ULTRAVIOLET SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF AMITRIPTYLINE HYDROCHLORIDE

T. Durai Ananda Kumar*, Md. Simon Haque

Department of Pharmaceutical Chemistry, JSS College of Pharmacy, Mysuru, JSS Academy of Higher Education & Research, Mysuru, Karnataka, India; e-mail: tduraianandakumar@jssuni.edu.in

Amitriptyline hydrochloride (AMH) is a first-generation tricyclic antidepressant drug. Higher doses of AMH may lead to undesirable clinical conditions such as cardiac arrhythmia, anxiety (sleep disturbances), tachycardia, convulsion, and hyperglycemia. Hence, the accurate analytical method for the quantification of AMH is crucial. The reported AMH quantification methods are less sensitive and require chromogenic reactions. A newly developed spectrophotometric method for the quantification of AMH in the aqueous medium (greener) without using chromogenic conditions is reported. The λ_{max} of AMH is found to be 239 nm, and the method has been validated according to the International Conference on Harmonization Q2A guidelines. The linearity of the developed method is within the range 0.5–2.5 μg/mL with the correlation coefficient (r^2) of 0.9949, which indicates the higher sensitivity of the detection compared with the reported spectrophotometric methods. The percentage recovery (98–102%), precision, limit of detection (0.0266 μg/mL), and limit of quantification (0.0806 μg/mL) data also ensure the method efficiency. The forced degradation studies under the influence of various external factors also suggested the validity of the proposed method for routine analysis. The method was extended for the quantification of AMH present in marketed formulations.

Keywords: *amitriptyline hydrochloride, UV spectrophotometric method, method validation, forced degradation.*

УФ-СПЕКТРОФОТОМЕТРИЧЕСКИЙ МЕТОД ОПРЕДЕЛЕНИЯ ГИДРОХЛОРИДА АМИТРИПТИЛИНА

T. D. A. Kumar*, M. S. Haque

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Фармацевтический колледж Академии высшего образования и исследований JSS, Майсуру, Карнатака, Индия; e-mail: tduraianandakumar@jssuni.edu.in

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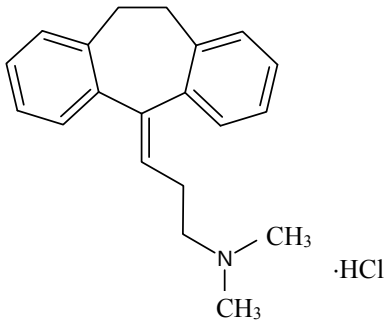
Предложен спектрофотометрический метод количественного определения амитриптилина гидрохлорида (АМГ) в водной среде без использования хромогенных условий. Метод проверен в соответствии с рекомендациями Международной конференции по гармонизации (ICH) Q2A. Для АМГ получено λ_{max} = 239 нм. Линейность метода в диапазоне 0.5–2.5 мкг/мл при коэффициенте корреляции r^2 = 0.9949 свидетельствует о более высокой чувствительности обнаружения АМГ по сравнению с известными спектрофотометрическими методами. Данные о процентном восстановлении (98–102%), точность, предел обнаружения (LOD) 0.0266 мкг/мл и предел количественного определения (LOQ) 0.0806 мкг/мл также указывают на его эффективность. Исследования вынужденной деградации под действием различных внешних факторов свидетельствуют о возможности использования предложенного метода для рутинного анализа. Метод расширен для количественного определения АМГ в коммерческих препаратах.

Ключевые слова: *амитриптилина гидрохлорид, УФ-спектрофотометрический метод, валидация метода, принудительная деградация.*

Introduction. Amitriptyline hydrochloride (AMH) is a dibenzocycloheptadiene class of a first-generation tricyclic antidepressant classified by the World Health Organization. AMH inhibits the re-uptake of norepinephrine and serotonin, downregulates the adrenergic and serotonin receptors, and produces an antidepressant effect [1]. AMH also exerts analgesic [2], adrenergic uptake inhibition [3], anticholinergic [4], anti-inflammatory [5], and sedative functions [6]. AMH is an approved neurotherapeutic for the treatment of migraine and neuropathic pain (fibromyalgia, postherpetic neuralgia) and less commonly for insomnia [7, 8]. AMH is a tertiary amine of a dibenzo[a,d]annulene derivative and is chemically known as 3-(10,11-dihydro-5H-dibenzo[a,d]cycloheptene-Δ5-ylidene)-N,N-dimetyhl-1-propanamine hydrochloride



