GERF Bulletin of Biosciences
June 2011, 2(1):1-5

Research Article

Identification, synthesis and spectral characterization of impurities in process development of Escitalopram

Srinivas Gangula,[1] Naveen Kumar Kolla,[1] Chandrashekar R. Elati,[1] Ashok. Dongamanti,[2*] Rakeshwar Bandichhor[1*]

[1]Integrated Product Development, Innovation Plaza, Dr.Reddy's Laboratories Ltd, Bachupalli, Qutubullapur, Rangareddy District-72, Andhra Pradesh, India.
[2]Department of Chemistry, Osmania University, Hyderabad-07, India

Abstract

Synthesis and identification of five process-related impurities of escitalopram are described. Structures of all the four impurities were identified by employing LC-MS (liquid chromatography-mass spectrometry) and subsequently characterized by other spectral techniques e.g. IR and NMR. Based on the spectral data, the structures of these impurities were characterized as 1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-2-benzofuran-5-carboxamide 4, 1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydro-2-benzofuran-5-carboxylicacid 5, *N*-{3-[5-cyano-1-(4-fluorophenyl)]-1,3-dihydro-2-benzofuran-1-yl]propyl}formamide 6 and 1-(4-fluorophenyl)-1-[3-(methyl amino)propyl]-1,3-dihydro-2-benzofuran-5-carbonitrile 7.

Keywords: Escitalopram, impurities, spectroscopy, identification, characterization and synthesis

Introduction

Escitalopram **1**, (*S*)-(+)-1-[3-(Di methyl amino) propyl] 1-(4-fluorophenyl) 1, 3-dihydro-5-isobenzofurancarbonitrile) is a selective and potent serotonin reuptake inhibitor (SSRI). It has widely been used for the treatment of depression and anxiety disorders (Baumann, 1996; Hyttel *et al.*, 1992 ; Hyttel and Larsen 1985 ; Sanchez *et al.*, 2004; Sorbera *et al.*, 2001; Nageswara *et al.*, 2006) . During the analysis of escitalopram, four unknown impurities **4** to **7** as shown in Fig. 1 were detected consistently in almost all the batches consisting of area percentage ranging from 0.05 to 0.15%, in a gradient High Performance Liquid Chromatography (HPLC) method (Ref. 7). As per stringent regulatory requirements (ICH guidelines), it is mandatory to profile all the impurities particularly those are > 0.1% for any API to get approved and commercialized (ICH Guideline Q3A, 2002).

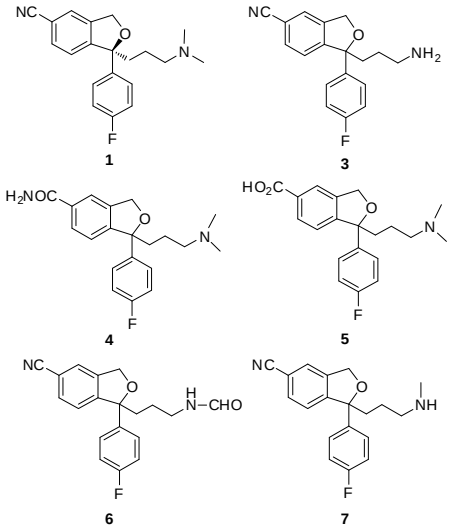


Fig 1. Structure of escitalopram **1** and impurities **3-7**

Herein, we describe our efforts to synthesize and characterize the impurities of escitalopram by using various spectroscopic techniques.

*Corresponding authors: ashokdou@gmail.com, rakeshwarb@drreddys.com

Material and Methods

General Procedures

^{1}H NMR spectra were recorded on a Gemini 200 MHz NMR Spectrometer; the chemical shifts are reported in δ ppm relative to TMS. The IR spectra were recorded in the solid state as KBr dispersion using Perkin Elmer FT-IR Spectrophotometer. The mass spectra were recorded on Shimadzu LCMS-QP8000, LC-MS and AB-4000 Q-trap LCMS/MS.

