Comparative Analytical Study for Determination of Acetylsalicylic Acid in Bulk and in Pharmaceutical Formulations

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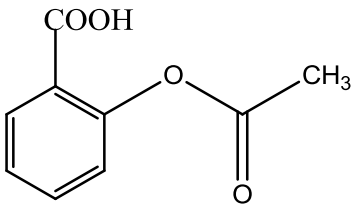
Abstract

A new sensitive colorimetric method for determination of aspirin tablet in aqueous solution. The method is depending on the formation of a yellow colored azo dye by diazotization of 2, 4-dichloroaniline followed by azo-coupling reaction between the resulting product and acetylsalicylic acid. The lambda max of azo dye at 527nm. Beer's low was found to be obeyed in the concentration range of 2-25µg/mL with rang of molar absorptivity between (2935) $Lmol^{-1}cm^{-1}$.Aspirin tablet from different sources industrial companies (SDI, NDI) using high performance liquid chromatography (HPLC) with reversed-phase (ODS-C18) column at low wave length of Uv-visible detection (280nm).the acetylsalicylic acid was eluted for 5 minute at flow rate 2ml/min. The retention time of acetylsalicylic acid was observed at 4.11 minutes. The concentration of the different above companies were gave good relationship between area under the peak (AUP) and acetylsalicylic acid content (p1> 0.001). The comparing of the all values of aspirin tablets in (SDI, NDI) between the above methods were evaluated a good accuracy while the new colorimetric spectrophotometric method for the determination of acetylsalicylic acid (ASA) in pharmaceutical formulations developed procedure was successfully applied to the rapid determination of acetylsalicylic acid in commercial pharmaceutical preparations. The unique features of this procedure are that determination can be carried out at room temperature and analysis time is short. The newly developed method is simple, inexpensive and efficient for use in the analysis of a large number of samples.

Keywords: Acetylsalicylic acid,2,4-dichloroaniline azo coupling reaction, pharmaceutical analysis.

Introduction

Aspirin is a [2-(acetyloxy) benzoic acid] [1]. Sympathicomimetic drug, which was first introduced in medicine by dresser in 1899 [2]. It is prepared by treating acetylsalicylic with acetic anhydride [3,4]. A [2-(acetyloxy) benzoic acid] with formula structural explain below is widely used as anti-inflammatory in the treatment of respiratory diseases in humans [5, 6].



Structure formula of acetylsalicylic acid.

Various analytical method have been reported for determination of tablets ASA [7]; using analysis technique [8]. Including chromatographic method HPLC and GC/MS [9, 10], the potentiometers, flow-injection spectrophotometric [11], diazotization and coupling [12, 13], in current study describes assay sensitive colorimetric method of ASA in tablets [14].

Several analytical methods were developed to prepared aspirin alone or with other drugs [15, 16] including liquid chromatography, UV derivative spectrophotometric method [17].

In this research a new simple and sensitive colorimetric method developed in aqueous solution [18, 19]. Based on the formation of yellow colored azo dye by diazotization of 2, 4-dichloroaniline followed the resulting produced and alkali aspirin solution [20]. The proposed method has the advantage of being rapid and simple.

Experimental

Instruments and Equipment:

1.HPLC, knauer advanced scientific instrument pump 1000 (manager 5000) including the parts of detector PDA (photo Diod array), auto sampler3900 computerized/Chroma gate, column L1C18 (ODS) octadecyl silane chemically bound to porous silica or ceramic micro particles, 3-10µm in Diameter (250 x 4.6mm). Sonycate/karlkotl vibrater. PH meter

microprocessor pH meter. (HANNA No.210). BUK scientific, model 500, infrared spectrophotometer, balance mettle Toledo/AB20y, Switzerland. UV-visible spectrophotometer/Cary 100 cone (varian).

2. Double-beam UV-Visible spectrophotometer model (UV-1650 PC) SHIMADZU (Japan), interfaced with computer via a SHIMADZU UV probe data system program (Version1.10), using 1.00 cm quartz cell was used for measuring the absorption single, Ultra sonic devise (ultrasonicator) for dissolving samples, (SONOREX), (W.Germany), Ultra-pure water manufacturing device, (TORAYPURE), model uv-08 (Japan).
3. The IR spectra were measured as (KBr disc) were recorded with Shimadzu-FTIR 8300 spectrophotometer.

