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Application News

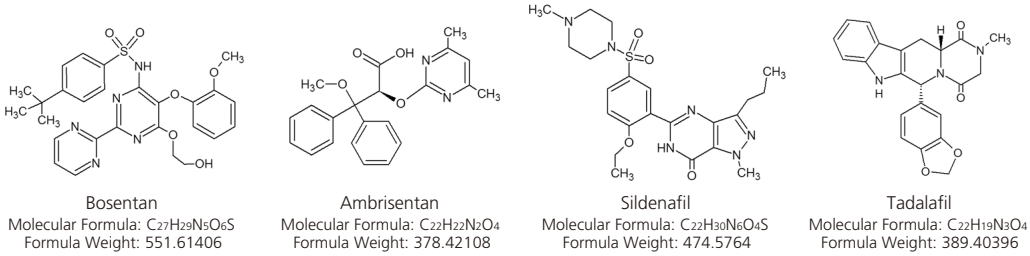
No.C101

Liquid Chromatography Mass Spectrometry

High-Speed, High-Sensitivity Analysis of Drugs in Plasma Using Triple Quadrupole LC/MS/MS (LCMS-8040)

This Application News introduces examples of high-speed, high-sensitivity analysis of four drug substances, including the endothelin receptor antagonists (ERA) bosentan and ambrisentan, and the phosphodiesterase-5 (PDE-5) inhibitors sildenafil and tadalafil. All of these substances are used as therapeutic agents for the treatment of pulmonary arterial hypertension (PAH), and the analysis was conducted by LC/MS/MS using ESI in positive mode.[1)]

PAH is a condition in which there is a rise in pulmonary artery pressure due to narrowing of the lumens of the small arteries peripheral to the pulmonary artery which carries blood from the heart to the lungs. The right ventricle of the heart, which pumps poorly oxygenated blood to the lungs, cannot withstand the high pressure. PAH, the result of high pressure applied to the right ventricle over a long period, is an intractable condition characterized by reduced function which eventually leads to right ventricular heart failure. In recent years, therapeutic agents that dilate the blood vessels have been developed, and their use is linked to favorable clinical outcomes. In order to conduct pharmacokinetics studies using these therapeutic agents in conjunction with other drugs, rapid trace analysis of pharmaceuticals in plasma is required. Use of the high-sensitivity, high-selectivity LC/MS/MS method drastically reduces the amount of sample required, thereby permitting faster and simpler pretreatment and the development of a more efficient work flow.



