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# Identification, synthesis, and strategy for reduction of process related potential impurities in sildenafil citrate.

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**ABSTRACT**

Seven potential process related impurities were detected during the impurity profile study of drug substance, Sildenafil Citrate **(1)**. Simple high performance liquid chromatography and liquid chromatography-mass spectrometry methods were used for the detection of these process impurities. Based on the synthesis and spectral data (MS, IR, $^{1}$H NMR, $^{13}$C NMR), the structures of these impurities were characterized as Chloro Impurity-**A (6)**, Methyl ester Impurity-**B (7)**, Acid Impurity-**C (8)**, Desethyl Impurity-**D (11)**, N-Oxide Impurity-**E (12)**, Dimer Impurity-**F (14)** and Desmethyl Impurity-**G (15)**. Among these impurities, Impurity-**A** and Impurity-**B** have been identified and synthesized first time. The reported synthetic routes of impurities **D, E,** and **F** are novel. Potential causes for the formation of these impurities are discussed and strategies to minimize their formation are also described.

**Keywords*:*** Sildenafil citrate; process related impurities; synthesis; characterization; stratergy to reduce impurities

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## INTRODUCTION

Sildenafil Citrate is marketed as VIAGRA which is developed by a group of pharmaceutical chemists working at Pfizer's research facility in England [1, 2]. It is a pyrazolo and pyrimidine derivative related to acetalildenafil, phospodiesterase inhibitor[3-7]. Sildenafil Citrate is used as common treatment for erectile dysfunction. Sildenafil citrate, is chemically known as 1-[4-ethoxy-3-(6, 7-dihydro-1-methyl-7-oxo-3-propyl-1*H*-pyrazolo [4, 3-*d*] pyrimidin-5-yl phenylsulfonyl]-4-methylpiperazine Citrate. It is generally believed that the active pharmaceutical ingredient (API) can be contaminated by impurities, which may influence the quality and safety of the drug. It has been found that the process employed in manufacturing of Sildenafil citrate(**1)** can result in the formation of certain impurities at a level of ≥ 0.10% (HPLC area).[8] One major concern is to maintain the quality of the API while reducing the level of the impurities. It is therefore significant to isolate and characterize the corresponding impurities during the manufacturing process.

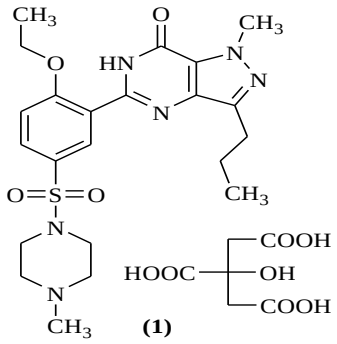
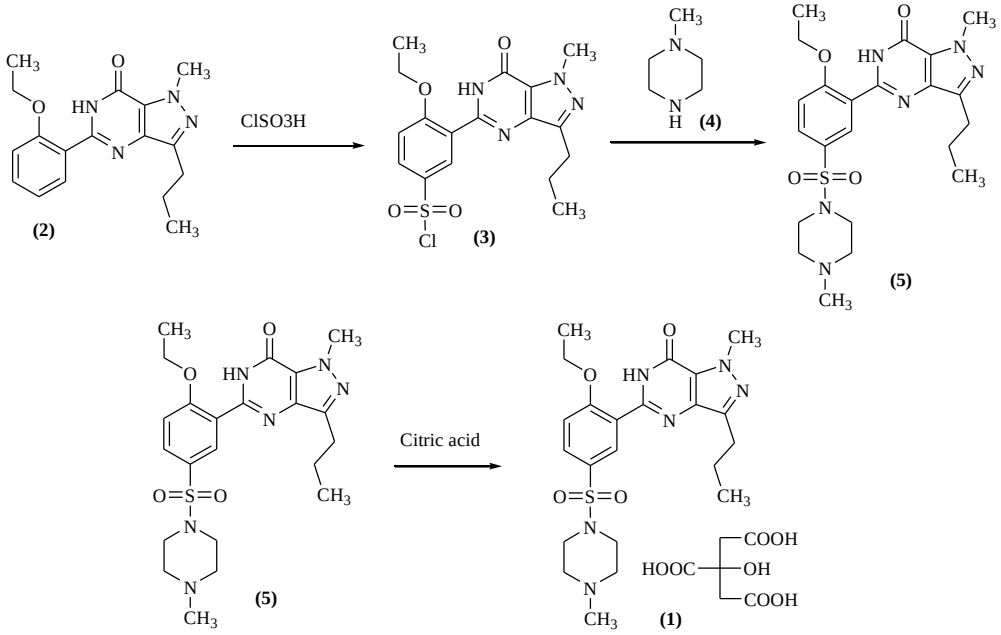