1-(3-aminopropyl)-1-(4-fluorophenyl)-1, 3-dihydro-2-benzofuran-5-carbonitrile (3)

a) ***Preparation of 3-chloropropan-1-amine solution in toluene:*** To a solution of aqueous sodium hydroxide solution (100 g, in 600 ml of water) were added 3-chloropropan-1-amine hydrochloride (95.1 g, 0.732 mol) and toluene (1500 ml) at 25-35 °C. The mixture was stirred for 10-15 min. and the organic layer was separated. Resulting aqueous layer was extracted with toluene (2 x 600 ml) and the combined organic layers were directly used for alkylation step. **b)** ***Alkylation with 3-chloropropan-1-amine free base:*** Dimethylsulfoxide (DMSO) (500 ml) and potassium tertiary butoxide (81.9 g, 0.732 mol) were charged into a 2.0 L round bottomed flask under nitrogen atmosphere. The reaction mixture was heated to 65-70 °C for 60 min. Thereafter, in a separate vessel, 1-(4-fluorophenyl)-1, 3-dihydroisobenzofuran-5-carbonitrile **2** (100 g, 0.41 mol) was dissolved in dimethylsulfoxide (300 ml) and added drop wise to the above reaction mixture over 10-15 min. at 30-35 °C. To the resulting reaction was added the above prepared 3-chloropropan-1-amine solution in toluene (part a) rapidly in a 10 min duration and maintained at 40-45 °C for 1h. After completion of the reaction (content of **2** by TLC), the reaction mixture was slowly added to a pre-chilled water (1 L) over 30 min. at 10-15 °C. The organic layer was separated and the aqueous layer was extracted into toluene (3 x 600 ml). The combined toluene layers were treated with aq. HCl solution (48 ml of conc. HCl in 1000 ml of water) and the resulting acidic solution was washed with toluene (4 x 500 ml). The resulting acidic aqueous layer was neutralized with caustic lye solution (30 ml) to a pH of 10.5 and the product was extracted into toluene (3 x 500 ml). The final organic layer was washed with hot water (3 x 300 ml) and then the solvent was removed under vacuum to obtain impurity **3** as a residue. The oxalate salt of impurity **3** was obtained from the residue by precipitation using oxalic acid (37.8 g, 0.30 mol) and ethyl acetate (900 ml) as a solvent in a 57 % (92.8 g) yield 97 % purity by HPLC. mp 120.2 °C ; IR (cm^{-1}): 3371 (N-H, -O-H stretching), 3063 (aromatic C-H stretching), 2959 (aliphatic C-H stretching), 2229 (-CN stretching), 1720 (-C=O, stretching) 1603, 1503 (aromatic C=C stretching), 1225 (-C-N stretching), 1405 (aliphatic C-H bending), 1224, (C-O stretching), 834, 709 (aromatic C-H bending); ^{1}HNMR (200 MH$_Z$, DMSO-d$_6$): δ 1.4-1.5 (m, 2H), 2.25 (m, 2H), 2.85 (t, 2H), 4-5 (br, NH$_2$), 5.2 (qt, 2H, *J* = 12.4 MHz), 7.16 -7.22 (m, 2H), 7.60 -7.61 (m, 2H), 7.75-7.8 (m, 2H), 7.85 (s, 1H); ^{13}CNMR (200 MH$_Z$, DMSO-d$_6$): δ 22.3, 37.2, 48.1, 71.2, 90.6, 115.2, 118.8, 123.3, 125.7, 126.9, 132.1,139.9, 140.1, 149, 163.8, 165; HRMS (TOF MS): M$^+$: Found 297.1532 (free base), $C_{18}H_{17}N_2OF.C_2H_2O_4$ requires 386 (with oxalate salt).

