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MULTIVARIATE CALIBRATION TECHNIQUE AIDED UV SPECTROPHOTOMETRIC METHOD FOR THE ESTIMATION OF ACITRETIN IN PHARMACEUTICAL DOSAGE FORM: ASSESSMENT OF GREENNESS PROFILE

DHIVAKARAN M, SEETHARAMAN R*, KAVITHA J, KOKILAMBIGAI K S AND KUPPURAJ P

Department of Pharmaceutical Analysis, SRM College of Pharmacy, SRM Institute of Science and Technology, Kattankulathur, Chengalpattu District, Tamil Nadu - 603203, India

***Corresponding Author: Dr. Seetharaman R: E Mail: seetharr@srmist.edu.in; seerampharm@gmail.com**

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ABSTRACT

The present work aims to develop a eco-friendly UV spectrophotometric method using a multivariate calibration technique for the estimation of Acitretin in pharmaceutical capsules. For more accurate measurements, the multivariate calibration approach measures the sample absorbance at different wavelengths. The UV spectrophotometric method was developed and method validation was performed. All the validation parameters comply with ICH norms. The proposed Multivariate Calibration technique can be applied to the estimation of Acitretin. The results were treated statistically. The analytical Eco scale, Agree metrics, and Green analytical procedure index was used to assess the method's greenness scores.

Keywords: Acitretin, Multivariate calibration technique, Pharmaceutical formulations, ICH guidelines, Validation.

INTRODUCTION

Acitretin (**Figure 1**) is a second-generation tretinoinderivative **[1]**. It has replaced etretinate in retinoid therapy of psoriasis because of its better pharmacokinetic profile, including a significantly shorter half-time **[2–4]**. It is widely used in the systemic treatment of severe forms of psoriasis and other skin

disorders **[2]**. Acitretin mechanism of psoriasis treatment is to regulate epidermal keratinocytes, reduce cohesion and increase skin dryness, suppress psoriatic lesion location of abnormal keratinocyte proliferation and differentiation **[5]**. Acitretin is limited by its teratogenicity and therefore considered inappropriate in most female patients of childbearing age. Common side effects include mucocutaneous dryness and elevated triglycerides **[6]**.

Acitretin is chemically (2E,4E,6E,8E)-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethylnona-2,4,6,8-nanotetraenoic acid. The molecular formula is $C_{12}H_{26}O_3$ with the molecular weight of 326.43 **[7]**. The primary method for the determination of acitretin in pharmaceutical has been the high performance liquid chromatography (HPLC) reported by the Pharmacopoeia of the People's Republic of China (2005) **[8]**. A literature survey revealed that few publications are reported for estimation of Acitretin in pharmaceutical preparations as well as in biological fluids **[9, 10]**. The literature review referred to the Multivariate Calibration technique (MVC) using UV spectrophotometry, which haven't reported. Hence, the present method deals with the development of the eco-friendly UV spectrophotometric aided MVC technique for the estimation of Acitretin.

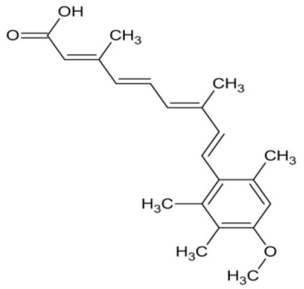


Figure 1: **Chemical structure of Acitretin**

The MVC approach for acitretin was used to decrease instrumental error and increase efficiency. The method is easy, inexpensive and can be applied to bulk chemical and pharmaceutical dosage forms. For exact findings, MVC employs straight regression algorithms ranging between the wavelengths of 5-10nm **[11]**. In this study, we discussed the use of a UV spectral MVC approach with minimal mathematical content for estimating Acetritin in pharmaceutical dosage forms. As a result, to ensure the sensitivity in comparison to the traditional ultraviolet (UV) approach, five distinct wavelengths were chosen. The algorithm techniques of MVC's statistics multivariate data are converted into univariate data using the following equations **[12]**. If the absorbance of a sample(x) is measured at five different wavelengths (λ), that is, at 340,345,350,355 and 360nm, the following equation can be produced for each selected wavelength.

