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Ultra Fast Analysis of Amino Acids in Cultured Cell Extracts Using UHPLC/MS/MS

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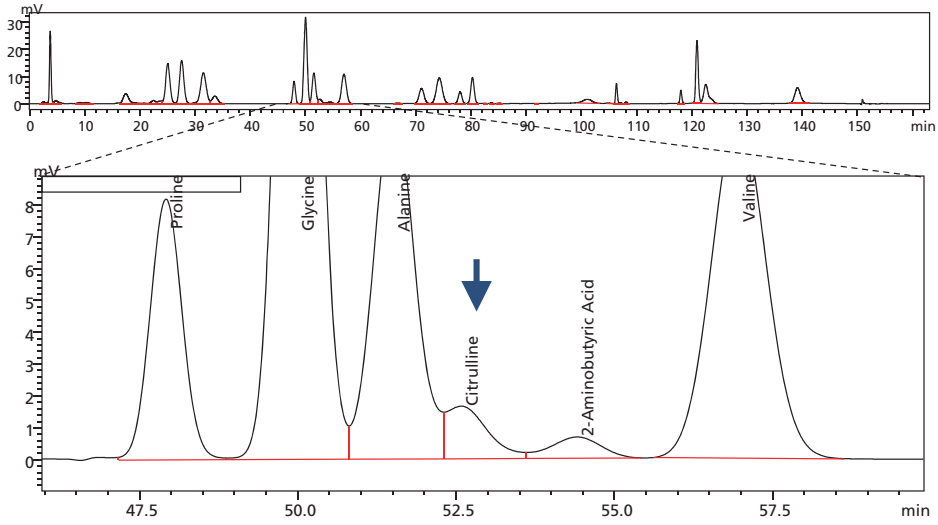
Introduction

An essential aspect for the research of metabolic behavior involves the analysis of amino acids (AAs), which is typically carried out using ion-exchange HPLC with post-column derivatization. Recently, not only relatively high concentrations of AAs such as proteinogenic AAs but also relatively low concentrations of AAs and related compounds such as non-proteinogenic AAs and dipeptides are also monitored as significant targets for this purpose.

In case of biological samples, however, interferences by other compounds which involve high concentrations of AAs significantly affect the sensitivity for low concentrations of AAs due to low selectivity of this traditional method. Moreover this method requires over two hours for the trace analysis of AAs including related compounds (Fig. 1).

Herein, we describe highly selective and ultra fast trace analysis method of AAs using reversed-phase UHPLC/MS/MS with pre-column derivatization specifically aimed at detection of low concentrations of AAs in biological samples , along with its application for the analysis of cultured cell extracts.



Interference to low concentrations of AAs by matrix

Fig. 1 Ion-exchange post-column derivatization method (Sample: Rat plasma)

Methods and Materials

Preparation

Samples were prepared using the EZ:faast amino acid kit (Phenomenex). After addition of 3 internal standards and solid phase extraction, samples were derivatized using alkylchloroformate which modifies N-terminal and C-terminal of AAs (Fig. 2). This preparation takes about 7 minuites.

