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Il Farmaco 60 (2005) 689–699
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High performance liquid chromatographic determination of oxeladin citrate and oxybutynin hydrochloride and their degradation products

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Received 12 October 2004; received in revised form 1 June 2005; accepted 7 June 2005

Available online 14 July 2005

Abstract

Two high performance liquid chromatographic (HPLC) methods are presented for the determination of oxeladin citrate (OL) and oxybutynin hydrochloride (OB) and their degradation products. The first method was based on HPLC separation of OL from its degradation product using a Nucleosil C_{18} column with a mobile phase consisting of acetonitrile –0.1% phosphoric acid (60:40 v/v). The second method was based on HPLC separation of OB from its degradation product using a VP-ODS C_{18} column with a mobile phase consisting of acetonitrile/0.01 M potassium dihydrogen phosphate/diethylamine (60:40:0.2). Quantitation was achieved with UV detection at 220 nm based on peak area. The two HPLC methods were applied for the determination of OL or OB, their degradation products, methylparaben and propylparaben in pharmaceutical preparations. The proposed methods were used to investigate the kinetics of acidic and alkaline degradation processes of OL and OB at different temperatures and the apparent pseudofirst-order rate constant, half-life and activation energy were calculated. The pH-rate profiles of degradation of OL and OB in Britton–Robinson buffer solutions within the pH range 2–12 were studied.

Keywords: Oxeladin citrate; Oxybutynin hydrochloride; HPLC; Kinetics of degradation; pH-rate profile

1. Introduction

Oxeladin citrate (OL), α, α-diethylbenzeneacetic acid 2-[2-(diethylamino)ethoxy]ethyl ester citrate, is a centrally acting cough suppressant for non-productive cough [1]. The literature survey reveals spectrophotometric methods using bromothymol blue [2], quercetin [3] and TLC method [2] for determination of OL in pharmaceutical preparations. Another spectrophotometric method through complexation with methyl orange [4], GC [5] and GC–MS [6] were applied for determination of OL in hematological specimens. Characterization of impurities in OL was reported using orthogonal chromatographic systems [7].

Oxybutynin hydrochloride (OB), α-cyclohexyl-α-hydroxybenzeneacetic acid 4-(diethylamino)-2-butynyl ester hydrochloride, is an anticholinergic drug used in treatment of urinary incontinence [8]. The official methods for determination of OB are non-aqueous titration using standard perchloric acid as titrant [9], potentiometric acid–base titration [10,11] in pure form, HPLC in tablets [9,10] and spectrophotometry using bromocresol green in syrup and oral solution [9]. Separation of OB enantiomers was carried out by capillary electrophoresis and liquid chromatography (LC) using chiral selectors [12–16]. HPLC was used to study the physicochemical properties [17] and determination [18] of OB in pharmaceuticals. OB was determined in biological samples using LC [19,20], GC [21,22] and high-performance frontal analysis [23].

OL and OB are ester type drugs susceptible to hydrolysis. However, no method has been reported for the assay of OL in the presence of its degradation products while there is a stability indicating method for the determination of OB by reversed-phase ion-pair LC with two counter-ions in the eluent using oxyphencyclimine hydrochloride as internal standard [18].

The aim of this work was to develop simple stability indicating method for the determination of OL, OB and their degradation products using HPLC to investigate the kinetics of the acidic and alkaline degradation processes and to calculate the activation energy for OL and OB.

The proposed method was found to be easier than the published method [18] for the determination of OB in presence

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doi:10.1016/j.farmac.2005.06.001

of its degradation product, whereas there is no need for using neither internal standard nor two counter-ions in the eluent but only one counter ion is used. The published method [18] determined neither degradation product of OB nor coexisting preservatives. Moreover, the proposed method is the first publication for determination of OL, its degradation product and coexisting preservatives namely methylparaben (MP) and propylparaben (PP).

2. Experimental

2.1. Instrumentation

The HPLC (Shimadzu, Kyoto, Japan) instrument was equipped with a model series LC-10 ADVP pump, SCL-10 AVP system controller, DGU-12A Degasser, Rheodyne 7725i injector with a 20 μl loop and a SPD-10AVP UV–VIS detector, separation and quantitation were made on a 150 × 4.6 mm (i.d.) Nucleosil 100-5 C_{18} column (5 μm particle size), Macherey–Nagel, Germany for OL and 250 × 4.6 mm (i.d.) VP-ODS Shim-pack column (4.6 μm particle size) for OB. The detector was set at λ 220 nm. Data acquisition were performed on class-VP software.

The IR spectrophotometer used was a Bruker Vector 22, Germany.

PMR spectra were recorded on a Varian Gemini 200 PMR spectrometer (200 mHz), USA.

2.2. Materials and reagents

Pharmaceutical grade of OL (CU Chemie Uetikon GmbH, Germany), OB (Vinar integration, India), MP and PP (Nipa, Britain) were used and certified to contain 99.8%, 99.9%, 99.8%, 99.9%, respectively. Acetonitrile used was HPLC grade (BDH, Poole, UK). Potassium dihydrogen phosphate, diethylamine, sodium hydroxide, phosphoric, hydrochloric, citric and boric acids were analytical grade.

Oxeladine syrup, batch number 4103 (Pharaonia pharmaceuticals, Alexandria, Egypt) was labeled to contain 200 mg OL, 50 mg MP, and 25 mg PP per 100 ml; Paxeladine syrup, batch number 130068, and capsule, batch number 070028 (The Arab Drug Co, Under License of beaufour, France) were labeled to contain 200 or 40 mg of OL per 100 ml syrup or capsule, respectively; Detronin syrup, batch number 4104 (Pharaonia pharmaceuticals) was labeled to contain 100 mg OB, 100 mg MP, and 20 mg PP per 100 ml; Uripan syrup, batch number 020235, and tablets, batch number 020814 (The Egyptian Co. for chemicals and pharmaceuticals, ADWIA, 10th of Ramadan City, Egypt) were labeled to contain 100 or 5 mg of OB per 100 ml syrup or tablet, respectively, were used.

2.3. HPLC conditions

The mobile phases were prepared by mixing acetonitrile and 0.1% phosphoric acid in a ratio 60:40 v/v for OL, or acetonitrile and 0.01 M potassium dihydrogen phosphate and diethylamine in a ratio of 60:40:0.2 v/v for OB. The flow rate was 1.5 ml min^{-1}. All determinations were performed at ambient temperature. The injection volume was 20 μl.

2.4. Preparation of the alkali-induced degradation product

One gram of OL or OB was refluxed separately with 100 ml 0.1 M sodium hydroxide at 100 °C for 3 h. Subsequently the pH of the solution was adjusted to 2.0 or 4.0 using 1 M hydrochloric acid to precipitate the degradation product of OL or OB, respectively. The precipitate was filtered and dried under vacuum. The dried precipitate was analyzed by IR and NMR and found to be α, α-diethylbenzeneacetic acid (DA) as a degradation of OL or α-cyclohexyl-α-hydroxy-benzeneacetic acid (CA) as a degradation of OB.

