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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATION OF ABIRATERONE ACETATE IN PHARMACEUTICAL FORMULATION BY UV AND RP-HPLC

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ABSTRACT

A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the determination Abiraterone Acetate in pharmaceutical dosage form. The column used was Hypersil ODS C18 (4.6 x 150mm, 5 µm,) in isocratic mode, with mobile phase containing phosphate buffer adjusted the pH-3.0 with orthophosphoric acid and Acetonitrile ratio of (50%:50%) the flow rate was 1.0 mL/ min and eluents was monitored at 254nm. The retention time Abiraterone was 4.858 min. The linearity for Abiraterone was in the range of 10-30 µg/ml respectively. The recovery of Abiraterone was found to be 99.5%, respectively. The proposed method was validated and successfully applied to the estimation of Abiraterone in tablet dosage form. Mass and FTIR analysis performed to determine Abiraterone Acetate in pharmaceutical dosage form.

KEYWORDS: **Abiraterone Acetate, RP-HPLC, Validation**

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INTRODUCTION

Abiraterone is a derivative of steroidal progesterone and is an innovative drug that offers clinical benefit to patients with hormone refractory prostate cancer. Abiraterone is administered as an acetate salt prodrug because it has a higher bioavailability and less susceptible to hydrolysis than Abiraterone itself. Abiraterone is a drug used in combination with prednisone in metastatic castration-resistant prostate cancer (formerly hormone-resistant or hormone-refractory prostate cancer) i.e., prostate cancer not responding to androgen deprivation or treatment with antiandrogens. It is formulated as the prodrugAbiraterone acetate and marketed under the trade name Zytiga. Intas Pharmaceuticals has recently started marketing Abiraterone acetate under the trade name Abiratas, Cadila Pharmaceuticals has recently started marketing Abiraterone acetate under the trade name Abretone & by Glenmark Pharmaceuticals as Abirapro. After an expedited six-month review, Abiraterone was approved by the U.S. Food and Drug Administration (FDA) in April 2011.[1,2] In Phase III trials, it extended median survival to 14.8 months versus 10.9 months placebo, and the trial was stopped because of the successful outcome. It is indicated for use in combination with prednisone as a treatment for metastatic castration-resistant prostate cancer. It has received FDA (28 April 2011), EMA (23 September 2011), MHRA (5 September 2011) and TGA (1 March 2012) approval for this indication. [3,4,5,6] In Australia it is covered by the Pharmaceutical Benefits Scheme when being used to treat castration-resistant prostate cancer and given in combination with prednisone/prednisolone (subject to the conditions that the patient is not currently receiving chemotherapy, is either resistant or intolerant of docetaxel, has a WHO performance status of <2, and his disease has not since become progressive since treatment with PBS-subsidised Abiraterone has commenced).[7] Abiraterone, the active metabolite of abiraterone acetate, inhibits CYP17A1, which manifests as two enzymes, 17α-hydroxylase (IC50 = 2.5 nM) and 17,20-lyase (IC50 = 15 nm) (six-fold more selective for inhibition of 17α-hydroxylase over 17,20-lyase)[9], that are expressed in testicular, adrenal, and prostatic tumor tissues. CYP17 catalyzes two sequential reactions: (a) the conversion of pregnenolone and progesterone to their 17α-hydroxy derivatives by its 17α-hydroxylase activity, and (b) the subsequent formation of dehydroepiandrosterone (DHEA) and androstenedione, respectively, by its 17,20-lyase activity[10].DHEA and androstenedione are androgens and precursors of testosterone. Inhibition of CYP17 activity by abiraterone thus decreases circulating levels of androgens such as DHEA, testosterone, and dihydrotestosterone (DHT). Abiraterone also acts as an antagonist of the androgen receptor (AR) (to some extent) and as an inhibitor of the enzymes 3β-hydroxysteroid dehydrogenase, CYP11B1 (steroid 11β-hydroxylase), and other CYP450s (e.g., CYP1A2, CYP2C9, and CYP3A4).[8,11] Abiraterone acetate is able to reduce serum testosterone levels to less than 1 ng/dL (i.e., undetectable)[9], and decreases the weights of the prostate gland, seminal vesicles, and testes, in accordance with its antiandrogen action.[12] Literature survey revealed that only a few analytical methods such as liquid chromatography-high performance liquid chromatography (HPLC) method have been reported.[13] Hence, a new sensitive and efficient HPLC method was developed and validated for the assay of the drug in injection. The structure of Abiraterone is shown in (Fig 1).

