

Application of UV-Visible Spectrophotometric Method for the Estimation of Ciprofloxacin HCl and Levofloxacin Hemihydrate (Antibiotics) in Marketed Drugs

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(Received on 27th February 2019, accepted in revised form 24th February 2020)

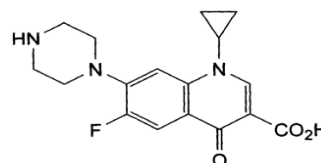
Summary: Ciprofloxacin HCl, 1-Cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid, is a synthetic broad antibacterial compound belonging to the group of fluoroquinolones which is antimicrobial drug act through the inhibition of DNA-gyrase, an enzyme that is critical to bacterial chromosome replication. It is used in a wide range of infections of the urinary, respiratory and gastrointestinal tracts, as well as in skin structure and ocular infections. Levofloxacin Hemihydrate is a fluoroquinolone, has a broad-spectrum antibiotic activity against gram-positive and gram-negative bacteria and used in respiratory and urinary tract infections. Various Pakistani branded antibiotic drug samples containing Ciprofloxacin HCl and Levofloxacin Hemihydrate were collected from local market and their working standards from the material suppliers of Karachi city. Samples were analyzed by using UV-Vis spectrophotometer. Then the method was validated by linearity, accuracy, precision (reproducibility, intermediate precision) and range which suggested that method is appropriate and proper to determine the potency of these various brands of Ciprofloxacin HCl and Levofloxacin Hemihydrate. The analytical data suggest that the drug samples analyzed were within the limit of the Pharmacopeia requirement and criteria.

Keywords: Ciprofloxacin HCl, Levofloxacin Hemihydrate, Reference solution, UV-Vis Spectroscopy, Validation.

Introduction

The evaluations of the quality of the pharmaceutical products ensure their bioavailability and impart most advantageous therapeutic activity. Bacteria are treated with antibiotic drugs (respiratory tract contaminations, whooping cough and pneumonia), which do not work against virus (common cold, the influenza) [1]. Fluoroquinolones are the man made latest class of antibiotics from "floxacin" origin. The commonly used quinolones are not well absorbed and are used to treat urinary tract diseases [2]. Fluoroquinolones may cause diarrhoea, swelling and heartburn, pain of abdomen, loss of hunger, feeling ill, scratchy skin rash, cough [3]. However, these drugs are not completely absorbed and metabolized in human bodies and are excreted in excess amounts through urine and feces in their active pharmacological forms. According to reports, Fluoroquinolones are the most frequently detected antibiotics in the world followed by other classes of antibiotics such as tetracycline, sulfonamides, and macrolides in the aqueous environment [4]

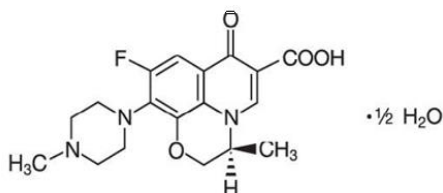
Ciprofloxacin HCl, a synthetic drug is 1-Cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1, 4-dihydroquinoline-3-carboxylic acid, broad antiseptic compound belongs to fluoroquinolones [5, 6].



Scheme-I: Structural formula of Ciprofloxacin HCl.

Levofloxacin Hemihydrate is a fluoroquinolone antibiotic. It has a wide range of spectrum antibiotic action in contrast to gram-positive and gram-negative microorganisms and used in respiratory and urinary tract contaminations [7]. It is the levo isomer Ofloxacin. Chemically it is the (S)-9-fluoro-2, 3-dihydro-3-methyl-10-(4-methyl-1-piperazin-1-yl)-7-oxo-7H-pyrido 1, 4-benzoxazine-6 carboxylic acid hemihydrates [8, 9]. It inhibits microbial type II and IV topoisomerases and DNA gyrase. These outcomes effects on the component breaking on a bacterial genetic material (super coiling and resealing). Later DNA duplication and record is reserved [10]. Adverse effects are also reported, increased risk of dysglycemia with the use of fluoroquinolones was observed in both patients with and without diabetes [11].

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Scheme-2: Structural formula of Levofloxacin Hemihydrate.

