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Research Article

Synthesis and Characterization of Potential Impurities of Sildenafil

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Abstract: Sildenafil citrate **1** is a pyrazolopyrimidone derivative, used for the treatment of male erectile dysfunction. During the process development of sildenafil **2**, four process related potential impurities were detected in the final crude material ranging from 0.01 to 0.15% by HPLC. The present work describes the identification, synthesis and characterization of four impurities (desmethyl sildenafil **6**, sulfonic acid **7**, sildenafil dimer **8,** and sildenafil N-oxide **9**) by their respective spectral data (IR, ^{1}H NMR, ^{13}C NMR, DEPT and MS). Our study will be of immense help to others to obtain chemically pure sildenafil.

Introduction

The safety of a drug product is not only dependent on the toxicological properties of the active drug substances (API), but also on the impurities formed during the various chemical transformations. Therefore, identification, quantification, and control of impurities in the drug substance and drug product are important parts of drug development for obtaining marketing approval. It is more challenging for an organic chemist to identify the impurities which are formed in very small quantities in a drug substance. Since most of the time it is very difficult to identify and control impurities within acceptable levels in the process, extra purification steps may then be necessary thereby making the process less competitive. More often than not, the syntheses of impurities are not described in the literature which makes it even more difficult for the organic chemist who must then design a synthesis, which is time consuming. The development of a drug substance is incomplete without the identification of an impurity profile involved in the process. Thus, in our study we explored the formation, identification, synthesis and characterization of impurities found in the preparation of sildenafil **2** (Scheme 1). This study will be of immense help for organic chemists to understand the potential impurities in sildenafil synthesis and thereby obtain the pure compound.

Sildenafil[1-7] is a selective inhibitor of cyclic guanosine monophosphate (cGMP) specific phosphodiesterase type 5 (PDE5) and is the

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first agent with this mode of action for the treatment of male erectile dysfunction a disease more commonly known as male impotence. Sildenafil is used as its citrate salt and is designated chemically as 1-[[3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-4-methyl piperazine citrate. This product was developed by Pfizer and is currently being marked under the trade name Viagra. International Conference on Harmonization (ICH) guidelines recommended that all the unknown impurities that are present in the drug substance at a level of $\geq$0.10% need to be identified and characterized.[8,9] As sildenafil citrate **1** is an important drug substance and to obtain information on product profile, a comprehensive study was under taken on the impurities generated during the process development of **1**.

Materials and Methods

All solvents and reagents were used as received without further purification. The ^{1}H NMR spectra were recorded on a Varian Mercury plus 400 MHz FT NMR spectrometer, the chemical shifts are reported on δ ppm relative to TMS. The ^{13}C NMR and DEPT spectra were recorded on a Varian Mercury plus 200 MHz FT NMR spectrometer, the chemical shifts are reported on δ ppm relative to $CDCl_3$. The IR spectra were recorded in the solid state as KBr dispersion using Perkin Elmer FT-IR spectrometer. The mass spectra were recorded on Shimadzu LCMS-QP8000 and Micro-mass LCT Premier XE mass spectrometer. Elemental analysis for CHN was performed on Perkin Elmer model 2400 CHN/O analyzer.

Synthesis of Sildenafil (2)

