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Sensitive Bromatometric Determination Of Finasteride In Pharmaceuticals Based On Complex-Formation Reactions

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ABSTRACT

Two highly sensitive spectrophotometric methods are described for the determination of finasteride in pure form and tablet form. The methods are based on the bromination of finasteride by a measured excess of *insitu* generated bromine followed by the determination of residual bromine by two different redox-complexation reaction schemes. In one procedure (method A), the residual bromine is treated with an excess of iron (II) and the resulting iron (III) is complexed with thiocyanate and measured at 470 nm. The second approach (method B) involves treating the unreacted bromine with a calculated and known excess amount of iron (II) and complexing the remaining iron(II) with orthophenanthroline at a raised pH followed by measurement at 510 nm. In both methods, the amount of bromine reacted corresponds to the amount of finasteride. In method A, the absorbance is found to decrease linearly with increasing concentration of finasteride (r = -0.9997) whereas a linear increase in absorbance (r = 0.9999) resulted in method B. The systems obey Beer's law for 0.5-4.0 and 0.5-6.25 μg/ ml for method A and method B, respectively. The calculated apparent molar absorptivity values are 9.94×10^4 and 6.68×10^4 l mol/ cm for method A and method B, respectively, and the corresponding Sandell sensitivity values are 0.0038 and 0.0056 μg/cm^2. The limits of detection (LOD) and quantification (LOQ) are also reported for both methods. Accuracy, and intra-day and inter-day precision of the methods were established as per the present ICH guidelines. The proposed methods were applied successfully to the determination of finasteride in tablets and the results were found to agree closely with the label claim. The results were also compared statistically with those obtained by a reference method by applying the Student's t-test and F-test. The excipients did not interfere in the determination. The accuracy of the methods were further confirmed by performing recovery tests *via* standard addition method.

KEYWORDS

Finasteride;
Determination;
Spectrophotometry;
Bromate-bromide;
Complexation reactions.

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INTRODUCTION

Finasteride (FNS), chemically known as N-(1,1-dimethylethyl)-3-oxo-(5α, 17β)-4-azaandrost-1-ene-17-carboxamide (Figure 1)[1] is an antiandrogen which acts by inhibiting 5-alpha reductase, the enzyme that converts testosterone to dihydrotestosterone[2]. It is used in benign prostatic hyperplasia (BPH) in low doses and in prostate cancer in higher doses. Additionally, it is registered in many countries for male pattern-baldness. Many papers have been published on the determination of FNS in biological fluids[3] particularly in human plasma using high performance liquid chromatography (HPLC)[4-11] with different detector systems. HPLC is the single most widely used technique for the determination of FNS in pure drug and related substances[12,13], tablets[14-16] and capsules[17]. HPLC has also been applied in stability indicating[18] and storage stability[19] studies. Ilango *etal* [20] have recently reported a uv-spectrophotometric method for the determination of FNS in tablet preparations and the method is reported to be applicable over 5-25 μg/ml concentration range. Other techniques including HPTLC[21], mid-infrared spectrophotometry[22] and polorography[23] devoted to assay in formulations are also found in the literature. The drug is official in Martindale Extra Pharmacopeia[24] and United States Pharmacopoeia[25] and the latter describes HPLC procedure for assay in pure drug and in tablets.

Visible spectrophotometry, because of simplicity, sensitivity, reasonable accuracy and precision, and speed has withstood the test of time and remained competitive with the newer analytical methods. There is only one report[26] on the visible spectrophotometric determination of FNS in pharmaceuticals and the method is based on the measurement of oxidative couple formed with 3-methyl-2-benzothiazolinone hydrazone (MBTH) in the presence of iron(III) chloride, the colored product peaking at 446 nm. Although the method is fairly sensitive (2- 10 μg/ml), the chromogen formed is poorly stable, and the method employs an expensive reagent (MBTH). Further, of the reported HPLC methods, the method of Ziyang *et al*[12] is quite sensitive with the detector response being linear in the concentration range 0.604-6.04 μg/ml; all other methods lack the sensitivity expected of HPLC. In addition, the procedures require either derivatization of the compound or selective detectors and elaborate multi step extractions. Added to this, the technique requires expensive instrumental set up and not accessible to many laboratories in developing and under developed countries. Even the polarographic methods are inadequately sensitive with the determination ranges being 8-40 and 2-40 μg/ml using direct current and differential pulse modes, respectively.

