

Different Mathematical Spectrophotometric Methods for Determination of Ampyrone in Presence of Its Acid Degradation Product

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ABSTRACT

Three simple spectrophotometric methods namely; area under the curve, simultaneous equation method, and modified simultaneous equation method were developed and validated for the determination of Ampyrone with a the presence of its acidic product using zero order spectra without prior separation techniques. The linearity was found in the range of (10-50 mg/L) and (10-55 mg/L) for Ampyrone and its acidic product, respectively. The recovery percentage was found to be in the range from 99.6 to 100.65 for the area under curve method and 96.64 to 104.8 for the simultaneous equation method and its modified version which showed good accuracy and precision for three proposed methods.

KEYWORDS: Area under curve; Ampyrone; simultaneous equation; modified simultaneous equation; Acidic product.

الخلاصة

استخدمت ثلاث طرق طيفية بسيطة وهي؛ المساحة تحت المنحنى، طريقة المعادلة المتزامنة وطريقة المعادلة المتزامنة المعدلة والتحقق من صحتها لتحديد Ampyrone بوجود مركبه الحامضي وباستخدام أطيايف الترتيب الصفري دون الحاجة الى استخدام عمليات الفصل المسبقة. كان مدى الخطية بين (10-50 ملغ / لتر) وبين (10-55 ملغ / لتر) للأمبيرون ومنتجه الحامضي، على التوالي. تم العثور على نسبة الاسترداد في حدود 99.6 إلى 100.65 للمساحة تحت طريقة المنحنى ومن 96.64 إلى 104.8 لطريقة المعادلة المتزامنة وإصدارها المعدل الذي أظهر دقة جيدة للطرق الثلاثة المقترحة.

INTRODUCTION

Spectrophotometric methods are the most commonly employed techniques which attracted the attention of the researchers and analysts everywhere. Owing to the common availability of instrument, speed, precision and accuracy. When compared with other methods of analysis such as electrophoresis and chromatography, the spectrophotometric techniques are offered simplified operations and far more economical [1, 2].

The area under the curve and Simultaneous equation method were used for estimation components without using any computer programs (like derivative, Continuous wavelet transform and mean centre) and did not need preliminary separation steps. They can be applied with simple mathematical calculations [3].

Ampyrone is called 4-Aminoantipyrine (4-amino-1,5-dimethyl- 2-phenylpyrazole-3-one) and its analogues have shown a wide range of biological activities such as antirheumatic antiviral, anti-inflammatory, analgesic and antipyretic[4,5]. It used as a colorimetric detection for determination Cu^{2+} in aqueous solution[6], Fe^{+2} in water[7] and determination of phenolic drugs via oxidative coupling reaction forming coloured products[8,9]. There are many methods for the determination of Ampyrone including solid-phase spectrophotometry[10], electrochemical method based on fabrication of multi-walled carbon nanotube electrodes for the estimation toxic drugs Ampyrone[11], electrochemical method by using graphite pencil electrode[12], LC/Mass spectrometry[13] and capillary electrophoresis[14].

This work presented three spectrophotometric methods for the determination of the minor component Ampyrone with a presence of its acidic product based on zero order spectra, such as: area under curve method, simultaneous equation method and its modified version.

The new methods are very simple, inexpensive, specific and do not require any prior extraction or separation steps and provide reliable results with high sensitivity, accuracy and robustness.

EXPERIMENTAL

Apparatus

Uv-Visible spectrophotometer (model 1650 PC, SHIMADZU, Japan) with the software program and two matched 1-cm quartz cells.

Chemicals and reagents

Standard pure powder of Ampyrone (4-Aminoantipyrene) (Sigma-Aldrich) ($C_{11}H_{13}N_3O$) and hydrochloric acid(BHD) prepared by 2N aqueous solution.

Preparation Standard Solution of 4-Aminoantipyrene

Ampyrone 100 mg/L (M.wt=203.24 gm mol⁻¹) was prepared by dissolving 0.025 gm of 4-aminoantipyrene in 100 mL of distilled water and completed to the mark in volumetric to 250 mL with the same solvent.

Preparation Standard Solution of Acidic Product

Acidic product of Ampyrone was prepared according to Abbas R. F method [15].