To a stirred mixture of **4** (10 g, 0.024 mol) in acetone (50 mL), was added **3** (5.8 g, 0.058 mol) and stirred at 25 - 35 ^{o}C for 2 h. The separated solid was filtered and the wet compound thus obtained was dissolved in dichloromethane (100 mL) followed by washed with water (50 mL) then with 25% aqueous ammonia (50 mL). The organic layer concentrated to dryness under reduced pressure followed by the resulted product was triturated with toluene (50 mL) to afford the title compound **2** as white crystalline solid (10.4 g, yield 90%, purity by HPLC 99.85%). IR (cm^{-1}): 3311 (NH), 1689 (Amide C=O), 1247 (C-N), 1350 and 1171 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.2 (br, 1H), 7.85 (d, *J* = 2.4 Hz, 1H), 7.81 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.38 (d, *J* = 8.8 Hz, 1H), 4.2 (q, *J* = 7.2 Hz, 2H), 4.1 (s, 3H), 2.9 (b, 2 x 2H), 2.77 (t, *J* = 7.2 Hz, 2H), 2.3 (t, *J* = 4.8 Hz, 2 x 2H), 2.14 (s, 3H), 1.74 (m, 2H), 1.32 (t, *J* = 6.8 Hz, 3H), 0.93 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR/(DEPT) (200 MHz, $CDCl_3$) δ 159.2, 153.5, 146.9, 146.3, 138.3, 131.6(+), 131.1(+), 128.9, 124.4, 121.0, 112.9(+), 66.0(-), 54.0(-), 45.9(-), 45.6(+), 38.1(+), 27.7(-), 22.2(-), 14.5(+), 14.0(+); Mass (*m/z*): 475.4 (M^+ + 1); Anal. Calcd for $C_{22}H_{30}N_6O_4S$: C, 55.68; H, 6.37; N, 17.71. Found: C, 55.72; H, 6.33; N, 17.66.

5-[2-Ethoxy-5-(piperazine-1-sulfonyl)-phenyl]-1-methyl-3-propyl-1,6-dihydropyrazolo[4,3-d]pyrimidin-7-one (6)

To a solution of piperazine (6.3 g, 0.073 mol) in dichloromethane (30.0 mL), solution of **4** (5.0 g, 0.012 mol) in dichloromethane (250.0 ml) was added dropwise at 25 - 35 ^{o}C and the resulted mixture was stirred for 3 h. Water (50.0 mL) was added and stirred for 15 min then layers were separated. The organic layer was washed with water (20.0 mL) followed by concentrated to dryness under reduced pressure below 60 ^{o}C to afford the title compound **6** as white crystalline solid (4.9 g, yield 87%, purity by

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HPLC 97.9%). IR (cm^{-1}): 3309 (NH), 1689 (Amide C=O), 1249 (C-N), 1348 and 1167 (SO_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.8 (d, *J* = 2.4 Hz, 1H), 7.8 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.2 (d, *J* = 8.8 Hz, 1H), 4.4 (q, *J* = 7.2 Hz, 2H), 4.2 (s, 3H), 3.05 (t, *J* = 5.6 Hz, 2 x 2H), 2.9 (m, 2 x 2H), 2.8 (t, *J* = 7.2 Hz, 2H), 1.9 (m, 2H), 1.64 (t, *J* = 7.2 Hz, 3H), 1.0 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR/(DEPT) (200 MHz, $CDCl_3$) δ 159.2, 153.5, 146.8, 146.3, 138.3, 131.5(+), 131.0(+), 129.0, 124.4, 121.0, 112.9(+), 66.0(-), 46.8(-), 45.3(-), 38.1(+), 27.7(-), 22.2(-), 14.5(+), 14.0(+); Mass (*m/z*): 461.4 (M^+ + 1); Anal. Calcd for $C_{21}H_{28}N_6O_4S$: C, 54.77; H, 6.13; N, 18.25. Found: C, 54.81; H, 6.17; N, 18.21.

4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6,7-dihydro-1*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)-benzenesulfonic acid (7)

