Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 10, 2015

Original Article

STUDY ON THE INTERACTION OF AMITRIPTYLINE HYDROCHLORIDE AND MALONIC ACID BY SPECTROPHOTOMETRIC AND SPECTROFLUORIMETRIC METHODS IN TABLETS: APPLICATION TO CONTENT UNIFORMITY TESTING

TAGHREED ABDU-EL-FATTAH MOHAMMED

Department of Analytical Chemistry, National Organization for Drug Control and Research (NODCAR),6-Abu Hazem street, Pyramids Ave. P. O. Box 29, Post Code 35521, Giza, Egypt
Email: dr.taghreednodcar@gmail.com

Received: 06 Jul 2015 Revised and Accepted: 08 Aug 2015

ABSTRACT

Objective: The objective of this research to study the interaction between amitriptyline hydrochloride in drug products via reaction with mixed acids anhydrides: Application to Content Uniformity Testing**.**

Methods: Malonic acid anhydride (MAA), is a labeling reagent known to react with tertiary amines and forming stable condensation colored product which can be measured either spectrophotometrically, or spectrofluorometrically. Different experimental parameters were established by varying each in turn while keeping others constant. These factors include; effect of concentration of MAA, effect of diluting solvent, effect of heating temperature and time.

Results: Linearity of the proposed methods was found to be between 0.5–10 µg/ml µg/ml with good correlation coefficient (0.9998) for spectrophotometrically method, and 0.05–6 µg/ml with good correlation coefficient (0.9998) for spectrofluorometrically method. LOD was found to be 0.168 µg/ml and 0.016 µg/ml, and LOQ was calculated to be 0.510 µg/ml and 0.050 µg/ml for two methods respectively. The % recovery of the proposed methods was found to be 100.06 %-100.07 %. The method was found to be precise as the values of % RSD obtained for both intraday and interday, precision studies were found to be<2.0 %. The molar absorptivity, Sandell sensitivity and Quantum yield were calculated. Also the activation energy, enthalpy, entropy, and Gibbs free energy change of the reaction were evaluated. The methods were applied to the analysis of commercial tablet Tryptizol (10 mg, 25 mg). The study was extended to content uniformity testing.

Conclusion: The present study described the successful evaluation of MAA reagent in the development of simple and rapid spectrophotometric and spectroflourimetric methods for the accurate determination of APT in drug substance and drug products.

Keywords: Amitriptyline HCl, Malonic acid, Spectrophotometric, Spectrofluorimetric, Activation energy, Quantum yield, Content uniformity.

INTRODUCTION

Amitriptyline HCl (APT) is, 3-(10, 11-Dihydro-5H-dibenzo [a, d] cyclohepten-5-ylidene)-N, N dimethyl-1-propanamine [1], (fig. 1.) It is a tricyclic antidepressant used in the case of anxiety and also exerts an anticholinergic activity [2].

APT is official in, BP and USP. The BP [3] and USP [4] describe potentiometric and HPLC methods, respectively for estimation of APT. A literature survey revealed near-infrared spectroscopy method [5]. Determination by UV spectrophotometric method [6-9] and chromatographic methods of APT with other antipsychotic agents like nortriptyline, prochlorperazine maleate, and Chlordiazepoxide in human blood [10-13], thin layer chromatographic methods of APT with other antipsychotic agents [14-16]. Fluorescence quenching effect and chemiluminescence analysis used for determination of APT [17, 18]. APT HCl determined by chemometric method, capillary electrophoresis method and flow-injection potentiometric method [19-21].

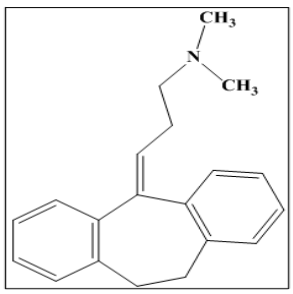


Fig. 1: Structure of amitriptyline hydrochloride

So the aim of this work is to develop a comparative study of recent, simple and sensitive validated methods that are of lower cost than the reported HPLC methods. Due to the high sensitivity of the proposed methods, the methods are ideally suited for content uniformity testing.

Malonic acid anhydride (MAA) is a labeling reagent known to react with tertiary amines and forming stable condensation colored product which can be measured either spectrophotometrically [22] or spectro fluorometrically [23]. Malonic acid anhydride has been utilized for determination of several drugs containing a tertiary amino group such as tramadol, acebutolol and dothiepin [24].

METHODS AND MATERIALS

SHIMADZU UV-2450 PC Series Spectrophotometer (Japan) with two matched 1 cm quartz cells using the following spectral parameters: Scan mode: absorbance, Speed: fast and Slit width: 2 nm.

Fluorescence spectra and measurement were recorded using an Agilent Cary Eclipse Fluorescence Spectrophotometric, equipped with the xenon flash lamp, grating excitation and emission monochromators for all measurements and an Agilent Cary Eclipse recorder. Slit width for both monochromators was set at 10 nm.

Amitriptyline hydrochloride (APT) was kindly supplied by Future Pharmaceutical Industries, Egypt, with a purity of 100.06% as determined by the official method [4].

Acetic acid anhydride El-Nasr Pharmaceutical Chemicals Co, (ADWIC).

