Automated Parallel Derivatization Strategy to Improve Metabolites Analysis in Liquid Chromatography-Mass Spectrometry

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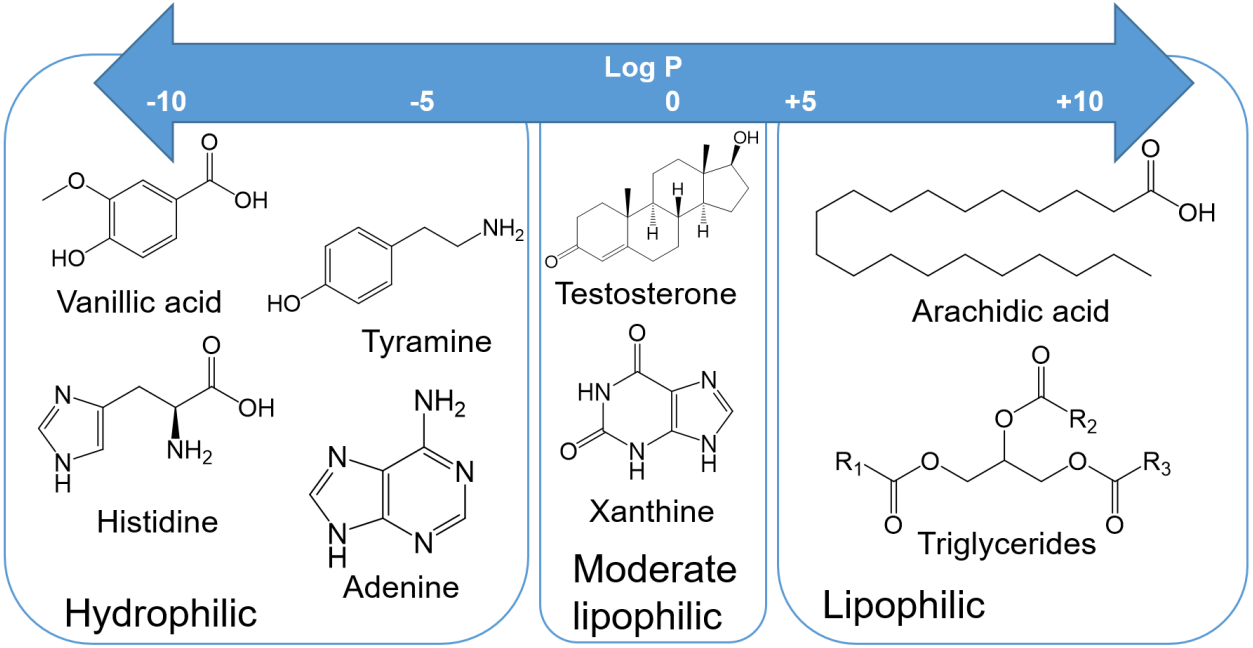
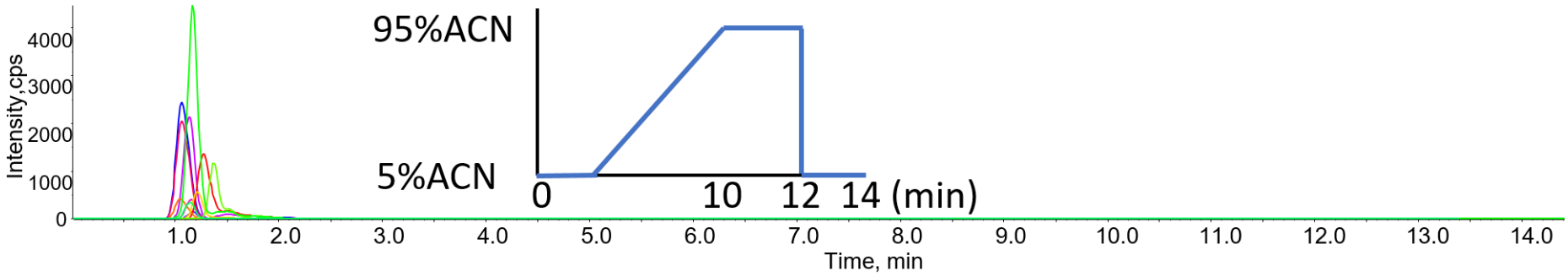
Liquid Chromatography-Mass Spectrometry (LC-MS) for polar metabolites

Human Metabolome Database (HMDB) contains 114,098 metabolite entries



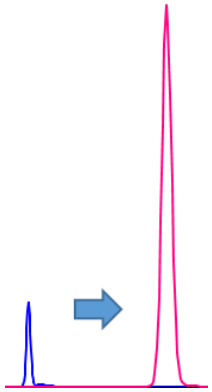
LC-MS analysis of polar metabolites



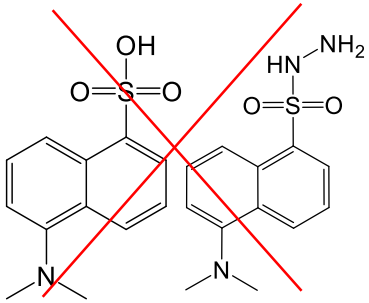
Benefits and constraints of chemical derivatization in LC-MS

Chemical Derivatization

- Enhanced retention time in LC
- Improved MS response factor
- Lower limit of detection and quantification
- Signature fragments for QUAL/QUANT



