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DEVELOPMENT AND VALIDATIONRP-HPLC METHOD FOR THE SIMULTANEOUS DETERMINETION OF LOPERAMIDE HYDROCHLORIDE AND NORFLOXACIN IN PHARMACEUTICAL FORMULATION

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Keywords:

Loperamide Hydrochloride, Norfloxacin, RP-HPLC, Validation

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ABSTRACT: A reverse-phase liquid chromatographic (RP-HPLC) method was developed for the determinations of loperamide hydrochloride and norfloxacin in their marketed formulation and bulk. The separation was carried out using mobile phase of triethylamine and acetonitrile (50:50%) with pH 4.The pH adjusted with orthophosphoric acid. The column used was Capcell pack C18 Column (250mn x 4.6mm, 5µm) and flow rate of 1 ml/min. Detection carried out at 213 nm. The retention time loperamide hydrochloride 5.6 and norfloxacin were found to be 2.1 min respectively. Developed method was validated according to ICH guideline. Linearity was observed at concentration rang of 2-6 µg/ml for loperamide hydrochloride and 200-600 mg/ml for norfloxacin. The regression equation were found to be Y=48615x-435565 and Y=72087x-14016 the correlation coefficient (r^2) 0.9996 and 0.9988 norfloxacin and loperamide hydrochloride respectively. The percentage RSD for the method precision was found to be less than 2%. The accuracy is found in 98-101 %. The proposed method is precise, accurate, selective and rapid for simultaneous determination of loperamide hydrochloride and norfloxacin.

INTRADUCTION: Loperamide hydrochloride synthetic piperidine derivative, it is an opioid drug effective against diarrhea resulting from gastroenteritis or inflammatory bowel disease. IUPAC name is 4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl] - N, N - dimethyl - 2,2-Di-phenylbutanamide hydrochloride. Norfloxacin IUPAC name is 1-ethyl-6-fluoro-4-oxo-7-piperazin-1-yl-1H-quinoline-3-carboxylic acid. It is gyrase of the bacterial DNA.

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Objective of Study: Literature survey revealed that numbers of method have been reported in literature for the individual analysis of norfloxacin and loperamide hydrochloride by UV spectrophotometric and RP-HPLC method. RP-HPLC and UV methods are available in literature for simultaneous determination of norfloxacin with other drugs.

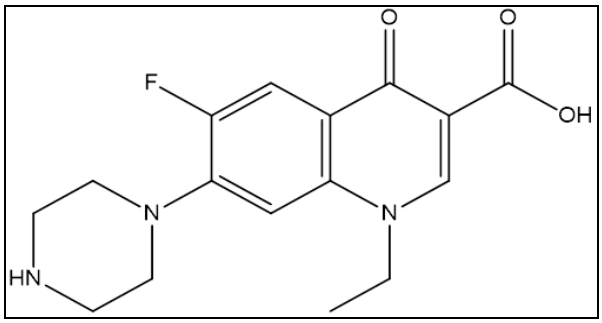

FIG.1: NORFLOXACIN

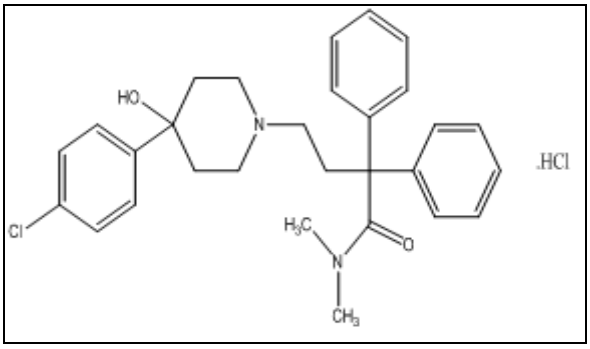

FIG.2: LOPERAMIDE HYDROCHLORIDE

RP-HPLC and UV spectrometric methods are available in literature for determination of loperamide hydrochloride with other drugs.

However, there is no reported RP-HPLC method available for simultaneous estimation of loperamide hydrochloride and norfloxacin.

The aim of the present work was to develop simple, economic, accurate, specific and precise RP-HPLC methods for simultaneous estimation loperamide hydrochloride and norfloxacin in combined pharmaceutical formulation and validation of newly developed analytical methods.

MATERIAL AND METHODS:

Apparatus and Software:

A shimadzu HPLC instrument (LC solution software) equipment with UV detector, Auto sampler injector system, C18 column (250mm x 4.6mm, 5µm) were used. Other equipment was used digital pH meter (LABINDIA PICO+), Pricisa weighing balance, Sonicator (PCI analysis) and Millipore assembly.

Reagent and Chemical: Standard bulk drug sample loperamide hydrochloride and norfloxacin were provided by Holden Medical Laboratories Sinnar(MS).

