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Short communication

Simultaneous microdetermination of bosentan, ambrisentan, sildenafil, and tadalafil in plasma using liquid chromatography/tandem mass spectrometry for pediatric patients with pulmonary arterial hypertension

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ABSTRACT

A simultaneous, selective, sensitive, and rapid liquid chromatography/tandem mass spectrometry (LC–MS/MS) method was developed and validated for the quantification of bosentan, ambrisentan, sildenafil, and tadalafil in 50 μL of human blood plasma. Diluted plasma samples were extracted using a solid-phase extraction procedure with 2% formic acid and methanol. The four drugs were separated by high-performance liquid chromatography using a C18 column and an isocratic mobile phase running at a flow rate of 0.2 mL/min for 5 min. The drugs were detected by a tandem mass spectrometer with electrospray ionization using deuterated compounds as internal standards. Calibration curves were generated over the linear concentration range of 2–1000 ng/mL in plasma with a lower limit of quantification of 2 ng/mL for all compounds. Finally, this validated method was applied to a clinical pharmacokinetic study in pediatric patients with pulmonary arterial hypertension (PAH) following the oral administration of PAH drugs. These results indicate that this method is suitable for assessing the risk/benefit of combination therapy in the pediatric population and useful for therapeutic drug monitoring for PAH treatment.

1. Introduction

Pulmonary arterial hypertension (PAH) is a severe and progressive disease that has no cure and can lead to heart failure and death [1]. The three major pathways involved in the development and progression of PAH, i.e., the endothelin-1, nitric oxide (NO), and prostacyclin pathways, are targeted by currently available therapies.

Combination therapy using these drugs is a key management method for PAH [2]; however, the risks and benefits of this strategy are unclear [3]. Prostacyclin and its analogs, which can be administered intravenously, subcutaneously, or by inhalation, are generally reserved for patients with severe disease or for those who are progressing on oral drugs. In addition, beraprost sodium, an oral prostacyclin analog, is available for the treatment of mild PAH in some countries [4]. The use of these drugs in combination is adjusted on the basis of patient condition or the treatment environment. Therefore, there is no standard procedure for combination therapy concerning PAH [5].

Although some methods to determinate the plasma concentrations of PAH drugs have been reported [6–9], further improvement of the detection methods in terms of simultaneity, detection time in high-performance liquid chromatography (HPLC), sample amount, and discontinuous determination (e.g., gradient elution) is required. Under these circumstances, it is important to develop a more convenient quantification method for individual concentrations of these drugs in plasma to assess both the effectiveness of combination therapy and the interactions among these drugs. Moreover, the amount of blood that must be sampled often

