A Simple HPLC-DAD Method for Determination of Adapalene in Topical Gel Formulation

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Abstract

A simple stability indicating high-performance liquid chromatography method for the analysis of adapalene in pharmaceutical gel formulation is developed and validated. An isocratic separation is performed using a Merck RP-8 (150 mm × 4.6 mm i.d., particle size 5 m) column and a mixture of acetonitrile–water (67:33, v/v, pH adjusted to 2.5 with phosphoric acid) as the mobile phase. The detection is achieved with a photodiode array detector at 321 nm. The specificity of the method is verified by subjecting both the reference substance and the pharmaceutical form to hydrolytic, oxidative, photolytic, and thermal stress conditions. There is no interference from the excipients of the formulation on the determination of adapalene in gel. The response is linear over the concentration range of 8.0–16.0 µg/mL (r > 0.999) with a limit of detection and quantification of 0.04 and 0.14 µg/mL, respectively. The mean recovery is 100.8%. The RSD values for the intra- and inter-day precision studies are < 1.2%. The method is validated by reaching satisfactory results for linearity, selectivity, specificity, precision, accuracy, robustness, and system suitability.

Introduction

Acne is an extremely common disease, with a prevalence of 80–85% among adolescents, and 15–30% of cases need intense medical treatment (1). Acne patients may suffer from social, psychological, and emotional disabilities similar to those reported by patients with chronic disabling asthma, epilepsy, diabetes, back pain, or arthritis (2). In recent years, the pathogenesis of this widespread disease has been well understood. The distribution of acne is primarily on the face, chest, and back areas, where there are the greatest concentrations of pilosebaceous glands. The most important pathophysiological factors are altered follicular growth and differentiation, as well as sebaceous hyperplasia, because they combine to induce the microcomedone (the precursor of almost all acne lesions) (3).

The introduction of retinoids in the 1970s for the clinical management of *acne vulgaris* may be considered revolutionary (4). Nowadays, topical retinoids are a vital part of almost any acne regimen (5). They are recommended as first-line therapy for mild to moderate inflammatory acne and also for maintenance therapy. They act against comedones and microcomedones and have direct anti-inflammatory effects (3). Retinoids can be defined as a group of substances with the ability to bind retinoid receptors and activate the retinoid pathway. They are classified in three generations, based on their chemical structures (5).

Adapalene is a third generation synthetic retinoid used in the treatment of acne. It is a highly lipophilic compound, derived from naphthoic acid (5), and chemically designated as 6-[3-(1-adamantyl)-4-methoxyphenyl]-2-naphthoic acid (6) (Figure 1). On the market since 1995, it is available in two topical dosage forms: gel (0.1%, 0.3%) and cream (0.1%) (4). In comparison with tretinoin, a first generation retinoid, adapalene presents similar (7), or even better, efficacy (8) and improved tolerability (7,8), which makes it clinically advantageous (9). Also, adapalene is more stable to light and oxidation by benzoyl peroxide than tretinoin (10). Adapalene systemic absorption is minimal, and no evidence of teratogenicity has been reported (5).

Methods for the determination of adapalene using solid-phase extraction and gradient high-performance liquid chromatography (HPLC) with UV and fluorescence detection were published (11,12). However, these methods were intended for the analysis of biological samples and require sample pretreatment. In the present study, the quality control was focused, so the method was suitable for the routine analysis of both raw material and pharmaceutical product. Besides, it possessed a stability-indicating nature, which meant that it was capable of detecting
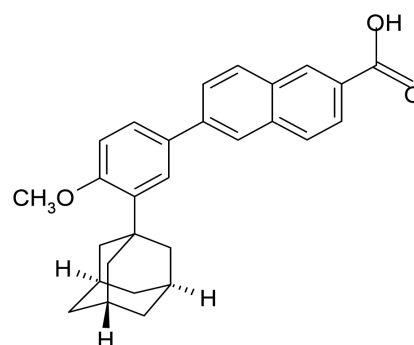

Figure 1. Chemical structure of adapalene.