Year of Experiment: 2015

Analysis of Capsule Formulation:

Twenty capsules were weighed accurately and powdered. Powder equivalent to 200 mg norfloxacin and 2 mg loperamide hydrochloride was weighed and transferred to a 100 ml volumetric flask. It was dissolved in 60 ml diluent and sonicated for 30 minutes. Then the volume was adjusted up to the mark with the same solvent and mixed well. Then it was first filtered through a 0.45µm whatman filer paper. A final concentration of 200µg/ml of norfloxacin and 2µg/ml of loperamide hydrochloride were prepared. Each sample solution was injected into sample injector of HPLC two (n=2) under chromatographic condition as described above.

Area of peak was measured at 213 nm. The amount of drug present in the sample was determine from peak area of norfloxacin and loperamide hydrochloride present in the pure mixture respectively and analysis of marketed formulations shows in table below.

TABLE 1: ASSAY OF FORMULATION

Name	% Assay	% RSD	Retention time
Norfloxacin	100.74	0.098	2.1
Loperamide HCL	98.35	0.003	5.9

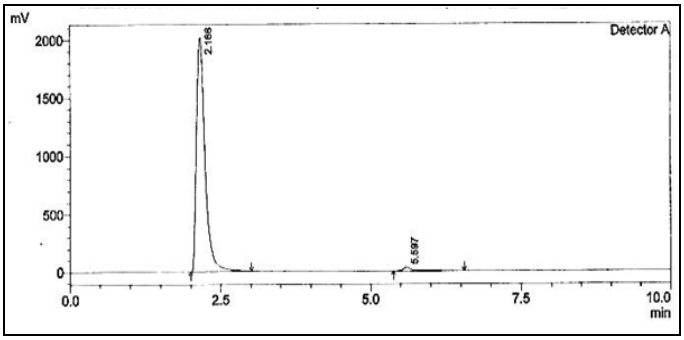

FIG. 3: TYPICAL HPLC CHROMATOGRAPHY OF NORFLOXACIN AND LOPERAMIDE HCL

Preparation of Mobile Phase and Stock Solution:

Acetonitrile: Buffer (Transfer 3 gm. of triethylamine and 1 ml of orthophosphoric acid and 550 ml HPLC grade water and mix.)50:50 and adjust pH 4 with orthophosphoric acid. Diluent as 0.1% orthophosphoric acid: acetonitrile (85:15). Norfloxacin and loperamide hydrochloride weighing about 200 mg and 20 mg of drug respectively and dissolve in 100 and 200 ml diluent to get 2000 ang/ml 100µg/ml of norfloxacin and loperamide hydrochloride.

Method Validation:

The developed method was validated by validation parameter such as system suitability, linearity, precision, accuracy, robustness.

Linearity:
The working solutions were prepared by dilution of aliquots of the stock solutions with diluent to reach the concentration ranges 200-600µg/ml and for norfloxacin and loperamide hydrochloride respectively. Triplicate injection foe each concentration were injected and peak area were recorded. Calibration curve were plotted for both drugs by taking the peak area on y- axis and concentration on x axis. The calibration curve was constructed and evaluated by its coefficient of determination (r^2).

Accuracy:
Accuracy indicated the deviation between the mean value found and the true value. Accuracy was determined by means of recovery experiments by addition of active drug to placebo formulations. The accuracy was calculated from the test results as the percentage of the analyse recovered by the assay.

Robustness:
To verify the robustness of the method, the analysis was done under variables pH, mobile phase ratio, column temperature, wavelength and flow rate. Sample solution were injected and run under chromatic condition.

System suitability parameter:
The system was evaluated by analysing repeatability, retention time, tailing factor and theoretical plates of column.

RESULTS AND SISCUSSION:
All of the analytical validation parameter for the proposed method was determine according to International Conference on Harmonization (ICH) guidelines.

Linearity:
The linearity of this method was determined at ranging from 200-600µg/ml for norfloxacin and 2-6µg/ml for loperamide hydrochloride. The regression equation were found to be Y=48615x-435565 and Y=72087x-14016 the correlation coefficient (r^2) 0.9996 and 0.9988 norfloxacin and loperamide hydrochloride respectively.