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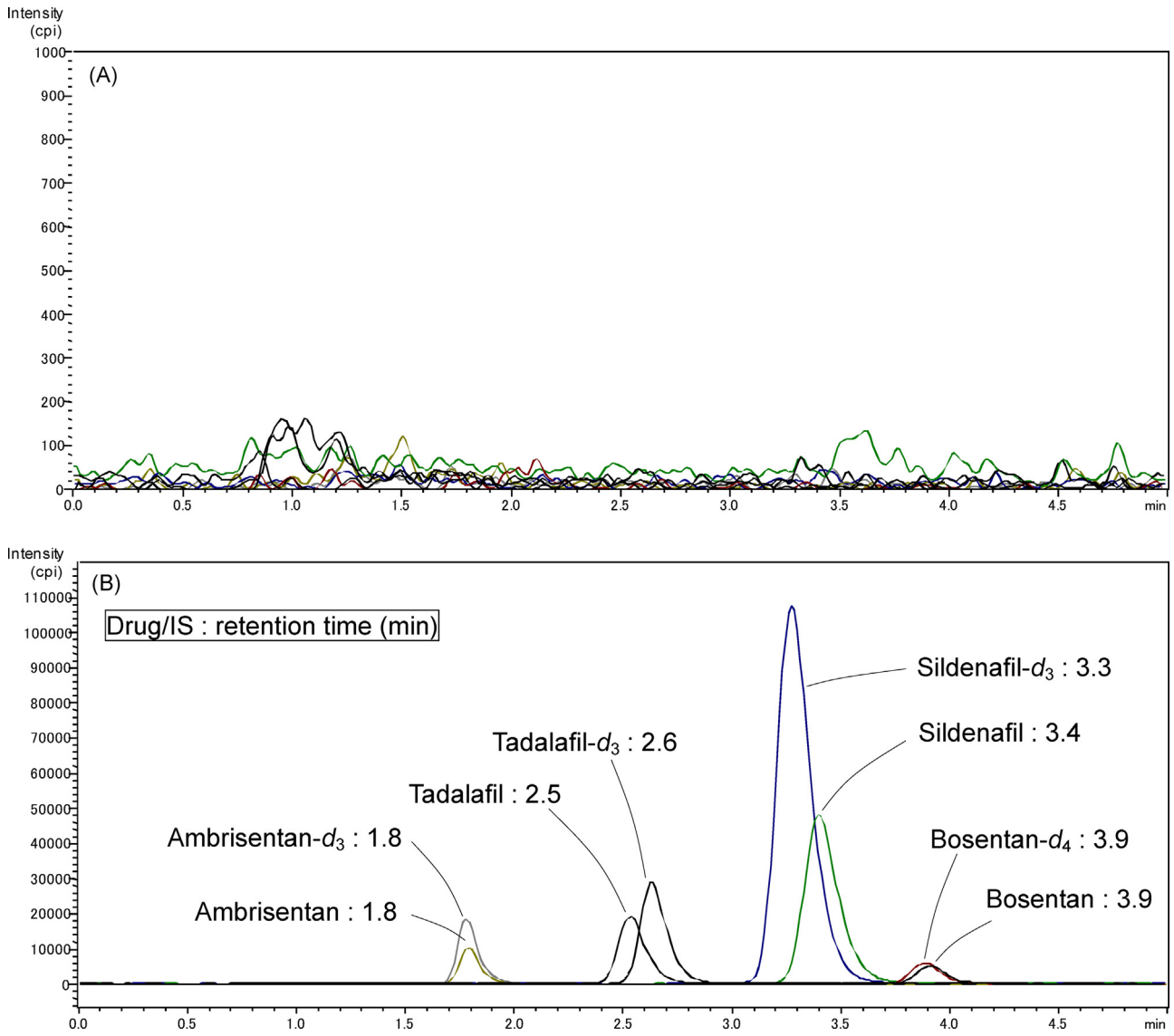

Fig. 1. Representative MRM chromatograms of (A) blank human plasma and (B) human plasma spiked with ambrisentan, tadalafil, sildenafil, bosentan and their ISs at 100 ng/mL each.

becomes an ethical problem in pharmacokinetics studies, which require frequent blood sampling, particularly in the pediatric population [10].

In this study, we developed a simultaneous quantification method with consecutive measurements of the PAH drugs bosentan, ambrisentan, sildenafil, and tadalafil that features a short detection time of 5 min and uses small amounts of blood.

2. Materials and methods

2.1. Chemicals and reagents

Bosentan, ambrisentan, sildenafil, tadalafil, and their deuterated derivatives (used as internal standards [ISs]) were purchased from Toronto Research Chemicals (North York, Ontario, Canada). Liquid chromatography–mass spectrometry (LC–MS)-grade methanol, acetonitrile, and formic acid were purchased from Sigma–Aldrich (St. Louis, MO, USA). Ammonium acetate solution was purchased from Wako Pure Chemical Industries (Tokyo, Japan). Milli-Q water, reagent-grade deionized water, and the Milli-Q water system were purchased from Millipore (Billerica, MA, USA). Blank human plasma was obtained from healthy volunteers. Solid-phase extraction (SPE) cartridges were supplied by Waters.

2.2. Standard solutions and sample preparation

Standard solutions of the four drugs were individually prepared at 1 mg/mL in methanol in polypropylene tubes. An IS solution

Table 1
Collision energy and MRM transitions for the four analytes and their corresponding internal standards.

	Collision energy (eV)	Precursor ion (m/z)	Product ion (m/z)
Tadalafil	16	390.2	268.1
Tadalafil-d_3	16	393.0	271.1
Bosentan	35	553.0	202.0
Bosentan-d_4	35	557.1	202.1
Sildenafil	40	475.1	58.1
Sildenafil-d_3	40	478.1	61.1
Ambrisentan	1	379.0	346.7
Ambrisentan-d_3	1	382.1	346.9

MRM, multiple reaction monitoring.