A heterogeneous mixture of water (50.0 mL) and **4** (5.0 g, 0.012 mol) was heated at 70 ^{o}C for 6 h. The reaction mixture was allowed to cool to 25 - 35 ^{o}C and filtered the undissolved **4** followed by washed with water (10.0 mL). The filtrate was concentrated to dryness under reduced pressure below 80 ^{o}C followed by leached the product thus obtained with hexane (50.0 mL) and dried at 60 ^{o}C to afford the title compound **7** as white crystalline solid (4.3 g, yield 90%, purity by HPLC 99.4%). IR (cm^{-1}): 3600 – 3300 (Sulfonic acid OH), 1701(Amide C=O); ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.7 (d, *J* = 2.4 Hz, 1H), 7.5 (d, *J* = 8.8, 1H), 6.9 (d, *J* = 8.8 Hz, 1H), 4.1 (s, 3H), 4.0 (q, *J* = 6.8 Hz, 2H), 2.7 (t, *J* = 7.2 Hz, 2H), 1.72 (m, 2H), 1.2 (t, *J* = 7.6 Hz, 3H), 0.92 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR/(DEPT) (200 MHz, DMSO-d_6) δ 160.0, 153.8, 148.1, 145.0, 137.8, 131.5(+), 130.0(+), 126.2, 124.4, 123.7, 113.3(+), 64.9(-), 37.8(+), 27.1(-), 21.7(-), 14.2(+), 13.8(+); Mass: (*m/z*): 393.2 (M^+ + 1); Anal. Calcd for $C_{17}H_{25}N_4O_5S$: C, 52.03; H, 5.14; N, 14.28. Found: C, 52.09; H, 5.18; N, 14.22.

Bis-1,4-[4-ethoxy-3-(6, 7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo [4,3-*d*]pyrimidin-5-yl)-benzenesulfonyl]-piperazine (8)

To a stirred solution of methanol (100.0 mL) and sodium hydroxide (0.5 g, 0.012 mol), was added **4** (5.0 g, 0.0125 mol) and stirred at 25 - 35 ^{o}C for 15 min. Piperazine (0.52 g, 0.006 mol) was added dropwise and stirred for 2 h. The separated solid was filtered and washed with methanol (10.0 mL). To the wet cake, water (50.0 mL) was added and stirred at 25 - 35 ^{o}C for 30 min. Filtered the solid, washed with water (10.0 mL) and dried at 75 ^{o}C to afford the title compound **8** as an off-white crystalline solid (4.0 g, yield 91.2%, purity by HPLC 97.8%). IR (cm^{-1}): 3315 (NH), 1690 (Amide C=O), 1250 (C-N), 1347 and 1168 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.0 (d, *J* = 2.8 Hz, 2 x 1H), 7.8 (dd, *J* = 8.8, 2.4 Hz, 2 x 1H), 7.25 (d, *J* = 8.4 Hz, 2 x 1H), 4.25 (q, *J* = 6.8 Hz, 2 x 2H), 4.2 (s, 2 x 3H), 3.1 (br, 4 x 2H), 2.8 (d, *J* = 7.6 Hz, 2 x 2H), 1.8 (m, 2 x 2H), 1.44 (t, *J* = 6.8 Hz, 2 x 3H), 1.0 (t, *J* = 7.2 Hz, 2 x 3H); ^{13}C NMR/(DEPT) (200 MHz, $CDCl_3$) δ 159.2, 153.5, 146.9, 146.3, 138.3, 131.6(+), 131.0(+), 129.0, 124.4, 121.0, 112.9(+), 66.0(-), 45.3(-), 38.1(+), 27.7(-), 22.2(-), 14.5(+), 14.0(+); Mass (*m/z*): 835.4 (M^+ + 1); Anal. Calcd for $C_{38}H_{46}N_{10}O_8S_2$: C, 54.66; H, 5.55; N, 16.78. Found: C, 54.59; H, 5.57; N, 16.74.

5-[2-Ethoxy-5-(4-methyl-4-oxo-piperazine-1-sulfonyl)-phenyl]-1-methyl-3-propyl-1,6-dihydro-pyrazolo[4,3-*d*]pyrimidin-7-one (9)

To a solution of **2** (5.0 g, 0.012 mol) in mixture of dichloromethane (50.0 mL) and methanol (25.0 mL), was added H_2O_2 (5.0 mL) , acetic acid (1.0 mL) and stirred at 25 -

