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Utility of cerium (IV) ammonium sulphate as oxidizing agent for spectrophotometric assay of oxybutynin hydrochloride in pharmaceutical preparations

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Abstract
Sensitive and accurate spectrophotometric methods have been developed for the assay of oxybutynin hydrochloride (OXB) in bulk drug and pharmaceutical preparations. The proposed methods are based on oxidation reaction of OXB with a known excess of cerium (IV) ammonium sulphate (CAS) as an oxidizing agent in acid medium followed by determination of unreacted oxidant by adding a fixed amount of dye e.g. amaranth (AM), rhodamine 6G (Rh6G) and indigo carmine (IC) followed by measuring the absorbance at 520, 530 and 610 nm, respectively. The effect of experimental conditions was studied and optimized. The beer's law was obeyed in the concentration ranges of 2.0-12, 1.0-10, and 2.0-14 µg mL^{-1} using AM, Rh6G and IC dyes, respectively with a correlation coefficient ≥ 0.9994. The calculated molar absorptivity values are 1.8347×10^4, 3.285×10^4 and 2.5097×10^4 L mol^{-1} cm^{-1} using AM, Rh6G and IC dyes, respectively. The limits of detection and quantification were reported. Intra-day and inter-day accuracy and precision of the methods have been evaluated. No interference was observed from the additives. The proposed methods were successfully applied to the assay of OXB in tablets preparations and the results were statistically compared with those of the reported method by applying Student's t-test and F-test. The reliability of the methods was further ascertained by performing recovery studies using the standard addition method.

Keywords: oxybutynin hydrochloride, spectrophotometry, ceric (IV) ammonium sulphate, dyes, tablets

Introduction
Oxybutynin hydrochloride is chemically designated as 4-(diethylamino)-2-butynyl (RS)-2-cyclohexyl-2-hydroxy-2-phenylacetate hydrochloride (Figure 1) [1]. It is an antimuscarinic drug with a great selection for the muscarinic receptors of the bladder. It is used in the management of urinary frequency, urgency, and incontinence in detrusor instability and in the treatment of nocturnal enuresis [2].

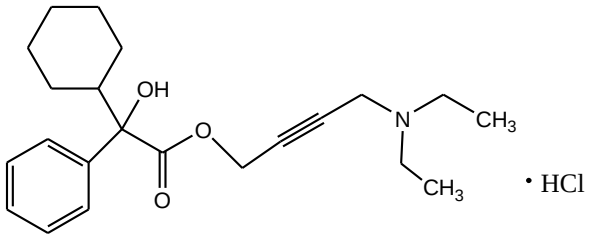


Fig 1: The chemical structure of oxybutynin hydrochloride (OXB).

The literature survey revealed that few methods have been performed for the determination of OXB such as spectrophotometry [3, 10], spectrofluorimetry [5], electrochemical methods [11, 12], HPTLC [9, 13] and HPLC [8, 14, 17]. However, most of these methods were complex, tedious, require expensive experimental setup and skilled personnel, suffer from time-consuming procedures, and are inaccessible to many laboratories in developing and under developed nations.

In contrast, spectrophotometry is considered as the most convenient analytical technique in most quality control and clinical laboratories, hospitals and pharmaceutical industries for the assay of different classes of drugs in pure form, pharmaceutical formulations and biological samples, due to its simplicity and reasonable sensitivity with significant economic advantages. Few spectrophotometric methods have been reported for the quantification of OXB in pharmaceutical formulations [3, 10] (Table 1). However, these previously reported methods suffer from one or other disadvantage such as poor sensitivity, depending on critical experimental variables, few methods require a rigid pH control and tedious and time-consuming liquid-liquid extraction step and use of expensive reagent or large amounts of organic solvents. For these reasons, it was worthwhile to develop a new, simple, cost effective, selective and sensitive spectrophotometric method for the determination of OXB in pure form and tablets.

Table 1: Comparison between the report spectrophotometric methods for determination of OXB.

