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A Validated RP-HPLC Method for the estimation of Loperamide Hydrochloride in Tablet dosage forms

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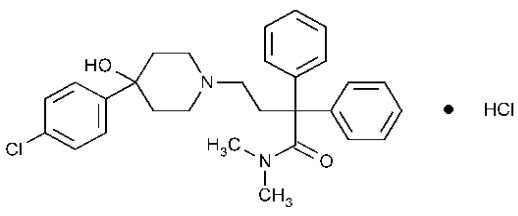
Abstract: A rapid and sensitive Reverse Phase High Performance Liquid Chromatographic [RP-HPLC] method was developed for the estimation of Loperamide Hydrochloride [LPD] in pure and its capsule dosage forms. The method was validated as per International Conference on Harmonization [ICH] guidelines. The mobile phase used in this study is a mixture of Tetrabutylammonium hydrogen sulphate buffer&Acetonitrile in the ratio of 70:30% v/v. Stationary phase was Zodiac C18 reverse phase column (150×4.6mm, 3µm) dimensions at 35ºc temperature. The analysis was performed with run time of 18.0 minutes at a flow rate of 1.00ml/min. The LPD was monitored at 220nm with UV detection and LPD was eluted at 3.65min. The method was linear (r2 =0.999) at concentration ranging from 25 to 150µg/ml, precise (intra-day relative standard deviation [RSD] and inter-day RSD values < 1.0%), accurate (mean recovery = 99.5%), estimated from linearity by regression respectively. The results showed that the proposed method is suitable for the precise, accurate and rapid determination of LPD in bulk, its capsule dosage forms.
Keywords: Loperamide Hydrochloride, RP-HPLC, Validation, Dosage form.

Introduction

Loperamide is a synthetic piperidine derivative, is an opioid drug effective against diarrhea resulting from gastroenteritis or inflammatory bowel disease. In most countries it is available generically and under brand names such as Lopex, Imodium, Dimor, Fortasec and Pepto Diarrhea Control. It was developed at Janssen Pharmaceutical.

Structure



IUPAC Name: 4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]- *N*,*N*-dimethyl-2,2- Di-phenylbutanamide.

Molecular Formula : $C_{29}H_{33}Cl\ N_2O_2$
Molecular Weight : 477.037
Category : Anti-diarrheal drug.
Solubility : It is soluble in methanol.

Mechanism of Action:

Loperamide is an opioid-receptor agonist and acts on the μ-opioid receptors in the myenteric plexus of the large intestine; by itself it does not affect the central nervous system like other opioids.

It works by decreasing the activity of the myenteric plexus, Loperamide also decreases colonic mass movements and suppresses the gastrocolic reflex. Loperamide molecules do not cross the blood-brain barrier in significant amounts, and, thus, it has no analgesic or euphoric properties. Any that do cross the blood-brain barrier are quickly exported from the brain by P-glycoprotein also known as multidrug resistance protein. Tolerance in response to long-term use has not been reported. However, loperamide has been shown to cause a mild physical dependence during preclinical studies, specifically in mice, rats, and rhesus monkeys. Symptoms of mild opiate withdrawal have been observed following abrupt discontinuation of long-term therapy with loperamide.

Comprehensive literature survey reveals that several analytical methods have been reported for the estimation of LPD which includes Reverse Phase HPLC [3,4], Spectrophotometry [1] , LC-MS[3,4,6] spectrofluorimetric,[2] electro kinetic chromatography [5].

Experimental:

Instrumental description:

High performance Liquid Chromatograph system equipped with pump, detector and injector [Agilent 1200 pumps with gradient mixer assembly]. Visible spectrophotometric detectors with a Data handling system EZ Chrome Elite software are used. A stainless steel column 100mm long, 4.6mm internal diameter filled with Octadecyl silane chemically bonded to porous silica particles of 3μm diameter. **Zodiac C18, 100X4.6, 3μm** is used.

Chromatographic conditions:

The mobile phase was delivered through the column at flow rate of 1.0mL/min. The column temperature was maintained at 35^{o}C.The sample injection volume was 10μL.Agilent dual λ absorbance detector or equivalent was set at wavelength of 220nm. The details of chromatographic conditions were presented in Table-1.01 for the determination of loperamide in bulk and pharmaceutical formulations.

Reagents:

Tetrabutylammonium hydrogen sulphate: AR Grade

Acetonitrile : HPLC grade

Water : Milli-Q grade

Preparation of solutions:

Preparation of Loperamide hydrochloride Standard solution:

Weighed 20mg of Loperamide hydrochloride working standard and transferred in to a 100 ml volumetric flask, dissolved and diluted with diluent. Filtered it through 0.45 μ or finer porosity membrane filter.

