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Research Article

DETERMINATION OF OXELADIN CITRATE IN THE PRESENCE OF TWO OF ITS DEGRADATION PRODUCTS BY HPTLC AND HPLC

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ABSTRACT

Objectives: -To study the hydrolytic and the oxidative degradation of oxeladin citrate.

-To prepare its alkaline induced hydrolytic degradation product; α,α-Diethyl benzeneacetic acid and its N-oxide degradation product; Diphenylmethoxy-N, N-dimethyl ethanamine N-oxide.

-To develop and validate accurate, selective, sensitive and precise HPTLC-densitometric and RP-HPLC methods for determination of oxeladin citrate in the presence of both; its hydrolytic and its N-oxide degradation products in bulk form and in pharmaceutical formulations.

Methods: Method A uses HPTLC-densitometry, depending on separation and quantitation of oxeladin citrate and its two degradation products on TLC silica gel 60 F254 plates, using acetone-ethyl acetate-methanol-acetic acid-sodium lauryl sulphate (6:5:3:0.5:0.2, v/v/v/v/w) as a developing system followed by densitometric measurement of the bands at 220 nm.

-Method B comprises RP-HPLC separation of the same components using a mobile phase consisting of methanol–water (50:50, v/v, pH 3.1 by trifluoroacetic acid) on a Phenomenex C_{18} column at a flow-rate of 2 mL/min and UV detection at 220 nm.

Results: Accurate, selective, sensitive and precise HPTLC-densitometric and RP-HPLC methods were developed and validated for determination of oxeladin citrate in the presence of both; its hydrolytic and its N-oxide degradation products. The proposed methods were successfully applied to the analysis of oxeladin citrate either in bulk powder or in pharmaceutical formulation without interference from other dosage form additives, and the results were statistically compared with the established method.

Conclusion: The proposed methods could be applied as stability-indicating methods for the determination of pure OL and in the presence of the mentioned degradation products either in bulk powder or in pharmaceutical formulations.

Keywords: Oxeladin citrate; HPTLC-densitometric method; RP-HPLC method.

INTRODUCTION

Oxeladin citrate (OL) is α,α-Diethyl benzeneacetic acid 2-[2-(diethylamino)ethoxy]ethyl ester citrate salt; the structural formula is shown in Fig. 1. It isa centrally acting cough suppressant for non-productive cough [1].

The literature survey reveals spectrophotometric methods using bromothymol blue [2] and quercetin [3] for determination of OL in pharmaceutical preparations. A spectrophotometric method depending on complexation with methyl orange [4] and GC methods [5,6] were applied for determination of OL in hematological specimens. A single RP-HPLC method [7] was able to determine OL in presence of Its hydrolytic degradate α,α-Diethyl benzeneacetic acid (DBA) using a mobile phase consisting of acetonitrile-0.01 M potassium dihydrogen phosphate-diethylamine (60:40:0.2). Therefore, the aim of this study was to develop new accurate, selective and sensitive stability-indicating methods for the determination of OL in the presence of DBA, and the N-oxide degradation product α,α-Diethyl benzeneacetic acid 2-[2-(diethylamino) ethoxy]ethyl ester N-oxide (NOX) which can be used for determination of OL either in bulk powder or in its pharmaceutical formulations.

MATERIALS AND METHODS

Materials

Pure standards

Oxeladin citrate was kindly supplied by Pharaonia pharmaceuticals Company, Alexandria, Egypt. Its purity was found to be 99.90% as labeled by the company.

Pharmaceutical formulation

Oxeladine® syrup (batch no.11228) and Paxeladine® syrup (batch no.030265) were both labeled to contain 200mg of Oxeladin citrate per 100 mL syrup and were manufactured by Paranoia Pharmaceuticals Company, Alexandria, Egypt, and The Arab Drug Co, ADCO, Under License of beaufour, France, respectively.

Chemicals and reagents

All reagents and chemicals used throughout this work were of analytical grade and were used without further purification. Methanol was of HPLC grade and obtained from ChromasolvW, Sigma-Aldrich Chemie Gmbh,Germany. Ethyl acetate and sodium hydroxide were from E. Merck, Germany. Hydrogen peroxide solution, sodium lauryl sulphate and acetone were from Al-Nasr Company, Egypt.

Instruments

A UV lamp with short wavelength 254nm (USA) was used, and a TLC scanner densitometer (Camag, Muttenz, Switzerland). The following requirements were taken into consideration: slit dimensions, 5x0.2 mm; scanning speed, 20 mm/s; spraying rate, 10/mL; data resolution: 100 mm/step.Pre-coated silica gel aluminum plates (20x20 cm; 60 F254) were obtained from Fluka, Sigma-Aldrich Chemie Gmbh, Germany. The sample applicator for TLC was a Linomat IV with a 100mL syringe (Camag, Muttenz, Switzerland).

Shimadzu class-LC 10 AD liquid chromatograph equipped with a UV–vis detector operated at a wavelength of 220 nm, a degasser (DGU-3A) and a data processor (C-R4A) were all from Shimadzu, Kyoto, Japan. The analytical column was a Phenomenex C_{18} ODS (Shimadzu, Kyoto, Japan), 25cm length, 4.6mm i.d. A Sonix TV ss-series ultrasonicator (USA) was used.

