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Flow injection-Spectrophotometric Determination of Phenylephrine Hydrochloride and Amoxicillin Trihydrate in Pharmaceutical Preparations

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Abstract

A batch and flow injection (FI) spectrophotometric methods have been developed for the determination of phenylephrine hydrochloride (PHP) and amoxicillin trihydrate (AMOX) in pharmaceutical preparations. The methods are based on coupling reaction of 2,4-dinitrophenylhydrazine (DNPH) with either PHP or AMOX in the presence of sodium hydroxide to form a red colored water-soluble products with absorption maxima at 525 and 515 nm for PHP and AMOX respectively. Optimum conditions for determining the drugs were investigated. Beer's law were obeyed over the concentration ranges of 2-50μg/ml PHP and 1-40 μg/ml AMOX for batch method, and of 30-1000 μg/ml PHP and50-1200μg/ml AMOX for FIA method. The limits of detection were 1.044 μg/ml PHP and 0.230 μg/ml AMOX for batch method, and for FI method were 14.21 μg/ml PHP and 34.00 μg/ml AMOX. The proposed methods were applied satisfactorilyto the determination of PHP and AMOX in pharmaceutical preparations. The procedures are characterized by its simplicity, accuracy and precision. The results obtained were in good agreement with those obtained using reference standard methods at the 95% confidence level.

Keywords: phenylephrine hydrochloride(PHP), amoxicillin trihydrate(AMOX), oxidative- coupling reaction, 2,4-dinitrophenylhydrazine (DNPH), spectrophotometric determination, Flow injection.

1. Introduction

Phenylephrinehydrochloride (PHP), [(R)-1-(3-hydroxyphenyl).2-(methylamino) ethanol hydrochloride], is a white crystalline powder, and belongs to the group of medicines called sympathomimetics [1]. It acts stimulating the alpha receptors in certain areas of the body. It is used locally, as decongestant, for non-specific and allergic conjunctivitis, sinusitis and nasopharyngitis [2]. PHP nasal drops are used for treating symptoms such as runny nose, sneezing, itching of the nose and throat [3]. Various methods have been reported in the literature for the analysis of PHP including spectrophotometry [4–6], spectrophotometry with chromogenic reagent [7], chromatography [8]. High-performance liquid chromatography [9–14], micellar liquid chromatography [15,16], and capillary zone electrophoresis [17] have also been reported for the determination of PHP.

