The Journal of Multidisciplinary Research (TJMDR)

Content Available at www.saap.org.in ISSN: 2583-0317

Development & determination of UV method for sildenafil & fluxetine in bulk drug & in formulation

B.Satya Prasad[1*], Dr J.N. Suresh Kumar[2], P. Sai Mounika[3], P. Balaji[3], R. ChaitanyaKrishna[3], T. Lakshmi Vasanthi[3], Y. Tirupati Rao[3], K. Hima Bindu[3]

1. Associate Professor, 2. Principal & Professor, 3. Research Students

1,2,3. Narasaraopeta Institute of Pharmaceutical Sciences, Narasaraopeta, Palnadu, A.P

Received: 07 Feb 2023 Revised: 24 Feb 2023 Accepted: 22 Mar 2023

Abstract

A simple, rapid, precise and highly selective spectrophotometric method was developed for simultaneous estimation of Sildenafil citrate and Fluoxetine Hydrochloride in tablet dosage form. This method, involves the measurement of absorbances of Sildenafil citrate and Fluoxetine Hydrochloride at the wavelengths of 282nm (λmax of Sildenafil citrate) and 249 nm (λmax of Fluoxetine Hydrochloride). Methanol was used as solvent. Linearity was observed in the concentration range of 10-18µg/ml for Sildenafil citrate and 3-8ug/ml. Fluoxetine Hydrochloride. The accuracy of the method was confirmed by recovery studies of tablet dosage forms and was found to be 98.1% and 99.2% for Sildenafil citrate and Fluoxetine Hydrochloride respectively. The method showed good reproducibility and recovery with % RSD less than 2. Thus, the proposed method was found to be rapid, specific, precise, accurate and cost-effective quality control tool for the routine analysis of Sildenafil citrate and Fluoxetine Hydrochloride in bulk and combined dosage form. A rapid and simple derivative spectrophotometric method was developed for simultaneous determination of Sildenafil citrate and Fluoxetine Hydrochloride in tablet dosage form. This method involves second order derivative spectroscopy using 282 nm and 249 nm as zero crossing points for Sildenafil citrate and Fluoxetine Hydrochloride respectively in methanol. Linearity was observed in the concentration range of 10-18µg/ml for Sildenafil citrate and 3-8ug/ml for Fluoxetine Hydrochloride. The accuracy of the method was found to be 98.21% and 99.2% for Sildenafil citrate and Fluoxetine Hydrochloride respectively. The method showed good reproducibility and recovery with % RSD less than 2.Thus, the proposed method was found to be rapid, specific, precise, accurate and cost-effective quality control tool for the routine analysis of sildenafil citrate and Fluoxetine Hydrochloride in bulk and combined dosage form. A selective, precise, isocratic and accurate stability indicating reverse phase high performance liquid chromatographic method was developed for the simultaneous determination of Sildenafil citrate and Fluoxetine Hydrochloride in the tablet dosage form. The method was validated for the linearity, accuracy, precision, robustness, system suitability as per ICH guidelines. Sildenafil citrate and Fluoxetine Hydrochloride were found to be linear in the range of 10-100 and 10-18µg/ml and 3-8ug/ml with the recoveries of 98.6% and 99.2%. The method was also applied for the determination of sildenafil citrate and Fluoxetine Hydrochloride in the presence of their degradation products formed under variety of stress conditions.

Keywords: - *Fluoxetine Hydrochloride, sildenafil citrate.*

***Corresponding Author**

B.Satya Prasad

DOI: https://doi.org/10.37022/tjmdr.v3i1.440

Produced and Published by

South Asian Academic Publications

Introduction

Pharmaceutical analysis is a branch of practical chemistry that involves a series of process for identification, determination, quantification and purification of a substance, separation of the components of a solution or mixture, or determination

of structure of chemical compounds. The substance may be a single compound or a mixture of compounds and it may be in any of the dosage form. The substance used as pharmaceuticals are animals, plants, microorganisms, minerals and various synthetic products.Analytical chemistry is the science that seeks ever improved means of measuring the chemical composition of natural and artificial materials [1,21]. The major stages of an analytical process are described as follows:

Steps in analytical cycle:

- To be effective and efficient, analysing samples requires expertise in:
- The chemistry that can occur in a sample
- The total amount of sample available Concentration range of analyte
- Analysis and sample handling methods for a wide variety of problems (the tools-of-the-trade)
- Accuracy and precision of the method Proper data analysis and record keeping

Analytical chemistry is the science of obtaining, processing and communicating information about the composition and structure of matter. In other words, it is the art and science of determining what matter is and how much it exists. Analytical chemistry also is concerned with developing the tools used to examine chemical compositions. It is concerned with the chemical characterization of matter both qualitatively and quantitatively. Qualitative analysis gives an indication of the identity of the chemical species in the sample and quantitative analysis determines the amount of one or more of these components [2, 11].

