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

NOURUDDIN W. ALI1, M. ABDELKAWY2 AND NESSREEN S. ABDELHAMID3*

1-3Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Beni-Suef University, Abshaaed Shehata Ahmad Hegazy St, 62514, Beni-Suef, 1-3Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr El-Aini St, 11562, Cairo, Egypt. Email:www.nesserin salah@yahoo.com

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 (6:5:30:5:0.2, v/v/v/v/v) 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 C18 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 is a 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 complicated 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 degrade 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 Pharmaion 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 Poxeladine® 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 acetic acid 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, 10/20 mL; data resolution; 100 nm/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 100μL 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 C18 ODS (Shimadzu, Kyoto, Japan), 25cm length, 4.6mm i.d. A Sonix TV s-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 aceton–ethyl acetate–methanol–acetetic 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 degrade, 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 cold 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, v/v/v/v/w) 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**

**Application to pharmaceutical formulations Poxeladine® 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 of the two solutions 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 Poxeladine® and Oxeladine® syrups, then the same procedures of RP-HPLC and HPTLC densitometric methods were followed and the concentrations of OL was 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 degrade (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 degrade 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 both spectra at 1750 Cm⁻¹. The peak corresponding to the C-O bonds of the ester and ether groups at 1660 Cm⁻¹ is present in both OL, DBA at 1700 Cm⁻¹ 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 DBA whereas 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 corresponding 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
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 543 m/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 acid with different ratios. The best mobile phase was acetone-ethyl acetate-methanol-acetic acid-sodium lauryl sulphate (6:5:3:0.2:0.5, v/v/v/v/w). This selected mobile phase allowed good separation of the studied compounds with good Rf 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 Rf values (0.67), 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.

Scan 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 5x0.2 mm proved to be the slit dimension of choice that provides highest sensitivity.

The proposed HPTLC method is based on the difference in the Rf values of OL (Rf=0.42), DBA (Rf=0.19) and NOX (Rf=0.71). The two preservatives; MP and PP present in the syrups, have equal Rf 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 (tR) 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 5-200µg/mL⁻¹, 5-100µg/mL⁻¹ and 0.5-50µg/mL⁻¹ for OL, DBA and NOX, respectively in RP-HPLC. The regression equations were calculated and found to be:

For HPTLC

\[ Y_1 = 0.3930 C_1 + 0.0090 r_1 = 0.9999 \text{ for OL,} \]
\[ Y_2 = 0.0870 C_2 - 0.0010 r_2 = 0.9998 \text{ for DBA and} \]
\[ Y_3 = 0.1730 C_3 - 0.0020 r_3 = 0.9998 \text{ for NOX} \]

where \( Y_1, Y_2 \) and \( Y_3 \) are the integrated peak areas x 10^-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

\[ Y_1 = 0.0555 C_1 + 0.0562 r_1 = 0.9997 \text{ for OL,} \]
\[ Y_2 = 0.0098 C_2 + 0.0636 r_2 = 0.9999 \text{ for DBA and} \]
\[ Y_3 = 0.0301 C_3 + 0.0077 r_3 = 0.9999 \text{ for NOX} \]

where \( Y_2, Y_3 \) and \( Y_4 \) are the areas under the peaks x 10^-4, \( C_1, C_2 \) and \( C_4 \) 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.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HPTLC-densitometric method</th>
<th>RP-HPLC method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>0.1-2(µg/band)</td>
<td>5-200(µg/mL⁻¹)</td>
</tr>
<tr>
<td>Linearity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope</td>
<td>0.3930</td>
<td>0.0555</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.0090</td>
<td>0.0562</td>
</tr>
<tr>
<td>Correlation coefficient</td>
<td>0.9999</td>
<td>0.9997</td>
</tr>
<tr>
<td>Accuracy (mean ± SD)</td>
<td>100.235± 1.275</td>
<td>101.000± 1.070</td>
</tr>
<tr>
<td>Precision (RSD%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repeatability</td>
<td>0.492</td>
<td>0.415</td>
</tr>
<tr>
<td>Intermediate precision</td>
<td>0.749</td>
<td>0.733</td>
</tr>
</tbody>
</table>

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

*The interday precision (n = 3), average of three different concentrations repeated three times on three successive days.
Good lineairities are 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 Poxeladine® 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.

<table>
<thead>
<tr>
<th>Pharmaceutical preparation</th>
<th>HPTLC-densitometric method</th>
<th>RP-HPLC method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Taken (µg/band)</td>
<td>Found (%±SD)</td>
</tr>
<tr>
<td>Oxeladine® syrup (batch no.11228)</td>
<td>0.5</td>
<td>±0.202</td>
</tr>
<tr>
<td>Oxeladine® syrup (batch no.030265)</td>
<td>0.5</td>
<td>±0.216</td>
</tr>
</tbody>
</table>

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 and t 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.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HPTLC-densitometric method</th>
<th>RP-HPLC method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Obtained values</td>
<td>Reference value</td>
</tr>
<tr>
<td>Resolution (R)</td>
<td>DBA OL NOX</td>
<td>&gt;1.5</td>
</tr>
<tr>
<td>Selectivity factor (α)</td>
<td>0.32</td>
<td>0.30</td>
</tr>
<tr>
<td>Symmetry factor</td>
<td>1.01</td>
<td>1.02</td>
</tr>
<tr>
<td>Capacity factor (K')</td>
<td>4.26</td>
<td>1.38</td>
</tr>
<tr>
<td>Column efficiency (N)</td>
<td>432.6</td>
<td>961</td>
</tr>
<tr>
<td>HETP</td>
<td>0.058</td>
<td>0.026</td>
</tr>
</tbody>
</table>

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-diethylylamine (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.

<table>
<thead>
<tr>
<th>Items</th>
<th>HPTLC-densitometric method</th>
<th>RP-HPLC method</th>
<th>established method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>100.235</td>
<td>101.068</td>
<td>100.57</td>
</tr>
<tr>
<td>SD</td>
<td>1.275</td>
<td>0.938</td>
<td>0.833</td>
</tr>
<tr>
<td>N</td>
<td>7</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Variance</td>
<td>1.628</td>
<td>0.880</td>
<td>0.694</td>
</tr>
<tr>
<td>Student’s t-test</td>
<td>0.459(2.365)</td>
<td>0.459(2.262)</td>
<td>0.459(2.551)</td>
</tr>
<tr>
<td>F-value</td>
<td>2.346(2.827)</td>
<td>1.268(2.551)</td>
<td>1.268(2.551)</td>
</tr>
</tbody>
</table>

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

Figures in parentheses represent the corresponding tabulated values of F at P = 0.05.

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 oxeladin citrate in its pharmaceutical preparations; Paxeladine syrup (batch no.030265) and Oxeladine syrup (batch no. 11220).

<table>
<thead>
<tr>
<th>Items</th>
<th>HPTLC-densitometric method</th>
<th>RP-HPLC method</th>
<th>established method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>101.49</td>
<td>101.98</td>
<td>101.59</td>
</tr>
<tr>
<td>SD</td>
<td>0.216</td>
<td>0.202</td>
<td>0.178</td>
</tr>
<tr>
<td>N</td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Variance</td>
<td>0.047</td>
<td>0.041</td>
<td>0.032</td>
</tr>
<tr>
<td>Student's t-test (2.447)(^a)</td>
<td>1.57</td>
<td>1.34</td>
<td>1.072</td>
</tr>
<tr>
<td>F-value (4.284)(^b)</td>
<td>1.33</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)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

\(^7\) 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


