

Determination of the New Oxazolidinone Antibiotic Linezolid in Presence of Both its Alkaline and Oxidative Degradation Products Using Validated Stability-Indicating Chromatographic Methods as Per ICH Guidelines

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Abstract: Two specific, sensitive and precise stability-indicating chromatographic methods have been developed, optimized and validated for Linezolid (LIN) determination in presence of its alkaline- (ALK) and oxidative- (OXD) degradation products. The first method was based on thin layer chromatography (TLC) combined with densitometric determination of the separated bands at 254 nm. The separation was achieved using silica gel 60 F254 TLC plates and chloroform: ethanol (5:2, v/v) as a developing system. Good correlation was obtained between the integrated peak area ratios of the studied drug and its corresponding concentrations in the specified range. The second method was based on high performance liquid chromatography (HPLC) with ultraviolet detection, by which the proposed components were separated on a reversed phase C18 analytical column using isocratic elution system with 'acetonitrile (ACN) : 0.15% triethylamine (TEA); pH=3.5' (30 : 70, v/v). The flow rate was maintained at 1.0 mL·min⁻¹ and the detection wavelength was 254 nm. Different parameters affecting the suggested methods were optimized for maximum separation of the cited components. System suitability parameters of the developed methods were also tested. The suggested methods were validated in compliance with the ICH guidelines and were successfully applied for determination of LIN in its commercial tablets. Both methods were also statistically compared to a reported HPLC method with no significant difference in performance.

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1. Introduction

Antimicrobial resistance has become a significant nosocomial problem and contributed to the increasing importance of community-acquired infections. This phenomenon required the development and employment of new antimicrobial agents, effective against resistant strains. One approach for overcoming this resistance is the discovery and development of agents with a new mechanism of action. The oxazolidinones are a new class of antimicrobials with good activity against Gram positive bacteria; they possess a unique mechanism of bacterial protein synthesis inhibition[1]. Linezolid; chemically designated as LIN, is considered as the first available oxazolidinone antibacterial agent. It shows strong activity against Gram positive pathogens, including multidrug-resistant organisms such as methicillin-resistant *Staphylococcus aureus*, multidrug-resistant *Streptococcus pneumoniae*, vancomycin-intermediated *Staphylococcus aureus* and vancomycin-resistant *Enterococcus spp.* strains. LIN is currently the only antibacterial agent which can be administered orally (as well as intravenously) with

strong activity against MRSA. It may be particularly useful as an alternative to vancomycin, in patients whose renal function is impaired, in cases of patients with poor or lack of intravenous access and in patients who require outpatient therapy, or who do not tolerate glycopeptides[2].

Literature survey reveals some LC methods for the determination of LIN in tablets and in biological fluids [3-12]. Chiral LC method was also reported in the literature[13]. Three methods determined LIN in the presence of its alkaline-induced degradation products using TLC densitometry, first derivate and first derivate of ratio spectra methods[14]. Studies using capillary electrophoresis to determine LIN solution for infusion and elucidate the separation mechanism of LIN enantiomers were published [15-16]. Its electrochemical characterization was also reported [17].

With the intent of improving the quality of the active pharmaceutical ingredient (API) and its formulation, there is an increasing need of separation, identification, quantification, and characterization of the most probable and possible degradation products

generated under the various ICH guidelines for forced degradation[18]. These guidelines require the stress testing to be conducted to elucidate the inherent stability characteristics of the active substance. An ideal stability-indicating method is one that quantifies the drug and also resolves its degradation products. The proposed chromatographic methods were validated as per ICH guidelines[19].

The aim of the present work is to develop accurate, specific, reproducible, and stability-indicating methods for the simultaneous determination of LIN in presence of both its alkaline and oxidative degradation products for assessment of the drug stability in its dosage form. The unique feature and the novelty of the proposed chromatographic methods is that it is the first time that LIN was determined simultaneously in presence of these two degradation products together, Counter for; other reported methods which can resolve LIN from only ONE of its degradation products. This important feature of the adopted methods can resolve the intact drug in presence of more than one degradation product in the same sample, the problem which can't be solved by the already reported chromatographic methods developed in presence of only one type of degradation products. Other important features of the proposed stability-indicating chromatographic methods; are the higher sensitivity and the wider range of quantitation of LIN, which offer high flexibility in the determination of the intact drug at very low and high concentrations.

2. Experimental

2.1. Instruments

For TLC, The samples were spotted in the form of bands of width 6mm with a Camag 100 microlitre sample syringe; (Hamilton, Bonaduz, Switzerland) on precoated silica gel aluminium Plate 60 F254, (20 cm×10 cm) with 250 μ m thickness; (E. Merck, Darmstadt, Germany) using a Camag Linomat IV; (Camag, Muttenz, Switzerland). The mobile phase consisted of chloroform: ethanol (5:2, by volume). Linear ascending development was carried out in 20 cm×10 cm twin trough Automatic Developing Chamber ADC 2 chamber; (Camag, Muttenz, Switzerland). Densitometric scanning was performed on Camag TLC scanner III; (Camag, Muttenz, Switzerland) in the reflectance-absorbance mode at 254 nm for all measurements and operated by winCATS software version 3.15. The source of radiation utilized was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm. Concentrations of the chromatographed compounds were determined from the intensity of diffusely reflected light. Evaluation was via peak area ratios with second order polynomial regression.

