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Development of accelerate quantification analysis for hydrophilic metabolites using ionparing chromatography with a high-speed triple quadrupole mass spectrometer

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Introduction

Metabolomics, the comprehensive study of metabolites, allows for detailed phenotypic analysis through a combination of metabolite information. Among the many kinds of metabolic reactions, central pathways to energy metabolism are of the most biologically important pathways, and includes more than a hundred of hydrophilic compounds such as sugar phosphates, organic acids, and nucleotides. Ion-pare chromatography coupled to a triple quadrupole mass spectrometer is one of the techniques to analyze these hydrophilic metabolites. In order to improve throughput of analysis, high-speed with a quantitative capability is required from the mass spectrometer. In this study, we report a developed analytical system for hydrophilic metabolite using ion-pare chromatography with a high-speed triple quadrupole mass spectrometer.

Materials and Methods

Fig. 1 LCMS-8040 Triple Quadrupole Mass Spectrometer

UHPLC

Apparatus:	Nexera series UHPLC LCMS-8040 triple-quadrupole mass spectrometer
Mobile phase A:	10 mM tri-butyl ammonium and 15 mM acetic acid in water/methanol (97/3)
Mobile phase B:	methanol
Flow rate:	0.3 mL/min
Time program :	0%B (0-0.5 min) - 25%B (7.5 min) -90%B (11-11.5 min) - 0%B (11.6-15 min)
Analytical column:	L-Column ODSII (2 mm I.D. × 150 mmL., 3 µm)
Oven temp.:	40°C
Injection Vol.:	3 µL
LC-MS	
Apparatus:	LCMS-8040 triple-quadrupole mass spectrometer
Ionization:	ESI negative mode
DL Temp.:	250°C
HB Temp:	400°C
Drying Gas:	10 L/min
Nebulizing Gas:	2 L/min

Results

Quantitative analysis of standard samples

By using ion-paring chromatography coupled to high speed triple-quadrupole mass spectrometry, hydrophilic metabolites including sugar phosphates, organic acids, coenzymes, and nucleotides were analyzed successfully within 15 minutes (Fig. 2).

In comparison of several brands of ODS column, we chose L-Column ODS in order to favor separation of sugar phosphates. The qualitative capability of the chosen separation method in terms of limit of detection, dynamic range, carry over, and stability are summarised (Table 1).

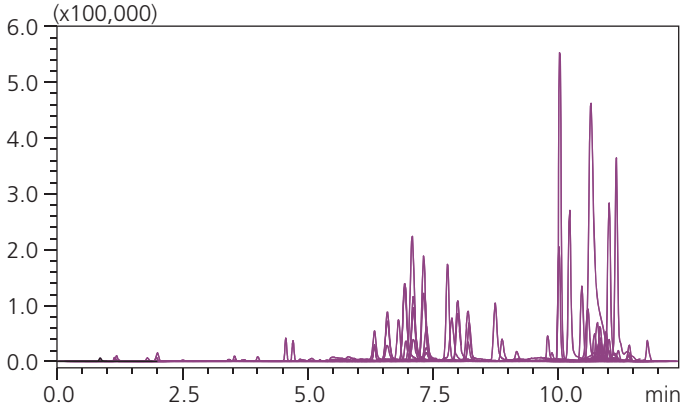

Fig. 2 Typical chromatograms of 96 standard compounds.

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Analysis of yeast extract

Yeast extracts were analyzed as an experimental model to confirm applicability of the developed method to biological samples. 75 components were detected successfully between concentration range 0.4 – 100,000 nM (compound dependant).

Linearity was also tested through serial dilution to confirm the range of linearity in which metabolites could be quantified (Fig. 4). These results show that this method can detect change of a metabolite quantitatively.

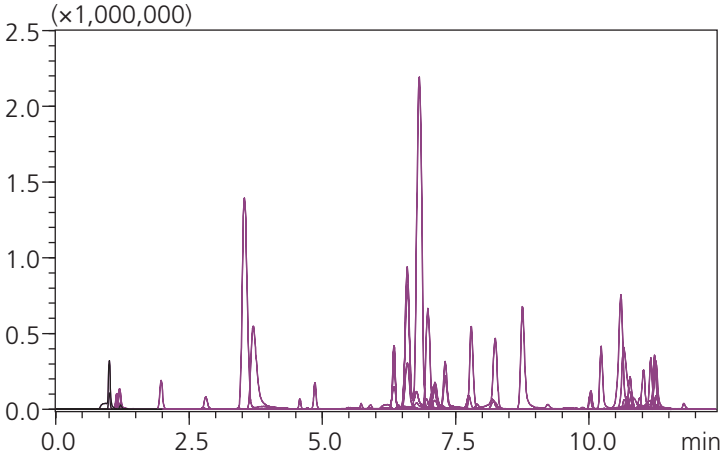

Fig. 3 Typical chromatograms of yeast extract.

