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Research Article

# A NOVEL VALIDATED STABILITY INDICATING RP RP-HPLC METHOD FOR DETERMINATION OF ACITRETIN IN BULK AND PHARMACEUTICAL FORMULATION

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**ABSTRACT**

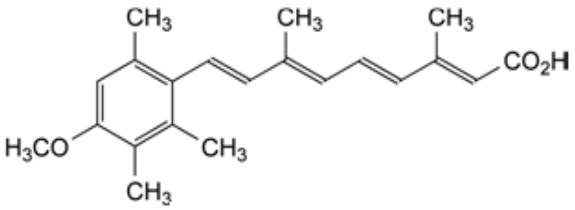
A simple, accurate and precise Stability indicating Reverse Phase –High Pressure Liquid Chromatography method for the estimation of Acitretin in pure and pharmaceutical dosage form has been reported. Quantitative estimation of Acitretin was done by using an isocratic Shimadzu HPLC instrument on Enable C18 column (250 mm x 4.6 mm, 5µ). The Instrument is equipped with binary pump and variable wavelength PDA detector. A 20µL Hamilton syringe was used for injecting the samples. Data was analyzed by using LC Solutions software. The mobile phase consists of Methanol: Acetonitrile 85:15 v/v. and at a flow rate of 1 milliliter/minute. The Acitretin was eluted at approximately 2.726 min minutes. The wavelength was found to be 360nm. A linear response was observed in the concentration ranges of 20-120µg/ml with a regression coefficient of 0.999. The developed method was validated with respect to specificity, precision (% RSD about 0.4%), linearity (linearity of range about (10-50 µg/mL), robustness, LOD and LOQ values were found to be 0.56 µg/ml and 1.69 µg/mL respectively.

**Keywords:** High performance liquid chromatography, Acitretin

## INTRODUCTION

**Acitretin** (trade names **Soriatane** and **Neotigason**) is a second-generation retinoid. It is taken orally, and is typically used for psoriasis. It is a metabolite of etretinate, which was used prior to the introduction of acitretin. Etretinate was discontinued because it had a narrow therapeutic index as well as a long elimination half-life ($t_{1/2}$=120 days), making dosing difficult. In contrast, acitretin's half-life is approximately 2 days. However, because acitretin can be reverse metabolized into exterminate which has an extremely long half-life, women must avoid becoming pregnant for at least three years[1] after discontinuing acitretin. Therefore, acitretin is generally not recommended for women of child-bearing age with a risk of becoming pregnant. Acitretin is an oral retinoid used in the treatment of severe resistant psoriasis. Because of the potential for problems and severe side effects it is generally used in only very severe cases of psoriasis that have been unresponsive to other treatments. It binds to nuclear receptor that regulates gene transcription. They induce keratinocyte differentiation and reduce epidermal hyperplasia, leading to the slowing of cell reproduction. Acitretin is readily absorbed and widely distributed after oral administration. A therapeutic effect occurs after two to four weeks or longer. Patients that have received the medication are advised against giving blood for at least three years due to the risk of birth defects. [2]

**Acitretin** [3-5]




## MATERIALS AND METHODS

Acetonitrile, Methanol, used were of HPLC grade and purchased from Merck Specialties Private Limited, Mumbai, India.

### Instrumentation

Quantitative estimation of Acitretin was done by using an isocratic Shimadzu HPLC instrument on an Enable C18 column (250 mm x 4.6 mm, 5µ). The Instrument is equipped with binary pump and variable wavelength PDA detector [5-7]. A 20µL Hamilton syringe was used for injecting the samples. Data was analyzed by using LC Solutions software. Shimadzu UV-Visible spectrophotometer was used for spectral studies. Degassing of the mobile phase was done by using a Loba ultrasonic bath sonicator. A Shimadzu balance was used for weighing the materials.

### Experimental conditions

Chromatographic separation achieved using an analytical column, Inertsil ODS 3V C18 column (250 mm x 4.6 mm, 5µ). Mobile phase was consisted of Methanol: Acetonitrile 85:15 v/v.

The elution was achieved isocratically at a flow rate of 1 mL/min with injection volume of 20 µL. Column temperature was maintained at 45°C and chromatograph was recorded at wavelength 360 nm.

**Preparation of standard stock solution**

Accurately weighed and transferred 10mg of Acitretin into 100ml clean dry amber colored volumetric flask. Add 3/4$^{th}$ volume of diluent, sonicated for 5 minutes and make up to the final volume with diluents. (Concentration of Acitretin is 100µg/ml). From that take 1ml, 2ml, 3ml, 4ml and 5ml in five different 10ml dried amber coloured volumetric flask and made up to the mark to all.

**Sample Solution**

20 capsules were weighed and average weight was calculated. Then the powder weight equivalent to 10mg of Acitretin was transferred into a 100mL dried amber coloured volumetric flask and 75mL of diluent added. Then sonicated for 15 min, further the volume made up with diluent and filtered through 0.45µ filter

