UNIVERSITÉ DE GENÈVE

ISPSO
INSTITUT DES SCIENCES PHARMACEUTIQUES DE SUISSE OCCIDENTALE

Ratisser plutôt que balayer

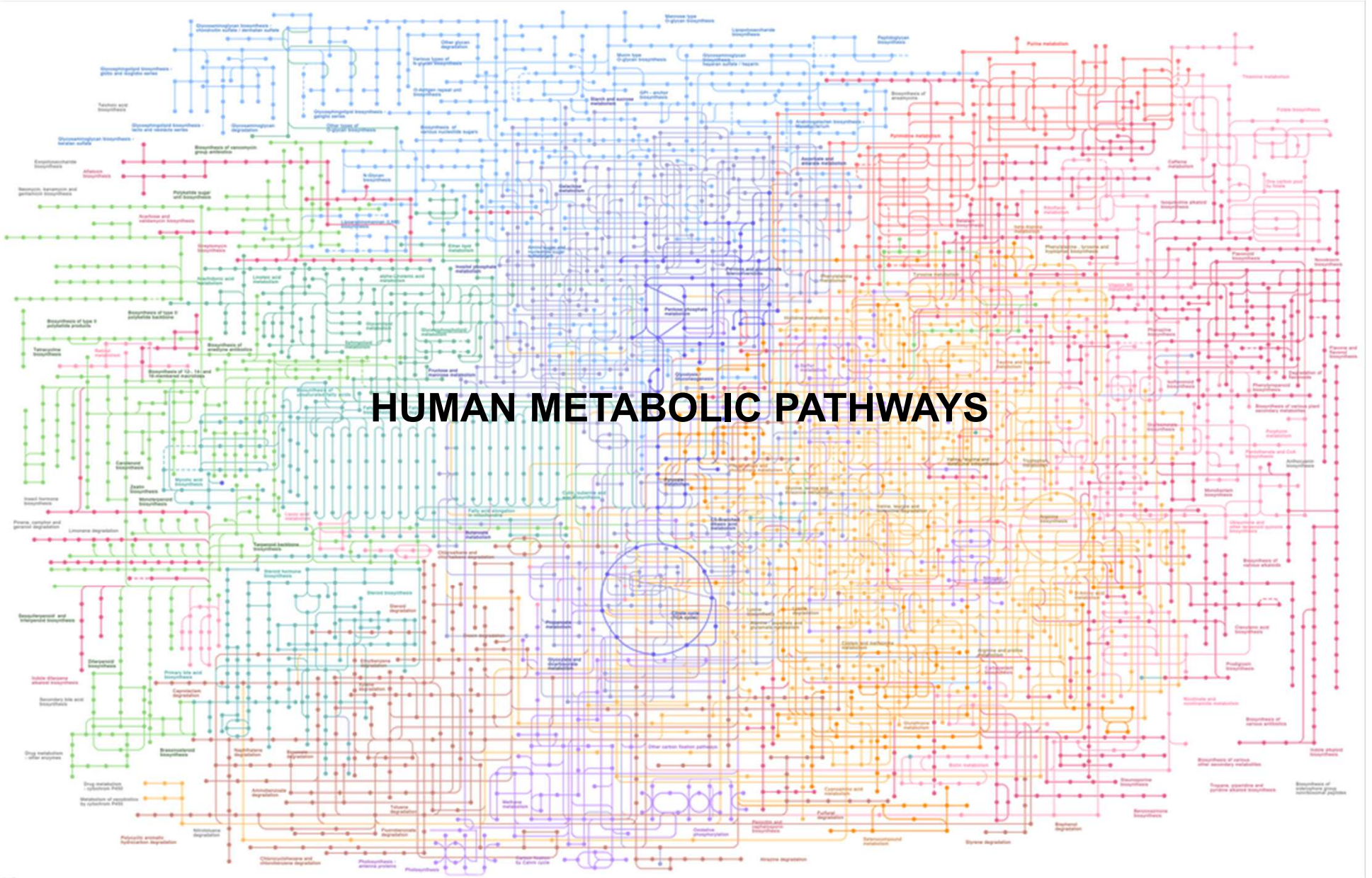
L'analyse multi-ciblée comme alternative de choix à l'analyse non-ciblée pour le profilage étendu des stéroïdes

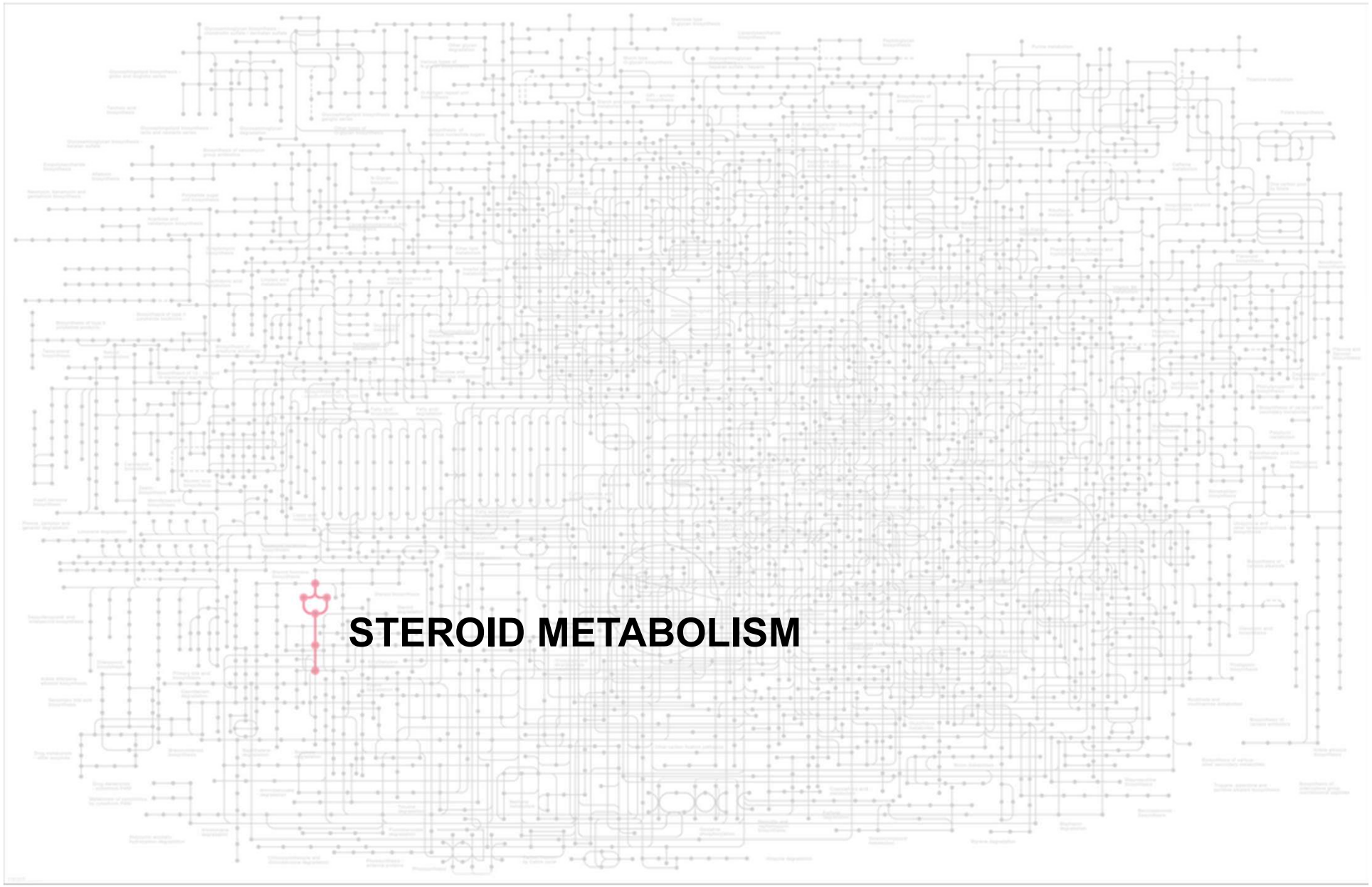
Dr. Mathieu Galmiche
Post-doctorant
Université de Genève
Section des sciences pharmaceutiques
Analyse biomédicale et métabolomique

28èmes Journées Scientifiques du ccCTA – 3 octobre 2025

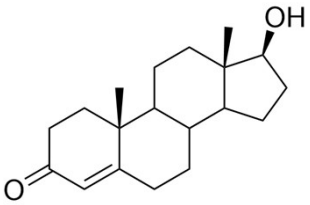
ccCTA

KEGG

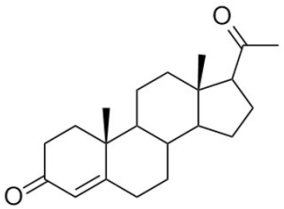



STEROID METABOLISM

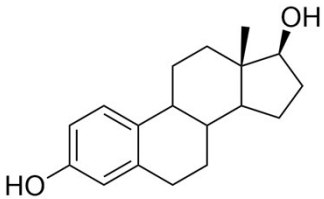
Several hundreds (thousands?) of steroid metabolites in humans



