Development and Validation of RP-HPLC Method For Quantitative Analysis Of Abiraterone In Pure and Pharmaceutical Dosage Form

P. Jitendra Kumar*1 Syed.Rafiya, Tezenile .Magh, T.Venkata Lakshmi, T.Vakya Mani, D.Rama Brahma Reddy1
Nalanda Institute OF Pharmaceutical Sciences, Siddharth Nagar, Kantepudi (V), Sattenapalli (M), Guntur District – 522438, Andhra Pradesh

ABSTRACT

A simple, Precised, Accurate method was developed for the estimation of Abiraterone by RP-HPLC technique. Chromatographic conditions used are stationary phase Azilent C18 (150mm x 4.6 mm, 5μ)Mobile phase 0.01%KH2PO4:Acetonitrile in the ratio of 60:40 and flow rate was maintained at 1.0 ml/min, detection wave length was 235 nm, column temperature was set to 30°C and diluent was mobile phase Conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 25% to150 % levels, R² value was found to be as 0.999. Precision was found to be 0.7 for repeatability and 0.2for intermediate precision. LOD and LOQ are 1.629μg/ml and 4.937μg/ml respectively. By using above method assay of marketed formulation was carried out 100.81% was present. Degradation studies of Abiraterone were done, in all conditions purity threshold was more than purity angle and within the acceptable range .Full length method was not performed ; if it is done this method can be used for routine analysis of Abiraterone

Keywords: HPLC , Abiraterone , Method development , ICH Guidelines
INTRODUCTION

Abiraterone is a derivative of steroid progesterone approved by the FDA for the treatment of prostate cancer. [1] The approval was accelerated based on progression-free survival, therefore confirmatory trials by the sponsor to demonstrate clinical efficacy in prostate cancer treatment are in progress of being conducted.[2-4] Abiraterone is marketed by under the brand name Zytiga. After an expedited six-month review, Abiraterone was approved by the U.S. Food and Drug Administration (FDA) in April 2011. It has received FDA (28 APRIL 2011), EMA (23 September 2011), MHRA (5 September 2011) and TGA 1(1 March 2012) approval for this indication. In the present study, a new RP-HPLC method was developed which shown high reproducibility and sensitivity. The developed method was validated as per ICH guidelines.[5-11]

MATERIALS AND METHOD

Standards and Chemical Used

Abiraterone was gift sample for Noverties company, Hyderabad. All the chemicals Acetonitrile HPLC Grade, HPLC grade Water.

Instrumentation

HPLC instrument used was of WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Software used is Empower. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was be used for measuring absorbance for Abiraterone solutions.

Preparation of Mobile phase:

Into a 1000ml cleaned volumetric flask, HPLC grade, acetonitrile 500ml and Potassium dihydrogen phosphate 500ml (0.01N) which are filtered through 0.25mm membrane filters by vacuum filtration were slowly added, mixed well and sonicated upto 20min. Cool the above solution. This solution is again sonicated to 10min. Cool the solution to room temperature and use for chromatography method.

Preparation of Standard stock solutions:

Accurately weighed 50mg of Abiraterone transferred 50ml of volumetric flask, and 3/4 th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution (1000µg/ml of Abiraterone).

Preparation of Standard working solutions (100% solution):

1ml of Abiraterone each stock solution was pipette out and taken into a 10ml volumetric flask and made up with diluents. (100µg/ml of Abiraterone)
Preparation of Sample stock solutions:
5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filter.(2500 µg/ml of Abiraterone)

Preparation of Sample working solutions (100% solution):
0.4ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. 100µg/ml of Abiraterone)

RP-HPLC METHOD DEVELOPMENT
Based on nature and solubility characteristics of Abiraterone, reverse phase mode of HPLC was selected for chromatography. Among different RP-HPLC stationary phases tried, C18 column was found to be optimum.