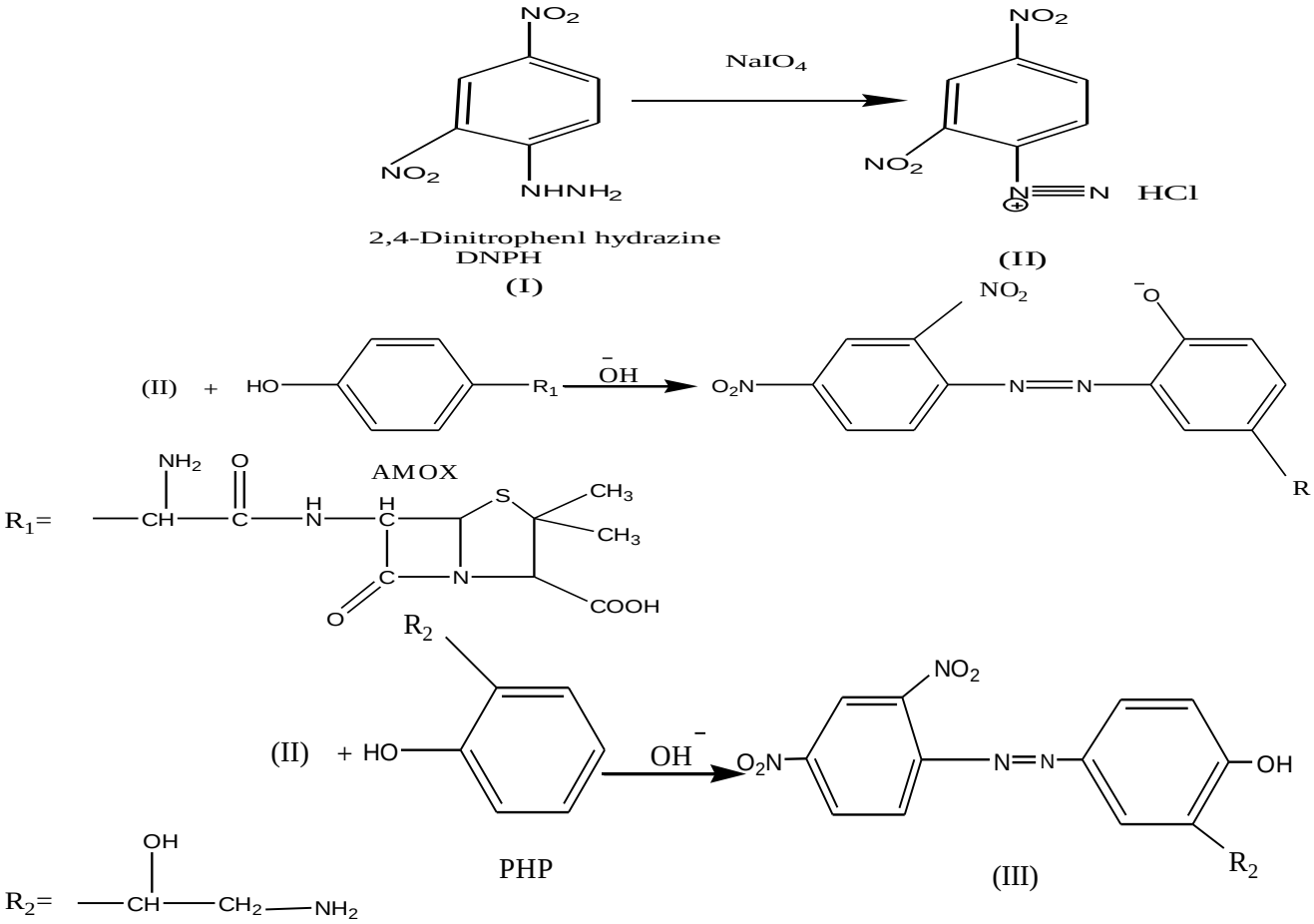
Amoxicillintrihydrate is chemically (6-[(*R*)-(-)-2-amino-2-(*p* hydroxyl phenyl) acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0]heptane-2-carboxylic acid trihydrate. It is one of the most widely used semisynthetic penicillins[1] in the treatment of acute bacterial sinusitis and community-acquired pneumonia [2]. A review of the literature revealed that many methods have been described for its determination in pharmaceutical formulation and biological fluids. They include HPLC [18-21], chemiluminescence [22-24],spectrofluorimetry [25], flow-injection analysis [26-31], voltammetry and polarography [32,33] and titrimetry [34], and spectrophotometric method[35 -39].

In the present paper, an automated procedure is proposed for the spectrophotometric determination of PHP and AMOX by coupling 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 PHP and 515nm for AMOX.

1.1. Reaction mechanism of the method

PHP and AMOX form a red colored product (λmax of 525 and 515 nm with a molar absorption coefficient of 5.784 x 10^3 and 6.29 x 10^3l mole^{-1} cm^{-1} respectively) with

DNPH in the presence of sodium periodate inalkaline medium. The absorption spectra of the colored products are given in Fig.(1). Under the reaction conditions, the reaction is based on the oxidation of DNPH with sodium periodateto produce diazoniumcation, The intermediate ofDNPH undergoes electrophilic substitution in alkaline medium with PHP or AMOX to form a colored product (III) according to Scheme (1).



Scheme (1) Proposed mechanism of the reaction between DNPH and AMOX or PHP.

