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Research Article

Spectrophotometric Determination Methyldopa and Salbutamol by Oxidative Coupling, Cloud Point and Flow Injection in Pharmaceutical Formulations

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ABSTRACT

Three methods study in this research simple, sensitive, an expensive and rapid Oxidative Coupling reaction, Cloud Point Extraction and Flow Injection spectrophotometric methods for determination Methyldopa and Salbutamol, the first method oxidative coupling reaction between the Methyldopa and thiosemicarbazide in presence of ferric nitrate anhydrous to yield dark green colored product that have absorbance at λ_{max} 460 nm. Salbutamol coupling reaction with 4-nitrophenyl hydrazine in presence the potassium Iodide and sodium hydroxide to product the violet colored has absorbance at λ_{max} 530 nm. Beer's law is obeyed from (1-50) µg/ mL with sandall's sensitivity (0.039,0.073), correlation coefficient (0.9998) and molar absorptivity (0.536×10^4,0.327×10^4) for Methyldopa and Salbutamol respectively. The secondly method to estimation the trace amount of phenolic drugs that product from the oxidative coupling reaction it is cloud point extraction, cloud point extraction enables the drugs to be precisely estimated under the optimal experimental conditions, the maximum absorption at λ_{max} (470,535) nm respectively. The concentration was range (0.25-6) µg/mL, molar absorptivity 0.510×10^5,0.483×10^5 and enrichment factor (9.51,14.72) respectively for Methyldopa and Salbutamol .Flow injection analysis is simple method to determination the phenolic is based on the measurement of absorption signal for product resulting from oxidative coupling reaction , study all experimental parameters chemical and physical to development and stability the colored of product .Total flow injection of 1.5mL/min was pumped and active material was detect at λ_{max} (460,530)nm respectively for Methyldopa and Salbutamol . In this the proposed methods were suxciffuly, applied to the determination Methyldopa and Salbutamol in pharmaceutical preparation.

Keywords: Oxidative Coupling, Phenolic Drugs, Flow Injection, Cloud Point Extraction, Spectrophotometric.

INTRODUCTION

Methyldopa is (β-3,4-dihydroxy phenyl-α-methyl alanine MD), It's a white to yellow-white powder or almost colorless, soluble in warm distilled water[1,2], Methyldopa have various pka value depend on the functional group that found in the structure such as $-NH_2$, -OH and COOH[3,4] the structure fig.1, several methods to estimation Methyldopa in pharmaceutical preparation titrimetric method, fluorimetry, kinetic measurement, potentiometric and flow injection method[5,6]. Salbutamol Sulfate (SBS) as (rs)-1-(4-hydroxy3-hydroxymethylphenyl)-2-(tert-butyl amino) ethanol sulfate, the salbutamol also known albuterol[7] fig. 2. Salbutamol is acted as it cardiovascular and bronchodilator in fluencies are smaller than it bronchodilator action drugs which is a common entity in high performance[8]. Salbutamol is prevalently used by athletes, anti-doping agent[9], Salbutamol in pharmaceutical used in various way to estimation oxidative coupling, diazotization and coupling[10], intrastation, nitration, charge transfer[11], HPLC, Electrophoresis, Gas chromatography and spectrophotometric method[12]. Oxidative Coupling reaction its reaction between two or more from organic compounds in presence of oxidant agent and the color product can be determining by different methods such as spectrophotometric, polar graphic and chromatographic[13,14], oxidative coupling are applied successfully in many fields such as pharmaceutical agricultural[15]. Cloud Point Extraction (CPE) it's introduced by watanbe and tanaka in 1978[16],CPE used to estimation various trace metals and drugs, the having considerable advantages such as simplicity cheapness, fast selectivity ,high enrichment factor and low toxicity to the environment than other extraction method that used the best organic solvents[17,18].Flow injection it's best technique characterized as simple, cheap, fast and selectivity to estimate the drugs[19,20], Flow injection analysis (FIA) system have many interest such as increase sample throughput, low reagent consumption, reduce waste production, and inexpensive equipment[21,22].

EXPERIMENTAL

Instruments

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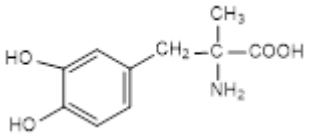

Figure 1: Methyldopa

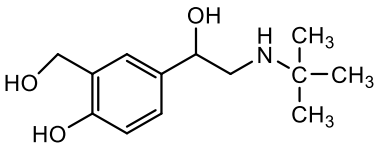

Figure 2: Salbutamol

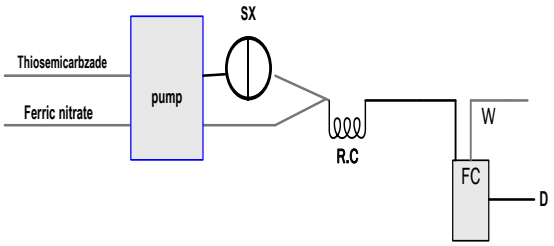

Figure 3

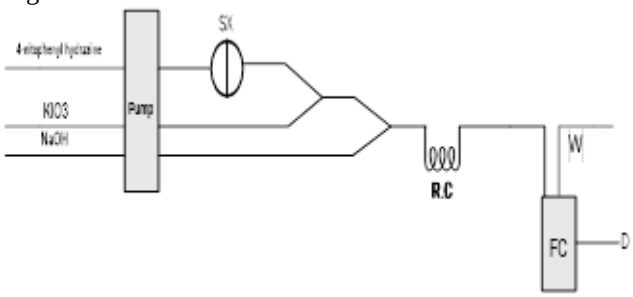

Figure 4

Figure 3,4: Scheme of employed flow system, P: peristaltic pump, R.C: reaction coil, S: sample injection, W: waste, FC: flow cell

