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Spectrophotometric Method Development and Validation for Estimation of Linezolid in Tablet Dosage Form

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Linezolid is oxazolidinone antibiotic. It inhibits bacterial protein synthesis by acting at an early step and a site different from that of other Antimicrobial agents. The aim of the present research work was to develop accurate and rapid Differential spectrophotometric (Method A) and Area under Curve (Method B) to determine Linezolid content in pharmaceutical formulation. Differential spectrophotometric method is simplest and rapid technique for altering the spectral properties of analyte with the adjustment of the pH by means of aqueous solutions of acid, alkali or buffers. Differential spectrophotometry based on the principle of ionization of functional groups and pH of the solution. The difference absorption spectra of equimolar solution of Linezolid in 0.1M hydrochloric acid in sample cell relative to 0.1M sodium hydroxide in reference cell were taken. Area under curve method involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths. Absorption maxima was found 258.27 nm (Method A) and area under curve was measured between253-263nm.The drug followed a linear relationship in the range of 4-20µg/ml; while the correlation coefficient was 0.999(Method A) and 0.9993 (Method B). The recovery was 100.01% ±0.17 (Method A) and 99.95 ± 0.33, the relative standard deviation for repeatability, intraday and interday was found to be less than 2%. These methods are found suitable for day to day analysis of linezolid in tablet dosage form.

Keywords: Linezolid (LNZ), Differential spectrophotometry, Area under curve (AUC), UV Spectrophotometer

Introduction

Linezolid (LNZ)N-{[(5S)-3-[3-fluoro-4-(morpholin-4-yl)phenyl]-2-oxo-1,3-oxazolidin-5-yl] methyl} acetamideis oxazolidinone antibiotic. It inhibits bacterial protein synthesis by acting at an early step and a site different from that of other AMAs. It binds to 23s fraction of the 50s ribosome and interfere with formation of the ternary N-formylmethionine-t-RNA-70s initiation complex. Binding of linezolid distorts the t-RNA binding site overlapping both 50s and 30s ribosomal subunits and stops protein synthesis before it starts (Tripathi, 2008). The empirical formula is $C_{16}H_{20}FN_3O_4$. Its molecular weight is 337.346 g/mol. A literature review revealed that analytical simple spectrophotometry (Prasanthi et al., 2012), HPLC (Prasanti et al., 2012), HPTLC (Agrawal et al., 2003) and capillary electrophoresis (Michalska et al., 2008) have been established for the estimation of LNZ in tablet dosageform; but there was no mention of a method based on differential spectrophotometric and AUC determination of LNZ. The selectivity and accuracy of spectrophotometric analysis of samples containing

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absorbing interferents may be markedly improved by the technique of difference spectrophotometry (Beckett et al.,2007). The essential feature of a difference spectrophotometric assay is that the measured value is the difference absorbance (ΔA) between two equimolar solutions of the analyte in different chemical forms which exhibit different spectral characteristics (Beckett et al., 2007). AUC method based on selection of the wavelength range; where drug shows maximum absorbance. So in this study simple, rapid, precise, and accurate spectrophotometric methods have been developed for the determination of Linezolid in pharmaceutical dosage form and validated as per the ICH guideline.

Materials and Methods

Reagents and Materials

Active Pharmaceutical Ingredient (API) of Linezolid supplied by ALEMBIC Pharmaceutical Limited, Vadodara, India). The pharmaceutical dosage form used in study was ALZOLID (label claim, LNZ 600 mg) manufactured by (ALEMBIC Pharmaceutical Limited, India). Methanol AR grade was purchased from (Finar Chemicals Pvt. Ltd, Ahmedabad, India), Sodium hydroxide (NaOH) pallets and Hydrochloric acid (36%) (Molychem, Mumbai, India) were used.

Instrumentation

UV-Visible double beam spectrophotometer (SHIMADZU 1700) with 10 mm matched quartz cells was used. All weighing were done on precision balance (REPTECH), and Ultrasonicsteri-cleaner was used (CYBERLAB) to degas the solutions.

Preparation of standard solutions

Stock solution of 1000 µg/ml of LNZ was prepared in methanol. From this solution 100µg/ml solution was prepared by dilution with methanol.

Preparation of 0.1N NaOH solution

Take 0.4g of NaOH pallets and dissolve it in first 60ml of distilled water and then dilute upto 100ml with same.

Preparation of 0.1N HCLsolution

Take 0.85ml of concentrated hydrochloric acid(36%) and dissolve it in 100ml of distilled water.

