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# Determination of Amitriptyline using Bromate-Bromide and two Dyes

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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

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## 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.

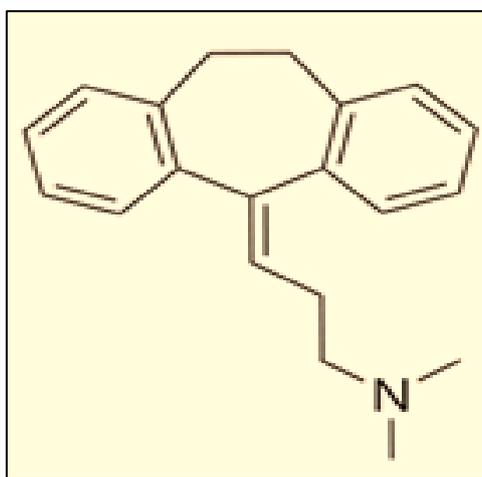


Fig. 1. Structures of Amitriptyline

## 2. Experimental

### 2.1 Apparatus

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

## 2.2 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

## 2.3 Solutions

A stock standard solution equivalent to  $1000 \mu\text{g mL}^{-1}$   $\text{KBrO}_3$  containing a large excess of  $\text{KBr}$  were prepared by dissolving accurately weighed 100 mg of  $\text{KBrO}_3$  and 1.0 g  $\text{KBr}$  in water and diluting to 100 mL in a volumetric flask. The above solution was diluted appropriately with water to get 10 and  $30 \mu\text{g mL}^{-1}$  concentrations. To prepare  $50 \mu\text{g mL}^{-1}$  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  $50 \mu\text{g mL}^{-1}$ . Hydrochloric acid ( $5 \text{ mol L}^{-1}$ ) was prepared by diluting 40.8 mL of concentrated acid to 100 ml with water and mixed well. A  $1000 \mu\text{g mL}^{-1}$  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  $100 \mu\text{g mL}^{-1}$ . The stock solution was diluted appropriately to get  $200 \mu\text{g mL}^{-1}$  dye solution with water. A stock standard containing  $500 \mu\text{g mL}^{-1}$  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  $10 \mu\text{g mL}^{-1}$  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 ( $10 \mu\text{g mL}^{-1}$  w. r. t  $\text{KBrO}_3$ ) and  $5 \text{ mol L}^{-1}$  hydrochloric acid. The flasks were stopped and let stand for 15 min with occasional shaking. Then 1 mL of  $50 \mu\text{g mL}^{-1}$  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-carmin (method B )

Varying aliquots of standard drug solution (0.5-5.0 mL; 25  $\mu\text{g mL}^{-1}$ ) 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. Accurately measured 1.5 of bromate – bromide solution (30  $\mu\text{g mL}^{-1}$  w. r. t  $\text{KBrO}_3$  ) was added to each flask followed by 1 mL of 5 mol  $\text{L}^{-1}$  hydrochloric acid. The flasks were stopped and let stand for 15 min with occasional shaking. Then 1 mL of 200  $\mu\text{g mL}^{-1}$  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

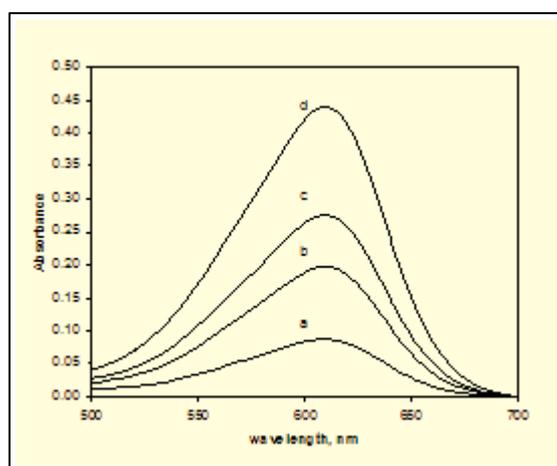
### 3.1 Spectrophotometric methods

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.

