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SIMULTANEOUS ESTIMATION OF LOPERAMIDE HYDROCHLORIDE AND NORFLOXACIN BY VALIDATED UV-SPECTROPHOTOMETRIC METHOD

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ABSTRACT

A simple, accurate, precise and reproducible spectrophotometric method has been developed and validated for the simultaneous estimation of Loperamide hydrochloride and Norfloxacin in combined capsule dosage form by Q-Absorbance ratio method. Q-Absorbance ratio method involves two wavelengths; the iso-absorptive point and λ_{max} of loperamide hydrochloride i.e. 231.5 nm and 222 nm respectively in methanol. The linearity was detected in the range of 5-35 µg/ml (R^2 = 0.999) for Loperamide hydrochloride and 1-7 µg/ml (R^2 = 0.998) for Norfloxacin. The accuracy existed between 98-102% and the %RSD was less than 2%. The developed method was validated for linearity, accuracy and precision as per ICH guidelines.

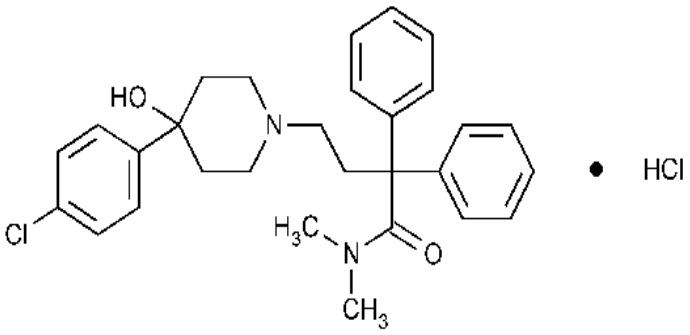
KEY WORDS: Loperamide hydrochloride, Norfloxacin, Q-Absorbance ratio method.

1. INTRODUCTION

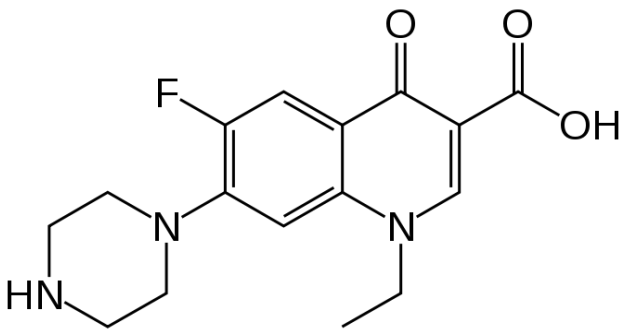
Loperamide hydrochloride synthetic piperidine derivative, is an opioid drug effective against diarrhea resulting from gastroenteritis or inflammatory bowel disease.[1] Rare side-effects associated with loperamide are paralytic ileus, dizziness and rashes.[2] IUPAC name is 4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]-N,N-dimethyl-2,2-Di-phenylbutanamide hydrochloride. Its formula is $C_{29}H_{34}Cl_2N_2O_2$ and molecular weight is 513.5.[3] Norfloxacin[4], chemically known as 1-ethyl-6-fluoro-4-oxo-7-piperazin-1-yl-1H-quinoline-3-carboxylic acid, is a fluorized quinolone, inhibits, like the other members of this group, the gyrase of the bacterial DNA. This effect is responsible for the bactericidal action of norfloxacin. Follows a selection of sensitive bacili: most enterobacteriaceae (E.coli, klebsiellas, etc.), Pseudomonas

aeruginosa, and many pathogenic enteric bacteria (Salmonella, Shigella, etc.), but also Neisseria (especially gonococci). Streptococci are partially resistant whereas anaerobic bacteria are completely resistant.[5]