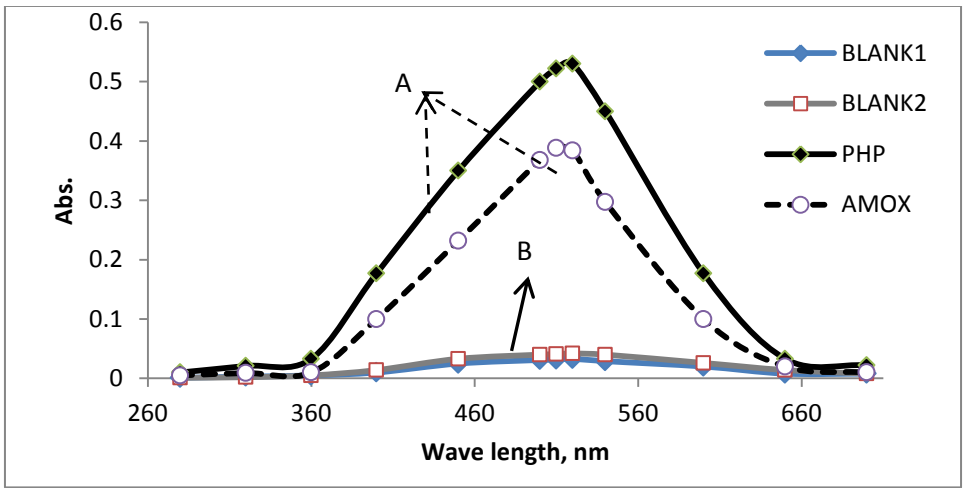


Fig.(1) Absorption spectra ofA (10 µgml^{-1}) of PHP and AMOX treated as described under procedure and measured against reagent blank and B the reagent blanks measured against distilled water.

2. Experimental

2.1. Apparatus

All spectral and absorbance measurements were carried out on a Shimadzu UV–VIS260 digital double beam recording spectrophotometer. A flow cell with 50 µl internal volume and 1 cm bath length was used for the absorbance measurements. A two-channel manifold (Fig.(2)) was employed for the FIA spectrophotometric determination of PHP Aand AMOX drug. A peristaltic pump (Ismatec, Labortechnik – Analytik, CH – 8152, Glatbrugg – Zurich – Switzerland) was used to transport the carries solutions. (Rheodyne, Altex 210, Supelco–USA) injection valvewas employed to provide appropriate injection volumes of standard solutions and samples. 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.

Channel A was used to transport mixture of DNPH and sodium periodate, channel B to transport sodium hydroxide solution. The sample was injected into the stream of the mixture of DNPH with sodium periodate solution, through the injection valve. Solutions were propelled by peristaltic pump with individual flow rate of 0.8ml min^{-1}. The absorbance was measured at 525 and 515 nm for PHP and AMOX respectively.

2.2. Reagent and materials

Analytical reagent grade chemicals and distilled water were used throughout. A stock solution of 20 mM DNPH(BDH) was prepared daily by dissolving 0.1445 gm in 2 ml concentrated H_2SO_4, and stir, thentransferinto a 100 ml calibrated flask and the solution was made up to the volume with distilled water. More dilute solutions (5 and 0.5 mM) were prepared by suitable dilutions with distilled water. Sodium periodate (BDH) 20mMwas prepared by dissolving 0.1967gm in30ml distilled water, thentransferinto a 100 ml calibrated flask and the solution was made up to the volume with distilled watersolution. More dilute solutions (5 and 0.5 mM) were prepared by suitable dilutions with distilled water. Sodium hydroxide (BDH)2M solution was prepared by dissolving accurate weights in distilled water. More dilute solutions were prepared by suitable dilutions with distilled water.

2.3. Preparation of standard solutions

Pure PHP and AMOX drugs samples were kindly providedfrom state company for Drug Industries and Medical Appliance, SDI, Samara, Iraq. Dosage forms were obtained from commercial sources. A1000 $\mu g.ml^{-1}$stock solutions of PHP and AMOX were prepared in distilled water. Serial dilutions with distilled water were made to cover the working range of the calibration graphs (Table (1)).

