BIOLOGY CENTRE CAS

MetaboAuto - A Novel Platform for Automated GC-MS Metabolomics

Petr Šimek

Biology Centre, Czech Academy of Sciences
Analytical Biochemistry and Metabolomics
Ceske Budejovice, Czech Republic

simek@bclab.eu

MetaboAuto for GC-MS Metabolomics

Outline

1. Basic GC-MS metabolomics workflows
2. Sample preparation for GC-MS MX

LLME– alkyl chloroformate (RCF) derivatization

(principle, features, workflows)

3. Metabolite coverage
4. Automation
5. Applications
    - 5.1. Urine metabolomics
    - 5.2. Plasma metabolomics
    - 5.3. Steroid-tocopherol profiling
    - 5.4. Chiral analysis
6. Conclusions and Perspectives
7. Acknowledgements

1. A typical GC-MS metabolomics workflow

Biological sample

↓

Addition of internal standards

↓

Elimination (precipitation) of polymeric structures (lipoproteins, proteins, sugars, cell structures)

↓

Volatilization of protic metabolites (derivatization by a suitable reagent excess)

↓

GC separation and MS detection

↓

Data processing and data mining

GC-MS Metabolomics

1. Typical features

- Excellent separation (isomers of small analytes)
- Highly sensitive and robust detection (RSD < 5%)
- Equipment – cost effective
- An automated workflow possible

Typical GC-MS MX restrictions

- For a limited metabolite set
- Sample preparation involving a derivatization step is essential

Three current basic derivatization approaches in GC-MS MX

1.1. Silylation + oximation

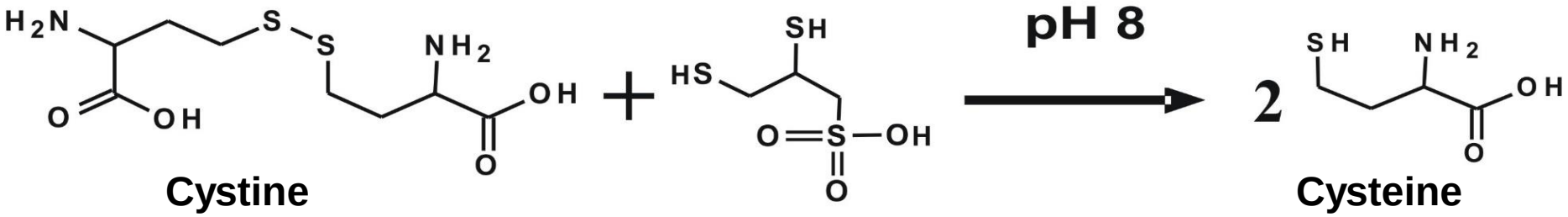
1.2. Alkyl chloroformates + liquid liquid microextraction (LLME)

1.3. Transmethylation of the lipidic FA acid residues

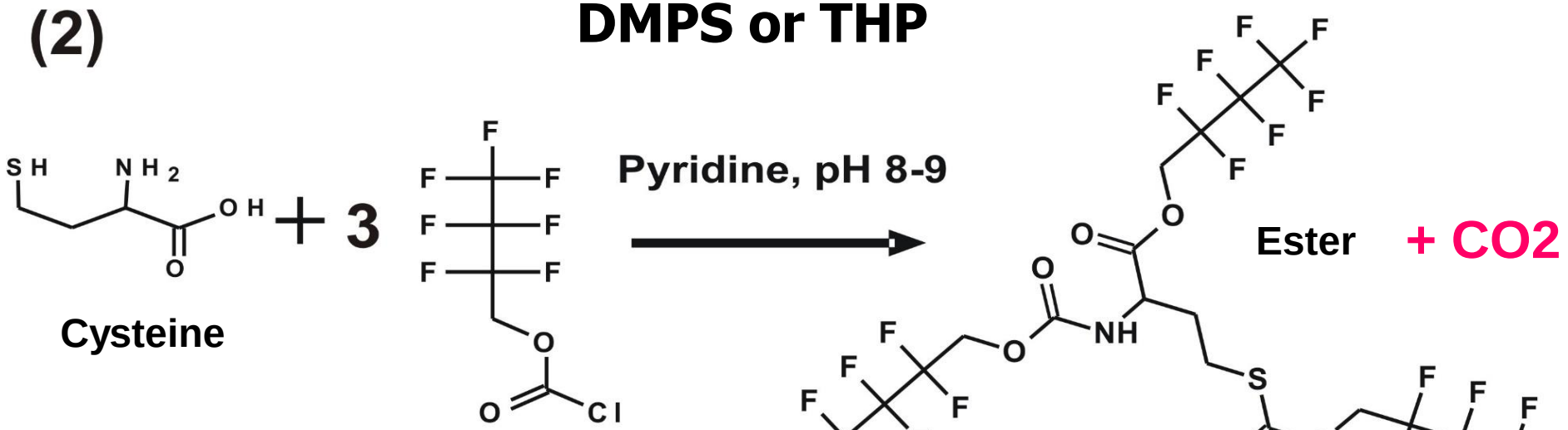
1.2. The alkyl chloroformate (RCF) derivatization

How does it work ?

(1)



(2)



Heptafluorobutylchloroformate

(HFBCF)

