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Use of charge-transfer complex formation for the spectrophotometric determination of nortriptyline

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Abstract

Three simple and selective methods are proposed for the determination of nortriptyline hydrochloride in bulk form and in tablets. The first two methods are based on the formation of charge-transfer complexes between the drug base as a n-donor and quinhydrone or *p*-chloranil as π-acceptor. The products exhibit absorption maxima at 497 and 560 nm in acetonitrile for quinhydrone and *p*-chloranil, respectively. The third method is based on the interaction of *N*-alkylvinylamine formed from the condensation of the free secondary amine group and acetaldehyde with *p*-chloranil to give a vinylamino substituted quinone. The coloured product exhibits an absorption maximum at 650 nm in dioxane. All variables were studied to optimize reaction conditions. Beer's law was obeyed and the relative standard deviations were found to be less than 1.5%. The methods have been applied to the analysis of nortriptyline hydrochloride in the bulk drug and in tablets.

Keywords: Nortriptyline hydrochloride; Charge-transfer complexes; Spectrophotometry; Tablet analysis

1. Introduction

Nortriptyline belongs to the family of tricyclic antidepressants, a group of drugs widely used for treating depressive diseases. Most existing methods for the determination of nortriptyline in bulk and in pharmaceutical formulations have been reviewed [1]. Among other methods described for assay of nortriptyline are direct UV-spectrophotometry [2,3], derivatives UV spectrophotometry [4,5], fluorimetry [6] and colourimetry [7,8]. The colourimetric methods involve ion-pair extraction with chloroform using Light Green FCF at pH 5 and Orange II at pH 2 [7] or charge-transfer complex with 7,7,8,8-tetracyanoquinodimethane (TCNQ) [8].

We describe new spectrophotometric methods based on the interaction between the secondary amine moiety of the nortriptyline as the n-donor and as π-acceptors quinhydrone, *p*-chloranil and *p*-chloranil with acetaldehyde. The selectivity, sensitivity and precision of the proposed methods and the application to Motival tablets were studied.

2. Experimental

2.1. Apparatus

A Shimadzu UV-1601 recording spectrophotometer with quartz cells of 1 cm optical path length was used.

2.2. Materials

Pharmaceutical grade nortriptyline and fluphenazine hydrochlorides (Squibb Co. USA) were used as working standards. Motival tablets (Squibb Co. Egypt), labelled to contain 10 mg nortriptyline and 0.5 mg fluphenazine hydrochlorides were purchased locally.

2.3. Reagents

The reagents used were of analytical-grade and solvents were always HPLC or spectroscopic grade. *p*-Chloranil (Aldrich) was prepared as 5×10^{-3} M solution in acetonitrile or dioxane. A 1.0 M solution of acetaldehyde (Merck) in dioxane was used. Quinhydrone (Merck) was prepared as 5×10^{-2} M solution in methanol.

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2.4. Preparation of sample solutions

A solution was prepared by dissolving an accurately weighed amount of 50 mg of nortriptyline or fluphenazine–HCl in about 20 ml of distilled water. The solution was quantitatively transferred into a separatory funnel, and the solution was rendered distinctly alkaline (to a pH of 11) by a dropwise addition of 0.5 N sodium hydroxide. The drug base was extracted with four 20 ml portions of dichloromethane, and the dichloromethane extract was filtered through a suitable filter paper containing a small amount of anhydrous sodium sulfate into a 100 ml standard flask and diluted to volume with dichloromethane to provide a standard equivalent to 500 μg ml^{-1} solution of drug.

For the preparation of a working solution in a solvent other than dichloromethane, an appropriate volume of solution corresponding to the required concentration was evaporated to dryness with a stream of nitrogen and the residue was dissolved in the desired solvent (acetonitrile or dioxane).

Nortriptylin solutions of 1×10^{-2} and 1×10^{-3} M were prepared as described above. An 1.0 mg ml^{-1} solution of fluphenazine was always added to *p*-chloranil and *p*-chloranil with acetaldehyde.

