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Short communication

Identification, synthesis, isolation and characterization of new impurity in metoprolol tartrate tablets

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ABSTRACT

A new unknown impurity was observed in accelerated stability studies of Metoprolol tartrate tablets. This impurity has been identified, synthesized and characterized through different spectral studies and confirmed as an adduct of lactose and Metoprolol formed by Maillard reaction.

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1. Introduction

Metoprolol tartrate is chemically known as bis [(2RS)-1-[4-(2-methoxyethyl) phenoxy]-3-[(1-methylethyl) amino]propan-2-ol](2R, 3R)-2,3-dihydroxybutanedioate having molecular weight 684.81. It is a selective β_1 receptor blocker and commonly used to treat high blood pressure, chest pain due to poor blood flow to the heart, and a number of conditions involving an abnormally fast heart rate [1–3]. In Metoprolol tartrate tablets, active ingredient is Metoprolol and lactose is one of the excipients that has been used as a diluent. Interactions and incompatibility studies between active pharmaceutical ingredient and excipient are very crucial part in the preformulation stage of the development of all dosage forms. Sometimes the potential physical and chemical interactions between drugs and excipients may lead to the formation of impurities that can influence the drugs therapeutic effects [4,5]. Therefore it is essential to carry out stability studies of drug product to predict, evaluate, and ensure drug product safety and efficacy [6].

While performing accelerated stability studies of Metoprolol tartrate tablets, formation of one new unknown impurity at a level greater than 0.1% was observed in HPLC analysis. According to International conference on Harmonisation (ICH) guidelines any unknown impurity present at or above 0.1% level in drug product should be well identified and characterized. Therefore this new unknown impurity was identified, synthesized and characterized by different spectroscopic techniques like LC–MS, ^{1}H, ^{13}C NMR, HSQC.

The present study, to the best of our knowledge, is the first that revealed the identification, synthesis and characterization of new unknown impurity that was found to be formed during accelerated stability studies of Metoprolol tartrate tablets.

2. Materials and methods

2.1. Samples and reagents

Metoprolol tartrate sample was obtained from manufacturing site of Ipca Laboratories Ltd. (Mumbai, India). Reagents used for analysis like trifluoroacetic acid, ammonium acetate, acetic acid (AR grade), acetonitrile (HPLC grade), D_2O and all chemicals used for synthesis were purchased from Merck (India) Limited. Milli-Q grade water has been used for the preparation of mobile phase.

2.2. High performance liquid chromatography

A Waters Model Alliance 2695 Separation Module (Waters Corporation, Milford, MA, USA) equipped with a Waters 2489 UV Detector was used for the studies. Data was processed through Waters empower software. The analysis was carried out on Waters Symmetry C_{18} column, 150 mm with i.d. 4.6 mm and 5.0 μm particle size with a mobile phase consisting of Isocratic mixture of buffer

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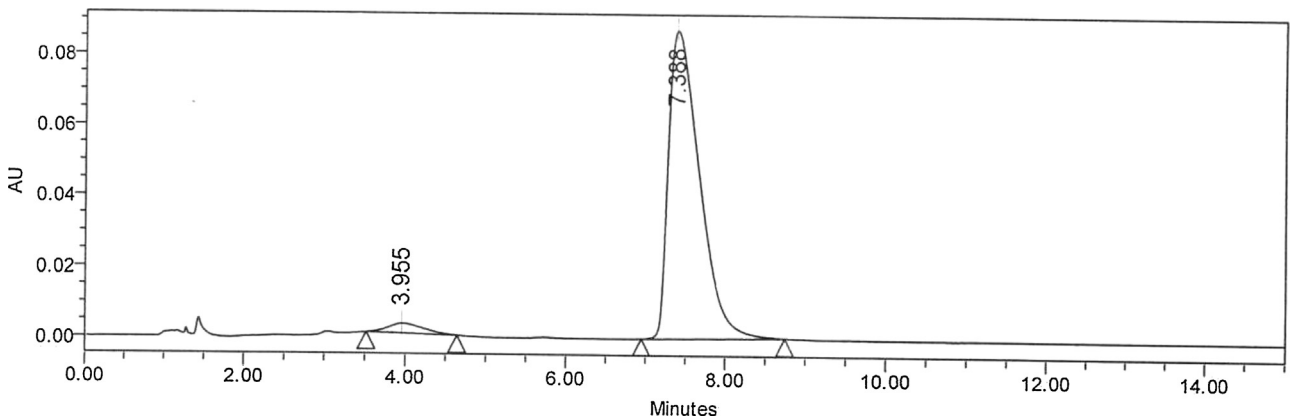

Fig. 1. HPLC chromatogram of Metoprolol tartrate tablet.

