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Identification, isolation, synthesis and characterization of impurities of quetiapine fumarate

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In the process for the preparation of quetiapine fumarate (**1**), six unknown impurities and one known impurity (intermediate) were identified ranging from 0.05–0.15% by reverse-phase HPLC. These impurities were isolated from crude samples using reverse-phase preparative HPLC. Based on the spectral data, the impurities were characterized as 2-[4-dibenzo[*b*,*f*][1,4]thiazepine-11-yl-1-piperazinyl]1-2-ethanol (impurity I, desethanol quetiapine), 11-[(*N*-formyl)-1-piperazinyl]-dibenzo[*b*,*f*][1,4]thiazepine (impurity II, *N*-formyl piperazinyl thiazepine), 2-(2-hydroxy ethoxy)ethyl-2-[2-[4-dibenzo[*b*,*f*][1,4]thiazepine-11-piperazinyl-1-carboxylate (impurity III, quetiapine carboxylate), 11-[4-ethyl-1-piperazinyl]dibenzo [*b*,*f*][1,4] thiazepine (impurity IV, ethylpiperazinyl thiazepine), 2-[2-(4-dibenzo[*b*,*f*][1,4]thiazepin-11-yl-1-piperazinyl)ethoxy]1-ethyl ethanol [impurity V, ethyl quetiapine), 1,4-bis[dibenzo[*b*,*f*][1,4]thiazepine-11-yl] piperazine [impurity VI, bis(dibenzo)piperazine]. The known impurity was an intermediate, 11-piperazinyl-dibenzo [*b*,*f*][1,4]thiazepine (piperazinyl thiazepine). The structures were established unambiguously by independent synthesis and co-injection in HPLC to confirm the retention times. To the best of our knowledge, these impurities have not been reported before. Structural elucidation of all impurities by spectral data (^{1}H NMR, ^{13}C NMR, MS and IR), synthesis and formation of these impurities are discussed in detail.

1. Introduction

Quetiapine fumarate (**1**) is an antipsychotic drug belonging to the chemical class of dibenzothiazepine derivatives. It is used as a hemifumarate salt. Its IUPAC name is 2-[2-(4-dibenzo[*b*,*f*][1,4]thiazepin-11-yl-1-piperazinyl)ethoxy ethanol, (E)-2-butenedioate (2 : 1) salt. A literature survey revealed various methods for the synthesis of quetiapine (Warawa et al. 2001). We synthesized the compound according to the Scheme (Warawa and Migler 1989) with modifications to make it simpler and commercially viable. Its molecular formula is $(C_{21}H_{25}N_3O_2S)_2 \cdot C_4H_4O_4$ and molecular weight is 883 amu as fumarate salt and 383 amu as base.

HPLC methods (Saracino et al. 2006; Mandrioli et al. 2002) and a HPLC-electrospray ionization mass spectrometric method (Zhou et al. 2004) were reported for the determination of quetiapine in human plasma.

During the preparation of **1** in the laboratory, six unknown impurities were detected in HPLC along with one known impurity. A comprehensive study was undertaken to isolate, synthesize and characterize these impurities by spectroscopic techniques. An impurity profile study is necessary for any final product to identify and characterize all the unknown impurities that are present in level of >0.1%. (ICH guideline, 2005). The present study describes the isolation, synthesis and characterization of related impurities of **1**.

2. Investigations, results and discussion

2.1. Detection and identification

Quetiapine fumarate was analysed by HPLC under the analytical conditions described below. The chromatogram displayed seven peaks at relative retention times compared to quetiapine at 0.80, 0.94, 1.08, 1.17, 1.65, 1.67 and 2.28. The LC-MS analysis showed six peaks having m/z values 339, 323, 427, 323, 411 and 504. They were isolated from crude samples of quetiapine by preparative HPLC. All the compounds were co-injected with quetiapine fumarate sample in HPLC to confirm the retention times. HPLC chromatogram of quetiapine fumarate spiked with all impurities is shown in the Fig. Synthesis and structural elucidation of these impurities are discussed in the following sections.

