JOURNAL OF PHARMACEUTICAL SCIENCE AND BIOSCIENTIFIC RESEARCH (JPSBR)

(An International Peer Reviewed Pharmaceutical Journal that Encourages Innovation and Creativities)

Analytical Method Development and Validation for Simultaneous Estimation of Cilostazole and Imipramine by RP HPLC

Sanket N.Patel, Divya Thakkar, Mandev B Patel

Department of Quality Assurance, A-One college of Pharmacy, Anasan, Ahmedabad, Gujarat, India

ABSTRACT:

A simple, rapid, economical, precise and accurate RP-HPLC method for simultaneous estimation of Cilostazole and Imipramine has been developed. A reverse phase high performance liquid chromatographic method was developed for the simultaneous estimation of Cilostazole and Imipramine.. The separation was achieved by LC- 20 AT C18 (25 cm × 0.46 cm) Hypersil BDS column and Buffer (pH 4.5)-Methanol (20:80)as mobile phase, at a flow rate of 1 ml/min. Detection was carried out at 222 nm. Retention time of Cilostazole and Imipramine were found to be 5.383 min and 3.153 min, respectively. The method has been validated for linearity, accuracy and precision. Linearity observed for Cilostazole 6-18 µg/ml and for Imipramine 6-18 µg/ml. The percentage recoveries obtained for Cilostazole and Imipramine were found to be in range of 99.61 ± 0.50 and 99.78 ± 0.65 respectively. Developed method was found to be accurate, precise and rapid for simultaneous estimation of Cilostazole and Imipramine.. The proposed method was successfully applied for the simultaneous estimation of both the drugs in commercial Combined dosage form.

Key words: Cilostazole, Imipramine, Simultaneous estimation, RP-HPLC Method, Validation.

Article history:

Received 10 March 2015
Revised 14 April 2015
Accepted 16 April 2015
Available online 01 September 2015

Citation:

Patel S. N., Thakkar D., Patel M. B. Analytical Method Development and Validation for Simultaneous Estimation of Cilostazole and Imipramine by RP HPLC. **J Pharm Sci Bioscientific Res**. 2015 5(5):411-417

For Correspondence:

Sanket Patel

Student of Quality Assurance,

A-One college of Pharmacy, Anasan, Ahmedabad, Gujarat, India.

Email: jpsbronline@rediffmail.com

(www.jpsbr.org)

INTRODUCTION:

Cilostazol is a medication used in the alleviation of the symptom of intermittent claudication in individuals with peripheral vascular disease. It is manufactured by Otsuka Pharmaceutical Co. under the trade name Pletal. Although drugs similar to cilostazol have increased the risk of death in patients with congestive heart failure, studies of significant size have not addressed people without the disease.

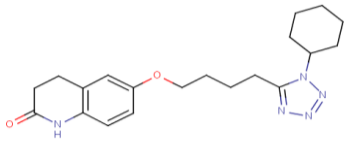

Figure 1:Chemical structure of Cilostazole

Imipramine, the prototypical tricyclic antidepressant (TCA), is a dibenzazepine-derivative TCA. TCAs are structurally similar to phenothiazines. This medication is used to treat depression. It is also used with other therapies for the treatment of nighttime bwetting (enuresis) in children

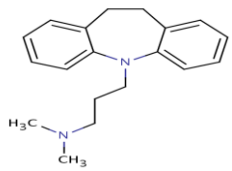

Figure 2:Chemical structure of Imipramine

Materials and methods

Materials

HPLC Thermo separation. Imipramine was procured from Alar laboratories. Cilostazole was procured from Zillion pharmaceuticals pvt. ltd. All solvents (HPLC grade) were obtained from Merck Chemicals.

Methods

Preparation of Standard Solution

(A) Imipramine standard stock solution: (120 µg/mL)

A 12 mg of Imipramine was weighed and transferred to a 100 mL volumetric flask. volume was made up to the mark with methanol.

(B) Cilostazole standard stock solution: (120 µg/mL)

A 12 mg of Cilostazole was weighed and transferred to a 100 mL volumetric flask. volume was made up to the mark with methanol.

(C) Preparation of standard solution of binary mixtures of Imipramine (12 µg/mL) and Cilostazole (12 µg/mL)

Take 1 mL from the Imipramine stock solution and 1mL from Cilostazole stock solution and transferred to 10 mL volumetric flask and volume made up to the mark by mobile phase which was used in particular trials.

