original articles

*Analysis of some antispasmodic drugs: oxyphencyclimine and glycopyrronium bromide**

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Introduction

Antispasmodic agents like oxyphencyclimine hydrochloride and glycopyrronium bromide (see Fig. 1) are mainly used in cases of intestinal, biliary and renal colics. Oxyphencyclimine was previously determined polarographically by Andrezej and ultraviolet spectrophotometrically by Kracmar *et al.*[1,2] Gas chromatographic methods have been used by Marozzi and Hackett.[3,4] Glycopyrronium was analysed spectrophotometrically in the ultraviolet region by Kracmar,[5] and by NMR spectroscopy by Takko.[6]

Irving and Markham utilized bromocresol green as a colour forming agent in the analysis of long chain tertiary alkylamines and quaternary ammonium salts.[7] The NF XIV published a colorimetric method for the analysis of glycopyrronium bromide injection and tablets using bromocresol purple; about 1000 µg glycopyrronium bromide was used in this determination.[8] The USP XX/NF XV described an ultraviolet spectrophotometric method for the analysis of oxyphencyclimine hydrochloride tablets; about 200 µg oxyphencyclimine hydrochloride was used.[9] USP XX and NF XIV applied non-aqueous titration methods for the determination of pure oxyphencyclimine hydrochloride and glycopyrronium bromide, respectively; about 100 mg of these compounds are used.

The purpose of this investigation was to employ sensitive and rapid methods of analysis that can be applied to determine oxyphencyclimine hydrochloride and glycopyrronium bromide in microquantities both as the pure compound and in dosage forms. The suggested methods depend on applying a dye-salt formation method and a citric acid–acetic anhydride reagent method for the determination of these antispasmodic drugs.

The dye-salt formation method was adopted for the microdetermination of oxyphencyclimine hydrochloride and glycopyrronium bromide both in pure form and in their dosage forms. The method is based on the reaction between the anionic dye tropeolin 000 (Fig. 2) and the tertiary amine group of oxyphencyclimine hydrochloride and the quaternary ammonium group in glycopyrronium to form an orange coloured dye-salt (ion pair) extractable with 1,2-dichloroethane.

Methods

APPARATUS

We used a pH meter (G 711, Schott-Geräte GmbH, Hofheim, FRG), a Beckman Model 26 spectrophotometer (Beckman Instruments International, Geneva, Switzerland) and a mechanical flask shaker (Stuart Checoslovake, Prague, Czechoslovakia; 300 oscillations/min) provided with a stopwatch.

MATERIALS AND REAGENTS

All materials and reagents used were of analytical grade. Pure oxyphencyclimine hydrochloride and oxyphencyclimine hydrochloride tablets (Daritran® 5 mg) were sup-

Key words
Analysis, quantitative
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Glycopyrronium bromide
Oxyphencyclimine

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Abstract

Oxyphencyclimine hydrochloride (via its tertiary amine group) and glycopyrronium bromide (via its quaternary ammonium group) were analysed by two different colorimetric methods. The first method depends on the dye-salt formation method using tropeolin 000 as the anionic dye and 1,2-dichloroethane as solvent for the extraction of the formed ion pairs. The coloured ion pairs have maximum absorption at 483-486 nm. The second method is based on the use of citric acid–acetic anhydride reagent. The coloured complex has maximum absorption at 562-565 nm.

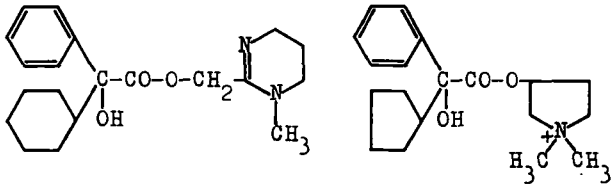

FIGURE 1
Structural formulae of oxyphencyclimine (left; 1,4,5,6-tetrahydro-1-methyl-2-pyrimidinemethanol α-phenylcyclohexaneglycolate ester) and glycopyrronium (right; 3-hydroxy-1,1-dimethylpyrrolidinium α-phenylcyclopentaneglycolate)

plied by Pfizer Co., Cairo, Egypt. Pure glycopyrronium bromide and glycopyrronium bromide tablets (Robinul® 2 mg) were supplied by Robinul Co., Richmond, USA.

