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Abstract

A simple and sensitive method was developed for spectrophotometric determination of finasteride, indapamide and tiemonium methyl sulphate in their pure form and in their pharmaceutical formulations. It was found that the studied drugs have the ability to reduce silver nitrate to silver nanoparticles (Ag NPs) in presence of sodium citrate as a stabilizing agent. Silver nanoparticles (Ag NPs) produce a very intense surface plasmon resonance peak at 423 nm that allows quantitative determination of the studied drugs. The calibration curves were linear with concentrations range of 0.50–5.00, 0.50-5.00 and 0.30-2.00 µg/mL for finasteride, indapamide and tiemonium methyl sulphate, respectively. The proposed method was successfully applied to determination of the studied drugs in their pharmaceutical formulations. Furthermore, content uniformity testing of the studied pharmaceutical tablets was also conducted.

Keywords: Silver nanoparticles, finasteride, indapamide and tiemonium methyl sulphate

Introduction

Finasteride is N-(1,1-Dimethylethyl)-3-oxo-4-aza-5a-androst-1-ene-17b-carboxamide **(Table 1).** It is an azasteroid that inhibits the type-2 isoform of 5α-reductase, the enzyme responsible for conversion of testosterone to the more active dihydrotestosterone, and therefore has anti-androgenic properties. It is given orally in a dose of 5 mg daily in the management of benign prostatic hyperplasia to cause regression of the enlarged prostate and to improve symptoms; it may reduce the incidence of acute urinary retention and the need for surgery [1]. Finasteride is official drug listed in British Pharmacopeia (BP) [2] and can be determined using HPLC based method. Several methods have been reported for finasteride determination including spectrophotometry [3-7], HPLC [8-10], HPTLC [11], voltammetry [12] and polarography [13].

Indapamide is 4-Chloro-N-[(2RS)-2-methyl-2,3-dihydro-1H-indol-1-yl]3sulphamoylbenzamide **(Table1)**. The drug exerts diuretic actions similar to thiazide diuretics, despite lacking thiazide moiety in the drug. It is used for hypertension and oedema associated with heart failure [1]. Indapamide is an official drug listed in BP [2], and can be determined using HPLC. Different techniques were reported for its determination including spectrophotometry [14-18], chromatographic methods [19-21] and voltammetry [22].

Tiemonium methyl sulphate is 4-[3-Hydroxy-3-phenyl-3-(2-thienyl)propyl]4-methylmorpho-linium methyl sulphate **(Table 1).** It is used for relief of visceral spasms [1]. Tiemonium methyl sulphate is not listed in any pharmacopeias. Thus a thorough literature search was performed on this drug. Most of the reported methods used spectroscopic techniques due to their simplicity [23-27]. Chromatographic methods [28,29] and electrochemical ones [30] were also used for the drug determination.

Recently silver nanoparticles have reported to have wide applications in various area of chemistry due to their unique optical properties [31-34]. In this work, we report a simple and sensitive method for determination of finasteride, indapamide and tiemonium methyl sulphate. This assay is based on spectrophotometric determination of silver nanoparticles at 423 nm, which were formed due to reduction of silver nitrate by aforementioned drugs in the presence of sodium citrate.

EXPERIMENTAL

Instrumentation

A single cell holder JENWAY 6715 UV/ Visible spectrophotometer (UK) equipped with 10 mm matched quartz cells was employed for all absorbance measurements a vortex mixer model VELP® Scientifica RX3, Europe. A centrifuge model Hettich Zentrifugen universal 320/320 R, Germany.

Materials and reagents

All solvents and reagents used were of the highest purity.Finasteride was obtained from SIGMA pharmaceutical industries, Egypt. Its purity was found to be 99.92 according to the comparison method [6].

Indapamide was obtained from Pharco pharmaceuticals, Alexandria, Egypt. Its purity was found to be 99.95 according to the comparison method [17]. Tiemonium methyl sulphate was obtained from Adwia Pharmaceutical Industries Co., Cairo, Egypt. Its purity was found to be 99.9% as reported from company. Its purity was found to be 100.40 according to comparison

method [23]. Silver nitrate ($AgNO_3$) was obtained from Morgan Speciality Chemicals Company. Its purity was found to be 99.5% as reported from company, Batch No 572070216 Sodium citrate was obtained from Fischer chemical, Fischer scientific UK limited, U.K. Sodium hydroxide was obtained from Alpha Chemicals for laboratory use. Its purity was found to be 98% as reported from company.

Pharmaceutical preparations

Prostride® capsules containing 5 mg finasteride per capsule (obtained from Adwia Pharmaceutical Industries Co., Cairo, Egypt) Batch No. 1603221

Hypotense® tablets containing 2.5 mg indapamide per tablet (obtained from the Arab Drug Company, Cairo, Egypt) Batch No. 210189

Normaten® tablets containing 2.5 mg indapamide and 50 mg captopril per tablet (obtained from Tenth of Ramadan For Pharmaceutical Industeries & Diagnostic Reagent (rameda), 6th of October city, Egypt) Batch No. 170439

Visceralgine® tablets containing 50 mg timonium methyl sulphate per tablet (obtained from Sedico Pharmaceutical Company, Giza, Egypt) Batch No. 0916299

Viscera® ampoules, containing 5 mg timonium methyl sulphate per 2 mL(Sedico Pharmaceutical Company, Giza, Egypt), Batch No. 1216287/A

Standard solutions

Stock standard solutions

A stock standard solution containing 1mg/mL of each drug was prepared separately in ethanol, methanol and water for finasteride, indapamide and tiemonium methyl sulphate respectively.

