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Research Article

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Identification, isolation and quantification of unknown impurity in tolterodine tartrate tablets by stability indicating HPLC method

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ABSTRACT

A sensitive gradient reverse phase high performance liquid chromatography (HPLC) method development for identification and quantification of impurities in pharmaceutical products is a challenging and innovative activity. The present investigation aims to the identification of new unknown impurity of Tolterodine tartrate formed in stability samples of the drug product at a level up to 0.5% by using a stability indicating HPLC method. This impurity molecular weight was identified by LC-MS and characterized by various spectroscopic techniques such as ^{1}H NMR, ^{13}C NMR, LC/MS/MS, elemental analysis and FT-IR. Based on the data obtained from spectroscopic tools, the impurity was named as, 6-methyl-4-phenylchroman-2-ol. The structure of this impurity was also established unambiguously, prepared by isolation and co-injected into HPLC to confirm the retention time. To the best of our knowledge, this impurity has not been reported elsewhere. Structural elucidation of the impurity by spectral data is discussed in detail.

Keywords: HPLC; Tolterodine tartrate; LC-MS; 6-methyl-4-phenylchroman-2-ol.

INTRODUCTION

Stability testing is used as a primary tool to determine and asses the expiration dating and storage conditions for pharmaceutical products. Stability testing includes long-term and accelerated conditions, where the product is stored at room temperature and humidity, high heat and controlled humidity conditions. Stability study results are the evidence of establishment and assurance of safety, quality and efficacy of the drug product. In order to determine the product stability, the appropriate physical, chemical, biological and microbiological testing must be performed. One of the evaluation criteria is the appearance of impurities during real time and accelerated stability studies.

Tolterodine tartrate is a new muscarinic receptor antagonist intended for the treatment of urinary urge incontinence and other symptoms related to unstable bladder [1,2], is chemically known as (R)-N, N-diisopropyl-3-(2-hydroxy-5-methylphenyl)-3-phenylpropanamine L-hydrogen tartrate. It is soluble in methanol, slightly soluble in ethanol [3] and practically insoluble in toluene. During the stability studies of drug product in the laboratory, several batches have been analyzed for purity by HPLC. Besides several known and unknown impurities, one additional compound at level >0.1% was detected by HPLC. As per the stringent regulatory requirements recommended by ICH, the impurities >0.1% must be identified and characterized [4].

Literature survey reveals that, spectrophotometric methods [5,6] and a few HPLC analytical methods for the stability-indicating and quantification of Tolterodine [7-9] and in plasma [10-13] for dosage form have been reported [3]. A thorough study has been under taken to optimize the HPLC method to establish the retention time of unknown impurity and followed by to synthesize and characterize this unknown impurity.

EXPERIMENTAL SECTION

Reagents and Materials

Tolterodine tartrate and test samples (1 mg and 2 mg Tolterodine tartrate tablets) and excipients were provided by Creative Organics Limited, Bangalore. Mono basic potassium salt (KH_2PO_4) and ortho phosphoric acid (H_3PO_4) were procured from Merck Chemicals Ltd, India. Acetonitrile and Triethylamine were from Sigma Aldrich Chemicals Pvt. Ltd., India and water was purified with a Milli-Q plus system from Millipore.

Stability conditions

The stability study was performed by packing Tolterodine tartrate tablets in blisters (PVC-PVDC/Aluminum) and stored in climatic chambers under the following conditions[3] of temperature and relative humidity (R.H.): chamber A; 25 ◦C and 60% R.H., chamber B; 30 ◦C and 60% R.H. and chamber C; 40 ◦C and 75% R.H. Samples were pulled out from climatic chambers and analyzed at a interval of 0, 3 and 6 months.

Instrumentation and chromatographic parameters

The analysis was performed on HPLC system (Waters Alliance separation module 2667) consisting of a quaternary gradient pump, an automatic injector, a Photo diode array / Ultraviolet (PDA/UV) detector and a variable temperature column oven. Data acquisition was carried out using Empower software. BDS Hypersil (From Thermo Scientific) C18, 250×4.6 mm and 5 µm HPLC column was used in this study. The baseline separation was achieved with a linear step gradient condition of buffer pH 4.5 in less than 50 min for the unknown impurity and Tolterdone. The mobile phase involved a variable composition of mobile phase A (20 mM KH_2PO_4 adjusted pH to 4.5 with H_3PO_4) and mobile phase B (Acetonitrile). The mobile phase was pumped through a column at a flow-rate of 0.7 mL/min (gradient program showed in Table 1) and the injection volume was used 20 µL. The oven temperature was set at 45 ◦C. The optimum wavelength selected was 205 nm which represents where all other impurities have suitable responses. The stressed samples were analyzed using a PDA detector covering range of 200-400 nm.

