IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
January 2015 Vol.:2, Issue:2

Two New UV-Visible Spectrophotometric Methods for the Determination of Nilutamide in Pure and their Tablets using 2,3-Dichloro-5,6-Dicyano-1,4-Benzoquinone (DDQ) and Para Chloranilic Acid (PCA)

P. S. Sarat[1] and D. Ramachandran*

*[1,]*Department of Chemistry, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India*

Submission: 2 January 2015
Accepted: 15 January 2015
Published: 25 January 2015

www.ijppr.humanjournals.com

Keywords: Nilutamide, UV-visible spectrophotometry and ICH guidelines

ABSTRACT

Two new simple, economical UV-visible spectrophotometric methods were developed for the assay of nilutamide in bulk drug and in tablet dosage form based on the formation of charge-transfer complexes between 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in Method –I and chloranilic acid (PCA) in Method –II and the studied drug (nilutamide). The optimum conditions for the analysis of the drug were established and the wavelength maxima (λ_{max}) for nilutamide in Method - I and Method - II was found to be 560 and 524 nm respectively. The linearity for Method - I and Method - II was found to be in the range of 10.0-50.0 and 8.0-40.0µg/mL respectively. The limit of detection for Method - I and method-II were found to be 0.0175 and 0.0180 respectively. The regression of the curve for method I was Y=0.0109x+0.0042 with coefficient of correlation 0.9997and Y=0.0111x +0.0052 for Method-II with coefficient of correlation 0.9997 respectively. The %RSD results for precision studies for Method - I and Method - II was found to be 0.479 and 0.761 and the percentage recovery for both methods (Method - I & Method - II) was found to be 99.94 and 99.80 respectively. The proposed methods could be effectively applied for the analysis of nilutamide in tablet pharmaceutical formulation for routine analysis with good precision and accuracy.

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. Nilutamide competes with androgen for the binding of androgen receptors, 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 through inhibition of androgen-dependent DNA and protein synthesis. It is sold in the local pharmacy as nilutamide tablets (Zydus Cadila Healthcare Ltd) each tablet contains 150mg of nilutamide.

Till to date no UV-Visible spectrophotometric methods have been yet reported in the literature for its determination in pharmaceutical dosage forms and this fact prompted the author to develop accurate and inexpensive UV-Visible spectrophotometric methods for routine determination of the nilutamide in pure and tablet dosage forms. The present paper describes two new UV-Visible spectrophotometric methods, which are based on reactivity of the secondary amine group of nilutamide with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in Method –I and chloranilic acid (PCA)in Method –II to produce colored species of reasonable stability.

EXPERIMENTAL

i. Instrumentation

a) 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.

b) Precision Balance

0.001g Readability and repeatability, 200g capacity analytical balance was used to weigh the required amount of the drug and the reagents.

ii. Chemical and reagents

Pure sample of nilutamide drug was obtained from Kekule Pharma Limited, Hyderabad, Telangana 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.

Method – I; DDQ solution (Sd-Fine, 0.2% w/v)

Prepared freshly just before use by dissolving 200mg of DDQ in 100mL of acetonitrile.

Method –II; PCA solution (Merck, 0.1%W/V)

Prepared by dissolving 100mg of PCA in 100mL of acetonitrile and kept in the dark when not in use.

iii. 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 solutions of concentrations of 200µg/mL for the Method-I and 160µg/mL for the Method -II respectively.

iv. Procedure for market formulations

About ten tablets of **nilutamide** [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 procedures described below respectively.

RESULTS AND DISCUSSIONS

I. METHOD DEVELOPMENT

Effective method development ensures that the laboratory resources and the reaction conditions were optimized. In this present section the optimum conditions for the color development of proposed methods were established by varying one parameter, keeping the

others at a time fixed and observing the effect produced on the absorbance of the colored species. In this accord, the following experiments were conducted and the conditions so obtained were incorporated in recommended procedures.

Method-I & II

In these two methods the optimum conditions were fixed basing on the study of effects of various parameters, such as volume of DDQ&PCA solution, volume of solvents used initially and subsequently for dilution and the stability of colored species after final dilution.

