Voltammetric reduction of finasteride at mercury electrode and its determination in tablets

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Abstract

Finasteride in hydroalcoholic solutions (ethanol/Britton-Robinson buffer, 30/70) exhibits cathodic response in a wide range of pH (−0.5 to 12) using differential pulse (DPP) and tast polarography (TP). The reduction peak of finasteride at acidic pH, is a catalytic proton peak resulting from a mechanism involving a first protonation of finasteride followed by the reduction of the protons combined with finasteride in order to regenerate finasteride and liberate hydrogen. Based on the catalytic hydrogen wave, a novel method for the determination of finasteride can be proposed. For analytical purposes we selected DPP technique in an ethanol/0.0625 mol L^{-1} H_2SO_4 (30/70) solution medium. In this condition the I_p varied linearly with finasteride concentration between 5×10^{-5} and 5×10^{-4} mol L^{-1}. Within-day and inter-day reproducibility's were adequate with R.S.D. values lower than 2%. The selectivity of the method was checked with both accelerated degradation trials and typical excipients formulations. The developed method was applied to the assay and the uniformity content of finasteride tablets and compared with the standard HPLC method. The DPP-developed method was adequate for the finasteride determination in pharmaceutical forms as that exhibited an adequate accuracy, reproducibility and selectivity. Furthermore, treatment of the sample was not required as in HPLC; the method is not time-consuming and less expensive than the HPLC ones.

Keywords: Finasteride; Differential pulse voltammetry; Reduction; Tablets

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1. Introduction

Finasteride (*N*-(1,1-dimethylethyl)-3-oxo-4-aza-5α-androst-1-ene-17α-carboxamide) (Fig. 1) is an inhibitor of the 5α-reductase cellular type 2 and it is used to treat prostatic alterations and androgenetic alopecia in men [1,2]. The drug is a member of the family of compounds known as 4-azasteroids [3]. 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 [4].

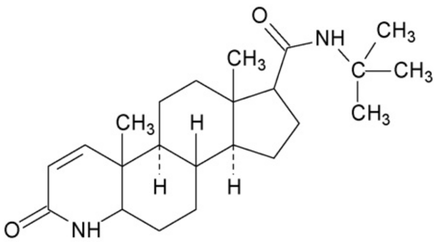

Fig. 1. Chemical structure of finasteride.

HPLC has been used to quantify finasteride in biological fluids [5–10] and also applied to bioequivalence studies [11,12]. The determination of finasteride in tablets has been carried out by using HPLC–UV [13,14], GC–FID [15] and recently a spectrophotometric method based on the formation of ion-pair complexes between the drug with a colorant and measured at the maximum wavelength has been published [16]. In addition, the LC determination of finasteride and its application to storage stability studies had been also described [17].

From the electrochemical point of view, Amer has described the polarographic behaviour of finasteride [18]. The results of the electrochemical behaviour described in that paper differ substantially from our findings. A detailed discussion is included.

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2. Experimental

2.1. Reagents and drugs

Finasteride (99.8% chromatographically pure) was supplied by Sanitas Laboratories (Santiago, Chile). Commercial capsules of Saniprosto® (declared amount per tablet 5.0 mg finasteride, Sanitas Laboratories. Santiago, Chile) were obtained commercially. Spironolactone (≥97%, Sigma–Aldrich).

All reagents were of analytical grade unless indicated otherwise. Phosphoric acid and acetonitrile HPLC grade (Merck) were used. Deionized water was obtained in the laboratory, using ionic interchanged columns (Milli-Q).

2.2. Solutions preparation

2.2.1. Buffer solutions

0.1 mol L^{-1} Britton-Robinson buffer (acetic acid–boric acid–phosphoric acid) for polarographic experiments was used, and desired pH was adjusted with concentrated solutions of NaOH. An ionic strength of 0.3 mol L^{-1} was adjusted with KCl.

2.2.2. H_0 scale

A modified Hammett's acidity scale (H_0) for solutions below pH 1.5 (using H_2SO_4–H_2O) was employed [19].

2.2.3. Stock drug solution

15.54 mg finasteride was dissolved and diluted up to 25 mL with ethanol, to obtain a final concentration around of 1.67×10^{-3} mol L^{-1} finasteride. The solution was protected from light by using amber glass material.