$A_{\lambda 221} = a \times C_x + k_1$ ……. (1)

$A_{\lambda 224} = b \times C_x + k_2$ ……. (2)

$A_{\lambda 227} = c \times C_x + k_3$ ……..... (3)

$A_{\lambda 230} = d \times C_x + k_4$ ……...…. (4)

$A_{\lambda 233} = e \times C_x + k_5$ ……..…….…. (5)

Whereas,

- A_λ= Absorbance of the sample;
- a, b, c, d, e = Slope of the straight regression functions of a sample;
- k_1, k_2, k_3, k_4, k_5 = Intercept of the straight regression;
- C_x = Concentration of the sample

The above five equations can be rearranged as:

$A_T = a \times C_x + b \times C_x + c \times C_x + d \times C_x + e \times C_x + K_T$……. (6)

Equation (6) can be re- arranged as:

$A_T = C_x (a + b + c + d + e) + K_T$ ……. (7)

Whereas,

- A_T = Sum of the absorbances acquired
- K_T = Sum of intercepts of regression equation

The concentration of the sample (X) in a solution can be calculated by using the equation

$$Cx = \frac{A_T - K_T}{(a+Ab+c+d+\ )} \quad \text{……. (8)}$$

Greenness evaluation techniques

The Globally Harmonized System of Classification and Labeling of Chemicals (GHS) established a set of pictograms with related signal words, and the analytical eco scale **[13]** is predicated on assigning penalty points relying over both quantity and number. The analytical eco scale approach considers each reagent, including its kind and quantity, potential occupational exposure, energy depletion, and waste. Penalty points are eliminated from a 100 point base score.

Analytical eco-scale = 100 - total penalty points……. (9)

The Green Analytical Procedure Index **[14]** (GAPI) is a visual depiction made up of five pentagons with distinctive colour coding. The colour coding in the pictogram corresponds to three levels of evaluation at each stage of an analytical technique. The colour coding used by GAPI to determine greenness spans from green to yellow to red, denoting the low, medium, and high environmental impacts connected with the analytical technique, respectively. J. Potka-Wasylka provided a succinct overview of GAPI in the year 2018 **[14]**. The third assessment methodology makes use of AGREE metrics' **[15]** special software for assessing the greenness profile. The result of the software is a circle with numbers around the edges that range from 1 to 12 and are oriented clockwise. These figures represent the 12 green analytical chemistry philosophies. Based on the inputs and their weight, the outputs of each of these 12 principles are rated from 0 to 1. This aggregate scale uses the colours red, yellow, and green to show different numbers. Red means zero, dark green means one or close to one, and yellow means a number between red and dark

green. A score that represents the level of greenness is produced by adding the 12 principles and the core.

MATERIALS AND METHODS

Instruments used

A LABINDIA UV 3092 model double beam UV-VISIBLE spectrophotometer (Gurugram, India) was used. It consists of an automatic eight-cell charger, Czerny-Turner monochromator optics sealed and coated with quartz, a deuterium lamp, and a tungsten lamp were used as a detector, which has a wavelength of 190-900 nm and a spectral bandwidth of 0.1-5.0nmwith a 0.1nm interval. The software used to run this instrument and produce data output is UV Win Lab Version 5.1.1. For weighing the materials, an analytical balance (AS 245, Mettler Toledo, India) was used, and for sonication purposes, a Labman Digital Ultra Sonicator (model LMUC-3, The barton, Australia) was used.

Reference Samples

Acitretin was kindly supplied by Ideal Analytical and Research Institution (Pondicherry, India)

Preparation of solutions

Standard stock solution preparation of Acitretin

Weigh accurately 10 mg of Acitretin and transfer it into a 100 ml volumetric flask. Dissolve it in 25 ml of ethanol, sonicate for 20 minutes, and then increase the volume to 100 mL with ethanol. The solution was filtered through Whatman grade 42 circular filter paper.

Working solutions of Acitretin

From the above stock solution, 3.5-6.5 µg mL^{-1}solutions were prepared by using Ethanol as a solvent.

Selection of wavelength for MVC

Across the wavelength range of 200 to 400 nm, the Acitretin working standard solutions were scanned against ethanol as the blank solution, which has maximum absorption at 350nm. Thus, the wavelength for MVC approach was around these absorption maxima, i.e., 340, 345, 350, 355, 360.

