International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 8, Issue 2, 2016

Original Article

STABILITY INDICATING RP-HPLC-PDA METHOD FOR DETERMINATION OF ABIRATERONE ACETATE AND CHARACTERIZATION OF ITS BASE CATALYZED DEGRADATION PRODUCT BY LC-MS

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Received: 10 Jul 2015 Revised and Accepted: 12 Dec 2015

ABSTRACT

Objective: The present work describes stability indicating (SI) RP-HPLC-PDA method for determination of abiraterone acetate (ABA) and characterization of its base catalyzed degradation product by LC-MS.

Methods: The separation was achieved by using column Kromasil C_{18} (250 mm × 4.6 mm, 4.0 µ) using acetonitrile (ACN): ammonium acetate buffer 10 mM, pH adjusted to 3.5 with acetic acid (AA) in the ratio of 10:90 % v/v as eluent. The Mobile phase flow rate was 0.6 ml/min and data integration was achieved at 235 nm.

Results: The retention time of ABA was 5.4±0.01 min. Linearity was found to be in the range of 5–30 µg/ml. The limit of detection and quantitation were 0.25 µg/ml and 0.75 µg/ml respectively, and percentage recovery of ABA was found to be 99.52 to 100.13 %. Mass spectral data of base degraded product of ABA shows a prominent molecular ion peak at m/z-391.5. Major fragmentation leads to formation of 10–Methyl 2,3,4,7,8,9,10,11,12,13,14,15-dodecanhydro-1H cyclopenta (α)phenanthren-3-ol as a degradant (D_2) at m/z-257.81, due to corresponding loss of $C_8H_{12}ON$. All the analytical validation parameters were determined and found in the limit as per ICH guidelines.

Conclusion: The results of the various validation studies showed that the LC method is fast, specific, accurate, reproducible, possessed significant linearity and precision. The drug was found to be stable under all the stress conditions except basic stress. Thus developed method reported first time is novel with a very short run time of 6 min.

Keywords: Abiraterone acetate, HPLC, Stability indicating assay, Forced degradation, Validation, LC-MS.

INTRODUCTION

ABA is chemically (3B 17-(3-Pyridinyl) androsta-5, 16 dien-3yl acetate) (fig. 1), is an antineoplastic agent with an orally active inhibitor of the steroidal enzyme CYP17A1. CYP17A1 is an enzyme that catalyzes the biosynthesis of androgen and is highly expressed in adrenal, testicular and prostatic tumor tissue. USFDA approved this compound in dec 2012 and currently marketed for the treatment of patients with metastatic castration-resistant prostate cancer (CRPC), which is a serious and aggressive type of prostate cancers [1].

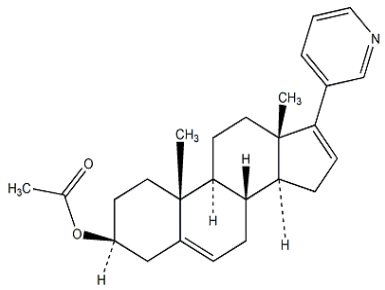


Fig. 1: Chemical structure of ABA

Review of literature for ABA reveals that there are few analytical methods reported for its determination. LC/MS/MS-ESI method is used for its determination in rat and human plasma [2]. But no article related to the stability indicating a chromatographic method for determination of the ABA by predicting possible degradation pathways has been reported. The International Conference on Harmonization (ICH) guideline entitled 'stability testing of new drug substances and products' requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance [3]. An ideal stability indicating method is one that quantifies the standard drug alone and also resolves its degradation products. Since HPLC is a routine analytical technique used due to its advantage of higher sensitivity, this technique was selected for stability indicating assay. The aim of the present work is to develop a fast, accurate, specific, and reproducible SI RP-HPLC-PDA method for determination of ABA in the presence of degradation products formed under different stress conditions and the characterization of base induced degradation product by LC-MS.

