Quantification of potential impurities present in testosterone undecanoate active pharmaceutical ingredient by stability indicating HPLC method using UV detector

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ABSTRACT

The study involves the development followed by validation of potential impurities in testosterone undecanoate by high pressure liquid chromatography (HPLC) technique. The chromatographic separation of potential impurities and degradation products were achieved in YMC pack C8 column (150 mm x 4.6 mm, 5 µm) using gradient elution method. For the gradient elution method, mobile phase-A and Mobile phase-B was used. Mobile phase-A was prepared using water and acetonitrile mixture in a ratio of (90:10, v/v) and mobile phase-B was only acetonitrile. Column temperature was kept at 35°C throughout the analysis. Wavelength of 240 nm were selected for the analysis. The current developed method is specific, linear, precise, and accurate. Specificity of the method was confirmed by peak purity analysis using photodiode array detector. Testosterone is the major degradant. The validation study was done as per the current ICH guidelines.

Keywords: Testosterone undecanoate, Testosterone replacement therapy, Male hypogonadism.

1. INTRODUCTION

Testosterone is one of the important male gonadal hormone and is essential for the development of male sex organs and secondary sexual characteristics in men. Low level of Testosterone in serum occurs due to inadequate function of testis which results male hypogonadism [1,2]. The symptom of hypogonadism includes erectile dysfunction, decrease bone density, decrease volume of ejaculate and increase body fat [3-5].

Testosterone has poor bioavailability and it undergoes liver metabolism so rapidly that it is difficult to maintain the normal serum testosterone level in hypogonadal patient [6-8]. To improve the bioavailability and phytokinetics, several ester derivative of testosterone are commercially developed and available in the market such as testosterone enantate, testosterone propionate, testosterone cypionate and testosterone undecanoate (TU).

Testosterone undecanoate (TU) is known as testosterone 17β-undecylate [9]. It is one of the important drugs used in testosterone replacement therapy. The administration route of TU is both oral as well as intramuscular. Oral formulation of TU (brand name Andriol) have less bioavailability (about 7%), thus it requires frequently daily oral dosing in combination with fatty meals to increase the bioavailability [10]. Nebido® is the long acting intramuscular injection of TU and is available in more than 80 countries. It was also approved in 2014 by the FDA of United States in the brand name of Aveed® [11-13].

Various research articles are reported for the

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development and analysis of testosterone esters [14-16]. However no HPLC method has been reported yet (including major pharmacopoeias USP, EP, BP, JP, and IP) for the determination of potential impurities in TU. Therefore it is important to have a stability indicating method for the quantification of potential impurities in TU.

The quality, efficacy and safety is significantly affected by the impurities present in the drug product [17]. Thus it is important to develop a stability indicating, specific and accurate method. As per the current ICH Guideline of stability testing of drug substances, forced degradation should be carried out to establish that the method is stability indicating and the analytical method should be validated [18-21]. The current work involves method development, forced degradation and method validation of TU.

2. Experimental

2.1. Materials and chemicals

Sample of TU and Imp-1, Imp-2, Imp-3, Imp-4, Imp-5 and Imp-6 were obtained from chemical research division, Ipca laboratories Ltd. (Vadodara, India). Water (HPLC grade) for the analysis was obtained from water purification system of Milli-Q plus from Millipore (Bedford, MA, USA). Acetonitrile was purchased from Merck India (Mumbai, India).

2.2. Instrumentation and HPLC conditions

The instruments used in the study includes: HPLC separation module (2695) with UV detector (2487) and photodiode array detector (2996) (Waters Corporation, Milford, MA, USA); a photo stability chamber model NEC-104RTS (Newtronic, Mumbai, India).

