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TRACE LEVEL QUANTIFICATION OF CYPIONYL CHLORIDE IN TESTOSTERONE CYPIONATE BY PRE COLUMN DERIVATIZATION WITH 2-BUTANOL BY GAS CHROMATOGRAPHY WITH ELECTRON IMPACT IONIZATION DETECTOR

Nagaraju Rajana[*1,3], P. Madhavan [1], J. Moses Babu [2], K. Basavaiah [3] and Dharamasoth Rama Devi [4]

Scientist in Analytical Research & Development [1], Custom Pharmaceutical Services, Dr. Reddy's Laboratories Ltd., Miyapur, Hyderabad - 500049, Telangana, India.
Integrated Product Development Organization [2], Dr. Reddy's Laboratories, Innovation Plaza, Bachupally - 500072, Telangana, India.
Department of Inorganic and Analytical Chemistry [3], AU College of Pharmaceutical Sciences [4], Andhra University, Visakhapatnam - 530003, Andhra Pradesh, India.

Keywords:

Cypionyl chloride, Gas chromatography, Electron impact ionization, Validation, Derivatization, Quantification

Correspondence to Author:
Nagaraju Rajana

Scientist in Analytical Research and Development, Custom Pharmaceutical Services, Dr. Reddy's Laboratories Ltd., Miyapur, Hyderabad - 500049, Telangana, India.
E-mail: nagarajurajana@drreddys.com

ABSTRACT: The author has been proposed the precolumn derivatization method for the trace level quantification of cypionyl chloride in testosterone cypionate, it maybe potential impurity in the testosterone cypionate, it has to quantify in the testosterone drug Substance. The method development of cypionyl chloride has been started with gas chromatography with FID detector. The detection of cypionyl chloride was very poor and detection was less even for derivatization with 2-butanol in GC-FID detector. The GC-MS Electron impact ionization detector with derivatization by 2-butanol was developed and validated for trace level quantification of cypionyl chloride in Testosterone cypionate. The LOQ of this method was 1.7 ppm and the LOD was 0.6 ppm. The validation parameters were done against the ICH Q2 R1 guidelines. The precision, linearity, accuracy and solution stability was met the ICH Q2 R1 guidelines.

INTRODUCTION: Cypionyl chloride is key starting material for the testosterone cypionate, it is one of the many synthetic versions of testosterone. testosterone enanthate is first popular and testosterone cypionate is the second most popular testosterone. Testosterone cypionate delivered to the body by an oil-soluble intramuscular injection. This type of injection is called as DEPO-Testosterone.

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Testosterone cypionate is basically esterified testosterone, so it has increased lipid solubility. Testosterone cypionate is an injection is excreted in urine as sulfuric acid and glucuronic acid conjugates of testosterone. It is useful for individuals under 18 years of age. Also it is not be used by women, especially those who are pregnant, because it causes damage to the fetus. Testosterone cypionate is used in place of natural testosterone in men, who are suffering from testosterone levels.

It is a prescription drug and legally speaking. The body cannot store testosterone, which means if you are suffering from a deficiency you will have to continue taking these injections at regular intervals and in proper doses, it is also used by athletes and body builders for its anabolic properties.

Testosterone cypionate can help promote fat loss, increase sex drive, help in gaining muscle mass, and increase the density of bone. Like testosterone, testosterone cypionate also has an anabolic and androgenic rating of 100. It has a long half-life and a slow release rate of about 8 - 10 days. It is similar to Testosterone enanthate in its effect and you can even switch between them [1]. The study of Genotoxic and cytotoxic effects of testosterone cypionate was done by many analytical techniques [2, 3], the physical, chemical and biological techniques were present in drug bank of testosterone cypionate [4], HPLC methods were available for the determination of Testosterone cypionate as drug substance and in drug product [5, 6], many LC-MS and GC-MS analytical methods were available for determination of the Testosterone and its esters [7 - 11].

