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Semi-preparative isolation and structural elucidation of an impurity in citalopram by LC/MS/MS

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ABSTRACT

Two impurities were detected in citalopram bulk drug substance by HPLC analysis. A new LC–ESI/MS method was developed for the identification of impurities. One of the impurities was found to be unknown and has not been reported previously. The structure of the unknown impurity was proposed on the basis of MS^n data obtained using ion trap mass analyzer and accurate mass obtained using Q-TOF mass analyzer. The impurity was isolated by semi-preparative HPLC. The structure of the impurity was confirmed as 1-(1,1-bis (4-fluorophenyl)-1,3-dihydroisobenzofuran-5-yl)-4-(dimethylamino) butan-1-one hydrobromide by using NMR and IR spectroscopy.

1. Introduction

Citalopram HBr (Fig. 1) is a selective serotonin reuptake inhibitors (SSRIs) and used as an antidepressant drug [1]. Serotonin (5-hydroxytryptamine) has been recognized as an agent that enhances platelets aggregation and as a neurotransmitter in the central nervous system (CNS). It is found in high concentration in enterochromaffin cells through the gastrointestinal tract, in platelets, and in specific regions of the CNS [2]. Additionally, citalopram is also used for the anxiety, panic and obsessive-compulsive disorder [3].

EP and USP describe HPLC method for citalopram and its related impurities [4,5]. Few LC/MS methods are also reported for characterization of trace level impurities of citalopram [6,7]. Determination of citalopram using flow injection-solid phase extraction with spectrofluorometric detection is reported [3].

Organic impurities can arise during the manufacturing process and storage of the drug substance and criteria for their acceptance upto certain limits are based on pharmaceutical studies, clinical trials or known safety data. As per regulatory guideline, the pharmaceutical studies using the sample of the isolated impurity can be considered for safety assessment [8]. Therefore, it is essential to isolate and characterize the unidentified impurities present in active pharmaceutical ingredients (APIs).

Different methods of synthesis of citalopram and its salt are reported in the literature [9–12]. In present work citalopram synthesized from one of the route [10] was analyzed by HPLC method [4]. Two impurities were detected in the bulk drug sample obtained by this process. One of the impurities was found to be unknown and had not been reported previously. Present paper describes the isolation and characterization of this unknown impurity present in the citalopram bulk drug sample.

2. Experimental

2.1. Materials and reagents

Citalopram API (crude and pure) and intermediate samples were obtained from Chemical Research Division, Ipca Laboratories Ltd., Mumbai, India. HPLC grade acetonitirile, liq. ammonia, methanol, THF, KH_2PO_4 and phosphoric acid was purchased from Merck India Limited. Glacial acetic acid was purchased from Qualigens India Limited. De-ionized water was prepared using milli-Q plus purification system (Millipore, Bradford, USA). N,N-dimethyloctylamine, potassium bromide (FTIR) grade and deuterated chloroform was purchased from Merck KGaA, Germany.

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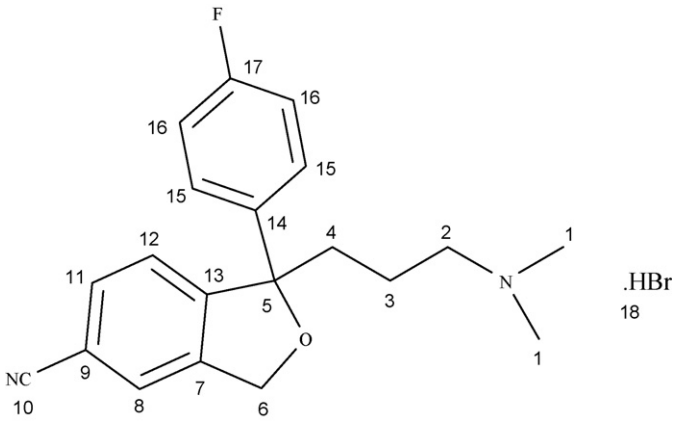

Fig. 1. Chemical structure of citalopram.

2.2. Mass spectrometry

The LC–ESI/MS^n analysis was carried out on LCQ-Advantage (Thermo Finnigen San Jose, USA) ion trap 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* 100–600. MS^n studies were carried out by maintaining normalized collision energy at about 30% with the mass range *m*/*z* 50–600. The LC unit was consisted of an Agilent 1100 series quaternary gradient pump with a degasser and auto sampler. A Kromasil C18 column (250 mm × 4.6 mm i.d., 5 μm akzo nobel, Sweden) was used for chromatographic separation. The mobile phase consists of a mixture of aqueous 0.065 M ammonium acetate and acetonitrile in the ratio 50:50 (v/v). The flow rate was maintained at 1.0 ml/min.

