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Qualitative and Quantitative Estimation by HPLC Method in Transdermal Formulations: A Technical Note

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ABSTRACT

A simple, selective, precise and stability-indicating Isocratic high-performance chromatographic method of analysis for adapalene as a model lipophylic drug in Transdermal (gel) formulations was developed. Mobile phase consisted of acetonitrile (ACN), tetrahydrofuran (THF) and phosphate buffer (PB) (pH-2.5; 0.01 M) in the ratio of 30:40:30 respectively. This mobile phase was found to give adequate results in terms of peak shape, symmetry, tangent and tailing. Retention time (RT) for adapalene was found to be 2.4 with run time of 5 minutes. Samples were subjected to acid, alkali hydrolysis, oxidation, thermal, humidity and photodegradation. The whole analysis was carried out at the wavelength of 272 nm. The linear regression analysis data for the calibration plots showed good linear relationship with r^2 = 0.9995 with respect to peak area in the concentration range of 14-26μg/ml.The mean value of correlation coefficient; slope and intercept were 0.9995, 9060.51 and 1282. The method was validated for precision, specificity, recovery and robustness, in accordance with ICH guidelines. The drug undergoes degradation under basic (alkaline) conditions. This indicates that the drug is susceptible to base hydrolysis. Statistical analysis proves that the method is reproducible, selective and accurate for the estimation of said drug. As the method could effectively separate the drug from its degradation product, it can be employed as a stability-indicating one.

Keywords*:* Adapalene analysis; HPLC method; validation, Stability indicating

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INTRODUCTION

Adapalene 6-(3-(1-adamantyl)-4-methoxy phenyl)-2-napthoic acid [1] is off white crystalline powder. It has been used to treat acne lesions. Extensive researches have revealed potent anti-inflammatory effects of adapalene. It is a modulator of cellular differentiation, keratinization and inflammatory processes all of which represent important features in the pathology of acne- vulgaris [2]. Mechanistically, adapalene binds to specific retinoic acid nuclear receptors but does not bind to the cytosolic receptor protein. Although the exact mode of action of adapalene is unknown, it is suggested that topical adapalene may normalize the differentiation of follicular epithelial cells resulting in decreased micro-comedone formation [3]. Adapalene is unstable at basic pH and undergoes alkaline hydrolysis in alkali / higher pH solution. Since no method is available for the analysis of adapalene in formulations in the literature [4], we have developed a very sensitive and accurate HPLC method which is stability indicating also. The International Conference on Harmonization (ICH) guideline entitled 'stability testing of new drug substances and products' requires the stress testing to be carried out to elucidate the inherent stability characteristics of the active substance [5]. Susceptibility to oxidation is one of the required tests. Also, the hydrolytic and the photolytic stability are required. An ideal stability indicating method is one that quantifies the drug *per se* and also resolves its degradation products. Nowadays, HPLC is becoming a routine analytical technique due to its advantages [6].The major advantage of HPLC is that several samples can be run quickly. It ensures accurate determination of minute quantities of sample. The aim of this work is to develop an accurate, specific, repeatable and stability-indicating method for the determination of adapalene as per ICH guidelines.

EXPERIMENTAL

Materials

Adapalene was purchased from Ranbaxy Research labs Gurgaon, India. All chemicals and reagents used were of analytical grade and were purchased from Ranbaxy Chemicals, India.

HPLC method development

The proposed study was an attempt to develop and validate an HPLC method for determination of adapalene in Adapalene gel.

During the development of the HPLC method, mobile phases investigated were phosphate buffer, acetonitrile, tetrahydrofuran in different ratios and with different pHs. Mobile phase selection was based on peak parameters i.e. height, asymmetry, tailing, baseline drift, run time, ease of preparation of the mobile phase, need for pH adjustment and cost (in that order). Keeping all these requirements in consideration, proposed chromatographic condition was found appropriate for quantitative determination.

Samples were prepared by dissolving 2 gm of gel in the diluent {mixture of acetonitrile and tetrahydrfuran (60:40)} which on further dilution contained 20 µg/ml of Adapalene.

