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ORIGINAL ARTICLE

Identification, synthesis and characterization of an unknown process related impurity in eslicarbazepine acetate active pharmaceutical ingredient by LC/ESI–IT/MS, ^{1}H, ^{13}C and ^{1}H–^{1}H COSY NMR

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Abstract A new impurity was detected during high performance liquid chromatographic (HPLC) analysis of eslicarbazepine acetate active pharmaceutical ingredient. The structure of unknown impurity was postulated based on liquid chromatography mass spectrometry using electrospray ionization and ion trap analyzer (LC/ESI–IT/MS) analysis. Proposed structure of impurity was unambiguously confirmed by synthesis followed by characterization using ^{1}H, ^{13}C nuclear magnetic resonance spectrometry (NMR), ^{1}H–^{1}H correlation spectroscopy (COSY) and infrared spectroscopy (IR). Based on the spectroscopic and spectrometric data, unknown impurity was characterized as 5-carbamoyl-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepin-10-yl propionate.

1. Introduction.

Eslicarbazepine acetate is chemically *S*-(−)-10-acetoxy-10,11-dihydro-5*H*-dibenzo[*b*,*f*] azepine-5-carboxamide acetate (Fig. 1). It is a prodrug to eslicarbazepine and an active metabolite of oxcarbazepine. Eslicarbazepine acetate is rapidly and extensively metabolized to eslicarbazepine which is responsible for

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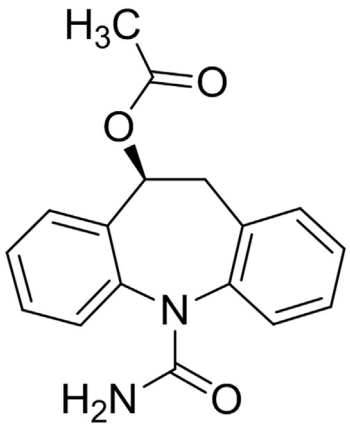

Fig. 1 The structure of eslicarbazepine acetate.

pharmacological activity. It is used as add-on therapy in refractory partial epilepsy and also in bipolar disorder [1–4].

Liquid chromatographic methods have been reported for the estimation of (*R*)-enantiomer in eslicarbazepine acetate and chromatographic conditions have been mentioned for the analysis of eslicarbazepine acetate [5,6]. A highly efficient and sensitive method for determination of potential impurities in eslicarbazepine active pharmaceutical ingredient and an isocratic stability indicating method for the determination of eslicarbazepine acetate and its impurities has been reported recently [7,8].

Objective of the current study was to identify, synthesize and characterize one unknown impurity detected consistently in several batches of eslicarbazepine acetate ranging from 0.05% to 0.08%. Regulatory agencies world over are demanding the characterization of unknown impurities to ensure their non genotoxicity, identification and control to establish the quality, safety and efficacy of drug substance. Further, International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use (ICH) guidelines indicate that unknown impurities at or above 0.05% in the drug substance require identification depending on the maximum daily dosage [9]. Therefore, it was felt necessary to characterize the unknown impurity observed in the drug substance. Unknown impurity was identified by LC–MS/MS data and evaluating the synthetic scheme of eslicarbazepine acetate. Proposed structure was further unambiguously confirmed by independent synthesis followed by characterization using MS, 1D NMR, 2D NMR and IR. To the best of our knowledge, this impurity has not been reported previously. A plausible mechanism for the formation and control of new impurity has also been proposed in this study.

