Journal of Pharmaceutical and Biomedical Analysis 53 (2010) 895–901

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Structural elucidation of process-related impurities in escitalopram by LC/ESI-MS and NMR

Bhanu Raman[a,*], Brajesh A. Sharma[a,b], Pradeep D. Ghugare[b], Sanjay Nandavadekar[b], Dharmendra Singh[b], Pravin K. Karmuse[b], Ashok Kumar[b]

[a] Department of Chemistry, K. J. Somaiya College of Science and Commerce, Vidyavihar (E), Mumbai 400077, India
[b] Ipca Laboratories Ltd., Chemical Research Division, Kandivli Industrial Estate, Kandivli (W), Mumbai 400067, India

ARTICLE INFO

Article history:
Received 21 April 2010
Received in revised form 9 June 2010
Accepted 19 June 2010
Available online 25 June 2010

Keywords:
Escitalopram
Impurities
LC/ESI-MS
Semi-preparative HPLC
NMR

ABSTRACT

Three impurities were detected in escitalopram bulk drug by HPLC–UV and LC/MS. These impurities were marked as ESC-I, -II and -III. Two of these impurities (ESC-II and -III) were unknown and have not been reported previously. Ion trap and Q-TOF mass analyzer were employed to carry out MS/MS and accurate mass analysis of these unknown impurities. Based on mass spectrometric data and synthetic specifics the structures of ESC-II and -III were proposed as *N*-(chloromethyl)-3-[5-cyano-1-(4-fluorophenyl)-1,3-dihydro-2-benzofuran-1-yl]-*N*,*N*-dimethylpropan-1-aminium and *N*-(chloromethyl)-4-[4-cyano-2-(hydroxymethyl)phenyl]-4-(4-fluorophenyl)-4-hydroxy-*N*,*N*-dimethylbutan-1-aminium respectively. The impurities were isolated by semi-preparative HPLC and structures were confirmed by NMR spectroscopy. The plausible mechanism for the formation of impurities is also discussed.

1. Introduction

Citalopram, ((*RS*)-1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile), a selective serotonin reuptake inhibitor (SSRI), is been demonstrated to be effective in the treatment of depression, panic disorder, premenstrual dysphoric disorder and obsessive–compulsive disorder, it is a racemate that comprises an *S*(+)-enantiomer, i.e. escitalopram (ESC) and an *R*(−)-enantiomer (*R*-citalopram) in a 1:1 ratio [1]. In studies using the individual enantiomers, the *S*-isomer has been shown to be responsible for essentially all the serotonin reuptake inhibition. In case of citalopram, the *S*(+)-enantiomer is greater than two orders of magnitude more potent than *R*(−)-enantiomer in vitro as an inhibitor of serotonin (5-hydroxytryptamine) uptake. Furthermore, escitalopram has very little effect on other receptors, making it most selective SSRI [2].

Few analytical methods for estimation of escitalopram in biological fluid samples have been reported [3–7]. Quantitative methods for related impurities and degradants for escitalopram APIs and formulations, using liquid chromatography, mass spectrometry and capillary electrophoresis have also been reported [8–11].

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 [12]. 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.

During process development studies of escitalopram, three impurities in the bulk drug samples were detected by current available analytical method [13] and out of which two were found to be unknown and not reported before [13,14]. 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. Materials and reagents

ESC bulk drug samples were obtained from Chemical Research Division, Ipca Laboratories Ltd. (Mumbai, India). HPLC grade acetonitrile was purchased from Merck Ltd. (Mumbai, India), ammonium formate from LOBA Chemie Pvt. Ltd. (Mumbai, India) and analytical reagent grade trifluroacetic acid (TFA) was purchased from Lancaster, England. De-ionized water prepared using

* Corresponding author.
E-mail address: drbhanuraman@rediffmail.com (B. Raman).

