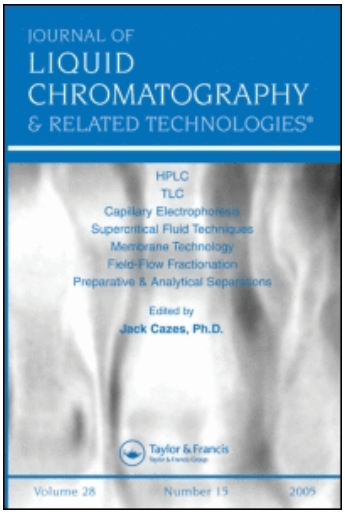

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High Performance Liquid Chromatographic Procedure for Retinoic Acid in Ophthalmic Solution

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC PROCEDURE FOR RETINOIC ACID IN OPHTHALMIC SOLUTION[1]

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ABSTRACT

A fast and simple procedure has been developed for the quantitation of retinoic acid in ophthalmic solutions. The procedure involves the use of reverse phase high performance liquid chromatography with a mobile phase containing an amine modifier. This procedure is specific for retinoic acid in the presence of its degradation products and formulation excipients. The method is linear over a wide range of concentrations.

INTRODUCTION

trans-Retinoic acid (3,7-dimethyl-9-(2,6,6-trimethyl-1-cyclohexene-1-yl)-2,4,6,8-nonatetraenoic acid) is required for normal physiological maintenance of growth and function of the corneal epithelium of the eye. The deficiency of this vitamin results in keratinization of the corneal epithelium and may lead to ulceration[2]. Recently, Tseng and Sommer, et al., demonstrated the efficacy of retinoic acid ointment for the treatment of various dry-eye disorders[3,4]. However, ointment

dosage forms interfere with visual activity, and may cause blurred vision. To overcome this problem, we solubilized trans-retinoic acid in aqueous solution in the presence of Tween-80 and analyzed the active ingredient. In this paper we describe the high pressure liquid chromatographic (HPLC) assay method.

Various HPLC (normal phase and reversed phase) and gas chromatographic methods are published in the literature for the determination of trans-retinoic acid[5-6], retinal mixtures[7], and vitamin A.[8] In some of these cases, the trans-retinoic acid was not baseline separated from its degradation products and impurities. In other cases, even though complicated gradient systems were used, trans-retinoic acid was not well separated from 13-cis-retinoic acid and other minor impurities. In the case of retinal, normal phase chromatography was used and in the case of vitamin A, derivatization followed by a gas chromatographic method was used. The procedure described in this paper is a simple isocratic method which is stability indicating and gives baseline separations of the minor impurities from the trans-retinoic acid.

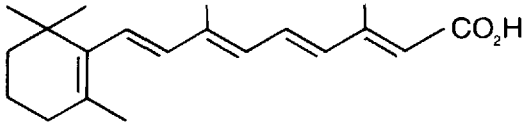
MATERIALS AND METHODS

Reagents and Chemicals

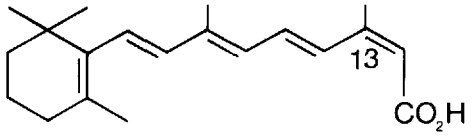
trans-Retinoic acid (1) and 13-cis-retinoic acid (2) (Figure 1) were purchased from Sigma Chemical Co. (P.O. Box 14508, St. Louis, MO 63178, USA). Acetonitrile was HPLC grade. Other reagents, such as boric acid, sodium borate, Tween-80, polyethylene glycol-300. etc., were reagent grade.

Equipment and Mobile Phase

Separations were carried out on a 15 cm x 0.46 cm Utrasphere-ODS column (Rainin Instruments Inc., Mack Road,



trans-retinoic acid (1)



13-cis-retinoic acid (2)

Figure 1: Structures of trans and 13-cis-retinoic acids.

Woburn, MA 01801, USA), particle size 5 um. The analytical column was protected with a guard column packed with pellicular 30-40μm ODS material. Waters' HPLC System equipped with model 712 automatic injector, model 590 multiple wavelength detector, and 840 data system was used for analysis. The mobile phase consisted of 70% acetonitrile and 30% water containing 5% (V/V) acetic acid and 0.02% (V/V) triethylamine as a modifier. The flow rate was generally 2 mL/min, sometimes being adjusted according to baseline resolution of the main peak from the interfering peaks. The detector was set at 254 nm (even though the λ max was 350 nm), so that the formulated solution could be injected without dilutions.

Preparation of Sample

A sample solution was prepared by mixing Tween-80 (5 g), polyethylene glycol-300 (5%), trans-retinoic acid (0.1 g), boric acid (0.3 g) and sodium borate (32 mg) and brought to

90 g with double distilled deionized water. The pH of the solution was adjusted to approximately 7 with 2.5 N sodium hydroxide solution, then the solution was brought to 100 g with double distilled deionized water.

Preparation of Standard

Stock standard solution was prepared by dissolving 130 mg of trans-retinoic acid in methanol. This solution was further diluted to give the desired concentration range of the samples. Standards were prepared fresh and protected from light.

Degradation Procedure

The trans-retinoic acid formulation was stored under the following conditions:

A. 10 mL solutions were stored in white plastic bottles (Arco K123 polypropylene 3% TiO_2) clear glass and amber glass bottles (USP Type 1).
B. The bottles were stored at 5°, 23°, and 40°C.
C. Some of the bottles were subjected to fluorescent light.