In establishing optimum conditions for the rapid formation of the stable charge transfer complexes, nilutamide was allowed to react with different volumes of the reagents (0.5-3.0mL of 0.2%DDQ or 0.5-3.0mL of 0.1% PCA). In both the cases, maximum absorbance values were obtained for sample only when 2.0mL of the reagent was used. At higher volumes of the above said reagents the greater absorbance for blank and lower absorbance for color complexes were observed. Finally 2.0mL of reagent (DDQ/PCA) was fixed and was used throughout the investigation. Acetonitrile was chosen as solvent in this assays as highly intense colored products were formed with respect to dichloromethane, acetone, 1,2-dichloroethane, 1,4-dioxane, methanol or ethanol as the complexes formed with these solvents gave very low absorbance values. In both the methods the formation of charge transfer complexation reaction was instantaneous and the absorbance values of nilutamide-DDQ and nilutamide-PCA complexes were stable for at least 12hrs, respectively **(Table.1&2)**.

II. RECOMMENDED PROCEDURES

Method-I; DDQ

Aliquots (0.5 -2.5mL) of a standard nilutamide (200µg/mL) solution were accurately transferred into a series of 10.0mL calibrated test tubes and the total volume was adjusted to 3.0mL by adding adequate quantity of acetonitrile to each tube. Then 2.0mL of 0.2% DDQ solution was added to each tube and the mixture was diluted to the volume with acetonitrile. After mixing the absorbance of the colored solution in each calibrated tube was measured at 560nm against a reagent blank (**Fig.4**).

Method-II; PCA

Different aliquots of standard nilutamide solutions (0.5 - 2.5mL;160µg/.mL) were accurately transferred into a series of 10.0mL calibrated tubes and the total volume was adjusted to 3.0mL by adding adequate quantity of acetonitrile. To each tube 2.0mL of 0.1% p-chloranilic acid was added, and the contents were mixed well and kept aside for 10min. The mixture was diluted to the volume with acetonitrile and the absorbance of the colored complex developed in each tube was measured at 524nm against a reagent blank prepared simultaneously. The concentration of the nilutamide was read from the standard graph using the Beer's law data (**Fig.5**).

III. METHOD VALIADTION

The developed procedures were fully validated according to International Conference on Harmonization (ICH) guidelines.

a) Spectral & Optical characteristics

The absorption spectra's developed for each proposed methods (Method - I and Method – II) were scanned on a spectrophotometer in the wavelength region of 340 to 900nm against similar reagent blank or distilled water. The results were graphically represented in **Fig.2 &3.** The Beer's law plots of these systems were recorded graphically (**Figs.4&5**). Regression analysis of the Beer's law data using the method of least squares was made and the slope (b), intercept (a) and correlation coefficient (r) for both proposed methods was calculated and the values are presented in **Table.3**. The optical characteristics such as Beer's law limits, molar absorptivity and Sandell's sensitivity values for the proposed methods were also given in **Table.3**.

b) Precision

The precision of proposed methods was ascertained from the absorbance values obtained by actual determination of six replicates of a fixed amount of nilutamide in total solution. The percent relative standard deviation and percent range of error (at 0.05 and 0.01 confidence limits) were calculated for the proposed methods (**Table.3**).

c) Accuracy

To determine the accuracy of each proposed method, different amounts of bulk samples of nilutamide within the Beer's law limits were taken and analyzed by the proposed methods. The results (percent error) are recorded in (**Table.4**).

d) Selectivity

The selectivity of the proposed method was checked by monitoring the drugs standard solutions in the presence of other ingredients which present in their tablets and the results obtained revealed that the response was not significant different from that results that obtained in case of pure drug in calibration curve.

e) Analysis of formulations

Commercial formulations (tablets) containing nilutamide were successfully analyzed by the proposed methods. The values obtained by the proposed and reference method [5] for formulations were compared statistically with F and t tests and found not to different significantly. The results F and t tests and recovery experiments for the proposed methods are summarized in **Table.5.**

f) Nature of the colored species

The mechanism of the reaction is based on the formation of donor-acceptor (DA) complex through the interaction between secondary amine moiety of the selected drug [nilutamide] acting as electron donor with DDQ (in Method-I) PCA (in Method-II) as electron acceptor. The mechanism of reaction is represented in **Figure.6**