MATERIALS AND METHODS

A Waters HPLC system consisting of a Water 2695 binary gradient pump, an inbuilt auto sampler, a column oven and Waters 2996 PDA detector was employed throughout the analysis. The data was acquired using Empower 2 software.and The column used was Hypersil ODS C18 (4.6 x 150mm, 5 μm,) Double beam Uv-visoble spectroscopy (Make:Labindia 3000) .sonerex sonicator was used for enhancing dissolution of the compounds. An Adwa digital pH meter was used for pH adjustment. Analytically pure Abiraterone was obtained as gift samples from Nishka labs, Hyderabad. Acetonitrile, methanol, water (E. Merck, Mumbai, India) were of HPLC grade, while ortho-phosphoric acid and potassium dihydrogen phosphate (S. D. Fine Chemicals, Mumbai, India) were of Analytical grade used for the preparation of mobile phase

(i) Preparation of mobile phase and stock solutions

Potassium dihydrogen phosphate was weighed (6.8 g) and dissolved in 1000 ml of water. Finally the pH was adjusted to 3.0 with ortho phosphoric acid. The solution was sonicated for 10 minutes and filtered using Whatman filter paper (No.1). For the estimation of Abiraterone from the injection, twenty tablets were taken and their contents were mixed thoroughly. Average weight was calculated. Tablet content or the powder equivalent to 100mg was weighed accurately and transferred into a 100ml volumetric flask, dissolved and dilute up to mark with diluent (1000 ppm). Mix well and filter through 0.45μm filter.

(ii) Chromatographic conditions

A reverse phase C18 column equilibrated with mobile phase phosphate buffer-methanol adjusted to pH 3.0 was used. Mobile phase flow rate was maintained at 1.0 mL/min and eluents was monitored at 254 nm. The sample was injected using a 20 μL fixed loop, and the total run time was 8.0 min. Appropriate aliquot of Abiraterone stock solutions was taken in different 10 ml volumetric flasks and diluted up to the mark with mobile phase to obtain final concentrations of 10, 15,20,25,30 μg/mL of Abiraterone The solution was injected using a 20 μl fixed loop system and chromatograms were recorded. Calibration curve was constructed by plotting average peak area versus concentrations and regression equation was calculated for Abiraterone

(iii)Uv –Vis Spectroscopy Conditions

The sample was prepared using mobile phase as a diluent and inserted into UV-Vis spectroscopy. Then record the spectrum. Its monitored at 254 nm. Appropriate aliquot of Abiraterone stock solutions was taken in different 10 ml volumetric flasks and diluted up

to the mark with mobile phase to obtain final concentrations of 10, 15,20,25,30 µg/mL of Abiraterone. Calibration curve was constructed by plotting average peak area versus concentrations and regression equation was calculated for Abiraterone

(iv) Determination of Abiraterone dosage form

For the estimation of Abiraterone from the injection, five injections were taken and their contents were mixed thoroughly. Average weight was calculated. Sample equivalent to 100mg was weighed accurately and transferred into a 100ml volumetric flask, dissolved and dilute up to mark with diluent. Take above solution 0.2 ml in 10 ml volumetric flask dilute up to mark with diluents (20ppm). Mix well and filter through 0.45µm filter. The solution was injected at above chromatographic conditions and peak areas were measured. The quantification was carried out by keeping these values to the straight line equation of calibration curve. The method was validated for accuracy, precision, specificity, and robustness. for both hplc and uv.

(v) Accuracy

The accuracy of the method was determined by calculating recovery of Abiraterone by the spiked method. Known amount of Abiraterone was added to a pre quantified sample solution, and the amount of Abiraterone was estimated by measuring the peak areas and by fitting these values to the straight-line equation of calibration curve.

(vi)Precision

The intraday and inter day precision study of Abiraterone was carried out by estimating the corresponding responses 5 times on the same day and on different days. The results were reported in terms of relative standard deviation. The Repeatability studies were carried out by estimating response of 5 different concentrations of Abiraterone and results are reported in terms of relative standard deviation (%RSD).

(vii) Specificity

Commonly used excipients were spiked into a pre weighed quantity of drugs. The chromatogram was taken by appropriate dilutions and the quantities of drugs were determined.

(viii) Robustness

Robustness of the method was studied by changing change in the chromatographic parameters: Effect of Variation in column oven temperature.± % 10 and the flow 0.9 and 1.1 ml/min instead of 1.0 ml/min.