0731-7085/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2010.06.019

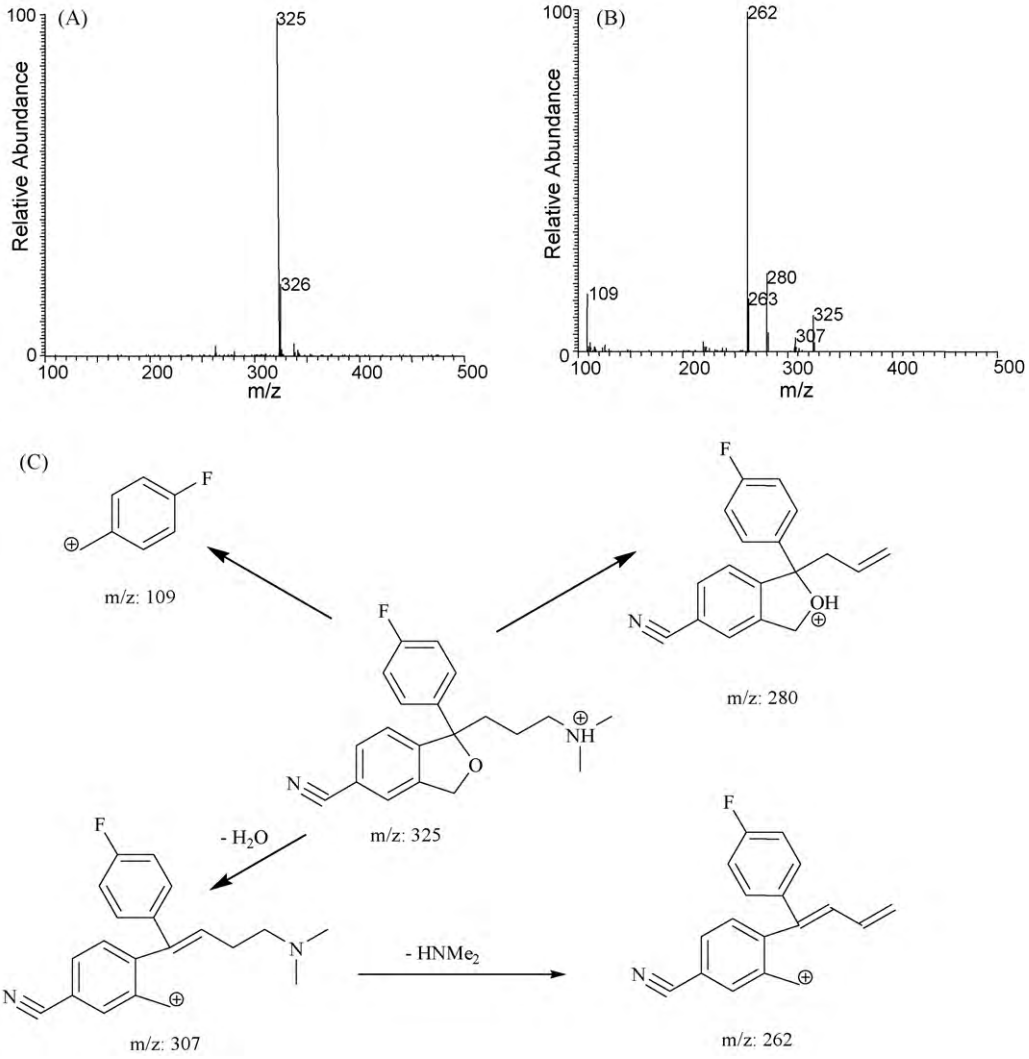


Fig. 1. Mass spectral data of escitalopram (A) mass spectrum of escitalopram (B) MS/MS spectrum of product ion peak at *m/z* 325 (C) Possible fragmentation mechanism of escitalopram.

milliQ plus purification system Millipore (Bradford, USA) was used throughout the studies. $CDCl_3$ 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 Waters XTerra C18 column (150 mm × 4.6 mm i.d., 3 μm particles) was used for chromatographic separations. The mobile phase consisting of (*A*) 1.5 g ammonium formate dissolved in 1000 ml of water and (*B*) acetonitrile, with timed gradient programme *T* (min)/*B* (%): 0/30, 10/30, 15/60, 20/55, 25/55, 30/30, 35/30. The flow rate was set to 1.0 ml/min with UV detector wavelength was fixed at 240 nm. The sample solution (1000 ppm) was prepared in mobile phase and 20 μl 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* 100–500. MS/MS studies were carried out by maintaining normalized collision energy at 35% with the mass range *m/z* 100–500.

Q-TOF Micromass spectrometer (Waters, Milford, MA, USA) was used for accurate mass determination. The mass resolution of the instrument was 6000. Leucine enkephalin ($C_{28}H_{37}N_5O_7$) was used as an external lock mass (556.2771 Da). The source block and desolvation lamp were kept at 90 and 180 °C respectively. The nebulizer and desolvation gas flows were 20 and 450 l/h. The instrument parameters in positive mode were; capillary voltage 3000 V, cone at 25 V, extractor at 2 V and MCP at 2700 V. Data acquisition and processing was done using masslinks (version 4.0) software.

2.3. Semi-preparative HPLC

The impurities were isolated using Waters Auto-purification system equipped with 2525 binary gradient pump, 2487 UV detector and 2767 sample manager (Waters, Milford, MA, USA). A Waters Xterra Prep MS C18 OBD column (250 mm × 19 mm i.d, particle size 10 μm) was used for chromatographic separation. A mixture of 0.1% ammonium hydroxide solution (pH adjusted to 3.3 with TFA) and acetonitrile in the ratio of (75/25, v/v) was used as mobile phase. The flow rate was 30 ml/min. The sample concentration was 100 mg/ml. The injection volume was 10 ml and the detector wavelength was set at 240 nm.

