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Development And Validation Of A HPLC Method For Adapalene And Benzoyl Peroxide In Bulk And Gel

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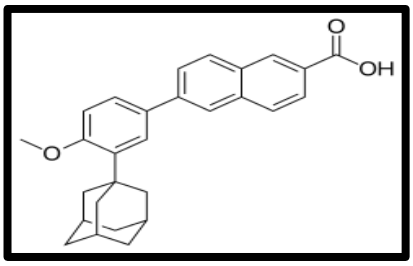
Abstract
The proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical for simultaneous estimation of Adapalene and Benzyol peroxide in semisolid dosage form. The developed method was validated in terms of accuracy, precision, linearity, robustness and ruggedness, and results will be validated statistically according to ICH guidelines. The Sample recoveries in all formulations were in good agreement with their respective label claims. From literature review and solubility analysis initial chromatographic conditions Mobile phase ortho phosphoric acid buffer : Methanol (30:70) were set (Buffer pH 3 adjusted with opa), symmetry C 18 (250×4.6mm, 5µ) Column, Flow rate 1.0 ml/min and temperature was ambient (30°C), eluent was scanned with PDA detector in system and it showed maximum absorbance at 254 nm. As the methanol content was increased Adapalene and Benzyol peroxide got eluted with good peak symmetric properties. The retention times for Adapalene and Benzyol peroxide was found to be 2.972 min and 3.548 min respectively. System suitability parameters were studied by injecting the standard five times and results were well under the acceptance criteria. Linearity study was carried out between 50% to150 % levels, R^2 value was found to be as 1.0 & 0.999. By using above method assay of marketed formulation was carried out, 99.83% & 98.89% was present in "Persol Plus Gel" market available .label claim was each gram of adapalene and benzoyl peroxide gel 0.1% / 2.5% contains 1 mg (0.1%) adapalene and 25 mg (2.5%) benzoyl peroxide in a white to very pale yellow, opaque, aqueous based gel.

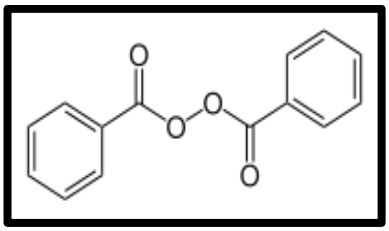
Keywords: Symmetry C18, Adapalene and Benzyol peroxide, HPLC

Introduction:

The molecular structure of the drug is given in Figure 1. Chemical formula: $C_{28}H_{28}O_3$, Molecular weight: 412.52 g/mol, Solubility: Sparingly soluble in water, completely soluble in methanol Category: Topical retinoid. 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 2. Chemical formula: $C_{14}H_{10}O_4$, Molecular weight: 242.23 g/mol, Category: radical initiator. 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. 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 [16-19], LC-MS, and spectrophotometric techniques [20,21].. The objective of this investigation was to develop simple accurate and economical procedures for simultaneous estimation of Adapalene and Benzoyl Peroxide dosage form



Experimental Work: Simultaneous estimation of Adapalene and Benzoyl Peroxide by HPLC:

Reagents and material: Potassium di-hydrogen-orthophosphate, Sodium perchlorate, Perchloric acid, Ortho phosphoric acid, - Merck (GR), Acetonitrile & Methanol (HPLC grade)

Instrumentation & chromatographic conditions: Chromatographic separation was performed on an WATERS HPLC instrument, software: Empower2, 2695 separation module. 2996 PDA 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 symmetry C_{18} (4.6 x 250mm, 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 weighing 6.8 gm of KH_2PO_4 into 1000ml volumetric flask dissolved in HPLC grade water and adjust pH up to 3 with ortho phosphoric acid. From the above prepared buffer take 300 ml (30%) and 700ml Methanol (70%) HPLC grade, were mixed and degassed in ultrasonic water bath for 5 minutes and was filtered through 0.45 µ filter under vacuum filtered and degassed same was used as diluent.