Malonic acid anhydride (Qualikems Fine Chemicals, Pvt. Ltd, and New Delhi 110060) was freshly prepared as 8% w/v in acetic acid anhydride for Method I and 0.8% w/v for Method II.

Ethanol 96% was obtained from Panera Quimica S. A. U.

Tryptizol tablets, were purchased from local pharmacies: batch #1210735, labeled to contain 10 mg, and batch #1210846, labeled to contain 25 mg APT HCl/tablet, manufactured by: Kahira Pharm. & Chem. IND. Co. Cairo-Egypt.

Preparation of solutions

Stock APT solution

An accurately weighed amount 10 mg of APT was quantitatively transferred into a 100-ml volumetric flask, dissolved in 80-ml ethanol 96%, completed to volume with the same solvent to obtain a stock solution of 0.1 mg/ml. The stock solution was found to be stable for at least two weeks when kept in a refrigerator. The stock solution was further diluted with ethanol to obtain working solutions in the range of 5-100 µg/ml for method (I) and 0. 5–60 µg/ml for method (II).

Tablets sample solution

Ten tablets were weighed and finely powdered. An accurately weighed amount of the powdered tablets equivalent to 10 mg of APT was transferred into a 100-ml volumetric flask, and dissolved in about 80-ml of ethanol. The contents of the flask were shaking well, sonicated for 30 min, and then completed to volume with ethanol. The contents were mixed well and filtered; the filtered solution was diluted quantitatively with ethanol to obtain suitable concentrations for the analysis by the proposed methods (I) and (II).

General procedures

Construction of the calibration curve

Spectrophotometric method (Method I)

Aliquots containing the drug (5–100 µg/ml) were quantitatively transferred to a set of screw capped tubes. The solvent was evaporated till dryness using a water bath then the tubes cooled under tap water. To each tube, 2.0 mL of 8% w/v MAA reagent was added and mixed well. The solutions were heated in thermostatically controlled water bath at 90 °C for 15 min. The solutions were cooled and quantitatively transferred to 10 mL volumetric flasks. Each flask was made up to volume with ethanol. The absorbance of the reaction product was measured at 485 nm against a reagent blank prepared simultaneously. The calibration graph was constructed by plotting the absorbance versus concentration of the drug (µg/ml). Alternatively, the corresponding regression equation was derived.

Spectro fluorimetric method (Method II)

Aliquots containing the drug (0. 5–60 µg/ml) were quantitatively transferred to a set of screw capped tubes. The mentioned procedure under Spectrophotometric method (Method I) was adopted using 0.8 % w/v MAA. The fluorescence intensity was measured at 560 nm after excitation at 480 nm. The calibration graph for the proposed method was constructed by plotting the fluorescence intensity versus the drug concentration (µg/ml). Alternatively, the corresponding regression equation was derived.

Procedure for content uniformity testing

The same procedure applied for the analysis of amitriptyline hydrochloride in tablets was adopted using one tablet as a sample. Ten different tablets were assayed and the uniformity of their contents was tested by applying the official USP [4]guidelines (Chapter 905: Uniformity of Dosage Units).

Determination of stoichiometric ratio

The stoichiometry of the reaction was done using the Job's method [25], and the limiting logarithmic method [26].

Job's method

The Job's method of continuous variation [25] was employed. Master equimolar (1×10^{-4}M) aqueous solutions of APT and MAA were prepared. Series of 10-ml portions of the master solutions of APT and AMM was made up comprising different complementary proportions (0:10, 1:9. 9:1, 10:0, inclusive) in 10-ml volumetric flasks. The solutions were further manipulated as described under Spectrophotometric method (Method I).

Limiting logarithmic method

The limiting logarithmic method [26] was employed. Two sets of experiments were carried out employing the general recommended procedures described above. The first set of experiments was carried using varying concentrations of the AMM (1.7×10^{-3}–13.6×10^{-3}M) at a fixed APT concentration (1×10^{-4}M). The second set of experiments was carried using varying concentrations of APT (7.5×10^{-6}-2.5×10^{-4}M) at a fixed concentration of AMM (1.7×10^{-3}M). The logarithms of the obtained absorbance for the reaction of APT with MAA were plotted as a function of the logarithms of the concentrations of the reagent and APT in the first and second sets of experiments. The slopes of the fitting lines in both sets of experiments were calculated.

RESULTS AND DISCUSSION

The reported spectrophotometric methods for APT is derivative spectrophotometric methods in which the Vierordt method by measuring the peaks amplitude at 240 nm [7]was used, where APT was determined by Ratio spectra derivative spectrophotometry measuring the peak amplitude at 218 nm [9].

