

RESEARCH

Open Access



Identification, synthesis, and characterization of potential genotoxic impurities of sildenafil citrate drug substance

P. Rajesh Reddy^{1*}, Sivanadh Musunuri², D. Rama Sekhara Reddy², V. Subrahmanyam Chittala¹, V. N. S. Murthy P¹ and K. Krishnamohan¹

Abstract

Background: Sildenafil is a selective inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5). Sildenafil enhances the effect of nitric oxide by inhibiting phosphodiesterase type 5, which is responsible for the degradation of cGMP in the corpus cavernosum. The possible genotoxic impurities of sildenafil were synthesized, i.e., sildenafil sulfonyl methyl ester, sildenafil sulfonyl ethyl ester, and sildenafil sulfonyl isopropyl ester. The present work describes the synthesis and characterization of these sulfonyl ester compounds related to sildenafil.

Results: All the synthesized sildenafil sulfonyl esters have proved to be beneficial for the pharmaceutical industry in view of the regulatory importance.

Conclusion: A simple, efficient, and repeatable method was developed for the preparation of sildenafil sulfonyl esters in view of the regulatory importance of the potential genotoxic impurities in the active pharmaceutical ingredient. A detailed study of various impurities in sildenafil was conducted. Different process-related sulfonyl esters in sildenafil were identified, synthesized, and characterized by using various spectroscopic techniques like liquid chromatography-mass spectrometry (LCMS), mass, ¹H NMR, and FT-IR. These efforts to synthesize and characterize them effectively have proved to be beneficial.

Keywords: Sildenafil, Genotoxic impurities, Sulfonyl esters, Synthesis, Characterization

Background

Sildenafil is chemically known as 5-[2-ethoxy-5-(4-methylpiperazinylsulfonyl) phenyl]-1-methyl-3-n-propyl-1,6-dihydro-7H-pyrazolo [4,3-d] pyrimidin-7-one. Sildenafil is used for the treatment of erectile dysfunction in male and it is also used for hypertension, and its citrate salt **1** is marketed by Pfizer under the brand names Viagra® and Revatio®

Impurities present in an active pharmaceutical ingredient (API) will influence drug effectiveness by the change of quality and safety. Impurities more than 0.1% [1] should be identified and characterized as per the International Conference on Harmonization (ICH) guidelines. To perform, co-injection studies and analytical performance characteristic studies, for example, specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ), system suitability testing, and relative retention factor [2] impurities, are required.

In view of the regulatory importance of the genotoxic impurities [3–5] in the API, a detailed assessment study

* Correspondence: rajeshpeketi1979@gmail.com

¹Research and Development Department, Monvi Labs, 3rd floor, Plot No. 97, Rd. Number 9, ALEAP Industrial Area, Gajularamaram, Hyderabad, Telangana 500090, India




Full list of author information is available at the end of the article

on potential genotoxic impurities in sildenafil was conducted. The genotoxic structure evolution was further confirmed by Derek and Sarah analysis.

Mutagenic assessment for the synthetic route to sildenafil citrate

Mutagenic impurity risk assessment perspective, raw materials, reagents, solvents, by-products, related substances, intermediates, and degradation products from the synthetic process of sildenafil citrate were assessed for potential mutagenic assessment by structure activity relationship (SAR) screening using the expert rule-based software Derek Nexus and statistics-based software Leadscape.

The key raw materials, reagents, and impurities of sildenafil citrate have been assessed for structural alert using the DEREK and SARA software, and the details of sildenafil sulfonyl esters are provided in the following table:

S. no.	Name of the compound	In silico analysis for mutagenicity		
		Derek	Sarah	Leadscape
1.	 Sildenafil sulfonyl methyl ester (6)	Structural alert	Structural alert	Structural alert
2.	 Sildenafil sulfonyl ethyl ester (7)	Structural alert	Structural alert	Structural alert
3.	 Sildenafil sulfonyl isopropyl ester (8)	Structural alert	Structural alert	Structural alert

The daily dose of sildenafil citrate **1** is 100 mg for long-term treatment, mutagenic impurity control for individual impurity would be 15 ppm, and total impurities would be 50 ppm based on the threshold of toxicological concern (TTC) rule.

During the process development of sildenafil citrate **1** in the laboratory, we prepared possible, novel sildenafil sulfonyl-related esters. In the present work, the genotoxic impurities of sildenafil were synthesized and characterized by various spectroscopic techniques.

Methods

Solvents and reagents were obtained from commercial sources, and these are used without purification. Triethyl orthoformate was purchased from AVRA chemicals. Sildenafil sulfonic acid and sildenafil sulfonyl chlorides are the intermediates received from the Monvi Laboratories having purity > 99% by HPLC. ¹H NMR and ¹³C NMR spectral data were performed on Bruker Avance 300-MHz, 500 MHz spectrometer in DMSO-d₆ and CDCl₃. The chemical shift values were reported on the δ scale in parts per million, downfield from tetramethylsilane as an internal standard. IR spectra were recorded in the solid

state using a Perkin Elmer FT-IR spectrophotometer. The mass spectrum was recorded using a PerkinElmer PE SCIEX-API 2000. LCMS was recorded by using SCIEX LC-MS/MS system.

Methyl 4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6,7a-dihydro-3aH-pyrazolo[4,3-d]pyrimidin-5-yl)benzenesulfonate (sildenafil sulfonyl methyl ester **6**)

Method 1

To a suspension of sildenafil sulfonic acid **3** (10 g, 25.5 mmol) in trimethyl orthoformate (20 mL), methanol (20 mL) was added at room temperature and stirred the reaction mass at reflux for 24 h. After completion of the reaction, the mass was concentrated to remove the solvent. The resulting residue was dissolved in dichloromethane (50 mL) and washed with DM water (50 mL). Finally, the organic layer was washed with aqueous sodium bicarbonate solution (50 mL). Concentrated the resulting organic layer and crystallized with ethyl acetate to obtain which colored compound **6** (6.5 g, 63%)

Method 2

To a suspension of sildenafil sulfonyl chloride **4** (10 g, 25.38 mmol) in dichloromethane (50 mL), methanol (1.62 g, 50.76 mmol) and pyridine (8 g, 101.52 mmol) were added at room temperature and stirred for 24 h at 25–30 °C. The reaction mass was washed with water (100 mL), aqueous hydrochloric acid (100 mL), and saturated sodium bicarbonate solution (100 mL) followed by water (100 mL). The organic layer was concentrated and crystallized with ethyl acetate to obtain white compound **6** (5.2 g, 50% yield); IR (KBr pellet, cm⁻¹): 3338, 1703, 1356, 1255, 1180; ¹H-NMR (DMSO, 300 MHz): 0.92–0.96 (t, 3H, CH₃), 1.32–1.36 (t, 3H, CH₃), 1.73–1.75 (m, 2H, CH₂), 2.49–2.50 (t, 2H, CH₂), 3.76 (s, 3H, CH₃), 4.17 (s, 3H, N-CH₃), 4.22–4.24 (q, 2H, OCH₂), 7.40–7.43 (d, 1H, Ar), 8.00–8.02 (m, 2H, Ar); and HRMS for C₁₈H₂₂N₄O₅S: (M+H)⁺ calcd 407.1344 found, 407.1389.

