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Application News

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Liquid Chromatography Mass Spectrometry

LC-MS/MS Analysis Using Coinjection Method with the SIL-30AC

Sample pretreatment takes time and introduces a potential for human error. For analyses that require sample pretreatment, automation of the pretreatment process is an effective means of improving throughput while maintaining repeatability. Automation also allows for analyses to be performed overnight, which is essential when screening a large number of samples.
As well as a powerful rinse mechanism that dramatically reduces carryover, the Nexera Series SIL-30AC autosampler includes a pretreatment function as standard, so large volume samples can be analyzed in succession automatically with no operator intervention. Here we introduce the automation of an internal standard method using this pretreatment function along with the measurement results, for the simultaneous analysis of amino acids and acylcarnitines in a dried blood spot (DBS).

Internal Standard Method

The internal standard method measures quantity based on the relative ratio of an internal standard substance to the target sample, and can correct variations depending on the injection volume and analytical environment. This method requires the internal standard solution be added to the target sample in advance of analysis, which introduces problems in terms of throughput and increased reagent consumption. By using the pretreatment function, it is possible to inject just the required volumes of internal standard solution and target sample automatically immediately prior to analysis. This eliminates the necessity of adding internal standard solution to the sample prior to analysis.

Pretreatment Program and Analysis

The SIL-30AC pretreatment function is controlled from the LabSolutions workstation. The pretreatment function can be implemented using a default template process, or the user can enter their own commands to design a more complex process. The pretreatment window is displayed by clicking [Pretreatment Program] on the autosampler tab in [Instrument Parameters View] in LabSolutions. The autosampler movement used during analysis described in this article is shown in Fig. 1, while the relevant LabSolutions pretreatment program setup window is shown in Fig. 2. The program commands shown in Fig. 2 describe the suction of 1 µL from the vial No.1 of the control vial rack (rack No.: 0), after which a set volume (iV) µL is drawn in from sample vial No. (sn) of rack No. (rn), and injected into the instrument.

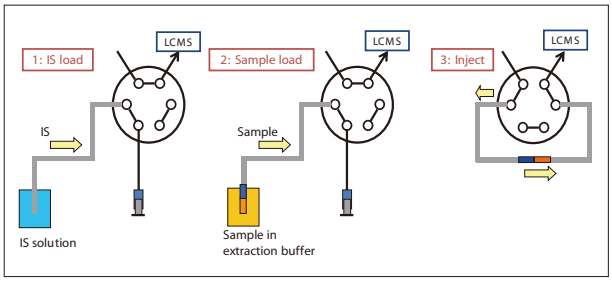

Fig. 1 Autosampler Movement

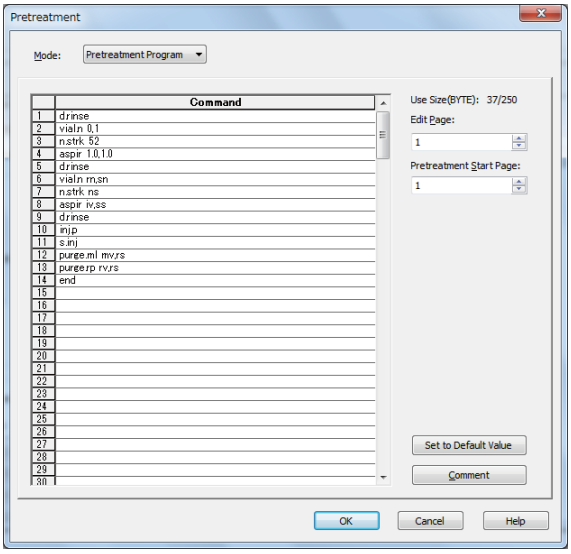

Fig. 2 Pretreatment Program Setup Window in LabSolutions

Simultaneous Analysis of Amino Acids and Acylcarnitines in a DBS—Sample Extraction and MS Analysis

LC-MS/MS can be used for rapid analysis of a multicomponent mixture of amino acids as indicators of amino acid metabolism and acylcarnitines as indicators of fatty acid metabolism. This type of screening is performed by putting liquid extracted from a DBS into a 96-well plate and performing automated analysis using an autosampler. Target materials are quantified by comparing the target material with an internal standard material added to the extracted liquid before analysis. For this article, we investigated a method of removing the pretreatment step of adding internal standard solution to the extracted liquid by utilizing an autosampler to perform coinjection of extraction liquid and internal standard solution, then using the LCMS-8040 triple quadrupole liquid chromatograph mass spectrometer for measurements.

A kit manufactured by ChromSystems was used in experiments. Except for addition of the internal standard solution to the extraction liquid, pretreatment was performed according to the protocol that came with the kit. The sample pretreatment method is shown in Fig. 3. The sample used was quality control DBS, which was prepared by soaking blood onto quality control filter paper. After cutting 3.0 mm diameter disks of DBS and placing them into a 96-well plate, pretreatment was performed according to the protocol. Flow injection analysis (FIA) was then performed using the sample extract after pretreatment. Multiple reaction monitoring (MRM) was used to measure the target compounds. Target compound quantitative analysis was performed using internal standard materials. The LC and MS conditions are shown in Table 1.

