Optimisation of LC-MS conditions for a metabolomic approach

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1. Introduction

Metabolomics is a rapidly growing area in the 'omics'-science. It endeavours to reliably separate and detect as many metabolites as possible in a single analysis. To this goal, we are developing an LC-MS tool to quantify metabolites in a relative way. Arabidopsis thaliana metabolites of different classes were chosen as representative compounds for evaluation of several chromatographic columns.

2. Aim

The main purpose was to create an lc method that has a high separation efficiency within an acceptable time. This means that the metabolites must be unravelled as much as possible in a single chromatographic run.

3. Materials

HPLC Conditions

CapLC Pump and Autosampler (Waters)
Columns:
- Inertsil® ODS-3 C18, 5µm, 150 x 1.0mm (LC Packings)
- Atlantis™ dC18, 3µm, 150 x 1.0mm (Waters)
- Zorbax® XDB C18, 3.5µm, 150 x 0.5mm (Agilent)

Mobile Phase:
- Eluent A: 100% water + 0.1% formic acid
- Eluent B: 90/10 acetonitrile/water + 0.1% formic acid

Flow rate: 40µl/min; 20µl/min (Zorbax XDB)
Injection volume: 10µl
Column temperature: 45°C

MS Conditions:

Mass Spectrometer: Q-TOF micro (Waters)
Ion Source: Lockspray™ in both positive and negative ion mode
Software: Masslynx 4.0
m/z-range: 50-1000
Capillary Voltage: 2500V
Cone Voltage: 30V

Metabolites:

Amino acids, carbohydrates, nucleotides, plant hormones, steroids, fatty acids, polyamines, carotenoids and others were used from stock solutions of 1mg/ml

Gradient profile: (**optimised!**)

4. Methods

▪In terms of metabolites, one of the problems one has to deal with is the predominantly polar character of the compounds. In view of the intended LC separation, we chose for a reversed phase chromatography which provides high separation efficiency based upon a stable and reproducible stationary phase.

▪A comparison between Atlantis dC18 (specialty reversed phase type material particularly suited for the retention of highly polar compounds), Inertsil ODS-3 and Zorbax XDB (modern types of reversed phase material, not specifically introduced for the retention of polar compounds) was set up.

▪Columns were evaluated by using a number of eluents and gradients, comparing the separation efficiency. Gradient elution was performed starting with a 100 percent aqueous phase as eluent A up to a 90/10 acetonitrile/water mixture (eluent B). Formic acid (0.1%) was chosen as mobile phase additive from a chromatographic as well as a mass spectrometric point of view. Thus it was possible to acquire samples in both positive and negative ion mode without changing eluents.

▪Optimal Q-TOF parameters were investigated for each compound, both in positive and negative ion mode, resulting in a consensus of acquisition parameters for all metabolites available.

