

## Review Article

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# STABILITY-INDICATING RP-HPLC METHOD VALIDATION FOR ACITRETIN AND ITS RELATED SUBSTANCES IN CAPSULES

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## ABSTRACT

A simple, specific, and precise high performance liquid chromatography method was developed and validated for the estimation of Acitretin in capsule dosage forms. Although the analytical method developed in-house, was based on monograph of Acitretin on British pharmacopoeia 2007, but it was modified to get better separation among Acitretin and its related substances. A Peerless Basic C<sub>18</sub> (CHROMATOPAK 250 x 4.6 mm, 5µm), in isocratic mode, with mobile phase containing Ethanol: Water: Glacial Acetic acid (7:2.97:0.03 v/v) was used. The flow rate was 1.0 ml/min and effluent was monitored at 360 nm. The retention time of Acitretin was 19.4 min. The validation study is carried out fulfilling the ICH guidelines in order to prove the fundamental criteria for validation: specificity, linearity, precision, accuracy, robustness and solution stability. Proposed method was successfully applied for the quantitative determination of Acitretin in capsule dosage form.

**Keywords:** *Acitretin; HPLC; Validation; Capsule.*

## INTRODUCTION

Psoriasis is a genetically determined disorder.<sup>1</sup> The estimated prevalence is 1.5% to 3% in the general population. There is wide ethnic and racial variation. It has a bimodal peak of incidence, at 16-22 years and 57-60 years. The treatment of a psoriatic patient needs to be tailor-made. Acitretin is the least toxic systemic treatment for psoriasis. It is an oral retinoid of choice used in the treatment of severe resistant psoriasis.<sup>2</sup>

Acitretin is a second generation retinoid. It is the major metabolite of etretinate with the advantage of a much shorter half-life when compared with etretinate.<sup>3</sup> Acitretin works by inhibiting the excessive cell growth and keratinisation (process by which skin cells become thickened due to the deposition of a protein within them) seen in psoriasis.<sup>4</sup> It, therefore, reduces the thickening of the skin, plaque formation and scaling.

Very few analytical methods have been reported to determine Acitretin in both pharmaceutical preparations and biological specimens. These included high-performance liquid chromatography with ultraviolet detection (HPLC-UV), and Gas chromatography with mass spectrometric detection GC-MS.<sup>5-8</sup> The test for assay method; developed in-house based on monograph of Acitretin on British pharmacopoeia 2007, with necessary modification to make the method simple and fast to quantify Acitretin and its degradation products in a final product, at the production level.<sup>9</sup> This work describes the validation parameters stated by the ICH guidelines<sup>10,11</sup> to achieve an analytical method with acceptable characteristics of suitability, reliability and feasibility.

## EXPERIMENTAL

### Materials

The reference standards and impurities standards namely Acitretin, Tretinoin, Acitretin related Compound A and, Acitretin related Compound B were kindly provided as gift sample by the Strides Arcolab Limited (Bangalore, India). Commercially available 10mg and 25mg Acitretin capsule (Soriatane Capsule) were purchased from local market. A placebo for the validation study was prepared with its declared excipients. All the chemicals used were of HPLC grade.

### Equipment

The chromatographic system used to develop this technique is a HPLC Agilent 1200 series featuring a column oven (G1316A), a quaternary pump (G1311A), an automatic injector (G1313A) and a variable wavelength detector (G1314A) and diode array detector (G1315B).

### Chromatographic conditions

Chromatographic separation of Acitretin and its related substances was performed using a Peerless Basic C<sub>18</sub> (CHROMATOPAK 250 x 4.6 mm, 5µm), made of stainless steel. The mobile phase consisted of ethanol, water and glacial acetic acid in the ratio of 7:2.97:0.03. The mobile phase filtered through a 0.45 µm membrane filter and was pumped through the column at a flow rate of 1.0 ml min<sup>-1</sup>. The injection volume to carry out the chromatography was set at 10µl with sample cooler temperature 10°C. The wavelength was fixed at 360nm with 30 min run time.

