Determination of phenolic drugs and their formulations via various analytical methods

Abood Nisreen Kais[1], Hassan Mohammed Jasim M.[1*] and AL-Da'amy Muneer A.[2]
1. Department of Chemistry, College of Science, Mustansiriyah university Baghdad, IRAQ
2. Department of Chemistry, College of Education for Pure Science, Karbala University, Karbala, IRAQ
*dr.moh2004@uomustansiriyah.edu.iq

Abstract

In this study, new spectrophotometric methods developed for estimation of Methyldopa and Salbutamol are described. The first method depends on the conversion of Methyldopa and Salbutamol to diazonium ion followed by reaction with 2-aminothiazole reagent in the alkaline medium. The formed azo dye has a Violet and Reddish-orange colour with absorption intensity at λ_{max} 565 and 490 nm. Between the concentration range 2.5-62 µg / ml the Beer's law is obeyed with correlation coefficient as 0.9998 and 0.9997, molar absorptivity as 0.382×10^4,0.497×10^4 $L.mol^{-1}.cm^{-1}$ and the detection limit as 0.182, 0.159 µg. mL^{-1}.

The second method is based on cloud point extraction (CPE) of a trace amount of the formed azo dye in the first method followed by measuring with a UV-visible spectrophotometer. Concentration range that obeyed the Beer's law was between 0.25-6 µg / mL, the correlation coefficient was 0.9997, molar absorptivity was 0.437×10^5,0.469×10^5 $L.mol^{-1}.cm^{-1}$, detection limit was 0.032,0.034 µg. mL^{-1}, pre-concentration factor was 25 and distribution coefficient was 265.03, 221.26 respectively for Methyldopa and Salbutamol. Third method Flow injection technique was conducting drugs measuring with a UV-visible spectrophotometer. Concentration range that obeyed the Beer's law was between 1-150 µg /mL, the correlation coefficient was 0.9998, molar absorptivity was 0.171×10^4,0.157×10^4 $L.mol^{-1}.cm^{-1}$, detection limit was 0.037, 0.045 µg. mL^{-1}. The proposed methods were applied and proved their compatibility for estimating Methyldopa and Salbutamol in pure and pharmaceutical samples by comparing the readings received with previous studies.

Keywords: Methyldopa, Salbutamol, Diazotization, Cloud point, Flow injection.

Introduction

Methyldopa (α-methyl-3-4-dihydroxy phenylamine) catecholamine is widely used in the control of moderate and as an antihypertensive agent[1] as in figure 1 and gestational hypertension, pregnancy and blood pressure[2], methyldopa can be determined using various analytical methods like high-performance liquid chromatography (HPLC) with UV detection, polarography and visible spectrophotometry, flow injection analysis (FIA) voltammetry[3,4], liquid Chromatography , electrochemical[5], kinetic measurement[6] and potentiometric[7]. Salbutamol Sulfate (SBS) as (rs)-1-(4-hydroxy3-hydroxymethylphenyl)-2-(tert-butyl amino) ethanol sulfate, the salbutamol also known as albuterol[8] as in figure 2, was used to prevent and treat the wheezing, shortness of breath[9,10]. Salbutamol was used to treat high blood potassium levels[11], salbutamol drug always used as inhaler or nebulizer[12]. Various methods were reported for the determination of salbutamol (SBS) such as High-Performance Liquid Chromatography, mass spectrometry, flow injection and pornographic[13,14].

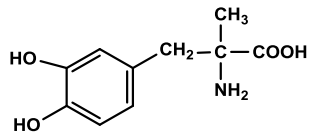

Figure 1: Methyldopa

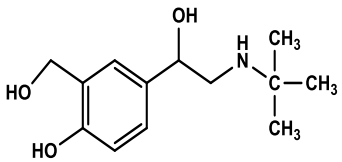

Figure 2: Salbutamol

Methyldopa and Salbutamol can be determined by the diazotization reaction. Azo-dye is very important dye consumed in food, paper and leather.[15] Diazotization method is inexpensive and very simple, the Azo-dye is very important families of dye that contains –N=N- band[16]. The various methods were found in extraction and determination of azo-dye such as liquid-liquid extraction (LLE)[17], solid phase micro extraction[1] and cloud point extraction[18]. Diazotization, Cloud point extraction and Flow injection methods were developed in this present work using to determine Methyldopa and Salbutamol in pharmaceutical formulations.

CPE has been successfully applied for extraction of a wide range of analyses from biological and environmental media including vitamin A and E[21]. Flow Injection analysis (FIA) methodology originally described by Ruzicka and Hansen[22] and flow injection methods are easy to use and inexpensive[23].

Material and Methods

Apparatus: All spectral and absorbance measurement were carried in single beam UV Visible spectrophotometer 160 equipped with 1cm and 0.5 cm quartz cell. An Ultrasonic and thermostatic water bath from Elma Hans Schmidbauer Gmbh and Co.KG were used coupled with extraction of samples. Flow injection configuration a three-channel manifold was employed with. Peristaltic pump (ALITEA, C4, made in Sweden) with polyvinyl chloride tube (0.8) mm internal diameter as in figure 3.

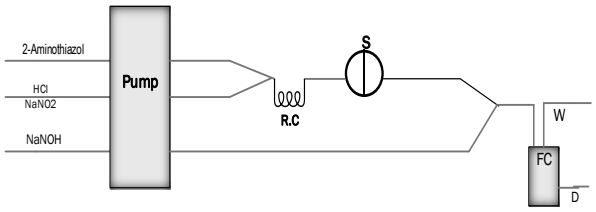

Figure 3: Scheme of employed flow system, P: peristaltic pump, R.C: reaction coil, S: sample injection, W: waste, FC: flow cell

Reagents: All chemicals were of analytical quality and were bought from Merck Ltd. (Darmstadt, Germany). However, Methyldopa and Salbutamol were purchased from the quality control laboratory (the general company for the manufacture of medicines and medical supplies - Samarra). Stock solutions (1000 $\mu g.m^{-1}$) of Methyldopa and Salbutamol were prepared by dissolving 0.1 gm of Methyldopa and Salbutamol in distilled water and dilution to the mark in 100 ml volumetric flask. Stock solution of 2-Aminothiazole (1000 $\mu g.m^{-1}$) was prepared by dissolving 0.1 gm of 2-aminothiazole in distilled water and dilution to the mark in 100mL volumetric flask, 25% NaOH (6.25 M),1%$NaNO_2$ (0.144M), 4% Urea ,10% Triton X-114,0.01M of CTBA (0.3644g in 100 mL in distilled water) and 5% w/v Na_2SO_4.

