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A comparative study for the Determination of aspartame in pharmaceutical preparations by Kinetic Spectrophotometric and Reverse Phase-High Performance Liquid Chromatography methods.

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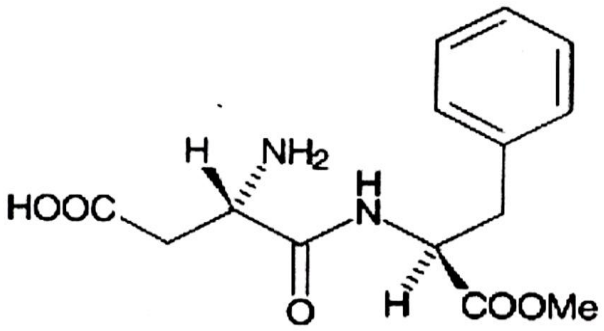
Abstract

This investigation involves development of a new kinetic spectrophotometric and a reverse phase-high performance liquid chromatography (RP-HPLC) methods for the determination of aspartame AS in pharmaceutical preparations. Spectrophotometric method was based on the oxidation of the drug AS with alkaline potassium permanganate. The reaction is followed spectrometrically by measuring the rate change of the absorbance of AS at 600nm. A fixed-time (at 48 min) method is adopted for determining the drug concentration. A linear calibration graph was in the range of 1-7μg.ml^{-1}, with a correlation coefficient of 0.9998, detection limit of 0.101μg.ml^{-1}, molar absorption coefficient is 5.2×10^4 L/mol.cm, Sandels sensitivity (S) 0.0056 μg/cm^2 and relative standard deviation RSD% of 1.40%. In HPLC method, the drug was analyzed using RP-HPLC method with a Zorbax ODS-C_{18} (15cm×4.6mm i.d); analytical column (5μm partical size) and Isocratic elution with a mobile phase containing 15% acetonitrile in 0.02M sodium acetate buffer (pH 5.4), at a flow rate of 1ml.min^{-1}, 20 μl sample loop, and the UV detector was set at λmax 220nm, Calibration graph was in the range of 10-70 μg.ml^{-1} with a correlation coefficient of 0.9991, detection limit of 1.09μg.ml^{-1} and a relative standard deviation of 0.91%.The two methods were applied successfully to determine the content of AS in pharmaceutical preparations with a recovery of 98.8-99.3%.

Introduction

Aspartame: (S)-3-amino-N-[(S)-1-(methoxy carbonyl)-2-phenyl ethyl](British pharmacopoeia,2000) Succinamic acid is a white crystalline powder.

The empirical formula for (AS) is $C_{14}H_{18}N_2O_5$ and its molecular weight is 294.3, it is used as sweetening agent and its structure is:



Many methods have been developed for the determination of Aspartame in various matrices such as pharmaceutical preparation, beverages, saccharin, foods, chocolate, soya-bean products, desserts, sweeteners, soft drinks and in fermented milk beverages. It has been determined in beverage by Ion chromatography(Qu,F.et al ,1999) (IC) and in chocolate by (Liquid chromatography)(Hagiwara et al., 1999). And in food,saccharin,milk, by HPLC(Zhang et al 1998,Deborde &Lamarque, 1990). Flow-injection analysis has been used for the determination of AS by using 50mM-Tris.HCl as buffer solution at pH 8.5 and packed column prouse, phenyl alanine(Prodolliet & Bruelhart, 1993). (Male & Luong, 1991)used FIA-spectrophotometer for the determination of (AS) in foods, saccharin and dietary products. (Pesek &Matyska, 1997) determined AS in foods, and caffeine in non-alcoholic beverages by capillary electrophoresis, Finally. (Nikolelis & Krull 1990) used enzyme electrode of the potentiometric Carbon dioxide sensor for the determination of AS in soft drink, beverages and sweeteners.

The present paper describe a spectrophotometric method for the determination of aspartame in pharmaceutical preparations based on the oxidation with alkaline potassium permanganate. The proposed method was applied successfully for the determination of the analyte in pharmaceutical preparations. And RP-HPLC method was compared successfully with kinetic spectrophotometry and were applied for the analysis of pharmaceutical preparations samples containing aspartame.

Experimental

A- Philips UV/Visible recording spectrophotometer model Pu 8770 with 1cm matched quartz cells is used for optical measurements.

B-HPLC apparatus

The analysis was performed on (CECil 1100 series) (Korea), solvent reservoirs of about 500ml capacity round bottle were used . High performance pumps, (pressure range 0-500 $Kg.cm^{-1}$) which delivers the mobile-phase from solvent reservoirs to the mixing cell. Injection valve fitted with 20µl sample loop. Separation of drugs were carried out on a(15cm×4.6mm i.d) stainless steel, 5µm particle size Zorbax ODS-C_{18}. The eluted compound was detected via uv-vis detector fitted with 8µl flow cell at 220nm.