Development of the methods

Method A: Differential Spectrophotometric Method

Selection of analytical wavelength

From stock solution of 100 µg/ml, take 1ml-1ml into two volumetric flasks and then make up the volume in one volumetric flask with 0.1N NaOH and in another volumetric flask with 0.1N HCL upto 10ml.Then spectra was scanned within range of 200-400nm by keeping acidic form (i.e. LNZ in 0.1N HCL) in reference cell and basic form (i.e. LNZ in 0.1N NaOH) in sample cell using 0.1N HCL in reference cell and 0.1N NaOH in sample cell as blank and then find out λ max (Figure 1).

Method B: Area Under Curve (AUC) Method

Selection of analytical wavelength range

For the selection of analytical wavelength range, 6µg/ml solution of LNZ was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200 nm to 400 nm. From the spectra of drug, area under the curve in the range of 253.0-263.0 nm

(Figure 2) was selected for the analysis. The calibration curve was prepared in the concentration range of 4-12µg/ml at their respective AUC range. By using the calibration curve, the concentration of the sample solution can be determined.

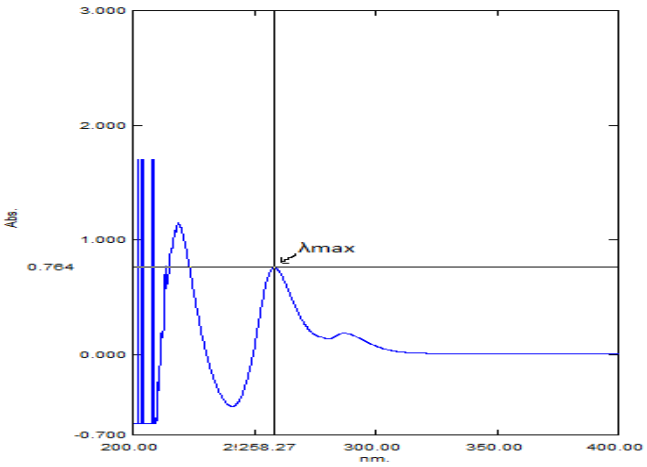

Fig. 1. λmax of Linezolid by Method A

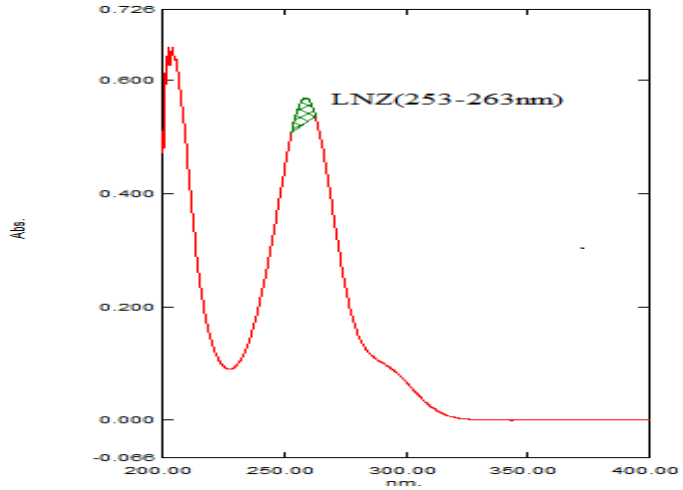

Fig. 2. Area under curve of linezolid (Method B)

Validation Parameters

The proposed method is validated according to the International Conference on Harmonization(ICH) guidelines**.**

Linearity

Aliquotes of 0.6ml, 0.9ml, 1.2ml, 1.5ml, 1.8ml were taken from 100µg/ml stock solution and transfer into 10ml volumetric flask and then volume was adjusted to the mark with 0.1N NaOH and 0.1N HCL. It gives 6, 9, 12, 15 and 18 µg/ml solutions. Then calibration curve was obtained by plotting absorbance →concentrations. Regression analysis was done for the slope, intercept and correlation coefficient values (Figure 3).

Repeatability

The precision of the methods was assessed by repeated scanning (n = 6) and measurement of the absorbance of solutions of LNZ (12µg/ml) for Method A and LNZ (8µg/ml) for Method B without changing the parameters for the respective methods. The repeatability was expressed in terms of relative standard deviation (% RSD).