CONCLUSION

Two simple, rapid and accurate UV-visible spectrophotometric methods was developed for the estimation of nilutamide using DDQ and PCA. The wavelength selected for the present analysis of nilutamide was 560 nm for DDQ and 524nm for PCA respectively. The spectra's and calibration curves obtained for nilutamide in the range of 10-50 for DDQ and 8.0-40µg/mL for PCA respectively. The recovery studies were also carried out to ensure the reproducibility and repeatability of the method of adding known amount of standard drug solution. In contrast, the proposed methods described in this paper were free from rigid experimental conditions and is characterized by simplicity, reasonable sensitivity, cost-effectiveness, and use of easily available

chemicals when compared to the existing spectrophotometric methods. Hence, it is concluded that the two developed UV-visible specrtophotometric methods can be effectively used for the routine analysis of nilutamide in bulk drug and tablet dosage form.

ACKNOWLEDGEMENT

The authors are grateful to the Kekule Pharma Limited, Hyderabad, Telanga state, India for gift samples nilutamide; Director, Bio Lee Life sciences Pvt Ltd, Hyderabad and Chemistry Dept. Acharya Nagarjuna University, Guntur (AP), India for providing necessary laboratory facility for this research work.

REFERENCES

1. Kassouf W, Tanguay S, Aprikian AG. Nilutamide as second line hormone therapy for prostate cancer after androgen ablation fails. J. Urol. 2003;169(5):1742-4.
2. Moguilewsky M, Bertagna C, Hucher M. Pharmacological and clinical studies of the antiandrogen Anandron (nilutamide). J Steroid Biochem.1987; (4-6):871-5.
3. Hsieh AC, Ryan CJ. Novel concepts in androgen receptor blockade. Cancer J. 2008;14(1):11-14.
4. Pendyala, L.; Creaven, P.J.; Huben, R.; Tremblay, D.; Bertagna, C. Pharmacokinetics of Anandron in patients with advanced carcinoma of the prostate, Cancer Chemother.Pharmacol., 1988, 22, 69–76.
5. George Lunn, HPLC methods for recently approved pharmaceuticals; Drug Analysis I, John Wiley & Sons, Inc., Hoboken, New Jersey,2005,448.

