Quantification of Polar Metabolites in Urine using Automated Parallel Derivatization and LC-SWATH/MS

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Introduction

Chemical derivatization has been used to improve MS sensitivity and LC retention time for metabolite profiling. However, for metabolite quantification, where a large number of different metabolites are analyzed, the number of commercially isotopically labelled internal standards is very limited. In addition, there is no single workflow which can provide large-scale metabolomics coverage in particular for polar metabolites. To overcome these limitations and to enable QUAL/QUANT analysis we describe an automated parallel derivatization approach based on two derivatization procedures followed by LC-MS analysis using data independent acquisition (SWATH/MS). Quantification is achieved with the use of ^{13}C labelled derivatization reagents.

Methods

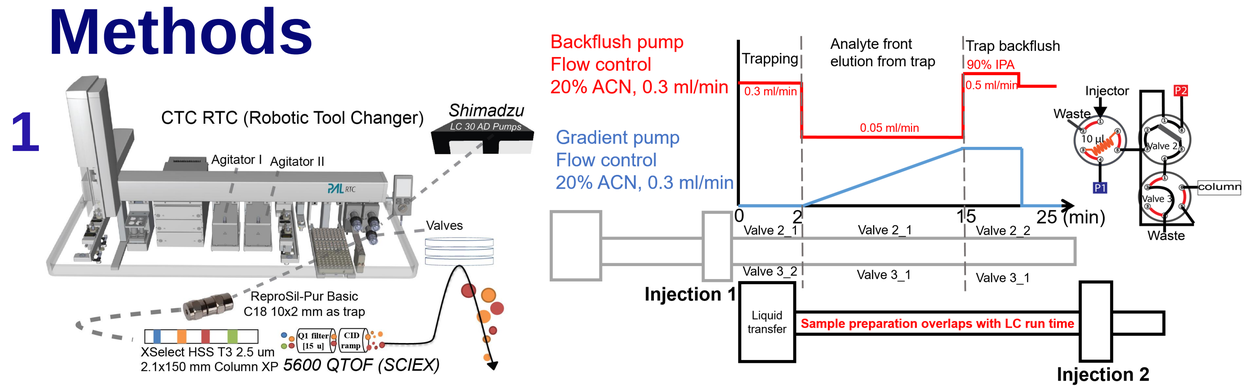

Figure 1. Schematic representation of RTC autosampler (CTC Analytics) with hyphenation to two LPG pumps (Shimadzu) and MS TTOF 5600 instrument (Sciex) used for the automated parallel derivatization in combination with a column switching setup which overlap with LC run time.

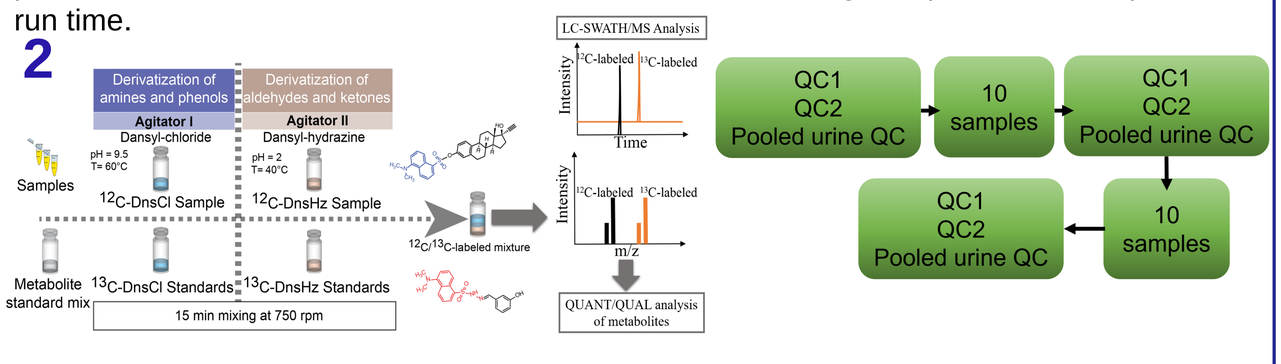

Figure 2. Workflow of the parallel derivatization samples with Dansyl-Cl/Dansyl-Hz and the 40 metabolite standard mix with ($^{13}C_2$) labelled Dansyl-Cl/ Dansyl-Hz for quantification. The analysis sequence of the urine samples from prostate cancer patients is given on the right.