All of the solutions were analyzed after two weeks of storage under the above conditions.

Assay Method

Ten uL of the standard or sample was injected three times to determine the relative standard deviation. Then each standard and sample was injected twice under the chromatographic conditions.

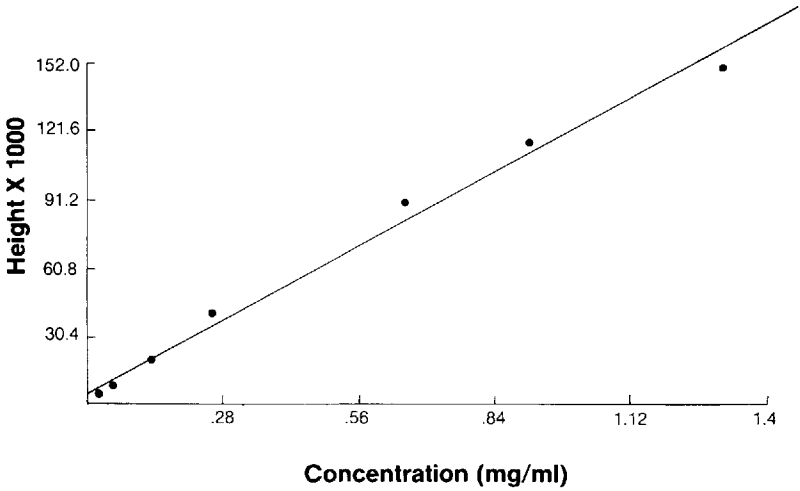


Figure 2: Calibration curve of trans-retinoic acid standard concentrations vs. peak heights.

Calculations and Limits of Detection

A calibration curve was drawn by plotting standard concentrations of trans-retinoic acid vs. peak heights (Figure 2). Correlation coefficients were found to be better than 0.99, the limit of detection under our conditions was found to be as low as 0.008 mg/mL. The procedure is linear from 0.008 mg/mL to 1.3 mg/mL. It should be noted, however, that one could use a higher wavelength, e.g., 350 nm in cases where one wants to detect lower amounts than stated here. Relative standard deviation of a triplicate injection of a standard sample was calculated to be less than 1%. Unknown concentrations were calculated from the calibration curve.

RESULTS AND DISCUSSION

Initially we attempted the separation of trans-retinoic acid from the impurities and degradation products using

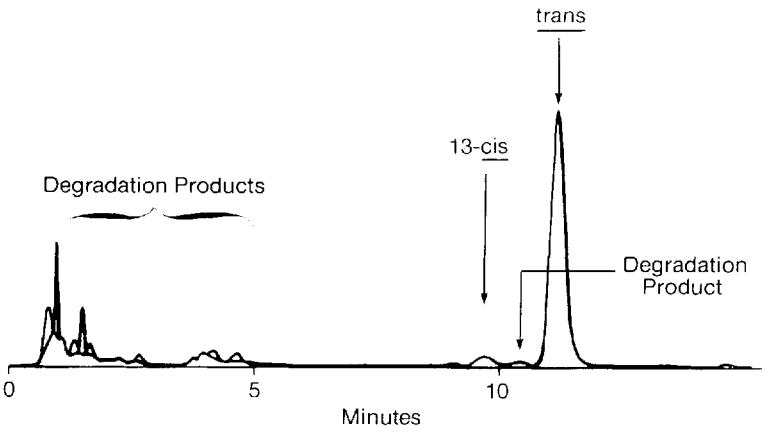


Figure 3: A typical chromatogram of a degraded sample of trans-retinoic acid.

acetonitrile and various concentrations of acetic acid. However, the peak shape was poor and retinoic acid could not be separated from the impurities, e.g., 13-cis-retinoic acid, and the other minor impurities. As shown in Figure 3, the addition of triethylamine (0.02%) as an amine modifier resulted in a baseline separation of the impurities and the peak shape was improved considerably. The result is an improvement over the literature procedures where 13-cis-retinoic acid and other minor impurities were not separated from the desired analyte, trans-retinoic acid[5-6].

Degradation Studies

The degradation products were examined using our present HPLC conditions to ensure that none would interfere with intact trans-retinoic acid. The results indicate that the degradation products and 13-cis-retinoic acid do not interfere with the trans-retinoic acid (Figure 3). The stability results are shown in Table 1 and summarized as follows: a) stability of

TABLE 1

Two-Week Stability of trans-Retinoic Acid Under Different Conditions

Temperature	Concentration (mg/mL)[a]		
	White Plastic Bottle	Clear Glass Bottle	Amber Glass Bottle
5°C	0.38	-	-
23°C	0.21	0.21	0.32
40°C	0.07	0.06	0.17
Fluorescent Light	0.21	0.13	0.29

[a] Initial value 0.69 mg/mL.

trans-retinoic acid is increased with decreasing temperature, b) stability also increases with absence of light (glass bottle vs. amber bottle), and c) there is no apparent advantage of storing the formulation in a white plastic bottle vs. a clear glass bottle (Table 1).

CONCLUSIONS

We have demonstrated that the present procedure is a simple, stability indicating method. This method, unlike other published procedures, does separate 13-cis-retonic acid, a contaminant of trans-retonic acid, and other minor degradation products, specifically the one that elutes in between 13-cis-retinoic acid and trans-retonic acid. This method is also linear over a wide range of concentrations. Additionally, we have found that the trans-retonic acid was found to be unstable in the ocular formulations under all conditions studied.

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