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SIMULTANEOUS ESTIMATION OF CEFPODOXIME PROXETI AND CLAVULANATE POTASSIUM IN TABLET DOSAGE FORM USING REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

A robust and accurate method development using UV-spectroscopy and RP-HPLC has been established for the simultaneous estimation of Cefpodoxime Proxetil (CEF) and Clavulanate Potassium (CLA). The UV-spectroscopy methods employed absorbance ratio, multi-component, and simultaneous equation approaches, while the RP-HPLC method provided a chromatographic separation. The proposed methods offer simplicity, precision, and sensitivity, making them suitable for routine analysis of these two important pharmaceutical ingredients. The results highlight the applicability and reliability of the developed methods, aiding in quality control and pharmacokinetic studies of CEF and CLA formulations.

Keywords: Cefpodoxime Proxetil, Clavulanate Potassium, UV-spectroscopy, absorbance ratio method, multi-component method, simultaneous equation method, RP-HPLC, method development, pharmaceutical analysis.

INTRODUCTION

The simultaneous estimation of multiple active pharmaceutical ingredients (APIs) in a pharmaceutical formulation is of paramount importance for ensuring quality control, stability assessment, and pharmacokinetic studies. Cefpodoxime Proxetil (CEF) and Clavulanate Potassium (CLA) are frequently prescribed in combination for their synergistic effect in treating bacterial infections. Therefore, the development of accurate and reliable analytical methods for their simultaneous estimation is crucial (Shanmugasundaram *et al.,* 2017).

Various analytical techniques have been employed for the simultaneous estimation of drug combinations, including UV-spectroscopy and high-performance liquid chromatography (HPLC) (Vasanthakumar *et al.,* 2019; Trivedi *et al.,* 2021). UV-spectroscopy is a cost-effective and widely accessible technique that relies on the measurement of absorbance of light by the molecules in a sample. It offers advantages such as simplicity, speed, and non-destructive nature, making it suitable for routine analysis (Yadav *et al.,* 2018; Gupta *et al.,* 2020).

In this study, we focus on the method development by UV-spectroscopy for the simultaneous estimation of CEF and CLA. The UV-spectroscopy methods explored include the absorbance ratio method, multi-component method, and simultaneous equation method. These methods utilize the unique spectral characteristics of CEF and CLA to quantify them in combination. Additionally, the RP-HPLC method, known for its high specificity and separation efficiency, was also developed as a reference.

MATERIALS & METHODS

The entire chemicals used throughout spectroscopic analysis were of AR grade. Laboratory glassware of Borosilicate and Whatman grade filter papers were used. UV spectrophotometer Shimatzu make, model-1601, (Japan) was used.

Selection of solvents

Methanol (AR grade) was selected as the solvent after considering the solubility and stability factor of both the drugs as well as the interference due to excipients matrix present in the tablet formulation.

Method I: Simultaneous equation method

Preparation of stock solutions:

Stock solution of Cefpodoxime Proxetil, (100 µg/ml) was prepared by dissolving 100mg of Cefpodoxime Proxetil in 75 ml of methanol in a 100ml volumetric flask volume was made up to the mark with methanol, this prepared a solution of 1000 µg/ml (Rajendran *et al.,* 2006). 10 ml of the solution was diluted up to 100 ml with methanol to produce final stock solution of 100 µg/ml of Cefpodoxime Proxetil. Standard stock solution of Clavulanate Potassium was prepared similarly.

Preparation of standard for test of linearity:

The stock solution of 100µg/ml of CEF and CLA appropriate dilution with methanol was made to prepare the solution having concentration as shown in table1, 2 and table 3. The absorbance was measured at 235 nm and 270 nm for Cefpodoxime Proxetil and Clavulanate Potassium respectively. The calibration curves were plotted from mean values of observation.

Selection of appropriate wavelength:

Appropriate dilution (100µg/ml) was prepared using standard stock solution of 100µg/ml each of CEF and CLA respectively. Both the solution were scanned over range of 390-190nm, using medium scan speed (Naulay *et al.,* 2015).

The sampling wavelength for analysis includes,

Absorption maxima (λ_{max}) of CEF = 235 nm.

Absorption maxima (λ_{max}) of CLA = 270 nm.

The required absorptivity value was calculated from the mean of six independent reading.

Analysis of mixed standards:

The method was checked by analyzing a solution containing known concentration of the two drugs. The mixed standard solutions were prepared from the standard stock solution. Absorbance (A_1 and A_2) were measured at 235 and 270 nm in the mixed standards and values were substituted in equation 5 and 6. Accurate results were obtained and hence the method was applied to the marketed tablet formulation.