METHOD VALIDATION

Chromatographic conditions and System Suitability Parameters:

- ✓ **Column**: C_{18} (25 cm × 0.46 cm) Hypersil BDS
- ✓ **Mobile Phase**: Buffer (pH 4.5)-Methanol (20:80)
- ✓ **Flow Rate** : 1.0 ml/min
- ✓ **Detection Wavelength**: 222 nm
- ✓ **Run time**: 7 min
- ✓ **Injection volume** : 20.0 µl

Table 1 System Suitability Parameters:

Parameters	Data observed	
	Imipramine	**Cilostazole**
Theoretical plates per column	7334	4447
Symmetry factor/Tailing factor	1.333	1.659
Resolution	9.846	

Selection of wavelength :

Standard solution of Imipramine (12 µg/mL) and Standard solution of Cilostazole (12µg/mL) were scanned between 200-400 nm using UV-visible spectrophotometer.

Both solutions were scanned between 200 - 400 nm.

Wavelength was selected from the overlay spectra of above solutions.

Method validation:

Linearity

The linearity for Imipramine and Cilostazole were assessed by analysis of combined standard solution in range of 6-18 µg/ml and 6-18 µg/ml respectively. 5,5.5,10,12.5,15 ml solutions were pipette out from the Stock solution of Imipramine (120 µg/ml) and Cilostazole (120 µg/ml) and transfer to 100 ml volumetric flask and make up with mobile phase to 6,9,12,15 and 18 µg/ml and 6,9,12,15 and 18 µg/ml for Imipramine and Cilostazole respectively

In term of slope, intercept and correlation co-efficient value. The graph of peak area obtained verses respective concentration was plotted.

Precision

Results should be expressed as Relative standard deviation (RSD) or coefficient of variance.

Repeatability

Standard solution containing Imipramine (12µg/ml) and Cilostazole (12µg/ml) was injected six times and areas of peaks were measured and % R.S.D. was calculated.

B. Intra-day precision

Standard solution containing (6,12,18 µg/ml) of Imipramine and (6,12,18 µg/ml) of Cilostazole were analyzed three times on the same day and % R.S.D was calculated.

C. Inter-day precision

Standard solution containing (6,12,18 µg/ml) of Imipramine and (6,12,18 µg/ml) of Cilostazole were analyzed three times on the different day and % R.S.D was calculated.

Accuracy

For Imipramine

6 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80% , 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 222 nm. The amount of Imipramine was calculated at each level and % recoveries were computed.

For Cilostazole

6 µg/ml drug solution was taken in three different flask label A, B and C. Spiked 80% , 100%, 120% of standard solution in it and diluted up to 10ml. The area of each solution peak was measured at 222 nm. The amount of Cilostazole was calculated at each level and % recoveries were computed.

LOD and LOQ

The LOD was estimated from the set of 3 calibration curves used to determination method linearity. The LOD may be calculated as,

LOD = 3.3 × (SD/Slope)
Where,
SD= Standard deviation of Y-intercepts of 3 calibration curves.
Slope = Mean slope of the 3 calibration curves.

The LOQ was estimated from the set of 3 calibration curves used to determine method linearity. The LOQ may be calculated as,

LOQ = 10 × (SD/Slope)
Where,
SD = Standard deviation of Y-intercepts of 3 calibration curves.
Slope = Mean slope of the 3 calibration curves.

Robustness

Following parameters were changed one by one and their effect was observed on system suitability for standard preparation.

1. Flow rate of mobile phase was changed (± 0.2 ml/min) 0.8 ml/min and 1.2 ml/min.

2. pH of Mobile phase was changed (± 0.2) 3.7 and 3.3.

3.Ratio of Mobile phase was changed(±2) Buffer: Methanol (18:82) and Buffer: Methanol (22:78)

Precision

Repeatability

Repeatability was determined by analyzing standard solution of Gresiofulvin having the concentration 20 µg/ml. Scanned these solutions six times in a day. The results were reported in terms of % RSD (relative standard deviation).

