SHIMADZU

LC-MS Application Data Sheet No. 035

Drug Analysis In Plasma By LC-MS:
ON-LINE Pretreatment Method
For Removal of Proteins

LC-MS is a popular analysis method in various fields due to its high selectivity. Previously, extensive deproteinization was required for the LC-MS analysis of drugs in biological samples and other samples containing proteins. A system has now been created that automates deproteinization and analysis of drugs in plasma.

Figure1 shows the flow diagram of the automatic deproteinization system. Use of the appropriate pretreatment mobile phase and pretreatment column permits trapping of the target components in the sample and elution of proteins from the column. A polyvinyl-alcohol reverse-phase pretreatment column and 10mM aqueous ammonium acetate solution as the mobile phase for pretreatment were adopted for this system.

The target components are trapped in the pretreatment column after the sample is injected by the auto-injector. After total elution of the proteins in the sample, the high-pressure column-switching valve is switched. Then, the trapped target components are eluted from the pretreatment column, introduced to the analytical column and detected by the mass spectrometer. This series of operations is conducted by a preset software program, to permit fully automatic analysis, including deproteinization. No complex tasks are required.

Figure 2 shows the example of an analysis of a sample equilibrated by the addition of drug “A”. The high-pressure column-switching valve was set to be switched five minutes after injection of the sample. Due to the effects of interfering components, the drug A peak could not be confirmed on the UV chromatogram. However, the SIM chromatogram is unaffected by the interfering components, and the drug A peak could be confirmed at a retention time of approximately 13.5 minutes.

This system also eliminates salts and other compounds from samples that influence the condition of the mass spectrometer.

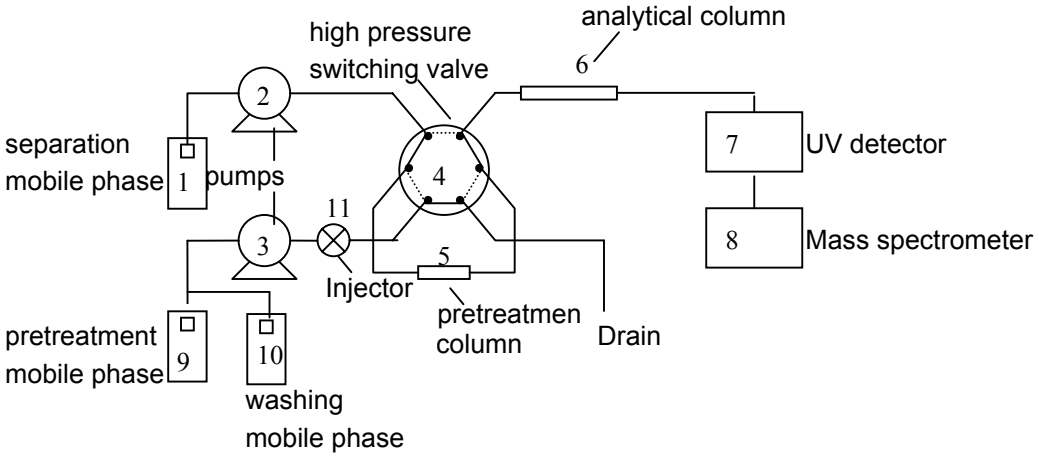

Figure 1
Schematic of Automated Deproteinizing System

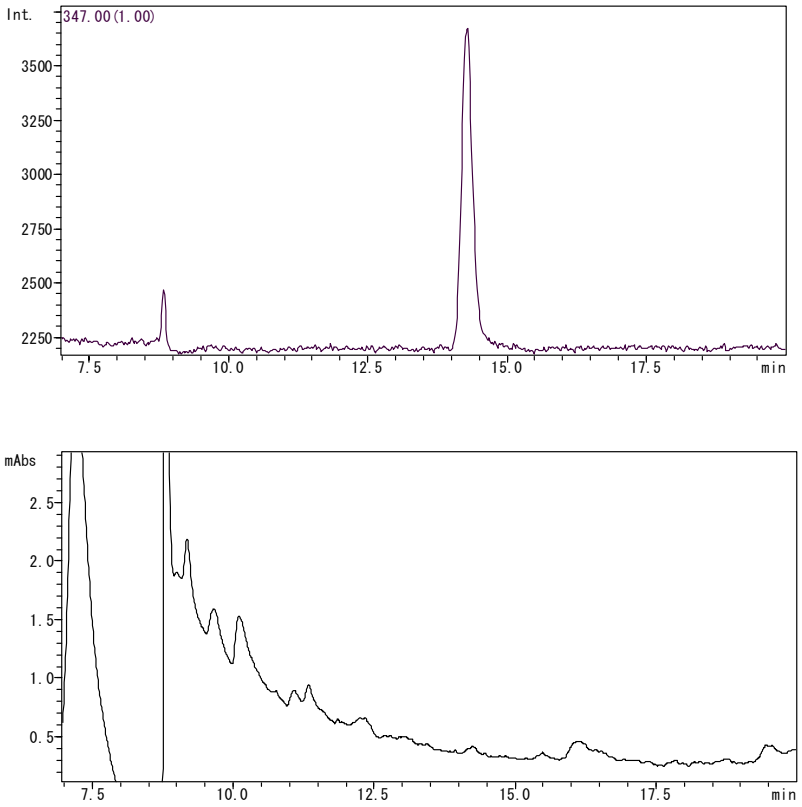

Figure 2. UV chromatogram (upper) and mass chromatogram (lower) of drug A containing plasma

Table 1 Analytical conditions

[Pretreatment]			
Column	: Shim-pack SPC-RP3 (4.0 mmI.D. x 30 mm)		
Mobile phase	: water containing 10mM ammonium acetate		
Flow rate	: 1mL/min		
Column temperature	: 40°C		
[Analysis]			
Column	: Shim-pack VP-ODS (2.0 mmI.D. x 150 mm)		
Mobile phase	: 55% acetonitrile - water containing 10mM ammonium acetate		
Flow rate	: 0.2 mL/min		
Injection volume	: 20uL		
Column temperature	: 40 °C		
Probe voltage	: +4.5 kV (ESI-Positive mode)		
CDL temperature	: 250°C	**BH temperature**	: 200°C
Nebulizing gas flow	: 4.5 L/min		
CDL voltage	: -30V		
Q-array DC voltage	: 35V	**Q-array RF**	:150V

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