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Electrochemical Study of Some Charge-Transfer Complexes Involving Amitriptyline and Nortriptyline and Determination of Drugs

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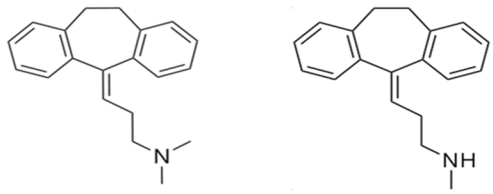
ABSTRACT

Spectrophotometric and electrochemical studies were successfully used in quantitative analysis of Amitriptyline Hydrochloride (ATH) and Nortriptyline Hydrochloride (NTH). Conductometric titration were carried out in equeous solution using iodide and dopamine and serotonine as titrant. Two new methods using spectrophotometry are dascribed for the determination of (ATH) and (NTH) with potassium bromate as the oxidizing agent and acid dyes, Methyl orange and Indigo carmine. Both spectrophotometric methods are based on the oxidation of mentioned drugs by a known excess of bromate in acid medium and in the presence of excess of bromide followed by estimation of surplus oxidant by reacting with either Indigo carmine (method A) or Methyl orange (method B) and measuring the absorbance at 609 or 507 nm.

KEYWORDS: Conductometry; Amitriptyline Hydrochloride; Nortriptyline Hydrochloride; Dopamine Hydrochloride; Serotonine Hydrochloride; Iodine.

1. INTRODUCTION

Amitriptyline hydrochloride and Nortriptyline Hydrochloride are extensively used in the treatment of emotional and psychiatric disorders in which the major symptom is depression, particularly endogeneous depression (Fig. 1.)



Nortriptyline Amitriptyline

Fig. 1. Structures of Amitriptyline and Nortriptyline

Various analytical procedures have been reported for determination of these drugs. The tricyclic drugs are basic compounds, a fact that posses certain difficulties for the chromatographer. Since the tricyclics posses basic pK values, they are ionized in acidic or neutral pH mobile-phase solutions, preventing good chromatographic separations. Analytical methods for the determination of theses drugs include Ultra-Violet spectrophotometry [1-2], polarography [3], Infrared immunoassay [4], high pressure liquid chromatography [5-6], capillary electrophoresis [7] and thin layer chromatography [8].

Investigation of charge-transfer complexes formation involving drugs, is very interest. The complexes are often characterized by an intense, broad, electronic absorption band either in the visible or in the UV region. Charge transfer reactions sometimes demonstrate no color change, no obvious charge transfer band in the absorption spectrum, therefore electrochemistry provides a supplementrary technique for charge-transfer systems [9-10].

In conductimetric titrations of charge-transfer complexes, a complex (DA), formed from the association of a donor (D) and an acceptor (A). The formation of charge transfer complexes can be followed by measuring changes in the conductivity of solution. The stoichiometry of the complexes be deduced from the mole fraction at the conductivity peak and the known concentrations of the stock solutions [11-13].

We are interested in the pharmaco-chemical study of amitriptyline and Nortriptyline. In terms of its mechanism of action, drugs inhibit serotonin and noradrenaline reuptake almost equally. These drugs are approved most commonly for the treatment of depression. In this work, conductimetric and spectroscopic methods have been employed to examine the interaction of drugs as a possible electron donor with several small molecules of biological importance including iodine, dopamine hydrochloride and serotonin hydrochloride. Iodine is known for its electron-accepting properties, which may be deduced from molecular orbital consideration [14-15]. Among

the small molecules, serotonin and dopamine are known to behave as electron acceptors toward a strong donor. Results from these studies will provide the fundamental chemical and pharmacological information required for possible structural modification of the drug to enhance the primary antiarrhythmic action and suppress undesirable side effects. An important goal of this work is to contruct a simple model system for elucidation of the biological reactions of ATH and NTH. Also, this paper describes simple, accurate, precise and sensitive methods for the determination ATH and NTH in pure sample and pharmaceutical preparations. The methods use bromine-generated in situ as the oxidizing agent and two dyes as either indicator or spectrophotometric reagents.