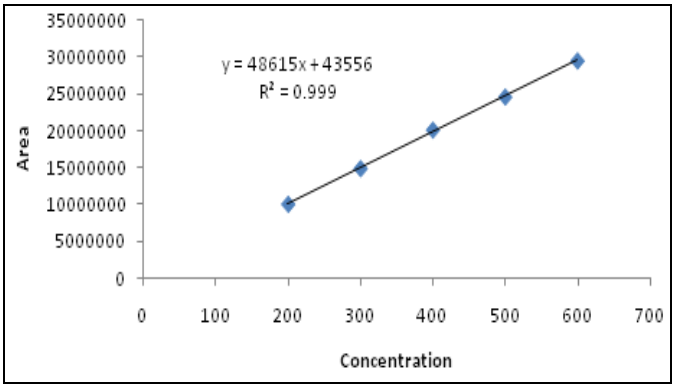

FIG. 4: CALIBRATION CURVE OF NORFLOXACIN

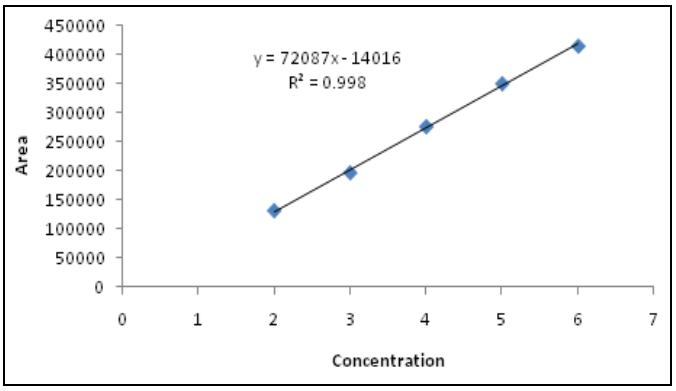

FIG.5: CALIBRATION CURVE OF LOPERAMIDE HCL

Precision:
The precision (measurement of intraday, interday, system precision and method precision) results showed good reproducibility with percent relative standard deviation (% RSD) was below 2.0%. This indicated that method was highly precise.

TABLE 2: DATA OF PRECISION

Precision	Norfloxacin (%RSD)	Loperamide HCl(%RSD)
Intraday	0.01	0.8
Interday	0.5	0.8
System precision	0.02	0.14
Method precision	0.04	0.12

Mean value of six determinations

Accuracy:
Recovery studies were performed to judge the accuracy of the method. The studies were carried out by adding a known quantity of pure drug to pre-analysed formulation and the proposed method was followed. From the amount of drug found, the percent recovery was calculated. Recovery study was carried out at five levels 50%, 75%, 100%, 125%. and 150% for the formulation concentration 200-600µg/ml for loperamide hydrochloride.

TABLE 3: DATA OF ACCURACY

Level Addition %	Loperamide HCl % recovery of pure drug	Norfloxacin % recovery of pure drug
50	98.12	99.75
75	98.10	99.78
100	98.86	99.75
125	98.93	99.52
150	98.24	99.68

Mean value of six determinations

System Suitability Test:

The parameter of system suitability study was presented in table. It was found that the average retention time norfloxacin and loperamide hydrochloride were found to be 2.2 min 5.6 min for five replicate injections respectively. The number of theoretical plates were found to be 4893 and 40679 for norfloxacin and loperamide hydrochloride respectively, which suggested an efficient performance of the column. The resolution was found to be both drugs and this parameter shown in **Table 4.**

TABLE 4: DATA OF SYSTEM SUITABLE PARAMETER

Parameters	Norfloxacin	Loperamide HCL
Retention time	2.1	5.9
Resolution	5.9	14.1
Tailing factor	1.75	1.02

Mean value of six determinations

Robustness:

Robustness was performed by small but deliberate variation in the chromatography conditions and was found to be unaffected by small variations like ±2% in volume of mobile phase composition, ±0.2% ml/min in flow rate of mobile phase and ±2% change in pH It was observed that there were no marked change in the criteria, which demonstrated that the proposed method was robust. These parameter shows in table.

TABLE 5: ROBUSTNESS

Parameters	Norfloxacin			Loperamide HCl		
	Retention time	Peak area	% RSD	Retention time	Peak area	% RSD
1. Change in mobile phase composition(v/v)						
48:52	2.1	20108876	0.01	6.4	253789	0.04
50:50	2.1	19722806	0.02	5.5	272322	0.01
52:48	2.1	20146565	0.05	5.4	274925	0.24
2. Change in mobile phase flow rate (ml/ min)						
0.9 ml/ min	2.4	21865488	0.06	6.2	272074	0.09
1 ml/ min	2.1	19720238	0.01	5.5	275525	0.20
1.1 ml/ min	1.9	17920175	0.20	5.1	247043	0.1
3. Change in wavelength						
211 nm	2.1	20529495	0.003	5.6	283454	0.06
213 nm	2.1	19725374	0.01	5.6	270786	0.06
215 nm	2.1	18594917	0.02	5.6	260963	0.03
4. Change in pH of mobile phase						
4.1	2.1	19877985	0.02	6.0	274949	0.26
4.0	2.1	19885361	0.06	5.6	275147	0.40
3.9	2.1	19981170	0.01	6.2	272335	0.70

Mean value of three determinations

CONCLUTION: The RP-HPLC method has been developed for the simultaneous estimation of norfloxacin and loperamide hydrochloride in their combined marketed formulation and bulk drugs. The method gave good resolution for both the drugs with a short analysis time below 10 minutes which enable rapid quantification for many sample in routine and quality control analysis. The developed method was validated. It was found to be simple, pricise, accurate and robust. The proposed method can be used for routine analysis norfloxacin and loperamide hydrochloride in combined dosage form.

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