



Research Article

Spectrophotometric Determination of Etilerfrine HCl, Salbutamol Sulphate and Tiemonium Methyl Sulphate Using Surface Plasmon Resonance Band of Gold Nanoparticles

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Abstract

A simple and sensitive method was developed for spectrophotometric determination of etilefrine hydrochloride, salbutamol sulphate and tiemonium methyl sulphate in pure form and in their pharmaceutical formulations. The method was based on reduction of gold solution to gold nanoparticles by the studied drugs in presence of sodium dodecyl sulphate as stabilising agent. Gold nanoparticles (Au NPs) showed a new absorption band at 530 nm that was used for quantitative determination of the cited drugs. Different variables were examined and optimised in the experiment as gold solution concentration, type of buffer, suitable pH, stabilising agent, order of addition, time and temperature of the reaction. Under optimum conditions, the calibration curves were linear with concentration ranges of 3.0-20.0, 5.0-18.0 and 2.0-26.0 µg/mL for etilefrine Hydrochloride, salbutamol sulphate and tiemonium methyl sulphate respectively. The method was applied successfully to determine the studied drugs in pure form and in their pharmaceutical dosage forms, exhibiting good reproducibility and accuracy.

Keywords: Gold nanoparticles; Etilerfrine Hydrochloride; Salbutamol sulphate; Tiemonium methyl sulphate

Introduction

Etilerfrine hydrochloride is [2-ethylamino-1-(3-hydroxyphenyl) ethanol hydrochloride] [1a]. It 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 [2a]. Different techniques were reported for the

determination of etilefrine hydrochloride including: spectrophotometry [3-7], spectro fluorimetry [8] and HPLC [9].

Salbutamol sulphate is [Bis [(1RS)-2-[(1,1-dimethylethyl) amino]-1-[4-hydroxy-3-(hydroxymethyl) phenyl] ethanol] sulphate] [1b]. It is a direct-acting sympathomimetic with mainly beta-adrenergic

activity and a selective action on beta2 receptors (a beta2 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 [2b]. Different methods were reported for determination of salbutamol sulphate including spectrophotometry [10-15], HPLC [16, 17], thin layer chromatography [18], capillary electrophoresis [19] and voltammetry [20].

Tiemonium methyl sulphate (TIM) is 4-[3-Hydroxy-3-phenyl-3-(2-thienyl) propyl]-4-methyl-morpholinium methylsulphate. It is quaternary ammonium antimuscarinics which is used in the relief of visceral spasms [2c]. The drug is not included in any official pharmacopeias till now. From the literature survey, there were few methods had been reported for its determination using spectroscopic methods [21-23]. Chromatographic methods had been applied [24, 25].

However, some of these methods are expensive, somewhat time consuming; they require expensive instruments, which make it unavailable in many laboratories for the analysis of the cited drugs in biological samples. It is important to develop a simple and sensitive method for their determination. Recently simple colorimetric methods have been widely used in analytical areas due to their low cost, simplicity and practically. Noble metal nanoparticles have attracted much interest in modern chemistry due to their unique optical properties. When gold nanoparticles and silver nanoparticles are dispersed in liquid media, solutions show a distinctive color attributed to their small size and they exhibit a strong UV-Vis extinction band. Gold nanoparticles have wide applications in various area of chemistry [26-27].

In this work, gold nanoparticles were synthesized using the reducing capacity of the cited drugs in presence of stabilizing agent. The synthesized nanoparticles were used for their quantitative determination in pure and pharmaceutical formulations.

Experimental Instrumentation

A single cell holder JENWAY 6715 UV/Visible spectrophotometer equipped with 10 mm matched quartz cells was employed for all absorbance measurements

Materials and reagents

All solvents and reagents used were of the highest purity.

Etilefrine hydrochloride, obtained from Chemical Industrial Development (Cid). Its purity was found to 100.26% according to the comparison method.

Salbutamol sulphate, obtained from Pharco Pharmaceuticals. Its purity was found to 100% according to the comparison method.

