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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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Characterization;
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Impurity

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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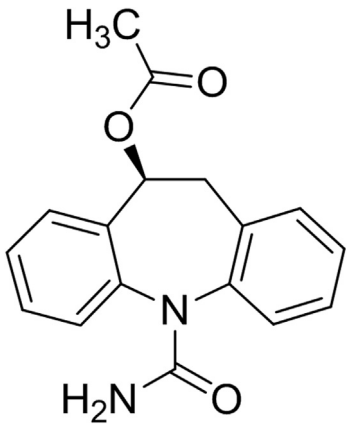

Fig. 1 The structure of eslicarbazepine acetate.

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).

The ^{1}H and ^{13}C chemical shift values were reported on the δ scale in parts per million (ppm) relative to deuterated chloroform ($CDCl_3$, 7.26 ppm, 77.0 ppm) and (DMSO, 2.50 ppm, 39.5 ppm). All spectra were recorded with sample spinning. 2D homonuclear shift correlation experiment (^{1}H–^{1}H–COSY) was performed with 20–90° and 90° high power gradient pulse.

2.5. *Fourier transform infrared spectroscopy (FT-IR)*

The IR spectrum was recorded in solid state as potassium bromide (KBr) powder dispersion using Nicolet FT-IR model AVATAR 370 (Thermo Electron Scientific Instruments, Madison, WI, USA) with a deuterated triglycine sulphate (DTGS) KBr detector. Data were collected between 400 and 4000 cm^{-1}, with a resolution of 4.0 cm^{-1}. A total of 16 scans were obtained and processed using the OMNIC software version 6.0.

3. Results and discussion

3.1. *Identification of unknown impurity by LC-ESI/MS/MS*

HPLC analysis of eslicarbazepine acetate using previously reported method [7] revealed the presence of one unknown impurity at relative retention time (RRT) 1.48 in several batches of eslicarbazepine acetate in the range of 0.05–0.08% along with three known impurities. Known impurities at RRTs 0.45, 0.75 and 1.19 were marked as Impurity-1, Impurity-2 and Impurity-3 respectively based on their elution order. The unknown impurity at RRT 1.48 was marked as Imp-A (Fig. 2A). It was observed that the RRT of unknown impurity was very close to one of the previously reported impurities at RRT 1.45 (Impurity-4), therefore the known impurity at RRT 1.45 was spiked in the sample and confirmed that there is adequate resolution between unknown impurity at RRT 1.48 (Imp-A) and the known impurity at RRT 1.45 (Fig. 2B). The sample was subjected for LC–MS analysis using the method described under Section 2.3.

Prior to the characterization of Imp-A, the mass spectral fragmentation of the parent drug molecule of eslicarbazepine acetate was investigated as depicted in Fig. 3A–C. This multistage mass fragmentation pattern of eslicarbazepine acetate along with its potential impurities has already been reported [7]. Imp-A showed its protonated molecular ion at *m/z* 311 (Fig. 3D) which is 14 Da higher than that of eslicarbazepine acetate. The MS^2 analysis of the parent ion at *m/z* 311, showed a prominent peak at *m/z* 237 which can be attributed to the neutral loss of propenone (CH_3–CH=C=O, 56 Da) due to the formation of metastable ion at *m/z* 255 followed by loss of water (H_2O, 18 Da) (Fig. 3D and E). This fragment ion at *m/z* 237 was