General Procedure of Diazotization: The excellent method was developed to prepare Azo Coupling by adding accurately 1 ml 1000 $\mu g.ml^{-1}$ 2-Aminothiazol in 20 ml volumetric flask immersed in ice bath 0-5ºC, add 0.75mL of (1:1) HCl and add gradually 1 mL of 1% $NaNO_2$, wait for 30 minutes then add 1mL 10% urea with shaking the solution to remove the excess of nitrite followed by adding 1 mL of 1000 $\mu g.\ mL^{-1}$ from Methyldopa and Salbutamol respectively, then add 2 ml of 25% NaOH for methyldopa and salbutamol finally brought to 20 ml with distilled water. The Azo-dye formed has violet, reddish-orange colored that gave absorbance at λ_{max} 565 and 490 nm respectively.

General procedure of Cloud Point Extraction for Phenolic Drugs: Different concentrations ranging from 0.25-6 µg /mL of the azo dye (Methyldopa, Salbutamol) were transferred to 15 mL centrifuge tubes, then 1 mL of Triton X- 114 10% v/v was added followed by addition of 2,1 mL of 0.01 M (CTAB), 2.5, 2 mL of 5% w/v Na_2SO_4, respectively and then distilled water was added to make total solution volume to 12.5 mL. Solutions were placed under ultrasonic for 2 minutes at room temperature followed by further ultra-sonication at 40, 50°C for 45 minutes. The resultant solutions were centrifuged for 5 minutes at 4000 rpm and then cooled in ice bath for 10 minutes to stabilize the micelle layer at the bottom of the centrifuge tube. The supernatant was removed and 0.5 ml of ethanol was added to dissolve the micelle layer. An absorption measurement was made on the prepared dye at λ max (565,490) nm against a reagent blank in UV-Vis spectrophotometer.

Procedure for pharmaceutical preparations: Methyldopa tablets provided from UK and from Lebanon were carefully weighed, the average weight of tables was extracted. The equivalent weight was dissolved in distilled water to ensure the complete solubility and then made up to flask 100 mL. The solution was filtered. Salbutamol tablets provided from UK 2 mg Tablets and from Salbutamol Tablets 2mg Product Gulf Pharmaceutical Ras Al Khaimah, U.A.E were carefully weighed, then the average weight of tablets was extracted.

General procedure of Flow Injection of Methyldopa and Salbutamol: 100 µl of drugs (Methyldopa and Salbutamol) were injected into carrier stream from mixing three channel, first channel used to carrier the (5.99×10^{-3}) M 2-aminothiazole , second channel carrier acid and sodium nitrite by T-shaped, the reaction is carried out via mixed well in 200 cm reaction coil, after that the mixture is allowed to pass through injector and the result product reacted with a stream of (1.00) M NaOH and the absorbances of the resulting violet and reddish-orange were measured at λ_{max} 565 and 490 nm respectively.

Results and Discussion

The preliminary investigation shows the diazotization reaction of 2-aminothiazole with nitrous acid and coupling with Methyldopa and Salbutamol the violet colored and reddish-orange product at λ_{max} (565 ,490) nm respectively in present sodium hydroxide. The absorption spectrum of the product against reagent blank as shown in figure 4 and figure 5.

Study of optimization reaction of diazonium salt: Various parameter affected absorption intensity of colored Azo-dye such as acid type, volume of acid, sodium nitrate concentration and reaction time. The effect of different acids was studied for the formation of diazonium salt and the results are shown in the table 1. The best volume of acid was 0.75 mL of HCl as shown in figure 6.The effect of the amount of $NaNO_2$ was studied by varying the volumes of 0.144M (1% w/v) $NaNO_2$ used from 0.5-2mL in the diazotization process and it was found that 0.1 mL gave the best absorption intensity as shown in figure 7. The reaction time was studied. It was observed that the duration time of 30 minutes was the optimum time to obtain the highest absorption intensity for Methyldopa and Salbutamol as shown in figure 8. To remove the excess of nitrous acid, a series of various volume 0-4 mL from 4% w/v urea was used,

the results indicated 1 mL is enough to remove the excess remain acid as in figure 9.

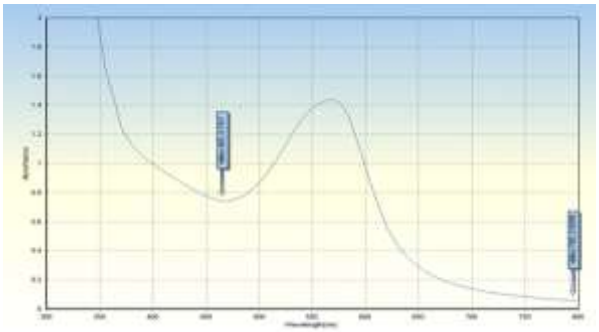

Figure 4: Absorption spectrum for 50 µg ml^{-1} Methyldopa with the reagent against the reagent blank under optimum conditions

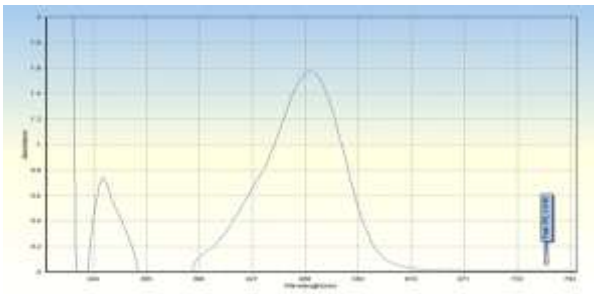

Figure 5: Absorption spectrum for 50 µg ml^{-1} Salbutamol with the reagent against the reagent blank under optimum conditions

Table 1
Effect of acid type

Type of acid	Abs. Methyldopa λ max 565 nm	Abs. Salbutamol λ max 490 nm
HCl	1.374	0.9504
H_2SO_4	0.923	1.456
HNO_3	0.758	0.573
CH_3COOH	0.486	0.243

The effect of different bases on the reaction of formation of Azo-dye, 25% w/v of NaOH, KOH, Na_2CO_3 and NH_4OH was studied. The results indicate that the best base was NaOH, also for fixing the optimum concentration of sodium hydroxide on the diazo- coupling mixture. Different volumes of 25% sodium hydroxide (0.5 to 3.5) mL were studied, the best absorbance was observed by the addition of 2 mL NaOH for Methyldopa and Salbutamol respectively as in figure 10.