Procedures

Forced degradation of OL:

Hydrolysis

25 mg of OL powder was separately dissolved in two flasks one containing 25 mL of 0.1N hydrochloric acid and the other containing

0.1 N sodium hydroxide. The solution containing 0.1N sodium hydroxide became turbid at the moment. The two solutions were refluxed for 6 hours, where complete degradation was achieved in the alkaline degradation solution, while in case of acidic degradation solution, no degradation took place as confirmed by thin layer chromatography (TLC) using acetone-ethyl acetate-methanol-acetic acid-sodium lauryl sulphate (6:5:3:0.5:0.2, v/v/v/v/w) as a developing system. The base degraded sample was filtered to remove the turbidity resulting from the alcoholic aliphatic byproduct, and then the solution was rendered acidic by 0.5 N hydrochloric acid then left to dry spontaneously at room temperature. DBA is then extracted with ether, followed by spontaneous drying of the solvent. The degradation product was identified by IR and mass spectrometry, and used for preparation of its stock solution. The acid degradation using 1N hydrochloric acid was tried, where no degradation took place.

Oxidation

25 mg of OL powder was separately dissolved in two flasks one containing 25 mL of methanolic solution of hydrogen peroxide (3%, v/v) and the other containing 25 mL of hydrogen peroxide solution (30%, v/v). The solutions were kept for 6 hours at room temperature in the dark in order to exclude the possible degradation effect of light. The resultant solutions were spotted on TLC plate along with standard OL. They were found to give the same degradate, NOX. The degradation was incomplete upon using 3% hydrogen peroxide, while in case of 30% hydrogen peroxide; the degradation was complete, where the solution was left for spontaneous drying of the solvent. The degradation product was identified by IR and mass spectrometry, and used for preparation of its stock solution.

Photolysis

25 mg of OL powder was dissolved in 25 mL methanol and the solution was exposed to both cool white lamp and UV lamp for 24 hours. The resultant solutions were spotted on TLC plate along with standard OL, where no degradation took place.

Standard solutions

For stock standard solutions of OL, DBA and NOX (1 $mgmL^{-1}$ in methanol), 0.1 g of OL, DBA and NOX were accurately weighed into three separate 100 mL volumetric flasks; 50 mL of methanol was added to each flask and shaken to dissolve, then the volume was made up to the mark with methanol. For working standard solutions of OL, DBA and NOX (100 μgmL^{-1}), aliquots equivalent to 10 mL of OL, DBA and NOX stock standard solutions (1 $mgmL^{-1}$) were transferred accurately into three separate 100 mL volumetric flasks, and then the volume was completed to the mark with methanol.

Laboratory prepared mixtures

Mixtures containing different ratios of OL, DBA and NOX were prepared using their respective working solutions in methanol.

HPTLC-spectrodensitometric method

Linearity and construction of calibration curves

Into three different series of 10 mL volumetric flasks, aliquots of OL, DBA and NOX were accurately transferred from their working solutions; the volume was then made up with methanol. A 10 µL aliquot of each solution was spotted as a band of 5mm width on a TLC plate (20x10 cm with 250 mm thickness) using a Camag Linomat IV applicator, such that we obtain a linearity range of 0.1-2 µg/band for each of the three drugs . The bands were applied at 7mm intervals and 10mm from the bottom and sides. A linear ascending chromatogram developing to a distance of 9 cm was performed in a chromatographic tank previously saturated for 30 min with the developing mobile phase consisting of acetone - ethyl acetate - methanol - acetic acid - sodium lauryl sulphate (6 : 5 : 3 : 0.5 : 0.2) at room temperature. The peak areas were recorded using scanning wavelength at 220 nm and the calibration curves were constructed by plotting the integrated peak areas versus the corresponding concentrations for each compound. The regression equations were computed.

Analysis of laboratory prepared mixtures of OL, DBA and NOX

Mixtures containing different concentrations of OL, DBA and NOX were analyzed by applying the above-described procedure.

RP-HPLC method

Linearity and construction of calibration curves

Different aliquots of OL, DBA and NOX were transferred from their respective working solution (100μgmL^{-1} in methanol) into three separate series of 10mL volumetric flasks, and the volumes were made up with the mobile phase such that we obtain final concentration ranges of 5-200μgmL^{-1} for OL, 0.5-50μgmL^{-1} for NOX and 5-100$\mu gmL^{-1}$for DBA. Each concentration was injected in triplicate. The chromatographic separation was carried out using methanol–water (50:50, v/v, pH 3.1 by trifluoroacetic acid) as a mobile phase on a Phenomenex C_{18} column at a flow rate of 2mL/min and UV detection at 220 nm. The chromatograms were recorded and the peak areas of OL, DBA and NOX were determined, the calibration curves relating peak areas to the corresponding concentrations were constructed for the three compounds and the regression equations were computed.

Application to pharmaceutical formulations Paxeladine® syrup and Oxeladine® syrup

An aliquot equivalent to 100mg of OL was transferred from each dosage form into a 100mL volumetric flask. Then, 75 mL of methanol was added to each and the two solutions were sonicated for 30 min, completed to volume with methanol then filtered. The two solutions were diluted with methanol to obtain 100 μgmL^{-1} working solutions for Paxeladine® and Oxeladine® syrups, then the same procedures of RP-HPLC and HPTLC densitometric methods were followed and the concentrations of OL were calculated from the computed regression equations.