The stock solution of each of DA and CA were prepared separately by dissolving 25 mg of each in 25 ml methanol.

2.5. Preparation of the acid-induced degradation product

One gram of OL or OB was refluxed separately with 100 ml 1 M hydrochloric acid at 100 °C for 12 h. Subsequently, the pH of the solution was adjusted to 2.0 or 4.0 using 1 M sodium hydroxide to precipitate the degradation product of OL or OB, respectively. The same procedure for separation of DA and CA previously described under preparation of the alkali-induced degradation product was followed.

2.6. Standard solutions and calibration graphs

Stock solutions were prepared by separately dissolving OL, OB, MP and PP in methanol to obtain concentration of 1000 μg ml^{-1} for each compound. The standard solutions were prepared by dilution of the stock solutions with the specified mobile phase to reach concentration ranges of 5–30 μg ml^{-1} for OL, OB and 1–30 μg ml^{-1} for MP, PP and 1–20 μg ml^{-1} for DA, CA. Triplicate 20 μl injections were made for each concentration and chromatographed under the specified conditions described previously. The peak area values were plotted against the corresponding concentrations. Linear relationship was obtained for each compound (Tables 1 and 2).

2.7. Sample preparation

2.7.1. For capsules and tablets

The contents of pellets of 20 Paxeladine capsules or twenty Uripan tablets were weighed and finely powdered. Accurately weighed portion of the powder equivalent to about 20 mg of OL or OB was separately extracted and diluted to 100 ml with methanol. The sample solutions were filtered.

2.7.2. For syrup

Accurately measured volume of syrup equivalent to 20 mg of OL or OB was diluted to 100 ml with methanol. The sample solutions were filtered.

Table 1
Characteristic parameters of the regression equations for OL, DA, MP and PP determined by the proposed HPLC method for OL

Parameters	OL	DA	MP	PP
Calibration range (μg ml^{-1})	5–30	1–20	1–30	1–30
Detection limit (μg ml^{-1})	4.66×10^{-3}	3.66×10^{-3}	4.07×10^{-3}	2.65×10^{-3}
Quantitation limit (μg ml^{-1})	15.53×10^{-3}	12.19×10^{-3}	13.54×10^{-3}	8.82×10^{-3}
Regression equation (Y)[a]:Slope (b)	6.67×10^{3}	10.44×10^{3}	30.24×10^{3}	24.75×10^{3}
Standard deviation of the slope (S_b)	15.71	19.28	62.08	33.10
Relative standard deviation of the slope (%)	0.24	0.18	0.21	0.13
Confidence limit of the slope [b]	6.64×10^{3} –6.70×10^{3}	10.39×10^{3}–10.48×10^{3}	30.11×10^{3}–30.37×10^{3}	24.68×10^{3}–24.82×10^{3}
Intercept (a)	-5.62×10^{2}	-2.36×10^{2}	5.69×10^{2}	2.32×10^{2}
Standard deviation of the intercept (S_a)	3.08×10^{2}	2.50×10^{2}	1.11×10^{3}	5.94×10^{2}
Confidence limit of the intercept [b]	(-1.22×10^{3})–0.99×10^{2}	(-7.72×10^{2})–2.98×10^{2}	(-2.16×10^{3})–3.30×10^{3}	(-10.39×10^{2})–15.03×10^{2}
Correlation coefficient (r)	0.9999	0.9999	0.9999	0.9999
Standard error of estimation	3.55×10^{2}	3.27×10^{2}	1.61×10^{3}	8.98×10^{2}

[a] $Y = a + bC$, where C is the concentration in μg ml^{-1} and Y is the peak area.
[b] 95% confidence limit.

Table 2
Characteristic parameters of the regression equations for OB, CA, MP and PP determined by the proposed HPLC method for OB

Parameters	OB	CA	MP	PP
Calibration range (μg ml^{-1})	5–30	1–20	1–30	1–30
Detection limit (μg ml^{-1})	1.69×10^{-3}	3.16×10^{-3}	1.40×10^{-3}	2.87×10^{-3}
Quantitation limit (μg ml^{-1})	5.64×10^{-3}	10.52×10^{-3}	4.66×10^{-3}	9.58×10^{-3}
Regression equation(Y) [a]:Slope (b)	21.16×10^{3}	49.06×10^{3}	56.97×10^{3}	55.01×10^{3}
Standard deviation of the slope (S_b)	18.09	78.32	40.29	79.87
Relative standard deviation of the slope (%)	0.09	0.16	0.07	0.14
Confidence limit of the slope [b]	21.12×10^{3}–21.20×10^{3}	48.86×10^{3}–49.25×10^{3}	56.87×10^{3}–57.07×10^{3}	54.81×10^{3}–55.21×10^{3}
Intercept (a)	-3.19×10^{2}	3.26×10^{2}	-3.67×10^{2}	-4.92×10^{2}
Standard deviation of the intercept (S_a)	3.55×10^{2}	9.77×10^{2}	7.26×10^{2}	14.37×10^{2}
Confidence limit of the intercept [b]	(-11.88×10^{2}) -5.49×10^{2}	(-20.65×10^{2}) -27.18×10^{2}	(-21.45×10^{2}) -14.09×10^{2}	(-40.09×10^{2}) -30.25×10^{2}
Correlation coefficient (r)	0.9999	0.9999	0.9999	0.9999
Standard error of estimation	3.85×10^{2}	14.04×10^{2}	10.49×10^{2}	21.13×10^{2}

[a] $Y = a + bC$, where C is the concentration in μg ml^{-1} and Y is the peak area.
[b] 95% confidence limit.

Further dilutions of the filtrated capsule, tablet or syrup samples were carried out with the specified mobile phase to provide solutions of 20 μg ml^{-1} for OL or OB. The general procedures for HPLC methods of OL or OB described under calibration were followed.

2.8. Kinetic investigation

Accurately weighed 60 mg of OL or OB were dissolved separately in 100 ml distilled water. Separate 5 ml aliquots of these solutions were transferred into separate stoppered conical flasks and mixed with 5 ml of 0.2 M sodium hydroxide or 2 M hydrochloric acid. The flasks were placed in a thermostatic oven at different temperatures (90, 85, 80, 75, 70 °C for acidic degradation of OL, OB and 90, 85, 80, 70, 60 °C for alkaline degradation of OL and 80, 70, 60, 50, 40 °C for alkaline degradation of OB) for different time intervals. At the specified time the contents of the flasks were neutralized to pH 7.0 using predetermined volumes of 1 M sodium hydroxide and 0.1 M hydrochloric acid solutions. The contents of the flasks were transferred into 100 ml volumetric flasks and diluted to volume with mobile phase. Aliquots of 20 μl of each solution were chromatographed under the conditions described above and the concentrations of the remaining OL or OB were calculated at each temperature and time interval.

