



Research Paper

Development and validation of Casodex In RP- HPLC

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ABSTRACT

Casodex is an antiandrogen sold under the brand name Bicalutamide. To quantify it, a brand new, fast, inexpensive, and isocratic switch level superior execution fluid chromatography (RP-HPLC) system was developed. The system uses a Phosphate cradle with a pH 4.0-Methyl liquor in the mix of 30-70 as a flexible stage. The developed methodology was accepted as per ICH recommendations. The development was completed in an isocratic fashion inside the portable stage, and chromatographic separation was achieved using a WATERS Partnership 2695 detachment module using Programming: have interaction 2 and 996 PDA Finder at room temperature, with linearity seen in the range of 10-50 g/ml.

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1. INTRODUCTION

HPLC is in like manner alluded to as unreasonable pressure fluid chromatography considering unnecessary strain is utilized to improvement the skim price and inexperienced division through compelling the telephone phase through at tons higher price. The stress is finished the usage of a siphoning device¹. The improvement of HPLC from old fashion phase chromatography is probably ascribed to the improvement of greater modest molecule sizes. greater modest molecule length is critical considering they provide extra noteworthy floor district over the traditional sizeable molecule sizes. The HPLC is the method of inclination with inside the place of insightful science, taking into consideration this approach is explicit, vigorous, direct, precise and proper and the predicament of discovery is low and also it offers the ensuing advantages².

HPLC is the preferred approach in the field of coherent innovation because to its specificity, robustness, speed, accuracy, and clarity; its negligible personality hindrance; and its many distinct benefits. rapidity (a lot of tests need to be able to be done in a short amount of time). The primary objective of proposed work is:

To develop new simple, sensitive, accurate and economical analytical method for the estimation of Casodex in bulk and marketed pharmaceutical dosage form.

To validate the proposed method in accordance with USP and ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the Casodex in bulk and marketed pharmaceutical dosage form.

2. MATERIALS AND METHOD

Chemicals

S.No	Chemical	Brand names
1	CASODEX (Pure)	Casodex.
2	Water for HPLC	LICHROSOLV (MERCK)
3	Acetonitrile for HPLC	Merck

Preparation of standard stock solution for Casodex

Fifty milligrammes (mg) of CASODEX are weighed and transferred to a fifty millilitre (ml) clean glass volumetric jar (thousand micrograms (g) per millilitre). It has been dissolved in methanol, and a similar dissolvable has been used to increase the volume to 50 ml³. We sifted and sonicated the aforementioned mixture for around ten to fifteen minutes. Second

stockpile item: using the above setup (1000 g/ml), remove 8 ml with a pipette and fill the remaining space with methanol to a volume of 100 ml (80 g/ml). The group went through a sonication process for 10-15 minutes. inventory 3: pipette off 10 ml from the aforementioned arrangement (80 g/ml), and add enough methanol to get the total to 100 ml (8 g/ml). After a few minutes of sonication, the arrangement was complete.

Instrumentation

S.No	Instruments And Glass wares	Model
1	HPLC	WATERS Alliance 2695 separation module, Software: Empower 2, 996 PDA Detector.
2	pH meter	Labindia
3	Weighing machine	Sartorius
4	Volumetric flasks	Borosil
5	Pipettes and Burettes	Borosil
6	Beakers	Borosil
7	Digital ultra sonicator	Labman

Preparation of working standard for Casodex

Weakening was done in order to obtain a consciousness range of 100–1200ng/mL by starting with a value of 0. After adding methanol to make up the volume to 10 ml, 5 ml, 1.5 ml, 2.0 ml, 3.0 ml, 4.5 ml, 5.5 ml, and 6.0 ml from the stock-3 association (8,000 ng/ml) were sonicated for 10 to 15 minutes.

The 10 mg (BIC) of test material was transferred to a volumetric flagon holding 10 mL of methanol, and the volume was adjusted accordingly. The concentration of BIC in the final product dropped to 10 g/mL. Twenty microliters (L) of a consistent volume were infused into the sample, and the maximum height was measured⁴.

Method development

One of the best methods for checking drug concentration is reversed-phase high-performance liquid chromatography (RP-HPLC). since high-performance liquid chromatography uses a wide variety of chromatographic components. Improvement of trial conditions is a complex interaction including the kind and centralization of natural modifier, pH, support molarity, temperature, and flow rate, etc. therefore, an environmentally friendly approach, such as an exploratory strategy to embellish chromatographic walls, is of paramount importance⁵. In light of the pKa of CASODEX and the fact that most RP-HPLC procedures were developed using cradles in conjunction with particle pair reagents at an acidic pH, the pH of the transportable level is chosen to be on the acidic side, in particular with trifluro acidic corrosive, for convenience.

