Spectrophotometric (flow injection and batch) methods for the determination of some catecholamine drugs in pharmaceutical preparations

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Abstract

A batch and flow injection (FIA) Spectrophotometric methods have been investigated for the determination of some catecholamine drugs in aqueous solution and in pharmaceutical preparations. The methods are based on the reaction of catecholamine drugs (methyl dopa (I) , adrenaline (II) and dopamine (III)) with N,N-dimethyl aniline in the presence of potassium metaperiodate in neutral media to form an orange water soluble stable dye that has a maximum absorption at λ_{max} 478,493 and 482nm for (I), (II) and (III) respectively . linearity was in the range of 1-40,0.5-30 and 0.5-20 $\mu g.ml^{-1}$ with a limit of detection (signal /noise=3) of 0.39 ,0.34 and 0.36 $\mu g.ml^{-1}$ for (I), (II) and (III) respectively by batch method and 5-60,5-100and 5-100 $\mu g.ml^{-1}$ with a limit of detection of 0.90,0.88 and 1.71 $\mu g.ml^{-1}$ for (I), (II) and (III) respectively by FIA method .The effect of chemical and physical parameters have been carefully considered and the proposed procedures were successfully applied to the determination of some catecholamine drugs in pharmaceutical formulation .

الخلاصة

يتضمن البحث تطوير طريقة طيفية بإستخدام اسلوب الحقن الجرياني بتقدير ادوية الكـاثيكول أمـين فـي المستحضرات الصيدلانية. تعتمد الطريقة على تفاعل تكوين معقد انتقال الشحنة بين هذه الادوية والكاشف العضـوي N,N – ثنائي مثيل انيلين بوجود بوتاسيوم ميثابيرأيوديت حيث يعطي ناتج برتقالي ذائب فـي المـاء ولـه اعلـى امتصاص عند طول موجي 478 و 493 و482 نانو ميز نسبةً الى المثيل دوبا (**I**) والادريتالين (**II**) والدوباثين (**III**) على التوالي. طيف قانون بير في مدى التراكيز 60-5 و 100-5 و 100-5 مايكروغرام . مل$^{-1}$ وكانـت

قيمة حد الكشف (s/n=3) مساوية الى 0.9 و 0.88 و 1.71 مايكرغرام . مل$^{-1}$ نسبةً الـى الـدواء (**I**) و(**II**) و(**III**) على التوالي. تم تطبيق الطريقة بنجاح في تقدير الادوية في المستحضرات الصيدلانية.

Introduction

Methyl dopa (I) , adrenaline (Π) and dopamine (Ш) belong to an important class of clinical drugs , catecholamine. They are widely used in the treatment of bronchialasthma , hypertension , Parkinson's disease , myocardial in farction and cardiac surgery (1).Suppliers formulated these drugs either in tablets or injection .The extensive use of catechol amine drugs require the development of a rapid , selective and accurate method that can be used in routine quality control . Several methods have been proposed for the determination of these drugs in biological specimens, They normally require the use of chromatograph with flourimetric or electrochemical detection (2). Catechol amine drugs in commercial formulations have been determined Spectrophotometrically using metaperiodate (3), Fe (Ш) and o-phenanthroline (4), molybdo phosphoric acid (5), isoniazid in the presence of N-bromosuccinamide (6) and thiourea Ferric nitrate (7) In the present paper , a FIA procedure is proposed for the Spectrophotometric determination of catecholamine drugs by reaction with N,N- dimethyl aniline in the presence of potassium metaperiodate in netural medium. The reaction was performed in both batch and FIA and the two approaches were compared. The coloured reaction products have been Spectrophotometrically measured at 478,493 and 482 nm for methyl dopa (I), adrenaline (Π), and dopamine (Ш) respectively.

Experimental

Apparatus

All spectral and absorbance measurements were carried out n on a shimadzua 260, uv –visible digital double beam recording Spectrophotometer using 1 cm matched silica cells.

In FIA , a cecil flow cell with 50 µl internal volume and 1cm bath length was used for the absorbance measurements . A Two channel manifold Fig. (1) was employed for the FI- Spectrophotometric determination of catecholamine drugs .

