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Extractive spectrophotometric method for determination of Dothiepin (Dosulepin) hydrochloride in pure and in pharmaceutical dosage forms

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ABSTRACT

Simple, rapid, sensitive and economical spectrophotometeric procedures have been described for the determination of anti-depressant drugs such as Dothiepin (Dosulepin) hydrochloride (D-HCl) and in bulk sample and in different dosage forms were investigated. The implemented procedures are based on the formation of a yellow colored ion-associates due to the interaction between the examined drugs D-HCl with Picric acid (PA), Chlorophenol red (ClPR), Bromthymol blue (BrTB), Bromcresol purple (BrCP) reagents. In this case the suitable recommended buffer solution has been used and the extraction was carried out using chloroform, then recording the optimum wavelength using visible spectrophotometer. Moreover, the optimum reaction conditions were carefully investigated whereas the Beer's law is obeyed within a concentration range of 16-46µg/mL. In addition we have determined the molar absorptivity, Sandell sensitivity and the optimum conditions for quantitative analysis of the investigated drugs. The correlation coefficient was = 0.9983 (n = 6) with a relative standard deviation (RSD) = 1.55 for five selected concentrations of the reagents. Therefore we have determined successfully the concentration of D-HCl drug in their pharmaceutical formulations up to 46µg/mL.

KEYWORDS

Dothiepin (Dosulepin) hydrochloride;
Pharmaceutical analysis;
Spectrophotometery;
Ion-associates.

INTRODUCTION

The selective drug in the current investigations has a wide range of applications in pharmaceutical chemistry; they are white crystalline powders that are easily soluble in water.

Dothiepin hydrochloride ($C_{19}H_{21}NSHCl$; D-HCl), (3(6H)dibenzo[b,e]thiepin-11-ylidene) propyl dimethylamine hydrochloride (Figure 1) is a tricyclic antidepressant with a noticeable action. Is indicated in the treatment of depression and anxiety[1], Several analytical methods have been applied determine Dothiepin hydrochloride (D-HCl) quantitatively in their dosage forms including spectrophotometric method[2,3], High

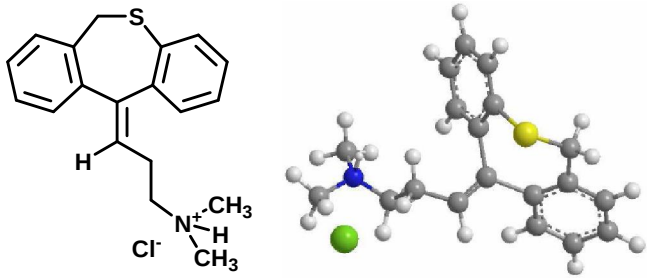

Figure 1 : Two and three dimentional structures of: Dothiepin hydrochloride.

Performance Liquid Chromatogrphy HPLC[4], capillary electrophoresis[5] and other spectrophotometric for another drugs[6-10].

EXPERIMENTAL

Apparatus

The electronic absorption spectral measurements of D-HCl (Figure 2) with selected reagents were recorded on Jenway 6505 UV-Vis spectrophotometer equipped with quartz cell of 1 cm optical path length with a resolution of 0.1 nm. The pHs of the prepared solutions were adjustment using Jenway 3510 pH meter. All spectroscopic measurements were carried out at room temperature (25 ± 2 °C). Moreover, doubly distilled water were obtained ELGA distillation apparatus model UHQ-II-MK3, UK.