2.5. Recommended methods

Method A. Aliquots of a solution of the drug in acetonitrile in the concentration range 0.12–1.20 mg, were transferred by pipette into 10 ml standard flasks, 2 ml of quinhydrone solution in methanol was added, the solution was well mixed and allowed to stand at 25 ± 1°C for 30 min. The solution was diluted to volume with acetonitrile. The absorbance was measured at 497 nm against a blank prepared similarly with 20% (v/v) methanol–acetonitrile replacing the standard or the sample solution.

Method B. Aliquots of a solution of the drug in acetonitrile, in the concentration range 0.2–2.0 mg, were transferred into 10 ml standard flasks, 1.0 ml of fluphenazine (1.0 mg ml^{-1}) and 1.0 ml of *p*-chloranil solutions in acetonitrile were added, the solution was well mixed and allowed to stand at room temperature (25 ± 1°C) for 20 min. The solution was diluted to volume with acetonitrile. The absorbance was measured at 560 nm against a blank prepared similarly.

Method C. Aliquots of a solution of the drug in dioxane (50–500 μg), were transferred into 10 ml standard flasks, 1.0 ml of fluphenazine (1.0 mg ml^{-1}), 1.0 ml of *p*-chloranil and 0.5 ml of acetaldehyde solutions in dioxane were added. The reaction mixture was well mixed and allowed to stand at room temperature (25 ± 1°C) for 30 min and then diluted to volume with dioxane. The absorbance was measured at 650 nm against a blank treated similarly.

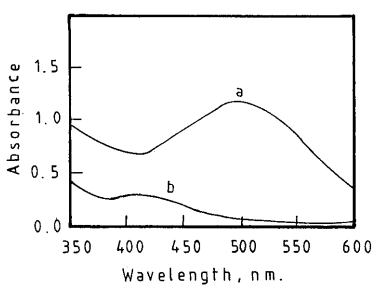

Fig. 1. Absorption spectra of (a) NR–QH complex (4×10^{-4} M), (b) reagent blank versus 20% (v/v) methanol in acetonitrile.

2.6. Procedure for nortriptyline tablets

An accurately weighed amount of powdered tablets equivalent to 50 mg of nortriptyline–HCl was dissolved in about 20 ml of distilled water. The solution was made alkaline with sodium hydroxide (0.5 M), and quantitatively transferred into a separatory funnel. The drug base was extracted and the procedure continued as described for the preparation of sample solutions. Different aliquots from the drug solution were measured by pipette and the method was continued as described for the recommended methods for the three reactions.

2.7. Stoichiometric relationship

Job's method of continuous variations [9] of equimolar solutions was employed: 1×10^{-2}, 2×10^{-3} and 1×10^{-3} M standard solutions of nortriptyline base and 1×10^{-2} M quinhydrone, 2×10^{-3} M *p*-chloranil and 1×10^{-3} M *p*-chloranil with 0.05 M acetaldehyde solutions were used. A series of solutions was prepared in which the total volume of drug and reagent was kept at 2 ml in the total volume of 10 ml. The reagents were mixed in various proportions and completed as directed under the recommended methods.

3. Results and discussion

Nortriptyline (NR) reacts with quinhydrone (QH) to give a red colour, as directed in BP [2], during its identification and distinction from amitriptyline. It therefore seemed worthwhile to examine the nortriptyline–quinhydrone complex in more detail to ascertain whether it could be used for the spectrophotometric determination of nortriptyline.

Quinhydrone in solution of 20% (v/v) methanol–acetonitrile displayed an absorption shoulder peak at about 420 nm (Fig. 1), while the nortriptyline showed a negligible absorption in the 300–700 nm region. Mixing the acetonitrile solution of nortriptyline with the methanolic solution of quinhydrone resulted in a

change of yellow colour of the quinhydrone to deep red. As a consequence, the absorption band of quinhydrone was shifted to a longer wavelength (bathochromic shift). The complex formation of nortriptyline and quinhydrone exhibited an absorption band at 497 nm (Fig. 1a). The interaction between nortriptyline (R–NH) and quinhydrone is a charge-transfer complexation reaction between the drug, the n-donor (D), and quinhydrone, the π-acceptor (A), followed by the formation of radical ions according to Scheme 1.

$$D^{\bullet\bullet} + A \rightarrow [D^{\bullet\bullet} \rightarrow A] \rightarrow D^{\bullet+} + A^{\bullet-}$$

DA complex radical ions (Scheme 1)

The dissociation of the DA complex is promoted by the high ionizing power of the solvent, acetonitrile [10]. The studies revealed that the colour development remained stable up to 20% (v/v) methanol in acetonitrile.