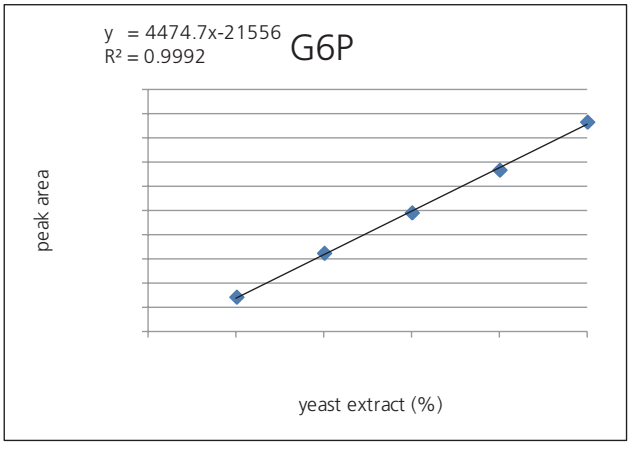

Fig. 4 Linearity between sample dilution to peak area.

Conclusions

Accelerated quantitative analysis of hydrophilic metabolites was developed that was able to analyse 96 components in 15 minutes. The result of analysis of yeast extract showed applicability of this method to future studies involving biological samples.

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	Compound	*m/z*	R.T.	LOD (nM)*	Linearity		RSD % at 1μM** (n=4)	
					Linear Range	R^2	R.T.	Peak area
1	Arginine	173.1>131.2	0.905	2.4	7.8 - 500	0.9924	0.14	8.1
2	Histidine	154>93.15	0.907	7.1	23.6 - 500	0.9979	0.25	3.6
3	4-Aminobutyrate	102>84	1.037	43.4	144.6 - 50000	0.9884	0.63	39.9
4	Serine	104>74.15	1.138	28.6	95.3 - 2000	0.9824	0.15	6.4
5	Asparagine	131>113.15	1.151	18.8	62.6 - 20000	0.9832	0.05	7.1
6	Glutamine	145>127.05	1.16	1.9	6.2 - 10000	0.9842	0.11	5.8
7	Hydroxyproline	130>84.15	1.179	8.6	28.8 - 1000	0.9800	0.21	14.0
8	Homoserine	118>100	1.182	1.4	4.8 - 1000	0.9895	0.17	1.4
9	Threonine	118>74.05	1.186	8.8	29.2 - 2000	0.9846	0.11	5.4
10	Leucine	130.1>84	1.201	16.5	55.1 - 1000	0.9935	0.31	15.4
11	Ribitol	151>89.1	1.243	59.4	198.1 - 5000	0.9868	0.08	5.1
12	Trehalose	341>89	1.302	27.8	92.6 - 20000	0.9897	0.42	17.4
13	Proline	114>68.1	1.331	55.3	184.3 - 5000	0.9957	0.66	31.9
14	Cytidine	242>109.15	1.845	1.3	4.2 - 50000	0.9901	0.03	1.8
15	Methionine	148>47.05	1.987	0.5	1.7 - 5000	0.9938	0.05	4.2
16	Theanine	173>155.25	2.006	3.0	10 - 20000	0.9850	0.09	2.7
17	Guanine	150>133.1	2.499	2.3	7.5 - 5000	0.9879	0.07	7.9
18	Isoleucine	130.1>45	2.578	134.3	447.6 - 50000	0.9902	0.35	32.8
19	Tyrosine	180>163.05	2.834	8.9	29.8 - 200000	0.9980	0.16	8.1
20	Amino adipic acid	160>116.2	3.345	7.2	24 - 100000	0.9880	0.26	5.0
21	Glutamate	146>102.2	3.477	44.6	148.8 - 200000	0.9894	0.18	9.2
22	Uridine	243>110.15	3.548	0.7	2.2 - 10000	0.9911	0.25	3.6