Q-TOF Micromass (Waters, Milford, MA, USA) spectrometer was used for accurate mass determination. The mass resolution of the instrument was 5773.8. Leucine enkephalin ($C_{28}H_{37}N_5O_7$) was used as a lock mass (556.8771 Da). The source block and desolvation lamp were kept at 150 °C and 300 °C, respectively. The nebulizer and desolvation gas flows were 20 l/h 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 unknown impurity was isolated from the crude sample of citalopram API using Waters auto-purification system consisting of 2525 binary gradient pump, a 2487 UV detector and 2767 sample manager (Waters, Milford, MA, USA). A Waters Symmetry C18 Column (100 mm × 30 mm i.d., particle size 5 μm), USA was used for semi-preparative isolation. The mobile phase consisting of mixture of liq. ammonia, water and acetonitrile in the ratio of (0.1:50:50, v/v/v) and apparent pH adjusted with glacial acetic acid to 7.0. The flow rate was maintained at 25 ml/min. The sample solution of 100 mg/ml was prepared by using mixture of acetonitrile and water (50:50, v/v) as a diluent. The injection volume was 1 ml and the detection was monitored at 225 nm.

2.4. NMR

The ^{1}H, ^{13}C NMR and DEPT-135 measurement of the isolated impurity was performed on a AVANCE 400 (Bruker, Fallanden, Switzerland) instrument at 300 K. The ^{1}H and ^{13}C chemical shift values were reported on the δ scale in ppm relative to $CDCl_3$-d 7.28 ppm and 77.0 ppm, respectively.

2.5. IR spectroscopy

The IR spectrum of isolated impurity 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 impurity by HPLC and LC/MS

The citalopram API samples prepared by known synthetic route [10] were analyzed by using HPLC method as described in USP [4].

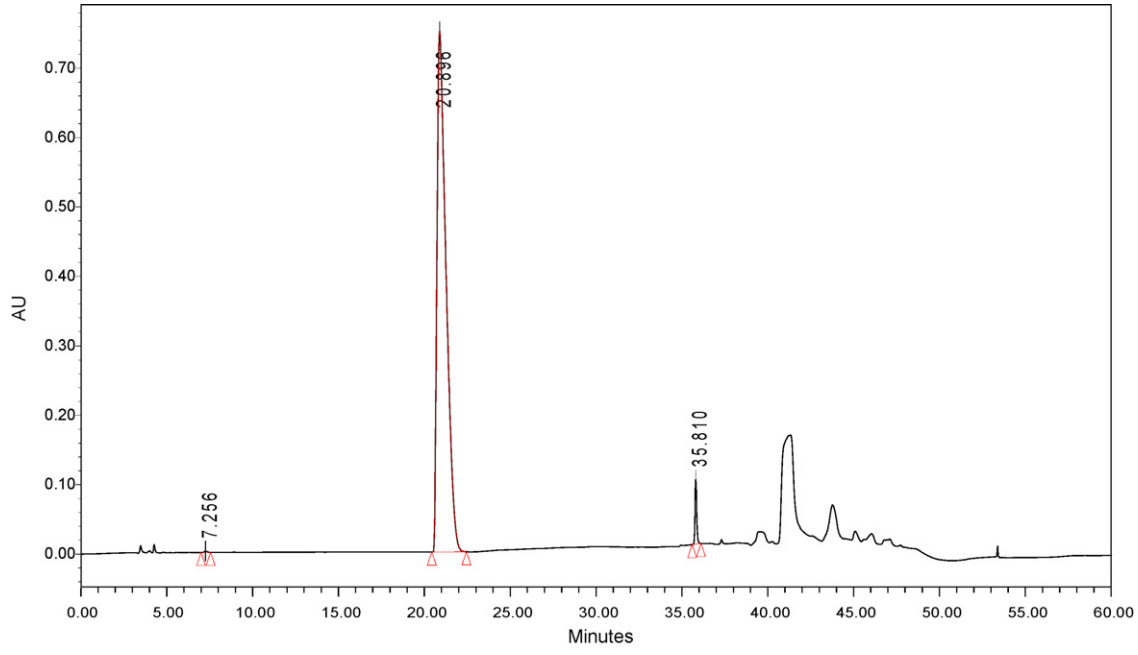

Fig. 2. Typical HPLC chromatogram of citalopram (RT 20.8) with impurity-I (RT 7.2) and II (RT 35.8).