RESULTS AND DISCUSSION

The main aim of this work was to establish accurate, specific, reproducible and sensitive stability-indicating methods for the determination of OL in presence of its alkaline induced degradation product (DBA) and its N-oxide degradation product (NOX) in its bulk powder and pharmaceutical dosage forms with satisfactory precision for good analytical practice.

Upon adding 0.2 M NaOH to OL, hydrolysis of the ester group took place resulting in an acidic degradate (in the form of sodium salt) and an immediately precipitated aliphatic byproduct which is then removed by filtration**,** Figure 1. When 0.2M HCl is added to the filtrate the free acid form of the degradate is liberated from its salt then the solution is left to dry spontaneously at room temperature. DBA is then extracted with ether, followed by spontaneous drying of the solvent.

The degradation product was identified by IR and mass spectrometry, where the peak corresponding to the aliphatic C-H bonds at 2980 Cm^{-1} is present in both (arrow 1). The peak corresponding to the esteric C=O appeared in the IR spectrum of OL at 1750 Cm^{-1}, while it appeared in the IR spectrum of DBA at 1700 representing the carboxylic acid C=O (arrow 2). The peak corresponding to the C-O bonds of the ester and ether groups at 1250 Cm^{-1} is present in the IR spectrum of OL while it is nearly absent in that of DBA (arrow 3) indicating the hydrolysis of the ester linkage, as shown in Figures 2 and 3.

The mass spectra of OL and DBA confirmed their identities as mass molecular ion peaks at 527 and 192m/z corresponded to the intact drug (OL) and its alkaline induced degradation product (DBA), respectively, as shown in Figures 4 and 5.

When 30% hydrogen peroxide solution was added to OL and left for Six hours, complete oxidation took place into the N-oxide form (NOX) as shown in Figure 6. The solution was evaporated at room temperature.

The degradation product (NOX) was identified by IR and mass spectrometry, where the peak corresponding to the aliphatic C-H bonds at 2980 Cm^{-1} is present in both (arrow 1). The peak corresponding to the esteric C=O appeared in both spectra at 1750 Cm^{-1} (arrow 2). The peak corresponding to the C-O bonds of the ester and ether groups at 1250 Cm^{-1} is present in both spectra

(arrow 3). The appearance of the peak corresponding to the N-oxide group at 1550 Cm^{-1} in the IR spectrum of NOX (arrow 4) indicated the oxidation process as shown in Figures 7.

The mass spectra of NOX confirmed its identity as mass molecular ion peak at 543m/z corresponded to N oxide degradation product (NOX), as shown in Figure 8.

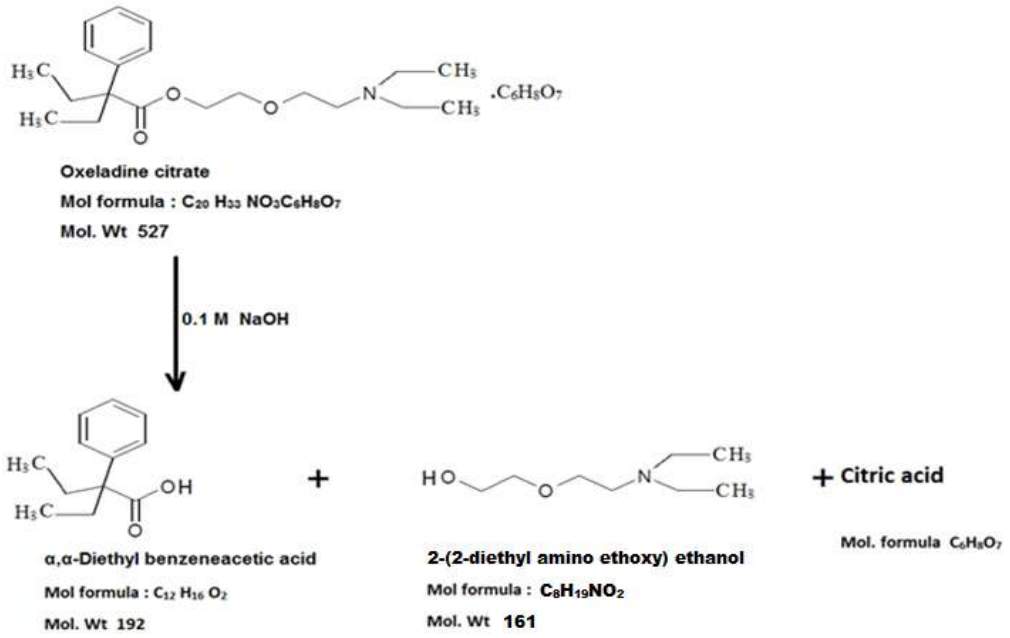

Fig. 1: It shows the scheme of the alkaline induced degradation of oxeladin citrate.

