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Determination of tretinoin in creams by high-performance liquid chromatography

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ABSTRACT

A stability indicating reversed-phase high-performance liquid chromatographic method has been developed to quantify tretinoin (all-*trans*-retinoic acid) in cream formulations. Tretinoin cream samples were dissolved directly in tetrahydrofuran and diluted for injection. Separation was accomplished on a 15 cm Nova-Pak C_{18} column using a tetrahydrofuran–phosphate buffer solvent system (42:58, v/v) and 1.0 ml/min flow-rate. The method is able to separate tretinoin from its degradation products formed under stressing conditions. Excellent precision and accuracy were found for the assay of tretinoin in the cream formulations.

INTRODUCTION

Tretinoin (all-*trans*-retinoic acid) is a compound which is effective for the topical treatment of acne and other skin disorders [1,2]. Tretinoin is also known to be relatively unstable, with the possibility of several types of degradation products being formed [3,4]. Therefore, a stability-indicating high-performance liquid chromatographic (HPLC) method is desired to quantitate tretinoin in cream formulations. The present analytical method in the United States Pharmacopeia (USP) [5] calls for the assay of tretinoin in creams, gels and topical solutions by a ultraviolet spectrophotometry. A normal-phase HPLC method is described for isotretinoin in tretinoin drug substance [5]. Additional HPLC methods for tretinoin and its degradation products/impurities in products and biologiacal samples have been described in the literature [3,6–11]. Although these methods are able to separate tretinoin and its many degradation poducts, none of the methods appeared to be directly applicable to our goal; to develop a quick and simple stability indicating assay for tretinoin in dermatological products. This paper reports a stability-indicating reversed-phase HPLC method for tretinoin which is able to separate the tretinoin and internal standard peaks from the light induced isomers, as well as hydrolysis and oxidation products formed during forced degradation studies. This method has been successfully applied to cream formulations of tretinoin.

EXPERIMENTAL

Materials

Tretinoin and isotretinoin (13-*cis*-retinoic acid) were obtained from BASF (Parsippany, NJ, U.S.A.). The purest lot (compared *vs.* USP standard) of tretinoin raw material was used as a reference standard. Tretinoin creams (0.1%, w/w) and cream placebos used in this study were prepared by our Pharmaceutical Product Development group. HPLC-grade tetrahydrofuran (THF) with and without stabilizer [0.025% butylated hydroxytoluene (BHT)] was obtained from J. T. Baker (Phillipsburg, NJ, U.S.A.). Unless otherwise noted, THF without stabilizer was used. Sodium phosphate monobasic monohydrate, potassium persulfate, sodium hydroxide (Certified, ACS grade), hydrochloric acid (reagent grade) and 85% phosphoric acid (HPLC grade) were obtained from Fisher Scientific (Fair Lawn, NJ, U.S.A.). Anthracene (Gold Label, 99.9%) was obtained from Aldrich (Milwaukee, WI, U.S.A.). Water used was passed through a Millipore Milli-Q Water System (Millipore, Bedford, MA, U.S.A.). Samples and mobile phase were filtered through Millipore type HV filters, 0.45 μm.

Sample preparation precautions

All tretinoin sample and solution preparations were performed under gold lights [12]. Low actinic glassware was used for all preparations unless otherwise noted. Samples for injection were placed in amber vials.

HPLC instrumentation and conditions

Two liquid chromatographic systems were used for the method development: a Hewlett-Packard 1090 system with a 1040 photodiode array detector and Model 85B computer (Hewlett-Packard, Palo Alto, CA, U.S.A.) and a Waters HPLC system comprised of a 6000A solvent delivery system, Model 712 WISP auto injector and Model 441 absorbance detector fixed at 365 nm (all from Waters Assoc., Milford, MA, U.S.A.). The detector sensitivity was set at 0.05 a.u.f.s. for the raw material specificity study and 0.02 a.u.f.s. for the cream analysis study. Detector output was monitored with a Houston Instruments Model D5000 recorder (Houston Instruments, Austin, TX, U.S.A.) set at 10 mV. Data acquisition was accomplished with a Hewlett-Packard 3350 series computer utilizing LAS software (Hewlett-Packard). The computation and plotting of absorbance ratio plots from the photodiode array detector were performed by modified HP 1040A software described earlier [13].

