FI-Spectrophotometric Determination of Adrenaline in Pharmaceutical Formulations Via Oxidative Coupling Reaction with Tyramine and Potassium metaperiodate

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Abstract

A batch and flow injection (FI) Spectrophotometric methods have been developed for the determination of adrenaline in aqueous solution and in pharmaceutical ampoules preparation .The methods are based on the reaction of adrenaline with tyramine in presence of potassium metaperiodate as oxidizing agent in neutral solution .The water soluble orange colour produced was measured at 475 nm .Linearty was observed from 0.6 – 25 and from 1 – 70 $\mu g.ml^{-1}$ adrenaline with quantification limits of 0.23 and 0.47 $\mu g.ml^{-1}$ adrenaline by batch and FI procedure respectively . The effect of chemical and physical parameters have been carfully considered and the proposed methods were successfully applied to the determination of adrenaline in ampoules formulation .

الخلاصة

تم تطوير طريقتين تقليدية وحقن جرياني طيفية لتقدير الادرينالين في المحاليل المائية و الحقـن الدوائية . تعتمد الطريقة على تفاعل الادرينالين مـع الكاشـف العضـوي التـايرمين و ميتـابيريودات البوتاسيوم كعامل مؤكسد في الوسط المتعادل . تم قياس الامتصاص للناتج البرتقالي اللون الـذائب فـي الماء عند الطول الموجي 475 نانوميتر . كانت حدود الخطية بين 0.6 – 25 و 1 – 70 مـايكروغرام . مل$^{-1}$ وحد كشف مقداره 0.23 و 0.47 مايكروغرام . مل$^{-1}$ من الادرينالين لكل من الطريقة التقليديـة وطريقة الحقن الجرياني على التوالي . كذلك تم دراسة العوامل الفيزيائية و الكيميائية التي تـؤثر علـى الطريقتين وتم تطبيق الطريقتين بنجاح في تقدير الادرينالين في مستحضراته الصيدلانية .

Introduction

Adrenaline [1–(3,4– dihydroxy phenyl) – 2 – methyl amino ethanol] is one of the catecol amine drugs that discovered in 1960 and was used as anti Glucoma and agonist [1]. Various methods have been reported for the determination of adrenaline. These include titrimetric[2,3], potentiometric[4], chromatographic[5], spectrophotometric [6-9] and flow injection analysis [10,11] .

Oxidative coupling organic reactions seems to be one of the most popular spectrophotometric methods for the determination of several drugs such as sulphonamide [12] , phenylephrine HCL [13] , methyldopa [14,15] , folic acid [16] and paracetamol [17].

In the present paper ,an automated method is proposed for the spectrophotometric determination of adrenaline by reaction with tyramine in

the presence of potassium metaperiodate in neutral medium. The reaction can be carried out in batch and in FIA and in this paper the two approaches are compared. The reaction products have been spectrophotometrically measured at 475 nm .

Experimental part

Materials used :

The adrenaline pure drug was obtained from Rhon opulence company / france . Where as the injection (1mg / 1ml) were from life pharma – Italy .

Adrenaline stock solution (1000 $\mu g.ml^{-1}$) :

0.1000 gm of adrenaline was dissolved in 10 ml of ethanol and completed the volume to 100 ml with distilled water in a volumetric flask of 100 ml .

Tyramine reagent (0.1 M) :

Tyramine reagent was prepared by dissolving 1.2108 gm in 100 ml of distilled water .

Potassium metaperiodate(0.1M) :

Potassium metaperiodate solution was prepared by dissolving 2.3000 gm of KIO_4 in 100 ml of distilled water . More dilute solutions were prepared by suitable dilutions.

Apparatus used :

All spectral and absorbance measurments were carried out on a shimadzu uv–visible 260 digital double beam recording spectrophotometer using 1 cm silica cell .

In FIA, a flow cell with 50 µl internal volume and 1 cm path length was used for the absorbance measurments. A two – channel manifold (Fig.1) was employed for the FIA spectrophotometric determination of adrenaline drug . A peristaltic pump (Ismats Labortechnik – Analytik , CH – 8152 , Glatbrugg – Zurrich – Switzerland) was used to transport the carrier stream .

(Rheodyne – USA) injection valve was employed to provide appropriate injection volumes of standard solutions and sample of drug . Flexible vinyl chloride tubing of 0.5 mm internal diameter was used for the peristaltic pump . Reaction coil (Rc) was of Teflon with internal diameter of 0.5 mm .

Channel 1 was used to transport potassium metapereiodate solution and channel 2 to transport tyramine solution . The sample was injected into the resulting stream of the mixture of tyramine with potassium metaperiodate solution , through the injection valve . Solution were propelled by peristaltic pump with individual flow rate of 2.25 ml . min , the absorbance measured at 475 nm .

