Journal of Pharmaceutical and Biomedical Analysis 56 (2011) 423–428

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Short communication

Identification and structural elucidation of an unknown impurity in carbamazepine active pharmaceutical ingredient by liquid chromatography–tandem mass spectrometry and semi-preparative chromatographic isolation

Saji Thomas[a,*], Chandra S. Mathela[b], Ashutosh Agarwal[a], Saroj Kumar Paul[a]

[a] Jubilant Life Sciences Ltd., Analytical Research Department, R&D Centre, C-26, Sector-59, Noida 201 301, Uttar Pradesh, India
[b] Department of Chemistry, Kumaun University, D.S.B. Campus, Nainital 263 001, Uttarakhand, India

ARTICLE INFO

Article history:
Received 6 January 2011
Received in revised form 16 May 2011
Accepted 19 May 2011
Available online 27 May 2011

Keywords:
Carbamazepine
Impurity
LC/MS/MS
NMR
Elemental analysis

ABSTRACT

Two impurities were detected in the HPLC analysis of crude carbamazepine active pharmaceutical ingredient. One of the impurities of the order of 0.5% was found to be unknown and has not been reported previously. A LC–MS compatible reverse phase isocratic method was developed and tandem mass spectrometry was performed using electrospray ionization source and ion trap mass analyzer. Isolation of unknown impurity was performed by semi-preparative HPLC followed by characterization using nuclear magnetic resonance spectroscopy (NMR), infrared spectroscopy (FT-IR) and elemental analysis (CHNS) confirmed its structure as tetrabenzo[b,f,b′f′]azepino[4′,5′:4,5]thieno[2,3-*d*]azepine-3,9-dicarboxamide. A plausible mechanism for the formation of this impurity is proposed.

1. Introduction

Carbamazepine (Fig. 1a) is a dibenzazepine derivative with antiepileptic and psychotropic properties. It is used to control secondarily generalised tonic–clonic seizures, partial seizures and in some primary generalised seizures. Carbamazepine is also used in the treatment of trigeminal neuralgia and has been tried with variable success in glossopharyngeal neuralgia and other severe pain syndromes associated with neurological disorders such as tabes dorsalis and multiple sclerosis [1–3].

HPLC methods for the determination of carbamazepine related impurities were reported in USP, Ph.Eur., BP and IP [4–7]. A GC–MS method for carbamazepine kinetic and chemical assessment of the UV/H_2O_2 treatment has been reported [8].

Determination of unknown organic impurities is the key to the production of high quality drug substances [9]. ICH guidelines indicate that impurities at or above 0.1% in the drug substance require identification [10]. Two impurities were detected in a laboratory sample of carbamazepine when analysed as per USP [4] method. One of the impurities exceeded the 0.1% identification threshold, which did not correspond to any of the previously reported impurities (Table 1) and called for structural identification. Chromatographic conditions mentioned in all the pharmacopoeias were identical, except flow rate in USP method was 1.5 mL/min against 2.0 mL/min in other pharmacopoeias and was found to be less suitable for LC/MS/MS analysis as well as semi-preparative isolation. Therefore a new LC–MS compatible method was developed, in order to get faster elution of the impurity, which was suitable for semi-preparative HPLC.

Combinations of spectrometric and spectroscopic techniques were employed to analyse the impurities during and after isolation. The structure of the unknown impurity in carbamazepine was additionally confirmed by semi-preparative HPLC isolation followed by its characterization by NMR, IR, MS and EA techniques. To the best of our knowledge this impurity has not been previously reported.

