METABOLOMICS SESSION

R&D IN METABOLOMICS

IMPLEMENTATION OF A METABOLOMICS BASED SCREENING MODEL TO DETECT ANABOLIC PRACTICES IN BREEDING ANIMALS

Orléans, 17-20 septembre 2012

Bruno LE BIZEC, Pf, Dr, HDR

F. COURANT, S. CHEREAU, F. MONTEAU,
C. JACOB, A-L ROYER, F. BOYARD-KIEKEN,
JP. ANTIGNAC, G. DERVILLY-PINEL

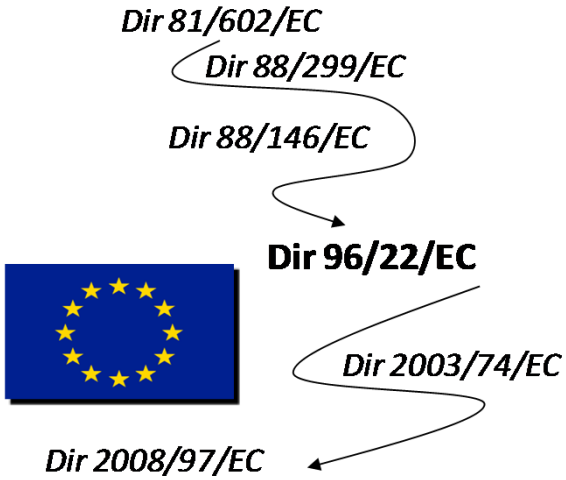
Laboratoire d'Étude des Résidus et Contaminants dans les Aliments
ONIRIS - France - www.laberca.org

1. Introduction

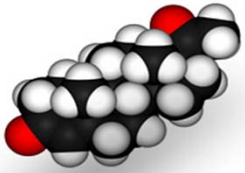
Where the current methods are challenged…

Member States shall prohibit:

(a) the administering to a farm or aquaculture animal, by any means whatsoever, of substances having a thyrostatic, oestrogenic, androgenic or gestagenic action and of beta-agonists;



Natural hormones



Major difficulties to screen for the illegal use of natural hormones

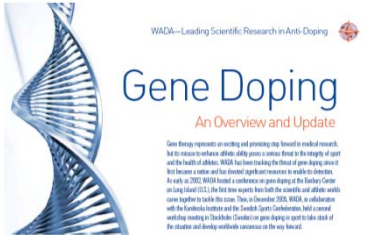
Designer drugs

One only searches for the known... &...
One only finds what is searched

Low doses cocktails

Same or superior effect (additional, synergetic)
But residues individually non detectable

Gene doping

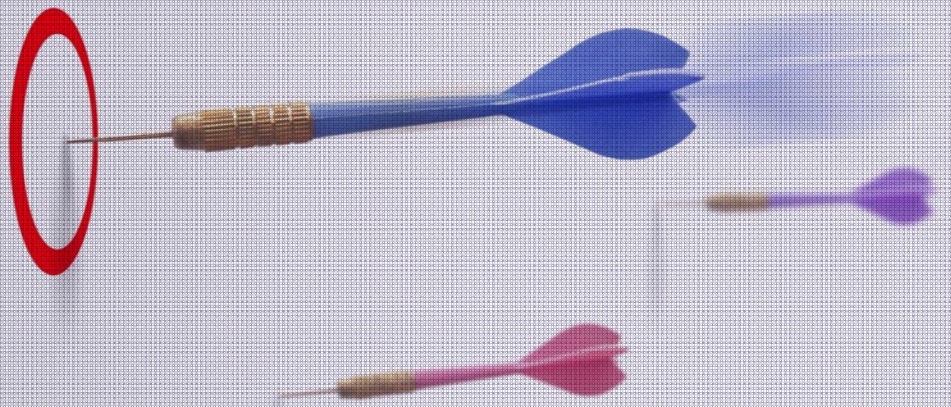


Undetectable by classical analytical strategies

Existing and emerging options to tackle these challenges

COMPOUND based approaches

Targeted approaches on a limited number of known chemical hazards

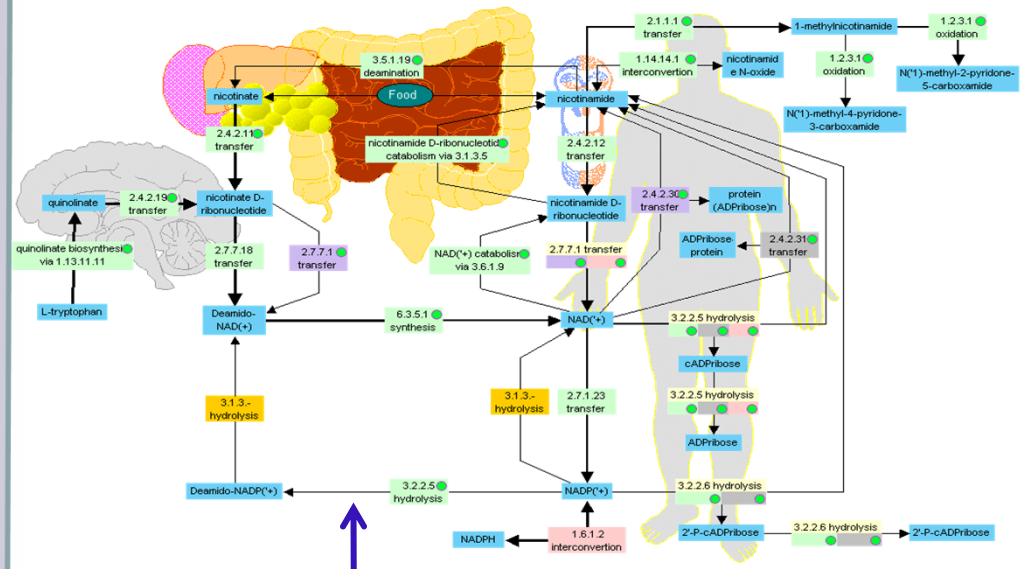


No signal
→ False negative

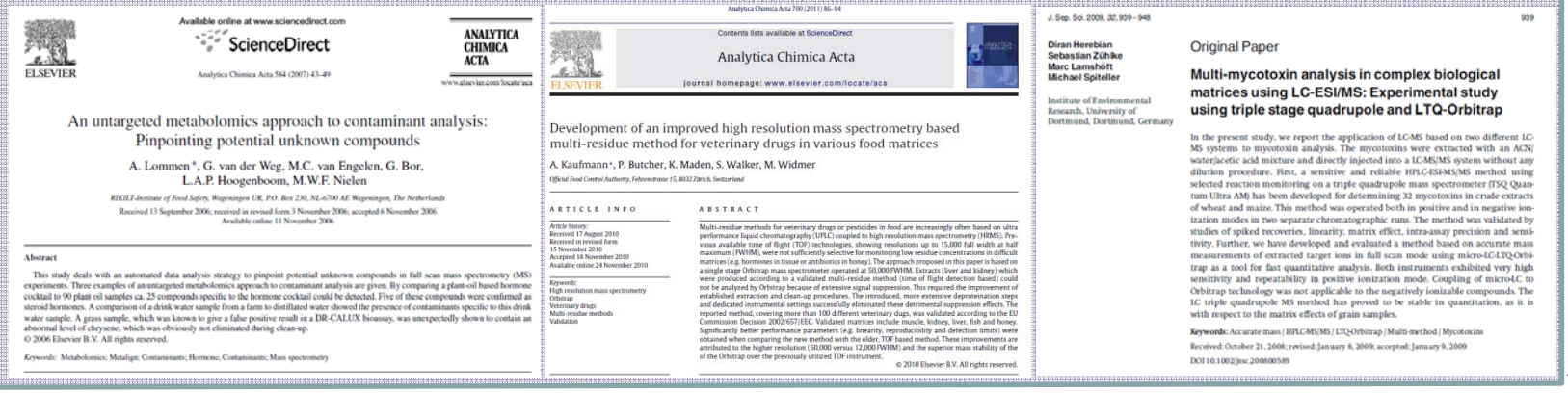
X?

EFFECT based approaches

Holistic observation of a biology system (food producing animals) when exposed to a chemical hazard



Untargeted approaches on the basis of a minimum set of known chemical hazards



Available online at www.sciencedirect.com
ScienceDirect
Analytica Chimica Acta 584 (2007) 43–49
ANALYTICA CHIMICA ACTA
www.elsevier.com/locate/aca

An untargeted metabolomics approach to contaminant analysis: Pinpointing potential unknown compounds

A. Lommen*, G. van der Weg, M.C. van Engelen, G. Bor, L.A.P. Hoogenboom, M.W.F. Nielen

RIKILT-Institute of Food Safety, Wageningen UR, P.O. Box 230, NL-6700 AE Wageningen, The Netherlands

Received 13 September 2006; received in revised form 3 November 2006; accepted 6 November 2006
Available online 11 November 2006

Abstract

This study deals with an automated data analysis strategy to pinpoint potential unknown compounds in full scan mass spectrometry (MS) experiments. Three examples of an untargeted metabolomics approach to contaminant analysis are given. By comparing a plant-oil based hormone cocktail to 90 plant oil samples ca. 25 compounds specific to the hormone cocktail could be detected. Five of these compounds were confirmed as steroid hormones. A comparison of a drink water sample from a farm to distillated water showed the presence of contaminants specific to this drink water sample. A grass sample, which was known to give a false positive result in a DR-CALUX bioassay, was unexpectedly shown to contain an abnormal level of chrysene, which was obviously not eliminated during clean-up.

© 2006 Elsevier B.V. All rights reserved.

Keywords: Metabolomics; Metalign; Contaminants; Hormone; Contaminants; Mass spectrometry

Analytica Chimica Acta 700 (2011) 86–94
Contents lists available at ScienceDirect
Analytica Chimica Acta
journal homepage: www.elsevier.com/locate/aca

Development of an improved high resolution mass spectrometry based multi-residue method for veterinary drugs in various food matrices

A. Kaufmann*, P. Butcher, K. Maden, S. Walker, M. Widmer

Official Food Control Authority, Fehrenstrasse 15, 8032 Zürich, Switzerland

ARTICLE INFO

Article history:
Received 17 August 2010
Received in revised form
15 November 2010
Accepted 18 November 2010
Available online 24 November 2010

Keywords:
High resolution mass spectrometry
Orbitrap
Veterinary drugs
Multi-residue methods
Validation

ABSTRACT

Multi-residue methods for veterinary drugs or pesticides in food are increasingly often based on ultra performance liquid chromatography (UPLC) coupled to high resolution mass spectrometry (HRMS). Previous available time of flight (TOF) technologies, showing resolutions up to 15,000 full width at half maximum (FWHM), were not sufficiently selective for monitoring low residue concentrations in difficult matrices (e.g. hormones in tissue or antibiotics in honey). The approach proposed in this paper is based on a single stage Orbitrap mass spectrometer operated at 50,000 FWHM. Extracts (liver and kidney) which were produced according to a validated multi-residue method (time of flight detection based) could not be analyzed by Orbitrap because of extensive signal suppression. This required the improvement of established extraction and clean-up procedures. The introduced, more extensive deproteination steps and dedicated instrumental settings successfully eliminated these detrimental suppression effects. The reported method, covering more than 100 different veterinary drugs, was validated according to the EU Commission Decision 2002/657/EC. Validated matrices include muscle, kidney, liver, fish and honey. Significantly better performance parameters (e.g. linearity, reproducibility and detection limits) were obtained when comparing the new method with the older, TOF based method. These improvements are attributed to the higher resolution (50,000 versus 12,000 FWHM) and the superior mass stability of the of the Orbitrap over the previously utilized TOF instrument.

© 2010 Elsevier B.V. All rights reserved.

J. Sep. Sci. 2009, 32, 939 – 948
939

Diran Herebian
Sebastian Zühlke
Marc Lamshöft
Michael Spiteller

Institute of Environmental Research, University of Dortmund, Dortmund, Germany

Original Paper

Multi-mycotoxin analysis in complex biological matrices using LC-ESI/MS: Experimental study using triple stage quadrupole and LTQ-Orbitrap

In the present study, we report the application of LC-MS based on two different LC-MS systems to mycotoxin analysis. The mycotoxins were extracted with an ACN/water/acetic acid mixture and directly injected into a LC-MS/MS system without any dilution procedure. First, a sensitive and reliable HPLC-ESI-MS/MS method using selected reaction monitoring on a triple quadrupole mass spectrometer (TSQ Quantum Ultra AM) has been developed for determining 32 mycotoxins in crude extracts of wheat and maize. This method was operated both in positive and in negative ionization modes in two separate chromatographic runs. The method was validated by studies of spiked recoveries, linearity, matrix effect, intra-assay precision and sensitivity. Further, we have developed and evaluated a method based on accurate mass measurements of extracted target ions in full scan mode using micro-LC-LTQ-Orbitrap as a tool for fast quantitative analysis. Both instruments exhibited very high sensitivity and repeatability in positive ionization mode. Coupling of micro-LC to Orbitrap technology was not applicable to the negatively ionizable compounds. The LC triple quadrupole MS method has proved to be stable in quantitation, as it is with respect to the matrix effects of grain samples.

Keywords: Accurate mass / HPLC-MS/MS / LTQ-Orbitrap / Multi-method / Mycotoxins

Received: October 21, 2008; revised: January 8, 2009; accepted: January 9, 2009

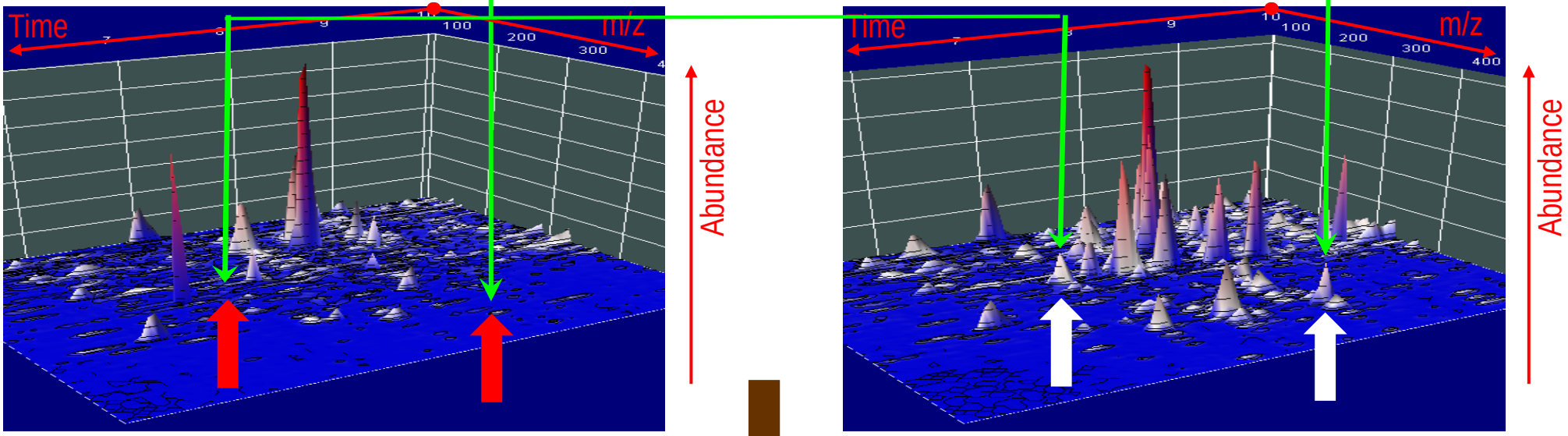
DOI 10.1002/jssc.200800589

The metabolome: a high resolution picture may reveal some differences

Comparison of signal abundances in 2 (or more) groups of samples

Sample(s) from controlled animal(s)

Sample(s) from treated animal(s)



Statistical criteria F (ANOVA) or t (student test) associated to a statistical confidence level (p-value)

1. Introduction

Expected level of information
and consequence on the MS technology

RESOLUTION

WEAK → MEDIUM → HIGH → ULTRA-HIGH

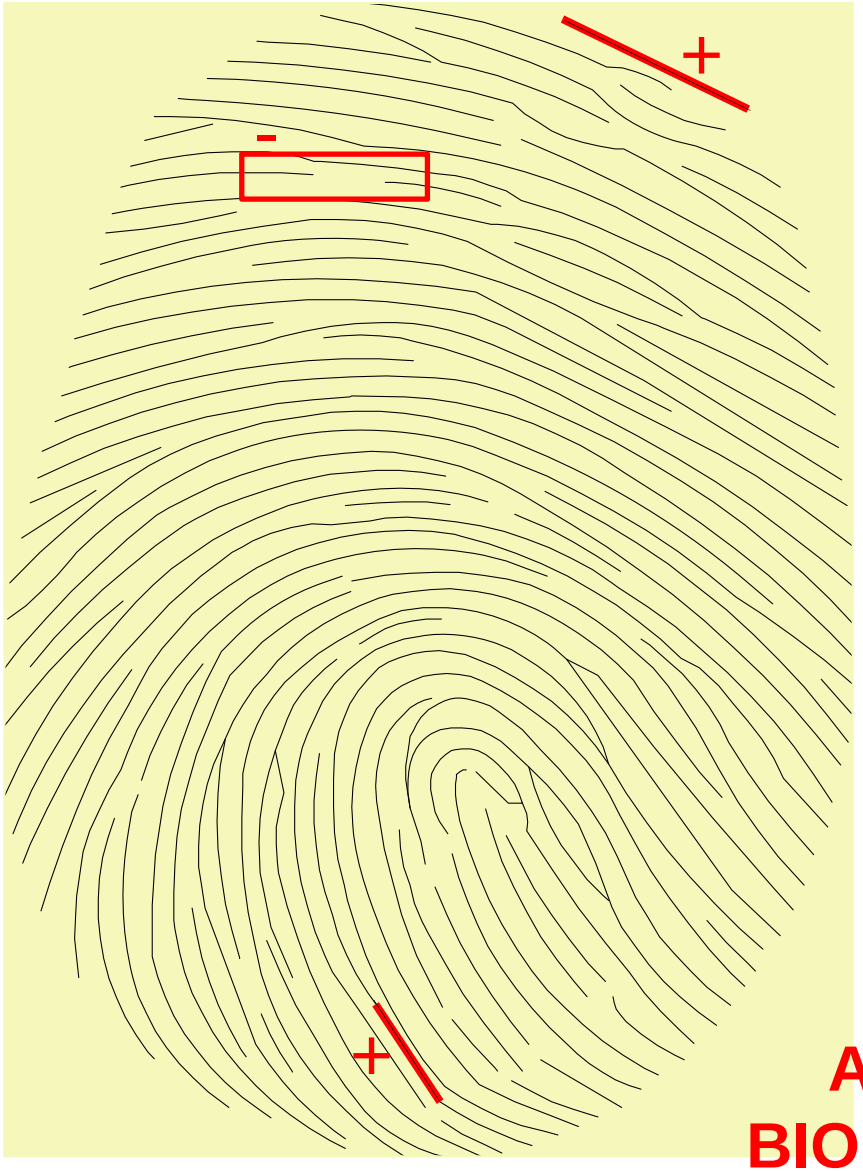
MASS SPECTROMETRY

QUADRUPOLE → TOF → FT-ORBITRAP → FT-ICR

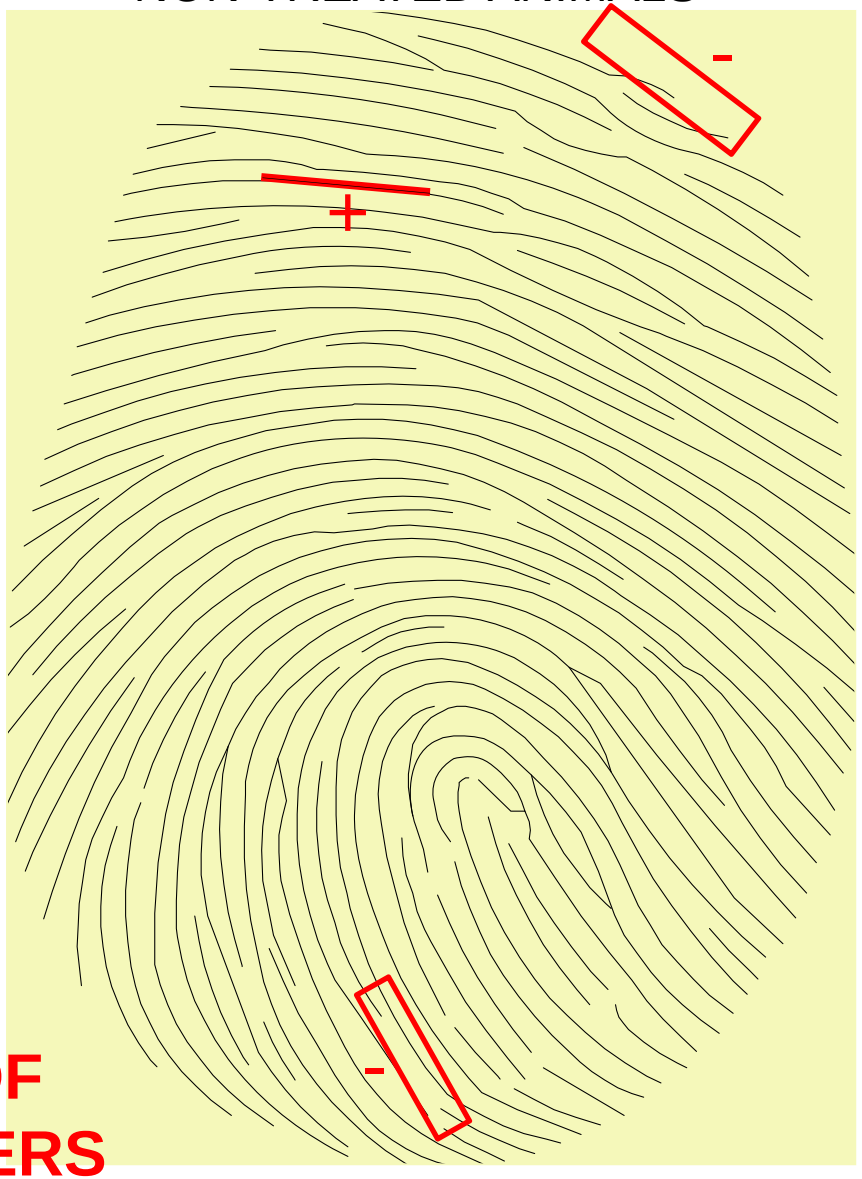
1. Introduction

A very high resolution picture processed with an *ad hoc* statistical tool may reveal even light differences