Chemicals and Reagents:

Pure acetylsalicylic acid $C_9H_8O_4$ F.W.180.2 standard material was obtained from state company of Drug industries and medical Appliances (Samara-Iraq-SDI and Nainwa-Iraq-NDI); all commercial drugs tablets contain 100 mg of acetylsalicylic acid.

Preparation of stock and working standard solution

a. A standard solution of 100µg ml^{-1} aspirin was freshly prepared by dissolving 0.01 gm of acetylsalicylic acid in 10ml absolute ethanol and then diluted with distilled water in 10ml; A 2, 4-dichloroaniline was obtained from (Merck) a standard solution of 100mg ml^{-1} was prepared by dissolving 0.01gm of 2, 4-dichloroaniline in 10ml absolute ethanol and then diluted with distilled water to 100ml.A sodium nitrite (99.8% purity) form (BDH) and stander solution was prepared. 4-sodium hydroxide of (98% purity) from (RDL), solution of 1M was prepared by dissolving 4 gm in 100 ml distilled water.

b. Acetonitrile of HPLC grade PAI PANREAC, Lot 2621072914, Barcelona-Espna; Glacial acetic acid BDH, Batch No.28118, England; Formic acid and Sodium heptane sulfunate and other materials are used of HPLC analytical grade were obtained from the center for drug research and quality control.

Procedure

General Procedure:

1. Assay of acetylsalicylic acid using 2, 4-dichloroaniline by diazotization:

The 2 ml of acetylsalicylic acid standard solution 100 µg/mL and 0.75 ml of 1M Sodium Hydroxide solutions were added to 5 ml of 2, 4-dichloroaniline and 0.5 ml of 1% sodium nitrate and 0.5 ml of 1M HCl were mixed and completed with distilled water to the mark in 10ml volumetric flask and shacked for 3 minutes, shaking and cooling ice bath for 3 minute, after 5 minutes the yellow color is completely developed and the absorbance measurement was carried out at a wavelength at 527nm, against a blank solution prepared in the same method but without a acetylsalicylic acid.

2. Assay of acetylsalicylic acid using UV-Visible spectrophotometry:

Ten tablets were weighed and weighed and finely powdered. A weighed amount of the powder containing 100 mg of acetylsalicylic acid equivalent to one tablet was dissolved in 10 ml absolute ethanol and then diluted with distilled in 50 ml of volumetric flasks and diluted up to the mark to obtain 100 µg/ml.

Oral solution 2 ml was taken from container containing 400 µg/ml of acetylsalicylic acid was transferred in to 100ml volumetric flasks and diluted up to the mark with distilled water. Working standard was prepared by suitable dilution and the recommended procedure was used for acetylsalicylic acid its determination.

3. Assay of acetylsalicylic acid by HPLC method:

The standard calibration curve was prepared after the acetylsalicylic acid was determined in the each tablet from different pharmaceutical companies.

The preparation of the calibration curve was used from the external standard acetylsalicylic acid usp reference standard and the stock solution was prepared from different dilution (0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6µg/ml) then injected in to HPLC to obtain the (AUP) of each concentration. While the chromatographic procedure was carried out by using, stainless steel column with a dimension of (250X4.6mm) that contain packing L1C18.

(ODS); diluted mixture was prepared of acetonitrile of acetonitrile and formic acid (99: l) was prepared. With the Mobile phase: 2 gram of sodium heptanesulfonate in a mixture of 850 ml of water and 150 ml of acetonitrile was dissolved, and adjust with glacial acetic acid to pH 3.4. Operate in mode LPG: Flow rate 2ml/min.Run time 5min. Injection volume 20µl. Detector wave length 280 nm (UV-Visible detector).

