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Novel reagents for the sensitive spectrophotometric determination of flutamide, an anticancer drug in pharmaceutical preparations

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Abstract

Simple and sensitive spectrophotometric methods for the determination of flutamide (FLA) in either pure form or in its pharmaceutical preparations are described. The first method is based on the diazotisation of reduced FLA, followed by coupling with alcoholic iminodibenzyl (IDB) in acid medium to give a purple coloured product having a λ_{max} of 570 nm. In the second method, the diazotisation of reduced FLA followed by coupling with 4-amino-5-hydroxy-2,7-naphthalenedisulphonic acid monosodium salt (AHND) in a buffer medium of pH 12, gives a red coloured product having a λ_{max} of 520 nm. Common excipients used as additives in pharmaceutical preparations do not interfere in the proposed methods. Both the methods are highly reproducible and have been applied to a wide variety of pharmaceutical preparations and the results compare favourably with the reported method.

Keywords: Flutamide; Spectrophotometry; Iminodibenzyl; AHND; Pharmaceuticals

1. Introduction

Flutamide (FLA), chemically 2-methyl-*N*-[4-nitro-3-(trifluoromethyl)phenyl] propanamide is widely used as antineoplastic (hormonal) and antiandrogen drug (Budavari et al., 1989). FLA is a powerful nonsteroidal androgen antagonist which is used to treat prostate cancer, is believed to block androgen receptor sites. 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 (Osol and Hoover, 1996). This new drug is recently included in the United States Pharmacopoeia which involves a chromatographic method for the analysis of the pure drug and FLA capsules (US Pharmacopeia XXIV, 1999). A survey of literature reveals that there are not many methods for the assay of FLA. The reported methods include polarography (Snycerski, 1989), gas-chromatogra-

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phy (Sane et al., 1993) and high performance liquid chromatography (HPLC) (Farthing et al., 1994). The polarographic, UV spectrophotometric and HPLC determination of FLA in tablets has been reported (Alvarez-Lueje et al., 1998). The spectrophotometric methods available for the determination of FLA include the formation of yellow colour with hydrochloric acid with λ_{max} of 380 nm suffers from various drawbacks (Zarapkar et al., 1996). We have recently reported three spectrophotometric methods for the determination of FLA using promethazine hydrochloride or resorcinol or (NEDA) *N*-(1-naphthyl)ethylenediamine dihydrochloride (Rangappa et al., 2000; Nagaraja et al., 2000). Two more spectrophotometric methods which have been recently published do not give much information about the optical characteristics makes use *p*-dimethylamino cinnamaldehyde or NEDA or chromotropic acid or resorcinol (Reddy et al., 2001a,b) and more over this is a repetition of our earlier work.

In continuation of our work on the spectrophotometric determination of organic compounds of pharmaceutical importance (Nagaraja et al., 1996, 1998, 2001), we succeeded in developing two more new coupling agents for the sensitive spectrophotometric determination of FLA based on the interaction of the reduced FLA with either iminodibenzyl to produce purple coloured product or 4-amino-5-hydroxy-2,7-naphthalenedisulphonic acid monosodium salt to produce a red coloured product. The methods offer the advantages of sensitivity, selectivity and rapidity without the need for extraction.

2. Materials and methods

2.1. Materials

A JASCO model UVIDEC-610 UV–Vis spectrophotometer with 1.0 cm matched cells was used. Pharmaceutical grade FLA was obtained as a gift sample from Cipla, India. IDB (Sigma, USA) and AHND (Merck, Germany) were used as received. $NaNO_2$ (BDH) and HCl (AR) were used. All other chemicals and solvents used were of analytical reagents grade. Deionized water was used to prepare all solutions and in all experiments. Commercial dosage forms were purchased from local sources.

2.2. Solutions

Accurately weighed (100 mg) FLA was transferred to 100 ml beaker containing 4.0 ml of methanol. 0.5 g of Zn dust and 4.0 ml of concentrated hydrochloric acid were added and the mixture is left for 30 min till the reaction ceases. Solution was filtered into 100 ml standard flask and made up to the mark. The working standard solution of reduced FLA containing 25 μg ml^{-1} was prepared by further dilution. A 0.5% solution of freshly prepared IDB in alcohol and AHND solution of 0.5% (0.5 g AHND in 5 ml concentrated HCl and diluted to 100 ml with water) was used. One percent aqueous solution of sodium nitrite, 2% aqueous solution of sulphamic acid, 1:1 sulphuric acid and NaOH–phosphate buffer of pH 12 were used for the experiment.

