International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.9, pp 756-768, 2017

Batch and Cloud Point Extraction Spectrophotometric Methods for the Determination of Two Types Catecholamine Drugs

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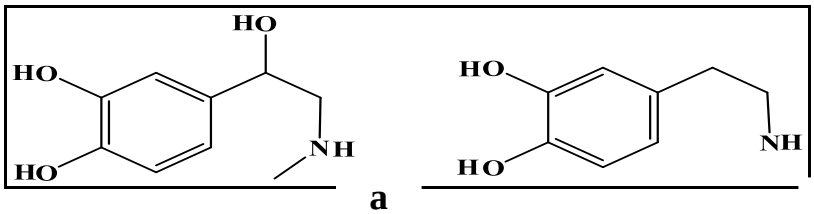
Abstract : Batch and cloud point spectrophotometric methods were developed for the estimation of catecholamine drugs. Batch method is based on diazotization of 2-aminothizole and coupling with adrenaline and dopamine respectively. The resulted dye gives medium violet colored with adrenaline that shows a maximum absorption at λ_{max}(565) nm, and faint violate colored with dopamine that shows a maximum absorption at λ_{max}(555) nm. The cloud point extraction method is based on separation and preconcentration of violet dye with UV-Visible spectrophotometry detects. The analytical data of the batch method are: the concentration range of(1.0 – 17.5),(1.0 –12.5) µg.ml^{-1},molar absorptivity of (1.7 x 10^4) (5.51 x 10^5) L.mol^{-1}.cm^{-1},Sandell's sensitivity value (0.0175) (0.061) µg.cm^{-1}, limit of detection (0.043)(0.038) µg.ml^{-1}RSD% (0.65%) (0.91%)for(99.83 ± 0.023%) and (99.998 ± 0.036%) for adrenaline and dopamine respectively. The analytical data of the cloud point extraction method were, concentration range of (0.25 – 5.0) µg. ml^{-1}, molar absorptivity of (4.8 x 10^4) (1.8 x 10^5) L.mol^{-1}.cm^{-1}Sandell's sensitivity value (6.1 x 10^{-3}) (0.01) µg.cm^{-1},limit of detection (0.019) (0.025) µg.ml^{-1}, RSD% (0.307%) and (0.445%)and the recovery were (100.03 ± 0.008%), (99.93 ± 0.009%) for adrenaline and dopamine respectively. In addition, the measurement enrichment factor (2.71), (2.46) and preconcentration factor (25) for adrenaline and dopamine respectively. The two methods were examined successfully for the estimation of adrenaline and dopamine in traditional drugs and urine.

Key words : Spectrophotometry, Catecholamine, Adrenaline, Epinephrine, Dopamine, 2-aminothiazole, and cloud point extraction.

Introduction

Adrenaline assumes a vital part in the central nervous system, neurotransmitters, and the capacity to transmit electrical driving forces between certain natural tissues and manage them is imperative to the work of the human body. consequently, they are utilized as a part of the treatment of different illnesses, for example, Parkinson's, Alzheimer's and Arthritis. [1. They affect the body's metabolism and change of their concentration levels in biological fluids may induce many diseases, such as Parkinson's disease, schizophrenia, and even tumors, including paraganglioma and pheochromocytoma [4-7].Moreover, catecholamine drugs are common emergency and usually used to treat anaphylactic shock, bronchial asthma, and organic heart disease [8].



a **b**

Figure1: (a) Adrenaline and (b) Dopamine.

Cloud point extraction (CPE) This method of separation, preconcentration, rapid, selective and sensitive procedure that has been extensively applied tomicro amounts of dye. CPE is considered the most versatile and simple method for separation and preconcentration of micro amounts of reagent and materials such as elements including [Au, Tl, Co, Mn, Cr, Zn, Ni, Pb, Cd, Cu, Ag, As, Se and Sb] [9-24]. CPEoffers advantages such as safety, low cost, high extraction efficiency, easy disposal of the surfactants, and low toxicity of the utilized reagents compared with classical organic solvents [25]. Azo dye can be in hydrophobic chalets that were produced after reactive under appropriate conditions [26].Many methods have been published for the calculation of adrenaline in pharmaceutical dosage and biological samples, such as electro-chemistry. [27,28] Chemiluminescence[29],flow injection[30], fluorometric[31].High-performance liquid chromatography (HPLC) and other chromatography techniquesHPTLC, LC-MS MS [32-35].Spectrophotometric methods have been carried out for the estimation of adrenaline using several reagents such as chloranil[36],ferric chloride in the presence of potassium hexacyanoferrate (III)[37],or n the presence(1,10-phenanthroline)[38].In this study, a new analytical method was developed for estimation of catecholamine, which is based on the reactions of diazotization reaction. statistical calculations proved that this study can be applied to live sample of medicines available in the local markets and show their success.

