Development of Analytical Method for Simultaneous Estimation of Adapalene and Benzoyl Peroxide in Gel Formulation by RP-HPLC

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Abstract

A simple, accurate, rapid and precise isocratic high-performance liquid chromatographic method has been developed and validated for simultaneous estimation of Adapalene and Benzoyl Peroxide in pharmaceutical gel formulation. The chromatographic separation was carried out at Analytical Technology HPLC instrument (Software: HPLC Work Station) equipped with Deuterium lamp as detector, HPLC pump and manual injecting facility programmed at 20μL capacity per injection was used. The stationary phase was Zodiac Column C8 (250mmx4.6mm, 5μm) at ambient temperature. Mobile phase containing Acetonitrile: Methanol (90:10). The mobile phase flow rate 1.0mL/min, Injection volume 20μL and detection was carried out at 245nm using UV-Detector. The retention time was found to be 3.7±0.1mins and 5.8±0.1mins Benzoyl Peroxide and Adapalene respectively.

The assay values of both were found to be well within the limits that is 98.42% and 99.19% for Adapalene and Benzoyl Peroxide respectively. The linearity of the proposed method was investigated in the range of 1.9-4.4μg/ml and 48-112μg/ml for Adapalene and Benzoyl Peroxide, respectively. Mean percentage recoveries were 98.94% for Adapalene and 99.28% for Benzoyl Peroxide. The LOD of Adapalene and Benzoyl Peroxide was found to be 0.3μg/mL and 4μg/mL whereas the LOQ was 1.2μg/mL and 20μg/mL respectively. Percentage relative standard deviation of percent assay values for replicate sample preparation was 0.25% for Adapalene e and 0.40% for Benzoyl Peroxide. The method was robust with respect to change in flow rate, temperature and composition of mobile phase. These analytical methods are also applicable in ordinary laboratories. It can also be adopted for quality control tests for these drugs in gel formulation.

Keywords : Adapalene; Benzoyl peroxide; Simultaneous Estimation; RP-HPLC

Introduction
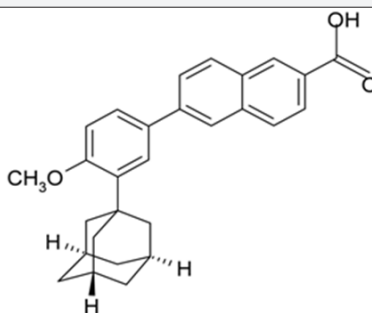
Chemical Name: Adapalene is chemically designated as 6-[3-(1-adamantyl)-4-methoxyphenyl]-2-napthoic acid. And its molecular formula is $C_{28}H_{28}O_3$ and molecular weight is 412.529g/mol.

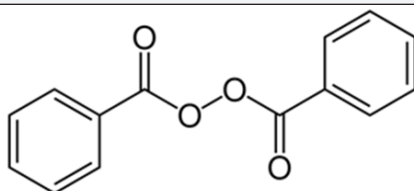**Figure 1:** Molecular structure of Adapalene.

Adapalene is a third-generation synthetic topical retinoid used in the treatment of mild-moderate acne and is also used to treat keratosis pilaris as well as other skin conditions. It is effective against acne conditions where comedones are predominant. It is a highly lipophilic compound, derived from napthoic acid, has both exfoliating and anti-inflammatory effects. Topical retinoids are a group of medicines derived from vitamin A. These compounds result in the proliferation and reduced

keratinisation of skin cells independent of their functions as a vitamin [1-4]. Adapalene in small concentrations is a moderator of cellular differentiation, keratinization, and inflammatory processes. It has both exfoliating and anti- inflammatory effects. The exact mode of action of Adapalene is unknown [5]. The molecular structure of the drug is given in Figure 1.

Benzoyl Peroxide is organic peroxide consist of two Benzoyl groups bridged by a peroxide link. Benzoyl Peroxide is an antibacterial agent with demonstrated activity against propionibacterium acnes. This action combined with the mild keratolytic effect of Benzoyl Peroxide is believed to be responsible for its usefulness in acne. It is available in concentration from 2.5-10% [6-8]. Benzoyl Peroxide works as a peeling agent. It increases skin turnover, clearing pores, and reducing the bacterial count as well as acting directly as an antimicrobial [9,10]. The molecular structure of the drug is given in Figure 2.

