SHIMADZU APPLICATION NEWS

LAAN-A-LC-E016

● HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

No.L315

Determination of Drugs in Blood Plasma by "Co-Sense for BA" (Part 6)

Co-Sense for BA, an automated pretreatment column-switching HPLC system for analyzing biological samples such as blood plasma and serum, is used as an effective tool in satisfying the requirements for high-throughput analysis in pharmacokinetics testing and metabolic research.
On the other hand, to further increase analysis throughput in recent years, high-throughput columns with 3μm and smaller diameter spherical particles and monolithic columns which consist of a single rod of silica-based material are becoming popular. In particular, since the inlet pressure in monolithic columns is low, analysis can be performed with high flow rate, making them applicable for high throughput analysis.
Introduced here is an analysis of drugs in plasma in an investigation of shortened analysis time using a monolithic type column as the analytical column. For more information on the Co-Sense for BA principle and related application examples, refer to Application News No. L285, 286, 293, 305 and 307.

■ System Configuration

Since the aim of this investigation is to drastically shorten the analysis time, portions of the standard Co-Sense for BA flow line were modified. Fig. 1 shows the flow line diagram.
- Addition of pump for pretreatment line washing
To quickly wash the pretreatment line following deproteinization, a pump was added (Pump 2) to deliver wash solution for the pretreatment line. Although switching between the pretreatment side mobile phase and wash solution is available via a mobile phase switching valve connected to the solvent delivery pump, in order to greatly shorten the analysis time, it was necessary to minimize the time required for mobile phase switching. Therefore, mobile phase switching was performed using Pump 2.
- Elimination of online dilution bypass
The online dilution bypass built into the Co-Sense for BA system is an effective tool for increasing recovery rates, however, this automated pretreatment process increases the overall analysis time. With shortening the analysis time as the top priority in this investigation, the dilution bypass was removed. Considering the pretreatment side mobile phase flow rate of 4.0mL/min and blood plasma sample injection volume of 10μL, this could allow the pretreatment time to be shortened to 0.5min.
- Addition of flow line switching valve before mass spectrometer
To avoid introduction of excessive blood plasma matrix into the mass spectrometer, a flow line switching valve (Valve B) was installed before the mass spectrometer to protect it from even a small amount of contamination.
- Installation of splitter just before the mass spectrometer
Since analysis mobile phase must be pumped at a high flow rate with a monolithic column, a splitter was installed just before the mass spectrometer.

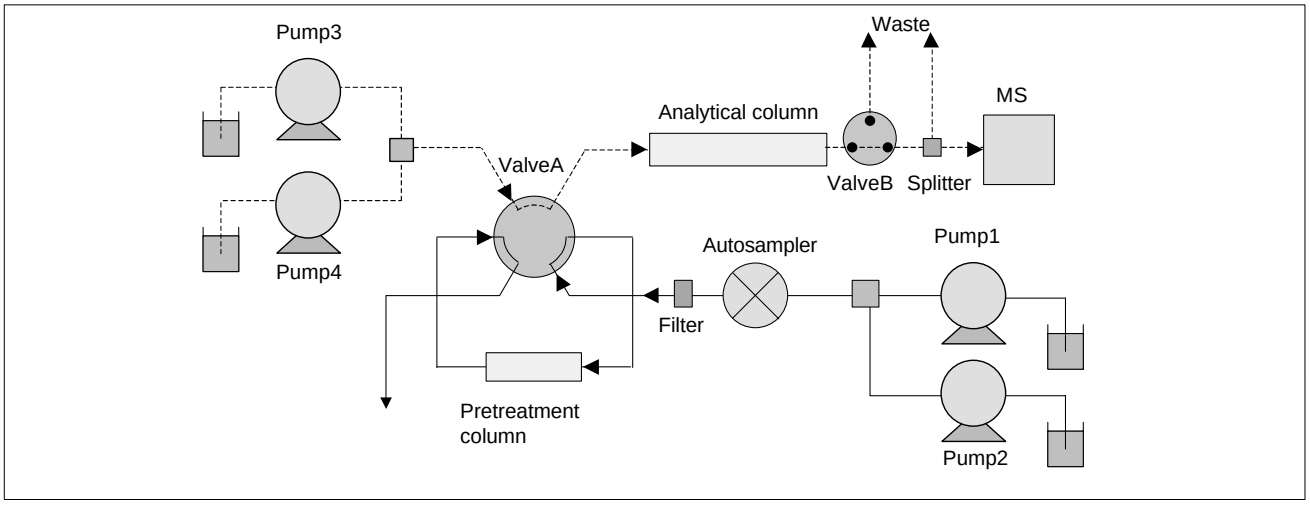

Fig. 1 Flow Diagram

■ Analysis of Drugs in Plasma

Metoprolol, propranolol, lidocaine and bupivacaine were used as test compounds. The analytical conditions used are shown in Table 1. The analysis mobile was split using a splitter (MS/drain), so that 1/7 of the volume was introduced into the mass spectrometer. From sample injection, deproteinization was conducted in 0.5min by the pretreatment column. The trapped test compounds were introduced into the analysis column via the flow line switching valve. After pretreatment, the pretreatment line was washed with water/actetonitrile (=1/4, v/v) at 6.0mL/min. One cycle of the analysis, including pretreatment and gradient elution of the sample, was completed in an analysis time shortened to 1.2 minutes (Fig. 2). Furthermore, a high 95% or greater recovery was obtained for all of the test compounds.

