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Identification and structural elucidation of process related impurities in duloxetine hydrochloride

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ABSTRACT

Three impurities were detected in duloxetine hydrochloride bulk drug by HPLC-UV and LC/MS. These impurities were marked as DLX-I, DLX-II and DLX-III. Two of the impurities (DLX-I and DLX-II) were unknown and have not been reported previously. An optimized method using liquid chromatography coupled with electrospray ionization ion trap mass spectrometry (LC/ESI-ITMS) in positive and negative ion mode has been developed to carry out structural identification of unknown impurities. Based on mass spectrometric data and synthetic specifics the structures of DLX-I and DLX-II were proposed as phenyl-3-(1-hydroxynaphthalen-4-yl)-3-(thiophen-2-yl)propylmethylcarbamate and phenyl-3-(1-hydroxynaphthalen-2-yl)-3-(thiophen-2-yl)propylmethylcarbamate respectively. The impurities were isolated by normal phase flash chromatography and structures were confirmed by NMR and FTIR spectroscopy. The existence of conformational isomer was confirmed by 2D NOESY NMR. The plausible mechanism for the formation of impurities is also discussed.

Key words: Duloxetine impurities; LC/ESI-MS; Negative ion mode; Conformational isomer; 2D NOESY.

1. INTRODUCTION

Duloxetine hydrochloride, (+)-(S)-*N*-Methyl-γ-(1-naphthyloxy)-2-thiophenepropylamine hydrochloride [1], an antidepressant, is a dual inhibitor of serotonin and norepnephrine reuptake. It can be used for major depressive disorder and for the management of diabetic peripheral neuropathic pain. It has got an edge over existing antidepressant such as fluoxetine and tomoxetine due to various features like improved efficacy, tolerability, safety, faster recovery, fewer side effects, low affinity for neuronal receptors and dual inhibiting nature [2-3].

Impurities are an extremely critical issue in the pharmaceutical industry especially due to the stringent regulations and manufacturing process. Impurity profile of an Active Pharmaceutical Ingredients (APIs) and evaluation of their toxicity effect is necessary step in developing a safe and effective drug and is essential for medical safety reasons [4]. Typically process-related impurities are unwanted chemicals that remain with the APIs and could be generated at any of the synthetic steps or contamination of any un-reacted molecule involved in the process development.

Brenna et. al. has discussed the isolation and characterization of phenolic impurity of duloxetine in commercial sample [5]. Liquid chromatographic method for stability assessment of intermediate and degradant is reported in few references [6-15].

During process development studies of duloxetine hydrochloride (DLX), three impurities in the bulk drug samples were detected by in-house analytical method and out of which two were found to be unknown and not reported before. In view of the fact that the impurity levels were above the acceptable limits of 0.1%, a comprehensive study was carried out using a suitable spectrometric and spectroscopic techniques.

2. Experimental

2.1. Chemicals

DLX bulk drug samples were obtained from Chemical Research Division, Ipca Laboratories Ltd. (Mumbai, India). Ammonium acetate from LOBA Chemie Pvt. Ltd. (Mumbai, India), acetonitirile (ACN) was obtained from Merck Ltd. (Mumbai, India). Glacial acetic acid was

purchased from Qualigens India Limited (Mumbai, India). De-ionized water prepared using miliQ plus purification system Millipore (Bradford, USA) was used throughout the studies. Ethyl acetate, Hexane, Potassium bromide (FTIR) grade, $CDCl_3$, DMSO-d_6 and D_2O were purchased from Merck KGaA (Darmstadt, Germany).

2.2. Liquid chromatography/ Mass spectrometry

The LC part consisted of an 1100 series HPLC (Agilent Technologies, Waldbronn, Germany) equipped with quaternary gradient pump, degasser and auto sampler. A Zorbax Rx-C8 column (250mm x 4.6mm i.d., 5μm particles) was used for chromatographic separations. The mobile phase composed of 7.7 g ammonium acetate dissolved in 1000 ml of water adjusted to pH 6.0 with glacial acetic acid (A) and acetonitrile (B) in a gradient mode (T_{min}/A:B; T_0/50:50; T_7/50:50; T_{15}/35:65; T_{25}/35:65; T_{30}/50:50, T_{35}/50:50). The flow rate was set to 1.0 mL per minute with UV detector wavelength was fixed at 225 nm. The sample solution (500 ppm) was prepared in mobile phase and 20µL was injected. The ESI-MS and MS/MS analysis was carried out on LCQ-Advantage (Thermo Finnigan, San Jose, CA, USA) ion trap mass spectrometer. The source voltage was kept at 3.0 kV and capillary temperature at 250 °C. Nitrogen was used as both sheath and auxiliary gas. Mass range was kept at m/z 100-800. MS/MS studies were carried out by maintaining normalized collision energy at 35% with the mass range m/z 100-800.

2.3. Flash chromatography

The impurities were isolated using a CombiFlash Companion system, consisting of a binary gradient pump and UV detector (Teledyne ISCO, Lincoln, Nebraska). A CombiFlash normal phase 40g disposable flash column (Teledyne ISCO, Lincoln, Nebraska) was used for chromatographic separation. A mixture of hexane and ethyl acetate was used as a mobile phase in the ratio of 95:5 (*v/v*), at a flow rate of 40 mL/min. The UV detector wavelength was fixed at 254 nm.