1mL from the reagent gave high absorbance at λ_{max} 565 and 490 nm respectively. Highest absorbance was formed with high sensitivity as listed in table 2.

Analytical Data: Under the optimized conditions, the absorbance of phenolic drugs increases linearly as the concentration of phenolic drugs increases.

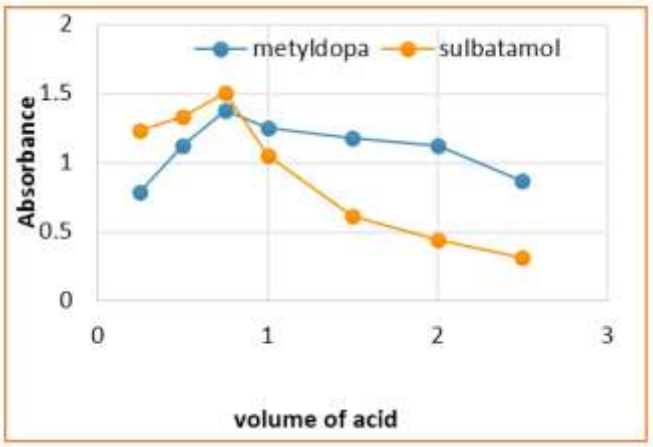

Figure 6: Effect volume of acid

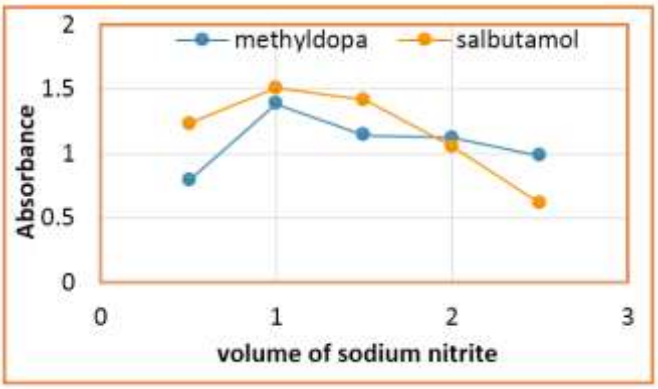

Figure 7: Effect of 1% sodium nitrite

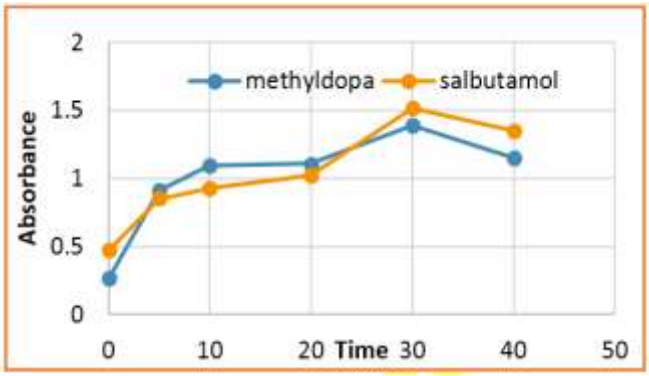

Figure 8: Effect of reaction time after addition of $NaNO_2$

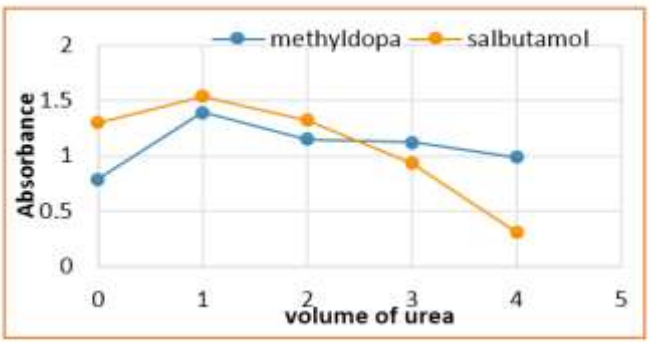

Figure 9: Effect volume of urea

Table 2
Effect of addition sequence on absorption intensity

Order addition	Abs. Methyldopa λ max 565 nm	Abs. Salbutamol λ max 490 nm
Salt + drug+ base	1.410	1.642
Salt+(drug +base)	1.311	1.453
Salt+ base + drug	0.987	1.091