In the second method (B) nortriptyline reacts with *p*-chloranil (CL) and gives a purple chromogen that exhibits a strong absorption maximum at 560 nm in acetonitrile (Fig. 2a). This band can be attributed to the formation of a charge-transfer complex between nortriptyline (n-donor) and *p*-chloranil (π-acceptor) followed by the formation of radical ions as directed in Scheme 1. Further support for the assignment was provided by comparison of the absorption band with that of the CL radical anion produced by the iodide reduction method [11].

The third method (C) is based on the condensation of the basic secondary nitrogen of nortriptyline in dioxane with acetaldehyde to form *N*-vinyl-nortriptyline which reacts with *p*-chloranil to give a blue nortriptyline-vinyl substituted quinone, that exhibits an absorption maximum at 650 nm (Fig. 3a). The reaction was based on the work of Bückley et al. [12] and had been utilized for the quantitation of some drugs containing a free secondary amine group in their molecular structures [13,14].

Acetonitrile was found to be the best solvent for method A or B and dioxane for method C, because they have high relative permittivities which ensure the maximum yield of radical ion species. The effect of the reagent concentration on the intensity of the colour developed at the selected wavelengths was ascertained using different millilitres of the reagent QH (method A) and *p*-chloranil CL (method B or C). It was found that 2 ml of 5×10^{-2} M QH, and 1.0 ml of 5×10^{-3} M CL solutions were sufficient for the production of maximum and reproducible colour intensity (Fig. 4a,b). While in the presence of acetaldehyde with CL (method C) (Fig. 4c), the absorbance increases up to 0.5 ml and then decreases at higher concentration of acetaldehyde, so, 0.5 ml of 1.0 M acetaldehyde solution was used for the highest sensitivity. The molar absorptivity, for example, in presence of acetaldehyde was found to be 6.32×10^3, whereas in its absence it was 1.59×10^3 as shown in Table 1.

Due to the presence of fluphenazine (FL) in the Motival tablets, it is necessary to study the effect of this drug on NR determination by the recommended methods. Although FL alone was too insensitive for the three previous reactions, it increases the colour reaction of the NR–CL complex, i.e. it causes interference. The effect of variation of FL concentration should be studied in the presence of NR.

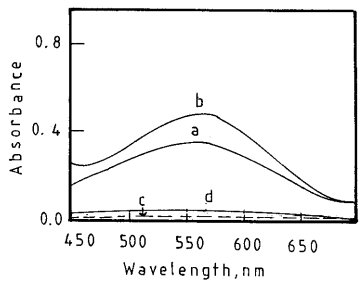

Fig. 2. Absorption spectra of (a) NR–CL complex (3×10^{-4} M), (b) (a) + FL (1.0 mg/10 ml), (c) and (d) reagent blanks for (a) and (b) respectively versus acetonitrile.

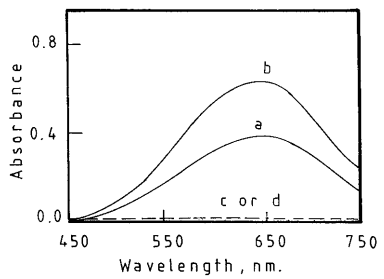

Fig. 3. Absorption spectra of (a) NR–CL complex (1×10^{-4} M) with acetaldehyde (0.05 M), (b) (a) + FL (1.0 mg/10 ml), (c) and (d) reagent blanks for (a) and (b) respectively versus dioxane.

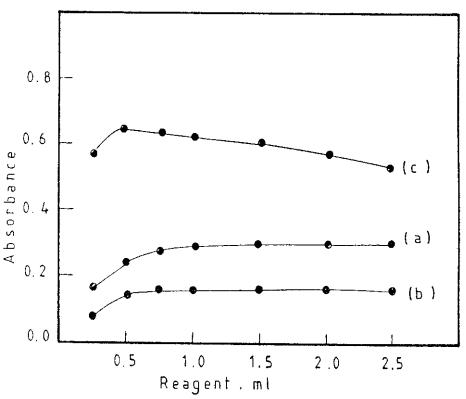

Fig. 4. Effect of the volume of (a) quinhydrone (5×10^{-2} M), (b) *p*-chloranil (5×10^{-3} M), (c) acetaldehyde (1 M) on absorption of the reaction product. [Drug] = 10^{-4} M, λ = 497 (a), 560 (b), 650 (c) nm.