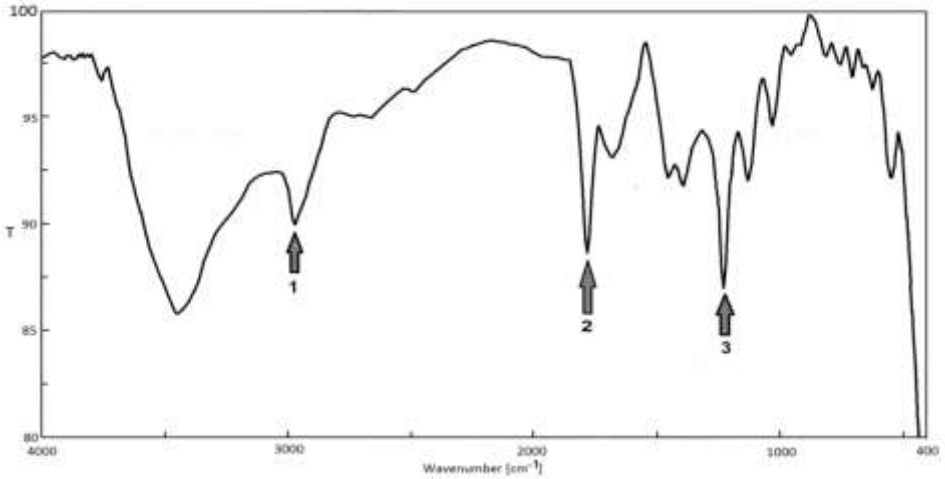

Fig. 2: It shows the ir spectrum of oxeladin citrate.

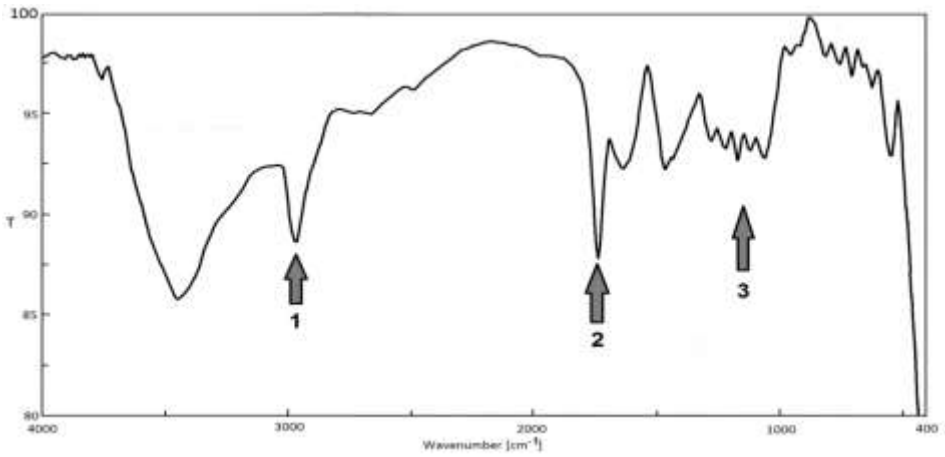

Fig. 3: It shows the ir spectrum of the alkaline induced degradation product of oxeladin citrate.

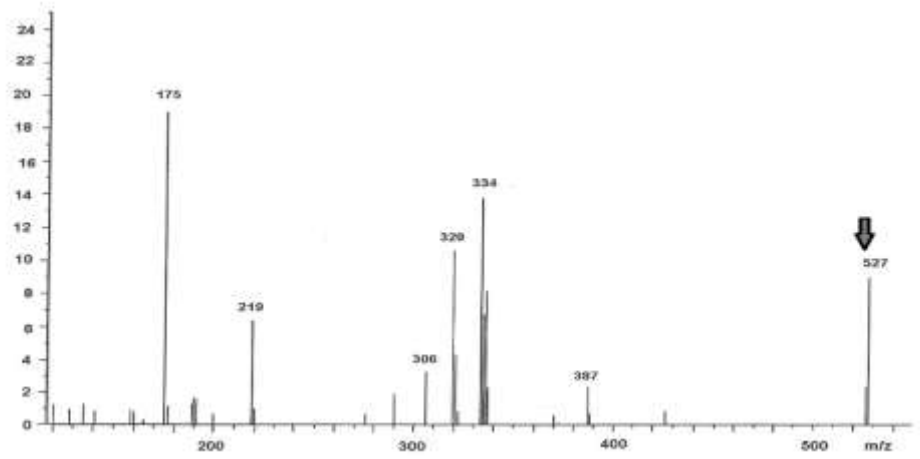

Fig. 4: It shows the mass spectrum of oxeladin citrate (Molecular Weight 527).

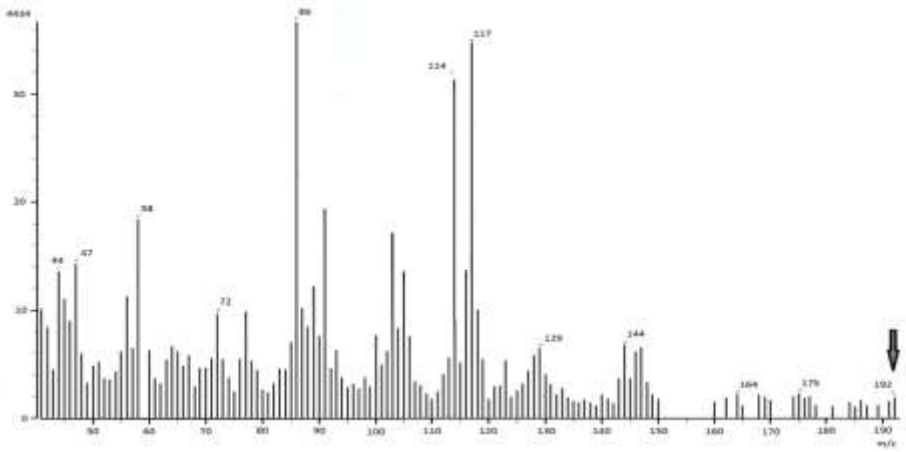

Fig. 5: It shows the mass spectrum of the alkaline induced degradation product of oxeladin citrate (Molecular Weight 192).