RESULT AND DISCUSSION

Selection of mobile phase The mobile phase Buffer(pH 4.5):Methanol (20:80v/v) was selected because it was found to ideally resolve the peaks with retention time (RT) 3.153 min and 5.383 min for Imipramine HCl and Cilostazole respectively and the same is shown in fig3

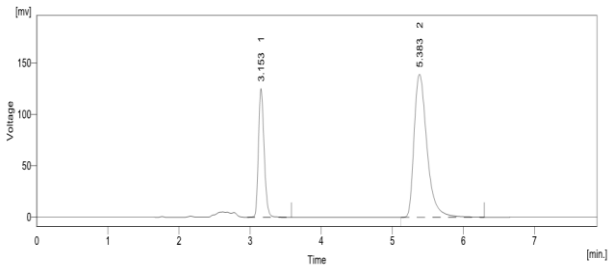

Figure 3 : Chromatogram of Imipramine and Cilostazole in Buffer (pH 4.5) : Methanol (20:80v/v) (Flow rate-1.0 ml/min).(final)

Linearity and Range

The linearity for Imipramine and Cilostazole were assessed by analysis of combined standard solution in range of 6-18 µg/ml and 6 -18 µg/ml respectively. Correlation co-efficient for calibration curve Imipramine and Cilostazole was found to be 0.996 and 0.998 Respectively.

The regression line equation for Imipramine and Cilostazole are as following: or
Cilostazole: **y = 149.8x + 68.76**
Imipramine : **y = 141.1x + 91.68**

Table:2 Linearity data for Imipramine

Sr. No	Concentration (µg/ml)	Area
1	6	737.485
2	9	1090.495
3	12	1492.054
4	15	1838.463
5	18	2267.736

Table:1 Linearity data for Cilostazole

Sr. No	Concentration (µg/ml)	Area
1	6	307.618
2	9	414.499
3	12	622.853
4	15	766.717
5	18	932.895

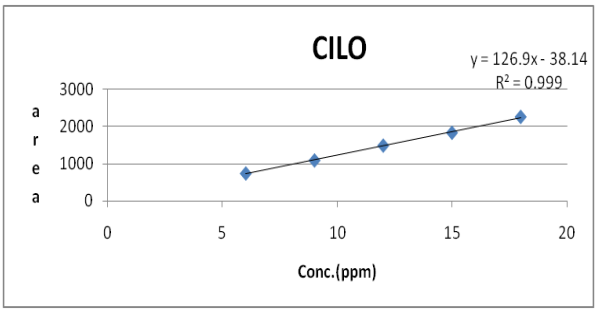

Figure 4: Calibration Curve of Cilostazole (6-18 µg/ml)

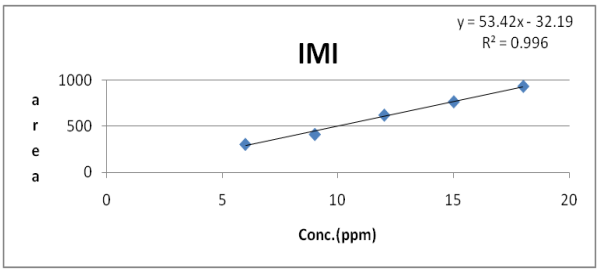

Figure 4:Calibration Curve of Cilostazole (6-18 µg/ml).

Precision

I. Repeatability

The data for repeatability of peak area measurement for Cilostazole and Imipramine based on six measurements of same solution of Cilostazole and Imipramine are depicted in table 3, 4. The % RSD for Cilostazole and Imipramine was found to be 0.903 and 0.427 respectively.

Table 3: Repeatability data for Cilostazole.

Cilostazole			
Conc (µg/ml)	**Area**	**Mean ± S.D (n=6)**	**% R.S.D**
12	1486.372 1458.613 1492.32 1495.324 1487.859 1490.828	1485.22±13.42	0.903

Precision

I. Repeatability

The data for repeatability of peak area measurement for Cilostazole and Imipramine based on six measurements of same solution of Cilostazole and Imipramine are depicted in table 17, 18. The % RSD for Cilostazole and Imipramine was found to be 0.903 and 0.427 respectively.

Table 4: Repeatability data for Imipramine.

Conc (µg/ml)	Area	Mean ± S.D (n=6)	% R.S.D
12	620.138 621.375 615.927 623.849 620.759 621.998	620.67 ±2.65	0.427

Interday precision

The data for interday precision for Cilostazole and Imipramine is shown in table 5. The % R.S.D. for Interday precision was found to be 0.878-1.856. for Cilostazole and 0.621-0.902 for Imipramine .

