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Determination of Ketoprofen by Direct Injection of Deproteinized Body Fluids into a High-Pressure Liquid Chromatographic System

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Abstract □ A rapid, sensitive, and specific determination of ketoprofen in human and animal deproteinized body fluids by reversed-phase high-pressure liquid chromatography is presented. The acid is detectable in amounts as low as 0.1 μg/ml. Limits of error are in the range of ±5.1% of the sample mean.

Keyphrases □ Ketoprofen—high-pressure liquid chromatographic analysis in various body fluids □ High-pressure liquid chromatography—analysis, ketoprofen in various body fluids □ Anti-inflammatory agents—ketoprofen, high-pressure liquid chromatographic analysis in various body fluids

A TLC procedure (1) for the determination of ketoprofen, 2-(3-benzoylphenyl)propionic acid, in body fluids overcame most limitations of other procedures (2). The search for new methods to shorten the time required to perform analyses and to improve sensitivity led to the present high-pressure liquid chromatographic (HPLC) procedure.

EXPERIMENTAL

Apparatus and Operating Conditions—A high-pressure liquid chromatograph[1] was equipped with two independent pumps, a reversed-phase column[2] (0.25 m × 4 mm), a variable wavelength spectrophotometer[3] to monitor the column effluent, and a liquid chromatograph terminal[4] to program chromatography conditions and record and integrate peak areas. Operating conditions were adjusted to give a mobile phase flow rate of 0.8 ml/min (operating resulting pressure of 32–38 atm). The column oven temperature was set at 40°. The detector wavelength was 255 nm (for quantitative evaluation) or 205 nm (for qualitative evaluation of metabolites).

Table I—Recovery of Ketoprofen Added to Human Plasma as Compared to Ketoprofen Dissolved in Phosphate Buffer (255 nm)

Ketoprofen Added, μg/ml	Relative Area		Recovery, %
	Buffer	Plasma	
1	16.760	13.946	83.9
	16.500	13.685	
	17.040	13.750	
	15.500	13.800	
	16.450	13.795	
2	33.000	31.822	90.4
	34.590	29.370	
	35.670	29.582	
	32.730	32.103	
	33.998	30.719	
4	68.700	64.428	90.3
	72.400	63.862	
	71.500	61.596	
	71.100	65.844	
	70.800	63.933	
8	140.320	127.430	90.7
	139.570	128.010	
	139.870	127.100	
	142.010	126.750	
	140.443	127.323	

Reagents—Methanol[5] for HPLC was used as received. Double-distilled water was filtered through a 0.2-μm pore size membrane filter[6]. The mobile phase was double-distilled water–methanol (85:15).

Preparation of Standards—Ketoprofen was dissolved in 96% ethanol or 0.15 *M* phosphate buffer at pH 8. Aliquots of ethanolic solutions equivalent to 1, 2, 4, and 8 μg were placed in glass-stoppered centrifuge tubes and evaporated to dryness under a gentle nitrogen stream. Then 1 ml of untreated body fluid was added to each tube and mixed well.

[1] Hewlett-Packard 1084A.
[2] Hewlett-Packard RP8-79918A.
[3] Hewlett-Packard 1030B.
[4] Hewlett-Packard 79850A.
[5] Merck.
[6] Sartorius type SM 11307.

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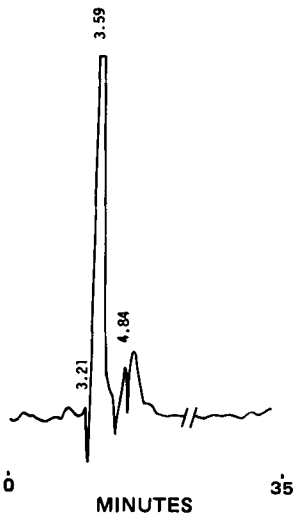

Figure 1—*Chromatogram of untreated deproteinized human plasma. The attenuation was 2^6 (64×10^{-4}/aufs), and the slope sensitivity was 0.45. UV monitoring was at 255 nm.*

These standards and the appropriate blanks were handled in the same manner as body specimens. For recovery determination, the same aliquots of standard dissolved in phosphate buffer were injected into the chromatograph without any manipulation.

