Original Article

Optimization and validation of spectrophotometric methods for determination of finasteride in dosage and biological forms

Abstract

Aim and Background: Three simple, accurate and sensitive spectrophotometric methods for the determination of finasteride in pure, dosage and biological forms, and in the presence of its oxidative degradates were developed. **Materials and Methods:** These methods are indirect, involve the addition of excess oxidant potassium permanganate for method A; cerric sulfate [Ce(SO4)2] for methods B; and N-bromosuccinimide (NBS) for method C of known concentration in acid medium to finasteride, and the determination of the unreacted oxidant by measurement of the decrease in absorbance of methylene blue for method A, chromotrope 2R for method B, and amaranth for method C at a suitable maximum wavelength, λ_{max}: 663, 528, and 520 nm, for the three methods, respectively. The reaction conditions for each method were optimized. **Results:** Regression analysis of the Beer plots showed good correlation in the concentration ranges of 0.12– 3.84 $\mu g\ mL_{-1}$ for method A, and 0.12–3.28 $\mu g\ mL_{-1}$ for method B and 0.14 – 3.56 $\mu g\ mL_{-1}$ for method C. The apparent molar absorptivity, Sandell sensitivity, detection and quantification limits were evaluated. The stoichiometric ratio between the finasteride and the oxidant was estimated. The validity of the proposed methods was tested by analyzing dosage forms and biological samples containing finasteride with relative standard deviation ≤ 0.95. **Conclusion:** The proposed methods could successfully determine the studied drug with varying excess of its oxidative degradation products, with recovery between 99.0 and 101.4, 99.2 and 101.6, and 99.6 and 101.0% for methods A, B, and C, respectively.

Key words: Biological samples, drug, dosage forms, redox reaction, spectrophotometry

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INTRODUCTION

Finasteride [Proscar, *N*-(1,1-dimethylethyl)-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide [Figure 1] is a 4-aza-3-oxosteroidal inhibitor of human 5α-reductase.[1-6] It is a member of the family of compounds referred to as 4-azasteroids. Its synthesis has been described;[7] the compound appears to have some potential as a therapeutic agent for benign prostatic hyperplasia.[8] The 4-azasteriods are a newly developed family of compounds that block the intracellular metabolism of testosterone and thereby enable the more potent androgen dihydrotestosterone to come into play[1-6] Today the most accepted mechanism brings over the interaction of finasteride with the NADP-5α reductase complex which is related with the redox properties of finasteride, and corresponds to a reduction of the drug in the double bond between the carbons 1 and 2 of the androstane ring; the dihydrofinasteride has been identified by mass spectrometry.[9] Despite its widespread use, little has been published concerning its quantitation.

Several methods for finasteride determination have been reported in the literature. Most of these studies have determined the concentration of finasteride employing gas-chromatography (GC)[10] and high-performance liquid chromatography (HPLC),[11-15] (HPLC-MS),[16,17] (HPLC-UV),[18,19] polarography,[20] voltammetry,[21] and spectrophotometry.[22-25]