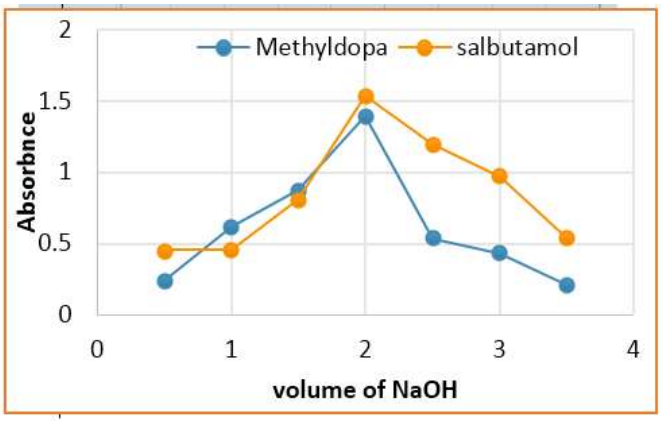

Figure 10: Effect of NaOH volume

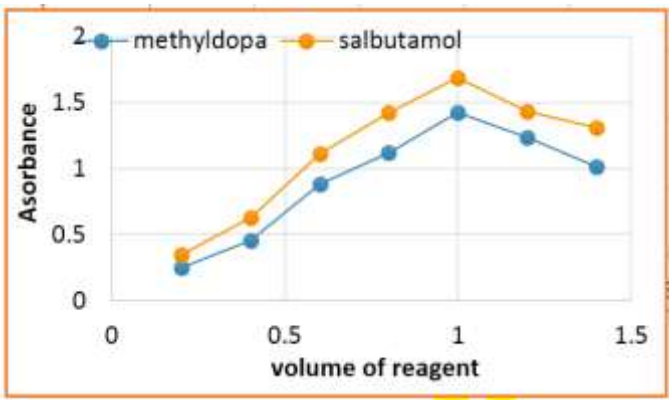

Figure 11: Effect of reagent volume

Accuracy and Precision: The accuracy was estimated by determination of the relative error, percentage and recovery.

Effect of Interference: Effect of interference was studied in drugs (Methyldopa and Salbutamol) by adding 1g from each interference to 50 µg of the drugs. The results are shown in table 6.

Study of optimization of cloud point extraction for phenolic drugs: A series of various volumes of 10% triton X-114 (0.25-2) mL to improve the cloud point extraction was observed. The results shown in figure14 reveal that series of various volume (0-3) mL of cationic surfactant was used to find the best volume of CTAB to give the high absorbance. The results are shown in figure 15.

The two parameters equilibration temperature and incubation were considered as the most important steps in cloud point extraction in order to ensure effective separation and pre concentration of phenolic drugs. The temperature was changed from 25 to 75 Cº and the incubation with heating times that ranged from 10 to 60 mint, the results are shown in figure 17 and 18 respectively.

Analytical data of cloud point extraction for phenolic drugs: Under the optimized conditions established by CPE procedure for the determination of Methyldopa and Salbutamol, linear calibration graph was established by plotting absorbance versus concentration of methyldopa and salbutamol (0.25-6) µg. mL^{-1}.

Accuracy and Precision: The accuracy was estimated by determining the relative error, percentage and recovery. Precision estimate determination for the percentage relative standard deviation RSD% is shown in the table 9.

Study of the optimum reaction conditions of Flow Injection: The optimization conditions of chemical parameters involved the concentration of reagent, concentration, sodium nitrate and sodium hydroxide concentration. Different concentration of acid HCl was used to obtain the best absorption on the flow injection for Methyldopa and Salbutamol respectively; the best concentration of acid was HCl (0.8) M. The amount of sodium nitrite has an effective role in this reaction. Different concentration of $NaNO_2$ used as the best concentration for Methyldopa and Salbutamol in flow injection, are shown in figure 21.

Different concentrations (0.2-2) M of NaOH were studied, the best absorption of the Methyldopa and Salbutamol in flow injection is shown in figure 22. The optimization of physical parameters was studied for the reaction coil. The length of the reaction was studied in the range from 30-230 cm, the results indicated that reaction coil length of 100 cm for the methyldopa and salbutamol gave maximum absorbance. Flow rate was studied using different rates used in the range (1-5) ml/min, the flow rate that gave maximum absorbance for methyldopa and salbutamol was 2 ml/min. Injection volume was studied with various sample loops in the range (50-200) µl. The best volume that gave maximum absorbance for methyldopa and salbutamol was 100 µl. The results of physical optimization conditions are shown in figures 23, 24 and 25 respectively.

Accuracy and Precision: The accuracy and precision were studied for the proposed method under optimum conditions using three different concentrations and measure absorbance at a minimum for five readings per concentration. The statistical analysis results exhibited in table 14 proved that the calculated t-values and F-values for Methyldopa and Salbutamol determination in different pharmaceuticals are less than t-tabulated and F-tabulated at 95% confidence interval and (n-1) degrees of freedom.

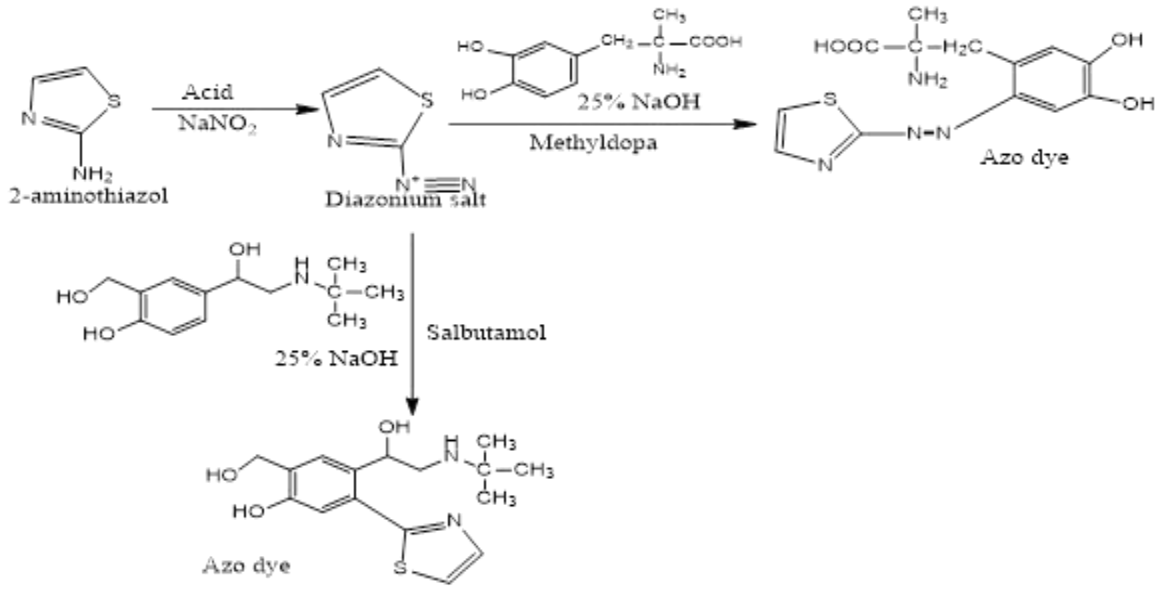

Figure 12: The suggest mechanism of reaction colored steps

Table 3
Characteristic parameter for the regression equation of the proposed diazotization method for methyldopa and salbutamol