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and quantifying adapalene in stressed samples, generated by forced decomposition studies (13,14), in accordance with the International Conference on Harmonisation (ICH) guidelines (14,15), the U.S. Food and Drug Administration (FDA) (16,17), and the United States Pharmacopeia (USP) (18). Therefore, this report presents a strategy which allows for the quantification of adapalene in topical gel formulation through a simple, fast, and isocratic HPLC–diode-array detection (HPLC–DAD) method with a short retention time, reaching excellent peak symmetry.

Experimental

Materials and chemicals

An adapalene reference substance (assigned purity of 99.22%) was obtained from Deg (São Paulo, Brazil). An adapalene gel form (0.1%, 30.0 g) was purchased from the market. The excipients of the formulation (carbomer, propylene glycol, poloxamer, disodium edetate, methylparaben, phenoxyethanol, and sodium hydroxide) were acquired from different distributors and were all of analytical grade. HPLC-grade acetonitrile was obtained from Tedia (Fairfield, CT). Purified water was prepared using Milli-Q Plus (Millipore, Bedford). All other reagents and chemicals were of analytical grade.

Instrumentation

The centrifugation step was accomplished with a Janetzki centrifuge, model T32 A (Berlin, Germany). The analysis was performed on a Shimadzu LC system (Kyoto, Japan), which consisted of a LC-10AD pump, an SPD-M10ADVP photodiode array (PDA) detector, a SLA-10ADVP system controller, a DGU-14A degasser, and a Rheodyne injector with a 20 μL loop. The data were acquired and processed using a CLASS-VP software (Version 6.1).

Photodegradation was carried out in a photostability UV chamber ($1.0 \times 0.17 \times 0.17$ m) with mirrors and equipped with a UV-A lamp (Orion, 352 nm, 30 W, 130 V); UV cuvettes (Ultra Vette, São Paulo, Brazil) were used as containers for the solutions. For thermal stability studies, a dry air oven (Biomatic, Porto Alegre, Brazil) was used.

Chromatographic conditions

The chromatographic separation was performed using a Merck RP-8 column (150 mm × 4.6 mm i.d., particle size 5 μm). The chromatographic analysis was performed isocratically at room temperature (25°C), using acetonitrile–water (67:33, v/v; the pH was adjusted to 2.5 with phosphoric acid) as the mobile phase, previously degassed using an ultrasonic bath. The flow rate was 1.4 mL/min, and the PDA detector was set at 321 nm. The injection volume was 20 μL for all solutions.

Standard and sample solution preparation

An adapalene reference substance was accurately weighed (10.0 mg) and dissolved in a 100-mL volumetric flask with 50 mL of ethanol. An accurately weighed amount of pharmaceutical gel corresponding to 10.0 mg of adapalene was dissolved in a 100-mL volumetric flask with 50 mL of ethanol. After that, the solu-

tions were submitted to an ultrasonic bath for 15 min. These solutions were diluted appropriately with ethanol to obtain a concentration of 100.0 μg/mL of analyte (stock solutions).

The subsequent dilutions were performed by diluting an appropriate volume of the stock solutions in the mobile phase. Before being analyzed by HPLC, sample solutions were centrifuged in tubes with caps at $6500 \times g$ for 5 min. This procedure assures the complete precipitation of the gel matrix components, whereas adapalene remains soluble in ethanol.

Method validation

Before performing any validation experiments, it should be assured that the system and the procedure were capable of providing data of acceptable quality (19). Therefore, the system suitability test was performed by injecting a standard solution. Theoretical plates, tailing, retention factors, and injection repeatability was determined. The developed chromatographic method was validated for specificity, linearity, precision, accuracy, and robustness (15–18).

Specificity/forced degradation studies

The specificity was investigated by the determination of the peak purity with the aid of a PDA detector, and analysis of interference from the excipients (the placebo solution) and the degradation products were generated by forced degradation studies. Sample solutions were exposed to oxidative (H_2O_2, 30%), acidic (HCl, 1 M), alkaline (NaOH, 1 M), thermal (80°C), and photolytic (UV-A radiation, 352 nm). The exposure time was at least 72 h. After the degradation period, all solutions were neutralized (if necessary) and diluted in the mobile phase to achieve a concentration of 12.0 μg/mL. The results were compared with the blank and standard solutions.