Stability of the solution

Solution stability studies were performed for Acitretin by storing prepared sample solutions at room temperature for 0-12 hours. The absorbance was measured at regular intervals of 0, 6, and 12 hrs.

Method Validation

For method validation, ICH Q2(R1) **[16]** norms were followed.

Linearity

Linearity of Acitretin was performed by suitable dilution of the stock solution with Ethanol to achieve concentrations ranging from 3.5-6.5µg mL^{-1} (3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5) in order to analyse linearity and spectral region. The absorbance of linearity solutions at the

appropriate wavelength was measured and analysed for the MVC method.

Limit of Detection and Limit of Quantification

The Limit of Detection (LOD) and Limit of Quantification (LOQ) were estimated for Acitretin based on the calibration curve slope and standard deviation of responses for a particular wavelength using the following formulae:

$$\text{LOD} = \frac{3.3 \text{ x standard deviation}}{\text{Slope}} \quad \text{..... (9)}$$

$$\text{LOQ} = \frac{10 \text{ x standard deviation}}{\text{Slope}} \quad \text{..... (10)}$$

Precision

The precision was evaluated and analysed for repeatability through intraday and interday precision. To test different levels of accuracy, a typical standard solution of Acitretin at a concentration of 5 µg $mL^{-1}$was used. Six solutions at five distinct wavelengths were analysed for the repeatability study. In the scenario of intravariation, at a specified time interval, the absorbance of prepared solutions was evaluated three times on a comparable day. Further, intravariation was accomplished by utilising the absorbance on three subsequent days.

Accuracy

The accuracy of the methodology for Acitretin was tested at 80%, 100%, and 120% of the pre-analysed sample solutions, and the percentages of recovery values were estimated.

Assay

Weigh accurately a quantity of Acitretin capsules equivalent to 10 mg of Acitretin, add 25 ml of ethanol and sonicate for 20 minutes. Add sufficient ethanol and make upto 100 mL. The solution obtained above is filtered and diluted with ethanol to attain 5 µg mL^{-1} concentration of Acitretin. The absorbance of the resulting solution is measured at 350 nm and the content of Acitretin is quantified.

RESULTS AND DISCUSSION

These standard solutions of Acitretin were scanned initially between 200-400 nm. The highest absorbance was recorded at 350 nm for Acitretin. To perform MVC, the wavelength was chosen at 350nm and the UV spectrum was recorded for standards and samples of Acitretin by taking ethanol as a blank. The spectra of 5 µg mL^{-1} standard Acitretin are represented in **Figure 2**.

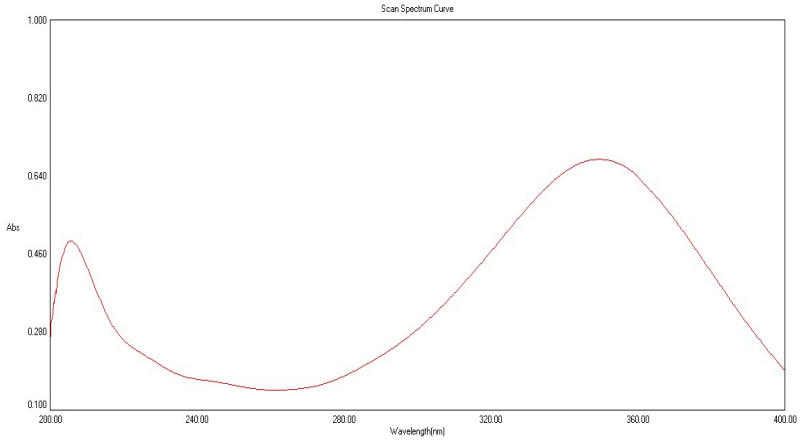


Figure 2: UV spectrum of standard Acitretin (5 µg mL^{-1}) using ethanol as a blank

Stability of solution

The results of solution stability of Acitretin is not major changes in the absorbance values as well the spectrum obtained at using the solution measured at 0, 6 and 12 hrs. The absorbance difference between the fresh standard solution and stored solutions were negligible and found to be less than 2%.