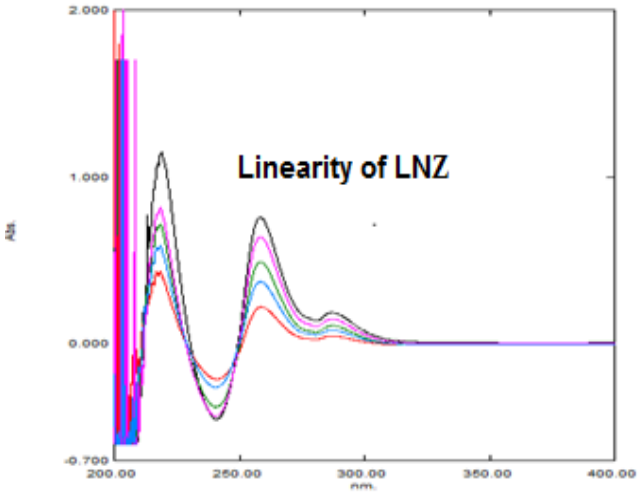

FIG. 3. Linearity curve for differential spectroscopy of Linezolid

Intermediate precision

The intra-day and inter-day precision of the proposed method was done by analyzing the corresponding responses three times on the same day and on three different days over a period of one week for three different concentrations of standard solutions of LNZ (9, 12 and 15µg/ml) for Method A and LNZ (6, 8 and 10 µg/ml) for Method B. The results were reported in terms of relative standard deviation (% RSD).

Accuracy (% recovery)

The accuracy of the method was performed by calculating %recovery of LNZ by the standard addition method. Known amounts of standard solutions of LNZ were added at 80, 100 and 120% levels to pre-quantified sample solutions of LNZ (12µg/ml) for Method A and LNZ (8 µg/ml) for Method B. At each level of the amount three determinations were performed. The amount of LNZ was estimated by applying obtained values to relative standard deviation (% RSD).

Sensitivity

Limit of detection (LOD) and limit of quantitation (LOQ) were calculated as 3.3 σ/S and 10σ/S respectively; where σ the standard deviation and S is the slop.

Analysis of Tablet Formulation

For analysis of commercial formulation; twenty tablets were weighed, average weight determined and crushed into finely powder. An accurately weighed quantity of powder equivalent to 100 mg of LNZ was transferred into 100 mL volumetric flask containing 50 mL methanol, gentle shaking was carried out for 5 min and ultrasonicated for 5 min. Then volume was adjusted to mark with same solvent and filtered through Whatmann filter paper (no.41) if necessary. From above solution 1.0 mL was transferred to 10 mL volumetric flask, volume was adjusted to the mark with same solvent. Now for Method A, take 1.5ml

from this solution into two volumetric flasks and then make up the volume in one volumetric flask with 0.1N NaOH and in another volumetric flask with 0.1N HCL upto 10ml. Then spectra was scanned within range of 200-400nm by keeping acidic form (i.e. LNZ in 0.1N HCL) in reference cell and basic form (i.e. LNZ in 0.1N NaOH) in sample cell using 0.1N HCL in reference cell and 0.1N NaOH in sample cell as blank and then absorbance note at 258nm (λ max). For Method B, take 1.0ml from stock solution of tablet in volumetric flask and then make up the volume with methanol. The analysis was repeated for three times. The absorbance was putted in regression equation of calibration curve and amount of LNZ was found. There was no interference from the excipients commonly present in the tablets. The low % R.S.D. value indicated the suitability of this method for routine analysis of LNZ in tablet dosage form.

Results and Discussion

Method A is differential spectrophotometric method. In which absorption maxima (λ max) at 258.27 nm (Figure 1). The calibration curve was linear in concentration range of 4-18µg/ml. Method B is the area under curve method. In this method the simple UV spectrum of Linezolid in methanol was obtained and area between two selected wavelengths measured. Area measured between 253nm and 263nm (Figure 2). The calibration curve was linear in concentration range of 4 – 18µg/ml. The proposed methods were found to be simple, sensitive, rapid, accurate, precise and economic for the routine analysis of linezolid in pharmaceutical formulations. Accuracy was determined by calculating the recovery, and the mean was determined (Table 1). Precision was calculated as repeatability, intraday and intermediate variation in term of % RSD for Linezolid. LOD values for Linezolid were found to be 0.1and 0.08µg/ml for method A and B respectively. LOQ values for Linezolid were found to be and 0.5 and 0.1µg/ml for method A and B respectively indicates sensitivity of the proposed methods. The methods were successfully used to determine the amounts of Linezolid present in tablets. The results obtained are in good agreement with the corresponding labeled amount (Table 2). Characteristic parameters and summary of validation parameters for both methods are given in Table 3. By observing the validation parameters, the methods were found to be sensitive, accurate and precise. Hence the methods can be employed for the routine analysis of Linezolid in tablet formulations.

