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Development and validation of spectroscopic methods for simultaneous estimation of combination of antibiotic agents

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ABSTRACT

A simple, sensitive rapid and reproducible UV and method has been developed and validated for simultaneous determination of Cefixime and Pefloxacin in bulk and pharmaceutical formulation. For development of UV method for Cefixime and Pefloxacin methanol was used as solvent and detection wavelength were found to be at 289.0 nm and 232.80 nm respectively. The method was found to linear in concentration range 10-35 µg/ml for both drugs the precision and repeatability results showed %RSD less than 2%. The LOD was found to be 0.6445 µg/ml and 1.7187 µg/ml for CEF and PEF respectively and LOQ was found to be 1.9531 µg/ml and 5.2083 µg/ml for CEF and PEF respectively. The method was validated as per ICH guidelines.

Keywords: Cefixime trihydrate, Pefloxacin mesylate, UV Spectroscopy, Combined Dosage Form.

INTRODUCTION

Cefixime is a aminothiazolyl, semi synthetic, broad spectrum third generation cephalosporin, active against gram positive and gram negative aerobic bacteria. This C₁₆H₁₅N₅O₇S₂·3H₂O is the chemical formula of Cefixime trihydrate with molecular weight 507.50 as trihydrate. Chemically, it is (6R, 7R)-7-[[[(Z)-2-(2-aminothiazole-4-yl)-2-[(carboxymethoxy) imino] acetyl] amino]-3-ethenyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid trihydrate [1-2].

The Cefixime trihydrate is a cephalosporin antibiotic. The third generation antibiotic acts by

inhibiting the synthesis of bacterial cell wall. The bactericidal action of cephalosporin is due to the inhibition of cell wall synthesis. It binds to the penicillin binding proteins (PBPs), inhibiting the final transpeptidation step of the peptidoglycan synthesis in the cell wall of the bacteria, thus inhibiting biosynthesis and arresting cell wall assembly resulting in bacterial cell death. It is commonly used in the treatment of susceptible infections including gonorrhoea, otitis media, pharyngitis and infections of respiratory-tract such as bronchitis, and urinary-tract.

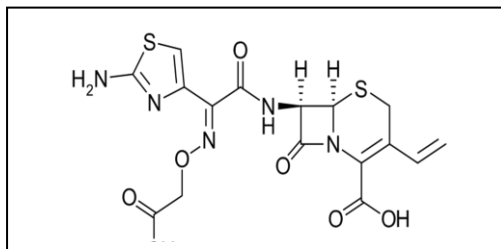


Fig .1 Structure of Cefixime

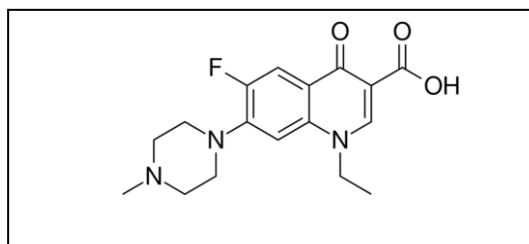


Fig.2 Structure of Pefloxacin

Pefloxacin is a broad-spectrum third generation fluoroquinolone synthetic antibiotic. Chemically pefloxacin is 1- ethyl-6-fluoro-7- (4-methyl- 1-piperazinyl)-4-oxo- 1, 4- dihydro-3-quinolone carboxylic acid and structurally it is analog of norfloxacin. Dihydrate mesylate salt of pefloxacin has been frequently used worldwide in the form of, parenteral infusion, oral tablet and topical eye drops [4].

