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Research Article

DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF CEFUROXIME AXETIL AND LINEZOLID IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORM BY RP- HPLCNaveen Kumar G S^{*1} and Srinivasa U²

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Abstract:

A new sensitive, accurate, precise and validated RP-HPLC method was developed for the concurrent assessment of cefuroxime axetil and linezolid in bulk drug and pharmaceutical dosage form. The wavelength selected for quantitation was 276 nm. The method has been validated for linearity, accuracy, precision, robustness, limit of detection and limit of quantitation. Linearity was observed in the concentration range of 2-12 µg/ml for cefuroxime axetil and 6-36 µg/ml for linezolid. For RP-HPLC, the chromatographic separation was achieved by systronics C18 (250×4.6 mm) 5 µm column using phosphate buffer (pH 7): methanol (60:40 v/v) as mobile phase with flow rate 1 ml/min. The retention time of cefuroxime axetil and linezolid were found to be 3.127 min and 11.986 min, respectively. The developed method was simple, specific and economic, which can be used for simultaneous estimation of cefuroxime axetil and linezolid in tablet dosage form.

Keywords: Cefuroxime axetil, Linezolid, RP-HPLC and Validation

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INTRODUCTION:

Cefuroxime Axetil is Chemically 1-acetyloxyethyl (6R,7R)-3-(carbamoyloxymethyl)-7-[[(2Z)-2-(furan-2-yl)-2-methoxyiminoacetyl]amino]-8-oxo-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylate[1].

Cefuroxime Axetil which is a potent antibiotic agent now a day's recommended as the oral therapy for bacterial infections. It belongs to cephalosporin family which has main effect to decrease bacterial infections, by interrupting the bacterial cell wall formation [2]. Linezolid is chemically N-(((5S)-3-(3-Fluoro-4-morpholinophenyl)-2-oxo-5-oxazolidinyl) methyl) Acetamide [4]. Linezolid as a oxazolidinone reduces growth of bacteria and helps in prevention of bacterial infections [3]. Cefuroxime Axetil and Linezolid used in high bacterial infection like kidney, urinary tract, meningitis, respiratory tract infections and Pneumonia, skin infections and infections. Cefuroxime Axetil is official in IP [4], USP-NF [5] and NF [6]. Literature survey reveals that many analytical methods are reported for determination of Cefuroxime Axetil and Linezolid [7-10] individually. However, no method is reported for development and validation for simultaneous estimation of these two drugs by reverse phase RP-HPLC. Hence, the purpose of the present work was to develop and validate the RP-HPLC method for simultaneous estimation of Cefuroxime Axetil and Linezolid in combined dosage form.

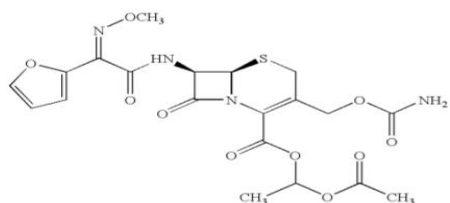


Fig 1: Structure of Cefuroxime Axetil

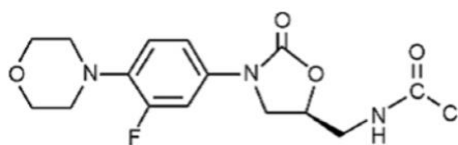


Fig 2: Structure of Linezolid

MATERIALS AND METHODS:**Instrument**

HPLC- LC100 UV- Detector, Model LC-100 Systronics.

Chemicals and Reagents

The bulk drug, Cefuroxime Axetil was obtained from Centurion Laboratories, Baroda and Linezolid was

obtained from nirlife Ltd, Ahmedabad. Fixed dose of combined dosage form of Cefuroxime Axetil 500 mg and Linezolid 600 mg were prepared in laboratory scale as pilot batch. Analytical grade methanol was procured from Merck Finechemicals (Mumbai).

Instrumentation and chromatographic conditions

Chromatographic separation was achieved by using systronics LC-100 high-performance liquid chromatography, equipped with degasser PGU-20A 5, variable wavelength programmable diode array detector UV, auto sampler SIL-20 AC HT, and column oven CTO-10 A5 VP. Pronto SILC 18, 250 × 4.6mm ID, 5 μm column using phosphate buffer (pH 7) : methanol (60:40 v/v) as mobile phase with flow rate 1 ml/min.

Preparation of mobile phase

Phosphate buffer, pH 7 was prepared by taking 50.0 ml of 0.2 M potassium dihydrogen phosphate in a 200 ml volumetric flask, to which 29.1 ml of 0.2 M sodium hydroxide was added and diluted further to the required volume with water. Six hundred millilitres of phosphate buffer pH 7 and 400 ml of methanol were mixed, sonicated for 10 min and filtered through 0.45 μm membrane filters and used as mobile phase.

