ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
23 (2000) 617–622

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

New spectrophotometric method for the determination of flutamide in pharmaceutical preparations

P. Nagaraja *, K.R. Sunitha, M.F. Silwadi

Department of Studies in Chemistry, Mysore University, Manasagangotri, Mysore 570 006, India

Received 11 November 1999; received in revised form 16 February 2000; accepted 19 February 2000

Abstract

A sensitive and simple spectrophotometric method for the determinations of reduction product of flutamide (FLA) is described. The method is based on the interaction of diazotized flutamide reduction product with *N*-(1-naphthyl) ethylenediamine dihydrochloride (NEDA) in neutral or resorcinol (RSL) in alkaline medium. Absorbance of the resulting chromophores is measured at 525 or 480 nm, respectively, and is stable for at least 7 days. The two coupling reagents are applied successfully for the determination of FLA in tablets. The common excipients used as additives in pharmaceutical preparations do not interfere with the determination. Results from the analysis of pure FLA and its commercial tablets by the proposed methods agree well with the reported method.

Keywords: Flutamide; Reduction; Diazotization; *N*-(1-naphthyl) ethylenediamine; Resorcinol; Spectrophotometry

1. Introduction

Flutamide (FLA) 2-methyl-*N*-[4-nitro-3 (trifluoro methyl) phenyl] propanamide is widely used as an antiandrogen drug [1]. It is a powerful nonsteroidal androgen antagonist. This drug and its primary hydroxy metabolite decrease metabolism of C-19 steroids by the cytochrome P-450 system at the target cells in the secondary sex organ [2]. This new drug is not yet included in U.S.P., B.P. or I.P. The reported analytical methods for the determination of FLA are chromatography [3], polarography [4], gas chromatography [5] and high performance liquid chromatography [6]. Zarapkar et al [7] first reported the spectrophotometric determination of FLA utilising the fact that it forms yellow colour when dissolved in hydrochloric acid with maximum absorbance at 380 nm. This method suffers from narrow range of determination, yellow colour formation require heating, application is restricted to only pure FLA drug and provide inadequate information on stability, sensitivity, interference and analytical data. However, this is the only spectrophotometric method available for comparison purpose, since this new drug is not yet included in any pharmacopoeia. Though the chromotographic and polarographic methods are sensitive requires complication procedure. Some of

* Corresponding author. Fax: +91-821-518835.
E-mail address: nagarajap@mailcity.com (P. Nagaraja).

0731-7085/00/$ - see front matter
PII: S0731-7085(00)00319-8

these methods are time consuming and suffer from lack of good sensitivity. A thorough literature survey on FLA shows that so far there is no more chromogenic reagents reported for the estimation of FLA using spectrophotometry.

In the present study we succeeded in developing new coupling agents for the sensitive and selective spectrophotometric determination of FLA based on the interaction of diazotized FLA reduction product with *N*-(1-naphthyl) ethylenediamine dihydrochloride (NEDA) or resorcinol (RSL) and the formation of intense pink colour in neutral aqueous medium or orange red colour in alkaline medium. This method offers the advantage of sensitivity, selectivity and rapidity without the need of extraction or heating.

2. Experimental

A JASCO model UVIDEC-610 UV–vis spectrophotometer with 1.0 cm matched cells was used.

Pharmaceutical grade FLA was obtained as a gift from Cipla, India, NEDA was from BDH, Poole, UK and RSL from SD fine, India. All other chemicals used were analytical reagent grade. Deionized water was used to prepare all solutions and in all experiments.

2.1. Solutions

Accurately weighed (50 mg) FLA was transferred to 100 ml beaker containing 1.0 ml of concentrated hydrochloric acid, 20.0 ml water and 0.5 g piece of zinc metal. The solution was cooled to room temperature and filtered. The filtrate was diluted to 100 ml in volumetric flask with water. The working standard solution of reduced FLA containing 25 μg ml^{-1} was prepared by further dilution. A 0.1% aqueous solutions of NEDA and RSL were freshly prepared and protected from sunlight. A 1% aqueous solution of sodium nitrite, 2% aqueous solution of sulphamic acid, 10% sodium hydroxide solution and 10 M sulphuric acid solution were used.

