*Section A-Research paper*
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# Quantification of sildenafil citrate in bulk and dosage form by HPTLC by using hydrotropic agents

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## ABSTRACT

This work is concerned with the method development and validation of sildenafil citrate as an active pharmaceutical ingredient by HPTLC (high-performance thin-layer chromatographic) technique by using hydrotropic agents. The pre-coated silica gel 60 $F_{254}$ aluminum plate was selected as the stationary phase, and the solvent system consisted of 10 % sodium benzoate, 10 % sodium acetate, 3.5 % citric acid (5 : 3 : 2 v/v/v) was used as developing solvents. Analysis of sildenafil citrate was carried out at 295 nm and being detected at an $R_f$ of 0.72. The developed method was validated for various parameters such as linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), and robustness. The correlation coefficient of sildenafil citrate was found to be 0.996. The calibration plot was linear between 500-1000 ug/mL, respectively. The average percentage recovery of sildenafil citrate was found to be 99.83 %. Intra and inter-day precision measured as RSD was less than 2%.

**Keywords**: High Performance Thin Layer Chromatography, Sildenafil citrate, Validation, LOD, LOQ.

## INTRODUCTION

Erectile dysfunction (impotence; inability to achieve or maintain an erection) in men is treated with sildenafil (Viagra). In adults and children of 1 year age and older with pulmonary arterial hypertension (PAH; high blood pressure in the vessels supplying blood to the lungs, causing shortness of breath, dizziness, and fatigue), sildenafil (Revatio) is used to increase the ability to exercise. The drug sildenafil belongs to the class known as phosphodiesterase (PDE) inhibitors. Duringsexual stimulation, sildenafil improves erectile dysfunction by boosting blood flowto the penis. An erection may result due to increased blood flow. In order to facilitate easy blood flow, sildenafil stimulates PAH by relaxing the blood vessels in the lungs [1]. The structure of sildenafil is shown in figure 1. The chemical name of sildenafil is 5-[2- ethoxy-5-(4-methylpiperazin-1- ylsulfonyl)phenyl]-1-methyl-3-propyl-1,6- dihydro-7H- pyrazolo[4,3-d]pyrimidin-7-oneand its formula is $C_{22}H_{30}N_6O_4S$.[2]

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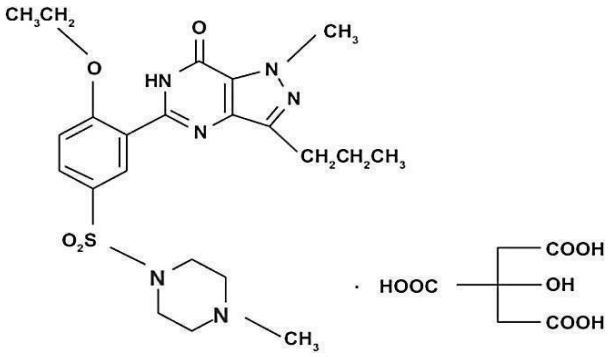

Figure 1. Structure of sildenafil [4]

Hydrotropes are surface-active, water-soluble chemicals that have a major impact on the solubility of pharmaceuticals which are poorly soluble, most likely as a result of the creation of organized assemblies in the solution. Due to the minimal hydrophobic portion of the molecule, problems associated with conventional surfactant solubilization, such as emulsification, do not occur with hydrotropes. Although many hydrotropes exhibit no indications of self-aggregation even at high concentrations, some may aggregate stepwise. Improvements in solubility of 1,000–10,000 times is possible. The requirement for employing high concentrations, which are frequently many moles in magnitude, is one of potential drawback of hydrotropes [3].

Rational and need of establishment of HPTLC has clearly defined in official guidelines at International conference of Harmonization (ICH)[5]. Previously there is no research paper published on the HPTLC method development and validation of Sildenafil Citrate by Hydrotropic Agents. So it is necessary to validate HPTLC method by hydrotropy. Hydrotropic agents are environment friendly, less hazardous as compared to typical organic solvents like methanol, toluene, ethyl acetate and are not volatile. They increase solubility by salt in and salt out technology.