Method	Wavelength (nm)	Beer's law (μg mL^{-1})	Molar absorptivity (l $mol^{-1}cm^{-1}$)	Detection limit (μg mL^{-1})	Reference
Tropaoline OOO (TPOOO)	480	1.0-7.5	1.954×10^4	0.062	[3]
Alizarin red S (ARS)	430	2.0-15	1.545×10^4	0.11	
2,3-dichloro-5,6-dicayno-p-benzoquinone (DDQ)	457	20 - 80	NA	0.205	[4]
2,5-dichloro-3,6-dihydroxy-p-benzoquinone (p-CLA)	520	30-160	NA	0.524	
Malonic acid anhydride in acetic acid anhydride	375	4.0–40	NA	1.12	[5]
Bromothymol blue (BTB)	414	2.5-12.5	0.0579	NA	[6]
Fe(III)/phenanthroline	510	0.8 - 4.8	NA	NA	[7]
Picric acid	344	NA	NA	NA	[8]
Bromocresol purple (BCP)	410	1.0-8.0	2.34×10^4	0.21	[10]
Bromophenol blue (BPB)	416	1.0-12	1.22×10^4	0.19	
CAS/ AM	520	2.0-12	1.8347×10^4	0.58	Proposed work
CAS/ Rh6G	530	1.0-10	3.285×10^4	0.27	
CAS/ IC	610	2.0-14	2.5097×10^4	0.51	

* NA: not available.

From the foregoing paragraphs, cerium (IV) ammonium sulphate (CAS) despite their strong oxidizing power, versatility, and high oxidation potential and stability in solution has not been applied for the assay of OXB in pure forms and tablets. Amaranth (AM), rhodamine 6G (Rh6G) and indigo carmine (IC) dyes are well known for their high absorptivity and have been utilized for estimation of excess oxidant.

The present work aims to develop a simple, rapid, sensitive, accurate, precise, cost-effective and validated spectrophotometric method for the estimation of OXB in pure and dosage forms. The method is based on the oxidation of the investigated drugs with slight excess of CAS in acidic medium. The unconsumed of oxidant is then estimated by adding a fixed amount of AM, Rh6G and IC dyes to form colored species which absorbs maximally at 520, 530 and 610 nm, respectively. The proposed methods have been demonstrated to be superior to the reported methods with respect to speed, simplicity, sensitivity, being accurate and precise, cost effectiveness, eco-friendliness and can be adopted by the pharmaceutical laboratories for industrial quality control.

Experimental

Apparatus

All absorption spectra were made using Varian UV–Vis spectrophotometer (Cary 100 Conc., Australia) equipped with 10 mm quartz cell was used for absorbance measurements. This spectrophotometer has a wavelength accuracy of ±0.2 nm with a scanning speed of 200 nm/min and a bandwidth of 2.0 nm in the wavelength range of 200–900 nm.

Materials and Reagents

All chemicals, solvents and reagents used in this work were of analytical reagent or pharmaceutical grade and all solutions were prepared fresh daily. Bidistilled water was used throughout the investigation.

Pure sample of oxybutynin hydrochloride (OXB) was kindly supplied by the Egyptian Company for Chemicals and Pharmaceuticals (ADWIA) (10^{th} of Ramadan City, Egypt), with a purity of 99.84±0.40% by applying the official method [10]. All pharmaceutical preparations were obtained from commercial sources in the local markets. Ditronin® tablets, labeled to contain 5.0 mg OXB per tablet, product of Pharaonia Pharmaceuticals Pharo Pharma Company (Alexandria, Egypt). Uripan® tablets, labeled to contain 5.0 mg OXB per tablet, product of ADWIA Co. S.A.E, 10^{th} of Ramadan City, Egypt were obtained from commercial sources.

Standard solution

A stock standard solution (100 μg mL^{-1}) of OXB was prepared by dissolving 10 mg of pure OXB in bidistilled water further diluted to 100 mL with bidistilled water in a 100-mL measuring flask. The standard solution was found stable for at least one week without alteration when kept in an amber colored bottle and stored in a refrigerator when not in use.