Ethyl 4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6,7a-dihydro-3aH-pyrazolo[4,3-d]pyrimidin-5-yl)benzenesulfonate (sildenafil sulfonyl ethyl ester **7**)

Method 1

To a suspension of sildenafil sulfonic acid **3** (10 g, 25.5 mmol) in trimethyl orthoformate (20 mL), ethanol (20 mL) was added at room temperature and stirred the reaction mass at reflux for 24 h. After completion of the reaction, the mass was concentrated to remove the solvent. The resulting residue was dissolved in dichloromethane (50 mL) and washed with DM water (50 mL). Finally, the organic layer was washed with aqueous sodium bicarbonate solution (50 mL). Concentrated the resulting organic layer and crystallized with ethyl acetate to obtain which colored compound **7** (6 g, 56%)

Method 2

To a suspension of sildenafil sulfonyl chloride **4** (10 g, 25.38 mmol) in dichloromethane (50 mL), ethanol (2.3 g, 50.76 mmol) and pyridine (8 g, 101.52 mmol) were added at room temperature and stirred for 24 h at 25–30 °C. The reaction mass was washed with water (100 mL), aqueous hydrochloric acid (100 mL), and saturated sodium bicarbonate solution (100 mL) followed by water (100 mL). The organic layer was concentrated and crystallized with ethyl acetate to obtain white compound **7** (5 g, 47% yield); IR (KBr pellet, cm^{-1}): 3305, 1692, 1356, 1245, 1182; $^1\text{H-NMR}$ (DMSO, 300 MHz): 0.92–0.96 (t, 3H, CH_3), 1.21–1.26 (t, 3H, CH_3), 1.32–1.36 (t, 3H, CH_3), 1.73–1.75 (m, 2H, CH_2), 2.76–2.81 (t, 2H, CH_2), 4.12–4.24 (m, 7H, N-CH_3 and $2\times\text{OCH}_2$), 7.38–7.42 (d, 1H, Ar), 7.99–8.01 (m, 2H, Ar); MS m/z : 421.1561 $[(\text{M}-\text{H})^-]$ HRMS for $\text{C}_{19}\text{H}_{22}\text{N}_4\text{O}_5\text{S}:(\text{M}+\text{H})^+$ calcd 421.1501 found, 421.1564.

Isopropyl 4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6,7a-dihydro-3aH-pyrazolo[4,3-d]pyrimidin-5-yl)benzenesulfonate (sildenafil sulfonyl isopropyl ester **8)**

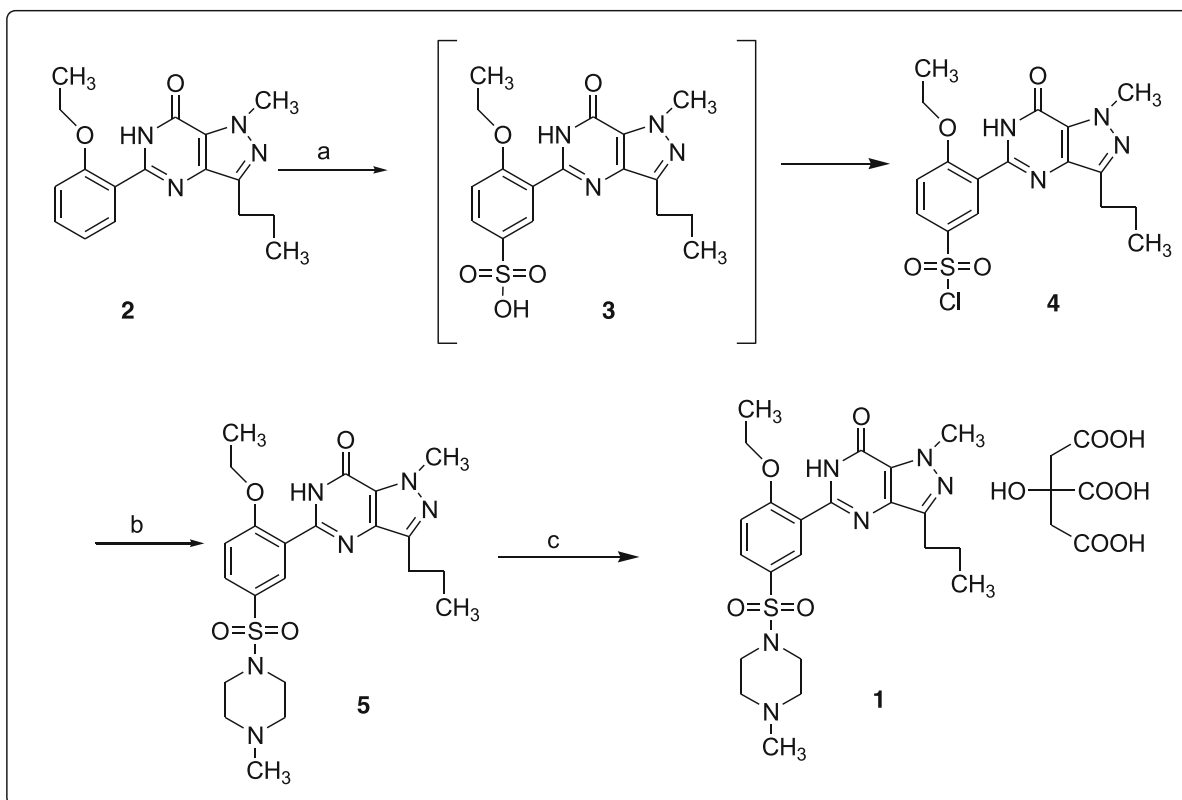
Method 1

To a suspension of sildenafil sulfonic acid **3** (10 g, 25.5 mmol) in trimethyl orthoformate (20 mL), isopropyl