For the dye-salt formation we used a 0.1% aqueous solution of tropeolin 000 (Prolabo, Paris, France); MacIlvaine's buffer solutions (pH 2.7-7.7), and Clark and Lub's buffer solutions (pH 8-10); and freshly washed, dried and distilled 1,2-dichloroethane (Prolabo).

The citric acid–acetic anhydride reagent method was performed using a freshly prepared saturated citric acid solution (2% wt/vol) in redistilled acetic anhydride (the fraction boiling at 140°C being used); isopropyl alcohol (Prolabo) purified by distillation over zinc dust and potassium hydroxide; and acetone (Prolabo) purified by distillation at 57°C.

PROCEDURES

Dye-salt formation method

To a series of 125 ml separating funnels sample solutions equivalent to 18-180 μg pure oxyphencyclimine hydrochloride (0.18 mg/ml) were transferred, and in another set of separating funnels sample solutions equivalent to 40-400 μg pure glycopyrronium bromide (0.2 mg/ml) were added. The stated volume of 0.1% aqueous solution of tropeolin 000 (Table I) was subsequently added. The volume was completed to 10 ml with buffer solution of optimum pH and extracted with 10 ml 1,2-dichloroethane by shaking for the required time using a mechanical shaker.

In the case of oxyphencyclimine hydrochloride the extract was filtered through a small piece of cotton wool saturated with the same solvent and collected in 10 ml volumetric flasks (concentration in extract: 1.8-18 μg/ml) while in the case of glycopyrronium bromide three extractions were necessary using three successive portions of 1,2-dichloroethane (10, 10 and 5 ml). The three extracts were filtered on the same piece of cotton wool and collected in 25 ml volumetric flasks (final concentration 1.6-16 μg/ml). The absorbances were determined at the respective wavelength of maximum absorption (Table I).

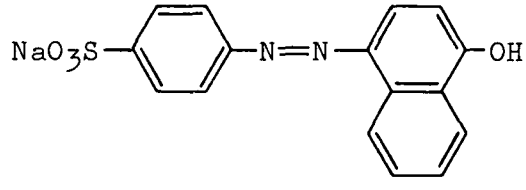

FIGURE 2
Tropeolin 000: α-naphtholazobenzene-*p*-sulfonic acid (sodium salt)

TABLE I
Optimum conditions for dye-salt method

	Oxyphencyclimine hydrochloride	Glycopyrronium bromide
λ_{max}	483 nm	486 nm
Amount of 0.1% tropeolin 000	2 ml	5 ml
pH of buffer solution	3	2.6
Number of extractions	1*	3†
Shaking time	1 min	2 min
Ratio of aqueous: organic phases	1:1	1:1
Final volume of coloured extract	10 ml	25 ml
Stability of the colour	over two hours	over two hours
Obedience to Beer's law	1.8-18 μg/ml	1.6-16 μg/ml
Sensitivity	≤ 1.8 μg/ml	≤ 1.6 μg/ml

* Ten millilitres of 1,2-dichloroethane are used for the extraction.
† Two successive portions of 10 ml 1,2-dichloroethane and a third one of 5 ml were used.

Twenty Daritran® tablets were powdered and an amount equivalent to 18 mg oxyphencyclimine hydrochloride was transferred to a 100 ml volumetric flask. The flask was shaken with warm distilled water and made up to volume with distilled water. An aliquot was centrifuged for 15 min. Different amounts of the prepared tablet solution equivalent to 36-108 μg oxyphencyclimine hydrochloride were analysed as mentioned before.

Robinul® tablets were treated in the same way as Daritran® tablets, using an amount of powdered tablets equivalent to 20 mg glycopyrronium bromide. Different portions of the prepared tablet solution equivalent to 80-280 μg glycopyrronium bromide were analysed as before.