Preparation of Standard Stock Solution: 10 mg of Adapalene and 10mg of Benzoyl peroxide were accurately weighed and transferred into a 10 ml clean dry volumetric flask, about 7 ml of diluent was added, sonicate to dissolve it completely and the volume was made up to the mark with the same solvent to give a concentration of 1000 µg/ml. (Stock solution) Further 0.2 and 0.1 ml were pipette out from the above stock

solution into a 10ml volumetric flask and diluted up to the mark with diluent to give a concentration of 20 µg/ml and 10µg/ml respectively.

Preparation of combined stock solution: mix 2ml each of above stock solution into 25 ml volumetric flask & make up the volume upto mark with diluent

Preparation of Sample preparation: transfer an accurately weighed amount of 1.0 gm gel into 25 ml. Of clean dry volumetric flask and adde 10 ml of diluent was added to it and was shaken by mechanical stirrer and sonicate for about 30 minutes by shaking at intervals of five minutes each and was diluted up to the mark with diluent. Taken 2 ml from above solution into 25 ml volumetric flask & dilute upto mark with diluent. The solution was filtered through 0.45 µm filter before injecting into HPLC system.

Preparation of Placebo: The amount of powdered inactive ingredient supposed to be present in gel were accurately weighed and transferred in to 10 ml volumetric flask, 7 ml of diluent was added and shaken by mechanical stirrer and sonicated for about 30 minutes by shaking at intervals of five minutes and was diluted up to the mark with diluent and allowed to stand until the residue settles before taking an aliquot for dilution. 0.1 ml of upper clear solution was transferred to a 10 ml volumetric flask and diluted with diluent up to the mark and the solution was filtered through 0.45 µm filter before injecting into HPLC system.

Method Validation:

As per ICH guideline the method was validated and following parameters were evaluated, along with Ruggedness [22-25]. Analysis of sample was carried out using the above method and the result are tabulated in Table 1.

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.

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 2 to 4ppm for Adapalene and 10 to 30ppm for Benzoyl Peroxide and its correlation coefficient is 1.0 and 0.999, and linearity graph is shown in Figure 6 &7 and values were given in Table 3 & 4.

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 50%, 100% and 150% levels. Each level was repeated three times. The contents of Adapalene and Benzoyl Peroxide found by proposed method is shown in the Table 5&6 respectively. The mean recoveries of Adapalene and Benzoyl Peroxide were 99.60% and 98.73% respectively which shows there is no interference from excipient

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&8 which should be less than 2%

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 6.37µg/mL respectively which was given in the Table 9.

Robustness It 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 % Methanol (organic phase) 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 10

Results:

