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CHROMATOGRAPHIC METHOD FOR THE PURIFICATION OF FULVESTRANT IN PHARMACEUTICAL FORMULATION

C.A.Jyothirmayee
Ch.S.D.St.Theresa's College for Women
INDIA

K.Sri Latha
Ch.S.D.St.Theresa's College for Women
INDIA

A.R.N.L.Sirisha
Ch.S.D.St.Theresa's College for Women
INDIA

ABSTRACT

This paper describes rapid, sensitive and specific method for the purification of fulvestrant in pharmaceutical preparations and also high performance liquid chromatography (HPLC) method. In column chromatography the purity of the compound is very accurate in Petroleum ether and ethyl acetate or Toluene and ethyl acetate.HPLC method was used to study the degradation behavior. Fulvestrant was subjected to degradation under the conditions of hydrolysis (acid and alkali), oxidation (30% H_2O_2). The linearity was established over the concentration range.

KEY WORDS: Fulvestrant, Column Chromatography, HPLC Stability studies.

INTRODUCTION

Fulvestrant[1], 7-alpha-[9-(4,4,5,5,5-penta fluoropentylsulphinyl) nonyl]estra-1,3,5-(10)- triene-3,17-beta-diol, is a new estrogen receptor antagonist available for the treatment of hormone receptor-positive metastatic breast cancer in postmenopausal women . Although tamoxifen has been a great asset in the treatment of breast cancer, some of its features make it less than ideal . Moreover, tamoxifen also increases the risk of endometrial cancer. For these reasons, there has been considerable interest in developing alternative hormonal treatments for breast cancer.

Fulvestrant is an estrogen receptor antagonist with no known agonist effects; its mechanism of action works by down-regulating the estrogen receptor. It has a unique mode of action that offers the potential for continued hormonal treatment in patients and also offers potential therapeutic advantages over aromatase as it has been reported that it is similar to anastrozole in its primary efficacy. Fulvestrant has low aqueous solubility and has been developed as a long-acting, oil-based formulation for being used as a once-monthly intramuscular injection. This parenteral depot formulation provides adequate bioavailability and offers potential compliance advantages over existing breast cancer treatment. Intramuscular administration can offer

sustained plasma drug concentration, and will also be less affected by vomiting and subsequent tablet loss than oral agents.

LITERATURE REVIEW

Fulvestrant exists as a mixture of two diastereomers which are epimeric at the sulphur atom of the side chain. These two diastereomers are known as Fulvestrant Sulfoxide A and Fulvestrant Sulfoxide B. No synthetic route for the synthesis of one pure diastereomer is described in the literature or in the proposed process. The present invention proposes to solve this need by providing a method for efficiently separating the diastereomers of fulvestrant. For example, it may be applied to pure fulvestrant having a mixture of sulfoxide A and sulfoxide B using a chiral system.

METHODOLOGY:

CHROMATOGRAPHIC PURIFICATION METHOD(COLUMN CHROMATOGRAPHY**):**

The method involves the packing of a column with silica gel(about 50gms) dried under nitrogen . Now the purified fractions by dissolving fulvestrant sulfoxide A or fulvestrant sulfoxide B in organic solvent to form a mixture and precipitating from the mixture fulvestrant sulfoxide 10 A or fulvestrant sulfoxide B were loaded on the top of the column. Then the column is eluted with Petroleum ether and Ethyl acetate(15ml Ethyl acetate and 85ml Petroleum ether) for the first fraction and (30ml Ethyl acetate and 70ml of Petroleum ether) for the second fraction which is continuously monitored with TLC.

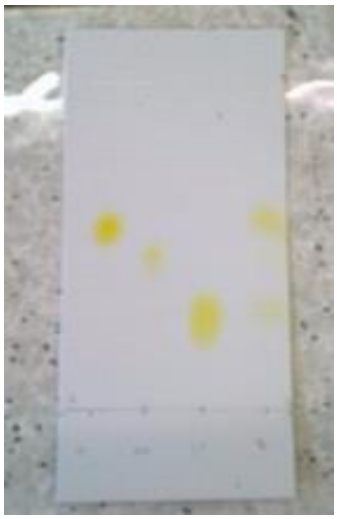

Fig:1

On the TLC plate the spots are visualised in UV chamber or Iodine blower. The chromatogram is developed in Methanol ,Chloroform(1:9). The two fractions (FST-A &FST-B) were separately collected , evaporated and dried under high vaccum and nitrogen.