2.3. Procedure

Aliquots of the working standard solution of reduced FLA (10–400 μg for IDB; 10–200 μg for AHND) were transferred into 25 ml calibrated flasks. For IDB method, 2 ml of 1% $NaNO_2$ was added, cooled in an ice bath and added 2 ml of 2% sulphamic acid, cooled, followed by the addition of 3 ml of 0.5% alcoholic IDB along with 4 ml of alcohol, left for 5 min and diluted to the mark with 1:1 H_2SO_4. After mixing the solution thoroughly, the absorbance was measured at 570 nm against the corresponding reagent blank within 35 min and calibration graph was constructed. For AHND method, 1 ml of 1% $NaNO_2$ was added, cooled and 1 ml of 2% sulphamic acid was added, cooled, the solution was swirled followed by the addition of 1 ml of 0.5% AHND and 4 ml of buffer of pH 12. The solution was heated on a water bath for 15 min, cooled to room temperature and the solution was diluted to the mark with water. The solution was mixed well and the absorbance was measured at 520 nm against the corresponding reagent blank and calibration graph was constructed.

2.4. Procedure for the assay of FLA in commercial samples

Twenty tablets were powdered and mixed thoroughly. An amount equivalent to 50 mg of FLA was taken dissolved in 4 ml of methanol and the substance was subjected to reduction using zinc and HCl. The filtrate was made up to 100 ml and an aliquot of this solution was treated as described above for the pure sample either by IDB method or by AHND method.

3. Results and discussion

3.1. Spectral characteristics

The reduced FLA was diazotized in acidic medium and coupled with IDB in alcohol medium to form a purple coloured product of λ_{max} 570 nm or coupled with AHND in buffer medium to get a red coloured product of λ_{max} 520 nm. These wavelengths were used for all measurements. The absorption spectra of FLA reaction products formed are shown in Fig. 1. The corresponding reagent blanks have practically negligible absorbance at these wavelengths.

3.2. Optimum reagents concentration

Various concentration and volume ranges for all the reagents were studied. However, the following are the optimum concentration and volume ranges. The recommended volume for all the reagents is mentioned in Section 2.2.

For the IDB method, it was found that a 1% solution of $NaNO_2$ in the range of 1–3 ml, a 2% solution of sulphamic acid in the range of 1–3, 2–4 ml of 0.5% alcoholic IDB and 3–5 ml of alcohol were necessary to achieve maximum colour intensity. For AHND method, 1–2 ml of 1% $NaNO_2$, 1–2 ml of 2% sulphamic acid, a 0.5%

