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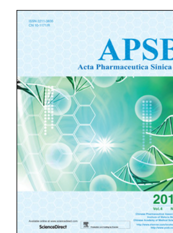

ORIGINAL ARTICLE

Assessment by HPLC of the degradation behavior of acitretin under hydrolytic, oxidative, photolytic and thermal stress conditions

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KEY WORDS

Acitretin;
Degradation kinetics;
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Validation

Abstract Acitretin is a photosensitive oral retinoid with very limited data available on its degradation. The official HPLC method for acitretin determination was insufficient to resolve the degradation products generated during stability studies. Therefore, an isocratic RP-HPLC–UV method was developed for the determination of acitretin in the presence of its related impurities and degradation products. Efficient chromatographic separation was achieved on a Thermo beta-basic column C18 (100 mm × 4.6 mm, 5 μm) with mobile phase containing 0.3% (*v*/*v*) glacial acetic acid with acetonitrile (ACN) and isopropyl alcohol (IPA) in an isocratic ratio of 70:30 at a flow rate of 1.0 mL/min with the eluent monitored at 360 nm. The method was validated for specificity, linearity, precision, accuracy and robustness. The calibration plot was linear over the concentration range of 50–150 μg/mL with a correlation coefficient (r^2) of 0.999. The proposed method was used to investigate the degradation kinetics of acitretin under the different degradative conditions. The degradation rate constant (K), half-life ($t_{1/2}$), and t_{90} were calculated. Degradation of acitretin followed pseudo-first-order kinetics. The drug was found to be less stable under acidic and photolytic degradation conditions: the photolytic degradation constants for acitretin in sunlight and UV light were 0.002698% and 0.0008402% min^{-1}, respectively. The LOD for acitretin and the known impurities were at a level below 0.02%. The method shows consistent recoveries for ACTR (99.8%–101.2%) and also for its known impurities (97.2–101.3%). The method was found to be accurate, precise, linear, specific, sensitive, rugged, robust, and useful for characterizing the stability of this chemical.

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1. Introduction

Acitretin (ACTR; (all-*E*)-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid, Fig. 1), an oral retinoid, is used as a first line treatment for psoriasis[1]. Acitretin works by inhibiting the excessive cell growth and keratinization seen in psoriasis. It therefore reduces the thickening of the skin, plaque formation and scaling[2].

Regulatory requirements for the identification, quantification, and control of impurities in drug substances and their formulated products are now being explicitly defined, particularly through the International Conference on Harmonization (ICH). It is also recommended by ICH that all routine impurities at or above 0.1% level should be identified through appropriate analytical methods[3–5]. ACTR is cited in the European Pharmacopoeia to contain the imp-A ((2*Z*,4*E*,6*E*,8*E*)-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethylnona-2,4,6,8-tetraenoic acid, Fig. 1) and imp-B (ethyl (all-*E*)-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethylnona-2,4,6,8-tetraenoate, Fig. 1)[6]. Therefore, it was thought worth determining the impurities of ACTR to ensure the quality, efficacy and safety of the final pharmaceutical formulation. To this end, a method for analyzing ACTR in the presence of its two known impurities (imp-A and imp-B) was developed. Tretinoin, all *trans*-retinoic acid ((all-*E*)-3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4,6,8-nonatetraenoic acid, Fig. 1), is likely to be present in formulations of acitretin[6]. Therefore, in addition to the known impurities of ACTR, tretinoin was also analyzed in this study.

A literature survey revealed that few publications are reported for estimation of ACTR in pharmaceutical preparations as well as in biological fluids. Surber and Laugier et al.[7] have reported an HPLC method for the determination of ACTR in human plasma and further applied it to a pharmacokinetic study. Liquid chromatography with tandem mass spectrometry methods has been used for quantitation of ACTR in human plasma either alone[8], in combination with its metabolite[9] or in combination with other drugs[10]. A spectrofluorimetric method has been reported for the determination of acitretin in pharmaceuticals in the concentration range of 30.0–1100 ng/mL[11]. The official HPLC method[6] for ACTR analysis includes a C18 stationary phase with 0.3% (*v*/*v*) solution of glacial acetic acid (GAA) in a mixture of water and ethanol in the ratio of 8:92 (*v*/*v*) as mobile phase at reduced flow rate (*i.e.,* 0.6 mL/min). The method results in a long run time to elute ACTR and its known impurities, and did not provide stability information essential for regulatory submission. The run time could be shortened by increasing the flow rate.