AMH is a white crystalline compound and freely soluble in water and alcohol. The molecular formula of AMH is $C_{20}H_{23}N$. HCl, the molecular weight is 277.403 g/mol, the log$P$ = 5.1, and the pK_a value of AMH is 9.76 (basic drug) [9]. AMH is officially included in the Indian Pharmacopoeia, British Pharmacopoeia, and United States Pharmacopeia.

Higher doses of AMH may lead to undesirable clinical conditions such as cardiac arrhythmia, anxiety (sleep disturbances), tachycardia, convulsion, and hyperglycemia [10, 11]. This factor, besides the regular quality control analysis, demands special attention. Spectrophotometric [12–14], spectrofluorometric [15], flow injection potentiometry [16, 17], HPLC [18–20], and GC-MS [21] methods were reported for the quantification of AMH (both for single-dose and combination products). However, the chromatographic methods are much less sensitive, laborious, and hence not suitable for routine analysis. Similarly, the reported spectrometric methods require chromogenic reactions [12–14], along with heating, and are less sensitive (higher linearity range).

Hence, the development of a sensitive and cost-effective ultraviolet (UV)-spectrophotometric analytical method for quantifying AMH present in the pharmaceutical dosage form without using chromogenic reaction is crucial. Drug chemical stability on storage decides the safety and efficacy aspects [22]. Forced degradation studies are useful in determining the stability of the drug [23]. In light of the above-mentioned facts, we attempted to develop a simple UV-spectrophotometric method for the determination of AMH. The present paper describes the development, validation, force degradation studies, and assay of AMH present in pharmaceutical formulations.

Methods. A Shimadzu 1700 AD (Shimadzu, Japan) and a Shimadzu 1800 AD double-beam UV-visible spectrophotometer (Shimadzu, Japan) with 1-cm matched quartz cells that measure 2 nm in spectral width and have a 0.5-nm wavelength accuracy were used for the measurement of absorbance. A Shimadzu AUX200 analytical balance (Shimadzu, Japan) was used for weighing the samples, and a GT SONIC ultra sonicator (Antech, India) was used for the preparation of solutions. Milli-Q water was obtained from Millipore Direct-Q® 3 UV water purification system (filter >0.22 μm). Visual Melting Range Apparatus (Lab. India, India) and Shimadzu FT-IR 8400s (Shimadzu, Japan) were used for the drug authentication studies.

All the analytical grade chemicals and solvents were purchased from Sd Fine Chemicals, Mumbai, India. The pure form of AMH (API) was obtained from RL Fine Chem, Bengaluru, India, as a gifted sample. AMH tablets (Tryptomer, Amitone; 10 mg) were used in the investigations.

The standard stock solution of AMH (1000 μg/ml) was prepared by dissolving accurately weighed 10 mg of AMH in Milli-Q water and diluted with Milli-Q water in a 10-mL volumetric flask. Dilution of 1 mL of stock solution with Milli-Q water in a 10 mL volumetric flask produced a working standard solution (100 μg/mL). Further serial dilutions were carried out to obtain the working standard solutions within the range 0.5–2.5 μg/mL.

Aliquots of working standard solutions of AMH (1 to 5 mL) were transferred into 10-ml volumetric flasks, and the volume was made with Milli-Q water. The resulting solutions were scanned within the range 200–400 nm against the blank at room temperature. The absorption maximum (λ_{max}) and molar absorptivity were found to be 239 nm and 0.07×10^5, respectively. The amount of AMH present in the aliquots was calculated using the regression equation.

The proposed spectrophotometric quantification method for AMH was validated according to the International Conference on Harmonization (ICH) guidelines [24].

The linearity of the proposed method was verified by analyzing AMH with a concentration range of 0.5–2.5 μg/mL. The calibration curve was drawn by plotting the absorbance of AMH against the corresponding concentrations. The coefficient of regression (r^2), slope and *Y*-intercept of the calibration curve were calculated using the regression equation.

