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NOVEL METHODS FOR THE VISIBLE SPECTROPHOTOMETRIC DETERMINATION OF PIPAZETHATE HCl AND CHLORPHENOXAMINE HCl IN PURE AND TABLET DOSAGE FORMS

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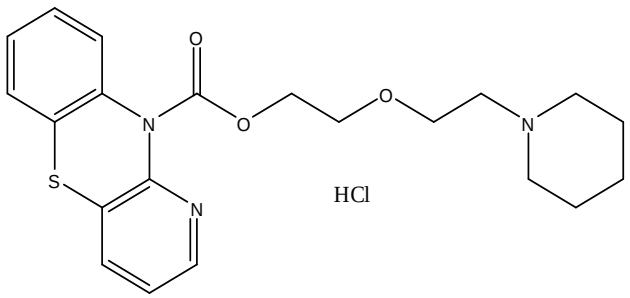
ABSTRACT

Three rapid, simple, precise and sensitive visible spectrophotometric methods (A-C) have been developed for the determination of pipazethate hydrochloride (PZ-HCl) and Chlorphenoxamine hydrochloride (CPA-HCl) in pure and Tablet Dosage Forms. Both methods (A-C) involves the formation of intense yellow ion-association complex between drug(s) and either of picric acid (PA) or phenol red (PR) or chlorophenol red (CPR) reagents followed by extraction with methylene chloride. The ion-associates exhibit absorption maxima at 359, 393 and 405 nm for PZ-HCl and at 357, 388 and 400 nm for CPA-HCl with PA, PR and CPR, respectively. The calibration curves resulting from the measurements of absorbance–concentration relations (at the optimum reaction conditions) of the extracted ion-association complexes are linear over the concentration range 3.05-43.60, 8.72-104.64 and 3.27-49.05 µg/mL for PZ-HCl and 3.40-47.64, 8.51-88.48 and 3.40-40.84 µg/mL for CPA-HCl with PA, PR and CPR, respectively. The molar absorptivities and Sandell's sensitivities of the reaction products were calculated. In methods (A-C) the slope, intercept, correlation coefficient, relative standard deviation (RSD), detection and quantitation limits were also calculated. No interference was observed from common excipients present in pharmaceutical formulations. The results are well compared to those obtained by the reference methods using Students t- and F-tests. Therefore, the present methods are suitable for the drugs determination, as they are accurate and precise to a high extent.

INTRODUCTION: Pipazethate hydrochloride (PZ-HCl) (**Fig. 1**) [1], chemically, it is 2-(2-Piperidinoethoxy)ethyl pyrido[3, 2- b][1, 4]benzothiazine- 10- carboxylate hydrochloride [2]. Medically, it is a centrally acting cough suppressant which also has some peripheral actions in non-productive cough. It has a bronchodilator effect which reduces the increased resistance to expiration during paroxysms of cough.

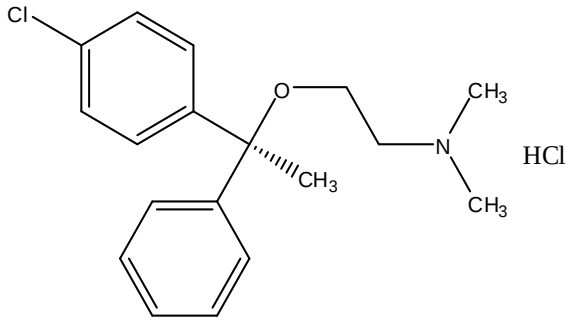


$C_{21}H_{25}N_3O_3S$, HCl = 436.0

FIG. 1: CHEMICAL STRUCTURE OF PZ-HCl

In recent years, a very few analytical methods appeared in the literature for the determination of PZ-HCl in various types of samples such as high-performance liquid chromatography (HPLC) [3], visible spectrophotometry [4, 5] and electrochemical method such as conductimetric method [6]. These methods involve a time-consuming extraction procedures or heating and require strictly con trolled reaction conditions. Many of these methods are less sensitive.

Chlorphenoxamine hydrochloride (CPA-HCl) (**Fig. 2**), [1] chemically, it is 2-(4-Chloro-α-methylbenzhydryloxy)-NN-dimethylethylamine hydrochloride [2]. Medically, it has antimuscarinic and antihistaminic properties. It has been used in nausea, vomiting, and vertigo, and was formerly used in the symptomatic treatment of parkinsonism.



$C_{18}H_{22}ClNO$, HCl = 340.3

FIG. 2: CHEMICAL STRUCTURE of CPA-HCl

In recent years, a few analytical methods have been reported on the determination of CPA-HCl in Pure and in tablet dosage forms such as Thin layer chromatography (TLC) (densitometric method) [7], UV/visible spectrophotometry [8-12], Atomic-absorption spectrometry (AAS) [13] and electrochemical method such as Polarographic method [14]. These methods are time-consuming, expensive costly, complicated and require strictly con trolled reaction conditions. Many of these methods are less sensitive.

Thus, the aim of the present work was to investigate economical, simple, precise, sensitive and environmental friendly three analytical methods (A-C) for the determination of PZ-HCl and CPA-HCl using visible spectrophotometry. Both methods (A-C) involves the formation of intense yellow ion-association complex between drug(s) and either of picric acid (PA) or phenol red (PR) or chlorophenol red (CPR) reagents by extraction with proper water-immiscible organic solvent. The results obtained from the proposed methods also have been statistically compared using Students t- and F-tests to the reference methods. They also have the advantage of being cheaper than the reported methods.

Experimental:

Materials and Reagents: PZ-HCl and CPA-HCl standards were kindly supplied as a gift samples by Egyptian International Pharmaceutical Industries Co. Cairo, Egypt (E.I.P.I.CO.) and used without further purification and purity was confirmed by thin layer chromatography and by melting point measurements. Commercial tablets of Pipazethate hydrochloride such as Selgon tablets containing 20 mg pipazethate hydrochloride and Allergex tablets containing 20 mg chlorphenoxamine hydrochloride were purchased from local drug market. Picric acid (PA) reagent (**Fig. 3**) from Arablab chemicals, phenol red (PR) (**Fig. 4**) and chlorophenol red (CPR) (**Fig. 5**) reagents from Merck chemicals. All other chemicals, solvents and reagents used were obtained from commercial sources and were of analytical reagent grade. Doubly distilled water was used throughout for final washings and preparations of all aqueous solutions. Freshly prepared solutions were always employed.

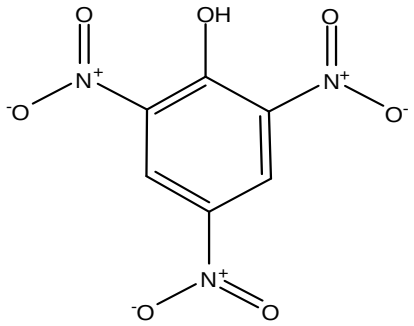


FIG. 3: CHEMICAL STRUCTURE OF PA

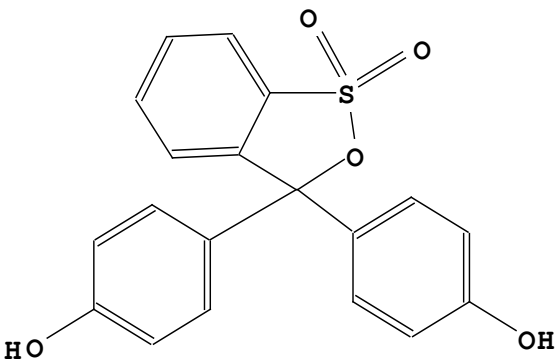


FIG. 4: CHEMICAL STRUCTURE OF PR

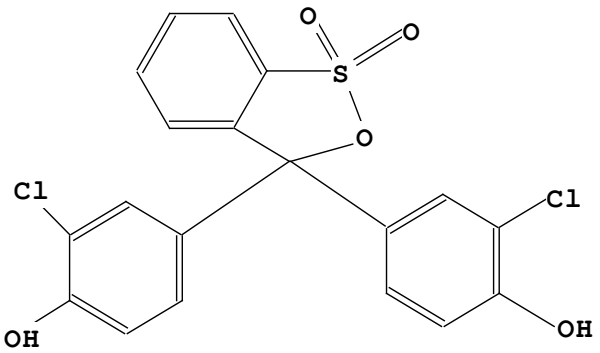


FIG. 5: CHEMICAL STRUCTURE OF CPR

Instruments and apparatus: All spectrophotometric measurements were carried out by using UV-Visible Diode Array spectrophotometer (Hewlett Packard-Model 8452A), in 1.0 cm quartz cells, was connected to PC computer and Hewlett Packard DeskJet printer. The pHs of the prepared solutions were adjusted using Jenway pH–meter. Moreover, the doubly distilled water was obtained ELGA apparatus model, UHQ-II-MK3, UK. Temperature adjustment during experiments was carried out with controlled temperature Water Bath (MLW) Model, W11-TGL, GBR. Automatic Pipettes were used to measure the very small volumes whereas glass micropipettes and burettes were used to measure the large volumes.

