SHIMADZU APPLICATION NEWS
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● LIQUID CHROMATOGRAPHY MASS SPECTROMETRY
No.C57

Application using Analytical / Preparative LC-MS System (Part 1)

Normally, as a large quantity of sample is used in preparative isolation, chromatography is conducted beforehand using a small amount of sample to preserve as much as possible for fractionation. Here we introduce an example of measurement using a system consisting of parallel analytical and preparative flow lines to integrate these analyses into a combined operation.

Preparative LC-MS is used as a tool for purification of compounds, and accordingly, ease of use without the need for specialized knowledge is required. Therefore, this report also introduces an example in which PsiPort Browser software is used as a facilitating tool in LC-MS analysis.

■ System Configuration for Analysis and Preparative Isolation

Fig.1 shows a flow diagram of the parallel analytical and preparative systems. Switching between the analysis and preparative systems is conducted quickly and surely via the flow selection valve.

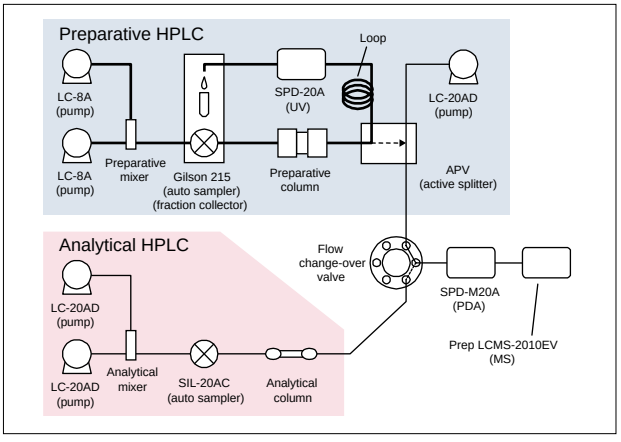


Fig.1 Flow Diagram of Preparative and Analytical LC-MS System

■ Operation Using PsiPort Browser

PsiPort Browser is a software application for web-based instrument control, data review and report generation using just a few mouse clicks. The operator need only select a method created beforehand by a system administrator, and specify the sample vial positions. Moreover, the instrument status is always indicated for easy verification, and the analytical results can be checked using any web browser (Internet Explorer, etc.).