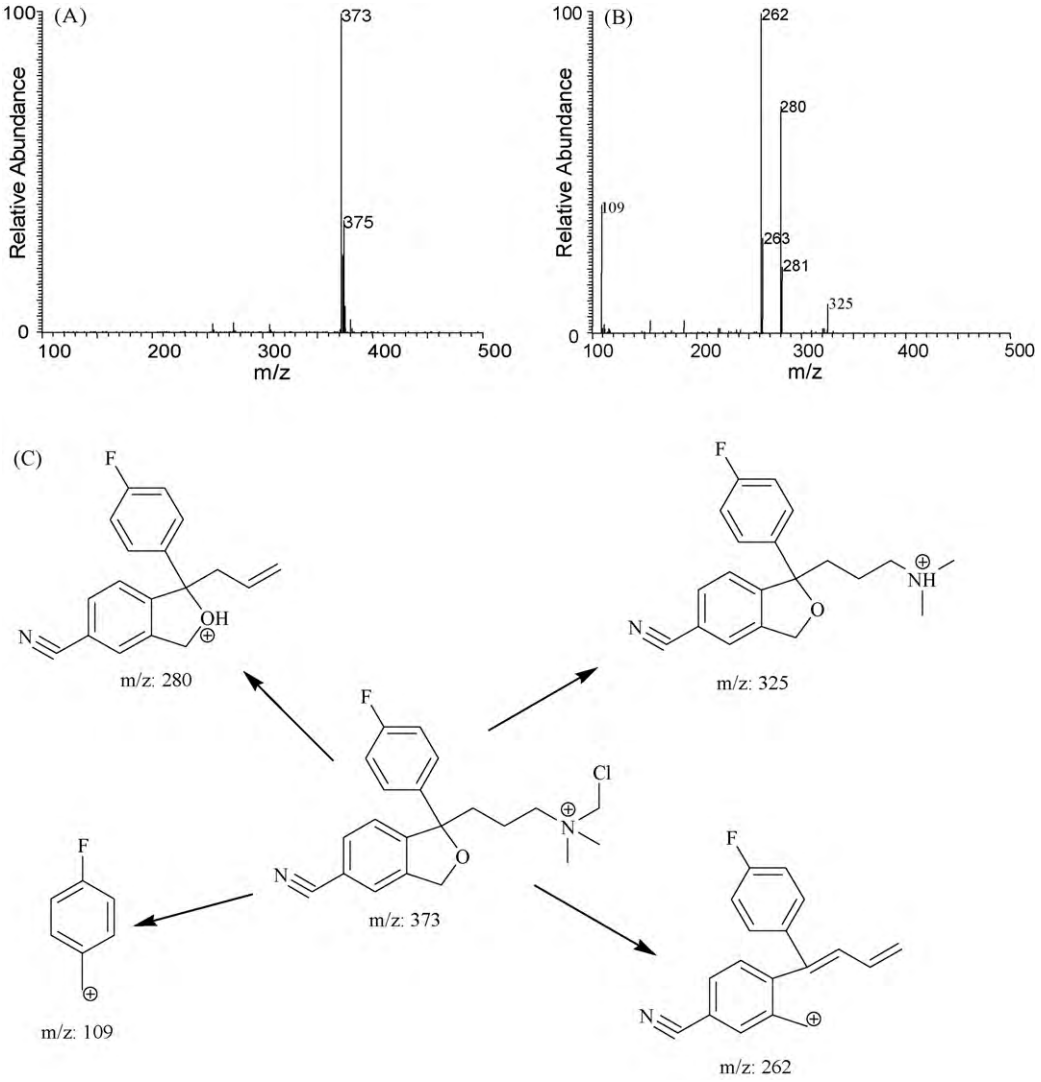


Fig. 2. Mass spectral data of ESC-II (A) mass spectrum of ESC-II (B) MS/MS spectrum of product ion peak at *m/z* 373 (C) Possible fragmentation mechanism of ESC-II.

2.4. NMR

The ^{1}H, ^{13}C measurements of the isolated impurities and ESC 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 and HSQC) 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 ^{1}H 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.

3. Result and discussion

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

The HPLC analysis of ESC samples using USP method [13], revealed the presence of three impurities, marked as ESC-I (RT 4.3 min), ESC-III (RT 4.9 min) and ESC-II (RT 11.0 min) along with principle peak (RT 10.2 min).

