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The Use of Integrated Analytical Tools for Determination of Cyclopentolate and Naphazoline Hydrochlolrides in Pure and Pharmaceutical Preparations

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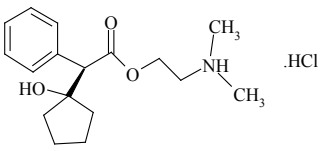
Abstract

Simple and sensitive spectrophotometric methods were described for the determination of cyclopentolate (CPH) and naphazoline hydrochlorides (NPZ) drugs in pure form and in pharmaceutical preparations based on ion pair and charge transfer complexation reactions, respectively. The first method is based on the reaction of the CPH drug with Mo(V)-thiocyanate in hydrochloric acid medium and dyestuff reagents namely bromophenol blue (BPB), bromocresol green (BCG) and bromocresol purple (BCP). The ion pair complexes formed were quantitatively extracted into dichloroethane, chloroform and methylene chloride in case of Mo(V)-thiocyanate, BPB, and BCP reagents, respectively. The second method is based on charge transfer complex formation between NPZ (electron donor) and TCNQ (π-acceptor reagent). All the experimental variables were optimized. The calibration graphs are rectilinear in the concentration ranges 5.00-250.0, 0.93-56.07, 0.93-56.07 and 1.86-56.07 μg mL^{-1} for CPH using Mo(V)-thiocyanate, BPB, BCG and BCP reagents, respectively, and 2.00-240.0 μg mL^{-1} for NPZ using TCNQ reagent. The Sandell sensitivity (S), molar absorptivity, correlation coefficient and regression equations were calculated. The limits of detection (LOD =5.54, 0.51, 0.32, 0.54 and 3.19 using Mo(V)-thiocyanate, BPB, BCG ,BCP and TCNQ reagents, respectively) and limits of quantification (LOQ = 7.55, 1.70, 1.05, 1.80 and 5.60 using Mo(V)-thiocyanate, BPB, BCG , BCP and TCNQ reagents, respectively) are calculated. The law values of standard deviation and relative standard deviation reflect the accuracy and precision of the proposed methods. The two methods can be applied to the analysis of the two drugs in eye drops, with no evidence of interference from excipients. There was no significant difference between each of the two methods and the official one.

Keywords: Cyclopentolate; Naphazoline hydrochlorides; Ion-pair formation; Charge transfer; Spectrophotometry

Introduction

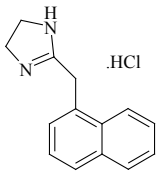
Cyclopentolate hydrochloride (CPH) has the IUPAC name 2-(dimethylamino)-ethyl-1-hydroxy-α-phenylcyclopentaneacetate hydrochloride of molecular weight 327.85 g/mol and molecular formula $C_{17}H_{25}NO_3$.HCl. Cyclopentolate is an anticholinergic agent that induces relaxation of the sphincter of the iris and the ciliary muscles. When applied topically to the eyes, it causes a rapid, intense pupillary dilation (mydriasis) and paralysis of accommodation (cycloplegia) that is maximal in 15 to 60 minutes. Recovery usually occurs within 24 hours.



Structure of cyclopentolate hydrochloride.

The methods available for the determination of CPH include capillary column gas chromatography [1] and spectrophotometric methods [2-4].

Naphazoline hydrochloride (NPZ) has the IUPAC name 2-(1-naphthylmethyl)-2-imidazoline monohydrochloride of molecular weight 246.74 g/mol and molecular formula $C_{14}H_{14}N_2HCl$. It acts as an ocular vasoconstrictor constricts the vascular system of the conjunctiva. It is presumed that this effect is due to direct stimulation action of the drug upon the alpha adrenergic receptors in the arterioles of the conjunctiva resulting in decreased conjunctival congestion. Naphazoline belongs to the imidazoline class of sympathomimetics.



Structure of naphazoline hydrochloride

A number of studies were described for the determination of NPZ in both pure and pharmaceutical samples including micellar electrokinetic chromatography [5,6], high-performance liquid chromatographic (HPLC) [7], spectrophotometric [8-13] and potentiometric methods [14].