2.9. pH-rate profile

Accurately weighed 60 mg of OL or OB were transferred separately into 100 ml volumetric flask and diluted to volume with Britton–Robinson buffer solutions [24]. The pH values of Britton–Robinson buffer solutions used for the measurement of the pH-rate profile of the degradation of OL or OB are within pH 2–12 in one unit pH intervals. The pH values of these buffer solutions were checked before and after the reaction, and were unchanged. Separate 5 ml aliquots of the buffer solution containing OL or OB were transferred into stoppered conical flasks. The flasks were placed in a thermostatic oven at 90 °C for different time intervals. At the specified time interval the contents of the flasks were neutralized to pH 7.0 using 1 M sodium hydroxide or 1 M hydrochloric acid solutions. The contents of the flasks were transferred into 100 ml volumetric flasks and diluted to volume with mobile phase. Aliquots of 20 μl of each solution were chromatographed under the conditions described above and the con-

centrations of the remaining OL or OB were calculated at each pH value and time interval.

3. Results and discussion

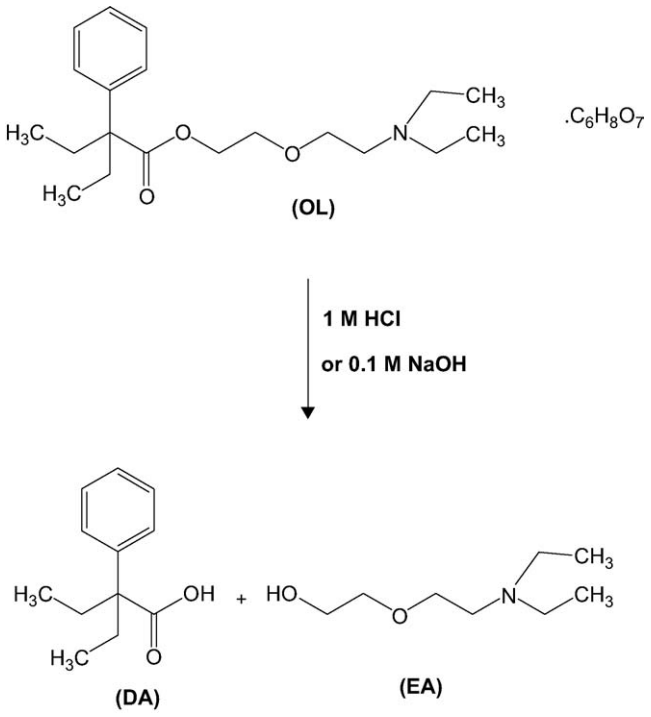
3.1. Identification of the degradation products

When OL or OB were boiled with 0.1 M sodium hydroxide for 3 h or 1 M hydrochloric acid for 12 h, DA or CA could be isolated from the reaction mixture as degradation products of OL or OB, respectively. The suggested pathways for the degradation of OL and OB in 0.1 M sodium hydroxide and 1 M hydrochloric acid are presented in Schemes 1 and 2, respectively.

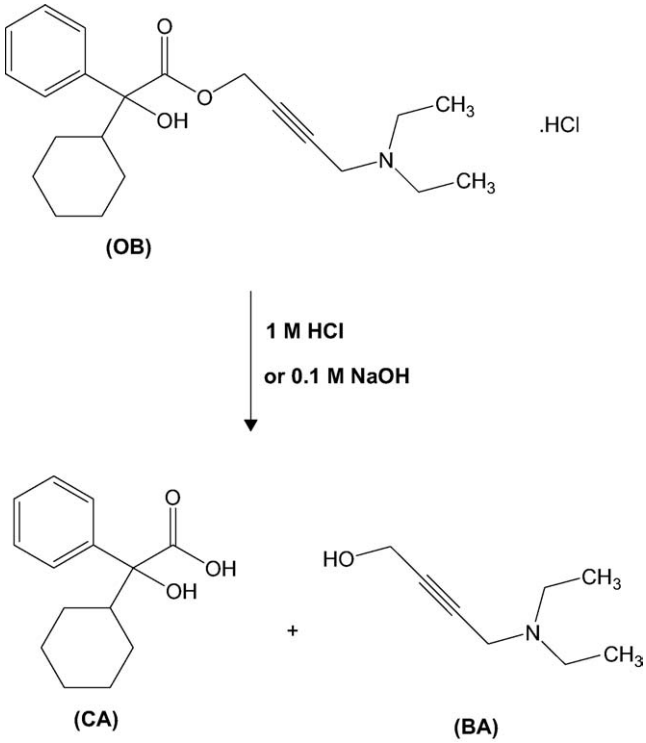
The assignments of the degradation products DA and CA as α, α-diethylbenzeneacetic acid and α-cyclohexyl-α-hydroxy-benzeneacetic acid, respectively, were based on the comparison of the IR and PMR spectral data of the purified specimens, separated from the degradation reaction, with those of the intact OL and OB, respectively.

The IR spectrum (KBr) of OL was characterized by the absorption frequency of C=O ester band at 1731 cm^{-1} and C–N stretching band at 1217 cm^{-1}. On the other hand the IR spectrum (KBr) of DA revealed the OH association, C=O stretching and in-plane C–O bending of the COOH at 2250–3500, 1703 and 1303 cm^{-1}, respectively. Moreover, the spectrum lacked the characteristic ester C=O and C–N stretching bands of OL.

The PMR spectrum of OL in deuterated chloroform was characterized by the appearance of the aromatic protons at δ 7.20–7.29 ppm, quaternary N^+H proton of citrate salt at δ



Scheme 1.



Scheme 2.

10.08 ppm (exchangeable singlet, 1H), protons of the *d*iethyl chain at δ 0.71 ppm (triplet, 6H, C**H**$_3$–CH_2–C) and δ 2.03 ppm (quartet, 4H, CH_3–C**H**$_2$–C); protons of the diethylamino chain at δ 1.17 ppm (triplet, 6H, C**H**$_3$–CH_2–N) and δ 2.80 ppm (quartet, 4H, CH_3–C**H**$_2$–N); and protons of ethoxy ethyl chain at δ 3.06 ppm (triplet, 2H, O–CH_2–CH_2–O– CH_2–C**H**$_2$–N), δ 3.55 ppm (triplet, 2H, O–CH_2–CH_2–O– C**H**$_2$–CH_2–N), δ 3.62 ppm (triplet, 2H, O–CH_2–C**H**$_2$–O– CH_2–CH_2–N) and δ 4.18 ppm (triplet, 2H, O–C**H**$_2$–CH_2–O– CH_2–CH_2–N). On the other hand the PMR spectrum of DA in the same solvent was characterized by the appearance of the aromatic protons at δ 7.32–7.37 ppm and protons of the *d*iethyl chain at δ 0.79 ppm (triplet, 6H, C**H**$_3$–CH_2–C) and δ 2.13 ppm (quartet, 4H, CH_3–C**H**$_2$–C). Moreover, the spectrum lacked the characteristic signals of 2-(diethylamino) ethoxyethyl chain protons and quaternary N^+H proton of OL.

