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SPECTROPHOTOMETRIC AND SPECTROFLUORIMETRIC DETERMINATION OF AMINEPTINE AND NORTRIPTYLINE THROUGH TERNARY COMPLEX FORMATION WITH EOSIN AND Cu (II)

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ABSTRACT

Two sensitive, spectrophotometric and spectrofluorimetric procedures are developed for the determination of two tricyclic antidepressant (amineptine hydrochloride and nortriptyline hydrochloride). Both methods are based on the formation of ternary complex between copper (II), eosin and the two cited drugs. Spectrophotometrically, the complex was estimated by two methods, the first method depends on the extraction of the ternary complex with chloroform. The complex showed an absorption maximum at 524 and 502 nm. for amineptine and nortriptyline, respectively. The second spectrophotometric method depends on the direct measurement of the complex after addition of sodium lauryl sulphate (for amineptine hydrochloride) or methyl cellulose (for nortriptyline hydrochloride) as surfactant at pH 3.6. Under the optimum conditions, the ternary complexes showed an absorption maximum at 550 nm for amineptine and nortriptyline. The solution of the ternary complex obeyed Beer's law in concentration range 2 - 14 and 6 - 20 μg ml^{-1} with the first method, 0.8 - 8 and 1.6 - 9.6 μg ml^{-1} with the second method for amineptine and nortriptyline, respectively. The proposed methods were applied to the determination of the two cited drugs in pharmaceutical tablets. A fluorescence quenching method for the determination of the two tricyclic antidepressant by forming this ternary complex was also investigated for the propose of enhancing the sensitivity of the determination.

INTRODUCTION

Amineptine hydrochloride 7-[(10,11- Dihydro -5H-dibenzo[a,d]cyclohepten -5-yl) amino] heptanoic acid and nortriptyline 3-(10,11-Dihydro -5H-dibenzo [a,d] cyclohepten-5-ylidene)-N-methyl-1-propanamine are tricyclic antidepressant used widely in endogenous depression and may also be effective in some cases of reactive depression [1] . The few reported methods in the literature for the determination of the non official drug, amineptine are HPLC [2-4] and GC-MS [5] . For nortriptyline varity of HPLC methods have been described for the routine clinical determination [6,7] . Few photometric and fluorimetric methods have been reported for the determination of nortriptyline. They usually involve ion-pair extraction into dichloromethane or chloroform, and use Chrome Azurol S [8] , anthracene -2-sulphonate [9] or tetrabromosulphone fluorescein [10] as the reagent with photometric [8] or fluorimetric [9,10] . In view of the fact that amineptine hydrochloride did not possess colorimetric or fluorimetric methods, it is useful to have a simple colorimetric and fluorimetric methods for its determination.

This paper reports simple, sensitive and accurate spectrophotometric and fluorimetric methods for the determination of the two cited drugs. The two methods are based on the chelate-forming ability of the two drugs with copper (II) with the subsequent formation of the ternary complex with eosin (sodium salt of 2,4,5,7-tetrabromofluorescein).

EXPERIMENTAL

Apparatus

-Shimadzu 1601 UV recording spectrophotometer

-Shimadzu RF-1501 spectrofluorophotometer.

- UV lamp-shot wavelength 254 nm

- TLC plates 20 x 20 cm with 0.25 mm thickness gel 60 F_{254} (E. Merck)

Materials and reagents

All the reagent were of analytical grade. Double distilled water was used

Amineptine hydrochloride pure drug and Survector® tablets (labelled to contain 100 mg amineptine hydrochloride per tablet) were obtained from Servier Egypt Industries Limited.

Nortriptyline hydrochloride pure drug and Motival tablets (labelled to contain 10 mg nortriptyline hydrochloride and 0.5 mg fluphenazine hydrochloride per tablet) were obtained from Bristol-Myers Squibb, Egypt.

Eosin (PS PARIC Scientific limited), 0.1% solution in distilled water.

Copper (II) sulphate , 0.2% solution in distilled water.

Sodium lauryl sulphate 0.5% w/v in distilled water.

Methylcellulose, 0.1% w/v in distilled water (with the aid of heat)

Developing system; Cyclohexane / toluene / diethylamine (75 : 15 : 10).

Phthalate buffer pH 3.6, to 250 ml of 0.2 potassium hydrogen phthalate, 11.94 ml of 0.2 M HCl was added, the solution was diluted to 1000 ml with water.

