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Research Article

Development and Validation RP-HPLC Method For Simultaneous Estimation of Cefuroxime Axetil and Linezolid In Combined Dosage Form

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ABSTRACT

A simple, specific and accurate Reverse Phase High Performance Liquid Chromatography Method was developed for the simultaneous determination of Cefuroxime Axetil and Linezolid in combined dosage form. The using Kromasil C-8 (250×4.6 mm, 5 µm) column in Isocratic mode, with Mobile Phase Acetonitrile : Phosphate buffer, pH=4.0 (60:40 % v/v) (pH 4.0 adjusted with Orthophosphoric acid). The Flow Rate was 0.9 ml/min and effluents were monitored at 268 nm. The Retention Time of were found to be Linezolid and Cefuroxime Axetil 6.860 min and 8.840 min respectively. The Linearity for Cefuroxime Axetil and Linezolid were found to be 2.5-12.5 µg/ml and 3-15 µg/ml respectively. The Recoveries of Cefuroxime Axetil and Linezolid were found to be 101.66 – 101.90% and 98.14 - 101.15 % respectively. The proposed method was validated and successfully applied for the estimation of Cefuroxime Axetil and Linezolid in combined dosage form.

Key-words: Cefuroxime Axetil, Linezolid, RP-HPLC.

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INTRODUCTION:

Cefuroxime, like the penicillins, is a beta-lactam antibiotic. By binding to specific penicillin-binding proteins (pbps) located inside the bacterial cell wall, it inhibits the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins; it is possible that cefuroxime interferes with an autolysin inhibitor[2].

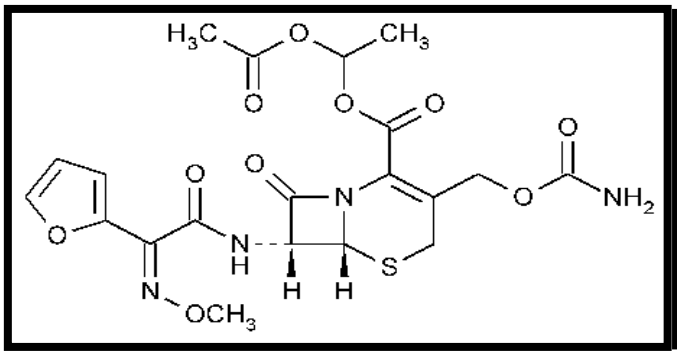

Figure 1: Structure of Cefuroxime Axetil

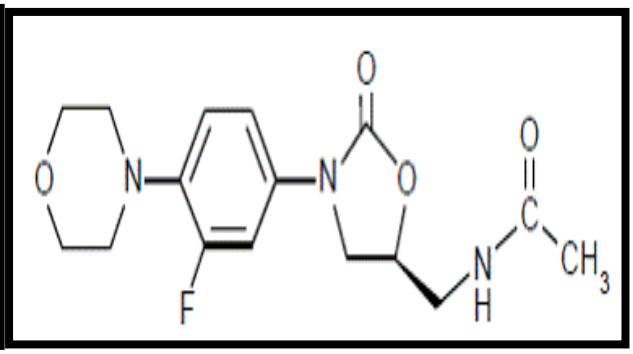

Figure 2: Structure of Linezolid

Linezolid is a synthetic antibacterial agent of the oxazolidinone class of antibiotics. It has in vitro activity against aerobic gram positive bacteria, certain gram negative bacteria and anaerobic microorganisms. It selectively inhibits bacterial protein synthesis through binding to sites on the bacterial ribosome and prevents the formation of a functional 70s-initiation complex. Specifically, Linezolid binds to a site on the bacterial 23s ribosomal RNA of the 50s subunit and prevents the formation of a functional 70s initiation complex, which is an essential component of the bacterial translation process Linezolid is also a reversible, nonselective inhibitor of monoamine oxidase. Therefore, Linezolid has the potential for interaction with adrenergic and serotonergic agents[3].

Cefuroxime Axetil and Linezolid used in high bacterial infection like kidney, urinary tract, meningitis, respiratory tract infections and Pneumonia, skin infections and infections.