Linearity

The developed method linearity findings for Acitretin were identified a concentration range of 70 - 130% for 5 µg mL^{-1} (3.5-6.5 µg mL^{-1}) according to ICH Q2 R1 guidelines. The spectra for Acitretin were represented in **Figure 3**. The calibration curve was developed by measuring the absorbance of diluted standard solutions at five distinct wavelengths (340, 345, 350, 355, 360).

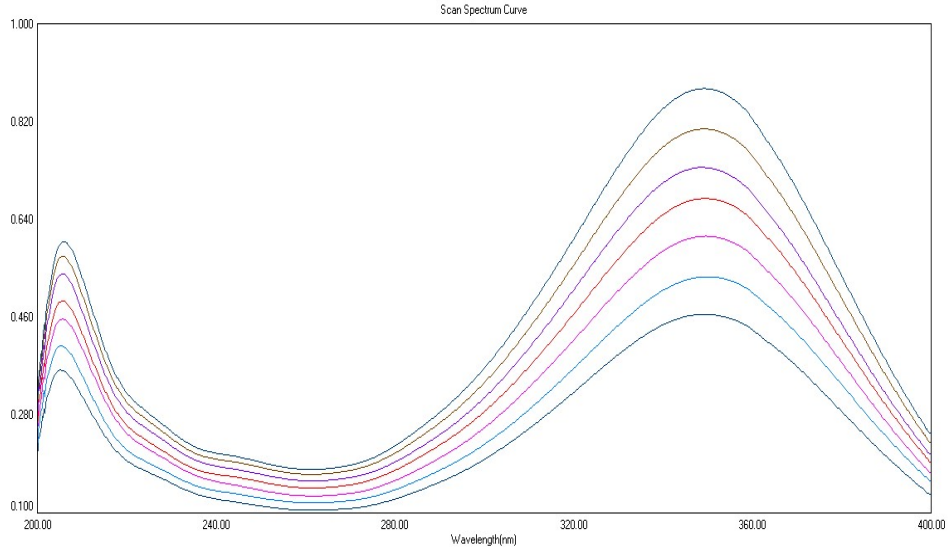
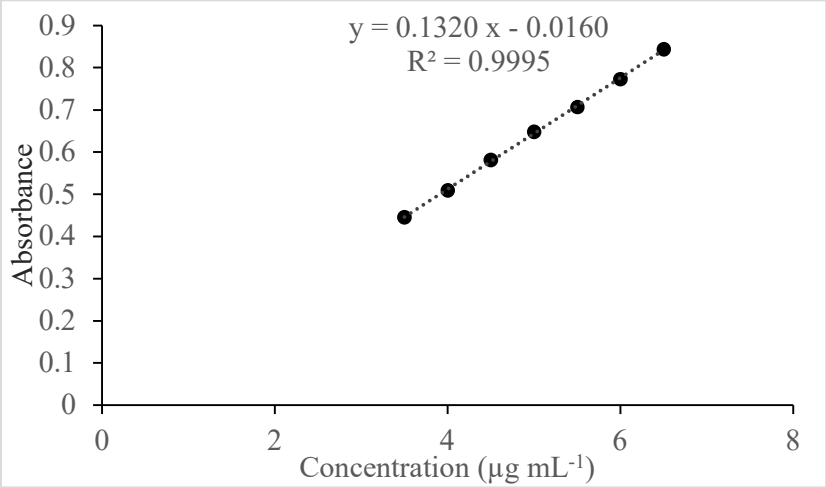
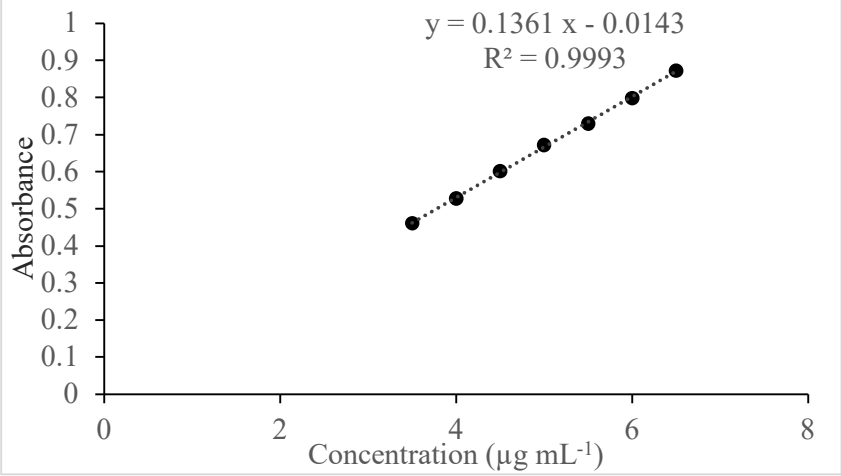