Standard stock solution:

Solution of 0.2 mg ml^{-1} were prepared by dissolving 20 mg of amineptine hydrochloride or nortriptyline hydrochloride in 2 ml methanol in 100 ml volumetric flask and diluting to volume with distilled water. the solution was stable for at least 1 weak.

General procedures

Spectrophotometric method:

Method 1 (Extractive spectrophotometric procedure):

Appropriate volumes of the standard solution in the concentration range 0.02 - 0.14 mg of amineptine or 0.06 - 0.2 mg of nortriptyline were placed in 50-ml separating funnels. The volume of each solution was adjusted to 10 ml with distilled water. Five milliliters of copper(II) sulphate solution was added followed by 0.8 ml of eosin solution. The complex was extracted with 3 x 3 ml portions of chloroform. The solution was shaken for 1 min. each time and the chloroform layer was passed through a layer of anhydrous sodium sulphate into 10 ml volumetric flask. The volume of the chloroform layers was made up to 10 ml, and the absorbance was measured at 524 nm for amineptine and 502 nm for nortriptyline against blank in which the drug is omitted.

Method II (Spectrophotometric procedure using surfactants):

Appropriate volumes of the standard solution in the concentration range 0.02- 0.2 mg of amineptine or 0.04 - 0.24 mg of nortriptyline were pipetted into two sets of 25 ml volumetric flasks. A1.5 ml of 0.1% eosin solution, 1 ml of 0.2% copper (II) sulphate, 1.5 ml 0.5% sodium lauryl sulphate or 1 ml 0.1% methyl cellulose, for amineptine or nortriptyline, respectively and 3 ml of the buffer solution pH 3.6, were added to the flasks, in this order. The mixture was diluted to volume with water, homogenized by shaking, allow to stand for 15 min. at room temperature (25 ± 5°C) and the absorbance of the solution (solution A) was measured at 550 nm against a similarly prepared eosin copper(II) sulphate and buffer solution (solution B).

Fluorimetric procedure:

Volumes of the above solutions (in concentration of 2.5 - 80 µg for the two cited drugs) were pipetted into two 10 ml volumetric flasks and diluted to volume with water. The difference in the relative fluorescence intensities between solutions A and B at a 545 nm emission wavelength with excitation at 308 nm was measured.

The concentration of amineptine or nortriptyline in the analyte solutions can be determined by reference to corresponding calibration graphs, which had been constructed previously according to the regression equations (Table 1).

Assay of pharmaceutical tablets:

For amineptine hydrochloride

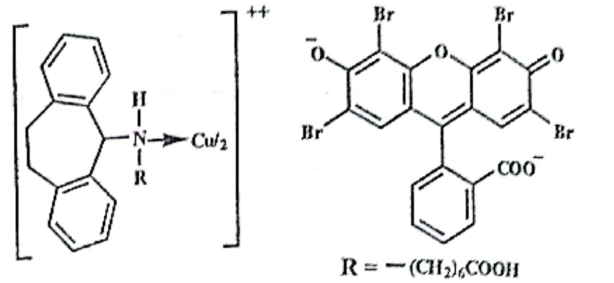
Five tablets of servector tablet were powdered and a quantity of the powder equivalent to 20 mg of amineptine hydrochloride was dissolved by shaking with 2 ml methanol followed by 40 ml of water. The solution was filtered through filter-paper into a 100 ml volumetric flask and then diluted to volume with water. The assay of amineptine HCL content was completed as described above in general procedures.

For nortriptyline hydrochloride

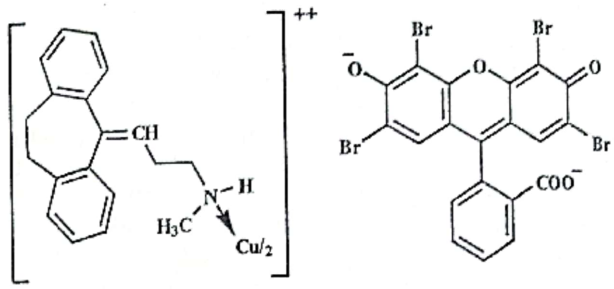
Five tablets were washed from the colour coat using distilled water, dried, weight and powdered. A quantity of powder tablets equivalent to 20 mg nortriptyline was dissolved by shaking with 10 ml methanol. 200µl (equivalent to 400 µg of nortriptyline) of this solution was applied in band form to TLC plate. The plate was placed in chromatographic tanks previously saturated for 1 hour with developing mobile phase and then air dried. The spot corresponding to nortriptyline were visualized under UV light at 254 nm, scraped, extracted with 1 ml methanol followed by 7 ml water, filtered and the volume was completed to 10 ml with water. The assay of nortriptyline HCl content was completed as described above in general procedures.