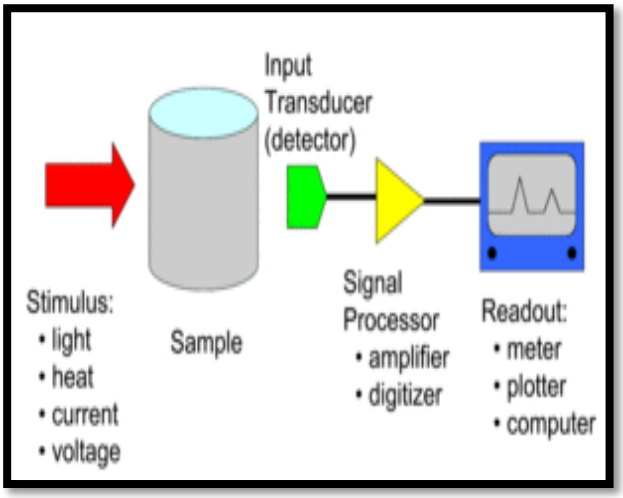
Analytical methods can be separated into classical and instrumental. Classical methods use separation techniques such as precipitation, (extraction, and distillation and qualitative analysis by colour, odour, or melting point. The quantitative analysis is achieved by measurement of weight or volume. Instrumental methods use anapparatus to measure physical qualities of the analyte such as (light((absorption, fluorescence, or conductivity. The separation of material is accomplished by using chromatography or electrophoresis methods3.

The various physical properties employed for analysis with their instrumental methods are given in the table below:

Physical properties employed for instrumental methods

Sl. No.	Physical property measured	Instrumental method based on the measurement of property
1	Absorption of Radiation	Spectrophotometry (X-ray, UV, Visible, IR): Colorimetry, Atomic absorption, NMR.
2	Emission of Radiation	Emission spectroscopy (X-ray, UV, Visible, IR): Flame photometry, Fluorescence (X-ray, UV, Visible)
3	Scattering of Radiation	Turbidimetry, Nephelometry
4	Refraction of Radiation	Refractometry
5	Diffraction of Radiation	X-ray, electron Diffraction methods.
6	Rotation of Radiation	Polarimetry
7	Electrical Potential	Potentiometry
8	Electrical Conductance	Conductometry
9	Electrical Current	Polarography, Amperometry titrations
10	Mass-to-charge ratio	Mass spectrometry

Instrumental Analysis:



BLOCK DIAGRAM OF AN ANALYTICAL INSTRUMENT SHOWING THE STIMULUS AND MEASUREMENT OF RESPONSE

UV-SPECTROPHOTOMETRY

Spectroscopy is the measurement and interpretation of electromagnetic radiation absorbed or emitted when the molecules or atoms or ions of a sample move from one energy state to another energy state.Spectroscopy is a general methodologythat can be adapted in many ways to extract the information you need (energies of electronic, vibrational, rotational states, structure and symmetry of molecules, dynamic information). Ultraviolet-Visible Spectrophotometry is one of the most frequently employed techniques in pharmaceutical analysis. It involves the measurement of the amount of Ultraviolet (190-380nm) radiation by a substance in a solution. A compound or drug which possesses conjugated double bond absorbs UV radiation at a specific wavelength and this character of the drug is specific for a fixed solvent system. The wavelength at

which maximum absorption occurs is called λmax. It is independent of concentration. For a drug to be measured by the ultraviolet analytical method, it should follow the Beer's-Lambert's law [4, 22]. The concentration of an analyte in solution can be determined by measuring the absorbance at some wavelength and applying the Beer-Lambert Law.

SPECTROPHOTOMETRIC ASSAY

Assay of single component sample:

Single standard (or) Double-point standardization

It involves the measurement of the absorbance of a sample solution and of a standard solution of the reference substance. The concentration of the substance in the sample is calculated from the proportional relationship that exists between absorbance and concentration5,13.

$$C_{test} = \frac{A_{test} \times C_{std}}{A_{std}}$$

C_{std} = Concentration of the standard solution

C_{test} = Concentration of the sample

A_{test} and A_{std} = Absorbances of the sample and standard solutions.

Calibration graph method

In this procedure the absorbance's of a number of standard solutions of the reference substance at concentrations encompassing the sample concentrations are measured and a calibration graph is constructed. The concentration of the analyte in the sample solution is read from the graph as the concentration corresponding to the absorbance of the solution [6,14,23].