Based on literature survey, it was found that drugs have been analysed by few methods like UV Spectrophotometry Method, RP-HPLC, HPTLC, LC-MS etc in bulk and pharmaceutical dosage form and human plasma individually and combination with other drug but there is no reported method for simultaneous estimation of Cefuroxime Axetil and Linezolid in synthetic mixture.

INSTRUMENTAL:

- HPLC (Shimadzu)
  Model: SPD-20A, LC-20AD
  Column: Kromasil C-8 (250×4.6 mm, 5 µm) column
  Detector: U.V Detector
  Software: Spinchrome
- Hamilton Syringe
- Analytical Weighing Balance (Wensar DAB-220)
- Sonicator (Equitron)
- Digital pH Meter (Systronic)
- High Vacuum Pump (Parag engineering)

CHEMICALS AND MATERIALS:

- Acetonitrile – Avantor Performance Material India Ltd. (HPLC grade)
- Methanol – Finar Ahmedabad. (HPLC grade)
- Water – Astron Chemical India. (HPLC grade)
- OPA (75% Ortho Phosphoric Acid) - AR Grade, Astron Chemical India.
- Cefuroxime Axitil was obtain from Centurion Laboratory, Vadodara
- Linezolid was obtain from Nir life, Ahmedabad

SELECTION OF DETECTION WAVELENGTH:

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. At 268 nm both drug give good peak height and shape. So, 268 nm was selected for simultaneous estimation of Cefuroxime Axetil And Linezolid in Combined Dosage Form.

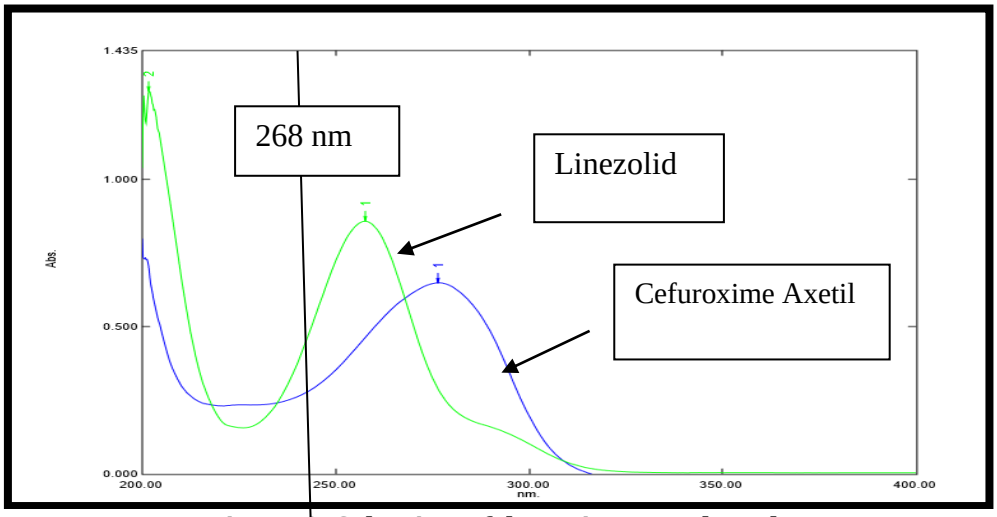

Figure 3: Selection of detection wavelength

SELECTION OF MOBILE PHASE:

Various mobile phases, such as Methanol: Water, Acetonitrile: Water, ACN: Water : Methanol in different proportion was tried. The combination of Acetonitrile: Phosphate buffer, pH=4.0 (60:40 % v/v) pH 4.0 adjusted with Orthophosphoric acid provided optimum polarity for proper migration, separation and resolution of Cefuroxime Axetil and Linezolid. Under these conditions, the eluted peaks were well defined and resolved. Absorbance is measured at wavelength 268 nm. Flow rate is 0.9 ml/min and Run time is 15 min.