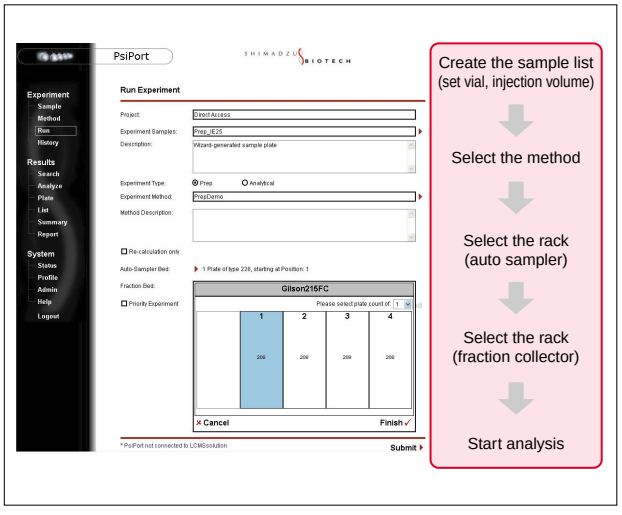


Fig.2 Analysis Startup Window using PsiPort Browser

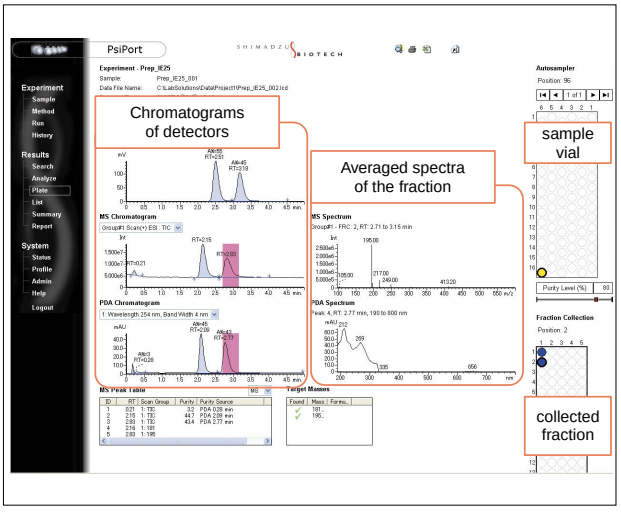


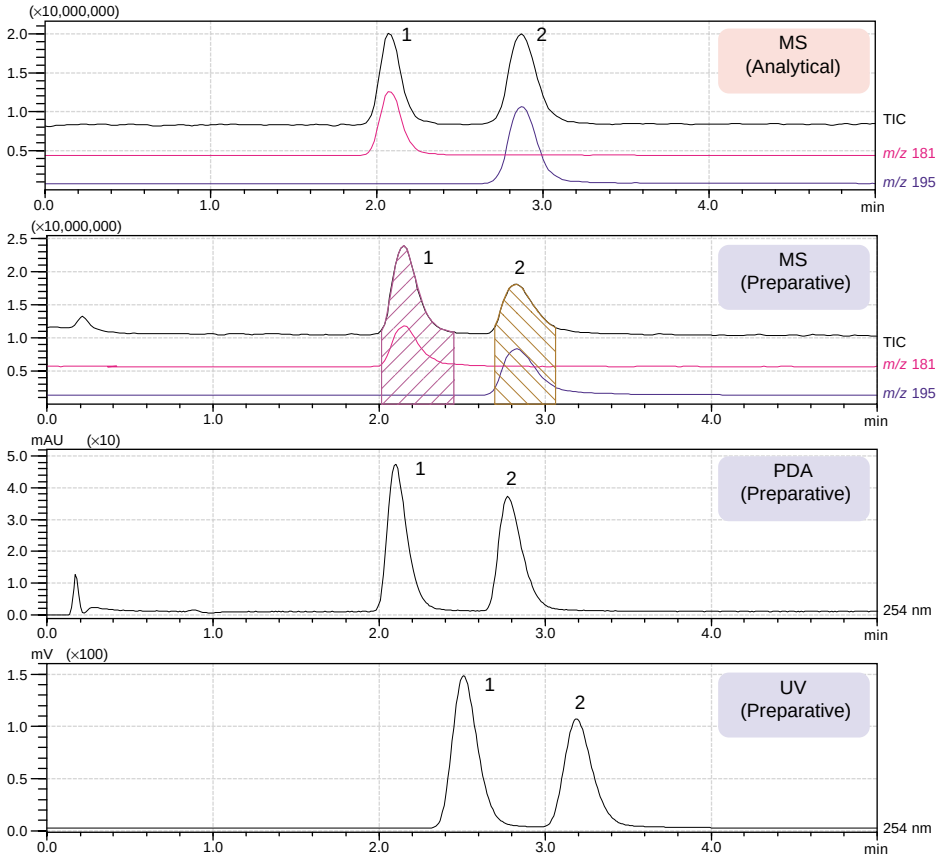
Fig.3 Results Display using PsiPort Browser

■ Analysis and Preparative Isolation of Caffeine and Theophylline

Here we introduce examples of measurement of caffeine and theophylline as test compounds. In order to obtain results of equivalent resolution in both analysis and preparative operations, columns having the same packing material and same length were selected, and the same linear velocity flow rate setting was used for both.

Using the analytical conditions shown below, the theophylline protonated molecule ($[M+H]^+$) *m/z* 181 was detected at a retention time of 2.07 minutes, and the caffeine protonated molecule ($[M+H]^+$) *m/z* 195 was detected at a retention time of 2.87 minutes. Moreover, under the larger scale preparative conditions, the same retention behavior was observed for each compound, and both compounds were fractionated accordingly.

In this way, by investigating the preparative conditions and determining the presence of the target compounds and impurities at the analytical scale, fractionation can be accomplished efficiently, without wasting precious sample.



[Peaks]
1: theophylline, 2: caffeine

Fig.4 Analysis and Preparative Isolation of Theophylline and Caffeine

Table 1 Analytical and Preparative Conditions

Preparative column	: Gemini 5 µm C18 Axia packed (50 mmL. × 21.2 mmI.D.)	Split ratio	: 1 / 500
Analytical column	: Gemini 5 µm C18 (50 mmL. × 2.0 mmI.D.)	Probe voltage	: +4.5 kV (ESI-Positive mode)
Mobile phase	: Water containing 0.1% formic acid / Acetonitrile = 75 / 25	Nebulizing gas flow	: 1.5 L/min
Flow rate	: 20 mL/min (preparative condition)	Drying gas pressure	: 0.1 MPa
	0.2 mL/min (analytical condition)	CDL temperature	: 250 ˚C
Injection volume	: 100 µL (preparative condition)	Block heater temperature	: 200 ˚C
	2 µL (analytical condition)	CDL, Q-array voltage	: using Default values
Column temperature	: Room temperature	Scan range	: *m/z* 100-500 (1.0 sec)
Make-up flow	: Methanol (0.2 mL/min)		

NOTES:
✻This Application News has been produced and edited using information that was available when the data was acquired for each article. This Application News is subject to revision without prior notice.

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