2.4. Procedure

2.4.1. General batch procedure

Into a series of 25 ml volumetric flasks, 1.5 ml of DNPH (5 mM) and 1.5ml of sodium periodate (5 mM) were introduced for the determination of PHP, and 1ml of DNPH (5mM) and 1 ml of sodium periodate (5 mM) for the determination of AMOX. An increasing volume of phenolic drugs working solutions (100 μgml^{-1}) were transferred to cover the range of the calibration graphs (Table (1)).4ml of sodium hydroxide (0.5 M) was added for AMOX drug and 3 ml of sodium hydroxide was used for PHP drug . 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 PHP and AMOX respectively versus the reagent blank prepared in the same way but containing no phenolic drugs. A calibration graphs were drawn and the regression equations were calculated. For the optimization of conditions and in all subsequent experiments were carried out on 10 $\mu g\ mL^{-1}$ of PHP and AMOX.

2.4.2. General FIA procedure

Working solutions of PHP and AMOX 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 0.5 mM DNPH and 0.5 mM sodium periodate solution and was then combined with a stream of 0.5 M sodium hydroxide with a flow rate of 0.8ml min^{-1} in each channel (Fig.(2)). The resulting absorbance of the colored dye was measured at λ_{max} and a calibration graph was prepared over the range cited in Table (1). Optimization of conditions

was carried out on 100 µgml^{-1} of PHP or AMOX respectively.

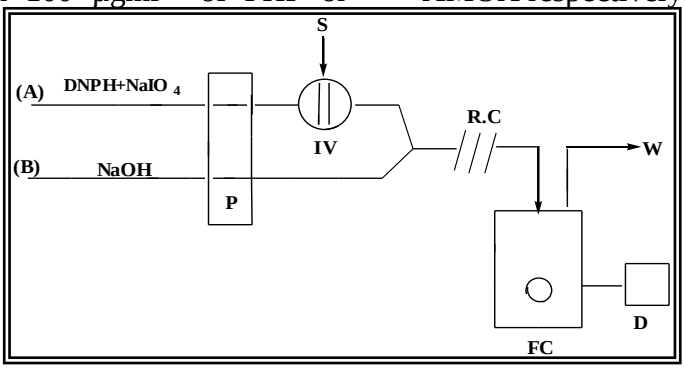


Fig.(2) Manifold employed for FIA-Spectrophotometric determination of PHP and AMOX with DNPH and sodium periodate solution in alkaline medium where: IV, Injection valve ; R.C,. Reaction Coil ; S , Sample ; P, Peristaltic pump ; FC , Flow cell ; D, Detector ; W, Waste.

2.4.3. Procedure for AMOX in capsules and vials

An accuracy weighed amount of 10 powdered capsules(500mg AMOX per each capsules) or mixed content of 10 vials (500 mg AMOX per vial) equivalent to 100 mg of the pure drug was transferred into a 100 ml calibrated flask (to prepare1000 µg.ml^{-1}) and completed to the mark with distilled water. The flask with its contents was shaked well and filtered and aliquot of 250 µg of stock solutions in final volume of 25 ml were used for analysis. More dilute solutions of pharmaceutical preparations for batch and FIA procedures were made up by simple dilution with distilled water, and the measurement was carried out as described earlier under general procedure.

*2.4.4. Procedure for***PHP in***nasal drops*

The contents of three bottles of nasal drops (0.25% of PHP) were mixed. An aliquot corresponding to 50 mg of PHP (10mL) was diluted to 50 ml with distilled water in a volumetric flask to obtain 500µgml^{-1} of PHP. Further appropriate solutions of pharmaceutical preparations for batch and FIA procedures were made by simple dilution with distilled water.

3. Results and Discussion

The factors affecting on the sensitivity and stability of the colored product resulting from the oxidative coupling reaction of PHP and AMOX 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.

3.1. Batch spectrophotometric determination

The best experimental conditions for the determination of PHP and AMOX 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 8 ml) by adding various volumes of their solutions to a fixed concentration of PHP and AMOX (250 µgml^{-1}in a final volume 25 ml)) and measuring the absorbance at maximum wave length. The obtained results show that 1.5 ml of 5 Mm DNPH, 1.5 ml of 5 mM sodium periodate and 3 ml of 0.5 mM of sodium hydroxide are the volumes that can give a higher absorption intensity and stability of the dye product at 525 nm for 10 µgml^{-1} PHP, and 1ml of 5 Mm DNPH, 1ml of 5 mM sodium periodate and 4 ml of 0.5 mM of sodium hydroxide are the volumes that can give a higher absorption intensity and stability of the dye product at 515 nm for 10 µgml^{-1} of AMOX. 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 Jops̀ method of continuous variation [40], a molar ratio of 1:1 drugs to DNPH was obtained by the applied method as shown in Fig.(3). The stability constants of the dye products were calculated [41] by comparing the absorbance of a solution containing stoichiometric amount of PHP or AMOX 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 1.843×10^5 and 3.75×10^5 l mol^{-1} for each of PHP and AMOX respectively.