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35 ^{o}C for 72 h. Aqueous sodium sulfite solution (50.0 mL, 10% solution) was added and stirred for 30 min. The layers were separated and washed the organic layer with water (50.0 mL) followed by concentrated to dryness under reduced pressure. Acetone (25.0 mL) was added and stirred for 30 min. Filtered the product and dried at 60 ^{o}C to afford the title compound **9** as white crystalline solid (5.0 g, yield 84%, purity by HPLC 98.7%). IR (cm^{-1}): 3288 (NH), 1712 (Amide C=O), 1245 (C-N), 1348 and 1170 (SO_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.9 (br, 1H), 8.8 (d, *J* = 2.4 Hz, 1H), 7.8 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.18 (d, *J* = 8.8 Hz, 1H), 4.4 (q, *J* = 6.8 Hz, 2H), 4.2 (s, 3H), 3.7 (m, 2H), 3.4 (m, 2 x 2H), 3.25 (s, 3H), 3.2 (m, 2H), 2.9 (t, *J* = 7.2 Hz, 2H), 1.8 (m, 2H), 1.6 (t, *J* = 6.8 Hz, 3H), 1.0 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR/(DEPT) (200 MHz, $CDCl_3$) δ 159.7, 153.6, 146.8, 146.3, 138.2(+), 131.3, 131.1(+), 128.4, 124.4, 121.7, 113.3(+), 66.1(-), 65.2(-), 60.8(+), 40.8(-), 38.2(+), 27.6(-), 22.2(-), 14.5(+), 14.0(+); Mass (*m/z*): 491.3 (M^{+}+1); Anal. Calcd for $C_{22}H_{30}N_6O_5S$: C, 53.86; H, 6.16; N, 17.13. Found: C, 53.92; H, 6.20; N, 17.17.

Results and Discussion

During the laboratory optimization of sildenafil citrate **1**, HPLC analysis of sildenafil revealed that, four impurities were detected in the range of 0.05% to 0.15%. According to ICH (International Conference on Harmonization) guidelines the amount of acceptable level for known and unknown impurities in a final drug candidate must be less than 0.15% and 0.10% respectively. In order to meet the stringent regulatory requirements, the impurities need to be indentified and characterized. An in-house LC gradient method[10] was developed for the separation of sildenafil and its process related potential impurities. LC-MS analysis of crude **2** (Figure 1) indicated that, molecular weight of four impurities desmethyl sildenafil **6**, sulfonic acid **7**, sildenafil dimer **8**, and sildenafil N-oxide **9** as 460.4, 392.2, 834.4 and 490.3 respectively. By taking a lead from this LC-MS data, a comprehensive study was undertaken to synthesis and characterizes these impurities. Based on the spectral data (MS, IR, ^{1}H NMR, ^{13}C NMR & DEPT), the structure of these impurities were characterized as 5-[2-Ethoxy-5-(piperazine-1-sulfonyl)-phenyl]-1-methyl-3-propyl-1,6-dihydro pyrazolo [4,3-*d*] pyrimidin-7-one **6**, 4-Ethoxy-3-(1-methyl-7-oxo-3-propyl-6,7-dihydro-1*H*-pyrazolo[4,3-*d*] pyrimidin-5-yl)-benzenesulfonic acid **7**, Bis-1,4-[4-ethoxy-3-(6, 7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo [4,3-*d*] pyrimidin-5-yl)-benzenesulfonyl]- piperazine **8**, and 5-[2-Ethoxy-5-(4-methyl-4-oxo-pipera- zine -1-sulfonyl)-phenyl]-1-methyl-3-propyl-1, 6-dihydro-pyrazolo[4,3-*d*]pyrimidin-7-one **9**. The impurities **7**, **8** and **9** are new compounds reported for the first time in our process. All the four impurities spectral data were compared with **2** for better visualization of four similar molecules.