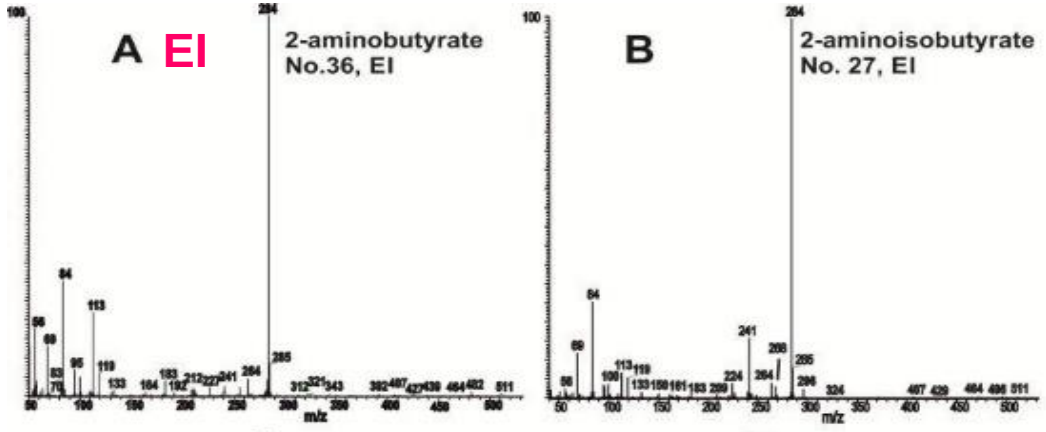
1.2. Fluoroalkyl chloroformates

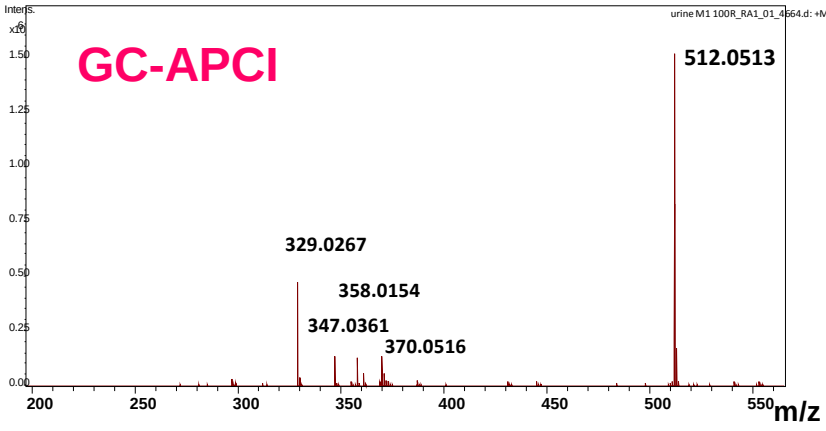
- HFBCF metabolites as highly volatile as ethyl- derivatives

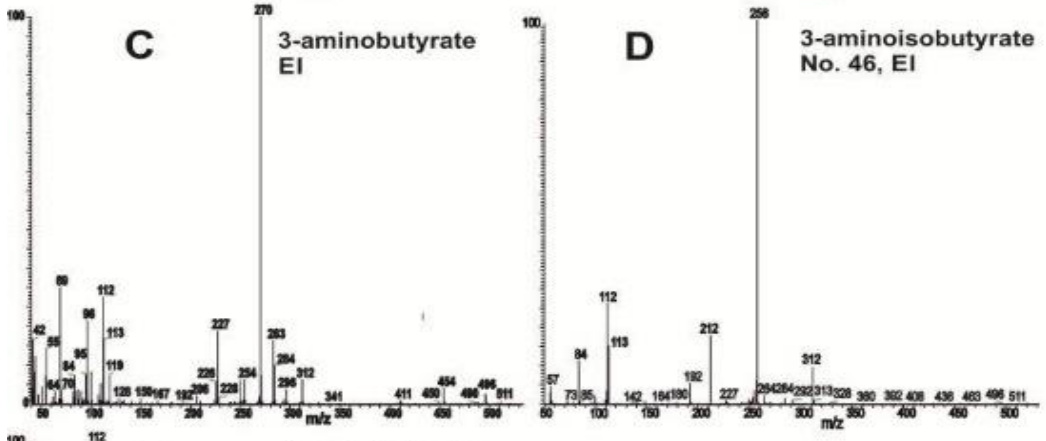
  (+182 Da ester, + 226 Da carbamate, (thio) carbonate
- Heptafluorobutyl moiety – less polar than butyl!
- Extraction into isooctane
- Reaction < 5s in situ in aqueous environment, ceased
- Defined stable reaction products (esters, carbonates, carbamates)
- Much lower matrix effects than any other GC-MS based metabolomics method
- Excellent separation
- Excellent mass spectral properties