2.4. Nuclear magnetic Resonance

The ^1H, ^{13}C measurements of the isolated impurities and DLX were performed on AVANCE 400 (Bruker, Fallanden, Switzerland) instrument at 300 K. DEPT spectral editing was used to identify the presence of methyl and methine groups as positive peaks and the methylenes as negative peaks. 2D NMR experiments (DQF-COSY, HMBC HSQC and NOESY) were also performed using the same instrument. The exchangeable proton was identified by a D_2O exchange experiment. Sample concentration was 6.0 mg in 0.6 ml. All the samples were prepared in $CDCl_3$. The ^1H and ^{13}C chemical shift values were reported on the δ scale in ppm relative to $CDCl_3$ (7.28 ppm) and (77.0 ppm) respectively for DLX and DLX-I; DMSO-d_6 (2.49 ppm) and (39.5 ppm) for DLX-II.

2.5. FTIR spectroscopy

The IR spectrum of isolated impurities was recorded in the solid state KBr powder dispersion using a Spectrum-One FT-IR spectrometer (PerkinElmer, Beaconsfield, UK).

3. RESULT AND DISCUSSION

3.1. Detection of impurities by HPLC and LC/ESI-MS

DLX bulk drug samples prepared during process development studies by one of the known, economical and feasible synthetic route [11] showed three impurities, when analysed by HPLC method as described in above mentioned *Liquid chromatography/ Mass spectrometry* method. Impurities were marked as DLX-I (RT 17.8 min), DLX-II (RT 20.8 min) and DLX-III (RT 4.1 min) along with principle peak (RT 14.2 min) as depicted in a typical chromatogram (Fig.1).

To investigate further, the bulk drug samples were analysed by LC/ESI-MS method described in *Liquid chromatography/ Mass spectrometry* section. Mass spectra were recorded in both positive and negative ion modes. DLX and DLX-III showed good response in positive ion mode, whereas the DLX-I and DLX-II showed good responses in negative ion mode. The positive ion ESI-MS spectrum of DLX exhibited $[M+H]^+$ ion peak at m/z 298 (Fig.2a). DLX-III showed $[M+H]^+$ isotopic peaks for single chlorine with ~32 % relative abundance at m/z 157 and m/z 159. At the same time both DLX-I and DLX-II analysed in negative ion ESI-MS mode showed $[M-H]^-$ ion peak at m/z 416 (Fig 3a & 4a).

This mass spectral data manifested molecular mass of DLX (m/z 297) and DLX-III (m/z 156 with single chlorine present in the structure). Based on mass spectral data, DLX-III is identified as phenyl chloroformate, a key reactant used during synthesis of DLX. This was further confirmed by co-injecting the reference material of the said intermediate. In further observation, the molecular mass of DLX-I (m/z 417), DLX-II (m/z 417) understood as positional isomer and an odd molecular mass of impurities implies the existence of an odd number of nitrogen atom. Mass of DLX-I and DLX-II was found to be matching with the mass of carbamate intermediate [16], which is one of the important intermediate, which on further reaction with sodium hydroxide gives duloxetine. But the

Figure - 1: Typical HPLC chromatogram of showing DLX (RT 14.2) with DLX-III (RT 4.1), DLX-I (RT 17.8) and DLX-II (RT 20.8).

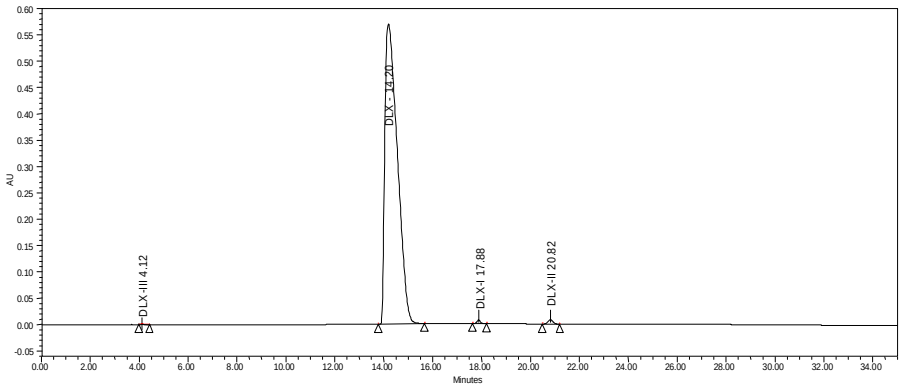
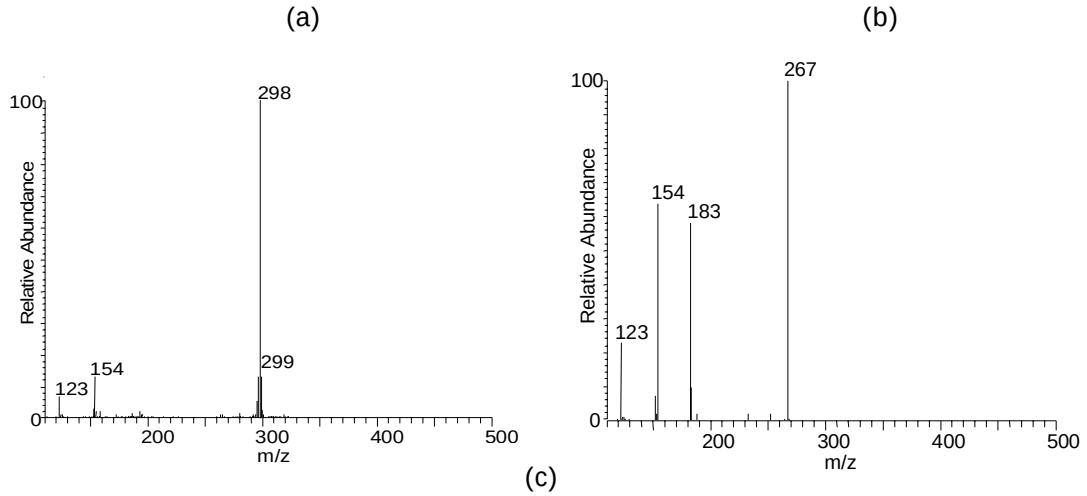