TREATED



NON-TREATED ANIMALS



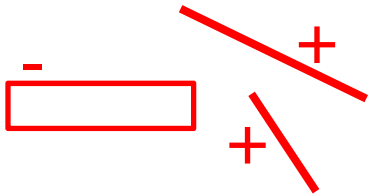
A SET OF BIOMARKERS

1. Introduction

Use of markers for predicting animal appurtenance to a group

TREATED ANIMAL

REFERENCE BIOMARKERS' PROFILE



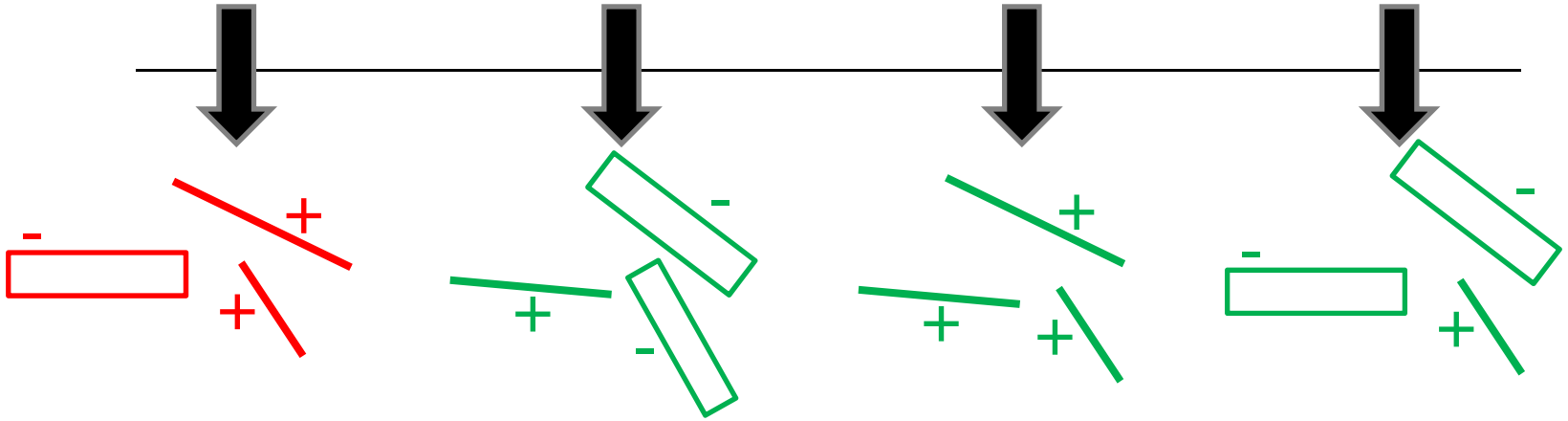
SAMPLE from ANIMAL 1

SAMPLE from ANIMAL 2

SAMPLE from ANIMAL 3

SAMPLE from ANIMAL 4

BIOMARKERS QUANTITATION



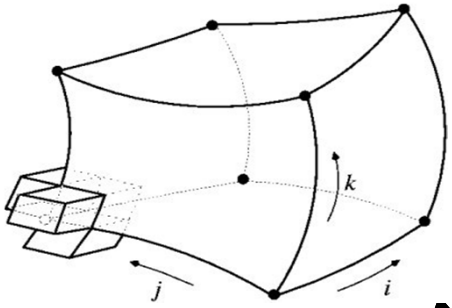
SAMPLE STATUS ?

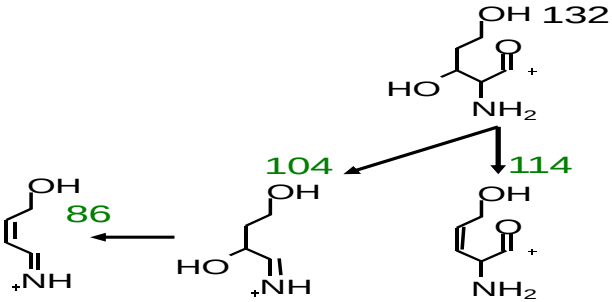
SUSPECT

COMPLIANT

1. Introduction

Principle, detection of diagnostic markers and further use

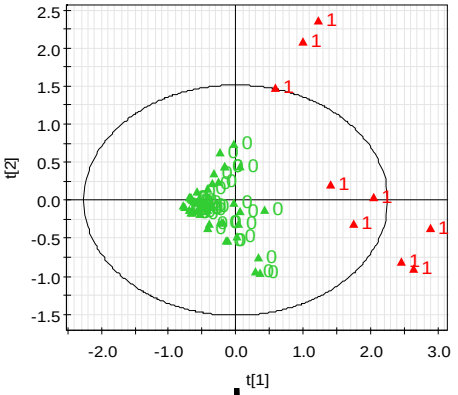




BIOMARKERS IDENTIFICATION

CANDIDATE BIOMARKER(S)

VALIDATION

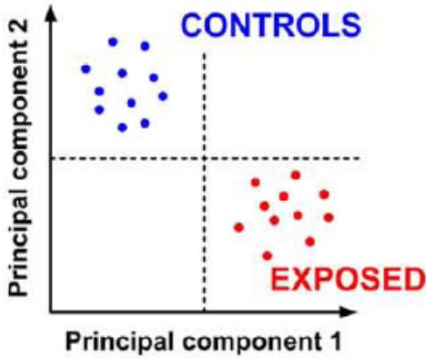


DATA PROCESSING
(XCMS, Peak picking, grouping and RT adjustment)

EXTRACTION DILUTION,...

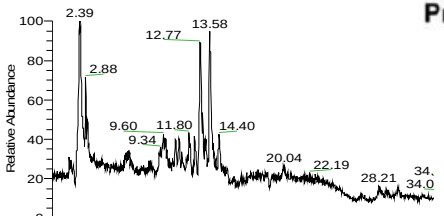
Urine

MULTIVARIATE STATISTICS
(supervised or not)



SAMPLE PREPARATION

FINGERPRINTING



ANIMAL EXPERIMENT

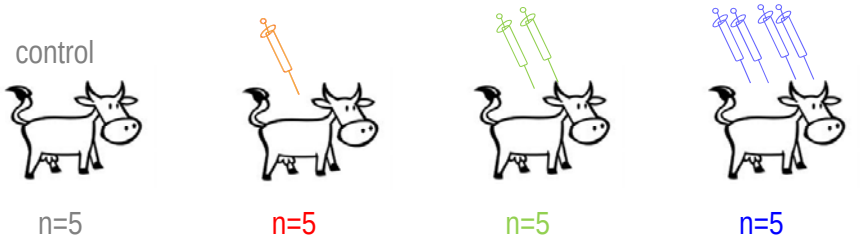
BIOMARKER(S)

UTILISATION

1. INTRODUCTION

2. ILLUSTRATIONS

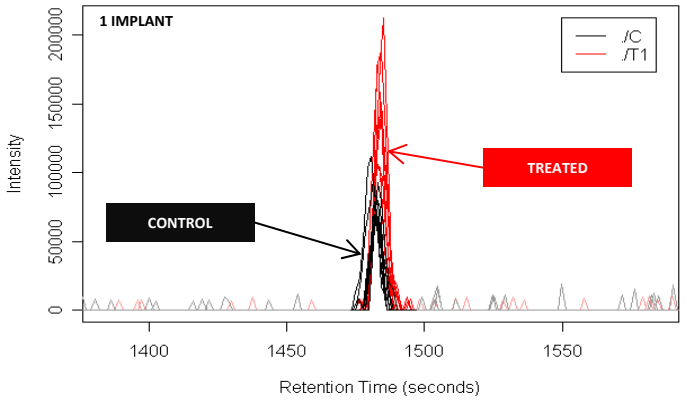
3. CONCLUSION AND PERSPECTIVES

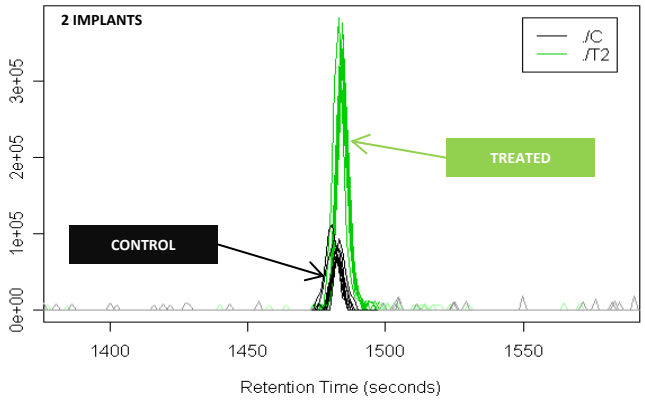


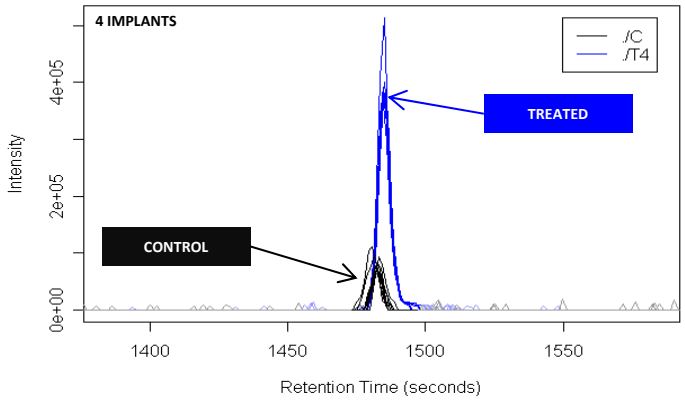
Untargeted, adult bovines, kidney, steroids,
Validation of candidate biomarkers

Revalor ® implants
(140 mg trenbolone acétate + 24 mg estradiol)

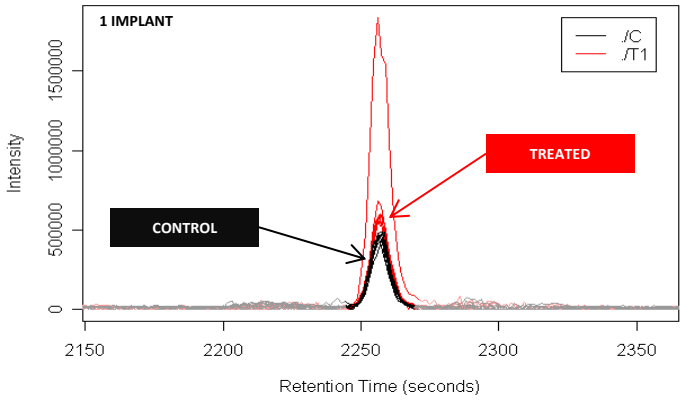
m/z : 597.1 ; RT : 24.7 min

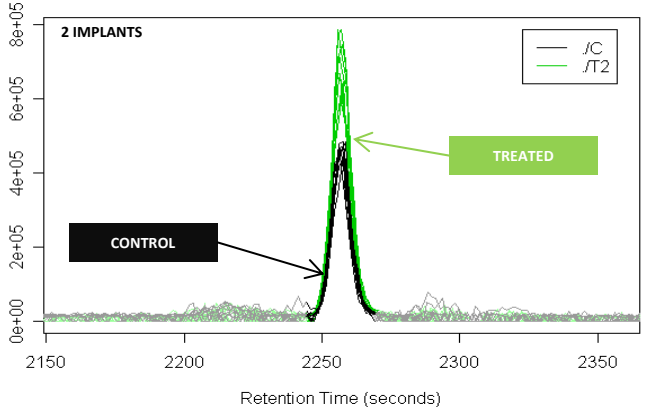


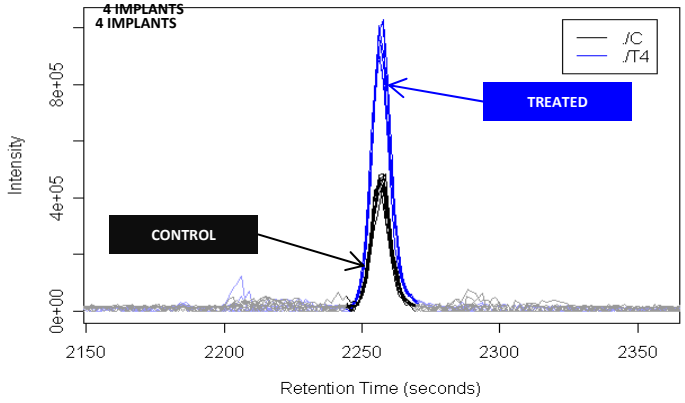


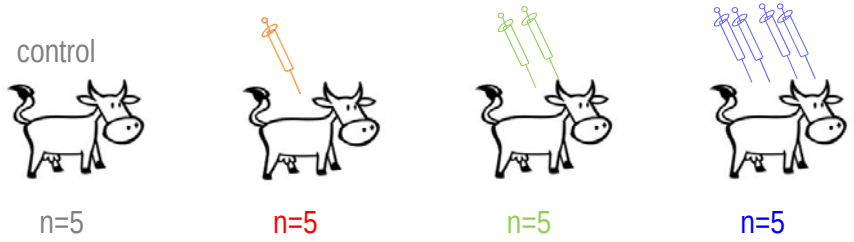


m/z : 781.5 ; RT : 37.6 min





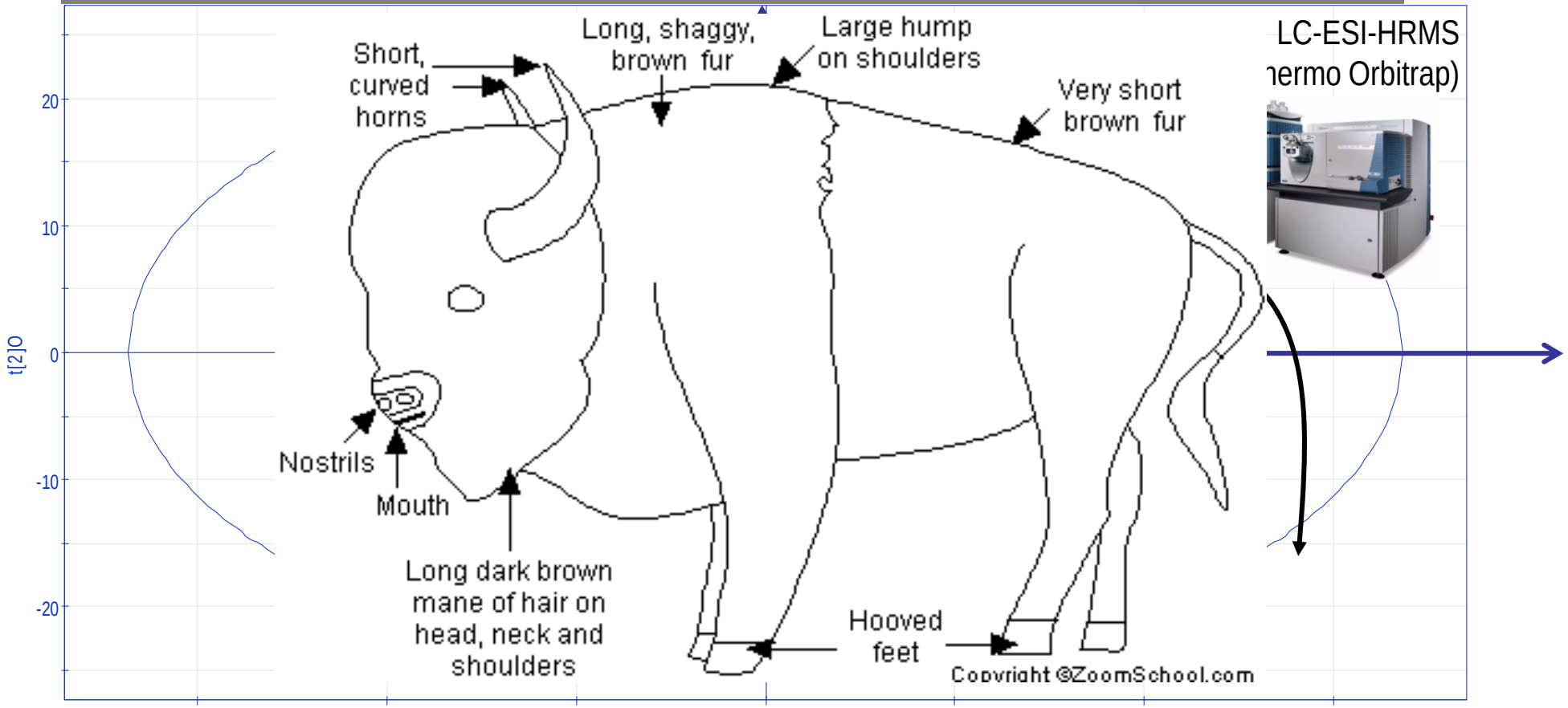




Untargeted, adult bovines, kidney, steroids,
Observation of the data, group separation

Revalor ® implants
(140 mg trenbolone acetate + 24 mg estradiol)

OPLS performed on metabolic fingerprints recorded on kidney samples collected at D90 - hexane extract



LC-ESI-HRMS
hermo Orbitrap)

Axis = Equation involving different biomarkers explaining the difference (variability) between the groups

Sample preparation directly impacts the dimension and the nature of the fingerprinting

Liquid matrices

- 80°C

Plasma/Serum

Urine

Normalization

Connective role
High dynamic [ ] range (>10^5).
100's of molecules < 1KDa and >1KDa.
Many large proteins.