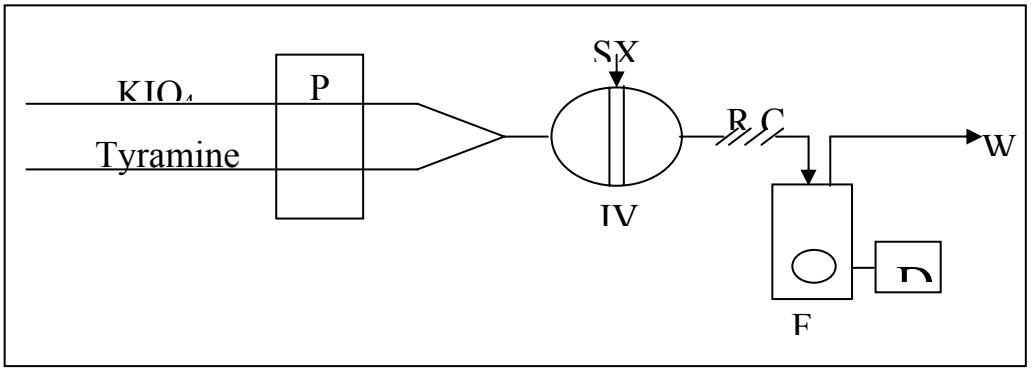


Fig (1): Manifold employed for FI-Spectrophotometric determination of adrenaline with tyramine and potassium metaperiodate .where IV. Injection valve, Rc .Reaction coil, SX. Sample, P.Peristaltic pump. D. Detector, W. Waste

Procedure for the batch method:

Into a series of 25 ml calibrated flask , transfer increasing volumes of adrenaline (10 $\mu g.ml^{-1}$) . Add 1.5 ml of $1x10^{-2}$ M of potassium metaperiodate solution , fllowed by 3.5 ml of $5x10^{-3}$ M of tyramine solution . Dilute the solution to the mark with distilled water and allow the reaction mixture to stand for 20 min at room temperature . Measure the absorbance at 475 nm against a reagent blank prepared in the same way but containing no adrenaline . The colour of the formed dye is stable for about 120 min . For the optimization of condations and in all subsequent experiments , a solution of 10 $\mu g.ml^{-1}$ adrenaline was used and the final volume was 25 ml .

Procedure for the FIA method:

Sample containing different concentrations of adrenaline drug were prepared by simple dilution with distilled water of the stock solution (1000 $\mu g.ml^{-1}$). The FIA spectrophotometric measurments were carried out using the manifold shown in Fig . 1 , employing 0.001 M of tyramine and 0.005 M potassium metaperiodate with a flow rate of 1.13 ml .min in each channel . 150 µl of samples and standard solutions were injected and the absorbance of the resulting dye product was measured at 475 nm . Optimizations of conditions were carried out on 20 $\mu g.ml^{-1}$ of adrenaline .

Results and discussion

Batch Spectrophotometric determination:

When a diluted aqueous solution of adrenaline was mixed with tyramine and potassium metaperiodate in neutral medium , an intense orange colour forms immediately and become stable after 20 min . The colour has a maximum absorption at 475 nm . Fig . 2 .shows the spectra of the orange colour formed and of the reagent blank . The best experimental condations for the determination of adrenaline were established for potassium metaperiodate (from 0.0001 – 0.008 M) and tyramine (from 0.0001 – 0.01 M) by altering one variable at a time and studing the absorbance at 475 nm as a function of time . The obtained results show that 0.0006 M of potassium metaperiodate and 0.0007 M of tyramine are the concentrations that can give a higher absorption intensity at 475 nm for 250 of adrenaline in a final volume of 25 ml .

The development of the colour of adrenaline from a mixture containing 10 $\mu g.ml^{-1}$ in 0.0006 M potassium metaperiodate and 0.0007 M tyramine gave evidence that the colour develops during the first 20 min . and remains stable for more than 120 min .

The effect of temperature on the colour intensity of the dye was studied . In pratice , high absorbance was obtained when the colour was developed at room temperature (25 C) than when the calibrated flask were plased in an ice – bath at (0 C) or in a water bath at (50 C) .

The stoichiometry of the reaction was investigated using molar ratio method [18] . The result obtained (Fig . 3) show a 1:1 drug to tyramine product was formed at 475 nm .

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

Table 1: Analytical features of the procedures developed for the determination of adrenaline

parameter	Batch method	FIA method
Regression equation	Y=0.0163X-0.001	Y=0.0054X+0.0126
Linear range ($\mu g.ml^{-1}$)	0.6 – 25	1 – 70
Correlation coefficient	0.9999	0.9999
Limit of detection (s/n=3) ($\mu g.ml^{-1}$)	0.23	0.47
RSD% for 10 $\mu g.ml^{-1}$	1.20	0.78
Recovary % for 10 $\mu g.ml^{-1}$	99.65	100.94
Molar absorptivity($L.mol^{-1}.cm^{-1}$)	$2.99x10^3$	$0.98x10^3$
λ_{max} (nm)	475	475
Through – put (hr^{-1})		120

FI Spectrophotometric determination:

The batch method for determination of adrenaline was adopted as a basis to develop F1 procedure , using the manifold indicated in Fig.1.The absorbance intensity of the coloured product at 475 nm has been improved by studying the effect of the different FI parameters on the reaction between adrenaline and tyramine in the presence of potassium metaperiodate such as tyramine concentration (from 0.0001-0.01 M) , potassium metaperiodate (from 0.0001-0.05 M), flow rates (from 0.15-2.5 ml/min.in each channel) , length of the reaction coil (from 25 - 250 cm) and injection volume (from 50-250 µL) . The results obtained showed that a concentration of 0.001 M and 0.005 M were optimum for tyramine and potassium metaperiodate respectively . A flow rate of 1.13 ml/min. in each channel , a reaction coil of length of 70cm and an injection volume of 150 µL were the best conditions which provided the highest absorbance at 475 nm with the lowest blank value .

