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Simple and Inexpensive Methods Development for Determination of Venlafaxine Hydrochloride from Its Solid Dosage Forms by Visible Spectrophotometry

K. RAGHUBABU[*1], L. SHANTI SWARUP[1], B. KALYANARAMU[2] M. N. RAO[3], AND C. RAMDAS[3]

[1]Department of Engineering Chemistry, AU College of Engineering (A), Andhra University, Visakhapatnam-530003, AP (India)
[2]Department of Chemistry, M. R. College for Men (A), Vizianagaram-535002, AP (India).
[3]M/s Tyche Industries, Hyderabad, AP (India).

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Abstract: Two simple, sensitive and cost effective visible spectrophotometric methods (M1 and M2) have been developed for the determination of venlafaxine hydrochloride from bulk and tablet dosage forms. The method M1 is based on the formation of green colored coordination complex by the drug with cobalt thiocyanate which is quantitatively extractable into nitro benzene with an absorption maximum of 626.4 nm. The method M2 involves internal salt formation of aconitic anhydride, dehydration product of citric acid [CIA] with acetic anhydride [Ac2O] to form colored chromogen with an absorption maximum of 561.2 nm. The calibration graph is linear over the concentration range of 10-50 µg/mL and 8-24 µg/mL for method M1 and M2 respectively. The proposed methods are applied to commercial available tablets and the results are statistically compared with those obtained by the reference method and validated by recovery studies. The results are found satisfactory and reproducible. These methods are applied successfully for the estimation of the venlafaxine hydrochloride in the presence of other ingredients that are usually present in dosage forms.

Keywords: Anti-depressant, Acetic anhydride, Citric acid, CTC, Colorimetric, Nitrobenzene, Regression analysis.

Introduction

Venlafaxine hydrochloride (VX) is a bicyclic third generation antidepressant chiral compound of phenethyl amine type with a novel chemical structure [1, 2] (Fig.1).

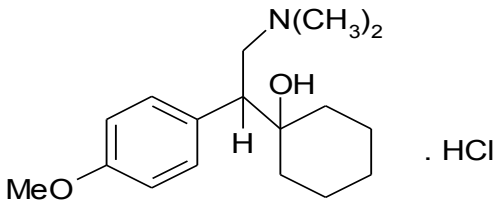

Figure 1. Chemical structure of Venlafaxine hydrochloride.

It is chemically designated as (R/S)-1-[2-(dimethyl amino)-1-(4-methoxy phenyl)ethyl] cyclohexanol hydrochloride salt and is usually categorized as a serotonin-nor epinephrine reuptake inhibitor (SNRI) but it has been referred to as a serotonin-norepinephrine–dopamine reuptake inhibitor. It weakly inhibits the reuptake of dopamine. VX is well absorbed, with peak plasma concentrations occurring approximately 2 hours after dosing. It is extensively metabolized, to O-desmethyl venlafaxine, the only major active metabolite. It has the empirical formula of $C_{17}H_{27}NO_2$.HCl. Its molecular weight is 313.87. The drug is official in BP [3] and suggests potentiometric method for the determination of VX in bulk and tablet formulations.

Various methods have been reported for the estimation of venlafaxine hydrochloride in biological matrices such as plasma, which include the use of liquid chromatography (LC) with UV detection[4], LC with electro spray ionization mass spectrometry[5], LC with coulometric detection[6], LC with fluorimetric detection[7,8], LC with diode array detection[9,10], gas chromatography-mass spectrometry (GC–MS) [11], LC-MS[12], LC–MS–MS[13,14], and for the estimation in serum by using LC[15], HPLC methods[16-22], HPLC-MS/ESI [23], voltammetry[24],Capillary Electrophoresis[25,26], Flow injection analysis[27]. Stability indicating methods have also been reported for its in vitro determination in gastric and intestinal fluids [28] and pharmaceutical formulations [29], few UV [30-33] and Visible Spectrophotometry [34-38]. However analytical important functional groups in VX have not been exploited properly in developing visible spectrophotometric methods and most of the previous methods involve sophisticated equipments which are costly and pose problems of maintenance.