Filtration

Secretary fluids.
Extreme dynamic [ ] range (>10^{11}).
Thousands of molecules < 1KDa.
Many small proteins
High enzyme activities.

Solid matrices

Muscle

Kidney fat

Lyophilization

Liquid/Solid Extraction

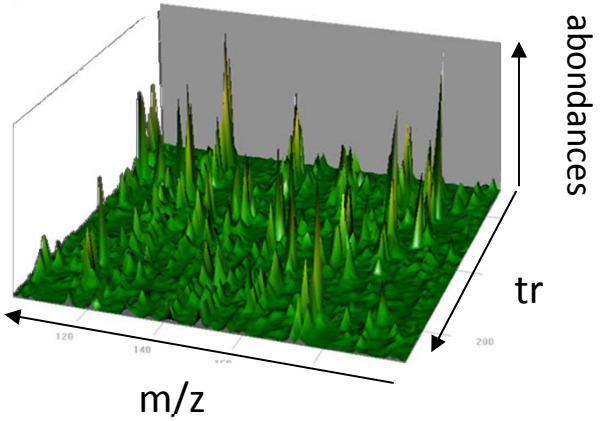
CH_3OH/Water
CH_3OH

CH_3CN
Acetone

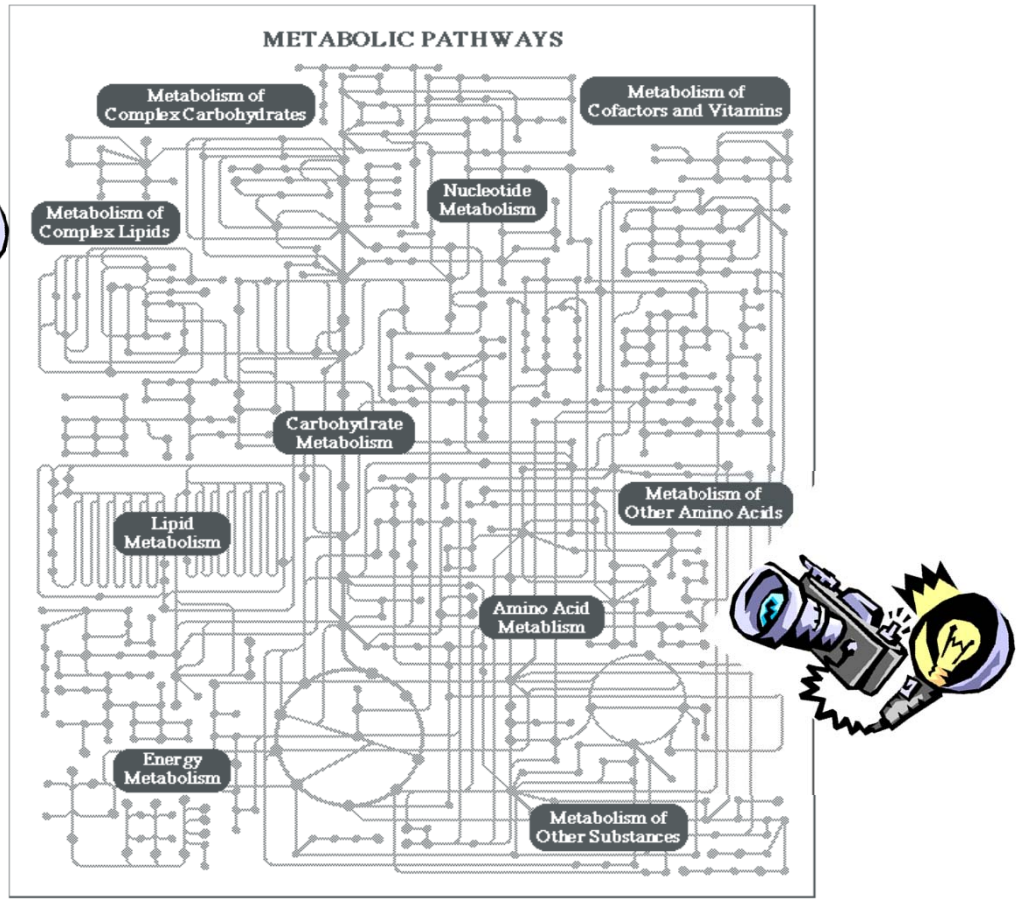
DCM
Hexane

+ **POLARITY** -

Richesse d'information: Nombre d'ions



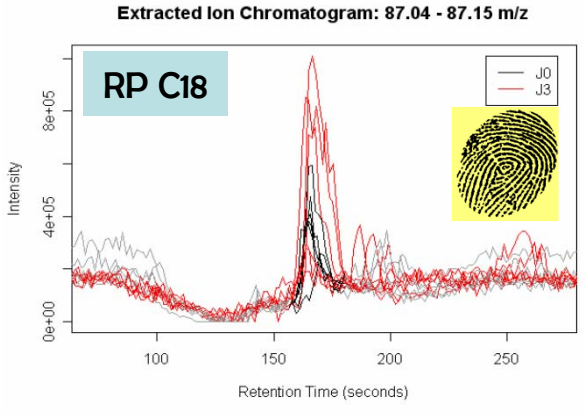
Spécificité: Fraction du métabolome

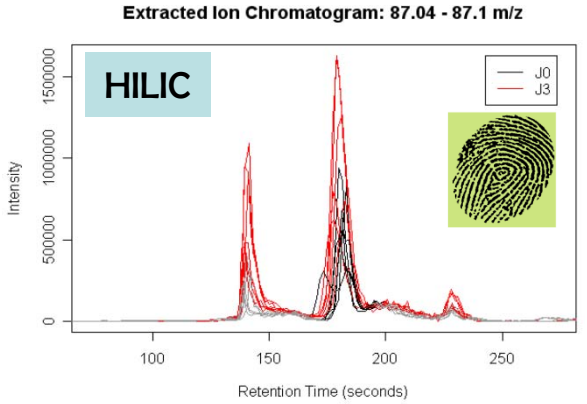


	Richesse d'information	Spécificité
Méthanol	1955 ions	Sucres, acides aminés, acides gras
Méthanol + ultrasons	2010 ions	Sucres, acides aminés, acides gras
Acétonitrile	1791 ions	Acides gras, acides organiques
Chloroforme/ méthanol	1872 ions	Acides aminés, acides gras
CME *Phase Chloroforme*	1854 ions	Acides gras, stéroïdes, acides organiques
CME *Phase aqueuse*	2029 ions	Sucres, acides aminés

Signal acquisition: plenty of possibilities, but not without Consequences on the characterized metabolome

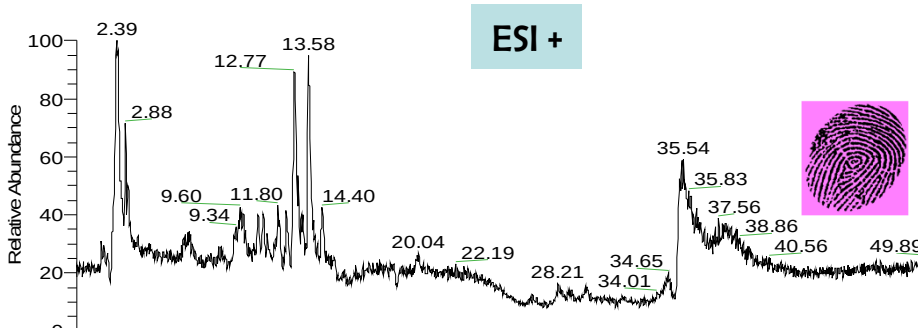
SEPARATION

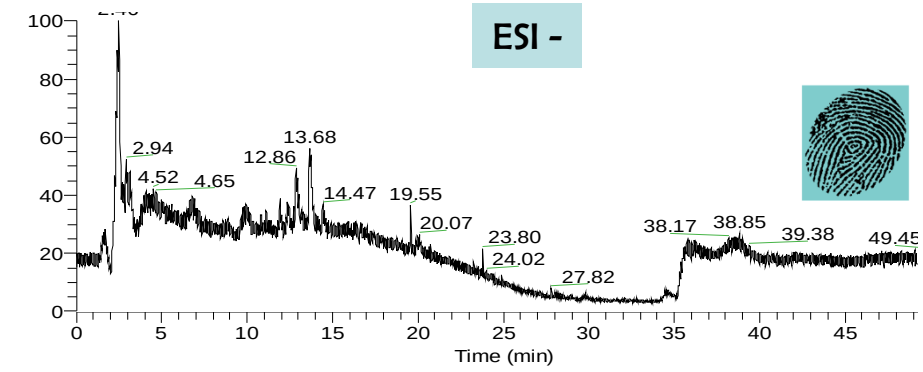




Or DI, GCxGC, LCxLC....

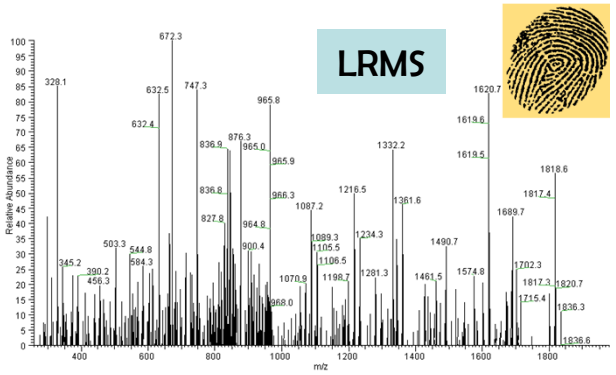
IONIZATION

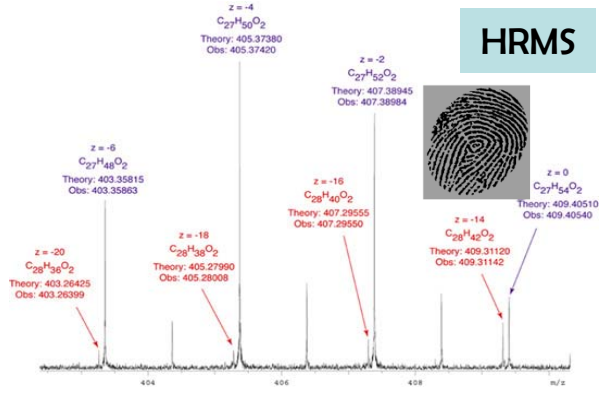




Or APCI, APPI, EI, PCI, NCI...

ACQUISITION





Or LRMS, HRMS, HRMS^n
MSn, IMS...

Does the mass spectrometers define the biomarker(s)? Gika et al. Anal Chem (2010) 82(19): 8226-8234.

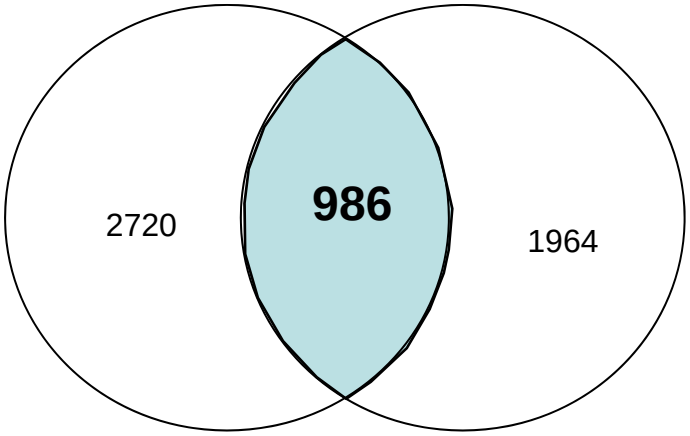
Bovine treated with Revalor-XS implant
200 mg TbAc + 40 mg E2

N=32 / sampling day
(16 control, 16 treated animals)

Filtration at 10 Kda
Normalization at 1.013 c

SYNAPT (Q-TOF, H2DMS)
ESI+, m/z 65-1000

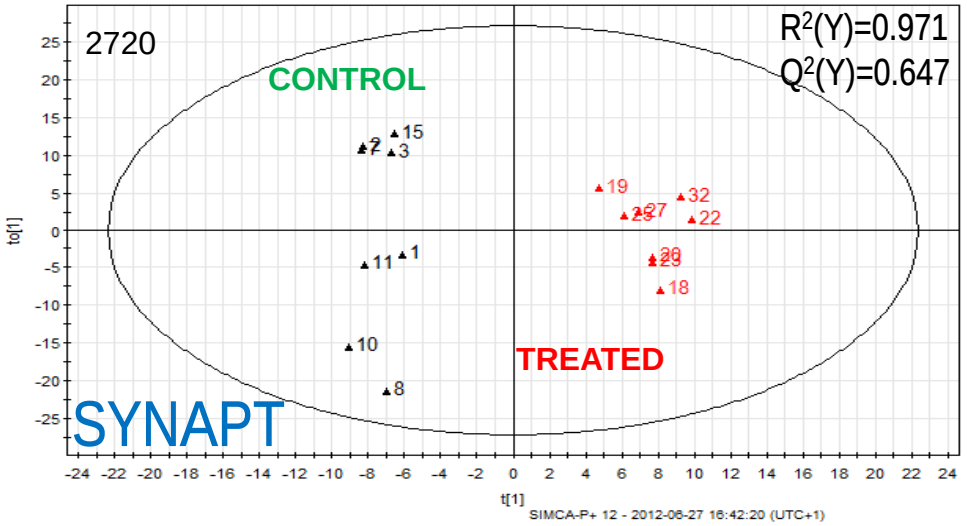
EXACTIVE (ORBITRAL TRAP)
ESI+, m/z 80-1000

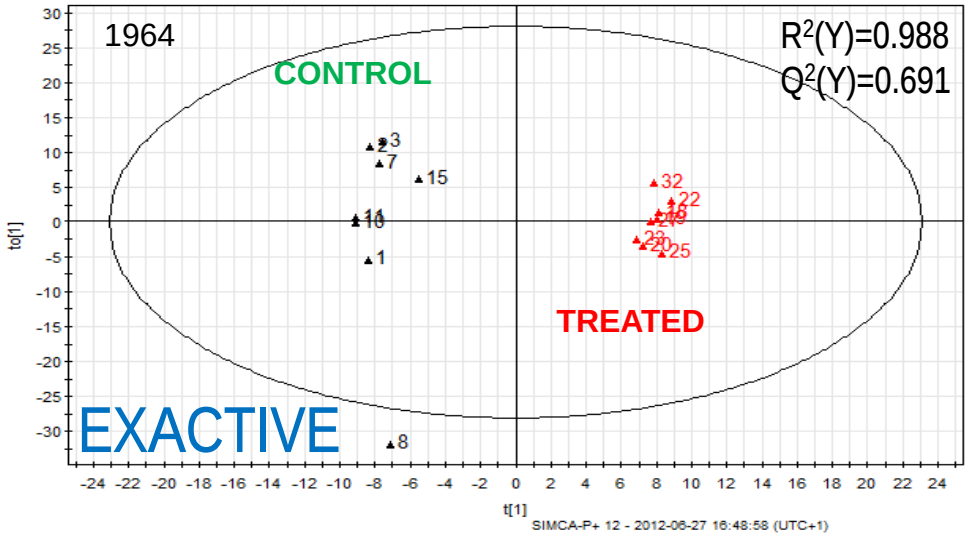


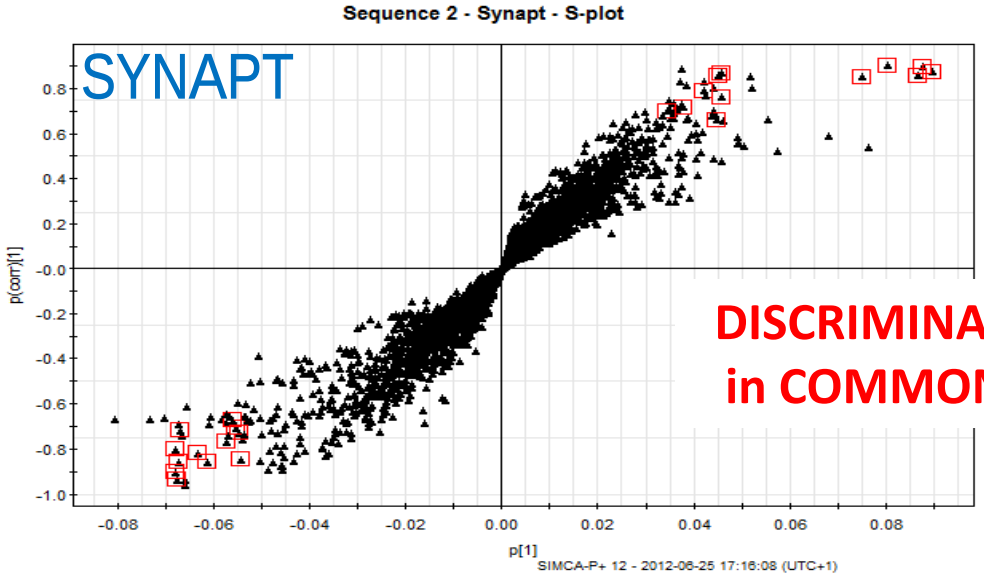
2720 ions as constituting the acquired metabolic fingerprints

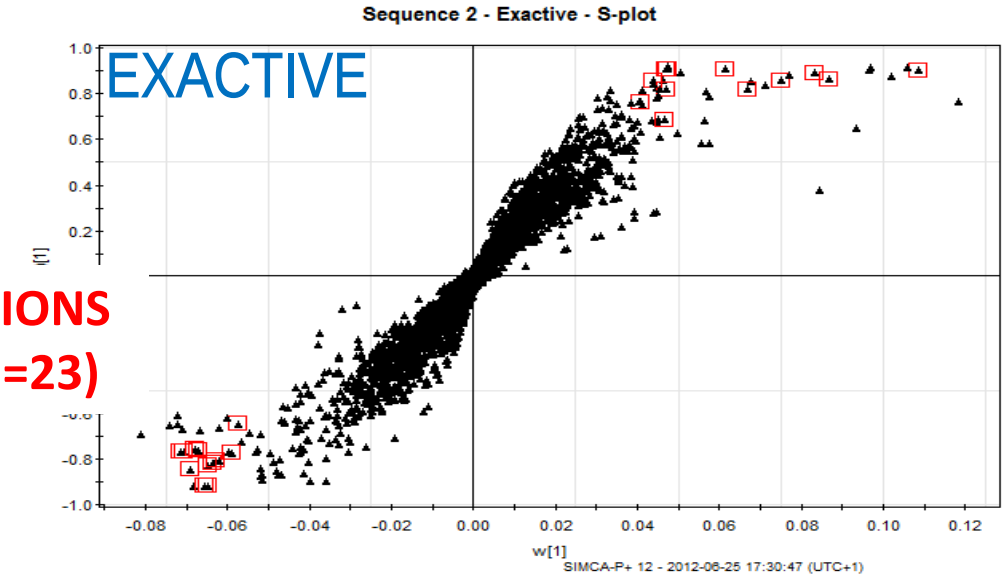
1964 ions as constituting the acquired metabolic fingerprints

Does the mass spectrometers define the biomarker(s)? Gika et al. Anal Chem (2010) 82(19): 8226-8234.