Aquity 50 x 2.1 mm, 1.7 phase was used to accomplish the initial preliminary work, and the results were tested for reproducibility. Too

rapid top elution and an incorrect plate count of less than 2000 prevent the achievement of a symmetrical peak shape, leading to the selection of the Aquity 100 x 2.1mm 1.7 section. It was shown that the most agreeable ratio for dividing up Salt degradant tops while maintaining a flow rate of 0.5ml/minute and a temperature of 40°C is 52:48 for flexible levels An and B, respectively. The absorbance maxima at 270nm were validated by PDA testing from 200 to 400nm for the CASODEX popular and test association that was ultimately chosen for analysis⁶.

Excellent grade pinnacle shape, following and plate counts with agreeable region is finished with 2µL infusion extent with insignificant enlarge with briefest run season of 2.5 minutes. Portable An and B concentrations were mixed and used as a diluent in a time-based extraction procedure, yielding the necessary instance accuracy after 20 minutes of sonication.

3. RESULT AND DISCUSSION

The final mobile phase combination selected which shows system suitability parameter was Phosphate buffer pH 4.0-Methanol in the ratio of 30-70 in standard drug. System suitability parameters are shown in table

Retention time [min]	Area	Height	Assymetry	Theoretical plate
3.303	2263.226	387.261	1.318	6940

Table 1 System suitability parameters for final selected trial

The absorption spectra of BIC significantly overlap. To optimize the LC parameters, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry for BIC was obtained with a mobile phase consisting of methanol: acetonitrile (25:75 v/v), pH 3.8 adjusted using 10% o-phosphoric acid. Quantification of the drug was performed at 277 nm. Resolution of the components with clear baseline separation was obtained.

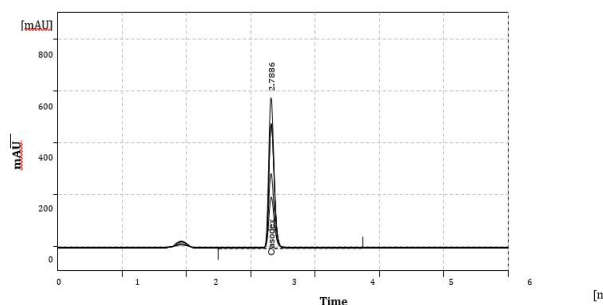


Figure no.1 Linearity chromatogram of casodex

Accuracy

The recoveries obtained were 99.96 % for BIC, respectively (Table 2). The high values indicate that the method was accurate. The recovery studies showed that the results were within acceptable limits, above 99.5% and below 100.5%.

Method precision

Precision study was carried out using parameter like method repeatability study which showed that results were within acceptable limit 0.0446 i.e. % RSD below 2.0 indicating that the method is reproducible.

Parameter	CASODEX
Linearity Range ($\mu\text{g/mL}$)	10-50
Detection Wavelength (nm)	277
Slope $\pm$ SD	369.65
Intercept $\pm$ SD	42.3
Correlation coefficient	0.9998

Table 2. Precision analysis

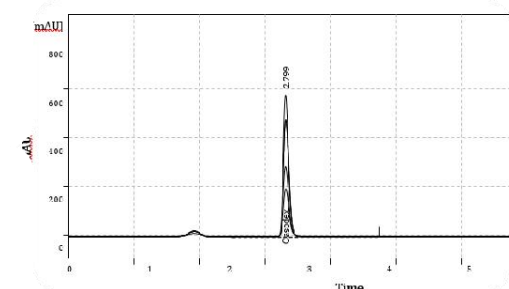


Figure no.2 Regression analysis of the calibration curves for BIC in the proposed HPLC Method.

Table 4 Intraday precision at 10 $\mu\text{g/ml}$

STANDARD	AREA
1	2367.69
2	2856.368
3	2960.882
Average	2261.646667
Standard deviation	5.699601506
%RSD	0.252011138

Table 5 Intraday precision at 20 $\mu\text{g/ml}$

STANDARD	AREA
1	2267.69
2	2256.368
3	2260.882
Average	2261.646667
Standard deviation	5.699601506
%RSD	0.252011138

Table 6 Intraday precision at 30 $\mu\text{g/ml}$

STANDARD	AREA
1	3390.793
2	3366.973
3	3383.848
Average	3380.538
Standard deviation	12.25010918
%RSD	0.362371587

Interday Precision

The area, average area, standard deviation and %RSD at 10 $\mu\text{g/mL}$, 20 $\mu\text{g/mL}$ and 30 $\mu\text{g/mL}$ respectively for interday precision.