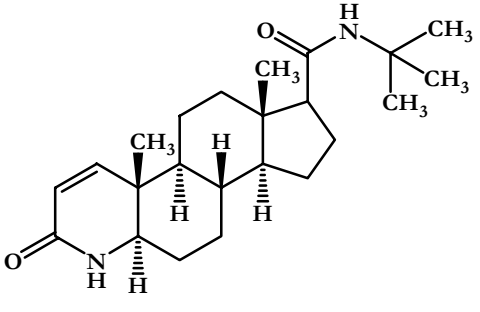

Figure 1: Structure of finasteride

In previous papers, we have reported the successful use of bromate-bromide reagent for the sensitive spectrophotometric determination of a variety of pharmaceuticals[27-35]. The work communicated in this paper is aimed at developing sensitive and cost-effective spectrophotometric methods for the assay of FNS in pharmaceuticals. The methods make use of bromate-bromide mixture and are based on the bromination of finasteride by a measured excess of *insitu* generated bromine followed by the determination of residual bromine by two different redox-complexation reaction schemes. The methods when applied to assay in tablets were found to give accurate and precise results.

EXPERIMENTAL

Apparatus

All absorbance measurements were made with a Systronics Model 106 digital spectrophotometer equipped with matched 1-cm quartz cells.

Reagents and standards

All used chemicals were of analytical reagent

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grade purity and all solutions were prepared in distilled water.

Bromate-bromide mixture(20 and 40 μg/ml in $KBrO_3$)

A stock standard solution equivalent to 1000 μg/ml $KBrO_3$ and a large excess of KBr was first prepared by dissolving accurately weighed 100 mg of $KBrO_3$ and 1g of KBr in water and diluting to the mark with water in a 100 ml calibrated flask. This was diluted stepwise to obtain working concentrations containing 20 and 40 μg/ml $KBrO_3$ for use in method A, and method B, respectively.

Ferrous ammonium sulphate, FAS (400 and 350 μg/ml)

A stock solution equivalent to 0.01 M FAS was prepared by dissolving about 400 mg of the salt (S.D. Fine Chem, Mumbai, India) in 50 ml of water containg 1ml of dil H_2SO_4 and diluted to 100 ml with water, and standardized[36] using pure potassium dichromate. The stock solution was then diluted appropriately with water to get 400 μg /ml (for method A) and 350 μg /ml (for method B) FAS.

Ammonium thiocyanate (3M)

Prepared by dissolving 23 g of the salt (S.D. Fine Chem, Mumbai, India) in 100 ml of water.

1,10-Phenanthroline (0.25%)

Aqueous solution was prepared by dissolving 250 mg of the complexing agent (S.D.Fine Chem, Mumbai, India) in 100 ml of water with the aid of heat.

Hydrochloric acid(5M)

Concentrated hydrochloric acid (S.D. Fine Chem., Mumbai, India; sp. gr. 1.18) was diluted appropriately with water to get 5 M acid.

Standard solution of finasteride

Pharmaceutical grade finasteride was received from Cipla Ltd, Bangalore, India. which was reported to be 99.8% pure, as gift and was used as received. A stock standard solution equivalent to 1000 μg/ml FNS was prepared by dissolving accurately weighed amount of pure drug in 50 ml of glacial acetic acid and diluting with water to a definite volume. The same solution (1000 μg /ml FNS) was futher diluted with water to get working concentrations of 20 and 25 μg/ml for use in spectrophotometric method A, method B, respectively. The standard solutions were kept in amber coloured bottle and stored in a refrigerator when not in use.