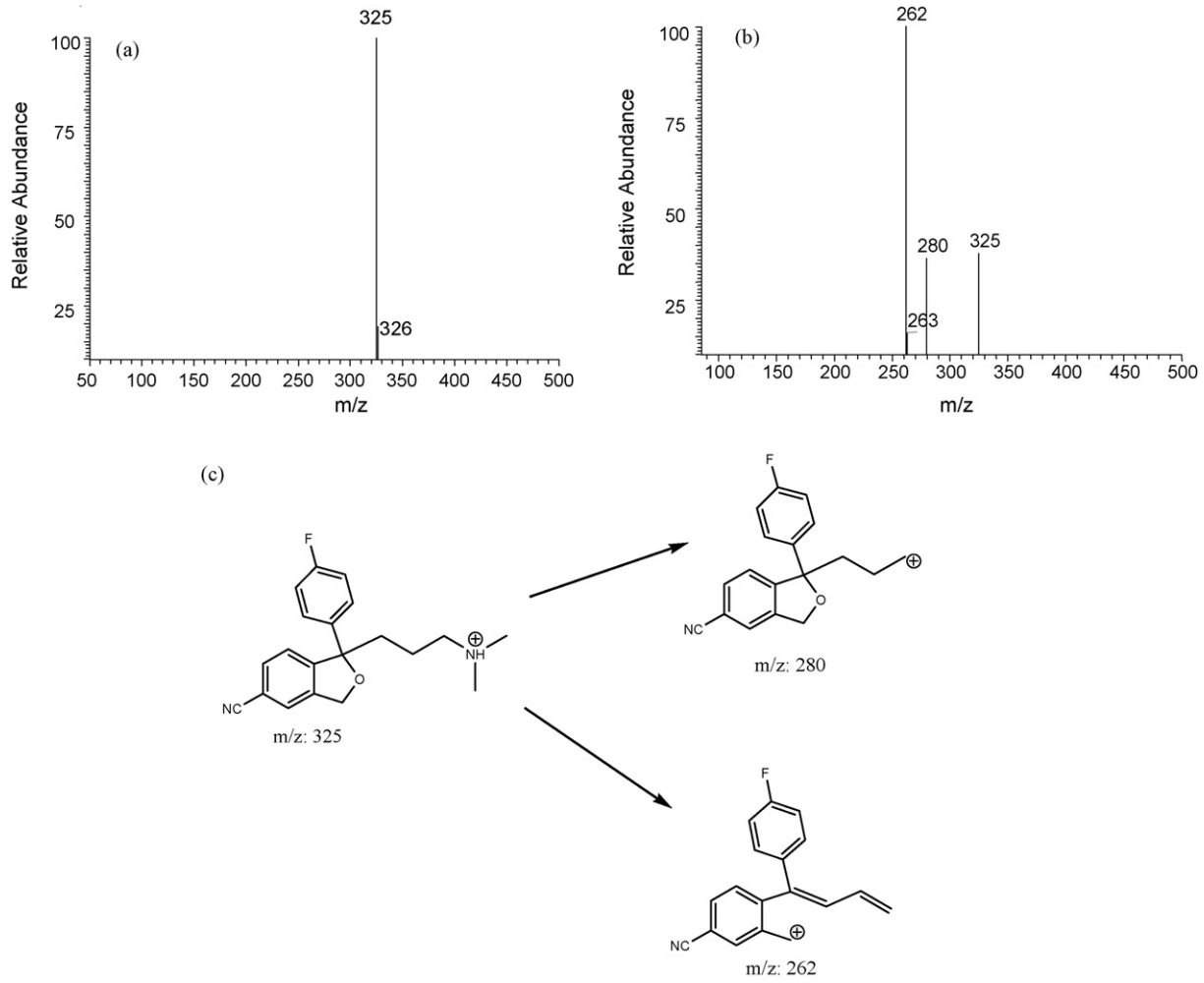

Fig. 3. MS and MS/MS data of citalopram. (a) Mass spectrum of citalopram, (b) MS/MS spectrum of product ion and (c) mechanism of formation of fragment ions with *m*/*z* 325.

The analysis revealed the presence of two impurities ranging from 0.02 to 1.0% (by area normalization). The impurities were marked as impurity-I (RT 7.2 min) and impurity-II (RT 35.8 min) respectively (Fig. 2).

To further investigate these impurities, a new LC/MS compatible method described in Section 2.2 was developed. Mass spectral data showed protonated molecular ion peaks at *m*/*z* 325, *m*/*z* 343 and *m*/*z* 422 for citalopram (Fig. 3a), impurity-I and impurity-II (Fig. 4a) respectively. The isotopic peak for carbon of citalopram, impurity-I and impurity-II appeared at *m*/*z* 326, 344 and 423, respectively. On the basis of RRT with respect to citalopram and the mass spectral data, the impurity-I having molecular ion peak at *m*/*z* 343 is identified as 1-(3-dimethylaminopropyl)-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carboxamide, i.e. citalopram related compound A [4].

