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Original Article

DETERMINATION OF ETILEFRINE HYDROCHLORIDE, FENOTEROL HYDROBROMIDE, SALBUTAMOL SULPHATE AND ESTRADIOL VALERATE USING SURFACE PLASMON RESONANCE BAND OF SILVER NANOPARTICLES

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ABSTRACT

Objective: The aim of this work was to evaluate a simple, sensitive, effective and validated procedure for the determination of etilefrine hydrochloride, fenoterol hydrobromide, salbutamol sulphate and estradiol valerate.

Methods: In this study the method based on the ability of the cited drugs to reduce Ag^{+}ions to silver nanoparticles (Ag-NPs) in the presence of polyvinyl pyrrolidone (PVP) as a stabilizing agent producing very intense surface plasmon resonance peak of Ag-NPs (λ_{max} = 417-425 nm). The plasmon absorbance of the Ag-NPs allows the quantitative spectrophotometric detection of the cited drugs.

Results: The calibration curves were linear with concentrations range of 0.4-0.8, 0.1-0.9, 0.8-2 and 1.6-9.6 µg/ml for the cited drugs. Apparent molar absorptivity, detection and quantitative limits were calculated.

Applications of the proposed methods to representative pharmaceutical formulations are successfully presented; also the proposed method was applied for the determination of estradiol valerate in human urine samples.

Conclusion: The extracellular synthesis of nanoparticles was fast and eco-friendly; moreover, the method doesn't require various elaborate treatments and tedious extraction procedures.

Keywords: Silver nanoparticles, Etilefrine hydrochloride, Fenoterol hydrobromide, Salbutamol sulphate, Estradiol valerate.

INTRODUCTION

Etilefrine hydrochloride [2-Ethylamino-1-(3-hydroxyphenyl) ethanol hydrochloride] [1], is a direct-acting sympathomimetic with beta1-agonist properties, and some alpha-and beta2-agonist actions. It is used for the treatment of hypotensive states [2]. Different techniques were reported for the determination of etilefrine hydrochloride including: spectrophotometry [3-6], spectro flourimetry [7], automated sequential injection spectro photometry [8], Flow-injection spectrophotometry [9], flow-injection chemiluminometric assay [10] and HPLC [11].

Fenoterol hydrobromide [(1RS)-1-(3, 5-dihydoxyphenyl)-2- [[(1RS) -2-(4-hydroxyphenyl)-1-methylethyl]amino]ethanol hydrobromide] [1], is direct-acting sympathomimetic with beta-adrenoceptor stimulant activity largely selective for beta2 receptors (a beta2 agonist). It is used as a bronchodilator in the management of reversible airways obstruction, as occurs in asthma and in some patients with chronic obstructive pulmonary disease [2]. Various analytical methods were applied for the determination of fenoterol hydrobromide in raw material, pharmaceuticals and biological fluids. These methods include liquid chromatography [12, 13], HPLC [14], gas chromatography [15], voltammetry [16], electrophoresis [17], spectrophotometry [18, 19] and spectroflourimetry [20].

Salbutamol sulphate [Bis [(1RS)-2- [(1, 1-dimethylethyl) amino]-1-[4-hydroxy-3-(hydroxmethyl) phenyl] ethanol] sulphate] [1], is a direct-acting sympathomimetic with mainly beta-adrenergic activity and a selective action on beta2 receptors (a $beta_2$ agonist. It is used as bronchodilators in the management of reversible airways obstruction, as in asthma and in some patients with chronic obstructive pulmonary disease. It also decreases uterine contractility and may be given as the sulfate to arrest premature labour [2]. Different methods were reported for determination of salbutamol sulphate including spectrophotometry [21-26], HPLC [27, 28], liquid chromatography [29], thin layer chromatography [30], capillary electrophoresis [31] and voltammetry [32].