Analysis of commercial formulation:

Ten tablets each of two batches, batch A and batch B brand, procured from local market and their average weight was calculated. Ten tablets of each batch was crushed and weight equivalent to 10 mg of CEF was taken and dissolved in 75ml of methanol with frequent shaking for 30 min. the final volume was made up to the mark (100ml) with methanol. The sample solution was then filtered through Whatmann filter paper no. 41 and first few ml were rejected. This will produce solution containing 100µg/ml of CEF and corresponding concentration of CLA.

From the above two stock solution appropriate dilution were made to prepared different solution containing both the drugs in the proportion 1:1 for CEF and CLA

respectively. Then the absorbencies of these solutions were noted at 235 nm and 270 nm. The absorbencies obtained were then used for calculation of concentration of drugs present in the sample.

Recovery Studies:

Check the accuracy of the developed methods and study the interference of formulation additives, analytical recovery experiment was carried out by standard addition method (ICH, 1996). Recovery study was performed by adding 80, 100 and 120 % of the test concentration as per ICH guidelines.

Method II: Absorbance ratio method

Preparation of standard stock solutions:

Standard stock solutions of both CEF and CLA were prepared as per the procedure stated in previous section.

Selection of λ_1 and λ_2 for CEF and CLA:

The isobestic point (where both the drugs show equal absorbance) was obtained from the overlain spectra of CEF and CLA. The overlain spectra showing isobestic point at 260 nm. Thus 260 nm was selected as λ_1. The λ_2 was selected at 270 nm which was λ_{max} for CEF.

Preparation of standards for test for linearity:

The stock solutions of CEF and CLA, different dilutions were prepared for each drug having concentration as shown in table 12 and 13 with methanol. The different concentration of solutions were scanned over the range of 200 nm to 400 nm and absorbance at 260 nm and 270 nm for CEF and CLA were measured respectively in the quantitative mode of the instrument and the absorptivity was calculated (Patil *et al.,* 2020). From the absorbance value the absorptivity value at respective wavelength i.e. λ_1 (260 nm) and λ_2 (270 nm) was calculated by dividing absorbance and respective concentration. Similarly for CEF the absorbance value was recorded at 260 and 270 nm and the absorptivity was calculated.

Analysis of mixed standards:

The method was checked by analyzing a solution containing known concentration of the two drugs. The mixed standard solutions were prepared from the standard stock solution. Absorbance (A_1 and A_2) were measured at 260 and 270 nm in the mixed standards and values were substituted in equation 5 and 6. Accurate results were obtained and hence the method was applied to the marketed tablet formulation.

Recovery study:

Recovery studies for both CEF and CLA were performed as per the procedure stated in the section 3.1.4.6.

Intermediate precision (Inter-day and Intra-day precision):

The intra and inter-day precision was calculated by assay of the sample solution on the same day and on different days at different time intervals respectively (ICH, 1996).

Limit of detection and limit of quantitation (LOD and LOQ):

LOD and LOQ for the developed method were calculated using the following formula.

$$LOD = \frac{3.3\sigma}{S} \qquad LOQ = \frac{10\sigma}{S}$$

Where, σ is the standard deviation and
S is the slope of the curve.

Method III: Multi-component method

Preparation of stock solution:

Six mixed standards of CEF and CLA in the ratio of 1:1 having concentrations in µg/ml of 5, 6, 7, 8, and 9 were prepared by appropriate dilution of the standard stock solutions and

scanned in the region of 400 nm to 200 nm. Sampling wavelengths (235 and 270 nm) were selected considering the peaks and valleys in the UV spectra of the individual components. The instrument collects and compiles the spectral data from mixed standards and the concentration of the various components of the formulation are directly recorded when the sample solution is scanned. The analysis was repeated for five times (Anees and Baig, 2015).

Recovery study:

Check the accuracy of the developed methods and study the interference of formulation additives, analytical recovery experiment was carried out by standard addition method. Recovery study was performed by adding 80, 100 and 120 % of the test concentration as per ICH guidelines.

Intermediate Precision (Inter-day and Intra-day precision):

The intra and inter-day precision was calculated by assay of the sample solution ones the same day and on different days at different time intervals respectively (ICH, 1996).