Table 1
Analytical parameters for the complexation of nortriptyline with QH, CL and CL with acetaldehyde

Parameter	Method		
	QH	CL	CL with acetaldehyde
λ_{max} (nm)	497	560	650
Beer's law limits (μg ml^{-1})	12–120	15–180	5–50
Molar absorptivity (l mol^{-1} cm^{-1})	2.94×10^3	1.59×10^3	6.32×10^3
Sandell sensitivity (μg cm^{-2})	0.102	0.189	0.047
Slope (specific absorptivity)	0.0098	0.0053	0.0210
Intercept	0.0013	0.0002	0.0020
Correlation coefficient (r)	0.9999	0.9999	0.9996
Relative standard deviation (%, $n = 6$)	0.15	0.55	0.59
Ringbom optimum concentration range (μg ml^{-1})	17.8–100	25.1–177.8	7–47

An investigation of the effect of FL concentration on the complex produced in methods A, B and C showed that the shape of the absorption spectrum and the position of the absorption maximum of these reactions do not vary with the addition of FL, while the absorbance increases in the case of methods B and C (Fig. 2b, Fig. 3b). In method A, the increase in FL concentration even to 10-fold excess to NR did not affect the intensity of the developed colour or its stability. As shown in Fig. 5b,c it was found that the addition of 1.0 mg of FL in the total volume of 10 ml is necessary to carry out methods B and C and overcome its interference in the determination of NR in the Motival tablets. In addition, the sensitivity and the stability of the complex were raised to more than 24 h.

The optimum reaction time was determined by following the colour development at ambient temperature (25 ± 1°C). Complete colour development was attained after 30, 20 and 30 min for QH, CL and CL with acetaldehyde, respectively, and the colour remained stable for 4 h for QH and more than 24 h for the last two methods (B and C).

Job's continuous variations graph for the reaction between NR and QH or CL, shows that 1:1 and 2:1 complexes (NR:reagent) are formed using QH and CL (in absence or presence of acetaldehyde), respectively (Fig. 6). This finding was anticipated by the presence of one basic or electron donating centre (–NH) in the nortriptyline drug.

4. Analytical parameters

Under the experimental conditions described, standard calibration curves for nortriptyline with different reagents were constructed by plotting absorbance versus concentration. Conformity with Beer's law was evident in the concentration range of the final dilution cited in Table 1. The molar absorptivity, the Sandell sensitivity and the linear regression equation for each method are listed in Table 1. The correlation coefficients were between 0.9996–0.9999 indicating good linearity. For more accurate results, Ringbom plots [15] for optimum concentration range were obtained (Table 1).

Six replicate determinations at different concentration levels were carried out to test the precision of the methods (Table 2). The relative standard deviations

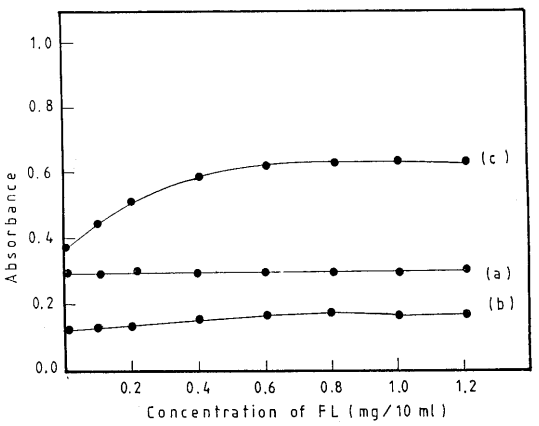

Fig. 5. Effect of FL concentration on the reaction of 10^{-4} NR with (a) QH, (b) CL and (c) CL with acetaldehyde.