Preparation of Standard Solutions: For methods A, B and C, standard stock solutions 0.01 M of PZ-HCl and CPA-HCl were freshly prepared by dissolving the appropriate weights of 1.09 g (PZ-HCl) and 0.8508 g (CPA-HCl) in least amount of warm water then the solutions were made up to 100 mL with distilled water. Successive dilutions were prepared for carrying out the subsequent studies.

Standard stock solution 0.01 M of PA was freshly prepared by dissolving the appropriate weight of 0.5728 g in least amount of warm water then the solution was made up to 100 mL with distilled water. Successive dilutions were prepared for carrying out the subsequent studies. Similarly, standard stock solutions 0.01 M of PR and CPR were freshly prepared by dissolving the appropriate weights of (0.8860 g and 1.0582 g, respectively), in least amount of methanol then the solutions were made up to 100 mL with distilled water. Successive dilutions were prepared for carrying out the subsequent studies.

Recommended Procedures for the determination of PZ-HCl and CPA-HCl (Calibration Standards): For method A, B and C, 2.5 mL of 0.001 M (PA or CPR) or 0.005 M PR were added in acid medium to a solution of PZ-HCl using the concentration range of 3.05-43.60 µg/mL (PA), 3.27-49.05 µg/mL (CPR) and 8.72-104.64 µg/mL (PR) of PZ-HCl (n=5) were transferred into a series of 125 mL separating funnels. Methylene chloride (10 mL) was added to each of the separating funnel, the contents were shaken well for two minutes and left at room temperature for a minute. The two phases were allowed to separate and the methylene chloride layer was passed through anhydrous sodium sulphate.

The absorbances of the yellow ion-association complexes were measured at 359, 393 and 405 nm for PA, PR and CPR, respectively, against corresponding reagent blank. This blank was prepared in the same manner without the addition of PZ-HCl. A calibration curves were plotted (**Fig. 20**).

In a similar way, 2.5 mL of 0.001 M (PA or CPR) or 0.005 M PR were added in acid medium to a solution of CPA-HCl within concentration range of 3.4-47.64 µg/mL (PA), 3.4-40.84 µg/mL (CPR) and 8.51-88.48 µg/mL (PR) of CPA-HCl (n=5). Using the same procedures described for PZ-HCl. The absorbance of the extract was measured at λ_{max} 357, 388 and 400 nm for PA, PR and CPR, respectively, against corresponding reagent blank. A calibration curves were plotted (**Fig. 21**). The results of PZ-HCl and CPA-HCl ion-associates with PA or PR or CPR correlated to Beer's law are presented in **Table 2**.

Determination of PZ-HCl and CPA-HCl in tablets: For methods A, B and C, ten tablets of selgon and allergex were accurately weighed separately and finely powdered and mixed. A portion of the powder equivalent to the average weight of one tablet was transferred into a 100 mL volumetric flask and 30 mL of distilled water was added. The content of the flask was sonicated for 15 min. and filtered through Whatmann No. 41 filter paper to separate out the insoluble excipients. The residues were washed thoroughly with distilled water. Then take aliquot of the filtrate made up to 100 mL volume with distilled water in volumetric flask.

Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant and diluting them with distilled water to give final concentrations.

Then the absorbance of these solutions was measured against reagent blank at λ_{max}. The amount of PZ-HCl or CPA-HCl per tablet was calculated using the calibration curve method. A standard addition method was also used to confirm the accuracy and recoveries.

RESULTS AND DISCUSSION: Both methods (A-C) involves the formation of intense yellow ion-association complex between drug(s) and either of PA or PR or CPR reagents followed by extraction with methylene chloride. Many drugs are easy to be determined by spectrophotometry based on color. Optimum reaction conditions for quantitative determination ion-association complexes of PZ-HCl and CPA-HCl with PA, PR and CPR reagents, respectively, were established via a number of following preliminary experiments.

Selection of suitable Wavelength: The absorption spectra of the formed ion-association complexes were measured in the visible region within 300-700 nm wavelength range against blank reagent prepared in the same manner without the addition of the drug. The PZ-HCl ion-associates with PA, PR and CPR reagents, λ_{max} of 359, 393 and 405 nm have been obtained, respectively as shown in **Fig. 6**. For CPA-HCl ion-associates, λ_{max} of 357, 388 and 400 nm with PA, PR and CPR reagents have been obtained, respectively as shown in **Fig. 7**.

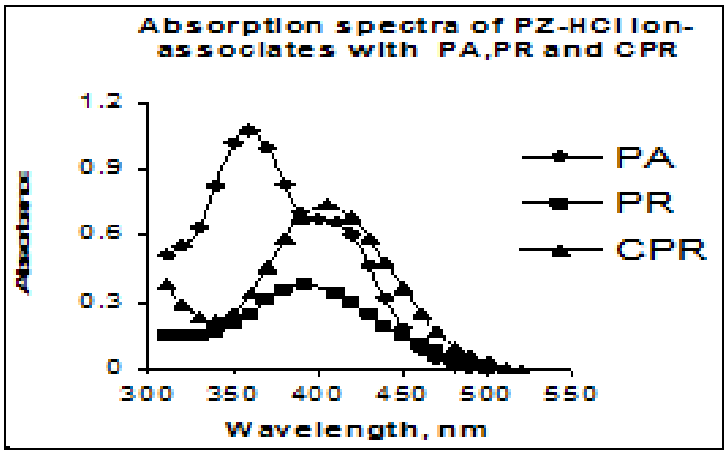


FIG. 6: ABSORPTION SPECTRA OF PZ-HCl ION-ASSOCIATES WITH PA, PR AND CPR

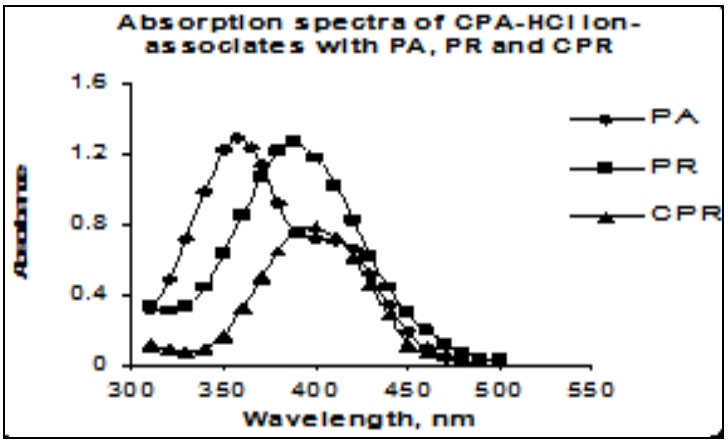


FIG. 7: ABSORPTION SPECTRA OF CPA-HCl ION-ASSOCIATES WITH PA, PR AND CPR

Effect of extracting solvents: The polarity of the solvent affects both extraction efficiency and absorpitivity of the ion-associates. Therefore, several water-immiscible organic solvents including n-hexane, petroleum ether, cyclohexane, carbon tetrachloride, toluene, benzene, diethyl ether, methylene chloride and chloroform were investigated.

