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Development and Validation of a New Simple and Rapid UV Spectroscopic Method for Cefalexin and Potassium-Clavulanate in Pure and Dosage Form

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Abstract: ***A novel, rapid, specific, and economic UV spectrophotometric method has been developed using distilled water as the solvent for the simultaneous estimation ofcefalexin (CEP) and potassiumclavulanate(PTC) content in bulk and pharmaceutical dosage formulations. The method was linear in the range of 15-75 µg/mL of CEP and 05-25 µg/mL for PTC. The absorbance was measured at 262nm and 212nm for cephalexin and potassium clavulanate respectively and exhibited a good correlation coefficient (R^2=0.9998) for CEP and (R^2=0.9979) for PTC. This method was successfully applied to the determination of cefalexin and potassium clavulanate content in bulk and pharmaceutical dosage formulations and the results were in good agreement with the label claims. The method was validated statistically and by recovery studies for linearity, accuracy, repeatability, reproducibility, and robustness. The obtained results proved that the method can be employed for the routine analysis of cefalexin and potassium clavulanate in bulks as well as in commercials.***

Keywords: Cefalexin, Potassium clavulanate, UV spectroscopy, Pharmaceutical dosage form

1. Introduction

Cefalexin a beta-lactam antibiotic from the cephalosporin family is bactericidal and inhibits the synthesis of the peptidoglycan layer of the bacterial cell membrane [1]. Because cephalexin is very similar to d-alanyl-d-alanine, an aminoalkanoic acid found on the peptidoglycan layer of the plasma membrane, it can irreversibly bind to the PBP binding site, which is crucial for cell membrane synthesis [2]. It is most active against gram-positive cocci and has moderate activity against some gram-negative bacilli. However, some bacterial cells have the beta-lactamase enzyme, which hydrolyzes the beta-lactam ring, rendering the drug inactive. This contributes to the antibacterial resistance to cefalexin [3] [4]. Clavulanate potassium, the salt of clavulanic acid, is a beta-lactam drug and works as a mechanism-based beta-lactamase inhibitor [5]. It contains a beta-lactam ring within its structure that irreversibly binds beta-lactamases, preventing them from inactivating certain beta-lactam antibiotics with efficacy in the treatment of gram-positive and gram-negative susceptible infections. Although not effective as an antibiotic on its own, when combined with penicillin group antibiotics it can overcome antibiotic resistance in bacteria that secrete beta-lactamase which otherwise inactivates most penicillins. These agents are not active against all beta-lactamase enzymes. It is important to note that beta-lactamase inhibitors do not increase the intrinsic activity of the antibiotic or extend the spectrum of action of the drug. The inhibitors simply bind beta-lactamase enzymes, allowing the drug to kill the bacteria [6]. A review of the literature shows that both cephalexin and potassium clavulanate are official in Indian pharmacopeia [7] [8]. Few chromatographic methods have been reported for the determination of cefalexin and potassium clavulanate in pharmaceutical preparations containing other active ingredients [9] [10]. Other techniques are under development for the determination of the latter compounds with various other active ingredients, including spectrophotometry [11] [12] [13], polymer membrane electrodes [14] [15], electrochemistry [16] and HPTLC [17] [18] [19]. There is an increasing number of publications describing the chromatographic method for the determination of cephalexin and potassium clavulanate with other active ingredients [20] [21].

The present study aimed to develop a unique, sensitive, accurate, rapid, and relatively simple method for the simultaneous quantification of cefalexin and potassium clavulanate in raw materials and pharmaceutical formulations by the UVspectroscopic method.

The absorption correction method is useful mainly when λ_{max} of two drugs are found to be near or very difficult to get isosbestic point whereby we cannot choose simultaneous equation method, q absorbance method, this method is found to be very useful in such cases where one drug has zero/no absorbance at the $\lambda_{max}$of the other, while at another wavelength λ_2 both the drugs shows absorbance.

2. Experimental

2.1 Materials

Working reference standards of cefalexin (CEP) and potassium clavulanate (PTC) were supplied by Lupin Laboratories Ltd. (India) and Alkem Laboratories Ltd.

(India), respectively. The chemical structures of these compounds are shown in Figure 1. Methanol was purchased from SD fine-chem Limited (India).The reagents were of analytical grade. Tablets were purchased from the Indian market, containing cefalexin 375mg and potassium clavulanate 125mg per tablet (SPORIDEX CV 375).

