Flow Injection- Spectrophotometric Determination of Salbutamol Sulphate and Pyridoxine Hydrochloride Using 2,4-Dinitrophenylhydrazine

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Abstract

A batch and flow injection (FI) methods with spectrophotometric detection is proposed for determining of two of phenolic drugs: salbutamol sulphate(SLB)and pyridoxine hydrochloride(PYD) in pure and in pharmaceutical formulations. The methods utilized an oxidative-coupling reaction based upon oxidation of 2,4-dinitrophenylhydrazine (DNPH) with sodium periodate, where an electrophilic intermediate (diazonium salt of the reagent) is produced, which couples with either SLB or PYD in the presence of sodium hydroxide yielding a highly colored condensation product. The absorbance is measured after 15min at 525and515 nm for SLB and PYD respectively. Calibration graphs for both batch and FIA methods were linear over the concentration ranges of 1-24µg/ml for SLB and 0.4-16µg/ml for PYD, and of 30-1100µg/ml for SLB and20-1200µg/ml for PYD respectively. A detection limits of 0.580µg/ml SLB and 0.150µg/ml PYD for batch method, and 15.32µg/ml SLB and12.21µg/ml PYD for FIA method. The proposed methods were applied successfully to the determination of SLB and PYD in pharmaceutical preparations and were compared statistically with reference methods by means of *t*-test and *F*- test and were found not to differ significantly at 95% confidence level. The procedures are characterized by its simplicity, accuracy and precision. The results obtained were in good agreement with those obtained using reference methods for comparison.

Keywords: Salbutamol sulphate (SLB), Pyridoxine hydrochloride(PYD),Oxidative-coupling reaction, 2,4-dinitrophenylhydrazine (DNPH), Spectrophotometric determination , Flow injection

التقدير الطيفي–الحقن الجرياني لكبريتات السالبيوتامول وهيدروكلوريد البايردوكسين باستخدام 2،4– ثنائي نايتروفنيل هيدرازين

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الخلاصة

يتضمن البحث تطوير طرق طيفية بسيطة للتقدير الكمي للمقادير الضئيلة من كبريتات السالبيوتامول وهيدروكلوريد البايردوكسين في المحاليل النقية والمستحضرات الصيدلانية باستخدام المطياف الضوئي بطريقتي الدفعة والحقن الجرياني .اعتمدت الطرق على تفاعل الازدواج التاكسدي حيث يتم اكسدة كاشف 2،4–ثنائي نايتروفنيل هيدرازين باستخدام بيرايودات الصوديوم وتكوين مادة وسطية الكتروفيلية(ملح الدايازونيوم

للكاشف) ومن ثم تدخل تفاعل الازدواج مع كبريتات السالبيوتامول اوهيدروكلوريد البايردوكسين في وسط قاعدي حيث تتكون نواتج ملونة مستقرة. قيست الامتصاصية بعد15دقيقة من بدء التفاعل عند طول موجي 525 و515 نانوميتر لكل من كبريتات السالبيوتامول وهيدروكلوريد البايردوكسين على التوالي .تشير منحنيات الامتصاص مقابل التركيز بان قانون بير ينطبق ضمن مدى التركيز 1−24 و 0,4−16 مايكروغرام.مل$^{-1}$ من كبريتات السالبيوتامول وهيدروكلوريد البايردوكسين لطريقة الدفعة ،اما بطريقة الحقن الجرياني فكان مدى التركيز من 30−1100 و 20− 1200 مايكروغرام.مل$^{-1}$من كبريتات السالبيوتامول وهيدروكلوريد البايردوكسين على التوالي وبحد كشف 0,580 و0,150. مايكروغرام.مل$^{-1}$ من كبريتات السالبيوتامول وهيدروكلوريد البايردوكسين لطريقة الدفعة واما لطريقة الحقن الجرياني فكانت 15,32 و 21,12 مايكروغرام.مل$^{-1}$لكل من كبريتات السالبيوتامول وهيدروكلوريد البايردوكسين على التوالي .تمت دراسة الظروف المثلى للتفاعل وجميع المتغيرات الكيميائية والفيزيائية بدقة وطبقت الطريقتين بنجاح على المستحضرات الصيدلانية الحاوية على كبريتات السالبيوتامول وهيدروكلوريد البايردوكسين .وقورنت النتائج التي تم الحصول عليها مع نتائج طرق التحليل القياسية للادوية اعلاه وبطريقة احصائية (اختبار tواختبار F) واظهرت النتائج عدم وجود فرق جوهري بين نتائج الطرق المقترحة ونتائج الطرق القياسية.