CHROMATOGRAPHIC CONDITION:

Column: Kromasil C-8 (240 mm × 4.6 mm, 5 µm)
Mobile phase: ACN : potassium dihydrogen Ortho phosphate buffer (pH 4.0 adjusts with Ortho phosphoric acid) (60 / 40 %/v/v)
Flow rate: 0.9 ml/min
Run time: 15 min
Detection wavelength: 268 nm
Detector: U.V Detector
Injection volume: 20 µl

PREPARATION OF STANDARD STOCK SOLUTION:

Cefuroxime Axetil (100 µg/ml):

Accurately weighed Cefuroxime Axetil (10 mg) was transferred to a 100 ml volumetric flask, and diluted upto the mark with mobile phase to obtain a standard stock solution (100 µg/ml).

Linezolid (100µg/ml):

Accurately weighed Linezolid (10 mg) was transferred to a 100 ml volumetric flask, and diluted upto the mark with mobile phase to obtain a standard stock solution (100 µg/ml).

ASSAY:

Preparation and analysis of Tablet:

For analysis of Cefuroxime Axetil and Linezolid in tablet, twenty tablets (Oratil LZ) which contain 500 mg of Cefuroxime Axetil and 600 mg of Linezolid was taken .Powder weight equivalent to 10 mg of drug was dissolved in a 100 ml volumetric flask and made up to half mark with ACN. It was sonicated till drug dissolve and was made up to mark with methanol (**Stock solution)**. The Stock solution contains 100 µg/ml Cefuroxime Axetil of and 120 µg/ml of Linezolid.

From above stock solution pipette out 0.75 ml and transferred in to a 10 ml volumetric flask and the volume was adjusted up to the mark with Mobile phase to make final concentration of Cefuroxime Axetil 7.5 µg/ml and Linezolid 9 µg/ml.

ANALYTICAL METHOD VALIDATION:

The developed method was validated with respect to linearity, accuracy, precision, limit of detection and limit of quantification in accordance with the ICH Q2 (R1) guideline.

- **SPECIFICITY:**

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

- **LINEARITY & RANGE:**
  - The linearity of Cefuroxime Axetil and Linezolid was found to be in the range of 2.5-12.5 µg/ml and 3-15 µg/ml respectively. Plot the calibration curve of area vs concentration (µg/ml). Linearity of both the drugs was checked in term of slope, intercept and correlation coefficient.

Preparation of calibration curve Aliquots of stock solution of Cefuroxime Axetil (100 µg/ml) 0.25, 0.5, 0.75, 1.0 and 1.25 ml and Linezolid (100 µg/ml) 0.3, 0.6, 0.9, 1.2 and 1.5 ml were pipette out in same five different 10 ml volumetric flasks and further diluted with mobile phase to obtain the concentration of about 2, 4, 6, 8 and 10 µg/ml for Cefuroxime Axetil and 4, 8, 12, 16, and 20 for Linezolid. 20 µl of each solution were injected into HPLC system and analyzed. Calibration curve was obtained by plotting respective peak area Vs Concentration in µg/ml and regression equation was obtained.

- **PRECISION:**

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: Intermediate (Intraday) precision, Reproducibility (Interday precision), Repeatability.

1) Intraday Precision: Solutions containing 5.0, 7.5, 10 µg/ml of Cefuroxime Axetil and 6, 9, 12 µg/ml of were analyzed three times on the sameday and %R.S.D was calculated.
2) Interday Precision: Solutions containing 5.0, 7.5, 10 µg/ml of Cefuroxime Axetil and 6, 9, 12 µg/ml of Linezolid were analyzed on three different successive days and %R.S.D was calculated.
3) Repeatability: Solutions containing 7.5 µg/ml of Cefuroxime Axetil and 9.0 µg/ml of Linezolid were analyzed for six times and %R.S.D. was calculated.

- **LIMIT OF DETECTION (LOD):**

Limit of detection can be calculated using following equation as per ICH guidelines.
LOD = 3.3 x (σ/ S)
Where, σ = standard deviation of the Y intercept of calibration curve
S = Mean slope of the corresponding calibration curve.

- **LIMIT OF QUANTIFICATION (LOQ):**

Limit of quantification can be calculated using following equation as per ICH guidelines.
LOQ = 10 x (σ/ S)
Where, σ = standard deviation of the Y intercept of calibration curve
S = Mean slope of the corresponding calibration curve.