The IR spectrum (KBr) of OB was characterized by the absorption frequency of C=O ester band at 1743 cm^{-1} and C≡C band at 2069 cm^{-1}, C–N stretching band at 1210 cm^{-1}, NH stretching of HCl salt at 2563, 2480 cm^{-1} and broad OH association band at 2250–3500 cm^{-1}. On the other hand, the IR spectrum (KBr) of CA revealed the C=O stretching and in-plane C–O bending of the COOH at 1714 and 1294 cm^{-1}, respectively; alcoholic and carboxylic OH broad association bands at 2250–3500 cm^{-1}. Moreover, the spectrum lacked the characteristic ester C=O, C≡C, C–N stretching and NH stretching bands of OB.

The PMR spectrum of OB in deuterated chloroform was characterized by the appearance of the aromatic protons at δ 7.22–7.59 ppm, alcoholic OH proton at δ 3.56 ppm (exchangeable singlet, 1H), quaternary N^+H proton of hydrochloride

salt at δ 12.28 ppm (exchangeable broad singlet, 1H); and protons of the 2-butynyl chain at δ 4.76 ppm (singlet, 2H, O–C**H$_2$**–C≡C–CH$_2$) and δ 3.85 ppm (singlet, 2H, CH$_2$– C≡C–C**H$_2$**–N). On the other hand the PMR spectrum of CA in the same solvent was characterized by the appearance of the aromatic protons at δ 7.17–7.84 ppm and alcoholic OH proton at δ 4.60 ppm (exchangeable broad singlet, 1H), CH_2 protons of C_3, C_4,C_5 of cyclohexyl at δ 1.12 ppm (multiplet, 6H), CH_2 protons of C_2, C_6 of cyclohexyl at δ 1.66 ppm (quartet, 4H) and CH proton of C_1 of cyclohexyl at δ 2.25 ppm (multiplet, 1H). Moreover, the spectrum lacked the characteristics signals of 2-butynyl chain protons and quaternary N^+H proton of OB.

3.2. Assay parameters

The UV absorption spectra of OL with its degradation product DA or OB with its degradation product CA; and MP and PP are overlapped (Figs. 1 and 2). The absorption bands of OL or OB are extensively overlapped with DA or CA spectra, respectively. The simultaneous determination of OL, DA, MP, PP, or OB, CA, MP, PP by conventional, derivative and derivative ratio spectrophotometry is hindered by strong spectral overlap throughout the wavelength range. The method used to resolve a complex mixture of such compounds is mainly HPLC.

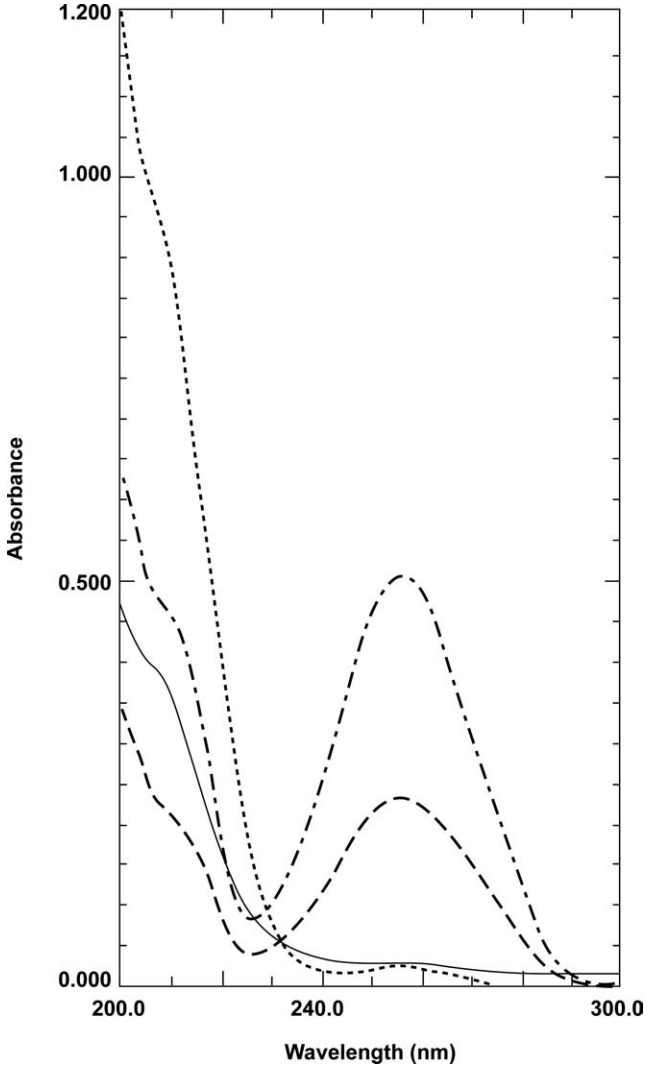


Fig. 1. UV absorption spectra of 20 μg ml^{-1} of OL (——), 20 μg ml^{-1} of DA (------), 5 μg ml^{-1} of MP (– – –) and 2.5 μg ml^{-1} of PP (– –) in distilled water.

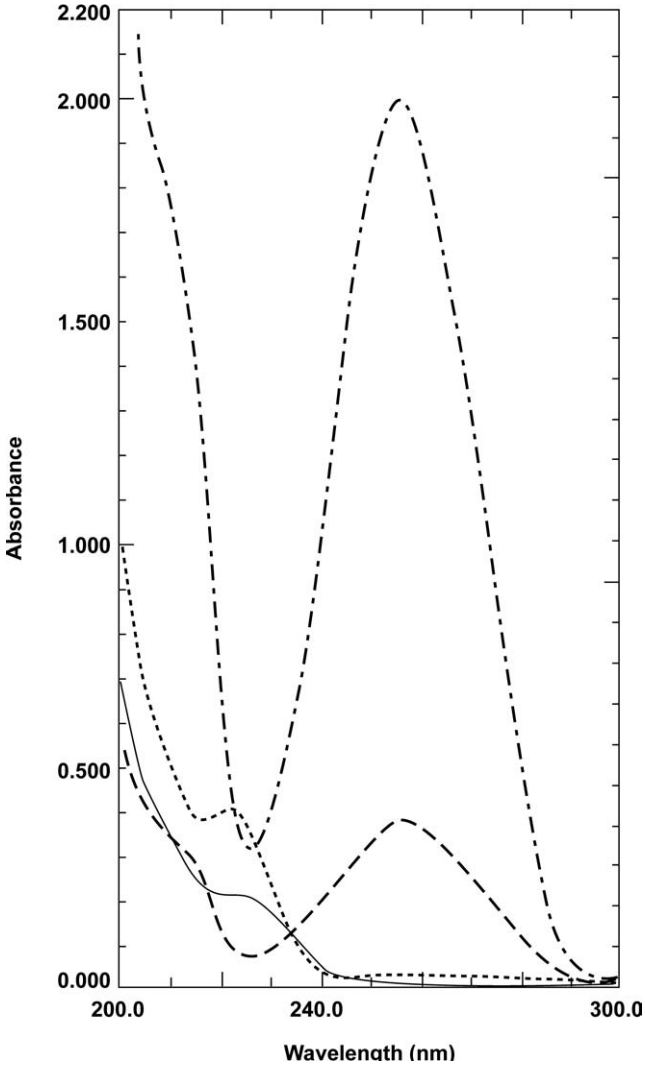


Fig. 2. UV absorption spectra of 20 μg ml^{-1} of OB (——), 20 μg ml^{-1} of CA (------), 20 μg ml^{-1} of MP (– – –) and 4 μg ml^{-1} of PP (– –) in distilled water.