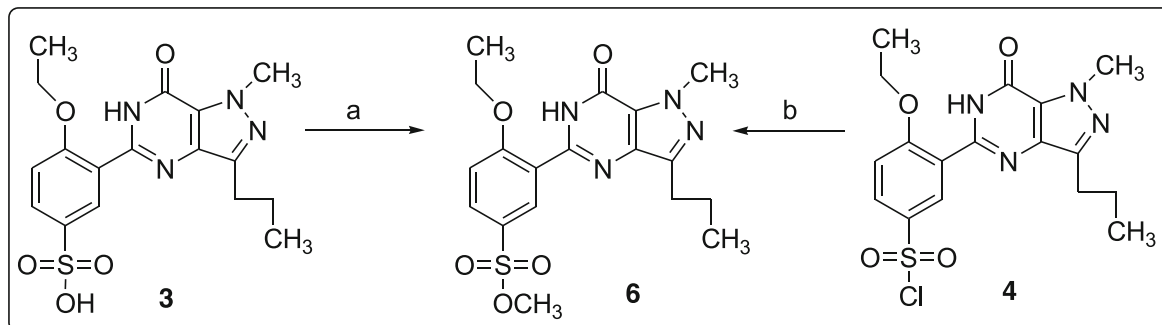
alcohol (20 mL) was added at room temperature and stirred the reaction mass at reflux for 24 h. After the completion of the reaction, the mass was concentrated to remove the solvent. The resulting residue was dissolved in dichloromethane (50 mL) and washed with DM water (50 mL). Finally, the organic layer was washed with aqueous sodium bicarbonate solution (50 mL). Concentrated the resulting organic layer and crystallized with ethyl acetate to obtain which colored compound **8** (6.5 g, 63%)

Method 2

To a suspension of sildenafil sulfonyl chloride **4** (10 g, 25.38 mmol) in dichloromethane (50 mL), isopropanol (3 g, 50.76 mmol) and pyridine (8 g, 101.52 mmol) were added at room temperature and stirred for 24 h at 25–30 °C. The reaction mass was washed with water (100 mL), aqueous hydrochloric acid (100 mL), and saturated sodium bicarbonate solution (100 mL) followed by water (100 mL). The organic layer was concentrated and crystallized with ethyl acetate to obtain white compound **8** (5.5 g, 50% yield); IR (KBr pellet, cm^{-1}): 3310, 1706, 1330, 1248, 1179; $^1\text{H-NMR}$ (DMSO, 300 MHz): 0.91–0.96 (t, 3H, CH_3), 1.23–1.25 (d, 6H, $2\times\text{CH}_3$), 1.32–1.36 (t, 3H, CH_3), 1.70–1.78 (m, 2H, CH_2), 2.76–2.81 (t, 2H, CH_2),



Scheme 1 Reported synthetic scheme of sildenafil citrate¹. Reagents and conditions: (a) chlorosulfonic acid, (b) *N*-methylpiperazine and dichloromethane; and (c) citric acid, water, and methanol



Scheme 2 Synthetic scheme of sildenafil sulfonyl methyl ester **6**. Reagents and conditions: (a) trimethyl orthoformate, methanol, and dichloromethane and (b) pyridine, methanol, and dichloromethane

4.17 (s, 3H, CH₃), 4.21–4.24 (q, 2H, OCH₂), 4.70–4.72 (m, 1H, CH(CH₃)₂), 7.37–7.40 (d, 1H, Ar), 7.98–8.01 (m, 2H, Ar); C₂₀H₂₆N₄O₅S:(M+H)⁺calcd 435.1657 found, 435.1703.

Results

Sildenafil citrate **1** has been synthesized by known literature methods [6–8]. Our process for the

synthesis of sildenafil citrate **1** is shown in Scheme 1. Sildenafil was prepared by reacting 5-[2-ethoxyphenyl]-1-methyl-3-*n*-propyl-1,6-dihydro-1*H*-pyrazolo [4,3-*d*] pyrimidin-7-one (sildenafil cyclized) **2** with chlorosulfonic acid to produce 5-(5-chlorosulfonyl-2-ethoxyphenyl)-1-methyl-3-propyl-1*H*-pyrazolo [4,3-*d*] pyrimidin-7(6*H*)-one (**4**, sildenafil