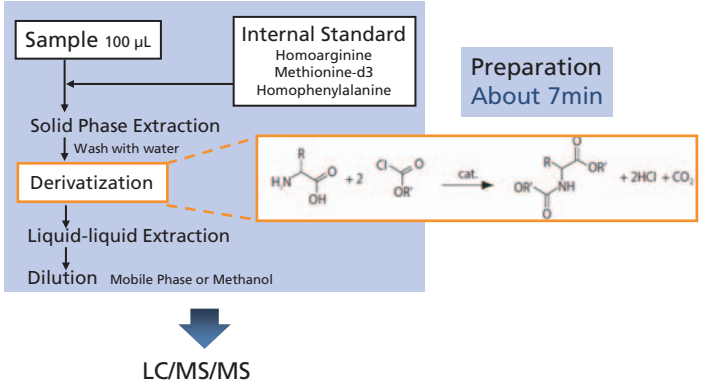


Fig. 2 Procedure of EZ:faast AAs kit preparation

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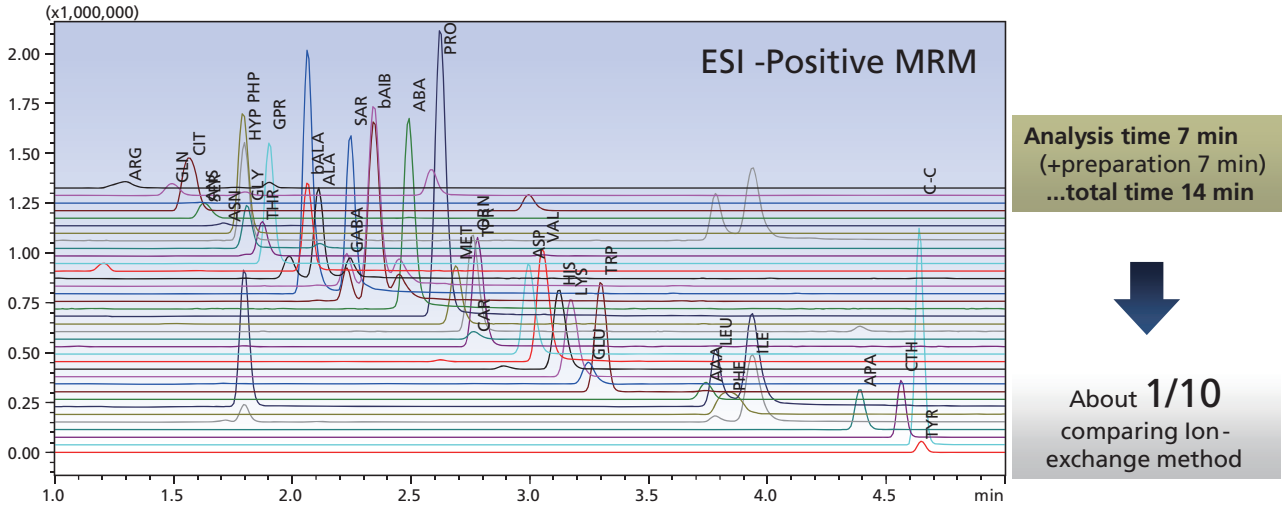
Instruments and analytical methods

Reversed-phase core-shell type column Kinetex C18 (Phenomenex) and a UHPLC system (Shimadzu Nexera) coupled with a triple quadrupole type mass spectrometer (Shimadzu LCMS-8030) was used for ultra fast analysis of AAs.

Using our analytical method, 36 AAs and related compounds were analyzed in 7 minuites (Fig. 3). This means total analysis time including preparation was 14 minutes, significantly shorter than that of traditional HPLC methods.

Column :Phenomenex Kinetex C18 2.1x150 mm, 1,7 µm
Mobile Phase :5 mmol/L Ammonium Formate - Water/Methanol
Gradient Prog. :B conc. 50%(0 min)->65%(1-3 min)->90%(4-4.5 min)->50%(4.51-7 min)
Flow rate: :0.4 mL/min
Column Temp. :50°C
Inj. Vol. :2 µL



Analysis time 7 min
(+preparation 7 min)
...total time 14 min

About **1/10**
comparing Ion-exchange method

Fig. 3 MRM chromatograms of 36 AAs standard

Table 1 shows calibration curve ranges, correlation coefficient (R) and reproducibility (CV%) of 36 AAs.
Most of AAs were detected selectively with MRM and lower limits of calibration curves were ranged from 2 to 500 pmol/mL.

Table 1 Calibration curves and reproducibility of 36 AAs

	MRM transition	calibration curve range (pmol/mL)	R	CV %
Arg	303.2>70.1	20 - 80000	0.9934	4.5
Gln	275.2>171.9	50 - 80000	0.9953	3.7
Ans	369.2>309.2	200 - 80000	0.9892	7.7
Cit	304.2>156.1	50 - 80000	0.9907	1.2
Ser	234.1>146.0	50 - 10000	0.9938	3.8
Asn	243.1>115.1	50 - 10000	0.9926	4.1
Pro-Hyp	357.2>156.0	5 - 40000	0.9991	1.9
Hyp	260.1>172.0	10 - 4000	0.9959	1.0
Gly	204.1>118.0	500 - 10000	0.9991	1.7
Thr	248.1>159.9	50 - 10000	0.9964	7.5
Gly-Pro	301.2>157.9	2 - 10000	0.9980	10.1
βAla	218.1>98.0	5 - 10000	0.9948	7.1
Ala	218.1>130.0	100 - 80000	0.9941	2.1
GABA	232.2>130.0	100 - 10000	0.9780	1.2
Sar	218.1>115.9	100 - 80000	0.9932	1.8
βAIBA	232.2>129.9	2 - 10000	0.9976	6.9
ABA	232.2>143.9	2 - 10000	0.9963	8.3
Pro	244.2>156.0	50 - 10000	0.9993	1.8