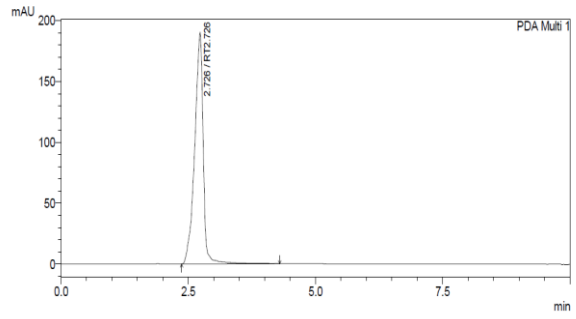



**Figure 1: Chromatogram of standard**

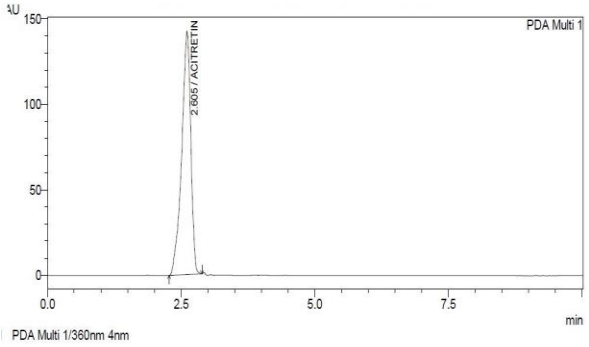



**Figure 2: Chromatogram of sample**

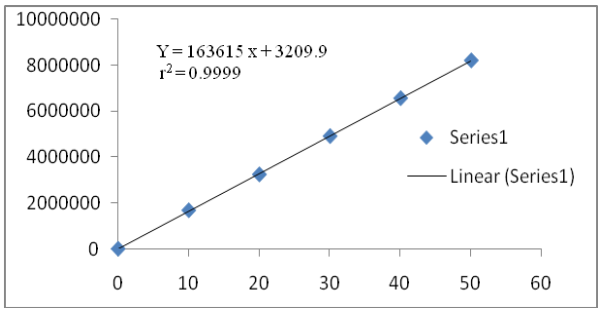



**Figure 3: Calibration curve**

**Table 1: Results for Precision**

| S.No | Peak area of Acitretin |
|---|---|
| 1 | 4902528 |
| 2 | 4881563 |
| 3 | 4937516 |
| 4 | 4953427 |
| 5 | 4873254 |
| Mean | 4909658 |
| S.D | 34846.8 |
| %RSD | 0.70 |

**Table 2: Recovery of Acitretin**

| % Recovery | Recovery Level (%) | Fixed concentration (µg/ml) | Spiked concentration (µg/ml) | Recovered concentration (µg/ml) |
|---|---|---|---|---|
| 99.9 | 80 | 10 | 8 | 7.99 |
| 100.1 | 100 | 10 | 10 | 10.02 |
| 100.2 | 120 | 10 | 12 | 12.05 |

**Table 3: Robustness**

| Flow rate | Peak area of Acitretin | Retention time |
|---|---|---|
| 0.8 | 6355040 | 3.45 |
| 1.2 | 4181095 | 2.30 |

## RESULTS AND DISCUSSION

**Method development**

Some important parameters like pH of the mobile phase, concentration of the acid or buffer solution, etc., were tested for a good chromatographic separation. Trials showed that mobile phase with reverse phase $C_{18}$ column gives symmetric and sharp peaks. After the optimization of chromatographic conditions, estimation of Acitretin as carried out by the developed RP-HPLC method. Standard solution of drug was injected separately and chromatogram of Acitretin was recorded in Figure 1. Now the sample solution was injected separately and chromatogram was recorded until the reproducibility of the peak areas were satisfactory.

**Validation**

HPLC method was validated [7-9] according to the International Conference on Harmonization Guidelines (ICH Q2B, validation of analytical procedures, methodology). The method was validated for parameters such as system suitability, linearity, precision, accuracy, and robustness.

**Linearity**

From the stock solutions of 0 Acitretin.2 ml, 0.4ml, 0.6ml, 0.8ml, 1.0ml and 1.2ml were taken in six different 10 ml volumetric flasks and diluted with the mobile phase to the give the concentrations from 10-50 µg/ml. These Solutions were injected into the chromatographic system and the response was recorded in Figure 2.

**Precision**

To study precision, six replicate standard solutions of Acitretin (100µg/ml) were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was shown in Table 1.

**Accuracy**

The accuracy of the method was determined by recovery experiments. The recovery studies were carried out by comparing method of three individual standards with each of three samples with same procedure from the formulation and injecting. The percentage recovery and percentage relative standard deviation of the percentage recovery were calculated and presented in Table 2. From the data obtained, added recoveries of standard drugs were found to be accurate.

**Limit of Detection**

Limit of Detection (LOD) is defined as lowest concentration of analyte that can be detected, but not necessarily quantified, by the analytical method. Limit of detection is determined by the analysis of sample with known concentration of analyte and by establishing the minimum level at which the analyte can be reliably detected and it was found to be 0.56 µg/ml of Acitretin.

**Limit of Quantification**

Limit of quantification (LOQ) is the concentration that can be quantitated reliably with a specified level of accuracy and precision. LOQ was found to be 1.69 µg/mL of Acitretin

**Robustness**

Robustness of the developed method was demonstrated by purposely altering the experimental conditions. Robustness of method was carried out with variation of mobile phase ±0.2%, flow rate ±0.2 ml/min. It indicates that there was no effect on the results, hence the developed method is said to be more robust and shown in Table 3.

**Specificity**

Specificity is the ability of the analytical method to measure the analyte free from interference due to other components. Specificity was determined by comparing test results obtained from analyses of sample solution containing ingredients with that of test results those obtained from standard drug. Chromatograms for standard & samples were recorded and they represent no interference.

**System Suitability**

System suitability tests were carried out on freshly prepared standard stock solution of Bamifylline and it was calculated by determining the standard deviation of by injecting standard solutions in six replicates at frequent time interval.

**CONCLUSION**

The proposed method was found to be simple, precise, accurate, linear, robust and rapid for determination of Acitretin in bulk and its pharmaceutical dosage form. The developed method gave good resolution with short analysis time.

**ACKNOWLEDGEMENT**

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