Reagents

A stock solution of 5.0×10^{-3} mol L^{-1} cerium (IV) ammonium sulphate (CAS) (E-Merk, Darmstadt, Germany) was freshly prepared by dissolving 316.2 mg of [$CeN_4H_{20}S_4O_{18}$, M. Wt. = 632.55 g mol^{-1}] in the least amount of H_2SO_4 (2.0 mol L^{-1}) then completed to the mark in a 100-mL calibrated flask with the same acid and kept in a dark bottle and a refrigerator when not in use. H_2SO_4 solution (2.0 mol L^{-1}) was prepared by adding 10.8 mL of concentrated acid (Merck, Darmstadt, Germany, 98%, Sp. Gr. 1.84) to bidistilled water, cooled to room temperature, transfer to 100 mL with measuring flask, diluted to the mark and standardized as recorded [18]. A stock solution (1000 μg mL^{-1}) amaranth (AM), rhodamine 6G (Rh6G), and indigo carmine (IC) were first prepared by dissolving accurately weighed 112 mg of each dye (Sigma-aldrish, 90 % dye content) in bidistilled water and diluting to volume in a 100-mL calibrated flask. The solution was then diluted 5.0-fold to get the working concentration of 200 μg mL^{-1} of each dye.

Recommended procedure

Different aliquots (0.2-1.2 mL), (0.1-1.0 mL) and (02-1.4 mL) of a standard 100 μg mL^{-1} OXB solution using AM, Rh6G and IC methods, respectively, were transferred into a series of 10 mL calibrated flasks followed by adding 1.0 mL of H_2SO_4 (2.0 mol L^{-1}**)** and 2.0 mL of (5.0×10^{-3} mol L^{-1}) CAS solution for all dyes. The flasks were stoppered, and the contents were mixed well, and the flasks were kept in boiled water bath for

5.0 min with occasional shaking. Finally, the solution was cooled and 1.0 and 1.5 mL of (AM or IC) and Rh6G dye solution (200 µg mL^{-1}) were added to each flask and mixed well, and then the volume was diluted to the mark with bidistilled water. The decrease in color intensity of dye were measured after 5.0 min against reagent blank solution treated similarly omitting TOL drug at their corresponding λ_{max} 520, 530 and 610 nm for AM, Rh6G and IC, respectively. The concentration of unknown was determined in each case from calibration graph which obtained by plotting the concentration of OXB against the decrease in absorbance of dye at the corresponding λ_{max}.

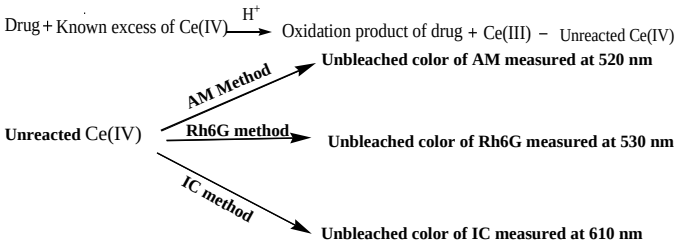
Assay procedure for tablet formulations

The contents of twenty tablets were weighed accurately and ground into a fine powder. An accurate weight of the powdered tablets equivalent to 10 mg OXB was dissolved in bidistilled water with shaking for 5.0 min and filtered using a Whatman No. 42 filter paper. The filtrate was diluted to the mark with bidistilled water in a 100-mL measuring flask to give 100 µg mL^{-1} stock solution of OXB for analysis by the proposed methods. A convenient aliquot was then subjected to analysis by the spectrophotometric procedures described above. Determine the nominal content of the tablets using the corresponding regression equation of the appropriate calibration graph.

Results and Discussion

Absorption spectra

Many dyes are irreversibly destroyed to colorless species by oxidizing agents in acid medium [18]. Cerium (IV) ammonium sulphate (CAS), because of its high oxidation potential and excellent solution stability, has been widely used as an effective analytical reagent in spectrophotometric methods for the determination of many pharmaceutical compounds [19-22]. The analytical reactions involved two steps; the first one was concerned with oxidation of the studied drugs with a known excess of CAS in acidic medium at room temperature (25 ±2 °C). The second step involved the determination of the residual CAS via its reaction with a fixed amount of AM, Rh6G or IC dyes and measuring the absorbance at the respective λ_{max}. The tentative reaction scheme of spectrophotometric methods is shown in Scheme 1. In all methods, the absorbance increased linearly with increasing concentration of OXB. The latter methods make use of the bleaching action of oxidant on dyes, the discoloration being caused by the oxidative destruction of the dye.