It inhibits DNA gyrase and topoisomerase IV, the bacterial enzymes, which are responsible for transcription and replication of DNA of bacteria. The primary quinolone target for gram-negative bacteria is DNA gyrase while topoisomerase IV for gram-positive organisms. [5] Systemically PEF is prescribed in uncomplicated cystitis in women, uncomplicated gonococcal urethritis in males and in gastrointestinal and genitourinary tract (gram-negative bacterial infections) [6]. Some methods have been reported for determination of Pefloxacin in pharmaceutical formulation bulk, and biological fluids such as vibrational spectroscopy (VIB) [7], atomic absorption spectroscopy (AAS) [8-9], spectrofluorimetry (SF) [8], UV-spectroscopy (UV) [10], visible spectroscopy (VS) [11-18], voltammetric study (VL) [19-20], microbiological assay (MA) [21-23], potentiometric titration [23], enzyme-linked immunosorbent assay (ELISA) [24-25] capillary electrophoresis (CE) [26] NIR-spectroscopy [27] colorimetric method[28] HPLC[29-32]. All the above methods have their

applications and limitations. Some of analytical methods (CE, MA, AAS, ELISA, HPLC, RP-HPLC) even have good sensitivity and selectivity. But the methods are time consuming; complex and costly thus are not suitable for assay of Pefloxacin. Besides this, in visible spectroscopic and colorimetric methods the use of various dyes and organic solvents results in non-eco-friendly, time consuming procedures and sometimes are proved carcinogenic.

MATERIALS AND METHODS

Reagents and Chemicals

Methanol (AR Grade), Acetonitrile, Water HPLC grade, potassium hydroxide orthophosphoric acid and potassium dihydrogen ortho phosphate were provided by Merck Pharmaceuticals Ltd Hyderabad. Pure Standard gift sample of Cefixime Trihydrate (CEF) and Pefloxacin Mesylate was provided by Richer Pharmaceuticals.

Instrument

Shimadzu UV/Vis-2450 and UV/Vis- 1800 double beam UV/Vis spectrophotometer with a fixed slit width of 2 nm, 1 cm quartz cells was used for recording derivative spectra of standard and test samples. Sartorius CD2250 balance was used for weighing the samples.

Preparation of Stock Solutions

Cefixime trihydrate

Weigh and transfer 100 mg of Cefixime trihydrate working standard into 100 ml of volumetric flask, add 10 ml of methanol and sonicated to dissolve for 10 minutes and dilute to volume with diluents. The Wavelength of the Cefixime was found to be 289.00 nm and Absorbance is 0.617.

Pefloxacin mesylate

Weigh and transfer 200mg of Pefloxacin mesylate working standard into 100 ml volumetric

flask add 10 ml of methanol and sonicated to dissolve for 10 minutes and dilute to volume with diluent. The Wavelength of the Pefloxacin was found to be 232.80 nm and Absorbance is 0.352.

Preparation of mixture

Crush to powder 20 tablets, weigh and transfer the tablet powder equal to 430 mg of into 100 mL volumetric flask add 20 mL of methanol, sonicated to dissolve for 10 minutes and dilute to volume with diluent. Filter the solution through whatman filter. Further dilute 2.5 ml to 100ml with diluents. The wavelength of the mixture was found to be 254.20 nm and absorbance is 0.492.

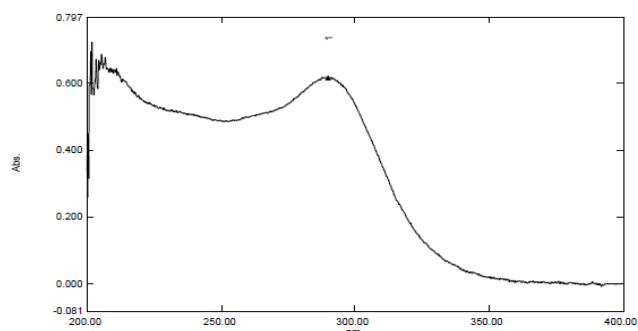


Fig. 3-UV Spectra of Cefixime

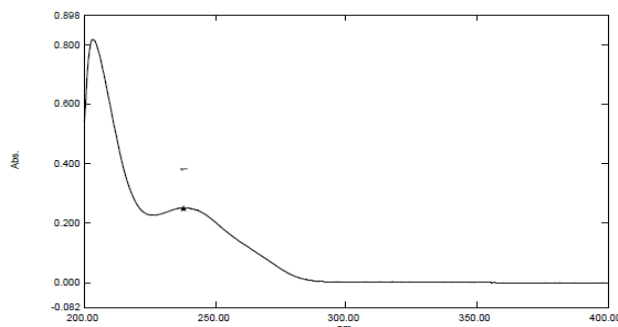


Fig .4:- UV Spectra of Pefloxacin.