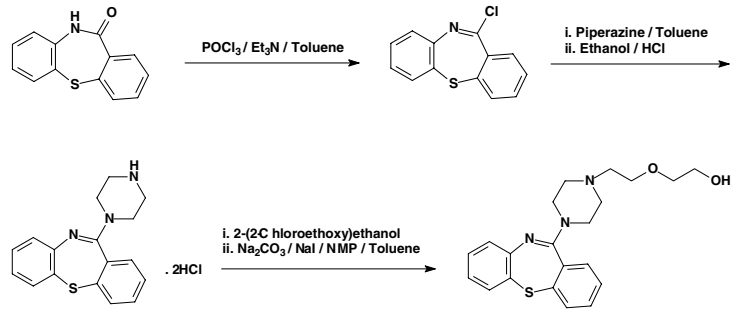
Quetiapine fumarate was synthesized as shown in the Scheme, impurities I–VI were synthesized as described in the Experimental section and characterized.

2.2. Characterization and origin of impurities

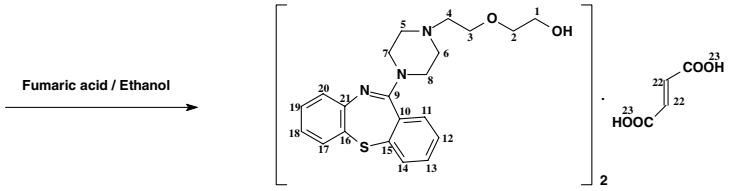
2.2.1. Impurity I

The ESI mass spectrum of impurity I displayed the protonated molecular ion at m/z 340. Therefore the molecular weight of this impurity was considered as 339 which was less by 44 amu than quetiapine. The odd molecular weight

Scheme



Piperazinyl thiazepine (2)



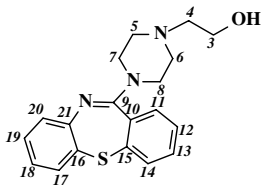
Quetiapine fumarate (1)

indicated the presence of odd number of nitrogens which in turn indicated the intactness of dibenzo[*b*,*f*][1,4]thiazepine piperazinyl ring in this impurity structure. Presence of broad signals at 3.70 ppm (4 H) and 4.34 ppm (4 H) in ^{1}H NMR spectrum was attributed to piperazinyl ring. Two triplets were observed in impurity spectrum at 3.39 ppm and 3.89 ppm corresponding to $2 \times CH_2$ signals instead of $4 \times CH_2$ groups present in the side chain of quetiapine. In ^{13}C NMR two CH_2 signals were observed at 61.1 ppm and 73.1 ppm instead of four CH_2 signals. Based on the above spectral data observations, the structure of impurity I was characterized as 2-[4-dibenzo[*b*,*f*][1,4]thiazepine-11-yl-1-piperazinyl] l-2-ethanol (desethanol quetiapine).

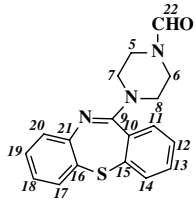
2-[2-Chloroethoxy]ethanol is a raw material for the preparation of quetiapine. 2-Chloroethanol may be present as an impurity in this raw material. During the alkylation step in the preparation of quetiapine, alkylation of piperazinyl thiazepine with the impurity, 2-chloro ethanol leads to the formation of impurity I (desethanol quetiapine).

2.2.2. Impurity II

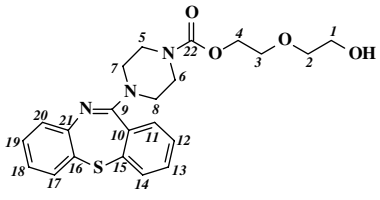
ESI mass spectrum of impurity II exhibited a protonated molecular ion peak at m/z 324, indicating the molecular weight as 323. The molecular weight of impurity II was 28 amu more than that of the intermediate, 4-piperazinyl dibenzo[*b*,*f*][1,4]thiazepine (**2**), m/z 295. MS fragmentation peaks were observed at m/z 296 and 251. ^{1}H NMR spectrum of this impurity showed signals similar to those of **2** and in addition one new signal was observed at 8.13 ppm integrated to one proton which was not exchangeable. This data suggested the attachment of a −CHO group on piperazine NH. Based on the spectral data, the structure of impurity II was characterized as 11-[(*N*-formyl)-1-piperazinyl]-dibenzo[*b*,*f*][1,4]thiazepine (*N*-formyl piperazinyl



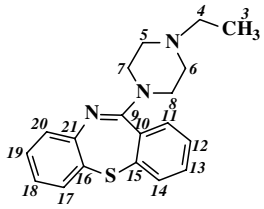
Impurity I (Desethanol quetiapine)



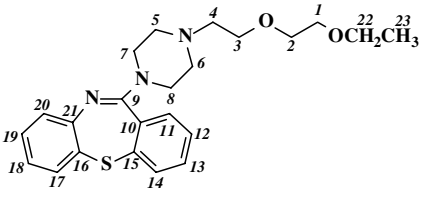
Impurity II (N-Formyl piperazinyl thiazepine)