Assay of Multi-Component samples:

The assay of components of mixture sample can be done by following methods:

Simultaneous Equation method [15,24]
Absorbance Ratio method
Geometric Correction method
Difference spectrophotometry
Derivative spectrophotometry

The basis of all the spectrophotometric techniques for multicomponent samples is the property that at all wavelengths,The absorbance of a solution is the sum of absorbances of the individual components orthe measured absorbance is the difference between the total absorbance of the solution in the sample cell and that of the solution in the reference (blank) cell.If a sample contains two absorbing drugs (X and Y) each of which absorbs at the λmax of the other, it may be possible to determine both drugs by the technique of simultaneous equation [7,15].

The information required is:

The absorptivities of X at λ1 and λ2, ax1 and ax2 respectively.

The absorptivities of Y at λ1 and λ2, ay1 and ay2 respectively.

The absorbances of the diluted sample at λ1 and λ2, A1 and A2 respectively.

Let Cx and Cy be the concentrations of X and Y respectively in the diluted sample.

Two equations are constructed based upon the fact that the sum of individual absorbance of X and Y.

$$\text{At } \lambda 1,\ A1 = ax1bcx + ay1bcy$$

$$\text{At } \lambda 2,\ A2 = ax2bcx + ay2bcy$$

$$Cx = \frac{A2ay1 - A1ay2}{ax2ay1 - ax1ay2}$$

$$Cy = \frac{A1ax2 - A2ax1}{ax2ay1 - ax1ay2}$$

Using the above equations, concentrations of individual components in a mixture can be determined.

METHOD VALIDATION

Validation is defined as follows by different agencies.

Food and Drug Administration (FDA)8,16**:**

Establishing documentation evidence, which provides a high degree of assurance that specific process will consistently produce a product meeting its predetermined specification and quality attributes.

World Health Organization (WHO) [9,25**]:**

Action of providing that any procedure, process, equipment, material, activity or system actually leads to the expected results.

European Committee (EC) [10,16]

Action of providing in accordance with the principles of good manufacturing practice, that any procedure, process, equipment material, activity or system actually led to the expected results. In brief validation is a key process for effective Quality Assurance

Analytical method validation [17,26]

Analytical monitoring of a pharmaceutical product or of specific ingredients within the product is necessary to ensure its safety efficacy throughout all phases of its shelf life. Such monitoring is in accordance with the specifications elaborated during product development.Analytical method validation is the corner stone of process validation without a proven measurement system it is impossible to confirm whether the manufacturing process has done what it purports to do. All new analytical methods developed are validated.

Steps followed for validation procedures18

Proposed protocols or parameters for validations are established

1. Experimental studies are conducted
2. Analytical results are evaluated
3. Statistical evaluation is carried out
4. Report is prepared documenting all the results

The International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use has developed a consensus text on the validation of analytical procedures. The document includes definitions for eight validation characteristics.

MATERIALS &METHODS [19,27]

a) Pure drug samples: The drug sample of SILDENAFIL CITRATE AND FLUOXETINE HYDROCHLORIDE was received as a gift sample from KP labs, Hyderabad respectively.

b)Marketed product: The formulation was received as a gift from local Pharmacy.

c) Chemicals and solvents used

Fluoxetine hydrochloride

Sildenafil citrate

Methanol

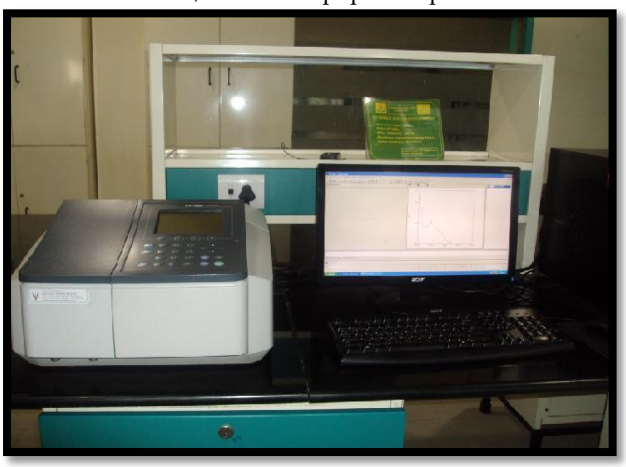
d)Instruments

Schimadzu Digital Electronic Balance- BL 220H.

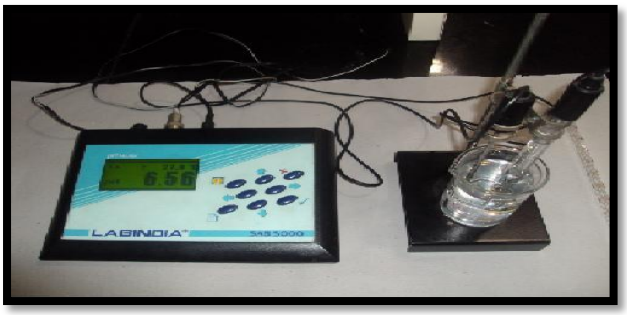
Lab India SAB 5000, pH meter.

