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Der Pharma Chemica, 2012, 4(4):1404-1407
(http://derpharmachemica.com/archive.html)

ISSN 0975-413X
CODEN (USA): PCHHAX

Spectrophotometric Simultaneous Determination of Dapoxetine and Sildenafil in Combined Tablet Dosage Form by Absorbance Corrected Method.

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ABSTRACT

One simple, economical, precise and accurate method is described for the simultaneous determination of Dapoxetine and Sildenafil in combined tablet dosage form. The method is Absorption Corrected Method. The amplitudes at 237.54 nm and 325.92 nm in the Absorption Corrected Method were selected to determine DPT and SL, respectively in combined formulation. The method was validated by following the analytical performance parameters suggested by the International Conference on Harmonization (ICH). All validation parameters were within the acceptable range. Under experimental conditions described, calibration curve, assay of tablets and recovery studies were performed. A critical evaluation of proposed method was performed by statistical analysis of data where slope, intercept, correlation coefficient is shown in Table (1). As per the ICH guidelines, the method validation parameters checked. Beer's law is obeyed in the concentration range of 5-25 µg/ml for DPT and 8-40 µg/ml for SL by the given method.

KEY WORDS: Absorbance corrected Method, Dapoxetine, and Sildenafil.

INTRODUCTION

Dapoxetine (Fig.1), is N,N-dimethyl-3-(naphthalene-1-yloxy)-1-phenylpropan-1-amine.It is a selective serotonin reuptake inhibitor (SSRI) delays the ejaculation reflex and extends the intravaginal ejaculatory latency time (IELT)[7,8]. It is mainly used in premature ejaculation [4]. Sildenafil (Fig.2), is 1-[4-ethoxy-3-(6, 7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)phenylsulfonyl]-4-methlpiperazine[9]. It inhibits the enzyme PDE5 by occupying its active site. Sildenafil protects cyclic guanosine monophosphate (cGMP) from degradation. It is used topically for the treatment of ejaculatory dysfunction [2]. Detailed literature survey reveals that, it was found that dapoxetine can be estimated by chiral recognition of dapoxetine enantiomers with methylated gamma-cyclodextrin [5,6] and influence of sildenafil was estimated on plasma profiles of male albino rats (hormonal and biological study)[3]. To date, there have been no published reports about the simultaneous estimation of DPT and SL by UV spectrophotometer in standard drug and in pharmaceutical dosage forms. This present study reports for the first time simultaneous UV spectrophotometric estimation of DPT and SL by absorbance corrected method in standard drug and in pharmaceutical dosage forms. The proposed method was optimized and validated as per the International conference on harmonization (ICH) guidelines [1].

MATERIALS AND METHODS

Instrumentation
An UV-Visible double beam spectrophotometer (Varian Cary 100) with 10mm matched quartz cells was used. All weighing were done on electronic balance (Model Shimadzu AUW-220D).

Reagents and chemicals
Pure drug sample of dapoxetine, % purity 99.86 and sildenafil, % purity 99.92 was kindly supplied as a gift sample by Emcure Pharmaceutical Pvt.Ltd. Pune, India. These samples were used without further purification. Tablet used for analysis was SUSTIMAX (Batch No.FMA11001) manufactured by Emcure Pharmaceutical Pvt. Ltd. Pune, containing dapoxetine hydrochloride 30 mg and sildenafil citrate 50 mg per tablet.

Preparation of Standard Stock Solutions and Calibration Curve
Standard stock solutions of pure drug containing 1000 µg/mL of DPT and SL were prepared separately in methanol. Standard stock solutions were further diluted with methanol to get working standard solutions of analytes in the concentration range of 5-25 µg/mL and 8-40 µg/mL DPT and SL of , respectively and scanned in the range of 200-400nm. First derivative amplitudes of spectrum, by using the above mentioned procedures, were used to prepare calibration curves for both the drugs.

Preparation of Sample Solution and Formulation Analysis
Twenty tablets were weighed accurately and a quantity of tablet powder equivalent to 30 mg of DPT and SL was weighed and dissolved in the 30 mL of methanol with the aid of ultrasonication for 10 min and solution was filtered through Whatman paper No. 41 into a 100 mL volumetric flask. Filter paper was washed with solvent, adding washings to the volumetric flask, volume was made up to the mark with methanol. The solution was suitably diluted with methanol to get 30 µg/mL of D and 50 µg/mL of SL.

Theoretical aspects
Absorption Corrected Method
λ max of DPT and SL was determined by scanning the drug solution in UV Spectrophotometer in the range 200 - 400 nm at 0.5 band width and 600 nm/min scan speed and was found to be at 237.54nm and 325.92 nm respectively. Sildenafil also showed absorbance at 325.92 nm, while dapoxetine did not show any interference at 325.92nm [Fig 3]. To construct Beer's plot for DPT and SL, stock solutions of 1000 µg/ml of both the drugs were prepared in methanol and working standard dilutions were made in methanol using stock solution of 1000 µg/ml. Also Beer's plot was constructed for DPT and SL in solution mixture at different concentration (5:8,10:16,15:24,20:32,25:40 µg/ml) levels. Both the drugs followed linearity individually and in mixture within the concentration range 5-25 µg/ml and 8-40 µg/ml for DPT and SL, respectively.

Recovery studies
The accuracy of the proposed method was checked by recovery studies, by addition of standard drug solution to preanalysed sample solution at three different concentration levels (80 %, 100 % and 120 %) within the range of linearity for both the drugs. The basic concentration level of sample solution selected for spiking of the drugs standard solution was 30 µg/ml of dapoxetine and 50 µg/ml of sildenafil for given this method.