Working standard solutions

The stock standard solution of each drug was diluted separately, by the same solvent of each drug, to obtain the concentration of 10 µg /ml.

General procedures

In a 5 mL volumetric flask appropriate amounts of silver nitrate, sodium citrate, cited drugs (finasteride, indapamide, tiemonium methyl sulphate), and sodium hydroxide, only with finasteride, were added to make up the volume with distilled water. Each solution was heated in water at suitable temperature for appropriate times. Absorbance was measured at the suitable wavelength against reagent blank treated similarly **Table 2.**

Assay of pharmaceutical preparations

A-Assay tablets

1-Assay of hypotense® and Normaten® tablets

Ten tablets were weighed and pulverized. Then, the powder accounting for 10 mg of drugs was transferred in 10 mL volumetric flask. The powder was dissolved using 1 mL of 0.05M HCl

and diluted to mark using methanol. Solutions were filtered and neutralized with 1 mL of 0.05M NaOH then further diluted to 10µg /mL. Aliquots from this solution were used for subsequent experiments.

2-Assay of visceralgine® tablets

Ten tablets were weighed, pulverized into fine powder, in 10 mL volumetric flask specific quantity of powdered tablets equivalent to 10.0 mg pure drugs were dissolved, diluted to mark using methanol and sonicated for 30 minutes. Solutions were filtered and then further diluted to 10µg /mL. Aliquots from this solution were used for subsequent experiments.

B-Assay of prostride® capsules

The contents of ten capsules were emptied, in 10 mL volumetric flask an accurately weighed amount of finasteride equivalent to 10 mg was dissolved and diluted to the mark using ethanol. The drug solution was filtered and further diluted to 10µg /mL and aliquots from this solution were used for subsequent experiments.

C-Assay of viscera® ampoule

Specific volumes of ampoule solutions equivalent to 10.0 mg pure drug were placed in 100.0 mL volumetric flask, diluted to 100.0 mL with 50% methanol (v/v). The drug solution was then diluted to 10µg /mL and aliquots from this solution were used for subsequent experiments.

Procedures for Content Uniformity Testing

Each of the ten tablets of hypotense, and capsules of Prosteride were weighted accurately. Each tablet or capsule was considered a sample and analyzed as previously mentioned under assay of pharmaceutical preparations. The content of drug present in tablets was calculated as percent of the label claim for each tablet or capsule. The percent drug content of label claim was assessed to see if it complies with acceptance criteria.

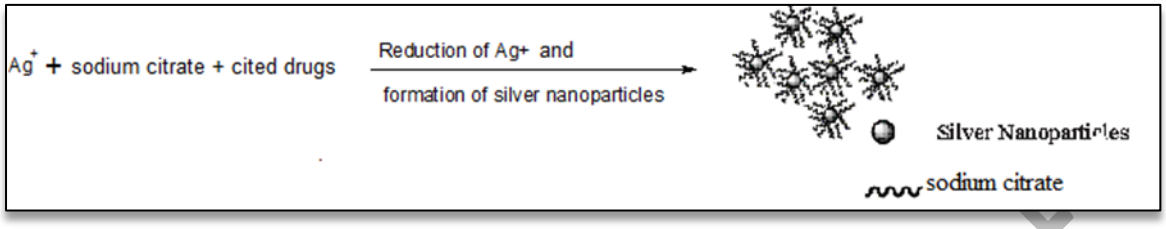
Procedures for application of tiemonium methyl sulphate into plasma

In a centrifuge tube mix 0.5 mL of 20µg/mL tiemonium methyl sulphate solution with 0.5 mL of plasma using vortex . Add 4.0 mL of acetonitrile solution to the tube, mix well for 1 minute and centrifuge for 30 min at 5000 rpm. The supernatant was passed through cellulose acetate syringe filter. In 5 mL volumetric flask, add 0.15mL of the supernatant to different concentration of pure drug. The procedures were completed as previously mentioned under general procedures.

RESULTS AND DISCUSSION

In recent years, silver nanoparticles have been reported in many applications. They have gained much interest in chemical analysis due to their high extinction coefficient and cost effectiveness of the analysis. At an alkaline medium; finasteride, indapamide and tiemonium methyl sulphate were reduced by silver nitrate solution to silver nanoparticles, which were stabilized by sodium citrate solution. The principle of this assay was based on reduction of silver nitrate to silver nanoparticles by the analytes. **Scheme 1**

Silver nanoparticles exhibit a well-known absorption band at 423 nm that have successfully been utilized in determination of the cited drugs **Figure 1**.



Scheme. 1 Reduction of silver ions by the cited drugs

Optimization of experimental variables:

Effect of silver nitrate

In order to find optimum concentration of silver nitrate, different concentrations from 0.5 to 30 mM were examined as shown in **Figures 2, 3**, **Table 2**.

Effect of Stabilizer type and concentration

Stabilization of silver nanoparticles is very important to prevent their aggregation. Nanoparticles stabilization is achieved by two mechanisms: electrostatic and steric stabilization. Electrostatic stabilization is caused by the repulsion between particles, (e. g., sodium citrate) while steric stabilization is achieved by surrounding the metal center by surfactants or polymers (e. g., PVP). In this study poly vinyl pyrrolidone (PVP), sodium citrate, sodium dodecyl sulphate (SDS), cetyl trimethyl ammonium bromide (CTAB) and methyl cellulose were tried as stabilizing agent. Sodium citrate was selected as the best stabilizer for prevention of Ag NPs agglomeration **Figure 4-6, Table 2.**

Effect of NaOH concentration

There was an increase in absorbance by increasing NaOH concentration till 0.001 M. Beyond this concentration, addition of NaOH shows decrease in absorbance, and formation of black precipitates, most likely due to formation of silver oxide.