In order to assess the possible analytical applications of the proposed methods. The effect of some common excipients frequently found with AMOX drug in pharmaceutical formulations, such as sucrose, glucose, fructose, lactose, starch, talc and magnesium stearate was studied by analyzing synthetic sample solutions containing 10 $\mu g ml^{-1}$ of AMOX and excess amounts (10-fold excess) of each excipient, none of these substances interfered seriously.

The regression equations obtained, from a series of PHP or AMOX 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 PHP and AMOX determination in order to make an effective comparison between the two approaches. Also the accuracy and precision of the proposed method were studied (Table (2)).

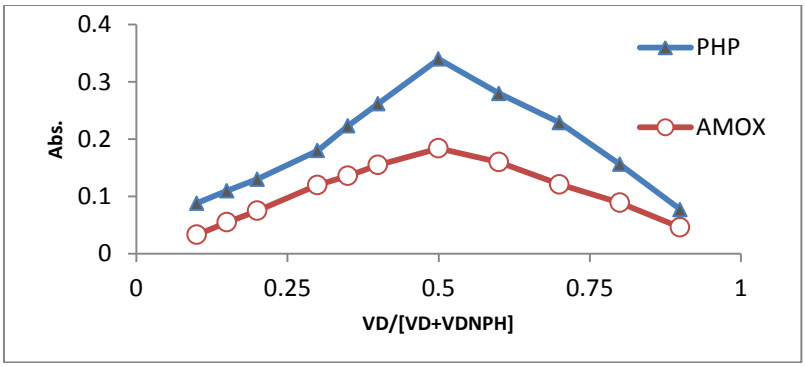


Fig.(3) Study of the mole ratio of the reaction between PHP, AMOX and DNPH.

Table (1)

Analytical characteristics of the procedures developed for the determination of PHP and AMOX.

Parameter	*Batch procedure*		*FIA procedure*	
	PHP	*AMOX*	*PHP*	*AMOX*
Regression equation	y=0.0278x+0.1168	y=0.0137x+0.0669	y=0.0007x+0.1444	y=0.0008x+0.160
Linear range (µg mL^{-1})	2-50	1-40	30-1000	50-1200
Correlation coefficient	0.9968	0.9988	0.9975	0.9968
Limit of detection (s/n=3) $\mu g mL^{-1}$	1.044	0.230	**14.21**	**34.00**
Reproducibility %	**<1.12**	<1.21	**<0.99**	**<0.90**
Average of recovery,%	**99.89**	**99.50**	**99.72**	**100.1**
Sandells̀ Sensitivity ($\mu g.cm^{2-}$)	0.0352	0.0666	0.143	0.125
Through-put (hr^{-1})	**4**	**4**	28	28

3.2. FIA spectrophotometric determination

The batch method for the determination of PHP and AMOX were adopted as a basis to develop FIA procedure. The manifolds used for the determination of each of PHP and AMOX were so designed to provide different reaction conditions for magnifying the absorbance signal generated by the reaction of PHP and AMOX 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 (Fig.(2)). The influence of different chemicaland physical FIA parameters on the absorbance intensity of the colored product was optimized as follows.

3.2.1. 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 0.5 mM was found to be the most suitable concentration for obtaining maximum absorbance (Fig. (4-a, b)), and was chosen for further use. Sodium hydroxide was found necessary for developing the colored product and increase its stability. The effect of sodium hydroxide was studied in the concentration range of 0.1–1M and a greatest absorbance intensity was obtained with 0.5 M of sodium hydroxide for determination of PHP and AMOX respectively (Fig.(5)).