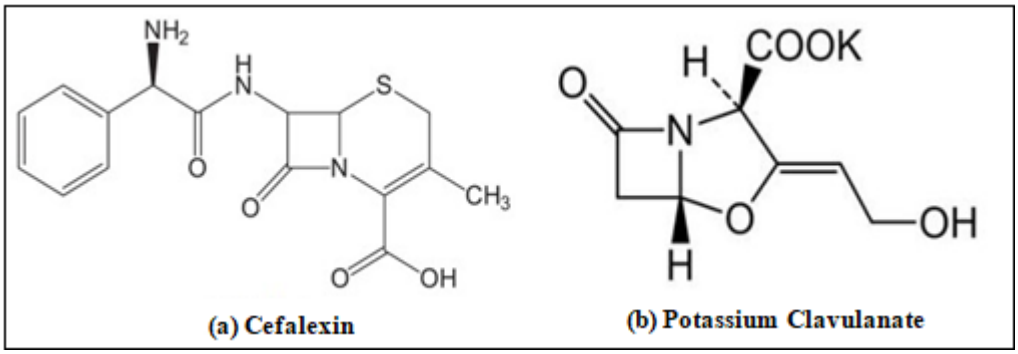

Figure 1: Chemical structure of (a) Cefalexin and (b) Potassium Clavulanate

2.2. UV-VISIBLE Spectrophotometer and Conditions

The UV visible spectrophotometer consisted of SHIMADZU (Japan) model UV-1800 a double beam spectrophotometer, equipped with a dual lamp (deuterium lamp and Tungsten lamp), Samples of all analytes were monitored at 262nm and 212nm using a Photomultiplier tube absorbance detector (190–800 nm) using a matched quartz cell (1cm path and 4.5 mL volume). Each spectroscopy was analyzed and integrated automatically using the UV probe Software version 2.34. The solvent system consisted of Distilled water

2.3. Standard solutions

Standard stock solutions of cefalexin (300µ/mL) and potassium clavulanate (100 µg/mL) were prepared by direct weighing of standard substance with subsequent dissolution in water.

Precaution: Preparing a fresh solution of potassium clavulanate is suggested, as the drug gets degraded easily. The prepared solution could be kept under refrigeration at 2-8^0c for a maximum of 3 days for getting satisfactory results.

2.4. Determination of wavelength of absorbance

30 mg of CEP and10 mg of PTC were accurately weighed and transferred into 100ml volumetric flasks separately. Dissolved in distilled water and made up to the volume of 100ml with the same after sonication for 6min 20sec and kept for refrigeration. These solutions were observed to contain 300µg/ml, and 100µg/ml of CEP and PTC respectively. The standard stock solutions of CEP and PTC were further diluted with distilled water to get the concentration of 30 µg/ml and 10 µg/ml of CEP and PTC respectively and the solutions were scanned between the range 190 - 400 nm in 1cm cell against distilled water as blank after 15 min standby, and the overlain spectra were recorded and shown in(Fig.2).

2.5. Calibration graphs

A series of working standard drug solutions equivalent to 15– 75 µg/mL for CEP and 5–25 µg/mL for PTC was prepared by diluting the stock standard solution with the solvent system. To construct the calibration curve five replicates of every standard solution The absorbance of CEP at 262nm and PTC at 212nm was measured. Then, the measured absorbance of CEP and PTC was plotted against the corresponding concentration to get the calibration graph. (Fig 3)

2.6. Assay procedure for dosage forms

Twenty tablets were weighed and the average weight was found. The tablets were triturated to a fine powder after peeling of film coating. An accurately weighed quantity of powder equivalent to 30mg of CEP, and 10mg of PTC was transferred into a 100 ml volumetric flask and diluted with 50ml of distilled water initially, The solution was sonicated for 6 minutes 20 sec, then made up to the volume with the same. The solution is then filtered through Whatman filter paper and kept for refrigeration. From the stock solution, further dilutions were made by diluting 1 ml into 10 ml with distilled water to obtain a 30µg/ml solution of CEP and 10µg/ml of PTC theoretically. From which CEP and P were was calculated by absorption correction method. The absorbance of the sample solution was measured at 262nm and 212nm with distilled water as blank after 15 min standby and calculated by using the equations;

Cx=A1/ax1…(Eq.1)

Cy =A2 –ax2 cx/ay2… (Eq.2)

Where,

- A1 and A2 are the absorbance of sample solution at 262nm and 212nm, respectively.
- ax1and ax2, absorptivity coefficients of CEP at 262nm and 212nm, respectively.
- ay2, absorptivity coefficient of PTC at 212nm.

2.7. Method validation

The method was validated using ICH guidelines by determining the following parameters: linearity, accuracy, precision, robustness, ruggedness, detection limit, and quantification limit.

3. Results and Discussion

3.1 Analysis of Formulation

Simultaneous estimation of CEP and PTC in formulation by UV spectroscopic method was carried out using optimized conditions by absorption correction method.The pure sample solutions were prepared and absorbance were recorded. The recorded CEP and PTC standard absorbance graphs are given in Figure 2.The assay procedure was repeated three times.The percentages of individual drugs found in formulations, mean, and relative standard deviation in formulations were calculated and presented in Table 1.