A 150 × 3.9 mm Waters Nova-Pak C_{18} column (4 μm particle size) was used for method related separations in this study. A guard column (Nova-Pak C_{18} Cartridge, Waters Assoc.) was attached prior to the analytical column. Dilute phosphoric acid was prepared by diluting 10 ml of 85% phosphoric acid to 100 ml with water. The mobile phase was prepared by mixing 420 ml of prefiltered THF and 580 ml of prefiltered phosphate buffer. The phosphate buffer was prepared by dissolving 1.38 g of $NaH_2PO_4 \cdot H_2O$ in 1000 ml of water and adjusting to pH 3.0 with dilute phosphoric acid. A mobile phase flow-rate of 1.0 ml/min and injection volume of 25 μl were used for analysis.

Tretinoin degradation (specificity) studies

Tretinoin samples for demonstrating specificity were prepared in the following manner:

Oxidation studies. A 40-mg amount of tretinoin was transferred to a 100-ml volumetric flask along with 10 ml of THF and 10 ml of 0.025 *M* potassium persulfate. The sample was reacted for 12 h, after which it was diluted to volume with stabilized THF and filtered. An aliquot was prepared for analysis by pipetting 5 ml into another 100-ml volumetric flask and diluting with 40 ml of stabilized THF, 0.5 ml of dilute phosphoric acid and water to volume.

Light degradation studies. A 20-ml volume of a 0.40 mg/ml solution of tretinoin in THF was transferred to a 50-ml clear glass screw cap test tube. The sample was irradiated with light (approximately 1000 ft.-candles) for 10 min. A 5.0-ml aliquot of the degraded sample was prepared for analysis as described above (see section *Oxidation studies*).

Heat degradation studies. A 40-mg amount of tretinoin was transferred to a 100-ml volumetric flask and stored in a 50°C constant temperature oven for 47 weeks (the sample was purged with fresh air weekly). It was diluted to volume with THF and a 5.0-ml aliquot of the degraded sample was prepared for analysis as previously described.

Base degradation studies. A 40-mg amount of tretinoin was transferred to a 100-ml volumetric flask along with 20 ml of 0.025 *M* sodium hydroxide. The sample was allowed to react for 12 h after which it was neutralized with 2 ml of 0.25 *M* HCl and diluted to volume with THF. A 5.0-ml aliquot of the degraded sample was prepared for analysis as previously described.

Acid degradation studies. A 40-mg amount of tretinoin was transferred to a 100-ml volumetric flask along with 20 ml of 0.1 *M* HCl. The sample was stored for 47 weeks at room temperature after which it was diluted to volume with THF. A 5.0-ml aliquot of the degraded sample was prepared for analysis as previously described.

Tretinoin cream sample preparation

Internal standard solution. A 100-mg amount of anthracene reference standard was dissolved in 100 ml of THF.

Tretinoin standard solution (0.0040 mg/ml). A 100-mg amount of tretinoin reference standard was accurately weighed into a 250-ml volumetric flask and dissolved in and diluted to volume with stabilized THF. A first dilution was made by pipetting 5 ml of the above solution and 10.0 ml of internal standard solution into a 100-ml volumetric flask and diluting to volume with stabilized THF. A working standard solution was prepared by diluting 5 ml of this solution with 10 ml of stabilized THF, 0.1 ml of dilute phosphoric acid and water to 25.0 ml.

Cream sample preparation. An amount of cream containing 1.0 mg of tretinoin was accurately weighed into a 50-ml volumetric flask. A 5.0-ml aliquot of internal standard solution and 20 ml of stabilized THF were added. The flask was shaken to disperse the cream and the mixture was diluted to volume with stabilized THF. A 5.0-ml volume of this solution was further diluted to 25 ml with 10 ml of stabilized THF, 0.1 ml of dilute phosphoric acid and water to volume. The sample was filtered through a 0.45-μm disposable filter before analysis.