- **ACCURACY:**
  - The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Recovery studies were carried out by addition of standard drug to the sample at three different concentration levels 50%, 100%, 150%. This performance was done in triplicate. The amount of Cefuroxime Axetil and Linezolid were calculated at each level and % recoveries were calculated by measuring the peak area and fitting the values in equation.

➢ **ROBUSTNESS:**

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.
It should show the reliability of an analysis with respect to deliberate variation in method parameter.
In case of liquid chromatography, examples of typical variations are:

- Influence of variations of pH in mobile phase;
- Influence of variations in mobile phase composition;
- Different columns (different lots and/or suppliers)
- Temperature
- Flow rate

➢ **SYSTEM SUITABILITY TEST:**

A system suitability test is an integral part of liquid chromatography. They are used to verify that resolution and reproducibility of chromatography system are adequate for the analysis to be done. The test includes the Resolution, Column efficiency, Tailing factor and Theoretical plates.

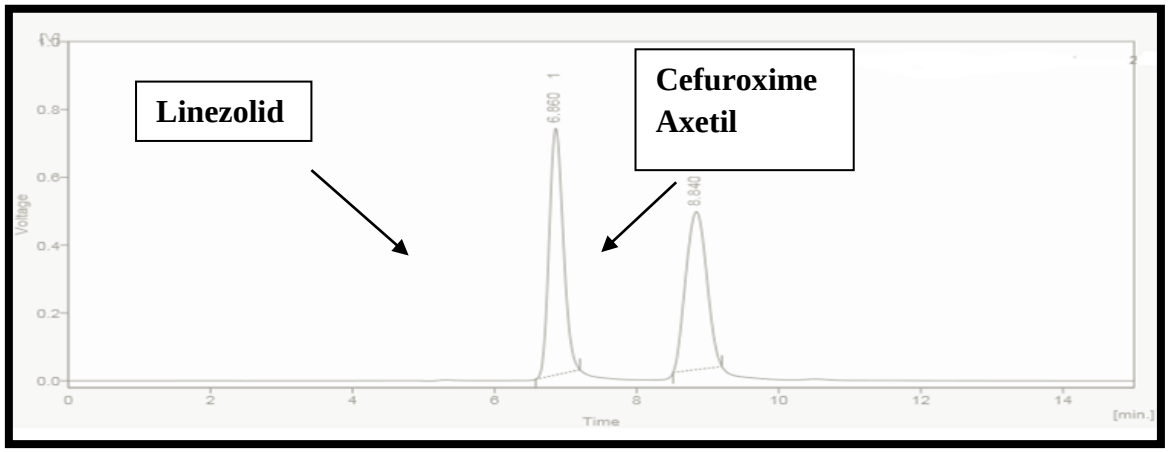

Figure 3: Chromatogram of Cefuroxime Axetil (2.5 µg/ml) and Linezolid (3.0 µg/ml) in ACN: potassium dihydrogen Ortho phosphate Buffer (pH 4.0) (60: 40 %v/v)