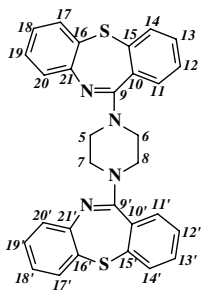
Impurity III (Quetiapine carboxylate)



Impurity IV (N-Ethylpiperazinyl thiazepine)



Impurity V (Ethyl quetiapine)



Impurity VI (Bis (dibenzo) piperazine)

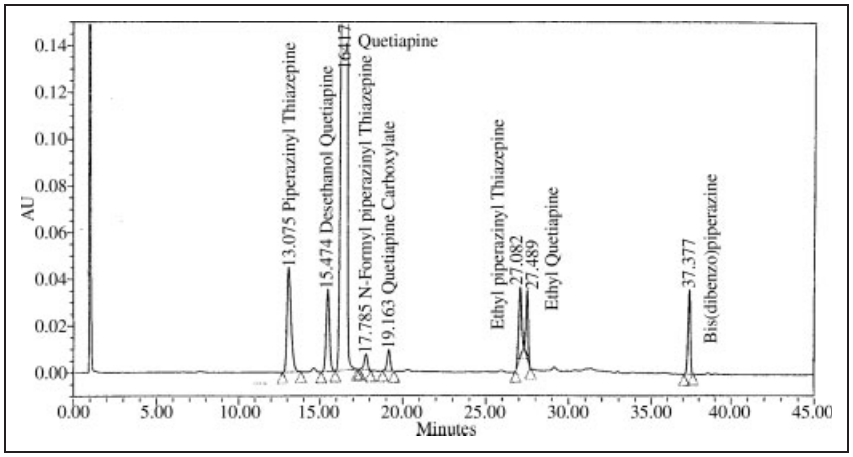


Fig.:
LC-Chromatogram of Quetiapine fumarate sample spiked with impurities

thiazepine). This impurity arises when the alkylation of piperazinyl thiazepine with 2-chloroethoxyethanol is carried out in *N,N*-dimethyl formamide.

2.2.3. *Impurity III*

ESI mass spectrum of impurity III displayed a protonated molecular ion peak at m/z 428, indicating the molecular weight of the compound as 427 which is 44 amu more than quetiapine. MS fragmentation peaks were observed at m/z, 384, 340 and 324. After thorough study of ^{1}H NMR and ^{13}C NMR spectra of this impurity, it was found to be as carboxy group attachment. It was observed from the ^{1}H NMR spectrum that there was no change in the number of protons compared to quetiapine and the signal corresponding to methylene protons attached to piperazine ring has shifted downfield from 3.50 ppm to 4.14 ppm. An additional signal was observed at 161.5 ppm in the ^{13}C NMR spectrum and it was confirmed as quaternary carbon from DEPT experiment. From the above spectral data, the structure of impurity III was characterized as 2-(2-hydroxy ethoxy)ethyl-2-[2-[4-dibenzo[*b*,*f*][1,4]thiazepine-11-piperazinyl-1-carboxylate (quetiapine carboxylate). This impurity arises when the alkylation of piperazinyl thiazepine is carried out in the presence of sodium carbonate (quetiapine carboxylate).

2.2.4. *Impurity IV*

ESI mass spectrum of this impurity exhibited a protonated molecular ion peak at m/z 324 indicated the molecular weight of this impurity as 323. Molecular weight of this impurity is 28 amu more than that of 11-piperazinyl thiazepine (intermediate). This odd molecular weight indicated the presence of odd number of nitrogen atoms which in turn indicated the intactness of piperazinyl thiazepine ring. MS fragmentation peaks were observed at 296 and 253 amu. The signals corresponding to $-O-CH_2-CH_2-OH$ group of quetiapine molecule were absent in impurity IV. Additionally, ^{1}H NMR spectrum of this impurity showed a

Table 1: Comparative ^{1}H NMR assignments for quetiapine fumarate and its impurities