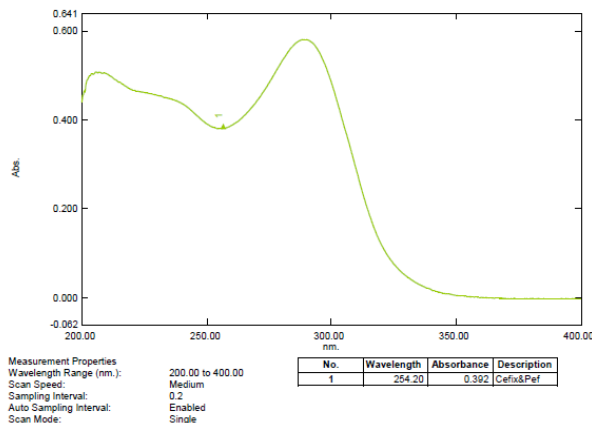


Fig. 5:- Overlain absorption spectra of Cefixime and Pefloxacin

Determination of absorptivity at analytical Wavelength

Preparation of standard solutions

Weighed and transferred 100 mg of Cefixime trihydrate and 200mg of Pefloxacin mesylate working standard into 100 ml volumetric flask,

added 20 ml of methanol and sonicated to dissolve for 10 minutes and diluted to volume with methanol. Further 2.5 ml of the above solution is diluted to 100ml with methanol.

Absorptivity for drug was calculated by using following equation A (1%, 1cm).

$$\text{Absorptivity} = \frac{\text{Absorbance}}{\text{Conc. (g/100ml)}}$$

Validation of Simultaneous Equation Method

The proposed methods were validated by studying parameters such as accuracy, precision, linearity, limit of detection (LOD), limit of quantitation (LOQ).

Linearity

Selection of linearity range

For each drug, appropriate aliquots of standard stock solutions were transferred to a series of 10

ml, volumetric flasks. The volume was made up to the mark with methanol to obtain standard solution for CEF, of concentrations 10-30 µg/mL and for PEF 20-70 µg/ml. The absorbance of each of these solutions were measured at the selected wavelengths i.e. 289 nm and 232 nm and plotted against their concentrations.