Introduction

Salbutamol sulfate, 4-[2-(*tert*-butylamino)-1-hydroxyethyl]-2 (hydroxyl methyl) phenol (SLB) is a selective β2-agonist antiasthmatic. Its primary action is to stimulate adenyl cyclase which catalyzes the formation of cyclic adenosin monophosphate[1]. The drug is official in *European Pharmacopoeia*[2], and *British Pharmacoeia*[3]. According to literature, several analytical methods have been developed for SLB determination in the dosage forms and biofluids, including HPLC(4–6) and spectrophotometric methods, including oxidative coupling reaction[7], direct oxidation or nitration reactions(8,9), forming a charge transfer complex[10] besides to using different reaction systems(11,12). Most of these methods require extensive sample preparation prior to the measurement step, some are less sensitive and some other are relatively complicated in terms of assay procedure or equipment requirement for analysis.

Pyridoxine hydrochloride(PYD) namely vitamin B6 ,is chemically 3-hydroxy-4,5-bis-(hydroxylmethyl)-2-picoline hydrochloride. It is a water soluble vitamin and involved principally in amino acid, carbohydrate and fat metabolism. It is required for the formation of haemoglobin. It is responsible mainly for the transference of amino acid groups and the maintenance of body cells, acting as a coenzyme [13]. Its lack results in skin and nervous system changes and certain types of anemia. A variety of methods for the determination of PYD have been developed including HPLC (14-17), TLC(18-20), micellar electrokinetic capillary chromatography[21], spectrophotometry [22,23], and mass spectrometry [24].

Visible absorption spectrophotometry was applied in pioneering works on flow injection analysis. Through all the thirty-three years of development of FIA, UV-Vis spectrophotometry has been and currently is the most common detection used in FIA [25]. UV-Vis spectrophotometry is the technique of choice for FIA pharmaceutical applications, as it offers the advantages of simple and low cost instruments that are available at all laboratories.

In the present paper, an automated procedure is proposed for the spectrophotometric determination of two of phenolic drugs: SLB and PYD by reaction with 2,4Dnitrophenyl hydrazine (DNPH). The reaction can be carried out in batch and in FIA and in this paper the two approaches are compared. The reaction product has been spectrophotometrically measured at 525 nm for SLB, and 515nm for PYD.

Experimental

Apparatus

All spectral and absorbance measurements were carried out on a Shimadzu UV–Vis 260 digital double beam recording spectrophotometer using 1-cm path length quartz cells and the measurements were performed at 25ºC.

The flow injection configuration employed is outlined in Figure 1. A quartz flow cell with 50 µl internal volume and 1 cm bath length was used for the absorbance measurements.

A two-channel manifold peristaltic pump (Ismatec, Labortechnik – Analytik, CH – 8152, Glatbrugg – Zurich – Switzerland) was used to propelling the solutions. Samples were injected using a rotating Rheodyne(Altex 210, Supelco – USA) with a sample loop of 150µl.Flexible vinyl tubing of 0.5mm internal diameter was used for the peristaltic pump. Reaction coil (RC) was of Teflon with internal diameter of 0.5 mm. The absorbance was measured at 525 and515 nm for SLB and PYD respectively.

Reagent and Materials

All reagents used were of analytical-reagent grade and were obtained from BDH, UK. Distilled water was used throughout, DNPH was daily prepared as 20mM solution by dissolving 0.1981gm in 50mL distilled water, and working solutions were prepared by appropriate dilution of the stock solution.

Sodium periodat 20mM solution, was prepared by dissolving 0.2138 gm in 50mL distilled water and working solutions were prepared by appropriate dilution of the stock solution. Sodium hydroxide 2M solution was prepared by dissolving 8.000 gm in 100mL distilled water and working solutions were prepared by appropriate dilution of the stock solution

Preparation of Standard Solution

Pure SLB and PYD drugs samples were kindly provided from state company for Drug Industries and Medical Appliance, SDI, Samara, Iraq. Dosage forms were obtained from commercial sources. A 1000µg.mL^{-1} stock solutions of SLB (1.73mM) and PYD (4.86mM) were prepared in distilled water. Serial dilutions with distilled water were made to cover the working range Table 1

Preparation of Pharmaceutical Samples

Twenty tablets of each drugs was weighed, powdered and mixed thoroughly. Similarly, content of 10 vials of PYD were also mixed. A quantity equivalent to 100 mg of the pure drug was transferred into a 100 mL volumetric flask. The drugs were dissolved in distilled water, shaken well and made up to the volume with distilled water. The resultant solutions were filtered and an aliquot of 250µgmL^{-1} of stock solutions in final volume of 25mL were used for analysis. For syrup samples**,** an aliquot corresponding to 50 mg of SLB was diluted to 50mL with distilled water in a volumetric flask. More dilute solutions of pharmaceutical preparations for batch and FIA procedures were made up by simple dilution with distilled water.