Position[a]	Quetiapine fumarate δ (ppm), multiplicity	Impurity-I δ (ppm), multiplicity	Impurity-II δ (ppm), multiplicity	Impurity-III δ (ppm), multiplicity	Impurity-IV δ (ppm), multiplicity	Impurity-V δ (ppm), multiplicity	Impurity-VI δ (ppm), multiplicity
1	3.43 (t, 2H)	–	–	3.45 (t, 2H)	–	3.44 (m, 2H)	–
2	3.49 (t, 2H)	–	–	3.47 (t, 2H)	–	3.52 (m, 2H)	–
3	3.55 (t, 2H)	3.82 (t, 2H)	–	3.61 (t, 2H)	1.29 (t, 3H)	3.56 (t, 2H)	–
4	2.52 (t, 2H)	3.27 (t, 2H)	–	4.14 (t, 2H)	3.18 (t, 2H)	3.40 (t, 2H)	–
5	2.50–2.54						
6	(brm, 4H) and	3.48 and 3.76	3.42–3.73	3.42 and 3.48	3.20–3.75	3.20–3.75	3.03.65
7	3.43–3.48	(2brs, 8H)	(m, 8H)	(2brs, 8H)	(brm, 8H)	(brm, 8H)	(brm, 8H)
8	(brm, 4H)						
9	–	–	–	–	–	–	–
10	–	–	–	–	–	–	–
11	7.55 (m, 1H)	7.68 (m, 1H)	7.55 (d, 1H)	7.55 (d, 1H)	7.68 (m, 1H)	7.69 (m, 1H)	7.56 (m, 2H)
12	7.40 (m, 2H)	7.60 (m, 2H)	7.34–7.36	7.44 (m, 2H)	7.52–7.60	7.52–7.60	7.44–7.48
13			(m, 2H)		(m, 2H)	(m, 2H)	(m, 4H)
14	7.45 (m, 1H)	7.62 (m, 1H)	7.42 (d, 1H)	7.47 (m, 1H)	7.62 (m, 1H)	7.62 (m, 1H)	7.46 (m, 2H)
15	–	–	–	–	–	–	–
16	–	–	–	–	–	–	–
17	7.37 (dd, 1H)	7.54 (dd, 1H)	7.40 (dd, 1H)	7.38 (dd, 1H)	7.54 (dd, 1H)	7.54 (dd, 1H)	7.38 (dd, 2H)
18	7.18 (ddd,1H)	7.35 (brm, 1H)	7.23 (ddd, 1H)	7.18 (ddd, 1H)	7.35 (brm, 1H)	7.35 (brm, 1H)	7.20 (ddd, 2H)
19	6.88 (ddd, 1H)	7.15 (ddd, 1H)	6.95 (ddd, 1H)	6.91 (ddd, 1H)	7.16 (brd, 1H)	7.16 (brd, 1H)	6.90 (ddd, 2H)
20	6.99 (dd, 1H)	7.35 (brm, 1H)	7.10 (dd, 1H)	7.01 (dd, 1H)	7.35 (brm, 1H)	7.35 (brm, 1H)	7.01 (dd, 2H)
21	–	–	–	–	–	–	–
22	6.62 (s, 2H)	–	8.13 (s, 1H)	–	–	3.89 (brm, 2H)	–
23	–	–	–	–	–	1.08 (t, 3H)	–

s, singlet; d, doublet; dd, doublet of a doublet; ddd, doublet of a double doublet; m, multiplet; brs, broad singlet; brm, broad multiplet; q, quartet; t, triplet
[a] Refor Scheme for numbering of quetiapine fumarate

Table 2: Comparative ^{13}C NMR and DEPT assignments for quetiapine fumarate and its impurities

