Spectrophotomitric Determination of Paracetamol by using either Vaniline or $KMnO_4$ oxidizing agents

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Abstract

Two simple and sensitive Vis spectrophotometric methods were developed for the determination of paracetamol and compared with the specific and rapid UV spectrophotometric method at 243nm for the simultaneous quantification in standard solutions and tablets. The first method involves determination of paracetamol by using a vanaline as a reagent in the presence of 1M HCl to produce a stable yellow- orange color Schiff base absorbs at 392 nm after fraction of it by 4M H_2SO_4.The second method involves using alkaline potassium permanganate as an oxidizing agent produucing a stable blue-green color absorbs at 606 nm after fraction of it by 4M Na0H ,The paracetamol pKa value (9.537) was calculated from absorbance measurement at particular wave length in acidic, basic and buffer solution (pH=9) the result approch to the true value.

تقدير الباراسيتامول طيفيا باستخدام الفانيلين وبرمنكنات االبوتاسيوم كعوامل مؤكسدة

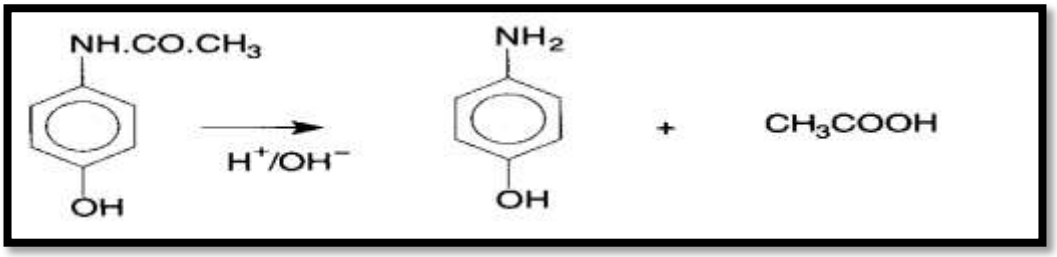
عيسى محمد ثلج الجبوري

الخلاصة

تم تطوير طرائق بسيطة وذات حساسية عالية تعتمد على الطيف المرئي لتقدير الباراسيتامول في مستحضراته الصيدلانية ومقارنتها بطريقة طيف الاشعة فوق البنفسجية (UV) مباشرتا عند الطول الموجي(nm،الطريقة الاولى تتضمن تكسير العقار بحامض الكبريتيك(4M) ومن ثم تفاعله مع الفانيلين كالديهيد بوجود حامض الهيدروكلوريك لتكوين قاعدة شيف الملونة باللون الاصفر- البرتقالي ويظهر امتصاص عند الطول الموجي 392 nm اما الطريقة الثانية فتتضمن التحلل القاعدي باستخدام هيدروكسيد الصوديوم(4M) ومن ثم اكسدته ببرمنكنات البوتاسيوم لتعطي محلول ذات لون ازرق- اخضر يظهر امتصاص عند الطول الموجي 606nm والتي يمكن تقديرهما طيفيا في المنطقة المرئية، تم تقدير ثابت التفكك pKa (9.537) باستخدام الامتصاصية عند قراءتها في ثلاثة اوساط كيميائية حامضي وقاعدي وفي محلول منظم عند pH =9 وكانت النتائج قريبة من النتائج الحقيقية.

Introduction

Paracetamol (acetaminophen) and systematic (IUPAC) name: N-(4-hydroxyphenyl) acetamide is a pharmaceutical compound widely used as analgesic and antipyretic[1,2]. It belongs to the class of drugs known as aniline analgesic.[3,4] There are several methods have been reported for the quantification of paracetamol in pharmaceutical formulations using thin layer chromatography, high performance liquid chromatography[5,6], immunological method[7], FT-Raman spectroscopy[8,9] and flouriscence spectrophotometry[10]. These methods vary in accuracy and precision, analytical UV-Visible spectrophotometer is an essential tool for any quality control laboratory. It is useful for identity, purity and quantitative analysis assays of raw materials[11,12]. Various color reactions have been proposed for the determination of paracetamol, including indophenol dye, and Schiff`s base formation, nitration and subsequent chelation, oxidation, oxidative coupling.[13]



(Hydrolysis of paracetamol)

There are two functional group the first one a neutral amide group and the second a very weak acid phenolic group[14] that can be reacted with other compounds. The objective of the present study should have two methods ,the first method is based on the preliminary hydrolysis of paracetamol by H_2SO_4 to p-aminophenol and coupling of the latter with vaniline compound to form of a yellow Schiff base. The second spectrophotometric method are based on the oxidation of paracetamol by $KMno_4$ aftere hydrolysis of it in NaOH to form green color.