Procedure

General Batch Procedure

Into a series of 25 mL volumetric flasks, , 1.5mL of DNPH(5mM) and 1.5mL of sodium periodate(5mM) for the determination of SLB and 1mL of DNPH and 1 mL of sodium periodate were introduced for the determination of PYD. An increasing volume of phenolic drugs working solutions (100µgmL^{-1}) were transferred to cover the range of the calibration curves. Then 3mL of sodium hydroxide was used for SLB drug solutions and 4mL of sodium hydroxide (0.5M) was added for PYD drug solutions. The solutions were diluted to the mark with distilled water, mixed well and left for 15min at room temperature (25 C^{o}). The absorbance was measured at 525nm and 515nm for SLB and PYD respectively versus the reagent blank prepared in the same way but containing no phenolic drugs. A calibration curves were drawn and the regression equations calculated. For the optimization of conditions and in all subsequent experiments were carried out on 10 µg mL^{-1} of SLB (0.0173mM) and PYD(0.0486mM).

Procedure for The Stoichiometric Method (molar ratio*)*

The stoichiometry of the formed product was investigated by continuous variation (Job's method) method. The method was applied by placing 1 to 9 mL of 0.5mM solutions of the phenolic drugs (SLB and PYD) into a series of 25mL volume flasks; this was followed by placing 9 to 1 mL of 0.5 mM reagent (DNPH), other reagents were added and proceeds as described under the general batch procedure.

General FIA Procedure

Working solutions of SLB and PYD in the range cited in Table 1 were prepared from stock solutions. A 150 µL portion of the drugs solutions were injected into the stream of the mixture of 0.5mM DNPH and 0.5mM sodium periodate solution and was then combined with a stream of 0.5 M sodium hydroxide. Solutions were propelled by peristaltic pump with individual flow rate of 0.8mL min^{-1} in each

channel Figure 1**,** Channel A was used to transport mixture of DNPH and sodium periodate, channel B to transport sodium hydroxide solution.). The resulting absorbance of the colored dye was measured at λ_{max} and a calibration curve was prepared over the range cited in Table.1. Optimization of conditions was carried out on 100µgmL^{-1} of SLB and PYD drug respectively

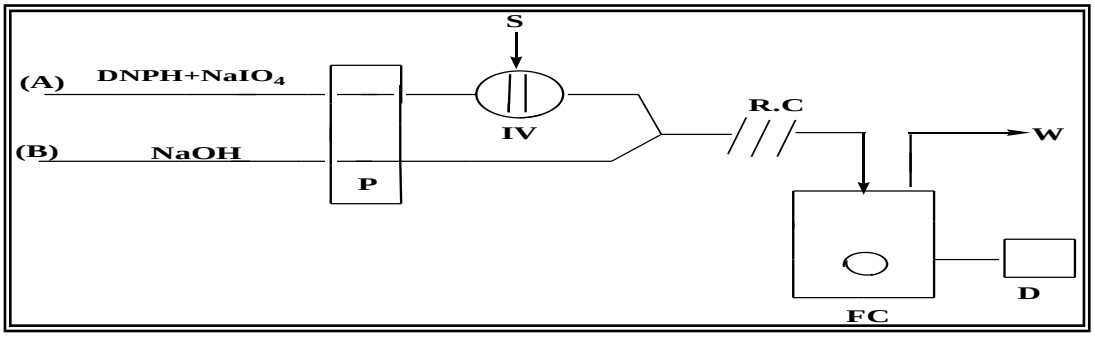


Figure 1- Manifold employed for FIA-Spectrophotometric determination of SLB and PYD with DNPH and sodium periodate solution in alkaline medium where: IV, Injection valve ; R.C, .Reaction Coil ; S , Sample ; P, Peristaltic pump ; FC ,Flow cell ; D, Detector ; W,Waste.