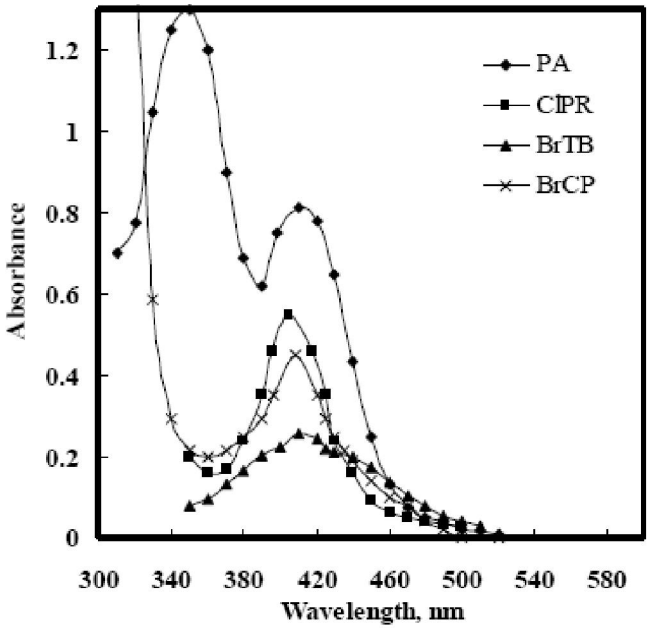

Figure 2 : The electronic absorption measurements in the visible region for: Dothiepin hydrochloride ion-associates with picric acid, chlorophenol red, bromthymol blue and bromcresol purple.

Materials

(1) Dothiepin (Dosulepin) hydrochloride and Prothiaden capsules (25 mg/cap) were obtained through the courtesy of Kahira Pharmaceutical and Chemical Industries, Cairo, Egypt. All chemicals and reagents are of analytical grade. Chlorophenol red (ClPR), bromthymol blue (BrTB) and bromcresol purple (BrCP) are products of Merck chemical company. Picric acid (PA) is a product of Arablab chemicals while; sodium acetate trihydrate, acetic acid and anhydrous sodium sulfate are of Merck Chemical Company. The common solvents of chloroform, benzene, n-hexane, petroleum ether, toluene, cyclohexane and diethyl ether were purchased from Lab-Scan. The D-HCl drug, solvents as well as the reagents have been used as supplied without further purifications.

(2) Preparation of Stock and Standard Solutions of $2.0x10^{-3}$ M were prepared with doubly distilled water. Acetate buffer solutions were made of a mixture of 0.1 M acetic acid (1050 g/L) and 0.1 M sodium acetate trihydrate (13.6 g/L) On the other side we prepare Phosphate buffer solutions were made of a mixture of 0.1M disodium hydrogen phosphate (14.2g/L), 0.1M HCl and 0.1M NaOH. as seeing below.

(A) Acetate buffer solution (pH 3.0)

1.0 L of acetate buffer solution of pH was prepared by adding of 982.3 mL of 0.1 M acetic acid solution to 17.7 mL of 0.1 M sodium acetate.

(B) Acetate buffer solution (pH 4.0)

1.0 L of acetate buffer solution of pH was prepared by adding of 947.0 mL of 0.1 M acetic acid solution to 153.0 mL of 0.1 M sodium acetate.

(C) Acetate buffer solution (pH 5.0)

1.0 L of acetate buffer solution of pH was prepared by adding of 357.0 mL of 0.1 M acetic acid solution to 643.0 mL of 0.1 M sodium acetate.

(D) Acetate buffer solution (pH 6.0)

1.0 L of acetate buffer solution of pH was prepared by adding of 52.2 mL of 0.1 M acetic acid solution to 947.8 mL of 0.1 M sodium acetate.

(E) Phosphate buffer solution (pH 7.0)

1.0 L of Phosphate buffer solution of pH was prepared by adding of 756.0 mL of 0.1 M disodium hydrogen phosphate solution to 244.0 mL of 0.1 M hydrochloric acid.

(F) Phosphate buffer solution (pH 8.0)

1.0 L of Phosphate buffer solution of pH was prepared by adding of 955.1 mL of 0.1 M disodium hydrogen phosphate solution to 44.9 mL of 0.1 M hydrochloric acid.

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(G) Phosphate buffer solution (pH 9.0)

1.0 L of Phosphate buffer solution of pH was prepared by adding of 955.0 mL of 0.1 M disodium hydrogen phosphate solution to 45.0 mL of 0.1 M hydrochloric acid.

(H) Phosphate buffer solution (pH 10.0)

1.0 L of Phosphate buffer solution of pH was prepared by adding of 966.4 mL of 0.1 M disodium hydrogen phosphate solution to 33.6 mL of 0.1 M sodium hydroxide.