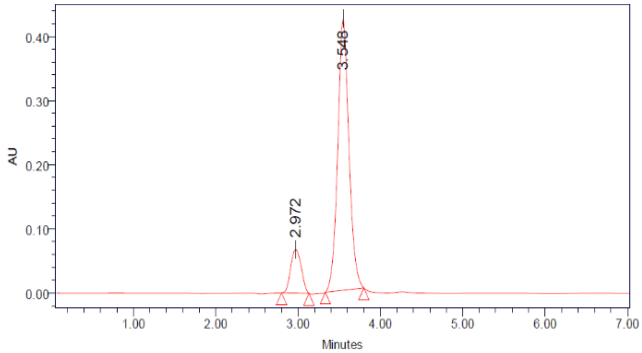


Fig 3: Standard Chromatogram for Optimized Method

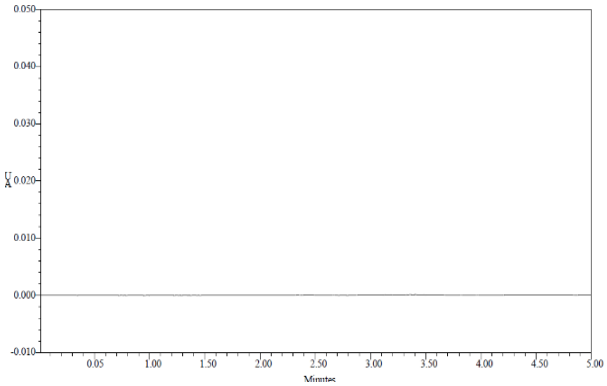


Fig 4: Chromatogram of Blank

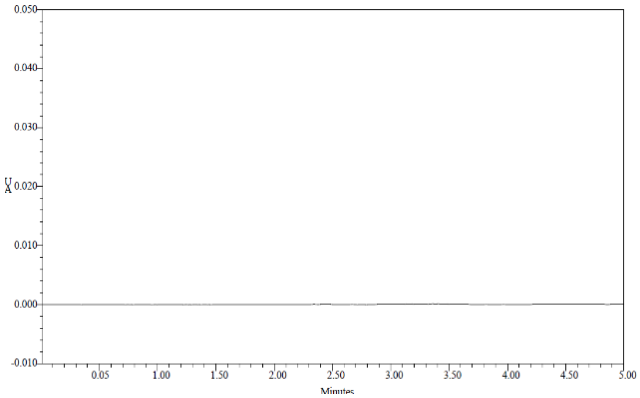


Fig 5: Chromatogram for Placebo

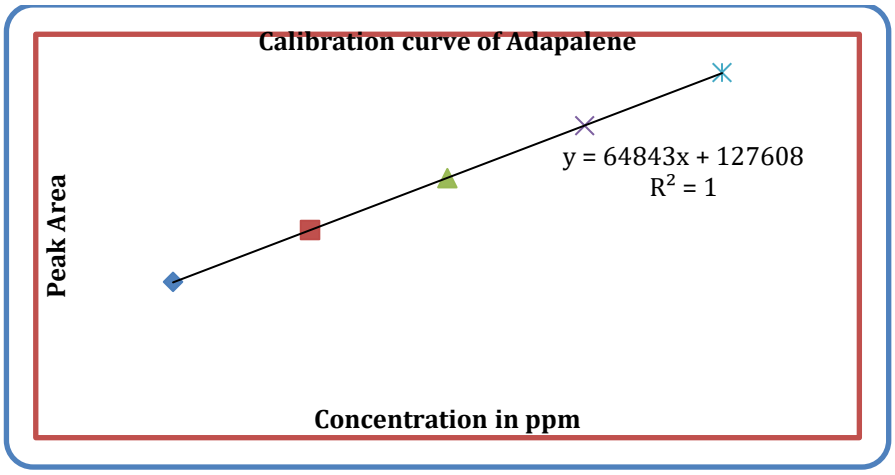


Fig 6: Calibration curve of Adapalene

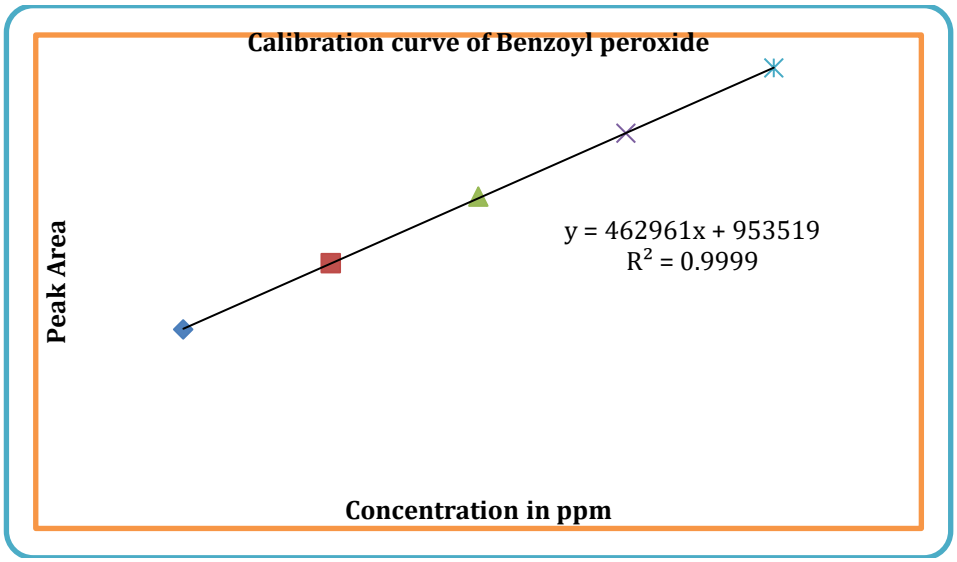


Fig 7: Calibration curve of Benzoyl peroxide

Table 1: % of Assay found in dosage form "Persol Plus Gel".

