AJPS, 2010, Vol. 8, No.2

Accepted 10 September 2010

Spectrophotometric Determination of Aspirin in Pure Form and Pharmaceutical Formulations using Oxidation–reduction reaction

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الخلاصة:

طريقة بسيطة, عالية الحساسية وذات دقة عالية لتقدير الاسبرين بشكل نقي او في المستحضرات الصيدلانية من خلال تكوين معقد الزرقة البروسية.

تضمنت الطريقة تفاعل الاسبرين المتحلل في محيط حامضي مع مزيج كلوريد الحديديك وبوتاسيوم سداسي سيانيد الحديديك وتكوين معقد ملون لتقدير الاسبرين طيفيا عند اقصى طول موجي 765 نانوميتر.تم تحديد الظروف الفضلى للتفاعل للحصول على اعلى حساسية واطول استقرارية. عند الظروف الفضلى للامتصاصية لمعقد الزرقة البروسية وجد ازدياد بالخطية مع ازدياد تركيز الاسبرين والموثق من خلال قيمة معامل الارتباط.مدى التركيز المستخدم 0,5– 7 مايكروغرام مل$^{-1}$وبحدود كشف 0,2267مايكروغرام مل$^{-1}$وبانحراف قياسي نسبي 0,025.

طبقت الطريقة المقترحة بدقة وضبط عالي وبنجاح لتقديرنسبة الاسبرين بشكل نقي اوفي المستضرات الصيدلانية وتم قارنة النتائج الاحصائية باستخدام اختباري وقد وجد ان ان قيمهما اقل من قيمهما الواردة بالطريقة المستخدمة في تقديره بالدستور البريطاني .

Abstract:

A simple, sensitive and accurate spectrophotometric method of determination of aspirin in pure form and pharmaceutical formulation.

The method is based on the formation of Prussian blue (PB) complex. The reaction between the acidic hydrolysis product of the aspirin with the mixture of $FeCl_3$ and potassium hexacyanoferrate (III) was evaluated for the spectrophotometric determination of the aspirin. The maximum absorbance of the colored complex occurred at λ=765nm. Reaction conditions have been optimized to obtain PB complex of high sensitivity and longer stability. Under optimum conditions the absorbance of the PB complex where found to increase linearly with increase in concentration of the aspirin, which corroborated with correlation coefficient value. The concentration ranges are 0.5-7µg mL^{-1} with detection limit 0,2267µg mL^{-1} and relative standard deviation 0,025%. The proposed method was successfully applied to determine of the selected aspirin in

Accepted 10 September 2010

pure form and pharmaceutical formulations with good precision and accuracy compared to standard method as revealed by t- and F- values and the results obtained agree well with the labeled contents.

Key word: Aspirin, spectrophotometric, oxidation–reduction reaction.

Introduction

Acetylsalicylic acid (Aspirin) has now enjoyed safe and effective use for 100 years, initially as an analgesic, antipyretic, antithrombic and anti-inflammatory agent. Low-dose aspirin also helps prevent life-threating vascular complications connected with, such as retinopathy and nephropathy [1].

The various methods such as UV [2], spectrofluorimetry [3, 4] HPLC [5, 6] RP-HPLC [7], FT-IR/ATR spectrometry [8] LC-MS [9] and SWV [10] have been reported for the determination of acetylsalicylic acid. However, an extensive survey of the literature revealed that there is no method available for the determination of aspirin in pure form and pharmaceutical formulation by oxidation – reduction reaction.

The formation of PB complex is a classical qualitative test used to detect Fe^{-2} using hexacyanoferate (III) ion [11,12]. PB complex used for the determination of some antibiotic [13,14] and non-steroidal anti-inflammatory drug[15].

The aim of the present work to study the redox reaction in developing simple, accurate, sensitive and reproducible assays to determination aspirin in pure form and pharmaceutical formulation using Prussian blue (PB) reaction.

Materials and Methods:

Apparatus

Spectral and absorbance measurements were made on UV 1650 Shimadzu spectrophotometer by using 1 cm quartz cell.