Linearity and range

To evaluate the linearity of the method, five standard solutions were prepared (8.0, 10.0, 12.0, 14.0, and 16.0 μg/mL). The linearity study was performed over three days. The peak areas were plotted versus the respective concentrations, and a linear regression analysis was used to obtain the equation and correlation coefficient.

Precision

Precision was determined by repeatability (intra-day precision) and intermediate precision (inter-day precision) and was expressed as the relative standard deviation (RSD) of a series of measurements. The repeatability was evaluated by assaying six samples at the same concentration (12.0 μg/mL) during the same day. The intermediate precision was evaluated by repeating the studies on three different days and comparing the obtained results.

Accuracy

In accordance with ICH (15) and USP (18), accuracy can be determined by adding known quantities of the analyte to the drug product. Therefore, known amounts of the adapalene reference substance (2.0, 4.0, and 6.0 μg/mL) were added to the sample solutions (8.0 μg/mL), yielding final concentrations of 10.0, 12.0, and 14.0 μg/mL. The accuracy was then calculated as

the percent recoveries of the added known amounts of the adapalene reference substance. These studies were performed on three different days.

Robustness

The robustness was tested by the modifications in the experimental conditions. The chromatographic parameters (peak retention time, theoretical plates, tailing, the capacity factor, and the repeatability) were evaluated for different flow rates (1.3 and 1.5), acetonitrile concentrations (66 and 68%), and a Merck RP-8 column (120 mm × 4.6 mm i.d., particle size 5 μm).

Results and Discussion

Method development

Different mobile phases and columns were tested. The best conditions were achieved with a Merck RP-8 (150 mm × 4.6 mm i.d., particle size 5 μm) column and a mixture of acetonitrile–water (67:33, v/v; the pH was adjusted to 2.5 with phosphoric acid) as the mobile phase. The flow rate was 1.4 mL/min. The absorption spectrum of adapalene showed good response at 321 nm. Therefore, the PDA detector was set at this wavelength. The injection volume was 20 μL for all solutions. An important target of method development was to exclude interference from excipients and the degradation products. Interference from inactive ingredients was investigated by the analysis of the adapalene standard solution (12.0 μg/mL), the excipient solution, and the solvent (ethanol). No peak was observed for the excipient solution and the solvent, whereas one peak was obtained with the standard solution, corresponding to adapalene with a retention time (tr) of 6.8 min. Chromatograms for the standard, excipient, and the solvent are shown in Figure 2. Interference from the degradation products was evaluated by forced degradation studies. The peak purity values for adapalene in the chromatograms of the stressed samples are in the range of 0.999 to 1.000, indicating homogeneous peaks.

Method validation

The results for the system suitability test demonstrate that the chromatographic system was able to provide reliable data. The theoretical plates (N = 7154), asymmetry (A = 1.19), retention factor (k' = 2.61), and the relative standard deviation (RSD) of repeated injections ($n = 5$) (RSD = 0.41%) were in agreement with the recommendations (16).

Specificity/stress degradation studies

The typical chromatograms for the standard and the sample solutions before being stressed were similar to that presented in Figure 2. The typical chromatograms obtained for the gel form stressed samples