3.2.1. For OL, DA, MP, PP

The developed HPLC method has been applied for the determination of OL, DA, MP and PP. To optimize the HPLC assay parameters, the mobile phase composition was studied. A satisfactory separation was obtained with a mobile phase consisting of acetonitrile/0.1% phosphoric acid (60:40 v/v) at ambient temperature. Increasing acetonitrile concentration to more than 80% led to inadequate separation of the four compounds. At lower acetonitrile concentration (< 40%) separation was occurred but with excessive tailing for OL. Quantitation was achieved with UV detection at 220 nm based on peak area. The specificity of the HPLC method is illustrated in Fig. 3 where complete separation of

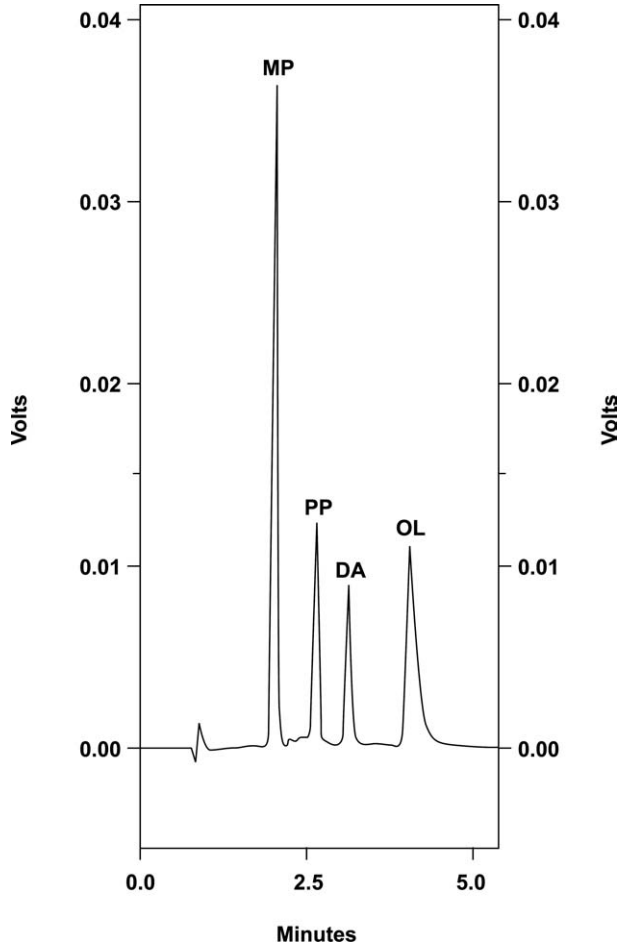


Fig. 3. HPLC chromatogram of 20 μl injection of laboratory-prepared mixture of 5 μg ml^{-1} of MP, 2.5 μg ml^{-1} of PP, 5 μg ml^{-1} of DA and 20 μg ml^{-1} of OL.

OL, DA, MP and PP was noticed. The average retention time ± (standard deviation) S.D. for MP, PP, DA and OL were found to be 2.1 ± 0.004, 2.7 ± 0.006, 3.3 ± 0.004 and 4.1 ± 0.005 min, respectively, for 10 replicates.

3.2.2. For OB, CA, MP, PP

A satisfactory separation For OB, CA, MP and PP was obtained with a mobile phase consisting of acetonitrile/0.01 M potassium dihydrogen phosphate/diethylamine (60:40:0.2 v/v) at ambient temperature. Increasing acetonitrile concentration to more than 80% led to inadequate separation of the four compounds. At lower acetonitrile concentration (< 40%) separation was occurred but with excessive tailing for OB and CA.

The reversed-phase liquid chromatographic separation of basic drugs such as OB often involves the interaction with residual silanol groups, resulting in poor chromatographic performance [18]. The addition of an organic amine such as diethylamine to the aqueous–organic eluent greatly improves the sharpness of the OB peak. Quantitation was achieved with UV detection at 220 nm based on peak area. The specificity of the HPLC method is illustrated in Fig. 4 where complete separation of OB, CA, MP and PP was noticed. The average

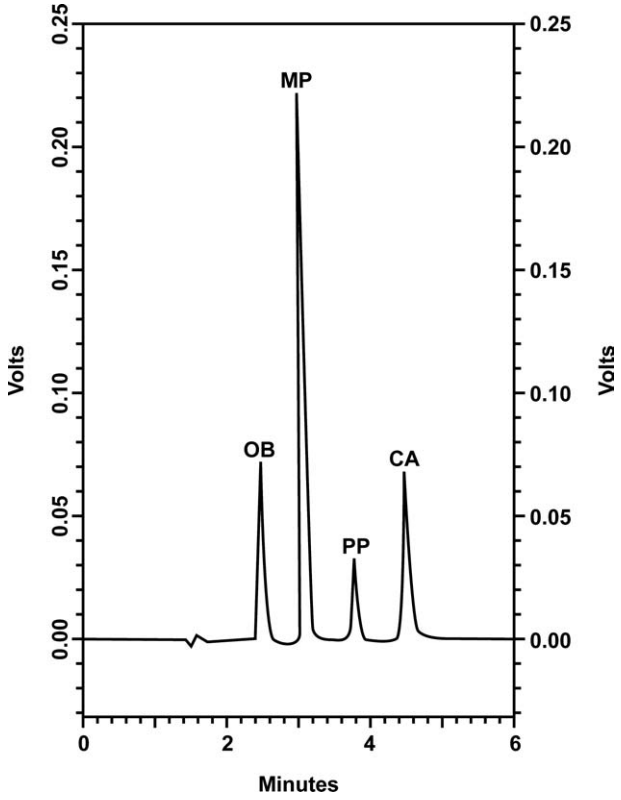


Fig. 4. HPLC chromatogram of 20 μl injection of laboratory-prepared mixture of 20 μg ml^{-1} of OB, 20 μg ml^{-1} of MP, 4 μg ml^{-1} of PP and 10 μg ml^{-1} of CA.

retention time ± S.D. for OB, MP, PP and CA were found to be 2.4 ± 0.003, 3.1 ± 0.005, 3.8 ± 0.004 and 4.5 ± 0.006 min, respectively, for 10 replicates.

3.3. Analysis of pharmaceutical products

The proposed HPLC method was applied to the determination of the studied compounds in commercial pharmaceutical products. Seven replicates determinations were made. Satisfactory results were obtained for each compound in a good agreement with the label claims (Tables 3 and 4). Typical chromatograms obtained for the quantitative analysis of the studied compounds in pharmaceutical products were very similar to that presented in Figs. 3 and 4, except that DA and CA could not be detected. The results of OL and OB in pharmaceutical products, obtained by the proposed method, were compared with the published spectrophotometric and HPLC methods [2,18] for determination of OL and OB, respectively. Statistical comparison of the results was performed with regards to accuracy and precision using Student's *t*-test and the *F*-ratio at 95% confidence level (Tables 3 and 4). There was no significant difference between the results.