The manifold included a four channel peristaltic pump [Ismatec, labortechnik-Analytic CH-8152 Glatbrugg , Zurich , Switzerland]

To transport the carrier stream . (Rhenodyen – USA) injector valve was used to provide appropriate injection volumes of standard solution

and samples. Flexible vinyl chloride tubing of 0.8 mm internal diameter was used for the peristaltic pump .The reaction coil (RC) was made from a teflon with an internal dimeter of 0.5 mm . Channel A Fig. (1) was used to transport N,N-dimethyl aniline while channel B for potassium meta periodate .The drug sample was injected into the resulting stream of the mixture of N,N-dimethyl aniline with potassium metaperiodate solution through the injection valve. Solutions were propelled by the peristaltic pump with an individual flow rates of 2.25 ,1.20,1.50 $ml.min^{-1}$ for (I), (II) and (III) respectively and the absorbance was measured at λ_{max} 478 ,493 and 482 nm for (I), (II) and (III) respectively.

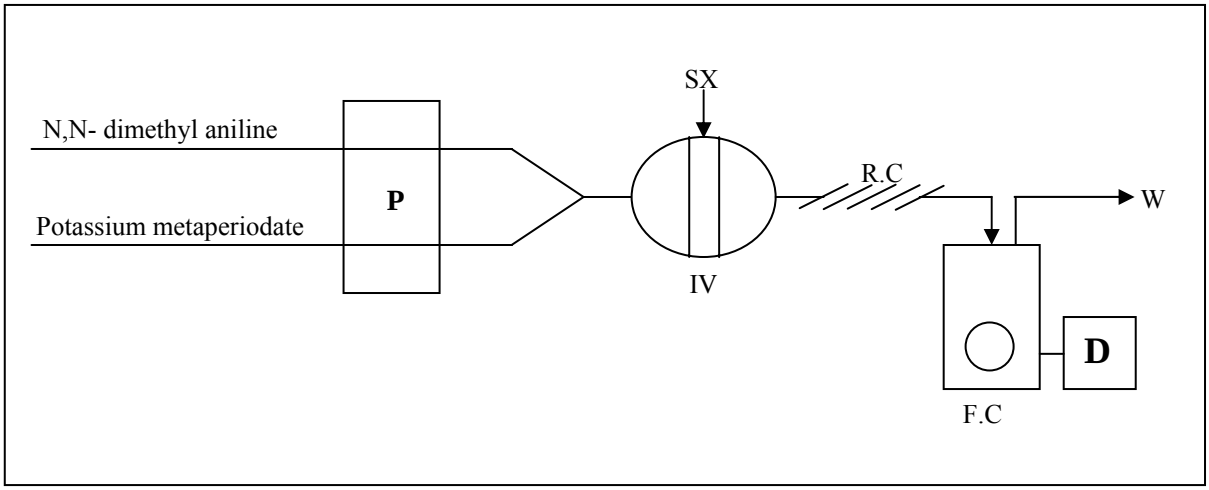

Fig. 1: Manifold employed for FIA spectrophotometric determination of catechol amine drugs with N, N-Dimethyl aniline and potassium meta periodate. Where: FC:Flow cell. IV: injection valve. RC: reaction coil. S: drugs sample (catechol amine drugs). P: peristaltic pump. D. Detector. W: waste

regents

methyl dopa and aldomate tablets 250 mg (State company for Drug Industries and medical appliance , SDI , Samara , Iraq) ., dopamine hydrochloride (Fluka) , dopamine hydrochloride injection 200 mg/5ml (Biological Italy lab ., Novate, milano , Italy) , adrenaline (Fluka), adrenaline injection $mg.ml^{-1}$ (life pharm ,Italy) and potassium metaperiodate (BDH) .All other chemicals used were of analytical reagents grade . Distilled water was used to prepare all solutions .

Solutions

Freshly prepared aqueous solution of the pure drugs (100 $\mu g.ml^{-1}$) of methyl dopa, adrenaline and dopamine hydrochloride (protected from sun light) were used as standard solutions for the analytical purpose . Aqueous solution of 0.01 M potassium

mteaperiodate and 0.01 M N,N-dimethyl aniline, dissolved in a minimum amount (10 ml) of ethanol and diluted to 100 ml with distilled water were used . More diluted solutions were prepared by suitable dilution .

Pharmaceutical preparation

Tablets

Ten tablets of methyl dopa were weighed and finally powdered using a mortar A weighed amount of the powdered 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 .

Ampoules

samples containing different concentration of adrenaline and dopamine . HCl drugs were prepared by simple dilution of the drug with distilled water .

