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Application News

No. L519

Supercritical Fluid Chromatography

Analysis of Choline and Acetylcholine in Rat Cerebrospinal Fluid Samples Using the Nexera UC-MS/MS System

Choline, which is a structural element of cell membranes, and acetylcholine, which is known as a neurotransmitter, are both familiar compounds in the field of bioanalysis. Since acetylcholine is biosynthesized in the body from choline, it is possible to estimate the quality of internal activity by monitoring both of these compounds. This article focuses on the SFC analysis of these compounds in a rat cerebrospinal fluid sample by direct injection of the cerebrospinal fluid to the Nexera UC SFC system. Also introduced is automatic extraction and analysis of a cerebrospinal fluid sample impregnated into filter paper, in consideration of convenience and durability for storage and transport, using the Nexera UC online SFE-SFC-MS/MS system.

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SFC-MS/MS Analysis

A CN column provided favorable separation of choline and acetylcholine in SFC-MS/MS analysis. Calibration curves were created from the peak area values from six times repeated analyses for each of the three concentrations of 10, 100, and 1000 µg/L. Good linearity was obtained and the quantitation limit (LOQ, ASTM method) was 30 µg/L for choline and 10 µg/L for acetylcholine. Table 1 lists the conditions of SFC-MS/MS analysis. Fig. 1 shows the structural formula of choline and acetylcholine and Fig. 2 shows the obtained calibration curves.

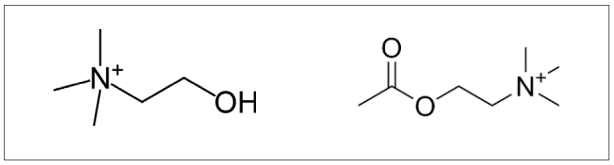

Fig. 1 Structure of Choline (Left) and Acetylcholine (Right)

Table 1 SFC-MS/MS Analytical Conditions

Column	:	Inertsil CN-3 250 mm L. × 4.6 mm I.D., 5 µm
Mobile phase	:	A) Supercritical fluid of CO_2 B) Modifier: Methanol containing 20 mmol/L ammonium formate / water =95/5 (v/v)
Time program	:	B Conc. 10 % (0 min) → 25 % (10 min) → 50 % (10.1-12 min) → 10 % (12.1-15 min)
Flow rate	:	2.5 mL/min
Column temp.	:	40 °C
Injection volume	:	1 µL
BPR pressure	:	10 Mpa
BPR temp.	:	50 °C
Detector	:	LCMS-8050 (ESI, MRM mode)
Make-up	:	Methanol
Make-up flow rate	:	0.2 mL/min
MRM transitions	:	(+) *m/z* 104.1>60.1 (for choline) (+) *m/z* 146. 1>87.1 (for acetylcholine)

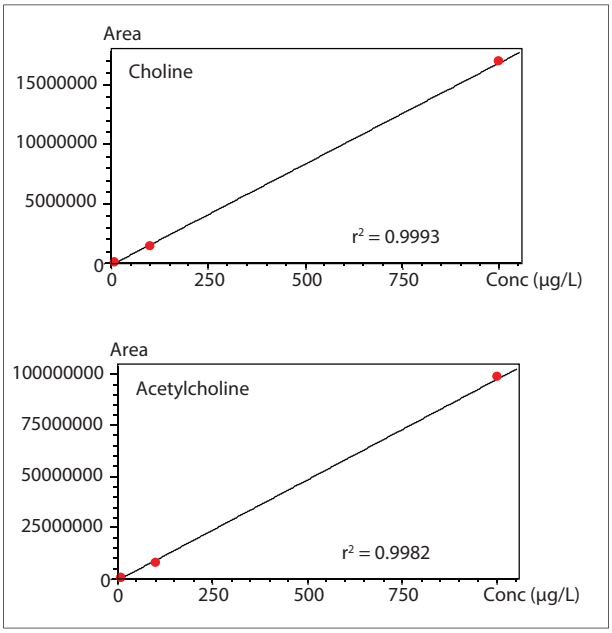

Fig. 2 Calibration Curves of Choline and Acetylcholine

The retention time and peak area repeatabilities after six repetitions at each concentration of 10, 100, and 1000 µg/L was confirmed at calibration curve creation and the results are summarized in Table 2. The linearity (r^2) was 0.9993 for choline and 0.9982 for acetylcholine. Fig. 3 shows the MRM chromatograms for 100 µg/L.

