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Spectrophotometric determination of some anti-tussive drugs and its applications to pharmaceutical formulations

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ABSTRACT

Four simple, sensitive and reproducible spectrophotometric methods for the determination of some Anti-tissuve drugs, pipazethate hydrochloride (PiCl), dextromethorphan hydrobromide (DEX) and butamirat citrate (BT) in bulk samples and in pharmaceutical formulations are described. The first and second methods, are based on the charge-transfer complex formation of DEX and BT as n-donors and 2,3-dichloro-5,6 dicyano-p-benzoquinone (DDQ) or p-chloranilic acid (p-CA) as π-acceptors to give highly coloured species. The coloured products are measured spectrophotometrically at 465 and 462 for DEX and BT, respectively using DDQ (Method A) and at 530 and 525 nm for DEX and BT nm, respectively using p-CA (Method B). The third method is based on the oxidation of the studied drugs with ammonium metavanadate in sulphuric acid medium resulting in the development of a greenish blue colour at 759, 765 and 766 nm for PiCl, DEX and BT, respectively (Method C). The fourth method is based on the formation of an ion-association complex with alizarin red S as chromogenic reagents in acidic medium, which is extracted into chloroform. The complexes have a maximum absorbance at 425 and 428 nm for DEX and (PiCl or BT), respectively (Method D). Regression analysis of Beer-Lambert plots showed a good correlation in the concentration ranges of 20-240μg ml^{-1} for DDQ (Method A), 30-360μg mL^{-1} for p-CA (Method B), 0.05-0.6 mg mL^{-1} for ammonium metavanadate (Method C) and 2.0-24μg mL^{-1} for alizarin red S (Method D). For more accurate analysis, Ringbom optimum concentration ranges were calculated. The molar absorptivity, Sandell sensitivity, detection and quantification limits were calculated. Applications of the procedures to the analysis of various pharmaceutical preparations gave reproducible and accurate results. Further, the validity of the procedures was confirmed by applying the standard addition technique.

KEYWORDS

Pipazethate hydrochloride;
Dextromethorphan hydrobromide;
Butamirat citrate;
DDQ;
p-CA;
Ammonium vanadate;
Alizarin red S.

INTRODUCTION

Pipazethate hydrochloride (PiCl), 10H-pyrido[3,2-b][1,4]benzothiadiazine-10-carboxylic acid 2-(2-piperidinoethoxy)ethyl ester[1] is a bronchodilator that suppresses irritative and spasmodic cough by inhibiting the excitability of the cough center and the peripheral neural receptors in the respiratory passage. The response to the drug takes about 10–20 min and lasts for 4–6 h (SCHEME 1). Pipazethate has been determined using

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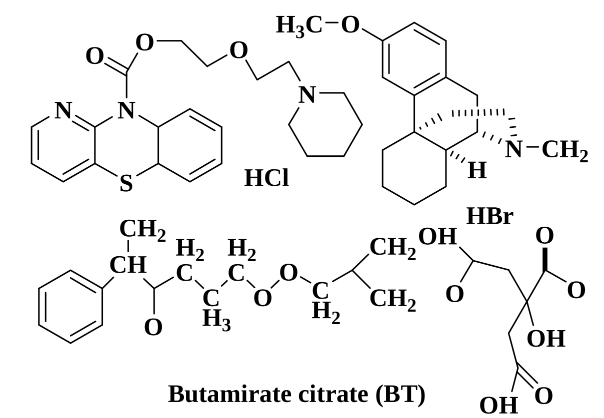


SCHEME 1: Chemical structure of the drugs under study

a limited number of techniques including spectrophotometry[2-6], TLC[7], HPLC[8], Conductimetry[9] and ISE[10]. PiCl was used in determination of Mo (VI) in alloy steels and soil samples[11].

Dextromethorphan hydrobromide (DEX), [(+)-3-Methoxy-17-methyl-9α, 13α, 14α-morphinan hydrobromide monohydrate] is a cough suppressant, used for the relief of non-productive cough; it has a central action on the cough centre in the medulla[12] (SCHEME 1). Different methods reported for the determination of DEX in the bulk drug, in the dosage forms with other drugs in cough-cold products and in biological samples. HPLC have been reported[13,14], spectrophotometry[4,5], the first and second-derivative technique UV-spectrophotometry[15-18], capillary electrophoresis[19,20], GC[21] and LC[22,23].

Butamirate citrate, 2-(2-diethylaminoethoxy)ethyl 2-phenyl butamirate dihydrogen citrate (BT), is widely used as a central cough suppressant[24,25]. The drug is not described officially in any pharmacopoeia. A literature survey revels that a few previous methods are described in the literature referring to both the relative bioavailability of different butamirate citrate preparations after single dose oral administration[26], the determination of compound using an optical compensation method[27] and determination of butamirate citrate in cough preparations by derivative UV spectrophotometry and HPLC[28].

In the present investigation, we investigate the development of three accurate, reproducible and adequately sensitive spectrophotometric methods for determination PiCl, DEX and BT based on the formation of charge-transfer complex of DEX and BT with 2,3-dichloro-5,6 dicyano-p-benzoquinone (DDQ) (Method A) and p-chloranilic acid (p-CA) (Method B), on the oxidation of the three studied drugs with Ammonium vanadate in sulphuric acid medium (method C) and on the formation of ion-association complexes between the three studied drugs and alizarin red S under reaction conditions used (method D). The proposed methods have been successfully applied in pure and in pharmaceutical formulations and favorably comparable with those of the official or reported methods.

EXPERIMENTAL

Apparatus

All the absorption spectral measurements were made using Kontron 930 (UV-Visible) spectrophotometer (German) with scanning speed 200 nm/min, and band width 1.0 nm equipped with 10 mm matched quartz cells.

Hanna pH-meter instrument (Portogal) (HI: 9321) was used for checking the pH of acetate buffer solutions of pH values 2.50-5.6 were prepared as recommended previously[29].

Material and reagents

All chemicals used were of analytical grade, and all of the solutions were freshly prepared in doubly distilled water.