Experimental

Apparatus:

The present work was carried out on shimadzu UV-Vis spectrophotometer model no. 1800, with 1 cm matched quartz cells. All the weighing measurements were made by Sartorius BL210 S AG Germany digital electronic balance and the pH value was mesured by pH meter Jenway 3310, UK.

Materials

Paracetamol standard was provided by SDI. Paracetamol tablets containing 500 mg Paracetamol and the inactive ingredient used in drug matrix were obtained from market. The chemicals are of analytical grade H_2SO_4, HCl, $KMnO_4$, NaOH, vaniline, are obtained from different companies.

Method 1

Standard preparation

Standard stock solution of paracetamol was prepared by;

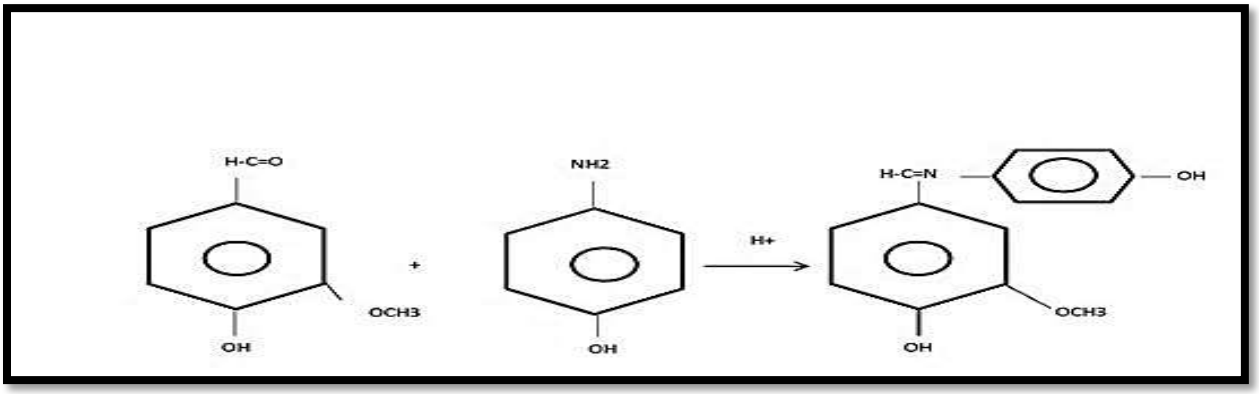
1. Weigh 0.100 g paracetamol into a beaker and dissolve in 40 ml 4M sulphuric acid.
2. Sonicated with heating at 60^{o}C for 30 min.
3. Transfer quantitatively to a 100 ml volumetric flask and make up to volume with deionised water.

Test sample preparation;

Ten tablets were weighed(5160mg) and powdered. Powder equivalent to 100 mg of paracetamol was weighed and mixed with 40 ml of 4M sulphuric acid and sonicated with heating to a temperature of a 60C for 30 min. After complete dissolution, the cooled solution was filtered through a Whatman No 40 filter paper. The solution was made up to the mark with distilled water in a100 ml volumetric flask.

Procedure for the determination of paracetamol by using vaniline reagent ;

An aliquot of paracetamol standard solution (0.5ml, 1ml, 2ml, 4ml) was mixed with 1 ml of 0.5M HCl and 10mg of vaniline, to a give a stable yellow coloured product. The mixture was made up to 25 ml in a volumetric flask with dis.water to produce a concentration (2%, 4%, 8%, 16%) respectively, This solution was scanned in the range 370-700 nm. The wavelengths of maximum absorbance of paracetamol was found to be at 392 nm. The formation of schiff base is shown in the following reaction;



(Schiff base Reaction)

Method 2

Standard and sample solution preparation ;

As in method 1 but NaOH is used as a solvent instead of H_2SO_4

Procedure for the determination of paracetamol using $KMnO_4$ as areagent ;

An aliquot of paracetamol standard solution (0.25 ml, 0.5ml, 1ml, 2ml) was mixed with1ml of 0.1 M HCl and 1.0 ml of 0.5 $KMnO_4$, to a give a stable green colored product. The mixture was made up to 25 ml with distilled water in a volumetric flask to produce a concentration(1% ,2%, 4% , 8%) mg respectively. This solution was scanned in the range 370-700 nm. The wavelengths of maximum absorbance of paracetamol was found to be at 606 nm.

