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Novel methods for the visible spectrophotometric determination of diltiazem HCl and levamisole HCl in pure, tablet dosage forms and biological fluids

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ABSTRACT

Two rapid, simple, precise and sensitive extractive spectrophotometric methods (A and B) have been developed for the determination of diltiazem hydrochloride (DT-HCl) and levamisole hydrochloride (LM-HCl) in pure, tablet dosage forms and biological fluids. Both methods (A and B) involves the formation of intense yellow ion-association complex between drug(s) and either of bromocresol purple (BCP) or chlorophenol red (CPR) reagents followed by extraction with methylene chloride. The ion-associates exhibit absorption maxima at 399 and 402 nm for DT-HCl and at 405 and 406 nm for LM-HCl with BCP 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 2.26—27.06 and 2.26—48.48 µg/mL for DT-HCl and 1.20—16.86 and 2.41—32.51 µg/mL for LM-HCl with BCP and CPR, respectively. The molar absorptivities and Sandell's sensitivities of the reaction products were calculated. In methods A and B the slope, intercept, correlation coefficient, relative standard deviation (RSD), detection and quantitation limits were also calculated (n=5) for DT-HCl and LM-HCl. No interference was observed from common excipients present in pharmaceutical formulations. The results are well compared to those obtained by the reference methods using t- and F-tests. Therefore, the present methods are suitable for the drugs determination, as they are sensitive and precise to a high extent.

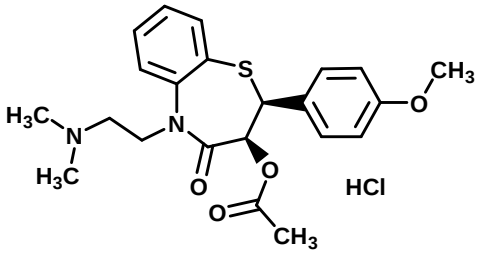
KEYWORDS

Visible spectrophotometry;
Diltiazem HCl;
Levamisole HCl;
Bromocresol purple;
Chlorophenol red;
Tablets;
Biological samples.

INTRODUCTION

Diltiazem hydrochloride (DT-HCl) (Figure 1),[1] chemically, it is (2S,3S)-5-(2-Dimethylaminoethyl)-2,3,4,5-tetrahydro-2-(4-methoxyphenyl)-4-oxo-1,5-benzothiazepin-3-yl acetate hydrochloride[2]. Medically, it is a benzothiazepine calcium-channel blocker. It is a peripheral and coronary vasodilator property. DT-HCl inhibits cardiac conduction, particularly at the sino-atrial and atrioventricular nodes[2, 3]. Pharmacokinetics, It is

rapidly absorbed (approx. 90%) from the gastrointestinal tract after oral administration, but undergoes extensive first-pass hepatic metabolism via deacetylation, N-demethylation, O-demethylation and oxidative deamination. Peak plasma concentrations occur about 3 to 4 hours after a dose by mouth. About 2 to 4% of a dose is excreted in urine as unchanged diltiazem with the remainder excreted as metabolites in urine, bile and faeces.

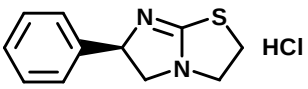


$C_{22}H_{26}N_2O_4S$, HCl = 451.0

Figure 1 : Chemical structure of DT-HCl

A number of methods are available for DT-HCl determination in various types of samples. These including high-performance liquid chromatography (HPLC)[4-6] and microbiological assay methods were reported for the determination of diltiazem in biological fluids such as plasma, serum and urine[7-10], gas chromatograph (GC)[11-13], high-performance thin layer chromatography (HPTLC)[14], capillary electrophoresis (CE)[15-17], electrochemical methods[18-21]. A few methods have been reported on the determination of DT-HCl as visible spectrophotometry[22-24]. Several of these above mentioned methods require the use of hazardous and expensive chemicals which make the process not only a challenge for the environment but too much complicated, time consuming and expensive costly.

Levamisole hydrochloride (LM-HCl) (Figure 2),[1] chemically, it is (S)-2,3,5,6-tetrahydro-6-phenylimidazo[2,1-b]thiazole hydrochloride[2]. Medically, it is used as an anthelmintic and as an adjuvant in malignant disease. LM-HCl is active against intestinal nematode worms and appears to act by paralysing susceptible worms which are subsequently eliminated from the intestines. In particular, LM-HCl is effective in the treatment of ascariasis. It is also used in hookworm infections[2, 3]. Pharmacokinetics, It is rapidly absorbed from the gastro-intestinal tract. Maximum plasma concentrations are attained within 1.5 to 4 hours. It is extensively metabolized in the liver. The plasma half-life for levamisole is 3 to 4 hours and for the metabolites is 16 hours. It is excreted mainly in the urine as metabolites and a small proportion in the faeces. A bout 70% of a dose is excreted in the urine over 3 days, with about 5% as unchanged levamisole.



$C_{11}H_{12}N_2S$, HCl = 240.8

Figure 2 : Chemical structure of LM-HCl

A survey of literature has revealed several analytical methods for the determination of LM-HCl in various types of samples, including high-performance liquid chromatography (HPLC)[25] and microbiological assay methods were reported for the determination of livamisole in biological fluids such as plasma, serum and urine[26-30], gas chromatography (GC)[31], Thin layer chromatography (TLC)[32], A fluorescence[33], Atomic absorption spectrometric[34-37], electrochemical methods[38-41], oscillopolarographic titration[42] and turbidimetric method and flow-injection[43]. A few methods have been reported on the determination of LM-HCl as visible spectrophotometry[44-46]. Several of these above mentioned methods require the use of hazardous and expensive chemicals which make the process not only a challenge for the environment but too much complicated, time consuming and expensive costly.

Thus, the aim of the present work was to investigate economical, simple, precise, sensitive and environmental friendly two analytical methods A and B for the determination of DT-HCl and LM-HCl using visible spectrophotometry. Both methods (A and B) involves the formation of intense yellow ion-association complex between drug(s) and either of bromocresol purple (BCP) or chlorophenol red (CPR) reagents followed by extraction with proper water-immiscible organic solvent. It was also aimed to apply the developed methods for accurate analysis of DT-HCl and LM-HCl not only in their pure forms and their tablet dosage forms but in biological fluids as well (serum and urine samples). The results obtained from the proposed methods also have been statistically compared using t- and F-tests to the reference methods. They also have the advantage of being cheaper than the reported methods.

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EXPERIMENTAL

Materials and Reagents

DT-HCl and LM-HCl standards were kindly supplied as a gift samples by Egyptian International Pharmaceutical Industries Co. Cairo, Egypt (E.I.P.I.CO.) and Kahira Pharmaceuticals and Chem. Industries CO. Cairo, Egypt, respectively and used without further purification and purity was confirmed by thin layer chromatography and by melting point measurements. Commercial tablets of DT-HCl such as Altiazem tablets containing 60 mg DT-HCl and commercial tablets of LM-HCl such as Ketrex tablets containing 40 mg LM-HCl were purchased from local drug market. Bromocresol purple (BCP) (Figure 3) and chlorophenol red (CPR) (Figure 4) 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.