This paper introduces two spectrophotometric methods for the determination of CPH and NPZ. The first method is based on the reaction of the CPH drug with Mo(V)-thiocyanate in hydrochloric acid medium, bromophenol blue (BPB), bromocresol green (BCG) and bromocresol purple (BCP) reagents via the protonated nitrogen atom

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of the drug. In case of using Mo(V)-thiocyanate, Mo(V) formed by the reduction of Mo(VI) with ascorbic acid, combines with ammonium thiocyanate to form a red Mo(V)–thiocyanate binary complex in hydrochloric acid medium. The drug under investigation was added to the red binary complex and the ion pair formed was extracted in dichloroethane (based on the fact that red Mo(V)–thiocyanate binary complex does not extracted in dichloroethane) and measured spectrophotometrically against reagent blank. The second method is based on charge transfer complex formation between NPZ (electron donor) and TCNQ (π-acceptor reagent). The proposed methods were applied successfully to the determination of CPH and NPZ either pure or in dosage forms, with good accuracy and precision. The results were compared with those given by the official methods.

Material and Methods

Chemicals and solutions

All chemicals and reagents used were of analytical reagent grade. They included cyclopentolate hydrochloride (CPH) provided by SEDICO, Egypt, and naphazoline hydrochloride (NPZ) provided by Misr Company for Pharmaceutical Industry, Egypt. Reagents used included 7,7,8,8-tetracyanoquinodimethane (TCNQ) (supplied from Aldrich) and dyestuffs namely bromocresol purple (BCP) (purchased from Fluka, Switzerland), bromocresol green (BCG) and bromophenol blue (BPB) (were purchased from Win lab, U.K). Sulphuric, hydrochloric and nitric acids were supplied from Merck. Absolute ethanol was supplied from Adwic, while n-propanol and acetonitrile (AR) were supplied from Aldrich. Chloroform, methanol, acetone, tetrahydrofuran, 1,4–dioxane, n-pentanol, methylene chloride and petroleum ether were supplied from El-Nasr Company.

1 mg mL^{-1} Stock solutions of cyclopentolate and naphazoline hydrochlorides were prepared by dissolving the accurate weighed amount from each drug in a definite volume of water and methanol, respectively, to get the required concentration. Dilute solutions were prepared by accurate dilution from the stock solution to get the desired concentrations.

10% (w/v) Solutions of each of ascorbic acid and ammonium thiocyanate were prepared by dissolving the accurate weight (10 g) of each substance in 100 mL bidistilled water. 0.02% (w/v) Ammonium molybdate solution was prepared by dissolving the accurately weighed (0.02 g) of ammonium molybdate in bidistilled water. 0.02% (w/v) of 7,7,8,8-tetracyanoquinodimethane (TCNQ) reagent was prepared by dissolving the accurate weighed amount of 20 mg of TCNQ in 100 mL acetonitrile.

The dyestuffs were used as 0.02% solutions of bromocresol purple (BCP), bromophenol blue (BPB) and bromocresol green (BCG), all in 10% (v/v) ethanol.

4 mol L^{-1} Acid solutions (HCl, H_2SO_4 and HNO_3) were prepared by accurate dilution with bidistilled water from concentrated solutions. All solutions must be protected from light by keeping them in dark coloured quick fit bottles during the whole work.

Colircusi (eye drops) was supplied from United Company of Distribution (UCD), Cairo, Egypt. Each 1 mL of sterile solution contains 10 mg cyclopentolate hydrochloride. Neozoline (Eye/Nasal drops) was produced by Amoun Pharmaceutical Company, El-Obour City, Cairo, Egypt. Each 100 mL contains 50 mg naphazoline hydrochloride.

The water was always twice distilled from all glass equipments. Redistillation was carried out from alkaline permanganate solution.

Instrument and conditions

The spectrophotometric measurements were carried out using the manual Unico 1200 spectrometer (United Products and Instruments, Inc.) in the wavelength range from 325-1000 nm.