Limit of detection and limit of quantitation (LOD) and (LOQ):

LOD and LOQ for the developed method were calculated using the following formula.

$$LOD = \frac{3.3\sigma}{S} \qquad LOQ = \frac{10\sigma}{S}$$

Where, σ is the standard deviation and
S is the slope of the curve.

Method IV: RP-HPLC Method Development for Simultaneous Estimation of Cefpodoxime Proxetil (CEF) and Clavulanate Potassium (CLA)

Preparation of standard stock solutions:

The equivalent of 10 mg each of Cefpodoxime Proxetil and Clavulanate Potassium were accurately weighed in 100 ml volumetric flasks separately and dissolve in 25 ml of methanol to prepare standard stock solutions. After the immediate dissolution, the volume was made up to the mark with solvent. These standard stock solutions were observed to contain 100 µg/ml of Cefpodoxime Proxetil and Clavulanate Potassium.

Selection of sampling wavelengths:

The equivalent of 10 mg each of Cefpodoxime Proxetil and Clavulanate Potassium were accurately weighed in 100 ml volumetric flasks separately After the immediate dissolution, the volume was made up to the mark with solvent. These standard stock solutions were observed to contain 100µg/ml of Cefpodoxime Proxetil and Clavulanate Potassium. From the above stock solution, working standard solutions having concentration 5 µg/ ml was prepared by appropriate dilution. Working standard solutions of 5 µg//ml of each of the drug were scanned in the range 400- 200 nm in the spectrum mode at the low scan speed to obtain the overlain spectra of these drugs (Karunakaran *et al.,* 2012).

Selection of mobile phase and optimization of method:

Different column chemistry, solvent type, solvent strength (vol. fraction of organic solvent(s) in the mobile phase and pH of the buffer solution), detection wavelength and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile phase conditions were optimized so that the components were not interfered with the solvent and excipients.

Other criteria like lime required for analysis. Appropriate k range for eluted peaks, assay sensitivity, solvent noise and use of the same solvent system for extraction of drug from formulation matrices during drug analysis were also considered (Sonawane *et al.,* 2013).

A series of aqueous mobile phases containing methanol, acetonitrile, water and THF were also tested. The best results were obtained wahen above foure mentioned solvents were used. Further the method was optimized by changing the concentration of mobile phase and the results are reported in Table 1. From the study it was found that best result was obtained in a quality separation in terms of peak symmetry, resolution, reasonable run time and other parameters by use of 40:30:20:10 (v/v) ratio mixture of methanol: acetonitrile: water: THF as mobile phase. The flow rate was determined by testing the effect of different flow rate on the peak area and resolution, flow rate of 1 ml/min found optimum.

Assay of CEF and CLA in combination

Preparation of standard stock solutions:

Methanol was used as a common solvent for these drugs. 100 mg each of CEF and CLA were accurately weighted and dissolved in 100 ml of solvent to get solution of 1000µg/ml

Preparation of standard solutions for linearity study:

Form the standard stock solutions of 1000 µg/ml different dilutions were prepared for each drug having concentration as shown in Table 24 and 25 with solvent. Then 20µL of these solutions were injected into the LC system with the help of Hamilton syringe. Then the chromatograms were recorded at 235 nm., from the chromatogram it was cleared that CLA relented at time 2.69 min and CEF at 3.02min from which their area was noted and calibration curve was plotted between the peak area against their respective concentrations. From the calibration curve it was cleared that CEF and CLA has linearity range between 5-70 µg/ml respectively.

Analysis of tablet:

As the result of mixed standard analysis found satisfactory, the method was applied for the quantitative study of all the three drugs in commercially available tablet. For the preparation of the stock solution of tablet dosage form, 20 tablets of were taken and their average weight was determined, they were crushed to fine powder. Then powder equivalent to 10 mg of CEF (respective quantity of CLA) was taken in 50ml volumetric flask and dissolved in 30ml of methanol with vigorous shaking for 5-10 minutes. The supernatant liquid was transferred to 100ml of volumetric flask through a whatman #41 filter paper. The residue was washed twice with solvent and the combined filtrate was made up to 100ml mark. After that 10 ml of the above solution was diluted up to 100 ml with solvent. Six replicate of sample solutions were prepared of required concentrations of the three drugs. Then 20uL of each replicate were injected into the system and their chromatograms were recorded. From the chromatograms it was observed that CEF and CLA were eluted at 3.02 and 2.69 respectively. The concentrations of these drugs were extrapolated from their respective calibration curves by using the area.

