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DIRECT COLORIMETRIC ASSAY OF NILUTAMIDE IN PURE AND FORMULATIONS USING BENZIDINE REACTION

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ABSTRACT

A direct colorimetric assay method was developed for the assay of nilutamide in pure and in its dosage form using simple benzidine reaction. The present proposed method is based on the benzidine reaction between nilutamide containing gem poly halogen compound in alkaline medium results in the opening of pyridine ring takes place followed by intense red color development with benzidine in formic acid medium exhibiting absorption maximum at 558nm and obeying Beer's law in the concentration range of 12-60µg/mL. Statistical analysis has been carried out for the proposed method that revealed good precision and high accuracy. The direct colorimetric method developed by the author could be successfully extended to the commercial pharmaceutical formulations of other drugs containing gem halides functional groups.

KEY WORDS: Nilutamide, Linearity, Validation

INTRODUCTION

Nilutamide [1-4] (Figure.1), 5,5-dimethyl-3-[4-nitro-3-(trifluoromethyl)phenyl]-2,4-imidazolidinedione is an antineoplastic hormonal agent belong to the family of Azolidines primarily used in the treatment of prostate cancer. It competes with androgen for the binding of androgen receptors through the inhibition of androgen-dependent DNA and protein synthesis, consequently blocking the action of androgens of adrenal and testicular origin that stimulate the growth of normal and malignant prostatic tissue that results in growth arrest or transient tumor regression. It is sold in the local pharmacy as niltamide tablets (Zydus Cadila Healthcare Ltd) each tablet contains 150mg of nilutamide.

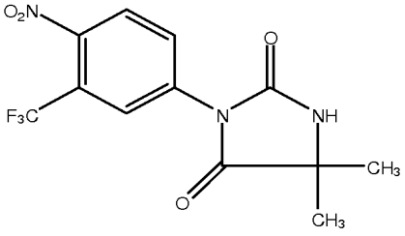

Figure.1.Molecular structure of Nilutamide

Till to date no UV-Visible spectrophotometric methods have been reported in the literature for its determination in pharmaceutical dosage forms and this fact prompted the author to develop accurate and inexpensive direct colorimetric method for the determination of the nilutamide in pure and tablet dosage forms. The present paper describes a new direct colorimetric method, which is based on reactivity of the gem halide group of nilutamide with benzidine in alkaline medium to produce colored species with reasonable stability.

EXPERIMENTAL

INSTRUMENTATION: UV-Visible Spectrophotometer: A UV-VIS Spectrophotometer Elico (SL-160 model) with 1.0cm matched quartz cuvettes was used for all spectral and absorbance measurements. An 0.001g readability and repeatability, 200g capacity analytical balance was used to weigh the required amount of the drug and the reagents.

CHEMICAL AND REAGENTS: Pure sample of nilutamide drug was obtained from Kekule Pharma Limited, Hyderabad, Telanga state, India and commercial formulation in the brand name nilutamide (Zydus Cadila Healthcare Ltd) each tablet contains 150mg of nilutamide was purchased from local pharmacy. All the chemicals and reagents used are of analytical grade and solutions were prepared in double distilled water.

BNZ solution(Loba; 3.0%, w/v): Prepared by dissolving 3.0gm of benzidine in 100mL of 88% HCOOH. KOH solution(qualigens; 10% w/v): Prepared by dissolving 10gms of KOH in 100mL of distilled water

PREPARATION OF STOCK AND WORKING STANDARD SOLUTIONS: Stock solution (1.0mg/mL) of nilutamide was prepared by dissolving 100mg of the drug in 10mL of DMSO and made up to 100mL with distilled water to get a clear solution. An appropriate volume of this stock solution was diluted step wise to get the working standard solution of concentration of 240µg/mL for the present cited proposed method respectively.

PROCEDURE FOR MARKET FORMULATIONS: About ten tablets of niltamide [Each tablet containing 150mg of nilutamide purchased from local pharmacy were pulverized to fine powder. Then powder equivalent to 100mg of nilutamide was accurately weighed and transferred into a 100mL calibrated flask, 10mLof DMSO was added and the content shaken thoroughly for 15-20 min and later the volume was finally diluted to the mark with double distilled water, mixed well and filtered through Whatman filter paper No 41. Aliquots of this filtrate were accurately diluted with distilled water as per the working standard solutions and this solution was used for the determination of nilutamide as per the proposed procedure described below.

RESULTS AND DISCUSSIONS

METHOD DEVELOPMENT [OPTIMIZATION OF REACTIONS CONDITIONS]: The experimental factors affecting color development, reproducibility, sensitivity, and conformity with Beer's law in developing this method were investigated and results so obtained were incorporated in the proposed procedure (Table.1). It was found that, 5.0mL of pyridine, 2.0mL of KOH solution and 5.0mL of Benzidine solution were necessary to achieve colored product of maximum intensity.

