WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 4, 1786-1794. Research Article ISSN 2277– 7105

Q-ABSORBANCE RATIO METHOD FOR SIMULTANEOUS ESTIMATION OF CILOSTAZOL AND IMIPRAMINE IN COMBINED DOSAGE FORM

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Article Received on
15 Fab 2015,

Revised on 10 March 2015,
Accepted on 02 April 2015

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ABSTRACT

The present manuscript describes simple, sensitive, accurate, precise and economical Q-absorbance ratio method for the determination of cilostazol and imipramine in combined dosage form. Absorbance ratio method uses the ratio of absorbances at selected wavelengths, one which is an isoabsorptive point and other being the λ-max of one of the two components. Cilostazol and imipramine show an isoabsorptie point at 233.400 nm in methanol. The second wavelength used is 258 nm, which is the λ-max of cilostazol in methanol. The linearity was obtained in the concentration range of 10-35 µg/ml for cilostazol and imipramine.

KEY WORDS: Cilostazol, imipramine, absorbance ratio method, isoabsorptive point, simultaneous

INTRODUCTION

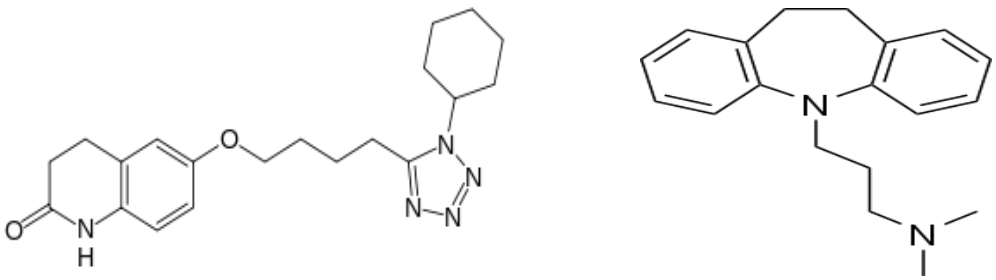
Cilostazol is a selective inhibitor of 3-type phosphodiesterase (PDE3) with therapeutic focus on increasing cAMP. It inhibits platelet aggregation and is a direct arterial vasodilator. Its main effects are dilation of the arteries supplying blood to the legs and decreasing platelet coagulation.[1] Cilostazol is chemically 6-[4-l(-cyclohexyl-lH-tetrazol-5-yl-butoxyl] 3-4-dihydro-2(1H)-quinolinone (Figure 1).[2]

Several methods have been reported for cilostazol alone and in combination with other drugs.[3-9]

Imipramine is a dibenzazepine derived tricyclic antidepressant which acts by inhibition of serotonin and nor epinephrine reuptake within synaptic clefts in the central nervous system, thus increasing brain levels of these neurotransmitters. Imipramine is a tricyclic antidepressant that continues to be widely used in the therapy of depression. Imipramine can cause mild and transient serum enzyme elevations and is rare cause of clinically apparent acute cholestatic liver injury.[10] Chemically imipramine is 3-(10,11-dihydro-5H-dibenzo[b,f]azepin- 5-yl)-N,N-dimethyl-propan-1-amine (Figure 1).[11]

Liturature survey revealed many methods for imipramine in alone or combination with several other drugs.[12-15]

The combination of these two drugs is not official in any pharmacopoeia; hence no official method is available for the simultaneous estimation of cilostazol and imipramine in their combined dosage forms. Literature survey does not reveal any simple Spectrophotometric method for simultaneous estimation of Cilostazol and Imiprmine in combined dosage forms. The present communication describes simple, sensitive, rapid, accurate, precise and cost effective spectrophotometric method based on Q-absorbance ratio method. The method was validated as per ICH guidelines.[16]



(A) (B)

Figure 1: Structure of (A) Cilostazol (B) Imipramine

MATERIALS AND METHODS

Materials

Cilostazol and imipramine were gifted from Pure Chem Pvt. Ltd, Ankleshwar, Gujarat and Unimark Remedies Pvt. Ltd, Ahmedabad, Gujarat. LASTINEM Injection (Cilostazol 125mg and Imipramine 125mg) was purchased from local market. All the reagents used were of AR grade.