2.2.4. Work solution

A 3-mL aliquot of the stock solution was taken and then diluted to 10 mL with mobile phase or Britton-Robinson buffer solution, for HPLC or DPP, respectively.

2.3. Apparatus

2.3.1. Voltammetric analyzer

Differential pulse polarographic (DPP), tast polarographic (TP) experiments were performed with an automatized assembly BioAnalytical System, composed by a CV-50 W potentiostat polarographic system (CGME), coupled to a GATEWAY 2000 PC and acquisition and treatment data system BAS CV-50 W v. 2.0

A dropping mercury electrode (BAS) as the working electrode, a platinum wire (Metrohm type 6.0322.000) counter electrode and an Ag/AgCl (BAS) reference electrode were employed. The operating conditions were: sensitivity between 1 and 10 μA; drop time 300 ms; potential range 0 to −1700 mV; pulse retard 17 ms; pulse height 50 mV.

2.3.2. HPLC

Measurements were carried out using a Waters assembly equipped with a model 600 Controller pump and a model 996 Photodiode Array Detector. The acquisition and treatment of data were made with the Millenium v. 2.1 software. As chromatographic column a Bondapak/Porasil C18 column of 3.9 mm × 150 mm was used. As column guard a C18 Bondapak (30 mm × 4.6 mm) was employed. The injector was a 20 μL Rheodyne valve. UV detection at 240 nm was employed and the column was kept at constant temperature using a Waters column heater cartridge model 600.

An isocratic elution composed of a solution consisting of acetonitrile/2.5 mmol L^{-1} phosphoric acid (50/50, v/v) mobile phase was used. The flow was 1.5 mL min^{-1} and the working temperature was kept constant at 45 ± 1 °C. In these conditions, finasteride exhibited a retention time of 3.94 ± 0.04 min. This chromatographic system was based on USP assay for finasteride [13].

2.4. Analytical procedure

2.4.1. Calibration curve preparation

2.4.1.1. Polarography. By diluting the finasteride stock solution with ethanol/0.0625 mol L^{-1} sulfuric acid (30/70, v/v), working solutions ranging between 1×10^{-5} and 5×10^{-4} mol L^{-1} were prepared.

2.4.1.2. HPLC. By diluting the finasteride stock solution with mobile phase, working solutions ranging between 3×10^{-6} and 3×10^{-5} mol L^{-1} were prepared. The solutions were injected and chromatographed according to the working conditions previously given. UV detector was operated at $\lambda = 240$ nm.

2.4.2. Synthetic samples

Excipients (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) were added to the drug for recovery studies, according to manufacturer's batch formulas for finasteride tablets.

2.4.3. Composite assay for finasteride tablets

Not less than 15 finasteride tablets were weighed and finely powder and then an accurately weighed quantity of the powder, equivalent to about 5 mg of finasteride were transferred to a 25-mL volumetric flask with aid of ethanol, sonicated and fill to volume with ethanol. The solution was divided into two; one for DPP assay and the second one centrifuged for 10 min at 4000 rpm, and the clear liquid was used for HPLC assay.

2.4.4. Individual tablet assay procedure

2.4.4.1. Polarography. For this study no less than 10 commercial tablets of finasteride (Saniprosto®, amount declared 5.0 mg finasteride per tablet) were used. Each tablet was independently suspended in 10-mL ethanol with sonication to assure the complete dissolution of the drug and diluted to a final volume of 25.0 mL with the same solvent. A 3-mL aliquot of each solution was taken and diluted to 10 mL with 0.0625 mol L^{-1} sulfuric acid, to obtain a finasteride concentration of 1.6×10^{-4} mol L^{-1}. Each sample solution was transferred to a polarographic cell, bubbled with nitrogen during 5 min and recorded at least twice from −900 to −1400 mV. The mg amount of finasteride in the sample solution was calculated from the prepared standard calibration curve.