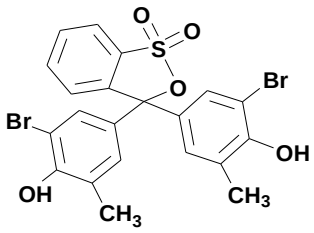

Figure 3 : Chemical Structure of BCP

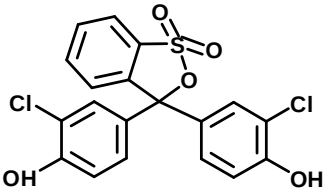

Figure 4 : Chemical Structure of CPR

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. Centrifugation of body fluids samples was carried out with Centrifuge (5702R) Model, Eppendorf AG 22331 Hamburg, Germany. 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 and B, standard stock solutions 0.01 M of DT-HCl and LM-HCl were freshly prepared by dissolving the appropriate weights of 1.1275 g (DT-HCl) and 0.602 g (LM-HCl) 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. Standard stock solutions 0.01 M of CPR and 0.001 M of BCP were freshly prepared by dissolving the appropriate weights of (0.1351 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 DT-HCl and LM-HCl (Calibration standards)

For methods A and B, 2.5 mL of 0.001 M BCP or 0.005 M CPR were added in acid medium to a solution of DT-HCl using the concentration range of 2.26-27.06 µg/mL (BCP) and 2.26—48.48 µg/mL (CPR) of DT-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 399 and 402 nm for BCP, CPR, respectively, against corresponding reagent blank. This blank was prepared in the same manner without the addition of DT-HCl. A calibration curves were plotted (Figure 19). In a similar way, 2.5 mL of 0.001 M BCP or 0.005 M CPR were added in acid medium to a solution of LM-HCl within concentration range of 1.20—16.86 µg/mL (BCP) and 2.41—32.51 µg/mL (CPR) of LM-HCl (n=5). Using the same procedures described for DT-HCl. The absorbance of the extract was measured at λ_{max} 405 and 406 nm for BCP and CPR, respectively, against corresponding reagent blank. A calibration curves were plotted (Figure 20). The results of DT-HCl and LM-HCl ion-associates with BCP or CPR correlated to Beer's

law are presented in TABLE 2.

Sample analysis

Determination of DT-HCl and LM-HCl in tablets

For methods A and B, ten tablets of altiazem and katrex 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 whatman 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 DT-HCl or LM-HCl per tablet was calculated using the calibration curve method. A standard addition method was also used to confirm the accuracy and recoveries.

Determination of DT-HCl and LM-HCl in serum samples

In methods A and B, drug-free serum samples were obtained from Bahteem Hospital from throw four volunteers and stored in 4°C. Each individual was instructed not to use any medication before one day of serum withdrawing. Because untreated serum samples can lead to turbid solutions when these are added an amount of DT-HCl or LM-HCl stock solution, a deproteinization process with appropriate amount of trichloroacetic acid was employed. The precipitated proteins were separated by centrifugation for 10 min at 4000 rpm. The clear supernatant layer was transferred into a 50 mL volumetric flask, and the protein-free serum was directly analyzed without any pretreatment. According to the experimental procedures described earlier (section 2.4), the samples were processed for visible spectrophotometric determination of DT-HCl or LM-HCl by standard addition method. The results were summarized in TABLE 7.

Determination of DT-HCl and LM-HCl in urine samples

For methods A and B, collection of urine samples from four volunteers was conducted in the same way as described elsewhere[47]. Each individual was instructed not to use any medication before two weeks of urine collection. The urine samples were then collected in thoroughly washed and clean plastic bottles in the morning time from full bladder without any dose of DT-HCl or LM-HCl. It may be necessary to adjust the pH or centrifuge the urine for 10 min to remove the suspended matter before determination. According to the experimental procedures described earlier (section 2.4), the samples were processed for visible spectrophotometric determination of DT-HCl or LM-HCl by standard addition method. The results were presented in TABLE 7.

RESULTS AND DISCUSSION

Both methods (A and B) involves the formation of intense yellow ion-association complex between drug(s) and either of BCP or CPR reagents followed by extraction with methylene chloride. Many drugs are easy to be determined by spectrophotometry based on colour. Optimum reaction conditions for quantitative determination ion-association complexes of DT-HCl and LM-HCl with BCP and CPR reagents 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 DT-HCl ion-associates with BCP and CPR reagents, λ_{max} of 399 and 402 nm have been obtained, respectively as shown in Figure 5. For LM-HCl ion-associates, λ_{max} of 405 and 406 nm with BCP and CPR reagents have been obtained, respectively as shown in Figure 6.

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.

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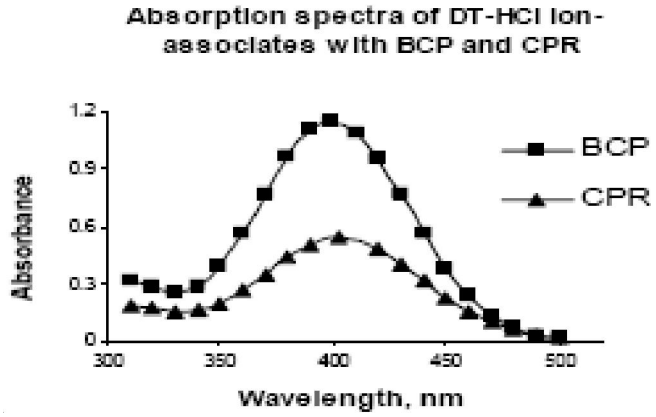

Figure 5 : Absorption spectra of DT-HCl ion-associates with BCP and CPR

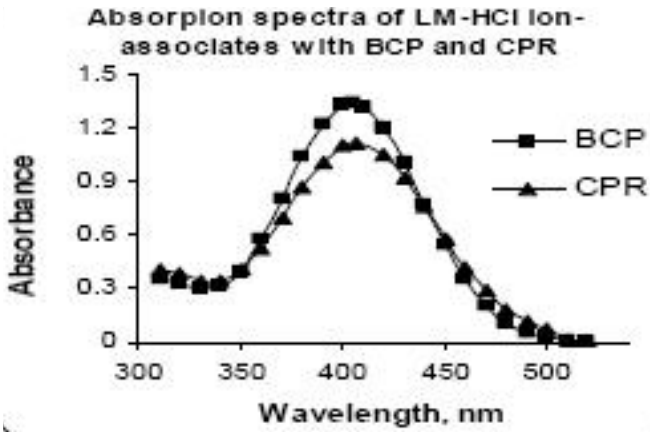

Figure 6 : Absorption spectra of LM-HCl ion-associates with BCP and CPR

The most convenient solvent for DT-HCl and LM-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. Figures. 7 and 8 summarize the effect of extracting solvents on the formed ion-associates.

Effect of pH

To investigate the optimum medium conditions to determine DT-HCl and LM-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 colour of drug–reagent ion-associates against the chosen reagents. In DT-HCl and LM-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 2-5 for BCP and CPR, as shown in Figures. 9 and 10.