Citric acid–acetic anhydride reagent method

To a series of test tubes different amounts of the alcoholic sample solutions (1 mg/ml; methanol is used for the preparation of the oxyphencyclimine hydrochloride solution and ethanol for the glycopyrronium bromide

TABLE II
Optimum conditions for citric acid–acetic anhydride reagent method

	Oxyphencyclimine hydrochloride	Glycopyrronium bromide
λ_{max}	562 nm	565 nm
Amount of reagent	12 ml	12 ml
Time of heating at 90°C	25 min	25 min
Stability of the colour	over two hours	over two hours
Obedience to Beer's law	1-12 μg/ml	2-20 μg/ml
Sensitivity	≤ 1 μg/ml	≤ 2 μg/ml

TABLE III
Analysis of pure oxyphencyclimine hydrochloride and glycopyrronium bromide

Method	Oxyphencyclimine HCl		Glycopyrronium bromide	
	taken	recovery (%)*	taken	recovery (%)*
Dye-salt method*	5.4 µg/ml	100.0	3.2 µg/ml	100.6
	9.9 µg/ml	99.1	7.2 µg/ml	99.3
	11.7 µg/ml	100.4	9.6 µg/ml	101.0
	13.5 µg/ml	99.5	13.6 µg/ml	100.2
	15.3 µg/ml	99.7		
Citric acid–acetic anhydride method†	3.0 µg/ml	100.7	6.0 µg/ml	100.0
	5.0 µg/ml	100.4	10.0 µg/ml	101.0
	7.0 µg/ml	99.6	14.0 µg/ml	99.3
	9.0 µg/ml	100.0	18.0 µg/ml	99.8
USP XX‡	100 mg	99.0		
	200 mg	99.0		
	300 mg	99.7		
	400 mg	100.0		
NF XIV§			100 mg	101.6
			300 mg	99.4
			500 mg	99.6
			800 mg	99.6

* Mean of three determinations.
† Final concentrations in extract.
‡ Non-aqueous titration; 1 ml 0.1 N perchloric acid = 0.03809 g oxyphencyclimine hydrochloride.
§ Non-aqueous titration; 1 ml 0.1 N perchloric acid = 0.03983 g glycopyrronium bromide.

solution) were transferred and then heated to dryness. The residue was heated with 12 ml citric acid–acetic anhydride reagent for 25 min in a water bath at about 90°C with occasional shaking. After cooling, the red-violet liquid was transferred to a 50 ml volumetric flask in the case of oxyphencyclimine hydrochloride and to a 25 ml volumetric flask in the case of glycopyrronium bromide and made up to volume with acetone. Absorbances were determined at 562 nm for oxyphencyclimine hydrochloride and at 565 nm for glycopyrronium bromide against a similarly treated blank (Table II).

Twenty Daritran® tablets were powdered and an amount equivalent to 50 mg oxyphencyclimine hydrochloride was transferred to a 50 ml volumetric flask. A

TABLE IV
*Analysis of Daritran® tablets**

Method	Taken	Recovery (%)†	Added‡	Recovery (%)†
Dye-salt method	3.6 µg/ml	99.3	3.6 µg/ml	98.6
	5.4 µg/ml	97.6	5.4 µg/ml	99.1
	9.0 µg/ml	98.9	7.2 µg/ml	100.0
	10.8 µg/ml	98.4	7.2 µg/ml	98.6
Citric acid–acetic anhydride method	4.0 µg/ml	99.0	4.0 µg/ml	101.0
	6.0 µg/ml	100.0	6.0 µg/ml	99.7
	8.0 µg/ml	101.3	4.0 µg/ml	101.0
	10.0 µg/ml	100.0	2.0 µg/ml	101.0
Pfizer method§	5 mg	99.1‖		

* Daritran® tablets contain 5 mg oxyphencyclimine hydrochloride and 250 mg meprobamate per tablet.
† Mean of three determinations.
‡ Added in the form of a solution containing the equivalent amount of pure oxyphencyclimine hydrochloride.
§ The method according to Pfizer Co. is a UV-spectrophotometric method. The oxyphencyclimine content is calculated as follows:

$$\text{mg/tablet} = \frac{(\text{absorbance at 258 nm}) - (\text{absorbance at 280 nm})}{8.25 \times \text{weight of sample (g)} \times 0.92}$$

(0.92 is a correction factor determined by Pfizer Co.).
‖ Mean of four determinations.