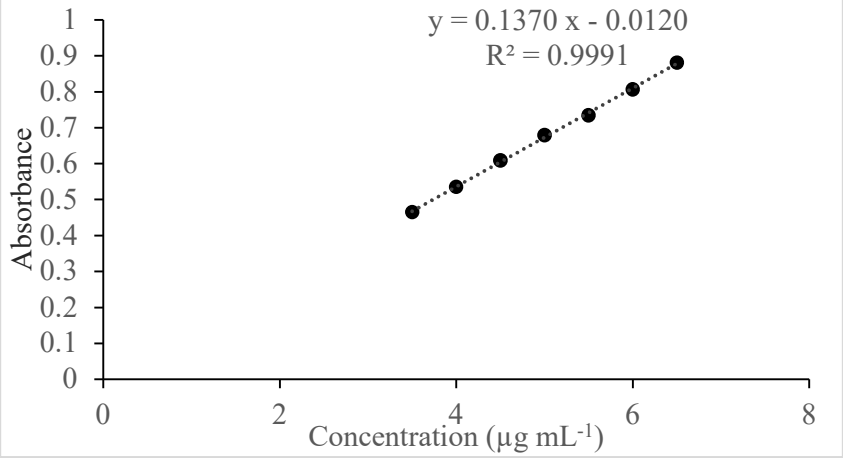
Figure 3: Linearity spectrum of Acitretin (3.5-6.5 µg mL^{-1}) using ethanol as a blank



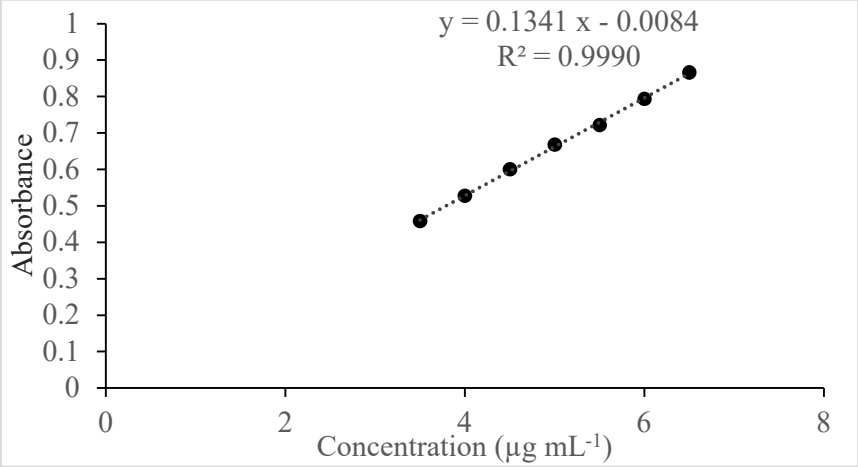
Multivariate calibration graph at 340 nm



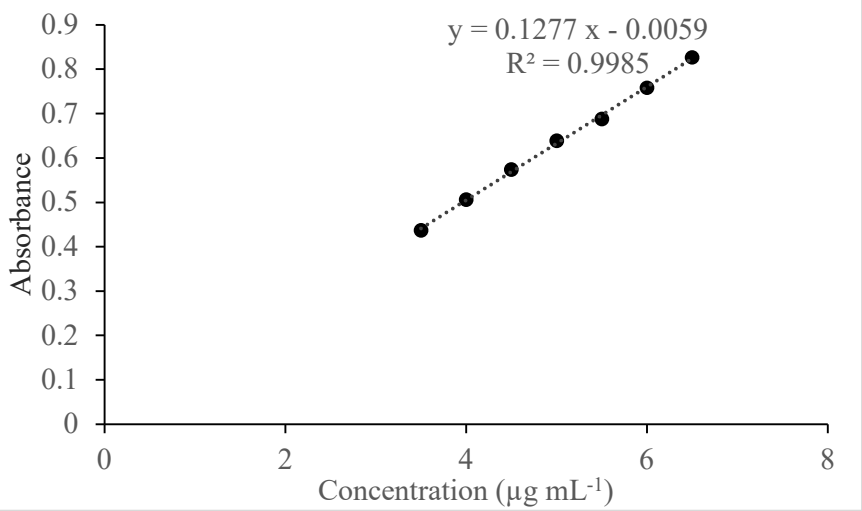
Multivariate calibration graph at 345 nm