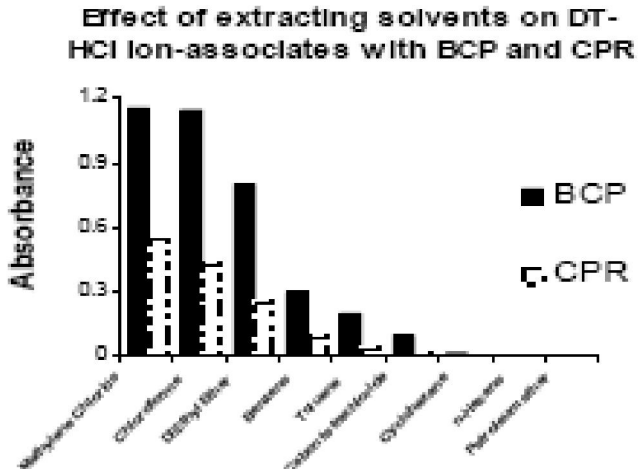

Figure 7 : Effect of extracting solvents on DT-HCl ion-associates with BCP and CPR

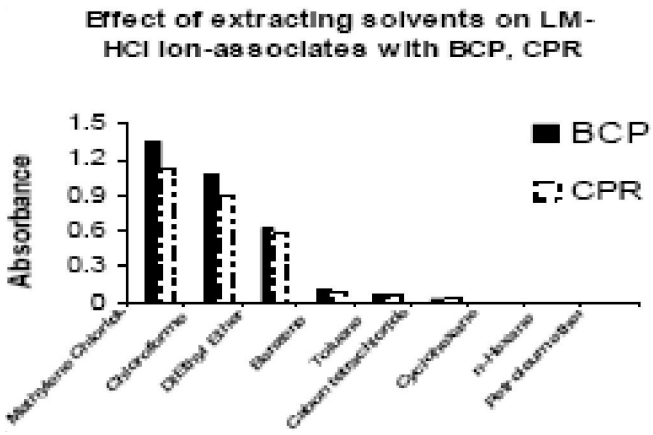

Figure 8 : Effect of extracting solvents on LM-HCl ion-associates with BCP and CPR

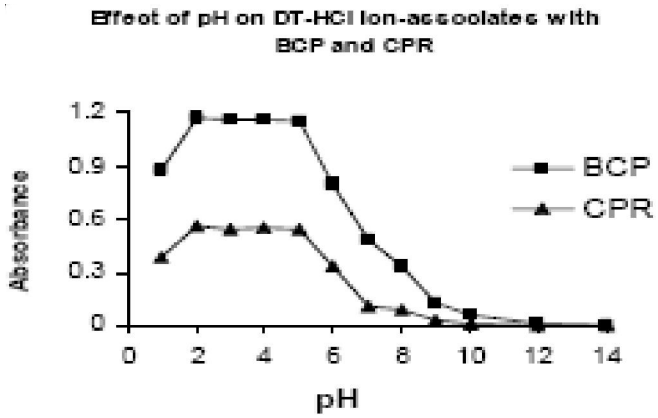

Figure 9 : Effect of pH on DT-HCl ion-associates with BCP and CPR

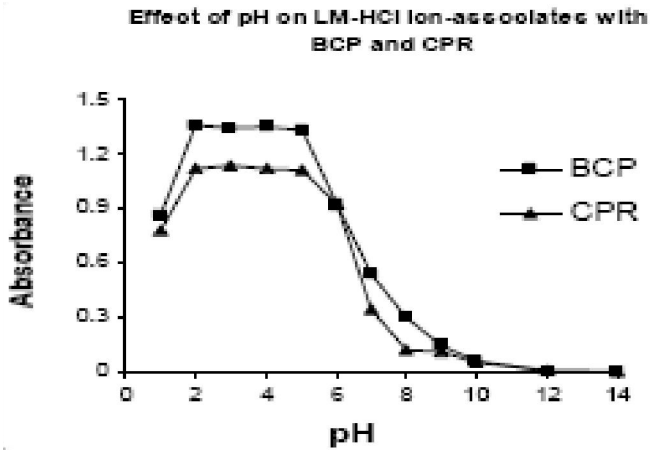

Figure 10 : Effect of pH on LM-HCl ion-associates with BCP and CPR

At pHs less than 2, the absorbance decrease may be attributed to the formation of diprotonated species of the drug. In case of pH < 5 the absorbance decrease due to the formation of free base of the drug which are insoluble in water and precipitates during the mixing.

Effect of reagent concentration

The effect of reagent concentration was tested by using varying amounts (1-6) mL of 0.001 M BCP with 1 mL of 0.0002 M (DT-HCl or LM-HCl) and 0.005 M CPR with 1 mL of 0.001 M (DT-HCl or LM-HCl).

After implementing the optimum pH condition for DT-HCl and LM-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 0.001 M BCP and 5 mL 0.005 M CPR were sufficient for good colour 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 DT-HCl and LM-HCl are represented graphically in Figures. 11 and 12, respectively. The developed colour remained of DT-HCl ion associates was stable for 24 and 21 hours for BCP and CPR at λ_{max} of 399 and 402 nm, respectively. Similarly, the ion-associates of LM-HCl are formed almost instantaneously. Moreover, the developed colour for LM-HCl remained stable for 24 and 24 hours for BCP and CPR, at λ_{max} of 405 and 406 nm, respectively. After these intervals, a decrease in colour intensity occurred in ion associated of DT-HCl and LM-HCl.

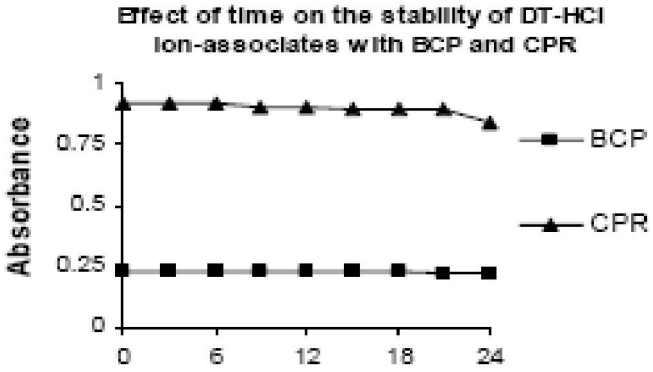

Figure 11 : Effect of time on the stability of DT-HCl ion-associates with BCP and CPR

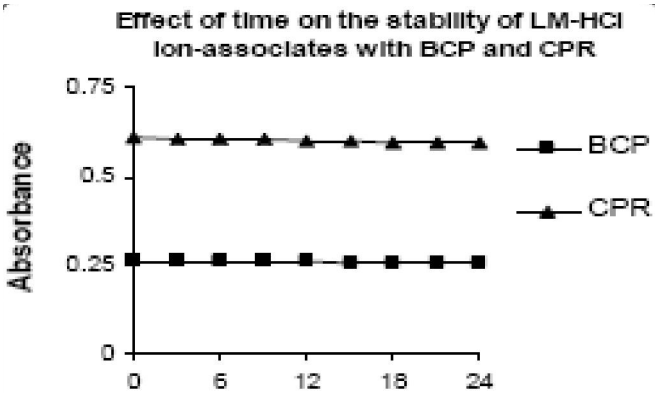

Figure 12 : Effect of time on the stability of LM-HCl ion-associates with BCP and CPR

Effect of temperature

Under the 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 DT-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 and 45°C for BCP and CPR, respectively as represented by its absorptivity at the recommended (λ_{max}). Similarly, the ion-associates of LM-HCl are formed instantaneously with all reagents at room temperature 25± 5 °C and remain constant up to 45°C and 45°C for BCP and CPR, respectively. The effect of temperature

on the stability of ion–associates of DT-HCl and LM-HCl are shown in Figures. 13 and 14.