These methods were lack the simplicity which is found usual in this technique. The absorption spectrum of APT was recorded against ethanol (fig.2). It was found that APT exhibits maximum absorption peak (λ_{max}) at 240 nm and the molar absorptivity (ε) at this wavelength was 1.1×10^4l/ml/cm. Because of the blue shifted λ_{max} of APT, its determination in the pharmaceutical formulations based on the direct measurement of its absorption for ultraviolet light is susceptible to potential interferences from the co-extracted excipients. Therefore, derivatization of APT to a more red-shifted derivative was necessary. APT contains a tricyclic ring system with an alkyl amine substituent on the central ring. For which many chromogenic reagents are available for color-producing reactions. In the present study was devoted to investigate the reaction between APT and MAA, and employed this color reaction in the development of a new simple and rapid spectrophotometric method for determination of APT in its tablets. The reaction between APT and MAA was performed, and the absorption spectrum of the reaction product was recorded against reagent blank. The product was highly yellow-colored exhibiting λ_{max} at 485 nm (fig.2). Obviously, the λ_{max} of APT-MAA derivative was red-shifted from the underivatized APT by 245 nm. As well, the value of ε (sensitivity) was greatly enhanced to be 3.2×10^5l/mol/cm. The following paragraphs describe the optimization of the reaction conditions.

In the spectrofluorimetric method, the same product exhibits fluorescence at 560 nm after excitation at 480 nm but in this method used 0.8% w/v MAA, as shown in (fig. 3).

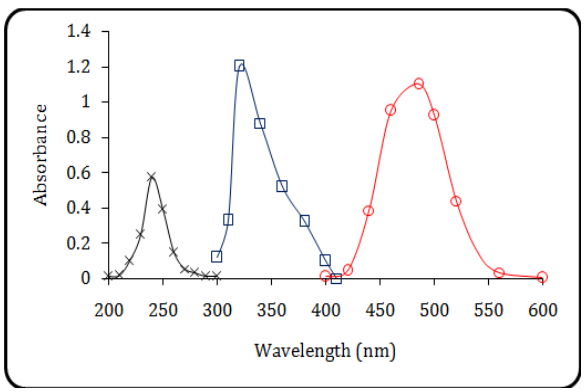


Fig. 2: Absorption spectra of: (a) APT (10µg/ml) in ethanol, (b) the reaction product of APT (10µg/ml) with (8% w/v MAA) in ethanol, (c) blank solution of MAA

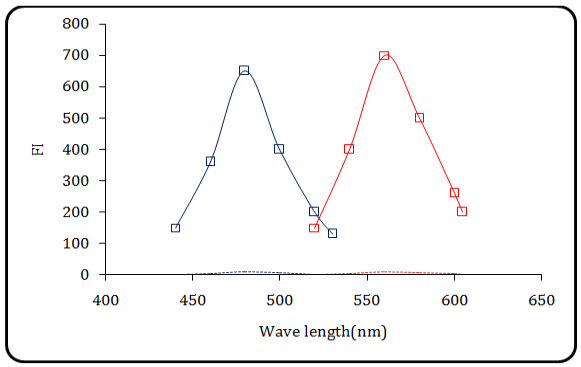


Fig. 3: Fluorescence spectra of (a, b) reaction product of APT (6µg/ml) with (0.8% w/v) MAA, (a `, b`)blank of (0.8% w/v) MAA

Optimization of reaction conditions

Different experimental parameters were carefully studied namely: effect of concentration of MAA, effect of diluting solvent, effect of heating temperature and time.

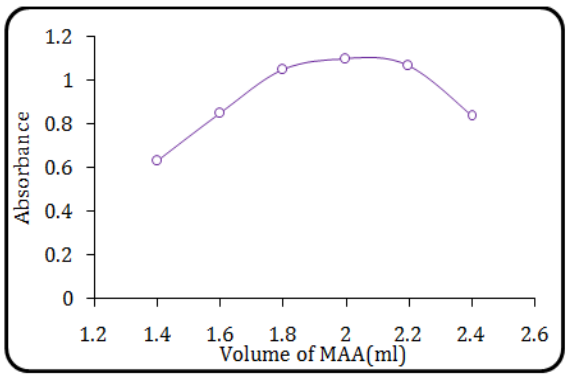


Fig. 4: Effect of volume of MAA (8%w/v) on the absorbance value of the reaction product of APT (10µg/ml) with MAA

Effect of volume of MAA

The influence of the concentration of MAA was studied using different volumes of 8% (w/v) and 0.8% (w/v) solution of MAA in acetic anhydride for spectrophotometric and spectrofluorimetric methods respectively. It was found that, increasing volume of the reagent produced a proportional increase in the absorbance of the reaction up to 1.8 mL. However, no further increase in absorbance was observed upon increasing the volume of the reagent up to 2.2 mL, after which a gradual decrease in the absorbance value of the reaction product. Therefore, 2±0.2 mL of 8% (w/v) and 0.8 % (w/v) MAA solution was chosen as the optimal volume of the reagent for methods I and II respectively, (fig. 4).

Effect of diluting solvent

Upon diluting the reaction solutions with ethanol, transparent solution was obtained indicating the solubility of the APT-MAA product in ethanol, and the possibility of using ethanol as a diluting solvent. In order to select the most appropriate solvent for diluting the reaction solutions, different solvents were tested and compared with ethanol; methanol, isopropanol, acetonitrile, and 1,4-dioxane. The highest readings were obtained upon such as ethanol was used for dilution. However, the use of isopropanol, acetonitrile decrease in the absorbance value of the reaction product, 1, 4 dioxane led to very turbid solution. Methanol and ethanol gave almost absorbance value. However, the reproducibility upon using methanol was found to be adversely affected. Of all the solvents studied, the highest absorption intensity with maximum product stability was attained upon using ethanol as diluting solvent. Therefore, ethanol was chosen an optimum diluting solvent throughout this approach, as shown in (fig. 5).

