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Development and Validation of IndirectVisible Spectrophotometric Method for Doxepin and Dothiepinin Pureandthe Tablet Dosage Forms

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ABSTRACT

The aim of the present work is to develop a simple, sensitive, accurate and rapid visible spectrophotometric method for the quantitative estimation of doxepin and dothiepin in both pure and tablet dosage forms. The developed method is based on the oxidation of DOX and DOT by Ce(IV)inan acid medium followed by reacting it with leuco crystal violetto form a violetcoloreddye which showed absorption maxima (λ_{max}) at 590 nm. Beer's law was obeyed in the concentration range of 0-60µg mL^{-1}in both the drugs. The recovery ranged from 99.26-100.02% for DOX and from 99.64 – 100.36% for DOT. The regression coefficient was found to be 0.998 and 0.996for DOX and DOT,respectively.The limits of detection (LOD) and quantification (LOQ) values are calculated according to ICH guidelines. The method developed was successfully applied for the determination of DOX and DOT in dosage forms.

Keywords: Indirect visible spectrophotometry, doxepin, dothiepinand tablet dosage forms..

INTRODUCTION

Chemically, doxepin hydrochloride is (E)-3-(dibenzo [b,e]oxepin-11(6H)-ylidene)-N,N-dimethylpropan-1-amine hydrochloride, whichis used as a psychotropic agent with tricyclic antidepressant and anxiolytic properties (Fig. 1.a). Doxepin is also used for the treatment of sleep maintenance, and the tradename of doxepin for this indication is Silenor. It displays a potent central anticholinergic activity and can inhibit both norepinephrine and serotonin (5-HT) reuptake in synapses in the brain.[1]Dothiepin hydrochloride is chemically known as (E)-3-(dibenzo[b,e]oxepin-11(6H)-ylidene)-N,N-dimethylpropan-1-amine hydrochloride and is a tricyclic antidepressant (TCA). Dothiepin, a thio analogue of amitriptyline, has been used extensively in Europe during the past 15 years. It is a safe and effective agent for the treatment of major depressive disorder. Although the onset of action is comparable to that of other tricyclic antidepressants, dothiepin may cause fewer intolerable side effects and have less cardiotoxicity than these other compounds(Fig. 1.b).[2]

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Figure 1.a) Doxepin Hydrochloride b)Dothiepin Hydrochloride

In view of their pharmaceutical importance, considerable work has been done for the detection and quantification of DOX and DOT. Different analytical methods appearin the literature for the assay of DOX and DOT in biological fluids and in pharmaceutical formulations. Some of these methods include spectrophotometry[3-10],spectrofluorimetry[9-11], HPLC[12], and gas chromatography (GC)[13]which have been reported for their determination in various samples. The reported HPLC and GC techniques require expensive experimental set up and are not affordable in every laboratory for routine analysis. Existing spectrophotometric methods are less sensitive, less accurateand costly reagents and also need extraction stepswhich use toxic organic reagents. In order to overcome these demerits, there is a need to develop a sensitive, accurate and cost effective spectrophotometric method for their determination.

The present paper reports a simple, fairly sensitive, accurate and validated spectrophotometric procedure for the assay of DOX and DOT in pure form and in tablets.

Materials and Methods

Apparatus

All absorbance measurements were performed using a Systronics Model 166 digital spectrophotometer provided with 1-cm matched quartz cells. An Elico 120 digital pH meter was used for pHmeasurements.

Material and Reagents

Analytical reagent grade chemicals and reagents were used, and double distilled water was used throughout the experiment.

i. Standard DOX and DOT solutions.The pure grade DOX and DOT, certified to be 99.99% pure werereceived from Raja LaxmiFine Chemicals India Ltd.,Bangalore,India,as a gift sample and were used as received. A stock standard solution equivalent to 100 µg $mL^{-1}$of DOX and DOT were prepared by dissolving 10 mg of the pure drug in separate 100 mL calibrated flasks using distilled water.

ii. Standard Ce(IV) solution.Standard Ce(IV) solution (1000 µg mL^{-1}) was prepared by dissolving 0.3916g ammonium ceric nitrate (BDH,Anal R) in 100mL water containing 0.5mL of conc.HNO_3.A working standard solution was prepared to yielda suitable dilution of a standard solution as and when required.

iii. Leuco crystal violet.Leuco crystal violet (LCV,0.2%) wasprepared by dissolving 250mg of LCV (Sigma-Aldrich,Steinhein,Germany)in 200mL of water containing 3mL of 85% phosphoric acid to a 1000mL volumetric flask and shaken gently until the dye dissolved. The contents of the flask were then diluted to the mark with distilled water.

iv. Sulfuric acid.0.05M and 0.5M concentrations were used.

v. Acetate buffer. Acetate buffer (pH -4.0) was prepared by dissolving 13.6g of sodium acetate tri-hydrate in 80mLof water. The solution pH was adjusted to 4.0 with acetic acid, and the mixture was diluted to 100mL with water.