Materials

- Pure grade pipazethate hydrochloride and its pharmaceutical preparations (Selgon, tablets 20 mg and drops 40 mg/ml) were provided by the Egyptian International Pharmaceutical Industries Company (EIPICO), Egypt.
- Pure Dextromethorphan HBr (DEX) and its pharmaceutical preparations, Tussilar tablets(10 mg) and Tussilar drops (1.0 g DEX /15 mL) kindly supplied by Kahira Pharm. & Chem. Ind. Company, Egypt.
- Pure grade butamirat citrate and its pharmaceutical preparations (Sinecod, drops 5 mg/ml)was kindly donated by NORVATIS PHARMA S.A.E., Egypt, under licence from Norvatis Consumer Health SA, Nyon, Switzerland.

Standard solutions

For methods A and B

Standard stock solutions of DEX and BT were prepared by dissolving 50 mg in 5.0 mL acetonitrile and the volume was diluted to the mark in a 100 mL calibrated flask with the same solvent.

For methods C and D

Stock solution of PiCl, DEX and BT (1.0 mg ml^{-1}) was freshly prepared by dissolving 100 mg of pure material in 20 ml bidistilled water and completed to 100 ml with bidistilled water in 100 ml calibrated flask. Working solutions were obtained by further dilution of the stock solutions with water.

Reagents

- 2,3-dichloro-5,6 dicyano-p-benzoquinone (DDQ), 2 mg ml^{-1} (Merck-Schuchardt, Munich, Germany) and p-CA, 4 mg ml^{-1} (Fluka, Switzerland) and (5×10^{-3} M) from both reagents in acetonitrile and the solutions were freshly prepared (daily).
- Ammonium metavanadate, 3 % w/v solution, prepared by dissolving 3 gm in boiling 50% v/v sulphuric acid and diluting to 100 mL with the same solvent.
- Alizarin red S, 3,4-dihydroxy-9, 1-dioxo-2-anthracene sulfonic acid (I). A stock solution (2×10^{-3} M) was prepared by dissolving the appropriate weights of ARS in doubly distilled water. Chloroform (Aldrich).

General procedures

Methods A (Using DDQ)

Aliquots of the standard solutions of DEX and BT containing (0.2-1.8) and (0.2-2.4 mg) of DEX and BT, respectively were transferred into in a 10-ml calibrated flask. Add 2 ml 0.25% of reagent solution and heat in a water-bath at 60 ± 2^0C for 10 and 15 min for DEX and BT, respectively. Cool and then dilute to volume with acetonitrile and measure the absorbance at 465 and 462 nm for DEX and BT, respectively against a reagent blank prepared in the same manner.

Methods B (Using p-CA)

Aliquots of the standard solutions of DEX and BT containing (0.4-3.6 mg) and (0.3-2.7 mg) of DEX and BT, respectively were transferred into in a 10-ml calibrated flask. Add 2.5 and 3 ml 0.25% of reagent solution and heat in a water-bath at 60 ± 2^0C for 10 min for DEX and BT, respectively. Cool and then dilute to volume with acetonitrile and measure the absorbance at 525 and 530 nm for DEX and BT, against a reagent blank prepared in the same manner.

Methods C (Using ammonium metavanadate)

Aliquots of standard solution equivalent to 0.5-5.0 mg DEX, 1-6 mg PiCl and 0.5-4.5 mg BT were transferred into a 10 mL volumetric flask. 3 and 2 mL of 3 % w/v ammonium metavanadate for DEX and (PiCl or BT), respectively were added followed by 2 mL of concentrated sulphuric acid. The mixture was mixed well and boiled gently for 10 and 20 min for (DEX or BT) and PiCl, respectively in water bath. The mixture was cooled and diluted to volume with bidistilled water and The absorbance was measured at 759, 765 and 766 nm for PiCl, DEX and BT, respectively against blank (omitting the addition of drug).

Method D (Extractive method using ARS)

A 0.2-2.4 mL portions of 100μg mL^{-1} DEX, PiCl and BT were transferred into a series of 50 ml of separating funnels; then 3 mL of acetate buffer of pH 3.2 and 3.0 and 3.0 mL of (2×10^{-3} M) ARS for DEX and (PiCl or BT), respectively were added. The total volume was adjusted to 10 mL by adding distilled water. Two 5 mL portions of chloroform was added to each separating funnel and the contents were shaken for exactly 2.0 min. The two phases were allowed to separate and the chloroform layer was passed through anhydrous sodium sulphate and the absorbance was measured at 425 and 428 nm for DEX and (PiCl or BT), respectively against the reagent blank (omitting the addition of drug). A calibration graph was drawn or regression equation calculated.

Applications for pharmaceutical formulations

Procedure for tablets

At least ten tablets of the studied drugs were weighed into a small dish, powdered and mixed well. A portion equivalent to 10 mg was weighed and dissolved in 100 mL distilled water (Methods A and B) and acetonitrile (Method C and D), shaken well and filtered through a sintered glass crucible (G4). An aliquot of the dug solution was then treated as described above.

Procedure for drops

The contents of 5.0 bottles of drops of the studied drugs were mixed. An accurate volume equivalent to 1.0 g of DEX, 40 mg of PiCl and 5 mg of BT was transferred to a 100 ml measuring flask and completed to the mark with bidistilled water (Methods A and B) and acetonitrile (Method C and D). This solution was further diluted stepwise to the requisite concentration 100µg ml^{-1} of the studied drugs with the same solvent and analyzed as described under the general procedure described above.

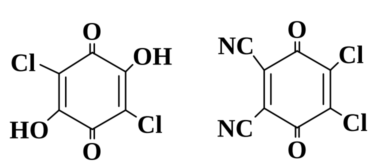
Stoichiometric relationship

Job's method of continuous variations was employed a 1×10^{-3} M standard solution of DEX, PiCl and BT and $1{\times}10^{-3}$ M solution of ARS or 5×10^{-3} M standard solution of DEX and BT and $5{\times}10^{-3}$ M solution of DDQ or *p-CA* under consideration were used. A series of solutions were prepared in which the total volume of drug and reagent was kept at 2.0 ml. The reagents were mixed in various proportions and diluted to volume in a 10-ml calibrated flask with the appropriate solvent following the above mentioned procedures.