2. EXPERIMENTAL

2.1. Apparatus

A JASCO model V-530 UV-Vis spectrophotometer with 1 cm matched cell was used for electronic spectral measurements. Conductance and capacitance measurements wetre made using a Metrohm 660 conductivity meter. A dip-type conductivity cell, made of platinum black, was used. The electrodes were cleaned after every second or third titration using an electrochemical procedure.

2.2. Materials

All chemicals used were of analytical reagent grade and distilled water was used to prepare all solutions. Pure drug sample was provided by Arasto pharma. Chem. Co. Tehran, Iran and used as received. Iodine and dopamine hydrochloride was obtained from MERCK chemicals. Serotonin was purchased from Sigma chemical Co.

2.3. Solutions

A stock standard solution equivalent to 1000 μg mL^{-1} KBrO3 containing a large excess of KBr were prepared by dissolving accurately weighed 100 mg of KBrO3 and 1.0 g KBr in water and diluting to 100 mL in a volumetric flask. The above solution was diluted appropriately with water to get 10 and 30 μg mL^{-1} concentrations. To prepare 50 μg mL^{-1} methyl orange first a 500 mg of dye in water was prepared and diluting to mark in a 100 mL calibrated flask and filtered. This was diluted 10-fold to obtain a working concentration of 50 μg mL^{-1}. Hydrochloric acid (5 mol L^{-1}) was prepared by diluting 40.8 mL of concentrated acid to 100 ml with water and mixed well. A 1000 μg mL^{-1}solution of Indigo-Carmine was prepared by dissolving 125 mg (80% purity) in water and diluting to 100 mL in a volumetric flask. This was further diluted 10-fold to get a working concentration of 100 μg mL^{-1}. The stock solution was diluted appropriately to get 200 μg mL^{-1} dye solution with water. A stock standard containing 500 μg^{-1} solutions of ATH or NTH were prepared by dissolving accurately weighed 50 mg of pure drug and dissolving in 30 mL deionized water into a 100 mL volumetric flask and volume was made up to 100 mL using de-ionized water. Solutions of 10^{-2} M Iodine and dopamine hydrochloride and Serotonin hydrochloride were prepared.

2.4. Conductivity Titrations

A solution of the donor (ATH, NTH) were titrated into a known volume of an acceptor in the cell. The titration was usually stopped after the addition of 30-40 mL of donor. A complete range of conductance and capacitance values is thus obtained for mole fractions of donor ranging from 0 to 1. After each addition of 0.1 ml of titrant, the solutions were stirred for 2 min and left for 2 min in order to attain equilibrium. Titration was performed at 298 K. Considering the change in volume, the observed values were corrected by multiplication by a factor (V+v)/V, where V was the volume of the original solution and v was the volume of added titrant. For the minimizing this correction, the concentration of the titrant was 5-6 times higher than that of the drugs. A graph of corrected conductivity versus the volume of titrant added was constructed and the end point was determined.

2.5. Spectrophotometry with methyl orange (method A)

Aliquots (0.5-5.0 mL) of 10 μg mL^{-1} drugs solution were accurately measured into a series of 10 mL calibrated flasks and the total volume was adjusted to 5 mL with water. To each flask was added 1 mL each of bromate-bromide solution (10 μg mL^{-1} w. r. t $KBrO_3$) and 5 mol L^{-1} hydrochloric acid. The flasks were stopped and let stand for 15 min with occasional shaking. Then 1 mL of 50 μg mL^{-1} methyl orange solution was added to each flask and diluted to the mark with water. The absorbance of each solution was measured at 507 nm against a reagent blank after 10 min.

2.6. *Spectrophotometry with indigo-carmine (method B)*

Varying aliquots of standard drugs solution (0.5-5.0 mL; 25 μg mL^{-1}) were transferred into a series of 10 mL calibrated flasks by means of a micro burette and the total volume was brought to 5 mL 1 by adding water. Accuratly measured 1.5 of bromate – bromide solution (30 μg mL^{-1} w. r. t $KBrO_3$) was added to each flask followed by 1 mL of 5 mol L^{-1} hydrochloric acid. The flasks were stopped and let stand for 15 min with occasional shaking. Then 1 mL of 200 μg mL^{-1} indigo carmine solution was added to each flask and diluted to the mark with water. The absorbance of each solution was measured at 610 nm against a reagent blank after 10 min. In the methods, the concentration of the unknown was read from the calibration graph or calculated from the regression equation obtained from Beer's law data .