Reagents

Reagents of analytical grade and distilled water were used through the work.Solutions were prepared by appropriate dissolution of reagents as shown in table(1).The aspartame stock solution (100µg/ml)was prepared by dissolving 0.01gm AS in 100ml of distilled water. Sodium acetate(0.02M) solution for HPLC analysis using sodium acetate which was prepared by dissolving 0.820gm of pure material in 500 ml of distilled water.Phosphoric acid, (H_3PO_4) 85% Fluka to adjust the(pH). Methanol, (CH_3OH) HPLC grade, acetonitrile, (CH_3CN) HPLC grade are used. Finally, solutions of lower concentrations AS 100µg/ml were prepared by appropriate dilution of stock solution with distilled water.

Pharmaceutical preparation

-(For spectrophotometric method)

Canderal tablets:provided from(SDI) Samara-Iraq.

Ten tablets were grinded well and a certain portion of the final fine powder was accurately weighed to give an equivalent to 18 mg of Aspartame. The resulting solution was dissolved with distilled water and filtered by using a Whatman filter paper No.4 to avoid any suspended particles. Then 1ml of the prepared prepared solution was transferred into a 100ml of volumetric flask and made up to the mark with distilled water forming a solution of $100\mu g.ml^{-1}$ concentration. The same procedure was adopted for preparation of duclaryl tablets provided from (Bilim Company, Turkey).

-(for HPLC method)

ten tablets were grinded well and the powder was weighed accurately 50 mg and dissolved with 50ml mobile phase (sodium acetate and acetonitrile), and filtered. Then 1ml of the prepared solution was

transferred into a 25 ml of volumetric flask and made up to the mark with mobile phase forming a solution of 100 µg/ml concent.

General procedure for the spectrophotometric method.

Initial rate method:Aliquots of 0.01M $KMnO_4$ solution (1.0ml) and 1M NaOH solution(2.0ml) were transferred into a 10ml volumetric flask.

Accurate volume of the working solution of AS 0.1-1.1ml were added to the flasks respectively and the volumes were diluted with distilled water.The contents of the mixture were shaken well and immediately transferred to the spectrophotometric cell at room temperature. The absorbances were recorded at 600nm as a function of time against reagent blank.In the second procedure, the absorbance was measured at a fixed time of (48min) and was plotted against the final concentration of (AS) and the content of the druge was calculated from either the calibration graph or regression equation.

Results and discussion

Part (1):Kinetic spectrophotometric determination of Aspartame in pharmaceutical preparation.

In an alkaline medium, potassium permanganate oxidizes AS, resulting in the formation of manganate ion(Sabah and Scriba 1998), which showed an absorption peak at 600nm.

Because the intensity of the color increased with time, a kinetically based method was elaborated for the determination of AS in dosage forms.

The various experimental parameters affecting the formation of the reaction product were optimized as follows:

Effect of $KMnO_4$ concentration

To study the effect of the $KMnO_4$ concentration, aliquots of (AS) containing 100µg.ml^{-1} were transferred into a series of 10ml volumetric flasks, followed by addition of varying volumes of 0.01M $KMnO_4$ 0.1-1.1ml and 2.0ml of 1M NaOH solution. The absorbance at 600nm was measured at a fixed time of 48minutes. It is apparent from Fig.(1) that the absorbance increased with increasing volume of the $KMnO_4$, and becames constant at 0.9ml and so then 1.0ml of $KMnO_4$ was used as the optimal volume.

Effect of the NaOH concentration

The influence of the NaOH concentration on the formatin of MnO_4^{2-} was examined critically. Fig.(2) shows that the maximum absorbance was obtained with 1.8ml of the 1M NaOH, so the optimum volume of 2.0ml has been recommended for the subsequent experiments.

Effect of oxidation time

At a preselected fixed time, the absorbance of the solution containing varying amount of AS was measured at 600nm. Calibration graphs were constructed by plotting the absorbance against the initial concentration of AS at fixed time of 6,12,18,24 and 48 minutes. It is apparent from Fig.(3) that the most acceptance value was obtained at a fixed time of 48min., and therefore it was considered to be the most suitable time interval for performing measurements under this condition.

Final absorption spectrum of aspartame

A clear peak of MnO_4^{2-} at 600nm, Fig.(4) show the spectra before and after the oxidation of AS by 0.01M $KMnO_4$ solution in an alkaline medium of 1M NaOH against their blanks.

