A new mode for an on-line determination of Amiloride in pure and pharmaceutical preparation using CFIA with homemade Ayah $3S_{BGR}x3$- 3D solar cell microphtometer analyzer.

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Abstract:

A new, simple, accurate, fast and sensitive spectrophotometric method has been developed for the analysis of amiloride in pure and pharmaceutical formulations by continuous flow injection analysis. The method was based on the oxidation of the drug with Ce(IV)sulfate in acidic medium to formed a pinkish-red color species which determined using homemade Ayah $3S_{BGR}$ x3-3D solar cell flow injection photometer. Optimum conditions were obtained using a high intensity green light emitted diode as a source. The linear dynamic range for the instrument response versus amiloride concentration was 0.0005- 10 mmol.L^{-1} while the L.O.D was of 9.471 ng / sample. The correlation coefficient (r) was 0.9824 while percentage linearity (r^2%) was 96.50%. RSD% for the repeatability (n=8) was lower than 1.5% for the determination of amiloride, at concentration of 0.5 mmol.L^{-1}. The method was applied successfully for the determination of amiloride in pharmaceutical preparation. A comparison was made between the newly developed method and the classical method (UV-Vis spectrophotometry at wave length 540nm) of analysis 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 company 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 no significant difference between two methods at 99% but a significant difference at 95% confidence level.

Key words: Amiloride, flow injection analysis, spectrophotometry.

نمط جديد للتقدير الأني للأميلورايد في المستحضرات الصيدلانية والنقية بأستخدام التحليل بالحقن الجرياني المستمر عن طريق استخدام محلل طيفي مايكروي مصنع محليا Ayah $3S_{BGR}$ x3-3D solar cell.

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الخلاصة:

طورت طريقة طيفية جديدة ، بسيطة، سريعة، مظبوطة، وحساسة لتحليل الاميلورايد في صيغته النقية والمستحضرات الصيدلانية بأستخدام التحليل بـالحقن الجرياني المستمر . استندت الطريقة على اكسدة العقار بكبريتات السيريوم (VI) في الوسط الحامضي لتكوين اصناف ملونة وردي- محمر والتي قدرت بأستخدام

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مطياف الحقن الجرياني مع Ayah $3S_{BGR}$ x3−3D solar cell المصنع محليا. تم دراسة الظروف الفضلى التي تم التوصل اليها بأستخدام ثنائي الوصلة باعث للضوء الاخضر وبشدة عالية كمصدر للتشعيع . كان المدى الخطي الفعال لعلاقة تغير الاستجابة الألية مع تركيز الاميلورايد 0.0005 − 10 مللي مول.لتر$^{-1}$ بينما حدود الكشف 9.471 نانوغم / انموذج. وان معامل الارتباط (r) 0.9824 بينما نسبة الخطية r^2 % = 96.50 % . الانحراف القياسي النسبي المؤي (RSD%) للتكرارية اقل من 1.5 % لمحلول الاميلورايد بتركيز 0.5 مللي مول .لتر$^{-1}$ (8=n) طبقت الطريقة بنجاح لتقدير الاميلورايد في المستحضرات الصيدلانية. اجريت مقارنة بين الطريقة المطورة والطريقة التقليدية (مطيافية UV−Vis) عند طول موجي 540 نانو متر للتحليل باستخدام منحني الاضافات القياس من خلال استخدام اختبار t− المزدوج. تبين انه لايوجد فرق جوهري بين القيمة الرسمية وقيمة t− المحسوبة من الطريقة المطورة عند فاصل ثقة 95% بالاضافة الى اجراء مقارنة بين الطريقتين وحساب قيمة t− ولوحظ انه لايوجد فرق جوهري بين الطريقتين عند مستوى قناعة 99% وفرق جوهري عند مستوى قناعة 95%.

Introduction:

Amiloride(Am) (3,5-diamino - *N* - (diaminomethylene) - 6-chloropyrazine - carboxamide monohydrochloride), figure-1 is an orally administered potassium - sparing diuretic agent that is widely used therapeutically mainly in combined formulations with hydrochlorothiazide[1] . One possible explanation for this observation is that Am can enter the channel lumen, thereby forming a molecular plug that obstructs the flow of permeant ions. In addition, permeant cations, such as Na^+, and poorly permeant cations, such as K^+, interact competitively with Am in this tissue [2] . Am is a potent, reversible inhibitor of ion movement through epithelial sodium channels [3] . In the toad urinary bladder, the Am-induced current inhibition is voltage dependent: transmembrane voltages that would tend to drive the positively charged Am from the mucosal solution into the membrane(channel) increase its apparent affinity for its binding site [4] . Am restrains the sodium – potassium interchange in kidneys by sealing the distal membrane to promote sodium excretion and potassium re-absorption [5]. Because of its potassium-sparing ability it is used in the treatment of hypokalemia induced by thiazide or other kaliuretics in patients with congestive heart failure or hypertension and prophylaxis [6]. It is also used as a therapeutic drug and a pharmacological tool [7]. This natriuretic agent can be functional as a doping substance. In sports, diuretics are abused mainly for rapid diminution of corporal weight gain for different weight categories and for the reduction of the concentration of medical drugs in urine by diluting the latter by means of the rapid production of an elevated volume of urine, leading to a smaller possibility of detecting other doping substances . Owing to the uncontrollable use of Am , the International Olympics Committee (from 1990) has included it in the list of forbidden substances [8]. The therapeutic and doping dose of Am varies from 5 to 20 mg per day (one administration only) [9]. Am was found to interact with adenosine A1receptors in calf brain at a site distinct from the ligand binding site [10] . It is a specific reversible inhibitor of sodium transport [11] . And blocks the Na^+ / H^+ exchange pathway [12] . It is an inhibitor of urokinase-type plasminogen activator [13]. Literature survey reveals that various analytical methods have been reported for the determination of Am in pure and pharmaceutical formulations which include, spectrophotometric method[14,15] ,capillary zone electrophoresis[16] , Atomic Emission spectrometry[17], high performance liquid chromatography (HPLC) [18-20].

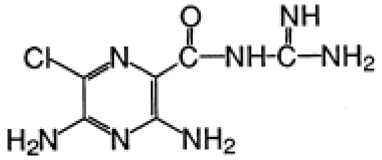

Figure 1- chemical structure of amiloride

This method based on the oxidation of Am by Ce(IV) sulfate in acidic medium . The oxidation product yields a pinkish red color measured at 525nm. The procedure is simple, rapid and is proposed for the control analysis of the drug in pure and pharmaceutical preparation as an alternative analytical procedure.

Experimental:

Chemicals and reagents:

All chemicals were used of analytical-reagent grade while distilled water was used to prepare the solutions. A standard solution of Am ($C_6H_8ClN_7O$, M.Wt 229.6g.mol^{-1}, SID, 0.02M) was prepared by dissolving 1.148 g in 250 ml methanol (absolute 99.5 % , Fluka-Garantie) . A 0.1M stock solution Ce(IV) sulfate ($Ce(SO_4)_2$ M.Wt 332.298 g.mol^{-1} , Hopkin & Williams LTD) was prepared by dissolving 16.614 g in 500 ml of sulphuric acid 1M, (H_2SO_4, M.Wt 98g.mol^{-1}, 18 M, sp.g 1.84g.ml^{-1} , percentage 96%, BDH,) (standardized with solution Na_2CO_3) was prepared by diluting 27.7ml of the sulphuric acid in distilled water to final volume 500ml by using 500mL volumetric flask.

Sample preparation:

Thirteen tablets were weighted, crushed and grinded. Tablets contain 5 mg of Am were weighted 2.97332 g (equivalent to 114.8 mg of active ingredient) (5 mmol.L^{-1}), and 2.7459 g (equivalent to 57. 4 mg of the active ingredient) (2.5 mmol.L^{-1}) for Amiloride, Actavis , and Modurtic, Algorith respectively. Dissolved in little methanol, followed by filtration to get rid of dissolved materials and completed the volume to 100 ml with methanol.