The chromatographic column YMC pack C8 (150 mm x 4.6 mm, 5 µm) was used in the analysis (YMC Co., LTD Kyoto, Japan). For the gradient elution method, mobile phase-A and Mobile phase-B were used. Mobile phase-A was prepared using water and acetonitrile mixture in a ratio of (90:10, v/v) and mobile phase-B was only acetonitrile; column temperature was maintained at 35°C throughout the analysis; flow rate was 1.0 mL/min; injection volume was 10 µL; detector wavelength was fixed at 240 nm; sample temperature was 25°C. Diluent was a mixture of water and acetonitrile in a ratio of (30:70, v/v). Gradient program is tabulated in Table (1).

Table (1)
Linear gradient program

Time/minutes	Solution-A, %	Solution-B, %
0	55	45
5	50	50
10	20	80
20	10	90
30	10	90
40	55	45
50	55	45

2.3. Preparation of sample, stock solution for validation and forced degradation samples

A 500 µg/mL of test sample of TU was prepared by transferring about 25.0 mg of sample into 50 mL volumetric flask, 10 mL of acetonitrile was added, sonicated for 1.0 min and made upto the volume with diluent. A 1000 µg/mL of individual stock solution was made by transferring about 25.0 mg each of TU, imp-1,

imp-2, imp-3, imp-4, imp-5 and imp-6 into 7 separate 25 mL volumetric flask, added 10 mL of acetonitrile, sonicated for 1.0 min and made upto the volume with diluent. 0.75 mL of each of above individual stock solutions was pipette out into 20 mL volumetric flask and made upto the volume with diluent. This solution was labeled as standard stock solution (37.5 µg/mL). Further from this standard stock solution, remaining required solutions of different concentrations were prepared for validation. 25 mg each of sample was weighed and transferred into 3 separate 50 mL volumetric flasks and labeled as 1, 2 and 3. 10 mL of acetonitrile was added into each volumetric and the sample was dissolved. 5 mL of 0.5 N hydrochloric acid solution, 0.5 N sodium hydroxide and 5.0 % hydrogen peroxide solution was also added into the volumetric flask 1, 2, and 3 respectively. The flasks were kept in water bath at 60°C for 4 h, 20 min and 6 h respectively. The excess of acid or base in volumetric flask 1 and 2 were neutralized and made upto the mark with diluents. Corresponding blank solutions were prepared. Thermal degradation was performed on solid sample at 50°C for 48 h. Photolytic degradation was performed by spreading the sample on petri dish and kept in a photo stability chamber model NEC-104RTS (Newtronic) to get the light intensity of 1.2 million Lux hours for white light and 200 W h/m^2 for ultraviolet region.

3. Results and discussion

3.1. HPLC method development

The objective of the chromatographic method was to get the baseline separation between all impurities and also to elute TU before 30.0 min to reduce the run time. Impurities were labeled on the basis of their elution time. Structure and details of TU and its impurities of are listed in Table (2). The λ_{max} of TU and its potential impurities were found to be 240 nm as showed in Fig. 1. The method development was initiated using water and acetonitrile in a ratio of (50:50, v/v) as mobile phase by isocratic elution method, using YMC pack C8 (150 mm x 4.6 mm, 5 µm). The peak of TU was not eluted upto 100 min. The mobile phase was then modified to water and acetonitrile in a ratio of (30:70, v/v) in which the peak of TU elutes in about 40.0 min. After several experiments the method was finally developed using linear gradient program. For the gradient elution method, mobile phase-A and Mobile phase-B was used. Mobile phase-A was prepared using water and acetonitrile mixture in a ratio of (90:10, v/v) and mobile phase-B was prepared using acetonitrile only. YMC pack C8 column (150 mm x 4.6 mm, 5 µm) was used. The column temperature was kept at 35°C and detector wavelength was selected as 240 nm throughout the analysis. The TU peak eluted at about 22 min with the base line separation of all impurities (Fig. 2).