Multivariate calibration graph at 350 nm



Multivariate calibration graph at 355 nm



Multivariate calibration graph at 360 nm

Best-fit values	340 nm	345 nm	350 nm	355 nm	360 nm
Slope	0.1320	0.1361	0.1370	0.1341	0.1277
Y-intercept when X=0	0.0160	0.0143	0.0120	0.0084	0.0059
R^2	0.9995	0.9993	0.9991	0.9990	0.9985
Deviation from zero	Significant	Significant	Significant	Significant	Significant

Table 1: Linearity values for proposed method of Acitretin

Limit of Detection and Limit of Quantification

The LOD and LOQ for Acitretin was calculated from the linearity slope, which has been confirmed by different sample analyses. The LOD for Acitretin was calculated from the average of all the absorbance, which was found to be 0.1030 µg mL^{-1}**.** The LOQ for Acitretin was calculated from the average of all the absorbance, which was found to be 0.3121µg mL^{-1}**.**

Precision

The system precision spectra for Acitretin are represented in **Figure 4**. The interday precision spectra for Acitretin are

represented in **Figure 5**. The intraday precision spectra were represented in **Figure 6** for Acitretin. The % RSD of system precision, interday and intraday precision, was determined for Acitretin. It was found to be less than 2%, which shows that the approach method is precise. The outcomes are represented in **Table 2** for Acitretin. The proposed method shows good precision compared to the values obtained from various precision methods.