2.4.4.2. HPLC. For this study no less than 10 commercial tablets of finasteride (Saniprosto®, amount declared 5.0 mg finasteride per tablet) were used. The content of each tablet was independently suspended in 10-mL ethanol with sonication to assure the complete dissolution of the drug and diluted to a final volume of 25.0 mL with the same solvent. Each one of the above solutions was centrifuged by 10 min at 4000 rpm, and then an aliquot of 0.5-mL supernatant was taken and diluted to a 10-mL volume with mobile phase. The mg amount of finasteride in the sample solution was calculated from the corresponding prepared standard calibration curve. This procedure was adapted from the finasteride assay on Pharmacopoeia [13].

2.4.5. Selectivity studies [20]

2.4.5.1. Degradation trials. Hydrolysis. 3 mL of finasteride stock solution were transferred to a 10-mL distillation flask and adding (a) 7-mL water for neutral hydrolysis, (b) 1 mL 1 mol L^{-1} HCl for acid hydrolysis, or (c) 1 mL 1 mol L^{-1} NaOH for alkaline hydrolysis. Then each solution was completed to 10-mL volume with water and boiled for 1 h at reflux.

Photolysis. 10 mL of 1×10^{-3} mol L^{-1} finasteride ethanol solution was bubbled for 2 min with nitrogen and transferred to a black box and then irradiated with UV light (UV Black–Ray long wave ultraviolet lamp, UVP model B 100 AP (50 Hz, 2.0 A) with a 100 W Par 38 Mercury lamp equipped with a 366 nm filter) at a distance of 15 cm for 8 h (1.2×10^{19} quanta s^{-1}, determined by using the potassium ferrioxalate chemical actinometer) [21].

Thermolysis: Circa 2.1 mg was heated at 105 °C for 5 h.

Appropriate volumes of each obtained solution from the degradation trials or the corresponding mg from thermolysis in raw material assay were taken and completed to a final volume with ethanol and 0.0625 mol L^{-1} sulfuric acid to obtain a theoretical concentration of 6×10^{-5} mol L^{-1} finasteride (30% ethanol in final solution). Samples from these studies were stored at −20 °C and protected from light prior to polarographic analysis. Each sample was analyzed by duplicate.

2.4.6. Statistic analysis

Comparison between different techniques, as well as the comparison with standard deviations was carried out by means of the Student's *t*-test, and using significance limits between 95% and 99% of confidence [22,23].

3. Results and discussion

Finasteride in hydroalcoholic solutions (ethanol/Britton Robinson buffer, 30/70) exhibits cathodic response in a wide range of pH (2–12) using DPP and TP (Fig. 2A). As can be seen, the polarographic response in DPP mode at pH 2 is very well defined and peak currents are notoriously higher than the peak currents at pH > 3. With the aim to investigate the possibility to obtain better resolution of the polarographic peak, H_0 scale was explored. In super acid media (Fig. 2B), well-resolved peaks and high peak current values were obtained. On the other hand, at pH > 2, both the peak shape changes and peak current diminishes. As can be seen in Fig. 2A, the DP polarogram evidences a shoulder in alkaline pH and in TP a new wave appears (insert in Fig. 2A).