**Figure. 1. Structure of Sildenafil Citrate (1).**

Early experimental studies suggested that synthetic method outlined in (Scheme-1) is the most advantageous and convenient route for the synthesis of (**1)** [9-10]. Compound (**2)** is first converted to the corresponding Chloride intermediate (**3)** by Chlorosulphonation. Then, Chloride intermediate (**3)** is reacted with N-methyl piperazine (**4)** to give Sildenafil (**5)**. In the last step, the free base (**5)** reacts with Citric acid to produce its Citric acid salt (**1)**. A few impurities are identified; they can be produced by degradation, incomplete reactions, and side reactions. Contaminated starting ingredients, however are also major source of the impurities in (**1)**, such as Chlorosulphonic acid, which generally contains **2** impurities (see Scheme-2 for the impurities investigated in this work).




**Scheme-1 Synthesis of Sildenafil Citrate**

It is extremely challenging to identify impurities that formed in very small quantities in drug substances. The mechanism is also not straightforward due to the complexity of the conditions: multiple reactants and multiple impurities can coexist. This is strongly influenced by the reaction conditions. To solve the puzzle, the potential impurities have been explored and synthesized. A mechanism of formation is here proposed and a practical strategy for lowering the concentration of impurities in the product is presented.

## RESULTS AND DISCUSSION

HPLC was used to separate the impurities during the process of synthesizing (**1**). Seven impurities were identified and are listed in figure-**3**. Their structures have been determined by MS, $^{1}$H NMR, and $^{13}$C NMR. It is essential to understand how these impurities are introduced in the drug, the mechanism by which the impurities form, and the strategies for detecting and assessing impurities. These points are elaborated upon as follows.

Sildenafil citrate **(1)** HPLC analysis revealed that seven 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 must be less than 0.15% and 0.10% respectively. In order to meet the stringent regulatory requirements, the impurities need to be identified and characterized. An in house HPLC gradient method was developed for the separation of sildenafil and its process related potential impurities. In some batches at RT 29.10 min shows impurity about 0.1%.To identify this impurity LCMS has been done. LCMS of impurity-**A** (Supporting) indicated that, molecular weight as 347 (M+H). By taking a lead from this data, a comprehensive study was undertaken to synthesize and characterize this impurity. Also impurity-**B** has been prepared and characterized by spectral data (MS, IR, 1HNMR $^{13}$C NMR).

A typical HPLC chromatogram of a Sildenafil Citrate with seven impurities spike (Supporting) was recorded as described in the Experimental Section, and the target impurities under study were marked as Acid Impurity-**C (8)** (RT): 2.889 min), Desethyl Impurity-**D (11)** (RT): 4.752 min), N-Oxide Impurity-**E (12)** (RT): 6.578 min), Desmethyl Impurity-**G (15)** (RT): 9.735 min), Methyl ester Impurity-**B (7)** (RT): 23.930 min), Dimer Impurity-**F (14)** (RT): 29.511 min) and Chloro Impurity-**A (6)** (RT): 31.545 min). The spectroscopic data of impurity-**A** to impurity-**G** were compared with those of Sildenafil Citrate.