TIM, obtained from Adwia Pharmaceutical Industries Co., Egypt. Its purity was found to be 99.9% as reported from company and 100.22% according to the comparison method.

Gold (III) solution: 1g of chloroauric acid (Fischer chemical, Fischer scientific UK limited, U.K.) was dissolved in distilled water. The solution was made up to the mark in 100 mL volumetric flask.

Phosphate buffer (pH 7.5, 0.2 mol) was prepared by dissolving 27.22 g of potassium dihydrogen orthophosphate in 930 mL of water, adjusting pH of the solution to 7.5 with a 30% w/v solution of potassium hydroxide and adding sufficient water to produce 1000 mL phosphate buffer [1].

A solution of 2.0 g/L sodium dodecyl sulphate (SDS) (Sigma Aldrich, Germany).

Pharmaceutical preparations

Effortil® tablets containing 5.0 mg etilefrine hydrochloride per tablet, obtained from Chemical Industrial Development (Cid) under the licence of Boehringer Ingelheim, Germany, batch No. 01160039.

Effortil® drops containing 7.5 mg etilefrine hydrochloride per gram solution (obtained from Chemical Industrial Development (Cid) under the licence of Boehringer Ingelheim, Germany), batch No. 12160292.

Salbovent Fotre® tablets containing 4.8 mg salbutamol sulphate per tablet (obtained from Alex. Co. for Pharmaceuticals and Chemical Industries), batch No. 6172028.

Farcolin® respiratory solution containing 0.121 g salbutamol sulphate per 20.0 mL solution (obtained from Pharco Pharmaceuticals), batch No. 1404.

Ventolin® syrup containing 2.0 mg salbutamol sulphate per 5.0 mL (obtained from Galaxosmithkline),

batch No. 7221009.

Salbovent® ampoule containing 0.6025 mg salbutamol sulphate per 1.0 mL (obtained from Alex. Co. for Pharmaceuticals and Chemical Industries), batch No. 2418008.

Visceralgine® tablets containing 50.0 mg Timonium methylsulphate per tablet (obtained from Sedico Pharmaceutical Company, Giza, Egypt), batch No. 0916299.

Viscera® ampoules containing 5.0 mg Timonium methylsulphate per 2.0 mL (Sedico Pharmaceutical Company, Giza, Egypt), batch No. 1216287/A.

Standard solutions

10.0 mg of etilefrine hydrochloride, salbutamol sulphate and TIM were weighed accurately and transferred into a 10.0 mL standard flask, dissolved and made up to mark with bidistilled water and then further diluted to 100 µg/mL for the studied drugs.

General procedure

Different aliquots of the studied drug solutions were added into 5.0 mL volumetric flask containing appropriate volumes of phosphate buffer solution of pH 7.5, gold(III) (2%) solution and SDS solution according to a certain sequence. The contents were heated in boiling water bath for appropriate times and then diluted to mark with distilled water. After cooling the solution to room temperature; absorbance was

measured at suitable wavelength against reagent blank treated similarly (Table 1).

Assay of pharmaceutical preparations

Assay of tablets

effortil® and salbovent Fotre® tablets

Ten tablets were weighed, pulverized into fine powder, in 10.0 mL volumetric flask specific quantity of powdered tablet drugs equivalent to 10.0 mg pure drug were dissolved and diluted to mark using distilled water. Solutions were filtered and then further diluted to 100 µg/mL with distilled water. Procedures were completed as in general procedures by applying the standard addition technique.

visceralgine® tablets

Ten tablets were weighed, pulverized into fine powder, in 10.0 mL volumetric flask specific quantity of powdered tablet drugs equivalent to 10.0 mg pure drug were dissolved and diluted to mark using methanol then sonicated for 30 min. Solutions were filtered and then further diluted to 100.0 µg/mL with distilled water. Procedures were completed as in general procedures by applying the standard addition technique.

