High Performance Thin Layer Chromatographic Method for Estimation of Linezolid in Tablets

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A simple and sensitive high performance thin layer chromatography (HPTLC) method has been developed for the quantitative estimation of linezolid in its single component tablet formulations (600 mg). Linezolid was chromatographed on Silica Gel 60 F_{254} TLC plate using methanol: benzene (2:8 v/v) as mobile phase. Linezolid

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showed Rf value 0.45 ± 0.03 and scanned at 258 nm using a Camag TLC scanner 3. The method was validated in terms of linearity (200-1400 ng/spot), precision (intra-day variation, 0.75 to 1.26% and inter-day variation, 1.2 to 2.8%), accuracy (97.8 to 100.4%) and specificity. The limit of detection and limit of quantification for linezolid were found to be 16.7 ng/spot and 50.7 ng/spot, respectively. The developed method was successfully used for the assay of linezolid tablet formulations. The method was found to be simple, sensitive, specific, accurate and precise and can be used for the routine quality control testing of linezolid marketed formulations.

Key words: Linezolid, HPTLC, oxazolidinone, validation, recovery

Chemically, linezolid is N-[(5S)-3-[3-Fluoro-4-(4-morpholinyl)phenyl]-2-oxo-5-oxazolidinyl]methyl] acetamide[1]. Linezolid is the first drug of the class oxazolidinone antibacterial approved in US, UK and Canada for treatment of gram-positive infection[2]. Linezolid is not official in any pharmacopoeia; hence no official method is available for its estimation. Literature survey reveals spectrophotometric[3-4], HPLC[5-7], capillary electrophoresis[8] and HPTLC[9] method for the estimation of linezolid in biological fluids as well as in pharmaceutical formulations. The present study describes simple, sensitive, accurate, precise and specific HPTLC method for the estimation of linezolid in tablet formulations.

Linezolid working standard was procured as a gift sample from Alembic Pharmaceuticals Ltd., Vadodara, India. Silica Gel 60 F_{254} TLC plates (10×10 cm, layer thickness 0.2 mm, E. Merck, Germany) were used as stationary phase. Two single component film coated tablet formulations of linezolid (600 mg) (formulation A-Linospan tablets, manufactured by Cipla Pharmaceutical Ltd., Daman, India and formulation B-Linox tablets, manufactured by Glenmark Pharmaceuticals Ltd., Mumbai, India) were purchased from a local pharmacy. Methanol and benzene (AR, Ranbaxy Ltd., New Delhi, India) were used for mobile phase preparation and as solvents.

A Camag HPTLC system (Switzerland) comprising of Camag Linomat V semiautomatic sample applicator, Camag TLC Scanner 3, Camag twin-through chamber (10×10 cm), Camag winCATS software, Hamilton syringe (100 µl), Sartorius CP224S analytical balance, Sonicator (Frontline FS-4, Mumbai, India) were used in the study.

Linezolid (10 mg) was weighed accurately and transferred to a 10 ml volumetric flask. It was dissolved in and diluted to mark with methanol. One millilitre of above solution was further diluted to 10 ml with methanol to obtain the final concentration 100 µg/ml of linezolid. Twenty tablets (each containing 600 mg linezolid) were weighed and finely powdered. The powder equivalent to linezolid (10 mg) was weighed accurately and dissolved in 5 ml of methanol. The solution was sonicated for 10 min and then was filtered through Whatman filter paper No. 41. The residue was washed thoroughly with methanol. The filtrate and washing were combined in a 10 ml volumetric flask and diluted to mark with methanol. The solution (1 ml) was further diluted to 10 ml to have concentration of linezolid equivalent to 100 µg/ml.

The chromatographic estimations were performed using following condition; stationary phase, precoated Silica Gel 60 F_{254} aluminum sheets (10×10 cm) (pre-washed with methanol and dried in air); mobile phase, methanol:benzene (2:8 v/v); chamber saturation time, 30 min; temperature, 28±4°, migration distance, 80 mm; wavelength of detection, 258 nm; slit dimensions, 4×0.1 mm; scanning speed, 5 mm/s. Following spotting parameter were used - band width, 4 mm; space between two bands, 4 mm and spraying rate, 10 s/µl.

Six microlitres of standard solution of linezolid (100 µg/ml) was applied on TLC plate under nitrogen stream using semiautomatic spotter. The plate was dried in air and developed up to 80 mm at constant temperature using mixture of methanol: benzene (2:8 v/v) as mobile phase in Camag twin-trough chamber previously saturated with mobile phase for 30 min. The plate was removed from the chamber and dried in air. Photometric measurements were performed at 258 nm in absorbance/reflectance mode with Camag TLC Scanner 3 using winCATS software incorporating the track optimization option.