Schimadzu UV-1800, UV/Vis-Spectrophotometer.

Ultrasonic cleaner, Life care equipmentspvt.ltd.



UV SPECTROPHOTOMETER



PH METER

SHIMADZU DIGITAL ELECTRONIC BALANCE – BL 220H

FAST CLEAN ULTRASONICATOR

METHODOLOGY: UV SPECTROPHOTOMETRY20

Experimental

A Shimadzu-1800 UV / Vis double beam Spectrophotometer with 1 cm matched quartz cells was used for all spectral measurements. All chemicals used were of A.R.grade from S.D.Fine-chem, Merck, Fischer scientific, and Spectrochem, Mumbai. Authentic drug samples of SILDENAFIL citrate and FLUOXETINE hydrochloride was given as a gift sample by KP LABS, kothapet. Tablets of MONTAIR-FX (fluoxetine and sildenafil) were procured from local market.

UV Spectrophotometric Determination of sildenafil citrate and fluoxetine hydrochloride using methanol: It is an UV Spectrophotometric method which involves the determinationof sildenafil citrate and fluoxetine hydrochloride in bulk drug and pharmaceutical formulations. Sildenafil citrate has an absorption maximum at282 nm. It obeys Beer's law in the concentration range of 1-10 µg/ml. fluoxetine hydrochloride has an absorption maximum at 249 nmand it obeys beer's law in the concentration range of 10-90 µg/ml.

Method Development [28]

1. Solvent selection:

Solutions of silfenafil citrate and fluoxetine hydrochloride were prepared in different solvents like methanol, ethanol, acetonitrile, 0.1M HCl and 0.1M NaOH. UV spectrum of each were recorded by scanning between 200-400 nm.

An overlain spectra sildenafil citrate and fluoxetine hydrochloride were prepared in different solvents like methanol, water etc. as shown in the figures 6.1 and 6.2. Better absorbances were observed for both drugs when methanol is used as a solvent. Hence methanol is selected as a solvent for the present study.

Standard solution of sildenafil citrate:

Standard stock solution:

Accurately weighed 10 mg of sildenafil citrate was transferred into clean, dry 100 mL volumetric flask and dissolved with sufficient volume of methanol. The volume was made up to 100 mL with methanol to get concentration of 100µg/mL.

Standard solution of fluoxetine hydrochloride29**:**

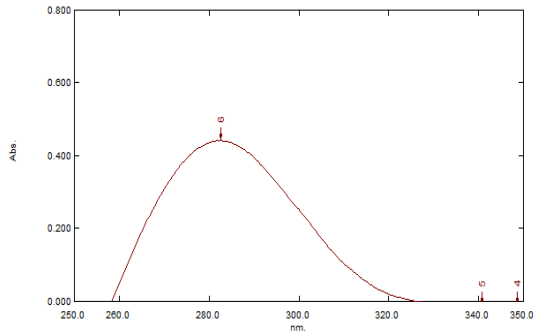
Standard stock solution:

Accurately weighed 10 mg of sildenafil was transferred into clean, dry 100 mL volumetric flask and dissolved with sufficient volume of methanol. The volume was made up to 100 mL with methanol to get concentration of 100µg/mL.

Selection of analytical wavelength [30]

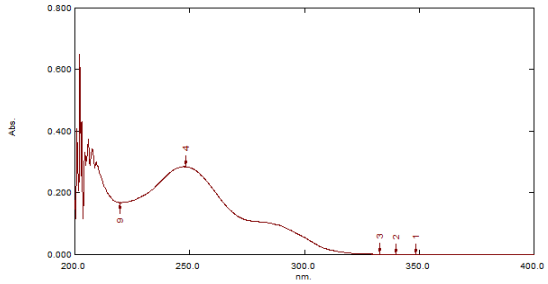
Appropriate dilutions of standard solutions of both the drugs were scanned in the UV range of 200 to 400 nm, using methanol as blank. The peaks were observed at 282 nm for sildenafil citrate and 249 nm for fluoxetine hydrochloride obtained were noted and the peak having highest absorbance was taken as wavelength maxima.