MATERIALS AND METHODS

Chemicals and reagents

Active Pharmaceutical ingredient (API) of ABA (purity 98.84 %) was received as gift sample from Sun Pharmaceuticals Ltd., Ahmednagar. The pharmaceutical tablet dosage form formulated in Laboratory was used in this study. HPLC Grade Methanol and ACN were supplied by Merck Specialties Pvt. Ltd., Mumbai. Analytical grade acetic acid (AA) was supplied by Labin Chemicals Pune. Analytical grade ammonium acetate was supplied by Analab Fine Chemicals Pvt. Ltd., Mumbai. Double distilled water prepared in the laboratory was used for the study.

Instrumentation and chromatography conditions

The chromatographic separation was performed on a Waters Binary gradient HPLC system, Japan, with the automatic injection facility was employed for this work. The detector consists of a Waters 2998 Photodiode Array Detector (PDA) and operated at 254 nm. The software used was Empower 2. The column used was Kromasil C_{18}

Column (250 mm × 4.6 mm, 4.0 µ). Chromatographic analysis was carried out at 40 °C temperature. The drug and degradation products were separated with a mobile phase consisting of ACN: ammonium acetate buffer 10 mM pH-3.5 (adjusted using AA). The mobile phase was sonicated and filtered through 25 mm, 0.45 µ nylon membrane filter. The flow rate was 0.6 ml/min, injection volume was 30 µl.

Preparation of standard and sample solution

A standard stock solution of ABA was prepared by dissolving and diluting 100 mg of the drug to 100 ml with ACN. Aliquots from this solution were suitably diluted with mobile phase to get the working standard solutions of ABA in the concentration range of 5–30 µg/ml with the mobile phase. For the preparation of sample solution, twenty tablets were weighed and finely powdered. An accurately weighed tablet powder according to label claim equivalent 100 mg was accurately weighed and transferred into a 100 ml volumetric flask containing 80 ml of ACN and sonicated for 20 min. This solution was further diluted to get final concentration15 µg/ml. The solution was filtered through 25 mm 0.45 µ nylon membrane filter. The resulting solution was injected into the column and peak area was recorded for quantitation.

Validation of RP-HPLC method

The method was validated using standard sample of ABA with the label claim of 250 mg by the determination of specificity, linearity and range, precision, accuracy, limit of detection, limit of quantification and robustness following ICH method validation guidelines [4].

System suitability

The system suitability test was performed to ensure that the complete testing system was suitable for the intended application. A standard solution containing 15 µg/ml of ABA was injected six times. The parameters measured were peak area, retention time, theoretical plates and tailing factor.

Linearity

Linearity test solutions were prepared by diluting a standard stock solution of ABA at concentration levels of 5-30 µg/ml. The solutions were injected six times and six curves were constructed. The slope and intercept were calculated.

Accuracy

Accuracy was carried out by selecting a drug concentration to 80, 100, and 120 % of label claim were present. At each level three determinations were carried out and the results obtained were compared with expected results.

Precision

The precision of the method was verified by intraday and intermediate precision studies. The intraday precision of the method was checked by analyzing test solution 10, 15 and 20 µg/ml of ABA which three times. The intermediate precision of the method was checked by repeating assay of 10, 15, 20 µg/ml of ABA three times in three sequential days. The intraday and intermediate precision of sample applications and measurement of peak area for the drug were expressed in terms of % relative standard deviation (% RSD).

Specificity

Specificity is the ability of the method to determine the analyte response in the presence of its potential impurity. The stability indicating the capability of the method was established from the peak purity of ABA in the degraded sample. Forced degradation studies of the drug substance can help to identify the likely degradation products which can assist to establish the degradation pathways and the stability of the molecule. ABA was degraded under different stress conditions like acidic, alkaline, oxidative hydrolysis, UV light, dry heat and neutral conditions [5].

a. Acid degradation studies

To 5 ml of stock solution, 5 ml of 1M hydrochloric acid was added and solution kept aside for 5 h. at 45 °C. The resultant solution was suitably diluted to obtain final conc. of 15µg/ml. 30µl solutions were injected into the system, and the chromatograms were recorded to assess the stability of the sample.

b. Base degradation studies

To 5 ml of stock solution, 5 ml 0.1 M sodium hydroxide was added and solution kept aside for 5 h. at 45 °C. The resultant solution was suitably diluted to obtain 15 µg/ml. 30 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample.