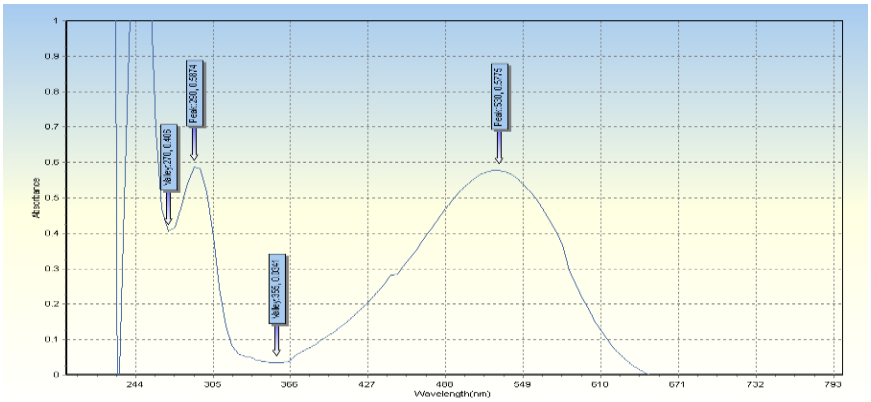

Figure 5: Absorption spectrum of oxidative coupling for methyldopa with the reagent against the reagent blank under optimum conditions.

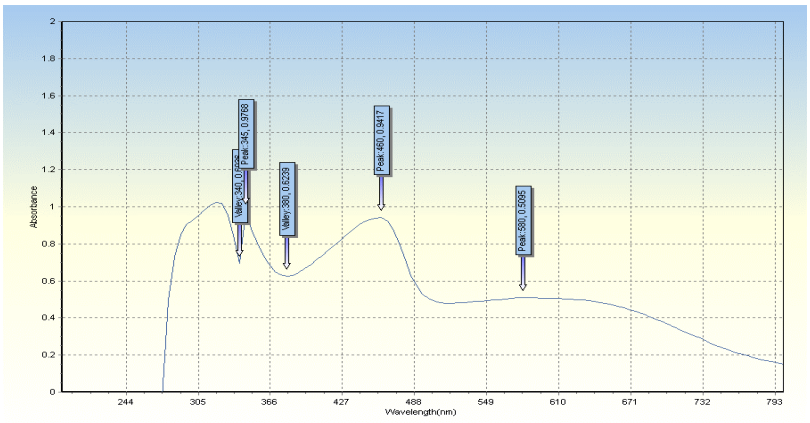

Figure 6: Absorption spectrum of oxidative coupling for Salbutamol with the reagent against the reagent blank under optimum conditions.

All spectral and absorbance measurement were carried in signal beam UV Visible spectrophotometer 160. Equipped with 1cm and 0.5 cm quarts' cell. An Ultrasonic and thermostatic water bath from Elma Hans Schmidbauer Gmbh and Co.KG used coupled with Extraction of samples. A centrifuge (Shanghai surgical instrument factory,80-2, Shanghai, Germany) that use to complete the separation of CPE, PH meter, type inoLap 7110 and Electronic Balance Mettle AE 200. The Flow injection configuration A three channel manifold Fig.3 for Methyldopa and Fig.4 for Salbutamol was employed for (FIA). Peristaltic pump (ALITEA, C4, made in Sweden) with polyvinyl chloride tube (0.8) mm internal diameter was used for the peristaltic pump and Reaction Coil

Reagents

Table 1: Effect order addition.

No. order	Order addition of Methyldopa	Absorbance of Methyldopa	Order addition of salbutamol	Absorbance of Salbutamol
1	O+R+D	0.067	R+O+D+B	0.089
2	O+D+R	0.084	R+O+B+D	0.090
3	R+D+O	0.133	R+D+O+B	0.512
4	D+R+O	0.879	D+R+O+B	0.322
5	R+O+D	0.341	D+O+R+B	0.311

D=Drug, R=Reagent, O=Oxidant, B=Base

Table 2: Characteristic parameter for the regression equation of the proposed oxidative coupling method for methyldopa and salbutamol.

Parameter	Methyldopa	Salbutamol
λ max(nm)	460	530
color	Green	Violet
linearity range µg/mL	1-50	1-50
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	0.536×10^4	0.327×10^4
Sandell's sensitivity ($\mu g/cm^2$)	0.039	0.073
Correlation coefficient ®	0.9998	0.9998
Regression equation	Y=0.0254x - 0.0223	Y=0.0137x-0.0065
Slope(b)	0.0254	0.0137
Intercept(a)	-0.0223	-0.0065
Analytical sensitivity ($\mu g.ml^{-1}$)	0.065	0.064
Limit of detection (µg/mL)	0.118	0.218
Limit quantification µg/mL	0.389	0.729
C.L. for the slope($b\pm ts_b$)at 95%	0.0254±0.00968	0.0137±0.00472
C.L. for the intercept($a\pm ts_a$) at 95%	0.0223±0.01984	0.0065±0.01124
Standard error for regression line ($S_{y/x}$)	0.005612	0.00318

All Chemicals were analytical grade. stock solutions (1000 µg/mL) Methyldopa and Salbutamol were prepared by dissolving 0.1 gm of Methyldopa and Salbutamol in distilled water and dilution to the mark in 100mL volumetric flask. Stock solution of thiosemicarbzide (1000 µg/mL) were prepared by dissolving 0.1 g of thiosemicarbazide in distilled water and dilution to the mark in 100mL volumetric flask.

-preparation 1 M NaOH was prepared by dissolving 10 g of NaOH in 250 mL distilled water, 4-nitro phenyl hydrazine 0.2M was prepared by dissolving 3.06g in 2mL of concentrated Sulfuric acid in 100mL distilled water, 0.1 M KIO_3 was prepared by dissolving 2.14 g of KIO_3 in 100 mL distilled water, $Fe(NO_3)_3.9H_2O$ (0.1) M was prepared by dissolving 2.41g in 1mL from HNO_3 in 100mL distilled water, 10% Triton X-114,0.01M of CTBA (0.3644gm in 100 mL in distilled water and 5% w/v Na_2SO_4.