Expired batches of Oxeladine syrup and Detronin syrup stored at ambient temperature under normal conditions were analyzed by the proposed HPLC method. The DA and CA as degradation products of OL and OB, respectively, were found. The mean percentage of OL ± S.D. and the mean concentration of DA ± S.D. ($n = 7$) were found to be 90.5 ± 0.62 and

Table 3
Determination of OL, DA, MP and PP in laboratory-prepared mixtures and commercial pharmaceutical products using the proposed HPLC method

	Mean found ± S.D. [a]				
	Proposed HPLC method				Published method [2] for OL
	OL	DA	MP	PP	
Laboratory-prepared mixtures	100.0 ± 0.38	99.9 ± 0.44	100.1 ± 0.51	100.0 ± 0.31	
Oxeladine syrup	102.5 ± 0.41		105.9 ± 0.34	103.8 ± 0.43	102.2 ± 0.62
t	1.06				(2.18) [b]
F	2.28				(4.28) [b]
Recovery [c]	100.1 ± 0.45	100.0 ± 0.41	99.9 ± 0.44	100.1 ± 0.33	
Paxeladine syrup	97.4 ± 0.35		97.5 ± 0.36	98.2 ± 0.44	97.1 ± 0.56
t	1.20				(2.18) [b]
F	2.56				(4.28) [b]
Recovery [c]	99.9 ± 0.52	100.2 ± 0.43	100.1 ± 0.51	99.8 ± 0.49	
Paxeladine capsules	103.3 ± 0.51				103.7 ± 0.72
t	1.20				(2.18) [b]
F	1.99				(4.28) [b]
Recovery [c]	100.2 ± 0.55	99.9 ± 0.43			

[a] Mean and S.D. percentage recovery from the label claim amount.
[b] Theoretical values for *t* and *F* at *P* = 0.05.
[c] For standard addition of 50% of the nominal content.

Table 4
Determination of OB, CA, MP and PP in laboratory-prepared mixtures and commercial pharmaceutical products using the proposed HPLC method

	Mean found ± S.D. [a]				
	Proposed HPLC method				Published method [18] for OB
	OB	CA	MP	PP	
Laboratory-prepared mixtures	100.1 ± 0.31	99.8 ± 0.44	100.0 ± 0.55	99.9 ± 0.39	
Detronin syrup	97.6 ± 0.39		101.9 ± 0.39	102.1 ± 0.50	97.5 ± 0.48
t	0.43				(2.18) [b]
F	1.51				(4.28) [b]
Recovery [c]	100.2 ± 0.42	100.0 ± 0.47	99.8 ± 0.31	100.1 ± 0.54	
Uripan syrup	101.9 ± 0.45				101.7 ± 0.52
t	0.77				(2.18) [b]
F	1.33				(4.28) [b]
Recovery [c]	100.1 ± 0.45	100.2 ± 0.51			
Uripan tablets	103.0 ± 0.39				102.8 ± 0.45
t	0.89				(2.18) [b]
F	1.33				(4.28) [b]
Recovery [c]	99.9 ± 0.48	100.0 ± 0.42			

[a] Mean and S.D., percentage recovery from the label claim amount.
[b] Theoretical values for *t* and *F* at *P* = 0.05.
[c] For standard addition of 50% of the nominal content.

188.1 ± 1.42 μg ml^{-1}, respectively, in expired Oxeladine syrup. While, the mean percentage of OB ± S.D. and the mean concentration of CA ± S.D. ($n = 7$) were found to be 89.2 ± 0.51 and 106.8 ± 0.95 μg ml^{-1}, respectively, in expired Detronin syrup.

3.4. Kinetic investigation

The kinetics of degradation of OL and OB were investigated in 0.1 M sodium hydroxide and 1 M hydrochloric acid, since the decomposition rates of OL or OB at lower strengths of hydrochloric acid were too slow to obtain reliable kinetic data. A regular decrease in the concentration of intact OL and OB with increasing time intervals was observed. At the selected temperatures, the acidic and alkaline degradation processes followed pseudo first-order kinetics (Figs. 5 and 6). From the slopes of the straight lines it was possible to calculate the apparent first-order degradation rate constant and the

Table 5
Degradation rate constant (K_{obs}) and half-life ($t_{1/2}$) for OL and OB in 1 M hydrochloric acid

Temperature (°C)	$K_{obs}(h^{-1})$		$t_{1/2}(h)$	
	OL	OB	OL	OB
90	0.2809	0.0460	2.467	15.065
85	0.1911	0.0320	3.626	21.656
80	0.1315	0.0218	5.269	31.788
75	0.0875	0.0151	7.918	45.894
70	0.0575	0.0101	12.052	68.613

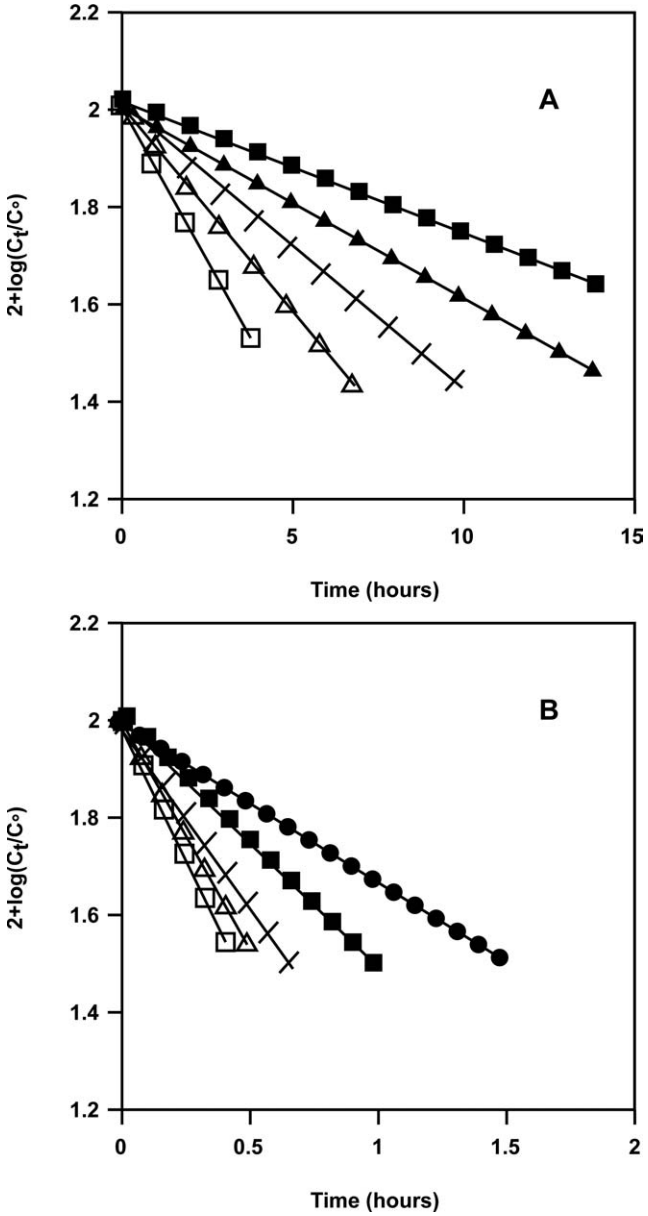


Fig. 5. Pseudo first-order plots for the degradation of OL in 1 M hydrochloric acid (a) and 0.1 M sodium hydroxide (b) at various temperatures. Key: 90(□); 85(Δ); 80(×); 75(▲); 70 (■); and 60 °C (•); C_t, concentration at time t, and C_0, concentration at time zero.