The HPLC system used is LaChrom Elite® (VWR-Hitachi International GmbH, Darmstadt, Germany), which comprised L-2130 model pump, equipped with L-2400 model UV detector and a 20- μ L volume injection loop. LaChrom Elite® HPLC system was controlled by EZChrom Elite Software Chromatography Data System, version 3.3.1 SP1. A Phenomenex C18 column (250 X 4.6 mm, 5 μ m i.d.) was used as the stationary phase. The mobile phase consisted of 'ACN: 0.15% TEA; pH=3.5' (30: 70, v/v). The flow rate was set to 1.0 mL·min⁻¹ and UV detection was carried out at 254 nm. The mobile phase and samples were filtered using 0.45 μ m membrane filter. Mobile phase was degassed by ultrasonic vibrations prior to use. All determinations were performed at ambient temperature. The samples were injected with a 100- μ L Hamilton analytical syringe. IR spectra were recorded on Perkin Elmer 1600 USA Spectrometer, using KBr discs and values were represented in cm^{-1} . Electron impact mass spectrometric spectrum was carried out using direct inlet unit (DI-50) in the Shimadzu QP-5050 GC-MS.

2.2. Chemicals and Solvents

All chemicals used throughout the work were of analytical grade and solvents were of spectroscopic and HPLC grade: ACN (Riedel-de Haen, Seelze, Germany), Methanol (Riedel-de Haen, Seelze, Germany), ortho-phosphoric acid 85 % (Merck, Darmstadt, Germany), Ethanol 96 % (Merck, Darmstadt, Germany), Chloroform (Merck, Darmstadt, Germany), TEA (Fluka, Neu-Ulm, German); 0.15% aqueous solution, Hydrochloric acid 35.4 % (Merck, Darmstadt, Germany); 3N and 5N aqueous solutions, Sodium hydroxide (Merck, Darmstadt, Germany); 2N and 5N aqueous solutions, Hydrogen peroxide 30 % (Fluka, Neu-Ulm, Germany); 3% aqueous solution.

2.3. Samples

Pure Standard

Linezolid (with certified purity of 99.50%) was kindly supplied by Al-Debeiky Pharmaceutical Company, Cairo, Egypt.

Pharmaceutical preparation

Averozolid® tablets (Batch No. 1104128) labeled to contain 600 mg of LIN per tablet and manufactured by Al-Debeiky Pharmaceutical Company for Averroes Pharma, were purchased from the local market.

Degraded samples

Three degraded solutions "acid (ACD), alkaline (ALK) and oxidative (OXD)" of LIN, having concentrations of (1.0 mg mL⁻¹) were prepared by dissolving 100 mg of the drug in 10mL of methanol then mixing with thirty milliliters of "3 N HCl, 2 N NaOH and 3% H₂O₂", respectively; heating in thermostatic water bath at 80°C for a period of 24 hrs,

12 hrs and 24 hrs, respectively. Cooling, neutralizing with 5 N NaOH and 5 N HCl for ACD and ALK, respectively and then the volume was completed with methanol for all the degraded solutions. These stock solutions were used for TLC. While, Further dilution was done from each degraded solution using the mobile phase [ACN: 0.15% TEA; pH=3.5 (30: 70, v/v)] to get concentration of ($100 \mu\text{g mL}^{-1}$) which was used as working solution for HPLC. Complete degradation was assessed by the disappearance of the intact peak in HPLC. The obtained degradation products were subjected to IR and MS analyses for subsequent structure elucidation.

2.4. Solutions

For TLC, stock solution of LIN (1.0 mg mL^{-1}) was prepared in methanol. While for HPLC, further dilution was done using the mobile phase [ACN: 0.15% TEA; pH=3.5 (30: 70, v/v)] to get concentration of ($100 \mu\text{g mL}^{-1}$) which was used as working solution.

2.5. Laboratory-prepared mixtures

For TLC, solutions containing different ratios of drug and its degradation products in methanol were prepared from their respective stock solutions. For HPLC, the solutions were prepared from the working solutions and diluted with the mobile phase.

3. Procedure

3.1. Construction of TLC Calibration Curve

The standard solutions were prepared by dilution of LIN stock solution (1.0 mg mL^{-1}) with methanol to reach a concentration range of 50–800 $\mu\text{g mL}^{-1}$. Ten microlitres from each standard solution were spotted in triplicate on the plate to obtain final concentration $0.5\text{--}8 \mu\text{g band}^{-1}$. The plate was developed on previously described mobile phase to a distance of about 8 cm. The chromatogram was scanned at 254 nm. The average peak area ratios obtained for each concentration of LIN to that of external standard ($2 \mu\text{g band}^{-1}$) were plotted versus their corresponding concentrations, to obtain the calibration graph and the regression equation was then computed.