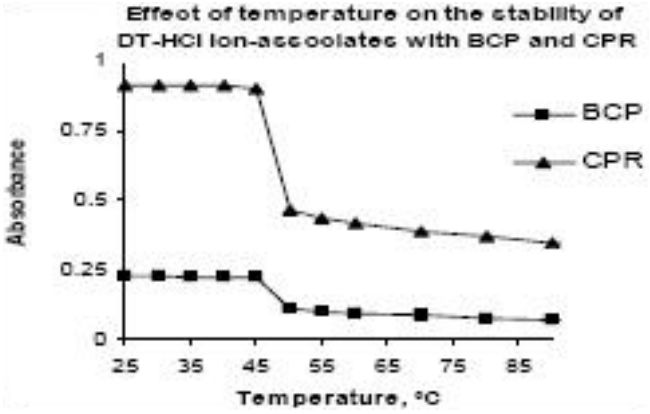

Figure 13 : Effect of temperature on the stability of DT-HCl ion-associates with BCP and CPR

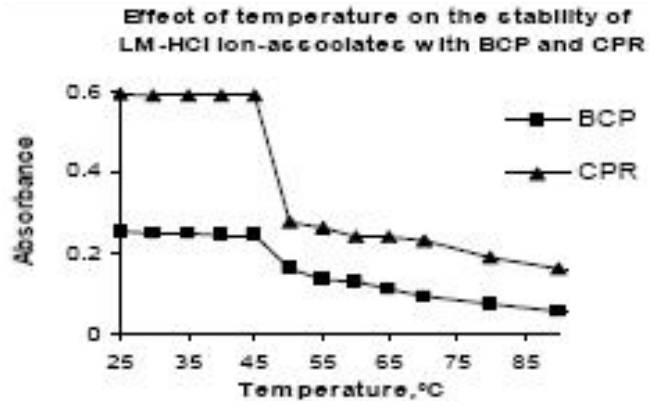

Figure 14 : Effect of temperature on the stability of LM-HCl ion-associates with BCP and CPR

The stoichiometry of the ion-associates

Aided by spectrophotometeric measurements, the stoichiometries of the ion-associates of DT-HCl and LM-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[48]. At the optimum conditions described earlier for DT-HCl and LM-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 Figures. 15 and 16.

Two straight lines were intersecting at the molar ratio of 1 in case of BCP and CPR which reflects the formation of 1:1 ratio of (drug: reagent) for all ion-associates.

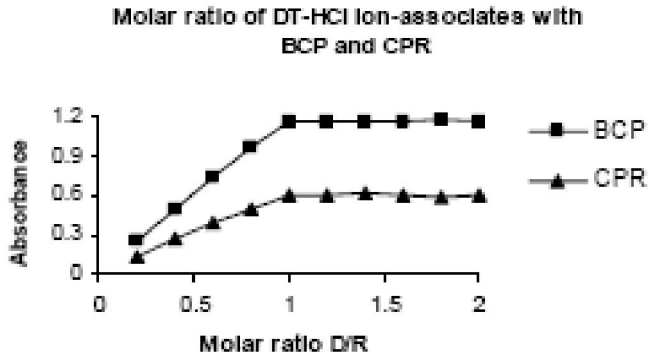

Figure 15 : Molar ratio of DT-HCl ion-associates with BCP and CPR [D=drugs and R=reagents]

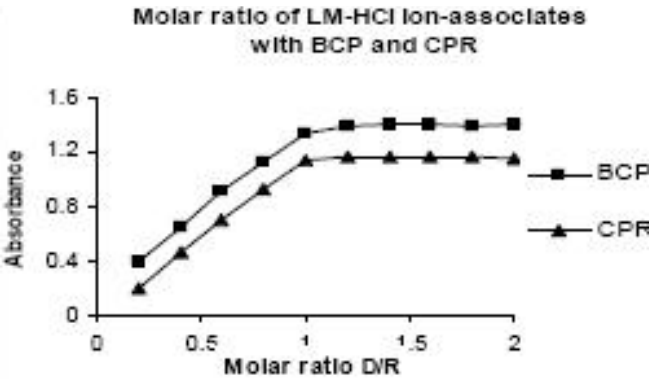

Figure 16 : Molar ratio of LM-HCl ion-associates with BCP and CPR [D=drugs and R=reagents]

The continuous variation method

The modification of Job's[49] continuous variation method performed by Vosburgh and Cooper[50] 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 Figures. 17 and 18, respectively.

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

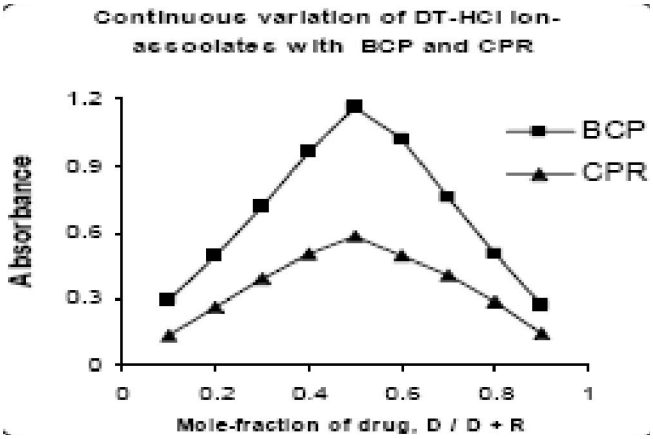

Figure 17 : Continuous variation of DT-HCl ion-associates with BCP and CPR

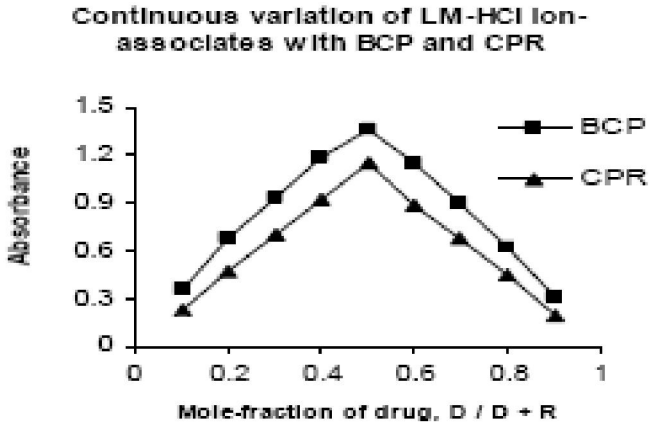

Figure 18 : Continuous variation of LM-HCl ion-associates with BCP and CPR

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[51-53] the structures of positive protonated nitrogen atom of DT-HCl and LM-HCl compounds were proposed.

In the second, the nature of the binding of reagents to each drug in the presence of equal amount of BCP or CPR was determined by the molar ratio[48] and the continuous variation methods[49, 50]. The results indicated that a 1:1 ratio of (drug: reagent) for all ion-associates are formed as shown in Figures. 15-18.