Preparation of Calibration Graph

Aliquots of standard DOX and DOT solutions (0.0, 0.5, 1.0,…,6.0 mL) were transferred separately into a series of 10 mL standard flasks. To this, 0.6 mL of Ce(IV) (50 µg mL^{-1}) and 0.5 mL each of 0.5 M H_2SO_4 and 0.025% LCV were added. The reaction mixture was kept in a water bath (40 ºC) for 5 minand cooled to room temperature before the contents were diluted to the mark with acetate buffer of pH - 4.0 and mixed well. The

absorbance of the formed dye was measured at 590 nm against distilled water. For each drug, a blank was prepared similarly omitting the drug and its absorbance was measured against distilled water. The decrease in absorbance corresponding to the consumed cerium(IV) and in turn, to the drug concentration, was obtained by subtracting the absorbance of the blank solution from that of the test solution. The calibration graph was drawn by plotting the difference in absorbance (test and blank solution) of the formed dye against the concentration of the DOX and DOT. The amounts of the DOX and DOT were determined from the concurrent calibration curve or regression equation.

Procedure for Tablets

In order to determine the contents of DOX and DOT in commercial dosage forms, two brands of tablets containing SPECTRA Ranbaxy (Solus) 25 mgand DOTHIP (Micro Synapse) 25 mg, which were used in the investigation, were purchased from local commercial sources and the contents of ten tablets were weighed accurately and ground into a fine powder. Anamount of the powder equivalent to 25 mg of DOX and 25 mg of DOT was weighed separately and transferred into 100mL calibrated flasks and 50mLof water was added. The content was shaken for about 30 min; the volume was diluted to the mark with water and mixed well and filtered using Whattman no.42 filter paper. The filtrate containing DOX and DOTseparately at a concentration of 100µg $mL^{-1}$was subjected to analysis by the procedure described above.

Results and Discussion

In this methodCe(IV) oxidized LCV to violet colored crystal in a sulfuric acid medium (pH -1.0-2.3) uponheating (at 40ºC) in a water bath for 5 min. The violet color of the formed dye was developed in an acetate buffer medium (pH -3.7-4.3) showing a maximum absorption at 590 nm. The reaction pathway for the proposed method is shown in Scheme1. Based on the above observations, a simple spectrophotometric method forthe determination of DOX and DOT was developed and validated as per the current ICH guidelines.

Optimization of Experimental Parameters

The various experimental parameters affecting the formation of the reaction product were optimized.

Effect of Time

The influence of the reaction time on the absorbance of the product was studied on 10µg $mL^{-1}$of DOX and DOTseparatelywith LCV as mentioned under the general procedure. The optimum reaction time was found to be 5min for this method.

Effect of Acidity and Temperature

The oxidation of LCVby Ce(IV) was studied. Of the various acids (sulfuric, hydrochloric and phosphoric) studied, sulfuric acid was found to be the best acid for the system. Constant absorbance readings were obtained in the 0.1-1.5 mL range of 0.5M sulfuric acid [(or) pH -1.0-2.3] at temperature 40ºC for 5min for this method. An increase inthe pH above 4.3markedly affected the stability and sensitivity of the dye. Color development did not take place belowpH-1.0 for this method. Hence, a volume of 0.5mL of 0.5M sulfuric acid in a total volume of 10mLwas used in all subsequent work.

Effect of Reagent Concentration and Buffer Media

The optimum concentration of LCV leading to maximum color stability was found to be 0.5mL of LCV reagent per 10mL of the reaction mixture. The absorbance values were measured in the pH range of 3.7-4.3 for this method. This could be achieved by adding acetate buffer of pH-4.0 for thismethod. The formed colored dye was stable for more than a week.