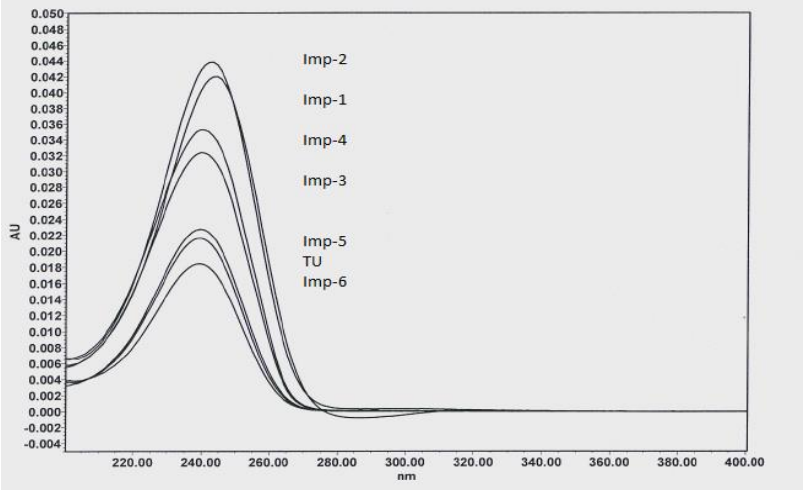
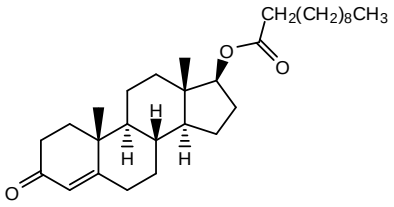
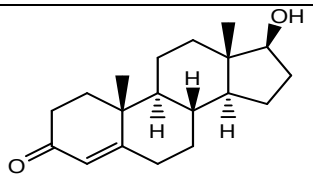
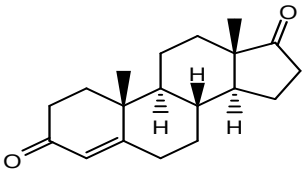
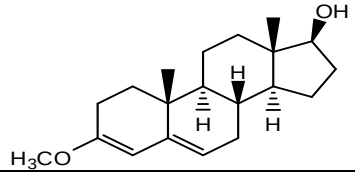
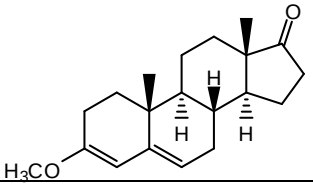
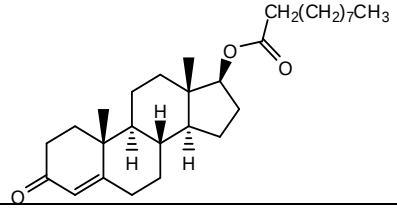
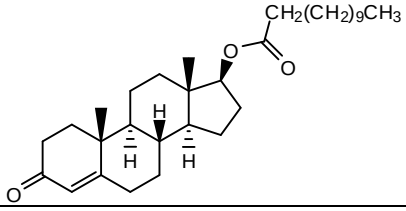


Figure (1): The λ_{max} of Testosterone Undecanoate and its potential impurities

Table (2)
Structure and details of TU and its impurities

S.no	Name	Structure	Code	Source
1	Testosterone Undecanoate		TU	Drug
2	Testosterone		Imp-1	Process and degradation Impurity
3	Androstenedione		Imp-2	Process impurity
4	3-Methoxy testosterone		Imp-3	Process impurity
5	3-Methoxy Androstenedione		Imp-4	Process impurity
6	Testosterone decanoate		Imp-5	Process impurity
7	Testosterone laurate		Imp-6	Process impurity