Šimek P et al, Anal Chem 2008, J. Chromatogr. A 2016

1.2. Fluoroalkyl chloroformates

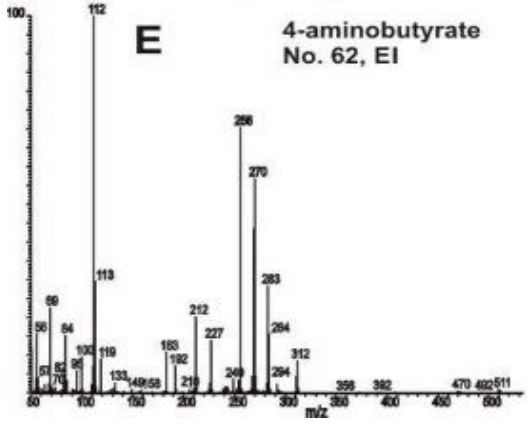


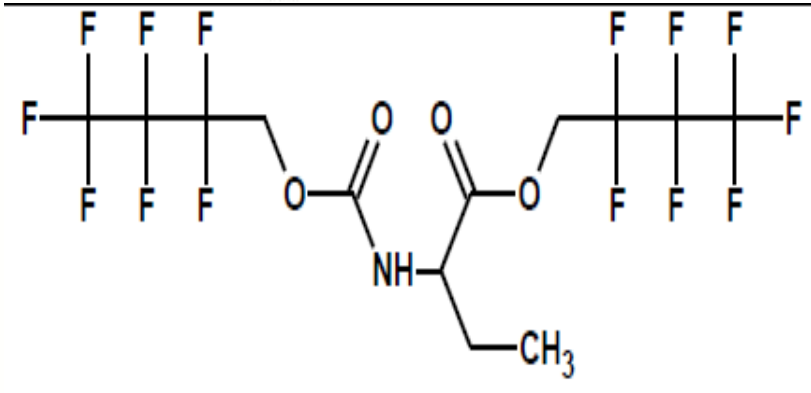




^{19}F m/z 18.9984

7*F Δ m/z = -11.2 mDa

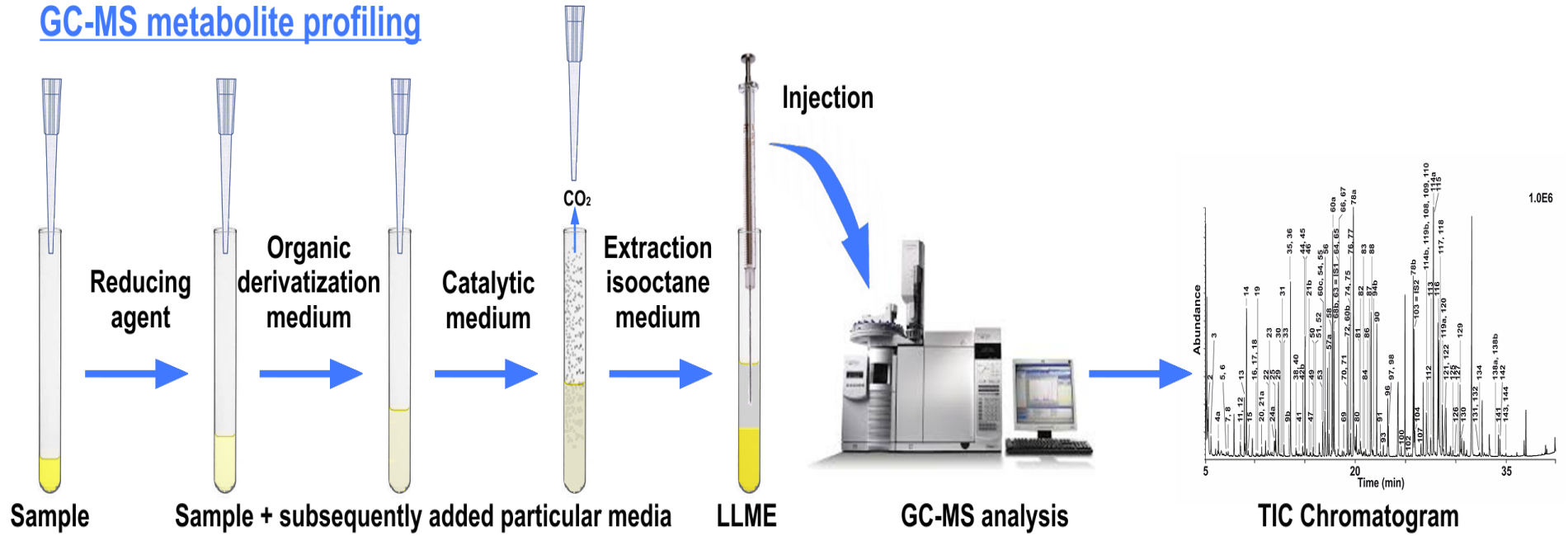




No. 36
2-Aminobutyrate
HMDB ID: HMDB00452
MOLECULAR FORMULA:
$C_{13}H_{11}F_{14}NO_4$
Monoisotopic Mass = 511.046453 Da

GC-MS Metabolomics

2. The Sample Preparation Workflow



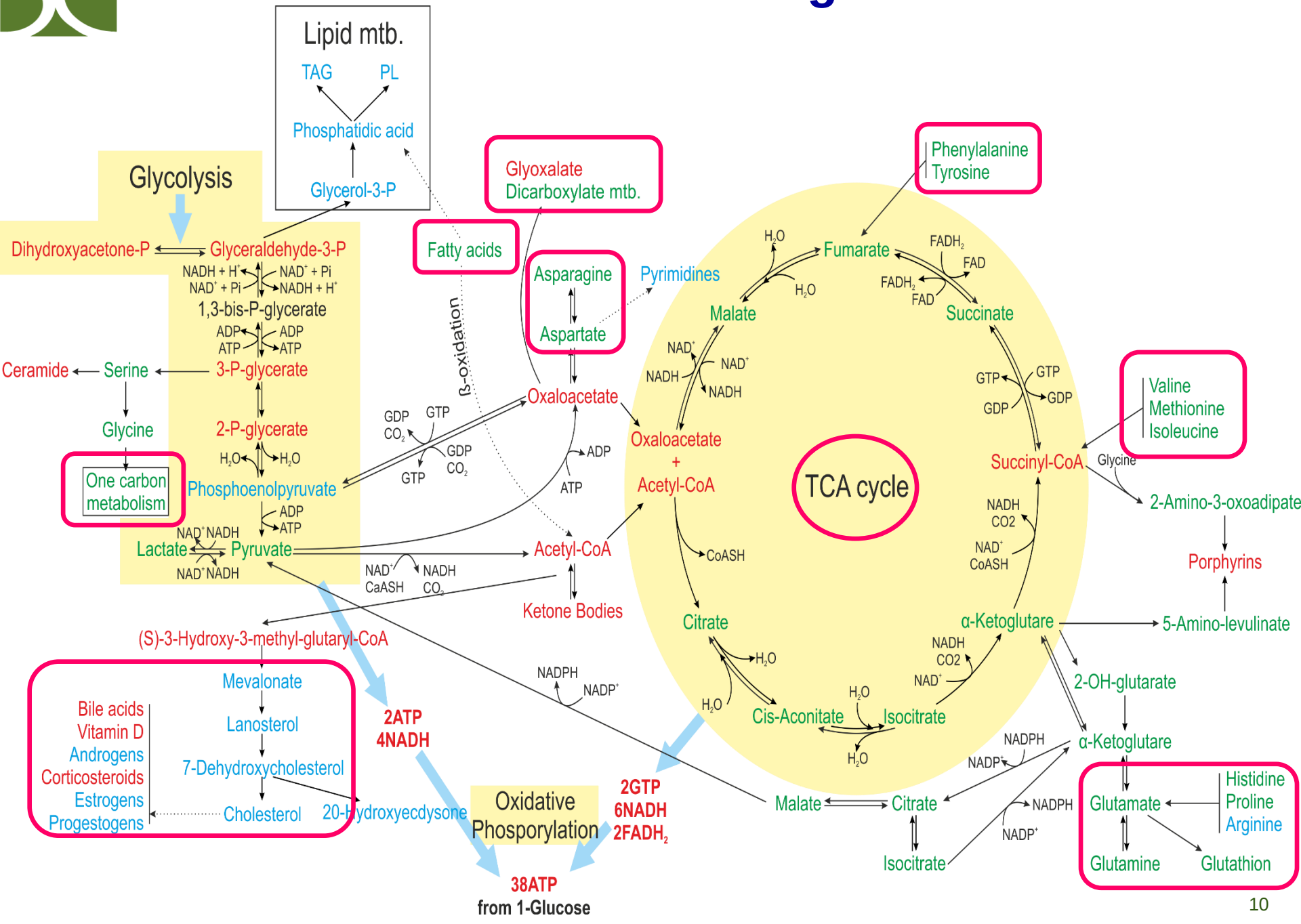
- **Precipitation** (Hušek P, Šimek P et al, JPBA 2012)
- **Reducing the disulfide bonds** (release of thiols, Švagera Z et al, ABC 2012)
- **Derivatization conditions** (Hušek P, Šimek P et al, JCA 2011)
- **Extraction** (Řimnáčová L, Šimek P et al, JCA 2014, JCA 2016)