Figure – 2: Mass spectral data of DLX in positive ion mode (a) mass spectrum of DLX (b) MS/MS spectrum of product ion peak at m/z 298 (c) Possible fragmentation mechanism of DLX.



(c)

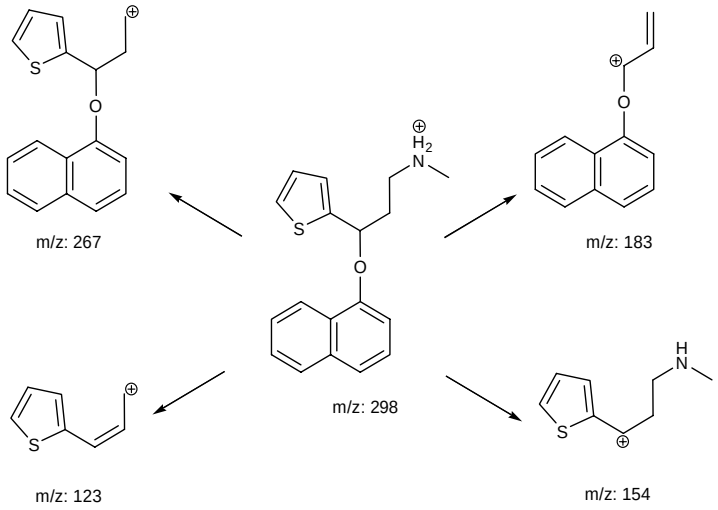


Figure - 3: Mass spectral data of DLX-I in negative ion mode (a) mass spectrum of DLX-I (b) MS/MS spectrum of ion peak at m/z 416 (c) Possible fragmentation mechanism of DLX-I.

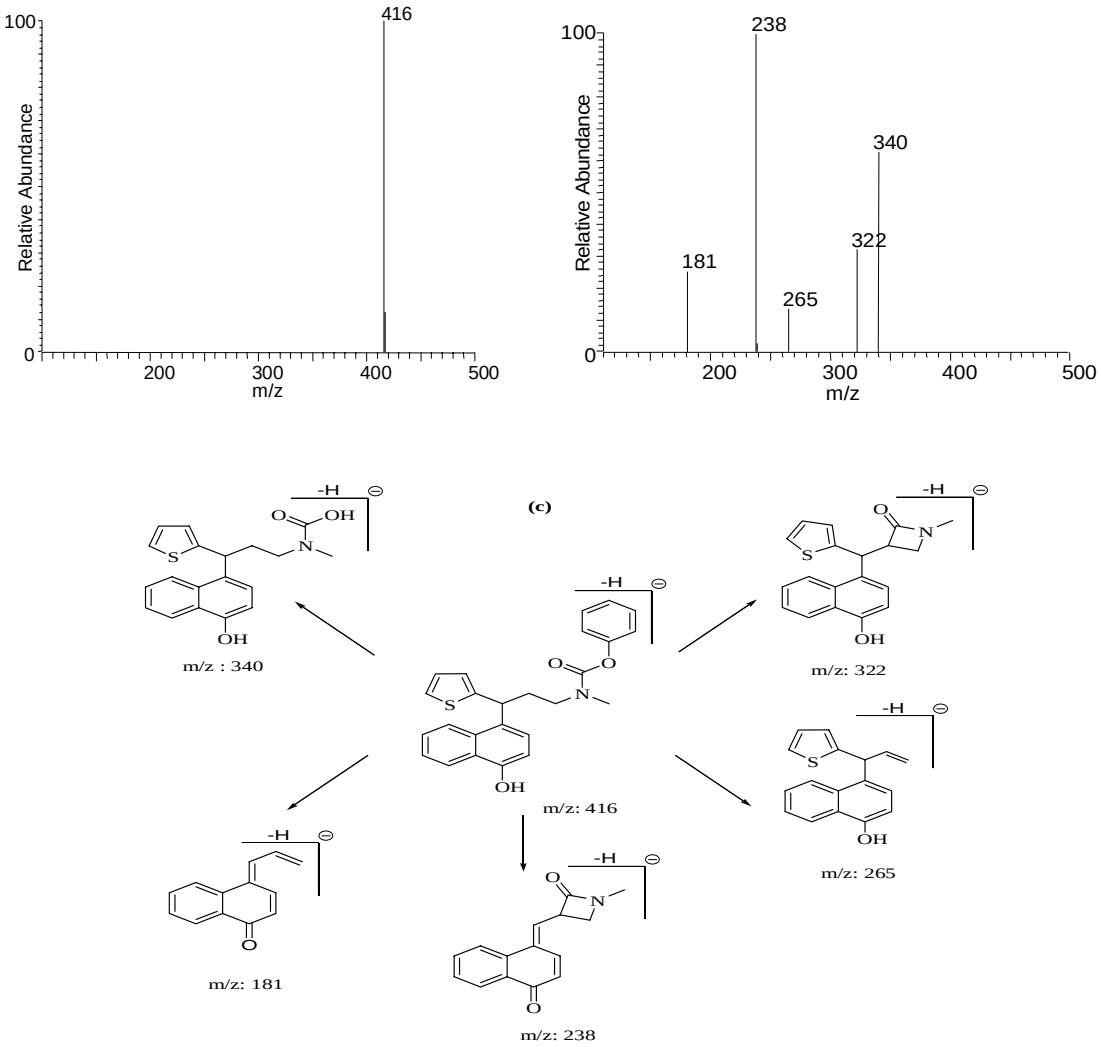
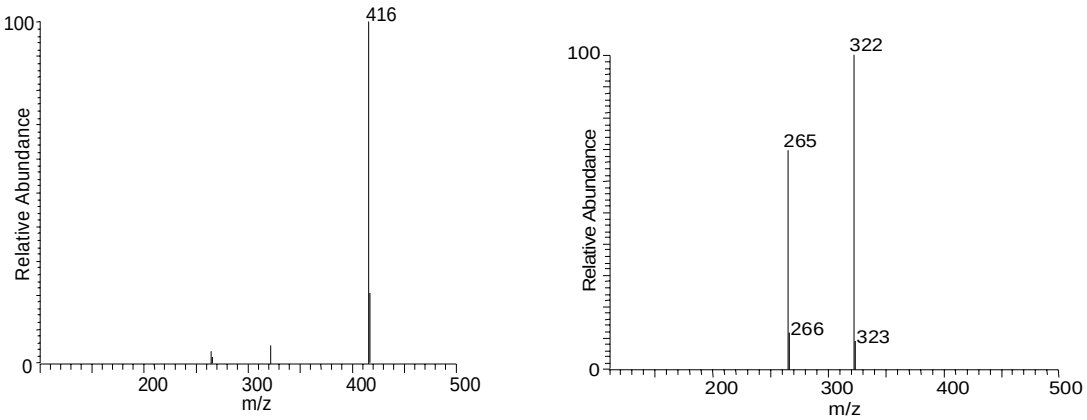