Both drugs are official in Indian Pharmacopoeia 2010[6], British Pharmacopoeia 2009[7] and United State Pharmacopoeia.[8] Literature survey revealed that RP-HPLC, Liquid Chromatography, UV Spectrophotometric and other methods[9-15] were reported for the estimation of Loperamide Hydrochloride and RP-HPLC, HPTLC and other spectrophotometric methods were reported for the estimation of Norfloxacin.[16-20] As per literature survey, no analytical method has been reported for simultaneous estimation of Loperamide Hydrochloride and Norfloxacin in pharmaceutical dosage forms. In presented research work, we had developed a novel, simple, accurate, sensitive, reproducible, economical analytical method to estimate Loperamide Hydrochloride and Norfloxacin in their combined dosage form in routine analysis. Chemical structures of drugs shown in Fig. 1



(A)



(B)

Figure 1: Chemical Structure of (A) Loperamide Hydrochloride and (B) Norfloxacin

2. MATERIALS AND METHODS

2.1 Reagents and Chemicals: All the reagents used in this assay were analytical grade. Capsules were purchased from local market.

2.2 Instruments: Shimadzu UV/Vis-1700 double beam UV/Vis spectrophotometer and Lab India UV/Vis-3000^{+} double beam spectrophotometer with a fixed slit width of 2 nm, 1 cm quartz cells was used. Class 'A' volumetric glassware were used.

2.3 Preparation of standard solution:

2.3.1 Preparation of stock solution of LOP: Weighed accurately about 50 mg of LOP & transferred into 50 ml volumetric flask, 30 ml of methanol was added and sonicated for about 15 min then diluted up to the mark with methanol to get a stock solution having strength 1000 µg/ml.

2.3.2 Preparation of working standard solution of LOP: 100 µg/ml solution of LOP was prepared by diluting 1 ml stock solution to 10 ml with methanol and diluted further to get the concentration range of 5, 10, 15, 20, 25, 30, 35 µg/ml of LOP.

2.3.3 Preparation of stock solution of NF: Weighed accurately about 50 mg of NF & transferred into 50 ml volumetric flask, 30 ml of methanol was added and sonicated for about 15 min then diluted up to the mark with methanol to get a stock solution having strength 1000 µg/ml.

2.3.4 Preparation of working Standard Solution of NF: 100 µg/ml solution of NF was prepared by diluting 1 ml stock solution to 10 ml with methanol. This solution was diluted further to get the concentration range of 1, 2, 3, 4, 5, 6, 7 µg/ml of NF.

2.4 Absorbance ratio method

The absorbance ratio method is a modification of the simultaneous equation method. It depends upon the principle that, for a substance which obeys beer's law at all wavelengths, the ratio of absorbance at any two wavelengths is constant value independent of concentration or path length. This ratio is called Q value.[21]

$$C_x = \frac{Q_M - Q_Y}{Q_X - Q_Y} \times \frac{A_1}{ax_1}$$

$$C_y = \frac{Q_M - Q_Y}{Q_X - Q_Y} \times \frac{A_2}{ay_2}$$

Where, $Q_0 = A_2/A_1$; $Q_X = a_{x2}/a_{x1}$; $Q_Y = a_{y2}/a_{y1}$, A_1 and A_2 are the absorbance of diluted samples at λ_1 and λ_2, a_{x1} and a_{x2} are the absorptivity of X. a_{y1} and a_{y2} are the absorptivity of Y.

2.4.1 Selection of wavelength

For Q-Absorbance method iso-absorptive point at 231.5 nm and the λ_{max} of LOP 222 nm was selected as working wavelength. Fig. 2

2.5 Optical parameter determination: It is an integral part of UV-Vis method development. The tests are based on the concept that the equipment, electronics analytical operations and sample to be analyzed constitute an integral system that can be evaluated as such. Optical parameters were calculated before starting the experiment. It was determined by taking %RSD of the absorbance of five standards solution. Various optical parameters were determined like wavelength maxima, molar absorptivity, sendell's sensitivity, intercept, slope and correlation coefficient. The data obtained is given in table 1.