Procedure for the batch Method

Into aseries of 25 ml calibrated flasks , transfer increasing volume of catecholamine drug solutions (40 $\mu g.ml^{-1}$). Add 2.50 ml of $5*10^{-2}$ M potassium metaperiodate solution , followed by 1.25 ml of $1*10^{-2}$ M of N,N-dimethyl aniline solution .Dilute the solution to the mark with distilled water and allow the reaction mixture to stand for 10 min at room temperature. Measure the absorbance at λ_{max} 478, 493 and 482 nm for (I), (П) and (Ш) respectively against a reagent blank prepared in the same way but containing no catechol amine drug. The colour of the resulting dye is stable for about 120 min. For the optimization of conditions and in all subsequent experiments, a solution of 10 $\mu g.ml^{-1}$ catechol amine was used and the final volume was 25 ml.

Procedure for the FIA Method

Samples containing different concentrations of catechol amine drugs were prepared by simple dilution with distilled water of the stock solution (100 $\mu g.ml^{-1}$) . the FIA spectrophotometric measurements were carried out using the mainfold show in Fig. (1) , employing 0.001 , 0.001 and 0.0025 M of N,N-dimethyl aniline and 0.005 ,0.005 and 0.0075 M potassium metaperiodate with a flow rate of 1.12 , 0.6 and 0.75 $ml.min^{-1}$ in each channel for (I), (П) and (Ш) respectively.

One hundred microliter samples and standard solutions were injected and the absorbance of the resulting dye product was measured at λ_{max} 478 , 493 and482 nm for (I), (П) and (Ш) respectively .

Results and Discussion

Batch spectrophotometric determination

Catecholamine drugs (methyl dopa, adrenaline and dopamine.HCl) react with N,N-dimethyl aniline in the presence of potassium metaperiodate and in neutral media to form an orange –red

colour product that can be measured at λ_{max} 478, 493 and 482 nm for (I), (II) and (III) respectively (Fig.2) . The absorbance is directly related to the concentration of the catecholamine drugs and can be used for their spectrophotometric determination. The best experimental conditions for the determination of catechol amine drugs were established for N,N-dimethyl aniline (from $2*10^{-5}$ to $2*10^{-3}$ M) and potassium meta periodate (from $1*10^{-5}$ to $1*10^{-2}$ M) by altering one variable at a time and studying the absorbance at the above wave lengths as a function of time. The results showed that $5*10^{-4}$ M of N,N-dimethyl aniline and $5*10^{-3}$ M of potassium metaperiodate gave the highest absorbance for 10 $\mu g.ml^{-1}$ catecholamine.

The development of the colour of catechol amine drugs from a mixture containing 10 $\mu g.ml^{-1}$ of mythyl dopa in 0.0005 M N,N-dimethyl aniline and 0.005 M potassium metaperiodate gave evidence that the colour develops during the first 10 min . and remains stable for more than 120 min.

The effect of temperature on the colour intensity of the dye was studied . In practice , high absorbance was developed at room temperature ($25C^o$) than when the calibrated flasks were placed in an ice –bath at ($0C^o$) or in a water bath at ($60C^o$).

The stoichiometry of the reaction was investigated using molar ratio method (8). The results obtained (Fig .3) show that a 1:1 drug to reagent product was formed . A charge transfer complex may be presumed to be taking plase involving electron transfer from the highest occupied Л molecular orbital of arylamine to the lowest empty molecular orbital of the oxidized catecholamine drug. The stability of the complex was $3.28*10^{5,}$ $5.62*10^5$ and $3.1*10^5$ $l.mole^{-1}$ for (1), (II) and (III) respectively.

Similar overlapping of Л – Л* orbital were reported in the 1:1 charge – transfer complex formed between chloranil and N,N,N,N tetra methyl – p- phenylene diamine (9) .

In order to assess the possible analytical applications if the proposed methods , the effect of some common excipients frequently found with catecholamine drugs in pharmaceutical preparation such as sucrose, glucose , lactose, starch , talc and magnesium stearate was studied by anlyzing synthetic sample solution containing 10 $\mu g.ml^{-1}$ of catecholamine and excess amounts (10 fold excess) of excipients, none of these substances interfered seriously.

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

Table 1 Analytical features of the procedures developed for the determination of catechol amine drugs .