Procedure A (determination of cyclopentolate HCl using Mo (V)–thiocyanate and dyestuff reagents (BPB, BCG, BCP): 3 mL of Mo(VI) ion (0.02% (w/v)) was mixed with 3 mL of 4M HCl, 4 mL of ammonium thiocyanate solution (10% (w/v)) and 3 mL of ascorbic acid solution (10% (w/v)) and were placed in 100 mL capacity separating funnel. After 15 min, different concentrations (5.00-250 μg mL^{-1}) of CPH (1 mg mL^{-1}) were added. The mixture was diluted to 30 mL with bidistilled water and mixed well. After another 10 min, 10 mL of dichloroethane was added twice with 5 mL portions and the solution mixture was shaked vigorously for one min. The solution allowed to being separated into two phases. The organic layer was collected in 10 mL measuring flask. The determination of CPH using dyestuff reagents (BPB, BCG, BCP) involves the preparation of series of solutions in which the concentration of reagent was kept constant at 30, 30 and 60 μg ml^{-1} using (0.02 %) (BPB, BCP and BCG reagents, respectively), while that of the drug was regularly varied from 0.93-56.07 of $5.7x10^{-4}$ mol L^{-1} drug solution, then the solution was completed to 10 mL in case of BCG and to 20 mL bidistilled water in case of BPB and BCP and the reaction mixture was left in a 50 mL separating funnel. After the selected time for each complex formation, the ion-pairs were collected in 10 ml measuring flask using 10 mL methylene chloride in case of BCG and BCP and 10 mL (5 mL x 2 times) chloroform in case of BPB, after shaking for one minute. the absorption spectra of the resulted ion pair solutions were scanned in the wavelength range 350-600 nm from which the best wavelength for each ion-pair was selected, against a blank solution. The linear part of the absorbance-drug concentration curve represents the concentration range within which Beer's law was valid.

Procedure for eye drop: An accurately known volume of eye drop solution equivalent to 100 mg of CPH was prepared in 100 mL bidistilled water. The procedure mentioned above was followed where different concentrations of CPH in the range 5.00-250 and 9.35-37.40 μg mL^{-1} were added using Mo(V)-thiocyanate and dyestuff reagents (BPB, BCG, BCP), respectively. The drug concentrations were calculated from the standard calibration graph prepared under identical conditions.

Procedure B: determination of naphazoline HCl using TCNQ: 1 mL of 0.02% (w/v) TCNQ, was added to different concentrations (2-240 μg mL^{-1}) of NPZ. The mixtures were completed up to 5 mL with acetonitrile. The absorbance of the coloured CT complex product was scanned in the wavelength range 600-900 nm from which the best wavelength for drug was selected .

Procedure for eye drop: Different concentrations of naphazoline HCl (60-150 μg mL^{-1}) were added to 1 mL of 0.02% (w/v) TCNQ reagent. The volumes were made up to the mark with acetonitrile in 5 mL calibrated measuring flask. The absorbance was measured at λ_{max} = 842 nm for naphaz.HCl using TCNQ reagent, against reagent blank.

Results and Discussion

Determination of cyclopentolate HCl

Using Mo(V)-thiocyanate reagent: The ion pair formed between cyclopentolate hydrochloride and Mo(V)–thiocyanate is extractable with dichloroethane with absorption maximum at 470 nm as shown in Figure 1. It is found that, the reduction probability of Mo(VI) to Mo(V) may occur by ascorbic acid or by SCN^- in acidic medium. The sensitivity

and stability of Mo(V)–thiocyanate binary complex is enhanced considerably by using ascorbic acid. Ascorbic acid gives reproducible value and masks many interfering ions [15]. It is found that 30 mg mL^{-1} of 10 % ascorbic acid are sufficient for complete conversion of 0.06 mg mL^{-1} of 0.02 % (w/v) Mo(VI) to Mo(V). Also, it was found that 40 mg mL^{-1} of 10% ammonium thiocyanate gave the maximum pronounced effect on the absorbance of the ion–pair used in the determination of cyclopentolate HCl. Equation (1) represents the reaction of Mo(VI) with ammonium thiocyanate in 3 mL of 4 mol L^{-1} HCl and in presence of ascorbic acid. The effect of time and temperature on the formation of the ion-pair is studied. The absorbance of Mo(V)–thiocyanate binary complex is stable after 15 minutes, while Mo(V)–thiocyanate-drug ion-pair needs 10 min at 25°C for their complete formation.

