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RESEARCH ARTICLE

DETERMINATION OF ACITRETIN IN PHARMA-CEUTICAL FORMULA-TIONS BY HPLC METHOD

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Abstract: Acitretin is in a group of medicines called retinoids. It is used in severe skin disorders like psoriasis and it is official in USP. Psoriasis is a skin disease affecting millions of person's worldwide. A simple, precise and rapid HPLC method was developed for the estimation of Acitretin in pharmaceutical dosage forms. The method was carried out on a Purospher BDSC18 column(250×4.6mmid, 5µm) column using a mixture of acetic acid Buffer (pH adjusted to 4): methanol: tetrahyrofuran [12:85:3]v/v/v as a mobile phase. The flow rate was 1 ml/min and Ultra-violet detection was carried out at 354 nm. Every part of determination was performed at ambient column temperature. The retention time was 4.313 min for Acitretin. The developed method was validated for parameters like specificity, accuracy, precision, robustness as per International Conference on Harmonization guidelines. Linearity for Acitretin was in the range of 30-180µg/ml and correlation coefficient was found to be 0.999. The percentage recovery was found to be in the limit of 98.9-99.8 %. Statistical analysis of the results has been carried out revealing high accuracy and good precision. Hence this method can be of use and value for the quality control department of pharmaceutical companies manufacturing these formulations without any interference due its sensitivity, simplicity and selectivity.

Key words: Acitretin, HPLC, Validation, ICH.

INTRODUCTION:

Acitretin (ACT) is in a group of medicines called retinoids. It is used in severe skin disorders like psoriasis [1-2]. Psoriasis is a skin disease affecting millions of person's worldwide. It is clinically categorized by erythematous, rounded plaques and sharply demarcated papules covered by silvery micaceous scale, epidermal hyper proliferation overlying immune mediated dermal inflammation, leading to profound adverse effects on patient's physical, social and mental wellbeing. Acitretin is used to treat Darier's disease. Acitretin works by inhibiting the excessive cell growth and keratinization seen in psoriasis [3-4]. It therefore reduces the thickening of the skin, plaque formation and scaling. Oral Acitretin is currently indicated for the treatment of severe psoriasis in adults, but its use is limited by systemic side effects and teratogenicity.

A literature survey reported that few publications are reported for estimation of acitretin in pharmaceutical preparations as well as in biological fluids by application of spectroflourimetry and LCMS/MS [5-12]. There is no HPLC method was available for determination of acitretin with faster elution time in pharmaceutical preparations (capsules). Hence an attempt was made to develop and validate a new, simple, precise, accurate and especially time saving method which is suitable for quality control in the pharmaceutical industry.

EXPERIMENTATION:

Apparatus:

- HPLC - LC 10 AT VP Shizmadzu
- pH meter- CYBER SCAN 510 (Elico)
- UV-Visible Spectrophotometer - UV-1700 (Shimadzu)
- Digital balance- (Sartorius)
- Sonicator- MODEL 2200MH (Shimadzu)
- Vacuum filter- MF-6126 (Millipore)

Materials and Reagents:

All chemicals and reagents used throughout the work were of analytical grade. All other solvents used were of HPLC grade. HPLC grade Milli Q water was used throughout the experiment work. acitretin was received as gift samples from Reddys Labs Hyderabad, India, respectively. Acetic cap® capsules were purchased from local market.

Mobile phase Preparation

The mobile phase was prepared by mixing in the ratio of

acetic acid Buffer (pH adjusted to 4): methanol: tetrahyrofuran [12:85:3].v/v/v. Then this solution was filtered through 0.45µ membrane filter and further the air bubbles are removed by sonication.

Preparation of standard drug stock solutions

The standard stock solutions of ACT (1mg/ml) was prepared by suitable dilutions.

Preparation of linearity solutions.

The linearity standard solutions were prepared from standard stock solutions. Pipette out 0.3, 0.6, 0.9, 1.2,1 .5 ml from ACT stock solution and transfer in 10 ml standard volumetric flask and make up to10 ml with mobile phase to acquire that the linearity concentrations of 30-150 µg/ml of Acitretin. Then the samples were filtered by a 0.45µm nylon membrane filter before injecting in to the column.