For routine analysis, simple, rapid and cost effective visible spectrophotometric methods are required and preferred. The main purpose of the present study was to establish relatively simple, sensitive, validated and inexpensive visible spectrophotometric methods for the determination of VX in pure form and in pharmaceutical dosage forms. So the authors have made some attempts in this direction and succeeded in developing two methods based on the reaction between the drug and cobalt thiocyanate [39] (M_1) or drug and citric acid-acetic anhydride reagent [40] (M_2). These methods can be extended for the routine assay of VX formulations.

Experimental

A Systronics UV/Visible spectrophotometer model -2203 with10mm matched quartz cells was used for all spectral measurements. A Systronics µ- pH meter model-362 was used for pH measurements. All the chemicals used were of analytical grade.

Reagents and Chemicals

CTC ($2.50x10^{-1}$M, solution prepared by dissolving 7.25 g of cobalt nitrate and 3.8 g of ammonium thiocyanate in 100mL distilled water), Citrate buffer pH(2.0) (prepared by mixing 306ml of 0.1M trisodium citrate with 694mL of 0.1M HCl and pH was adjusted to 2.0) were prepared for method M_1.

Citric acid monohydrate (Prepared by dissolving 1.2 grams of (1.2%, $6.245X10^{-2}$M) Citric acid in 5 mL methanol initially followed by dilution up to 100mL with acetic anhydride) and Acetic anhydride **(**SD Fine chemicals) were used for Method M_2.

Preparation of Standard and Sample Drug Stock Solution

An accurately weighed quantity of VX (pure or tablet powder) equivalent to 100mg was mixed with 5mL of 10% Na_2CO_3 solution and transferred into 125ml separating funnel. The freebase released was extracted with 3x15mL portion of chloroform and the combined chloroform layer was brought up to 100mL with the same solvent to get 1mg/mL VX drug stock solution in free base form. This free base stock solution was further diluted step wise with the same solvent to get the working standard solution concentrations [M_1-500 µg/mL, M_2-200 µg/mL].

Procedure /Assay

Method M_1

Aliquots of standard VX solution (0.5mL-2.5mL, 500µg/mL in free base form) were delivered into a series of 125mL separating funnels. Then 2.0mL of buffer solution (pH 2.0) and 5.0mL CTC solution were added. The total volume of aqueous phase in each separating funnel was adjusted to 15.0mL with distilled water. To each separating funnel 10.0mL of nitrobenzene was added and contents were shaken for 2 minutes. The two phases were allowed to separate and absorbance of nitrobenzene layer was measured at 626.4nm against a similar reagent blank (Fig-2 showing absorption spectra).The colored product was stable for 1 hour. The amount of VX in the sample solution was computed from its calibration graph (Fig-3 showing Beer's law plot).

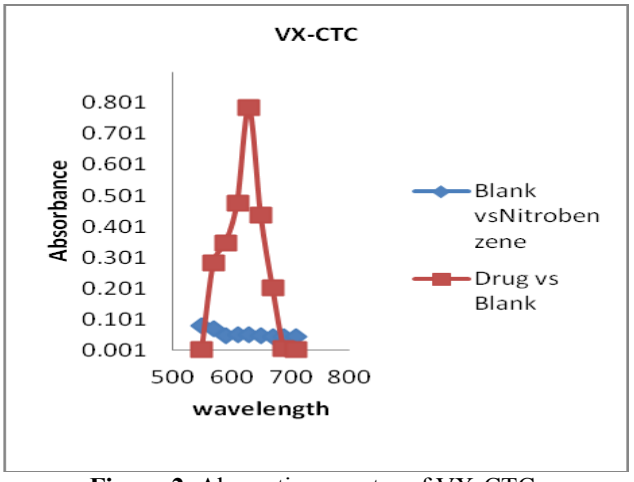

Figure 2. Absorption spectra of VX-CTC.

