SHIMADZU

LC-MS Application Data Sheet No. 044

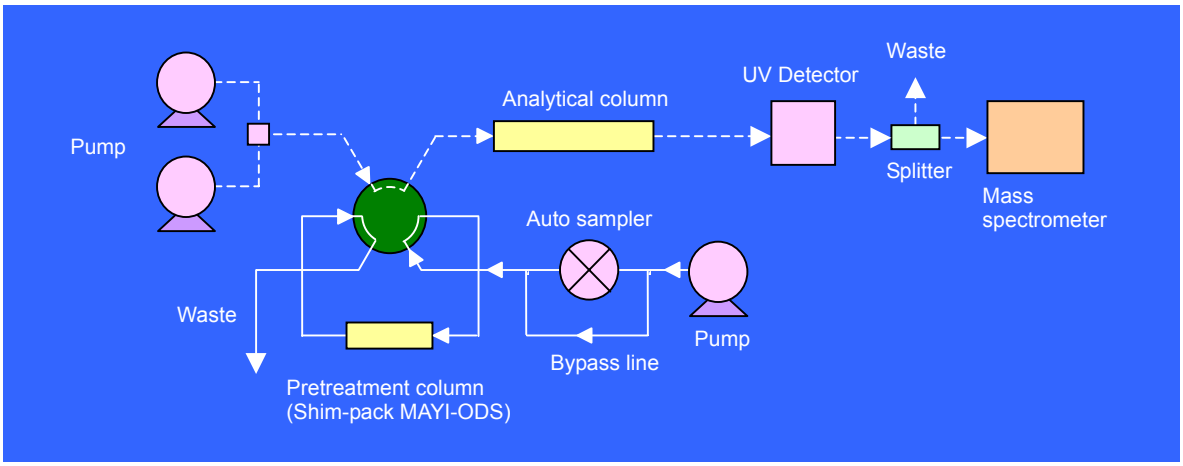
Drug Analysis using Co-Sense for BA LC-MS System (1)

HPLC and LC-MS are used to analyze drugs in bio-samples and enzyme reaction liquids in pharmacokinetics testing and metabolic research. Large amounts of proteins and ionic substances contained in these samples are removed through pretreatment such as addition of organic solvents and solid-phase extraction. However, these pretreatment procedures prolong the time required for analysis, as well as cause problems such as variations in the measured values due to devision in manual operation. The Co-Sense for BA, comprised of unique configuration employing the column switching technology and pretreatment column, resolves these problems and enhances the efficiency of analysis by automatically conducting all processes from sample pretreatment to analysis.

Fig.1 shows the flow diagrams of the Co-Sense for BA. The sample injected from the auto sampler is introduced into the Shim-pack MAYI-ODS pretreatment column. Proteins and ionic substances are not retained, the drugs permeate into the pores of the column packing and are retained by the hydrophobic layer inside (see (a)). The flow channel is then switched to introduce the mobile phase for analysis and guide the drugs from the pretreatment column to the analytical column (see (b)).

The use of the MS as the detector will enhance the selectivity, leading to higher sensitivity and reduction in the time required for analysis.

(a) Pretreatment



(b) Analysis

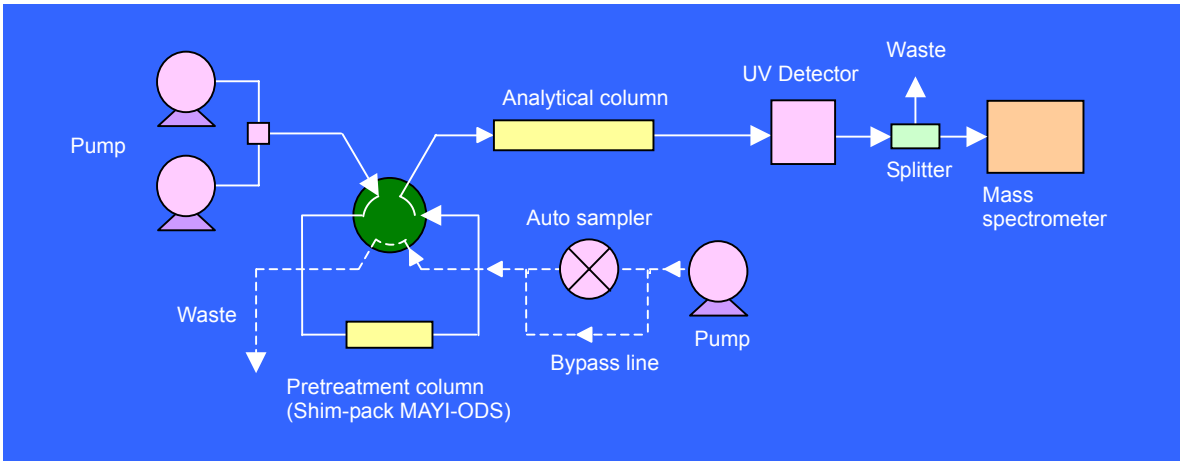


Fig. 1 Flow diagrams of Co-Sense for BA LC-MS system

Fig.2 shows an example of the simultaneous analysis of 5 drug components (1ug/mL of each, rat blood serum spiked). The whole cycle of the analysis was completed in 5 minutes (1 minute for protein removal and 4 minutes for gradient analysis). The reproducibility and recovery rate are shown in Table 1. Measurements could be conducted selectively.