Found that DT-HCl and LM-HCl reacts with BCP or CPR forming ion-associated compounds through the electrostatic attraction between positive protonated nitrogen atom of DTH^+ and LMH^+ and BCP^- or CPR^- anions. Charts 1 and 2 summarize probable reaction mechanism for the formation of ion-association complexes of DT-HCl and LM-HCl with BCP or CPR.

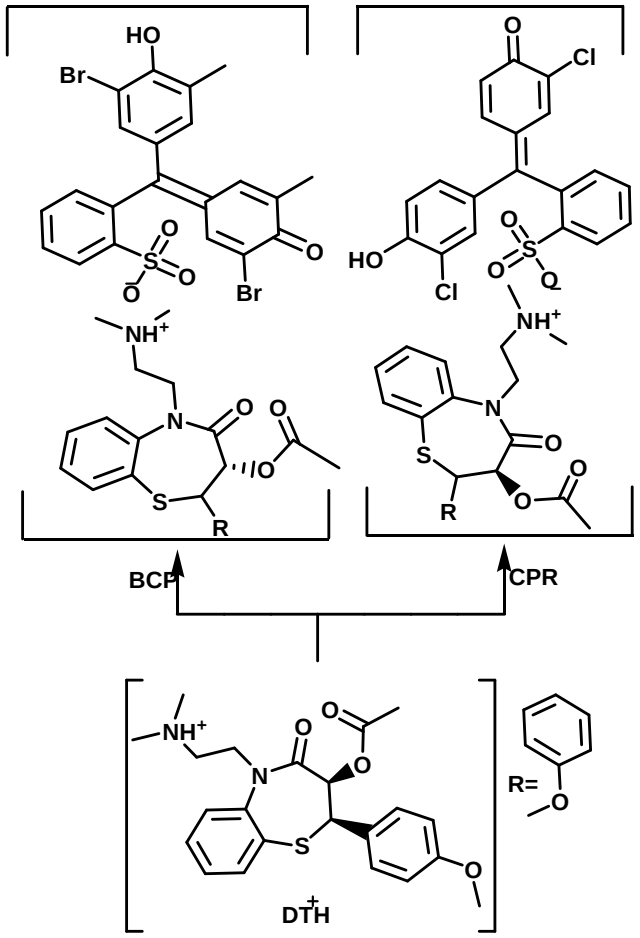

Chart 1 : Probable reaction mechanism for the formation of ion-association complexes of DTH^+ with BCP^- and CPR^-

TABLE 1 : Optimal condition for the extraction of DT-HCl and LM-HCl ion-associates with BCP and CPR

Parameters	*DT-HCl*		*LM-HCl*	
	DT-BCP	DT-CPR	LM-BCP	LM-CPR
λ max, nm	399	402	405	406
Extracting solvents	Cl_2CH_2	Cl_2CH_2	Cl_2CH_2	Cl_2CH_2
Colour of extract	Yellow	Yellow	Yellow	Yellow
pH range	2–5	2–5	2–5	2–5
Stability of extracts,h.	24	21	24	24
Temperature on the stability, °C	45	45	45	45
The stoichiometry of the ion-associates	1:1	1:1	1:1	1:1

Validity of the Beer's Lambert law

The spectrophotometric determination of compounds of DT-HCl and LM-HCl with BCP and CPR were carried out using appropriate concentration range to ensure the obedience to Beer's law (Figures 19 and 20).

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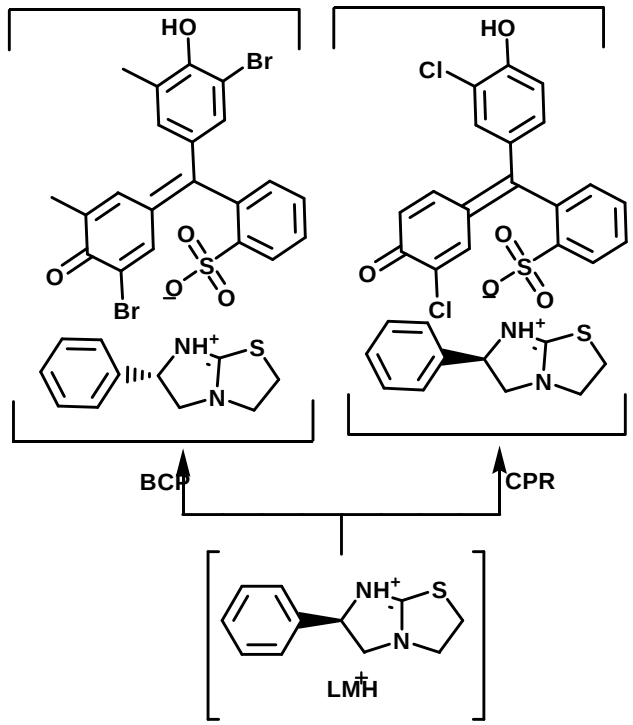

Chart 2 : Probable reaction mechanism for the formation of ion-association complexes of LMH^+ with BCP^- and CPR^-

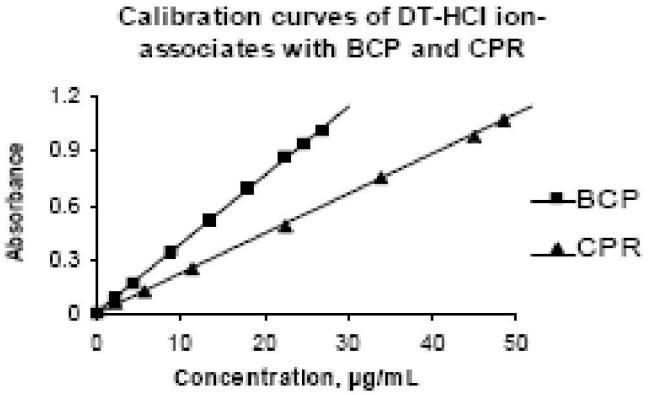

Figure 19 : Calibration curves of DT-HCl ion-associates with BCP and CPR

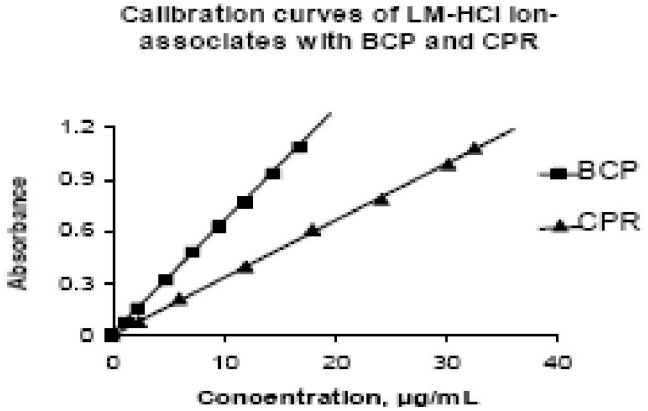

Figure 20 : Calibration curves of LM-HCl ion-associates with BCP and CPR

After optimization, the systems obeyed Beer's law in the concentration range of 2.26-27.06 and 2.26—48.48 µg/mL for DT-HCl and 1.20-16.86 and 2.41-32.51 µg/mL for LM-HCl with BCP and CPR, respectively. The apparent molar absorptivities (ε) were found to be 1.703×10^4 and 9.919×10^3 L mol^{-1} cm^{-1} for DT-HCl and 1.551×10^4 and 8.002×10^3 L mol^{-1} cm^{-1} for LM-HCl with BCP and CPR, respectively and also Sandell's sensitivities were calculated to be 2.6×10^{-2} and 4.4×10^{-2} µg cm^{-2} for DT-HCl and 1.6×10^{-2} and 3×10^{-2} µg cm^{-2} for LM-HCl ion-associates with BCP and CPR reagents, respectively.

