ISSN : 0974-7419 Volume 11 Issue 2

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 11(2) 2012 [49-55]

Simultaneous determination of biperiden and chlorphenoxamine in various dosage forms by spectrophotometry

F.A.A.Issa[1], S.M.EL-Helbawy[2], A.M.Abed EL-Magied[2*]

[1]Chemistry Department, Faculty of Science, Menoufia University, (EGYPT)

[2]Medico-Legal organization, Ministry of Justice, Cairo, (EGYPT)

E-mail : Ahmed.ma70@yahoo.com

Received: 7th June, 2011 ; Accepted: 7th July, 2011

ABSTRACT

Simple and rapid spectrophotometric procedures were established for quantization of biperiden hydrochloride (BPN) and chlorphenoxamine hydrochloride (CPA). The procedures are based on the reaction between the examined drugs BPN and CPA and picric acid (I), alizarin (II), bromothymol blue (III) and chlorophenol red (IV) producing ion-associates which can be measured at the optimum wavelength. The optimization of the reaction conditions was investigated. Beer's law is obeyed in the concentration ranges 7.8–122.9 µgml?1. The molar absorptivity, Sandell sensitivity are also calculated. The correlation oefficient was ¡Ý0.9998 (n = 4) with a relative standard deviation (R.S.D.) ¡Ü1.35 for four determinations. The methods are successfully applied to determine of BPN and CPA in pharmaceutical formulations.

KEYWORDS

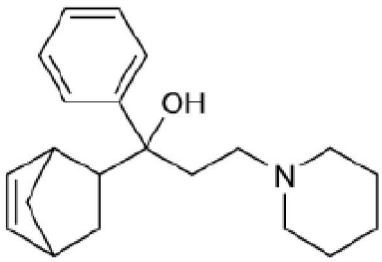
Spectrophotometry;
Ion-associates;
Drugs;
Biperiden;
Chlorphenoxamine.

INTRODUCTION

Biperiden hydrochloride (BPN) is α-Bicyclo[2.2.1]hept–5–en–2–yl-α-phenyl–1–piperidinepropanol. Chlorphenoxamine hydrochloride (CPA) is 2- [1 - (4 - Chlorophenyl) - 1 – phenylphenylethoxy] -*N*,*N*-dimethylethanamine. They are **a** weak peripheral anticholinergic[1, 2]. agent. It has, therefore, some antisecretory, antispasmodic, mydriatic, and antihistaminic effects. The mechanism of action of centrally active anticholinergic drugs such as BPN, CPA are considered to relate to competitive antagonism of acetylcholine at cholinergic receptors in the corpus striatum, which then restores the balance[3, 4]. Several methods have been applied for the determination of biperiden hydrochloride and chlorphenoxamine hydrochloride in dosage forms and in biological fluids. The different techniques used in this action include Spectrophotometry[5, 6], High Pereformance Liquid Chromatography[7], Gas Chromatography[8], Polarography[9], and cyclic voltammetery[10].

The chemical structure of the studied drugs

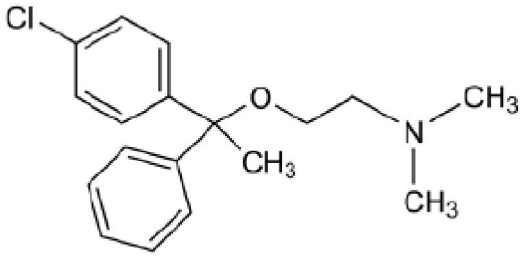
α-Bicyclo[2.2.1]hept–5–en–2–yl-α-phenyl–1–piperidine propanol



BIPERIDEN

Full Paper

2-[1-(4-Chlorophenyl)-1–phenylphenylethoxy]-*N*,*N*-dimethylethanamine



CHLORPHENOXAMINE

EXPERIMENTAL

Apparatus

The spectral measurements were carried out by using U.V visible diode array (Hewlett Packard-model 8452A) with a 10mm quartz cell.