Insert Scheme 1 here

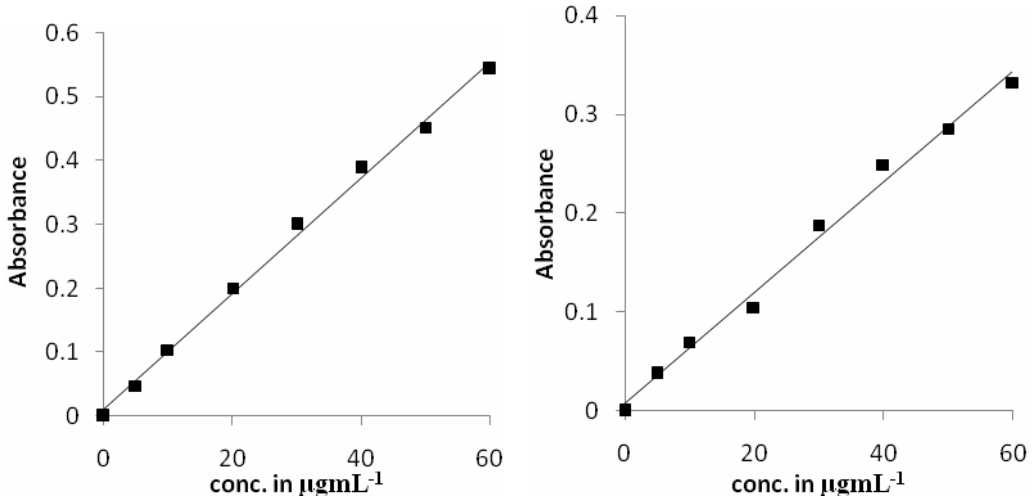
Method Validation

Theproposed method has been validated forlinearity, sensitivity, precision, accuracy, selectivity and recovery.

Linearity and Sensitivity

The analytical parameters for the spectrophotometric determination of DOX and DOT by the proposed method are given in Table 1. Under optimum conditions, a linear relation was obtained between absorbance and concentration of DOX and DOT in the range 0-60µg $mL^{-1}$for both DOX and DOT (Fig.2.a, 2.b). The calibration graph is described by the equation:$Y = a + bx$, where y = absorbance, a = intercept, b = slope and x = concentration, obtained by the

method of least squares. The correlation coefficient, intercept and slope for the calibration data and sensitivity parameters such as apparent molar absorptivity and Sandell'ssensitivity values, the limits of detection and quantification calculated as per the current ICH guidelines[14] are summarized in Table 1. The limits of detection (LOD) and quantification (LOQ) were calculated according to the same guidelines using the formulas:LOD=3.3σ/s and LOQ=10σ/s,where σ is the standard deviation of reagent blank determinations and s is the slope of the concentration curve.



(a) **(b)**

Figure 2. Calibration curves for a) DOX and b) DOT

Table 1.Analytical and regression parameters of the proposed method.

Parameter	**DOX**	**DOT**
λ_{max},nm	590	590
Beer's law range (μgmL^{-1})	0 – 60	0 – 60
Molar absorptivity(ε) ($Lmol^{-1}cm^{-1}$)	3.05×10^3	2.06×10^3
Sandell's sensitivity ($\mu g\ cm^{-2}$)	0.1036	0.1610
Intercept (a)	0.0109	0.0078
Slope (b)	0.0091	0.0056
Regression coefficient (r)	0.9980	0.9958
S_a	0.01897	0.01726
S_b	0.0003	0.0003
LOQ (μgmL^{-1})	1.4444	1.6990
LOD (μgmL^{-1})	0.3468	0.5607

*$y=a+bx$, where c is the concentration of DOX and DOT in $\mu g\ mL^{-1}$,y is the absorbance at therespective λ_{max}., S_a is the standard deviation of the intercept, and S_b is the standard deviation of the slope.

Accuracy and Precision

The within a day studies of the precision and accuracy was evaluated by performing replicate analysis of drug samples at three different concentrations (low, medium and high) (Table 2) within the working limits, each being repeated five times. The R.E. (%) and R.S.D. (%) values were found for the developed method and was found to be satisfactory. The percentage recovery values ranged between 97.5 - 100.1% with a relative standard deviation of less than 3%. The analytical results obtained from this investigation are summarized in Table 2.

Table 2.Evaluation of accuracy and precision.

Method	DRUG taken μgmL^{-1}	DRUG found* μgmL^{-1}	RE %	SD μgmL^{-1}	SEM μgmL^{-1}	RSD %	ROE** %
DOX	10	9.736	2.64	0.103	0.039	1.053	±1.05
	30	29.965	0.12	0.118	0.045	0.395	±0.395
	50	49.766	0.47	0.429	0.162	0.861	±0.861
DOT	10	9.966	0.34	0.086	0.032	0.8623	±0.862
	30	29.434	1.89	0.249	0.094	0.8462	±0.845
	50	49.657	0.69	0.256	0.097	0.5148	±0.514

RE:relative error; SD:Standard deviation; SEM:Standard error of mean; RSD:Relative standard deviation; ROE:Range of error.