The linear regression equations (with intercepts and slopes) and correlation coefficients of the mean of five consecutives calibration curves of DT-HCl and LM-HCl with BCP and CPR reagents, respectively are given in TABLE 2.

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 DT-HCl and LM-HCl, respectively. 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 DT-HCl and LM-HCl were evaluated and recorded in TABLE 2. Theses data indicated that the developed methods have a good repeatability (were lower than 10%).

The Limit of Detection (LOD) and Limit of Quantification (LOQ) of DT-HCl and LM-HCl by the proposed methods were determined using standard 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[54].

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 hydroxypropylmethyl cellulose, recovery studies were out. Under the experi-

TABLE 2 : Features of the calibration curves of DT-HCl and LM-HCl ion-associates with BCP and CPR (N=5)

Features	DT-HCl		LM-HCl	
	Values for BCB	Values for CPR	Values for BCB	Values for CPR
Number of data points	8	7	8	7
Beer's law verification range, µg /mL	2.26–27.06	2.26–48.48	1.20–16.86	2.41–32.51
Molar absorpitivity (ε) [L mol^{-1} cm^{-1}]	1.703 x 10^4	9.919 x 10^3	1.551 x 10^4	8.002 x 10^3
Sandell's sensitivity [µg cm^{-2}]	2.6 x 10^{-2}	4.4 x 10^{-2}	1.6 x 10^{-2}	3 x 10^{-2}
Regression equation (Y^a)	Y = a + bC	Y = a + bC	Y = a + bC	Y =a + bC
Slope (b)	0.0374	0.0217	0.0640	0.0329
Intercept (a)	0.004	0.006	0.005	0.006
Correlation coefficient (r^2)	0.9999	0.9997	0.9998	0.9997
RSD[b] (%)	0.24–2.15	0.36–3.45	0.88–3.52	0.53–3.46
Limit of Detection, LOD, µg /mL	0.06	0.32	0.12	0.22
Limit of Quantification, LOQ, µg /mL	0.18	0.96	0.37	0.68

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

mental conditions employed, excipients in different concentrations were added to a known amount of 5 and 10 µg/mL for DT-HCl with BCP or CPR, respectively and to a known amount of 4 and 6 µg/mL for LM-HCl with BCP or CPR, respectively and analyzed according to recommended procedures described earlier (section 2.4).

Results of the recovery studies of DT-HCl and LM-HCl drugs and the above mentioned excipients are presented in TABLE 3. It was concluded that the excipients did not interfere with quantification of DT-HCl and LM-HCl drugs in these methods and the proposed methods could be considered specific. In addition, the recoveries in most cases were around 100% and the lower values of the RSD indicate the good precision of these methods, thus the procedures was able to determination of DT-HCl and LM-HCl drugs in the presence of excipients. In the proposed methods, there were no needs for pre-separation and only centrifugation was applied to make the solution clear.

Sample analysis

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 DT-HCl and altiazem 60 mg /tablet as well as for pure LM-HCl and katrex 40 mg/tablet. [From the amount of drug found, percentage recovery was calculated and accuracy was assessed as the per-

TABLE 3 : Determination of DT-HCl and LM-HCl ion-associates with BCP and CPR in presence of excipients (each value is result of five separate determinations)

Excipients	Amount taken of DT-HCl (µg /mL)		Amount taken of LM-HCl (µg /mL)	
	5	10	4	6
	% Recovery ±SD BCP	% Recovery ±SD CPR	% Recovery ±SD BCP	% Recovery ±SD CPR
Microcrystalline cellulose	100.27±1.60	100.20±0.95	99.42±2.02	99.50±0.60
Lactose	99.53±0.61	100.13±1.25	100.75±1.64	100.83±1.17
Povidone	99.20±1.058	99.20±0.66	99.67±1.88	99.72±0.63
Starch	100.07±1.62	100.17±1.05	99.83±0.88	99.50±2.02
Magnesium stearate	100.40±1.20	100.47±1.34	100.58±1.28	100.28±1.84
Sucrose	98.80±0.92	100.30±1.31	99.25±0.75	100±1.26
Glucose	99.53±1.62	99.27±1.12	100.50±1.39	100.39±1.34
Hydroxypropyl methylcellulose	99.60±1.44	100.67±1.10	100.58±1.77	99.72±0.84

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TABLE 4 : Accuracy and recovery data for the developed methods (each value is 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		
DT-HCl	Pure solution	BCP	04.88-5.14	5.02±0.10	2.00	100.44±2.01	0.44
			09.79-0.22	10.03±0.18	1.79	100.32±1.80	0.32
			19.74-20.11	19.95±0.16	0.79	99.73±0.79	-0.27
		CPR	09.86-10.25	10.07±0.14	1.40	100.72±1.41	0.72
			19.62-20.30	19.95±0.28	1.40	99.76±1.40	-0.24
			34.71-35.30	35.01±0.24	0.69	100.02±0.69	0.02
	Altiazem Tablets	BCP	04.90-5.10	4.98±0.08	1.62	99.68±1.62	-0.32
			09.91-10.25	10.06±0.14	1.42	100.62±1.42	0.62
			19.55-20.11	19.92±0.22	1.11	99.62±1.11	-0.38
		CPR	09.78-10.20	09.96±0.19	1.86	99.58±1.85	-0.42
			19.69-20.11	19.93±0.17	0.86	99.65±0.86	-0.35
			34.55-35.10	34.8±0.23	0.65	99.43±0.65	-0.57
LM-HCl	Pure solution	BCP	03.91-04.10	04.01±0.08	2.04	100.30±2.04	0.30
			07.88-08.16	08.04±0.11	1.39	100.45±1.40	0.45
			11.85-12.20	12.03±0.14	1.19	100.28±1.19	0.28
		CPR	05.79-06.11	05.99±0.13	2.22	99.97±2.22	-0.03
			11.77-12.25	12.01±0.19	1.60	100.07±1.60	0.07
			23.79-24.26	24.05±0.19	0.77	100.21±0.77	0.21
	Katrex Tablets	BCP	03.87-04.08	03.97±0.09	2.20	99.35±2.18	-0.65
			07.88-08.11	07.99±0.10	1.20	99.98±1.20	-0.03
			11.78-12.25	12.01±0.19	1.59	100.05±1.59	0.05
		CPR	05.86-06.08	05.95±0.08	1.40	99.20±1.39	-0.80
			11.82-12.21	11.96±0.15	1.24	99.7±1.24	-0.30
			23.59-24.15	23.91±0.22	0.93	99.62±0.93	-0.38