Preparation of sample solution

Twenty capsules every single acetic cap® (Dr. Reddys), containing 10 mg of ACT were accurately weighed and find out their average weight. The powder equivalent to 10mg of ACT was taken in 100ml of standard volumetric flask and extracted with 50 ml of methanol. The sample was kept in an ultrasonic bath for 20 min and additional diluted to 100ml by using mobile phase. Then this sample is filtered through 0.2micron membrane filter. From this solution 5ml is dilute to10ml to get a final concentration of 50 µg/ml of ACT. All the solutions were protected from light.

Assay standard solution preparation

A working standard solution having concentration of 50 µg/ml of ACT was prepared from the above standard drug stock solution

Results and Discussion

Method Optimization

A number of trials were made to find out the ideal solvent system (mobile phase) for eluting the drug. The trails carried out by using mobile phase containing Methanol: water (50:50), Methanol: water (70:30), methanol: acetonitrile: water (30:30:40), methanol: acetonitrile: water (50:35:15). These trails showed poor resolution. To improve the resolution acetic acid buffer with ethanol system is used and got better peak shapes and further more to improve the resolution and reduce the tailing tetra hydrofuran also introduced, after many trails finally filtered and degassed the mixture of acetic acid buffer (pH adjusted to 4): methanol: Tetrahyrofuran [12:85:3] v/v/v was designated as a appropriate mobile phase and the detection was carried out at 354 nm. The obtained standard, sample and blank chromatograms were reported in Figure1.

VALIDATION OF THE METHOD

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[13].

Specificity:

The specificity of the proposed HPLC method was proved by its ability to determine the Acitretin in its formulation confirming that, there was no interference.

Linearity

To check linearity, standard graphs were built with six concentration of Acetretin in the range of 30-150 µg/ml of ACT were prepared in triplicates. The linearity was calculated by least square regression method and results are reported in Table.1and linearity overlain spectra are presented in Fig .2 and Calibration graph presented in Fig.3.

Table 1.Linearity data for Acitretin

Parameters	Acitretin
Detection wavelength	354
Linearity range(µg/ml)	30-180
Regression equation Y=mx+C (Slope(m))	452.36
Intercept(C)	766.05
Correlation coefficient	0.9994
Limit of Detection(µg/ml)	1
Limit of Quantification(µg/ml)	3

Limit of Detection and Limit of Quantification (sensitivity)

The limit if detection (LOD) was calculated from the linearity curve using the formula

LOD= 3.3X {Residual Standard deviation/Slope}.

The LOD for ACT was confirmed to be 1.0 µg/ml.

The Limit of quantification (LOQ) was calculated from the linearity curve using the formula.

LOQ= 10X {Residual Standard deviation/Slope}

The LOQ for ACT was confirmed to be3.0 µg/ml

Accuracy:

It was performed by adding 80%, 100%, 120% of pure standard drug of ACT to previously analyzed tablet powder sample and mixtures were reanalyzed by the proposed method. For every single concentration three sample sets were prepared and injected in duplicate and results were reported in Table.2 and it confirms the accuracy of the method .

Precision

Then system precision was determined by using working standard ACT solution containing 50µg/ml of ACT was injected 6 times and recorded the response of peak areas.