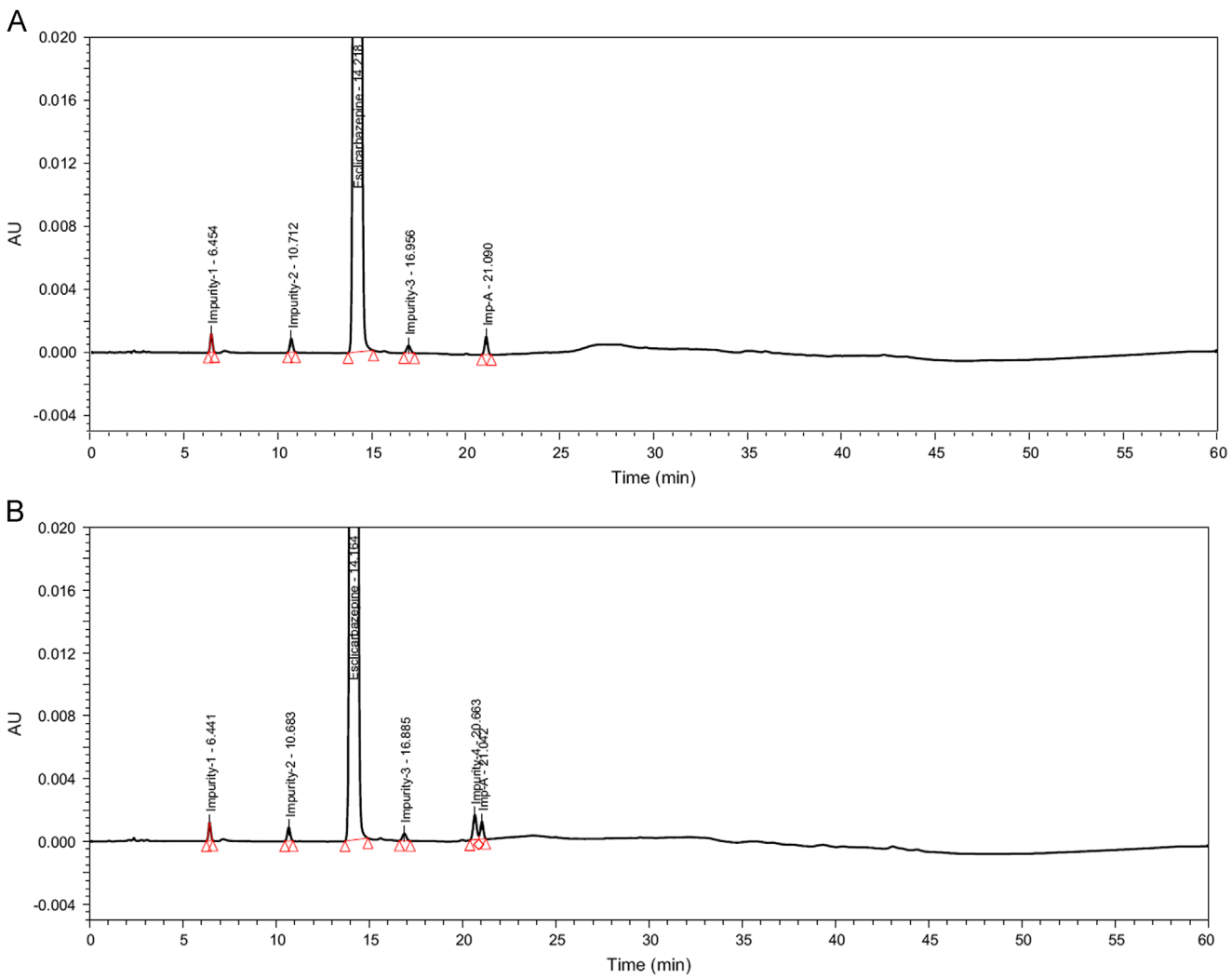

Fig. 2 Chromatogram of (A) eslicarbazepine acetate sample and (B) eslicarbazepine acetate sample spiked with impurity-4.

