AN IMPURITY PROFILE STUDY OF LAMOTRIZINE

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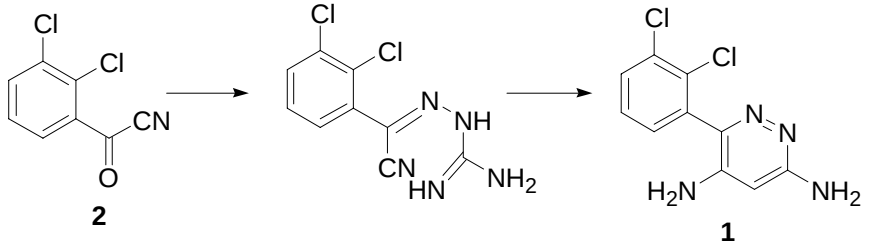
ABSTRACT

*In the process for the preparation of Lamotrizine **1** and its intermediate **2**, identified five potential impurities in intermediate **2**, ranging from 0.05-0.10% were detected in HPLC, based on the mass spectral data obtained by LC-MS analysis of all these impurities molecular weight is same and are characterized as isomeric impunities of **2**, as 2,4-dichlorobenzoylcyanide (**Imp-A**), 2,5-dichlorobenzoylcyanide (**Imp-B**), 2,6-dichlorobenzoylcyanide (**Imp-C**), 3,4-dichlorobenzoylcyanide (**Imp-D**), and 3,5-dichlorobenzoylcyanide (**Imp-E**). In the process of conversion of intermediate **2** to Lamotrizine **1**, the same impurities also converted, reflected in the final API and characterized as 3,5-diamino-6-(2,4-dichlorophenyl)triazine (**Imp-F**), 3,5-diamino-6-(2,5-dichlorophenyl)triazine (**Imp-G**), 3,5-diamino-6-(2,6-dichlorophenyl)triazine (**Imp-H**), 3,5-diamino-6-(3,4-dichlorophenyl)triazine (**Imp-I**), and 3,5-diamino-6-(3,5-dichlorophenyl)triazine (**Imp-J**).*

Keywords: lamotrizine; impurities, identification; characterization; synthesis; spectroscopy

INTRODUCTION

Lamotrizine (**1**, Lamictal®), is an antiepilepry[1] product was launched by Glaxo in 1990, in Ireland as their first world market. This product has USFDA approval in 1994, and launched in 1995. Lamotrigine is in phase-III trials for epilepsy in Japan. It has been approved in 15 countries for the treatment of seizures associated with Lennox astacert syndrome. Lamotrigine is well tolerated, the only side effect is an increase in colds and other viral illness. Lamotrizine **1**, chemically known as 3,5-diamino-6-(2,3-dichlorophenyl)triazine (**1**). Preparation of Lamotrizine **1** involves reaction of intermediate **2**, with amino guanidine followed cyclization (Scheme-1).[2]



Scheme-1: Synthesis of Lamotrizine **1**

EXPERIMENTAL

The synthesized impurities were used to validate the liquid chromatography. The validation performance was carried out in line with the International Conference on Harmonization (ICH) requirements.

Samples

The investigated samples of Lamotrigine bulk drug and intermediate were obtained from Dr. Reddy's Laboratories Ltd., Bulk Actives-III, Hyderabad, India.

High performance liquid chromatography for Formula-I

Agilent 1100 series equipped with variable wavelength UV detector was used. An in-house LC method was developed for the analysis of Lamotrizine and its intermediate, the C18 column (COSMOSIL MS-II C-18 250 x 4.6 mm x 5, Merk Laboratories Pvt Ltd.) with a mobile phase consisting of a mixture of 0.01M ammonium acetate and methanol in the ratio of 500:500 (v/v)(pH 4.5) was used with UV detection 210 nm at flow rate of 0.7 ml/min for the resolution of all impurities. The data was recorded using Waters Empower software. This LC method was able to separate these impurities, which ranged from 0.05 to 0.10% in the presence of parent compound and detection of impurities.

