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Development and validation of bioanalytical methods for Imidafenacin (KRP-197/ONO-8025) and its metabolites in human plasma by liquid chromatography–tandem mass spectrometry

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Abstract

Imidafenacin (KRP-197/ONO-8025, IM), 4-(2-methyl-1*H*-imidazol-1-yl)-2,2-diphenylbutanamide, is a new antimuscarinic agent currently under application for the indication of treatment of overactive bladder in Japan. We developed and validated the sensitive and selective bioanalytical methods for the extremely low levels of IM and its metabolite, M-2 (Method 1), M-4 (Method 2) and M-9 (Method 3) in human plasma using liquid chromatography–tandem mass spectrometry (LC–MS/MS). In each method, plasma sample was extracted by solid phase extraction, separated on a semi-micro high performance liquid chromatography column and detected by tandem mass spectrometer with an atmospheric pressure chemical ionization or ionspray interface. Selected reaction monitoring mode was used for quantification. Each method was found to have acceptable accuracy, precision, stability, selectivity and linearity over the concentration range of 10–500 pg/mL for IM and M-2, 10–1000 pg/mL for M-4 and 50–5000 pg/mL for M-9. Using these analytical methods, concentration profiles of IM and its metabolites in human plasma were successfully determined even in the low pg/mL levels after oral administration of IM at the therapeutic dosage of 0.1 mg.

Keywords: Imidafenacin; KRP-197; ONO-8025; Metabolites; Human-plasma; LC–MS/MS

1. Introduction

Micturition is mediated through the actions of several neurotransmitters affecting tone of the smooth muscle in the urinary bladder. Acetylcholine (ACh), in particular, plays an important role in contracting the reservoir and relaxing the outlet through activation of ACh receptors [1]. Compounds with high affinity for the muscarinic ACh receptors, such as propiverine, tolterodine, oxybutynin, darifenacin and solifenacin have been used in the management of overactive bladder (OAB) [2,3].

Imidafenacin (KRP-197/ONO-8025, IM), 4-(2-methyl-1*H*-imidazol-1-yl)-2,2-diphenylbutanamide (Fig. 1), is an antagonist for muscarinic ACh receptor currently under application for the indication of treatment of OAB in Japan, and is scheduled for launch into the market before long. IM has high affinity for M_1 and M_3 receptor subtypes [4–6]. Therefore, IM inhibits bladder contraction by mediating antagonism to M_3 receptor and regulates ACh release by inhibiting prejunctional facilitatory M_1 subtype [7–10].

Administration of IM (0.1 mg twice daily) improved symptoms of OAB with good tolerability and safety in Phase III clinical trial conducted in Japan. Because its therapeutic dosage is about 10–1000 times lower compared with those of other anticholinergics [11], the quantification methods with selectivity and sensitivity sufficient for determining the extremely low level (pg/mL) of concentrations of IM and three main metabolites; M-2, M-4 and M-9 in human plasma are indispensable in order to consider the efficacy and safety of these compounds after administration of IM in humans.

In this study, we developed and validated the highly selective and sensitive analytical methods for determination of IM, M-2, M-4 and M-9 in human plasma using liquid chromatography–tandem mass spectrometry (LC–MS/MS). Then, the methods were applied to determination of the concentrations at pg/mL