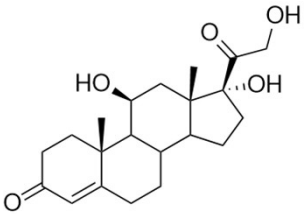
Androgens



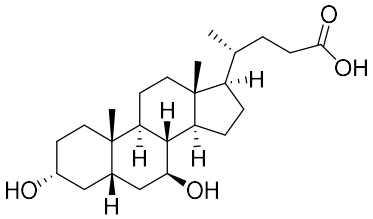
Progestogens



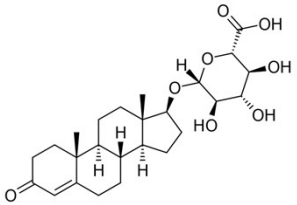
Estrogens



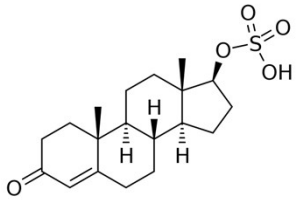
Corticosteroids



Bile acids



Glucuronides



Sulfates

Several hundreds (thousands?) of steroid metabolites in humans : SteroidOMICS

metabolites 2019 MDPI

Article

DynaStI: A Dynamic Retention Time Database for Steroidomics

Santiago Codesido [1,*], Giuseppe Marco Randazzo [1,2], Fabio Lehmann [3], Víctor González-Ruiz [1,4], Arnaud García [1], Ioannis Xenarios [3,5], Robin Liechti [3], Alan Bridge [3], Julien Boccard [1,4] and Serge Rudaz [1,4]

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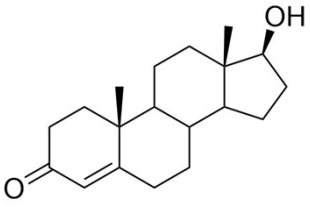
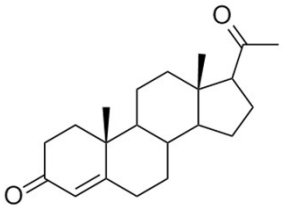
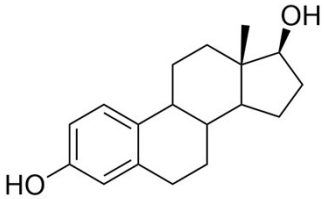
5% B → 95% B : 14 minutes



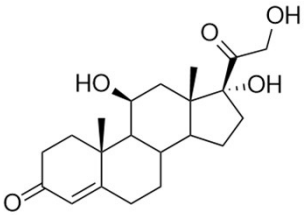
Androgens



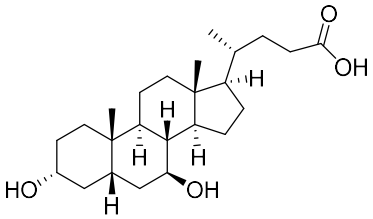
Progestogens



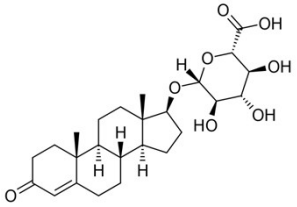
Estrogens



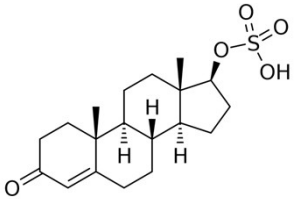
Corticosteroids



Bile acids



Glucuronides



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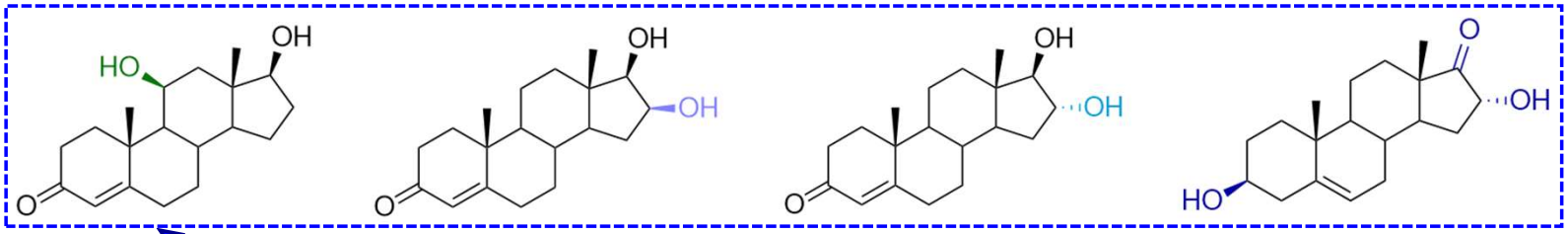
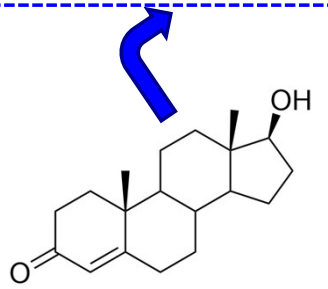
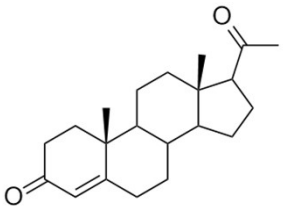


Isomers



Androgens



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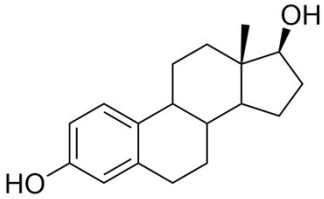
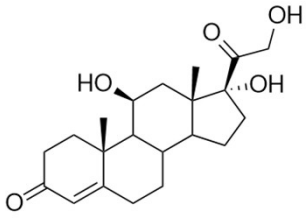


Estrogens



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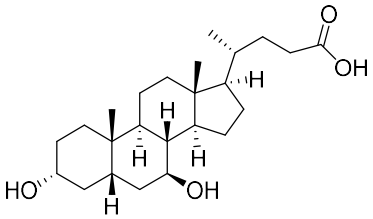

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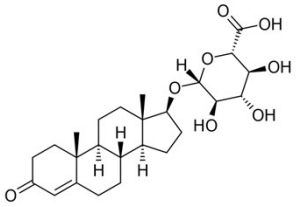
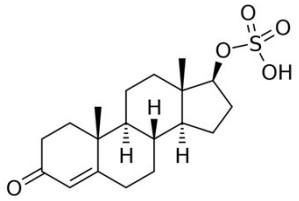
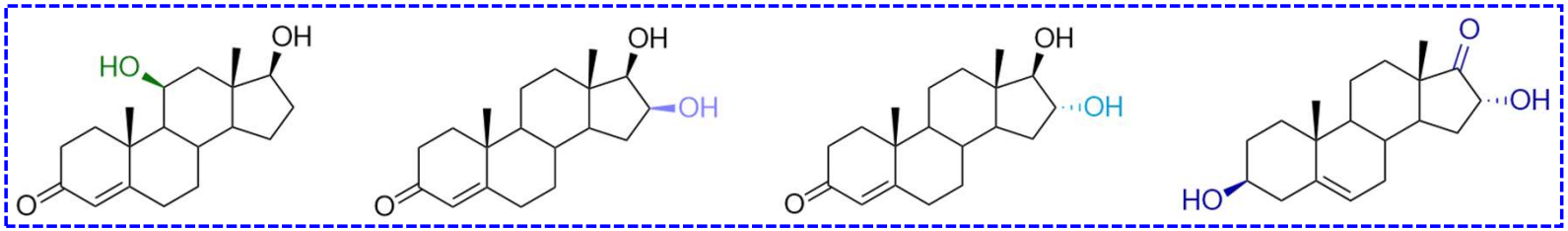


Glucuronides



Sulfates

High Resolution and Mass Accuracy will not help you there…



Produced in the adrenal. Precursor of potent '11-keto' androgens

Produced in the liver. Inactive metabolite (elimination product)

Produced in the liver. Inactive metabolite (elimination product)

Produced in the liver, ovaries and placenta. Estrogen precursor

Isomers

m/z = 305.2112

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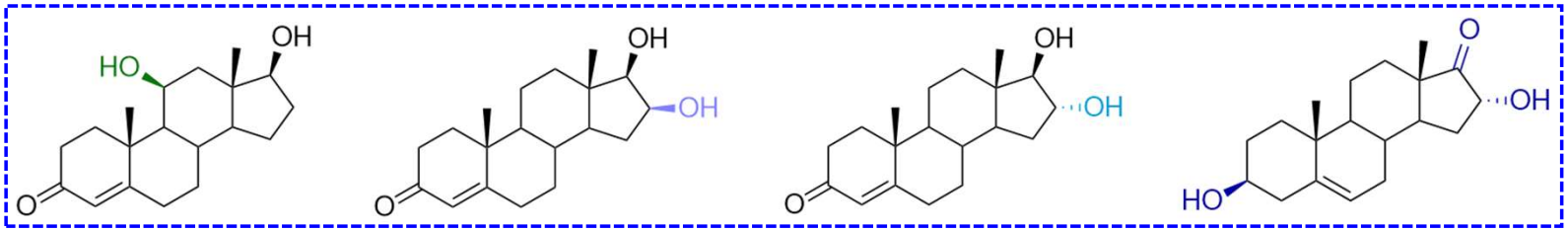

