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Research Article

A VALIDATED STABILITY-INDICATING RP-HPLC ASSAY METHOD FOR BOLDENONE UNDECYLENATE AND ITS RELATED SUBSTANCES

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ABSTRACT

A validated stability-indicating reversed-phase high performance liquid chromatography (RP-LC) method was developed for the determination of boldenone undecylenate and its related impurities in bulk samples. The stress degradation study including acid, base, H_2O_2, humidity, thermal and photolytic conditions were performed on bulk samples of boldenone undecylate as per International Conference on Harmonization (ICH) guidelines to show the stability-indicating capability of the method. The chromatographic method was optimized using the stress degradation samples and the impurities spiked solution. An acceptable resolution between the peaks which corresponds to process-related impurities and degradation products from the analyte was achieved on Waters X-Bridge C-18 (3.5 µm; 150 x 4.6 mm) stationary phase column. The mobile phase composed a mixture of water and acetonitrile using gradient elution. The flow rate was 1.0 mL min^{-1} and the detection wavelength was carried out at 254 nm. The mass balance in each case was in between 99.5 to 99.9, indicating that the developed method was stability-indicating. Validation of the developed method was carried out as per ICH requirements.

Keywords: Boldenone undecylenate, RP-HPLC stability method, development and validation.

INTRODUCTION

Boldenone undecylenate, chemically named as 1,4-androstadiene-3-one-17β-ol and is a derivative of testosterone. Equipoise is the trade name for the veterinary drug boldenone undecylenate. It is an injectable steroid which exhibits strong anabolic and moderately androgenic steroid[1,2]. It is primary used in veterinary medicine and most commonly with horses to improve performance, accelerate growth, increase appetite and/or enhance libido. Boldenone undecylenate also increases hemoglobin and hematocrit, in clear the number and the percentage of red blood cells[3]. It is typically dosed in the range of 200-400 mg per week, in weekly injections. The high-performance liquid chromatography (HPLC) is a well-established reliable technique used in controlling the quality and consistency of active pharmaceutical ingredients (API's) and dosage forms. According to current good manufacturing practices all drugs must be tested with a stability indicating assay method before release. Stress testing of the drug substance can help to identify the likely degradation products, which can in turn help establish the degradation pathways and the intrinsic stability of the molecule and validate the stability-indicating power of the analytical procedures used. To our knowledge, no stability-indicating HPLC method for the quantification of boldenone undecylenate is available in the literature. The present research work describes a suitable stability-indicating HPLC method to determine of boldenone undecylenate and its related impurities in bulk samples. The method was validated with respect to specificity, limit of detection (LOD) and quantitation (LOQ), linearity, precision, accuracy and robustness to show the stability-indicating power of the method and also to ensure the compliance in accordance with ICH Guidelines[4]. The developed method has been applied for the analysis of drug which was stored at long term and accelerated stability studies to show stability-indicating of the method.

Experimental

Chemicals and Reagents

Samples of boldenone undecylenate and its six process impurities (Fig. 1) were received from (Xavier pharmaceuticals Ltd, Mumbai, India). HPLC grade acetonitrile (ACN) was purchased from Rankem (Mumbai, India). HPLC-grade methanol (MeOH) was obtained from Rankem (Mumbai, India).

High-purity water was prepared by using Millipore Milli-Q plus purification system. The mobile phase was filtered through a 0.45 µm PTFE filter (Millipore, USA) and degassed before use.