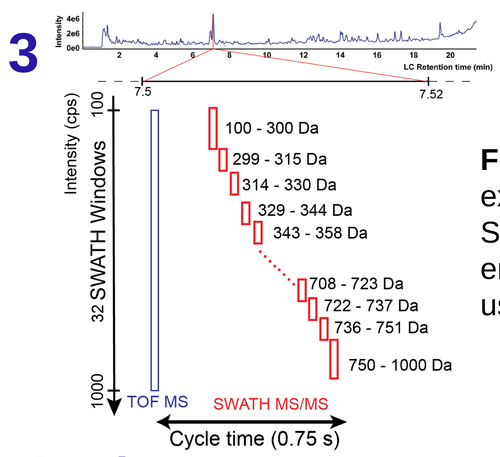

Figure 3. SWATH DIA method including a TOF MS experiment from 100-1000 Da in addition to 32 Q1 SWATH MS windows of 15 units size. The collision energy of 40 eV with an energy spread of 30 eV was used.

Analytes

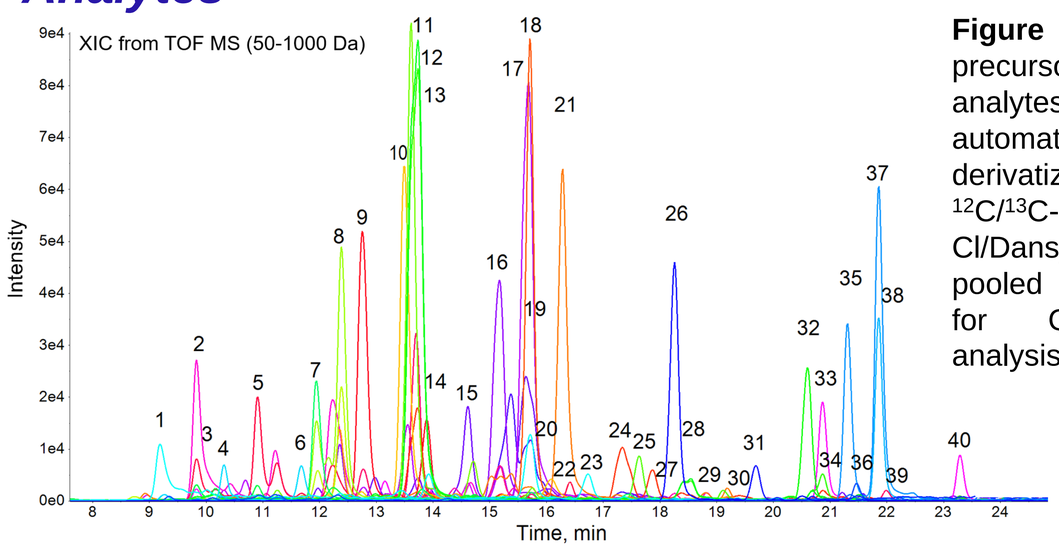

Figure 4. XIC of precursor ions of 40 analytes after automated parallel derivatization using $^{12}C/^{13}C$-Dansyl-Cl/Dansyl-Hz of a pooled urine sample for QUAL/QUANT analysis.

No	Compound	MW (g/mol)	Derivative	m/z derivative	No	Compound	MW (g/mol)	Derivative	m/z derivative
1	L-Glycine	75.07	DNS-Cl	309.0901	21	L-histidine	155.20	DNS-Cl	389.1278
2	Pyruvic acid	88.06	DNS-Hz	336.1011	22	L-arginine	174.20	DNS-Cl	408.1940
3	L-Alanine	89.09	DNS-Cl	323.1063	23	Oxoglutaric acid	147.02	DNS-Hz	394.1071
4	L-Tyrosine	181.2	DNS-Cl	415.1313	24	L-Glutamic acid	147.10	DNS-Cl	381.1108
5	2-aminobutyric acid	103.06	DNS-Cl	337.1211	25	Taurine	125.01	DNS-Cl	359.0731
6	L-valine	117.2	DNS-Cl	351.1377	26	Caffeic acid	180.04	DNS-Cl	648.1812
7	L-threonine	119.1	DNS-Cl	353.1165	27	L-methionine	149.20	DNS-Cl	383.1091
8	L-tryptophan	204.23	DNS-Cl	438.1475	28	L-serine	105.10	DNS-Cl	339.1006
9	L-phenylalanine	165.2	DNS-Cl	399.1369	29	Citrulline	175.10	DNS-Cl	409.1541
10	S-phenyl-L-cysteine	197.05	DNS-Cl	431.1094	30	L-Aspartic acid	133.10	DNS-Cl	367.1216
11	5-aminolevulinic acid	131.06	DNS-Hz	365.1530	31	L-proline	115.10	DNS-Cl	349.1219
12	Trans-4-hydroxy-L-proline	131.06	DNS-Cl	365.1166	32	Tyramine	137.18	DNS-Cl	604.1914
13	L-asparagine	132.05	DNS-Cl	366.1120	33	α-Ketoisovaleric acid	116.05	DNS-Hz	364.1325
14	L-carnosine	226.23	DNS-Cl	460.1653	34	Daidzein	254.06	DNS-Cl	721.0873
15	Acetaminophen	151.17	DNS-Cl	385.1217	35	Estradiol	272.38	DNS-Cl	506.2756
16	2,4-Dihydroxybenzoic acid	154.03	DNS-Cl	388.0849	36	Ethinyl estradiol	296.41	DNS-Cl	530.3666
17	4-hydroxybenzoic acid	138.03	DNS-Cl	372.0898	37	Estrone	270.37	DNS-Cl	504.2302
18	L-lysine	146.20	DNS-Cl	613.2125	38	Testosterone	288.21	DNS-Hz	535.2294
19	Vanillic acid	168.04	DNS-Cl	402.0999	39	L-Cystine	240.30	DNS-Cl	474.1132
20	4-hydroxyphenylpyruvic acid	180.16	DNS-Hz	414.1006	40	Androstenedione	286.19	DNS-Hz	781.5202