3.2. Construction of HPLC Calibration Curve

The standard solutions were prepared by dilution of LIN working solution ($100 \mu\text{g mL}^{-1}$) with the mobile phase to reach a concentration range of 5–80 $\mu\text{g mL}^{-1}$. Twenty microlitres by triplicate were injected for each concentration and chromatographed under HPLC conditions described above. The peak area ratios obtained for each concentration of LIN to that of external standard ($20 \mu\text{g mL}^{-1}$) were plotted against the corresponding concentrations to obtain the calibration graph and the regression equation was computed.

3.3. Assay of Laboratory-Prepared Mixtures

The peak areas of the laboratory-prepared mixtures were scanned and processed as described above for each of the proposed TLC or HPLC methods, respectively. The concentration of LIN in each mixture was calculated using the specified regression equations.

3.4. Application to Pharmaceutical Preparation

Ten tablets of Averozolid® were finely powdered. A portion of the powdered tablets equivalent to 600 mg of LIN was transferred into 100-mL volumetric flask, sonicated for 20 min with 50 mL methanol, then the volume was completed with the same solvent and filtered to prepare stock solution, having concentration 6 mg mL^{-1} . For TLC, Aliquots of 0.25, 0.5 and 1.0 mL were transferred from the prepared solution to 10-mL volumetric flasks and diluted with methanol; then ten microlitres from each dilution were applied onto TLC plates in triplicate, whereas for HPLC analysis, aliquot of 1.0 mL was transferred from the prepared solution to 10-mL volumetric flask and diluted with HPLC mobile phase; then from this solution, second dilution was done by transferring aliquots of 0.25, 0.5 and 1.0 mL to 10-mL volumetric flasks and the volume was completed with the mobile phase; then twenty microlitres from each final dilution were injected in triplicate. The general procedures described above for each method were followed to determine the concentration of LIN in its pharmaceutical preparation.

3.5. Application of standard addition technique

To check the validity of the proposed chromatographic methods, the standard addition technique was applied. Three portions of the previously powdered tablets, each claimed to contain 600 mg of LIN were accurately weighed and mixed with 300, 600 and 900 mg of the pure standard LIN, separately. Each spiked sample was transferred to 100 mL volumetric flask, sonicated for 20 minutes with 50 mL methanol then the volume was adjusted with the same solvent and filtered, to obtain three spiked solutions of concentrations (9, 12 and 15 mg mL^{-1} , respectively). For TLC, 0.5 mL from each spiked sample was separately transferred to 10-mL volumetric flask and diluted with methanol; then 10 μL from each dilution were applied onto TLC plates in triplicate. Whereas for HPLC analysis, aliquot of 1.0 mL was transferred from each of the spiked solutions to 10-mL volumetric flasks and diluted with HPLC mobile phase; then each solution was further diluted by transferring aliquot of 0.5 mL to 10-mL volumetric flask and the volume was completed with the mobile phase; then 20 μL from each final dilution were injected in triplicate. The chromatographic procedures; described under 3.2., were then preceded.

4. Results and Discussion

Development of analytical methods for the determination of pharmaceuticals in presence of their degradation products without previous chemical separation is always a matter of interest.

LIN was subjected to acid, alkaline and oxidation stress testing. The drug underwent degradation under acidic and alkaline conditions with the same degradation product through hydrolysis of amide linkage as suggested by Lopes and Salgado[8]. Therefore, NaOH was used as the hydrolyzing agent according to ICH guidelines[18]. While, N-oxide of the morpholine moiety in LIN structure was suggested as the oxidative degradation product via oxidation on the basis of the studies of Satyanarayana Raju et al[20]. The Chemical structures of LIN and its suggested degradation products; ALK and OXD are illustrated in (Fig. 1).

The assignment of LIN degradation was based on comparison of IR and MS spectral data; shown in (Fig. 2 and Fig. 3), for the degradation products with that of the intact drug. The IR spectrum of LIN indicates the appearance of two main bands at 3382 and 1658 cm⁻¹ corresponding to N-H amide and C-O amide, respectively. The IR spectrum of ALK, showed the absence of C-O amide and appearance of a broad band at 3652-3250 cm⁻¹ corresponding to primary amine NH₂ group. The band in the IR spectrum of OXD observed at 1231cm⁻¹ together with adjacent absorptions at 1254 and 1301 cm⁻¹; these are assigned to the N-O stretch, because this vibration is accompanied by a large change in dipole moment and polarizability [21]. Structure elucidation using MS showed molecular ion peak at *m/z* 338 corresponding to the intact drug, while the molecular ion peak of ALK was at *m/z* 295 and that of OXD was at *m/z* 354. Thus, MS spectra ascertained the suggested structures for LIN degradation products under alkaline and oxidation conditions.