2.2. Procedure

Aliquots of the working standard solution of FLA (2.5–175 μg) were transferred into 25 ml calibrated flasks; 1 ml of 10 M H_2SO_4 was added to each. After cooling in an ice-bath, 1.0 ml of sodium nitrite solution (1%) was added with swirling. The solutions were allowed to stand for 5 min; then 2.0 ml of sulfamic acid solution (2%) was added. The solutions were swirled and allowed to stand for 10 min. Next 4.0 ml volume of coupling reagent solution NEDA (0.1%) or RSL (0.1%) was added, after 5 min the solutions were diluted to the mark with water for NEDA or with NaOH (10%) for RSL. After mixing the solution thoroughly, the absorbance was measured at 525 nm for NEDA or 480 nm for RSL against the corresponding reagent blank and calibration graphs were constructed.

2.3. Procedure for assay of FLA in commercial samples.

Twenty tablets were powdered and mixed thoroughly. An amount equivalent to 50 mg of FLA was taken and the substance was subjected to reduction using hydrochloric acid and zinc. The filtrate was made up to 100 ml and an aliquot of this solution was treated as described above for the determination of FLA.

3. Results and discussion

3.1. Spectral characteristics

The FLA was reduced in the presence of acid and zinc to form amino derivative of flutamide. The reduced FLA was diazotized in acidic medium and coupled with NEDA in neutral aqueous medium to form pink coloured dye of λ_{max} 525 nm or coupled with RSL in alkaline medium to form orange–red coloured dye of λ_{max} 480 nm. These wavelengths were used for all measurements. The absorption spectra of FLA reaction products formed and the reagent blank are shown in Fig. 1.

3.2. Optimum reagents concentration

For the diazotization-coupling reaction, the use of sulfuric acid solution as the reaction medium was found to give more satisfactory results than hydrochloric acid solutions. It was found that a 10 M solution of sulfuric acid in the range of 0.5–1.5 ml, a 1% solution of sodium nitrite in the range of 0.5–2 ml, a 2% solution of sulfamic acid in the range of 1–4 and 2–6 ml of 0.1% NEDA or RSL were necessary to achieve maximum colour intensity. Hence, 1.0 ml of sulfuric acid, 1.0 ml sodium nitrite and 2.0 ml of sulphamic acid, were selected for diazotization. A 4.0 ml of NEDA or RSL was used as coupling agent to obtain maximum intensity and stability of the colour. The colour intensity increases slightly in the first 3 min, but then remains constant for 7 days.

The study of the effect of acidity on the diazotization-coupling of reduced FLA showed that the acidity is moderately critical. Higher acidity could not be ascertained because at this very strong acidity the excess nitrite could not be destroyed by sulfamic acid and as a result an intense colour was produced by the nitrosation of the NEDA (purplish) or RSL (yellow). Maximum intensity and stability of the colour were obtained with addition of 0.5–1.5 ml of 10 M sulphuric acid and 1.0 ml of sulphuric acid is recommended. Other mineral acids were tested and found unsatisfactory.

When 1.0 ml of 1% solution of sodium nitrite was used the excess of nitrite could be removed by the addition of 2.0 ml of 2% sulphamic acid solution. An excess of sulphamic acid has no effect on colour.