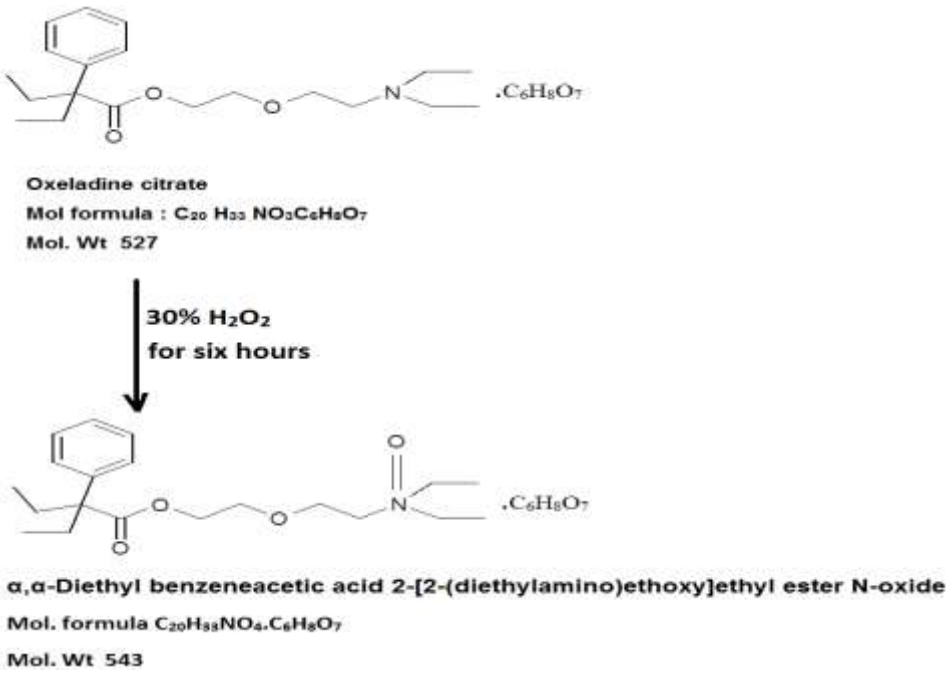

Fig. 6: It shows the scheme of the oxidative degradation of oxeladin citrate.

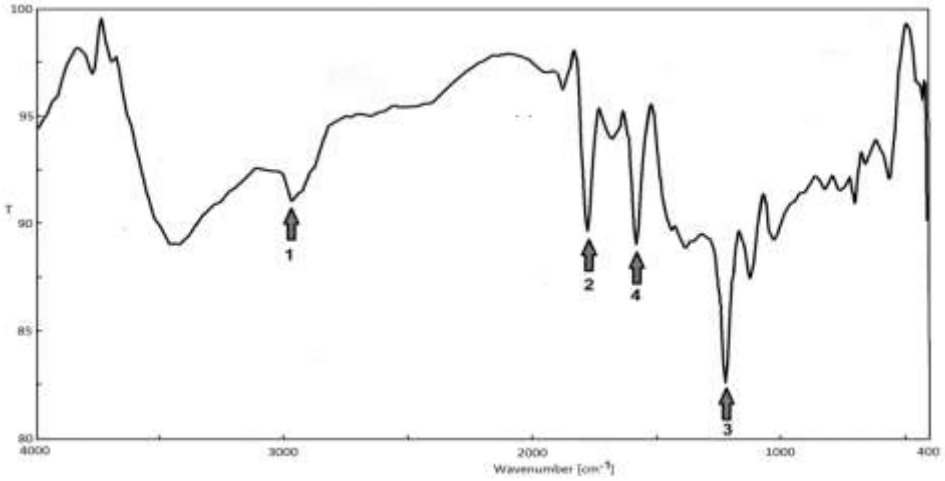

Fig. 7: It shows the ir spectrum of the n-oxide degradation product of oxeladin citrate

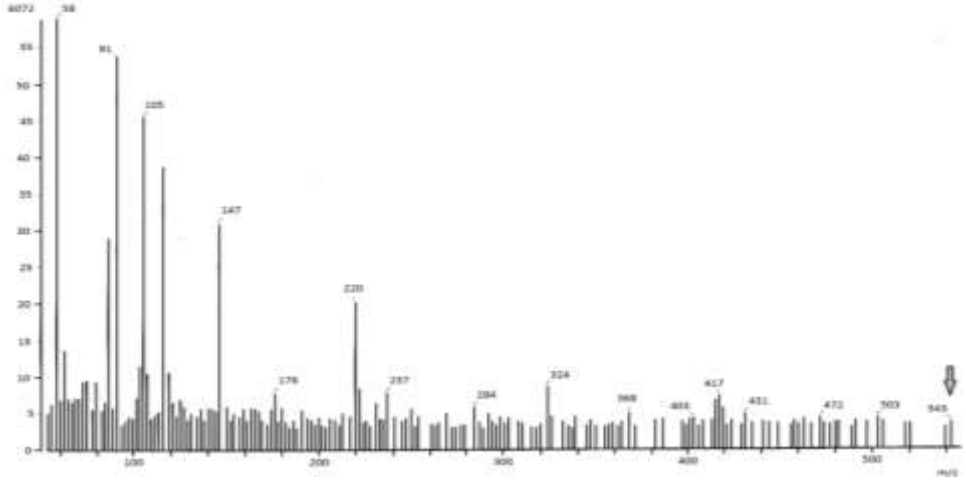

Fig. 8: The mass spectrum of the n oxide degradation product of oxeladin citrate (Molecular Weight 543)

HPTLC-densitometric method

HPTLC-densitometry is a useful technique for the resolution and determination of drug mixtures. This technique offers a simple way to quantify directly on TLC plates by measuring the optical density of the separated bands. The amounts of compounds are determined by comparison with a standard curve from reference materials chromatographed simultaneously under the same conditions [8]. To improve separation of bands, it was necessary to investigate the effect of different variables. Determination of the optimum parameters for maximum separation was carried out as described below.