Figure - 4: Mass spectral data of DLX-II in negative ion mode (a) mass spectrum of DLX-II (b) MS/MS spectrum of ion peak at m/z 416 (c) Possible fragmentation mechanism of DLX-II.

(a) (b)



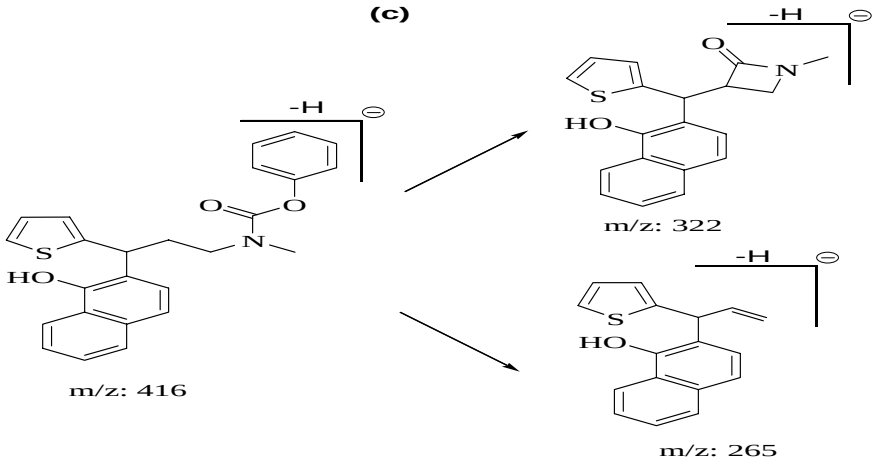
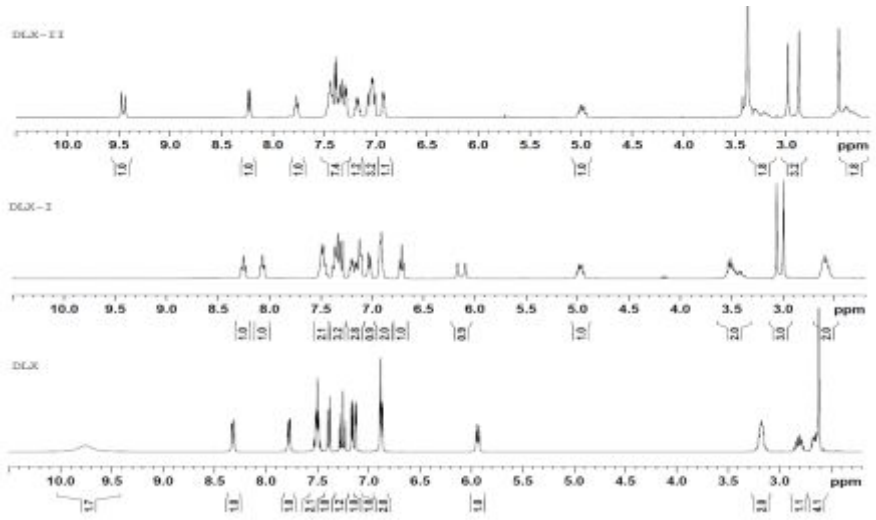


Figure - 5: Overlaid ^{1}**H** NMR spectrum of DLX, DLX-I in $CDCl_3$ and DLX-II in DMSO-d_6.