Recommended analytical conditions

According to the results obtained previously, the optimum conditions for the determination of AS using a kinetic spectrophotometric method are given in table(2).

Calibration graph

A linear calibration graph for Aspartame Fig.(5) under the optimized conditions was obtained.

Beer's law is obeyed over the concentration range of (1.0-7.0) $\mu g.ml^{-1}$ with a correlation coefficient of 0.9998 molar absorbance $\epsilon_{max} 5.2\times10^4$ $L.mol^{-1}.cm^{-1}$ and Sandels sensitivity(S) 0.0056 $\mu g.cm^{-2}$.

The relative standard deviation of the method was 1.40% for aspartame based on 6 replicate determinations.

Table(3)shows summary of analytical data for the determination of Aspartame using spectrophotometric method.

Application to pharmaceutical preparations

The proposed method was applied for the determination of aspartame in tablets. Good precision and recovery were obtained. The method was successfully compared with the British Pharmacopoeia standard method. The results obtained are summarized in table(4).

Part(2):Determination of aspartame using RP-HPLC method.

The determination of the aspartame by RP-HPLC is based on the isocratic elution(i.e.seperation in which the mobile phase composition remains unchanged) of the species on a ODS-RP-column.

Optimization of Experimental conditions

1-Effect of Percentage of organic modifier in the mobile phase

The amount of organic modifier present in the mobile phase will influence analytes that are retained by adsorption on to the stationary phase. Acetonitrile was used as a typical mobile phase modifier in this study. It was mixed with 5% (v/v) 0.02M sodium acetate as buffer solution at (pH5.4).

The results obtained (Fig.6) indicate that the retention times of aspartame decreased as the percentage of acetonitrile raised from 5 to 25%.

The best results were obtained when the percentage of acetonitrile in the mobile phase is 15%.

2-Effect of pH using 0.02M sodium acetate buffer

In general the (t_R) value of each species can be correlated with the values of pKa of the solute molecule. To study the effect of the pH the mobile phase on the elution of the studied compound, the pH of the mobile phase was varied from 5.0 to 7.0. Fig.(7) show that the retention time of aspartame decreased with decreasing pH of the mobile phase. A good separation of the investigated drug and short time of analysis was obtained when the pH of mobile phase is 5.4.

3-Effect of flow rate of the mobile phase

To investigate the effect of the flow rate on the retention time (t_R) of aspartame, the composition of the mobile phase was held constant with 15% acetonitrile (pH5.4) at $30C^0$. The results on Fig.(8) show that the retention time of the aspartame decreased with increasing the flow rate. The aim of choosing the optimum flow rate is to perform the analysis in a short time, which in turn prevents solute band broading; this finally leads to increase column efficiency(Sawyer et al 1984). A flow rate of 1ml/minute was selected to obtain maximum resolution in a suitable analysis time.

4-Effect of column temperature

Generally, increasing column temperature in RP-chromatography decrease the retention time of separation bands and increasing column efficiency by decreasing mobile phase viscosity, which is in turn lowering

the column head pressure(Knox and Majors 1975). The effect of column temperature in the range of 30 to 45C^0 on the retention time asymmetry of the peak and the sensitivity of the drug under study was investigated. The relation between log$\grave{k}$ and 1/T was plotted, and the plot obtained Fig.(9) shows that $\grave{k}$ of drug under study decrease with increasing column temperature and the optimum column temperature chosen to be 30C^0.

Recommended analytical conditions :

According to the results obtained previously the optimum experimental conditions established for the reversed-phase HPLC determination of aspartame in pharmaceutical preparations are given and summarized in table(5).

Calibration graph:

A linear calibration graph for the determination of Aspartame was obtained in the range (10-70)µg.ml^{-1} Fig.(10). The analytical data obtained from the calibration graph are summarized in table (6). Indicate good precision and accuracy of the proposed method.

Application of the developed Rp-HPLC method for the determination of Aspartame in some pharmaceutical preparations

Two types of tablets containing aspartame were analyzed using the developed method and the results were compared with the British Pharmacopoeia standard method. The results obtained are shown in table(7).

Comparison the two methods

The two proposed methods was compared as shown in table (8).

Table(1) Preparation of some solutions

Substance	Molar concentration(M)	Dissolved weight(gm)	Final volume(ml)
Sodium hydroxide	1.0	4	100
Potassium permanganate	0.01	0.158	100
Sodium carbonate	0.1	10.599	1000
Sodium oxalate	0.1	13.39	1000

Table(2) Optimum conditions for the determination of aspartame using the spectrophotometric method.