The explanation of this observation can be as follow; the reaction between analyte and silver nitrate results in the formation of protons, as a result, the removal of these protons can enhance the formation of Ag-NPs. F**igures 7,8, Table 2.**

Effect of temperature and time of heating

It was observed that the reaction rate increased with increasing the temperature. Heating the solution in water bath at 95, 85 and 100^0C for 50, 25 and 20 min was sufficient to produce maximum color intensities for finasteride, indapamide and tiemonium methyl sulphate respectively **Figures 9, 10 Table 2.**

Order of addition

The sequence of addition of reactants could influence the rate of silver nanoparticles formation. Out of several reagents studies, the most suitable sequence was drug, sodium citrate, silver nitrate then NaOH for finasteride, drug, sodium citrate then silver nitrate for indapamide while silver nitrate, sodium citrate, drug for tiemonium methyl sulphate F**igure 11 Table 2.**

Method validation

Method validation was performed according to the ICH guidelines[35].

Linearity:

The linearity range of the cited drugs was (0.50-5.0 µg/mL), (0.50-5.0µg/mL) and (0.30-2.0µg/mL) for finasteride, indapamide and tiemonium methylsulphate respectively. Regression equation parameters were calculated. The small values of intercepts, relative standard deviation, standard error and high value of correlation coefficient indicated good linearity of the method. All these data were listed in **Tables 3 and 4.**

Sensitivity:

The LOD and LOQ were calculated according to the following equation: LOD = 3.3 * (σ/s) and LOQ = 10 * (σ/s)

Where, σ = the standard deviation of blank and s = slope of the calibration curve. Their values were listed in **Table 3** indicating sensitivity of the proposed method.

Accuracy and precision

Accuracy

To ascertain the accuracy of the proposed method, the obtained results were compared with the reported methods. Statistical comparison of the results was performed using student-t-test and

F-test at 95% confidence level **Table 5**. No significant differences were found between the proposed methods and the reported ones.

Precision

Precision was determined by analyzing three different concentrations of each drug three successive times in the same day (intra-day). The same concentrations were assayed in three different day (inter-day).The relative standard deviation and percentages relative error (Er%) were calculated **Table 6** using the following equation:

$$Er\% = [(found - added)/added] \times 100$$

Good results and acceptable relative standard deviations were obtained.

Selectivity

Selectivity of the method was checked by analyzing different mixtures of the cited drugs with some common excipients as lactose, sodium dodecyl sulphate, calcium carbonate, sodium chloride, sucrose, magnesium stearate and talc. Results showed some interferences from presence of magnesium stearate which could be overcame by extraction with methanol for indapamide and tiemonium methyl sulphate or ethanol for finasteride for tablets filtration **Table 7.**

Robustness and ruggedness

Robustness was examined by evaluating the effect of small variations in the experimental parameters on the analytical performance of the proposed method. The variation of the studied parameters were analyzed according to the following: volume of silver nitrate solution (0.45, 0.40, 0.35 for finasteride, 1.1, 1.0, 0.9 mL for indapamide, 0.35, 0.30, 0.25 mL for tiemonium methyl sulphate), sodium citrate volume (0.11, 0.10, 0.09 mL for finasteride, 0.45, 0.40, 0.35mL for indapamide and 0.55, 0.50, 0.45mL for tiemonium methyl sulphate) and NaOH volume (0.11, 0.10, 0.09mL for finasteride and 0.22, 0.20, 0.18 mL for tiemonium methyl sulphate). It was found that these variations had negligible influence on the results **Table 8.** Ruggedness was tested using two different instruments. The results were calculated as recovery ± %RSD. The low values of %RSD indicated the reproducibility of the method as shown in **Table 8.**

Analytical applications

In assay of hypotense® and normaten® tablets, first we got a low recovery of indapamide but when we used 1.0 mL of 0.05 M HCl in dissolution medium, we got satisfactory results. This can be explained by a paper published by **Nishath Fathima et al** [36]. They studied mechanisms of drug excipients interaction showing that a physical interaction occurs between primary amine drugs, indapamide, and microcrystalline cellulose, an excipient in its tablet dosage forms. For low dose drugs it can lead to dissolution failures and this can be remedied by carrying out dissolution using 0.05 MHCl). The proposed method was applied to determine the studied drugs in their pharmaceutical dosage forms with satisfactory results obtained. Also spiking of tiemonium methyl sulphate into plasma and good extraction from it proved the suitability of the proposed method **Figure 12, Table 9-10.**

Content Uniformity Test

Due to the sensitivity of the proposed method, the method is ideally suited for content uniformity testing. The steps of the test were adopted according to the USP [37] procedure. The acceptance value (AV) was calculated and it was found to be smaller than the maximum allowed acceptance value (L1). The results demonstrated drug uniformity for finasteride and indapamide as shown in **Table 11.**

Conclusion

Application of silver nanoparticles as chromogenic agent has been demonstrated in this work for optical determination of finasteride, indapamide and tiemonium methyl sulphate. The proposed method was found to be simple, sensitive and easily applicable to analysis of the cited drugs in their pharmaceutical dosage forms with good accuracy and precision. The method is based on the reaction of $AgNO_3$ by the cited drugs in presence of sodium citrate and slightly basic medium to synthesize silver nanoparticles.