Apparatus and manifold:

The flow system used for the determination of Am, shown schematically in figure-2 , peristaltic pump two channels variable speed (Ismatec , Switzerland). Valve 6 – port medium pressure injection valve (I D E X corporation , USA) with sample loop (1 mm i.d. Teflon ,variable length) . the instrument response was measured by Ayah $3S_{BGR}$ x 3 – 3 D solar cell continuous flow injection analysis microphotometer (homemade) by using super bright blue470 nm , green 525nm and red light 635 nm emitted diode (LED) as source , three solar cell as a detector. The output signals were recorded by potentiometric recorder (Siemens, Germany)(1- 5 Volt,1000-5000 mV) . Peak height was measured for each signal. UV-Vis Spectrophotometer digital double beam type (UV- Vis spectrophotometer, UV-1800, shimadzu, Japan) was also used to scan the spectrum of colored species using 1cm glass cell.

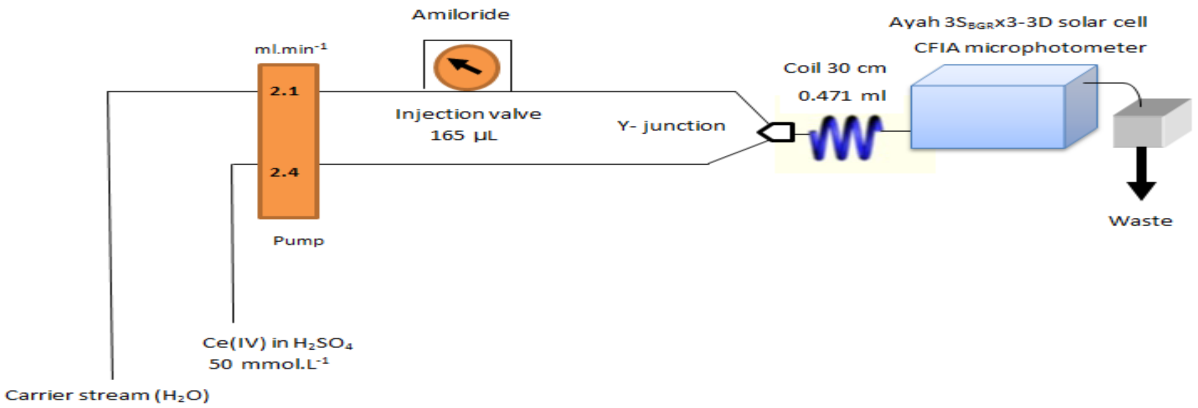

Figure 2-schematic diagram of Continuous Flow Injection Analysis system with Ayah $3S_{BGR}$ x 3 - 3D solar cell CFIA microphotometer.

Methodology:

The whole reaction of manifold system for Am determination by direct oxidation of Am with Ce(IV) sulfate in acidic medium to form colored species was shown in scheme (1) . The manifold system is composed from two lines: The first line supplied distilled water at 2.1 ml.min^{-1} which leads to the injection valve for carrying Am sample with 165 µ L sample volume (loop length 21 cm , with 1mm I.D.) . The second line supplied Ce(IV) sulfate 50 mmol.L^{-1} in acidic medium (500 mmol.L^{-1} of H_2SO_4) at 2.4 ml.min^{-1} . Both of lines meet at junction (Y- junction) with an outlet for reactants product from pinkish – red colored species which passes through Ayah $3S_{BGR}$ x3- 3D solar cell CFIA microphotometer and the variation of response was monitored using green light emitted diode LED (525 nm) throughout the reaction to obtain transducer energy response in mV versus time . Each solution was assayed triplicate. A proposed mechanism of oxidation of Am by Ce(IV)sulfate in acidic medium is presented in scheme (1) [21- 24].

Schematic (1): proposed mechanism of reaction between Am and Ce(IV) sulfate in acidic medium .

Results and discussion:

Scanning of spectrum for Am – Ce(IV) sulfate – H_3O^+ .

A study was carried out to obtain λ_{max} for the colored product (pinkish –red) by the oxidation of Am (2 mmol.L^{-1}) with Ce(IV)sulfate (10 mmol.L^{-1}) in acidic medium (H_2SO_4, 500 mmol.L^{-1}) using a homemade Ayah $3S_{BGR}x3$-3D solar cell CFIA microphotometer that works with three selected colors mainly three light emitting diode [Blue 470 nm , Green 525 nm , and Red 635 nm] . A sample of (165µL) was injected through the valve, figure-2, using two lines of the manifold reaction system. The transducer energy response of which was recorded on x-tpotentiometric recorder as shown in figure-3 which indicate the profile of the response and intensity. It can be seen that a smooth profile and high intensity can be obtained using super bright green LED at 525 nm which will be used as a source throughout this work.

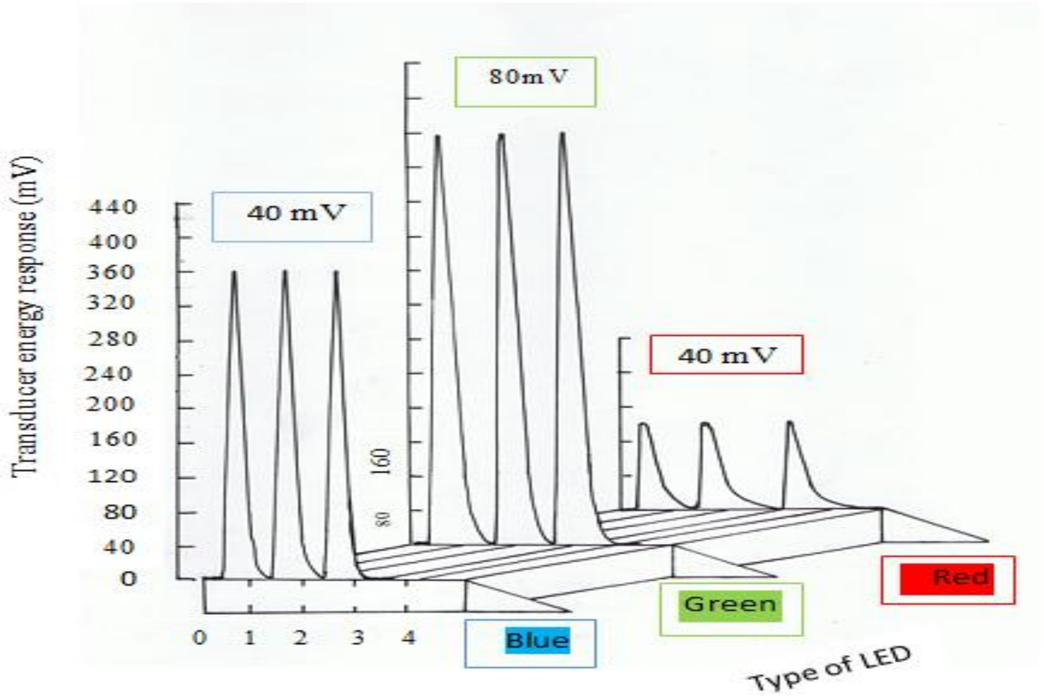

Figure 3- variation of type of the light emitting diode [blue 470 nm, green 525 nm, and red 635 nm] on transducer energy response profile versus time for three successive injected samples of Am .

Optimum conditions for oxidation of Am with Ce (IV) Sulfate in acidic medium.

