



New Mode for the On-Line Determination of Amiloride in Pure and Pharmaceutical Preparation Using CFIA Via the Use of Linear Array from Six LED (Snow White) with One Solar Cell in a Homemade Ayah 6Sx1-T-1D Solar CFI Analyser

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Abstract

A newly flow injection-turbidimetric method characterized by its speed and sensitivity has been developed for the determination of Amiloride in pure and pharmaceutical preparations. It is based on the formation of yellowish white precipitate for the Amiloride-phosphomolybdic acid ion pair in aqueous medium. Turbidity was measured by Ayah 6Sx1-T-1D solar cell CFI analyser via the attenuation of incident light from the surfaces precipitated particles at 0-180°. The chemical and physical parameters were investigated. Linear dynamic range for the attenuation of incident light versus Amiloride concentration was of 0.005-10 mmol.L⁻¹, with the correlation coefficient (r) of 0.9986, while the percentage linearity (r²%) was 99.71%. The L.O.Q was of 23.14 µg/sample, while L.O.D (S/N=3) = 5.74 ng/sample from the stepwise dilution for the minimum concentration in the calibration graph. The R.S.D% at 3, 9 mmol.L⁻¹ Amiloride is less than 0.5% (eight replicates) using 50 µL sample volume. Through put was of 40 sample.hr⁻¹. The method was applied successfully for the determination of Amiloride in pharmaceutical preparations. A comparison was made using the standard additions method via the use of paired t-test. It showed that there was no significant difference between the quoted value of each individual paired t-test with calculated t-value at 95% confidence interval from developed method. In addition to comparison between two methods and calculate t-value, it was noticed that there was no significant difference between the two methods at α=0.05 (95% confidence level).

Keywords: Amiloride, flow injection analysis, turbidity, pharmaceutical preparation

نمط جديد للتقدير الانلي للاميلورايد في المستحضرات الصيدلانية والنقية باستخدام التحليل بالحقن الجرياني المستمر عن طريق مصفوفة خطية لست ثنائي وصلة باعث للضوء الابيض الثلجي مع خلية شمسية في محلل لقياس التعكسية Ayah 6Sx1-T-1D solar CFI

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الخلاصة

طورت طريقة جديدة بالحقن الجرياني لقياس التعكسية وتميزت بالسرعة والحساسية لتقدير الاميلورايد في المستحضرات الصيدلانية والنقية. استندت على تكوين راسب ابيض مصفر للمزدوج الايوني: اميلورايد-حامض موليبيدات الفسفوريك في الوسط المائي. تم قياس التعكسية بوساطة محلل الحقن الجرياني المستمر Ayah

6Sx1-T-1D solar cell-CFI analyser عن طريق توهين الضوء المسلط من على سطوح دقائق الراسب بزاوية 0-180°. تم دراسة الظروف الفضلى من المتغيرات الفيزيائية والكيميائية. المدى الخطي الفعال لعلاقة توهين الضوء الساقط ضد تركيز الاميلورايد 0.005-10 مللي مول.لتر⁻¹ مع معامل ارتباط (r): 0.9986 ونسبة الخطية (r²)=99.71%. معامل التقدير الكمي (L.O.Q) : 23.14 مكغم/انموذج بينما حد الكشف L.O.D (S/N=3) 5.74 نانوغرام/انموذج من التخفيف التدريجي لاقل تركيز في منحنى المعايرة. الانحراف

القياسي النسبي المئوي لتركيز 3 و 9 مللي مول.لتر⁻¹ من الاميلورايد اقل من 0.5% (تكرارية لثمان مرات) باستخدام حجم لانموذج محقق 50 مايكرو لتر. معدل النمذجة 40 انموذج/ساعة. طبقت الطريقة بنجاح لتقدير الاميلورايد في المستحضرات الصيدلانية. اجريت مقارنة باستخدام منحنى الاضافات القياسية و اختبار t المزدوج ولوحظ عدم وجود فرق جوهري بين القيمة الرسمية للاختبارات الانفرادية وقيمة t-المحسوبة عند فاصل ثقة 95% للطريقة المطورة بالاضافة الى اجراء مقارنة بين الطريقتين ولوحظ عدم وجود فرق جوهري بينها عند $\alpha=0.05$ (مستوى الثقة 95%).

Introduction

Amiloride (AM) is chemically known 3,5-diamino-6-chloro-N-(diaminomethylene)Pyrazine-2-carboxamide which works by directly blocking the epithelial sodium channel there by inhibiting sodium reabsorption in the distal convoluted tubules and collecting ducts in the kidneys ,this promotes the loss of sodium and water from the body, but without depleting potassium [1] though Amiloride has mild hypotensive activity, the medication is generally used concurrently with a thiazide or loop diuretic to prevent or treat diuretic-induced hypokalemia [2].

Amiloride has been effectively used to treat both primary and secondary hyperaldosteronism, such as with hepatic cirrhosis although spironolactone is generally considered more effective. Although this drug is usually given with a saluretic, hyperkalemia may occur when the drug is given with potassium supplementation or an ACE-inhibitor or the patient has renal insufficiency, diabetes or is elderly [3]. An increase in serum potassium causes depolarization of cardiac and skeletal muscle cell. Such depolarization is particularly harmful to cardiac cell, leading to abnormal impulse conduction specifically hyperkalemia effects electrical myocardial conduction causing arrhythmias, bradycardia and hypotension [4]. The structure of Amiloride is depicted in figure-1.

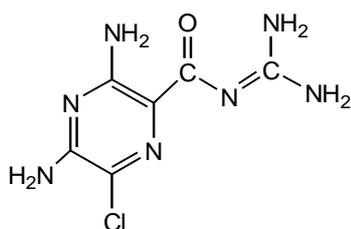


Figure.1- Structure of Amiloride

Amiloride is available in the market as combination drug with furosemide such as Amifru Tab., It is also available in combination with atenolol & hydrochlorothiazide namely Beta-Biduret Cap, BP-Loride tab [5,6].