Results and Discussion

Absorption Spectra

SLB and PYD form a red colored product (λmax of 525 and 515 nm with a molar absorption coefficient of 3.61 x $10^4$and 1.03 x 10^4 L Mole^{-1} Cm^{-1} respectively) with DNPH in the presence of sodium periodate in alkaline medium. The absorption spectra of the colored product are given in Figure 2. Under the reaction conditions, the reaction is based on the oxidation of DNPH with sodium periodate to produce diazonium cation, The intermediate of DNPH undergoes electrophilic substitution in alkaline medium with the phenolic drugs of SLB and PYD to form a colored product(III) according to Scheme -1**.** The factors affecting on the sensitivity and stability of the colored product resulting from the oxidative coupling reaction of SLB and PYD with DNPH and sodium periodate in alkaline medium were carefully studied. The colored dye product was only formed in alkaline medium, therefore, the effect of different alkaline solutions were studied such as sodium acetate, sodium carbonate, ammonium hydroxide and sodium hydroxide. Maximum sensitivity and stability were obtained only when the reaction was carried out in the presence of sodium hydroxide solution.

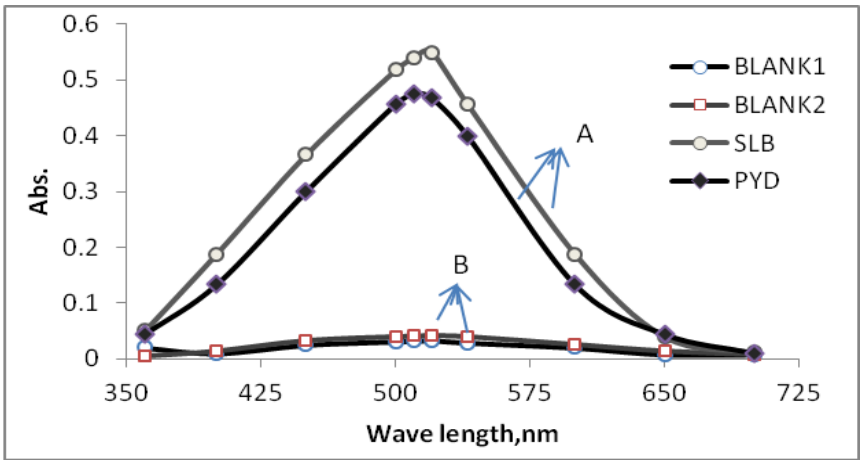
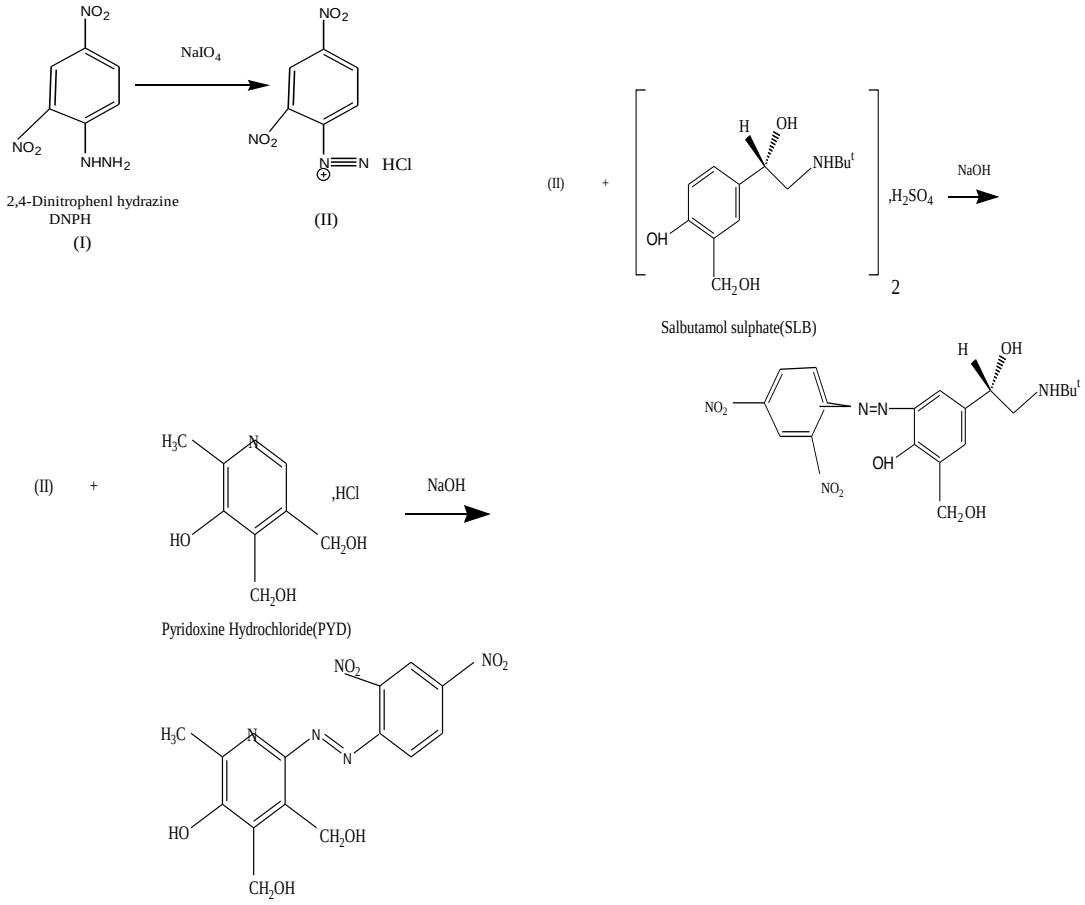