Recovery Studies

Average recovery found out to be 101.112, 99.862 and 98.573 respectively, standard deviation was 0.9340987, 0.9819543 and 0.9852619 and %R.S.D was 0.9238260, 0.9833150 and 0.9995260. Percentage recovery was found to be within limits. The

representative chromatograms obtained during recovery studies are obtained for 80% spiking with standard and for 100% spiking with standard and for 120% spiking with standard and for sample for recovery and standard for recovery respectively.

Spiking of standard	Area of sample spike with standard	Area of sample	Net area of standard	Area of standard
80%	2056.222	1133.024	923.198	1144.716
	2052.145	1133.024	919.121	1144.716
	2068.573	1133.024	935.549	1144.716
100%	2283.841	1133.024	1150.817	1144.716
	2263.255	1133.024	1130.231	1144.716
	2281.372	1133.024	1148.348	1144.716
120%	2499.043	1133.024	1366.019	1144.716
	2489.807	1133.024	1356.783	1144.716
	2472.39	1133.024	1339.366	1144.716

Table 7 Area of sample and standard

Robustness

Robustness of the method was studied by making deliberate changes in few parameters viz; change in mobile phase composition, pH, and flow rate. standard deviation was found to be Bellow 1 and % RSD is less than 2 for all results.

The robustness study was carried out and the method was found to be robust. The flow rate by increasing or decreasing by 0.2 at 20 $\mu\text{g/mL}$ and their respective chromatograms are given in for increase in flow rate for three determinations and for decrease in flow rate for three determinations⁷. Table shows effect of change in mobile phase ratio composition by 2% variation at 20 $\mu\text{g/mL}$ and their respective chromatograms are given in for change in mobile phase ratio by 2% for three determinations for change in mobile phase ratio by 2% (from 30-70 to 28-72) for three determinations and table 26 and table 27 shows the effect of change in pH by increasing or decreasing by 0.2 at 20 $\mu\text{g/mL}$ and their respective chromatograms are given for increase in pH for three determinations and for decrease in pH for three determinations⁸.

System Suitability Test

A sample solution of 10 $\mu\text{g/mL}$ of BIC (n=5) was prepared and same was injected, then the system suitability parameters were calculated from the chromatogram. The parameters, retention times, resolution factor, tailing factor and theoretical plates were evaluated.

Assay studies

Assay results shows the area of standard, area of samples, % assay, average assay, standard deviation and %R.S.D of assay. which shows label claim, result (actual) found, assay, average of assay, standard deviation and %R.S.D of drug and contains chromatograms obtained for the sample for first, second and third determination and contains chromatogram of the standard⁹.

S.No	Area of standard	
1.	2276.718	
S.No	Area Of Samples	% Assay
1.	2132.55	93.66772696
2.	2153.959	94.60807179
3.	2110.886	92.7161818
Average assay		93.66399352
Standard deviation		0.945950522
% RSD of assay		1.009940412

Table 8 Area of standard,area of samples ,average assay,standard deviation and % R.S.D of assay

4.CONCLUSION

The proposed RP-HPLC method presented in this paper has compensations of simplicity, precision and convenience for separation and quantitation of BIC in combination and can be used for the assay of their particular dosage form. Moreover, the proposed method is a stability indicating assay method that can determine BIC.

For routine analytical purpose it is always necessary to establish methods capable of analysing huge number of sample in short period of time with good accuracy and precision. Spectrophotometric and chromatographic technique can generate large amount of quality data which serves as a highly powerful and convenient analytical tool. Although HPLC method is more specific than spectrophotometric method in providing quality data. In present work RPHPLC methods were developed for estimation of antibacterial drug Casodex¹⁰. Literature surveys revealed that no HPLC method was available to quantify Casodex Hence, it will be thought worth to establish a newer validated HPLC method for the determination of Casodex as per ICH guidelines. In this method C18 column was used. The selected mobile phase was sodium acetate buffer (pH 4.0 adjusted with glacial acetic acid): acetonitrile in the ratio of 35:65%, v/v. At the flow rate 1ml/min. The drug was injected and found retention time of 8.557 minutes respectively. After developing method, it is validated by using different parameters such as linearity, selectivity, accuracy, precision, LOD, LOQ, system suitability and recovery studies. The developed method showed linearity with correlation coefficient of $r^2 = 0.9998$. In interday and intraday precision, the %RSD values are in good precise in the developed method.

5.CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

6. ACKNOWLEDGEMENT

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