Now they were sent to HPLC. In HPLC the column may have a packing particle of a size of about 3 µm to about 10 µm and preferably, the column has a packing particle a size of about 5 µm. Preferably, when using a chiral column system, the first mobile phase is n-hexane, and the second mobile phase is isopropanol. The first mobile phase may be present in an amount of about 75% to about 95% by volume and the second mobile phase is present in an amount of about 5% to about 25% by volume. Preferably, the first mobile phase is present in an amount of about 85% by volume and the second mobile phase is present in 5 an amount of about

15% by volume. The method of separating fulvestrant diastereomers using the chiral column may further comprise crystallizing fulvestrant sulfoxide A or fulvestrant sulfoxide B from Typically, the second mobile phase is acetonitrile, tetrahydrofuran, or methanol. The result of the purity percentage of either of the isomers of FST is 99.5.

A rapid, simple, stability-indicating, and validated RP-UPLC method was developed with 7 min of run time for the quantification of fulvestrant in oil-based injection formulations. This is the first stability-indicating method with the capability of resolving all the fulvestrant degradation impurities in the drug products. The method was validated for system suitability, linearity, precision, accuracy, specificity, intermediate precision, ruggedness, robustness, and solution stability.

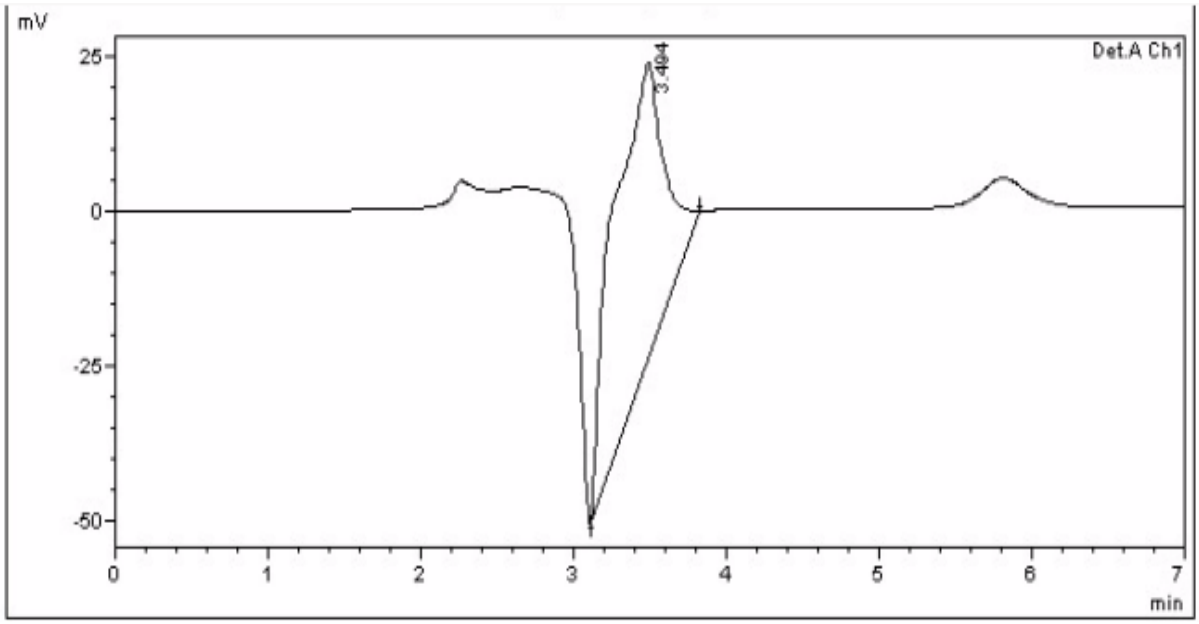

Fig:2

RESULTS

Instrument :Shimadzu, Column & Packing: Zorbax SB-C8, 3.5 µm, 150 x 4.6 mm

Mobile Phase A H_3 PO_4 0.05% in Water

Mobile Phase B Acetonitrile

Gradient

Time (min) Runtime 80 minutes

Post time 10 minutes

Detector λ= 220 nm

Column temperature 40 ^{0}C

Injection Volume 10 µL

Diluent Methanol,Acetonitrile50:50 (v/v)

When using a reverse phase column, the eluant system is a non-linear gradient. In other words, the amount of each of the two mobile phases varies over time. Typically, the mobile phase is a two phase system comprising a first mobile phase and a second mobile phase. Typically, the first mobile phase is water or a buffered aqueous solution. 5 Preferably, the first mobile phase is water. Buffered aqueous solutions suitable for the H3 PO4 (Sol. 85%) 0.1% in water; trifluoroacetic acid 0.1% or 0.01% in water; formic acid 0.1% in water. Typically, the chiral column temperature is from about 1O0C to about 4O0C, and preferably the column temperature is about 30 0C to about 35 0C. Typically, the flow rate is about 0.2 ml/min to about 5 ml/min. preferably, the flow rate is about 0.6 to about 1.3 ml/min, and more preferably about 0.75 ml/min to about 0.9 ml/min. The detector for the system can be any UV system that is commercially available. Typically, the detector is set to 220 