Reagents

Picric acid 2,4,6 tri nitro phenol (I), Alizarin 1,2 di hydroxy anthraquinone (II), bromothymol blue 3,3 dibromothymol sulphonaphthalein (III) and chlorophenol red 5,5'- dichlorophenol sulphonaphthalein (IV) were Merck products. A stock solution (1¡Á 10^{-3} M) was prepared by dissolving the appropriate weights of I and II in doubly distilled water, while III and IV were dissolved in the least volume alcohol then completed by distilled water. Biperiden hydrochloride (provided from Kahira Pharm company Egypt), chlorphenoxamine hydrochloride (provided from Eipico company Egypt). And their pharmaceutical formulation (i.e. Akiniton 2 mg, and alleregex tablets).

General procedure

Into 50 ml separating funnel, 5ml of (1 ¡Á 10^{-3} M) of I, II, III and IV were added by pipette, different volumes of solution containing (1 ¡Á10^{-3} M) of drugs of BPN and CPA were added and the volume was made up to 10 ml with distilled water . The formed ion–associates was extracted with 10 ml chloroform by shaking for two minutes and allowed to separates into two phases. The organic layer was collected and dried with anhydrous sodium sulphate then complete to 10 ml chloroform. The absorbance of the extract was measured at the recommended wavelength as recorded in TABLE.1 against reagent blank prepared in the same way without addition of the examined drugs. All measurements were carried out at room temperature (25 ¡À 2 °C).

Application to various dosage forms

At least 5 tablets of the drug were weighed into a small dish, powdered and mixed well. A portion equivalent to 100 mg was weighed and dissolved in 100 ml water, shaken well and filtered through a filter paper to give clear solution. The clear solution was diluted to 250 ml with water in a 250 ml calibrated flask. The drug content of this solution was obtained by applying the general procedure to aliquot containing different volumes of solution containing of (1 ¡Á 10^{-3} M) drugs as described above.

Stoichiometric relationship

Job's method of continuous variation was employed, of (1 ¡Á 10^{-3} M) standard solution of BPN, CPA and of (1 ¡Á 10^{-3} M) solution of reagents (I–IV) were used. A series of solutions were prepared in which the total volume of drug and reagent was kept constant at 5.0 ml. The reagents were mixed in various proportions and diluted to volume in 10 ml calibrated flask with the appropriate solvent following the above mentioned procedures.

RESULTS AND DISCUSSION

Several parameters such as reagent concentration, sequence of addition, effect of extracting solvent, effect of pH, effect of time, were optimized to achieve high sensitivity, stability and reproducible results.

Optimization

Most favorable conditions were examined to achieve maximum colour intensity in the quantitative determination of the examined drug (BPN and CPA). The absorption spectra of BPN and CPA and their ion–associates with picric acid, alizarin, bromothymol blue and chlorophenol red under the optimum conditions are recorded in TABLE 1. The absorption band of BPN and CPA ion–associates are located at 418, 422, 420, and 415 nm, 418, 428, 421 and 422 nm with reagents (I–IV), respectively. However, in all instances, the absorbance was measured at those /Ëmax against reagent blank; the influence of each of the following variables on the reaction was tested. Figure (1,2).

TABLE 1 : Characteristics and analytical data of BPN and CPA ion-associates with (I – IV) reagents.

Parameters	BPN				CPA			
	I	II	III	IV	I	II	III	IV
λ max (nm)	418	422	420	415	418	428	421	422
Beer's law up to (µg/mL)	17.56-122..9	17.56-122.9	8.8-61.6	8.8-61.6	15.8-110.6	15.8-110.6	7.9-55.3	7.9-55.3
Molar absorpitivity (ε) [$Lmol^{-1}cm^{-1}$]	$0.38x10^4$	$0.33x10^4$	0.5x10	$0.53x10^4$	$0.36x10^4$	$0.3x10^4$	$0.96x10^4$	$0.49x10^4$
Sandell sensitivity [µg cm^{-2}]	0.083	0.094	0.062	0.059	0.097	0.11	0036	0.071
Regression equation*								
Intercept	0.001	0.046	0.045	0..03	0.032	0.025	0.010	0.054
Slope	0.012	0.014	0.016	0.018	0.011	0.009	0.016	0.016
Correlation coefficient®	0.9999	0.9999	0.9987	0.9996	0.9998	0.9997	0.9997	0.9996

