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Determination of ipratropium bromide in vials using kinetic and first-derivative spectrophotometric methods

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Abstract

Two sensitive and accurate spectrophotometric methods are presented for the determination of ipratropium bromide (IPB). The first method, kinetic method, is based on the alkaline oxidation of IPB with $KMnO_4$. At a fixed time of 20 min, the formed manganate ion is measured at 608 nm. The concentration of IPB is calculated using the regression equation for the fixed-time method, at 20 min. The determination of IPB by fixed-concentration and rate-constant methods is feasible with regression equations obtained, but the fixed-time method was found to be more applicable. The second method uses first-derivative (D_1-) spectrophotometry for the determination of IPB at 254–268 nm. The applicability of the proposed methods was examined by analyzing Atrovent® unit dose vials and the percentage recoveries were 100.01 ± 1.16, 100.02 ± 0.97, for kinetic and D_1- methods, respectively.

Keywords: Ipratropium bromide; Kinetic method; First-derivative spectrophotometry

1. Introduction

Ipratropium bromide (IPB), chemically known as, (1R, 3r, 5S, 8r)-8-isopropyl-3-[(±)tropyloxy]-tropanium bromide, is a quaternary ammonium compound with anticholinergic properties [1] (Fig. 1).The drug is used by inhalation, in the treatment of obstructive airways disease and allergic rhinitis [2–4].

Screening of the literature revealed that IPB was determined using HPLC in aerosol [5–7] or in nebulizer solution [8]. Radio-receptor assays were developed for the determination of IPB in human plasma and urine [9,10]. A non-aqueous titration method was also reported for the determination of IPB [11]. The BP (1998) reported a HPLC method for the assay of IPB in pressurised inhalation [12]. No spectrophotometric methods, concerning the determination of IPB in pharmaceutical preparations were reported.

Alkaline $KMnO_4$ was used for the determination of different organic compounds [13]. Derivative spectrophotometry is an analytical technique, which offers a convenient solution to a number of analytical problems, especially its potential for increasing the detection sensitivity of minor spectral features [14,15].

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IPB is devoid of structural conjugation and it is therefore characterized by its low ability to absorb light in the UV-region ($A(1\%, 1\ cm) = 40$ at 254 nm, in water). The aim of the present work was the development of simple and sensitive analytical methods for the assay of IPB in pharmaceutical preparations. A kinetically-based method, was de-

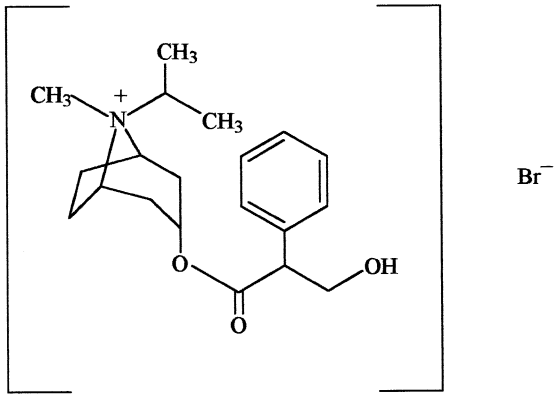


Fig. 1. Structure formula of ipratropium bromide (IPB).

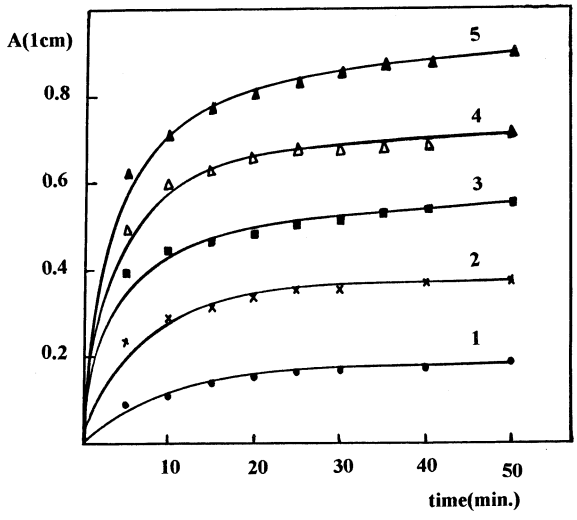


Fig. 2. Absorbance versus time graphs for the reaction between IPB and $KMnO_4$, at room temperature: showing the dependence of the reaction on IPB concentration. Concentrations of IPB: (1) 2.3×10^{-6} M; (2) 6.9×10^{-6} M; (3) 1.15×10^{-5} M; (4) 1.84×10^{-5} M; (5) 2.3×10^{-5} M. NaOH, 0.05 M and $KMnO_4$, 3.04×10^{-3} M.