Mass spectrometry

Mass spectra were obtained using an AB 4000 Trap LCMS/MS mass spectrometer with energy set to 4500 V. The samples were introduced via HPLC pump Agilent 1100 Series Auto sampler. The source temperature maintained at 250°C and 400°C respectively.

NMR spectroscopy

Characterization of impurities was achieved by using NMR Varian unity plus 200/400 MHz instrument. The samples were dissolved in DMSO-d_6 or $CDCI_3$, containing 0.03%v/v TMS in 5-mm NMR tube. TMS was used as an internal reference standard for the proton experiment. All experiments were conducted at 25°C, and no shift relaxation agents were employed. The ^{1}H-NMR chemical shift values were reported on the δ scale in ppm, relative to TMS (δ=0.00) respectively.

FT-IR spectroscopy

The IR spectra for impurities were detected in the solid state as KBr dispersion using Perkin Elmer 1650 FT IR spectrophotometer and reported in cm^{-1}.

RESULTS AND DISCUSSION

Detection of impurities

A typical analytical LC chromatogram of a production batch of Lamotrizine **1** and intermediate **2**, bulk drug recorded using the LC method as shown in fig.1 & fig.2. The target impurities under study are marked as **Imp-A** to **Imp-J**. These impurities were synthesized by synthetic method and co-injected; the RT's of all impurities are exactly matching with all the impurities present in the compound.

Synthesis of impurities

2,3-Dichlorobenzoylcyznide **2** and its isomers were synthesized as per the scheme shown in Scheme-2. The significant quantities required for these impurities for confirmation of structure, validation, and use as an analytical standard for further analysis studies.

General procedure for the preparation of Imp-A to Imp-E

A mixture of xylene (560 ml), CuCN (55.7 g, 0.62 mol) and KI (96.5 g, 0.58 mol) were heated to 145°C, added a solution of dichlorobenzoyl chloride (50 g, 0.23 mol) in xylene (200 ml) slowly over a period of 3 – 4 h and the contents were stirred for 15 h at 145°C. The reaction mixture was cooled to 30°C. The separated solid was filtered from the reaction mass and washed with xylene. The filtrate was concentrated under reduced pressure and isolated in hexane (550 ml). The obtained solid was dried at below 50°C, and characterized.

Imp-A: ^{1}**H-NMR** ($CDCl_3$, 200 MHz, δ ppm): 7.48 (d, 1H, CH), 7.50 (dd, 1H, CH), 8.10 (d, 1H, CH); **IR** (KBr, cm^{-1}): 2222 (-CN) and 1686 (C=O); MS (m/z): 199 M^+ (with two chlorine abundance) which corresponding to the molecular formula $C_8H_3Cl_2$ NO.

Imp-B: ^{1}**H-NMR** ($CDCl_3$, 200 MHz, δ ppm): 8.00 (d, 1H, CH), 7.68 (d, 1H, CH), 8.15 (s, 1H, CH); **IR** (KBr, cm^{-1}): 2227 (-CN) and 1701 (C=O); **MS** (m/z): 199 M^+ (with two chlorine abundance) which corresponding to the molecular formula $C_8H_3Cl_2$ NO.

Imp-C: ^{1}**H-NMR** ($CDCl_3$, 200 MHz, δ ppm): 7.30-7.45 (m, 1H, CH), 7.30-7.45 (m, 1H, CH), 7.30-7.45 (m, 1H, CH); **IR** (KBr, cm^{-1}): 2221 (-CN) and 1793 (C=O); **MS** (m/z): 199 M^+ (with two chlorine abundance) which corresponding to the molecular formula $C_8H_3Cl_2$ NO.