2.6 Method Validation: The method was validated with respect to linearity, precision, accuracy, robustness, LOD & LOQ and specificity.[22]

2.6.1 Linearity: Test solutions were prepared form standard stock solutions to get the final concentration of LOP and NF in a range of 5-35 µg/ml and 1-7 µg/ml respectively and analyzed in five replicates. Absorbance vs. respective concentration for LOP and NF were plotted as the calibration curve shown in Fig. 3

2.6.2 Precision: The inter-day precision study was performed on three different days i.e. day1, day2 and day3 at three different concentration levels, (n=9). The intra-day precision study was performed on the same day at 3 time intervals and at three different concentration levels (n=9). The %RSD of the obtained values was calculated and shown in Table 2.

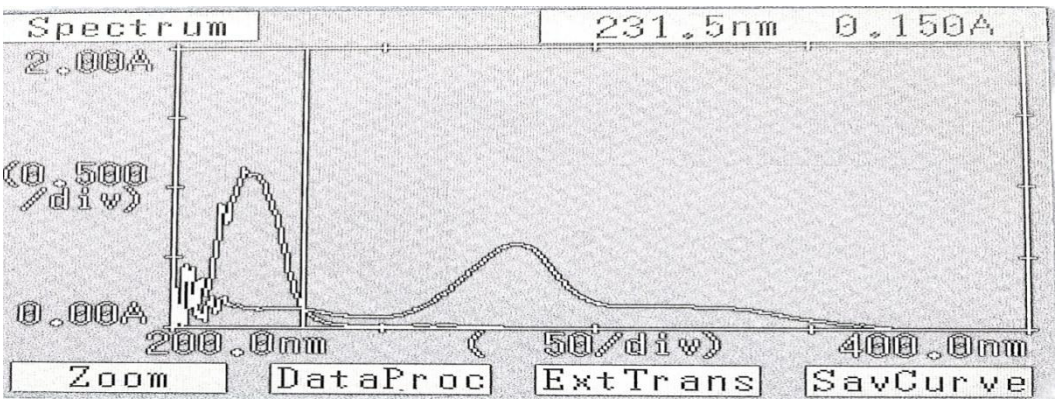
2.6.3 Accuracy: The accuracy of the method was evaluated as triplicate at three concentration levels (80, 100 and 120%), and the percentage recoveries were calculated and shown in Table 3.

2.6.4 Robustness: The robustness of method was performed by change in wavelength. Three replicates were made for the concentration 15 µg/ml of LOP and 5 µg/ml of NF and absorbance was record. The result is expressed in % RSD and shown in Table 4.

2.6.5 Limit of Detection and Limit of Quantitation: The LOQ and LOD were based on the standard deviation of the response and the slope of the constructed calibration curve (n=3), as described in International Conference on Harmonization guidelines Q2 (R1) and results are shown in Table 5.

2.6.7 Specificity: Specificity predicts the interference of excipients or impurities in analysis of pharmaceutical dosage form. Specificity was done by spiking the drug substance with appropriate levels of excipients and demonstrating the assay results that were unaffected by the presence of these extraneous materials. Placebo preparation was done by homogeneous mixing of excipients like lactose, starch, magnesium stearate and talc etc. and % interference shown in Table 5.

Application of Method on Marketed Formulation: Accurately weighed twenty capsules of NORSTREP each of which containing 200 mg NF, 2 mg LOP. A quantity of powder equivalent to 200 mg NF and 2 mg LOP were accurately weighed, the difference in ratio of NF and LOP is very high so to achieve the ratio in level, the mixture was spiked with 298 mg of pure drug of loperamide hydrochloride and transferred to standard 100 ml volumetric flask and added 60 ml of methanol. The followed prepared solution was sonicated for 10 minutes by using ultra sonication and made up to mark with methanol. Aliquot portion of this solution was further diluted to achieve final concentration. The solution was filtered with a Whattman filter paper no.1. First 5 ml portion of filterate was discarded and then absorbance was measured at respective wavelengths. The content was calculated by using the proposed absorbance ratio method equation and results are tabulated in Table 7.