Position[a]	Quetiapine fumarate		Impurity-I		Impurity-II		Impurity-III		Impurity-IV		Impurity-V		Impurity-VI	
	^{13}C δ (ppm)	DEPT	^{13}C δ (ppm)	DEPT	^{13}C δ (ppm)	DEPT	^{13}C δ (ppm)	DEPT	^{13}C δ (ppm)	DEPT	^{13}C δ (ppm)	DEPT	^{13}C δ (ppm)	DEPT
1	61.1	CH_2	–	–	–	–	61.1	CH_2	–	–	66.4	CH_2	–	–
2	73.1	CH_2	–	–	–	–	73.1	CH_2	–	–	70.4	CH_2	–	–
3	68.7	CH_2	58.6	CH_2	–	–	68.8	CH_2	9.6	CH_3	69.7	CH_2	–	–
4	57.8	CH_2	56.1	CH_2	–	–	66.0	CH_2	51.3	CH_2	55.6	CH_2	–	–
5														
6	46.7 and	$4 \times CH_2$	46.0, 51.3	$4 \times CH_2$	46.3, 51.0	$4 \times CH_2$	44.0	$4 \times CH_2$	46.4, 50.0	$4 \times CH_2$	45.7 51.4	$4 \times CH_2$	45.5, 47.6	$4 \times CH_2$
7	53.4		and 51.6		and 51.6				and 50.3		and 51.8		and 50.5	
8														
9	160.9	–	162.1	–	162.0	–	161.0	–	162.3	–	161.8	–	163.1 and 163.2	–
10	139.6	–	136.5	–	140.0	–	139.6	–	140.6	–	140.3	–	137.8 and 137.9	–
11	132.8	CH	134.0	CH	134.0	CH	132.8	CH	134.1	CH	133.5	CH	133.6 and 134.0	$2 \times CH$
12	129.8	CH	130.2	CH	130.3	CH	129.9	CH	130.3	CH	130.6	CH	130.2 and 130.4	$4 \times CH$
13	129.9	CH	130.7	CH	130.7	CH	130.0	CH	130.8	CH	131.3	CH	131.0	CH
14	132.1	CH	133.3	CH	133.3	CH	132.3	CH	133.4	CH	133.3	CH	132.8	CH
15	128.1	–	128.0	–	–	–	128.1	–	127.7	–	127.5	–	129.0	–
16	134.3	–	134.2	–	–	–	134.3	–	135.8	–	131.7	–	135.4	–
17	132.9	CH	135.1	CH	134.0	CH	133.9	CH	135.3	CH	133.9	CH	134.7 and 134.9	$2 \times CH$
18	130.1	CH	131.9	CH	132.1	CH	130.2	CH	132.0	CH	131.7	CH	132.2	$2 \times CH$
19	123.5	CH	126.1	CH	126.4	CH	123.8	CH	126.2	CH	126.6	CH	126.9 and 127.1	$2 \times CH$
20	126.0	CH	129.5	CH	130.3	CH	125.8	CH	129.7	CH	130.4	CH	128.9 and 129.2	$2 \times CH$
21	149.4	–	142.0	–	143.0	–	149.4	–	140.6	–	143.5	–	140.8	–
22	135.2	–	–	–	162.3	CH	156.0	–	–	–	65.5	CH_2	–	–
23	167.4	–	–	–	–	–	–	–	–	–	9.6	CH_3	–	–

[a] Refer Scheme for numbering of quetiapine fumarate

Table 3: FT-IR spectral data for quetiapine fumarate and its impurities

S. No.	Compound	IR (KBr) absorption bands, (cm^{-1})
1	Quetiapine fumarate	3320 (m) OH stretch, 3074, 3014 (w) aryl CH stretch, 2946, 2929, 2898, 2870 (m) aliphatic CH stretch, 1600, 1573 (s) aryl C=C stretch and C=N stretch, 1414 (s) CH_2 bend, 795, 768 (m) aryl CH out-of-plane bend.
2	Impurity-I	3060, 3004 (w) aryl CH stretch, 2951, 2884 (m) aliphatic CH stretch, 1622, 1572 (s) aryl C=C stretch and C=N stretch, 1443 (s) CH_2 bend, 779, 767 (m) aryl CH out-of-plane bend.
3	Impurity-II	3070 (w) aryl CH stretch, 2950, 2890 (m) aliphatic CH stretch, 1620, 1570 (s) aryl C=C stretch and C=N stretch, 1440 (s) CH_2 bend, 770, 765 (m) aryl CH out-of-plane bend.
4	Impurity-III	3065 (w) aryl CH stretch, 2975, 2886 (m) aliphatic CH stretch, 1620, 1572 (s) aryl C=C stretch and C=N stretch, 1446 (s) CH_2 bend, 780, 755 (m) aryl CH out-of-plane bend.
5	Impurity-IV	3063 (w) aryl CH stretch, 2979, 2895 (m) aliphatic CH stretch, 1627, 1573 (s) aryl C=C stretch and C=N stretch, 1430 (s) CH_2 bend, 782, 767 (m) aryl CH out-of-plane bend.
6	Impurity-V	3070 (w) aryl CH stretch, 2923, 2855 (m) aliphatic CH stretch, .1621, 1574 (s) aryl C=C stretch and C=N stretch, 1455 (s) CH_2 bend, 772, 751 (m) aryl CH out-of-plane bend.
7	Impurity-VI	3052 (m) aryl CH stretch, 2989, 2978, 2953, 2844 (w) aliphatic CH stretch, 1602, 1575 (s) aryl C=C stretch and C=N stretch, 1397 (s) CH_2 bend, 1245 (s) C-N strech, 1005, 759, 738 (s) aryl CH out-of-plane bend.

