Flow injection- Spectrophotometeric Determination of some Catecholamine Drugs in Pharmaceutical Preparations via Oxidative Coupling Reaction with p-Toluidine and Sodium periodate

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Abstract

A new spectrophotometric flow injection method has been established for the determination of some catecholamine drugs [methyl dopa I, dopamine.HCl II and adrenaline III]. The method is based on the oxidative coupling reaction of catecholamine with p-toluidine and sodium periodate to form an orange water-soluble dye product that has a maximum absorption at 480 nm. Linearity was observed in the range of 1-50, 2-50 and 5-70, with a limit of detection (signal/noise=3) of 0.4, 0.2 and 0.7 μg ml^{-1} for I, II, and III respectively. The method was applied successfully to the determination of I, II and III in pharmaceutical preparations with a good precision and accuracy.

INTRODUCTION

In recent years more and more strict regulations related to the quality control for pharmaceuticals led to increasing demands on the automation of analytical assays carried out in appropriate control laboratories. At the same time, during twenty-five years of the existence, the flow injection analysis (FIA) techniques (1) become a versatile instrumental tool that contributed substantially to the development of the automation in pharmaceutical analysis."This was documented by a number of reviews on the use of FIA in the analysis of drugs (2-4)"

Oxidative coupling organic reactions seem to be one of the most suitable FIA spectrophotometric determination of drugs such as sulphonamids (5, 6) paracetamol (7), methyldopa (8), folic acid (9) and phenylephrine. HCl (10). Catecholamines have been determined by visible spectrophotometry after reaction with metaperiodate (11), chloranil and fluoranil (12), Fe (III) and o-phenanthroline (13), palladium chloride (14), ammonium metavanadate (15) and isoniazid in the presence of N-bromosuccinimide (16).

The purpose of the present investigation is to develop a simple and

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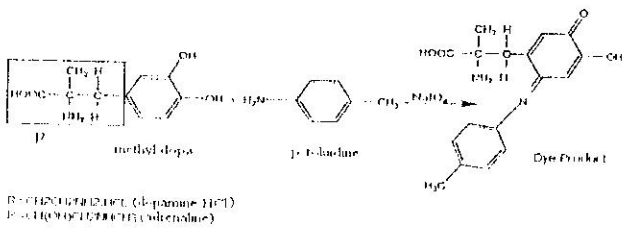
sensitive method for the determination of some catecholamine drugs (methyl dopa, dopamine.HCl and adrenaline) in pharmaceutical preparations using oxidative coupling reaction and FIA-spectrophotometer. The proposed methods is based on the reaction of the catecholamine drugs with p-toluidine in the presence of sodium periodate and in neutral medium to form an intense orange colored product which shows an absorption maximum at 480 nm. No previous published reports on the reaction mechanism have been appeared and the following reaction scheme may be proposed for catecholamine--p-toluidine in the presence of sodium periodate:



Scheme (1)

Experimental

Apparatus:

All spectral and absorbance measurements were carried out on a Shimadzu uv-visible 260 digital double beam recording spectrophotometer A flow cell with 50 µl internal volume and 1cm path length was used for the absorbance measurements. A two-channel manifold (Fig.1) was employed for the FIA spectrophotometric determination of catecholamine drugs. A peristaltic pump (Ismatec, Labortechnik – Analytik, CH – 8152, Glatbrugg – Zurrich- Switzerland) was used to transport the carries solutions. (Rheodyne-USA) injection valve was employed to provide appropriate injection volumes of standard solutions and samples. Flexible vinyl 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 A was used to transport -toluidine and channel B to transport sodium periodate solution. The sample was injected into the stream of the mixture of p-toluidine with sodium periodate solution, through the injection valve. Solutions were propelled by peristaltic pump with individual flow rate of 1.5 ml $min.^{-1}$. The absorbance was measured at 480 nm.

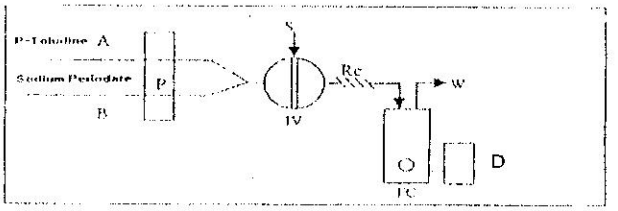


Fig (1).Manifold employed for FIA-Spectrophotometric determination of Catecholamine drugs with P-toluidine and Sodium periodate where:
IV. Injection valve, Re.Reaction Coil, S. Sample, P.Peristaltic pump, FC . Flow cell, D.Detector, W.Waste.