As per the knowledge of author no separate analytical method available for the determination of cypionyl chloride in testosterone cypionate. The proposed GC-MS method is unique to trace level quantification of cypionyl chloride in Testosterone cypionate. This method was not possible in Liquid chromatography with any type of detector *i.e.* UV-Visible, ELSD, RI, Mass (ESI or APCI) detector because when the contact of cypionyl chloride with aqueous mobile phase, it may convert to Cypionyl acid and also this impurity highly polar and UV inactive. Hence the trace level quantification can be done by using Gas chromatography with mass spectrometer **Fig. 1**.

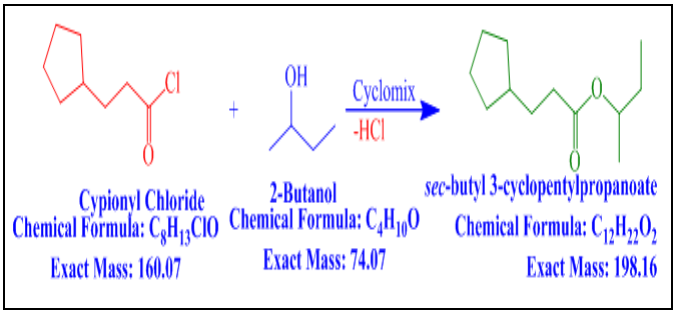

FIG.1: SCHEMATIC REPRESENTATION OF DERIVATIZATION OF CYPIONYL CHLORIDE WITH 2-BUTANOL

MATERIALS AND METHODS:

Chemicals and Reagents: Analytical grade 2-butanol, Methanol, 2-propanol, Ethanol and Acetonitrilewere purchased from Merck Mumbai, India, Cypionyl chloride and Testosterone cypionate for research, were obtained from Dr. Reddy's laboratories Ltd., Hyderabad India.

Instrumentation and Chromatographic Conditions: Agilent 7890A Gas chromatograph equipped with 5975C Mass selective detector, the GC method conditions were DB-5 MS (30 m × 0.32 mm × 0.25 µm), temperature program 80 °C (0 min hold) to 240 °C (12 min hold) @ 20 °C / min to 280 (9.0 min hold) @ 40 °C / min, injector temperature: 200 °C, Auxiliary temperature: 280°C, diluent: 2-Butanol anhydrous, carrier gas: 1.2 mL/min (Helium) Split ratio: 15 : 1, Injection Volume : 1.0 µL, run time : 30.0 min, retention time of Cypionyl chloride (related butyl ester): 4.9 min, wash vials solvent: Acetonitrile. The mass conditions were ion source: Electron impact ionization, source temperature: 230 °C, quadrupole temperature: 150 °C, EMV mode: Gain factor, solvent delay: 4.3 min, timed events off: 6.2 min, resolution: Low, ions / dwell time: 125/100 (ms), 143/100 (ms) and 169/100 (ms).

Standard and Sample Preparation:

Preparation of Cypionyl Chloride (Underivative) Stock Solution-1: Weighed 49.10 mg of Cypionyl chloride into a 50 mL volumetric flask containing 10 mL of 2-butanol, dissolved by cyclomixing and made up to the mark with the acetonitrile.

Preparation of Cypionyl Chloride (Underivative) Stock Solution-2: Transferred 0.5 mL stock solution-1 into a 25 mL volumetric flask, containing 10 mL diluent and made up to the mark with acetonitrile.

Preparation of Linearity Solutions Solution (derivatized): Pipetted out 0.24 mL, 0.36 mL, 0.48 mL, 0.72 mL and 0.96 mL from standard stock solution-2 and transferred into five separate 50 mL volumetric flasks containing 10 mL of 2-butanol. Mixed it well and made up to the mark with 2-butanol to get the concentration of 1.9 ppm, ppm, 2.8 ppm, 3.7 ppm, 5.6 ppm and 7.6 ppm with respect to nominal analyte concentration of 50 mg/ mL

Preparation Spiked Solution: Weighed about 250 mg of test sample and transferred into 5 mL of volumetric flask containing 3.0 mL of 2-butanol

and 0.022 mL of stock-2 solution of standard and made up to the mark with diluent.

Preparation Test Solution: Weighed about 250 mg of test sample and transferred into 5 mL of volumetric flask containing 3.0 mL of 2-butanol and made upto the mark with diluent.