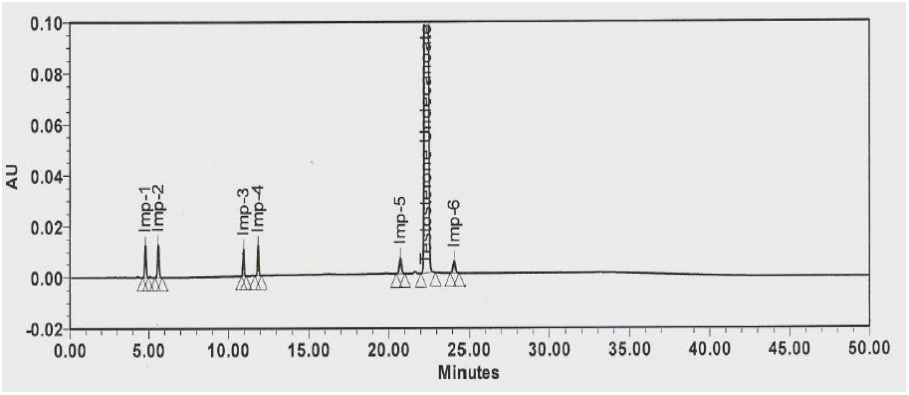


Figure (2): Chromatogram of spiked impurities in Testosterone Undecanoate

3.2. Forced degradation study

The forced degradation of TU was done in acidic, basic, thermal, oxidative and photolytic conditions. The degradation of TU was observed in acidic and basic conditions. In both the conditions, the impurity formed was identified as imp-1. Slight degradation was observed in oxidation condition. There was no impact of thermal and photolytic conditions on TU. The peak purity results of the analyte peak obtained from the Photodiode array detector in all the stress samples confirmed that method is stability indicating. The chromatograms are showed in Fig. 3 and data is tabulated in Table (3).

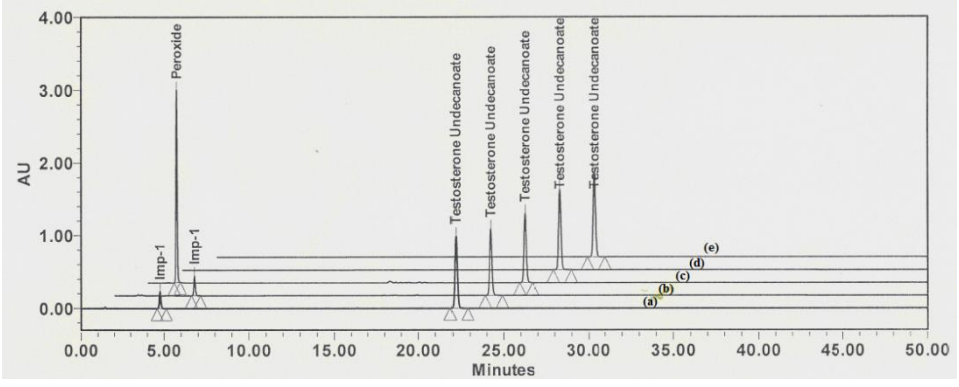


Figure (3): Chromatogram of forced degradation studies of Testosterone Undecanoate: (a) acid hydrolysis, (b) base hydrolysis, (c) oxidative degradation, (d) thermal degradation, (e) photolytic degradation

Table (3)
Percentage of degradation under different conditions using Testosterone sample at 500 µg/mL

Condition	Degradation %
Acidic (0.5N HCl, 60°C, 4 h)	13.3
Basic (0.5N NaOH, 60°C, 20 min)	16.2
Oxidation (5% H_2O_2, 60°C, 6 h)	5.3
Thermal (50°C, 48 h)	0.05
Photolytic (Photolytic chamber, 10 days, 25°C)	0.03

4. Method validation

As per ICH guidelines the method must be validated to establish specificity, solution stability, sensitivity, linearity, precision, accuracy and robustness. The method validation was carried out for imp-1, imp-2, imp-3, imp-4, imp-5, imp-6 and TU. A summarized result of method validation was tabulated in Table (4).

