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Development and Validation of UV Spectrophotometric Methods for Simultaneous Estimation of Levofloxacin and Prednisolone Acetate in Synthetic Mixture

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ABSTRACT

Two simple, rapid, accurate, precise and economical procedures spectrophotometric methods have been developed and validated for simultaneous estimation of Levofloxacin (LEF) and Prednisolone Acetate (PRA) in Synthetic Mixture. The first Method is Simultaneous Equation method, which is based on determination of LEF at 224.20 nm and PRA at 241.40 nm in methanol, respectively. The second Method was based on derivative spectrophotometric method involving the both the drugs at their respective zero crossing point (ZCP). The first order derivative spectrum was obtained in methanol and the determinations were made at 240.90 nm (ZCP of PRA) and 262.78 nm (ZCP of LEF) for estimation of LEVO and PRA respectively. The linearity was obeyed in the concentration range of 6-18 µg/mL for LEF and 4-12 µg/mL for PRA. Standard deviation and percent of relative standard deviation were calculated and found within limits. The mean percent of recovery were evaluated at 0%, 80%, 100% and 120% concentration levels and found to be within range. The methods can be routinely adopted for quality control of these drugs in Synthetic mixture. The methods were validated as per ICH guidelines.

INTRODUCTION

Levofloxacin is chemically, (2S)-7-fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1 azatricyclo [7.3.1.0^{5},^{13}] trideca-5(13),6,8,11-tetraene-11-carboxylic acid. Levofloxacin is used as an antibiotic to combat the infection. It inhibits bacterial type II topoisomerases, topoisomerase IV and DNA gyrase. Levofloxacin, like other fluoroquinolones, inhibits the A subunits of DNA gyrase, two subunits encoded by the gyrase gene. This results in strand breakage on a bacterial chromosome, supercoiling, and resealing; DNA replication and transcription is inhibited.

Prednisolone acetate is chemically, 11β, 17α-dihydroxy-3, 20-dioxopregna,-1,4-dien-21-yl acetate. After cell surface receptor attachment and cell entry, Prednisolone acetate is used to treat the caused eye condition of inflammation or injury. It works by relieving symptoms of swelling, redness and itching. It enters the nucleus where it binds to and activates specific nuclear receptors, resulting in an altered gene expression and inhibition of proinflammatory cytokine production.

The combination is effectively used for treatment of bacterial keratitis i.e. bacterial corneal ulcer generally infected due to *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

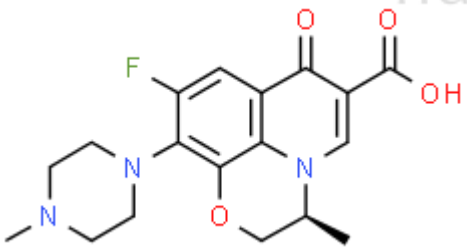

Figure: 1 Levofloxacin

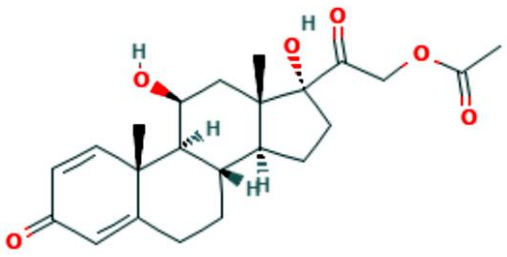

Figure: 2 Prednisolone Acetate

MATERIAL AND METHOD

Instrumentation

UV-Visible spectrophotometer: An UV-Visible spectrophotometer Shimadzu (UV-1800) with 1cm matched quartz cells was used for the spectral and absorbance measurements.

Digital balance: A REPTECH-RA123 digital Weighing balance was used for weighing purposes.

Preparation of standard stock solution

An accurately weighed standard powder of 10 mg of LEF and PRA were transferred in 10 ml volumetric flask separately, dissolved and diluted up to the mark with methanol AR grade, to get final concentration 1000 µg/mL of LEF and PRA. From the above stock solution 100 µg/mL was prepared by diluting 2.5 ml of stock solution to 25 ml with methanol.

