

DEVELOPMENT AND VALIDATION OF ADAPALENE AND DAPSONE IN BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC AND ITS DEGRADATION

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Abstract

The present paper describes a simple, accurate and precise reversed phase High Performance Liquid Chromatography (HPLC) method for rapid and simultaneous quantification of Adapalene and Dapsone. The chromatographic separation was achieved on X-Bridge C₈ (150x4.6mm, 3.5 μ) column. Mobile phase contained a mixture of 1mg Hexane Sulphonic acid of pH=5.0 adjusted with OPA is used as buffer and Acetonitrile in the ratio of 40:60 v/v, flow rate 1.0ml/min and UV detection at 249nm. The proposed method shows a good linearity in the concentration range of 1-15 μ g/ml of Adapalene and 50-750 μ g/ml of Dapsone under optimized conditions. Precision and recovery study results are in between 98-102%. In the entire robustness conditions %RSD is below 2.0%. Degradation has minimum effect in stress condition and solutions are stable up to 24hrs. This method is validated for different parameters like Precision, linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ), ruggedness, robustness and forced degradation study were determined according to the ICH Q2B guidelines. All the parameters of validation were found to be within the acceptance range of ICH guidelines.

Key words: RP-HPLC, Adapalene and Dapsone.

INTRODUCTION

Adapalene is a third-generation topical retinoid [1, 2] primarily used in the treatment of mild-moderate acne [3], and is also used off-label [4] to treat keratosis pilaris [5] as well as other skin conditions [6]. It is effective against acne conditions where comedones [7] are predominant. Per the recommendations of the Global Alliance on improving outcomes of Acne, retinoids such as adapalene are considered first line therapy in acne treatment and are to be used either independently or in conjunction with benzoyl peroxide and/or an antimicrobial agent for maximum antimicrobial agent for maximum efficacy [8, 9]. Furthermore, adapalene like other retinoids increases the efficacy and penetration of other topical acne medications that are used in conjunction with topical retinoids as well as hastens [10] the improvement of the post-inflammatory hyper pigmentation [11] caused by acne. In the long term, It can be used as maintenance therapy [12]. While adapalene is often thought to be the least efficacious of the retinoid medications, two multicenter randomized double –blinded studies showed that the use of adapalene 0.1% lotion for 12weeks improved inflammatory and non-inflammatory acne as well as reduced the total lesion count from baseline in those patients in the treatment group.

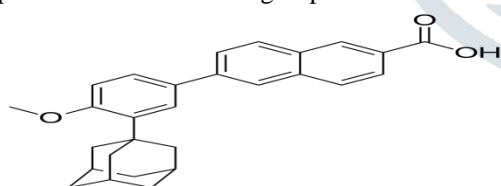


Fig. 1: Structure of Adapalene

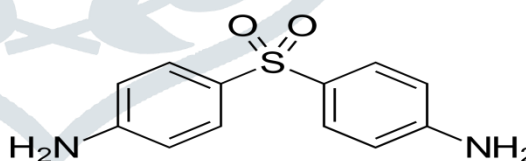


Fig. 2: Structure of Dapsone

Dapsone, also known as diamino diphenyl sulfone (DDS) is an antibiotic commonly used in combination with rifampicin and clofazimine for the treatment of leprosy [13]. It is a second-line medication for the treatment and prevention of pneumocystis pneumonia [14] and for the prevention of toxoplasmosis [15] in those who have poor immune function. Additionally, it has been used for acne, dermatitis herpetiformis and various other skin conditions. Dapsone is available both topically and by mouth. Severe side effects may include a decrease in blood cells, red blood cell [16] breakdown especially in those with glucose-6-phosphate dehydrogenase deficiency (G-6-PD), or hypersensitivity. Common side effects include liver inflammation and a number of types of skin rashes. While the safety of use during pregnancy is not entirely clear some physicians recommended that it be continued in those with leprosy. It is of the sulfone [18] class. It is on the World Health Organization's [19] list of essential medicines, the most effective and safe medicines needed in a health system [20]. The form, which is taken by mouth, is available as a generic drug and not very expensive.

MATERIALS AND METHODS

Chemicals

Acetonitrile, Ortho Phosphoric acid (OPA) and water (HPLC grade), Hexane sulphonic acid were purchased from Merck (India) Ltd. Worli, Mumbai, India. All APIs of adapalene and dapsone as reference standards were procured from Torrent Pharma, Ahmadabad.

Equipment

Waters alliance -2695 chromatographic system consisting of quaternary pump, PDA detector-2996 and chromatographic software Empower-2.0 was used.