RESULTS AND DISCUSSION

Method A and B (charge transfer methods)

The selected drugs were considered as electron-donors when they reacted with selected acceptors (DDQ and p-CA). they produce a new band of absorption intensity at a suitable λ_{max}. which was characteristic for each complex. These new bands wee used for a quantitative determination of them (TABLE 1).



DDQ p-CA

The reaction of DEX and BT with DDQ results in the formation of an intense orange-red colour, which exihibits three maxima at 580, 545 and 465 for DEX or at 578, 546 and 462 nm for BT. The 465 and 462 nm bands, having the highest absorption intensity, was selected for construction of Beer's plot (Figure 1). The predominant colour with DDQ is from the orange-red radical anion DDQ$^-$, which was probably formed by the dissociation of an original donor-acceptor (DA) complex with the cited drugs.

$$D^* + A \rightleftharpoons (D^{**}\text{-}A) \xrightarrow[\text{DA complex}]{\text{Polar solvent}} D^{*-} + A^{*-}$$

In addition to the DDQ radical anion, the reaction of the cited drugs with p-CA results in the formation of an intense purple colour with a maximum absorption at 525-530 nm (Figure 2). The experimental conditions should be carefully selected.

Choice of solvent

Different solvents such as acetone, methanol, ethanol, methylene chloride, acetonitrile and chloroform

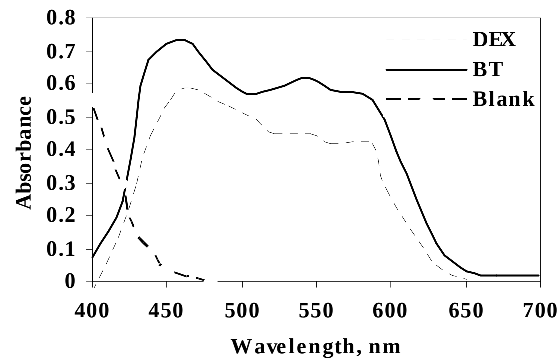


Figure 1: Absorption spectra of reaction products of DDQ with 40 µg ml^{-1} DEX and 100µg ml^{-1} BT against blank solution

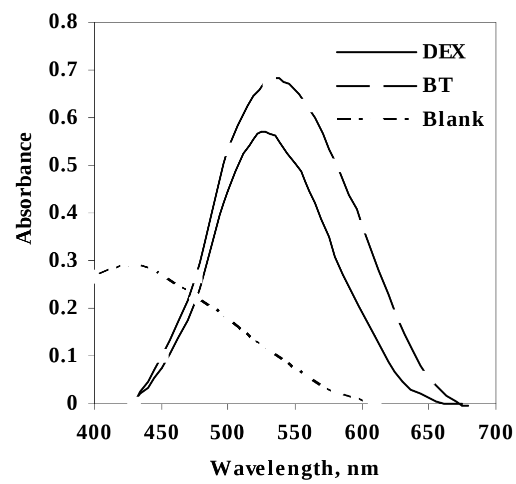


Figure 2: Absorption spectra of reaction products of *p*-CA with 80µg ml^{-1} DEX and 150µg ml^{-1} BT against blank solution.

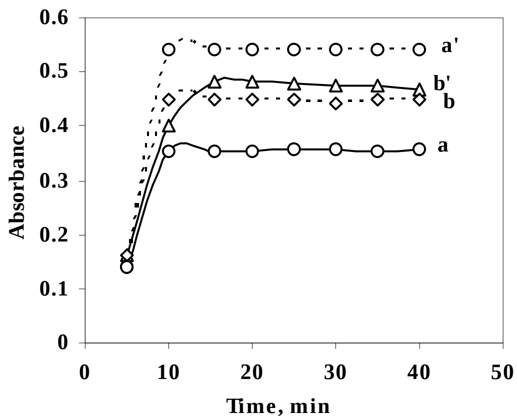


Figure 3: Effect of time on the colour intensity at 60°C for the studied drugs complexes with: a- DEX-DDQ, b-BT-DDQ, a'- DEX-p-CA, b'- BT-p-CA

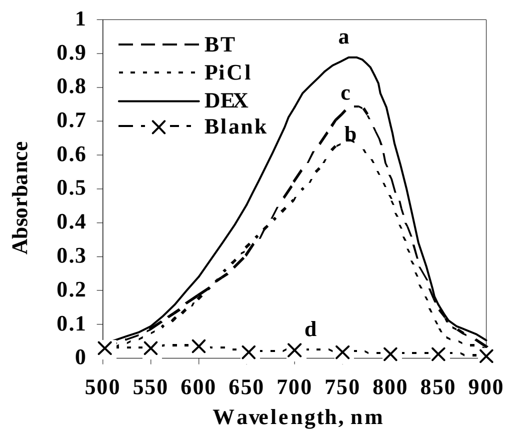


Figure 4: Absorption spectra of reaction product between; a- 1 mL of 1 mg mL^{-1} DEX and 3 mL of 5 % w/v ammonim vanadate (——), b- 1 mL of 1 mg mL^{-1} PiCl and 2 mL of 5 % w/v ammonim vanadate (———), c- 1 mL of 1 mg mL^{-1} BT and 2 mL of 5 % w/v ammonim vanadate (———), d- 5 % w/v ammonim vanadate (Reagent blank) (……)

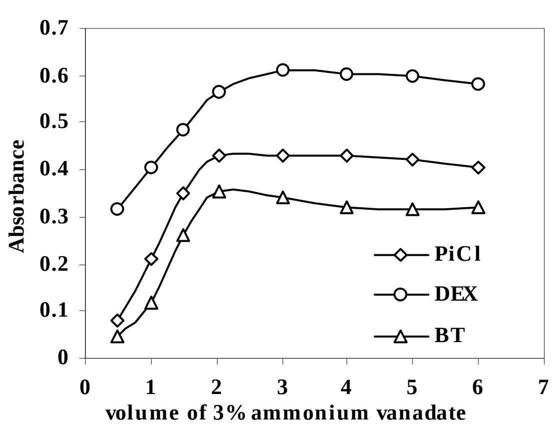


Figure 5: Effect of volume of 3 % w/v ammonium vanadate on the absorbance of the cited drugs (1 mg mL^{-1})

were examined. Acetonitrile afforded the maximum sensitivity when compared to all solvents examined, a property, which is known to promote the dissociation of the original charge-transfer complexes to the radical ions.