Type of Drug	Amount of drugs µg /ml		Relative Error %	Recovery %	Average Recovery%	RSD% (n=5)
	Taken	Found				
Methyldopa	10	9.88	-1.20	98.8	98.33	0.04
	20	19.56	-2.20	97.8		0.22
	30	29.52	-1.6	98.4		0.11
Salbutamol	10	9.98	-0.2	99.8	100.70	0.09
	20	20.10	0.5	100.5		1.15
	30	29.97	-0.1	99.90		0.16

Table 4
The accuracy and precision of proposed method for estimation of pure samples

Parameter	Methyldopa	Salbutamol
λ max(nm)	565	490
Color	Violet	Reddish -Orange
linearity range(µg.ml$^{-1)}$	2.5-62	2.5-62
Molar absorptivity (L.mol^{-1} cm^{-1})	0.382×10^4	0.497×10^4
Sandell's sensitivity (µg, cm^{2-})	0.055	0.048
Correlation coefficient ®	0.9998	0.9997
Regression equation	Y=0.0181x-0.0012	Y=0.0208x+0.0052
Slope(b)	0.0181	0.0208
Intercept(a)	-0.0012	0.0052
Analytical sensitivity µg/ml	0.047	0.048
Limit of detection (µg.ml^{-1})	0.182	0.159
Limit quantification (µg.ml^{-1})	0.552	0.481
C.L. for the slope (b±ts$_b$)at 95%	0.0181±0.0007576	0.0208±0.001224
C.L. for the intercept (a±ts$_a$ at 95%	-0.0012±0.02348	0.0052±0.037824
Standard error for regression line (Sy/x)	0.005965	0.009608

Table 5
The accuracy and precision of proposed method for estimation of commercial pharmaceuticals

Type of Drugs	Amount of drugs mg		Relative Error %	Recovery %	Average % Recovery	RSD% (n=5)
	Taken	Found				
Methyldopa Tablets 250mg product by Actavis UK	250	251.60 249.90 249.40	0.64 -0.04 -0.24	100.64 99.96 99.76	100.12	0.12 0.24 0.35
Methyldopa Tables250mg product byAlgorithm S.A.L. Zouk Mosbeh, Lebanon	250	250.70 252.10 249.80	0.28 0.84 -0.08	100.28 100.84 99.92	100.34	0.46 0.27 0.14
Salbutamol Tablets 2mg product by Actavis UK.	2	1.976 2.041 1.988	-1.20 2.05 -0.60	98.8 102.05 99.4	100.00	0.23 0.17 0.02
Salbutamol Tables 2mg Product Gulf Pharmaceutical Ras Al Khaimah, U.A.E.	2	1.989 2.020 1.978	-0.55 1.00 -1.10	99.5 101.00 98.90	99.80	0.21 0.04 0.21

Table 6
Effect of interference compound on pure drug.

Interference Compound	Recovery % Of methyldopa	Recovery % Of Salbutamol
Sucrose	99.76	100.10
Lactose	100.48	99.46
Maltose	100.62	99.62
Fructose	99.35	99.94
Starch	99.60	100.21

Table 7
Effect type of electrolyte salt

Type of salt	Abs. Methyldopa λ max 570 nm	Abs. Salbutamol λ max 495 nm
NaCl	0.245	0.216
CH_3COONa	0.316	0.367
Na_2SO_4	**0.814**	**0.818**
KCl	0.406	0.551

Table 8
Characteristic parameter for the regression equation of the proposed CPE method.

Parameter	Methyldopa	Salbutamol
λ max(nm)	570	495
color	Violet	Red
linearity range($\mu g.ml^{-1}$)	(0.25-6)	(0.25-6)
Molar absorptivity (L.mol1-.cm^{-1})	0.437×10^5	0.469×10^5
Sandell's sensitivity (µg, cm^{2-})	0.0048	0.005
Correlation coefficient ®	0.9997	0.9997
Regression equation	Y=0.207x – 0.044	Y=0.196x+0.0148
Slope(b)	0.207	0.196
Intercept(a)	-0.044	0.0148
Analytical sensitivity ($\mu g.ml^{-1}$)	0.505	0.656
Limit of detection ($\mu g.ml^{-1}$)	0.032	0.034
Limit quantification ($\mu g.ml^{-1}$)	0.097	0.102
Enrichment Factor(EF)	10.0	11.0
Pre-concentration factor(PF)	25	25
Distribution coefficient(D)	265.03	221.26
C.L. for the slope($b\pm ts_b$)at 95%	0.207 ±0.010815	0.196±0.008827
C.L. for the intercept($a\pm ts_a$) at 95%	-0.044±0.032515	0.0148±0.026551
Standard error for regression line ($S_{y/x}$)	0.0091	0.0074

Table 9
The accuracy and precision of proposed method for estimation of pure samples

Type of Drugs	Amount of drugs µg ml^{-1}		Relative Error %	Recovery %	Average Recovery%	RSD% (n=5)
	Taken	Found				
Methyldopa	2	1.98	-1.00	99.00		0.15
	5	4.99	-0.2	99.80	99.71	0.12
	6	6.02	0.33	100.33		0.03
Salbutamol	2	1.99	-0.5	99.50		0.06
	5	5.11	2.2	102.2	100.17	0.13
	6	5.93	-1.16	98.83		0.19

Table 10
The accuracy and precision of proposed method for estimation of commercial pharmaceuticals

