SHIMADZU APPLICATION NEWS

LAAN-A-LC-E007A

● HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

No.L307A

Direct Injection of Blood Plasma for the Determination of Drugs using "Co-Sense for BA" (Part 5)

The "Co-Sense for BA" biosample analysis system allows the direct injection of filtered blood plasma or blood serum samples into HPLC for analysis. Its major feature is the automation of the deproteinization and other pretreatment processes. Application News No. L285, L286, L293, and L305 covered the operating principle and applications of "Co-Sense for BA."

This Application News introduces examples of the analysis of drugs in blood plasma with Co-Sense for BA LCMS, which uses an LCMS-2010A Single-quadrupole Liquid Chromatograph Mass Spectrometer as the detector. This system is extremely effective for samples with a complex matrix, such as biosamples, and is expected to reduce analysis times.

■ Analysis of Basic Drugs

Formic acid, acetic acid, trifluoroacetic acid, and combinations of their ammonium-salt aqueous solutions and acetonitrile, are often used for the analysis of basic drugs by ESI.

A neutral mobile phase such as ammonium acetate results in greater sample carryover of highly hydrophobic drugs than an acidic mobile phase. The decision on whether to use an acidic or neutral mobile phase must be based on consideration of ionization efficiency, sample carryover, recovery, and separation.

Measurements on five basic drugs using a formic acid mobile phase are introduced here. They were monitored with the detector in ESI-positive mode using selected ion monitoring (SIM) on the $[M+H]^+$ ions of each drug component (see Fig. 1). The diagram indicates that each component was satisfactorily detected. Approximately ten minutes was required for each analysis, with two minutes for pretreatment, three minutes for gradient elution, two minutes to wash the analysis column, and two minutes for initial equalization, plus the Autosampler SIL-HT operation time and needle outer surface rinse time.

The examples presented in this Application News were all analyzed using a system with the flowpath configuration shown in Fig. 4. The installation of a switching valve upstream of the pretreatment pump allowed elimination of residual matrix and sample carryover by the washing liquid. A 1/4 (v/v) mixture of 0.1% formic acid/acetonitrile was used as the washing liquid to flush the pretreatment lines during analysis.

Table 1 Analytical Conditions

For Sample Injection	
Column	: Shim-pack MAYI-ODS (10mmL. × 4.6mmI.D.)
Mobile Phase	: Water/Acetonitrile = 95/5(v/v) containing 0.1% formic acid
Flow Rate	: 3.0mL/min
Dilution Factor	: 8
For Separation	
Column	: Chromolith SpeedRod (50mmL. × 4.6mmI.D.)
Mobile Phase	: A: Water/Acetonitrile = 95/5(v/v) containing 0.1% formic acid B: Acetonitrile containing 0.1% formic acid Linear gradient B30%→90%(2→5min)
Flow Rate	: 0.5mL/min
Temperature	: 40˚C
Probe Voltage	: 4.5kV (ESI-positive mode)
Nebulizing Gas	: 1.5L/min
Drying Gas	: 0.1MPa

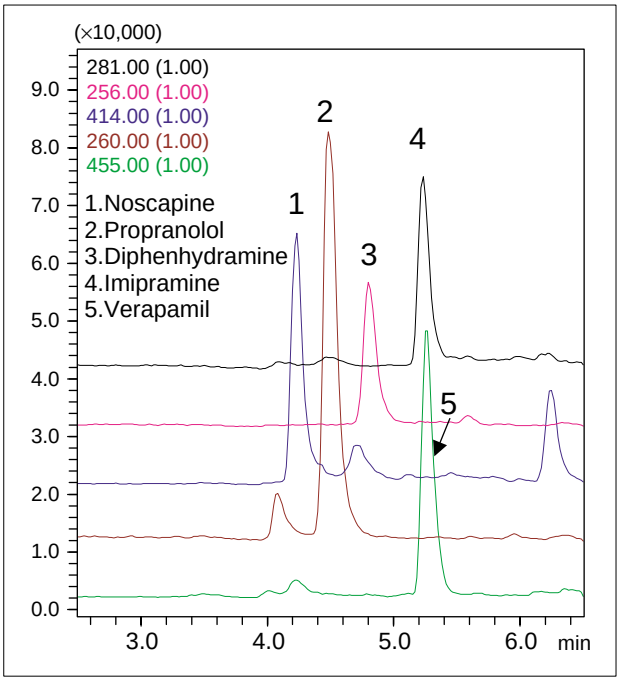

Fig.1 SIM Chromatogram of 5 Basic Drugs in Blood Plasma (10ng/mL spiked, 50μL injected)

■ Analysis of Ketoprofen and Warfarin

Warfarin and Ketoprofen are detected well in the ESI positive mode. 1/4 (v/v) mixture of water/acetonitrile was used to wash the pretreatment lines.