The mass spectral data obtained for the impurity-II is not matching with any of the reported impurities. Based on the HPLC and LC/MS spectral data, the impurity-II which is getting eluted late is inferred to be unknown.

3.2. Structural elucidation by mass spectrometry

The accurate mass measured on Q-TOF Micromass instrument of citalopram and impurity-II were 325.3254 Da and 422.1931 Da, respectively. In order to determine the molecular formula of impurity-II, these figures of measured masses were plugged into elemental composition calculator. Setting reasonable limits i.e. carbon 0–30, hydrogen 0–30, nitrogen 0–5, oxygen 0–5, and fluorine 0–3. The search revealed several theoretically possible molecular formulae. The closest possible molecular formula for protonated molecular ion of impurity-II ($C_{26}H_{26}F_2NO_2^+$) was selected on the basis of lowest difference in mass (millidalton) between theoretical (422.1926 Da) and observed (422.1931 Da) values.

Prior to characterization work on the impurity-II, it is logical to understand LC/MS/MS data for citalopram, the parent drug molecule. The MS^2 study of citalopram has been reported previously [7]. The MS^2 spectra obtained for the citalopram molecule showed two prominent peaks at *m*/*z* 280 and 262 (Fig. 3b). The formation of product ion at *m*/*z* 280 can be attributed to the loss of dimethyl amine (45 amu). The parent ion *m*/*z* 325 also gave fragment at *m*/*z* 262 (mass difference 63 amu) which can be attributed to the loss of dimethyl amine along with a water molecule (Fig. 3c).

An odd molecular mass of impurity-II implies the existence of an odd number of nitrogen atoms. The MS^2 analysis of impurity-II showed daughter ion peak at *m*/*z* 377 (Fig. 4b) resulted from parent ion *m*/*z* 422 (Fig. 4a). This product ion peak is further subjected to MS^3 analysis, which gave daughter ion peak at *m*/*z* 349, 335, 307 and 253, respectively (Fig. 4c). The ion *m*/*z* 349 and 335 further subjected to MS^4 analysis, both these ions gave product ion peak at *m*/*z* 307 (Fig. 4d).