From the preceding details it is apparent that a validated method is required to be developed which would provide simultaneous determination of ACTR in the presence of its reported impurities, and also provide an indication of ACTR stability. Thus, the aim of current study was to develop and validate a LC method for the determination of ACTR and its known impurities (imp-A, imp-B and tretinoin) along with its degradation products when provided in a capsule dosage form, in accordance with the ICH guidance document[12].

2. Materials and methods

2.1. Reagents and chemicals

Qualified standards of ACTR and its related imp-A imp-B were a gift from Torrent Research Centre (Ahmadabad, India). Analytical/HPLC grade chemicals and solvents were obtained from Ranbaxy Fine Chemicals Limited (Delhi, India).

2.2. Chromatography apparatus and conditions

The chromatograph consisted of a HP-Agilant 1100 HPLC System with G1311A Quaternary Pump, G1315A Diode Array Detector and variable wavelength detector, a G1313A Autosampler, and a G1322A Vacuum Degasser. The data were evaluated by HP Chemstation Software.

Optimum separation conditions were obtained with a Thermo Beta basic C18 (100 mm × 4.6 mm, i.d. 5.0 μm, 150 Å pore size) column with mobile phase consisting of ACN:IPA:GAA in the ratio of 70:29.7:0.3 (*v*/*v*/*v*) with column oven temperature maintained at 25 °C and elution monitored by a UV detector at 360 nm.

All measurements were performed with an injection volume of 20 μL of sample dissolved in a diluent:acetonitrile and isopropyl alcohol in ratio of 80:20.

2.3. Preparation of solutions

2.3.1. Preparation of resolution solution

As enumerated in the official procedures for analysis of ACTR[6], tretintoin, imp-A and imp-B were dissolved in 60 mL of diluent in a 100 mL volumetric flask. The standard solution was sonicated at 10 ± 2 °C for 10 min. The volume of the clear solution obtained was made up to 100 mL with the diluent to give the resolution

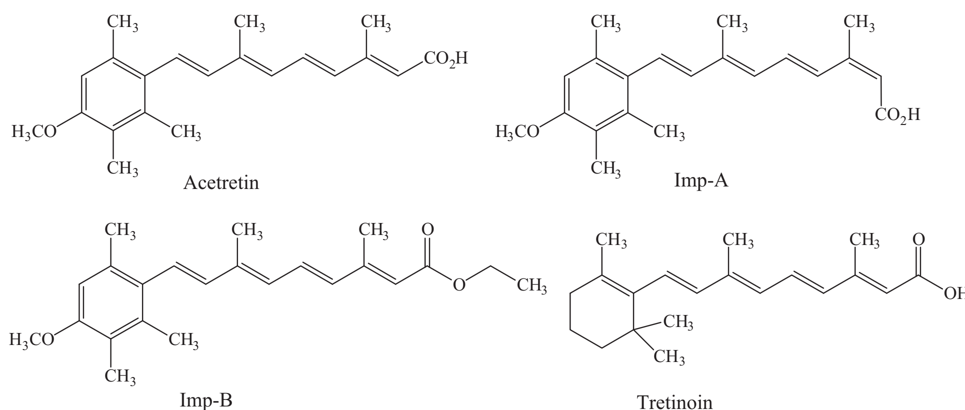

Figure 1 Chemical structures of acetretin, imp-A, imp-B and tretinoin.

solution containing 100 μg/mL ACTR, and 10 μg/mL each of imp-A and imp-B.

2.3.2. *Preparation of laboratory mixture solutions*

Appropriate amounts of ACTR, tretinoin, imp-A, imp-B and excipients equivalent to the average weight of the capsule powder were transferred to a 100 mL volumetric flask. About 60 mL of diluent was added and sonicated for 10 min at 10 °C with intermittent shaking and diluted to volume with the diluent to contain 100 μg/mL ACTR and 1.0 μg/mL each of known impurity. This solution was filtered through a 0.45 μm Nylon 66-membrane filter and used for the analysis.

2.3.3. *Preparation of sample solution*

The average weight of 20 capsules was determined. The fill of 20 capsules was removed and an amount equivalent to 10 mg of the active pharmaceutical ingredient (ACTR) was transferred to a 100 mL volumetric flask. Diluent (60 mL) was added and the flask solution was sonicated for 10 min at 10 °C with intermittent shaking and diluted to volume with the diluent. This solution was filtered through a 0.45 μm Nylon 66-membrane filter and used for the analysis. A similar method was employed to prepare the placebo solution.