Estradiol valerate [3-Hydroxyestra-1, 3, 5(10)-trien-17b-yl pentanoate] [1], is the most active of the naturally occurring oestrogens. Estradiol is primarily used as menopausal hormone replacement therapy and may also be used as replacement therapy for female hypogonadism or primary ovarian failure. Several analytical methods were reported for the determination of estradiol valerate including spectrophotometric [33-36], gas chromatography [37], capillary electrophoresis [38], liquid chromatography [39], flow injection chemiluminescence [40] and electrochemical methods [41].

Nanoparticles are one of the novel drug delivery systems, which can be of potential use in controlling and targeting drug delivery as well as in cosmetics textiles and paints. Nanoparticles have many advantages; it can be administrated by parenteral, oral, nasal, ocular routes, by attaching specific ligands on to their surfaces, nanoparticles can be used for directing the drugs to specific target cells, improving the stability and therapeutics index and reduce toxic effects. Nanoparticles have many applications e. g. cancer therapy, intra cellular targeting, vaccine adjuvant, DNA delivery, ocular delivery.

Nanoparticles made of silver and gold have been the focus of research for many decades as a result of their intriguing optical properties. When dispersed in liquid media, these nanoparticles exhibit a strong UV–visible extinction band that is not present in the spectrum of the bulk metal. Recently silver nanoparticles were developed for sensitive and selective detection of nebivolol [42], fexofenadine [43] and some catecholamines [44, 45].

In this work nanoparticles were used in quantitative determination of drugs; a simple, sensitive, effective and validated procedure for the determination of etilefrine hydrochloride, fenoterol hydrobromide, salbutamol sulphate and estradiol valerate was developed.

MATERIALS AND METHODS

Instrumentation

A Shimadzu UV and visible recording spectrophotometer (UV 260) with matched 10 mm quartz cell was employed for all absorbance measurements.

A JEOL-1010 Transmission Electron Microscope at 80 KV, Japan was employed for Transmission Electron Microscopy (TEM) examination at Regional Center for Mycology and Biotechnology (RCMB) at Al-Azhar University.

Materials and reagents

Chemicals used were of the highest purity; Etilefrine hydrochloride (obtained from Chemical Industrial Development (Cid). Fenoterol hydrobromide (obtained from Sigma Pharmaceutical Industries). Salbutamol sulphate (obtained from Pharco Pharmaceuticals). Estradiol valerate (obtained from Chemical Industrial Development (Cid)). Silver nitrate, 0.02M aqueous solution, polyvinylpyrrolidone (PVP), 0.14% aqueous solution, Sodium hydroxide, 0.0025M aqueous solution.

Pharmaceutical preparations

Effortil® tablets containing 5 mg etilefrine hydrochloride per tablet (obtained from Chemical Industrial Development (Cid) under the licence of Boehringer Ingelheim, Germany).

Effortil® Drops containing 7.5 mg etilefrine hydrochloride per gm solution (obtained from Chemical Industrial Development (Cid) under the licence of Boehringer Ingelheim, Germany).

Pronotrol® Syrup containing 2.5 mg fenoterol hydrobromide per 5 ml (obtained from Sigma Pharmaceutical Industries).

Salbovent Fotre® tablets containing 4.8 mg salbutamol sulphate per tablet (obtained from Alex. Co. for Pharmaceuticals and Chemical Industries

Farcolin® respiratory solution containing 0.121 g Salbutamol sulphate per 20 ml solution (obtained from Pharco Pharmaceuticals).

Ventolin® Syrup containing 2 mg salbutamol sulphate per 5 ml (obtained from Galaxosmithkline).

Cyclo-progynova® white tablets containing 2 mg estradiol valerate per tablet and brown tablets containing 2 mg estadiolvalerate+0.5 mg norgestrel per tablets (obtained from Bayer Schering Pharma, Germany).