General procedure of Oxidative Coupling

The best method was developed to prepared oxidative coupling reaction of Methyldopa by adding 1mL ,1000 µg/mL Methyldopa,1mL ,1000 µg/mL thiosemicarbazide and 1mL (0.1) M $Fe(NO_3)_3.9H_2O$, that gave the dark green colored at λ_{max} (460) nm. Salbutamol (1ml,1000 µg/mL coupling with 1 mL ,0.2 M 4-nitrophenyl hydrazine, 1 mL, 0.1 M KIO_3 in presence 0.5ml ,1M NaOH that gave violet colored at λ_{max} (530) nm, finally placed in 20 mL flask complete with distilled water. The oxidative coupling formed absorbance dark green, violet colored at λ_{max} (460.530) nm for Methyldopa and Salbutamol respectively.

General Procedure of Cloud Point Extraction for Phenolic Drugs

The various concentration (0.25-6) µg/mL from the dye of oxidative coupling reaction result transfer 1mL from this product into 15mL of centrifuge tubes ,10% v/v Triton X-114 1 mL add, 2 mL of 0.01 M of surfactant (CTBA) , 2 mL from 5% Na_2SO_4 respectively, after that complete the volume 12.5 mL with distilled water. Test tube put into ultrasonic thermostatic water bath let it 2 mint to mix the mixture and water bath 40,50ºC for 50 min. Formation Cloudy and it is separation into two phase after that transfer to centrifuge to complete separation two-layer organic phase and aqueous phase, decant the aqueous and add 0.5 mL ethanol to organic phase to dissolve the dye. 1cm cell was used to determination the dye, prepared the blank under in the same way.

General Procedure of Flow Injection

A Methyldopa and Salbutamol solution was prepared by different concentration 1-150 µg/mL from the standard working solution 1000 µg/mL. A 100µL portion Methyldopa was injected into stream of 0.2 M thiosemicarbazide the mixture reacted with stream (1.5×10^{-2}) M $Fe(NO_3)_3.9H_2O$, the reaction is carryout via mixed well in 50 cm reaction coil Figure3. Salbutamol 100µL was injected into carrier stream which product from mixing three channel the first channel carrier (0.2) M 4-nitrophenyl hydrazine the mixed react with oxidative agent 0.1 M KIO_3, then the mixture reacts with third channel stream 2 M NaOH the product carryout via mixed well 50 cm reaction coil Figure4, the resulting of Methyldopa and Salbutamol dark green and violet at λ_{max} (460 ,530) nm respectively.

Procedure for Pharmaceutical preparations

Methyldopa

Tables provide from UK and from Lebanon were weight, finely powder from each type then dissolved in distilled water and filtered a solution to separate the ono-dissolved

Table 3: 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	5	4.88	-2.4	97.6	99.48	0.19
	15	15.21	1.4	101.4		0.12
	20	19.89	-0.55	99.45		0.21
Salbutamol	5	5.09	1.8	101.8	100.79	0.15
	15	14.89	-0.73	99.27		0.98
	20	20.26	1.3	101.3		0.18

Table 4: The accuracy and precision of proposed method for estimation of commercial pharmaceutical

Type of Drugs	Amount of drugs mg		Relative Error %	Recovery %	Average Recovery %	RSD% (n=5)
	Taken	Found				
Methyldopa Tablets		252.60	1.04	101.04		0.14
250mg product by	250	249.95	-0.02	99.98	100.29	0.24
Actavis UK		249.66	-0.136	9986		0.15
Methyldopa Tables250mg		251.72	0.69	100.69		0.36
product byAlgorithm S.A.L.	250	250.10	0.04	100.04	100.17	0.17
Zouk Mosbeh, Lebanon		249.48	-0.21	99.79		0.44
Salbutamol Tablets		1.988	-0.6	99.4		0.63
2mg product by	2	2.033	1.5	101.5	99.33	0.27
Actavis UK.		1.942	-2.9	97.1		0.31
Salbutamol Tables 2mg		1.975	-1.25	98.75		0.11
Product Gulf Pharmaceutical	2	2.060	3.00	103.00	99.88	0.74
Ras Al Khaimah, U.A.E		1.958	-2.10	97.9		0.11

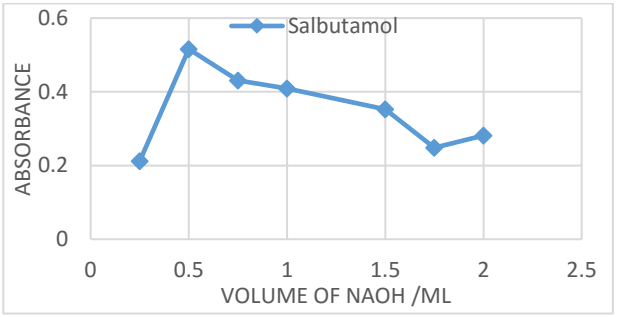

Figure 7: Effect volume of NaOH

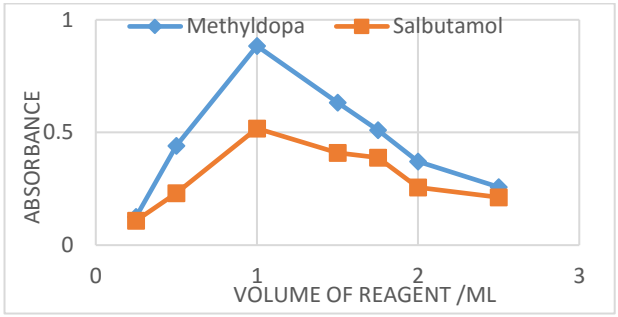

Figure 8: Effect volume of reagent

Table 5: Effect of interference compound on pure drug.

Interference compound	Recovery % Of Methyldopa	Recovery % Of Salbutamol
Sucrose	99.88	100.66
Lactose	100.28	99.76
Maltose	101.62	100.62
Fructose	99.94	99.98
Sodium Benzoate	98.99	99.44
Starch	101.60	101.21

component, the solution transfers into 100mL flask and complete with distilled water.