Antibiotic resistance effects the efficiency of medicines and chemicals [12]. The bacteria continuously multiply, causing extra destruction [13]. Antimicrobial resistance may be creating in bacteria, viruses, fungi and parasites, antimicrobial (Tuberculosis, MRSA, Gonorrhoea, E. coli, HIV, Candida, Malaria) resistance [14]. Recently proved that the development of new molecules complex of fluoroquinolones is a potential key target for controlling neuroinflammation [15]

This study was aimed to evaluate and specify the Ciprofloxacin HCl and Levofloxacin hemihydrate in tablet dosage forms of 250 mg of various brands by UV-Vis Spectrophotometric method and identify maximum wavelength for the quantification of Ciprofloxacin HCl and Levofloxacin hemihydrate from tablets then validated the method by linearity, accuracy, precision (reproducibility, intermediate precision) and range.

Experimental

Material and methods

Collection of samples

Antibiotic drug samples of different brands were purchased from the local market of Khairpur Mir's, Sindh for the UV-Visible spectrophotometric analysis and the samples were labeled as brands 1, 2, 3 4, 5 and so on, while the working standards of Ciprofloxacin HCl and Levofloxacin hemihydrate were obtained from their specific material suppliers at Karachi.

Preparation of standard of Ciprofloxacin HCl

Weigh and transfer accurately 50 mg of working standards in 50 mL of volumetric flask. Add 35 mL of water and sonicate to dissolve the material completely. Then make up the volume with water up to mark. Transfer 5 mL of this solution to 50 mL of volumetric flask and make up the volume with water. Again transfer 5 mL of this solution to 50 mL of volumetric solution up to the mark with water for the final standard solution.

Measure the absorbance at wavelength range of (200–600) nm using UV-Vis spectrophotometer (UV-Vis Spectrophotometer double beam Super Aquarius CECIL CE 9500). The maximum wavelength was taken at which the Reference solution (RS) showed maximum absorbance [15, 16].

Preparation of samples of Ciprofloxacin HCl

Weigh 20 tablets and grind them in a pestle mortar as a fine powder. Average weight of each variety of tablets was transferred to 250 mL of volumetric flask and 200 mL of water was added in it. Samples were sonicated to dissolve the material completely. Volume was adjusted with water by rigorous shaking 5 mL of above filtrate was transferred to 50 mL of the volumetric flask, and volume was adjusted. Again 5 mL of above sample was taken to 50 mL of volumetric flask and adjusted up to the mark with water and saved as final sample solution for further analysis [4].

Calculation Formula

$$\% \text{ of required materials in samples} = \frac{A_s}{A_{rs}} \times 100$$

where A_s = the absorbance of sample solution.

A_{rs} = the absorbance of standard solution.

Preparation of standard of Levofloxacin hemihydrate

Weigh and transfer accurately 25 mg of standard in 100 mL of volumetric flask and add 80 mL of 0.1 N HCl and sonicate to dissolve the material make the volume with 0.1 N HCl up to the mark. Transfer 2 mL of above solution to 50 mL of volumetric flask and volume was made with 0.1 N HCl. This was the final standard solution. Measure the absorbance of standard and samples at wavelength 200-600 nm to determine the wavelength on maximum absorbance [15].

Preparation of samples of Levofloxacin hemihydrate

20 tablets were weighed and grinded in a pestle mortar as a fine powder, $1/5^{\text{th}}$ of average weight of each brand of tablet was taken in 200 mL volumetric flask, 150 mL of 0.1 N HCl was added and then sonicated to dissolve the material completely adjust volume up to the mark. 2 mL of above solution was transferred into 50 mL of volumetric flask and then volume was marked with 0.1 N HCl. This was the final sample solution. Sample solutions of each brand of tablets, were measured spectrophotometrically at wavelength 200-600 nm to determine the maximum absorbance [7, 17].

Table-1: List of antibiotic drug samples.

