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ORIGINAL PAPER

Determination of Oxybutynin in Pharmaceuticals *via* Reaction with Mixed Acids Anhydrides: Application to Content Uniformity Testing

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Abstract Sensitive and simple spectrophotometric (Method I) and spectrofluorimetric (Method II) methods were developed and validated for the determination of oxybutynin HCl (OXB) in its dosage forms. The method was based on the reaction of OXB with malonic acid anhydride in acetic acid anhydride to form a highly yellow colored product that was measured at 375 nm spectrophotometrically. The same reaction product exihibits strong fluorescence that was measured at 440 nm after excitation at 390 nm. The factors affecting formation and stability of the reaction product were carefully studied and optimized, and the reaction mechanism was postulated. The absorbance-concentration plot is rectilinear over the range 4–40 μg/mL with LOD of 1.12 μg/mL and LOQ of 3.39 μg/mL. The fluorescence-concentration plot is rectilinear over the range 0.5–6 μg/mL with LOD of 0.11 μg/mL and LOQ of 0.33 μg/mL. The method was applied to the analysis of commercial tablets Detronin® and Uripan®. Statistical comparison of the results with those of the reference method revealed good agreement and proved that there were no significant difference in the accuracy and precision between the two methods respectively. The study was extended to content uniformity testing.

Keywords Oxybutynin · Malonic acid anhydride · Mixed anhydrides · Spectrophotometry · Spectrofluorimetry · Dosage forms

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Introduction

Oxybutynin (Fig. 1) is is 4-(Diethylamino)but-2-ynyl (*RS*)-2-cyclohexyl-2-hydroxy-2-phenylacetate hydrochloride [1]. It is an antimuscarinic drug with a great selectivity for the muscarinic receptors of the bladder. It is used in the management of urinary frequency, urgency, and incontinence in detrusor instability and in treatment of nocturnal enuresis [2]. A survey of the literature revealed that few methods have been reported for the determination of OXB including spectrophotometry [3, 4], differential pulse polarography [5] and HPLC methods [6–8]. The reported spectrophotometric methods for OXB [3, 4] in spite of being spectrophotometric method but they lack the simplicity which is found usual in this technique.

So the aim of this work is to develop a comparative study of recent, simple and sensitive validated methods that are of lower cost than the reported HPLC methods. Also to develop spectrophotometric and spectrofluorimetric methods able to be applied to content uniformity testing.

Malonic acid anhydride (MAA), is a labeling reagent known to react with tertiary amines and forming stable condensation colored product which can be measured either spectrophotometrically [9] or spectrofluorometrically [10]. Malonic acid anhydride has been utilized for determination of several drugs containing a tertiary amino group such as acyclovir and praziquantel [11], terfenadine [12], tramadol, acebutolol and dothiepin [13]. It was further used for the determination of many alkaloids either in their pure form or in their formulations and biological fluids such as, Pilocarpine, promethazine hydrochloride [9], cocaine base, atropine sulphate, strychnine hydrochloride, lidocaine hydrochloride and scopolamine hydrobromide [14] and

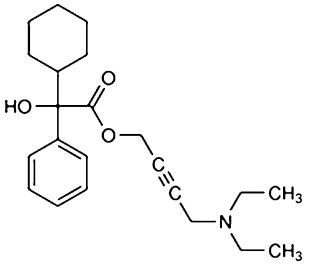


Fig. 1 Structural formula of the studied drug

also was used for the determination of triethylamine, tripentylamine, N,N-diethylaniline and pyridine [15].

Experimental

Apparatus

- Spectrophotometric analysis was carried out on a Shimadzu(Kyoto, Japan) UV-1601 PC, UV–Visible double-beam spectrophotometer with matched 1 cm path-length quartz cells. Absorption spectra were recorded on a fast scan speed, setting slit width to be 1 nm and sampling interval to be auto.
- Spectrofluorimetric measurements were made using Perkin Elmer LS 45 Luminescence Spectrometer equipped with 150 Watt Xenon arc lamp and quartz cell (1 cm).