Chemical variables:

Effect of Ce (IV) Sulfate concentration:

The study was carried out using series of solutions prepared by different concentration of Ce (IV) sulfate (5 -100 mmol.L^{-1}) in volumetric flask (25ml) diluted by sulphuric acid (500 mmol.L^{-1}) at fixed concentration of Am (5 mmol.L^{-1}), injection volume was (165µL) and flow rate (2.1, 2.4 ml.min^{-1}) for the carrier stream and Ce(IV) sulfate respectively . The obtained results were tabulated in Table. 1, Summarizes the average of three successive readings with relative standard deviation and confidence interval of the average response at 95% confidence ($\propto = 0.05$) .The data obtained showing the optimum concentration of Ce (IV) sulfate was 50mmol.L^{-1}, figure-4 shows the plot of the results as it was obtained from Ayah 3S$_{BGR}$ x3 -3D solar cell CFIA microphotometer. It was noticed too an increase in the response of the colored species with increasing Ce (IV) Sulfate concentration up to 50 mmol.L^{-1} , while at higher concentration (more than 50 mmol.L^{-1}) a decrease in response was noticed, It really might be due to increase of the density of the colored product which work as an internal filter that really prevent the remaining light intensity after absorption process by the colored species to the solar cell detector in addition to, at high concentration might be due to the formation of small colored colloidal precipitate, that might formed in front of the detector causing a distortion of the peak maximum.

Table 1- variation of Ce (IV) sulfate concentration on the transducer energy response of Am – Ce(IV)-H_3o^+ system .

[Ce(SO$_4$)] mmol.L^{-1}	transducer energy response expressed as peak heights (n=3) , Y_i (mV)	Average transducer energy response expressed as peak heights (n=3) ȳi (mV)	RSD %	Confidence interval of The average response $\bar{y}i \pm t_{0.05/2}\, \sigma_{n-1}/\sqrt{n}$
5	536,544,540	540	0.74	540 ± 9.94
10	736,736,736	736	0	736 ± 0
50	1120,1144,1132	1132	1.06	1132 ± 29.81
70	1064,1072,1068	1068	0.37	1068 ± 9.94
100	832,864,848	848	1.89	848 ± 39.74

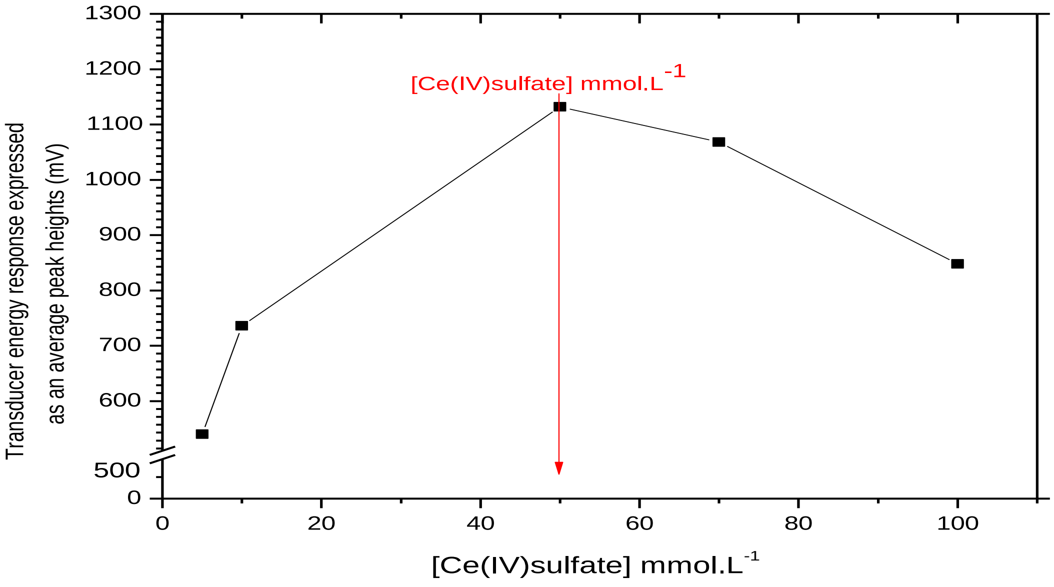

Figure 4- variation of Ce(SO$_4$)$_2$ concentration on transducer energy response using 165µL , 5 mmol.L^{-1} of Am.

Effect of sulphuric acid on Am- Ce(IV) – H_3O^+ system :

A series of solutions were prepared by different concentrations of sulphuric acid (10 -1000 mmol.L^{-1}) in volumetric flask (10ml) containing optimum concentration of Ce(IV) sulfate (50mmol.L^{-1}) and fixed concentration of Am (5mmol.L^{-1}) with sample volume (165µL). The results obtained shown in Table. 2 summarizes the average response of three successive injections. Since sulphuric acid act as catalyst for the oxidation of Am by Ce(IV)sulfate producing a colored species , therefore the increase in its concentration results in an increase in peak height and gave a regular and sharp response up to 500 mmol.L^{-1} as shown in figure-5, while an increase above 500 mmol.L^{-1} will decrease in the response due to the probability of dissociation of colored species, therefore 500 mmol.L^{-1} was chosen as the optimum concentration of sulphuric acid as a catalyst for the oxidation of Am by Ce(IV) sulfate.

Table 2- variation of sulphuric acid concentration on the transducer energy response of colored species using 5 mmol.L^{-1}, 165 µL of Am.

[H_2SO_4] mmol.L^{-1}	transducer energy response expressed as peak heights (n=3) , Y_i (mV)	transducer energy average response expressed as peak heights (n=3) $\bar{y}i$(mV)	RSD %	Confidence interval of The average response $\bar{y}i \pm t_{0.05/2}\ \sigma_{n-1}/\sqrt{n}$
10	904,896,900	900	0.44	900 ± 9.94
50	1112,1115,1118	1115	0.27	1115 ± 7.45
100	1136,1120,1128	1128	0.71	1128 ± 9.88
500	1160,1144,1176	1160	1.38	1160 ± 39.75
1000	752,740,750	747.33	0.86	747.33 ± 15.9

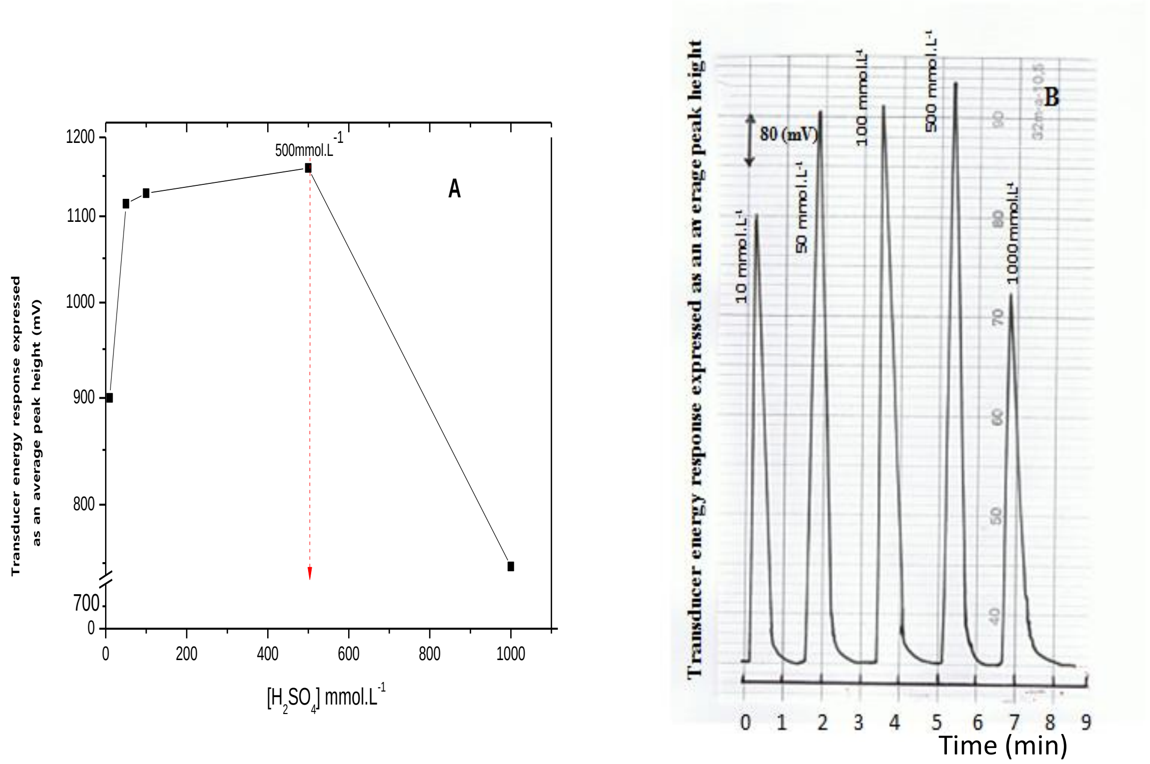

Figure 5- variation of the acidic medium (H_2SO_4) on: (A) transducer energy response (mV), (B) response profile using Ayah $3S_{BGR}X$ 3-3D solar cell CFIA microphotometer