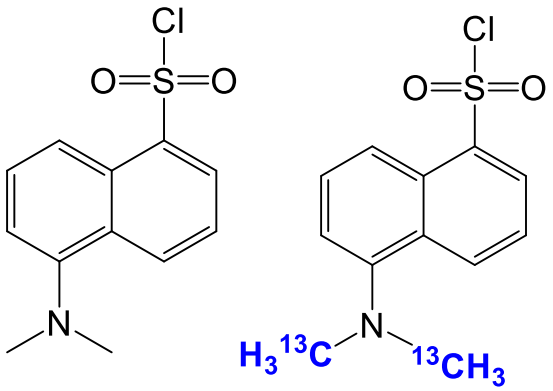
Drawbacks



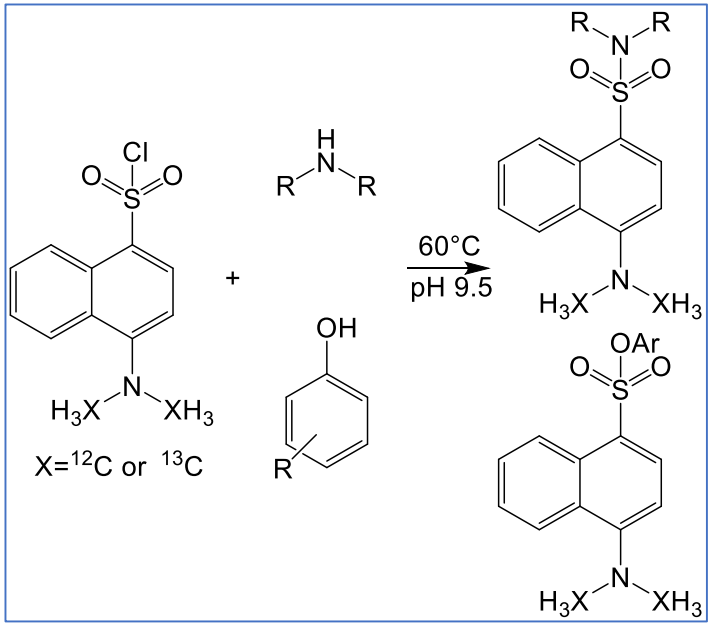
- Excess of derivatization agent
- Side reaction products
- Reaction kinetic is analyte dependent
- No single derivatization reagent for multifunctional compounds

Derivatization chemistry

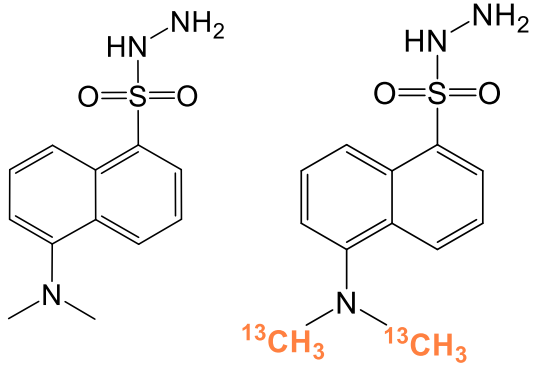
Dansyl chloride



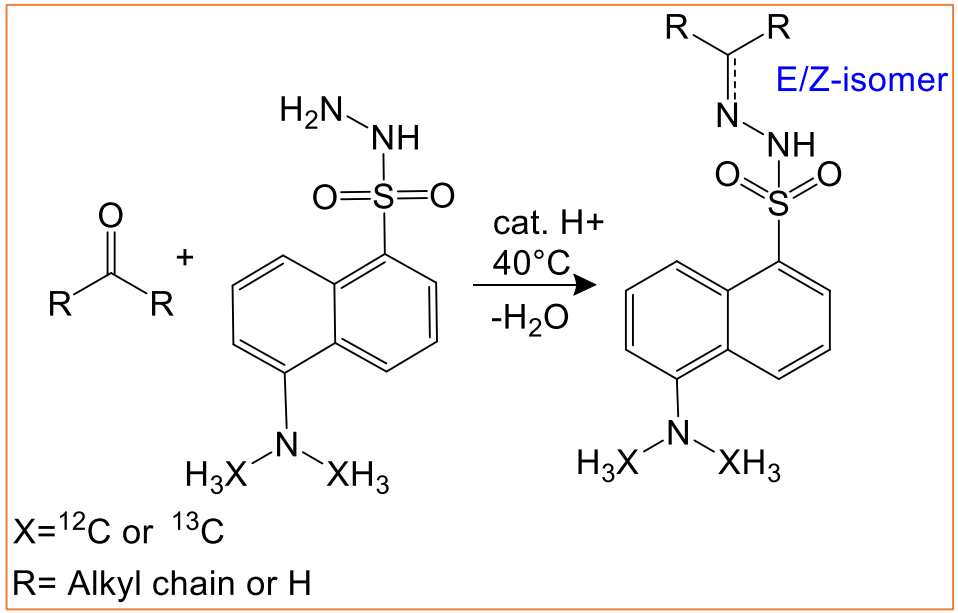
Dansylation reaction



Dansylhydrazine

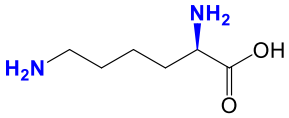
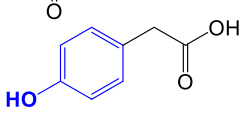
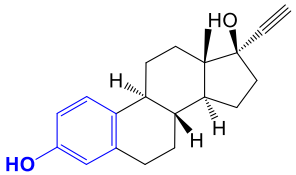
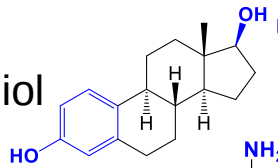
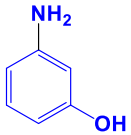
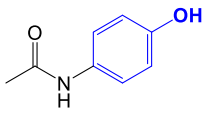


Hydrazone formation

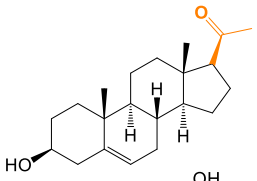
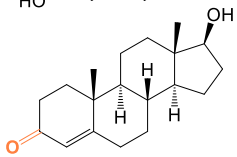
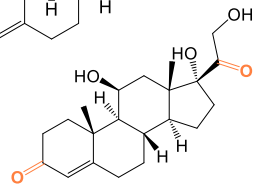
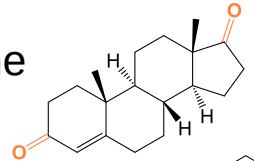
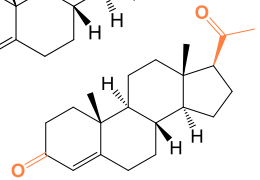


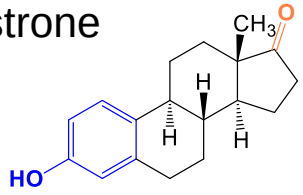
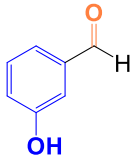
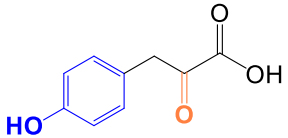
Model compounds