PROPOSED PROCEDURE: Aliquots of standard solution of nilutamide (0.5 - 2.5mL, 240µg/mL) were placed separately in a series of 20mL test tubes and total volume in each tube was made to 3.0mL with distilled water. Then 5.0mL of pyridine and 2.0mL of KOH solution were added successively and heated for 4min in a boiling water bath. All the tubes were cooled rapidly in an ice bath for 4min. After cooling, 5.0mL of supernatant pyridine layer was collected in to a series of 10mL calibrated tubes respectively and 5.0mL of benzidine solution was added to each tube, mixed thoroughly and the absorbance's of the colored species formed in each tube were measured at 560nm against reagent blank and the amount of drug (nilutamide) in a sample solution was obtained from Beer – Lamberts plot (Figure. 3).

METHOD VALIDATION

OPTICAL CHARACTERISTICS: The absorption spectra developed for the proposed method was scanned on a spectrophotometer in the wavelength region of 340 to 900nm against similar reagent blank or distilled water. The wavelength maxima (λmax) for nilutamide were found to be 558nm respectively and the results were graphically represented in Fig.2.

LINEARITY AND RANGE: The linearity of the proposed method for nilutamide was determined at five concentration levels ranging from 12µg/mL to 60µg/mL and a calibration curve was constructed by plotting response factor against various concentrations of nilutamide (Figure.3). The slope and intercept value for calibration curve of nilutamide was Y=0.01732x+0.0049 (R^2=0.9999) for the proposed method showing an excellent correlation exists between response factor and concentration of drug within the concentration range indicated above (Table.2).

TABLE.1:OPTIMUM CONDITIONS ESTABLISHED IN PROPOSED

PARAMETER	OPTIMUM RANGE	CONDITIONS IN PROCEDURE	REMARKS
λ_{max} (nm)	540 - 565	558	--
Effect of volume of pyridine required (mL)	4.0-6.0	5.0	5.0mL of pyridine was found to be adequate even for the upper limit of Beer's law
Effect of conc and volume (mL) of KOH	9.0 - 11.0% 1.8 - 2.2	10% 2.0	2.0mL of 10% alkali was found optimum to complete the reaction of gem poly halogen with pyridine.
Effect of heating and cooling times (min)	3.0 - 5.0 3.0 - 5.0	4.0 4.0	A minimum of 3.0 min heating in boiling water bath and 3.0min cooling in ice bath were found optimum and below 3.0min the reaction was incomplete and found low absorbance values.
Effect of conc of acidic medium for the benzidine reagent	80 - 88%	88%	Concentrated 88% formic acid gave good intensity of colored complex. The diluted formic acid resulted in erratic results.

TABLE.2: RESULTS OF STATISTICAL ANALYSIS OF THE PROPOSED METHOD

PARAMETER	RESULTS
λ_{max} (nm)	558
Beer's law limits (µg/mL)	12.0 - 60.0
Molar absorptivity (1/mol/cm)	4.124×10^3
Sandell's sensitivity (µg/cm^2/0.001 absorbance unit)	3.932×10^{-2}
Optimum photometric range (µg/mL)	13.5 – 56.0
Regression equation (Y=a+bc):slope (b)	0.0074
Intercept (a)	0.0052
Correlation coefficient (r)	0.9997
Relative standard deviation (%)*	0.968
0.05 level	0.809
0.01 level	1.198
LOD	0.0180

* Average of six determinations considered

TABLE.3: THE RESULTS OF (ACCURACY STUDIES) OF THE PROPOSED METHOD

Nilutamide in tablet µg.mL^{-1}	Pure Nilutamide added µg.mL^{-1}	Total found µg.mL^{-1}	Pure Nilutamide recovered %±SD*
24.0	5.0	29.98	99.94

TABLE.4: ASSAY OF NILUTAMIDE IN FORMULATION

SAMPLE	LABELLED AMOUNT (mg)	*AMOUNT OBTAINED (mg) BY THE PROPOSED METHOD	REFRENCE METHOD	%RECOVERY OF PROPOSED METHODS*
Tablet - 1	150	149.95	149.98	99.97

*Average of six determinations

SENSITIVITY (LOD): The limit of detection (LOD) is defined as the lowest concentration of an analyte that an analytical process can reliably differentiate from back-ground levels. In present study, LOD was evaluated based on the standard deviation of the response and the slope of the corresponding curve and the value of LOD for proposed method are given in Table.2 revealing good sensitivity.

PRECISION AND ACCURACY: The precision of the method was demonstrated by intraday variation studies. In the intraday studies, six repeated injections of standard and sample solutions were made and the response factor of drug peaks and percentage RSD were calculated (Table.2). The accuracy of the method was determined by recovery experiments. The recovery studies were carried out six times and the percentage recovery and standard deviation of the percentage recovery were calculated and presented in (Table.3).