Contents	Label Claim	Found %w/w	Assay % of Label	Peak area
Adapalene	0.1% w/w	0.10%	99.83%	937329
Benzoyl Peroxide	2.5% w/w	2.48%	98.89%	1278360

Table 2: System Suitability Parameter

Parameter	Adapalene	Benzoyl Peroxide
% RSD (n = 3)	0.257	0.40
Theoretical Plates	2281	2496
Resolution Factors	1.824	8.475
Tailing factor	1.6	1.51
Retention time	2.806	3.886

Table 3: Linearity and Statistical analysis data for Adapalene.

S.No	Linearity Level	Concentration	Area
1	I	2.0 ppm	192854
2	II	2.5 ppm	257325
3	III	3.0 ppm	321500
4	IV	3.5 ppm	386548
5	V	4.0 ppm	452458
Correlation Coefficient (R^2)			1.0

Table 4: Linearity and Statistical analysis data for Benzoyl Peroxide

S.No	Linearity Level	Concentration	Area
1	I	10 ppm	1411084
2	II	15 ppm	1881427
3	III	20 ppm	2351809
4	IV	25 ppm	2802175
5	V	30 ppm	3265515
Correlation Coefficient (R^2)			0.999

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

Sample No.	Spike Level	Amount (µg/ml) added	Amount (µg/ml) found	% Recovery	Mean % Recovery	%Mean Recovery
1	50 %	5	4.9	98%	100%	**99.60%**
		5	5.1	102%		
		5	5	100%		
2	100 %	10	9.88	98.8%	99.13%	
		10	9.91	99.1%		
		10	9.95	99.5%		
3	150 %	15	14.89	99.2%	99.69%	
		15	14.86	99.0%		
		15	14.82	99.79%		

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

Sample No.	Spike Level	Amount (µg/ml) added	Amount (µg/ml) found	% Recovery	Mean % Recovery	% Mean Recovery
1	50 %	20	4.9	98%	100%	**99.73%**
		20	5.1	102%		
		20	5	100%		
2	100 %	25	9.88	98.8%	99.31%	
		25	9.91	99.1%		
		25	9.95	99.5%		
3	150 %	30	14.89	99.2%	99.89%	
		30	14.86	99.0%		
		30	14.99	99.79%		

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

Injection No	Peak Area	R_t
1	1231404	2.808
2	1233196	2.806
3	1231008	2.805
4	1238575	2.807
5	1232407	2.804
Mean	1233318	
SD	3061.06	
%RSD	0.2481	
Injection No	**Peak Area**	**R_t**
1	912412	3.882
2	913062	3.880
3	909642	3.801
4	916881	3.882
5	914005	3.880
Mean	913200.4	
SD	2621.886	
%RSD	0.287	

Table 8: system precision result of the proposed RP-HPLC Method Benzoyl Peroxide

Table 9: Sensitivity table of Adapalene & Benzoyl Peroxide

Molecule	LOD	LOQ
Adapalene	0.3µg/mL	4.0 µg/mL
Benzoyl Peroxide	1.2µg/mL	6.37 µg/mL

Table 10: Robustness data for Adapalene and Benzoyl Peroxide**.**

S. No.	Condition	%RSD of Adapalene	%RSD of Benzoyl Peroxide
1	Flow rate (-2) 0.6ml/min	0.20	0.28
2	Actual Flow rate **0.8** ml/min	0.25	0.40
3	Flow rate (+2) 1.0ml/min	0.28	0.45
4	Mobile phase (-5%) 33.5B:66.5A	0.24	0.41
5	Actual Mobile phase **30A:70B**	0.25	0.40
6	Mobile phase (+5%) 26.5B:73.5A	0.28	0.39
7	Temperature (-) 25°C	0.25	0.40
8	Actual Temperature **30°C**	0.25	0.40
9	Temperature (+) 35°C	0.25	0.40

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