The most convenient solvent for PZ-HCl and CPA-HCl ion associates which exhibit the maximum absorbance, high extraction power and stable colours is methylene chloride.

In all cases the aqueous to organic phase ratio of 1:1.5 was the most suitable for the ion-associate extraction. Complete extraction was attained by using single portion of 10 mL solvent upon using the above reagents. **Fig. 8 and 9** summarize the effect of extracting solvents on the formed ion-associates.

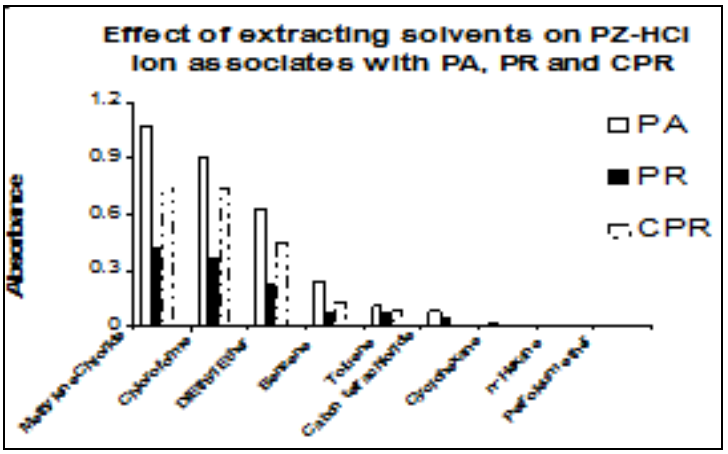


FIG. 8: EFFECT OF EXTRACTING SOLVENTS ON PZ-HCl ION-ASSOCIATES WITH PA, PR AND CPR

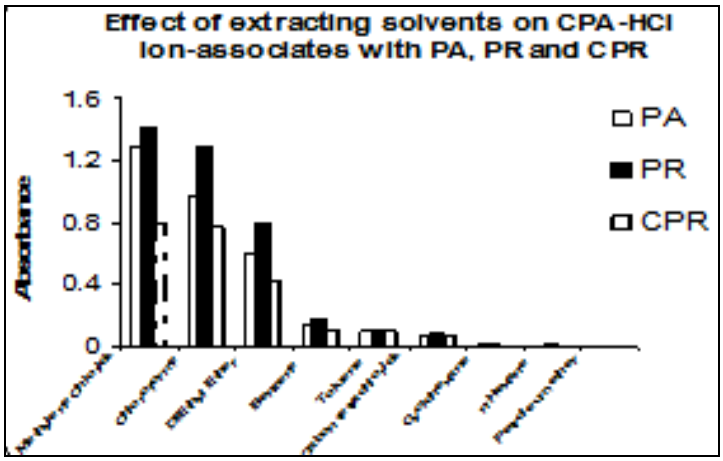


FIG. 9: EFFECT OF EXTRACTING SOLVENTS ON CPA-HCl ION-ASSOCIATES WITH PA, PR AND CPR

Effect of pH: To investigate the optimum medium conditions to determine PZ-HCl and CPA-HCl, quantitatively the effect of pH was studied by using a series of solutions (HCl/NaOH) in the pH range of 1-14, for developing the best color of drug-reagent ion-associates against the chosen reagents. In PZ-HCl and CPA-HCl, the optimum pH range for complete formation of the ion-associates showed that highest absorbance values, at their respective λ_{max} were found to be in the ranges 2-4 for PA, 2-6 for PR and 2-5 with CPR, as shown in Figs. 10 and 11, respectively.

At pH less than 2 for PZ-HCl and PZ-HCl, the absorbance decrease may be attributed to the formation of diprotonated species of the drug. In case of pH > 4 or pH > 6 or pH > 5 the absorbance decrease due to the formation of free base of the drug(s) which are insoluble in water and precipitates during the mixing.

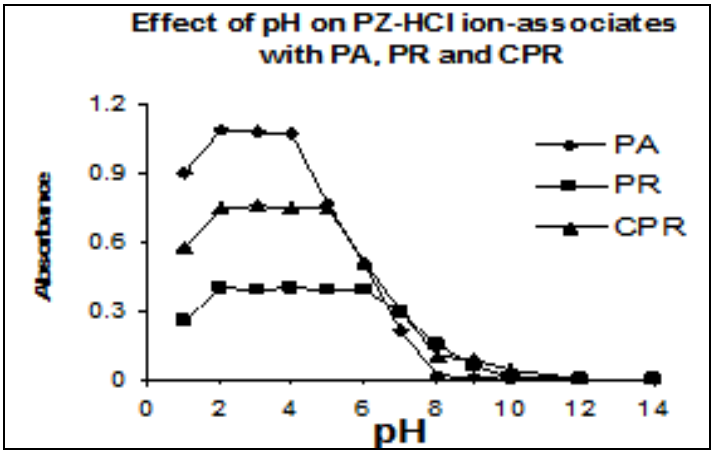


FIG. 10: EFFECT OF PH ON PZ-HCl ION-ASSOCIATES WITH PA, PR AND CPR

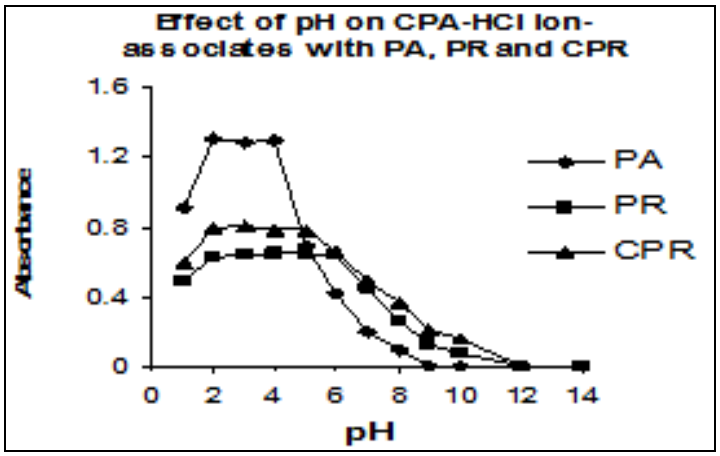


FIG. 11: EFFECT OF PH ON CPA-HCl ION-ASSOCIATES WITH PA, PR AND CPR

Effect of Reagent Concentration: The effect of reagent concentration was tested by using varying amounts (1-6) mL of 0.005 M (PR or CPR) with 1 mL of 0.001 M PZ-HCl and 0.001 M PA with 1 mL of 0.0002 M PZ-HCl and 0.001 M (PA or CPR) with 1 mL of 0.0002 M CPA-HCl and 0.005 M PR with 1 mL of 0.001 M CPA-HCl. After implementing the optimum pH condition for PZ-HCl and CPA-HCl, the formed ion-associate was completely extracted with single portion of 10 mL methylene chloride. The mixture was shaked for 2 minutes. The results showed that 5 mL of 0.005 M (PA or PR or CPR) were sufficient for good color intensity with maximum absorption of the investigated ion-associates.

Effect of Time: Under the above mentioned conditions the effect of time on the formation of the ion-associates was studied by measuring absorbance of the extracted ion-associates with increasing time intervals. The results showed that the ion-associates are formed almost instantaneously.

The effect of time on the stability of the ion–associates of PZ-HCl and CPA-HCl are represented graphically in **Fig. 12 and 13**, respectively. For PZ-HCl, the developed color remained stable for 21, 21 and 18 hours for PA, PR and CPR at λ_{max} of 359, 393 and 405 nm, respectively. Similarly, the ion-associates of CPA-HCl are formed almost instantaneously. Moreover, the developed color for CPA-HCl remained stable for 24, 18 and 21 hours for PA, PR and CPR, at λ_{max} of 357, 388 and 400 nm, respectively. After these intervals, a decrease in color intensity occurred in ion associated of PZ-HCl and CPA-HCl.