The precision studies describe the repeatability and ruggedness of the proposed method by measuring the absorbance of multiple aliquots of the analyte. The precision studies measure the intraday (repeatability) and interday (ruggedness) variations in the determinations. The precision was calculated by measuring the absorbance of three different concentrations of AMH (0.5, 1.5, and 2.5 μg/mL) and six times per concentration consecutively for two days.

The lowest amount of the analyte detected by the method is known as a limit of detection (LOD), and the lowest amount of the analyte that can be quantified by the method is known as a limit of quantification (LOQ). LOD and LOQ for the analytes were calculated using the following equations.

$$\text{LOD} = 3.3\ \sigma/S,$$

$$\text{LOQ} = 10\ \sigma/S,$$

where σ is standard deviation of *Y*-intercepts of regression line and *S* is slope of the calibration curve.

The closeness of agreement between a measured value and a true value explains the accuracy of the method. The method accuracy was determined through standard addition (80, 100, and 120%) and recovery studies. The method measures the absorbance of a pre-quantified 2 μg/mL of AMH solution spiked with an extra 80, 100, and 120% of the standard AMH solution at 239 nm in triplicate. The percentage recovery of AMH was calculated using the formula: % recovery = (amount found / amount added)×100.

The reliability of the method under deliberate variations is known as robustness. In the present analysis, the measurement of the analyte is performed by varying the wavelength of determination (237 and 241 nm). The ability of the method to reproduce results under a variety of conditions, such as different laboratories, analysts, and instruments, is known as ruggedness. The present analysis utilized two different instruments (Shimadzu 1700 AD and Shimadzu 1800 AD double-beam UV-visible spectrophotometer) and two analysts for the investigations.

The ability of the method to measure the analyte in the presence of degradation products is known as specificity. Forced degradation studies by changing the pH of the medium provide specificity details about the developed method. In the present investigation, acid-induced, base-induced, hydrogen peroxide-induced, and photolytic degradation studies are included. The degradation studies were also performed under neutral conditions to evaluate the exact influence of degradation agents (acid and base). The working stock solution of AMH was diluted to produce a 3-μg/mL concentration and was used for the force degradation studies. Hydrochloric acid (0.1 and 0.5 N), sodium hydroxide (0.1 and 0.5 N), hydrogen peroxide (30%), sunlight, and Milli-Q water (neutral condition) exposure were used for the degradation studies of the analyte. The absorbance of the analyte under the specific degradation condition was measured for every 1-h interval for the total duration of 4 h. The amount of drug degradation was calculated by measuring the absorbance of the solution at 239 nm.

Ten tablets of Tryptomer and Amitone containing 10 mg of AMH were accurately weighed separately and ground into fine powder. A weight of the tablet powder equivalent to 10 mg of AMH was transferred into a 10-mL volumetric flask containing Milli-Q water, dissolved with Milli-Q water, and filtered through Whatman's filter paper (No. 42) to produce a stock solution of 1000 μg/ml. Further dilution of the stock solution with Milli-Q water produced a 10-μg/mL concentration for measuring the absorbance.

Results and discussion. The structural authentication of AMH was assessed through melting point determination, absorption maximum (λ_{max}), and Fourier transform infrared spectroscopy (FT-IR) studies. The AMH melting point was found to be 197°C, the aqueous AMH solution showed λ_{max} at 239 nm, and the FT-IR studies also featured the characteristic bands.

A linear correlation (Beer's-Lamberts law is obeyed) between absorption and AMH concentration (0.5 to 2.5 μg/mL) was found through regression analysis (Fig. 1). The regression equation was $y = 0.0640x$ – – 0.0012, where y and x are the absorbance and the concentration (μg/mL) of AMH, respectively. The optical characteristics, namely, coefficient of regression ($R^2 = 0.9949$), slope (0.0640), and Y-intercept (0.0012) were calculated. The LOD and LOQ values were calculated as per ICH guidelines and found to be 0.0266 and 0.0806, respectively (λ_{max} = 239 nm, molar absorptivity $\varepsilon = 0.07\times10^5$, linearity 0.5–2.5 μg/mL, correlation equation (r^2) = 0.9949, intercept = 0.0640, limit of detection (LOD) = 0.0266 μg/mL, limit of quantification (LOQ) = 0.0806 μg/mL). These findings highlight the sensitivity of the proposed method.