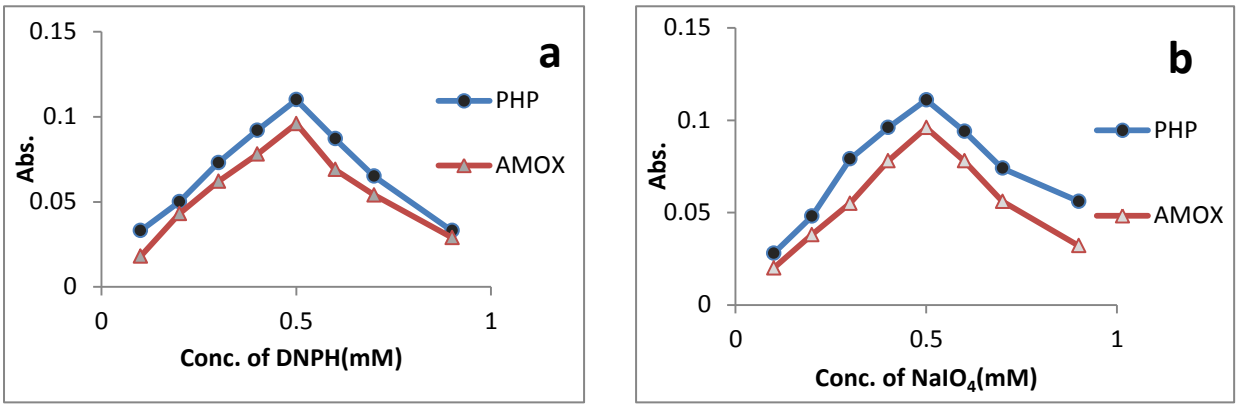


Fig.(4) a-effect of conc. of DNPH, b-effect of conc. of $NaIO_4$ on the colored reaction product .

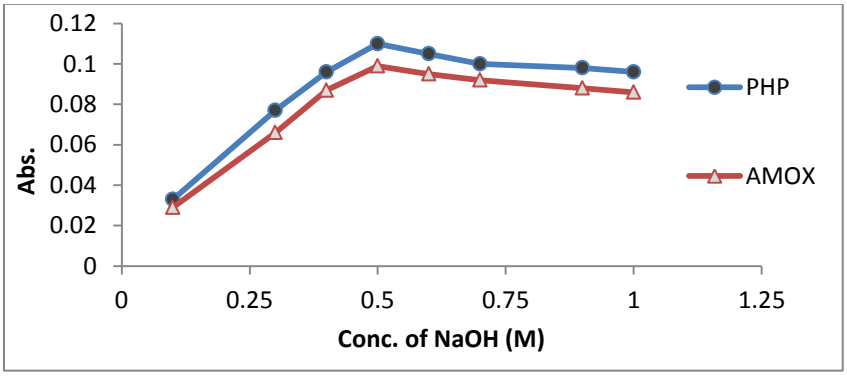


Fig.(5) Effect of the concentration of sodium hydroxide in M.

3.2.2. 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 0.8 $ml.min^{-1}$ (0.4 ml min^{-1} in each line) gave the highest absorbance as shown in Fig.(6) and was used in all subsequent

experiments. The volume of the injected sample was varied between 50 and 300 µl using different length of sample loop. The results obtained showed that injected sample of 150 µl gave the best absorbance (Fig.(7)). 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 Fig.(8) and was used in all subsequent experiments.

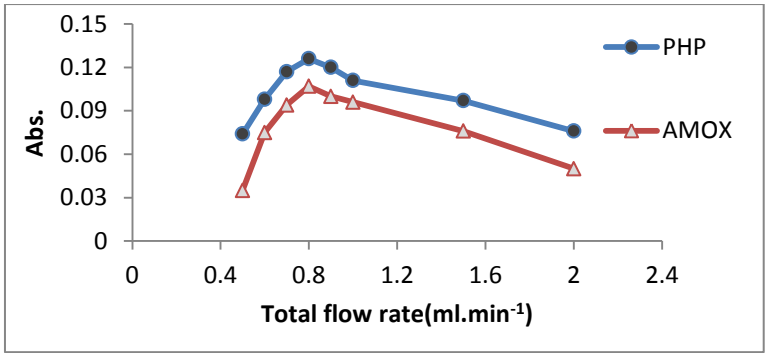


Fig.(6) Effect of the total flow rate (ml min^{-1}).