w – weak, s – strong, m – medium

$-CH_3$ triplet at 1.29 ppm and $-CH_2$ quartet at 3.18 ppm corresponding to ethyl group. This was supported by the presence of methyl and methylene signals at 9.0 ppm and 53.0 ppm in ^{13}C NMR spectrum. Based on the above spectral data, the structure of impurity IV was characterized as 11-[4-ethyl-1-piperazinyl]dibenzo[*b*,*f*][1,4]thiazepine (*N*-ethyl-11-piperazinyl thiazepine). Ethanolic hydrochloride was used to isolate piperazinyl thiazepine as dihydrochloride salt. Ethanolic hydrochloride may contain ethyl chloride which may alkylate piperazinyl thiazepine to yield this impurity (*N*-ethyl piperazinyl thiazepine).

2.2.5. Impurity V

ESI mass spectrum of this impurity displayed a protonated molecular ion peak at m/z 412 indicating the molecular weight of this impurity as 411 which was 28 amu higher than that of quetiapine. Molecular weight suggested that the impurity was formed due to the substitution of ethyl group on quetiapine. MS fragmentation peak was observed at 324 amu. In ^1H NMR spectrum of this impurity all the signals corresponding to quetiapine structure were present and in addition methyl signal was observed as triplet at 1.08 ppm and methylene signal as quartet at 3.53 ppm. In ^{13}C NMR spectrum the corresponding signals were observed at 9.6 ppm and 65.5 ppm. Based on the above spectral data, the structure of impurity V was determined as 2-[2-(4-dibenzo[*b*,*f*][1,4]thiazepin-11-yl-1-piperazinyl) ethoxy]1-ethyl ethanol (ethyl quetiapine). 2-[2-Chloroethoxy]ethoxy ethane may be an impurity present in the raw material, 2-[2-chloroethoxy] ethanol. This impurity arises during alkylation of piperazinyl thiazepine with 2-[2-chloroethoxy] ethoxy ethane (ethyl quetiapine).

2.2.6. Impurity VI

ESI mass spectrum impurity VI exhibited a protonated molecular ion peak at m/z 505, indicating the molecular weight of impurity as 504. The even molecular weight suggested that the even number of nitrogens are present in this impurity. The molecular weight of this impurity was 209 amu higher than that of piperazinyl thiazepine (quetiapine intermediate, m/z, 295). The difference molecular weight 209 amu corresponding to the dibenzo[*b*,*f*][1,4] thiazepine group. These mass values suggested that another dibenzo[*b*,*f*][1,4]thiazepine group was attached to piperazinyl NH of the quetiapine intermediate **2**. Sixteen aryl protons were present in the ^1H NMR spectrum, corresponding to two dibenzothiazepine groups and eight protons were observed at 3.4 ppm, corresponding to one piperazine group. In ^{13}C NMR spectrum, the number of carbon signals in aryl region were doubled, supported the presence of two dibenzo thiazepine groups. Based on the above spectral data, the structure of impurity VI was characterized as 1,4-bis[dibenzo [*b*,*f*][1,4]thiazepine-11-yl]piperazine (bis(dibenzo) piperazine). During the preparation of piperazinyl thiazepine intermediate from 11-chloro-dibenzo[b,f][1,4]thiazepine and piperazine, alkylation of both the nitrogen atoms of piperazine leads to the formation of bis(dibenzo) thiazepine.

2.2.7. Spectroscopic data

The ^1H and ^{13}C NMR chemical shift values of quetiapine fumarate and all impurities are presented in Tables 1 and 2 FT-IR spectral data are given in Table 3.

3. Experimental

3.1. Synthesis of the impurities

The investigated samples of quetiapine bulk drug and crude samples were synthesized in APL Research Centre (a unit of Aurobindo Pharma Ltd., Hyderabad, India). All impurities were isolated from crude samples by preparative HPLC. All the reagents used for analysis were procured from Merck (India) limited.