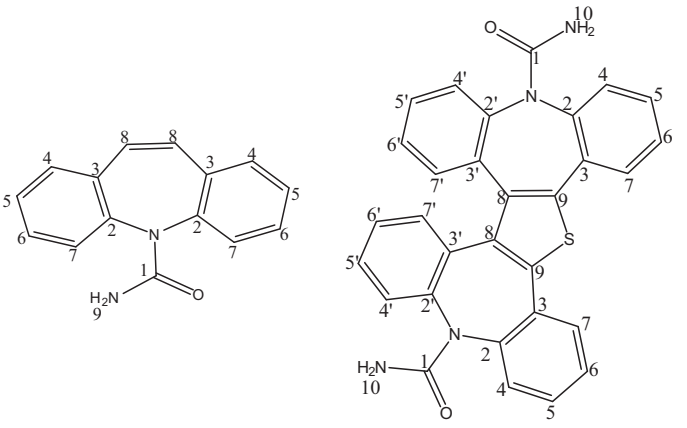
2. Experimental

2.1. Materials and reagents

Sample of carbamazepine API (Batch No. CBZ18/RD/09-crude) and iminodibenzyl standard (Batch No. IDB-std) were obtained from Jubilant Life Sciences Limited (Mysore, India). De-ionized water was prepared using a Milli-Q plus water purification system

* Corresponding author. Tel.: +91 120 4362210; fax: +91 120 2580033.
E-mail address: saji_thomas@jubl.com (S. Thomas).

0731-7085/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2011.05.030



(a) Carbamazepine (b) Impurity-B

Fig. 1. (a) Carbamazepine and (b) impurity-B. Numbering has been assigned only for NMR characterization.

from Millipore (Bradford, PA, USA). HPLC grade methanol, acetonitrile and tetrahydrofuran were purchased from Merck India Limited (Mumbai, India). Chloroform-d and dimethyl sulphoxide-d6 (for NMR) were purchased from Sigma–Aldrich Corporation (St. Louis, MO, USA). Analytical reagent grade acetic acid, formic acid and triethylamine were purchased from Qualigens India Limited (Mumbai, India). Potassium bromide FT-IR grade was purchased from Merck KGaA (Darmstadt, Germany).

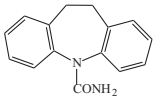
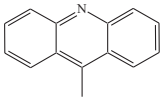
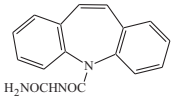
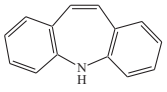
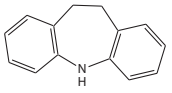
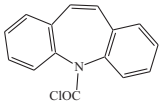
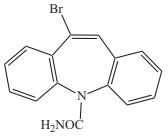
2.2. High performance liquid chromatography

Samples were analysed on a Waters alliance 2690 separation module equipped with 2487 UV detector (Waters Corporation, Milford, MA, USA) using a Nucleosil cyano column (250 cm × 4.6 cm, i.d. 5 μm particles, Macherey Nagel GmbH & Co. KG, Duren, Germany). For chromatographic separations as per USP method [4], tetrahydrofuran–methanol–water were mixed (3:12:85, v/v/v). To 1000 mL of this solution, 0.2 mL of anhydrous formic acid and 0.5 mL of triethylamine were added and a flow rate of 1.5 mL per min was used. 0.10 g of sample was dissolved in methanol and diluted to 50.0 mL with the same solvent. 10.0 mL of this solution was diluted to 20.0 mL with water. The injection volume was 20 μL and the wavelength of detector was set to 230 nm. The column was maintained at 25 °C throughout the analysis.

2.3. Semi-preparative HPLC

The unknown impurity was isolated from crude sample of carbamazepine using a Shimadzu semi-preparative HPLC system consisting of LC-8A binary gradient pump, a SPD-10AVP UV detector, SIL-10AP auto sampler and FRC-10A fraction collector (Shimadzu Corporation, Kyoto, Japan). An Inertsil ODS-3 column (GL Sciences Inc., Tokyo, Japan) (250 cm × 1.9 cm, particle size 10 μm) was used for semi-preparative isolation. A LC isocratic method was used, consisting of a mixture of water–methanol (30:70, v/v) as the mobile phase at a flow rate of 18 mL/min. A sample solution of 50 mg/mL was prepared using methanol as the diluent. The injection volume was 1 mL and the detection was monitored at 230 nm. The collected fractions (between retention

Table 1
List of Pharmacopoeial impurities in carbamazepine.