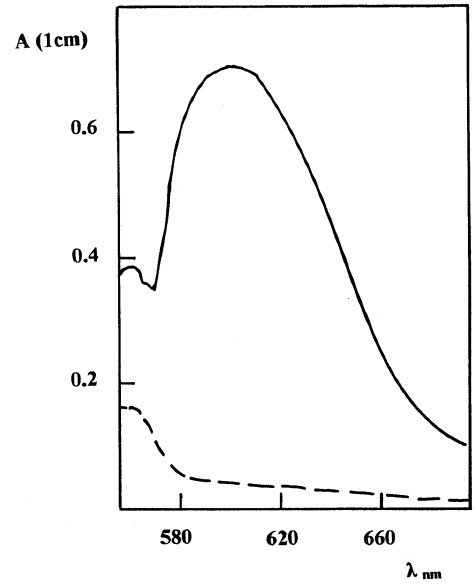
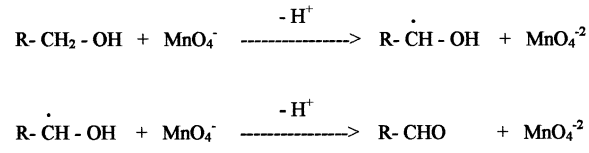


Fig. 3. Absorption spectra (solid line) for the coloured product of 1.84×10^{-5} M IPB with $KMnO_4$, reagent blank (dashed line).



Scheme 1. Proposed reaction between IPB and $KMnO_4$ in 0.05 M NaOH at room temperature.

Table 1
Logarithms of rates for different concentrations of IPB at constant concentrations of 0.05 M NaOH and 3.04×10^{-3} M $KMnO_4$ (0.48 mg ml^{-1}) at room temperature

log(rate), $\Delta A/\Delta t$	log[IPB] (M)
−4.38	−5.64
−3.92	−5.34
−3.56	−5.04
−3.52	−4.94
−3.40	−4.86
−3.27	−4.74
−3.17	−4.64

veloped through the oxidation of the drug with alkaline $KMnO_4$ at room temperature. Furthermore, IPB was determined by measuring its D_1-value at 254–268 nm.

Table 2
Values of κ' calculated from slopes of $\log A$ versus t graphs multiplied by -2.303 for different concentrations of IPB at constant concentrations of 0.05 M NaOH and 3.04×10^{-3} M $KMnO_4$ at room temperature

κ' (s^{-1})	[IPB] (M)
-5.41×10^{-4}	4.60×10^{-6}
-3.84×10^{-4}	6.90×10^{-6}
-2.26×10^{-4}	9.20×10^{-6}
-1.84×10^{-4}	1.38×10^{-5}
-1.48×10^{-4}	1.84×10^{-5}

Table 3
Values of reciprocal time taken at fixed absorbance for different rates of variable concentrations of IPB at constant concentrations of 0.05 M NaOH and 3.04×10^{-3} M $KMnO_4$ at room temperature

$(1/t)$ (s^{-1})	[IPB] (M)
1.389×10^{-3}	9.20×10^{-6}
3.876×10^{-3}	1.38×10^{-5}
5.555×10^{-3}	1.84×10^{-5}
8.333×10^{-3}	2.30×10^{-5}

Table 4
Regression equations at different fixed-times for IPB concentrations in the range 4.6×10^{-6}–2.3×10^{-5} M at constant concentrations of 0.05 M NaOH and 3.04×10^{-3} M $KMnO_4$ at room temperature