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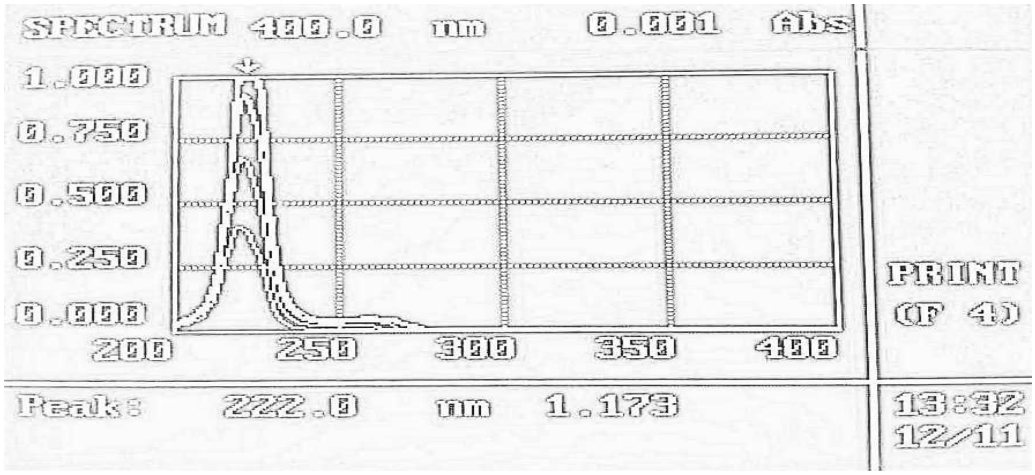

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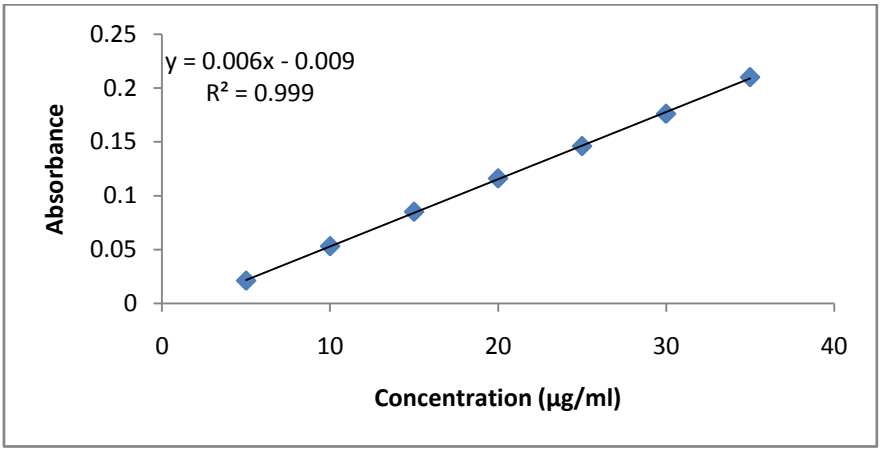
Figure 2: (A) Overlain spectra of Loperamide HCl and Norfloxacin; (B) λmax of Loperamide HCl at 222 nm



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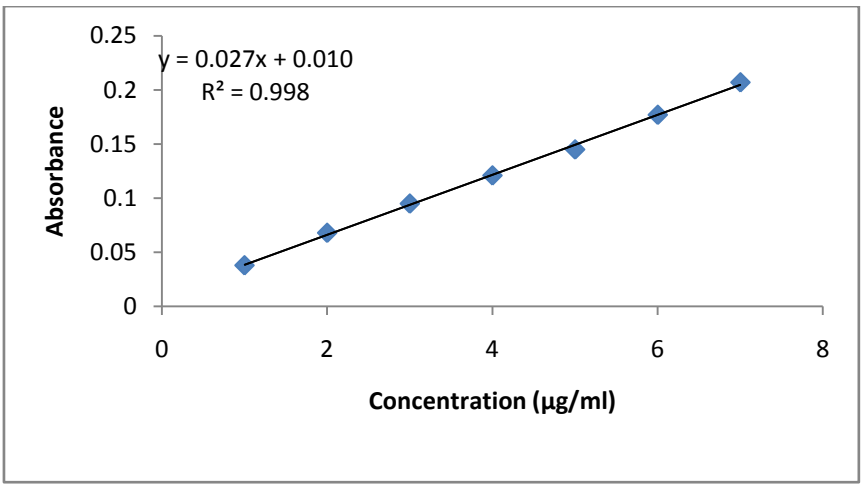