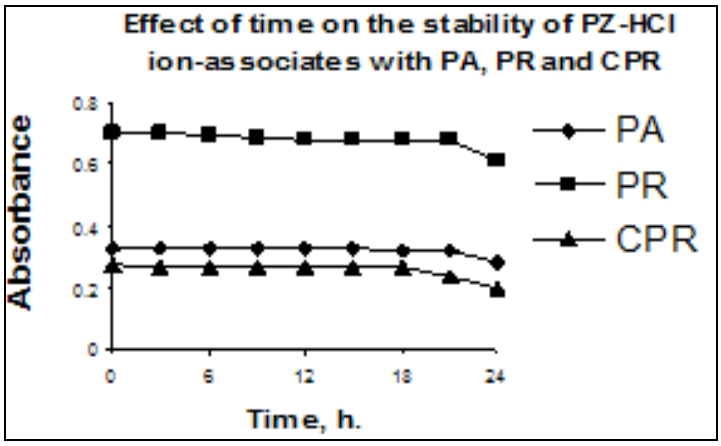


FIG. 12: EFFECT OF TIME ON THE STABILITY OF PZ-HCl ION-ASSOCIATES WITH PA, PR AND CPR

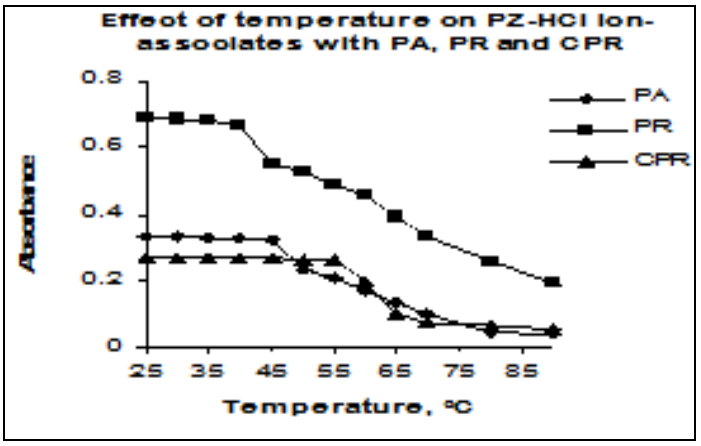


FIG. 14: EFFECT OF TEMPERATURE ON THE STABILITY OF PZ-HCl ION-ASSOCIATES WITH PA, PR AND CPR

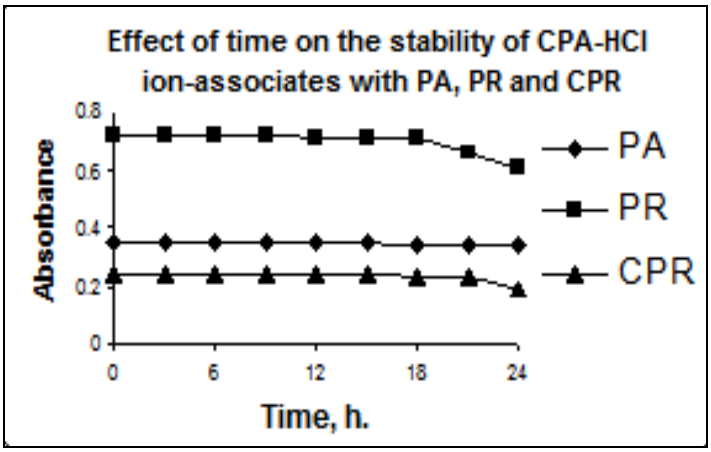


FIG. 13: EFFECT OF TIME ON THE STABILITY OF CPA-HCl ION-ASSOCIATES WITH PA, PR AND CPR

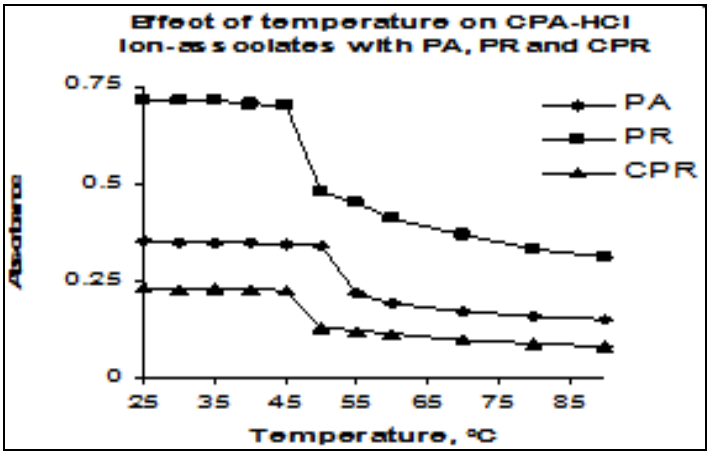


FIG. 15: EFFECT OF TEMPERATURE ON THE STABILITY OF CPA-HCl ION-ASSOCIATES WITH PA, PR AND CPR

Effect of Temperature: Under afore mentioned conditions (solvents, pH, reagent concentration and time), the effect of temperature on the formation of the ion-associates was studied by measuring the absorbance of the extracted ion-associates at a temperature range of 25-90°C.

For PZ-HCl, the results showed that the ion-associates are formed almost instantaneously in all cases at room temperature 25±5°C and remain constant up to 45°C, 40°C and 55°C for PA, PR and CPR, respectively as represented by its absorptivity at the recommended (λ_{max}). Similarly, the ion-associates of CPA-HCl are formed instantaneously with all reagents at room temperature 25±5°C and remain constant up to 50°C, 45°C and 45°C for PA, PR and CPR, respectively. The effect of temperature on the stability of ion–associates of PZ-HCl and CPA-HCl are shown in **Figs. 14 and 15**, respectively.

The Stoichiometry of the Ion- Associates: Aided by spectrophotometeric measurements, the stoichio-metries of the ion-associates of PZ-HCl and CPA-HCl with selected reagents were investigated by the aid of the following spectrophotometeric.

The Molar Ratio Method: The molar ratio method was described by Yoe and Jones [15]. At the optimum conditions described earlier for PZ-HCl and CPA-HCl ion-associates with their proper reagents, a series of solutions were prepared in which the reagent contents was kept constant, while that of the drug regularly varied. The absorbancies of the resultant extracts were measured at the corresponding λ_{max} of the ion-associates. The absorbance values were plotted against the molar ratio of drug/reagent as shown in **Figs. 16 and 17**, respectively. Two straight lines were intersecting at the molar ratio of 1 in case of PA, PR and CPR which reflects the formation of 1:1 ratio of (drug: reagent) for all ion-associates.

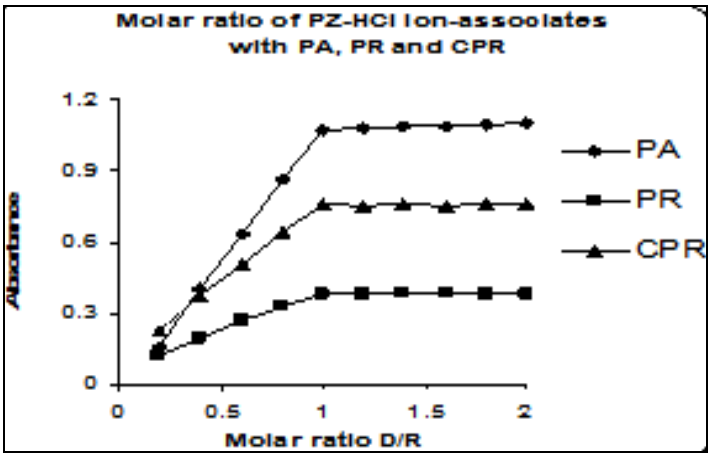


FIG. 16: MOLAR RATIO OF PZ-HCl ION-ASSOCIATES WITH PA, PR AND CPR [D=DRUGS AND R=REAGENTS]