3. Metabolite Coverage

- > 153 protic urinary metabolites individually derivatized by alkyl chloroformates (ethyl-, heptafluorobutyl)
- Reaction products investigated by GC-HRMS and LC-HRMS methods
- From the set:
  - 120 (78 %) - a single dominating product
  - 25 (16 %) - two peaks
  - 2 - more peaks (2-methylcitrate, citrate)
  - 5 - n.d.
- 132 validated in artificial urine (2 IS)
- 112 determined in the 25 µl urine aliquots (100 urines of healthy controls)

Pilot study: Hušek P, Šimek P et al (JCA 2016)

3. Metabolite Coverage



4. Automation of the GC-MS workflow

Urine / serum extract, 25 µL

↓1

1-Reducing medium (25 µL, 1 min)

↓2

2-Basic medium (25 µL, I.S., 200 µmol/L)

↓3

3-Reaction medium (50 µL)

↓4

4-Catalytic medium (25 µL, vortexing)

↓5

4-Catalytic medium (ibid, 25 µL, vortexing)

↓6

5-Organic medium (50 µL)

↓7

6-Acidic medium (25 µL)

↓8

Organic phase transfer (ca 25 µL)

↓9

Inject, sls, 1 µL

↓10

GC-MS, LC-MS instrumentation analysis

4. Automation of the GC-MS workflow

Manual sample preparation

4. Automation of the GC-MS workflow

The MetaboAuto concept

Full control of 30 operations (dilution, transfer, vortexing, liquid phase withdrawal

Base - RTC autosampler arm with exchangeable syringes

4. Automation of the GC-MS workflow

The MetaboAuto Video

https://www.youtube.com/watch?v=X0LaJzf7Evo

Full control of 30 particular operations (addition of internal standards, dilution, transfer, vortexing, liquid phase withdrawal