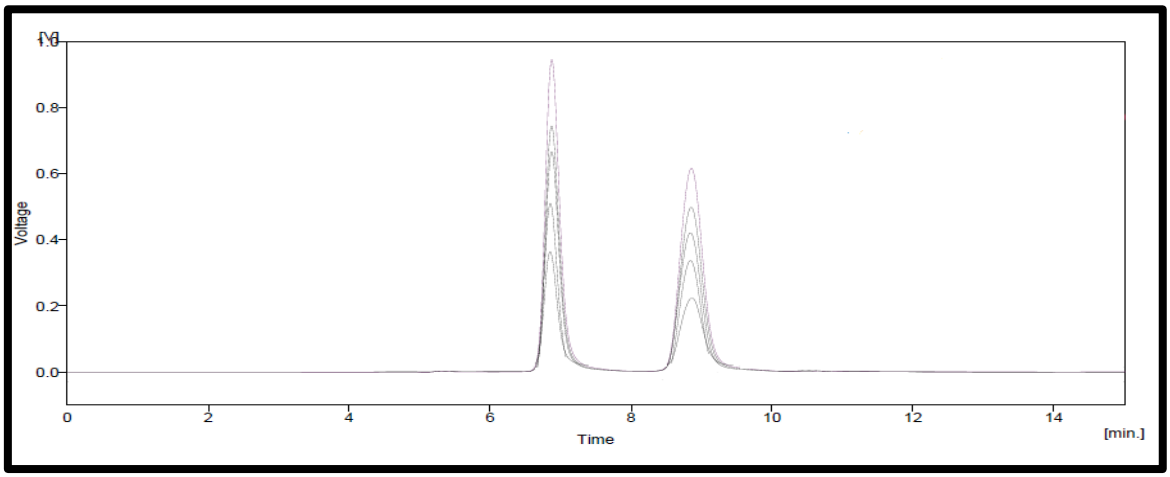

Figure 4: Overlay chromatogram of Cefuroxime Axetil (2.5-12.5 µg/ml) and Linezolid (3-15 µg/ml)

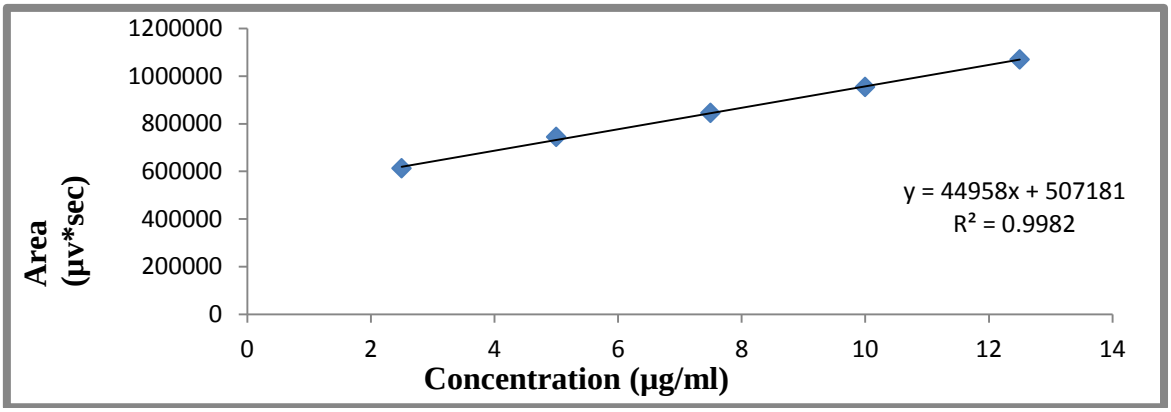

Figure 5: Calibration curve of Cefuroxime Axetil (2.5-12.5 µg/ml)

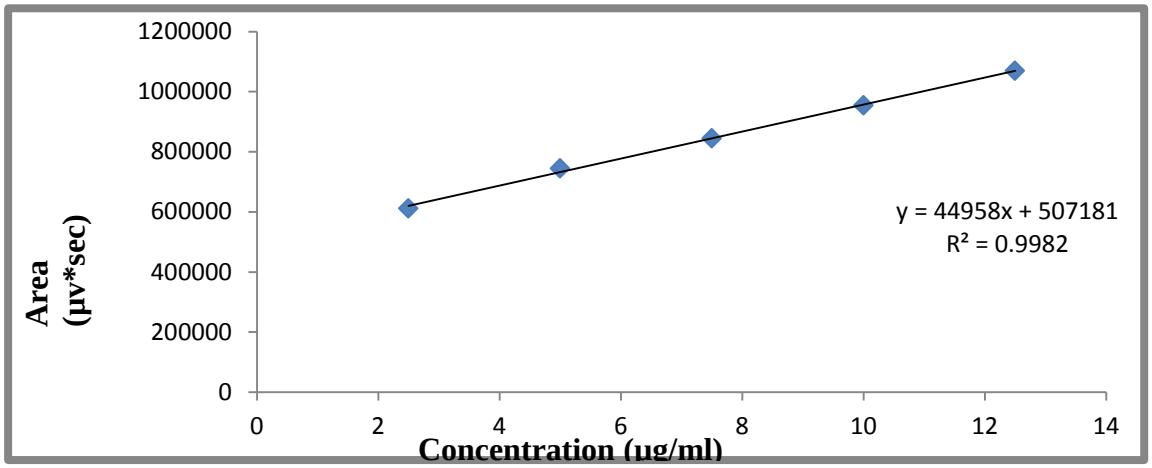

Figure 6: Calibration curve of Linezolid(3-15 µg/ml)