Represents the maximum absorbance of sildenafil citrate at 282 nm using methanol as a solvent



REPRESENTS THE MAXIMUM ABSORBANCE OF FLUOXETINE

HYDROCHLORIDE AT 249 NM USING METHANOL AS A SOLVENT



an x1: Absorptivity of sildenafil citrate at 282 nm

an y1: Absorptivity of fluoxetine hydrochloride at 249 nm

A1: Absorbance of sildenafil citrate at 282 nm

A2: Absorbance of fluoxetine hydrochloride at 249 nm

Validation of spectrophotometric method:

Linearity and Range

Standard stock solution sildenafil citrate:

Accurately weighed 10 mg of sildenafil citrate was transferred into a clean and dry 100 mL volumetric flask and dissolved with sufficient volume of methanol. The volume was made up to 100 mL with methanol to get concentration of 100µg/mL.

Working standard solution:

Aliquots of solution from standard solution were pipetted in volumes of 1.0, 1.2, 1.4, 1.6, 1.8, in ten different 10 mL volumetric flasks. The volume was made up with the methanol to obtain concentration ranging from 10-18 µg/mL.

Standard stock solution of fluoxetine hydrochloride:

Accurately weighed 10 mg of fluoxetine hydrochloride was transferred into a clean and dry 100 mL volumetric flask and dissolved with sufficient volume of methanol. The volume was made up to 100mL with methanol to get concentration of 100 µg/mL. Aliquots of solution from standard solution were pipetted in volumes of 0.3,0.4,0.5,0.6,0.8. in different 10 mL volumetric flasks. The volume was made up with the methanol to obtain concentration ranging from 3-8 µg/mL.

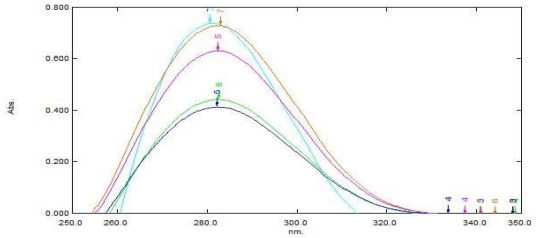
Determination:

Absorbance of workingstandard solutions for sildenafilcitrate was measured at 282nm and fluoxetine hydrochloride was measured at 249nm. Graph of concentration (on X-axis) Vs mean response (on Y- axis) was plotted for both the drugs separately.Sildenafil citrate was found to be the linear in the concentration range of 10-18 µg/mL and fluoxetine hydrochloride was found to be linear in the concentration range of 3-8 µg/mL. Calibration curves were plotted using concentration range v/s absorbance. Correlation coefficient, slope and intercepts.

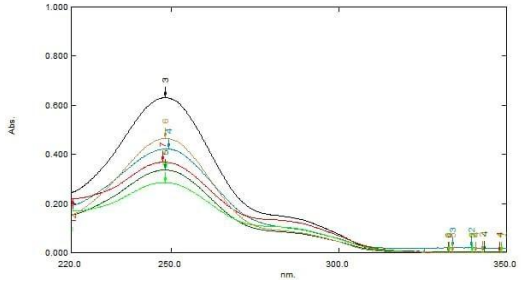
Calibration values of sildenafil

DRUG	CONCENTRATION	Calibration
Sildenafil	10	0.339
Sildenafil	12	0.472
Sildenafil	14	0.675
Sildenafil	16	0.726
Sildenafil	18	0.798
Fluoxetine	3	0.284
Fluoxetine	4	0.336
Fluoxetine	5	0.361
Fluoxetine	6	0.427
Fluoxetine	7	0.463

Calibration values of fluoxetine overlay spectra of sildenafil



Overlay spectra of fluoxetine



LOD is defined as the lowest concentration of the analyte that can be detected, but not necessarily quantified, by the analytical method.LOQ is defined as the lowest concentration of the analyte that can be determined with acceptable accuracy and precision by the analytical method.LOD and LOQ values for fluoxetine and sildenafil are determined by based on standard deviation of the response to slope values.

Based on standard deviation of the response and slope

The **limit of detection (LOD)** may be expressed as

$$LOD = 3.3\ \sigma\ \sigma/S$$

Where $\sigma\ \sigma$ = the standard deviation of the response

S = slope of calibration curve of analyte

The **limit of quantitation (LOQ)** may be expressed as

$$LOQ = 10\ \sigma\sigma/S$$

Where $\sigma\ \sigma$ = the standard deviation of the response

S = slope of calibration curve of analyte

Accuracy: (Recovery studies)

Accuracy is the closeness of the test results obtained by the method to the true value. In order to ensure the suitability and reliability of the proposed method, recovery studies were carried out. This recovery studies were carried out by using standard addition method. To an equivalent quantity of formulation powder (10 µg), 9.2 µg of fluoxetine sodium was added to it (standard addition method), so that the sample contains 10 µg of fluoxetine sodium and 10 µg of sildenafil. Then a known quantity of fluoxetine and sildenafil were added to 50%, 100% & 150% level and contents were reanalyzed by proposed method. The % recovery and %RSD were calculated and reported in table 2.