### Formation of Related compounds

The chloro group attached to benzene ring of **(2)** undergoes chlorination under drastic conditions leads to impurity-**A**. This impurity was quantitatively synthesized by purging Hcl gas with **(2)**. The mass spectrum of impurity-**A** displayed a protonated molecule peak at MS *m/z* 346 [M+H]+ , 347 which 34 amu more than that of **(2)**.In $^{1}$H NMR spectrum, all signals are similar to that of **(2)** except an appreciable down field chemical shift of protons attached to benzene ring. Based on synthetic methodology and spectral analysis, the structure of impurity-**A** was characterized as-(5-chloro-2-ethoxyphenylyl)-1-methyl-3-propyl-1H- pyrazolo [4,3-d] pyrimidin-7(6H)-one.

The O-methyl group attached to compound **(3)** in methanol with sodium methoxide and leads to impurity-**B (7)**. The mass spectrum of impurity-**B (7)** displayed a protonated molecule peak at *MS m/z 406[M + H] +, 407* which 4 amu less than that of **(3)**. Replacement of chlorine atom to O-methyl group. In $^{1}$H NMR spectrum, all signals are similar to that of **(3)** except an appreciable up field chemical shift of methyl protons. Based on synthetic methodology and spectral analysis, the structure of impurity-**B (7)** was characterized as 4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6, 7-dihydro-1H-pyrazolo [4, 3-d] pyrimidin-5-yl) benzene sulfonic methyl ester.

The hydroxy group attached to compound **(3)** in wet solid due to drying, acidic material with water leads to impurity-C **(8)**. The mass spectrum of impurity-C **(8)** displayed a protonated molecule peak at MS *m/z* 392[M + H] +, 393which 18 amu less than that of **(3)**.Replacement of chlorine atom to Hydoxy group. In $^{1}$H NMR spectrum, all signals are similar to that of **(3)** except an appreciable up field chemical shift of hydoxy proton. Based on synthetic methodology and spectral analysis, the structure of impurity-C **(8)** was characterized as 4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6, 7-dihydro-1*H*-pyrazolo [4, 3-d] pyrimidin-5- yl) benzene sulfonic acid. The ethyl groups attached to oxygen atom on benzene ring of **(2)** undergo degradation under drastic conditions and leads to impurity-**D (11)**. This impurity was quantitatively synthesized by using

aqueous HBr to form compound **(9)**. Then chlorosulfonation of **(9)** to form **(10)** and condensation of **(10)** with *N*-Methyl piperazine **(4)** to form impurity-D **(11).**The mass spectrum of impurity-D **(11)** displayed a protonated molecule peak at MS *m/z* 446[M + H]+,447 which 29 amu less than that of Impurity **(2)**. In $^{1}$H NMR spectrum, all signals are similar to that of **(2)** except an appreciable down field chemical shift of protons attached to oxygen. Based on synthetic methodology and spectral analysis, the structure of impurity-**D (11)** was characterized as 5-(2-hydroxy-5-(4-methylpiperazin-1-ylsulfonyl) phenyl)-1-methyl-3-propyl-1*H*-pyrazolo [4, 3-d] pyrimidin-7(6H)-one. The impurity-**E (12)** is a peracid degrading impurity. The methyl group attached to nitrogen atom in piperazine moiety of (**5)** undergoes aerial oxidation under drastic conditions. This impurity was quantitatively synthesized by peroxide assisted oxidation of **(5).**The mass spectrum of (**12)** displayed a protonated molecule peak at *m/z* 491 which is 16 amu more than that of **(5)**. In $^{1}$H NMR spectrum, all signals are similar to that of **(5)** except an appreciable down field chemical shift of protons attached to terminal nitrogen of N-Methyl piperazine moiety. This suggested that methyl group attached nitrogen atom of N-Methyl moiety of **(5)** could be oxidized. Based on synthetic methodology and spectral analysis, the structure of impurity-**E (12)** 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.