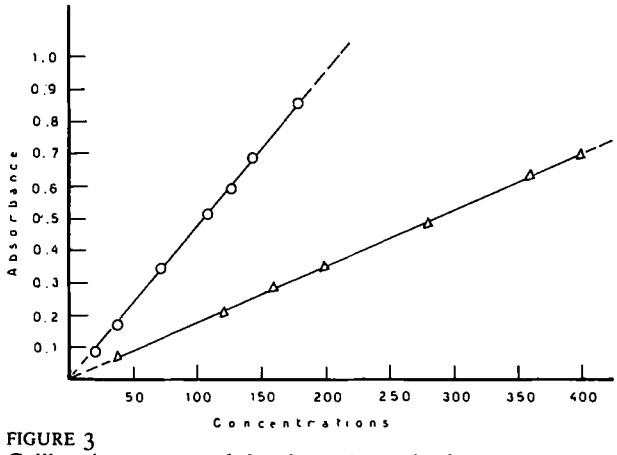

FIGURE 3
Calibration curves of the dye-salt method.
○: oxyphencyclimine hydrochloride (µg/10 ml);
△: glycopyrronium bromide (µg/25 ml)

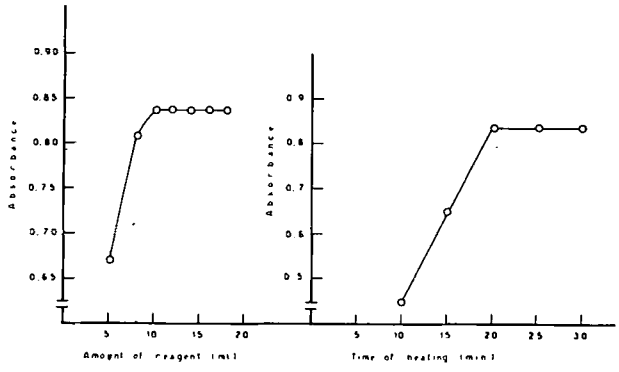

FIGURE 5
Optimum conditions for the determination of glycopyrronium bromide with citric acid–acetic anhydride method

sufficient amount of methanol was added to dissolve the active ingredient as completely as possible. The volume was made up with methanol followed by filtration in a dry flask through dry filter paper and a dry funnel. The first few millilitres of the filtrate were rejected and the appropriate aliquots (as mentioned in Table III) were transferred to a series of test tubes; the experiment was completed as before (Table I). The same procedure was followed for Robinul® tablets. An amount of powdered tablets equivalent to 25 mg glycopyrronium bromide was transferred to 25 ml volumetric flasks using ethanol for dissolving glycopyrronium bromide and the experiment was completed as before.

Pfizer method for the determination of Daritran® tablets

An amount of powdered tablets equivalent to 20 mg oxyphencyclimine hydrochloride was extracted with a mixture of chloroform and methanol (3+1). The volume was made up to 10 ml using the same solvent mixture. The required aliquot was evaporated to dryness, the residue dissolved in 25 ml methanol and the absorbances measured at 258 nm and 280 nm (Table IV).

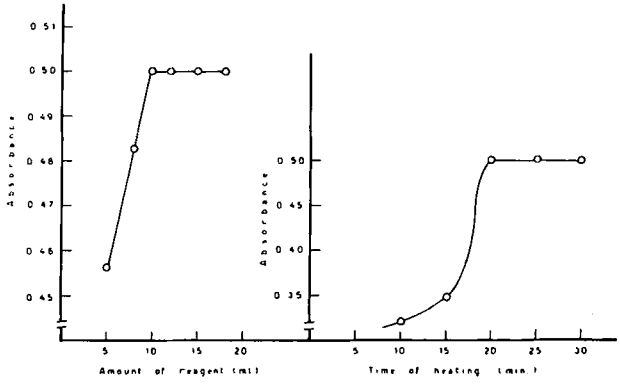

FIGURE 4
Optimum conditions for the determination of oxyphencyclimine hydrochloride with citric acid–acetic anhydride method