nm and/or 240 nm. The HPLC procedure was carried out to develop a stability-indicating method for quantification of fulvestrant. The major challenges faced in the current study were as follows: the resolution between the impurities and fulvestrant peak with less run time, selection of an appropriate mobile phase composition with an isocratic method to obtain optimum resolution between the impurity peaks, optimization of the test concentration to get the desired LOQ levels for the fulvestrant, and the low LOD and LOQ values indicating the method sensitivity. In addition, the HPLC method can be considered specific, where it can be used for drug substance separation and quantification of drug product from its degradation products and placebo peaks.

Method validation was performed as per the guidelines. The linearity of the proposed UPLC method was performed. Linear relationships between concentration and high regression coefficients were obtained. Intra-day and inter-day precision was also evaluated. In addition, good results in terms of LOD, LOQ, robustness, and selectivity were obtained.

DISCUSSION:

Recovery of the sample from HPLC*:* To determine the accuracy of the HPLC methods and to study the interference of formulation additives, the recovery was checked as three different concentration levels. Analytical recovery experiments were performed by adding the known amount of pure drugs to pre-analyzed samples of commercial dosage form. The recovery values were calculated by comparing the concentration obtained from the spiked samples with actual added concentrations. These values are also listed in.

Recovery of fulvestrant in pharmaceutical preparation

Commercial Preparation	Method	n	Found(mg) Mean±SD	Recovery	% RSD [a]	Confidence Interval
2.5mg/ml	HPLC	6	252.5 ± 2.05	102.0	0.81	2.48 – 2.5

Table1

CONCLUSIONS

The method of separating fulvestrant diastereomers using the chiral column further comprised crystallizing fulvestrant sulfoxide A or fulvestrant sulfoxide B from

Typically, the second mobile phase is acetonitrile, tetrahydrofuran, or methanol. The result of the purity percentage of either of the isomers of FST is 99.5.

The HPLC was equipped with a PDA detector at λ= 2 20 nm. After running the sample through the HPLC, each isomer was separated. The retention time of the fulvestrant sulfoxide A was 3.4 min; and the retention time of the fulvestrant sulfoxide B was 3.95 min.

Method validation was performed as per the guidelines. The linearity of the proposed UPLC method was performed. Linear relationships between concentration and high regression coefficients were obtained. Intra-day and inter-day precision was also evaluated. In addition, good results in terms of LOD, LOQ, robustness, and selectivity were obtained.

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