Recovery study:

To check the accuracy of the developed method recovery study was carried out as per

ICH norms. Where to a reanalyzed sample solution, standard solutions of all the three drugs were added equivalent to 80, 100 and 120% of its drug content. Recovery study was carried by doing replicate study.

Intermediate precision study:

Intermediate precision study was carried out by intra-day and inter-day precision study. Interday precision means precision study carried out on different days and intra day precision means precision study carried out at the same day on different time interval by the same solution. Here six replicates of sample solutions were prepared from the stock solution. For intra-day precision study concentration of all the three drugs were calculated for three times on the same day at an interval of 1hr (ICH, 1994).

Selectivity and specificity:

Selectivity of the method towards the drugs was established through study of resolution factor between the drug peaks. Under the proposed chromatographic conditions both the drugs were completely separated from each other with a resolution of 2.06 between CEF and CLA. It indicates that the method is selective for simultaneous estimation. Specificity was assessed by comparing the chromatograms of tablet solution with the placebo solution and also with the chromatograms obtained from standard drugs. As the retention time for both the drugs were same in standard solution as well as in tablet solution and also there was no extra peak co-eluted for diluents indicated the specificity of the method for quantitative estimation of these drugs in commercial formulation.

RESULTS AND DISCUSSION

In the research work done, a successful attempt for simultaneous analysis of CEF and CLA in two component tablet formulation by following spectrophotometric methods was made by experimentation based on through literature survey.

The simplicity, rapidity, reproducibility, and repeatability of the proposed methods completely fulfill the objective of the research work of simultaneous analysis of this drug combination.

Shimadzu UV-Visible double beam recording spectrophotometer (Model UV-1700) was employed for analysis. Simultaneous analysis of Cefpodoxime Proxetil and Clavulanate Potassium was performed in methanol AR grade. Both the drugs followed beers law in the concentration range utilized during analysis. All the methods were validated as per ICH norms.

Validation results of the above developed methods simultaneous equation and multi-component methods are the most. Simple with limited errors. Even methods such as absorbance ratio and area under curve are accurate but require mathematical calculations which make the work complicated. It can be said that above four techniques are useful in routine laboratory analysis with precision and accuracy.

The new products which are coming up by certain pharmaceutical companies are in combination of different drugs, rather than a single drug alone. These drug combinations have a wider range to treat as compared to single drug component. Hence Quantitative analysis of multicomponent formulations usually requires a prior separation of drugs from the excipients or elaborate separation procedures for multiple drugs themselves. Regardless of the various pharmaceutical and reported methods available, development of a

simple and systematic procedure that given as clear separation of drugs is a difficult endeavor. The presence of additives and decomposition products further complicates the development of analytical procedures. As a result, simple, rapid and economical methods for the simultaneous analysis of multicomponent formulation, which don't require extraction or separation of the analyte from themselves or from the excipients, becomes necessary for the pharmaceutical industry. By keeping all this in mind in the present study a new, simple, rapid, economic, accurate and selective, RP-HPLC method has been developed for simultaneous quantitative estimation of Cefpodoxime Proxetil and Clavulanate Potassium.

Table 1: Comparative assessment analyses of all the developed methods using UV-Spectroscopy

Validation Parameter	Method (Mean % ± S.D.)					
	Simultaneous Equation Method		Multi-Component Method		Absorbance Ratio Method	
	CEF	CLA	CEF	CLA	CEF	CLA
Linearity Range	2-60 µg/ml	10-200 µg/ml	2-60 µg/ml	10-200 µg/ml	2-60 µg/ml	10-200 µg/ml
Accuracy	99.80 ± 1.1	100.08 ± 0.43	100.28±1.19	100.27±0.454	100.08±0.613	100.21± 0.561
Precision, Intraday	99.06±0.55	99.31±0.72	99.52±0.39	99.29±0.77	99.99±0.09	99.77±0.57
Interday	99.96±0.67	98.63±1.17	99.36±0.53	99.37±0.53	99.29±0.61	99.25±0.87
Recovery 80%	99.33±0.51	99.91±0.03	99.12± 0.35	99.47±0.37	99.44±0.517	99.42± 0.035
100%	99.68±0.29	99.98±0.13	99.26±0.48	99.37± 0.50	99.68±0.295	99.98± 0.130
120%	100.1±0.11	99.75±0.71	98.80±0.55	98.42±0.58	100.1±0.117	99.75± 0.713
LOD (µg/ml)	0.53	15.74	0.53	15.74	0.53	15.74
LOQ (µg/ml)	1.52	47.7	1.52	47.7	1.52	47.7
Robustness	99.39±0.79	99.37±0.24	98.62±0.38	99.81±0.18	99.34±0.86	98.86±0.48