2.4. *System suitability*

System suitability parameters were evaluated to verify that the analytical system was working properly and would give accurate and precise results. Parameters such as peak asymmetry factor, tailing factor, resolution between imp-A and ACTR and RSD (%) of theoretical area obtained from two resolution solutions (in triplicate) were evaluated.

2.5. *Filter-compatibility studies*

A laboratory mixture solution was subjected to filter-compatibility studies. The solution was filtered using Whatman® filter paper No. 42 and 0.45 μm Nylon 66-membrane filter. Another laboratory mixture solution was centrifuged (unfiltered). Chromatography was performed on these three solutions, in triplicate, and difference between concentrations of each component in filtered and unfiltered sample solutions was calculated.

2.6. *Analytical method validation*

2.6.1. *Specificity*

Specificity is the ability of the method to measure the analyte response in the presence of its potential impurities and degradation products. The specificity studies were carried out in two parts namely part-A and part-B. In specificity part-A, separation and resolution were observed between diluent, known impurities, degradation product(s), placebo solution, and ACTR standard solution.

Whereas, in specificity forced degradation studies for acitretin were designed and executed as per guidelines described in the literature[13]. The forced degradation rate constant was calculated using Graphpad Prism® Version 5.01 software. To prevent any possible photodegradation all experiments were performed in dimmed light.

2.6.2. *Linearity*

Linearity test for the method was performed according to the guidelines described by ICH. Appropriate aliquots of ACTR stock solution were diluted with the diluent to appropriate concentrations and the linearity of ACTR was determined; the range was inclusive of concentrations at 50%, 80%, 100%, 120% and 150% of test concentration.

A calibration curve was drawn by plotting the peak areas of ACTR *versus* its corresponding concentration. The process was repeated for three consecutive days in the same concentration range. Values of coefficient of regression, slope and Y-intercept of the calibration curve were calculated.

2.6.3. *Precision*

Six solutions containing ACTR (100 μg/mL) were prepared separately. Chromatography was performed and the value of RSD (%) was calculated considering peak area for ACTR. Similarly, intermediate precision of the method was also evaluated by another analyst, on a different day in the same laboratory.

2.6.4. *Limit of detection (LOD) and limit of quantification (LOQ)*

The LOD and LOQ for ACTR and all related substance (RS) were estimated by signal-to-noise ratio, 3:1 and 10:1, respectively, injecting a series of six diluted solutions of ACTR and its RS in known concentrations.

2.6.5. *Accuracy*

Recovery studies were performed in triplicate at concentration levels of 50%, 100% and 150% of ACTR (100 μg/mL) to evaluate the accuracy of the proposed method. Solutions for the purpose were prepared by standard addition of ACTR stock solution to laboratory mixture solution.

2.6.6. *Stability of laboratory mixture solution*

The stability of the ACTR stock solution (100 μg/mL) and the laboratory mixture solution was evaluated at regular intervals for 24 h at room temperature and at 10 °C. The difference in areas of the respective peaks in the chromatograms was calculated.

2.6.7. *Robustness*

The method was performed with small variations such as a composition of the mobile phase (±2% of IPA), a change to the mobile phase flow rate (±0.2 mL/min), and increasing the temperature from normal (±5 °C). Chromatograms of six replicas of the laboratory mixture solution were obtained and the effect of each deliberate change was evaluated by applying system suitability parameters and calculating value of RSD (%) for each deliberate change.

2.7. *Application of the validated method*

2.7.1. *Laboratory mixture*

The validated method was applied to a known laboratory solution of ACTR. A series of laboratory solutions containing 8.0, 10.0 and 12.0 μg/mL of ACTR were prepared and the concentration of the analyte was back-calculated.

2.7.2. *Assay of ACTR in marketed dosage form*

A marketed formulation containing ACTR (10 mg of ACTR as the active pharmaceutical ingredient) was diluted to a concentration of

10 μg/mL with diluent. Each of the solutions was prepared in triplicate and chromatograms were recorded and the percent assay was calculated.

3. Results and discussion

3.1. Development of the chromatographic method

The official HPLC method[6] to analyze ACTR and its RS could not be used to determine drug stability, and using these methods to analyze samples of ACTR capsules treated with acid, alkali, hydrogen peroxide, heat and light and spiked with laboratory mixture solution did not yield satisfactory results. The methods were not able to produce sufficient resolution between degradation products with the RS. The chromatogram of sample containing degradation products generated by photolytic stress showed elution of acitretin related imp-A and degradation products at relative retention time (RRT) 0.943 and RRT 1.06 which did not meet the acceptance criteria for peak purity. Additionally, the method applied to samples of alkaline degradation yielded a chromatogram displaying co-elution of degradation products at the same RRT. Moreover, the official HPLC method for assay of ACTR yields a >60 min elution time for imp-B (Fig. 2a).