S. No.	Name of impurity	Pharmacopoeial code			Structure
		EP	BP	USP	
1	10,11-Dihydrocarbamazepine	Impurity-A	Impurity-A	Related Compound A	
2	9-Methylacridine	Impurity-B	Impurity-B	–	
3	N-Carbamoylcarbamazepine	Impurity-C	Impurity-C	–	
4	Iminostilbene	Impurity-D	Impurity-D	Iminostilbene	
5	Iminodibenzyl	Impurity-E	Impurity-E	–	
6	5-Chlorocarbonyliminostilbene	Impurity-F	Impurity-F	–	
7	10-Bromocarbamazepine	Impurity-G	–	–	

EP, European Pharmacopoeia; BP, British Pharmacopoeia; USP, United States Pharmacopoeia.

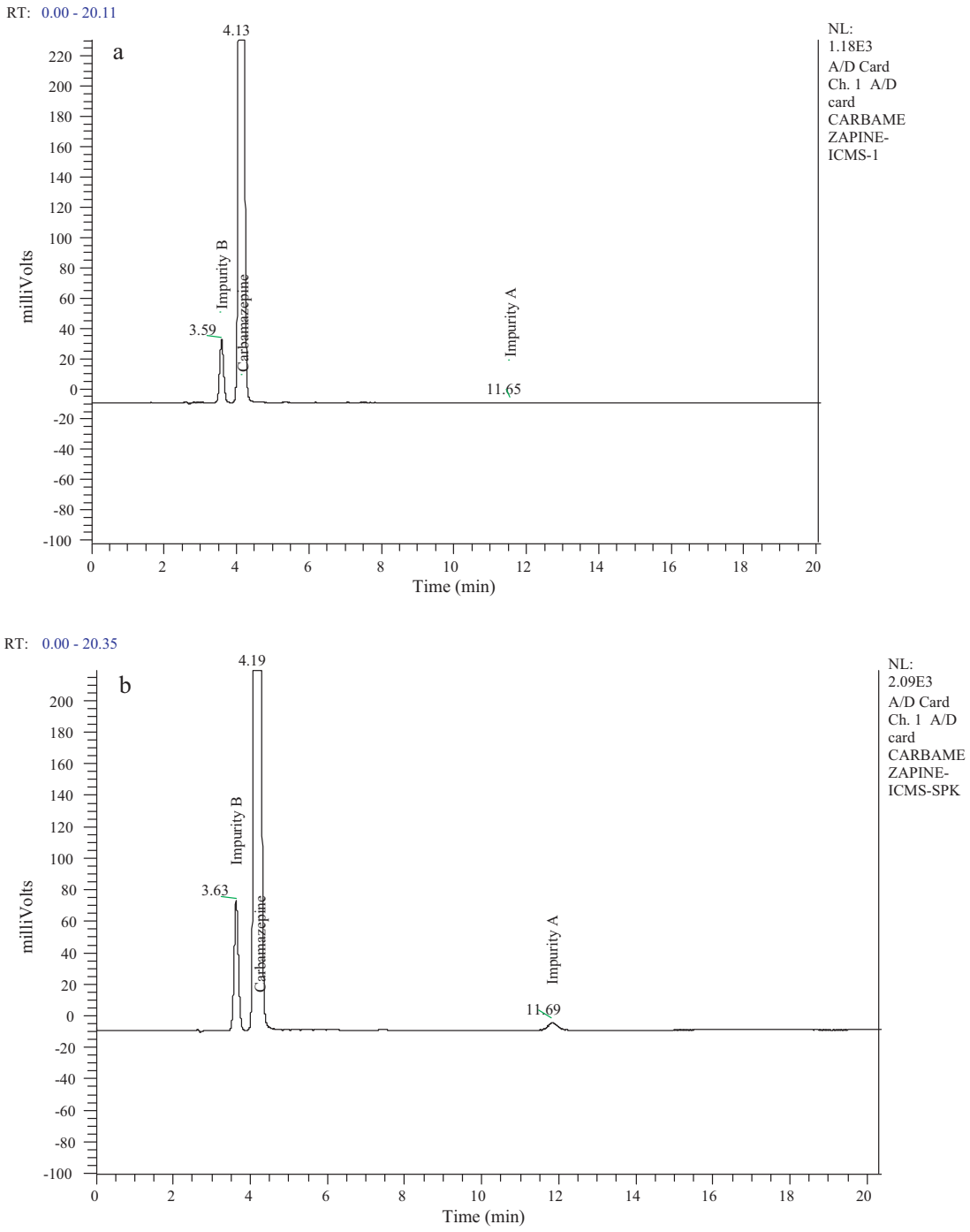


Fig. 2. (a) Chromatogram of carbamazepine in LCMS method. (b) Chromatogram of carbamazepine spiked with impurity-A and impurity-B in LCMS method.

time 1.4 min and 2.0) were pooled and freeze dried under high vacuum consisting of Virtis advantage lyophilizer (SP Scientific, New York, USA).

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

The MS and MS/MS studies were performed on Thermo LCQ-Advantage (Thermo Electron, San Jose, CA, USA) using electrospray ionization source and ion trap mass spectrometer. The source voltage was maintained at 3.0 kV and the capillary temperature at 250 °C. Nitrogen was used as both sheath and auxiliary gas. The mass to charge ratio was scanned across the range of *m/z* 50–1000. MS/MS studies were carried out by keeping normalized collision energy at 25% and an isolation width of 1 amu. The HPLC consisted of Waters alliance 2690 separation module equipped with 2487 UV detector and column oven. A C18 column (Inertsil ODS-3 250 mm × 4.6 mm, i.d. 5 μm particles) was used for chromatographic separation. The mobile phase consisted of a mixture of water–methanol–trifluoroacetic acid (30:70:0.05, v/v/v). The flow rate was maintained at 1.0 mL/min.

2.5. NMR Spectroscopy

^{1}H and ^{13}C NMR spectra were recorded at 399.957 and 100.432 MHz respectively, using a Bruker AVANCE 400 MHz