ventolin® syrup

2.5 mL syrup was placed in 10.0 mL volumetric flask and diluted to 10.0 mL with distilled water. Procedures were completed as in general procedures

Table 1 Analytical parameters and spectral data for determination of etilefrine hydrochloride (HCl), salbutamol sulphate and TIM through gold nanoparticles formation

Parameter	Etilefrine HCl	Salbutamol sulphate	TIM
λ_{max} (nm)	530	530	529
Volume of phosphate buffer, pH 7.5 (mL)	0.5	0.5	-
Volume of SDS, 0.2% w/v (mL)	1.5	0.5	1.0
Volume of gold solution, 2% v/v (mL)	2.0	1.5	2.0
Temperature (°C)	100	100	100
Time of heating (min)	20	20	10
Linearity range (µg/mL)	3.0-20.0	5.0-18.0	2.0-26.0
Apparent molar absorptivity* (/mol/cm)	9.04×10^3	1.4600×10^4	1.56×10^4
Limit of detection (LOD) (µg/mL)	0.895	1.625	0.610
Limit of quantification (LOQ) (µg/mL)	2.984	4.924	2.032
Regression equation:			
Slope (b)	0.0422	0.0310	0.0271
Intercept (a)	-0.0059	-0.0562	0.0808
Correlation coefficient (r)	0.9999	0.9999	0.9999

*Calculated on the basis of the molecular weight of the drug.

applying standard addition technique.

effortil® and farcolin® drops

A specific volume of drop solutions equivalent to 10.0 mg pure drug were placed in 100.0 mL volumetric flask and diluted to 100.0 mL with distilled water for etilefrine HCl and salbutamol sulphate. Procedures were completed as in general procedures by applying the standard addition technique.

salbovent® and viscera® ampoule

A specific volume of ampoule solutions equivalent to 10.0 mg pure drug were placed in 100.0 mL volumetric flask and diluted to 100.0 mL with distilled water for salbovent and with 50% methanol (v/v) for viscera. Procedures were completed as in general procedures by applying the standard addition technique.

Spiking of TIM into plasma and their extraction from plasma (By protein precipitation method)

From the standard solution containing 1000 µg/mL of TIM, 0.5 mL was pipetted out and spiked into 0.5 mL of plasma in a centrifuge tube. Then, 4 mL of acetonitrile was added to the tube and centrifuged for 30 min at 5000 rpm. The supernatant liquid was collected in another tube and 0.1 mL supernatant was added to different concentrations of pure drug and the procedures were completed as in general procedures applying standard addition technique.

Results and Discussion

Nanoparticles made of silver and gold have been the focus of research for many decades due to their intriguing optical properties. In this study, an aqueous gold solution that contained SDS, as stabilizer and phosphate buffer solution was added to etilefrine HCl, salbutamol sulphate and TIM solutions. Upon addition of the cited drugs which acted as the reducing agent, gold ions reduced to gold nanoparticles and then the absorbance characteristic to the plasmon of the Au-NPs was observed (530 nm) (Fig. 1).

Effect of pH

The reaction between the studied drugs and the gold solution was a redox reaction, and since H⁺ was produced from the reaction between the cited drugs and gold ions, its removal could accelerate the reduction of gold ions. With different buffer media as the acetate,

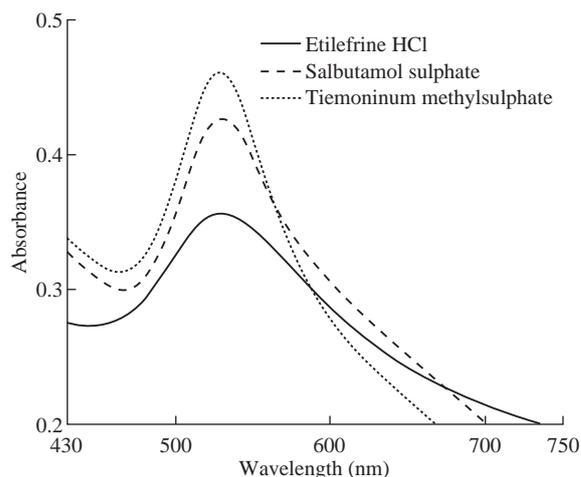


Fig. 1 Absorbance spectra of the gold nanoparticles formed in the presence of 8.5 µg/mL etilefrine HCl, 15.5 µg/mL salbutamol sulphate and 14 µg/mL TIM.