Literature survey reveals that several analytical methods have been reported for the determination of Am alone or in combination with other drugs including: spectrophotometry [7], Dual stopped-flow spectrophotometric [8]. Am and atenolol were simultaneously determined by HPLC [9], reversed phase liquid chromatography [10], derivative spectroscopy [11-13]. In addition to determination of some diuretics using oxidative coupling reaction [14].

In this paper, simple and sensitive turbidimetric methods for the analysis of Am were described. The methods are based on the formation of ion-pair complex of amiloride with phosphomolybdic acid in aqueous medium. Turbidity was measured via the attenuation of incident light by the effect of the presence of the solid particulate at 0-180° using of Ayah 63x1-T-1D solar-CFI analyser [15] which

supplied with linear array of six super snow white light emitting diode as a source and one solar cell as a detector. The negative signal from attenuation can be recorded.

Experimental

Chemicals

All chemicals were used of analytical reagent grade. Distilled water was used to prepare all the solutions. Freshly prepared solutions were always used. A standard solution of Am ($C_6H_8ClN_7O$, M.wt. 229.6 $g.mol^{-1}$, SDI, 0.02 $mol.L^{-1}$) was prepared by dissolving 1.148g in 250 mL methanol (absolute 99.5%, Fluka-Garantie). A stock solution phosphomolybdic acid (PMA) ($H_3PMo_{12}O_{40}$, 1825.25 $g.mol^{-1}$, BDH, 0.1 $mol.L^{-1}$) was prepared by dissolving 18.2525 g/100 mL distilled water. Nitric acid, 1 $mol.L^{-1}$, by diluting 127 mL of 70% HNO_3 (sp.gr. 1.42 $g.mL^{-1}$, BDH) with water to 2 L in a calibrated flask, sulphuric acid, 1 $mol.L^{-1}$, by diluting 111 mL of 96% H_2SO_4 (sp.gr. 1.84 $g.mL^{-1}$, BDH) with water in a 2L calibrated flask. Hydrochloric acid, 1 $mol.L^{-1}$, dilute 176.50 mL of 35% HCl (sp.gr. 1.18 $g.mL^{-1}$, BDH) to 2 L distilled water. Each acid was standardized against standard solution of 1 $mol.L^{-1}$ from sodium carbonate.

Sample Preparation

Thirteen tablets were weight, then crushed and grinded. Tablets containing 5 mg of Am were weighted 3.08812 g, 6.13262 g (equivalent to 114.8 mg of active ingredient, 5 $mmol.L^{-1}$) for Amiloride-Actavis and Modurtic-Algorithm respectively. Dissolved in to 30 mL of methanol. The solution was filtered to get rid of undissolved materials, the residue was washed with methanol and completed the volume to 100 mL with the same solvent .

Apparatus and Manifold

The flow system used for the determination of Am is shown schematically in figure.2, peristaltic pump with two channels and variable speed (Ismatec, Switzerland). Injection valve with valve 6-port medium pressure (IDEX corporation, USA) and sample loop (1 mm I.D., Teflon, Variable length) were used. The instrument response was measured by Ayah 6Sx1-T-1D solar cell CFI analyser (homemade) [15] which uses six snow white LED for irradiation of the flow cell at 2 mm path length. One solar cell used as a detector for collecting signals output via sample segment passes through 60 mm length of flow cell. The output signals were recorded by potentiometric recorder (Siemens, Germany) (1-500) volt or (1-500) mV. Using peak height mode for each signal. UV-Vis spectrophotometer digital double beam type UV-1800, Shimadzu, Japan was used to scan the spectrum of Am using 1 cm quartz cell.

Methodology

The whole manifold system for the reaction of Am-phosphomolybdic acid to form yellowish white precipitate as an ion pair complex is composed of two lines as shown in figure-2. The first line is the carrier stream (distilled water) at 1.5 $mL.min^{-1}$ flow rate which leads to the injection valve to carry Am sample, 50 μL ; while the second line supplied with phosphomolybdic acid (3 $mmol.L^{-1}$) at 1.8 $mL.min^{-1}$. Both of lines meet at a junction (Y-junction), with an outlet for reactants product, from which passes through a homemade Ayah 6Sx1-T-1D solar cell CFI analyser to obtain transducer energy response in mV versus time. Each solution injected was assayed in triplicate. The response was recorded on x-t-potentiometric recorder to measure the turbidity via the attenuation of incident light at 0-180°. A proposed mechanism of ion-pair for Am-PMA in aqueous medium is presented in Scheme 1. [16,17].

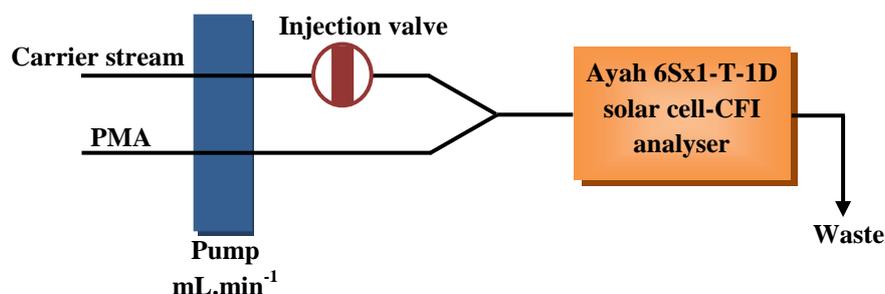
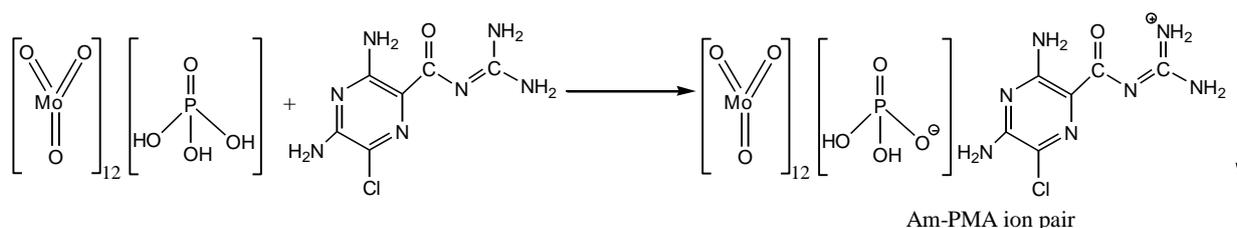