Results and Discussions

1. Spectrophotometry method

The result of this investigation indicated that the reaction between acetylsalicylic acid with 2, 4-dichloroaniline in the presence of sodium nitrite and hydrochloric acid yield highly soluble colored condensation products which can be utilized as a suitable assay procedures for acetylsalicylic acid; these color products have maximum absorption at 527 nm while the blank at these wave length show zero absorbance a Fig.(1). The influence of various reaction variables on the color development was tested to establish the most favorable conditions.

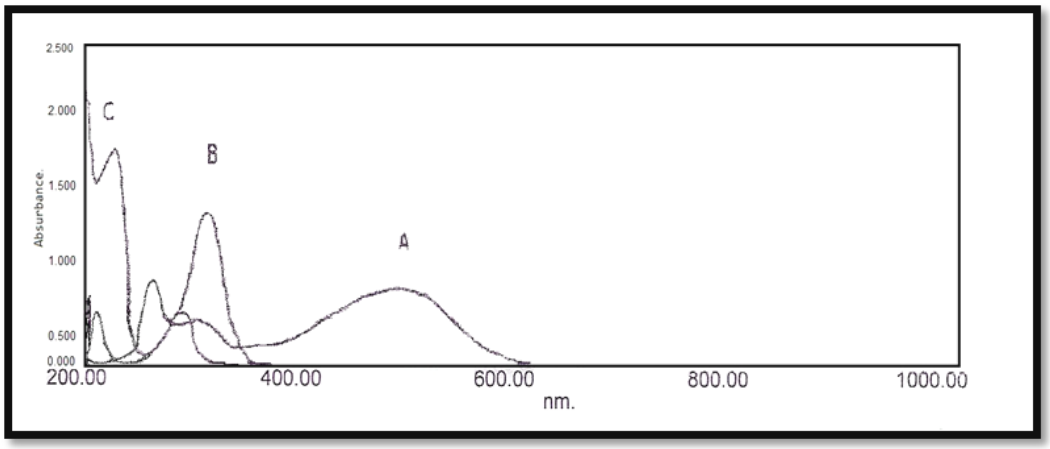


Fig. (1) (A) Absorption spectrum of the azo dye formed, acetylsalicylic acid (20mg/ml) and 2, 4-dichloroaniline (b) Absorption spectrum of the 2, 4-dichloroaniline (c) Absorption spectrum of acetylsalicylic acid.

The linear calibration graph for acetylsalicylic acid is obtained in Fig.(2).

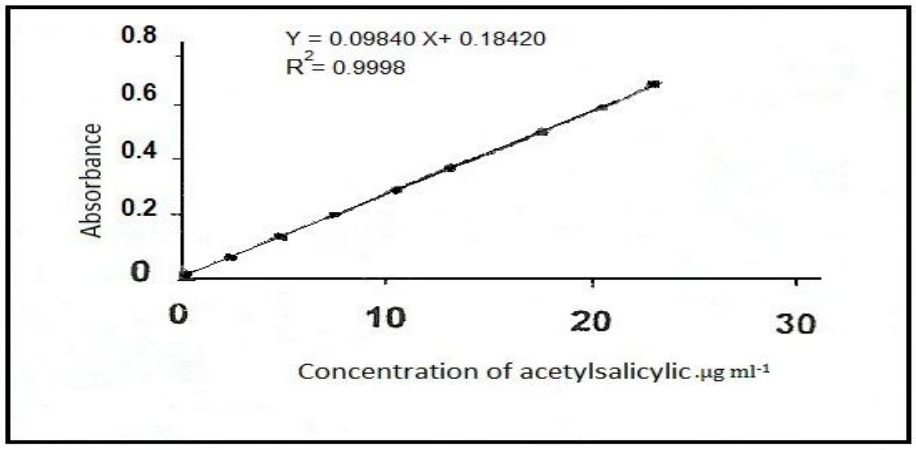


Fig. (2) The calibration curve of acetylsalicylic acid by UV spectrophotometry.

Which shows that the beer's low was obeyed over the concentration rang of 2-25 µg/ml with correlation coefficient and the conditional absorptivity of the yellow product formed was found (2935) $Lmol^{-1}cm^{-1}$. The results was reported in Table (1).