1-[3-(dimethylamino) propyl]-1-(4-fluorophenyl)-1, 3-dihydro-2-benzofuran-5-carboxamide (4)

To a solution of escitalopram free base **1** (10 g, 30.8 mmol) in *tert*-butanol (100 ml) was added KOH flakes (5.2 g, 92.5 mmol). The resulting solution was heated to 65-75 °C and stirred for 4-5 h. After cooling the reaction mass to 25 °C, the reaction mixture was saturated with water (100 ml) and was extracted into toluene (2 x 50 ml). The organic phase was washed with water (2 x 20 ml) and removed under vacuum. The obtained crude **4** was isolated as solid in IPA/*n*-heptane mixture in a 65% (6.9 g) yield and 98% purity by HPLC. IR (cm^{-1}): 3347 ((-N-H stretching), 3181 (aromatic C-H stretching), 2949 (aliphatic C - H stretching), 1666 (-C=O, stretching) 1619, 1506 (aromatic C=C stretching), 1219 (-C-N stretching), 1384 (aliphatic C-H bending), 1219, (C–O stretching), 830 (aromatic C-H bending) ; mp: 99.5 °C; ^{1}HNMR (200 MH$_Z$, DMSO-d$_6$): δ 1.2-13 (m, 2H), 2.2 (m, 2H), 2.3 (s, 6H), 2.5 (m, 2H), 5.18 (t, 2H, *J* = 12.8 MHz), 6.9 (br, 2H), 7.0 (m, 1H), 7.2 (m, 1H), 7.40 (d, 1H, *J* = 1.6 MHz), 7.45 (dd, 1H, *J* = 2.0 MHz), 7.67 (d, 1H, *J* = 8.4 MHz), 7.72 (m, 2H); ^{13}CNMR (200 MH$_Z$, DMSO-d$_6$) δ 22.2, 39.1, 45.3 (2C), 59.5, 71.2, 90.9, 114.8, 115.2, 115.3,126.7, 127.0 (2C), 133.1, 139.9, 140.0, 148.2, 163.0; HRMS (TOF MS): M$^+$: Found 343.1824, $C_{20}H_{24}N_2O_2F$ requires 343.1822.

1-[3-(dimethylamino) propyl]-1-(4-fluorophenyl)-1, 3-dihydro-2-benzofuran-5-carboxylicacid (5)

To a suspension of escitalopram free base **1** (10 g, 30.8 mmol) in water (30 ml) was added KOH flakes (8.4 g, 154.3 mmol). The resulting mixture was stirred at reflux temperature for 12 - 15 h. The reaction mass was cooled to 25 °C, neutralized with 20% HCl. There after the reaction mass was extracted with toluene (2 x 50 ml). The toluene layers were washed with brine (1 x10 ml), water (2 x 10 ml) and concentrated under reduced pressure to obtain **5** as solid in a 69 % (7.3 g) yield and 98 % purity by HPLC. mp: 110.5 °C; IR (cm^{-1}): 3410 (-OH stretching), 3181 (aromatic C-H stretching), 2949 (aliphatic C-H stretching), 1705.0 (-C=O, stretching) 1619, 1506 (aromatic C=C stretching), 1384 (aliphatic C-H bending), 1223, (C-O stretching), 836, 718 (aromatic C-H bending). ^{1}HNMR (200 MH$_Z$, DMSO-d$_6$): δ 1.4 -1.5 (m, 2H), 2.2 (m, 2H), 2.5 (s, 6H), 2.85 (m, 2H), 5.0 (t, 2H, *J* = 12.8 MHz), 7.15 - 7.20 (m, 2H), 7.55 -7.60 (m, 2H, 7.81 (s, 1H), 7.85 - 7.90

(d, 2H, *J* = 8.4 MHz); ^{13}CNMR (200 MH_Z, DMSO-d_6) δ 20.8, 38.2, 43.5 (2C), 57.7, 71.2, 90.1, 114.7, 115.2, 115.3,126.7, 127.0 (2C), 133.9, 138.6, 141.3, 146.8 and 163.0; HRMS (TOF MS): M^+: Found 344.1647, $C_{20}H_{23}NO_2F$ requires 344.1662.