4.1. TLC method

A sensitive stability-indicating TLC method was suggested for the determination of LIN in presence of its degradation products. The method was based on the difference in the migration rates of the three components by the used developing system. Different developing systems of different composition were tested in order to obtain optimum separation. Satisfactory separation was achieved upon using chloroform: ethanol (5:2, v/v). This system was found to give compact sharp bands for the three cited components with suitable R_f values. In order to minimize band diffusion, the optimum bandwidth chosen was 6 mm. Different scanning wavelengths were tested, where scanning at 254 nm was suitable providing good sensitivity for the three components with a single plate scan. Fig. 4 shows a typical chromatogram of the three components.

4.2. HPLC method

A simple, accurate, and selective HPLC method had been investigated and validated for quantitative analysis of LIN in presence of ALK and OXD. The LC procedure was optimized with a view to develop a quantitative and stability-indicating method in a convenient time analysis and with high quality separation of the three components. The chromatographic operational conditions were selected by considering the peak resolution and retention times of the three eluted components. Parameters affecting the efficiency of the chromatographic separation had been tested and optimized in a trial to obtain the maximum separation of the cited components. Different mobile phases were tested in order to find the best conditions for separation of LIN in presence of its degradation products. The optimal composition of the mobile phase was determined to be 'ACN: 0.15% TEA; pH=3.5' (30: 70, v/v). Reasonable separation with good resolution and suitable analysis time was obtained upon using flow rate of 1 mL·min⁻¹. Under the optimum chromatographic conditions, ALK, OXD and LIN were eluted at 2.73, 3.21, and 4.52 min, respectively, as shown in Fig. 5.

4.3. Method Validation

ICH guidelines[19] for method validation were followed for validation of the suggested methods.

Linearity and Range

Under the previously described experimental conditions, linear relationships were obtained by plotting the drug concentrations against the average peak area ratios obtained for each concentration of LIN to that of external standard (2 µg band⁻¹ for TLC method and 20 µg mL⁻¹ for HPLC method). The corresponding concentration ranges, calibration equations, LOD and LOQ and other statistical parameters are listed in Table1.

Accuracy

The accuracy of the investigated methods was validated by analyzing pure samples of LIN in triplicates. The concentrations of the active drug were calculated from the regression equations. Good results are shown in Table 1.

Precision

Precision was evaluated by calculating intra- and inter-assay precision by repeating the assay of three different concentrations; in triplicate, three times in the same day and assaying the same selected concentrations on three successive days using the developed chromatographic methods and calculating the RSD%. Results in Table 1 indicate satisfactory precision of the proposed methods.

Specificity

The specificity of the adopted methods was proven by the analysis of laboratory-prepared

mixtures containing different percentages of the degradation products. For TLC method, the bands of the active drug in the prepared mixtures were confirmed by comparing their R_f values with a standard solution. While for HPLC method, R_t values of LIN peak revealed in the chromatograms obtained by running the prepared mixtures were compared with those of a standard solution. The proposed methods were found to be specific for LIN determination in presence of up to 90% of its degradation products as presented in Table 2.

System Suitability Testing

In order to validate the suggested chromatographic methods, an overall system suitability testing was done to determine if the operating systems were performing properly. Good results were obtained as shown in Tables 3 (a) and (b).

Robustness

The influence of small changes in the chromatographic conditions were studied to evaluate the robustness of the developed methods such as small changes in TLC mobile phase ratio; chloroform: ethanol (5.2: 1.8 and 4.8: 2.2, v/v) and deliberate

variations in HPLC mobile phase ratio ('ACN: 0.15% TEA; pH=3.5' (28: 72 and 32: 68, v/v) and pH value of TEA (at 3.4 and 3.6). Results presented in Table 4 indicate that the capacity of the utilized methods remain unaffected by these small deliberate variations, providing an indication for reliability of the proposed chromatographic methods during routine work.

Application to commercial tablets

The suggested methods were successfully applied for determination of LIN in Averozolid® tablets. The results shown in Table 5 are satisfactory and with good agreement with the labeled amount. Applying the standard addition technique, no interference due to excipients was observed as shown from the results in Table 5.

The results obtained by applying the proposed chromatographic methods were statistically compared with those of the reported method by Lopes and Salgado [8] used for LIN analysis. It is concluded that; with 95% confidence, there is no significant difference between them since the calculated *t* and *F* values are less than the theoretical values; as presented in Table 6.

