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First Order Derivative Spectrophotometric Method for Estimation of Loperamide Hydrochloride in Bulk and Pharmaceutical Dosage Form

[1]Pradnya Patil *, Vaishali Rakibe, Tejal P. Patil, Dr. Rajanikant. T. Kakde, Jadhav M. M.
Assistant Professor, Department of Pharmaceutical Chemistry, Siddhi`s Institute of Pharmacy, Nandgaon,Thane.
Assistant Professor, Department of Pharmaceutical Chemistry, Mahatma Gandhi Vidyamandir Pharmacy College Panchavati, Nashik 03.
Assistant Professor, Department of Pharmaceutical Chemistry, Siddhi`s Institute of Pharmacy, Nandgaon,Thane
Principal, Siddhi`s Institute of Pharmacy, Nandgaon, Thane
Assistant Professor, Department of Pharmaceutics, Siddhi`s Institute of Pharmacy, Nandgaon, Thane

Abstract:- A sensitive first order derivative method has been developed and validated for the determination of Loperamide hydrochloridein formulations using Methanol: HCL as a solvent. The drug showed maximum absorbance at 259 nm and amplitude measured in the range of 257 nm-275 nm. The drug obeyed linearity in the range of 400-1400 µg/mL. The present method was validated as per International Conference on Harmonization guidelines. Percent recovery for Loperamide hydrochloride was obtained in the range of 97.90 - 99.99 % , indicates accuracy and % RSD < 2, indicates precision of the method. The results showed that the proposed method is suitable, precise, accurate and rapid for determination of Loperamide hydrochloride in bulk, its tablet dosage forms.

I. INTRODUCTION

Loperamide Hydrochloride (4-(p-chlorophenyl)-4-hydroxy-N,N-dimethyl-diphenyl-1-piperidine bytyramide monhydrochloride) is a white and in powder form which slightly soluble in water, Its freely soluble in alcohol and methanol. Its chemical formula is $C_{29}H_{34}Cl_2N_2O_2$. It is a piperidine derivative that reduces intestinal mobility and thus widely used for the control and symptomatic relief of diarrhoea. In addition, it has been reported that loperamide hydrochloridealso show slightly anti-hyperanalgesic activity. It reduces pain and do not show any side effect on central nervous system.

An opioid medication called loperamide hydrochloride, a synthetic derivative of piperidine, works well to treat diarrhoea brought on by inflammatory bowel illness or gastroenteritis. It is sold both generically and under brand names including Lopex, Imodium, Dimor, Fortasec, and Pept in the majority of the world's nations. The development took place at Janssen Pharmaceutical.

The intestinal wall directly absorbs loperamide hydrochloride due to its antidiarrheal properties. Loperamide hydrochloride, like morphine and other m-receptor agonists, lengthens the intestinal transit time by acting on the myenteric plexus in the longitudinal muscle layer, which reduces propulsive activity and enhances non-propulsive activity. In patients with ileo-anal pouches, loperamide hydrochloride also improves nighttime continence and enhances the tone of the anal sphincter.

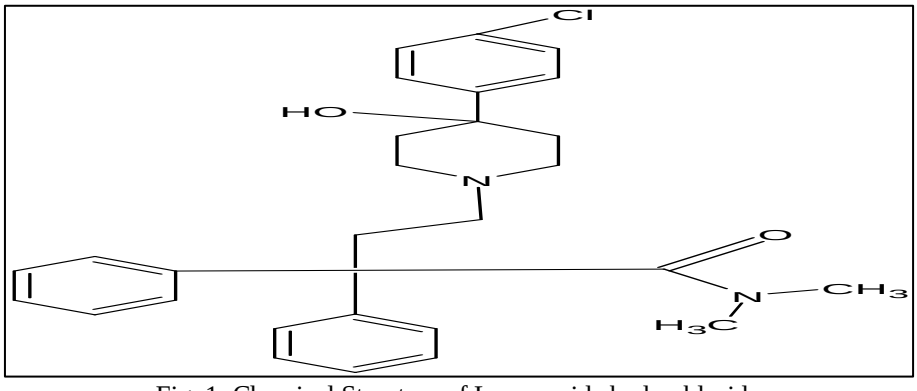

Fig. 1: Chemical Structure of Loperamide hydrochloride

II. EXPERIMENTAL

A. Instrumentation

A Shimadzu double beam UV/Visible spectrophotometer (Model-UV 2600) with a fixed slit width of 1 nm and UV-Probe system software was utilized for the investigation.