Type of Drugs	Amount of drugs gm		Relative Error %	Recovery %	Average Recovery %	RSD% (n=5)
	Taken	Found				
Methyldopa Tablets		251.06	0.424	99.576		0.51
250mg product by	250	250.11	0.044	100.044	99.86	0.26
Actavis UK		249.89	-0.044	99.96		0.17
Methyldopa Tables		249.98	-0.008	99.99		0.10
250mg product by	250	250.20	0.08	100.08	100.036	0.16
Algorithm S.A.L.		250.10	0.04	100.04		0.18
Zouk Mosbeh, Lebanon						
Salbutamol Tablets		2.021	1.05	101.05		0.42
2 mg product by	2	2.052	2.6	102.60	101.11	0.21
Actavis UK.		1.994	-0.3	99.70		0.05
Salbutamol Tables 2mg	2	2.033	1.65	101.65	101.93	0.09
Product Gulf Pharmaceutical		1.988	-0.6	99.40		0.03
Ras Al Khaimah, U.A.E		2.095	4.75	104.75		0.22

Table 11
Characteristic parameter for the regression equation of the FIA method

Parameter	Methyldopa	Salbutamol
λ max(nm)	565	490
Color	Violet	Reddish-Orange
linearity range(µg.ml$^{-1)}$	(1-150)	(1-150)
Molar absorptivity (L.mol^{-1} cm^{-1})	0.172×10^4	0.157×10^4
Sandell's sensitivity (µg, cm^{2-})	0.124	0.152
Correlation coefficient ®	0.9998	0.9998
Regression equation	Y=0.0081x-0.0077	Y=0.0066x-0.0048
Slope(b)	0.0081	0.0066
Intercept(a)	-0.0077	-0.0048
Analytical sensitivity (µg.ml^{-1})	0.014	0.017
Limit of detection (µg.ml^{-1})	0.037	0.045
Limit quantification (µg.ml^{-1})	0.122	0.150
C.L. for the slope(b±ts$_b$)at 95%	0.0081±0.00343	0.0066±0.000259
Standard error for regression line (Sy/x)	0.00799	0.006021

Table 12
The accuracy and precision of proposed method for estimation of pure samples

Type of Drug	Amount of drugs µg /ml		Relative Error %	Recovery %	Average Recovery%	RSD% (n=5)
	Taken	Found				
Methyldopa	10 30 50	9.88 30.21 49.89	-1.2 0.7 -0.22	98.80 100.7 99.78	99.76	0.14 0.32 0.21
Salbutamol	10 30 50	10.09 29.89 50.26	0.9 -0.36 0.52	100.9 99.63 100.52	100.35	0.19 1.05 0.16

Table 13
The accuracy and precision of proposed method for estimation of commercial pharmaceuticals

Type of Drugs	Amount of drugs gm		Relative Error %	Recovery %	Average Recovery %	RSD% (n=5)
	Taken	Found				
Methyldopa Tablets 250mg product by Actavis UK	250	250.21 248.99 250.11	0.084 -0.404 0.044	100.084 99.59 100.04	99.90	0.27 0.04 2.68
ethyldopa Tables 250mg product by Algorithm S.A.L. Zouk Mosbeh, Lebanon	250	249.81 249.98 252.04	-0.076 -0.008 0.816	99.92 99.99 100.82	100.24	0.11 0.05 0.31
Salbutamol Tablets 2 mg product by Actavis UK.	2	2.02 1.96 2.03	1.00 -2.00 1.50	101.00 98.00 101.5	100.16	0.10 0.73 0.76
Salbutamol Tables 2mg Product Gulf Pharmaceutical Ras Al Khaimah, U.A.E	2	2.003 1.99 1.99	0.15 -0.50 -0.55	100.15 99.50 99.45	99.70	2.12 0.14 1.61

Table 14
The comparison of the proposed method with stander method using t and F- Statistical test at 95% confidence level.

Pharmaceutical preparation	Rec% Batch method	Value		Rec% FIA method	Value		Standard method [25]
		t	F		t	F	Rec %
Methyldopa pure	98.33	0.495 (2.131)	2.940 (19.00)	100.69	0.0951 (2.131)	18.72 (19.00)	101.17
Methyldopa Tablets250mg product by Actavis UKMethyldopa Tables	100.12			99.90			101.17
250mg product byAlgorithm S.A.L.Zouk Mosbeh, Lebanon	100.34			100.24			98.20
Salbutamol pure	100.70	1.757 (2.131)	2.522 (19.00)	100.42	1.716 (2.131)	4.237 (19.00)	100.00
Salbutamol Tablets 2mg product by Actavis UK.	100.00			100.16			99.30
Salbutamol Tables 2mg Product Gulf Pharmaceutical Ras Al Khaimah, U.A.E	99.80			99.70			98.50