Formation of related compounds

The related compound **6** was formed due the presence of piperazine as an impurity in **3** which is used in the synthesis of **2**, and this impurity was quantitatively synthesized by reaction of **4** with piperazine (Scheme 2). The mass spectrum of **6** exhibited a protonated molecule peak at *m/z* 461.4, which is 14 amu less than that of **2**. In ^{1}H NMR spectrum, a singlet signal at 2.2 ppm with three proton integration assigned to methyl protons of N-methyl piperazine moiety of **2** was absent and rest of the signals are similar to that of **2**. These observations suggest that methyl group was lost from **2** and it was further confirmed in DEPT spectrum by displayed the shortage of one positive signal at 45.6 ppm corresponding to methyl group. IR spectrum displayed characteristic absorption peaks,

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amide NH at 3309 cm^{-1}, amide carbonyl at 1689 cm^{-1}, sulfone at 1348 cm^{-1} and 1167 cm^{-1}. Based on the synthetic methodology and spectral analysis, the structure of **6** was confirmed as 5-[2-Ethoxy-5-(piperazine-1-sulfonyl)-phenyl]-1-methyl-3-propyl-1,6-dihydropyrazolo [4,3-*d*] pyrimidin-7-one.

Related substance **7**, a potential impurity which is hydrolyzed product formed during the aqueous work-up and subsequent isolation of **2**. This impurity was synthesized by reacting **4** with water at 70 ^{o}C (Scheme 3). The mass spectrum of **7** displayed a protonated molecule peak at *m/z* 393.2, which is 82 amu less than that of **2**. In ^{1}H NMR spectrum, broad signals at 3.1 ppm and 2.6 ppm corresponding to four methylene group and a singlet signal at 2.2 ppm corresponding to methyl group protons of N-methyl piperazine moiety of **2** was disappeared and this was further confirmed in DEPT spectrum by displayed the shortage of 4 negative signals and one positive signal. These observations suggested that sulfonyl chloride group in **4** is hydrolyzed to sulfonic acid group. In IR spectrum, a characteristic broad absorption peak between 3600 – 3300 cm^{-1} confirms the presence of OH absorption of SO_3H group. Based on the synthetic methodology and spectral analysis, the structure of **7** was characterized as 4-Ethoxy - 3 - (1-methyl-7-oxo-3-propyl-6,7-dihydro-1*H*-pyrazolo[4,3-*d*] pyrimidin-5-yl)-benzenesulfonic acid.

The related substance **6** formed in the synthesis of **2** was further reacted with **4** to afford impurity **8** and this was synthesized by reacting **4** with piperazine (Scheme 4). Mass spectrum of **8** displayed a protonated molecule peak at *m/z* 835.4. The higher molecular weight suggests that impurity **8** could be a dimer. The IR spectrum of **8** was similar to that of **2**. In ^{1}H NMR spectrum, piperazine ring methylene group protons are appeared as broad singlet signal at 3.1 ppm with eight proton integration, which attributes that both ends of piperazine ring nitrogen atoms could be attached with same chemical environment. This suggested both ends of nitrogen atom of piperazine ring attached with one molecule of **4** and its molecular weight was matched well with the protonated molecule ion observed at *m/z* 835.4 in the mass spectrum. Based on the synthetic methodology and spectral analysis, the structure of **8** was characterized as Bis-1, 4-[4- ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-5-yl)-benzenesulfonyl] piperazine.

The impurity **9** is a peroxide degradent impurity. The methyl group attached to nitrogen atom in piperazine moiety of **2** undergo aerial oxidation under drastic conditions and leads to impurity **10**, and this impurity was quantitatively synthesized by peroxide assisted oxidation of **2** (Scheme 5). The mass spectrum of **9** displayed a protonated molecule peak at *m/z* 491.3 which is 16 amu more than that of **2**. In ^{1}H NMR spectrum, all signals are similar to that of **2** except an appreciable downfield chemical shift of protons attached to terminal nitrogen of N-methyl piperazine moiety. This suggested that methyl group attached nitrogen atom of N-methyl piperazine moiety of **2** could be oxidized. Based on synthetic methodology and spectral analysis, the structure of **9** was characterized as 5-[2-Ethoxy-5-(4-methyl-4-oxo-piperazine-1-sulfonyl)-phenyl]1-methyl-3-propyl-1,6-dihydro-pyrazolo[4,3-*d*]pyrimidin-7-one.

Conclusion

In conclusion, we have identified, synthesized and characterized four process related potential impurities **6**, **7**, **8**, and **9** of Sildenafil.