S.No	Names of drugs (250mg)	Manufacturers	Purity claimed (%)
1	Auxin	Novartis Pharma, Pakistan	100
2	Hiflox	Hilton Pharma, Pakistan	100
3	Novidat	Sami Pharma, Pakistan	100
4	Inoquin	Barrett Hodgson, Pakistan	100
5	Quinoflox	Bosch Pharma, Pakistan	100
6	Leflox	Getz Pharma, , Pakistan	100
7	Levoxin	The Searle company, Pakistan	100
8	Levomerc	Merck Quetta Pakistan	100
9	Cravit	Hilton Phrama, Pakistan	100
10	Levoday	Davis Pharma, Pakistan	100
11	Levocil	CCL Pharmaceutical , Pakistan	100

Linearity

Linearity of an analytical procedure is its ability within a given range to obtain test results which are directly proportional to the concentration of analyte in the sample. Solutions prepared in the range of different concentrations 80%, 90%, 100%, 110%, 120% and were used for the linearity calibration graph.

Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value that is accepted either as a conventional true value or an accepted reference value and the value found. Usually a minimum of three determinations at each of three concentrations across the intended range is recommended [17].

Precision

The precision of an analytical technique shows the closeness of process (degree of scatter) between serial of measurements increased from several samples of the same standardized sample under given situations [19, 20].

a) Intermediate Precision

Intermediate precision expresses within-laboratory variations as different days with different analysts or instruments, etc [19].

b) Reproducibility

Reproducibility is generally recognized by means of an inter-laboratory experiment [19].

Range

The range of an analytical process is the interval between the upper and lower level of analyte. The range of the procedure is validated by verifying that the analytical procedure provides acceptable linearity, accuracy and precision when applied to sample

containing analyte at the extremes of the range as well as within the range [18, 19].

Results and Discussion

The potency of various brands of samples which contains Ciprofloxacin HCl are shown in Table 2-4 and Fig 1-6. The samples selected for the testing having maximum value found 107.36% and minimum 95.70%. All samples are in limit of assay followed by British Pharmacopeia (B.P), United States Pharmacopeia (USP). Limit for assay is 90% to 110% [19, 20].

Linearity

Linearity of the graph shows that co-efficient correlation value showing that they have a linear or straight line which shows that different concentration of samples are analysed, and each concentration gives accurate results having R^2 values nearer to 1 that complies with the limit of test which follow the limits of BP, USP.

Accuracy

Accuracy confirmed by increasing the concentration of samples there is no any effect on it. In the 80% sample solution give results very close to 80%, in 100% sample solution give results very close to 100% and in 120% sample solution give results very close to 120%. So, from above results we can examine that all the results are in limit with straight line and RSD% is not more than 2% followed by BP, USP [19, 20].

Precision (Reproducibility and intermediate precision)

In the precision, reproducibility of the tests shows that different samples prepared have same concentration. By preparing different samples of same concentration it was observed that there is no any difference in results by repetition of samples having uniformity in analysed samples and confirmed their reliability. Graphical representation demonstrates that all are in the limit of assay.

Table-2: Assay calculations for ciprofloxacin HCl and Levofloxacin Hemihydrate (%).

Calculation of assay for ciprofloxacin HCl (wave length= 275 nm)					Calculation of assay for Levofloxacin Hemihydrate (wave length= 293 nm)			
S. No:	Name of Brand	Abs: of Sp	Abs: of Std:	Result%	Name of Brand	Abs: of Sp	Abs: of Std:	Result%
1	Axcin 250 mg Tab:	1.03	0.978	105.32	Leflox 250 mg Tab:	0.892	0.898	99.33
2	Algocin 250 mg Tab:	1.038	0.978	106.13	Levoxin 20 mg Tab:	0.899	0.898	100.11
3	Hiflox 250 mg Tab:	0.973	0.978	99.49	Levomeric 250 mg Tab:	0.868	0.898	96.66
4	Novidat 250 mg Tab:	1.008	0.978	103.07	Cravit 250 mg Tab:	0.879	0.898	97.88
5	Inoquin 250 mg Tab:	1.05	0.978	107.36	Levoday 250 mg Tab:	0.87	0.898	96.88
6	Quinoflox 250 mg Tab:	0.936	0.978	95.70	Levocil 250 mg Tab:	0.885	0.898	98.55
7	-	-	-	-	Lexa 250 mg Tab:	0.959	0.898	106.79