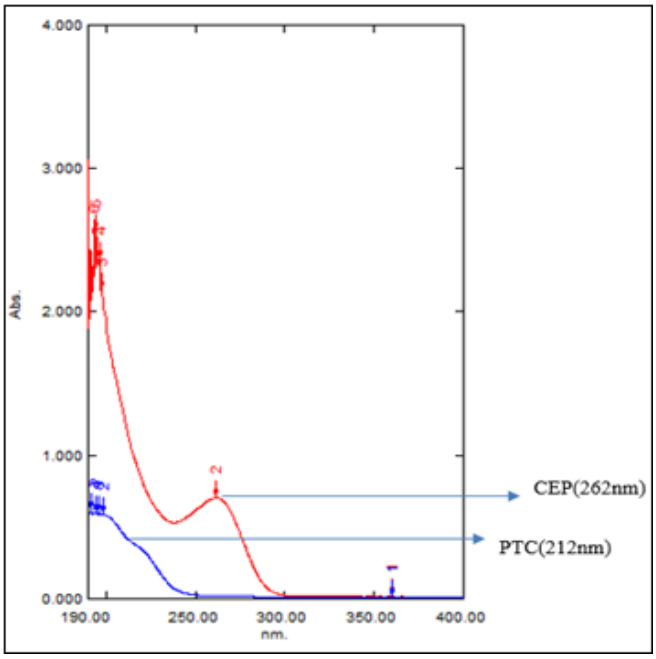

Figure 2: Absorbance graph of pure (a) cefalexin and (b) potassium clavulanate

Table 1: Assay result of the formulation.

Sample	Drug	Test Concentration (µg/ml)	*Estimated amount (mg) (mean±SD)	% purity	% RSD
Formulation (SPORIDEX CV 375)	CEP (label claim 375mg)	30	30.35±0.18	101.19	0.60
	PTC (label claim 125mg)	10	10.03±0.08	100.10	0.75

*Triplicate performance

3.3. Linearity and limits of quantification and detection

The calibration curves for CEP and PTC were linear over the concentration range of 15–75 µg/mL and 05–25 µg/mL, respectively (Figure 3). The regression equations' correlation coefficients (r) were greater than 0.997 in all cases. The minimum level at which the investigated compounds can be reliably detected (limit of detection, LOD) and quantified (limit of quantitation, LOQ) was determined experimentally. The LOD value was found to be 2.60 and 1.56 µg/mL, while LOQ was found to be 7.89 and 4.74 µg/mL for CEP and PTC respectively, Table 2.

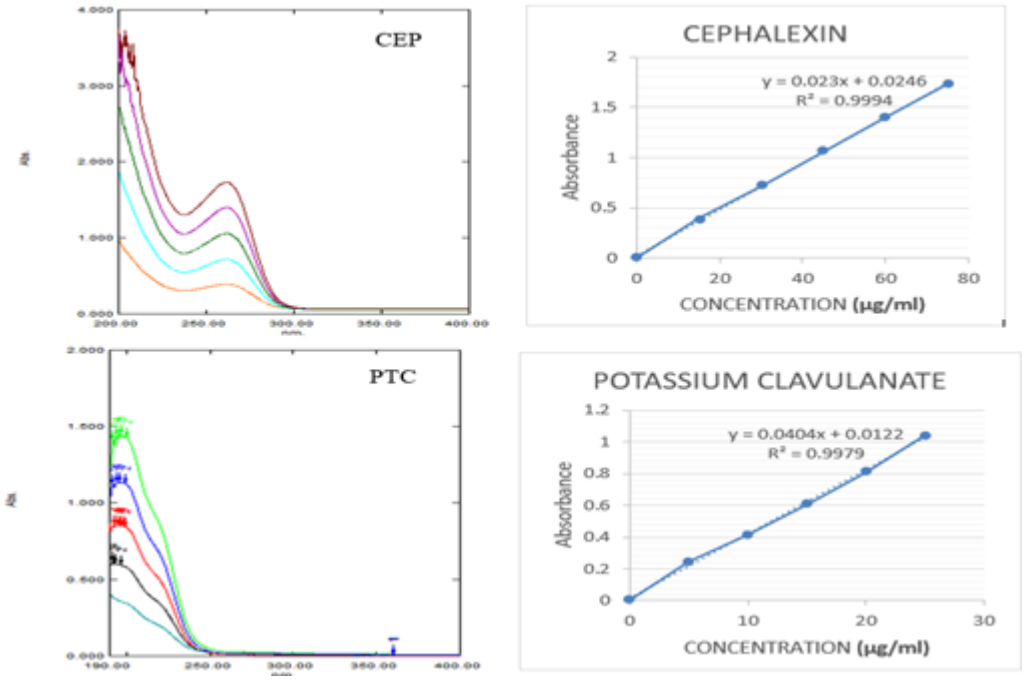

Figure 3: Calibration graph of cefalexin and potassium clavulanate

Table 2: Linearity data

CEP		PTC	
Conc.(µg/ml)	Absorbance	Conc.(µg/ml)	Absorbance
15	0.392	5	0.242
30	0.721	10	0.414
45	1.061	15	0.604
60	1.403	20	0.809
75	1.736	25	1.037