Stoichiometry of the ion–pair: The nature of the binding of Mo(V) to each drug in the presence of excess amount of ammonium thiocyanate is determined by the continuous variation and molar ratio methods [16,17] in order to check the stochiometric ratio of the ion pair. The data obtained are shown in Figure 2. The results indicate that a 1:1 [Mo(V)-thiocyanate]: [drug] ion–pair is formed through the electrostatic attraction between positive protonated drug and thiocyanate negative complex. The proposed structure of the formed ion pair is given in scheme (1).

Table 1 shows the analytical parameter data obtained for the determination of CPH using Mo(V)-thiocyanate reagent. The absorbance-concentration curve is found to be rectilinear and Beer's law is obeyed in the concentration range 5-250 µg ml^{-1}. Above this limit, negative deviation was observed, which can be explained by a possible association of the species formed in solution to give the final possible

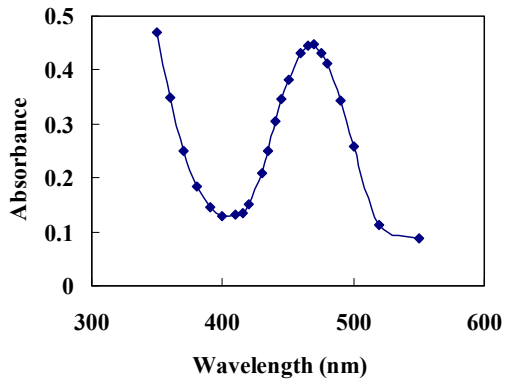


Figure 1: Absorption spectrum of cyclop.HCl-Mo(V)-thiocyanate ion pair.

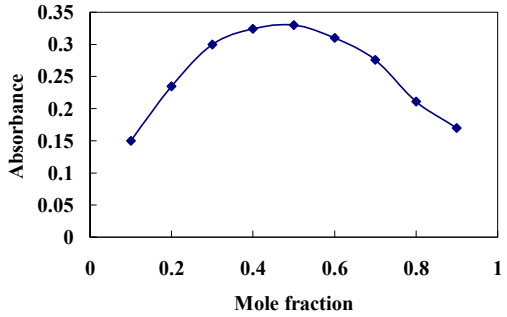
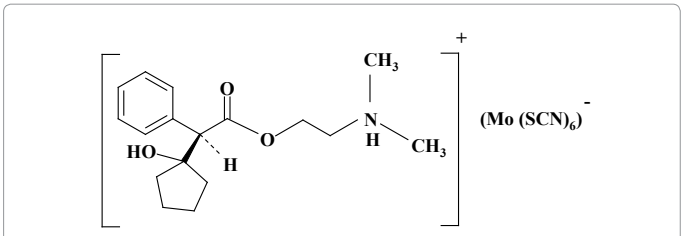


Figure 2: Stoichiometric ratio of the reaction of Mo(V)–thiocyanate with the cited drug at λ_{max} = 470 nm using continuous variation method.



Cyclop.HCl -Mo (V)

Scheme 1: Proposed structure of the ion pair.

Parameters \ Drug	cyclopentolate hydrochloride				Naphazoline hydrochloride
	Mo(V)-thiocyanate	BPB	BCG	BCP	TCNQ
λ_{max} (nm)	470	400	400	400	842
t (min.)	10	10	7	10	60
T (°C)	25	20	20	20	40
Conc. Range (µg mL^{-1})	5.00-250.0	0.93-56.07	0.93-56.07	1.86-56.07	2.00-240.0
ε ($L.mol^{-1}.cm^{-1}$)	4.600 x10^3	4.600 x10^3	4.610x10^3	5.515x103	0.79x10^4
S (µg cm^{-2})	0.066	0.066	0.066	0.055	0.51
A = mC + z,					
m	0.0206	0.0206	0.0333	0.0195	0.0033
z	0.1339	0.1339	0.2036	0.2105	0.0653
r	0.9982	0.9982	0.9963	0.9977	0.993
Percent recovery					
LOD (µg mL^{-1})	100.0-101.0	98.40-100.5	98.98-100.0	98.00-100.0	98.67-100.0
LOQ (µg mL^{-1})	5.54	0.51	0.32	0.54	3.19
SD	7.55	1.70	1.05	1.80	5.60
RSD (%)	0.04–1.20	0.03-0.17	0.06-0.15	0.04-0.05	0.04-0.19
	0.80–2.49	0.30-3.23	0.267-1.07	1.61-2.15	0.07-0.20