Preparation of Standard Stock Solution

Stock solutions were prepared by weighing 5 mg each of Cefuroxime Axetil and Linezolid. The weighed drugs were transferred to two separate 50 ml volumetric flasks. Volumes were made up to the mark with mobile phase to obtain a solution containing 100 μg/ml of Cefuroxime Axetil and Linezolid. The HPLC analysis was performed on reversed-phase high-performance liquid chromatographic system with isocratic elution mode using a mobile phase of methanol: phosphate buffer pH 7 (60:40 v/v) on a Pronto SILC, C18 column (250×4.6 mm, 5 μm particle size) with 1 ml/min flow rate at 276 nm using UV detector.

Calibration curves for Cefuroxime Axetil and Linezolid

Tablets contain Cefuroxime Axetil and Linezolid in a ratio of 1:3. Appropriate aliquots of Cefuroxime Axetil and Linezolid stock solutions were taken in different 10 ml volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of 2-12 μg/ml and 6-36 μg/ml of Cefuroxime Axetil and Linezolid, respectively. The solutions were injected using a 20 μl fixed loop system and chromatograms were recorded. Calibration curves were constructed by plotting average peak areas versus concentrations and regression equations were computed for both the drugs (**Table 1**).

Table 1: Linear regression data for calibration curve

Parameters (units)	Cefuroxime Axetil	Linezolid
Linearity range ($\mu\text{g/ml}$)	2-12	6-36
r ²	0.9969	0.9983
Slope	45859	20292
Intercept	11946	21158

Analysis of marketed formulations

Twenty tablets were weighed, powdered, a quantity of powder equivalent to 200 mg of was transferred to 100 ml volumetric flask and dissolved using mobile phase up. The solution was filtered through 0.2 μm Nylon membrane filter paper. Ten millilitres above solution was transferred to 100 ml volumetric flask and diluted up to mark with mobile phase (100 $\mu\text{g/ml}$). The sample solution was prepared to give final concentrations of 6 $\mu\text{g/ml}$ and 18 $\mu\text{g/ml}$ for Cefuroxime Axetil and Linezolid, respectively. Twenty microlitres of the above sample solution was injected into HPLC and peak areas were measured under optimized chromatographic conditions.

Method Validation

The method of analysis was validated as per the recommendations of ICH^[11] for the parameters like accuracy, linearity, precision, detection limit, quantitation limit and robustness. The accuracy of the method was determined by calculating percentage recovery of Cefuroxime Axetil and Linezolid. For both the drugs, recovery studies were carried out by applying the method to drug sample to which known amount of Cefuroxime Axetil and Linezolid had been added (standard addition method). At each level of

the amount six determinations were performed and the results obtained were compared.

Intraday and Interday precision

Intraday and interday precision study of Cefuroxime Axetil and Linezolid was carried out by estimating the corresponding responses 3 times on the same day and on 3 different days for the concentration of 6 and 18 $\mu\text{g/ml}$ of Cefuroxime Axetil and Linezolid, respectively.

Limit of detection and limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated using following formula: $\text{LOD}=3.3(\text{SD})/\text{S}$ and $\text{LOQ}=10(\text{SD})/\text{S}$, where SD is standard deviation of response (peak area) and S is the average of the slope of the calibration curve.

System suitability tests

System suitability tests are an integral part of chromatographic method, which are used to verify reproducibility of any chromatographic system. To ascertain its effectiveness, certain system suitability test parameters were checked by repetitively injecting the drug solution at the concentration level 6 and 18 $\mu\text{g/ml}$ for Cefuroxime Axetil and Linezolid, respectively to check the reproducibility of the system and the results are shown in **Table 2**.

Table 2: Summary of validation and SST parameters

Parameters (units)	Cefuroxime Axetil	Linezolid
Linearity range ($\mu\text{g/ml}$)	2-12	6-36
Correlation coefficient	0.9969	0.9983
LOD ($\mu\text{g/ml}$)	0.365	1.897
LOQ ($\mu\text{g/ml}$)	1.083	5.817
Recovery (%)	97.62-101.86	100.09-101.02
Precision (%RSD)		
Interday (n=3)	0.588-0.831	0.581-0.784
Intraday (n=3)	1.323-1.846	0.942-1.468
Robustness	Robust	Robust
Retention time \pm %SD (min)	3.413 \pm 0.017	11.983 \pm 0.108
Resolution	-	19.592
Theoretical plates	2612	5443
Tailing factor (asymmetry factor)	1.073	0.923

LOD: limit of detection, LOQ: limit of quantitation, SD: standard deviation, RSD: relative standard deviation

Robustness

For robustness evaluation of HPLC method, a few parameters like flow rate and percentage of methanol in the mobile phase were deliberately changed. One factor was changed at one time to estimate the effect. Each factor selected was changed at three levels (-1, 0, +1) with respect to optimized parameters. Robustness of the method was done at the concentration level 6 and 18 $\mu\text{g/ml}$ for Cefuroxime Axetil and Linezolid, respectively.