Figure.1 Typical chromatogram of blank, standard and sample of Acitretin

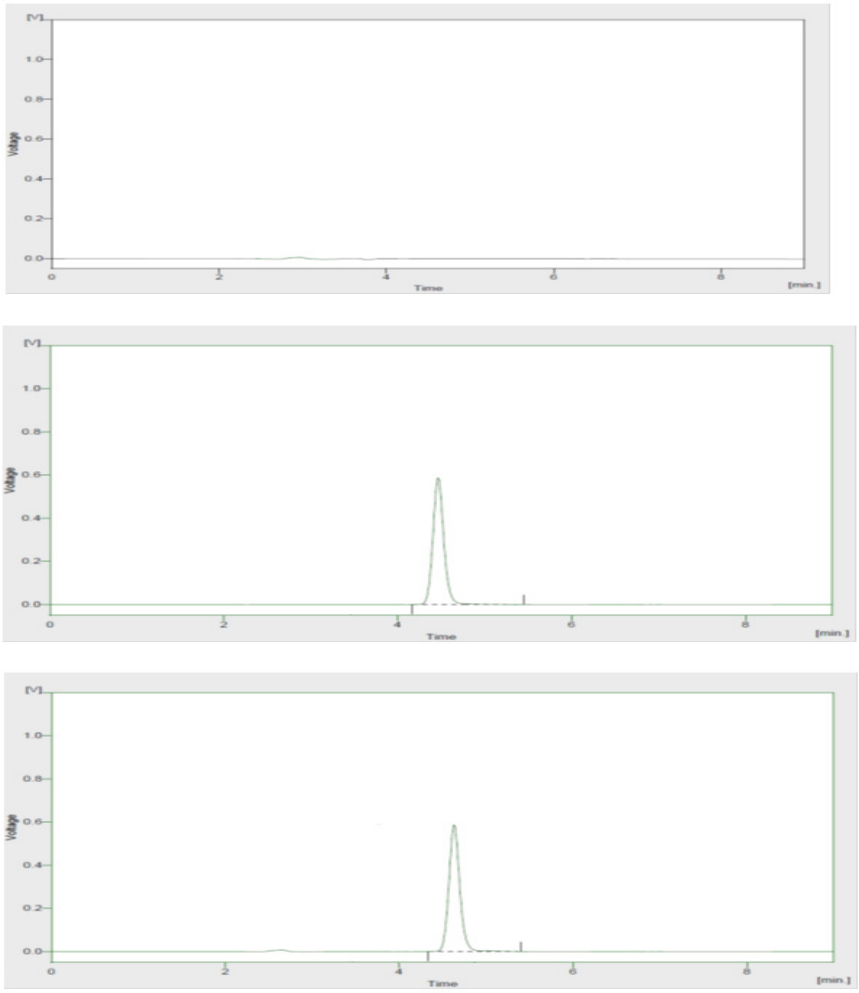

Figure.2 Standard Calibration curve of Acitretin

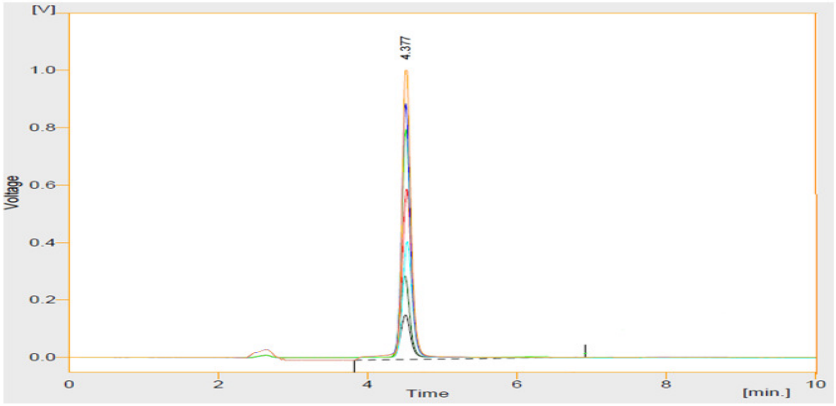

Figure.3 Standard Calibration curve of Acitretin

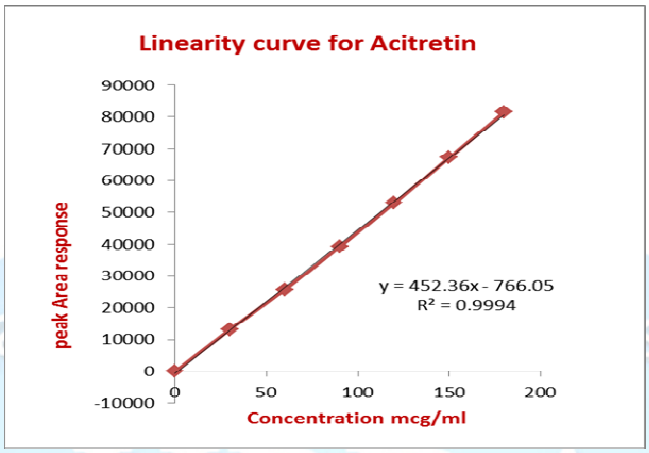

Table: 2. Results of recovery studies of Acitretin

Level in %	Amount added (µg/ml)	Added Amount Recovered (µg/ml)	Recovery%	Mean Recovery	RSD%
80% Spl1	40	39.98	99.98	99.99	1.241
80% Spl2	40	39.97	99.97		
80% Spl3	40	39.99	99.99		
100% Spl1	50	50.11	100.21	100.21	1.471
100% Spl2	50	50.14	100.24		
100% Spl3	50	50.15	100.19		
120% Spl1	60	59.99	99.99	99.98	0.541
120% Spl2	60	59.96	99.93		
120% Spl3	60	60.01	100.0		