Procedures

Method A

Aliquots of standard 20 μg/ ml FNS solution in the range of 0.25 to 2.0 ml were measured accurately with the help of a microburette and transferred into a series of 10 ml calibrated flasks, and the total volume was brought to 2 ml by adding requisite quantity of water. One ml of 5 M HCl was added to each flask followed by 1 ml of BrO_3^- - Br^- mixture (20 μg/ml in $KBrO_3$) the content mixed well and allowed to stand for 10 min with periodic shaking. Then, 1 ml of 400 μg/ml FAS was added and mixed. After 5 min, 1 ml of 3M ammonium thiocyanate was added, diluted to the mark with water, mixed and absorbance of each solution was measured at 470 nm against a water blank.

Method B

Varying aliquots (0.25, 0.5, 1.0……2.5 ml) of standard (25 μg/ml) FNS solution were accurately measured and transferred into a series of 10 ml calibrated flasks by means of a micro burette and the total volume was adjusted to 2.5 ml by adding water. To each flask was added 1 ml of 5 M HCl followed by 1ml of BrO_3^- - Br^- mixture (40 μg /ml in $KBrO_3$). The content was mixed well and let stand for 5 min with occasional shaking. Then, 1ml of 350 μg/ml FAS solution was added to each flask and mixed well; after 10 min, 1 ml each of 0.25% 1,10-phenanthroline and 1:1 NH_3 solution were added, the volume was diluted to the mark with water and mixed. The absorbance of each solution was measured at 510 nm after 15 min against a reagent blank.

In either method, calibration graph was prepared by plotting the absorbance as a function of concentration, and the concentration of the unknown was read from the calibration graph or calculated from the regression equation derived using the Beer's law data.

Procedure for tablets

Forty tablets were accurately weighed and ground into a fine powder. A quantity of the powder equivalent to 100 mg of FNS was accurately weighed into a 100 ml calibrated flask, 50 ml of glacial acetic acid added and shaken for 20 min; the volume was finally diluted to the mark with water, mixed well and filtered using a Whatman No. 42 filter paper. The filtrate (1000 µg/ml FNS) was appropriately diluted with water to get 20 and 25 µg/ml FNS concentrations and analysed by spectrophotometric methods by taking convenient aliquots (1 or 2 ml).

RESULTS AND DISCUSSION

Bromate-bromide mixture has been a valuable oxidimetric reagent for the determination of many organic and inorganic substances, and has been widely used in assay of several pharmaceutical substances[27-35]. The proposed methods are indirect and are based on the determination of residual bromine after allowing the reaction between FNS and *insitu* generated bromine to go to completion, and rely on two different reaction schemes.

Method development

Method A

Complex formation involving iron (III) and thiocyanate is a well-known reaction that has been widely used for the trace level determination of iron [37]. The present method is based on the bromination of FNS by a known excess of *insitu* generated bromine in HCl medium, reduction of the residual bromine by a fixed amount of iron (II) in the acid medium and subsequent formation of iron (III) – thiocyanate complex followed by absorbance measurement of the complex at 470 nm. When a fixed amount of BrO_3^- - Br^- is made to react with increasing amounts of FNS, there occurrs a concomitant fall in the bromine concentration. When the unreacted bromine is reduced by a fixed amount of iron (II), there will be a proportional decrease in the absorbance of iron(III)-thiocyanate complex on increasing the concentration of FNS (Figure 2) which formed the basis for the assay of drug by the proposed method.