Effect of acceptor concentration

The optimum concentrations that give maximal colour formation in case of DDQ method was 2.0 ml of 0.2% w/v DDQ solution in acetonitrile. While for p-CA 2.5 and 3.0 ml of 0.25% w/v p-CA solution in acetonitrile were found to be sufficient for the production of maximum and reproducible colour intensity. Higher concentrations of the reagent did not affect the colour intensity with DEX ad BT, respectively (TABLE 1).

Effect of time and temperature

The optimum reaction time was determined by following the colour intensity at ambient temperature (25 $\pm 2^0$C). Complete colour development was attained after 45 min for DDQ and 50 min for *P*-CA complexes. On raising the temperature to 60 $\pm 2^0$C for 10-15 min using DDQ and 10 min using *P*-CA, the complete colour development was obtained (Figure 3). The colour remained stable for 2.5 and 3.0 h for DDQ and P-CA reagent complexes.

Methods C (Using ammonium metavanadate)

The method has been used for the quantitative determination of DEX, PiCl and BT by oxidation with ammonium metavanadate in sulphuric acid medium resulting in the development of greenish blue colour at 759, 765 and 766 nm for PiCl, DEX and BT, respectively which was attributed to the vanadium (IV) produced by reduction of vanadium (V) by the selected drug (Figure 4). The optimum conditions for the formation of greenish blue colour were studied:

Effect of reagent concentration

It was found that 3 and 2 mL of 3 % w/v ammonium metavanadate was the most suitable concentration for carrying out the assay for PiCl and (DEX or BT), respectively (Figure 5).

Effect of heating time

Gentle boiling on a water bath for 5-30 min. showed that 20 min was sufficient to produce maximum colour intensity for DEX, PiCl and BT, respectively(Figure 6).

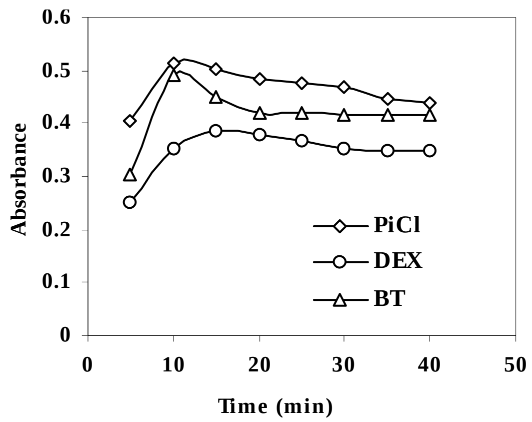


Figure 6: Effect of heating time on the absorbance of the cited drugs (1 mg mL^{-1}) with and 3 % w/v ammonim vanadate

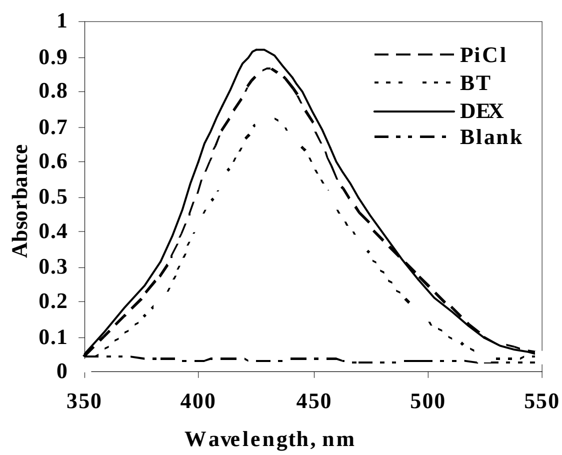


Figure 7: Absorption spectra of (10μg mL^{-1}) of the studied drugs with (2 × 10^{-3} M) ARS (.......)

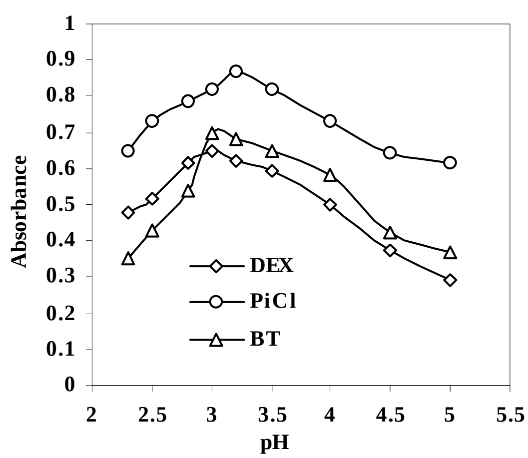


Figure 8: Effect of pH on the absorbance of (10 μg ml^{-1}) of the studied drugs with 3 ml of ARS

Effect of sulphuric acid concentration

Different concentrations (10, 30, 50, 70, 90 and 98% v/v) were tried and it was found that 2 mL of concentrated sulphuric acid (98%) gave best results.

Method D (Extractive method using ARS)

Ion-pair complex extraction spectrophotometry has been frequently used for the quantitative determination of many pharmaceutical compounds. Therefore, in the present investigation, ARS as an anionic dye forms a yellow coluored ion-pair complex with PiCl, DEX and BT in acidic pH, which is extracted into chloroform and can be measured at 425 and 428 nm, DEX and (PiCl or BT), respectively (Figure 7).