Table 2 Analytical Conditions

For Sample Injection	
Column	: Shim-pack MAYI-ODS (10mmL. × 4.6mmI.D.)
Mobile Phase	: A: 10mM (Ammonium) formate buffer (pH=3.7)
	: B: Acetonitrile
	A/B= 90/10(v/v)
Flow Rate	: 3.0mL/min
Dilution Factor	: 8
For Separation	
Column	: Shim-pack FC-ODS (75mmL. × 4.6mmI.D.)
Mobile Phase	: A: 10mM (Ammonium) formate buffer (pH=3.7)
	B: Acetonitrile
	Linear gradient B50%→90%(2→5min)
Flow Rate	: 0.8mL/min
Temperature	: 40˚C
Probe Voltage	: 4.5kV (ESI-positive mode)
Nebulizing Gas	: 1.5L/min
Drying Gas	: 0.2MPa

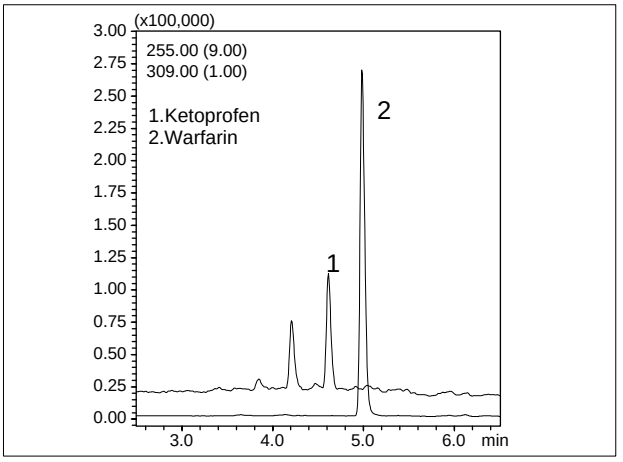

Fig.2 SIM Chromatogram of Warfarin and Ketoprofen in Blood Plasma (100ng/mL spiked, 10μL injected)

■ Analysis of Ibuprofen

Ibuprofen is detected well in the ESI negative mode. 1/4 (v/v) mixture of water/acetonitrile was used as the washing liquid.

Table 3 Analytical Conditions

For Sample Injection	
Column	: Shim-pack MAYI-ODS (10mmL. × 4.6mmI.D.)
Mobile Phase	: 10mM Ammonium acetate /Acetonitrile =95/5(v/v)
Flow Rate	: 3.0mL/min
Dilution Factor	: 8
For Separation	
Column	: Shim-pack FC-ODS (75mmL. × 4.6mmI.D.)
Mobile Phase	: A: 10mM Ammonium acetate
	B: Acetonitrile
	Linear gradient B40%→90%(2→4min)
Flow Rate	: 0.6mL/min
Temperature	: 40˚C
Probe Voltage	: -3.5kV (ESI-negative mode)
Nebulizing Gas	: 1.5L/min
Drying Gas	: 0.2Mpa

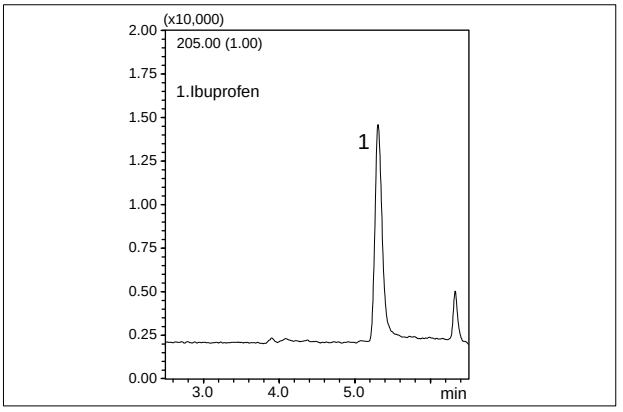

Fig.3 SIM Chromatogram of Ibuprofen in Blood Plasma (100ng/mL spiked, 50μL injected)

■ Flow Diagram of the System

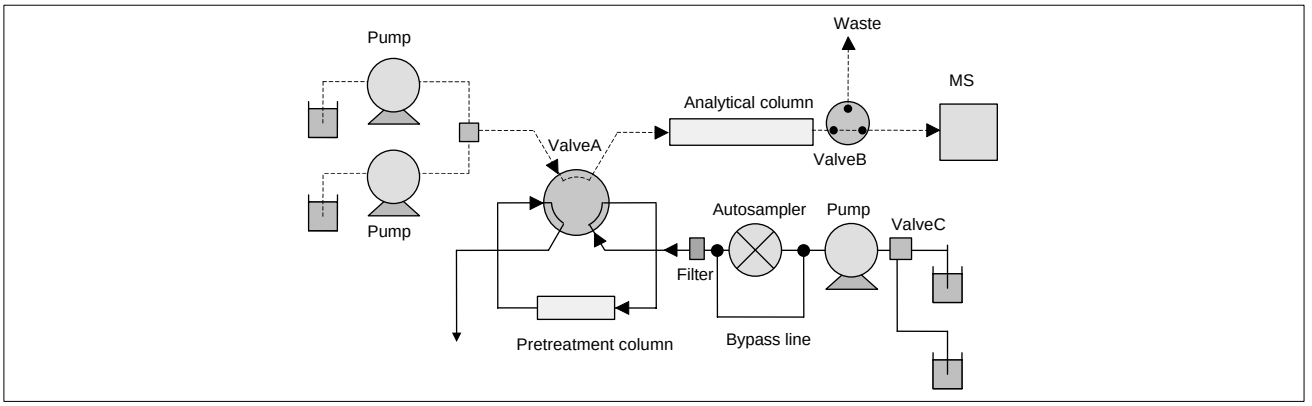

Fig.4 Flow Diagram of the System

*Data presented here was not acquired using instruments approved under the Japanese Pharmaceutical Affaires Law

SHIMADZU

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