Salbutamol

Tablets from UK 2mg Tables and from Salbutamol Tables 2mgProduct Gulf Pharmaceutical Ras Al Khaimah, U.A.E that carefully weighed, average weight of tables was extracted. The mean weights were finely powder equivalent weight dissolve into distilled water and complete solubility transfer to flask 100mL.

RESULTS AND DISCUSSION

The preliminary investigation shows that the oxidative coupling reaction of Methyldopa with thiosemicarbazide in presences oxidizing agent to product color dye dark green, Salbutamol oxidative coupling reaction with 4-nitrophenyl hydrazine, oxidizing agent in presences sodium hydroxide to product the violet colored at λ_{max} (460 ,530) nm respectively. Fig.5 and Fig.6 The absorption spectrum of the product against reagent blank.

Study Optimization Reaction of Oxidative Coupling Reaction

Different parameters that effect on the intensity colored dye have been select such as order addition, natural of medium, temperature effect, concentration of coupling reagent, amount of oxidizing agent, effect the concentration sodium hydroxide and oxidation time.

Table 6: Characteristic parameter for the regression equation of the proposed CPE method.

Parameter	Methyldopa	Salbutamol
λ max(nm)	470	535
color	Yellow	Violet
linearity range µg/mL	0.25-6	0.25-6
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)	0.510×10^5	0.483×10^5
Sandell's sensitivity µg/ cm^2	0.004	0.005
Correlation coefficient ®	0.9997	0.9997
Regression equation	Y=0.2415x – 0.0698	Y=0.2017x-0.0563
Slope(b)	0.2415	0.2017
Intercept(a)	-0.0698	-0.0563
Analytical Sensitivity µg/mL	0.480	0.480
Limit of detection µg/mL	0.024	0.029
Limit quantification µg.mL	0.079	0.098
Enrichment Factor(EF)	9.51	14.72
Preconcentration factor(PF)	25	25
Distribution coefficient	265.03	221.26
C.L. for the slope($b\pm ts_b$)at 95%	0.2415±0.0137	0.2017±0.0123
C.L. for the intercept($a\pm ts_a$) at 95%	-0.0698±0.0414	-0.0563 ±0.03696
Standard error for regression line ($S_{y/x}$)	0.011583	0.01034

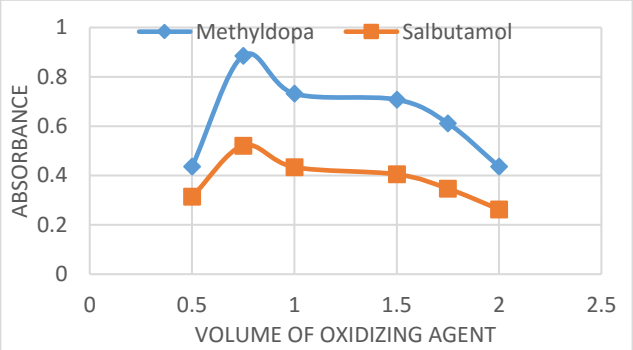

Figure 9: Effect volume of oxidizing agent.

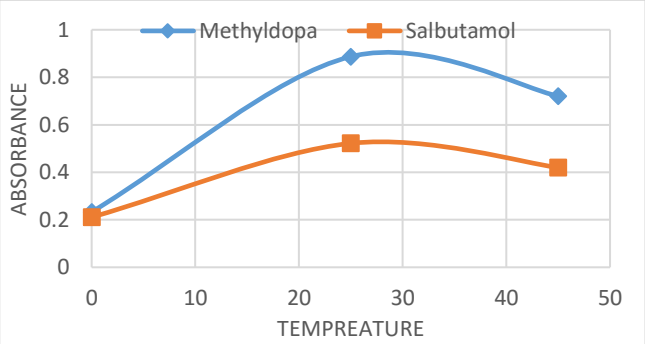

Fig.10: Effect Temperature

Various order addition was studied and it is found the sequence addition (D+R+O), (R+D+O+B) for Methyldopa and Salbutamol respectively, these gave the higher absorption of colored, so it depended into experiments the results in Table 1.

The oxidative coupling reaction effect by the natural of medium, different medium (acidity, basicity and neutral) was studied, the results were oxidative coupling for the Methyldopa was accrue in neutral medium and Salbutamol oxidative coupling reaction accrue in basicity medium these result gave the best intense colored product at λ_{max} (460 ,530) nm. Study effect different volumes (0.25-2) mL, 1M of NaOH for Salbutamol in 20 mL volumetric flask with keeping other conditions constant to improve the Oxidative coupling reaction efficiency. In this volume of base therefore used in subsequent studies, the result in figure 7.

Difference volumes (0.25-2.5) mL of oxidative reagent (0.01) M thiosemicarbazide, (0.2) M 4-nitrophenylhydrazine for Methyldopa and Salbutamol respectively was studied and the best volume of reagent to give the highest intense colored of product was 1 ml, the results in Figure 8.

The best t volume oxidizing agent $Fe(NO_3)_3.9H_2O$ (0.1) M, KIO_3(0.1) M was studied by adding various volumes (0.25-2) ml of oxidizing agent to volumetric flask 20 ml, the best volume gave highest absorbance was 1.5 ml, the results in Figure 9. The effect of temperature using different temperature (0, 25, 45) ºC, with keeping other conditions constant to improve the Oxidative coupling reaction efficiency show Figure 10.

Analytical Data

Under the optimized of oxidizing coupling reaction for phenolic drugs, preparation the calibration graph by using various concentration (1-50) µg /mL for the Methyldopa and Salbutamol were conducted and the linearly regression equation correlation to determination (R), slop (a) and intercept (b).