## MATERIAL AND METHODS

### Chemicals and reagents

Sildenafil citrate working standard of pharmaceutical grade was procured from Cipla PVT Ltd, Mumbai. Allother required chemical's such as nicotinamide , sodium benzoate, sodium acetate, citric acid, distilled water was used from institute.

### Instrumentation

Chromatography was performed on 20 x 10 cm Aluminum TLC plates 60F254 precoated with 250 µm layersof silica gel. Samples were applied in the form of bands, under a continuous flow of nitrogen, by means of a Camag Linomat V (Switzerland) sample applicator fitted with 100 µL applicator syringe (Hamilton micro-syringe). A constant application rate of 0.1 µL per second was used and the distance between the adjacent bands that is 20mm was optimized. The plates were then conditioned for 10 minutes in a presaturated twin-trough glass chamber (20 x 10 $cm^2$). Saturation time was 30 minutes. The spotted plate was then dipped in mobile phase (10% sodium benzoate: 10% sodium acetate: 3.5% citric acid (5:3:2 v/v/v) and ascending development was performed to a distance of around 80 mm from the point of application at ambient temperature. Subsequently, plates were dried in a current of air without the help of

an air dryer, and spots were visualized in Camag UV cabinet copper formed at 263 nm with Camag TLC scanner operated in reflectance-absorbance mode and controlled by Win Cats software. The slit dimensions(4 x 0.2 mm) were also optimized and kept constant throughout the analysis**.**

### Chromatographic conditions

Following are suitable chromatographic conditions maintained for suitable RF and spotting. Standard solutions were injected by pressure by using sample applicator: 3 µL First position X = 13.00 mm Position Y =

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9.00 mm solvent front position = 80 mm Plate format = 200 x 100 mm Track distance = 20mmBand length = 8 mm saturation time = 30 min.

**Preparation of Standard**

Firstly 5 % w/v of Nicotinamide was prepared by dissolving 2.5 grams of nicotinamide in 50 ml of distilled water. The final concentration of this solution will be 5 % weight by Volume. The stock solution of Sildenafil Citrate was prepared by dissolving accurately about 10mg of Sildenafil with 10 mL of 5%w/v of Nicotinamide . Aliquots of this solution were suitability diluted with methanol to get working standard solutions of Sildenafil citrate with a 500µg/mL concentration. A calibration curve was plotted between concentrations against their respective area for Sildenafil The calibration curve found that Sildenafil citrate has a linearity range between 500-1000 ug/mL concentration [6].

**Preparation of sample solution**

25 tablets of 250 mg each containing 50 mg of sildenafil citrate were weighed, opened and the powder was weighed equivalent to 100 mg (400 mg with excipients). Powder was taken into a 100 ml clean volumetric flask and to which about 50 ml of 5% w/v Nicotinamide was added. Flask was sonicated up to10 min to completely dissolve the solids and then diluted up to the mark with diluent. The final concentration of was the stock solution was 1000 ug/ml. Then further dilution was done to 9 ml to 10 ml [6,7].

**Solubility study**

It was found that sildenafil having a crystalline structure. It was very soluble in 5% Nicotinamide .Trials of different hydrotropic agents were taken with different concentrations

**Solubility trials**

Table 1. Solubility trials

| SN | Solvent | Inference |
|---|---|---|
| 1 | 1 % sodium benzoate | Not soluble |
| 2 | 2% sodium benzoate | Not soluble |
| 3 | 2% sodium benzoate | Not soluble |
| 4 | 4% sodium benzoate | Not soluble |
| 5 | 5% sodium benzoate | Stability issue |

**Optimization and development of mobile phase**

Using the systematic mobile phase optimization three mobile phases were selected i.e., 10% sodium benzoate-10%, sodium acetate-3.5%, citric acid (5:3:2 v/v/v) whereby the latter resulted in the best separation. The optimal wavelengths of 366 nm for sildenafil were obtained by spectra recording.