From this standard stock solution, different aliquots were transferred into 10 ml volumetric flask and volume was made up to the mark with Methanol. This solution was used as a working standard solution.

Selection of analytical wavelength

The 15 µg/mL solution of LEF was prepared in methanol and spectrum was recorded between 200-400 nm Similarly 10 µg/mL solutions of PRA was prepared in methanol and spectrum was recorded between 200-400 nm. The overlain spectrum of both drug were recorded.

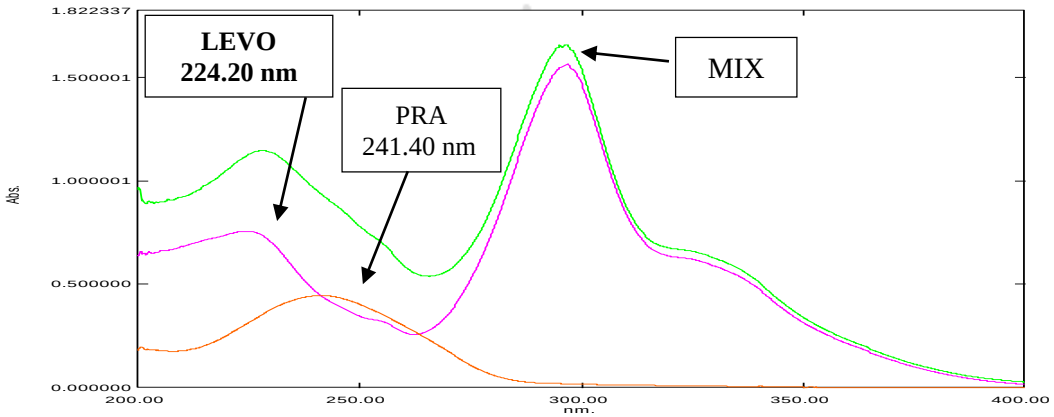

Figure: 3 Overlain spectra of LEF (15 µg/mL) and PRA (10 µg/mL) in methanol

Preparation for calibration curve

For construction of calibration curve, two series of different concentration in range of 6-18 µg/mL for LEF and 4-12 µg/mL for PRA were prepared in Methanol from stock solution. These solutions were scanned in range of 200-400 nm and absorbances were measured at selective wavelength and calibration curve were plotted for absorbance vs. concentration.

Method I (Simultaneous Equation Method)

Two wavelengths selected for the method are 224.20 nm (λ1) and 241.40 nm (λ2) that are absorbance maxima of LEF and PRA respectively in Methanol .Standard stock solution(s) of 100 µg/mL each of LEF and PRA were prepared separately in Methanol. The stock solutions of both the drugs were further diluted separately with to get a series of standard solutions of 6-18 µg/mL of LEF and 4-12µg/mL of PRA. The absorbance was measured at the selected wavelengths and absorptivities (A 1%, 1 cm) for both the drugs were determined as mean of three independent determinations. Concentrations in the sample were obtained by using following equations:

$$Cx = (A2\ ay1 - A1\ ay2) / (ax2\ ay1 - ax1\ ay2) \ldots\ldots\ldots (1)$$

$$Cy = (A1\ ax2 - A2\ ax1) / (ax2\ ay1 - ax1\ ay2) \ldots\ldots\ldots (2)$$

A1 and A2 are the absorbance of sample solutions at 241.40 and 224.20 nm, respectively.

ax1 and ax2 are E (1%, 1 cm) of PRA at 241.40 and 224.20 nm.

ay1 and ay2 (77.5, 560.0) are E (1%, 1 cm) of LEF at 241.40 and 224.20 nm.

Cx and Cy are concentrations of LEF and PRA in mg/mL in sample solution. The values of Cx and Cy were calculated by putting the values of A1 and A2 to solve the simultaneous Eqs. 1 and 2.