Table-1: Gradient program for HPLC system

Time in Minutes	Mobile phase A%	Mobile phase B%
0.01	65	35
10	50	50
20	50	50
30	30	70
35	65	35
50	65	35

Standard and sample preparation

A mixture of water and acetonitrile (50:50 v/v) was used as a diluent for standard and samples solution preparation. Tolterodine tartrate standard solution was prepared by exactly weighing about 25 mg and diluting to obtain a solution with a concentration of about 0.00125 mg/mL. Two formulations were analyzed: one containing 1 mg Tolterodine tartrate and the other one with 2 mg of Tolterodine tartrate per tablet. For quantification, both samples were prepared with each solution having a nominal concentration of 0.25 mg/mL of Tolterodine tartrate.

Isolation and identification of unknown impurity

Isolation of unknown impurity attempt was performed initially by injecting higher load of stability sample solution in to the chromatographic column and the effluent was collected in multiple times. The solution was pre-concentrated and analyzed in LC-MS and established the molecular weight. Based on the molecular weight, attempts were made and synthesized the unknown impurity.

LC/MS analysis

Investigations were carried out using an Agilent 1100 LC/MSD Trap XCT Plus. MS spectrometer was equipped with Electrospray ion source and ion trap mass analyzer. The MS optimized parameters were used in this study are:

positive polarity; drying gas flow and temperature were 6 L/min and 300 ◦C, respectively, and nebulizador pressure was 16 psi.

Spectroscopic analysis

During characterization of unknown impurity several spectroscopic tools have been used. IR experiments were performed with a FT-IR spectrometer (Perkin-Elmer Spectrum 2000, Wellesley, MA, USA) on the isolated and the synthesized compound. Data acquisition recorded from 4500 to 450 cm^{-1}. A 1% (w/w) on KBr as transparent material pressed discs. The NMR experiments were acquired on BRUKER 300 MHz instrument at 25 deg. in DMSO and the chemical shift values were reported on the d scale relative to TMS.

Method validation

The HPLC method was validated after the optimization of several chromatographic parameters using a BDS C18 column, 5 µm particle size, 250×4.6 mm. The baseline separation was achieved with a linear step gradient condition of buffer pH 4.5 and a run time of 45 min. The mobile phase consisted of buffer pH 4.5 as mobile phase A and acetonitrile as mobile phase B. The analysis was performed by optimized flow rate of 0.7 mL/min with a proposed gradient program and UV detection at 205 nm. The injection volume was set 10 µL and oven temperature was kept at 30 ◦C in throughout the method validation activity. Standard and samples were prepared to a final theoretical concentration of 0.00125 mg/mL and 0.25 mg/mL of Tolterodine tartrate respectively. The selectivity and sensitivity was established by analyzing solutions containing the excipients and known impurities of Tolterodine tartrate drug substance in diluent. The diluent injection run shown that there was no peak at the retention time corresponding to the Tolterodine and impurities. The linearity was established for unknown impurity from LOQ to 400% of specification level (0.5%) and accuracy was proved between 10 to 400 %. The percentage recovery and RSD values were calculated. Method robustness was investigated by varying several chromatographic conditions and the method was found robust. The limit of detection and limit of quantification for unknown impurity was established by injecting a series of diluted unknown impurity and established the RSD values.

Method validation summary

Parameter	Results
Method precision	%RSD for recoveries from six preparations <5.0%
Linearity	Correlation coefficient: >0.999 for all known impurities
Accuracy	>99%
Specificity	Specific and selective to all known impurities and hence stability indicating.

Multiple stressed samples prepared and analyzed as a part of forced degradation study using PDA detector by a sample scan range of 200-400 nm. This study has proven that method is specific with respect to the all known and unknown peaks.