Table (1)
Summery of linearity studies of ASA.

Parameters	***Value***
Linearity range(µg/ml)	2-25
Regression equation	Y=0.09840+0.18420
Slop	0.09840
intercept	0.18420
Correlation coefficient(r)	0.9999
Linearity(%r^2)	99.98
Molar absorptivity (L $mol^{-1}cm^{-1}$)	2935

The precision and accuracy of aspirin were carried out through analysis of (n=3) of different companies showed in Table (2).

Table (2)
Summary of linearity studies of ASA in U.V spectrophotometry method.

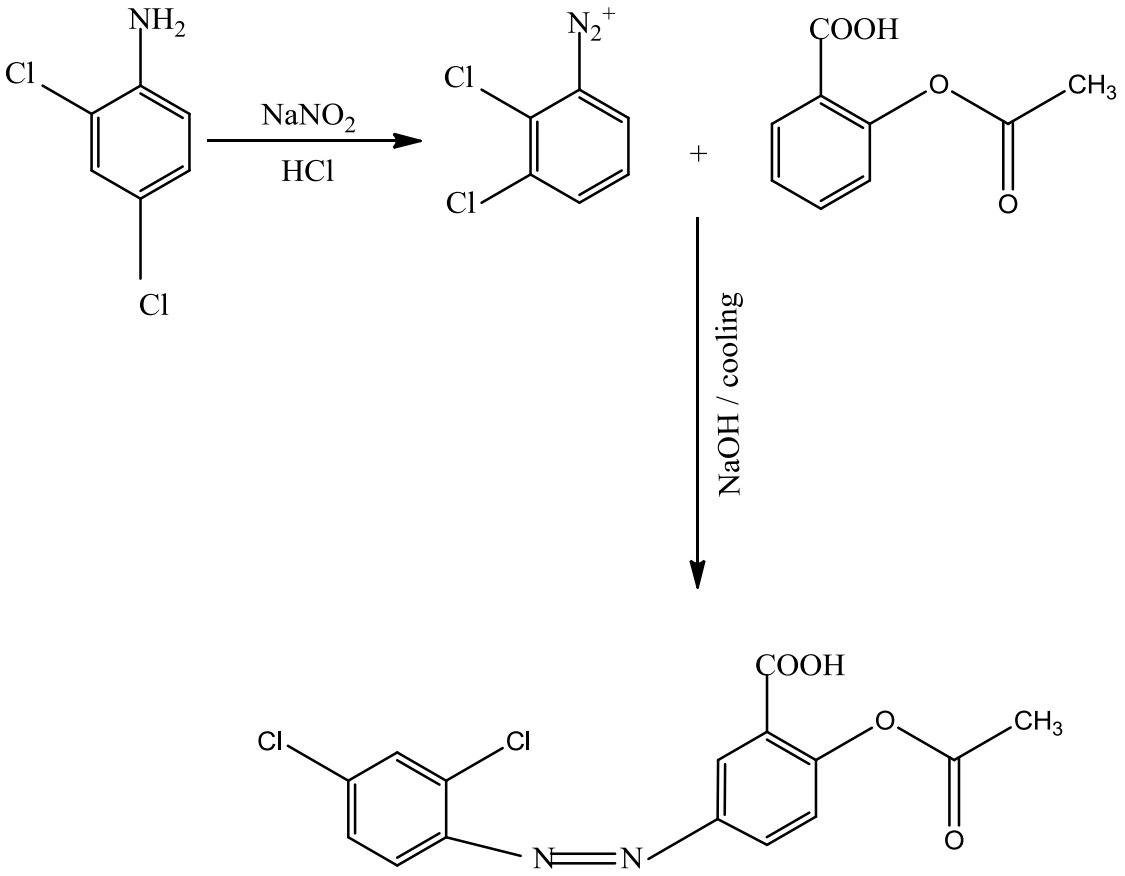
Compounds	***Amount (µg/ml) added***	***Amount (µg/ml) Found***	***RSD%***	***Recovery%***	$E_{re.}$*%*	***C.L.***
NDI	0.5	0.465	0.069	93.0	-7	$0.475\pm4.5x10^{-2}$
SDI	0.5	0.486	0.074	97.2	-2.8	$0.486\pm6.7x10^{-2}$