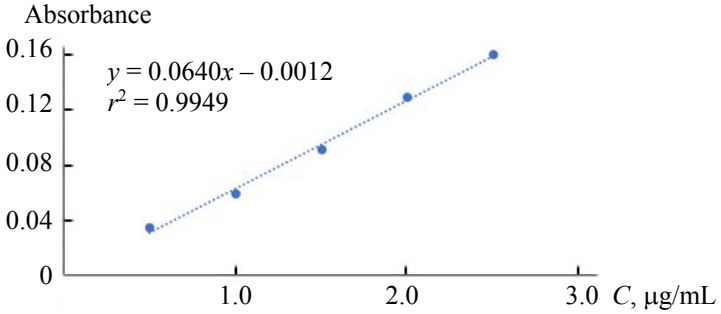


Fig. 1. Linearity curve for amitriptyline hydrochloride.

The precision studies describe the repeatability and ruggedness of the proposed method by measuring the absorbance of multiple aliquots of the analyte. The precision studies measure the intraday (repeatability) and interday (ruggedness) variations in the determinations. The precision was calculated by measuring the absorbance of three different concentrations of AMH (0.5, 1.5, and 2.5 μg/mL) and six times per concentration (Table 1).

The method accuracy was determined by a standard addition method (80, 100, and 120%) through recovery studies. The method shows a consistent percentage recovery of drugs, ranging from 99.23 to 100.51%. (Table 2).

TABLE 1. Regression Analysis: Intra-day and Inter-day Precision Analysis (n = 6)

AMH, μg/mL	Intra-day precision		Inter-day precision	
	AMH found, μg/mL	%RSD	AMH found, μg/mL	%RSD
0.5	0.56±0.031	1.489	0.49±0.036	1.795
1.5	1.45±0.025	1.066	1.388±0.028	0.589
2.5	2.53±0.037	0.653	2.46±0.029	0.260

N o t e. %RSD percentage relative standard deviation.

TABLE 2. Regression Analysis: Accuracy Data

% Recovery level	Pure drug added, μg/mL	% Recovery	% MR (±SD)	%RSD
80	1.5 1.5 1.5	100.00 99.22 98.45	99.23±0.775	0.7812
100	2 2 2	97.67 100.78 99.23	98.97±1.550	1.5665
120	2.5 2.5 2.5	101.55 100.78 99.22	100.51±1.184	1.1780

N o t e. Analyte concentration 2 μg/mL; MR – mean recovery, SD standard deviation, %RSD percentage relative standard deviation.

The method robustness was investigated by measuring the absorbance of the analyte at two different wavelengths (237 and 241 nm). The results indicate that the minor variations in the identified parameter do not significantly influence the method sensitivity and indicate the method reliability (Table 3). The method ruggedness was evaluated by quantifying using two different UV/visible spectrophotometers (Shimadzu 1700 AD and Shimadzu 1800) and two analysts. The percentage relative standard deviation (%RSD) values were within limits and indicated the sensitivity of the proposed method (Table 3).

TABLE 3. Regression Analysis: Robustness and Ruggedness Data (n = 6, 1.5 µg/mL)

Parameters	Robustness		Ruggedness	
	237 nm	241 nm	Shimadzu 1700	Shimadzu 1800
Absorbance ± SD	0.085±0.005	0.086±0.001	0.905±0.007	0.885±0.007
% RSD	0.602	1.402	0.781	0.798

N o t e. SD standard deviation, %RSD percentage relative standard deviation.