Table 5: Interday precision data for estimation of Cilostazole and Imipramine

	Cilostazole			Imipramine		
SR. NO.	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
1	6	726.05 ± 13.47	1.856	6	305.51± 2.76	0.902
2	12	1476.57± 16.16	1.094	12	618.08± 4.21	0.681
3	18	2216.87± 19.47	0.878	18	926.53± 5.75	0.621

Intraday precision

The data for intraday precision for Cilostazole and Imipramine is shown in table 6. The % R.S.D. for intraday precision was found to be 0.818-1.220 for Cilostazole and 0.504-0.888 for Imipramine HCl.

Table 6 : Intraday precision data for estimation of Cilostazole and Imipramine

	Cilostazole			Imipramine		
SR. NO.	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D	Conc. (µg/ml)	Area Mean ± S.D. (n=3)	% R.S.D
1	6	727.86±8.88	1.22	6	305.20± 2.71	0.888
2	12	1474.78±14.88	1.009	12	616.84± 3.11	0.504
3	18	2213.83±18.12	0.818	18	924.50± 6.74	0.729

Accuracy

Accuracy of the method was confirmed by recovery study from marketed formulation at three level of standard addition. The results are shown in table 7 and 8. Percentage recovery for Cilostazole was 99.61-99.85 %, while for Imipramine,it was found to be in range of 99.78-100.07%.

Table 7 : Recovery data for Cilostazole.

SR. NO.	Conc. Level (%)	Sample amount (µg/ml)	Amount Added (µg/ml)	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	6	4.8	4.76	99.24	99.85 ± 0.76
2		6	4.8	4.78	99.62	
3		6	4.8	4.83	100.69	
4	100 %	6	6	5.97	99.55	99.61 ± 0.50
5		6	6	5.95	99.15	
6		6	6	6.01	100.14	
7	120 %	6	7.2	7.14	99.17	99.70 ± 0.53
8		6	7.2	7.18	99.70	
9		6	7.2	7.22	100.23	

Table 8 : Recovery data for Imipramine.

SR. NO.	Conc. Level (%)	Sample Amount	Amount Added	Amount recovered (µg/ml)	% Recovery	% Mean Recovery ± S.D
1	80 %	6	4.8	4.74	98.85	100.07 ± 1.12
2		6	4.8	4.85	101.07	
3		6	4.8	4.81	100.29	
4	100 %	6	6	5.95	99.15	99.78 ± 0.65
5		6	6	6.03	100.45	
6		6	6	5.98	99.74	
7	120 %	6	7.2	7.22	100.27	99.79 ± 0.49
8		6	7.2	7.15	99.28	
9		6	7.2	7.19	99.80	

LOD and LOQ

Calibration curve was repeated for five times and the standard deviation (SD) of the intercepts was calculated. Then LOD and LOQ were calculated as follows:

LOD = 3.3 * SD/slope of calibration curve

LOQ = 10 * SD/slope of calibration curve

Where,

SD = Standard deviation of intercepts

Limit of Detection :

Table 9: Limit of Detection data for Cilostazole and Imipramine.

Imipramine	Cilostazole
LOD = 3.3 x (SD / Slope)	LOD = 3.3 x (SD / Slope)
= 3.3 x (24.14/53.42)	= 3.3 x (23.31/126.9)
= 1.491 µg/ml	= 0.606 µg/ml

Limit of Quantitation :

Table 10: Limit of Quantitation data for for Cilostazole and Imipramine.

Imipramine	Cilostazole
LOQ = 10 x (SD / Slope)	LOQ = 10 x (SD / Slope)
= 10 x (24.14/53.42)	= 10 x (23.31/126.9)
= 4.519 µg/ml	= 1.836µg/ml

Robustness

The effect of changes was found to be within the acceptance criteria as shown in table 25 and table 26**.** The % RSD should Be less than 2%.

Analysis of marketed formulation by developed method.

Applicability of the proposed method was tested by analyzing the commercially available Injectable formulation Lastinem .The results are shown in table 13.