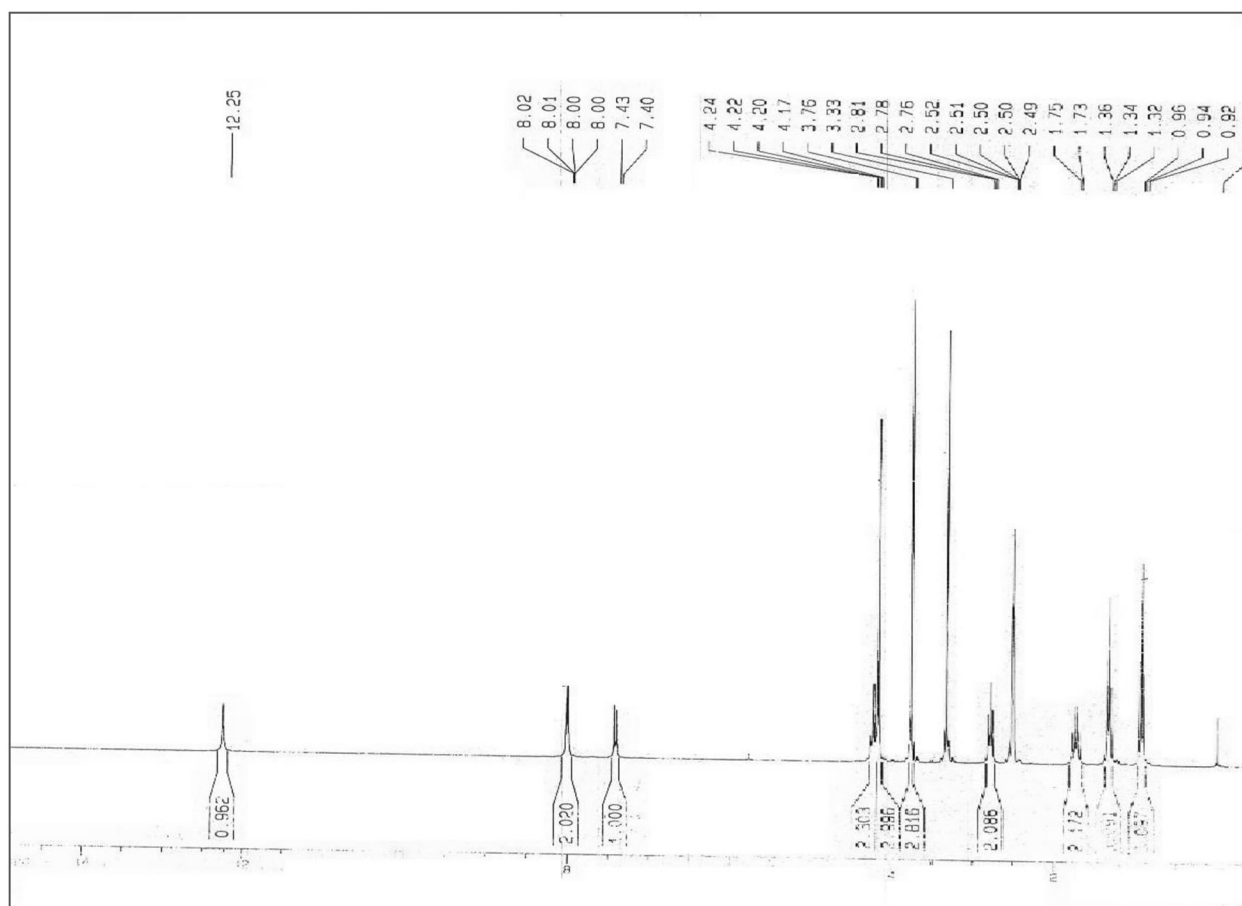


Fig. 1 NMR spectrum of sildenafil sulfonyl methyl ester

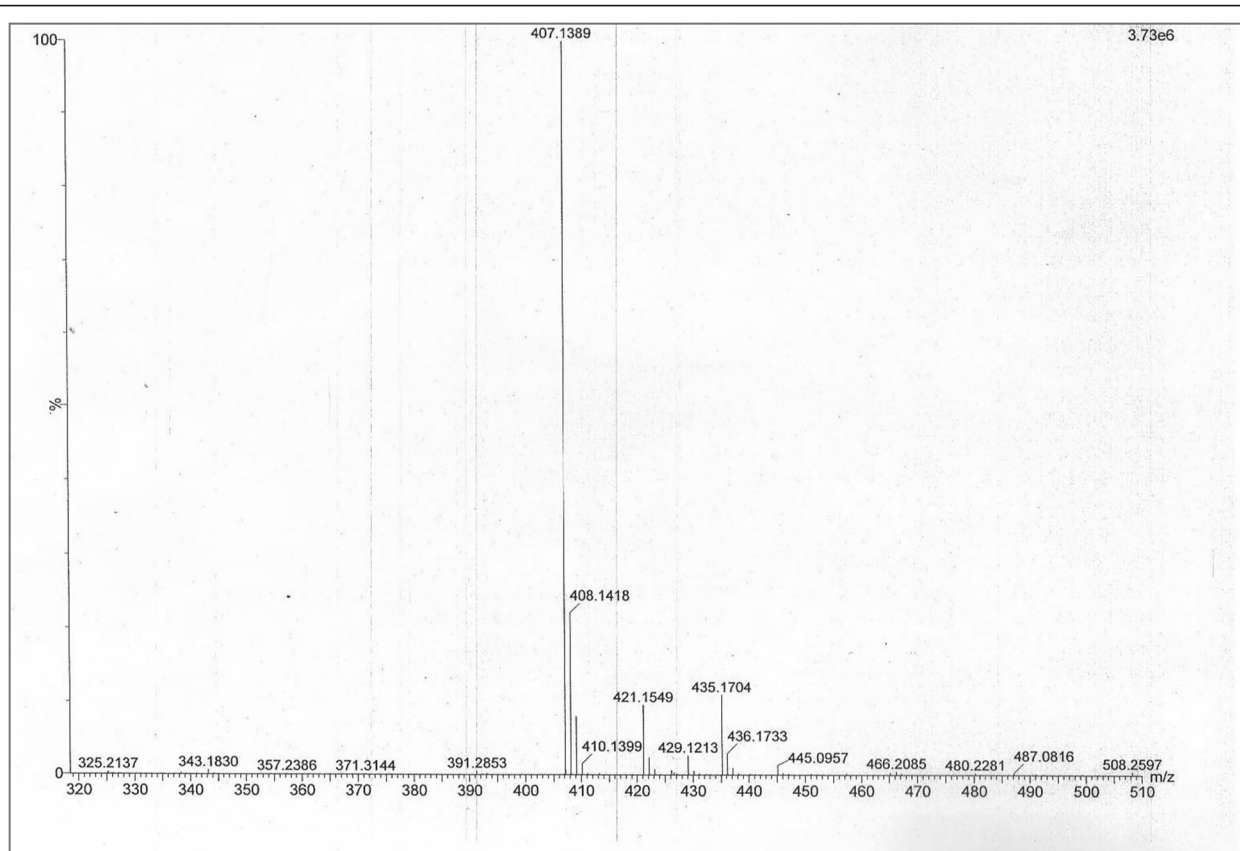
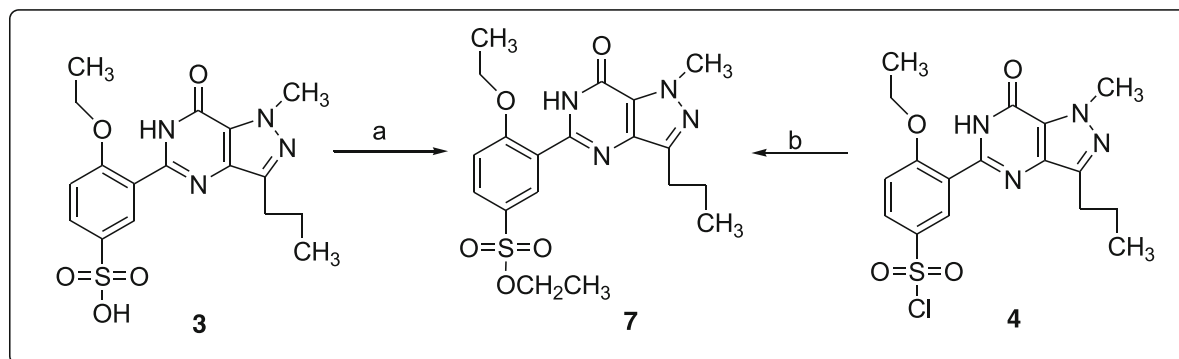


Fig. 2 HRMS spectrum of sildenafil sulfonyl methyl ester

sulfonyl chloride), which is further converted to 5-[2-ethoxy-5-(4-methylpiperazinylsulfonyl) phenyl]-1-methyl-3-n-propyl-1,6-dihydro-7H-pyrazolo [4,3-d] pyrimidin-7-one (sildenafil) **5** by reacting with *N*-methylpiperazine. Sildenafil is treated with citric acid in water to produce sildenafil citrate **1**.