c. Peroxide degradation studies

To 5 ml of stock solution, 5 ml of 30 % hydrogen peroxide (H_2O_2) was added and the solutions were kept aside for 5 h. at 45 °C and resultant solution was suitably diluted to obtain 15 µg/ml. 30 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample.

d. Thermal degradation studies

The standard drug solution was placed in the oven at 45 °C for 24 h. To study dry heat degradation, the resultant solution was diluted to obtain final conc. 15 µg/ml. 30 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

e. Photostability studies

The photochemical stability of the drug was also studied by exposing the sample solution to UV Light for 12 h. For HPLC study, the resultant solution was suitably diluted to obtain final conc. 15 µg/ml. 30 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample

f. Neutral degradation studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 5 h. at 45 °C. For HPLC study, the resultant solution was suitably diluted to obtain 15 µg/ml. 30 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Robustness

The robustness of an analytical procedure refers to its ability to remain unaffected by small and deliberate variations in method parameter and provides an indication of its reliability for the routine analysis. The robustness of the method were determined by analyzing a sample by varying parameters such as column temperature (40±2 °C), flow-rate (0.6±0.1 ml/min), pH (3.5±0.2) and wavelength (235±2 nm).

LOD and LOQ

LOD and LOQ were calculated as per ICH guidelines as 3.3 σ/S and 10 σ/S, respectively, where σ is the standard deviation and S is the average of the slopes of the calibration plots.

Method stability

To confirm the stability of standard and tablet sample solutions during the analytical process, both solutions were analyzed over a period of 3 d at room temperature (25±0.5 °C).

LC-MS analysis

The Base induced degradation product was identified using LC-MS analysis. To characterize the base degradation products solution degraded under basic condition was subjected to LC-MS analysis. LC-MS studies were performed on thermo scientific LC-MS instrument using Agilent, USA (Model No 6460) ZORBAX eclipse plus C18 column [100 mm (L) x 4.6 mm (D), 2.7 µm]. The flow rate was 0.5 ml/min and detection was performed using Triple-Quadrupole ESI.

RESULTS AND DISCUSSION

In this work an analytical LC method was developed and validated for the determination of ABA in bulk drug. To prove the method was stability indicating the drug was assayed in the presence of its

degradation products obtained under different stress conditions. The base degraded product was characterized by mass spectral studies.

Method development

In initial trials, different combinations of ACN, methanol, phosphate and ammonium acetate buffer were tried. As part of method development trial 1 (fig. 2) (T_1) with mobile phase methanol: phosphate buffer (10 mM) (40:60 % v/v) pH-4.35, at flow rate 1 ml/min. Using column Kromasil C_{18} was observed. In this trial baseline disturbance was observed and the peak was broad with unacceptable tailing. Further changes in flow rate, pH, and mobile phase (MP) composition were carried out form observations of these trials it was concluded that the mobile phase needs to be changed. Therefore, trial 2 was carried out with changes in MP, phosphate buffer was replaced with acetate buffer. Trail 2 was carried out with MP ACN: ammonium acetate buffer (70:30 % v/v) pH-6.2, at a flow rate 1 ml/min on column Kromasil C_{18}. In T_2 tailing was observed and less plate count observed.

Therefore, changes in pH and flow rate were carried out. Finally the optimized condition for ABA mobile phase consisting of ACN: 10 mM Ammonium acetate buffer (10:90 v/v) pH-3.5 (adjusted using AA) at a flow rate 0.6 ml/min and injection volume is 30 µl gave a sharp peak of ABA and, in conclusion, this system was optimized. It was observed that the developed chromatographic condition provides better separation of ABA (5.43 min) and its degraded products, which also gave a symmetric peak of the compound. The run time of the method was 10 min. The % RSD value calculated for the peak area was 0.86 %, tailing factor was 1.23 and no. of theoretical plates was 3477 [6].