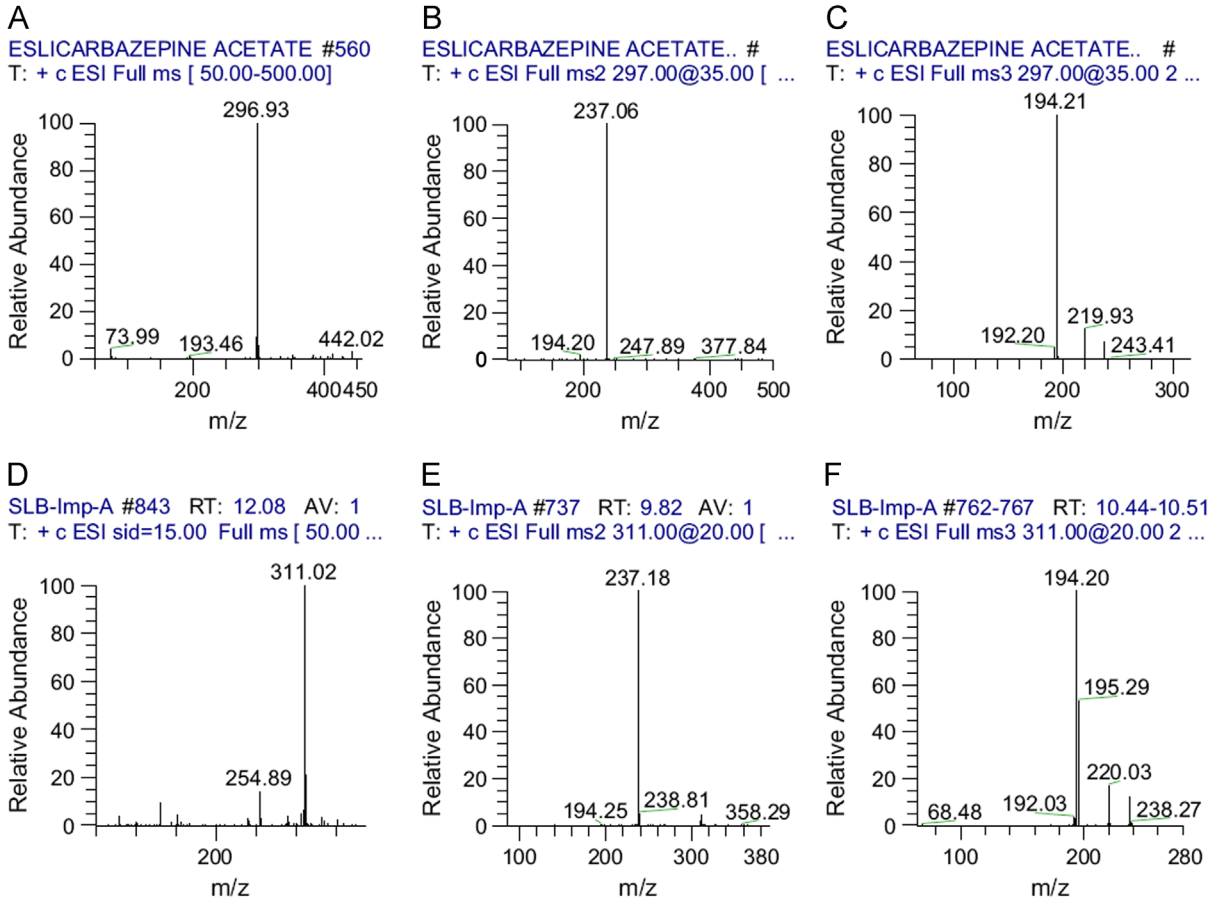

Fig. 3 (A) Mass spectrum of eslicarbazepine acetate showing [M+H] at *m/z* 297, (B) MS^2 spectrum of eslicarbazepine acetate, (C) MS^3 spectrum of eslicarbazepine acetate, (D) mass spectra of Imp-A showing $[M+H]^+$ (at *m/z* 311), (E) MS^2 spectrum of Imp-A, and (F) MS^3 spectrum of Imp-A.

characterized as dibenzo[*b*,*f*]azepine-5-carboxamide and was subjected to further fragmentation. In the MS^3 stage the precursor ion at *m/z* 237 underwent neutral loss of iminomethanone (NH=C=O, 43 Da) and ammonia (NH_3, 17 Da) to produce daughter ions at *m/z* 194 and *m/z* 220 respectively (Fig. 3F). A critical comparison between the MS^2 and MS^3 spectra of eslicarbazepine acetate and that of Imp-A indicated that the dibenzo[*b*,*f*]azepine-5-carboxamide is common to both of the molecules and it was suspected that Imp-A contains an additional methylene group in the acetate side chain. The mechanistic pathway indicating the formation of the daughter ions is outlined in Fig. 4A and B. The knowledge of eslicarbazepine acetate synthetic scheme led to propose the molecular structure of the unknown impurity based on LC–MS/MS data. The last step of synthesis involves acetylation of eslicabrazepine using acetic anhydride. It was suspected that formation of Imp-A was due to the presence of propionic anhydride in acetic anhydride and the most plausible structure of Imp-A was proposed as 5-carbamoyl-10,11-dihydro-5*H*-dibenzo[*b*,f]azepin-10-yl propionate. The synthetic scheme of plausible formation of Imp-A is depicted in Fig. 4C. Based on the proposed structure Imp-A was independently synthesized and used for further structural confirmation by IR, ^1H, ^{13}C and ^1H–^1H COSY–NMR techniques.