Phenols and amines

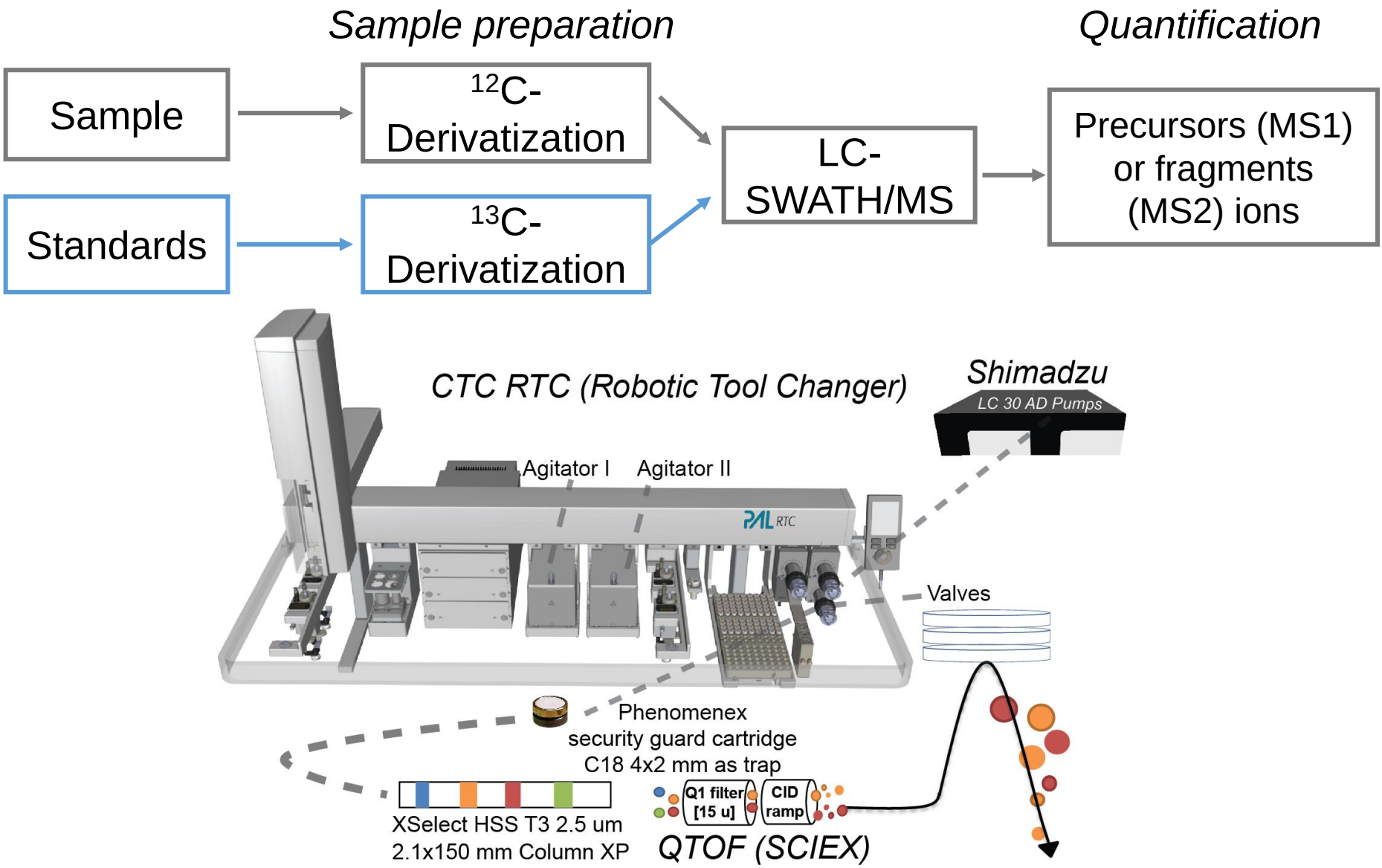
- L-Lysine 
- 4-phenylacetic acid 
- Ethinyl estradiol 
- Estradiol 
- 3-aminophenol 
- Acetaminophen 

Aldehydes and ketones

- Pregnenolone 
- Testosterone 
- Hydrocortisone 
- Androstenedione 
- Progesterone 

- Estrone 
- 3-Hydroxybenzaldehyde 
- 4-Hydroxyphenylpyruvic acid 

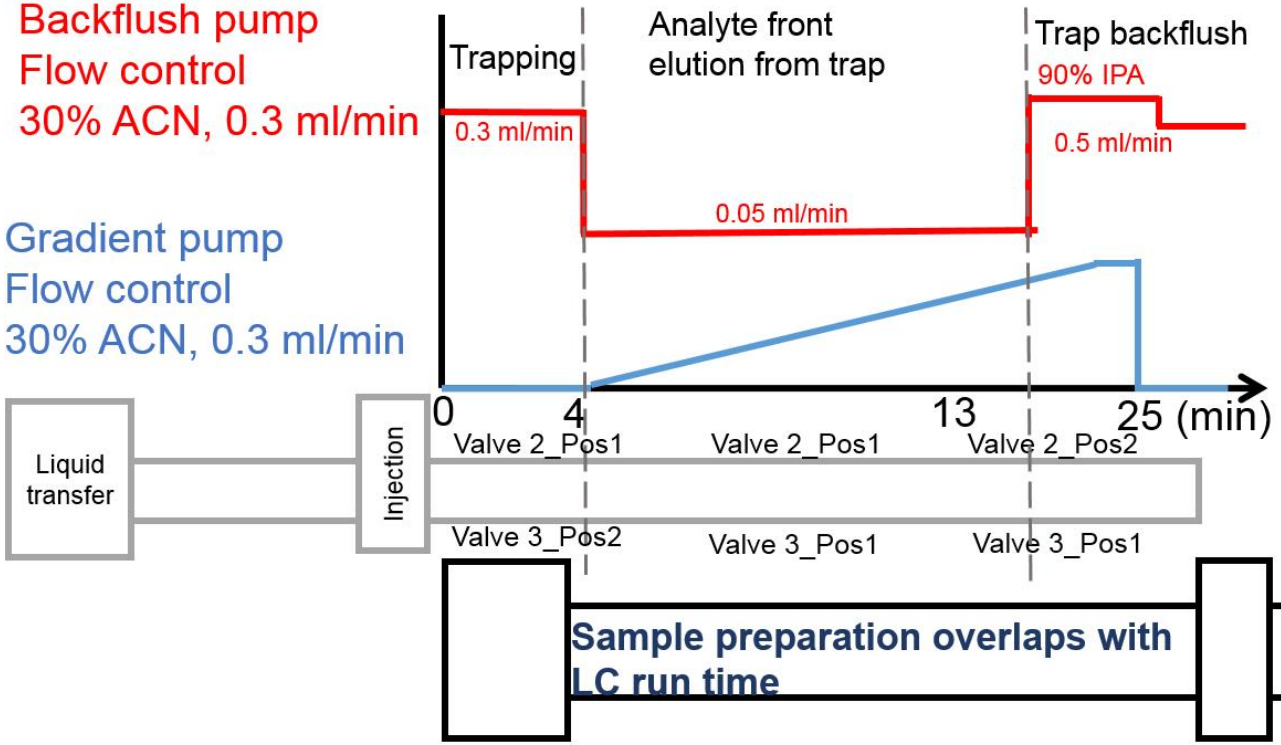
Automated Parallel derivatization using Dansyl-Cl and Dansyl-Hz