*A = a +bc where c is the concentration µg/mL

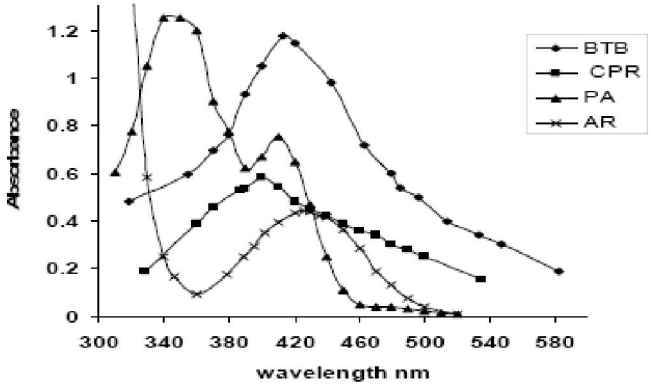


Figure 1 : Absorption spectra of BPN ion-associates with AR, PA, CPR, and BTB

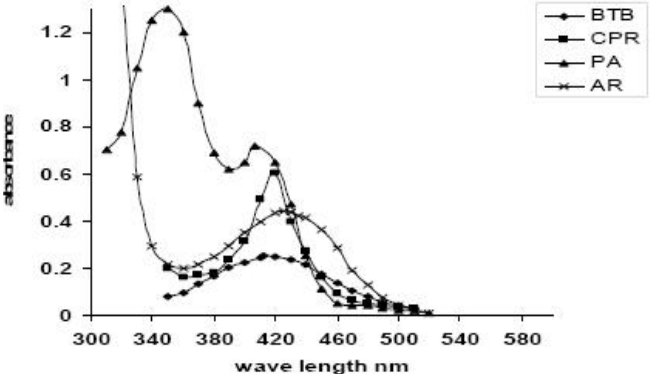


Figure 2 : Absorption spectra of CPA ion-associates with AR, PA, CPR and BTB.

Effect of the extracting solvent

The polarity of the solvent affects both extracting efficiency and absorptivity of the ion–associates. Various water – immiscible organic solvents were used (methylene chloride, chloroform, benzene, n-hexane, cyclohexane and diethyl ether). The most convenient solvent found to give the maximum colour intensity and extracting power of ion –associates was chloroform for

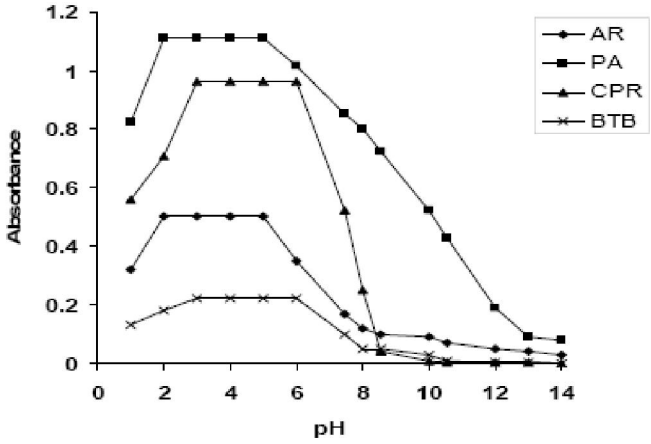


Figure 3 : Effect of pH on BPN ion-associates with AR, PA, CPR and BTB.

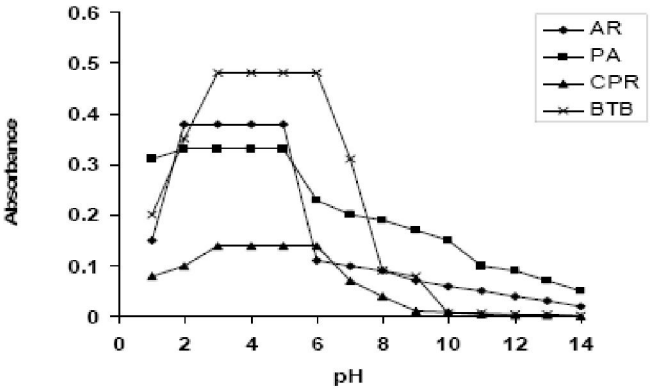


Figure 4 : Effect of pH on CPA ion-associates with AR, PA, CPR and BTB

BPN and CPA. The study revealed that a volume ratio of 1-1 (aqueous – organic) was the most suitable for the ion–associates extraction.

Effect of the reagent concentration

The effect of reagent concentration was tested by using varying amountes 1-5 ml of (1 ¡Á 10^{-3} M) solu-

Full Paper

TABLE 2 : Evaluation of accuracy of the proposed method for determination of BPN and CPA.