Scheme 1: The suggested reaction pathway for the proposed spectrophotometric methods using CAS and dyes.

Optimization of the reaction conditions

The optimum conditions for the assay procedures and color development for each method have been established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species.

Effect of acid type and concentration

To investigate the effect of acid concentration, different types of acids were examined (HCl, H_2SO_4, H_3PO_4, HNO_3 and CH_3COOH) to achieve maximum yield of redox reactions. Better results were suitable in sulphuric acid (H_2SO_4) (2.0 mol L^{-1}) with CAS as oxidant. The effect of H_2SO_4 concentration on the reaction between TOL and CAS was studied by varying the volume of H_2SO_4 (2.0 mol L^{-1} H_2SO_4) from 0.25-3.0 mL, keeping the concentration of oxidant and drug fixed. The results indicated that, at 1.0-2.0 mL of H_2SO_4 (2.0 mol L^{-1}), there were almost same absorbance values were obtained in the presence of TOL (Figure 2.). At the acid volumes less than 1.0 mL, reaction led to go slower and incomplete. Therefore, 1.0 mL of H_2SO_4 (2.0 mol L^{-1}) was the optimum volume for subsequent studies for OXB.

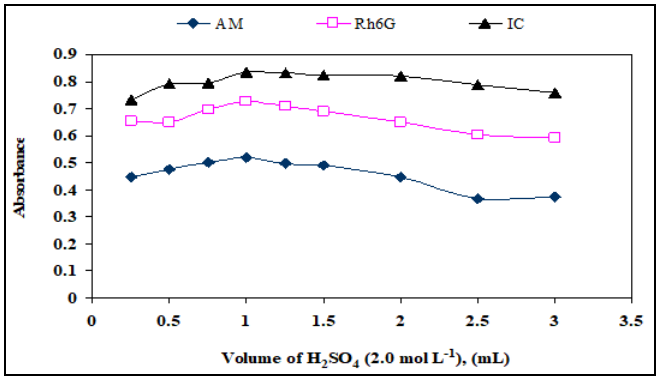


Fig 2: Effect of volume of H_2SO_4 (2.0 mol L^{-1}) on the absorbance of OXB (10 µg mL^{-1}) with CAS (5.0 x 10^{-3} mol L^{-1}) and (200 µg mL^{-1}) AM, Rh6G and IC dyes

Effect of CAS concentration

To investigate the optimum concentration of CAS, different volumes of oxidant were treated in the range of 0.25–3.0 mL with a fixed concentration dyes in optimum acidic medium and the absorbance was measured at optimum wavelength. The results indicate that the maximum and constant absorbance was achieved with 2.0 mL of CAS (5.0×10^{-3} mol L^{-1}) solution was taken as the optimum concentration for all measurements (Figure 3).

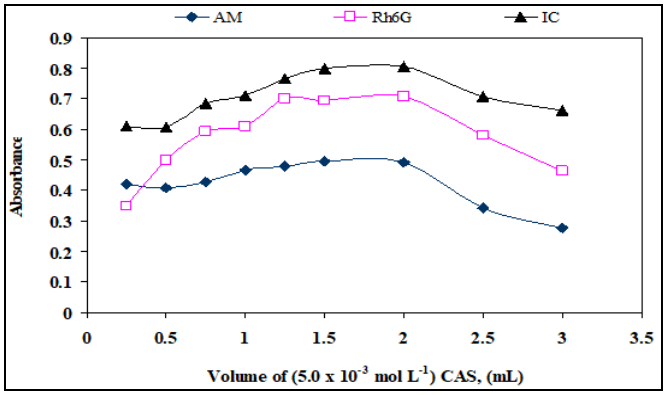


Fig 3: Effect of volume of CAS oxidant on the absorbance of the reaction product: (OXB (10 µg mL^{-1}); dyes (200 µg mL^{-1}) in optimum acidic medium).