half-life at each temperature for both acidic and alkaline degradation processes of OL and OB (Tables 5–7). Plotting log K_{obs} values versus $1/T$, the Arrhenius plots (Fig. 7) were obtained, which were found to be linear in the temperature range 70–90 °C for the acidic degradation of OL and OB, 60–90 °C and 40–80 °C for alkaline degradation of OL and OB, respectively. The activation energy was calculated to be 19.56 and 18.67 kcal mol^{-1} for acidic degradation process, and 9.65 and 11.25 kcal mol^{-1} for alkaline degradation process of OL and OB, respectively.

The pH-rate profiles of degradation of OL and OB in Britton–Robinson buffer solutions were studied at 90 °C (Fig. 8). Britton–Robinson buffer solutions were used throughout the entire pH range in order to avoid possible effects of different buffer species. The apparent first-order degradation rate constant and the half-life were calculated for each pH value (Table 8). OL and OB were found to be most stable at a pH of 4.0.

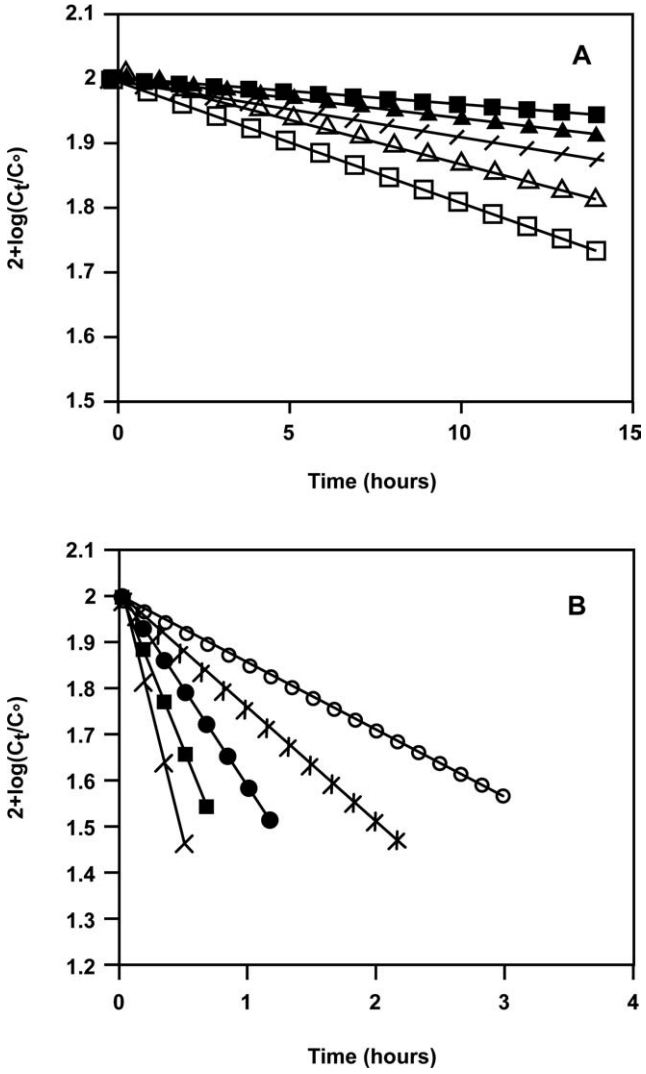


Fig. 6. Pseudo first-order plots for the degradation of OB in 1 M hydrochloric acid (a) and 0.1 M sodium hydroxide (b) at various temperatures. Key: 90(□); 85(Δ); 80(×); 75(▲); 70 (■); 60 (•); 50 (*); and 40 °C (O);C_t, concentration at time t, and C_0, concentration at time zero.

3.5. Validation of the method

3.5.1. Linearity

The linearity of the HPLC detector response for determination of OL, OB, DA, CA, MP and PP was evaluated by

Table 6
Degradation rate constant (K_{obs}) and half-life ($t_{1/2}$) for OL in 0.1 M sodium hydroxide

Temperature (°C)	K_{obs}(h^{-1})	$t_{1/2}$(h)
90	2.6714	0.259
85	2.2569	0.307
80	1.8424	0.376
70	1.2321	0.562
60	0.8060	0.859

Table 7
Degradation rate constant (K_{obs}) and half-life ($t_{1/2}$) for OB in 0.1 M sodium hydroxide

Temperature (°C)	$K_{obs}(h^{-1})$	$t_{1/2}$ (h)
80	2.4609	0.281
70	1.5542	0.445
60	0.9442	0.733
50	0.5504	1.259
40	0.3189	2.173

analyzing a series of different concentrations of each compound. Eight concentrations were chosen, ranging between 5 and 30 μg ml^{-1} for OL and OB, 1–30 μg ml^{-1} for MP and PP; and 1–20 μg ml^{-1} for DA and CA. Each concentration was repeated three times; this approach provided information on the variation in peak area between samples of same concentration. The linearity of the calibration graphs was validated by the high value of the correlation coefficient and the intercept value, which was not statistically ($P < 0.05$) different from zero. Characteristic parameters for regression equations obtained by least squares treatment of the results are given in Tables 1 and 2.

3.5.2. Precision

For evaluation of the precision estimates, repeatability and intermediate precision were performed at three concentration levels for each compound. The data for each concentration level were evaluated by one-way ANOVA. An 8 days × 2 replicates design was performed. Statistical comparison of the results was performed using the *P*-value of the *F*-test. Three univariate analyses of variance for each concentration level were made. Since the *P*-value of the *F*-test is always greater than 0.05, there is no statistically significant difference between the mean results obtained from one level of day to another at the 95% confidence level.