Figure.2- Schematic diagram of FIA Ayah 6Sx1-T-1D solar cell CFI analyser for determination of Am



Scheme 1-Mechanism of reaction between of Am & PMA

Results and Discussion

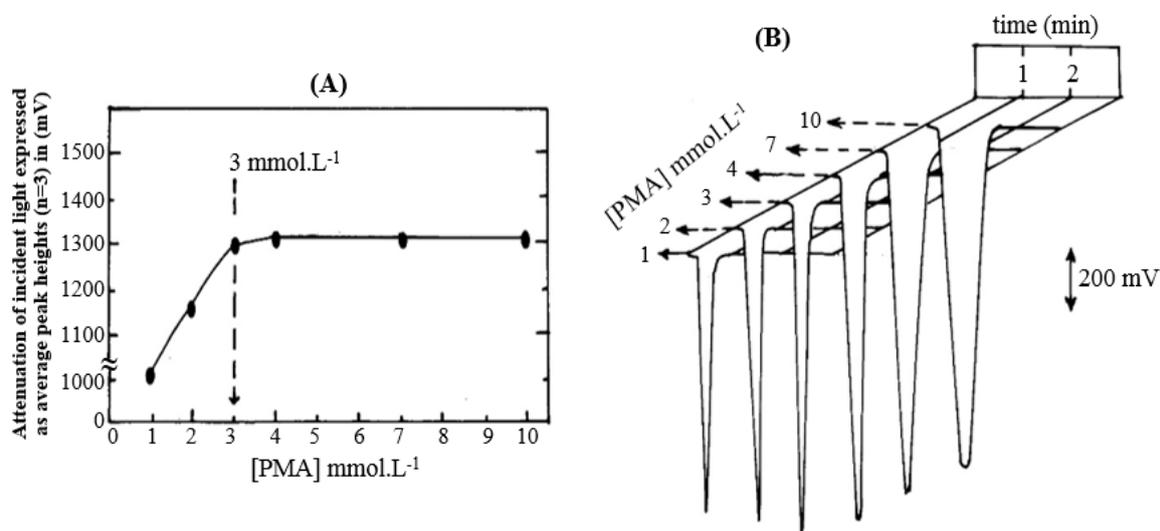
Study of the Optimum Parameters

The flow injection manifold system as shown in figure-2 was used to optimize the chemical and physical variables in order to obtain optimum conditions for the determination of Am by turbidity for Am-PMA system. They were studied by fixed variable optimization i.e. single variable was studied each at a time.

Chemical Variables

Effect of Phosphomolybdic acid

A series of PMA solutions were prepared ranging (1-10) mmol.L⁻¹ to establish the optimum concentration that can be used. At constant concentration of Am (6 mmol.L⁻¹) and 30 μ L sample volume were used. The study was conducted that 3 mmol.L⁻¹ of PMA was necessary to achieve the maximum attenuation of incident light as shown in figure-3-A. It can be shown that an increase in PMA might be cause an increase in particles density due to accumulation effect of precipitate particles up to 3 mmol.L⁻¹, following this concentration there was a slightly increase of the attenuation of light in addition to broadening in the peak maxima as shown in figure-3-B. Therefore 3 mmol.L⁻¹ PMA concentration was chosen as the optimum concentration that used for further experiment.



A: Attenuation of incident light

B: response profile using 6 mmol.L⁻¹ of Am, 30 μ L

Figure.3-Variation of PMA concentration on:

Effect of Acidic Media

The precipitation of Am with PMA was studied in different media (nitric acid, hydrochloride acid & sulphuric acid) at 10 mmol.L⁻¹ concentration in addition to the aqueous phase medium. The data obtained were plotted as shown in figure-4, in which can be seen that there were no significant excess in response height obtained from Ayah 6Sx1-T-1D solar cell CFI analyser for different acids used even though hydrochloric acid leads to decrease of response, it might be attributed to dissociation of

the precipitate particles. It was concluded that distilled water can be used equally compared to various acid used.

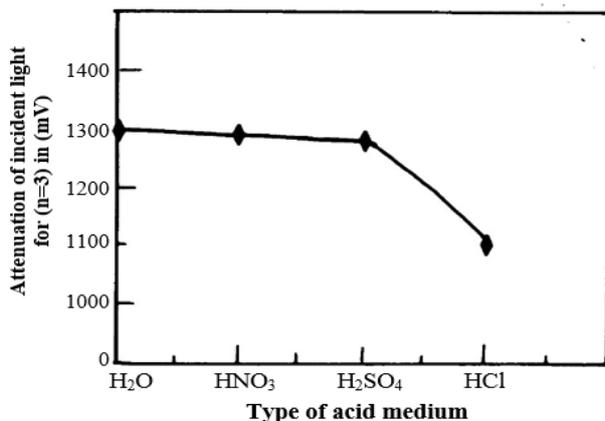


Figure.4-Effect of acid medium on the formation of ion pair for Am-PMA using 6 mmol.L⁻¹ of Am, 30 μ L sample volume and PMA (3 mmol.L⁻¹)

Effect of Polarity of the Carrier Stream

A variable ratio of methanol-water ($V_{\text{meth}}-V_{\text{H}_2\text{O}}$) as a carrier stream with fixed optimum parameters of PMA (3 mmol.L⁻¹) and 30 μ L sample volume at Am at 6 mmol.L⁻¹ were used. Figure-5 shows that the use of distilled water gave a regular profile response with acceptable sensitivity. While using mixture from methanol-water was observed a liberation of bubble in course of the formation of precipitation particulate leading to irregular distorted response profile. Therefore distilled water was chosen as the optimum carrier stream to conduct the reaction pattern. Table-1, shows the summary of results.