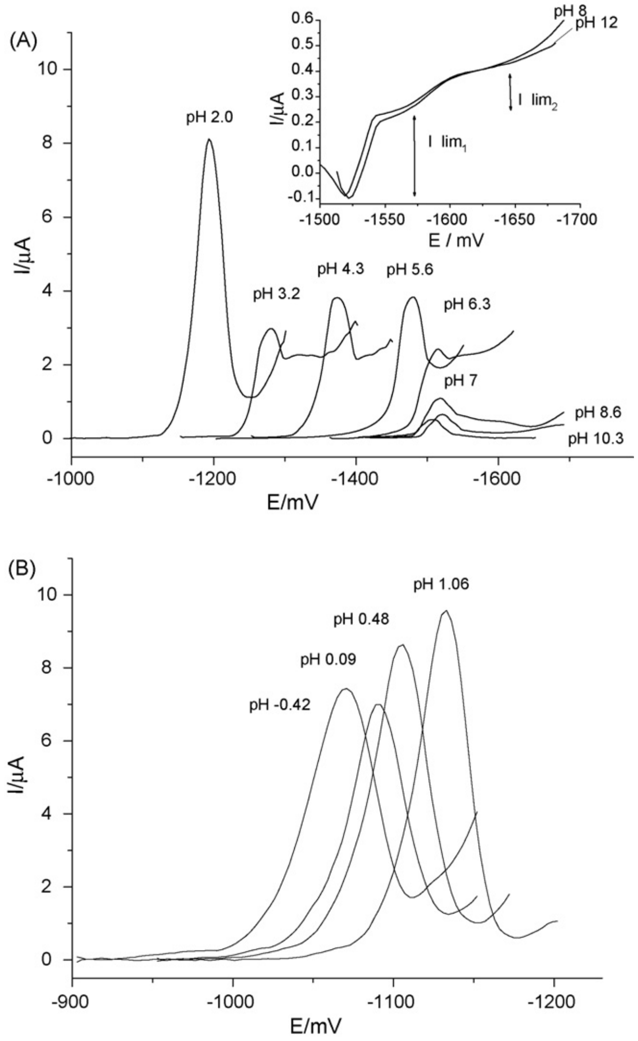

Fig. 2. DPP and TP (A) 5×10^{-4} mol L^{-1} finasteride solution at different pH. (A) pH 2–12, ethanol/Britton-Robinson buffer, 30/70. (B) pH < 2, ethanol/0.0625 mol L^{-1} sulfuric acid, 30/70.

From the DPP curves we can study the dependence of peak potentials and peak current with pH. E_p–pH plots exhibit three main zones. The first two, between pH −0.5 and 2 and pH 2 and 6, are pH-dependent. The third zone, above pH 6, is pH independent. As can be seen in Fig. 3A, the peak potential remains stable between pH 6 and 12. Furthermore, peak current dramatically decreases while the pH increases (Fig. 3B).

From the pH behaviour it is possible to conclude that the peak current is extraordinarily pH sensitive, changing about eight folds between pH 2 and 7. On the other hand, the value of

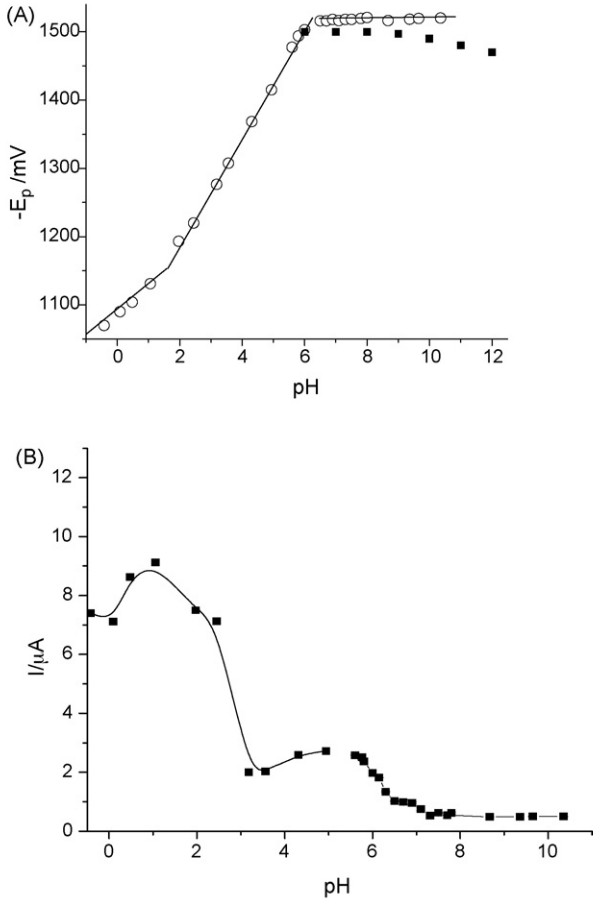

Fig. 3. (A) Peak potential evolution of 5×10^{-4} mol L^{-1} finasteride solution with pH ((○) our results; (■)Amer' results). (B) Peak current evolution with pH.

the peak potential is very near the hydrogen discharge. Both of these aspects are strongly indicative that the process obeys the catalytic hydrogen wave [24].