Materials and Reagents

All reagents and solvents were of Analytical Reagent grade.

- Pure sample of oxybutynin hydrochloride (OXB) was kindly supplied by the Egyptian Company for Chemicals and Pharmaceuticals (ADWIA) (10th of Ramadan City, Egypt), with a purity of 100.06% as determined by the official method [1].
- Acetic acid anhydride was obtained from (Merck, Darmstadt, Germany).
- Malonic acid anhydride (Aldrich Chemical Co. Ltd., USA) was freshly prepared as 10% w/v in acetic acid anhydride for Method I and 1% w/v for Method II.
- Ethanol was obtained from (Merck, Darmstadt, Germany).
- The following tablets containing the drug were purchased from local pharmacies:
- Ditronin® tablets, batch # 1370002, labeled to contain 5 mg OXB HCl/tablet, product of Pharaonia Pharmaceuticals Company (Alexandria, Egypt).
- Uripan® tablets, batch # 091044, labeled to contain 5 mg OXB HCl/tablet, product of ADWIA Co. S.A.E, 10th of Ramadan City, Egypt.

Standard Solution

Standard stock solution of OXB was prepared by dissolving 40.0 mg of the drug in 10 mL of ethanol in 100 mL volumetric flask and completing to the volume with the same solvent. This solution was stable for at least 7 days when kept in the refrigerator. Serial dilution with the same solvent was performed to obtain the appropriate concentration range.

General Procedure

Construction of the Calibration Curve

Spectrophotometric Method (Method I)

Aliquots containing the drug (4–40 μg/mL, final concentration) were quantitatively transferred to a set of screw capped tubes. The solvent was evaporated till dryness using a water bath then the tubes cooled under tap water. To each tube, 2.0 mL of MAA reagent (10% w/v) was added and mixed well. The solutions were heated in thermostatically controlled water bath at 80°C for 25 min. The solutions were cooled and quantitavely transferred to 10 mL volumetric flasks. Each flask was made up to volume with ethanol. The absorbance of the reaction product was measured at 375 nm against a reagent blank prepared simultaneously. The calibration graph was constructed by plotting the absorbance versus the final concentration of the drug (μg/ mL). Alternatively, the corresponding regression equation was derived.

Spectrofluorometric Method (Method II)

Aliquots containing the drug (0.5–6 μg/mL, final concentration) were quantitatively transferred to a set of screw capped tubes. The mentioned procedure under Spectrophotometric method (Method I) was adopted but using 1% (w/v) MAA. The fluorescence intensity was measured at 440 nm after excitation at 390 nm. The calibration graph for the proposed method was constructed by plotting the corrected fluorescence intensity versus the final concentration of the drug (μg/mL). Alternatively, the corresponding regression equation was derived.

Application

Procedure for Commercial Tablets

Ten tablets were finely pulverized and weighed. A weighed quantity of the powdered tablets equivalent to 40 mg of OXB was transferred into a 100 mL volumetric flask, about 80 mL of ethanol was added and the flask was sonicated for 30 min. The volume was completed to the mark with ethanol, mixed

well and filtered. Aliquots containing the drug in the final concentration ranges 4–40 μg/mL for spectrophotometric method or 0.5–6 μg/mL for spectrofluorimetric method were analyzed as described under "*Construction of the Calibration Graph*". The concentration of the drug was determined either from the calibration curve or using the corresponding regression equation.

Procedure for Content Uniformity Testing

The same procedure applied for the analysis of oxybutynin hydrochloride in tablets was adopted using one tablet as a sample. Ten different tablets were assayed and the uniformity of their contents was tested by applying the official USP [16] guidelines (*Chapter 905: Uniformity of Dosage Units*).

Results and Discussion

The reported spectrophotometric methods for OXB is derivative spectrophotometric methods in which the first derivative of ratio spectra (DD1) was used by measuring the peaks amplitude at 216 nm [3] and the second derivative of ratio spectra (DD2) was used, where OXB was determined by measuring the peak amplitude at 236.5 nm [4]. These methods in spite of being spectrophotometric method but they lack the simplicity which is found usual in this technique.