0.025 gm of pure Ampyrone was refluxed with 250 mL 2N HCl for 60 min. After cooling, the solution was evaporated to dryness by oven at 70 °C, the solid residue was extracted with ethanol, filtered into 250 mL volumetric flask completed to the mark with distilled water to obtained a solution contain an acidic product derivative 1000 mg/L of Ampyrone [16,17], transferred 25 mL of this solution into 250 mL volumetric flask, complete to the mark with distilled water to make solution contain acidic product derivative 100 mg/L of Ampyrone (Figure 1).

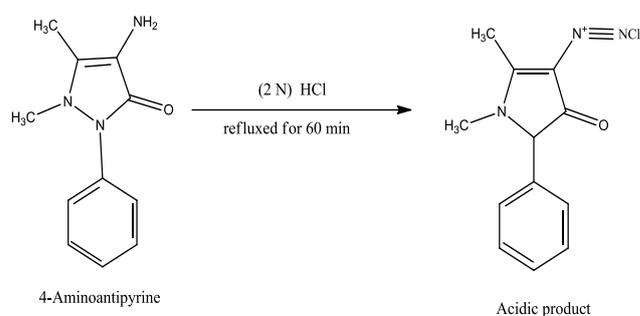


Figure 1. Scheme for the acid degradation

Preparation of Mixtures

To series of binary mixtures solutions of pure Ampyrone standard and its acidic product were prepared; the first series was prepared by using a concentration 30 mg/L of acidic product with three different concentrations (15, 25 and 35 mg/L) of pure Ampyrone, while the second series of mixture was prepared by using a concentration of 25 mg/L of pure Ampyrone with three difference concentrations (20, 30 and 40 mg/L) of the acidic product. The zero order and area under the curve spectra for Ampyrone and its acidic product were recorded as described under the linearity for each proposed method.

Area under Curve Method (AUC)

Aliquots from Ampyrone and its acidic product (100 mg/L equivalent to 10-50 mg/L and 10-55 mg/L, respectively). Were prepared into two sets of 20 mL volumetric flasks and completed to the mark with distilled water. Each set was scanned in the range of 200-400 nm to obtained the zero order absorbance. AUC for the wavelength ranges selected for estimation of Ampyrone and its acidic product are 235-245 nm ($\lambda_1-\lambda_2$) and 246-262 nm ($\lambda_3-\lambda_4$), the absorptivity values were calculated as, $y = AUC$ of Ampyrone or its acidic product in the range (235-245 nm or 256-262nm) / concentration of these components (in mg/L). Mixed standard solutions were prepared of Ampyrone and its acidic product and their AUC were measured at the selected wavelength range [18,19].

Simultaneous Equation Method

In this study working standard solutions were prepared over the concentration range 10-50 mg/L and 10-55 mg/L for Ampyrone and its acidic product, respectively. And the absorbance at 243 nm (λ_{max} of Ampyrone) and 257 nm (λ_{max} of acidic product) were selected

and measured for the formation of simultaneous equations[20,21].

$$C_{\text{Ampyrone}} = \frac{A_2 a y_1 - A_1 a y_2}{a x_2 a y_1 - a x_1 a y_2} \quad (1)$$

$$C_{\text{acidic product}} = \frac{A_1 a x_2 - A_2 a x_1}{a x_2 a y_1 - a x_1 a y_2} \quad (2)$$

Where, C_x is the concentrations of Ampyrone in mg/L, C_y is the concentrations of acidic product in mg/L, A_1 is the absorbance of the mixture at 243 nm (λ_{max} of Ampyrone), A_2 is the absorbance of the mixture at 257 nm (λ_{max} of acidic product), $a x_1$ is the absorptivities of Ampyrone at 243 nm (λ_{max} of Ampyrone), $a x_2$ is the absorptivities of Ampyrone at 257 nm (λ_{max} of acidic product), $a y_1$ is the absorptivities of acidic product at 243 nm (λ_{max} of Ampyrone) and $a y_2$ is the absorptivities of acidic product at 257 nm (λ_{max} of acidic product).

Modified Simultaneous Equation Method

Using the same readings in simultaneous equations method and using the following equations, Ampyrone and its acidic product were determined [22,23].

$$C_{\text{Ampyrone}} = \frac{A_1}{a x_1} \times \frac{b - m}{b - a} \quad (3)$$

$$C_{\text{acidic product}} = \frac{A_2}{a y_2} \times \frac{b(m - a)}{m(b - a)} \quad (4)$$

Where, $m = \frac{A_2}{A_1}$, $a = \frac{a x_2}{a x_1}$ and $b = \frac{a y_2}{a y_1}$.