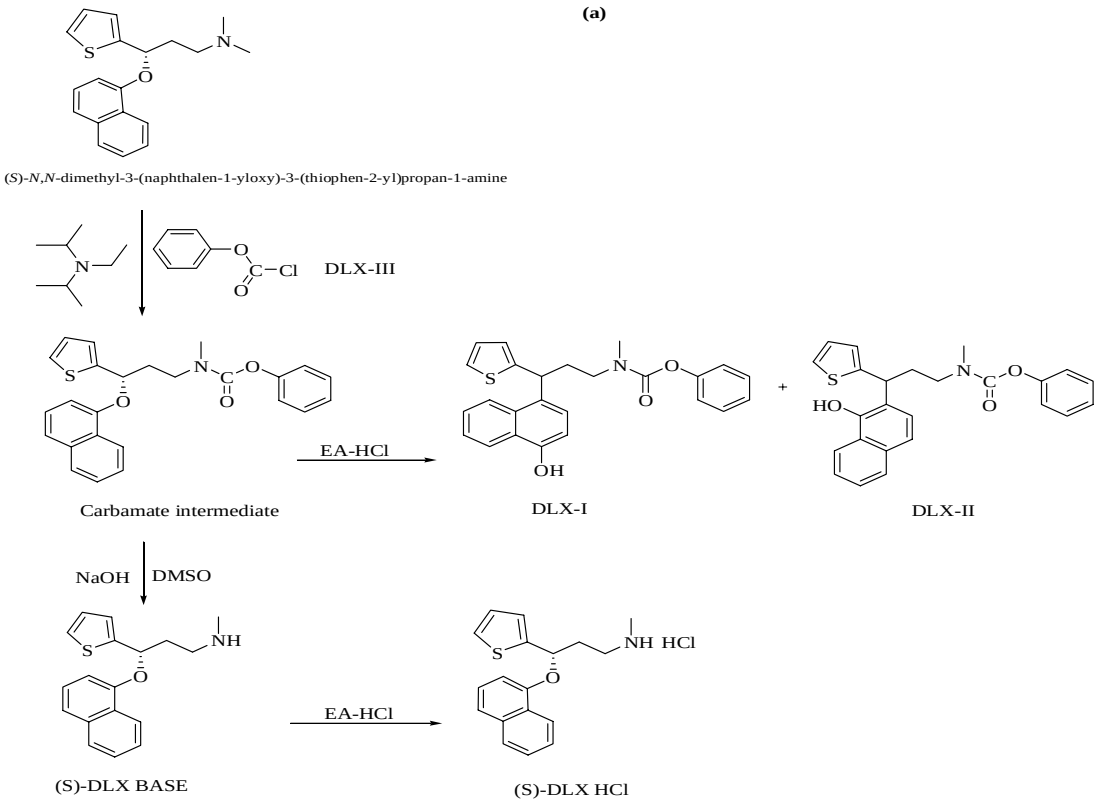
retention time of carbamate intermediate was found to be different than DLX-I and DLX-II. Additionally it was also observed that the impurities were not matching with any of the reported impurities [5-15]. Hence these impurities are inferred to be unknown, and were taken for structural elucidation.

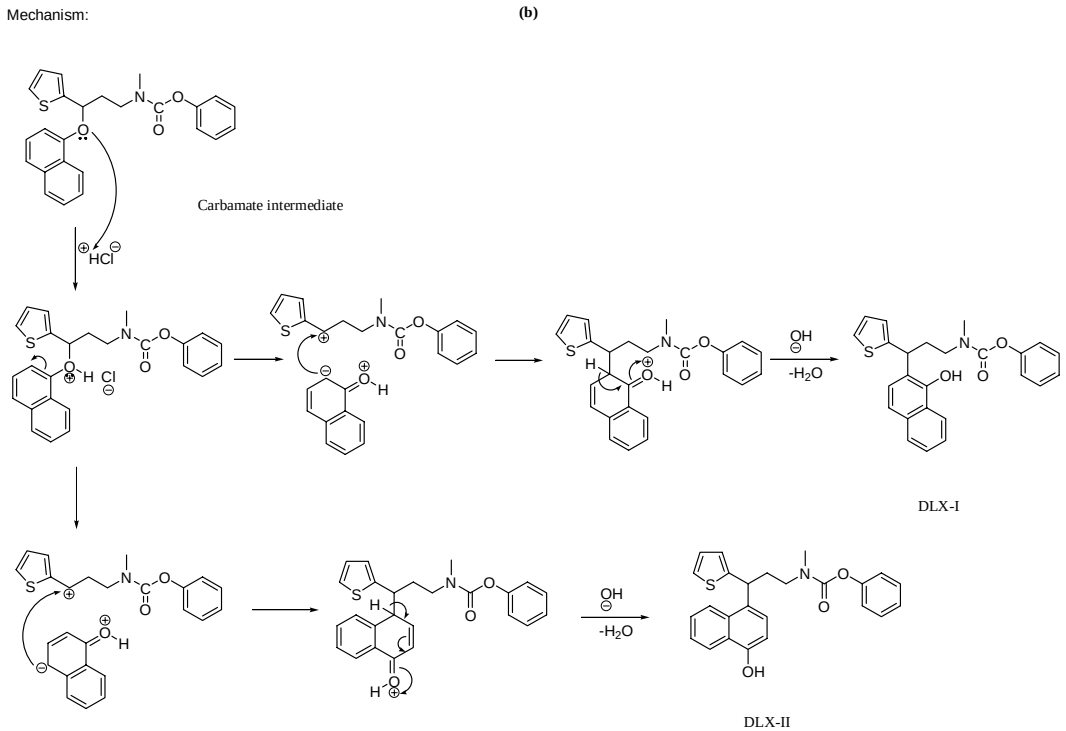
3.2. Structural elucidation by LC/ESI-MS