For the identification of impurities the available USP method was not suitable for LC/MS. The ESC bulk drug samples were analysed by newly developed LC/ESI-MS method described in Section 2.2. This mass spectral data manifested molecular mass of ESC (*m/z* 325), ESC-I (*m/z* 343), ESC-II (*m/z* 373) and ESC-III (*m/z* 391). Based on mass spectral data, ESC-I is identified as 4-((*R*)-4-(dimethylamino)-1-(4-fluorophenyl)-1-hydroxybutyl)-3-(hydroxymethyl)benzonitrile, a precursor of ESC. This was further confirmed by co-injecting the reference material of the said intermediate. ESC-II and ESC-III showed isotopic peaks for single chlorine with ~32% relative abundance at *m/z* 375 and *m/z* 393, respectively. Since ESC-II and ESC-III do not match with any of the reported impurities [13,14], these impurities are inferred to be unknown, and were taken for structural elucidation.

3.2. Structural elucidation by Q-TOF and LC/ESI-MS

Accurate mass measured on Q-TOF Micromass instrument for ESC, ESC-II and ESC-III were found to be 325.1711, 373.1477 and 391.1583 Da respectively. Based on the ^{37}Cl isotopic pattern obtained from mass spectral data, the elemental calculator was set with reasonable limits of carbon 0–30, hydrogen 0–30, chlorine 0–1, nitrogen 0–5, oxygen 0–5, and fluorine 0–3 (as the unknowns possibly contains these elements). The search revealed several possibilities. However, the most plausible molecular formulae for ESC-II ($C_{21}H_{23}ClFN_2O^+$) and ESC-III ($C_{21}H_{25}ClFN_2O_2^+$) were