Based on the synthetic process of sildenafil citrate, there is a possibility of the formation of sildenafil sulfonyl esters due to the usage of alcohol and its intermediates like sildenafil sulfonyl chloride (**4**) and sildenafil sulfonic acid (**3**). So many references [9–12] are available for sildenafil-related substances and its

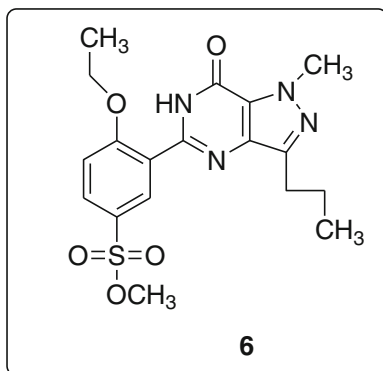


Scheme 3 Synthetic scheme of sildenafil sulfonyl ethyl ester **7**. Reagents and conditions: (a) trimethyl orthoformate, ethanol, and dichloromethane and (b) pyridine, ethanol, and dichloromethane

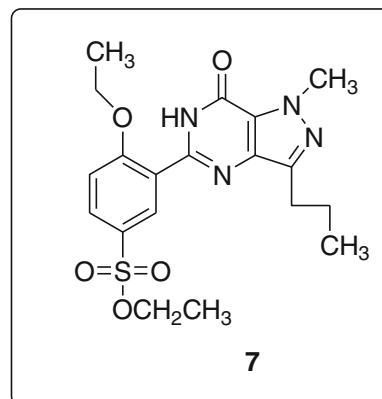
analogs. To the best of our knowledge, sildenafil sulfonyl ester identification and preparation are not reported anywhere until now.

The chemical names of the sildenafil sulfonyl esters are as follows:

1. Methyl 4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6,7a-dihydro-3aH-pyrazolo[4,3-d]pyrimidin-5-yl)benzenesulfonate (**6**, sildenafil sulfonyl methyl ester)



2. Ethyl 4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6,7a-dihydro-3aH-pyrazolo[4,3-d]pyrimidin-5-yl)benzenesulfonate (**7**, sildenafil sulfonyl ethyl ester)



3. Isopropyl 4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6,7a-dihydro-3aH-pyrazolo[4,3-d]pyrimidin-5-yl)benzenesulfonate (**8**, sildenafil sulfonyl isopropyl ester)

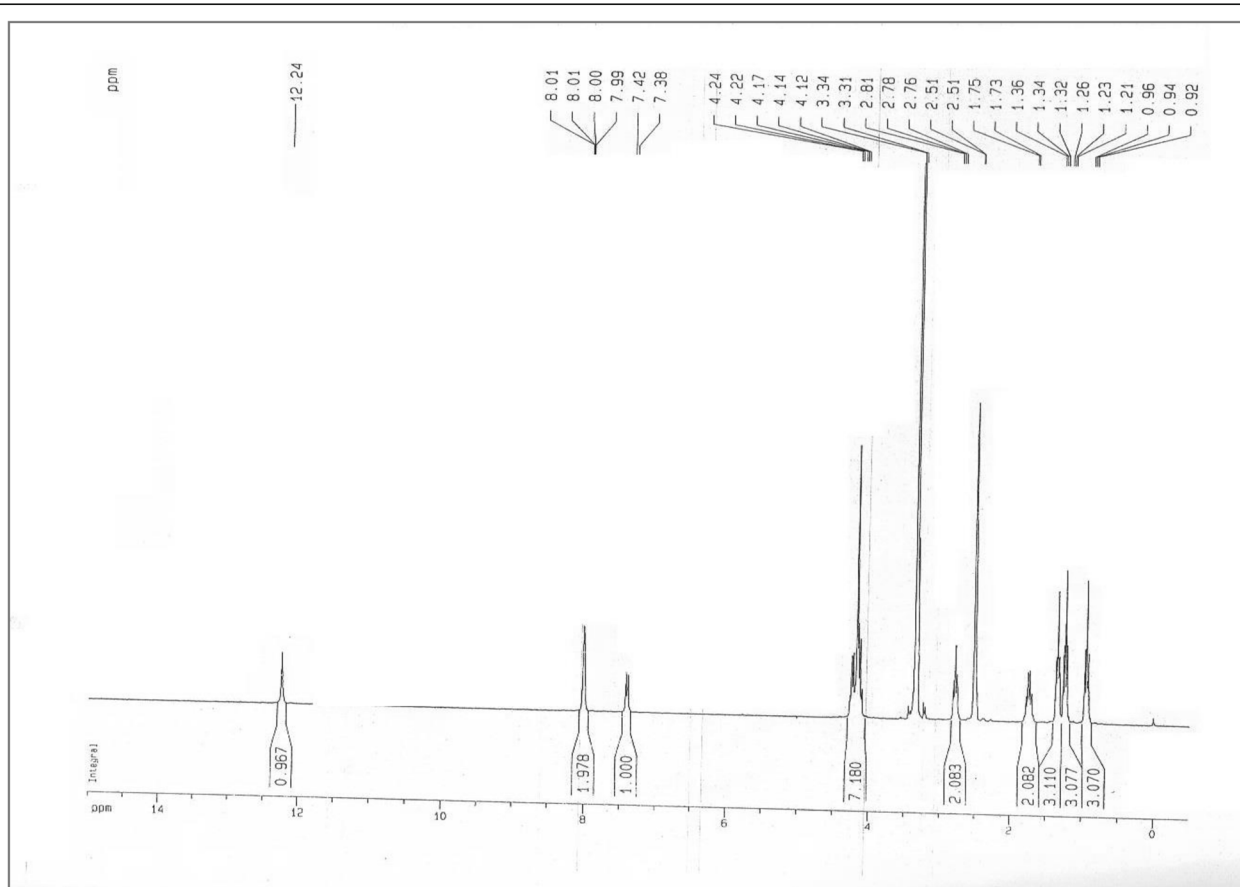
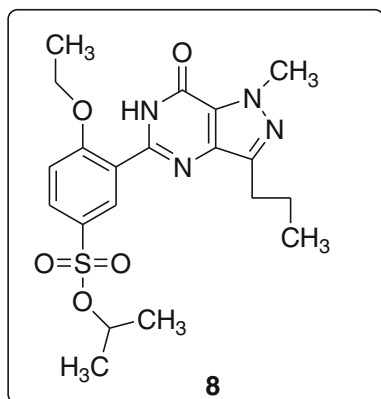


Fig. 3 NMR spectrum of sildenafil sulfonyl ethyl ester



Discussion

Methyl 4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6,7a-dihydro-3aH-pyrazolo[4,3-d]pyrimidin-5-yl)benzenesulfonate (sildenafil sulfonyl methyl ester 6)

Sildenafil sulfonyl methyl ester **6** was prepared in two ways, i.e., reacting sildenafil sulfonyl chloride **4** with methanol in the presence of pyridine in dichloromethane gives compound **6** and the other way [13] is treating sildenafil sulfonic acid **3** with trimethyl orthoformate in methanol (as shown in Scheme 2).