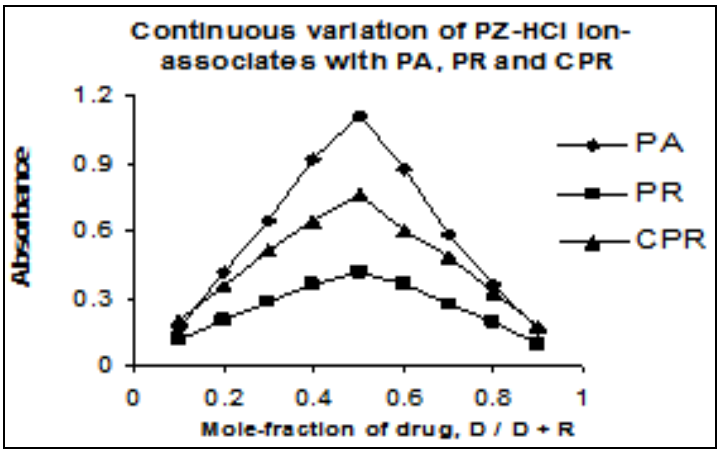


FIG. 18: CONTINUOUS VARIATION OF PZ-HCl ION- ASSOCIATES WITH PA, PR AND CPR

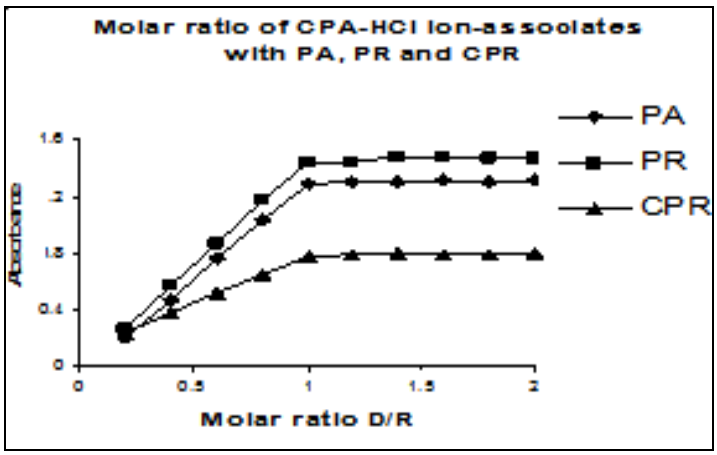


FIG. 17: MOLAR RATIO OF CPA-HCl ION-ASSOCIATES WITH PA, PR AND CPR [D=DRUGS AND R=REAGENTS]

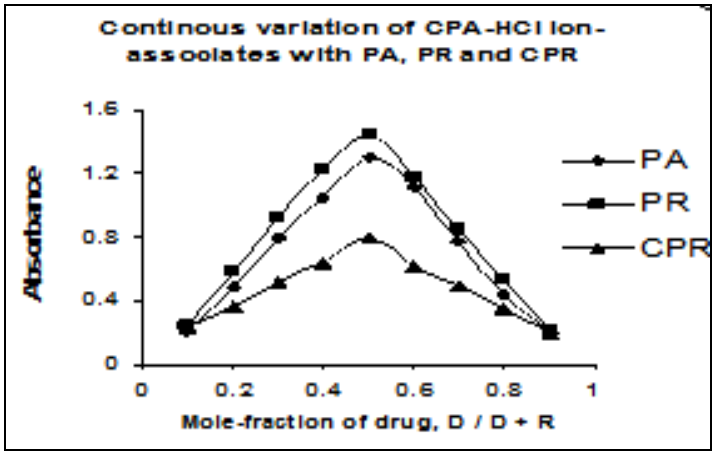


FIG. 19: CONTINUOUS VARIATION OF CPA-HCl ION-ASSOCIATES WITH PA, PR AND CPR

The Continuous Variation Method: The modification of Job's [16] continuous variation method performed by Vosburgh and Cooper [17] was utilized for investigating the reaction between drug and reagent. A series of solutions was prepared by mixing equimolar solutions of the drug and reagent in varying proportions while keeping the total molar concentration constant. The absorbance spectra of the resultant extracts were measured at the respective λ_{max} of the ion-associates to determine the absorbance. Then a plot of the absorbance against the mole fraction of the drug was constructed and presented graphically in **Fig. 18 and 19**, respectively.

The curves exhibit a maximum at mole fraction 0.5 with PA, PR and CPR indicating the formation of 1:1 (drug: reagent) for the proposed ion-associates.

Probable reaction mechanism for the formation of Ion-Association Complexes: In the first, aided by Chem Draw Ultra (Cambridge Soft Chem. Office, Ultra 2006 Versions 10.0), equipped with additional GAMES software [18, 19], the structures of positive protonated nitrogen atom of PZ-HCl and CPA-HCl compounds were proposed. In the second, the nature of the binding of reagents to each drug in the presence of equal amount of PA or PR or CPR was determined by the molar ratio [15] and the continuous variation methods [16, 17]. The results indicated that a 1:1 ratio of (drug: reagent) for all ion-associates are formed as shown in **Fig. 16-19.**

Found that PZ-HCl and CPA-HCl reacts with PA or PR or CPR forming ion-associated compounds through the electrostatic attraction between positive protonated nitrogen atom of PZH^+ and $CPAH^+$ and PA^- or PR^- or CPR^- anions. **Charts 1 and 2** summarize probable reaction mechanism for the formation of ion-association complexes of PZ-HCl and CPA-HCl with PA or PR or CPR.