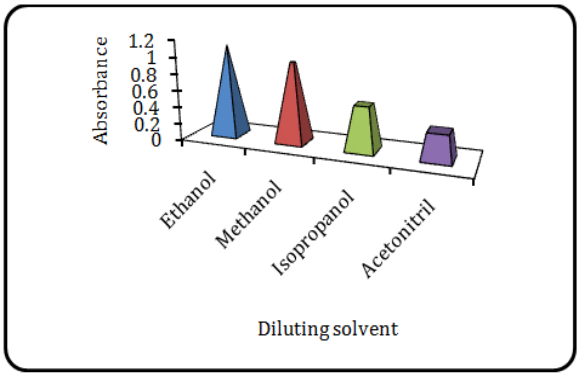


Fig. 5: Effect of diluting solvent on the absorbance of the reaction product between APT (10µg/ml) with MAA (8%w/v)

Effect of heating temperature and time reaction

Different temperature settings were tested to ascertain the temperature after which the reaction product attained its maximum absorbance values. Different temperatures were tested using a thermostatically controlled water bath ranged from 40–100 °C. It was found that increasing the temperature resulted in a gradual increase in the absorbance value of the reaction product up to 90 °C and then remained constant to 100 °C. Therefore, the reaction was performed at 90 °C within 15±2 min (fig. 6).

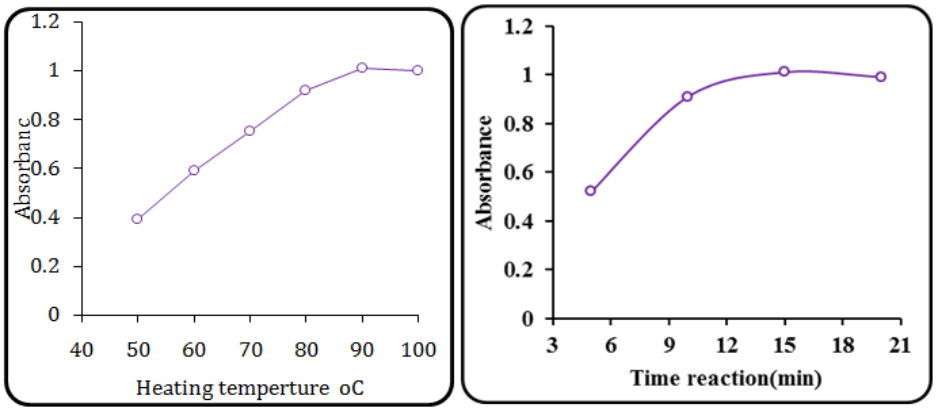


Fig. 6: Effect of heating temperature and reaction time on the absorbance value of the reaction product of APT (10µg/ml) with MAA (8%w/v)

Stability of the chromogenic

Under the optimum condition, the reaction between APT-MAA was complete after 15 min at heat temperature 90°C, and the absorbance no longer changed after standing for up to 20 min. It was found that the absorbance of the chromogenic remains stable for at least 1 h. This allowed the processing of large batches of samples, and their comfortable measurements with convenience. This gives the high throughput property to the proposed method when applied for analysis of large number of samples in quality control laboratories.

Stoichiometry, Kinetics and mechanism of the reactions

Under the optimum conditions, the stoichiometry of the reaction between APT and MAA was investigated by Job's [25]and limiting logarithmic [26] methods. The symmetrical bell shape of Job's plot (not shown data) indicated that the APT: MAA ratio was 1:1. In the limiting logarithmic method, two straight lines were obtained (fig. 7). The slopes of these lines were 1.04and 1.05, confirming the 1:1 ratio for the reaction. Based on this ratio, the reaction pathway was postulated to be proceeded as shown in scheme 1.

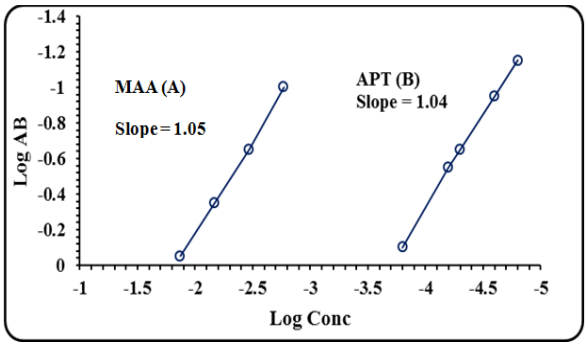
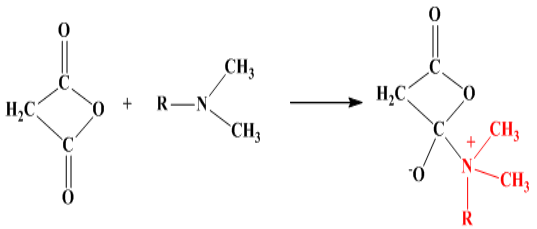


Fig. 7: Limiting logarithmic plot for molar reactivity of APT with MAA. C and A are the concentration and absorbance, respectively. For generating the first line (A), MAA: [1.7×10^{-3}-13.6×10^{-3} M]; APT: [1×10^{-4}M]. For generating the second line (B), APT [7.5×10^{-6}–2.5×10^{-4} M]; MAA: [1.7×10^{-3}M]