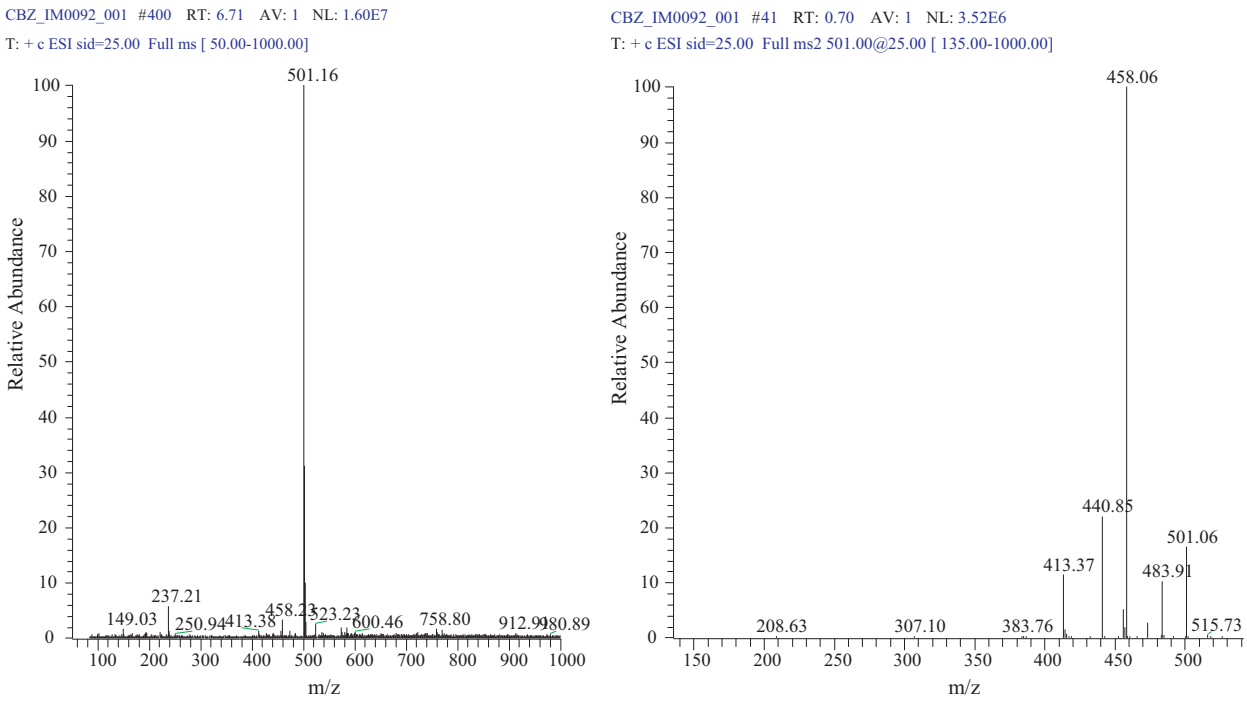


Fig. 3. (a) MS spectrum of impurity-B and (b) MS/MS spectrum of impurity-B.

spectrometer (Bruker, Fallanden, Switzerland) equipped with a 5 mm BBO probe and a z-gradient shim system. The ^{1}H spectra were recorded with 1 s pulse repetition time using 30° flip angle, while ^{13}C spectra were recorded with power gated decoupling using 30° flip angle with repetition time of 2 s. Samples were dissolved in dimethyl sulphoxide-d6. The ^{1}H and ^{13}C chemical shift values were reported on the δ scale in ppm relative to DMSO-d6 (2.50 ppm). All spectra were recorded with sample spinning.

2.6. FT-IR spectroscopy

The IR spectrum was recorded in the solid state as KBr powder dispersion using Nicolet FT-IR model AVTAR 370 (Thermo Electron Scientific Instruments, Madison, WI, USA) with a DTGBS 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.

2.7. Elemental analysis

Elemental analysis (C, H, N, S) was carried out using an elemental analyzer model Vario EL *III* with TCD detector (Elementar Analysensysteme GmbH, Hanau, Germany). Samples were weighed in a tin boat, to which tungsten oxide was added and neatly packed. The sample in tin boat was loaded in an auto sampler tray and was dropped into the combustion tube automatically at a temperature of 1200 °C. Complete combustion of sample was ensured with a special oxygen jet injection.

3. Results and discussion

3.1. Detection of impurities by HPLC

Carbamazepine API sample prepared by a known synthetic route [11–13] was analysed using HPLC method as described in USP [4]. The analysis revealed the presence of two impurities ranging from 0.01% to 0.5% (by area normalization method) which were marked as Imp-A (RT 67.8 min) and Imp-B (RT 95.9 min) respectively.

3.2. Identification of impurities by LC/MS/MS

To further investigate impurities A and B, a new mass spectrometry compatible HPLC method was developed, as described in Section 2.4. Use of high organic content and different column chemistry in the chromatographic conditions drastically reduced the retention time of carbamazepine and impurities in the newly developed LCMS method. Mass spectral data showed protonated molecular ion peaks at *m*/*z* 237, *m*/*z* 196 and *m*/*z* 501 for carbamazepine, impurity-A and impurity-B respectively. On the basis of RRT and mass spectral data, impurity-A having molecular ion peak at *m*/*z* 196 was identified as iminodibenzyl. The mass spectral data obtained for impurity-B at RT 95.8 min did not match with any of the known impurities. Based on the HPLC and LC/MS spectral data, impurity-B was inferred to be unknown. The typical chromatogram of carbamazepine sample highlighting the retention time of these impurities is shown in Fig. 2a. Isolated impurity-B and impurity-A standards were spiked in carbamazepine sample and further confirmed the retention time of these impurities (Fig. 2b).