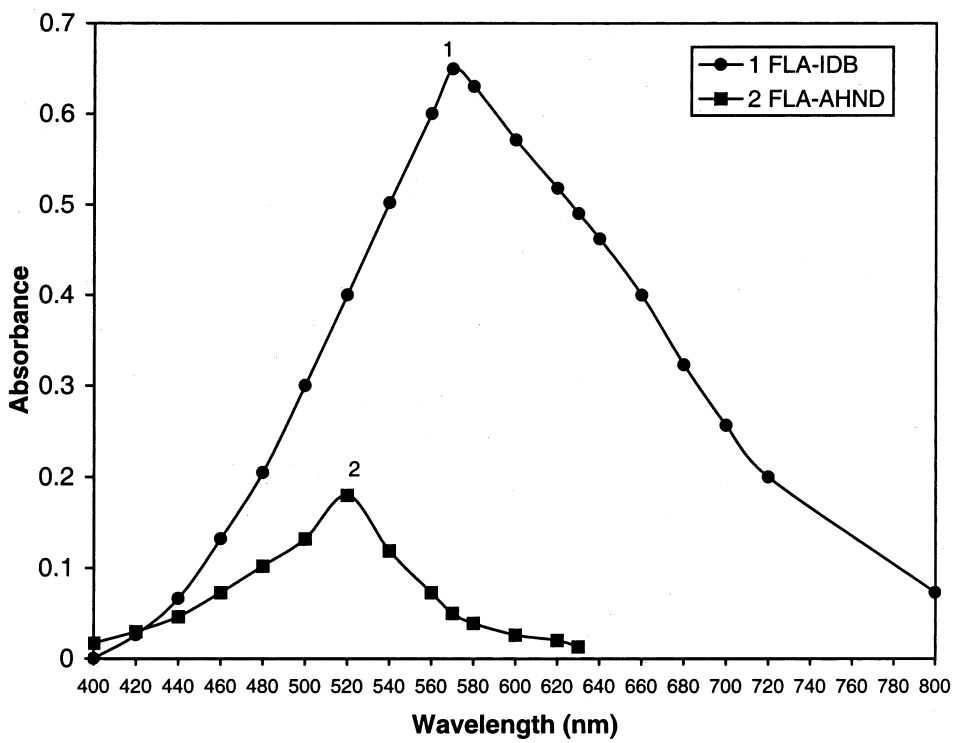


Fig. 1. Absorption spectra of ^{1}FLA–IDB reaction product. Initial concentration of FLA = 8 μg ml^{-1}. ^{2}FLA–AHND reaction product. Initial concentration of FLA = 4 μg ml^{-1}.

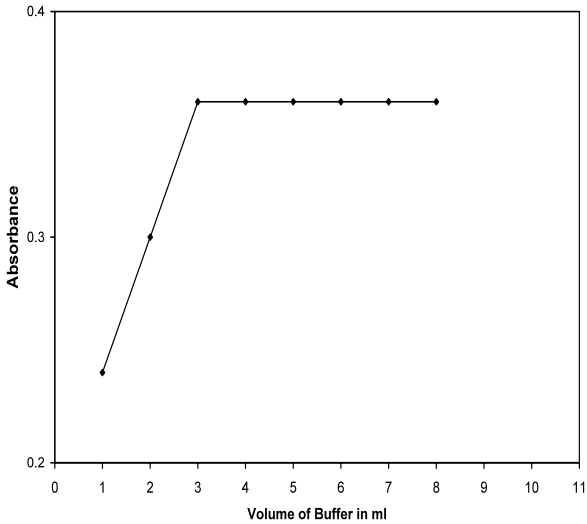


Fig. 2. Effect of buffer (pH 12) on the FLA–AHND reaction.

AHND in the range of 0.5–2 and 3–8 ml of buffer of pH 12 were required to get maximum colour intensity. Fig. 2 shows the effect of volume of buffer with absorbance. In both the methods, the excess of nitrite could be removed by the addition of sulphamic acid solution. Addition of excess of sulphamic acid solution has no effect on absorbance values. In the IDB method, excellent results were obtained by using 1:1 H_2SO_4 for dilution compared to other acids and solvents.

In the case of AHND 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.

3.3. Quantification

Beer's law is obeyed over the FLA concentration range of 0.4–16 μg ml^{-1} for IDB and 0.4–8.0 μg ml^{-1} for AHND as coupling agents. Limit of Quantification (LOQ) is determined by taking the ratio of standard deviation (σ) of the blank with respect to water and the slope of calibration curve (s) multiplied by a factor 10. That means, LOQ is approximately 3.3 times Limit of Detection (LOD). Naturally, the LOQ slightly crosses the lower limit of the Beer's law range. But, LOD is well below the lower limit of the Beer's law range. The upper limit of the Beer–Lambert range is determined by a plot of absorbance against concentration at the value of λ_{max}. Beyond this limit, the correlation results were really affected. Hence, the measurements were excluded above these limits to keep the relationship linear. The optical characteristics and precision data are given in Table 1.