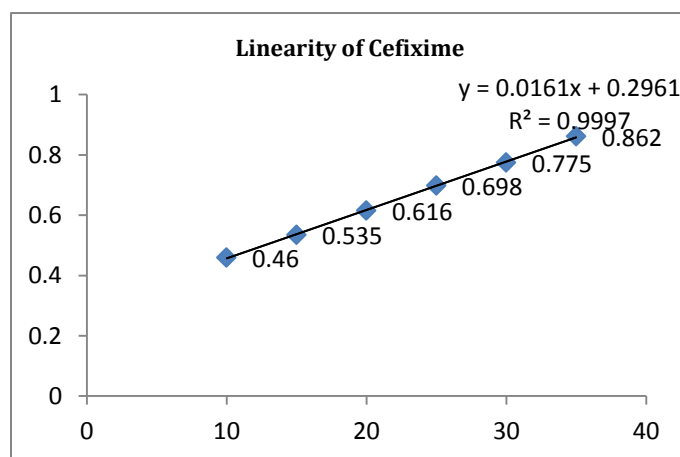


Fig. 6:-Calibration curve of Cefixime at 289 nm

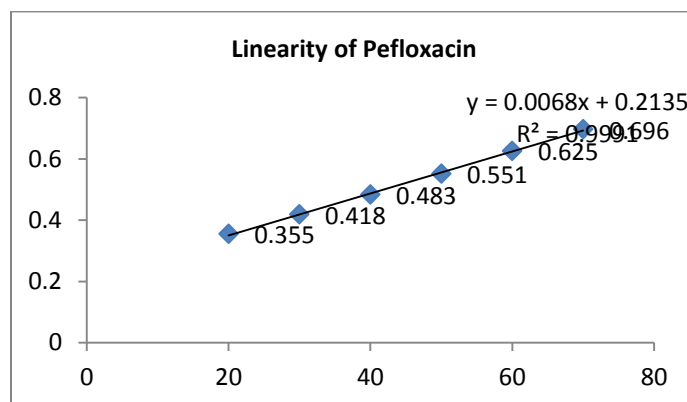


Fig. 7:-Calibration curve of Pefloxacin at 232 nm

Precision

Sample solution of substance 25 µg/ml. of CEF and 50 µg/ml of PEF were used for studying the degree of precision of the developed method. Repeatability is given by intraday and interday precision. Intraday precision was determined by analyzing the one concentration of drug for six times in the same day. Inter day precision was determined by analyzing the three different concentration of the drug daily for three days.

Accuracy

For accuracy of method, recovery studies were carried out by applying a known amount of standard CEF and PEF at a level of 80%, 100%, and 120% to the sample solution (standard addition method). Three determinations were performed at each level and the results obtained were compared with the expected results.

Limit of Quantitation and Limit of Detection (LOD and LOQ)

The LOD and LOQ were calculated using following equations as per International conference on harmonization guideline,

$$\text{LOD} = 3.3\sigma/S$$

$$\text{LOQ} = 10\sigma/S$$

Where σ is standard deviation of the response and S is the slope of regression equation.

RESULT AND DISCUSSION

Linearity

The linear regression data showed a good linear relationship over the concentration range of 10-35 µg/ml. with correlation coefficient $R^2=0.999$ and $y=0.016$ at 289 nm for Cefixime and $R^2=0.999$ and $y=0.006$ at 230nm for Pefloxacin.

Results are shown in table and calibration curve are given in figure.

Table .1: - Standard calibration curve table for Cefixime and Pefloxacin

Sr. No.	Conc. (µg/mL)	Absorbance of		
			Cefixime	Pefloxacin
			289 nm	232 nm
1	10	20	0.46	0.355
2	15	30	0.535	0.418
3	20	40	0.616	0.483
4	25	50	0.698	0.551
5	30	60	0.775	0.625
6	35	70	0.862	0.696

Table .2: - Comparative table for Cefixime and Pefloxacin Linearity

Sr. No.	Parameter	289nm	232nm
		CEF	PEF
1	Beer's Lambert (µg/mL.)	10-35	20-70
2	Regression Equation ($y=mx+c$)	$Y=0.006x+0.213$	$Y=0.016x+0.296$
3	Correlation Coefficient (R^2)	0.999	0.999
4	LOD	0.6445	1.7187
5	LOQ	1.9531	5.2083

Absorptivity

Table .3:-Absorptivity measurement of Cefixime

Sr. No.	Wt. of Cefixime (mg)	Conc. (mg/ mL)	Absorbance		Absorptivity	
			289nm	232nm	289nm	232nm
1	20.1	0.0100	0.810	0.3875	81.0	38.75
2	20.5	0.0101	0.808	0.3896	80.0	38.57
3	19.5	0.0102	0.810	0.3800	81.0	37.25
4	20.3	0.0104	0.809	0.3880	80.9	37.30
5	20.2	0.0100	0.809	0.3900	80.9	39.00