Results

Method performance

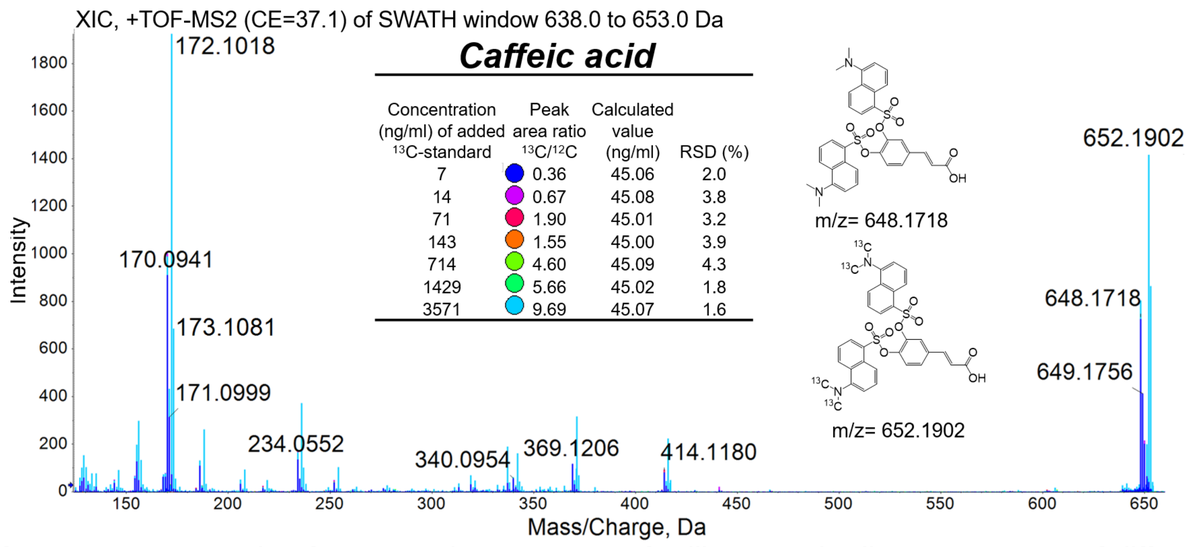

Figure 5. An example of HR-SRM/MS spectrum for illustrate the linear response of different endogenous metabolites in urine to the addition of increasing concentration of ^{13}C-standards metabolites for further quantification using single point calibration at MS1 or MS2 level.

