EMPRO

Current Research in Pharmaceutical Sciences

Available online at www.crpsonline.com

ISSN: 2250 – 2688

Received: 29/01/2022
Revised: 20/02/2022
Accepted: 28/02/2022
Published: 06/04/2022

Shivanand Yadav and Neeraj Sharma
Faculty of Pharmacy, Bhagwant University, Ajmer, Rajasthan, India

Correspondence

Dr. Neeraj Sharma
Faculty of Pharmacy, Bhagwant University, Ajmer, Rajasthan, India

Email: neerajsharma236@gmail.com

DOI: 10.24092/CRPS.2022.120104

Website: www.crpsonline.com

Quick Response Code:

Development of Reverse Phase High Performance Liquid Chromatography Method for Simultaneous Estimation of Sildenafil Citrate and Depoxetine Hydrochloride in Pharmaceutical Formulation

Shivanand Yadav and Neeraj Sharma

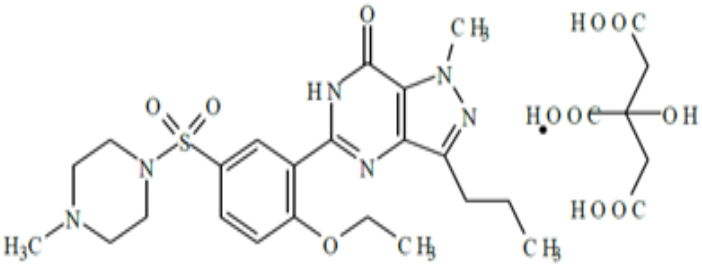
ABSTRACT

Simple, accurate and precise reversed-phase high-performance liquid chromatographic (RP-HPLC) methods for simultaneous estimation of sildenafil citrate (SIL) and depoxetine hydrochloride (DAP) in combined tablet dosage form have been developed and validated. The RP-HPLC method uses a Shimadzu – 1800, Software Version – UV Prob 2.33 with BDS hypersil C_{18} column and mixture of Buffer pH 4.0 and ACN in the ratio of 40:60 as the mobile phase. The detection was carried out using a UV–Visible Detector: Shimadzu SPD–20AT diode array detector set at 229 nm. Linearity of chromatographic method was found in the concentration range of 50 - 150 ppm for Sildenafil and 30 - 90 ppm for Dapoxetine respectively. % recovery for SIL was found to be 99.3 – 100.5 %**,** while for DAP it was found to be in range of 99.1 – 100.4 % in HPLC methods respectively. Both methods may be used for routine analysis of the drugs in a pharmaceutical formulation. Results of analysis were validated statistically.

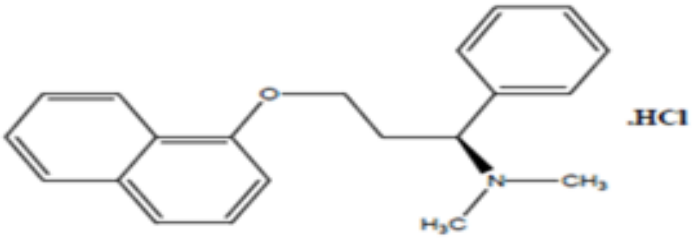
Key words: Linearity, Validation, HPLC, Sildenafil, Depoxetine

1. INTRODUCTION

Sildenafil Citrate (SIL) is chemically known as 1-[[3-(6,7-Dihydro -1-methyl- 7-oxo-3-propyl -1H-pyrazolo [4,3-d] pyrimidin-5-yl) -4-ethoxyphenyl]sulphonyl]-4-methyl piperazine citrate belongs to the selective inhibitor of cyclic guanosine monophosphate (cGMP)- specific phosphodiesterase type 5 inhibitor. Depoxetine Hydrochloride (DAP) is a new short-acting SSRI in development for the on-demand treatment of PE. It is believed to delay the timing of ejaculation via modulation of the expulsion reflex at a supraspinal level. The chemical structures of SIL and DAP are shown in Fig.1.



SIL



DAP

Fig. 1 Chemical structures of SIL and DAP

Literature survey revealed that several analytical methods have been reported for the determination of simultaneous determination of Tadalafil and Dapoxetine Hydrochloride in combined tablet dosage form. The principle for dual wavelength method is "the absorbance difference between two points on the mixture spectra is directly proportional to the concentration of the component of interest". The method was based on determination of Dapoxetine Hydrochloride at the absorbance difference between 280.0 nm and 295.4 nm and Tadalafil at the absorbance difference between 255.0 nm and 298.2 nm (Gunjan Amin *et al*. 2012.