The mass spectrum showed a molecular ion at m/z 407.1389 amu $[(M+H)^+]$. The NMR spectrum showed a singlet at δ 3.76, corresponding to the OCH_3 , confirming the assigned structure **6** (Figs. 1 and 2).

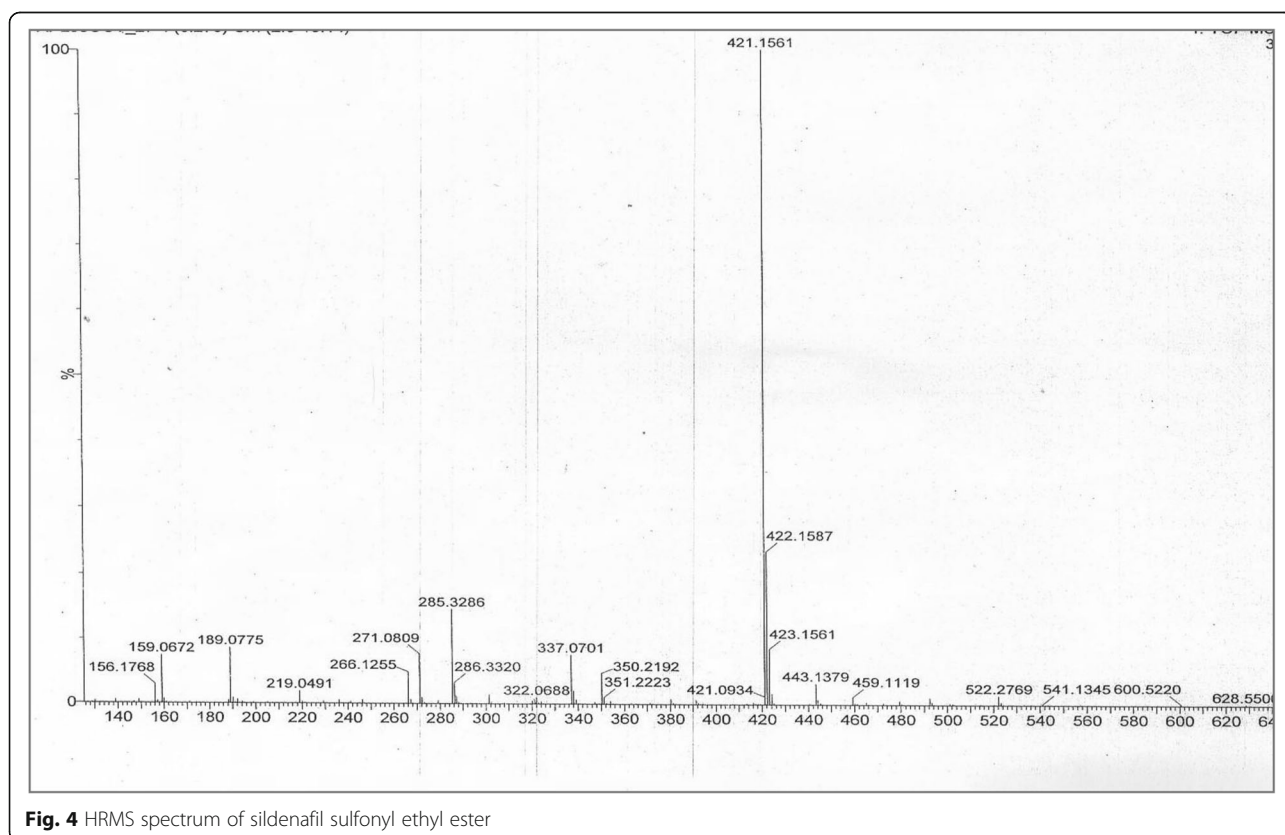
Sildenafil sulfonyl chloride may react with methanol during the preparation of sildenafil citrate and would result in the formation of sildenafil sulfonyl methyl ester.

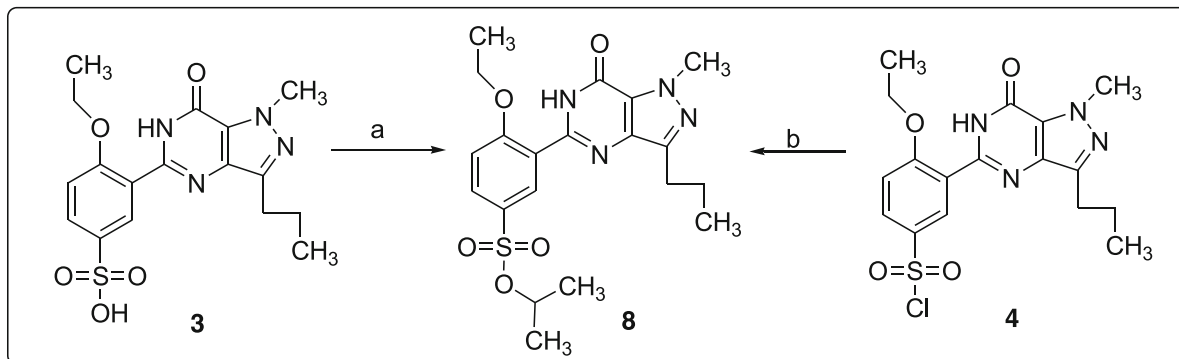
Sildenafil sulfonyl methyl ester **6** should be controlled to 15 ppm in sildenafil citrate drug substance based on the ICH M7 guidelines.

Ethyl 4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6,7a-dihydro-3aH-pyrazolo[4,3-d]pyrimidin-5-yl) benzenesulfonate (sildenafil sulfonyl ethyl ester 7)

Sildenafil sulfonyl ethyl ester **7** was prepared in two ways, i.e., reacting sildenafil sulfonyl chloride **4** with ethanol in the presence of pyridine in dichloromethane gives compound **7**, and the other way is treating sildenafil sulfonic acid **3** with trimethyl orthoformate in ethanol (as shown in Scheme 3).