Standard solutions

- Solutions of 100 µg/ml of etilefrine hydrochloride, fenoterol hydrobromide and salbutamol sulphate were prepared by dissolving 10 mg of the pure drug in bidistilled water then further dilution to 5, 5, 10 µg/ml for etilefrine hydrochloride, fenoterol hydrobromide and salbutamol sulphate respectively.
- Solution of 100 µg/ml of estradiol valerate was prepared by dissolving 10 mg of the pure drug in acetonitrile then further dilution to 20 µg/ml.

General procedure

In 5 ml volumetric flask, appropriate amounts of silver nitrate, PVP, different concentrations of the cited drugs and appropriate amounts of NaOH were added, completed to 5 ml with bidistilled water, and then heated in the water bath at 90 °C for appropriate times. Absorbance was measured at the suitable wavelength against reagent blank treated similarly. (table 1).

Table1: Analytical parameters for determination of etilefrine HCl, fenoterol HBr, salbutamol sulphate and estradiol valerate through silver nanoparticles formation

Parameter	Etilefrine HCl	Fenoterol HBr	Salbutamol sulphate	Estradiol valerate
λ_{max} (nm)	417	417	419	425
Volume of Silver nitrate (0.02 M)	0.5 ml	0.7 ml	0.7 ml	1 ml
Volume of PVP (0.14 %)	1 ml	0.5 ml	0.7 ml	0.5 ml
Volume of NaOH (0.0025M)	0.7 ml	1 ml	0.7 ml	0.7 ml
Temperature	90°C	90°C	90°C	90°C
Time of reaction	35 min.	20 min.	20 min.	30 min.
Beer's law limits (µg/ml)	0.4–0.8	0.1–0.9	0.8–2	1.6–9.6

Assay of pharmaceutical preparations

A-Assay of tablets

1. for Effortil tablets: Ten tablets were weighed, pulverized into fine powder, specific quantity of powdered drugs equivalent to 10 mg pure drug were dissolved in distilled water, solutions were filtered and diluted to 100 ml with distilled water then further dilution to 5 µg/ml. Procedures were completed as in general procedures.

2. for salbutamol tablets: Ten tablets were weighed, pulverized into fine powder, specific quantity of powdered drugs equivalent to 10 mg pure drug were dissolved in distilled water, solutions were filtered and diluted to 100 ml with distilled water then further dilution to 10µg/ml. Procedures were completed as in general procedures applying standard addition technique.

2. for cycloprogynova tablets (white and brown): Ten tablets were weighed, coat removed, and pulverized into the fine powder. Specific quantity of powdered tablets equivalent to 10 mg pure drug was dissolved in acetonitrile. Solutions were filtered and diluted to 100 ml with acetonitrile then further dilution to 20 µg/ml. Procedures were completed as in general procedures applying standard addition technique.

B-Assay of syrup 1

For bronotrol syrup: in 125 ml separating funnel, 5 ml of syrup and 5 ml of saturated NaOH was placed and extracted with 30 ml methylene chloride. The organic layer was collected in the beaker and allowed to evaporate till dryness. The residue was dissolved in 1 ml methanol and diluted to 25 ml bidistilled water then further dilution to 5 µg/ml. Procedures were completed as in general procedures applying standard addition technique.

2. for salbutamol syrup: 2 ml syrup was placed in 100 ml volumetric flask and diluted to 100 ml with distilled water then further dilution to 10 µg/ml. Procedures were completed as in general procedures applying standard addition technique.

C-Assay of drops

Specific volumes of drops solutions equivalent to 10 mg pure drug were placed in 100 ml volumetric flask and diluted to 100 ml with distilled water then further dilution to 5 and 10 µg/ml for etilefrine HCl and salbutamol sulphate respectively. Procedures were completed as in general procedures.

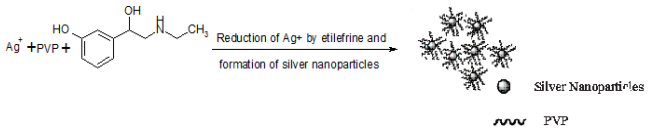
D-Assay of estradiol in Urine samples

Freshly voided urine samples were obtained from four healthy volunteers. Each sample was filtered through a 0.2-mm membrane to remove particulate matter. 0.5 ml estradiol valerate standard solution (50µg) was spiked in 50 ml urine sample.