Effect of polarity of the carrier stream:

Using variable ratio of methanol / water ($v_{(meth.)\ ml}$ / $v_{(water)\ ml}$) as a carrier stream with fixed optimum parameters of Ce (IV) sulfate 50 mmol.L^{-1} in 500 mmol.L^{-1} H_2SO_4 and 165 µL sample volume from Am (5 mmol.L^{-1}) figure-6 which shows that the use of distilled water gave a regular profile response with acceptable sensitivity and regularity. While the use of mixture from methanol-water; was observed liberation of bubble in course of the formation of colored species leading to irregular distorted response profile. Therefore distilled water was chosen as the optimum carrier stream to conduct the reaction pattern. Table. 3 shows summary of the results.

Table 3- variation of variable ratio of methanol / water as a carrier stream on response profile and sensitivity.

methanol/water V_{ml} / V_{ml}	transducer energy response expressed as peak heights (n=3) , Y_i (mV)	transducer energy average response expressed as peak heights (n=3) $\bar{y}i$(mV)	RSD %	Confidence interval of The average response $\bar{y}i \pm t_{0.05/2}\ \sigma_{n-1}/\sqrt{n}$
0	1000,1004,996	1000	0.40	1000 ± 9,93
5	824,820,828	824	0.49	824 ± 9.93
10	936,934,938	936	0.21	936 ± 4.97
15	904,904,892	900	0.76	900± 17.19
20	912,916,908	912	0.43	912 ± 9.93
25	1040,1040,1050	1043.33	0.55	1043.33 ± 14.33

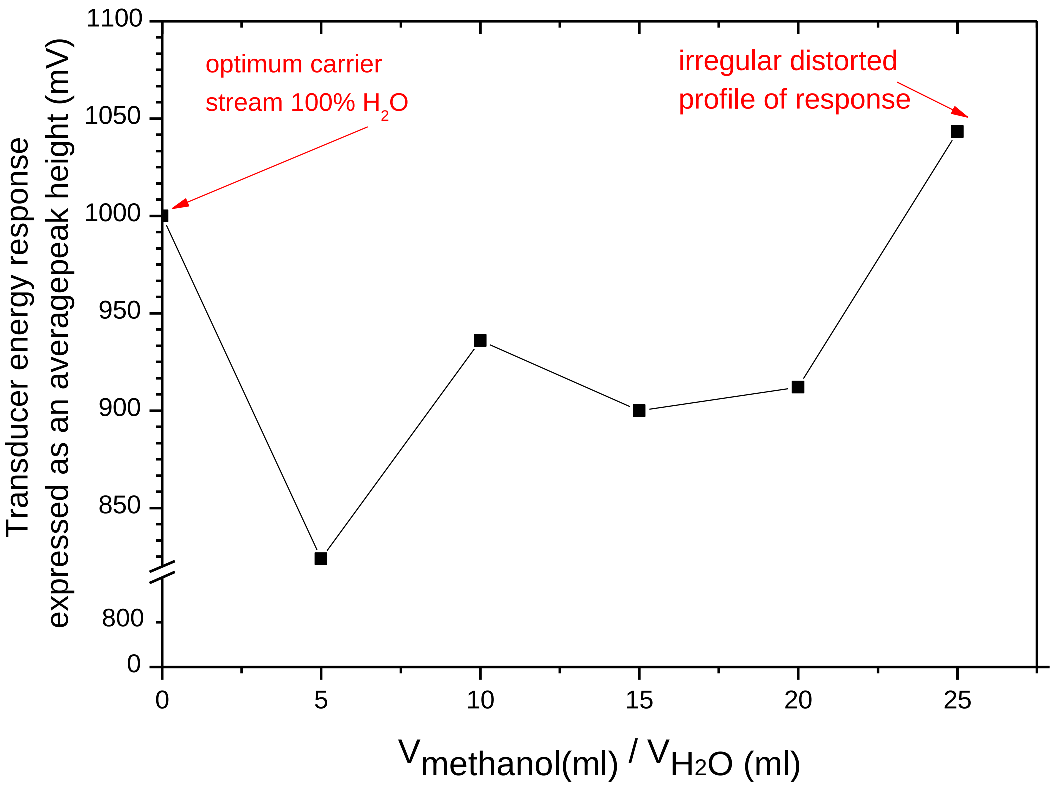

Figure 6- variation of variable ratio of the carrier stream (V $m_{ethanol}$ / V_{H2O}) on the transducer energy response (mV), for determination of Am by Am- Ce(IV)- H_3O^+ system.

Physical variables

Flow rate:

Using optimum concentration of the reactant: Ce (IV) of 50 mmol.L^{-1} in 500 mmol.L^{-1} H_2SO_4 and 5 mmol.L^{-1} of Am for the optimization of flow rate that ranged from 0.4 – 3.1 ml.min^{-1} for Ce (IV) sulfate, and 0.4 – 2.7 ml.min^{-1} for the carrier stream. Using 165µL of sample volume. The results are tabulated in Table. 4. It was noticed that at low flow rates, there were an increase in peak base width (Δt_b) as shown in figure-7.This might be due to the dispersion and dilution which causes an irregular response . While at higher flow rate (> 1.1, 1.2 ml.min^{-1} for the carrier stream and Ce(IV)sulfate respectively), although the effect of physical parameter was very crucial on the response obtaining regular response and very sharp maxima. Figure-7 shows that the best flow rate for the completion of the reaction of Am with Ce(IV)sulfate was 2.1 , 2.4 ml.min^{-1} for the carrier stream and Ce (IV) sulfate respectively to obtain a regular response , narrower Δt_b ,and minimize the consumption of reactants solutions . The time required from the moment of departure of the sample from the injection valve to the measuring cell is 18 sec.

Table 4- variation of flow rate (ml.min^{-1}) on the transducer energy response (mV).