The highly coloured product resulting from the reaction of the mixed anhydride of malonic and acetic acids with tertiary amines is suitable for spectrophotometric determination of tertiary amines [13]. Oxybutynin being tertiary amino compound yields a highly coloured yellow product at 375 nm on heating with malonic-acetic anhydride reagent at 80°C (Fig. 2). The same product exhibits fluorescence at 440 nm after excitation at 390 nm (Fig. 3).

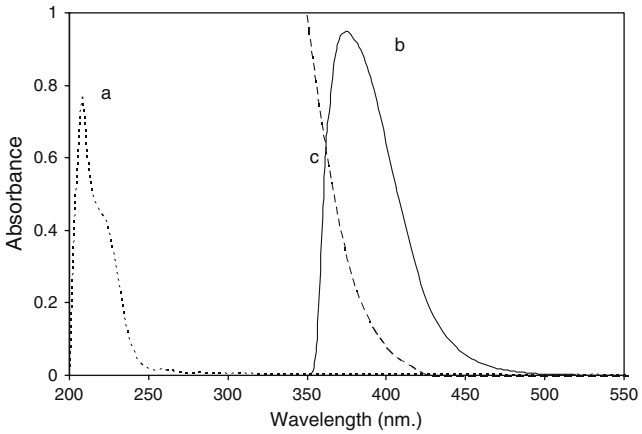


Fig. 2 Absorption Spectra of: **a** OXB (40 μg/mL) in ethanol. **b** The reaction product of OXB (40 μg/mL) with 10% MAA in ethanol. **c** Blank solution of MAA

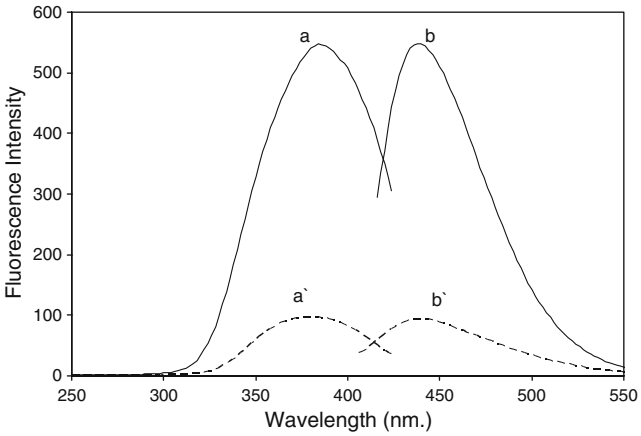


Fig. 3 Fluorescence spectra of: **a**, **b** reaction product of OXB (6.0 μg/mL) with 1% w/v MAA. **a`**, **b`** blank,1% w/v MAA

Optimization of Experimental Parameters

Different experimental parameters were established by varying each in turn while keeping others constant. These factors include; effect of concentration of MAA, effect of diluting solvent, effect of heating temperature and time.

Effect of Volume of MAA

The influence of the concentration of MAA was studied using different volumes of 10% (w/v) and 1% (w/v) solution of MAA in acetic anhydride for spectrophotometry and spectrofluorimetry respectively. It was found that, increasing volume of the reagent produced a proportional increase in the absorbance of the reaction product up to 1.8 mL. However, no further increase in absorbance was observed upon increasing the volume of the reagent up to 2.2 mL, after which further increase produced a gradual decrease in the absorbance value of the reaction product. Therefore, 2±0.2 mL of 10% (w/v) and 1% (w/v) MAA solution was chosen as the optimal volume of the reagent for methods I and II respectively (Fig. 4).

Effect of Heating Temperature and Time

Different temperature settings were tested to ascertain the temperature after which the reaction product attained its maximum absorbance values. Different temperatures were tested using a thermostatically controlled water bath ranged from 50–90°C. It was found that increasing the temperature resulted in a gradual increase in the absorbance value of the reaction product up to 75°C and then remained constant to 85°C. Therefore, the reaction was performed at 80°C within 25±5 min (Fig. 5).