Figure 2- Absorption spectra of A (10 µgml^{-1}) of SLB and PYD treated as described under procedure and measured against reagent blank and B the reagent blanks measured against distilled water.



Scheme 1- Proposed mechanism of the reaction between DNPH and SLB or PYD

Batch Spectrophotometric Determination

The best experimental conditions for the determination of SLB and PYD were established for DNPH 5mM (from 0.3 to 2 mL), sodium periodate5mM (from 0.2 to 2 mL) and sodium hydroxide0.5M (from 0.5 to 8ml) by adding various volumes of their solutions to a fixed concentration of SLB and PYD (10 µg mL^{-1}) and measuring the absorbance at maximum wave length. The obtained results show that 1.5mL of 5Mm DNPH, 1.5mL of 5mM sodium periodate and 3ml of 0.5mM of sodium hydroxide are the volumes that can give a higher absorption intensity and stability of the dye product at 525 nm for 10µgmL^{-1} SLB, and 1mL of 5Mm DNPH, 1mL of 5mM sodium periodate and 4ml of 0.5mM of sodium hydroxide are the volumes that can give a higher absorption intensity and stability of the dye product at 515 nm for 10µg$mL^{-1}$of PYD. Experimental results revealed that the color intensity reach a maximum after the drug solution had been reacted with DNPH and sodium periodate in alkaline medium for 10 min., therefore, a 15 min. development time was suggested as the optimum reaction time and remained stable for 120 min. The order of addition of the reagents is an essential part of the experiment, it was found that the order of addition of the reagent cited under general procedure gave maximum color intensity and a minimum absorbance of the blank and was used in all subsequent experiments.

The effect of temperature on the color intensity of the dye was studied. In practice, high absorbance was obtained when the color was developed at room temperature (25◦C) than when the calibrated flasks were placed in an ice-bath at (0◦C) or in a water bath at (50◦C).

The stoichiometry of the reaction was studied using equimolar concentrations of the drugs and

DNPH at constant sodium periodate and sodium hydroxide concentrations, adopting Jobs̀ method of continuous variation [26], a molar ratio of 1:1 drugs to DNPH was obtained by the applied method as shown in Figure 3**.** The stability constants of the dye products were calculated [27] by comparing the absorbance of a solution containing stoichiometric amount of SLB or PYD and DNPH with that of solution containing five-fold excess of DNPH reagent. The stability constants of the dye products in water under the described experimental conditions were 8.19 x $10^5$and 4.56 x 10^5l $mol^{-1}$for each of SLB and PYD respectively.

In order to assess the possible analytical applications of the proposed method, the effect of some common excipients frequently found with the drugs in pharmaceutical formulations, such as sucrose, glucose, fructose, lactose, starch, talc and magnesium stearate was studied Table 2, by analyzing synthetic sample solutions containing 10µgml^{-1} of SLB or PYD and excess amounts (10-fold excess) of each excipient, none of these substances interfered seriously.

The regression equations obtained, from a series of SLB and PYD standards, and the analytical figures of merits of this procedure are summarized in Table 1 in which are also summarized the main performance of the flow procedure developed for SLB and PYD determination in order to make an effective comparison between the two approaches.