Method Validation

Table-2: Linearity

Algocin 250 mg Tablets (Wave Length= 275 nm)					Leflox 250 mg Tablets (Wave Length= 293 nm)			
S. No:	Percent	Abs: of Sp:	Abs: of Std:	Result%	S.No.	Percent	Abs: of Sp:	Result%
1	80	0.797	0.996	80.02	1	80%	0.716	80.00
2	90	0.896	0.996	89.96	2	90%	0.805	89.94
3	100	0.996	0.996	100	3	100%	0.895	100.00
4	110	1.096	0.996	110.04	4	110%	0.986	110.17
5	120	1.195	0.996	119.98	5	120%	1.074	120.00

Table-3: Accuracy

Algocin 250 mg Tablets (Wave Length= 275 nm)					Leflox 250 mg Tablets (Wave Length= 293 nm)			
S. No:	Percent	Absorbance	Abs: of Std:	Result%	S. No:	Percent	Abs: of Sp:	Result%
1	80%	0.824	1.031	79.92	1	80%	0.754	80.10
2	100%	1.032	1.031	100.10	2	100%	0.91	101.68
3	120%	1.236	1.031	119.88	3	120%	1.075	119.98

Table-4: Precision

(a) Reproducibility:

Algocin 250 mg Tablets (Wave Length= 275 nm)					Leflox 250 mg Tablets (Wave Length= 293 nm)				
S. No:	Sample	Absorbance of Sample	Absorbance of Standard	Result	S. No:	Sample	Abs: Sp:	Abs: Std:	Result%
1	Sp: 1	1.058	1.07	98.88	1	Sp: 1	0.909	0.895	101.56
2	Sp: 2	1.07	1.07	100.0	2	Sp: 2	0.901	0.895	100.67
3	Sp: 3	1.10	1.07	102.80	3	Sp: 3	0.907	0.895	101.34
4	Sp: 4	1.08	1.07	100.93	4	Sp: 4	0.896	0.895	100.11
5	Sp: 5	1.11	1.07	103.74	5	Sp: 5	0.887	0.895	99.11
6	Sp: 6	1.09	1.07	101.87	6	Sp: 6	0.897	0.895	100.22
7	Sp: 7	1.08	1.07	100.93	7	Sp: 7	0.893	0.895	99.78
8	Sp: 8	1.069	1.07	99.91	8	Sp: 8	0.899	0.895	100.45
9	Sp: 9	1.09	1.07	101.87	9	Sp: 9	0.904	0.895	101.00

(b). Intermediate Precision

Algocin 250 mg Tablets (Wave Length= 275 nm)								
Day 1, Analysis 1				Day 2 Analysis 1				
S. No:	Abs: Sp	Abs: Std:	Result%	S. No:	Abs: Sp:	Abs: Std:	Result%	
1	1.086	1.11	97.84	1	1.09	1.11	98.20	
2	1.15	1.11	103.60	2	1.26	1.11	98.20	
3	1.16	1.11	104.50	3	1.18	1.11	106.31	
Day 1, Analysis 2				Day 2, Analysis 2				
S. No:	Abs: Sp:	Abs: Std:	Result%	S. No:	Abs: Sp:	Abs: Std:	Result%	
1	1.09	1.11	98.20	1	1.092	1.111	98.38	
2	1.15	1.11	103.60	2	1.156	1.111	104.14	
3	1.14	1.11	102.70	C	1.146	1.111	103.24	

(b). Intermediate Precision: Leflox 250 mg Tablets

Day 1, Analysis 1				Day 1, Analysis 2			
S.No:	Abs: Sp	Abs: Std:	Result	S.No:	Abs: Sp:	Abs: Std:	Result%
1	0.911	0.896	101.67%	1	0.91	0.894	101.79
2	0.911	0.896	100.89%	2	0.907	0.894	101.45
3	0.892	0.896	99.55%	3	0.896	0.894	100.22
Day 2, Analysis 1				Day 2, Analysis 2			
S.No:	Abs: Sp:	Abs: Std:	Result	S.No:	Abs: Sp:	Abs: Std:	Result%
1	0.906	0.895	101.23%	1	0.908	0.894	101.56
2	0.907	0.895	101.34%	2	0.903	0.894	101.01
3	0.899	0.895	100.45%	3	0.899	0.894	100.56