Parameter	Batch method			Flow injection method		
	Methyl dopa	Adrenaline	Dopamine	Methyl dopa	Adrenaline	Dopamine
Regression equation	Y=0.0165x+0.005	Y=0.0187x+0.0049	Y=0.0181x+0.0026	Y=0.033x+0.0115	Y=0.0034x+0.0037	Y=0.0007x+0.0049
Linear range (µg/ml)	1-40	0.5-30	0.5-20	5-60	5-100	5-100
Correlation coefficient	0.9997	0.9998	0.9997	0.9997	0.9999	0.9997
Limitof detection (s/n=3) µg/ml	0.39	0.34	0.36	0.90	0.88	1.71
RSD% for 20µg/ml	0.80	1.02	1.00	0.50	0.36	0.66
Recovery% 20µg/ml	99.8	101.10	101.2	101.8	100.22	101.7
Sample through-put/hr	15	15	15	120	120	120

I - Spectrophotometric determination

The batch method for the determination of catechol amine was adopted as a basis to develop FIA procedures . The FIA manifold used for the determination of catechol amine drugs was so designed to provide different reaction conditions for magnifying the absorbance signal generated by the reaction of catechol amine drug with N,N-dimethyl aniline and potassium metaperiodate. Maximum absorbance intensity was obtained when the sample was injected into a stream of mixed N,N-dimethyl aniline with potassium metaperiodate (Fig.1) . the absorbance intensity of the colour product has been improved by studying the effect of different FIA parameters on the reaction between catechol amine and N,N-dimethyl aniline in the presence of potassium metaperiodate such as N,N-dimethyl aniline concentration (from $1*10^{-4}$ - $1*10^{-2}$ M) ,potassium metaperiodate (from $1*10^{-4}$ - $5*10^{-2}$ M) , flow rate (from 0.15-2.5 $ml.min^{-1}$ in each channel) , length of the reaction coil (from 25-125 cm) and injection volume (from 50-250 µl) . The results obtained showed that a concentration of 0.0001,0.001 and 0.0025 M of N,N-dimethyl aniline gave the highest absorbance for (I), (П) and (Ш) respectively (Fig.4). and aconcentration of 0.005 ,0.005 and 0.0075 M of Potassium metaperiodate gave the best result for (I), (П) and (Ш) respectively (Fig.5). Aflow rate of 2.25, 1.20 and 1.50 $ml.min^{-1}$ gave the highest absorbance for (I), (П) and (Ш) respectively (Fig.6), reaction coil length of 50 cm (Fig.7) and an injection volume of 100 µl (Fig.8) were the best conditions which provide the highest absorbance with the lowest blank value . A standard calibration line , obtained for series of catechol amine standards and the main analytical figures of merit of the developed procedures are indicated in Table.1. The increase in the temperature of the reaction coil dose not increase the absorbance and cause a degradation of the coloured product and low sensitivity and stability of the reaction product.

Analytical Applications

The developed methodology is very adequate for the determination of (I), (П) and (Ш) in aqueous solution and in pharmaceutical preparation samples at a concentration level of traces (P.P.m) and without requiring any previous separation step . Moreover the proposed procedures are very economical when compared to other methods such as those based on the use of HPLC .

In compression of the batch with FIA procedures the latter 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 (Table .1).

The precision of the method was evaluated by analyzing pure sample of (I),(II) and (III) and agood recovery was obtined (Table.1) . The propose method was applied successfully to the analysis of some dosage forms containing catecholamine drugs . The resultes in Table .2 are in accordance with those obtained by the official spectrophotometric method (10).Statistical analysis (11) , F and T - test reveals that ther is no significant

difference in precision and accuracy between the proposed and the official spectrophotometric methods .

Finally , in comparison with other possible spectrophotometric methods (12-14) , the proposed procedures are simple , selective and dose not require temperature or pH control .

Table .2 Application of the proposed methods to the determination of chatechol amine drugs in pharmaceutical preparation .

Drug sample	Amount of drug µg/ml	Batch method		FI method		Standard method (9)
		Recovry%	RSD%	Recovry%	RSD%	
Aldomate (SDI)	40	99.38	1.2	101.8	0.58	101.17
Aldomate (ASIA)	40	100.87	1.36	99.1	0.66	
Adrenaline ampoules	20	100.45	1.46	100.89	0.68	100.0
Dopamine HCl Ampoules	20	101.2	1.21	101.73	0.87	102.5

Conclusion

The proposed method is simple , accurate and precise . It allows the determination of the studied catechol amine drugs in aqueous and in pharmaceutical preparations. Drugs solution can be analyzed at a rate of 120 sample per hour through FIA .