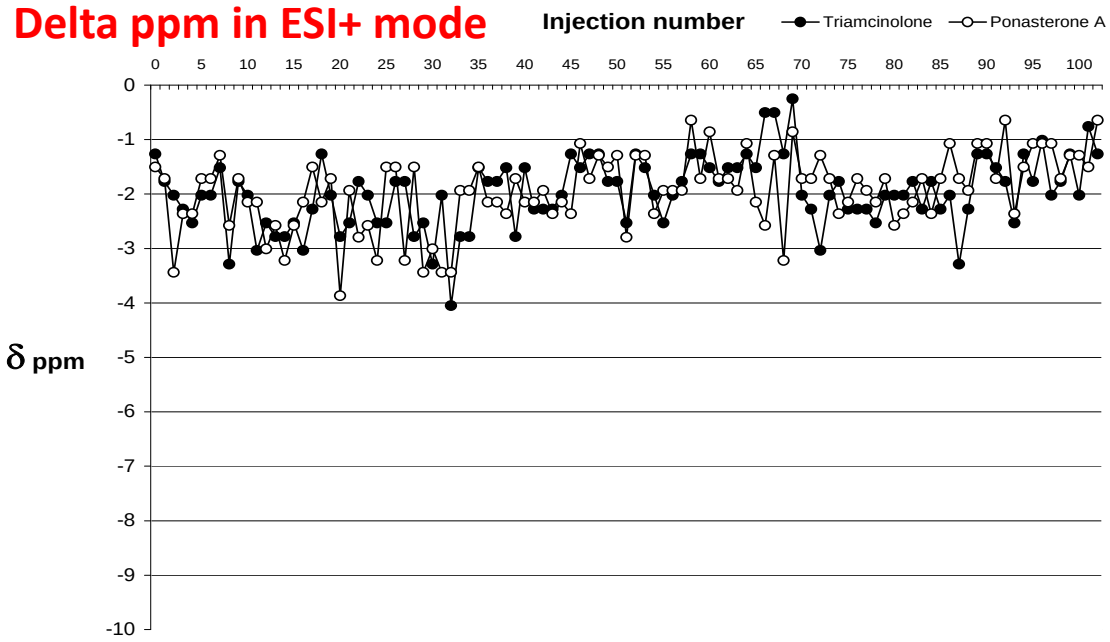




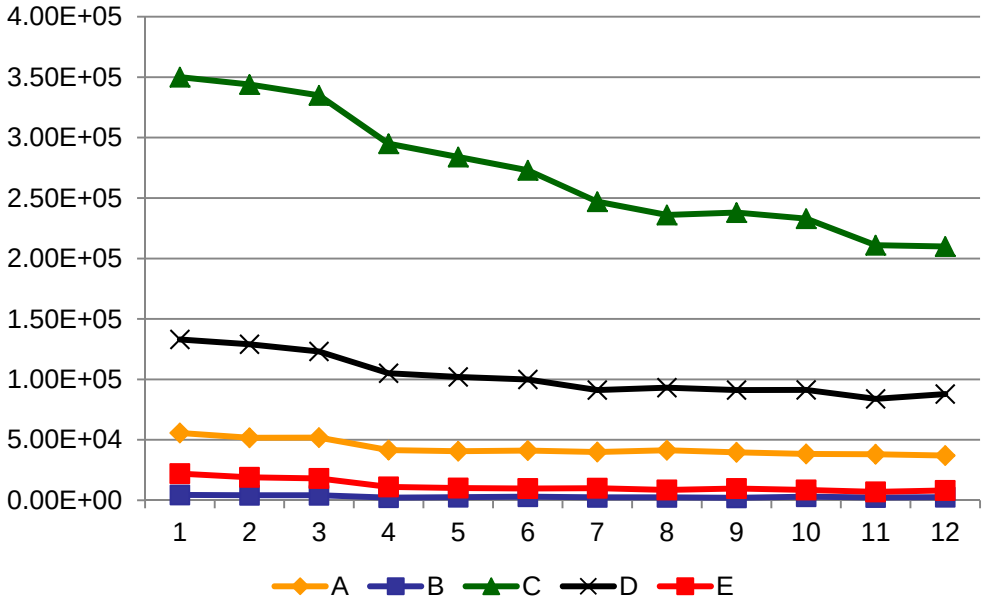


DISCRIMINANT IONS in COMMON (n=23)

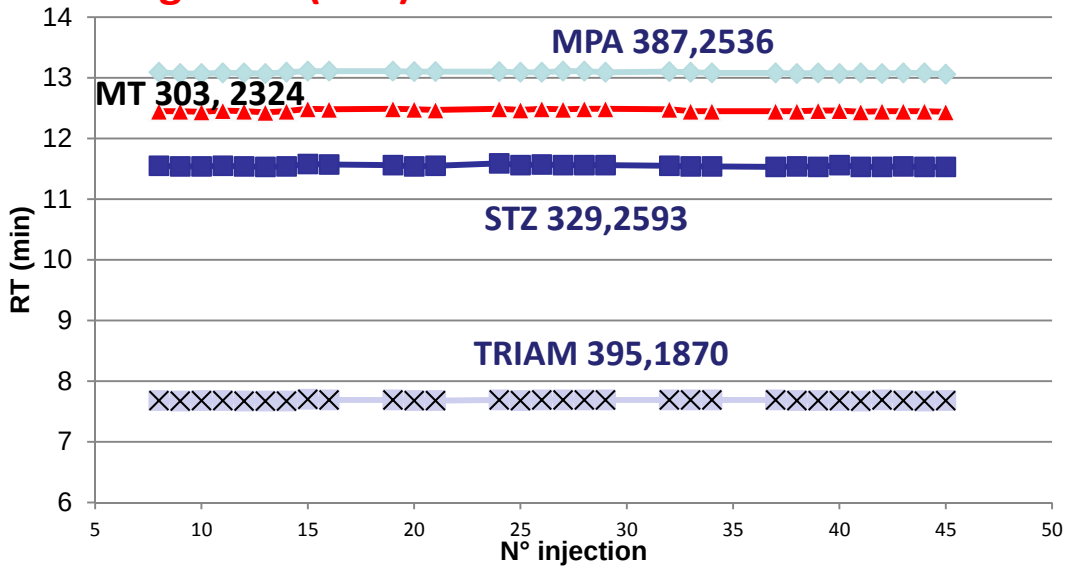
Delta ppm in ESI+ mode



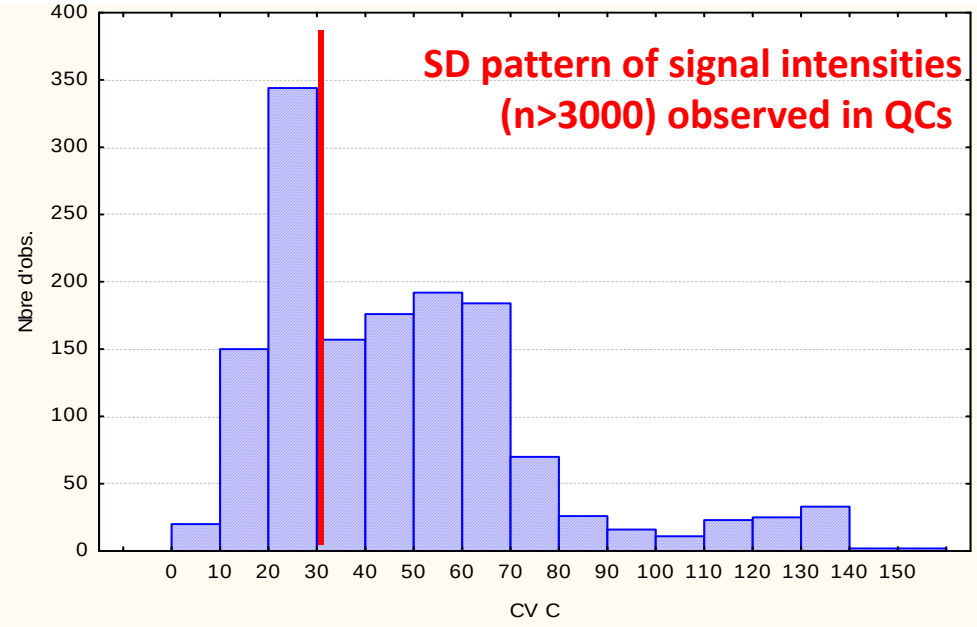
Monitoring of abundances



Monitoring of RT (min) of Ext Std

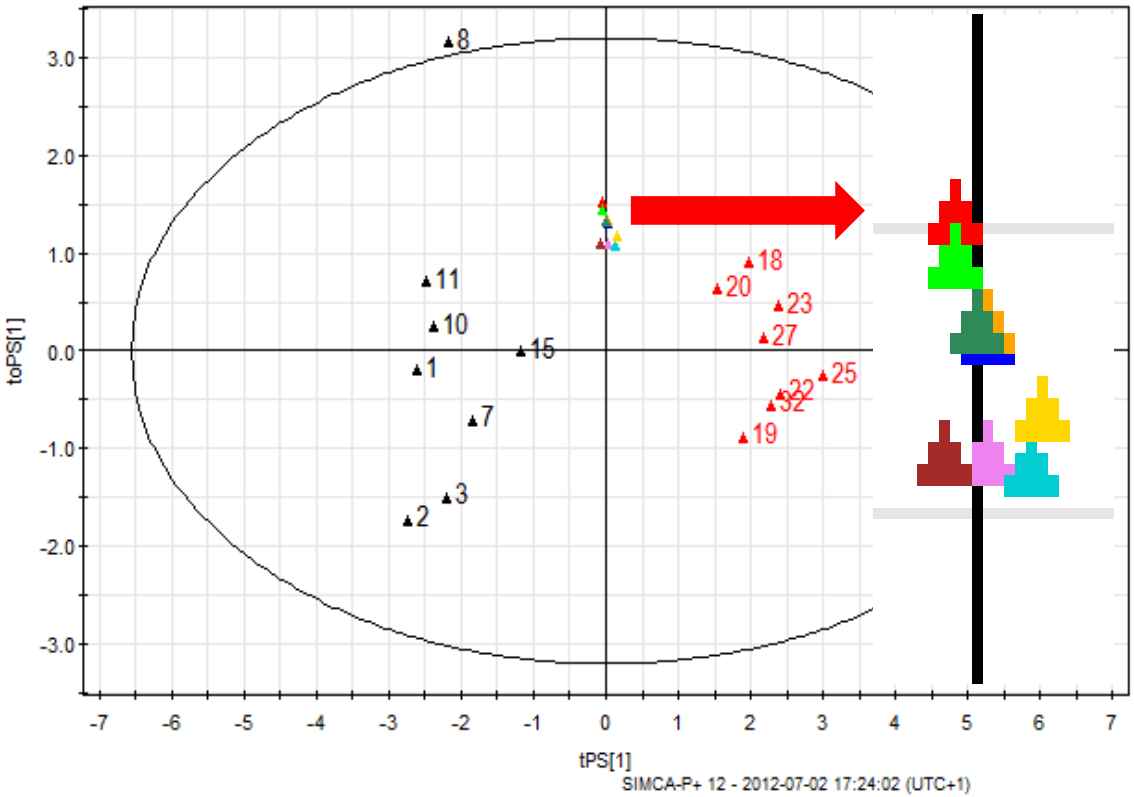


SD pattern of signal intensities (n>3000) observed in QCs



➢ OPLS-DA model based on selected variables

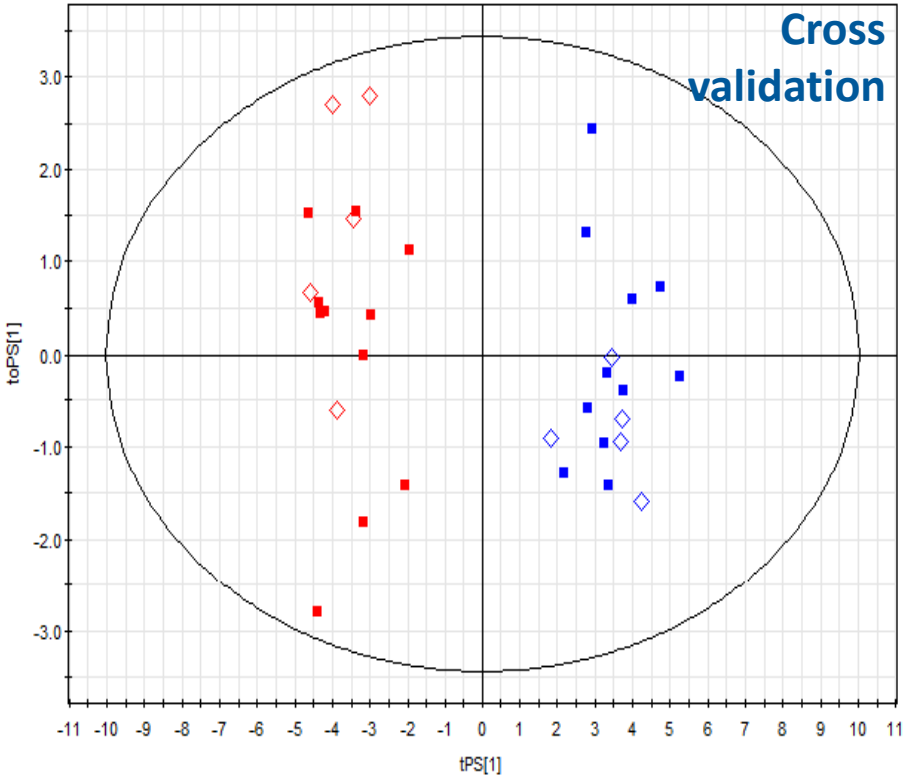
(Fold change up to 1.5 and P-value less then 0.05)

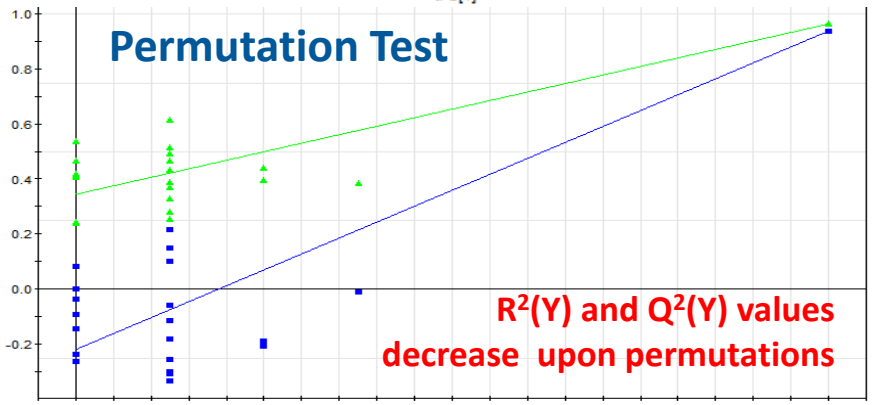


CV- ANOVA → suggests that the model is significant with a P-value $1.67E^{-13}$

➢ Candidate biomarkers robustness

■ OPLS Model ◇ Prediction Set



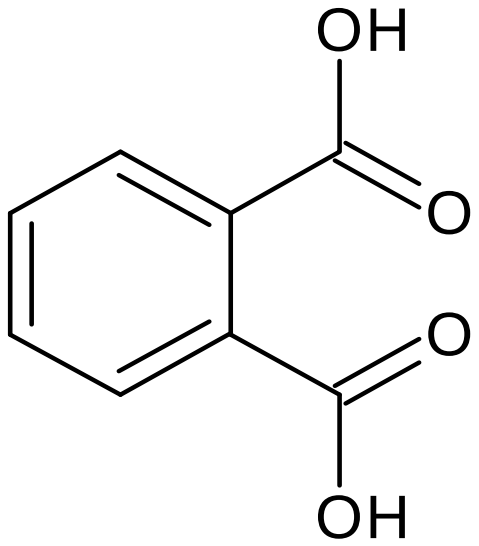


XCMS

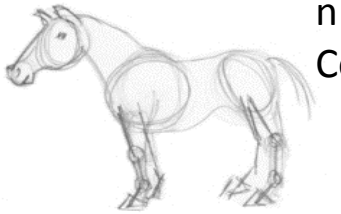
name	fold	tstat	pvalue	anova
M155T427	8.21002385	7.34062951	0.00038998	0.00011103
M154T427	11.0439706	7.26770818	0.00075516	0.00018432
M181T667	1.89111638	4.95814418	0.00115956	0.03680928
M269T651	2.61734154	5.06900243	0.0046466	0.00160242
M202T353	6.30632103	4.80249581	0.0066701	0.01674084

LABERCA data bank

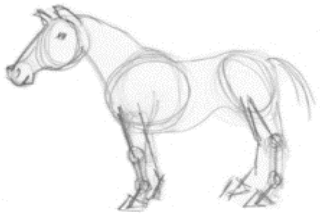
	A	B	AO	AP	AQ	AR	AS	AT	AU
1		Data file							
2		Statut							
3	Rank	Raw Data	CV% QC	Moyenne TEN	Moyenne DO	delta MZ (mn	delta TR (s)	Putative identification	
49	1	M489T640	18.54	1037120.46	3001810.63	1.51E+01	5		
50	2	M205T197	20.81	433496546	1313244856	6.98E-01	37	ID#99/ [L-tryptophan+H]+	
51	3	M955T639	10.22	13455.5412	4497297.28	1.08E+01	5		
52	4	M373T700	16.75	210028.873	66505560.1	1.10E-01	5	ID#81/ [cholic acid-2(H2O)+H]+	
53	5	M188T197	20.89	352324325	1040749063	5.44E-02	37		
54	6	M377T413	6.90	10507383.9	443568393	3.69E+00	21	ID#101/ [riboflavin+H]+	
55	7	M932T639	19.96	11751.0685	52793383.7	6.96E-01	4		
56	8	M206T197	21.08	49705952.7	155301501	5.84E-01	37		
57	9	M146T197	22.15	4818132.97	16169765.5	7.82E-01	37		
58	10	M934T639	20.75	1767.52729	10753889.1	1.12E+01	5		
59	11	M523T621	9.65	281843.181	4795557.97	1.69E+00	4		
60	12	M376T413	9.26	6160.58391	3790220.39	2.77E+01	5		
61	13	M195T381	21.02	20324046.8	814351273	8.20E+00	7		
62	14	M754T413	10.44	2915.77917	7375029.97	1.01E+00	4		
63	15	M196T381	21.41	1853450.82	68752152.2	3.04E-01	6		
64	16	M951T640	4.18	16273859.3	56598396.7	1.11E+02	13		