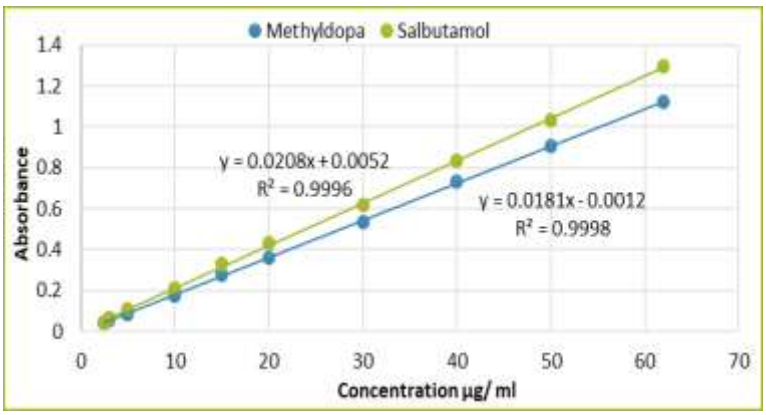

Figure 13: calibration graph of methyldopa and salbutamol in diazotization

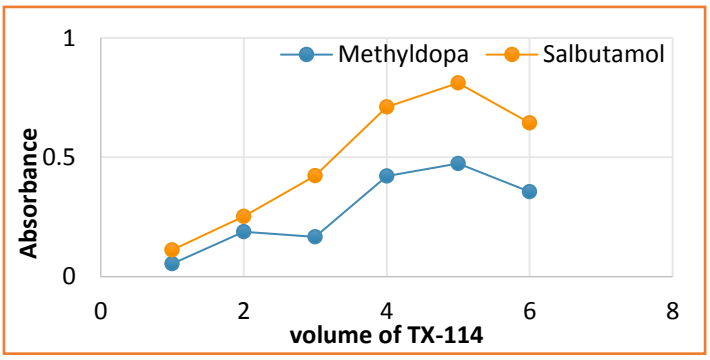

Figure 14: Effect volume of (10% v/v) Triton X-114

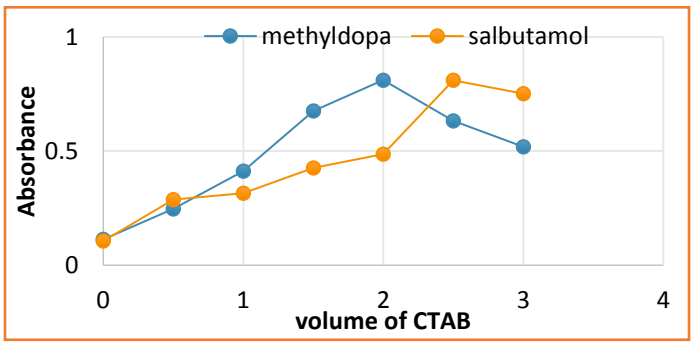

Figure 15: Effect volume of surfactant CTAB

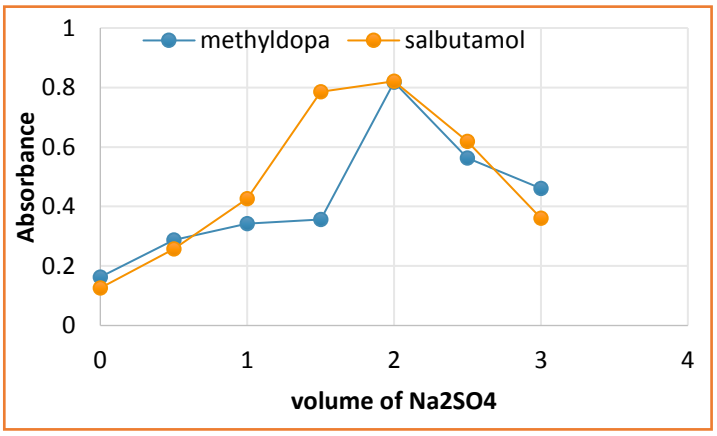

Figure 16: effect Volume of Na_2SO_4

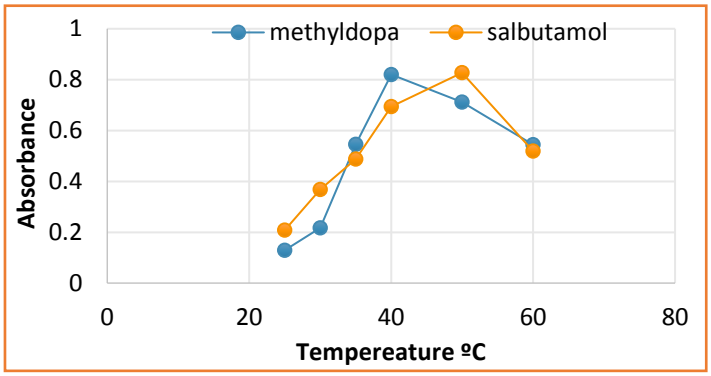

Figure 17: Effect of Temperature

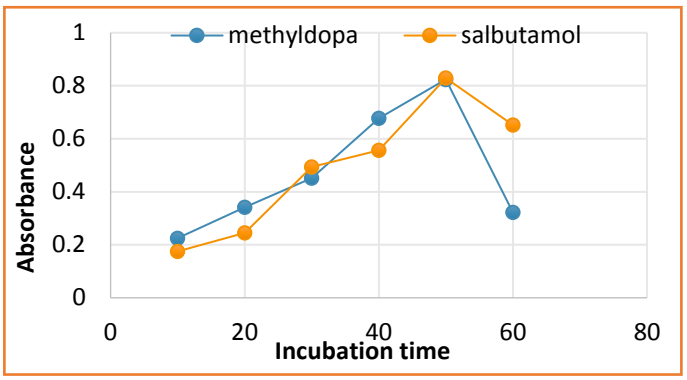

Figure 18: Effect of Incubation time

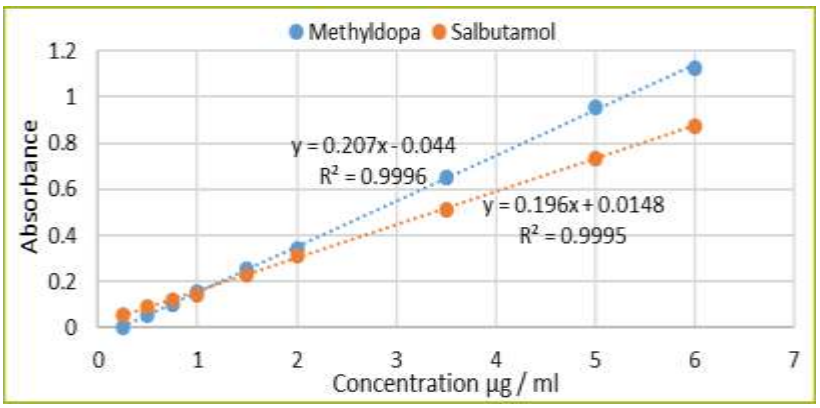

Figure 19: Calibration graph of cloud point extraction of methyldopa and salbutamol