Table 1: Analytical parameters for the determination CPH and NPZ by the proposed method.

product. The mean recovery values obtained amount in the range 100-101%. The correlation coefficient of the data obtained is 0.997. The Sandell sensitivity (S) is found to be 0.21. The limit of detection (LOD) and quantification (LOQ) are found to be 5.54 and 7.55, respectively. The SD is found to be 0.04–1.20 and the RSD are 0.80–2.49%. The low values of the relative standard deviation indicate the high accuracy and precision of the method. This is supported also by the calculated values of Sandell sensitivity; it indicates the high sensitivity of the method.

Using BPB, BCG and BCP reagents: The utility of BPB, BCG or BCP reagents as ion-pairing reagents in assay of CPH is investigated. The spectra of the reaction products show characteristic λ_{max} at 400 nm (Figure 3). The experimental conditions were established by varying one variable and observing its effect on the absorbance of the coloured species. The effect of varying the concentration of 0.02% w/v BPB, BCG or BCP reagents on the intensity of the coloured product was studied. It was found that maximum absorption was obtained when concentrations mentioned under procedure A were added. Several organic solvents were tried for extraction of the formed complexes such as chloroform, methanol, acetone, tetrahydrofuran, 1,4–dioxane, n-pentanol, methylene chloride, dichloroethane and petroleum ether. 10 ml of chloroform (5 ml for each) was preferred for selective extraction of the formed ion pair from the aqueous phase with successful results in case of BPB, while reproducible absorbance readings are obtained after extraction by 10 ml of methylene chloride (10ml x 1) in case of BCG and BCP reagents and one minute shaking time. The optimum reaction time was determined at ambient temperature. It was found that the optimum time for the completion of the reaction of CPH is 10, 10 and 7 minutes for BPB, BCP and BCG reagents, respectively. The developed colours were stable for at least 24 h.

Stoichiometry of the formed ion–pairs: Continuous variation [16] and the molar ratio methods [17] were applied for determining the stoichiometric ratio between CPH and BPB, BCG or BCP reagents. The results indicate that 1:1 ion–pairs are formed with the investigated drug using BPB, BCG or BCP reagents (Figure 4). The ion–pairs are formed through the electrostatic attraction between the positive protonated drug and the negative ion of reagents used as shown by the proposed structures given in scheme (2) [18]. The analytical parameters obtained are listed in Table 1.

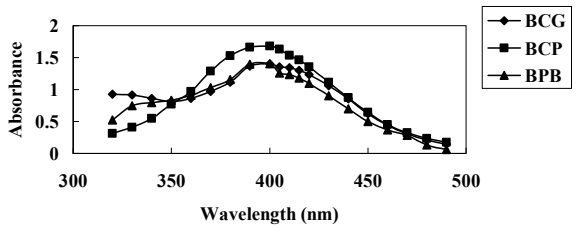


Figure 3: Absorption spectra of cyclop.HCl ion pairs using BPB, BCG and BCP reagents.

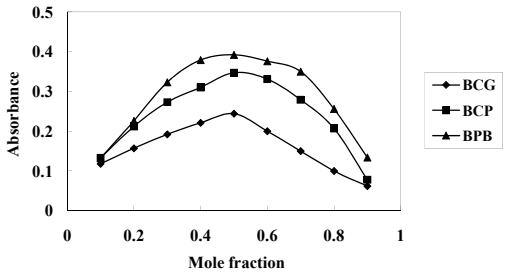
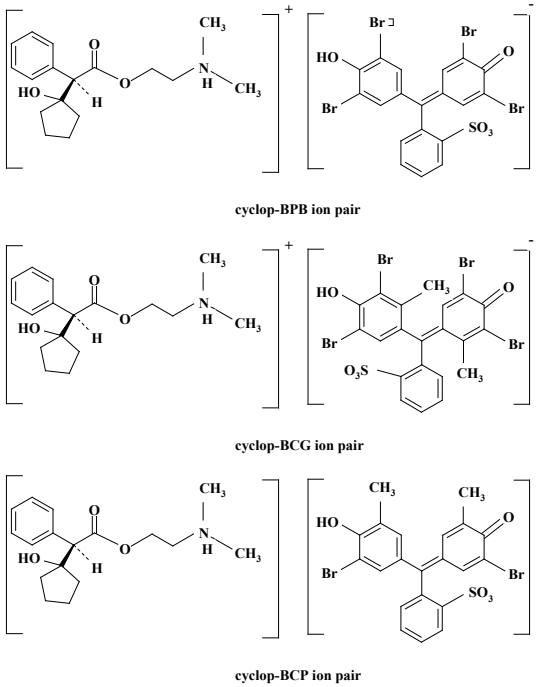