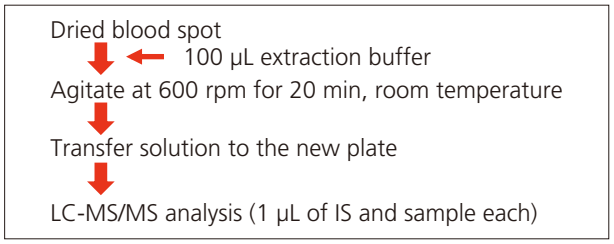

Fig. 3 Pretreatment Protocol

Table 1 Analytical Conditions

Mobile Phase	: 0.1 % HCOOH - H_2O / CH_3CN (2/8)
Time Program	: 0.1 mL/min (0 min) → 0.075 mL/min (0.1 min) → 0.5 mL/min (0.66-1 min)
Injection Volume	: 1 µL
Analysis Time	: 1 min
Ionization Mode	: ESI (+)
Probe Voltage	: +4.5 kV
Nebulizing Gas Flow	: 3.0 L/min
Drying Gas Flow	: 12.0 L/min
DL Temperature	: 250 ℃
Block Heater Temperature	: 400 ℃

Example Analysis Results

Results of sample measurement performed by the coinjection method and the conventional method (pretreatment performed according to the kit protocol) are shown in Table 2. Three spots were cut from each of Level-Ⅰ and Level-Ⅱ DBS quality control filter papers of differing concentrations, treated, and then target compounds were measured with n=10. The same measurements were performed over 3 days, and the mean values and ratios compared to data sheet target values were calculated. By using Shimadzu Neonatal Solution, which is software that supports the analysis of amino acids and acylcarnitines, we were able to calculate the concentration of target compounds automatically.

Results were not only confirmed to be within the range described in the data sheet (data not included), but also checked for conformance with target values as shown by the coinjection method results in Table 2. Results obtained by the coinjection method were then compared with results obtained by the conventional method for equivalence.

While the internal standard solution used with the coinjection method was prepared at 5 times the concentration of that used with the conventional method, the coinjection method uses 1/100th the volume per analysis (1 µL used by co-injection method, 100 µL used by conventional method), and therefore consumes 1/20th the volume of internal standard solution overall. In this way, by using a coinjection method that utilizes the pretreatment function of the SIL-30AC, it is possible to not only simplify the pretreatment process, but also reduce reagent consumption during analysis.

Table 2 Results of Analysis

Level-Ⅰ

Component	Average (µmol/L)		Target (µmol/L)	Ratio (average/target)	
	Coinjection method	Conventional method*		Coinjection method	Conventional method*
Ala	423.99	420.76	411.00	1.03	1.02
Arg	67.05	66.80	70.00	0.96	0.95
Asp	198.19	185.51	201.00	0.99	0.92
Cit	68.67	68.83	69.00	1.00	1.00
Glu	404.47	395.23	418.00	0.97	0.95
Gly	407.26	391.81	394.00	1.03	0.99
Leu	374.94	368.78	372.00	1.01	0.99
Met	70.59	69.09	71.00	0.99	0.97
Orn	211.86	202.91	208.00	1.02	0.98
Phe	152.56	158.20	167.00	0.91	0.95
Pro	320.11	325.46	360.00	0.89	0.90
Tyr	198.58	199.28	202.00	0.98	0.99
Val	262.67	258.42	262.00	1.00	0.99
C0	50.33	50.16	49.40	1.02	1.02
C2	28.30	28.11	28.10	1.01	1.00
C3	6.35	5.89	6.02	1.06	0.98
C4	1.13	1.03	1.08	1.04	0.96
C5	0.61	0.65	0.64	0.96	1.02
C6	0.51	0.49	0.51	1.01	0.97
C8	0.56	0.55	0.54	1.04	1.01
C10	0.53	0.51	0.51	1.03	1.00
C12	0.47	0.48	0.47	0.99	1.02
C14	0.52	0.50	0.51	1.01	0.98
C16	4.94	4.86	5.00	0.99	0.97
C18	2.61	2.61	2.56	1.02	1.02

Level-Ⅱ

Component	Average (µmol/L)		Target (µmol/L)	Ratio (average/target)	
	Coinjection method	Conventional method*		Coinjection method	Conventional method*
Ala	672.15	722.38	714.00	0.94	1.01
Arg	138.89	146.69	141.00	0.99	1.04
Asp	446.06	490.05	446.00	1.00	1.10
Cit	250.10	274.29	270.00	0.93	1.02
Glu	689.73	808.85	742.00	0.93	1.09
Gly	939.76	977.15	1006.00	0.93	0.97
Leu	616.42	652.52	644.00	0.96	1.01
Met	254.81	269.46	257.00	0.99	1.05
Orn	493.23	548.32	555.00	0.89	0.99
Phe	547.77	602.35	564.00	0.97	1.07
Pro	732.41	772.55	701.00	1.04	1.10
Tyr	525.85	595.79	580.00	0.91	1.03
Val	463.26	499.90	482.00	0.96	1.04
C0	99.33	110.00	107.00	0.93	1.03
C2	67.13	70.10	69.70	0.96	1.01
C3	14.83	14.96	15.40	0.96	0.97
C4	4.53	4.56	4.69	0.97	0.97
C5	2.37	2.71	2.71	0.87	1.00
C6	2.40	2.56	2.48	0.97	1.03
C8	2.40	2.39	2.50	0.96	0.96
C10	2.18	2.36	2.27	0.96	1.04
C12	2.11	2.26	2.20	0.96	1.03
C14	2.16	2.20	2.23	0.97	0.99
C16	12.60	13.08	13.30	0.95	0.98
C18	8.22	8.77	8.49	0.97	1.03

* Conventional method: Pretreatment was performed according to the kit protocol, and analysis was performed without using the SIL-30AC pretreatment program.

Notes: The equipment mentioned in this article has not been approved/certified as medical equipment based on the Japan's Pharmaceutical and Medical Device Act.
The analytical method described in this article cannot be used for diagnostic purposes.

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