In order to get sharp peak with base line separation from interfering peaks carried out a number of experiments by varying the composition of solvents and mobile phase flow rate. To have an ideal separation of the drug under isocratic conditions, mixtures of solvents like methanol, water and acetonitrile with or without different buffers in different combinations were tested as mobile phase. A mixture of 0.01N KH2PO4 : Acetonitrile (50:50) (v/v) was proved to be the most suitable of all the combinations, since the chromatographic peak obtained was better defined and resolved and almost free from tailing . This chromatographic conditions for the estimation of Abiraterone was discussed in table.

| Parameter       | Condition                                                |
|-----------------|----------------------------------------------------------|
| Mobile phase    | 0.01N KH2PO4: Acetonitrile (50:50) (V/V)                |
| Pump mode       | Isocratic                                               |
| Diluents        | Mobile phase                                            |
|                  | ODC C18 Column (150 x 4.6 mm, 5µ)                       |
| Column Temp     | 30°C                                                     |
| Wavelength      | 230nm                                                    |
| Injection Volume| 10µL                                                     |
| Flow rate       | 1.0 ml/min                                               |
| Run time        | 10min                                                    |

RESULTS AND DISCUSSION

Analysis of Formulation
The sample solution was injected and a chromatogram was recorded. The injections were repeated five times and the peak areas were recorded. The amount of drug present in the
pharmaceutical formulation was calculated using standard calibration curve (concentration in µg/ml was taken on X –axis and average peak area on Y –axis). A representative chromatogram has been given in Figure. 1

**Validation of The Proposed Method**

As an integral part of analytical method development is validation. The proposed method was validated as per ICH guidelines.

![Chromatogram](image)

**Figure 1: Chromatogram of Abiraterone**

**Linearity**

To demonstrate the linearity of assay method, inject 5 standard solutions with concentrations of about 25 ppm to 150ppm of Abiraterone. Plot a graph to concentration versus peak area. Slope obtained was y = 14912x + 5759 and Correlation Co-efficient was found to be 0.999 and Linearity plot was shown in table

| Linearity Level(%) | Concentration (ppm) | Peak Area       |
|--------------------|---------------------|-----------------|
| 0                  | 0                   | 0               |
| 25                 | 25                  | 375311          |
| 50                 | 50                  | 741258          |
| 75                 | 75                  | 1152591         |
| 100                | 100                 | 1498279         |
| 125                | 125                 | 1872841         |
| 150                | 150                 | 2228723         |
Precision:

**Repeatability:**
Six working sample solutions of 20ppm are injected and the % Amount found was calculated and %RSD was found to be 0.2

**Intermediate precision:**
Five working sample solutions of 20ppm are injected on the next day of the preparation of samples and the % Amount found was calculated and %RSD was found to be 0.3

**Ruggedness**
The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC 2965 SYSTEM, Azlient HPLC. By different operators using different columns of similar type like Hypersil C\(_{18}\) Hichron C\(_{18}\). It was observed that there were no marked changes in the chromatograms, which demon started that the RP-HPLC method developed, is ruggedness.

**Limit of Detection and Limit of Quantification**
A Calibration curve was prepared using concentrations in the range of 5-30 µg/ml (expected detection limit range). The standard deviation of Y-intercepts of regression line was determined and kept in following equation for the determination of Detection limit and Quantitation limit. The results were reported in table 3.

\[
\text{Limit of detection} = \frac{\sigma \times 3.3}{S}
\]

\[
\text{Limit of quantification} = \frac{\sigma \times 10}{S}
\]
Where,

\[ \sigma = \text{the standard deviation of the response.} \]

\[ S = \text{the slope of the calibration curve} \]

### Table 3. Limit of Detection and Limit of Quantification for Abiraterone

| Parameter                  | Values  |
|---------------------------|---------|
| Limit of Quantification   | 0.151 µg/ml |
| Limit of Detection        | 0.050 µg/ml |

**Accuracy:** The accuracy of the method was determined by standard addition method. A known amount of standard drug was added to the fixed amount of pre-analyzed sample solution. Three Concentrations of 50%, 100%, 150% are Injected in a triplicate manner and %Recovery was calculated as 100.15%. And chromatograms were shown in fig 3 Recovery ranging from 99.12 to 101.04% were obtained by the proposed method.