Experimental

Instruments:

The main instruments used in this work are Shimadzu UV-Visible spectrophotometer, UV-160 using 1 cm quartz cell, PH meter, type inoLab7110.Waterbath BS-11 (Korea) was used in the study.

Reagents:

All chemicals were reagent gradesupplied by Sigma Aldrich. Stock solution of2-aminothiazole(1000) $\mu g.ml^{-1}$, adrenaline standard solution (1000) $\mu g.ml^{-1}$anddopamine standard solution (1000) $\mu g.ml^{-1}$.

General Procedure of Diazotization Reaction:

Developed batch method of diazoniumdye was prepared by adding accurately (0.80) ml(1000)$\mu g.ml^{-1}$ of2-aminothiazolein(20.0) ml to calibrate flask in an ice bath at $\geq$ (4)C^0. Add (0.30) ml of (1:1) H_2SO_4; then add gradually (1.0) mlof (1%) ($NaNO_2$) wait for (10) min. Add (1.0) ml of (4%) urea with shake well.[Starch paper was used for detection of unreacted of ($NaNO_2$)],followed by adding(1.0) ml of (1000) $\mu g.ml^{-1}$ of catecholamine solutions. Then add (1.50) ml of (50%) KOH and completely with distilled water. The absorbance of the violet colored products were measured at λ_{max}(560, 555) nm for adrenaline and dopamine respectively against their reagent blank.

General procedure of CPE:

Transferred (20.0) ml of diazonium dye of catecholamine from the procedure above of with multi concentration (0.25 – 5.0)$\mu g.ml^{-1}$ into test tube and (1.0) ml of (10%) (V/V) Triton X-114, (2.0) ml of surfactant hexadecyltrimethylammonium bromide(0.10) $mol.L^{-1}$ and (2.0) ml of (5%) (Na_2SO_4). Transferred the mixture into a hot water bath for one hour at (65) C^0 to form a cloudy solution and separates the mixture into two phases. Then puts the mixture in ice bath the organic phase settled at the bottom decant the aqueous phase. Used (0.50) ml of ethanol to dissolve the dye. Cell(1.0) cm that use for estimating azo dye spectrophotometry. A blank solution was prepared in the same way.

General Procedure of Urine Sample preparation:

Samples of urine were gathered using plastic vials,(0.5)ml of HCl concentration was added to the urine sample for adjustment of catecholamine. Collected samples were examined by using the new method, to (1.0)ml of the diluted sample with (0.025) M of EDTA solution in a ratio 1:1. The methods used in general procedure of azo diazotization above.

Results and Discussion

The preliminary investigation shows that the reaction of diazonium salt of 2-aminothiazole coupling with catecholamine gate the chart. A violet colored product with an absorption maximum at (565 and 555) nm was formed when adrenaline and dopamine coupling with diazotized 2-aminothiazolein the presence of potassium hydroxide. The absorption spectrum of the resulting product against reagent blank was shown in (Fig.2).

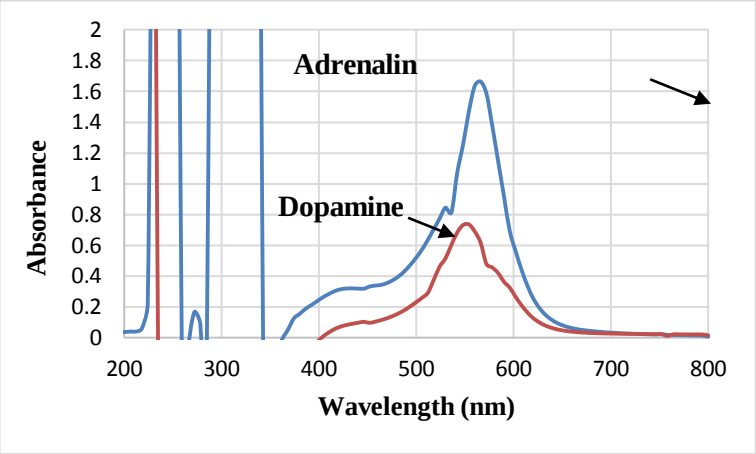


Figure (2): Absorption spectra of adrenaline against reagent blank

Optimization of experimental conditions of diazonium salt:

The optimum reaction conditions for determination of catecholamine drugs were established doing a number of preliminary experiments. The absorbance of a series of solutions was measured by varying one parameter and fixing the other parameters at (565, 555) nm for adrenaline and dopamine respectively.