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**Mobile phase optimization and development process**

Table 2. Optimization of mobile phase

| SN | Mobile Phase | Ratio | Spotting | Inference |
|---|---|---|---|---|
| 1 | 10% sodium benzoate + 10% sodium acetate + 5 % sodium caprylate | 5:3:2 | No spotting | Nil |
| 2 | 10% sodium benzoate + 10 % sodium acetate + 1 % citric acid | 5:3:2 | No spotting | Nil |
| 3 | 10% sodium benzoate + 10 % sodiumacetate + 1.5% citric acid | 5:3:2 | No spotting | Nil |
| 4 | 10% sodium benzoate + 10 % sodiumacetate + 2 % citric acid | 5:3:2 | No spotting | Nil |
| 5 | 10% sodium benzoate + 10 % sodiumacetate + 3% citric acid | 5:3:2 | Fronting | Nil |
| 6 | 10% sodium benzoate + 10 % sodiumacetate + 3.5 % citric acid | 5:3:2 | Clear Spotting | Detection at 0.7 Rf Value |

**Selection of working wavelength (max)**

The UV spectrum of 0.5 µg/ml of sildenafil in 5% w/v nicotinamide spectrum was recorded by scanning in the range (200 nm to 400 nm) From the UV spectrum wavelength selected as 263 nm. The spectrum was shown in Figure 3 [8].