Table .4:-Absorptivity measurement of Pefloxacin

Sr. No.	Wt. of Pefloxacin(mg)	Conc. (mg/ mL)	Absorbance		Absorptivity	
			250nm	277nm	250nm	277nm
1	40.2	0.01000	0.223	0.375	22.3	37.5
2	40.5	0.01002	0.222	0.374	22.1	37.3
3	39.5	0.00996	0.225	0.376	22.5	37.7
4	40.2	0.00998	0.224	0.375	22.4	37.5
5	39.9	0.01028	0.224	0.372	22.4	37.2

Table .5: - Results of estimation of Cefixime and Pefloxacin in tablet formulation

Sr. No	Conc. (µg/mL)		Absorbance*		Amount found(mg)		%Amount found*		%RSD	
	CEF	PEF	CEF	PEF	CEF	PEF	CEF	PEF	CEF	PEF
1	20	40	0.809	0.223	20.15	40.04	105	101	0.8587	0.2586

*Mean of 3 determinations

Precision

The precision and repeatability results showed %RSD less than 2%.The RSD values of CEF was found to be 0.56% and 0.148% of intraday and inter

day respectively and the RSD values of PEF was 0.053% and 0.054% of intraday and inter day respectively. This reveals that the given method is precise.

Table .6:-Determination of Intraday and Interday precision for CEF

Intraday precision of CEF						
Sr. No	Conc. (µg/mL)	Absorbance	Amount Found(mg)	%Amount Found	SD	*%RSD
1	10	0.693	100.00	97.50	0.0030	0.56
Interday precision of CEF						
1	10	0.629	100.00	99.80	0.1483	0.1484

*Mean of 6 determinations for intraday precision and Average of 3 determinations for Interday precision.

Table .7:-Determination of Intraday and Interday precision for PEF

Intraday precision of PEF						
Sr. No	Conc. (µg/mL)	Absorbance	Amount Found(mg)	%Amount Found	SD	*%RSD
1	40	0.316	39.80	99.50	0.0519	0.0532
Interday precision of PEF						
1	40	0.327	39.92	99.80	0.0529	0.0540

*Mean of 6 determination for intraday precision and Average of 3 determinations for Interday precision.

Accuracy

Recovery studies were carried out by applying a known amount of standard CEF and PEF at a level of 80%, 100%, and 120% to the sample solution

and the expected results are reported in the table 8. The results of recovery studies indicate the proposed method is highly accurate.

Table .8: - Accuracy of CEF at 289 nm

Sr. No.	%Addition	Conc.(µg/mL)	Absorbance	Amount recovered	%Recovered
1	80	8	0.544	5.44	78.52
2	100	10	0.688	6.88	99.23
3	120	12	0.839	8.39	121.00

Mean of 3 determinations

Table .9: - Accuracy of PEF at 232 nm

Sr. No.	%Addition	Conc.(µg/mL)	Absorbance	Amount recovered	*%Recovered
1	80	5	0.436	4.36	79.53
2	100	10	0.545	5.45	99.39
3	120	15	0.661	6.61	120.53

*Mean of 3 determinations

LOD and LOQ

The LOD was found to be 0.6445µg/ml and 1.7187 µg/ml for CEF and PEF respectively and

LOQ was found to be 1.9531 µg/ml and 5.2083 µg/ml for CEF and PEF respectively.

Table .10:-Determination of CEF and PEF

Sr. No.	Name	Concentration.(µg/mL)	
		CEF	PEF
1	Limit of detection (LOD)	0.6445	1.7187
2	Limit of Quantization(LOQ)	1.9531	5.2083

SUMMARY AND CONCLUSION

UV method was developed and validated as per ICH guidelines for the estimation of Cefixime and Pefloxacin.

For development of UV method for Cefixime and Pefloxacin methanol was used as solvent and detection wavelength were found to be at 289.0 nm and 232.80 nm respectively. The method was found to linear in concentration range 10-35 µg/mL for both drugs. The method was validated as per ICH guidelines. The precision and repeatability results

showed %RSD less than 2%. The LOD was found to be 0.6445µg/mL and 1.7187 µg/mL for CEF and PEF respectively and LOQ was found to be 1.9531 µg/mL and 5.2083 µg/mL for CEF and PEF respectively.