23	Aspartate	132>88.05	3.685	48.2	160.6 - 200000	0.9906	0.31	12.8
24	Thymine	125>42.05	4.003	17.6	58.5 - 50000	0.9888	0.18	4.2
25	Inosine	267>135.15	4.559	1.2	3.9 - 20000	0.9894	0.16	2.4
26	Guanosine	282.1>150.2	4.706	1.0	3.5 - 10000	0.9976	0.15	1.9
27	Phenylalanine	164>103.15	4.854	57.3	190.9 - 20000	0.9880	0.14	5.7
28	Shikimate	173>93.15	4.905	9.5	31.8 - 50000	0.9865	0.07	5.8
29	Glycerate	105>75.15	5.088	44.1	147.2 - 20000	0.9889	0.14	7.4
30	Thymidine	241.1>42.05	5.249	19.1	63.7 - 20000	0.9872	0.10	10.5
31	Glycolate	75>47.05	5.336	457.8	1526.1 - 20000	0.9975	0.12	9.8
32	Glyoxylate	73>45.05	5.577	689.3	2297.5 - 100000	0.9988	1.24	30.1
33	Inositol	179>87	6.34	744.4	2481.4 - 50000	0.9962	0.16	14.7
35	D-glucose/galactose	179>89.05	6.346	19.3	64.2 - 50000	0.9979	0.09	4.3
34	Lactate	89>43.1	6.348	71.6	238.6 - 10000	0.9744	0.09	3.3
36	Pyroglutamate	128>84.1	6.438	45.5	151.8 - 10000	0.9819	0.07	6.8
37	Glucose-6-phosphate	258.9>97.05	6.444	81.1	270.2 - 10000	0.9922	0.06	2.0
38	PIPES	301>193.25	6.637	0.6	2.1 - 10000	0.9958	0.07	1.1
39	Ribose-5-phosphate	229.1>96.95	6.759	21.8	72.7 - 10000	0.9993	0.10	5.0
40	Sedoheptulose-7-phosphate	288.9>97.1	6.867	4.3	14.3 - 10000	0.9875	0.14	2.9
41	Fructose-6-phosphate	258.9>97.1	6.927	88.1	293.7 - 10000	0.9895	0.17	4.2
42	Tryptophan	203.1>116.15	6.978	0.4	1.2 - 10000	0.9852	0.07	3.8
43	α-Glycerophosphate	171.1>79.1	7.074	7.6	25.3 - 10000	0.9912	0.19	3.7
44	Glucose-1-phosphate	258.9>79.05	7.113	149.4	497.9 - 10000	0.9925	0.21	3.2
45	Glyceraldehyde-3-phosphate	168.90>97.10	7.3	463.1	1543.8 - 100000	0.9919	0.54	21.0
46	Erythrose-4-phosphate	198.9>97.2	7.5	357.6	1191.9 - 100000	0.9885	0.75	8.8
47	Ribulose-5-phosphate	229>97.1	7.622	35.5	118.3 - 50000	0.9969	0.15	6.0
48	β-Glycerophosphate	170.9>79.05	7.753	9.6	32 - 20000	0.9868	0.13	1.2
49	Orotate	155>111.15	7.84	8.5	28.3 - 10000	0.9930	0.05	2.0

