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Determination of Amitriptyline using Bromate-Bromide and two Dyes

Azar Bagheri Gh[1*], Mahnaz Bagheri[2]

[1] Department of Chemistry, Center Tehran Branch, Islamic Azad University, Tehran, Iran

[2] Institutes of Standards and Industrial Research of Iran, Tehran, Iran

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Abstract:

Spectrophotometric studies were successfully used in quantitative analysis of Amitriptyline Hydrochloride (ATH) Two new methods using spectrophotometry are described for the determination of (ATH) 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: Spectrophotometric, Amitriptyline Hydrochloride, Methyl orange

(*) Corresponding Author: e-mail: azar.bagheri@iauctb.ac.ir

1. Introduction:

Amitriptyline hydrochloride is extensively used in the treatment of emotional and psychiatric disorders in which the major symptom is depression, particularly endogenous depression. (Fig.1). various analytical procedures have been reported for determination of this drug. The tricyclic drug is basic compounds, a fact that possess certain difficulties for the chromatographer. Since the tricyclic's possess basic pK values, they are ionized in acidic or neutral pH mobile-phase solutions, preventing good chromatographic separations. Analytical methods for the determination of these drugs include Ultra-Violet spectrophotometry [1-4] polarography,[5-7] Infrared immunoassay [8] high pressure liquid chromatography, [9-11] capillary electrophoresis [12-14] and thin layer chromatography [15].This paper describes simple, accurate, precise and sensitive methods for the determination ATH and 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.

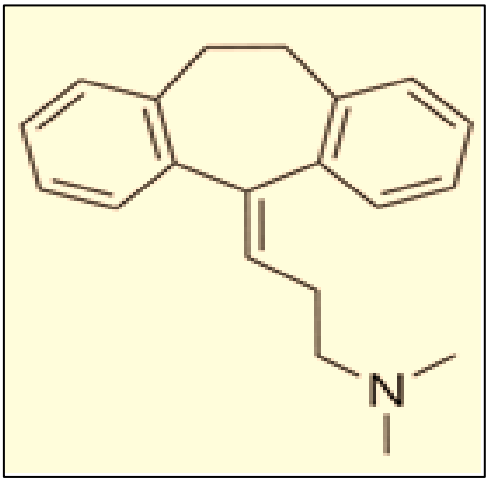

Fig. 1. Structures of Amitriptyline

2. Experimental

2.1 Apparatus

A JASCO model V-530 UV-Vis spectrophotometer with 1 cm matched cell was used for electronic spectral measurements.

2.2Materials

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

2.3 Solutions

A stock standard solution equivalent to 1000 μg mL -1 $KBrO_3$ containing a large excess of KBr were prepared by dissolving accurately weighed 100 mg of $KBrO_3$ 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 - concentrations. To prepare 50 μg mL -1methyl 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 -1solution 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 -1dye solution with water. A stock standard containing 500 μg -1 solutions of ATH 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.

2.4 Spectrophotometry with methyl orange (method A)

Aliquots (0.5-5.0 mL) of 10 μg mL -1 drug 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.

2.5 Spectrophotometry with indigo-carmine ((method B)

Varying aliquots of standard drug 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 l by adding water. Accuratly measured 1.5 of bromate – bromide solution (30 μg mL -1 w. r. t KBrO3) 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.

2.6 Assay procedure for tablets

A quantity of finely ground tablet powder equivalent to 50 mg of drug 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 Discussion

3.1 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 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 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:KBrO3.

3.2 Method development

When ATH 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 drug. 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 drug 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 λmax is observed with increase concentration of the drug, as shown by the correlation coefficients of method A and B, respectively (Fig. 2&3).

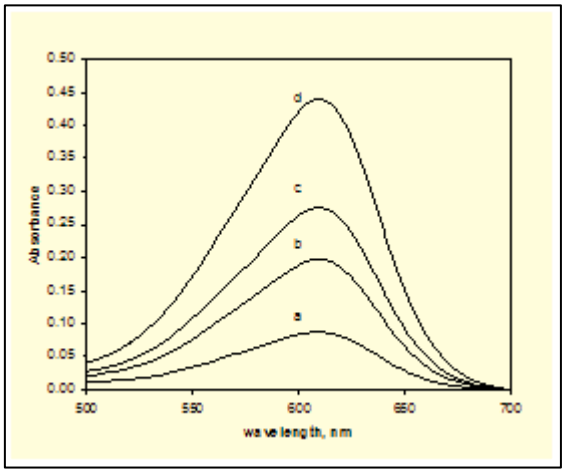

Fig 2. Absorption spectra of 200 $\mu g / ml$ indigo carmine in the presence of 30 $\mu g / ml$ bromate. (a) without ATH ; (b) with 2.5 $\mu g / ml$ ATH; (c) with 5 $\mu g / ml$ ATH and (d) with 10 $\mu g / ml$ ATH or.