Time (min)	Regression equation (A)	Correlation coefficient (r)
10	$-0.0233+32978.26C$	0.9958
20	$-0.0107+35630.43C$	0.9995
30	$-0.0065+37369.56C$	0.9988
40	$0.00055+37663.04C$	0.9978
50	$0.0233+37934.78C$	0.9988

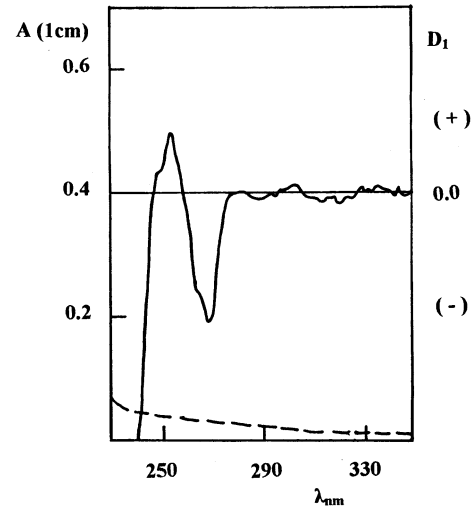


Fig. 4. Zero-order (dashed line), 10 μg ml^{-1}, and D_1- (solid line), 25 μg ml^{-1}, spectra of IPB in distilled water.

Table 5
Determination of IPB in Atrovent® vials, by fixed-time and first-derivative (D_1-) methods

	% Recovery		
	Fixed-time		First-derivative (D_1-)
Mean* ± S.D	100.01 ± 1.16		100.02 ± 0.97
RSD (%)	1.16		0.97
t**		0.023	
F**		1.450	

* Average of five determinations.
** Theoretical values of t and F at $P=0.05$ are 2.31 and 6.93, respectively.

2. Experimental

2.1. Apparatus

The study used a PYE UNICAM, PU 8800, UV/VIS spectrophotometer (Philips), using 1.00 cm quartz cells. The optimized operating conditions for recording the first-derivative spectra were: scan speed 120 nm min^{-1}, chart speed 20 nm cm^{-1}, spectral slit width 2 nm, response 20 s and an ordinate maximum-minimum of ± 5.

2.2. Materials

All materials used were of analytical reagent grade. Reference IPB was kindly provided by Boehringer Ingelheim, Germany and was used as received. Atrovent® unit dose vials, for inhalation, (batch number 738714) were purchased from a local market in Riyadh.

2.3. Reagents and solutions

IPB standard solution, 0.5 mg ml^{-1}, was prepared in distilled water.

A working solution (2.3×10^{-4} M) was prepared by dilution of 5 ml of standard solution to 25 ml with distilled water.

Potassium permanganate solution, 12 mg ml^{-1}, was prepared in distilled water.

Sodium hydroxide solution, 0.5 M was prepared and kept as a stock solution.

2.4. Construction of calibration graphs

2.4.1. Kinetic spectrophotometric method

An aliquot (0.4 ml) of $KMnO_4$ solution and 1.0 ml of 0.5 M NaOH solution were placed in 10 ml calibrated flasks. Accurate volumes of working solution (2.3×10^{-4} M) of IPB, over the concentration range 4.6×10^{-6}–2.3×10^{-5} M, were added and the solutions were diluted to volume with distilled water. At fixed time of 20 min, the absorbance was measured directly at 608 nm against an appropriate blank.

2.4.2. First-derivative method

Accurate volumes of standard IPB solution, 0.5 mg ml^{-1}, were transferred into 1 0 ml calibrated flasks and diluted to volume with water, to give final concentrations in the range 10–35 μg ml^{-1}. The D_1- spectrum was recorded for each solution, over the wavelength range of 350–220 nm, against water as a blank.

2.5. Procedure for vials

2.5.1. Kinetic spectrophotometric method

An accurately measured 1.0 ml aliquot, of the mixed contents of ten ampoules, was transferred into a 10 ml calibrated flask and diluted to volume with water. An aliquot (1 ml) of this solution was transferred into 10 ml calibrated flask containing 0.4 ml of $KMnO_4$ solution and 1 ml NaOH solution. At a fixed time of 20 min, the absorbance was measured at 608 nm against an appropriate blank. The nominal content of the vial was calculated from the calibration graph.