RESULTS AND DISCUSSION

Optimization of the mobile phase was performed based on asymmetric factor and peak area obtained forAbiraterone. The mobile phase phosphate buffer-methanol adjusted to pH 3.0 using ortho phosphoric acid was found to be satisfactory and gave symmetric peak for Abiraterone the retention time for Abiraterone was 4.858 min respectively (Fig 2).and spectrum of Abiraterone shown in (Fig 3)

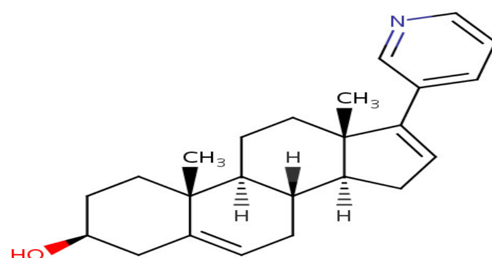

Figure 1
Structure of Abiraterone

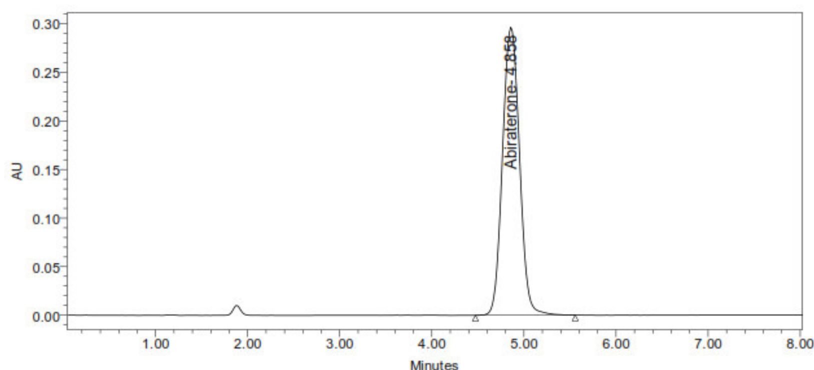

Figure 2
HPLC chromatogram of Abiraterone in optimized chromatographic conditions

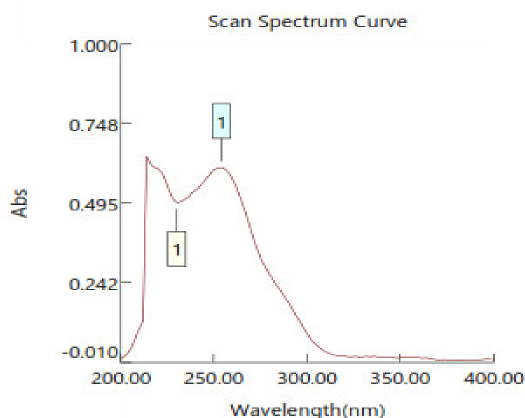

Figure 3
UV Spectrum of Abiraterone in optimized conditions

The calibration curve for Abiraterone was obtained by plotting the peak area of Abiraterone versus the concentration of Abiraterone over the range of 5-25 µg/mL, and it was found to be linear with r2 = 0.999. The validation parameters are summarized in (Table-1). The recovery Abiraterone was found to be 99.5% respectively. The system suitability test parameters are shown in (Table-1). The liquid chromatographic method was applied to the determination of Abiraterone in dosage form. The results for Abiraterone were comparable with the corresponding labeled amount.

Table 1
Validation parameters and data for proposed methods

Validation parameter	Results	
	(HPLC)	*(UV-VIS)*
Linearity	5-25 µg/mL	5-25 µg/mL
Regression coefficient (r^2)	0.999	0.999
*Accuracy (% recovery)	99.80%	99.20%
** System Precision (%RSD)	1.16	1.57
** Method precision(%RSD)	1.39	0.98
** Rugudness (%RSD)	0.97	1.54
Assay value (%)	99.80%	99.3
System suitability parameter		
Tailing factor	1.7	
Number of theoretical plates	2507	

***** Replicates of three concentration levels (in three determinations);***
***** Five repetitive injections of same homogeneous sample***

CONCLUSION

Proposed study describes a new UV and RP-HPLC method for the estimation of Abiraterone using simple mobile phase with low buffer concentration compared to the reported method. The method gives short analysis time (<5 min). The method was validated and found to be simple, sensitive, accurate and precise in both UV and HPLC analytical methods. Percentage of recovery shows that the method is free from interference of the excipients used in the formulation. Therefore, the proposed method can be used for routine analysis of Abiraterone in dosage form.

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CONFLICT OF INTEREST

Conflict of Interest: Conflict of interest declared none.

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