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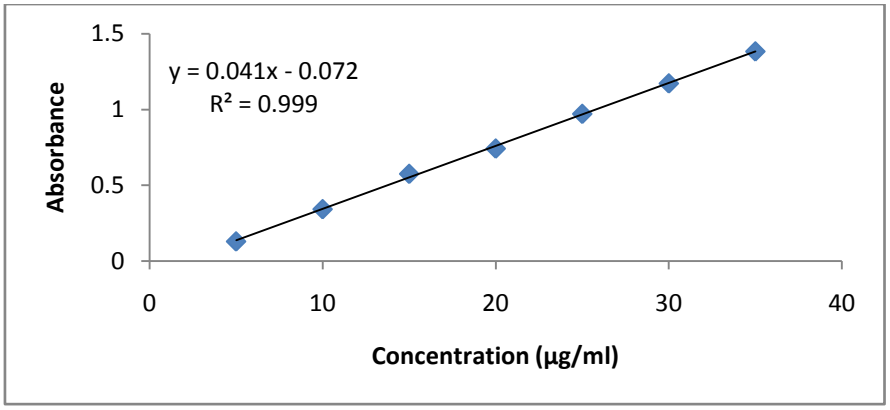

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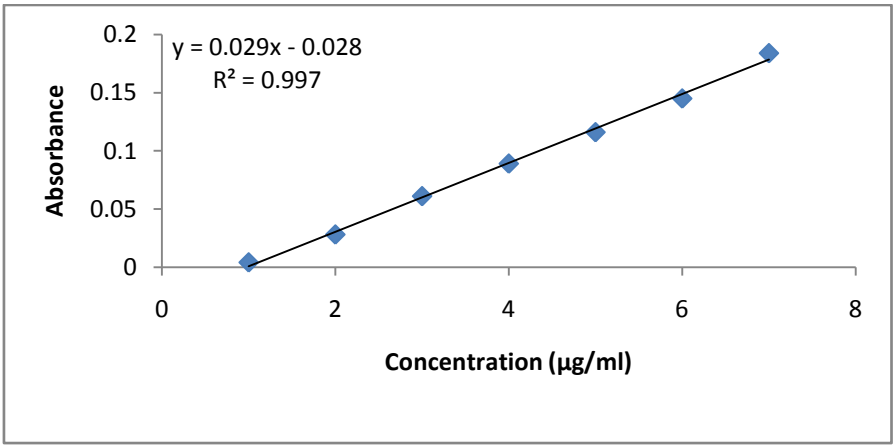

(D)

Figure 3: (A) Calibration curve of Loperamide HCl at 231.5 nm (iso-absorptive point); (B) Calibration curve of Norfloxacin at 231.5 nm (iso-absorptive point); (C) Calibration curve of Loperamide HCl at 222nm (λmax of loperamide) (D) Calibration curve of Norfloxacin at 222nm (λmax of loperamide)

Table 1: Optical parameters of LOP and NF

Optical Parameters	LOP		NF	
Wavelength maxima (nm)	222	231.5	222	231.5
Beer's law limits (µg/ml)	5-35	5-35	1-7	1-7
Molar Absorptivity ($L.mol^{-1}.cm^{-1}$)	18543.66	2836.57	6930.66	10125.79
Sandell's sensitivity ($\mu g.cm^{2}$)	0.027691	0.181028	0.046071	0.031533
Regression equation (y= mx+c)	y = 0.041x - 0.072	y = 0.006x - 0.009	y = 0.029x - 0.028	y = 0.027x + 0.010
Correlation coefficient (r^2)	0.999	0.999	0.997	0.998
Slope, m	0.041	0.006	0.029	0.027
Intercept, c	0.072	0.009	0.028	0.010