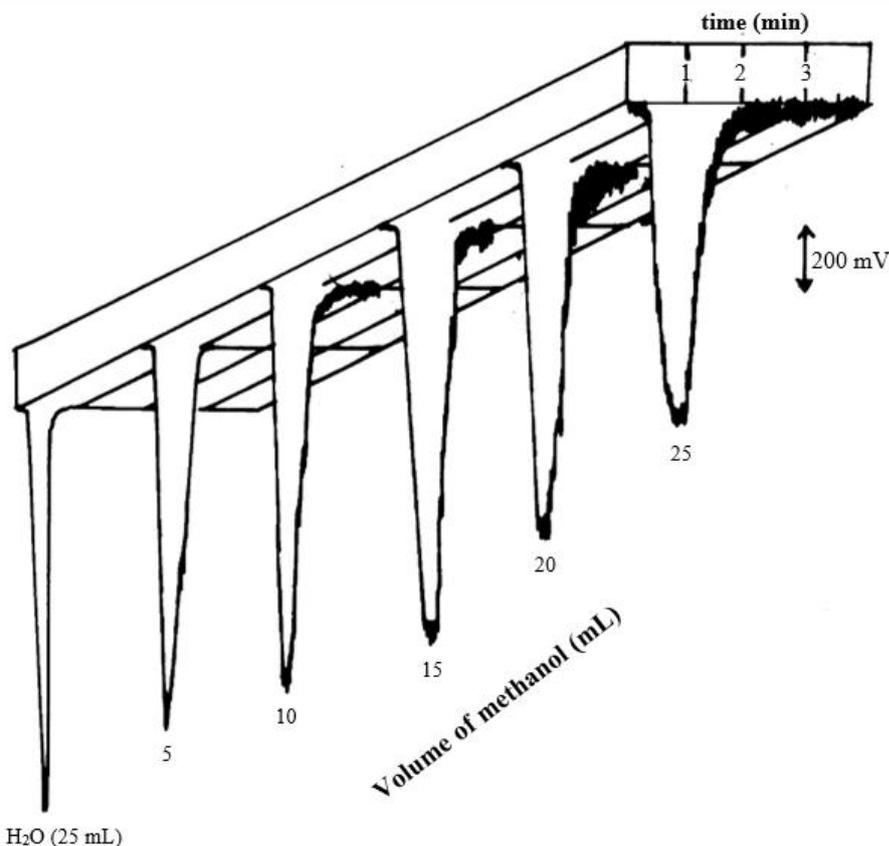


Figure.5- Effect of variation methanol (mL):H₂O (mL) volume ratio

Table 1-Variation of ratio of methanol-water as a carrier stream on response heights

Volume of methanol (mL)	Volume of water (mL)	Attenuation of incident light expressed as average of peak heights (n=3) \bar{y}_i (mV)	RSD%	Confidence interval of the average response $\bar{y}_i \pm t_{\frac{0.05}{2}, n-1} \frac{\sigma_{n-1}}{\sqrt{n}}$
0	25	1325	0.189	13050 \pm 6.21
5	20	1280	0.182	1280 \pm 6.09
10	15	1287	0.25	1287 \pm 8.82
15	10	1300	0.35	1300 \pm 11.30
20	5	1189	0.42	1189 \pm 12.42
25	0	1036	0.50	1036 \pm 12.92

Physical Variables

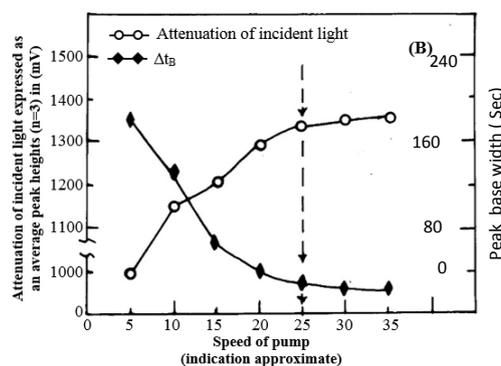
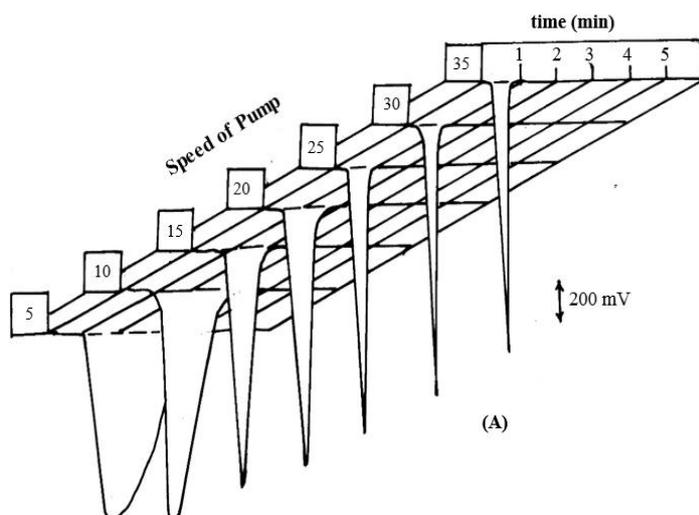
Flow Rate

Using optimum concentration of the reactants: PMA (3 mmol.L⁻¹) and 6 mmol.L⁻¹ of Am for the optimization of flow rate that ranged from 0.5-2 mL.min⁻¹ for carrier stream and 0.5-2.5 mL.min⁻¹ for PMA with 30 μ L of sample volume. The results are tabulated in table-2. It was noticed that at low flow rates, there were an increase in peak base width (Δt_B) as shown in figure-6-A. This mostly attributed to the dispersion and dilution which causes to a largement of sample segment of the precipitate product. While at higher flow rate (i.e: > 1.5, 1.8 mL.min⁻¹ for the carrier stream and PMA respectively), although the effect of physical parameter was very crucial on the response which in turn causes in obtaining of regular response and sharp maxima, but it is not very high due to departure speed of precipitate particles from measuring cell at a short time. Therefore, the best flow rate for the completion of the reaction between Am and PMA was 1.5 and 1.8 mL.min⁻¹ for carrier stream and PMA line respectively, to obtain a regular response, narrower Δt_B and minimize the consumption of reactants solution as shown in figure-6-B.