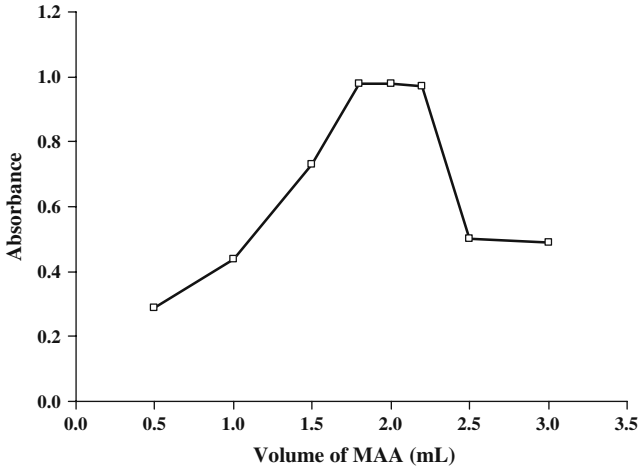


Fig. 4 Effect of volume of MAA 10% (w/v) on the absorbance value of the reaction product of OXB (40 μg/mL) with MAA

Effect of Diluting Solvent

The effect of different diluting solvents was investigated in order to choose the most suitable one for the study. Methanol, acetone and ethanol were tested. Dilution with acetone resulted in a decrease in the absorbance value of the reaction product. Methanol and ethanol gave the same absorbance value. However, the reproducibility upon using methanol was found to be adversely affected. Of all the solvents studied, the highest absorption intensity with maximum product stability was attained upon using ethanol as diluting solvent. Therefore, ethanol was chosen as optimum diluting solvent throughout this approach.

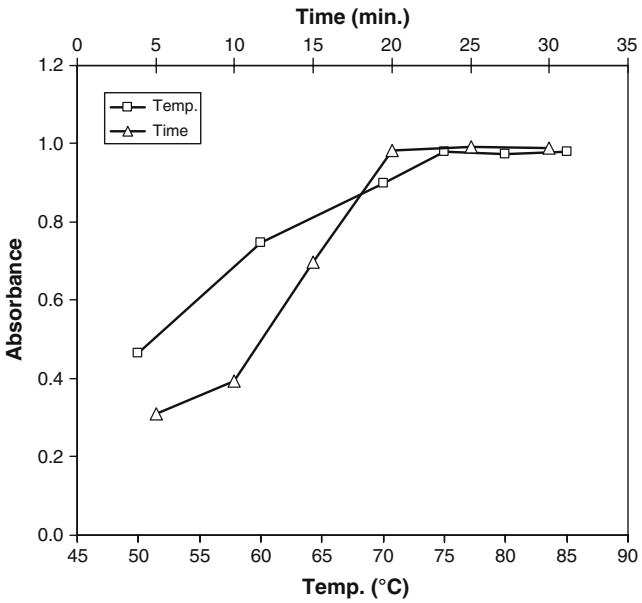


Fig. 5 Effect of heating temperature and time on the absorbance value of the reaction product of OXB (40 μg/mL) with MAA

Analytical Parameters for Oxybutynin

Validation of the Proposed Methods

The validity of the methods was tested regarding linearity, specificity, accuracy, repeatability and precision according to ICH Q2B recommendations [17].

Linearity

The absorbance-concentration plot was found to be linear over the range of 4–40 $\mu g.mL^{-1}$ with minimum detection limit (LOD) of 1.12 μg/mL. The fluorescence-concentration plot was found to be linear over the range of 0.5–6 μg/mL with minimum detection limit (LOD) of 0.11 μg/mL (Table 1)

Linear regression analysis of the data gave the following equations:

$$A = 0.0019 + 0.024\,C \quad (r = 0.9996)$$
$$F = 5.83 + 75.25\,C \quad (r = 0.9998)$$

Where A is the absorbance in 1-cm cell, C is the concentration of the drug (μg/mL), F = fluorescence intensity, r is the correlation coefficient.

