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Note

Stability-indicating reversed-phase high-performance liquid chromatographic assay for fenretinide drug substance

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Vitamin A and its retinoid analogues are necessary for the growth and differentiation of epithelial tissues. As such these compounds have manifested biological activity in the treatment of various skin disorders, including cystic acne[1,2], keratinization disorders such as Darier's disease[3], psoriasis[4] and ichthyosis[5], and basal cell carcinoma[6]. Further, retinoids demonstrate in rodents prophylactic effects on a variety of epithelial tumors.

Fenretinide, N-(4-hydroxyphenyl)retinamide, is a synthetic retinoid which has demonstrated activity in prevention of breast cancer in the rat while displaying no evident liver toxicity[7]. This compound is currently undergoing clinical trials in the U.S.A.

A stability-indicating, specific assay for fenretinide was necessary for evaluation of the drug substance used for preclinical and clinical supplies. Other workers who have analyzed natural or synthetic retinoids from biological fluids have used detector wavelengths near the maximum absorbance of the drug[8,9]. These procedures are necessary for metabolism studies, but do not exhibit a sufficient linear range or accuracy for use with bulk drug substance unless large volume dilutions are employed. The present paper describes a high-performance liquid chromatographic (HPLC) method for the evaluation of fenretinide, and presents data documenting the linearity, specificity, precision, sensitivity and stability-indicating quality of the method.

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 (Waters Assoc.) and Model 4100 integrator was used. The HPLC column was a Zorbax® ODS 5-μm particle size 25 cm × 4.6 mm I.D., and the mobile phase was acetonitrile–acidified water (pH 2.0) (75:25). The water was adjusted to pH 2.0 using analytical-reagent-grade acetic acid. The acetonitrile was HPLC grade. The solvent and column temperatures were ambient and the flow-rate was held at 2.0 ml/min.

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Solutions, standard and sample preparation

Acidified water. HPLC-grade water was adjusted to pH 2.0 with acetic acid by monitoring with a pH meter.

Sample solvent. A volume of 750 ml of acetonitrile was added to 250 ml of water.

Mobile phase. A volume of 750 ml of acetonitrile was added to 250 ml of acidified water and thoroughly degassed by vacuum. Helium sparging may be substituted for degassing.

Sample or standard. About 25 mg of sample or standard were accurately weighed into a separate 60-ml amber bottle and dissolved in exactly 50.0 ml of sample solvent.

Spiked standard. About 25 mg of a fenretinide standard and 0.3 mg of 13-*cis*-fenretinide were weighed into the same 60-ml amber bottle and dissolved in 50 ml of sample solvent.

Assay procedure

The instrument was set as previously indicated and the column equilibrated for at least 15 min with the mobile phase flowing. An injection of the sample solvent was made to obtain a blank chromatogram. The system suitability was determined by changing the recorder sensitivity to about 0.128 a.u.f.s. and the chart speed to about 1.0 cm/min and injecting 20 μl of the spiked standard solution. The resolution obtained between the fenretinide and 13-*cis*-fenretinide should be at least 1.4 by using the standard resolution equation as found in the United States Pharmacopoeia.

The precision of the system was determined using the relative standard deviation of response factors (area/μg) for the injections of the standard solution. Typically the percent relative standard deviation was less than 2.0%.

Calculations

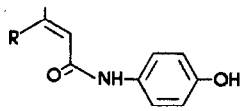
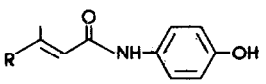
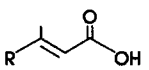
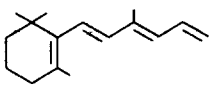
Integrated sample area counts were normalized for the amount of compound injected (area/μg sample), divided by normalized standard area counts (area/μg standard) and multiplied by the percent purity of the standard to determine the percent assay. Sample and standard weights were determined on a dried basis. Impurities were calculated in a similar fashion, with a sensitivity factor (F) added; this factor, which is specific for any individual impurity, accounts for the difference in sensitivity between fenretinide and the individual compound under analytical conditions (see Table I for specific F values). Unknown impurities were assigned a sensitivity factor of 1.0.