No.	parameter	Value
1-	Conc.of $KMnO_4$	0.01M
2-	Conc.of NaOH	1M
3-	Vol.of $KMnO_4$	1ml
4-	Vol.of NaOH	2.0ml
5-	Fixed time	48min
6-	Temperature	at Room temp.
7-	λmax	600nm

Table(3) analytical data for the determination of aspartame using spectrophotometric method.

Analytical data	Value
Linear range	1.0-10.0μg.ml^{-1}
Correlation Coefficient	0.9998
Regression equation	Y=0.0309x+0.3639
RSD%	1.40%
Detection limit (D.L)	0.101 μg.ml^{-1}
Molar absorbance ϵ_{max}	5.2×10^4 L.mol^{-1}.cm^{-1}
Sandels sensitivity (S)	0.0056 μg.cm^{-2}

Table(4): Application of the spectrophotometric proposed method for the determination of aspartame in pharmaceutical preparations.

Sample	Recovery%		RSD%
	Proposed method*	Standard method	
Pure aspartame	99.30	99.15	1.46
Canderel tablets	99.21	99.20	1.40
Duclaryl tablets	98.90	99.01	1.42

***"Each results is the average of three determinations"**

Table(5)The recommended analytical conditions for the determination of aspartame using RP-HPLC system.

Parameter	Recommended value
Column	Zorbax ODS-C_{18} (15cm×4..6mm i.d)
Organic modifier	15% Acetonitrile
Buffer	0.02 M Sodium acetate
pH	5.4
Flow rate	1.0 $ml.min^{-1}$
Column temperature	$30C^{0}$
Detector	U.V detector at 220nm
Injected sample volume	20 µl

Table (6): Analytical data for the determination of aspartame using RP-HPLC method.

Analytical data	Value
Linear range	10.0-70.0 $\mu g.ml^{-1}$
Correlation coefficient	0.9991
Regression equation	Y=424.29x+0.074
RSD%	0.91%
Average Recovery	98.8%
Detection limit (D.L)	1.09 $\mu g.ml^{-1}$

Table(7):Application of the proposed method for the determination of aspartame in pharmaceutical preparations.

sample	Recovery%		RSD%
	Proposed method	Standard method	
Pure aspartame	98.87	99.01	0.93
Canderel tablets	98.89	99.10	0.91
Duclaryl tablets	98.75	99.13	0.89

*Each results is the average of three determinations

Table(8):The statistical comparison of results for the spectrophotometric and RP-HPLC method.

The method	Regression equation	Linearity (µg/ml)	Correlation Coefficient (r)	Recovery%	RSD%	Detection limit (µg/ml)
spectrophotometric	Y=0.0309 x+0.3639	1.0-7.0	0.9998	99.3-99.7	1.40	0.101
RP-HPLC	Y=424.29 x+0.074	10-70	0.9991	98.3-98.8	0.91	1.09

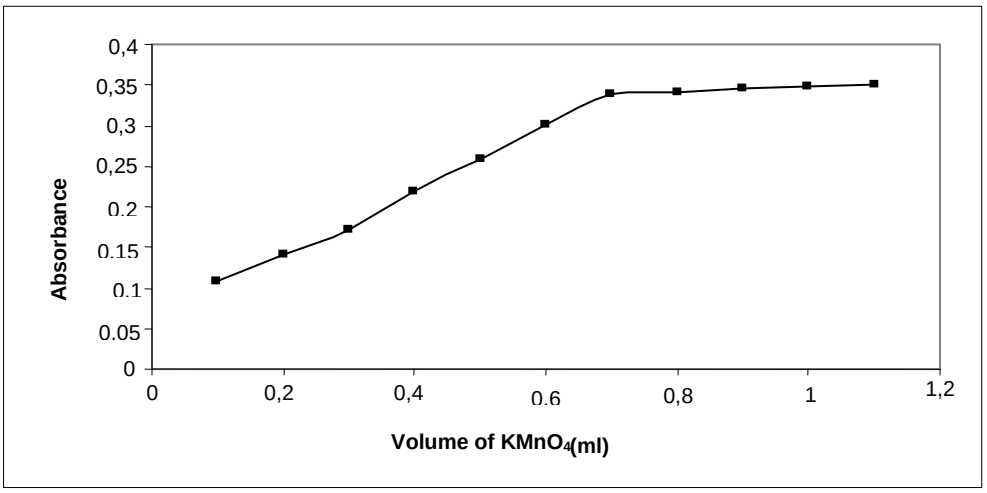


Fig (1) : Effect of the volume of 0.01M $KMnO_4$ on the intensity of color produced for the reaction (Aspartame10 µ g / ml , 2.0 ml of 1 M NaOH)