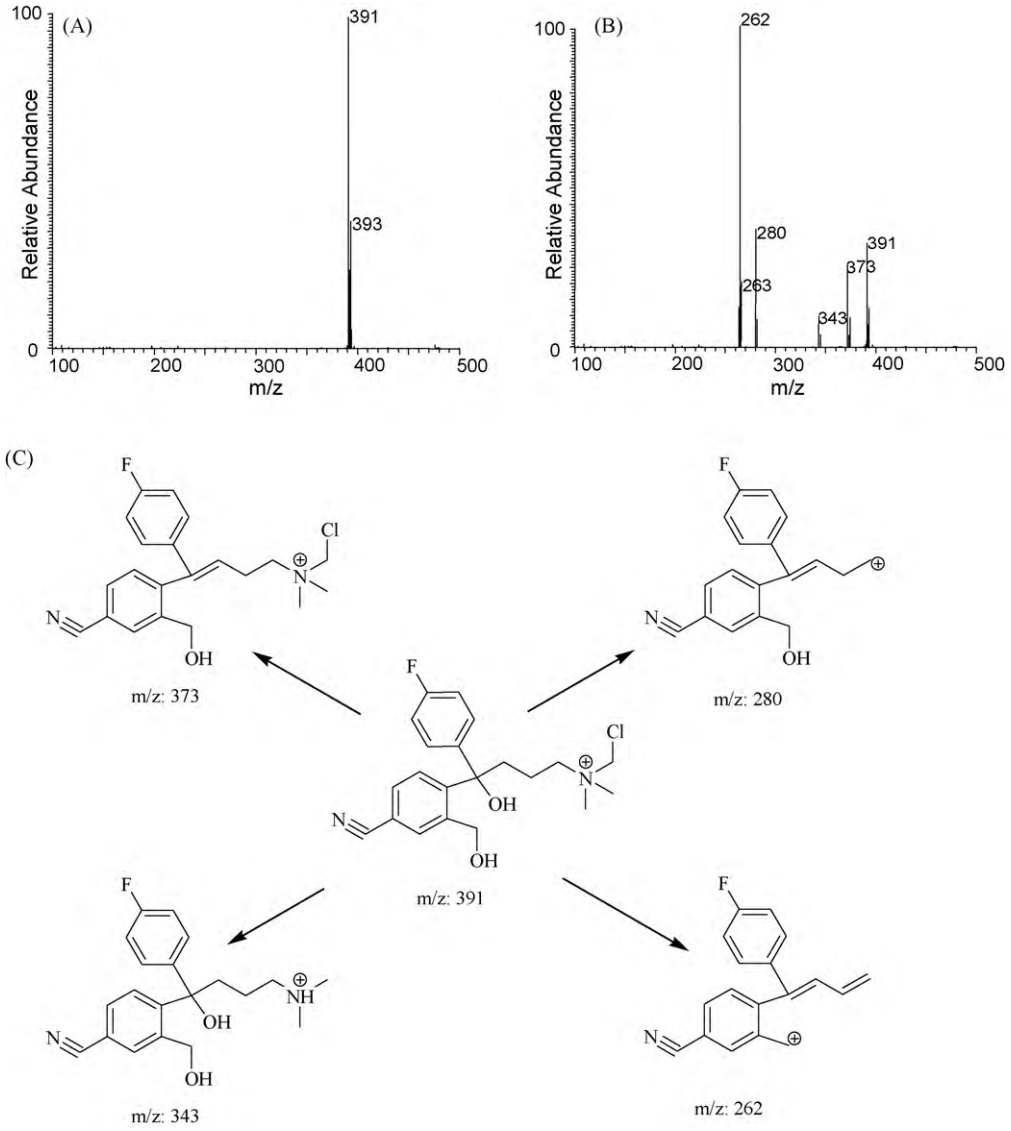


Fig. 3. Mass spectral data of ESC-III (A) mass spectrum of ESC-III (B) MS/MS spectrum of product ion peak at *m/z* 391 (C) Possible fragmentation mechanism of ESC-III.

selected on the basis of lowest difference in mDa (milliDalton) values in experimental and theoretical masses.

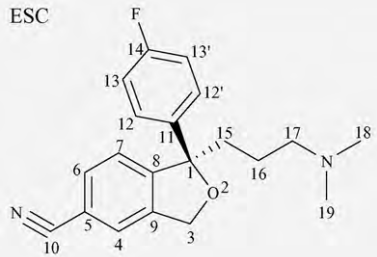
It is important to understand the fragmentation pattern of the parent drug molecule, i.e. ESC, and therefore taken for further studies using MS/MS. MS^2 of parent ion at *m/z* 325 of ESC yielded daughter ion peaks at *m/z* 307, *m/z* 280, *m/z* 262 and *m/z* 109 (Fig. 1A and B). The formation of product ion peak at *m/z* 307 and *m/z* 280 can be attributed to the loss of water molecule (−18 Da) and *N*-methylmethanamine (−45 Da), respectively. The peak appeared at *m/z* 262 can be produced by elimination of *N*-methylmethanamine along with water molecule (−63 Da). The product ion peak at *m/z* 109 could be assigned for 4-fluoro-1-methylbenzene. The proposed fragmentation mechanisms are depicted in Fig. 1C.

MS^2 spectra of ESC-II showed four daughter ion peaks at *m/z* 325, *m/z* 280, *m/z* 262, and *m/z* 109 (Fig. 2A and B). The formation of daughter ion peak at *m/z* 325 can be attributed to the loss of methyl chloride (−48 Da). The peak at *m/z* 280 can be due to the loss of 1-chloro-*N*, *N*-dimethylmethanamine (−93 Da). The odd molecular mass and missing ^{37}Cl pattern of daughter ion peak at *m/z* 280 indicates that the chlorine and nitrogen atom is part of neutral leaving moiety. The formation of daughter ion peak at *m/z* 262 (−111 Da) can be attributed to the loss of 1-chloro-*N*,*N*-dimethylmethanamine group along with water molecule. The *m/z* 109 (−264 Da) can be explained as 4-fluoro-1-methylbenzene as in case of ESC. Based on MS/MS studies and synthetic specifics [14], the structure for ESC-II can be proposed as *N*-(chloromethyl)-3-[5-cyano-1-(4-fluorophenyl)-1,3-dihydro-2-benzofuran-1-yl]-*N*,*N*-dimethylpropan-1-aminium. The fragmentation behavior of ESC-II can be explained by the mechanism given in Fig. 2C.

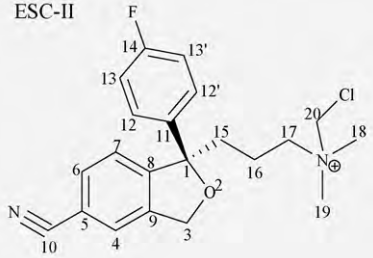
MS^2 analysis of parent ion at *m/z* 391 of ESC-III also showed four daughter ion peaks at *m/z* 373, *m/z* 343 *m/z* 280 and *m/z* 262 (Fig. 3A and B). The formation of product ion peak at *m/z* 373 can be due to loss of water molecule (−18 Da). The daughter ion peak at *m/z* 343 may formed due to the loss of neutral leaving chloromethyl moiety (−48 Da). The product ion peak at *m/z* 280 can be attributed to 1-chloro-*N*,*N*-dimethylmethanamine along with water molecule (−111 Da) and daughter ion peak at *m/z* 262 can be attributed to the loss of two water molecule along with 1-chloro-*N*,*N*-dimethylmethanamine group (−129 Da). The isotopic pattern for single chlorine was observed in the fragment at *m/z* 373, whereas it is absent in the *m/z* 343, *m/z* 280 and *m/z* 262. It is evident from reaction specifics that the dichloromethane is used as a solvent for purification at final

Table 1
^{1}H, ^{13}C and DEPT NMR data of ESC, ESC-II and ESC-III.