The addition of reagents:

The optimum result was obtained by the addition of reagent and other chemicals to formation the azo dye are given by the following sequence volumes were taken 5ml of 100µg ml^{-1} 2, 4-dichloroaniline, 0.5 ml of 1M HCl, 0.5ml of 1% sodium nitrate 2ml of 100µg ml^{-1} of acetylsalicylic acid and 0.75ml of sodium hydroxide. That the best of the order of addition of reagent and other chemicals which gave azo dye had high intensity and high absorbance at the wavelength 527 nm.

Composition of the formula structure:

Acetylsalicylic acid form colored product after coupling with an electrophilic of 2, 4-dichloroaniline diazotation ion in the presence of alkaline medium the Mechanism of the Reaction following



4-(2',4'-dichloro azo phenyl) acetyl salcylic acid

The composition of the formula structure of azo dye was studied by the mole ratio method [19]. A break of 1:1 suggested the formation of acetylsalicylic acid with 2, 4-dichloroaniline Fig.(3).

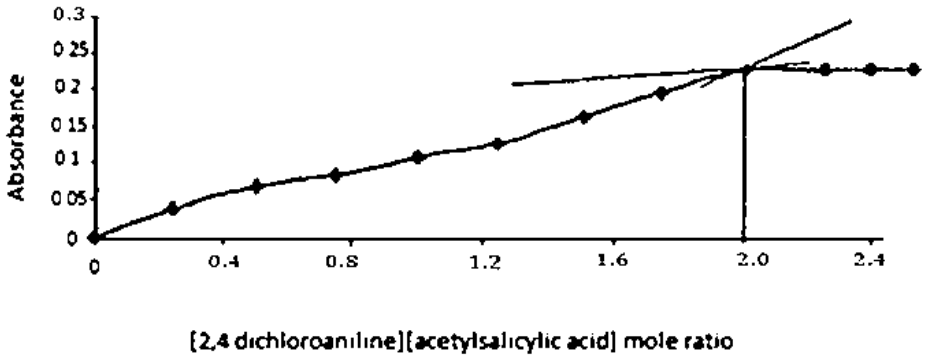


Fig. (3) Mole ratioof the azo dye of acetylsalicylic acid with 2, 4-dichloroaniline.

The apparent stability constants were calculated by comparing the absorbance of solution containing a twice amount of aspirin with 2, 4-dichloroaniline that of a solution containing five-fold excess of reagent. The average conditional stability constant of the dye in water, under the described experimental conditions is $0.38x10^4$. The color intensity reached maximum after formation of azo dye of acetylsalicylic acid. The color obtained was stable for at least 24 hours then used (FTIR) for the identification of the complex formed that the measurements obtained show in Fig. (4)