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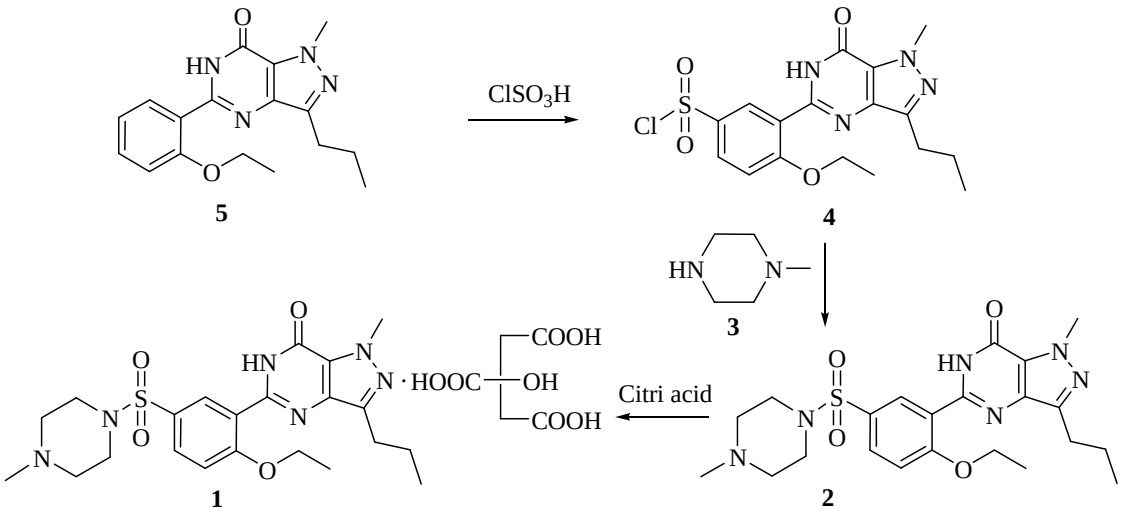
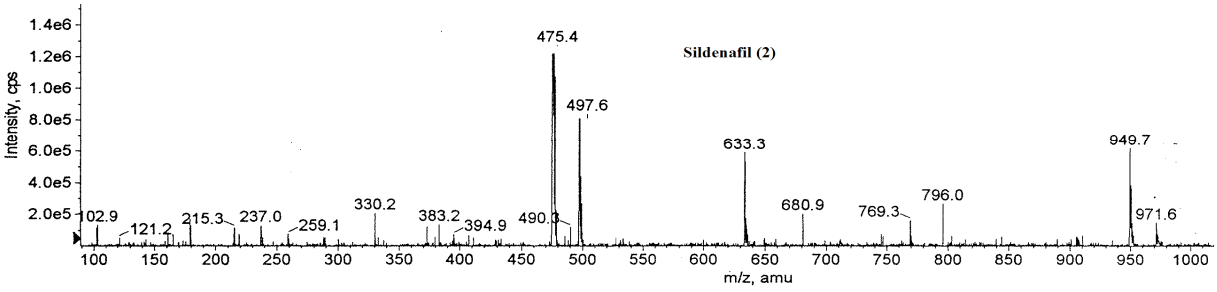
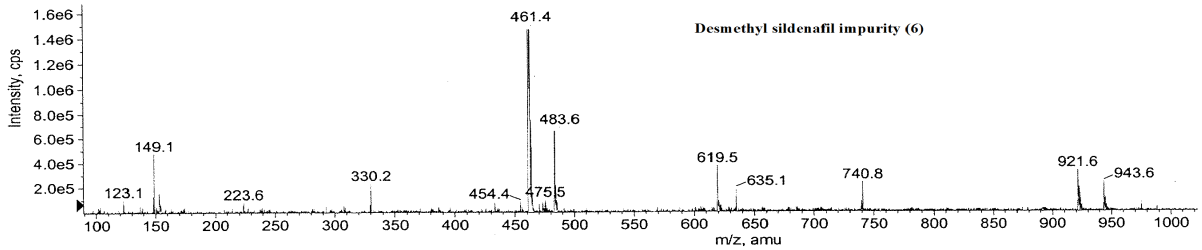