Materials and Reagents

All reagents used were analytical grade and water was always double distilled.

Pure samples

Aspirin pure grade was provided by SID- Samara factory.

Standard Stock Solution

Stock solutions of aspirin were prepared by dissolving 100 mg of pure drug in methanol, followed by dilution to 100ml with the same solvent to obtain 1mg mL^{-1} standard solutions.

Market samples

Aspirin tablets, labeled to contain 100 mg. They were obtained from commercial sources in the local market.

Accepted 10 September 2010

Reagents

Anhydrous $FeCl_3$ (Merck) and potassium hexacyanoferrate (III) (Merck) 0.2% (w/v) were prepared in bidistiled water. Sulphuric acid (10M) was prepared by adding 555 ml of concentrated acid (sp.gr. 1.83) to 445 ml of bidistilled water with cooling.

Recommended analytical procedure

Method

Aliquots (0.2-1.8 ml) of 100 µg ml^{-1} standard solutions of aspirin were transferred using micro burette into a series of 10 ml volumetric flask, and the total volume adjusted to 3 ml by adding bidistilled water. Then, 2 ml each of $FeCl_3$ (0.2%) and potassium hexacyanoferrate (III) (0.2%) were prepared for each flask, mixed well and left to stand for 10 min. Finally, 1 ml of 10M sulphuric acid was added to each flask, diluted to the mark with bidistilled water and mixed well. The absorbance values were measured at λ=765nm against a reagent blank prepared similarly. A calibration graphs were drawn by plotting the absorbance against the drug concentration.

Analytical of pharmaceutical formulations

Ten tablets were accurately weighed and powdered. An accurately weighed quantity equivalent to 10 mg aspirin was dissolved in 10 ml of methanol and transferred to 100 ml calibrated flask. The contents of the flask were shaken for 10min, and then make up to the mark with methanol. The general procedure was then followed for concentration ranges already mention for method.

Results and Discussion:

Fe (III) salts play a prominent role in the spectrophotometric determination of some pharmaceutical drugs [16-17]. Formation of PB complex has been employed in quantitative detection of Fe (II). A deep blue complex is formed by the reaction between Fe (II) and potassium hexacynoferrate (III) [12]. Aspirin are oxidized by Fe(III) ion and Fe(II) ion is produced (Eq. 1) in acidic medium. The produced Fe(II) ion react with potassium hexacynoferrate(III) and formed a colored complex is called Prussian blue (PB) (Eq. 2) with maximum absorbance 765nm (Fig. 1A). The intensity of color increase with time at room temperature; therefore, a spectrophoometric method was adopted for the determination of the drug. The chromogenic reagent i.e. Fe (III) mixed with potassium hexacyanoferrate (III) in acidic media did not show any strong absorbance in the visible region of the spectrum (Fig. 1B).

Accepted 10 September 2010

$$\text{Aspirin} + Fe^{+3} \xrightarrow{\text{acid}} Fe^{+2} + \text{Oxidized products} \quad (1)$$

$$Fe^{+2} + \text{Hexacyanoferrate}^{+3} \longrightarrow \text{Prussian blue (PB)} \quad (2)$$

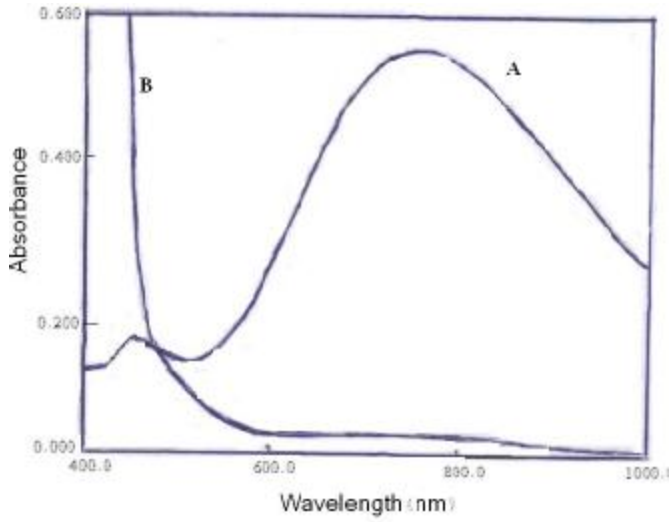

Fig-1: Absorption spectra of: (A) Reaction product of Aspirin drug with $FeCl_3$/ potassium hexacyanoferrate (III) system. (B) Reagent blank