RESULTS AND DISCUSSION

A cream placebo analyzed by the above method exhibited no interfering peaks;

TABLE I

RELATIVE RETENTION TIMES OF MAJOR DEGRADATION PEAKS COMPARED TO TRETINOIN

Relative retention time		
Light	Oxidation	Base
–	0.129	–
–	0.179	0.178
–	0.247	0.246
–	0.318	0.316
–	0.452	–
–	–	0.483
0.785	–	–
0.803	–	–
0.864	–	–
0.946	–	–

therefore removal of the components of the cream formulation was not necessary and the sample could be directly dissolved and diluted for analysis.

The stability-indicating nature of this method was demonstrated by chromatographing the artificially degraded tretinoin solutions previously described and collecting data at multiple wavelengths using photodiode array detection. Table I gives the retention times of the observed degradation products relative to tretinoin. Several peaks were found in each assay (except for the heat-degraded tretinoin sample), with products from light degradation studies eluting generally later than those from

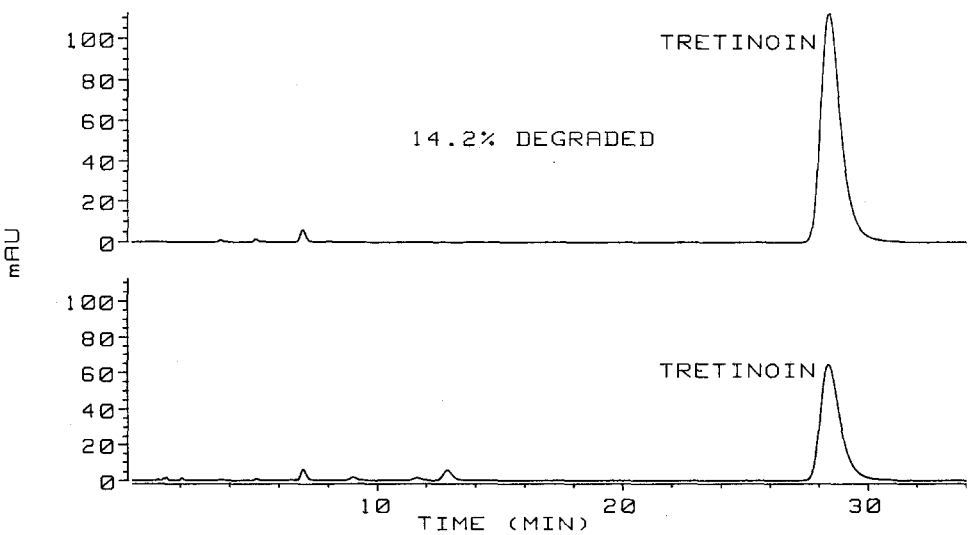

Fig. 1. Chromatograms of tretinoin solution oxidized by potassium persulfate. Injection volume, 25 μl (0.5 μg). Detection wavelengths, (top) 365 nm and (bottom) 320 nm.