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### **Solutions**

Resolution solution was prepared by dissolving 10 mg of Acitretin Standard, 1 mg of Acitretin related compound A into a 100.0 ml volumetric flask. To the resulting solution 25 ml of Tetrahydrofuran was added and sonicated to dissolve after that it was diluted to 100ml with 3 % w/v SLS. Standard solution of 100µg/ml was prepared by the same procedure.

Stock sample solution was prepared by taking and mixing average weight of 20 capsules. Sample solution of 100µg/ml was prepared by the same procedure from the stock sample solution.

### **Validation study**

#### **Specificity**

For the specificity study, diluent, standard solution preparation, Acitretin related compound A, Acitretin related compound B, Tretinoin, sample solution and sample spiked with impurities were separately injected into the HPLC system. Another study was carried out to check the absence of interference by excipients, which take part in the pharmaceutical preparation (placebo solution).

Within the study of specificity, a series of degradation studies were carried out where the samples were subjected to different degrees of stress. The samples corresponded to placebo and final product and were subjected to stress conditions as 0.1N NaOH, 0.1N HCl (keeping the solution to both, environmental temperature and 80°C for 2 min) and repeated in neutral stress condition (Water, environmental temperature and 80°C for 2 min). The powder was also subjected to the effect of temperature (80°C for 4 hours), UV light during at 254nm and 360nm for 8 hours and, sunlight for 4 hours. The samples were also subjected to an oxidation treatment with 3.3%v/v Hydrogen peroxide heated to 80°C for 2 min. After the stress assays, the samples were analyzed as shown in the chromatographic conditions.

#### **Stability in analytical solution**

The stability in analytical solution was evaluated by injecting the sample and the standard at regular intervals at room temperature (25°C) and sample cooler temperature (10 °C).

#### **Filter compatibility study**

Filter compatibility studies were carried out using % Difference of sample filtered through Whatmann no. 42 filter and 0.45µ membrane filter from centrifuged sample.

#### **Linearity**

To carry out this study, six levels of concentration within the range 50–150% of the work-concentration (100µgml<sup>-1</sup>) were prepared. Each of the levels of concentration were prepared in triplicate, individually

weighing the amount of active and the corresponding amount of placebo (three independent calibration equations were obtained). The experimental results were represented graphically, obtaining a calibration graph and carrying out the corresponding statistic study (ANOVA).

### **Precision**

For the precision study four different tests were carried out. The first one consisted of checking instrumental system precision, where a sample corresponding to a concentration of 100 µgml<sup>-1</sup> was injected six times, consecutively into the chromatograph, repeating the operation on a second day. The second test consisted of testing the standard solution precision where three solutions were prepared for both 50% and 150% and seven solutions at 100% of the work concentration, studying the relative standard deviation (S.D.) obtained for the response factor (relationship between the area obtained and the studied concentration). The third test consisted of checking the precision of the method, operating as described in the standard solution previously mentioned: seven individual samples were prepared and the relative standard deviation (R.S.D.) was studied for the response factor obtained. Lastly, the intermediate precision was studied. Preparing the samples according to the precision of the method and studying the variability, which takes place when the same analyst works on different days or when the analysts change.

### **Accuracy (recovery method)**

The recovery method was studied at concentration levels of 50% (three samples), 100% (seven samples) and 150% (three samples) where a known amount of the active ingredient was added to a determined amount of placebo and the amount of Acitretin recovered in relation to the added amount was calculated. This study was carried out on basis of the method described above.

### **Robustness**

The study of robustness was carried out to evaluate the influence of small but deliberate variations in the chromatographic conditions for the determination of Acitretin in capsule (percentage Acitretin). The %RSD of five replicate injections was determined. The factors chosen for the study were the wavelength (nm), column oven temperature (°C), flow rate (ml min<sup>-1</sup>) and mobile phase (percentage acetonitrile).