Produced in the adrenal.
Precursor of potent
'11-keto' androgens

RT = 8.25 min

Produced in the liver.
Inactive metabolite
(elimination product)

RT = 8.3 min

Produced in the liver.
Inactive metabolite
(elimination product)

RT = 7.55 min

Produced in the liver,
ovaries and placenta.
Estrogen precursor

RT = 8.3 min

Isomers

m/z = 305.2112

metabolites

MDPI

Article

DynaStI: A Dynamic Retention Time Database for Steroidomics

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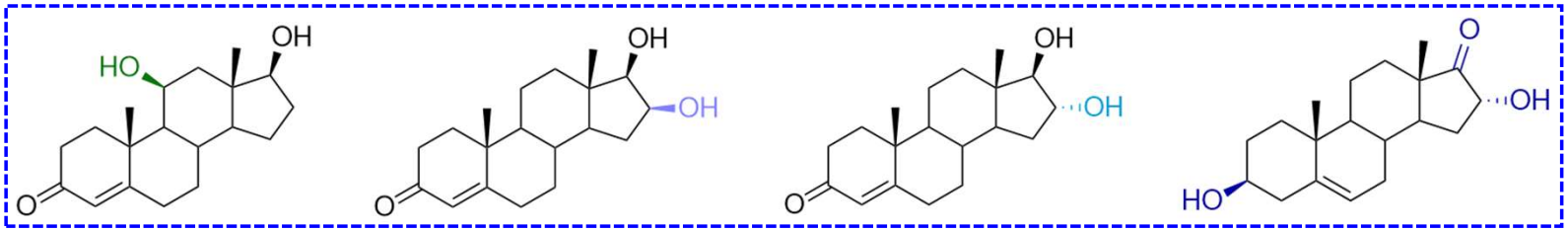

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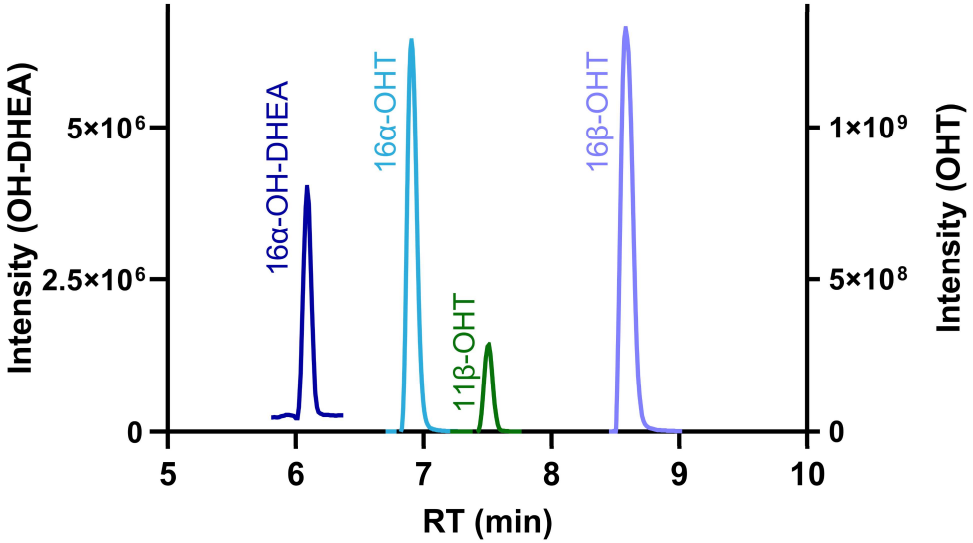
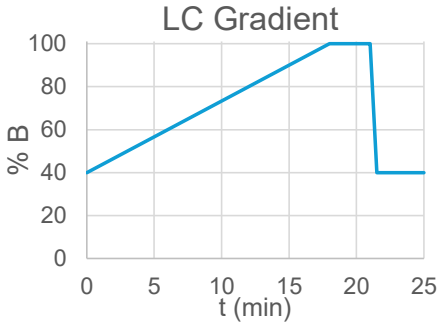


Column: Restek Inert **Biphenyl** 100 x 2.1 mm, 1.8 µm
Guard: Inert **Biphenyl** 5 x 2.1 mm, 2.7 µm

A : H_2O + 0.01 % FA
B : MeOH + 0.01 % FA
0.4 mL/min
25 minutes



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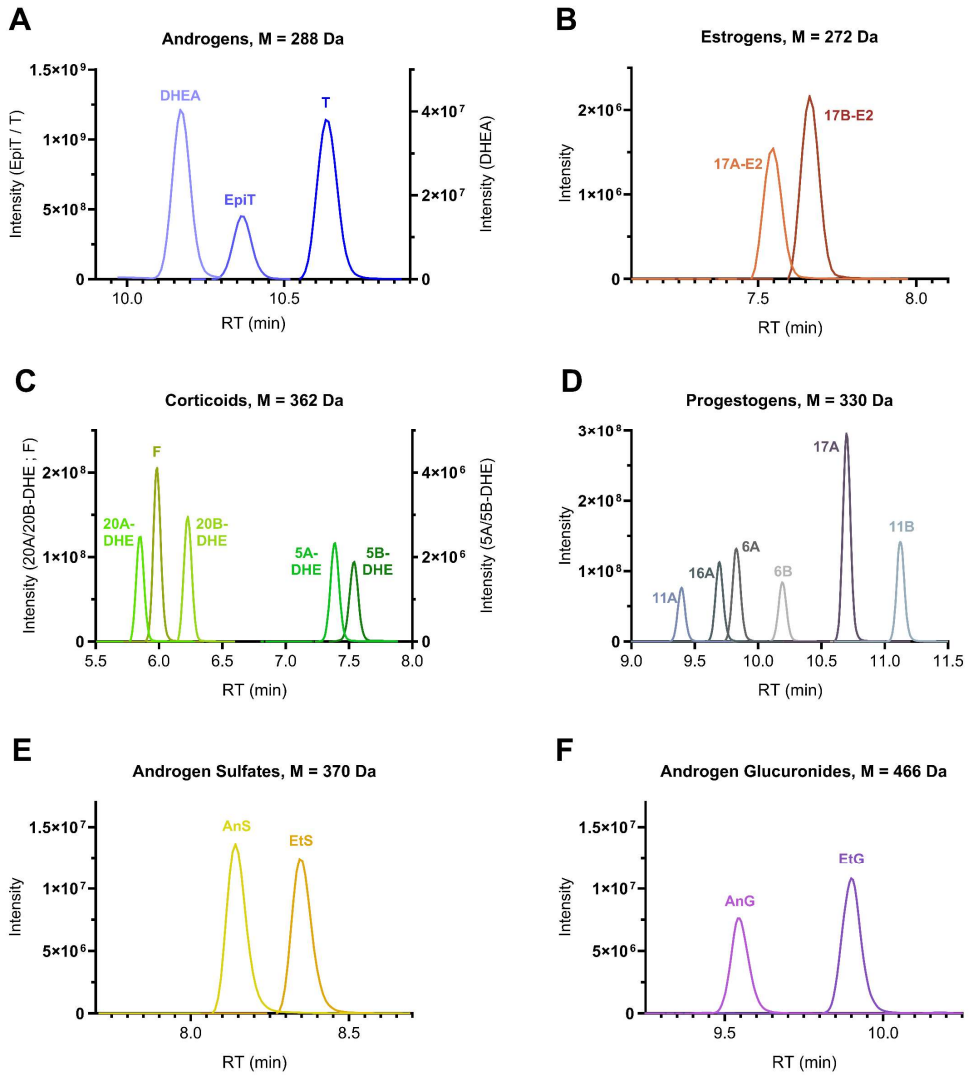

M.Galmiche et al., *Journal of Separation Science*, **2024**, 47(16), 2400436
M.Galmiche et al., *Journal of Separation Science*, **2025**, 48(4), e70147