RESULT AND DISCUSSION:

Method Development and Optimization: Method development of Cypionyl chloride was started with Gas chromatography with ALS injector, the response is very poor with neat injection and injector was blocked, hence started the development with pre column derivatization with 2-butanol and other alcohols also like methanol, ethanol and 2-Propanol, but response was less in GC-FID experiment. The study was extended to Gas chromatography with Electron impact ionization.

The Scan mode of the cypionyl ester was observed as 198 m/z but very less abundant and the abundant peaks were as 169, 143 and 125 m/z mass numbers, then chosen the selected ion monitoring for quantifying the Cypionyl chloride as cypionyl butyl ester **Fig. 2** but other esters showing all ions are very less abundance **Fig. 3**.

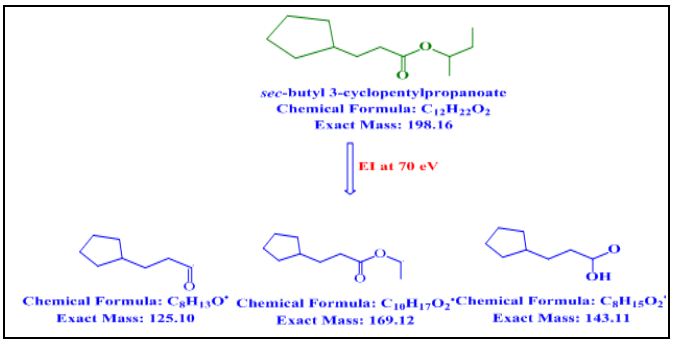

FIG. 2: PROPOSED FRAGMENTATION PATTERN OF CYPIONYL CHLORIDE BUTYL ESTER

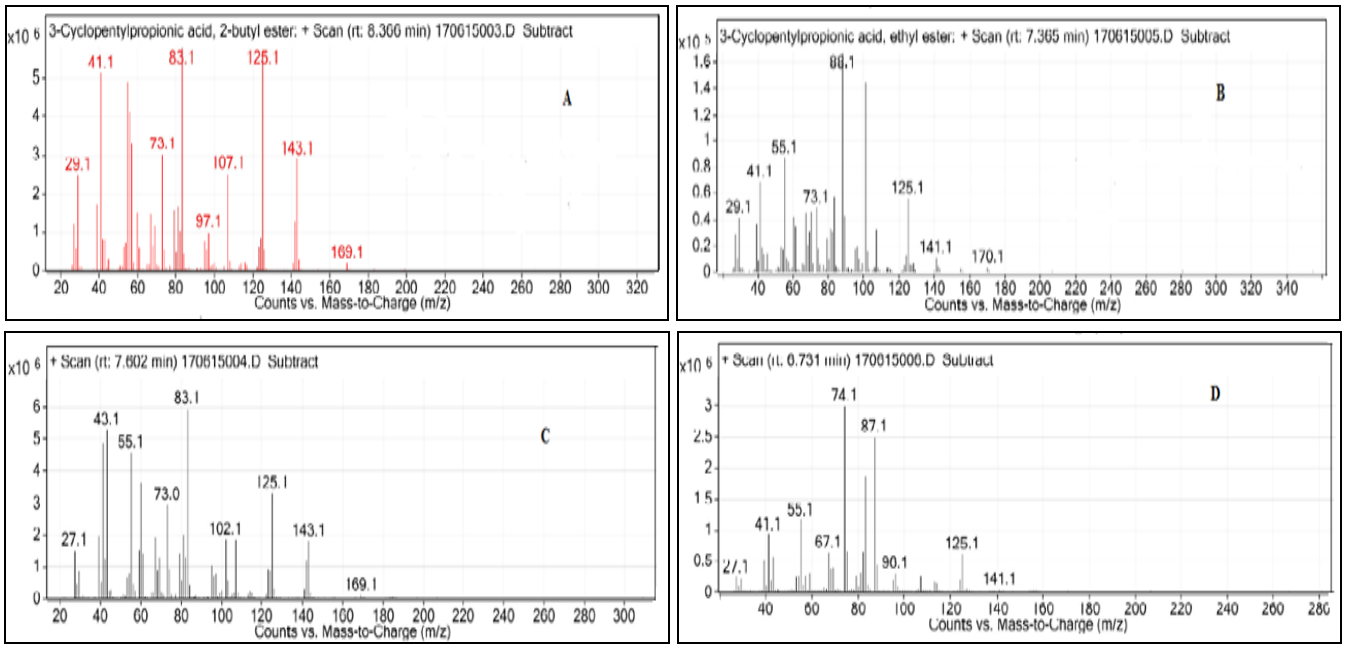

FIG. 3: SCAN MODE M/Z OF A) BUTYL ESTER B) ETHYL ESTER C) ISOPROPYL ESTER D) METHYL ESTER

Screened different stationary phases from non-polar to polar columns, the dimethyl poly siloxanes stationary phase (DB-1 column) was used for screening the all esters, the peak elution was late and, peak shape was broad. The mid polar column with 5% phenyl and 95 dimethyl arylene siloxane column was selected (HP-5, DB-17 and DB-5 MS column), and optimized with different oven program conditions, the peak shape was observed good and recovery study was done in Testosterone cypionate with DB-5 MS.30 m × 0.32 mm × 0.25 µm. 80 °C (0 min hold) to 240 °C (12 min hold) @ 20 °C / min to 280 (9.0 min hold) @ 40 °C / min, injector temperature: 200 °C, Auxiliary temperature: 280° C, diluent: acetonitrile and derivatization solvent as 2-butanol anhydrous, carrier gas: 1.2 mL/min (Helium) Split ratio: 15: 1, Injection Volume: 1.0 µL, run time: 30.0 min, retention time of Cypionyl chloride (related butyl ester): 4.9 min, wash vials solvent: Acetonitrile. The mass conditions were ion source: Electron impact ionization, source temperature: 230 °C,

quadrupole temperature: 150 °C, EMV mode: Gain factor, solvent delay: 4.3 min, timed events off: 6.2 min, resolution: Low, ions / dwell time: 125/100 (ms), 143/100 (ms) and 169/100 (ms) **Fig. 4**.

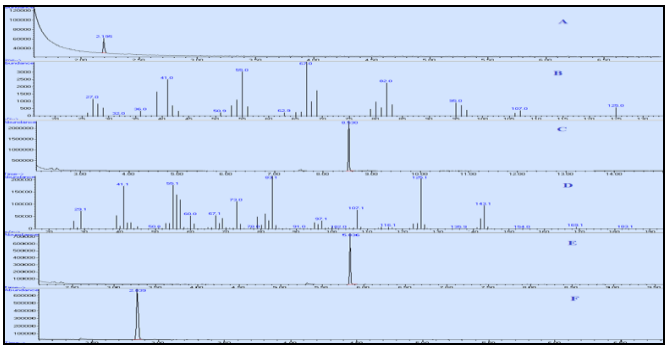

FIG. 4: A) TIC OF CYPIONYL CHLORIDE B) MASS SPECTRUM OF CYPIONYL CHLORIDE WITH HP-5 COLUMN C) TIC OF BUTYL ESTER WITH HP-5 COLUMN D) MASS SPECTRUM OF BUTYL ESTER WITH HP-5 COLUMN E) TIC OF BUTYL ESTER WITH HP-5 COLUMN F) TIC OF BUTYL ESTER WITH DB-17 COLUMN

Method Validation: Method validation study was done for Cypionyl chloride in Testosterone cypionate by pre column derivitization with Gas chromatography and Electon impact ionization detector the as per ICH Q2 (R1) as part of limit test and Validation of compendial procedures-USP (1225).

Specificity: Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, and matrix. The specificity of Cypionyl chloride as butyl ester by spiking 3.8 ppm in Testosterone cypionate sample (50 mg/mL) and injected in GC-MS-EI in both Scan and SIM mode. In scan mode the homogeneity of peak was passed and selected mass numbers observed in SIM mode *i.e.* 125/100 (ms), 143/100 (ms) and 169/100 (ms). The Testosterone cypionate peak was not observed in TIC because the Testosterone cypionate was high boiler and it not was entered the column.