(I) Phosphate buffer solution (pH 11.0)

1.0 L of Phosphate buffer solution of pH was prepared by adding of 965.3 mL of 0.1 M disodium hydrogen phosphate solution to 34.7 mL of 0.1 M sodium hydroxide.

General procedure

Into 50 ml separating funnel, 5.0 mL ($2.0x10^{-3}$ M) PA, ClPR, BrTB and BrCP were added to different volumes of solution containing ($1.0x10^{-3}$ M) D-HCl. In both cases 2.0 mL of buffer solution were added and the volume was made up to 10 mL with distilled water. The formed ion-associates was extracted using a separating funnel with 10 mL chloroform by shaking for two minutes and allowed to separates into two phases. The organic layer was collected and dried with anhydrous sodium sulfate then complete to 10 ml chloroform. The absorbance of the extract was measured at the recommended wavelength (λ_{max}) as recorded in TABLE 1. The blank was prepared using the same method in absence of the examined drug.

TABLE 1 : Characteristics and analytical data of dothiepin hydrochloride (D-HCl) ion associates with picric acid, chlorophenol red, bromthymol blue and bromcresol purple.

Parameters	D-HCl /PA	D-HCl /ClPR	D-HCl /BrTB	D-HCl /BrCP
λ max (nm)	410	405	410	408
Beer's law up to (µg/mL)	46	26	16	16
Molar absorpitivity (ε) [$Lmol^{-1}$ cm^{-1}]	$8.5x10^3$	$1.32x10^4$	$2.42x10^4$	$2.42x10^4$
Sandell sensitivity [µg cm^{-2}]	$3.9x10^{-2}$	$2.5x10^{-2}$	$1.37x10^{-2}$	$1.37x10^{-2}$
Colour of ion-pair	Yellow	Yellow	Yellow	Yellow
Regression equation*				
Intercept	0.0068	0.0057	0.0029	0.0003
Slope	0.0243	0.0410	0.0668	0.0681
Correlation Coefficient	0.9998	0.9996	0.9990	0.9983
Optimum condition				
Extracting solvents	chloroform	chloroform	chloroform	Chloroform
pH range	5-6	5-6	5-6	5-6
Time on the stability	12	14	18	16
Temperature on the stability	70	65	60	70
The stoichiometry of the ion-associates	1:1	1:1	1:1	1:1

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

Application to various dosage forms

Four capsules (100 mg) D-HCl drug were weighed into a small dish, powdered and mixed well, then dissolved in 100 mL distilled water, a turbid solution was shaken well and filtered through a filter paper to obtain a clear solution. Then, the clear solution was diluted with distilled water in a 250 mL calibrated measuring flask. The drug content of this solution was obtained by applying the general procedure to aliquot containing different volumes of solution drugs as described above.

Stoichiometric relationship

Job's method of continuous variation method[12] was employed, $1.0x10^{-3}$ M solution of D-HCl drugs was mixed with $1.0x10^{-3}$ M solution of each selected reagent. A series of solutions were prepared in which the total volume of drug and reagent was kept constant (5.0 mL). The reagents were mixed with each drug in various proportions along with the chosen buffer solution, which then diluted in 10.0 mL calibrated flask with the appropriate solvent following the above mentioned procedures.

RESULTS

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

Optimization

We aimed to determine the most favorable conditions to achieve maximum color intensity of D-HCl drug. Therefore, we have investigated the effects of pH, solvent and its polarity, sequence of mixing, time and temperatures to achieve the optimum conditions to aid in accurate quantitative analysis for these drugs. The optimum wavelength of maximum intensity ($_{max}$) of D-HCl (TABLE 1) and their ion associates with PA, ClPR, BrTB and BrCP reagents are recorded at the choozen optimum conditions. The absorption band of D-HCl with PA, ClPR, BrTB and BrCP ion-associates are located at 410, 405, 410 and 408 nm, respectively. It worth mentioning that, the maximum absorbencies (λ_{max}) were recorded and tested against reagent blank (prepared in the same manner without the addition of drug) to study the influence of each of the following variables on the formed ion associates between drugs and reagents.