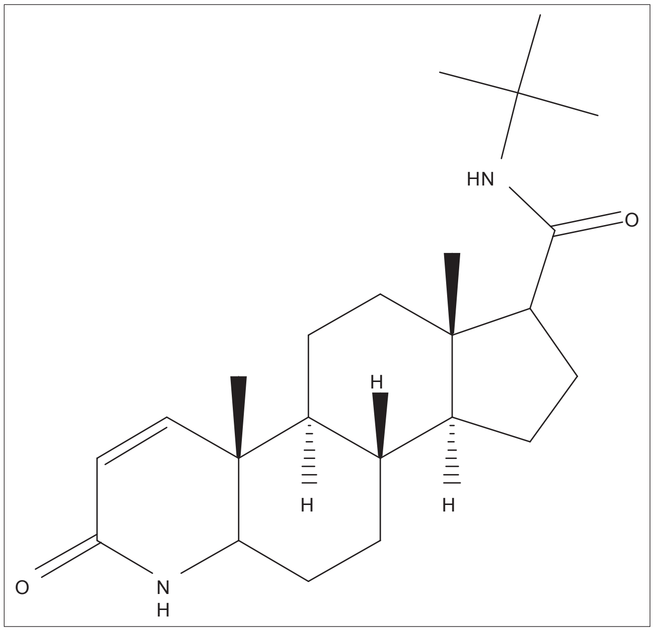

Figure 1: Chemical structure of finasteride

In recent years, stringent quality control in the pharmaceutical industries has given rise to a growing need for simple, selective and sensitive analytical methods for their determination in pure and in dosage forms. Spectrophotometry has always provided analytical techniques characterized by instrumental simplicity, moderate cost and portability. These features make spectrophotometric techniques particularly suitable for the determination of trace concentrations of clinically important compounds. Regarding the interest and widespread use of spectrophotometric detectors, such as, in liquid chromatographic systems, it is intriguing that they had never been used for the analysis of finasteride depending on its redox properties.

Oxidation-reduction reactions have been used as the basis for the development of simple and sensitive spectrophotometric methods for the determination of many pharmaceutical compounds.[26-30] None of these reagents have not been previously used for the spectrophotometric analysis of finasteride. For these reasons, the present study was dedicated to investigate the application of redox reaction in the direct spectrophotometric analysis of finasteride in bulk drug, dosage forms and in biological samples.

MATERIALS AND METHODS

Apparatus

All the absorption spectral measurements were made using Perkin Elmer Lambda 12 and JASCO V-530 (UV-Visible) (UV-Vis) spectrophotometers equipped with 10-mm matched quartz cells, a scanning speed of 400 nm/min, and a band width of 2.0 nm.

Material and reagents

All chemicals used were of analytical or pharmaceutical grade purity, and water was doubly distilled. Pure finasteride and its *prostride* capsules were kindly provided by Egyptian Company for Chemicals and Pharmaceuticals (ADWIA), Cairo, Egypt. Finasteride pure sample was used as received; (purity 99.68%). Stock solution, 100 μg mL^{-1}, was prepared by dissolving 10 mg finasteride in methanol and was further diluted with the same solvent. Working solutions of lower concentration were prepared by serial dilutions.

A stock (5.0×10^{-4} M) solution of $KMnO_4$ (Aldrich), was freshly prepared by dissolving an accurate weight in bidistilled water, and standardized as recommended.[31] A solution of cerium(IV) sulfate (3.0×10^{-3} M, May and Baker) was prepared by dissolving a known weight of $Ce(SO_4)_2$ in a small amount of warm 1.0 M H_2SO_4 in a 250-mL measuring flask, and then diluting with the same acid to the mark. An aqueous solution of *N*-bromosuccinimide (100 μg mL^{-1}, Aldrich) was freshly prepared. A solution of 5.0 M HCl was prepared and standardized prior to use, as recommended previously.[31]

Aqueous solutions of methylene blue (MB; 10^{-4} M, Merck), and chromotrope 2R (C2R; 5.0×10^{-3} M, Aldrich), and amaranth (AM; 2×10^{-3} M, Aldrich), were prepared by dissolving an appropriate weight in 100 mL bidistilled water.

Analysis of pure samples

Methods A

To a series of 10 mL calibrated flasks, containing upto (1.2–38.4 μg mL^{-1}) aliquots of finasteride, 0.8 mL of 5.0×10^{-4} M $KMnO_4$ and 0.8 mL of 0.2 M H_2SO_4 were transferred, and the solutions were diluted to 5.0 mL. The solution was heated in a water bath at 60 ± 1 °C for 5.0 min, the mixture was cooled and 2.0 mL of 10^{-4} M MB was added. The volume was completed to 10 mL with bidistilled water. The decrease in color intensity MB was measured spectrophotometrically against a blank solution containing the same constituent except drug treated similarly, at their corresponding λ_{max} 663 nm. The concentration range was determined by plotting the concentration of finasteride against absorbance at the corresponding maximum wavelength.

Methods B

Aliquots containing 1.2–32.8 μg mL^{-1} finasteride were transferred to a series of 10 mL calibrated flasks, and 0.6 mL 3×10^{-3} M $Ce(SO_4)_2$ containing 1.0 M H_2SO_4 was added. The solution was boiled in a water bath for 7.0 min. The mixture was cooled, and 0.40 mL 5×10^{-3} M C2R was mixed. The volume was diluted to 10 ml with water. A decrease in color intensity C2R was measured at their corresponding λ_{max} 528. The concentration range was determined by plotting concentration of finasteride against absorbance at the corresponding λ_{max}.

