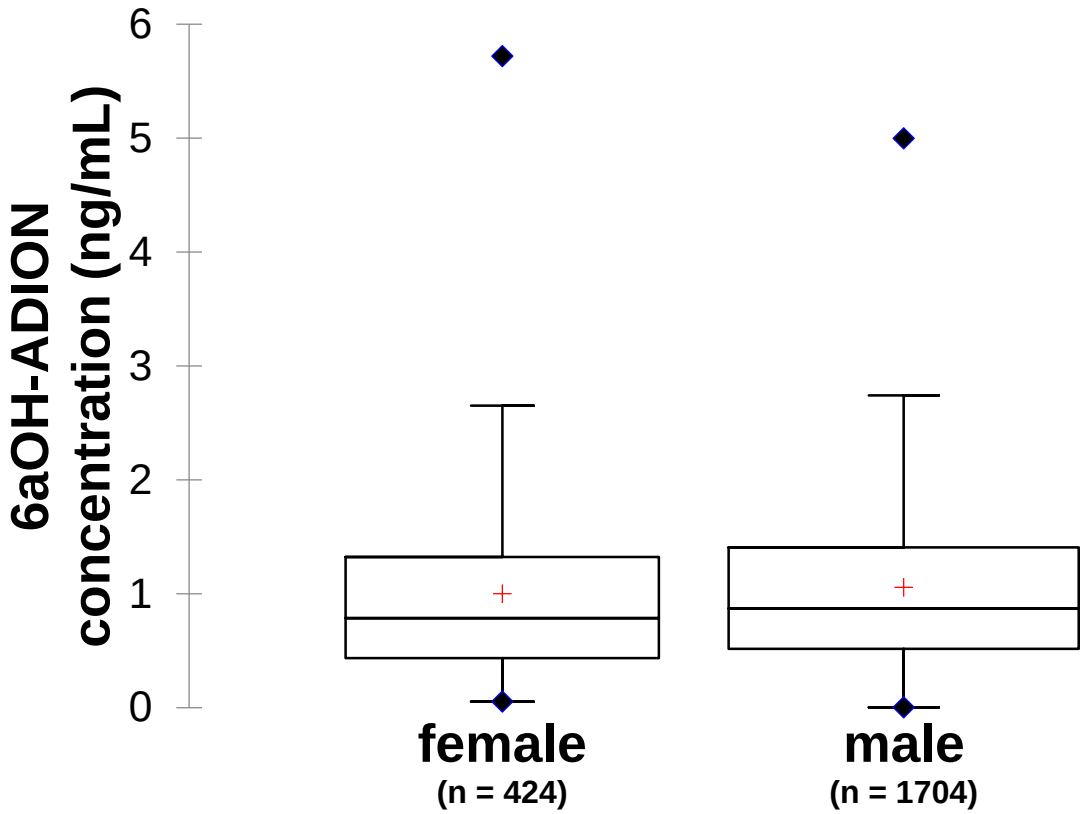


2128 routine doping control samples

99.9% reference limit (95% CI) = 4.9 ng/mL ~ 5 ng/mL

GHENT UNIVERSITY

REFERENCE POPULATION



GHENT UNIVERSITY

Analytica Chimica Acta 1168 (2021) 338610

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

A uniform sample preparation procedure for gas chromatography combustion isotope ratio mass spectrometry for all human doping control relevant anabolic steroids using online 2/3-dimensional liquid chromatography fraction collection

