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Research Article

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Identification, synthesis and characterization of principal process related potential impurities in Diazepam

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ABSTRACT

Six process related impurities of Diazepam were identified, synthesized and characterized. Their structures were verified by synthesis, comparison of the spectra and chromatographic (HPLC) retention data of the synthesized materials.

Keywords: Characterization, benzodiazepine, anxiolytic, anticonvulsant, hypnotic, sedative, skeletal muscle relaxant.

INTRODUCTION

In the present era, there is a tremendous upsurge for the impurity profiling for the pharmaceutical[1-4] and agrochemical[5] products. As per regulatory guidelines for any products it is important to fix the limit for any specified and unspecified impurity in the product with their characterization, identification and quantification by analytical method. If we know the structure of impurity it is easy to avoid or minimize percentage of the impurity.

The control of pharmaceutical impurities is currently a critical issue to pharmaceutical industry. In this publication our work deals with identification, synthesis and characterization of impurities / process related substances in Diazepam as per EP or USP monograph.

Diazepam first marketed as Valium by Hoffmann-La Roche, is a benzodiazepine drug. It is commonly used to treat a wide range of conditions, including anxiety, panic attacks, insomnia, seizures, muscle spasms, restless legs syndrome, alcohol withdrawal syndrome, benzodiazepine withdrawal syndrome, opiate withdrawal syndrome, and Ménière's disease[6,7]. It may also be used before certain medical procedures to reduce tension and anxiety, and in some surgical procedures to induce amnesia. It possesses anxiolytic, anticonvulsant, hypnotic, sedative, skeletal muscle relaxant, and amnestic properties.

Diazepam is a core medicine in the World Health Organization's Essential Drugs List, the minimum medical needs for a basic health-care system. Diazepam, first synthesized by Leo Sternbach, has been one of the most frequently prescribed medications in the world since its launch in 1963[8]. Diazepam also synthesized by modified methods[9,10] in later years. Various degradation pathway of Diazepam[11,12] is available in literature. There are six potential impurities (Scheme-I) known in Diazepam product. These impurities are also mention EP and USP pharmacopeia. Since these impurities are process related impurities, enrichment by degradation of Diazepam product was not possible and therefore there was no option than to synthesize these impurities in pure form.

EXPERIMENTAL SECTION

Thin-layer chromatography (TLC) were run on silica gel 60 F254 pre-coated plates (0.25 mm, Merck, Art. 5554)

and spots were visualized inside an UV cabinet under short UV. Infrared spectra were recorded on IR Affinity-1, Shimadzu. ^{1}H-NMR spectra were recorded on Bruker Advance III 400 MHz with TMS as an internal standard. Mass spectra were obtained using LC-MS API-2000, ABSciex. All solvents and reagents were purchased from Aldrich (India) and S. D. Fine Chemicals, Mumbai. The solvents and reagents were used without purification.

Experimental procedures

Synthesis of 7-chloro-5-phenyl-1*H*-benzo[e][1,4]diazepin-2(3*H*)-one (3), impurity A:

Step 1: To a solution of glycine (10.0 g, 0.13 mole) in ethanol (100 ml) was added thionyl chloride (10.6 ml, 0.15 mole, 1.1 eq.) at 0-5 °C dropwise over 15 min. Reaction mixture was then slowly warm to room temperature further stirred at same temperature for 2 h. Reaction was concentrated under reduced pressure to get glycine ethyl ester hydrochloride (**1**) (12.5 g, 67.20%) as pale yellow solid.