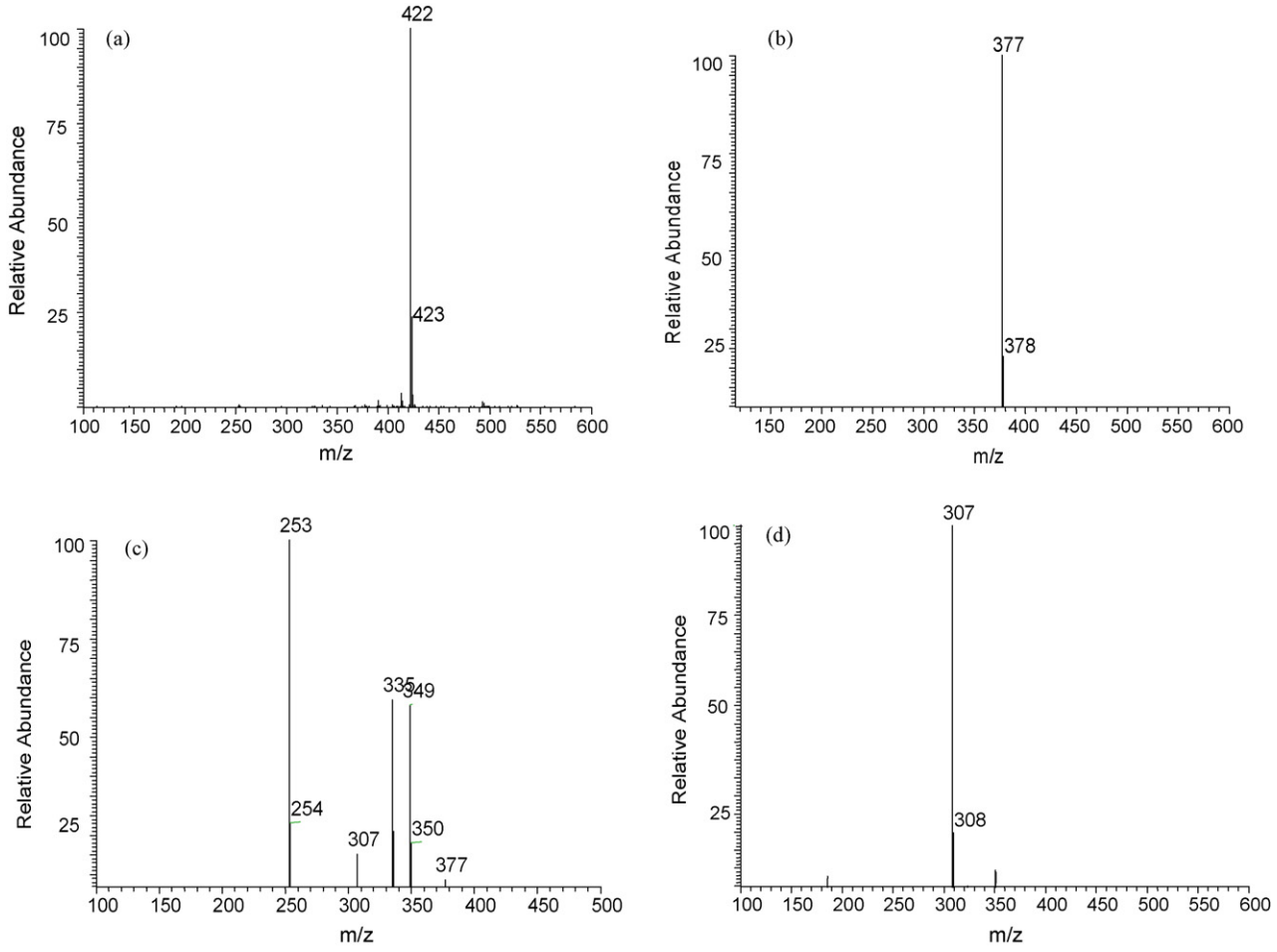

Fig. 4. MS and MS/MS data for citalopram impurity-II. (a) Mass spectrum of impurity-II, (b) MS/MS spectrum of precursor ion at *m/z* 422, (c) MS^3 spectrum of precursor ion *m/z* 377 and (d) MS^4 spectrum of precursor ion *m/z* 349.

A daughter ion at *m/z* 377 in MS^2 analysis obtained by the loss of –N $(CH_3)_2$ (45 amu). Formation of fragments at *m/z* 349, 335, 307 and 253 in MS^3 analysis can be attributed to the loss of $-C_2H_4$ (28 amu), $-C_3H_6$ (42 amu), $-C_4H_6O$ (70 amu) and $-C_7H_5FO$ (124 amu) respectively. The product ion obtained at *m/z* 307 from parent ion *m/z* 349 in MS^4 spectra is due to the loss of –CO (28 amu), while the MS^4 of *m/z* 335 peak resulted into fragment ion peak at *m/z* 307 which is attributed towards loss of $-CH_2CO$ (42 amu). Based on LC/MS/MS and LC/Q-TOF/MS analysis, the structure of impurity-II can be rationalized as [1-(1,1-bis (4-fluorophenyl)-1,3-dihydroisobenzofuran-5-yl)-4-(dimethylamino) butan-1-one hydrobromide] (Fig. 5). The proposed fragmentation mechanism is given in Fig. 6.

3.2.1. Isolation of impurity by semi-preparative HPLC

The crude samples of citalopram containing impurity-II in the range of 1.0–10.0% (area normalization) were subjected to semi-preparative isolation. The targeted unknown impurity was isolated by using conditions as described in Section 2.3. Citalopram and the impurity were eluted at about 1.5 min and 3.7 min, respectively. The fractions were collected manually between retention times 3.50 min and 4.00 min and checked by analytical HPLC mode. The fraction showing the presence of impurity above 95% were mixed together and concentrated to dryness under high vacuum. The HPLC purity of the isolated impurity was found to be above 97%. This isolated solid impurity was used for spectral characterization without any further purification.

3.2.2. Structural confirmation by NMR and IR

The NMR spectral data of citalopram and impurity-II were compared. The ^1H NMR spectrum of citalopram is showing two multiplet signals at 6.89–6.93 ppm and 7.38–7.42 ppm, which corresponds to four protons. However the impurity-II spectrum is showing two multiplet signals at 6.98–7.02 ppm and 7.24–7.30 ppm, which corresponds to eight protons (Table 1). Importantly the number of carbon signals in ^{13}C spectra of citalopram and impurity-II is same. This reveals the presence of two magnetically equivalent fluorophenyl groups in the impurity-II structure. Inference of the

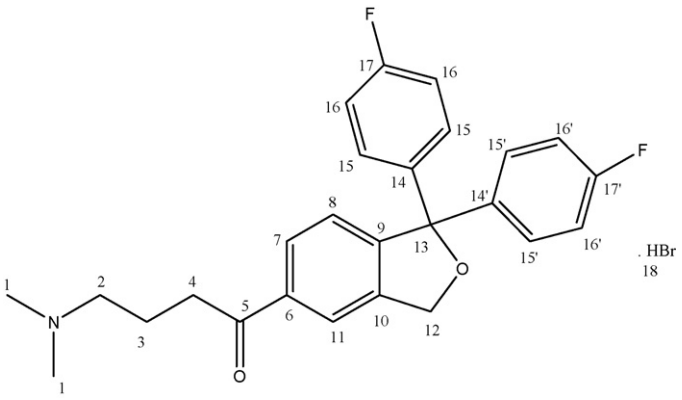

Fig. 5. Structural formula for impurity-II of citalopram.