Accuracy studies

Drugs	Amount taken (mg)	Amount added (mg)	Total amount found (mg)	% Recovery	% RSD
Sildenafil	100	20	120	98.45	0.12
		40	140	98.95	0.09
		60	160	99.75	0 07
Fluoxetine	30	10	40	98.75	0.16
		20	50	99.25	0.14
		30	60	99.85	0.18

Precision

It provides an indication of random error results and was expressed as coefficient of variation (CV) or relative standard deviation (RSD). The precision of the method was established by system precision and method precision. Variations of results within the same day (intra-day), variation of results between days (inter-day) were analysed.

1) System precision:

Intraday precision was determined by using drug concentrations 5 & 6 µg/ml for fluoxetine sodium, 40 & 60 µg for sildenafil and it was analysed three times in a day. Same procedure was followed for three different days to study the inter day precision and those values were reported.

Intraday precision

Concentration (µg/ml)		Absorbance		%* RSD	
		FLU	SIL	FLU	SIL
FLU	SIL	249 nm	282 nm	249 nm	282 nm
3	10	0.283	0.339	0.18	0 32
		0.284	0.341		
		0.284	0.339		
4	12	0.336	0.472	0.16	0.12
		0.335	0.472		
		0.335	0.473		

2) Method precision:

Intraday precision was determined by using a sample solution of drug (11 µg/ ml)and it was analysed three times in a day. Same procedure was followed for six different days to study the Inter day precision and those values were reported in

Interaday precision

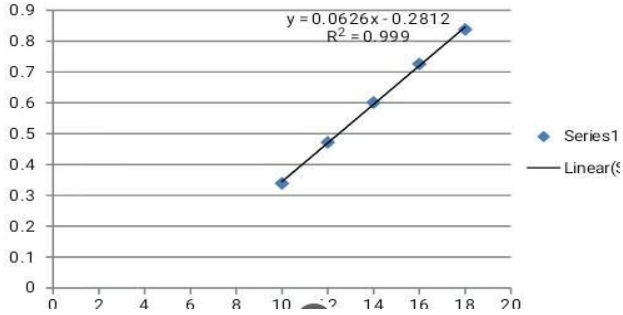
Concentration (µg/ml)		Absorbance		%* RSD	
		FLU	SIL	FLU	SIL
FLU	SIL	249 nm	282 nm	249 nm	282 nm
3	10	0.283	0.339	0.192	0.323
		0.282	0.338		
		0.284	0.338		
4	12	0.335	0.472	0.16	0.11
		0.336	0.473		
		0.337	0.472		

Repeatability of measurement

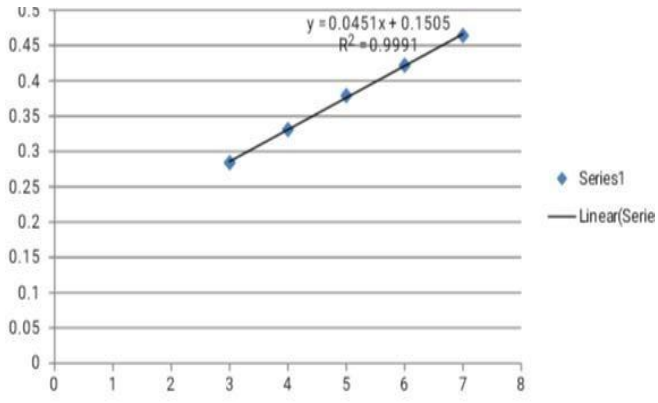
* Mean of six observations

Concentration (µg/ml)		Absorbance			% *RSD	
		FLU	SIL		FLU	SIL
		249 nm	282 nm		249 nm	282 nm
3	10	0.284	0.339	0.192	0.323	
		0.283	0.338			
		0.283	0.340			
		0.285	0.339			
		0.284	0.339			
		0.285	0.341			

Concentration (µg/ml)		Absorbance			% *RSD	
		FLU	SIL		FLU	SIL
		249 nm	282 nm		249 nm	282 nm
4	12	0.336	0.472	0.16	0.11	
		0.336	0.472			
		0.335	0.473			
		0.336	0.473			
		0.337	0.472			
		0.335	0.473			