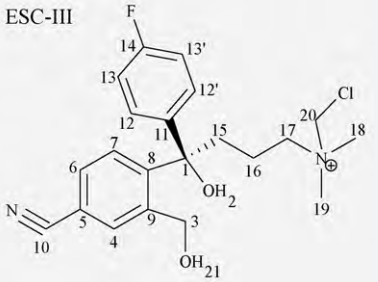
ESC



ESC-II



ESC-III



Position	Integration	δ (ppm)	Multiplicity, J (Hz)[a]	^{13}C (δ in ppm), J (Hz)[b]	DEPT[c]
1	–	–	–	90.4	–
2	–	–	–	–	–
3	2H	5.15	dd (42.6, 13.4)	71.2	CH_2
4	1H	7.55	s	132.0	CH
5	–	–	–	111.7	–
6	1H	7.55	m	125.1	CH
7	1H	7.46	m	122.7	CH
8	–	–	–	139.6	–
9	–	–	–	148.4	–
10	–	–	–	118.4	–
11	–	–	–	138.3 (2.9)	–
12, 12′	2H	7.45	m	126.5 (8.1)	CH
13, 13′	2H	6.97	m	115.4 (21.2)	CH
14	–	–	–	161.9 (246.6)	–
15	2H	2.36	m	37.6	CH_2
16	2H	1.73	m	19.1	CH_2
17	2H	3.09	t (7.6)	57.5	CH_2
18	3H	2.70	s	42.7	CH_3
19	3H	2.70	s	42.7	CH_3

Position	Integration	δ (ppm)	Multiplicity, J (Hz)[a]	^{13}C (δ in ppm), J (Hz)[b]	DEPT[c]
1	–	–	–	90.4	–
2	–	–	–	–	–
3	2H	5.13	dd (56.6, 13.1)	71.4	CH_2
4	1H	7.52	s	132.3	CH
5	–	–	–	112.0	–
6	1H	7.62	d (8.2)	125.4	CH
7	1H	7.53	d (8.2)	123.0	CH
8	–	–	–	139.9	–
9	–	–	–	148.4	–
10	–	–	–	118.6	–
11	–	–	–	138.4 (2.9)	–
12, 12′	2H	7.46	m	126.7 (8.1)	CH
13, 13′	2H	7.04	m	115.7 (22.0)	CH
14	–	–	–	162.2 (246.6)	–
15	2H	2.19	m	37.1	CH_2
16	2H	1.70	m	17.8	CH_2
17	2H	3.72	m	62.3	CH_2
18	3H	3.35	s	49.4	CH_3
19	3H	3.36	s	49.4	CH_3
20	2H	5.59	s	68.7	CH_2

Position	Integration	δ (ppm)	Multiplicity, J (Hz)[a]	^{13}C (δ in ppm), J (Hz)[b]	DEPT[c]
1	–	–	–	77.2	–
2 (OH)	1H	3.27	–	–	–
3	2H	4.06	dd (98.4, 13.46)	62.3	CH_2
4	1H	7.67	d (1.5)	134.2	CH
5	–	–	–	111.4	–
6	1H	7.60	dd (8.2, 1.5)	130.9	CH
7	1H	7.69	d (8.2)	126.9	CH
8	–	–	–	141.3	–
9	–	–	–	149.1	–
10	–	–	–	118.4	–
11	–	–	–	140.6 (2.9)	–
12, 12′	2H	7.21	m	127.3 (8.1)	CH
13, 13′	2H	6.94	m	115.0 (21.2)	CH
14	–	–	–	161.7 (246.6)	–
15	2H	2.20	m	38.3	CH_2
16	2H	1.57	m	17.1	CH_2
17	2H	3.53	m	63.4	CH_2
18	3H	3.14	s	48.9	CH_3
19	3H	3.15	s	48.9	CH_3
20	2H	5.15	dd (14.3, 13.46)	68.7	CH_2
21 (OH)	1H	3.27	–	–	–

s, singlet; d, doublet; m, multiplet; dd, doublet of doublet; t, triplet.

[a] ^{1}H–^{1}H coupling constants.

[b] ^{13}C–^{19}F coupling constants.

[c] Hybridization (degree of bonding) of carbon atoms.