Method C

To a series of 10 mL calibrated flasks, containing aliquots of finasteride (1.4–35.6 μg mL^{-1}), 1.2 mL 100 μg mL^{-1} NBS, 1.5 mL 5.0 M HCl, and 1.8 mL 1.0% KBr were transferred, and the solutions were diluted to 7.0 ml. After 5.0 min, 0.5 mL 2.0×10^{-3} M AM was added, mixed throughout, and diluted to the mark with water. The absorbance was measured at 520 nm against a blank solution prepared in the same manner without the drug. A calibration graph was prepared by plotting absorbance of the AM against the finasteride concentration. The amount of finasteride in unknown sample was calculated from its calibration curve.

Preparation of degradation products

A suitable amount (0.1 g) of finasteride was dissolved in 10 mL 0.1 M HCl, and then 1.0 mL 15% H_2O_2 was added. The solution was boiled in a water bath for 45 min and then diluted in a 100 mL measuring flask to the mark with bidistilled water. The stock solution was diluted quantitatively to obtain degraded samples of the required concentrations.

Analysis of capsules

Weigh the contents of 10 capsules was weighed and mixed well. To a quantity of the powder capsules equivalent to 10 mg of the drug, 20 mL methanol was added, filtered into a 100-mL measuring flask, washed the filter paper with another 20 mL methanol, and then diluted with the same solvent to the mark. The above procedures (A – C) were preceded and the finasteride content per capsule was determined either from the calibration graph or from the regression equation.

Plasma sample preparation

Drug-free human plasma was purchased from Zagazig University Hospital (Zagazig, Egypt). Plasma samples from volunteers who had taken Prostride capsule were frozen within 1 h of collection, and kept frozen until analyzed.

Plasma (0.5 mL) spiked with finasteride (analyte) was mixed with 1 mL 0.05 M ammonium formate buffer. Five millimeters of chloroform was added and the solution was shaken for 5.0 min. The aqueous layer was removed and organic layer was centrifuged for 10 min (10 krpm). The organic layer was evaporated using nitrogen gas, and the residue was dissolved in 1-mL methanol. A portion of this solution was then treated as described above in three procedures (A – C).

RESULTS AND DISCUSSION

This work was conducted to establish simple spectrophotometric methods for the determination of finasteride, which contains a tertiary amino group and pyridine ring. The presence of these groups makes this compound liable to atmospheric oxidation, forming an oxidative product. The structural activity relationship shows that these oxidative degradates are inactive. For this reason the establishments of methods that quantitatively determine the pure drug in the presence of its degradation product are of great pharmaceutical value. The absorption spectra of the reaction products in methods A, B, and C showed characteristic λ_{max} value at 663, 528 and 520 nm, respectively.

An analytical procedure based on the specific reactivity of the tertiary amino group and pyridine ring was investigated. The method involves two steps namely:

1. Oxidation of finasteride with $KMnO_4$, $Ce(SO_4)_2$ and NBS in acidic medium.
2. Determination of unreacted oxidant by measuring the decrease in absorbance of MB, C2R and AM at their λ_{max} 663, 528 and 520 nm, respectively.

Optimization

The influence of each of the following variables on the reaction was tested.

Method A

The influence of $KMnO_4$ concentration was studied in the range from 10^{-5} to 10^{-4} M, as final concentration. The optimum results were obtained with 4×10^{-5} M; higher concentration of $KMnO_4$ caused the color to disturbed as shown in Figure 2.

Different types of acid were examined (HCl, H_2SO_4, H_3PO_4, CH_3COOH and HNO_3). The most suitable acid to achieve maximum yield of redox reaction was found to be sulfuric acid. Moreover, various volumes of H_2SO_4 were tested and found to be 0.8 mL of 0.2 M.