Scheme 1: Proposed reaction pathway between MAA and APT under the described reaction conditions

For kinetic study using the optimum conditions, the absorbance-time curves for the reaction of APT at several concentrations (1.3 × 10^{-4} – 1.9 × 10^{-4} M) with a fixed concentration of MAA (1.7 × 10^{-3} M) were generated, and the initial reaction rates (K) were determined from the slopes of the curves. The logarithms of the reaction rates (Log K) were plotted as a function of logarithms of APT concentration (log C). As seen in (fig. 8), a straight line with a slope value of 1.06 was obtained by fitting the data to the following equation:

logK = logK' +n logC. Where K is the reaction rate, K' is the rate constant, C is the molar concentration of APT and n (slope of regression line) is the order of the reaction. The values of the slope (≈1) confirmed that the reaction was first order. However, under the optimized reaction conditions, the concentrations of MAA were in much more excess than that of APT in the reaction solution. Therefore, the reaction was regarded as pseudo-first order reaction.

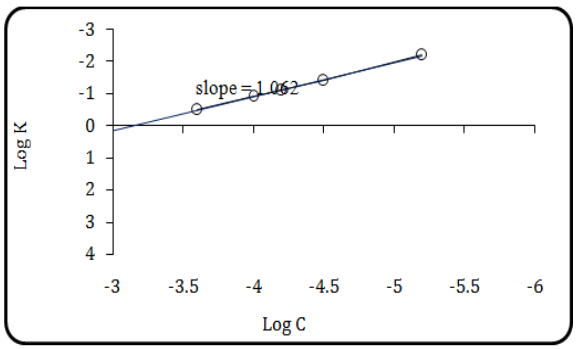


Fig. 8: Linear plot for log C versus log K for the kinetic reaction of APT with MAA. C is the APT concentration (1.3 × 10^{-4} – 1.9 × 10^{-4} M) and *K* is the reaction rate (second^{-1})

The apparent rate constant, Activation energy and quantum yield

The absorbance-time curves at three different temperatures (30, 50, 70 and 90°C) were generated using fixed concentrations of APT (1.5 × 10^{-4} M) and MAA (1.7 × 10^{-3} M). From these curves, the apparent rate constants were calculated. These rates were found to be 6.92 × 10^{-4}, 6.7 × 10^{-4}, 6.35 × 10^{-4} and 6.11× 10^{-4} second^{-1} at 30, 50, 70, and 90°C, respectively. The activation energy, defined as the minimum kinetic energy that a molecule possess in order to undergo a reaction, was determined using Arrhenius equation [27]: where k is the apparent rate constant, A is the frequency factor, Ea is the activation energy, T is the absolute temperature, and R is the gas constant. By plotting logK as a function of 1/T, a straight line with a slope value of 1.25 = – Ea/2.303 R. From this data, E* the activation energy, the entropy ΔS*, enthalpy of Δ H* and free energy ΔG* of activation were calculated using the following equation:

$$\Delta S^* = 2.303\ [\log (Ah/kT)]\ R$$

$$\Delta H^* = E^* - RT$$

$$\Delta G^* = H^* - Ts\ \Delta S^*$$

Arrhenius[27] equation	E* the activation energy	the entropy ΔS*	enthalpy of Δ H*	free energy ΔG*	A (s^{-1})
gas constant (R=2.004) (Kcal. mol^{-1})	5.76	-0.022	0.013	-6.51	6.48 x 10^9

Because of this low activation energy, the nucleophilic substitution reaction between APT and MAA could be easily taken place, and MAA could be used for determination of APT.

Determination of fluorescence Quantum Yield of amitriptyline HCl reaction product with MAA was obtained applying the following equation:

Yu = Ys. Fu/Fs. Au/As [28]

Where s and u are stand for reference and APT respectively, F is the area under the corrected emission spectrum, A is the absorbance at exciting wavelength (<0.05, 1 cm cell), Ys is the reference quantum yield: 0.543 for quinine sulfate in 0.1N sulfuric acid. The concentration was selected so that the absorbance was less than

0.05 to minimize error arising from inner effect. The fluorescence quantum yields obtained in the present work were: 0.29 for APT-MAA reaction product.

The result reveals the spectrofluorimetric method was more sensitive than Spectrophotometric method 10 fold.

Validation of the proposed method

The validity of the methods was tested regarding linearity, specificity, accuracy, repeatability and precision according to ICH Q2B recommendations [29].

Linearity

The absorbance-concentration plot was found to be linear over the range of 0.5–10 µg. ml^{-1} with the minimum detection limit (LOD) of 0.168 µg/ml. The fluorescence-concentration plot was found to be linear over the range of 0.05–6 µg/ml with the minimum detection limit (LOD) of 0.016 µg/ml, table 1. Linear regression analysis of the data gave the following equations:

A = 0.0759+0.1024C (r = 0.9998)

F = 0.3394+115.49C (r = 0.9998)

Where A is the absorbance, C is the concentration of the drug (µg/ml),

F = fluorescence intensity, r is the correlation coefficient

The limits of quantification (LOQ) and, The limits of detection (LOD) were calculated according to ICH Q2B [29] using the following equations:

LOQ = 10Sa/b LOD = 3.3Sa/b Where

Sa The standard deviation of the intercept of the regression line, b Slope of the calibration curve. The results are shown in table 1. The proposed methods were evaluated by studying the accuracy as percent relative error (% Error), table 1. The small values of % Error and % RSD indicates high accuracy and high precision of the proposed methods.