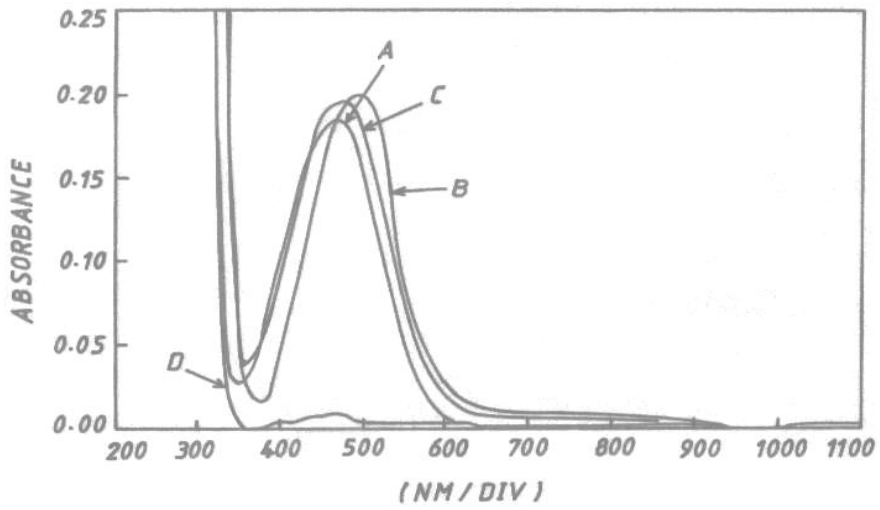

Fig. 2: Absorption spectra of (A) 20 μg.ml^{-1} of Methyl Dopa (B) 20 μg.ml^{-1} Adrenaline (C) 20 μg.ml^{-1} Dopamine treated as described under procedure and measured against blank and (D) the reagent blank measured against distilled water

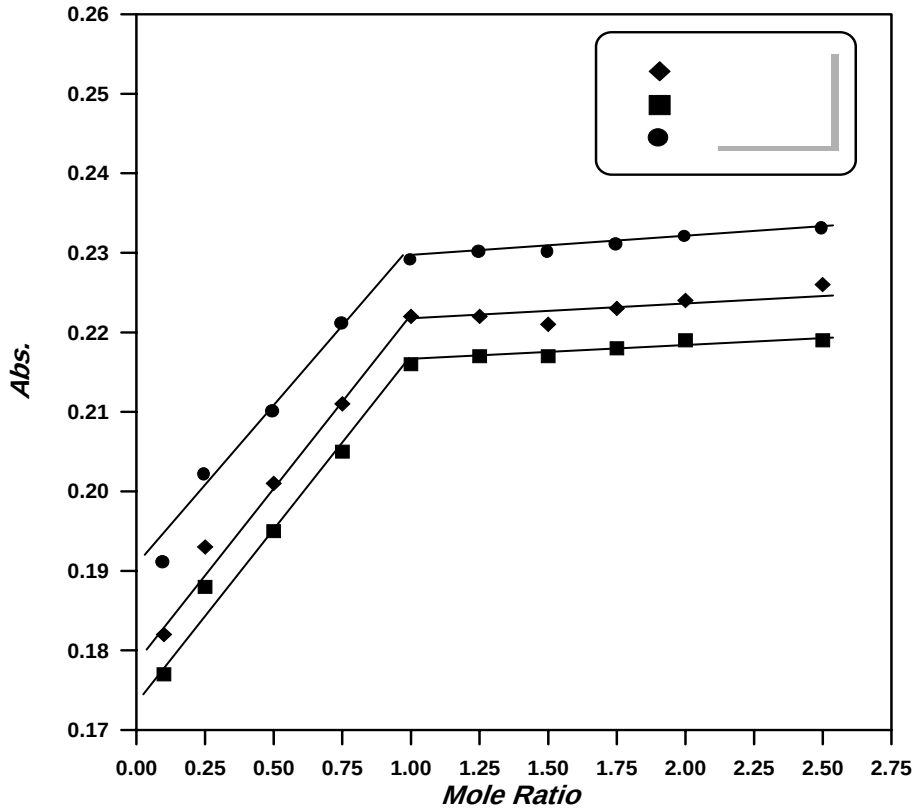

Fig. 3: Study of the mole ratio of the reaction between Catechola mine drags and N, N-dimethyle aniline

