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Note

Stability-indicating reversed-phase high-performance liquid chromatographic assay for fenretinide in soft gelatin capsules and concentrated corn oil suspensions

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Fenretinide, an analogue of vitamin A, has been undergoing clinical trials for the treatment of various skin disorders. The use of oils to enhance bioavailability of pharmaceuticals has been reported[1,2]. A delivery system employing a suspension of fenretinide in corn oil within a soft gelatin capsule has been developed to enhance the bioavailability of fenretinide in animals over a dry powder preparation; this was subsequently used in human clinical trials.

The high-performance liquid chromatographic (HPLC) evaluation of the bulk drug substance has been previously reported[3]. In the present paper we describe the procedure for the handling and evaluation of 25-, 100- and 175-mg soft gelatin capsules and 25% suspension concentrates of fenretinide in a corn oil matrix.

EXPERIMENTAL

Materials, equipment and liquid chromatographic conditions

A DuPont 850 high-performance liquid chromatograph equipped with a DuPont automatic sampler with a 20-μl loop and a fixed-wavelength 254 nm detector and Model 4100 integrator were used. The HPLC column was 25 cm × 4.6 mm I.D., packed with Zorbax® ODS, 5 μm particle size, and the mobile phase was acetonitrile–acidified water, pH 3.0 (90:10). The water was adjusted to pH 3.0 with analytical reagent grade acetic acid, and the acetonitrile was HPLC grade. The solvent and column temperatures were ambient and the flow-rate was 2.0 ml/min.

Solutions, standard and sample preparation

Mobile phase. A volume of 100 ml of acidified water (pH 3.0) was added to 900 ml of acetonitrile and the mixture was thoroughly degassed.

Sample solvent. A volume of 650 ml of acetonitrile was added to 350 ml of isopropanol and the mixture was thoroughly mixed.

Standard. About 24 mg of a fenretinide reference standard were accurately weighed into a 100-ml light-resistant volumetric flask and diluted to volume with sample solvent.

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Spiked standard. About 30 mg of a fenretinide standard and 0.3 mg of 13-*cis*-fenretinide were weighed into the same 100-ml light-resistant volumetric flask and diluted to volume with sample solvent.

Diluted spiked standard. A 50.0-ml volume of the spiked standard solution was diluted to 100 ml with sample solvent.

Capsule samples

With a scalpel or sharp scissors, ten capsules were cut and placed into a beaker wrapped with foil to minimize exposure to light. The shells and cutting tool were rinsed several times, and the washings were quantitatively transferred to a light-resistant volumetric flask. An aliquot of this solution was pipetted into a second volumetric flask and diluted to volume so that the final concentration was about 0.24 mg/ml.

Suspension concentrate sample

An amount of suspension equivalent to 250 mg of fenretinide was accurately transferred to a light-resistant 250-ml volumetric flask and diluted to volume. An aliquot of this solution was pipetted into a second volumetric flask and diluted to volume so that the final concentration was about 0.24 mg/ml.

Assay procedure

The instrument was assembled and set as previously described, and the column equilibrated for at least 20 min with the mobile phase flowing. The system suitability was determined using resolution, injection precision and detector linearity.

Acceptable results for the resolution, precision and detector linearity criteria were required before samples were analyzed. The standard solutions were interspersed with the samples if a large number of analyses were to be performed.

The percent label for fenretinide or weight percent impurity in the capsules was determined using the areas of the sample and standard, the weight of the standard and the theoretical concentration of the sample solution.

The percent label fenretinide or weight percent impurity in a suspension concentrate was determined using the areas of the sample and standard, the theoretical and actual amount of sample, and the actual and theoretical amount of standard.

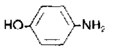
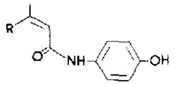
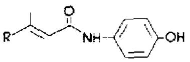
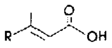
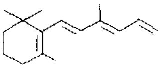
RESULTS AND DISCUSSION

The described HPLC assay procedure was used to assay for fenretinide in soft gelatin capsules and suspension concentrates. Table I presents structures, retention times, sensitivity factors, detection limits, and capacity factors for fenretinide and its potential decomposition products.