Urine, growth hormone



n = 90
Control

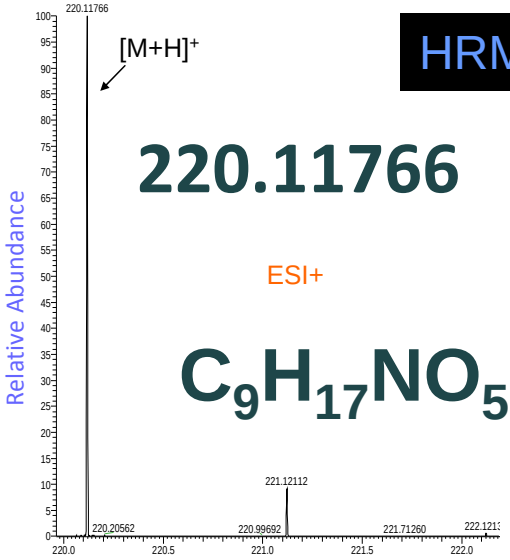


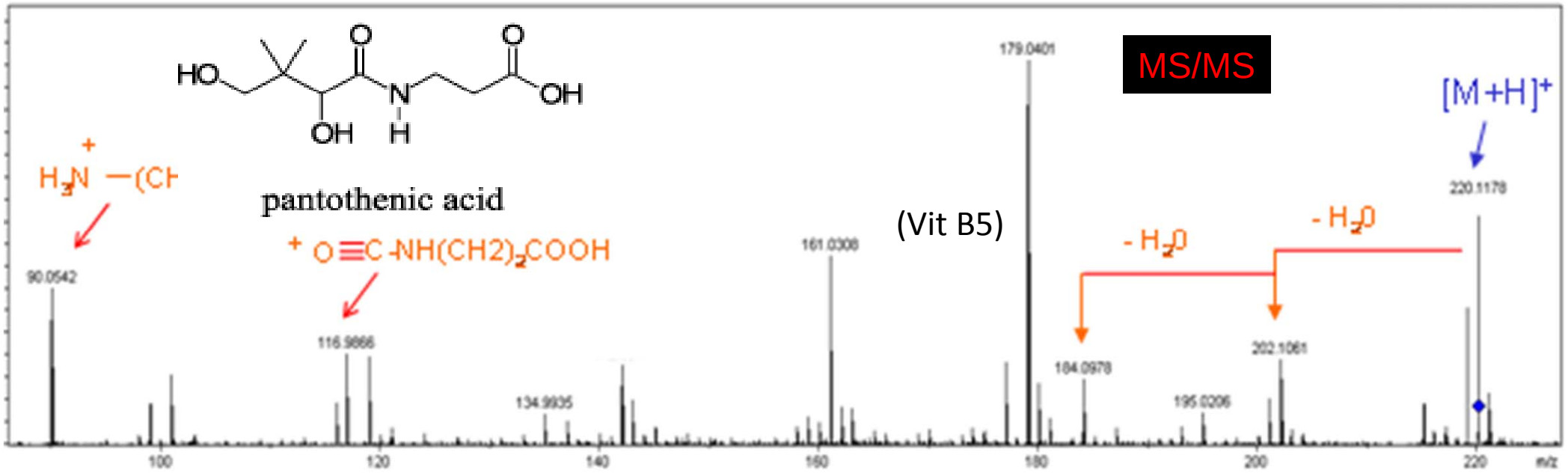
n = 160
Treated

reGH

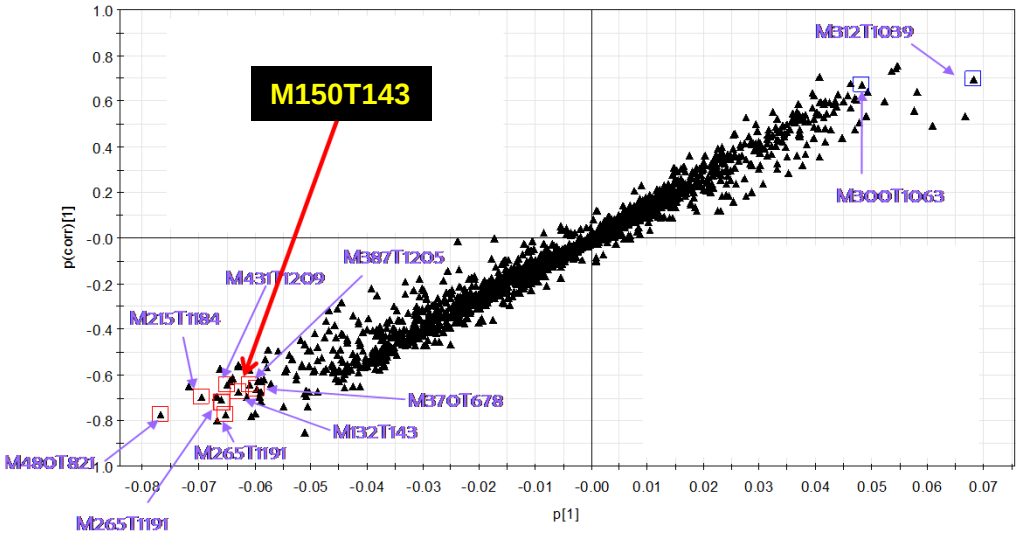
(18 µg.kg^{-1} bw/d → [0-15]d)

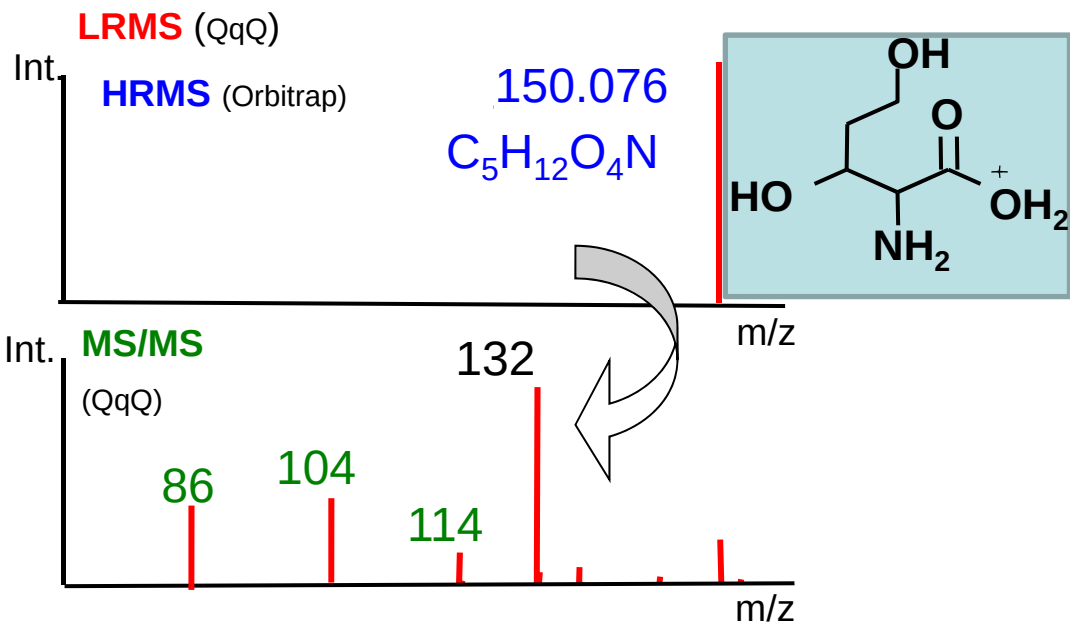
Kieken et al., Anal. Bioanal. Chem. 2009;394:2119-2128.

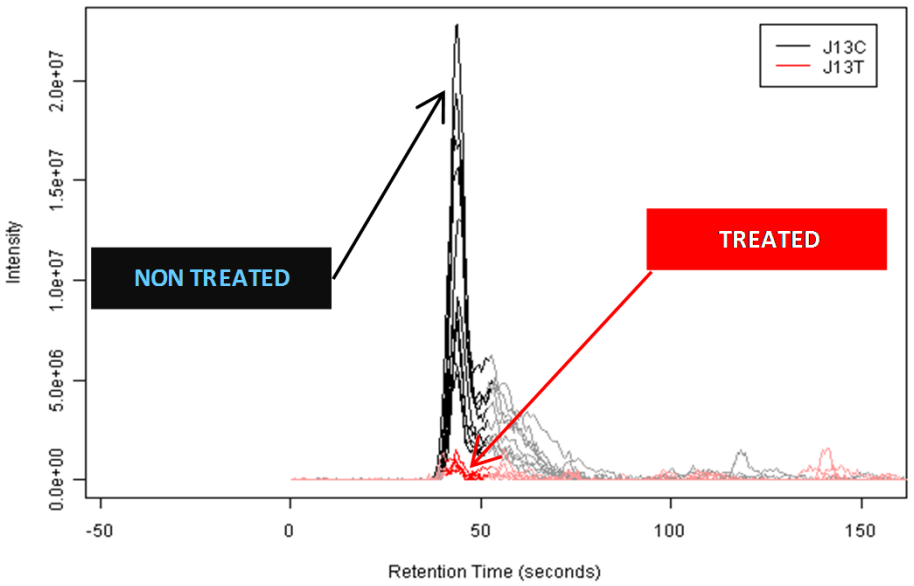




Untargeted, calves, urine, β-agonists
Identification of biomarkers







BMC Bioinformatics

BioMed Central

Research article

Open Access

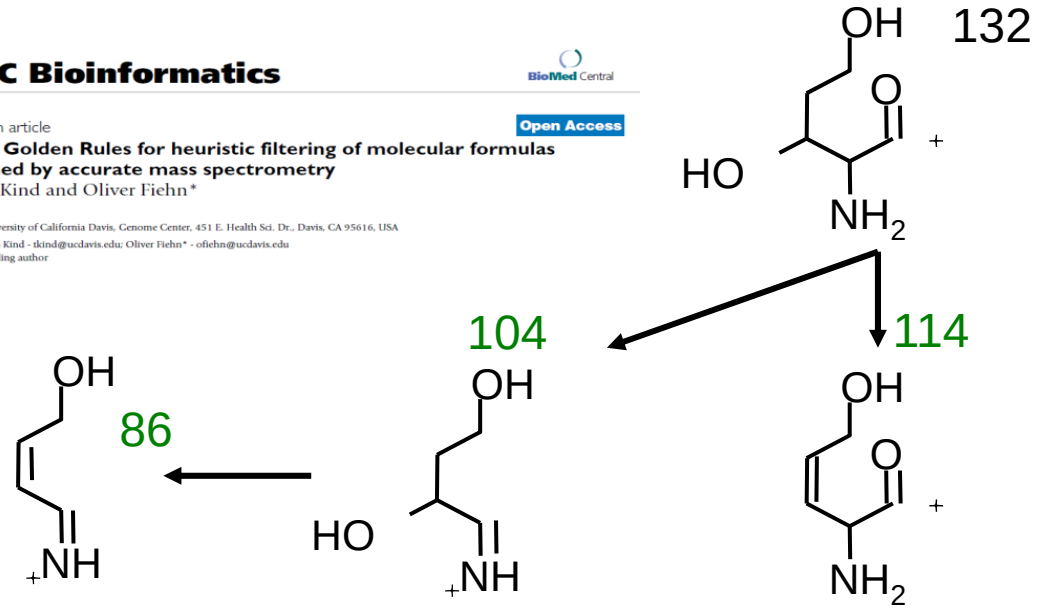
Seven Golden Rules for heuristic filtering of molecular formulas obtained by accurate mass spectrometry

Tobias Kind and Oliver Fiehn*

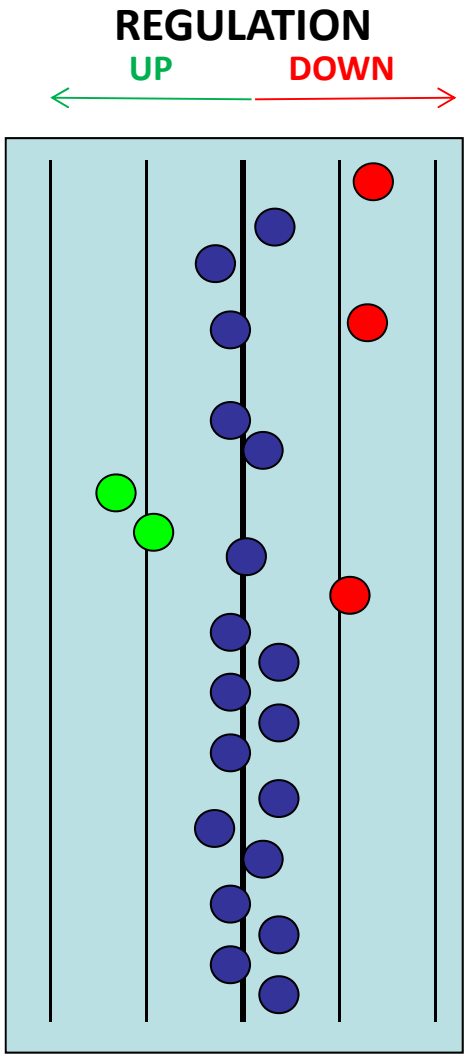
Address: University of California Davis, Genome Center, 451 E. Health Sci. Dr., Davis, CA 95616, USA

Email: Tobias Kind - tkind@ucdavis.edu; Oliver Fiehn* - ofiehn@ucdavis.edu

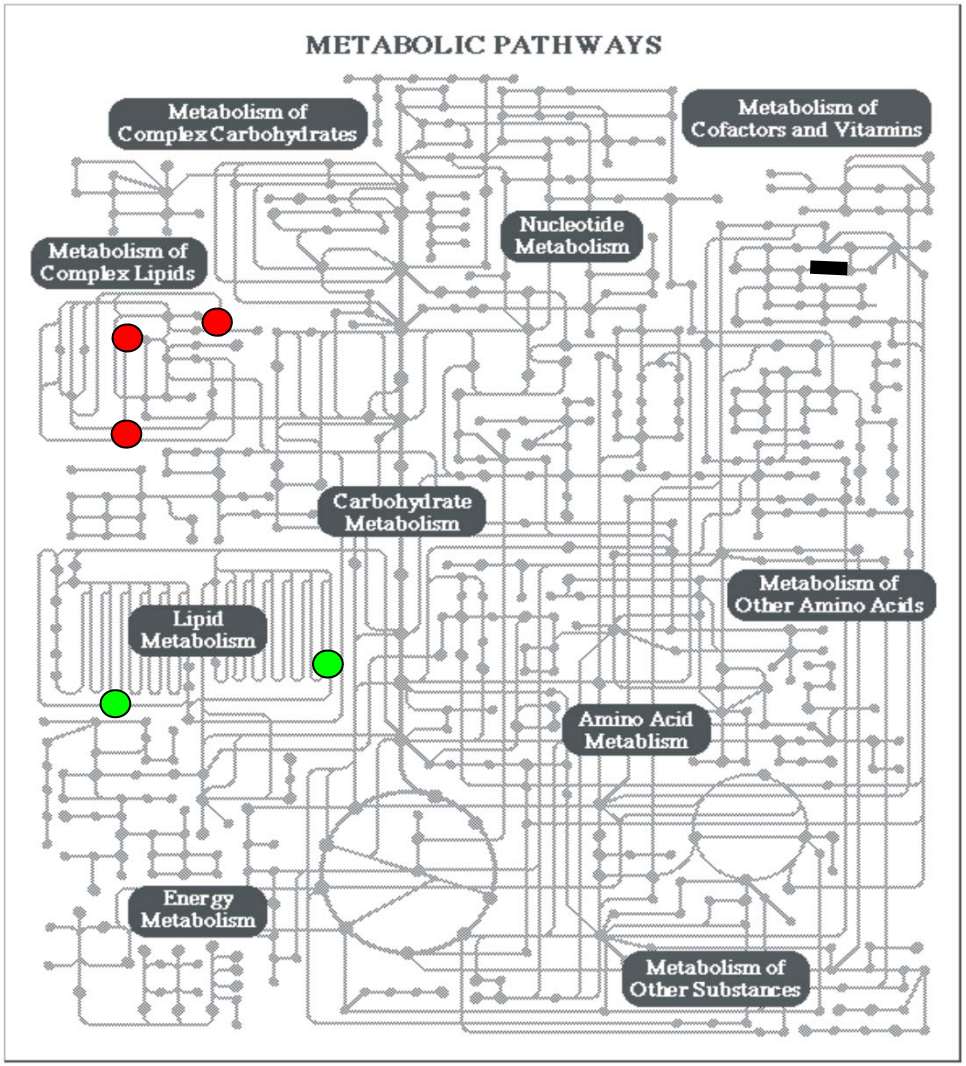
* Corresponding author



Biochemical profile map of the metabolic pathways:
understand what has been modified



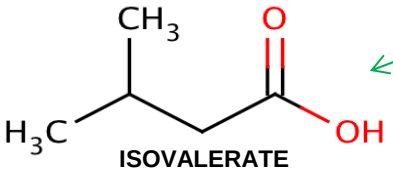
Markers' profile



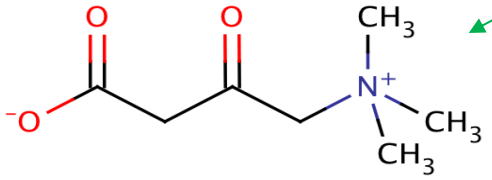
ONIRIS ONIR-02-10VW - Calf Anabolic Steroid

Heat map of statistically significant biochemicals profiled in this study. Shaded cells indicate p≤0.05 (red indicates that the mean values are significantly higher for that comparison; green values significantly lower). Blue-bolded text indicates 0.05<p<0.10.

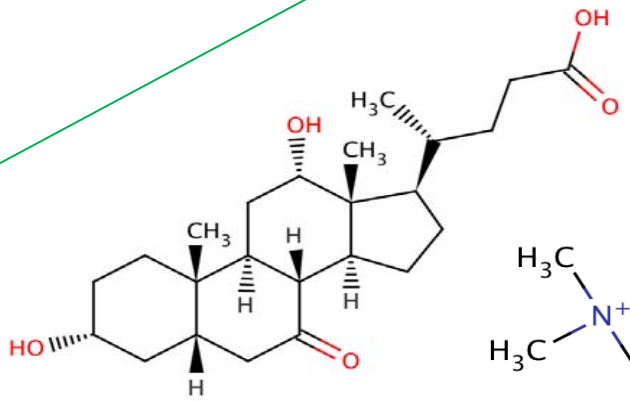
PATHWAY SORT	SUPER PATHWAY	SUB PATHWAY	BIOCHEMICAL NAME	PLATFORM	COMP ID	Kegg	HMDB	TREATED DAY 4 vs CONTROL DAY 4	TREATED DAY 4 vs TRATED DAY -7
504			sebacate (decanedioate)	LC/MS neg	32388	C08277	HMDB0001	1,36	0,68
505			azelate (nonanedioate)	LC/MS neg	18362	C08261	HMDB0001	1,20	0,67
505,8			dodecanedioate	LC/MS neg	32388	C0267	HMDB0006	1,27	0,59
521,4		Fatty acid, beta-oxidation	suberylglycine	LC/MS neg	35419		HMDB0090	1,33	0,64
561,12		Fatty acid metabolism (also BCAA metabolism)	propionylglycine	LC/MS pos	31932		HMDB0079	0,78	0,71
561,14			butyrylglycine	LC/MS pos	31850		HMDB0085	0,94	1,08
561,16		Fatty acid metabolism	valerylglycine	LC/MS pos	36768		HMDB0901	0,82	1,02
561,17			isovalerate	LC/MS neg	34732	C0826	HMDB0071	3,05	0,60
561,18			hexanoylglycine	LC/MS neg	35436		HMDB0070	1,31	0,77
563		Carnitine metabolism	3-dehydrocarnitine*	LC/MS pos	32654	C0263	HMDB1215	**0,56**	0,39
588	Lipid	Bile acid metabolism	cholate	LC/MS neg	22842	C0069	HMDB0061	0,81	0,62
591			glycocholate	LC/MS pos	18476	C01921	HMDB0013	0,94	0,51
592			taurocholate	LC/MS neg	18497	C05122	HMDB0000	0,98	0,42
597			3-dehydrocholate	LC/MS pos	31900		HMDB0050	1,27	0,56
598			deoxycholate	LC/MS neg	1114	C0448	HMDB0063	0,41	**1,20**
601			7-ketodeoxycholate	LC/MS neg	31904		HMDB0034	2,25	1,07
607			7,12-diketolithocholate	LC/MS pos	32323			1,94	0,83
619		Glycerolipid metabolism	ethanolamine	GC/MS	1497	C00189	HMDB0014	1,01	0,51
620			phosphoethanolamine	GC/MS	12102	C0034	HMDB0020	0,93	0,32
622			glycerol	GC/MS	15122	C00116	HMDB0131	1,20	0,54
623			choline	LC/MS pos	15506	C00114	HMDB0000	1,74	0,63
624			glycerol 3-phosphate (G3P)	GC/MS	15365	C0009	HMDB0122	1,19	0,37
630		Inositol metabolism	myo-inositol	GC/MS	19334	C00137	HMDB0210	0,92	0,47
630,1			chiro-inositol	GC/MS	37112			1,65	1,04
630,5			pinitol	GC/MS	37086	C0384		0,80	0,62
710		Mevalonate metabolism	3-hydroxy-3-methylglutarate	GC/MS	531	C03761	HMDB0037	1,48	1,04
729		Sterol/Steroid	dehydroisoandrosterone sulfate (DHEA-S)	LC/MS neg	32425	C0455	HMDB0103	1,30	0,69
777		Purine metabolism, (hypo)xanthine/inosine	allantoic acid	LC/MS neg	33434	C0049	HMDB0120	2,15	0,66
779			adenine	GC/MS	554	C00147	HMDB0000	1,11	0,76