Developing system: Different developing systems of different compositions and ratios were tried for separation, e.g. chloroform–methanol with different ratios, hexane–methanol with different ratios, chloroform–ethyl acetate with different ratios, and chloroform–methanol–acetic acidwith different ratios. The best mobile phase was acetone - ethyl acetate-methanol-acetic acid-sodium lauryl sulphate (6:5:3:0.5:0.2, v/v/v/w).This selected mobile phase allowed good separation of the studied compounds with good R_f values without tailing of the separated bands. This mobile phase was also able to separate the three components from the two preservatives; methyl paraben (MP) and propyl paraben (PP) which have equal R_f values (0.87), as shown in Figure 9.

Band dimensions: Different band dimensions were tested in order to obtain sharp and symmetrical separated peaks. The optimum band width chosen was 5mm and the space between bands was 7mm.

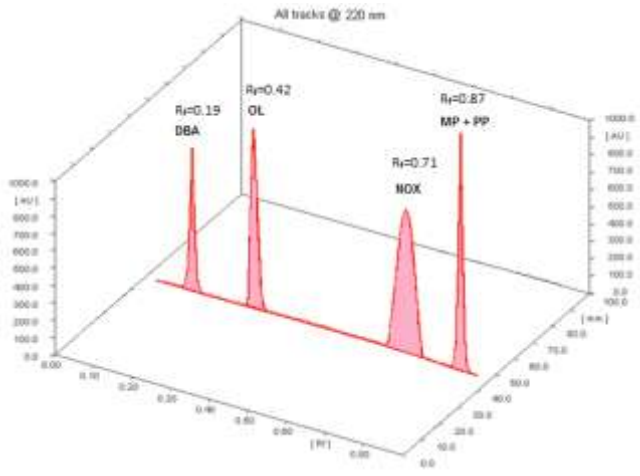

Fig. 9: It shows a thin-layer Chromatogram of separated peaks of (a) Oxeladin citrate, (b) Alkaline-Induced Degradation Product, (c) N oxide degradation product and (d) Methyl Paraben and Propyl Paraben.

Scanning wavelength: Different scanning wavelengths were tried, where 220nm was the most suitable wavelength for all drugs, at which peaks were sharper and symmetrical and minimum noise was obtained. At this wavelength maximum sensitivity for all drugs was achieved.

Slit dimensions of scanning light beam: The slit dimensions of the scanning light beam should ensure complete coverage of band dimensions on the scanned track without interference of adjacent bands. Different slit dimensions were tested, and 5x0.2mm proved to be the slit dimension of choice that provides highest sensitivity.

The proposed HPTLC method is based on the difference in the R_f values of OL (R_f=0.42), DBA (R_f=0.19) and NOX (R_f =0.71). The two preservatives; MP and PP present in the syrups, have equal R_f values, 0.87.

RP-HPLC method

A validated isocratic RP-HPLC method with UV detection was developed for the separation and quantitation of OL and its two degradates; DBA and NOX and it was applied to the pharmaceutical formulations.

To optimize the proposed RP-HPLC method, it was necessary to test the effects of different parameters that affect the sensitivity, selectivity and the efficiency of the chromatographic separation.

The mobile phase: Different mobile phases with different compositions and polarities were tried to achieve the chromatographic separation. Complete separation between OL, DBA and NOX was achieved using methanol–water. Also the effect of methanol–water ratio was studied to improve resolution. Methanol–water in the ratio (50:50, v/v) was found to be the most suitable, which also allowed the separation of the two preservatives; methyl paraben and propyl paraben which have equal retention times (9.3 min), Figure 10.

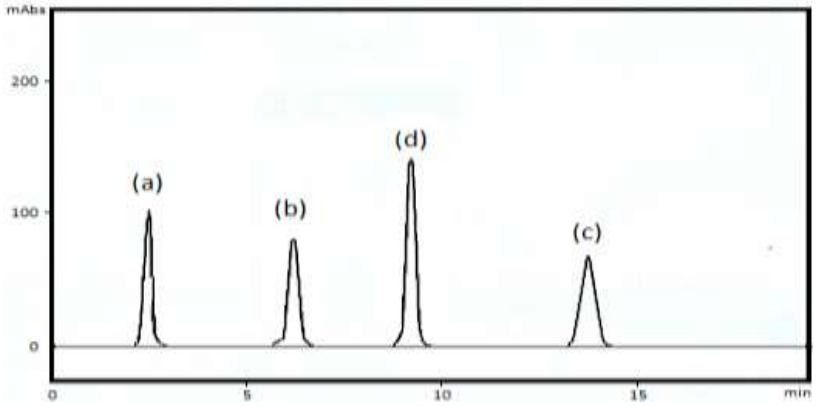

Fig. 10: It shows RP-HPLC Chromatogram of separated peaks of (a) Oxeladin citrate, (b) N Oxide Degradation, (c) Alkaline-Induced Degradation Product and (d) Methyl Paraben and Propyl Paraben.

pH effect: The effect of pH on the separation was tested by using buffers of different pH values. It was found that using buffer rather than water had no effect on the peak shapes or resolution, but adjustment of the pH of the solution to 3.1 using trifluoroacetic acid had a good effect on the chromatographic separation.