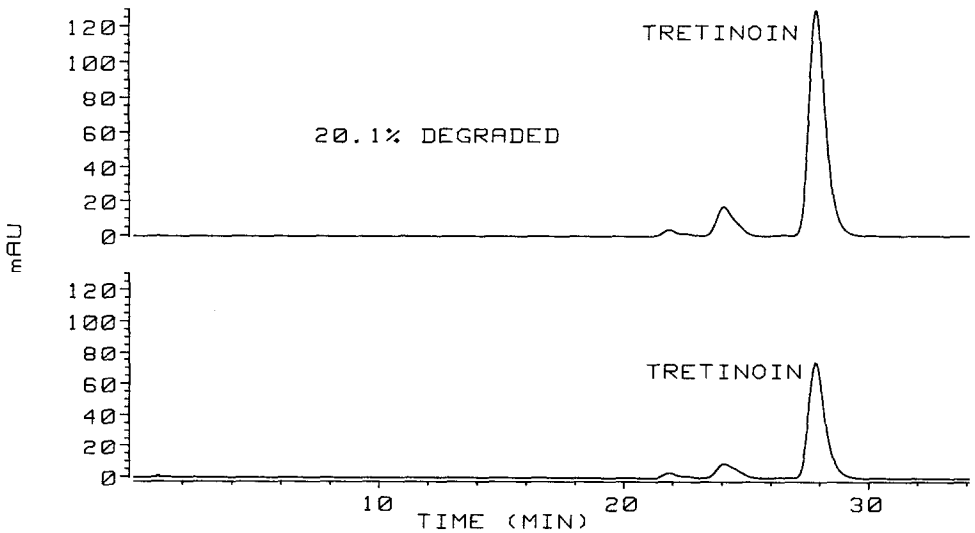

Fig. 2. Chromatograms of light-degraded tretinoin solution. Injection volume, 25 μl (0.5 μg). Detection wavelengths, (top) 365 nm and (bottom) 320 nm.

oxidation and base degradation studies. Although the heat-degraded sample exhibited a lower assay value (27.4% degraded) when compared to a standard, no additional peaks were displayed in the chromatograms at the analytical wavelength. Samples stored in acid showed no degradation after 47 weeks. Sample chromatograms of an oxidized and a light degraded sample are shown in Figs. 1 and 2.

Absorbance ratio plots were computed to determine the absence (or presence) of co-eluting impurities with the tretinoin peak by ratioing the absorbance values of the analytical wavelength against several other wavelengths. A typical absorbance ratio plot of a light degraded sample prepared by comparing 365 nm *vs.* 320 nm is shown in Fig. 3. The linearity of this plot (along with others at different wavelengths, data not shown) for this sample and also of others degraded by oxidation and by base confirms that the method described in this paper is stability-indicating.

Optimization of chromatography: effect of mobile phase concentration

The effects of changes in the mobile phase THF–phosphate buffer concentration ratio on the separation of tretinoin and isotretinoin is shown in Table II (isotretinoin was used because it is an easily formed degradation product of tretinoin). The values shown in Table II were obtained by separating a mixture of 0.02 mg/ml of tretinoin and 0.002 mg/ml isotretinoin over a range of 41–48% THF. For the actual assay method, 42% THF was chosen because it gave the best separation of all isomers/degradation products from tretinoin. Whereas this percentage provides good separation, it also presents a rather long run time of 35 min. This time was found to be necessary to separate a minor light isomerization product which is found directly preceeding the tretinoin peak in Fig. 2. If this product is not present, a substantial reduction in assay time may be accomplished by increasing the THF concentration (*e.g.*, to 46%) without