Chromatographic conditions

Chromatographic separation was carried out in isocratic mode at room temperature using X-Bridge C₈ (150x4.6mm, 3.5 μ) column. The mixture of Hexane Sulphonic acid of pH=5 adjusted with OPA: Acetonitrile 40:60 v/v at a flow rate of 1.0ml/min was used as a mobile phase. The injection volume was 10 μ l and eluent was monitored at 249nm using PDA detector. The run time was 7min.

Selection of wavelength

The absorption spectra of solution of adapalene and dapsone were scanned over the range of 200-400nm by using PDA detector and the spectra was recorded. The spectrum was shown in fig.3.

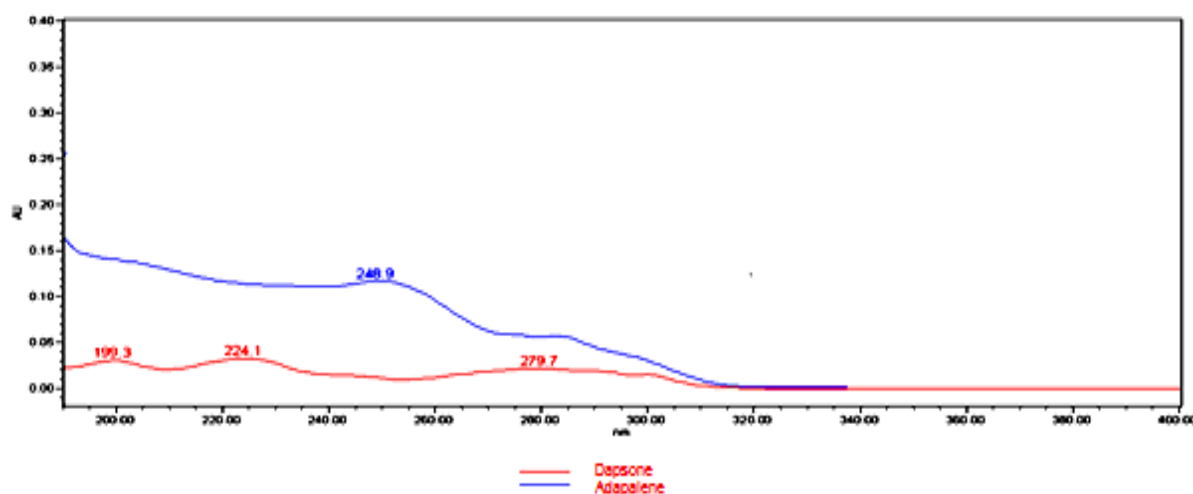


Fig. 3: PDA Spectrum of adapalene and dapsone

Preparation of Standard solution:

Weigh accurately 10mg of adapalene and 500mg of dapsone working standards were taken into a 100ml volumetric flask, add 70ml of diluent and sonicated for 10min to dissolve the components, makeup to the mark with mobile phase. Further diluted 5ml of above solution to 50ml volumetric flask and diluted to volume with diluent.

Preparation of Sample solution

Weigh 10mg of adapalene and 500mg of dapsone equivalent weight of sample and transferred into 100ml volumetric flask and add 70ml of diluent and sonicated for 30min to dissolve the components and centrifuge for 30min then diluted up to the mark with diluent. Further dilute 5ml of above solution to 50ml diluent and it was filtered through 0.45 μ nylon syringe filter.

Validation Procedure

The analytical method was validated as per ICH Q2 (R₁) guidelines for the parameters like system Precision, specificity, accuracy, precision, linearity, robustness, limit of detection (LOD), limit of quantification (LOQ) and forced degradation.

System Suitability

System suitability parameters were measured to verify the system performance. The parameters including USP plate count, USP tailing and % RSD are calculated and found to be within the limits.

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of other components, which may be expected to be present in the sample and standard solution. It was checked by examining the chromatograms of blank samples and samples spiked with adapalene and dapsone.

Accuracy

Accuracy is the closeness of the test results obtained by the method to the true value. It was assessed by the recovery studies at three different concentration levels. In each level, a minimum of three injections were given and amount of the drug present, percentage recovery and related standard deviation were calculated.

Precision

Precision of an analytical method is the degree of agreement among individual test results. It was studied by analysis of multiple sampling of homogeneous sample. The precision of the present method was assessed in terms of repeatability, intra-day and inter day variations. It was checked by analyzing the samples at different time intervals of the same day as well as on different days.

Linearity and range

Linearity of an analytical method is its ability to obtain results directly proportional to the concentration of the analyte in the sample within a definite range. The six series of standard solutions were selected for assessing linearity range. The calibration curve was plotted using peak area versus concentration of the standard solution and the regression equations were calculated. The least squares method was used to calculate the slope, intercept and correlation coefficient.