Linearity: Linearity of optimized method was studied and satisfactory with the all range of concentrations *i.e.* 1.9 ppm to 7.2 ppm (50 mg/mL was the test sample concentration). The linearity graph was drawn between the peak areas versus the concentration of butyl ester. The correlation coefficient (R), intercept (C), slope (m), were calculated by using linear regression type. The correlation coefficient was 0.998 for cypionyl butyl ester at the range from of 1.9 ppm to 7.2 ppm. This linearity mass range can be used to quantify the cypionyl butyl ester in Testosterone cypionate sample.

Precision: The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision study was done at 1.7, 3.8, 5.7 ppm with different preparation of same homogeneous solution and the % RSD were 0.8, 1.2, 1.9 respectively and the developed method was precise at limit of quantification, 3.8 ppm and 5.7 ppm and it can used for quantification at 1.7 ppm in Testosterone cypionate drug substance **Fig. 5** and **Fig. 6**.

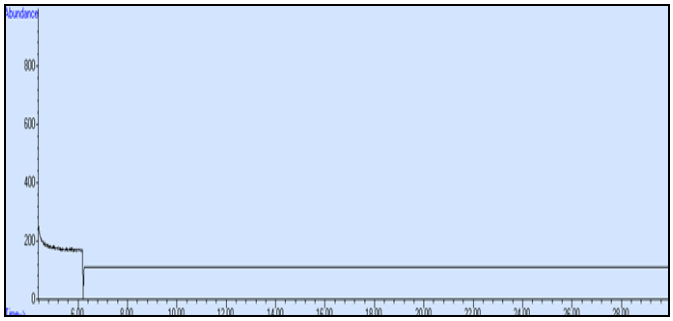

FIG. 5: SELECTED ION MONITORING BLANK TIC

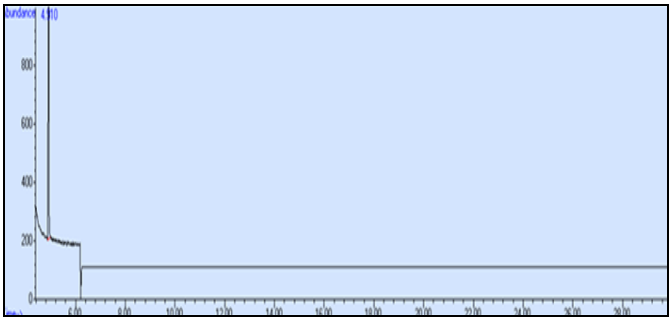

FIG. 6: SELECTED ION MONITORING 3.8 PPM TIC

Accuracy: The accuracy of an analytical procedure expresses 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. The accuracy study was performed at 1.7, 3.8, 5.7 ppm in active pharmaceutical ingredients. The average % recovery for cypionyl chloride in Testosterone cypionate were 95.3, 100.7, 103.6 at 1.7, 3.8, and 5.7 ppm respectively **Fig. 7, 8** and **9**.