The related substance impurity-**F** (**14)** formed in the synthesis of **(5)** due to traces of **(13).** This was synthesized by reacting **(3)** with **(13)** (Scheme-5). Mass spectrum of **(14)** displayed a protonated molecule peak at *MS m/z 833[M - H] +, 833*. The higher molecular weight suggests that impurity **(14)** could be dimer. The IR spectrum of **(14)** was similar to that of **(5).** In $^{1}$HNMR spectrum, piperazine ring methyl group protons are absent. Shows eight proton of piperazine ring, 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 **(3)** and its molecular weight was matched ion observed at *m/z* 835 in the mass spectrum. Based on the synthetic methodology and spectral analysis, the structure of **(14)** was characterized as Bis-1, 4-[4-ethoxy-3-(6, 7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo [4, 3-d] pyrimidi-5yl)-benzenesulfonyl] piperazine.

The related substance **(15)** impurity-**G** formed in the synthesis of **(5)** due to traces of **(13).** This was synthesized by reacting **(3)** with **(13)** (Scheme 5). Mass spectrum of **(15)** displayed a protonated molecule peak at MS *m/z* 460[M + H] +, 461. The molecular weight suggests that impurity **(15)** could be desmethyl impurity. The IR spectrum of **(15)** was similar to that of **(5)**.In $^{1}$HNMR spectrum, piperazine ring methyl group protons are absent. Eight proton of piperazine ring, shows attached with nitrogen. Based on the synthetic methodology and spectral analysis, the structure of **(14)** was characterized as5-(2-ethoxy-5-(piperazin-1-ylsulfonyl) phenyl)-1-methyl-3-propyl-1H-pyrazolo [4, 3-d] pyrimidin-7(6H)-one.

**Impurities Identification and Possible Pathways**.

As shown in Fig 2, impurity-**A** observed in some batches at RT 29.10 min was found to be present at levels greater than 0.05%. For identification its GC-MS has been taken which shows peak at [347] M+H and chlorine pattern so prepared impurity-**A** by purging HCl gas as shown in (scheme 2). Synthesized and characterized impurity was also confirmed by co-injecting in HPLC with the observed sample. Impurity-**B** is prepared by reacting chloro Compound (**2)** with methanol. Impurities **A** and **B** are the newly identified impurities of Sildenafil Citrate. These two impurities were believed to arise in the preparation of chloro compound **(3)** as shown in (Scheme 1). Impurity-**C** formed by heating chloro compound **(3)** with water. Impurities **D**, **E**, **F**, and **G** are structural analogues of (**1)**, where the N-methyl piperazine group has been replaced by chloro group. It is reasonable to believe that the ethyl moiety in impurity-**D** is vanished by drastic condition, the impurity-**D** was found to be present at levels greater than 0.05% in the crude product (**1)**. Impurities **E**, **F**, and **G** were present at lower concentrations. They are also caused by the presence of other impurities (piperazine) in the N- methyl piperazine (**4)** or peracid in solvents.

**Impurities Synthesis and Control**.

After identification of possible pathways for the formation of the impurities, the characteristics of the impurities which can influence the quality and safety of the drug were reevaluated. One path has been presented for the synthesis of all the investigated impurities. This model explains the quantities found and satisfies the analytical needs of regulatory requirements. However, the details of the formation of these impurities are not reported in previous studies, even though impurity-**A** has been identified as related