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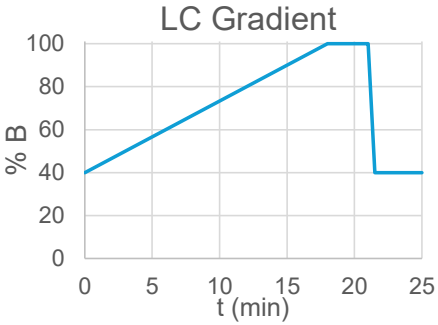
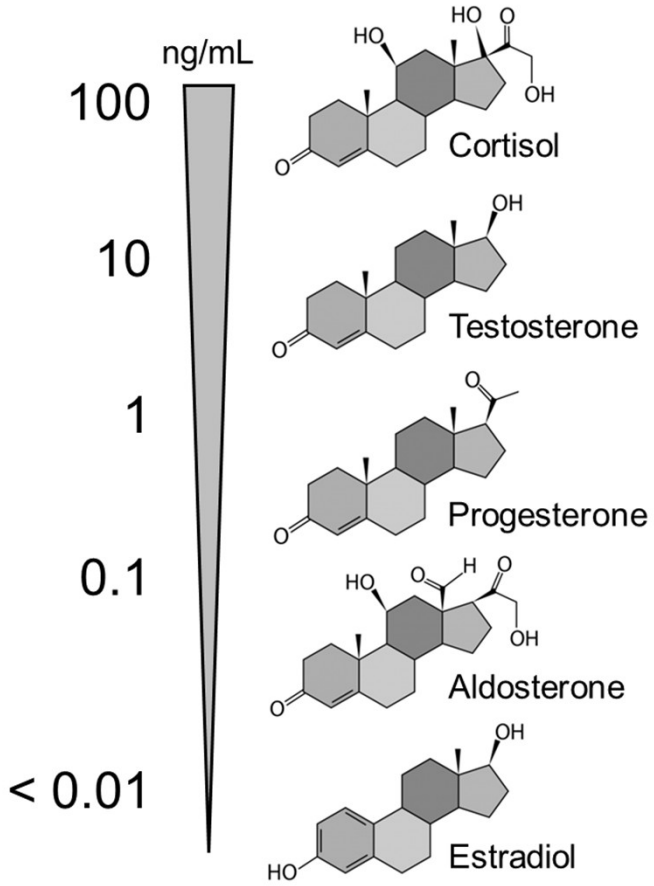


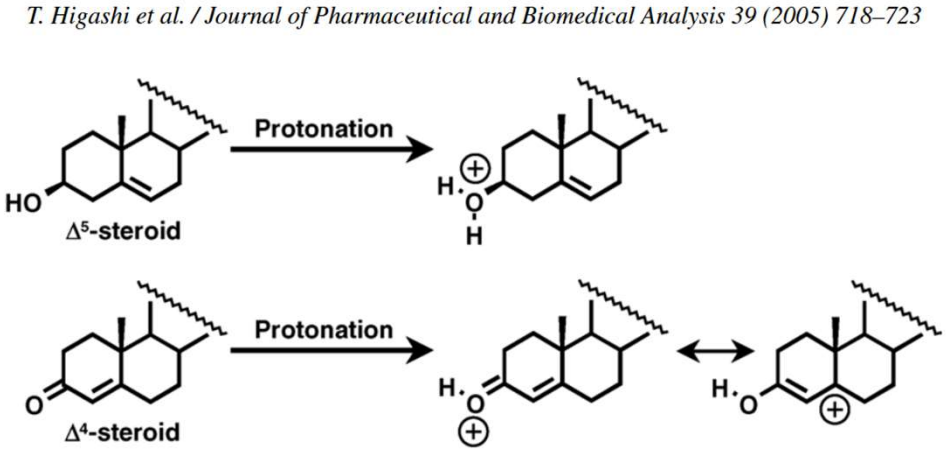
Are you sure there is no ‘David’ peak hidden under the ‘Goliaths’ ?

Endogenous concentrations ✖ **Ionization efficiencies**



adapted from the courtesy of Sergey Girel

Dynamic range



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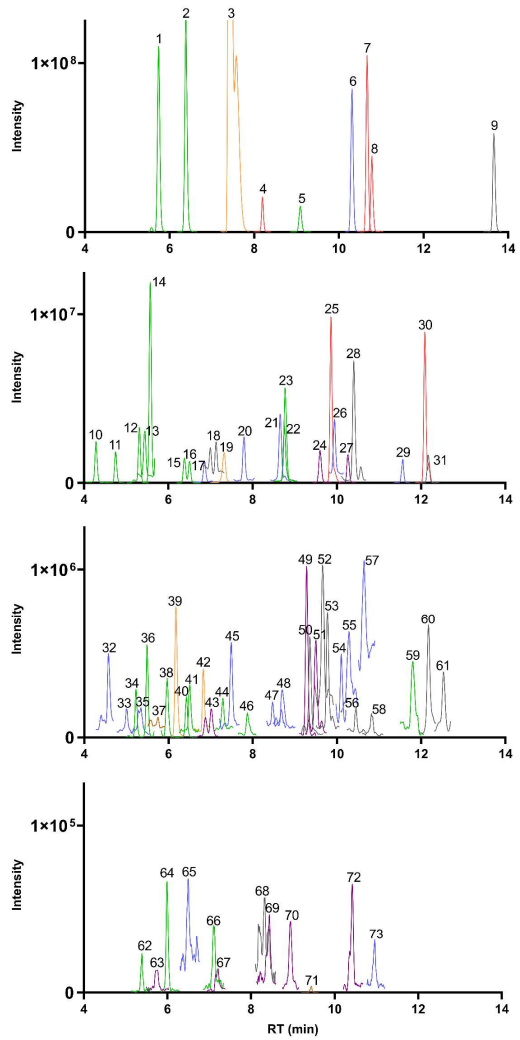


1) Cortisol
2) Cortisone
3) Androsterone Sulfate
4) Glycoursodeoxycholic Acid
5) Corticosterone
6) Testosterone
7) Glycochenodeoxycholic Acid
8) Cholic Acid
9) Progesterone

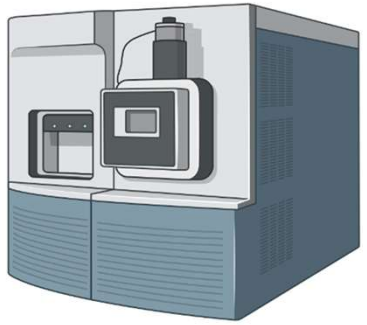
NIST

Dynamic range

10) 18-hydroxycortisol
11) 20α-dihydrocortisol
12) 20β-dihydrocortisol
13) 3α,5β-tetrahydroaldosterone
14) 20α-dihydrocortisone
15) 5α-dihydrocortisol
16) 5β-dihydrocortisol
17) 16α-hydroxytestosterone
18) 16α-hydroxypregnenolone
19) Testosterone Sulfate
20) 11-ketotestosterone
21) 11β-hydroxyandrostenedione
22) 11-deoxycortisol
23) 11-dehydrocorticosterone
24) Etiocholanolone Glucuronide
25) Ursodeoxycholic Acid
26) Dehydroepiandrosterone
27) Pregnanediol Glucuronide
28) 17α-hydroxyprogesterone
29) Androstenedione
30) Chenodeoxycholic Acid
31) 20α-dihydroprogesterone

32) 6α-hydroxytestosterone
33) 7β-hydroxy-DHEA
34) 3α,5α-tetrahydrocortisol
35) 7α-hydroxy-DHEA
36) 3α,5β-tetrahydrocortisol
37) 2-hydroxyestradiol
38) 20β-dihydrocortisone
39) Estrone Sulfate
40) 20β-cortolone
41) 3α,5β-tetrahydrocortisone
42) Epitestosterone Sulfate
43) 11-ketoetiocholanolone Glucuronide
44) Aldosterone
45) 11β-hydroxytestosterone
46) Allotetrahydro-11-dehydrocorticosterone
47) 16α-hydroxyandrosterone
48) 11-ketoandrostenedione
49) Androsterone Glucuronide
50) 16α-hydroxyprogesterone
51) Testosterone Glucuronide
52) Pregnenediol
53) 17α,20α-dihydroxyprogesterone
54) Epitestosterone
55) 19-nortestosterone
56) Pregnenetriol
57) Androsterone
58) 11β-hydroxyprogesterone
59) 11-deoxycorticosterone
60) Allopregnenolone
61) 5α,20α-tetrahydroprogesterone

62) 20β-cortol
63) Estradiol Glucuronide
64) 20α-cortolone
65) 7α-hydroxyandrostenedione
66) 5α-dihydrocortisone
67) Estrone Glucuronide
68) 17α-hydroxypregnenolone
69) DHEA Glucuronide
70) Epitestosterone Glucuronide
71) Estrone
72) 5α-dihydrotestosterone Glucuronide
73) 5α-dihydrotestosterone



Androgens | Progestogens | Estrogens | Corticosteroids | Bile acids | Sulfates | Glucuronides

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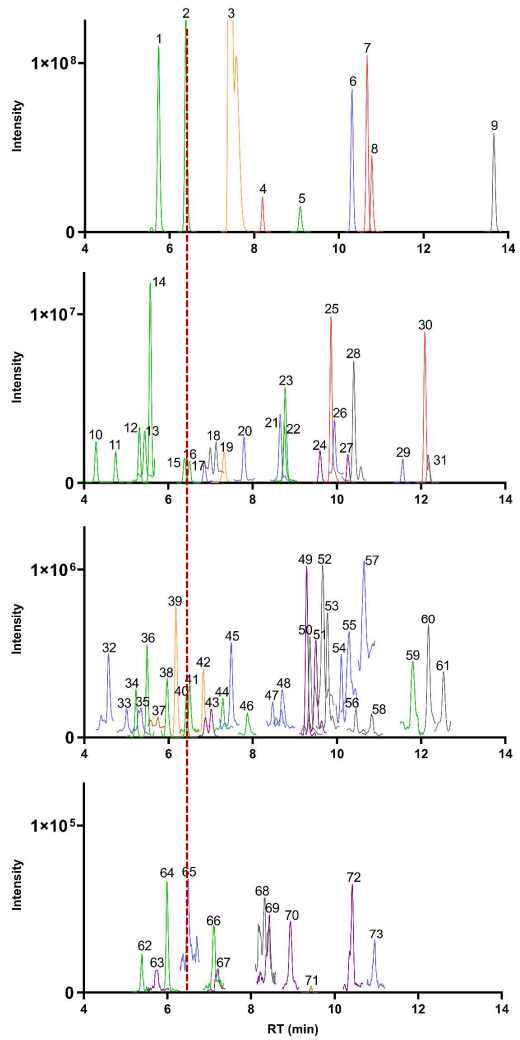