Cost effective

24h/7d, 76 samples/day

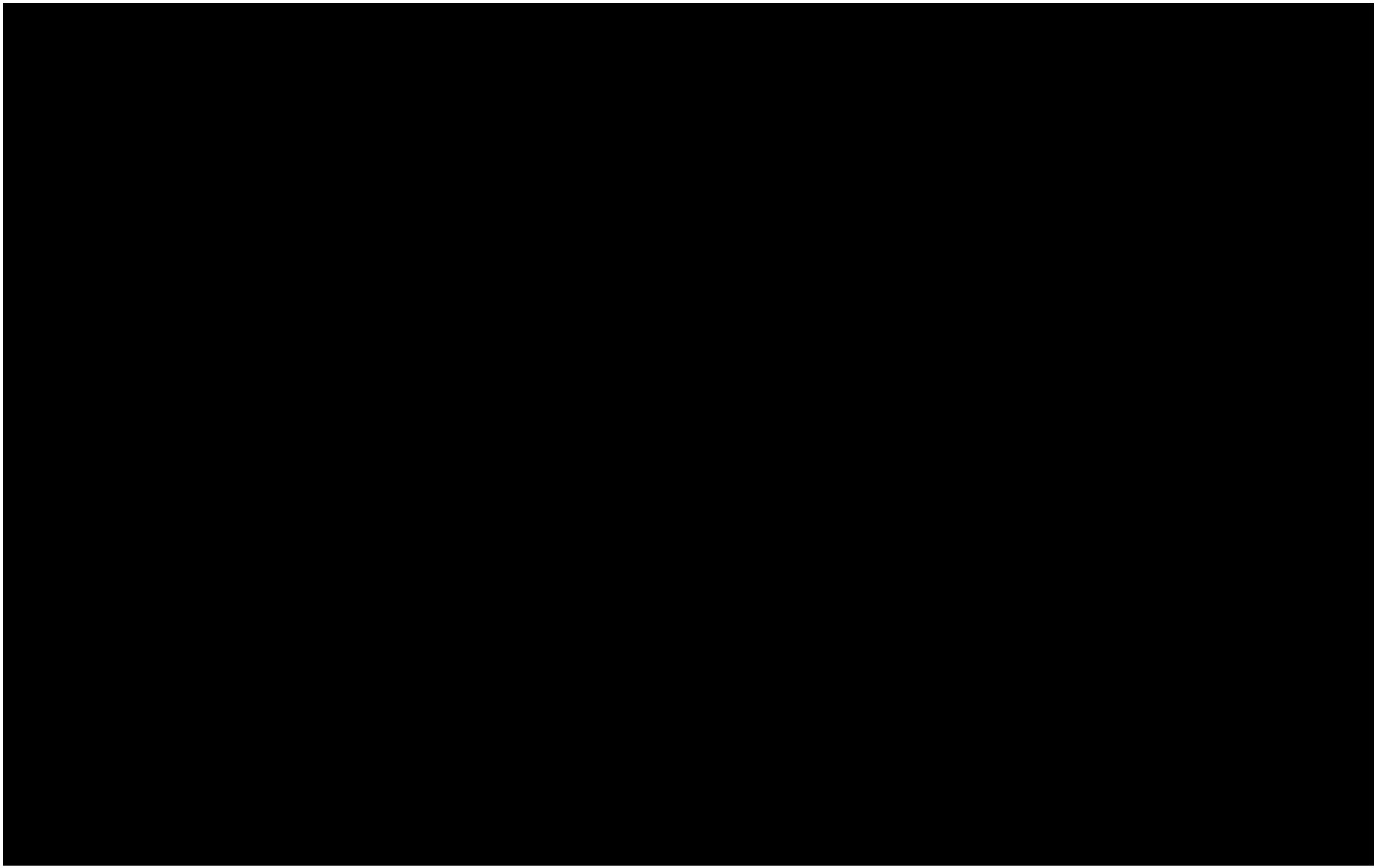
Mounted on a GC-MS system

Stand-alone robotic workstation

Poster No. 1 for further discussion

4. Automation of the GC-MS workflow

The MetaboAuto Video



5. Applications

5.1. Urine GC-MS metabolomics