3.2. *Synthesis of Imp-A*

Five grams of eslicarbazepine and 50 mL of dichloromethane, 1 g of dimethyl aminopyridine, 20 mL of triethylamine and 1 mL of pyridine were charged and stirred at 25–30 °C for 20 min. 10 mL of propionic anhydride was mixed with 10 mL of dichloromethane and then added to the reaction mixture over a period of 1 h maintaining the temperature at 25–30 °C. Temperature was raised to 40–45 °C and maintained for 3 h. The progress of reaction was monitored by HPLC. After reaction completion, reaction mass was cooled to 25–30 °C and the pH of the reaction mass was adjusted to 4.0 using dilute hydrochloric acid. Dichloromethane layer was separated, washed with water, dried over sodium sulphate and concentrated to obtain 3.8 g of 5-carbamoyl-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepin-10-yl propionate (73.0% yield). HPLC purity of isolated material was found to be 98.5%. The synthesized Imp-A was co-spiked with eslicarbazepine acetate sample and confirmed that the retention time of Imp-A in eslicarbazepine sample and co-spiked sample was exactly the same (Fig. 5). The MS and MS^2 spectra obtained for synthesized Imp-A using direct infusion mode and from on-line LC–MS/MS analysis were also found to be exactly the same. Synthesized impurity was used for further characterization by IR and NMR. Formation of Imp-A can be eliminated in the drug substance by controlling the level of propionic anhydride content in commercial samples of acetic anhydride.

3.3. *Structure elucidation of Imp-A by IR, ^1H, ^{13}C and ^1H–^1H COSY–NMR*

The ^1H and ^{13}C NMR spectral data of Imp-A were compared with those of eslicarbazepine acetate. The significant difference was observed in the shielded region where the chemical shift of methyl signal of eslicarbazepine acetate (δ 2.07, s and δ 21.0) was absent