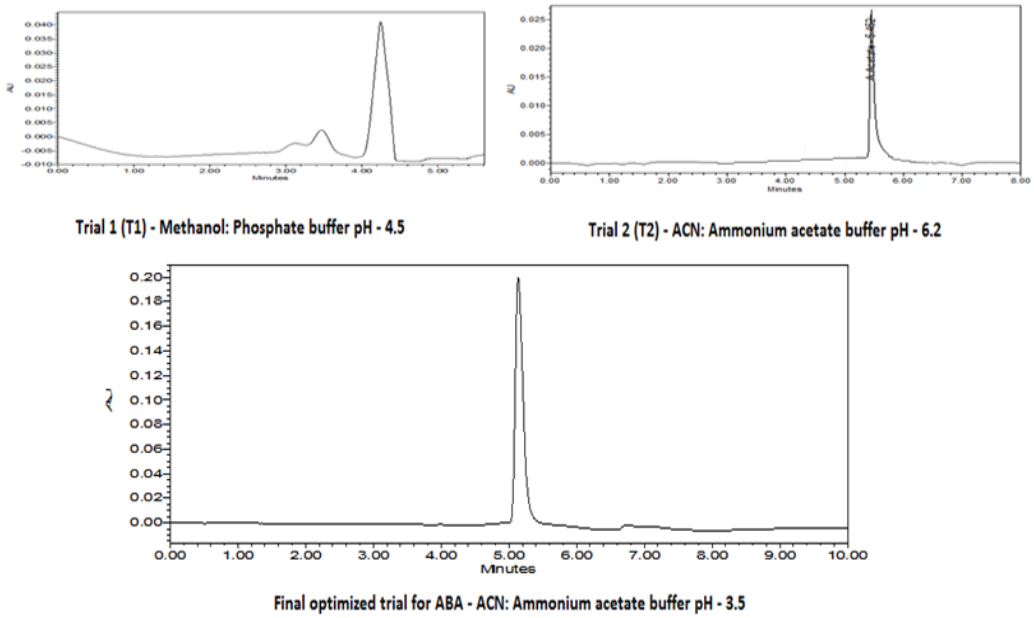


Fig. 2: Trial 1 (T_1), Trial 2 (T_2) and final optimized condition of ABA

System suitability

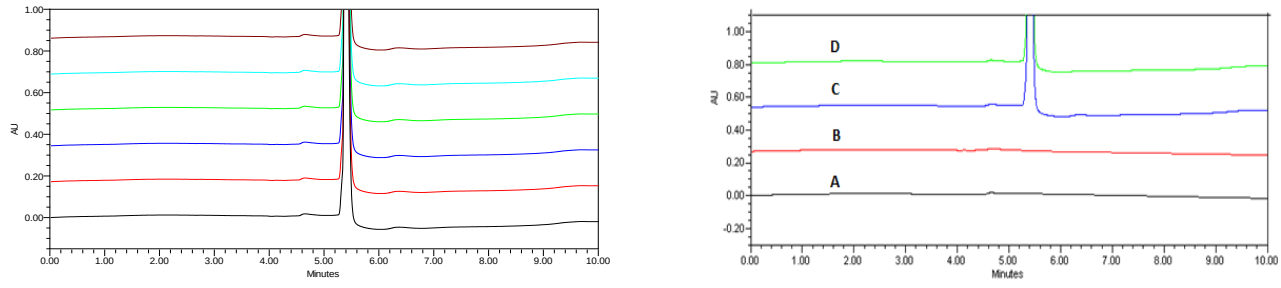
The experimental results showed that the parameters tested were within the acceptable range (% RSD<2.0 %) indicating that the system was suitable for the analysis intended. Overlay chromatogram for system suitability is shown in fig. 3 (a).

Validation of method

Validation of method was performed as per ICH Quality guidelines on validation, analytical procedures (Q2B).

Linearity

The developed method was found to be linear at concentrations ranging from 5-30 µg/ml of ABA. The calibration curves of the ABA were constructed by plotting area response (y) against the concentration of drug (x) and typically described by the following regression analysis equation: y= 36258x+8239.3, r^2= 0.9991. The results showed that an excellent correlation existed between peak areas and concentration of the drug within the concentration range indicated above and confirmed the linearity of the standard curves.



(a) **(b)**

Fig. 3: (a) System suitability overlay and (b) representative overlay chromatogram A mobile phase, B-placebo, C-Standard and D-formulation

Method sensitivity

The method sensitivity was calculated as per the formula given in the introductory part of analytical validation. The results for method sensitivity LOD and LOQ was found to be 0.28 µg/ml and 0.75 µg/ml.