Effect of dye concentration

The effect of dye concentration on the intensity of the color developed was carried out to obtain the optimum concentration of dyes that produces the maximum and reproducible color intensity by reducing the residual of CAS. The effect dye concentration was studied using different volumes (0.25–3.0 mL) of the studied dyes (200 µg mL^{-1}) AM, Rh6G and IC. It was observed that maximum color intensity of the oxidation products was achieved with 1.0 and 1.5 of (AM or IC) and Rh6G dye solution, respectively (Figure 4). The color was found to be stable up to 12 h.

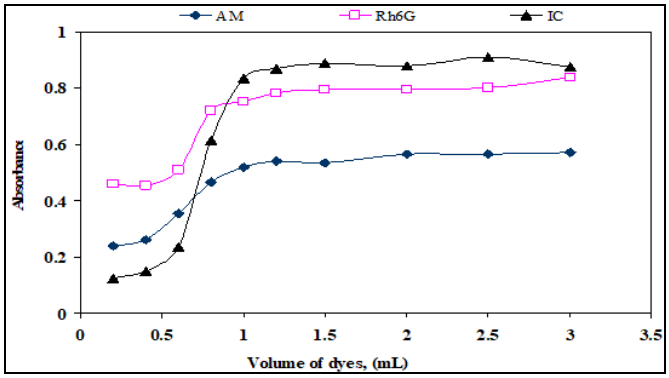


Fig 4: Effect of volume of dyes on the absorbance of the reaction product: (OXB (10 µg mL^{-1}); CAS (5.0×10^{-3} mol L^{-1}) in optimum acidic medium).

Effect of temperature and mixing time

The effect of temperature was studied by heating a series of sample and blank solutions at different temperatures ranging from 25 to 100 °C in water bath. It was found that raising the temperature accelerate the oxidation process and give reproducible results, so maximum color intensity was obtained in boiling water bath. The effect of mixing time required completing oxidation of TOL and for reducing the excess oxidant was studied by measuring the absorbance of sample solution against blank solution prepared similarly at various time intervals 2.0-20 min. It was found that the contact times gave constant and reproducible absorbance values at 5.0 min in boiling water bath (Figure 5). After oxidation process, 5.0 min standing time was found necessary for the complete bleaching of the dye color by the residual CAS and the absorbance of the unreacted dye was stable for at least 12 h, thereafter.

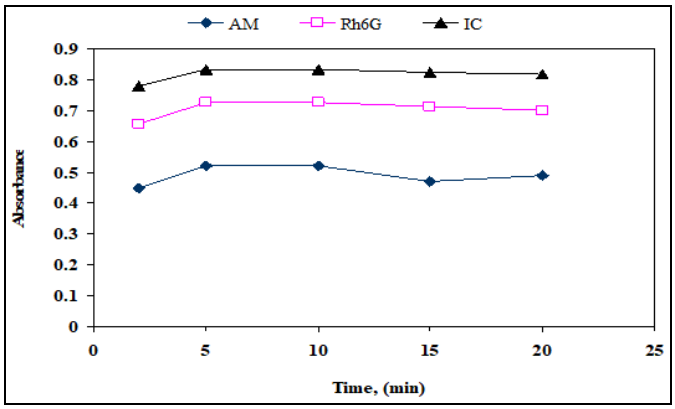


Fig 5: Effect of time on the absorbance of the reaction product: (OXB (10 µg mL^{-1}); CAS (5.0×10^{-3} mol L^{-1}) and dyes (200 µg mL^{-1}) in optimum acidic medium).

Effect of sequence of addition

After optimizing all other experimental variables, further experiments were performed to ascertain the influence of sequence of addition of reactants on the color development by measuring the absorbance. The optimum sequence of addition was OXB-H_2SO_4-CAS-dye. Other sequences gave lower absorbance values under the same experimental conditions.

Method validation

The proposed methods have been validated for linearity, sensitivity, precision, accuracy, selectivity and recovery.