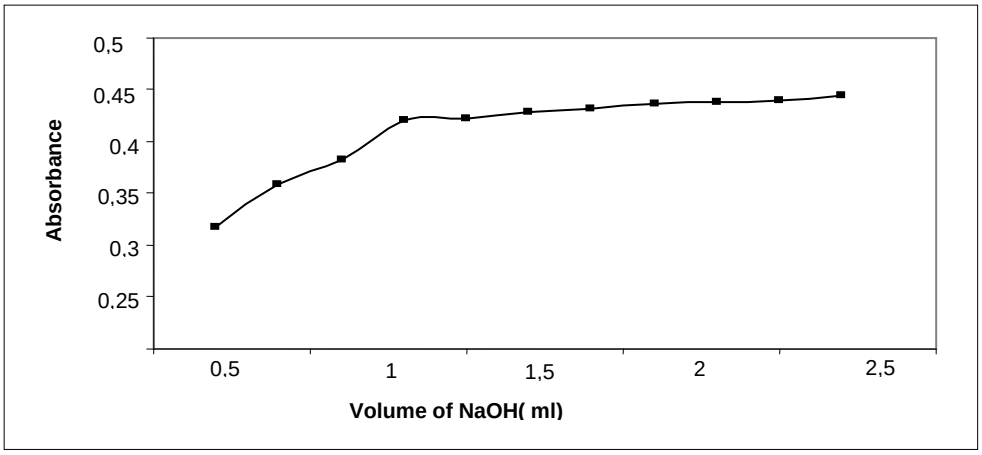


Fig (2) : Effect of the volume of 1M NaOH on the intensity of color produced for the reaction (Aspartame 10 µ g /ml , 1.0 ml of 0.01 M KMnO4)

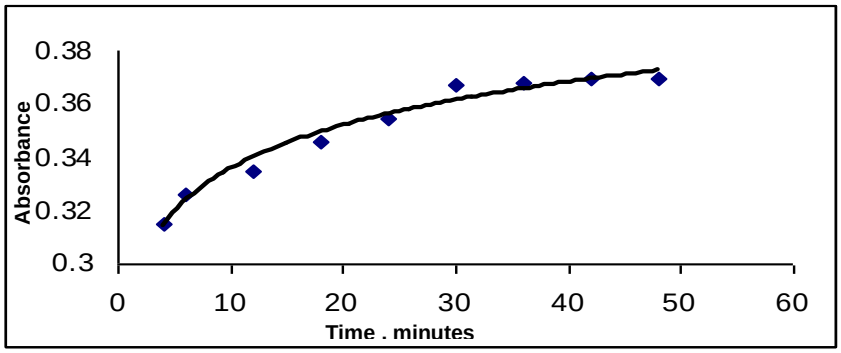
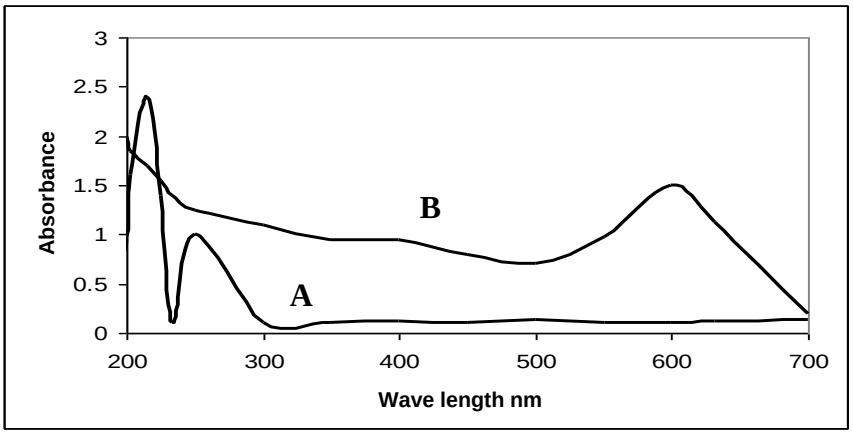
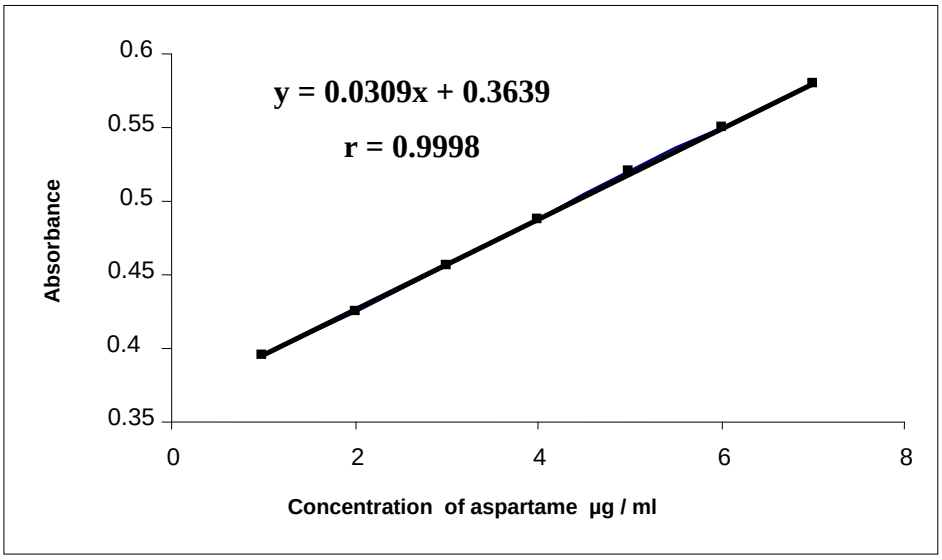