Reagents:

Methyl dopa and aldomate tablets, 250 mg (State Company for Drug Industries and Medical appliance, SDI, Samara-Iraq), dopamine hydrochloride (Fluka), dopamine hydrochloride injections 200 mg/5ml (Biologici Italy lab. Novate-Milano-Italy), adrenaline (Fluka), adrenaline injections 1 mg ml^{-1} (Lifepharm-Italy) and sodium periodate (BDH). All other chemicals used were of analytical-reagent Grade. Distilled water was used to prepare ll olutions xcept -toluidine which was dissolved in a minimum amount of ethanol and the volume was completed with distilled water.

Solutions:

Freshly prepared aqueous solution of the pure drugs (100 µg ml-1) of methyl dopa,dopamine hydrochloride and adrenaline (protected from sun light) were used as the standard solution for analytical purposes. Aqueous solutions of 0.2 w/v% p-toluidine and 0.01M sodium periodate were used. More dilute solutions were prepared

Pharmaceutical preparation Tablets

Ten tablets of methyl dopa were weighed and finally powdered using a mortar. A weighed amount of the powder equivalent to 100 mg of the pure methyl dopa was dissolved in hot water, cooled and made up to 100 ml with distilled water. The resulting solution was filtered off and was treated as described under recommended procedure.

Recommended procedure

Samples containing different concentrations of catecholamine drugs were prepared by simple dilution with distilled water of the stock solution (100 µg ml-1). The FIA spectrophotometric measurements were carried out using the manifold shown in Fig.1, employing 0.008 w/v% of p-toluidine and 0.4 mM sodium periodate with a flow rate of 1.5 ml min-1 in each channel .150 µl of samples and standard solutions were injected and the absorbance of the resulting dye product was measured at 480 nm.

Optimizations of conditions were carried out on 20 ىg ml-1 of methyl dopa.

Results and Discussion

Catecholamaine drugs (methyldopa,dopamine.HCl and adrenaline) react with p-toluidine in the presence of sodium periodate and in neutral media to form an intense orange colour product that can be measured at 480 nm (Fig.2) the absorbance is directly related to the concentration of catecholamine drugs and can be used for their spectrophotometric determination. The development of the colour product depends on the reaction conditions and was optimized as follows:

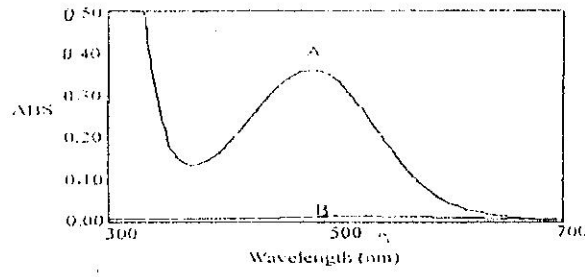


Fig.2 :Absorption spectra of A (20 µg ml^{-1}) of metyl dopa treated as described under procedure and measured against reagent blank and B the reagent blank measured against distilled water.

Manifold Designs

The FI manifold used for the determination of catecholamine drugs was so designed to provide different reaction conditions for magnifying the absorbance signal generated by the reaction of catecholamine drug with p-toluidine and sodium periodate. Maximum absorbance intensity was obtained when the sample was injected into a stream of mixed p-toluidine with sodium periodate (Fig 1).

Concentration of p-toluidine

The effects of various concentrations p-toluidine were investigated.A concentration of (0.008 w/v %) gave the highest absorbance and was chosen for further use. The results are shown in Fig (3).