Method II (First Order Derivative Method)

Zero Crossing 1st Derivative spectrophotometric method was developed for simultaneous estimation of LEF and PRA in their binary mixture. The zero order spectrum was processed to obtain first-derivative spectrum. The two first derivative spectra were overlaid which shows that PRA showed zero crossing at 240.90 nm, while LEV showed zero crossing at 262.78 nm. The determinations were made at 262.78 nm for PRA (ZCP of LEF) and 240.90 nm for LEV (ZCP of RRA).The zero order and first order overlaying spectra are presented in Figs. 3 and 5, respectively. Linearity was observed over concentration range of 6-18 µg/mL for LEF and 4-12 µg/mL for PRA. The proposed Zero Crossing 1st Derivative method is found to be simple, specific, accurate, precise, robust, rapid and economical.

METHOD VALIDATION

The proposed methods were validated accordance to ICH Q2 (R1) guidelines for linearity, precision, accuracy, limit of detection, limit of quantification. The results are shown in table 1.

1. Linearity and Range:

The linearity response was determined by analysing 5 independent levels of calibration curve in the range of 6-18 µg/mL and 4-12 µg/mL for LEF and PRA respectively (n=5). The calibration curve of absorbance vs. concentration was plotted and correlation coefficient and regression line equations for LEF and PRA were calculated.

1. Precision

i. Repeatability

Aliquots of 1.2 ml of working stock solution of LEF (100 µg/mL) and 0.8 ml of working stock solution of PRA (100 µg/mL) were taken into two separate series of 10 ml volumetric flask and volume was made upto mark with methanol to give a solution containing 12 µg/mL and 8 µg/mL of LEF and PRA. Solution was analysed six times (n=6) and % R.S.D. was calculated.

ii. Intraday Precision

Aliquots of 0.9, 1.2, and 1.5 ml of working stock solution of LEF (100 µg/mL) were taken into series of 10 ml volumetric flask. Aliquots of 0.6, 0.8 and 1.0 ml of working stock solution of PRA (100 µg/mL) were taken into series of 10 ml volumetric flask. Using methanol, volume was made upto mark to give a solution containing 9, 12 and 15 µg/mL of LEF and 6, 8 and 10 µg/mL of PRA. Solution were analysed for three times (n=3) on the same day within short interval of time and % R.S.D. was calculated.

iii. Interday Precision

Aliquots of 0.9, 1.2, and 1.5 ml of working stock solution of LEF (100 µg/mL) were taken into series of 10 ml volumetric flask. Aliquots of 0.6, 0.8 and 1.0 ml of working stock solution of PRA (100 µg/mL) were taken into series of 10 ml volumetric flask. Using methanol, volume was made upto mark to give a solution containing 9, 12 and 15 µg/mL of LEF and 6, 8 and 10 µg/mL of PRA. Solution were analysed for three times (n=3) on three different days and % R.S.D. was calculated.

2. Accuracy

Preparation of synthetic mixture solution

Synthetic mixture of 75 mg equivalent of LEF was taken into 10 ml of volumetric flask. Methanol was added and sonicated for 2-3 mins and volume was made upto mark with methanol. Solution was filtered through Whatmann filter paper no. 42. Thus, resulting solution gave 1500 µg/mL of LEF and 1000 µg/mL of PRA. From the above solution, 1.0 ml was pipette out and transferred to 10 ml volumetric flask and volume was made upto mark with methanol in order to give a solution containing LEF(150µg/ml)+PRA(100µg/ml).

3. LOD and LOQ

The LOD (Limit of Detection) was estimated from the set of 5 calibration curves that were used to determine linearity of the method. The LOD was calculated by using the formula: **LOD = 3.3 × S.D. /Slope**

Where,

S.D. = Standard deviation of the Y – intercepts of 5 calibration curves

Slope = Mean slope of 5 calibration curves

The LOQ was estimated from the set of 5 calibration curves that were used to determine linearity of the method. The LOQ was calculated by using the formula:

LOQ = 10 × S.D./Slope

Where,

S.D. = Standard deviation of the Y – intercepts of 5 calibration curves

Slope = Mean slope of 5 calibration curves

RESULT AND DISCUSSION

UV spectrophotometric methods were found to be simple, accurate, economic and rapid for routine simultaneous estimation of LEF and PRA in pharmaceutical dosage forms. For Simultaneous equation method, Linearity was obtained in concentration range of 6–18 µg/mL of LEV and 4-12 µg/mL of PRA, with regression 0.9968 and 0.9980, intercept 0.0875 and 0.0112 and slope 0.0563 and 0.0429 for LEF and PRA respectively. Recovery was in the range of 98.62 – 100.90 %; the value of standard deviation and % R.S.D. were found to be < 2 %; shows the high precision of the method. In first order derivative method, Linearity was obtained in concentration range of 6–18 µg/mL of LEV and 4-12 µg/mL of PRA, with regression 0.9968 and 0.9975, intercept 0.0018 and 0.0005 and slope 0.001 and 0.001 for LEF and PRA respectively. Recovery was in the range of 98.56 – 101.25 %; the value of standard deviation and % R.S.D. were found to be < 2 %; shows the high precision of the method.

Method I : Simultaneous Equation Method

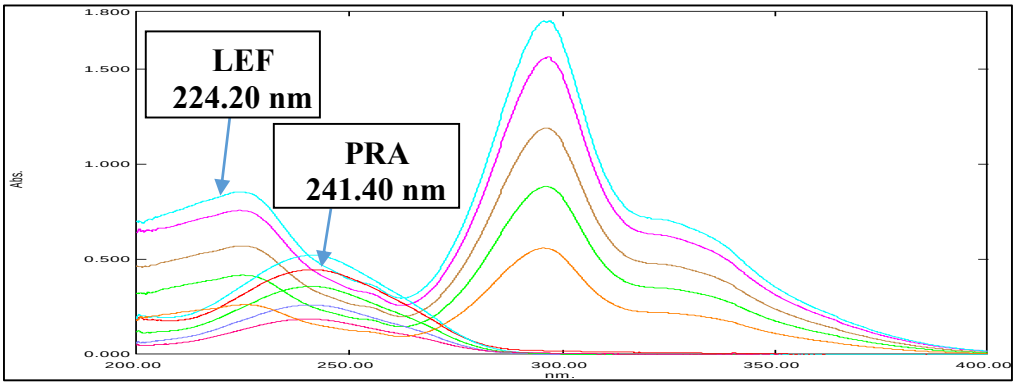

Figure: 4 Calibration curve of LEF (6-18 µg/mL) and PRA (4-12 µg/mL)