It was important to understand the fragmentation pattern of the parent drug molecule i.e. DLX, and therefore taken for further studies using MS/MS. MS^2 of parent ion at m/z 298 of DLX gave product ion peaks at m/z 267, m/z 183, m/z 154 and m/z 123 (Fig. 2b). The formation of product ion peak at m/z 267 can be attributed to the loss of methylamine (298-31 Da) and m/z 183 can be attributed to the loss of methylamine with thiophene ring (298-115 Da). Product ion peak at m/z 154 (298-144 Da) is formed due to the loss of naphthol ring and m/z 123 ion peak is formed due to the loss of naphthol ring along with methyl amine group (298-175 Da). The proposed fragmentation mechanisms are depicted in Fig. 2c.

MS^2 spectra of ion peak at m/z 416 of DLX-I showed product ion peaks at m/z 340, m/z 322, m/z 265, m/z 238 and m/z 181 (Fig. 3b). The formation of product ion peak at m/z 340 can be attributed to the loss of phenyl group (416-76 Da). The peak at m/z 322 can be formed due to the loss of phenyl group with one oxygen and structural rearrangement (416-94 Da). Loss of phenyl carbamate group can give m/z 265 (416-151 Da). Fragment ion peak at m/z 238, an odd molecular mass indicating nitrogen atom is not the part of neutral leaving moiety, possibly formed due the loss of phenyl and thiophene ring with rearrangement (416-178 Da). The formation of product ion peak at m/z 181 (416–235 Da) can be attributed to the loss of phenyl carbamate and thiophene ring. Based on MS/MS studies; especially m/z 238 and m/z 181 fragments, synthetic specifics [16] and possible rearrangement [5], the structure for DLX-I can be proposed as phenyl-3-(1-hydroxynaphthalen-4-yl)-3-(thiophen-2-yl)propylmethylcarbamate. The fragmentation behavior of DLX-I can be explained by the mechanism given in Fig. 3c.

Figure - 6: (a) Plausible scheme of formation of DLX, DLX-I and DLX-II (b) Plausible mechanism of formation of impurities.





MS^2 analysis of parent ion at m/z 416 of DLX-II showed only two fragment ion peaks at m/z 322 and m/z 265 (Fig. 4b). The peak at m/z 322 can be formed due to the loss of phenyl group with one oxygen followed by rearrangement (416-94 Da) and loss of phenyl carbamate group can give m/z 265 (416-151 Da). The difference in number of fragment ion in DLX-II as compared to DLX-I, is possibly due to different isomeric position. This is possible if the rearrangement [5] has taken place at different position as compared to DLX-I. Taken together the structure of DLX-II can be proposed as phenyl-3-(1-hydroxynaphthalen-2-yl)-3-(thiophen-2-yl)propylmethylcarbamate. The formation of fragment ion can be rationalized using the mechanism depicted in Fig. 4c.

3.3. Isolation of impurity by flash chromatography

DLX mother liquor samples collected during synthesis containing impurities in the range of 3-8 % (area normalization) were subjected to flash chromatography. Samples were loaded four times on silica gel (230-400 mesh) and eluted through flash column. The desired impurities were isolated using method discussed in *Flash chromatography* section. DLX-II and DLX-I were eluted at 5.2 min and 12.7 min respectively. The fractions were collected manually between times 5.5 min and 6.5 min for DLX-II and between times 13.0 min and 14.5 min for DLX-I. The isolated fractions were distilled under vacuum using rotary evaporator and reanalyzed by HPLC in order to check the retention times and purity. The HPLC purity of DLX-II and DLX-I checked by the method described in *Liquid chromatography/ Mass spectrometry* section, were found to be above 98.5% and 98.0% each. These isolated solid impurities were used for spectral characterization without any further purification.

