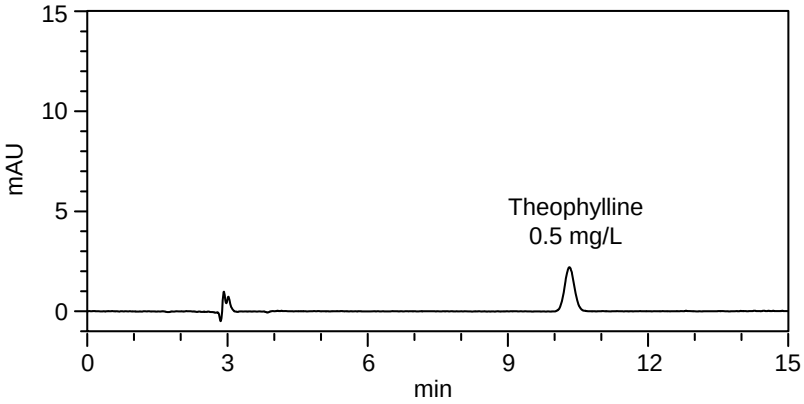
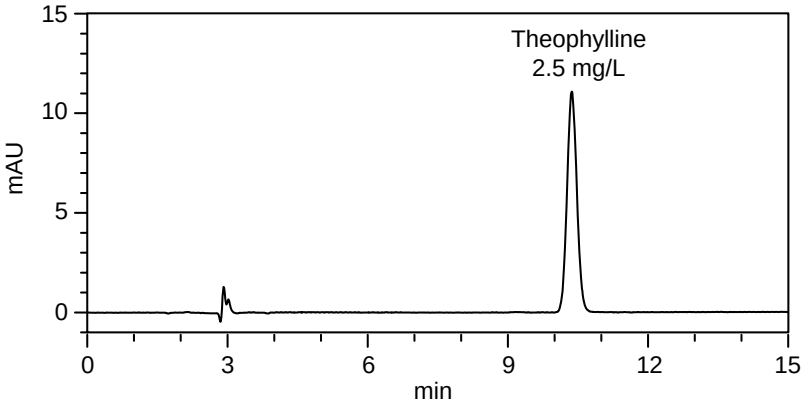
■ Purity Test by High-sensitivity DAD (Theophylline)

AS/LC-022

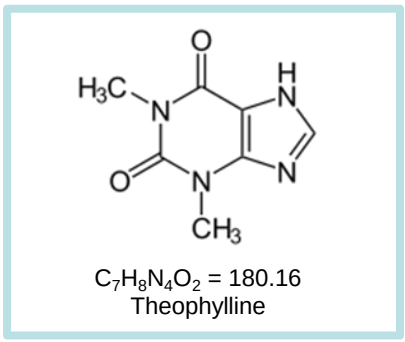
The Japanese Pharmacopoeia is the “government-designated standard for the properties, qualities, etc. of drug products” and is composed of general notices, general rules for crude drugs, general rules for preparations, general tests, processes and apparatus, and other drug related articles. Under the general tests, processes and apparatus section, there is a section of reagents and test solutions, and the test methods for each are specified.
Presented here is the analysis of theophylline, which is listed in the section of reagents and test solutions under the general tests, processes and apparatus section of the revised Japanese Pharmacopoeia 16th Edition. HPLC-UV is used for the purity test (related substances). The system suitability is confirmed first based on the specified analytical conditions and then, the related substances are confirmed. For the confirmation of the related substances, it is necessary to accurately understand very small peaks and thus, the detector performance is an important factor. With the Hitachi high-speed liquid chromatograph Chromaster 5430 DAD, low noise and low drift can be achieved, which allows for analysis with high sensitivity.

■ Confirmation of System Suitability





[Analysis of Theophylline]



$C_7H_8N_4O_2$ = 180.16
Theophylline

[Structural Formula of Theophylline]

<Analytical Conditions>
Column : Inertsil® ODS-3 (5 µm)
: 6.0 mm I.D. × 150 mm (GL Sciences Inc.)
Eluent : 1% acetic acid / methanol = 4 / 1 (v/v)
Flow rate : 1.0 mL/min
Column temperature : 40°C
Detection wavelength : DAD 220 - 400 nm (270 nm)
Injection vol. : 20 µL

* Adjust the flow rate so that the retention time of theophylline is about 10 minutes.

[System Suitability]
Under the System suitability for Purity (Related Substances) in the revised Japanese Pharmacopoeia 16th Edition, the “Test for required detection,” “System performance,” and “System repeatability” are specified. The specification values and the analysis results are summarized below. The results satisfy the requirements for all specifications.