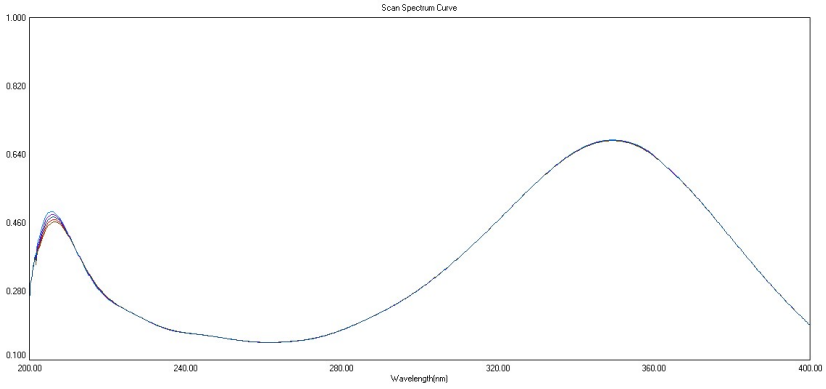


Figure 4: System precision overlay spectra of Acitretin

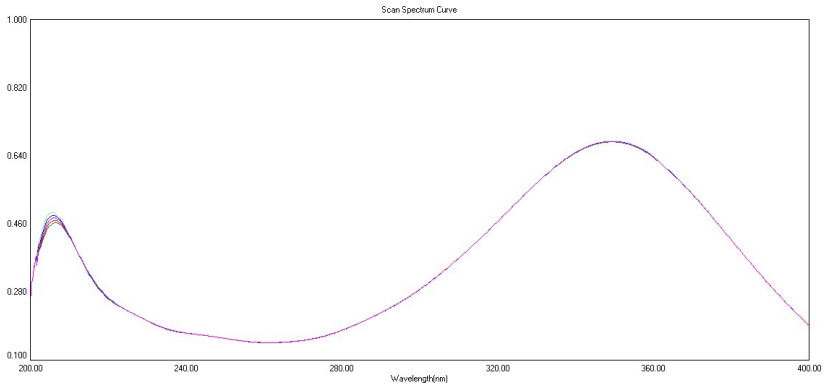


Figure 5: Interday precision overlay spectra of Acitretin

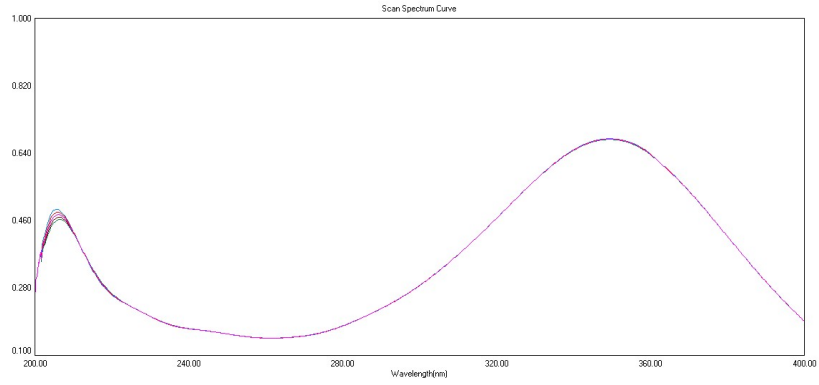


Figure 6: Intraday precision overlay spectra of Acitretin

Table 2: System precision, Interday and Intraday precision data for the proposed method of Acitretin

	System precision	Intraday and interday precision		
	Absorbance of standard for 5 µg mL^{-1}	% Recovery of sample equivalent to 5 µg mL^{-1} of sample		
		Day 1	Day 2	Day 3
1	0.678	99.31	98.89	98.59
2	0.681	98.91	99.3	99.54
3	0.667	99.35	99.21	99.53
4	0.672	99.30	99.52	99.58
5	0.689	99.50	99.19	99.34
6	0.692	99.41	98.76	99.57
Mean	0.680	99.30	99.15	99.36
SD	0.010	0.20	0.28	0.39
%RSD	1.42	0.20	0.28	0.39
CI	0.0077	0.1627	0.2218	0.3093

Accuracy

The accuracy of Acitretin was tested at 80, 100, and 120%. The overlay spectra for Acitretin is in **Figure 7**. The results are shown in **Table 3** for Acitretin and the obtained results were found to be within limits.

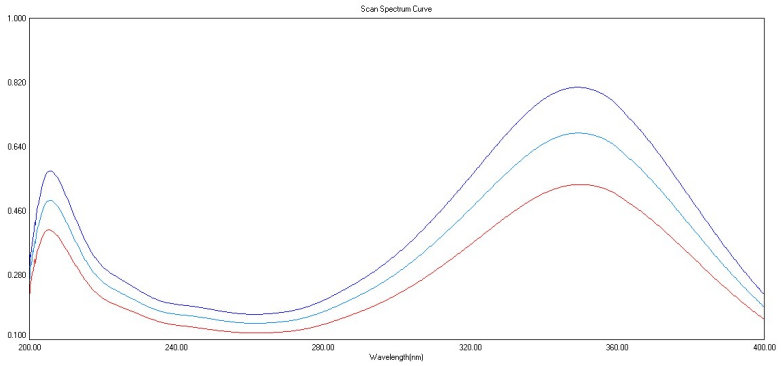


Figure 7: Overlay spectra of accuracy of Acitretin at 80, 100, 120 % spiking

Table 3: Accuracy data for proposed method of Acitretin

Concentration levels (%)	Amount present	Amount added (µg mL^{-1})	Amount recovered (µg mL^{-1})	Mean % Recovery	SD
80	2.5	1.5	3.91	97.92	0.7637
100	2.5	2.5	4.89	97.80	0.8000
120	2.5	3.5	5.95	99.06	0.5091

Assay of marketed formulations:

The quantification of Acitretin in the capsules formulation was examined using the suggested spectrophotometric method. For three replicates, the commercial capsules UV absorption spectrum was achieved. The pharmaceutical formulation does not have a significant loss in terms of high analytical recovery values after the extraction and filtration process. The findings, which demonstrate that the new approach performs better than the prior methods, are shown in **Table 4** for Acitretin.