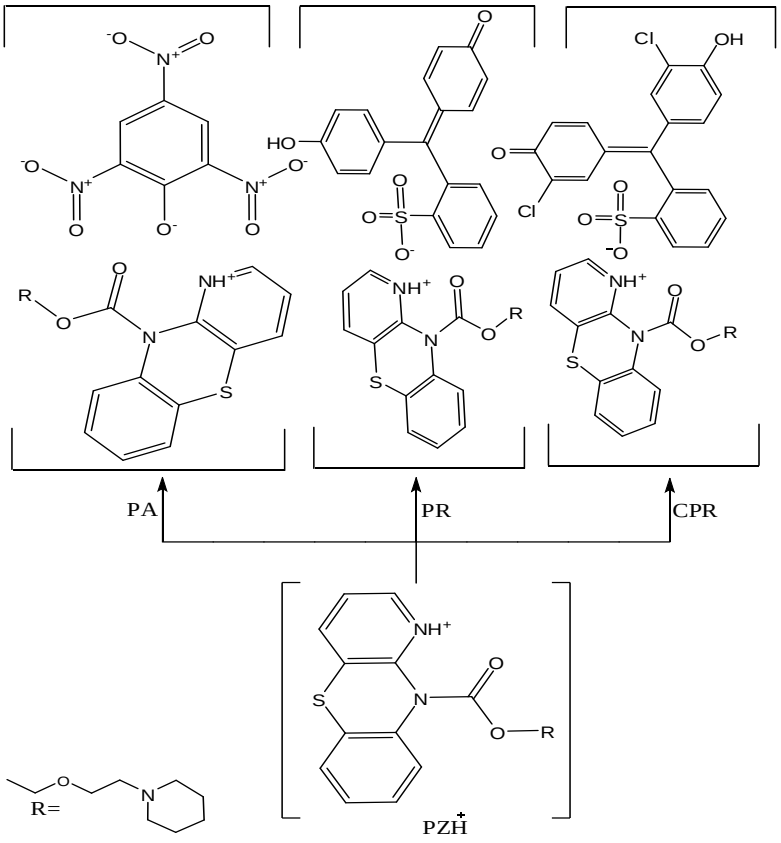


CHART 1: PROBABLE REACTION MECHANISM FOR THE FORMATION OF ION-ASSOCIATION COMPLEXES OF PZH^+ WITH PA^-, PR^- AND CPR^-

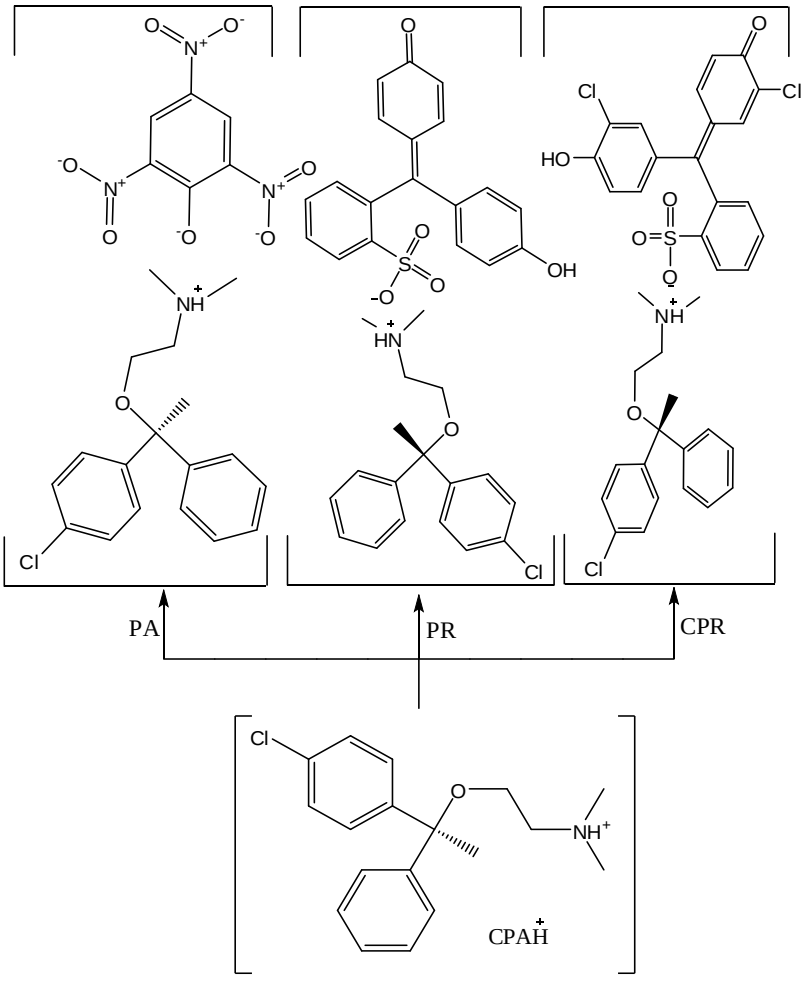


CHART 2: PROBABLE REACTION MECHANISM FOR THE FORMATION OF ION-ASSOCIATION COMPLEXES OF $CPAH^+$ WITH PA^-, PR^- AND CPR^-

TABLE 1: OPTIMAL CONDITION FOR THE EXTRACTION OF PZ-HCl AND CPA-HCl ION-ASSOCIATES WITH PA, PR AND CPR

Parameters	PZ-HCl			CPA-HCl		
	PZ-PA	PZ-PR	PZ-CPR	CPA-PA	CPA-PR	CPA-CPR
λ max, nm	359	393	405	357	388	400
Extracting solvents	Cl_2CH_2	Cl_2CH_2	Cl_2CH_2	Cl_2CH_2	Cl_2CH_2	Cl_2CH_2
Colour of extract	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
pH range	2–4	2–6	2–5	2–4	2–6	2–5
Stability of extracts, h.	21	21	18	24	18	21
Temperature on the stability, °C	45	40	55	50	45	45
The stoichiometry of the ion-associates	1:1	1:1	1:1	1:1	1:1	1:1

Validity of the Beer's Lambert Law: The spectrophotometric determination of PZ-HCl and CPA-HCl were carried out using appropriate concentration range to ensure the obedience to Beer's law. The obedience of absorbance PZ-HCl ion-associates with PA, PR and CPR to Beer's law is shown in **Fig. 20**. Similarly, the results of CPA-HCl ion-associates with PA, PR and CPR correlated to Beer's law are presented in **Fig. 21**.

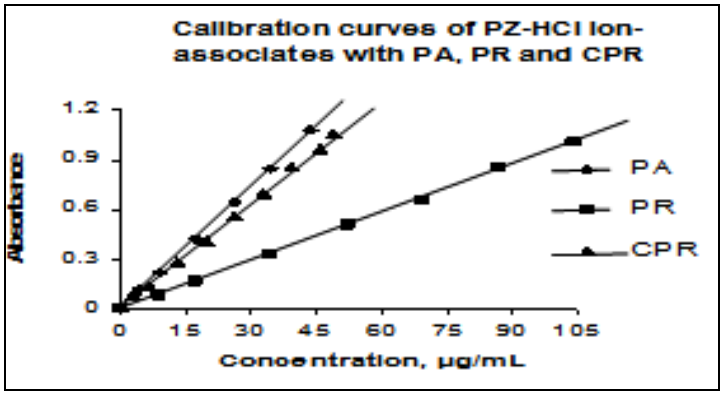


FIG. 20: CALIBRATION CURVES OF PZ-HCl ION-ASSOCIATES WITH PA, PR AND CPR

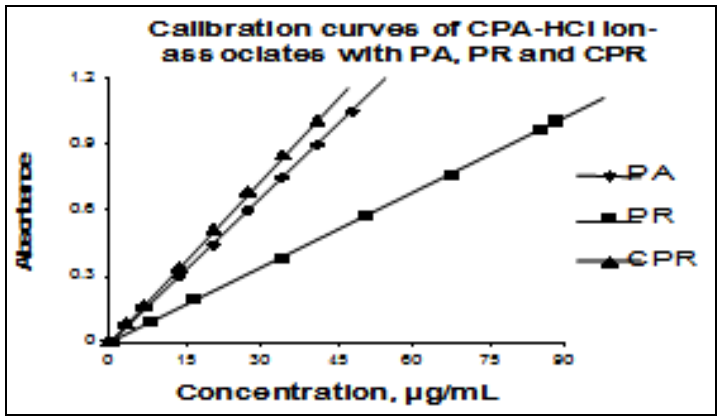


FIG. 21: CALIBRATION CURVES OF CPA-HCl ION-ASSOCIATES WITH PA, PR AND CPR

For PZ-HCl, the linear concentration ranges were found in the range 3.05–43.60, 8.72–104.64 and 3.27–49.05 µg/mL with PA, PR and CPR reagents, respectively as shown in **Table 2**. The molar absorptivities (ε) were calculated to be 1.105 x 10^4, 4.212 x 10^3 and 9.624 x 10^3 L mol^{-1} cm^{-1} for PZ-HCl ion-associates with PA, PR and CPR reagents, respectively and also Sandell's sensitivities calculated to be 3.9 x 10^{-2}, 10.4 x 10^{-2} and 4.5 x 10^{-2} µg cm^{-2} for PZ-HCl ion-associates with PA, PR and CPR reagents, respectively.

For CPA-HCl, the linear concentration ranges were found in the range 3.40–47.64, 8.51–88.48 and 3.40–40.84 µg/mL with PA, PR and CPR reagents, respectively as shown in Table 2. The molar absorptivities (ε) were calculated to be 7.674 x 10^3, 3.718 x 10^3 and 8.617 x 10^3 L mol^{-1} cm^{-1} for CPA-HCl ion-associates with PA, PR and CPR reagents, respectively and also Sandell's sensitivities calculated to be 4.04 x 10^{-2}, 9.2 x10^{-2} and 3.9 x10^{-2} µg cm^{-2} for CPA-HCl ion-associates with PA, PR and CPR reagents, respectively.

The regression equations (Y = a + bC where Y = absorbance, a = intercept, b = slope and C = concentration in µg/mL), calculated from the calibration graphs (N=5) using Kalied graph program, were evaluated and recorded in Table 2 for PZ-HCl and CPA-HCl. The intercepts of the lines were very small indicating that there is no systematic difference between the determined and expected concentrations within the investigated range using the current methods. The all RSD values from PZ-HCl and CPA-HCl were evaluated and recorded in **Table 2**. These data indicates that the developed methods have a good repeatability (were lower than 10%).