Results and discussion

Screening of anionic dyes and organic solvents for a possible dye-salt method of analysis for oxyphencyclimine hydrochloride and glycopyrronium bromide revealed that several dye-salt combinations are promising. Tropeolin 000 and 1,2-dichloroethane were chosen because this system gives the highest colour intensity. The accuracy and the sensitivity of the method are affected by several factors, including:

- amount of dye;
- effect of pH;
- ratio of aqueous:organic phases;
- number of extractions;
- time of shaking.

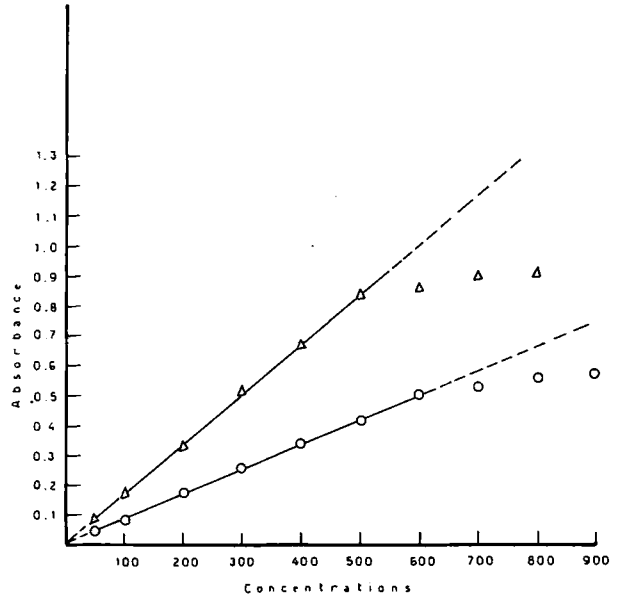

FIGURE 6
Calibration curves of the citric acid–acetic anhydride method. ○: oxyphencyclimine hydrochloride (µg/50 ml);
△: glycopyrronium bromide (µg/25 ml)

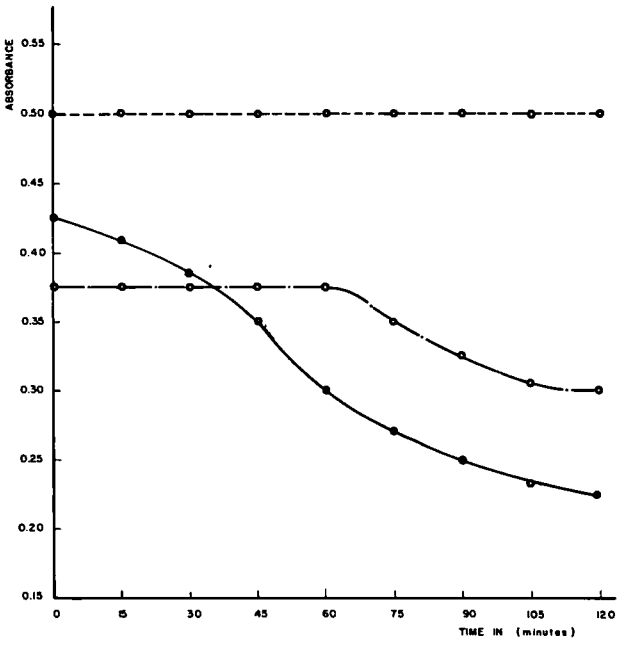

FIGURE 7
Stability of the coloured complex of oxyphencyclimine hydrochloride and effect of solvents (o – – o: acetone; ●—●: ethanol; o –·– o; isopropanol)

