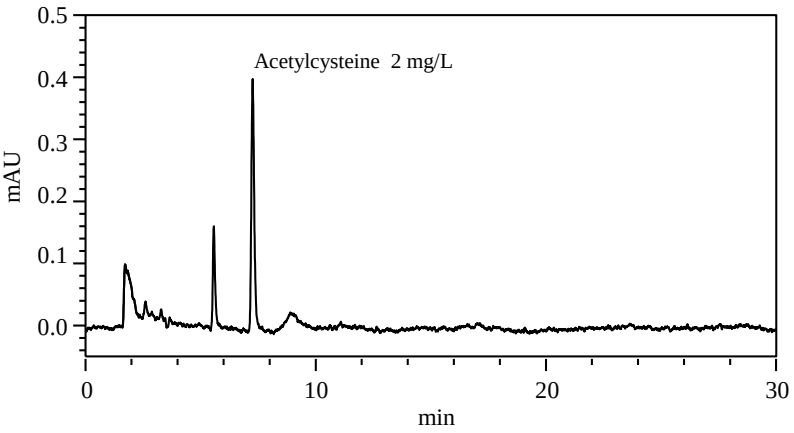
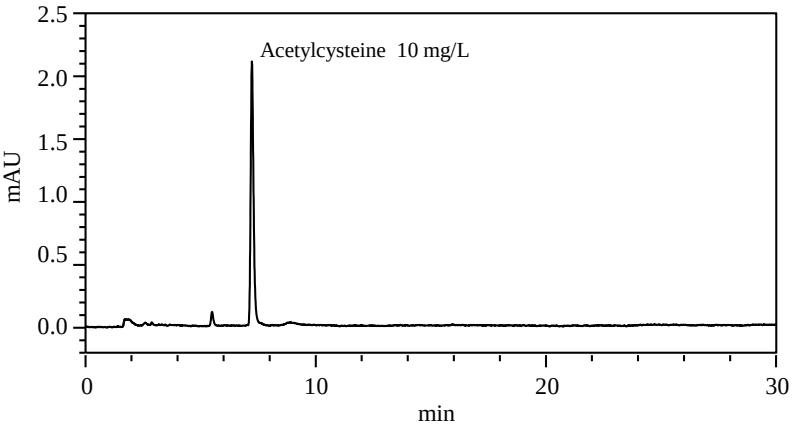
■ Analysis Example for Drugs in the Japanese Pharmacopoeia Sixteenth Edition (Acetylcysteine)

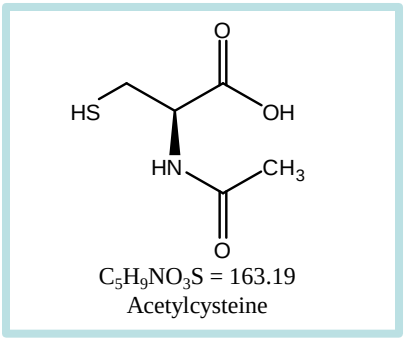
More than 100 drugs as well as their test methods were added to the Japanese Pharmacopoeia Sixteenth Edition, which became effective in April of 2011.
Presented here is an HPLC-UV method for analysis of one of the newly listed items, acetylcysteine, as well as related substances. First, the system suitability was confirmed based on the specified test conditions and then the test for related substances was performed. In the test for related substances, it is necessary to analyze very small peaks and therefore, detector performance becomes an important factor. The Hitachi high-speed liquid chromatograph "Chromaster" 5410 UV Detector achieves low noise and low drift, allowing for the required high sensitivity analysis.
In this application, a general reagent acetylcysteine was used as the model sample of this drug.

■Confirmation of System Suitability





[Analysis Example of Acetylcysteine]



$C_5H_9NO_3S$ = 163.19
Acetylcysteine

[Structural Formula of *N*-Acetyl-L-cysteine]

<Analytical Conditions>

Column	:	HITACHI LaChrom C18 (5 μm) 4.6 mm I.D. × 250 mm
Elluent	:	Dilute phosphoric acid (1 →2500) / Acetonitrile = 19 / 1 (v/v)
Flow rate	:	1.1 mL/min
Column temperature	:	40ºC
Detection wavelength	:	UV 220 nm
Injection vol.	:	10 μL

* Adjust the flow rate so that the retention time of acetylcysteine is about 7 minutes.

[System Suitability]
Under the system suitability for purity (6) related substance in the Japanese Pharmacopoeia Sixteenth Edition, the "Test for required detection," "System performance," and "System reproducibility" are specified. The specification values and the analysis results obtained are summarized below. The results indicate that the requirements for all items were satisfied.