Item		Specification value for system suitability	Analysis result
Test for required detection	(Peak area of 0.5 mg/L / Peak area of 2.5 mg/L) × 100 (%)	15 - 25	20.0
System performance	Number of theoretical plates (2.5 mg/L)	NLT 3000	11592
	Symmetry factor (2.5 mg/L)	NMT 1.5	1.06
System repeatability	Relative standard deviation of peak area (n = 6) (2.5 mg/L)	NMT 3.0%	0.099%

■ Purity Test by High-sensitivity DAD (Theophylline)

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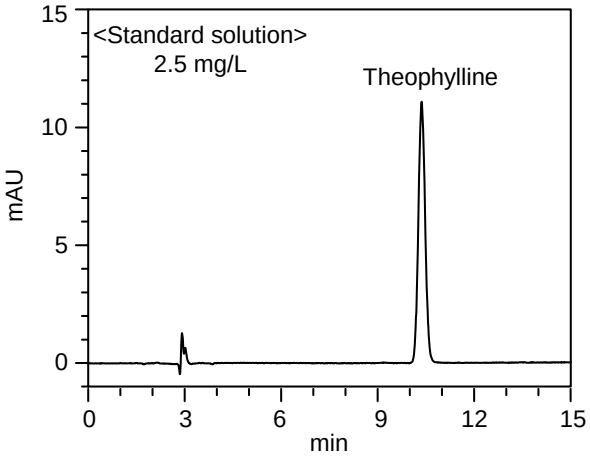
■ Purity Test (Confirmation of Related Substances)



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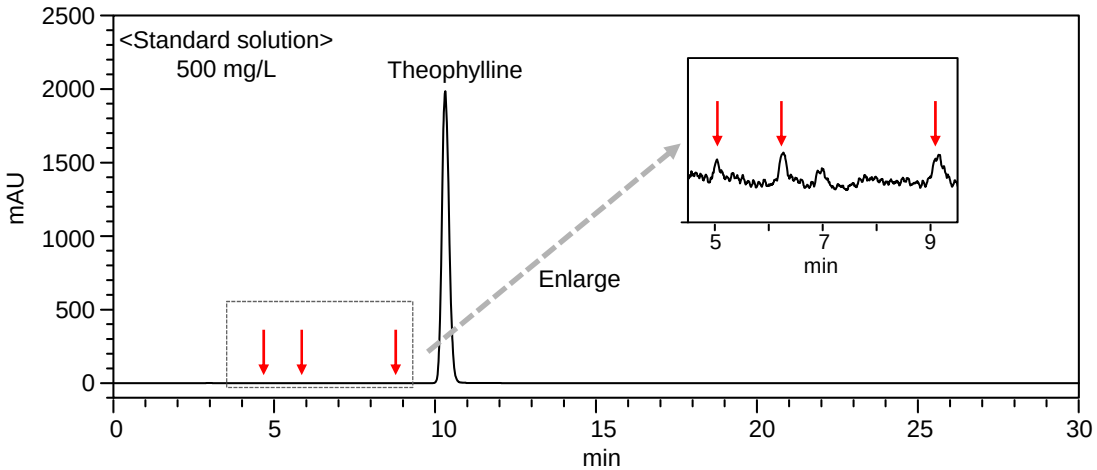

[Analysis Example of Theophylline]

**Several very small peaks other than theophylline peak were observed.
The area of each of these very small peaks was about 0.001% of the area of theophylline peak.
This indicates that the simultaneous analysis of the major component and trace components is possible.**

**[Confirmation of Related Substances]
According to the method for the Purity (Related substances) of theophylline reagent described in the revised Japanese Pharmacopoeia 16th edition, the result is to be determined based on two types of data obtained at different concentrations. The result is to be evaluated by comparing the theophylline peak area of the low concentration solution (standard solution: 2.5 mg/L) and the total area of the peaks other than theophylline peak in the high concentration solution (sample solution: 500 mg/L). As a result, the numerical value satisfying this requirement was obtained.**

Item	Specification value	Analysis result
Confirmation of related substances	(1) Peak area of theophylline in standard solution (2.5 mg/L) > (2) Total area of peaks other than theophylline in sample solution (500 mg/L) (However, the peak area measurement range is about 3 times of the retention time of theophylline)	○ ((1) 162037 > (2) 1092)

Main system configuration: Chromaster 5110 pump, 5210 autosampler, 5310 column oven, 5430 DAD

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