In 250 ml separating funnel the urine sample and 5 ml saturated NaOH were placed and extracted with 150 ml methylene chloride. The organic layer was collected in the beaker, allowed to evaporate till dryness and residue was dissolved in 10 ml acetonitrile. Procedures were completed as in general procedures applying standard addition technique.

RESULTS ANDDISCUSSION

Nanoparticles made of silver and gold have been the focus of research for many decades due to their intriguing optical properties. The systems in this study consist of an aqueous $AgNO_3$ solution that includes polyvinylpyrrolidone (PVP), as stabilizer, at an alkaline medium. Etilefrine hydrochloride, fenoterol hydrobromide, salbutamol sulphate and estradiol valerate act as effective reducing agents for the reduction of silver metal salt (Ag^+) to the Ag-NPs without added any seeds (Scheme 1).



Scheme 1: Reduction of silver ions by etilefrine HCl

In the absence of reducing agents, there is no absorption peak in visible region (380-700 nm). Upon addition of cited drugs which act as reducing agent silver ions reduced to silver nanoparticles and then the absorbance characteristic to the plasmon of the Ag-NPs is observed (417-425 nm). (fig. 1)

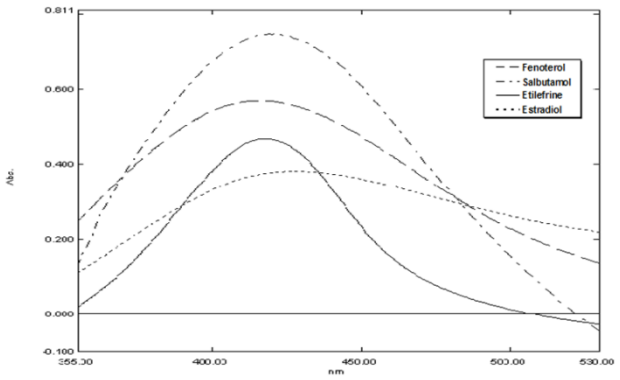


Fig. 1: Absorbance spectra of the silver nanoparticles formed in the presence of: -0.5 µg ml^{-1} etilefrine HCl, -0.5 µg ml^{-1} fenoterol HBr, -1.6 µg ml^{-1} salbutamol sulphate.-4 µg ml^{-1}estradiol valerate

Formation of silver nanoparticles was confirmed by TEM image which indicate nanopaticles formation in size of 11±2.57 nm. (fig 2)

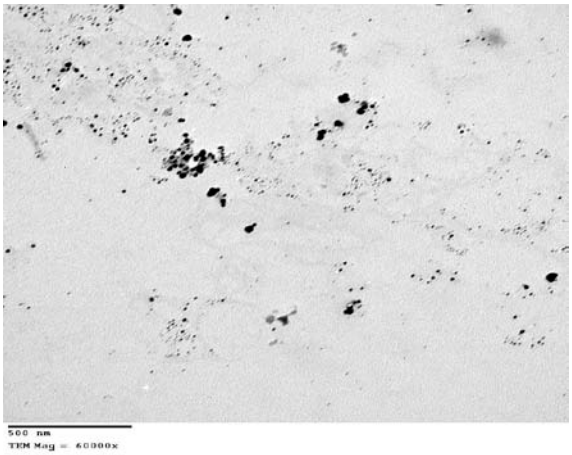


Fig. 2: TEM image for silver nanoparticles Optimum conditions affecting the reaction were studied