Figure 4: Stoichiometric ratio of the reaction of cyclop.HCl drug with BPB, BCG and BCP reagents using the continuous variation method.



Scheme 2: Structures of CPH-BPB, -BCG and -BCP ion pairs.

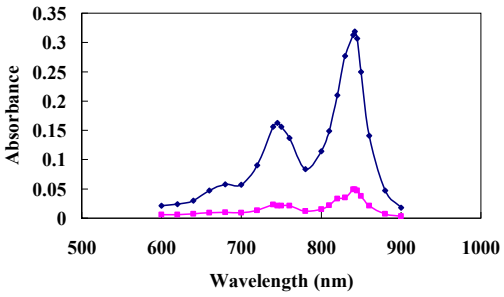
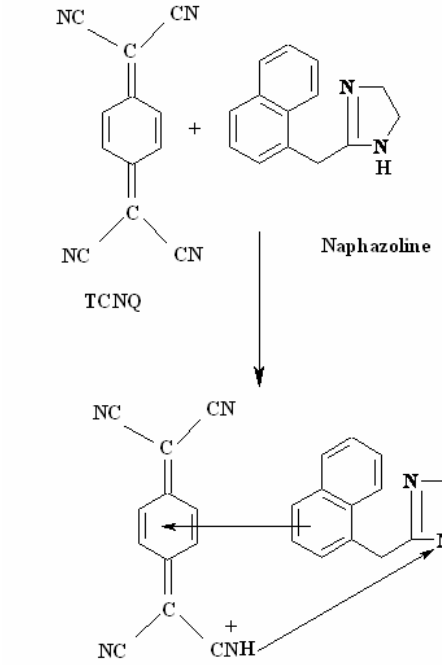


Figure 5: Absorption spectra of (a) TCNQ in acetonitrile and (b) CT complex of naphaz.HCl with TCNQ.

Organic solvents	A	ε L mol^{-1} cm^{-1}
Acetonitrile	0.319	0.79 x 10^4
Acetone	0.716	1.77 x 10^4
Methanol	1.303	3.22 x 10^4
1,4-Dioxane	0.015	0.04 x 10^4
Tetrahydrofuran	0.784	1.94 x 10^4
Cyclohexanone	0.306	0.76 x 10^4
Ethanol	1.414	3.49 x 10^4
2-Propanol	0.862	2.13 x 10^4
Chloroform	0.610	1.51 x 10^4

Table 2: The molar absorptivity values of NPZ-TCNQ CT complex in different solvents.



Scheme 3: Structure of NPZ-TCNQ CT complex.

Determination of naphazoline.HCl

The utility of TCNQ as charge transfer reagent in the assay of NPZ is investigated. The spectrum of the reaction product shows characteristic absorption band at λ_{max} at 842 nm (Figure 5). The experimental conditions were established by varying one variable and observing its effect on the absorbance of the coloured species. The effect of the

Compound	[Drug], Taken µg mL^{-1}	[Drug], Found µg mL^{-1}	Percentage Recovery (%)	SD*	RSD*
cyclop.HCl **Mo(V)-thiocyanate:**	150.0 200.0 250.0	150.0 199.3 249.8	100.0 99.65 99.90	0.24 0.76 0.84	3.60 0.38 1.19
BPB	18.60 28.04 37.38	18.60 28.28 37.38	100.0 100.9 100.0	0.02 0.02 0.01	2.84 2.69 1.07
BCG	9.35 18.60 28.04	9.35 19.08 28.04	100.0 102.5 100.0	0.01 0.03 0.029	1.95 2.87 2.07
BCP	18.60 28.04 37.38	18.60 28.54 39.19	100.0 101.8 104.8	0.013 0.024 0.026	2.25 2.91 2.77
naphaz.HCl **TCNQ**	60.00 100.0 150.0	61.75 100.0 149.3	102.9 100.0 99.50	0.05 0.06 0.01	0.08 0.06 1. 90

* Mean values for five experiments carried out on four days.

