SHIMADZU APPLICATION NEWS

LA190-E015B

● HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

No.L293B

Direct Injection of Blood Plasma for the Determination of Drugs using "Co-Sense for BA" (Part3)

The biosample analysis system "Co-Sense for BA" features the capability of automated pretreatment of biological samples such as blood plasma and blood serum. The principle of the system and application examples were introduced in Application News L285 and L286.

The Co-Sense for BA consists of two flow paths; a pretreatment flow path for injecting the sample and removing proteins, and an analytical flow path for separating and detecting the target substances. When using the "Co-Sense for BA" system, therefore, the pretreatment and the analytical conditions need to be separately optimized.

This Application News introduces examples of setting an analytical program, and optimizing the analytical conditions.

■ Analytical Program

Fig. 1 shows the chromatogram of diazepam contained in blood plasma, analyzed using the "Co-Sense for BA". The analytical conditions and analytical program are shown in Table 1 and Fig. 2 respectively. The upper part of Fig. 2 indicates the concentration of methanol in the mobile phase for sample separation (upper) and the flow rate of the mobile phase for sample injection (lower). The lower part of Fig. 2 indicates the switching timings of the high pressure flow channel selection valve.

By switching the high pressure flow channel selection valve three minutes after starting the analysis as shown in Fig. 2, macro molecules such as proteins can be removed from the system, and only compounds with smaller molecules are introduced into the analytical column.

The methanol concentration in the analytical column is kept at 60% until the diazepam is eluted, and after diazepam elution, the methanol concentration is increased to 85% to remove the impurities remaining in the columns. Depending on the analysis target, gradient elution may be conducted. The flow rate of the mobile phase for sample injection was 2 mL/min at first, but after the high pressure flow channel selection valve was switched over, it was reduced to 0.2 mL/min to lower its consumption.

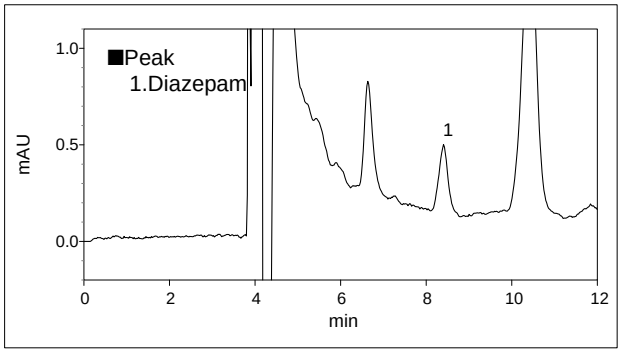

Fig.1 Chromatogram of Diazepam in Blood Plasma (0.2μg spiked, 50μL injected)

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 312nm

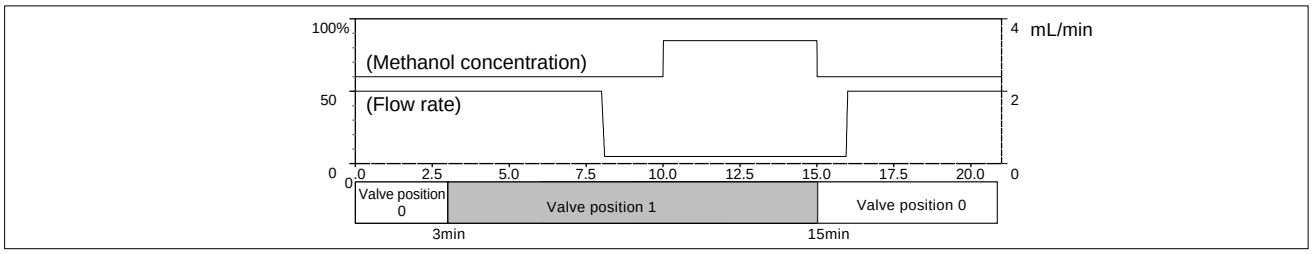

Fig.2 Analytical Program

■ Effect of Mobile Phase for Sample Injection on Drug Recovery Rate

Drugs contained in blood plasma are often bonded to proteins. Therefore, it is necessary to select a mobile phase for sample injection that promotes the separation of the drugs from the proteins. The drugs and proteins are bonded ionically or hydrophobically. They can be effectively separated by controlling the pH level, ion intensity, and organic solvent concentration of the mobile phase.