Acknowledgements

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Figures

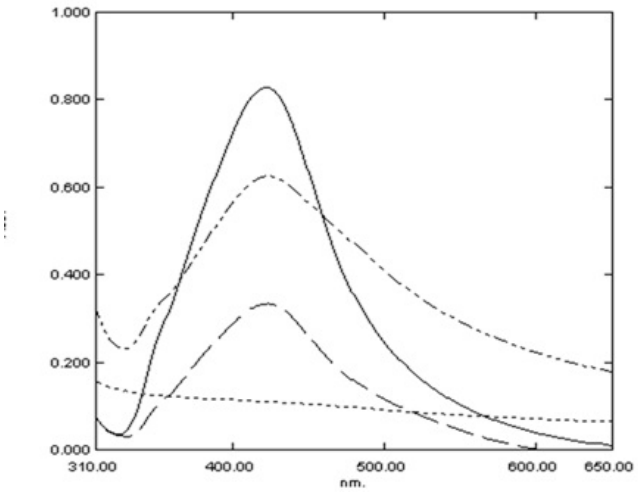


Fig. 1: Absorbance spectra of the silver nanoparticles formed in the presence of 4 µg/ml finasteride, 1.8µg/ml indapamide and 1.25µg/ml tiemonium methyl sulphate.

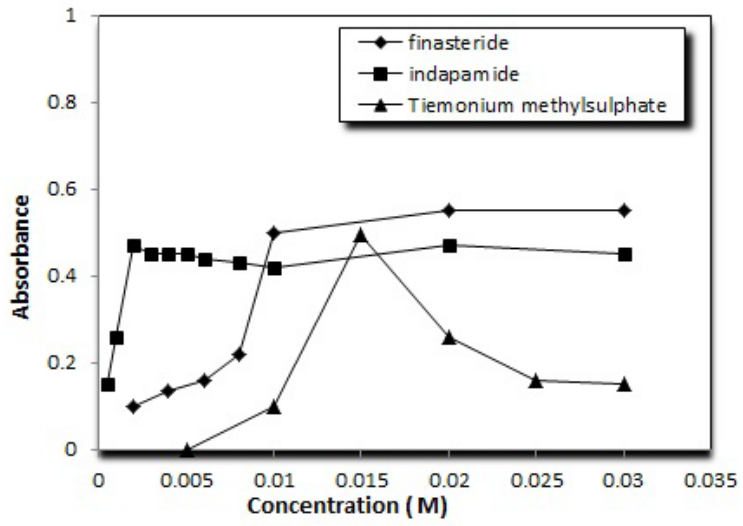


Fig. 2 Effect of concentration of silver nitrate solution on the absorbance of silver nanoparticles formed through reaction of sodium citrate and NaOH solution in presence of 4µg/ml finasteride, 4.5 µg/ml indapamide and 2 µg/ml tiemonium methyl sulphate

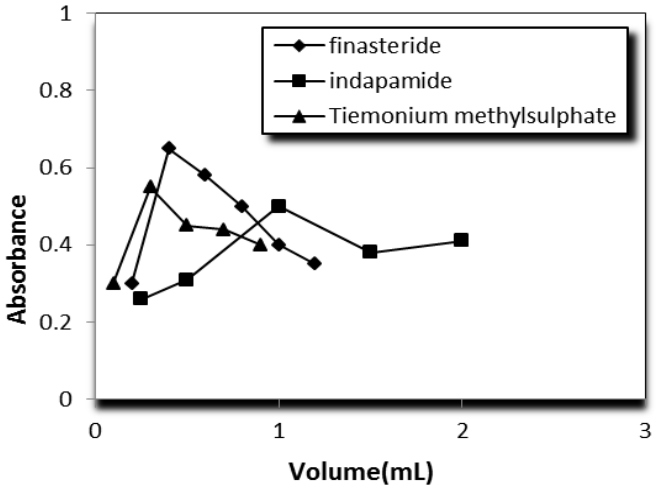


Fig. 3 Effect of volume of silver nitrate solution on the absorbance of silver nanoparticles formed through reaction of sodium citrate and NaOH solution in presence of 4µg/ml finasteride, 4.5 µg/ml indapamide and 2 µg/ml tiemonium methyl sulphate

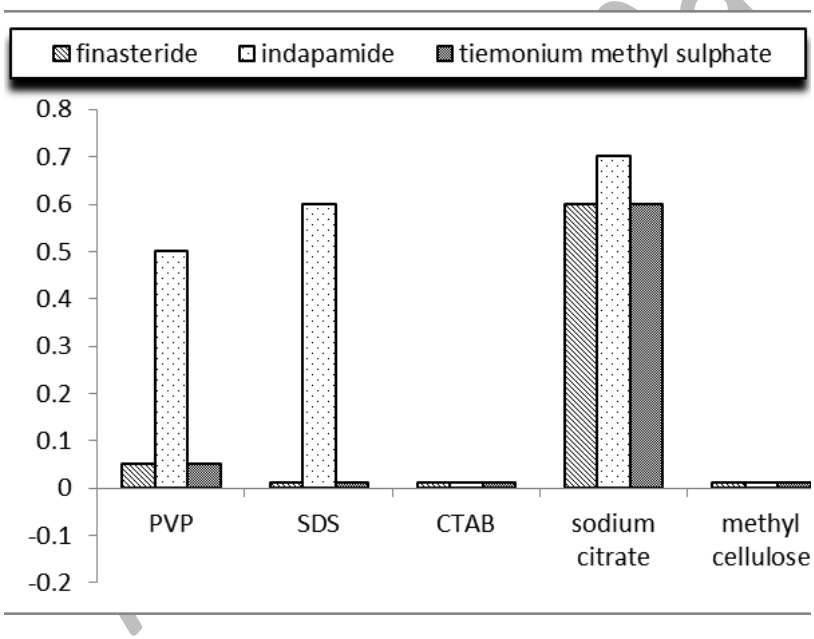


Fig. 4 Effect of stabilizer types on the absorbance of silver nanoparticles formed through reaction of silver nitrate and NaOH solution in presence of 4µg/ml finasteride, 4.5 µg/ml indapamide and 2 µg/ml tiemonium methyl sulphate