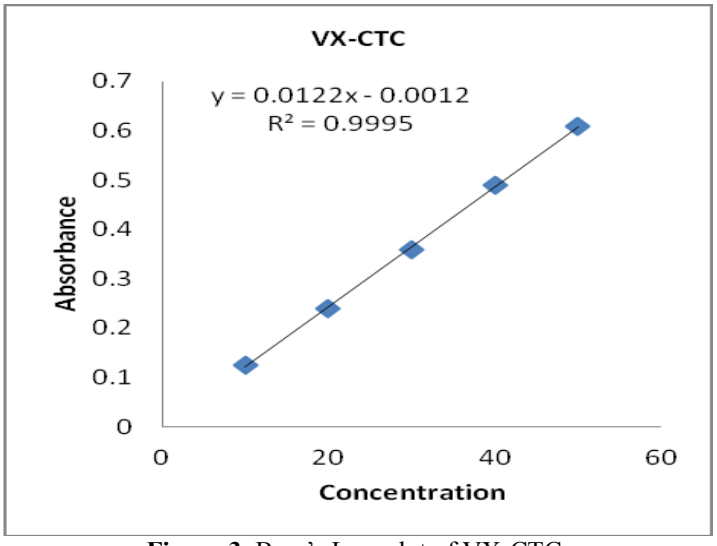

Figure 3. Beer's Law plot of VX-CTC.

Method M_2

Aliquots of standard VX drug solution [1.0-3.0mL;200µg/mL in free base form] in chloroform were taken into a series of 25mL graduated tubes and gently evaporated in a boiling water bath to dryness. To this, 10mL of citric acid- Acetic anhydride reagent was added and the tubes were immersed in a boiling water bath for 30 minutes then the tubes were cooled to room temperature and made up to the mark with acetic anhydride. The absorbance of the colored solutions was measured after 15minutes at 561.2 nm against the reagent blank (Fig-4 showing absorption spectra) within the stability period of 15-60min.The amount of VX was computed from its calibration graph (Fig-5 showing Beer's law plot).

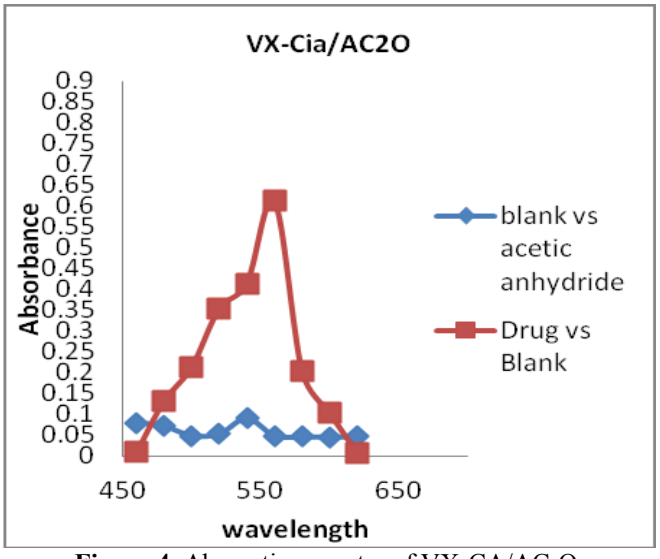

Figure 4. Absorption spectra of VX-CA/AC_2O.

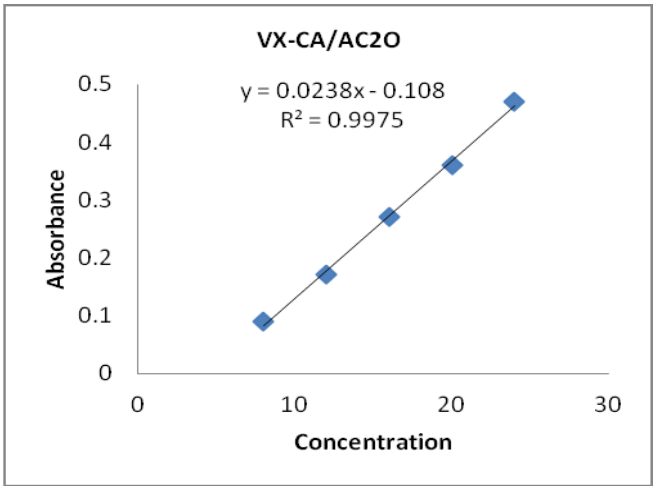

Figure 5. Beer's Law plot of VX-CA/AC_2O.