Table 2 shows the changes in the recovery rate of acidic drugs when the buffer type, pH, and ion type of the mobile phase were changed. When using a 100 mM acetate (Na) buffer solution (pH = 4.7) or 100 mM phosphate (Na) buffer solution (pH = 2.1), high recovery rates were obtained for all drugs.

Table 3 shows the changes in the recovery rate of basic drugs when the ion intensity and organic solvent concentration of the mobile phase were changed. For chlorpheniramine and propranolol, the higher the ion intensity, the higher the recovery rate. The recovery rate of imipramine, which has relatively high hydrophobicity, increases by adding acetonitrile to the mobile phase.

Table 2 Recovery of Acidic Drugs in Plasma (2μg/mL spiked each, 50μL injected)

Mobile phase for sample injection	Recovery (%)		
	Ketoprofen	Naproxen	Warfarin
100mM Acetate (Na) buffer<pH=4.7>/Acetonitrile =9/1 (v/v)	98	100<	100<
100mM Phosphate (Na) buffer <pH=2.1>/Acetonitrile = 9/1 (v/v)	97	98	98
100mM Ammonium acetate	100<	75	99

Table 3 Recovery of Basic Drugs in Plasma (0.5μg/mL spiked each, 50μL injected)

Mobile phase for sample injection	Recovery (%)				
	Lidocaine	Chlorpheniramine	Propranolol	Diphenhydramine	Imipramine
10mM Ammonium acetate	100<	80	87	93	78
100mM Ammonium acetate	99	99	91	96	80
100mM Ammonium acetate / Acetonitrile = 95/5 (v/v)	99	100<	99	100<	86
100mM Ammonium acetate / Acetonitrile = 90/10 (v/v)	-	-	-	-	95

■ Drug Elution with Standard Separation Conditions

To set the separation conditions for the analytical column, it is effective to study the elution properties of the target drug in advance. Table 5 shows the elution times of various drugs when analyzed under the conditions given in Table 4. The mobile phase and gradient parameters can be optimized for effective separation of impurity peaks due to substances inherent in blood plasma.

Table 4 Analytical Conditions

For Sample Injection
- Column : Shim-pack MAYI-ODS (10mmL.×4.6mmI.D.)
- Mobile Phase : Optimized Buffer/Acetonitrile Solution for each drug
- Flow Rate : 2.0mL/min
- Dilution Factor : 8

For Separation
- Column : Shim-pack VP-ODS (150mmL.×4.6mmI.D.)
- Mobile Phase : A: Buffer solution (shown in Table 5)
  B: Methanol
  Linear gradient B 15%→85% (4-19min.)
- Flow Rate : 1.0mL/min
- Temperature : 40˚C
- Detection : SPD-M10A*VP*

Table 5 Retention Index

	Mobile phase A (min)	Mobile phase B (min)	Mobile phase C (min)
Lidocaine	13.3	21.5	15.0
Noscapine	14.7	21.6	19.6
Chlorpheniramine	15.6	21.2	16.6
Propranolol	16.9	20.7	16.8
Diphenhydramine	17.2	22.1	17.1
Phenytoin	17.2	17.0	17.1
Isopropylantipyrine	17.7	17.7	17.8
Chlorpropamide	17.8	15.2	17.7
Verapamil	17.8	21.9	17.9
Carbamazepine	17.8	17.8	17.8
Acetohexamide	18.3	15.7	19.2
Reserpine	18.6	22.7	
Imipramine	18.8	24.4	18.9
Nifedipine	18.7	19.0	18.9
Ketoprofen	19.5	16.7	18.7
Naproxen	20.1	16.4	
Diazepam	20.2	20.2	20.2
Warfarin	20.5	15.3	19.3
Phenylbutazone	21.0	17.2	
Ibuprofen	22.2	19.2	21.3

Mobile phase A : 20mM Phosphate (Na) buffer <pH=2.5>
containing 100mM Sodium perchlorate
Mobile phase B : 20mM Phosphate (Na) buffer <pH=6.9>
Mobile phase C : 100mM Acetate (Na) buffer <pH=4.7>

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

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