3.4. Accuracy and precision

The precision and accuracy of the method were determined by analysis of samples for the drug mixture. The specificity of the method was determined. The recovery studies were carried out three times and the percentage recovery and percentage relative standard deviation was calculated and presented in Table 3. Inter-day assay variation was evaluated by analyzing these samples in replicates of three on 3 different days, andIntra-day assay variation was evaluated by analyzing these samples in replicates of three on an identical day, Table 4. The standard deviation, relative standard deviation, and recovery of different amounts tested were determined. The accuracy of the method is indicated by the excellent recovery and therefore the precision is supported by the low standard deviation.

Table 3: Accuracy data of analysis for the determination of cefalexin and potassium clavulanate

Drug	Theoretical% target level	*Amount recovered(mg) (mean±SD)	% Recovery	% RSD
CEP (label claim 375mg)	80	54.57±0.12	101.05	0.24
	100	58.34±0.08	97.24	0.16
	120	65.86±0.08	99.8	0.14
PTC (label claim 125mg)	80	17.57±0.10	100.10	0.57
	100	20.22±0.10	101.12	0.53
	120	21.75±0.11	98.90	0.54

*Triplicate performance

Table 4: Precision data of analysis for the determination of cefalexin and potassium clavulanate

Drug	Day/ Hour	Amount taken(µg/ml)	Intra-day		Inter-day	
			* %Content	%RSD	* %Content	%RSD
CEP	1	30	100.97	0.21	99.52	0.46
	2		101.00		99.80	
	3		100.62		100.42	
PTC	1	10	99.79	0.76	100.25	0.28
	2		101.31		99.92	
	3		100.49		100.47	

*Triplicate performance

3.5 Robustness and ruggedness

The method was validated for robustness by producing minor changes to the solvent system for further dilution of stock solution from water to methanol. Ruggedness was also determined by performing an analysis of the sample solution following the recommended procedures by 3 different analysts. Table 5-6

Table 5: Robustness data of analysis for the determination of cefalexin and potassium clavulanate

Drug	Parameter altered	Amount taken(µg/ml)	*Amount recovered (mg)	% Content	% RSD
CEP	Solvent system (Methanol)	30	29.85±0.41	99.51	1.40
PTC		10	9.97±0.04	99.76	0.31

*Triplicate performance

Table 6: Ruggedness data of analysis for the determination of cefalexin and potassium clavulanate

Drug	Parameter altered	Amount taken(µg/ml)	*Amount recovered (mg)	% Content	% RSD
CEP	Analyst 1	30	30.02±0.15	100.06	0.74
	Analyst 2		29.44±0.41	98.13	
	Analyst 3		29.76±0.70	99.21	
PTC	Analyst 1	10	9.99±0.02	99.99	0.45
	Analyst 2		9.92±0.07	99.25	
	Analyst 3		9.91±0.13	99.17	

*Triplicate performance

Conclusion

Conclusively, the UV spectroscopic method described in this paper is novel-specific, sensitive, rapid, and straightforward to perform. The proposed method enables the simultaneous determination of cefalexin and potassium clavulanate. The sample recoveries from all formulations were in good agreement with their respective label claims, which suggested non-interference of formulations excipients within the estimation. Moreover, this method is economical, and simple concerning analysis time as compared to sophisticated techniques. The method provided excellent specificity and linearity with a limit of quantification of 7.89 and 4.74 µg/mL and a limit of detection of 2.60 and 7.89 µg/mL for CEP and PTC, respectively. The key advantage of this method is the wide range selection of linearity. Hence the method can be convenient for routine quality control of drugs in the combined dosage form. Analytical parameter data for the estimation of cefalexin and potassium clavulanate is given in Table 7.

Table 7: Analytical parameter data for the estimation of cefalexin and potassium clavulanate

Parameter	Cefalexin	Potassium clavulanate
Detection wavelength	262nm	212nm
Beer's Law limit	15-75(µg/ml)	5-25(µg/ml)
Regression equation	y = 0.023x + 0.0246	y = 0.0404x + 0.0122
Correlation coefficient (r^2)	0.9994	0.9979
Slope	0.023	0.0408
Intercept	0.0246	0.0122
LOD (µg/mL)	2.606 µg/mL	1.564 µg/mL
LOQ (µg/mL)	7.897 µg/mL	4.742 µg/mL

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Conflicts of interest
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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