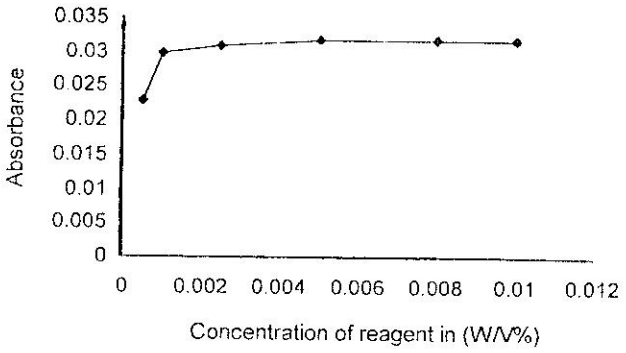


Fig.3 Effect of the concentration of p-toluidine On the coloured reaction product

Concentration of oxidizing agent concentration

It was observed that the reaction between methyl dopa and p-toluidine depends on the oxidation process with sodium periodate. The effects of various concentration of sodium periodate were similarly studied. A concentration of (0. 4 mM) gave the best results and minimum blank value as shown in Fig (4) and was considered as optimum value.

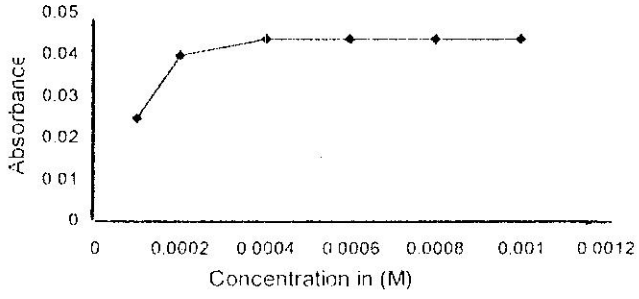


Fig.4 Effect of the concentration of sodium periodate in (M)

Effect of flow rate

The effect of flow rate on the sensitivity of the coloured reaction product was investigated in the range of 1-6 ml/min. The results obtained showed that total flow rate of 3 ml/min (1.5 ml min-1.in each line) gave the highest absorbance as shown in Fig (5) and was used in all subsequent experiments

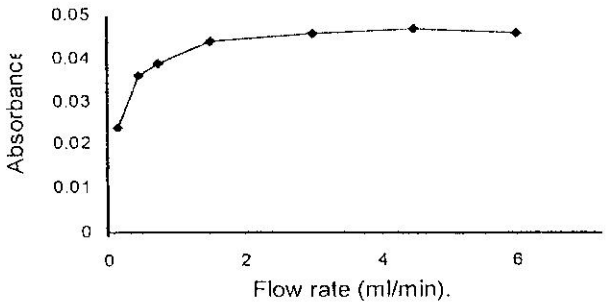


Fig.5 Effect of the total flow rate (ml/min)

Effect of reaction coil length

Coil length is an essential parameter that affected on the sensitivity of the coloured reaction product and was investigated in the range of 25-150 cm The result obtained showed that a coil length of 50 cm gave the highest absorbance as shown in Fig (6) and was used in all subsequent experiments.

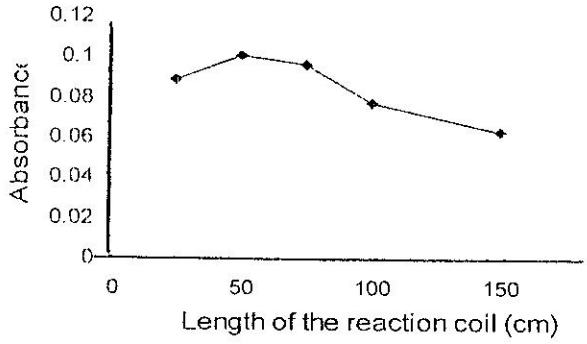


Fig.6 Effect of the length of the reaction coil in (cm)

Effect of injected sample volume

The effect of sample volume was investigated by injection of a volume of different lengths of sample loop. It was found that the absorbance was increased as the injected volume was increased up to 200 µl. The results

obtained showed that injected sample of 150 µl gave the best absorbance (i-e contains 150 µg/150 µl) as shown in Fig (7) and was used in the recommended procedure.