Chemical names

(i) Boldenone undecylenate: (1,4-androstadiene-3-one-17β-ol) (Mol. Wt: 452.57)

(ii) Imp-A: Boldenone free base (Mol. Wt: 286.41)

(iii) Imp-B: Testosterone (Mol.Wt: 288.42)

(iv) Imp-C: Boldenone propionate (Mol.Wt: 342.21)

(v) Imp-D: Testosterone propionate (Mol.Wt: 344.21)

(vi) Imp-E: 17-beta-Undecylenate-androsta-1,4, 6 triene-3–one (Mol.Wt: 450.65)

(vii) Imp-F: Testosterone undecylenate (Mol.Wt: 454.68);

(viii) Imp-G: 8R,9S,10R,13S,14S,17S)-10,13-dimethyl-3-oxo-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3H-cyclopenta[a]phenanthren-17-yl 2-(non-8-en-1-yl)-3-oxotridec-12-enoate; 2.1 RRT (Mol.Wt: 618.93)

Instrumentation

The HPLC method development, validation and forced degradation studies were done using Agilent 1100 series liquid chromatographic system (Agilent Technologies, Waldbornn, Germany) with photodiode array (PDA) detector. The output signal was monitored and processed by using chemstation software (designed by Agilent Technologies, Waldbronn, Germany) on Lenovo computer (Digital equipment co.).

The intermediate precision was carried out on Waters LC system (Milford, MA, USA) equipped with 2695 separation module and a 2487 dual wavelength detector. Photo stability studies were carried out in a photo stability chamber (Sanyo, Leicestershire, UK). Thermal stability study was performed in a dry air oven (Cintex, Dadar, India).

Chromatographic conditions

A simple liquid chromatographic method was developed for the determination of boldenone undecylenate, its process related impurities and degradation products. The chromatographic separation was achieved on a Waters X-Bridge C18 (3.5 µm; 150 x 4.6 mm) using the solvent A composed of water and acetonitrile in the ratio of 90:10 %v/v, and solvent B composed of ACN.