Effect of pH

The effect of pH was studied by extracting the coloured complex in the presence of various buffers such as NaOAc-HCl (pH=1.99-4.92), NaOAc-AcOH (pH=2.5-5.5) and potassium hydrogen phthalate–HCl (pH=2.2-4.6). The results are shown in (Figure 8.) The resulting data at higher pH values shows that the extract of ion-pair extraction decreases drastically, most probably due to the interference of the $H3O^+$ and ARS, and as a result diminishes the complexation power. Thus, the maximum colour intensity and constant absorbance were observed in acetate buffer (NaOAc-AcOH) of pH 3.2 and 3.0 for DEX and (PiCl or BT), respectively with a buffer volume of 3.0 ml of buffer for final 10 mL solution was used in further studies (Figure 8).

Choice of organic solvent

The effect of the extracting solvent used both on extraction efficiency and colour intensity was examined. Chloroform, dichloromethane, dichloroethane, toluene and carbon tetrachloride proved useful solvents; chloroform was selected due to the more stability of the extracted coloured product and considerably lower extraction abilities of the reagent blank. Consequently The optimum volume of the organic phase and the number of extraction times were also studied. Maximum absorbance was obtained by using 10 mL of chloroform during two 5 mL steps extraction.

Effect of shaking time

The extraction was studied by shaking different samples on a shaker and varying the shaking time for 0.5-5.0 min for the ion pair complexes. It was found

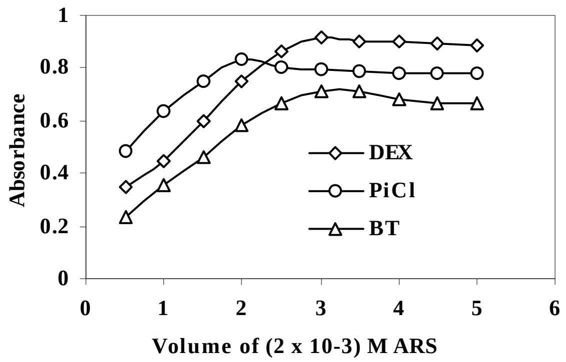


Figure 9: Effect of ARS reagent on the absorbance of the studied drugs (10μg ml^{-1})

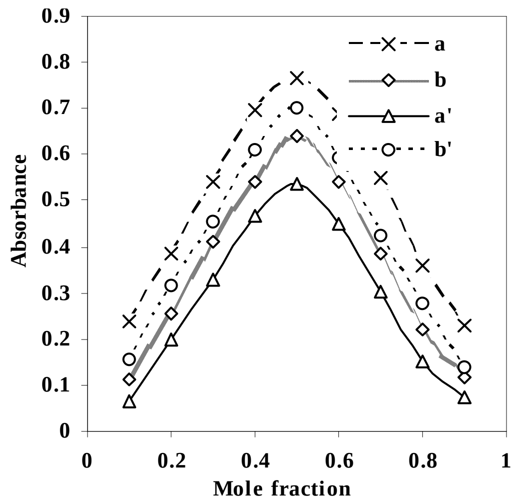


Figure 10: Continuous variation graph for the reaction of the studied drugs complexes with: a- DEX-DDQ, b-BT-DDQ, a'- DEX-p-CA, b'- BT-p-CA

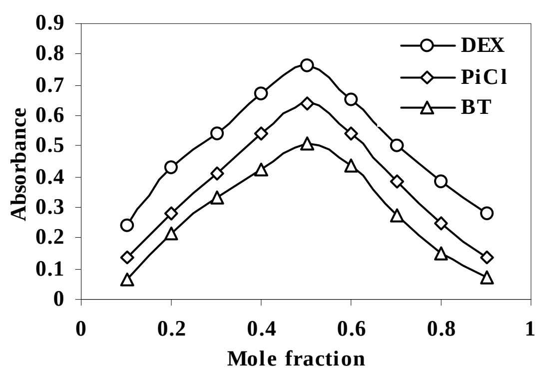


Figure 11: Job's method of continuous variation graph for the reaction of drugs with ARS [drug]=[ARS]= 1×10^{-3} M

that the absorbance remained constant over this time period for all systems. A shaking time of 2.0 min was adopted for all extractions. It was further observed that the yellow extracts remained stable for at least 24 h. The intensity of ion-pairs extraction were found to be stable in the temperature range 20-40^{0}C. Hence room temperature (25 ± 2^{0}C) was used.

Effect of reagent concentrations

The influence of the concentration of the ARS solution on the extraction of DEX, PiCl and BT was studied. The result obtained from the extraction of 10μg mL^{-1} drugs in the presence of various concentrations of ARS showed that the absorbance values of ion-pair complex in organic phase increases with the increasing of ARS in the aqueous phase. A maximum extraction of ion-pair complexes occurs when the volume of reagent reaches to 3.0 and 2.0 mL of (2.0×10^{-3} M) ARS for (DEX or PiCl) and BT, respectively. A further excess of the reagent has no considerable effect on the fraction of the complex extracted (Figure 9).

Sequence of addition

The optimum sequence was defined by following to colour intensity and maximum absorbance on changing the sequences of addition of drug, reagent and buffer. The best condition was "drug-reagent-buffer-solvent" for the highest absorbance and stability. Other sequences needed longer time in addition to lower stability.

Stoichiometric ratio

The stoichiometric ratio of the reactants was determined by molar ratio and continuous variation methods[30,31]. Job's continuous variation graph for the reaction between DEX and BT and DDQ or P-CA reagents shows that the interaction occurs on an equimolar basis. The reaction occurs through the formation of a charge-transfer complex (1:1) (Figure 10). The results obtained showed that the composition of the ion-pair complex was equimolar (1:1) (drug : reagent) are given in (Figure 11).