Table 11: Robustness data for Cilostazole

SR NO.	Area at Flow rate (- 0.2 ml/min)	Area at Flow rate (+ 0.2 ml/min)	Area at pH (-0.2)	Area at pH (+0.2)	Area at Mobile phase (-2)	Area at Mobile phase (+2)
1	1541.424	1449.225	1523.48	1422.33	1525.002	1446.318
2	1518.268	1418.468	1507.271	1397.652	1493.831	1423.192
3	1553.2	1467.029	1541.424	1434.351	1536.797	1465.564
% R.S.D	1.156	1.700	1.121	1.319	1.462	1.468

Table 12: Robustness data for Cilostazole

SR NO.	**Area at Flow rate (- 0.2 ml/min)**	**Area at Flow rate (+ 0.2 ml/min)**	**Area at pH (-0.2)**	**Area at pH (+ 0.2)**	**Area at Mobile phase(-2)**	**Area at Mobile phase(+2)**
1	638.198	598.712	623.896	585.104	631.271	597.512
2	645.626	608.284	639.393	595.814	637.474	607.063
3	648.109	612.011	643.165	598.319	641.234	611.397
% R.S.D	0.800	1.131	1.607	1.183	0.790	1.173

Table 13 : Analysis of marketed formulation.

Vial	mg/Vial powder		Assay (% of label claim*) Mean ± S. D.	
	Cilostazole (mg)	Imipramine (mg)	% Cilostazole	% Imipramine
Lastinem	125	125	99.26±1.125 2.218124	98.89 ± 1.21

The assay results were comparable to labeled value of each drug in Injectable dosage form. These results indicate that the developed method is accurate, precise, simple and rapid. It can be used in the routine quality control of dosage form in industries.

CONCLUSION

The proposed RP-HPLC method is simple, precise, accurate, economic and rapid for the determination of Imipramine and Cilostazole in bulk drug and in combined Injectable dosage form.

Analysis of authentic sample containing Imipramine and Cilostazole showed no interference from the common additives and excipients.

It can be successfully adopted for routine quality control analysis of Imipramine and Cilostazole in Injectable dosage form without any interference from common excipients and impurity.

ACKNOWLEDGEMENT

Words of gratefulness are expressed for God; My family; Dr. Mukesh Goyal; A-one college of Pharmacy, Anasan, Ahmadabad, India; Staff members of A-one college of Pharmacy; Dr. Mandev B Patel for providing all kind support.

REFERENCES

1. Cilostazole, “Drug bank”, http://www.drugs.com/mmx/cilostazol.html
2. Imipramine, “Drug bank” http://www.drugs.com/pro/cilostazol.html
3. Cilostazole http://www.medicinenet.com/cilostazol-oral/article.htm
4. Imipramine http://www.webmd.com/drugs/2/drug-8664/imipramine-oral/details
5. Snyder L., Kirkland JJ., Glajch JL., “ Practical HPLC Method Development”, Wiley- Interscience Publication. 2nd Edn, Pp. 1- 9,722-723.
6. Chatwal, GR., Sham, AK., “ Instrumental Methods Of Analysis”, New Delhi, Himalaya Publishing House, 5th Edn, 2002 Pp. 256.
7. Ahuja, S. And Scypinski S., “ Handbook Of Modern Pharmaceutical Analysis”, Volume - III, Elsexier Publication , 2009, Pp. 349.
8. Dong M W., “Modern HPLC for Practicing Scientists” New Jersey, A Wiley Interscience Publication. (2006)
9. Sharma BK., , “Instrumental Methods Of Chemical Analysis”, Krishna Prakashan Media (P)Ltd., 27th Edn, 2011, Pp. C-10,C-286.
10. Jeffery G., Bassett J., Mendham J. And Denney R., 6th Edition,” Vogel’s Textbook Of Quantitative Chemical Analysis”, Longman Scientific & Technical, 2009 Pp.615,628- International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use. Validation of Analytical Procedures: Text and Methodology ICH Q2 (R1). 2005. Beckkett AH. And Stenlake JB.; Practical Pharmaceutical Chemistry, Part-II; 4th Edition; CBS Publisher & Distributors, 2007, pp. 257-336,157-173.
11. Skoog DA., Holler FJ., and Crouch SR.; Instrumental Analysis, 8th Edition, Cengage learning India Pvt.Ltd, 2009, pp. 378-407.
12. Chatwal GR.and Anand SK., Instrumental methods of chemical analysis, 5th Edition; Himalaya publishing house, New Delhi, 2008, pp. 2.149-1.184,2.624-2.639.