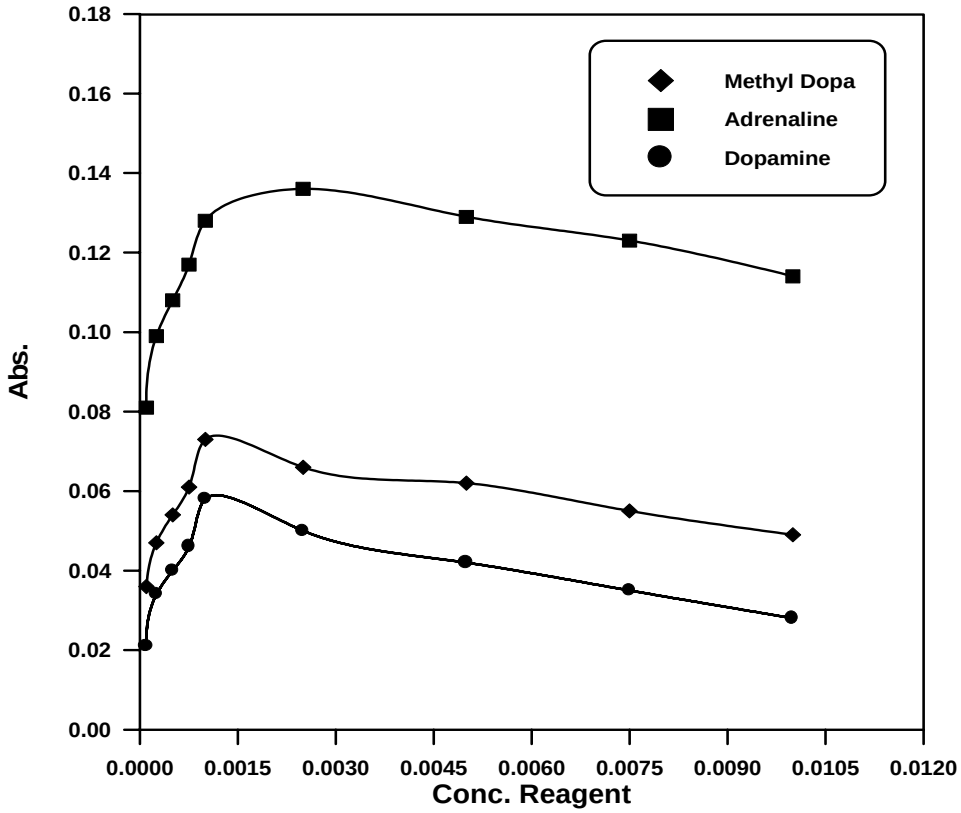

Fig. 4: Effect of the concentration of N, N-dimethyle aniline on the colored reaction product