2.7. Assay procedure for tablets

A quantity of finely ground tablet powder equivalent to 50 mg of drugs was accurately weighed in to a 100 ml

calibrated flask, 60 ml de-ionized water was added and shaken for 20 min; the volume was finally diluted to the mark with water, mixed well and filtered using a whatmann No. 42 filter paper. A convenient aliquot was then subject to analysis by either method.

3. RESULTS AND DISSCUTIONS

3.1. Conductometric titrations

The role of non-covalent molecular interactions is accorded only fleeting implicit acknowledgement in some of the literature of medical biochemistry and physiology. That such precisely controlled reaction sequences as oxidation, phosphorylation or the complicated and elegant regulatory cascades that follow the action of catecholamines such as adrenaline, could ever occur as simple collision or diffusion rate limited processes, is unimaginable. The role of non-covalent molecular interactions is accorded only fleeting implicit acknowledgement in some of the literature of medical biochemistry and physiology. Furthermore, when the effects of a drug on metabolism are traced, one should give due consideration to non-covalent molecular interaction. Drugs, therapeutic or otherwise, may cause gross alteration of biological functions. Their effects are dose-dependent and, in many cases, reversible. Here is a situation that leads naturally to a theory of drug action, central to which is the existence of a generalized non-covalent association, or charge transfer complexation. We studied the in vitro action of heterocyclic compounds, such as ATH or NTH, on Iodine and dopamine and Serotonin.

The conductometric titration curves of ATH and NTH by dopamine, seretonin and Iodide in the investigated range of concentrations are presented in Fig. 2. Mole ratios for drugs with all titrans (calculated from the curves and point). Mole ratios of drugs:titrants are 1:3, 1:3 and 3:1 for dopamine, serotonin and I_2, respectively.