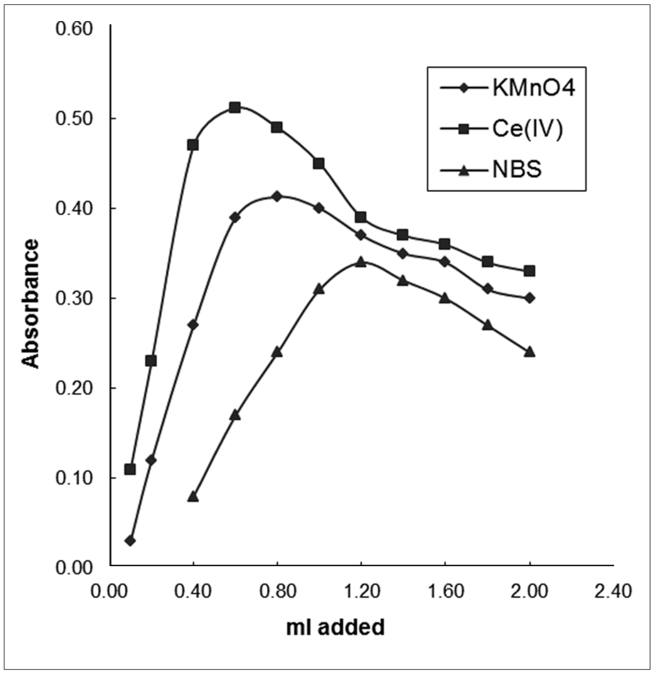

Figure 2: Effect of oxidant volume on the redox reaction with 2.0 mg mL and#8722;1 finastride.

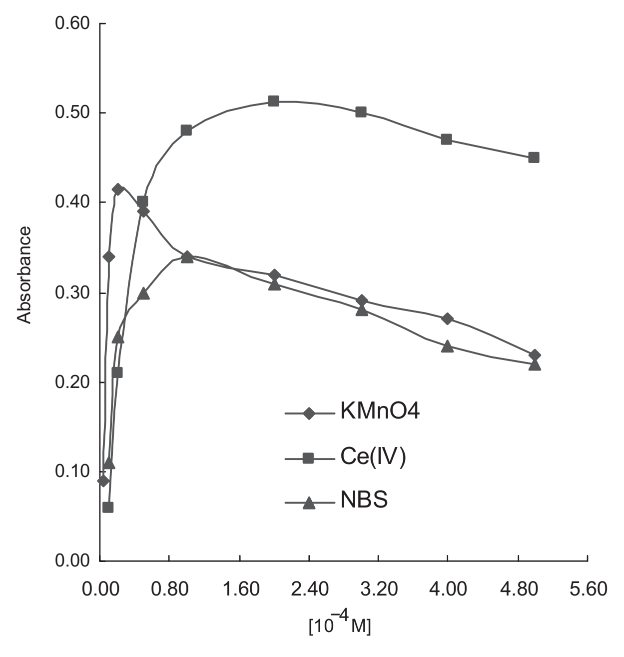

Figure 3: Effect of dye concentration on the redox reaction of 2.0 mg mL and#8722;1 of finastride.

The oxidation process of finasteride is catalyzed by heating in water bath of 60 ± 1°C. The time required to complete the reaction is 5.0 min. After oxidation process, the solution must be cooled at least for 3.0 min before addition of MB. The effect of time after the addition of dye indicated that shaking for 1.0 min is sufficient to give reliable results. The optimum volume of dye used for production of maximum color intensity is 2.0 mL of 10^{-4} M MB [Figure 3].

The stoichiometry of the reaction between finasteride and $KMnO_4$ was investigated by the molar ratio method. Experimental results showed that the molar ratio of finasteride to $KMnO_4$ is 1: 2. The excess $KMnO_4$ reduced the color intensity of MB through disruption of the conjugation system in the dye. The color of dye remains constant in absorbance for at least 48 h, and then decreases slightly afterwards.

Methods B

Cerium(IV) sulfate reacts with finasteride, giving a number of oxidized products according to the functional groups present in finasteride and the experimental conditions. The unreacted Ce(IV) oxidizes C2R to form colorless products. The remaining C2R is then measured spectrophotometrically at its corresponding λ_{max} 528 nm.