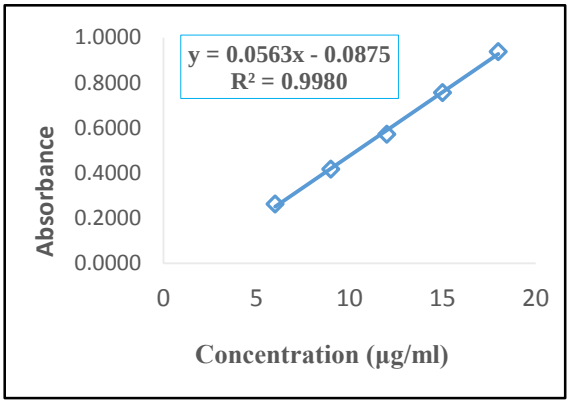

Figure: 5 Calibration curve of LEF at 224.20 nm

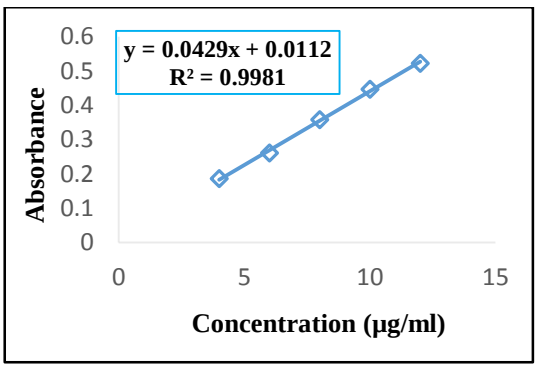

Figure: 6 Calibration curve of PRA at 241.40 nm

Method II : First Order Derivative Method

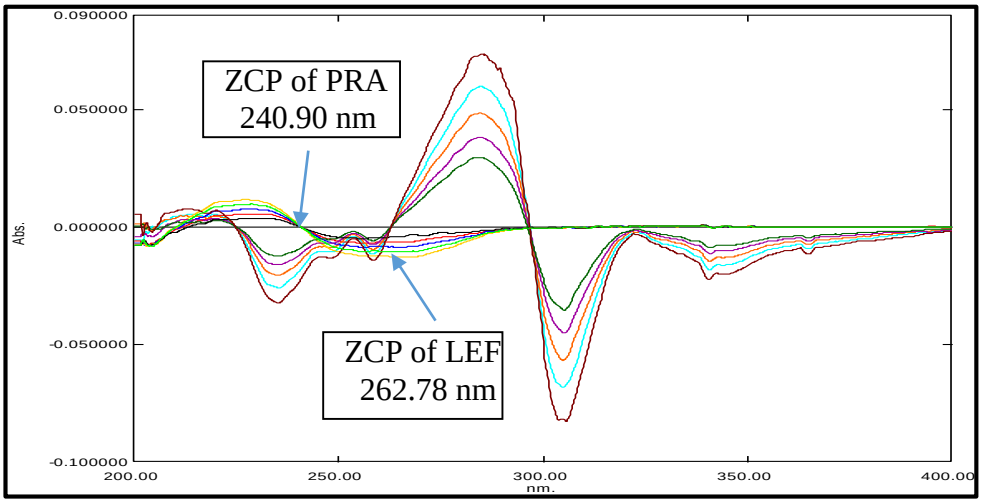

Figure: 7 Calibration curve of LEF (6-18 µg/mL) and PRA (4-12 µg/mL)

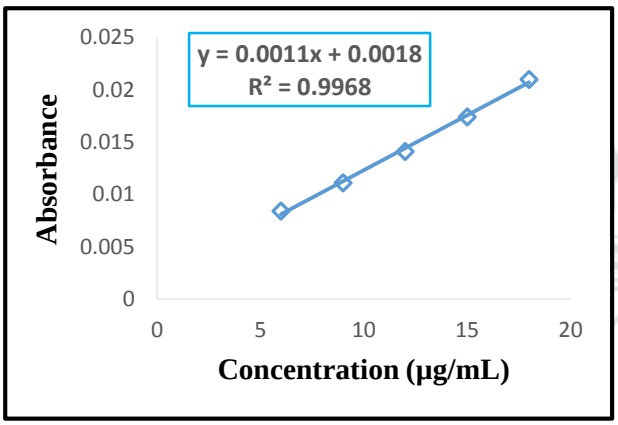

Figure: 8 Calibration curve for LEF at 240.90 nm (ZCP of PRA)

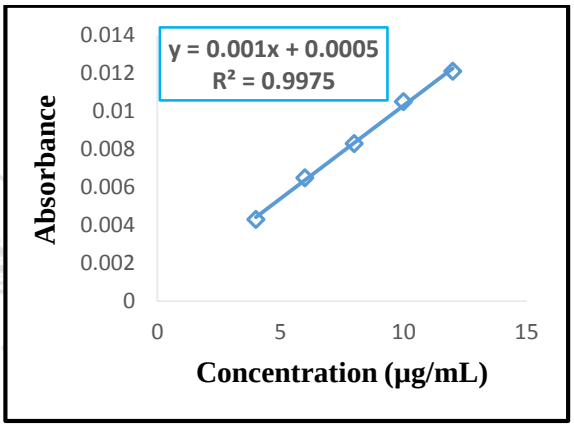

Figure: 9 Calibration curve for PRA at 262.78 nm (ZCP of LEF)

Table: 1 Linear regression analysis and optical characteristics of LEF and PRA

Parameter	UV Spectroscopy			
	Method I		*Method II*	
	LEF	PRA	LEF	PRA
Analytical Wavelength (nm)	224.20	241.40	240.90	262.78
Beer's law limit (µg/mL)	6-18 µg/mL	4-12 µg/mL	6-18 µg/mL	4-12 µg/mL
Coefficient of Correlation(r2)	0.9980	0.9981	0.9968	0.9975
Slope	0.0563	0.0429	0.001	0.001
Intercept	0.0875	0.0112	0.0018	0.0005
LOD (µg/mL)	0.2476	0.0303	0.0982	1.0844
LOQ (µg/mL)	0.7503	0.0919	0.2976	3.2863