3.3. Structural elucidation of impurity-B

The full scan spectra of impurity-B showed its protonated molecular ion at *m*/*z* 501. The MS^{2} analysis of impurity-B showed daughter ion peak at *m*/*z* 458 and other prominent peaks at *m*/*z* 484, *m*/*z* 441 and *m*/*z* 413 (Fig. 3a and b). The product ions at *m*/*z* 484 and 458 can be attributed to neutral losses of ammonia (17 amu) and iminomethanone (HN=C=O, 43 amu) respectively. The formation of product ions at *m*/*z* 441 and *m*/*z* 413 can be rationalized by considering the fragmentation pattern as depicted in Fig. 4a. Loss of similar neutral moieties both from carbamazepine and impurity-B indicated the presence of same functional groups in both molecules. Based on LC/MS/MS analysis this impurity was suspected to be the dimer impurity of carbamazepine and the most plausible structure for this impurity

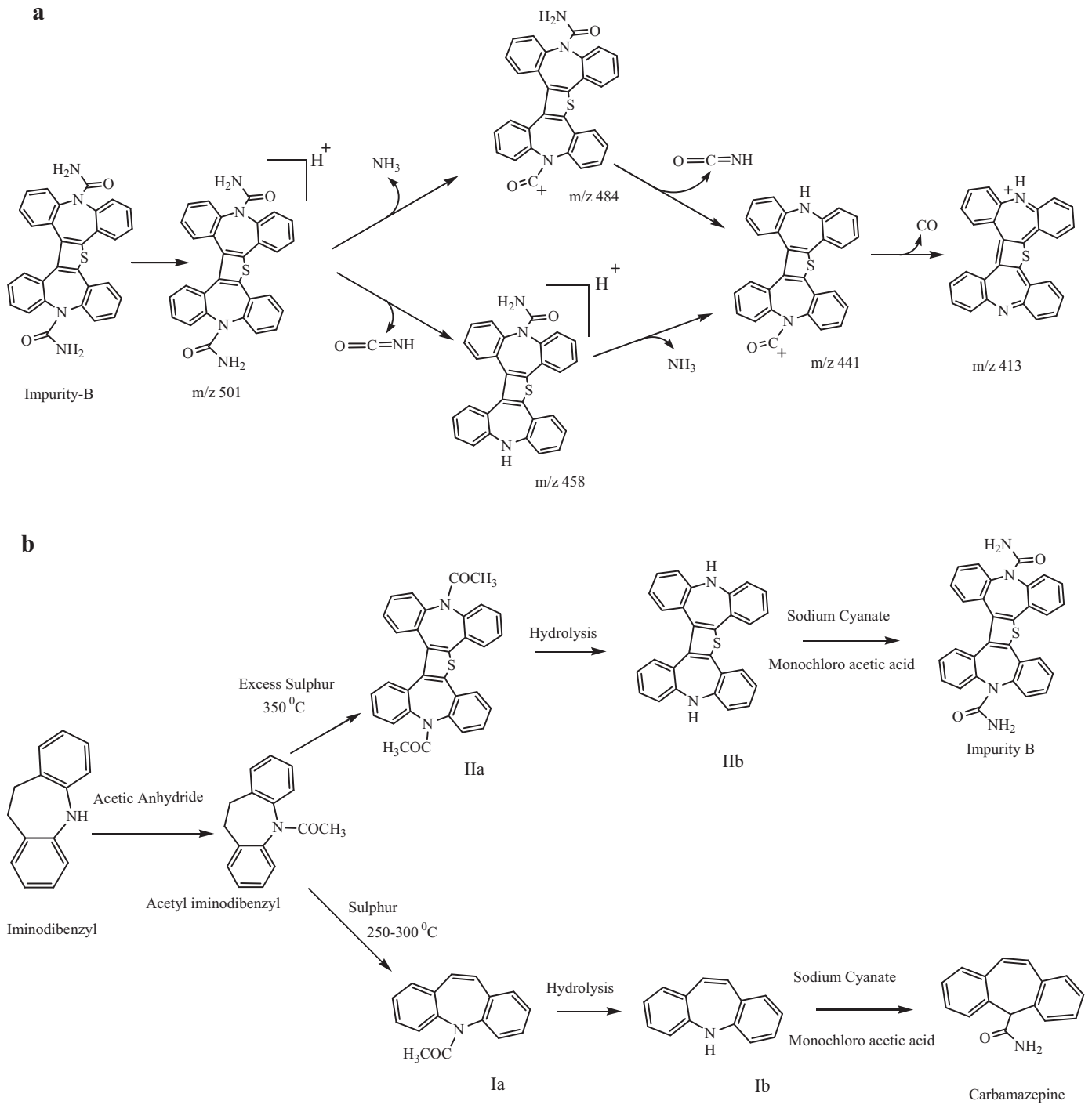


Fig. 4. (a) Plausible scheme for fragmentation of impurity-B. (b) Synthetic scheme of carbamazepine showing the formation of impurity-B.