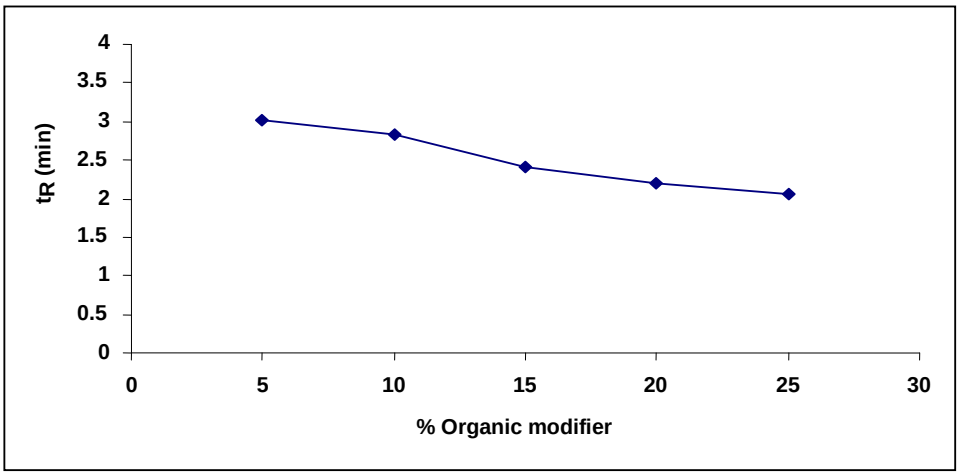
Fig (3) : Effect of time on the oxidation of AS by 0.01M KMnO4.



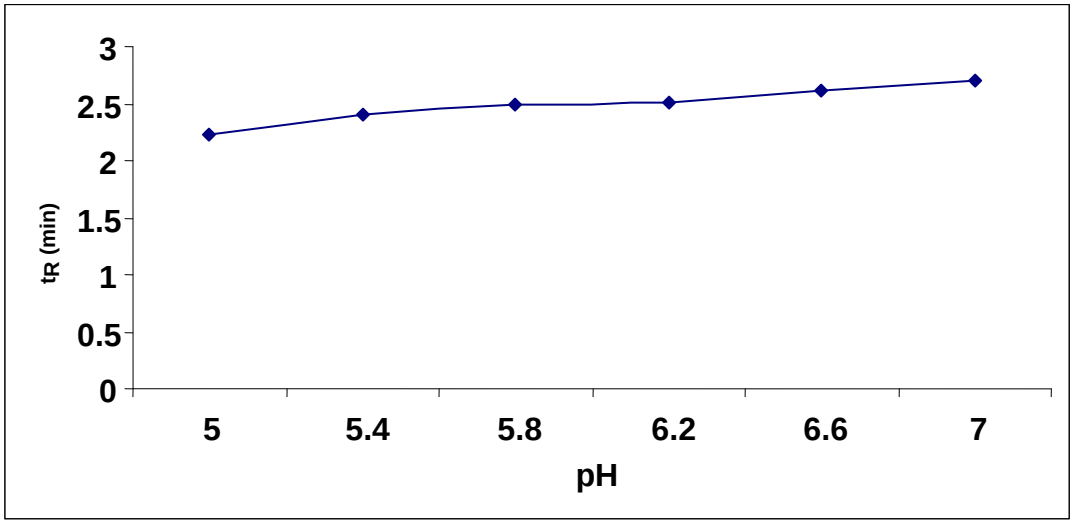
Fig(4) A: The absorbance spectrum for aspartame before oxidation with blank solution.
B: The absorbance spectrum after the oxidation of aspartame with KMnO4 solution in alkaline media NaOH.