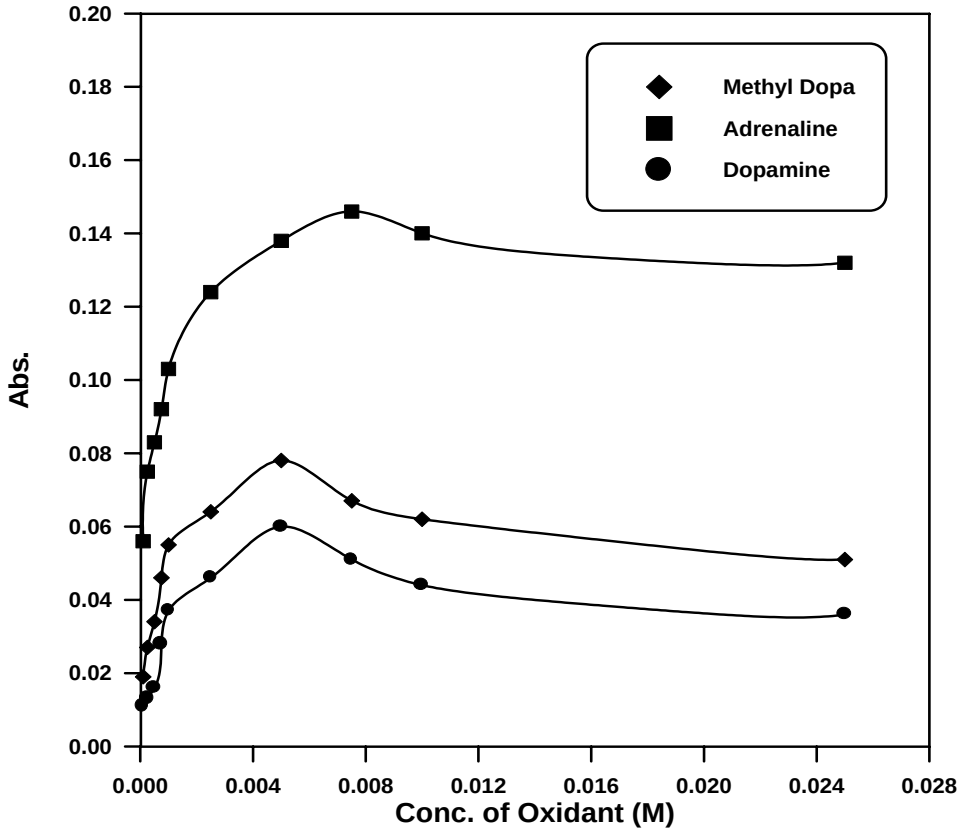

Fig. 5: Efeect of the concentratio of Potassium meta periodate

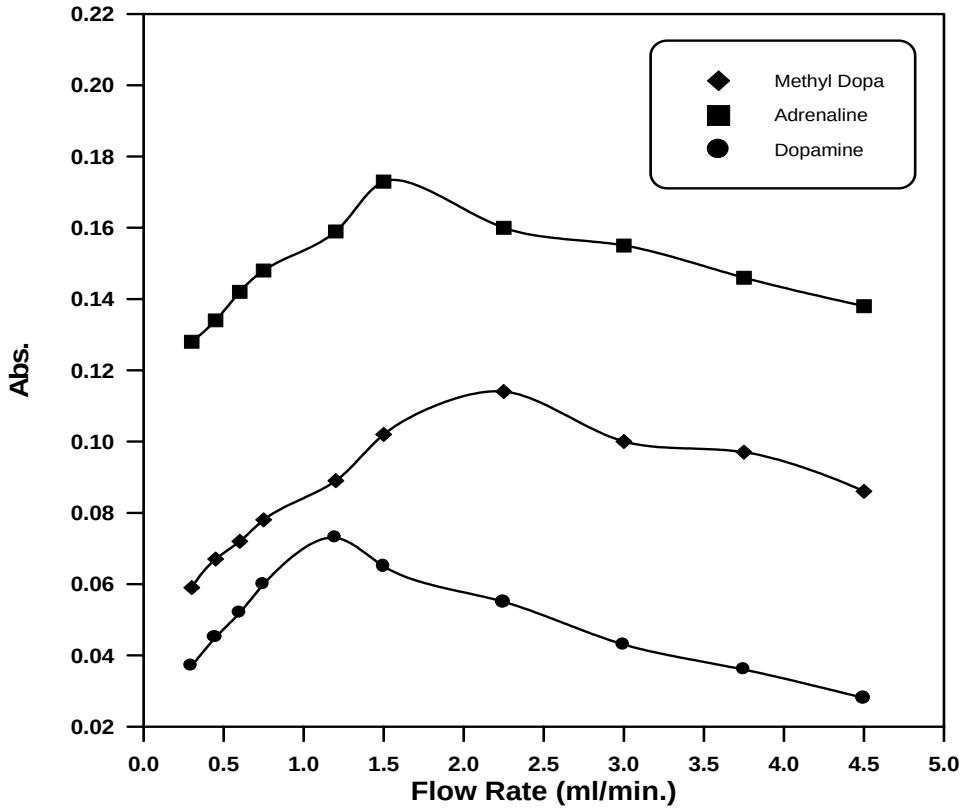

Fig. 6: effect of total flow rate (ml/min.)