Preparation of Sample—A 1-ml aliquot of human or rat plasma or rat inflammatory exudate [prepared according to Fukuhara and Tsurufuji (3)] was mixed with 10 ml of absolute ethanol and centrifuged at 2000 rpm for approximately 10 min. The supernate was flash evaporated to dryness and taken up with 1 ml of 0.15 *M* phosphate buffer at pH 8. The sample was shaken for 20 min at 37°, cooled, and filtered through a 0.2-μm pore size membrane filter. A 50-μl aliquot of the filtrate was injected in the chromatograph.

Increased sensitivity could be obtained by taking up the sample with 0.5 ml of phosphate buffer and filtering it through a homemade filter having a dead volume of only 100 μl and/or injecting 200 μl of the filtrate into the chromatograph instead of the customary 50 μl.

Calculation—Calibration curves were prepared by plotting the integrated areas of standards against the relative concentrations. Values for unknown concentrations of ketoprofen in biological specimens were calculated from the slope of the standard curve.

Column Purge—To preserve column efficiency daily after analyses, it was advantageous to set the oven temperature at 55° and to wash the column with double-distilled water for 10 min, with water–methanol (1:1) for 10 min, and with methanol for 10 min.

Precision Parameter Determination—To assess the precision of the assay, 10 complete determinations were carried out on the same day on one rat plasma sample collected 1 hr after oral ketoprofen.

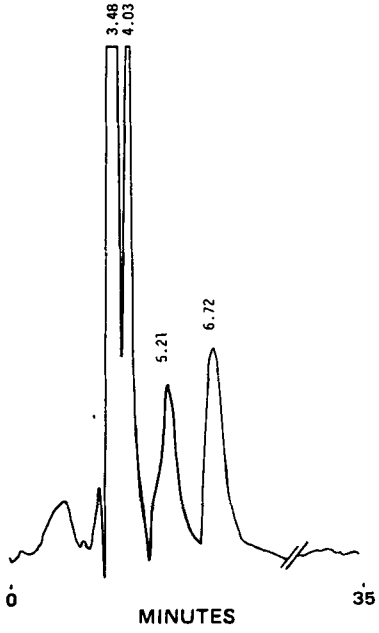

Figure 2—*Chromatogram of untreated deproteinized human plasma. UV monitoring was at 205 nm.*

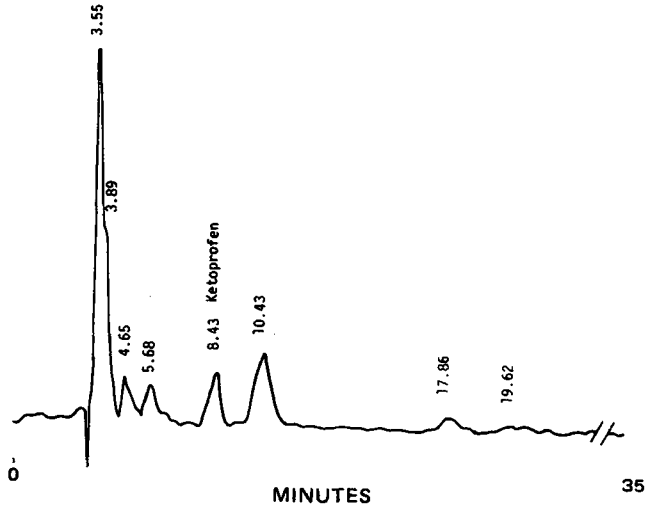

Figure 3—*Chromatogram of deproteinized human plasma 1 hr after oral administration of ketoprofen. UV monitoring was at 255 nm.*

Limits of error of the sample mean were calculated from the relative standard deviation according to Saunders and Fleming (4).

Clinical Utility of Assay—To evaluate the clinical utility of the present procedure, plasma samples from five patients receiving ketoprofen at 40 mg po were assayed.

RESULTS AND DISCUSSION

Percent recoveries of known amounts of ketoprofen added to plasma are shown in Table I. Chromatograms of untreated human plasma at 255 and 205 nm are shown in Figs. 1 and 2, respectively. In Figs. 3 and 4, chromatograms obtained at 255 and 205 nm, respectively, show typical profiles of ketoprofen in human plasma 1 hr following administration.