The effect of acid type was studied by using a number of acids (HCl, H_2SO_4, HNO_3 and CH_3COOH) for the formation of diazonium salt the finding observed that the sulfuric acid gave a higher absorption and this indicates its high ability to give proton and the formation of nitrous acid as shown in the Table (1). The best concentration of sulfuric acid was estimated in the range of 0.1 to 0.6 normality and the concentration of (0.275) N for adrenaline and (0.458) N for dopamine gave the best absorbance is as shown in (Fig.3).

Table (1): Effect of acid type

Type of acid	Ab. Adrenaline	Abs. dopamine
HCl Conc.	0.7974	0.3831
H_2SO_4Conc.	1.0019	0.4532
CH_3COOH Conc.	0.4838	0.2676
HNO_3Conc.	0.776	0.0884

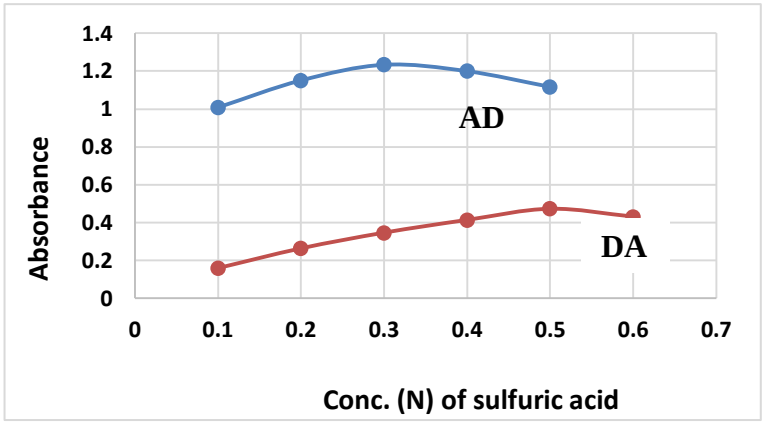


Figure (3): Effect of acid concentration

Sodium nitrite salt concentration has an effective role in this reaction so it has been studied and calculated the most effective concentration which leads to rapid and integrated reaction The results indicated that the best volume of salt was 1.0ml of ( ) M of salt for both drugs as shown in Figure 4.The best time to complete the reaction and the formation of the diazonium salt was 10 minutes,different periods of time were studied between 10-60 minutes. Figure 5 shows the practical results in this study .

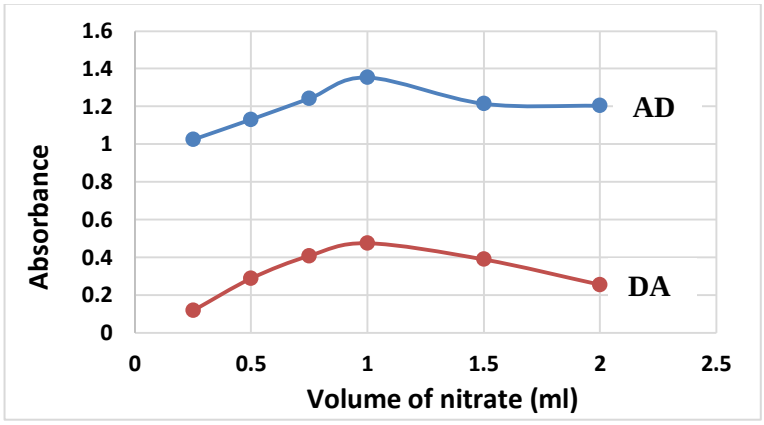


Figure (4): effect volume of sodium nitrite

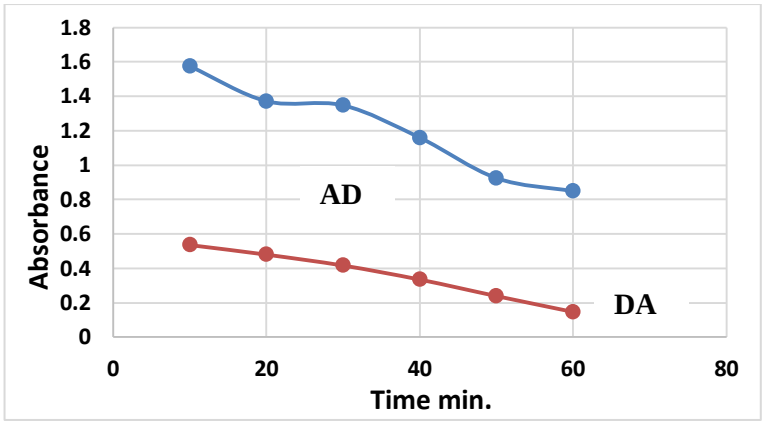


Figure (5): Effect the time after added $NaNO_2$ 1%

In these reactions it is necessary to use urea and choose the best concentration of it as its presence helps to eliminate the unreacted of sodium nitrite salt. The results showed that adding 4 ml of 4% ml urea solution is sufficient and useful in this reaction. The results were shown in Figure 7.