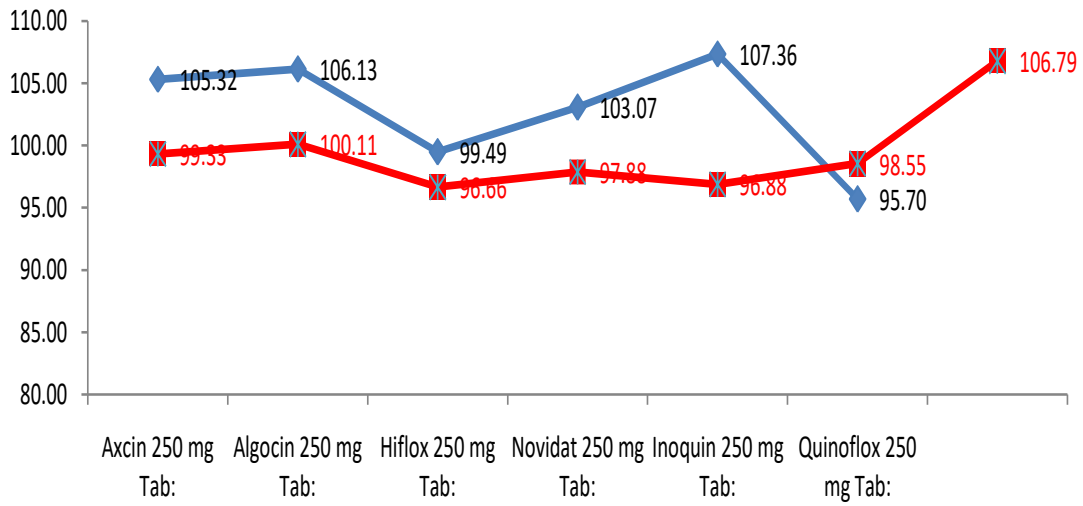


Fig. 1: Calculation of Ciprofloxacin HCl (wave length= 275 nm) and Levofloxacin Hemihydrate (wavelength = 293nm).

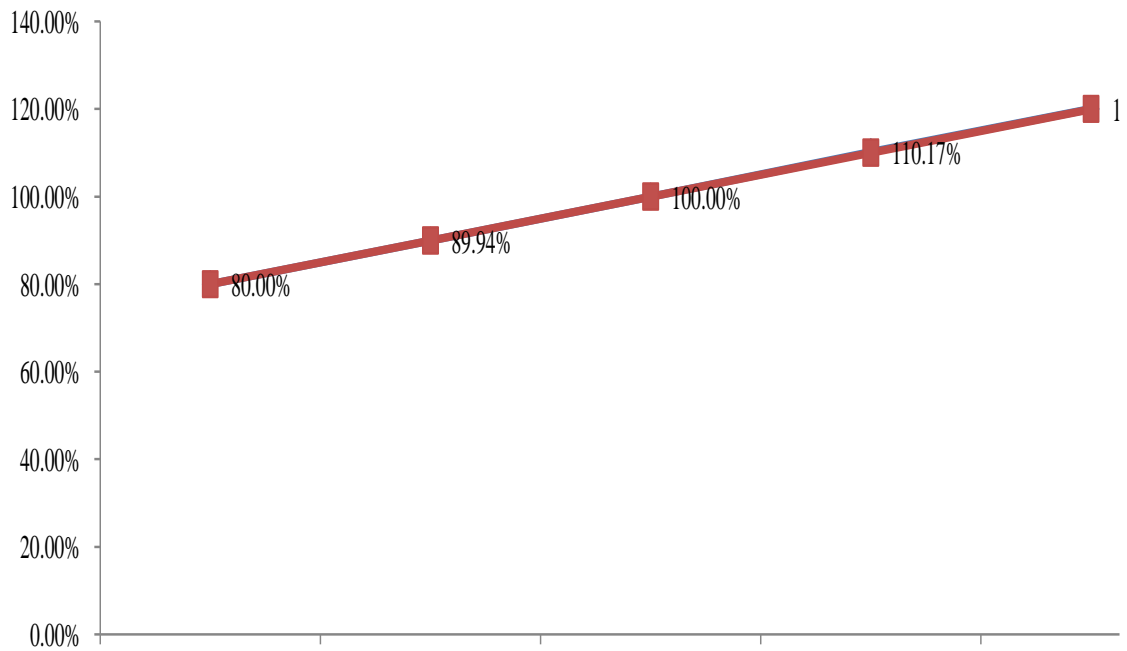


Fig. 2: Linearity: Leflox 250 mg Tablets (Wave Length= 293 nm) and : Algocin 250 mg Tablets (Wave Length= 275 nm).