2.5.2. First-derivative method

An aliquot consisting of 1 ml of the mixed contents of ten ampoules was transferred into 10 ml calibrated flask and diluted to volume with water. The D_1- spectrum was recorded over the wavelength range 350–220 nm against water as a blank. The nominal content of the vial was calculated from the calibration graph.

3. Results and discussion

3.1. Kinetic spectrophotometric method

3.1.1. Kinetics and optimization of the reaction conditions

The reaction between IPB and $KMnO_4$ in alkaline solution yields a green color as a result of manganate species, which absorbs at 608 nm. As the intensity of color increases with time, it was deemed useful to elaborate a kinetically-based method for the determination of IPB. In order to come to this conclusion, the reaction was investigated under various conditions of reagent concentration and alkalinity.

At room temperature, the reaction rate increased substantially, as revealed by the intensification of the developed color, suggesting higher analytical sensitivity. Therefore, room temperature was selected as the optimum temperature.

The reaction rate and maximum absorbance increased with increasing $KMnO_4$ concentration and ultimately the adoption of 480 μg ml^{-1} $KMnO_4$ in the final solution proved to be adequate for the maximum concentration of IPB used in the calibration curve. The influence of NaOH concentration on the reaction rate was studied between 0.005–0.2 M. It was found that increas-

ing NaOH concentration increases the reaction rate with maximum absorbance being reached in a shorter time. It was also observed that there was no significant difference in the absorbances of reactant solutions at NaOH concentration above 0.05 M; while decreasing NaOH concentration resulted in lower absorbance values. Therefore, 0.05 M NaOH was chosen as the most suitable concentration.

The rate of reaction was also found to be IPB dependent. The rates were followed at room temperature with various concentrations of IPB in the range 2–10 μg ml^{-1}, keeping $KMnO_4$ and NaOH constant at high concentrations as above.

The graphs shown in Fig. 2, clearly indicate that the reaction rate obeys the following equation:

$$\text{Rate} = \kappa'[\text{IPB}]^n \quad (1)$$

where κ' is the pseudo-order rate constant and n is the order of the reaction.

Fig. 3 shows the absorption spectrum of the colored species obtained by reacting IPB and alkaline $KMnO_4$ at room temperature. The molar ratio was found to be 1:2 for IPB:$KMnO_4$.

Apparently, the reaction proceeds in two steps. The first step being fast and the second is the rate-determining step. Scheme 1 represents a proposed mechanism for the reaction between IPB and $KMnO_4$, in NaOH solution, at room temperature.

The rate could be estimated as $\Delta A/\Delta t$ [16], where A is the absorbance and t is the time in s. Taking logarithms of rates and concentrations (Table 1), Eq. (1) is transformed into:

$$\log(\text{rate}) = \log \Delta A/\Delta t = \log \kappa' + n \log[\text{IPB}] \quad (2)$$

Regression of log(rate) versus log[IPB] gave the regression equation:

$$\log(\text{rate}) = -2.36 + (1.18) \log C$$

with a correlation coefficient (r) = 0.9952.

Hence $\kappa' = 4.366 \times 10^{-3}$ s^{-1} and the reaction is first order ($n = 1$) with respect to IPB.

3.1.2. Evaluation of kinetic methods

The determination of IPB under the optimized experimental conditions mentioned above, where $KMnO_4$ concentration was at least 48 times the concentration of IPB and NaOH concentration was at least 200 times the initial concentration of IPB, would result in pseudo-zero-order conditions with respect to their concentrations. However, the rate will be directly proportional to IPB concentration in a pseudo-first-order rate equation as follows:

$$\text{Rate} = \kappa'[\text{IPB}] \quad (3)$$

Several experiments were then carried out to obtain IPB concentration from the rate data according to Eq. (3). Initial rate, rate constant, fixed-concentration and fixed-time methods [17,18], were tried and the most suitable analytical method was selected taking into account applicability, sensitivity (i.e. the slope of the calibration graph), the intercept and the correlation coefficient (r).