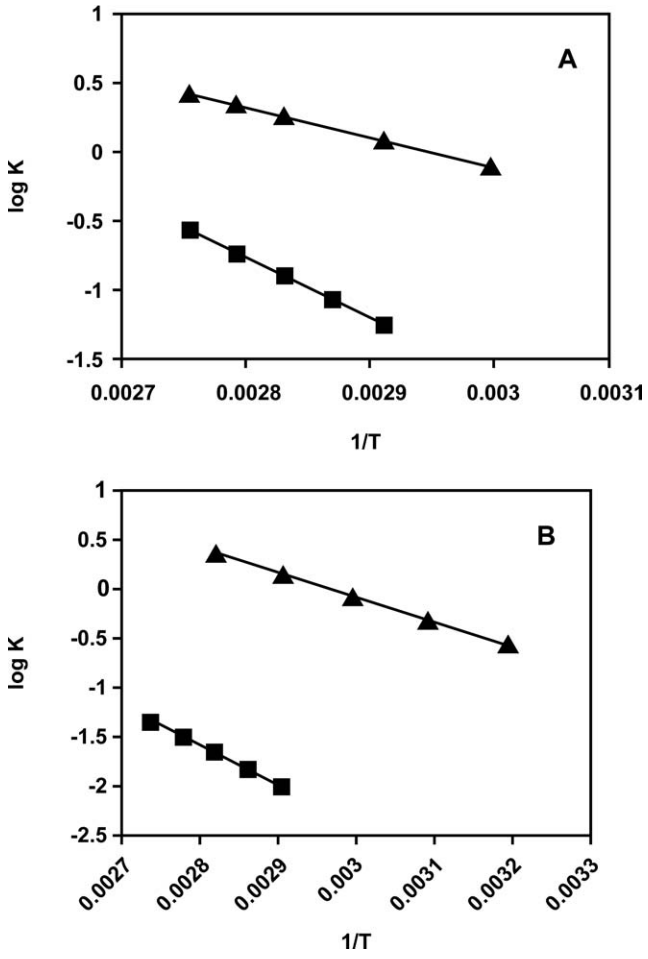


Fig. 7. Arrhenius plots for the degradation of OL (a) and OB (b) in 1 M hydrochloric acid (■) and 0.1 M sodium hydroxide (▲).

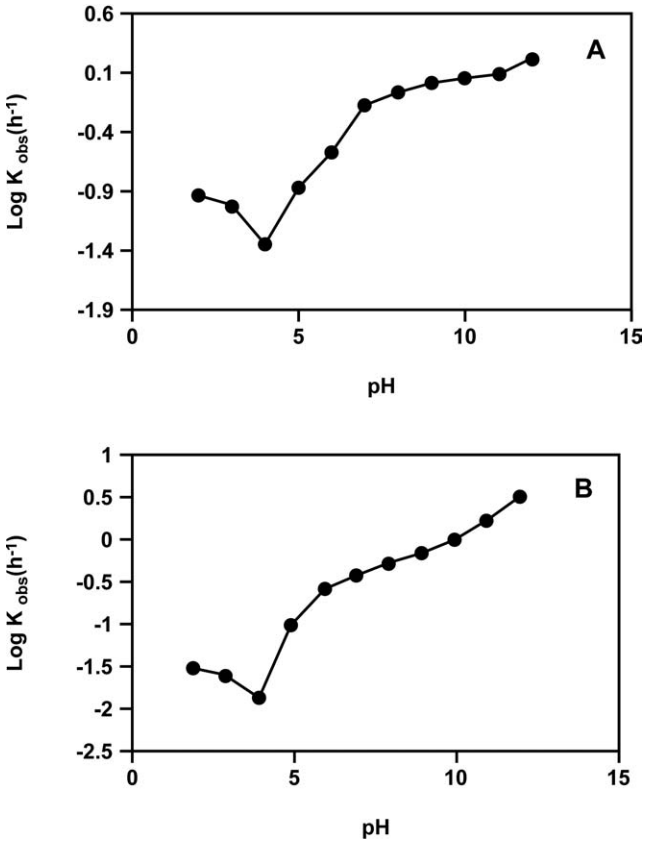


Fig. 8. pH-rate profile for the decomposition of OL (a) and OB (b) in Britton–Robinson buffer at 90 °C.

3.5.3. Range

The calibration range was established through consideration of the practical range necessary, according to each com-

Table 8
Degradation rate constant (k_{obs}) and half-life ($t_{1/2}$) for OL and OB in Britton–Robinson buffer at different pH values and a temperature of 90 °C

pH	k_{obs} (h^{-1})		$t_{1/2}$ (h)	
	OL	OB	OL	OB
2	0.1179	0.0260	5.877	26.653
3	0.0948	0.0211	7.310	32.843
4	0.0232	0.0121	29.870	57.272
5	0.1382	0.0806	5.014	8.598
6	0.2789	0.2176	2.484	3.184
7	0.7017	0.3224	0.987	2.149
8	0.9074	0.4480	0.763	1.546
9	1.0916	0.5990	0.634	1.157
10	1.1998	0.8686	0.577	0.798
11	1.3219	1.4740	0.524	0.470
12	1.7825	2.8170	0.388	0.246

pound concentration present in pharmaceutical product, to give accurate, precise and linear results. The calibration range of each compound is given in Tables 1 and 2.

3.5.4. Detection and quantitation limits

According to ICH recommendations [25], the approach based on the S.D. of the response and the slope was used for determining the detection and quantitation limits. The theoretical values were assessed practically and are given in Tables 1 and 2.

3.5.5. Selectivity

Method selectivity was achieved by preparing different laboratory-prepared mixtures of OL, DA, MP, PP or OB, CA, MP, PP at various concentrations within the linearity range. The laboratory-prepared mixtures were analyzed. Satisfactory results were obtained (Tables 3 and 4), indicating the high selectivity of the proposed method for determination of the studied compounds.

The proposed method is highly selective towards OL, OB, MP, PP, DA and CA. Regarding 2-[2-(diethylamino)ethoxy] ethyl alcohol (EA) and 4-(diethylamino)-2-butynyl alcohol (BA), it is assumed that their ultraviolet absorption characteristics are relatively low to be detected by the assay conditions used in this work.

3.5.6. Accuracy

This study was performed by addition of known amounts of OL, OB, their degradation products (DA, CA), MP and PP to a known concentration of the commercial pharmaceutical products (standard addition method). The resulting mixtures were assayed and the results obtained for added compounds were compared with the expected results. The excellent recoveries of standard addition method (Tables 3 and 4) suggested the good accuracy of the proposed methods.

Extreme degradation of OL and OB in aqueous solution was achieved by acid and alkaline hydrolysis. No peaks interfering with the elution of OL, OB, MP, and PP were observed in both cases.

The influence of the commonly used syrup, capsule and tablet excipients (glycerin, propylene glycol, sucrose, sorbitol, ethanol, citric acid, sodium citrate, sunset yellow, lactose, starch, magnesium stearate, talc, microcrystalline cellulose) was investigated before the determination of the studied compounds in pharmaceutical products. No interference could be observed with the proposed methods.

3.5.7. Robustness

Variation of the organic strength of the mobile phase by ± 2% did not have a significant effect on chromatographic resolution of the studied compounds.

3.5.8. Stability

The studied compound solutions in mobile phase exhibited no chromatographic changes for 3 h when kept at room temperature, and for 8 h when stored refrigerated at 5 °C.

4. Conclusion

The proposed HPLC method provides simple, accurate and reproducible quantitative analysis for the determination of OL, OB, their degradation products (DA, CA), MP and PP in pharmaceutical products, without any interference from the excipients. It was found that OL and OB are rapidly degraded in alkaline medium, while they are more stable in acidic medium. The most stability of OL and OB was found to be at pH 4.0.

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