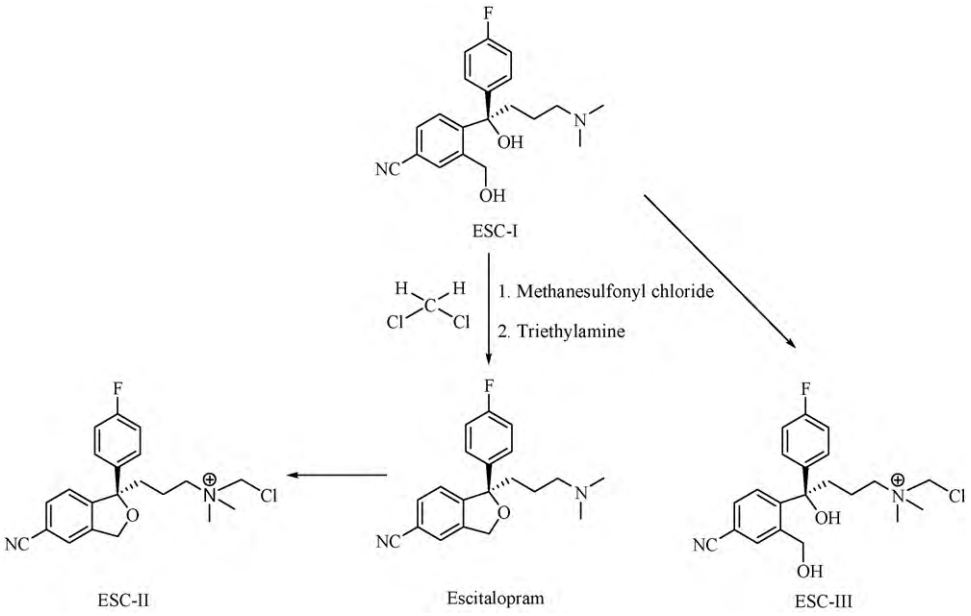


Fig. 4. Plausible mechanism of formation of escitalopram, ESC-II and ESC-III.

step. Taken together the structure of ESC-III can be proposed as *N*-(chloromethyl)-4-[4-cyano-2-(hydroxymethyl) phenyl]-4-(4-fluorophenyl)-4-hydroxy-*N*,*N*-dimethylbutan-1-aminium. The formation of daughter ion fragments can be rationalized using the mechanism depicted in Fig. 3C.

3.3. Isolation of impurity by semi-preparative liquid chromatography

Mother liquor (ML) samples obtained during development stages were used for semi-preparative isolation using method discussed in Section 2.3. ESC, ESC-II and ESC-III were eluted at 8.2, 19.7 and 25.3 min respectively. The fractions were collected manually between times 7.5 and 8.5 min for ESC-II and between times 24.5 and 25.5 min for ESC-III. The isolated fractions were freeze dried and reanalyzed by HPLC in order to check the retention times and purity. The HPLC purity of ESC-II and ESC-III checked by the method described in Section 2.2, were found to be above 97.5 and 98.0% each. These isolated solid impurities were used for spectral characterization without any further purification.

3.4. Structural confirmation by NMR

^{1}H and ^{13}C NMR spectral data of ESC-II showed two extra aliphatic protons and an additional methylene carbon atom as compared to ESC. While the ESC-III showed four extra aliphatic protons and an additional carbon atom in ^{13}C NMR as compared to ESC. ESC-II spectral data showed singlet at 5.59 ppm integrating for two protons attached to methylene carbon atom at 68.7 ppm and ESC-III showed doublet of doublet signal at 5.15 ppm integrating for two protons attached to methylene carbon atom at 68.7 ppm. These strong downfield shifts of signals as compared to ESC proton NMR signals, confirms the change in electronic environment due to chlorine. Downfield chemical shift of H17 proton of ESC-II and ESC-III as compared to ESC is because of electron deficient neighboring nitrogen, which is due to the addition of methyl chloride group to the nitrogen atom of aliphatic chain. The noticeable change in H3 proton chemical shift position of ESC-III as compared to ESC and ESC-II was observed due to opening of five membered dihydroisobenzofuran ring. In the HMBC spectrum, long-range correlation between δ 5.15 (H3) and δ 90.4 (C1) of ESC and δ 5.13 (H3) and δ 90.4 (C1) of ESC-II indicated that both C1 and C3 were connected to the same oxygen atom. However, no long-range correlation was observed between δ 4.06 (H3) and δ 77.2 (C1) of ESC-III. This indicated that the five membered dihydroisobenzofuran is now opened. This leads into formation of two hydroxyl groups at H2 and H21 position, giving signal at 3.27 ppm for two protons, which is confirmed by D_2O exchange analysis. The ESC, ESC-II and ESC-III proton and carbon position assignment based on DEPT, ^{1}H–^{1}H COSY (DQF) and ^{1}H–^{13}C HETCOR (HMBC and HSQC) is given in Table 1 along with confirmed structures.