The method precision was determined by sample of same concentration was injected the working sample solution containing 50µg/ml of ACT of tablet sample six times recorded the response of the peak area. The peak areas and percentage relative standard deviation were calculated and presented in tables 3. Precision study also evaluated by intermediate precision. From the data obtained, the developed RP-HPLC method was found to be precise.

Standard and sample solution stability

Standard and sample solution stability was evaluated at room temperature and refrigerator temperature for 24h. The relative standard deviation was found below 2.0%. It showed that both standard and sample solution were up to 24h at room temperature and refrigerator temperature.

Robustness:

Robustness of the method was determined by making slight changes in the chromatographic conditions. It is studied by altering the composition of mobile phase i.e., organic modifier percentage, buffer pH and flow rate by ±0.05 ml/min analyzing six samples from a homogeneous batch. The changes did not affect the results indicating that the proposed method is robust under these chromatographic conditions.

Ruggedness

The ruggedness of the method was determined by carrying out the experiment on different instruments like Shimadzu HPLC (LC2010 A4T), Water Alliance HPLC 2695 by different operators using similar chromatographic conditions.T he samples were analyzed for each conditions and the assay content of the both the analytes were estimated. It can be observed from the results in table.4 that the values are well within acceptance limits of 99.96-100.11% for ACT with a RSD of less than 2.0% for both ACT. The above experiments indicated that the method delivers consistent and reliable results.Table.4.

System Suitability

System suitability testing is an integral part of many analytical procedures. Table 5 shows the summary of system suitability parameters of the presented study. All the results were fall in the acceptable limits, and it confirms the system suitability of the method.

Analysis of formulations

The HPLC method developed is sensitive and specific for

Table .3 Precision Results for Acitretin

Precision	System precision*			Method Precision*			Intermediate precision*		
In % Values	Limit	Average	RSD	Limit	Average	RSD	Limit	Average	RSD
Acitretin	2.0		0.128	98-102	100.55	0.392	3	98-102	1.11

*Average of six determinations,

Table:4 Ruggedness of the method.

Parameters	% Assay For ACT	%RSD
Analyst 1(n=3), %	99.99	0.895
Analyst 2(n=3), %	100.11	1.128
Instrument 1(n=3), %,	99.98	0.327
Instrument 2(n=3), %	99.96	0.254

Table 5. System suitability study of proposed HPLC method

S.NO	Parameters	ACT	Acceptance criteria
1	Retention time(min)	4.372	
2	RSD of replicate injections	0.128	Not more than 2%
3	Asymmetric factor	0.94	Not more than 2
4	Theoretical plates(N)	5305	Not less than 2000
5	Resolution factor		More than 2
6	Linearity Range(µg/ml)	30-180	--
7	Limit of detection (µg/ml)	1	--
8	Limit of Quantification(µg/ml)	3	--

Table: 6 Analysis of Formulations

Drug	Labelled Amount(mg)	Amount of mg/Tablet found*	% of Label claim	%RSD*
Acitretin	10	10.79±0.50	100.24±0.05	0.63

the estimation of Acitretin. Also the method is validated for different parameters, hence has been applied for the estimation of drug in pharmaceutical dosage forms. Tablets (containing Aectac CAP® 10 mg of (Dr.Reddys) were evaluated for the amount of ACE present in the formulation. Each sample was analyzed in triplicate and the amount of Acitretin was found to be 100.79% one of the tablet excipients interfered with the analyte and results are reported in Table.6.

Conclusion

A novel simple chromatographic method developed for estimation of acetretin in bulk and pharmaceutical dosage form according to ICH recommended conditions. The total analysis time completed within 5 min respectively and the high column efficiency was confirmed from the large number of theoretical plates (>2000). The proposed method is sensitive enough for the quantitative detection of analytes in the pharmaceutical preparation. Since, all the results within the limit, the developed analytical method deliberated as validated and suitable for routine quality control of their bulk drug and its dosage forms.

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