Imp-D: ^{1}**H-NMR** ($CDCl_3$, 200 MHz, δ ppm): 7.8 (d, 1H, CH), 7.40-7.45 (d, 1H, CH), 7.80-7.45(dd, 1H, CH); **IR (KBr, cm^{-1}):** 2223 (-CN) and 1793 (C=O) **MS** (m/z): 199 M^+ (with two chlorine abundance) which corresponding to the molecular formula $C_8H_3Cl_2$ NO.

Imp-E: ^{1}**H-NMR** ($CDCl_3$, 200 MHz, δ ppm): 7.94 (d, 1H, CH), 7.84 (d, 1H, CH), 7.89 (d, 1H, CH); **IR** (KBr, cm^{-1}): 2223 (-CN) and 1763 (C=O); **MS** (m/z): 199 M^+ (with two chlorine abundance) which corresponding to the molecular formula $C_8H_3Cl_2$ NO.

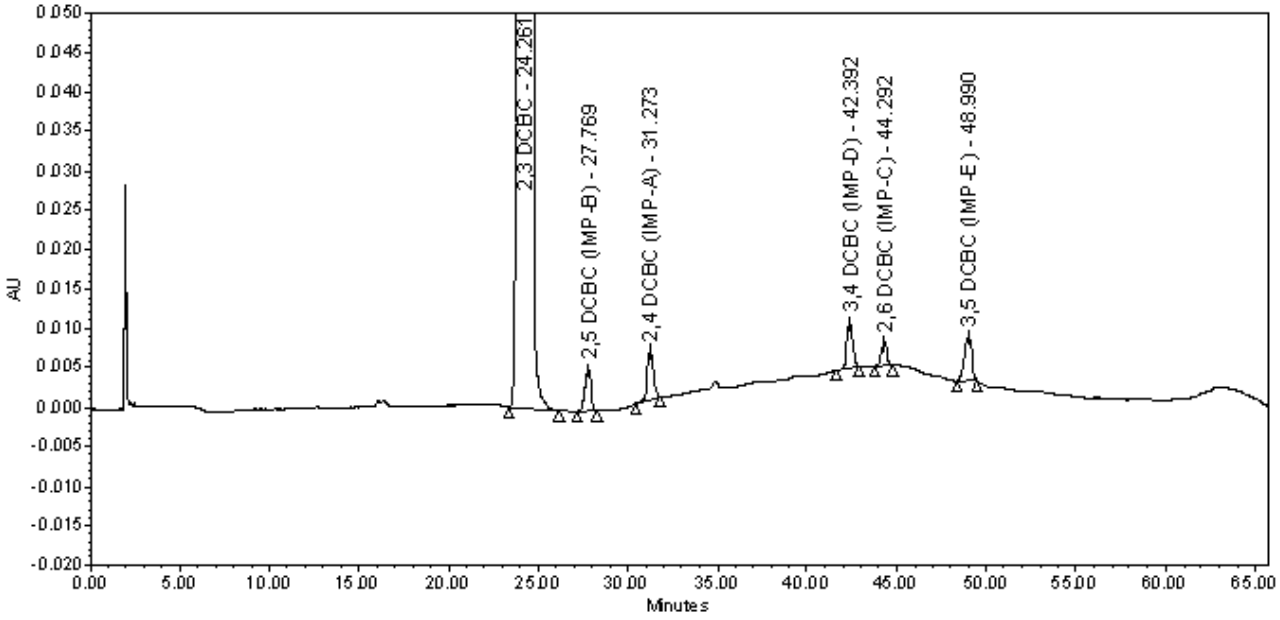


Fig.1. HPLC chromatogram of Lamotrizine intermediate **2** spiked with isomeric impurities