Table 1: Linearity Data Cefuroxime Axetil (2.5-12.5 µg/ml) and Linezolid (3-15 µg/ml)

CEFUROXIME AXETIL			LINEZOLID		
Conc. (µg/ml)	**Mean Peak Area (µV.s) ± S.D. (n=6)**	**% RSD**	**Conc. (µg/ml)**	**Mean Peak Area (µV.s) ± S.D. (n=6)**	**% RSD**
2.5	611453.7 ± 5680.37	0.9289	3.0	439468.7 ± 4380.57	0.9967
5.0	744081.7± 6860.51	0.9220	6.0	6893345.5 ± 5409.05	0.7846
7.5	844107± 6957.011	0.8241	9.0	872430.8 ± 6801.25	0.7795
10	953441± 7375.63	0.7735	12	1076661 ± 8166.10	0.7584
12.5	106875.0± 8164.96	0.7639	15	1285552± 8169.80	0.6351

Table 2: Precision Study for Cefuroxime Axetil

CEFUROXIME AXETIL		
INTRADAY PRECISION		
Conc. (µg/ml)	**Mean Peak Area (µV.s) ± S. D. (n=3)**	**% R.S.D**
5	744081.7 ± 6860.51	0.9220
7.5	844107 ± 6957.01	0.8241
10	953441 ± 7375.63	0.7735
INTERDAY PRECISION		
Conc. (µg/ml)	**Mean Peak Area (µV.s) ± S. D. (n=3)**	**% R.S.D**
5	743748.3 ± 7284.68	0.9794
7.5	844273.7 ± 7082.84	0.8389
10	953607.7 ± 7332.57	0.7689
REPEATABILITY		
Conc. (µg/ml)	**Mean Peak Area (µV.s) ± S. D. (n=6)**	**% R.S.D**
7.5	844271 ± 7082.84	0.8338

Table 2: Precision Study for Linezolid

LINEZOLID		
INTRADAY PRECISION		
Conc. (µg/ml)	**Mean Peak Area (µV.s) ± S. D. (n=3)**	**% R.S.D**
5	744081.7 ± 6860.51	0.9220
7.5	844107 ± 6957.01	0.8241
10	953441 ± 7375.63	0.7735
INTERDAY PRECISION		
Conc. (µg/ml)	**Mean Peak Area (µV.s) ± S. D. (n=3)**	**% R.S.D**
6	689501.2 ± 5635.09	0.8172
9	872597.5 ± 6891.11	0.7897
12	1076661 ± 8166.10	0.7584
REPEATABILITY		
Conc. (µg/ml)	**Mean Peak Area (µV.s) ± S. D. (n=6)**	**% R.S.D**
9	689334.5 ± 5409.05	0.7846

Table 4: Recovery Study Data

Name of Drug	% Level of Recovery	Test Amt. (µg/ml)	Amt. of Drug Spiked (µg/ml)	Total Amount Recovered (µg/ml)	% Recovery ± R.S.D. (n=3)
Cefuroxime Axetil	50	5	2.5	7.58	101.14 ± 577.35
	100	5	5	9.8	98.17 ± 5773.5
	150	5	7.5	12.39	99.12 ± 1154.70
Linezolid	50	6	3	9.12	101.38 ± 707.10
	100	6	6	12.1	100.9 ± 4242.64
	150	6	9	15.16	101.26 ± 391.69

Table 5: LOD and LOQ Data

Drug Name	CEFUROXIME AXETIL	LINEZOLID
Standard Deviation	335.012	651.99
Slope	44891	69685.67
LOD (µg/ml)	0.024	0.030
LOQ (µg/ml)	0.246	0.308