Interferences

To test the efficiency and selectivity of the proposed analytical methods to pharmaceutical formulations, a systematic study under the optimum experimental conditions was made for the effect of the additives and excipients (e.g. lactose, glucose, fractose, calcium hydrogen phosphate, magnesium stearate and starch) that are usually present in dosage forms. The criterion of interference was an error of not more than ±3.0% in

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TABLE 1: Optimum conditions for the colour development of DEX and BT using DDQ and p-CA methods

Parameters	DDQ		*p*-CA	
	DEX	BT	DEX	BT
Conc. Of acceptor (% w/v)	2.0 ml 0.2%	2.0 ml 0.2%	2.5 ml 0.25%	3.0 ml 0.25%
Reaction time (min) at (60± 2° C)	10	15	10	10
Stability of the complex	2.5 hours	2.5 hours	3.0 hours	3.0 hours
λ_{max} (nm)	465	462	525	530
Beer's law limits $\mu g\ ml^{-1}$	20-180	20- 240	40-360	30- 270
Ringbom optimum range $\mu g\ ml^{-1}$	28-160	30-200	55-320	45-240
Molar absorpitivity ($L\ mol^{-1}\ cm^{-1}$) $\times 10^3$	2.79	2.49	1.01	1.896
Sandell's Sensitivity, $\mu g\ cm^{-2}$	0.133	0.20	0.367	0.26
Detection limits $\mu g\ ml^{-1}$	0.31	0.36	0.26	0.42
Regression equation*				
Slop (b)	0.0059	0.0035	0.0021	0.0029
Intercept (a)	0.0973	0.0936	0.0673	0.0764
Correlation coefficient (r)	0.9997	0.9995	0.9993	0.9991
% Relative standard deviation[a]	0.69	1.023	1.196	1.263

*:A = a + b C, where A is the absorbance, a is the intercept, b is the slope and C is the concentration of drug in $\mu g\ ml^{-1}$. [a]Relative standard deviation for six determinations.

TABLE 2 : Spectral characteristics of the coloured products of the studied drugs with with Ammonium vanadate and ARS

Parameters	Ammonium vanadate			ARS		
	DEX	PiCl	BT	DEX	PiCl	BT
pH	Conc. sulphuric acid	Conc. sulphuric acid	Conc. sulphuric acid	3.2	3.0	3.0
λ_{max} (nm)	765	759	766	425	428	428
Beer's law limits	0.05-0.5 $mg\ ml^{-1}$	0.1- 0.6 $mg\ ml^{-1}$	0.05-0.45$mg\ ml^{-1}$	2.0-24 $\mu g\ ml^{-1}$	2.0- 20 $\mu g\ ml^{-1}$	2.0-18 $\mu g\ ml^{-1}$
Ringbom optimum range	0.1-0.46$mg\ ml^{-1}$	0.15-0.55 $mgml^{-1}$	0.07-0.36$mg\ ml^{-1}$	3.5-22 $\mu g\ ml^{-1}$	4.0 -18 $\mu g\ ml^{-1}$	3.0-16 $\mu g\ ml^{-1}$
Molar absorpitivity ($L\ mol^{-1}\ cm^{-1}$)	9.02×10^2	7.076×10^2	9.817×10^2	2.234×10^4	1.819×10^4	3.092×10^4
Sandell's Sensitivity, $ng\ cm^{-2}$	0.411$\mu g\ cm^{-2}$	0.616$\mu g\ ml^{-1}$	0.509$\mu g\ ml^{-1}$	16.58 $\mu g\ cm^{-2}$	23.97 $\mu g\ cm^{-2}$	16.16 $\mu g\ cm^{-2}$
Detection limits	0.23 $mg\ ml^{-1}$	0.21 $mg\ ml^{-1}$	0.58	0.16 $\mu g\ ml^{-1}$	0.21 $\mu g\ ml^{-1}$	0.32
Regression equation*						
Slop (b)	0.0022	0.0015	0.0014	0.0605	0.0425	0.0495
Intercept (a)	0.0293	0.0252	0.080	0.0022	-0.0048	0.0733
Correlation coefficient (r)	0.9991	0.9994	0.9990	0.9992	0.9997	0.9985
% Relative standard deviation [a]	0.657	0.936	0.942	0.813	1.14	1.21

*: A = a + b C, where A is the absorbance, a is the intercept, b is the slope and C is the concentration of drug in $\mu g\ ml^{-1}$. [a] Relative standard deviation for six determinations

absorbance. Experiments showed that there was no interference from additives and excipients, for the examined methods. Also, there was no interference from common degradation products results from oxidation of DEX, PiCl and BT or from thermal and hydrolytic degradation, which are likely to occur at normal storage condition.

Analytical data

A calibration graph was constructed using a standard solution of PiCl, DEX and BT. Under the optimum experimental conditions, a linear relationship existed between the absorbance and concentration of the drugs in the concentration ranges listed in (TABLES 1 and 2)[32]. The correlation coefficients, intercepts and slopes of the calibration graph for the studied drugs are calculated. For more accurate results, Ringbom optimum concentration ranges are calculated and listed in (TABLES 1 and 2). The detection limit was also determined (3 s/m), where s=standard deviation of the blank and m=slope of the calibration graph according to IUPAC definitions[33].

The mean molar absorptivity and Sandell sensitivity as calculated from Beer's law are presented in (TABLES 1 and 2). In order to determine the accuracy and precision of the method, solutions containing four

TABLE 3: Evaluation of accuracy and precision of the proposed methods for the studied drugs