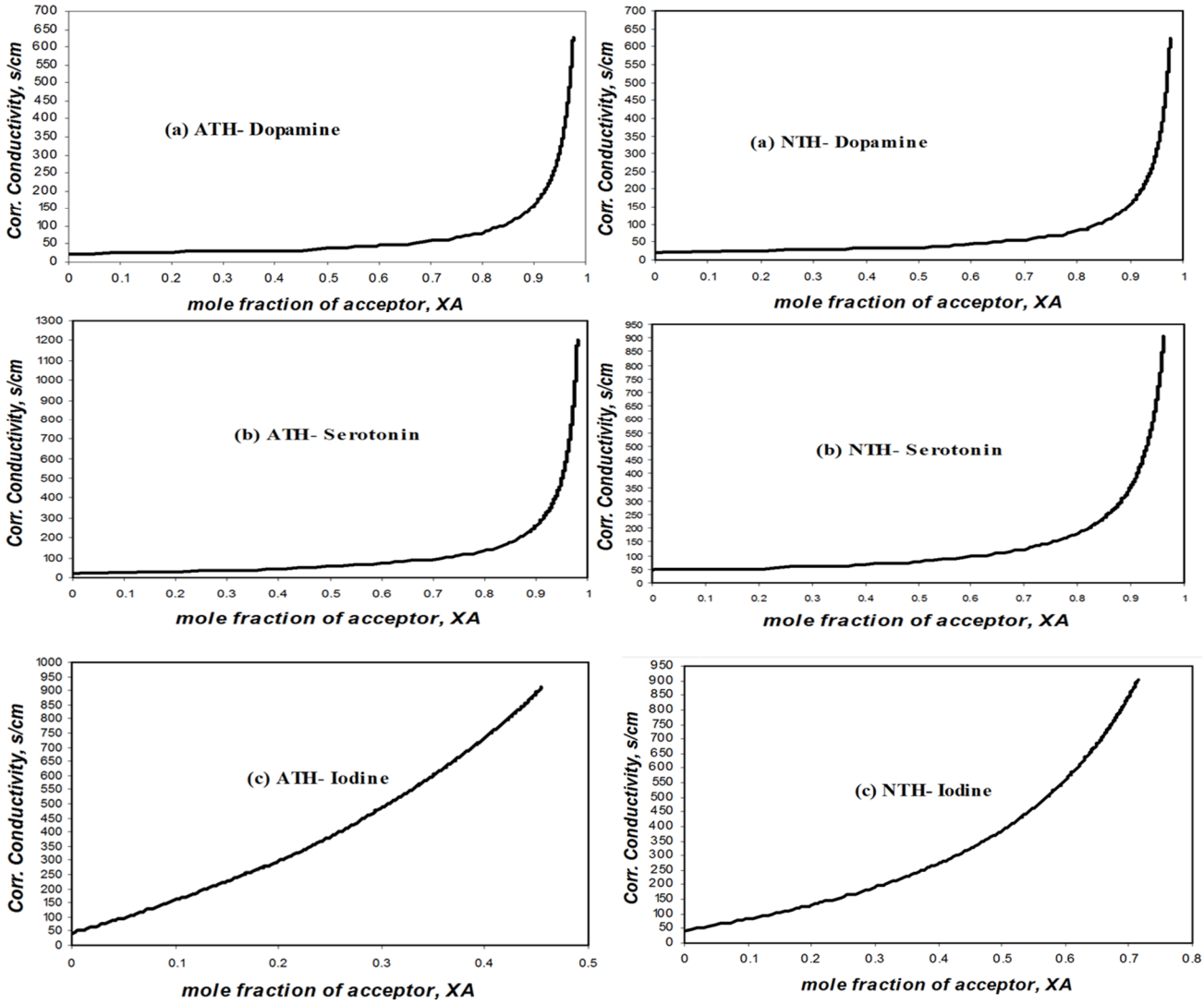


Fig. 2. Plots of data from conductivity titrations carried out at 25 °C. The stock solution concentrations were each in ATH or NTH, 0.2 mM; dopamine hydrochloride, 10 mM in (a) and ATH or NTH, 0.2 mM; serotonin hydrochloride, 10 mM in (b) and ATH or NTH, 0.8 mM; iodine, 1.0 mM in (c).

3.2. Spectrophotometric methods

Bromate-Bromide mixture is a valuable oxidimetric reagent widely used in the assay of several pharmaceutical substances both by titrimetric and spectrophotometric methods [16-17]. The proposed spectrophotometric methods are indirect and based on the determination of the residual bromine after allowing the reaction between ATH or NTH and a measured amount of bromine to be complete. The surplus bromine was determined by reacting it with a fixed amount of either methyl orange or indigo carmine dye.

The amounts of bromine reacted correspond to the amount of ATH and NTH which formed the basis for the assay of each of mentioned drug and both of reaction were found to follow a 2:3 stoichiometry for drug:$KBrO_3$.

3.3. Method development

When ATH and NTH treated with a fixed and known amount of bromate- bromide solution in acid medium (producing in situ bromine), the produced bromine; acting as an oxidizing agent; react with equivalent amount of the studied drugs. In presence of methyl orange and indigo carmine dyes, the remaining amount of bromine oxidize these dyes to colorless products. The proposed spectrophotometric methods based on the measurement of the color of the unreacted concentrations of dyes. As the concentration of the drugs increase, the remaining amount of bromine decrease and subsequent the measured amount of the dye increase. A proportional increase in the absorbance at the respective λ_{max} is observed with increase concentration of the drug, as shown by the correlation coefficients of method A and B, respectively (Fig. 3&4).