Table 1 Performance data of the proposed methods

Parameter	Spectrofluorimetric method	Spectrophotometric method	Official method [1]
Concentration range (μg/mL)	0.5–6	4–40	
LOD (μg/mL)	0.11	1.12	
LOQ (μg/ mL)	0.33	3.39	
Correlation coefficient (r)	0.9998	0.9996	
Slope	75.3	0.024	
Intercept	−5.8	0.002	
$S_{y/x}$	3.48	1.0×10^{-2}	
S_a	2.51	8.2×10^{-3}	
S_b	0.71	3.2×10^{-4}	
% Error	0.35	0.51	
%RSD	0.86	1.24	
Mean found (%)	100.10	100.56	100.35
± SD	0.86	0.51	0.77
Student's t-value	0.425 (2.37)	0.263(2.37)	
Variance ratio *F*-test	1.254 (19.30)	2.653 (5.79)	

$S_{y/x}$ = standard deviation of the residuals.

S_a = standard deviation of the intercept of regression line.

S_b = standard deviation of the slope of regression line

% Error = RSD% / √ n.

Values between parentheses are the tabulated t and F values respectively, at p=0.05. [18]

The limits of quantification (LOQ) and The limits of detection (LOD) were calculated according to ICH Q2B [17] using the following equations:

$$LOQ = 10S_a/b \quad LOD = 3.3S_a/b$$

Where

S_a The standard deviation of the intercept of regression line

b Slope of the calibration curve.

The results are shown in Table 1.

The proposed methods were evaluated by studying the accuracy as percent relative error (% Er) (Table 1) and precision as percent relative standard deviation (% RSD) and the results are shown in Table 1. The small values of % Er and % RSD indicates high accuracy and high precision of the proposed methods.

Accuracy

To prove the accuracy of the proposed methods, the results of the assay of OXB, both in pure form and in pharmaceutical preparations were compared with those of the official comparison methods. Statistical analysis [18] of the results obtained by the proposed and comparison or official method using Student's *t*-test and variance ratio *F*-test showed no significant differences between them regarding accuracy and precision, respectively (Tables 1 and 2). The official method [1] for the pure drug is based on a potentiometric titration with 0.1 M NaOH after dissolving the powdered drug in a mixture of ethanol and 0.01 M HCl. The comparison method for determination of OXB tablets is based on measuring the absorbance of the methanolic solution of OXB at 208 nm [19].

The validity of the methods was evaluated by Statistical analysis of the regression lines regarding the standard deviation of the residuals ($S_{y/x}$), the standard deviation of the intercept (S_a) and standard deviation of the slope (S_b). The results are given in Table 1. The small values of the figures point out to the low scattering of the points arround calibration graph and to the precision of the methods.

Precision

Intra-day

The repeatability was tested by applying the proposed method for the determination of three concentrations of OXB in pure form on three successive times. The low values of standard deviations, % Error indicate high accuracy of the proposed methods , while low values of % RSD indicates high precision of the proposed methods (Table 2).

Inter-day Precision

Inter-day precision was tested by repeated analysis of the drug in pure form using three different concentrations for a period of three successive days. The low values of standard deviations, % Error indicate high accuracy of the proposed methods, while low values of

Table 2 Validation of the proposed methods for the determination of OXB in pure form

Sample concentration	% recovery (repeatability)	% recovery Intermediate precision
Method I		
10 μg/mL		
X^-	101.16	101.70
± SD	0.78	0.64
%RSD	0.78	0.64
% Error	0.45	0.36
20 μg/mL		
X^-	99.97	99.69
± SD	2.18	0.99
%RSD	2.18	0.99
% Error	1.26	0.57
30 μg/mL		
X^-	100.54	99.64
± SD	1.46	1.06
%RSD	1.45	1.06
% Error	0.84	0.62
Method II		
2 μg/mL		
X^-	99.50	98.90
± SD	0.89	0.88
%RSD	0.89	0.88
% Error	0.36	0.36
4 μg/mL		
X^-	99.97	100.16
± SD	0.68	0.69
%RSD	0.68	0.69
% Error	0.28	0.28
6 μg/mL		
X^-	100.39	100.75
± SD	0.88	0.89
%RSD	0.88	0.89
% Error	0.36	0.35