## **RESULTS AND DISCUSSION**

### **System suitability**

The chromatographic separation, as explained above, was carried out with a Peerless Basic C<sub>18</sub> (CHROMATOPAK 250 x 4.6 mm, 5µm). To evaluate the chromatographic parameters (capacity factor-K', number of theoretical plates, asymmetry of the peaks,

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tailing factor and resolution between two consecutive peaks; peaks of void and analyte) the chromatogram obtained for the test standard, was used. The capacity factor ( $K'$ ) of the analyte peak was 2.158. The number of theoretical plates (per meter of the column) was 9,896. The results obtained for the asymmetry of the analyte peak and the tailing factor, parameters were 0.800 and 1.129, respectively.

### Stability of the analytical solution

The results obtained in the study of the solution (both, reference and sample solution) where it can be noticed that solutions were stable for 17 h at room temperature (25°C) and 19hr for sample cooler temperature (10°C), as during this time the result does not decrease below the minimum percentage (95%).

### Validation study

#### Specificity

Comparing substances, raw material and standard reference (Acitretin CRS) in the same chromatogram, it was noticed that both eluted at the same retention time and therefore, it was concluded that it was the same substance. The excipients did not interfere in the estimation and when it was evaluated for the spectral purity in the diode array detector, the purity of the peak constituting for Acitretin passed the test. The present peak purity index of Acitretin was 0.999999 with single point threshold of 0.999988 and the peak purity index was calculated as 11. The purity index, threshold and minimum index were calculated using Agilent Chemstation Software. The results indicated that the method was specific.

Another study carried out to check the selectivity of the method was the degradation test carried out under different stress conditions. During the study of stress with 1N HCl, the presence of an unknown degraded product was noticed, which eluted at 17.3 min approximately with relative %age area of about 7.76%. When 1N NaOH was used as a stress media, and the solution was kept at room temperature, no unknown peak appeared. When the sample was subjected to 80° C temperature, after 4 h of study, no unknown peak appeared, whereas in the samples subjected to high RH (79%) a degraded peak appeared which eluted at 17.1 min with relative %age area of about 0.24%. When the samples were subjected to an oxidation treatment, with H<sub>2</sub>O<sub>2</sub> 3.3%, peak appeared which eluted at 16.9 min with relative %age area of about 5.41% corresponding to unknown degraded products. When KMnO<sub>4</sub> was used, no known or unknown degraded peak appeared. After treating the sample with UV light, no apparent change was noticed.

It can be concluded that none of the peaks that could be generated by the stress treatment interfere with the peak corresponding to the active, therefore showing it was a selective method and suitable for routine work.

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### Linearity

The equation of the regression curve obtained (with all the values) relating the tested concentrations and the response obtained correspond to  $y = 79.364x - 128.98$  and a correlation coefficient of 0.999, which is higher than the value established at the beginning of the study, which corresponds to 0.9982. In addition, the analysis of residuals show that the values are randomly scattered around zero, which shows good fit to the linear model. To evaluate whether the  $y$  (79.364x-128.98) intercepts were significantly different from zero, the P-value was determined. For Acitretin P value is 0.3862. Hence it is statistically equal to zero. In addition, the origin is within the lower and the upper limit of the 95 % CI that gives high degree of confidence to the value obtained for intercept as shown in Table 1. Moreover, the value of the intercept is less than 5 % of the area response at 100 % level.

Table 1. Statistical results for linearity

	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	128.9809	135.9190	0.9490	0.3862	-220.4101	478.3719

### Precision

In the study of the instrumental system precision where, a R.S.D. of 0.18% was obtained for retention time, and of 0.25% for the area obtained corresponding to the first day, being 0.22 and 0.31% for the second day, respectively (with  $n = 6$  number of analyses per day). The inter-day study ( $n = 20$  analyses) carried out showed a R.S.D. of 0.21% for the area obtained and 0.55% for retention time. In all these cases the R.S.D. obtained was far below 1%, the limit percentage set for the precision study of the instrumental system, thus showing that the equipment used for the study worked correctly for the developed analytical method, and being highly repetitive. The precision study for standard solution ( $n = 6$  analyses) showed a R.S.D. of 1.093% for the response factor. For the study of the precision of the method ( $n = 6$ ) the value of R.S.D. corresponded to 0.54%.