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NIST

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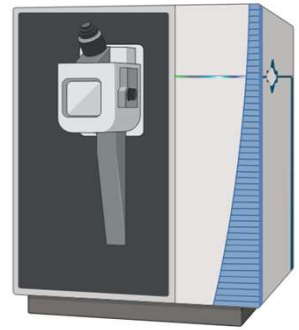
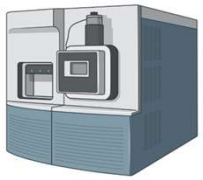
Androgens | Progestogens | Estrogens | Corticosteroids | Bile acids | Sulfates | Glucuronides



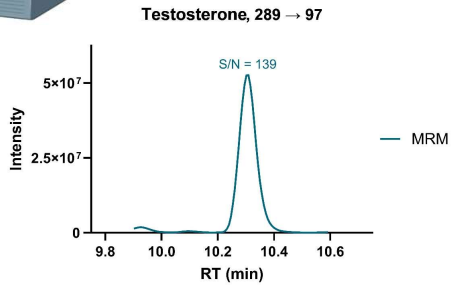
In data-dependent untargeted metabolomics, such low-intensity peaks will not be fragmented, hampering structural elucidation.

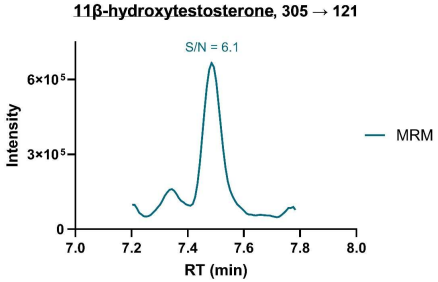
Sniffing out the ultratraces …



2.5 ng/mL

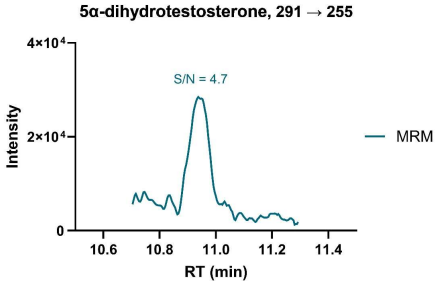
8.7 nmol/L



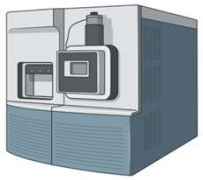


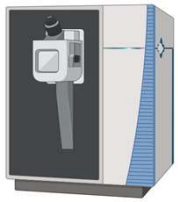
0.2 ng/mL

0.6 nmol/L



Sniffing out the ultratraces …





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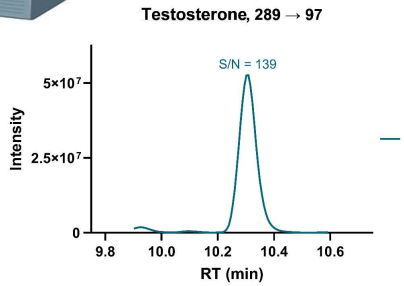
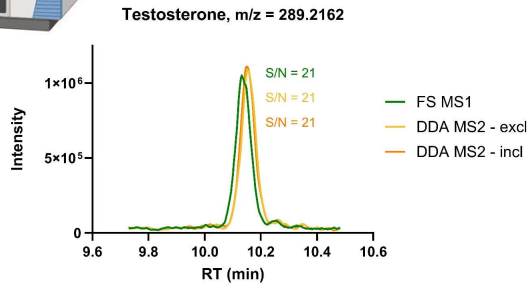
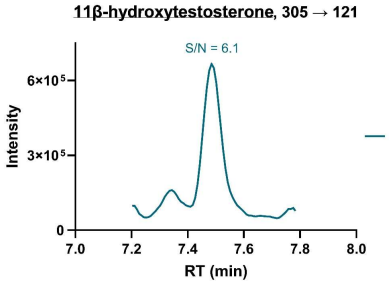
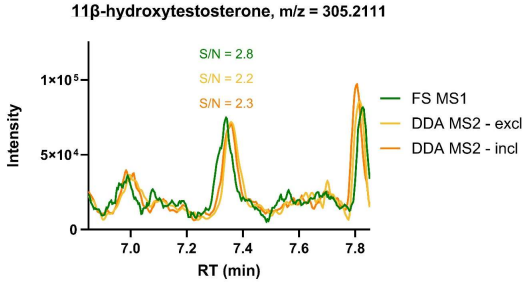