### Table 3 Accuracy data

| % Level | Amount Spiked (µg/mL) | Amount recovered (µg/mL) | % Recovery | Mean % Recovery |
|---------|-----------------------|--------------------------|------------|----------------|
| 50%     | 10                    | 10.104                   | 101.04     | 100.15%        |
|         | 10                    | 10.023                   | 100.23     |                |
|         | 10                    | 10.040                   | 100.40     |                |
| 100%    | 20                    | 20.181                   | 100.90     |                |
|         | 20                    | 20.057                   | 100.28     |                |
|         | 20                    | 20.005                   | 100.02     |                |
| 150%    | 30                    | 30.039                   | 100.13     |                |
|         | 30                    | 29.771                   | 99.24      |                |
|         | 30                    | 29.737                   | 99.12      |                |

**Robustness:** Small Deliberate change in the method is made like Flow minus, flow plus, Mobile phase minus, Mobile phase plus, Temperature minus, Temperature Plus. %RSD of the above conditions are calculated.

### Table 4 Robustness Data

| Parameter              | % RSD |
|------------------------|-------|
| Flow Minus             | 1.6   |
| Flow Plus              | 0.6   |
| Mobile phase Minus     | 0.9   |
| Mobile phase Plus      | 1.0   |
| Temperature minus      | 0.8   |
| Temperature plus       | 0.5   |

**System Suitability**
A Standard solution of Abiraterone working standard was prepared as per procedure and was injected five times into the HPLC system. The system suitability parameters were evaluated from standard Chromatograms obtained by calculating the % RSD of retention time, tailing factor, theoretical plates and peak areas from five replicate injections are within range and Results were shown in table 5

CONCLUSION

A convenient, rapid, accurate, precise RP-HPLC method has been developed for estimation of Abiraterone. The proposed method followed the ICH guidelines. The proposed method can be used for the routine analysis of Abiraterones in bulk preparations of the drug and in pharmaceutical dosage forms without interference of excipients.

REFERENCES

1. H. Beckett and J. B. Stenlake. “Practical Pharmaceutical Chemistry”, Volume I and II, CBS Publishers & Distributors, New Delhi, India, 2000.
2. G. Ramana Rao, S. S. N. Murthy and P. Khadgpathi. Gas chromatography to pharmaceutical analysis (Review). Eastern Pharmacist. 30(353): 35 (1987). S
3. Jala Chandra Reddy et al., Development and validation of a novel RP-HPLC method for stability-indicating assay of Abiraterone acetate, Journal of Liquid Chromatography & Related Technologies, 2016, 39(7): 354-363.
4. Gadhave r. V. et al., stability indicating rp-hplc-PDA method for determination of abiraterone acetate and characterization of its base catalyzed degradation product by lc-ms, International Journal of pharmacy and pharmaceutical sciences, 2016, 8( 2).
5. Mukthinuthalapati Mathrusri Annapurna et al., Stability Indicating RP-HPLC method for the determination of Abiraterone (An Anti-Cancer Drug), Research Journal of Pharmacy and Technology, 2018, 11(7): 3007-3012.
6. ARUN KUMAR KUNA et al., Analytical Method Development And Validation For The Estimation Of Abiraterone Acetate In Pharmaceutical Formulation By Uv And Rp-Hplc, International Journal Of Pharma And Bio Sciences, 2016, 7(2):57-61.
7. Mukthinuthalapati Mathrusri Annapurna et al., New Spectrophotometric Techniques for the determination of Abiraterone acetate (A Steroidal Inhibitor) in Tablets, Journal of Chemical and Pharmaceutical Sciences, 2017, 10 (2);768-770.
8. Anjaneyulu Reddy et al., A validated stability indicating RP-HPLC method development for anti-cancer drug enzalutamide in bulk and pharmaceuticals,
international journal of pharmaceutical sciences and drug research, 2019,11(3): 85-90.

9. Kuna AK et al., Analytical Method Development and Validation for the Estimation of Abiraterone and its Impurity in Pharmaceutical Formulation By RP-HPLC, Scholars Research Library, 2018, 10 [9]: 19-31.

10. S. Vijay Kumar et al., Validated RP-HPLC/UV method for the quantitation of Abiraterone in rat plasma and its application to a pharmacokinetic study in rats, Biomedical Chromatography, 2017, 35(2): 203-207.

11. Juana Rodriguez et al., A Rapid, Direct and Validated HPLC- Fluorescence Method for the Quantification of Abiraterone and Abiraterone Acetate in Urine and Serum Samples from Patients with Castration- Resistant Prostate Cancer, Current Pharmaceutical Analysis, 2018, 14(3): 233 – 238.