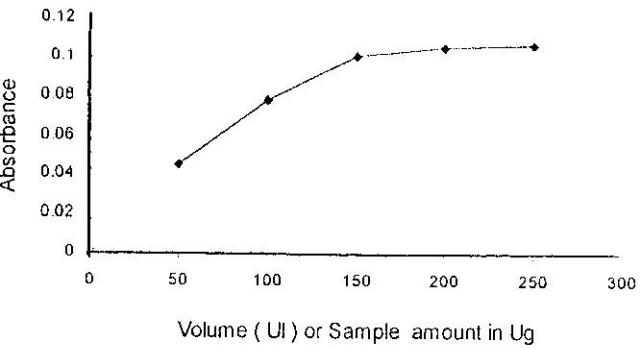


Fig.7 Effect of the ingected sample volume in (µl)

Interference studies

In order to assess the possible analytical applications of the proposed FIA method.The effect of some common excipients frequently found with catecholamine drugs in pharmaceutical formulations, such as sucrose, glucose, fructose, lactose, starch, sodium chloride, talc and magnesium stearate was studied by analyzing synthetic sample solutions containing 20 µg ml-1 of methyl dopa and excess amounts (10-fold excess) of each excipient, none of these substances interfered seriously.

Nature and stability constant of the dye product (17)

The stoichiometry of the reaction was investigated using molar ratio method (17) and FIA technique under the optimized conditions. The results obtained (Fig .8) shows a 1:1 drug to reagent product was formed. The formation of the dye may probably occur as given in scheme 1.

The stability constants of the dye product using FIA [obtained by following the equation cited in (17)] are given in Table (1) , which indicate a stable dye products are formed through the reaction of catecholamine drugs with p-toluidine and in the presence of Sodium periodate.

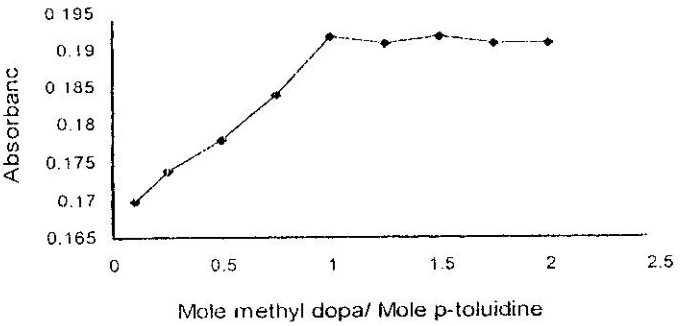


Fig.8 Study of the mole ratio of the reaction between methyl dopa and p-toluidine

Determination of the studied Catecholamine drugs

Under the described experimental condition, a series of standard solutions cover the concentration ranges cited in Table 1 was pumped, each as three replicates, to test the linearity of the calibration graph. A plot of the absorbance versus concentration of the studied catecholamine drugs were linear over the ranges given in Table 1. Linear regression analysis of the results, the correlation coefficient of the linear ranges and limit of detection are shown in Table 1.

The precision of the method was evaluated by analysing pure sample of Catecholamine drugs (Table 1). Finally the proposed method was fast with a sample through-put of 180 injection/ hr.

Table.1 Spectral characteristics and analatical data of some catecolamine drugs.

Parameters	Methyl dopa	Dopamine.HCl	Adrenaline
Colour of the reaction Product	Orange	Orange	Orange
λ max (nm)	480	480	480
Beer's low (µg/ml)	2-50	1-50	5-70
Limit of detection (s/n=3) In (µg/ml)	0.40	0.20	0.70
Regression equation	y= 0.0031x +0.0263	y= 0.003x +0.02	y= 0.003x -0.0044
Correlation coefficient	0.9898	0.9888	0.9988
Relative standard deviation RSD% for 20µg/ml	0.37	0.20	0.10
Recovery,% for 20 µg/ml	99.75	98.5	100.5
Mole ratio of the product (Drug/o-Toludine)	1:1	1:1	1:1
Stability constant of the Product (L/mole)	$1.50*10^6$	$1.8*10^6$	$8.06*10^6$
Through put (hr^{-1})	180	180	180

The proposed method was applied successfully to the analysis of some dosage forms containing Catecholamine drugs. The results in Table 2 are in accordance with those obtained by the official spectrophotometric method (18)

Statistical analysis (19) F-and T-test reveals that there is no significant difference in precision and accuracy between the proposed and the official spectrophotometric methods. Finally, in comparison with other possible spectrophotometric methods (20-22), the proposed procedure s imple, selective and does not require temperature or pH control.

Table 2. Application of the proposed and official methods to the determination of some Catecholamine drugs in its dosage forms.