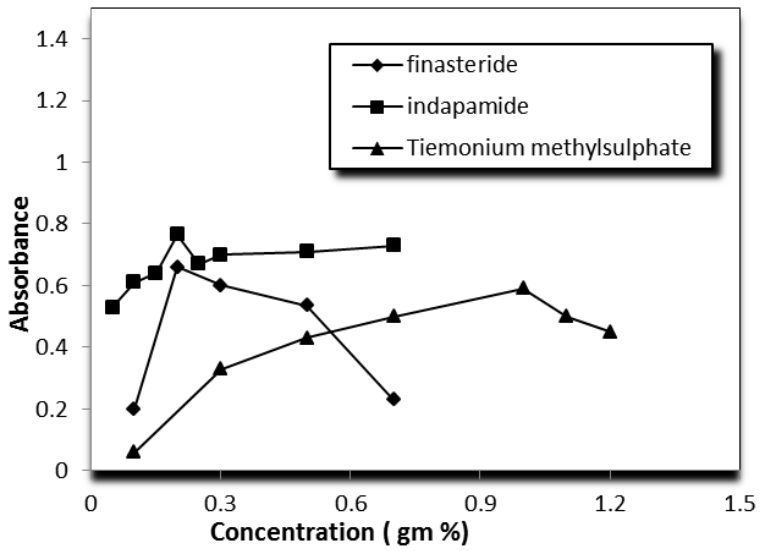


Fig. 5 Effect of concentration of sodium citrate solution on the absorbance of silver nanoparticles formed through reaction of silver nitrate and NaOH solution in presence of 4µg/ml finasteride, 4.5 µg/ml indapamide and 2 µg/ml tiemonium methyl sulphate

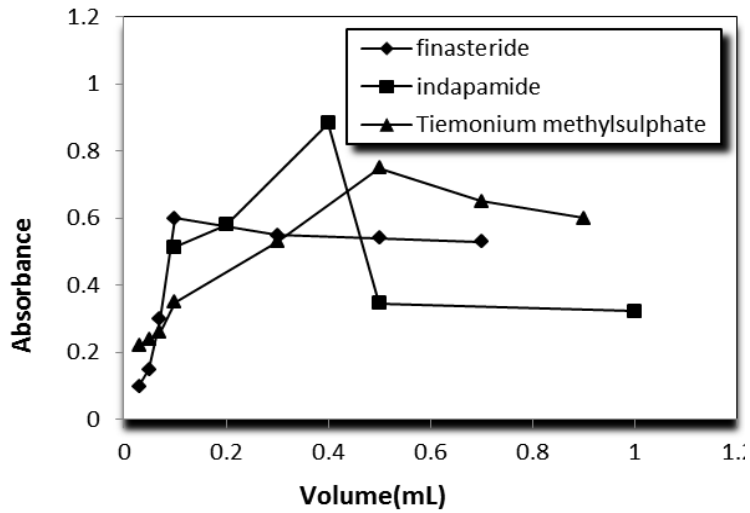


Fig. 6 Effect of volume of sodium citrate solution on the absorbance of silver nanoparticles formed through reaction of silver nitrate and NaOH solution in presence of 4µg/ml finasteride, 4.5 µg/ml indapamide and 2 µg/ml tiemonium methyl sulphate

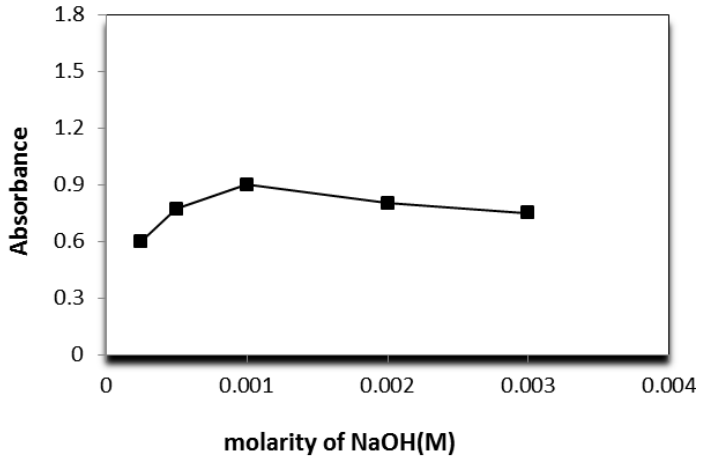


Fig. 7. Effect of concentration of NaOH solution on the absorbance of silver nanoparticles formed through reaction of silver nitrate and sodium citrate solution in presence of 4µg/ml finasteride

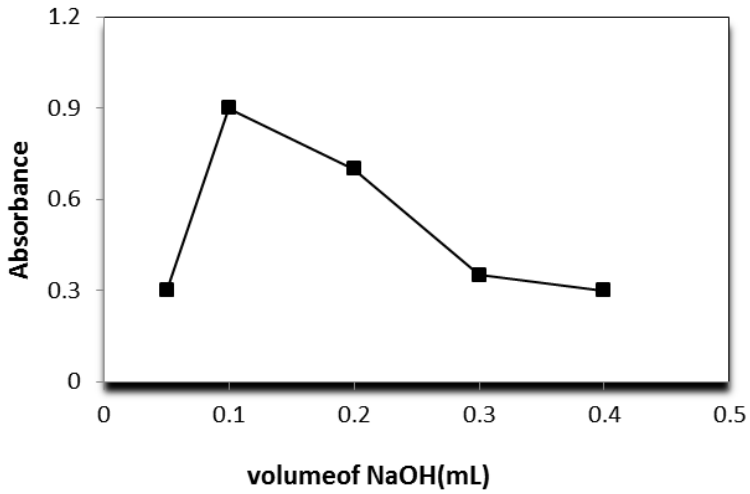


Fig. 8. Effect of volume of NaOH solution on the absorbance of silver nanoparticles formed through reaction of silver nitrate and sodium citrate solution in presence of 4µg/ml finasteride