Chemical Name: Benzoyl Peroxide is chemically designated as dibenzoyl peroxide. And Its molecular formula is $[C_6H_5C(O)]_2O_2$ and molecular weight is 242.23g/mol.

Figure 2: Molecular structure of Benzoyl Peroxide.

Combination therapy with a topical retinoid and an antimicrobial agent, which addresses the majority of the causative factors of acne, is considered a first-line treatment option for almost all patients. Adapalene has also been shown to retain its efficacy when applied at the same time as Benzoyl Peroxide due to its more stable chemical structure [11-15]. A detailed literature survey for Benzoyl Peroxide and Adapalene revealed that the determination of an individual compound or in combination with other drugs has been reported using HPLC [16-21], LC-MS, and spectrophotometric techniques [22,23]. But there is no method which describes the simultaneous determination of Adapalene and Benzoyl Peroxide and from gel dosage form meant for external application. The objective of this investigation was to develop simple accurate and economical procedures for simultaneous estimation of Adapalene and Benzoyl Peroxide from a gel dosage form.

Experimental Work

Simultaneous estimation of Adapalene and Benzoyl Peroxide by HPLC

Reagents and material

Adapalene RS, Benzoyl Peroxide RS, Acetonitrile (HPLC grade), Tetra Hydrofuran, Methanol

Instrumentation and chromatographic condition

Chromatographic separation was performed on an Analytical technology HPLC instrument (Software: HPLC Work Station) equipped with Deuterium lamp as detector, HPLC pump and manual injecting facility programmed at 20μL capacity per injection was used. Detection was carried out at 245nm using UV Detector. The separation was achieved on the ODS Zodiac Column C8 (250mmx4.6mm, 5μm) at ambient temperature. The elution was carried out isocratically at flow rate of 1mL/min.

Preparation of mobile phase

Mobile phase was prepared by mixing 90 volumes of Acetonitrile and 10 volumes of Methanol. The mobile phase was ultrasonicated, filtered through 0.45μm membrane filter, and degassed.

Diluent

Use the mixture of Acetonitrile and Tetrahydrofuran (55:45).

Preparation of Standard Stock Solution

A.   Standard stock solution for adapalene:

Weigh and transfer about 10.0mg Adapalene RS in 50mL volumetric flask sonicate to dissolve and make up the volume with the diluent to mark. Further dilute 5mL in 25mL with diluent.

#### B.   Standard stock solution for benzoyl peroxide:

Weigh accurately transfer about 25.0mg Benzoyl Peroxide RS in 25mL volumetric flask sonicate to dissolve and make up the volume with the diluent to mark.

Combined standard solution

Mix 2mL each of above standard stock solution in to 25mL volumetric flask and dilute to mark with diluent.

Sample solution: Weigh accurately gel about 1.0g in to a 25.0mL volumetric flask, and add 15.0mL of diluent, The solutions were sonicated for 20 minutes on ultra-sonicator, dilute to volume with diluent. Take 2mL from above solution in 25mL volumetric flask and dilute to mark with diluent.

Procedure: Filter both Sample and Standard Solution with 0.2μ filter paper and inject 20μL (Figure 3a-3d).

Figure 3a : Filter both Sample and Standard Solution with 0.2 µ filter paper and inject 20µL.