B. Materials and Reagents

- *Pure standard*

The International Drug Agency of Pharmaceutical Industry Co. and Chemical Industries Development Co., both located in Cairo, Egypt, provided certified pure loperamide hydrochloride.

➢ *Chemicalsand Reagents*

All the chemicals such as methanol and hydrochloric acid (AR grade) were obtained from Nashik Chemicals Pvt Ltd in Satpur, Nashik. Distilled water was used through out the study. Commercial Loperamide hydrochloride Tablets (LOPAMIDE) used for estimation was manufactured by Torrent Pharmaceuticals containing 2 mg of drug in each Tablet.

➢ *Pharmaceutical formulation*

Lopamide tablets were labeled to contain 2mg of Loperamide hydrochloride per tablet, Tablet was manufactured by Torrent Pharmaceuticals.

➢ *Preparationof 0.1N HCL*

8.18 mL of concentrated hydrochloric acid were homogenized after being diluted with 1000 mL of distilled water.

➢ *Preparation of Solvent*

Mobile phase was created by mixing 0.1N HCL with methanolin a ratio of 45 mL to 5 mL for 50 mL.

➢ *Preparation of Standard Stock Solution*

A 50 mL volumetric flask containing 100 mg of Loperamide hydrochloride was precisely weighed, then 20 mL of methanol :0.1N HCL was added, and the mixture was sonicated for five minutes. The drug was well dissolved, and methanol and 0.1N HCL were added to make a volume of 50 mL, yielding a concentration of 2000 µg/mL.

➢ *Pharmaceutical formulation preparation*

10 tablets of LOPAMIDE were finely powdered and well mixed. An amount equivalent to 100 mg weighed, and transferred into the 50 mL Volumetric flask and dissolved in solvent (methanol: 0.1N HCL). ultrasonication was carried out for one and half hour, filtered, cooled and make up the volume using methanol: 0.1N HCL. (2000 µg/mL).

➢ *Selection of Wave length*

When scanned in the near UV area with a 10µg/mL solution of loperamide hydrochloride, the drug's highest absorbance was seen at 259 nm.

- **Method: First order derivative spectro photometry**

The first order derivative spectra showed maxima at 257 n mand minimaat 275 nm. The derivative amplitudes were calculated by considering the maxima and minima of the curve from the concentration range 400-1400µg/mL. The graph was plotted by using amplitude against concentration and regression equation was calculated.

III. VALIDATION

The criteria of the International Conference of Harmonization (ICH) were followed in the development and validation of the approach. The following parameters were identified: linearity, accuracy, precision, detection limit, quantitation limit, robustness.

A. Linearity

Through analysis of a series of solutions made with methanol (0.1N HCL), the linearity was assessed. The approach followed the concentration range of 400–1400 µg/mL while adhering to Beer–Lambert's law. Table 1 contains the absorbance data, and Figure 1 displays the calibration curve for the first order derivative.

B. Precision

By measuring the triplicates of three distinct concentrations (800, 1000, and 1200 µg/mL) within the linearity range on the same day (intraday precision) and on different days (inter-day precision), the method's precision was determined. The results of the calculation of the percentage relative standard deviation (%RSD) are displayed in Table 2.

C. Accuracy

The accuracy of the novel method was evaluated using three different levels of recovery trials with the standard addition method. Pure drug solution was added to the formulation solution at 80%, 100%, and 120% after a pre-analysis. The results are shown in Table 3.

D. Limit of Detection

The lowest concentration of analyte in the sample that can be identified but may not always be quantified as an accurate number is known as the detection limit of a particular analytical method. The standard deviation of the y-intercepts of the regression lines and the slope value were used to calculate the detection limit (DL). The standard deviation of the response, denoted by σ, and the slope of the standard curve, represented by S, can be used to express the detection limit as LOD=3.3σ/S.

E. Quantitation Limit

The lowest concentration of analyte in a sample that can be quantitatively identified with appropriate precision and accuracy is known as the quantitation limit of a particular analytical process. The formula used to calculate the quantitation limit was QL=10σ/S, where σ represents the response's standard deviation and S denotes the standard curve's slope.