Analysis of Clinical samples

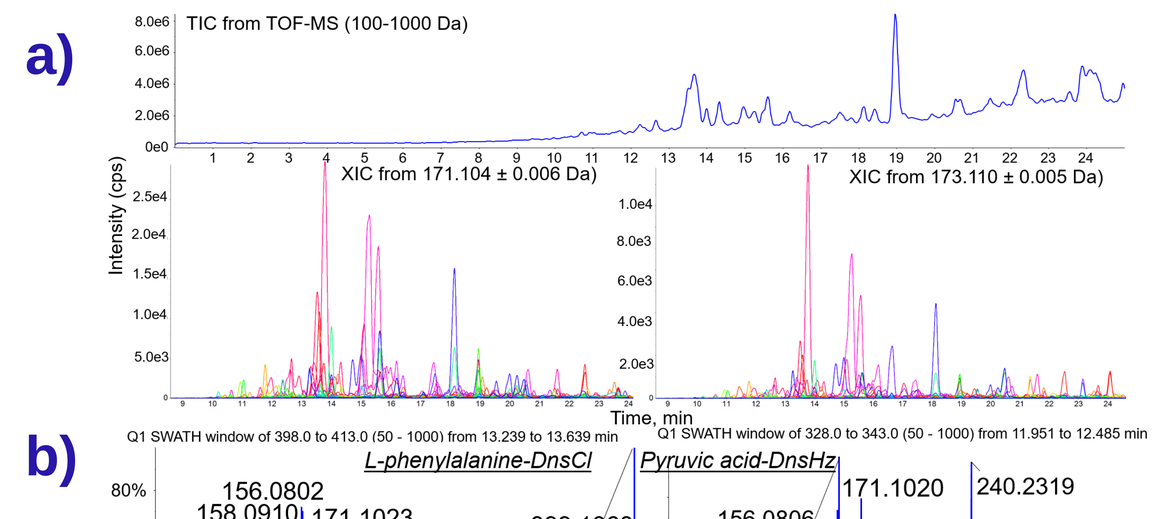

Figure 6. Derivatized urine sample from prostate cancer patients using $^{12}C/^{13}C$-Dansyl-Cl/Dansyl-Hz. (a)TIC and overlayed XICs from all SWATH Q1 windows of tag-fragment *m/z* 171 (left) and *m/z* 173 (right) that represent common fragments of derivatized metabolites belong to same chemical group. (b) HR-SRM/MS spectrum of derivatized L-phenylalanine and pyruvic acid identified and quantified in the sample using differential isotope labelling $^{12}C/^{13}C$ of precursor ions (MS1 level) or fragments ions (MS2 level).

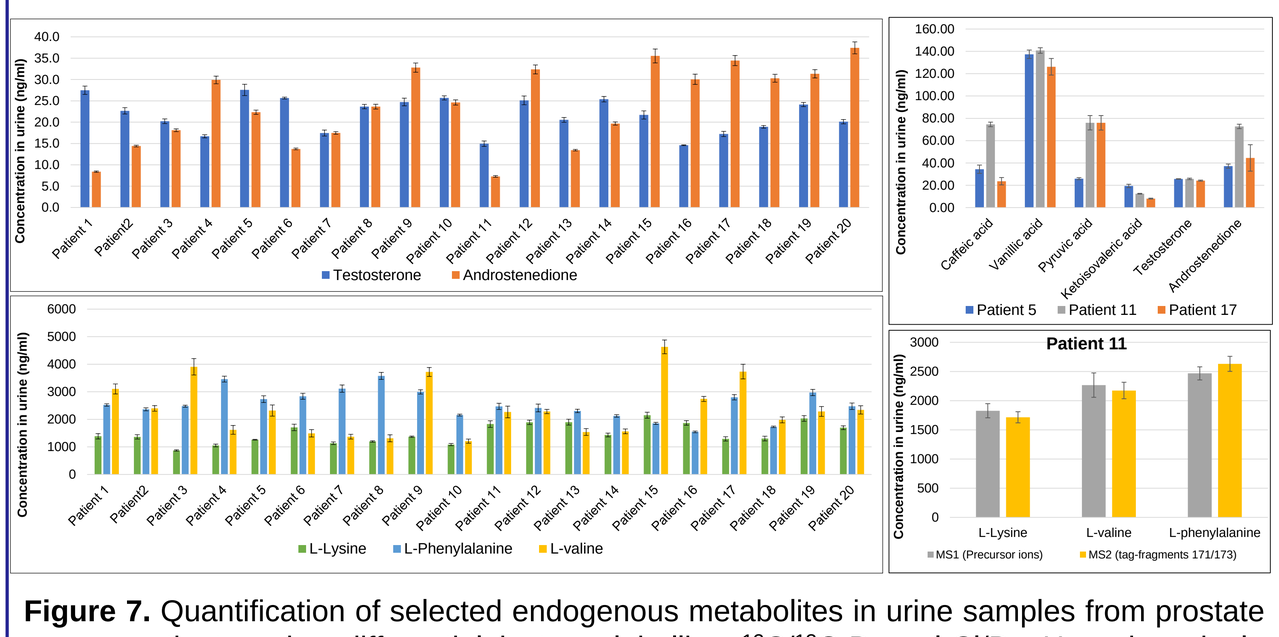

Figure 7. Quantification of selected endogenous metabolites in urine samples from prostate cancer patients using differential isotope labelling $^{12}C/^{13}C$-Dansyl-Cl/DnsHz using single point calibration and quantified at MS1 or MS2 level.

Conclusions

Automated parallel derivatization combined with SWATH/MS acquisition enables:

Reproducible quantification of multifunctional metabolites (aldehydes, ketones, amines and phenols) in a single LC-MS analysis.

QUAL/QUANT analysis of metabolites in biological samples using analogous ^{13}C labeled derivatization reagents and MS1 and MS2 level.

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 with the help of tag fragment ions.

References

1. Guo, K. and L. Li, Anal. Chem., 2009. **81**(10): p. 3919-3932.
2. Zhao, S., et al., Anal. Chem., 2017. **89**(12): p. 6758-6765.