Results and Discussion

In developing these methods, systematic studies of the effects of various parameters were undertaken by varying one parameter at a time and controlling all others fixed (OVAT method). The effect of various parameters such as time, volume and strength of reagents, pH buffer solution and order of addition of reagents, stability period and solvent for final dilution of the colored species were studied and the optimum conditions were established. Among the various water immiscible organic solvents (C_6H_6, $CHCl_3$, dichloro methane, nitro benzene, chloro benzene and CCl_4) tested for the extraction of colored coordinate complex into organic layer, nitrobenzene was preferred for selective extraction of colored complex from organic phase in method M_1. Different solvents like acetic anhydride, acetic acid, methanol, ethanol, and isopropanol were also used as diluents but acetic anhydride was found to be ideal for final dilution in method M_2. The ratio of organic to aqueous phase was found to be 1:1.5 by slope ratio method for method M_1. The optical characteristics such as Beer's law limit, Sandell's sensitivity, molar absorptivity, percent relative standard deviation, (calculated from the six measurements containing $3/4^{th}$ of the amount of the upper Beer's law limits) were calculated and the results are summarized in Table-1.

Recovery experiments indicated the absence of interference from the commonly encountered pharmaceutical excipients present in formulations. The proposed methods are found to be simple, sensitive and accurate and can be used for the routine quality control analysis of VX in bulk and dosage forms.

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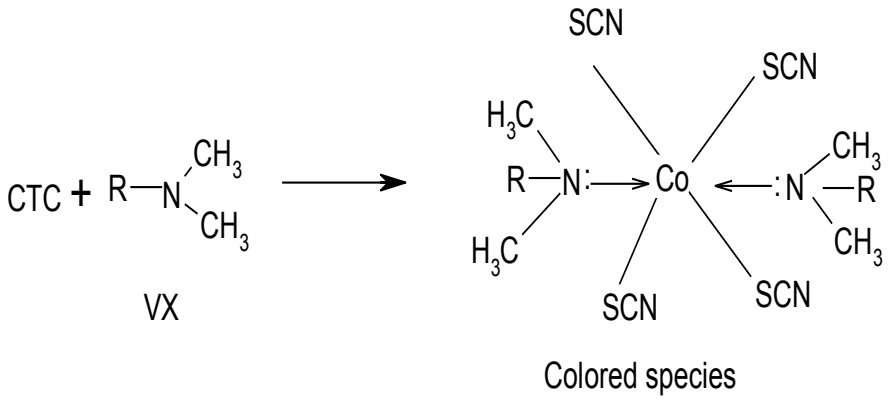
Chemistry of Colored Species

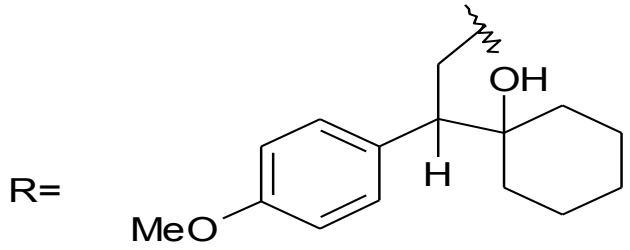
In method M_1 the green color species formed is the coordination complex of the drug (electron donor) and the central metal of cobalt thiocyanate, which is extractable into nitro benzene from aqueous solution and in method M_2 red-violet color internal salt of aconitic anhydride is formed when VX was treated with CTC or CIA/Ac_2O reagents. The formations