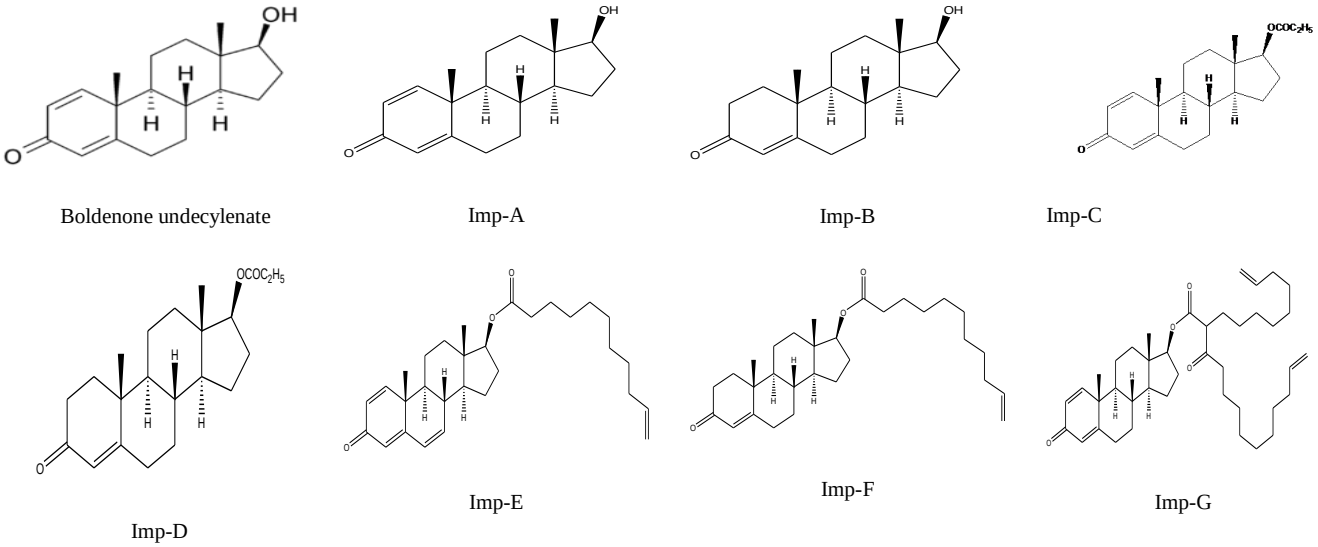


Fig. 1: Structures of boldenone undecylenate and its related impurities

Table 1: precision at LOQ level

Preparation	Area						
	Imp-A	**Imp-B**	**Imp-C**	**Imp-D**	**Imp-E**	**Imp-F**	**Imp-G**
1	1.202	1.339	1.719	1.305	1.392	2.618	2.319
2	1.237	1.296	1.693	1.459	1.439	2.827	2.167
3	1.193	1.359	1.697	1.309	1.305	2.392	2.162
4	1.247	1.337	1.625	1.549	1.538	2.629	2.381
5	1.262	1.380	1.723	1.372	1.473	2.473	2.271
6	1.198	1.417	1.739	1.482	1.382	2.903	2.091
Average	1.2229	1.3547	1.6993	1.4127	1.4215	2.6403	2.2318
Std deviation	0.0294	0.0413	0.0402	0.0995	0.0807	0.1970	0.1098
% RSD	2.4	3.1	2.4	7.0	5.7	7.5	4.9

Table 2: Results of accuracy for related substances and assay

Compound	% Level	Spiked quantity (µg mL^{-1})	Recovered quantity (µg mL^{-1})	% Recovery
Boldenone undecylenate	50	155	156	100.6
	100	306	303	99.0
	150	455	452	99.3
Imp-A	0.05	0.147	0.145	98.6
	0.10	0.305	0.302	99.0
	0.15	0.445	0.439	98.7
Imp-B	0.05	0.144	0.142	98.6
	0.10	0.304	0.295	97.0
	0.15	0.447	0.443	99.1
Imp-C	0.05	0.156	0.153	98.1
	0.10	0.305	0.302	99.0
	0.15	0.455	0.449	98.7
Imp-D	0.05	0.152	0.145	95.4
	0.10	0.307	0.299	97.4
	0.15	0.457	0.442	96.7
Imp-E	0.05	0.155	0.146	94.2
	0.10	0.306	0.292	95.4
	0.15	0.447	0.422	94.4
Imp-F	0.05	0.145	0.137	94.5
	0.10	0.308	0.295	95.8
	0.15	0.446	0.433	97.1
Imp-G	0.05	0.152	0.144	94.7
	0.10	0.307	0.295	96.1
	0.15	0.456	0.443	97.1

A gradient program was performed and as follows: time (min)/% B, 0/25, 15/85, 30/100, 35/100 at a flow rate of 1.0 mL min^{-1} with a post run time of 5 min. The column temperature was maintained at 40°C and the detection was carried out at 254 nm. The test concentration was 1000 µg mL^{-1} and the injection volume was 5 µL. The MeOH was used as a diluent for the preparation of standard and test samples.

Preparation of standard and sample solutions

The standard solution of boldenone undecylenate was prepared at a concentration of 1.0 mg mL^{-1} by dissolving an appropriate amount of drug substance in MeOH. Stock solutions of each impurity were prepared at concentration of 1.0 mg mL^{-1} in MeOH. The analyte

concentration of boldenone undecylenate was fixed as 1.0 mg mL^{-1}. The sample solutions were prepared by diluting the stock solutions to attain the required concentration of impurities and drug substance using methanol as diluent.

MATERIALS AND METHODS

The described HPLC method has been extensively validated for related substances as per ICH guidelines[7].

Stress studies/specificity

Specificity is the ability to assess unequivocally the analyte in the presence of its potential impurities[5] which may be expected to be present like impurities, degradants, matrix, etc[6]. The specificity of the developed HPLC method for boldenone undecylenate was established in presence of its known impurities, namely Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G and its degradation products. The ability of the method to separate all the compounds was assessed by evaluating the resolution between the peaks corresponding to the various compounds to show the stability-indicating ability and specificity of the proposed HPLC method.

The stress conditions employed for degradation studies as per ICH recommendation include photolytic, thermal, oxidation and hydrolysis with acid and base. The photolytic stress study was performed for 11 days at 200 Watt hrs square $meter^{-1}$ of UV light, 1.2 million lux hrs of visible light. Acidic stress was performed in 0.1 N HCl at ambient temperature for 24 hrs.