Table (4)
Method Validation summary data

Parameter	Imp-1	Imp-2	Imp-3	Imp-4	Imp-5	Imp-6	TU
Retention Time (RT)	4.78	5.60	10.95	11.86	20.73	24.11	22.36
Relative RT	0.21	0.25	0.49	0.53	0.93	1.08	1.00
Resolution	--	4.75	32.57	6.21	45.80	6.49	6.47
Symmetry factor	1.09	1.07	1.04	1.04	1.03	1.09	1.05
Response factor	0.71	0.65	0.78	0.73	0.99	1.08	1.00
Linearity	0.9999	0.9999	0.9998	0.9998	0.9997	0.9998	0.9997
Detection Limit (µg/mL)	0.0452	0.0453	0.0454	0.0453	0.0453	0.0451	0.0452
Quantitation Limit (µg/mL)	0.151	0.151	0.151	0.151	0.151	0.150	0.151
Intra-day precision (n=6, % RSD)	0.545	0.267	0.271	0.345	0.889	0.515	--
Inter-day precision (n=6, % RSD)	0.745	0.556	0.350	0.692	0.763	0.711	--
Accuracy at LOQ (n=3, %)	96.2	93.2	84.1	86.0	95.7	94.5	--
Accuracy at 100 (n=3, %)	102.9	102.3	100.4	99.6	101.0	99.7	--
Accuracy at 150 (n=3, %)	100.1	99.5	99.2	99.3	99.0	96.8	--

4.1. Specificity

Specificity was the first parameter of the method. It demonstrated that the analyte peak was free from any interference from the impurities as well as from the blank. Specificity was proved by injecting blank, sample and spiked sample with impurities. The TU peak was well resolved from six impurities and no blank interference was observed. Forced degradation was also performed to prove that the method was specific as well as stability indicating.

4.2. Solution stability

Solution stability of TU and impurities were established by injecting spiked and unspiked sample solution in HPLC vial at 25°C in auto sampler. Area of each impurity was checked and found that the percentage difference from initial (0 h) and after 24 h was less than 4.0 hence the sample solution was stable upto 24 h at 25°C.

4.3. Sensitivity

The value of limit of detection (LOD) and limit of quantitation (LOQ) defines the sensitivity of the method. Lower the value, higher the sensitivity. The LOD and LOQ for each impurity (imp-1, imp-2, imp-3, imp-4, imp-5 and imp-6) and TU were determined and found to be in the range of 0.0452 µg/mL to 0.0454 µg/mL and 0.150 µg/mL to 0.151 µg/mL respectively.

4.4. Linearity and range

A linearity solution was prepared by diluting the standard stock solution of desired concentration. Linearity of the method was determined from LOQ to 150% of impurity limit [0.15% of the drug concentration

(500 µg/mL)].The value of correlation coefficient for all impurities were higher than 0.995, which showed that the method has good correlation between the peak response and concentration of impurities (Fig. 4).