The proposed Spectrophotometric method was found to be simple, precise and accurate for determination of CEF and PEF in bulk and pharmaceutical dosage form. The method utilizes easily available and cheap solvent for analysis of CEF and PEF.

Based on the results observed, it was concluded analysis of Cefixime and Pefloxacin.
that proposed method can be used for routine

REFERENCES

- [1]. British pharmacopoeia (B.P).Cefixime trihydrate, volume 1, 2010, 410-411.
- [2]. .Martindale, Thirty –Sixth edition, Edited by Sean C Sweetman. Royal Pharmaceutical Society of Great Britain (RPS) Publishing, UK. 2009.
- [3]. Bergogne-Brrzin, E. Pefloxacin. International Journal of Antimicrobial Agents, 1, 1991, 29-46.
- [4]. Goodman LS, Gilman AG, Rall TW, Nies AS, Taylor P, eds. Goodman and Gilman's the pharmacological basis of therapeutics. New York: Pergamon Press, 8, 1990, 1057–60.
- [5]. Clarke AM, Zemcov SJV, Campbell ME. In vitro activity of Pefloxacin compared to enoxacin, norfloxacin, gentamicin and new β -lactams. J Antimicrobial Chemother, 15, 1985, 39
- [6]. Salem, H. Spectrofluorimetric, atomic absorption spectrometric and spectrophotometric determination of some fluoroquinolones. Am J Appl Sci, 2, 2005, 719–729.
- [7]. Salem, H. Colorimetric and atomic absorption spectrometric determination of some fluoroquinolone derivatives. Sci Pharma, 72, 2004, 51–71.
- [8]. Deng B, Li L, Shi A, Kang Y. Pharmacokinetics of Pefloxacin mesylate in human urine using capillary electrophoresis electrochemiluminescence detection. J Chromatogr B Analyt Technol Biomed Life Sci, 877(24), 2009, 2585-8.
- [9]. Xie Y., Song Y, Zhang Y, Zhao B. Near-infrared spectroscopy quantitative determination of Pefloxacin mesylate concentration in pharmaceuticals by using partial least squares and principal component regression multivariate calibration. Spectrochimica Acta Part A: Molecular and Bimolecular Spectroscopy, 75(5), 2010, 1535-1539.
- [10]. Ahmad A. K. S., Kawy M. A., Nebsen M. Spectrophotometric and Spectrofluorimetric determination of Pefloxacin. Anal Lett, 30, 1997, 809–820.
- [11]. Jelikić-Stankov M, Veselinović D, Malesev D, Radović Z. Spectrophotometric determination of Pefloxacin in pharmaceutical preparations. J Pharm Biomed Anal, 7(12), 1989, 1571-7.
- [12]. Avadhanulu A B, Pantulu A R R. Spectrophotometric determination of Pefloxacin in its dosage forms. Indian Drugs, 31(6), 1994, 258-262
- [13]. Mostafa, S. El-Sadek M., Alla, E. A. Spectrophotometric determination of ciprofloxacin and Pefloxacin through charge transfer complexation. J Pharm Biomed Anal, 27, 2002, 133-142.
- [14]. Mostafa, S. El-Sadek M., Alla, E. A. Spectrophotometric determination of enrofloxacin and Pefloxacin through ion-pair complex formation. J Pharm Biomed Anal, 28, 2002, 173-180.
- [15]. Kuchekar B. S., Kale A. A. Shinde G. S. Shaikh A. M. Shinde D. B. Extractive spectrophotometric determination of pefloxacin in pharmaceutical dosage forms. Indian Drug, 40, 2003, 471-473.
- [16]. Basavaiah K., Prameela, H. C. Quantitative determination of pefloxacin mesylate by residual-base neutralization method. J Serb Chem Soc, 69, 2004, 403–410.
- [17]. Radi A, El Ries MA, Kandil S. Spectroscopic and voltammetric studies of pefloxacin bound to calf thymus double-stranded DNA. Anal Bioanal Chem, 381(2), 2005, 451-455.
- [18]. Basavaiah K, Prameela HC, Somashekar BC. Spectrophotometric determination of pefloxacin mesylate in pharmaceuticals. Acta Pharm, 57(2), 2007, 221-30.
- [19]. Beltagi AM. Determination of the antibiotic drug pefloxacin in bulk form, tablets and human serum using square wave cathodic adsorptive stripping voltammetry. J Pharm Biomed Anal, 31(6), 2003, 1079-88.
- [20]. Uslu B, Topal BD, Ozkan SA. Electro analytical investigation and determination of pefloxacin in pharmaceuticals and serum at boron-doped diamond and glassy carbon electrodes. Talanta, 74(5), 2008, 1191-200.
- [21]. Montay G, Goueffon Y, Roquet E Absorption, distribution, metabolic fate, and elimination of pefloxacin mesylate in mice, rats, dogs, monkeys and humans. Antimicrob Agents Chemother 25, 1984, 463.
- [22]. Bertazzoni Minelli E, Benini A, Muner A, Bassi C, Abbas H, Pederzoli P. Pefloxacin penetration into human necrotic pancreatic tissue. J Antimicrob Chemother, 38(2), 1996, 237-43.