phosphate and borate were tried within a wide range of pH from pH 1 to pH 12.5. The optimum buffer medium was phosphate buffer at pH 7.5. The absorbance of drugs increased up to pH 7.5 and then started to decrease, which might be attributed to the formation of gold hydroxide. Thus, 0.5 mL of phosphate buffer was sufficient for the reaction of etilefrine and salbutamol, while TIM reduced gold solution with no need of buffer (Fig. 2 and 3; Table 1).

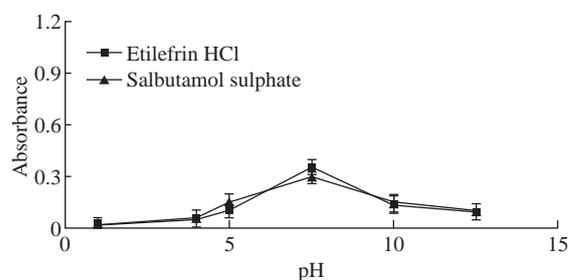


Fig 2 Effect of variation of pH on the absorbance of gold nanoparticles formed through the reaction of gold solution and SDS in presence of 15 µg/mL etilefrine HCl and 18 µg/mL salbutamol sulphate.

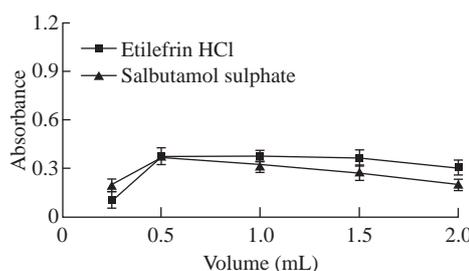


Fig 3 Effect of volume of buffer solution on the absorbance of gold nanoparticles formed through the reaction of gold solution and SDS in presence of 15 µg/mL etilefrine HCl and 18 µg/mL salbutamol sulphate.

Effect of stabilizer

Stabilization of the formed gold nanoparticles was very crucial to prevent their aggregation. Different surfactants such as SDS, cetyl trimethyl ammonium bromide (CTAB) and methyl cellulose were tried. In presence of CTAB, the intensity of absorbance of Au NPs decreased in comparison with when SDS was used. This might be attributed to the agglomeration of nanoparticles; hence, SDS was the best one that prevented aggregation through steric stabilization. Different concentrations and volumes of SDS were added to the reaction mixture. Additions of 1 mL, 0.5 mL and 1.5 mL of 0.2% SDS were chosen as the optimum value for etilefrine HCl, salbutamol sulphate and TIM respectively (Fig. 4 and 5; Table 1).

Effect of gold solution concentration

Maximum absorbance values were obtained using 2.0 mL, 1.5 mL and 2.0 mL of 2% v/v gold solution for etilefrine HCl, salbutamol sulphate and TIM respectively (Fig. 6 and 7; Table 1).

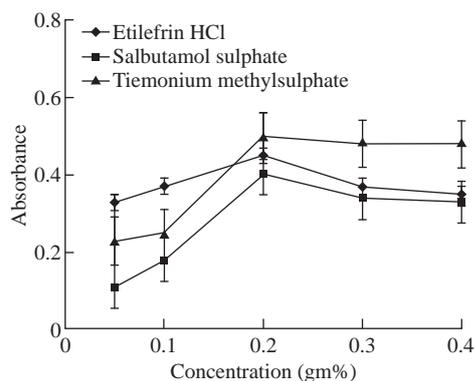


Fig. 4 Effect of concentration of SDS solution on the absorbance of gold nanoparticles formed through the reaction of buffer and gold solution in presence of 15 $\mu\text{g/mL}$ etilefrine HCl, 18 $\mu\text{g/mL}$ salbutamol sulphate and 20 $\mu\text{g/mL}$ TIM.