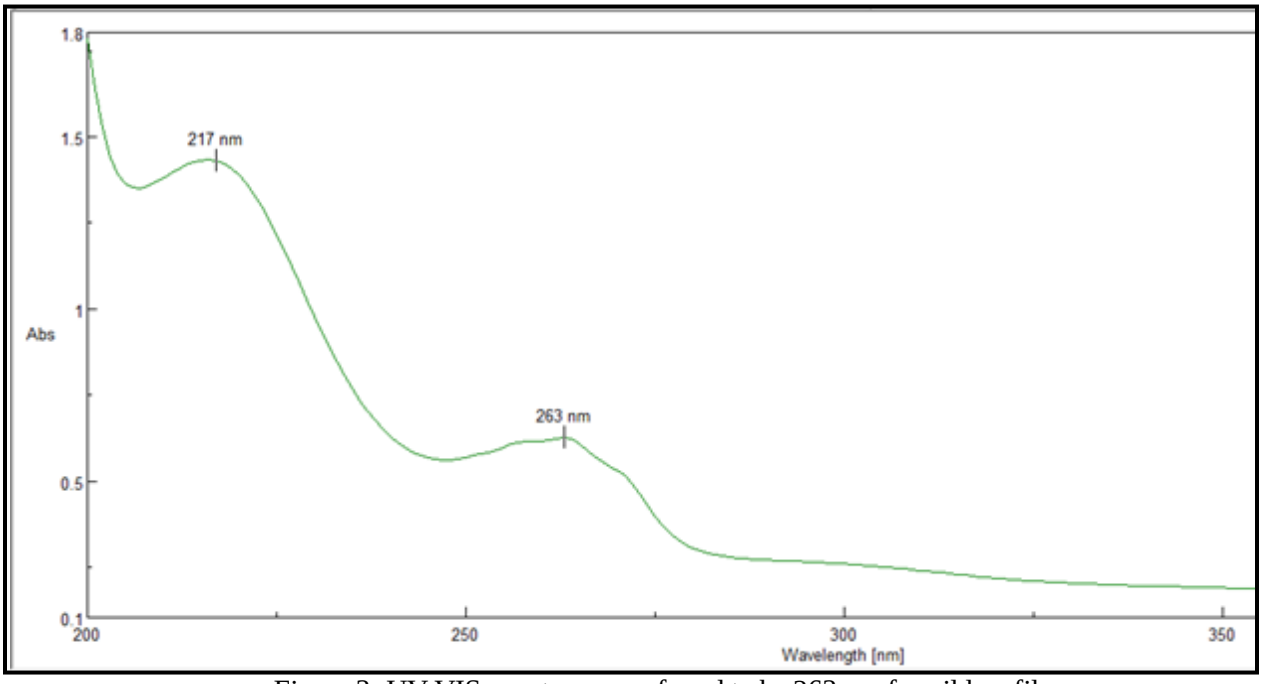


Figure 3. UV VIS spectrum was found to be 263 nm for sildenafil

**Method Validation Linearity**

The linearity of the method was done at five equal frequent concentrations by diluting the standard stocksolution to give solution over the range of 500-1000 µg/ml of sildenafil. A calibration curve was

constructed at six linear concentrations of sildenafil (500 to 1000 µg/mL). Solutions were injected into the chromatographic system, after getting the results plotted a graph concentration versus an area to evaluate correlation coefficient [9]. Acceptance criteria: Correlation coefficient not less than 0.99.

**Accuracy and Recovery**
Three levels of accuracy solutions (80%, 100%, and 120% accuracy) are prepared. The preparations were made separately for each level. 80%, 100% &120% In the same way that samples were prepared, solutions were made with various medication weights and a constant concentration of sample preparation. Acceptance standards: % the ideal recovery range is 98.0–102.0. Acceptance criteria: The recover values for the mean of three preparations should have a % RSD of 2.0 [10].

**Precision**
Using the six different sample preparations as described above, precision and validation parameters were tested. Six samples were separately injected into the chromatographic apparatus, and the individual sample assay percentage was calculated. Intermediate precision (intraday and interday) studies and repeatability studies were also performed. Acceptance criteria: % assay should be 95.0 to 105 & % RSD for six preparations assay should be ≤ 2.0 [8]
.

**Robustness**
Small but intentional changes were made to the optimized method parameters to test the suggested technique's robustness. The effects on the $R_f$ value of medications were investigated by making minor adjustments to the mobile phase composition, volume, and time of chamber saturation with the mobile phase. A small (0.1 mL for the component) alteration was made to the mobile phase's chemical makeup. The robustness of the procedure was tested in accordance with ICH guidelines [10]. Acceptance criteria: System suitability should be within the acceptance criteria.

**Limit of LOD and LOQ**
The LOD and LOQ of the developed method were correlated by using the standard method [9]. Acceptancecriteria: ≤ 2

## RESULT AND DISCUSSION

**Linearity**
The HPTLC method was optimized for validation and method development of sildenafil. The mobile phase 10% sodium benzoate: 10% sodium acetate: 3.5% citric acid (v/v/v) resulted in very good resolution and sharp peaks of RF 0.72 for sildenafil. It was observed that, methanol followed by drying and activation andpresaturation of TLC chamber with mobile phase for 30 mins confirmed greater repeatability results. The linearity graph was shown in figure 4

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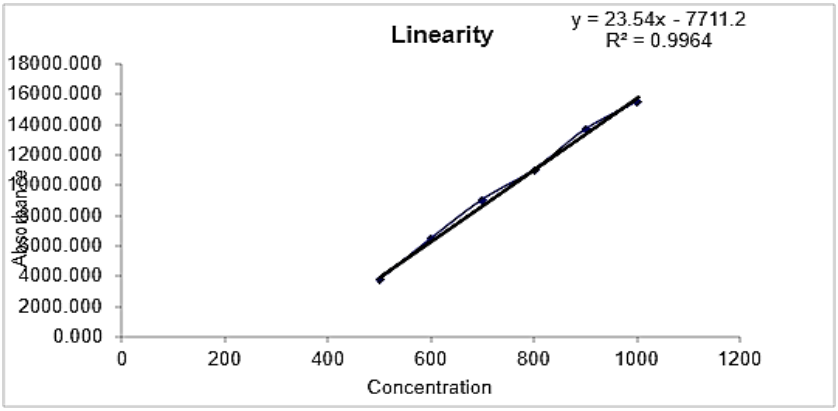