Scanning wavelength: Different wavelengths were studied for detection of the effluent; it was found that detection at 220 nm gave maximum sensitivity for the three compounds.

Flow rate: Different flow rates were also tested to provide good separation within acceptable run time. The best flow rate used was 2 mL/min. Finally a satisfactory separation was obtained upon using the optimum experimental conditions.

The retention time (t_R) of OL, NOX and DBA were 2.6 min, 6.2 min and 13.7 min, respectively, with no interference among the peaks, as shown in Figure 10.

Method validation

The linearity of proposed methods was evaluated and it was evident in the ranges of 0.1-2µg/band for all components in HPTLC and in the ranges of 5-200µgmL^{-1}, 5-100µgmL^{-1} and 0.5-50µgmL^{-1} for OL, DBA and NOX, respectively in RP-HPLC. The regression equations were calculated and found to be:

For HPTLC

Y_1 =0.3930 C_1 + 0.0090 r_1=0.9999 for OL,

Y_2 =0.0870 C_2 - 0.0010 r_2=0.9998 for DBA and

Y_3 =0.1730 C_3 - 0.0020 r_3=0.9998 for NOX

whereY_1, Y_2 and Y_3 are the integrated peak areasx10^{-5} , C_1, C_2 and C_3 are the concentrations in micrograms per band, and r_1, r_2 and r_3 are the correlation coefficients for OL, DBA and NOX, respectively.

For RP-HPLC

Y_1 =0.0555 C_1 + 0.0562 r_1=0.9997 for OL,

Y_2 =0.0098 C_2 + 0.0636 r_2=0.9999 for DBA and

Y_3 =0.0301 C_3 + 0.0077 r_3=0.9999 for NOX

whereY_1, Y_2 and Y_3 are the areas under the peaks x10^{-5} , C_1, C_2 and C_3 are the concentrations in µg/mL, and r_1, r_2 and r_3 are the correlation coefficients for OL, DBA and NOX, respectively.

Table 1: It shows the results of assay validation parameters of the proposed methods for determination of oxeladin citrate in presence of its alkaline induced and its N oxide degradation products.

Parameters	HPTLC-densitometric method	RP-HPLC method
Range	0.1-2(µg/band)	5-200(µgmL^{-1})
Linearity		
Slope	0.3930	0.0555
Intercept	0.0090	0.0562
Correlation coefficient	0.9999	0.9997
Accuracy (mean ± SD)	100.235± 1.275	101.000± 1.070
Precision (RSD%)		
Repeatability [a]	0.492	0.415
Intermediate precision [b]	0.749	0.733

[a]The intraday precision (n = 3), average of three different concentrations repeated three times within one day.

[b]The interday precision (n = 3), average of three different concentrations repeated three times on three successive days.

Good linearitiesare evident from the high values of the correlation coefficients and low values of intercepts, Table 1.

The precision of proposed methods was evident, as shown in Table 1. The methods accuracy was checked by applying the methods for determination of pure samples of the studied compounds. The concentrations were calculated from the corresponding regression equations and the results are shown in **Table 1**. Accuracy was further assessed by applying the standard addition technique on Paxeladine® and Oxeladine® syrups, for which good recoveries were obtained, revealing no interference from excipients and good accuracy of the methods, Table 2.

Table 2: It shows the results of determination of oxeladin citrate in its pharmaceutical formulations by the proposed methods and application of standard addition technique.

Pharmaceutical preparation	HPTLC-densitometric method			RP-HPLC method		
	Taken (µg/band)	Found (%±SD)	Standard addition technique (mean±SD)	Taken (µgmL^{-1})	Found (%±SD)	Standard addition technique (mean±SD)
Oxeladine® syrup (batch no.11228)	0.5	101.93 ±0.202	100.44 ±0.916	30	101.96 ±0.270	99.35 ± 0.591
Paxeladine® syrup (batch no.030265)	0.5	101.49 ±0.216	99.81 ± 1.034	30	101.60 ± 0.178	100.17 ±1.380

Specificity of the proposed methods is evident from the HPTLC and RP-HPLC chromatograms in Figures 9 and 10, which also show no interference from methyl paraben and propyl paraben which have the same R_f and t_R values and used as preservatives in the syrups.

The robustness of the proposed methods was evaluated through studying the effects of different factors on HPTLC densitometric and RP-HPLC methods to obtain the optimum parameters for complete separation.

System suitability testing for HPTLC densitometric and RP-HPLC methods was based on the concept that the equipment, electronics, analytical operations and samples constitute an integral system that can be evaluated as a whole. System suitability is used to ensure system performance before or during the analysis of the drugs. System suitability was checked by calculating the capacity factor (K), tailing factor(T), column efficiency (N), selectivity factor (α) and resolution (R), and the system was found to be suitable, as shown in Table 3.