This paper describes simple, accurate, precise, and sensitive reversed-phase (RP)-HPLC methods for simultaneous determination of SIL and DAP in a combined tablet dosage form. The proposed methods were optimized and validated according to International Conference on Harmonization (ICH) guidelines**.**

2. MATERIALS AND METHODS

2.1 Drugs and chemicals

2.1.1 Chemicals and Reagents Used

- Sildenafil cirtate (Standard)
- Dapoxetine hydrochloride (Standard)
- Triethyleamine (TEA)
- SUSTIMAX (Marketed Formulation)

- Sildenafil cetrate – 50 mg
- Dapoxetine hydrochloride – 30 mg (per tablet)

- Methanol (HPLC Grade)
- Acetonitrile (HPLC Grade)
- Ortho-phosphoric acid (HPLC grade)
- Water (HPLC grade)

2.1.2 Instruments

- Analytical balance (Mettler –Toledo - AG 285)
- UV–Visible Spectrophotometer (Shimadzu – 1800, Software Version – UVProb 2.33)
- Vacuum Pump (Samarth Instruments Pvt. Ltd.)
- Digital pH meter (Mettler –Toledo - 780)
- Ultrasonic Bath (Cadmach Ltd, India JAC 4020)
- Milli-Q water dispenser (Watpure – 4326)
- Whatman filter paper GF/A (Filter concept Pvt. Ltd.)
- Cellulose Acetate Filter, 0.45 µm (Filter concept Pvt. Ltd.)
- HPLC System
  - Liquid Chromatography: Shimadzu ChemStation - CHT 2010
  - UV–Visible Detector: Shimadzu SPD–20AT
  - Analytical Column:Luna C18 (250 mm × 4.6 mm, 5 µm)
  - Data Processor: Spinchrome CFR
  - Injector: Rheodyne – (Fixed Capacity Loop of 20 µl)
  - Syringe: Hamilton, 25 µl

2.2 Method: RP-HPLC method

The mobile phase was selected on the basis of best separation, peak purity index, peak symmetry, theoretical plate etc. So number of trials were taken for the selection of mobile phase. After trials Buffer : ACN (40:60) was selected.

(a) **S**tandard stock solutions: Standard stock solutions having concentration of 100µg/mL were prepared by dissolving drugs separately in the mobile phase.

(b) Preparation of the calibration curves: The linear regression analysis data for the calibration plots showed a good linear relationship over the concentration range of 0.25-4 µg/mL for Tadalafil and 0.75-12 µg/mL for Dapoxetine Hydrochloride respectively. The mean values of the correlation coefficient, slope

and intercept were 0.9995 ± 1.27, 133612 ± 0.72 and 49109 ± 1.21 for Tadalafil and 0.9992 ± 0.78, 155825 ± 0.87 and 132842 ± 0.54 for Dapoxetine Hydrochloride, respectively. The method was validated for precision, robustness and recovery.

(c) Procedure for analysis of tablet formulation: Twenty tablets of commercial tablets were taken and their average weight was determined, they were crushed to fine powder. Then powder equivalent to 10 mg of SIL (respective quantity of DAP) was taken in 50 ml volumetric flask and dissolved with mobile phase. The supernatant liquid was transferred to 100 ml of volumetric flask through a whatman #41 filter paper. After that 10ml of the above solution was diluted up to 100ml with mobile phase. The sample solution was injected, and the peak areas were recorded. A representative chromatogram is given in **Fig. 2**.

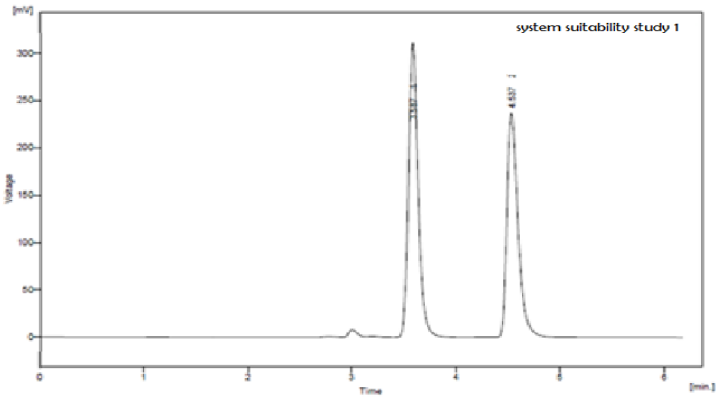


Fig. 2 Chromatogram of SIL and DAP in tablet dosage form

(d) Recovery studies: To study the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample at 3 different levels: 80, 100, and 120% of the target concentration.