The mass spectrum showed a molecular ion at m/z 421.1561 amu $[(M+H)^+]$. The NMR spectrum showed a triplet at δ 1.34 and quartet at δ 4.22 corresponding to the CH_3 and OCH_2 , respectively, confirming the assigned structure **7** (Figs. 3 and 4).





Scheme 4 Synthetic scheme of sildenafil sulfonyl isopropyl ester **8**. Reagents and conditions: (a) trimethyl orthoformate, isopropyl alcohol, and dichloromethane and (b) pyridine, isopropyl alcohol, and dichloromethane

Sildenafil sulfonyl chloride may react with ethanol during the preparation of sildenafil citrate and would result in the formation of sildenafil sulfonyl ethyl ester.

Sildenafil sulfonyl ethyl ester **7** should be controlled to 15 ppm in sildenafil citrate drug substance based on the ICH M7 guidelines.

Isopropyl 4-ethoxy-3-(1-methyl-7-oxo-3-propyl-6,7a-dihydro-3aH-pyrazolo[4,3-d]pyrimidin-5-yl) benzenesulfonate (sildenafil sulfonyl isopropyl ester **8)**

Sildenafil sulfonyl isopropyl ester **8** was prepared in two ways, i.e., reacting sildenafil sulfonyl chloride **4** with isopropyl alcohol in the presence of pyridine in