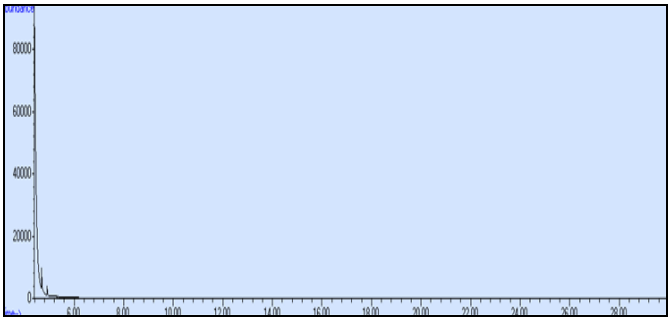

FIG. 7: TESTOSTERONE TEST SAMPLE TIC

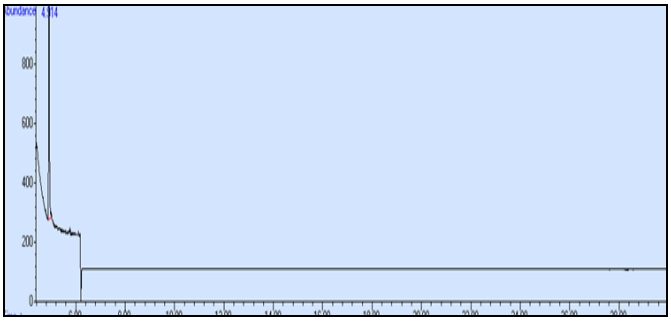

FIG. 8: ACCURACY AT 3.8 PPM TIC

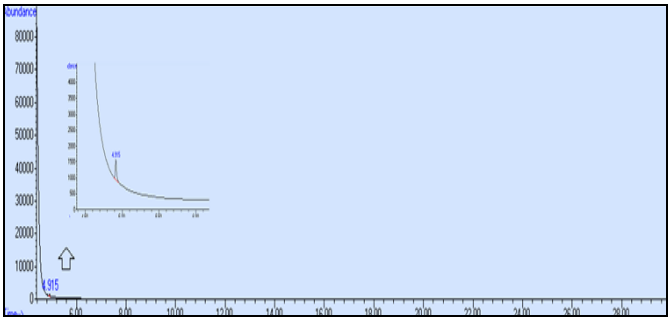

FIG. 9: ACCURACY AT LOQ TIC

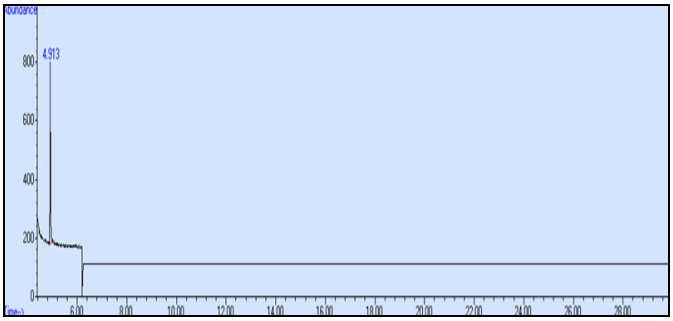

FIG. 10: SELECTED ION MONITORING LOQ TIC

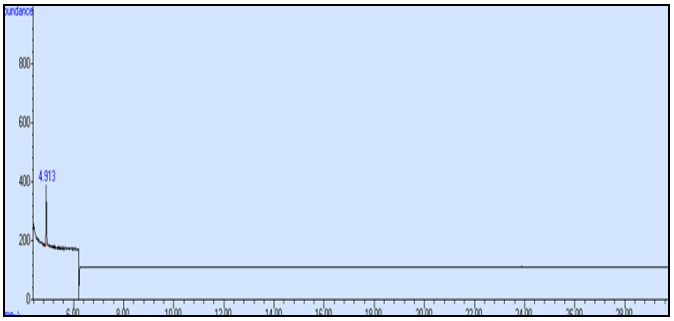

FIG. 11: SELECTED ION MONITORING LOD TIC

TABLE 1: VALIDATION SUMMARY

Validation parameter Regression equation	Results
Slope	4824
Intercept	744
R value	0.998
R2 value	0.995
LOD & LOQ	
LOD (in ppm)	0.6
LOQ (in ppm)	1.7
Precision	
Precision at LOQ n = 6 (% RSD)	0.8
Precision at 3.8 ppm n = 6 (% RSD)	1.2
Precision at 5.7 ppm n = 6 (% RSD)	1.9
Intermediate Precision at 3.8 ppm n = 6 (% RSD)	1.5
Cumulative Precision at 3.8 ppm n = 12 (% RSD)	2.3
Recovery	
% Recovery at 1.7 ppm	95.3
% Recovery at 3.8 ppm	100.7
% Recovery at 5.7 ppm	103.6
Solution stability	
Standard solution stability (% Variation at 18 hrs)	0.2
Spiked solution stability (% Variation at 18 hrs)	-1.7

LOD: Limit of detection; LOQ: Limit of quantification.

Limit of Detection (LOD), Limit of Quantification (LOQ) and Precision at LOQ: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy, ICH Q2 (R1), the LOD and LOQ values for the Cypionyl chloride in the Testosterone cypionate was performed by STEYX method and the LOD and LOQ values were 1.7 ppm and 0.6 ppm respectively. The LOQ precision was done by injecting (n = 6) LOQ solution and % RSD values were calculated **Fig. 10** and **11**, less than 1.0%, results were captured in **Table 1**.