Figure 3b

Figure 3c

Figure 3d
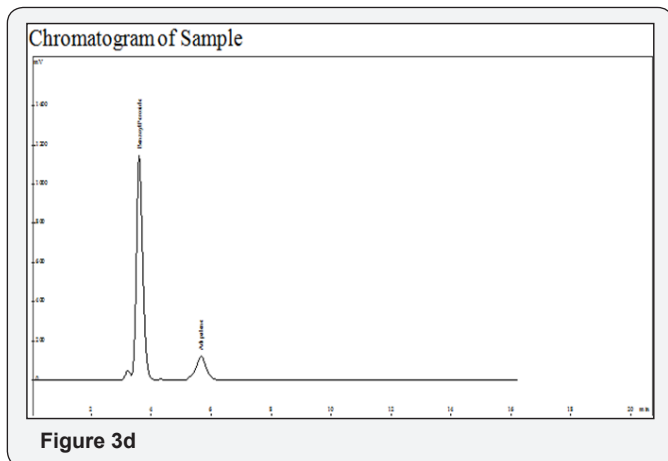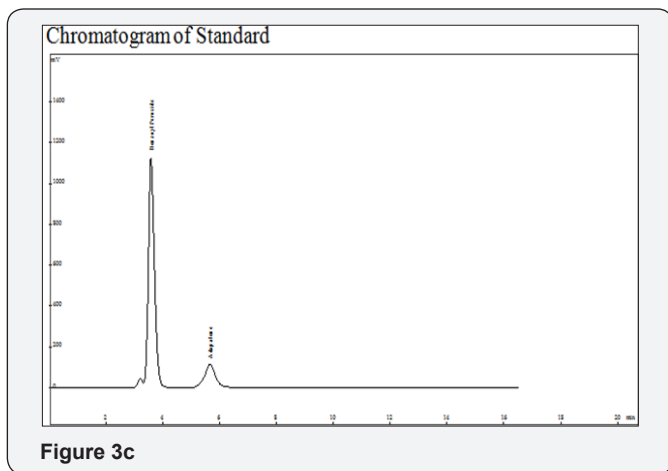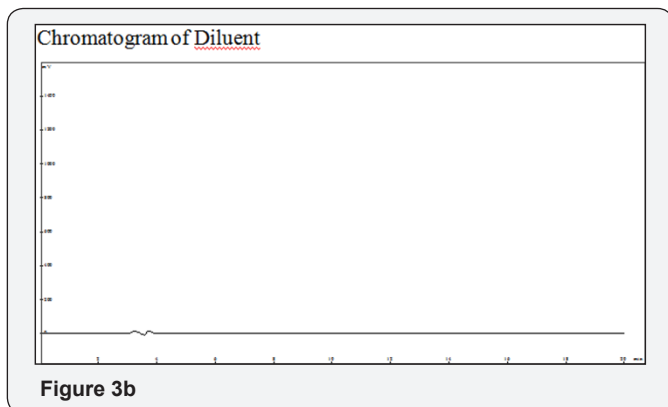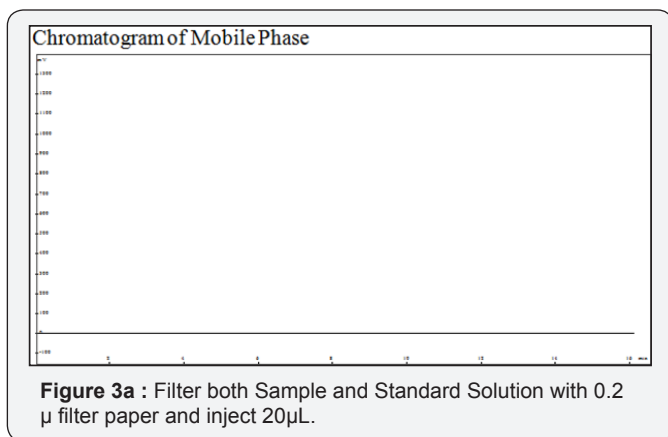
Method Validation

As per ICH guideline the method was validated and following parameters were evaluated, along with Ruggedness [24-29]. Analysis of sample was carried out using the above method and the result are tabulated in Table 1.

Table 1: Analysis of sample in house production batch.

| Contents	Label Claim	Found %w/w	Assay % of Label Amount
Adapalene	0.1% w/w	0.10%	98.42%
Benzoyl Peroxide	2.5% w/w	2.48%	99.29%

System Suitability Studies

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. In that the column efficiency, resolution and peak tailing factor were calculated for the standard solutions (Table 2). The values obtained demonstrated the suitability of the system for the analysis of this drug combination.

Table 2: System Suitability Parameter.