Optimization of Experimental Conditions

Effect of volume of potassium hexacyanoferrate (III)

It was found that increasing the volume of (0.2%) potassium hexacyanoferrate (III) would increase the absorbance of the reaction product up to 0.4ml, after which potassium hexacyanoferrate (III) has no effect on the absorbance as shown in Fig.2.

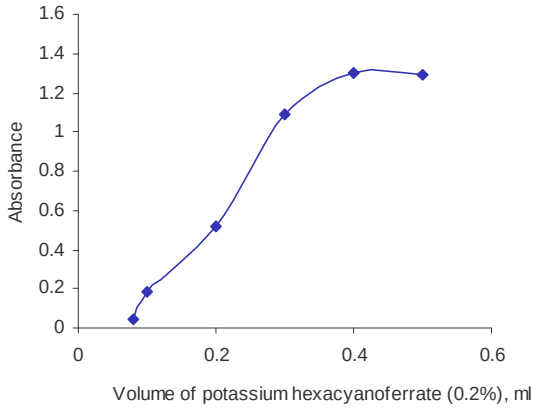

Fig-2: Effect of volume of (0.2%) of potassium hexacyanoferrate on the absorbance intensity of Aspirin drug at 765nm

Accepted 10 September 2010

Effect of volume of ferric chloride

The maximum absorbance increased with increase ferric chloride volume. It was found that 0.4 ml of (0.2%) of ferric chloride was adequate for the maximum absorbance (Fig. 3)

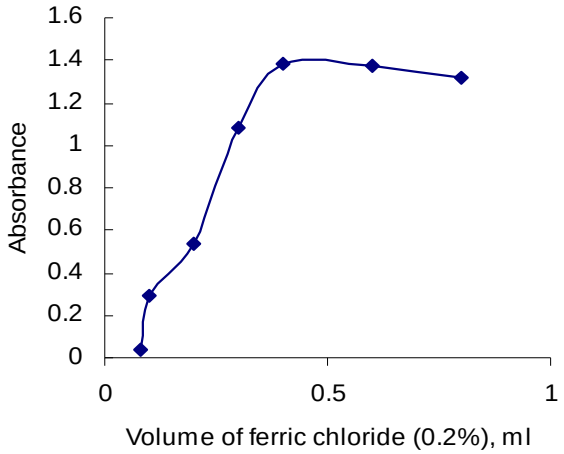

Fig-3: Effect of (0.2%) ferric chloride on the absorbance intensity of Aspirin drug using 0.4 ml (0.2%) potassium hexacyanoferrate at 765nm

Effect of diluting solvents

The effect of diluting solvents was studied. It was found that water was the best solvent for dilution. Using methanol or ethanol however, resulted in precipitation of the colored complex.

Effect of acid

It was found that constant absorbance was observed in the presence of sulphuric acid. Also, nitric and hydrochloric acids were also tried, but the absorbance reading of the colored complex did not remain stable for more than 10 min.

Effect of heating time

The effect of heating time on the redox complexation reactions was studied. It was found that 25 min was sufficient for completion of the reaction (Fig-4).