are shown in Figure 3. No decomposition was seen after the exposure of the reference substance and the pharmaceutical gel form to the heat condition. Under storage in H_2O_2, 30% (v/v) at room temperature for 72 h, the reference substance was significantly more stable than the gel form with 0.4 and 1.23% of decomposition, respectively. Under this condition, the peak observed at 1.20 min could be attributed to the hydrogen peroxide stabilizer (acetanilide), because it was also detected in the blank solution. The photolytic condition presented the highest rate of decomposition: 48 and 22.2% for the reference substance and the gel form, respectively. In an acidic condition, the reference substance was less stable than the gel form with 10.6 and 0.78% of decomposition, respectively. In an alkaline condition, no significant decomposition was observed for both samples (< 1%).
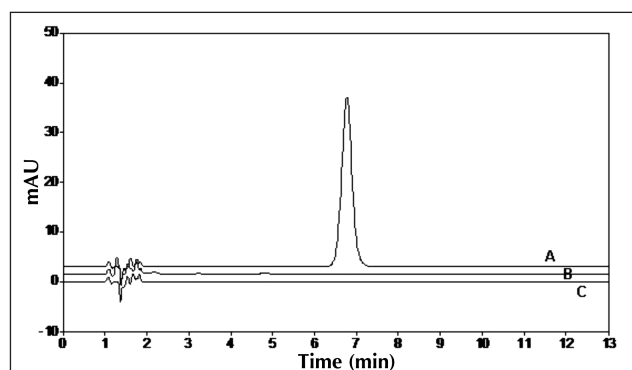
Figure 2. Chromatograms obtained for (A) the standard solution of adapalene at 12.0 μg/mL, (B) the solutions of the excipients, and (C) ethanol. Chromatographic conditions: RP-18 column (125 mm × 4.6 mm, 5 μm packing, Merck); flow rate: 1.4 mL/min; mobile phase: acetonitrile–water (67:33, v/v; the pH was adjusted to 2.5 with phosphoric acid); UV detection at 321 nm.

Figure 3. Representative chromatograms of adapalene (12.0 μg/mL) obtained from degradation studies. (A) Acid hydrolysis (1.0 M HCl), (B) nase hydrolysis (1.0 M NaOH), (C) thermal degradation (80°C), (D) photolysis (UV 352 nm), and (E) oxidation (H_2O_2 30%, v/v). The exposure time was at least 72 h.

Degradation products did not appear on the chromatograms in the conditions of the present study. Nevertheless, the peak purity values for adapalene, obtained with a PDA detector, were 0.9999–1.0000 for both the reference substance and the pharmaceutical gel form solutions, indicating homogeneous peaks, and thus establishing the specificity of the assay. Table I presents the results of the forced degradation studies using the proposed method, showing the degradation percentage and the purity of the adapalene peak in the chromatogram.

Linearity

Linearity parameters were obtained by plotting the concentration versus the peak area, and show good linearity in the 8.0–16.0 μg/mL range. A linear regression analysis was performed on the resultant curve. The representative linear equation was y = 46154x + 12220, and the correlation coefficient (r = 0.9999) was highly significant. The validity of the assay was verified by means of Analysis of Variance (ANOVA), and the results showed that the regression equation was linear with no deviation from linearity. The LOD and LOQ were 0.04 and 0.14 μg/mL, respectively.

Precision

The intra-day precision was assessed by assaying the samples in three different days by the same analyst, presenting the following results: 100.3 ± 0.54%, 100.0 ± 0.56%, and 99.3 ± 0.20% (mean ± RSD) for the raw material, and 100.8 ± 1.34%, 99.4 ± 0.65%, and 101.7 ± 0.38% for the gel form. The RSD for intra- and inter-day precision was 0.50% and 1.19% ($n = 18$), respectively.

Accuracy

The results obtained for the accuracy study show that the proposed method is accurate. The recovery ranged from 98.5 to 101.8%, and the average recovery was equivalent to 100.8%. The RSD for accuracy was 1.06%.

Robustness

The robustness of adapalene was similar for the reference substance and the pharmaceutical gel form. The values for these parameters were satisfactory in accordance with the literature (15–17). None of the alterations caused a significant effect on the determination of adapalene, indicating robustness of the method. The results of the robustness for the pharmaceutical gel form are shown in Table II.

Conclusion

The developed stability-indicating HPLC assay is simple, rapid, and selective for the determination of adapalene in a gel dosage form. It was fully validated, reaching excellent results for the parameters of linearity, accuracy, precision, robustness, and system suitability. Among the significant advantages of this method are a simple mobile phase, a short run time, and an isocratic elution. Adapalene was successfully isolated from the gel matrix without need of sophisticated procedures, and good analyte recoveries were obtained. This method is suitable for the routine analysis of adapalene in a gel dosage form.