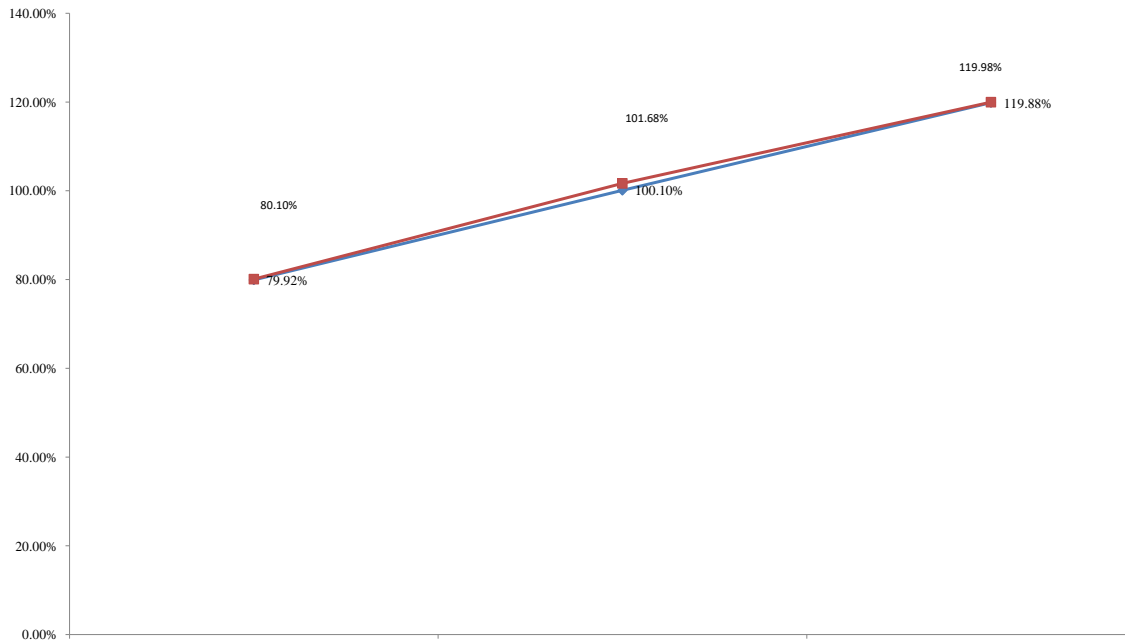


Fig. 3: Accuracy: Alginic 250 mg Tablets (wave Length= 275 nm) and Leflox 250 mg Tablets(Wave Length= 293 nm).