RESULTS AND DISCUSSION

During analysis of stability samples an unknown impurity at RRT 2.00 found in 3rd month stability samples analysis at a level of about 0.5%. The unknown impurity has shown a characteristic UV spectrum with maximum at about 205 nm as shown in Fig. 1. In addition, it has shown increasing in percentage level at increasing storage time, temperature and humidity. The percentage impurity level has reached maximum of 2% after 6 months in blister packing stability samples and that made necessary of identification and characterization of unknown impurity. The low content (in total weight) of Tolterodine tartrate in the tablet and, consequently, the small amount of degradation product present in the sample presented a challenge. For this reason an additional accelerated degradation study was carried out in order to generate a greater content in the target compound. Tolterodine tartrate API and a synthetic mixture tablet corresponding to 1 mg dose were subjected to two different treatments. In the first one, samples were put in to an oven at 105 ◦C for 16 h in opened vials. In the second one sample were exposed to humidity coming from water placed in the bottom of a container in the same conditions.

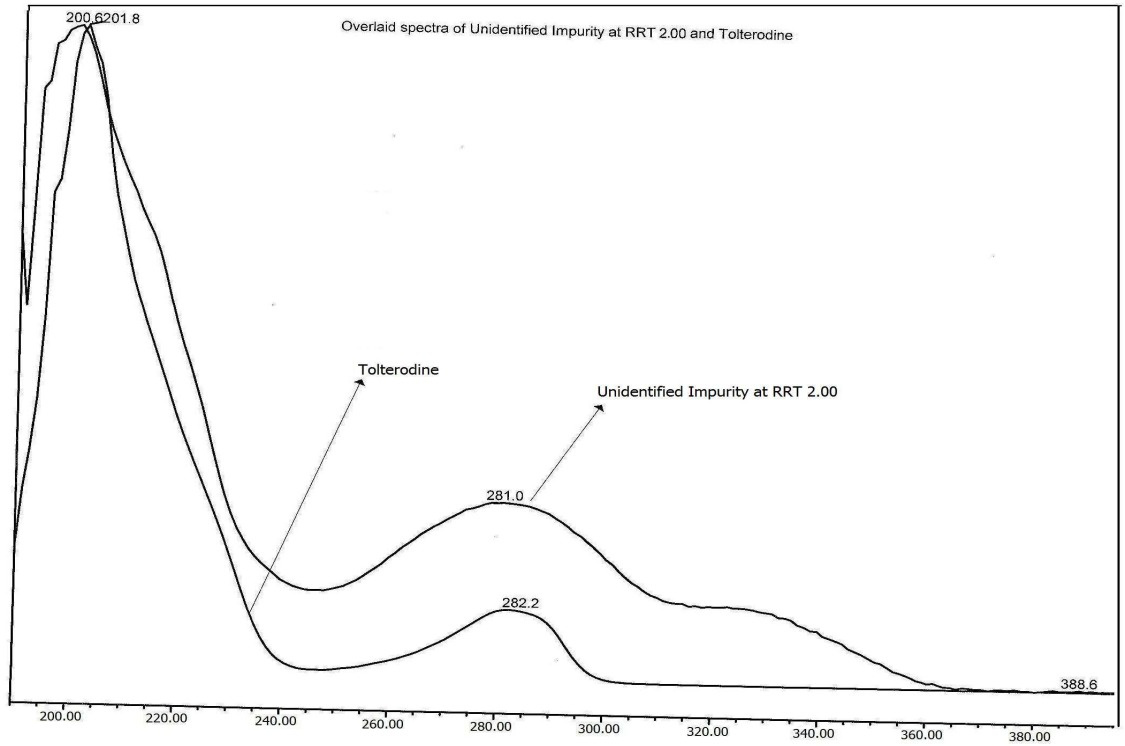

Fig.-1: UV spectrum of impurity and Tolterodine tartarate.

In accordance with the results observed in samples from the climatic chambers, temperature and humidity proved to play an important role in the generation of this degradation product. However, API exposed to similar conditions did not produce this impurity, thus proving the excipients used in the formulation could be played a major role in generating this impurity.

Characterization by spectroscopic tools

It is evident that the tool which provides the primary chemical information was LC/MS with using of volatile buffer as a mobile. Therefore, the 20mM mono basic phosphate solution at pH 4.5 was substituted in the mobile phase with a 20mM ammonium acetate buffer at pH 4.5 keeping other chromatographic parameters constant. The results obtained revealed the equivalent selectivity offered by both mobile phases.

The IR spectrum of the synthesized unknown impurity (Fig. 2) shown a band at 3437.50 cm^{-1} due to –OH stretching, bands at 3050.83 and 3027.33 cm^{-1} due to –C-H stretching (aromatic), bands at 2695.11 and 2924.92 cm^{-1} due to $–CH_3$ stretching (aliphatic), and bands at 1600.34, 1588.55, 1494.45 and 1452.95 cm^{-1} are due to C=C stretching (aromatic).