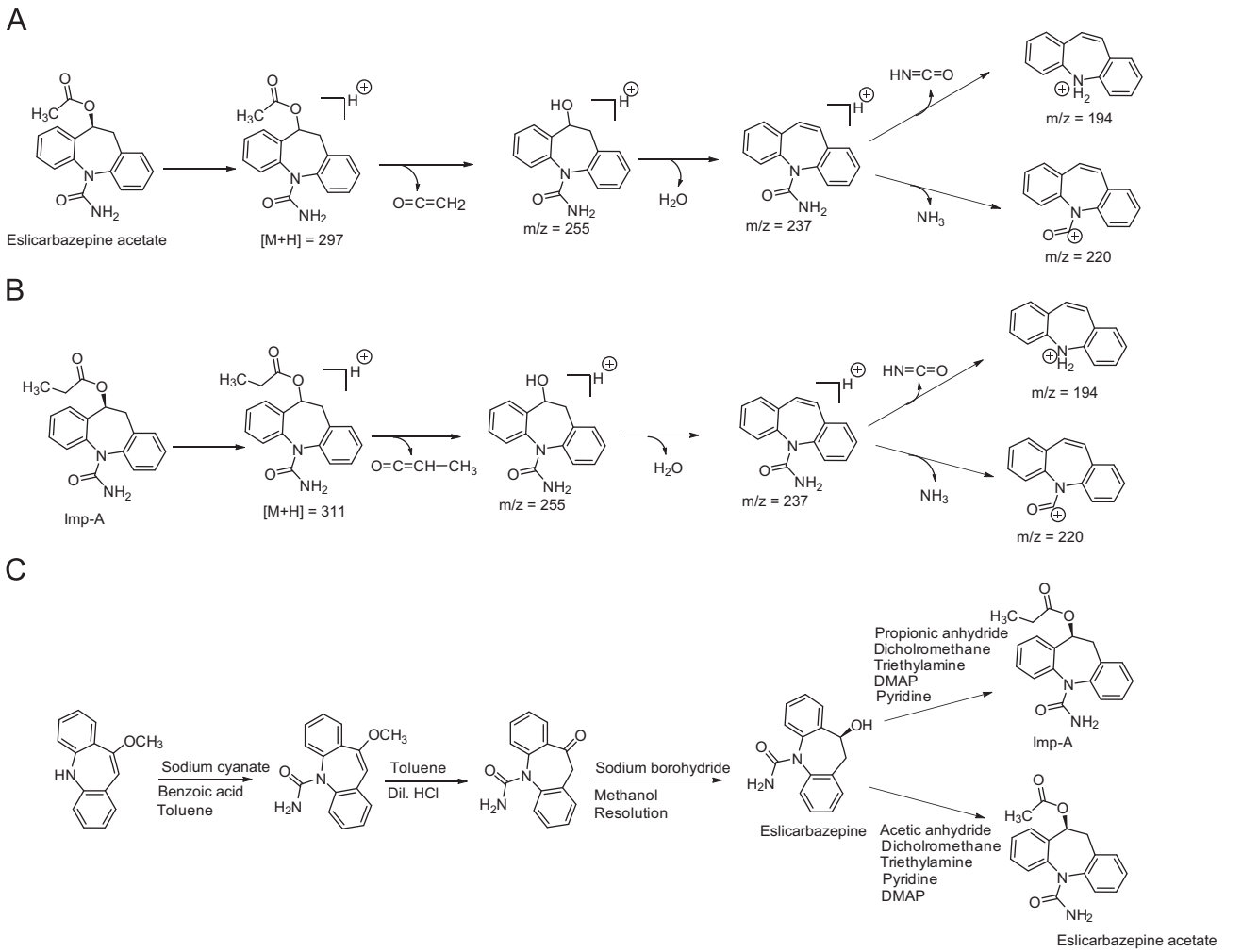

Fig. 4 Fragmentation pathway showing the formation of product ions of (A) eslicarbazepine acetate, (B) Imp-A and (C) synthetic scheme of eslicarbazepine acetate and pathway for the formation of Imp-A.

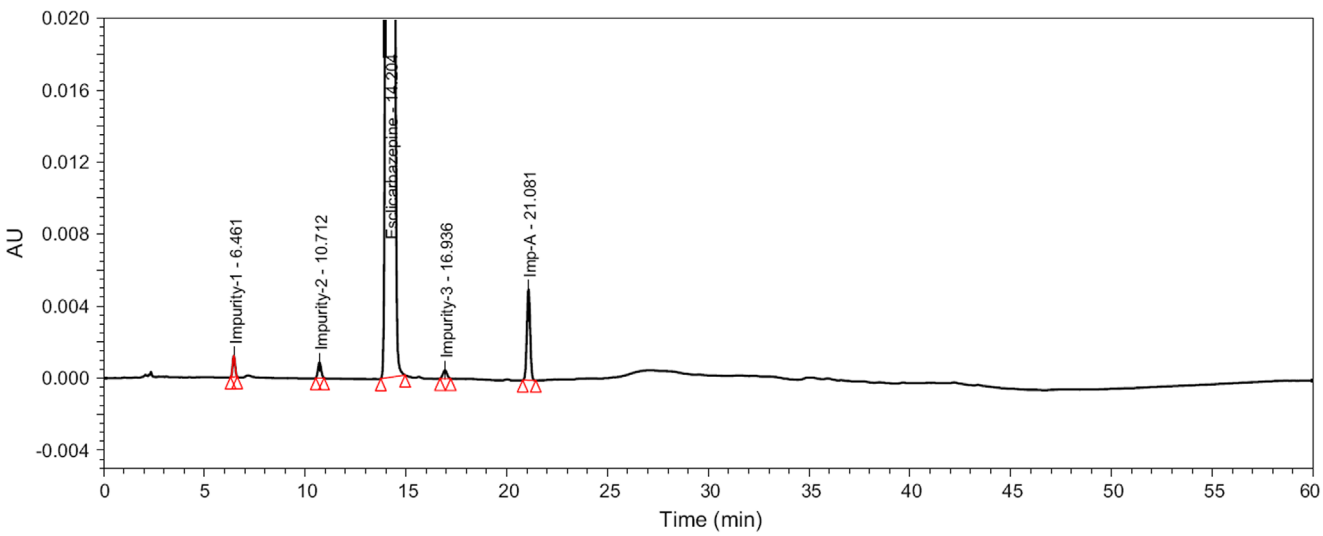

Fig. 5 Chromatogram of eslicarbazepine acetate sample spiked with synthesized Imp-A.

with the noticeable appearance of two signals (δ 2.36, q, 1.16, t in ^{1}H NMR and δ 27.8, 9.0 in ^{13}C NMR respectively) with an ethyl group pattern in Imp-A (Fig. 6A). The ^{1}H NMR of Imp-A showed a total of 18 protons. The exchangeable amide protons were observed at δ 4.80. Eight aromatic protons were observed as a second order multiplet at δ 7.34. ^{13}C NMR supports the presence