3.4. Structural confirmation by NMR and FTIR

^1H and ^{13}C NMR spectral data of DLX were compared with DLX-I and DLX-II. DLX showed seven aliphatic protons within 2.5 to 3.5 ppm range. Signal for one proton at 5.92 ppm appeared as multiplet. The corresponding ^{13}C signal for this proton appeared at 73.1 ppm. Based on downfield chemical shift it was assigned as H11. Aromatic region showed ten protons and broad singlet for two protons. These broad protons were assigned for NH and HCl after D2O exchange analysis. DLX-I and DLX-II also showed seven aliphatic protons similar to DLX. But the signal pattern was more complex, as few of the peaks were having split signals (Fig. 5). Signal for one proton appeared at 4.95 and 5.00 ppm with corresponding ^{13}C signal at 38.7 and 35.8 ppm in DLX-I and DLX-II respectively. This upfield chemical shift compared to H11 of DLX confirmed that the H11 of DLX-I and DLX-II are not directly attached to electronegative oxygen. DLX-I and DLX-II showed one D_2O exchangeable proton at 6.09 and 9.49 ppm respectively. Both also showed fourteen protons in aromatic region, which was four extra than DLX. This confirmed the presence of additional aromatic ring. Proton signal pattern for naphthyl ether group in DLX were not similar to DLX-I and DLX-II, hence it confirmed the change in chemical environment in and around the naphthyl ring. FTIR characteristic signals appeared at 3318, 1697, 1264 and 1206 cm^{-1} for DLX-I and 3289, 1699, 1262 and 1207 cm^{-1} for DLX-II. These signals corresponds to –OH, -C=O, -O-Ph and C-N stretching vibration respectively. Taking reference from mass spectral data and possible naphthyl ether to naphthol rearrangement [5] it was established that exchangeable proton is –OH and substituting one of the proton of naphthyl ring of carbamate intermediate. On further investigating impurities by 2D NOESY analysis, it was confirmed that the split pattern of ^1H and ^{13}C was because of conformational isomers. This was due to presence of carbamate group in the structure and the rotation around their -N-C(=O) bond is restricted because of its partial double bond character [17-18]. The DLX, DLX-I and DLX-II proton and carbon position assignment based on DEPT, ^1H-^1H COSY (DQF) and ^1H-^{13}C HETCOR (HMBC and HSQC) is given in Table 1 along with confirmed structures.

3.5. Synthesis of impurities

20 g carbamate intermediate was charged in a round bottom flask followed by 100 mL EA-HCl (~ 12%) at room temperature. This mixture was stirred for 2 h. After completion, the reaction mixture was neutralized to pH 7.2 with 10% NaOH solution. The obtained aqueous solution was extracted with dichloromethane (50 mL x 2). The entire organic layer collected and dried over sodium sulfate. Further it was concentrated on rotary evaporator. This crude semi-solid mass containing DLX-I (30%) and DLX-II (35%) was subjected to conventional column chromatography using (9:1, *v/v*) hexane and ethyl acetate as eluent. The appropriate fractions were collected separately for DLX-I and DLX-II.

3.6. Formation of impurities

The key intermediate, phenyl-3-(naphthalen-1-yloxy)-3-(thiophen-2-yl) propylmethyl carbamate (carbamate intermediate) in presence of NaOH and DMSO gives duloxetine base. Finally duloxetine base is then treated with ethyl acetate-HCl (about 12%) to give DLX. During conversion of duloxetine base

Table -1: ^{1}H and ^{13}C NMR interpretation data of DLX, DLX-I in $CDCl_3$ and DLX-II in DMSO-d_6.

	DLX				DLX-I				DLX-II			
Position	Integration	δ (ppm)	Multiplicity *J* (Hz)[a]	^{13}C (δ in ppm)	Integration	δ (ppm)	Multiplicity *J* (Hz)[a]	^{13}C (δ in ppm)	Integration	δ (ppm)	Multiplicity *J* (Hz)[a]	^{13}C (δ in ppm)
1	1H	7.46	m	125.3	1H	7.43	m	126.6	1H	7.34	m	127.1
2	1H	7.46	m	126.4	1H	7.43	m	124.7	1H	7.34	m	125.0
3	1H	7.75	m	127.5	1H	8.23	m	122.7	1H	7.34	m	128.0
4	-	-	-	134.4	-	-	-	125.0	-	-	-	125.4
5	-	-	-	125.7	-	-	-	132.4	-	-	-	133.5
6	1H	8.30	m	121.0	1H	8.04	m	122.8	1H	8.27	m	122.7
7	1H	7.37	d,8.53	121.8	-	-	-	151.0	1H	7.20	-	125.4
8	1H	7.22	t,7.92	125.5	1H	6.69	m	108.0	1H	7.06	m	120.3
9	1H	6.86	m	107.2	1H	7.31	m	131.1	-	-	m	124.9
10	-	-	-	152.4	-	-	-	132.4	-	-	-	149.2
11	1H	5.92	m	73.1	1H	4.95	m	38.7	1H	5.00	m	35.8
12	2H	2.80	m	34.7	2H	2.57	m	34.7	2H	2.38	m	33.7
13	2H	3.15	m	46.0	2H	3.49	m	48.2	2H	3.25	m	47.7
14	3H	2.61	m	32.9	-	-	-	154.9	-	-	-	154.2
15	-	-	-	142.9	-	-	-	151.3	-	-	-	151.7
16	1H	7.12	m	125.6	1H	7.01	m	121.6	1H	7.34	m	122.3
17	1H	6.86	m	126.7	1H	7.31	m	124.2	1H	7.34	m	129.6
18	1H	7.15	m	125.4	1H	6.88	m	124.2	1H	7.34	m	124.3
19	2H	9.75	brs	-	1H	7.31	m	124.2	1H	7.34	m	129.6
20	-	-	-	-	1H	7.10	m	121.6	1H	7.34	m	122.3
21	-	-	-	-	-	-	-	148.8	-	-	-	149.0
22	-	-	-	-	1H	7.10	m	125.2	1H	6.97	m	127.1
23	-	-	-	-	1H	6.88	m	126.6	1H	7.06	m	125.3
24	-	-	-	-	1H	7.10	m	123.5	1H	7.06	m	125.0
25	-	-	-	-	3H	2.99 & 3.06	(s)*	35.0	3H	2.93 & 3.04	(s)*	34.9
26	-	-	-	-	1H	6.09 & 6.16	(s)*	-	1H	9.49 & 9.53	(s)*	-

s- singlet; d- doublet; m- multiplet; dd- doublet of doublet; t- triplet; [a] ^{1}H-^{1}H coupling constants; * Two singlets.

to DLX, the un-reacted trace amount of carbamate intermediate carried over to final stage undergoes the rearrangement reaction in presence of acidic condition to form DLX-I and DLX-II (Fig. 6a). The mechanism of formation is depicted (Fig. 6b). This type of rearrangement is well discussed [5].