Lenka Honesova *, Peter Van Eenoo , Michael Polet

GHENT UNIVERSITY

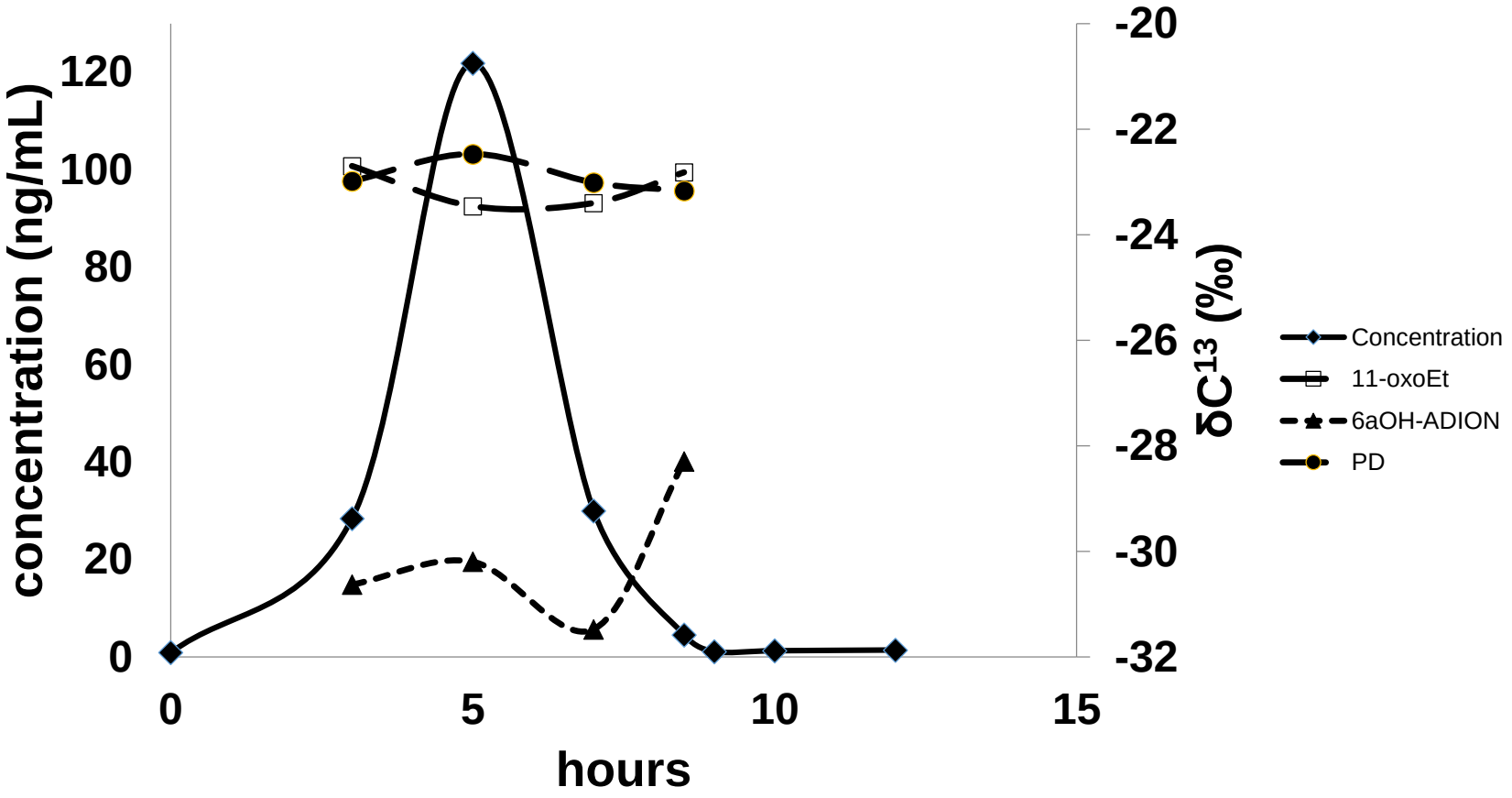
ENDOGENOUS ^{13}C VALUES

Only 4 out of 2128 samples contained a sufficiently high 6aOH-ADION concentration for IRMS