Ion-associates	Pure solution (µg/mL)			Tablets (µg/mL)		
	Taken	Found	Recovery %	Taken	Found	Recovery %
BPN-I	20	20.08	100.4	20	19.70	98.5 0
	30	29.80	99.33	30	29.55	98.50
	40	40.10	100.25	40	39.50	98.75
	50	50.04	100.08	50	49.45	98.90
BPN-II	20	20.05	100.25	20	19.65	98.25
	30	29.90	99.66	30	29.45	98.16
	40	40.02	100.05	40	39.60	99.00
	50	49.80	99.60	50	49.55	99.10
BPN-III	20	19.95	99.75	20	20.05	100.25
	30	30.04	100.10	30	29.60	98.66
	40	39.85	99.62	40	39.55	98.87
	50	50.04	100.08	50	49.60	99.20
BPN-IV	20	20.01	100.05	20	19.85	99.25
	30	29.95	99.83	30	29.50	98.33
	40	40.09	100.22	40	40.10	100.25
	50	49.90	99.80	50	49.65	99.10
CPA-I	20	19.85	99.25	20	19.87	99.36
	30	29.90	99.66	30	29.95	99.83
	40	39.92	99.80	40	39.93	99.84
	50	49.75	99.50	50	50.14	100.28
CPA-II	20	19.90	99.50	20	20.04	100.2
	30	29.95	99.83	30	29.88	99.60
	40	39.80	99.50	40	39.70	99.25
	50	49.75	99.50	50	49.84	99.68
CPA-III	20	20.02	100.1	20	19.78	98.90
	30	29.80	99.33	30	30.08	100.03
	40	40.10	100.25	40	39.55	98.87
	50	49.90	99.80	50	50.04	100.08
CPA-IV	20	20.08	100.4	20	19.85	99.25
	30	29.95	99.83	30	29.80	99.33
	40	40.05	100.12	40	40.08	100.20
	50	50.04	100.08	50	49.44	98.88

tion of BPN and CPA. The result shows that 2.5 ml of (1 ¡Á 10^{-3} M) solution of the reagent (I – IV) were sufficient to achieve the maximum absorbance value.

Effect of pH

The effect of pH on the formation of ion–associates was studied using stock solution of sodium hydroxide and hydrochloric acid (0.5 M). 5 ml of (2 ¡Á 10^{-3} M) of reagent was mixed with 1ml (5 ¡Á 10^{-4} M) of drug solution and adding drops of HCl or NaOH to adjust pH then the volume was made up to 10 ml with distilled water. The optimum pH range for BPN and CPA was 2 – 6 pH for (I – IV) reagents, respectively. Figure (3, 4).

Effect of temperature

The effect of temperature on the formation and stability of ion–associates was studied by measuring the absorbance of the extracted ion–associates at temperature range form 25-70°C. The results show that the ion-associates were formed almost instantaneously at room temperature (25 ± 2 ?C) and remain constant up to 45°C for reagents I-IV. Figure (5, 6).

Effect of time

The effect of time on the formation and stability of ion–associates was studied by measuring the absorbance of the extracted ion–associates at increasing time intervals. The results show that the developed color remained stable for 14 hr. with reagents from I - IV after these intervals, slight decrease in color intensity occurred Figure (7, 8).

Effect of sequence of mixing

The optimum sequence of mixing was (reagent-drug –solvent). For production the highest colour intensity and shortest time for maximum absorbance, while other sequences needed longer time in addition to lower stability.

The stoichiometry of the ion–associates

The stoichiometry of the ion–associates formed

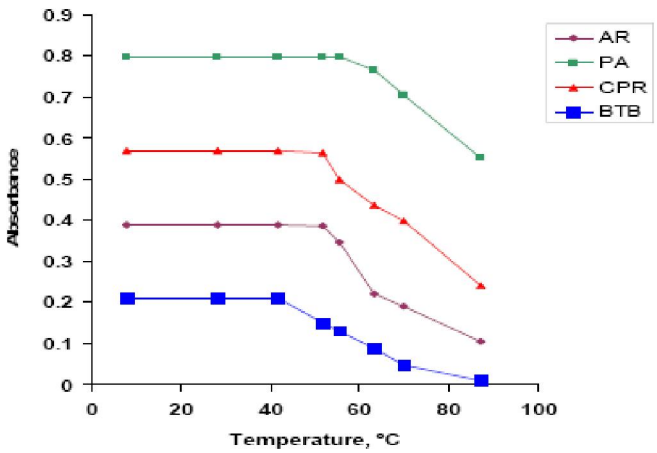


Figure 5 : Effect of temperature on the stability of BPN ion-associates with AR, PA, CPR,and BTB.