ACTR, p*K*a 4.79[14], was slightly soluble in selected analytical solvents like ACN, tetrahydrofuran (THF), IPA and methanol. ACTR was practically insoluble in water. The chromatographic conditions were optimized by different means (using different columns, different buffers and different organic phases). The goal of the method under development was envisaged to be capable of eluting wide range of compounds of different polarities, with excellent efficiency and sufficient band spacing in a shorter run time. During development of chromatographic method elution was performed using C18 columns having pore size of about 150 Å. Mobile phase consisting ACN, IPA, 50 mmol/L potassium phosphate buffer and various volumes of GAA in water was used preliminary in isocratic elution. The chromatogram showed co-elution of imp-A with ACTR. Increasing the proportion of ACN in the mobile phase resulted in rapid elution of acitretin with co-elution of impurity. Replacement of the potassium phosphate component with GAA yielded a mobile phase ACN:IPA:GAA in the ratio of 80:19.7:0.3 (*v*/*v*/*v*) and at a flow rate 1.0 mL/min gave optimum resolution in separate peaks of ACTR and its RS, although tailing was observed in few peaks. However, very late elution of tretinoin and imp-B was observed, making the official HPLC method time-consuming. Therefore, the proportion of IPA was increased from 20% to 30% of mobile phase. The final mobile phase was ACN:IPA:GAA in the ratio of 70:29.7:0.3 (*v*/*v*/*v*). The mobile phase was mixed and eluted at 1.0 mL/min by the system and the column temperature was maintained at 25 °C.

3.2. System suitability

Chromatographic separation was performed with a Thermo Beta basic C18 (100 mm × 4.6 mm, i.d. 5.0 μm) column with the above-mentioned mobile phase and a representative chromatogram is shown in Fig. 2b, which displays a tailing factor less than 1.5 for all the peaks and a resolution of 10.3 for impurity-A with respect to ACTR. The RSD (%) of the peak areas of six injections ACTR standard solution was 1.6.

Tailing factor, a parameter that ICH guidelines consider as a factor to be controlled, was within the established limits. The resolution factor between two consecutive peaks represents approximately twice the minimum request to be considered. The results of system suitability studies are shown in Table 1.

3.3. Filter compatibility studies

The results of the filter compatibility studies for unfiltered and filtered methods are tabulated in Table 2, and indicate that either a 0.45 μm filter or a Whatman filter can be used for regular analysis.

3.4. Specificity

The HPLC chromatograms recorded for ACTR alone and with its RS, blank, and placebo preparations displayed a single, non-overlapped peak for ACTR. The resolution factor obtained between the peak for ACTR and other peaks was more than 2.1 and the tailing factor of the peak for ACTR and the RS was always in the range 1.03–1.50. Thus, the HPLC method presented in this study is selective for ACTR and also for the other related compounds, which might co-exist as impurities. The degradation kinetics of ACTR is shown in Fig. 3.