Effect of NaOH concentration

The influence of pH on Ag+reduction by the cited drugs is expected since they have a hydroxyphenyl group which can lose H+during oxidation and o-quinone formation process (Scheme 1). Because buffered condition failed to obtain silver nanoparticles we added NaOH for provide enough alkalinity. By addition of NaOH, absorbance increases up to a known concentration of NaOH then decreases with the formation of black precipitate which might be due to the Ag2O formation. Thus, 0.7 ml of 0.0025M NaOH was selected as the optimum NaOH concentration for etilefrine hydrochloride, salbutamol sulphate and estradiol valerate. While 1 ml of 0.0025M NaOH was sufficient for fenoterol hydrobromide

Effect of Silver nitrate concentration

Maximum absorbance values were obtained using 0.5, 0.7, 0.7 and 1 ml of 0.02M silver nitrate for Etilefrine hydrochloride, fenoterol hydrobromide, salbutamol sulphate and estradiol valerate respectively.

Effect of Stabilizer type and concentration

An important issue in the preparation of metal nanoparticles is the choice of the capping agent used to protect or stabilize the nanoparticle colloidal metals from agglomeration. Size and morphologies of nanoparticles are depending significantly on capping materials. Nanoparticles stabilization is achieved according to the two basic modes: electrostatic and steric stabilization [46]. Electrostatic stabilization is caused by the columbic repulsion between particles, caused by the electrical double layer formed by ions adsorbed at the particle surface (e. g., sodium citrate) and the corresponding counter ions. Steric stabilization is achieved because of the coordination of steric ally demanding organic molecules and polymers that act as protective shields on the metallic surface (e. g., PVP). In this study PVP and sodium citrate were selected as the stabilizer for preventing of silver nanoparticles agglomeration in which the PVP was better used in compare to sodium citrate. 1, 0.7, 0.7, 0.5 ml of 0.14% PVP were optimum for etilefrine hydrochloride, fenoterol hydrobromide, salbutamol sulphate and estradiol valerate.

Effect of temperature and time of heating

Heating in water bath at 90 °C for 35, 20, 20 and 30 min was sufficient to produce maximum color intensities for etilefrine hydrochloride, fenoterol hydrobromide, salbutamol sulphate and estradiol valerate.

Method validation

Linearity

Under the described experimental conditions standard calibration curves with good linearity for silver nanoparticles formed using etilefrine hydrochloride, fenoterol hydrobromide, salbutamol sulphate and estradiol valerate were constructed by plotting absorbance against concentration.

A linear correlation was found. The concentration ranges, molar absorptivity, correlation coefficient, intercept and slope for the calibration curve were calculated. Also relative standard deviation, analytical standard error, detection and quantification limits were calculated and listed in tables (2, 3).

The validity of the proposed method was assessed by its application to the determination of the cited drugs in their pharmaceutical preparations, and in urine samples for estradiol valerate tables (4, 5, 6).

Student's t-test and F-test(at 95% confidence level) were applied to the results obtained compared with that obtained from official methods.(1) Results showed that there are no significant differences between the proposed and official methods. Results of different statistical treatment of the data are shown in table (7).

Accuracy and precision

Accuracy and precision were carried out by six determinations at two different concentrations of the four drugs in the same day (intra-day), and in six different days (inter-day). Percentage relative standard deviation (R. S. D.%) as precision and percentage relative error (Er%) as accuracy of the suggested method was calculated.

The percentage relative error calculated using the following equation:

Er% = [(founded − added)/added] × 100

The results of accuracy and precision [table (8)] show that the proposed methods have good repeatability and reproducibility

Table 2: Spectral data for determination of etilefrine HCl, fenoterol HBr, salbutamol sulphate and estradiol valerate through silver nanoparticles formation