Table 3 Application of the proposed methods to the determination of OXB in different tablets

Preparation	Method I % found	Method II % found	Reference method [19] % found
[a]Ditronin® tablets 5.0 mg OXB HCl/tablet	100.22	99.93	101.05
	99.45	102.50	99.89
	100.00	99.01	99.54
X̄±SD	99.89±0.40	100.48±1.8	100.16±0.79
Student's *t* test	0.528	0.280	
Variance ratio F test	3.971	5.238	
[a]Uripan® tablets 5.0 mg OXB HCl/tablet	101.06	98.45	99.33
	100.23	100.04	100.87
	99.25	100.80	100.25
X̄±SD	100.18±0.91	99.76±1.2	100.15±0.77
Student's *t* test	0.044	0.469	
Variance ratio F test	1.367	2.395	

[a] Ditronin® tablets, batch # 1370002, labeled to contain 5.0 mg OXB HCl/tablet, product of Pharaonia Pharmaceuticals Company (Alexandria, Egypt).

[b] Uripan® tablets, batch # 091044, labeled to contain 5.0 mg OXB HCl/tablet, product of ADWIA Co. S.A.E, 10th of Ramadan City, Egypt.

The tabulated values of t and F are (2.78) and (19.00) respectively, at $p=0.05$ [18]

Each result is the average of three separate determinations

% RSD indicates high precision of the proposed methods (Table 2).

Sensitivity

The sensitivity of the proposed spectrofluorimetric method was revealed by high change in response with the small change in concentration and this was confirmed from high value of the slope.

Selectivity

The selectivity of the method was investigated by observing any interference encountered from the common tablet excepients, such as talc, lactose, starch, avisil, gelatine, and magnesium stearate. These excepients did not interfere with the proposed methods. As revealed by a blank experiment using tablets additives but omitting OXB.

Robustness of the Method

The robustness of the proposed methods was examined against small, deliberate variations in the experimental parameters such as the change in the volume of (10%w/v) and (1%w/v) MAA, (2±0.2 mL), the change in the

Table 4 Results of content uniformity testing of OXB in tablets using the proposed methods

Parameter	Tablet no.	Percentage of the label claim (Method I)		Percentage of the label claim (Method II)	
		Ditronin®[a]	Uripan®[b]	Ditronin®[a]	Uripan®[b]
Data	1	101.12	99.45	98.10	101.29
	2	99.84	99.63	99.24	100.54
	3	99.65	102.50	99.36	99.10
	4	100.59	101.26	101.23	102.36
	5	102.34	102.34	101.80	97.56
	6	101.25	97.91	100.95	100.82
	7	100.23	100.25	101.23	101.24
	8	98.57	99.44	99.35	99.25
	9	97.95	99.56	98.56	97.89
	10	99.60	98.68	99.00	99.63
Mean (X`)		100.09	100.10	99.88	99.97
S.D.		1.30	1.50	1.30	1.55
% RSD		1.29	1.50	1.30	1.55
% Error		0.41	0.47	0.41	0.49
Acceptance value (AV) [16]		3.12	3.60	3.12	3.72
Max. allowed AV (L1) [16]		15.00	15.00	15.00	15.00

[a] Ditronin® tablets, batch # 1370007, labeled to contain 5.0 mg OXB HCl/tablet, product of Pharaonia Pharmaceuticals Company (Alexandria, Egypt)

[b] Uripan® tablets, batch # 0911211, labeled to contain OXB HCl equivalent to 5.0 mg OXB/tablet, product (ADWIA) Co. S.A.E, 10th of Ramadan City, Egypt

heating temperature, (80±5°C) and the change in the heating time, (25±5 min). These minor changes that may take place during the experimental operation did not affect the absorbance or fluorescence of the reaction product. That indicated the reliability of the proposed method during its routine application for the analysis of OXB.