The Limit of Detection (LOD) and Limit of Quantification (LOQ) of PZ-HCl and CPA-HCl by the proposed methods were determined using calibration curves and recorded in Table 2. [LOD and LOQ were calculated as 3.3 σ/S and 10 σ/S, respectively, where S is the slope of the calibration curve and σ is the standard deviation of y- intercept of regression equation [20].

TABLE 2: FEATURES OF THE CALIBRATION CURVES OF PZ-HCl AND CPA-HCl ION-ASSOCIATES WITH PA, PR AND CPR (N=5)

Features	PZ-HCl			CPA-HCl		
	Values for PA	Values for PR	Values for CPR	Values for PA	Values for PR	Values for CPR
Number of data points	7	7	9	8	7	7
Beer's law verification range, µg /mL	3.05–43.60	8.72–104.64	3.27–49.05	3.4–47.64	8.51–88.48	3.4–40.84
Molar absorpitivity (ε) [L mol^{-1} cm^{-1}]	1.105 x 10^4	4.212 x 10^3	9.624 x 10^3	7.674 x 10^3	3.718 x 10^3	8.617 x 10^3
Sandell's sensitivity [µg cm^{-2}]	3.9 x 10^{-2}	10.4 x 10^{-2}	4.5 x 10^{-2}	4.04 x 10^{-2}	9.2 x10^{-2}	3.9 x10^{-2}
Regression equation (Y^a	Y = a + bC	Y = a + bC	Y = a + bC	Y = a + bC	Y = a + bC	Y = a + bC
Slope (b)	0.0244	0.0097	0.0214	0.0216	0.0113	0.0247
Intercept (a)	0.008	-0.002	0.007	0.010	-0.006	0.007
Correlation coefficient (r^2)	0.9998	0.9995	0.9998	0.9998	0.9998	0.9999
RSD[b)] (%)	0.76-2.53	0.20-2.55	0.69-3.09	0.89-3.11	0.19-2.11	0.48-2.76
Limit of Detection, LOD, µg /mL	0.40	0.75	0.38	0.53	1.04	0.51
Limit of Quantification, LOQ, µg /mL	1.22	2.28	1.15	1.62	3.14	1.54

a) Y=a+bC (where C is the concentration of analyte, µg /mL and Y is absorbance), b) Calculated from five determinations.

Interferences study: To study the potential interference problems from the commonly used excipients and other additives which may be present in the pharmaceutical preparations such as microcrystalline cellulose, lactose, povidone, starch, magnesium stearate, sucrose and hydroxypropylmethylcellulose, recovery studies were out. Under the experimental conditions employed, excipients in different concentrations were added to a known amount of 9, 20 and 9 µg/mL for PZ-HCl with PA, PR and CPR, respectively and to a known amount of 9, 16 and 8 µg/mL for CPA-HCl with PA, PR and CPR, respectively and analyzed according to recommended procedures described earlier. Results of the recovery analysis of PZ-HCl and CPA-HCl drugs and the above mentioned excipients are presented in **Table 3**. It was concluded that the excipients did not interfere with quantification of PZ-HCl and CPA-HCl drugs in these methods and the proposed method could be considered specific. In addition recoveries in most cases were around 100% and the lower values of the RSD indicate the good precision of the method, thus the procedures was able to determination of PZ-HCl and CPA-HCl drugs in the presence of excipients. In the proposed method, there were no needs for pre-separation and only centrifugation was applied to make the solution clear.

TABLE 3: DETERMINATION OF PZ-HCl AND CPA-HCl ION-ASSOCIATES WITH PA, PR AND CPR IN PRESENCE OF EXCIPIENTS (EACH VALUE IS A RESULT OF FIVE SEPARATE DETERMINATIONS)

Excipients	Amount taken of PZ-HCl (µg/mL)			Amount taken of CPA-HCl (µg/mL)		
	9	20	9	9	16	8
	% Recovery ±SD PA	% Recovery ±SD PR	% Recovery ±SD CPR	% Recovery ±SD PA	% Recovery ±SD PR	% Recovery ±SD CPR
Microcrystalline cellulose	99.55±1.42	99.33±0.93	100.18±0.95	99.70±0.36	99.62±0.39	99.80±1.42
Lactose	99.33±0.62	100.03±1.57	99.70±1.079	99.45±0.69	100.17±1.24	100.59±1.44
Povidone	100.04±0.84	99.95±1.05	100.15±0.84	100.44±1.02	100.42±0.97	99.96±1.37
Starch	99.44±0.62	99.97±1.42	100.15±0.74	99.45±0.80	100.23±1.09	100.17±1.21
Magnesium stearate	100.41±1.63	99.32±0.67	99.33±0.55	100.15±1.62	99.96±1.10	100.71±0.44
Sucrose	100.78±1.06	100.13±0.60	100.18±1.11	100.07±1.079	100.13±1.66	100.13±1.88
Glucose	99.00±0.73	99.72±0.86	99.70±0.74	100.30±1.45	99.37±1.38	99.75±1.39
Hydroxypropyl methylcellulose	100.52±1.07	99.55±0.70	99.93±1.11	99.89±1.35	99.58±0.51	100.09±1.58

Analysis of tablets: Recovery studies were performed to judge the accuracy of the proposed method. Five replicate determinations, using selected reagents, three different concentrations of pure PZ-HCl and selgon 20 mg /tablet as well as for pure CPA-HCl and allergex 20 mg/tablet were investigated. [From the amount of drug found, percentage recovery was calculated and accuracy was assessed as the

percentage relative error (Bias %) between the measured mean concentrations and added concentrations at the same concentration of PZ-HCl and CPA-HCl]. And then, the results of the accuracy and recovery studies of PZ-HCl and CPA-HCl in their pure forms and their tablet dosage forms are summarized in **Table 4**.

To give additional support to accuracy of the developed assay method, standard addition method was done. In this study, three different concentrations of pure PZ-HCl and CPA-HCl drugs with PA, PR and CPR reagents, respectively were added to a known pre-analyzed formulation samples (PZ-HCl and CPA-HCl tablets) and the total concentrations were determined using the proposed methods (n=5).

The percent recovery of the added pure drug was calculated as, % recovery =$[(C_v–C_u)/C_a]\times100$, where C_v is the total drug concentration measured after standard addition, C_u is drug concentration in the formulation and C_a is drug concentration added to formulation. Therefore, the results of the analyses and recovery studies of PZ-HCl and CPA-HCl with PA, PR and CPR reagents are given in **Table 5**.

The applicability of the proposed methods for the determination of PZ-HCl and CPA-HCl drugs in their pure forms and their commercial dosage forms (tablets) was examined by analyzing marketed product. The results of the proposed methods were statistically compared with reference method [21] and summarized in **Table 6**. It is evidence from tables that the calculated t-test value and F-test values [22] are less than the theoretical ones at 95% confidence level, indicating no significant difference between the methods compared. Based on the foregoing, the proposed methods are highly sensitive, precise, simple and rapid and are successfully applied for the quality control of pure PZ-HCl and CPA-HCl drugs and their pharmaceutical dosage forms (tablets).