RESULTS AND DISCUSSION

The HPLC assay procedure described was used to quantitate fenretinide, retinoic acid, 13-*cis*-fenretinide and any unidentified impurities. Fenretinide was quantitated *versus* a standard of known purity and a weight percent on a dried basis was calculated. If present, retinoic acid and 13-*cis*-fenretinide impurities can be quantitated by converting their area percents to weight percents by the use of sensitivity factors. These sensitivity factors (F) were obtained experimentally by chromatographing known concentrations of authentic samples of these compounds and di-

TABLE I

STRUCTURES, RETENTION TIMES, SENSITIVITY FACTORS AND DETECTION LIMITS OF POTENTIAL IMPURITIES OF FENRETINIDE

Identity	*Structure*	*Approx. retention time (min)*	*Sensitivity factor (F)*	*Detection limit (μg)**
13-*cis*-Fenretinide		10	1.7	0.005
Fenretinide		11	1.0	–
Retinoic acid		14	0.84	0.01
R =				

* 10 μg of fenretinide is normally injected.

viding their response factors (peak area/μg injected) by the fenretinide response factor. In the case of unidentified impurities, a sensitivity factor of 1.0 was used.

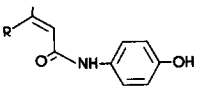
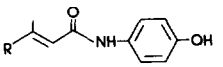
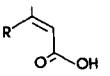
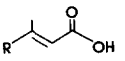
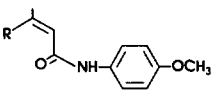
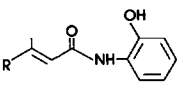
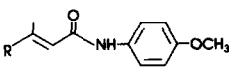
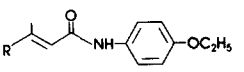
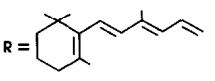
The structures, retention times, sensitivity factors and detection limits of the identified potential impurities as well as fenretinide are given in Table I demonstrating the method to be specific and sensitive. Table II lists structures and retention times of fenretinide and structurally similar retinoids.

The linearity of the method is shown in Fig. 1 demonstrating the fenretinide response to be linear and passing through the origin. The amount of fenretinide normally injected into the HPLC column (10 μg) for the assay is well within the linear range at 254 nm. At a detector wavelength of 365 nm the fenretinide linear range extends only to approximately 4 μg.

The stability-indicating capability of the HPLC method is demonstrated in Fig. 2 which shows the HPLC chromatogram of a fenretinide sample stressed with excessive heat. Degradation due to prolonged exposure at elevated temperature (*i.e.*, 60°C for one month) is indicated by a decrease in the fenretinide assay value and increased levels of impurities. The fenretinide assay value for this stressed sample was 98%. Further exposure to excessive heat (80°C for one month) leads to substantial increases in the early eluting impurities as seen in Fig. 3 which shows a stressed sample of fenretinide with an assay value of 84%. Retinoids in general are sensitive to light and will begin decomposition upon exposure. The rate and degree of decomposition varies with the particular compound. Fenretinide in solution is susceptible to decomposition if exposed to light. The chromatograms of these solutions show a

TABLE II

STRUCTURES AND RETENTION TIMES OF FENRETINIDE AND STRUCTURALLY SIMILAR RETINOIDS

Identity	*Structure*	*Retention time (min)*
13-*cis*-Fenretinide		9.5
Fenretinide		10.3
13-*cis*-Retinoic acid		11.1
Retinoic acid		13.7
N-(4-Methoxyphenyl)-13-*cis*-retinamide		20.2
N-(2-Hydroxyphenyl)retinamide		20.4
N-(4-Methoxyphenyl)retinamide		21.5
N-(4-Ethoxyphenyl)retinamide		27.3
		

similar pattern of degradation and decreased assay values as seen in the heat-stressed samples.

The precision of the HPLC assay was determined by repetitive injections of one solution containing fenretinide at its normal concentration and two potential degradation products at 0.5% levels relative to fenretinide. Table III presents this data and the percent relative standard deviation.

The stability of the column packing material has been excellent, with little deterioration of peak shape or retention time. Columns which had been used heavily for many months produced the same chromatography as new columns.

