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Development and validation of stability indicating HPLC method for adapalene and benzoic acid in pharmaceutical Gel formulations

M.Mudasir[1]*, N.Tabassum[1], J.Ali[2], R.Jan[3]

[1]Department of Pharmaceutical Sciences, University of Kashmir, Srinagar-190006, (INDIA)

[2]Department of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard University, New Delhi-110062, (INDIA)

[3]Department of Clinical Biochemistry, Sher-i-Kashmir Institute of Medical Sciences, Soura, Srinagar-190009 (INDIA)

Tel : 09469402091

E-mail : mudasirmohamad@gmail.com

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ABSTRACT

A simple, selective, precise and stability-indicating high-performance chromatographic method of analysis for adapalene and benzoic acid in pharmaceutical (gel) formulations was developed. Mobile phase consisted of acetonitrile (ACN), tetrahydrofuran (THF) and phosphate buffer (PB) (pH-3.0; 0.01 M). This mobile phase was found to give adequate results in terms of peak shape, symmetry, tangent and tailing. Retention time (RT) for adapalene and benzoic acid was found to be 8.8 (±1) and 2.2 (±1) respectively. Samples were subjected to acid, alkali hydrolysis, oxidation, thermal, humidity and photodegradation. The whole analysis was carried out at timed wavelength of 230 nm and 272 nm for benzoic acid and adapalene respectively. The linear regression analysis data for the calibration plots showed good linear relationship with r^2 = 0.9995 and 0.9998 for adapalene and benzoic acid respectively with respect to peak area respectively in the concentration range of 14-26µg/ml for adapalene and 28-52µg/ml for benzoic acid. The mean value of correlation coefficient; slope and intercept were 0.9995, 9060.51 and 1282, for adapalene and 0.9998, 10185.77 and 1302 for benzoic acid respectively. The method was validated for precision, specificity, recovery and robustness, in accordance with ICH guidelines. The drug undergoes degradation under basic and thermal conditions. This indicates that the drug is susceptible to base hydrolysis, and thermal degradation. 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.

1. 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 microcomedone formation[3,4]. Adapalene is unstable at basic pH and undergoes alkaline hydrolysis in alkali / higher pH solution. Benzoic acid undergoes thermal degrada-

tion when exposed to heat in solution as well as in solid form. Since no method is available for the analysis of adapalene in formulations in the literature[5], 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[6-7]. 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[8]. 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.

2. EXPERIMENTAL

2.1. 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.

2.2. HPLC method development

The proposed study was an attempt to develop and validate an HPLC method for determination of adapa lene with benzoic acid in Adapalene-Azelaic acid 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 20µg of adapalene and 40µg of benzoic acid.

TABLE 1: Composition of mobile phase in gradient method

S.no.	Time (min)	Buffer	Organic phase
1	0	60	40
2	3	60	40
3	10	20	80
4	11	60	40
5	15	60	40

The chromatographic column used was Inertsil ODS-3V (150 × 4.6) 5µ. The number of theoretical plates was more than 30,000 (for adapalene) and 4000 (for benzoic acid) and RSD was less than 2% for the entire study. Column oven Temperature was 30^0C. Mobile phase selected was phosphate buffer and organic (ACN: THF, 60:40) in the ratio of 60:40 (pH-3.0). Gradient methods for adapalene in Adapalene-Azelaic acid gel was used, the ratio are given in the TABLE 1. 10µl samples (20µg/ml of adapaline and 40µg/ml of benzoic acid) were injected. Flow rate was 2.0 ml/min. Samples were run for 15 min at a wavelength at 230 nm and 272 nm for benzoic acid and adapalene respectively. The HPLC method was further validated as per ICH guidelines 1996[9,10].

2.3. Method validation:

2.3.1. Linearity

The linearity for adapalene and benzoic acid were determined over the range of 14-26µg/ml and 28-52 µg/ml which was 70-130% of standard conc.(20µg/ml and 40µg/ml) respectively.

2.3.2.Precision

2.3.2.1. System precision

Six replicate injection of standard solution containing 20µg and 40µg/ml of adapalene and benzoic acid were injected into the HPLC system.