LOD and LOQ

LOD is the lowest amount of analyte in a sample that can be detected while LOQ is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy. LOD and LOQ were separately determined based on the calibration curves. The LOD and LOQ for adapalene and dapsone were determined by injecting progressively low concentrations of standard solutions using the developed RP-HPLC method. The LOD and LOQ for calculated as 3.3s/n and 10s/n respectively as per ICH guidelines where s/n indicates signal-to-noise ratio.

Stress Degradation

Stress degradation should be no interference between the peaks obtained for the chromatogram of forced degradation preparations. Stress degradation studies were performed as per ICH guidelines Q₁A (R₂). The degradation peaks should be well separated from each other and the resolution between the peaks should be at least 1.0 and the peak purity of the principle peaks shall pass. Forced degradation studies were performed by different types of stress conditions to obtain the degradation of about 20%.

Robustness

The robustness of an analytical procedure is a measure of its ability to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness study was performed by injecting standard solution into the HPLC system and altered chromatographic conditions such as flow rate (± 0.2), organic content in the mobile phase ($\pm 10\%$). The separation factor, retention time and peak asymmetry were calculated by determining the effect of the modified parameters.

RESULTS AND DISCUSSION

Method Validation

In this method system suitability, linearity, precision, accuracy, LOD, LOQ, robustness, forced degradation are validated for the selected drugs adapalene and dapsone drugs. The proposed method having standard solution and sample solution chromatograms are shown.

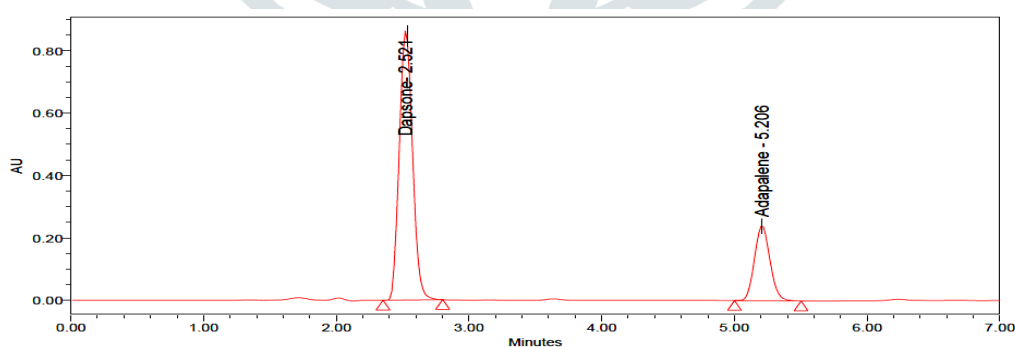


Fig. 3: Chromatogram of standard

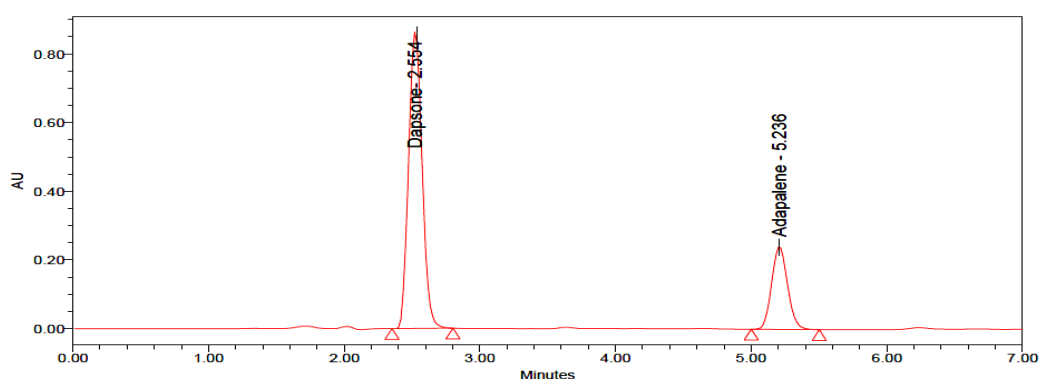


Fig. 4: Chromatogram of sample

System suitability

The HPLC system was stabilized for 60min to get a stable baseline. Six replicate injections of the standard solution containing 10µg/ml of adapalene and 500µg/ml of dapsone were assessed to check the system suitability. The number of theoretical plate count for adapalene and dapsone were 4852 and 5542 respectively. Tailing factor for adapalene and dapsone were 1.12 and 1.09 respectively. All the parameters were found to be within limit.

Linearity

Linearity of the method was evaluated by preparing a standard solution containing 10µg/ml of adapalene and 500µg/ml of dapsone. Sequential dilutions were performed to the given solutions at 10, 25, 50, 100, 125 and 150% of the target concentrations. These were injected and the peak areas are used to plot calibration curves against the concentration. The correlation coefficient values of these analytes were 0.999. The results were shown in table 1.