_N_-{3-[5-cyano-1-(4-fluorophenyl)]-1,3-dihydro-2-benzofuran-1-yl]propyl} formamide (6)

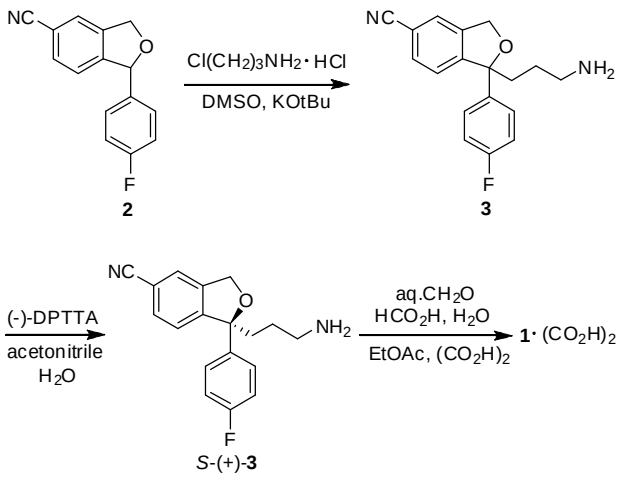
To a solution of impurity **3** (10 g, 33.7 mmol) in toluene (100 ml) was added formic acid (3.3 g, 67.5 mmol). The mixture was stirred at 110 °C under azeotropic condition till required amount of water is collected. After cooling to 25 °C, water (40 ml) was added and then neutralized with 10% NaOH solution. The organic phase was washed with brine (20 ml), water (2 x 20 ml), and then dried over Na_2SO_4. Then the final organic layer was concentrated under vacuum to obtain a crude sample of **6** in a 55 % (6 g) yield and 96 % purity by HPLC. IR (cm^{-1}): 3307 (N-H stretching), 3063 (aromatic C-H stretching), 2959 (aliphatic C-H stretching), 2230 (-CN stretching), 1668 (-CHO, stretching) 1603, 1503 (aromatic C=C stretching), 1225 (-C-N stretching), 1405 (aliphatic C-H bending), 1224, (C - O stretching), 835, 734 (aromatic C-H bending); mp 110 °C; ^{1}HNMR (200 MH_Z, DMSO-d_6) δ 1.4-1.5 (m, 2H), 2.25 (m, 2H), 2.85 (m, 2H), 5.2 (m, 2H), 7.20 -7.21 (m, 2H), 7.60-7.61 (m, 2H), 7.75 (m, 2H), 7.78 -7.79 (m, 2H), 7.95 (m, 1H); ^{13}CNMR (200 MH_Z, DMSO-d_6) δ 24.1, 37.5, 38.6, 71.02, 90.5, 115.2, 118.8, 123.0, 123.1, 125.5, 126.8, 126.9 (2C), 131.1, 139.9, 140.2,160, 163.7; HRMS (TOF MS): M^+: Found 325.1350, $C_{19}H_{18}N_2O_2F$ requires 325.1352.

1-(4-fluorophenyl)-1-[3-(methylamino) propyl]-1, 3-dihydro-2-benzofuran-5 carbonitrile (7)

To a solution of **3** (10 g, 33.7 mmol) in toluene (200 ml) was added benzaldehyde (4.2 g, 40.5 mmol) and refluxed at azeotropic conditions till required amount of water is collected. To the resulting clear solution was added a solution of dimethylsulfate (DMS) (5.1 g, 40.0 mmol) in toluene (20 ml) drop wise at 110 °C. The resulting mixture was stirred at 110 °C for another 3 h. After cooling to 25 °C, the reaction mass was thoroughly washed with brine solution (1 x 20 ml), water (2 x 20 ml), and then dried over Na_2SO_4. The organic phase was concentrated under reduced pressure and the resulted residue **7** was converted to oxalate salt in ethyl acetate in a 75 % (10 g) yield and 97 % purity by HPLC. IR (cm^{-1}): 3341 (-O-H and N-H stretching), 3063 (aromatic C-H stretching), 2959 (aliphatic C - H stretching), 2231 (-CN stretching), 1720 (-C=O, stretching) 1603, 1503 (aromatic C=C stretching), 1225 (-C-N stretching), 1405 (aliphatic C-H bending), 1225, (C-O stretching), 836, 720 (aromatic C-H bending); mp 127 °C; ^{1}H NMR (200 MH_Z, DMSO-d_6): δ 1.4-1.5 (m, 2H), 2.25 (m, 2H), 2.85 (t, 2H), 5.2 (qt, 2H, *J* = 12.4 MHz), 7.16 -7.22 (m, 2H), 7.60 -7.61 (m, 2H), 7.75-7.8 (m, 2H), 7.85 (s, 1H); ^{13}CNMR (200 MH_Z, DMSO-d_6): δ 20.7, 37.0, 48.1, 71.2, 90.4, 115.2, 118.8, 123.1, 125.7, 126.9, 132.1,139.9, 140.1, 149, 161.3, 165; HRMS (TOF MS): M^+: Found 311.1571 (free base), $C_{19}H_{19}N_2OF.C_2H_2O_4$ requires 400 (with oxalate salt).