Drug form	Proposed method Recovery,%*	RSD,%*	Official Method Recovery,%
Methyl dopa (Tablet)			
Aldomate (250 mg)	101.20	0.31	98.30
Aldomate (250 mg)	98.69	0.21	98.02
Dopamine HCl (Injection 200 mg/5ml)	100.80	0.43	101.70
Adrenaline	99.70	0.54	99.00

* For five determinations of 20 µg ml^{-1}.

References

1. Ruzicka, J.and Hansen E.H., 1988. Flow Injection Analysis, Wiley , New York.
2. Karlicek, R., Solich p., Polasek M., 1994. J.Flow Injection Anal., 11. 45.
3. Calatayud, J.M. and Mateo J.V.G., pharm. Technol Internat., 4 1992 17.
4. Catayud, J.M., 1996. Flow Injection Analysis of pharmaceuticals.Automation in the laboratory , Taylar and Francis , London
5. Al-Abachi, M.Q., Salih E.S., and Salem M.S., 1990. Fresenius J.Anal.Chem.337: 408.
6. Al-Abachi, M.Q., Salih E.S., Al-Ghabsha T.S., 1990. Microchemical Journal 41: 64.
7. Al-Abachi, M.Q., Al-Ward, H.S., 2001. National Journal of chemistry 4: 548.
8. Al-Abachi, M.Q., Farid, Y.Y and Hamza. M.J.2002..National Journal of chemistry 8: 520.
9. Al-Abachi, M.Q.,Al-Abachi, R.S., 2002. National Journal of chemistry 8: 527
10. Al-Abachi, M.Q., Hussan M.J. and Mustafa M.A., 2003. National Journal of chemistry 9: 79.
11. El-Kommos, E.M. Mohamed, F.A. and Khedr, S.A, 1990 . J.Assoc.off.Anal.Chem. 73: 516
12. Al-Ghabsha, T.S., Al-Sabha, T.N. and Saleem, M.S. 1994. J.Techn. Res., 49
13. Issopoulos, P.B., 1990. Fresenius, J. Anal. Chem., 336 124.
14. Zivanovic, L., Vasiljevic, M. 1991. and Kustrin, J.Pharm. Biomed. Anal.9: 1157
15. San.R.T., Bhounsule, G.I and Sawant, S.V., 1987. Indian Drugs, 24: 207
16. Nagaraja, P., Srinivasa Murthy, K.C., Rangappa, K.S. 1998 . and

Made Growda, N.M., Talanta, 46: 39.
17. Hargis, L.G, Analatical chemistry, 1988, Prentice-Hall Inc., New Jersey.
18. British pharmacopoeia, 1993.crown copyright, London Department of Health, Scotish home and Health department,.
19. Sanders, D.H and Murph, A.F., Statistics. 1976. Mc.Graw-Hill, New York,.
20. Berzas. J.J, Lemus, J.M and Buitrago. P. 1995 .Anal.Chim. Acta 300: 293.
21. Idem, Fr. 1995.J.Anal.Chem. 353: 221.
22. Nagaraja, P., Murthy, K.C.S., Rangappa, N.S. and Gowda, N.M., 1998. Ind J.Pharm.Sci. 60: 99.

التقدير الطيفي بالحقن الجرياني لتحليل بعض ادوية الكاتيكول امين في المستحضرات الصيدلانية بوساطة الإقتران التأكسدي مع بارا توليدين وبيريودات الصوديوم

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الخلاصة

يتضمن البحث تطوير طريقة طيفية بإستخدام إسلوب الحقن الجرياني لتقدير بعض عقاقير الكاتيكول امين (المثيل دوبا (I) والدوبامين هيدروكلورايد (II) والأدرينالين (III) في المستحضرات الصيدلانية. تعتمد الطريقة على تفاعل الإزدواج التأكسدي بين عقاقير ادوية الكاتيكول امين وكاشف باراتوليدين وبيريودات الصوديوم , حيث يعطي ناتج برتقالي ذائب في الماء يعطي اعلى امتصاص عند طول موجي 480 نانوميتر. وكان مدى إطاعة قانون بير بين 2-50, 1-50, 5-50 مايكروغرام/مل وبحدود كشف (s/n=3) مقداره 0.7,0.2,0.4 مايكروغرام/مل لكل من (I) و(II) و(III) على التوالي. تم تطبيق الطريقة بنجاح في تعيين (I) و(II) و(III) في المستحضرات الصيدلانية بدقة ومظبوطية جيدتين.