Table 1: Results of quantitative determination of amitriptyline hydrochloride

Parameter	Spectrophotometric method	Spectrofluorimetric method	Official USP method[4]
Concentration range	0.5–10.0 (µg/ml)	0.05–6.0 (µg/ml)	
LOD (µg/ml)	0.168	0.016	99.97
LOQ (µg/ml)	0.510	0.050	±0.038
Correlation coefficient (r)	0.9998	0.9998	
Slope	0.1024	155.94	
Intercept	0.0759	-0.3394	
S_b	1.71×10^{-3}	1.749	
S_a	9.67×10^{-3}	5.006	
$S_{y/x}$	5.83×10^{-3}	3.956	
% Error	0.031	0.038	
% RSD	0.075	0.094	
Mean found (%)	100. 06	100.07	
±SD	±0.075	±0.094	
Student's *t*-value	1.5 (2.228)*	0.98 (2.228)*	
Variance ratio *F*-test	2.52 (5.1)*	3.47 (5.1)*	
Molar absorptivity (L mol^{-1} cm^{-1})	3.2×10^5		
Sandell sensitivity (µcm^{-2}/0.001 abs unit)	5.6×10^{-3}		
Quantum Yield		0.29	

Sb = standard deviation of the slope of regression line, Sa = standard deviation of the intercept of regression line, Sy/x = standard deviation of the residuals, % Error = RSD%/√ n., * Values between parentheses are the tabulated t and F values respectively, at p=0.05.

Accuracy

To prove the accuracy of the proposed methods, the results of the assay of APT, both in drug substance and in drug product were compared with those of the official comparison method. Statistical analysis of the results obtained by the proposed and comparison or official method using Student's *t*-test and variance ratio *F*-test showed no significant differences between them regarding accuracy and precision, respectively as shown in tables 1 and 2.

The validity of the methods was evaluated by Statistical analysis of the regression lines regarding the standard deviation of the residuals (S*y/x*), the standard deviation of the intercept (S*a*) and standard deviation of the slope (S*b*).

The results are given in table 1. The small values of the fig. point out to the low scattering of the points around calibration graph and to the precision of the methods.

Precision

The repeatability (Intra-day) was tested by applying the proposed method for the determination of three concentrations of APT in drug substance on three successive times. The low values of standard deviations, % Error indicate high accuracy of the proposed methods, while low values of % RSD indicates high precision of the proposed methods, table 2.

The Intermediate precision (Inter-day precision) was tested by repeated analysis of the drug in drug substance using three different concentrations for a period of three successive days. The low values of standard deviations, % Error indicate high accuracy of the proposed methods, while low values of.

Robustness and ruggedness

The robustness of the proposed methods was examined against small, deliberate variations in the experimental parameters such as the change in the volume of (8%w/v) and (0.8%w/v) MAA, (2±0.2 mL), the change in the heating temperature, (90±2 °C) and the change in the heating time, (15±2 min). These minor changes that may take place during the experimental operation did not affect the absorbance or fluorescence of the reaction product. That indicated the reliability of the proposed method during its routine application for the analysis of APT, as shown in table 3.

Ruggedness was also tested by applying the proposed methods to the assay of APT using the same operational conditions but using two different instruments at two different laboratories and different elapsed time. Results obtained from lab-to-lab and day-to-day variations were reproducible, as the relative standard deviations (RSD) did not exceed 2%.

Applications to drug product

The proposed methods were then applied to the determination of APT in its tablets. The results are shown in table 4.

The results of the proposed methods were statistically compared with those obtained using the reference method USP [4]. Statistical

analysis of the results, using Student's *t*-test and variance ratio *F*-test revealed no significant difference between the performance of the proposed and reference methods regarding the accuracy and precision, respectively, table 4.

Table 2: precision of the proposed methods for the determination of APT in drug substance

Sample concentration		Repeatability (% recovery)	Intermediate precision (%recovery)
Spectrophotometric method (I)			
2(µg/ml)	mean±SD	99.97±1.16	100.52±1.46
	%RSD	1.159	1.460
	% Error	0.433	0.596
4(µg/ml)		99.64±0.99	101.2±0.66
		0.990	0.659
		0.331	0.269
6(µg/ml)		100.16±0.87	99.99±0.95
		0.869	0.950
		0.355	0.388
Spectrofluorimetric method (II)			
1(µg/ml)	mean±SD	100.08±1.25	100.11±1.19
	%RSD	1.249	1.189
	% Error	0.510	0.486
3(µg/ml)		99.99±0.621	100.30±0.952
		0.621	0.952
		0.254	0.388
5(µg/ml)		100.09±0.911	100.32±1.65
		0.911	1.650
		0.372	0.673

Table 3: Robustness and ruggedness assay conditions on the analytical performance of the proposed methods (method I and method II) for determination of APT using MAA reagent