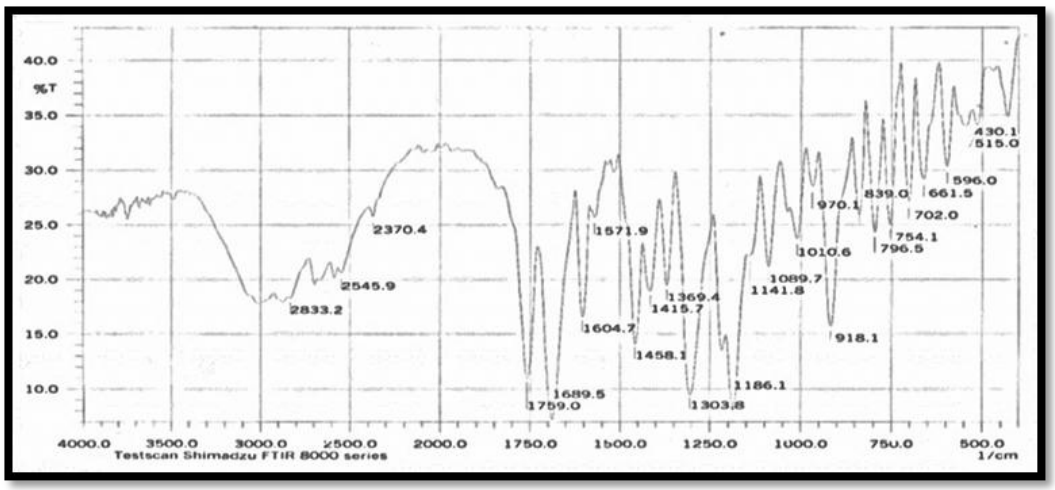


Fig. (4) The (FTIR) absorption (cm^{-1}) of the complex formed.

The reaction is followed by disappearance of (NH_2) absorption band at (3100-3400) cm^{-1} with appearance of (N=N) absorption band at (1450-1460) cm^{-1}.

2. HPLC method:

The different solutions were prepared of acetylsalicylic acid these dilutions injected in to HPLC system was obtained using area under the peak (Aup) Fig. (5) Showing the results of the calibration curve follows the straight-line equation of ASA.

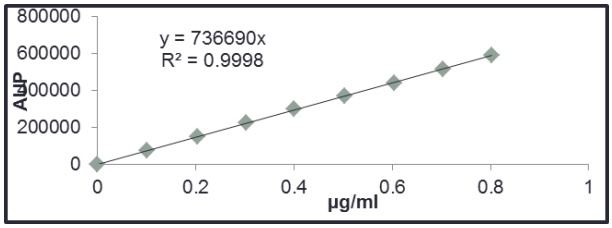


Fig.(5) Calibration Curve of acetylsalicylic acid in HPLC method.

Linear regression analysis of calibration data gave in Table (3) with Correlation coefficients close to unity.

Table (3)
Summery of linearity studies of ASA.

Parameters	*Value*
Linearity range(µg/ml)	0.1-0.8
Regression equation	Y=736690x
Slop	736690
Intercept	0
Correlation coefficient(r)	0.9999
Linearity(%r^2)	99.98

The highly significant linear correlation of the area under the peak (Aup) on the concentration indicated by the high value of r^2 and r, which are close to the highest value of the perfect correlation; this ensures the accuracy of the work and the qualification of the HPLC device.The following Chromatogram of 0.5 µg/ml of standard solution of acetylsalicylic acid is shown below in Fig. (6).

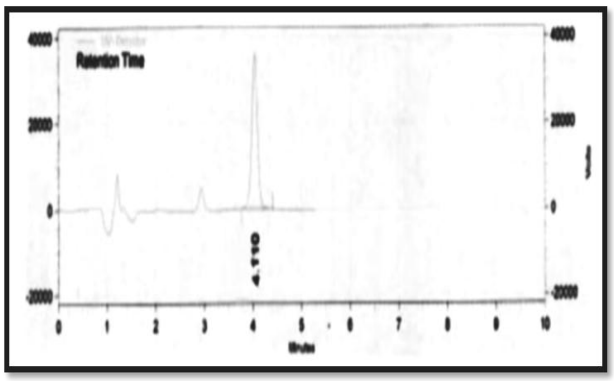


Fig. (6) The chromatogram of standard solution of acetylsalicylic acid retention time of acetylsalicylic acid (TR) is 4.110min.

The precision and accuracy were carried out through analysis of (n=3) of different companies showed in Table (4).

Table (4)
Summary of linearity studies of ASA in HPLC method.

Compounds	*Amount (µg/ml) added*	*Amount (µg/ml) Found*	*RSD %*	*Recovery %*	$E_{re.}$%	*Aup*	*C.L.*
NDI	0.5	0.475	0.072	95	-5	349927	$0.475 \pm 4.5 \times 10^{-2}$
SDI	0.5	0.490	0.076	98	-2	360978	$0.490 \pm 4.5 \times 10^{-2}$

Effect of Organic solvent

The effect of organic solvent such us methanol, ethanol, ether and distill water were studied by using in the dilution and measuring the absorbance were found 0.39, 0.316, 0402, 0.476 respectively. So we used distill water because it had best absorbance for it is abundance.