3.1.3. Initial-rate method: pseudo-zero-order method

In this method, graphs of the rate (at the beginning of the reaction) versus IPB concentration were not easy to obtain, because the first step of the reaction was too fast to follow, so tangents of the curve were not easy to draw. This method was therefore abandoned.

3.1.4. Rate-constant method

Graphs of log (absorbance) versus time for IPB concentration in the range 4.60×10^{-6}–1.84×10^{-5} M were plotted and all appeared to be rectilinear. Pseudo first-order-rate constants (κ') corresponding to different IPB concentrations (C) were calculated from the slopes, multiplied by -2.303 and are presented in (Table 2). Regression of C versus κ' gave the equation:

$$\kappa' = -5.73 \times 10^{-4} + 26.16C \quad (r = 0.8854)$$

The value of r indicates poor linearity which is probably due to slight changes in the temperature.

3.1.5. Fixed-concentration method

Reaction rates were recorded for different IPB concentrations in the range 9.20×10^{-6}–2.30×10^{-5} M. A pre-selected value of the absorbance

was fixed and the time was measured in s. The reciprocal of time ($1/t$) versus the initial concentrations of IPB (Table 3) was plotted and the following equation of the calibration graph was obtained:

$$1/t = -3.09 \times 10^{-3} + 489.26C \quad (r = 0.9959)$$

The range of IPB concentration giving the most satisfactory calibration graph with the above equation was limited (4–10 μg ml^{-1}) and showed poor linearity, which could be a disadvantage.

3.1.6. Fixed-time method

Reaction rates were determined for different concentrations of IPB. At a preselected fixed-time, which was accurately determined, the absorbance was measured. Calibration graphs of absorbance versus initial concentration of IPB were established at fixed times of 10, 20, 30, 40 and 50 min with the regression equations assembled in Table 4. It is clear that the slope increases with time and the most acceptable values of r and the intercept were obtained for a fixed time of 20 min, which was therefore chosen as the most suitable time interval for measurement. The detection limit [19], was 8.05×10^{-7} M (0.35 μg ml^{-1}) while the quantification limit [20], was 2.69×10^{-6} M (1.17 μg ml^{-1}).

3.2. First-derivative method

The zero-order and the D_1-spectra of IPB in the wavelength range 350–220 nm are shown in Fig. 4. No absorption maximum was detected in the zero-order spectrum, therefore, conventional UV spectrophotometry cannot be used for quantitation of the drug. Meanwhile, the D_1-spectrum displayed a trough at 268 nm and a maximum at 254 nm.

A calibration curve was constructed, by measuring the peak-trough amplitude, at 254–268 nm. Regression analysis indicated a linear relationship between the D_1-values and concentration in the range 10–35 μg ml^{-1}. The linear regression equation was:

$$D_1 = 0.0486 + 0.132C \quad (r = 0.9996)$$

The detection limit [19], was 0.195 μg ml^{-1} while the quantification limit [20], was 0.652 μg ml^{-1}.

3.2.1. Assay of vials

The validity of the proposed methods for pharmaceutical preparations and the effect of possible interferences were studied by assaying Atrovent® unit dose vials, for inhalation, (labelled to contain 500 μg IPB per 2 ml).

The fixed-time method was applied to the determination of IPB in the supplied drug formulation. The concentration of IPB was calculated using the corresponding regression equation, shown in Table 4, at the fixed-time of 20 min. The results obtained were compared with those obtained by the proposed D_1-method at 254–268 nm. The results are accurate and precise, as indicated by the excellent percentage recovery and RSD percentage (Table 5).

Application of the t- and F-tests showed no significant difference in accuracy and precision between the kinetically-based method and the D_1-method.

However, the kinetically based method is more sensitive than the D_1-method.

4. Conclusion

In conclusion, the proposed kinetic and D_1-methods provide simple and sensitive methods suitable for the quality control analysis of IPB in dosage forms. Furthermore, the proposed method does not require the elaboration of treatment and procedures, which are usually associated with chromatographic methods.

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