4. CONCLUSION

A simple LC/ESI-MS method was developed for identification of process related unknown impurities of duloxetine hydrochloride bulk drug. The structures of impurities were proposed on the basis of LC/MS-MS, fragmentation mechanism and synthetic specifics. Isolation of unknown impurities has been carried out by using normal phase flash chromatography and structures were confirmed by NMR and FTIR spectroscopy. The formation of impurities was rationalized by plausible mechanism.

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5. REFERENCES

1. The Merck Index, Fourteenth ed. Whitehouse Station, Merck **&** Co., New Jersey 2006.
2. Wernicke JF, Raskin J, Rosen A, Pritchett YL, D'Souza DN, Iyengar S., Knopp K and Le TK. Duloxetine in the long-term management of diabetic peripheral neuropathic pain: An open-label, 52-week extension of a randomized controlled clinical trial, Current Therapeutic Research, 2006 ; 67:283-304.
3. Kamal A, Khanna GBR, Ramu R and Krishnaji T. Chemoenzymatic synthesis of duloxetine and its enantiomer: Lipase catalyzed resolution of 3-hydroxy-3-(2-thienyl) propanenitrile, Tetrahedron Lett. 2003; 44:4783–4787.
4. ICH International conference on harmonization, Impurities in New Drug Substances Q3A (R2) 2006.
5. Brenna E, Frigoli S, Fronza G, Fuganti C and Malpezzi L , Isolation and characterisation of a phenolic impurity in a commercial sample of duloxetine, Journal of Pharmaceutical and Biomedical Analysis.2007; 43:1573–1575.
6. Soni P, Mariappan TT and Banerjee UC. High-performance liquid chromatographic method for the simultaneous estimation of the key intermediates of duloxetine. Talanta., 2005; 67:975–978.
7. Reddy PRM, Sreeramulu J, Naidu PY and Reddy AR. Stability indicating fast LC for the Simultaneous estimation of Intermediates and degradants of duloxetine hydrochloride. Chromatographia, 2010; 71:95-100.
8. Laha TK, Mishra G and Sen S. A Validated Stability Indicating Reversed Phase High Performance Liquid Chromatographic Method of Duloxetine and Characterization of its Degradation Products through Retro-Synthesis. Pharm Anal Acta (Scientific Reports). 2012; 1: 1-6.
9. Raman NVVSS, Harikrishna KA, Prasad AVSS, Reddy KR and Ramakrishna KJ. Determination of duloxetine hydrochloride in the presence of process and degradation impurities by a validated stability-indicating RP-LC method, J. of Pharma. and Biomedical Analysis, 2010; 51: 994–997.
10. Patel SK, Patel NJ, Prajapati AM, Patel DB and Patel SA. Stabilityindicating RP-HPLC Method development and validation for Duloxetine Hydrochloride in Tablets. Journal of AOAC International, 2010; 93: 123-132.
11. Sinha VR, Anamika, Kumria R and Bhinge JR. Stress degradation studies on duloxetine hydrochloride and development of an RPHPLC method for its determination in capsule formulation, Journal Chromatographer Science, 2009; 47: 589-93.
12. Raman NVVSS, Harikrishnaa KA, Prasada AVSS, Reddya KR and Ramakrishna K. Determination of duloxetine hydrochloride in the presence of process and degradation impurities by a validated stability-indicating RP-LC method, J. Pharmaceutical and Biomedical analysis, 2010; 51: 994-997.
13. Chhalotiya UK, Bhatt KK, Shah DA and Baldania SL. Development and validation of a stability-indicating RP-HPLC method for duloxetine hydrochloride in its bulk and tablet dosage form, Science Pharma., 2010; 78: 857-868.
14. Reddy PB. Validation and stability indicating reverse phase-high performance liquid chromatography for the determination of duloxetine in tablets, International Journal of Chem Tech Research, 2009; 1: 602-605.
15. Jansen PJ, Oren PL, Kemp CA and Baertschi SW. Characterization of impurities formed by interaction of duloxetine HCl with enteric polymers hydroxypropyl methylcellulose acetate succinate and hydroxypropyl methylcellulose phthalate, Journal of pharmaceutical science, 2000; 87: 81–85.

16. Siyan RS, Gohel SKV and Singh GP. Progress for preparation of duloxetine hydrochloride, United States Patent Application Publication, US 2010/0105925 A1. 2010.
17. Smith BD, Goodenough-Lashua DM, D Souza CJE, Norton KJ and Schmidt. Substituent Effects on the Barrier to Carbamate C-N Rotation., Tetrahedron Letter, 2004; 45: 2747–49.
18. Ledneczki I, Forgo P, Kiss JT, Molnar A and Palinko I. Conformational behavior of acetamide derivatives studied by NMR spectroscopic and computational methods, Journal of Molecular Structure, 2007; 349: 834–836.