(e) Precision: Precision of the method was checked using 3 replicate readings at 3 concentration levels of within range expressed as RSD values.

2.3 Statistical analysis

Means, standard deviation (SD), Relative standard deviation (RSD), and linear regression analyses were calculated using Microsoft Excel 2003.

3. RESULTS AND DISCUSSION

Analytical method development and simultaneous estimation of Sildenafil citrate and Dapoxetine hydrochloride and its validation by RP-HPLC, various mobile phases like water, ACN, and different pH buffer solutions were tried in various proportions. Different temperature, different flow rate and different columns were tried and finally optimized chromatographic conditions are shown below;

Optimized Chromatographic Conditions

- Column : BDS hypersil C_{18} (250 × 4.6 mm) 5µm
- Detector : 229 nm
- Injection Volume : 20 µL
- Flow Rate : 1.0 mL/min
- Temperature : 25ºC
- Run Time : 10 minute

Table 1 Regression analysis of calibration curves of method.

Parameters	**Method**	
	SIL	**DAP**
Intercept	34.1	34.2
Slope	20.10	29.87
SD[c]	2719.3	4588.1
LOD (ppm)	5.59	3.78
LOQ (ppm)	16.97	11.45

[a] Detection wavelength for HPLC method.
[b] y = mx + c, where y is the absorbance and x is the concentration (µg/mL).
[c] SD = standard deviation.

Table 2 System suitability parameters for RP-HPLC method.

Parameters	**SIL**	**DAP**
Calibration range, µg/mL	2-40	8-150
Theoretical plate number	7556	8786
HETP[a]	0.0073	0.0040
Tailing factor	1.52	1.86
Capacity factor (k')	0	1.78
Resolution	-	5.287

[a] HETP = Height equivalent to theoretical plate, cm

4. CONCLUSION

The proposed RP-HPLC methods were found to be simple, fast, accurate, precise, and sensitive. Thus, it may be used for routine analysis of SIL and DAP in combined tablet dosage form.

REFERENCES

1. Sethi PD. et al., HPLC Quantitative Analysis of Pharmaceutical Formulation. CBS Publishers & Distribution Pvt. Ltd., New Delhi, 1997; Vol-1: page no.1-32, 182-184.
2. Hobart H. Willard. Instrumental methods of analysis. CBS publishers & distributors, New delhi. 2004; 7th edition: PP:-1-5.
3. Mendham J, Denny RC, Barnes JD and Thomas MJK. Vogel's Textbook of Quantitative Chemical Analysis. India: Dorling Kindersley (India) Pvt. Ltd. 2008; 6th ed.: pp 5-11, 244-271.
4. Kar A.: Pharmaceutical Drug Analysis. New age international Pvt Ltd., New Delhi. 2005; 2nd ed.: pp. 1-4, 452-467.
5. Skoog DA. and West DM. Principles of Instrumental Analysis. Sauners college, Philadelphia. 1980; 2nd ed: pp.1-4, 690-699.
6. Analytical Chemistry, 2012. Available from: www.en.wikipedia.org/wiki/Analytical chemistry.
7. Chatwal G. R. and Anand S.: Instrumetnal Methods of Chemical Analysis. Himalaya Publishing House, Mumbai. 2000: pp 180-98.
8. Brown P. and Deantonis K.: Handbook of Instrumental Techniques for Analytical Chemistry. Prentice Hall. Inc., New Jersey. 1997; F. (ed.): pp.2-7.147-157.
9. Sharma BK. Instrumental Method of Chemical Analysis. GOEL Publishing House, New Delhi. 2006; 25th ed.: pp 678-682, 2.625-2.639.
10. Okafo GN and Roberts JK. Development of achiral separation method in Pharmaceutical analysis. Wiley India Pvt. Ltd., New Delhi. 2008: pp 90, 31-48.
11. Munson JB. Pharmaceutical Analysis Modern Methods. International Medical Book Distributors, Mumbai. 2001: pp 378.
12. Willard HH, Merritt LL, Dean JA and Settle FA. Instrumental Method of Analysis. CBS Publishers and Distributors, New Delhi. 2001;7th ed.: pp 170.
13. "High-performance liquid chromatography", November 2011. Available from:www.en.wikipedia.org/wiki/Highperformance liquid chromatography.
14. Schirmer RE. Modern Methods of Pharmaceutical Analysis. CRC Press, Floride. 2000; 2nd ed.: pp 67-72, 239-384.
15. Sethi PD. High Performance Liquid Chromatography: Quantitative Analysis of Pharmaceutical Formulations. CBS Publishers, New Delhi. 2001: p.13-4.