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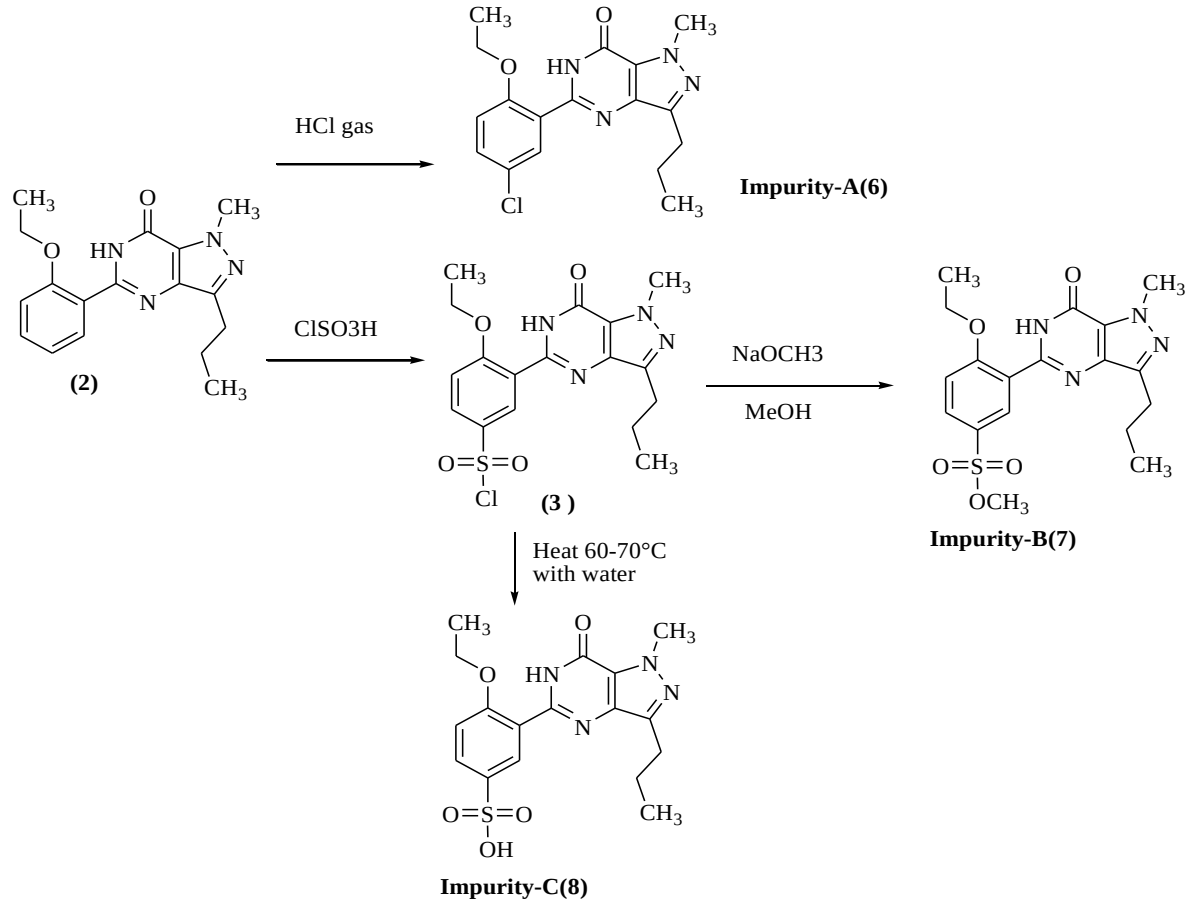
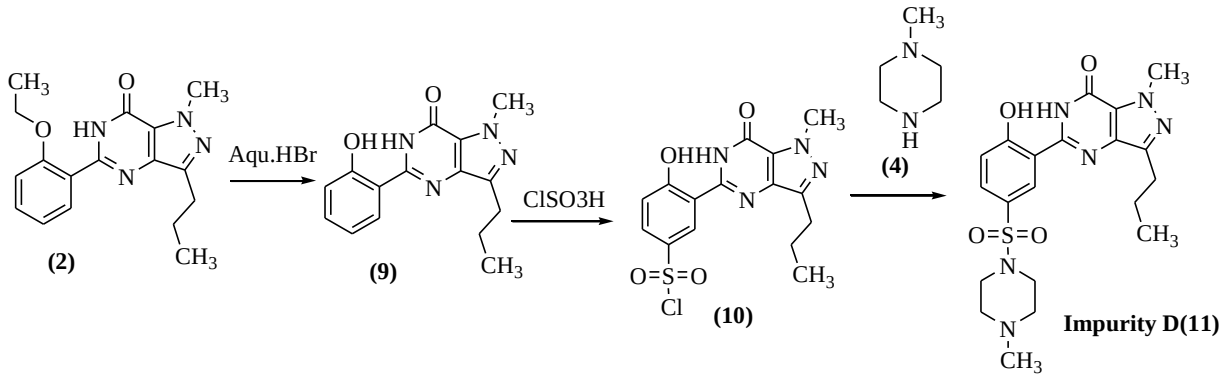
substance by GC-MS. Compared to the product (**1)**, replacement of the chloride moiety with chlorine or methyl ester can produce impurities **A** and **B**. It is desirable that impurities **A** and **B** can be synthesized from the same starting material (compound **2**) of product (**1**). As shown in (Scheme 2), Impurity-**C** formed by heating chloro compound **(3)** with water. Impurity-**C** is removed by water slurry in stage-1. Compound (**5)** was oxidized with $H_2O_2$ in methylene chloride at 0-5°C; impurity-**D** can be furnished by neutralization with aqueous sodium carbonate solution. Impurity-**E** was synthesized by treating compound (**2)** with Aqu.HBr for desethylation. Chlorosulphonation of Desethylated material quenched and isolated. Compound (**4)** added to the isolated compound in methylene chloride. Finally, the coupling of compound (**3)** with piperazine 0.5 mole and 1.0 mole in methylene chloride to give the desired impurity-**F** and **G** respectively. All the above impurities formed below 0.1 levels removed at Sildenafil Citrate salt isolation.