Table 1 Analytical Conditions

For Sample Injection	
Column	: Shim-pack MAYI-ODS (10mmL. × 4.6mm I.D.)
Mobile Phase	: 10mM Ammonium acetate /Acetonitrile =95/5(v/v)
Flow Rate	: 4.0mL/min
Injection Volume	: 10μL
Extraction Time	: 0.5min
For Separation	
Column	: Chromolith SpeedROD RP-18e (50mmL. × 4.6mm I.D.)
Mobile Phase	: A: 10mM Ammonium acetate B: Acetonitrile B25%(0min)→B90%(0.3min)→B90%(0.9min)→B25%(0.9min)→Stop(1.2min)
Flow Rate	: 4.0mL/min
Mixer	: 2.6mL
Split	: 1/7(MS/drain)
Temperature	: 40˚C
Probe Voltage	: +4.5kV (ESI-positive mode)
Nebulizing Gas	: 1.5L/min
Drying Gas	: 0.2MPa

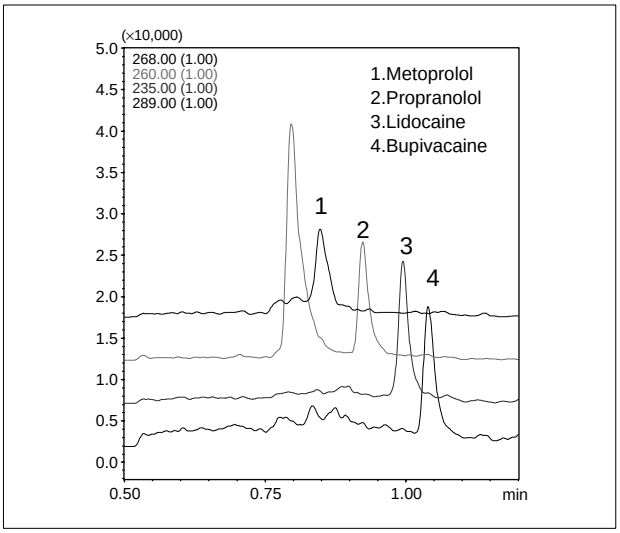

Fig. 2 SIM Chromatogram of Four Drugs in Plasma (5ng/mL spiked, 10μL injected)

■ Repeatability and Linearity

With these analytical conditions, in evaluating the elution time and area value repeatability (n=5), good repeatability was obtained despite the acute gradient (Table 2, Table 3). In addition, in checking the linearity in the range from 5ng/mL to 100ng/mL, contribution ratio (R^2) of 0.9998 or higher was obtained for each compound, demonstrating a good linearity. Here, the linearity for metoprolol is shown in Fig. 3.

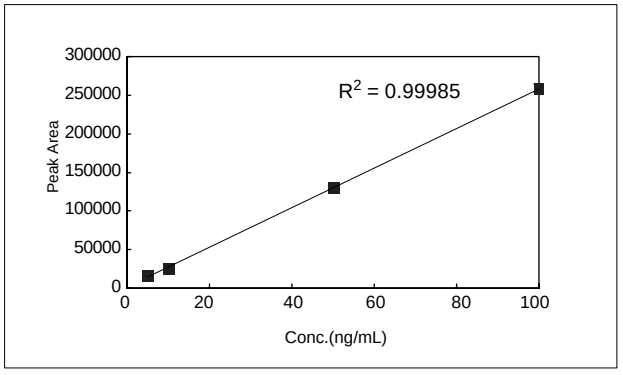

Fig. 3 Linearity of Metoprolol (Added to plasma at 5, 10, 50 and 100ng/mL; 10μL injected)

Table 2 Repeatability of Retention Time (100ng/mL standard; 10μL injected)

	Metoprolol	Propranolol	Lidocaine	Bupivacaine
CV(%)	0.20	0.18	0.22	0.24

Table 3 Repeatability of Peak Area (Added to plasma at 5, 10 and 100ng/mL; 10μL injected)

		Metoprolol	Propranolol	Lidocaine	Bupivacaine
CV (%)	100ng/mL	3.7	0.9	1.4	1.0
	10ng/mL	2.5	4.1	2.3	3.4
	5ng/mL	9.4	6.4	6.0	2.6

*The publishing data was not obtained using instruments approved by pharmaceutical regulations.

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