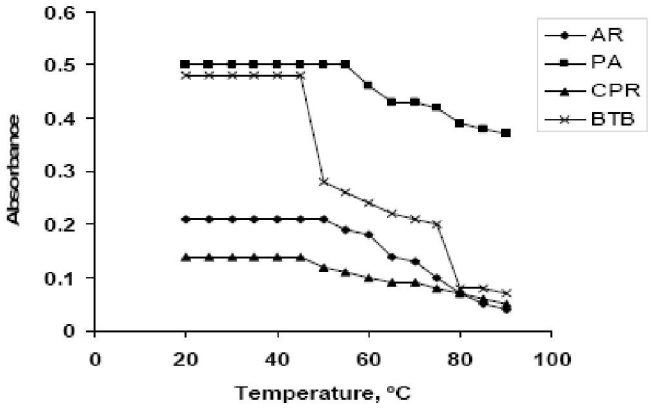


Figure 6 : Effect of temperature on the stability of CPA ion-associates with AR, PA, CPR and BTB.

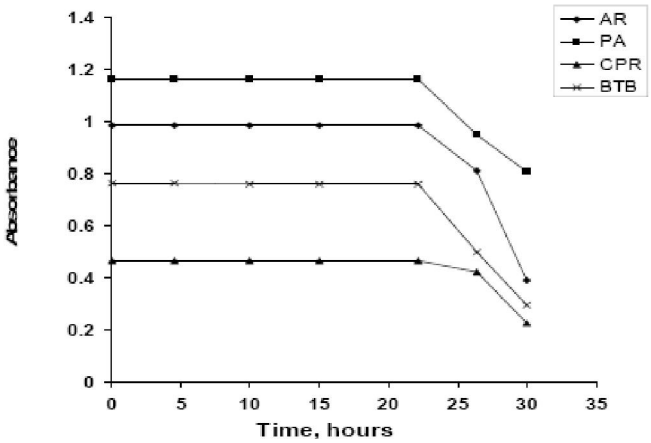


Figure 7 : Effect of time on the stability of BPN ion – associates with AR, PA, CPR, BTB.

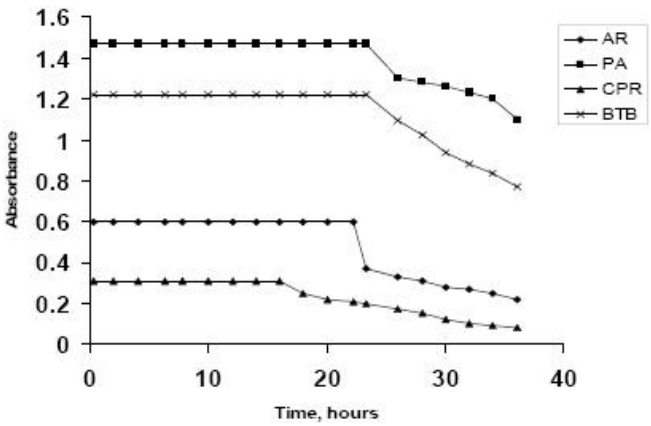


Figure 8 : Effect of time on the stability of CPA ion – associates with AR, PA, CPR, BTB.

between drugs under investigation and reagents (I–IV) was investigated by applying the continuous variation method[11] and molar ratio method[12]. Figure (9, 10, 11, 12) The result indicates the existence of [1:1] charge transfer complex at a definite ¡Ëmax recorded in TABLE 1.