[a] **Predicted concentration of DT-HCl and LM-HCl with BCP and CPR were calculated by linear regression equations.**

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

TABLE 5 : Results of standard addition method for DT-HCl and LM-HCl with BCP and CPR (each value is result of five separate determinations)

Method	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)
BCP	04.92	05.00	9.92	100.08±1.85	03.98	04.00	07.95	100.35±2.10
	04.92	10.00	14.86	99.40±2.00	03.98	08.00	11.94	99.53±0.96
	04.92	20.00	24.87	99.74±1.43	03.98	12.00	15.99	100.08±0.91
CPR	09.92	10.00	19.88	99.56±1.96	05.97	60.00	11.97	100.03±2.14
	09.92	20.00	29.82	99.48±1.66	05.97	12.00	17.88	99.25±1.42
	09.92	35.00	44.84	99.76±1.09	05.97	24.00	29.99	99.49±1.64

The percent recovery of the added pure drug was calculated as, % recovery = $[(C_v–C_u)/C_a] \times 100$, 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.

centage relative error (Bias %) between the measured mean concentrations and added concentrations at the same concentration of DT-HCl and LM-HCl]. And then, the results of the accuracy and recovery studies of DT-HCl and LM-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 DT-HCl and LM-HCl drugs with BCP and CPR reagents, respectively were added to a known pre-analyzed formulation samples (DT-HCl and LM-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 DT-HCl

TABLE 6: Statistical evaluations of obtained data from DT-HCl and LM- HCl (pure drugs) and pharmaceutical formulations (tablets) containing DT-HCl and LM-HCl by the proposed and reference methods

Statistical values	DT-HCl Pure solution BCP	CPR	Reference method	DT-HCl Altiazem Tablets BCP	CPR	Reference method	LM-HCl Pure solution BCP	CPR	Reference method	LM-HCl Katrex Tablets BCP	CPR	Reference method
N	5	5	3	5	5	3	5	5	3	5	5	3
X, Recovery (%)	100.16	100.17	99.78	99.97	99.55	100.05	100.35	100.08	99.91	99.79	99.51	100.03
S.D.	1.54	1.20	0.73	1.38	1.15	0.61	1.47	1.52	0.73	1.61	1.13	0.65
R.S.D. (%)	1.53	1.20	0.73	1.38	1.16	0.61	1.47	1.52	0.73	1.62	1.14	0.65
F- value												
F^c	4.41	2.68		5.04	3.51		4.05	4.35		6.12	3.03	
F^t	6.94	6.94		6.94	6.94		6.94	6.94		6.94	6.94	
t-value												
t^c	0.55	0.73		0.13	0.97		0.67	0.25		0.33	1.03	
t^t	2.776	2.776		2.776	2.776		2.776	2.776		2.776	2.776	

N: number of determination, $\bar{X}$: 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.

and LM-HCl with BCP and CPR reagents are summarized in TABLE 5.

The applicability of the proposed methods for the determination of compounds of DT-HCl and LM-HCl in their pure forms and their commercial dosage forms (tablets) was examined by analyzing marketed products. The results of the proposed methods were statistically compared with reference methods[55] and summarized in TABLE 6. It is evidence from tables that the calculated t-test value and F-test values[56] 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 DT-HCl and LM-HCl drugs and their pharmaceutical dosage forms (tablets).

Analysis of serum and urine samples

There are many advantages in measuring quantities of drugs and their metabolites identified in the screening methods, particularly with respect to problems of interpretation. The proposed methods depend upon the reaction between the suggested reagents with unchanged drug exists in serum and urine. The present methods were safely used in serum and urine analysis for quantitative determination of compounds of diltiazem and levamisole in serum and urine samples after GC/MS identification.

TABLE 7 : The recovery of DT-HCl and LM-HCl with BCP and CPR in serum and urine samples (N=5)

Drug	Method	Sample	The added (µg /mL)	Found (µg /mL) ±S.D	Recovery (%)	R.S.D. (%)
DT-HCl	BCP	Serum	03.00	02.87±0.07	95.53	2.49
			06.00	05.69±0.11	94.80	1.85
			12.00	11.53±0.18	96.08	1.57
	BCP	Urine	04.00	03.86±0.10	96.55	2.70
			08.00	07.80±0.15	97.55	1.92
			16.00	15.65±0.30	97.83	1.94
	CPR	Serum	04.00	03.76±0.12	93.90	3.20
			08.00	07.54±0.13	94.20	1.77
			12.00	15.31±0.16	95.66	1.06
	CPR	Urine	07.00	06.73±0.14	96.20	2.07
			14.00	13.49±0.28	96.34	2.09
			28.00	27.18±0.54	97.08	1.99
LM-HCl	BCP	Serum	02.00	01.91±0.04	95.50	1.85
			05.00	04.81±0.10	96.24	1.97
			09.00	08.64±0.12	95.98	1.42
	BCP	Urine	03.00	02.90±0.05	96.80	1.55
			06.00	05.82±0.10	97.06	1.64
			10.00	09.89±0.09	98.94	0.93
	CPR	Serum	04.00	03.76±0.10	94.10	2.71
			08.00	07.68±0.10	95.98	1.24
			16.00	15.31±0.15	95.68	0.96
	CPR	Urine	05.00	04.83±0.08	96.68	1.71
			10.00	09.74±0.11	97.38	1.15
			20.00	19.45±0.17	97.26	0.88

According to the experimental procedures mentioned earlier (sections 2.5.2 and 2.5.3), the standard addition method was used for quantitative determination of compounds of diltiazem and levamisole in serum and urine samples within the concentration range of 2.26-27.06 and 2.26—48.48 µg/mL for DT-HCl and

1.20-16.86 and 2.41-32.51 µg/mL for LM-HCl with BCP and CPR, respectively.

The results of analysis and recovery studies are given in TABLE 7, in which recoveries in most cases were around 100% and the lower values of the RSD indicate the good precision of the method values.

CONCLUSION

The proposed methods A and B are simple, rapid, precise and sensitive compared to the reported methods. The utility of the proposed methods for the determination of compounds of DT-HCl and LM-HCl in their pure forms, tablet dosage forms and biological fluids (serum and urine samples) 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 DT-HCl and LM-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, tablet dosage form and biological fluids depend on simpler spectrophotometric measurements in visible region using chemical reagents which are available.

REFERENCES

[1] The Merck Index, an Encyclopedia of Chemicals, Drugs and Biologicals Merck, N.J. Rahway, USA, 13th edition **(2001)**.

[2] K.Parfitt; ed. Martindale, "The Extra Pharmacopoeia", the Pharmaceutical Press, London, 36 th edition **(2009)**.

[3] A.C.Moffat; 3th edition "Clarks Isolation and Identification of Drugs", Pharmaceutical Press, London **(2004)**.

[4] M.A.Garcia, C.Solans, J.J.Aramayona, L.J.Fraile, M.A.Bregante, J.R.Castillo, Talanta, **47**, 1245-1254 **(1998)**.

[5] Y.Shah, S.Khanna, V.S.Dighe, K.C.Jindal, Indian Drugs, **27**, 363-364 **(1990)**.