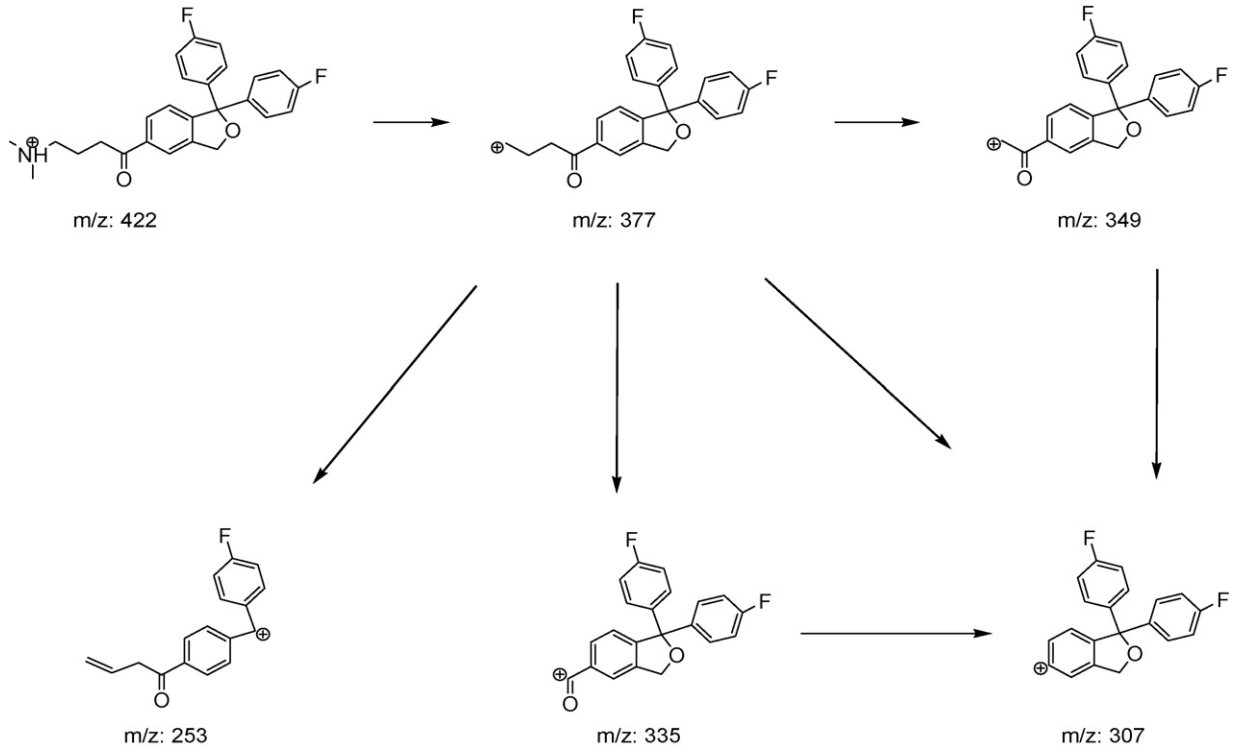

Fig. 6. Mechanism of formation of fragment ions with *m*/*z* 422.

presence of fluorophenyl group in the impurity-II structure is also supported by the accurate mass data, ($C_{26}H_{26}F_2NO_2^+$). It is also observed that the signal at 5.18 ppm in the impurity was giving singlet pattern, whereas in case of citalopram a doublet splitting pattern is obtained. This is due to symmetrical environment developed by two fluorophenyl rings in impurity-II as compared to that of citalopram. Further the signal at 118.2 ppm in ^{13}C NMR spectra of citalopram is due to –C≡N group is observed. The similar signal is found to be missing in impurity-II. Instead, a signal appeared at 197.3 ppm can be attributed to –C=O group. In FTIR (Fig. 7) the citalopram shows peak at 2229 cm^{-1} which is signal from –C≡N. However the impurity-II shows peak at 1681 cm^{-1} which confirms the presence of –C=O group.