RESULTS AND DISCUSSION

Ternary complexes of general formula ($L_NM_XS_Y$) have been widely used in spectrophotometric analysis [11-16]. For the ternary complexes dealt with in this paper is that their main ligand L is either amineptine or nortriptyline, the second ligand S is eosin and M is copper (II) metal (Scheme 1). These triple complexes are extractable with chloroform, whereas the binary systems (copper-drug and copper - eosin) where not extractable.



amineptine



nortriptyline

Scheme 1 Stable ternary complex

Extractive spectrophotometric procedure:

The experimental conditions were established by varying one variable and observing its effect on the absorbance of the coloured product:

Five milliliters of 0.2% w/v copper (II) sulphate solution and 0.8 ml of 0.1% w/v eosin solution was found optimum to maximize the colour intensity.

It was found that three extractions each for 1 min were necessary for the quantitative estimation of the complex. The colour of the ternary complex in the chloroform was quit stable for at least 24 h.

To prove the formation of a ternary complex between copper(II) (A), eosin (B) and the drugs (amineptine or nortriptyline) (C), the interaction of the three component may be considered as

either $AB + C \leftrightarrow AC + B$ (1)

or $AB + C \leftrightarrow ABC$ (2)

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Table 1 : Optical characteristics and statistical data of the regression equations for ternary complex formation with amineptine hydrochloride and nortriptyline hydrochloride.

Parameters	Amineptine hydrochloride			Nortriptyline hydrochloride		
	Spectrophotometeric methods		Fluorimetric method	Spectrophotometeric methods		Fluorimetric method
	Method I	Method II		Method I	Method II	
Beer's law (μgm^{-1})	2-14	0.8-8.0	0.25-8.0	6-20	1.6-9.6	0.25-8.0
Molar absorptivity ($mol^{-1}cm^{-1}$)	$3.2x10^4$	$4.3x10^4$	-	$1.3x10^4$	$29x10^4$	-
Regression equation						
Intercept (a)	0.0121	0.0231	43.922	0.0110	0.0024	32.483
Slop (b)	0.0857	0.1122	4.8368	0.0446	0.0967	-1.7111
Correlation coefficient (r)	0.9989	0.9992	0.9988	0.9989	0.9997	0.9995
Relative coefficient (%)	1.981	0.854	0.747	1.683	0.985	0.779
Standard analytical error	0.074	0.062	0.055	0.078	0.064	0.049

A series of absorption spectra have been done for each component, separately, and to their mixtures under the experimental conditions, discussed above, in both aqueous and organic solvent. The spectra revealed that aqueous solution of eosin (B) absorbs in the visible region at λ_{max} 517 nm, while neither copper (II) sulphate (A) nor the drugs (C) have absorbance in the visible region. The mixture (AB) has the same maximum absorbance as that of (A) and (B) separately, also the mixture (AC) has the same maximum absorbance as that of (A) and (C), separately.

According to these considerations, and to the finding that the complexes formed have absorption maximum at 524 nm for amineptine and 502 nm for nortriptyline, the absorbance was not be additive, but it would be a ternary complex system, ABC, having different properties from that of AB or AC. Practically, extraction of aqueous solutions of the separate components with chloroform gave no absorption maxima in the visible region, while that of the ternary mixture, in the same solvent, gave a predominant absorption spectrum with (max at 524 nm for amineptine and 502 nm for nortriptyline (Fig.1)

Spectrophotometric procedure using surfactants

The absorption spectra of the ternary complex (drug-Cu-eosin) (solution A) and the blank solution (Cu-eosin) (solution B) were scanned in the range 430 - 630 nm. It was found that, on addition of the cited drugs to eosin - Cu (II) solution, a difference in absorbance was obsorved around 550 nm (Fig 2). The absorption difference was proportional to the concentration of either amineptine or nortriptyline.

To optimize the assay parameters, the effects of pH, reaction time, effect of temperature, concentration of surfactant, eosin and copper (II) sulphate on the absorbance of the ternary complex formed were studied. The effect of pH on the absorbance of the ternary complex studied at 550 nm. The absorbance of the drug -Cu (II) -eosin complex solution was investigated over a the pH ranges 2.2 - 5.2. The optimum absobance was achieved at pH from 3.6 to 4.0.