can be assigned as tetrabenzo[b,f,b′f′]azepino[4′,5′:4,5]thieno[2,3-*d*]azepine-3,9-dicarboxamide (Fig. 1b). The proposed mass fragmentation pattern was further supported by previously reported values [14].

CHNS data obtained for the unknown impurity showed nitrogen 11.23%, carbon 72.04%, sulphur 6.46% and hydrogen 4.06% against theoretical values of nitrogen 11.19%, carbon 71.98%, sulphur 6.41% and hydrogen 4.03%. CHNS data confirmed the proposed structure based on LC/MS/MS data.

The NMR spectral data of carbamazepine and impurity-B were compared. The ^{1}H NMR spectrum of carbamazepine showed a singlet at 5.21 ppm which corresponds to two exchangeable amide protons and another singlet at 6.91 ppm, which corresponds to the two protons at 8 positions. However, impurity-B showed a singlet at 5.95 ppm, which corresponds to four exchangeable amide protons. These indicate two magnetically equivalent carboxamide groups in impurity-B. It was also observed that the singlet at 6.91 ppm in carbamazepine spectrum was absent in the impurity-B spectrum, which indicates that there are no protons present at the 8 position. Furthermore the signals at 157.45 ppm in carbamazepine and at 156.31 in impurity-B, characteristic of carbonyl carbon in ^{13}C NMR spectrum confirm the presence of C=O group in both structures.

Proton NMR of carbamazepine showed a multiplet ranging 7.28–7.45 for eight protons (two sets of four symmetrically

substituted protons) viz. 4, 5, 6, 7 along with a broad singlet for two amide protons (δ 5.23) exchangeable with D_2O. ^{13}C NMR data are as follows: 157.46 (1), 139.68 (2), 129.12 (3), 130.16 (4), 128.46 (5), 134.69 (6), 127.40 (7), 139.68 (8).

Proton NMR of impurity-B showed a multiplet ranging 7.28–7.48 for sixteen protons (two sets of eight symmetrically substituted protons each side to the thiophene linkage) viz. 4, 5, 6, 7, 4′, 5′, 6′, 7′ along with a broad signal for four amide protons at δ 5.96 exchangeable with D_2O. ^{13}C NMR data are as follows: 156.31 (1), 142.12 (2), 129.14 (3), 127.03 (4), 133.43 (5), 128.31 (6), 130.10 (7), 135.93 (8), 137.69 (9), 142.12 (2′), 129.55 (3′), 127.03 (4′), 133.43 (5′), 128.72 (6′), 130.91 (7′). The proposed NMR assignments were further supported by previously reported values [15,16].

FT-IR spectrum of carbamazepine displayed characteristic absorptions at 3465 cm^{-1}, 3340 cm^{-1} (NH stretching), 1677 cm^{-1} (amide, C=O stretching), 1605 cm^{-1}, 1594 cm^{-1} (aromatic, C=C stretching). The impurity-B spectrum displayed characteristic absorptions at 3481 cm^{-1}, 3398 cm^{-1} (NH stretching), 1664 cm^{-1} (amide, C=O stretching), 656 cm^{-1} (C–S stretching), 1586 cm^{-1}, 1575 cm^{-1} (aromatic, C=C stretching). ^1H NMR, ^{13}C NMR and FT-IR spectral data confirm the proposed structure for impurity-B.