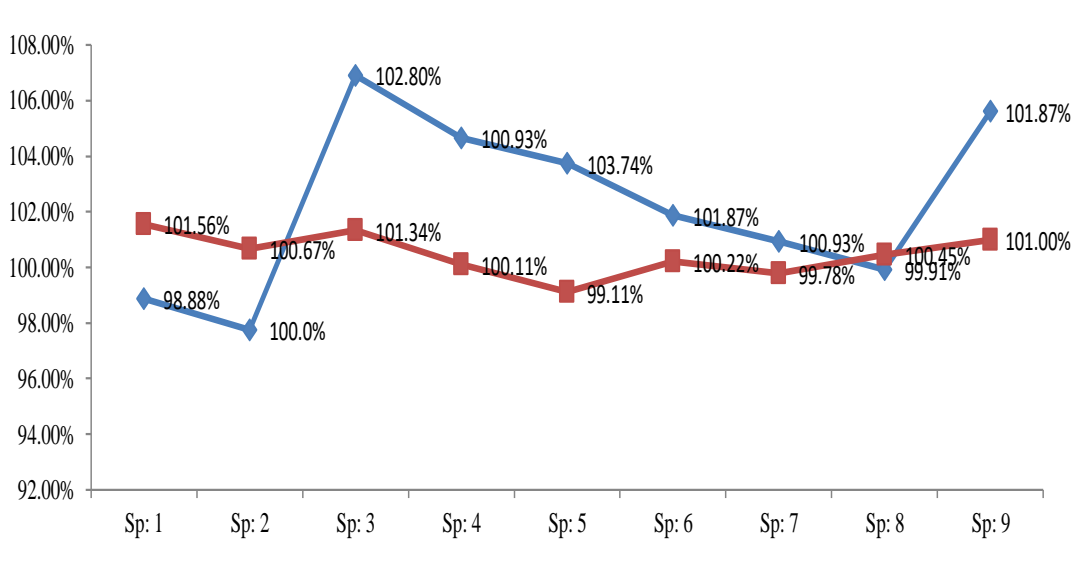


Fig. 4: Precision: Alginic 250 mg Tablets (Wave Length= 275 nm).

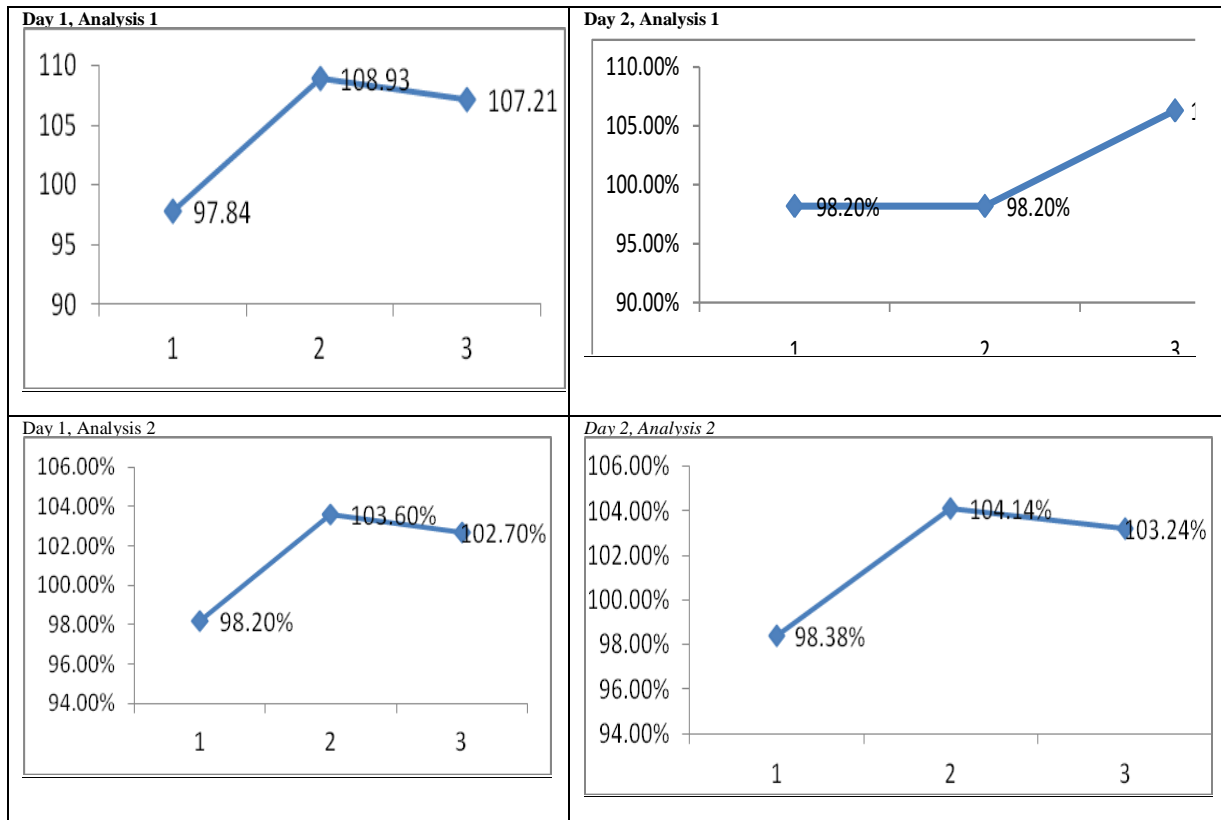


Fig:5 Intermediate Precision: Algoicin 250 mg Tablets (Waveiength= 275 nm)