The study in basic solution was carried out in 0.01 N NaOH at ambient temperature for 24 hrs. The oxidation study was carried out in 3% hydrogen peroxide for 24 hrs. The drug sample was exposed to heat at 105°C for 7 days. Samples were withdrawn at appropriate times and subjected to LC analysis after suitable dilution (1000 µg mL^{-1}) to evaluate the ability of the proposed method to separate boldenone undecylenate from it's degrade products. Photodiode array detector was employed to ensure the homogeneity and purity of boldenone undecylenate peak in all the stressed sample solutions. The assessment of mass balance in the degraded samples was carried out to confirm that the amount of impurities detected in stressed samples matched with the amount present before the stress was applied[8].

System suitability test

System suitability test is an integral part of chromatographic methods and used to verify that the resolution and reproducibility of the chromatographic system are adequate for the analysis to be performed.

Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ of boldenone undecylenate and its related impurities were determined by diluting known concentrations of boldenone undecylenate and its related impurities that would yield

a signal to noise ratio of 3:1 and 10:1 respectively[9,10]. Precision was carried out at LOQ level by preparing six individual preparations of boldenone undecylenate with its related impurities at LOQ level, and calculated the percentage RSD for the areas of boldenone undecylenate and its related impurities. The accuracy at LOQ level was also carried out by preparing three recovery solutions of boldenone undecylenate with its related impurities at LOQ level and

calculated the percentage recovery for areas of all related impurities.

Linearity

The linearity of method was established at two different levels. The assay linearity was performed by preparing five different solutions of boldenone undecylenate from 50 to 150% w/w (i.e. 500, 750, 1000, 1250 and 1500 µg mL^{-1}) with respect to analyte concentration of 1000 µg mL^{-1}. The linearity at low level was performed by preparing six different solutions corresponding to LOQ to 200% with respect to the impurity specification level of 0.10% (i.e. LOQ, 0.05, 0.075, 0.10, 0.15 and 0.20% w/w) of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G and boldenone undecylenate. The peak area versus concentration data was performed by least-square linear regression analysis. The correlation coefficient, slope, intercept and % y-intercept of the calibration curves were computed. The relative response factor (RRF) of each impurity was determined by dividing the slope of each impurity with the slope of boldenone undecylenate.

Precision

The assay method precision was evaluated by carrying out six independent assays of test samples of boldenone undecylenate against qualified working standard and calculated the percentage of RSD. The precision of the related substance was checked by injecting six individual measures of boldenone undecylenate which were performed with 0.10 % w/w of each of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F and Imp-G to the reference of target analyte concentration (1000 µg mL^{-1}). The content of each impurity were determined for each of the preparation, and the method precision was evaluated by calculating the percentage RSD of six impurity content. The experiments with a different analyst, column and instrument in the same laboratory were performed in order to certain the intermediate precision or ruggedness of the developed HPLC method.

Accuracy

The accuracy of an analytical procedure expresses as the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found[11]. The accuracy of the assay was evaluated in triplicate at three concentration levels, i.e. 50, 100 and 150 µg mL^{-1} of boldenone undecylenate and the percentage recovery at each level was calculated. Standard addition and recovery experiments were conducted to determine the accuracy of related substance in the same method for the quantification of all seven impurities in boldenone undecylenate. The study was carried out in triplicate at 0.05, 0.10 and 0.15% w/w of the target specification limit. The percentage recoveries for Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F and Imp-G were calculated from the original amount spiked, and the amount of the same impurities was calculated against the main peak diluted to impurity specification level with RRF correction.

Selectivity

The selectivity of the method was established from the resolution of the boldenone undecylenate from the nearest peak and also among all the other peaks. All the degradants and impurities were separated amongst as well as from analyte with a resolution of greater than 1.5, show the selectivity of the method.