(ammonium acetate–acetic acid; pH 3.7; 0.06 M) and acetonitrile in the ratio of 84:16 (v/v). The column temperature was maintained at ambient condition. Injection volume was 100 μL. The flow rate was maintained at 1 ml/min and column eluent was monitored at 280 nm for 15 min.

2.3. Preparative high performance liquid chromatography

A Waters Model Alliance 2555 Separation Module (Waters Corporation, Milford, MA, USA) equipped with a Waters 2489 UV Detector and Empower software was used. The column used for separation was Waters Symmetry C_{18} column, 250 mm with i.d. 30 mm and 5.0 μm particle size with a mobile phase consisting of mixture of water (pH 3; adjusted with TFA) and acetonitrile in the ratio of 80:20 (v/v). Injection volume was 5 ml containing 2.5 g of reaction mass. The flow rate was maintained at 15 ml/min and column eluent was monitored at 280 nm for 15 min.

2.4. Mass spectrometry

The High Resolution Mass Spectra was obtained from a Thermo Scientific Q-Exactive-Orbitrap mass spectrometer (Waltham, Massachusetts, United States) with 17,500 resolutions. Sample was prepared in isocratic mixture of acetonitrile and water (50:50, v/v). Sample was introduced by syringe pump and ionization was achieved by HESI ionization source in the positive ion detection mode. Nitrogen was used as both the sheath gas and the auxiliary gas at 30 psi and 10 psi respectively. HESI source parameters were set as capillary temperature 320 °C, spray voltage of 4 kV and heater temperature 250 °C. Scanning of sample was done over mass range of 150–1000 . Operating software used was Thermo Xcalibur 3.0.63. MS/MS studies were carried out by maintaining normalized collision energy at about 25% with the mass range *m/z* 269.10–969.20.

2.5. Nuclear magnetic resonance spectroscopy

The ^{1}H, ^{13}C and HSQC (2D) NMR experiments were performed on AVANCE 400 (Bruker, Fallanden, Switzerland) instrument at 300 K. Distortionless enhancement by polarization transfer (DEPT) spectral editing revealed the presence of methyl and methine groups as positive peaks while the methylenes as negative peaks. Sample was run in D_2O.

3. Results and discussions

3.1. Detection of unknown impurity

Metoprolol tartrate tablets were analyzed by the HPLC method as described in Section 2.2. One new unknown impurity was observed at RT 3.955 along with Metoprolol at RT 7.388 (Fig 1). The positive ESMS spectrum (Fig 2) of these tablets exhibited molecular ion peak as $[M+H]^+$ at *m/z* 268.19 (MS-RT 5.55) which corresponds to Metoprolol, the main active compound of the tablet. Unknown peak at MS-RT 3.06 revealed two *m/z* 630.25 and 592.29. The *m/z* of 630.25 being the potassium adduct of the impurity with molecular weight 591.65. This indicates the possibility of unknown impurity to be an adduct of Metoprolol and lactose that can be formed by simple condensation reaction between Metoprolol (m. wt = 267.36) and lactose (m. wt. = 342.30) with the loss of one water molecule (Maillard reaction) [7–9]. Possible structure of Metoprolol lactose adduct is shown in (Fig 3). To confirm the new impurity as an adduct of Metoprolol and lactose, synthesis has been carried out under defined reaction conditions (Section 3.2). The impurity was isolated by preparative liquid chromatography as described in Section 2.3. The obtained impurity was fully characterized with the help of spectral techniques like ^{1}H NMR, ^{13}C NMR, HSQC (2D NMR) and MS.

3.2. Synthesis and isolation of Metoprolol lactose adduct

Metoprolol tartarate (10 mmol) and lactose (20 mmol) were mixed in N, *N*-Dimethyl acetamide and ethanol in presence of triethylamine (10 mmol) and refluxed for 38 h at 90 °C. Reaction mass was distilled completely and the crude sample of reaction mass containing 5-7% of required impurity was subjected to preparative liquid chromatography as mentioned in Section 2.3. The HPLC purity of isolated impurity was more than 95% and was used for spectral characterization without any further purification.

3.3. Structure elucidation of impurity

Mass spectrometry was performed for this isolated impurity by ESI technique in positive mode. The positive DI-MS spectrum (Fig 4) of isolated impurity exhibited molecular ion peak as $[M+H]^+$ at *m/z* 592.29 and as sodium adduct $[M+Na]^+$ at *m/z* 614.28. The MS/MS data displayed a dominant fragment at *m/z* 574.28 which is 17 amu less than the molecular ion peak indicating a removal of the hydroxyl group. This indicates that the impurity is Metoprolol-lactose adduct as proposed. The high resolution mass proposed the probable molecular formula $C_{27}H_{45}NO_{13}$.

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