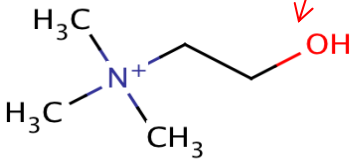
ISOVALERATE



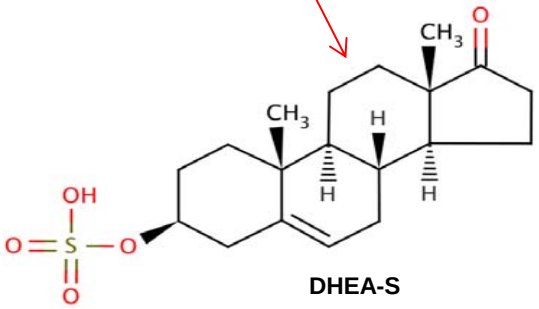
3-DEHYDROCARNITINE



7-KETODEOXYCHOLATE



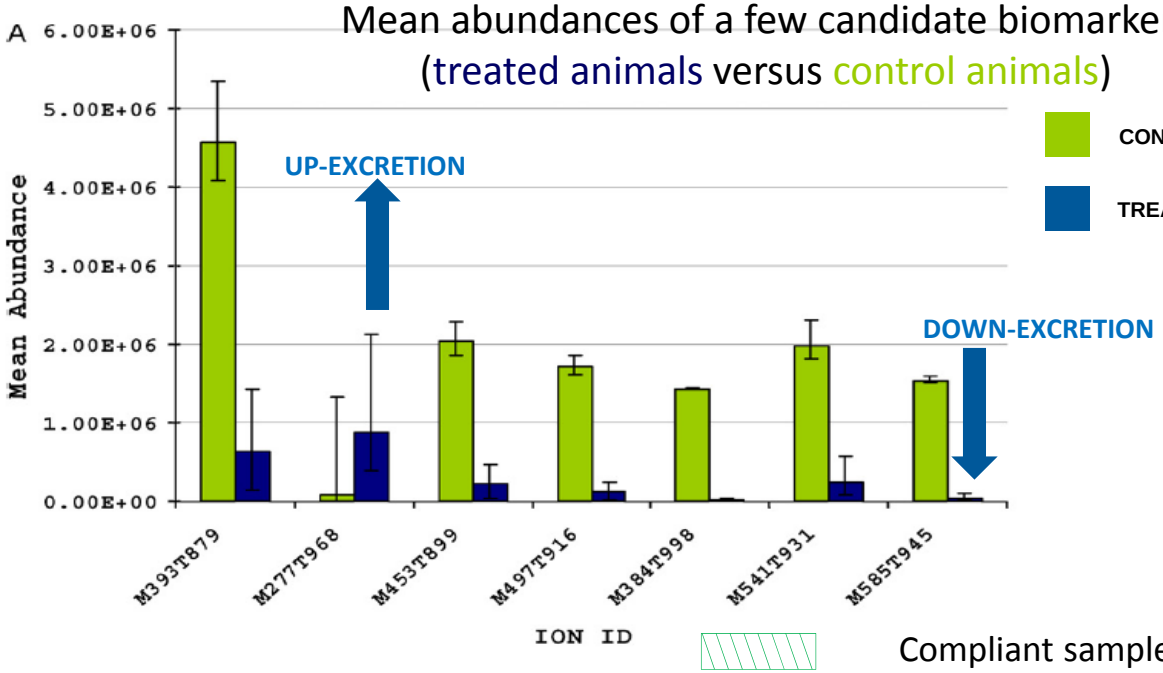
CHOLINE



DHEA-S

Mean abundances of a few candidate biomarkers
(treated animals versus control animals)

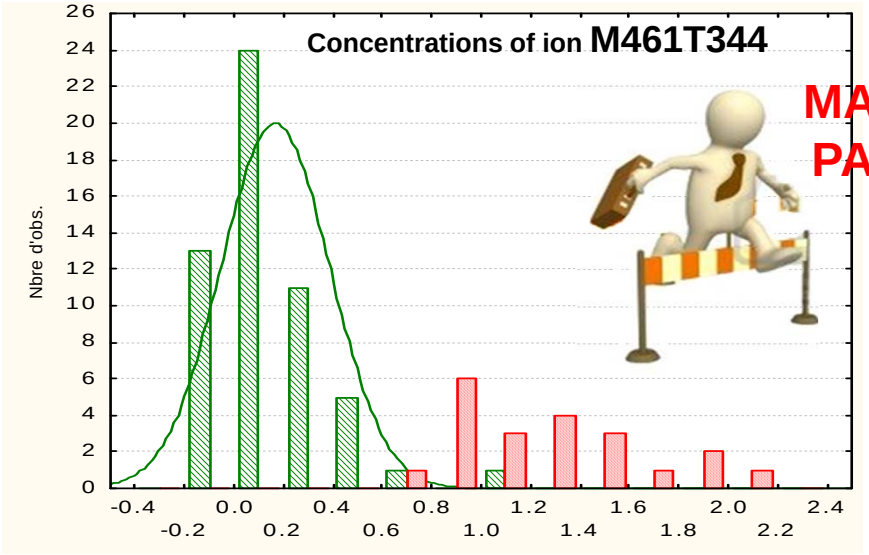
Untargeted, adult bovines, kidney, steroids,
Validation of candidate biomarkers

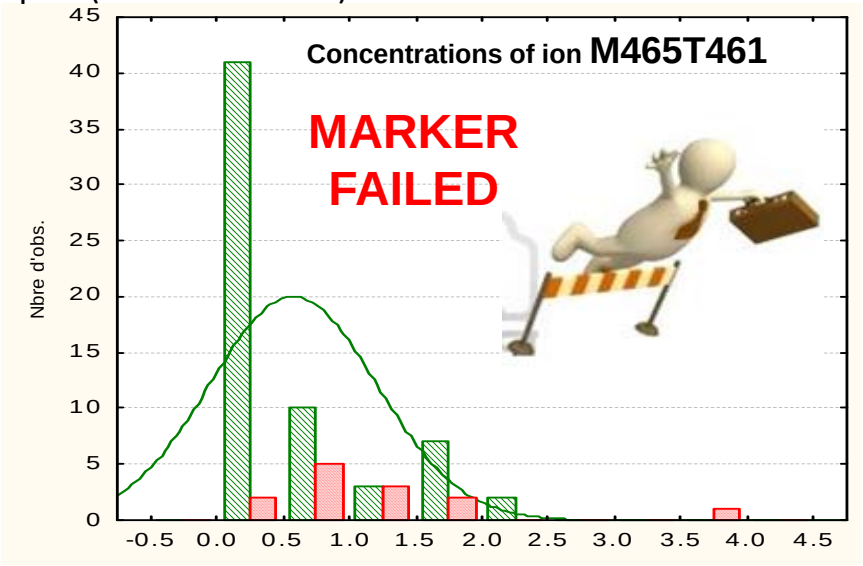


NEEDS FOR A LARGE INCREASING OF THE NUMBER OF ANIMALS IN THE CONTROL POPULATION

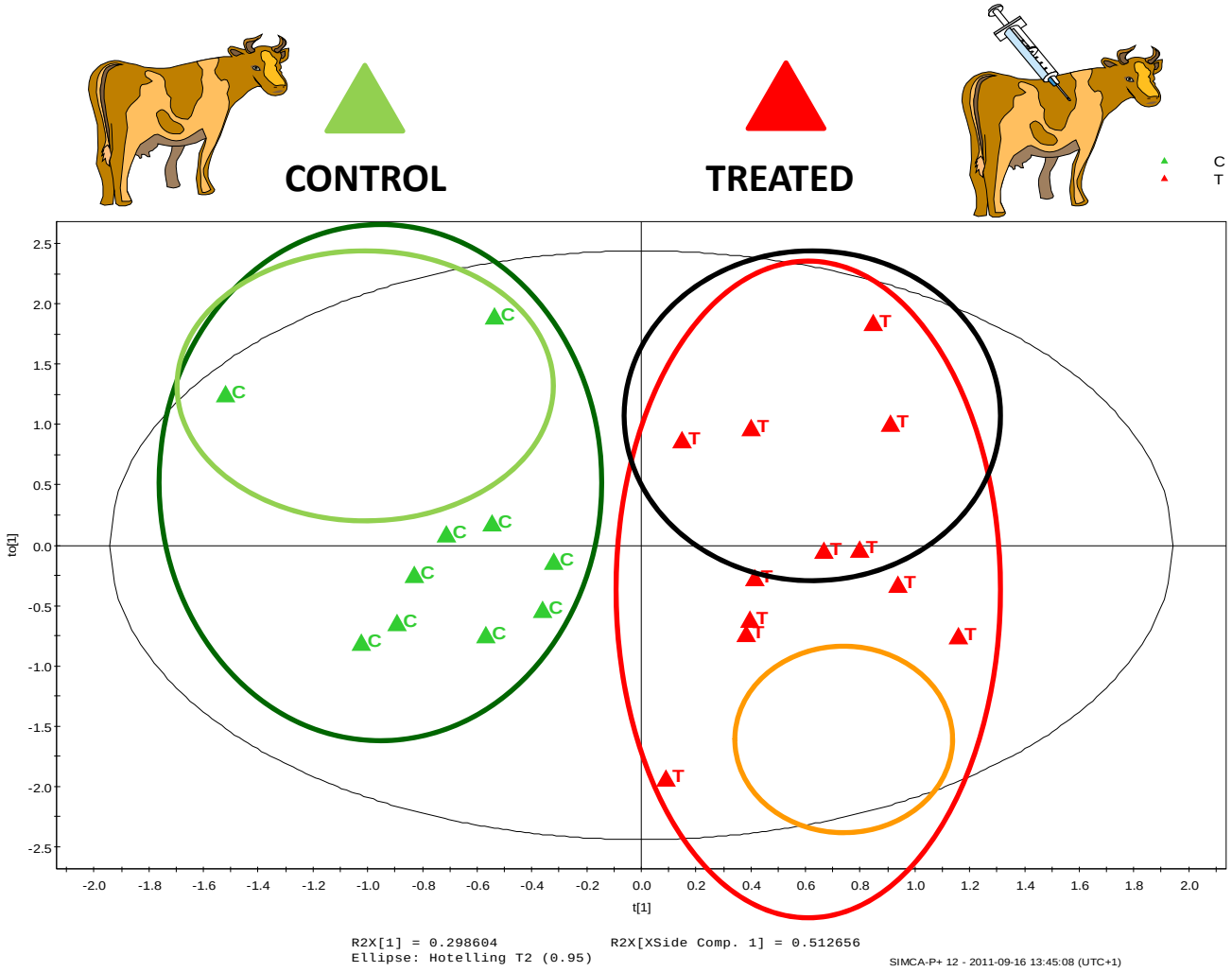
Compliant sample (control population)

Non compliant samples (treated animals)





Untargeted, adult bovines, kidney, steroids,
Validation of candidate biomarkers



NEEDS FOR ADDITIONAL TREATED ANIMAL EXPERIMENTS FOR ROBUSTNESS AND SELECTIVITY

Validated model

BIOCOP, NT + E2Bz, set 1 of calves just after 1st IM, NL

Prediction, same experiment

BIOCOP, NT + E2Bz, set 1 of calves just after 2nd IM (2-weeks after), NL

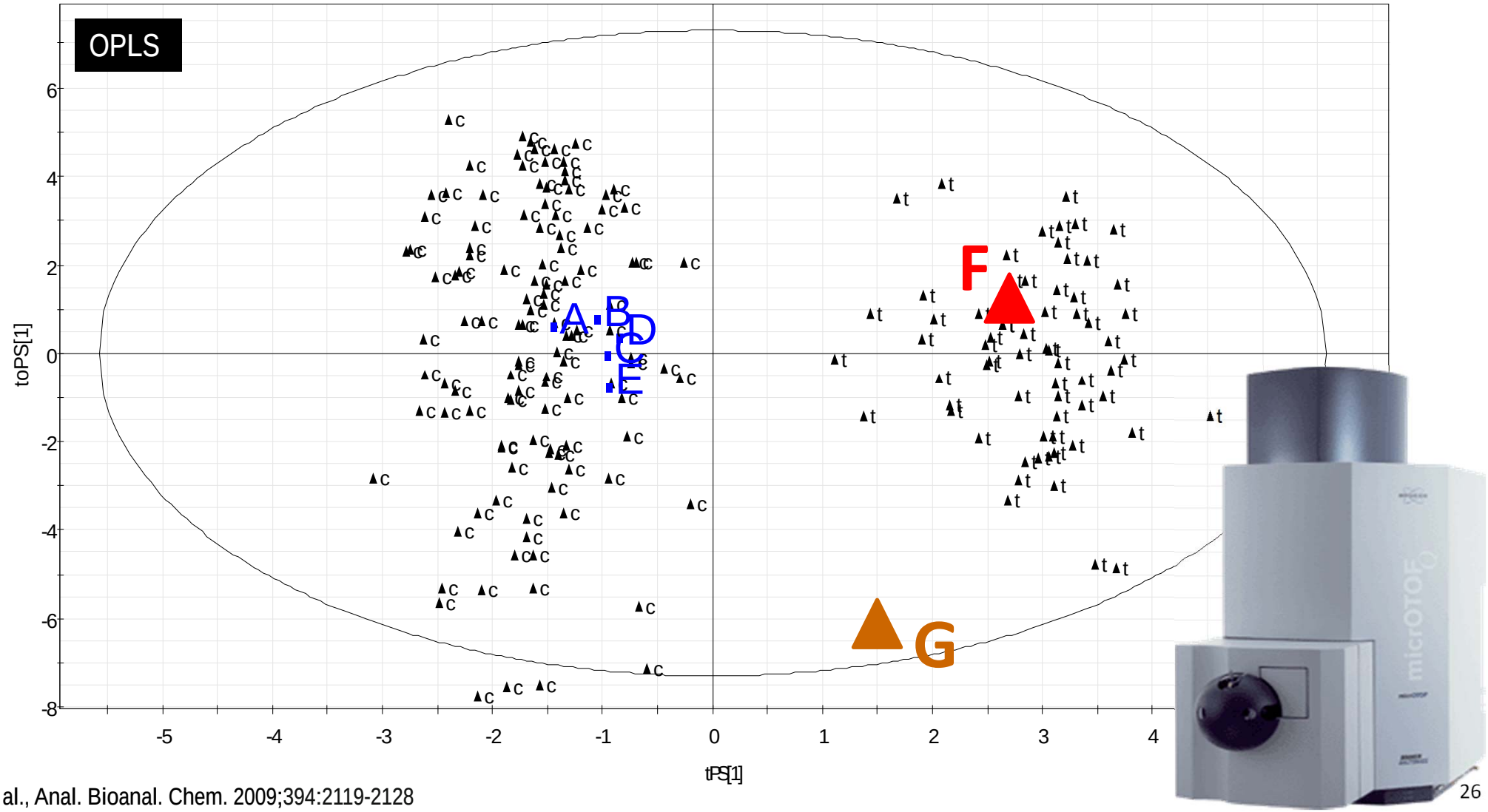
Prediction different experiment & same drug

MOONBANK, E2Bz, set 2 of steers just after one IM, UK

Dervilly-Pinel et al., Drug Testing & Analysis, 2012; 4(1):1-11

Untargeted, urine, growth hormone
Metabolic monitoring of 80 biomarkers, biological passport

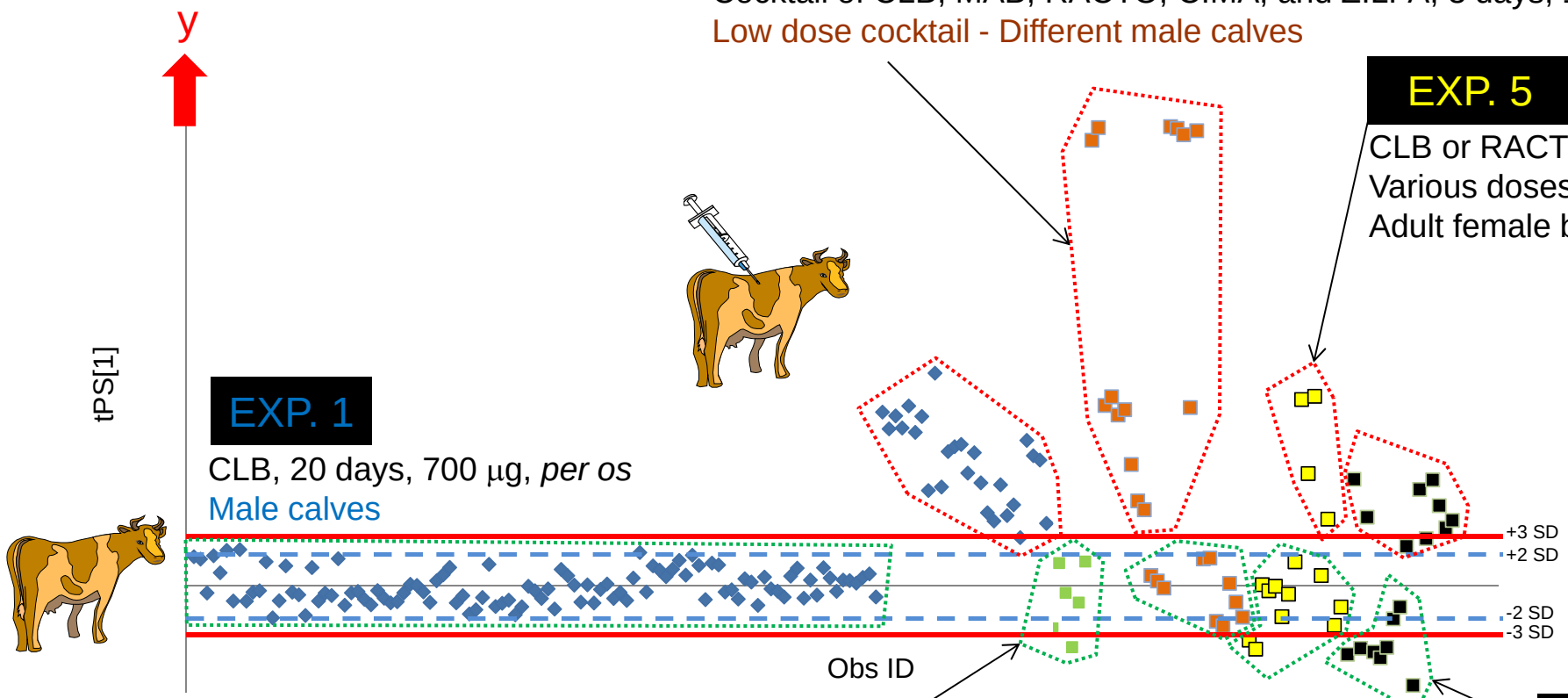
Prediction of unknown horse racing urine samples A, B, C, D, E..... and F .... or G



Kieken et al., Anal. Bioanal. Chem. 2009;394:2119-2128

Untargeted, calves, 200 urine samples, β-agonists
Prediction, validation: attempt to assess False Negative, False Suspect

EQUATION
y= - 0.91 x [M1] + 0.44 x [M2] + 0.37 x [M3]



EXP. 1
CLB, 20 days, 700 µg, *per os*
Male calves

EXP. 2
CLB, 5 days, 500 µg, *per os*
Same drug
Different male calves

EXP. 3
Cocktail of CLB, MAB, RACTO, CIMA, and ZILPA, 3 days, 100 µg each, *per os*
Low dose cocktail - Different male calves

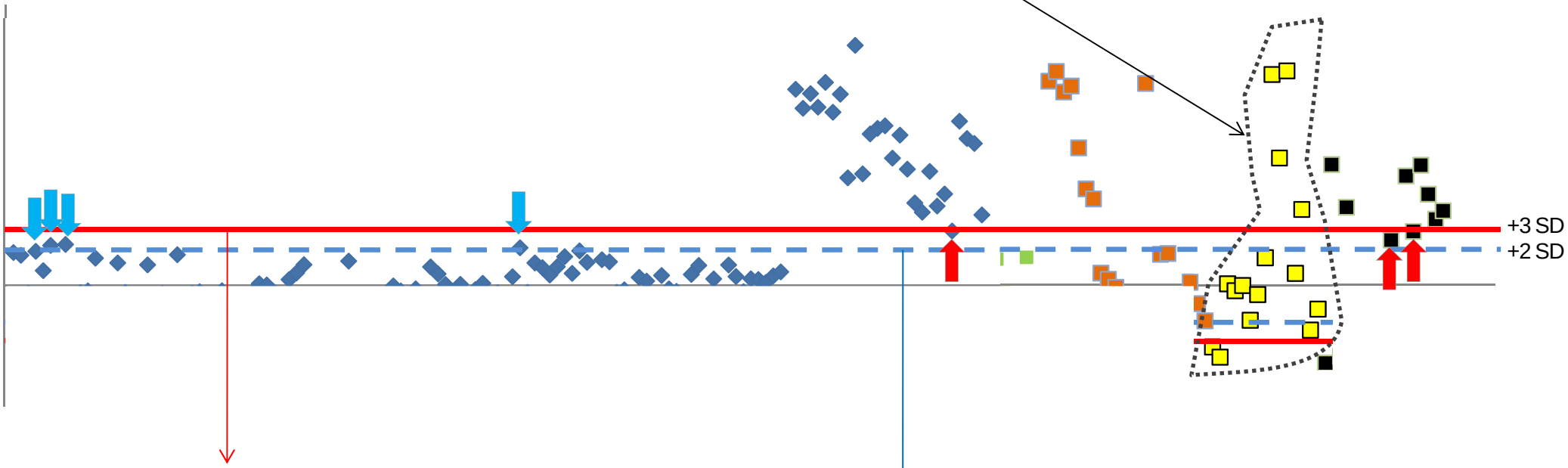
EXP. 4
National Monitoring Plan
Randomized bovines (gender, age, location...)