Moreover, the unknown impurity has been completely characterized by using IR and LC-MS, further study has been employed by using NMR and confirmed the structure. All results obtained by independent identification techniques demonstrate that the correct assignation of the structure proposed here, as shown in Fig. 3. In fact, in our study Tolterodine API was stable to the most common degradation factors but its degradation, in tablets kept in blisters isolated from light, was moderate with heat and humidity, and it was completely dependent on the presence of excipients. Further studies will be necessary to determine the excipient or mixture responsible for the degradation path way or to predict reaction mechanism. Based on the above data the impurity structure is identified as 6-methyl-4-phenylchroman-2-ol.

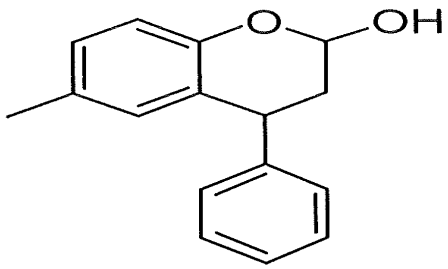

Fig.-3: The proposed structure of the unknown impurity (6-methyl-4-phenylchroman-2-ol).

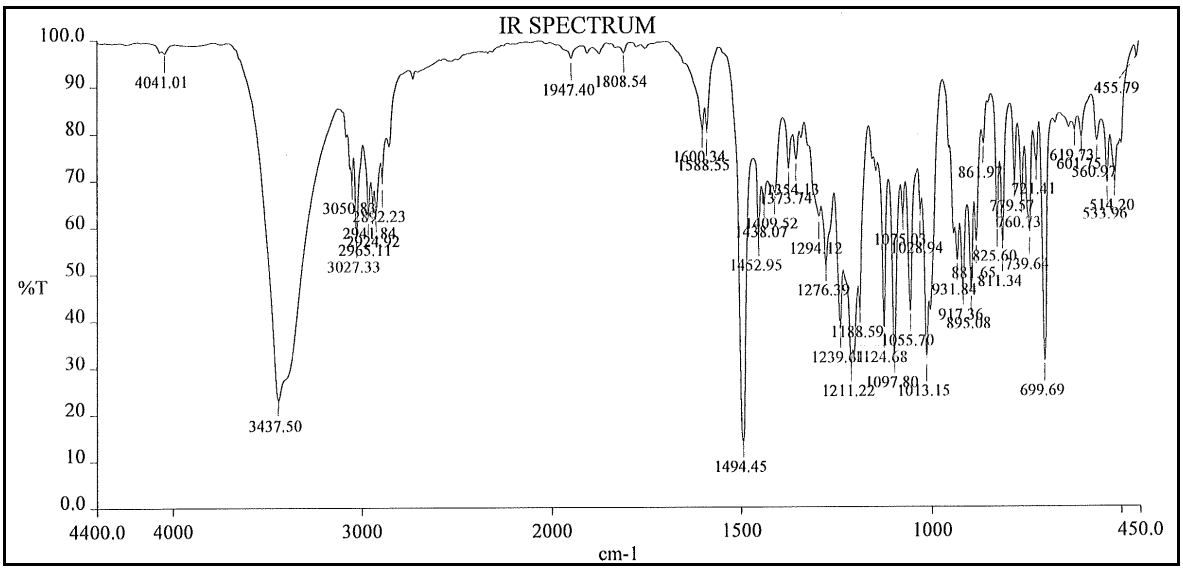

Fig.-2: IR spectrum of Tolterodine tartarate and unknown impurity.

CONCLUSION

The analytical method used for the identification and characterization of unknown impurity at RRT 2.00 in the Tolterodine tartrate tablets is a very useful tool for the monitoring of drug product quality. An efficient isolation method, a set of elucidation techniques, as well as the synthesis of the compound has been successfully applied to the identification of a degradation product that appears in Tolterodine tartrate tablets. Accelerated degradation studies have proven that this impurity, identified as, 6-methyl-4-phenylchroman-2-ol, is generated from the interaction between Tolterodine and excipients under high temperature and humidity conditions, but is independent of the presence of light. A rapid HPLC method with UV detection has been developed that allows the determination of this impurity. This method has been properly validated and it has been shown that it is reliable, being linear, accurate and precise.

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