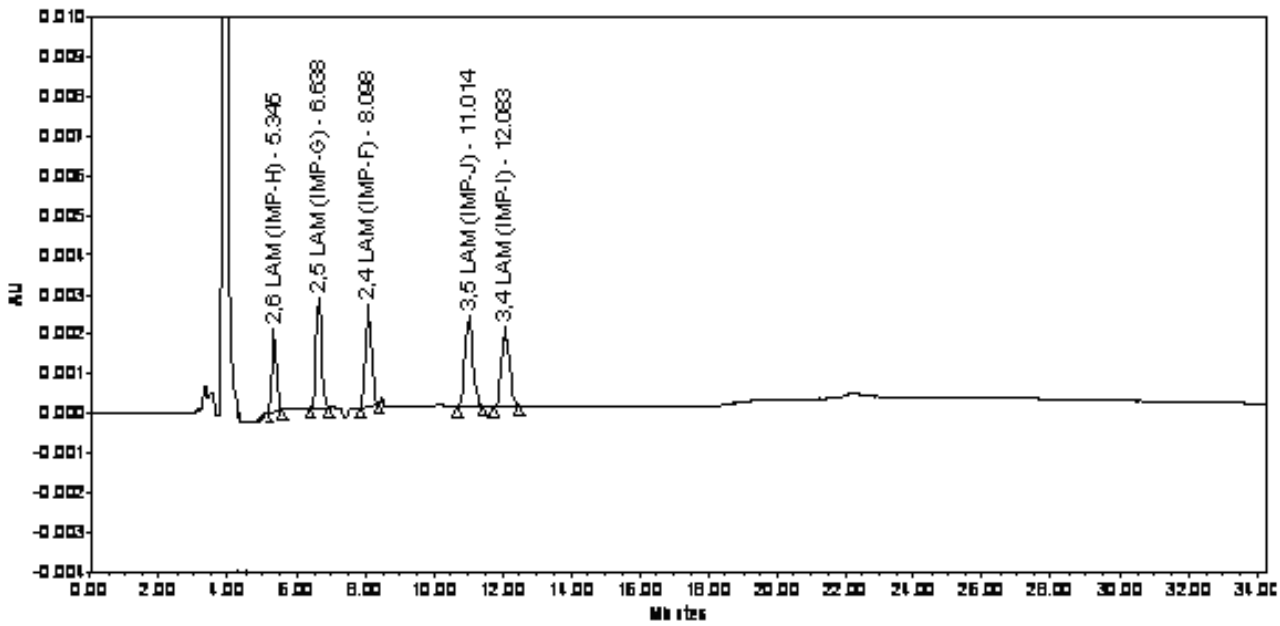
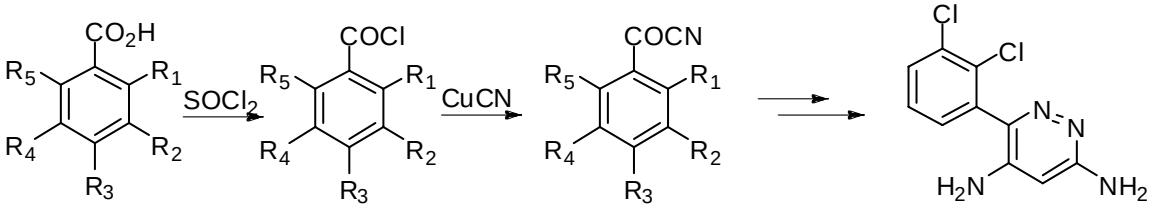