S/N ÷ 7



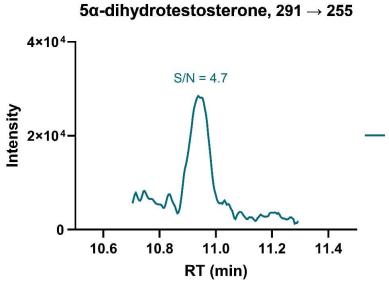


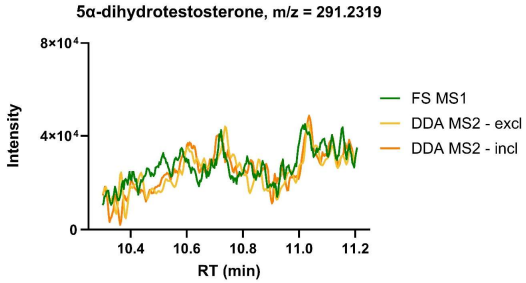
S/N ÷ 3



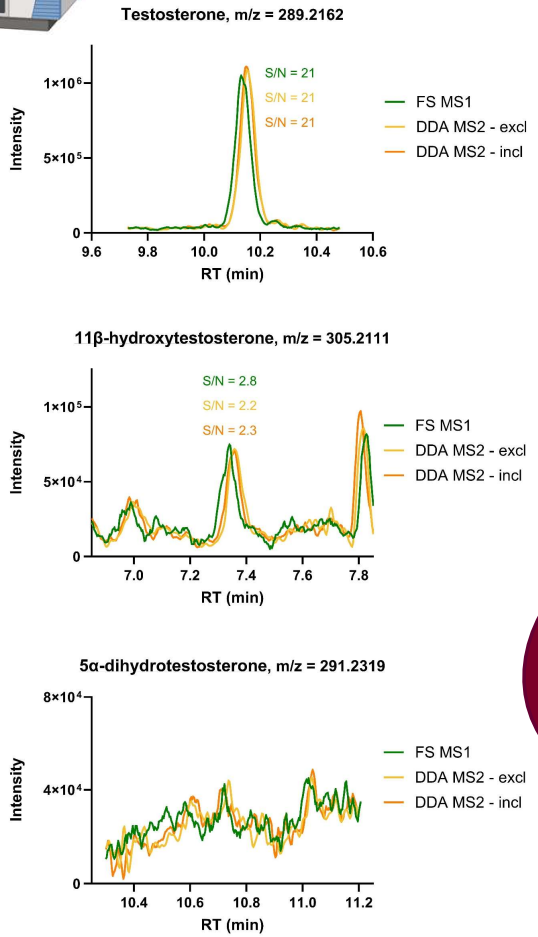
0.2 ng/mL

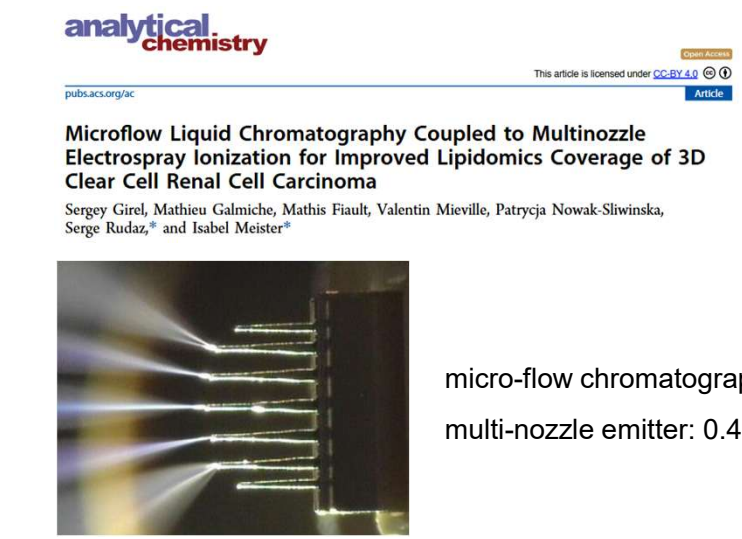
0.6 nmol/L





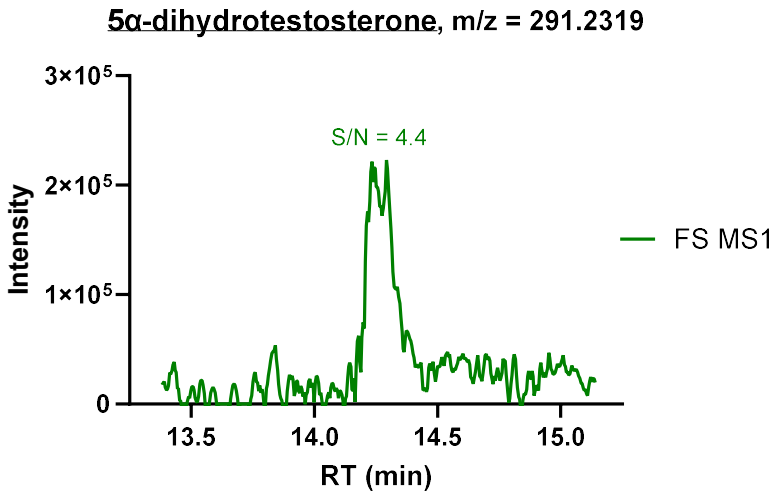
Sniffing out the ultratraces …





micro-flow chromatography: 2.0 µL/min

multi-nozzle emitter: 0.4 µL/min per nozzle

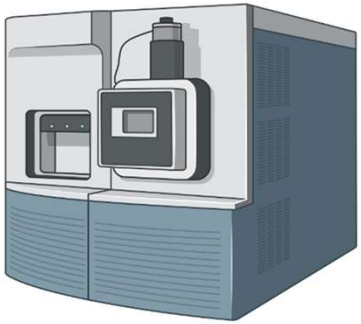


Multiplexing has its limits …

185 compounds

2 to 4 MRM transitions per compound

Positive / Negative polarity switching



Multiplexing has its limits … that we can circumvent

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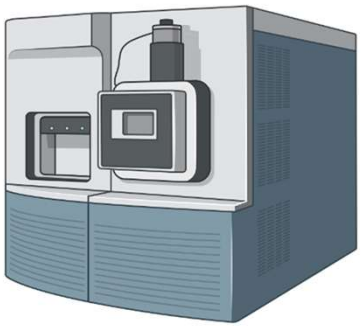

1. Injection of group-pooled QCs

   Separately in positive and negative ionization modes

Multiplexing has its limits … that we can circumvent

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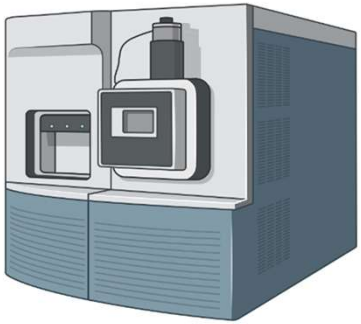
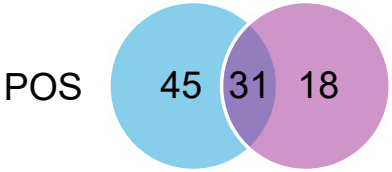


1. Injection of group-pooled QCs

   Separately in positive and negative ionization modes

2. Construction of a cohort-tailored MRM database with tentative identifications



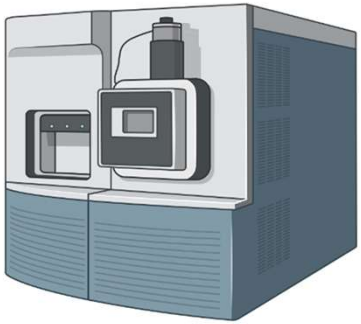
94 total hits

Multiplexing has its limits … that we can circumvent

185 compounds

2 to 4 MRM transitions per compound

Positive / Negative polarity switching



1. Injection of group-pooled QCs

   Separately in positive and negative ionization modes

2. Construction of a cohort-tailored MRM database with tentative identifications

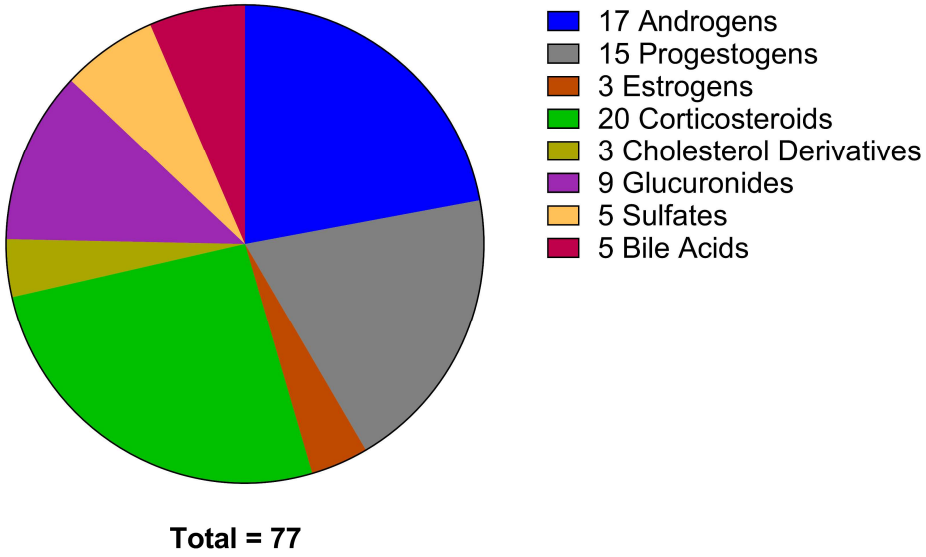
   POS 

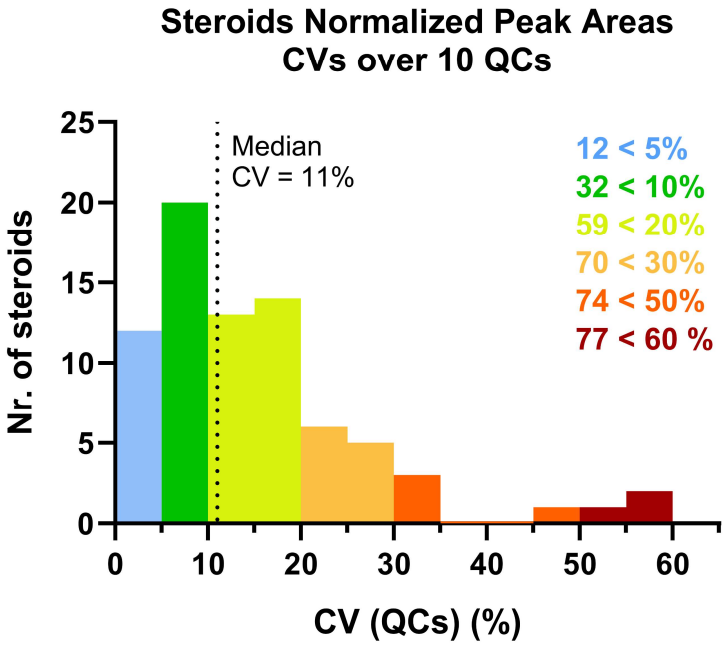
   NEG 94 total hits

3. Acquisition of the entire cohort with the specific list of MRM transitions for the 94 tentative identifications, with polarity switching

Multi-targeted data can still be handled like untargeted data

70 manually curated steroid detections





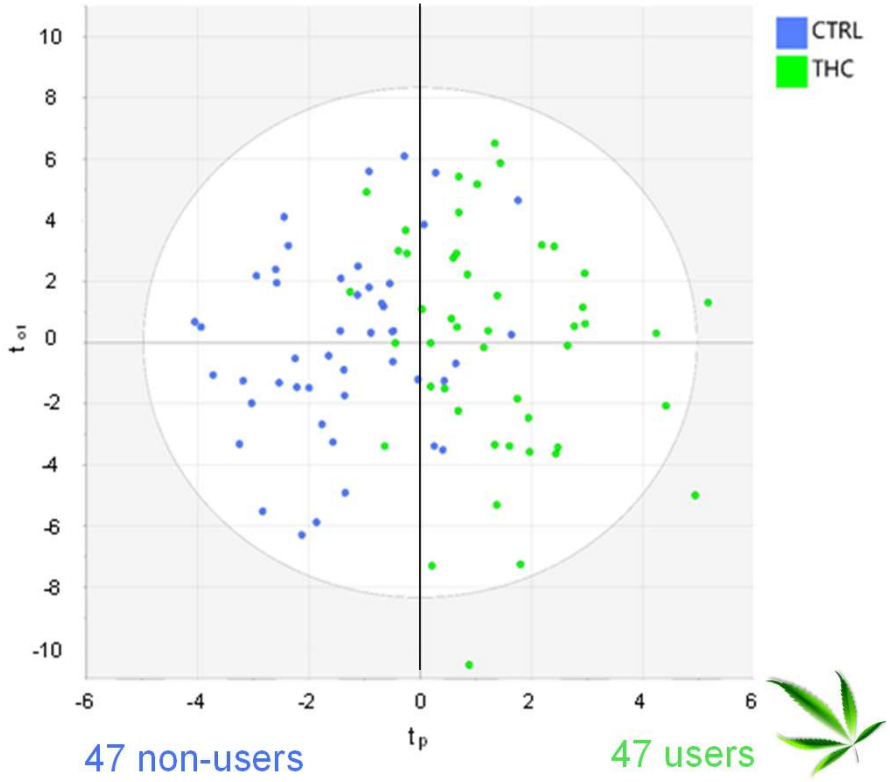
Multi-targeted data can still be handled like untargeted data