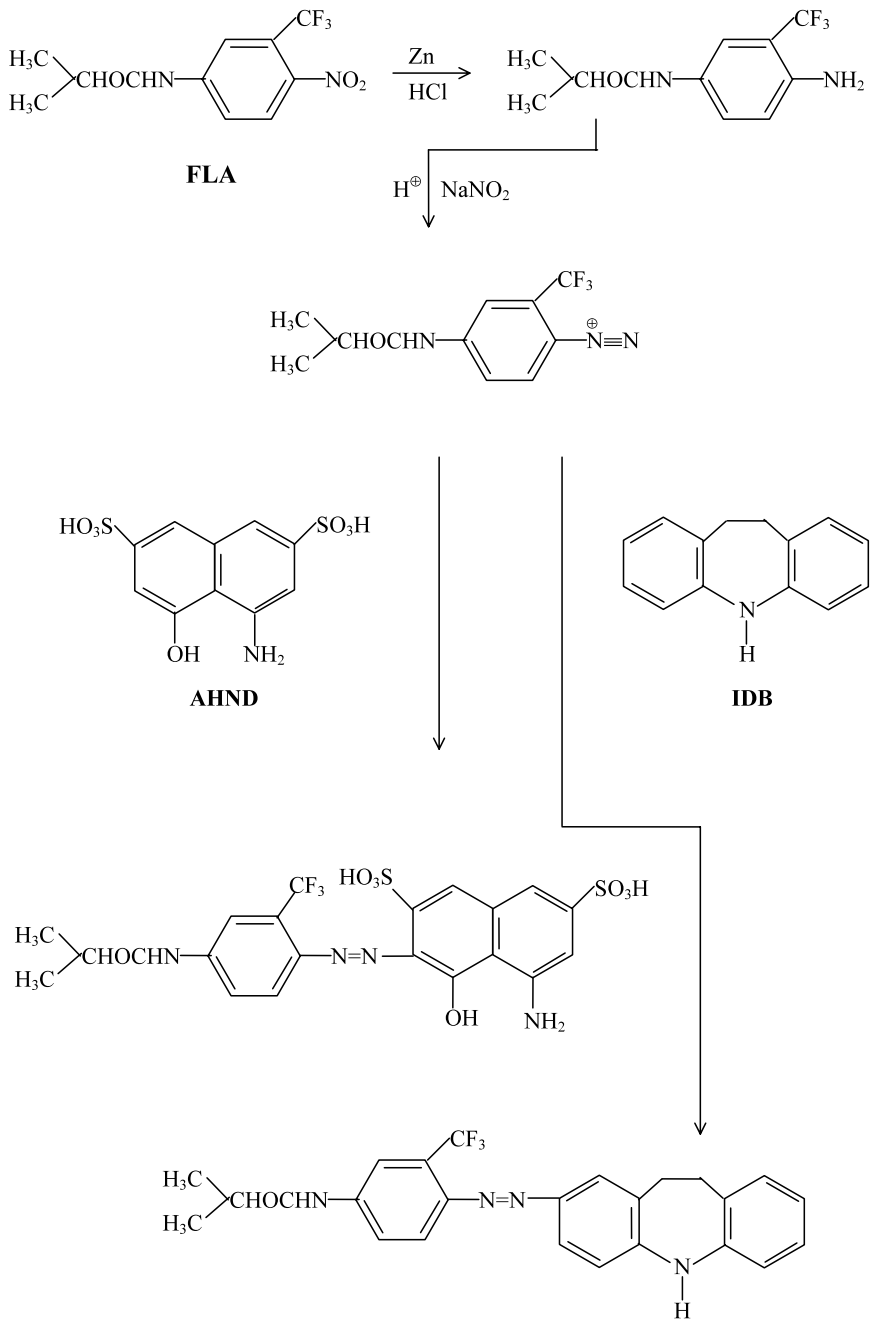
3.4. Reaction sequence

In an acidic medium, nitrite reacts with reduced FLA to form diazonium salt. The salt is then coupled either with IDB in presence of alcohol to yield a purple product with λ_{max} of 570 nm or with AHND in presence of buffer of pH 12 to give a red coloured product with λ_{max} of 520 nm. The reaction mechanisms for the formation of the products are shown in Scheme 1.

Table 1
Optical characteristics and precision data

Parameters/characteristics	IDB	AHND
Colour	Purple	Red
λ_{max} (nm)	570	520
Stability	35 min	5 days
Beer's law range (μg ml^{-1})	0.4–16.0	0.4–8.0
Limit of detection (μg ml^{-1})	0.1318	0.2882
Limit of quantification (μg ml^{-1})	0.4393	0.9607
Molar absorptivity (1 mol^{-1} cm^{-1})	0.301×10^5	0.217×10^5
Sandell's sensitivity (μg cm^{-2})	0.00933	0.0127
Optimum photometric range (μg ml^{-1})	0.8–14.0	0.8–6.0
Regression equation (y)[a]		
Slope (b)	0.0794	0.04543
Intercept (a)	0.0138	−0.0017
Correlation coefficient (r)[b]	0.9930	0.9937
Relative standard deviation[c](%)	0.7887	1.1266
Range of error	±1.09	±1.56

[a] $y = bx + a$, where x is the concentration in μg ml^{-1}.
[b] $n = 10$.
[c] Ten replicates.



Scheme 1.