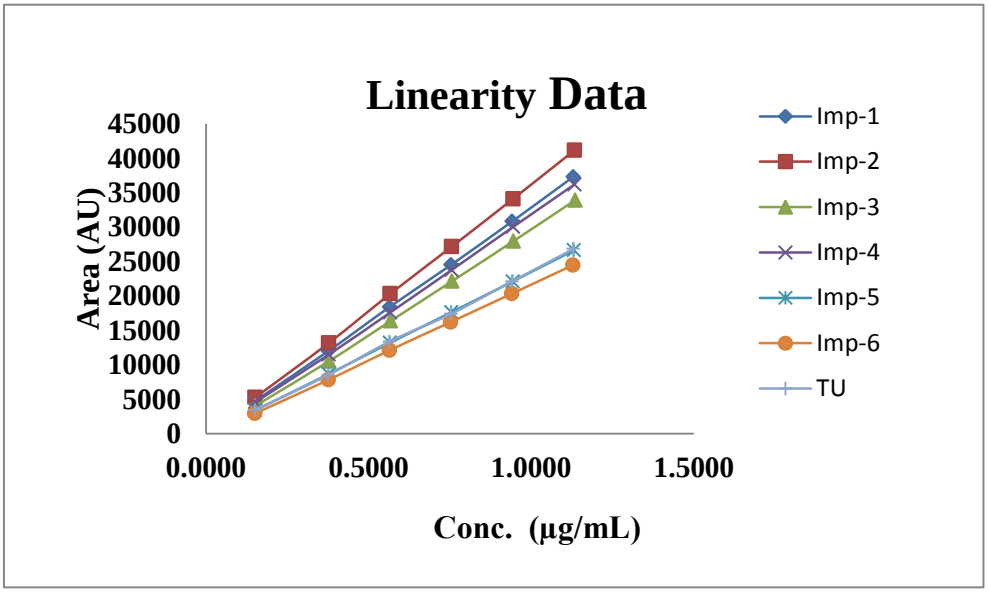


Figure (4): Linearity data of all impurities and Testosterone Undecanoate

4.5. Accuracy

Recovery studies demonstrated accuracy of the method. In accuracy, a known amount of TU sample (500 µg/mL) was spiked with known concentration of impurities in triplicate at LOQ level (0.03% of the drug substances), 100% level (0.15% of the drug substances) and 150% level (0.225% of the drug substances). The percentage recovery for each impurity was calculated by comparing the added concentration with recovered concentration. The percentage recovery was found in the range of 84.1% to 102.9%.

4.6. Precision

The Intra-day precision of the method was established by injecting six separate preparations of TU (500 µg/mL) spiked with 0.15% (0.75 µg/mL) of each impurity. The inter-day precision was also done in same lab by different analyst on different instrument and on different dates. The percentage RSD for each impurity was found below 1.0% in both the precision.

4.7. Robustness

The studies showed that there was no impact on the method even by deliberately changing the chromatographic parameters (i.e. column temperature, flow rate and mobile phase composition). It was observed that the resolution among all the impurities and TU peak was higher than 4.0. This showed the robustness of the method.

5. Conclusion

The above study demonstrates that the developed HPLC method was suitable for the determination of potential impurities of TU. The method is simple, specific, sensitive, precise, linear and accurate. The validation study was done as per the current ICH guidelines and can be used for routine as well as stability studies.

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التحديد الكمي للشوائب المحتملة الموجودة في المكون الصيدلاني undecanoate التستوستيرون النشط عن طريق الاستقرار بالإشارة إلى طريقة HPLC باستخدام كاشف للأشعة فوق البنفسجية

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ملخص

تتضمن الدراسة التطوير الذي يليه التحقق من الشوائب المحتملة في التستوستيرون undecanoate بواسطة تقنية الفصل الكروماتوجرافي السائل عالي الضغط (HPLC). تم تحقيق الفصل الكروماتوجرافي للشوائب المحتملة ومنتجات التحلل في العمود C8 لحزمة YMC (150 مم × ٤,٦ مم ، ٥ ميكرومتر) باستخدام طريقة شطف التدرج. بالنسبة لطريقة شطف التدرج ، تم استخدام الطور المتحرك A ومرحلة B المحمولة. تم تحضير الطور المتحرك A باستخدام خليط من الأسيتونتريل بالماء بنسبة (٩٠:١٠ ، v / v) وكان الطور المتحرك B فقط acetonitrile. تم الحفاظ على درجة حرارة العمود عند ٣٥ درجة مئوية خلال التحليل، وتم اختيار طول موجة ٢٤٠ نانومتر للتحليل. إن الطريقة المتطورة الحالية محددة، وخطية، ودقيقة. تم تأكيد خصوصية الطريقة من خلال تحليل نقاء الذروة باستخدام كاشف صفيف ضوئي. التستوستيرون هو التدهور الرئيسي. تم إجراء دراسة التحقق وفقًا لإرشادات ICH الحالية.

الكلمات الدالة: التستوستيرون undecanoate، العلاج باستبدال التستوستيرون، قصور الغدد التناسلية الذكور.

تاريخ استلام البحث ٢٠١٧/١٠/٣١ وتاريخ قبوله للنشر ٢٠١٨/٦/١١.