Table 2: Intra-day and inter-day precision studies of LOP & NF

Concentration (µg/ml)		Intra-day precision*		% RSD*		Inter-day precision*		% RSD*	
LOP	NF	LOP	NF	LOP	NF	LOP	NF	LOP	NF
10	4	0.064	0.123	0.89	1.69	0.065	0.121	0.89	0.47
15	5	0.082	0.142	1.21	1.07	0.082	0.145	1.41	0.69
20	6	0.103	0.173	0.97	1.16	0.101	0.181	0.99	1.15

**Mean of nine replicates (n=9)*

Table 3: Accuracy study of LOP and NF by standard-addition method

Amt. of Sample (µg/ml)		Spiked Concentration (µg/ml)		Recovery* (%)		% RSD*	
LOP	NF	LOP	NF	LOP	NF	LOP	NF
100%	100%	80%	80%	99.5	100.7	1.17	0.75
100%	100%	100%	100%	99.9	99.5	0.67	0.39
100%	100%	120%	120%	100.4	99.7	1.05	0.62

**Mean of three replicates (n=3)*

Table 4: Robustness

S.No	Wavelength		Active drug in %		Percentage deviation	
	LOP	NF	LOP	NF	LOP	NF
1.	231.5	231.5	99.9	99.5	0.67	0.1
2.	230.5	230.5	99.9	100	0.68	0.4

Table 5: LOD and LOQ

Parameters	LOP		NF	
Wavelength maxima (nm)	222	222	231.5	231.5
LOD	0.08	0.11	0.55	0.19
LOQ	0.37	0.52	1.66	0.56

Table 6: Specificity

No. of aliquots	Excipients absorbance (mean)		% Interference	
	LOP	NF	LOP	NF
1.	0.004	0.002	0.11	0.27
2.	0.006	0.004		

Table 7: Analysis of LOP and NF in marketed formulation

Brand name	Label claim (mg/capsule)		Found (mg/capsule)		Percentage purity	
	LOP	NF	LOP	NF	LOP	NF
NORSTREP	2	200	1.99	198	99	99.5

Where, no. of aliquots = 3

3. RESULT AND DISCUSSION

The present work is precise and accurate for the estimation of LOP and NF in combined dosage form. The calibration curve was linear over the concentration range 5-35 µg/ml for LOP and 1-7 µg/ml for NF. Regression equation was found y = 0.041x - 0.072, y = 0.006x - 0.009, with correlation coefficient (r^2) 0.999, 0.999 at 222 nm (λmax of LOP) and 231.5 (isobestic point) respectively for LOP and regression equation was found y = 0.029x - 0.028, y = 0.027x + 0.010 with correlation coefficient (r^2) 0.997, 0.998 at 222 nm (λmax of LOP) and 231.5 (isobestic point) respectively for NF as shown in table 1. The %RSD in inter-day precision study was found 1.21 (LOP), 1.07 (NF) at isobestic point. The %RSD in intra-day precision study was found 0.082 (LOP), 0.145 (NF) at isobestic point shown in table 2. The %recovery in accuracy study was found between 98-102% and the %RSD was less than 2% shown in table 3. The % RSD in robustness study was less than 2%, this indicates that the method is precise, accurate and robust shown in table 4. The LOD was found 0.08 µg/ml (LOP), 0.11 µg/ml (NF) at 222 nm (λmax for LOP) and 0.55 µg/ml (LOP) and 0.19 µg/ml (NF) at 231.5 nm (isobestic point). The value of LOQ was found 0.37 µg/ml (LOP), 0.52 µg/ml for (NF) at 222 nm (λmax for LOP) and 1.66 µg/ml (LOP) and 0.56 µg/ml (NF) at 231.5 nm (isobestic point) shown in table 5. This indicates that the method is accurate, precise and robust as per ICH guidelines. The % interference of excipients was also checked and found within the limits shown in table 6. The method was successfully applied for marketed preparation and the result was shown in table 7.

4. CONCLUSION

In this method the selected drugs showed good linearity, sample recovery was within limits and the assay of the capsule was in good agreement with the label claim and suggested non-interference of formulation excipients in the estimation. So it can be concluded that the developed method was novel, simple, accurate, precise, reproducible and economical, which can be used to estimate LOP and NF in their combined dosage form in routine analysis.

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