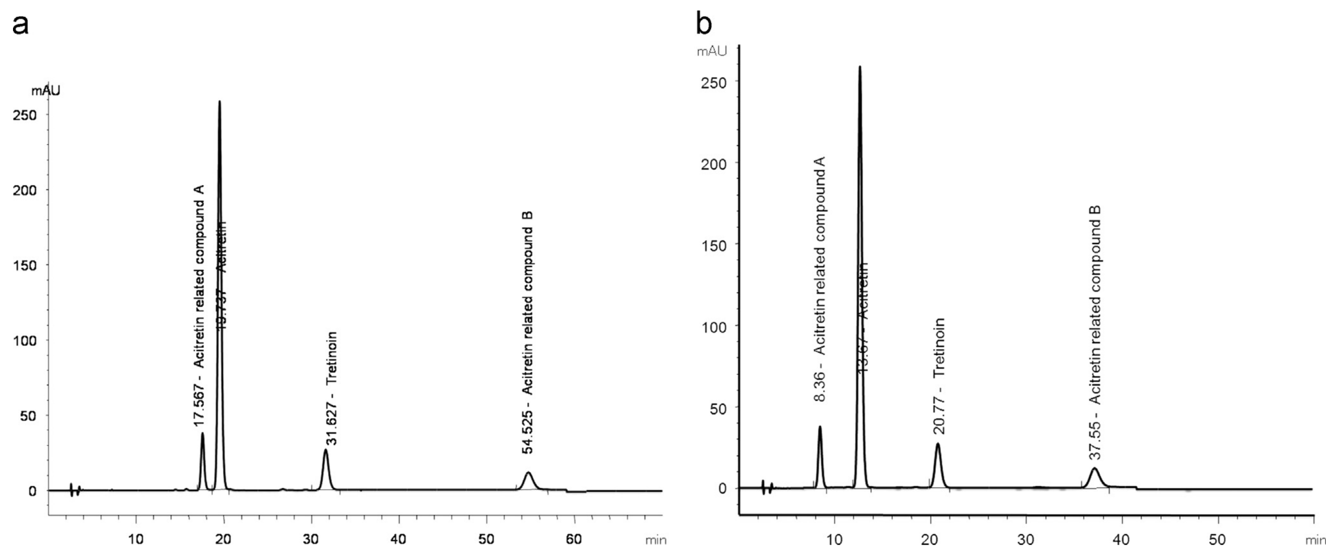

Figure 2 The chromatograms of ACTR and its RS in official method (a) and under optimized HPLC conditions (b).

Table 1 System suitability studies for resolution solution of ACTR and its RS ($n=6$) in the optimized ion pair-HPLC method.

System suitability parameter	USP limit	ACTR	imp-A	imp-B	Tretintoin
Retention time	–	13.61	8.36	37.55	20.77
RSD (%) of R_t	–	0.56	0.30	0.15	0.64
RSD (%) of peak area	≤ 2.0	0.65	1.90	1.26	0.50
Peak asymmetry factor (at 10%)	≤ 1.5	1.15	1.01	1.08	1.21
Capacity factor (k')	5–20	5.35	2.90	16.54	8.70

Table 2 Difference in percent peak area of filtered sample solutions with unfiltered sample.

Compound	Difference with unfiltered sample %			
	Set-1		Set-2	
	0.45 μm filter	Whatman filter	0.45 μm filter	Whatman filter
ACTR	1.5	−1.7	−0.4	3.7
Tretintoin	−1.7	−2.7	−0.5	−0.1
imp-A	−1.8	4.2	−1.0	−0.2
imp-B	−1.6	6.6	−0.9	1.0
Total impurity	−1.0	−0.7	−0.8	0.0

HPLC results of specificity part (forced degradation studies) of ACTR suggested the degradation behavior tabulated in Table 3.

3.4.1. *Degradation under acidic conditions*

ACTR was observed to be degraded to about 40% under acidic conditions, when treated with 1 mol/L HCl for 6 h at room temperature. imp-A was obtained as the major degradation product (12.52%) and eluted at 0.60 RRT as shown in Fig. 4a. ACTR was found to be relatively stable ($\approx$95.0% remaining) under less intense stress conditions (0.1 mol/L HCl for 6 h at room temperature) with imp-A as the only degradation product. The degradation rate constant under acidic condition (1 mol/L HCl for 6 h at room temperature in dark) was $1.26 \times 10^{-6}\% \text{ min}^{-1}$ with degradation half-life more than 6 days.

3.4.2. *Degradation under basic conditions*

ACTR was found to be degraded to 70.0% when treated with 1 mol/L NaOH for 6 h at room temperature. The chromatogram (Fig. 4b) displayed imp-A as a major degradation product (10.06%), eluting at 0.60 RRT. ACTR was found to be stable ($\approx$98.5% remaining) under less alkaline conditions (0.1 mol/L NaOH for 1 h at 80 °C) with imp-A as the only degradation product. Results of the degradation studies suggest long term storage of the drug leads to degradation, with a fall in the content of ACTR and a corresponding rise in imp-A.

3.4.3. *Degradation under oxidative conditions*

The drug was degraded to 80% in the presence of peroxide (3% H_2O_2 at 80 °C for 1 h) with imp-A as a major degradation product eluting at 0.62 RRT. The chromatogram (Fig. 4c) displayed more than 3 degradation products. ACTR was relatively stable (98.02% remaining) under milder oxidative conditions (3% H_2O_2 at 80 °C for 10 min).