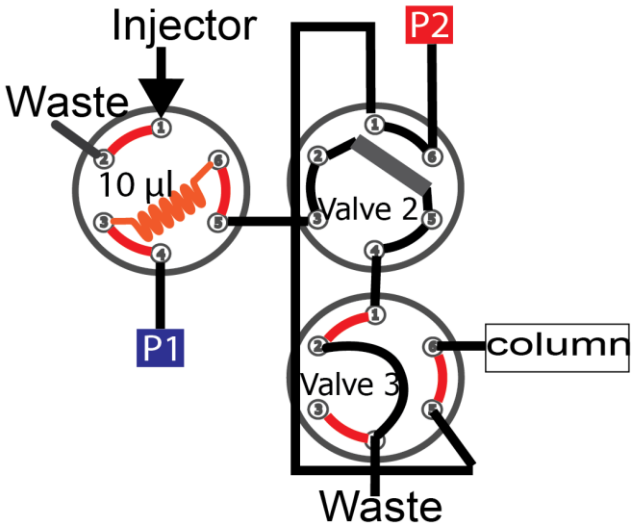


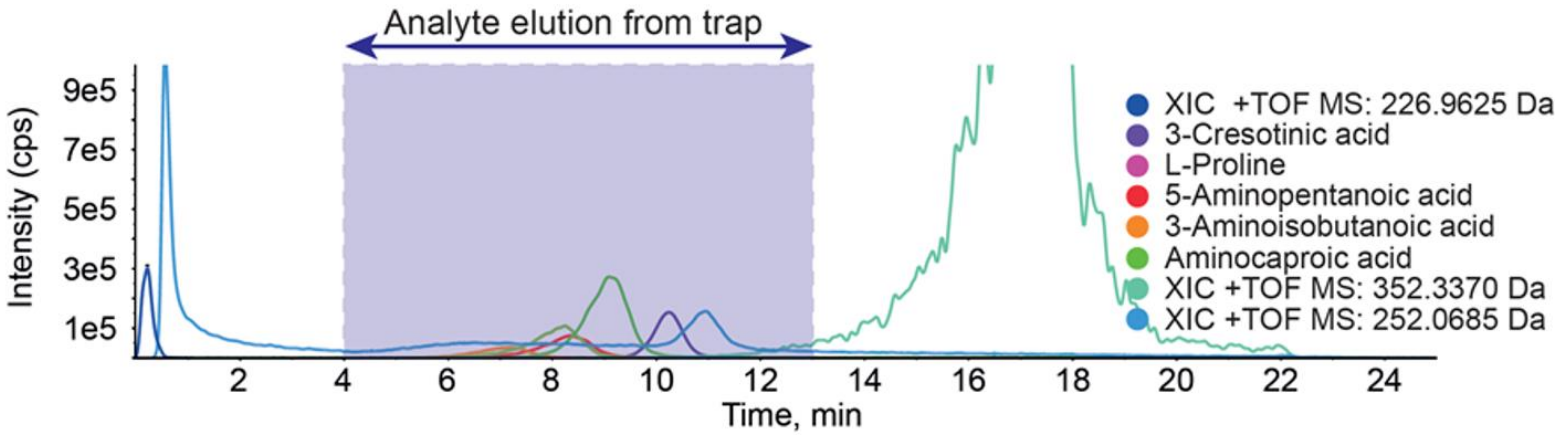
Dual column switching setup

Backflush pump
Flow control
30% ACN, 0.3 ml/min

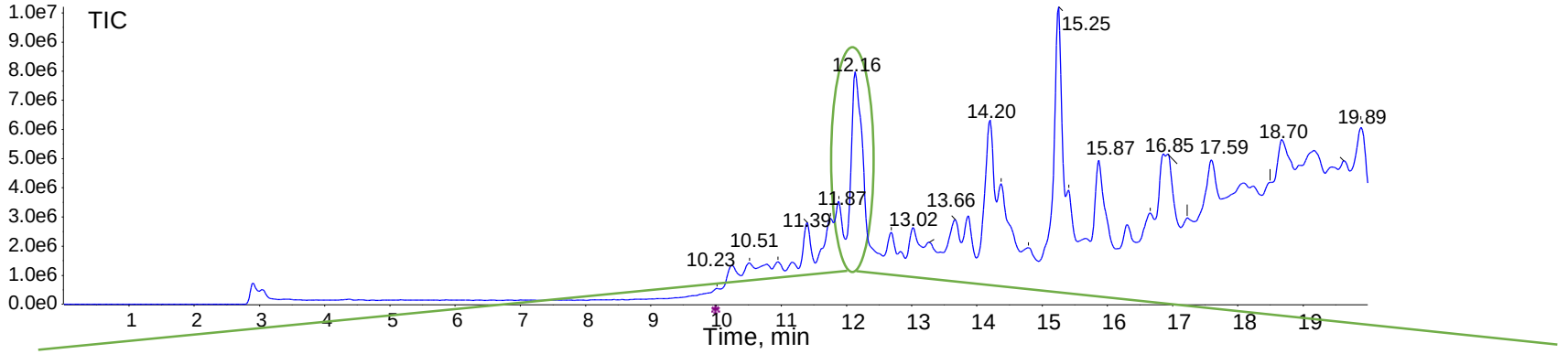
Gradient pump
Flow control
30% ACN, 0.3 ml/min