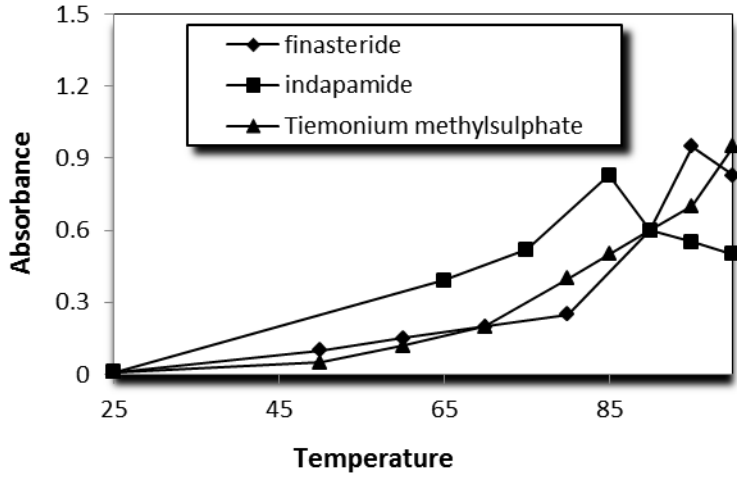


Fig. 9 Effect of temperature on the absorbance of silver nanoparticles formed through reaction of silver nitrate, sodium citrate and NaOH solution in presence of 4µg/ml finasteride , 4.5 µg/ml indapamide and 2 µg/ml tiemonium methyl sulphate

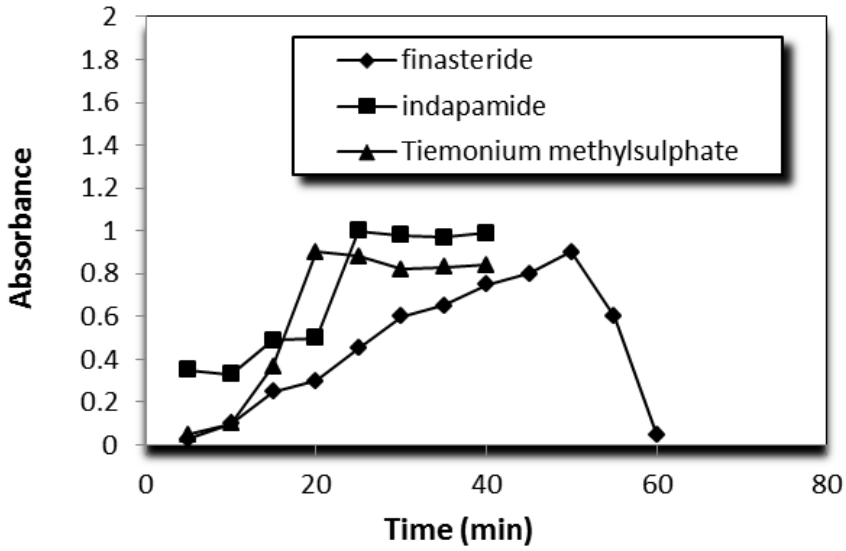


Fig. 10 Effect of time of heating on the absorbance of silver nanoparticles formed through reaction of silver nitrate, sodium citrate and NaOH solution in presence of 4µg/ml finasteride, 4.5 µg/ml indapamide and 2 µg/ml tiemonium methyl sulphate

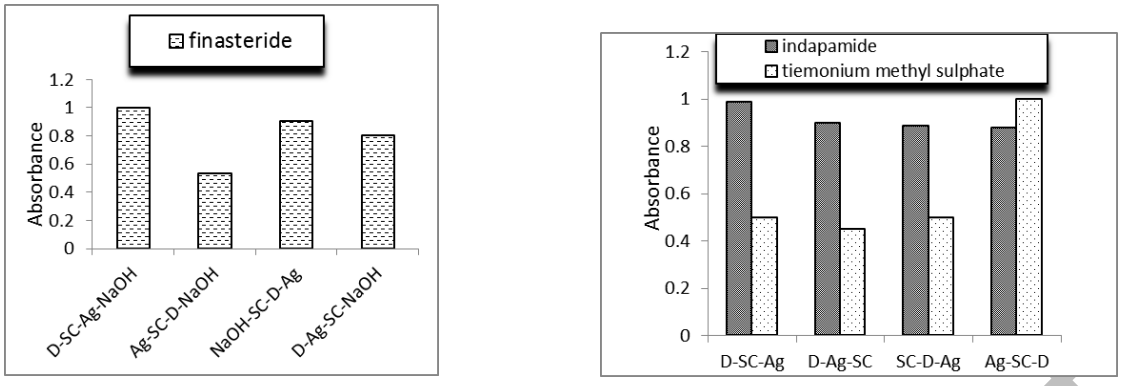


Fig. 11: Effect of order of addition of silver nitrate, sodium citrate and NaOH solutions to: 4µg/ml finasteride, 4.5 µg/ml indapamide and 2 µg/ml tiemonium methyl sulphate