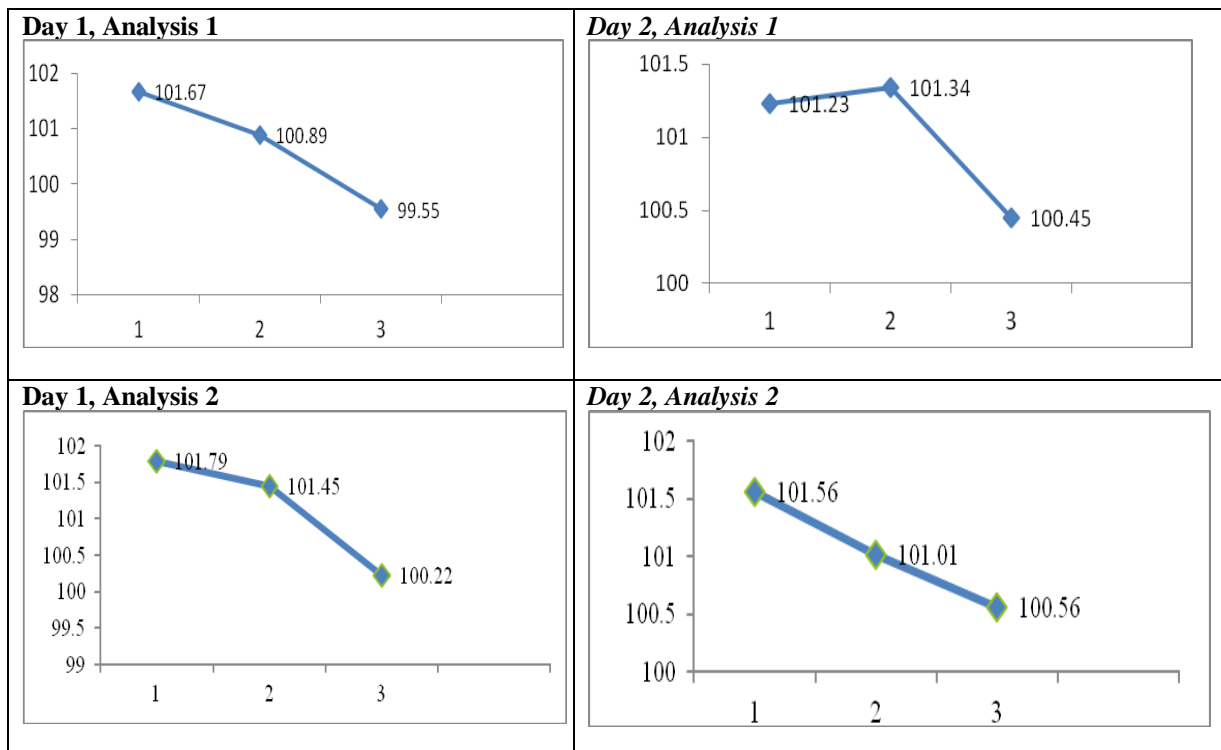


Fig: 6: Intermediate Precision: Leflox 250 mg Tablets (Wave Length= 293 nm).

The intermediate precision, in selected samples were analysed on day 1 and 2 with different interval of time. The results show that there is no any difference in results which were obtained in analysis 1 and analysis 2 on the same day. The obtained results were in the limit of assay and meets with criteria [19, 20].

Range:

The range of an analytical procedure is the interval between the upper and lower level of analyte and found that the procedure is validated by verifying that the analytical procedure provides acceptable precision, accuracy and linearity when applied to sample containing analyte at the extremes of the range as well as within the range [20, 21].

Conclusions

The maximum wavelength $\lambda = 275$ and 293nm are optimized for various brands of Ciprofloxacin HCl and Levofloxacin Hemihydrate respectively for assay determination. The method validation parameters (Linearity, Accuracy, Precision (a)Intermediate precision (b)Reproducibility and Range) are appropriate and proper to determine the potency of various brands of Ciprofloxacin HCl and Levofloxacin hemihydrate tablets. The analytical data suggests that the brand samples analyzed were within the limit of the Pharmacopeia requirement and criteria.

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