**Scheme2. Synthesis of impurity A, B and C**




**Scheme 3. Synthesis of impurities D**

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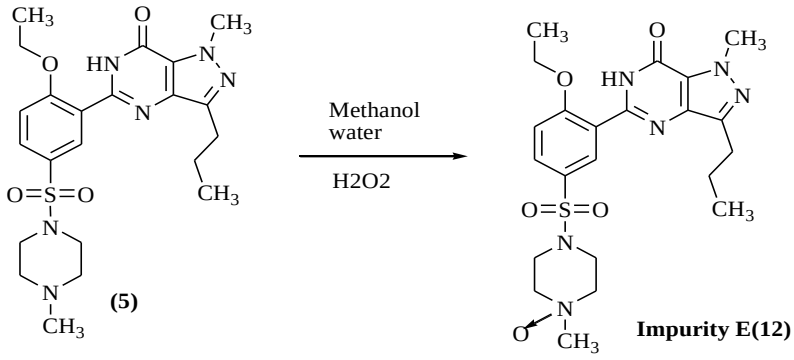
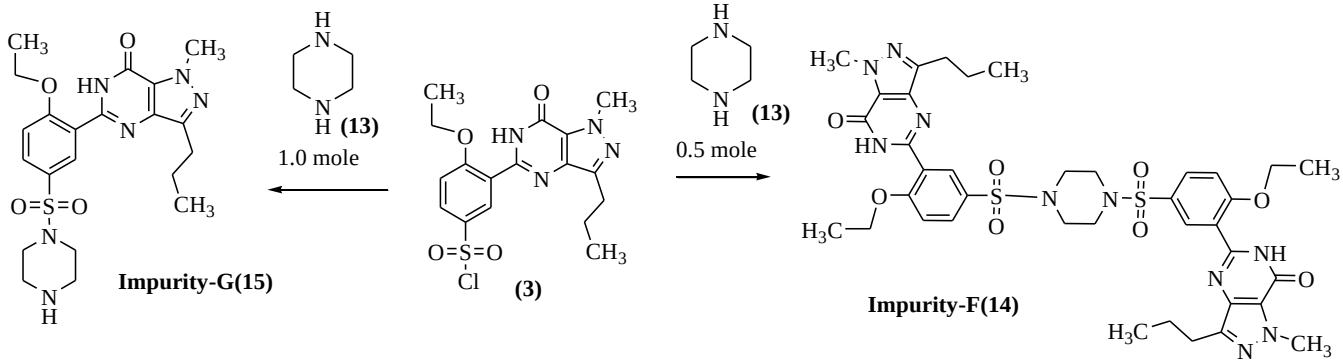



**Scheme 4. Synthesis of impurity E**




**Scheme 5. Synthesis of impurities F and G**

During the process development of sildenafil citrate (Scheme 1), the final API was observed with several process related impurities. To study the impurity profile of sildenafil citrate, an in-house HPLC gradient method was developed for the separation of sildenafil and its process related potential impurities. HPLC analysis of crude sildenafil revealed that there are seven major impurities associated with the product (**1**). To further substantiate the assigned structure, these compounds were independently synthesized (Scheme 2) and compared with the isolated samples. A typical HPLC chromatogram of sildenafil citrate spiked with seven impurities shown in Figure-**3**. To improve the safety and quality of the drug, the concentrations of these impurities must be reduced to levels accepted by the International Conference on Harmonization (ICH).