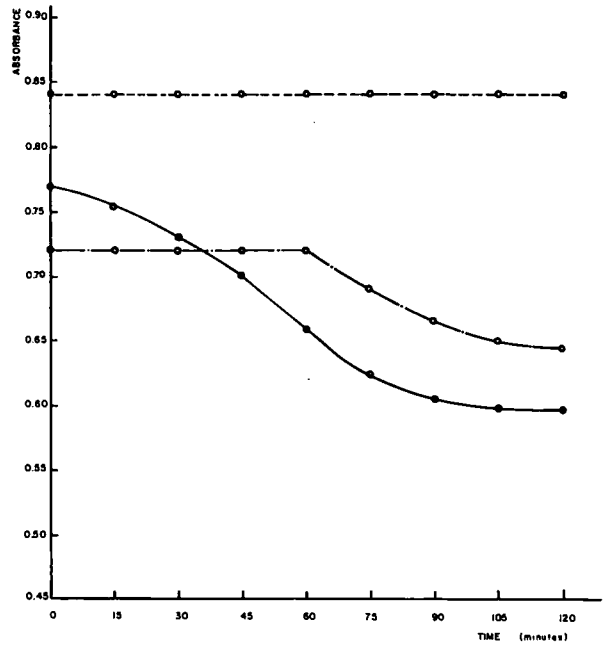

FIGURE 8
Stability of the coloured complex of glycopyrrolate and effect of solvents (o – – o: acetone; ●—●: isopropanol; o –·– o; ethanol)

These factors were thoroughly investigated and optimum conditions for complete dye-salt (ion pair) formation, highest sensitivity and maximum absorption are carefully selected (Table I). Under the proposed conditions of reaction, the extracts follow Beer's law over the concentration ranges of 1.8-18 μg/ml oxyphencyclimine HCl [$y = -0.002 + 0.0047x$; $r = 0.999$ (n = 8), where y = absorbance and x = concentration in μg/ml] and 1.6-16 μg/ml glycopyrronium bromide [$y = 0.0015 + 0.0017x$; $r = 0.999$ (n = 7)] (Fig. 3). The sensitivity of the method was found to be ≤ 1.8 μg and ≤ 1.6 μg for oxyphencyclimine hydrochloride and glycopyrronium bromide, respectively.

Another microanalytical method has been applied for the colorimetric determination of oxyphencyclimine hydrochloride and glycopyrronium bromide both in pure form and in tablets. The method is based on the reaction of oxyphencyclimine hydrochloride and glycopyrronium bromide with citric acid–acetic anhydride reagent to form red-violet complexes. This method was developed by Palumbo for the determination of tertiary amines and some alkaloids.[10] In the present investigation, the best

TABLE V
*Analysis of Robinul® tablets**

Method	Taken	Recovery (%)†	Added‡	Recovery (%)†
Dye-salt method	3.2 μg/ml	102.5	3.2 μg/ml	100.0
	4.8 μg/ml	101.7	6.4 μg/ml	98.8
	8.0 μg/ml	100.3	4.8 μg/ml	100.6
	11.2 μg/ml	100.2	2.4 μg/ml	100.0
Citric acid–acetic anhydride method	4.0 μg/ml	100.0	4.0 μg/ml	99.0
	8.0 μg/ml	100.5	8.0 μg/ml	100.0
	10.0 μg/ml	100.0	10.0 μg/ml	100.0
	12.0 μg/ml	100.8	8.0 μg/ml	101.5
NF XIV method§	100 μg/ml	99.9‖		

* Robinul® tablets contain 2 mg glycopyrronium bromide.
† Mean of three determinations.
‡ Added in the form of a solution containing the equivalent amount of pure glycopyrronium bromide.
§ The NF XIV method is a colorimetric method using bromocresol purple at pH 5.3 and extraction with chloroform. Absorbance is determined at 410 nm.
‖ Mean of four determinations.