Table 1: Final Chromatographic Conditions

S.No.	Chromatographic Parameter	Condition
1	Column	Kromasil C-18(100*4.6)5µ
2	Column Oven Temperature	40°C
3	Detection wavelength	272nm
4	Run Time	5 minutes
5	Elution Mode	Isocratic
6	Composition of Mobile Phase	a) Buffer: b) Organic(30:40) a) Phosphate buffer(10mM),pH 2.5 b) ACN:THF
7	Injection Volume	20 µl
8	Flow Rate	1.5ml/min
9	Diluent	ACN: THF:60:40

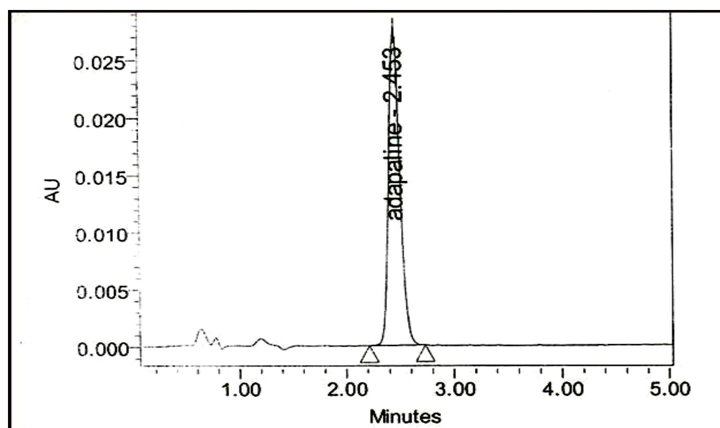

FIGURE: CHROMATOGRAM OF ADAPALENE

The chromatographic column used was Kromasil C-18(100*4.6)5µ. The number of theoretical plates was more than 30,000 for Adapalene and RSD (relative standard deviation) was less than 2% for the entire study. Column oven Temperature was 40°C. Mobile phase selected was phosphate buffer and organic (ACN: THF, 30:40) in the ratio of 30:70 (pH-2.5). Isocratic method for adapalene in Adapalene gel was used. 10 µl samples (20 µg/ml of Adapalene) were injected. Flow rate was 1.5 ml/min. Samples were run for 5min at a wavelength at 272nm (Table -1 and Figure). The HPLC method was further validated as per ICH guidelines 1996.

Method validation

Linearity

The linearity for adapalene was determined over the range of 14-26 µg/ml which was 70-130% of standard concentration (20 µg/ml).

Precision

System Precision

Six replicate injection of standard solution containing 20 µg of adapalene were injected into the HPLC system.

Method precision

Six sample of a single batch of adapalene were prepared as described in section 2.2 and analyzed by proposed method.

Recovery studies

Known amount of placebo for adapalene was taken and spiked with adapalene standard at three levels (80%, 100% and 120%) with respect to adapalene in triplicate. The samples were prepared and analyzed as per proposed method.

Robustness

The robustness was determined by injecting three-sample solution at each different condition with respect to control condition. Robustness of the method was checked by varying the instrumental conditions such as flow rate (± 10%), organic content in mobile phase ratio (± 2%), wavelength of detection (± 5 nm), column oven temperature (± 5°C) and change in pH of buffer (± 0.2%). Sample solution was injected in each condition and assay % of adapalene was calculated.

Specificity

Placebo (gel base) of adapalene was taken and solution prepared similar to sample solution. The solution was analyzed as per proposed method. Sample solution was also analyzed as per proposed method.

Ruggedness

Ruggedness of the method was verified by analyzing samples (prepared and analysed as described in sec 2.2) of the single batch of adapalene by two different analysts using different instruments on different days.

Stress degradation study

A stress degradation study was carried out in adapalene gel according to the ICH guidelines.

Hydrolytic and Oxidative degradation

Sample (gel) and Placebo (gel base) were separately treated with 1N hydrochloric acid, 0.2 N sodium hydroxide and 30% hydrogen peroxide solutions. Solutions of these samples were prepared as per the conditions given in Table 8, followed by analysis as per the proposed method.

Thermal degradation

Samples and placebo (gel base) were subjected to thermal degradation by keeping at 105°C for 24 hr, followed by analysis as per proposed method.

Photolytic degradation

Photolytic degradation study was carried out by exposing the sample and placebo (gel base) to light in photolytic chamber at 500W/m^2 for 24 hr, followed by analysis as per proposed method.