Linearity and sensitivity

Under the optimum conditions a linear correlation was found between absorbance at λ_{max} and the concentration of OXB in the ranges of 2.0-12, 1.0-10 and 2.0-14 µg mL^{-1} using AM, Rh6G and IC methods, respectively. The calibration graph is described by the equation:

$$A = a + b\,C \qquad (1)$$

Where A= absorbance, a= intercept, b= slope and C= concentration in µg mL^{-1}, obtained by the method of least squares. Correlation coefficient, intercept and slope of the calibration data are summarized in Table 2. For accurate determination, Ringbom concentration range [23] was calculated by plotting log concentration of drug in µg mL^{-1} against transmittance % from which the linear portion of the curve gives an accurate range of microdetermination of OXB and represented in Table 2. Sensitivity parameters such as

apparent molar absorptivity and Sandell's sensitivity values, as well as the limits of detection and quantification, were calculated as per the current ICH guidelines [24] and illustrated in Table 2. The high molar absorptivity and lower Sandell's sensitivity values reflects the good and high sensitivity of the proposed methods. The validity of the proposed methods was evaluated by statistical analysis [25] between the results achieved from the proposed methods and that of the reported method. Regarding the calculated Student's *t*-test and variance ratio *F*-test (Table 2), there is no significant difference between the proposed and reported method [10] regarding accuracy and precision.

The limits of detection (LOD) and quantification (LOQ) were calculated according to the same guidelines using the formulas [24, 25]:

$$LOD=3.3\sigma/s \text{ and } LOQ=10\sigma/s \qquad (2)$$

Where σ is the standard deviation of five reagent blank determinations, and is the slope of the calibration curve.

Table 2. Analytical and regression parameters of proposed oxidation spectrophotometric methods for determination of OXB.

Parameters	AM	Rh6G	IC
Beer's law limits, µg mL^{-1}	2.0-12	1.0-10	2.0-14
Ringboom limits, µg mL^{-1}	4.0-10	3.0-8.0	4.0-12
Molar absorptivity, x 10^4 L mol^{-1} cm^{-1}	1.8347	3.285	2.5097
Sandell sensitivity, ng cm^{-2}	21.47	11.99	15.70
Regression equation[a]			
Intercept (a)	0.0052	0.0087	0.0019
Standard deviation of intercept (S_a)	0.05	0.042	0.064
Slope (b)	0.045	0.079	0.0643
Standard deviation of slope (S_b)	0.042	0.054	0.038
Correlation coefficient, (r)	0.9995	0.9994	0.9994
Mean ± SD	99.25±0.70	99.50±0.80	99.70±0.95
RSD%	0.71	0.80	0.95
RE%	0.74	0.84	1.0
Limit of detection, µg mL^{-1}	0.58	0.27	0.51
Limit of quantification, µg mL^{-1}	1.93	0.90	1.70
Calculated *t*-value [b]	0.69	0.72	0.57
Calculated *F*-value [b]	1.65	1.38	1.95

[a] $A = a + bC$, where C is the concentration in µg mL^{-1}, A is the absorbance units, a is the intercept, b is the slope.
[b] The theoretical values of t and F are 2.57 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom (p= 0.05).

Accuracy and precision

To evaluate the precision of the proposed methods, solutions containing three different concentrations of OXB were prepared and analyzed in six replicates. The analytical results obtained from this investigation are summarized in Table 3. Lower values of the relative standard deviation (RSD %) and percentage relative error (RE %) indicate the precision and accuracy of the proposed methods. The percentage relative error is calculated using the following equation:

$$\%\,R.E. = \left[\frac{found - taken}{taken}\right] \times 100 \qquad (3)$$

The assay procedure was repeated six times, and percentage relative standard deviation (RSD %) values were obtained within the same day to evaluate repeatability (intra-day precision) and over five different days to evaluate intermediate precision (inter-day precision).

For the same concentrations of drugs inter- and intra-day accuracy of the methods was also evaluated. The percentage recovery values with respect to found concentrations of each drug were evaluated to ascertain the accuracy of the methods. The recovery values close to 100% as compiled in Tables 3 shows that the proposed methods are very accurate.