Linearity of sildenafil citrate



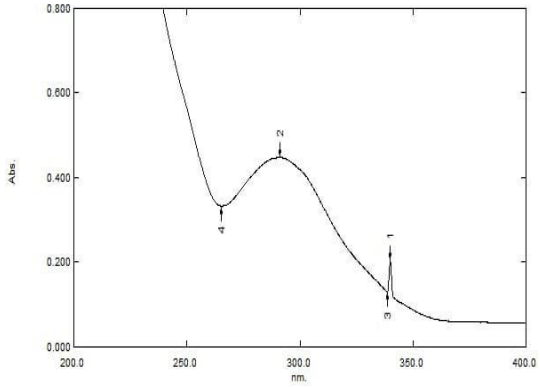
Linearity of fluoxetine hydrochloride

ANALYSIS OF FORMULATION

Sample stock solution:

Twenty tablets each containing 10 mg of fluoxetine sodium and 120 mg of sildenafil were accurately weighed and the average weight was calculated and finely powdered. A quantity equivalent to 10 mg of sildenafil was weighed and dissolved in methanol in 100ml volumetric flask and to it 9.2mg of fluoxetine sodium was added (standard addition method). Sonicated to dissolve it completely and make volume up to the mark with diluent. Mixed well and filtered through a 0.45µm filter to get the final concentration of 100µg/ml. Those values were reported.

Analysis of formulation



Analysis of marketed formulation

Drugs	Labelled amount, mg tablet^{-1}	Amount found, mg tablet^{-1}
Sildenafil	100	100
Fluoxetine	20	20

Ruggedness data of sildenafil citrate and fluoxetine hydrochloride

Drug Sildenafil(nm)	Parameter	% Found	% RSD
280	**Analyst-1**	98.75	0.18
283	**Analyst-1**	99.25	0.20

Robustness data of sildenafil citrate and fluoxetine hydrochloride

Drug(Fluoxetine)nm	**Parameter**	**% Found**	**% RSD**
248	**Analyst-2**	**98.35**	**0.312**
250	**Analyst-2**	**99.15**	**0.332**

Summary and Conclusion

The direct UV method development for the analysis of sildenafil citrate and fluoxetine hydrochloride. can be applied for the routine analysis of formulation.The UV spectroscopic method was found to be a rapid, specific, precise, accurate and cost-effective quality control tool for the routine analysis of sildenafil citrate and fluoxetine hydrochloride in bulk and tablet dosage form..

For the simultaneous estimation of sildenafil citrate and fluoxetine hydrochloride, the UV spectroscopic method was developed and validated according to ICH guidelines. Hence, this method can be applied for the routine analysis of formulation. The developed isocratic LC method offers simplicity, selectivity, precision and accuracy. In the proposed method symmetrical peaks

with good resolution were obtained and this method is validated according to ICH guidelines. Hence, it can be applied for routine analysis of formulation.The proposed method was validated as per the ICH guidelines. The precision was measured in terms of repeatability, which was determined by sufficient number of aliquots of a homogeneous sample. The % RSD was found and lie within the range of ± 4.0. This showed that the precision of the method was satisfactory. The results showed that the recovery of sildenafil citrate and fluoxetine hydrochloride by the proposed the method was satisfactory.