Fig.2: HPLC chromatogram of isomeric impurities of Lamotrizine **1**



Imp-A : R_1,R_3 = Cl; R_2,R_4,R_5 = H **Imp-F** : R_1,R_3 = Cl; R_2,R_4,R_5 = H

Imp-B : R_1,R_4 = Cl; R_2,R_3,R_5 = H **Imp-G** : R_1,R_4 = Cl; R_2,R_3,R_5 = H

Imp-C : R_1,R_5 = Cl; R_2,R_3,R_4 = H **Imp-H** : R_1,R_5 = Cl; R_2,R_3,R_4 = H

Imp-D : R_2,R_3 = Cl; R_1,R_4,R_5 = H **Imp-I** : R_2,R_3 = Cl; R_1,R_4,R_5 = H

Imp-E : R_2,R_4 = Cl; R_1,R_3,R_5 = H **Imp-J** : R_2,R_4 = Cl; R_1,R_3,R_5 = H

Scheme-2: Synthesis of isomeric impurities of **1**& **2**

General procedure for the preparation of Imp-F to Imp-J

A mixture of DM water (10 ml), aminoguinide bicarbonate (3.5 g, 0.025 mol) was stirred at 10-15°C, added con. H_2SO_4 (18.5g, 0.0188 mol) at below 45°C over a period of 30-45 min. then added a solution of dichlorobenzoyl cyznide (5.0 g , 0.025 mol) in acetonitrile (10 ml) at below 45°C over a period of 45 min. followed by the reaction mixture stirred at same temperature for 90-120 min., at room temperature for 24 h, and at 10-15°C for 60-90 min. The obtained solid filtered and washed with DM water (50 ml). The wet cake was taken in to a solution of NaOH (2 g, 0.05 mol) in methanol (35 ml), heated to reflux temperature for 2-3 h, distilled off solvent complete under vacuum at below 60°C under reduced pressure and isolated in water (30 ml), finally crystallized in water (30 ml) and characterized.

Imp-F: ^{1}H-NMR (DMSO-d_6, 400 MHz, δ ppm): 7.7 (s, 1H, CH), 7.42-7.55 (dd, 2H, CH); **IR** (KBr, cm^{-1}): 3344, 3128, 1560, 1503, 1441, 1149, 811, 747; **MS** (m/z): 257 M^+ (with two chlorine abundance) which corresponding to the molecular formula $C_9H_7Cl_2N_5$.

Imp-G: ^{1}H-NMR (DMSO-d_6, 400 MHz, δ ppm): 7.45 (s, 1H, CH), 7.52-7.7 (dd, 2H, CH); **IR** (KBr, cm^{-1}): 3497, 3128, 1645, 1560, 1503, 1441, 1149, 1093, 807; **MS** (m/z): 257 M^+ (with two chlorine abundance) which corresponding to the molecular formula $C_9H_7Cl_2N_5$.

Imp-H: **^{1}H-NMR** (DMSO-d_6, 400 MHz, δ ppm): 7.1-7.4 (t, 1H, CH), 7.2-7.3 (d, 2H, CH); **IR** (KBr, cm^{-1}): 3379, 3128, 1606, 1560, 1503, 1428, 791, 730; **MS** (m/z): 257 M^+ (with two chlorine abundance) which corresponding to the molecular formula $C_9H_7Cl_2N_5$.

Imp-I: ^{1}H-NMR (DMSO-d_6, 400 MHz, δ ppm): 6.9 (s, 1H, CH), 7.4-7.55 (dd, 2H, CH); **IR** (KBr, cm^{-1}): 3307, 3121, 1615, 856, 806; **MS** (m/z): 257 M^+ (with two chlorine abundance) which corresponding to the molecular formula $C_9H_7Cl_2N_5$.

Imp-J: ^{1}H-NMR (DMSO-d_6, 400 MHz, δ ppm): 7.5 (s, 1H, CH), 7.6 (s, 1H, CH), 7.9 (s, 1H, CH); **IR** (KBr, cm^{-1}): 3497, 3362, 1615, 1514, 871, 820; **MS** (m/z): 257 M^+ (with two chlorine abundance) which corresponding to the molecular formula $C_9H_7Cl_2N_5$.

CONCLUSION

The results from the various physiochemical techniques confirm the molecular structures of proposed isomeric impurities of 2,3-dichlorobenzoyl cyanide **2** and Lamotrizine **1**, based on the analytical and the sequence of the preparations, the structures of all the impurities were established.

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"The activist is not the man who says the river is dirty. The activist is the man who cleans up the river."

-Ross Perot