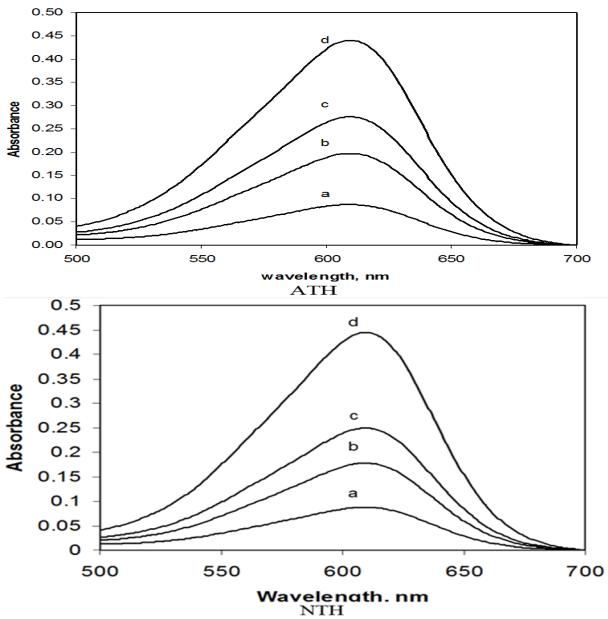


Fig 3. Absorption spectra of 200 mg/ml indigo carmine in the presence of 30 mg/ml bromate. (a) without ATH or NTH; (b) with 2.5 mg/ml ATH or NTH; (c) with 5 mg/ml ATH or NTH and (d) with 10 mg/ml ATH or NTH.

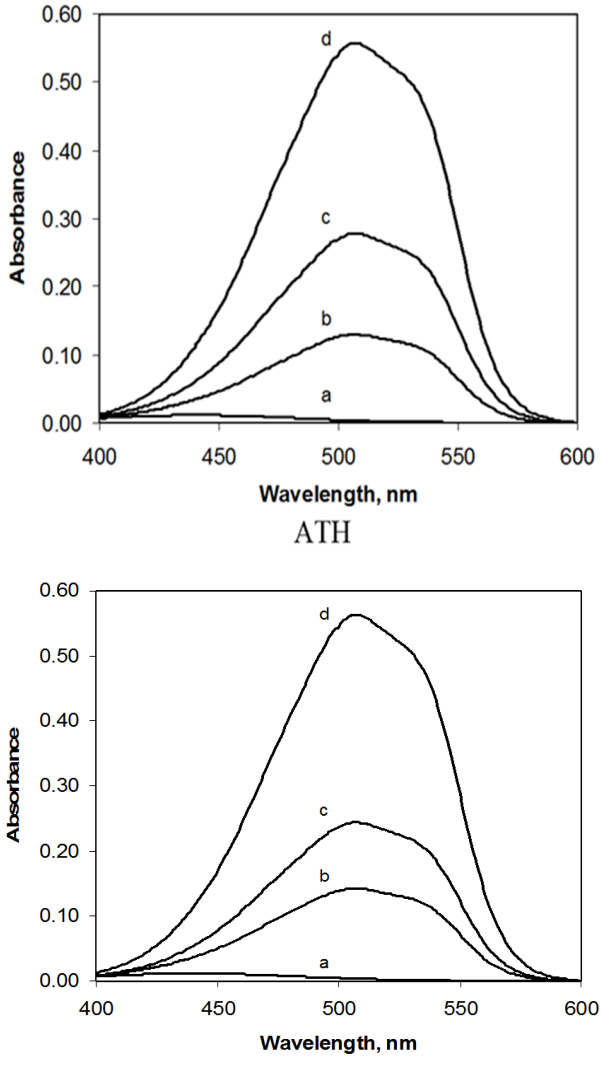


Fig. 4. Absorption spectra of 5 methyl orange in the presence of 1 bromate. (a) without ATH, NTH; (b) with 1 ATH, NTH; (c) with 2 ATH, NTH and (d) with 4 ATH, NTH.

Preliminary experiments were performed to fix upper limits of the dyes that could be measured spectrophotometrically and these were found to be 20 and 5 μg mL^{-1} for indigo carmine and methyl orange, respectively. A bromate concentration of 1.0 μg mL^{-1} in the presence of an excess of bromide was found to irreversibly destroy the red colour of 5 μg mL^{-1} methyl orange, whereas 4.5 μg mL^{-1} bromate was required to destroy 20 μg mL^{-1} indigo-carmine under similar concentration of bromide. The reaction was complete in 15 min in both methods and contant time is not critical and any delay up to 30 min in either method had no effect on the absorbance of the measured colour was constant for several days even in the presence of the reaction product. For both steps, vis, the reaction between insitu bromine and drugs and bleaching of dyes by bromine, hydrochloric acid medium was found to be ideal. Two mL of 5 M hydrochloric acid and 1 mL of 2 M hydrochloric acid in a total volume of ~3- 4

mL were adequate for the bromination step in method A and B, respectively.