3.4.4. *Degradation under photolytic conditions*

ACTR was found to be highly unstable when exposed to 1.2 million Lux hours with near-UV energy ≥ 200 Wh/m^2; the chromatogram is shown in Fig. 4d. The chromatogram displayed imp-A as major degradation product (12.06%) showing elution at 0.61 RRT. The degradation rate constant was $0.0008402\% \text{ min}^{-1}$. Results of the degradation studies suggest long term storage of the drug under photolytic conditions leads to degradation, with a fall in the content of ACTR and a corresponding rise in degradation products.

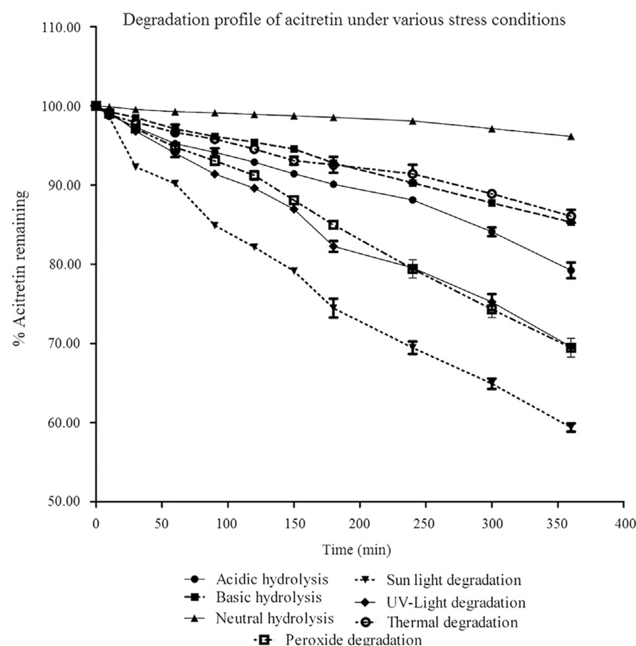

Figure 3 The degradation behavior of ACTR under different conditions.

3.4.5. *Thermal degradation*

Unalterd drug content was reduced to about 90% upon dry heat degradation (80 °C for 8 h) with an unknown degradation product

Table 3 Kinetics of ACTR degradation under different conditions.

	Acidic hydrolysis	Basic hydrolysis	Neutral hydrolysis	Sunlight degradation	UV-Light degradation	Thermal degradation	Peroxide degradation
One phase decay	Unclear	Unclear	Unclear	Exponential	Exponential	Exponential	Unclear
ACTR remaining (%)	41.1	72.8	95.7	66.7	78.9	91.5	79.0
Peak purity of ACTR peak	0.9997	0.9999	0.9999	0.9999	0.9998	0.9999	0.9996
Y	99.29	99.88	100.0	99.31	99.94	99.36	100.2
Plateau	−41,260	−44,000	−24,110	36.10	−14.43	46.08	−96,750
K (% min^{-1})	1.26×10^{-6}	9.055×10^{-7}	3.950×10^{-7}	0.002698	0.0008402	0.0007584	8.794×10^{-7}
Half-life (min)	~547,079	~765,481	1.755×10^{6}	256.9	825.0	913.9	788,212
R^2	0.9809	0.9916	0.9631	0.9934	0.9933	0.9676	0.9925
Absolute sum of squares	15.38	3.886	1.007	23.92	13.56	12.17	15.72
Sy.x	0.8997	0.4522	0.2302	1.122	0.8449	0.8004	0.9096