RESULTS AND DISCUSSION:

The mobile phase consisting of methanol: phosphate buffer pH 7 (60:40, v/v), at 1 ml/min flow rate was optimised which gave two sharp, well-resolved peaks with minimum tailing factor for Cefuroxime Axetil and Linezolid (**fig. 3**). The retention times for Cefuroxime Axetil and Linezolid were 3.127 min and 11.986 min, respectively. UV overlain spectra of both Cefuroxime Axetil and Linezolid showed that both

drugs absorbed appreciably at 276 nm, so this wavelength was selected as the detection wavelength. The calibration curve for Cefuroxime Axetil and Linezolid was found to be linear over the range of 2-12 $\mu\text{g/ml}$ and 6-36 $\mu\text{g/ml}$, respectively. The data of regression analysis of the calibration curves is shown in **Table 1**. The proposed method was successfully applied to the determination of Cefuroxime Axetil and Linezolid in their combined tablet dosage form. The results for the combination were comparable with the corresponding labeled amounts (**fig. 3**).

The LOD for Cefuroxime Axetil and Linezolid were found to be 0.365 and 1.897 $\mu\text{g/ml}$, respectively, while LOQ were 1.083 and 5.817 $\mu\text{g/ml}$, respectively. The results for validation and system suitability test parameters are summarized in **Table 2**. Results for robustness evaluation for both the drugs are presented in **Table 2**. Insignificant differences in peak areas and less variability in retention times were observed.

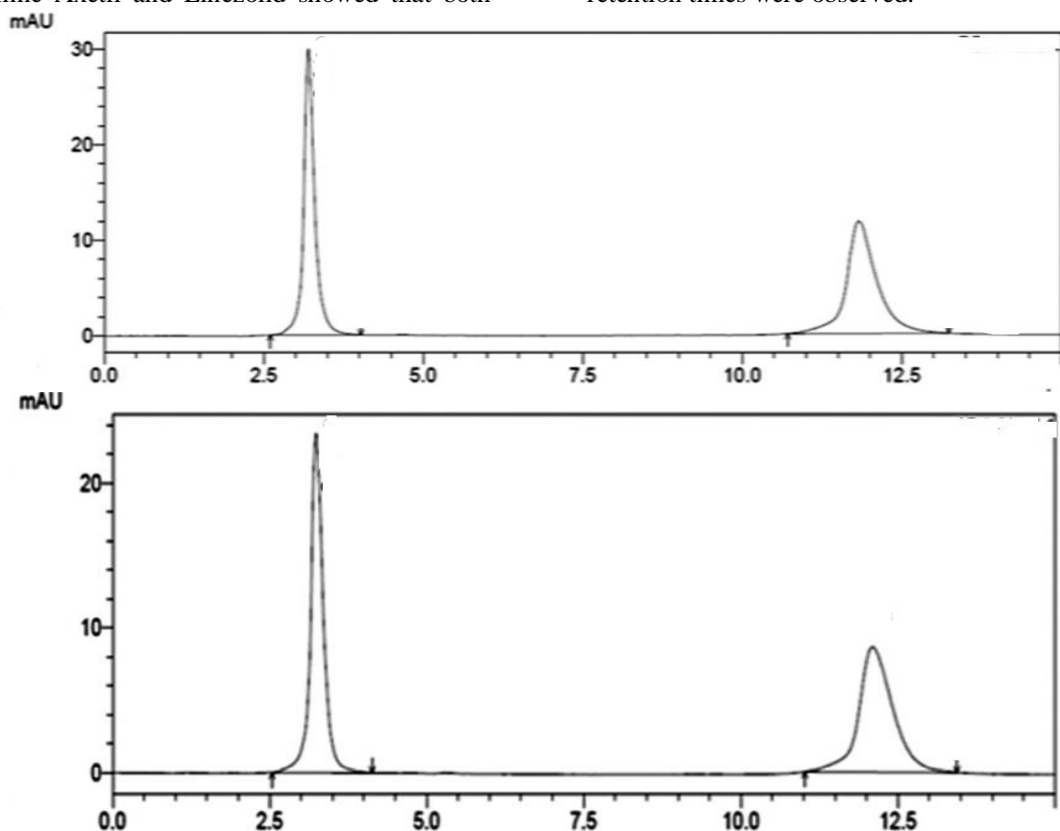


Fig 3: Chromatogram of mixture Cefuroxime Axetil and Linezolid (A) and chromatogram of market formulation of Cefuroxime Axetil and Linezolid (B).

CONCLUSION:

Proposed RP-HPLC method is specific, accurate and precise for the simultaneous determination of cefuroxime axetil and Linezolid from pharmaceutical dosage form. The described method is suitable for routine analysis and quality control of pharmaceutical preparations containing these drugs either as such or in combination.

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