Table II. Robustness Evaluation for Adapalene Pharmaceutical Gel Form ($n = 3$)

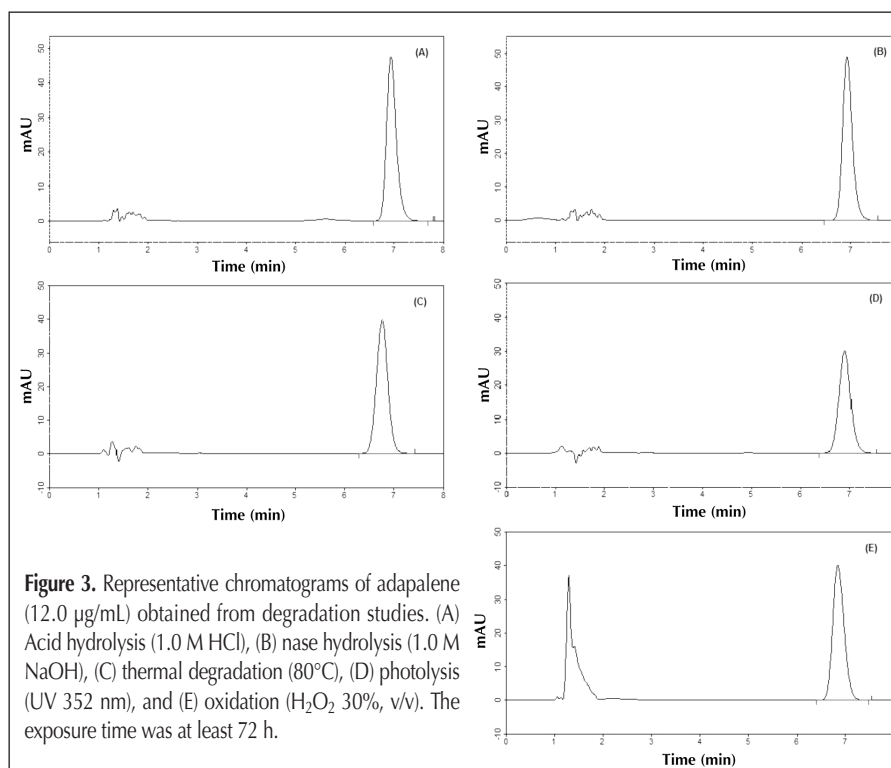| Parameter	t_R[†] (min)	T[‡] (≤ 2)	N[§] (> 2000)	K[††] (> 2)	(%)[‡‡]
Proposed method*	6.89	1.21	6996	2.59	100.0
Flow rate (1.3 mL^{-1})	7.33	1.24	7148	2.89	100.8
Flow rate (1.5 mL^{-1})	6.43	1.12	5212	2.95	99.6
Acetonitrile (66%)	7.12	1.26	3755	2.74	99.6
Acetonitrile (68%)	6.27	1.21	4241	2.18	100.4
Different column	6.55	1.08	4662	2.37	102.0
RSD (%)					0.91

* Mobile phase with acetonitrile–water (67:33, v/v), with pH adjusted with phosphoric acid. The column used was a Merck RP-18 (125 × 4.6 mm i.d., 5 μm particle size)
† Retention time ‡ Tailing factor
§ Theoretical plate number †† Retention factor
‡‡ Of label claim

Table I. Results of Analysis of Forced Degradation Study Using Proposed Method

Stress condition	(%) Degradation*	RSD (%) ($n = 3$)	(%) Degradation[†]	RSD (%)	Peak purity[‡]
Acid hydrolysis (1 M HCl, 72 h)	10.6	1.48	0.78	1.25	1.0000
Basic hydrolysis (1 M NaOH, 72 h)	0.2	0.97	N.D.[§]	–	1.0000
Oxidation (30% H_2O_2, 72 h)	0.4	1.05	1.23	1.32	1.0000
Heat (80°C, 72 h)	N.D.	–	N.D.	–	1.0000
Photolytic degradation (72 h)	48.2	1.60	22.2	1.75	0.9999

* Values of reference substance solution.
† Values of pharmaceutical gel form solution.
‡ Peak purity values in the range of 0.9999–1.0000 indicate a homogeneous peak.
§ ND = No degradation.

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