Step 2: Crude product (**1**) (4.0 g, 0.0286 mole) and 2-amino-5-chlorobenzophenone (**2**) (5.0 g, 0.022 mole) was dissolved in dry pyridine (100 ml). Reaction mixture was heated to reflux at 120 °C and stirred at same temperature for 18 h. Completion of reaction was monitored by TLC (3:7, ethyl acetate:hexane, R_f=0.25), reaction mixture was concentrated under reduced pressure to obtain residue. To the residue was added water (100 ml) and extracted with diethyl ether (2×50 ml). Combined organic layer dried over sodium sulfate and filtered. The filtrate was concentrated under reduced pressure to obtain desired crude product. Crude product was purified by silica column chromatography to give (**3**) (2.02 g, 34.83%) as off white solid. HPLC purity=99.97%. Melting point=214.7 ºC. IR (cm^{-1}) 3196 (N-H), 2958-3066 (aromatic C-H), 1678 (amide C=O), 1477, 1367 (aliphatic C-H bending), 1359 (C-O asymmetric stretching), 815 (C-Cl stretching), 736, 700 (Ar-H bending). MS: m/z 271 $[M+H]^+$, 293 [M+Na]. ^{1}H-NMR ($CDCl_3$) δ 9.0 (1H, bs, N-H), 7.6 (2H, m, Ar-H), 7.53-7.52 (2H, m, Ar-H), 7.5-7.48 (3H, m, Ar-H), 7.38 (1H, dd, Ar-H), 4.55 (2H, s, -CH_2).

Synthesis of 7-chloro-2-methoxy-5-phenyl-2,3-dihydro-1*H*-benzo[e][1,4]diazepine (4), impurity F: To a solution of (**3**) (1.0 g, 0.0037 mole) and potassium carbonate (0.63 g, 0.0044 mole, 1.2 eq.) in acetonitrile (20 ml) was added iodomethane (0.3 ml, 0.0044 mole, 1.2 eq.) at 10-15 °C. Reaction mixture was then heated at 50 °C, stirred at 50 °C for 25 h. Reaction progress was monitored by TLC (2:8, ethyl acetate:hexane, R_f =0.45), reaction mixture was then concentrated under reduced pressure to obtain residue. To the residue was added water (20 ml) and extracted with ethyl acetate (2×15 ml). Combined organic layer dried over sodium sulfate and filtered. The filtrate was concentrated under reduced pressure to obtain desired crude product. Crude product was purified by silica column chromatography to give (**4**) (0.6 g, 58%), as off white sticky solid. HPLC purity=95.60%. IR (cm^{-1}) 2958-3066 (aromatic C-H), 1678 (amide C=O), 1481, 1398 (aliphatic C-H bending), 1321 (C-O stretching), 812 (C-Cl stretching), 740, 696 (Ar-H bending). MS: m/z 285 $[M+H]^+$, 307 [M+Na]. ^{1}H-NMR ($CDCl_3$) δ 7.60-7.52 (2H, m, Ar-H), 7.50-7.42 (3H, m, Ar-H), 7.40-7.29 (3H, m, Ar-H), 4.84-4.81 (1H, dd, CH-H), 3.78-3.75 (1H, dd, CH-H), 3.39 (3H, s, O-Me).

Synthesis of 2-(methylamino)-5-chlorobenzophenone (7), impurity D:

Step 1: *N*-(2-benzoyl-4-chlorophenyl)acetamide (5): To a solution of **(2)** (10.0 g, 0.0432 mole) in absolute ethanol (30 ml) was added acetic anhydride (8.27 g, 0.0810 mole) in absolute ethanol (70 ml) dropwise at 0 ºC over 30 min. Reaction mixture then warmed to room temperature and stirred for 16 h at same temperature. Progress of the reaction was monitored by TLC (0.5:9.5, ethyl acetate:hexane, R_f=0.5), reaction mixture was then concentrated under reduced pressure to get yellow solid as crude product. Crude product was recrystallized using ethyl acetate and hexane to get (**5**) (10.2 g, 86%), as off white solid, single spot by TLC.

Step 2: *N*-(2-benzoyl-4-chlorophenyl)-*N*-methylacetamide (6): To a solution of sodium hydride (1.75 g, 0.0438 mole) in anhydrous DMF (10 ml) was added a solution of (**5**) in anhydrous DMF (20 ml) dropwise at 0-5 ºC. Reaction mixture was then warmed to room temperature and stirred for 1 h. Methyl iodide (7.72 g, 0.0548 mole) was then added drop wise over 15 min. Reaction mixture then further stirred for 5 h at room temperature. Reaction progress was monitored by TLC (1:9, ethyl acetate:hexane, R_f= 0.1). Water (100 ml) was added and extracted using ethyl acetate (3×100 ml). Combined organic layer dried over sodium sulfate and filtered. The filtrate was concentrated under reduced pressure to obtain desired crude product. Crude product was purified by silica gel column chromatography to get (**6**) (6.5 g, 62%) as a viscous oil, single spot by TLC.