Aliquots of 2, 4, 6, 8, 10, 12 and 14 µl of standard linezolid solution (100 µg/ml) were spotted on precoated TLC plate using semiautomatic spotter under nitrogen stream. The TLC plate was developed

and photometrically analyzed as described under chromatographic separation. The calibration curve was prepared by plotting peak area versus concentration (ng/spot) corresponding to each spot.

The method was validated in terms of linearity, accuracy, inter-day and intra-day precision, specificity, repeatability of measurement of peak area as well as repeatability of sample application. The limit of detection and limit of quantification were also determined.

Eight microlitres of sample solution from formulation A and B (100 µg/ml) were applied separately on TLC plate, developed and scanned as described in chromatographic separation. The amount of linezolid present in the sample solution was determined by fitting area values of peak corresponding to linezolid into the equation of line representing calibration curve of linezolid.

Linezolid is soluble in methanol; therefore methanol was selected as solvent. The formulation was dissolved in methanol with sonication for 10 min to assure complete release of drug from the formulation matrix. The mixture of methanol:benzene (2:8 v/v) could resolve linezolid spot with better peak shape. Combination of methanol and benzene offered optimum migration (Rf = 0.45±0.03) and resolution of linezolid from other components of formulation matrix. Even saturation of TLC chamber with mobile phase for 30 min assured better reproducibility and better resolution.

Linearity range for linezolid was found in the concentration range of 200 to 1400 ng/spot, with a correlation coefficient of 0.9945. The average linear regression equation was represented as Y= 3.635X+2071.5, where X= concentration of linezolid in ng/spot and Y= peak area. The limit of detection and limit of quantification for linezolid were found to be 16.7 ng/spot and 50.7 ng/spot, respectively.

The intra-day precision (RSD) was calculated for standard linezolid (200-1400 ng/spot) for 5 times on the same day. The inter-day precision (RSD) was calculated for standard linezolid (200-1400 ng/spot) for 5 times over a period of one week. The intra-day and inter-day coefficients of variation were found to be in the range of 0.75-1.26% and 1.5-2.8%, respectively. These values indicate that the method is precise.

Precision of the instrument was checked by repeated scanning of the same spot (800 ng/spot) of linezolid seven times without changing position of the plate and %CV for measurement of peak area was found to be 0.34%. Repeatability of sample application was checked by spotting 8 µl of linezolid standard solution seven times on TLC plate (n=7) and %CV for peak area was found to be 2.7%. Both the %CV, for measurement of peak area and sample application (less than 1% and 3%, respectively) ensures proper functioning of HPTLC system.

Accuracy of the method was evaluated by calculating recovery of linezolid by standard addition method at 3 different levels of the calibration curve (n=5). The % recovery was found to be 97.8 to 100.4 ensuring that the method is accurate.

The method was found to be specific for linezolid. The specificity of the method was ascertained by analyzing standard drug and the samples. The spot for linezolid in the sample was confirmed by comparing the Rf value and spectra of the spot with that of standard. None of the formulation excipients were interferes in the quantification of linezolid at this Rf value.

Different validation parameters for the proposed HPTLC method for determining linezolid content are summarized in Table 1. This method was applied to determine the content of linezolid in two different market samples of single component linezolid tablets. The content and percentage of linezolid in two different market samples were found to be 597.2 mg, 99.5±1.26% and 585.9 mg, 97.7±1.65%, respectively (n=5). The results are in agreement with the labeled value of linezolid in tablet dosage forms (Table 2). The results indicate that the proposed HPTLC method was found to be simple, sensitive, specific, precise

TABLE 1: SUMMARY OF VALIDATION PARAMETERS OF LINEZOLID

Parameters	Results
Linearity range (ng/spot)	200-1400
Correlation co-efficient	0.9945
Precision (%CV)	
Intra-day (n=5)	0.75-1.26
Inter-day (n=5)	1.5-2.8
Repeatability of peak area (n=7)	0.34
Repeatability of sample application (n=7)	2.7
%Recovery (n=5)	97.8-100.4
Limit of detection (ng/spot)	16.7
Limit of quantification (ng/spot)	50.7
Specificity	Specific

TABLE 2: ANALYSIS OF LINEZOLID TABLET DOSAGE FORMS

Formulation	Labeled claim (mg)	Amount found* (mg)	% Assay*±SD
Tablet 1	600	597.2	99.5±1.26
Tablet 2	600	585.9	97.7±1.65

*Each value is an average of five determinations. SD is standard deviation

and accurate for the estimation of linezolid in tablet formulations.

The authors are thankful to Alembic Pharmaceuticals Ltd, Vadodara, India for providing the gift sample of linezolid and S. K. Patel College of Pharmaceutical Education and Research for providing all the facilities to carry out the work.

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Accepted 26 July 2007
Revised 12 February 2007
Received 28 March 2006
Indian J. Pharm. Sci., 2007, 69 (4): 571-574