2.3.2.2 Method precision

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

2.3.3. Recovery studies

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

2.3.4. 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 and benzoic acid was calculated.

2.3.5. Specificity

Common placebo (gel base) of adapalene and benzoic acid were 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.

2.3.6. Ruggedness

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

2.4. Stress degradation study

A stress degradation study was carried out in adapa lene and benzoic acid gel according to the ICH guidelines[11].

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 11, 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.

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

INJ= Injection, CC = Correlation Coefficient

TABLE 3: Linearity of benzoic acid

Sample id	Conc. (µg/ml)	Area counts (µv*sec)		
		inj # 1	inj # 2	mean
L-1	28.12	288863	292881	290872
L-2	32.14	331958	330048	331003
L-3	36.16	366664	366084	366374
L-4	40.18	412033	412496	412265
L-5	44.20	451006	450579	450793
L-6	48.22	488055	488275	488165
L-7	52.22	533526	537652	535589
			Slope	10185.77
			Intercept	1302
			CC	0.99986

INJ= Injection, CC = Correlation Coefficient

Humidity degradation

Humidity degradation study was carried out by exposing the sample and placebo (gel base) to 92% relative humidity at 25^{0}C for 24 hr, followed by analysis as per proposed method. Using the peak purity test, the purity of adapalene and benzoic acid peak was checked at every stage of above-mentioned studies. TABLE 11 shows the final degradation of adapalene and benzoic acid achieved.

3. RESULTS AND DISCUSSION

3.1. 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, 60:40) in the ratio of 60:40 (pH-3.0).

3.2. Validation of the method

3.2.1. Linearity

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A good linear regression, with R^2 value of 0.9995 and 0.9998 for adapalene and benzoic acid was obtained respectively.

3.2.2. Precision

Method Precision for six duplicate samples of adapalene and benzoic acid showed % RSD of 1.22 and 2.01 respectively. Results are shown in TABLES 4 and 5. The % RSD value indicates that the method has an acceptable level of precision. (Acceptance criteria: RSD should not be more than 2%).This indicates that the proposed method had good system and method precision.

TABLE 4: System precision for adapalene and benzoic acid

inj #	Area counts (µv*sec)	
	Adapalene	Benzoic acid
1	193924	439839
2	196253	436354
3	195803	435745
4	194798	434909
5	194579	433214
6	195157	434438
Mean	195086	435750
SD	845	2278
RSD (%)	0.43	0.52

TABLE 5: Method precision for adapalene and benzoic acid

Sample	Mean area counts (µv*sec)		Adapalene		Benzoic acid	
	Adapalene	Benzoic acid	Assay (%w/w)	% Assay	Assay (%w/w)	% Assay
MP-1	183530	398827	0.100	100.00	0.162	81.00
MP-2	176183	379683	0.097	97.00	0.152	76.00
MP-3	178430	385598	0.098	98.00	0.155	77.50
MP-4	177599	382277	0.098	98.00	0.154	77.00
MP-5	181168	391100	0.100	100.00	0.157	78.50
MP-6	178774	385701	0.099	99.00	0.155	77.50
		Mean	0.099		0.156	
		SD	0.001		0.003	
		%RSD	1.22		2.01	

3.2.3. Robustness of the method

The mean, standard deviation, and RSD are shown in TABLES 6 and 7. 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%).

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

3.2.4. Specificity

Both Sample as well as placebo (gel base) was analyzed as per the proposed method. Since No interference from placebo, (Figures 1 and 2) was observed at the retention time of adapalene and benzoic acid peaks. Peak purity plots (Figure 3) also indicates that peaks of adapalene and benzoic acid are pure and don't have any co-eluting peaks. Therefore, it is concluded that the method is specific.