Instrumentation

Digital analytical balance (Shimadzu ATX 224), UV-Visible Spectrophotometer (Shimadzu 1601), IR Spectrophotometer(FTIR 8400s).

Method Development

Selection of solvent

Both the drugs are soluble in methanol. The overlain spectra of cilostazol and imipramine when overlapped which show feasibility of using this solvent for spectrophotometric analysis for Q-absorbance ratio method of these drugs. Therefore methanol was selected as solvent.

Preparation of Standard Solutions

10 mg of standard cilostazol and imipramine were weighed and transferred to 100 ml separate volumetric flasks and dissolved in methanol. The flasks were shaken and volumes were made up to mark with methanol to give a solution containing 100 µg/ml each of cilostazol and imipramine.

Sample preparation for determination of cilostazol and imipramine in combined dosage form

First weighed finely powdered equivalent to 125mg of cilostazol and 125 mg of imipramine were transferred to a 100 ml volumetric flask. Solvent was added up to the mark. Sonicated for 15 minute and filtered through Whatman filter Paper No: 41. 1 ml of aliquot was taken and transferred to volumetric flask of 100 ml capacity. Volume was made up to the mark with the methanol. Then 0.8 ml was taken and diluted upto 100 ml to get solution containing cilostazol 10 µg/ml & 10 µg/ml imipramine. After that solution was used for the estimation of cilostazol and imipramine.

Determination of maximum wavelength and isoabsorptive point

Absorbance ratio method uses the ratio of absorbances at two selected wavelengths, one which is an isoabsorptive point and other being the λ-max of one of the two components. From the overlay spectra of two drugs, it is evident that cilostazol and imipramine show an isoabsorptive point at 233.400 nm. The second wavelength used is 258 nm, which is the λ-max of imipramine (Figure 2). Working standard solutions having different concentration for cilostazol and imipramine were prepared in methanol and the absorbances at 233.400 nm (isoabsorptive point) and 258 nm (λ-max of cilostazol) were measured and absorptivity coefficients were calculated using calibration curve.

The concentration of two drugs in the mixture can be calculated using following equations.

$CX = [(QM – QY) / (QX - QY)] \times A1/ax1$

$CY = [(QM – QX) / (QY - QX)] \times A1/ay1$

Where, A1 and A2 are absorbances of mixture at 233.400 nm and 258 nm; ax1 and ay1 are absorptivities of cilostazol and IMI at 233.400 nm; ax2 and ay2 are absorptivities of cilostazol and IMI respectively at 258 nm; QM = A2 / A1, QX = ax2 / ax1 and QY = ay2 / ay1.

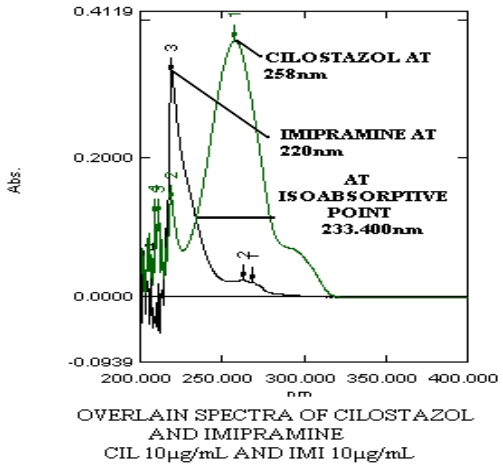


Figure 2: The overlain spectra of cilostazol andiImipramine.

VALIDATION OF METHOD

Linearity (calibration curve)

Aliquot portions cilostazol and imipramine were separately transferred into 10 mL volumetric flasks. The volume was adjusted to the mark with methanol to obtain different concentrations. The absorbances of solution were then measured at 258 nm and 233.400 nm. The calibration curves were constructed by plotting absorbances versus concentration and the regression equations were calculated.