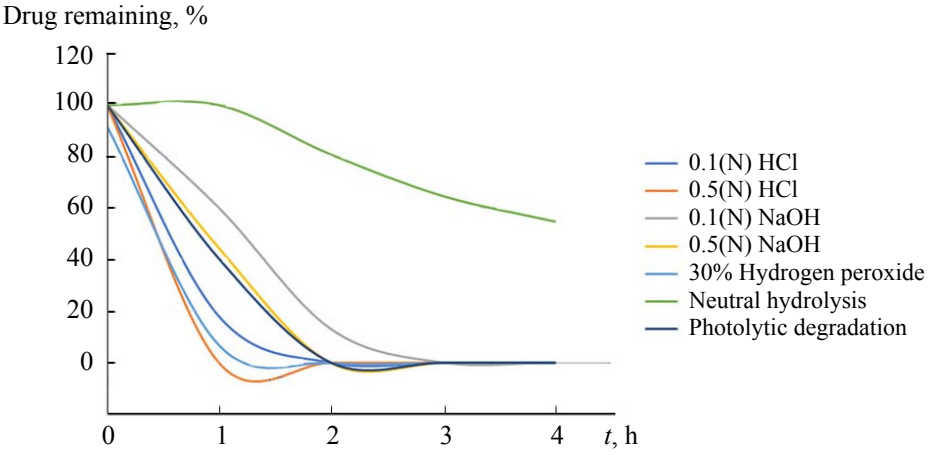


Fig. 2. Forced degradation graph for amitriptyline hydrochloride.

Specificity. In acid degradation studies, complete degradation of AMH is observed within 2 and 1 h for 0.1 N and 0.5 N HCl, respectively. In 0.1 N NaOH medium, the complete degradation was effect within 3 and 2 h for 0.5 N NaOH. The oxidative degradation studies with hydrogen peroxide (30%) are more sensitive and degradation completed within 1 h. In a neutral medium (Milli-Q water), AMH was found to be stable for the total duration and in photolytic degradation AMH was stable for 2 h (Table 4).

TABLE 4. Specificity Analysis: Forced Degradation with Acid, Base, Oxidizing Agent and Light

Medium	Parameter	Time, h				
		0	1	2	3	4
0.1 N HCl	Absorbance	0.195	0.034	0		
	Concentration, µg/mL	3	0.55	0		
	Degradation, %	0	82	100		
0.5 N HCl	Absorbance	0.194				
	Concentration, µg/mL	3	0			
	Degradation, %	0	100			
0.1 N NaOH	Absorbance	0.193	0.111	0.021		
	Concentration, µg/mL	3	1.8	0.34	0	
	Degradation, %	0	40	87	100	
0.5 N NaOH	Absorbance	0.195	0.083			
	Concentration, µg/mL	3	1.31	0		
	Degradation, %	0	56.33	100		

Continue Table 4

Medium	Parameter	Time, h				
		0	1	2	3	4
30% H_2O_2	Absorbance	0.175				
	Concentration, μg/mL	2.76	0			
	Degradation, %	8	100			
Neutral	Absorbance	0.195	0.191	0.156	0.121	0.102
	Concentration, μg/mL	3	3	2.4	1.9	1.6
	Degradation, %	0	0	19.2	36.3	46.2
Photons	Absorbance	0.194	0.076			
	Concentration, μg/mL	3	1.2	0		
	Degradation, %	0	59.7	100		

The developed and validated method was utilized for the quantification of Tryptomer and Amitone brands containing 10 mg of AMH. The amount of drug detected from the Tryptomer and Amitone was 9.85±0.01 and 9.92±0.01 mg, respectively (Table 5). The %RSD calculated (0.012 and 0.058) indicates the greater efficiency of the method in the detection of AMH.

TABLE 5. Assay Studies for Amitriptyline Hydrochloride Present in Tablet Formulations

Brand name	Label claim, mg	Amount found, mg ± SD	%Assay	%RSD
Tryptomer	10	9.85±0.01	98.5	0.012
Amitone	10	9.92±0.01	99.2	0.058

N o t e. SD standard deviation, %RSD percentage relative standard deviation.

Conclusions. The method discussed in this paper gives a straightforward way of analyzing amitriptyline hydrochloride under regular laboratory conditions without the need for any derivatization or complex chemical reaction. Milli-Q water was used throughout the study as a diluent, and it provides excellent sensitivity. The wavelength of 239 nm was used for the study, and the linearity was found to be within the range 0.5–2.5 μg/mL. The validation of the method is carried out according to the ICH Q2A guidelines. By this method, the marketed formulation was analyzed, and the assay was found to be 99.2 and 98.5%. Hence, this method can be applied as a routine quality control assay procedure for amitriptyline hydrochloride.

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