	MRM transition	calibration curve range (pmol/mL)	R	CV %
Met	278.1>190.0	2 - 4000	0.9934	11.4
Orn	347.2>287.0	50 - 80000	0.9925	4.2
Car	441.2>284.0	20 - 10000	0.9909	9.5
Tpr	262.1>173.9	5 - 10000	0.9997	3.7
Asp	304.2>216.0	50 - 80000	0.9954	4.1
Val	246.2>157.9	50 - 80000	0.9930	3.8
His	370.2>196.0	50 - 80000	0.9971	5.2
Lys	361.2>301.0	50 - 80000	0.9911	1.8
Glu	318.2>171.9	50 - 80000	0.9886	2.4
Trp	333.2>245.0	2 - 10000	0.9968	7.9
AAA	332.2>244.0	20 - 10000	0.9949	2.9
Leu	260.2>172.0	10 - 80000	0.9947	2.3
Phe	294.2>205.9	5 - 80000	0.9918	5.3
Ile	260.2>129.9	50 - 80000	0.9946	0.9
APA	346.2>198.0	10 - 80000	0.9941	1.9
Cth	479.2>230.0	2 - 80000	0.9963	4.6
C-C	497.2>248.0	2 - 10000	0.9984	4.0
Tyr	396.2>308.0	50 - 10000	0.9971	2.1

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Analysis of cultured cell extracts

The applicability of our methods for biological samples was demonstrated using cultured cell extracts of human colon cancer cell and human fibroblast cell.
Fig 4 shows extraction flow of cultured cell.

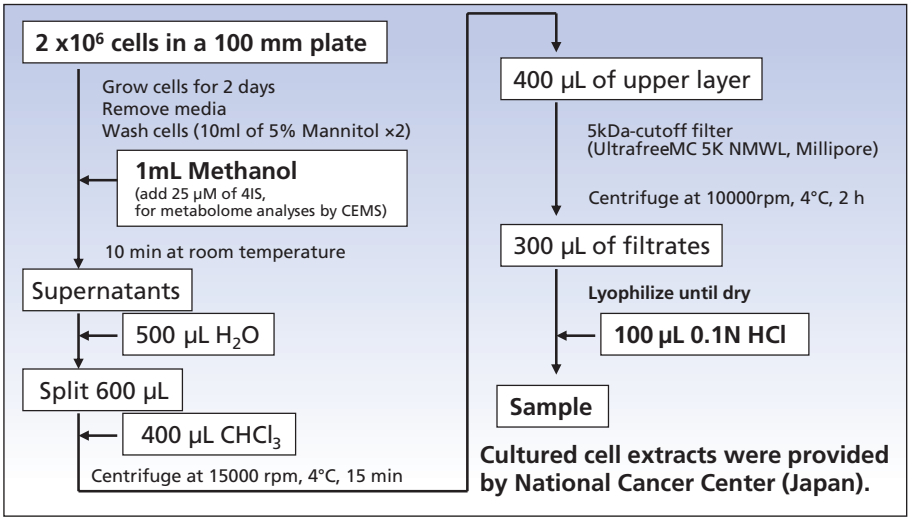

Fig. 4 Extraction flow of cultured cell

As a result, pmol/mL range of AAs such as prolylhydroxyproline, carnosine and aminoadipic acid were detected from human colon cancer cells as isolated peaks besides coexistence of nmol/mL range of AAs such as glutamine, glycine and alanine (Fig. 5). Also in the case of human fibroblast cells, any severe interference was not confirmed. Table 2 shows quantification results of each cultured cell extracts.