The symbols are identical with those cited in simultaneous equations method.

The ratio "m" must be calculated for each determination of C_{Ampyrone} and $C_{\text{acidic product}}$ while "a" and "b" are constant. The advantage of this method was determined of "a" and "b" once and No need to re-measure or re-calculate for each determination. Therefore, this method is simple and fast; gives an advantage over the simultaneous equations method.

RESULTS AND DISCUSSIONS

The proposed methods for estimation of Ampyrone and its acidic product were found to be lowest cost, fast and using eco-friendly solvents without prior separation.

Area under Curve Method

Cramer's rule was used for calculating the concentration of Ampyrone as shown in equation 5 and 6 after the area under curve for Ampyrone and its acidic product were recorded at the wavelength of (235-245) and (256-262) nm (Figure 2). The following equations show the concentrations of Ampyrone in presence of its acidic product:

$$AUC = 2.389 C_{\text{Ampyrone}} + 2.286 C_{\text{acidic product}} \quad (5)$$

at 2.35-2.45 ($\lambda_1 - \lambda_2$)

$$AUC = 1.333 C_{\text{Ampyrone}} + 1.502 C_{\text{acidic product}} \quad (6)$$

at 2.35-2.45 ($\lambda_1 - \lambda_2$)

Where C_{Ampyrone} is the concentrations of 4-aminoantipyrine(mg/L), $C_{\text{acidic product}}$ is the concentrations of its acidic product in (mg/L), the absorptivity values of Ampyrone 2.389 at ($\lambda_1 - \lambda_2$), the absorptivity values of Ampyrone 2.286 at ($\lambda_3 - \lambda_4$), 1.333 is the absorptivity values of acidic product at ($\lambda_1 - \lambda_2$) and the absorptivity values of acidic product 1.502 at ($\lambda_3 - \lambda_4$)

The calibration curves were constructed for AUC method by plotting the AUC over the two selected wavelength range vs. Ampyrone and its acidic product concentration in mg/L (Figure 3). The linearity studies and statistical results are summarized in Table 1.

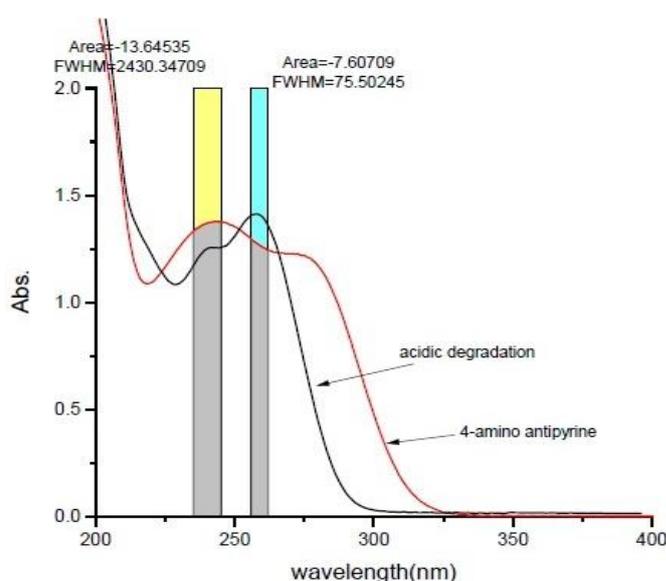


Figure 2. Area under curve method of the zero order absorption spectra of Ampyrone 30 mg/L and acidic product 30 mg/L.

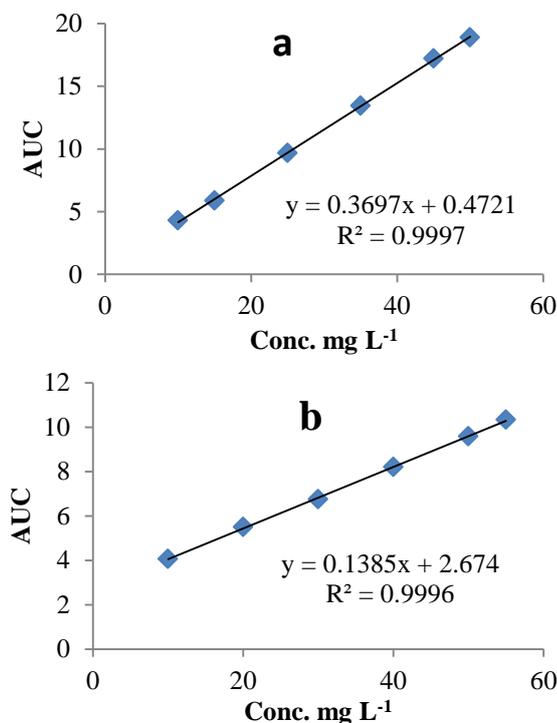


Figure 3. Calibration curve of area under curve method for, a. Ampyrone 10-50 mg/L at area (235-245 nm), b. its acidic product 10-55 mg/L at area (256-262 nm).