- [23]. Sullam, P M, Täuber, M G, Hackbarth, C J, Chambers, H F, Scott, K G, Sande, M A. Pefloxacin therapy for experimental endocarditis caused by methicillin-susceptible or methicillin-resistant strains of *Staphylococcus aureus*. *Antimicrob Agents Chemother*, 27(5), 1985, 685–687.
- [24]. European Pharmacopoeia, 5th ed., European Directorate for the Quality of Medicines, Council of Europe, Strasbourg 2004, 2193-94. 32 British Pharmacopoeia, Her Majesty's Stationery Office, 2(5), 2010, 1623-24
- [25]. Deng B, Li L, Shi A, Kang Y. Pharmacokinetics of pefloxacin mesylate in human urine using capillary electrophoresis electro chemiluminescence detection. *J Chromatogr B Analyt Technol Biomed Life Sci*, 877(24), 2009, 2585-8. 16
- [26]. Xie Y., Song Y, Zhang Y, Zhao B. Near-infrared spectroscopy quantitative determination of Pefloxacin mesylate concentration in pharmaceuticals by using partial least squares and principal component regression multivariate calibration. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 75(5), 2010, 1535-1539.
- [27]. Sane RT, Dighe V, Bapat VV, Gangrade MG. Extractive colorimetric method for the determination of pefloxacin mesylate dihydrate from pharmaceutical preparations. *Indian J Pharm Sci.*, 53, 1991, 64–6.
- [28]. Lacarelle B, Le Guellec C, Morel A, Albanese J, Alazia M, Ballereau M, Llurens M, Bruno R, Francois G, Durand A. Monitoring of pefloxacin serum concentrations in intensive care unit patients: comparison of a new immunoassay with high-performance liquid chromatography. *Ther Drug Monit*, 16(2), 1994, 209-13.
- [29]. Montay G, Tassel JP. Improved high-performance liquid chromatographic determination of pefloxacin and its metabolite norfloxacin in human plasma and tissue. *J Chromatogr*, 339(1), 1985, 214–218.
- [30]. Cochereau-Massin I, Bauchet J, Faurisson F, Vallois JM, Lacombe P, Pocidalo JJ. Ocular kinetics of pefloxacin after intramuscular administration in albino and pigmented rabbits. *Antimicrob Agents Chemother*, 35(6), 1991, 1112-5.
- [31]. Munera MI, Cuesta F, Abadia A, Vasquez J, Restrepo M. Determination of pefloxacin concentration in mesenteric lymph nodes by high-performance chromatography. *Antimicrob Agents Chemother*, 38(3), 1994, 632-4.