Assay

The results obtained from the assay of the test and standard solutions in compliance with for original conditions. Assay of 20 tablets of label claims 250 mg mean drug content and % RSD was found to be 98.02 % and 0.11 %.

Accuracy

The result of recovery studies at various levels shows that the recovery is between 99.52 to 100.13 % (limit 98-102 %). It indicates that there is no interference in the analysis of the drug from the excipients in the formulation. The results of recovery studies of the formulation are shown in table 1.

Precision

The Intraday and interday precision of sample applications and measurement of peak area for the drug were expressed in terms of % relative standard deviation (% RSD). For the inter-day variation studies, 3 replicates of standard solutions were analyzed on 3 consecutive days and percentage RSD were calculated. The results obtained for Intraday and Inter-day variations are shown in table 2.

Specificity

For the evaluation of the specificity of the RP-HPLC-PDA method, the stress degraded standard solution was injected into RP-HPLC under the proposed chromatographic conditions overlay chromatogram for mobile phase-A, placebo-B, Standard-C and formulation–D is shown in fig. 3 (b). Procedures for degradation studies were described in material and method part of forced degradation study.

The chromatograms of ABA of various degradation conditions are shown in fig. 4, 5, 6, 7, 8, and 9. ABA most degraded in base degradation and degradants observed at a retention time (t_R) 4.10, 4.79, 6.24, 7.19 min. In acidic, oxidative, UV, thermal and neutral minor degradation observed. The above results showed that the developed RP-HPLC-PDA method was specific for the determination of ABA in pharmaceutical preparations. A summary of degradation of ABA is shown in table 3 [7, 8].

Table 1: Accuracy study data

Recovery Level (%)	Base level amount (µg/ml)	Qty. Spiked (µg/ml)	Total Qty (µg/ml)	Amount recovered (µg/ml)	(%) Recovery	(%) R. S. D
80%	7.5	5	12.5	12.44	99.20%	0.19
100%	7.5	7.5	15	15.02	100.26%	0.23
120%	7.5	1	17.5	17.46	99.46%	0.12

Table 2: Interday and Intraday precision of ABA (n=3)

Standard Conc.	Intraday precision			Interday precision		
	10 µg/ml	15 µg/ml	20 µg/ml	10 µg/ml	15 µg/ml	20 µg/ml
Replicates Peak area						
1	346958	536587	718467	346901	535946	718276
2	348823	535688	721361	348823	534913	721569
3	347894	534639	719684	347821	536284	719275
Mean	347892	535638	719684	347848	535714	719706
SD	932.5	974.9	1501.6	961.2	714.2	1688.4
% RSD	0.26	0.18	0.20	0.27	0.13	0.23

Acid degradation

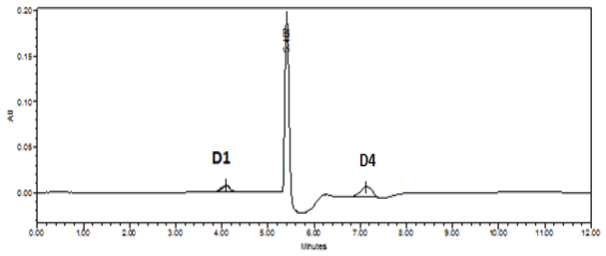


Fig. 4: Chromatogram of acid (1 M HCl for 5 h. at 45 °C) treated ABA t_R-5.4, t_R–4.08 (D1), 7.20 (D4) min

Peroxide degradation

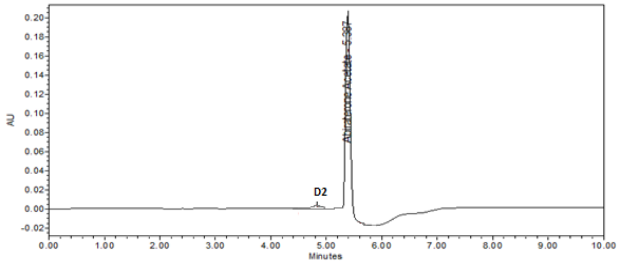


Fig. 6: Chromatogram of H_2O_2 (5 h. at 45 °C) treated ABAt_R-5.4 min, t_R-4.82 (D_2) min