In case of NEDA as a coupling agent dilution of the coloured solution with different solvents like water, methanol, ethanol, acetic acid and acetonitrile have been tested. Results showed that water gives maximum intensity and stability of the colour. In case of RSL as a coupling agent the orange red colour is formed in alkaline medium. It was found that a 5–15% of NaOH as diluents was necessary for the achievement of maximum

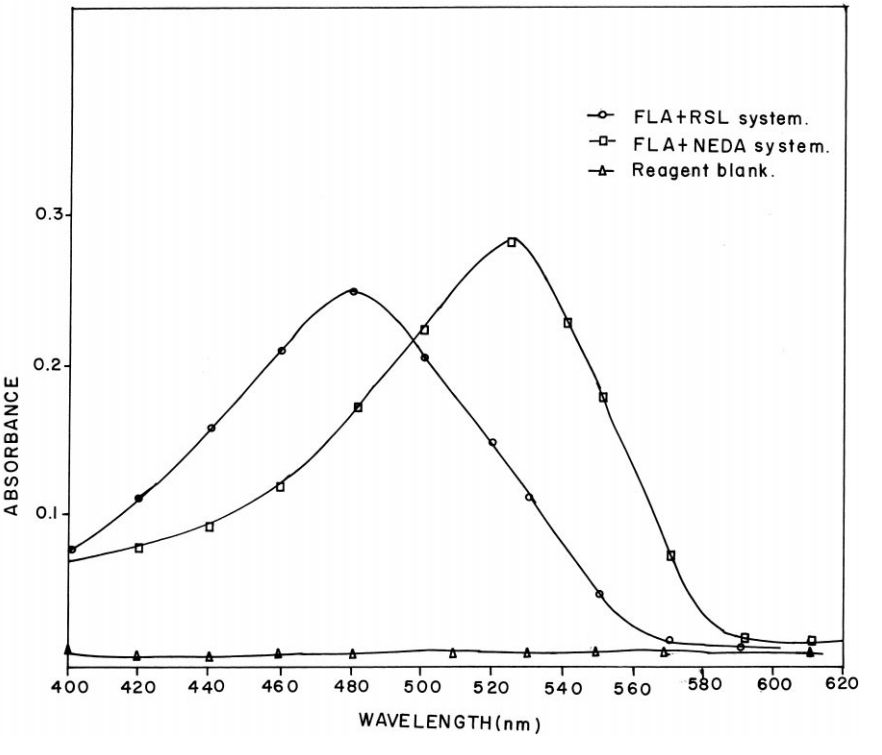


Fig. 1. Absorption spectra of FLA reaction products. Initial concentration of FLA 3.0 μg ml^{-1}.

Table 1
Parameters for the spectrophotometric determination of FLA

Optical character	Values of NEDA	Values of RSL
Beer's Law range (μg ml^{-1})	0.25–7	0.25–6
Molar absorptivity (1 mol^{-1} cm^{-1})	2.57×10^4	2.07×10^4
Specific absorbance	9.3×10^2	7.5×10^2
Sandel's sensitivity (μg cm^{-2})	0.0107	0.0133
Regression equation (y)[a]		
Slope (a)	0.257	0.189
Intercept (b)	0.0041	0.0305
Correlation coefficient (r)	1.002	0.9998
R.S.D. (%)[b]	1.22	1.44
% Range of error[b] (95% confidence limits)	±0.28	±0.29

[a] $y = ax + b$, where x is the concentration (μg ml^{-1}) for FLA.
[b] Relative standard deviation ($n = 5$).

colour intensity. Hence, 10% NaOH was selected for all further studies.

It was found that a 0.1% concentration of NEDA or RSL in the range 2–6 ml was necessary for the maximum intensity and stability of the colour. Hence, 4.0 ml of NEDA or RSL was selected for further studies.

3.3. Quantification

Beer's law is obeyed over the FLA concentration range 0.1–7.0 μg ml^{-1} for NEDA or RSL as a coupling agents. Molar absorptivity, specific absorbance, Sandel sensitivity, percentage relative standard deviation, correlation co-efficient, intercepts and slopes for the calibration data of NEDA and RSL by the suggested method are given in Table 1.

