SHIMADZU APPLICATION NEWS

LA190-E003A

● HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

No.L286A

Direct Injection of Blood Plasma for the Determination of Drugs using "Co-Sense for BA" (Part 2)

The "Co-Sense for BA" biosample analysis system combines a newly developed Shim-pack MAYI-ODS pretreatment column with column switching technology and a unique on-line dilution bypass to automate and enhance the accuracy of deproteinization pretreatment operations. This Application News introduces examples of the analysis of drugs in blood plasma using Co-Sense for BA.
See Application News No. L285 for information on the operating principle and features of Co-Sense for BA.

■ Establishing the Analytical Conditions

As drugs in blood plasma are frequently bonded to proteins, deproteinization can result in lower drug recovery. Consequently, a mobile phase for sample injection that promotes to weaken the bond between proteins and drugs must be selected for Co-Sense for BA. Controlling the pH and ionic strength of the mobile phase for sample injection and the addition of organic solvents are effective because drugs and proteins are bonded through ionic or hydrophobic interactions. In this example, acetic acid (sodium) buffer solutions with and without the addition of up to 10% acetonitrile were used as the mobile phase for sample injection. The injected blood plasma sample is automatically diluted 8 times by the mobile phase for sample injection in the dilution bypass.
The Shim-pack MAYI-ODS pretreatment column selectively excludes macromolecules such as proteins and effectively traps drugs and other low-molecular compounds.
An SPD-M10A*VP* photodiode array detector was a useful tool for selecting the optimal wavelength to improve selectivity between drugs and impurities.
The examples introduced below are analyses of drug standards spiked in filtered human blood plasma using Co-Sense for BA. Refer to Tables 1 to 4 for details about the individual analytical conditions.

■ Analysis of Warfarin in Plasma

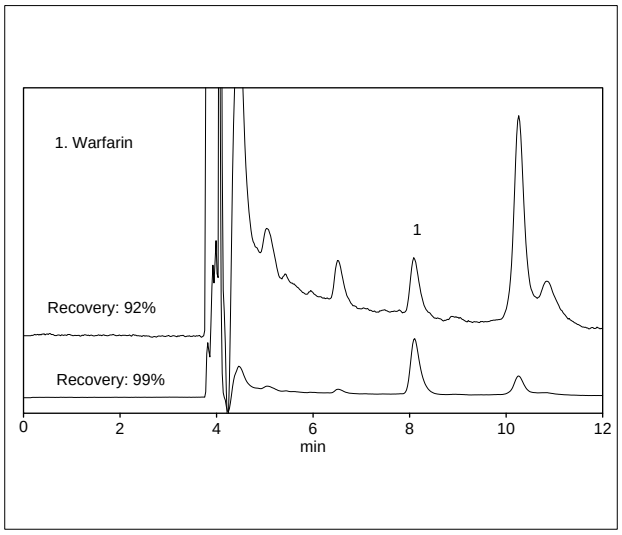

Fig. 1 Chromatogram of Warfarin in Plasma (upper: spiked 0.1μg/mL, 50μL injected; lower: spiked 1μg/mL, 50μL injected)

Table 1 Analytical Conditions

For Sample Injection	
Column	: Shim-pack MAYI-ODS (10mmL.×4.6mmI.D.)
Mobile Phase	: A : 100mM Acetate (Na) buffer <pH=4.7>
	B : Acetonitrile
	A/B = 95/5 (v/v)
Flow Rate	: 2.0mL/min
Dilution Factor	: 8
For Separation	
Column	: Shim-pack FC-ODS (75mmL.×4.6mmI.D.)
Mobile Phase	: A : 20mM Phosphate (Na) buffer <pH=2.5>
	B : Methanol
	A/B = 40/60 (v/v)
Flow Rate	: 1.0mL/min
Temperature	: 40˚C
Detection	: SPD-M10A*VP* at 315nm

■ Analysis of Naproxen in Plasma

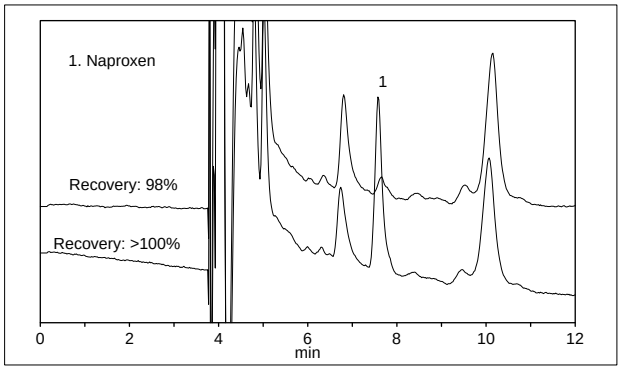

Fig. 2 Chromatogram of Naproxen in Plasma (upper: spiked 0.1μg/mL, 50μL injected; lower: spiked 1μg/mL, 50μL injected)