5. Results

		Zorbax XDB				Inertsil ODS-3				Atlantis dC18			
component	log D (pH3)	mean t_R	mean $t_{R\ rel.}$	CV%	k'	mean t_R	mean $t_{R\ rel.}$	CV%	k'	mean t_R	mean $t_{R\ rel.}$	CV%	k'
lysine	-5.34	3.67	0.10	29.57	0.03	2.91	0.36	8.50	0.14	3.45	0.49	0.00	0.16
leucine	-1.87	5.28	1.71	1.35	0.48	7.00	4.45	1.43	1.74	5.89	2.93	0.59	0.99
glutamaat	-3.19	3.82	0.25	16.88	0.07	3.34	0.78	1.48	0.31	3.71	0.75	3.07	0.25
serine	-3.58	3.70	0.13	43.95	0.04	3.22	0.66	1.74	0.26	3.65	0.68	1.69	0.23
glutamylcysteine	-3.72	4.49	0.92	4.35	0.26	5.60	3.05	1.15	1.19	4.88	1.91	1.09	0.65
s-adenosylmethionine	----	3.88	0.31	16.41	0.09	3.10	0.55	3.66	0.21	3.65	0.68	3.37	0.23
lactose	-5.18	3.79	0.22	15.75	0.06	3.55	1.00	4.51	0.39	3.83	0.87	1.15	0.29
galactose	-3.13	3.77	0.20	17.32	0.06	3.33	0.78	0.00	0.30	3.54	0.57	4.38	0.19
xylulose	-2.80	6.02	2.45	3.40	0.69	6.88	4.33	2.18	1.70	7.20	4.24	0.82	1.43
glucose-6-fosfaat	-6.08	----	----	----	----	----	----	----	----	----	----	----	----
maltotriose	-7.24	----	----	----	----	3.88	1.33	0.00	0.52	4.13	1.16	2.48	0.39
ADP-glucose	-7.41	4.17	0.60	10.66	0.17	7.01	4.46	0.81	1.74	5.44	2.48	0.93	0.84
xanthine	-0.84	4.79	1.22	2.96	0.34	6.99	4.44	1.63	1.74	7.17	4.21	1.37	1.42
adenine	-0.61	4.16	0.59	12.22	0.17	3.97	1.42	1.41	0.55	4.78	1.81	0.64	0.61
uracil	-0.84	4.35	0.78	3.93	0.22	5.31	2.75	1.38	1.08	5.15	2.18	0.53	0.74
IMP	-4.59	4.54	0.97	4.31	0.27	7.44	4.89	1.06	1.91	6.51	3.55	0.86	1.20
UMP	-5.75	4.19	0.62	12.28	0.17	7.04	4.49	1.48	1.76	5.20	2.24	1.69	0.76
CDP	-7.51	4.03	0.46	5.43	0.13	6.74	4.18	0.73	1.64	4.14	1.18	0.85	0.40
GTP	-7.85	4.19	0.62	12.28	0.17	7.55	5.00	6.24	1.96	6.29	3.33	1.95	1.12
zeatine	0.06	9.31	5.74	3.63	1.61	8.73	6.18	0.00	2.42	9.29	6.33	0.71	2.14
gibbereline GA3	-0.37	14.43	10.86	0.91	3.04	17.39	14.83	0.16	5.81	15.87	12.90	0.29	4.36
jasmijnzuur	2.28	22.03	18.46	0.13	5.17	25.88	23.33	0.09	9.13	23.30	20.34	0.25	6.87
abscisinezuur	1.90	19.41	15.84	0.13	4.44	22.95	20.40	0.10	7.99	20.80	17.84	0.06	6.02
linoleenzuur	6.63	26.79	23.22	0.07	6.51	27.47	24.92	0.08	9.76	27.34	24.37	2.25	8.23
brassinolide	3.46	26.22	22.65	0.09	6.34	26.88	24.32	0.09	9.52	26.74	23.78	0.02	8.03
cadaverine	-5.04	3.71	0.14	46.84	0.04	2.75	0.20	0.00	0.08	3.25	0.29	12.03	0.10
spermidine	-6.99	3.66	0.09	44.44	0.03	2.66	0.10	49.02	0.04	3.11	0.15	47.30	0.05
chorismineZuur	-0.43	15.27	11.70	0.58	3.28	17.80	15.25	0.20	5.97	18.18	15.22	0.10	5.14
coumarinezuur	1.50	12.75	9.18	1.15	2.57	16.54	13.99	0.11	5.48	15.27	12.31	0.64	4.16
THF	-3.14	8.93	5.36	4.01	1.50	9.11	6.55	0.18	2.57	8.52	5.56	0.58	1.88
				11.25 ±13.92				3.08 ±9.06				3.16 ±8.79	

Tabel : logD (calculated with Pallas 3.0®; mean t_R, mean t_R relative, CV% and peak capacity for each metabolite (positive ion mode; n=3); mean CV% ± standard deviation for each column.

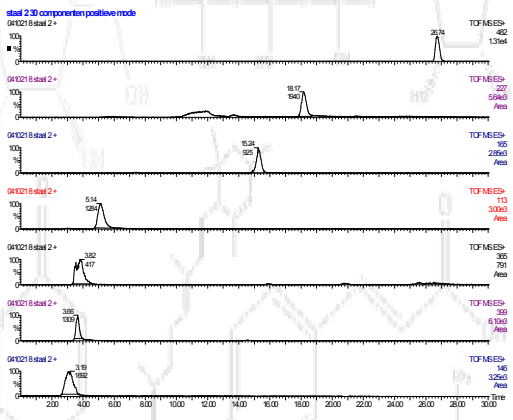

Figure: Mass chromatograms of spermidine (m/z 146), S-adenosylmethionine (m/z 399), lactose (m/z 365 → Na^+), uracil (m/z 113), coumaric acid (m/z 165), chorismic acid (m/z 227) and brassinolide (m/z 482) on an Atlantis dC18 column, in positive ion mode.

6. Conclusion

▪Peak performance was very similar for Atlantis dC18 and Inertsil ODS-3 columns. These were insensitive to dewetting problems and showed very reproducible retention. The Zorbax XDB column showed less dewetting resistance and thus less reproducibility.

▪At the end, we chose for the Atlantis dC18 column because some compounds only showed up using this column, especially in negative ion mode.

▪We succeeded in achieving a good separation of polar and apolar metabolites in a broad polarity range / limited time gradient.

▪It nevertheless has to be stressed that complete separation was never the ultimate goal. Indeed, this separation will now be extended towards biological (plant) extracts resulting inevitably in major overlap of the many metabolite peaks, even while we succeeded in making as much as possible use of the whole polarity range within a 30 minutes separation. Single MS detection using high resolution data will alleviate identification uncertainty of coeluting metabolites.

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