Table 2-Effect of the variation of flow rate on the measuring of attenuation of incident light

Pump speed indication approximate	Flow rate (mL.min-1)		Attenuation of incident light expressed as average of peak heights (n=3) \bar{y}_i (mV)	RSD%	Confidence interval of the average response $\bar{y}_i \pm t_{\frac{0.05}{2}, n-1} \frac{\sigma_{n-1}}{\sqrt{n}}$	Δt_B peak base width (sec)	t* (sec)
	Carrier stream	PMA line					
5	0.5	0.5	920	0.26	920 \pm 5.84	180	60
10	0.9	1.0	1150	0.17	1150 \pm 4.97	126	50
15	1.1	1.3	1200	0.17	1200 \pm 4.92	66	40
20	1.3	1.5	1290	0.15	1290 \pm 4.78	60	27
25	1.5	1.8	1332	0.094	1332 \pm 3.11	36	20
30	1.8	2.0	1350	0.10	1350 \pm 3.35	27	18
35	2.0	2.5	1353	0.19	1353 \pm 6.26	24	16

t* (sec): time for the departure of sample segment from injection valve reaching to the flow measuring cell.



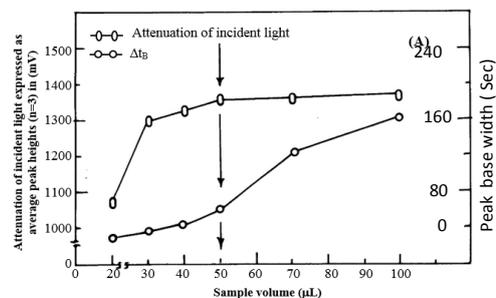
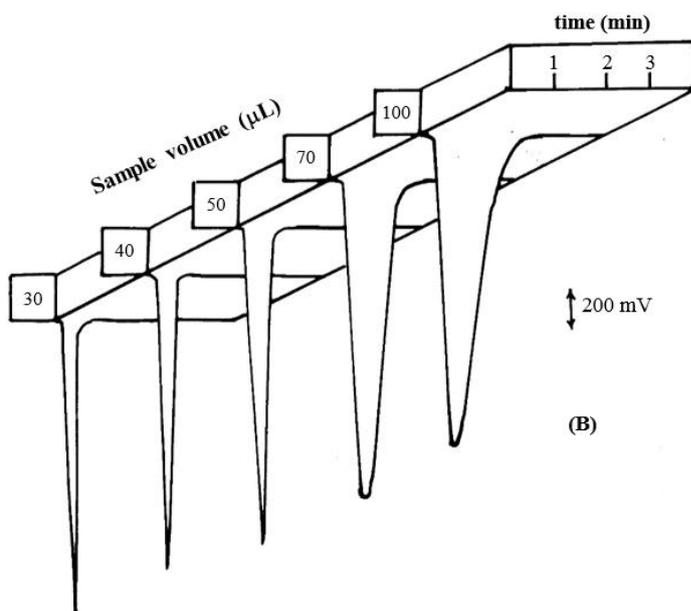
A: response profile

Figure.6-Variation of flow rate on:

B: Attenuation of incident light and Δt_B

Sample Volume

Using the optimum flow rate of $1.5 \text{ mL}\cdot\text{min}^{-1}$ for carrier stream and $1.8 \text{ mL}\cdot\text{min}^{-1}$ for PMA solution ($3 \text{ mmol}\cdot\text{L}^{-1}$), concentration of Am $6 \text{ mmol}\cdot\text{L}^{-1}$ with a various volumes ($20\text{-}100 \mu\text{L}$) were injected using open valve mode. The plot of change in sample volumes vs. attenuation of incident light and Δt_B is shown in figure-7-A. It was noticed that an increase of sample volume up to $50 \mu\text{L}$ led to a significant increase in response height and more perceptible than low sample volume. While a larger sample volume (i.e. more than $50 \mu\text{L}$) even though it gave a slightly higher response but wider in Δt_B and response maxima which was might be attributed to the continuous relatively longer time duration of precipitate particles segment in front of the detector and the increase of particles size causing a reverse movement of particles leading to obtain irregular response and broad in the peak maxima as shown in figure-7-B. Therefore; $50 \mu\text{L}$ was chosen as an optimum sample volume.



A: Response profile

Figure.7- Variation of sample volume segment of Am on:

B: Attenuation of incident light and Δt_B

Purge Time

Using different purge time for the sample segment (i.e. using 2-40 sec) allowed time for the Am (6 mmol.L^{-1}) sample to pass through the injection valve (injection mode) followed by turning the injection valve to the load position. Optimum sample volume of $50 \mu\text{L}$ was used. Figure-8 shows the continuation of the increase in the height of response and Δt_B with increase of injection time up to 20 sec. Thereafter, was no significant differences in height of response. At less 20 Sec, a decrease in response may be attributed to the incomplete purge time of sample from sample loop in the injection valve. Therefore; 20 sec as a purge time was chosen as optimum time to the complete purge of sample from loop for the next studies.