Preparation of calibration curve

From the respective stock solution (100mg /100ml) different concentration of 2, 4, 8 and 16% mg/ml paracetamol in first method and 1, 2,4 and 8% mg/ml paracetamol in second method were prepared and measured at 392 nm respectively. Calibration curve were plotted as absorbance vs concentration and their linearity range was determined (Fig. 1, 2).

A	C%
0.093	2
0.186	4
0.364	8
0.636	16

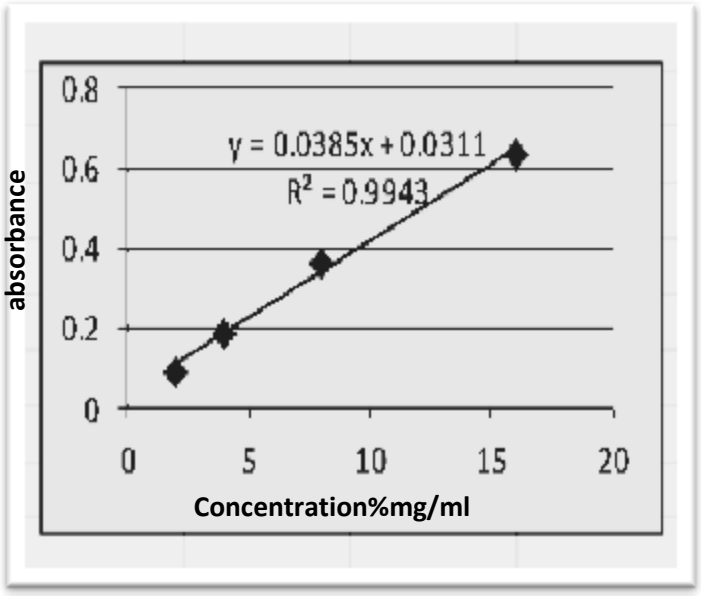


Fig. (1):- Calibration plot for the estimation of paracetamol with vaniline .

A	C%
0.152	1
0.308	2
0.61	4
1.241	8

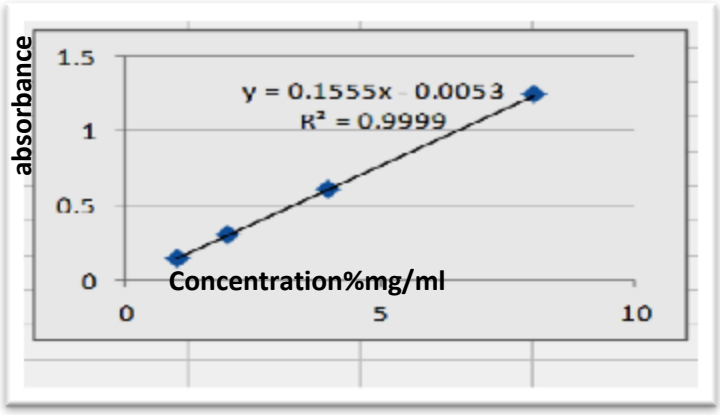


Fig.(2):- Calibration plot for the estimation of paraceatmol with $KMnO_4$.

Results and Discussion

The specific color reaction between paracetamol and vaniline in first method and with KMnO4 in the second method are studied in various concentration ranges of the reagent and different pH media. At higher concentrations of the acid or base the color appeared, but rapidly faded away, the value of the pH was recommended as 4-5 for the first method and 9-10 for the second method. At wave length 606nm and 392nm absorbance measurements were recorded after 20 minutes and found to be stable (Fig 3,4). An amount of 10mg of the vanilin and 1ml of 0.5 KMnO4 exhibited stable color in the two methods. Quantification of samples were performed using calibration curves prepared by standards solution at a series of

concentration for two procedures as described in Fig 1and 2. Was the linearity range was (2 -16 mg %) for first method and (1- 8mg %) for second method. Calibration curve is used to find out the concentration of the unknown analyte concentrations in samples by using the equation of straight line,where y= 0.0385X+0.0311 and y= 0.1555-0.0053 for two methods as in table 1 and 2 bellow.

Table (1):- Sample and related reading and actual Conc.% in first method.