3.1.1. Synthesis of impurity I, desethanol quetiapine hydrochloride

11-Piperazinylthiazepine dihydrochloride (5 g, 0.0136 mol) reacted with 2-chloro ethanol (1.2 g, 0.0149 mol) in the presence of sodium carbonate (8.7 g, 0.0821 mol), 1-methyl-2-pyrrolidine (8 ml, 0.0771 mol) and a catalytic amount of sodium iodide (0.05 g, 0.0003 mol) in toluene (40 ml) at 95–100 °C for about 24 h and the reaction was monitored by TLC. Upon completion of reaction the reaction mass was washed with water (2×25 ml). The organic layer was concentrated completely under reduced pressure at 50–55 °C. The residue was dissolved in ethanol (40 ml) and pH of the reaction mass was adjusted to ~2.0 with ethanolic HCl (10 ml, ~20% w/w). The precipitated product was filtered, washed with isopropylether (15 ml) and dried at 55–60 °C to yield 4.7 g of desethanol quetiapine.

3.1.2. Synthesis of impurity II, N-formyl piperazinyl thiazepine

11-Piperazinyl thiazepine dihydrochloride (10 g, 0.027 mol), sodium carbonate (17.25 g, 0.163 mol), sodium iodide (0.1 g, 0.0006 mol) and 2-chloro-

ethoxy ethanol (3.8 g, 0.03 mol) were added to *N,N*-dimethylformamide (25 ml) at room temperature. The reaction mass was heated to ~100 °C and stirred for 8 h while monitoring the process by HPLC. There after, the reaction mass was cooled to room temperature and poured into water (200 ml). The product was extracted with ethyl acetate (2 × 75 ml) and the organic extract was washed with water (2 × 80 ml). The organic layer was concentrated completely under the reduced pressure at 50–55 °C. The residue contains ~5% of this impurity which was isolated by preparative HPLC.

3.1.3. Impurity III, quetiapine carboxylate

Impurity III was isolated from mother liquors obtained during the preparation of compound **1**.

3.1.4. Synthesis of impurity IV, N-ethyl-11-piperazinyl thiazepine

11-Piperazinyl thiazepine dihydrochloride (5 g, 0.0136 mol) reacted with ethyl bromide (2.4 g, 0.020 mol) in the presence of sodium carbonate (8.7 g, 0.0821 mol) and dimethylformamide (15 ml) at room temperature for 2 h and the reaction was monitored by TLC. The reaction mass was poured into water (200 ml) and extracted with ethylacetate (2 × 80 ml). The organic layer was washed with water (2 × 50 ml) and concentrated completely under reduced pressure at 50–55 °C. The resulting residue was dissolved in ethanol (40 ml) and treated with ethanolic HCl (10 ml, 20% w/w) at pH 2.0. Isopropylether (20 ml) was added dropwise to isolate the product. The product was stirred at room temperature for 1 h. The product was filtered, washed with isopropylether (5.0 ml) and dried at 55–60 °C to yield 4.2 g of title compound.

3.1.5. Impurity V, synthesis of ethyl quetiapine

Ethyl bromide (5.3 g, 0.0486 mol) was added dropwise to a mixture of quetiapine fumarate (10 g) and sodium hydroxide (2.9 g, 0.073 mol) in dimethylformamide (50 ml) at 15–17 °C. The reaction mass was stirred at 15–20 °C for 8 h and the reaction mass was monitored by HPLC until completion. Water (250 ml) was added to the reaction mass and extracted with methylene chloride (2 × 100 ml). The organic layer was washed with water (2 × 50 ml) and concentrated under reduced pressure. The residue was dissolved in ethanol (50 ml) and ethanolic HCl (20 ml, ~20% w/w) was added dropwise. The precipitated product was stirred for 1 h. The product was filtered, washed with ethanol (5 ml) and dried at 40–45 °C to yield 6 g of product containing ~91% of the desired product by HPLC.

3.1.6. Impurity VI, synthesis of bis(dibenzo)thiazepine

11-Chloro-dibenzo[*b*,*f*][1,4]thiazepine (20.5 g, 0.0835 mol) was added in small portions to a stirred mixture of piperazine (14.4 g, 0.167 mol) in toluene (160 ml) at ~50 °C. The reaction was heated to ~100 °C, stirred for 4 h and the reaction was monitored by HPLC until disappearance of starting material. The reaction mass was cooled to ~20 °C and filtered the salts. The toluene filtrate was washed with water (4 × 100 ml). The organic layer was concentrated completely under reduced pressure at 50–55 °C. The residue contains ~12% of bis(dibenzo)thiazepine impurity which was isolated by preparative HPLC.