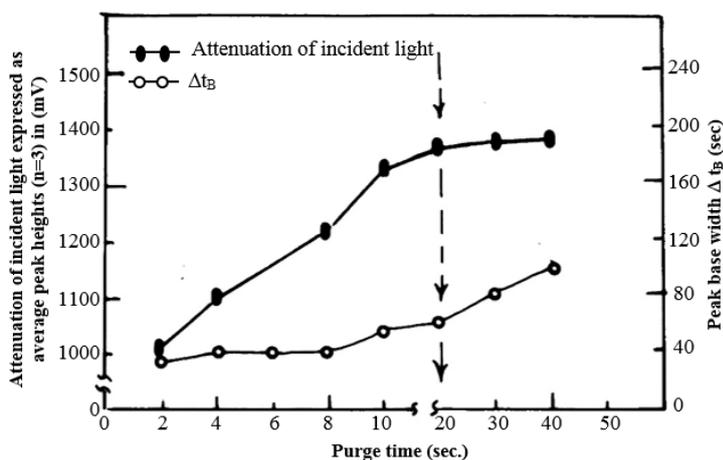


Figure 8- Variation of purge time on the attenuation of incident light

Incident Light Intensity

Intensity of light source was studied by using the optimum physical and chemical parameters achieved in previous section; with 6 mmol.L^{-1} of Am, $50 \mu\text{L}$. Variable intensity of light source was used 1.05 – 2.5 Volt by variation of light intensity channel in Ayah 6Sx1-T-1D solar cell-CFI analyser operation where read by AVO-meter. Figure-9, shows that an increase on the attenuation of incident light with increased intensity of source light (snow white). The intensity of 2.35 Volt was selected as the optimum voltage that can be supplied to give a better transducer energy response.

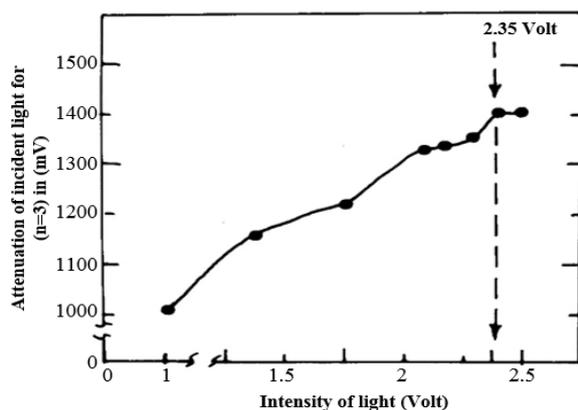


Figure 9-Effect of variation of light intensity on the attenuation of incident light

Calibration Curve

Using the optimum conditions previously established, a series of solutions for Am ($0.005\text{-}20 \text{ mmol.L}^{-1}$) were prepared. Each measurement was repeated three times. Attenuation of incident light for average peak height (mV) was plotted against the concentration of Am. A straight-line graph figure-10 from $(0.005\text{-}10) \text{ mmol.L}^{-1}$ of Am was obtained. Above 10 mmol.L^{-1} of Am, the value for correlation coefficient will deviate from linearity which might be attributed to the accumulation of

precipitate particles that cause a long duration of the precipitate in the flow cell and an increased exposure time in front of the detector leading to increase in the Δt_B and broadening in the response maxima. All results of the linear regression analysis [18-20] was summarized in table-3. At was shown that the correlation coefficient, linearity percentage, straight line equation and the calculated t-value at 95% confidence level of 58.66 which larger than tabulated t-value indicating clearly that the linearity against non-linearity is accepted.

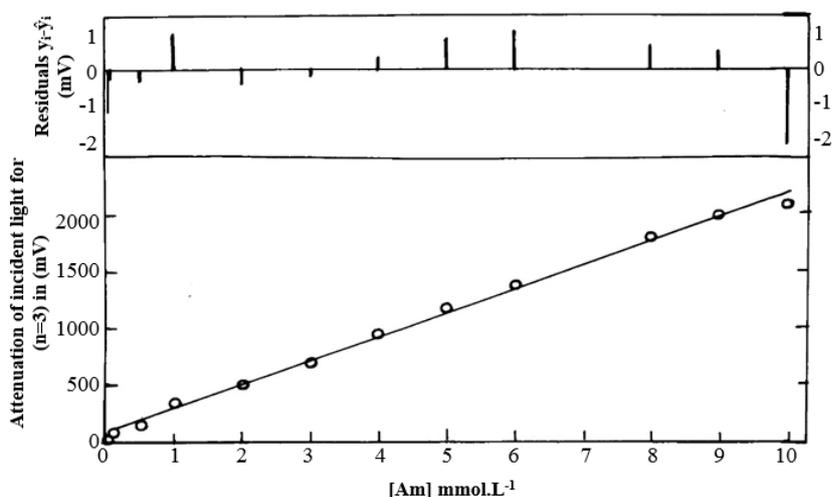


Figure 10-Calibration graph and residuals ($y_i - \hat{y}_i$) in mV using Ayah 6Sx1-T-1D solar cell-CFI analyser.

Table 3-Summary of calibration graph results for the determination of Am using Am-PMA system by the proposed method .

Measured [Am] mmol.L ⁻¹	Linear dynamic range mmol.L ⁻¹ n=12	\hat{y} (mV)= $a \pm S_a t + b \pm S_b t$ [X] at confidence interval 95%, n-2	r, r ² %	at 95%, n-2	
				t _{tab.}	t _{cal} = $\frac{ t \sqrt{n-2}}{\sqrt{1-r^2}}$
0.005-20	0.005-10	99.03±42.03+209.07±7.94[X]	0.9986 99.71%	2.228	<< 58.66

[X]: Amiloride concentration (mmol.L⁻¹)

Limit of Detection (L.O.D)

Three different approaches were used, gradual dilution of lowest concentration in the calibration graph, or detection based on the numerical value of slope and from the linear regression plot. Table-4 tabulated all these calculation value of detection limit for 50 μL sample volume as well as the limit of quantitation (LOQ).