2. Materials and method

2.1. Materials and reagents

Sample of eslicarbazepine acetate active pharmaceutical ingredient and standards of Imp-1 ((10*S*)-10-Hydroxy-10,11-dihydro-5*H*-dibenzo[*b*,*f*] azepine-5-carboxamide), Imp-2 (10-Oxo-10, 11-dihydro-5*H*-dibenzo [*b*,*f*]azepine-5-carboxamide), Imp-3 (10-Acetoxy-5*H*-dibenzo[*b*,*f*]azepine-5-carboxamide) and Imp-4 (5-Acetyl-5,11-dihydro-10*H*-dibenzo[*b*,*f*]azepin-10-one) were obtained from Chemical Research and Development Department, Jubilant Life Sciences Limited (Noida, India). Deionized water was prepared using a Milli-Q Plus water purification system from Millipore (Bedford, MA, USA). HPLC grade acetonitrile, analytical reagent grade (AR) potassium dihydrogen phosphate, ammonium bicarbonate, hydrochloric acid and orthophosphoric acid were purchased from Merck India Limited (Mumbai, India). Deuterated chloroform ($CDCl_3$) and deuterated dimethyl sulfoxide (DMSO-d6) were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). Potassium bromide of Fourier transformed infrared spectroscopy (FT-IR) grade was purchased from Merck KGaA (Darmstadt, Germany). Laboratory reagent grade dichloromethane, dimethyl aminopyridine, triethylamine, propionic anhydride and sodium sulphate were purchased from S.D Fine Chemicals (Mumbai, India).

2.2. High performance liquid chromatography (HPLC)

Samples were analyzed on a Waters alliance 2690 separation module equipped with 2487 UV detector (Waters corporation, MA, USA) using a symmetry shield RP-8 (250 mm × 4.6 mm, 5 µm, Waters corporation, MA, USA). Mobile phase A consisted of 10 mM potassium dihydrogen phosphate adjusted to pH 5.00 ± 0.05 with orthophosphoric acid–acetonitrile (95:5, v/v) and mobile phase B consisted of acetonitrile–water (80:20, v/v) in gradient mode (T_{min}A:B) $T_0$70:30, T_{15}65:35, T_{20}50:50, T_{40}30:70, T_{55}70:30, and T_{60}70:30. The flow rate was set at 1.0 mL/min. The injection volume was 10 µL for a sample concentration of 400 µg/mL prepared in diluent (mobile phase A–acetonitrile, 50:50, v/v). Detector wavelength was fixed at 215 nm and the column temperature was maintained at 35 °C throughout the analysis.

2.3. Liquid chromatography–tandem mass spectrometry (LC–MS/MS).

The equipment and chromatographic conditions used for LC–MS investigation were exactly the same as described under Section 2.2. Mobile phase A consisted of 10 mM ammonium bicarbonate–acetonitrile (95:5, v/v) and mobile phase B consisted of acetonitrile–water (80:20, v/v) in gradient mode (T_{min}A:B) $T_0$70:30, T_{15}65:35, T_{20}50:50, T_{40}30:70, T_{55}70:30, and T_{60}70:30. The flow rate was set at 1.0 mL/min. The injection volume was 10 µL for a sample concentration of 400 µg/mL prepared in diluent (mobile phase A–acetonitrile, 50:50, v/v). Detector wavelength was fixed at 215 nm and the column temperature was maintained at 35 °C throughout the analysis.

The MS and MS/MS studies were performed on Thermo LCQ-Advantage and Xcalibur software (Thermo Electron, San Jose, CA, USA) using electrospray ionization source and ion trap mass spectrometer. The typical electrospray source conditions were spray voltage 5 kV, capillary voltage 15–20 V, heated capillary temperature 250 °C, tube lens offset voltage 20 V, sheath gas (N_2) pressure 20 psi and helium was used as damping gas. In the full scan MS^2 mode, collision energies of 15–35 eV and isolation width of 5 a.m.u. were used. The excitation time was 30 ms and the isolated ions were then subjected to a supplementary alternative current (AC) signal to resonantly excite causing collision induced dissociation (CID).

2.4. Nuclear magnetic resonance spectroscopy (NMR)

^1H and ^{13}C NMR spectra were recorded at 399.957 MHz and 100.432 MHz, respectively, using a Bruker AVANCE 400 MHz spectrometer (Bruker, Fallanden, Switzerland) equipped with a 5 mm BBO probe and a z-gradient shim system. Samples were dissolved in deuterated chloroform ($CDCl_3$) and dimethyl sulfoxide (DMSO-d6).