3.4. Reaction sequence

In an acidic medium nitrite reacts with reduced FLA to form diazonium salt. The salt is then coupled with NEDA to yield a pink azo dye of λ_{max} 525 nm. When the diazonium salt is allowed to react with RSL in the presence of NaOH, an orange red dye of λ_{max} 480 nm is formed. The reaction mechanism is shown in Scheme 1.

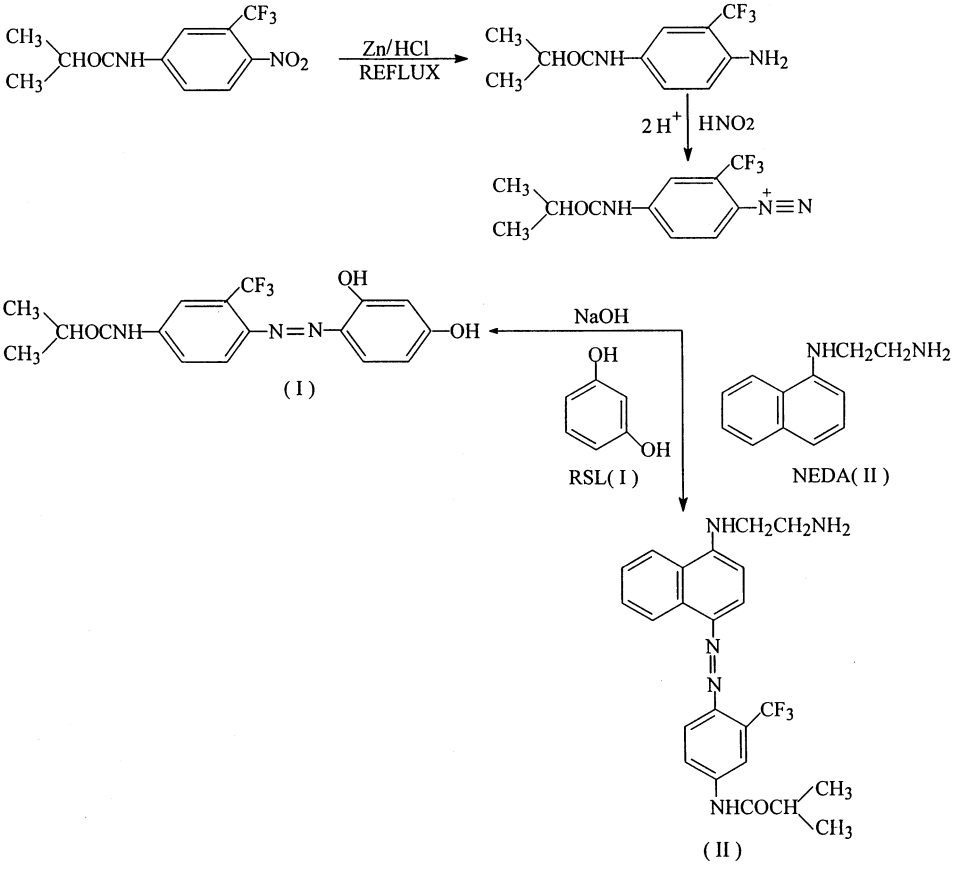
3.5. Stability

The diazotization of FLA reduced product is complete in 5 min at room temperature. The diazotized salt is then stable for ~25–35 min and then deteriorates slowly. Cooling in ice increased the time needed for diazotization and did not eliminate deterioration. Hence, the reaction is studied at room temperature. The azo products resulting from the suggested method were studied at different temperatures. It was found that the absorbency values remain constant in the temperature range 5–90°C. Hence, room temperature was recommended for the coupling reaction. The pink and orange red azo products were stable for more than 7 days and results were reproducible.

3.6. Interference

Under the diazotization reaction conditions used, other amines such as aniline, morpholine, piperidine, etc. give a positive reaction. However, the problem of interferences does not arise in the analysis of the commercially available FLA dosage forms. The effects of additives associated with the FLA in its formulations were investigated using the developed method. This method does not suffer any interference from common excipients, additives and other substances such as magnesium stearate, glucose, dextrose, lactose, starch, gum acacia, carboxymethyl cellulose, sodium alginate, vitamin B_6 and ascorbic acid. The results are given in Table 2.