Accepted 10 September 2010

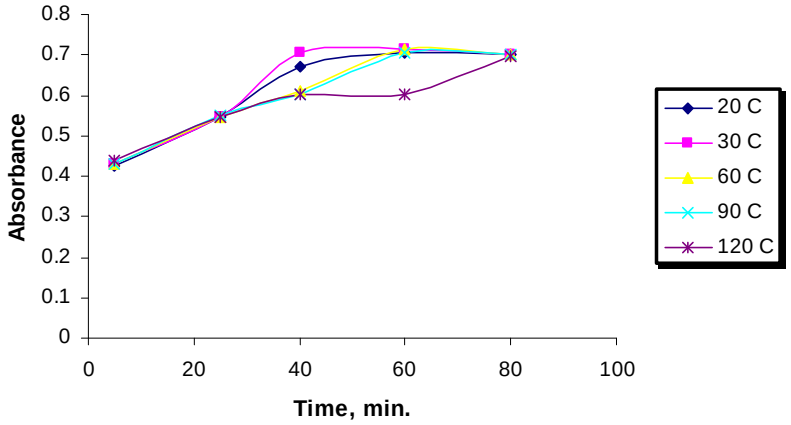

Fig-4: Effect of time (min) on the absorbance of the formed complexes of Aspirin drug with the temperature (20, 30, 60, 90,120°C)

Effect of time on stability

The absorbance of the colored complexes was found to be stable at room temperature for more than 24 hrs.

Effect of order in which reagents were added

After fixing all other parameters, a few other experiments were performed to order: drug, ferricyanide and iron(III), followed by sulphuric acid after full color development, gave maximum absorbance and stability, and for this reason the same order of addition was followed throughout the investigation.

Calibration curve

After optimizing the reaction conditions describe above, calibration curve (Fig. 5) for Aspirin was constructed by plotting absorbance of PB complex and the concentration of the Aspirin drug. The calibration curve was linear in the concentration range of 0.5-7 µg ml^{-1}.

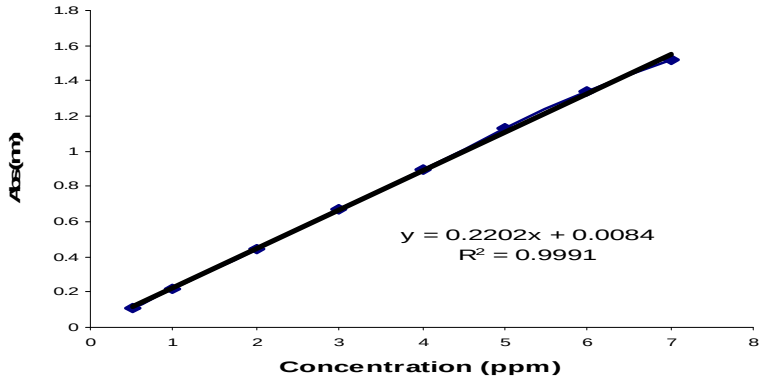

Fig-5: Calibration curves of Aspirin

The limit of quantification (LOQ) was determined by establishing the lowest concentration that can be measured, below which the calibration curve is non linear and was found 0.7557 µg ml^{-1}. The limit of detection (LOD) was

Accepted 10 September 2010

determined by evaluating the lowest concentration of the analyte that can be readily detected and was found to be 0.22671 ml μg^{-1}.

The proposed method was tested for linearity, specificity, precision and reproducibility. Linear regression equation was obtained. The regression plots showed a linear dependence of absorbance values on drug concentration over the range cited in table 1. The table also shows the slope and intercept. Validation of the method was evaluated by statistical analysis of the regression data regarding standard deviation of the residual ($S_{y/x}$), the standard deviation of intercept (S_a) and standard deviation of the slop (S_b). The small values given point to the low scattering of the points of the calibration curve. The proposed method was evaluated by studying the accuracy as present relative error and precision as percent relative standard deviation (RSD %) as shown in Table 1.

Parameter	
Concentration range (µg ml^{-1})	0.5 – 7.0
limit of detection (LOD) (µg ml^{-1})	0.2267
limit of quantitation (LOQ) (µg ml^{-1})	o.7557
Correlation coefficient (r)	0.9995
Linearity percentage, (r^2 %)	99.91
Test for a significant correlation, t*	258
Slope	0.0084
Intercept	0.2202
$S_{y/x}$	0.01664
S_a	0.00274
S_b	0,01129
RSD %	0.025
Molar absorpitivity, ε ($L.mol^{-1}.cm^{-2}$)	0.0018
Sandell′s sensitivity, S (mg. cm^{-2})	0.003

Table-1: Performance data of the proposed method

* t = 2.306 at confidence level 95% and (n-2) = 8 degrees of freedom

Accuracy and precision

In order to determine the accuracy and precision of the proposed method, solution containing three different concentrations of aspirin were prepared and analyzed in five replicates. The relative standard deviation as precision and percentage relative error (E %) as accuracy of the suggested method were calculated at 95% confidence levels.