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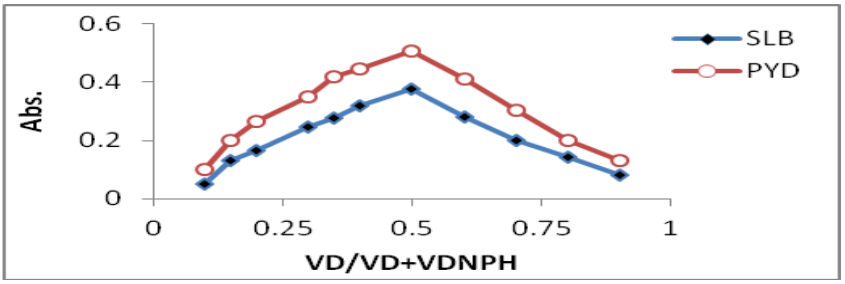

Figure 3- The mole ratio of the reaction between SLB, PYD and DNPH

Table 1- Analytical characteristics of the procedures developed for the determination of SLB and PYD

Parameter	Batch procedure		FIA procedure	
	SLB	PYD	SLB	PYD
Regression equation	y=0.0626x+0.046	y=0.0489x+0.077	y=0.0009x+0.192	y=0.0008x+0.16
Linear range (µg mL^{-1})	1-24	0.4-16	30-1100	20-1200
Correlation coefficient	0.9998	0.9996	0.9998	0.9997
Limit of detection (s/n=3) µg mL^{-1}	0.580	0.150	12.21	15.32
Reproducibility %	<1.06	<0.99	<0.87	<0.82
Average of recovery,%	100.21	98.46	99.85	100.01
Sandell̀s Sensitivity (µg.cm^{2-})	0.01597	0.01996	0.111	0.125
Sampling frequency ($hour^{-1}$)	4	4	28	28

Table 2 - Effect of excipients (100 µg mL^{-1}) on the recovery of Phenolic drugs (10 µg mL^{-1})

excipient	SLB			PYD		
	Conc. µg.ml^{-1}	E%*	Rec%*	Conc. µg.ml^{-1}	E%*	Rec%*
Lactose	10.15	+1.50	101.50	9.98	-0.20	99.80
Talc	9.95	-0.43	99.57	10.05	+0.50	100.50
Starch	9.89	-1.02	98.98	9.88	-1.20	98.80
Mg stearate	9.88	-1.26	98.74	10.11	+1.10	101.10
PVP	10.12	+1.17	101.17	10.08	+0.80	100.80

*Average of five determinations

FIA Spectrophotometric Determination

The batch method for the determination of SLB and PYD were adopted as a basis to develop FIA procedure. The manifolds used for the determination of each of SLB and PYD were so designed to provide differentreaction conditions for magnifying the absorbance signalgenerated by the reaction of SLB and PYD drugs with DNPH and sodium periodate in sodium hydroxide medium. Maximum absorbance intensity was obtained when the sample was injected into a stream of mixed DNPH with sodium periodate and was then combined with the stream of sodium hydroxide Figure 1. The influences of different chemical and physical FIA parameters on the absorbance intensity of the colored product were optimized as follows.

Optimization of Reagents Concentration

The effect of different concentrations of mixture of DNPH and sodium periodate was investigated, while keeping other conditions constant. It was found that a mixture of DNPH and $NaIO_4$ of 0.5mM were found to be the most suitable concentration for obtaining maximum absorbance Figure 4a,b and was chosen for further use. Sodium hydroxide was found necessary for developing the colored product, the effect of sodium hydroxide was studied in the concentration range of 0.1M–1M and a greatest absorbance intensity was obtained with 0.5mM Figure 5.

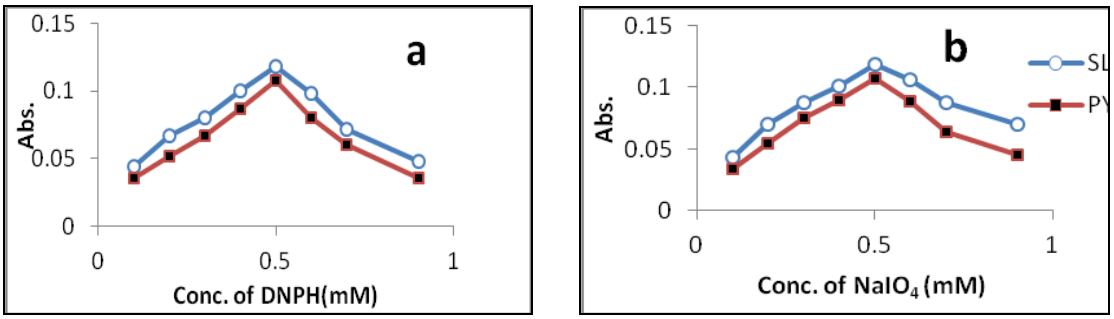

Figure 4- a-Effect of conc. of DNPH, b-effect of conc. of $NaIO_4$ on the colored reaction product.