Scheme 1: Synthesis of sildenafil citrate **1**





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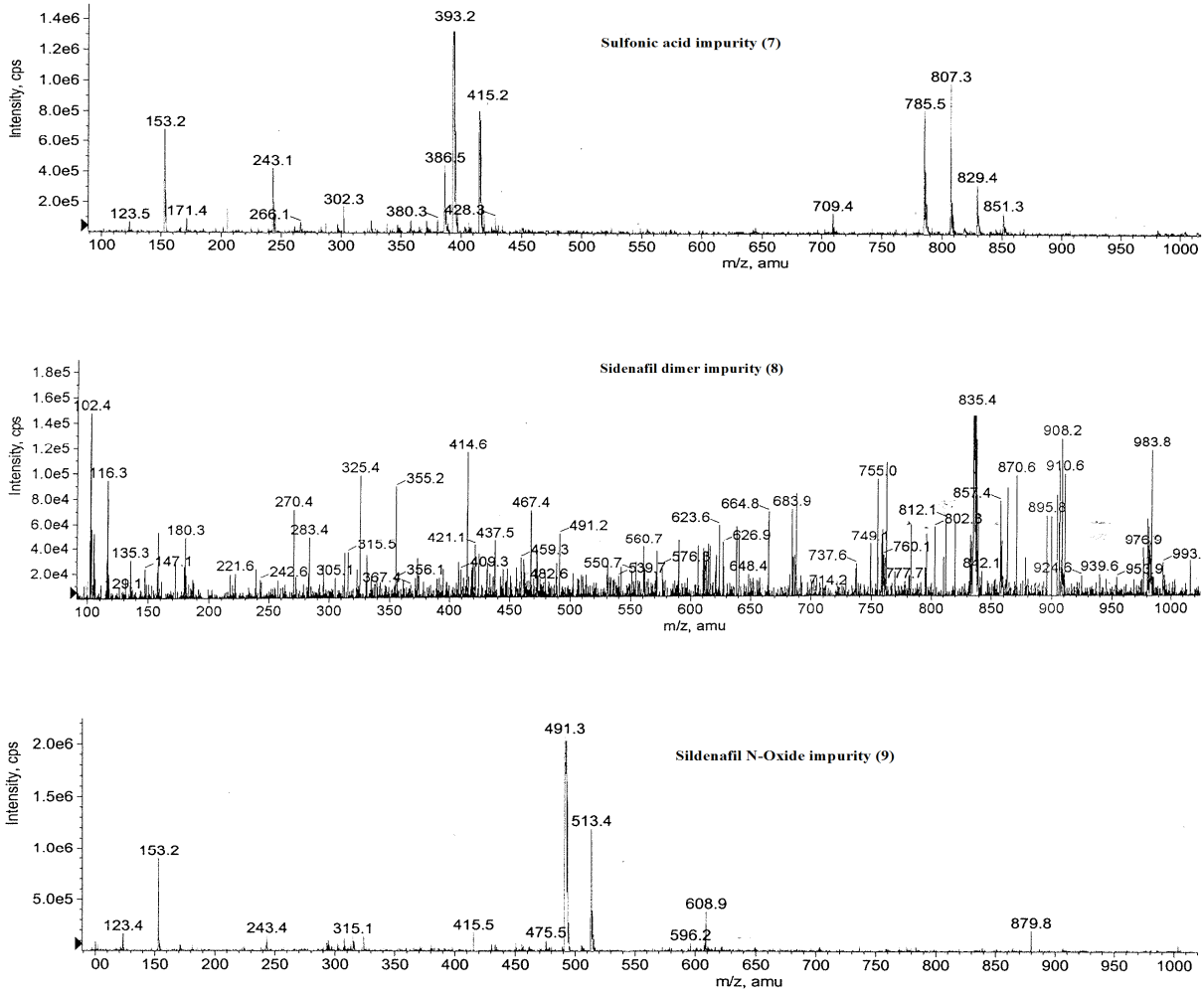
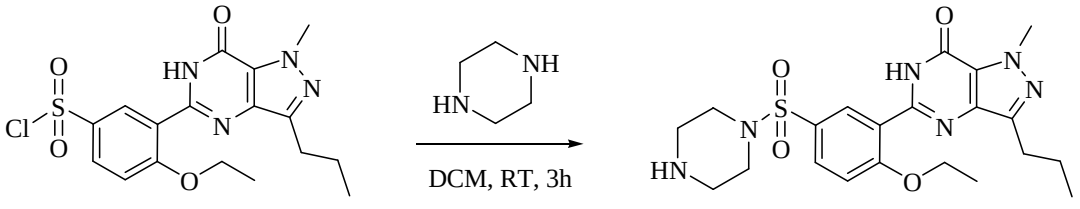