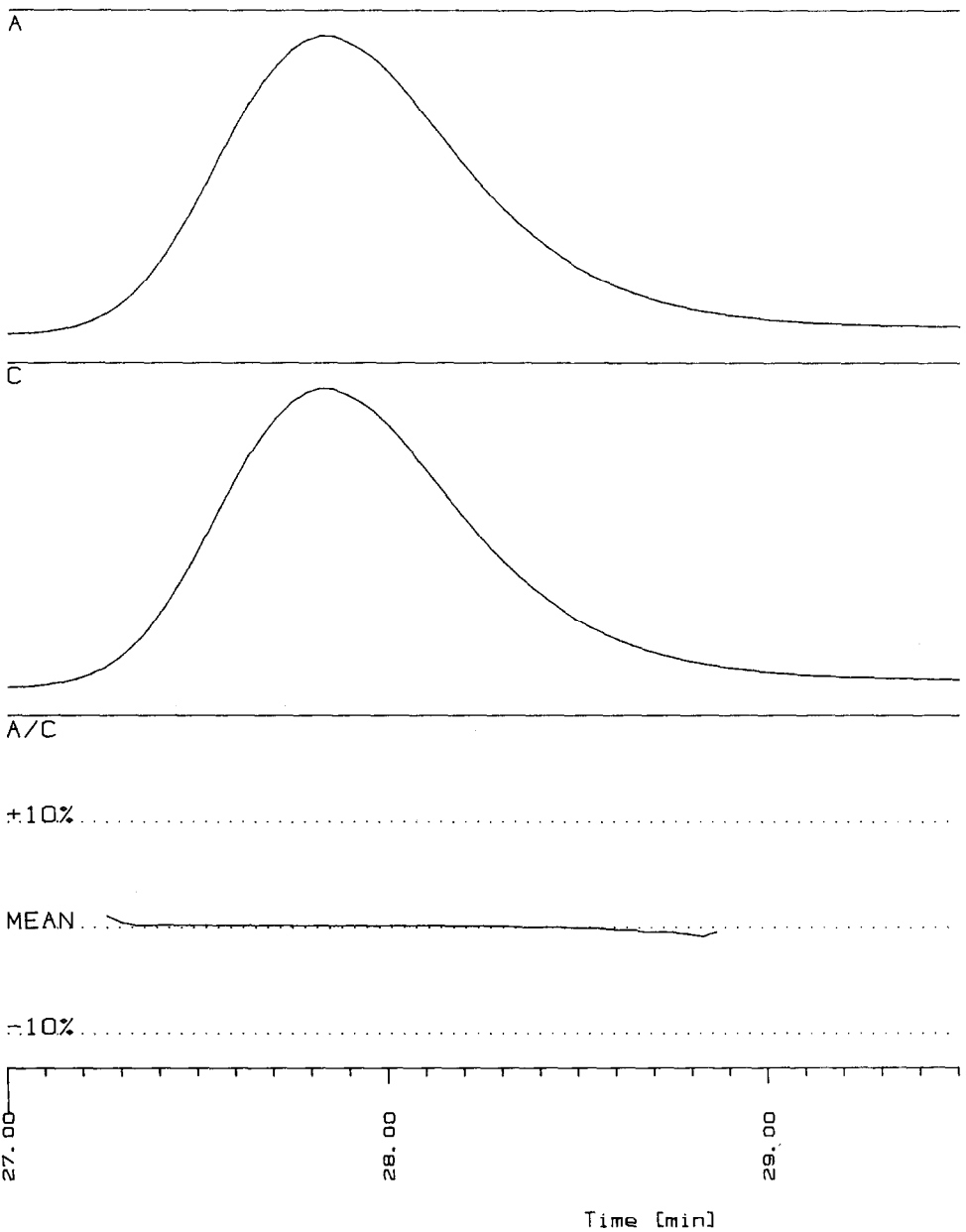

Fig. 3. Absorbance ratio plot of a solution of light-degraded tretinoin sample. Detection wavelengths, 365 nm (A) and 320 nm (C).

sacrificing the separation of tretinoin from all other degradation products as it was found that baseline separation between tretinoin and isotretinoin was achieved even at tretinoin retention times of less than 10 min (see Table II). In typical chromatograms of

TABLE II

EFFECT OF MOBILE PHASE CONCENTRATION ON THE SEPARATION OF ISOTRETINOIN AND TRETINOIN

THF (%)	Retention time (min)		Relative retention time[a]	Chromatographic resolution
	Tretinoin	Isotretinoin		
41	31.5	27.2	0.86	3.1
42	26.1	22.7	0.87	2.9
43	21.5	18.8	0.87	2.4
44	18.0	15.9	0.88	2.5
45	15.2	13.6	0.89	2.4
46	12.9	11.6	0.90	2.3
47	11.0	10.0	0.91	2.3
48	9.5	8.7	0.92	1.9

[a] Retention time of isotretinoin/retention time of tretinoin.

degraded cream sample (storage at 40°C for 16 weeks), the only degradation product found was isotretinoin (Fig. 4). It should also be noted that whereas previous papers described improved separation of light-induced isomers [4,11], the goal of this study was to develop a stability-indicating HPLC assay which would separate any isomers/degradation products formed from tretinoin and internal standard (anthracene). Anthracene was chosen as the internal standard due to its relative inexpensiveness, lack of interference and stability (as opposed to using a retinoid for an internal standard).