Item		Specification values for system suitability	Analysis results
Test for required detection	(Peak area of 2 mg/L / Peak area of 10 mg/L) × 100 (%)	15 - 25	19.6
System performance	Number of theoretical plates (10 mg/L)	NLT 15000	17554
	Symmetry factor (10 mg/L)	NMT 1.5	1.29
System reproducibility	Relative standard deviation of peak area (n = 6) (10 mg/L)	NMT 2.0%	0.58%

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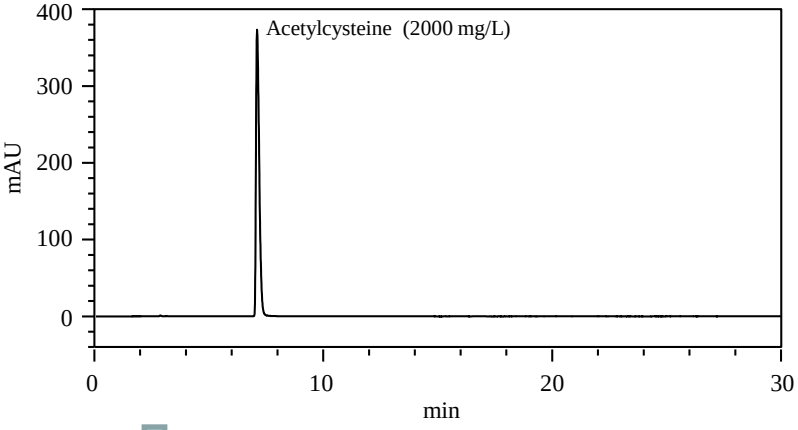
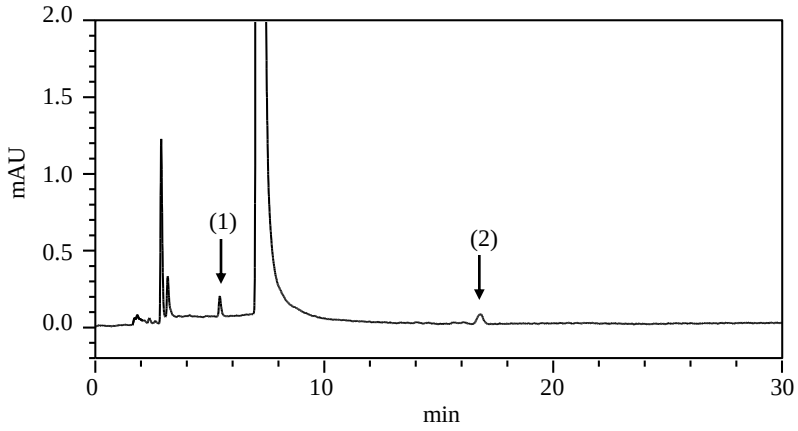
■Test for Relative Substances



Enlarge



[Analysis Example of Acetylcysteine]

<Analytical Conditions>

Column	:	HITACHI LaChrom C18 (5 μm) 4.6 mm I.D. × 250 mm
Eluent	:	Dilute phosphoric acid (1 → 2500) / Acetonitrile = 19 / 1 (v/v)
Flow rate	:	1.1 mL/min
Column temperature	:	40°C
Detection wavelength	:	UV 220 nm
Injection vol.	:	10 μL

* Adjust the flow rate so that the retention time of acetylcysteine is about 7 minutes.

Multiple peaks representing the related substances of acetylcysteine were found.
Each of the very small peaks, (1) and (2), has an area equivalent to about 0.03% of the area of acetylcysteine (2000 mg/L). In this example, the S/N of these peaks are respectively 28 and 13, indicating that sufficiently reliable peak heights and areas are obtained.

[Test for Related Substances]
Under the purity (6) related substance in the Japanese Pharmacopoeia Sixteenth Edition, two types of numerical values are specified for the peaks other than acetylcysteine peak. The specification values and the analysis results obtained are summarized below. The results indicate that the requirements for all items were satisfied.

	Specification values for related substances	Analysis results
Areas of peaks other than acetylcysteine	Each related substance is NMT 0.3%	Each related substance is NMT 0.16%
	Total is NMT 0.6%	Total is NMT 0.31%

(2000 mg/L, 10 μL injection)

Main system configuration: Chromaster
5110 pump, 5210 autosampler, 5310 column oven, 5410 UV detector

NOTE: These data are an example of measurement; the individual values cannot be guaranteed.