Solution stability and mobile phase stability

The solution stability of boldenone undecylenate in presence of its related impurities at specification level was carried out by leaving

the solution in a tightly capped volumetric flask at room temperature (25 ± 2°C) on a laboratory bench top for 48 h. The content of all related impurities were determined at a 6 h interval up to the study period 48 h. The mobile phase stability was carried out by evaluating the content of all related impurities in boldenone undecylenate sample solution which was spiked with known impurities at specification level. The sample solution was prepared freshly at each 6 h interval up to 48 h, while the same mobile phase was used during the study period.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters provides an indication of its reliability during normal usage. To determine the robustness of the developed HPLC method, deliberate changes were made from original experimental conditions. The effect of the flow rate was studied at 0.8 and 1.2 mL min^{-1}, instead of 1.0 mL min min^{-1}.

The effect of mobile phase A composition was studied at 89% and 91%, instead of 90%. The effect of the column temperature was studied at 35°C and 45°C, instead of 40°C. In all the three parameters (flow rate, temperature and mobile phase composition) the

theoretical plates, the tailing factor of analyte and the resolution between critical pair i.e. between analyte and Imp-E were computed.

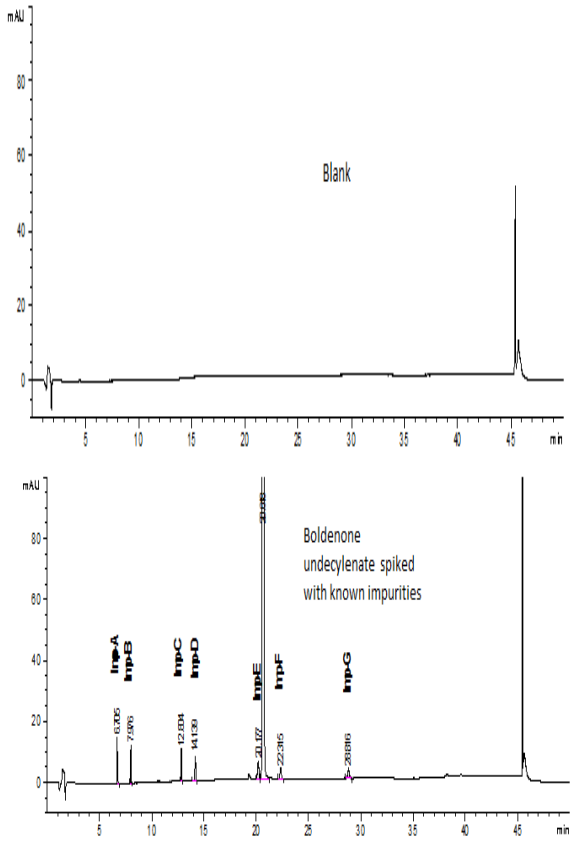
RESULTS AND DISCUSSION

Development and optimization of HPLC method

The impurities, Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F and Imp-G are the potential impurities of boldenone undecylenate drug substance. The main objective of the chromatographic method was to separate Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G and the degradation products from the analyte, boldenone undecylenate. The Imp-E was co-eluted with analyte peak by using different stationary phases, such as C18, cyano and phenyl using various mobile phases, such as trifluoro acetic acid and acetate buffers with organic modifiers, including ACN and MeOH.

Selection of column has played a critical role in achieving the separation of Imp-E from analyte peak. Initially the method development was initiated by using 0.05% trifluoro acetic acid in water as mobile phase A and 0.05% trifluoro acetic acid in ACN as mobile phase B with a gradient program T/%B: 0/60, 25/100, 35/100, 36/60, 40/60 post run: 5 min at a flow rate of 1.2 mL min^{-1} on a 100 mm length 4.0 mm internal diameter column and 3.0 µm particle size C18 stationary phase. When an impurity-spiked solution was injected, for the analyte peak system suitability parameters are very poor and the resolution between the Imp-E and analyte was also very poor.