Table 1: Results of Regression and Assay Validation Parameters of the Proposed Chromatographic Methods for Determination of LIN in presence of ALK and OXD:

Validation parameters	TLC Method	HPLC Method
Linearity Range	0.5-8 ($\mu\text{g band}^{-1}$)	5-80 ($\mu\text{g mL}^{-1}$)
Slope (X^2 coefficient)	-0.0266	---
Slope (X coefficient)	0.4533	0.0498
Intercept	0.2036	-0.0006
SE of Slope (X^2 coefficient)	0.0184	---
SE of Slope (X coefficient)	0.0120	0.0155
SE of Intercept	0.0014	0.0003
Correlation coefficient (r)	0.9998	0.9999
LOD	0.13 ($\mu\text{g band}^{-1}$)	1.35 ($\mu\text{g mL}^{-1}$)
LOQ	0.41 ($\mu\text{g band}^{-1}$)	4.11 ($\mu\text{g mL}^{-1}$)
Accuracy (Mean% \pm SD)	99.49 \pm 0.688	99.05 \pm 0.836
Precision (RSD)	<i>Intra-day</i> 0.728 <i>Inter-day</i> 1.493	0.511 1.129

Table 2: Specificity of the Proposed Stability-Indicating Chromatographic Methods

TLC Method			% Recovery* of LIN
LIN ($\mu\text{g band}^{-1}$)	ALK ($\mu\text{g band}^{-1}$)	OXD ($\mu\text{g band}^{-1}$)	
4.5	0.25	0.25	99.53
3.5	0.75	0.75	101.52
2.5	1.25	1.25	98.99
1.5	1.75	1.75	98.61
0.5	2.25	2.25	99.18
Mean% \pm SD			99.57 \pm 1.141

HPLC Method			% Recovery* of LIN
LIN ($\mu\text{g mL}^{-1}$)	ALK ($\mu\text{g mL}^{-1}$)	OXD ($\mu\text{g mL}^{-1}$)	
45.0	2.5	2.5	99.41
35.0	7.5	7.5	100.31
25.0	12.5	12.5	99.51
15.0	17.5	17.5	99.96
5.0	22.5	22.5	100.51
Mean% \pm SD			99.94 \pm 0.481

Mean of three determinations.

Table 3 (a): System Suitability Parameters for the Proposed TLC Method

Parameters		Obtained values
Retardation Factor (R_f)	ALK	0.20
	OXD	0.43
	LIN	0.88
Resolution R_s	OXD to ALK	2.42
	LIN to OXD	4.87
Tailing factor T	LIN	0.95
	ALK	1.12
Capacity Factor (K')	OXD	3.94
	LIN	9.55
	OXD to ALK	3.53
Selectivity (α)	LIN to OXD	2.42

Table 3 (b): System Suitability Parameters of the Proposed HPLC Method

Parameters		Obtained values
Retention time (R_t , min)	ALK	2.73
	OXD	3.21
	LIN	4.52
Resolution (R_s)	OXD to ALK	2.19
	LIN to OXD	4.71
Tailing factor (T)	LIN	1.15
Asymmetry Factor	LIN	1.02
Capacity Factor (K')	ALK	4.00
	OXD	5.44
	LIN	8.50
Selectivity (α)	OXD to ALK	1.36
	LIN to OXD	1.56
Injection repeatability*	LIN	0.795
Theoretical plates (N)	LIN	6181.21
Height Equivalent to Theoretical Plate ($HETP$)	LIN	0.0040 cm plate ⁻¹

*RSD% for five injections

Table 4: Results from Robustness Testing of the Proposed Chromatographic Methods

Method	Conditions	Obtained values for LIN			
		R_f	T		R_s^*
TLC	Mobile phase composition (chloroform: ethanol)				
	5.2:1.8	0.87		0.96	4.79
	4.8:2.2	0.89		0.94	4.93
HPLC	Mobile phase composition (ACN: 0.15% TEA; pH=3.5)	R_t	N	T	R_s^*
	28:72	4.45	6167.50	1.17	4.65
	32:68	4.59	6254.45	1.15	4.78
	pH of 0.15% TEA				
	3.4	4.51	6170.35	1.13	4.68
	3.6	4.57	6094.57	1.16	4.75

* Resolution of the nearest degradation product (OXD) relative to the intact drug (LIN)

Table 5: Quantitative Determination of LIN in its Pharmaceutical Preparation by the Proposed Chromatographic Methods and Application of Standard addition technique

Pharmaceutical preparation	Claimed amount per tablet	Standard addition technique			
		TLC Method			
Averozolid® tablets, B.N.: 1104128	600 mg LIN	Pharmaceutical preparation taken ($\mu\text{g band}^{-1}$)	Authentic added ($\mu\text{g band}^{-1}$)	% Recovery* of LIN	
		3.0	1.5	99.81	
			3.0	100.62	
			4.5	100.45	
TLC Method		Mean% \pm SD		100.29 \pm 0.424	
HPLC Method		HPLC Method			
Mean% \pm SD	99.75 \pm 1.287	Pharmaceutical preparation taken ($\mu\text{g mL}^{-1}$)	Authentic added ($\mu\text{g mL}^{-1}$)	% Recovery* of LIN	
HPLC Method		30.0	15.0	99.99	
			30.0	100.75	
Mean% \pm SD	100.58 \pm 0.951		45.0	101.25	
		Mean% \pm SD		100.66 \pm 0.638	

* Mean of three determinations

Table 6: Statistical Comparison between the Reported Method and the Proposed Chromatographic Methods for the Determination of LIN.