Both studies with values far below the value established (0.7%) at the beginning of the study. For the intermediate precision, a study carried out by the same analyst working on different days ( $n = 6$  number of analyses per day). The results were given both individually and as a whole observing that the inter-day R.S.D. corresponded to 0.85. The same study was carried out for different analysts ( $n = 6$  number of samples per analyst) obtaining a R.S.D. of 0.72%. Both results together with the individual results are below the established limit according to the AOAC (2.7%), thus showing that the proposed analytical technique has a good intermediate precision.

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### Accuracy (recovery method)

The results obtained for the accuracy study (recovery method) from 9 samples studied (n = 3 for 50%, n = 3 for 100% and n = 3 for 150%) indicated that the mean of the recovery was 100.35%, and R.S.D. was 0.39%. The experimental t-value of the recovery percentage was 1.041. It is far below the specification limit of 2.17, established in the tabulated t (95% level of probability). Therefore, it can be concluded that the recovery study of the active in the matrix for the developed method for the assessment of the active in final product was correct, and therefore, the proposed analytical method was sufficiently accurate.

### Robustness

The factors chosen for the study were the wavelength (nm), column oven temperature (°C), flow rate (ml min<sup>-1</sup>), mobile phase (percentage acetonitrile). The developed method was found to be sufficiently robust for those deliberate changes as shown in Table 2. The overall %RSD of robustness for the optimized method was 0.85%. Thus, the optimized method was robust for the all deliberate changes.

Table 2. Robustness Parameters

SYSTEM SUITABILITY PARAMETERS	Change in organic phase ratio (±2.0%)			Change in Temperature (±5°C)			Change in Flow Rate (±0.2 ml/min)		
	70 : 30	68 : 32	72 : 28	35°C	30°C	40°C	1.0	0.8	1.2
The RSD for area response obtained from five replicate injections of Standard Solution should be NMT 2.0%.	0.25	0.04	0.07	0.25	0.20	0.10	0.25	0.04	0.08
Tailing factor for Acitretin peak should be NMT 2.0 in Standard Solution Preparation.	1.15	1.22	1.22	1.15	1.15	1.20	1.15	1.15	1.15
Theoretical plate of Acitretin peak should NLT 2000 in Standard Solution Preparation	11749	10558	10872	11682	10090	10557	11749	12212	9550
Resolution between Acitretin Related substance A and Acitretin should be NLT 1.5.	2.83	2.76	2.71	2.83	2.82	2.61	2.83	2.90	2.56

### Detection limit and Quantitation limit

According to the study carried out with the seven linearities and graphically summing up the results of the relative standard deviation versus the concentration, the quantitation limit is set at 0.30µg/ml which corresponds to a 0.30% of the work concentration (100µg/ml) showing that this point is linear, precise and exact, the detection limit is set at 0.10 µg/ml, a concentration equivalent to 0.1% of the work concentration and which could not demonstrate its linearity, precision and accuracy.

### CONCLUSION

A simple and quick analytical method has been developed to be applied in routine determination of Acitretin and its degraded products in capsule. The method proposed by HPLC to determine Acitretin in capsule has been proved linear, precise, accurate and selective manner to be applied in routine analysis and in quality control of Acitretin capsule. It has been proved that it was selective, linear between 50 and 150% of the work concentration (100µg/ml) for Acitretin and

between the quantitation limit and 150% for degraded products, with a correlation coefficient higher than 0.998, exact, precise, accurate and robust regarding flow rate, mobile phase, and temperature.

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