Table 4-Summary of limit of detection of different approaches at 50 μL sample volume and optimum parameters

Gradual dilution for the minimum concentration	Based on the value of slope $x = \frac{3S_B}{\text{slope}}$	Theoretically	
		Linear equation \hat{y}_i (mV) = $y_B + 3S_B$ L.O.D	L.O.Q \hat{y}_i (mV) = $y_B + 10S_B$
5.74 ng	4.94 ng	6.94 μg	23.14 μg

x= value of L.O.D. based on slope

S_B = standard deviation of blank solution

y_B= average response for the blank solution (equivalent to intercept in straight line equation)

L.O.D = limit of detection, L.O.Q = Limit of quantitation

Repeatability

The repeatability was studied via measurements of R.S.D.% for some selected concentration of Am ($n=8$) tabulated in Table-5. The response profile at concentration 3, 9 mmol.L^{-1} of eight successive injected sample measurements is shown in figure-11 which noticed the kind of response-time for the used of concentration.

Table 5-Repeatability of Am results at optimum parameter

[Am] mmol.L^{-1}	\bar{y}_i (mV) $n=8$	Repeatability R.S.D.%	Confidence interval of the mean at 95%, $n-1$ \bar{y}_i (mV) $\pm t_{\frac{0.05}{2}, n-1} \frac{\sigma_{n-1}}{\sqrt{n}}$
3	720	0.17	720 ± 1.03
9	1800	0.13	1800 ± 1.94

$$t_{\frac{0.05}{2}, n-1} = 2.365, n = 8$$

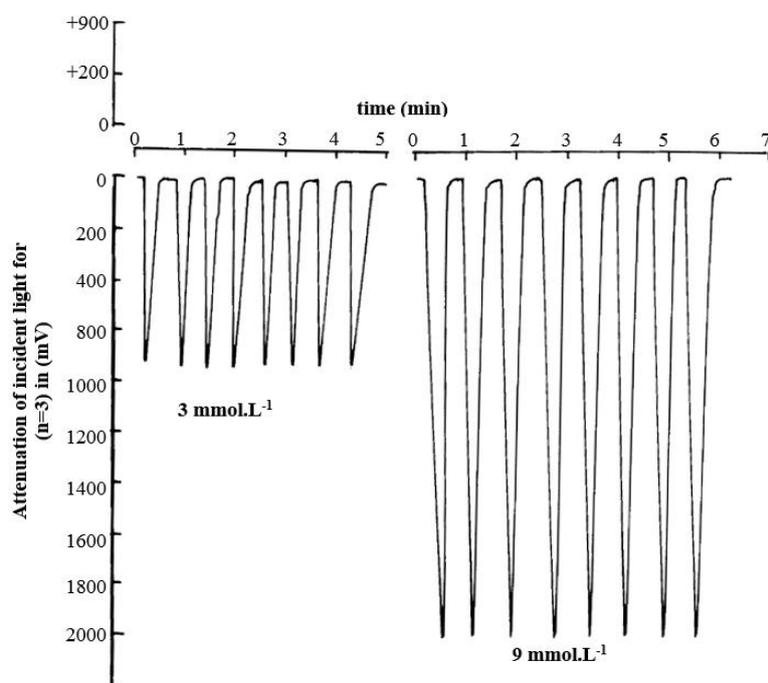


Figure 11-Response-time (min) for eight successive repeatable measurements of Am (3, 9 mmol.L^{-1})

Analysis of Pharmaceutical Preparation

The CFIA via attenuation of incident light expressed as (T_{0-180°) method using Ayah 6Sx1-T-1D solar cell CFI analyser achieved in this work used for the analysis of Am in two different of pharmaceutical preparations (Amiloride-Actavis and Moduretic-Algorithm). Each sample containing 5 mg of Am (active ingredient); and was compared with classical spectrophotometric method via the measurement of absorbance at $\lambda_{\text{max}} = 361 \text{ nm}$ [21] for Am as shown in figure-12. Linearity of the calibration curve was obtained for the concentration of Am ranged 0.05-5 mmol.L^{-1} and the summary of results were tabulated in table- 6. The standard additions method was applied by prepared a series of solutions from each pharmaceutical drug via transferring 5 mL (5 mmol.L^{-1}) to five volumetric flask (100 mL), followed by the addition of (0.1, 2, 3 and 4) from 20 mmol.L^{-1} standard solution of Am in order to have the concentration (0.25, 0.45, 0.65, 0.85 and 1.05) mmol.L^{-1} for the preparation of standard addition calibration graph. The measurements were conducted by both methods.

Results were mathematically treated for standard additions method. The results were tabulated in table-7 at confidence interval 95%.

Paired t-test was used as shown in table-8-A,B, which shows a comparison-treatment of data were subjected at two different paths:

First: comparing individual mean with quoted value [22-23] as described by the manufacturer. Having reference value of 5 mg to be compared with practically found value using Ayah 6Sx1-T-1D solar cell CFI analyser. Table-8-A shows that t-test value obtained assuming:

For actavis company

$$H_0 : \mu_{actavis} = \mu_5$$

$$H_1 : \mu_{actavis} \neq \mu_5$$

For algorith company

$$H_0 : \mu_{algorith} = \mu_5$$

$$H_1 : \mu_{algorith} \neq \mu_5$$

i.e. H_0 = Null hypothesis, H_1 = Alternative hypothesis.

Indicate that

Null hypothesis for actavis & algorith company that there were no significant difference between quoted value and founded value since $t_{cal.}$ less than $t_{tab.}$ (4.303) at 95% confidence interval

Second: A paired t-test was conducted between the sample from two different manufacturer by either method of analysis i.e. using proposed method with classical method.

Our hypothesis is as follows:

$$\text{Null hypothesis} = H_0 = \mu_{prop. meth.} = \mu_{class. meth.}$$

$$\text{Alternative hypothesis } H_1 = \mu_{prop. meth.} \neq \mu_{class. meth.}$$

A t-value for n-1 degree of freedom = 12.7

Any value of t calculated should be less than 12.7 in order to accept H_0 i.e. there is no significant difference between the two method of analysis.

Calculate t-value = 1.41 for n-1 at $\alpha=0.05$ (95%), two tailed test indicated that $1.41 < 12.7$, therefore H_0 is accepted in favour of H_1 as shown that in table-8-B.