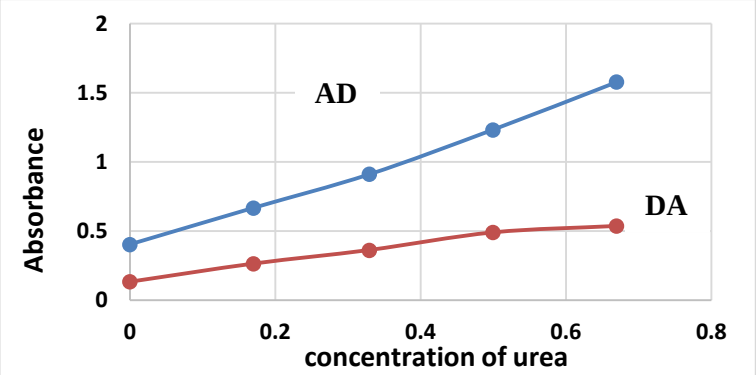


Figure (6): Concentration of urea

The type of base used in such reactions and their concentration is of utmost importance as the literature indicates that the reaction of the formation of Azo dyes is comprised of solutions in a medium with Ph between 6.5-7.5,for this study bases of (NH_4OH, NaOH and KOH) were selected to complete this study. The study showed that the best base is KOH and the best volume was 1.5 ml of 0.6M.Table 2 and Fig 7 show the results of the study.

Table (2):Effect type of base

Type of base	**Abs. adrenaline**	**Abs. dopamine**
NH_4OH	1.3736	0.3724
NaOH	1.5653	0.5357
KOH	1.5878	0.6481

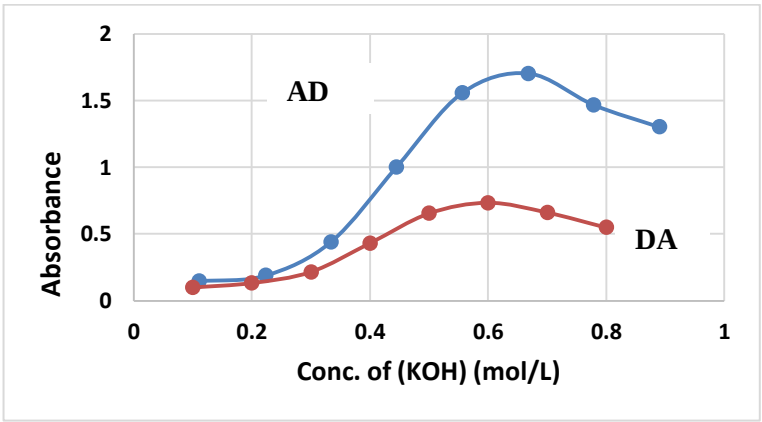


Figure (7): Effect Concentration of base.

The effect of the sequence of addition of reagents on the reaction product was studied and showed that (Table 3) the best addition sequence contained the following the order(Salt + catecholamine + base).

Table (3):Study of sequence addition

Sequence addition	**Abs. Adrenaline**	**Abs. Dopamine**
Salt + catecholamine + base	1.7141	0.7353
Salt + base + catecholamine	1.3311	0.6478
Salt + (catecholamine + base)	0.8113	0.2582

The mole ratio methods were used to determine the association of the drugs under study with the 2-aminothiazol reagent. It was found that the ratio of the drugs with the reagent was 1: 1 and the results were shown in Figure 9.

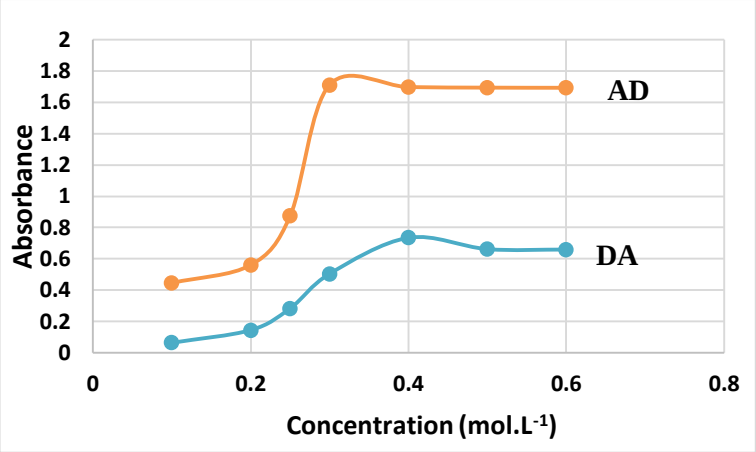


Figure (8) mole ratio study

The stability of the azo dye prepared in this research was studied using the optimal conditions obtained from the previous results. The stable dye was found to be more than one day, Table (4) shows the finding of this study.