Base degradation

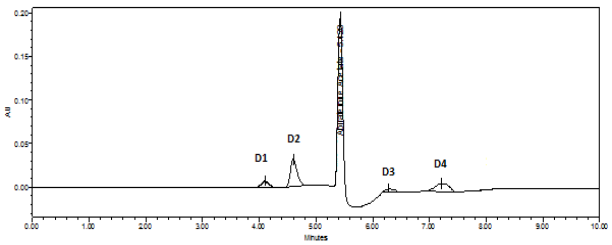


Fig. 5: Chromatogram of alkali (0.1 M NaOH for 5 h. at 45 °C) treated ABAt_R-5.43 min, $t_{R\text{-}4.09}$ (D1), 4.79 (D2), 6.24 (D3), 7.21 (D4) min

Heat degradation

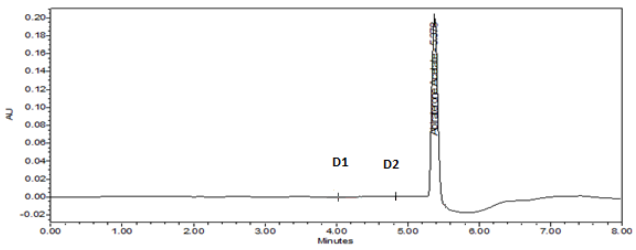


Fig. 7: Chromatogram of dry heat (24 h. at 45 °C) treated ABA t_R 5.4 min, t_R–4.03, 4.83 min

UV degradation

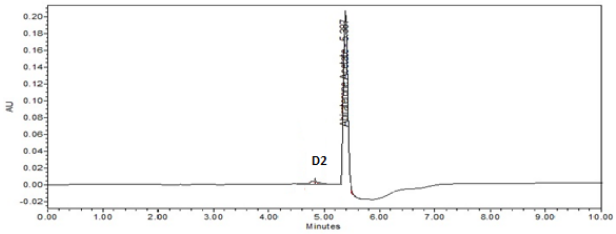


Fig. 8: Chromatogram of photochemically (UV light, 12 h.) treated ABA t_R-5.4 min, t_R–4.80 min

Neutral degradation

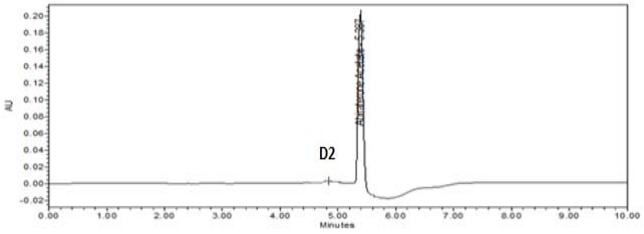


Fig. 9: Chromatogram of neutral (5 h. at 45 °C) ABAt_R-5.4 min, t_R–4.81 min

Table 3: Summary of degradation of ABA

Stress condition	% degraded	Amount recovered	Degraded products t_R (min)	Peak purity data (peak anglepeak threshold),
1M HCL (5 h at 45°C)	4.65 %	95.35 %	4.08, 7.20	0.27, 1.34
0.1M NaOH (5 h at 45°C)	10.08 %	89.92 %	4.09, 4.79, 6.24, 7.19	0.24, 1.32
H2O2 (5 h at45°C)	0.94 %	99.06 %	4.82	0.28, 1.36
Dry heat (24 h at 45°C)	3.97 %	96.03 %	4.03, 4.83	0.25, 1.31
UV light (12 h)	1.08 %	98.92 %	4.80	0.23, 1.29
Neutral (5 h at 45°C)	0.74 %	99.26 %	4.81	0.26, 1.33

t_R = Retention time, peak purity = Peak angle<peak threshold.