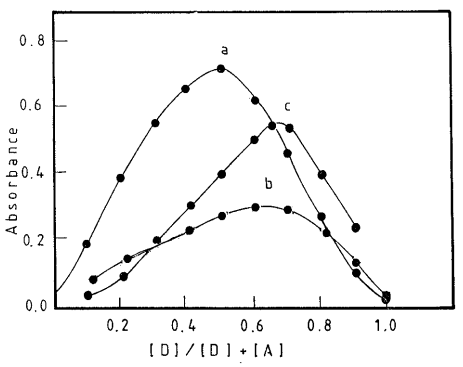

Fig. 6. Continuous variations plot for (a) [NR] + [QH] = 2×10^{-3} M $\lambda = 497$ nm, (b) [NR] + [CL] = 4×10^{-4} M, $\lambda = 560$ nm, (c) [NR] + [CL] = 2×10^{-4} M, $\lambda = 650$ nm.

Table 2
Tests of precision of the methods on samples of pure nortriptyline

Method	Nortriptyline			Standard error	Confidence limits ($P = 0.05$)
	Taken (μg ml^{-1})	Found ± SD [a] (μg ml^{-1})	RSD (%)		
QH	30	30.05 ± 0.19	0.63	0.077	30.05 ± 0.20
	40	39.95 ± 0.36	0.90	0.147	39.95 ± 0.38
	50	50.08 ± 0.32	0.64	0.131	50.08 ± 0.34
	60	59.92 ± 0.41	0.68	0.167	59.92 ± 0.43
	Mean		0.71		
CL	25	25.1 ± 0.24	0.10	0.098	25.1 ± 0.25
	50	50.2 ± 0.31	0.62	0.126	50.2 ± 0.33
	100	100.5 ± 0.48	0.48	0.196	100.5 ± 0.50
	150	149.6 ± 0.67	0.45	0.273	149.6 ± 0.70
	Mean		0.41		
CL + acetaldehyde	10	10.06 ± 0.13	1.23	0.053	10.06 ± 0.14
	20	19.90 ± 0.23	1.16	0.094	19.90 ± 0.24
	30	29.93 ± 0.35	1.17	0.143	29.93 ± 0.37
	40	40.02 ± 0.43	1.07	0.176	40.02 ± 0.45
	Mean		1.16		

[a] $n = 6$.

Table 3
Spectrophotometric determination of nortriptyline hydrochloride in Motival tablets

Sample	Nortriptyline HCl				
	Taken (μg ml l^{-1})	Found ± SD (%) [a]			
		Method A	Method B	Method C	UV spectrophotometric method [3]
Motival tablet [b]	30	100.10 ± 0.77	100.15 ± 0.95	100.07 ± 0.65	99.98 ± 56
		$t = 0.309$	0.378	0.257	(2.228) [c]
		$f = 1.891$	2.878	1.347	(5.050) [c]
	40	99.55 ± 0.62	99.59 ± 0.59	99.68 ± 0.59	99.75 ± 0.68
		$t = 1.417$	0.435	0.435	
		$f = 1.47$	1.328	1.328	
	50	99.90 ± 0.58	100.01 ± 0.73	99.95 ± 0.63	100.1 ± 0.8
		$t = 0.202$	0.204	0.361	
		$f = 1.905$	1.201	1.612	

[a] Mean ± standard deviation of six determinations.
[b] Motival tablets containing 10 mg nortriptyline hydrochloride and 0.5 mg fluphenazine hydrochloride per tablet (Bristol-Mayers Squibb Egypt).
[c] Values in parentheses are the critical values at $P = 0.05$.

were found to be less than 1.5%, indicating reasonable repeatability of the selected methods.

Statistical comparison for the results of the proposed and USP methods (Table 3) was performed with regard to accuracy and precision using the student t-and f-ratio tests. At 95% confidence level, the calculated t- and f-values did not exceed the theoretical values, indicating that there is no significant difference between the proposed methods and the USP method with regard to accuracy and precision. However, the principal advantage of the proposed methods is their suitability for the routine quality control of the drug alone and in tablets without fear of interference caused by the excipients expected to be present in tablets. The good agreement of the results indicates the suitability of these spectrophotometric methods for the determination of nortriptyline in the presence of fluphenazine (Motival tablets). In addition, the use of these spectrophotometric procedures leads to considerable savings in cost and time.

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