Table (4):Study of stability of azo dye

Time (minutes)	*Abs. Adrenaline*	*Abs. Dopamine*
Immediately	1.6995	0.741
10	1.7153	0.753
20	1.7131	0.752
30	1.7122	0.748
40	1.7122	0.748
50	1.7118	0.737
60	1.7115	0.733
24 hours	1.6561	0.699

Calibration graph:

The absorbance of catecholamine increases linearly as the concentration of catecholamine increases, the calibration graph was gained from the series of standard solution for adrenaline and dopamine the linearity, regression equation, correlation of determination (R), slope (b), and intercept (a). Moreover, different parameters of the analytical achievement of the proposed method are briefly shown in the (Fig.9) and Table (5).

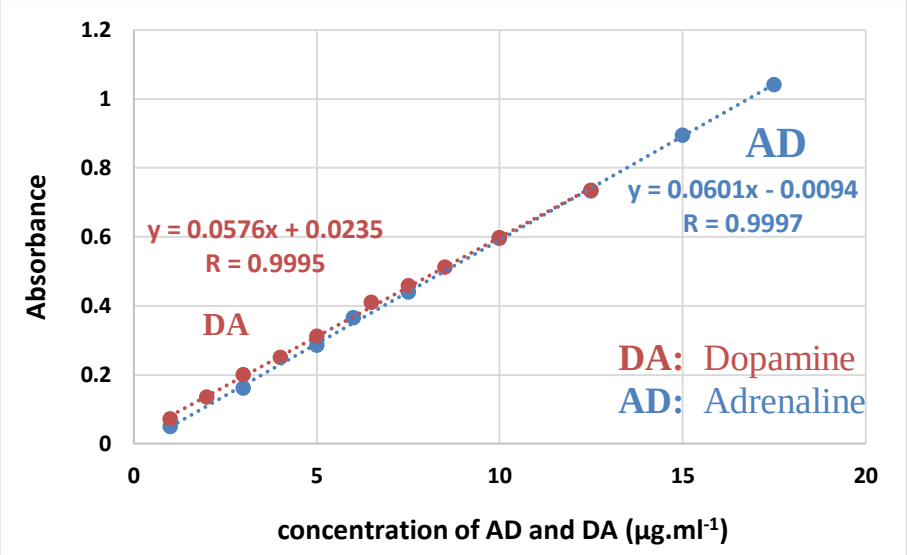
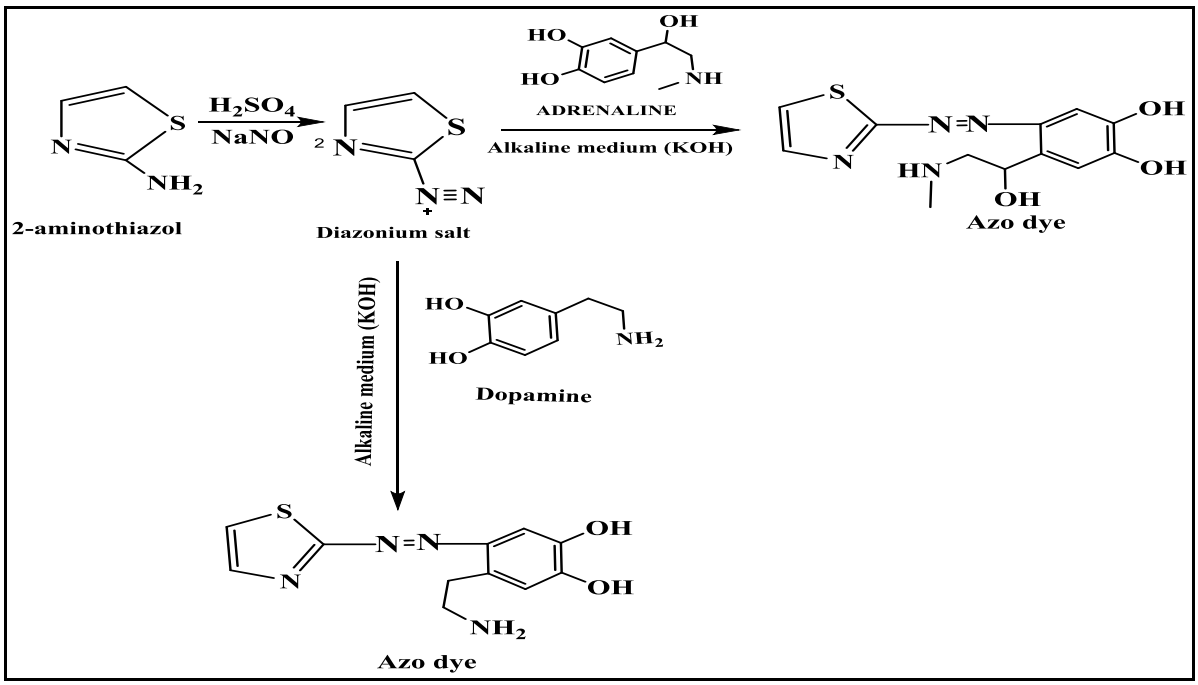