Effect of the extracting solvent

The solvent polarity affects both the extracting efficiency and the molar absorptivity (ε) of the formed ion associates. Therefore, we have used various water immiscible organic solvents like chloroform, benzene, n-hexane, petroleum ether, toluene, cyclohexane and diethyl ether to investigate the solvent effect on the extraction of drug against the reagents. The most convenient solvent is chloroform; it provides maximum color intensity (absorbance ~ 0.7) as well as powerful extraction of ion associates for both D-HCl drug. Moreover, toluene and benzene could be also useful, however their maximum absorbance ~ 57% and 50% of chloroform for D-HCl. Other solvents n-hexane, cyclohexane, petroleum ether and diethyl ether are not recommended for D-HCl (Figure 3) drug.

Effect of pH

As stated earlier different stock of acetate buffer solutions were prepared with pH's of 3, 4, 5 and 6 to account for the effect of pH on the formation of ion associates. Initially 5.0 mL of $2x10^{-3}$ M of reagent was mixed with 1.0 mL ($5x10^{-4}$ M) of the drug solution, then 2.0 mL of Acetate buffer was added to adjust the pH followed by dilution with distilled water in 10.0 mL calibrated measuring flask. The adjusted optimum pH was found to be 5-6 for D-HCl.

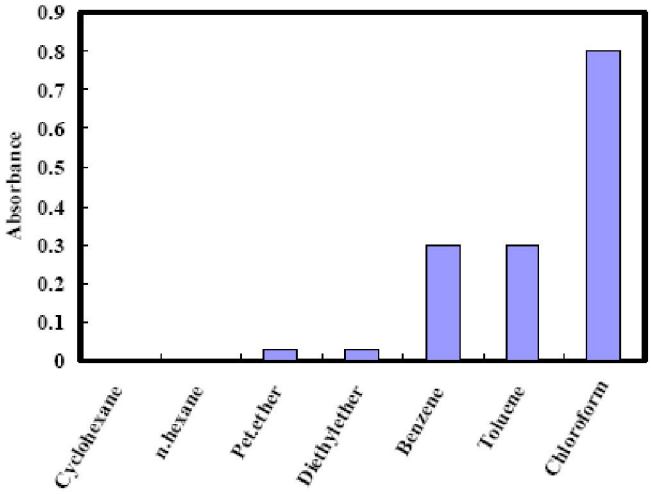

Figure 3 : Effect of extracting solvents on: Dothiepin hydrochloride ion-associates with picric acid, chlorophenol red, bromthymol blue and bromcresol purple.

Effect of temperature and time

The effect of temperature and time on ion associates formation and stability was studied by measuring the absorbance of the extracted ion associates at different temperatures ranged form 25 to 90 °C and at increasing time intervals, respectively. The results show that the ion associates were formed almost instantaneously at room temperature (25 ± 2 °C) and remain stable up to 70 °C with all reagents. In addition, the developed color remains stable for 24 hrs. with reagents after one day a slight decrease in the color intensity of the ion associates was observed.

Effect of mixing sequence

The optimum sequence of mixing was found to be drug, reagent, buffer, and then solvent, which allow the highest color intensity and shortest time to obtain maximum absorbance. On the other hand, other sequences rather the one given above requires more time longer time in addition to lower stability of the ion associates.

The stoichiometry of the ion-associates

The stoichiometric ratio of the D-HCl ion-associ-

ates formed between drug of interest and the selected reagents has been determined by implementing the molar ratio method[11] and continuous variation method[12]. The result indicates the existence of 1:1 at a definite λ_{max} recorded in (TABLE 1).

Specificity

No interference was observed during the quantitative determination of D-HCl drug with all reagents in presence of different additives such as lactose, glycerol, propylene glycol, sugar and starch which are present in its pharmaceutical preparations.

LINEARITY

Beer's law is obeyed in the concentration range 1-46 of Dothiepin hydrochloride (D-HCl; Figure 6) with PA, ClPR, BrTB and BrCP reagents.