In order to establish the optimum conditions, investigations were carried out to achieve maximum color development in the quantitative determination of finasteride. The influence of each of the following variables on the reaction was tested. The most suitable acid to be used with $Ce(SO_4)_2$ was found to be sulfuric acid of 1.0 M concentration, presented as 6.0% (v/v) total volume in the reaction mixture.

The influence of Ce(IV) concentration was studied in the range from 5×10^{-5} - 5×10^{-4} M, as final concentration. The optimum results were obtained with 0.6 mL of 5.0×10^{-4} M; higher concentration of Ce(IV) caused the color to disturbed as shown in Figure 2.

The oxidation process of finasteride with $Ce(SO_4)_2$ is catalyzed by heat and reaches maximum at 100 °C. The time required to complete the reaction is 7.0 min. The optimum volume of C2R used for the production of maximum and reproducible color intensity is 0.4 mL of 1.0×10^{-4} M C2R in case of NBS [Figure 3]. The effect of time after the addition of C2R indicated that shaking for 1.0 min is sufficient to give reliable results for C2R.

The stoichiometry of the reaction between finasteride and $Ce(SO_4)_2$ was investigated by the molar ratio method. Experimental results showed that the molar ratio of finasteride to $Ce(SO_4)_2$ is 1: 3. The excess Ce(IV) reduced the color intensity of C2R through disruption of the conjugation system in the dye. The color of C2R remains constant in absorbance for at least 48 h, and then decreases slightly afterwards.

Method C

NBS reacts with finasteride, resulting in oxidation, substitution, or addition, depending on the functional groups present in the drug, probably a mixture of products, with reproducible data under specified experimental conditions. The excess NBS reacts with AM dye (bromination reaction) to form colorless products. Different volumes of 100 μg mL^{-1} NBS were examined, and the optimum amount was 1.2 ml; the results were highly agreeable at this concentration level [Figure 2]. The remaining AM dye was then measured spectrophotometrically at λ_{max} 520 nm.

To ascertain the optimum conditions for method C, several experiments were conducted to achieve the optimum parameters through the effects of types of acid concentration, time, KBr concentration, sequence of additions and dye concentration. It was established that 1.5 mL 5-M HCl (as optimum acid), 1.8 ml 1% KBr, and 0.5 mL 2.0×10^{-3} M AM dye [Figure 3] are required for maximum color development and more intensive absorbance. The reaction takes place completely in the presence of KBr after 5 min of mixing. Finasteride – NBS – HCl – KBr is the optimum sequence of addition. The effect of time after the addition of AM dye indicated that shaking for 1 min is sufficient to give reliable results.

In order to investigate the molecular ratio between finasteride and NBS at the selected conditions, the molar ratio method was studied. Experimental results showed that the molar ratio of finasteride to NBS is 1: 1. The excess NBS reduces the intensity of red color through disruption of the conjugation system AM. The remaining color of AM remains constant in absorbance for at least 48 h, and then decreases slightly afterwards.

Validation of the proposed methods

Calibration curves and linearity

Under the optimum reaction conditions described above, the calibration curves for finasteride with the different analytical reagents employed in the present work were constructed. The regression equations for the results were derived using the least squares method. In all cases, Beer's law plots ($n = 6$) were linear with very small intercepts and good correlation coefficients in the general concentration range of 0.12 - 3.84 μgmL^{-1} [Table 1]. For more accurate analysis, Ringbom optimum concentration range were evaluated to be 0.25 - 3.60, as recorded in Table 1.

Sensitivity

Statistical analysis of the results obtained [Table 1], indicated that the proposed methods were accurate and precise. The limits of detection (LOD) and limits of quantification (LOQ) were determined[32] using the formula:

$$\text{LOD or LOQ} = \kappa SD_a / b,$$

where κ = 3 for LOD and 10 for LOQ, SD_a is the standard deviation of the intercept, and b is the slope. Based on the basis of six replicate measurements, the limits of detection were 35, 33 and 0.41 ng mL^{-1} and the limits of quantification were 0.12, 0.11 and 0.14 μgmL^{-1}, using methods a, B, and C, respectively. Both LOD and LOQ values confirmed the sensitivity of the proposed methods.