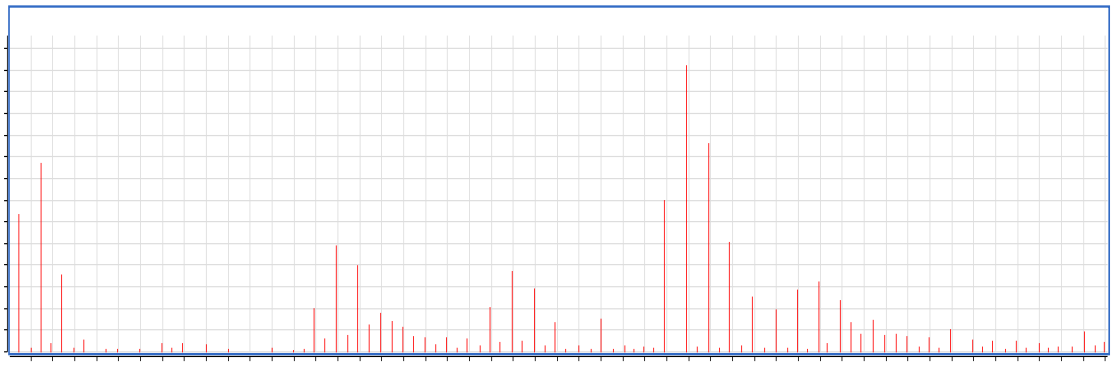


Fig. 10: Mass spectra of base degradant D_2

Mass spectral analysis of base degraded product

The mass spectral fragmentation pattern of the ABA shows a prominent molecular ion peak at m/e-391.5, which however is not the base peak. As shown in fig.10, under base hydrolysis lead to formation of degradant (D_2) 10-Methyl 2,3,4,7,8,9,10,11,12, 13,14,15-dodecanhydro-1H-cyclopenta (α) phenanthren-3-ol) at m/z-257.81, due to corresponding loss of C_8 H_{12} O N as shows in fig. 11 [9-11].

Robustness

The parameters included for observation of change level are temperature, flow rate, pH and wavelength. Each factor selected was changed at three levels (-1, 0, 1). One factor was changed at one time to estimate the effect. Results are given in table 4. In significantly less variability in retention times and peak areas were observed.

Method stability

The result showed that both the retention time and the peak area of ABA were unchanged (% RSD less than 1.2 %) and no significant degradation was observed within 3 d which was sufficient for performing the analytical process.

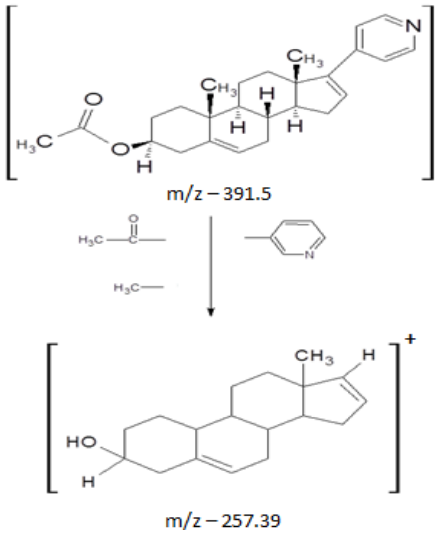


Fig. 11: Degradation pathway of ABA under base hydrolysis degradant (D_2)

Table 4: Robustness study data (n=3)

Parameter count	Change level	Area	Retention time (min	% Assay, % RSD
Temperature (40±2 °C)	38 °C	536710	5.40	100.19 %, 0.15 %
	42 °C	541218	5.42	101.02 % 0.12 %
Flow rate (0.6±0.1 ml/min)	0.5 ml/min	535769	5.48	100.02 % 0.18 %
	0.7 ml/min	532359	5.31	99.39 %, 0.37 %
PH	3.3	543057	5.46	101.36 %, 0.12 %
(3.5±0.2)	3.7	535029	5.41	99.88 %, 0.87 %
Wavelength	233	531811	5.38	99.8 %, 0.25 %
(235±2 nm)	237	543984	5.42	99.69 %, 0.19 %

