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Rapid Communication

HPLC determination of the stability of retinoic acid in gel formulation

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Summary

A new formulation of retinoic acid gel was prepared for the topical therapy of Kaposi's sarcoma. The chemical stability of retinoic acid was studied for 6 months at 4°C. A method was developed for its rapid and sensitive separation and quantitation using high-performance liquid chromatography (HPLC). This preparation was shown to be stable for 6 months used refrigerated storage conditions.

Tretinoin (all-*trans*-retinoic acid; vitamin A acid) has been applied for local treatment of several skin diseases and aged skin (Boyd, 1989). It can control and prevent cutaneous malignancy but has been shown to be a skin irritant and the therapeutic concentration is within the varying range of side effects concentration (Orfanos et al., 1987). We have formulated vitamin A acid gel in a new vehicle, i.e. oil of sweet almond, to improve its therapeutic tolerance, while increasing dosage. The purpose of the present study was to determine the chemical stability of retinoic acid gel 1% new formulation during a period of 6 months. A clinical study was carried out for the topical effect of this retinoid on Kaposi's sarcoma lesions.

The formulation of retinoic acid gel 1% used the following components: retinoic acid (Bonnassis-Paris, France) 1 g; oil of sweet almond (Carilene-Montesson, France) 93 g; Cab-O-Sil (Cabot-Neuilly sur Seine, France) 6 g. The retinoic acid and oil of sweet almond were mixed with Cab-O-Sil. The gel was aseptically packed.

The assay of retinoic acid was based on HPLC. We propose a simple and rapid technique for a specific and sensitive measurement of retinoic acid in gel formulation.

The operating conditions were as follows. Column: Technopack C18, 10 μm particle size, 300 × 3.9 mm i.d. (Wellington house, Macclesfield, U.K.); Injector: Spectroflow 480 Kratos (20 μl); Detector: Spectroflow 757 Kratos; Recorder: Enica 21 Delsi; Pump: Spectroflow 400 Kratos.

The wavelength was set at 343 nm. An amount of 400 ml of mobile phase was prepared fresh daily by mixing 380 ml ethanol and 20 ml water. The mobile phase was degassed by an ultrasonic cleaner (Branson Chelton, U.S.A.) for 10 min before beginning and pumped out at a constant flow rate of 0.5 ml/mn

Calibration curve: A calibration curve was con-

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structed daily by appropriate dilution of a solution of retinoic acid gel 1%, 1 g in 100 ml cyclohexane (Fluka Chemika). The concentration range was 100–16.6 μg/ml. Calibration curves were generated by making triplicate 20 μl injections of the standard concentration. Average peak area was plotted vs concentration.

Stability studies: Retinoic acid gel was stored in a refrigerator at 4° C. Triplicate samples of gel were removed for analysis after intervals of 1, 3 and 6 months storage. One sample was taken for analysis immediately after preparation to give the initial ($t = 0$) assay value. Further samples were subjected to sterility tests immediately after preparation and 1, 3 and 6 months storage. pH was measured in the preparation (50 ml) of each with a pH meter (pHm 82, standard pH meter, Radiometer Copenhagen, sensitivity 0.01 pH unit), calibrated before each measurement with known standard solutions of pH 4 and 7. The initial pH was tested as well as after 6 months storage.

Validation of HPLC assay: The relationship between peak area and retinoic acid concentration over the concentration was linear and the equation of the least squares regression line was

$$y = 137640x - 6691$$

$$r^2 = 0.9989 \ (n = 10)$$

Our HPLC method showed good day to day reproducibility. The results of the analysis (10 replicate analysis of each on 2 consecutive days) are shown in Table 1.

Stability studies: There was no loss of retinoic acid from the gel preparation stored at 4° C over 6 months (Table 2). Sterility tests carried out immediately after preparation and again after 6 months refrigerated storage revealed no microbial contamination. The pH values of retinoic acid gel had not changed. In conclusion, it can be stated that the formulation of retinoic acid 1% gel in oil of sweet almond and Cab-O-Sil can be stored for at least 6 months without degradation at 4° C.

Clinical study: We demonstrated clinical responses in patients' skin lesions treated topically with 1% tretinoin gel for three months. Histological studies of skin lesions from the patients showed redifferentiating cells after treatment. These data suggest that retinoic acid might play a role in the treatment of this disease (Bonhomme et al., 1990)

TABLE 1

Reproducibility of retinoic acid determination

Mean retinoic acid (μg/ml)	Standard deviation (μg/ml)	Standard deviation (%)
102	0.02	2.0
55	0.01	1.8

TABLE 2

Assay results at 4° C

Storage time (months)	Retinoic acid concentration (μg/ml)	Retinoic acid as % of initial concentration
0	102	100
1	99	97
3	105	103
6	102	100
pH value		
Initial	4.7	
6 months	4.65	

The average of three experiments is shown.

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