Figure 4. Linearity Graph

Table 3. Calibration parameters

| | |
|---|---|
| **Equation** | **y = 23.54x-7711.2** |
| **Slope** | **23.54** |
| **Intercept** | **7711.2** |
| **Regression** | **0.9964** |

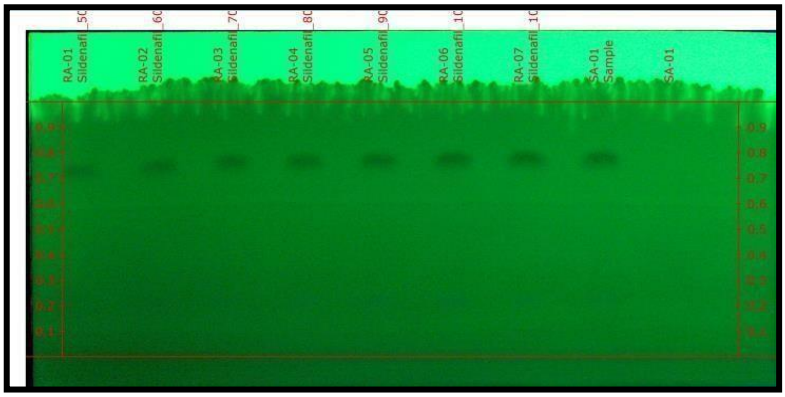

Figure 5. TLC plate of sildenafil detection

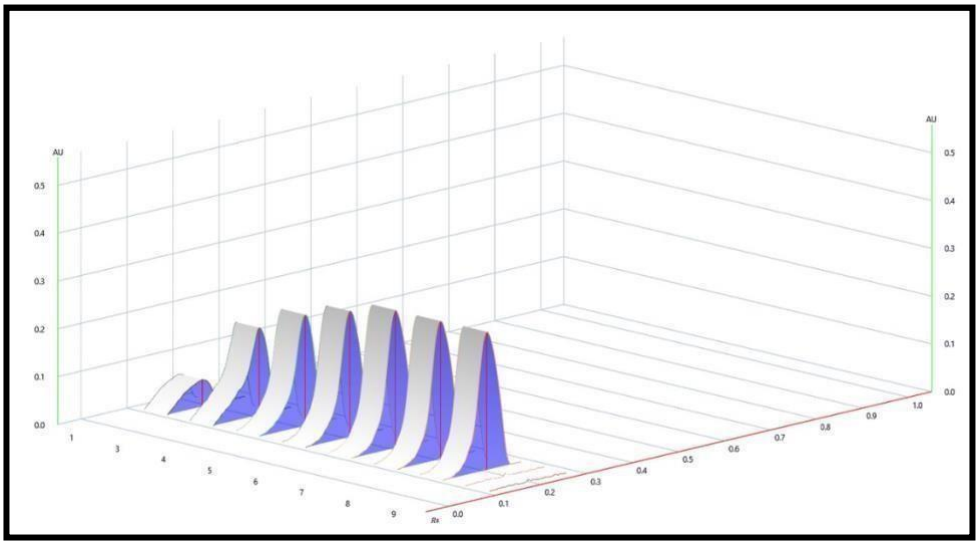

Figure 6. 3D graph of sildenafil having linearity

**Accuracy**

To determine the accuracy of the said methods accuracy study has been carried out as per according toICH guidelines. The determination was performed in triplicate at each level as shown in table 4.

Table 4: Recovery study

| SN | Level (%) | Amount taken (µg/ml) | Amount added (µg/ml) | Abs Mean±SD | Amount recovered Mean±SD | % Amount recovered Mean±SD |
|---|---|---|---|---|---|---|
| 1 | 80 | 700 | 560 | 21995.67±0.01 | 560.75±0.30 | 100.17±0.94 |
| 2 | 100 | 700 | 700 | 25195.667±0.00 | 696.88±0.14 | 99.55±0.35 |
| 3 | 120 | 700 | 840 | 28524±0.00 | 838.28±0.14 | 99.79±0.29 |

**Precision**

The precision study was performed as per ICH guideline acceptance criteria for each parameter is given above.