Sample	Sex	Concentration of 6aOH-ADION (ng/mL)	$\delta^{13}C$ value(‰)			$\Delta\delta^{13}C$ value(‰)	
			6aOH-ADION	11-oxoEt	PD	11-oxoEt – 6aOH-ADION	PD – 6aOH-ADION
A	F	5.7	-24.10	-24.83	-24.16	-0.73	-0.06
B	M	4.9	-22.52	-23.05	-22.68	-0.53	-0.16
C	M	4.6	-23.97	-23.08	-23.39	0.90	0.58
D	M	4.4	-23.61	-23.55	-22.73	0.06	0.88
					Average	-0.07	0.31
					Standard deviation	0.73	0.50

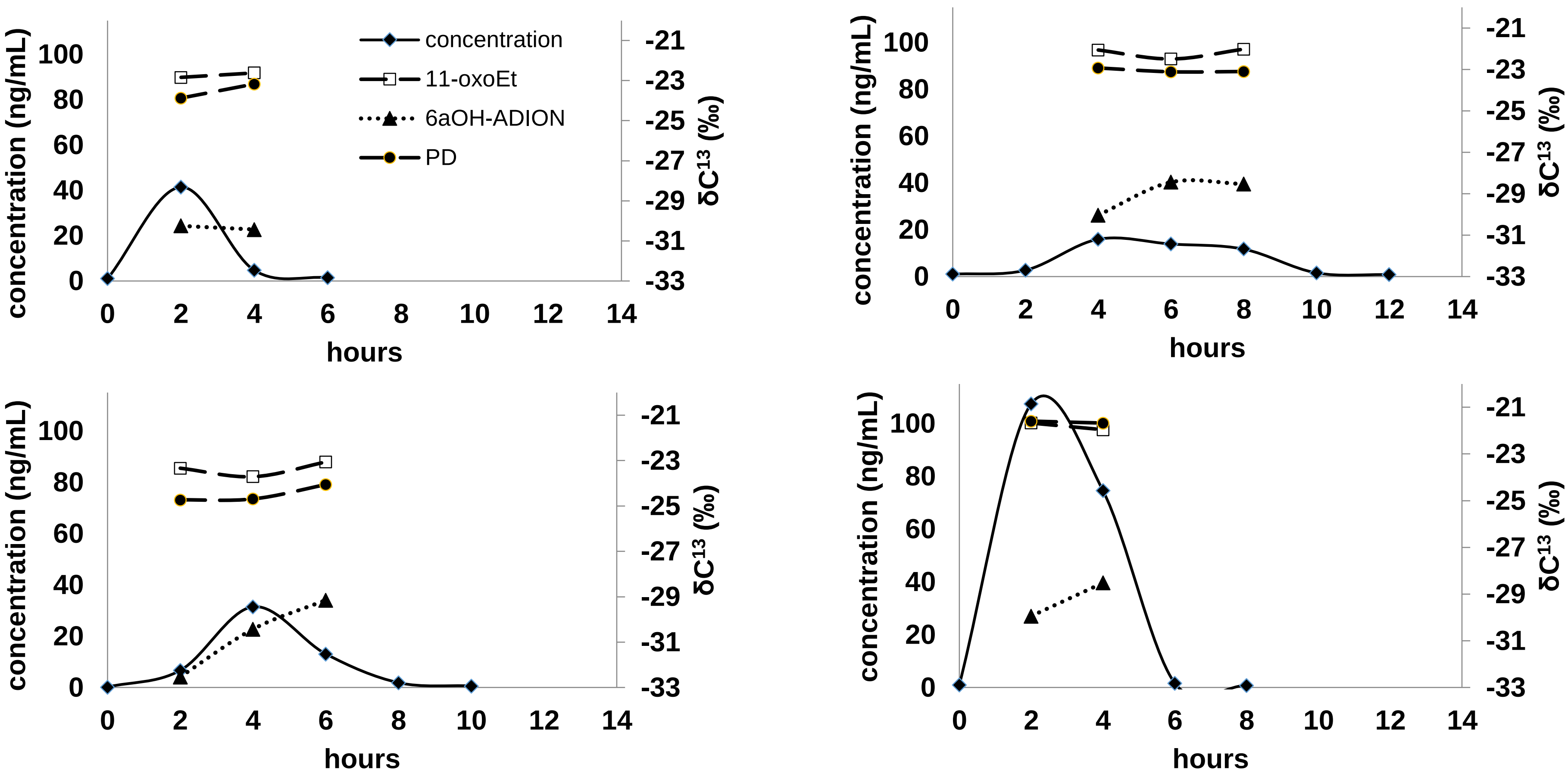
GHENT UNIVERSITY

EXCRETION STUDY ADION



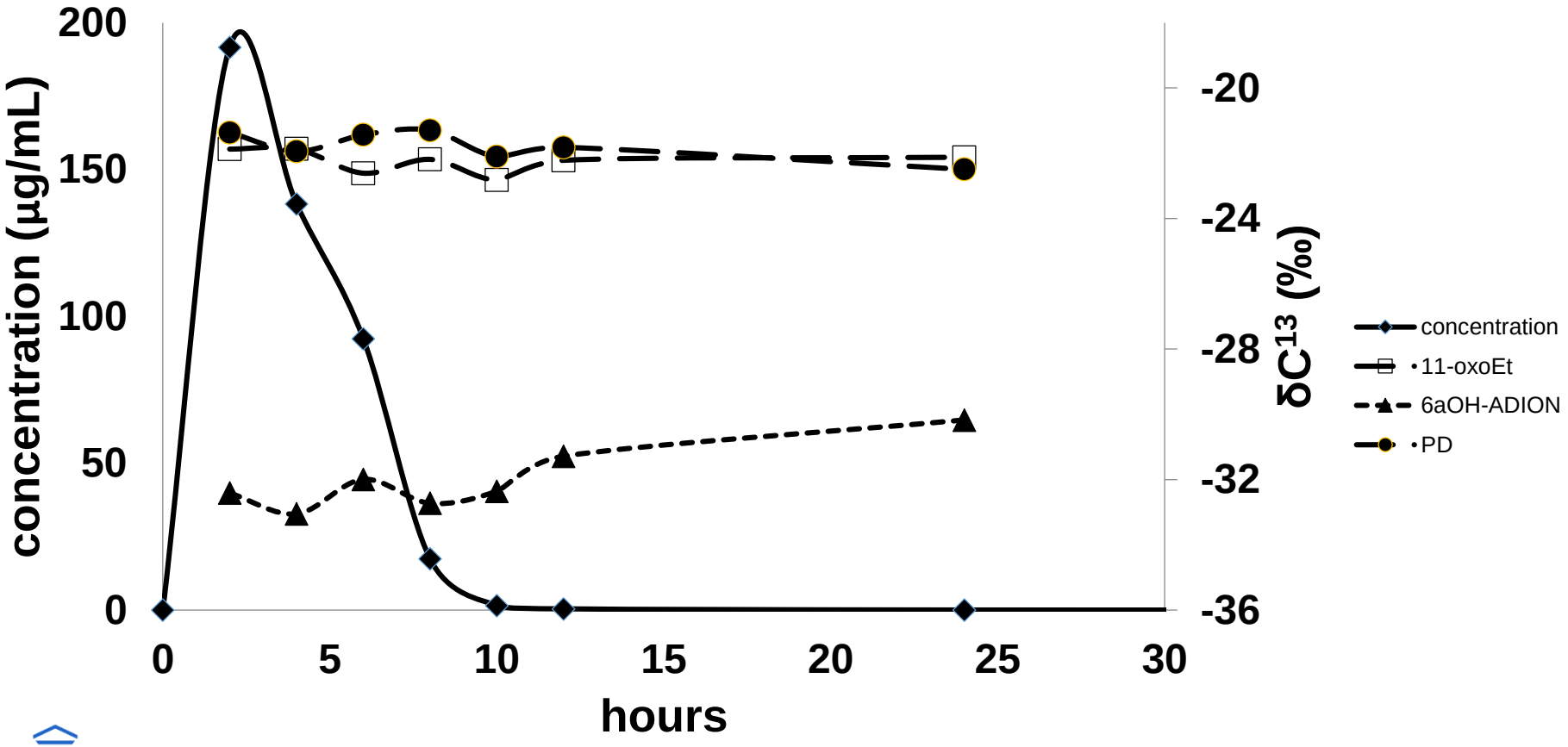
GHENT UNIVERSITY

EXCRETION STUDY TU



GHENT UNIVERSITY

EXCRETION STUDY 6OXO-ADION



GHENT UNIVERSITY

EXCRETION STUDIES

In all samples were 6aOH-ADION was elevated, at least one classic steroid profile parameter was elevated as well.

$\Rightarrow$ IRMS would have been recommended anyway

$\Rightarrow$ why monitor 6aOH-ADION?

GHENT UNIVERSITY

SUSPICIOUS SAMPLE

	A	Et	aab-diol	bab-diol	T	EpiT	DHEA	DHT	ADION	Formestane	7bOH-DHEA	6aOH-ADION	PD	11-oxoEt
Concentration (ng/mL)	3236	1330	58	13	3.4	8.7	12	2.8	0.6	8.5	4.9	17	43	