Method precision (repeatability)

The precision of the instrument was checked by repeated scanning and measurement of absorbance of solutions (n = 3) for cilostazol and imipramine (10 µg/ml for both drugs) without changing the parameter of the proposed spectrophotometry method.

Intermediate precision (reproducibility)

The intraday and interday precision of the proposed method was determined by analyzing the corresponding responses 3 times on the same day and on 3 different days over a period

of 1 week for 3 different concentrations of standard solutions of cilostazol and imipramine (10 µg/ml). The result was reported in terms of relative standard deviation (% RSD).

Accuracy (recovery study)

The accuracy of the method was determined by calculating the recoveries of cilostazol and imipramine by the standard addition method. The known amounts of standard solutions of cilostazol and imipramine were at added at 50, 100 and 150 % level to prequantified sample solutions of cilostazol and imipramine (10 µg/ml for cilostazol and 10 µg/ml for imipramine). The amounts of cilostazol and imipramine were estimated by applying obtained values to the respective regression line equations.

Limit of detection and limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH) guidelines.

$LOD = 3.3 \times \sigma/S$

$LOQ = 10 \times \sigma/S$

Where, σ = the standard deviation of the response and

S = slope of the calibration curve.

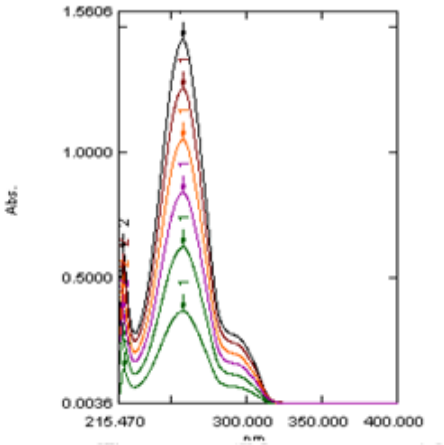
RESULTS AND DISCUSSION

The two wavelengths were used for the analysis of the drugs were 258 nm (λ-max of cilostazol) and 233.400 nm (isoabsorptive point) at which the calibration curves were prepared for both the drugs. For cilostazol, Beer- Lambert's law is obeyed in concentration range of 10 -35 µg/ml at 258 nm (Table 1, Figure 3,5). For imipramine, the Beer- Lambert's law is obeyed in concentration range of 10-35 µg/ml at 233.40 nm (Table 1, Figure 4, 6). The % recoveries were found to be in the range of 98.45 – 99.54 % for cilostazol and 98.03-101.94 % for imipramine (Table 2). The precision of method was determined by repeatability, intraday and interday precision and was expressed as the % RSD, which indicated good method precision (Table 1).

The Limit of detection for cilostazol and imipramine was found to be 0.026 µg/ml and 0.069 µg/ml respectively. Limit of quantification for cilostazol and imipramine was found to be 0.077 and 0.209 µg/ml respectively (Table 1).

The proposed spectrophotometric method was successfully applied to cilostazol and imipramine combined dosage form. The cilostazol and imipramine content in marketed injection formulation was found to be 98.02 % and 100.70 % respectively (Table 3).

The result of the analysis of pharmaceutical formulation by the proposed method is highly reproducible and reliable and it is in good agreement with the label claim of the drug. The method can be used for the routine analysis of the cilostazol and imipramine in combined dosage form without any interference of excipients.



Fugure 3: Linearity spectra of cilostazol at 258 nm (10-32 µg/ml).