Parameter	Adapalene	Benzoyl Peroxide
Precision of the method (n = 5)	0.25	0.40
Theoretical Plates	2125	5652
Resolution Factors	1.824	8.475
Tailing factor	1.000	1.000
Retention time	3.724	5.840

Linearity

Figure 4 : Linearity Graph of Adapalene.
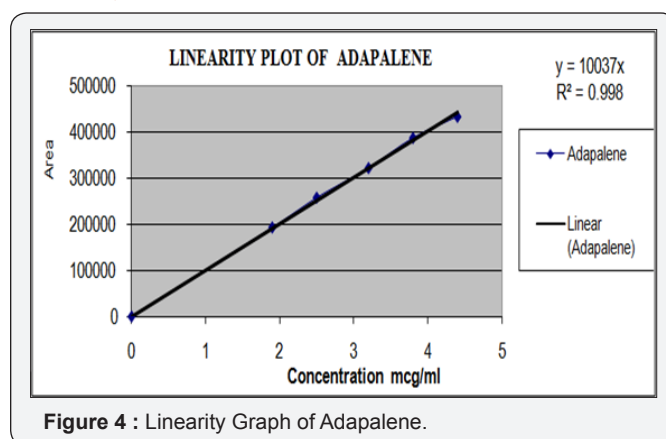
Linearity of the method was established by analysis of combined standard solution. The range of an analytical procedure is the interval between the upper and lower concentrations (amounts) of analyse in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. Linearity of the proposed method was established by using series of standard solutions of Adapalene and Benzoyl Peroxide these studies are repeated in triplicate with

different stock solutions. The curve obtained by concentration on X-axis and peak area on Y-axis against showed linearity in the concentration range of 1.9 to 4.4μg/mL for Adapalene and 48 to 112μg/mL for Benzoyl Peroxide and its correlation coefficient is 0.998 and 0.999, and linearity graph is shown in Figure 4 & 5 (Table 3 & 4).
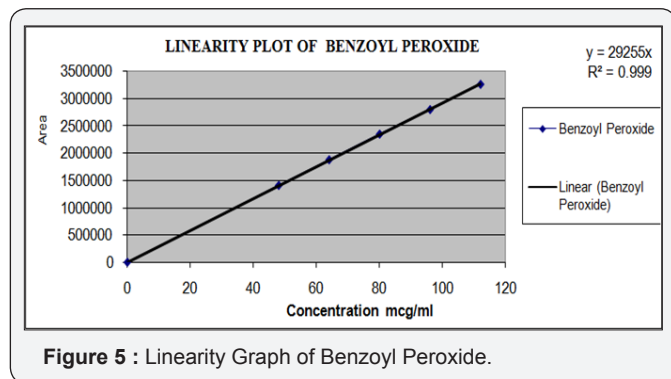

Figure 5 : Linearity Graph of Benzoyl Peroxide.

Table 3: Linearity and Statistical analysis data for Adapalene.

S. No		Concentration (μg/mL)	Area
1.		1.9μg/mL	192854
2.		2.5μg/mL	257325
3.		3.2μg/mL	321500
4.		3.8μg/mL	386548
5.		4.4μg/mL	452458
Correlative Coefficient(r^2)			0.998

(Table 6).

Table 4: Linearity and Statistical analysis data for Benzoyl Peroxide.

S. No.	Concentration (μg/mL)	Area
1.	48μg/mL	1411084
2.	64μg/mL	1881427
3.	80μg/mL	2351809
4.	96μg/mL	2802175
5.	112μg/mL	3265515
Correlative Coefficient(r^2)		0.999

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection and limit of quantification of the developed method were determined by injecting progressively low concentration of the standard solutions using the developed RP-HPLC method. The LOD of Adapalene and Benzoyl Peroxide was found to be 0.30μg/mL and 4.0μg/mL respectively. The LOQ is the smaller concentration of the analyte response that can be quantified accurately the LOQ was 1.2μg/mL and 20μg/mL respectively.

Recovery Studies

To study the accuracy and reproducibility of the proposed method recovery experiments were carried out. A fixed amount of pre-analyzed sample was taken and standard drug was added at 80%, 100% and 120% levels. Each level was repeated three times. The contents of Adapalene and Benzoyl Peroxide found by proposed method is shown in the Table 5. The mean recoveries of Adapalene and Benzoyl Peroxide were 99.08% and 98.94% respectively which shows there is no interference from excipient

Table 5: Accuracy (by Recovery) data for the proposed RP-HPLC method for Adapalene.