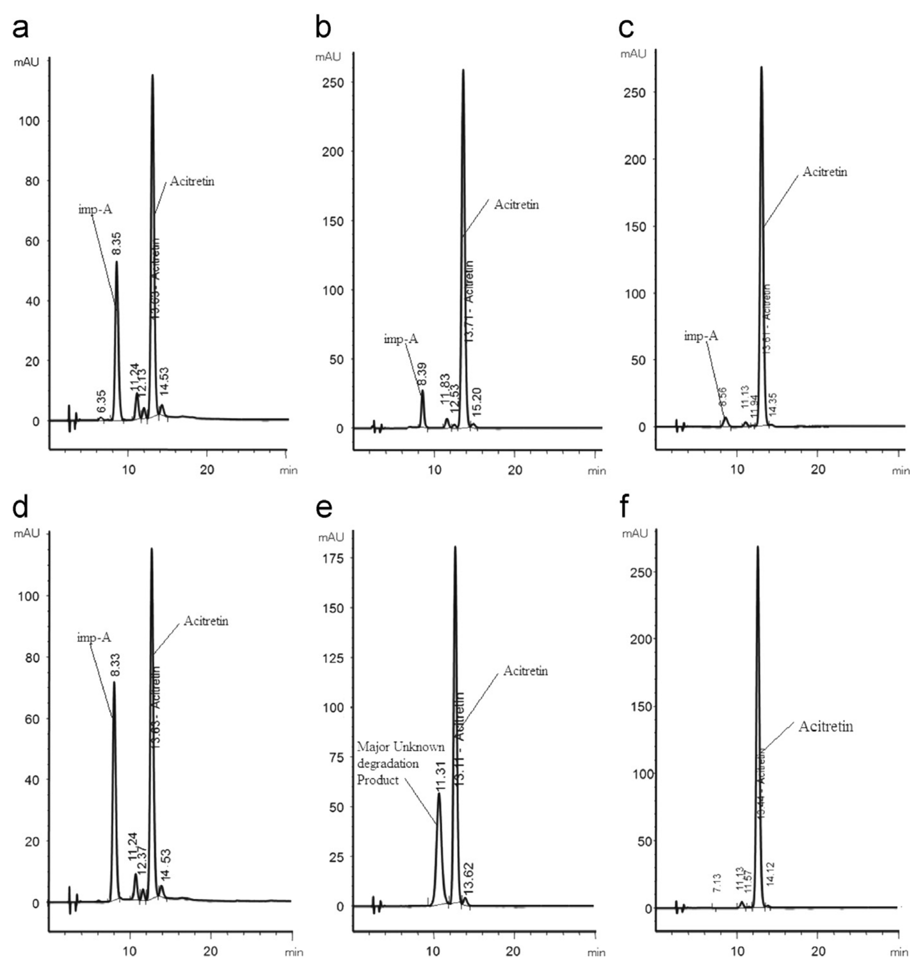

Figure 4 The chromatogram of the sample with acid-treated (a), alkali-treated (b), peroxide-treated (c), photolytically-treated (d), heat-treated (e), and neutral-treated (f).

eluting at 0.86 RRT. The chromatogram (Fig. 4e) displayed two degradation products.

3.4.6. *Degradation under neutral conditions*

ACTR was found to lose about 4.25% under neutral conditions (refluxed in water for 2 h at 80 °C) and two unknown degradation products (at RRT 0.82 and 0.86) were formed under these conditions; the chromatogram is shown in Fig. 4f.

3.5. *Linearity*

Calibration curves for ACTR and its RS, examined in pure solutions as well as in the Laboratory mixture solution, were found to be linear; correlation coefficients were ≥0.997 in all cases. Table 4 lists the linearity parameters of the calibration curves for ACTR and RS in Laboratory mixture. UV-relative

Table 4 Range of linearity ACTR and its RS.

Compound	Linearity range (μg/mL)	R^2	Slope	Intercept	Standard error	*t*-Stat	*P*	F_R
ACTR	0.35–1.50	0.999	34.62	−0.80	0.39	−2.05	0.06	
Tretintoin	0.20–1.50	0.998	32.76	−0.33	0.323	−1.01	0.33	0.95
imp-A	0.20–1.50	0.999	53.87	−0.67	0.49	−1.37	0.197	1.56
imp-B	0.27–1.50	0.998	33.44	−0.65	0.38	−1.74	0.10	0.97

Table 5 LOD and LOQ results for ACTR and its RS.

Compound	LOD		LOQ	
	Concentration (μg)	RSD (%) of injection ($n=6$)	Concentration (μg)	RSD (%) of injection ($n=6$)
ACTR	0.12	11.4	0.35	6.9
Tretintoin	0.07	9.8	0.2	4.3
imp-A	0.07	10.9	0.2	5.3
imp-B	0.1	16.8	0.27	7.7

Table 6 Intra-day and intermediate precision of ACTR and its RS (RSD (%) of $n=6$ injections).

Compound	Intraday precision		Intermediate precision
	System precision	Method precision	Different day
ACTR	0.3	1.0	0.9
Tretintoin	0.8	1.7	0.7
imp-A	0.9	1.2	1.0
imp-B	0.4	1.9	1.7
Total impurity	0.8	1.9	1.4

Table 7 Accuracy results of ACTR and its related substances in the term of RSD(%) of mean recovery.