3.5. Formation of impurities

It is evident that 4-((*R*)-4-(dimethylamino)-1-(4-fluorophenyl)-1-hydroxybutyl)-3-(hydroxymethyl) benzonitrile (ESC-I) cyclized in presence of methanesulfonyl chloride and triethylamine (TEA) to form ESC. ESC is then extracted into dichloromethane (DCM; Fig. 4). The ESC-II is formed due to interaction of traces of DCM with ESC and ESC-III is formed by interaction of traces of un-reacted ESC-I with DCM. This type of interaction of tertiary amine group with halogenated hydrocarbon solvents is well studied [15].

3.6. Conclusion

A simple LC/ESI-MS method was developed for identification of process-related unknown impurities of escitalopram bulk drug. The structures of impurities were proposed on the basis of LC/MS–MS, accurate mass obtained by Q-TOF analysis, fragmentation mechanism and synthetic specifics. Isolation of unknown impurities has been carried out by using semi-preparative liquid chromatography and structures were confirmed by NMR spectroscopy.

Acknowledgements

The authors wish to thank, the management of Ipca Laboratories, for providing necessary facility.

References

[1] C. Sanchez, K.P. Bogeso, B. Ebert, E.H. Reines, C. Braestrup, Escitalopram versus citalopram: the surprising role of the R-enantiomer, Psychopharmacology 174 (2004) 163–176.

[2] W.J. Burke, C.J. Kratochvil, Stereoisomers in psychiatry: the case of escitalopram, Primary Care Companion J. Clin. Psychiary 4 (2002) 20–24.
[3] M. Kosela, C.B. Eapa, M. Ameya, P. Baumann, Analysis of the enantiomers of citalopram and its demethylated metabolites using chiral liquid chromatography, J. Chromatogr. B 719 (1998) 234–238.
[4] S. Andersen, T.G. Halvorsena, S.P. Bjergaarda, K.E. Rasmussena, L. Tanumb, H. Refsumb, Stereospecific determination of citalopram and desmethylcitalopram by capillary electrophoresis and liquid-phase microextraction, J. Pharm. Biomed. Sci. 33 (2003) 263–273.
[5] S.S. Singh, H. Shah, S. Gupta, M. Jain, K. Sharma, P. Thakkar, R. Shah, Liquid chromatography–electrospray ionisation mass spectrometry method for the determination of escitalopram in human plasma and its application in bioequivalence study, J. Chromatogr. B 811 (2004) 209–215.
[6] C. Greiner, C. Hiemake, W. Bader, E. Haen, Determination of citalopram and escitalopram together with their active main metabolites desmethyl(es-)citalopram in human serum by column-switching high performance liquid chromatography (HPLC) and spectrophotometric detection, J. Chromatogr. B 848 (2007) 391–394.
[7] N. Jonannesson, J. Bergquist, Rapid on-line extraction and quantification of escitalopram from urine using sol–gel columns and mass spectrometric detection, J. Pharm. Biomed. Sci. 43 (2007) 1045–1048.
[8] R.N. Rao, A.N. Raju, D. Nagaraju, Development and validation of a liquid chromatographic method for determination of enantiomeric purity of citalopram in bulk drugs and pharmaceuticals, J. Pharm. Biomed. Sci. 41 (2006) 280–285.
[9] R.N. Rao, A.N. Raju, Enantiospecific assay of citadiol—a key intermediate of escitalopram by liquid chromatography on Chiralpak AD-H column connected with UV and polarimetric detectors in series, J. Pharm. Biomed. Sci. 43 (2007) 311–314.
[10] B. Sungthong, P. Jac, G.K.E. Scriba, Development and validation of a capillary electrophoresis method for the simultaneous determination of impurities of escitalopram including the R-enantiomer, J. Pharm. Biomed. Sci. 46 (2008) 959–965.
[11] S.R. Dhaneshwar, M.V. Mahadik, M.J. Kulkarni, Column liquid chromatography–ultraviolet and column liquid chromatography/mass spectrometry evaluation of stress degradation behavior of escitalopram oxalate, J. AOAC Int. 92 (2009) 138–147.
[12] ICH Guideline, Impurities in New Drug Substances Q3A (R2), October 25, 2006.
[13] http://www.usp.org/pdf/EN/pendingStandards/escitalopramOxalate.pdf.
[14] S.G. Uttarwar, B.N. Gawli, United States Patent Application Publication, US 2006/0189816 A1 (August 2006).
[15] A.H. Gainza, Reaction of halogenated hydrocarbon solvents with tertiary amines: spectrophotometric and conductimetric study, Int. J. Chem. Kinet. 36 (2004) 500–509.