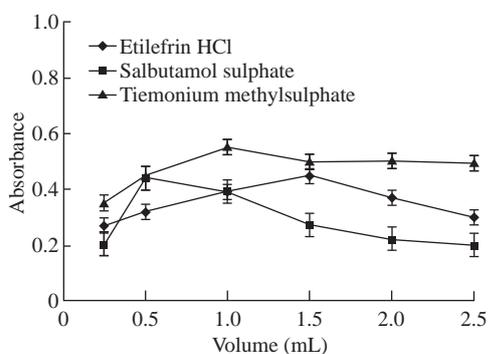


Fig. 5 Effect of volume of SDS solution on the absorbance of gold nanoparticles formed through the reaction of buffer and gold solution in presence of 15 $\mu\text{g/mL}$ etilefrine HCl, 18 $\mu\text{g/mL}$ salbutamol sulphate and 20 $\mu\text{g/mL}$ TIM.

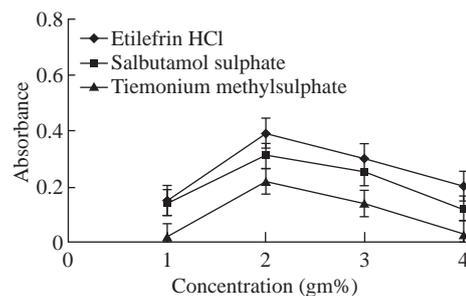


Fig. 6 Effect of concentration of gold solution on the absorbance of gold nanoparticles formed through the reaction of buffer and SDS in presence of 15 $\mu\text{g/mL}$ etilefrine HCl, 18 $\mu\text{g/mL}$ salbutamol sulphate and 20 $\mu\text{g/mL}$ TIM.

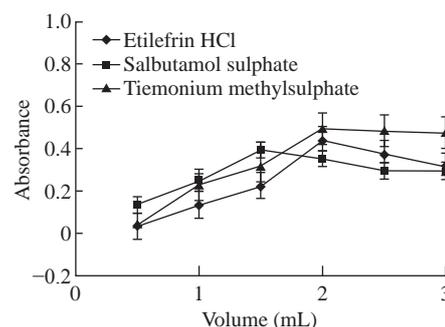


Fig. 7 Effect of volume of gold solution on the absorbance of gold nanoparticles formed through the reaction of buffer and SDS in presence of 15 $\mu\text{g/mL}$ etilefrine HCl, 18 $\mu\text{g/mL}$ salbutamol sulphate and 20 $\mu\text{g/mL}$ TIM.

Effect of temperature and time of heating

Heating in water bath at 100 $^{\circ}\text{C}$ for 20, 20 and 10 min was sufficient to produce maximum color intensities for etilefrine HCl, salbutamol sulphate and TIM respectively (Fig. 8 and 9; Table 1).

Effect of order of addition

The sequence of addition of the constituents for formation of gold nanoparticles was studied. The most suitable order was drug, gold, SDS and buffer for etilefrine HCl, drug, buffer, SDS and gold for salbutamol sulphate, while drug, SDS and gold was

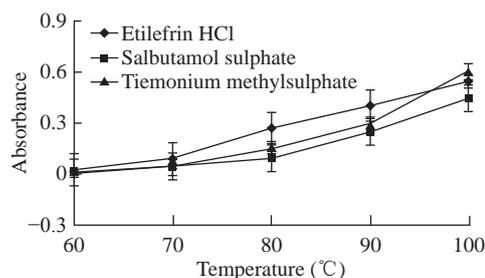


Fig. 8 Effect of temperature on the absorbance of gold nanoparticles formed through reaction of buffer, SDS and gold solution in presence of 15 $\mu\text{g/mL}$ etilefrine HCl, 18 $\mu\text{g/mL}$ salbutamol sulphate and 20 $\mu\text{g/mL}$ TIM.

found the best sequence for tiemonium sulphate. Other sequences gave lower absorbance values (Fig. 10; Table 1).