Table 1: Linearity study results

Analyte	Linearity range	Equation of calibration curve	Correlation coefficient
Adapalene	1-15µg/ml	$Y = 5406.6x + 260.7$	0.9994
Dapsone	50-750µg/ml	$Y = 3962.3x + 66812$	0.9994

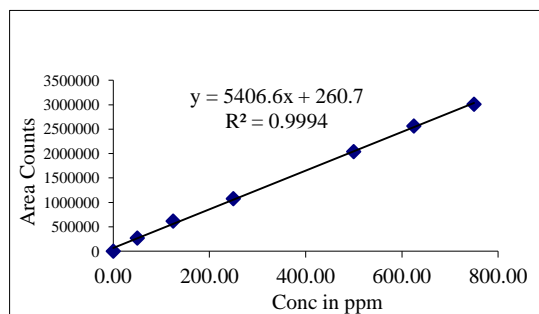


Fig. 5: Linearity plot for Adapalene

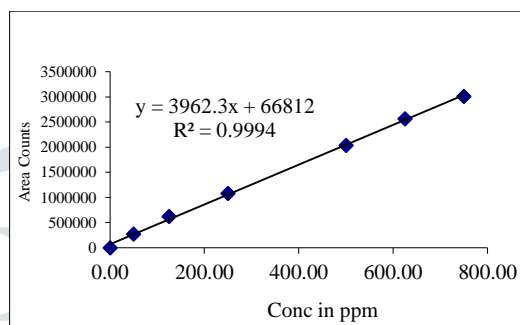


Fig. 6: Linearity plot for Dapsone

LOD and LOQ

LOD and LOQ minimum concentration level at which the analyte can be reliably detected, quantified by using the standard formulas. LOD values for adapalene and dapsone were 0.01µg/ml and 0.5µg/ml. LOQ values for adapalene and dapsone were 0.1µg/ml and 5µg/ml.

Precision

Method precision was investigated by the analysis of six separately prepared samples of the same batch. From these six separate samples solution was injected and the peak areas obtained used to calculate mean and percentage RSD values. The present method was found to be precise as %RSD of the less than 2.0%. The results are given in table 2.

Table 2: Method Precision results

Analyte	Amount present	% RSD
Adapalene	10	0.62
Dapsone	500	0.41

Accuracy

Accuracy was determined by recovery studies which were carried out in three different concentration levels (50%, 100% and 150%). APIs with concentration 5, 10 and 15µg/ml of adapalene and 250, 500 and 750µg/ml of dapsone were prepared. As per the test method the test solution was injected to three preparations each spike level and the assay was performed. The percentage recovery values were found to be in the range of 98-102%. The results are given in table 3.

Table 3: Accuracy (recovery) study results

% of target concentration	Adapalene (% Recovery)	Dapsone (% Recovery)
50	99.7	99.8
100	99.9	100.1
150	100.2	100.4
Mean (% Recovery)	99.9	100.1

Ruggedness

Ruggedness of the method was studied and showed that chromatographic patterns did not significantly change when different HPLC system, analyst, column. The value of percentage of RSD was below 2% exhibits the ruggedness of the developed method.

Robustness

Robustness of the method was found to be %RSD should be less than 2%. Slightly variations were done in the optimized method parameters like flow rate ($\pm 20\%$), Organic content in mobile phase ($\pm 10\%$). The results are given in table 4.

Table 4: Robustness results

Drug Name	Flow Plus (1.2ml/min)	Flow Minus (0.8ml/min)	Organic Plus (A66:34)	Organic Minus (A54:46)
	%RSD			
Adapalene	0.58	0.61	0.69	0.78
Dapsone	0.34	0.28	0.36	0.42

Forced Degradation

Forced degradation conditions such as acidic, basic, oxidation, thermal and water stress were attempted as per ICH Q1A (R2). The effect of assay on their results are shown below table 5.

Table 6: Forced Degradation results

Degradation	Adapalene (% Degradation)	Dapsone (% Degradation)
Acid	15.6	17.7
Alkali	18.9	15.8
Peroxide	17.9	16.3
Reduction	17.8	15.3
Thermal	11.7	15.8

CONCLUSION

This method described the quantification of adapalene and dapsone in bulk and pharmaceutical formulation as per ICH guidelines. The developed method was found to be accurate, precise, linear and reliable. The advantage lies in the simplicity of sample preparation and the less expensive reagents were used. In addition two compounds are eluted within 7mins. The proposed HPLC conditions ensure sufficient resolution and the precise quantification of the compounds. Statistical analysis of the experimental result indicates that the precision and reproducibility data are satisfactory. The developed chromatographic method can be effectively applied for routine analysis in drug research.

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