3.4. Analytical parameters

A linear correlation was found between absorbance at λ_{max} and concentration of ATH or NTH (Table1,2). Correlation coefficient, intercept and slopes for the calibration data are also presentation in Table 1,2. The graphs showed negligible intercept and the described by the equation:

$$Y = a + bx$$

(where Y = absorbance of cm^{-1} layer of solution; a = intercept; b = slope and X = concentration in $\mu g\ mL^{-1}$). Regression analysis of Beer's law data using the method of least squares was made to evaluate of a, b and correlation coefficient (R^2) for each system and the values are presented in Table 1, 2.

The optical characteristics such as Bear's law limits, molar absorptivity and sensitivity values of methods are also given in Table 1, 2. The limit of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines [18] are also presented in Table 1, 2 and reveal the very high sensitivity of methods.

$$\text{LOD} = \frac{3.3\sigma}{S} \quad \text{and} \quad \text{LOQ} = \frac{10\sigma}{S}$$

where σ is the standard deviation and S is the slop of the calibration curve for four determinations at each level. The range, standard deviation (S.D.) and RSD (%) are given in Tables of 3, 4.

Accuracy and reliability of the methods were further ascertained through recovery studies. To a fixed and known amount of drugs in tablets powder, pure ATH or NTH were added at three different levels and the total were found by the proposed methods. Recoveries of the pure drugs added to tablets powder reveal that absorbance measurement in spectrophotometric methods were not affected by tablets excipients such as talc, starch, sodium alginate and calcium gluconate and calcium dihydrogen orthophosphate.

The comparision of the actual difference between the mean and the true value $(\bar{x} - \mu)$ with the largest difference that could be expected as a result of indetermediate error $(\pm ts/\sqrt{n})$ is made in the last two columns of Table 3, 4. Comparison of the difference between the determined value and the true value with the independent error recorded lower values indicating that no significant difference between the mean and true values in Table 3, 4.

Table1. Optical parameters of Amitriptyline hydrochloride.

Parameter	Method A	Method B
λ_{max} (nm)	609	507
Beer's law limits ($\mu g\ ml^{-1}$)	1.25-12.5	0.5-5
Molar absorptivity ($l\ mol^{-1}\ cm^{-1}$)	9.8×10^3	4.2×10^4
Limit of detection (LOD)[a] ($\mu g\ ml^{-1}$)	0.19	5.4×10^{-3}
Limit of quantification (LOQ) ($\mu g\ ml^{-1}$)	0.59	0.016
Regression equation (Y)[c]		
Slope	0.0311	0.1334
Intercept	0.11	0.004
Correlation coefficient	0.99	0.992

Table2. Optical parameters of Nortriptyline hydrochloride.

Parameter	Method A	Method B
λ_{max} (nm)	609	507
Beer's law limits ($\mu g\ ml^{-1}$)	1.25-12.5	0.5-5
Molar absorptivity ($l\ mol^{-1}\ cm^{-1}$)	9.93×10^3	4.03×10^4
Limit of detection (LOD)[a] ($\mu g\ ml^{-1}$)	0.18	5.37×10^{-3}
Limit of quantification (LOQ) ($\mu g\ ml^{-1}$)	0.55	0.016
Regression equation (Y)[c]		
Slope	0.0331	0.1345
Intercept	0.091	0.0073
Correlation coefficient	0.9899	0.9947

4. CONCLUSIONS

Conductimetric measurements are used successfully in quantitative titration of systems in which the conductance of the solution varies before and after the equivalence point. The reagent concentration in each titration must not be less than 10 times that of the drug solution in order to minimize the dilution effect on the conductivity throughout the titration. In this case, the titration curve can be represented by two lines intersecting at the end point. The optimum concentration of the reagent was 10^{-2} M dopamine and serotonine and 10^{-3} iodine in titration of ATH and NTH. Concentrations less than these limits led to unst-