Simultaneous Equation Method and Modified Simultaneous Equation Method

The overlay spectrum of Ampyrone and its acidic product (Figure 4) shows that there was interference in quantitation of individual Ampyrone at their λ_{max} due to absorption of its acidic product at that particular wavelength. So, the simultaneous equation method and its modified version method were developed for determination of Ampyrone and its acidic product from the prepared mixture. The calibration curves (Figure 5) for this method was drawn by plotting the absorbance at the two selected λ_{max} wavelengths vs. Ampyrone and its acidic product concentrations in mg/L. The results of the statistics calculation are presented in Table 1.

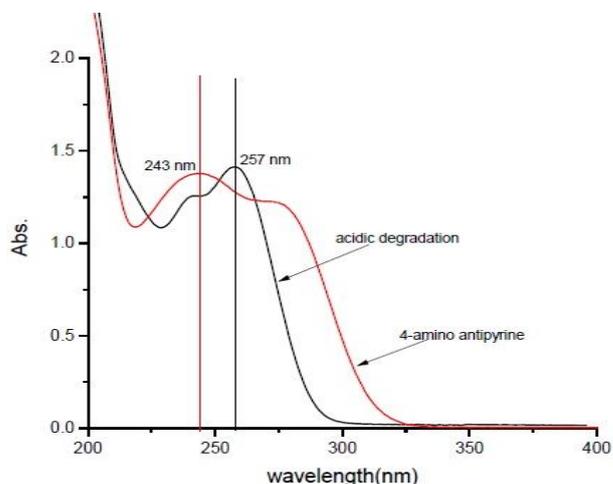


Figure 4. Zero order absorption spectra of Ampyrone 30 mg/L and acidic product 30 mg/L.

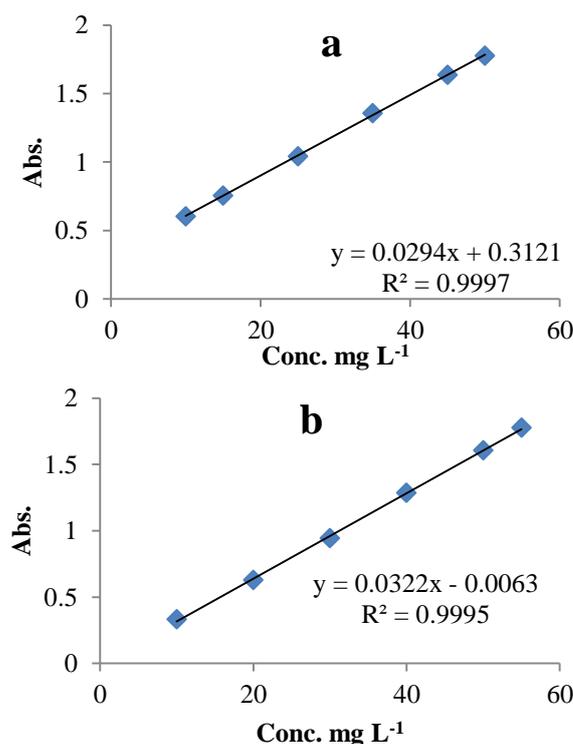


Figure 5. Calibration curve of simultaneous equation method for, a. Ampyrone 10-50 mg/L at λ_{max} (243 nm), b. its acidic product 10-55 mg/L at λ_{max} (257 nm).

Table 1. Statistical data of the proposed methods.

Validation parameters	AUC		Simultaneous equation method and its modified version	
	Ampyrone	acidic product	Ampyrone	acidic product
Wavelength (nm)	235-245	256-262	243 nm	257 nm
Linearity range (mg/L)	10-50	10-55	10-50	10-55
Equation	Y=0.369x+0.472	Y=0.138x+2.674	Y=0.029x+0.312	Y=0.032x-0.006
R ²	0.999	0.999	0.999	0.999
Slope	0.369	0.138	0.029	0.032
Intercept	0.472	2.674	0.312	-0.006
Mean±SD	100.363±2.048	100.458±1.218	101.451±0.887	101.106±2.466
Standard deviation of the intercept (S _a)	0.026	0.029	0.014	0.019
LOD mg/L	0.24	0.702	1.67	2.02

R² = Coefficient of determination, LOD = limit of detection = 3.3 × S_a / S, L = 10 × S_a / S, where, S = is the slope of the corresponding linearity graph, and S_a = the standard deviation of intercepts of regression lines.