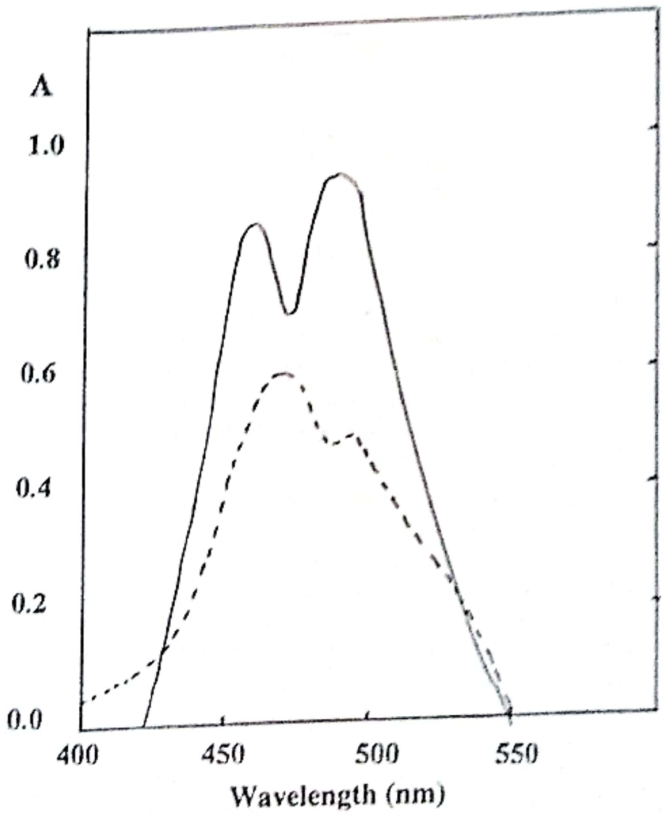

Figure 1 : Absorption spectra of the ternary complex of $7\mu gml^{-1}$ amineptine (-------) and 20 (gml-1 norpriptyline (_____) with eosin and Cu(II). (Method I)

In order to examine the effect of temperature and reaction time on the absorbance of the ternary complex, the above mentioned procedure was carried out at different temperatures (room temperature, 50, 60, 70 and 80°C) using thermostatic water bath. Maximum and constant absorbance was obtained at room temperature (25±5°C) after 15 min from the addition of the reaction contents, excessive heat decrease the absorbance sharply.

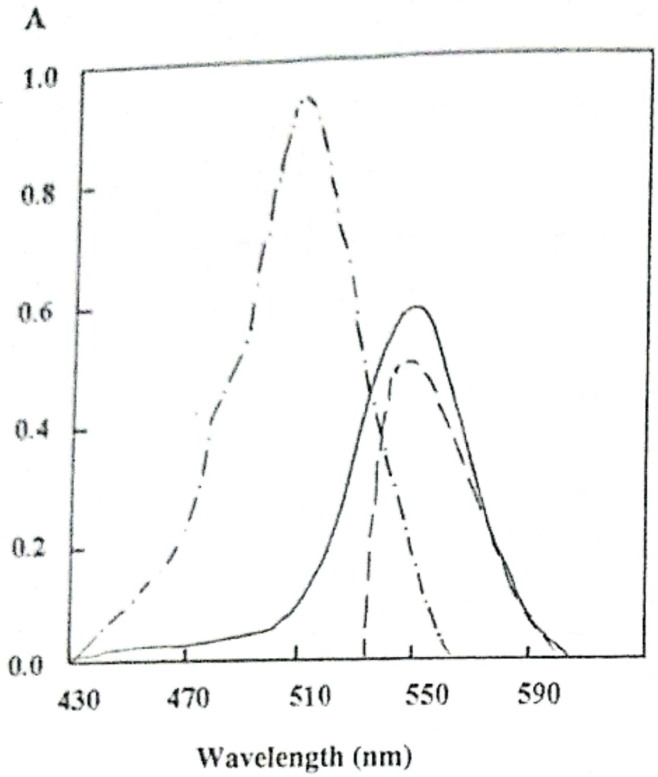

Figure 2: Absorption spectra of the ternary complex of 4 μgml^{-1} amineptine (-------) and 6 μgml^{-1} norpriptyline (_____) with eosin and Cu (II), absorption spectrum of eosin and Cu(II) solution (-.-.-.-.-) (Method II).