Figure (9): Calibration graph of AD and DA



Scheme I: The suggest a mechanism of reaction colored steps

Table (5): Optical characteristics and statistics for the proposed method

Parameters	Adrenaline	Dopamine
λ_{max}(nm)	565	555
Color	Violet	Violet
PH	13	13
Linearity range (µg.ml^{-1})	1.0-17.5	1.0-12.5
Molar absorptivity (L.mol^{-1}.cm^{-1})	1.7 x 10^4	5.51x10^5
Sandell's sensitivity (µg.cm^{-2})	0.0175	0.061
Correlation coefficient (r)	0.9997	0.9995
Regression equation	y = 0.0601x - 0.0094	y = 0.0576x + 0.0235

Slope	0.0601	0.0576
Intercept	-0.0094	0.0235
Analytical sensitivity ($\mu g.ml^{-1}$)	0.18	0.272
limit of Detection ($\mu g.ml^{-1}$)	0.043	0.038
limit of Quantification ($\mu g.ml^{-1}$)	0.1419	0.1254
C.L. for the slope ($b \pm ts_b$) at 95%	0.0601 ± 0.0013	0.0576± 0.00078
C.L. for the intercept ($a \pm ts_a$) at 95%	-0.0094 ± 0.0129	0.0235± 0.0055
Mean %Rec. ± S.D	99.83 ± 0.023	99.998 ± 0.036
Mean RSD%	0.65%	0.909%

Accuracy and precision

At three different concentrations of adrenalineand dopamine were determined, the accuracy and precision of the calibration graph Table (6) indicated an accept Table of accuracy and precision.

Table (6): Accuracy and precision for present method.

Catecholamine	**Amount of azo dye ($\mu g.ml^{-1}$)**		**Relative Error %**	**Recovery (100 + E %)**	**RSD%***	**RSD%****
	Taken	**Found**				
Adrenaline	1	0.995	-0.499	99.5	1.496	0.65
	7.5	7.488	-0.016	99.98	0.253	
	17.5	17.527	0.002	100.003	0.201	
Dopamine	2	1.998	-0.001	99.999	1.318	0.909
	5	5.023	0.005	100.005	1.182	
	10	9.918	-0.008	99.99	0.228	

(*) mean of (5) replicate
(**) mean of (15) replicate

Application

Preparation the medicinal solutions containing catecholamine drugs with a concentration of (1000)$\mu g.ml^{-1}$,the following steps were followed in the preparation of the calibration curve and the results shown in Table (7) were obtained, indicating that the proposed method could be successfully applied to pharmaceutical and biologic preparations.

Table (7): Application for drugs of catecholamine.

Sample	**Found**	**Relative Error %**	**Recovery (100 + E %)**
Adrenaline ampule 1mg/ml Chemical Company of Malaysia (MCC)	0.993	-0.007	99.993
Dopamine 40mg/ml Turkey	39.764	-0.056	99.94
Dopamine 40mg/ml Germany	39.984	-0.0004	99.9996

The mean of (5) replicates

Optimization of experimental conditions of cloud point extraction:

The conditions of PH, concentration and volume of (%10) TX-114, concentration and volume of surfactant finally the type and concentration of salt were used to study the factors influencing the extraction of

azo dye which synthesized in this study in order to improve additional method for assessing dopamine and adrenalin.

The result was appeared that (Table 8), The azo dye was formed in alkaline medium and gives the best cloud point extraction at PH =13 different temperature (25-75) C^0 for (25) min incubation period were tested, the results showed a higher absorbenc when the temperature was (65) C^0 for (45) min, as shown Table (9).

Table (8): Effect of PH

PH	Abs. Adrenaline	Abs. Dopamine
2	0.098	0.117
5	0.119	0.234
7	0.223	0.318
10	0.487	0.389
13	0.616	0.491

Table (9): Effect of time and temperature on the extraction

Time / Minutes	Abs. Adrenaline	Abs. Dopamine
25	0.013	0.184
35	0.038	0.252
45	0.196	0.397
55	0.394	0.486
65	0.642	0.513
75	0.511	0.477

The effect of(%10) TX-114 concentration was studied by using various volumes of (%10) TX-114 toimprove the cloud point extraction efficiency was (1.0) ml of (%10) TX-114 as shown in (Fig.10).