3.2.3. Formation of impurity

During the synthesis of citalopram, an intermediate Ia formed by condensation of 5-cyanophthalide with excess of 4-fluorophenyl magnesium bromide (GR-I) at faster rate of addition using THF as a solvent. This intermediate on reaction with 3-dimethylamino propyl magnesium chloride (GR-II) and cyclization by using H_2SO_4 leads to formation of impurity-II.

3.2.4. Synthesis of impurity

For synthesis, intermediate Ia was prepared by treating twice mole GR-I, which further treated with GR-II and cyclized by H_2SO_4 to yield impurity-II. The schematic diagram is depicted in Fig. 8.

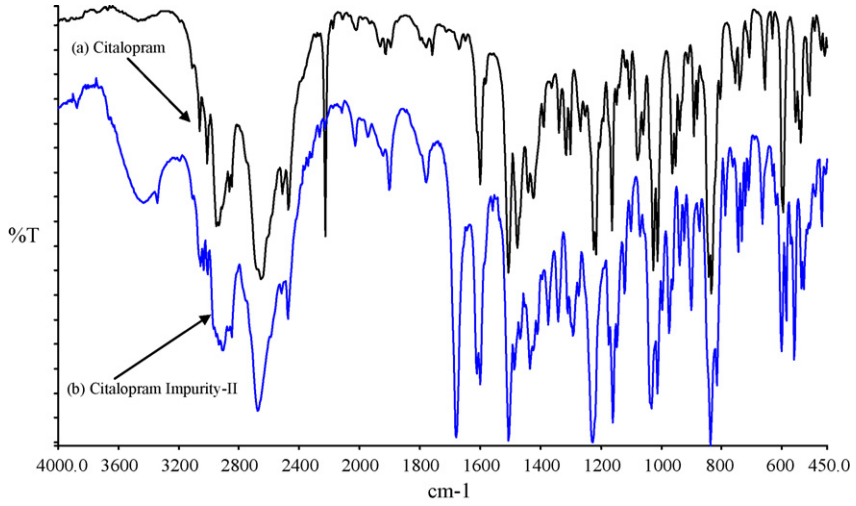

Fig. 7. Overlaid FTIR spectra of (a) citalopram and (b) citalopram impurity-II.