## EXPERIMENTAL

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Advance Spectrometer 300 MHz FT-NMR spectrometer with TMS as an internal standard. Chemical shift (d values) and coupling constants (*J* values) are given in ppm and Hz, respectively. ESI mass spectra were performed on Thermo Finnigan LCQ Classic Mass Spectrometer. Uncorrected melting points were determined on an electrothermal melting point apparatus. Solvents and reagents were used without any pretreatment. Reaction progress and chemical purity were evaluated by Gradient HPLC analysis using a column Hypersil BDS C-18, 5µm (250×4.6mm)or equivalent with a mobile phase consisting of A: Buffer 3.85 g ammonium acetate with 1000ml water pH=7.5 ±0.1 with ammonia solution and filter through 0.45µ filter paper. B: Water and acetonitrile (3:7, v/v), with a timed gradient program of T/%B: 0/35, 15/35, 30/80, 40/80, 45/35, 50/35, with a flow rate of 1 ml/min and UV detection at 240nm was used. This HPLC method was able to detect all the impurities.

All the procedures and spectral data has been incorporated in Supporting Information.

## CONCLUSION

In summary, seven observed potential impurities of Sildenafil Citrate (**1**) were synthesized by new and easy work up method and characterized by LC-MS, FT-IR, $^{1}H$ NMR and $^{13}C$ NMR techniques. The origins of

formation impurities **A–G** during the preparation of (**1)** were also mapped out. In addition, a strategy to lower the concentrations of these impurities to levels accepted by ICH is here proposed. This information would be immensely useful for process chemists working in this area.

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**REFERENCES**

[1] A.S. Bell, D.Brown, N.K. Terrett, U.S. Patent 5250534, 1993.
[2] *(a)* M. Boolell, M.J.Allen, S.A. Ballard, Int. J. Impot. Res. 8, 47 (1997).
(b) S. Gepi-Attee, G.J. Muirhead , A.M. Naylor , I.H. Osterloh , C. Gingell, Br. J. Urol. 78, 257 (1996)
[3] H.W. Hamilton, D.F. Ortwine, D.F. Worth, J.A. Bristol, J. Med.Chem., 30, 91 (1987).
[4] V. Braude, J. Aronhime, S. Shabat, U.S.Patent 0182066 A1, 2005.
[5] P.J. Dunn, Org.Process Res. Dev., 9, 88 (2005).
[6] (a) I. Goldstein et al. N. Engl. J. Med., 338, 1397 (1998).
(b) A. A. Badwan, L. Nabulsi, M.M. Al Omari, N. Daraghmeh, M. Ashour, (2001) in: G. Harry Briran (Ed.), Sildenafil Citrate Monograph, 27, Academic Press, New York 339.
[7] N.K. Terrett, A.S. Bell, D. Brown, P. Ellis, Bioorg. Med. Chem. Lett., 6, 1819 (1996).
[8] ICH harmonized tripartite guideline, Impurities in New Drug Substances Q3A (R2), current step 4th version, October 25, 2006. Communication
[9] (a) R.R. Hivarekar, S.S. Deshmukh, N.K. Tripathy, *Org.Process Res. Dev*., 16, 677 (2012).
(b) A.C. Mali, S.S. Ippar, M.B. Bodke, N.S. Patil, V.T Mathad,, Org. Process Res. Dev., 17, 863 (2013).
(c) S. Mohanarangam, B. Satyanarayana, R.C. Elati, B. Vijayabhaskar, P. Pratap Reddy, *P.* J. Chin. Chem. Soc., 58, 841 (2011).
[10] N. Daraghmeh, A.A. Badwan, M. Al Omari, A.M.Y. Jaber, J. Pharm. Biomed. Anal., 25,483 (2001)