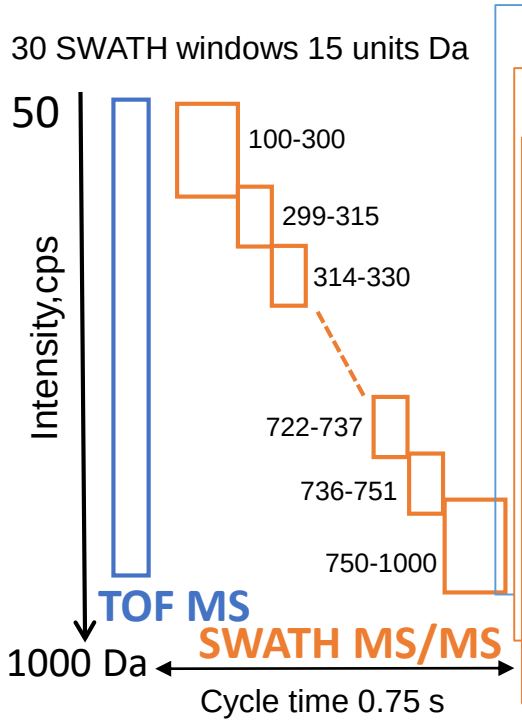


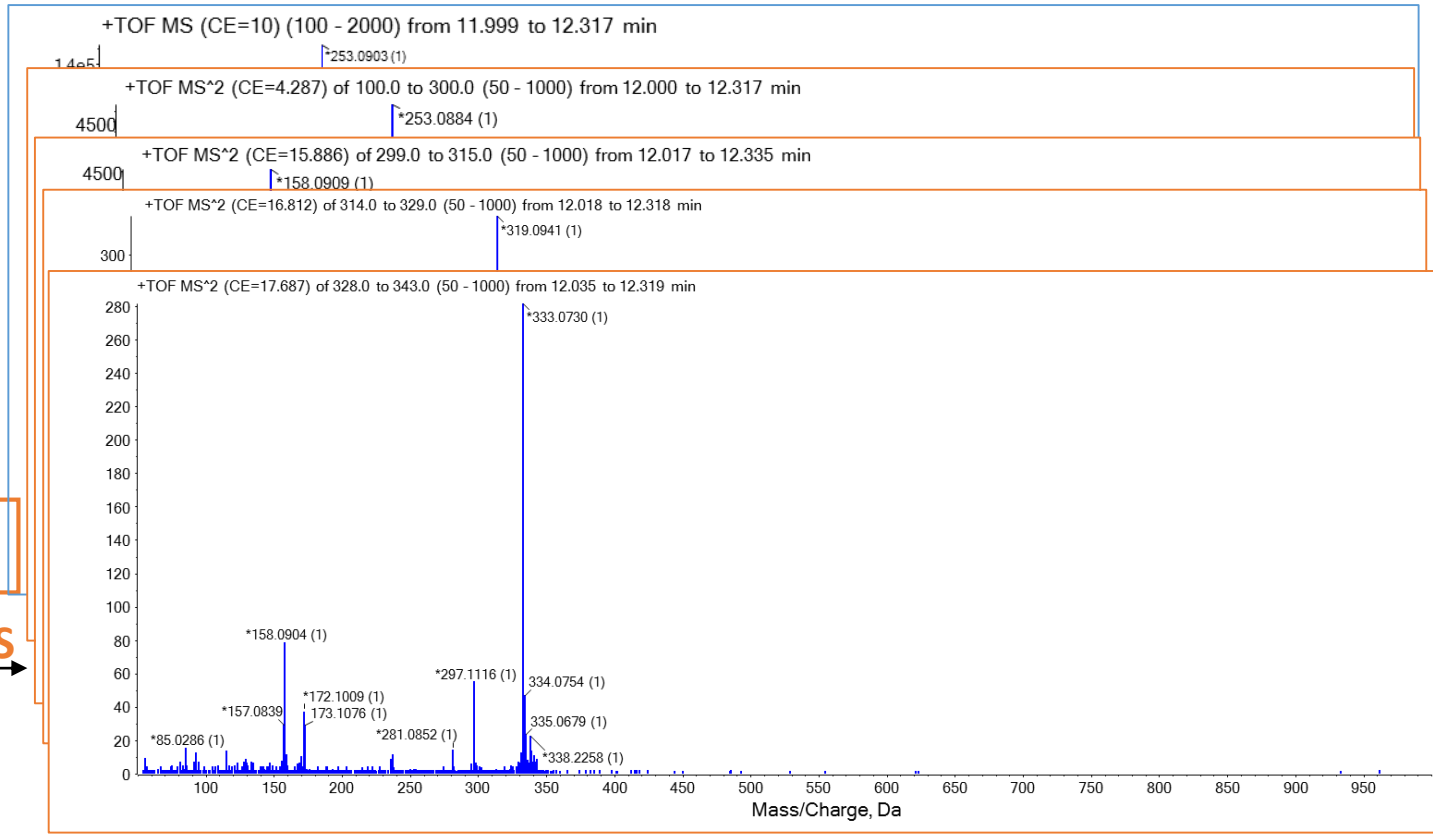




SWATH-MS, Data Independent acquisition

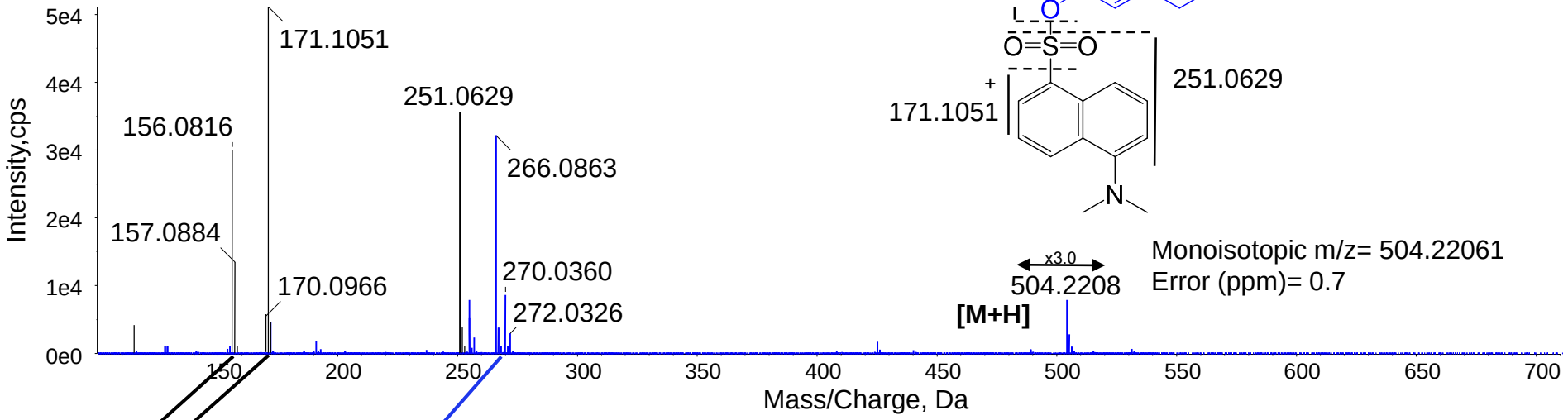




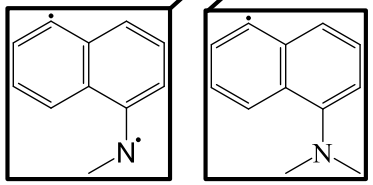


Fragmentation pattern in ESI-SWATH/MS

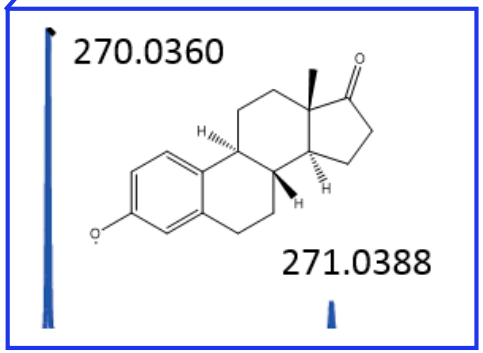
SWATH Q1 window 497.0 to 512.0 Da



Monoisotopic m/z= 504.22061
Error (ppm)= 0.7

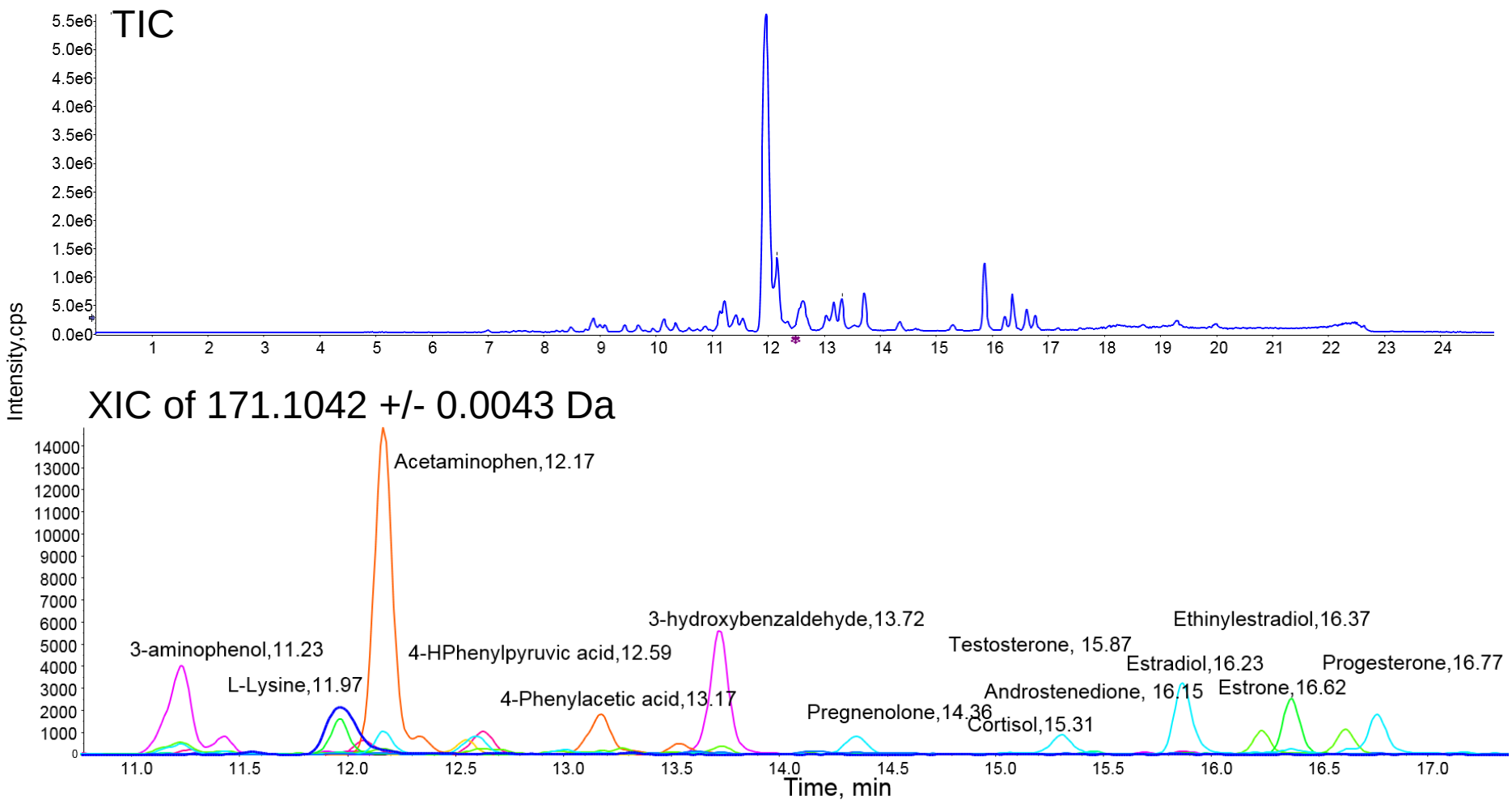