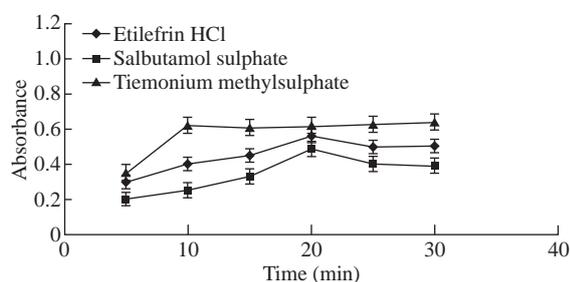


Fig. 9 Effect of time of heating on the absorbance of gold nanoparticles formed through reaction of buffer, SDS and gold solution in presence of 15 $\mu\text{g/mL}$ etilefrine HCl, 18 $\mu\text{g/mL}$ salbutamol sulphate and 20 $\mu\text{g/mL}$ TIM.

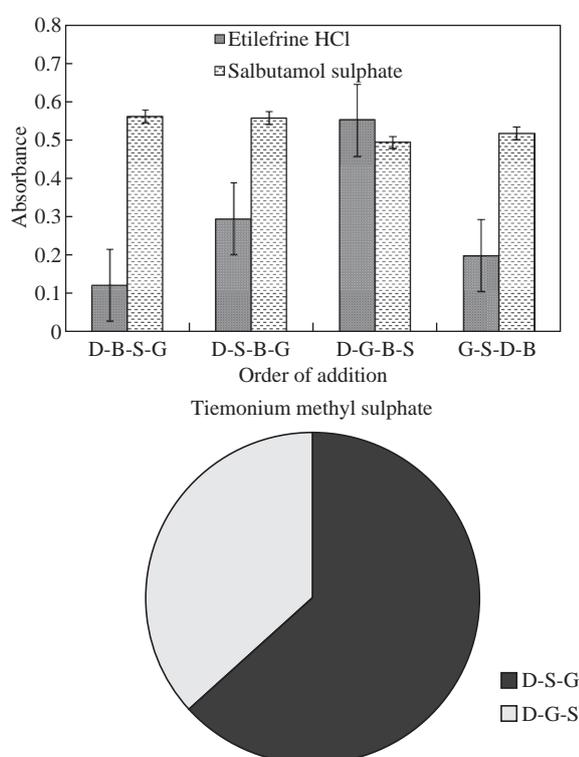


Fig. 10 Effect of order of addition of phosphate buffer, SDS and gold solution to 15 $\mu\text{g/mL}$ etilefrine HCl, 18 $\mu\text{g/mL}$ salbutamol sulphate and 20 $\mu\text{g/mL}$ TIM. *D = drug; B = buffer; S = SDS; G = gold solution.

Method validation

Linearity

Under the optimum conditions described, calibration curves for determination of the cited drugs by the proposed method were constructed by plotting absorbance against drug concentrations. Beer's law plots were linear over the range of 3-20 $\mu\text{g/mL}$, 5-18 $\mu\text{g/mL}$ and 2-26 $\mu\text{g/mL}$ for etilefrine HCl, salbutamol sulphate and tiemonium methylsulphate

respectively, with small intercepts and good correlation coefficients indicating good linearity over the working concentration ranges. Molar absorptivity, relative standard deviation, analytical standard error, detection and quantification limits were also calculated and listed in Table 1.

Sensitivity

The LOD and LOQ were evaluated using the following equations according to guidelines by the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH): $\text{LOD} = 3.3\sigma/s$, and $\text{LOQ} = 10\sigma/s$, where σ = the standard deviation of replicate blank responses (under the same conditions as for sample analysis), and s = the slope of the calibration curve. LODs and LOQs were calculated and listed (Table 1).