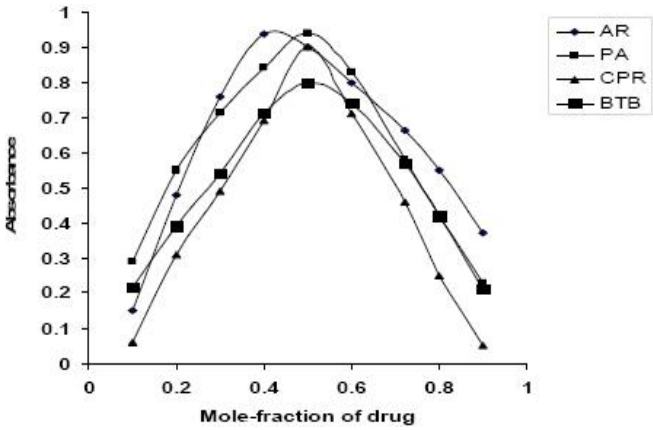


Figure 9 : Continuous variation of(BPN) ion-associates with AR, PA, CPR and BTB.

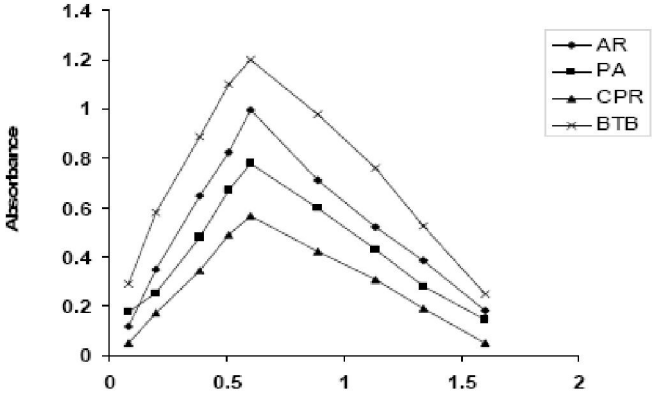


Figure 10 : Continuous variation of CPA ion-associates with AR, PA, CPR and BTB.

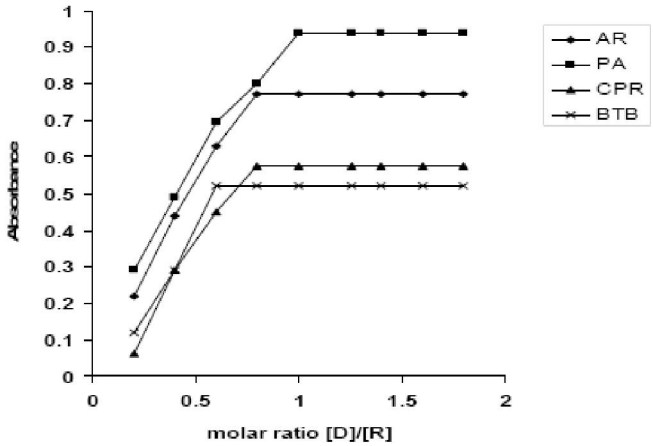


Figure 11 : Molar ratio of BPN ion-associates with AR, PA, CPR and BTB

Interference

No interference was observed in the determination of BPN and CPA with different reagents (I–IV) in the presence of different additives such as lactose, glycerol, propylene glycol, sugar and starch. Which are present in its pharmaceutical preparations.

Full Paper

TABLE 3 : Statistical treatment of data obtained for BPN and CPA applying the proposed methods in comparison with the Pharamacopial method

Parameters	Pharamacopial method	I	II	III	IV
Pure solution (BPN)					
X±SD	100.06±0.87	99.99±0.91	99.89±0.99	99.87±0.85	99.97±1.4
n	3	4	4	4	4
t value*		0.28	0.58	1.17	2.1
F value		1.82	2.52	2.27	1.74
Tablets (BPN)					
X±SD	100±0.92	98.66±0.93	98.62±0.95	99.24±0.88	99.22±1.38
n	3	4	4	4	4
t value*		0.57	1.42	1.04	0.90
F value		1.56	1.82	2.25	1.96
Pure solution (CPA)					
X±SD	100.06±0.9	99.55±1.04	99.58±1.09	99.87±1.23	100.01±1.4
n	3	4	4	4	4
t value*		0.28	0.58	1.17	2.1
F value		1.82	2.52	2.27	1.74
Tablets CPA))					
X±SD	100.02±0.80	99.82±0.98	99.68±0.95	99.47±1.25	99.40±01.44
n	3	4	4	4	4
t value*		1.89	1.05	1.76	2.58
F value		1.38	1.57	1.27	1.09