EXP. 5
CLB or RACTO or CLB+RACTO,
Various doses
Adult female bovines

Untargeted, calves, 200 urine samples, β-agonists
Prediction, validation: attempt to assess False Negative, False Suspect

Equation
y= - 0.91 x [M1] + 0.44 x [M2] + 0.37 x [M3]

EXP. 5
CLB (x1) or RACTO (x1) or
CLB+RACTO (1/10 + 1/10)
Adult female bovines



Hypothesis 1 → mean values +3 sd,
FALSE NEGATIVE SCORE → 3 samples, 1.5%
FALSE SUSPECT → 0 samples, 0 %

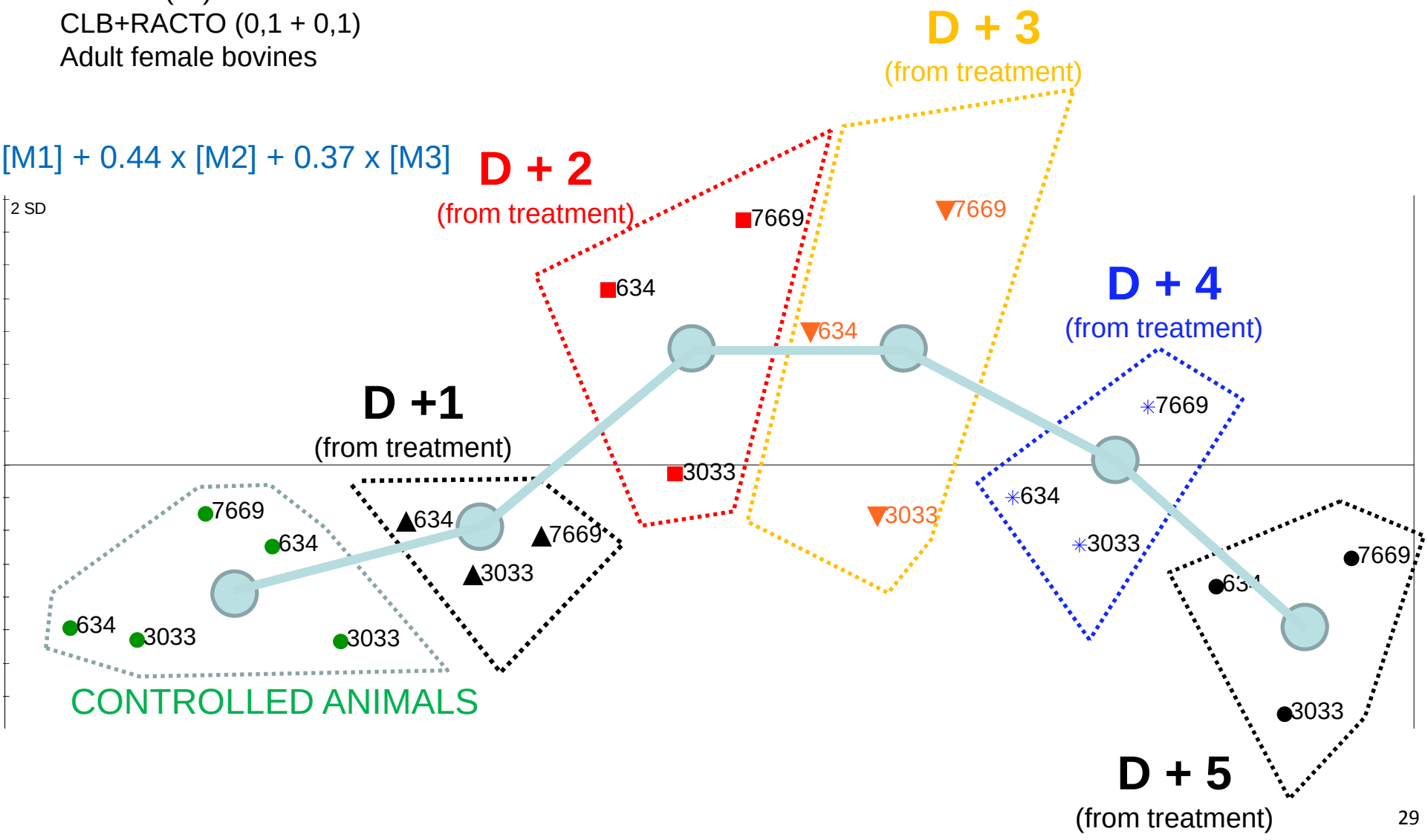
Hypothesis 2 → mean values +2 sd,
FALSE NEGATIVE SCORE → 0 samples, 0 %
FALSE SUSPECT → 4 samples, 2 %

Untargeted, calves, urine samples, β-agonists
Different treatments with β-agonists, kinetic observations

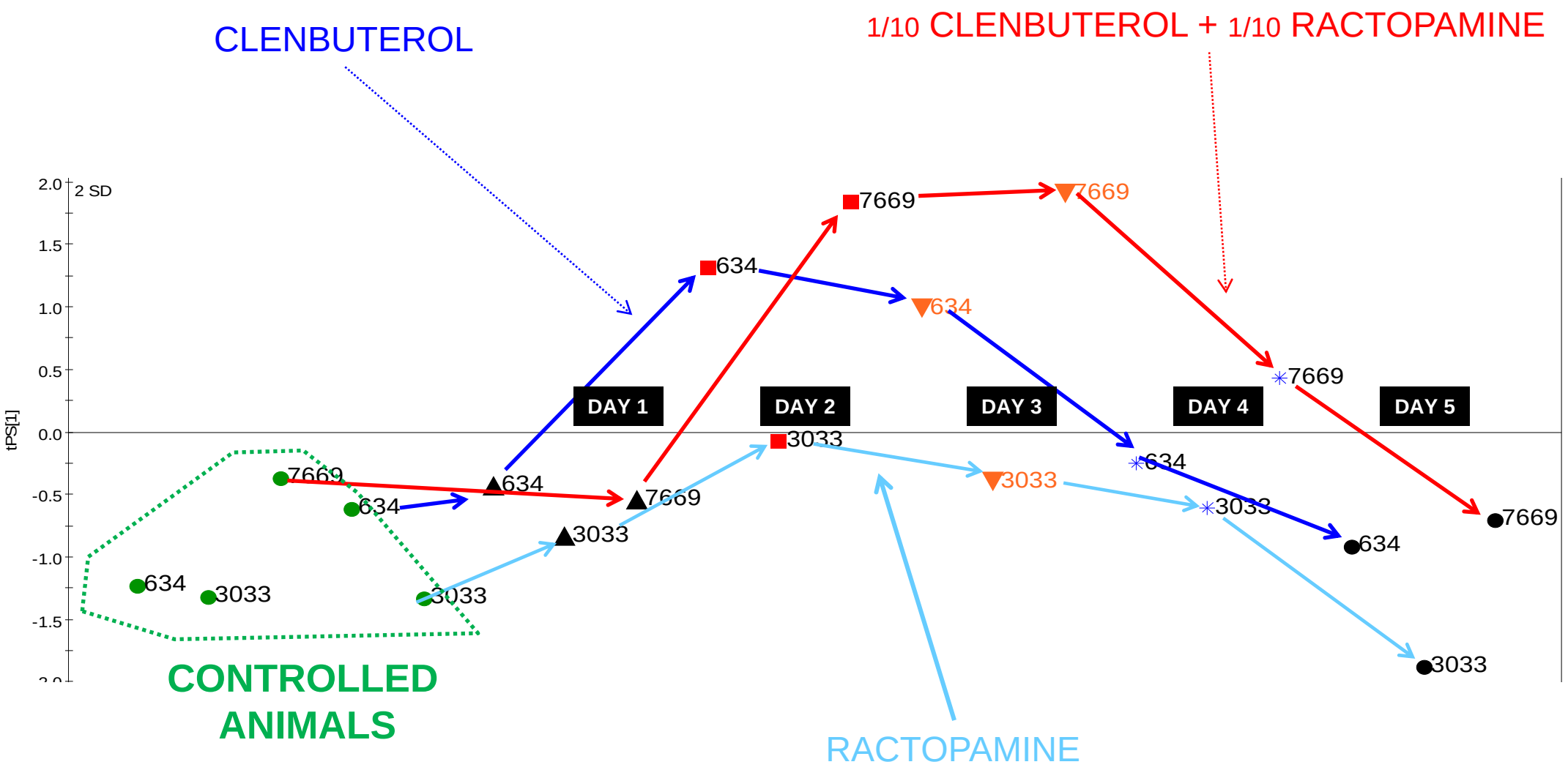
EXP. 5

CLB (x1) or
RACTO (x1) or
CLB+RACTO (0,1 + 0,1)
Adult female bovines

Equation
$y = -0.91 \times [M1] + 0.44 \times [M2] + 0.37 \times [M3]$



Untargeted, calves, urine samples, β-agonists
Validation, kinetic observations



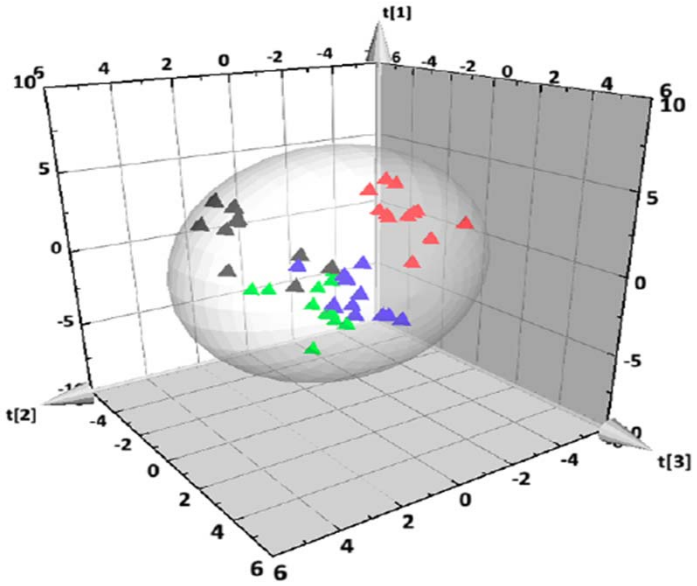
1. INTRODUCTION

2. ILLUSTRATIONS

3. CONCLUSION AND PERSPECTIVES

3. Conclusion and perspectives

From the proof of concept
to the daily use of a robust method…

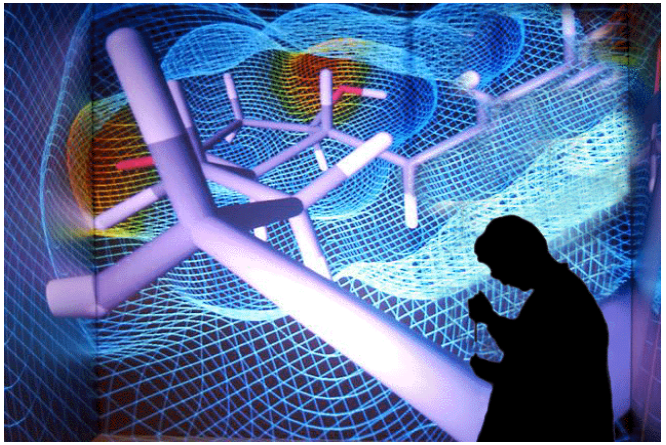


DESCRIPTIVE +PREDICTIVE MODELS (PROTOTYPES) AVAILABLE FOR MOST ANABOLIC FAMILIES

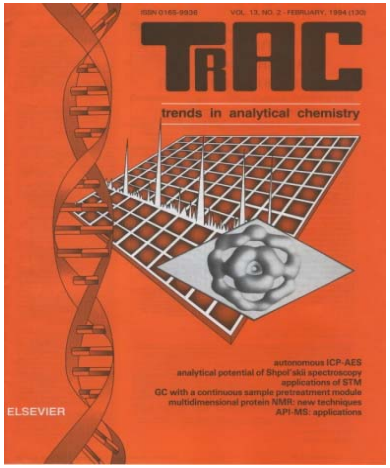
FIRST CANDIDATE BIOMARKERS FOR A COUPLE OF APPLICATIONS

?

BUT… SOME QUESTIONS STILL REMAINING



READY TO BE USED IN OFFICIAL CONTROLS (EXPERIMENTAL STAGE)



Trends in Analytical Chemistry, Vol. 29, No. 11, 2010 Trends

Targeted and untargeted profiling of biological fluids to screen for anabolic practices in cattle

G. Pinel, S. Weigel, J.-P. Antignac, M.H. Mooney, C. Elliott, M.W.F. Nielen, B. Le Bizec

Trends Trends in Analytical Chemistry, Vol. 30, No. 2, 2011

Mass spectrometry-based metabolomics applied to the chemical safety of food

J.-P. Antignac, F. Courant, G. Pinel, E. Bichon, F. Monteau, C. Elliott, B. Le Bizec

3. Conclusion and perspectives

Regulatory issues… notions of biomarkers not clearly mentioned in the current EU regulation (food safety)

FOOD SAFETY

COUNCIL DIRECTIVE 96/23/EC

of 29 April 1996

on measures to monitor certain substances and residues thereof in live animals and animal products and repealing Directives 85/358/EEC and 86/469/EEC and Decisions 89/187/EEC and 91/664/EEC

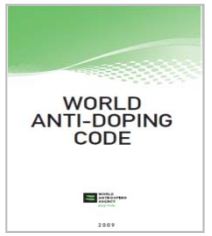
Article 1

This Directive lays down measures to monitor the substances and groups of residues listed in Annex I.

Article 2

'residue' shall mean a residue of substances having a pharmacological action, of their metabolites and of other substances transmitted to animal products and likely to be harmful to human health;

HUMAN ANTIDOPING



Article 2.1.2

Sufficient proof of an anti-doping rule violation is established either by the presence of a prohibited substance, its metabolites or **markers** […]

WADA's Executive Committee approved WADA's Athlete Biological Passport Operating Guidelines on December 1, 2009.

HORSERACING

Article 6, §11

[…] The finding of **any scientific indicator** of administration or other exposure to a prohibited substance is also equivalent to the finding of the substance […]

New version approved in April 2009 "International Agreement on Breeding, Racing and Wagering"

3. Conclusion and perspectives

Transcriptome profiling

Analytica Chimica Acta 638 (2009) 106–113

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Identification of potential gene expression biomarkers for the surveillance of anabolic agents in bovine blood cells

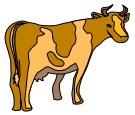
Irmgard Riedmaier*, Ales Tichopad, Martina Reiter, Michael W. Pfaffl, Heinrich H.D. Meyer

Physiology Weihenstephan, Technische Universitaet Muenchen, Weihenstephaner Berg 3, 85354 Freising, Germany

Irmgard Riedmaier

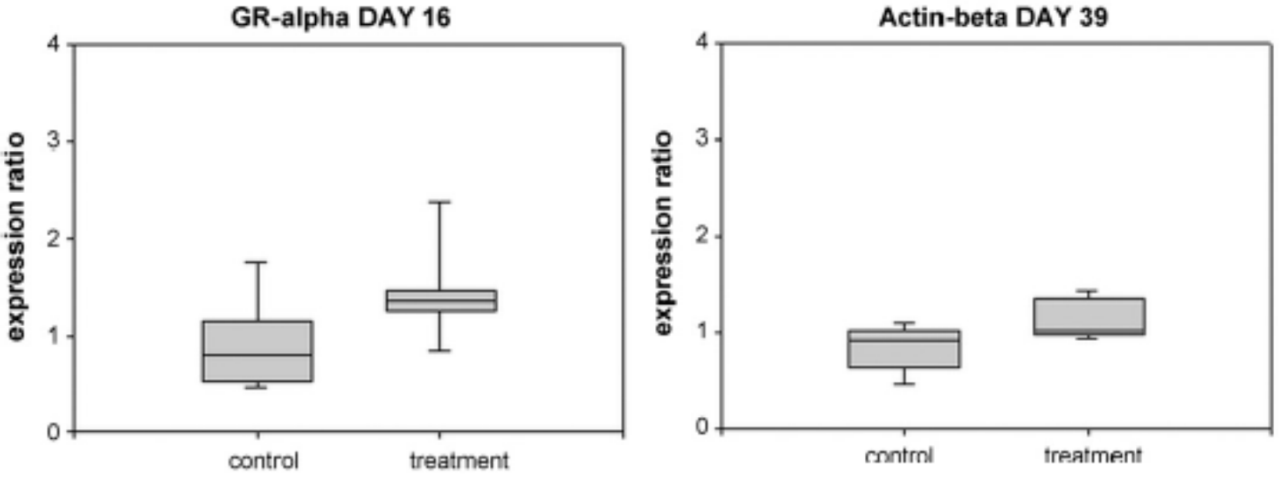
Gene group	Gen		Sequenz
Steroid receptors	AR	for	CCT GGT TTT TCA ATG AGT ACC GCA TC
		rev	TTG ATT TTT CAG CCC ATC CAC TGG A
	ERalpha	for	AGG GAA GCT CCT ATT TGC TCC
		rev	GGT GGA TGT GGT CCT TCT C
	ERb	for	TTA GCC ATC CAT TGC CAG CC
		rev	GCC TTA CAT CCT TCA CAC GAC
	GRa	for	TTC GAA GAA AAA ACT GCC CAG C
		rev	CAG TGT TGG GGT GAG TTG TG
	FasL	for	CAT CTT TGG AGA AGC AAA TAG
		rev	GGA ATA CAC AAA ATA CAG CCC
	Fas	for	TGT TGT CAG CCT TGT CCT CC
		rev	GTT CCA CTT CTA GCC CAT GTT C
	bcl-2	for	ATG ACT TCT CTC GGC GCT AC
		rev	CCG GTT CAG GTA CTC GGT CA