F. Robustness

An analytical procedure's resilience to tiny, intentional changes in method parameters is measured by its robustness, which also indicates how reliable it is under typical operating conditions. We alter both the instrument and the solvent concentration in this suggested procedure.

G. Assay

The previously prepared solution was used for the analysis of commercial tablets (LOPAMIDE). The absorbance was measured at 259 nm against the blank, and the amplitude was determined between 257 and 275 nm. Table 4 presents the findings.

IV. RESULTS AND DISCUSSION

The simple and economical UV spectro photometric method has been developed using methanol: 0.1N HCL after optimization using various solvents. The validation of the methods was carried out as per the ICH guidelines and the results obtained are discussed below.

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A. Linearity

Linear relationships were obtained between amplitude of first derivative spectra versus the corresponding concentrations. Method obeyed linearity in the range of 400-1400 µg/mL. The linear regression equation was found to bey=0.000353x – 0.00493.

Table 1: Linearity observations of Loperamide hydrochloride

Concentration (µg/ml)	Maxima (At 257)	Minima (At 275)	Amplitude
400	0.074	0.078	0.152
600	0.105	0.111	0.216
800	0.142	0.149	0.291
1000	0.175	0.184	0.359
1200	0.205	0.218	0.424
1400	0.241	0.257	0.499

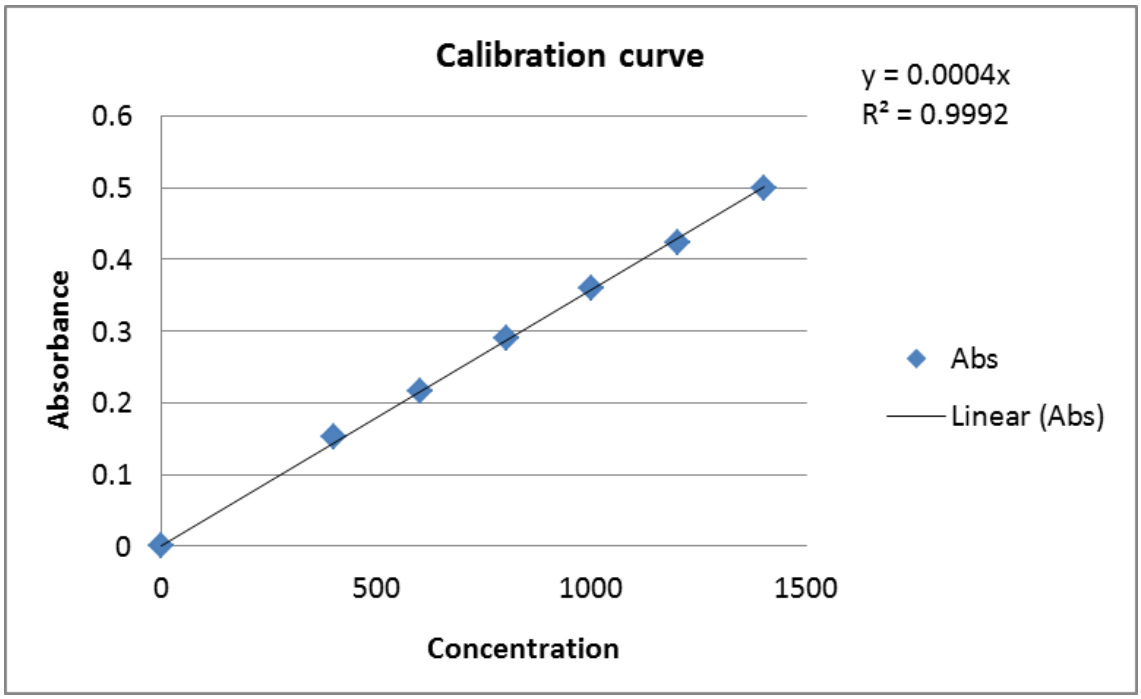

Fig. 2: Calibration cure of LOPERAMIDE HYDROCHLORIDE

➢ *Analysis of marketed tablet formulation(Assay)-*

The label stated for the commercial version of loperamide hydrochloride was calculated using a line equation derived from the calibration plot. Table No. 2 displayed the tablet analysis results, and Table No. 3 displayed the statistical validation.