Multivariate Analysis: OPLS-DA

$R^2Xp = 0.06$

$R^2Y = 0.49$

$Q^2 = 0.19$



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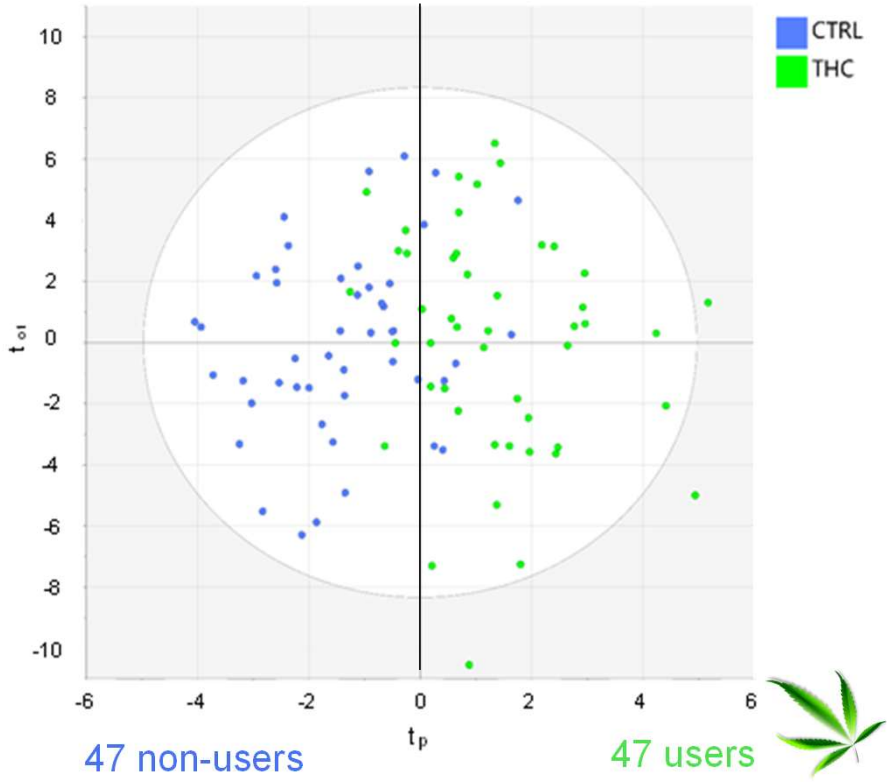
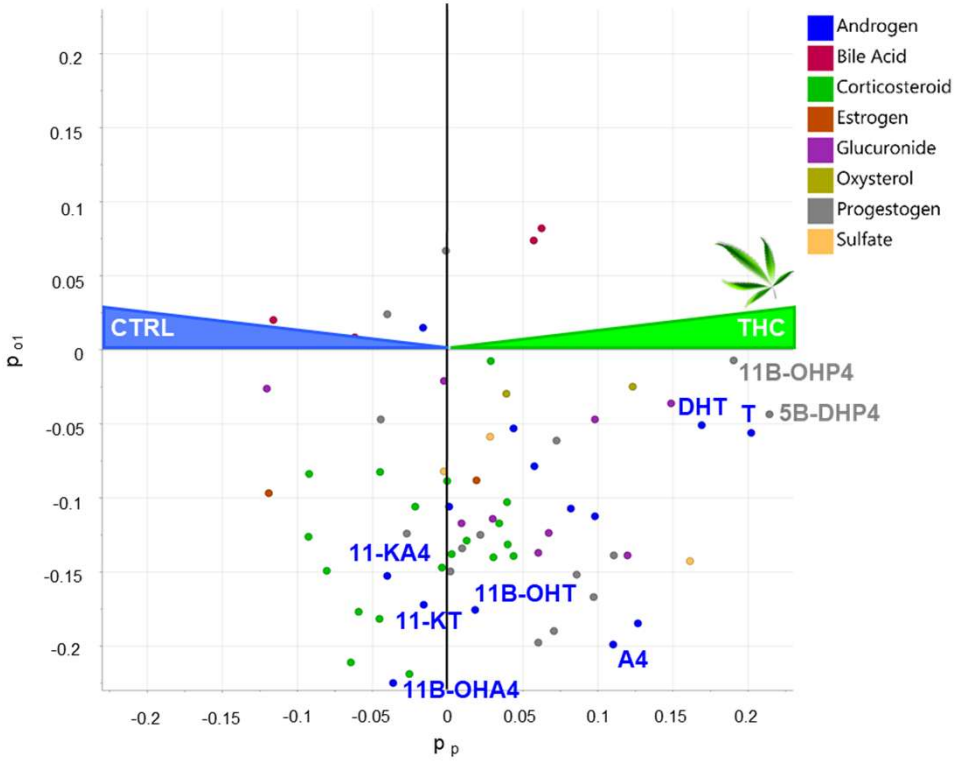
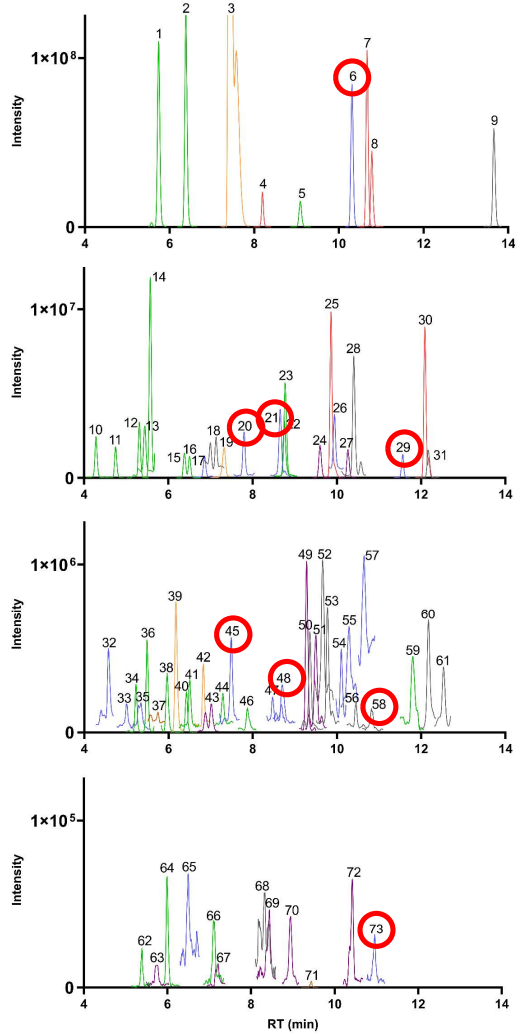




Which signals are the most biologically meaningful?



1) Cortisol
2) Cortisone
3) Androsterone Sulfate
4) Glycoursodeoxycholic Acid
5) Corticosterone
6) Testosterone
7) Glycochenodeoxycholic Acid
8) Cholic Acid
9) Progesterone

10) 18-hydroxycortisol
11) 20α-dihydrocortisol
12) 20β-dihydrocortisol
13) 3α,5β-tetrahydroaldosterone
14) 20α-dihydrocortisone
15) 5α-dihydrocortisol
16) 5β-dihydrocortisol
17) 16α-hydroxytestosterone
18) 16α-hydroxypregnenolone
19) Testosterone Sulfate
20) 11-ketotestosterone
21) 11β-hydroxyandrostenedione
22) 11-deoxycortisol
23) 11-dehydrocorticosterone
24) Etiocholanolone Glucuronide
25) Ursodeoxycholic Acid
26) Dehydroepiandrosterone
27) Pregnanediol Glucuronide
28) 17α-hydroxyprogesterone
29) Androstenedione
30) Chenodeoxycholic Acid
31) 20α-dihydroprogesterone

32) 6α-hydroxytestosterone
33) 7β-hydroxy-DHEA
34) 3α,5α-tetrahydrocortisol
35) 7α-hydroxy-DHEA
36) 3α,5β-tetrahydrocortisol
37) 2-hydroxyestradiol
38) 20β-dihydrocortisone
39) Estrone Sulfate
40) 20β-cortolone
41) 3α,5β-tetrahydrocortisone
42) Epitestosterone Sulfate
43) 11-ketoetiocholanolone Glucuronide
44) Aldosterone
45) 11β-hydroxytestosterone
46) Allotetrahydro-11-dehydrocorticosterone
47) 16α-hydroxyandrosterone
48) 11-ketoandrostenedione
49) Androsterone Glucuronide
50) 16α-hydroxyprogesterone
51) Testosterone Glucuronide
52) Pregnenediol
53) 17α,20α-dihydroxyprogesterone
54) Epitestosterone
55) 19-nortestosterone
56) Pregnenetriol
57) Androsterone
58) 11β-hydroxyprogesterone
59) 11-deoxycorticosterone
60) Allopregnenolone
61) 5α,20α-tetrahydroprogesterone

62) 20β-cortol
63) Estradiol Glucuronide
64) 20α-cortolone
65) 7α-hydroxyandrostenedione
66) 5α-dihydrocortisone
67) Estrone Glucuronide
68) 17α-hydroxypregnenolone
69) DHEA Glucuronide
70) Epitestosterone Glucuronide
71) Estrone
72) 5α-dihydrotestosterone Glucuronide
73) 5α-dihydrotestosterone

Androgens | Progestogens | Estrogens | Corticosteroids | Bile acids | Sulfates | Glucuronides

Summary: Targeted ϟ Untargeted metabolomics for human health

- Annotation is a critical bottleneck in HRMS-based metabolomics. It has been for years.
- Targeted approaches provide unequivocal compound identity, even without HRMS.