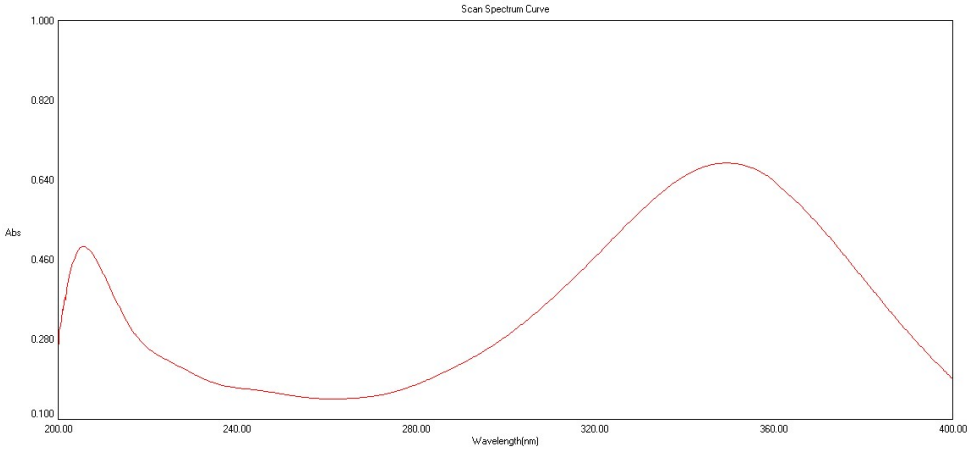


Figure 8: UV spectrum of sample Acitretin (5 µg mL^{-1}) using ethanol as a blank

Table 4: Assay results for marketed formulation of Acitretin

Marketed formulation	Label claim (mg)	Mean ± SD (n=3)	% RSD
Batch - 1	10	9.90 ± 0.02	0.1542
Batch - 2	10	9.92 ± 0.05	0.5040

Evaluation of Greenness Profile

The results of greenness profile for the proposed methods were evaluated. The results of analytical scale is shown in **Table 5**, while the results agree metrics and GAPI is depicted in **Figure 9** and **Figure 10**.

Table 5: Summary of Eco scale penalty points for the proposed method

Description	Penalty points	Total Penalty Points	Score
Ethanol	4	4	96
Instrument	0		
Occupational hazard	0		
Waste	0		

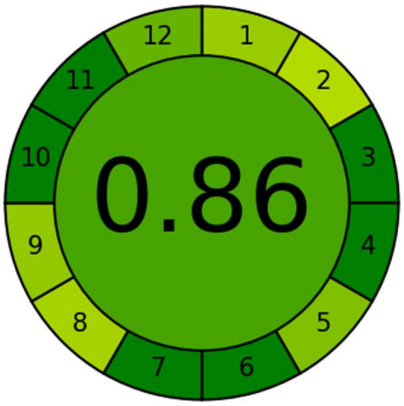


Figure 9: Agree metrics output for the proposed method

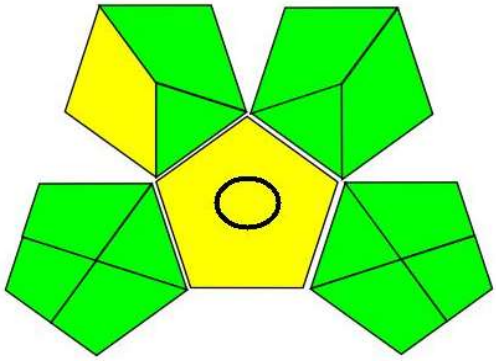


Figure 10: GAPI Pictogram for the proposed method

CONCLUSION:

The proposed multivariate calibration approach was a simple, novel, accurate, precise technique for the estimation of Acitretin. It is highly recommended to develop a new strategy for routine acitretin analysis. When compared to ICH recommendations, all validation parameters were assessed and found to be within acceptable bounds. The proposed method possess an ideal greenness profile assessed by analytical ecoscale, GAPI and agree metrics. The proposed method shall be used for routine simultaneous determination of acitretin as an alternative to time-consuming and expensive separation techniques.

ETHICAL STATEMENT

This study does not involve experiments on animals or human subjects

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article exists.

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