3.2.5. Recovery studies

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 shown in TABLES 8 and 9 indicate that the method has an acceptable level of accuracy. (Acceptance criteria: recovery should be in the range of 98-102%)

3.2.6. Ruggedness

The mean, SD, RSD for two set of data are shown in TABLE 10. (Acceptance criteria: Overall RSD should not be more than 2%).The results were found to be

TABLE 6: 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
1	0.099	0.100	0.101	0.095	0.094	0.097	0.097	0.097	0.097	0.098
2	0.097	0.093	0.098	0.095	0.097	0.096	0.095	0.096	0.096	0.097
3	0.097	0.097	0.099	0.092	0.092	0.097	0.097	0.099	0.095	0.096
Mean	0.100	0.100	0.100	0.090	0.090	0.100	0.100	0.010	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
%RSD	1.180	3.510	2.000	2.220	3.330	0.000	1.000	2.000	1.000	1.000
Overall mean	-	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.1000	0.100
Overall SD	-	0.002	0.002	0.002	0.003	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
-	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6	Set 7	Set 8	Set 9	Set 10

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

TABLE 7: Robustness of Benzoic acid

S. no.	Assay of benzoic acid (%w/w)									
	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6	Set 7	Set 8	Set 9	Set 10
1	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.180
2	0.190	0.190	0.190	0.190	0.190	0.190	0.200	0.190	0.190	0.180
3	0.180	0.190	0.190	0.180	0.180	0.180	0.190	0.180	0.180	0.170
Mean	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.180
SD	0.006	0.000	0.000	0.006	0.006	0.006	0.006	0.006	0.006	0.006
%RSD	3.160	0.000	0.000	3.160	3.160	3.160	3.160	3.160	3.160	3.330
Overall mean		0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.190	0.180
Overall SD		0.004	0.004	0.005	0.005	0.005	0.005	0.005	0.005	0.008
Overall RSD		2.00	2.00	2.63	2.63	2.63	3.16	3.00	2.63	4.00

Set 1 : Control sample; Set 2 : Sample wavelength 225 nm; Set 3 : Sample wavelength 235 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. 25°C ; Set 9 : Sample temp. 35°C ; Set 10 : Sample buffer pH 2.8.; Set 11 : Sample buffer pH 3.2

TABLE 8: 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

TABLE 9: Accuracy for benzoic acid

Sample	Mean area counts (µv*sec)	Amt. recovered (mg)	Amt. added (mg)	Actual amt. added (mg)	% Recovery
80%-Rec-1	731848	2.721	3.230	2.726	99.81
80%-Rec-2	739945	2.751	3.230	2.726	100.91
80%-Rec-3	728962	2.710	3.230	2.726	99.40
100%-Rec-1	909573	3.381	4.011	3.385	99.87
100%-Rec-2	899283	3.343	4.011	3.385	98.75
100%-Rec-3	906354	3.369	4.011	3.385	99.51
120%-Rec-1	1091869	4.059	4.847	4.091	99.22
120%-Rec-2	1082737	4.025	4.847	4.091	98.38
120%-Rec-3	1094229	4.068	4.847	4.091	99.43
				Mean	99.48
				SD	0.718
				%RSD	0.72

within the limits thus confirming that the method is rugged.