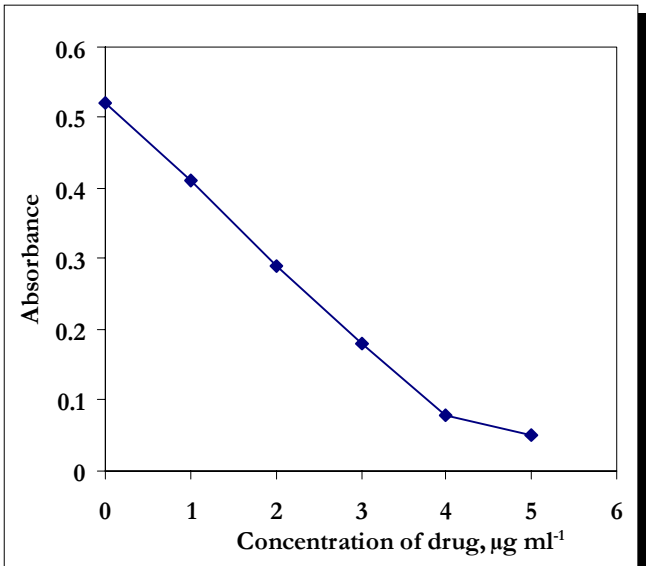

Figure 2: Beer's law curve for method A

The conditions for the determination of iron (III) with thiocyanate are well established [37]. Hence, various parameters associated with the bromination of FNS by *insitu* generated bromine, and subsequent reduction of residual bromine by iron(II) were optimized. The sequence of the reactions was found to be rapid and quantitative in HCl medium and found to be complete in 10 min under the described experimental conditions. One ml of 5 M HCl in a total volume of 4 ml was found optimum. The reduction of the residual bromine by iron (II) and complex formation reaction of iron (III) with thiocyanate was also found to be feasible in the same medium.

Fixing 5.5 µg/ml as the upper limit of iron (III) that could be determined by thiocyanate method [37], 200 µg of bromate in the presence of excess of bromide would be required to oxidize 400 µg FAS. Although a fixed amount of iron(II) is not really required, large amounts are undesirable since iron(II) tends to undergo aerial oxidation. Hence, a fixed amount of iron(II) (400 µg) enough to reduce the total *insitu* generated bromine was employed. The formation of iron (III)- thiocyanate complex was instantaneous and the colour was stable for at least 60 min in the presence of the reaction product.

Method B

The spectrophotometric method based on the use of orthophenanthroline as the chelating agent con-

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tinues to be one of the sensitive methods for the determination of iron (II) in a variety of matrices. This reaction coupled with the bromination character of BrO_3^- - Br^- mixture in acid medium has been made use in developing a sensitive indirect assay method for FNS.

The drug in varying amounts, when treated with a fixed and known amount of BrO_3^- - Br^- mixture, consumes the latter in proportionate amounts, and there will be a concomitant decrease in the amount of bromine. When the decreasing amounts of bromine are reacted with a fixed amount of iron(II), there occurs a proportional increase in the concentration of iron(II). This is indicated by the increase in the absorbance of the orthophenanthroline complex formed with residual iron(II). The absorbance measured at 510 nm is found to increase linearity with FNS concentration (Figure 3) serving as the basis for the assay procedure.

HCl medium was found to ideal for bromination reaction. This reaction was found to be rapid and quantitative when 1ml of 5 M HCl was used in a total volume of ~6 ml, and hence, 1ml of 5 M HCl was used throughout. Besides this reaction, even the oxidation of iron (II) by the residual bromine was found to proceed quantitatively in same acid concentration, and contact times of 5 and 10 min were found optimum for the two steps, although any delay up to 30 min in adding FAS had no effect on the final absorbance measurement.

