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Measurement of tamsulosin in human serum by liquid chromatography–tandem mass spectrometry☆

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ABSTRACT

A simple, sensitive and robust method to extract tamsulosin from human serum, and quantify by liquid chromatography–tandem mass spectrometry (LC–MS/MS) was developed and validated and is applicable as a measure of compliance in clinical research. Tamsulosin was extracted from human serum (100 μL) via liquid–liquid extraction with methyl *tert*-butyl ether (2 mL) following dilution with 0.1 M ammonium hydroxide (100 μL), achieving 99.9% analyte recovery. Internal standard, d9-finasteride, was synthesised in-house. Analyte and internal standard were separated on an Ascentis® Express C18 (100 mm × 3 mm, 2.7 μm) column using a gradient elution with mobile phases methanol and 2 mM aqueous ammonium acetate (5:95, v/v). Total run-time was 6 min. Tamsulosin was quantified using a triple quadrupole mass spectrometer operated in multi-reaction-monitoring (MRM) mode using positive electrospray ionisation. Mass transitions monitored for quantitation were: tamsulosin m/z 409 → 228 and d9-finasteride m/z 382 → 318, with the structural formulae of ions confirmed by Fourier transform ion cyclotron resonance mass spectrometry (within 10 ppm). The limit of quantitation was 0.2 ng/mL, and the method was validated in the linear range 0.2–50 ng/mL with acceptable inter- and intra-assay precision and accuracy and stability suitable for routine laboratory practice. The method was successfully applied to samples taken from research volunteers in a clinical study of benign prostatic hyperplasia.

1. Introduction

Benign prostatic hyperplasia (BPH) is a highly prevalent disorder in older men which causes lower urinary tract symptoms and in severe cases can lead to urinary retention and renal tract complications [1]. Tamsulosin (Fig. 1) is an α1 adrenergic antagonist, targeting uro-specific $\alpha1_A$ and $\alpha1_D$ receptors, and is an important therapy for many BPH patients [2]. In clinical studies of response to pharmacological intervention establishing compliance with study medication is important. Traditional methods of establishing compliance such as a 'pill count' at the end of a study can be complemented by measurement of drug in serum.

Tamsulosin levels in those treated with the 0.4 mg modified release formulation are reported to be between 11.8 ng/mL [3] and 16.1 ng/mL [4] after a single dose, and 10 ng/mL after 21 days dosing [5]. Measurement of tamsulosin levels has been reported from plasma by HPLC [6,7], LC–MS [8] and LC–MS/MS [3,9–12], with key features of these methods described in Table 1. HPLC alone precludes additional specificity and sensitivity afforded by its use in conjunction with mass spectrometry, and in all assays described requires undesirably large (1–1.5 mL) sample volumes. While LC–MS methods have been described, it is now increasingly recognised that tandem mass spectrometry (such as LC–MS/MS) with the monitoring of 2 mass transitions is the gold standard of analyte measurement. Electrospray ionisation has been used more commonly, but atmospheric pressure ionisation (APCI) was used successfully, by Qi et al. [9]. The combination of an LC–MS/MS approach, minimal sample volume, simple extraction method and

Abbreviation: BPH, benign prostatic hyperplasia.