Table 3: Results of intra-day and inter-day accuracy and precision study for OXB obtained by the proposed CAS method.

Method	Taken (µg mL^{-1})	Recovery %	Precision RSD % [a]	Accuracy RE %	Confidence Limit [b]
	Intra-day				
AM	4.0	99.00	0.50	-1.0	3.96± 0.021
	8.0	99.90	0.85	-0.10	7.992 ± 0.071
	12	100.70	1.10	0.70	12.084 ± 0.14
Rh6G	3.0	100.30	0.73	0.30	3.009 ± 0.023
	6.0	99.50	1.15	-0.50	5.97 ± 0.072
	9.0	100.50	1.40	0.50	9.045 ± 0.133
IC	4.0	99.70	1.10	-0.30	3.988 ± 0.046
	8.0	100.30	0.90	0.30	8.024 ± 0.076
	12	99.10	1.60	-0.90	11.892 ± 0.20

AM	Inter-day				
	4.0	100.30	0.90	0.30	4.012 ± 0.034
	8.0	99.60	1.20	-0.40	7.968 ± 0.10
	12	99.00	1.30	-1.0	11.88 ± 0.162
Rh6G	3.0	100.60	0.75	0.60	3.018 ± 0.024
	6.0	101.0	0.90	1.00	6.06 ± 0.057
	9.0	99.40	1.50	-0.60	8.946 ± 0.141
IC	4.0	99.50	0.80	-0.50	3.98 ± 0.033
	8.0	98.80	1.60	-1.20	7.904 ± 0.133
	12	100.90	1.70	0.90	12.108 ± 0.216

[a] *RSD%, percentage relative standard deviation; RE%, percentage relative error.*
[b] *Mean ± standard error.*

Robustness and ruggedness

Robustness was examined by evaluating the influence of small variation of method variables, including concentration of analytical reagents and reaction time on the performance of the proposed methods. In these experiments, one parameter was changed whereas the others were kept unchanged, and the recovery percentage was calculated each time. The analysis was performed with altered conditions by taking three different concentrations of drugs and it was found that small variation of method variables did not significantly affect the procedures as shown by the RSD values in the range of 0.60-2.50%. This provided an indication for the reliability of the proposed methods during its routine application for the analysis of OXB and so the proposed spectrophotometric methods are considered robust. Ruggedness was expressed as the RSD and was also tested by applying the proposed methods to the assay of OXB using the same operational conditions but using three different instruments as well as three different anaysts. The inter-analysts RSD were in the ranges 0.50-2.30%, whereas the inter-instruments RSD ranged from 0.90-2.60% suggesting that the developed methods were rugged. The results are shown in Table 4.

Table 4: Results of method robustness and ruggedness.

Methods	**Nominal amount concentration (µg mL^{-1})**	**RSD%**			
		Robustness		**Ruggedness**	
		Variable alerted [a]			
		Acid volume (n=3)	**Reaction time (n=3)**	**Different analysts (n=3)**	**Different instruments (n=3)**
AM	4.0	0.80	1.10	0.50	0.90
	8.0	1.20	1.60	1.40	1.30
	12	2.10	2.50	1.90	2.0
Rh6G	3.0	1.30	0.75	0.90	1.0
	6.0	1.50	1.50	1.50	1.50
	9.0	2.0	1.90	2.10	2.30
IC	4.0	1.20	0.60	1.50	1.40
	8.0	1.60	1.70	2.10	1.80
	12	2.20	2.40	2.30	2.60

[a] *Volume of (2.0 mol L^{-1}) H_2SO_4 is (1.0±0.2 mL) and reaction time is (5.0±2.0 min) (after adding CAS) were used.*

Recovery studies

To ascertain the accuracy, reliability and validity of the proposed methods, recovery experiment was performed through standard addition technique. This study was performed by spiking three different levels of pure drug (50, 100 and 150% of the level present in the tablet) to a fixed concentration of tablet powder solution (pre-analysed) and the total concentration was found by the proposed methods. The determination with each level was repeated three times and the percent recovery of the added standard was calculated from:

$$\textbf{\% Recovery} = \frac{[C_F - C_T]}{C_p} \times 100 \qquad (4)$$

Where C_F is the total concentration of the analyte found, C_T is a concentration of the analyte present in the tablet preparation; C_P is a concentration of analyte (pure drug) added to tablets preparations. The results of this study presented in Table 5 revealed that the accuracy of the proposed methods was unaffected by the various excipients present in tablets which did not interfere in the assay.