D=drug, Ag=silver nitrate, SC=sodium citrate

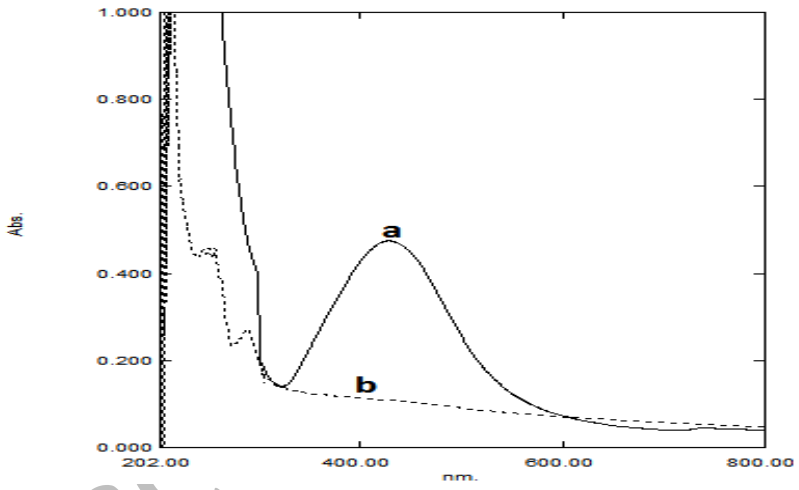


Fig.12: (a) Plasma sample spiked with 0.90 µg/mL tiemonium methyl sulphate using the proposed method, (b) Blank plasma

Table (1): chemical structure of the studied drugs:

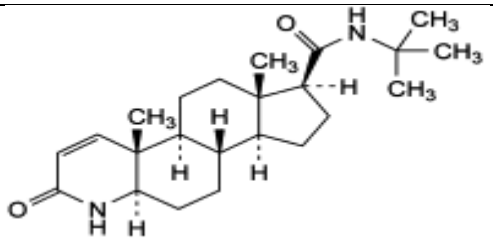
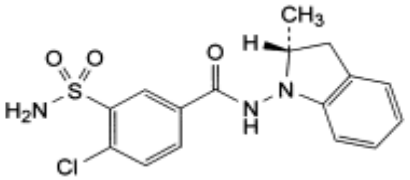
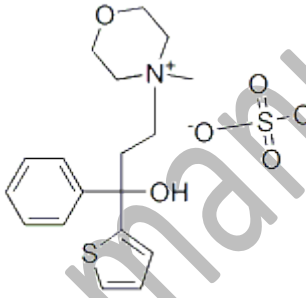
Finasteride	
Indapamide	 and enantiomer
Tiemonium methyl sulphate	

Table (2): Analytical parameters for determination of finasteride, indapamide and tiemonium methyl sulphate through silver nanoparticles formation

Parameter	Finasteride	Indapamide	Tiemonium methyl sulphate
λmax (nm)	422	423	423
Concentration of $AgNO_3$ (M)	0.01	0.002	0.015
Volume of $AgNO_3$ (ml)	0.40	1.00	0.30
Concentration of sodium citrate (w/v)	0.2%	0.2%	1%
Volume of sodium citrate (ml)	0.10	0.40	0.50
Concentration of NaOH (M)	0.001	------	-----
Volume of NaOH (ml)	0.10	-----	------
Temperature(^{0}C)	95.00	85.00	100.00
Time of heating (min)	50.00	25.00	20.00

Table (3): Spectral data for determination of cited drugs using silver nanoparticles formation

Parameter	Finasteride	Indapamide	Tiemonium methylsulphate
Linearity range (µg/ml)	0.50-5.0	0.50-5.0	0.30-2.00
Apparent molar absorptivity*($mol^{-1}cm^{-1}$)	7.52×10^4	6.85×10^4	2.20×10^5
Limit of detection LOD (µg/ml)	0.151	0.163	0.093
Limit of quantification LOQ (µg/ml)	0.503	0.543	0.309
Regression equation**:			
Slope (b)	0.1988	0.1842	0.4945
Intercept (a)	0.006	0.007	0.0127
Correlation coefficient (r)	0.9999	0.9999	0.9999

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

** A=a + bc

Table (4): Determination of finasteride, indapamide and tiemonium methylsulphate through silver nanoparticles formation

	Finasteride		Indapamide		Tiemonium methylsulphate	
	Taken µg/mL	Recovery* %	Taken µg/mL	Recovery* %	Taken µg/mL	Recovery* %
	0.50 1.00 2.00 2.50 3.00 3.50 5.00	99.60 100.10 99.09 101.41 99.60 99.74 100.00	0.50 1.00 2.00 3.00 3.50 4.00 4.50 5.00	100.98 100.98 99.89 99.53 99.74 99.48 100.49 100.22	0.30 0.50 0.60 0.90 1.00 2.00	99.29 98.81 100.20 100.51 100.57 99.83
Mean* ±SD	99.93±0.727		100.16±0.604		99.87±0.702	
N	7		8		6	
V	0.529		0.365		0.493	
R.S.D.	0.728		0.603		0.703	
S.E.	0.275		0.214		0.287	

* Mean of three different experiments

Table (5): Statistical analysis of results obtained by the proposed and the comparison methods.

drug	Finasteride		Indapamide		Tiemonium methyl sulphate	
Items	Proposed method	Comparison method[6]	Proposed method	Comparison method[17]	Proposed method	Comparison method[23]
Mean ± SD	99.93±0.727	99.92±0.731	100.16±0.604	99.95±0.652	99.87±0.702	100.40±0.753
Variance	0.529	0.535	0.365	0.426	0.493	0.567
N	7	5	8	5	6	6
Student-t-test	0.023(2.228) *	------	0.591(2.201) *	-----	1.261(2.228) *	-----
F-test	1.011(4.53) *	-------	1.167(4.12) *	-------	1.150(5.05) *	------

*Theoretical values of t and F at p = 0.05

Table (6): Precision data for the determination of the cited drugs by the proposed method.