Humidity degradation

Humidity degradation study was carried out by exposing the sample and placebo (gel base) to 92% relative humidity at 25°C for 24 hr, followed by analysis as per proposed method. Using the peak purity test, the purity of adapalene peak was checked at every stage of above-mentioned studies. Table 8 shows the final degradation of adapalene achieved.

RESULTS AND DISCUSSION

Development of the optimum mobile phase

During the development of the HPLC method, mobile phases investigated were Phosphate buffer, acetonitrile, tetrahydrofuran in different ratios and with different pHs. Mobile phase selection was based on peak parameters i.e. height, asymmetry, tailing, baseline

drift, run time, ease of preparation of the mobile phase, need for pH adjustment and cost (in that order). Mobile phase selected was buffer and organic (ACN: THF, 30:40) in the ratio of 30:70 (pH-2.5).

Validation of the method

Linearity

Table 2: Linearity of Adapalene

Sample id	Conc. (µg/ml)	area counts (µv*sec)		
		inj # 1	inj # 2	mean
L-1	14.08	129544	132206	130875
L-2	16.09	148620	149797	149209
L-3	18.10	164079	164498	164289
L-4	20.12	183985	183741	183863
L-5	22.13	203347	201844	202596
L-6	24.14	21436	215784	215310
L-7	26.15	239400	240445	239923
			Slope	9060.51
			Intercept	1282
			CC	0.9995

A good linear regression, with R^2 value of 0.9995 for Adapalene was obtained [Table-2].

Precision

Table 3: System Precision for Adapalene

Inj #	Area Counts (µv*Sec)
	Adapalene
1	193924
2	196253
3	195803
4	194798
5	194579
6	195157
Mean	195086
SD	845
RSD (%)	0.43

Table 4: Method Precision for Adapalene

Sample	Mean Area Counts (µv*Sec)		
	Adapalene	Assay (%W/W)	% Assay
MP-1	183530	0.100	100.00
MP-2	176183	0.097	97.00
MP-3	178430	0.098	98.00
MP-4	177599	0.098	98.00
MP-5	181168	0.100	100.00
MP-6	178774	0.099	99.00
		Mean	**0.099**
		SD	**0.001**
		%RSD	**1.22**

Inj= Injection

Precision for six duplicate samples of adapalene showed % RSD of 0.43 & 1.22 for system & method precision respectively. The % RSD value indicated that the method has an acceptable level of precision. (Acceptance criteria: RSD should not be more than 2%) [Table 3 &4].

Robustness of the method

Robustness of the method is indicated by the overall RSD value between the data of set-1 and data at each variable condition. (Acceptance criteria: Over all RSD should not be more than 5%).

Table 5: Robustness of Adapalene

S. No.	assay of adapalene (%w/w)										
	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6	Set 7	Set 8	Set 9	Set 10	Set 11
1	0.099	0.100	0.101	0.095	0.094	0.097	0.097	0.097	0.097	0.098	0.100
2	0.097	0.093	0.098	0.095	0.097	0.096	0.095	0.096	0.096	0.097	0.097
3	0.097	0.097	0.099	0.092	0.092	0.097	0.097	0.099	0.095	0.096	0.098
Mean	**0.100**	0.100	0.100	0.090	0.090	0.100	0.100	0.010	0.100	0.100	0.100
SD	**0.001**	0.003	0.002	0.002	0.003	0.000	0.001	0.002	0.001	0.001	0.002
%RSD	**1.180**	3.510	2.000	2.220	3.330	0.000	1.000	2.000	1.000	1.000	2.000
OVERALL MEAN		**0.100**	**0.100**	**0.100**	**0.100**	**0.100**	**0.100**	**0.100**	**0.1000**	**0.100**	**0.100**
OVERALL SD		**0.002**	**0.002**	**0.002**	**0.003**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**
OVERALL RSD		**2.000**	**2.000**	**2.000**	**3.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.000**	**1.00**

set 1 : control sample; set 2 : sample wavelength 267 nm; set 3 : sample wavelength 277 nm; set 4 : sample flow rate 1.8 ml/min.; set 5 : sample flow rate 2.2 ml/min. ; set 6 : sample organic minus 2%.; set 7 : sample organic plus 2%.; set 8 : sample temp. 25ºc.; set 9 : sample temp. 35ºc.; set 10 : sample buffer ph 2.8.; set 11 : sample buffer ph 3.2

The method was found to be robust and no significant changes were observed. The average % RSD was found to be within the acceptable limits [Table-5].