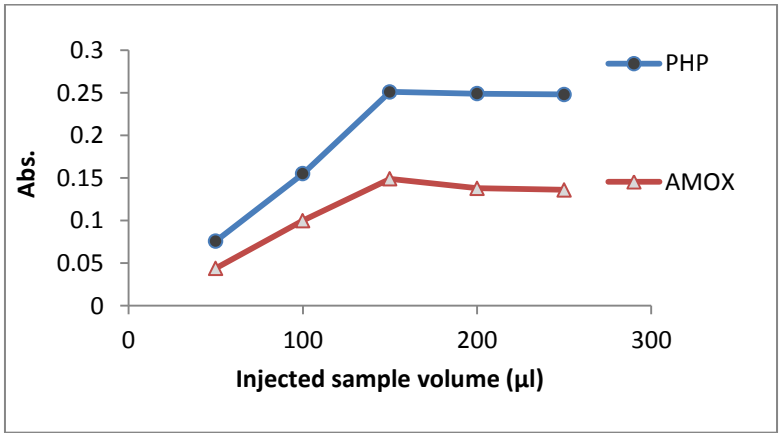


Fig.(7) Effect of the injection volume (µL).

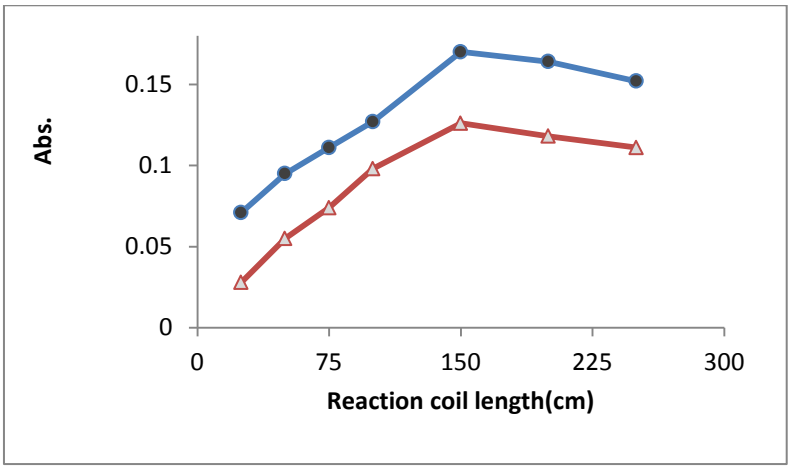


Fig.(8) Effect of the length of the reaction coil(cm).

A standard calibration line, obtained for a series of PHP and AMOX standards and the main analytical figures of merits of the developed procedure are indicated in

Table (1). The accuracy and the precision of the proposed method were studied (Table (2)).

3.3. Analytical application

The proposed methods were applied successfully to the analysis of some pharmaceutical preparations containing PHP and AMOX. The results summarized in Table (3) are in accordance with those obtained by the official standard methods [2].

Finally, statistical analysis [42], *F*- and *T*-test, reveals that there is no significant difference in precision and accuracy between the proposed methods and the official methods.

Table (2)
The accuracy and precision of the proposed method (Batch and FI methods).

Phenolic drug	Batch method					Flow injection method				
	Conc. μgml^{-1}		E%	Rec.%	RSD%	Conc. μgml^{-1}		E%	Rec.%	RSD%
	Present	Found				Present	found			
PHP	8	7.92	-1.0	99.0	1.12	40	39.8	-0.30	99.70	0.96
	16	15.88	-0.11	99.89	0.98	100	99.8	-0.11	99.89	0.98
	32	32.09	+0.29	100.29	0.87	400	400.1	+0.02	100.02	0.68
AMOX	8	7.89	-1.35	98.65	1.211	60	59.8	-0.18	99.82	0.90
	16	16.07	+0.48	100.48	0.967	120	120.1	+0.48	100.48	0.83
	24	24.09	+0.41	100.41	0.715	600	600.1	+0.41	100.41	0.54

Table (3)
Application of the proposed and official methods to the determination of some PHP and AMOX drugs in dosage forms.