Table 2 Repeatabilities of Choline and Acetylcholine Standards (n = 6)

		Retention time (%RSD)	Peak area (%RSD)
Choline	10 µg/L	0.22	7.5
Choline	100 µg/L	0.05	1.7
Choline	1000 µg/L	0.07	2.2
Acetylcholine	10 µg/L	0.07	5.7
Acetylcholine	100 µg/L	0.06	4.2
Acetylcholine	1000 µg/L	0.07	6.0

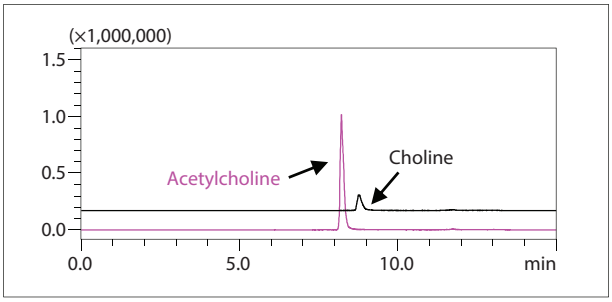

Fig. 3 Choline and Acetylcholine Standards (100 µg/L)

Next, by employing the microdialysis method in which biological compounds are continuously sampled from an awake animal via the semipermeable membrane of a minute dialytic probe connected to a pump, cerebrospinal fluid was sampled from a rat and directly delivered to SFC analysis. The injection volume of cerebrospinal fluid was set to 1 µL due to concerns regarding the miscibility between the aqueous sample and low polar supercritical carbon dioxide, which is the main component of the mobile phase used in SFC. With respect to acetylcholine, the LOQ determined according to the ASTM method was about 10 µg/L. Since the calculated concentration was less than the LOQ, only peak identification was performed. As shown in Table 3, the retention time and peak area repeatabilities were favorable for the six repeated analyses of choline. Fig. 4 shows the chromatograms resulting from SFC analysis of the cerebrospinal fluid sample.

Table 3 Choline Quantitative Value in Rat Cerebrospinal Fluid Sample and Repeatabilities (n = 6)

	Retention time (%RSD)	Peak area (%RSD)
Choline (Concentration 229.6 µg/L)	0.10	3.1

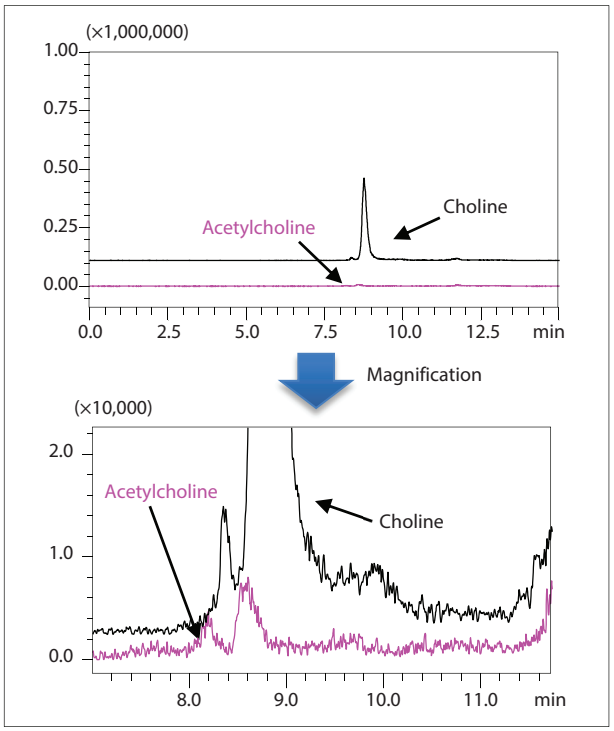

Fig. 4 SFC Analysis of Choline and Acetylcholine in a Cerebrospinal Fluid Sample

Online SFE-SFC-MS/MS Analysis

Next, a sample was prepared by impregnating cerebrospinal fluid sample into filter paper and drying the paper. SFE-SFC-MS/MS analysis was then performed on the sample. The convenience of this method is gaining attention not only because of easy of sample handling but also because of improved miscibility concerns between a mobile phase of low polar supercritical carbon dioxide and an aqueous sample solvent containing a biological sample. Table 4 lists the conditions used in online SFE-SFC-MS/MS analysis.