Step 3: Solution of **(6)** (6.0 g, 0.0209 mole) in 6N HCl solution (206 ml) was heated to 90 ºC, stirred for 12 h at 90 ºC to obtain clear solution. Reaction progress was monitored by TLC (1:9, ethyl acetate:hexane, R_f= 0.8). Reaction mixture was cooled to room temperature and basified to pH 8 using aqueous 50% sodium hydroxide solution, extracted using ethyl acetate (3×75 ml). Combined organic layer dried over sodium sulfate and filtered. The filtrate was concentrated under reduced pressure to get (**7**) (3.80 g, 74%) as yellow colored hygroscopic solid. HPLC purity=99.03%. Melting point=113.6 ºC. IR (cm^{-1}) 3223 (N-H), 2958-2866 (aliphatic-H), 1666 (amide C=O), 1504,

1456, 1367 (aliphatic-H bending), 1253 (C-O-C asymmetric stretching), 761, 748. MS: m/z 274 $[M+H]^+$, 296 [M+Na]. ^{1}H-NMR ($CDCl_3$) δ 8.46 (1H, bs, N-H), 7.55 (2H, m, Ar-H), 7.53-7.52 (2H, m, Ar-H), 7.5-7.48 (3H, m, Ar-H), 6.7 (1H, dd, Ar-H), 2.96-2.95 (3H, d, N-Me).

Synthesis of 6-chloro-1-methyl-4-phenyl quinazolin-2-(1*H*)-one (9) impurity E:
Step 1: Reaction mass consisting mixture of (**2**) (2.0 g, 0.0086 mole) and urea (1.05 g, 0.0174 mole) was heated to 200 ºC for 45 min. Reaction progress was monitored by TLC (1:1, ethyl acetate: hexane, R_f= 0.10). Reaction mixture was cooled to 10 ºC solid formation observed filtered, washed with hot ethanol and dried under vacuum to get 6-chloro-4-phenylquinazolin-2(1*H*)-one (**8**) (2.0 g, 91.0%).

Step 2: To a solution of (**8**) (2.0 g, 0.007 mole) in of DMF (40 ml) was added sodium hydride (0.37 g, 0.0156 moles) at 0-5 ºC. Methyl iodide (0.73 ml, 0.0117moles) was added at 0-5 ºC. Reaction mixture warmed to room temperature and stirred for 3 h. Reaction progress was monitored by TLC (1:1 ethyl acetate: hexane, R_f=0.25). Water (100 ml) was added and extracted using ethyl acetate (3×100 ml). Combined organic layer dried over sodium sulfate and filtered. The filtrate was concentrated under reduced pressure to obtain desired crude product. Crude product was purified by silica gel column chromatography to get (**9**) (0.65 g, 28%) as off white solid. HPLC purity=98.76%. Melting point=223.6 ºC. IR (cm^{-1}) 3223 (N-H), 2958-2866 (aliphatic-H), 1666 (amide C=O), 1504, 1456, 1367 (aliphatic-H bending), 1253 (C-O-C asymmetric stretching), 761, 748. MS: m/z 246 $[M+H]^+$. ^{1}H-NMR ($CDCl_3$) δ 7.83 (1H, s, Ar-H), 7.82-7.77 (3H, m, Ar-H), 7.59-7.52 (3H, m, Ar-H), 7.53-7.48 (1H, m, Ar-H), 3.78 (3H, s, N-Me).