Drug	Added (µg/ml)	Intra-day				Inter-day			
		found±SE (µg/ml)	Recovery %	RSD%	Er%	found±SE (µg/ml)	Recovery %	RSD%	Er%
Finasteride	2.00	2.00± 1.030	100.02	1.784	0.02	1.940± 1.109	97.00	1.980	-3.00
	3.00	3.023± 1.213	100.77	2.085	0.77	3.015± 1.245	100.49	2.145	0.49
	5.00	4.899± 1.236	97.99	2.185	-2.01	4.866± 1.209	97.32	1.209	-2.68
Indapamide	1.00	1.003± 1.187	100.25	2.050	0.25	1.004± 0.829	100.43	1.430	0.43
	3.00	2.980± 1.271	99.35	2.216	-0.65	2.990± 1.255	99.65	2.182	-0.35
	5.00	5.047± 0.958	100.94	1.643	0.94	5.052± 1.232	101.05	2.112	1.05
Tiemonium methyl sulphate	0.50	0.497± 1.104	99.35	1.924	-0.65	0.500± 1.168	100.02	2.022	0.02
	1.00	1.006± 1.168	100.57	2.011	0.57	1.006± 1.284	100.57	2.212	0.57
	2.00	2.015± 1.046	100.77	1.799	0.77	2.010± 0.892	100.50	1.537	0.50

Table 7: Analysis of the cited drugs by the proposed method in presence of some common excipients.

Tolerance Molar ratio (M:M)*	Recovery %**						
	finasteride		indapamide	tiemonium methyl sulphate			
	Lactose	Sodium dodecylsulphate	Lactose	Calcium carbonate	Lactose	Sodium chloride	Sucrose
1:1	99.09	101.61	98.53	102.75	100.51	97.14	98.26
1:10	95.32	100.35	95.01	103.88	99.61	96.91	99.61
1:50	96.58	101.61	95.28	103.43	98.26	96.01	100.51
1:100	97.08	98.84	100.16	102.75	96.69	95.11	103.65
Other excipients	finasteride		indapamide	tiemonium methyl sulphate			
Magnesium stearate(40µg/ml)	94.06		93.11	93.76			
Talc(40µg/ml)	99.09		-------	102.75			

*Drug: Excipients, finasteride 2 µg/ml ($5.3x10^{-4}$ M), indapamide 2 µg/ml ($5.4x10^{-4}$ M) and tiemonium methyl sulphate 0.9 µg/ml ($2x10^{-4}$ M)

** Mean of three determinations.

Table (8): Method robustness and ruggedness expressed as (recovery±%RSD).

	Robustness				Ruggedness
	Parameters altered				Inter-instruments(n=2)
Drugs	Taken µg/ml	Volume of silver nitrate solution	Volume of sodium citrate	Volume of NaOH	JENWAY6715UV/Vis. -ShimadzuUV1800 PC
Finasteride	3.50	99.26± 0.836	98.30± 1.462	101.32± 1.636	100.46± 1.012
Indapamide	3.00	100.49± 1.226	98.50± 0.943	-------	98.62± 1.297
Tiemonium methyl Sulphate	0.90	99.01± 1.511	100.58± 1.569	---------	99.49± 1.757

Table (9): Application of the proposed method for determination of the cited drugs in their pharmaceutical formulations:

Drug	Prostride®capsules			hypotense®tablets			Normaten® tablets		
Statistic	Taken µg/ml	Added µg/ml	Recovery* %	Taken µg/ml	Added µg/ml	Recovery* %	Taken µg/ml	Added µg/ml	Recovery* %
	1.00	-----	100.10	1.00	----	98.81	1.00	---	96.09
		1.25	102.21		2.00	98.53		1.00	97.18
		1.50	103.62		2.50	99.46		2.50	97.50
		2.25	101.50		3.00	100.25		3.00	97.54
		2.75	102.25		3.50	99.89		4.00	96.91
		3.00	103.79		4.00	100.98			
Mean±S.D.	102.67±0.990			99.82±0.911			97.28±0.299		
N	5			5			4		
V	0.979			0.829			0.089		
S.E	0.443			0.407			0.134		

* Mean of three different experiments

Table 10: 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* %
	0.30	----	102.66	0.30	---	99.29	0.30	---	97.27
		0.30	100.64		0.40	99.75		0.30	97.94
		0.60	98.52		0.50	101.23		0.60	96.16
		0.70	100.33		0.70	100.91		0.70	95.71
		0.80	101.69		0.90	101.85		0.80	96.13
		1.50	101.42		1.30	99.14		1.70	97.58
		1.60	101.40		1.40	99.86			
Mean*±SD	100.67±1.175			100.46±1.036			96.70± 0.990		
N	6			6			5		
V	1.380			1.074			0.979		
S.E.	0.480			0.423			0.404		

* Mean of three different experiments

Table 11: Results of content uniformity testing of finasteride and indapamide tablets using the proposed methods

Parameter	Percentage of the label claim	
	finasteride	indapamide
	96.13	99.21
	97.33	102.94
	97.73	103.38
	104.00	96.19
	102.67	95.00
	96.00	104.64
	98.80	99.68
	101.47	103.68
	103.20	95.65
	97.87	99.56
Mean (X)	99.52	99.99
± S.D.	3.03	3.57
% RSD	3.04	3.57
% Error	0.96	1.13
Acceptance value (AV)	7.26	8.56
Max. allowed AV (L1)	15	15