Table 4 Online SFE-SFC-MS/MS Conditions

Vessel	:	0.2 mL (1 µL of sample was added to filter paper)
Extractant	:	A) Supercritical fluid of CO_2 B) Methanol containing 20 mmol/L ammonium formate / water = 95/5 (v/v) A/B = 9/1 (v/v)
Flow rate	:	2.5 mL/min
Extraction time	:	Static (0-3 min) – Dynamic (3-6 min) – Static (6-8 min) - Dynamic (8-11 min) – Static (11-13 min) – Dynamic (13-16 min)
BPR pressure	:	10 Mpa
Extraction temp.	:	60 °C
Time program	:	B Conc. 10 % (16 min) → 25 % (26 min) → 50 % (26.1-28 min) → 10 % (28.1-31 min)

* SFC-MS/MS conditions are identical to Table 1 except for the time program.

Fig 5. shows the result obtained from online SFE-SFC-MS/MS analysis of a sample created by dropping 1 µL of 100 µg/L standard solution onto filter paper (GA-200 by ADVANTEC). Fig. 6 shows the result obtained by processing the rat cerebrospinal fluid sample in the same manner. The peak obtained for acetylcholine was small like the SFC analysis result, however, since the baseline noise level was improved in comparison, improved LOQ was obtained. Because the S/N value of corresponding peak to acetylcholine was more than 15 based on the baseline noise determined by ASTM method, a simple quantitative calculation was made based on the 100 µg/L standard data in the same way as the more concentrated choline. The obtained choline concentration of 297 µg/L was close to the SFC result and suggested that extraction in online SFE was performed efficiently. For acetylcholine, a calulation result of 1.7 µg/L was obtained from the peak area.

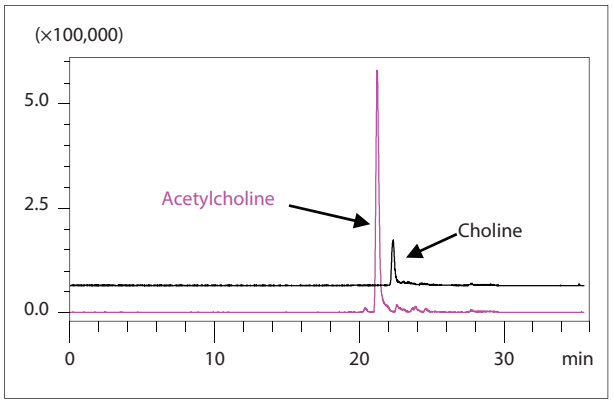

Fig. 5 Online SFE-SFC Analysis of Choline and Acetylcholine Standards

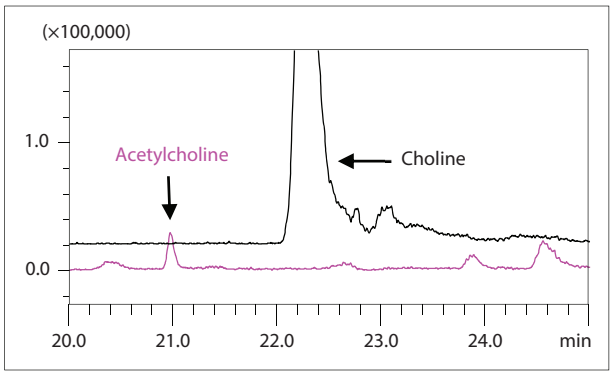

Fig. 6 Online SFE-SFC Analysis of Choline and Acetylcholine in a Cerebrospinal Fluid Sample

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