The effects of additives associated with the FLA in its formulations were investigated using the developed method. This method does not suffer any interference from common excipients and additives such as magnesium stearate, glucose, ascorbic acid, talc, starch, lactose, sodium alginate and dextrose.



Scheme 1.

Table 2
Determination of FLA[a] in the presence of excipients and other substances

Material	Amount (mg)	% Recovery of FLA ± R.S.D.[b]
Magnesium stearate	40	99.9 ± 0.85
Glucose	40	99.7 ± 0.94
Lactose	40	99.8 ± 0.96
Dextrose	40	101.0 ± 0.95
Starch	40	99.4 ± 1.14
Gum acacia	40	99.0 ± 1.02
Talk	40	99.8 ± 0.96
Vitamin B_6	20	99.0 ± 1.04
Ascorbic acid	20	100.2 ± 0.75
Carboxy methyl cellulose	40	99.8 ± 1.00
Sodium alginate	40	98.8 ± 2.20

[a] 3 μg ml^{-1} of FLA taken.
[b] Average of five determinations.

3.7. Application

The reproducibility of the method was checked by ten replicate determinations at the 3 μg ml^{-1} level of FLA and the standard deviation was found to be between 1.2 and 2.4%. The applicability of the method for assay of pharmaceutical preparation was examined. The results of the assay of available tablets of FLA are summarized in Table 3. These results are highly reproducible. The results of the assay of tablets were cross-checked by the reported method [7].

4. Conclusions

The method is found to be simple, economical, selective and more sensitive than the reported method [7]. The statistical parameters and the

Table 3
Determination of FLA in pharmaceutical preparations

Trade name	Nominal drug content (mg)	Proposed method		Official method[a] found (%)	
		Found[b] (%)	Added (mg)	Recovery[b] (%)	
Cytamid[c]	250/tablet	99.96 ± 0.68	250	99.98 ± 1.8	101.7 ± 0.5
Drogenil[d]	250/tablet	100.04 ± 0.68	250	101.1 ± 2.4	99.2 ± 0.9

[a] Reported Zarapkar method.
[b] Average ± S.D. of five determinations.
[c] Marketed by Cipla Ltd.
[d] Marketed by Fulford.

recovery study data clearly indicate the reproducibility and accuracy of the method. Analysis of the authentic samples containing FLA showed no interference from the common additives and excipients. Hence, this approach could be considered for the determination of FLA in the Quality Control Laboratories.

Acknowledgements

One of the authors (K.R.S) thanks Mysore University for support of this work.

References

[1] S. Budavari, M.J. O'Neil, A. Smith, P.E. Heckelman, Merck Index, 11th edn., Merck & Co Inc, Rathway, NJ, USA, 1989, p. 658.
[2] A. Osol, J.E. Hoover, et al., Remington's Pharmaceutical Sciences, 18th edn., Merck Publishing Company, Easton, PA, 1996, p. 1152.
[3] Y.a.G. Ballon, O.I. Lazebnaya, I.S. Dukhovnaya, Khim. Farm. Zh. 19 (1985) 626–627.
[4] A. Syncerski, J. Pharm. Biomed. Anal. 7 (1989) 1513–1518.
[5] R.T. Sane, M.G. Gangrade, V.V. Bapat, S.R. Surve, N.L. Chonkar, Indian Drugs 30 (1993) 147–151.
[6] D. Farthing, D. Sica, I. Fakhry, D. Lowe-Watters, E.A. Cefali, G. Allan, Biomed. Chromatogr. 8 (1994) 251–254.
[7] S.S. Zarapkar, C.D. Damle, U.P. Halkar, Indian Drugs 33 (1996) 193–194.