Pump speed Indication approximate	Flow rate (ml.min^{-1})		Average transducer energy response expressed as peak heights (n=3) ȳi (mV)	RSD%	Confidence interval of the average response $\bar{y}i \pm t_{0.05/2}\,\sigma_{n-1}/\sqrt{n}$	Δt_b sec	t sec
	Carrier stream of Am	$Ce(SO_4)_2$ Line					
5	0.4	0.4	752	0.24	752 ± 4.55	240	66
10	0.7	0.7	864	0.27	864 ± 5.77	174	40
15	1.1	1.2	952	0.18	952 ± 0.45	120	36
20	1.4	1.6	1037	0.19	1037 ± 4.97	90	27
25	1.8	2.0	1088	0.02	1088 ± 0.55	84	20
30	2.1	2.4	1160	0.05	1160 ±1.44	60	18
35	2.4	2.8	1076	0.06	1076 ±1.69	54	15
40	2.7	3.1	1020	0.13	1020 ± 3.28	48	13

Δt_b (sec): peak base width

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

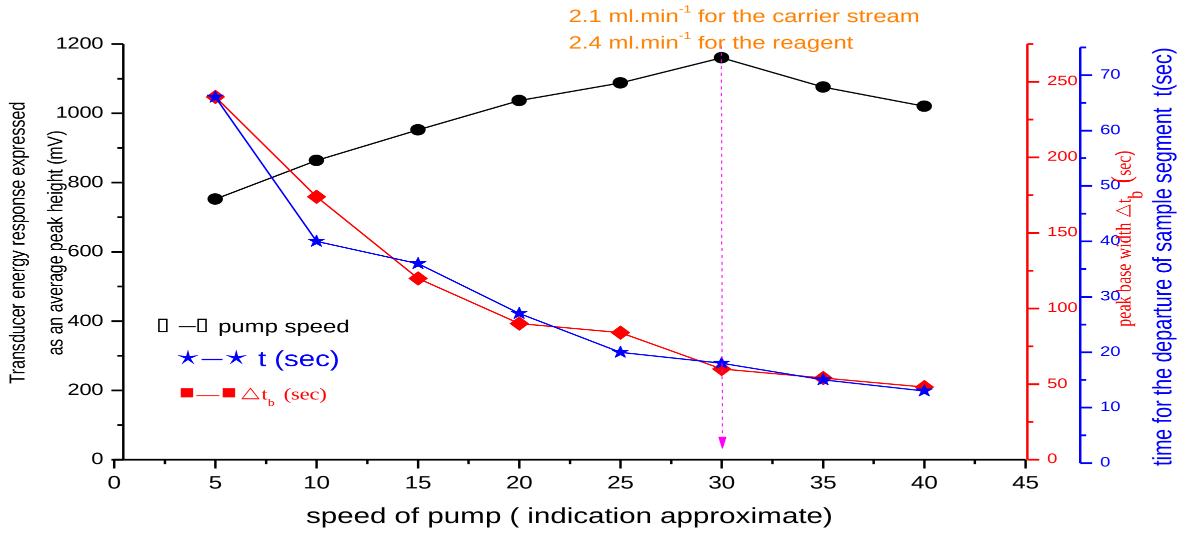

Figure 7-variation of flow rate on transducer energy response, peak base width (Δt_b sec), and time for the departure of sample segment from injection valve reaching to the flow measuring Cell (t sec).

Sample volume:

The effect of sample volume, using optimum flow rate 2.1 $ml.min^{-1}$ for the carrier stream and 2.4 $ml.min^{-1}$ for the Ce (IV) sulfate solution. A selected concentration 5 $mmol.L^{-1}$ of Am and optimum concentration of Ce(IV) sulfate (50 $mmol.L^{-1}$) using green light emitting diode as source for irradiation with variable sample volumes were studied. Variable sample volumes were obtained using sample loops to obtained 110, 156, 165, 211, 279 µL, successively with open valve technique. The obtained results are tabulated in Table. 5, while figure-8 shows these effects. It was noticed that an increase in sample volume led to an increase in the height of response profile without effecting on the response profile of obtaining up to the sample volume 165 µL. over 165 µL, there was a broadening at the peak maxima and an increase in the base width (Δt_b) this is illustrated in figure-8 which shows that the optimum volume was 165 µL for better response profile.

Table 5- variation of injected sample volume on transducer energy response at a selected Am concentration of 5 $mmol.L^{-1}$.

Loop length **(cm)**	Injected sample volume µL	Average transducer energy response expressed as peak heights (n=3) $\bar{y}i$ (mV)	RSD%	Confidence interval of The average response $\bar{y}i \pm t_{0.05/2}\, \sigma_{n-1}/\sqrt{n}$	Δt_b sec	t sec
14.00	110	733.33	0.21	733.333 ± 3.78	42	10.8
19.82	156	880.00	0.37	880.00 ± 7.99	48	11.4
21.00	165	1160.67	0.21	1160.67 ± 6.04	54	12
26.80	211	1100.33	0.29	1100.33 ±7.99	60	12.6
35.60	279.0	1096.00	0.31	1096.00 ± 8.5	66	18

Δt_b (sec):- peak base width.
t (sec) :- arrival time of the injected sample from the valve to the flow measuring cell .

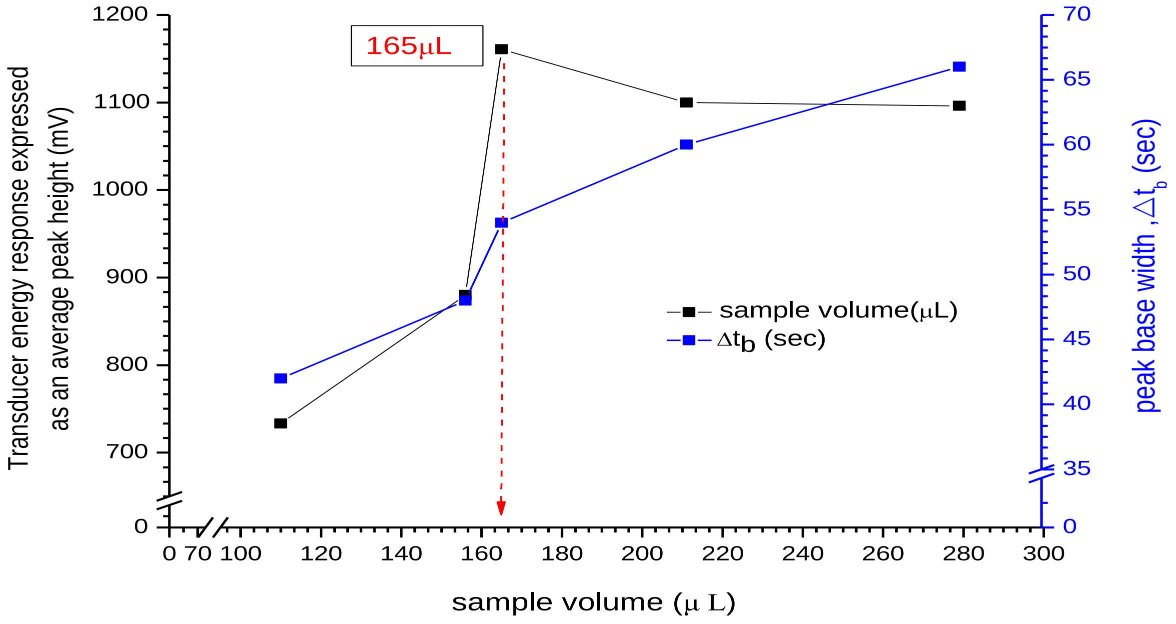

Figure 8- variation of sample volume segment of Am on transducer energy response.

Purge time:

Using different purge time for the sample segment i . e , using 2 – 20 sec allowed time for the Am sample to passing through the injection valve (injection mode) followed by turning the injection valve to the load position . Optimum sample volume of 165 µL was used. Figure-9 shows the continuation of the increase in the height of response and Δt_b with increase of injection time up to 15 sec, after that there was no significant differences in height of response. The decrease in response when using less than 15 sec was attributed to the incomplete purge time of sample from sample loop in the injection valve. Therefore, 15 sec as purge time was chosen as optimum time to the complete purge of sample from sample loop for the next studies and obtained a better response profile. The obtained results are tabulated in Table. 6.

Table 6- variation of purge time on the transducer energy response.

Purge time sec	Average transducer energy response expressed as peak heights (n=3) ȳi (mV)	RSD%	Confidence interval of The average response $\bar{y}_i \pm t_{0.05/2}\, \sigma_{n-1}/\sqrt{n}$	Δt_b sec
2	577.33	1.058	577.33 ± 15.179	42
4	808.67	0.999	808.67 ± 20.079	48
6	1000	0.8	1000.00 ±19.875	54
10	1025.67	0.587	1025.67 ± 14.973	54
12	1074.67	0.429	1074.67 ± 11.473	60
15	1160.23	0.425	1160.23 ± 11.473	60
20	1072	0.7	1072 ± 19.875	66

Δt_b (sec):- peak base width.

