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METHOD DEVELOPMENT AND VALIDATION OF CYPROTERONE ACETATE IN BULK AND PHARMACEUTICAL DOSAGE FORM USING RP-HPLC

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Abstract

Cyproterone acetate (CA) is an anti-androgenic drug with progestogenic activity. CA binds to the androgen receptor and prevents androgen-induced receptor activation in target tissues, inhibiting the growth of testosterone-sensitive tumor cells. The current method aims to develop an RP-HPLC method for the detection of CA in both its pure and formulated form. A simple and robust method was developed where separation was carried out with a mobile phase of Acetonitrile: Water at the ratio of 80:20 and flow rate of 1.5ml/min. The detection wavelength was 281 nm. The linearity was performed for a concentration range of 5-25 µg/ml and the regression coefficient were found to be 0.9991. All other validation parameters like accuracy, precision, LOD, LOQ, robustness & ruggedness were performed as per ICH guidelines and results were found to be within limits. The % assay was found to be 97.32 – 98.08%. Though many methods require complex mobile phases such as buffers, where pH must also be maintained, the current research work develops the method using a simple, inexpensive, and easily available mobile phase composition using milli-Q water and acetonitrile. Hence, the proposed data concludes that the developed RP-HPLC method can be used for routine quality control of CA in any formulation.

Keywords: Cyproterone acetate; HPLC; Method Development;

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INTRODUCTION

Cyproterone acetate (CA) is an anti-androgenic drug with progestogenic activity. It is frequently used in combination with other stable estrogens like Ethinyl estradiol (Scott & Soltero, 1987). CA is chemically 6-chloro-1β,2β-dihydro-17α-hydroxy-3`H-cyclopropa [1,2]-pregna1,4,6-triene-3,20-dione acetate and its structure is given in figure 1. It is used in males to treat prostatic cancer and to control libido in extreme hypersexual deviation. In females CA along with ethinyl estradiol is used for control of acne and idiopathic hirsutism (Segall et al., 2000).

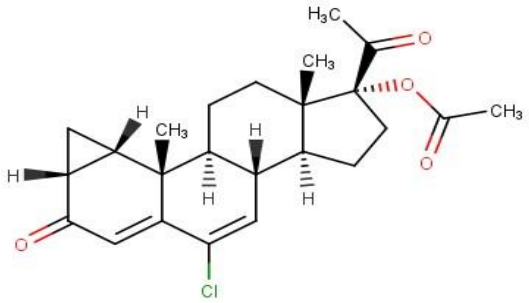

Figure 1: Structure of Cyproterone acetate

CA is a BCS class II compound with low solubility and strong permeability. CA has a poor GI absorption rate and is rapidly metabolised by various pathways (Hydroxylation, conjugation) (Christiaens et al., 2003). Cyproterone binds with the androgen receptor which prevents androgen-induced receptor activation in target tissues, inhibiting the development of testosterone-sensitive tumor cells. CA also has progestational agonist effects at the pituitary level, which reduces luteinizing hormone. This results in reduction of serum testosterone levels and testicular androgen secretion (PubChem, n.d.).

Upon a thorough literature review, few methods reported include determination of megestrol acetate and Cyproterone acetate in serum of patients with advanced breast cancer by HPLC (Dikkeschei et al., 1990), HPLC incorporating to short column and micellar mobile phase for determination of some contraceptive drugs (Chaida & Youngvises, 2019). HPLC method for the analysis of Cyproterone acetate in tablets (Cannell et al., 1981). The current study reports the development of the RP-HPLC method with better detection of CA in its pure and formulated form. Though many methods require complex mobile phases such as buffers, where pH must also be maintained, the current research work develops the method using a simple, inexpensive, and easily available mobile phase composition using milli-Q water and acetonitrile, which helps in faster elution of the drug(Shah et al., 2021). Thus, developed method is validated with all parameters as per ICH guidelines.

MATERIALS AND METHODS:

Materials

Acetonitrile of HPLC grade by Rankem, Water from milli-Q RO framework (Model- TLD-75-S) were used. Working standard of Cyproterone acetate was obtained as gift sample from Indian Pharmacopoeia Commission (IPC) New Delhi, India.