Accuracy and Precision

The relative error and recovery percentage for AUC method were calculated (Table 2). The recovery percentage values were found to be in the range between 99.56 and 100.6, indicating that the area under curve method is reliable and accurate.

The values of recovery percentage of the laboratory prepared mixture of Ampyrone with its acidic product (table-3) were found in the range between 96.64 - 104.8 indicating the recommended simultaneous equation method and its modified version are accurate. Table 4 shows the limit of detection of the two

proposed methods involving Ampyrone proved a good agreement with reference methods.

Table 2. The relative error and recovery percentage for estimation of Ampyrone and acidic product using Area under curve method.

Taken Mixture of Ampyrone + acidic product	Found(mg/L) Ampyrone	E. %	Rec. %
25 mg/L+ 20 mg/L	25.04	0.16	100.16
25 mg/L+ 30 mg/L	25.10	0.4	100.4
25 mg/L+ 40 mg/L	25.12	0.48	100.48
Taken Mixture of Ampyrone + acidic product	Found(mg/L) Acidic product	E %	Rec %
15 mg/L+30 mg/L	30.15	0.5	100.5
25 mg/L+30 mg/L	29.87	-0.43	99.56
35 mg/L+ 30 mg/L	30.18	0.6	100.6

$$E\%(Relative\ error\%) = \frac{found-taken}{taken} \times 100 \text{ and } Rec\%(Rrecovery) = E\% + 100.$$

Table 3. The relative error and recovery percentage for estimation of Ampyrone and acidic product using Simultaneous equation method and its modified version.

Taken Mixture of Ampyrone +acidic product	Found (mg/L) Ampyrone	A ₁ Abs. at 243 nm	A ₂ Abs. at 257 nm	m	a	b	E%	Rec.%
25 mg/L+20 mg/L	24.97	1.0415	1.1691	1.1225	1.1225	0.9688	-0.12	99.88
25 mg/L +30 mg/L	24.16	1.0811	1.2023	1.1121			-3.36	96.64
25 mg/L +40 mg/L	26.09	1.100	1.233	1.1209			4.38	104.38
Taken Mixture of Ampyrone +acidic product	Found (mg/L) acidic product	A ₁ Abs. at 243 nm	A ₂ Abs. at 257 nm	m	a	b	E%	Rec.%
15 mg/L+30 mg/L	29.89	0.9743	0.9439	0.9687	1.1225	0.9688	-0.36	99.63
25 mg/L+30 mg/L	29.56	0.9553	0.9243	0.9675			-1.44	98.56
35 mg/L+30 mg/L	30.67	0.9988	0.9676	0.9687			2.24	102.24

$$ax_1= 8467.6, ax_2= 9504.9, ay_1= 8146.3, ay_2= 7892.7, m=A_2/A_1, a= ax_2/ax_1, b= ay_2/ay_1, E\%(Relative\ error\%) = \frac{found-taken}{taken} \times 100 \text{ and } Rec\%(Rrecovery) = E\% + 100.$$

Table 4. Comparison the values of limit of detection of the proposed methods with the references methods.

Methods	LOD	Ref.
Electrochemical method based on fabrication of multi-walled carbon nanotube electrodes	1.63×10^{-10} M	[11]
Electrochemical method by using graphite pencil electrode	0.458×10^{-7} M	[12]
Area under curve spectrophotometric method determination of Ampyrone at wavelength 235-245 nm	0.239 mg/L	Proposed method
Simultaneous equation and its Modified version spectrophotometric methods determination of Ampyrone at 243 nm	1.138 mg/L	Proposed method

CONCLUSIONS

This work offered the application of simple, precise and do not need any progressing apparatus for the determination of Ampyrone with a presence its acidic product.

The area under the curve method, simultaneous equation method and its modified version are depended on zero-order spectra and suitable for computer programs that cannot make derivative spectrophotometry. The proposed methods have the advantages of being eco-friendly and do not

need any prior separation steps which save cost and reduce effort.

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