3.2. High performance liquid chromatography

A Waters Alliance 2695 separation module equipped with 2996 photodiode array detector with Empower pro data handling system [Waters corporation, MILFORD, MA 01757, USA] was used. The analysis was carried out on YMC Pack-C8, 150 mm long, 4.6 mm i.d., 5 µm particle diameter column. Mobile phase A was a mixture of phosphate buffer and acetonitrile in the ratio of 90 : 10 v/v, adjusted to pH 6.7 ± 0.05 with dilute orthophosphoric acid solution (phosphate buffer was prepared by dissolving 0.77 g of disodium hydrogen orthophosphate (anhydrous) and 0.57 g of potassium dihydrogen orthophosphate in 1000 ml of water). Mobile phase B was acetonitrile. UV detection was carried out at 225 nm and flow rate was kept at 1.5 ml/min. Column oven temperature was set at 45 °C and data acquired for 45 min. Pump mode was gradient and the program was as follows, Time (min)/A(v/v) : B(v/v); $T_{0.01}$/80 : 20, $T_{15.0}$/70 : 30, $T_{25.0}$/60 : 40, $T_{30.0}$/35 : 65, $T_{35.0}$/30 : 70, $T_{45.0}$/25 : 75, $T_{50.0}$/80 : 20, $T_{60.0}$/80 : 20.

3.3. Preparative liquid chromatography

A Shimadzu LC-8A preparative liquid chromatograph equipped with SPD-10A VP, UV-Vis detector [Shimadzu corporation, Analytical Instruments Division, Kyoto, Japan] was used. Hyperprep HS C18 (250 mm long × 21.2 mm i.d.) preparative column packed with 10 µm particle size was employed for isolation of impurities. The mobile phase consisted of (A) 0.1 M ammonium acetate solution and (B) acetonitrile. Flow rate was set at 20 ml/min and UV detection was carried out at 225 nm. The gradient program was as follows, time(min)/A(v/v) : B(v/v); $T_{0.01}$/98 : 2, $T_{20.0}$/90 : 10, $T_{35.0}$/80 : 20, $T_{50.0}$/70 : 30, $T_{60.0}$/60 : 40, $T_{75.0}$/50 : 50, $T_{90.0}$/25 : 75.

3.4. LC-MS/MS analysis

LC-MS/MS analysis was carried out using a Perkin Elmer triple quadrupole mass spectrometer (API 2000, PE SCIEX) coupled with a Shimadzu HPLC equipped with SPD 10 AT VP UV-VIS detector and LC 10 AT VP pumps. Analyst software was used for data acquisition and data processing. The turbo ion spray voltage was maintained at 5.5 kv and temperature was set at 375 °C. The auxillary gas and curtain gas used was high pure nitrogen. Zero air was used as nebulizer gas. LC-MS spectra were acquired from m/z 100–1000 in 0.1 amu steps with 2.0 s dwell time. The analysis was carried out using Hypersil BDS C18, 150 × 4.6 mm column with 5 µm particle dia. Mobile phase consisted of (A) 0.01 M ammonium acetate and (B) 1 : 1 mixture of acetonitrile and methanol. UV detection was carried out at 225 nm and flow rate was kept at 1.5 ml/min. Data acquisition time was 50 min. The gradient program was as follows, Time (min)/ A(v/v) : B(v/v); $T_{0.01}$/75 : 25, $T_{5.0}$/75 : 25, $T_{35.0}$/50 : 50, $T_{40.0}$/15 : 85, $T_{50.0}$/15 : 85.

3.5. NMR Spectroscopy

The ^{1}H NMR, ^{13}C NMR (proton decoupled) and DEPT spectra were recorded on Bruker 300 MHz spectrometer using DMSO-d_6 as solvent and tetramethylsilane (TMS) as internal standard.

3.6. Mass spectrometry

Mass spectra were recorded on a Perkin Elmer PE SCIEX-API 2000 mass spectrometer equipped with a Turboionspray interface at 375 °C. Detection of ions was performed in electrospray ionisation, positive ion mode.

3.7. FT-IR Spectroscopy

FT-IR spectra were recorded as KBr pellet on a Perkin-Elmer instrument model – spectrum one.

3.8. Isolation of impurities by preparative HPLC

All impurities were isolated by preparative HPLC from crude samples by using the conditions described above. Fractions collected were analyzed by analytical HPLC as per the conditions mentioned above. Fractions of >90% were pooled together, concentrated on Rotavapor to remove acetonitrile. The concentrated fractions were passed through the preparative column using water:acetonitrile (50 : 50) as mobile phase to remove the buffers used for isolation. Again the eluate was concentrated in a Rotavapor to remove acetonitrile. The aqueous solutions were lyophilized using freeze dryer (Virtis advantage 2XL).

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