Instrumentation

Shimadzu Prominence HPLC (Tokyo, Japan) system LC-20AT solvent delivery system with manual 20 µL loop capacity sample injector, UV detector SPD-20A and Lab solution CS software for data management was used. Hibar RP_{18} (250 mm × 4.6 mm i.d., 5 µm) was used as the stationary phase, and acetonitrile: water in an 80:20 ratio as the mobile phase at a flow rate of 1.5 ml/min with a detection wavelength of 281 nm.

PREPARATION OF SOLUTIONS

Preparation of standard solutions

10 mg of CA was accurately weighed and transferred into 10 ml volumetric flask. It

is then diluted up to the mark using acetonitrile (1mg/ml).

Preparation of working standard

From the above solution 0.1ml was pipetted and diluted up to 10 ml using acetonitrile (10 µg/ml).

Preparation of sample solution

10 tablets were accurately weighed and powdered. Powder equivalent to 10 mg of Cyproterone acetate was weighed and transferred into 100 ml volumetric flask, dissolve the contents with acetonitrile and filtered. This filtrate was further diluted using acetonitrile.

Method Validation

The developed method was validated using all the following parameters as per ICH guidelines (*Q2(R1) Guideline.Pdf*, n.d.) (Snyder et al., 2012);

Accuracy:

Accuracy was determined as the closeness of the obtained value to the true value. It was calculated using recovery studies. The concentrations used were LQC, MQC, HQC.

Precision:

Precision describes variations between individual values when determined repeatedly. Precision was carried out using inter-day and intra-day comparison studies with a concentration of LQC, MQC, HQC

Linearity:

Linearity is defined as the ability of the analytical method to produce results that are directly proportional to the concentration of analyte. Linearity was performed with five different concentrations covering the range of 5-25 µg/ml. Results were assessed using the regression coefficient.

Specificity:

Specificity is defined as the ability of the analytical method to detect analyte response in presence of impurities/excipients/interfering substances.

Limit of Detection (LOD) and Limit of Quantification (LOQ):

LOD and LOQ are defined as the lowest concentration at which the analyte can be determined and quantified. The signal to noise ratio of LOD is 1:3 and for LOQ is 1:10.

Ruggedness and Robustness:

The ability of the method to produce content results when slight alterations are given like change in a flowrate, wavelength, mobile phase ratio, change in column, change of instrument, change of analyst, etc.

Assay:

Tablets were weighed, powdered and the powder equivalent to 10 mg of CA was calculated, weighed and dissolved it in 100ml ACN. From this 5, 15, and 25 µg/ml concentration was prepared and assay was performed using optimized chromatographic conditions. The LQC (5µg/ml), MQC (15µg/ml) and HQC (25µg/ml) solutions were injected and a chromatogram was recorded (**Figure 4**) standard deviation and % of drug present in the formulation was calculated.

RESULTS AND DISCUSSION:

Accuracy:

Accuracy was performed for LQC, MQC and HQC concentration and % recovery were calculated. The % recovery was found to be 97.32 – 98.08 % (**Table 1**).

Precision:

Inter-day and Intra-day precision was performed for LQC, MQC and HQC. % RSD were calculated and it was found to be within limits (**Table 2**).

Specificity:

Sample solution was injected and no peaks were eluted at retention time of Cyproterone acetate. Hence the method

was found to be specific for the determination of Cyproterone acetate in the formulation.

Linearity:

Five concentrations ranging from 5-25 µg/ml (**Table 3**) and the method was found to be linear with a regression coefficient of 0.9991 (**Figure 3**).

LOD and LOQ:

Limit of Detection was found to be 25.88 ng/ml and Limit of Quantification was found to be 78.42 ng/ml. Therefore, the method was found to have adequate sensitivity.

System Suitability:

System suitability of the method was performed by calculating the chromatographic parameters like theoretical Plates**,** tailing factor, symmetry factor, %RSD of peak area response on the repetitive injection of standard solutions **(Table 4)**.

Ruggedness and Robustness:

Alterations were made with in the developed procedure, noticeable changes were not found. Hence, the developed method was found to be rugged and robust **(Table 5)**.

Assay:

Assay was performed for LQC, MQC and LQC concentration of CA formulation and %assay was calculated. The amount of CA present in the formulation was found to be 97.32 – 98.08% and it is within limits **(Table 1)**.