Precision

The precision of the methods (within-assay and between-assays) were determined at the finasteride concentrations cited in Table 2. The within-assay precision was assessed by analyzing six replicates of each sample as a batch in a single assay run, and the between-assays precision was assessed by analyzing the same sample, as triplicate, in two separate assay

Table 1: Analytical characteristics of the proposed methods

Parameter	Finasteride		
	$KMnO_4$	Ce(IV)	NBS
Acid	H_2SO_4	H_2SO_4	HCl
Dye	MB	C2R	AM
λ_{max} / nm	663	528	520
Stability / h	48	48	48
Beer's conc. range / μg mL^{-1}	0.12 –3.84	0.12 – 3.28	0.14 – 3.56
Ringbom optimum range / μg mL^{-1}	0.25 – 3.60	0.25 – 3.10	0.30 – 3.40
Detection limits /ng mL^{-1}	35	33	41
Quantification limits / μg mL^{-1}	0.12	0.11	0.14
Molar absorptivity / L mol^{-1} cm^{-1}	7.59×10^4	9.45×10^4	6.22×10^4
Sandell sensitivity /ng cm^{-2}	4.9	3.9	5.9
Regression equation [a]			
Slope	0.206	0.256	0.169
RSD % of slope	0.0091	0.0063	0.0080
Intercept	- 0.02	+ 0.005	- 0.07
Correlation coefficient	0.9994	0.9992	0.9996
RSD %	0.85	0.73	0.95
Range of error %	± 1.20	± 1.00	± 1.45
Calculated t–values (2.57) [b]	1.07	0.76	1.37
Calculated F- test (5.05) [b]	2.47	2.03	2.88

[a] A = a + bC, where C is the concentration in μg mL^{-1}; [b] Values in parentheses are the theoretical values for t- and F- values at; 95% confidence limits and five degrees of freedom.

runs. The relative standard deviations (RSD) were less than 1.0 % [Table 2]. This level of precision was adequate for the quality control analysis of finasteride.

Specificity and interference

The proposed spectrophotometric methods have the advantages that the measurements are performed in the visible region, away from the UV-absorbing interfering substances that might be coextracted from finasteride-containing dosage forms. Regarding the interference of the excipients and additives usually presented in pharmaceutical formulation (Indigo Carmine, sodium lauryl sulfate, magnesium stearate, starch sodium glycolate, lactose spray dried, carboxymethylcellulose PA 102, talc, titanium dioxide, microcrystalline cellulose, red iron oxide, yellow iron oxide, hydroxypropylcellulose and pregelanitizated starch), their is no interference indicating the high selectivity of the proposed methods and applicability to use for routine determination in pure and in dosage forms.

Ruggedness and robustness

The ruggedness of the proposed methods was assessed by applying the procedures using two different instruments in two different laboratories at different elapsed time. Results obtained from lab-to-lab and day-to-day variation was found to be reproducible as RSD did not exceed 0.95%. Robustness of the methods was assessed by evaluating the influence of small variation of experimental variables: concentrations of oxidant C ± 0.05 M, acids C ± 0.01 M, dye C ± 0.02 M, temperature ± 5 °C and reaction time t ± 5.0 min) on the analytical performance of the method. In these experiments, one experimental parameter was changed while the other parameters were kept unchanged, and the recovery percentage was calculated each time. The small variations in any of the variables did not significantly affect the results; recovery percentages were 99.0–101.40, 99.2 – 101.6, and 99.6 – 101.0 % for methods A, B, and C, respectively. This provided an indication for the reliability of the proposed methods during routine work.

Applications

Analysis of dosage forms

The obtained satisfactory validation results made the proposed methods suitable for the routine quality control analysis of finasteride and its dosage forms pharmaceutical formulations (*Prostride* capsules). The results obtained by the proposed methods were statistically compared with those obtained by the official pharmacopoeia method.[18] The obtained mean values of the labeled amounts ranged from 100.20 ± 1.20, 100.4 ± 1.20, and 100.3 ± 0.7%, using A, B, and C methods, respectively as recorded in Table 3. In the *t*- and F-tests, no significant differences were found between the calculated and theoretical values of both the proposed and the reported methods at 95% confidence level.[33] This indicated similar precision and accuracy in the analysis of finasteride in its formulations. It is evident from these results that all the proposed methods are applicable to the analysis of finasteride in its capsules with comparable analytical performance. However, the critical recommendations of these methods might be based on the experimental conditions and the ultimate sensitivity that determines the amount of specimen required for analysis. For example, the methods involving Ce(IV) are recommended whenever