Accuracy and Precision

The accuracy and precision were studied under the optimal conditions by measuring absorption n=5 at λ_{max} (460, 530) nm for Methyldopa and Salbutamol respectively for three different concentration of the drugs. The accuracy estimated by determination the relative error, percentage and Recovery. Precision estimate

Table 7: The accuracy and precision of proposed method for estimation of pure sample

Type of Drugs	Amount of drugs µg/ mL		Relative Error %	Recovery%	Average Recovery%	RSD% (n=5)
	Taken	Found				
Methyldopa	2	1.95	-2.5	97.50		0.16
	5	4.96	-0.80	99.20	99.57	0.18
	6	6.12	2.00	102.00		0.09
Salbutamol	2	1.97	-1.50	98.50		0.16
	5	5.09	1.80	101.8	99.98	0.23
	6	5.98	-0.33	99.67		0.15

The reaction path for Phenolic drugs may be written

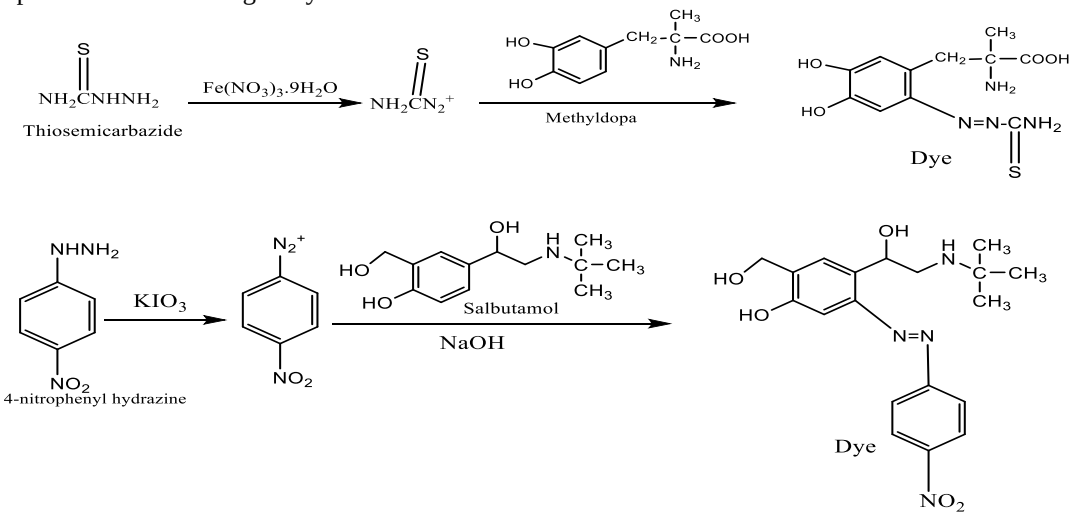

Figure 11: The suggest mechanism of reaction colored.

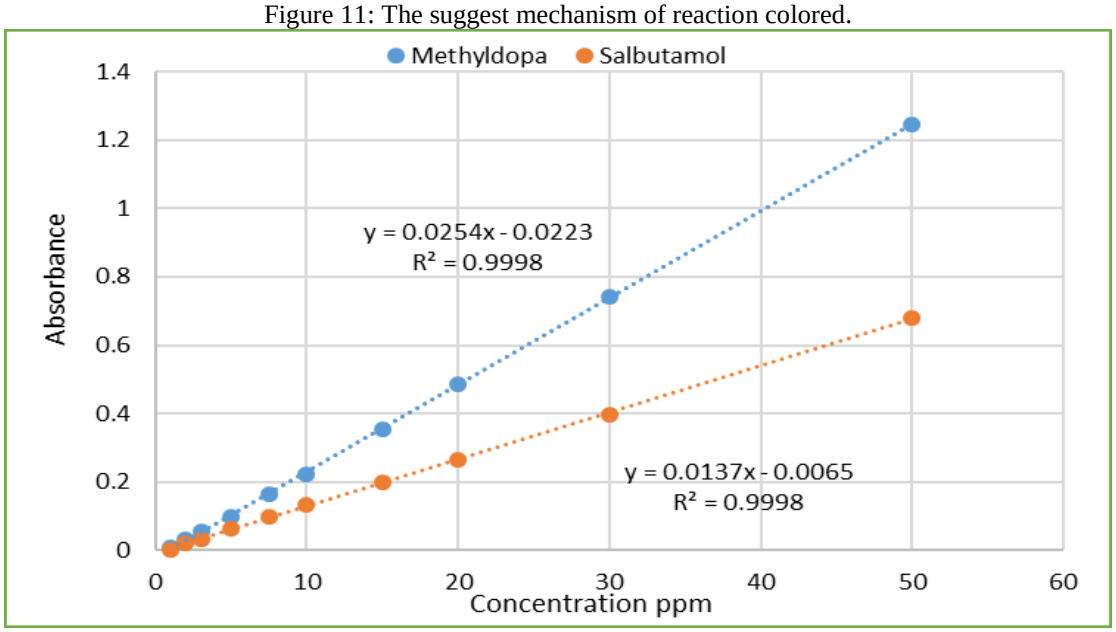

Figure 12: calibration graph of methyldopa and salbutamol in oxidative coupling method.

determination for the percentage relative standard deviation RSD%, as shown Table 3&4.

Effect of Interference

Interference expected was study for each drug Methyldopa and Salbutamol, have been studied the method to prepare the dye selectivity under optimum conditions and added 1ml (1.0 g,10000ppm) from interference such as Lactose, Sodium benzoate, Starch, Maltose, Fructose and Sucrose] with 1ml (0.1g, 1000 ppm) from each drug and the rest of addition are optimal conditions then diluted with distilled water in 20ml volumetric flask after that measured the absorbance, Table 5.

