SHIMADZU APPLICATION NEWS

LA190-E002A

● HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

No.L285A

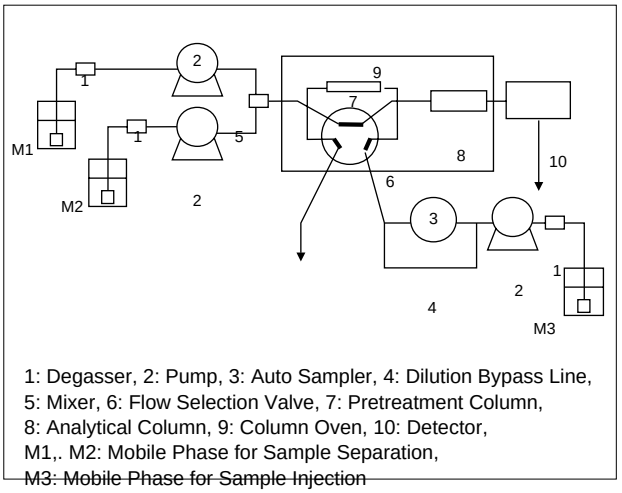
Direct Injection of Blood Plasma for the Determination of Drugs using "Co-Sense for BA" (Part 1)

Impurities such as proteins must be eliminated by pretreatment before HPLC analysis of drugs contained in biosamples, such as blood plasma and blood serum. Generally, deproteinization is conducted by centrifugal separation after adding a reagent, such as an organic solvent or acid. However, as this manual process complicates operations and may lower the analysis accuracy, an automatic deproteinization process is desirable.

The "Co-Sense for BA" biosample analysis system combines a newly developed pretreatment column with column switching technology and a unique on-line dilution bypass to automate and enhance the accuracy of pretreatment operations, such as deproteinization. This Application News introduces the operating principle and features of Co-Sense for BA.

■ Operating Principle of Co-Sense for BA

Co-Sense for BA is equipped with a newly developed pretreatment column "Shim-pack MAYI-ODS" and a dilution bypass, so it can automate deproteinization process for the analysis of drugs in blood plasma Fig. 1 shows a flow diagram for Co-Sense for BA. Shim-pack MAYI-ODS is an internal reversed-phase column with a hydrophilic polymer coating, constructed as shown in Fig. 2 Proteins and other macromolecules are blocked by the hydrophilic polymer coating and cannot enter the micropores. However, drugs can penetrate into the micropores, where they are trapped in the stationary phase. Consequently, the column selectively excludes macromolecules such as proteins.



1: Degasser, 2: Pump, 3: Auto Sampler, 4: Dilution Bypass Line,
5: Mixer, 6: Flow Selection Valve, 7: Pretreatment Column,
8: Analytical Column, 9: Column Oven, 10: Detector,
M1,. M2: Mobile Phase for Sample Separation,
M3: Mobile Phase for Sample Injection

Fig. 1 Flow Diagram

■ Analysis Procedure

When a sample such as pre-filtered blood plasma is injected by the autosampler (3), it is transported by the mobile phase for sample injection (M3) into the Shim-pack MAYI-ODS pretreatment column (7). The autosampler incorporates a dilution bypass line (4) to enhance the recovery of the drug from the proteins. (See next page for details.) When the high-pressure flow channel selection valve (6) is turned 60˚, the drug trapped in the Shim-pack MAYI-ODS column is transported for separation in the analytical column (7) by the mobile phases for sample separation (M1, M2).

* The numbers refer to numbers in Fig. 1.

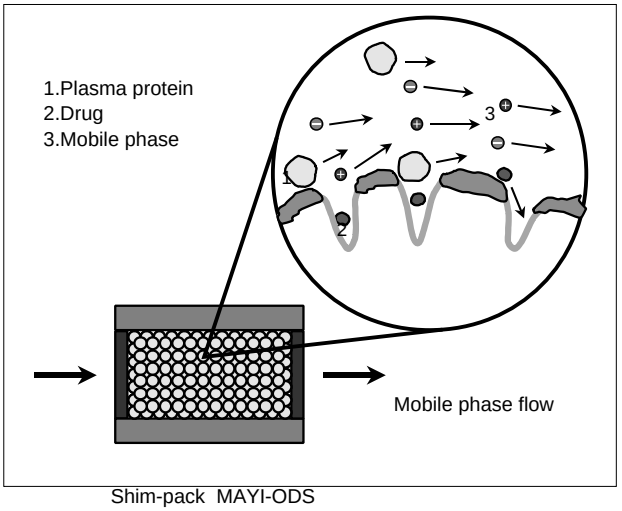


Fig. 2 Principle of Deproteinization

■ Deproteinization by Shim-pack MAYI-ODS

The analysis of blood plasma with added isopropylantipyrine is shown in Fig. 3 to illustrate the effectiveness of deproteinization by Shim-pack MAYI-ODS. Table 1 shows the analytical conditions.
The lower chromatogram in Fig. 3 indicates that protein elution occurs within three minutes. By switching the valve after protein elution, low-molecular compounds such as drugs can be selectively transferred to the analytical column to obtain the upper chromatogram in Fig. 3.