The effect of surfactants on the absorbance of the solution of the ternary complex was examined using various dispersing agents, such as sodium lauryl sulphate, methylcellulose, benzalkonium chloride, Tween 20 and Tween 80. Among the surfactants studied, best results were obtained in the presence of sodium lauryl sulphate for amineptine and methyl cellulose for nortriptyline

The effect of concentration of the reagents, eosin and copper (II) sulphate, on the absorbance of the ternary complex was studied. The optimum result was obtained using 1.5 ml of 0.1 % eosin and 1 ml of 0.2 % copper (II) sulphate solutions. The colour formed under the above mentioned optimum conditions was stable for at least 1 h.

Constitution of the ternary complex:

The nature of the ternary complex (drug-Cu (II)-eosin) was determined using Job's method of continuous variation [17] . The results of applying this method (procedure 1) can be summarized as follows: the [Cu (II): drug] ratio in the presence of excess eosin was 1:2 (Fig. 3a), while the [eosin : drug] ratio in the presence of excess Cu (II) sulphate was 1:2 (Fig. 3b) and the [eosin : Cu (II)] ratio in the presence of excess drug was 1:1 (Fig. 3c). Hence the composition of the ternary complex formed may be expressed as drug-Cu (II)-eosin (2 : 1: 1) (Scheme 1).

Study of the fluorimetric method:

It was found that the formation of the ternary complex reduced the fluorescence of solution B, so, a fluorescence quenching method for the determination of

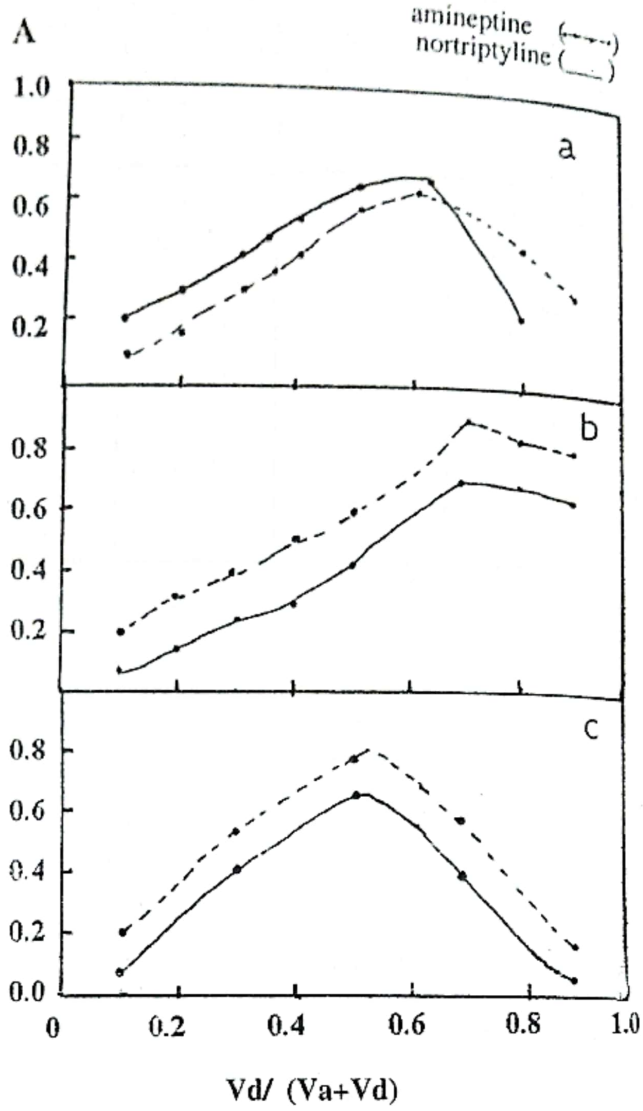

Figure 3 : **(a)** Continuous variation plots for dug: Cu (II) (5×10^{-4}) complex ratio in the presence of excess eosin (1.5 ml of 0.1% solution) Vd = drug and Va = Cu (II) **(b)** Continuous variation plots for dug: eosin (5×10^{-4}) complex ratio in the presence of excess Cu (II) (1 ml of 0.2% solution) Vd = drug and Va = eosin **(c)** Continuous variation plots for eosin : Cu(II) (2×10^{-4}) complex ratio in the presence of excess drug (2ml of 5×10^{-4}) Vd = eosin and Va = Cu (II).

amineptine and nortriptyline was developed. The uncorrected fluorescence spectra of solution A and B are shown in Fig. 4. On addition of amineptine or nortriptyline to solution B, the relative fluorescence intensity of the solution B decrease significantly. and the magnitude of the decrease was proportional to the concentration of the drug. In the development of the procedure for fluorimetric measurements, the same conditions as for the spectrophotometric method were adopted. The spectrophotometric and fluorimetric characteristics are summarized in table 1.