Table 3: It shows system suitability testing parameters of HPTLC-densitometric and RP-HPLC methods for determination oxeladin citrate in the presence of its alkaline-induced and its N oxide degradation products.

Parameters	HPTLC-densitometric method				Parameters	RP-HPLC method			
	Obtained values			Reference value		Obtained values			Reference value
	DBA	OL	NOX			OL	NOX	DBA	
Resolution (R)	4.7	5.5		R>1.5	**Resolution (R)**	4.5	7.7		R>1.5
Selectivity factor (α)	0.32	0.30		<1	**Selectivity factor (a)**	3.2	3.1		>1
Symmetry factor	1.01	1.02	0.98	~1	**Tailing factor (T)**	0.8	0.7	0.9	<1.5–2 or <2
Capacity factor (K')	4.26	1.38	0.41	0 <K' <10	**Capacity factor (k')**	1.6	5.2	12.7	1-10 acceptable
					Column efficiency (N)	432.6	961	4692	Increase with efficiency of the separation
					HETP[a]	0.058	0.026	0.005	The smaller the value the higher the column efficiency

[a]HETP, height equivalent to theoretical plate (cm/plate).

Table 4 shows statistical comparison of the results obtained by the proposed methods and the official method, which is an HPLC method that uses a mobile phase consisting of acetonitrile-0.01 M potassium dihydrogen phosphate-diethylamine (60:40:0.2) [7] when applied to pure OL. The calculated t and F values were smaller than the theoretical ones, indicating that there is no significant difference between the proposed method and the established method with respect to accuracy and precision.

Table 4: It shows the statistical comparison of the results obtained by the proposed methods and the established method for determination of pure oxeladin citrate.

Items	HPTLC-densitometric method	RP-HPLC method	established method[c]
Mean	100.235	101.068	100.57
SD	1.275	0.938	0.833
N	7	9	6
Variance	1.628	0.880	0.694
Student's t-test	0.593(2.365)[a]	0.300(2.262)[a]	
F-value	2.346(2.827)[b]	1.268(2.551)[b]	

[a]Figures in parentheses represent the corresponding tabulated values of t at P = 0.05.

[b]Figures in parentheses represent the corresponding tabulated values of F at P = 0.05

[c]HPLC method[7].

Table 5 shows statistical comparison of the results obtained by the proposed method and the official method[7] when applied to OL in Paxeladine® and Oxeladine® syrups. The calculated t and F values were smaller than the theoretical ones, indicating that there is no significant difference between the proposed method and the established method with respect to accuracy and precision.

Table 5: It shows the statistical comparison of the results obtained by the proposed methods and the established method for determination ofoxeladin citrate in its pharmaceutical preparations; Paxeladinesyrup(batch no.030265) and Oxeladine syrup (batch no. 11228).

Items	HPTLC-densitometric method		RP-HPLC method		established method	
	Paxeladine syrup	Oxeladine syrup	Paxeladine syrup	Oxeladine syrup	Paxeladine syrup	Oxeladine syrup
Mean	101.49	101.93	101.60	101.96	101.58	101.85
SD	0.216	0.202	0.178	0.270	0.174	0.236
N	6	6	6	6	6	6
Variance	0.047	0.041	0.032	0.073	0.030	0.055
Student's t-test (2.447)[a]	0.451	0.570	0.890	0.050		
F-value (4.284)[b]	1.57	1.34	1.072	1.33		

[a]Figures in parentheses represent the corresponding tabulated values of t at P = 0.05.

[b]Figures in parentheses represent the corresponding tabulated values of t at P = 0.05

[c]HPLC method[7].

CONCLUSION

The present work provides new sensitive, accurate and selective analytical techniques for the determination of OL in the presence of its alkaline induced degradation product as well as its N-oxide degradation product in bulk powder or in pharmaceutical formulation. Application of the proposed method to the analysis of OL in pharmaceutical formulations shows that neither the degradation products nor the excipients interfere with the determination, indicating that the proposed methods could be applied as stability-indicating methods for the determination of pure OL and in the presence of the mentioned degradation products either in bulk powder or in pharmaceutical formulations.

REFERENCES

1. Sweetman SC. Martindale—The Complete Drug Reference. 33rded. London: Pharmaceutical Press; 2002.
2. Knopp C, Korsatko W: Determination of oxeladin citrate in compound preparations. Pharmazie 37 (1982) 76–77.
3. Mohamed FA, Mohamed AM, Mohamed HA, Hussein SA: Utility of quercetin for determination of some tertiary amine and quaternary ammonium salts. Talanta 43 (1996) 1931–1939.
4. Gutman EB, Spatz H: Alternative methods for determining different drugs in haematological specimens, SAFYBI 22 (1982) 1752–1760.
5. Salvesen B, Haugland T: Determination of oxeladin in human sera by gas–liquid chromatography with thermionic detection. J. Chromatogr. 225 (1981) 463–468.
6. Lartigue-Mattei C, Galmier MJ, Chabard JL, Beyssac E, Aiache JM: Determination of oxeladin in human plasma by gas chromatography–mass spectrometry. Biomed. Chromatogr. 9 (1995) 32–35.
7. El-Gindy A: High performance liquid chromatographic determination of oxeladin citrate and oxybutinin hydrochloride and their degradation products. IlFarmaco 60 (2005) 689–699.
8. Grinberg N. Modern Thin Layer Chromatography. New York: Marcel Dekker; 1990.