Table 1 Analytical Conditions

For Sample Injection	
Column	: Shim-pack MAYI-ODS (10mmL.×4.6mmI.D.)
Mobile Phase	: 0.1% Phosphoric acid/Acetonitrile=95/5 (v/v)
Flow Rate	: 2.0mL/min
Dilution Factor	: 8
For Separation	
Column	: Shim-pack VP-ODS (150mmL.×4.6mmI.D.)
Mobile Phase	: A : water B : Acetonitrile Linear gradient B 5%→95%
Flow Rate	: 1.0mL/min
Temperature	: 40˚C
Detection	: SPD-10AV*VP* at 275nm for isoprropylantipyrine at 280nm for plasma protein

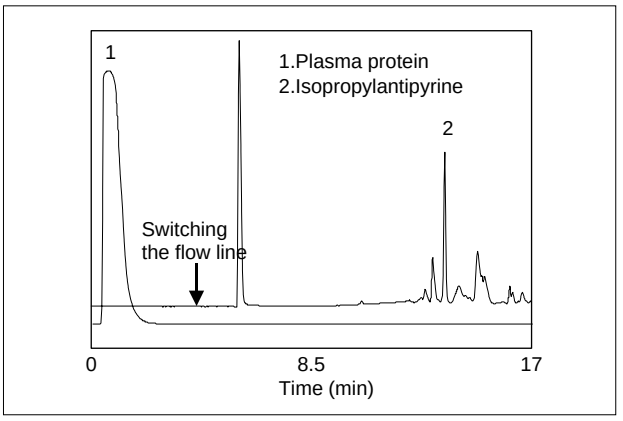


Fig. 3 Effect of Deproteinization (spiked 1μg/mL, 100μL injected)

■ Effects of On-line Dilution Bypass on Recovery

The injected sample was automatically diluted with mobile phase for sample injection(buffer solution with up to 10% organic solvent added)by eight times in a dilution bypass line to recover drugs reliably, even if drugs strongly bonded to proteins.
Fig. 4 shows the evaluation of the effects of the dilution bypass, using blood plasma with added indometacin, which strongly bonds to proteins. Table 2 shows only the analysis conditions that differ from Table 1. The recovery rate with no dilution bypass was approximately 50% for a 500μL sample injection, but improved to almost 100% when a bypass was provided.

Table 2 Analytical Conditions

Mobile phase	: A : 0.1% Trifluoroacetic acid in water B : 0.1% Trifluoroacetic acid in acetonitrile Linear gradient B 5%→95%
Flow Rate	: 1.0mL/min
Temperature	: 40˚C
Detection	: SPD-10AV*VP* at 315nm

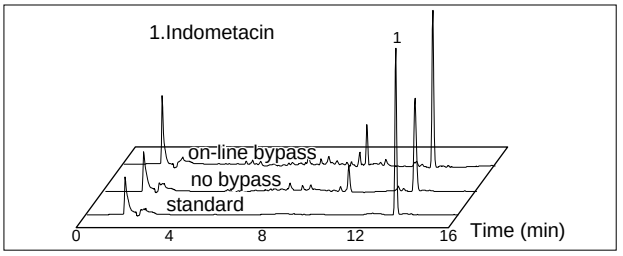


Fig. 4 Effect of On-line Dilution Bypass (spiked 1μg/mL, 500μL injected)

■ Repeatability

Phenytoin and carbamazepine were added to blood plasma samples to achieve a concentration of 1μg/mL. Table 3 lists the analytical conditions and Table 4 shows the results of repeatability evaluations using these samples.

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 = 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 Linear gradient B 50%→85%
Flow Rate	: 1.0mL/min
Temperature	: 40˚C
Detection	: SPD-10AV*VP* at 210nm for phenytoin at 300nm for carbamazepine

Table 4 Repeatability of Phenytoin and Carbamazepine

	Peak Area of Phenytoin	Peak Area of Carbamazepine
NO.1	238911	98548
NO.2	240042	98367
NO.3	237208	98706
NO.4	238317	98481
NO.5	239678	98152
Average	238832	98451
S.D.	1127.9	207.3
C.V. (%)	0.472	0.211

(Spiked 1μg/mL each, 50μL injected.)

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

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