Quantification, accuracy and precision:

A linear correlation was found between

absorbance and concentration in the ranges given in table 1. The correlation coefficients, intercepts and slopes for the calibration data for the two cited drugs are calculated using the least-squares method.

The precision and accuracy of the two methods were tested by estimating five replicates of the two cited drugs within the Beers law limits. The percentage standard deviation and the standard analytical error, can be considered to be very satisfactory (Table 1).

The utility of each method was verified by means of replicate measurements of the pharmaceutical formulations and recovery experiments. Recoveries were determined by adding standard drug to the pre-analyzed mixture of pharmaceutical preparations. The results of recovery experiments by the proposed methods are listed in Tables 2 and 3.

Table 2: Assay of amineptine hydrochloride in Survector tablets by the proposed method and refernce method [18] .

Statistics	Reference method λ_{max}	Spectrophotometeric methods		Fluorimetric method
		Method I	Method II	
Mean recovery	98.01	98.44	98.20	98.42
±SD	0.44	0.19	0.39	0.25
N	5	5	5	5
Variance	0.19	0.04	0.15	0.06
t-test		21=.16	0.72	1.88
		(2.306)	(2.306)	(2.306)
F-test		4.75	1.26	3.16
		(6.39)	(6.39)	(6.39)

Values in parenthesis are the tabulated values of t-and f-at p= 0.05
N is the number of experiments where each results is the average of triplicate measurements.

Table 3: Assay of nortriptyline hydrochloride in Motival tablets by the proposed method and refernce method [19] .

Statistics	Reference method λ_{max}	Spectrophotometeric methods		Fluorimetric method
		Method I	Method II	
Mean recovery	97.21	97.92	98.02	97.56
±SD	1.02	0.51	0.69	0.44
N	5	5	5	5
Variance	1.04	0.26	0.48	0.19
t-test		1.73	1.49	0.75
		(2.306)	(2.306)	(2.306)
F-test		4.00	2.16	5.47
		(6.39)	(6.39)	(6.39)

Values in parenthesis are the tabulated values of t-and f-at p= 0.05
N is the number of experiments where each results is the average of triplicate measurements.

The performance of the methods was assessed by calculation of the t- and F- values compared with the reference methods [18,19] and for The results showed that the calculated t- and F- values did not exceed the theoretical values (95% confidence limits for five degree of freedom), Tables 2 and 3. from which we can conclude that the proposed methods do not differ significantly from reference methods [18,19] .

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الملخص العربى

التقدير الطيفى واللصفى للامينبتين والنورتربتيلين من خلال تكوين معقد ثلاثى مع كاشف الأيوسين وفي وجود عنصر النحاس الثنائى

ماجدة محمد عياد ، هشام عزت عبداللطيف ، الهام أنور طه * ، وسوزان محمود سليمان *

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* الهيئة القومية للأبحاث والرقابة الدوائية - الجيزة - مصر

فى هذا البحث تم استحداث طريقتين أحداهما طيفية والأخرى لصفية لتعين بعض المركبات ثلاثية الحلقات والمستخدمه كمضادات للإكتئاب وهى مركب الأمينبتين والنورتربتيلين وذلك من خلال تكوين معقد ثلاثى مع كاشف الأيوسين وفى وجود عنصر النحاس الثنائى . هذا وقد تم فى الطريقة الطيفية قياس المعقد بطريقتين . الأولى بعد استخلاصه من الوسط المائى باستخدام مذيب الكلوروفورم وقياس اللون الناتج عن درجة ٥٢٤ ، ٥٠٢ نانوميتر للامينبتين والنورتربتيلين على الترتيب . بينما فى الطريقة الثانية تم إضافة بعض المواد نشيطة السطح لأذابة المعقد بدلاً من استخلاصه وتم قياس المعقد عند درجة ٥٥٠ نانوميتر وعند درجة حموضة ٣٫٦ درجة .

هذا وقد تم استخدام وتطبيق هذه الطرق على بعض المستحضرات الصيدلية المحتوية على هذه المركبات وقد كانت النتائج جيدة ومماثلة للطرق المنشورة فى الدوريات العلمية حيث أن مركب الأمينبتين غير دستورى.