Table 2: Precision of the proposed methods for analysis of finasteride (n = 6)

Oxidant	Taken μg mL^{-1}	Within-assays		Between-assays	
		Mean (μg mL^{-1} ± SD)	RSD%	Mean (μg Ml^{-1}± SD)	RSD%
KMNO$_4$	0.6	0.61 ± 0.49	0.84	0.59 ± 0.76	0.88
	1.2	1.17 ± 0.34	0.68	1.19 ± 0.45	0.65
	1.8	1.78 ± 0.50	0.88	1.81 ± 0.43	0.92
	2.4	2.43 ± 0.47	0.87	2.39 ± 0.59	0.95
	3.0	2.98 ± 0.37	0.74	3.01 ± 0.47	0.78
Ce(IV)	0.4	0.41 ± 0.32	0.45	0.39 ± 0.46	0.54
	0.8	0.81 ± 0.33	0.68	0.81 ± 0.54	0.74
	1.2	1.18 ± 0.27	0.59	1.21 ± 0.41	0.63
	2.4	2.38 ± 0.43	0.83	2.39 ± 0.36	0.87
	3.2	3.18 ± 0.45	0.94	3.21 ± 0.31	0.91
NBS	0.7	0.69 ± 0.41	0.87	0.71 ± 0.37	0.85
	1.4	1.41 ± 0.48	0.92	1.39 ± 0.61	0.94
	2.1	2.12 ± 0.52	0.91	2.09 ± 0.77	0.95
	2.8	2.82 ± 0.43	0.78	2.83 ± 0.49	0.87
	3.5	3.47 ± 0.53	0.94	3.54 ± 0.38	0.90

SD; Standard deviations, RSD; Relative standard deviations sensitive analysis is required; this because they have very high sensitivity.

Analysis of spiked plasma samples

The high sensitivity attained by the proposed methods allows the determination of the studied finasteride, in biological fluids. The method was used to determine the amount of finasteride in a healthy male 14 h after an intake of one capsule of *Prostride*, which contains 5.0 mg finasteride. Finasteride was detected and the results were summarized in Table 3.

CONCLUSIONS

The redox reaction of finasteride using potassium permanganate, ceric sulfate and N-bromosuccinimide as oxidant has been investigated. The decrease in color of MB, C2R and AM dyes were utilized in the development of simple, accurate, sensitive with good precision and accuracy spectrophotometric methods A, B, and C methods for the analysis of finasteride in pure form as well as in dosage and biological forms. With these methods, one can do the analysis at low cost without losing accuracy. The proposed methods can be used as alternative methods to the official ones for the routine determination of *Prostride* capsules. This encourages their successful use in routine analysis of finasteride in quality control laboratories and they involve very simple procedures.

Table 3: Determination of finasteride in capsules (5 mg/capsule) and spiked plasma by the proposed and official method[18]

Parameter	Prostride capsule			
	$KMnO_4$	Ce(IV)	NBS	Official
Recovery % [a]	100.2 ± 1.2	100.4 ± 1.2	100.3 ± 0.7	99.6 ± 1.8
± Standard Deviation	0.76	0.84	0.93	1.20
Number of experiments	6	6	6	6
Variance	0.81	0.89	0.95	1.11
t-test [b]	1.13	0.98	1.53	
F-value [b]	2.62	2.43	3.078	
	Spiked plasma pamples			
Mean recovery % [a]	99.5 ± 0.8	99.2 ± 1.0	99.0 ± 1.1	100.3 ± 1.5
± Standard Deviation	1.11	0.92	1.27	1.32
Number of experiments	6	6	6	6
Variance	1.02	0.78	1.16	1.23
t-test [b]	1.37	1.19	1.74	
F-value [b]	2.88	2.71	3.26	

[a]Average values of six determinations were used for the official and the proposed methods, respectively.
[b]Theoretical values for *t* and *F* at 95% confidence limit are 2.57 and 5.05, respectively.

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