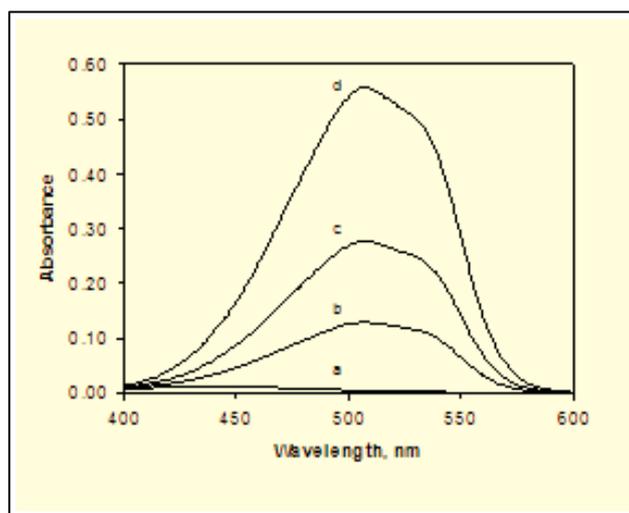
The amounts of bromine reacted correspond to the amount of ATH which formed the basis for the assay of each of mentioned drug and both of reaction were found to follow a 2:3 stoichiometry for drug:KBrO<sub>3</sub>.

### 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  $\lambda_{\max}$  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  $200 \mu\text{g}/\text{ml}$  indigo carmine in the presence of  $30 \mu\text{g}/\text{ml}$  bromate. (a) without ATH ; (b) with  $2.5 \mu\text{g}/\text{ml}$  ATH; (c) with  $5 \mu\text{g}/\text{ml}$  ATH and (d) with  $10 \mu\text{g}/\text{ml}$  ATH or.



**Fig. 3.** Absorption spectra of  $5 \mu\text{g/ml}$  methyl orange in the presence of  $1 \mu\text{g/ml}$  bromate. (a) without ATH,; (b) with  $1 \mu\text{g/ml}$  ATH,; (c) with  $2 \mu\text{g/ml}$  ATH, and (d) with  $4 \mu\text{g/ml}$  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  $5 \mu\text{g mL}^{-1}$  for indigo carmine and methyl orange, respectively. A bromate concentration of  $1.0 \mu\text{g mL}^{-1}$  in the presence of an excess of bromide was found to irreversibly destroy the red colour of  $5 \mu\text{g mL}^{-1}$  methyl orange, whereas  $4.5 \mu\text{g mL}^{-1}$  bromate was required to destroy  $20 \mu\text{g mL}^{-1}$  indigo-carmin under similar concentration of bromide. The reaction was complete in 15 min in both methods and constant 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 *in situ* 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.

### 3.3 Analytical parameters

A linear correlation was found between absorbance at  $\lambda_{\text{max}}$  and concentration of ATH (Table 1). 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):

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

(where  $Y$  = absorbance of  $\text{cm}^{-1}$  layer of solution ;  $a$  = intercept;  $b$  = slope and  $X$  = concentration in  $\mu\text{g}$

$\text{mL}^{-1}$ ). 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. The optical characteristics such as Beer'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.

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

Where  $\sigma$  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 ( $\bar{x} - \mu$ ) 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 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.

**Table 1.** Optical parameters of Amitriptyline hydrochloride.

Parameter	Method A	Method B
$\lambda_{\max}$ (nm)	609	507
Beer's law limits ( $\mu\text{g ml}^{-1}$ )	1.25- 12.5	0.5- 5
Molar absorptivity ( $1 \text{ mol}^{-1} \text{ cm}^{-1}$ )	$9.8 \times 10^3$	$4.2 \times 10^4$
Limit of detection (LOD) <sup>a</sup> ( $\mu\text{g ml}^{-1}$ )	0.19	$5.4 \times 10^{-3}$
Limit of quantification (LOQ) ( $\mu\text{g ml}^{-1}$ )	0.59	0.016
<i>Regression equation (Y)<sup>c</sup></i>		
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	$ \bar{X} - \mu $	$\pm ts/\sqrt{n}$	Amount taken	Rec (%)	SD.	RSD	$ \bar{X} - \mu $	$\pm ts/\sqrt{n}$
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 ( $\mu\text{g ml}^{-1}$ )	Rec (%)	SD.	RSD	$ \bar{X} - \mu $	$\pm ts/\sqrt{n}$	Amount taken ( $\mu\text{g ml}^{-1}$ )	Rec (%)	SD.	RSD	$ \bar{X} - \mu $	$\pm ts/\sqrt{n}$
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 be used as alternatives for rapid and routine determination of bulk sample and tablets as a part of industrial quality control.

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