The optical characteristics; Beer's law limits, molar absorptivities, Sandell's sensitivities[13] are summarized in TABLE 1 along with the results of regression analysis using the method of least square was made for the slope (b), intercept (a) and correlation coefficient (r) obtained from different concentrations.

METHOD VALIDATION

Results obtained were compared with those of the official methods along with the statistical outcomes. The

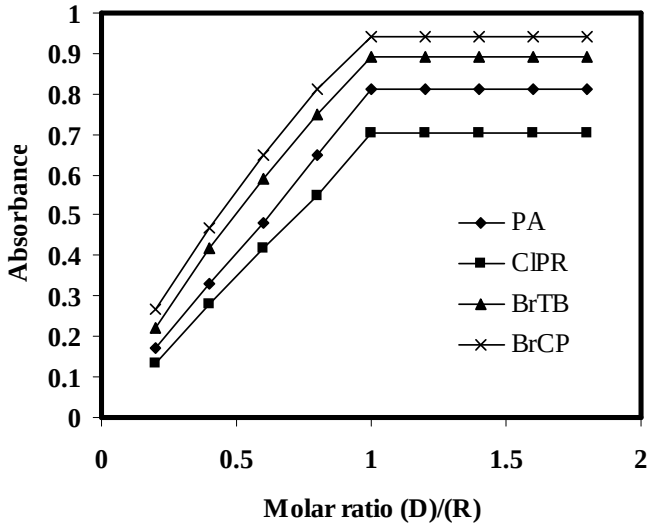

Figure 4 : Molar ratio of: Dothiepin hydrochloride ion-associates with picric acid, chlorophenol red, bromthymol blue and bromcresol purple. [D; for drugs and R; for reagent]

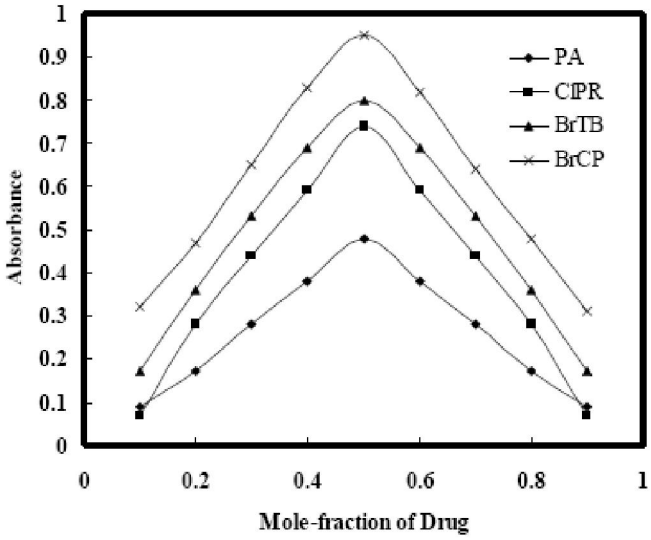

Figure 5 : Continuous variation of: Dothiepin hydrochloride ion-associates with picric acid, chlorophenol red, bromthymol blue and bromcresol purple.

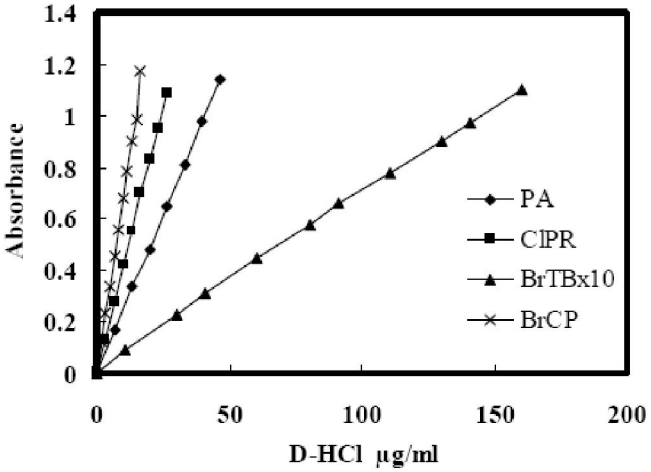

Figure 6 : Standard curves of: Dothiepin hydrochloride ion-associates with picric acid, chlorophenol red, bromthymol blue and bromcresol purple.