Parameter	Etilefrine HCl	Fenoterol HBr	Salbutamol sulphate	Estradiol valerate
Linearity range (µg/ml)	0.4–0.8	0.1–0.9	0.8–2	1.6–9.6
Apparent molar absorptivity* (mol^{-1} cm^{-1})	2.23×10^5	4.62×10^5	2.72×10^5	3.33×10^4
Sandell's sensitivity (mg/ml per 0001A)	1.02×10^{-1}	1.20×10^{-1}	4.72×10^{-2}	9.33×10^{-3}
Limit of detection LOD (µg/ml)	0.105	0.032	0.255	0.502
Limit of quantification LOQ (µg/ml)	0.319	0.098	0.771	1.522
Regression equation**:				
Slope (b)	1.7732	1.0418	0.4082	0.0908
Intercept (a)	-0.4209	0.0651	0.0842	0.0107
Correlation coefficient (r)	0.9994	0.9998	0.9998	0.9999

*Calculated on the basis of the molecular weight of the drug, ** A=a+bc

Table 3: Determination of etilefrine HCl, fenoterol HBr, salbutamol sulphate and estradiol valerate through silver nanoparticles formation

Statistics	Etilefrine HCl		Fenoterol HBr		Salbutamol sulphate		Estradiol valerate	
	Takenµg/ml	Recovery*%	Takenµg/ml	Recovery*%	Takenµg/ml	Recovery*%	Takenµg/ml	Recovery*%
	0.4	98.27	0.1	99.73	0.8	99.15	1.6	99.33
	0.45	101.14	0.2	100.73	1	101.62	3.2	99.22
	0.5	100.05	0.3	101.08	1.2	99.58	3.6	100.13
	0.55	100.79	0.4	99.08	1.4	99.36	4.8	101.68
	0.6	100.85	0.5	98.27	1.6	100.10	5.2	100.56
	0.65	99.08	0.6	100.77	1.8	100.28	5.6	99.40
	0.7	99.50	0.7	100.77	2	99.93	6	100.09
	0.8	100.17	0.8	100.06			9.6	100.07
			0.9	99.71				
Mean*±SD	99.98±0.983		100.02±0.927		100.00±0.818		100.01±0.773	
N	8		9		7		8	
V	0.967		0.859		0.668		0.597	
S. D.	0.983		0.927		0.818		0.773	
R. S. D.	0.984		0.927		0.817		0.773	
S. E.	0.348		0.309		0.308		0.258	

* Mean of three different experiments

Table 4: Determination of etilefrine HCl and fenterol HBr in their pharmaceutical formulations

Effortil tablets (Etilefrine HCl)		Effortil drops (Etilefrine HCl)		Syrup (Fenoterol HBr)		
Taken µg/ml	Recovery* %	Taken µg/ml	Recovery* %	Taken µg/ml	Added	Recovery* %
0.4	98.41	0.4	99.96	0.2	-	103.62
0.55	100.69	0.5	101.40		0.2	100.74
0.6	99.26	0.55	100.38		0.3	102.03
0.7	100.54	0.6	100.76		0.4	101.72
0.75	99.41	0.7	98.53		0.5	102.68
0.8	100.95				0.6	99.97
					0.7	100.63
Mean*±SD	99.88±1.002	100.21±1.076		101.30±1.016		
N	6	5		6		
V	1.003	1.157		1.033		
S. D.	1.002	1.076		1.016		
S. E.	0.409	0.481		0.415		

* Mean of three different experiments

Table 5: Application of standard addition technique for determination of Salbutamol sulphate in its pharmaceutical formulations

Tablets			Drops			Syrup		
Taken µg/ml	Added	Recovery* %	Taken µg/ml	Added	Recovery* %	Taken µg/ml	Added	Recovery* %
0.8	-	101.60	0.8	-	98.24	0.8	-	102.52.94
	0.8	99.46		0.8	99.15		0.8	98.85
	0.9	101.20		0.9	101.48		0.9	98.48

	1	99.17	1	101.37	1	99.66
	1.1	101.29	1.1	99.95	1.1	101.06
	1.2	99.99	1.2	101.22	1.2	99.18
Mean*±SD	100.22±0.980		100.63±1.030		99.45±1.003	
N	5		5		5	
V	0.961		1.062		1.006	
S. D.	0.980		1.030		1.003	
S. E.	0.438		0.461		0.449	