Results and Discussion

Escitalopram oxalate **1** was synthesized in the laboratory by a novel process (Sundaram *et al.*, 2005; Elati *et al.*, 2007), (Scheme 1) which involves the *C*-alkylation of 1-(4-fluorophenyl)-1, 3-dihydroisobenzofuran-5-carbonitrile **2** with 3-chloropropylamine to give a key intermediate **3**, which on further resolution with L-(-)-DPTTA yielded the (*S*)-1-(3-aminopropyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile *S*-(+)-**3**. Then the compound *S*-(+)-**3** was allowed to react with the mixture of aqueous formic acid and formaldehyde under Clarke-Eschweiler reaction conditions (Icke *et al.* 1945), that provided the crude escitalopram, which was further purified by oxalate salt formation to afford pharmaceutically acceptable escitalopram oxalate salt of **1**.

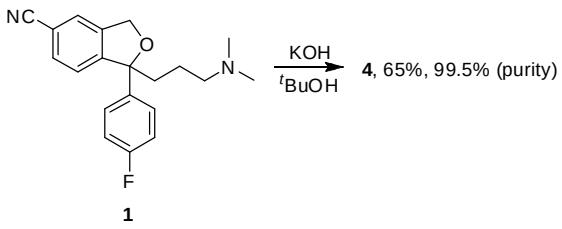


Scheme 1. Synthesis of oxalate salt of **1**

It was observed during the process development of escitalopram **1** oxalate that some of the batches were contaminated with impurities **4** to **7** in the range from 0.05% to 0.15%. These impurities were synthesized after identification by HPLC and detection of mass by LC-MS. It was necessary to synthesize these impurities **4 -7** in pure form for analytical method validation of escitalopram **1** as a bulk drug. Therefore, a comprehensive study was carried out to prepare these impurities.

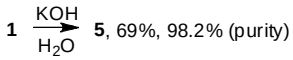
Impurity **3**, a key intermediate of escitalopram which was carried over till to the final step in the synthesis of **1**. Basic conditions were employed in the work of escitalopram synthesis. Under these basic conditions the cyanide moiety

present in the escitalopram was hydrolyzed and leads to the formation of amide impurity **4**. This impurity was synthesized by the reaction of compound **1** with potassium hydroxide base in tert- butanol solvent (Scheme 2).



Scheme 2. Synthesis of impurity **4**

The amide impurity **4** under basic conditions during workup afforded impurity **5** which was prepared by the reacting **1** with potassium hydroxide in water (Scheme 3).



Scheme 3. Synthesis of impurity **5**

Methylation of compound *S*-(+)-**3** with aqueous formic acid and formaldehyde forms escitalopram **1** (Scheme 1). The reaction proceeds through imine intermediate to afford **1**. Considering reaction path, mechanistically, as shown in Fig 2, the formation of impurities **6** and **7** can be envisioned. The *N*-formyl impurity **6** can undergo reduction at the expense of de-carboxylation to afford impurity **7**.