Drug form	Proposed methods				Official Method Rec.%
	Batch method		FIA method		
	Rec.%*	RSD%*	Rec.%*	RSD %*	
Nasophrine(PHP) Nasal Drops(SDI)	99.51	1.42	99.85	0.66	100.27
Amoxicillin trihydrate – 500mg Capsules(SDI)	99.46	1.25	99.73	0.71	99.80
Amoxicillin trihydrate 500mg Capsules(Global,UAE)	98.99	1.09	100.11	0.58	99.30
Amoxicillin- 500mg Capsules (Ajanta,India)	100.48	0.94	99.61	0.56	100.4
Amitron 500mg Injection,Spain	100.28	1.10	99.9	0.43	101.15

****For five determinations.***

Conclusions

The developed methodology is very adequate for the determinationof PHP and AMOX in aqueous solution and in pharmaceutical preparation samples at a concentration level of traces ($\mu g.ml^{-1}$) and without requiring a temperature or a pH control. Moreover, the proposed procedures are very economical when compared to other methods such as those based on the use of LC. In comparison of the batch with FIA procedure, the latter 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)). The precision of the methods was evaluated by analyzing pure samples of PHP and AMOX and a good recovery was obtained (Table (1)).

The proposed method is thus simple, rapid, precise and inexpensive, and hence can be used in routine analysis of PHP and AMOX in pharmaceutical preparation.

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الخلاصة

يتضمن البحث تطوير استخدام تقنية المطياف الضوئي بطريقة الدفعة والحقن الجرياني للتقدير الكمي لكل منالفنيل فرين هيدروكلوريدوالاموكسيسيلينتراي هبدريت في المستحضرات الصيدلانية .اعتمدت الطرق على تفاعل الازدواج بين الفنيل فرين هيدروكلوريداوالاموكسيسيلين تراي هبدريت مع كاشف ٢،٤–ثنائي نايتروفنيل هيدرازين في وسط قاعدي حيث تتكون نواتج ملونةوذائبة بالماء اعطت اعلى قمة امتصاص عند طول موجي ٥٢٥ و٥١٥ نانوميتر لكل من الفنيل فرين هيدروكلوريدوالاموكسيسيلين على التوالي .تشير منحنيات الامتصاص مقابل التركيز بان قانون بير ينطبق ضمن مدى التركيز ٢–٥٠ و ١–٤٠ مايكروغرام.مل$^{-١}$ من الفنيل هيدرازين هيدروكلوريدوالاموكسيسيلين لطريقة الدفعة ،اما بطريقة الحقن الجرياني فكان مدى التركيز من ٣٠– ١٠٠٠ و ٥٠– ١٢٠٠ مايكروغرام.مل$^{-١}$ من الفنيل هيدرازين هيدروكلوريدوالاموكسيسيلينعلى التوالي ،وبحدود كشف ١,٠٤٤ و ٠,٢٣٠. مايكروغرام.مل$^{-١}$ منالفنيل فرين هيدروكلوريدوالاموكسيسيلين لطريقة الدفعةولطريقة الحقن الجرياني كانت ١٤,٢١ و ٣٤ مايكروغرام.مل$^{-١}$ منالفنيل فرين هيدروكلوريدوالاموكسيسيلين على التوالي .تمت دراسة الظروف المثلى للتفاعلوجميع المتغيرات الكيميائية والفيزيائية بدقة وطبقت الطريقتين بنجاح على المستحضرات الصيدلانية الحاوية على الفنيل فرين هيدروكلوريدوالاموكسيسيلين.وقورنت النتائج التي تم الحصول عليها مع نتائج طرق التحليل القياسية للادوية اعلاه واظهرت نتائج المقارنة عدم وجود فرق جوهري بين نتائج الطرق المقترحة ونتائج الطرق القياسية.