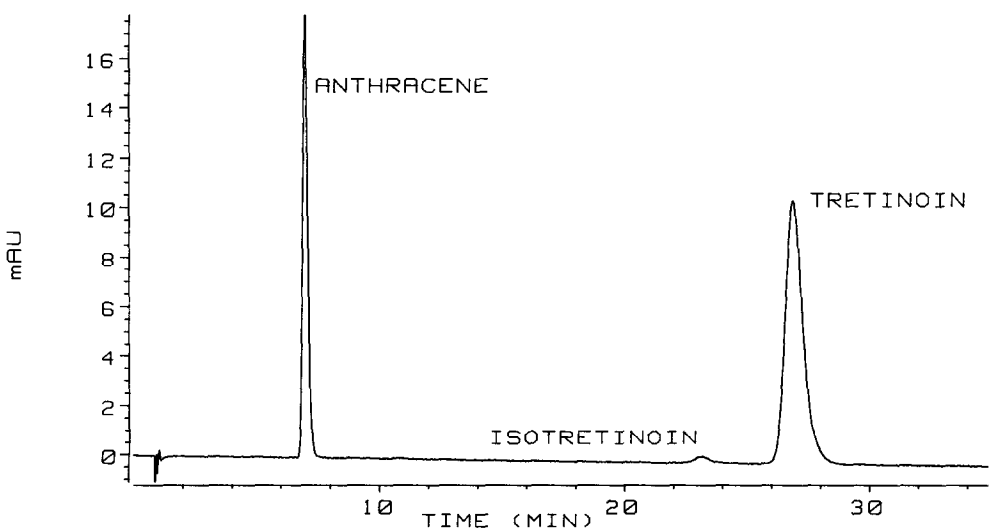

Fig. 4. Chromatogram of a tretinoin cream product stored at 40°C for 16 weeks containing 2.7% isotretinoin. Injection, 25 μl of cream sample preparation (0.106 μg of tretinoin). Detection at 365 nm.

Method validation for creams

The method for determining tretinoin in cream formulations was validated by determining three separate parameters; response linearity, accuracy and precision. Response linearity for tretinoin was determined by analyzing nine standard solutions covering the range of 10 to 300% (0.00040 to 0.012 mg/ml) of the normal sample concentration while keeping internal standard concentration constant. Linear regression analysis of the peak area ratio data gave a correlation coefficient (r) of 0.99997 and a y-intercept of 0.0060 (equivalent to 0.43% of the normal response).

The accuracy of the method was determined by doing standard recovery experiments. These experiments were carried out by spiking cream placebos with tretinoin solutions to simulate samples with tretinoin levels over the range of 50 to 200% of the normal value. The results of this study demonstrate excellent recovery with a mean value of 100.1% and a relative standard deviation of 0.9%. Also, the change in tretinoin level in sample showed no noticeable effect on the % recovery values.

The precision of the method was determined by assaying 0.1% (w/w) tretinoin cream using sample sizes over the range of 50 to 150% of the normal sample preparation weight (1.0 g). The results from these experiments give a mean value of 0.105% tretinoin (w/w) and a relative standard deviation of 1.0%. No effect of change in sample weight for analysis was observed.

CONCLUSIONS

A stability-indicating HPLC method for tretinoin in cream samples is described which is able to separate tretinoin from degradation products formed in both forced degradation studies of raw material and cream samples. The method showed excellent linearity, precision and accuracy for the assay of tretinoin in a cream product. This assay offers a prominent advantage over the current compendial method [5] in that it is able to quantitate both tretinoin and degradation products formed in the cream degradation studies and is thus stability-indicating.

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