Bosentan
Molecular Formula: $C_{27}H_{29}N_5O_6S$
Formula Weight: 551.61406

Ambrisentan
Molecular Formula: $C_{22}H_{22}N_2O_4$
Formula Weight: 378.42108

Sildenafil
Molecular Formula: $C_{22}H_{30}N_6O_4S$
Formula Weight: 474.5764

Tadalafil
Molecular Formula: $C_{22}H_{19}N_3O_4$
Formula Weight: 389.40396

Fig. 1 Structures of Drugs

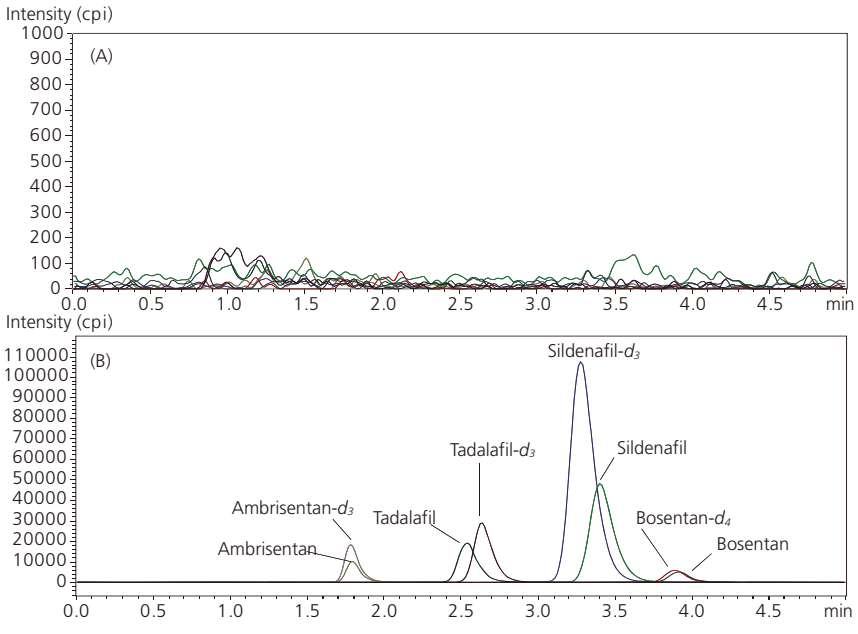


Fig. 2 Representative MRM Chromatograms of (A) Blank Human Plasma and (B) Human Plasma Spiked Standard Solutions and Respective ISs at 100 ng/mL Each

Fig. 1 shows the structures of the four substances, bosentan, ambrisentan, sildenafil and tadalafil. As internal standards, bosentan-d_4, ambrisentan-d_3, sildenafil-d_3 and tadalafil-d_3, respectively, were used. Fig. 2 shows the chromatograms representative of the four substances. A single high-speed analysis can be completed within 5 minutes, with ambrisentan eluting at 1.8 minutes, tadalafil at 2.5 minutes, sildenafil at 3.4 minutes, and bosentan at 3.9 minutes (analytical conditions are shown in Table 1).