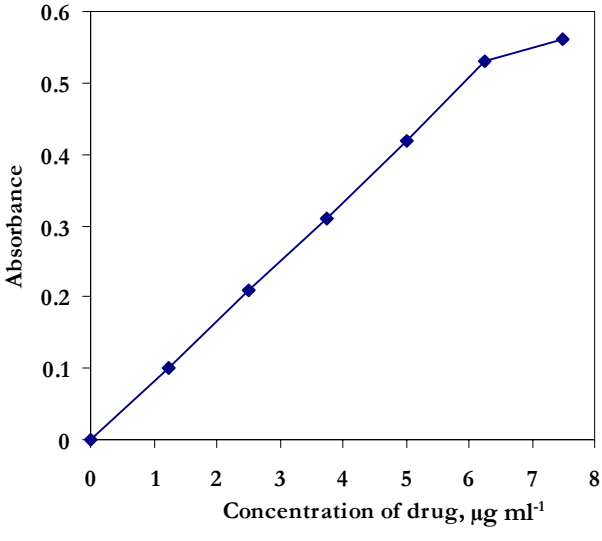

Figure 3: Beer's law curve for method B

Taking 5 µg/ ml as the upper limit of iron (II) that could be determined by orthophenanthroline method, 350 µg of FAS was used in this method. Quantitatively this was found to react with bromine equivalent to 400 µg of bromate . Hence, different amounts of FNS were reacted with 1 ml BrO_3^- - Br^- mixture equivalent to 40 µg/ml $KBrO_3$ before determining the unreacted bromine. This enabled to fix the concentration range of FNS that could be determined by the method. The complex was stable for several days even in the presence of the reaction product.

Method validation

Analytical parameters

A linear relation is found between the absorbance and concentration in the ranges given in TABLE 1. In method A, Beer's law is obeyed in the inverse manner. The calibration graphs are described by the equation: Y= a + bX (where Y=absorbance, a=intercept, b=slope and X=concentration in µg/ ml) obtained by the method of least squares. Correlation coefficients, intercepts and slopes for the calibration data are also presented in TABLE 1. Sensitivity parameters such as molar absorptivity and Sandell sensitivity values, and the limits of detection and quantification calculated according to ICH guidelines[38] are also compiled in TABLE 1.

TABLE 1: Analytical and regression parameters of spectrophotometric methods

Parameter	Method A	Method B
λ_{max}, nm	470	510
Beer's law limits, µg /ml	0.5-4.0	0.625-6.25
Molar absorptivity, l mol/ cm	9.94×10^4	6.68×10^4
Sandell sensitivity, µg/ cm^2	0.0037	0.0056
Limit of detection, µg/ml	0.10	0.05
Limit of quantification, µg/ml	0.31	0.16
Regression equation, Y*		
Intercept (a)	0.5165	-0.0052
Slope (b)	-0.1098	0.2143
Correlation coefficient, (r)	-0.9997	0.9989
S_a	0.00401	0.00287
S_b	0.00113	0.00134

***Y = a+bX, where Y is the absorbance and X concentration in µg/ml**
S_a. Standard deviation of intercept.
S_b. Standard deviation of slope.

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TABLE 2: Evaluation of accuracy and precision

Method	FNS taken, µg/ ml	FNS found,* µg/ ml	Range, µg/ ml	RE %	SD µg/ml	SEM	RSD, %	ROE,** %
A	1.0	0.97	0.07	3.0	0.014	0.0053	1.47	±1.46
	2.0	1.94	0.09	3.0	0.009	0.0034	0.50	±0.49
	3.0	2.89	0.12	3.7	0.017	0.0064	0.59	±0.58
B	2.0	1.93	0.08	3.5	0.056	0.0212	2.93	±2.92
	4.0	3.87	0.09	3.3	0.062	0.0234	1.60	±1.59
	6.0	5.94	0.14	1.0	0.048	0.0181	0.81	±0.80

RE. Relative error; SD. Standard deviation; RSD. Relative standard deviation; SEM. Standard Error of Mean.
*** Mean value of seven determinations**
**** At the 95% confidence level for 6 degrees of freedom.**

Accuracy and precision

To evaluate the accuracy and precision of the methods, pure drug solution at three different levels (within the Beer's law limits) was analysed, each determination being repeated seven times. The relative error (%) and the relative standard deviation(%) were less than 4 and indicate the high accuracy and precision of the methods (TABLE 2). For a better picture of reproducibility on a day-to-day basis, a series of experiments were performed in which standard drug solution at three different levels was determined each day for five days with all solutions being prepared afresh each day. The inter-day relative standard deviation values were in the range 1.5-3.5% and represented the best appraisal of the methods in routine use.

