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Short communication

Isolation, identification and characterization of degradant impurities in Tolterodine tartrate formulation

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ABSTRACT

During the stability study of Tolterodine tartrate drug product, two unknown impurities (Impurities I and II) were detected by ultra performance liquid chromatography (UPLC). Both impurities were isolated by preparative liquid chromatography and were subjected to mass and NMR spectral studies. Based on the spectral data, the Impurities I and II were characterized as N-(3-(2-hydroxy-5-methylphenyl)-3-phenylpropyl)-N,N-diisopropyl hydroxyl ammonium trifluoro acetate and 3-(2-hydroxy-5-methylphenyl)-N-isopropyl-3-phenylpropane-1-amine oxide respectively.

1. Introduction

Tolterodine tartrate ((R)-2-[3-[Bis(1-methylethyl)amino]-1-phenylpropyl]-4-methylphenol [R-(R*,R*)]-2,3-dihydroxy-butanedioate (1:1) salt, $C_{22}H_{31}NO{\cdot}C_4H_6O_6$) is an antimuscarinic (muscarinic receptor antagonist) drug that is used to treat overactive bladder, symptoms associated with voiding such as urge urinary incontinence, urgency, and frequency. It controls bladder incontinence by controlling contractions [1–3]. It acts by competitively antagonizing muscarinic receptors, inhibiting bladder contractions and reducing urinary frequency.

Stability testing [4] is used as a primary tool to determine and asses the expiration dating and storage conditions for pharmaceutical products. One of the evaluation criteria is the identification of impurities during real time and accelerated stability studies. As per the stringent regulatory requirements recommended by ICH, it is mandatory to identify and structurally characterize any impurity formed during production and stability testing, exceeding the identification threshold in the drug product [5–9]. An impurity profile study has to be carried out for any final product as per the regulatory requirements to identify and to characterize all the unknown impurities. To detect, isolate, identify and characterize the impurities, various instrumental analytical techniques [10–15] are routinely used.

A few HPLC methods have been reported for the stability-indicating quantification of Tolterodine [16–21] active pharmaceutical ingredient and dosage form. During the stability analysis two unknown impurities were detected, which were crossing the identification threshold. The present study describes the separation, isolation, identification and characterization of these impurities.

2. Experimental

2.1. Chemicals and reagents

Tolterodine tartrate was obtained from the R&D of Dr. Reddy's Laboratories Ltd., Hyderabad, India. HPLC grade acetonitrile, trifluoro acetic acid and ortho phosphoric acid were obtained from the Merck Co., Mumbai, India. Ultra pure water was collected from TKA Millipore water purification system. Potassium dihydrogen phosphate was procured from Rankem India Ltd. DMSO-d6 (for NMR) was obtained from Aldrich Chemical Co., USA.

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Table 1
LC conditions for analytical, preparative, LC–MS/MS and UPLC-TOF-MS analyses.

Technique	Mobile phase		Flow rate (ml/min)	Gradient program (T_{min}/%B)	Injection volume (μl)	Column
	Solvent (A)	Solvent (B)				
UPLC	Buffer[a]	Solvent A: ACN (10:90)	0.25	T_0/35; T_5/40; $T_{10\text{-}14}$/90 $T_{15\text{-}16}$/35	2.0	ACQUITY UPLC™ BEH shield, RP18 (2.1 × 100 mm, 1.7 μm)
Preparative	0.1% TFA	Solvent A: ACN (10:90)	5.0	T_0/35; T_{10}/55; $T_{15\text{-}20}$/95 $T_{22\text{-}25}$/35	500	XBridge™ Prep shield, RP 18 (10 × 250 mm, 5.0 μm)
LC–MS/MS	0.1% TFA	Solvent A: ACN (10:90)	1.0	T_0/35; T_4/40; T_{10}/45; T_{14}/50 T_{22}/60; $T_{27\text{-}31}$/80; $T_{32\text{-}40}$/35	5.0	ACE C18, (250 × 4.6 mm, 5 μm)
UPLC-TOF-MS	0.1% TFA	Solvent A: ACN (10:90)	0.25	T_0/35; T_5/40; $T_{10\text{-}14}$/90 $T_{15\text{-}16}$/35	2.0	ACQUITY UPLC™ BEH shield, RP18 (2.1 × 100 mm, 1.7 μm)

Column oven temperature and detection wavelength were 50 °C and 210 nm, respectively.
[a] Buffer: 0.01 M KH_2PO_4 buffer with the pH adjusted to 3.5 using 5% ortho phosphoric acid.