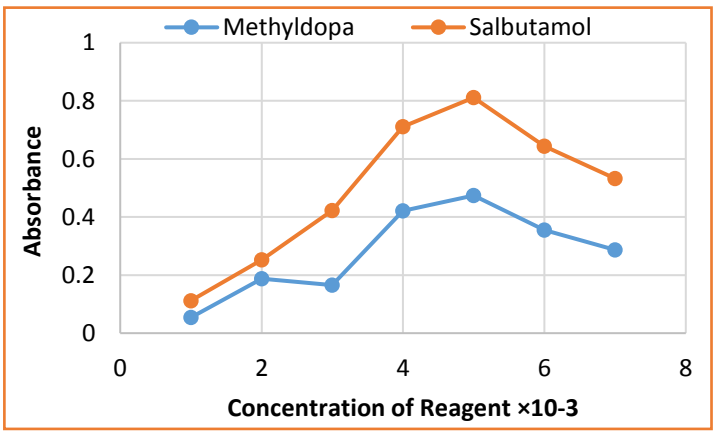

Figure 20: Effect concentration of 2-aminothiazole

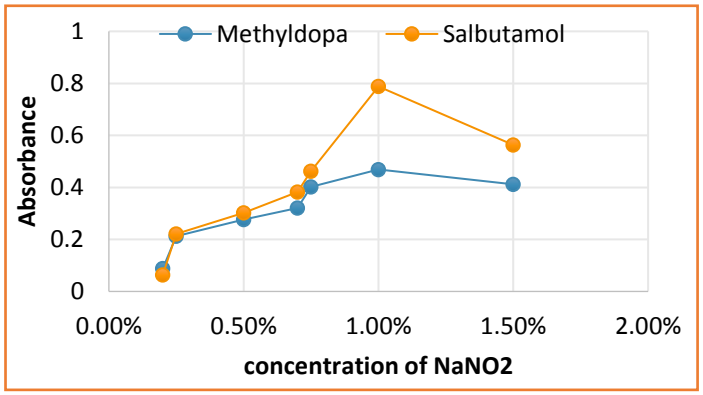

Figure 21: effect concentration of 1% $NaNO_2$

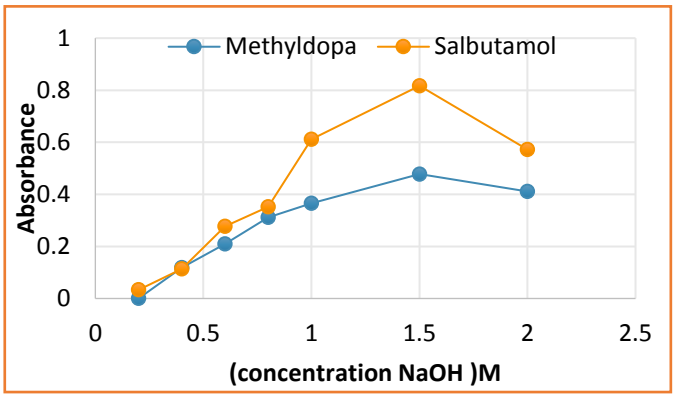

Figure 22: Effect concentration of NaOH

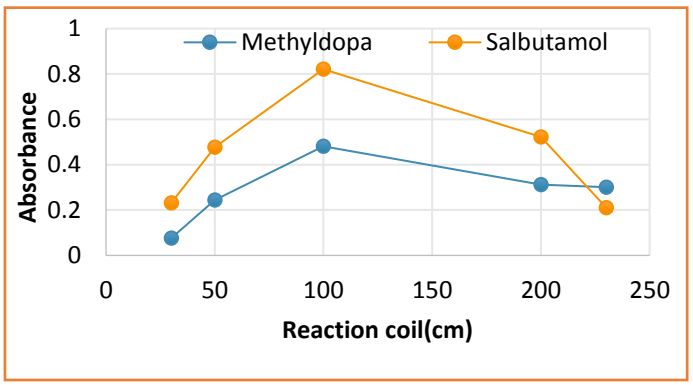

Figure 23: Effect of reaction coil

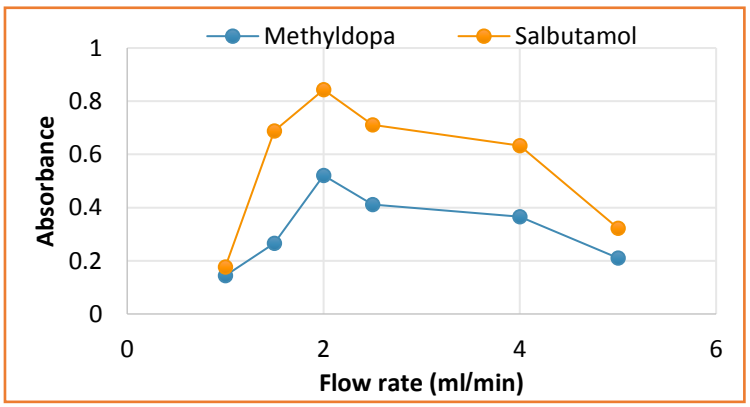

Figure 24: Effect of flow rate

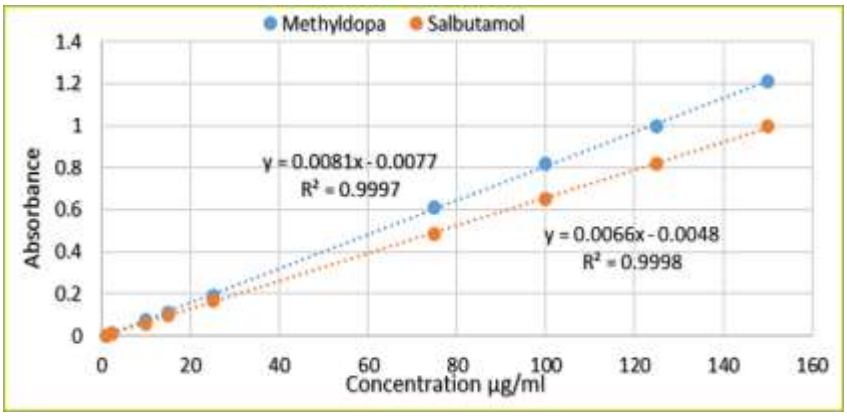

Figure 25: Calibration graph of Flow Injection of methyldopa and salbutamol

Conclusion

A simple, fast and sensitive spectrophotometric method has been developed for the determination of trace amounts of Methyldopa and Salbutamol with 2-aminothiazole. The first method including convention Methyldopa and Salbutamol to the colored product (Azo-dye) was measured by UV Visible Spectrophotometry. The second method pre-concentration of colored azo-dye and the third method FIA technique are very simple in this work and sensitive to determination the Methyldopa and Salbutamol. The proposed methods were used for the routine analysis of the drugs in the quality control.

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