References

1. Parker G. Beyond major depression. Psychol Med. 2010; 35: 467-474.
2. Erman ME, McCloskey MS, Fanning JR, Schumacher JA, Coccaro EF. "Serotonin augmentation reduces response to attack in aggressive individuals". Psychol Sci. 2009; 20 (6): 714–20.
3. Amsterdam JD, Shults J. Efficacy and Safety of Long-Term Fluoxetine Versus Lithium Monotherapy of Bipolar II Disorder: A Randomized, Double-Blind, Placebo-Substitution Study. Am J Psychiatry. 2008; 167(7):792–800.
4. Deepak S, JainAK, GuntaS, SanyalM, PandeUC andShrivastava P. "Spectroscopic analysis of Fluoxetine intablet dosage forms." J. Pharm. Biomed Anal. 2009, 26: 43-52.
5. Hai LZ, Wilson YL, Neil T. Fluoxetine: An overview of spectral data. Int. J. Chem Tech Res 2010; 2 (2): 1141-46.
6. Boolell M, S. Gepi-Attee, J.C. Gingell, and M.J. Allen, Br. J. Urol., 1996, 78, 257. Brock G., Sildenafil citrate (Viagra) Drugs Today, 2000, 36: 125-134.
7. Cooper J.D.H., Muirhead D.C, Taylor J.E, & P.R Baker, Journal of Chromatogr b, 1997, 701: 87-95.
8. Dinesh N.D, B.K. Vishukumar, P.Nagaraja, Gowda N.M..,and Rangappa K. S., J.pharm. Biomed.Anal, 2002, 29: 743.
9. DraghmehN, M. Al-omari, A.A. Badwan, and A.m. Ja-ber, J. Pharm. Biomed. Anal, 2001, 25:483
10. Aboul-Enein, M.Mohamed, Hefnawy, Jour-nal of Liquid Chromatography & Related Technolo-gies, 2003, 26: 2897-2908.
11. LowentritB.H t., Scardino P.T., Miles B.J., Orejuela F.J, Schatte E.C., Slawin K.M., Elliot S.P., and Kim E.D., J. Urol, 1999, 162: 1614.
12. Martel A.M., Agraul, X. Rabasseda, and R. Castaner, Drugs Fut, 1997, 22:138.
13. Attama AA et al. Studies on diclofenac β-cyclodextrin inclusion complexes. Journal of Pharmaceutical Research, July 2004; 3(3): 47-49.
14. Indop MA, Sunita C Bhosle, Tayade PT and Vavia PR. Evaluation of toxicity and antitumor effect of a hydroxypropyl β-cyclodextrin inclusion complex of quercetin. Ind. J. of Pharm. Sci., Aug, 2002; 64(4): 349 - 353.
15. Sunil Jambhekar, Casella R, Maher T. The physicochemical characteristics and bioavailability of indomethacin from β-cyclodextrin, HE β-CD and HPβ-CDs complexes. Inter.J.270, 2004,149-166.
16. Tayade NG, Nagarsenker MS. Preparation , characterization and evaluation of HPβ-CD inclusion complexes of antimalarial drug. Scientific abstract 54th IPC Pune, Sept.2002, 156.
17. Chowdary KPR, Vijaya Srinivas. Effect of polyvinyl pyrrolidone on complexation and dissolution rate of β and HPβ-CD complexes of celecoxib. Ind.J.Pharm.Sci. Sept-oct. 2006, 631-634.
18. Muller BW, Albers E. Effect of hydrotropic substances on the complexation of sparingly soluble drugs with cyclodextrin derivatives and the influence of cyclodextrin complexation on the pharmacokinetics of the drugs. J Pharm Sci. 1991; 80:599Y604.
19. Redenti E, Szente L, Szetli J. Drug/cyclodextrin/hydroxy acid multicomponent systems. Properties and pharmaceutical applications. J Pharm Sci. 2000; 89:1Y8.
20. Li P, Zhao L, Yalkowsky SH. Combined effect of cosolvent and cyclodextrin on solubilization of nonpolar drugs. J Pharm Sci. 1999; 88:1107Y1111.
21. Miyake K, Irie T, Arima H, et al. Characterization of itraconazole/ 2-hydroxypropyl-β-cyclodextrin inclusion complex in aqueous propylene glycol solution. Int J Pharm. 1999; 179:237Y245.
22. Rita Wadetwar, Kanchan Upadhye, SupamaBakhle, et al Production of cyclodextrin : Effect of PH, time and additives. Ind.J.Pharm.Sci. Julu-Aug. 2006, 520-523.
23. Shrivastava, B.,K.V. Gopaiah and G.S. Rao, 2019. Lipid-polymer based nanoparticles as a new generation therapeutic delivery platform for ulcerative colitis in vitro/in vivo evaluation. Int. J. Innov. Technol. Expl. Eng. Sci., 21: 42-46.
24. Gopaiah.k.v. Hydrotropic technique: a promising method to enhance aqueous solubility of

nimesulide and to reduce difficulties in bioavailability. Asian J. Pharm., 12:S1456-S1472.

25. Gopaiah, K.V, 2019. In vitro and In vivo anti-inflammatory potential of isolatedcompounds from ethyl acetate extracts of Tecomaria capensis. Int. J. Green Pharm., 12:S1-57.
26. Indian Journal of Pharmaceutical Sciences 81 (3), 569-574S.Bioactive Metabolites from Manglicolous Lichen Ramalinaleiodea (Nyl.) Nyl.VBTatipamula, H Polimati, KV GOPAIAH, AK Babu, S Vantaku, PR Rao.
27. Design, Formulation, and Evaluation of Sustained Release Tablets for Antihyperlipidemic Agent kvenkata Gopaiah, Asian journal of pharmaceutics 12 (04-supplymentry). 1-8.
28. Hydrotropic Technique: A Promising Method to Enhance Aqueous solubility of Nimesulide and to Reduce Difficulties in Bioavailability k.venkat Gopaiah, Asian journal of pharmaceutics 12 (04), S1-17.
29. Formulation Development and Evaluation of Metronidazole SustainRelease Tablets For Colon Specific Drug Delivery System.KV Gopaiah, D Prathyusha, M Srujana, V Kavitha, IJRAR-International Journal of Research and Analytical Reviews.
30. Formulation & Evaluation of Loratadine Hydrochloride Oral Disintegrating Tablets by Direct Compression Method by Using super DisintegratesM Srujana, D Prathyusha, V Kavitha, KV Gopaiah.