* Mean value of five determinations

**At the 95% confidence level for 6 degrees of freedom.

Application to Analysis of Commercial Samples

The validity of the proposed method was ascertained by the statistical comparison of the results obtained by a reference method [4]with the proposed method by applying Student's t-test for accuracy and F- test for precision in some commercial formulations. The results of an assay of DOX and DOTwerestatistically compared with the reference method [4]at the 95% confidence level and showed that there is no significant difference between the proposed and reference methods and the label claim(Table 3).

Table 3.Results of determination of DOX and DOT in tablets and statistical comparison with the reference method.

Tablet brand name	Nominal amount mg per tablet	Found*(% of nominal amount ± SD)		
		Reference method[5, 8]	DOX	DOT
SPECTRA Ranbaxy (Solus)	25 mg	100.4±0.85	99.59±0.59 t=0.88, F=2.07	-
DOTHIP (Micro Synapse)	25 mg	100.76±1.20	-	99.83±1.12 t=0.64, F=1.16

*Mean value of five determinations

Tabulated t and F-values at 95% confidence level are 2.77 and 6.39, respectively

Recovery Study

To test the applicability of the proposed method, recovery experiments were carried out by the standard addition method. In this study, pre-analyzed tablet powder was spiked with the pure drug at three different concentrations and the total was found by the proposed methods. Each determination was repeated three times. The recovery of the pure drug added was quantitative and revealed that co-formulated substances did not interfere in the determination. The results of recovery study are compiled in Table 4.

Table 4.Results f recovery experiments via the standard addition technique.

Tablet brand name	drug mg per tablet	Pure tablet added, µg mL^{-1}	Total found, µg mL^{-1}	Pure drug recovered*, %±SD
SPECTRARanbaxy (Solus), 25 mg	25	10	19.95	99.51±0.54
		20	29.85	99.26±0.89
		30	40.01	100.02±0.34
DOTHIP (Micro Synapse), 25 mg	25	10	20.04	100.35±0.25
		20	29.98	99.91±0.25
		30	39.89	99.64±0.17

** Mean value of three measurements*

Conclusions

The proposed method isa simple, rapid, accurate, precise and fairly sensitive spectrophotometric method that was developed for the determination of DOX and DOTin bulk drug and in tablets. Themethodrelieson the use of cheap chemicals and isbased on color reactions and highly sensitive when compared to highly expensive techniques such as HPLC/GC.From the calculated t- and F-values at the 95% confidence level, it is clear that the results obtained by the proposed method are in good agreement with those obtained by the reference method. The small values of R.E and R.S.D. indicate the reliability, accuracy and precision of the suggested procedure. The results obtained in Table 4are considered to be of high accuracy and can be successfully applied for the routine assay of DOX and DOT in pharmaceutical formulations.

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التطوير والتحقق من صحة الطريقة الطيفية المرئية الغير مباشرة للأشكال الدوائية للمستحضرات الدوائية Dothiepinin و Doxepin

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ملخـص

هدفت هذه الدراسة إلى تطوير طريقة طيفية مرئية وسريعة ودقيقة وحساسة وبسيطة للتقدير الكمي لـ Doxepin وDothiepining في كلا الشكلين الدوائيين (النقية، والأقراص الدوائية). تستند هذه الطريقة على اكسدة Doxepin وDothiepining في وسط حمضي متبوع بتفاعل مع الكريستال بنفسجي اللون الذي يظهر امتصاص الحد، وأقصاه 5900 نانومتر. تم اتباع قانون Beer في نطاق تركيز 0-65 مل في جميع المستحضرات الدوائية، وتتراوح نسبة الشفاء ما بين 99.26-100.02% بالنسبة لـ Dox و 99.64-100.36% لـ Dot. وكان معامل الانحدار 0.998 و0.996 بالنسبة لـ Dox و Dot على التوالي. وتم حساب حدود الكشف والمعايير الكمية وفقاً لمبادئ ICH التوجيهية ثم تطبيق الطريقة المطورة بنجاح لتحديد Dox و Dot في أشكال دوائية.

الكلمـات الدالـة: طيفية مرئية، دوكسيبين ودوثيبينين، أشكال أقراص الدواء.

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