Parameters	Recovery (%±SD)[a]	
	Method I	Method II
MAA concentration (%, w/v) 7.9%, 8.2% w/v	100.27±0.321	
MAA concentration (%, w/v) 0.7%, 0.9% w/v	100.38±0.247	
Volume of MAA (2±0.2 ml)	100.64±0.241	100.68±0.351
Temperature °C (88, 92 °C)	100.06±0.331	100.08±0.521
Reaction Time (min)(13,17 min)	100.67±0.214	100.81±0.341
[a]Values are mean of 3 determinations		

Table 4: Application of the proposed methods to the determination of APT in different tablets

Preparation	Method I % found	Method II % found	Official method USP[4] % found
Tryptizol® tablets 10.0 mg APT HCl/tab			
X^-±SD	100.04±0.09	100.01±0.024	100.10±0.221
Student's *t* test	0.984 (2.228)*	0.998 (2.228)*	
Variance ratio *F* test	1.620 (5.1)*	2.80 (5.1)*	
Tryptizol® tablets 25.0 mg ATP HCl/tab			
X^-±SD	100.06±0.132	100.06±0.082	100.12±0.214
Student's *t* test	1.02 (2.228)*	1.09 (2.228)*	
Variance ratio *F* test	2.60 (5.1)*	1.98 (5.1)*	

Tryptizol tablets, batch #1210735, labeled to contain 10 mg, and batch #1210846, labeled to contain 25 mg APT HCl/tablet., * Values between parentheses are the tabulated t and F values respectively, at p=0.05.

Selectivity

The selectivity of the method was investigated by observing any interference encountered from the common tablet excipients, such as talc, lactose, starch, avicil, gelatin, and magnesium stearate. These excipients did not interfere with the proposed methods. As revealed by a blank experiment using tablets additives but omitting APT.

Application to content uniformity testing

Due to the high sensitivity of the proposed methods and their ability to rapidly measure the absorbance of a single tablet extract with sufficient accuracy, the methods are ideally suited for content uniformity testing which is a time-consuming process when using conventional assay techniques.

The steps of the test were adopted according to the USP [4] procedure.

The acceptance value (AV) was calculated by the following formula:

$AV = [M–X]+KS$

Where M = reference value, K = acceptability constant, S = sample standard deviation.

Acceptance value here was found to be smaller than the maximum allowed acceptance value (L1). The results demonstrated excellent drug uniformity as shown in table 5.

Table 5: Results of content uniformity testing of APT in tablets using the proposed methods

Parameter	Tablet no.	Percentage of the label claim (Method I)		Percentage of the label claim (Method II)	
Data		Tryptizol® 10 mg/tab	Tryptizol® 25 mg/tab	Tryptizol® 10 mg/tab	Tryptizol® 25 mg/tab
	1	100.34	100.02	99.980	100.03
	2	101.02	100.03	99.970	100.50
	3	99.980	99.990	101.00	101.00
	4	100.74	100.21	101.00	100.23
	5	100.69	101.00	100.02	100.56
	6	99.980	100.45	100.05	99.990
	7	101.00	99.980	99.990	100.01
	8	101.44	100.61	100.14	99.980
	9	100.05	100.06	100.12	100.07
	10	100.06	100.00	100.00	100.20
Mean (X`)		100.53	100.24	100.23	100.26
SD		0.522	0.345	0.411	0.333
%RSD		0.519	0.344	0.410	0.332
%Error		0.212	0.141	0.167	0.135
Acceptance value (AV)		1.252	0.828	0.986	0.799
Max. allowed (AV)		15	15	15	15

Tryptizol tablets, batch #1210735, labeled to contain 10 mg, and batch #1210846, labeled to contain 25 mg APT HCl/tablet.

CONCLUSION

The present study described two alternative methods, the successful evaluation of MAA as an analytical reagent in the development of simple and rapid spectrophotometric and spectrofluorimetric methods for the accurate determination of APT in its drug products. The methods described herein have many advantages: it does not need expensive sophisticated apparatus, it is simple and rapid, and it has high sensitivity. The proposed methods used inexpensive reagents with excellent shelf life, and are available in any analytical laboratory. Therefore, the methods are practical and valuable for its routine application in quality control laboratories for analysis of AMI. The result reveals the spectrofluorimetric method was more sensitive than Spectrophotometric method 10 fold. In addition, the proposed methods are very suitable to be applied in content uniformity testing.