Table 5: Results of recovery experiments by standard addition method for the determination of OXB in tablets using the proposed methods.

Samples	Taken drug in Tablet (µg mL^{-1})	Pure drug Added (µg mL^{-1})	AM		Rh6G		IC	
			Total found (µg mL^{-1})	Recovery [a] (%) ± SD	Total found (µg mL^{-1})	Recovery [a] (%) ± SD	Total found (µg mL^{-1})	Recovery [a] (%) ± SD
Ditronin® tablets (5.0 mg)	4.0	2.0	5.964	99.40 ± 0.60	5.94	99.00 ± 0.80	5.946	99.10 ± 0.50
	4.0	4.0	8.04	100.50±0.75	7.936	99.20 ± 1.0	8.04	100.50 ± 0.9
	4.0	6.0	10.08	100.80±1.20	9.94	99.40 ± 1.30	9.90	99.00 ± 1.50
Uripan® tablets (5.0 mg)	4.0	2.0	5.91	98.50 ± 0.50	6.018	100.30±0.48	5.952	99.20 ± 0.40
	4.0	4.0	7.928	99.10 ± 0.90	7.896	98.70 ± 0.70	7.92	99.00 ± 0.80
	4.0	6.0	9.95	99.50 ± 1.40	9.96	99.60 ± 1.10	10.05	100.50±1.30

[a] *Average of six determinations.*

Application of pharmaceutical formulations

The proposed methods were applied to the determination of OXB in tablets. The results in Table 6 showed that the methods are successful for the determination of OXB and that the excipients in the dosage forms do not interfere. A statistical comparison of the results obtained from the assay of OXB by the proposed methods and the reported method [25] for the same batch of material is presented in Table 6. The results agree well with the label claim and were in agreement with the results obtained by the reported method [10]. When the results were statistically compared with those of the reported methods by applying the Student's t-test for accuracy and F-test for precision, the calculated t-value and F-value at 95% confidence level did not exceed the tabulated values for five degrees of freedom [25]. Hence, no significant difference between the proposed methods and the reported methods at the 95 % confidence level with respect to accuracy and precision.

Table 6: Results of analysis of tablets by the proposed methods for the determination of OXB and statistical comparison with the reported method [25].

Samples	Recovery [a] (%) ± SD			Reported method [25]
	AM	Rh6G	IC	
Ditronin® tablets	99.80 ± 0.60	99.10 ± 0.85	99.60 ± 0.30	99.40 ± 0.50
t-value [b]	1.15	0.68	0.77	
F-value [b]	1.44	2.89	2.78	
Uripan® tablets	99.00 ± 0.75	99.40 ± 0.60	99.50 ± 0.70	99.20 ± 0.40
t-value [b]	0.53	0.62	0.93	
F-value [b]	3.52	2.25	3.06	

[a] *Average of six determinations.*

[b] *The theoretical values of t and F are 2.571 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom (p = 0.05).*

Conclusions

A new, useful simple, rapid and cost-effective spectrophotometric methods have been developed for determination of OXB in bulk drug and in tablets using CAS as oxidizing agents and validated as per the current ICH guidelines. The present spectrophotometric methods are characterized by simplicity of operation, high selectivity, comparable sensitivity, low-cost instrument, they do not involve any critical experimental variable and are free from tedious and time-consuming extraction steps and use of organic solvents unlike many of the previous method reported for OXB. The assay methods have some additional advantages involve less stringent control of experimental parameters such as the stability of the colored system, accuracy, reproducibility, time of analysis, temperature independence and cheaper chemicals. These advantages encourage the application of the proposed methods in routine quality control analysis of OXB in pure and dosage forms. So, in the future research we recommend more studies on the application of the proposed methods for other pharmaceutical compounds,

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