Optimization of Cloud Point Extraction for Phenolic Drugs

Table 8: 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	252.06		0.82	100.82		0.41
250mg product by	250	249.11	-0.36	99.64	100.10	0.56
Actavis UK	249.59		-0.16	99.84		0.47
Methyldopa Tables	249.88		-0.05	99.95		0.16
250mg product by	250	250.88	0.35	100.35	99.90	0.26
Algorithm S.A.L.	248.50		-0.60	99.40		0.12
Zouk Mosbeh, Lebanon						
Salbutamol Tablets	1.97		-1.50	98.50		0.45
2 mg product by	2	2.02	1.00	101.00	99.00	0.321
Actavis UK.	1.95		-2.50	97.50		0.15
Salbutamol Tables 2mg	2.01		0.50	100.50		0.19
Product Gulf Pharmaceutical	2	1.97	-1.50	98.50	100.00	1.91
Ras Al Khaimah, U.A.E	2.02		1.00	101.00		0.82

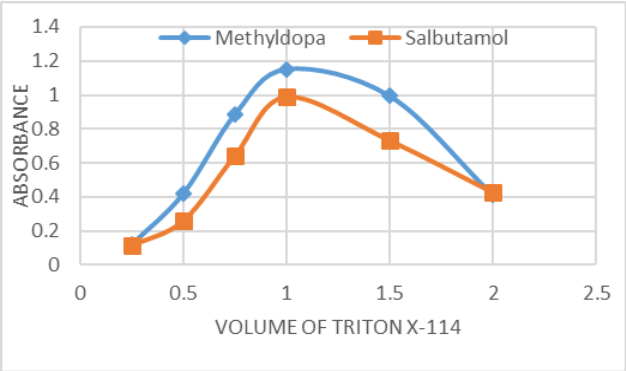

Figure 13: Effect volume of Triton X-114

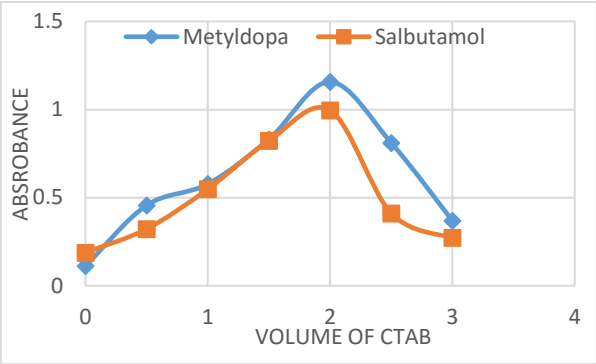

Figure14: Effect of cationic surfactant

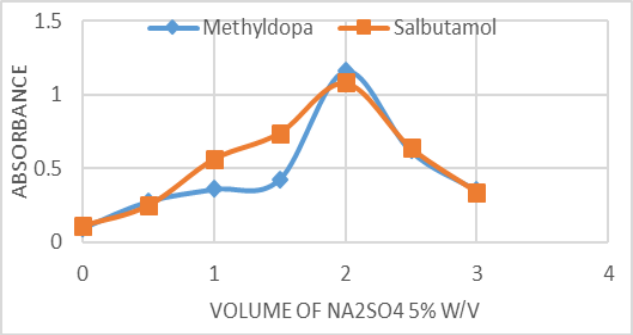

Figure 15: Effect of Na_2SO_4 5% w/v

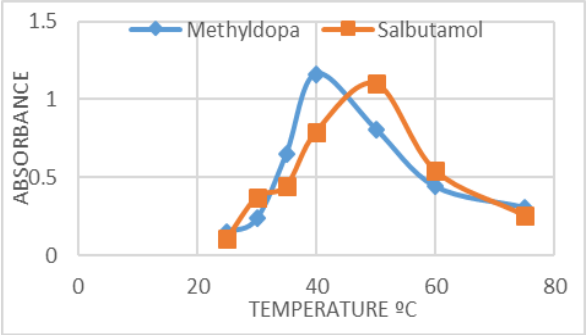

Figure16: Effect of equilibrium temperature

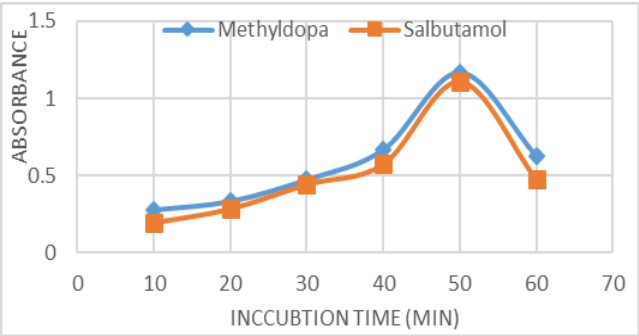

Figure 17: Effect of incubation time

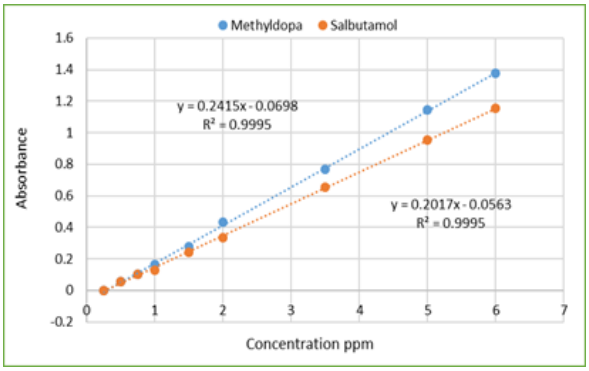

Figure 18: Calibration graph of cloud point extraction of methyldopa and salbutamol

Different parameter that effect on the intensity absorption of colored cloud point extraction such as Triton X-114, surfactant (CTBA), salt electrolyte, amount of salt, equilibrium temperature and Incubation time.

Different volumes of 10% Triton X-114 (0.25-2) mL studied to find the prefer volume of Triton X-114 was 1 mL the results in Figure13. (0.01) M Cationic surfactant was add, the various volume (0-3) mL of (CTBA) was studied the 2 ml of cationic was the best increase the efficiency of separation and increase the intensity absorbance, the results in Fig.14. Different salts (Sodium Sulphate, Sodium Chloride, Sodium acetate and Potassium Chloride) it studied at 5% the best electrolyte to cloud point extraction was Na_2SO_4. Various volumes (0- 3) mL of the salt was studied and the best volume 2 mL of salt Na_2SO_4 was gave the intents colored for Methyldopa and Salbutamol respectively, the results in Fig. 15. Influence the equilibrium temperature important formation the (CPE), prepare the dye need to heat for formation two phase, when the temperature increase above the required limited negatively that effect on the efficiency extraction, best temperature to give highest extraction for Methyldopa and Salbutamol was 40 and 50 ^oC respectively. The results in Fig.16. Incubation time of

Table 9: Characteristic parameter for the regression equation of the FIA method.