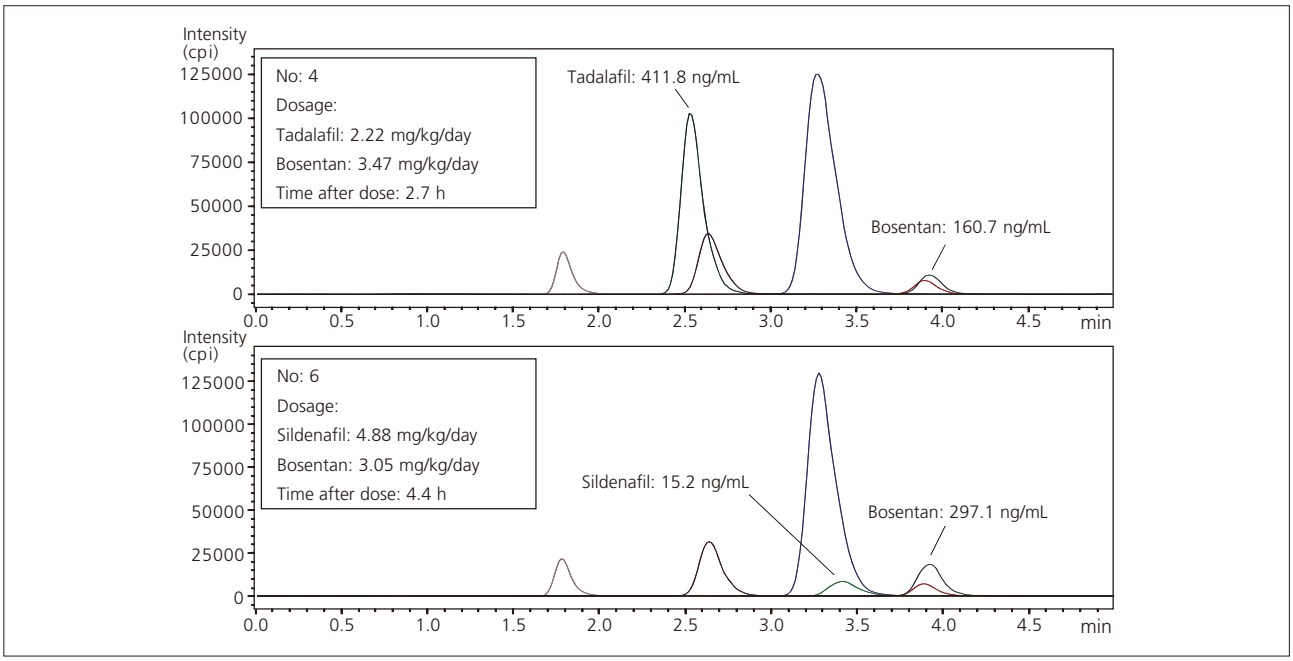


Fig. 3 Typical MRM Chromatograms of Plasma Samples

Typical chromatograms of drugs extracted from plasma are shown in Fig. 3, and the method for extracting the sample from plasma is shown in Fig. 4.
Use of the high-sensitivity LC/MS/MS method permitted analysis of the extract obtained from small amounts of plasma. Moreover, this drug extraction method is a simple method in which a solid phase extraction cartridge is used.
The extraction efficiencies from plasma using standard addition were 44.3 to 63.9 % in the case of ambrisentan, 78.9 to 88.5 % for tadalafil, 83.4 to 88.1 % for sildenafil, and 86.1 to 109.9 % for bosentan, for an overall extraction efficiency CV (%) of less than 17.7 %.
This method is one in which drug substances extracted from only 50 µL of plasma were analyzed at the rate of one analysis within 5 minutes using high-speed, high-sensitivity analysis, demonstrating its applicability to plasma pharmacokinetic studies.

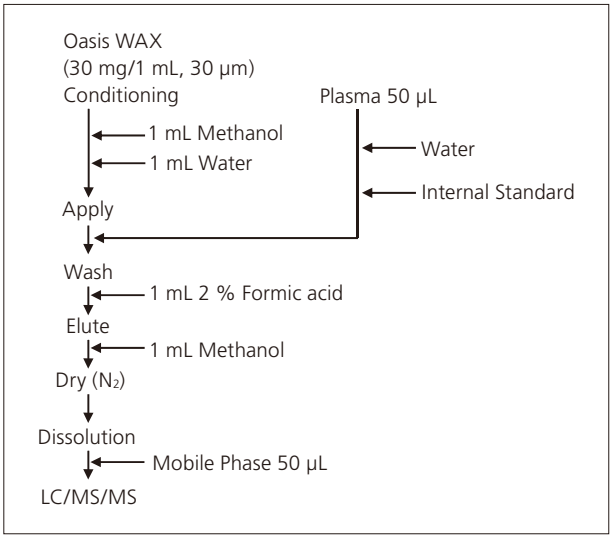


Fig. 4 Sample Extraction

Table 1 Analytical Conditions

Column	: Cadenza CD-C18 (75 mm L. × 2 mm I.D., 3 µm)
Guard Column	: Security Guard columns (Phenomenex, Torrance, CA)
Mobile Phase	: Acetonitrile / 5 mM Ammonium Acetate (45:55, v/v; pH adjusted to 5.0 with Acetic Acid)
Flowrate	: 0.2 mL/min.
Column Temperature	: 40 °C
Injection Volume	: 20 µL
Probe Voltage	: 4.5 kV (ESI-positive mode)
Block Heater Temperature	: 400 °C
MRM Transition	: Tadalafil; *m/z* 390.2>268.1, Tadalafil-d_3; *m/z* 393.0>271.1
	: Bosentan; *m/z* 553.0>202.0, Bosentan-d_3; *m/z* 557.1>202.1
	: Sildenafil; *m/z* 475.1>58.1, Sildenafil-d_3; *m/z* 478.1>61.1
	: Ambrisentan; *m/z* 379.0>346.7, Ambrisentan-d_3; *m/z* 382.1>346.9

[Reference]
1) Y. Yokoyama et al. Journal of Pharmaceutical and Biomedical Analysis 89 (2014) 227-232
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This product has not received the approval or certification as a medical device according to the Pharmaceutical Affairs Act. It is not for use in diagnostic procedures.

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