2.2. Chromatographic conditions (analytical)

Samples were analyzed on Waters ACQUITY™ UPLC system. A mixture of Mobile phase A and acetonitrile in the proportion of 50:50 (v/v) used as diluent for sample preparation. The optimized LC conditions are described in Table 1.

2.3. Chromatographic conditions (preparative)

Preparative isolation work was performed on an Agilent 1200 series preparative HPLC system which was equipped with an automated fraction collector and photodiode array detector and Chemstation software. A mixture of Mobile phase A and acetonitrile in the proportion of 50:50 (v/v) used as diluent for sample preparation. The optimized LC conditions are described in Table 1. The two impurity fractions were collected separately from several injections and pooled separately. The pooled fractions were concentrated by using Rotavapour (Model: Heidolph Laboratory 4002 control) under high vacuum. The aqueous solutions were lyophilized (Model: Virtis Advantage plus) to obtain the impurities.

2.4. Mass spectrometry (LC–MS/MS)

The electro spray ionization and MS–MS studies were performed on triple quadrupole mass spectrometer PE Sciex Model: API 3000. The positive and negative electro spray MS data was obtained by switching the capillary voltage between +5000 and −4500 respectively. The MS–MS data were generated with collision energy ramping from 30 V to 60 V in nitrogen atmosphere. A mixture of water and methanol in the proportion of 50:50 (v/v) was used as diluent for sample preparation and concentration of sample was 0.02 mg/ml. The optimized LC conditions are described in Table 1.

2.5. UPLC-TOF-MS

The UPLC-TOF-MS system consisted of an ACQUITY™ ultra performance liquid chromatography system and a Micro mass LCT Premier XE Mass Spectrometer (High sensitivity orthogonal time of-flight instrument, Waters, Millford, USA) equipped with a lock mass sprayer, operating in either the positive or negative ion mode. All analysis was acquired using the lock spray to ensure accuracy and reproducibility; leucine enkephalin was used as the lock mass. High resolution (W mode, FWHM 10500) positive polarity scan responses were collected from m/z 100 to 1000 at a rate of 1.0 s/scan. The concentration in a mixture of water and methanol in the proportion of 50:50 (v/v) used as diluent for sample preparation and concentration of sample was 0.02 mg/ml. The optimized LC conditions are described in Table 1.

2.6. NMR spectroscopy

NMR experiments (1D and 2D) were performed using a 500 MHz Unity INOVA NMR spectrometer (Varian) in DMSO-d6 at 25 °C as solvent. ^{1}H NMR measurements were carried out at 500 MHz, while ^{13}C NMR experiments were performed at 125 MHz. Proton and carbon chemical shifts were reported on δ scale in ppm, relative to tetramethyl silane (TMS) (δ = 0.00 ppm) and DMSO (δ = 39.50 ppm) as internal standards in ^{1}H and ^{13}C NMR spectra respectively. Standard pulse sequences provided by Varian were used for 1D and 2D NMR data.

2.7. Forced degradation study of Tolterodine tartrate drug product

Forced degradation studies were performed on the Tolterodine tartrate drug product with the intention of determining the conditions responsible for the formation of the degradation products.

2.7.1. Acid stressed degradation

200 mg equivalent of Tolterodine tartrate drug product was dissolved in 20 ml of methanol and water in the ratio of 1:1 HCl solution (5 ml of 0.1 N) was added and refluxed for about 4 h and neutralized.

2.7.2. Base stressed degradation

200 mg equivalent of Tolterodine tartrate drug product was dissolved in 20 ml of methanol and water in the ratio of 1:1. NaOH solution (5 ml of 0.1 N) was added and refluxed for about 4 h and neutralized.

2.7.3. Peroxide stressed degradation

200 mg equivalent of Tolterodine tartrate drug product was dissolved in 20 ml of methanol and water in the ratio of 1:1. Hydrogen peroxide (5 ml of 6% solution) was added and maintained at 70 °C for 4 h.

2.7.4. Thermal stressed degradation

200 mg equivalent of Tolterodine tartrate drug product was taken in a Petri dish. Water was sprinkled on the drug product and subjected to 105 °C for 24 h.

2.8. Sample preparation

Tolterodine tartrate sample was prepared at a concentration of 2 mg/ml in analytical diluent for analytical HPLC and 10 mg/ml in preparative diluent for the preparative HPLC analysis.