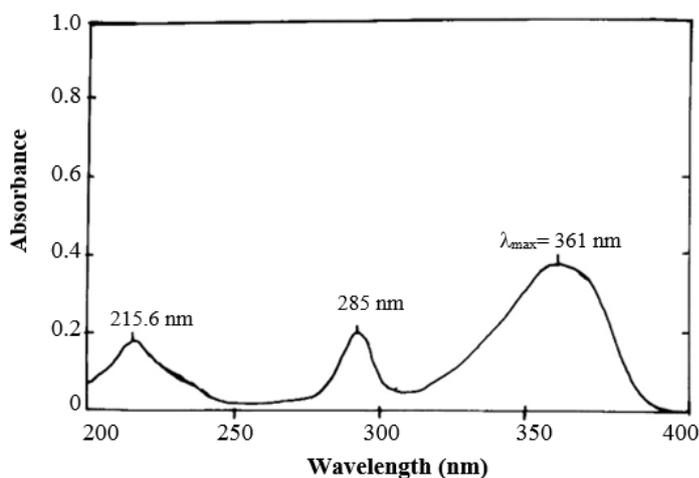


Figure 12-Absorbance spectra for Am by UV-Vis spectrophotometric type (UV-1800, Shimadzu) against methanol as a blank

Table 6-Summary of linear regression for the determination of Am using classical UV-Vis spectrophotometric method .

Measured [Am] mmol.L ⁻¹	Range calibration graph [Am] mmol.L ⁻¹	\hat{y} (mV)= $a \pm S_a t + b \pm S_b t$ [X] at confidence interval 95%, n-2	$t_{tab.}$	$t_{cal} = \frac{ t \sqrt{n-2}}{\sqrt{1-r^2}}$	$r, r^2\%$	Practically based on the gradual dilution for the minimum concentration
			at 95%, n-2			
0.005-6	0.005-5	0.132±0.052+0.321±0.0093[X]	2.228 << 30.89		0.9948 98.96%	11.48 µg

[X]: Am concentration in mmol.L⁻¹ , n=12

Table 7- Results for the determination of Am in pharmaceutical preparation using standard addition, with two methods, Ayah 6Sx1-T-1D solar cell CFI analyser and UV-Vis spectrophotometric method

Sample No.	Commercial name and Content and company, country	Confidence interval for average weight at 95% $\bar{w} \pm 1.96 \frac{\sigma_{n-1}}{\sqrt{n}}$ (g)	Sample weight equivalent to 5 mmol.L ⁻¹ of the active ingredient (114.8 mg)	Confidence interval for the theoretical content of active ingredient at 95% n=∞ (mg)	Equation of standard addition at 95% for n-2 $\hat{y}(mV) = a \pm S_a t + b \pm S_b t [X]$	r, r ² %	Practical content of active ingredient (mg)	Efficiency of determination Rec.%
					Ayah 6Sx1-T-1D solar cell CFI analyser			
UV-Vis spectrophotometric								
1	Amiloride 5 mg Actavis UK	0.1345±0.0021	3.08812	5 ± 0.078	396±42.23+1603±109.22x	0.9999 99.98	4.94	98.8
					0.32±0.021+1.26±0.71x	0.9936 98.73	5.08	101.6
2	Moduretic 5 mg Algorith Lebanon	0.2671±0.0014	6.13262	5 ± 0.026	268±59.23+1184±93.22x	0.9960 99.20	4.60	92.0
					0.43±0.025+1.58±0.29x	0.9928 98.56	5.44	108.9

\hat{y}_i = estimated vaule for attenuation of incident light or absorbance

$t_{\frac{0.05}{2}, \infty} = 1.96$ at 95%, $x = [Am]$ mmol.L⁻¹

Table 8:A-Results of paired t-test for the comparison between practical content of Am by proposed method with quoted value

Sample no.	Practical content of Am \bar{x} (mg)	Quoted value μ (mg)	$T_{tab.}$ at 95%, n-1	$t_{cal.} = \frac{(\bar{x} - \mu)\sqrt{n}}{\sigma_{n-1}}$
1	4.94	5	4.303 >> 0.074	
2	4.60	5	4.303 >> 0.489	

$t_{tab.} = t_{\frac{0.05}{2}, n-1} = t_{0.025, 2} = 4.303$ at 95% confidence level

$n = 3, /- t_{cal.} / = t_{cal.}$

Table 8:B- Paired t-test for comparison between proposed method (Ayah 6Sx1-T-1D solar cell CFI analyser) with classical UV-Vis spectrophotometric method using standard additions for the determination of Am

Sample no.	Practically content of Am (mg)		X_d	\bar{X}_d	σ_{n-1}	$t_{cal.} = \frac{\bar{X}_d \sqrt{n}}{\sigma_{n-1}}$	$t_{tab.}$ for 95%, n-1
	Proposed method	Classical method					
1	4.94	5.08	-0.14	-0.49	0.49	1.41 << 12.7	
2	4.60	5.44	-0.84				

$t_{tab.} = t_{\frac{0.05}{2}, n-1} = 12.7, /- t_{cal.} / = t_{cal.}, n = 2$

Conclusion

The turbidity measurement via the use of Ayah 6Sx1-T-1D solar cell-CFI analyser proposed a method for determining Am. It is characterized by accuracy, speed and sensitivity. The method is based on the precipitation of Am by PMA in aqua medium and measured turbidity via the attenuation of incident light at 0-180° using linear array of six snow white light emitting diode as a source and one solar cell as a detector. From the experiment point of view, the manipulation is very simple and sequential measurement was permitted with sample frequency up to 35 sample per hour. The proposing method, is used cheaper instruments and reagents comparing with the classical spectrophotometry [21]. The R.S.D% is less of than 1% and it was observed for all samples, indicating a satisfactory precision of the proposed method. The standard addition method was used to avoid matrix effects. Also this method can be applied to nano gram determination of Am in pure and the pharmaceutical preparations .It offers the advantages of a high sensitivity without the needing for heating or extracting. The statistical analysis was in a good agreement with those of British pharmacopoeia [22].

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