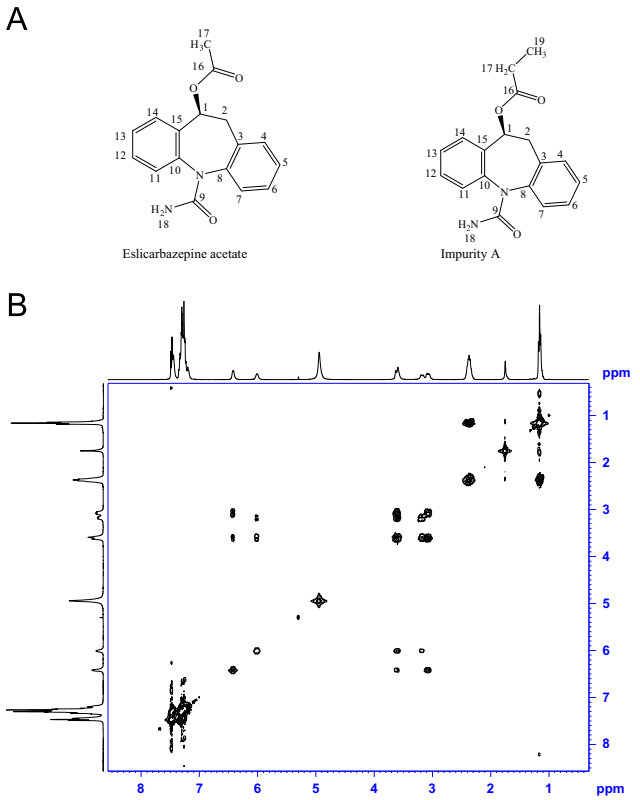

Fig. 6 (A) Structure of eslicarbazepine acetate and Imp-A (numbering is only for NMR characterization) and (B) ^{1}H–^{1}H COSY spectrum of Imp-A.

of propionyl ester by exhibiting the chemical shift values at δ 9.0 (–CH_3), 27.8 (–CH_2) and 173.6 (–O–C=O). Another downfield signal at δ 156.7 was assigned to the carbamoyl carbon. The assignments were further confirmed by ^{1}H–^{1}H COSY spectrum (Fig. 6B). The upfield coupling correlation was seen for ethyl group (–CH_3; δ 1.16 and –CH_2; δ 2.36). Another correlation was observed in the azepine ring between methylene (δ 3.11, 3.61) and methine protons (δ 6.20) while geminal coupling was observed between the methylene protons (δ 3.11, 3.61 H_AH_B) adjacent to the chiral centre. There is a single cross peak at δ 4.80 at the diagonal assigned for the exchangeable amide protons. A complex correlation was observed among the aromatic protons. Based on the ^{1}H, ^{13}C NMR and ^{1}H–^{1}H COSY spectral assignments the structure of Imp-A was confirmed.

Eslicarbazepine acetate (Fig. 6A): ^{1}H NMR (400 MHz, DMSO): 2.07 (s, 3H, 17), 3.07 (m, 2H, 2), 5.90 (s, 2H, 18, –NH_2), 6.18 (m, 1H, 1), 7.32 (m, 8H, 4, 5, 6, 7, 11, 12, 13, 14). ^{13}C NMR (100 MHz, DMSO): 21.0 (17), 35.5 (2), 70.1 (1), 127.4–133.8 (4, 5, 6, 7, 11, 12, 13, 14), 133.7 (3, 15), 141.4 (8, 10), 156.1 (9), 169.8 (16). IR: NH str. (3476.4), C=O str. (1726.2, ester; 1653.60, carbamoyl), C=C str. (1564.1, 1488.1).

Impurity A (Fig. 6A): ^{1}H NMR (400 MHz, $CDCl_3$): 1.16 (t, 3H, 19), 2.36 (q, 2H, 17), 3.11, 3.61 (m, 2H, 2), 4.80 (s, 2H, 18, –NH_2), 6.20 (m, 1H, 1), 7.34 (m, 8H, 4, 5, 6, 7, 11, 12, 13, 14). ^{13}C NMR (100 MHz, $CDCl_3$): 9.0 (19), 27.8 (17), 35.8 (2), 69.8 (1), 127.8–134.8 (4, 5, 6, 7, 11, 12, 13, 14), 138.9 (3, 15), 140.6 (8, 10), 156.7 (9), 173.6 (16). IR: NH str. (3474.3), C=O str. (1728.3, ester; 1662.0, carbamoyl), C=C str. (1566.0, 1487.2).

4. Conclusion

In this study, a new impurity detected in eslicarbazepine acetate drug substance was identified by LC–ESI/IT–MS and NMR. A proposed structure of impurity was confirmed by independent synthesis followed by structural elucidation using ^{1}H, ^{13}C, ^{1}H–^{1}H COSY, IR and MS techniques. Imp-A was characterized as 5-carbamoyl-10,11-dihydro-5*H*-dibenzo[*b*,*f*]azepin-10-yl propionate. This impurity was found to be highly potential in commercial scale and can be controlled in the drug substance by use of propionic anhydride free acetic anhydride.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jpha.2013.08.004.

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