- Biological/biochemical interpretation of unidentified features for health applications is very tricky.
  Processing of untargeted HRMS data is frequently performed with a ‘post-targeted’ approach.
- If so, there is no information gain compared to targeted metabolomics, and even likely some information loss due to decreased sensitivity.

- Even in ‘untargeted’ setups, chromatography defines the breadth of chemical coverage.
  Although ‘untargeted’, the study will still be limited to RP-amenable molecules, for example.
- On the other hand, ‘targeted’ does not mean restricted! It can be extended to hundreds of metabolites.

Combining untargeted screening and targeted absolute quantification: The future of metabolomics?

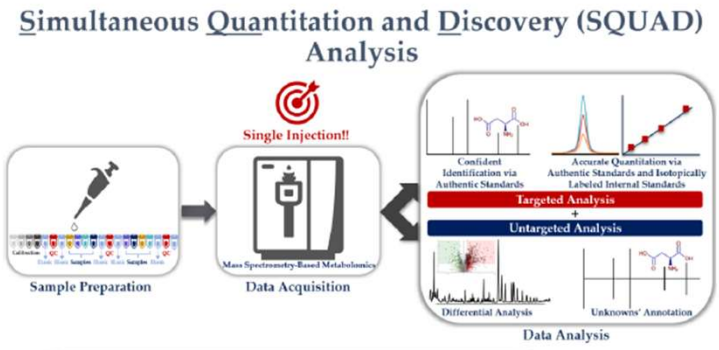
metabolites

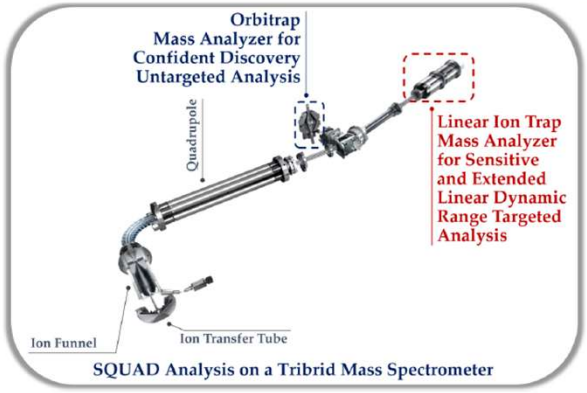
MDPI

Review

Simultaneous Quantitation and Discovery (SQUAD) Analysis: Combining the Best of Targeted and Untargeted Mass Spectrometry-Based Metabolomics

Bashar Amer *, Rahul R. Deshpande and Susan S. Bird





Analytica Chimica Acta 1331 (2024) 343314

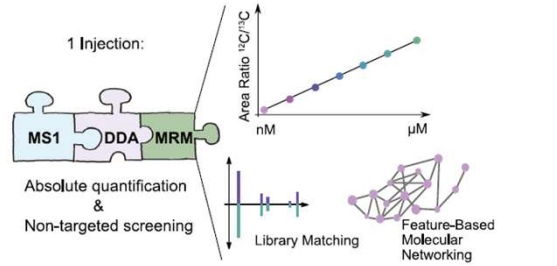
Triple acquisition mass spectrometry (TRAM) combining targeted and non-targeted metabolomics in a single run

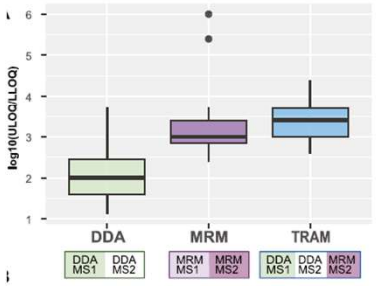
Lisa Panzenboeck [a,b], Harald Schoeny [a], Bruno Stelzer [a], Elisabeth Foels [a,b,c], Marvin Glas [a], Marlene Pühringer [a,b], Dorian Hirschmann [c], Daniela Loetsch [c], Christian Dorfer [c], Evelyn Rampler [a,b], Gunda Koellensperger [a,d,*]

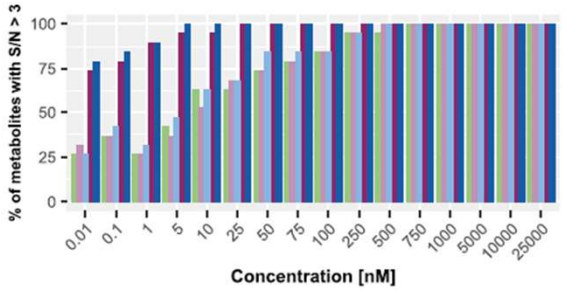
HIGHLIGHTS

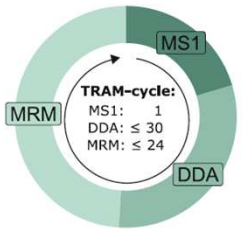
- High-speed triple acquisition strategy unifies targeted & non-targeted metabolomics.
- Absolute quantification & high number of DDA MS2 scans for individual samples.
- TRAM unites MS1, DDA-MS2 & MRM-MS2 data acquisition within a single injection.
- TRAM was benchmarked using SRM 1950 & cerebrospinal fluid of meningioma patients.
- Optimized MRM parameters & HILIC – retention times for more than 90 metabolites.

GRAPHICAL ABSTRACT

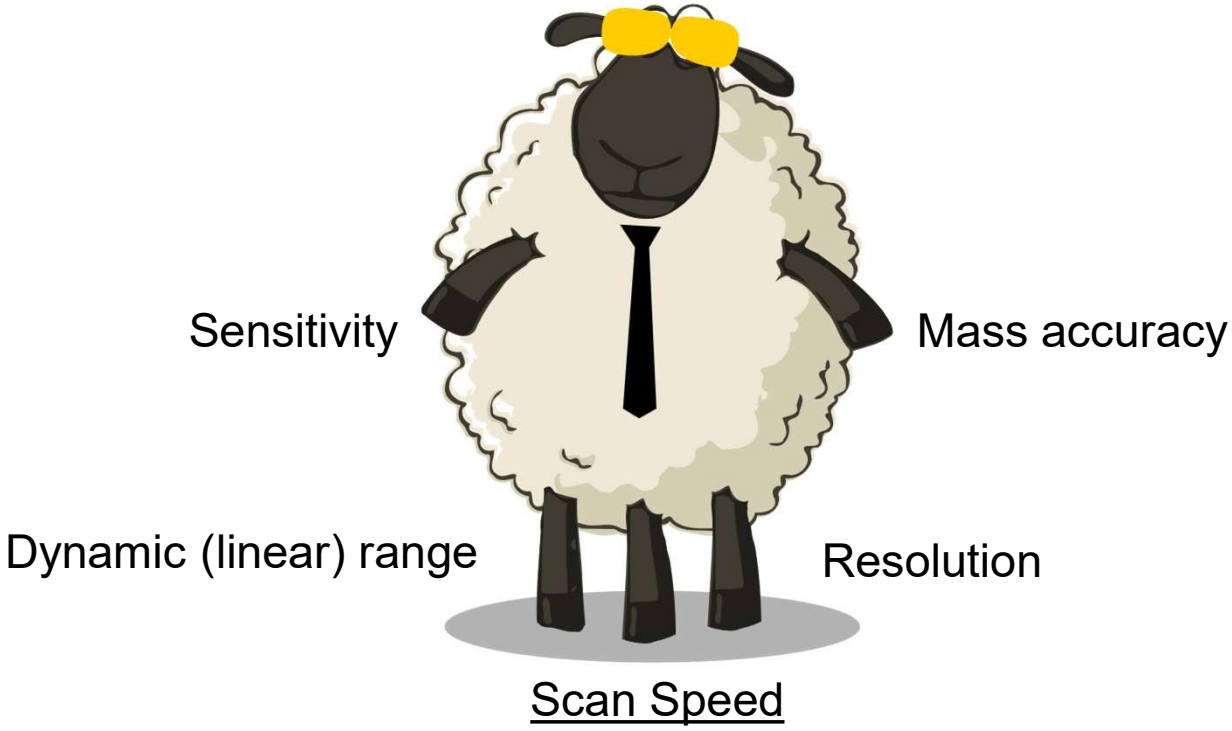








Combining untargeted screening and targeted absolute quantification: The future of metabolomics?



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