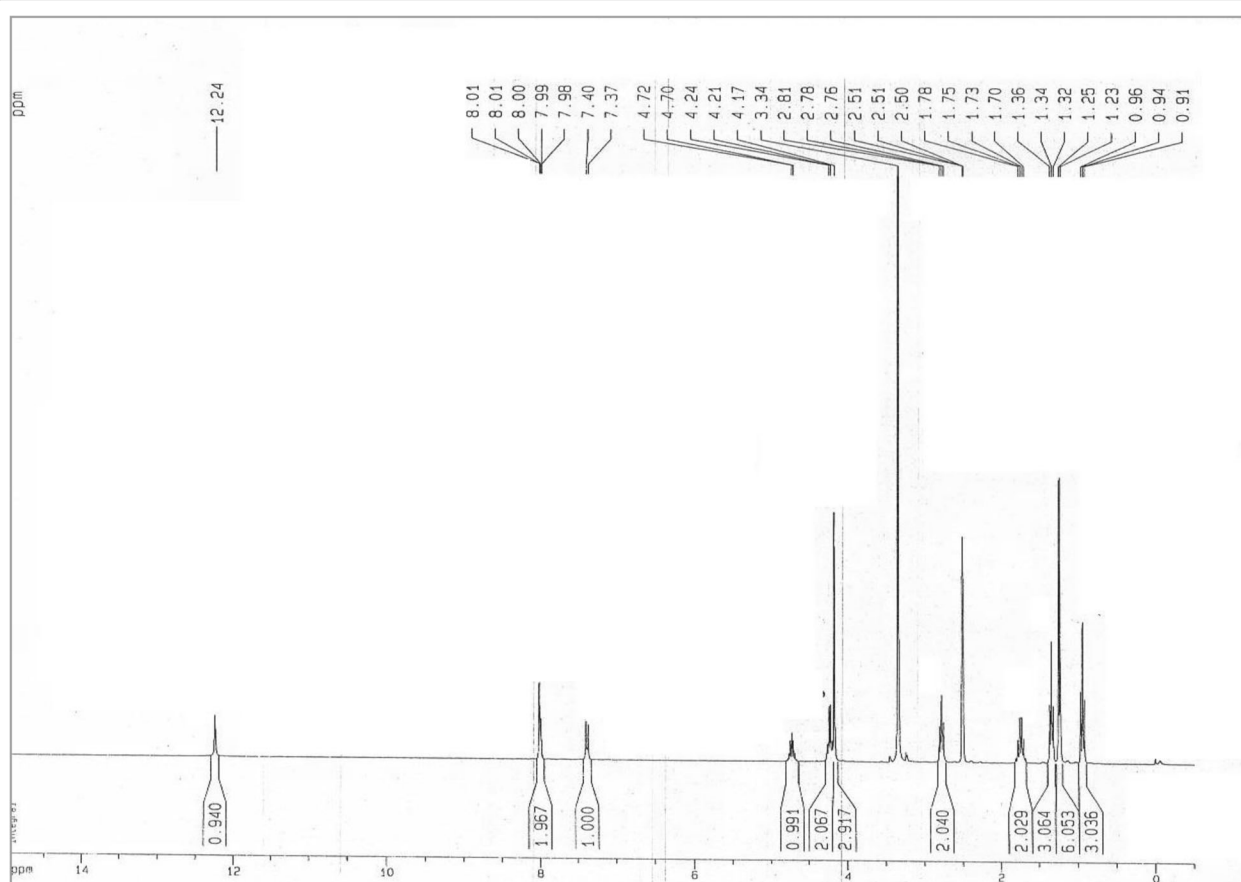


Fig. 5 NMR spectrum of sildenafil sulfonyl isopropyl ester

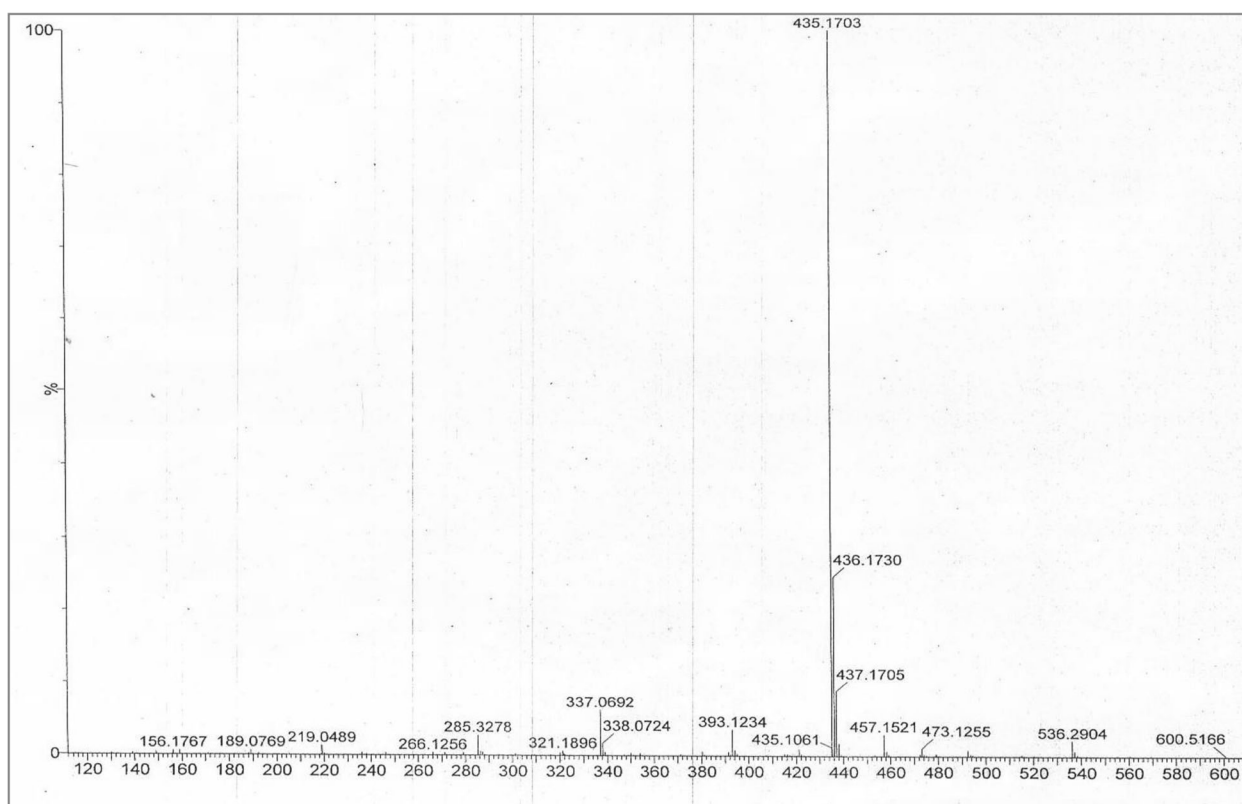


Fig. 6 HRMS spectrum of sildenafil sulfonyl isopropyl ester

dichloromethane gives compound **8**, and the other way is treating sildenafil sulfonic acid **3** with trimethyl orthoformate in isopropyl alcohol (as shown in Scheme 4).

The mass spectrum showed a molecular ion at m/z 435.1703 amu $[(M+H)^+]$. The NMR spectrum showed a doublet at δ 1.25 and multiplet at δ 4.72 corresponding to the $2 \times \text{CH}_3$ and OCH , respectively, confirming the assigned structure **8** (Figs. 5 and 6).

Sildenafil sulfonyl chloride may react with isopropanol during the preparation of sildenafil citrate and would result in the formation of sildenafil sulfonyl isopropyl ester.

Sildenafil sulfonyl isopropyl ester **8** should be controlled to 15 ppm in sildenafil citrate drug substance based on the ICH M7 guidelines

Further, sildenafil sulfonyl chloride (**4**) is also a potential genotoxic impurity, and it should be controlled to 15 ppm based on the TTC rule.

Conclusion

In conclusion, a detailed study of various impurities in sildenafil was conducted in view of the regulatory importance. Different process-related sulfonyl esters

in sildenafil were identified, synthesized, and characterized by using various spectroscopic techniques like liquid chromatography-mass spectrometry (LCMS), mass, ^1H NMR, and FT-IR. These efforts to synthesize and characterize them effectively have proved to be beneficial.

Acknowledgements

The authors wish to thank the management of Monvi Labs for carrying out this work and giving permission to publish it, and they are also thankful to the authorities of Krishna University and colleagues of the Analytical Research Department (ARD), Monvi Labs, for their cooperation.

Authors' contributions

RP performed the experiments and wrote the manuscript. SM and RD contributed to the research guidance. VS, VSNM, and KM contributed to the analytical evaluation. All authors read and approved the final manuscript.

Funding

No funding was available. This research did not receive any funding from funding agencies.

Availability of data and materials

All data provided in the manuscript is available upon request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

¹Research and Development Department, Monvi Labs, 3rd floor, Plot No. 97, Rd. Number 9, ALEAP Industrial Area, Gajularamaram, Hyderabad, Telangana 500090, India. ²Krishna University, Machilipatnam, Andhra Pradesh 521001, India.

Received: 3 March 2020 Accepted: 31 August 2020

Published online: 10 November 2020

References

1. International Conference on Harmonization (ICH) guidelines Q3A (R) impurities in New Drug Substances (2002) ICH guidelines, Geneva
2. International Conference on Harmonization (ICH) guidelines Q2B validation of analytical Procedure (1996) Methodology, Geneva
3. M7(R1) assessment and control of DNA reactive (mutagenic) impurities in pharmaceuticals to limit potential carcinogenic risk; ICH guidelines: 2018
4. Raman NVSSS, Prasad AVSS, Ratnakar Reddy K (2011) Strategies for the identification, control and determination of genotoxic impurities in drug substances: a pharmaceutical perspective. *J Pharm Biomed Anal* 55:662–667. <https://doi.org/10.1016/j.jpba.2010.11.039>
5. European medicines agency. Guideline on the specification limits for residues of metal catalysts or metal reagents, Doc. Ref. EMEA/CHMP/SWP/4446/2000, European Medicines Agency, 2008.
6. Bell A S, Brown D, Terrett N K. (1993) U.S. patent 5,250,534.
7. Dunn PJ (2005) OPRD. 9(1):88–97. <https://doi.org/10.1021/op040019c>
8. Raghavareddy A V, Srinivas G, Shantankumar K, Josephprabakar K, Sivakumaran M. Indian patent application no.2230/CHE/2010A.
9. Patel PV, Joshi N, Panchal DP (2012) *Heterocyclic Lett* 2(4):467–478
10. Saravanan M, Satyanarayana B, Pratapreddy P (2011) Synthesis and characterization of potential impurities of sildenafil. *Chem Biol Interface* 1(2): 177–184
11. Kumar IVS, Ramanjaneyulu GS, Bindu VH (2011) Synthesis of sildenafil citrate and process related impurities. *Lett Org Chem* 8(9):668–673
12. Ragahavareddy AV, Garaga S, Chandiran T, Badrinadhigupta P, Naidu A (2016) A facile, improved synthesis of sildenafil and its analogues. *Sci Pharm* 84:447–455. <https://doi.org/10.3390/scipharm84030447>
13. Padmapriya AA, Just G, Lewis NG (1985) A new method for the esterification of sulfonic acids. *Synth Commun* 15(12):1057–1062. <https://doi.org/10.1080/00397918508076842>

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Submit your manuscript to a SpringerOpen[®] journal and benefit from:

- Convenient online submission
- Rigorous peer review
- Open access: articles freely available online
- High visibility within the field
- Retaining the copyright to your article

Submit your next manuscript at ► [springeropen.com](https://www.springeropen.com)