* Mean of three different experiments

Table 6: Application of standard addition technique for determination of Estradiol valerate in its pharmaceutical formulations and in urine samples

White tablets			Brown tablets			Urine sample		
Taken µg/ml	Added	Recovery* %	Taken µg/ml	Added	Recovery* %	Taken µg/ml	Added	Recovery* %
0.8	-	101.60	0.8	-	98.24	0.8	-	102.52.94
	0.8	99.46		0.8	99.15		0.8	98.85
	0.9	101.20		0.9	101.48		0.9	98.48
	1	99.17		1	101.37		1	99.66
	1.1	101.29		1.1	99.95		1.1	101.06
	1.2	99.99		1.2	101.22		1.2	99.18
Mean*±SD	100.22±0.980		100.63±1.030			99.45±1.003		
N	5		5			5		
V	0.961		1.062			1.006		
S. D.	0.980		1.030			1.003		
S. E.	0.438		0.461			0.449		

* Mean of three different experiments

Table 7: Statistical data for determination of etilefrine HCl, fenoterol HBr, salbutamol sulphate and estradiol valerate through silver nanoparticles formation

Item	Etilefrine HCl		Fenoterol HBr		Salbutamol sulphate		Estradiol valerate	
	Official Method	Reported method	Official Method	Reported method	Official Method	Reported method	Official method	Reported method
Mean*±SD	100.06±0.655	99.98±0.983	99.08±1.209	100.02±0.927	99.71±1.116	100.00±0.818	99.93±1.017	100.01±0.773
N	6	9	4	9	4	7	5	8
V	0.429	0.967	1.460	0.859	1.245	0.668	1.034	0.597
S. D.	0.655	0.983	1.209	0.927	1.116	0.818	1.017	0.773
t		0.180 (2.160)*		0.066 (2.201)*		0.499 (2.262)*		0.161 (2.201)*
F		2.254 (3.690)*		1.700 (4.070)*		1.864 (4.760)*		1.732 (4.120)*

*Theoretical values of t and F at p = 0.05

Table 8: The intra-day and inter-day accuracy and precision data for determination of etilefrine HCl, fenoterol HBr, salbutamol sulphate and estradiol valerate through silver nanoparticles formation

	Intra-day					Inter-day				
	Taken, µg/ml	Found, µg/ml	Recovery*, %	RSD, %	Er, %	Taken, µg/ml	Found, µg/ml	Recovery* %	RSD, %	Er %
Etilefrine HCl	0.7	0.696	99.48	0.794	-0.52	0.7	0.695	99.23	1.006	-0.77
	0.8	0.801	100.18	0.582	0.18	0.8	0.802	100.21	0.961	0.21
Fenoterol HBr	0.8	0.802	100.22	0.842	0.22	0.8	0.804	100.46	1.062	0.46
	0.9	0.897	99.71	0.756	-0.29	0.9	0.894	99.37	1.035	-0.63
Salbutamol	1.82	1.806	100.32	0.899	0.32	1.8	1.806	100.35	1.118	0.34
sulphate	16	1.999	99.93	0.867	-0.07	2	1.998	99.91	1.100	-0.09
Estradiol valerate	5.6	5.576	99.59	1.131	-0.43	5.6	5.561	99.31	1.206	-0.69
	6	5.998	99.97	0.955	-0.03	6	5.991	99.85	1.063	-0.15

* Mean of six different experiments.

CONCLUSION

Application of silver nanoparticles as chromogenic agent has been demonstrated in this work for optical detection of the cited drugs based on the seedless production of Ag-NPs.

The proposed method is simple, sensitive, and inexpensive for their determination. This analytical protocol may be important green method for monitoring and optical detection of etilefrine hydrochloride, fenoterol hydrobromide and salbutamol sulphate in pure and pharmaceutical dosage forms. It can be used for routine

analysis of estradiol valerate in pure, pharmaceutical dosage forms and in urine samples.

CONFLICT OF INTERESTS

Declared None

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