[6] P.M.Lacroix, N.Beaulieu, T.D.Cyr, E.G.Lovering, J.Pharm.Sci., **78**, 243-246 **(1989)**.

[7] A.Bakkali, A.Barranco, R.M.A.Salces, E.Corta, L.A.Berrueta, B.Gallo, F.Vicente, J.I.Marquez, Chromatographia, **49**, 202-207 **(1999)**.

[8] P.K.Kunicki, D.Sitkiewicz; J.Liq.Chromatogr.Relat. Technol., **19**, 1169-1181 **(1996)**.

[9] G.Carignan, K.Carrier, S.Laganiere, M.Lessard, J.Chromatogr; Biomed.Appl., **672**, 261-269 **(1995)**.

[10] R.S.Chaudhary, S.S.Gangwal, M.K.Avachat, Y.N.Shah, K.C.Jindal; J.Chromatogr.Biomed.Appl., 125(**2 (J. Chromatogr., 614)**), 261-266 **(1993)**.

[11] R.T.Sane, S.M.Purandare, V.G.Nayak, M.D.Joshi, V.R.Bhate, V.R.Nerurkar, Indian Drugs, **26**, 623-625 **(1989)**.

[12] T.A.Kolbah, F.Plavsic; J.Pharm.Biomed.Anal., **8**, 915-918 **(1990)**.

[13] J.P.Clozel, G.Caille, Y.Taeymans, P.Theroux, P.Biron, J.G.Besner, J.Pharm.Sci., **73**, 207-209 **(1984)**.

[14] P.V.Devarajan, V.V.Dhavse; J.Chromatogr.Biomed.Appl., **706**, 362-366 **(1998)**.

[15] Y.Kuwahara, H.Nishi; Yakugaku Zasshi, **118**, 456-463 **(1998)**.

[16] C.Coors, H.G.Schulz, F.Stache; J.Chromatogr., **717**, 235-243 **(1995)**.

[17] H.Soini, M.L.Riekkola, M.V.Novotny; J.Chromatogr., **680**, 623-634 **(1994)**.

[18] Z.Z.Leng, X.Y.Hu, X.M.Jiang, R.J.Yu; Fenxi Huaxue, **23**, 332-335 **(1995)**.

[19] Z.Leng, X.Hu, P.Feng, W.Bai; Fenxi Yiqi, **3**, 15-18 **(1993)**.

[20] J.Wang, P.A.M.Farias, J.S.Mahmoud; Analyst (London), **111**, 837-839 **(1986)**.

[21] L.Cunningham, H.Freiser; Anal.Chim.Acta, **157**, 157-162 **(1984)**.

[22] N.Rahman, S.N.H.Azmi; J.Microchem., **65**, 39-43 **(2000)**.

[23] R.Bindu, A.K.Chandrasekharan; Indian Drugs, **31**, 168-169 **(1994)**.

[24] B.V.Kamath, K.Shivram, A.C.Shah; J.Pharm. Biomed.Anal., **11**, 407-409 **(1993)**.

[25] S.S.Zarapkar, U.P.Halkar, S.H.Rane; Indian Drugs, **36**, 517-520 **(1999)**.

[26] H.El-Kholy, B.W.Kemppainen; J.Chromatogr., **796**, 371-377 **(2003)**.

[27] M.Cherlet, S.De-Baere, S.Croubels, P.De-Backer; J.Chromatogr.Biomed.Appl., **742**, 283-293 **(2000)**.

[28] A.Cannavan, W.J.Blanchflower, D.G.Kennedy; Analyst (London), **120**, 331-333 **(1995)**.
[29] J.J.Garcia, M.J.Diez, M.Sierra, M.T.Teran; J. Liq.Chromatogr., **13**, 743-749 **(1990)**.
[30] E.Kouassi, G.Caille, L.Lery, L.Lariviere, M.Vezina; Biopharm.Drug Dispos., **7**, 71-89 **(1986)**.
[31] F.J.Schenck, L.V.Podhorniak, R.Wagner; Food Addit.Contam., **15**, 411-414 **(1998)**.
[32] J.Wang, Q.Pan, X.Su, X.Zhao, Yaowu Fenxi Zazhi, **10**, 12-15 **(1990)**.
[33] F.C.Gong, D.X.Wu, Z.Cao, X.C.He, Biosensors Bioelectronics, **22**, 423-428 **(2006)**.
[34] M.A.El-Ries, S.Khalil, J.Pharm.Biomed.Anal., **25**, 3-7 **(2001)**.
[35] S.Khalil, N.Borham, J.Pharm.Biomed.Anal., **22**, 235-240 **(2000)**.
[36] S.Khalil, M.M.El-Rabiehi, J.Pharm.Biomed.Anal., **22**, 7-12 **(2000)**.
[37] S.L.Ortiz, J.G.Mateo, J.M.Calatayud, J.Microchem., **48**, 112-117 **(1993)**.
[38] S.Sadeghi, F.Fathi, J.Abbasifar; Sensors Actuators Chem., **22**, 158-164 **(2007)**.
[39] S.C.Dhanesar; Am.Lab.(Shelton, Conn.), **30**, 48-50 **(1998)**.
[40] Z.Leng, X.Hu; Yaowu Fenxi Zazhi, **10**, 212-216 **(1990)**.
[41] G.Shen, D.Liao; Yaowu Fenxi Zazhi, **4**, 200-204 **(1984)**.
[42] X.Li, D.Pan; Yaoxue Tongbao, **23**, 737-739 **(1988)**.
[43] J.M.Calatayud, C.Falco; Talanta, **33**, 685-687 **(1986)**.
[44] A.M.El-Didamony; Spectrochimica Acta., **69**, 770-775 **(2008)**.
[45] W.Z.Liu, H.Chen; Fenxi Huaxue, **21**, 921-923 **(1993)**.
[46] L.Liang, Z.Tao; Yaowu Fenxi Zazhi, **12**, 238-239 **(1992)**.
[47] P.Jelena, K.R.Katarina, D.Zorica, J.Milica, I.Svetlana; Biophys.Drug Dispos., **24**, 309 **(2003)**.
[48] J.H.Yoe, A.L.Jones; Ind.Eng.Chem.Anal., Ed., **16**, 14 **(1944)**.
[49] **(a)** P.Job; Camp.Rend.(Paris), **180**, 928 **(1925)**; **(b)**Ann. Chim. (Paris), **9**, 133 **(1928)**; **(c)**Ann. Chim. (Paris), **6**, 97 **(1936)**.
[50] W.C.Vosburgh, G.P.Cooper; J.Am.Chem.Soc., **63**, 437 **(1941)**.
[51] D.M.Schnur, Y.H.Yuh, D.R.Dalton; J. Org. Chem., **54**, 3779 **(1989)**.
[52] B.L.Podlogar, D.J.Raber; **54**, 5032 **(1989)**.
[53] N.L.Allinger; J.Comput.Chem., **14**, 755 **(1993)**.
[54] Validation of Analytical Procedure Methodology ICH Harmonized Tripartite Guidelines, 1 **(1996)**.
[55] British Pharmacopoeia, HerMajasty's, Stationary Office, London **(2005)**.
[56] S.Dowdy, S.Weardern;" Statistics for Research", N.Y. Wiley **(1983)**.