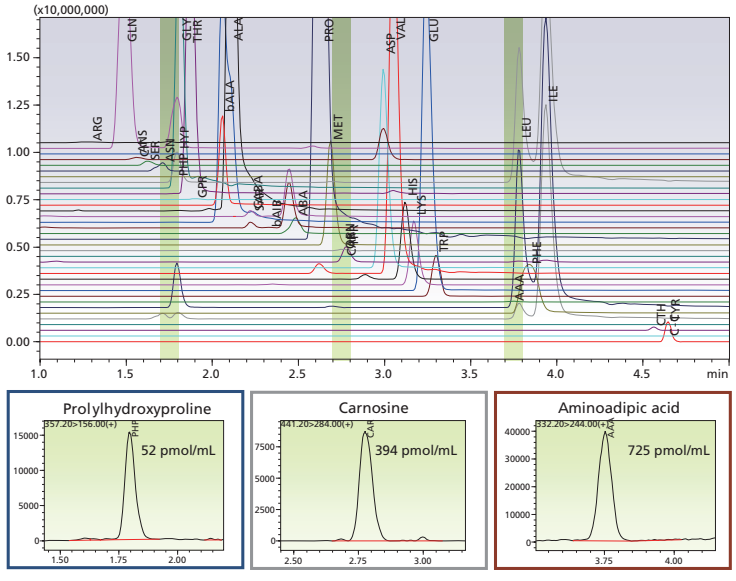

Fig. 5 MRM chromatograms of human colon cancer cells extract

Recovery test was also carried out for 8 amino acids which were detected at low concentration in each sample. Recovery ratio for those amino acids were in the range from 80 to 120 percent about both human colon cancer cells and human fibroblast cells (Fig. 6).

Table 2 Quantification result of cultured cell extracts

	Concentration in sample (pmol/mL)	
	human colon cancer cells	human fibroblast cells
Arg	6120	2710
Gln	915000*	84800
Ans	Tr	ND
Cit	861	Tr
Ser	6390	13200
Asn	66600*	2540
Pro-Hyp	52	66
Hyp	9500*	750
Gly	290000*	47800*
Thr	312000*	19100*
Gly-Pro	177	20
β Ala	17200*	1750
Ala	297000*	89500*
GABA	3430	297
Sar	Tr	Tr
β AIBA	35	Tr
ABA	2010	170
Pro	134000*	12800*
Met	45300*	4510
Orn	308	503
Car	394	29
Tpr	2980	243
Asp	47900	24700
Val	67700	11500
His	23300	3380
Lys	16600	5240
Glu	308000*	173000*
Trp	8790	1790
AAA	725	496
Leu	57900	10400
Phe	40716	6417
Ile	65600	10900
APA	ND	ND
Cth	1240	1840
C-C	4.2	ND
Tyr	39500*	8190

ND: not detected, Tr: trace amount
*reanalyzed after dilution

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This result indicates the matrix in cultured cell extracts didn't cause severe suppression effect nor enhancement effect.

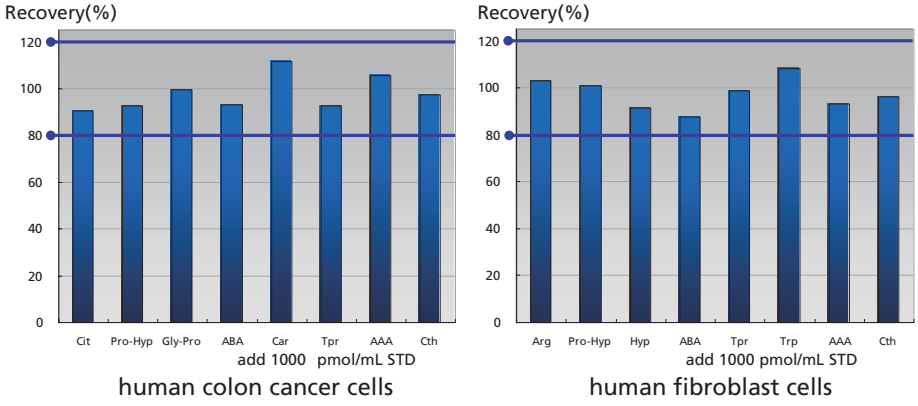

Fig. 6 Results of recovery test

Conclusions

- Ultra fast and highly selective analysis of amino acids using reversed-phase UHPLC/MS/MS with pre-column derivatization was investigated.
- 36 amino acids could be analyzed in 7 min for preparation and further 7min for instrumental analysis.
- In the cases of cultured cell extracts, low concentrations of amino acids were detected without severe interference from the matrix.

Acknowledgment

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