Solution Stability: The solution stability was performed to study the stability of the cypionyl chloride as ester at room temperature and the

Cypionyl chloride as butyl ester at room temperature was stable upto 48 h and the precision was within the range of 15% and the spiked solution of cypionyl chloride in Testosterone cypionate at 3.8 ppm was studied up to 18 hrs and the % variation from final to initial were 0.2 ppm and -1.7 ppm at standard solution stability and spiked solution stability. The results were captured in **Table 1**.

CONCLUSION: Based on the above method development study and validation for the limit test of Cypionyl chloride in Testosterone cypionate. The limit test method was precise, accurate, linear, robust, and rugged. The solution was stable at Limit of quantification by using Gas Chromatography and mass spectrometry. As the knowledge of author, this is first limit test method of cypionyl chloride in Testosterone cypionate with Limit of Quantification as 1.7 ppm and limit of detection as 0.6 ppm and the method can be used for intended purpose.

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CONFLICT OF INTEREST: No conflict of interest, Dr. Reddy's Laboratories IPDO IPM-00558 is allotted for the this article.

REFERENCES:

1. http://www.anabolicsteroids.net/testosterone-cypionate.php.
2. Genotoxic and cytotoxic effects of testosterone cypionate (deposteron®).
3. Pulmonary Eosinophilia Caused by Testosterone Cypionate.
4. https://www.drugbank.ca/salts/DBSALT001298.
5. Development of the fast, simple and fully validated high performance liquid chromatographic method with diode array detector for quantification of testosterone esters in an oil-based injectable dosage form.
6. Gupta VD: Quantitation of Testosterone in Suspension using High Performance Liquid Chromatography.
7. Determination of testosterone esters in serum by liquid chromatography – tandem mass spectrometry (LC-MS-MS) Erica Törnvall Final Thesis performed at National Board of Forensic Medicine.
8. Detectability of testosterone esters and estradiol benzoate in bovine hair and plasma following pour-on treatment.doi: 10.1007/s00216-009-3037-9.
9. Simona M, Marialinda F and Marcello C: Validation of a LC-MS-MS method for anabolic steroids in nutritional supplements.
10. http://www.pharmpress.com/files/docs/Sample%20chapter.pdf.
11. Marie B, Vincent C, Pascal K, Marion V, Laboratoire C, Illkirch F, Timothy J, Waters C, Manchester and Jean-Marc Joumier: UK Waters Corporations, St. Quentinen Yvelines, Screening for anabolic Steroids and their esters using hair analyzed by GC tandem quadrupole. France.http://www.waters.com/webassets/cms/library/docs/720002251en.pdf.
12. Raman NVVSS, Prasad AVSS and Reddy KR: Strategies for the identification, control and determination of genotoxic impurities in drug substances: A pharmaceutical industry perspective. J. Pharm. Biomed. Anal. 2011; 55: 662-667.
13. Dow LK, Hansen MM, Pack BW, Page TJ and Baertschi SW: The assessment of impurities for genotoxic potential and subsequent control in drug substance and drug product. J. Pharm. Sci. 2013, 102: 1404-1418.
14. Robinson, D. I. Control of Genotoxic Impurities in Active Pharmaceutical Ingredients: A Review and Perspective. Org. Proc. Res. Dev. 2010, 14, 946-959.
15. Elder DP, Lipczynski AM and Teasdale A: Control and analysis of alkyl and benzyl halides and other related reactive organohalides as potential genotoxic impurities in active pharmaceutical ingredients (APIs). J. Pharm. Biomed. Anal. 2008; 48: 497-507.
16. Bolt HM, Foth H, Hengstler JG and Degen GH: Carcinogenicity categorization of chemicals - New aspects to be considered in a European perspective. Toxicol. Lett. 2004; 151: 29-41.
17. European Medicine Agency (EMeA). Committee for Medicinal Products for Human Use (CHMP). Guideline on the Limits of Genotoxic Impurities. Available online: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002903.pdf (accessed on 16 January 2015).
18. ICH Guideline M7 on Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk. Available online: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2013/02/WC500139217.pdf (accessed on 16 January 2015).
19. ICH Q2 (R1). Validation of Analytical Procedures: Text and Methodology Q2(R1). Available online: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002662.pdf (accessed on 16 January 2015).

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