Synthesis of *N*-(2-benzoyl-4-chlorophenyl)-2-chloro-*N*-methylacetamide (10) impurity B: To a solution of **(7)** (1.5 g, 0.0061 mole) in acetonitrile (30 ml) was added potassium carbonate (1.27 g, 0.0092 mole) and stirred at room temperature for 15 min. Reaction mixture cooled to 5-10 ºC, was added chloro acetylchloride (0.83 g, 0.0073 mole) dropwise at 5-10 ºC. Reaction mixture was then warmed to room temperature and stirred for 10 h. at same temperature. Reaction progress was monitored by TLC (2:8, ethyl acetate:hexane, R_f=0.2). Water (100 ml) was added and extracted using ethyl acetate (2×50 ml). Combined organic layer dried over sodium sulfate and filtered. The filtrate was concentrated under reduced pressure to get off white solid crude product. Crude product was recrystallized using ethyl acetate and hexane to get (**10**) (1.43 g, 73%) as white colored solid product. HPLC purity=99.56%. Melting point=122.0 ºC. IR (cm^{-1}) 3059 (N-H), 2958-2866 (aliphatic-H), 1504, 1456, 1367 (aliphatic-H bending), 1253 (C-O-C Asymmetric stretching), 761, 748. MS: m/z 322 $[M]^+$, 346 [M+Na]. ^{1}H-NMR ($CDCl_3$) δ 7.55 (2H, m, Ar-H), 7.53-7.52 (2H, m, Ar-H), 7.5-7.46 (3H, m, Ar-H), 7.30 (1H, dd, Ar-H), 3.96-3.85 (2H, s, $-CH_2$), 3.0 (3H, s, N-Me).

Synthesis of 3-amino-6-chloro-1-methyl-4-phenylquinolin-2-(1*H*)-one (12) impurity C:
Step 1: Reaction mass consisting mixture of (**9**) (1.2 g, 0.0037 mole) in pyridine (10.20 ml, 0.1266 mole) heated to reflux, 115 ºC was stirred for 2 h at 115 ºC. Reaction mixture was cooled to room temperature, diethyl ether (10 ml) was added. Reaction mixture was then further cooled to 0-5 ºC and stirred for 15 min. at same temperature. Solid obtained was filtered, washed with diethyl ether (30 ml) to get (**11**) (1.50 g, 99.8%) as buff colored solid.

Step 2: To a solution of (**11**) (1.5 g, 0.0039 mole) in absolute ethanol (10 ml) was added hydrazine hydrate (2.0 ml). Reaction mixture then heated to reflux, 80 ºC was stirred for 3 h at 80 ºC. Reaction mass was then cooled to room temperature and concentrated under reduced pressure. Water (50 ml) was added and extracted using ethyl acetate (2×25 ml). Combined organic layer dried over sodium sulfate and filtered. The filtrate was concentrated under reduced pressure to obtain (**12**) (1.0 g, 94%) as orange colored solid. HPLC purity=99.29%. Melting point=136.2 ºC. IR (cm^{-1}) 3223 (N-H), 2958-2866 (aliphatic-H), 1627 (amide N-H), 1504, 1456, 1367 (aliphatic-H bending), 1253 (C-O-C asymmetric stretching), 761, 748. MS: m/z 285 $[M+H]^+$, 307 [M+Na]. ^{1}H-NMR ($CDCl_3$) δ 7.55 (2H, m, Ar-H), 7.6-7.55 (2H, m, Ar-H), 7.5-7.40 (3H, m, Ar-H), 7.38 (1H, dd, Ar-H), 4.5 (2H, s, NH_2), 3.84 (3H, s, N-Me).

RESULTS AND DISCUSSION

Six potential impurities are known for Diazepam API as mention in EP and USP monograph. All six impurities as mention in EP monograph are process related impurities, they form while API synthesis as a result of side reaction. In order to meet the stringent regulatory requirements, the impurities present in the drug substances must be identified and characterized. In present work these impurities are identified and synthesized in pure form. Diazepam impurity D as per EP is same as USP related compound A. Diazepam impurity C as per EP is same as USP related compound B. Scheme 1 represents synthetic scheme for all six impurities. Synthesis of impurity A, 7-chloro-5-phenyl-1*H*-benzo[e][1,4]diazepin-2(3*H*)-one (**3**) involve condensation of glycine ethyl ester hydrochloride (**1**) and 2-amino 5-chlorobenzophenone (**2**) in pyridine at reflux temperature. Impurity F, 7-chloro-2-methoxy-5-phenyl-2,3-dihydro-1H-benzo[e][1,4]diazepine (**4**) is O-methylated derivative of impurity A obtained by reacting same with iodomethane. Synthesis of impurity D involves N-methylation of 2-amino