Furthermore, in order to deep in the elucidation of the process involved in the reduction peak; finasteride was compared with a drug with similar structure such as spironolactone [24]. This drug produces a polarographic response via the 2e$^-$ and 2H$^+$ reduction of the carbonyl group. As can be seen in Fig. 4, the limiting currents of equimolar solutions of both compounds only are equal at pH > 9. At pH 2, the finasteride limiting current is about eight folds higher than spironolactone's one, which indicates that the electrochemical reduction of finasteride does not involve 2e$^-$ and 2H$^+$ in all pH range, such as was erroneously concluded in the previous work of Amer [18]. Securely, the increase of the limiting current of finasteride solutions at acidic pH indicates that at acidic pH the catalytic wave of protons is occurring. From the first work of Mairanovskii it is well known that sulfur- and nitrogen-containing organic compounds can act as catalyst diminishing hydrogen overpotential, thus, promoting a catalytic wave before the hydrogen of the medium [24].

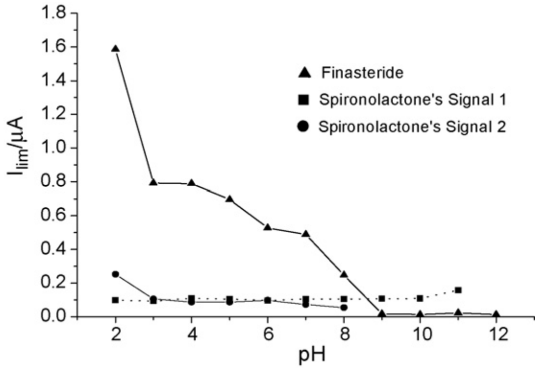

Fig. 4. Limiting current evolution with pH of finasteride and spironolactone (5×10^{-4} mol L^{-1} concentration solutions, ethanol/Britton-Robinson buffer, 30/70).

Consequently, in the case of finasteride, the catalytic wave is generated by the following steps. In a first step, finasteride (F) interacts with proton to form the protonated finasteride (FH$^+$), probably in the nitrogen at 4-position in the ring A. Then FH$^+$ was polarographically reduced to produce FH which in a bimolecular interaction regenerated finasteride and molecular hydrogen according to:

$$\mathrm{F} + \mathrm{H}^+ \rightleftarrows \mathrm{FH}^+$$

$$\mathrm{FH}^+ + \mathrm{e}^- \rightarrow \mathrm{FH}$$

$$2\mathrm{FH} \rightarrow 2\mathrm{F} + \mathrm{H}_2$$

Our results differ substantially from those previously described by Amer, who reported that the polarographic response of finasteride appears only between pH 6 and 12, and obviously he did not see the catalytic wave. Moreover, the ΔE_p reported by Amer between pH 6 and 12 is only about 30 mV (Fig. 3A). Likewise, in our results the peak current behaviour with pH is also in opposition with the Amer's description.

Based on the catalytic hydrogen wave, a novel method for the determination of finasteride can be proposed. For analytical purposes, we have selected the DPP technique working in ethanol/0.0625 mol L^{-1} H_2SO_4 (30/70) hydroalcoholic medium. In this condition the I_p varied linearly with finasteride concentration between 5×10^{-5} and 5×10^{-4} mol L^{-1}. Within-day and inter-day reproducibility's were adequate with R.S.D. values lower than 2%. In Table 1 the analytical parameters are summarized.

Table 1
Analytical parameters for the developed DPP method

Parameter	DPP
Within-day reproducibility, CV (%)[a]	0.17
Inter-day reproducibility, CV (%)[a]	1.06
Recovery (%)[b] ± S.D.	98.21 ± 1.84
Concentration range (mol L^{-1})	5×10^{-5}–5×10^{-4}
Calibration curve	$I_p = 19921.62[\mathrm{C}] - 0.0857$ ($n = 9$, $r = 0.9993$)
Detection limit (mol L^{-1})	7.59×10^{-6}
Quantitation limit (mol L^{-1})	1.53×10^{-5}

[a] Concentration level of 3.5×10^{-4} mol L^{-1}.
[b] Average on a concentration level of 1×10^{-4} mol L^{-1}.