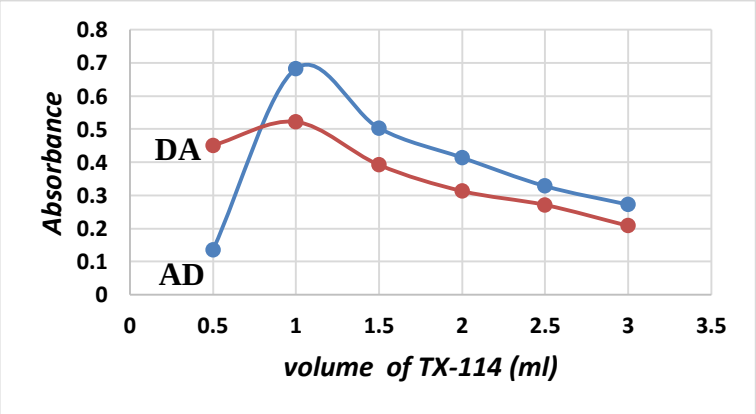


Figure (10): Effect volume of (%10) TX-114 on the extraction

The study showed that the presence of the cationic surfactant has a positive effect on the extraction efficiency of the cloud point and the strengthening of the intensity of the color and the sensitivity. So studied the best amount and concentration of cationic surfactant and it was found through the results that the ideal volume was 2ml of 0.1M of cationic surfactant, figure 11 shown the findings obtained.

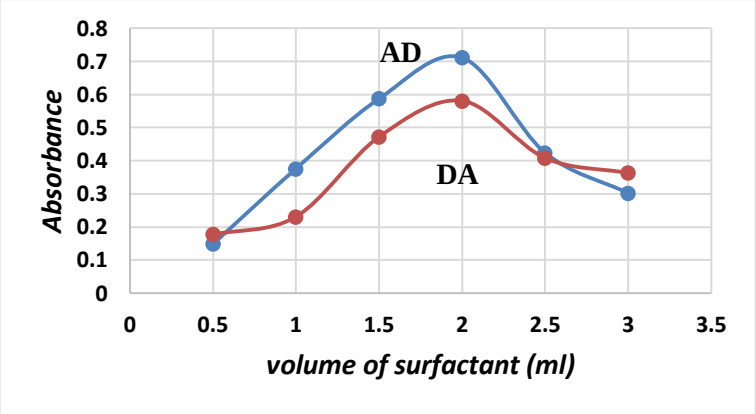


Figure (11): Effect volume of surfactant on the extraction

The effect of salt type was conducted by using a different salt such as ($NaNO_3$, NaCl, CH_3COONa, Na_2SO_4 and KCl) with a concentration of (5%) to improve the efficiency extraction of cloud point,the result observed that the best type of salt by studying the absorbance isNa_2SO_4 as shown in the Table (10). Also the volume of 5% of chosen salt was 2ml,Fig 12.

Table (10): Effect type of salt on the extraction

Type of salt 5%	**Abs. Adrenaline**	**Abs. Dopamine**
$NaNO_3$	0.263	0.231
NaCl	0.382	0.588
KCl	0.387	0.474
CH_3COONa	0.552	0.503
Na_2SO_4	0.778	0.644

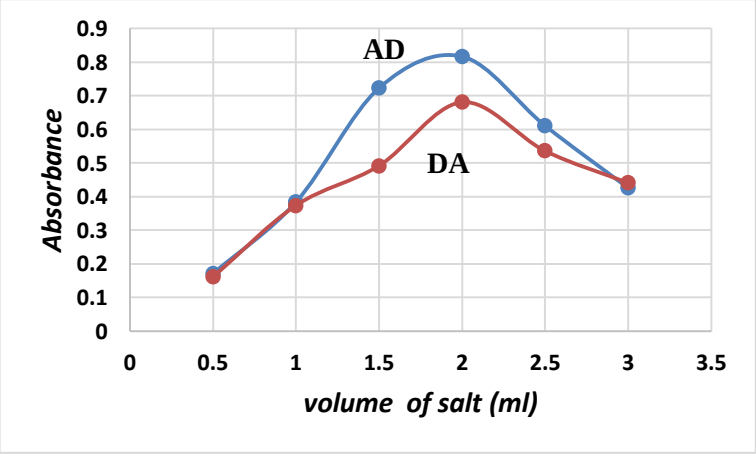


Figure (12): Effect volume of salt on the extraction

Calibration graph of cloud point extraction:

The concentration range of azo dye from (0.25 – 5.0) µg.ml^{-1} that is prepared under the optimized established conditions was linear with a correlation coefficient of (0.9998), (0.9996) and the Regression equation was(y= 0.1628x+0.0065), (y = 0.1418x - 0.0217)for adrenaline and dopamine respectively as shown in Fig. (13)