Table 2 Analytical Conditions

For Sample Injection	
Column	: Shim-pack MAYI-ODS (10mmL.×4.6mmI.D.)
Mobile Phase	: A : 0.1% Phosphoric acid
	B : Acetonitrile
	A/B = 95/5 (v/v)
Flow Rate	: 2.0mL/min
Dilution Factor	: 8
For Separation	
Column	: Shim-pack FC-ODS (75mmL.×4.6mmI.D.)
Mobile Phase	: A : 20mM Phosphate (Na) buffer <pH=2.5>
	100mM Sodium perchlorate
	B : Methanol
	A/B = 40/60 (v/v)
Flow Rate	: 1.0mL/min
Temperature	: 40˚C
Detection	: SPD-M10A*VP* at 330nm

■ Analysis of Six Drugs in Plasma

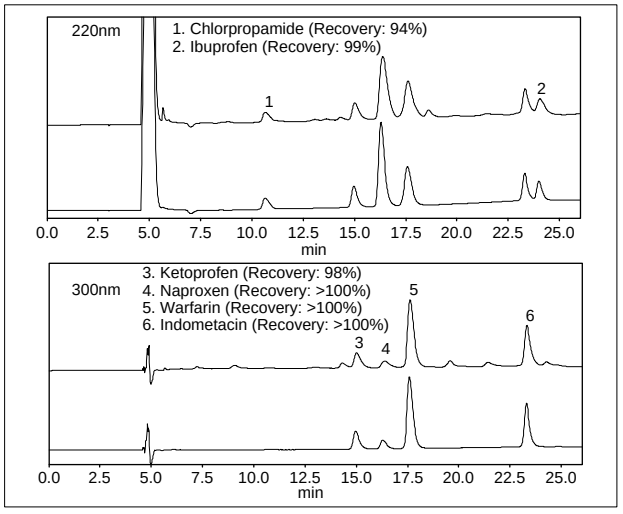

Fig. 3 Chromatogram of Six Drugs in Plasma (upper: spiked 2μg/mL each; lower: 2μg/mL standard; 100μL injected in all cases)

Table 3 Analytical Conditions

For Sample Injection	
Column	: Shim-pack MAYI-ODS (10mmL.×4.6mmI.D.)
Mobile Phase	: A : 100mM Acetate (Na) buffer <pH=4.7>
	B : Acetonitrile
	A/B = 90/10 (v/v)
Flow Rate	: 2.0mL/min
Dilution Factor	: 8
For Separation	
Column	: Shim-pack VP-ODS (150mmL.×4.6mmI.D.)
Mobile Phase	: A : 20mM Phosphate (Na) buffer <pH=2.5>
	B : Methanol
	Linear gradient B 50%→70% (4-19min.)
Flow Rate	: 1.0mL/min
Temperature	: 40˚C
Detection	: SPD-M10A*VP* at 220nm and 300nm

■ Analysis of Eight Drugs in Plasma

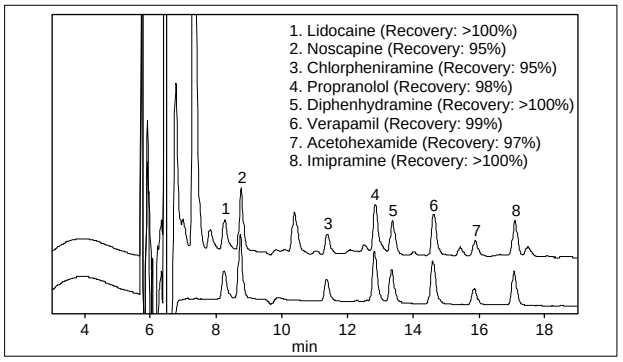

Fig. 4 Chromatogram of Eight Drugs in Plasma (upper: spiked 0.5μg/mL each; lower: 0.5μg/mL standard; 50μL injected in all cases)

Table 4 Analytical Conditions

For Sample Injection	
Column	: Shim-pack MAYI-ODS (10mmL.×4.6mmI.D.)
Mobile Phase	: 100mM Acetate (Na) buffer <pH=4.7>
Flow Rate	: 2.0mL/min
Dilution Factor	: 8
For separation	
Column	: Shim-pack VP-ODS (250mmL.×4.6mmI.D.)
Mobile Phase	: A : 20mM Phosphate (Na) buffer <pH=2.5>
	100mM Sodium perchlorate
	B : Methanol
	Linear gradient B 50%→70% (4-15min.)
Flow Rate	: 1.0mL/min
Temperature	: 40˚C
Detection	: SPD-M10A*VP* at 205nm

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

SHIMADZU

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