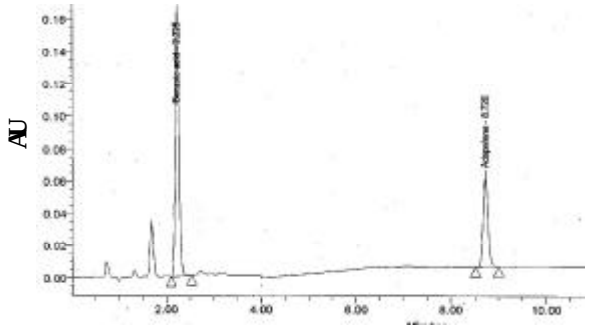

Figure 1: Chromatogram of adapalene and benzoic acid

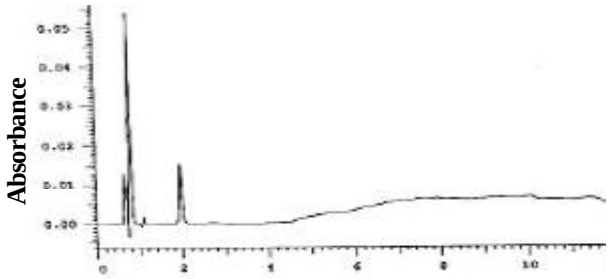

Figure 2: Chromatogram of placebo of adapalene and benzoic acid

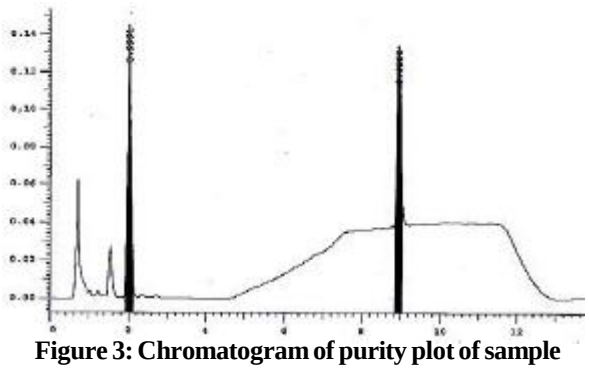

Figure 3: Chromatogram of purity plot of sample

3.3. Stress studies

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. Benzoic acid showed degradation by alkaline hydrolysis as well as thermal stress, the percentage degradation was 15.48 and 29.03 respectively which is again within limits. No degradation

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TABLE 10: Ruggedness for adapalene and benzoic acid

Sample no.	Mean area counts (µv*sec)				Assay (%w/w)			
	Adapalene		Benzoic acid		Adapalene		Benzoic acid	
	Set 1	Set 2	Set 1	Set 2	Set 1	Set 2	Set 1	Set 2
1	183530	431243	398827	916449	0.100	0.106	0.162	0.173
2	176183	412855	379683	875012	0.097	0.101	0.152	0.165
3	178430	402026	385598	852134	0.098	0.098	0.155	0.161
4	177599	402167	382277	848018	0.098	0.098	0.154	0.160
5	181168	416401	391100	869399	0.100	0.102	0.157	0.164
6	178774	411126	385701	859253	0.099	0.101	0.155	0.162
Mean	174175	399328	389237.6	878595.2	0.098	0.101	0.156	0.164
SD	696	2667	768	6219	0.001	0.003	0.003	0.005
%RSD	0.40	0.67	0.2	0.7	1.23	2.94	2.20	2.87
Overall mean					0.15		2.53	
Overall SD					0.002		0.001	
Overall %RSD					1.11		0.04	

TABLE 11: Final degradation study for adapalene and benzoic acid

Sample	adapalene area counts (µv*sec)	benzoic acid area counts (µv*sec)	Adapalene		Benzoic acid	
			Assay (%W/W)	Percent degradation	Assay (%W/W)	Percent degradation
Sample (1N Hcl 5 ml, Heat 120 min.80^{0}C)	211836	424162	0.103	-3.00	0.16	-5.16
Sample (0.2N NaOH 5 ml)	174198	348548	0.09	13.00	0.13	15.48
Sample (H_2O_2 30% 5 ml heat 120 min. 80^{0}C)	183691	397562	0.10	3.00	0.17	-6.45
Sample Thermal Deg. (105^{0}C, 2.5 hrs)	178551	280957	0.10	4.00	0.11	29.03
Sample Photolytic Deg.(2700 LUX, 24 hrs)	191829	399161	0.10	4.00	0.15	1.29
Sample Humidity Deg.(92% RH 25^{0}C, 24 hrs)	195461	396169	0.10	1.00	0.15	0.65
		Mean	0.099		0.145	
		SD	0.004		0.022	
		%RSD	4.544		14.951	

was achieved for the rest of the conditions employed.

4. CONCLUSION

A simple, easy and cost-effective stability indicating HPLC method for determination of adapalene and benzoic acid 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 and benzoic acid showed a retention time of 8.5-9.2 in Adapalene-Azelaic acid gel for adapalene and 1.8-2.2 for Benzoic acid. The number of theoretical plates was more than 30,000 and 4000 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 simultaneous estimation of adapalene and benzoic acid in pharmaceutical formulations.

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