No	ml 0f sample	Asorbance	Reading Conc.%	Actual Conc.%	Expected content in100mg
1	1	0.181	3.893	4	97.325
2	1.5	0.254	5.789	6	96.483
3	2.0	0.326	7.659	8	95.737

Table (2):- Sample and related reading, actual Conc.% in second method.

No	ml of sample	Asorbance	Reading Conc.%	Actual Conc.%	Expected content in 100mg
1	0.75	0.431	2.805	3	93.50
2	1	0.594	3.854	4	96.35
3	1.5	0.913	5.95	6	99.166

Fig. (3):- Spectrum of paracetamol standard in first method

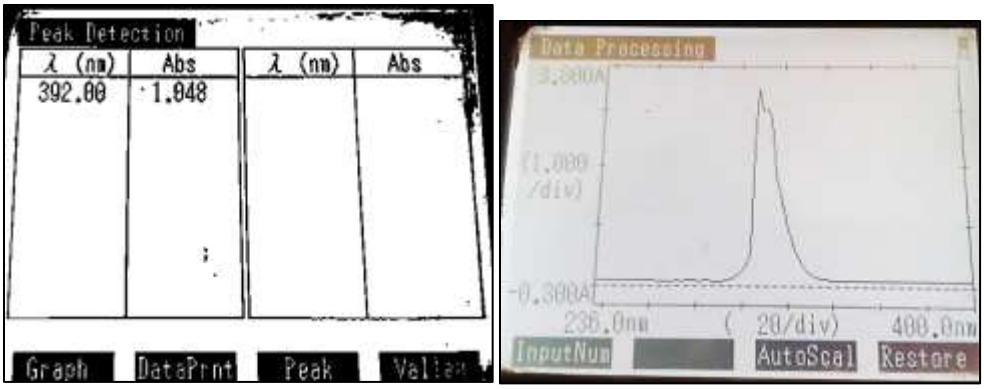


Fig (4):- Spectrum of paracetamol standard in seconed methods .

Direct determination of paracetamol by UV spectrophotometer:

In pharmaceutical analysis ,concentration and amounts are usually expressed in grams or milligrams than in moles , Beer–Lambert equationes is written in the following form; A=A(1%,1cm) C L[15,16] ,A is the measured absorbance , A(1%,1cm) is the absorbance of 1g/100ml solution in a 1cm cell; C is the concentration of the sample in g/100 ml, L is the path length in cm (1cm), then C=A/ A(1%,1cm)

Preparation of standard solution

1-The standard stock solutions of paracetamol was prepared by ;
dissolving 50 mg of drug in 100 ml of 0.1 M HCL to get a concentration of 50 mg/100 ml of paracetamol.
2- 2.5ml from the above prepared solution was diluted to100 ml with 0.1M HCl in a volumetric flask to get a concentration of 1.25 mg/100 ml of paracetamol.

Preparation of the sample

1. Five paracetamol tablets were weighed (2.541mg) and crushed to a fine powder.
2. Dissolve 0.250g of tablets powder with 250ml of 0.1M HCl and then made up to 500ml with 0.1 M HCl**.**
3. The extract is filtrated and 5ml of the solution is made up to 250 ml with 0.1M HCl**.** The above solutions are measured at 243nm to determine the paracetamol concentration .

Measurement of the A (1% 1cm)

1 . The absorbance of standard sample at 243 nm equal to 0. 81**.**
2. The equation A= A (1% 1cm) ×C×L, then the A(1% 1cm) =0.81/0.050=16.2
The dilution factor 2.5 (100 =40) then A (1% 11cm) =16.2×40 =648.

Measurement of paracetamol content in tablets

1. The absorbance of affixed concentration at 243nm is found to be 0.653 in 0.1M HCl.
2. Expected paracetamol content in tablet powder taken was
0.250 /2.541×5×500 = 245.96 mg**.**
3. Concentration in diluted tablet extract = 0.653/ 648=0.001007g /100ml,
0.0017 ×1000 =1.007 mg/100ml.
Dilution factor = 5 (250 =50) then 1.007×50 =50.385 mg/100ml
Volume of original tablets extract =500, therefore 50.385×5 =251.929 mg**,**
4. The % of the paracetamol content in the tablet = 251.929 / 245.96×100 =102.426 mg**.**