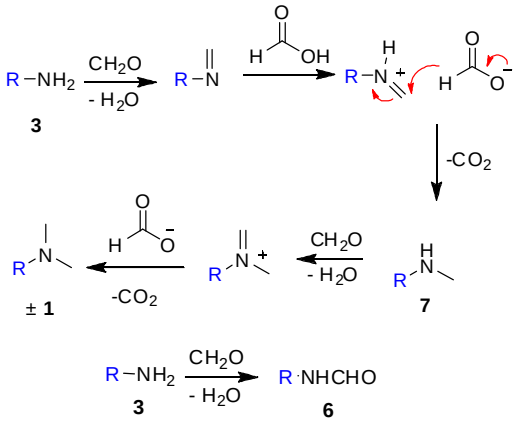
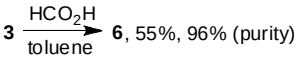


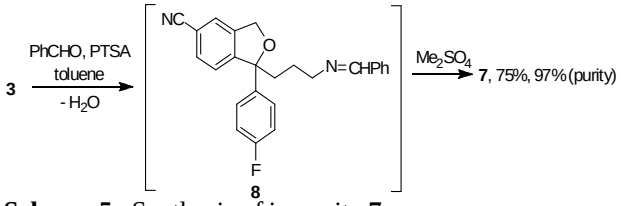
Fig 2. Mechanism of formation of impurities **6** and **7**

In order to prove the hypothesis impurity **6** was generated by the reaction of compound **3** with excess formic acid in toluene solvent as shown in Scheme 4.



Scheme 4. Synthesis of impurity **6**

Impurity **7** was synthesized by the reaction of **3** with benzaldehyde and *p*-Toluenesulfonic acid (PTSA) in toluene solvent followed by *N*-methylation and hydrolysis. Intermediate **8** was formed in the first step and subsequently subjected to methylation and hydrolysis to obtain impurity **7** as shown in Scheme 5.



Scheme 5. Synthesis of impurity **7**

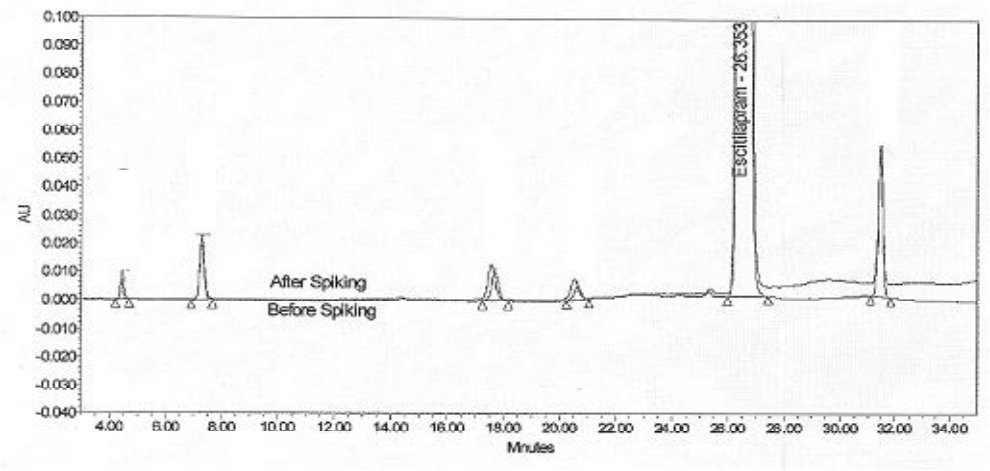
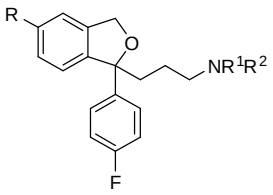


Fig. 3. HPLC chromatogram of **3** to **7** before and after spiking with crude sample of **1**

A typical analytical HPLC chromatogram of a laboratory batch of escitalopram **1** bulk drug is spiked with impurities (**3** to **7**) as shown in Fig. 2. As evident in chromatogram, all the impurities are found to be well resolved. We observed in the chromatogram that the impurities **3**, **4**, **5** and **7** are polar and impurity **6** is non-polar with respect to escitalopram.