Pharmaceutical Applications

The proposed methods were then applied to the determination of OXB in its tablets. The methods were tested for linearity, selectivity and accuracy according to ICH Q2B recommendations [17].

Accuracy

The results of the proposed methods were statistically compared with those obtained using the reference method [19]. Statistical analysis [18] of the results, using Student's *t*-test and variance ratio *F*-test revealed no significant difference between the performance of the proposed and reference methods regarding the accuracy and precision, respectively (Table 3).

Application to Content Uniformity Testing

Due to the high sensitivity of the proposed methods and their ability to rapidly measure the absorbance of asingle tablet extract with sufficient accuracy, the methods are ideally suited for content uniformity testing which is a time-consuming process when using conventional assay techniques. The steps of the test were adopted according to the USP [16] procedure. The acceptance value(AV) was calculated by the following formula: $AV = |M - X| + KS$

Where M = reference value , K = acceptability constant, S = sample standard deviation.

Acceptance value here was found to be smaller than the maximum allowed acceptance value (L1). The results demonstrated excellent drug uniformity as shown in Table 4.

Mechanism of the Reaction

The stoichiometry of the reaction was studied adopting the limiting logarithmic method [20]. It was performed in presence of excess of either reagent or drug. Plots of log absorbance versus each of log [MAA] or log [OXB] gave two straight lines, the slopes of them were 0.95 and 0.92 for MAA and OXB, respectively (Fig. 6). Hence, it was concluded that, the reaction proceeds in the ratio of 1:1. i.e, it is suggested that one molecule of the drug reacts with one molecule of MAA. Based on the observed

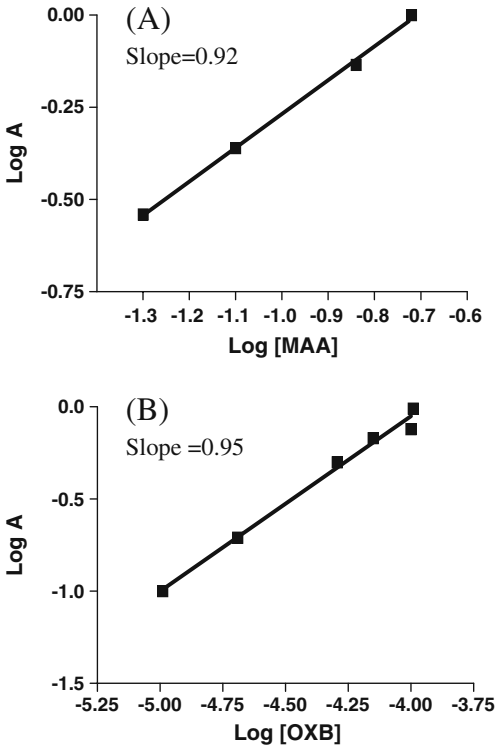
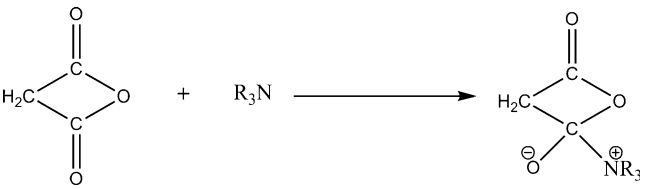


Fig. 6 Limiting logarithmic plots for the molar reactivity of OXB with MAA: **a** log A *vs* log [MAA]. **b** log A *vs* log [OXB]

molar ratio and by analogy to previous study [12], the mechanism of the reaction is postulated to proceed as shown in scheme 1.

Conclusion

The proposed spectrophotometric and spectrofluorimetric methods for the determination of OXB were found to be sensitive, accurate and simple. The methods were validated and proved to be selective, precise and robust, so they could be used for determination of OXB in pure form and pharmaceutical dosage forms. In addition, the proposed methods are very suitable to be applied in content uniformity testing. Thus, the proposed methods are suitable for routine analysis of OXB in quality control laboratories.



Scheme 1 Proposed reaction pathway between MAA and OXB under the described reaction conditions

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