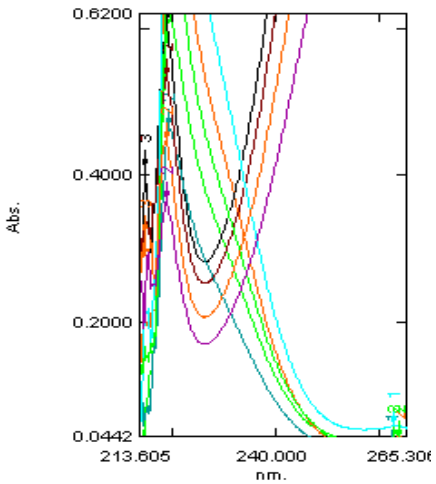
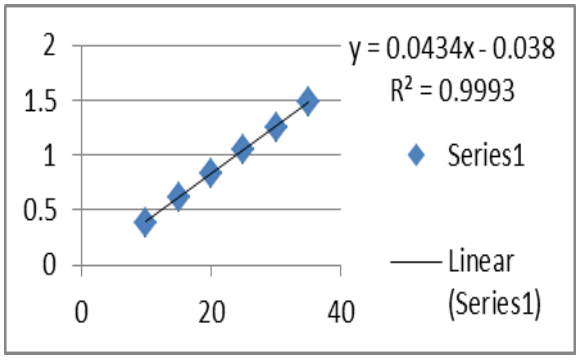


Figure 4: Fugure 2: Linearity spectra of imipramine at 233.400 nm (µg/ml).



Fugure 5: Calibration curve of cilostazol at 258 nm (10-32 µg/ml)

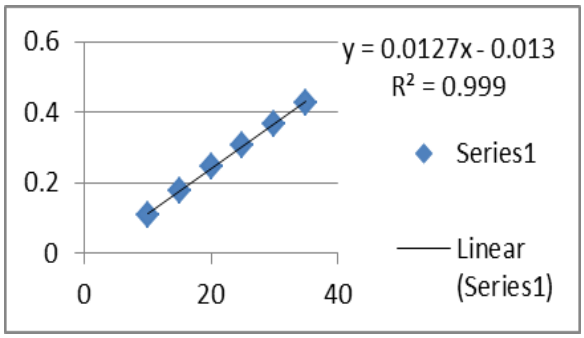


Figure 6: Calibration curve of imipramine at 233.400 nm (µg/ml).

Table 1: Regression analysis and summary of validation parameters for cilostazol and imipramine by Q-absorbance spectrophotometric method

Parameters		Cilostazol	Imipramine
		258nm	233.400
Linearity Range (µg/ml)		10-35	10-35
Regression equation		y = 0.0434x - 0.038 R² = 0.9993	y = 0.0127x - 0.013 R² = 0.999
Correlation co-efficient (r^2)		0.999	0.999
Accuracy (%)	80%	98.50	98.03
	100%	98.45	101.94
	120%	99.54	99.82
Precision (%RSD)	Intra day	0.1711	0.3472
	Inter day	0.2034	0.4847
Repeatability		0.0882	0.2432
LOD(µg/ml)		0.026	0.069
LOQ(µg/ml)		0.077	0.209

Table 2: Accuracy of Cilostazol and Imipramine (n=3)

Drug	% amount added	Amount taken	Amount added	Total Amount	Amount found	% recovery
Cilostazol	80%	10	08	18	17.73	98.50
	100%	10	10	20	19.69	98.45
	120%	10	12	22	21.89	99.54
Imipramine	80%	10	08	18	17.64	98.03
	100%	10	10	20	20.38	101.94
	120%	10	12	22	21.96	99.82

Table 3: Analysis of Cilostazol and imipramine by Spectrophotometric Method

Injection Formula	Labal Claim (mg)		Amount Found (mg)		% Lable claim	
Lastinem	**Cilostazol**	**Imipramine**	**Cilostazol**	**Imipramine**	**Cilostazol**	**Imipramine**
	125	125	122.50	125.87	98.02	100.70

CONCLUSION

These entire factors lead to the conclusion that the proposed method is accurate, precise, simple, sensitive, rapid, chip and can be applied successfully and routine simultaneous estimation of cilostazol and imipramine combined dosage form.

ACKNOWLEDGEMENT

The authors are thankful to Pure Chem Pvt. Ltd, Ankleshwar, Gujarat and Unimark Remedies Pvt. Ltd, Ahmedabad, Gujarat, for providing gift sample of cilostazol and imipramine for research.

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