Reagents	DEX				PiCl				BT			
	Taken	Recovery (%)	RSD[a] (%)	Confidence[b] limits	Taken	Recovery (%)	RSD[a] (%)	Confidence[b] limits	Taken	Recovery (%)	RSD[a] (%)	Confidence[b] limits
Ammonium Vanadate	mg ml^{-1} 0.2	99.60	0.562	0.199±0.001	mg ml^{-1} 0.1	100.24	0.693	0.1003±0.0008	mg ml^{-1} 0.1	99.97	0.723	0.0999±0.0008
	0.3	100.30	0.376	0.301±0.0012	0.2	99.75	0.558	0.1995±0.0012	0.2	100.08	0.658	0.2002±0.0014
	0.4	99.90	0.242	0.499±0.0013	0.3	99.92	0.382	0.2998±0.0012	0.3	100.14	0.520	0.3004±0.0016
	0.45	100.08	0.215	0.450±0.001	0.4	100.70	0.26	0.4028±0.0011	0.4	100.30	0.314	0.4012±0.0013
ARS	µ g ml^{-1} 5.0	100.20	0.72	5.01± 0.038	µg ml^{-1} 4.0	99.95	0.56	3.998±0.023	µg ml^{-1} 4.0	100.10	0.49	4.004±0.021
	10	100.65	0.34	10.065±0.036	8.0	99.80	0.48	7.984±0.402	8.0	100.25	0.42	8.02±0.035
	15	99.93	0.30	14.99±0.047	12	100.20	0.22	12.024±0.028	12	99.91	0.32	11.994±0.0403
	20	99.85	0.27	19.97±0.057	16	99.55	0.21	15.928±0.035	16	99.86	0.27	15.978±0.0453
DDQ	µ g ml^{-1} 40	99.97	0.36	39.99±0.15					µ g ml^{-1} 50	99.95	0.42	49.975±0.22
	80	100.35	0.21	80.28± 0.177					100	99.75	0.34	99.75±0.356
	120	100.10	0.17	120.12±0.214					150	100.27	0.18	150.41±0.284
	160	100.4	0.12	160.64±0.202					200	99.15	0.11	198.3±0.229
p-CA	µ g ml^{-1} 50	100.05	0.29	50.025±0.152					µg ml^{-1} 50	99.88	0.54	49.94±0.283
	150	99.83	0.20	149.75±0.314					100	100.07	0.47	100.07±0.494
	250	99.27	0.22	248.18±0.573					200	100.32	0.28	200.64±0.59
	350	99.65	0.15	348.78±0.549					250	99.59	0.13	248.98±0.34

[a]Relative standard deviation for six determinations; [b]95% confidence limits and five degrees of freedom.

TABLE 4: Determination of the studied drugs using the proposed methods compared with official or reference methods

Samples	Official methods	DDQ	p-CA	Ammonium vanadate	ARS
PiCl- Pure solution					
X ± SD	100.08± 1.06			100.60±0.942	99.65±1.137
N	6			6	6
Variance	1.124			0.89	1.88
SAE	0.433			0.385	0.56
t-value (2.57)*				0.81	0.61
F-value (5.05)*				1.27	1.15
DEX-Pure solution					
X ± SD	99.88 ± 0.783	99.28± 0.69	99.24± 1.196	99.35±0.653	100.30±0.815
N	6	6	6	6	6
Variance	0.613	0.476	1.43	0.426	0.664
SAE	0.32	0.28	0.49	0.27	0.33
t-value (2.57)*		1.28	1.00	1.16	0.83
F-value (5.05)*		1.29	2.33	1.44	1.08
BT- Pure solution					
X ± SD	99.42 ± 1.72	101.11± 1.03	99.72 ± 1.263	99.91± 0.942	99.99 ± 1.21
N	6	6	6	6	6
Variance	2.96	1.06	1.6	0.89	1.46
SAE	0.702	0.42	0.516	0.385	0.494
t-value (2.57)*		1.88	0.314	0.56	0.61
F-value (5.05)*		2.79	1.85	3.33	2.02

*Theoretical value at P= 0.05 at 95 % level & average of six determinations.

different concentrations of the studied drugs were prepared and analysed in quintuplicate. The measured standard deviations can be considered satisfactory, at least for the levels of concentrations examined. The repro-

TABLE 5: Determination of the studied drugs in pharmaceutical preparations using the proposed methods compared with official or reference methods

Samples	Official methods	DDQ	p-CA	Ammonium vanadate	ARS
Selgon tablets					
X ± SD	99.70 ± 1.163			100.25±1.046	100.05±0.876
N	6			6	6
Variance	1.35			1.094	0.77
SAE	0.475			0.43	0.36
t-value (2.57)*				0.79	0.54
F-value (5.05)*				1.24	1.76
Selgon Drops					
X ± SD	100.50 ± 1.364			100.15±1.058	99.96±1.172
N	6			6	6
Variance	1.86			1.12	1.37
SAE	0.557			0.43	0.48
t-value (2.57)*				0.45	0.70
F-value (5.05)*				1.66	1.35
Tussilar Tablets					
X ± SD	98.92± 0.854	100.03 ± 0.92	99.55 ± 1.084	99.80±0.661	99.60±1.15
N	6	6	6	6	6
Variance	0.73	0.85	1.18	0.44	1.32
SAE	0.35	0.38	0.44	0.27	0.47
t-value (2.57)*		1.98	1.021	1.82	1.06
F-value (5.05)*		1.16	1.61	1.67	1.81
Tussilar Drops					
X ± SD	99.22 ±1.39	100.15 ± 1.07	99.86 ± 0.974	100.02±1.31	99.56±1.58
N	6	6	6	6	6
Variance	1.93	1.145	0.95	1.72	2.50
SAE	0.57	0.44	0.40	0.535	0.645
t-value (2.57)*		1.19	0.84	0.94	0.36
F-value (5.05)*		1.69	2.037	1.13	1.29
Senicod Drops					
X ± SD	99.58 ± 0.85	99.83± 1.074	100.27± 0.873	100.07± 1.16	99.97 ± 0.961
N	6	6	6	6	6
Variance	0.723	1.15	0.762	1.35	0.924
SAE	0.35	0.44	0.356	0.474	0.39
t-value (2.57)*		0.41	1.27	0.762	0.68
F-value (5.05)*		1.6	1.055	1.86	1.28

Theoretical value at P= 0.05 at 95 % level & average of six determinations.

ducibility of the procedure was determined by running six replicate samples of the studied drugs. The analytical results obtained from this investigation are summarized in (TABLE 3). The percentage R.S.D. (≤0.97 %) can be considered to be very satisfactory.