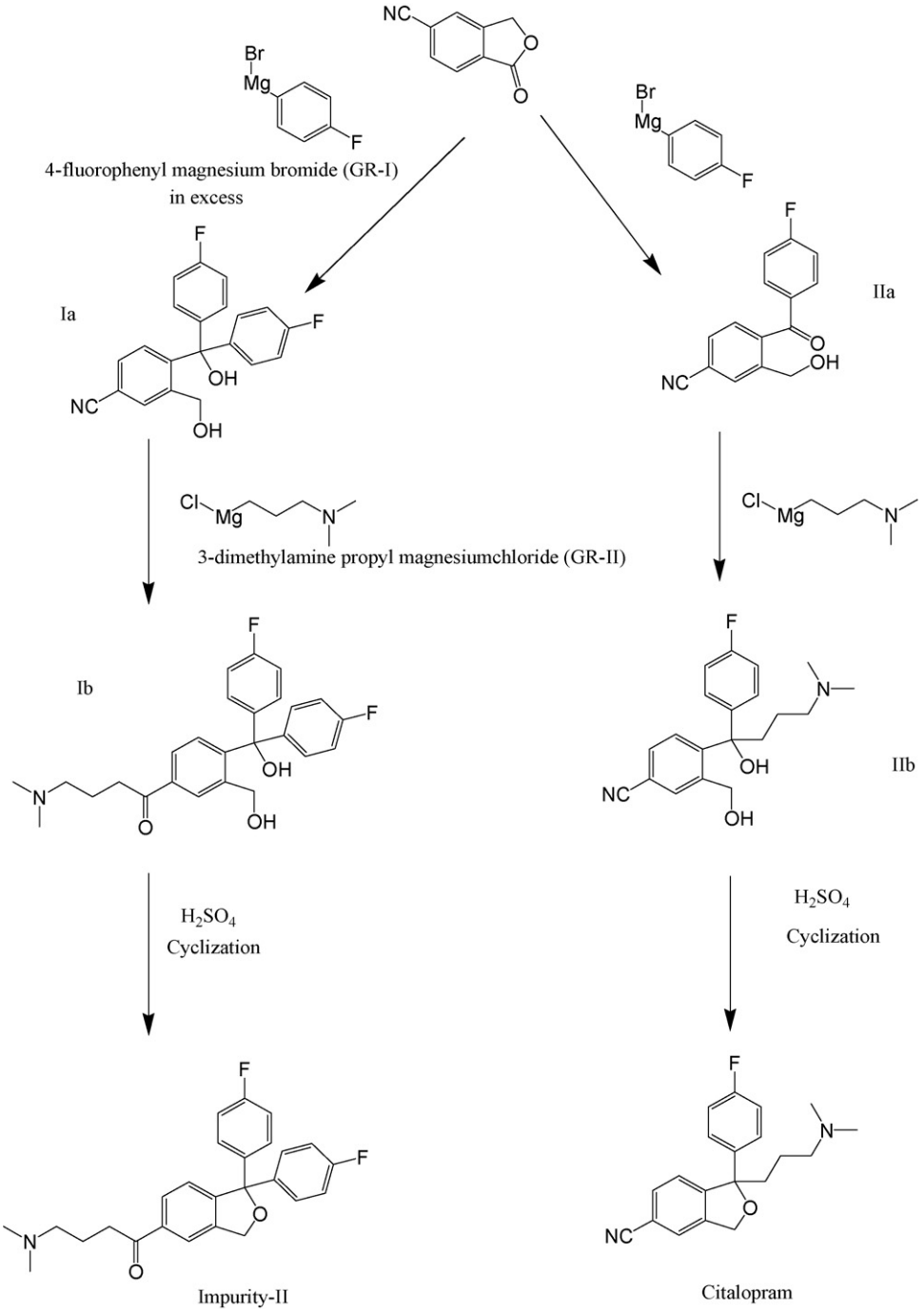

Fig. 8. Scheme for synthesis of citalopram showing the formation of impurity-II.

Table 1
Comparative NMR assignment of impurity-II and citalopram.

Impurity-II						Citalopram					
Position[a]	1H	δ (ppm)	J^c (Hz)	13C (chemical shift in ppm)	DEPT[d]	Position[b]	1H	δ (ppm)	J^c (Hz)	13C (chemical shift in ppm)	DEPT
1	6H	2.88	s	42.7	CH_3	1	6H	2.66	s	42.45	CH_3
2	2H	3.20–3.24	t, 7.61	57.1	CH_2	2	2H	3.05–3.09	t, 7.61	57.21	CH_2
3	2H	2.29–2.33	m	18.4	CH_2	3	2H	1.62–1.72	m	18.47	CH_2
4	2H	3.31–3.34	t, 6.40	35.5	CH_2	4	2H	2.25–2.36	m	37.34	CH_2
5	–	–	–	197.3	–	5	–	–	–	90.16	–
6	–	–	–	136.1	–	6	2H	5.04–5.18	–	71.04	–
7	1H	7.95–7.97	d, 7.92	127.8	CH	7	–	–	–	148.26	CH
8	1H	7.24–7.30	m	123.1	CH	8	1H	7.38–7.42	m	131.75	CH
9	–	–	–	149.1	–	9	–	–	–	111.37	–
10	–	–	–	140.2	–	10	–	–	–	118.20	–

Table 1 (*Continued*)

Impurity-II						Citalopram					
Position[a]	1H	δ (ppm)	J[c] (Hz)	13C (chemical shift in ppm)	DEPT[d]	Position[b]	1H	δ (ppm)	J[c] (Hz)	13C (chemical shift in ppm)	DEPT
11	1H	7.92	s	121.1	CH	11	1H	7.49	s	124.97	CH
12	2H	5.18	s	70.8	CH_2	12	1H	7.49	s	122.55	CH_2
13	–	–	–	91.8	–	13	–	–	–	139.50	–
14, 14′	–	–	–	139.1, 139.1	–	14	–	–	–	138.23, 138.26	–
15, 15′	4H	7.24–7.30	m	128.8, 128.9	CH	15	2H	7.38–7.42	m	126.30, 126.38	CH
16, 16′	4H	6.98–7.02	m	114.8, 115.0	CH	16	2H	6.89–6.93	m	115.04, 115.26	CH
17, 17′	–	–	–	160.8, 163.3	–	17	–	–	–	160.32, 162.84	–
18	1H	11.28	brs	–	–	18	1H	10.42	brs	–	–

[a] Refer the structural formula in Fig. 5.
[b] Refer the structural formula in Fig. 1.
[c] ^1H–^1H coupling constants.
[d] Hybridization (degree of bonding) of carbon atoms.

4. Conclusion

Isolation of an unknown impurity of citalopram has been carried out by using semi-preparative HPLC. The structural characterization of the isolated impurity was carried out by using LC/MS/MS and other modern spectroscopic (NMR and FTIR) techniques. The combined result of LC/MS/MS, NMR and FTIR confirmed the structure of unknown impurity as 1-(1,1-bis (4-fluorophenyl)-1,3-dihydroisobenzofuran-5-yl)-4-(dimethylamino) butan-1-one hydrobromide (Fig. 5).

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