CONCLUSION:

The developed RP-HPLC method was accurate, precise, and linear for the estimation of Cyproterone acetate in pure form and in its formulation. The retention time for the standard solution and sample solutions were found to be 3.885 and 3.980. The developed method was validated using all parameters like accuracy, precision, linearity, specificity, LOD, LOQ, ruggedness, and robustness. System suitability parameters were performed to determine column efficiency and peak symmetry. The developed method can be used for routine quality control analysis of CA in any formulation.

CONFLICT OF INTEREST STATEMENT:

The author declares that there are no conflicts of interest.

ACKNOWLEDGEMENT:

Authors thank the Indian Pharmacopoeia Commission (IPC), New Delhi for providing the Cyproterone acetate working standard as gift sample.

ABBREVATIONS:

CA - Cyproterone acetate, RP-HPLC – Reversed Phase High Performance Liquid Chromatography, ACN – Acetonitrile, LOD – Limit of Detection, LOQ – Limit of Quantification, %RSD – Percentage Relative Standard deviation, SD – Standard Deviation, ICH – International Council of Harmonisation, UV – Ultra violet, IPC - Indian Pharmacopoeia Commission, LQC – Lowest Quality Control, MQC – Middle Quality Control, HQC – Highest Quality Control.

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Table 1: Recovery studies of Cyproterone acetate

Actual Concentration	Amount found	% Recovery
5 µg/ml	4.86	97.32
15 µg/ml	14.56	97.11
25 µg/ml	24.52	98.08

Table 2: Precision studies of Cyproterone acetate

Sample	Inter day		Intra day	
	Amount found ±SD	%RSD	Amount found ±SD	%RSD
5 µg/ml	193112.7 ± 1939.532	1.004	193897 ± 1940.601	1.00
15 µg/ml	574346.3 ± 5123.045	0.891	572003.3 ± 5582.048	0.975
25 µg/ml	859720.7 ± 4355.182	0.506	854166.7 ± 5643.586	0.660

Table 3: Linearity

Concentration (µg/ml)	Mean Peak area (n=3)
5	195017
10	343600.7
15	516497.7
20	686184.3
25	862547.3

Table 4: System Suitability

Parameters	Values
Theoretical Plates	>4053
Tailing Factor	1.196
Symmetry factor	1.051
%RSD of peak area response	1.070

Table 5: Robustness and Ruggedness

Parameters		% RSD
Flow rate (ml/min)	1.6	0.163
	1.4	0.19
Wavelength (nm)	280	0.321
	282	0.665
Mobile phase ratio (Acetonitrile:Water)	75:25	0.251
	85:15	0.157