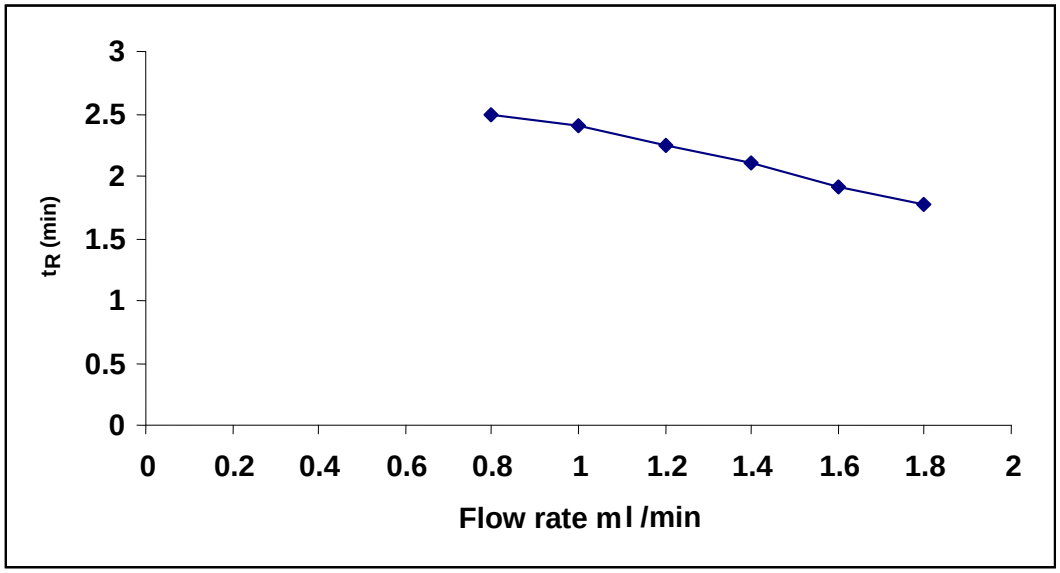
Fig(5):Calibration graph for the determenation of (AS) by the spectrophotometric method



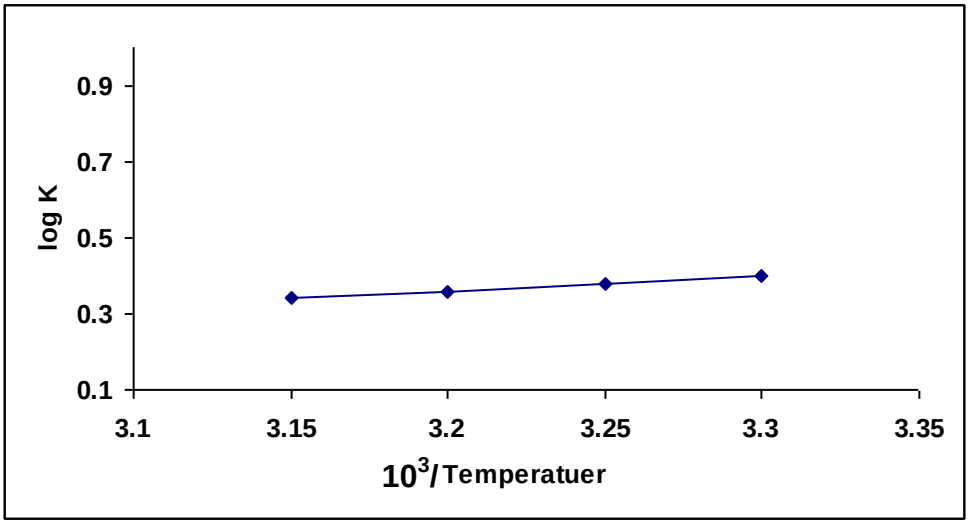
Fig(6): The effect of the organic modifier concentration on the retention of the analytes.



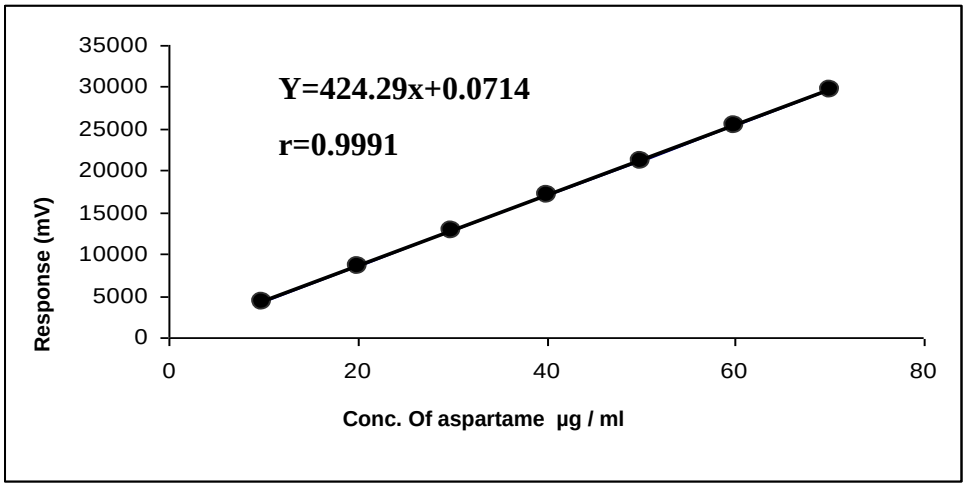
Fig(7): The effect of the pH of the mobile phase on the retention of the analytes.