A standard calibration line , obtained for a series of adrenaline standards and the main analytical figures of merit of the developed procedure are indicated in Table.

The increase in the temperature of the reaction coil does not increase the absorbance at 475nm and caused a degradation of the coloured product and low sensitivity and stability of the reaction products.

Analytical application:

The developed methodology is very adequate for the determination of adrenaline in aqueous solution and in pharmaceutical preparation samples at a concentration level of traces (p.p.m.) and without requiring any previous separation step nor a temperature or pH control . Moreover the proposed procedures are very economical when compared to other methods such as those based on the use of HPLC. Sample preparation was done by diluting the ampoules with deionized water .

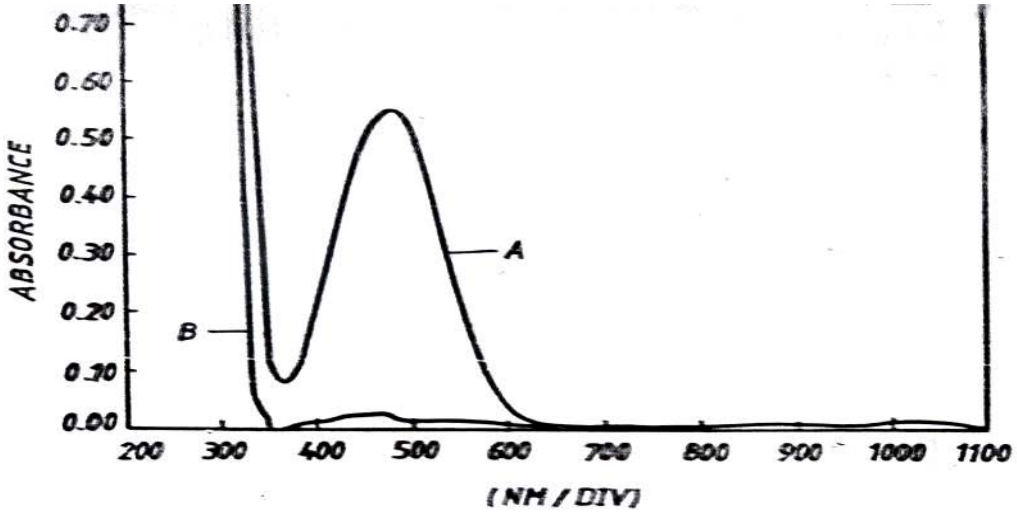
In comparison of the batch with FI procedure , the later is more convenient than the former method because of its speed (sample through-put of 120 injection/hr.) and wider linear range of the calibration graph (Table1) .

The precision of the method was evaluated by analyzing pure sample of adrenaline and a good recovery was obtained (Table 2). Finally the proposed method was applied successfully to the analysis of some ampoules containing adrenaline. The results in Table 2 are in accordance with those obtained by the standard method[19] .

Table . 2 Application of the proposed methods to the determination of Adrenaline in ampoules .

Drug sample	Amount of drugs taken µg.ml^{-1}	Batch method		FI method		Standard method
		Recovery* %	RSD* %	Recovery* %	RSD* %	Recovery* %
Pure adrenaline	10 µg.ml^{-1}	98.84	1.09	100.70	0.71	100
Ampoules Adrenaline	10 µg.ml^{-1}	101.34	1.16	99.25	0.75	
Ampoules Adrenaline	25 µg.ml^{-1}	98.74	1.24	99.20	0.84	

* Average of five determination



Fig(2) :Absorption spectra of A (25 µg. ml^{-1}) of Adrenaline treated as described under procedure and measured against reagent blank and B the reagent blank measured against distilled water.

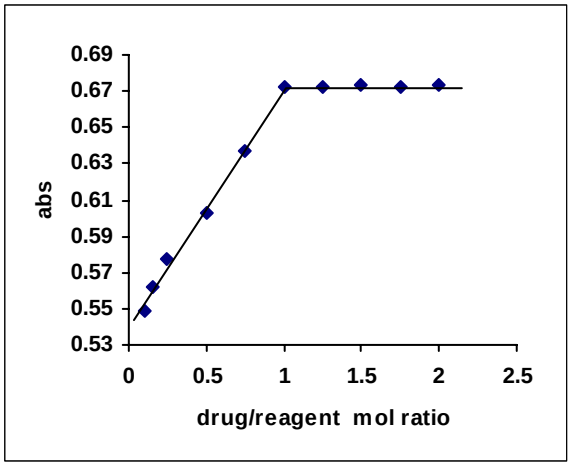


Fig (3): Molar ratio of adrenaline to reagent for the coloured product

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