Figure 1. LC-MS Spectrum of sildenafil and its potential impurities (**6**, **7**, **8** and **9**).



4 **6**

Scheme 2. Synthesis of impurity **6**.

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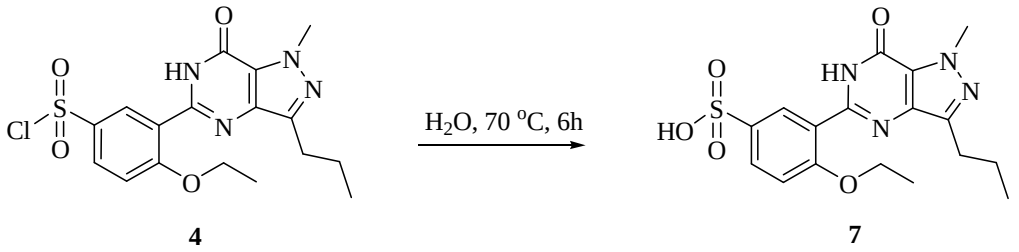
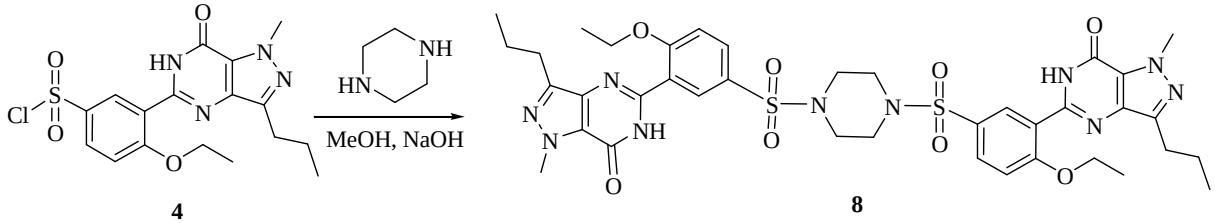


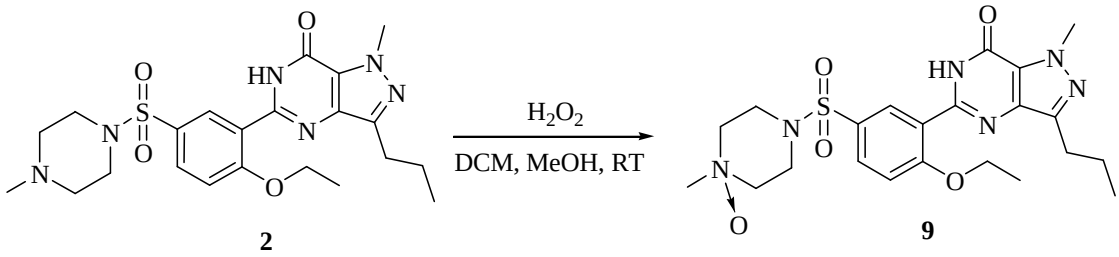
4 **7**

Scheme 3. Synthesis of impurity **7**.



4 **8**

Scheme 4. Synthesis of impurity **8**.



2 **9**

Scheme 5. Synthesis of impurity **9**.

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