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	Compound	*m/z*	R.T.	LOD (nM)*	Linearity		RSD % at 1μM** (n=4)	
					Linear Range	R^2	R.T.	Peak area
50	Fructose-1-phosphate	258.9>97.05	7.925	78.0	260.1 - 10000	0.9968	0.10	3.0
51	CMP	322>79.1	7.964	17.8	59.5 - 10000	0.9925	0.09	3.4
52	NAD	662.1>540.1	8.243	1.5	5 - 10000	0.9844	0.06	2.1
53	Pyruvate	87>43.05	8.275	128.3	427.7 - 20000	0.9871	0.05	9.9
54	Dihydroxy acetone-3-phosphate	168.9>97.05	8.391	5.6	18.7 - 20000	0.9873	0.09	1.1
55	UMP	322.9>97.1	8.661	11.4	37.9 - 20000	0.9917	0.05	5.0
56	GMP	362>79.1	8.995	30.7	102.3 - 50000	0.9909	0.08	9.9
57	Oxalacetate	131>87	9.31	8383.4	27944.7 - 500000	0.9961	0.02	4.6
58	TMP	321>79.1	9.719	9.3	30.9 - 50000	0.9966	0.04	3.2
59	AMP	346>79.05	9.811	30.5	101.6 - 100000	0.9945	0.03	10.5
60	Nicotinate	122>78.15	9.983	5.7	19.1 - 5000	0.9914	0.05	4.0
61	Pantothenate	218>88	10.022	1.0	3.4 - 5000	0.9934	0.04	8.0
62	Succinate	117>73.2	10.155	6.1	20.5 - 10000	0.9881	0.05	4.7
63	Fumarate	115>71.1	10.278	269.3	897.7 - 500000	0.9923	0.05	15.3
64	cAMP	328>134.1	10.465	0.7	2.3 - 50000	0.9900	0.03	3.7
65	Malate	132.9>115.2	10.578	5.9	19.8 - 10000	0.9959	0.04	4.8
66	UDP-glucose	564.8>323.1	10.712	0.6	2 - 5000	0.9919	0.03	5.5
67	2-Oxoglutarate	145>101.2	10.745	24.6	82 - 10000	0.9825	0.04	4.1
68	CDP	401.8>79.05	10.753	28.2	94 - 5000	0.9875	0.04	7.5
69	6-Phosphogluconate	275>79	10.771	8.8	29.2 - 2000	0.9836	0.07	20.4
70	ADP-glucose	588>346.15	10.806	48.2	160.8 - 5000	0.9870	0.06	8.0
71	GDP	442>79.1	10.806	1.0	3.5 - 500	0.9913	0.02	4.7
72	UDP	402.9>79.05	10.807	13.5	44.9 - 1000	0.9820	0.03	5.7
73	ADP-ribose	558>346.15	10.81	1.8	6.1 - 500	0.9969	0.03	7.2
74	NADP	741.8>620.1	10.811	4.8	16.1 - 2000	0.9722	0.03	3.9
75	KDPG	256.9>97.05	10.828	12.1	40.4 - 10000	0.9906	0.04	8.1
76	3-Phosphoglycerate	184.9>97.05	10.829	7.8	26.2 - 20000	0.9802	0.03	6.4
77	Fructose-2,6-bisphosphate	338.9>241.15	10.834	26.8	89.4 - 5000	0.9728	0.03	10.2
78	Fructose-1,6-bisphosphate	338.9>97.1	10.838	13.1	43.8 - 2000	0.9865	0.04	0.5
79	NADH	664>78.95	10.876	3.8	12.7 - 2000	0.9744	0.03	7.6
80	Ribulose-1,5-bisphosphate	308.9>97.05	10.887	15.5	51.6 - 10000	0.9785	0.02	7.7
81	Isocitrate	190.9>117	10.891	4.8	15.8 - 5000	0.9789	0.03	12.9
82	Citrate	190.9>87	10.892	45.4	151.3 - 2000	0.9915	0.09	11.7
83	ADP	425.9>79.1	10.913	23.1	77.1 - 2000	0.9938	0.02	7.2
84	Phosphoenolpyruvate	167>78.95	10.928	6.4	21.4 - 10000	0.9764	0.03	6.4
85	FMN	455>97.1	10.985	20.2	67.4 - 5000	0.9774	0.01	1.9
86	2-Isopropylmalate	175>115.2	10.998	5.3	17.6 - 5000	0.9976	0.02	2.9
87	FAD	783.9>97.1	11.155	3.1	10.4 - 1000	0.9909	0.01	3.3
88	CTP	481.9>159.1	11.171	44.1	147 - 50000	0.9840	0.10	14.3
89	GTP	521.9>159.05	11.185	76.2	254.1 - 50000	0.9963	0.15	11.0
90	NADPH	744>159	11.201	4.5	15 - 2000	0.9960	0.02	5.2
91	UTP	482.9>159.1	11.206	52.6	175.4 - 100000	0.9914	0.03	6.7
92	ATP	505.9>159.1	11.226	35.8	119.5 - 50000	0.9851	0.24	12.1
93	Coenzyme A	766.5>79	11.343	17.1	56.9 - 20000	0.9863	0.02	11.3
94	Malonyl coenzyme A	852.1>408.1	11.367	0.6	2.1 - 10000	0.9923	0.01	7.0
95	Acetyl coenzyme A	808>408	11.382	1.6	5.5 - 10000	0.9894	0.02	7.2
96	Succinyl coenzyme A	866>408	11.391	0.3	1.1 - 10000	0.9832	0.02	9.5

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