Table 6: Analysis of Cefuroxime Axetil and Linezolid in combined dosage form

Drug Name	Amount Drug Taken (µg/ml)	Amount Found (µg/ml) ± S.D. (n=3)	% Assay ± R.S.D. (n=3)
Cefuroxime Axetil	5	5.01 ± 576.19	0.1652 ± 100
Linezolid	6	6.02 ± 576.19	0.1652 ± 100.01

Table 7: Robustness Data of CEFUROXIME AXETIL and LINEZOLID

Condition	Variation	CEFUROXIME AXETIL %Assay ± R.S.D (n=3)	LINEZOLID %Assay ± R.S.D (n=3)
Flow Rate (1 ml ± 0.1 ml/ min)	0.8 ml/min	99.6 ± 6658.32	99.66 ± 4785.13
	0.9 ml/min	99.2 ± 4358.89	99.16 ± 4496.32
	1.0 ml/min	101.6 ± 1000	99.5 ± 4495.50
Detection Wavelength (257 nm ± 1 nm)	267 nm	101.8 ± 1527.52	98.46 ± 4241.22
	268 nm	99.2 ± 4358.89	99.82 ± 4496.32
	269 nm	99.6 ± 7234.17	98.46 ± 4967.36
Change in Mobile Phase Composition (%v/v/v)	59 : 41	101.8 ± 1154.70	99.83 ± 4026.27
	60 : 40	99.16 ± 4358.89	99.16 ± 4358.89
	61 : 39	99.6 ± 7000	99.66 ± 4967.56

Table 8: Summary of Validation Parameters

Sr. No.	Parameters	LINEZOLID	CEFUROXIME AXETIL
1	Linearity Range (µg/ml)	3-15	2.5-12.5
2	Regression Line Equation (y = mx + c)	y = 68650x + 25684	y = 44958x + 50718
3	Correlation Coefficient (R^2)	0.998	0.998
4	Intraday Precision (%RSD, n=3)	0.7846	0.8389
5	Interday Precision (% RSD, n=3)	0.7584 – 0.7846	0.7735 – 0.9220
6	Repeatability (% RSD, n=6)	0.7584 – 0.8172	0.7683 – 0.9794
7	LOD (µg/ml)	98.14 - 101.15	101.66 – 101.90
8	LOQ (µg/ml)	0.0308	0.0246
9	% Recovery Study (n=3)	0.308	0.246

RESULT:

A Reverse Phase Column proposed as a suitable method for the determination of Cefuroxime Axetil and Linezolid in combined dosage form.. The Chromatographic condition were optimized by changing the Mobile Phase Composition. Different ratios were experimented to optimize the Mobile Phase. Finally, Acetonitrile : Phosphate buffer, pH=4.0 (60:40 % v/v) (pH 4.0 adjusted with Orthophosphoric acid). The Flow Rate was 0.9 ml/min and effluents were monitored at 268 nm. The Retention Time of were found to be Linezolid and Cefuroxime Axetil 6.860 min and 8.840min respectively. as Mobile Phase which shows good resolution of Cefuroxime Axetil and Linezolid peak. The wavelength of detection selected was 268 nm, as the drug shows optimized absorbance at this wavelength. By our proposed method Retention Time of Linezolid and Cefuroxime Axetil 6.860 min and 8.840 min respectively and none of the Impurities were interfering in its assay.

DISCUSSIONS:

The statistical analysis of data and the drug recovery data showed that the method was simple, rapid, economical, sensitive, precise and accurate. It can thereby easily adopt for routine quality control analysis. The results of this analysis confirmed that the proposed method was suitable for determination of drug in Cefuroxime Axetil and Linezolid with virtually no interference of additives. Hence the proposed method can be successfully applied in estimation of Cefuroxime Axetil and Linezolid in combined dosage form.

CONCLUSION:

A simple, rapid, sensitive, accurate and precise RP-HPLC Method has been developed and validated for routine analysis of Cefuroxime Axetil and Linezolid in combined dosage form.The RP-HPLC method is suitable for simultaneous estimation of Cefuroxime Axetil and Linezolid in combined dosage form without interference of each other. The developed method was successfully applied in Synthetic Mixture. The proposed Method can be utilized for the routine analysis of Cefuroxime Axetil and Linezolid in combined dosage form.

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