Table 3: Between–day precision of the determination of CPH and NPZ drugs by the proposed methods.

Sample	Proposed		Official		% Recovery#		SD*	SD**
	[Drug] µg mL^{-1}		[Drug] mg mL^{-1}					
	Taken	Found	Taken	Found	Proposed	Official		
Colircusi: **Mo(V)-thiocyanate:**	150.0 250.0	149.8 250.1	10.00	10.17	99.87 100.0	101.7	0.013 0.012	0.30
BPB	18.60 37.38	18.70 37.50	10.00	10.17	100.5 100.3	101.7	0.02 0.03	0.30
BCG	18.60 37.38	18.40 37.28	10.00	10.17	98.92 99.50	101.7	0.08 0.05	0.30
BCP	18.60 37.38	18.70 37.80	10.00	10.17	100.5 101.1	101.7	0.02 0.02	0.30
Neozoline **TCNQ**	60.00 150.0	59.90 148.9	5.00	4.92	99.83 99.30	98.40	0.01 0.04	0.03

* Proposed method
** Official method
# Mean values for five replicates (n = 5)

Table 4: Spectrophotometric determination of CPH and NPZ in pharmaceutical preparations by the proposed and official titrimetric methods.

concentration of 0.02% w/v TCNQ on the intensity of the coloured product was studied. It was found that maximum absorption was obtained when concentration mentioned under procedure B was added. Several organic solvents were tried for enhancing the colour intensity of the formed CT complexes such as acetonitrile, methanol, ethanol, acetone, tetrahydrofuran, cyclohexanone, chloroform and 1,4–dioxane. The results obtained are given in Table 2. Although methanol, ethanol, tetrahydrofuran, acetone, 2-propanol and chloroform have high molar absorptivity than acetonitrile but the stability and reproducibility of the absorbance values of the CT complex are stable and reproducible in acetonitrile solvent. The optimum reaction time was determined at ambient temperature. It was found that, the optimum time for the completion of the reaction of NPZ is 60 minute. The developing colour was stable for at least 24 h. The stoichiometric studies revealed the formation of 1:1 [NPZ]: [TCNQ] CT complex as shown in scheme (3) [19]. Beer's law was valid over the concentration range stated in Table 1.

Quantification, accuracy and precision of the proposed methods

A linear correlation was found between absorbance and concentration in the ranges given in Table 1. The correlation coefficients, intercepts and slopes for the calibration data for the two cited drugs were calculated. The accuracy and precision of the proposed methods were established by measuring the content of CPH or NPZ in pure form at three different concentration levels. The between day precision of the proposed methods was performed by carrying out four replicate experiments at each concentration level within four days (Table 3). The results of standard deviation (SD), relative standard deviation (RSD) and recoveries by the proposed methods in Table 1 can be considered to be very satisfactory. Thus the proposed methods are very effective for the assay of CPH or NPZ in drug formulations. The applicability of the proposed methods for the determination of CPH or NPZ has been tested on commercially available pharmaceutical formulations and the data obtained are listed in Table 4. The results of the proposed methods were compared with those obtained by the official method [20,21].

Conclusion

The data given above reveal that the proposed methods are simple, accurate and sensitive with good precision and accuracy. With these methods, one can do the analysis in a short time at low cost without losing accuracy. The proposed methods can be used as alternative methods to reported ones for the routine determination of cyclopentolate and naphazoline hydrochlorides in the pure form and in pharmaceutical formulations.

References

1. Andermann G, Richard A J (1984) Determination of cyclopentolate by capillary column gas-liquid chromatography. High Res Chromatog and Chromatog Communi 3: 144-146.