Interference study

To investigate the effect of tablet fillers on the measurements involved in the methods, placebo analysis was carried out. A mixture containing lactose, starch, talc, magnesium stearate, sodium alginate and calcium gluconate in the ratio 80: 7 : 2.5: 0.5: 1:9 was extracted with water and filtered using a quantitative filter paper. The filtrate was subjected to analysis by the proposed methods and it was found that a positive relative error of 2.0 and 2.5% for method A, and method B, respectively, was obtained. From this study, it is apparent that the usual co-formulated substances seldom interfere in the methods.

Application to analysis of tablets

Commercially available tablets containing FNS were analysed by the proposed methods and the results are contained in TABLE 3. As can be seen, the results are in agreement with the label claim. The results were statistically evaluated by applying the Student's t-test and F-test after assaying the same batch tablets by the reference method[20] which consisted of the measurement of the absorbance of the drug solution in methanol at 206 nm. The calculated t- and F-values were less than the tabulated values at the 95% confidence level indicating that the proposed methods and the reference method have similar accuracy and precision.

TABLE 3: Results of determination of finasteride in formulations and statistical comparison with the reference method

Tablet brand name#	Nominal amount, mg	% found* ± SD		
		Reference method	Method A	Method B
FINAST[a]	5	102.6±1.54	101.2±0.98 t=1.76 F=2.47	100.8±1.12 t=2.14 F=1.89
FINCAR[b]	5	99.36±0.74	100.4±1.38 t=1.55 F=3.48	98.11±1.42 t=1.83 F=3.68
FISTIDE[c]	5	100.28±0.91	102.12±1.15 t=2.82 F=1.59	97.9±0.89 t=4.18 F=1.05

***Mean value of five determinations**
#Marketed by: a. Dr. Reddy's. Labs Ltd; b. Cipla. Ltd., India; c. Samarth. Pharm. Ltd.
Tabulated t-value at 95% confidence level is 2.77
Tabulated F-value at 95% confidence level is 6.39.

Recovery experiment

The accuracy and reliability of the methods were futher evaluated through recovery studies. To a fixed and known amount of tablet powder (pre-analysed), pure FNS was added at three different levels and the total was found by the proposed methods. The recoveries of the pure drug added to tablet powder were in the range of 96.8-102.5 (TABLE 4) and re-

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TABLE 4: Results of recovery study by standard addition method

Method	Formulation studied	FNS in formulation, µg	Pure FNS Added, µg	Total Found, µg	Pure FNS recovered %
A	FISTIDE 5 mg	10.21	7.5	17.61	98.61
		10.21	15.0	25.27	100.42
		10.21	30.0	40.73	101.72
B		19.58	10.0	29.26	96.8
		19.58	20.0	39.42	99.2
		19.58	40.0	60.58	102.5

****Mean value of Three determinations**

veal that the usual tablet excipients did not interfere in the assay methods.

CONCLUSIONS

Two new visible spectrophotometric methods for the assay of finasteride in pharmaceuticals using BrO_3^- - Br^- mixture as the brominating reagent have been developed and appropriately validated. Both methods are based on well characterised complexation reactions and are the most sensitive ever reported for FNS in pharmaceuticals. The stability of the coloured species and sensitivity of the reactions used are not critically dependent on any experimental variable. Results of assay of authentic samples indicate non-interference from tablet excipients. Besides, the methods are reasonably accurate and precise and hence can be conveniently used in routine use.

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