Table 3. Precision and accuracy of each drug in pure form

Compound name	Method B						Method C					
	Amount taken ($\mu g\ ml^{-1}$)	Rec (%)	S. D.	RSD	$\lvert\bar{x} - \mu\rvert$	$\pm ts/\sqrt{n}$	Amount taken ($\mu g\ ml^{-1}$)	Rec (%)	S.D.	RSD	$\lvert\bar{x} - \mu\rvert$	$\pm ts/\sqrt{n}$
ATH	2.5	105.60	0.003	1.51	0.14	1.0043	1	94.56	0.000	0.38	0.05	0.0007
	5	103.60	0.001	0.40	0.18	0.0016	3	100.61	0.000	0.09	0.02	0.0005
	7.5	95.97	0.000	0.23	0.30	0.0011	4	101.72	0.014	2.56	0.07	0.0207
	10	103.47	0.006	1.53	0.35	0.0095	5	98.52	0.000	0.07	0.07	0.0007
NTH	2.5	102.65	0.001	0.57	0.06	0.0014	1	99.08	0.002	1.57	0.01	0.0031
	5	96.72	0.001	0.47	0.16	0.0016	3	104.52	0.007	1.68	0.13	0.0104
	7.5	104.74	0.002	0.64	0.35	0.0033	4	102.60	0.005	0.86	0.10	0.0070
	10	105.22	0.000	0.08	0.52	0.0005	5	98.43	0.000	0.67	0.08	0.0005

Average value of three determination;
Tabulated t-value at 95% confidence limit 2.447.

Table 4. Precision and accuracy of each drug in dosage form

Compound name	Method B						Method C					
	Amount taken (μg ml^{-1})	Rec (%)	S. D.	RSD	$\lvert\bar{x}-\mu\rvert$	$\pm ts/\sqrt{n}$	Amount taken (μg ml^{-1})	Rec (%)	S.D.	RSD	$\lvert\bar{x}-\mu\rvert$	$\pm ts/\sqrt{n}$
ATH 10	2.5	104.17	0.002	0.93	0.10	0.0036	1	102.52	0.000	0.25	0.02	0.0009
	5	100.25	0.003	0.10	0.01	0.0050	3	101.37	0.000	0.15	0.04	0.0010
	7.5	101.10	0.003	0.65	0.08	0.0040	4	97.67	0.000	0.15	0.09	0.0012
	10	101.07	0.002	0.43	0.0	0.0031	5	100.30	0.002	0.22	0.01	0.0023
NTH 10	2.5	102.37	0.000	0.025	0.06	0.0010	1	98.40	0.001	0.45	0.02	0.0018
	5	102.75	0.004	0.94	0.13	0.0050	3	103.03	0.003	0.46	0.09	0.0038
	7.5	100.81	0.003	0.70	0.06	0.0046	4	101.22	0.002	0.23	0.05	0.0023
	10	99.61	0.003	0.58	0.04	0.0046	5	97.24	0.002	0.22	0.14	0.0025

Average value of three determination;
Tabulated t-value at 95% confidence limit 2.447.

able readings and more time were needed to obtain constant conductance values.

Two useful micro methods for the determination of ATH and NTH have been developed and validated. Both spectrophotometric methods are more sensitive than the existing UV and visible spectrophotometric and HPLC methods and are free from such experimental variables as heating or extraction step. Thus, they can used as alternatives for rapid and routine determination of bulk sample and tablets as a part of industrial quality control.

We have stablished that amitriptilyne or nortriptyline are complexing reagents which can form charge transfer complexes in vitro with a variety of biologically important small molecules. The donor-acceptor interactions and the stoichiometry of the complexes were studied by conductivity titration. An assessment of the free base form of drug shows that the association reaction between the donor and the acceptor is the major contributing equilibrium, justifying the use of the hydrochloride form of drug in investigating the drug donor-acceptor interactions.

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