Accuracy and precision

Accuracy

The accuracy of the proposed method was ascertained by determining pure samples of the cited drugs with reported methods. Statistical analysis of the results obtained by the proposed and comparison methods for the studied drugs showed that the calculated values didn't exceed the theoretical ones, which indicated no significant differences were found between the proposed method and comparison methods. Statistical comparison of the results was performed using Student's t-test and variance ratio F-test at 95% confidence level.

Precision

Intra-day precision (repeatability)

To determine intra-day precision of the proposed method, solutions containing three different concentrations (within the linearity ranges) of each drug in its pure form were prepared and analyzed by proposed method on three successive times in the same day. The values of relative standard deviation and percentages relative error ($\text{Er}\%$) of the suggested method was calculated using the following equation:

$$\text{Er}\% = \frac{\text{found} - \text{added}}{\text{added}} \times 100$$

Inter-day precision (intermediate)

To establish inter-day precision, three experimental replicates including three different concentrations (within the linearity ranges) of the cited drugs were carried out using proposed method over a period of three days. Relative standard deviation and percentages relative error ($\text{Er}\%$) were calculated.

Selectivity

To study the selectivity of the proposed method, interference liabilities were performed to explore the effect of common excipients that might be added during formulations. Under the experimental condition employed, to known concentrations of the cited drugs, the common excipients as lactose, calcium carbonate, sodium chloride, sucrose, magnesium stearate, talc and starch were added and analyzed. Results showed no interferences from the presence of these excipients (Table 2).

Robustness and ruggedness

Robustness

It was the measure of the capacity of proposed method to remain unaffected by small variations of the method variables. It was evaluated by making small incremental changes in one parameter while the others were kept unchanged as the volume of gold

solution (2.1, 2.0, 1.9 mL for etilefrine HCl, 1.6, 1.5, 1.4 mL for salbutamol sulphate and 2.1, 2.0, 1.9 mL for TIM), phosphate buffer volume (0.55, 0.50, 0.45 mL for etilefrine HCl and salbutamol sulphate) and SDS volume (1.6, 1.5, 1.4 mL for etilefrine HCl and salbutamol sulphate and 1.1, 1.0, 0.9 mL for TIM). The effect of the changes were studied on the absorbance by calculating recovery \pm %RSD, and the changes had negligible influence on the results, which provided an indication for the reliability of the proposed method during its routine application for analysis of the investigated drugs (Table 3).

Ruggedness

It was tested by applying the proposed method to the assay of the investigated drugs using the same procedures but using two different instruments. The obtained results were found to be reproducible as shown in (Table 3).

Table 2 Analysis of the cited drugs by the proposed method in presence of some common excipients

Tolerance molar ratio (M : M) *	Recovery%**					
	Etilefrine HCl		Salbutamol sulphate		TIM	
		Lactose	Calcium carbonate	Lactose	Sodium chloride	Sucrose
1:1	-	100.04	101.33	100.41	95.79	104.10
1:10	-	98.02	102.25	101.33	95.42	103.17
1:50	-	102.06	102.07	102.07	95.24	97.64
1:100	-	101.05	103.17	102.07	95.06	99.48
Other excipients	Etilefrine HCl	Salbutamol sulphate		TIM		
Magnesium stearate (40 μ g/mL)	95.66	96.01		95.79		
Talc (40 μ g/mL)	101.58	100.04		103.17		
Starch (40 μ g/mL)	98.62	96.01		-		

*Drug: Excipients, etilefrine HCl 16 μ g/mL (7.4×10^{-3} mol), salbutamol sulphate 16 μ g/mL (6.7×10^{-3} mol) and TIM 20 μ g/mL (4.7×10^{-3} mol).