GHENT UNIVERSITY

Research article

Drug Testing and Analysis

Received: 27 November 2013 Revised: 8 January 2014 Accepted: 8 January 2014 Published online in Wiley Online Library

(www.drugtestinganalysis.com) DOI 10.1002/dta.1618

Studies on the minor metabolite 6a-hydroxy-androstenedione for doping control purposes and its contribution to the steroid profile

Michael Polet,* Pieter Van Renterghem, Wim Van Gansbeke and Peter Van Eenoo

GHENT UNIVERSITY

PART THREE: 19NA

SUSPICIOUS CASES

Some 19NA cases that were likely to be an AAF, but turned out to be negative on IRMS.

7 ng/mL 19NA; AAF for some other AAS

19NA = -25,2 ‰

PD = -24,2 ‰

11oxoEt = -23,8 ‰

PD - 19NA = 1 ‰

11oxoEt - 19NA = 1,4 ‰

GHENT UNIVERSITY

SUSPICIOUS CASES

7 ng/mL 19NA; AAF for drostanolone

19NA = -22,8 ‰

PD = -22,5 ‰

11oxoEt = -22,8 ‰

PD - 19NA = 0,3 ‰

11oxoEt - 19NA = 0,0 ‰

GHENT UNIVERSITY

SUSPICIOUS CASES

4,7 ng/mL 19NA; AAF for some other AAS

19NA = -24,9 ‰

PD = -21,7 ‰

11oxoEt = -21,5 ‰

PD - 19NA = 3,2 ‰