The analytical results for accuracy and precision show that the method proposed have good repeatability and reproducibility as shown in Table 2.

Accepted 10 September 2010

No.	Conc. mg/ml		E%	Rec%	RSD%
	Present	Found			
1	0.5	0.502	0.4	101.4	0.199
2	4.000	4.010	0.25	101.25	0.025
3	7.000	7.026	0.371	101.371	0.0142

Table-2: Accuracy and precision data obtained by proposed method
* Average of five determinations

Application

The proposed method was applied to the determination of aspirin (tablet) in proprietary drugs purchased from local stores. The results, shown in Table 3, suggest that the method is suitable for the determination of aspirin and that the excipient in the dosage forms do not interfere.

Pharmaceutical Tablet	Conc. mg/ml		E%*	Rec%*	RSD%*
	Present	Found			
Germany	0.5	0.501	0.2	100.2	0.199
	4.0	4.001	0.025	100.025	0.025
	7.0	7.001	0.014	100.014	0.0143
Iraq	0.5	0.504	0.8	100.8	0.198
	4.000	4.031	0.775	100.775	0.0248
	7.000	7.056	0.8	100.8	0.0142
Egypt	0.5	0.502	0.4	100.4	0.199
	4.000	4.018	0.45	100.45	0.0249
	7.000	7.03	0.4	100.4	0.0142
Iran	0.5	0.502	0.4	100.4	0.199
	4.000	4.016	0.4	100.4	0.0249
	7.000	7.028	0.4	100.4	0.0142

Table-3: Application of the proposed to determination of aspirin in pharmaceutical tablet
* Average of five determinations

Applicability of the proposed method

The proposed method was applied to determination of aspirin in pharmaceutical formulations purchased from local stores.
The % recoveries of the studied drug compared with that obtained by the standard method [18] are given in Table 4.

Accepted 10 September 2010

The methods performance was assessed using the t-test for 95% confidence level with degree of freedom $n_1+n_2-2=8$ and variance ratio F-value test for 95% confidence level with degree of freedom n-1=4 compared with standard method.

Statistical analysis of the results showed that calculated t- and F- values at 95% confidence levels are less than the theoretical ones, confirming no significant differences between the performance of the proposed and standard method.

Conclusion:

Despite the great number of methods described in the literature for analysis of aspirin, the proposed method for the determination of aspirin in pharmaceutical samples has the advantage to be simple, sensitive, accurate and inexpensive. The method represented good accuracy and precision so that the respective relative standard deviation and relative error of prediction for drug were lower. The proposed method was applied successfully to analysis of drugs in tablets and thus is very appropriate for routine quality control analysis of drug.

Accepted 10 September 2010

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Accepted 10 September 2010

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Accepted 10 September 2010

No.	Standard method		Proposed method			Value	
Origin of pirin tablet	Rec%* Xi	$(X_i - \overline{X})^2$	Rec% $(Xi)_2$*	$(X_i - \overline{X})^2$	S	t -**	F-*
Pure	100	0.066254	100	0.12659364	0.46357	0.00172	1.0
Germany	100.078	0.0321824	99.913	0.07225344			
Iraqi	100.792	0.2857972	99.683	0.00150544			
Egypt	100.417	0.02547216	99.383	0.06822544			
Iran	100.4	0.02033476	99.243	0.16096144			
	$\overline{x}$ =100.3374	$\sum$=0.43004252	$\overline{x}$ =99.6444	$\sum$=0.4295394	$n_1+n_2-2=8$		$n_1-1=$ $n_2-1=$

Table-4: Comparison of the proposed method with standard method using t- and F- values.

* Average of five determinations.

** Theoretical t- and F- values at 95% confidence level 2.306 and 9.605, respectively