Added (%)	ACTR		Tretintoin		imp-A		imp-B	
	MR (%)	RSD (%)	MR (%)	RSD (%)	MR (%)	RSD (%)	MR (%)	RSD (%)
50	99.8	1.9	98.2	1.5	99.6	2.2	97.5	3.1
100	101.2	2.0	99.5	2.1	102.8	2.4	100.2	1.9
150	100.9	1.4	100.5	2.4	98.4	1.5	100.9	2.1
300	101.7	1.9	98.9	1.6	99.1	2.1	99.0	1.3

MR; mean recovery, $n=3$.

response factors (F_R) were calculated for each impurity using the following equation:

$$F_R = S_{impurity}/S_{ACTR}$$

where $S_{impurity}$ is the slope of the regression line for a given impurity and S_{ACTR} is the slope of the regression line for ACTR. Concentrations of ACTR and impurity were corrected. Statistical treatment of the linearity data of ACTR shows a linear response from lowest to highest level. In addition, the analysis of residuals shows values randomly scattered around zero, which fits well within the linear model. The origin of the linearity curve was within the lower and the upper limit of 95% and gives a high degree of confidence to the value obtained for intercept.

3.6. *LOD and LOQ*

LOD and LOQ, as measures of method sensitivity, were determined for degradation products and impurity was calculated by means of signal-to-noise ratio. The LOD and LOQ for ACTR and its RS are shown in Table 5. From the results it can be concluded that the proposed method can quantify small amounts of impurities in ACTR samples.

3.7. *Precision and repeatability*

The results obtained for repeatability studies and for intermediate precision are presented in Table 6. Values of RSD (%) for system

Table 8 Stability of ACTR and its related substances in analytical solution (1-day study).

Compound	Initial area	12 h		18 h		24 h	
		Area	Difference (%)	Area	Difference (%)	Area	Difference (%)
ACTR	36.15969	37.01846	2.4	37.59764	4.0	36.86213	1.9
Tretintoin	13.71313	14.14156	3.1	13.67648	−0.3	13.71130	0.0
imp-A	89.97086	89.69975	−0.3	91.34973	1.5	92.72081	3.1
imp-B	9.40208	9.34010	−0.7	9.03424	−3.9	9.74495	3.6
Total impurity	172.59161	172.62739	0.0	172.37648	−0.1	176.39990	2.2

Table 9 Effect of various specific changes on the system suitability parameters.

System suitability conditions		Ratio between the duplicate injection	RSD (%) for ACTR standard replicate injections
Change in flow rate (mL)	0.8	1.01	1.06
	1.0	0.97	1.06
	1.2	0.99	1.54
Change in column temperature (°C)	25	1.02	1.78
	30	0.97	1.06
	35	1.00	0.62
Change in IPA (%)	27.7	0.99	1.63
	29.7	0.97	1.06
	31.7	1.04	1.61

precision of ACTR and total impurities were 0.3 and 0.8, respectively. Method precision has a RSD (%) below 1.9 for repeatability and 1.4 for intermediate precision, which comply with the acceptance criteria.

3.8. Accuracy

The results are expressed as percent recoveries of the particular components in the samples. Table 7 shows the overall percent recoveries of ACTR and its six RS at 50%, 100%, 200% and 300% of the test concentration. The method shows consistent recoveries for ACTR (99.8%–101.2%). The related compounds showed overall percent recoveries ranging from 97.9% to 102.8% with RSD (%) ranging from 1.2 to 3.1.

3.9. Stability in analytical solution

The area change (%) in peaks of ACTR and all impurities was less than 2.0% and 5.0%, respectively. From the data shown in Table 8, it was concluded that standard and sample solutions may be used up to 24 h after preparation.

3.10. Robustness

Method robustness was checked after deliberate alterations of mobile phase composition, flow, pH and temperature which shows that the changes of the operational parameters do not lead to significant changes in the performance of the chromatographic system; results are displayed in Table 9. The tailing factor for ACTR and the RS always ranged from 1 to 1.5 and the components were well separated. The recoveries (%) of ACTR and RS were good and did not show a significant change when the critical parameters were modified. Considering the results of modifications in the system suitability parameters and the specificity of the method, we conclude that the method conditions are robust.

4. Conclusions

We developed an HPLC method for estimation of related substances for acitretin, analyzed in bulk drug and ACTR capsule as per ICH guidelines. The method was found to be specific for the estimation of known and unknown impurities and degradation products. The method can also be used to determine acitretin stability. The assay utilized a previously unreported set of conditions, including simple mobile phases, to effect separation without using an ion-pair reagent. LOD and LOQ, established by this method, are less than the previously reported methods. The method is found to be linear in the specified range, precise and robust. Accuracy of the method is also established for the formulation. Hence, the proposed method may be used for routine stability sample analysis.

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