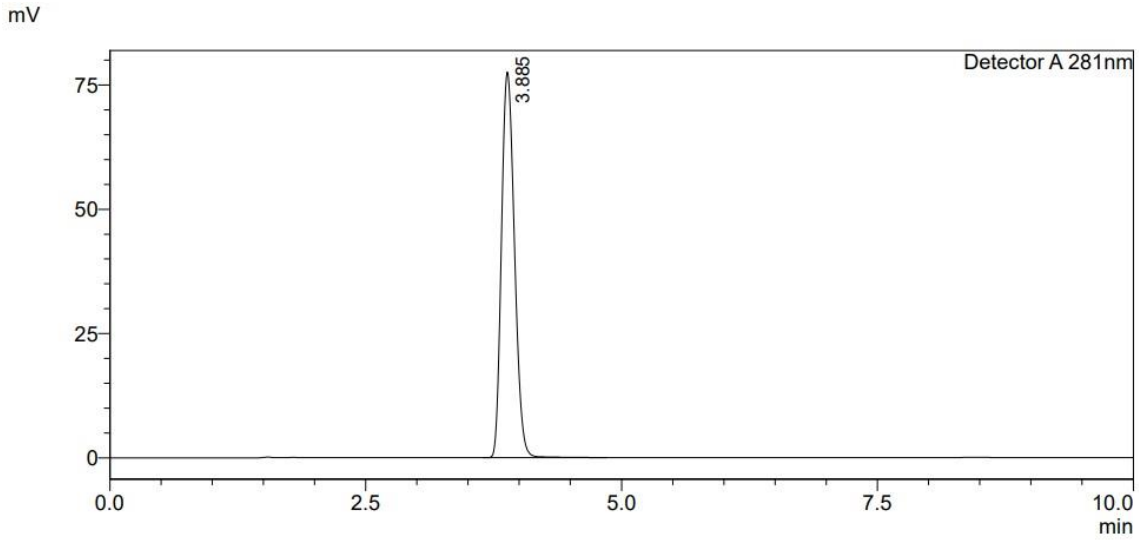

Figure 2: HPLC chromatogram of Cyproterone acetate Standard

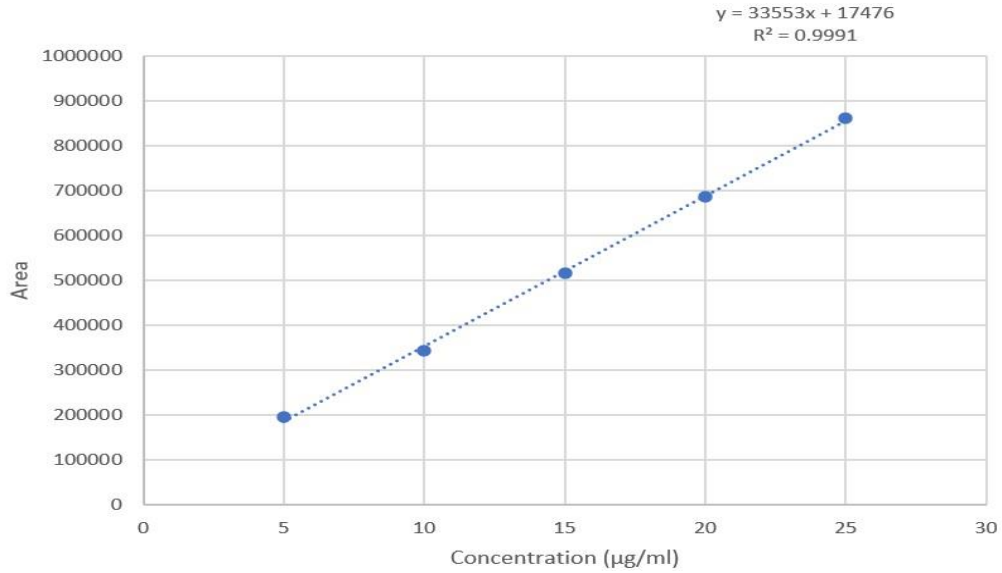

Figure 3: Calibration cure of Cyproterone acetate

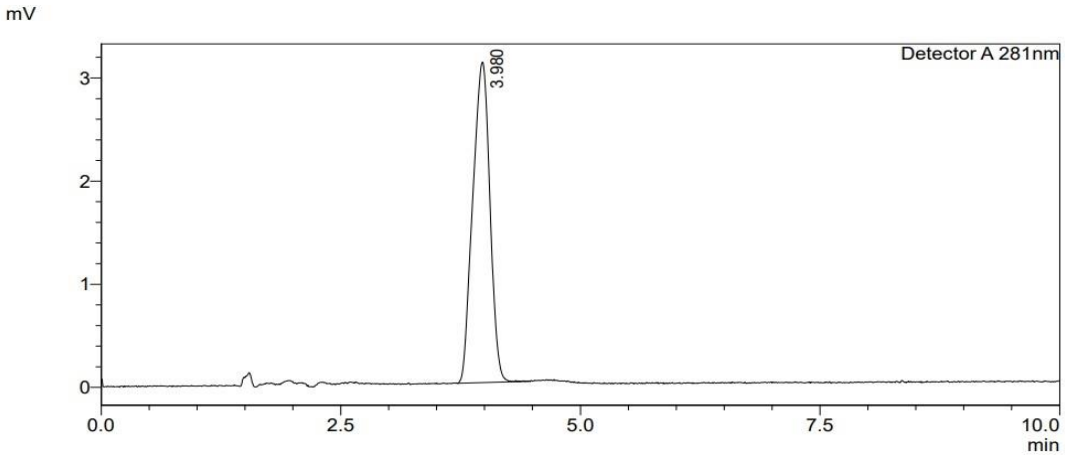

Figure 4: HPLC chromatogram of Cyproterone acetate Sample