Tag fragments ions

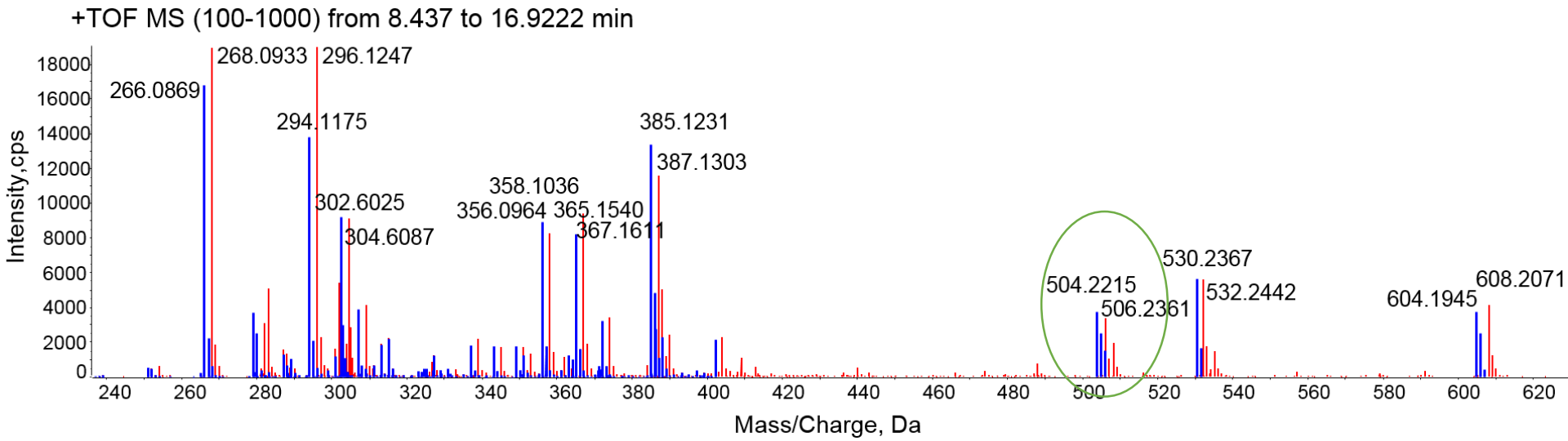


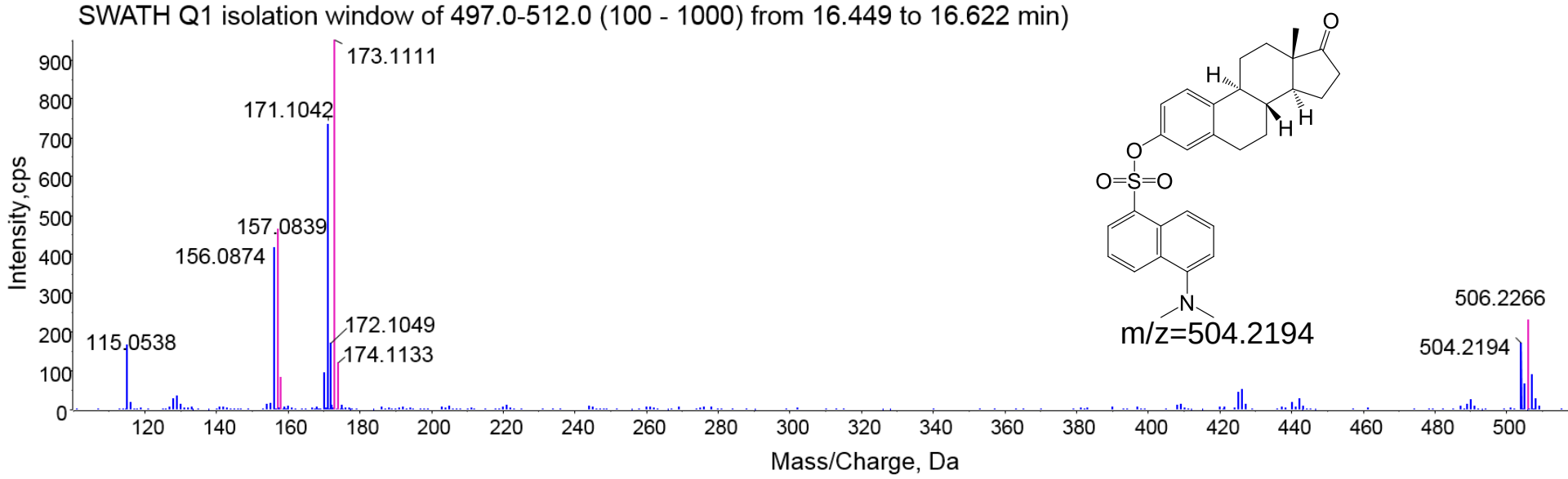
Analyte selective fragment

SWATH acquisition combined with derivatization strategy

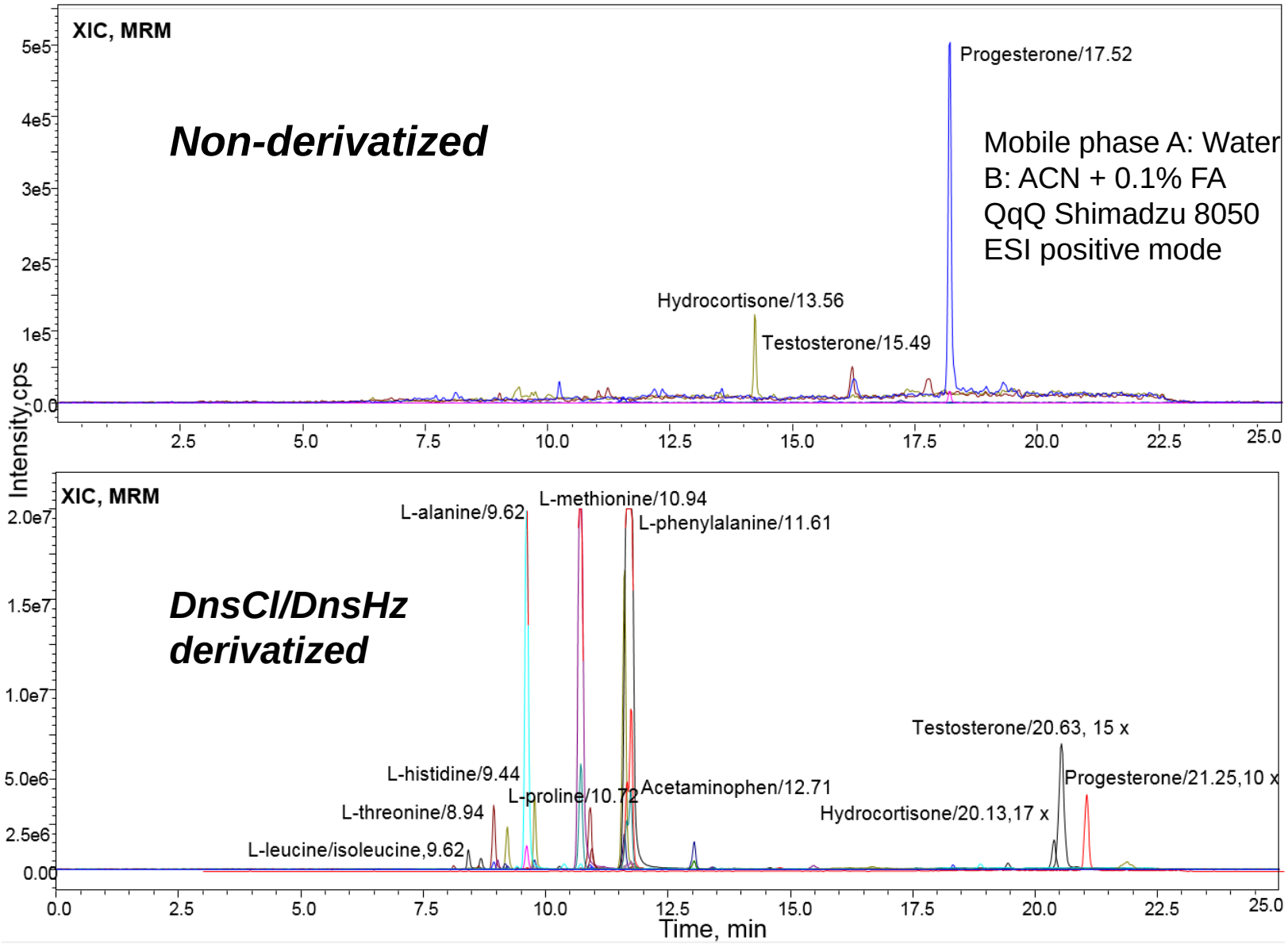


Differential Isotope Labeling Strategy (DIL), $^{12}C/^{13}C_2$-DnsCl/DnsHz





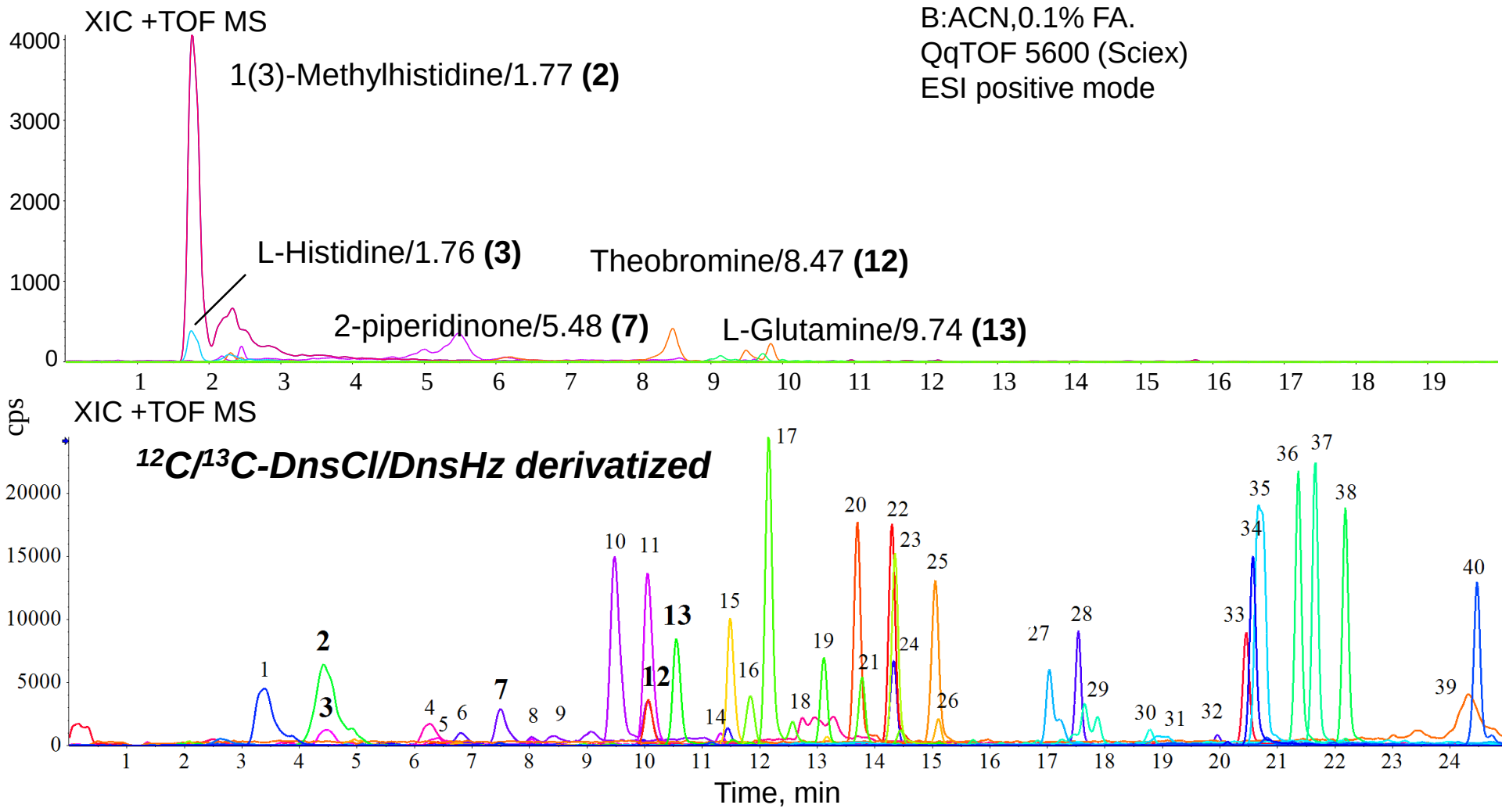
Application to plasma samples for QUAL/QUANT analysis



Application to urine samples for QUAL/QUANT analysis

Non-derivatized

Mobile phase A: H_2O,0.1 FA
B:ACN,0.1% FA.
QqTOF 5600 (Sciex)
ESI positive mode



CONCLUSIONS

Automated parallel derivatization combined with SWATH-MS acquisition enables:

- To profile multifunctional metabolites (aldehydes, ketones, amines and phenols) during a single LC run time.
- QUAL/QUANT analysis of metabolites in biological samples using analogous C^{13} labeled derivatization reagents.
- To generate valuable additional sample information of precursor, fragment ions and chemically related analytes within same batch.
- To facilitate post-acquisition identification of metabolites chemically related to tag fragment ions.

Acknowledgements

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PAL SYSTEM
Ingenious sample handling

AB SCIEX

SHIMADZU
Excellence in Science