ACKNOWLEDGEMENT

All my thanks are not enough to express my feeling of sincere gratitude to Dr. Elham Anwar Taha, professor of analytical chemistry, national organization for drug control and research (NODCAR), for her continuous encouragement, beneficial discussions, sincere help offered and for providing all the facilities during the work.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Mary Adele O'Neil. In: The Merck Index. Merck and Co. Inc., Whitehouse Station, New Jersey; 2006. p. 156-7.
2. Mishra L. Drug Today. Lorena Publications, (India) Inc., Delhi; 2006. p. 200-1.
3. British Pharmacopoeia. Vol. I. Her Majesty's Stationary office. London: UK; 2014. p. 151.
4. United States Pharmacopeia. 37th ed. The United States Pharmacopeia convention Inc., Twin brooks Parkway, Rockville; 2014. p. 1754.
5. Ulmschneider M, Penigault E. Non-invasive confirmation of the identity of tablets by near-infrared spectroscopy. J Anal 2000;28:336-46.
6. Pankaj S, Deepak S, Rajmani P. Simple, Rapid and Sensitive UV-Visible Spectrophotometric method for determination of antidepressant amitriptyline in pharmaceutical dosage forms. J Spectrosc 2013. doi.org/10.1155/2013/783457. [Article in Press]
7. Karpinska J, Suszynska J. The spectrophotometric simultaneous determination of amitriptyline and chlorpromazine hydrochlorides in their binary mixtures. Vierordt method. J Trace Microprobe Tech 2001;19:355-64.
8. Aman T, Kazi AA, Hussain MI, Firdous S, Khan IU. Spectrophotometric determination of amitriptyline-HCl in pure and pharmaceutical preparations. J Anal Lett 2000;33:2477-90.
9. Ozkan SA, Erk N, Senturk Z. Simultaneous determination of two-component mixtures in pharmaceutical formulations containing chlordiazepoxide by ratio spectra derivative spectrophotometry. J Anal Lett 1999;32:497-20.
10. Shen Y, Zhu RH, Li HD, Liu YW, Xu P. Validated LC-MS (ESI) assay for the simultaneous determination of amitriptyline and its metabolite nortriptyline in rat plasma: application to a pharmacokinetic comparison. J Pharm Biol 2010;53:735-9.
11. Yan M, Zh YG, Li HD, Chen BM, Ma N, Lei YQ, *et al*. Quantification of prochlorperazine maleate in human plasma by liquid chromatography-mass spectrometry: application to a bioequivalence study. J Chromatogr B: Anal Technol Biomed Life Sci 2009;877:3243-7.
12. Simonson L, Nelso K. Adsorption of amitriptyline hydrochloride in HPLC injector loops. J Liquid Gas Chromatogr 1992;10:533-5.
13. Vuppala PK, Boddu SP, Furr EB, McCurdy CR, Avery BA. Simple, sensitive, high-throughput method for the quantification of mitragynine in rat plasma using UPLC-MS and its application to an intravenous pharmacokinetic study. J Chromatogra 2011;74:703-10.
14. Lianza K, Sherma J. Application of an expanded model procedure for transfer of TLC screening methods for substandard and fake drugs designed for use in developing countries to quantitative hptlc-densitometry methods. J Liq Chromatogr Relat Technol 2013;36:2446-62.
15. Patel SK, Patel NJ. TLC determination of amitriptyline HCl, trifluoperazine HCl, risperidone and alprazolam in pharmaceutical products. J Chromatographia 2009;69:393-6.
16. Maslanka A, Krzek J. Densitometric high performance thin-layer chromatography identification and quantitative analysis of psychotropic drugs. J AOAC Int 2005;88:70-9.
17. Kaur K, Malik AK. Study on the fluorescence quenching reaction of amitriptyline and clomipramine hydrochlorides with eosin Y and its analytical application. J Fluoresc 2013;23:533-42.
18. Zhu QH. Chemiluminescence analysis of amitriptyline hydrochloride with a nanometer microreactor. J Fenxi Shiyanshi 2011;30:39-42.
19. Markopoulou CK, Malliou ET. Application of two chemometric methods for the determination of imipramine, amitriptyline an perphenazine in content uniformity and drug dissolution studies. J Pharm Biomed 2005;37:249-58.
20. Kou HS, Chen CC, Huang YH, Ko WK, Wu HL, Wu SM. Method for simultaneous determination of eight cyclic antidepressants by cyclodextrin-modified capillary zone electrophoresis: applications in pharmaceuticals. J Anal Chim Acta 2004;525:23-30.
21. El-Nashar RM, Abdel Ghani NT, Bioumy AA. Flow-injection potentiometric determination of amitriptyline hydrochloride. J Microchem 2004;78:107-13.

22. Thomas AD. Spectrophotometric determination of some drugs containing a tertiary amine group. J Pharm Pharmacol 1976;28:838–9.
23. Thomas AD. Spectrofluorimetric determination of some alkaloids containing a tertiary amino group. J Talanta 1975;22:865–9.
24. Abdellatef HE, El-Henawee MM, El-Sayed HM, Ayad MM. Spectrophotometric and spectrofluorimetric methods for analysis of tramadol, acebutolol and dothiepin in pharmaceutical preparations. J Spectrochim Acta Part A 2006;65:1087–92.
25. Job P. Advanced Physicochemical Experiments. 2nd edition. Oliner and Boyd, Edinburgh; 1964. p. 54.
26. Rose J. Advanced Physicochemical Experiments. Pitman, London; 1964.
27. Martin A, Swarbrick J, Cammarata A, Chun A. Physical Pharmacy: Physical Chemical Principles in the Pharmaceutical Sciences, Lea and Fibiger, Philadelphia, Pa, USA. 3rd edition; 1983.
28. Zircon M, Baranowski J, Bialkowski J. Baranowska L. J Sci Technol 2005;40:3137-48.
29. ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology, Q2 (R1), Current Step 4 Version, Parent Guidelines on Methodology Dated November 6;1996, Incorporated; 2005.