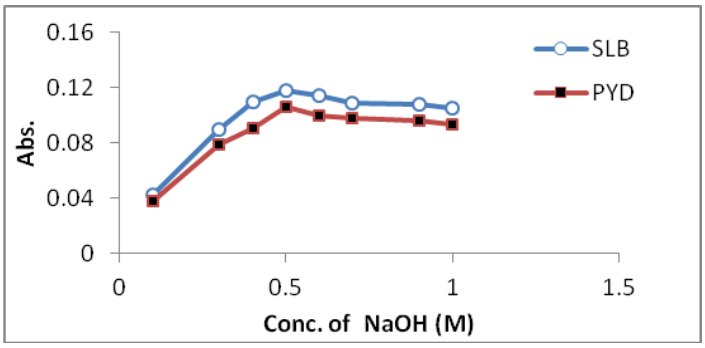

Figure 5- The effect of the concentration of sodium hydroxide (M)

Optimization of Manifold Parameters

The variables studied under the optimized reagent concentrations were the flow rate, the injected sample volume and the reaction coil length. The effect of total flow rate on the sensitivity of the colored reaction product was investigated in the range of 0.5-2 mL min^{-1}. The results obtained showed that a total flow rate of 0.8mL.min^{-1} (0.4 mL min^{-1} in each line) gave the highest absorbance as shown in Figure 6 and was used in all subsequent experiments. Coil length is an essential parameter that affected on the sensitivity of the colored reaction product and was investigated in the range of 25–250 cm The result obtained showed that a coil length of 150 cm gave the highest absorbance as shown in Figure 7 and was used in all subsequent experiments. The volume of the injected sample was varied between 50 and 300µL using different length of sample loop. The results obtained showed that injected sample of 150µL gave the best absorbance Figure 8. A standard calibration line, obtained for a series of SLB and PYD standards

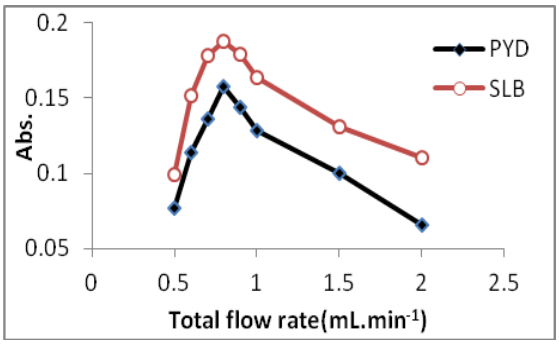

Figure 6- Effect of the total flow rate (mL min^{-1}).

and the main analytical figures of merits of the developed procedure are indicated in Table 1.