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

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

Parameter	Methyldopa	Salbutamol
λ max(nm)	460	530
Color	Green	Violet
linearity range µg/mL	1-200	1-200
Molar absorptivity ($L.mol^{-1}$ cm^{-1})	0.149×10^4	0.138×10^4
Sandell's sensitivity(µg/ cm^2)	0.141	0.172
Correlation coefficient	0.9998	0.9997
Regression equation	Y=0.0071x-0.005	Y=0.0058x-0.0024
Slope(b)	0.0071	0.0058
Intercept(a)	-0.005	-0.0024
Analytical sensitivity µg/ mL	0.014	0.014
Limit of detection µg/mL	0.0422	0.052
Limit quantification µg/mL	0.139	0.171
C.L. for the slope($b\pm ts_b$)at 95%	0.0071±0.00003	0.0058±0.00031
C.L. for the intercept($a\pm ts_a$) at 95%	-0.005±0.0294	-0.0024±0.2982
Standard error for regression line ($S_{y/x}$)	0.008039	0.008147

Table 13: 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		250.21	0.084	100.084		0.27
250mg product by	250	248.99	-0.404	99.59	99.90	0.04
Actavis UK		250.11	0.044	100.04		2.68
Methyldopa Tables		249.81	-0.076	99.92		0.11
250mg product by	250	249.98	-0.008	99.99	100.24	0.05
Algorithm S.A.L.		252.04	0.816	100.82		0.31
Zouk Mosbeh, Lebanon						
Salbutamol Tablets		2.02	1.00	101.00		0.10
2 mg product by	2	1.96	-2.00	98.00	100.16	0.73
Actavis UK.		2.03	1.50	101.5		0.76
Salbutamol Tables 2mg		2.003	0.15	100.15		2.12
Product Gulf Pharmaceutical	2	1.99	-0.50	99.50	99.70	0.14
Ras Al Khaimah ,U.A.E		1.99	-0.55	99.45		1.61