Interference Studies

The effect of interference by common organic compound was determined by measuring the absorbance of a dye solution containing 1 ml of $4X10^{-4}$ M of glucose and various amount of other species such as p-phenylen diamine, O-aminophenol,4-chloro nito aniline, resosenol, paracetamol, and starch. The results showed that 4-chloro nitro aniline major of common compound do interfere the results are given in Table (5).

Table (5)
Effect varies interference of organic compound on the absorption.

Interference	*Without addition*	*Glucose*	*o-aminophenol*	*resosenol*	*paracetamol*	*starch*	*4-chloro nitro aniline*	*p-phenylen diamine*
Absorbance	0.466	0123	0.137	0.198	0.168	0.195	0.217	0.135

Analytical application

Proposed method have been used of two type of drugs containing acetylsalicylic acid (tablet and oral) and they gave good accuracy and precision, the proposed method (UV-vis, HPLC) was compared with British pharmacopeias standard method, since F-test, T-test showed that three were no significant differences between the proposed method and official method [18]. The results obtained were tabulated in Table (6).

Table (6)

Determination of acetylsalicylic acid in pharmaceutical preparations by the proposed method and comparison with the British pharmacopeia method.

Procedure Applied	*Pharmaceutical Formulation**	*Recovery%*	*%E Relative Error*	*Relative Standard Deviation*%*
Proposed method UV-Vis	Tablets** acetylsalicylic acid	99.13	0.86	2.75
	Oral acetylsalicylic	99.55	0.45	3.25
British pharmacopeia method[18] UV-Vis	Tablets** Acetylsalicylic acid	100	-	-
	Oral acetylsalicylic	100	-	-
Proposed method HPLC	Tablets** acetylsalicylic acid	99.87	0.125	4.3
	Oral* acetylsalicylic	100.2	0.2	2.3
British pharmacopeia method[18] HPLC	Tablets* Acetylsalicylic Acid	100	-	-
	Oral** acetylsalicylic	101.2	1.2	2.55

mean of three determination marketed by SDI ** mean of three determination marketed by NDI.

Comparative Method

The procedures for comparative methods (HPLC and colorimetric method) have been described in the British Pharmacopoeia. That Acetylsalicylic acid with colorimetric method was detected a newly developed method is simple, inexpensive and efficient for use in the analysis of a large number of samples.

Conclusion

Despite the great number of methods described in the literature for the analysis of ASA, the proposed diazotization by azo-coupling spectrophotometric method for the determination of acetylsalicylic acid in pharmaceutical samples reported in this paper is simple, rapid, inexpensive, and thus is very appropriate for routine quality control analysis of active drug in the laboratories of hospitals, pharmaceutical industries and research institutions. The procedure is easier to execute and requires less sample handling than methods currently described in the literature. Statistical comparison of the results with the diazotization method showed good agreement and indicates no significant difference in accuracy and precision. So, the precision of the proposed method of analysis was found to be good (for drug samples RSD < 5%).

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الخلاصة

طريقة جديدة لونية حساسة لتحديد قرص الأسبرين في محلول مائي. أسلوب جديد يعتمد تشكيل لصبغ AZO الملونة الصفراء ومن مصادر مختلفة الشركات الصناعية (NDI،SDI) قد تم ايضا استخدام تقنية (HPLC) مع (ODS-C18) في طول موجة منخفضة من الأشعة فوق البنفسجية مرئية يصل الى280nm على حامض الصفصاف لمدة ٥ دقيقة في 2 min/ml معدل التدفق وقد لوحظ وقت الاحتفاظ لحبة الاسبرين في 4.11 nm وقد أعطى تركيز الشركات المذكورة أعلاه مختلف علاقة جيدة بين المنطقة الخاضعة لقمة (AUP) ومحتوى الحبة. جرى تقييم للمقارنة بين القيم لكل من أقراص الأسبرين مع الطيفية اللونية لتلك المستحضرات الصيدلانية والتي اعطت نتايج جيدة وجديدة يمكن ان تستعمل مع عديد من انواع الادوية المتوفرة لشركات دوائية اخرى.