Excessive heat, thereby forcing degradation, was used to demonstrate the stability-indicating properties of the method. Fig. 1 shows the HPLC chromatograms of fenretinide soft gelatin capsules stored at ambient temperature and stressed at 100°C overnight. Degradation was indicated in the stressed sample by a decrease in the fenretinide assay value to approximately 80% and increased levels of impurities. Fenretinide in solution is also sensitive to light. Because of this sensitivity the use of light-resistant containers for sample preparation, as well as for sample vials and

TABLE I

STRUCTURES, RETENTION TIMES AND SENSITIVITY FACTORS OF SOME POTENTIAL DEGRADATION PRODUCTS OF FENRETINIDE

Compound	*Structure*	*Retention time (min)*	*Capacity factor*	*Sensitivity factor*	*Detection limit (%)*
4-Aminophenol		1.5	0.47	1.1	0.16
13-*cis*-Fenretinide		4.5	3.5	1.7	0.16
Fenretinide		4.9	3.9	1.0	–
Retinoic acid		7.3	6.3	0.84	0.34
	R = 				

transfer lines, is required. The sensitivity of fenretinide drug substance to light and heat showed a similar pattern of decomposition and decreased assay values as seen in heat-stressed capsule samples. Retinoids in general are sensitive to light and heat; soft gelatin capsules have been used for many years to package and protect such light-sensitive pharmaceuticals.

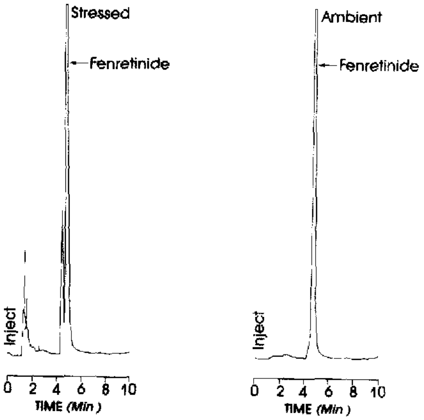


Fig. 1. HPLC chromatograms of a fenretinide soft elastic gelatin capsule stressed at 100°C overnight and a fenretinide soft elastic gelatin capsule stored at ambient temperature.

The response of fenretinide is linear from the origin to 22 μg. The amount of fenretinide chromatographed in the assay (4.8 μg) is well within the linear range. The potential impurities retinoic acid, *p*-aminophenol and 13-*cis*-fenretinide also showed linear responses over the range of interest. The use of 254 nm as a detection wavelength extends the linear response range of fenretinide, thereby allowing large samples to be taken and eliminating the need for large volumes of solvent or several dilutions. At a detector wavelength of 365 nm, the wavelength of maximum absorptivity, the fenretinide response is linear from the origin to only 4.5 μg.

The precision of the HPLC assay was determined and calculated to be 0.3% relative standard deviation for fenretinide. The recovery of fenretinide from the vehicle matrix was determined using a placebo spiked with approximately the labeled amount of fenretinide. Each sample was carried through the assay procedure. The average recovery was 101.2%.

The proportions of organic solvents in the sample solvent were chosen to assure complete and reproducible solubilization of the capsule contents, while minimizing baseline perturbation at the column void volume. Significant baseline shifts were considered to be deleterious to the integration of the potential decomposition product *p*-aminophenol.

This assay procedure has been successfully employed for over two years in studies of the stability of fenretinide suspensions contained in stainless-steel drums, polyethylene bags, or in soft gelatin capsules.

The use of oil vehicles in soft gelatin capsules to enhance the bioavailability of lipophilic drugs has been recognized. This results in lower administered doses necessary to achieve the desired clinical response. The procedures and techniques to handle and assay successfully such a product formulated in an oil matrix have been demonstrated.

REFERENCES

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