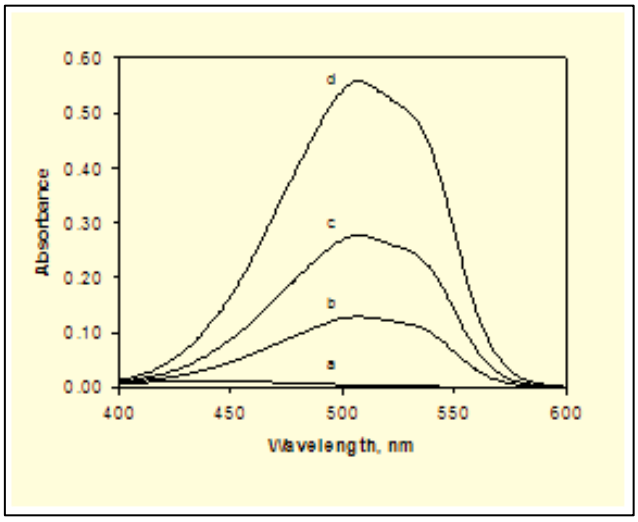

Fig. 3. Absorption spectra of $5^{\mu g/ml}$ methyl orange in the presence of $1^{\mu g/ml}$ bromate. (a) without ATH,; (b) with $1^{\mu g/ml}$ ATH,; (c) with $2^{\mu g/ml}$ ATH, and (d) with $4^{\mu g/ml}$ ATH,.

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 drug, 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.

3.3Analytical parameters

A linear correlation was found between absorbance at λmax and concentration of ATH (Table1). Correlation coefficient, intercept and slopes for the calibration

data are also presentation in Table 1. The graphs showed negligible intercept and the described by the equation (1):

$$Y = a + bx \qquad (1)$$

(where Y = absorbance of cm^{1-} layer of solution ; a = intercept; b = slope and X = concentration in μg

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 (R2) for each system and the values are presented in Table 1. The optical characteristics such as Bear's law limits, molar absorptivity and sensitivity values of methods are also given in Table 1. 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.

$$LOD = \frac{3.3\sigma}{S} \quad and \quad LOQ = \frac{10\sigma}{S} \qquad (2)$$

Where σ 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 2.

Accuracy and reliability of the methods were further ascertained through recovery studies. To a fixed and known amount of drug in tablets powder, pure ATH was 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 ($\bar{x}$ - μ) 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 2. 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 in dosage forms.

Table1. Optical parameters of Amitriptyline hydrochloride.

Parameter	Method A	Method B
λ_{max} (nm)	609	**507**
Beer's law limits (µg 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] (µg ml^{-1})	0.19	**5.4×10^{-3}**
Limit of quantification (LOQ) (µg 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**

Table 2 Precision and accuracy of drug in pure form

Compound name	method A						method B					
	Amount taken	Rec (%)	SD.	RSD	$\lvert \bar{X} - \mu \rvert$	$\pm ts/\sqrt{n}$	Amount taken	Rec (%)	SD.	RSD	$\lvert \bar{X} - \mu \rvert$	$\pm ts/\sqrt{n}$
ATH	2.5	105.60	0.003	1.51	0.14	0.0043	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	2.65	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**

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

Table 3. Precision and accuracy of drug in dosage form

Compound name	method B						method C					
	Amount taken (µg ml^{-1})	Rec (%)	SD.	RSD	$\lvert \bar{X} - \mu \rvert$	$\pm ts/\sqrt{n}$	Amount taken (µg ml^{-1})	Rec (%)	SD.	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**	**0.10**	**0.0031**	**5**	**100.30**	**0.002**	**0.22**	**0.01**	**0.0023**

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

4. Conclusions

Two useful micro methods for the determination of ATH 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.

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