Fig(8): The effect of the flow rate of the mobile phase on the retention of the analytes.



Fig(9): The effect of the column temperature on the retention of the analytes.



Fig(10): Calibration graph for the determenation of (AS) by the RP-HPLC method

Conclusion

In part(1) A simple Kinetic spectrophotometric method was developed for the determination of aspartame in pharmaceutical preparation by oxidation of the (AS) with alkaline $KMnO_4$. The results show good precision and accuracy for the determination of (AS) in the range of 1.0-7.0µg.ml^{-1} and Correlation Coefficient of 0.9998 with molar absorption coefficient 5.2×10^4 l/mol.cm, Sandels sensitivity (S) 0.0056µg/cm^2, detection limit 0.101µg.ml^{-1} and RSD% of 1.40% with 99.7% recovery.

In part (2) Include a simple and sensitive high performance liquid chromatographic (HPLC) method for the determination of Aspartame in pharmaceutical preparations.

The linearity of this method is 10.0-70.0 µg.ml^{-1}, correlation coefficient 0.9991 and RSD% was 0.91% with detection limit 1.09 µg.ml^{-1} and recovery 98.8%.

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دراسة مقارنة لتقدير الأسبارتام في المستحضرات الصيدلانية بالطريقة المطيافية الضوئية الحركية وطريقة كروماتوغرافيا السائل ذات الأداء العالي–الطور العكوس

عبد المجيد خورشيد أحمد ، كامران شكر حسين و مصطفى راجي أياد
كلية العلوم– جامعة كركوك

الخلاصة

تضمنت هذه الدراسة جزئين أساسيين، ضم الجزء الأول تطوير طريقة طيفية لتقدير دواء الأسبارتام تستند على أكسدة هذا الدواء مع برمنكنات البوتاسيوم القاعدية ($KMnO_4$) ومتابعة التفاعل طيفيا من خلال قياس التغير بالإمتصاص عند طول موجي (٦٠٠) نانوميتر وجد أن الزمن اللازم لإتمام التفاعل هو ٤٨ دقيقة ومدى الخطية يتراوح بين(1-7)مايكروغرام/مل ومعامل الإرتباط 0.9998، وحد الكشف مقداره 0.101 مايكروغرام/مل، ومعامل الإمتصاص المولاري 5.2×10^4 لتر/مول.سم، وحساسية دلالة ساندل (S) 0.0056 مايكروغرام/سم^2، أما قيم الإنحراف القياسي النسبي المئوي فكان ١,٤%. أما في الجزء الثاني فتم تطوير طريقة كروماتوغرافيا جديدة لتقدير العقار المذكور أعلاه بإستخدام طريقة كروماتوغرافيا السائل العالي الأداء الطور العكوس (RP-HPLC) بإستعمال عمود من نوع Zorbax (15cm×4.6mm i.d) ODS-C_{18} محشو بدقائق ذات أقطار ٥مايكروميتر بإزاحة أيزوكراتيكية Isocratic elution، وبطور متحرك مكون من مزيج يحتوي على ١٥% أسيتونايتريل ومحلول منظم بتركيز 0.02مولاري من خلات الصوديوم ذو دالة حامضية =5.4 وبسرعة جريان قدرها ١مل/دقيقة وحجم محقنة ٢٠مايكروليتر وبدرجة حرارة ٣٠م˚ وبإستخدام مكشاف المنطقة فوق البنفسجية والمرئية عند الطول الموجي ٢٢٠ نانوميتر.كانت الحدود الخطية للطريقة تتراوح بين ١٠–٧٠ مايكروغرام/مل وبمعامل إرتباط 0.9991 وإنحراف قياسي نسبي مئوي مقداره ٠,٩١% وحد كشف ١,٠٩ مايكروغرام/مل. طبقت هاتين الطريقتين المقترحتين بنجاح على المستحضرات الصيدلانية وكانت نسبة الإستعادة ٩٨,٨–٩٩,٣%.