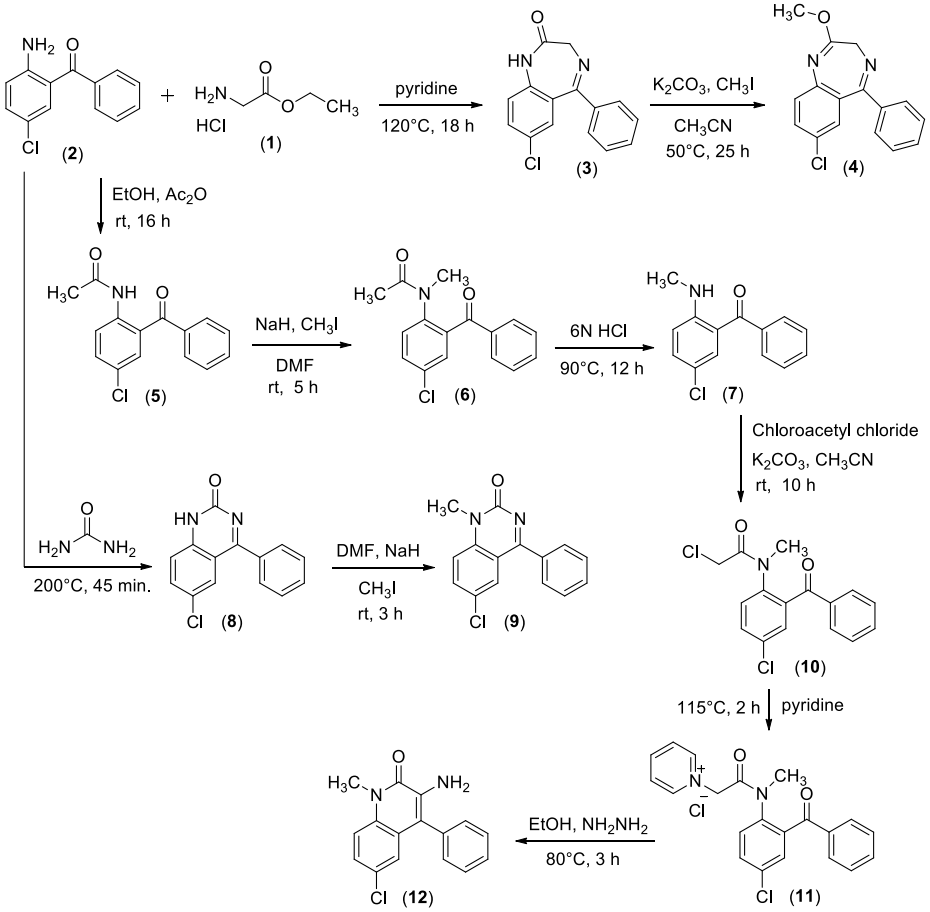
5-chlorobenzophenone (**2**) using methyl iodide *via* acyl protection. Synthesis of impurity E (**9**) obtained from neat condensation of 2-amino 5-chlorobenzophenone (**2**) and urea followed by methylation using methyl iodide to give quinazolin-2-one (**9**). Impurity B (**10**) derived from acylation of impurity D (**7**) using chloroaetyl chloride under basic condition. Impurity C (**12**) can be easily synthesized from Impurity B (**10**), first step is condensation with pyridine followed by reacting with hydrazine to obtain amino quinolin-2-one **(12)**. HPLC retention data for all six Diazepam impurities is as mention in Table 1.

Table 1: HPLC chromatography retention time data

Sr. No.	Impurity as per EP	RT in HPLC*
1	A	2.63
2	B	4.13
3	C	7.23
4	D	11.68
5	E	2.89
6	F	3.50

HPLC condition
Column: Novapack, 3.9×150 mm, C-18.
Mobile phase: Acetonitrile:MeOH:Water, (400:400: 200, v/v).
Detector wavelength: 254 nm.
Flow: 1.0 ml/ min.

Scheme 1: Synthetic reaction scheme for Diazepam impurities



CONCLUSION

Diazepam impurities are synthesized independently for their use in pharmaceutical industry for method development and validation. Synthesis of forced degradation impurities from API itself may not cost effective, but here all six impurities are process related impurities and hence their complete synthesis is required. These six compounds are used as an impurity standards of Diazepam, those can be further studied in various aspects.

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