Parameters	Method of comparison	Reported Method ^b	TLC Method	HPLC Method
	Mean	99.96	99.49	99.05
	S.D.	0.517	0.688	0.836
	Variance	0.268	0.473	0.699
	t-test ^a	---	1.22	2.07
	F-test ^a	---	1.77	2.61

^a The theoretical values of t and F at P = 0.05 are (2.31) and (6.39), respectively where n=5.

^b HPLC method using C18 column, flow rate 1.0 mL min⁻¹, mobile phase composed of 1% acetic acid : methanol : acetonitrile (50 : 25 : 25, by volume) and UV detection at 254 nm.

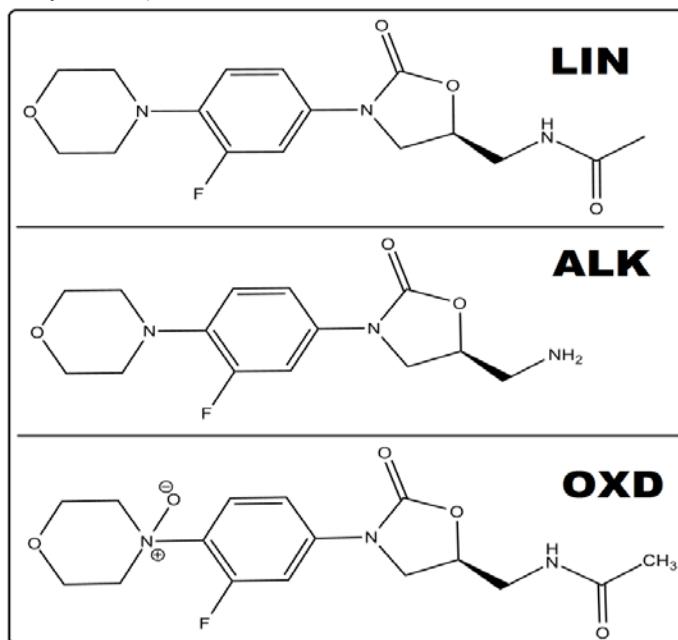


Fig. 1: Chemical structures of LIN, ALK and OXD