The Imp-E was almost co-eluted with the analyte hence the peak purity of analyte also failed. To improve the peak shape, mobile phase A was replaced with 10 mM ammonium acetate buffer and injected the impurity-spiked solution. Peak shape was slightly improved, but the Imp-E still posses less resolution from the analyte peak. Next trails were performed on cyano and phenyl columns but results were not so good, so C18 stationary phase was employed with water:ACN (90:10) as mobile phase A and acetonitrile as mobile phase B and different gradient programs to get better resolution of Imp-E from analyte and to avoid co-elution of all process related impurities and degradant impurities.



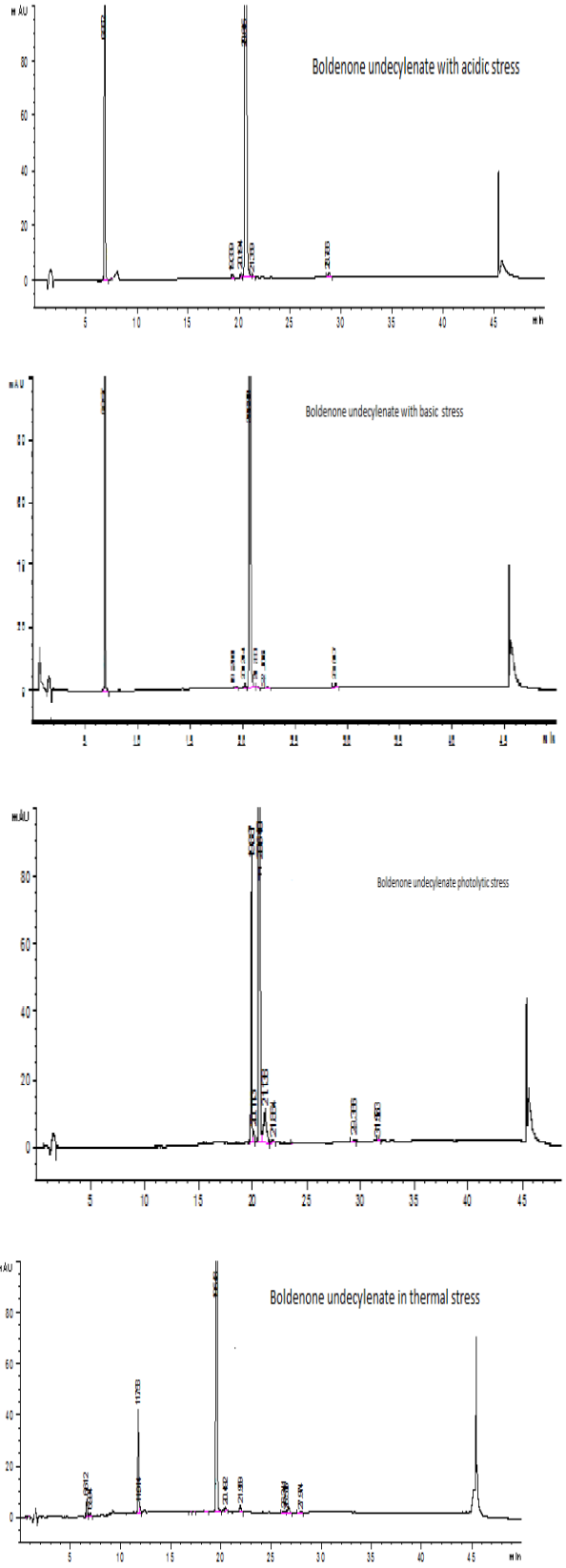


Fig. 2: Degradation chromatograms and impurities spiked solution of boldenone undecylenate

Chromatographic separation was successfully achieved on C18 stationary phase, Waters XBridge C18 (150 mm length, 4.6 mm internal diameter, 3.5 µm particle size) column having a stationary phase of tetraethylsiloxane and bis(triethoxysilyl)ethane hybrid

octadecylsilanes, and mobile phase system containing water:ACN (90:10) (mobile phase A) and ACN (mobile phase B), by employing a gradient program 0/25, 15/85, 30/100, 35/100 (time (min)/%B) at a flow rate of 1.0 mL min^{-1}.