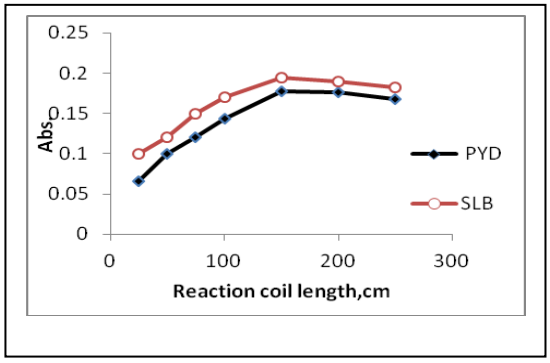

Figure 7- Effect of the length of the reaction coil in cm

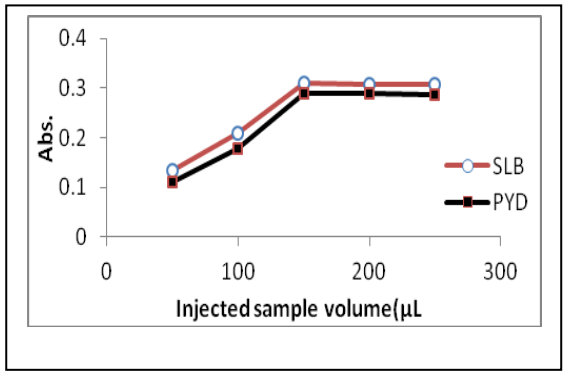

Figure 8- Effect of the injection volume in µL

Analytical application

The precision of the methods was evaluated by analyzing pure samples of SLB and PYD and a good recovery were obtained Table 1 The proposed methods were applied successfully to the analysis of some pharmaceutical preparations containing SLB and PYD, and they gave a good accuracy and precision as shown in Table 2. The results were in accordance with those obtained by the official methods [3]. Finally, statistical analysis[28], *F*- and *T*- test, reveals that there is no significant difference in precision and accuracy between the proposed methods and the official methodsTable 3.

Table 3 -Application of the proposed methods to the determination of SLB and PYD drugs in dosage forms

drug	Pharmaceutical preparation	Batch method					Flow injection method				
		Conc. µg.mL^{-1}		E%	Rec. %	RSD %	Conc. (µg.mL^{-1})		E%	Rec.%	RSD%
		Present	Found*				Present	Found*			
SLB	**Butadin** SDI, Iraq 2mg SLB/tablet	4	3.88	-2.83	97.17	1.856	40	40.095	+0.24	100.24	0.901
		8	7.95	-2.5	97.5	1.691	100	99.850	-0.15	99.85	0.749
		16	16.30	+1.88	1.88	0.983	400	400.233	+0.06	100.06	0.337
	Butadin Syrup SDI, Iraq 2mg/5ml	4	3.90	-0.62	99.38	1.749	40	39.750	-0.75	99.25	0.872
		8	8.04	+0.55	100.55	1.365	100	99.300	+0.30	100.30	0.587
		16	16.07	+0.44	100.44	0.921	400	400.400	+0.11	100.11	0.321
	Butalin Syrup (Julphar),U.A.E. 2mg/5ml	4	3.92	-1.90	98.10	1.121	40	39.800	-0.50	99.50	0.788
		8	8.09	+1.13	101.13	1.032	100	99.700	-0.31	99.69	0.579
		16	16.26	+1.69	101.69	0.897	400	399.200	-0.22	99.78	0.402
PYD	**Samavit B6 40** SDI, -Iraq 40mg/**tablet**	2	1.97	-1.50	98.5	1.163	60	59.300	-1.17	98.83	0.788
		4	4.95	-1.25	98.7	0.925	120	118.700	-1.08	98.92	0.614
		10	10.18	+1.88	101.88	0.827	600	601.000	+0.16	100.16	0.439
	PyridoxineHCl injections MEHECO Corp.,China 100mg/2mL	2	1.95	-2.50	97.50	0.948	60	59.400	-1.00	99.00	0.837
		4	3.96	-1.00	99.00	0.817	120	118.900	-0.92	99.08	0.671
		10	10.18	+1.87	101.87	0.748	600	602.000	+0.16	100.16	0.346

Table 4- The comparison of the proposed method with standardmethod using t- and F-statistical testsat 95% Confidence Level

Pharmaceutical preparation	Proposed methods						Standard method
	Rec.%* Batch method	Value		Rec.% FI method	Value		Rec.%
		t	F		t	F	
SLB pure	100	0.892 (2.447)	1.037 (9.227)	100.00	1.380 (2.447)	7.870 (9.227)	100.00
Butadin Tablets(SDI)	98.85			100.02			99.30
Butadin Syrup(SDI)	100.12			99.783			98.50
Butalin Syrup(Julphar)	100.30			99.657			99.80
PYDpure	100.00	0.153 (2.776)	1.962 (19.00)	100.00	0.093 (2.776)	2.936 (19.00)	100.00
Samavit tablets	99.71			99.30			98.80
PyridoxineHCl injections	99.10			99.416			99.80

Conclusions

The results obtained confirm that the proposed methods are simple, economical with reasonable precision and accuracy for the determination of SLB and PYD. The optical parameters and statistical comparison justify this method for application in routine drugestimation in pure and dosage forms. Also, the procedures do not involve any critical reaction conditions or tedious sample preparation steps. So, the recommended methods are well suited for the assay and evaluation of drugs in pharmaceutical preparation and can also be considered as a general method for the quantification of phenolic drugs(SLB and PYD). In comparison of the batch with FIA procedure, the later is more convenient than the former method because of its speed (sample through-put of 28 injection h^{-1}) and wider linear range of the calibration graph Table 1.

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