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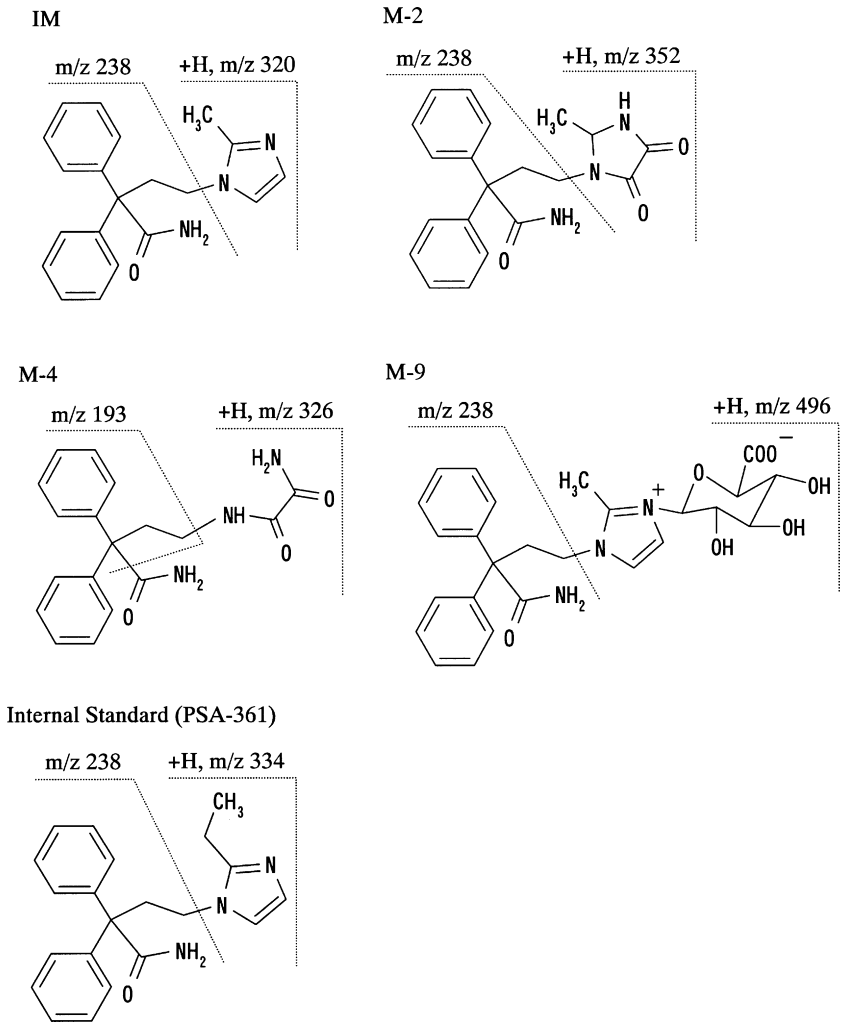

Fig. 1. Chemical structures of IM, its metabolites M-2, M-4, M-9 and the internal standard PSA-361. Broken lines indicate precursor and product ions obtained by collision-induced dissociation in LC–MS/MS system.

levels of IM and its metabolites in human plasma after oral administration of IM at the therapeutic dosage of 0.1 mg in healthy Japanese male volunteers.

2. Experimental

2.1. Materials

IM, M-2, M-4, M-9 and the internal standard (IS, PSA-361, Fig. 1) were synthesized at Kyorin Pharmaceutical Co. Ltd. Acetonitrile, methanol and distilled water were of high performance liquid chromatography (HPLC) grade. All other reagents were of analytical grade. Human control plasma was collected from healthy male volunteers or was purchased from Interstate Blood Bank (Memphis, TN, USA). Human control plasma for selectivity test was collected individually from six healthy male volunteers.

2.2. Instrumentation

LC–MS/MS instruments were separately applied to the three analytical methods for determination of IM and M-2 (Method 1), M-4 (Method 2) and M-9 (Method 3) as listed in Table 1. The triple-stage quadrupole mass spectrometers, API 300, API 4000 and API 3000 (Applied Biosystems, Foster City, CA, USA) were used as MS/MS units for Methods 1, 2 and 3, respectively, with an atmospheric pressure chemical ionization (APCI) interface: Heated-Nebulizer (Method 1) or an ionspray interface: TurboIonSpray (Methods 2 and 3). Agilent 1100 series (Agilent Technologies, Palo Alto, CA, USA) was used as an HPLC unit for the three methods.

2.3. Chromatographic conditions

For determination of IM and M-2 (Method 1), a semi-micro HPLC column, CAPCELLPAK C_{18} UG120 (5 μm, 2.0 mm i.d. × 150 mm, Shiseido, Tokyo, Japan) was used with a guard column, CAPCELLPAK C_{18} UG120 (5 μm, 2.0 mm i.d. × 10 mm, Shiseido, Tokyo, Japan). Mobile phase was the mixture of distilled water containing 0.1% formic acid and acetonitrile in the volumetric ratio of 75:25 and 65:35 for IM and M-2, respectively. The flow rate was 0.2 mL/min and the column temperature was maintained at 25 °C.