Table: 2 Results of Precision Study

	LEF	PRA	*Method I*		*Method II*	
	(µg/mL)	(µg/mL)	LEF	PRA	LEF	PRA
Intraday *(%RSD)	9	6	0.6070	0.7165	0.8765	0.9123
	12	8	0.8609	0.9799	0.6981	1.0319
	15	10	0.9884	1.0308	0.8403	1.0036
Interday *(%RSD)	9	6	0.9570	1.1044	1.0781	1.0006
	12	8	1.0443	1.2478	0.9415	1.2198
	15	10	1.2707	1.3132	1.0219	1.2046

Table: 3 Results of Recovery Study

Level	Amount of sample (µg/mL)		Amount of Std. spiked (µg/mL)		% Recovery	
	LEF	PRA	LEF	PRA	LEF	PRA
Method I						
0%	6	4	0	0	98.83%	95.50%
80%	6	4	4.8	3.2	100.64%	99.44%
100%	6	4	6	4	99.58%	98.62%
120%	6	4	7.2	4.8	100.90%	100.56%
Method II						
0%	6	4	0	0	100.83%	98.75%
80%	6	4	4.8	3.2	99.91%	99.86%
100%	6	4	6	4	101.66%	101.25%
120%	6	4	7.2	4.8	98.56%	100.11%

Table: 4 Assay Result of Synthetic Mixture

Drug	Actual Conc. (µg/mL)	*Method I*	*Method II*
LEF	15	99.53 ± 0.0078	99.43 ± 0.0045
PRA	10	99.80 ± 0.0107	99.80 ± 0.0130

SUMMARY OF VALIDATION PARAMETER FOR PROPOSED METHOD

Table: 5 Summary of Simultaneous Equation Method

Parameters	Levofloxacin	Prednisolone Acetate
Selected Wavelength range	224.20	241.40
Linearity (n=5)	6-18 (µg/ml)	4-12 (µg/ml)
Regression equation	y = 0.0563x - 0.0875	y = 0.0429x + 0.0112
Slope (m)	0.0563	0.0429
Intercept (c)	0.0875	0.0112
Regression Co-efficient (R^2)	0.9980	0.9981
Correlation Coefficient (r)	0.9989	0.9990
Repeatability (n=6) (% RSD)	0.4439	0.3150
Intraday precision (n=3) (% RSD)	0.6070-0.9884	0.7165-1.0308
Interday precision (n=3) (% RSD)	0.9570-1.2707	1.1044-1.3132
LOD (n=5)	0.2476	0.0303
LOQ (n=5)	0.7503	0.0919
% Recovery (n=3)	98.83-100.90 %	98.62-100.56 %
% Assay ± S.D. (n = 5)	99.53 ± 0.0078	99.80 ± 0.0107

Table: 6 Summary of first order derivative method

Parameters	Levofloxacin	Prednisolone Acetate
Zero Crossing Point	240.90	262.78
Linearity (n=5)	6-18 µg/ml	4-12 µg/ml
Regression equation	Y=0.0011x-0.0018	Y=0.001x-0.0005
Slope (m)	0.001	0.001
Intercept (c)	0.0018	0.0005
Regression Co-efficient (R^2)	0.9968	0.9975
Correlation Coefficient (r)	0.9984	0.9987
Repeatability (n=6) (% RSD)	0.3870	0.9051
Intraday precision (n=3) (% RSD)	0.6981-0.8765	0.9123-1.0319
Interday precision (n=3) (% RSD)	0.9415-1.0781	1.0006-1.2198
LOD (n=5)	0.0982 µg/ml	1.0844 µg/ml
LOQ (n=5)	0.2976 µg/ml	3.2863 µg/ml
% Recovery (n=3)	98.56-100.83%	98.75-101.25%
Assay (%) Mean ± S.D. (n = 3)	99.43 ± 0.0045	99.80 ± 0.0130

CONCLUSION

All the validation parameters for all the developed methods were studied as per the ICH guidelines. All the methods were found to be simple, accurate, Specific, Selective, Precise and reproducible. Hence, the methods can be used for routine analysis of both the drugs in their combined dosage form.