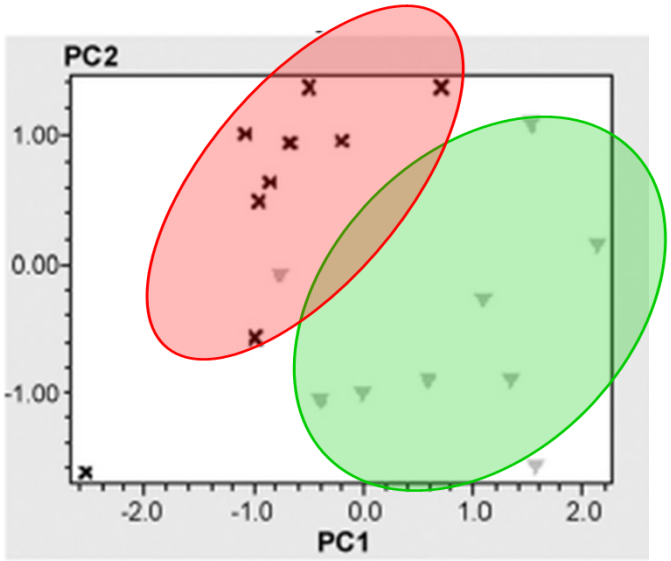
Trenbolone acetate 140 mg, Estradiol 14 mg, 9C & 9T



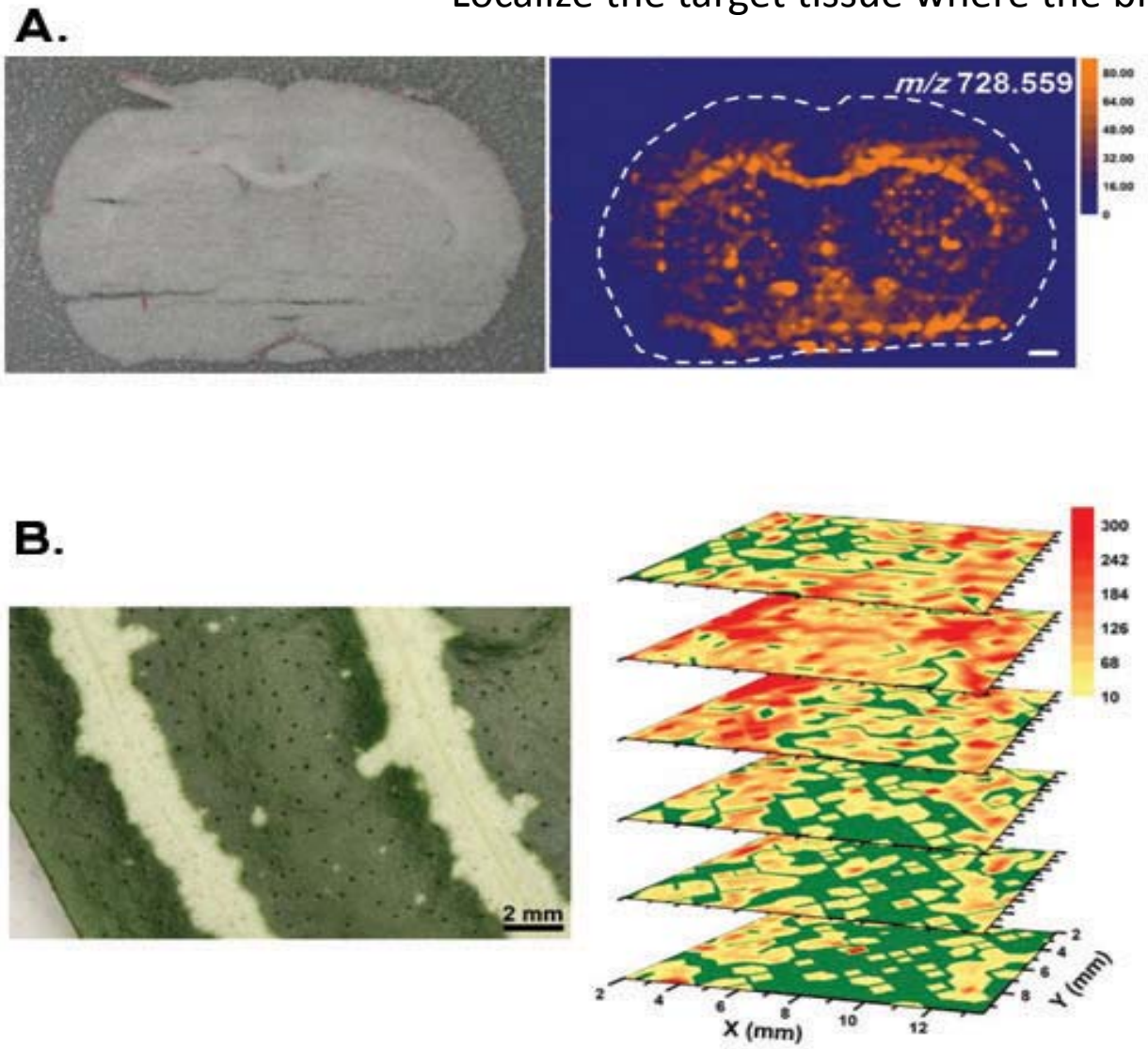
Blood cells

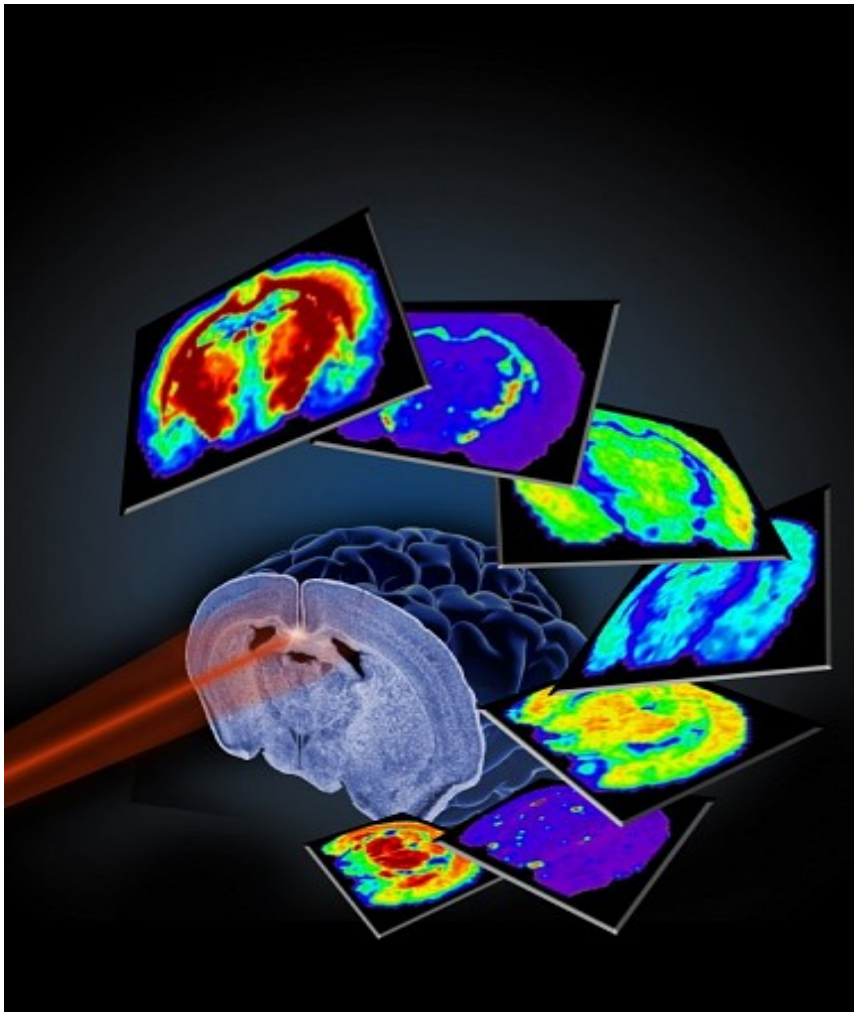
PCA based on 11 regulated genes





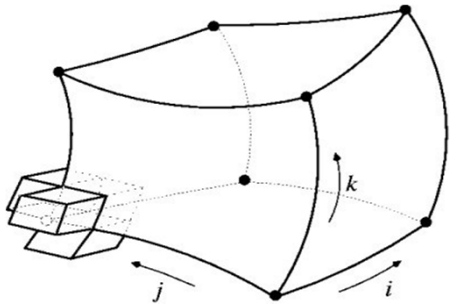
- Find candidate biomarkers in tissue
- Localize the target tissue where the biomarker accumulates





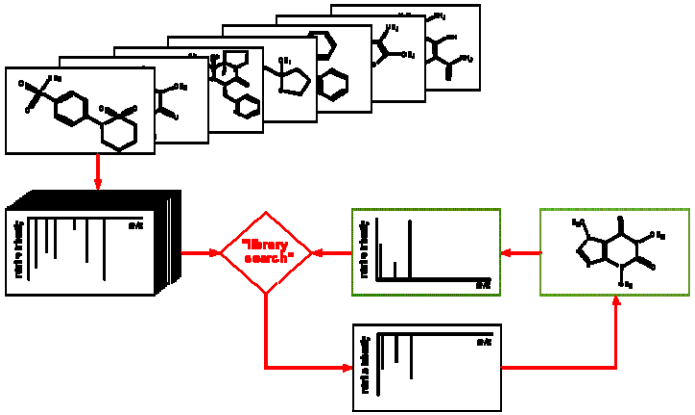
3. Conclusion and perspectives

Dedicated statistical tools (multi-block methods: CPCA, MB-PLS)

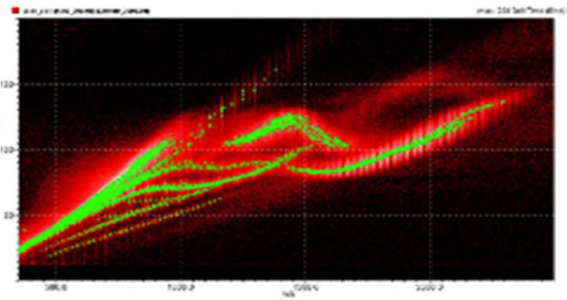


Technology: possible improvements

MS Libraries Covering Main Mammals Metabolomes

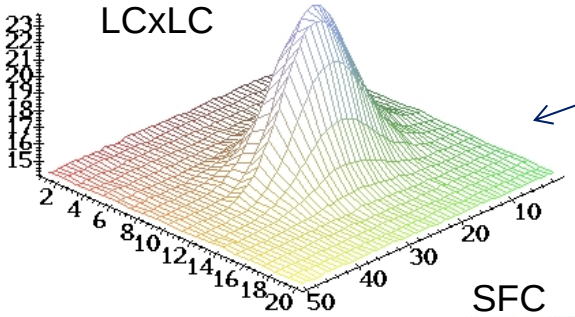


New MS Dimension (IMS)



Portable device

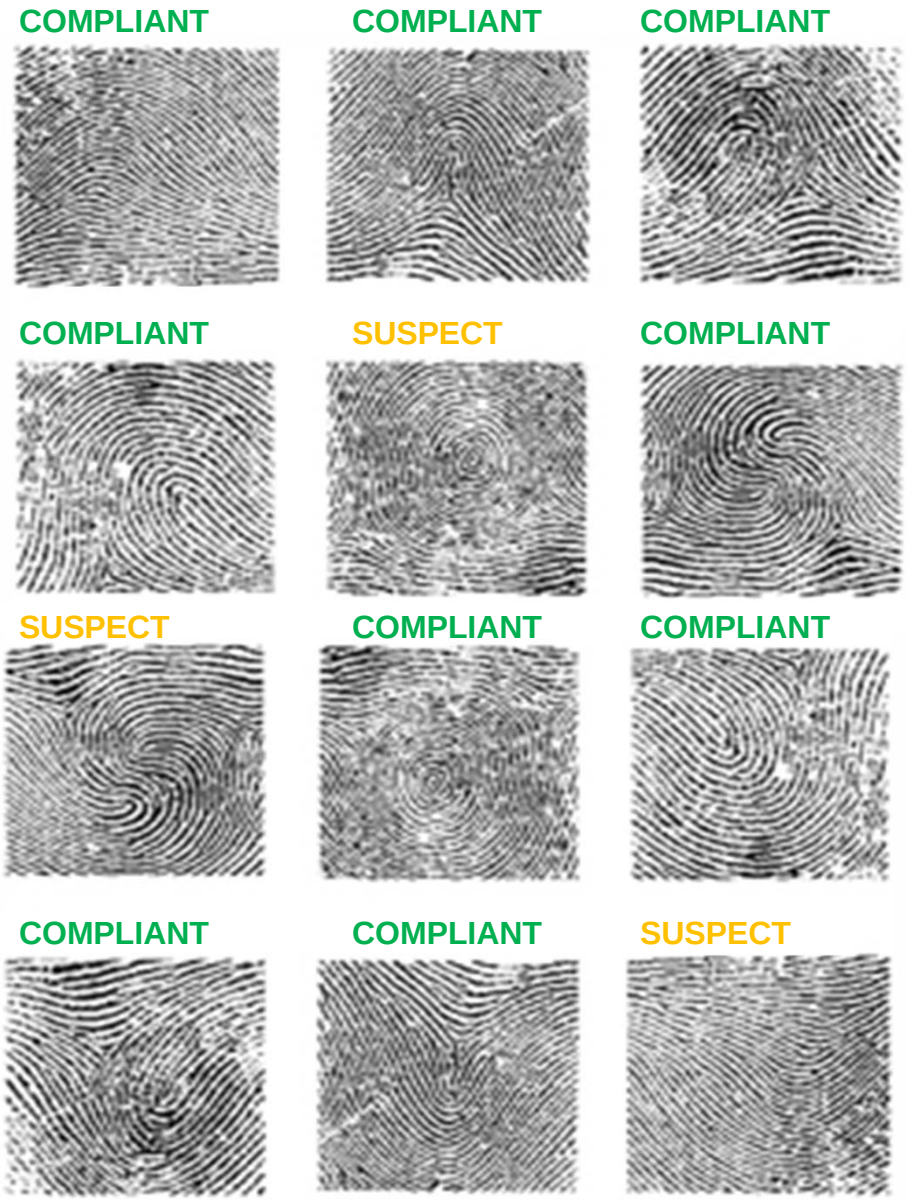
LCxLC



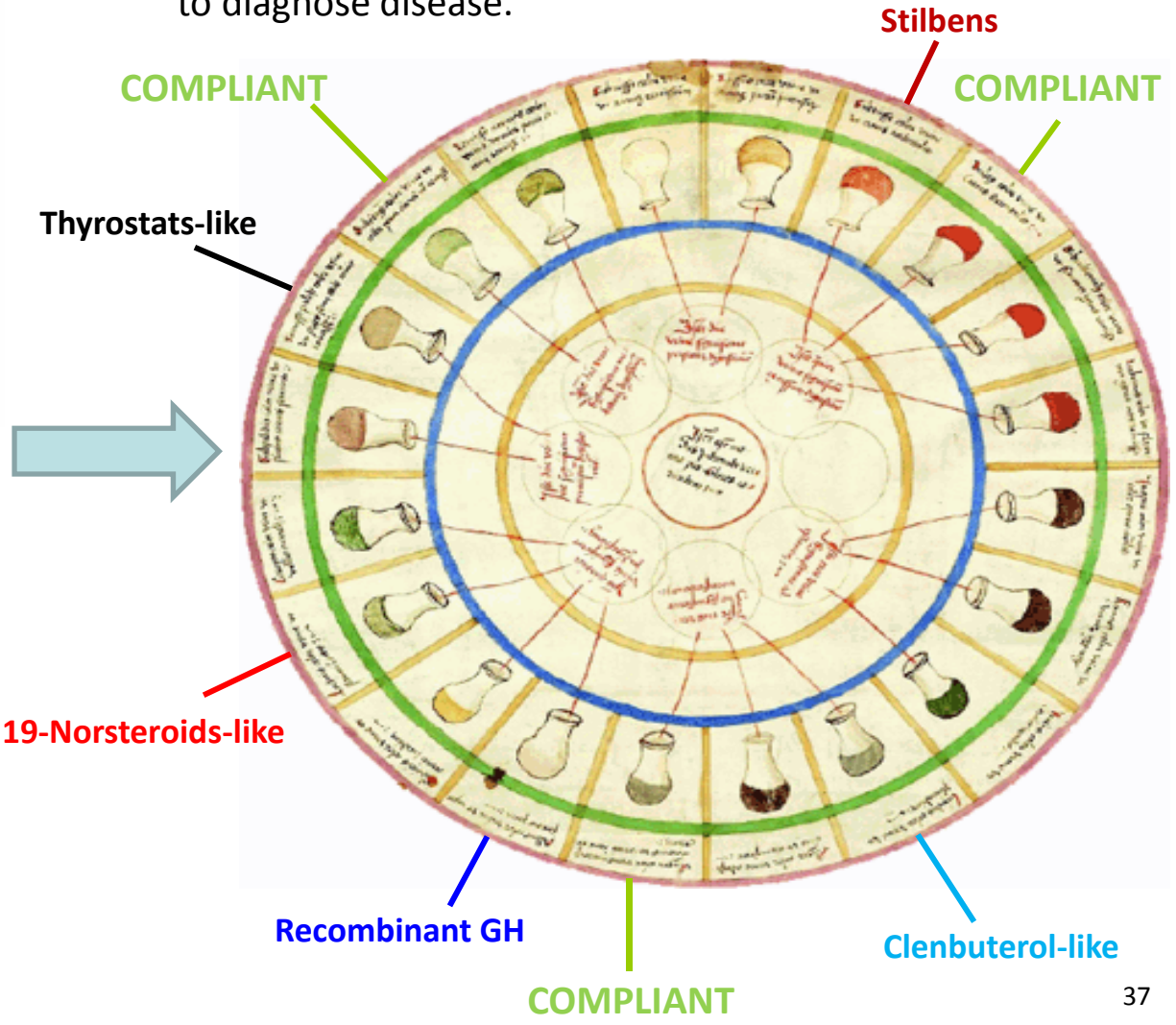
SFC

GCxGC

Benchtop Ultra HRMS with fast scanning facilities



This urine **wheel** was published in 1506 by Ullrich Pinder, in his book Epiphanie Medicorum. It describes the possible colours, smells and tastes of urine, and uses them to diagnose disease.



3. Conclusion and perspectives

Y. BONNAIRE
F. KIEKEN

M. MOONEY
C. ELLIOTT

M. NIELEN
A. LOMMEN

Comité Local d'Organisation

- Martine Cadène - CBM, Orléans
- Benoit Maunit - ICOA, Orléans
- Martine Beaufour - CBM, Orléans
- Christelle Briois - LPC2E, Orléans
- Cyril Colas - CBM-ICOA, Orléans
- Patrick Emond - Université de Tours
- Laëtitia Fougère - ICOA, Orléans
- Guillaume Gabant - CBM, Orléans
- Catherine Guette - CHR Angers
- Sandrine Lefeuvre - CHR Orléans
- Patricia Legland - CBM, Orléans
- Xavier Poisson - Technologie Servier, Orléans

Comité Scientifique

- Gérard Bolbach - IBI, Paris
- Martine Cadène - CBM, Orléans
- Brian T. Chait - Rockefeller University, NY
- Pierre Gareil - ENSCP, Paris
- Benoit Maunit - ICOA, Orléans
- Estelle Pujos-Guillot - INRA, Clermont-Ferrand
- Peter Roepstorff - University of Southern Denmark, Denmark
- Guillaume Van der Rest - LCP, Orsay
- Joëlle Vinh - ESPCI, Paris

LABERCA

F. COURANT
S. CHEREAU
E. BICHON
A-L ROYER
C JACOB
F. MONTEAU
J.-P. ANTIGNAC
G. DERVILLY-PINEL

METABOLOMICS SESSION

R&D IN METABOLOMICS

IMPLEMENTATION OF A METABOLOMICS BASED SCREENING MODEL TO DETECT ANABOLIC PRACTICES IN BREEDING ANIMALS

Orléans, 17-20 septembre 2012

Bruno LE BIZEC, Pf, Dr, HDR

F. COURANT, S. CHEREAU, F. MONTEAU,
C. JACOB, A-L ROYER, F. BOYARD-KIEKEN,
JP. ANTIGNAC, G. DERVILLY-PINEL

Laboratoire d'Étude des Résidus et Contaminants dans les Aliments
ONIRIS - France - www.laberca.org