Evaluation and optimization of rapid DDA and DIA screening methods for yeast sub-metabolome analysis on a high-resolution IM-Q-TOF mass spectrometer

Teresa Mairinger[1], Ruwan Kurulugama[2], Tim J. Causon[1], H. Drexler[1], George Stafford[2], John Fjeldsted[2], Stephan Hann[1]

[1]Department of Chemistry, University of Natural Resources and Life Sciences - BOKU Vienna, Muthgasse 18, 1190 Vienna, Austria
[2]Agilent Technologies , Santa Clara, CA

Overview

- We are aiming at the significant enhancement of analytical selectivity in non-targeted analysis by combining dansylation of metabolites and rapid UHPLC with **prototype quadrupole broad band isolation directed by ion mobility drift separation**.
- The optimized conditions were applied to **extracts of *Pichia Pastoris*** and the method was validated via standard addition of a multi metabolite mixture.
- Figures of merit **regarding linearity and precision of mass, precursor and fragment drift time as well as precursor/fragment ratios** were determined for selected compounds.

Introduction

The study of metabolic activities on a cellular level is key to successful optimization in biotechnological processes. The wide chemical diversity and highly complex matrices inherent to this area are still posing a challenge to current analytical approaches for metabolite screening. Even if dedicated front-end separation techniques combined with high resolution MS set the benchmark from an analytical point of view, the increasing number of samples and sample complexity demand for a compromise in terms of selectivity, sensitivity and high-throughput analyses. To tackle this challenge, different approaches involving a rapid chromatographic separation combined with drift tube ion mobility separation with HR-MS detection are tested for dansylated metabolites in yeast cell extracts.

Methods

Chemostat or Batch → Sampling + Quenching → Boiling EtOH extraction of metabolites → Derivatization *via* Dansylation → Analysis of metabolites *via* UHPLC-IM-QTOFMS

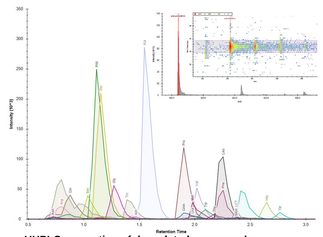
Derivatization

- 100 µL Sample + 100 µL buffer (0.5 M Na_2CO_3/ $NaHCO_3$, pH 9.53)
- Addition of 100 µL DNS-Cl (20 mg mL^{-1} in 90:10 ACN:Acetone)
- Agitation for 1 h @ 60 °C
- Removal of excess reagent via PSA
- Agitation for 15 h @ 60 °C

UHPLC

Column	Acquity UPLC BEH C18, 1.7 µm, 1.0 x 50 mm
Eluents	A: H_2O+ 0.1% FA, B: MeOH + 0.1% FA
Temperature	40 °C
Injection Vol	1 µL partial loop (5 µL)
flow:	300 µL min^{-1}

Gradient [min]	A /%	B /%	Flow /mL min^{-1}
0	70	30	0.3
0.5	70	30	0.3
3	0	100	0.3
3.9	0	100	0.3
4	70	30	0.3
5	70	30	0.3



UHPLC separation of dansylated compounds

DDA QTOF

Auto-MS/MS mode was employed for generation of fragment spectra of dansylated compounds

ESI source conditions

Gas Temperature	225 °C
Drying Gas	13 L min^{-1}
Nebulizer	40 Psig
Sheath Gas Temp	350 °C
Sheath Gas Flow	12 L min^{-1}
Vcap	4000 V
Nozzle	500 V
Fragmentor	400 V
Oct 1 RF Vpp	750 V

IMS Settings

Total drift time	60 ms
Trapping time	10 ms
Trap release time	300 µs
Drift Tube Entrance	1574 V
Drift Tube Exit	224 V
Rear Funnel Entrance	217.5 V
Rear Funnel Exit	45 V
IM Transients per frame	16
Cycle time	0.96 s

DIA IM-all ions

0 and ramped CE was applied in alternating ion mobility frames

Frame 1
No fragmentation
0 V CID Energy

Frame 2
With drift time directed ion fragmentation
Ramped CID Energy

Drift time directed ramps

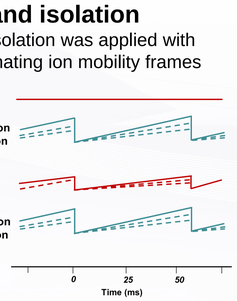
Drift time /ms	18	22	25	29	33	37	41	45	49	53
Collision energy ramp /V	16	18	19	21	22	24	25	26	28	29
Quad ramp (isolation with 70 u)	130	270	410	560	710	850	990	1130	1280	1420

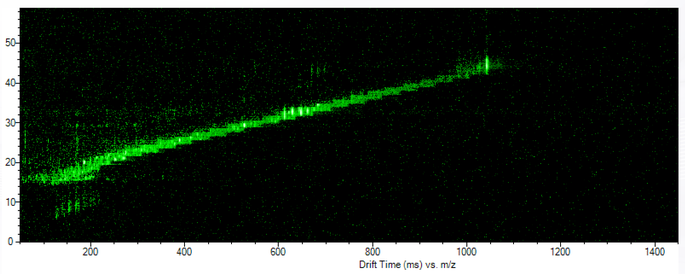
DIA IM-Q-broad band isolation

Quadrupole broad band isolation was applied with 0 and ramped CE in alternating ion mobility frames