The column temperature was maintained at 40° C and detection wavelength was set at 254 nm. The injection volume was 5 µL, with a sample loading of 0.001 mg. In the optimized conditions, the impurities namely Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G and drug substance boldenone undecylenate were well separated with a resolution of more than 1.5 and all degradation impurities were separated with resolution more than 1.5 from analyte peak. The typical chromatogram of boldenone undecylenate with its related impurities was shown Fig. 2. Te method was specific for boldenone undecylenate from its potential impurities, namely Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F and Imp-G and its degradant impurities.

Method validation

Forced degradation studies

No degradation was observed in stressed conditions when the analyte was subjected to oxidation (Fig. 2). The degradation of drug substance was observed under acidic, basic, photolytic and thermal conditions (Fig. 2). The drug substance of boldenone undecylenate under acid hydrolysis leads to the formation of an unknown degradation Impurity at 0.33 RRT. The boldenone undecylenate under base hydrolysis leads to the formation of unknown degradation impurity at 0.33 RRT. Slight degradation was observed in thermal stress conditions. The drug substance of boldenone undecylenate under photolytic stress conditions significant degradation was observed at 1.02 RRT. The peak purity index over the single point threshold obtained in all stressed samples for the analyte peak demonstrates the specificity of the HPLC method.

The assay of boldenone undecylenate is unaffected in the presence of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F, Imp-G and its degradation products. The mass balance in all stressed samples was nearly 99.0 % w/w, when the RRF of the degradant impurities was considered to be one. All the studies confirm the specificity and stability indicating ability of the developed HPLC method.

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD of boldenone undecylenate, Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F and Imp-G were 0.024, 0.030, 0.030, 0.048, 0.121, 0.242 and 0.199 µg mL^{-1}, respectively (of analyte concentration i.e, 1000 µg mL^{-1}) for 5 µL injection volume. The LOQ of boldenone undecylenate, Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F and Imp-G were 0.08, 0.10, 0.10, 0.16, 0.40, 0.80 and 0.66 µg mL^{-1} respectively (of analyte concentration i.e, 1000 µg mL^{-1}) for 5 µL injection volume. These limits of quantification levels of the impurities were helpful for the process research work to control the impurities at the accepted level during the optimization of the process. Precision study was carried at the LOQ level by injecting six individual preparations of imp-A, imp-B, imp-C, Imp-D, Imp-E, Imp-F and Imp-G and calculated the percentage RSD. The results were presented in Table 1.

Linearity

Linear calibration plot for the assay was obtained over the calibration ranges tested, i.e. 50-150 µg mL^{-1} and the correlation coefficient obtained was greater than 0.999. The values of slope, intercept and %Y-intercept of the calibration curves were determined. An excellent correlation existed between the peak area and concentration boldenone undecylenate for the assay determination. Linear calibration plot for related substances was obtained over the calibration ranges tested, i.e. LOQ to 20 µg mL^{-1} for Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F and Imp-G. The correlation coefficients obtained for all impurities were also greater than 0.999.

The values of slope, intercept and %Y-intercept of the calibration curves were determined. The RRF of each impurity was determined using the slope of the each impurity plot against the boldenone undecylenate plot. The Y-intercept of each plot was within the 3.0% of the response at 0.10% w/w level of each impurity, describing that the plot is going almost through the origin. This enables obtaining an exact value of RRF which will minimize the error in quantification of impurities.

Precision

The precision of an analytical procedure expresses the closeness of agreement among a series of measurements obtained from multiple samplings of the same homogenous sample under prescribed conditions. The RSD of assay results of boldenone undecylenate during the method precision study was within 0.3 % and the RSD of content of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F and Imp-G were within 3.8 %. The RSD of assay results obtained in the intermediate precision study was within 0.4% and the RSD content of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F and Imp-G were within 4.8%. Also the individual values fall well in the range of confidence interval of average, confirming good precision of the method.