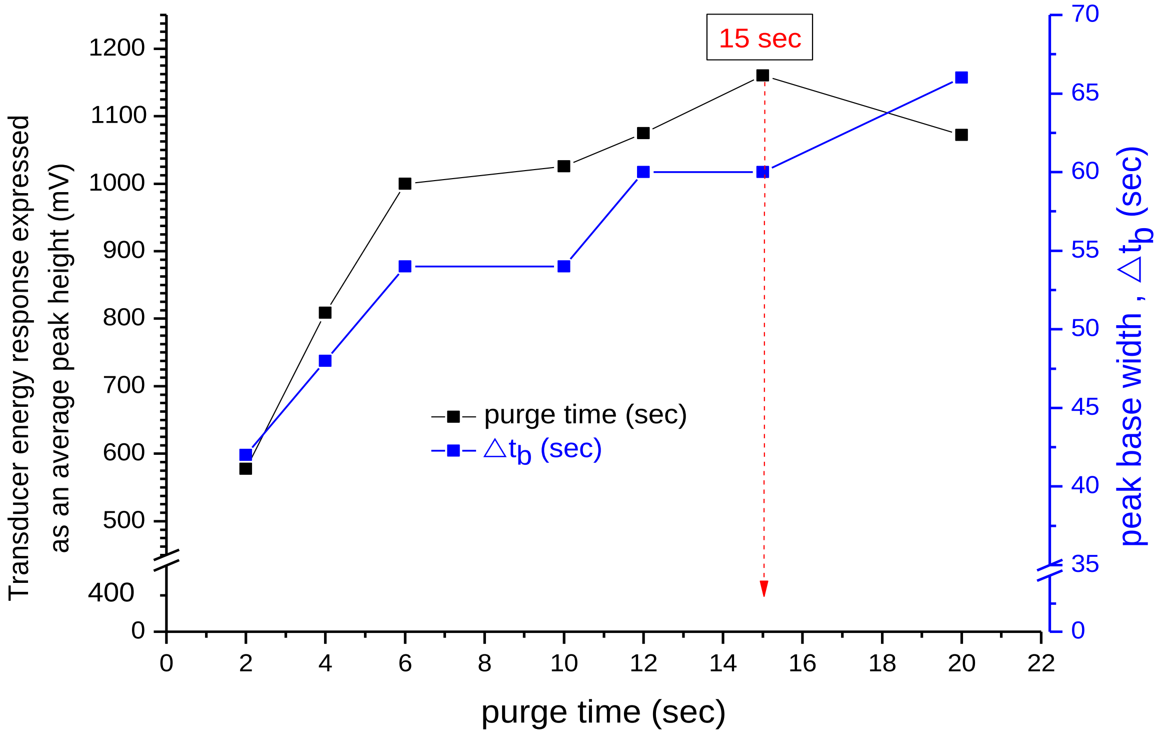

Figure 9- variation of purge time on the transducer energy response.

Effect of coil length:

Variable coil length 0 – 100 cm was studied , this range of length comprises a volume of 0 – 0.785 ml which connected after Y junction directly in flow system, figure-2. Optimum concentration of Ce(IV) sulfate (50 mmol.L^{-1}) in 500 mmol.L^{-1} of H_2SO_4 and 5 mmol.L^{-1} of Am with sample volume 165 µL were used. Table. 7 shows all the results obtained for effect of reaction coil on transducer energy response, Δt_b , and concentration of Am after dilution (column 9). Figure-10 shows that the best coil length for the completion of the oxidation of Am by Ce(IV) sulfate in acidic medium is 30 cm (235 µL) . While increasing of coil length more than 30 cm gave broadening of the peak maxima and increase of the Δt_b as shown in figure-10, which most probably attributed to the increase effected of dilution and dispersion on colored segment and continuous longer time duration of colored species in front of detector figure-2 shows manifold for determination of Am using coil after Y – junction .

Table 7- variation of coil length on energy transducer response.

Coil Length cm	Volume ml $r^2 \prod h$ r = 0.5 mm	Average transducer energy response expressed as peak heights (n=3) ȳi (mV)	RSD%	Confidence interval of The average response ȳi $\pm t_{0.05/2}\ \sigma_{n-1}/\sqrt{n}$	Δt_b sec	t sec	V_{add} ml) (	[Am] mmol.L^{-1} after dilution
0	0	1160	0.20	1160 ± 5.77	60	66	5.11	0.16
30	0.235	1250.67	0.26	1250.67 ± 7.99	65	72	5.56	0.15
60	0.471	1245.33	0.32	1245.33 ± 9.88	66	78	6.01	0.14
100	0.785	1205.33	0.25	1205.33 ± 7.43	72	114	8.71	0.09

Δtb (sec):- peak base width.
t (sec) :- arrival time of the injected sample from the valve till leave the flow measuring cell .
V $_{added}$ (ml): - volume that added depended on: t (sec), flow rate and sample volume.

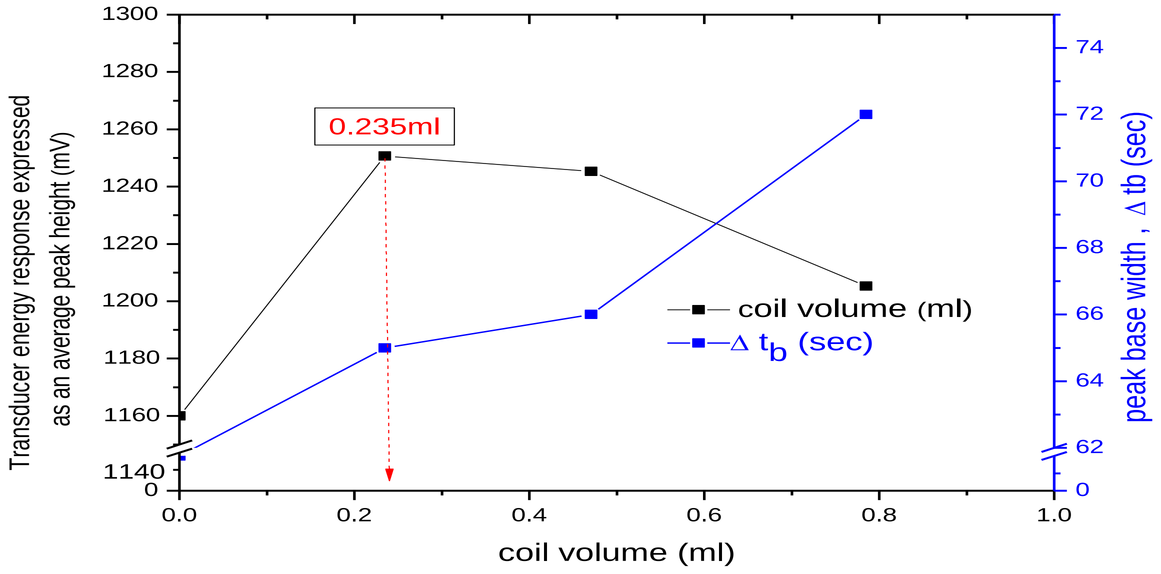

Figure 10- variation of coil length on the transducer energy response for determination of Am 5 mmol.L^{-1} with 165µL sample volume.

Calibration graph:

At the established optimum conditions, series of solution for Am (0.0005 - 12 mmol.L^{-1}) were prepared. Each measurement was repeated three times. Transducer energy response of the average peak height (mV) was plotted against the concentration of Am, a straight –line graph, figure-11, from (0.0005 – 10 mmol.L^{-1}) of Am was obtained . Above 10 mmol.L^{-1} the value for correlation coefficient will decrease and deviate from linearity most probably due to the high intensity of the colored species in front of detector and due to the effect of inner filter of colored species present in solution, which results from the oxidation of Am by Ce(IV)sulfate in acidic medium which might be due to a decrease in transmitted light. The obtained results were tabulated in Table. 8.