Frame 1
Quadrupole broad band isolation without fragmentation
0 V CID Energy
Quad Isolation Mass Position

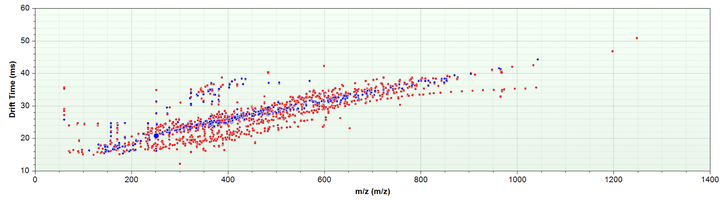
Frame 2
Quadrupole broad band isolation with drift time directed ion fragmentation
Ramped CID Energy
Quad Isolation Mass Position



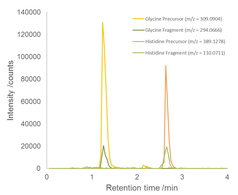


Drift time vs. m/z trend line for dansylated compounds

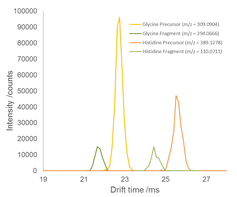
Results



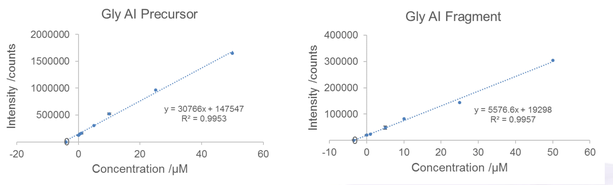
Molecular features detected in all ion mode (red) vs. drift time directed quadrupole isolation (blue) in ethanolic extract of the yeast *Pichia Pastoris*



Accurate mass and drift time extracted ion current of derivatized glycine and histidine precursor and fragment ions



Drift spectra of derivatized glycine and histidine precursor and fragment ions



	Glycine precursor ion				Glycine fragment ion					Histidine precursor ion				Histidine fragment ion				
	Number of transients	Drift time	*m/z*	Peak Area	Number of transients	Drift time	*m/z*	Peak Area	Ratio Fragment/ Precursor	Number of transients	Drift time	*m/z*	Peak Area	Number of transients	Drift time	*m/z*	Peak Area	Ratio Fragment/ Precursor
Sample		ms		counts*min		ms		counts*min	%		ms		counts*min		ms		counts*min	%
BBI_07_1µM_a_in_Pp	272	22.75	309.0898	145101	272	21.54	294.0664	6165	4.25	306	25.57	389.1267	56349	306	24.55	110.0715	24529	44
BBI_08_1µM_b_in_Pp	272	22.75	309.0969	140508	272	21.54	294.0740	5856	4.17	306	25.02	389.1349	52225	306	24.55	110.0738	29679	57
BBI_09_1µM_c_in_Pp	272	22.75	309.0894	143712	272	21.54	294.0670	6314	4.39	306	25.62	389.1264	51459	306	24.55	110.0712	29351	57
BBI_10_1µM_d_in_Pp	272	22.75	309.0899	140700	272	21.54	294.0652	6809	4.84	306	25.71	389.1266	55009	306	24.55	110.0710	25430	46
BBI_11_1µM_e_in_Pp	272	22.77	309.0900	142140	272	21.55	294.0666	5217	3.67	306	25.52	389.1265	53406	306	24.55	110.0711	27826	52
BBI_12_1µM_f_in_Pp	272	22.75	309.0896	137265	272	21.54	294.0662	4686	3.41	306	25.58	389.1275	47066	306	24.55	110.0712	25722	55
AVG		**22.75**	**309.0909**	**141571**		**21.54**	**294.0676**	**5841**	**4.12**		**25.50**	**389.1281**	**52585**		**24.55**	**110.0716**	**27089**	**52**
STD		**0.008**	**0.0029**	**2750**		**0.004**	**0.0032**	**773**	**0.51**		**0.245**	**0.0034**	**3243**		**0.000**	**0.0011**	**2170**	**5.7**
RSD /%		**0.04**		**1.9**		**0.02**		**13**	**12**		**0.96**		**6.2**		**0.00**		**8.0**	**11**

Analytical figures of merit of drift time directed quadrupole isolation obtained with a prototype broad band driver. Precision was calculated for 6 independent samples from ethanolic extracts of *Pichia Pastoris* spiked with 1 µM metabolite mixture.

Conclusions

- Drift time directed broad band isolation allows the determination of small metabolites over several orders of magnitude. In comparison to all ions mode we observe less artefacts, higher linearity and a larger working range.
- Fragment/precursor ratios obtained via alternating frames with quadrupole and collision energy ramping are stable over the working range. Evaluation of fragment intensities even extends the working range and is promising in the context of fold change analysis in non-targeted approaches.
- The combination of retention time, accurate mass of precursor and fragment ions and their respective CCS will lead to highest confidence in identity confirmation of unknown compounds.

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