TABLE VI
Statistical comparison of results

Method	Confidence interval*	Number of experiments	Variance	Student's t-value†
Oxyphencyclimine hydrochloride				
USP XX	99.4 ± 0.8	4		
Dye-salt method	99.7 ± 0.5	5	0.24	0.98 (2.365)
Citric acid–acetic anhydride method	100.2 ± 0.8	4	0.23	2.18 (2.447)
Daritran® tablets				
Pfizer method	99.1 ± 0.9	4		
Dye-salt method	98.6 ± 1.4	4	0.76	1.12 (2.447)
Citric acid–acetic anhydride method	110.1 ± 1.5	4	0.85	1.55 (2.447)
Glycopyrronium bromide				
NF XIV	100.0 ± 1.8	4		
Dye-salt method	100.3 ± 1.2	4	0.55	0.48 (2.447)
Citric acid–acetic anhydride method	100.0 ± 1.2	4	0.52	0.02 (2.447)
Robinul® tablets				
NF XIV	99.9 ± 0.8	4		
Dye-salt method	101.2 ± 1.8	4	1.28	1.58 (2.447)
Citric acid–acetic anhydride method	100.3 ± 0.6	4	0.17	1.52 (2.447)

* The confidence interval is calculated as the mean recovery ± t × SD/√n, where t is the t value of Student's t-test at 95% probability and n–1 degrees of freedom, SD is the standard deviation and n is the number of experiments.
† The theoretical values are indicated between brackets.

conditions for the citric acid–acetic anhydride reagent were studied including amount of reagent and time of heating (Table II, Fig. 4, 5).

Beer's law was obeyed over the concentration range of 1-12 μg/ml at 562 nm for oxyphencyclimine HCl [$y = 0.024 + 0.00073\,x$; $r = 0.99$ (n = 7)] and 2-20 μg/ml at 565 nm for glycopyrronium bromide [$y = 0.068 + 0.0013\,x$; $r = 0.98$ (n = 7)] (Fig. 6). The sensitivity of the method was found to be ≤ 1 μg/ml and ≤ 2 μg/ml for oxyphencyclimine hydrochloride and glycopyrronium bromide, respectively.

The effect of ether, isopropylalcohol and acetone on the sensitivity and the stability of the coloured complex was studied. Acetone was preferred since maximum absorbance reading and highest stability of the colour were achieved (Fig. 7, 8).

The two proposed methods have been successfully applied for the analysis of Daritran® and Robinul® tablets without any interference from included excipients (Tables III, IV and V).

The validity of the results has been assessed by applying the standard addition technique to solutions of Daritran® and Robinul® tablets, respectively. Results obtained are compared to USP XX and NF XIV methods (Tables III, IV and V). The USP XX method is not applied to Daritran® tablets due to its content of meprobamate so the Pfizer method is used as reference method in this case.

Statistical studies of the results for pure forms and tablets of these two antispasmodic compounds determined by the two proposed methods, show that there is no significant difference between the proposed procedures and the official USP XX or NF XIV and the Pfizer methods (Table VI).

Conclusion

In conclusion the proposed methods have the advantages of sensitivity, rapidity and simplicity over the official methods[8,9] and those previously published based on ultraviolet spectrophotometry,[2-5] or reaction with bromocresol green.[1] The ultraviolet spectrophotometric methods are liable to interference from tablet excipients. Concerning the Irving and Markham colorimetric method, using bromocresol green, the blank is dependent both on pH and on concentration of the excess reagent.

Concerning the official methods the NF XIV colorimetric method for the analysis of glycopyrronium bromide injection and tablets using bromocresol purple, necessitates a pretreatment procedure involving extracting the dye-buffer mixture with chloroform until the last extract is colourless. Moreover, the formed colour was extracted with four portions of chloroform.

The present work proved to be free from all these drawbacks, since no interference from included tablet excipients is noticed and therefore no prior

separation of active ingredients is required. Meanwhile the present procedures have the advantages of high sensitivity over the official methods as the lower limits of determination are about 1.8 µg/ml and 1.6 µg/ml for oxyphencyclimine hydrochloride and glycopyrronium bromide, respectively, in the case of the dye-salt method, and 1 µg/ml and 2 µg/ml for oxyphencyclimine hydrochloride and glycopyrronium bromide, respectively, in the case of the citric acid–acetic anhydride reagent method.

Thus the suggested methods can be used for routine analysis of these antispasmodic compounds because of their high sensitivity, rapidity and simplicity.

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