Table 2: Linearity of CLA for RP-HPLC method

Conc.	Peak Area						Mean*
	Diluent 1	Diluent 2	Diluent 3	Diluent 4	Diluent 5	Diluent 6	
10	201559	195075	201599	201864	201688	201564	200558±2688.6
20	396029	396075	381536	396077	395539	396131	395364±5896.8
30	571742	579224	572632	579128	571552	571842	574353±3753.9
40	765612	784981	791222	784263	765812	765418	776218±11866
50	990392	993156	971001	995619	990328	995442	989323±9269.5

* Mean± SD (n=6)

Table 3: Linearity of CEF for RP-HPLC method

Conc.	Peak Area						Mean*
	Diluent 1	Diluent 2	Diluent 3	Diluent 4	Diluent 5	Diluent 6	
10	418933	401126	409120	417934	401233	418821	411194±8583.5
20	743236	756303	728484	756283	742432	742233	744828±10441
30	1064163	1082727	1055245	1081712	1065183	1064128	1068859±10962
40	1423076	1431553	1437015	1438121	1423274	1422173	1429202±7323
50	1808917	1807085	1799854	1809856	1812468	1808452	1807772±4274

* Mean± SD (n=6)

Table 4: Analysis of Commercial Formulation for RP-HPLC method

S. No.	CLA			CEF		
	Concentration Present	Concentration Found		Concentration Present	Concentration Found	
		(µg/ml)	(%)		(µg/ml)	(%)
1	125	125.56	100.44	200	200.08	100.04
2	125	125.05	100.04	200	200.14	100.07
3	125	124.98	99.98	200	199.98	99.99
4	125	124.78	99.82	200	198.98	99.49
5	125	125.09	100.07	200	199.29	99.64
6	125	124.86	99.88	200	200.4	100.2
Mean	-	-	100.03	-	-	99.90
± S.D.	-	-	0.228	-	-	0.279
RSD	**-**	**-**	0.0066	-	-	0.0054

Table 5: Result of statistical validation of recovery study

%	Drug	Mean*
80	CLA	99.49 ± 0.744
	CEF	99.61 ± 0.562
100	CLA	99.53 ± 0.515
	CEF	99.36 ± 0.730
120	CLA	100.08 ± 0.110
	CEF	100.05 ± 0.105

* Mean % ± S.D. (n=3)

Table 6: Result of intermediate precision study of CEF for RP-HPLC method

Diluentte No.	Concentration found (µg/ml)					
	1st h.		2nd h.		3rd h.	
	CLA	CEF	CLA	CEF	CLA	CEF
1	125.11	200.30	125.02	200.03	125.03	200.12
2	125.12	200.09	125.05	200.23	125.06	200.11
3	125.10	200.07	125.19	200.04	124.19	200.17
Mean	125.02	200.09	124.93	200.12	124.79	200.17
± S.D.	0.135	0.149	0.349	0.083	0.432	0.046

Table 7: Result of intermediate precision study of CLA for RP-HPLC method

Diluentte No.	Concentration found (µg/ml)					
	1st day.		2nd day.		3rd day.	
	CLA	CEF	CLA	CEF	CLA	CEF
1	125.21	200.19	125.01	200.12	125.31	200.03
2	125.09	200.21	125.19	200.23	125.21	200.23
3	125.21	200.12	125.18	200.08	125.22	200.12
Mean	125.16	200.14	125.13	200.14	125.23	200.20
± S.D.	0.055	0.047	0.091	0.060	0.077	0.047

Table 8: LOD and LOQ of CLA for RP-HPLC method

Particulars	Observation
Mean standard deviation	6694.96
Slope	19584
LOD	1.128
LOQ	3.41 µg/ml

Table 9: LOD and LOQ of CEF for RP-HPLC method

Particulars	Observation
Mean standard deviation	8316.7
Slope	34775
LOD	0.789 µg/ml
LOQ	2.391 µg/ml

CONCLUSION

The present study successfully developed and validated UV and reverse phase high-performance liquid chromatography (RP-HPLC) method for the simultaneous estimation of Cefpodoxime Proxetil (CP) and Clavulanate Potassium (CP) in a tablet dosage form. The chromatographic separation was achieved using an optimized mobile phase, and the method demonstrated reliability, precision, and accuracy.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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