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REFERENCES:

1. Drug Profile, "Levofloxacin", May 2020, https://www.drugbank.ca/drugs/DB01137.
2. Drug Profile, "Levofloxacin", June 2020, https://pubchem.ncbi.nlm.nih.gov/compound/Levofloxacin.
3. Drug Profile, "Prednisolone acetate", June 2020, https://www.drugbank.ca/salts/DBSALT000254.
4. Drug Profile, "Prednisolone acetate", May 2020, https://pubchem.ncbi.nlm.nih.gov/compound/Prednisolone-acetate.
5. Sharma HL., and Sharma KK. Principle of Pharmacology; 2nd Edn; Paras Medical Publisher, New Delhi, 2012, pp 708-715.
6. Kashyap R, srinivasa U, Dadhaniya S. First order derivetive and dual wavelength spectrophotometry methods development and validation for simultaneous estimation of alogliptin and pioglitazone in but and dosage form. 2014(4). 21-31.
7. Nagamalleswari G. Quantitative analysis of Amoxicillin and Dicloxacillin in Combined Dosage Form by First Derivative and Simultaneous Equation Method in Application to the determination of Content Uniformity. IJAPA. 2014;4:53-57
8. Yadav N. and Goyal A. Simultaneous Estimation of Aliskiren and Amlodipine in Combined Tablet Formulation by Simultaneous Equation and First Derivative Spectroscopic Methods. Organic and medicinal chemistry international journal. 2018:1-5
9. Saudagar R, Saraf S. First order derivative simultaneous equation and area under the curve methods for estimation of domperidon maleate and rabeprazole sodium in tablet dosage form. Indian Drugs. 2006;43(5):388-392
10. Merukar SS, Mhaskar PS, Bavaskar SR, Burade KB, and Dhabale PN. Simultaneous spectrophotometric methods for estimation of levocetirizine and pseudoephedrine in pharmaceutical tablet dosage form. J. Pharm. Sci. Rese.2009;1:38–42,
11. Prabu SL, Shirwaikar A, Kumar C, and Kumar G. Simultaneous UV spectrophotometric estimation of ambroxol hydrochloride and levocetirizine dihydrochloride. Indian J. Pharm. Sci. 2008;70: 236–238.
12. Parmar K. Development and Validation of First-Order Derivative Spectrophotometry for Simultaneous Determination of Levocetirizine Dihydrochloride and Phenylephrine Hydrochloride in Pharmaceutical Dosage Form. Int. J. Spectrosc. 2013;1:1-6.
13. Validation of analytical procedures: Text and Methodology Q2 (R1); ICH Harmonized Tripartite Guideline. 2005, pp. 4-13.
14. ICH, editor Q2A, text on validation of analytical procedures. Fed Regist; 1995.
15. ICH, editor Q2B validation of analytical procedures: methodology. Fed Regist; 1997.
16. Lonikar N, Baby S. Mallikarjuna G. A Selective uv spectrophotometric analytical method to quantify the levofloxacin in bulk sample. Eur. J. pharm. Med. Res. 2016; 3: 351-353.
17. Patel P. Shah H. Patel K. Dr. Patel M. Development and validation of spectrophotometric methosds for Simultaneous estimation of cefixime trihydrate and levofloxacin Hemihydrate in their combined tablet dosage form. Int. J. Pharm. Res. Bio-Sci. 2012; 1: 502-512.
18. Hakan A. Sermin G. Spectrometric determination of levofloxacin and norfloxacin in tablets by multivariate calibration approach. Rev. Anal. Chem. 2010; 29: 81-91.
19. Tamilarasi G. Dr. Vetrichelvan. T. Dr. Vekappayya D. tharabai. R. yuvarajan.k. Spectrophtometric estimation of levofloxacin hemihydrate and Cefpodoxime proxetil in tablet. Int. J. Pharm. Sci. 2014; 6: 646-648.
20. Patil T. Pore Y. Development and Validation of Simultaneous UV Spectrophotometric Method for the Determination of Levofloxacin and Ambroxol in Tablets. J. Korean Chem. Soc. 2008; 52:622-629.