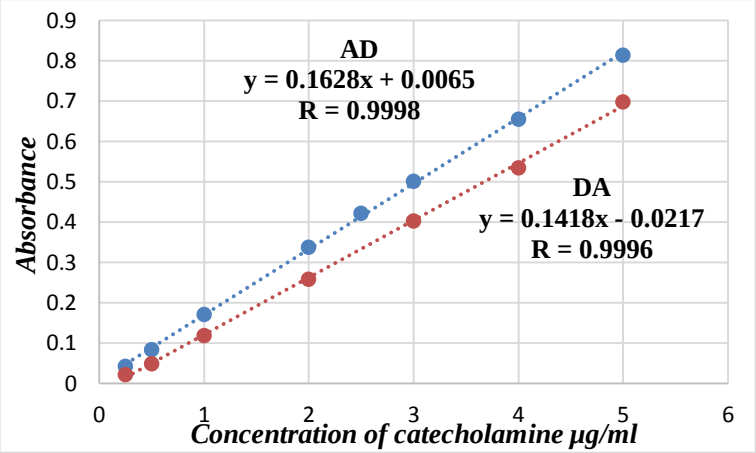


Figure (13): calibration graph concentration of azo dye (µg.ml^{-1})

Table (12): Optical characteristics and statistics for the cloud point extraction method.

Parameters	**Adrenaline**	**Dopamine**
λ_{max}(nm)	565	555
Color	Violet	Violet
PH	13	13
Linearity range (µ g.ml^{-1})	0.25 - 5	0.25 - 5
Molar absorptivity (L.mol^{-1}.cm^{-1})	4.8 x 10^4	1.8 x 10^5
Sandell's sensitivity (µ g.cm^{-2})	6.1 x 10^{-3}	0.01
Correlation coefficient (r)	0.9998	0.9996
Regression equation	y = 0.1628x + 0.0065	y = 0.1418x - 0.0217
Slope	0.1628	0.1418
Intercept	0.0065	-0.0217
Analytical sensitivity (µ g.ml^{-1})	0.592	0.549
limit of Detection (µ g.ml^{-1})	0.019	0.025
limit of Quantification (µ g.ml^{-1})	0.0627	0.0825
C.L. for the slope (b ± ts_b) at 95%	0.1628 ± 0.0095	0.1418 ± 4.4x10^{-3}
C.L. for the intercept (a ± ts_a) at 95%	0.0065 ± 0.0034	0.0217 ± 0.013
Mean %Rec. ± S.D	100.03 ± 0.008	99.993
Mean RSD%	0.307	0.445
Preconcentration factor	25	25
Enrichment factor	2.71	2.46

Accuracy and precision

At three different concentrations of azo dye was determined, the accuracy and precision of the calibration graph. Table (11) indicated an accept Table of precision and accuracy.

Table (11): Accuracy and precision for present method.

Catecholamine	Amount of azo dye ($\mu g.ml^{-1}$)		Relative Error %	Recovery (100 + E %)	RSD%*	RSD%**
	Taken	Found				
Adrenaline	2	2.01	0.52	100.52	0.547	0.307
	3	3.017	0.57	100.57	0.232	
	5	4.95	-0.996	99.004	0.142	
Dopamine	1	0.975	-0.025	99.975	0.824	0.445
	3	3.003	0.003	100.003	0.386	
	5	5.001	0.001	100.001	0.126	

(*) mean of (5) replicate
(**) mean of (15) replicate

Application

Preparation the medicinal solutions containing catecholamine drugs with a concentration of (1000)$\mu g.ml^{-1}$,the following steps were followed in the preparation of the calibration curve and the results shown in Table (13) were obtained, indicating that the proposed method could be successfully applied to pharmaceutical and biologic preparations.

Table (13): Application for drugs of catecholamine.

Sample	Found	Relative Error %	Recovery (100 + E %)
Adrenaline ampule 1mg/ml Chemical Company of Malaysia (MCC)	0.991	-0.009	99.991
Dopamine 40mg/ml Turkey	39.816	-0.005	99.995
Dopamine 40mg/ml Germany	39.993	-0.002	99.9998

the mean of (5) replicates

Conclusions

This research included the development of new spectral methods that are easy to obtain in terms of the availability of materials, and the laboratory conditions available to estimate the catecholamine drugs in biological fluids and drugs dosage. The diazotization reactions of 2-aminotiazole compound were used and coupled with catecholamine. Moreover, to invest the colored dye product from the above reaction, cloud-point extraction method to obtain the maximum possible analytical data and to eliminate possible interference during measurements. These methods were applied successfully for the estimation of catecholamine compounds in urine and drugs.

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