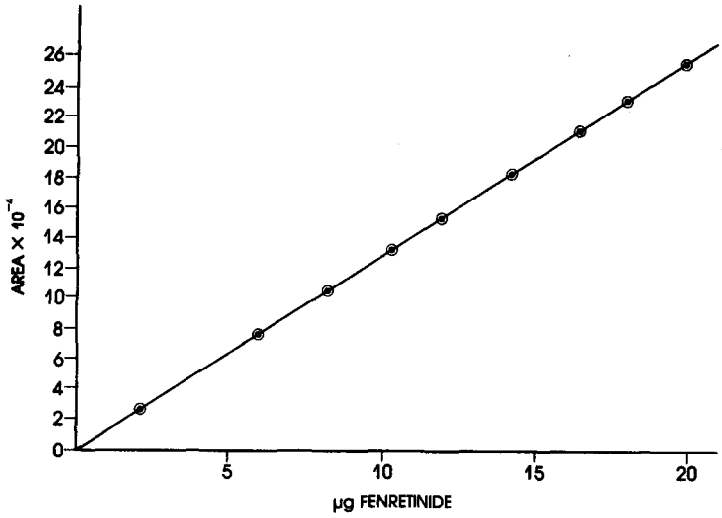


Fig. 1. Linearity of fenretinide response.

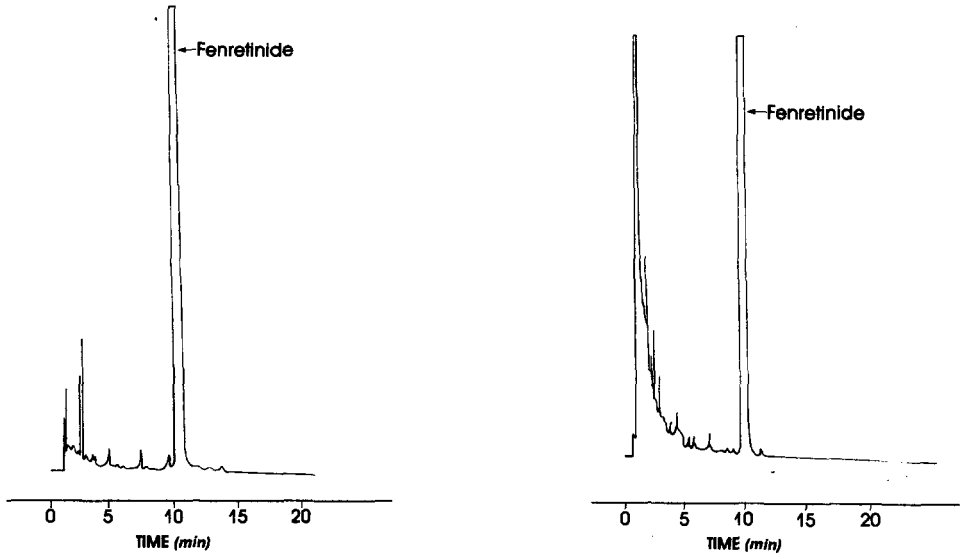


Fig. 2. Fenretinide sample stressed with excessive heat for one month at approximately 60°C (recorder sensitivity 0.04 a.u.f.s.).

Fig. 3. Fenretinide sample stressed with excessive heat for one month at approximately 80°C (recorder sensitivity 0.04 a.u.f.s.).

TABLE III

PRECISION DATA IN AREA/μg OBTAINED FOR FENRETINIDE, 13-*cis*-FENRETINIDE AND RETINOIC ACID AT THE 0.5% LEVEL

Injection No.	*Fenretinide*	*13-cis-Fenretinide*	*Retinoic acid*
1	128,590	1125	621
2	128,690	1020	640
3	128,490	1074	614
4	128,490	1005	650
5	128,670	1075	615
6	128,870	1052	601
7	128,970	1058	594
8	129,070	1023	616
9	128,640	–	–
10	128,940	–	–
11	129,040	–	–
12	128,940	–	–
13	128,670	–	–
14	128.870	–	–
15	128,870	–	–
16	128,570	–	–
% R.S.D.	0.0	3.6	3.0

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