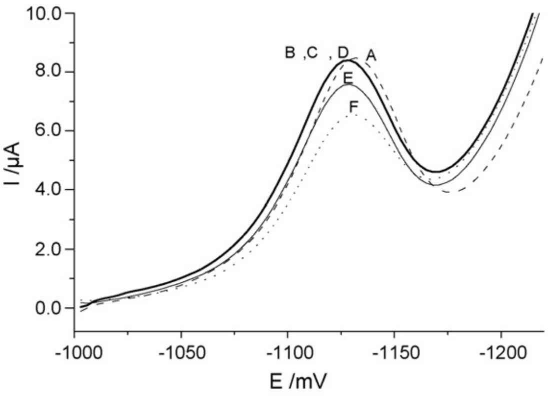

Fig. 5. DPP of selectivity trials: (A) 6×10^{-5} mol L^{-1} finasteride standard solution, (B) neutral hydrolysis, (C) thermolysis, (D) photolysis, (E) acid hydrolysis and (F) alkaline hydrolysis (ethanol/0.0625 mol L^{-1} sulfuric acid, 30/70).

Table 2
Individual tablet assay of finasteride[a]

Tablet	HPLC–UV		DPP	
	Found (mg)	Found (%)	Found (mg)	Found (%)
1	4.75	95	4.88	97.6
2	4.83	96.6	4.79	95.8
3	4.91	98.2	4.88	97.6
4	4.99	99.8	4.91	98.2
5	4.76	95.2	4.94	98.8
6	4.99	99.8	5.15	103.0
7	4.66	93.2	4.75	95.0
8	4.69	93.8	4.85	97.0
9	4.88	97.6	4.86	97.2
10	4.79	95.8	4.98	99.6
Average	4.83	96.5	4.89	98.0
S.D.	0.12	2.32	0.11	2.21
CV (%)	2.5	2.4	2.3	2.3

[a] Declared amount/tablet: 5.0 mg finasteride.

In order to check our proposed method for selectivity, we tried different degradation pathways for finasteride. Selectivity is a parameter that gives account of the capacity of the method of producing a signal due to only the presence of the analyte (finasteride) and consequently free of other interferences such as degradation products, metabolites or pollutants. We have used the following trials in order to prove the selectivity: hydrolysis (acidic, alkaline and neutral), photolysis and thermolysis [20].

When a finasteride solution was exposed to either acid or alkaline hydrolysis, the polarographic peak diminishes and no new signals appear in the polarograms (Fig. 5). Actually, hydrolysis of finasteride in acid media for 1 h produces a peak current reduction in approximately 17% and in alkaline media, about 40%. Furthermore, in order to check the selectivity of possible photodecomposition products, finasteride was exposed to 366-nm UV for 8 h. In this experiment, the finasteride's peak remains unaltered. Similar behaviour was obtained when finasteride was submitted to thermolysis or neutral hydrolysis test. According to the described results obtained in the selectivity trials, it can be concluded that the proposed differential pulse polarographic method is sufficiently selective, in order to be applied to finasteride quantification and also can be applied for further stability studies.

In order to obtain the precision and accuracy of the developed method, a recovery study was performed. These results (Table 1) reveal that the method has an adequate precision and accuracy and consequently, can be applied to the determination of finasteride in commercial dosage (tablets). Also, we can conclude from these experiments that typical excipients included in the drug 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) do not interfere with the selectivity of the method, and previous separation or extractions are not necessary.

The proposed DPP method was applied successfully to both a composite assay and an individual tablet assay, in order to verify the uniformity content of finasteride. With comparative purposes a HPLC analysis was also carried out (Table 2). In the composite assay 98.5% and 99.6% of the label claimed for DPP and HPLC, respectively were found, which fall within the percentages accepted by United Sates Pharmacopoeia (95.0–105.0%). Also, the content for all assayed tablets in the individual tablet assay fall in the range of 85.0–115.0% of label claim and no unit is outside the range of 75.0–125.0% of label claim, fulfilling the Pharmacopoeia requirement for uniformity content of tablets [13]. The results obtained in the uniformity content test by each applied method were compared by applying Snedecor *F*-test (variance proportion) and then the Student's *t*-test ($p < 0.05$, $n = 10$), and it was concluded that no significant differences exist between them and that they were statistically equivalent.

Finally, we can conclude that the DPP-developed method is enough for the finasteride determination in pharmaceutical forms, as it exhibits an adequate accuracy, reproducibility and selectivity. Furthermore, treatment of the sample is not required as in HPLC; the method is not time-consuming and less expensive than the HPLC ones.

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