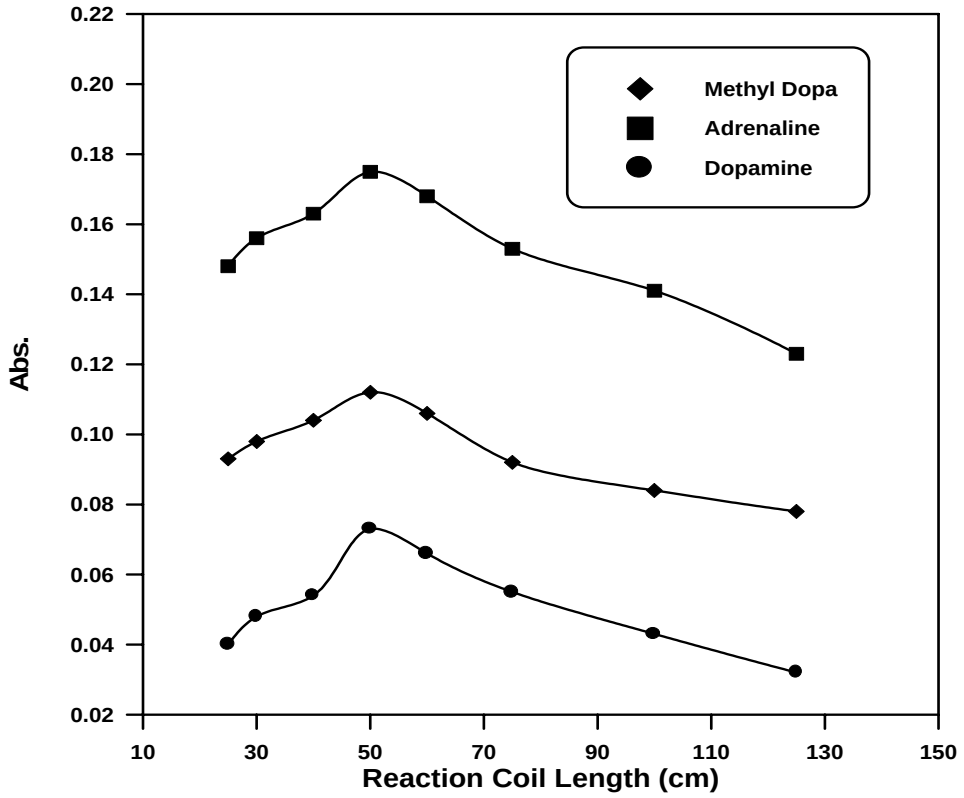

Fig. 7: Effect of the length of the reactio coil (cm)

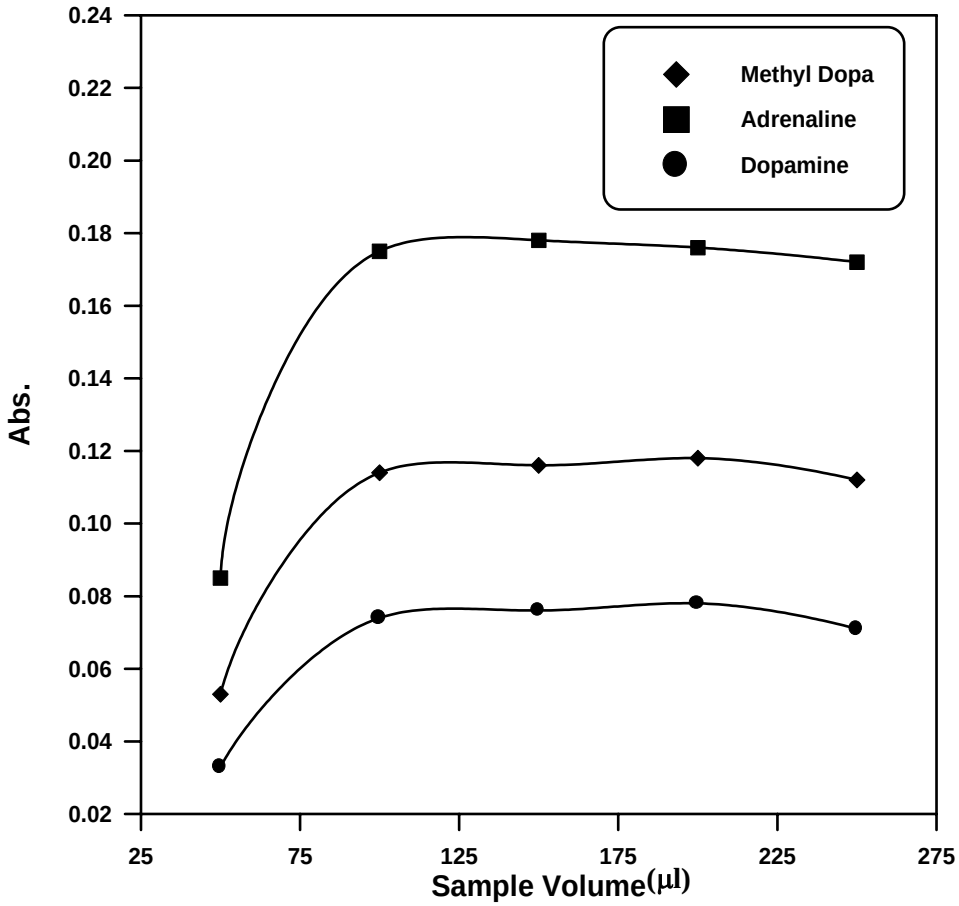

Fig. 8: Effect of the injected sample volume in (µl)

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