Theoretical value at 95% confidence level.
n: number of replicates

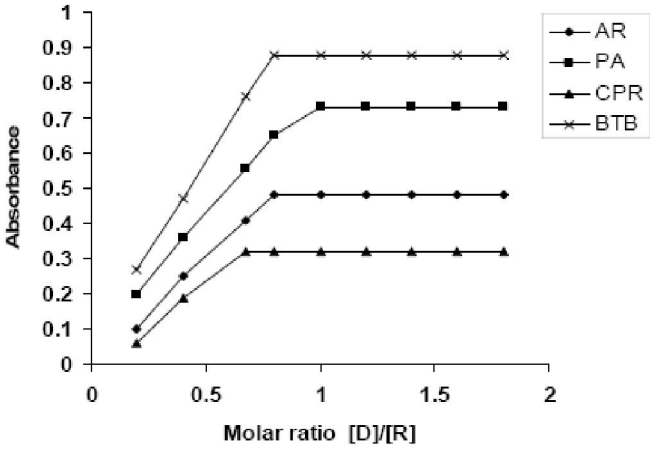


Figure 12 : Molar ratio of CPA ion-associates with AR, PA, CPR and BTB.

VALIDATION OF THE METHOD

Results obtained were compared with those of the official methods. The statistical treatment of obtained results revealed that there is no significant difference between both as shown in TABLE 3. Four replicate determination at different concentration levels were carried out to test the precision of the method. The recoveries in the range (98.16- 100.4 %)as in TABLE 2, reflecting high accuracy of the results, in addition to high precision indicated by very low values of standard deviation. The performance of the proposed method was assessed by calculation of t and f tests compared with the Pharamacopial method[13, 14]. Mean values were obtained with student's t and f testes at 95% confidence level for five degrees of freedom were in the accepted values.

CONCLUSIONS

The proposed method for the estimation of biperedien and chlorphenoxamine hydrochlorides with different reagents (I–IV) in pharmaceutical preparations was successfully applied to various dosage forms, the

results are recorded in TABLE 1 compared statistically with the official methods[13, 14]. High recoveries, high accuracy, in addition to the high precision indicated by very low values of relative standard deviations[15]. This finding indicates that the proposed methods are successful. Also this method is applicable to wide range of concentration, less time consuming and needs simple reagents which are available, thus offering an economic method for routine determination of the cited drugs.

REFERENCES

[1] A.C.Moffat; Ed. "Clarks Isolation and Identification of Drugs", Pharmaceutical Press, London, **(1994)**.

[2] K.Parfitt; Ed. Martindale, "The Extra Pharmacopoeia", The Pharmaceutical Press, London, 32nd Edition**, (1999)**.

[3] W.Lund, Ed. The Pharmaceutical Codex, Pharmaceutical Press, London, **(1994)**.

[4] Association of British Pharmaceutical Industry, Data Sheet Compendium, Datapharm Publications, London, **(1996)**.

[5] E.Dinç; I.M.Palabiyik, O.Ustündað, F.Yurtsever, F.Onur; J.Pharm.Biomed.Anal., **28(3-4)**, 591-600 **(2002)**.

[6] L.I.Bebawy, N.M.EL-Kousy; J.Pharm.Biomed. Anal.Volume **20(4)**, 663-670 **(1999)**.

[7] Y.P.Patela, U.J.Dhorda, Sundarsan, Talanta., **47**, 625-630, **(1998)**.

[8] C.Köppel, J.Tenczer, I.Arndt, K.Ibe; Arzneimittelforschung., **37(9)**, 1062-4 **(1987)**.

[9] A.Temizer, N.Ozaltin; Assoc.Off.Anal.Chem., **69(2)**, 192-5 **(1986)**.

[10] N.T.Abdel-Ghani, G.M.Abu-Elenien, S.H.Hussein; Journal of Applied Electrochemistry, **40(12)**, 2077-2090.

[11] P.Job; Ann,chim., **9**, 133 **(1928)**.

[12] J.H.Yeo, A.L.Jones; Ind.Eng.Chem.Anal.Edn., **16**, 111 **(1944)**.

[13] United States Pharmacopia, Twentieth Review, The National Formulary, Nineteenth Review, The United States Pharmacopeial Convention, Rockville, MD, **(2000)**.

[14] British Pharmacopoeia, HerMajasty's, Stationary Office, London, **(1998)**.

[15] S.Dowdy, S.Weardern; Statistics for Research", Wiley, NY, **(1983)**.