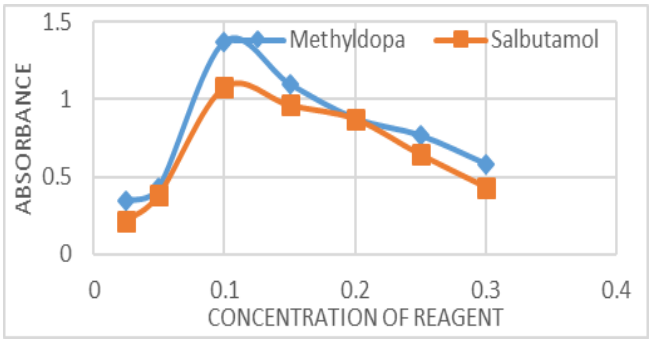

Figure 19: Effect concertation of reagent

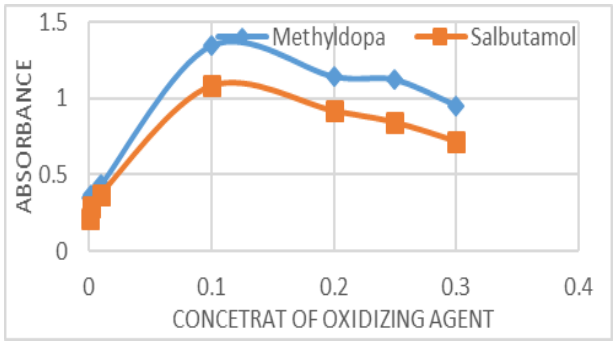

Figure 20: Effect concentration of oxidizing agent

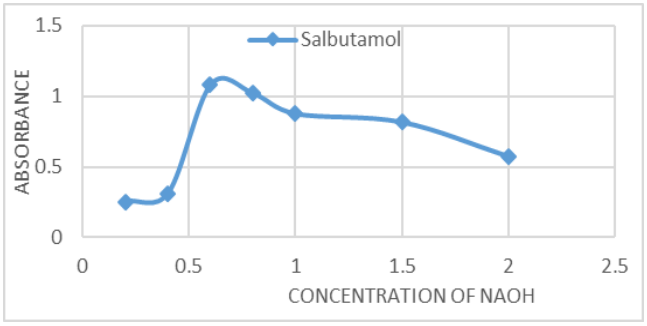

Figure 21: Effect concertation of NaOH

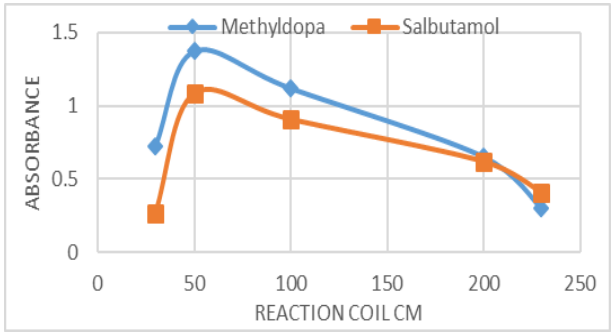

Figure 22: Effect of reaction coil

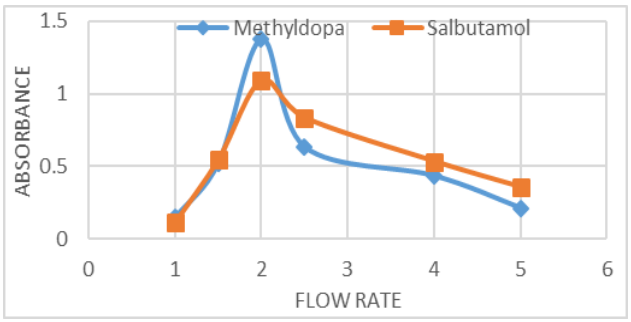

Figure 23: Effect of flow rate

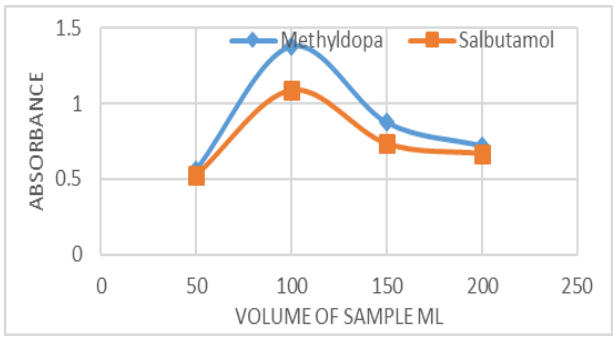

Figure 24: Effect volume of sample

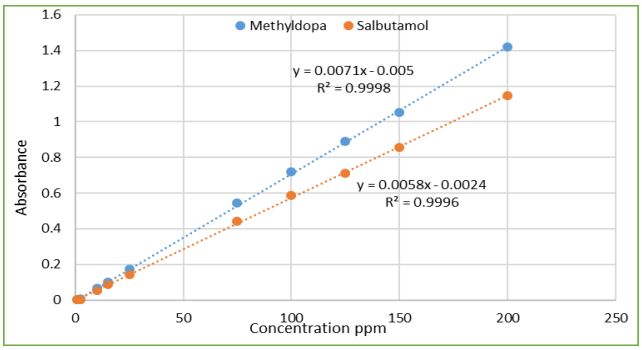

Figure 25: Calibration graph of Flow Injection of methyldopa and salbutamol

the extraction efficiency lead to increase the size of surfactant-rich phase, that increase the distribution ratio D and enrichment factor. Different time to incubation 10-60 min was studied the results in Fig. 17.

Calibration graph of the Cloud Point Extraction for Phenolic Drugs

Under optimal experimental conditions of CPE the calibration curve for the Methyldopa and Salbutamol, the linear calibration graph was established by plotting using various concentrate of phenolic drugs (0.25-6) µg /mL calibration graph was gained the linearly regression equation, correlation to determination (R), slop (a) and intercept (b). Figure 18.

Accuracy and Precision

The accuracy and precision of proposed methods under the optimal conditions were study by analyze five

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

Pharmaceutical preparation	Proposed methods						Standard method[23]
	Rec% Batch method	Value		Rec% FIA method	Value		Rec %
		t	F		t	F	
Methyldopa pure	98.99			98.98			100.23
Methyldopa Tablets250mg product by Actavis UK Methyldopa Tables 250mg product by Algorithm S.A.L.Zouk Mosbeh, Lebanon	100.29	0.335 (2.131)	9.244 (19.00)	99.90	0.641 (2.131)	7.611 (19.00)	99.88
	100.17			100.24			99.78
Salbutamol pure	99.89			99.87			100.44
Salbutamol Tablets 2mg product by Actavis UK.	99.33	1.545 (2.131)	1.119 (19.00)	100.16	0.831 (2.131)	1.695 (19.00)	99.96
Salbutamol Tables 2mg Product Gulf Pharmaceutical Ras Al Khaimah ,U.A.E	99.88			99.70			99.88

replicate of pure samples and pharmaceutical for three different concentrations the accuracy estimate for relative error percentage and Recovery. Precision used to estimation the RSD%, Table 7 &8.

Optimum Reaction Conditions of Flow Injection for determinate the Phenolic Drugs

Study Optimization of Chemical Parameter

Study all optimal conditions of the chemical parameters that include the concentration of reagent, concentration of oxidizing agent and concentrate of sodium hydroxide. Different concentration (0.025-0.3) M thiosemicarbazide, 4-nitro phenyl hydrazine for the Methyldopa and Salbutamol respectively was studied the highest intense colored was into (0.1) M, the results in Fig. 19. different concentration (1×10^{-3}- 3×10^{-1}) M of oxidizing agent $Fe(NO_3)_3.9H_2O$ and KIO_3 (0.1) M was the best concentrate of oxidizing agent respectively for Methyldopa and Salbutamol, the results in Fig.20. The Flow injection for the Methyldopa accrue in neutral medium, Salbutamol accrue into basicity medium the Sodium hydroxide use as the best base. Various concentrate of sodium hydroxide (0.2 – 2) M was studied the highest absorbance was into (0.6) M, the result in Fig.21.

Study Optimization of manifold Parameters

Difreent physical parameters was study such as reaction coil, length of the reaction coil range (30-230) cm, 50 cm the was best reaction coil gave high best absorbance at λ_{max} (460,530) for Methyldopa and Salbutamol Respectively Fig.22. Total flow rate (1-5) mL/ min was studied, best flow rate gave highest absorbance was 2 mL/ min, the result in Fig.23. The several volume of the injection sample studied between (50-200) µL, 100 µl volume best volume to give highest absorbance and was use in all subsequent experiments, the results in Fig.24

Calibration graph of Flow Injection for phenolic drugs

Under optimal experimental conditions, the curve of flow injection was prepared by plotting absorbance versus concentration of methyldopa and salbutamol 1- 200 µg/ mL, Fig.23. Characteristic parameter for the regression equation of the FIA method shown in Table9.

Accuracy and Precision

Study the accuracy and precision for proposed method, under optimum experimental conditions, three different concentrations, measured absorbance at a minimum for five readings perconcentration. Tables10 & 11. for commercial pharmaceuticals. The statistical analysis results exhibited in Table 12 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.

CONCLUSION

The application of oxidative coupling reaction of oxidative the phenolic drugs describe that the results of proposed method was simple and reasonable accuracy and precision for estimate Methyldopa and Salbutamol. Cloud point extraction was the best pre-concentration of trace drugs that advantages no toxicity, simple and cheap to estimate the phenolic drugs. Flow injection method is very simple in this work it is have various advantages such as low reagent consumption, low time to analysis and an inexpensive equipment. The proposed methods (Oxidative coupling reaction, Cloud point extraction and Flow injection) offered a good linearity, accuracy and precision and can be satisfactorily applied to the analysis for phenolic drugs in pharmaceutical formulation and used for routine analysis of drugs in quality control.

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