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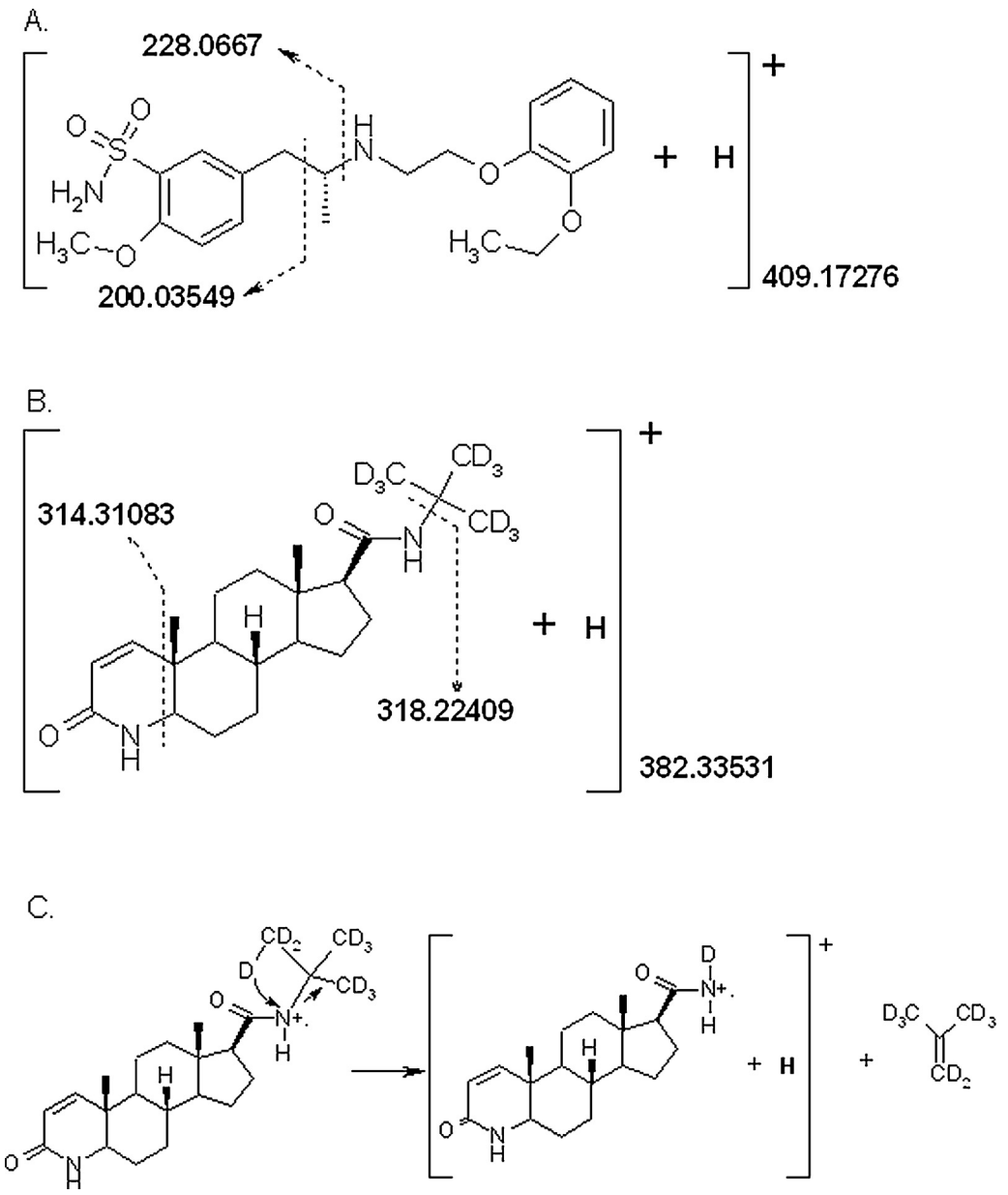

Fig. 1. Chemical structures and proposed fragmentation patterns for analyte and internal standard. Accurate masses shown for analyte quantifier and qualifier ions were confirmed to within 10 ppm of their theoretical monoisotopic mass. (A) Structure and proposed fragmentation pattern for tamsulosin. (B) Structure and proposed fragmentation for d9-finasteride. (C) Proposed mechanism for fragmentation of d9-finasteride into quantifier ion. From the charged radical parent ion a single deuterium shifts in a concerted transfer of 2 electrons from the deuterated *tert*-butyl amine to the amide nitrogen with loss of neutral d8-2-methylpropene (shown). A single electron radical transfer is also possible.

excellent analyte recovery was not achieved in any published method (Table 1). Therefore, we sought to develop an assay to measure tamsulosin from human serum with a simple extraction method, excellent analyte recovery and sufficient sensitivity to allow use of small sample volumes.

2. Experimental

2.1. Reagents and standards

All solvents were HPLC grade and chemicals were from Sigma–Aldrich (Dorset, UK) unless otherwise stated. The internal standard, d9-finasteride was synthesised in house (see Section 2.3). Sources of other chemicals were as follows: tamsulosin hydrochloride (AK Scientific, Mountain View, USA), water and ammonium hydroxide (35%, v/v) solution (Fisher Scientific, Loughborough, UK), methanol (VWR, Lutterworth, Leicestershire, UK), 4-aza-5α-androstan-1-en-one-16β-carboxylic acid (APAC pharmaceutical, LLC, Columbia, USA), and 2-amino-2methyl-d3-propane-1,1,1,3,3,3-d6 (CDN isotopes Inc., Quebec, Canada).

2.2. Biological samples

Pooled male human serum (collected from healthy men aged 17–45 years on no medications) was purchased (TCS Biosciences, Buckingham, UK) for use as blank matrix in method development, validation and standard curves (referred to as “drug-free serum”). For method application, serum was collected from 3 male subjects who had received at least 3 months of treatment with tamsulosin MR 0.4 mg daily (Synthon Hispania, Sant Boi de Llobregat, Spain).