TABLE 4: ACCURACY AND RECOVERY DATA FOR THE DEVELOPED METHODS (EACH VALUE IS A RESULT OF FIVE SEPARATE DETERMINATIONS)

Drug	Sample	Method	Predicted con. (µg/mL)[a]			Mean % recovery (±S.D)	Accuracy (%)[b]
			Range	Mean (±S.D)	% R.S.D		
PZ-HCl	Pure solution	PA	08.85-09.21	09.05±0.14	1.60	100.51±1.61	0.51
			17.73-18.30	18.06±0.25	1.36	100.36±1.37	0.36
			31.54-32.21	31.88±0.27	0.84	99.62±0.84	-0.38
		PR	19.73-20.20	19.95±0.19	0.94	99.73±0.93	-0.27
			39.49-40.31	39.99±0.31	0.79	99.98±0.79	-0.02
			79.66-80.22	79.96±0.25	0.31	99.95±0.31	-0.05
		CPR	08.85-09.25	9.02±0.16	1.75	100.20±1.76	0.20
			17.77-18.22	17.97±0.19	1.04	99.86±1.03	-0.14
			35.55-36.16	35.92±0.27	0.75	99.78±0.75	-0.22
	Selgon Tablet	PA	08.74-09.15	8.99±0.16	1.79	99.93±1.79	-0.07
			17.65-18.15	17.93±0.19	1.06	99.63±1.06	-0.37
			31.52-32.08	31.79±0.21	0.66	99.36±0.65	-0.64
		PR	19.65-20.20	19.91±0.24	1.20	99.55±1.19	-0.45
			39.29-40.21	39.77±0.38	0.97	99.44±0.96	-0.57
			79.31-80.05	79.69±0.29	0.37	99.62±0.36	-0.38
		CPR	08.85-09.16	08.99±0.13	1.38	99.84±1.38	-0.16
			17.59-18.1	17.90±0.19	1.08	99.45±1.07	-0.56
			35.48-36.14	35.74±0.26	0.73	99.29±0.72	-0.71
CPA-HCl	Pure solution	PA	08.84-09.14	09.01±0.13	1.41	100.09±1.41	0.09
			17.69-18.22	17.92±0.19	1.08	99.54±1.073	-0.46
			35.69-36.21	36.01±0.22	0.61	100.03±0.61	0.03
		PR	15.69-16.17	15.92±0.19	1.17	99.49±1.17	-0.51
			31.46-32.20	31.89±0.31	0.97	99.67±0.97	-0.33
			63.52-64.31	63.95±0.38	0.60	99.93±0.60	-0.08

		CPR	07.91-08.21	08.05±0.12	1.53	100.58±1.54	0.58
			15.74-16.10	15.92±0.15	0.93	99.5±0.93	-0.5
			31.69-32.25	31.97±0.22	0.69	99.89±0.69	-0.11
	Allergex Tablets	PA	08.79-09.08	08.95±0.11	1.24	99.4±1.23	-0.60
			17.59-18.21	17.89±0.25	1.37	99.38±1.36	-0.62
			35.56-36.25	35.88±0.29	0.81	99.67±0.80	-0.33
		PR	15.52-16.10	15.89±0.25	1.55	99.3±1.54	-0.70
			31.51-32.19	31.87±0.29	0.90	99.58±0.90	-0.42
			63.31-64.08	63.64±0.37	0.58	99.44±0.58	-0.56
		CPR	07.81-08.12	09.95±0.13	1.61	99.3±1.60	-0.70
			15.72-16.22	15.96±0.20	1.25	99.76±1.25	-0.24
			31.59-32.26	31.87±0.29	0.92	99.61±0.92	-0.34

[a] Predicted concentration of PZ-HCl and CPA-HCl with PA, PR and CPR were calculated by linear regression equation.

[b] Accuracy is given in % relative error (=100×[(predicted concentration–nominal concentration) / nominal concentration)].

TABLE 5: RESULTS OF STANDARD ADDITION METHOD FOR PZ-HCl AND CPA-HCl WITH PA, PR AND CPR (EACH VALUE IS A RESULT OF FIVE SEPARATE DETERMINATIONS)

Method	PZ-HCl				CPA-HCl			
	Conc. of drug In tablets (µg /mL)	Conc. of pure drug added (µg /mL)	Total conc. of drug found (µg /mL)	% Analytical Recovery (±S.D)	Conc. of drug in tablets (µg /mL)	Conc. of pure drug added (µg /mL)	Total conc. of drug found (µg /mL)	% Analytical recovery (±S.D)
PA	08.95	09.00	17.96	100.11±1.97	08.94	09.00	17.95	100.03±1.17
	08.95	18.00	26.90	99.72±1.33	08.94	18.00	26.85	99.49±1.20
	08.95	32.00	40.78	99.46±0.55	08.94	36.00	44.80	99.62±0.83
PR	19.86	20.00	39.77	99.54±1.28	15.90	16.00	31.79	99.30±1.68
	19.86	40.00	59.75	99.74±0.86	15.90	32.00	47.89	99.76±1.19
	19.86	80.00	99.51	99.68±0.81	15.90	64.00	79.61	99.54±0.79
CPR	08.94	09.00	17.90	99.58±1.51	07.94	08.00	15.95	100.18±1.48
	08.94	18.00	26.82	99.22±1.61	07.94	16.00	23.89	99.67±1.51
	08.94	36.00	44.72	99.38±0.86	07.94	32.00	39.84	99.68±0.87

The percent recovery of the added pure drug was calculated as, % recovery $=[(C_v–C_u)/C_a]\times100$, where C_v is the total drug concentration measured after standard addition; C_u , drug concentration in the formulation; C_a , drug concentration added to formulation

TABLE 6: STATISTICAL EVALUATIONS OF OBTAINED DATA FROM PZ-HCl AND CPA-HCl (PURE DRUGS) AND PHARMACEUTICAL FORMULATION (TABLETS) CONTAINING PZ-HCl AND CPA-HCl BY THE PROPOSED AND REFERENCE METHODS

Statistical values	PZ-HCl Pure solution			[21] Ref. method	PZ-HCl Selgon Tablets			[21] Ref. method	CPA-HCl Pure solution			[21] Ref. method	CPA-HCl Allergex Tablets			[21] Ref. method
	PA	PR	CPR		PA	PR	CPR		PA	PR	CPR		PA	PR	CPR	
N	5	5	5	3	5	5	5	3	5	5	5	3	5	5	5	3
X^-, Recovery (%)	100.16	99.89	99.95	100.22	99.64	99.54	99.53	99.95	99.89	99.69	99.99	100.31	99.48	99.44	99.56	99.39
S.D.	1.28	0.68	1.177	0.66	1.19	0.85	1.04	0.58	1.03	0.89	1.13	0.54	1.08	1.01	1.21	0.69
R.S.D. (%)	1.28	0.68	1.177	0.66	1.20	0.85	1.04	0.58	1.03	0.90	1.13	0.54	1.09	1.02	1.21	0.70
F- value:																
F^c	3.73	1.07	3.16		4.25	2.14	3.22		3.63	2.71	4.35		2.44	2.12	3.04	
F^t	6.94	6.94	6.94		6.94	6.94	6.94		6.94	6.94	6.94		6.94	6.94	6.94	
t-value:																
t^c	0.10	1.08	0.51		0.58	1.08	0.90		0.91	1.56	0.63		0.19	0.11	0.31	
t^t	2.77	2.77	2.77		2.77	2.77	2.77		2.77	2.77	2.77		2.77	2.77	2.77	

N: number of determination, X^-: mean recovery, S.D: standard deviation, F-value and t-value are theoretical values at 95% confidence level, F^c: calculated F-value, F^t: tabulated F-value, t^c: calculated t-value, t^t: tabulated t-value.

CONCLUSION: The proposed methods A, B and C are simple, rapid, precise, accurate and sensitive compared to the reported methods. The utility of the proposed methods for the determination of PZ-HCl and CPA-HCl compounds in their pure forms and their tablet dosage forms have been well demonstrated. The assay methods did not involve any stringent experimental conditions, and were also free from interference by common excipients. The mean values obtained and the calculated standard deviations are compared with those obtained by the reference methods, by applying the t- and F-tests. The results presented herein for PZ-HCl and CPA-HCl express excellent agreement and considered significant with those obtained using reference methods.

Hence, the proposed methods could be used for routine quality control. Thus, it clear that the present methods are of high accuracy, precision, speed and selectivity, beside being of low cost and easily applied for the determination of the drugs under investigation in pure form and tablet dosage form depend on simpler spectrophotometric measurements in visible region using chemical reagents which are available.

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