**Mean of three determinations.

Table 3 Method robustness and ruggedness expressed as (recovery \pm RSD%)

Drugs	Robustness				Ruggedness
	Parameters altered	Parameters altered			Inter-instruments (n=2)
	Taken (μ g/mL)	Volume of gold solution	Volume of phosphate buffer	Volume of SDS	JENWAY6715UV/Vis. -ShimadzuUV1800 PC
Etilefrine HCl	4.0	102.63 \pm 1.453	102.82 \pm 1.450	99.07 \pm 1.922	100.06 \pm 1.675
Salbutamol sulphate	14.0	97.20 \pm 1.216	97.28 \pm 1.441	97.20 \pm 1.216	97.05 \pm 1.679
TIM	20.0	99.91 \pm 1.128	-	98.75 \pm 1.483	99.48 \pm 1.311

Table 4 Determination of etilefrine HCl and salbutamol sulphate in their pharmaceutical formulations

Etilefrine HCl					Salbutamol sulphate								
Effortil tablets		Effortil drops		Salbovent Forte® tablets	Farcolin® respiratory solution		Ventolin® syrup		Salbovent® ampoule				
Taken (µg/mL)	Added (µg/mL)	Recovery* (%)	Added (µg/mL)	Recovery* (%)	Taken (µg/mL)	Added (µg/mL)	Recovery* (%)	Added (µg/mL)	Recovery* (%)	Added (µg/mL)	Recovery* (%)	Added (µg/mL)	Recovery* (%)
	-	95.50											
	3.0	99.45	-	104.98		-	100.77	-	104.65	-	104.00	-	104.65
	4.0	98.28	4.0	100.06		5.0	100.77	5.0	101.42	5.0	100.77	5.0	100.77
3.0	7.0	98.48	5.0	99.00	5.0	7.0	99.63	7.0	100.09	7.0	101.47	7.0	99.63
	9.0	98.18	7.0	101.18		9.0	98.28	9.0	100.43	9.0	99.00	9.0	99.35
	14.0	97.99	13.0	99.14		11.0	99.47	11.0	98.89	11.0	100.06	11.0	99.31
	16.0	96.40	15.0	99.35		13.0	99.06	13.0	99.06	13.0	99.31	13.0	99.31
Mean*± SD	98.13 + 0.989		99.75 + 0.899		99.44 + 0.910		99.98 + 1.041		100.12 + 1.024		99.77 + 0.687		
N	6		5		5		5		5		4		
V	0.978		0.809		0.828		1.084		1.048		0.472		
S.E.	0.404		0.402		0.407		0.466		0.458		0.344		

*Mean of three different experiments

Table 5 Determination of tiemonium methylsulphate in its pharmaceutical formulations and in plasma sample

Visceralgin® tablets			Visceralgin® ampoule			Plasma sample		
Taken (µg/mL)	Added (µg/mL)	Recovery* (%)	Taken (µg/mL)	Added (µg/mL)	Recovery* (%)	Taken (µg/mL)	Added (µg/mL)	Recovery* (%)
	-				95.20			96.31
	2.0	97.97		-	95.20		4.0	97.97
	4.0	96.31		4.0	95.45		6.0	96.06
4.0	6.0	96.13	4.0	6.0	96.96	2.0	8.0	95.11
	8.0	96.68		8.0	96.26		12.0	95.69
	10.0	97.88		14.0	95.99		14.0	96.52
		96.75		16.0			16.0	95.06
Mean* ± SD	96.75 + 0.682				95.97 + 0.692			96.07 ± 1.085
N	5				5			6
V	0.465				0.479			1.177
S.E.	0.305				0.309			0.443

Analytical applications

The proposed method was successfully applied to the assay of the studied drugs in their pharmaceutical formulations and in plasma sample for TIM without interference from common excipients. Satisfactory results obtained from the recoveries of the drugs were in good agreement with the label claim and proved the suitability of the proposed method (Table 4 and 5).

Conclusions

The proposed method was found to be simple, economic, selective and sensitive. The statistical parameters and recovery study data clearly indicated the reproducibility and accuracy of the methods. The proposed method was successfully applied for the determination of the studied drugs in their pharmaceutical formulation without interference from the common excipients.

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Conflict of Interests

The authors declare that no competing interest exists.

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