Accuracy Level %	S.No	Amount Added (mg)	Amount Recovery (mg)	Area	%Recovery	Mean%
80%	1	8.0mg	7.92mg	260865	99.00%	98.83%
	2	8.0mg	7.97mg	264682	99.62%	
	3	8.0mg	7.83mg	261554	97.87%	
100%	1	10.0mg	9.91mg	324851	99.10%	98.97%
	2	10.0mg	9.83mg	321522	98.31%	
	3	10.0mg	9.95mg	325458	99.52%	
120%	1	12.0mg	11.87mg	390541	98.91%	99.02%
	2	12.0mg	11.86mg	391452	98.83%	
	3	12.0mg	11.92mg	392658	99.33%	

Table 6: Accuracy (by Recovery) data for the proposed RP-HPLC method for Benzoyl Peroxide.

Accuracy		Amount Added	Amount Recovery	Area	%Recovery	Mean%
Level %		(mg)	(mg)			
	1	20.0mg	19.87mg	1882543	99.35%	
80%	2	20.0mg	19.70mg	1868524	98.51%	99.03%

	3	20.0mg	19.85mg	1873256	99.25%	
	1	25.0mg	24.73mg	2358491	98.92%	
100%	2	25.0mg	24.92mg	2351848	99.68%	99.38%
	3	25.0mg	24.89mg	2353235	99.56%	
	1	30.0mg	29.96mg	2823388	99.86%	
120%	2	30.0mg	29.82mg	2865454	99.40%	99.45%
	3	30.0mg	29.73mg	2843578	99.09%	

Precision Studies

Precision of method was studies by analysis of multiple sampling of homogeneous sample. The precision of analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous Sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. Precision should be investigated using homogenous authenticated sample. Precision Expressed as % RSD is given in Table 7 which should be less than 2%.

Table 7: system precision result of the proposed RP-HPLC Method.

Sample	Adapalene	Benzoyl Peroxide
Sample 1	99.15%	99.09%
Sample 2	98.63%	99.82%
Sample 3	98.85%	98.96%
Sample 4	99.11%	98.88%
Sample 5	99.24%	98.67%
%RSD	0.25%	0.40%

Robustness and Ruggedness of the Method

Robustness of the methodj

Robustness is a measure of its capacity to remain unaffected by small but deliberate variations in the chromatographic method parameters and provides an indication of its reliability. This was done by small deliberate changes in the chromatographic conditions at 3 different levels and retention time of Adapalene and Benzoyl Peroxide was noted. The factor selected were flow rate, Column Temperature and % Acetonitrile in the mobile phase. It was observed that there were no deliberate changes in the chromatogram, which demonstrated that the RP-HPLC method developed, are robust. Results describe in Table 8.

Table 8: Robustness of the method.

	Benzoyl peroxide	Adapalene
Between Instrument I and II		
Instrument	% Content	% Content
I	99.48%	99.82%
II	99.72%	98.73%
% Error	0.25%	0.56%
Between Instrument I and II		
Analist	% Content	% Content
I	99.22%	98.43%
II	98.61%	98.04%
% Error	1.06%	0.41%
Ruggedness of the method		

The USP guideline defines ruggedness as "the degree of reproducibility" of the test result obtained by the analysis of the same samples under a variety of normal test condition such as; different Laboratory, different analyst, different instrument etc. Here this was done by changing the instrument and analyst. Results, presented in the Table 9 that indicates the selected factors are remained unaffected by small variations of this parameter.

Table 9: Ruggedness of the method.

	Benzoyl peroxide	Adapalene
Between Instrument I and II		
Instrument	% Content	% Content
I	99.48%	99.82%
II	99.72%	98.73%
% Error	0.25%	0.56%
Between Instrument I and II		
Analist	% Content	% Content
I	99.22%	98.43%
II	98.61%	98.04%
% Error	1.06%	0.41%

Conclusion

Based on the results, it is concluded isocritic RP-HPLC method was successfully developed for the assay of Adapalene and Benzoyl Peroxide in topical pharmaceutical formulation. The developed method is selective, precise, accurate, linear and robust. The forced degradation data proved that the method is specific for the analytes and free from the interference of the placebo and degradation products. Moreover, it may be applied for the individual and simultaneous determination of Adapalene and Benzoyl Peroxide compounds in a pharmaceutical drug product and substance. It can be utilized for the determination of assay, blend uniformity, and content uniformity of pharmaceutical products. The developed methods were validated based on ICH guidelines and gave comparable results.

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