3.4. Formation of impurity

During the synthesis of carbamazepine, iminodibenzyl is acetylated to get acetyl iminodibenzyl, which on further reaction with excess sulphur at 350 °C gives intermediate IIa. Intermediate IIa on hydrolysis yields intermediate IIb which undergoes carboximadation in presence of sodium cyanate and monochloro acetic acid leading to the formation of impurity-B. The schematic diagram of formation of impurity is depicted in Fig. 4b.

4. Conclusion

LC–MS studies were performed to get molecular weight, establish its mass fragmentation profile and identify an unknown impurity in carbamazepine active pharmaceutical ingredient. Unknown impurity was isolated using semi-preparative HPLC. Studies were carried out using NMR, FT-IR and CHNS analyzer to further confirm the proposed structure and the molecular formula of unknown impurity could be deduced as $C_{30}H_{20}N_4O_2S$ and the corresponding structure was characterized as tetrabenzo[b,f,b′f′]azepino[4′,5′:4,5]thieno[2,3-*d*]azepine-3,9-dicarboxamide.

Acknowledgements

The authors are thankful to the management of Jubilant Life Sciences Limited for providing necessary facilities. Authors would like to thank Dr. Hawaldar Maurya, Mr. Dinesh Vishwakarma, Mr. Mohan Kumar, Mr. Raghavendra Desai Rao, Mr. I. Sridhar and Dr. Subhash Chandra Joshi for their co-operation in carrying out this work. We would also like to thank Dr. Dharamvir for continuous encouragement throughout the course of work.

References

[1] J.E.F. Reynolds (Ed.), Martindale – The Complete Drug Reference, thirty sixth ed., Royal Pharmaceutical Society of Great Britain, 2009, pp. 471–477.
[2] R.W. Evans, T.H. Clay, C.T. Gualtieri, Carbamazepine in pediatric psychiatry, J. Am. Acad. Child Adolesc. Psychiatry 26 (1987) 2–8.
[3] H. Liu, M.R. Delgado, Effect of valproate and falbamate on carbamazepine and its metabolites in epileptic children, J. Epilepsy 10 (1997) 37–41.
[4] United States Pharmacopoeia-32, vol. 2, 2009, pp. 1784–1785.
[5] European Pharmacopoeia, vol. 2, sixth ed., 2008, pp. 1411–1412.
[6] British Pharmacopoeia, vol. 1, 2008, pp. 384–385.
[7] Indian Pharmacopoeia, vol. 2, 2010, pp. 977–978.
[8] D. Vogna, R. Marotta, R. Andreozzi, A. Napolitano, M. d'Ischia, Kinetic and chemical assessment of the UV/H_2O_2 treatment of antiepileptic drug carbamazepine, Chemosphere 54 (2004) 497–505.
[9] E.M. Sheldon, J.B. Downar, Development and validation of a single robust HPLC method for the characterization of a pharmaceutical starting material and impurities from three suppliers using three separate synthetic routes, J. Pharm. Biomed. Anal. 23 (2000) 561–572.
[10] ICH Guidelines, Impurities in New Drug Substances Q3A (R2), October 25, 2006.
[11] W. Schindler, Riehen, Geigy Chemical Corporation, United States Patent US 2,948,718 (1960).
[12] G. Acklin, E. Aufderhaar, G. Kaupp, B. Raz, Ciba-Geigy Chemical Corporation, United States Patent US 4,847,374 (1989).
[13] K.D. Vyas, W.S. Jafri, A.K. Kulkarni, Max India Limited, United States Patent US 6,224,908 B1 (2001).
[14] E.M. Urrea, J. Radjenovic, G. Caminal, M. Petrovic, T. Vicent, D. Barcelo, Oxidation of atenolol, propranolol, carbamazepine and clofibric acid by a biological fenton-like system mediated by the white-rot fungs *Trametes versicolor*, Water Res. 44 (2010) 521–532.
[15] L.J. Kricka, A. Ledwith, Dibenz[b,f]azepine and related ring systems, Chem. Rev. 74 (1974) 101–123.
[16] F.F. Molock, D.W. Boykin, ^{13}C NMR studies on dienz[b,f]azepines, J. Heterocycl. Chem. 20 (1983) 109–112.