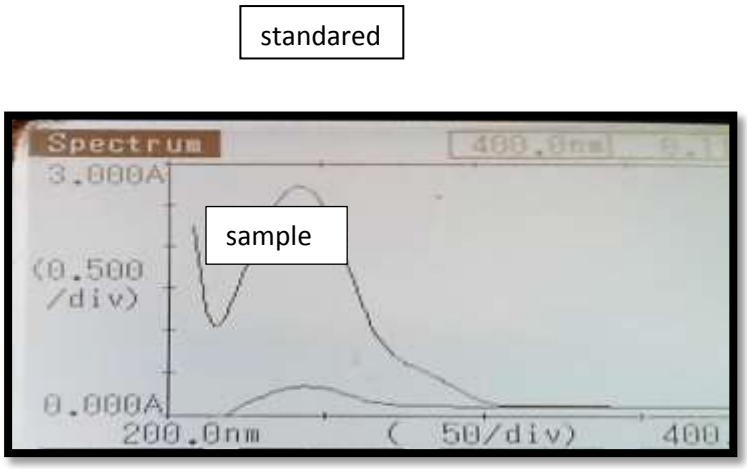


Fig.(5):- UV Spectrum of standard and sample of paracetamol.

Determination of pKa by UV-VIS Spectrophotometer

The absorbance shift in UV depending on the pH value is used for determination of pKa value by general equation of absorbance measurement at particular wave length.

pKa = pH + log Ai -A / A-Au when

A the absorbance in the buffer solution .

Ai the absorbance in NaOH (ionized form).

Au the absorbance in HCl (unionized form) .

0.1M HCl	0.1M NaOH	pH 9.0 Buffer solution
Au = 0.53	Ai= 1.73	A= 0.71

pKa measurement = 9.0 + log 1.33- 0.71/ 0.71 – 0.53 = 9.5371.

The pKa actual value =9.50 then the difference between the actual and measured value is 9.5371-9.50 = 0.0371.

Validation parameter of the developed methods

The validation of the results of the two methods were achieved using regression statistics (Fig1, Fig 2). The calibration curves for the two methods were linear, in the range (2 -16 %), (1 – 8.0%) indicating a good linearity, correlation coefficient (R^2) (0.9943, 0.9999) respectively. Six replicate samples of the same concentration (4%)of paracetamol were analyzed by first and second methods, (Table 3) summarizes the values obtained for the main parameters, from the standard deviation(s) it can concluded that 68% of the results of the analysis lies within the range 99.9±0.0587 or 99.9 ± 0.0470, Limits of detection (LOD) were 0.0448 mg, 0.0369 mg and limits of quantification (LOQ) were equal to (0.149, 0.121) respectively indicating for sensitive analysis. The percent recoveries equal to (96.50), (97.50) and the relative errors equal to 3.5%, 3.27% for the first and the second methods respectively indicating good accuracy of the proposed methods. The precision of an analysis is often expressed as the ± relative standard deviation(± RSD) wich equal to 1.4939 and 1.2147 for two methods indicating good precision, the

value's F table is 6.34 at 95% confidence level, Since In this case, F value for first method is 1.5 and for second method is 0.66 and since both value are less than the F table value of 6.34 so both methods are precise [11]**.** The critical value of (t- table) at the 99.9% confidence level for (4) Freedom degree is 8.61, since for first method t is 5.689 and 6.236 for the second method wich are less than t table value of 8.61, that means the methods are of good accuracy.

Table (3):-Validation parameters results of paracetamol of two methods.

methods	avereg e	SD	RSD	LOD	LOQ	Relative error	recovery	F-value	T- test
1	3.869	0.0578	1.4939	0.0448	0.149	3.5%	96.5%	1.50	5.689
2	3.869	0.0470	1.2147	0.0364	0.121	3.27%	96.7%	0.66	6.236

The method 2 having a linear response y = 0.1555x is four times more sensitive than the method**1** exhibiting a linear response y = 0.0385x. the limit of detection is due to a combination of range and sensitivity[17]. Since the assay content of sample in both Vis methods(392, 606 nm) is between 95 and 97 %. Compared with the UV (243 nm) method 102% thus thes methods can be used for any type of samples.

Conclusion

The proposed methods(M1,M2) could be applied successfully for determination of the studied compound in pure form as well as in different dosage forms. Moreover, small amounts of the studied compounds could be determined compared with the UV method. The proposed methods were simple, accurate, precise, sensitive, rapid and low cost, the UV method used directly to the analysis of paracetamol dosage forms without the need for separation or complex sample preparation such as extraction steps prior to the drug analysis. Thus the validated methods is suitable and can be used for the routine analysis.

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