**Intraday Precision**

Table 5. Intraday precision

| **Conc** | **Intraday** | | |
|---|---|---|---|
| | **Mean ± SD** | **Amount found** | **% Amount found** |
| 700 | 9116.00±0.00 | 713.1670337 | 101.8810048 |
| 800 | 10277.33±0.01 | 762.3510644 | 95.29388305 |
| 900 | 13720.00±0.01 | 908.1526343 | 100.9058483 |

**Interday precision**

Table 6. Interday precision

| Conc | Intraday | | |
|---|---|---|---|
| | **Mean ± SD** | **Amount found** | **% Amount found** |
| 700 | 9047±0.00 | 710.27 | 101.47 |
| 800 | 10795.33±0.01 | 784.29 | 98.04 |
| 900 | 13673±0.01 | 906.18 | 100.69 |

**Repeatability**

Table 7. Repeatability results

| Repeatability | | | | |
|---|---|---|---|---|
| **SN** | **Conc** | **Area** | **Amount found** | **% Amount found** |
| 1 | 700 | 9006 | 710.16 | 101.45 |
| 2 | 700 | 8915 | 706.30 | 100.90 |
| 3 | 700 | 8740 | 698.86 | 99.84 |
| 4 | 700 | 8970 | 708.63 | 101.23 |
| 5 | 700 | 8770 | 700.14 | 100.02 |
| 6 | 700 | 8844 | 703.28 | 100.47 |
| | | Mean | 704.56 | 100.65 |
| | | SD | 4.58 | 0.65 |
| | | % RSD | 0.65 | 0.65 |

**Robustness**

There were several parameters for doing robustness study. Slight change in mobile phase composition, volume, chamber saturation time also can bealternates for robustness study.

Table 8. Change in mobile phase composition

| **Mobile Phase** | | | **sodium benzoate:sodium acetate:citric acid 5:3:2** |
|---|---|---|---|
| SN | Conc | ng/Band | Area |
| 1 | 3 | 700 | 9006 |
| 2 | 3 | 700 | 8915 |
| 3 | 3 | 700 | 8740 |
| 4 | 3 | 700 | 8970 |
| 5 | 3 | 700 | 8770 |
| 6 | 3 | 700 | 8844 |
| | | Mean | 8874.17 |
| | | SD | 107.70 |
| | | % RSD | 1.21 |

Table 9. Change in mobile phase volume

| | | **sodium benzoate:sodium acetate:citric acid 5:4:1** | |
|---|---|---|---|
| **SN** | **Conc** | **ng/Band** | **Area** |
| 1 | 3 | 700 | 9000 |
| 2 | 3 | 700 | 8910 |

| | | | |
|---|---|---|---|
| 3 | 3 | 700 | 8737 |
| 4 | 3 | 700 | 8960 |
| 5 | 3 | 700 | 8767 |
| 6 | 3 | 700 | 8840 |
| | | Mean | 8869.0 |
| | | SD | 105.63 |
| | | % RSD | 1.19 |

Table 10. Change in duration of saturation

| SN | Conc | ng/Band | Duration of Saturation | | |
|---|---|---|---|---|---|
| | | | 20 min | 25 min | 30 min |
| 1 | 3 | 700 | 9006 | 9010 | 9085 |
| 2 | 3 | 700 | 8915 | 8916 | 8916 |
| 3 | 3 | 700 | 8740 | 8714 | 8774 |
| 4 | 3 | 700 | 8970 | 8960 | 8973 |
| 5 | 3 | 700 | 8770 | 8767 | 8771 |
| 6 | 3 | 700 | 8844 | 8845 | 8884 |
| | | Mean | 8874.17 | 8868.67 | 8900.50 |
| | | SD | 107.70 | 114.35 | 120.48 |
| | | %RSD | 1.21 | 1.29 | 1.35 |