Preparation of Loperamide hydrochloride Sample solution:

Weighed 20mg of Loperamide hydrochloride working standard and transferred in to a 100 ml volumetric flask, dissolved and diluted with diluent. Filtered it through 0.45 µ or finer porosity membrane filter.

Estimation of Loperamide from commercial formulations by the proposed method:

Twenty tablets are weighed to get the average weight and pulverized. The sample powder, claimed to contain 100mg of active ingredient was transferred into 100mL volumetric flask and dilute to volume with water. This solution was further diluted stepwise with water, as under preparation of standard solutions to get different required. The area under the curve, the drug content per each tablet was calculated.

Table.1.01: Chromatographic conditions

Parameters	**Method**
Mobile phase	**Mobile Phase A:** Dissolve 17g of Tetrabutylammonium hydrogen sulphate and dissolved in 1000 ml of HPLC grade water. **Mobile Phase B:** Acetonitrile.
Diluent	Mobile phase-A and Acetonitrile taken in the ratio 70:30
Stationary phase (Column)	**Zodiac C18, 100X4.6, 3µm**
Injection volume	10µL
Flow rate	1.0 mL/min
Column temperature (C^{O})	35^{0}C.
Column Pressure (psi)	1450
Detection wavelength (run)	220nm (UV)
Run time	18 min
Drug retention time	3.65 min

Procedure:

The composition and flow rate of the mobile phase was programmed from mother pump and the mobile phase buffer and acetonitrile (70:30) was passed through the 0.45µm membrane filter using millipore HPLC solvent filtration assembly, was delivered at 1.0mL/min for column stabilization. During this period, the base line was continuously monitored. The wavelength of detection was selected at 220nm. The prepared dilutions containing concentrations of Loperamide in the range 25.0 -150µg/mL were injected into the chromatograph. The stability of the solution of Loperamide during analysis was determined by repeated analysis of samples during the course of the experiment of the same day and also on different days after storing at laboratory bench conditions and in the refrigeration. Chromatogram parameters, retention time and asymmetry factor were standardized. A chromatogram indicating the separation of Loperamide is given in Fig 1.01.The retention time for Loperamide is 3.65min. The amount of the drug present in each pharmaceutical formulation was calculated through peak area ratio of component by making use of the standard calibration curve.

Validation of the developed method:

Specificity: The selectivity of the method was investigated by observing interferences between Loperamide and the excipients.

Linearity: Linearity studies were carried out by analyzing five separate solutions of drug prepared from stock solution in concentration range of 25.0 -150µg/mL. In this HPLC method the calibration curve was set up by plotting the lisinopril peak area ratios vs function of drug concentrations. The curves were linear over the range of 25.0 -150µg/mL (Fig.1.02) to get the target concentration of Loperamide in 10µL injected at a flow rate of 1.0mL/min.The correlation coefficient was 0.9999.

Recovery studies (Accuracy and Precision): Reproducibility of the proposed method was studied by five individual injections of the standard. The percent relative standard deviation was found to be 0.11 (n=5).

Precision of the method was determined by replicate analysis of five individual sample preparations in a similar manner as described earlier and the percent label claims were found to be 99.86%to 99.99% for Loperamide.

Recovery was determined by adding known amounts of the drug to the placebo. The recovery study was conducted in three different levels. Each solution was injected in triplicate. The percent recovery was calculated from the average of three replicates. In placebo preparations the percent recoveries were found between 99.86% to 99.99% for Loperamide. The results obtained are presented in Table - 1.02.