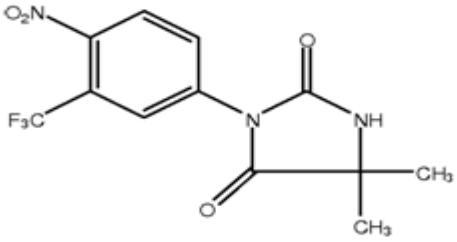

Figure.1.Molecular structure of Nilutamide

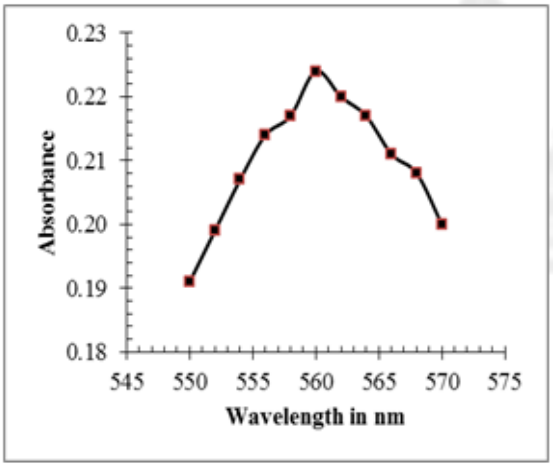

Figure.2. Absorption spectra of nilutamide with DDQ

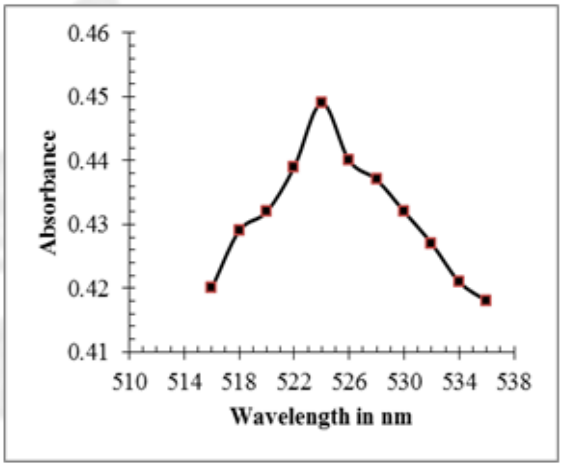

Figure.3. Absorption spectra of nilutamide with PCA

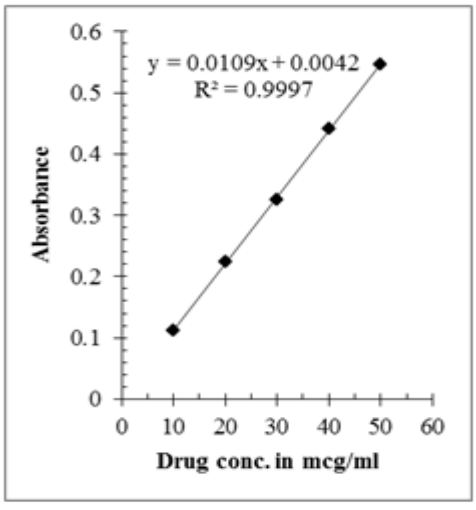

Figure.4.Beers law plot of nilutamide with DDQ

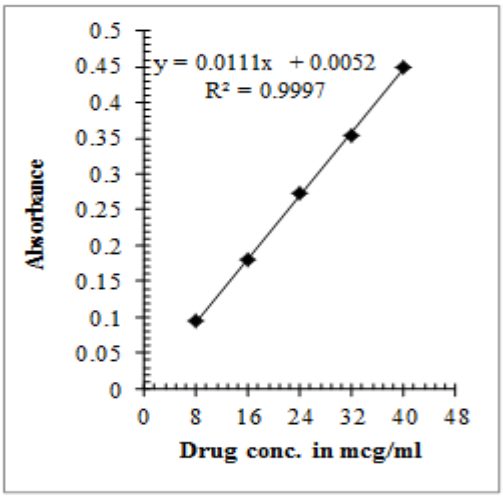

Figure.5.Beers law plot of nilutamide with PCA

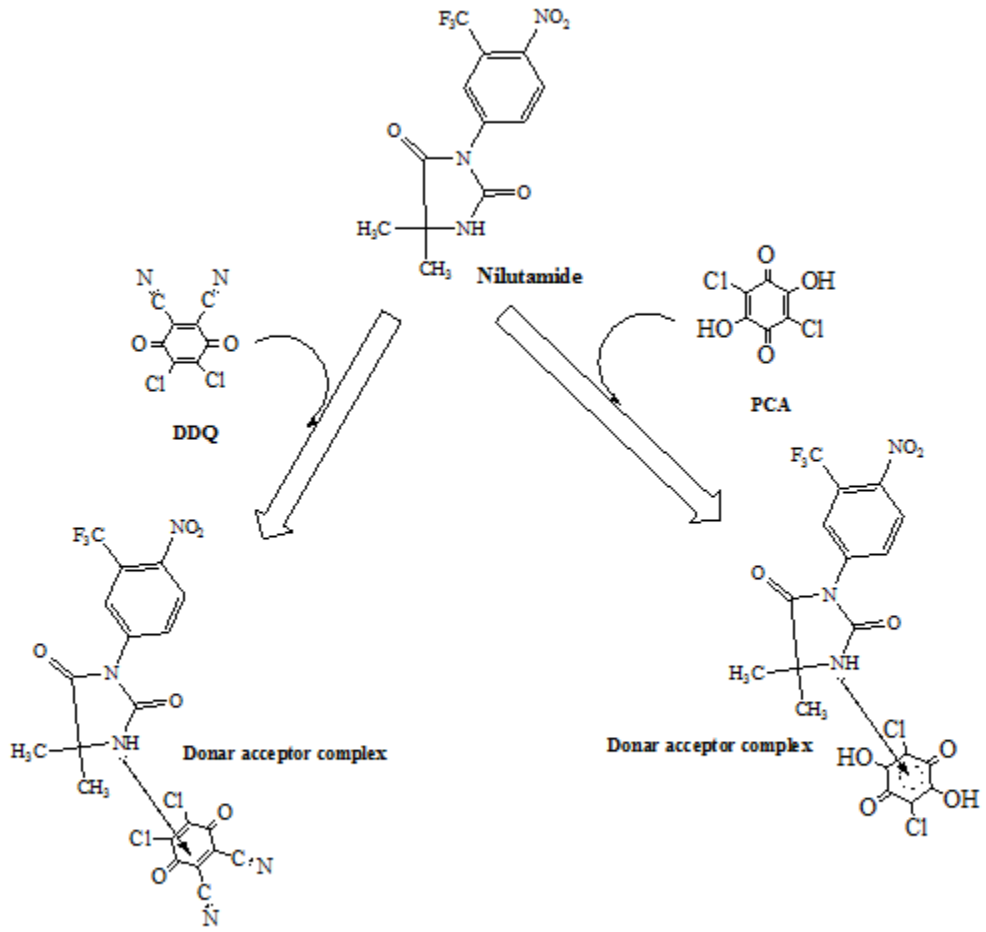

Figure.6.Reaction scheme of Nilutamide with DDQ (Method-I) and PCA(Method-II)

Table.1: Optimum conditions established in DDQ method

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	550 - 570	560	
Effect of volume of DDQ in CH_3CN.	1.0 - 1.5 mL	2.0 mL	Beyond upper and lower limits, the absorbance values were not constant and beyond 2.5mL leads to higher blank values
Solvent for final dilution.	-----	Acetonitrile	Acetonitrile has been found to be suitable for final dilution to give better absorbance values.
Stability of the colored species after final dilution.	12hrs	----	The intensity of the colored product begins to decrease slowly after 12hrs.

Table.2: Optimum conditions established in PCA method

Parameter	Optimum range	Conditions in procedure	Remarks
λ_{max} (nm)	490 - 540	524 nm	
Effect of volume of PCA reagent	1.5 - 2.5mL	2.0mL	Beyond 2.0mL blank absorbance values increased.
Solvent for final dilution		Acetonitrile	The absorbance of the test solution decreased when the solvents like methanol, ethanol, propanol, isopropanol or acetonitrile were used for final dilution.