Solution stability
Solution stability was checked by analyzing solution in fridge temperature and at room temperature by Method. Solution was stable for 12 hours at room temperature.

Method sensitivity (LOD and LOQ)
The values of LOD and LOQ were calculated by using σ (standard deviation of response) and b (slope of the calibration curve) and by using equations,

LOD = (3.3 x σ)/b and LOQ = (10 x σ)/b.

Precision of the Method
Method repeatability was determined by six times repetitions of assay procedure. For intra-day precision method was repeated 5 times in a day and the average % RSD was determined. Similarly the method was repeated on five

different days for inter-day precision and average % RSD was determined Table (1). Precision of analyst was determined by repeating study by another analyst working in the laboratory.

Specificity

Specificity is a procedure to detect quantitatively the analyte in the presence of component that may be expected to be present in the sample matrix. Commonly used excipients in tablet preparation were spiked in a pre-weighed quantity of drugs and then absorbance was measured and calculations done to determine the quantity of the drugs.

Robustness:

The robustness of the proposed method was tested by changing parameters such as wavelength range, slit width, temperature, shaking time etc. None of these variables significantly affected the absorbance of the drugs indicating that the proposed methods could be considered as robust.

RESULTS AND DISCUSSION

Under experimental conditions described, calibration curve, assay of tablets recovery studies, solution stability were performed. Using appropriate dilutions of standard stock solution, two solutions were scanned separately for this method. A critical evaluation of proposed method was performed by statistical analysis of data where slope, intercept, correlation coefficient are shown in Table (1). As per the ICH guidelines, the method validation parameters checked were linearity, accuracy and precision, specificity, roubustness & method sensitivity (LOD & LOQ). Beer's law is obeyed in the concentration range of 5-25 µg/mL and 8-40 µg/mL for DPT and SL, respectively. Correlation coefficient was greater than 0.999 for both the drugs. The proposed method was also evaluated by the assay of commercially available tablets containing DPT and SL. The results of formulation analysis were in the range of 100 ± 2 % and are presented in Table (1). Recovery was found in the range of 99.80 to 100.22% for DPT and 98.92 to 99.40 %, for SL by absorbance corrected method. The accuracy is evident from the data as results are close to 100 % and standard deviation is low. All the statistical data analysis was performed by using MIP Pharmasoft 1.0, software developed and validated in the Institute.

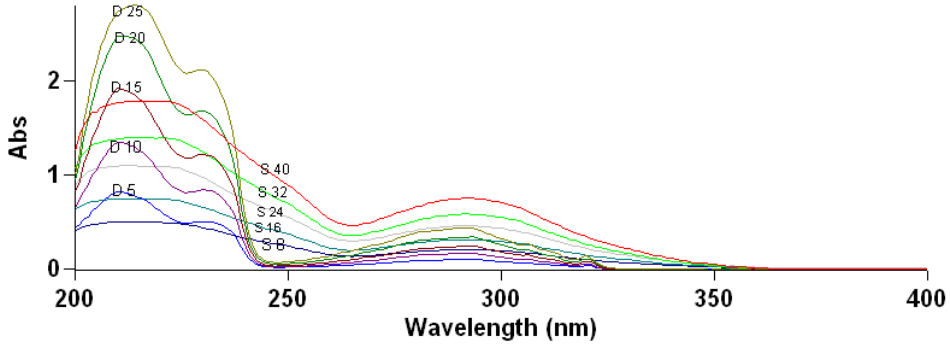

Fig 3: Simple overlay spectra of dapoxetine (5-25 µg/ml) and sildenafil (8-40 µg/ml) in methanol.

Table 1: Optical characteristics of the proposed methods and result of formulation analysis

Parameter		Dapoxetine	Sildenafil
wavelength (nm)		237.54nm	325.92nm
Beer's law limit (µg/mL)		5-25	8-40
Regression Equation*	**Slope (m)**	0.0401	0.0052
	Intercept (c)	0.1495	0.0353
Correlation coefficient (r)		0.9999	0.9994
Precision (%RSD)	**Repeatability (n=5)**	0.73	0.78
	Intra-day(3x5 9)))times0)0))	1.21	1.13
	Inter-day(3x5 days)	0.90	0.80
Formulation Analysis (% Assay, %RSD), n=6		100.40 ±0.45	101.85 ± 0.4
LOD	**(µg/mL)**	0.534	0.646
LOQ	**(µg/mL)**	1.574	2.575
Ruggedness (%RSD)	**Analyst I**	0.57	0.43
	Analyst II	0.56	0.81

RSD = Relative Standard Deviation, Y* = mX + c, where Y is the absorbance and X the concentration in micrograms per milliliter

Table 2: Result of accuracy study

Recovery Level	Analyte name	Amount Spiked (µg/mL)	% Mean Recovery, % RSD, n=6 Method
80%	DPT	12	100.22 0.78
	SL	19.2	99.40, 0.96
100%	DPT	15	99.80, 1.01
	SL	24	98.92, 1.55
120%	DPT	18	100.01, 0.18
	SL	28.8	98.95, 1.35

CONCLUSION

The validated spectrophotometric method employed here proved to be simple, economical, precise and accurate. Thus it can be used as IPQC test and for routine simultaneous determination of DPT and SL in tablet dosage form.

Acknowledgement
The authors wish to express their gratitude Emcure Pharmaceutical Pvt.Ltd. Pune, India, for the sample of pure Dapoxetine and Sildenafil. The authors are also thankful to the management of MAEER's Maharashtra Institute of Pharmacy for providing necessary facilities.

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