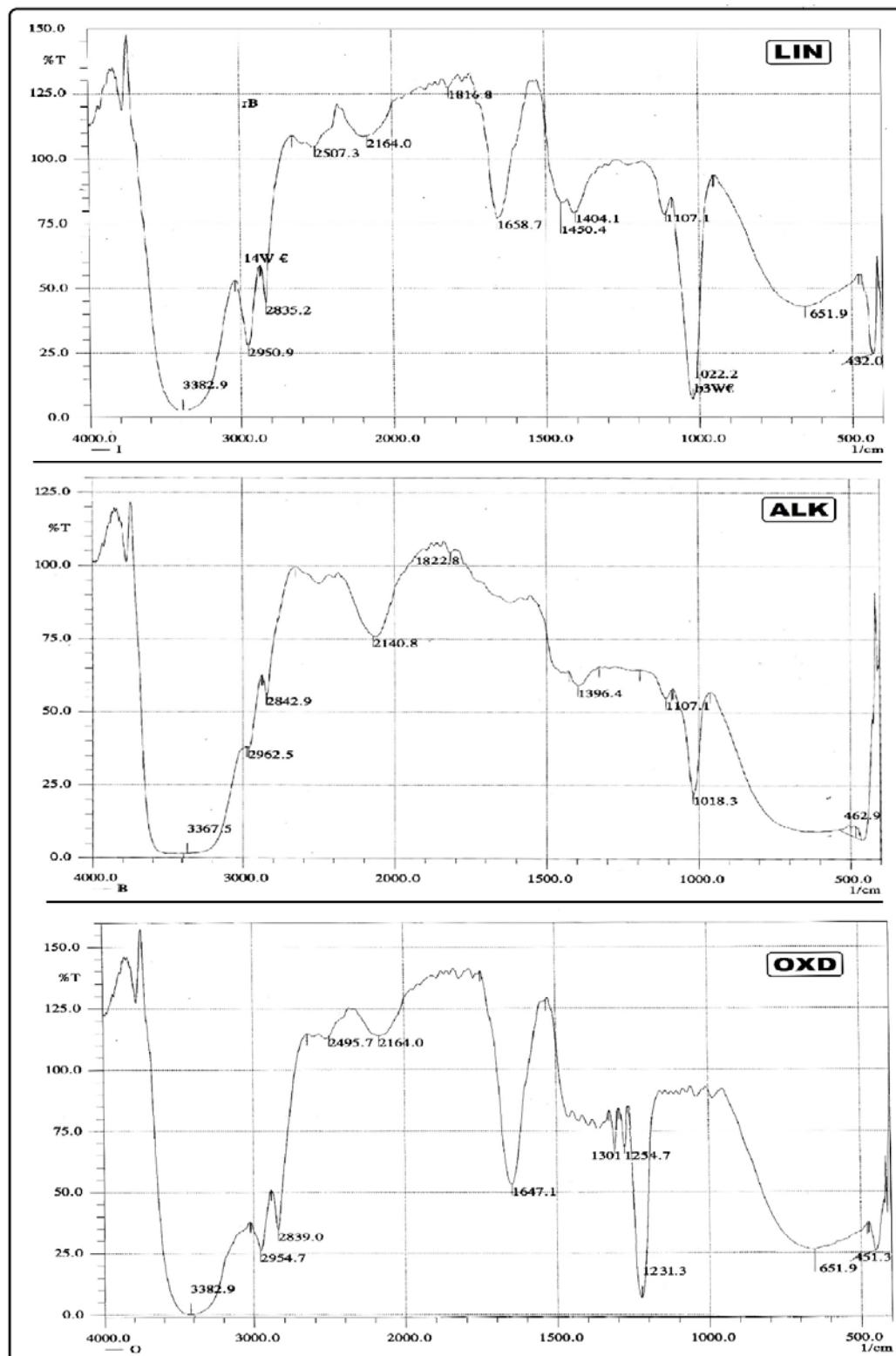


Fig. 2: IR Spectra of LIN, ALK and OXD

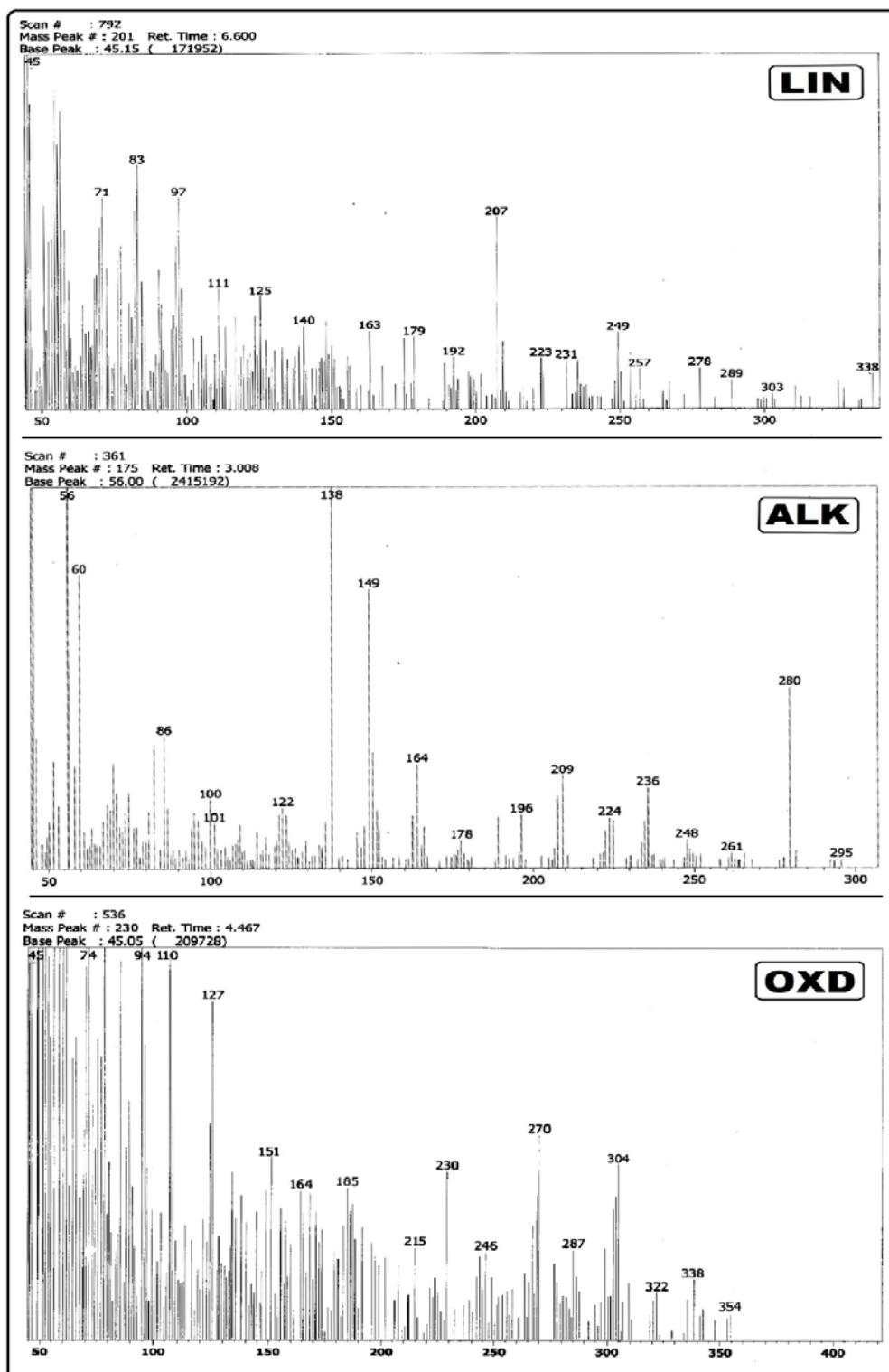


Fig. 3: MS Spectra of LIN, ALK and OXD

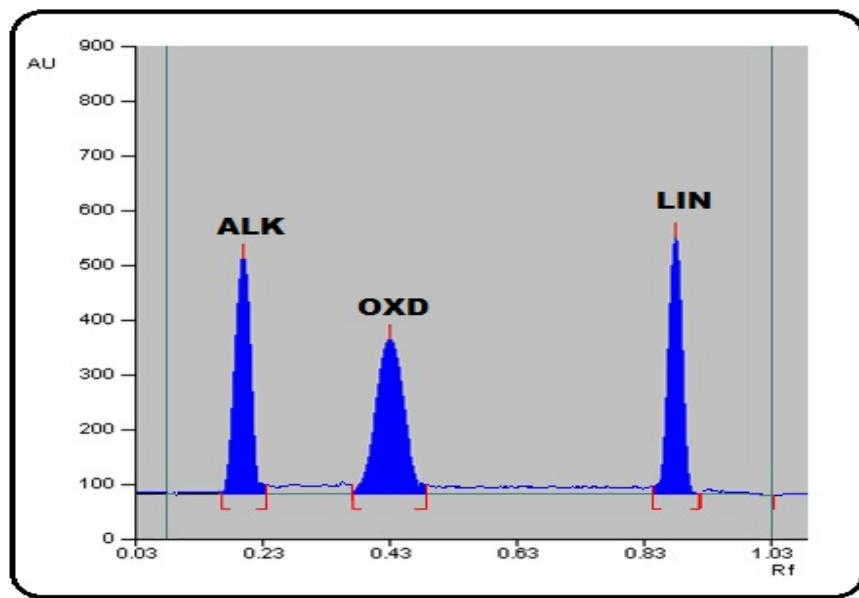


Fig. 4: Thin layer chromatogram of LIN, ALK and OXD mixture, using ‘Chloroform: Ethanol’ (5:2, by volume) at 254 nm.

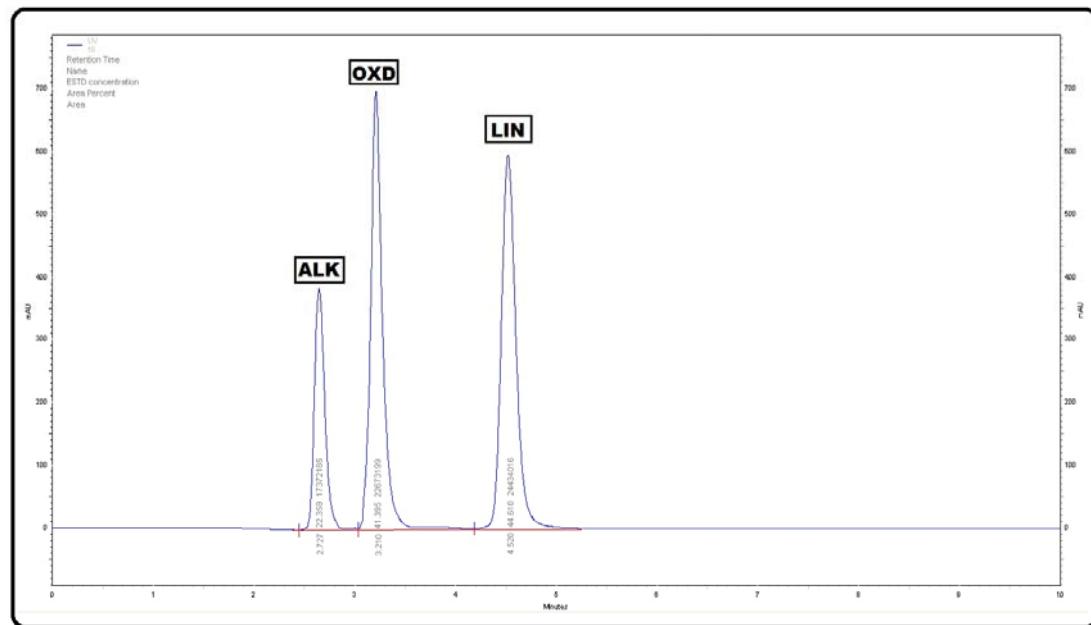


Fig. 5: HPLC chromatogram of LIN, ALK and OXD mixture, using ‘ACN - 0.15% TEA; pH=3.5’ (30: 70, v/v) at 254

5. Conclusion

The suggested chromatographic methods provide simple, accurate, and reproducible stability-indicating methods for the quantitative analysis of LIN in presence of its degradation products. The developed TLC method is highly sensitive and has the

advantages of short run time, large sample capacity, and use of minimal volume of solvents. The proposed HPLC method offers high specificity and good resolution between the three proposed components within suitable analysis time. The applied chromatographic methods could be useful for stability

investigation of the active drug and checking the extent of degradation in its pharmaceutical preparation.

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