Analytical applications

The proposed methods were successfully applied to determine PiCl, DEX and BT in dosage forms. The results obtained were compared statistically by Student's t -test (for accuracy) and variance ratio F-test (for precision) with the official method obtained by the pharmacopoeial methods for PiCl and DEX[1,12] (based on potentiometric titration using 0.1 M sodium hydroxide) and BT[24] (based on HPLC method) at 95% confidence level with five degrees of freedom as shown in (TABLES 4 and 5). The results showed that the t - and F- values were less than the critical value indicating that there was no significant difference between the proposed and official methods. The proposed methods were more accurate with high recoveries than the official method so the proposed methods can be recommended for routine analysis in the majority of drug quality control laboratories.

CONCLUSION

All the proposed methods were advantageous over other reported visible spectrophotometric methods with respect to their higher sensitivity, simplicity, reproducibility, precision, accuracy and stability of colored species. The proposed methods can be applied for routine analysis and in quality control laboratories for the quantitative determination of pipazethate hydrochloride (PiCl), dextromethorphan hydrobromide (DEX) and Butamirat citrate (BT) in bulk samples and in pharmaceutical formulations.

REFERENCES

[1] J.E.F.Reynolds, Martindale; 'The Extra Pharmacopoeia', Pharmaceutical Press, London, 30th edn., p.8140 **(1999)**.

[2] S.S.Zarapker, R.V.Rele, V.M.Shah; Indian Drugs, **24**, 445-449 **(1987)**.

[3] S.S.Zarapker, R.V.Rele, V.J.Doshi; Indian Drugs, **24**, 560-564 **(1987)**.

[4] R.El-Shiekh, F.Zahran, A.A.E.F.Gouda; Spectro chimica Acta A.**66**, 1279-1287 **(2007)**.

[5] A.S.Amin, R.El-Sheikh, F.Zahran, A.A.E.F.Gouda; Spectrochimica Acta, **A67**, 088-1093 **(2007)**.

[6] R.El-Shiekh, A.S.Amin, F.Zahran, A.A.E.F.Gouda; J.AOAC Int., **90**, 686-692 **(2007)**.

[7] H.D.Revanasiddappa, P.G.Ramappa; Indian Drugs **32**, 73-77 **(1995)**.

[8] H.D.Revanasiddappa, P.G.Ramappa; Indian Drugs, **32**, 534-536 **(1995)**.

[9] N.T.Abdel-Ghani, A.F.Shoukry, R.M.El-Nashar; Analyst, **126**, 79-85 **(2001)**.

[10] Y.M.Issa, A.F.Shoukry, R.M.El-Nashar, J.Pharm. Biomed.Anal.26, 379-386 **(2001)**.

[11] M.B.Melwanki, J.Seetharamappa, S.P.Masti; Anal.Sci., **17**, 1121 **(2001)**.

[12] United States Pharmacopoeia, 25th Review, The National Formulary, 19th Review, The United States Pharmacopoeia Convention, Rockville, MD, 975 **(2002)**.

[13] L.A.Shervington; Anal.Lett., **30**, 927-944 **(1999)**.

[14] E.K.Bendriss, N.Markoglou, I.W.Wainer; J. Chromatogr.B: Biomed.Sci.Appl., **754**, 209-215 **(2001)**.

[15] V.Tantishaiyakul, C.Poeaknapo, P.Sribun, K. Sirisuppanon; J.Pharm.Biomed.Anal., **17**, 237-243 **(1998)**.

[16] S.Ajay, T.Piyush; Indian Drugs **36**, 444-448 **(1999)**.

[17] S.Gangwal, P.Trivedi; Indian Drugs, **36**, 568-571 **(1999)**.

[18] M.S.Bratio, S.G.Kaskhedikar, S.C.Chaturvedi; Indian Drugs, **36**, 702-705 **(1999)**.

[19] L.Suntornsuk; Electrophoresis, **22**, 139-143 **(2001)**.

[20] M.R.Gomez, R.A.Olsina, L.D.Martinez, M.F.Silva; J.Pharm.Biomed.Anal., **30**, 791-799 **(2002)**.

[21] A.P.Argekar, S.V.Raj, S.U.Kapadia; Indian Drugs **35**, 24-28 **(1998)**.

[22] D.R.Jones, J.C.Gorski, M.A.Hamman, S.D.Hall; J. Chromatogr.B: Biomed.Sci.Appl., **678**, 105-111 **(1996)**.

[23] R.D.Bolden, S.H.Hoke, T.H.Eichhold, D.L. McCauley-Myers, K.R.Wehmeyer, J.Chromatogr. B: Analyt.Technol.Biomed.Life Sci., **772**, 1-10 **(2002)**.

[24] J.E.F.Reynolds, Martindale; 'The Extra Pharmacopoeia', 31st ed., The Pharmaceutical Press, Royal Pharmaceutical Society, London, **(1996)**.

[25] J.Elks, C.R.Ganellin; 'Dictionary of Drugs', Chapman and Hall, **(1990)**.

[26] H.Bohner, P.S.Janiak., V.Nitsche, A.Eichiger, H. Schutz; Int.J.Clin.Pharmacol.Ther., **35**, 117 **(1997)**.

[27] I.Dol., C.Altesor, M.Knochen; Quim.Anal. Barcelona, **15**, 148 **(1996)**.

[28] E.T.Mlliou, C.G.Antoniou, J.E.Koundourellis; Anal. Sci., **19**, 563-568 **(2003)**.

[29] Basset, J.R.C.G.H.Denny, Jeffery Mendham; 'Vogel Text Book of Quantitative Inorganic Analysis', Imperical Collage, London, 4th Edn., **350**, **(1986)**.

[30] H.T.S.Britton; Hydrogen Ions, Chapman and Hall, London, **(1952)**.

[31] P.Job; Ann.Chim., **9**, 133 **(1928)**.

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

[33] J.N.Miller, J.C.Miller; 'Statistics and Chemometrics for Analytical Chemistry', 5th Ed., Prentice Hall, England, **(2005)**.

[34] H.M.N.H.Irving, H.Freiser, T.W.West; 'IUPAC Compendium of Analytical Nomenclature', Denitive Rules, Pergamon Press, Oxford, **(1981)**.