3.5. Stability

The diazotization of reduced FLA is complete in 5 min at room temperature. The stability of the purple coloured product formed from FLA–IDB interaction is 35 min. After this time interval, the

colour intensity slowly decreases. Attempts to increase this time interval, by changing the reaction conditions was unsuccessful. However, a time interval of 35 min was sufficient enough to record the measurements. In contrast, the red product formed by the coupling reaction between FLA and AHND was stable for 5 days. An increase of temperature form 10–40 °C, did not affect the results in both the methods. However, a temperature of 30 °C is recommended for both the methods and reproducible results were obtained.

3.6. Interference

Under the diazotization reaction conditions used, other amines such as morpholine, aniline, piperidine, etc. gave a positive reaction. However, the problem of interferences does not arise in the analysis of commercially available FLA tablets. The effect of additives associated with the FLA in its formulations were investigated using the developed methods. The methods does not suffer any interference from common excipients and other substances. The analysis of interference with the excipients was conducted from the initial step, i.e. the reduction stage. However, the ratio of excipients and the active ingredient (FLA) really does not matter. The results are given in Table 2 for various excipients and the percentage recovery of the drug varied from 99.5 and 100.2.

3.7. Application

The reproducibility of the method was checked by ten replicate determinations at the 8 μg ml^{-1} level of FLA for IDB (4 μg ml^{-1} level of FLA for AHDN) and the relative standard deviation was found to be between 0.78 and 1.2%. The applicability of the method for the assay of pharmaceutical preparations was examined. The results of the assay of available tablets of FLA are summarized in Table 3. The actual weight of individual tablets is 750 mg, out of which the label claim of FLA is 250 mg. The analysis of the tablets was conducted from the reduction stage. The results are highly reproducible and the assay of tablets were cross checked by the reported method (Nagaraja et al., 2000) which agrees favourably.

3.8. Conclusions

The present methods are found to be simple, economical, selective and more sensitive than the few reported methods. The IDB method has a slight edge over AHND method with regard to sensitivity, while AHND method dominates over IDB method based on stability. The statistical parameters and the recovery study data clearly indicate the reproducibility and accuracy of the methods. Analysis of the authentic samples containing FLA showed no interference from com-

Table 2
Determination of flutamide in presence of excipients

Excipient	Amount of excipient added (mg)	% Recovery of flutamide ± % RSD[a]	
		IDB	AHDN
Carboxy methylcellulose	40	99.7 ± 0.6	99.8 ± 0.95
Dextrose	35	99.5 ± 0.5	99.7 ± 0.95
Starch	35	99.5 ± 0.6	99.8 ± 0.90
Glucose	40	99.7 ± 0.7	99.6 ± 0.80
Gumacacia	40	99.8 ± 0.8	99.7 ± 0.90
Lactose	40	99.5 ± 0.7	99.6 ± 0.80
Magnesium stearate	35	100.1 ± 0.8	100.2 ± 0.85
Talc	35	99.8 ± 0.7	99.7 ± 0.85
Sodium alginate	35	99.6 ± 0.7	99.5 ± 0.75
Stearic acid	35	99.7 ± 0.6	99.8 ± 0.80

8 μg ml^{-1} of FLA taken for IDB. 4 μg ml^{-1} of FLA taken for AHDN.
[a] Average of five determinations.

Table 3
Determination of flutamide in pharmaceutical preparations

Commercial formulations analysed	Total weight of the tablet (mg)	Label claim (mg)	Amount of drug found[a] in mg		
			Proposed method		Reported method[g]
			IDB method	AHND method	
Cytomid[b]	750	250	249.2 ± 0.75	249.3 ± 0.80	249.2 ± 0.80
Drogenil[c]	750	250	249.6 ± 0.80	249.5 ± 0.90	249.4 ± 0.80
Flutacare[d]	750	250	249.3 ± 0.75	249.4 ± 0.90	249.3 ± 0.70
Plutamide[e]	750	250	249.5 ± 0.70	249.6 ± 0.80	249.4 ± 0.80
Prostamid[f]	750	250	250.8 ± 0.80	250.7 ± 0.95	250.9 ± 0.90

[a] Average of five determinations ± relative standard deviation (%).
[b] Marketed by Cipla.
[c] Marketed by Fulford.
[d] Marketed by Criticare.
[e] Marketed by Torrent.
[f] Marketed by BDH.
[g] NEDA method Nagaraja et al. (2000).

mon additives and excipients. Hence, these methods could be considered for the determination of FLA in the Quality Control Laboratories.

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