Accuracy

The percentage recovery of Imp-A, Imp-B, Imp-C, Imp-D, Imp-E, Imp-F and Imp-G in bulk drug samples ranged from 85.0 to 115.0. The percentage recovery of boldenone undecylenate in bulk drug samples 99.3 to 100.6. All the individual recovery values of the assay and impurities were well in the confidence interval of mean values. Good recovery values reflecting the exact values of RRF for all impurities as well as the capability of method accuracy. The results were presented in Table 2.

Robustness

In all the deliberate varied chromatographic conditions carried out as described above (flow rate, mobile phase composition-A and column temperature), the tailing factor of boldenone undecylenate was less than 1.0, theoretical plates are more than 70,000 and the resolution between boldenone undecylenate and Imp-E was greater than 1.5. There was a very minor variation in the theoretical plates, resolution and tailing factor results observed in all the robustness conditions illustrating the robustness of the method. Though the higher column temperature shows a better tailing factor, it is preferable to run in nominal temperature when considering the durability of the column.

Solution stability and mobile phase stability

The RSD of assay of boldenone undecylenate during solution stability and mobile phase stability experiments were within 0.80 %. No significant changes were experienced in the content of any of the impurity during solution stability and mobile phase stability experiments. The accuracy of the assay at each time point against the initial value is between 99.5 and 100.6%. The accuracy of the content of each impurity against the initial value is between 96.4 and 103.3%. The solution stability and mobile phase stability experimental data confirms that sample solutions and mobile phase used were stable up to 48 h. It is an advantage that from the same run, the assay results and impurities quantification can be derived. This helps to reduce the analysis time and number of samples that can be analyzed till 48 h in the same sequence in the quality control during the regular analysis.

CONCLUSIONS

The developed simple HPLC method for related substance and assay determination of boldenone undecylenate is linear, precise, accurate and specific. The method was validated to the requirements of ICH and the results were satisfactory. The developed stability-indicating HPLC method can be used for the routine analysis of production samples and also to check the stability of bulk samples of Boldenone undecylenate during its storage.

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REFERENCES

1. Amer, J., American Medical Association, Council on Scientific Affairs. Medical and non-medical uses of anabolic-androgenic steroids; Medical Association, (1990); 264: 2923-2927.
2. Bahrke, M.S., Yesalosk, C.E. and Wright, J. E. Psychological and behavioral effects of testosterone levels and anabolic-androgenic steroids among males: a review. Sports Medicine, (1990); 10: 303-337.
3. Llewellyn, W. (2006*).* Anabolics 2006. Jupiter, FL: Body of Science
4. ICH, Text on validation of analytical procedures, Q2A (R1). International Conference on Harmonization, IFPMA, Geneva.
5. US Food and Drug Administration (2001) Guidance for industry: analytical procedures and methods validation: chemistry, manufacturing, and controls documentation. Draft Guidance. US Food and Drug Administration.
6. Q3A (R2) Current *Step 4* Version, impurities in new drug substances, October 2006
7. ICH Q2 (R1), Validation of Analytical Procedures: Text and Methodology, November 2005.
8. Bakshi, M. and Singh, S. "Development of validated stability-indicating assay methods-critical review, Journal of Pharmaceutical and Biomedical Analysis, (2002); 28: 1011–1040.
9. Shabir, G. A. Validation of high-performance liquid chromatography methods for pharmaceutical analysis. Understanding the differences and similarities between validation requirements of the US Food and Drug Administration, the US Pharmacopeia and the International Conference on Harmonization. Journal of Chromatography A, (2003); 987: 57–66.
10. Ribani, M., Bottoli, C. B. G., Collins, C. H., Jardim, I. S. F., Melo, L. F. C., Validation for chromatographic and electrophoretic methods, Quimica Nova, (2004); 27: 771-780.
11. Validation of Chromatographic methods, (1994) Reviewer guidance, Centre for Drug Evaluation and research (CDER) CMC 3.