11oxoEt - 19NA = 3,4 ‰

GHENT UNIVERSITY

19NA RESEARCH PROJECT

Sport Integrity Australia
Australian Sports Drug Testing Laboratory

Estimation of the prevalence of injectable nandrolone preparations in Australia with endogenous carbon isotope ratios

40 previously reported positive workplace urine samples from the period 2018 – 2021 containing 19NA

=> 28 had sufficient urine for 19NA IRMS analysis in the Ghent doping control laboratory

GHENT UNIVERSITY

19NA RESEARCH PROJECT

6 out 28 sample < 15 ng/ml => IRMS
22 out 28 sample > 15 ng/ml => AAF

23 out 28 samples (82%) in the endogenous range ($\delta^{13}C \geq -25.8‰$)

9 samples AAF
11 samples ATF
8 samples negative

GHENT UNIVERSITY

19NA RESEARCH PROJECT

A lot more nandrolone preparations with endogenous $\delta^{13}C$

Need to work closely with TA/RMAs

GHENT UNIVERSITY

ACKNOWLEDGMENTS

Thanks to…

WADA

PCC

Sport Integrity Australia

ASDTL

Colleagues of DoCoLab

Thank you for your attention!

GHENT UNIVERSITY

DoCoLab

wada

PARTNERSHIP FOR clean competition