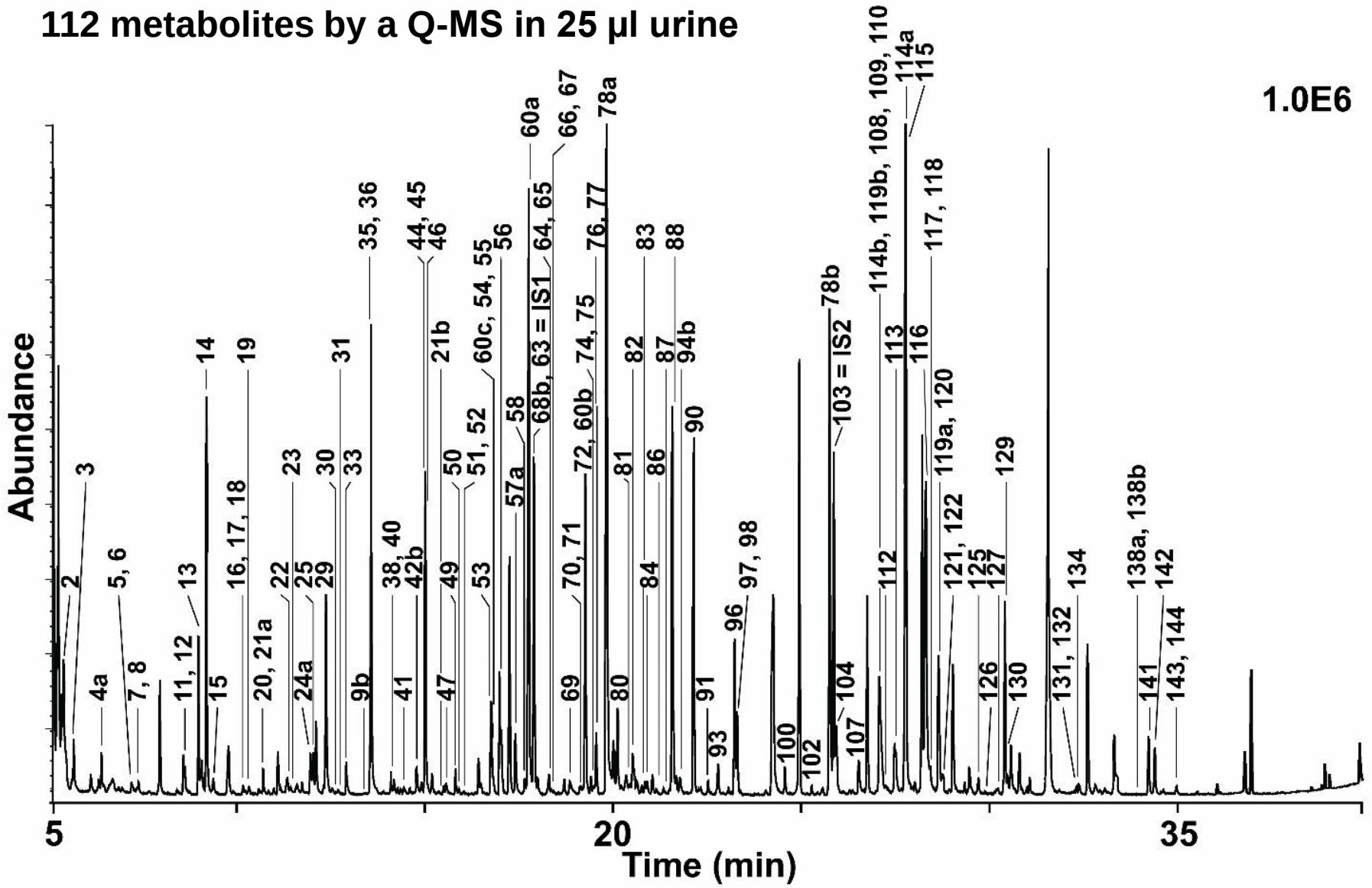
5.2. Plasma GC-MS metabolomics

5.3. Steroid-tocopherol GC-MS profiling

5.4. Chiral GC-MS analysis

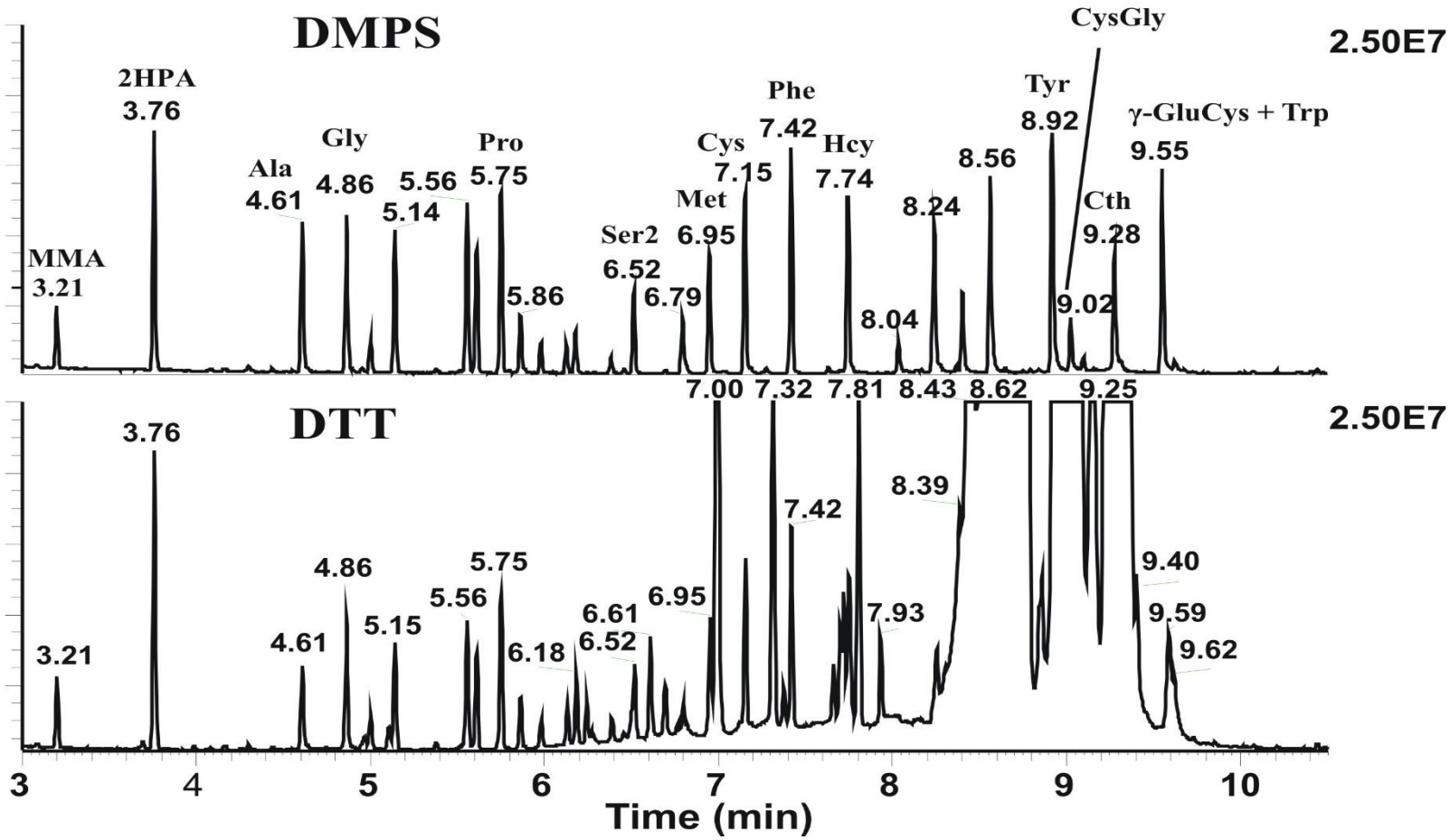
5.1. Urine Metabolomics

112 metabolites by a Q-MS in 25 µl urine



Hušek P, Šimek P et al (JCA 2016)