Table 8- summary of calibration graph results for the determination of Am using Am-Ce(IV) — H_3O^+

Measured [Am] mmol.L^{-1}	Linear dynamic range mmol.L^{-1} n = 14	Ŷi (mV) = a ± ts_a +b ± ts_b [Am] mmol.L^{-1} at confidence level 95% , n – 2	r r^2 %	t tab	t_{cal} $=\|r\|\sqrt{n-2}/\sqrt{1-r^2}$
0.0005 – 12	0.0005 – 10	98.21±99.49+197.29±23.62 [Am]mmol.L^{-1}	0.9824 96.50 %	2.179 <<18.19	

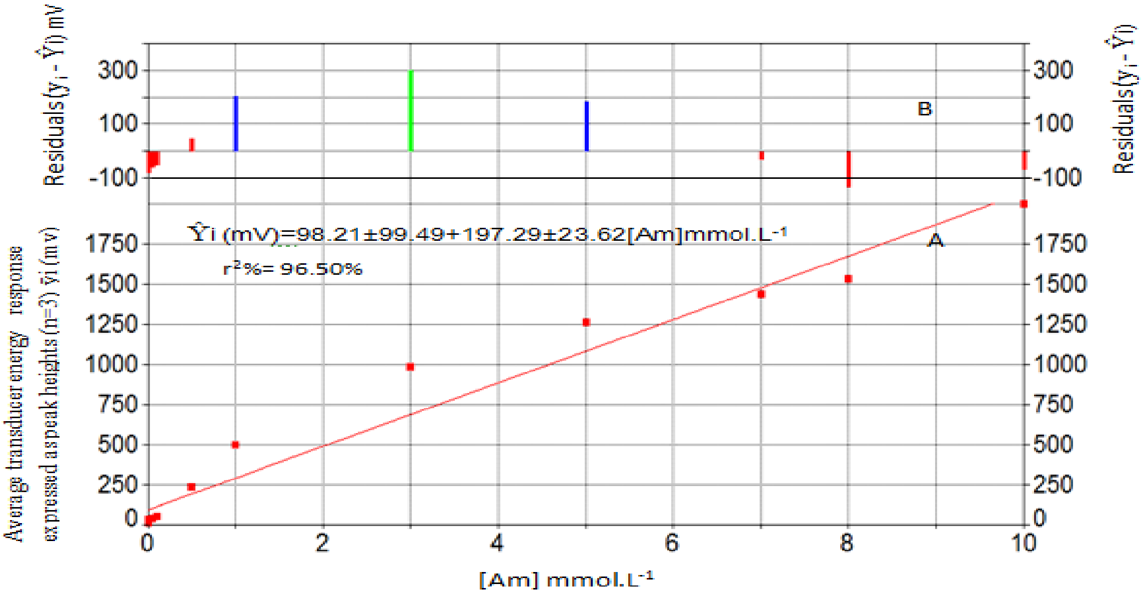

Figure 11- calibration graph for the variation of Am concentration on: - A: Energy transducer response expressed by linear equation using Ayah $3S_{BGR}$ x3 -3D solar cell CFIA microphotometer, B-residual (yi – Ŷi) , Yi: practical value , Ŷi: estimate value.

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 or from the linear regression plot. Table. 9 tabulated all these calculation value of detection limit for 165µL sample volume.

Table 9- summery of limit of detection based on different approaches at 165 µL sample volume.

Gradual dilution for minimum Concentration	Based on the value of slope X = $3S_B$ / slope	Linear equation Ŷi (mV) = Y_B+3S_B
0.25 µm 9.471 ng	18 µm 681.91 ng	2.10µm 79.60 ng

S_B: standard deviation of blank solution. , X= value of L.O.D based on slope.
Y_B: average response for the blank solution (equivalent to intercept in straight line equation).

Repeatability:

The repeatability of measurement and the efficiency of homemade Ayah 3S$_{BGR}$x 3S – 3D solar cell CFIA microphotometer were studied at fixed concentrations of Am, mainly with two concentrations of 5, 0.5 mmol.L^{-1} at optimum parameters. The repeated measurements for seven and eight successive injections were measured and obtained results were tabulated in Table. 10 which shows that the percentage relative standard deviation was less than 1.3, while figure-12 shows a kind of response-time profile for the used concentrations.

Table 10- Repeatability of Am results obtained for the formation of colored species from Ce(IV) sulfate –Am H3O$^+$ system.

[Am] mmol.L^{-1}	no. of injection	Average transducer energy response expressed as peak heights ȳi (mV)	RSD %	Confidence interval of The average response 95 % confidence Ý±$t_{0.05/2}$ σ_{n-1}/ $\sqrt{n}$
5	7	1250	0.186	1250 ± 2.15
0.5	8	242	1.248	242 ± 2.53

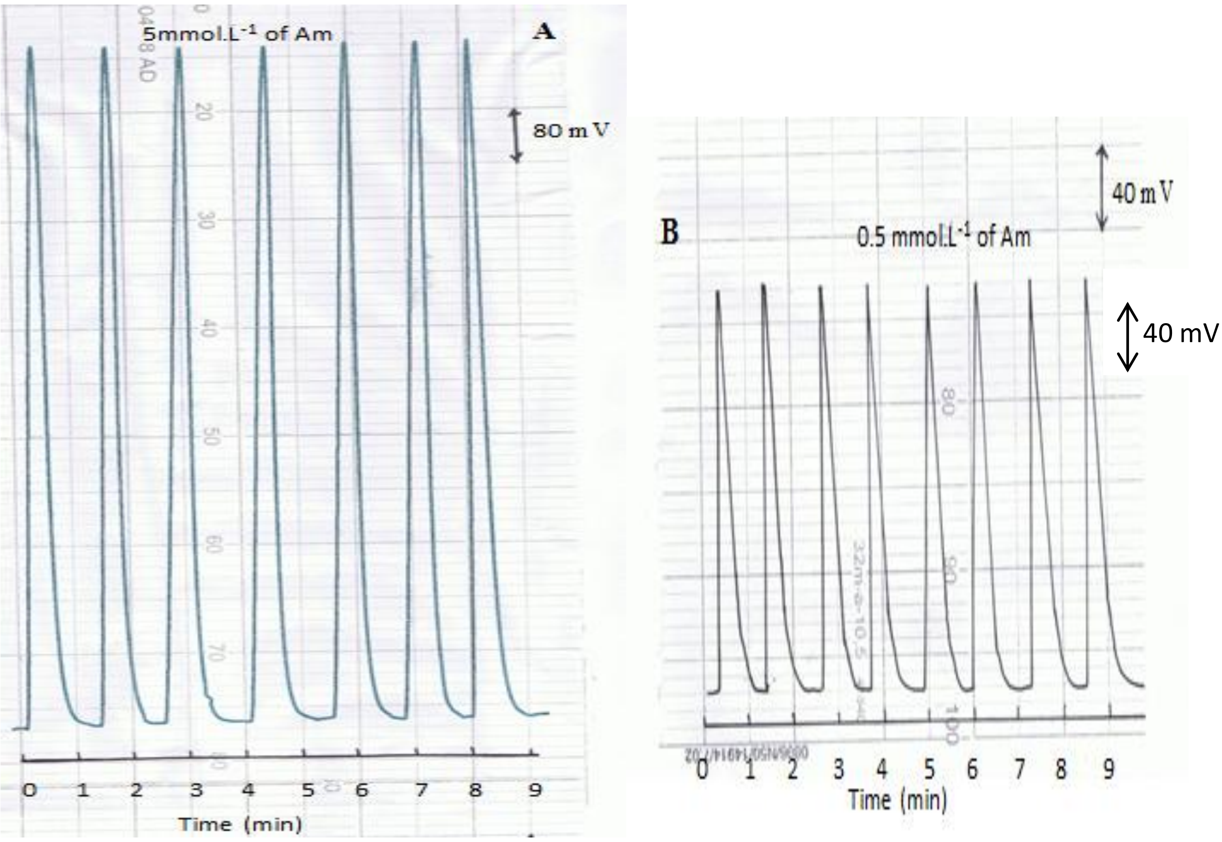

Figure 12- A Profile of successive repeatability measurements of Am (A: 5, B: 0.5, mmol.L^{-1}) using Ayah 3S$_{BGR}$ x3S-3D solar cell CFIA microphotometer.