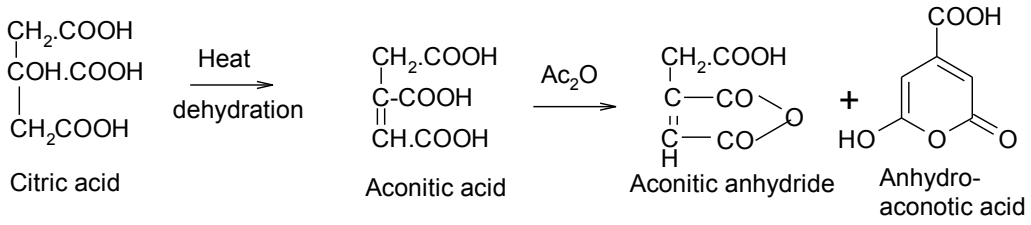
of colored species are due to the presence of the tertiary amino group in it. It is based on the analogy of tertiary amine as given in scheme (Fig-6).

Table 1. Optical characteristics, precision and accuracy of proposed methods.

Parameter	**Method M_1**	**Method M_2**
λ_{max}(nm)	626.4	561.2
Beer's law limit(µg/mL)	10-50	8-24
Sandell's sensitivity (µg/cm^2/0.001 abs. unit)	0.003342618	0.00237037
Molar absorptivity (Liter/mole/cm)	93899.44167	132413.9063
Correlation coefficient	0.999	0.997
Regression equation (Y)*		
Intercept (a)	-0.001	-0.108
Slope(b)	0.012	0.023
%RSD	0.9948	1.3752
% Range of errors(95% Confidence limits) 0.05 significance level 0.01 significance level	1.044 1.637	1.443 2.26
*Y = a + b x, where Y is the absorbance and x is the concentration of VX in µg/mL.		







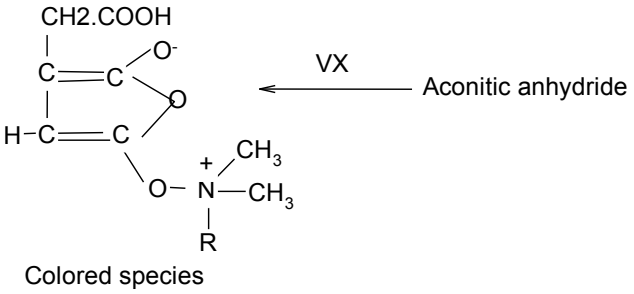


Figure 6. Probable Scheme for method M_1&M_2.

Table 2. Analysis of venlafaxine hydrochloride in pharmaceutical formulations by proposed and reference methods.

Method	*Formulations	Labeled Amount (mg)	Found by Proposed Methods			Found by Reference Method ± SD	#% Recovery by Proposed Method ± SD
			**Amount found ± SD	t	F		
A	Batch-1	37.5	36.30± 0.580	0.213	4.303	96.34±2.31	96.81 ± 1.55
	Batch-2	75	72.03± 0.553	0.768	1.499	95.80±0.60	96.05 ± 0.738
B	Batch-1	37.5	36.31± 0.416	0.2487	4.34	96.34±2.31	96.81 ± 1.11
	Batch-2	75	72.29± 0.842	1.92	3.48	95.80±0.60	96.38 ± 1.12

* Batch 1&2 from two different companies (Batch-1: Venlor-XR capsules of Cipla (India), Batch 2: Ventab XL tablets of Intas pharmaceuticals (India).

**Average ± Standard deviation of six determinations, the t- and f-values refer to comparison of the proposed method with reference method (UV). Theoretical values at 95% confidence limits t =2.57 and f = 5.05. # Recovery of 10mg added to the pre-analyzed sample (average of three determinations). Reference method (reported UV method) using double distilled water (λ_{max}=224 nm).

Conclusion

The reagents utilized in the proposed methods are cheap, readily available and the procedures do not involve any critical reaction conditions or tedious sample preparation. The proposed visible spectrophotometric methods are validated as per ICH guide lines and possess reasonable precision, accuracy, simple, sensitive and can be used as alternative methods to the reported ones for the routine determination of VX depending on the need and situation.

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