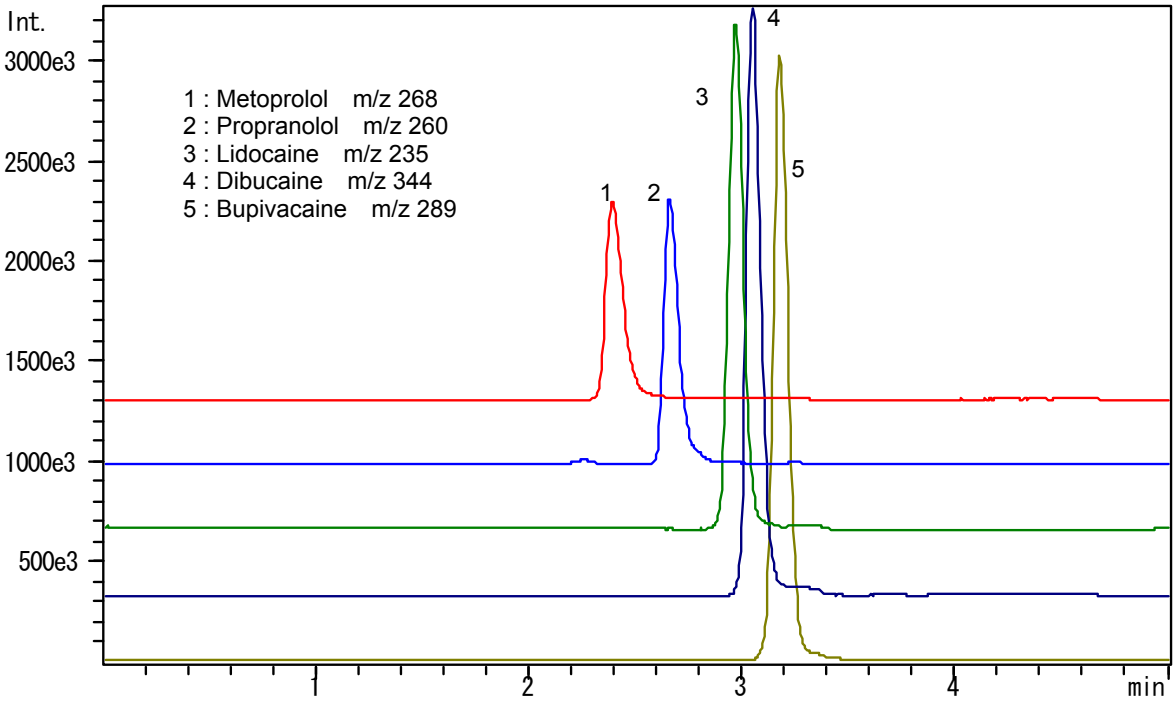


Fig.2 SIM chromatograms of drugs (1ug/mL, spiked, rat blood serum spiked)

Drug	m/z	RSD (%, peak area)	Recovery (%)
Metoprolol	268	1.0	95.0
Propranolol	260	1.4	97.4
Lidocaine	235	2.6	94.0
Dibucaine	344	2.5	107.7
Bupivacaine	289	1.5	97.1

Table 1 Reproducibility and recovery (n=5)

Table 2 Analytical conditions for LC-MS

Pretreatment	
Column	: Shim-pack MAYI-ODS (4.6 mmI.D. x 10 mmL)
Mobile phase	: 10mM ammonium acetate/acetonitrile = 95/5
Flow rate	: 3.0 mL/min
Column temperature	: 45 ℃
Injection volume	: 10 μL
Analysis	
Column	: Shim-pack GVP-ODS (4.6 mmI.D. x 10 mmL)
Mobile phase	: A : 10mM ammonium acetate, B : acetonitrile
Gradient program	: 5%B(0-0.5min) - 90%B(3-4min) - 5%B(4.01min) - STOP(5min)
Flow rate	: 0.8 mL/min, split ratio 1 : 3 (mass spectrometer : waste)
Column temperature	: 45 ℃
Probe voltage	: +4.5 kV (ESI-Positive mode)
Nebulizing gas flow	: 4.5 L/min

SHIMADZU CORPORATION **International Marketing Division**
3. Kanda-Nishikicho 1-chome, Chiyoda-ku, Tokyo 101-8448, Japan
Phone: 81 (3) 3219-5641 Fax. 81 (3) 3219-5710
Cable Add. SHIMADZU TOKYO