Stability of colored species after final dilution	12hrs	----	After 12hrs, the intensity of the colored product was found to decrease slowly with time.

Table.3. Results of the data analysis for the quantitative determination of nilutamide by the proposed methods

Parameter	DDQ	PCA
λ_{max} (nm)	560	524
Beer's law limits (µg/mL)	10.0 - 50.0	8.0 - 40.0
Molar absorptivity (1 $mol^{-1}.cm^{-1}$)	2.690×10^3	2.017×10^3
Sandell's sensitivity (µg.cm^{-2}/0.001 AU)	0.0282	0.0184
Optimum photometric range (µg/mL)	11.0-48.6	9.5 - 36.0
Regression equation (Y=a+bc) ;slope (b)	0.0109	0.0111
Intercept (a)	0.0042	0.0052
Correlation coefficient (r)	0.9997	0.9997
Relative standard deviation (%)*	0.479	0.761
% Range of error (confidence limits)		
0.05 level	0.401	0.636
0.01 level	0.593	0.942

* Average of six determinations considered

Table.4: Results of (accuracy studies) of nilutamide by the proposed visible spectrophotometric methods

Proposed methods	Nilutamide in tablet µg.mL^{-1}	Pure Nilutamide added µg.mL^{-1}	Total found µg.mL^{-1}	Pure Nilutamide recovered %±SD*
Method - I	30.0	5.0	34.98	99.94
Method - II	24.0	5.0	29.94	99.80

Table.5: Assay and recovery of nilutamide in dosage forms

Method	Pharmaceutical Formulation	Labeled Amount (mg)	Proposed Method			Found by reference method3±S.D	%Recovery by proposed methods*
			Amount found** (mg) ±S.D	t (value)	F (Value)		
Method -I	**NILTAMIDE**	150	149.95±0.10	0.131	2.56	149.96±0.16	99.99
Method - II			149.93±0.08	0.816	1.67		99.97

*Average ± standard deviation of six determinations the t and F- values refer to comparison of the proposed method. Theoretical values at 95 % confidence limits t = 2.365 and F = 4.88.
** Average of six determinations.