Table 2: Analysis of marketed formulation

Sr. no	Brand name	Concentration (µg/ml)	Amount found (µg/ml)	% amount found
1	LAPAMIDE	400	402.46	100.61
2		400	402.46	100.61
3		400	399.63	99.90
4		400	405.29	101.32
5		400	402.46	100.61
6		400	402.46	100.61

Table 3: Statistical validation of LOPERAMIDE HYDROCHLORIDE

Sr. no	Mean*	SD	%RSD	SE
1	0.147	0.000632	0.430	0.0002580

*Average of six determinations.

B. Precision

% RSD values for intraday and inter day precision studies were found to be in the range of which are within the acceptable limit (< 2.0%) indicating that the method is precise.

Table 4: Intraday Precision study

Sr. No	Conc. µg /ml (2hrs interval)	Mean	SD	%RSD
12 pm				
1	800	0.291	0.001154	0.396
2	1000	0.359	0.001154	0.321
3	1200	0.424	0.000577	0.136
2 pm				
1	800	0.288	0.001154	0.397
2	1000	0.366	0.000577	0.157
3	1200	0.424	0.000577	0.136
4 pm				
1	800	0.288	0.001154	0.400
2	1000	0.366	0.000577	0.157
3	1200	0.424	0.000577	0.490

*Average of three determinants.

C. Interday precisionstudy-

Table 5: Interday precision study

Sr. No	Conc. µg /ml (2hrs interval)	Mean	SD	%RSD
Day 1				
1	800	0.291	0.001	0.0291
2	1000	0.359	0.00208	0.55
3	1200	0.425	0.001	0.235
Day 2				
1	800	0.290	0.00152	0.526
2	1000	0.368	0.00057	0.156
3	1200	0.423	0.00057	0.136
Day 3				
1	800	0.288	0.00154	0.400
2	1000	0.367	0.000577	0.157
3	1200	0.424	0.00208	0.490

*Average of three determinants.

D. Accuracy

Accuracy was calculated as the percentage recoveries of different concentrations of pure drug and it was further confirmed by standard addition technique (Table. 6).

Table 6: Recovery study of Loperamide hydrochloride by first order method

Level of addition	Drug Conc. (µg/ml)	Standard added (µg/ml)	Total Conc. (µg/ml)	Conc. recovered (µg/ml)	% Recovery
80%	200	160	360	357.13	99.20
	200	160	360	359	99.99
	200	160	360	357.13	9920
100%	200	200	400	396.12	99.03
	200	200	400	393.12	98.28
	200	200	400	396.12	99.03
120%	200	240	440	430.79	97.90
	200	240	440	436.45	99.19
	200	240	440	436.45	99.19

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Table 7: Statistical validation study of Loperamide hydrochloride by first order method

Level of Addition	% Mean Recovery*	SD	%RSD	SE
80%	99.46	0.000577	0.44	0.000333
100%	98.78	0.000577	0.40	0.000333
120%	98.52	0.00115	0.73	0.000663

*Average of three determinant

E. Limits of detection and quantitation (LOD and LOQ)

LOD and LOQ were calculated. The low values of LOD and LOQ indicated the high sensitivity of the method (Table 1).

Table 8: LOD and LOQ

Parameters	Result
LOD (µg/mL)	0.94
LOQ (µg/mL)	2.85

F. Robustness

There was no significant change in absorbance by changing solvent concentration.

Table 9: Robustness study of Loperamide hydrochloride by first order method

Sr. no	Concentration(µg/ml)	Mean *	SD	%RSD
1	400	0.157	0.00265	1.68
2	400			
3	400			
4	400			
5	400			
6	400			

V. CONCLUSION

The ICH recommendations were followed in the development and validation of the UV spectrophotometric procedures. Based on the results, it can be said that the techniques for determining the concentration of loperamide hydrochloride are straightforward, affordable, exact, and accurate, and they can be used to successfully estimate the amount of medicine in API and formulation. Therefore, it is convenient to use the suggested methods for regular quality control analysis.

Table 9: Optical Characteristics.

Parameter	Method
λ(nm)	Maxima 257 Minima 275
Linearity(µg/mL)	400-1400 µg/mL
Correlationcoefficient(R^2)	y=0.000353x+0.00493
Regression Equation	0.999
Slope(m)	0.000353
Intercept(c)	0.00493
LOD(µg/mL)	0.94
LOQ(µg/mL)	2.85

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