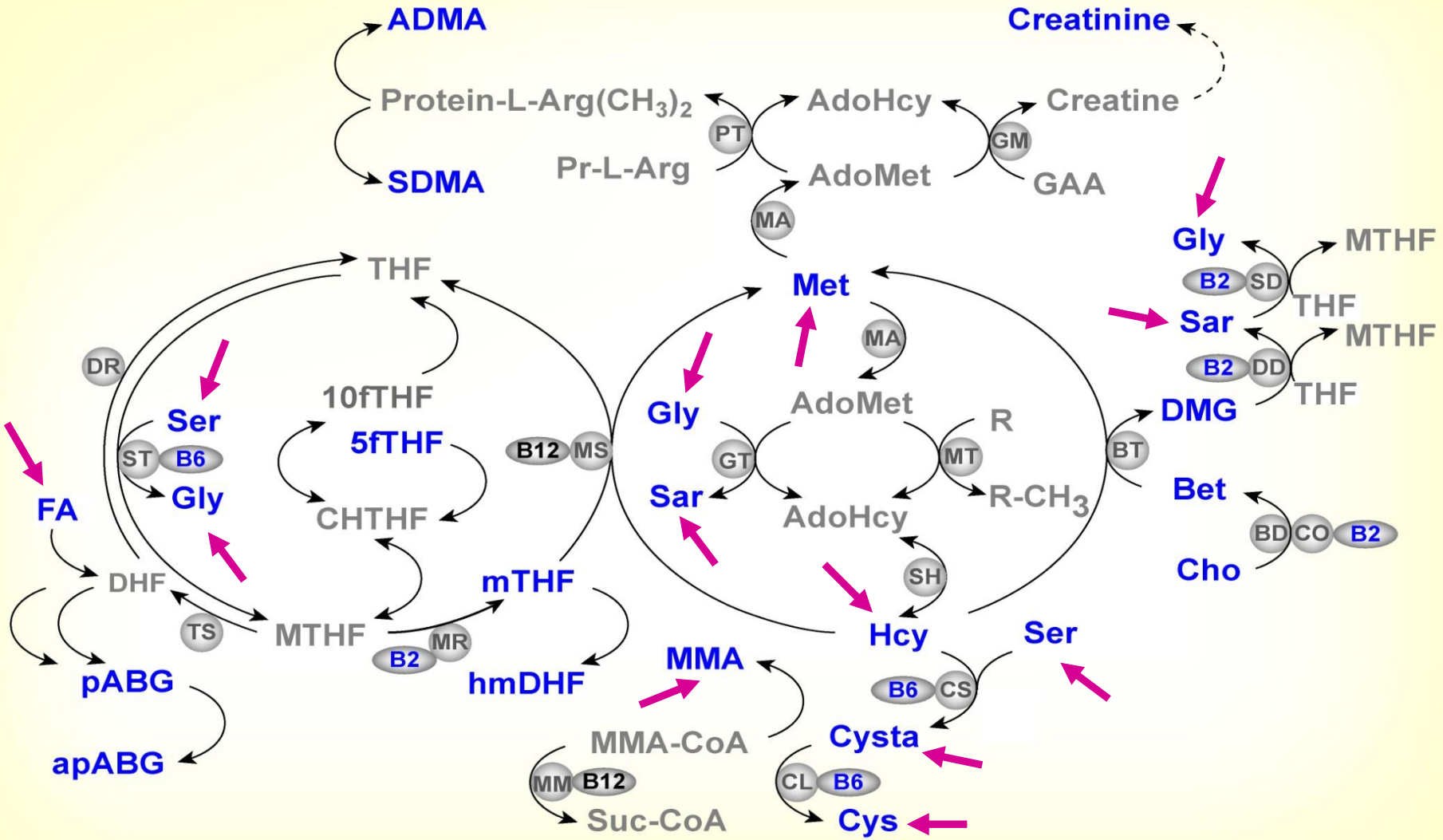
5.2. Plasma metabolomics



Šimek P et al, Anal Chem 2008

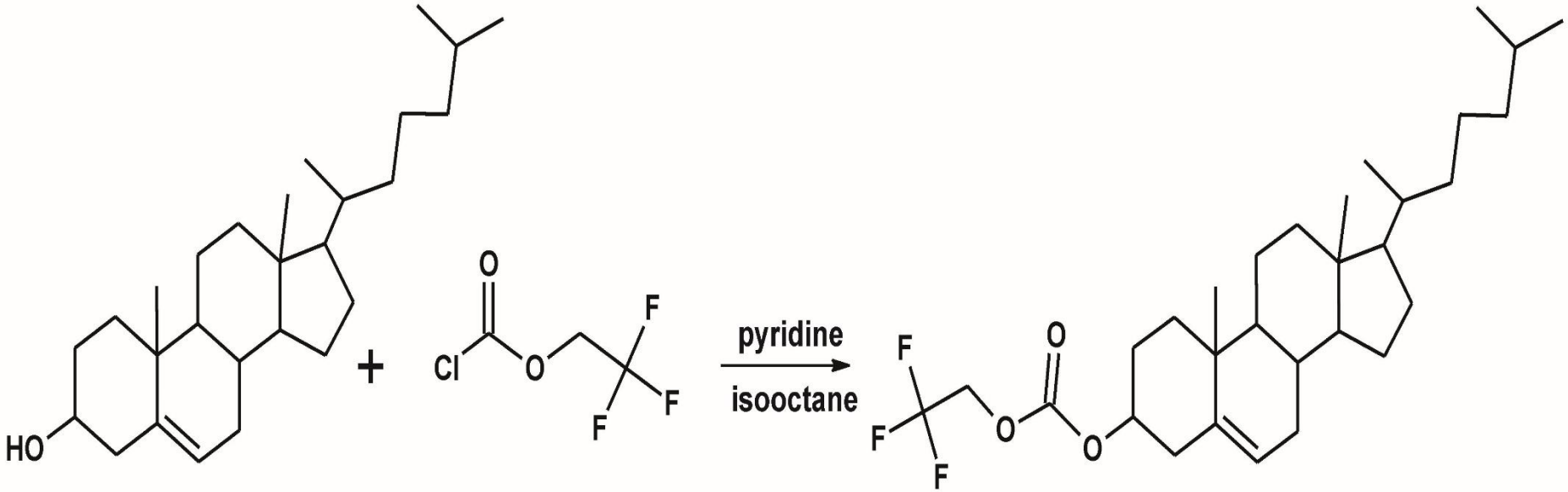
5.2. Plasma metabolomics

One carbon metabolism



Adapted according Ueland P et al, ABC 2010

5.3. Steroid-Tocopherol Profiling



Cholesterol
MM = 386.4

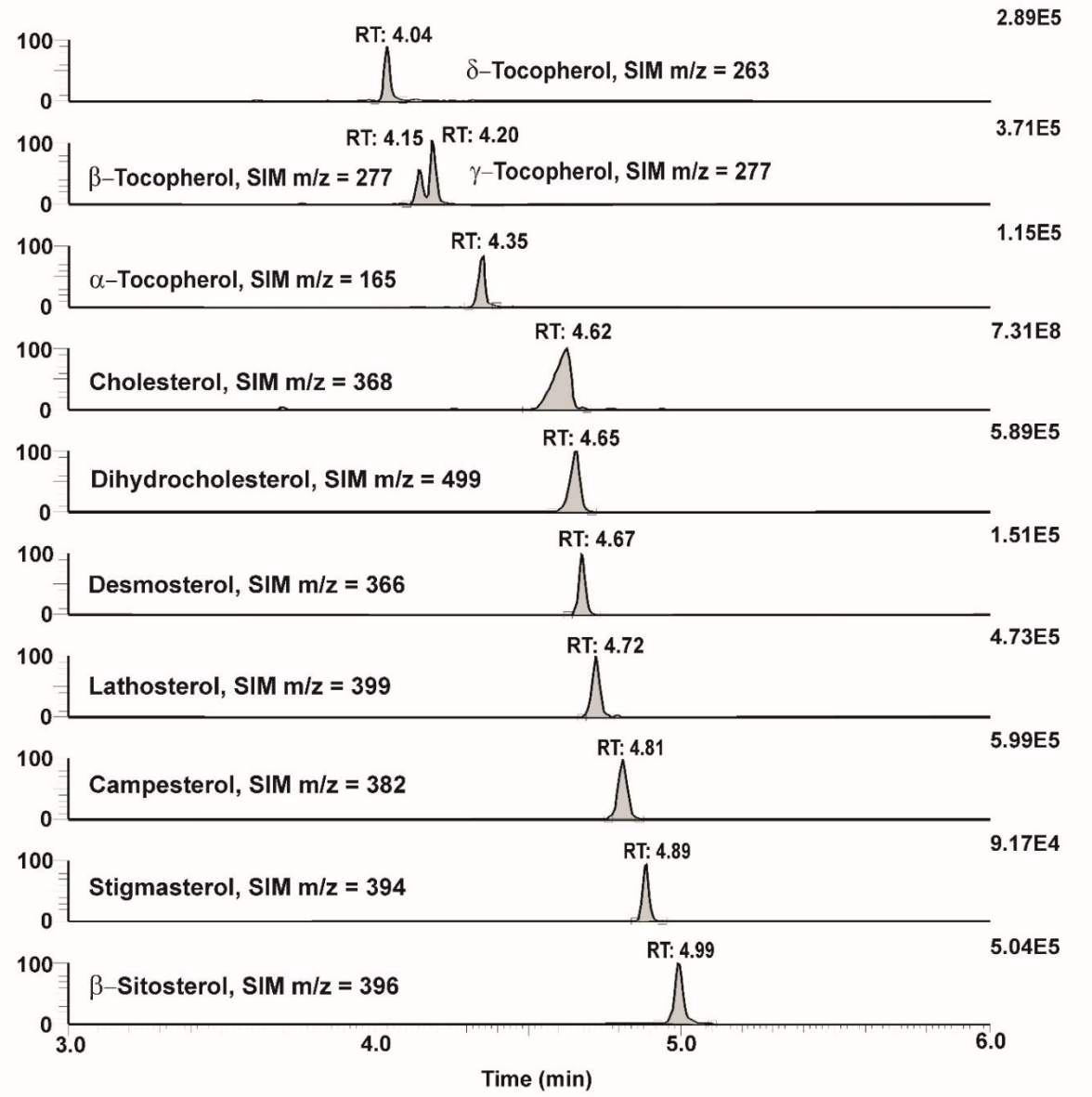
TFECF
MM = 161.9

TFEOC cholesterol
MM = 512.4

Anhydrous conditions

Řimnáčová L., Hušek P., Šimek P., JCA (2014)

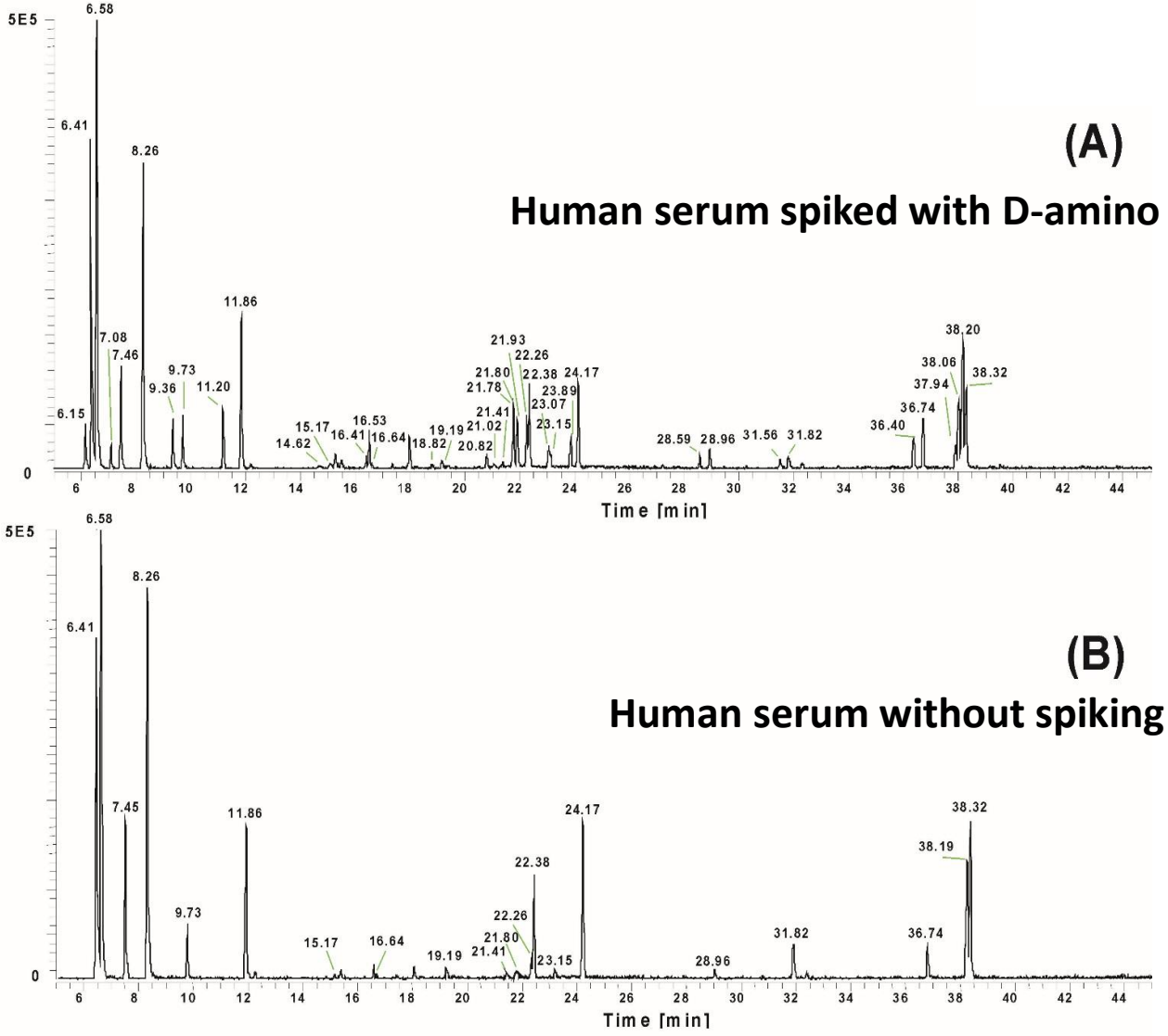
5.3. Steroid-Tocopherol Profiling



Řimnáčová L., Hušek P., Šimek P., JCA (2014)

5.4. Chiral analysis – 35 AA (R, S) enantiomers separated

Chirasil Val column



Added *D*-amino acids:

RT (min) - Name

6.15 - D-Ala

7.08 - D-Val

9.36 - D-Ile

11.20 - D-Leu

14.62 - D-Thr

16.41 - D-Asp

18.82 - D-Ser

20.84 - D-Met

21.02 - D-Gln

22.26 - D-Glu

22.38 - D-Phe

23.07 - D-Asn

23.89 - D-Cys

28.59 - D-HCy

31.56 - D-His

36.40 - D-Orn

37.97 - D-Lys

46.85 - D-Trp

Šimek P et al (2012), Methods in Molecular Biology, Amino acid analysis

6. Conclusions

- A new efficient, versatile GC-MS metabolomics platform
- Metabolites of the central metabolism
- Metabolites of the steroid and tocopherol metabolism
- Various biological matrices (plasma, urine)
- An option – chiral AA analysis
- **MetaboAuto**
  A fully automated GC-MS metabolomics analytical platform

7. Acknowlegements

The Research Team

Poster **Iva Opekarova et al**

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