Specificity

Both Sample as well as placebo (gel base) was analyzed as per the proposed method. No interference from placebo was observed at the retention time of adapalene peaks. Peak purity plots also indicates that peaks of adapalene is pure and don't have any co-eluting peaks. Therefore, it is concluded that the method is specific.

Recovery/Accuracy studies

Table 6: Accuracy for Adapalene

sample	mean area counts (µv*sec)	amt. recovered (mg)	amt. added (mg)	actual amt. added (mg)	% recovery
80%-Rec-1	329420	1.621	1.632	1.619	100.14
80%-Rec-2	331888	1.633	1.632	1.619	100.08
80%-Rec-3	326672	1.608	1.632	1.619	99.31
100%-Rec-1	410877	2.022	2.046	2.030	99.62
100%-Rec-2	405767	1.997	2.046	2.030	98.39
100%-Rec-3	409773	2.017	2.046	2.030	99.38
120%-Rec-1	486186	2.393	2.435	2.416	99.07
120%-Rec-2	478936	2.357	2.435	2.416	97.58
120%-Rec-3	486537	2.394	2.435	2.416	99.11
				Mean	**99.19**
				SD	**0.806**
				%RSD	**0.81**

amt = amount

The percent recoveries were in the range of 98.39 to 100.14% and 98.38 to 100.91% for the two components studied. Results indicated that the method has an acceptable level of accuracy. (Acceptance criteria: recovery should be in the range of 98-102%) [Table-6].

Ruggedness

Table 7: Ruggedness for Adapalene

Sample No.	Mean Area Counts (µv*Sec))		Assay (%W/W)	
	Adapalene		Adapalene	
	Set 1	Set 2	Set 1	Set 2
1	183530	431243	0.100	0.106
2	176183	412855	0.097	0.101
3	178430	402026	0.098	0.098
4	177599	402167	0.098	0.098
5	181168	416401	0.100	0.102
6	178774	411126	0.099	0.101
Mean	**174175**	**399328**	**0.098**	**0.101**
SD	**696**	**2667**	**0.001**	**0.003**
%RSD	**0.40**	**0.67**	**1.23**	**2.94**
Overall Mean			**0.15**	
Overall SD			**0.002**	
Overall %RSD			**1.11**	

The mean, SD, RSD for two set of data were calculated. (Acceptance criteria: Overall RSD should not be more than 2%).The results were found to be within the limits thus confirming that the method is rugged [Table-7].

Stress studies

Table 8: Final Degradation Study for Adapalene

Sample	Adapalene Area Counts (µv*Sec)	Adapalene	
		Assay (%W/W)	Percent degrada Tion
Sample (1N Hcl 5 ml, Heat 120 min.80°C)	211836	0.103	-3.00
Sample (0.2N NaOH 5 ml)	174198	0.09	13.00
Sample (H_2O_2 30% 5 ml heat 120 min. 80°C)	183691	0.10	3.00
Sample Thermal Deg. (105°C, 2.5 hrs)	178551	0.10	4.00

Sample Photolytic Deg.(2700 LUX, 24 hrs)	191829	0.10	4.00
Sample Humidity Deg.(92% RH 25°C, 24 hrs)	195461	0.10	1.00
		Mean	**0.099**
		SD	**0.004**
		%RSD	**4.544**

Adapalene degraded by alkaline hydrolysis with 0.2 N NaOH (5 ml) at room temperature. The percentage degradation was 13% i.e. within the limits and was stable for rest of the stress conditions. No degradation was achieved for the rest of the conditions employed [Table-8].

CONCLUSION

A simple, easy and cost-effective stability indicating HPLC method for determination of adapalene in gel formulation was developed using phosphate buffer: acetonitrile: tetrahydrofuran as the mobile phase. The method was thoroughly validated and was found to be accurate, precise, linear and robust. Adapalene showed a retention time of 2.45 in Adapalene gel. The number of theoretical plates was more than 30,000 and RSD was less than 2% for the entire study. To conclude, the present developed and validated HPLC method appears to be very sensitive, selective, precise, accurate, less time consuming, reproducible and thus suitable for routine analysis for the estimation of Adapalene in pharmaceutical formulations.

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