Table 11. Change in analyst

| SN | Analyst | Conc | Mean | Amount Found | % Amount Found | SD | RSD | % RSD |
|---|---|---|---|---|---|---|---|---|
| 1 | Analyst I | 700 | 8590.37 | 692.50 | 98.92927944 | 106.0015723 | 1.23 | 1.23 |
| 2 | Analyst II | 700 | 8769.73 | 700.12 | 100.0178015 | 9.204527871 | 0.10 | 0.10 |

**Limit of Detection and Limit of Quantitation**
LOD and LOQ are calculated using formulae LOD = 3.3* Average SD/Slope, LOQ=10* AverageSD/Sloperespectively. They were found out to be: LOD = 1.4, LOQ = 4.3

## CONCLUSION

For the identification and quantification of sildenafil citrate a new HPTLC approach has been created. The key characteristics of this approach are its low cost, increased speed, and satisfactory precision and accuracy. Solubility of sildenafil citrate was checked in various hydrotropic agents. Statistical analysis shows that the approach is sensitive, specific, and repeatable. The method was successfully validated in accordance with ICH requirements. Without excipient interference, it can be used for routine quality control analysis of sildenafil citrate as a bulk medication in tablet formulation.

## REFERENCES

1. The American Society of Health-System Pharmacists, National Library of medicine. Patient Medication Information
2. Drug Evaluation and Research, US Food and Drug Administration.
3. Lieberman H., Chapter 32 - Chemical and Physicochemical Approaches to Solve Formulation Problems, The Practice of Medicinal Chemistry (Fourth Edition) 2015,767-791
4. Yosra S.R., Magda A., Ossama

**ISSN 2063-5346**

A., Fabrication, appraisal, and transdermal permeation of sildenafil citrate-loaded nanostructured lipid carriers versus solid lipid nanoparticles, Elnaggar Internationaljournal of nanomedicine,2011;6:3195-205

5. ICH Guidance for Industry, Q1A(R2): Stability testing of New drug substances and products (Revision 2) , U.S. Department of health and Human Services, Food and Drug Administration, Centre for drug evaluation and Research (CDER) Centre for Biologics Evaluation and Research (CBER) Rockville, Md , USA,2003
6. Kashid S. K, Tapkir A. Chaudhari P., Analytical Method Development and Validation for Stability indicating HPTLC Method for assay of Stiripentol in Bulk and Dosage form, Journal of Applied Pharmaceutical Sciences and research, Volume 3, Issue 4, 2020
7. Shivashankar, V., Gandhimathi, M., Ravi T. K., RP-HPLC method development and validation for the analysis of dronedarone hydrochloride in tablet dosage form Journal of Pharmacreations vol 2(4)2015, 66-71
8. Prajapati A. C., Patel B. S., Badmanaban R., Development and Validation of HPTLC Method For Simultaneous Estimation of Sildenafil Citrate And Dapoxetine Hydrochloride in Combined Dosage Form, Journal of Pharmatutor Magazine Vol. 2, Issue 10 ISSN: 2347-7881
9. Patel D. C., Patel N., Dr. Sherikar O., Mehta P.,Development and Validation of RP–HPLC, HPTLCand UV-Visible Spectrophotometric Methods for Simultaneous Estimation of Alprazolam and Propranolol Hydrochloride in Their Combined Dosage Form, journal of Analytical Chemistry, 2014, Vol. 69, No. 7, pp. 674–680
10. Wasnik U., Nikalje R., Birajdar K., Lakhade S., Jadhav S., Kore, Harde M. Development and Validation of TLC-Densitometric Method for Simultaneous Estimation of Moxifloxacin HCL and Ketorolac Tromethamine in Bulk Drug and Marketed Formulation, Eur. Chem. Bull. 2023, 12(Special Issue 1), 2468-2479.