Analysis of pharmaceutical preparation:

The established method was used for the determination of Am in two different kind of Am from two different well known drug manufactures (Amiloride, 5 mg- Actavis) and (Moduretic,5 mg- Algorith) using Ayah 3S$_{BGR}$ x 3S – 3D solar cell CFIA microphotometer and compared with classical spectrophotometric method via the measurement of λ_{max} at (540 nm) . The standard additions method was applied by prepared a series of solutions from each pharmaceutical drug via transferring 0.42 mL

(5 mmol. L^{-1}) of Amiloride, Quated value-5 mg), and 0.9 mL (2.5 mmol. L^{-1}) of Moduretic, Quated value-5 mg), to five volumetric flask (25 ml) , followed by the addition of (0 , 0.0125 , 0.0625 , 0.0875 , and 0.125 mL) from 20 mmol.L^{-1} standard solution of Am in order to have the concentration range from 0 – 0.1 mmol.L^{-1} for the preparation of standard additions calibration plot . The measurements were conducted by both methods.

Results were mathematically treated for standard additions method and tabulated in Table. 11 at confidence interval 95 %.

Paired t – test was used as shown in Table. 12 which show a comparison – treatment of data were subjected to two different paths.

First approach: comparison of newly developed method with official quoted value (5 mg) as shown in table. 12 column (4) by calculated t – values with individual t – test.

It was found that there was no significant difference between the quoted value of each individual company with calculated t – value at 95 % confidence interval as the calculated t – value is less than critical tabulated t – value .

Second approach: comparison of obtained results from both methods with neglecting the difference of origin. It was noticed that there were no significant difference between two methods as shown in column 8 at 99% but significant difference between two methods at 95 % confidence interval. Table. 12 shows results of t – test for the new adopted method (Ayah $3S_{BGR}$ x 3 –3 D solar cell CFIA micro photometer) and classical method (UV – Vis spectrophotometric) for the determination of Am in pharmaceutical drugs.

Table 11- results for the determination of Am in pharmaceutical preparation using standard additions, by two methods Ayah $3S_{BGR}$ X3 -3D solar cell CFIA microphotometer and UV-Vis spectrophotometric method.

Sample No.	Commercial Name Quoted active ingredient country	Confidence interval average weight $\bar{W} \pm 1.96\, \sigma_{n-1}/\sqrt{n}$ at 95% (g)	Sample weight in 100 ml	Theoretical Content For the active Ingredient at95% n=∞ (mg)	equation of standard Addition curve at 95% , n -2 $\hat{Y}i= a\pm S_at + b\pm S_bt$ [x]	Practical concentration (mmol.L^{-1}) and what is Equivalent of active Ingredient (mg)at 95% mmol.L^{-1} / 25ml mmol.L^{-1} / 100ml	Practical Content for the active Ingredient at 95% n=3 (mg)	Efficiency of Determination Rec %
			Drawing volume / 25 ml					
1	Amiloride, 5 mg, Actavis, Barnstaple, , UK	0.1295 ±0.00027	2.97332 g Equivalent to 114.8 mg of the Active ingredient (5mmol.L^{-1}) in 100 ml	5 ± 0.0105	Ayah $3S_{BGR}$x3 – 3D solar cell CFIA microphotometer			
					UV – Vis spectrophotometer method			
			0.42 ml / 25 ml		114.42±32.74+ 1364.74±553.51 [x]	0.083 mmol.L^{-1} / 25ml 4.940 mmol.L^{-1} / 100ml 113.43 mg	4.940 mg ± 0.0110	98.81 %
					0.79±0.063+8.95± 1.15[x]	0.088/25ml 5.238/100ml 120.267mg	5.238 mg ± 0.976	104.76%
2	Moduretic, 5mg, ALGORIT H, Lebanon	0.23919 ±0.00011	2.7459gm equivalent to 57.40 mg of the Active Ingredient (2.5mmol.L^{-1}) In 100ml	5 ± 0.0250	165.23±30.38+1834.1 ±513.54[x]	0.019/25ml 2.528/100ml 58.038mg	5.06 mg ±1.064	101.11%
			0.9ml / 25 ml		0.134±0.041+1.35± 0.67[x]	0.096/25ml 2.6671/100ml 61.226mg	5.33 ±1.221	106.67%

$\hat{Y}i$ = estimated value for energy transducer response (mV) or absorbance,

X: [Am] mmol.L^{-1}. $t_{0.025, \infty} = 1.96$ at 95 %,

Table 12- paired t –test results for Ayah 3 S_{BGR} x 3S –3 D solar C F I A micro photometer (proposed method) with classical method using standard additions method for determination of Am in pharmaceutical preparation.

<table>
<tr><th rowspan="3">Sample no.</th><th colspan="2">A moment found □ (mg) ± RSD % at 95 % , n = 3</th><th colspan="2">(x-μ) $\sqrt{n}$ /σ_{n-1} Ayah $3S_{BGR}x3S$-3D solar CFIA with Quoted (5 mg)</th><th rowspan="3">X d mmol.L^{-1}</th><th rowspan="3">□ d</th><th rowspan="3">σ_{n-1}</th><th rowspan="3">t_{cal} = □d $\sqrt{n}$ / σ_{n-1} at 95% at 99%</th><th rowspan="3">t_{tab} at 95% at 99%</th></tr>
<tr><th rowspan="2">Proposed method Ayah $3S_{BGR}x3S$-3D solar cell CFIA microphotometer</th><th rowspan="2">UV-Vis method</th><th colspan="2"></th></tr>
<tr><th>t_{Cal}</th><th>t_{tab}</th></tr>
<tr><td>1</td><td>4.94 ± 0.73</td><td>5.24±0.98</td><td colspan="2">-0.0851l <<4.303 l *</td><td>- 0.3</td><td rowspan="2">-0.285</td><td rowspan="2">0.021</td><td colspan="2" rowspan="2">19.19 >> 12.7 At 95 % or 19.19 << 63.67 At 99 %</td></tr>
<tr><td>2</td><td>5.06 ±1.08</td><td>5.33 ±1.23</td><td colspan="2">0.095<<4.303</td><td>- 0.27</td></tr>
</table>

$|t| = t_{cal} << t_{tab}$ (4.303) at 95% , $t_{tab} = t_{0.01/2}$, n-1 = 63.66 *

$t_{tab} = t_{0.05/2}$, n-1 = 12.7

Conclusion:

A spectrophotometric CFIA is proposed method for determination of Am with its application in the quality control analysis of pharmaceutical formulations. The method based on the oxidation of Am by Ce(IV) sulfate in acidic medium forming pinkish red product . The experimental point of view, the manipulation is very simple and sequential measurement was permitted with sample frequency up to 30 samples per hour. The proposed method uses cheaper instrument and reagent. In this paper a more effective faster determination was achieved by Ayah $3S_{BGR}$ x 3-3D solar cell CFIA microphotometer , light emitted diode as source with a detection using solar cell . The standard additions method was used to avoid matrix effects . Also this method can be applied to micro determination of Am in pure as well as in pharmaceutical preparation and over high sensitivity without the need for heating or extraction.

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