2. Rao KVK, Reddy MN, Rao SS, Rao MEB (2001) Spectrophotometric determination of cyclopentolate hydrochloride in pharmaceutical dosage forms. J Instit Chem 5: 171-173.

3. Reddy MN, Rao KVK, Murthy TK, Reddy MD (2001) Extractive spectrophotometric determination of cyclopentolate hydrochloride. Asian J Chem 3: 1266-1268.

4. Rao KVK, Kumar MK, Mathivanan N, Kumar MS, Rao MEB, Rao SS (2002) Extractive spectrophotometric determination of cyclopentolate hydrochloride and tropicamide. Acta Ciencia Indica Chem3173-174.

5. Gallego JML, Arroyo JP (2003) Determination of prednisolone and the most important associated compounds in ocular and cutaneous pharmaceutical preparations by micellar electrokinetic capillary chromatography . J Chromatogr Bvol 1: 39-47.

6. Gallego JML, Arroyo JP (2003) Determination of prednisolone, naphazoline, and phenylephrine in local pharmaceutical preparations by micellar electrokinetic chromatography. J Sep Sci 26: 947-952.

7. Petr C, Dalibor Š, Petr S. (2006) Fast simultaneous spectrophotometric determination of naphazoline nitrate and methylparaben by sequential injection chromatography. Talanta, vol. 2, 70, 408-413.

8. Hman SO. (1987) Direct Determination of antazoline and naphazoline in mixtures. Drug Develop. Ind. Pharma., vol. 7, 13, 1257-1265.

9. Belal S, Elsayed MA, Abdel-Hamid ME, Abdine H. (2006) Utility of chloranil in assay of naphazoline, clemizole, penicillin G sodium, and piperazine. J. of Pharm. Sci., vol. 70, 2, 127–130.

10. Charles J, Ertucat M. (1999) Simultaneous determination of naphazoline nitrate and tetramethylthionine base in eye drops by first-derivative UV spectrophotometry. Anal. Lett., vol. 2, 32, 373–382 .

11. Goicoechea HC, Olivier AC (2002) Chemometric assisted simultaneous spectrophotometric determination of four-component nasal solutions with a reduced number of calibration samples. Anal. Chim. Acta, vol. 453, 2, 289-300.

12. Goicoechea H, Collado M, Satuf M, Olivieri AJ (2003) Complementary use of partial least-squares and artificial neural networks for the non-linear spectrophotometric analysis of pharmaceutical samples. Anal Bioanal Chem 3 460-465.

13. Souri E, Amanlou M, Farsam H, Afshari A. (2006) A Rapid Derivative Spectrophotometric Method for Simultaneous Determination of Naphazoline and Antazoline in Eye Drops. Chem. Pharm. Bull. (Tokyo), vol. 1, 54, 119-22.

14. Ghoreishi SM, Behpour M, Nabi M. (2006) A novel naphazoline-selective membrane sensor and its pharmaceutical applications. Sens. and Act. B, vol. 2, 113, 963-969.

15. Mohamed GG, Nour El-Dien FA, Khalil SM, Mohamed NA (2006) Spectrophotometric determination of trazodone, amineptine and amitriptyline hydrochlorides through ion-pair formation with molybdenum and thiocyanate. Spectrochim Acta A 65: 1221–1226.

16. Job P (1928) Formation and stability of inorganic complexes in solution. Annali di Chimica Applicata 9: 113–203.

17. Vosburgh WC, Cooper GR (1941) Complex ions. I. The identification of complex ions in solution by spectrophotometric measurements. J Am Chem Soc 63: 437–442.

18. Mohamed GG, Nour El-Dien FA, Khalil SM, El-Tantawy ASM (2010) Spectrophotometric determination of peptic ulcer sulfur-containing drugs in bulk form and in tablets. Drug Testing and Analysis 2: 28-36.

19. Nour El-Dien FA, Mohamed GG, Frag EY (2009) Utility of π-acceptor reagents for spectrophotometric determination of sulphonamide drugs via charge-transfer complex formation Chemical Papers 63: 646-653.

20. British pharmacopeia, Stationary Office London, 630, 2008.

21. British pharmacopeia, Stationary Office London, 1517, 2008.