DISCUSSION

New RP-HPLC-PDA method was developed and validated for ABA. Various chromatographic conditions were tried during method development. Method was validated for linearity, accuracy, precision, specificity, LOD, LOQ and robustness these parameters. To make the method more specific stress degradation of API was carried out using acid, alkali, oxidative, thermal, UV light and neutral stress conditions. As per ICH guidelines 10-30 % stress limit is acceptable. All these conditions sufficient for separation of the degraded product were shown. Further analyte peak was pure as shown by peak purity study. The method is linear within the concentration range 5-30 µg/ml as represented by the value of correlation coefficient (r^2) 0.991 and value of slope were less than 5 % value of the area of nominal concentration. The method is accurate as shown by the result of recovery study % recovery (100±2) and % RSD (<2) values were found within the limit. Robustness study indicate that there is not much variation in system suitability test (SST) parameters and % assay of analyte (limit 100±2 %) under planned variation chromatographic study parameter retention time (t_R), area, plate count (N), capacity factor (K´) and USP tailing were not considered for this study as these parameters were unaffected due to small chromatographic parameter variation as observed during development study. Degradation study shows that drug is more sensitive to alkali stress degradation. From mass spectral study the structure of one of the degraded base product was predicted which is major degradation product among all stress degraded products. The information is used to predict the alkali stress degradation pathway.

CONCLUSION

The results of the various validation studies showed that the RP-HPLC-PDA method is fast, specific, accurate, reproducible, possessed optimum linearity and precision. A stability indicating method was developed which separated all degradation products formed under different stress conditions and degradation pathway of ABA under basic hydrolysis are suggested. In base hydrolysis the major base degradation lead to the formation of the degradation product eluted at t_R-4.79 (D_2) which was then determined by LC-MS at a m/z value of 257.81, due to corresponding loss of C_8 H_{12} O N. The drug was found to be stable under all the stress conditions except basic stress. Thus developed method reported the first time is novel with a very short run time of 6 min.

ACKNOWLEDGEMENT

The authors would like to convey regards to Sun Pharmaceuticals Ltd., Ahmednagar, (India) for providing an API of ABA as gift samples. The authors also thank to Centre for Food Testing (CFT) Bharti Vidyapeeth University, Pune for providing LC-MS Facility and Department of Quality Assurance Techniques, MAEER's Maharashtra Institute of Pharmacy, Pune for providing necessary facilities to carry out this work.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Donnell A, Judson I, Dowsett M, Raynaud F, Dearnaley D, Mason M, *et al*. Hormonal impact of the 17alpha-hydroxylase/C(17, 20)-lyase inhibitor abiraterone acetate (CB7630) in patients with prostate cancer. Br J Cancer 2004;90:2317-25.
2. Gurav SD, Ranindra P, Junaid F, Hohd Z, Shriram R, Ramesh M. Development and validation of a highly sensitive method for the determination of abiraterone in rat and human plasma by LC-MS/MS-ESI: application to a pharmacokinetic study. Biomed Chromatogr 2012;26:761-8.
3. ICH guidelines for the stability of new drug substances and products. Q1A(R2) ICH, Geneva; 2005. p. 1-13.
4. ICH guidelines for validation of analytical procedures: text and methodology. Q2(R1) ICH, Geneva; 2005. p. 1-14.
5. Mandrupkar SN, Mulgund SV, Nagras MA. Development of validated stability indicating RP-HPLC method for estimation of acenocoumarol in bulk and tablet dosage form. Int J Pharm Sci Rev Res 2012;2:101-6.
6. Dong MW. Modern HPLC for practicing scientist. John Wiley and Sons Inc. Publication; 2006. p. 1-13, 193-221.
7. Boccardi G, Baertschi SW. Pharmaceutical stress testing--predicting drug degradation. Taylor and Francis, New York; 2005. p. 220.
8. Alsante KM, Hatajik TD, Lohr LL. Handbook of isolation and characterization of impurities in pharmaceutical. Academics Press: New York; 2003. p. 380.
9. Ceresole R, Rosasco MA, Forastieri CC, Segall AI. HPLC determination of Acenocoumarol and its major thermal degradation product. J Liq Chromatogr Relat Technol 2008;31:179-87.
10. Kollroser M, Schober C. Determination of coumarin-type anticoagulants in human plasma by HPLC-electrospray ionization tandem mass spectrometry with an ion trap detector. Clin Chem 2002;48:84-91.
11. Gupta A, Rawat S. Method development and hydrolytic degradation study of Doxofylline by RP HPLC and LC–MS/MS. Asian J Pharm Anal 2011;1:29-33.