comparison ensures that there is no significant difference between the current study and the official methods as shown in (TABLE 2). Six replicate determination at different concentration levels were carried out to test the precision and accuracy of the method. The recoveries were ranged from (99.80 to 100.14 % which reflect the high accuracy of the results, with reliable precision as indicated by very low values of standard deviation (TABLE 3). The performance of the proposed method was assessed by calculation of t and f tests compared with the Pharamacopial method[14,15]. Mean values were obtained with t and f testes at 95% confidence level for five degrees (n-1) = (6-1; i.e., six repli-

cate minus 1) of freedom were in the accepted values.

TABLE 2 : Concentration of dothiepin hydrochloride (D-HCl) in µg as determined from spectrophotometric methods.

Reagent	Pure solution			Prothiaden capsules			Reagent	Pure solution			Prothiaden capsules		
	Taken	Found	Recovery %	Taken	Found	Recovery %		Taken	Found	Recovery %	Taken	Found	Recovery %
PA	5.00	4.95	99.00	5.00	4.98	99.60	BrTB	2.00	2.01	100.50	2.00	1.98	99.00
	10.00	10.00	100.00	10.00	10.01	100.10		4.00	4.00	100.00	4.00	4.05	101.25
	15.00	15.02	100.13	15.00	14.99	99.93		6.00	6.03	100.50	6.00	5.98	99.66
	20.00	20.01	100.05	20.00	20.05	100.25		8.00	7.95	99.37	8.00	7.97	99.62
	25.00	24.98	99.92	25.00	25.03	100.12		10.00	9.98	99.80	10.00	10.05	100.05
	Mean recovery ± RSD* 99.82±0.415			Mean recovery ± RSD* 100±0.224				Mean recovery ± RSD* 100.034±0.431			Mean recovery ± RSD* 100.06±0.78		
ClPR	5.00	5.01	100.20	5.00	5.00	100.00	BrCP	2.00	1.99	99.50	2.00	2.02	101.00
	10.00	10.03	100.30	10.00	9.98	99.80		4.00	4.00	100.00	4.00	4.01	100.25
	15.00	14.97	99.80	15.00	15.02	100.13		6.00	6.01	100.16	6.00	5.98	99.66
	20.00	19.94	99.70	20.00	20.04	100.20		8.00	7.98	99.75	8.00	8.01	100.12
	25.00	25.01	100.04	25.00	24.89	99.56		10.00	10.03	100.30	10.00	9.97	99.70
	Mean recovery ± RSD* 100.008±0.228			Mean recovery ± RSD* 99.93±0.23				Mean recovery ± RSD* 99.94±0.286			Mean recovery ± RSD* 100.14±0.484		

TABLE 3 : Statistical treatment of data obtained for dothiepin hydrochloride applying the proposed methods in comparison with the pharmacopoeial method.

Parameters	Pharmacopoeia Method	D-HCl/PA	D-HCl/ClPR	D-HCl/ BrTB	D-HCl/ BrCP
Pure Solution					
X ± SD	99.80±0.219	99.82±0.415	100.01±0.228	100.03±0.431	99.94±0.286
n*	3	6	6	6	6
t value**		0.1178	2.234	0.65	1.212
F value		3.591	1.0809	1.65	1.710
Tablets					
X ± SD	99.92±0.223	100 ±0.224	99.93±0.232	100.006±0.783	100.14±0.484
n*	3	6	6	6	6
t value**		0.8295	0.1472	0.2562	1.1264
F value		1.0107	1.0888	12.3316	4.7197

[a]n is the number of replicates; [b]Theoretical value at 95% confidence level

CONCLUSIONS

The proposed method made use of a simple reagent, which most ordinary analytical laboratories can afford. The method is sufficiently sensitive to permit determinations as low as 1.0 µg/mL for Dothiepin hydrochloride (D-HCl) drug at the given optimum conditions. Unlike GC and HPLC procedures, the spectrophotometer is relatively simple to handle and affordable. The proposed method is simple, precise, accurate and convenient. Hence, the proposed methods should be useful for routine quality control purposes.

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