ANALYSIS OF PHARMACEUTICAL PREPARATIONS: Commercially available nilutamide tablets were subjected to analysis by the proposed method along with a reference method. The comparison of the results obtained with the proposed method and reference method[5] for dosage forms of nilutamide tabulated in Table.4 confirmed the suitability of the proposed method for commercial pharmaceutical formulations.

PROPOSED REACTION MECHANISM: The reaction of the gem trifluromethyl compound (nilutamide) in alkaline medium results in the opening of the pyridine ring and subsequent reaction with benzidine in formic acid medium resulted in the development of red color species (Figure.4).

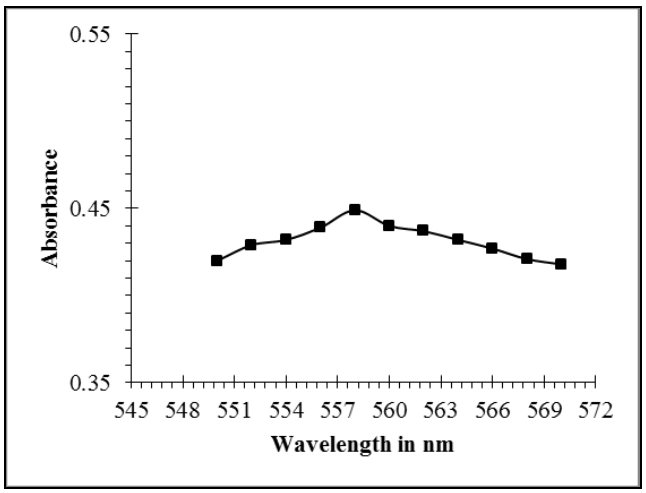

Figure.2.ABSORPTION SPECTRA OF NILUTAMIDE

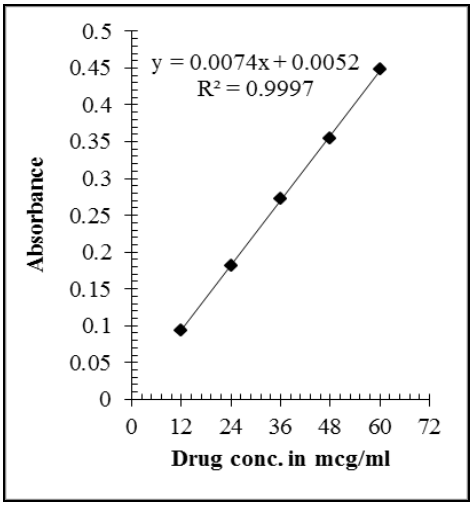

Figure.3.BEER'S LAW PLOT OF NILUTAMIDE

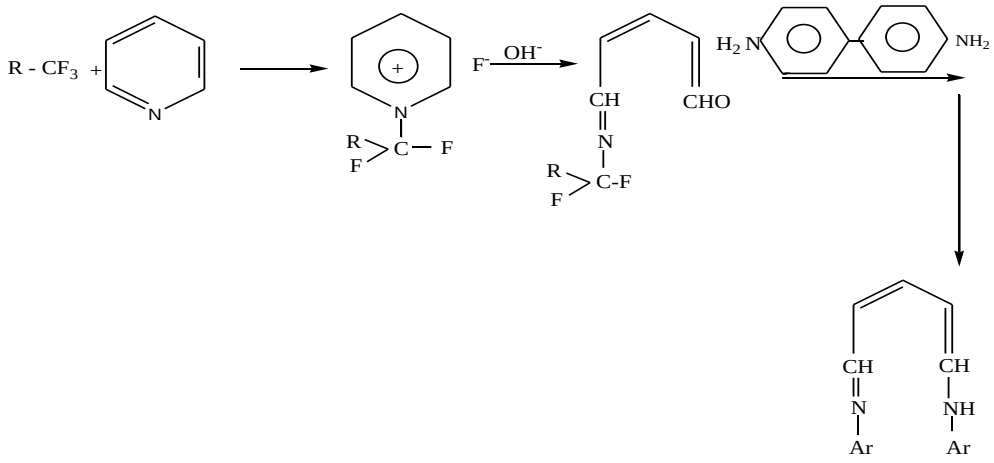

Figure.4.Reaction scheme of Nilutamide

CONCLUSIONS

The statistical parameters obtained from the proposed method developed by the author for determination of nilutamide in pure and market formulations indicated that the proposed method is simple, accurate, fast, precise and economical. The proposed method exhibited acceptable linearity that utilized a single step reaction and simple organic reagents. The proposed colorimetric method can be used as general method in the determination of nilutamide in pure and in market formulations since, there is no interference from the common excipients that might be found in market formulations.

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