Fig: 1.01 Model chromatogram for Loperamide

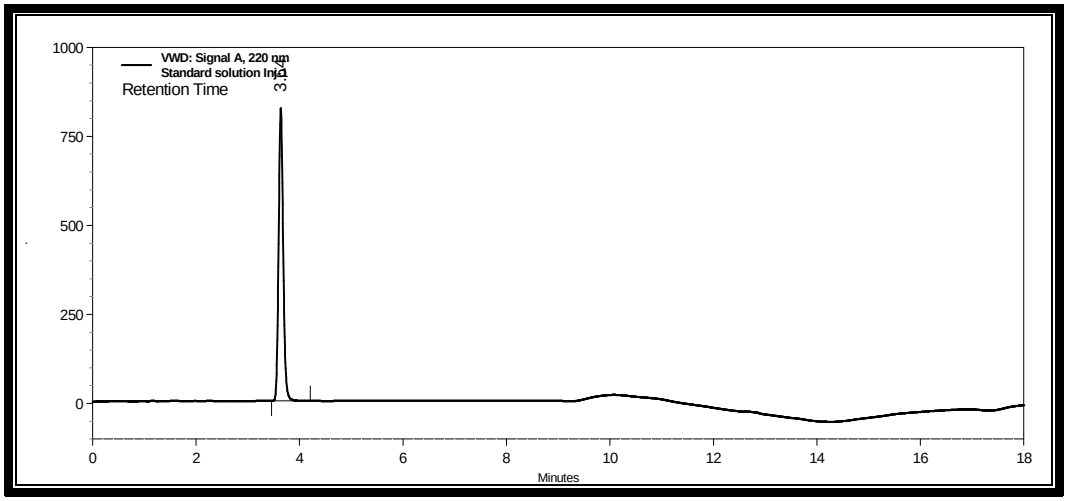


Fig: 1.02. Standard calibration graph of Loperamide

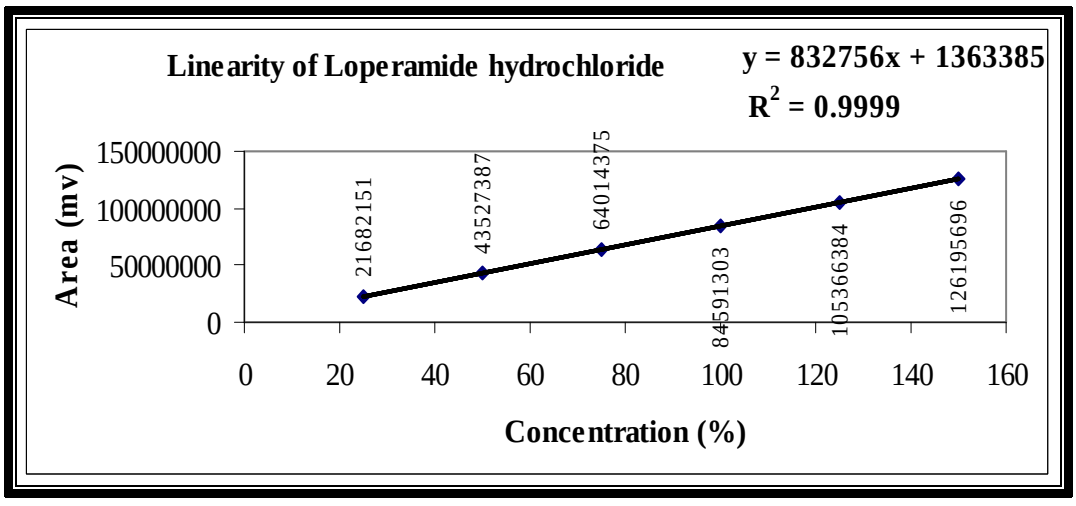


Table – 1.02: Linearity of Loperamide Hydrochloride

Level	Conc. (mg/ml)/ (%)	Inj-1	Inj-2	Inj-3	Avg	%RSD
Level-1	0.05	21728454	21635285	21682714	21682151	0.21
Level-2	0.1	43448878	43562426	43570857	43527387	0.16
Level-3	0.15	64063390	63950798	64028936	64014375	0.09
Level-4	0.2	84475253	84661271	84637385	84591303	0.12
Level-5	0.25	105172889	105526808	105399455	105366384	0.17
Level-6	0.3	126138251	126233319	126215519	126195696	0.04
R^2 Value			0.9990			
Correlation co-efficient			0.9999			
Intercept			1383671			
% of Y-Intercept			1.63571			
Slope			832067			

Results and Discussion:

Different mobile phases were employed for developing proposed HPLC method for the determination of Loperamide. Initially mobile phases consisting of buffer and acetonitrile in the ratio of 50:50 were tried. Zodiac C18, 100X4.6, 3µm was used. Early elution with tailing of peaks was observed. Then the composition of the mobile phase was changed to 70: 30 under these conditions peak shape and no tailing was observed. Loperamide was eluted at around 3.65min with symmetric peak shape. A typical chromatogram for Loperamide using Zodiac C18, 100X4.6, 3µm with mobile phase, composed of buffer: acetonitrile 70: 30 at 1.0mL/min flow rate. The λ_{max} of detection was fixed at 220nm, so that there was less interference from mobile phase with highest sensitivity according to UV analysis. The peak area ratio to the standard versus concentration was found to be linear. The developed method was validated according to the standard procedures and the results are tabulated in Table – 1.03.

Table – 1.03: Assay and Recovery of Loperamide

Pharmaceutical formulation	Labeled amount (mg)	Amount obtained by proposed method	% Recovery of proposed methods
Tablet	10	10.05	100.5 %

Conclusion:

The HPLC method for determining for Loperamide in pharmaceutical formulations was developed using mobile phase, composed of buffer: Acetonitrile 70:30 with 1.0mL/min flow rate. The λ_{max} of the chromatogram was detected with ultraviolet detector at 220nm. The calibration curve was linear over the range of 25.0 -150µg/mL. The correlation coefficient was found to be 0.9999. The proposed method is simple, sensitive and accurate with good precision is suitable for routine analysis of this drug in formulations.

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