R = CN, $R^1 = R^2 = CH_3$ (**1**)
R = CN, $R^1 = R^2 = H$ (**3**)
R = $CONH_2$, $R^1 = R^2 = CH_3$ (**4**)
R = CO_2H, $R^1 = R^2 = CH_3$ (**5**)
R = CN, $R^1 = H$, $R^2 = CHO$ (**6**)
R = CN, $R^1 = H$, $R^2 = CH_3$ (**7**)

Conclusions

In conclusion, we have identified, synthesized (starting from racemic substrates), developed robust analytical methods and characterized the four process related impurities present in escitalopram particularly those are close to 0.10%. The characterization of these compounds is based on LC–MS, IR and NMR spectral data followed by their independent syntheses in order to meet the regulatory requirements as per ICH guidelines.

Acknowledgements

The authors wish to thank the management of the Dr.Reddy's Laboratories Ltd. for supporting this work.

References

1. Baumann P (1996). Pharmacology and pharmacokinetics of citalopram and other SSRIs. *Int. Clin. Psychopharm. 11*, 5.
2. Hyttel J, Bogeso KP, Perregaard J and Sanchez C (1992). The pharmacological effect of Citalopram resides in the (S)-(+)-enantiomer. *J. Neural Transm.Gen. Sect., 88* : 157-160.
3. Hyttel J. and Larsen JJ (1985). Serotonin-selective antidepressants. *Acta Pharmacol. Toxicol*. 56: 146-153.
4. Sanchez C, Bogeso KP, Ebert B, Reines EH and Braestrup C (2004). Escitalopram versus citalopram: the surprising role of the R-enantiomer. *Psychopharmac*. 174 (2): 163 -176.
5. Sorbera LA, Revel L, Martin L, Castaner J (2001). Treatment of erectile dysfunction treatment of female sexual dysfunction phosphodiesterase 5 inhibitor. *Drug future*., 26(1): 15-19.
6. Nageswara Rao R, Narasa Raju A and Nagaraju D (2006). Development and validation of a liquid chromatographic method for determination of enantiomeric purity of citalopram in bulk drugs and pharmaceuticals. *J. Pharm. Biomed. Anal.*, 4: 280-285.
7. An in-house LC method was developed for the analysis of escitaloparm **1** and its impurities **3-7**. The analysis was carried out on Develosil ODS MG-5 columns, 250 x 4.6 mm, 5µ particle size with a mobile phase consisting of A: 8:2 (v/v) ratio of 0.01M of KH_2PO_4 in Mille Q water and acetonitrile, B: 3:7 (v/v) ratio of 0.01M of KH_2PO_4 in Mille Q water and acetonitrile. The time gradient program [t (min) / A (v/v)/ B (v/v) = 0/85/15, 5/85/15, 15/80/20, 20/65/35, 25/45/55, 30/45/55, 40/20/80, 54/20/80, 55/85/15, 60/85/15] was used with UV detection at 235 nm. The flow rate was set at 1.0 ml/min at 25 °C. This LC method was able to detect all these impurities. The data was recorded using Waters Millennium Software.
8. ICH Guideline Q3A (R), Impurities in New Drug Substances February 7 2002.
9. Sundaram V, Mathad VT, Elati RRC, Kolla NK, Vankawala PJ, Govindan S, Chalamala S, Gangula S (2005). Preparation of escitalopram. WO Patent 047274A1, 2005.
10. Elati C R, Kolla NK, Vankawala PJ, Gangula S, Chalamala S, Sundaram V, Bhattacharya A, Vurimidi H, Mathad V T (2007). Substrate Modification Approach to achieve efficient Resolution: Didesmethylcitalopram: A Key Intermediate for Escitalopram.*Org. Process Res. Dev.*, *11*,289.
11. Icke RN, Wisegarvu BB and Alles GA (1945). β-Phenylethyldimethylamine .*Organic Synthesis*, CV *3*, 723.