Table 1: Recovery Data for Proposed Methods

Method	Level	Amount of sample taken(µg/ml)	Amount of std. added (µg/ml)	Total amount (µg/ml)	Statistical Analysis		
					%Recovery	SD[a]	% RSD[b]
A	80%	12	9.6	21.6	100.16	0.57	0.570912
	100%	12	12	24	100.05	0.71	0.709645
	120%	12	14.4	26.4	99.83	0.79	0.791345
B	80%	8	6.4	14.4	99.96	0.07	0.069965
	100%	8	8	16	100.05	0.14	0.139874
	120%	8	9.6	17.6	99.83	0.78	0.782537

Method A is the Differential spectrophotometric method, Method B is the Area under curve method, SD[a]- standard deviation, RSD[b]-relative standard deviation

Conclusion

The proposed spectrophotometric methods provide simple, specific, precise, accurate and reproducible quantitative analysis for determination of LNZ in tablet dosage form. The methods were validated as per ICH guidelines in terms of linearity, accuracy, precision,

limits of detection (LOD) and quantification (LOQ) and ruggedness. The method can be used for routine analysis of LNZ in dosage form.

Table 2: Results of Analysis of Tablet Formulation

Method	Formulation	Label claim	Estimated amount (mg) ± SD	%purity ± SD[a] (n=3)
A	ALZOLID	600mg	598.5 ± 0.82	99.37 ± 0.68
B	ALZOLID	600mg	595.6 ± 1.48	99.41 ± 0.27

n is number of replicate and SD[a] - standard deviation.

Table 3: Regression Analysis Data and Summary of Validation Parameters for the Proposed Methods

Parameter	Method A	Method B
Beer's-Lambert's range (µg/ml)	4-18	4-18
Regression equation (y =mx + c)	0.044x-0.041	0.048x-0.011
Correlation coefficient (R^2)	0.999	0.9993
Slope (m)	0.044	0.048
Intercept (c)	-0.041	-0.011
Precision (SD[a] ± %RSD[b]) Repeatability(n=6) Intraday(n=3) Intermediate(n=3)	 0.0092±1.90 0.0075±1.51 0.0077±1.16	 0.0026±0.66 0.0023±0.78 0.0055±1.39
LOD[c](µg/ml)	0.18	0.08
LOQ[d](µg/ml)	0.54	0.24

[a]SD = Standard deviation. [b]RSD = Relative standard deviation. [c]LOD = Limit of detection.
[d]LOQ = Limit of quantification. n is number of determinations

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References

Agrawal H, Mahadik K, Paradkar A, Kaul N. 2003. Stability indicating HPTLC determination of linezolid as bulk drug and in pharmaceutical dosage form. Drug DevInd Pharm. 29: 1119-1126.

Beckett, A.H., Stenlake, J.B. 2007. Practical pharmaceutical chemistry, 4th ed. – part II. Noida (UP): CBS Publishers.

Goti PP, Savsani JJ, Patel PB. 2012. Spectrophotometric method development and validation for estimation of α-lipoic acid in tablet dosage form. Int J Pharm Pharm Sci. 4: 519-522.

ICH, Q2 (R1). 2005. Validation of Analytical Procedure: Text and Methodology, International Conference on Harmonization.

ICH, Q2A. 1994. Harmonised Tripartite Guideline, Test On Validation Of Analytical Procedures, IFPMA, In: Proceedings Of The International Conference On Harmonization, Geneva.

Indian pharmacopoeia. 2010. vol-II. Government of India, Ministry of Health and Family Welfare, Ghaziabad: Indian PharmacopoeialCommission : 959-960.

Lories I. 2003. Stability-indicating methods for the determination of linezolid in the presence of its alkaline-induced degradation products. Talanta. 60: 945-953.

Michalska K, Pajchel G, Tyski S. 2008. Determination of linezolid and its achiral impurities using sweeping preconcentration by micellar capillary electrophoresis.J Pharm Biomed Anal.48: 321-330.

Prasanti JK, Sundar SB. 2012. A validated RP-HPLC method for the determination of linezolid in pharmaceutical dosage forms. Int J Pharm Bio Sci. 3: 44 – 51.

Prashanthi P, Mateti A, Vanitha P, Thimmaraju MK, Raghunandan N. 2012. Development and validation of UV spectrophotometric method for the Estimation of Linezolid in bulk and pharmaceutical formulation. International Journal of Natural Products Research. 2: 57-60.

Sali MS, Barhate AL, Patil VD. 2010. Development and validation of area under curve and first derivative spectrophotometric methods for Ropinirole in tablet dosage form. Der Pharma Chemica. 2: 12-16.

Tripathi, K.D. 2008 Essential of medical pharmacology, 6thed. New Delhi (India): Jaypee Publishers.