The method is sensitive enough to reveal ketoprofen in concentrations as low as 0.1 μg/ml of body fluids. Limits of error were in the range of ±5.1% of the sample mean. Human plasma levels (mean ± *SE*) of ketoprofen at 1 and 2 hr following administration were 3.2 ± 0.4 and 2.8 ± 0.3 μg/ml, respectively.

Reversed-phase HPLC is particularly useful for the analysis of body fluids because endogenous polar components elute first and column efficiency is preserved. These characteristics permitted the development of an analytical procedure without time-consuming purification and extraction. In fact, it was sufficient to remove plasma protein that could subsequently interfere with the efficiency of the analytical system.

Body fluids other than plasma and inflammatory exudate were examined including human synovial fluid and rat bile, feces, and urine for which, however, further extraction and purifications steps were necessary.

Although 255 nm was used for quantitative evaluation, examination

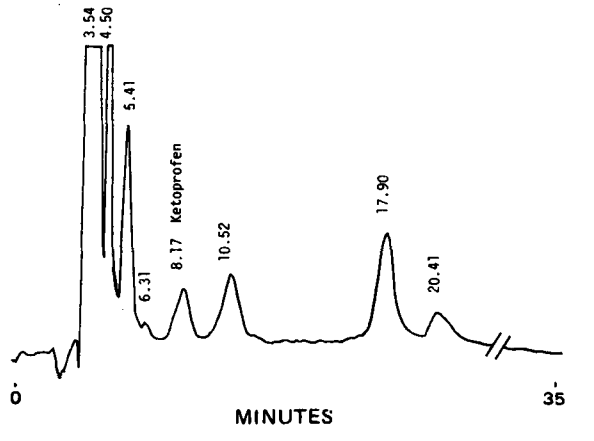

Figure 4—*Chromatogram of deproteinized human plasma 1 hr after oral administration of ketoprofen. UV monitoring was at 205 nm.*

of samples at 205 nm gave interesting qualitative information on the presence of metabolites of ketoprofen. In fact, at 205 nm, the number of metabolites detected was greater than at 255 nm. Such metabolites show a kinetic behavior quite dissimilar from that of ketoprofen. Previous work (5) supports the assumption that area peaks (detected at 205 and 255 nm) having a retention time longer than that of ketoprofen can be referred to hydroxylated metabolites whereas area peaks detectable only at 205 nm can be referred to compounds with modified unsaturated system.

This procedure appears to be useful for studies of separation and characterization of these metabolites. It also has the advantage of rapidity since a technician can process 20–30 samples in 1 day with an automatic sampler and a minimum of active involvement. In conclusion, high sensitivity, specificity, and rapidity, coupled with the ability to separate unchanged ketoprofen from its metabolites, make this procedure particularly suitable for study of the pharmacokinetics of ketoprofen in humans.

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Light Stability of Norethindrone and Ethinyl Estradiol Formulated with FD&C Colorants

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Received August 18, 1977, from the *Research Laboratories, Ortho Pharmaceutical Corporation, Raritan, NJ 08869.* Accepted for publication August 4, 1978. *Present address: School of Pharmacy, University of Wisconsin, Madison, WI 53706.

Abstract □ In general, light-sensitive tablets exhibit discoloration in the surface layer(s) only. A case is discussed where a quantitative interaction between a drug, ethinyl estradiol (in a combination tablet containing norethindrone and ethinyl estradiol), and a dye (FD&C Red No. 3) occurs, and discoloration exists throughout the tablet. The data suggest that accelerated light studies should be carried further than those dictated by predictive periods so that equilibrium levels can be deduced.

Keyphrases □ Norethindrone—stability in tablets, effect of FD&C dyes □ Ethinyl estradiol—stability in tablets, effect of FD&C dyes □ Dyes, FD&C—effect on stability of norethindrone and ethinyl estradiol in tablets □ Stability—norethindrone and ethinyl estradiol in tablets, effect of FD&C dyes □ Tablets—norethindrone and ethinyl estradiol, effect of FD&C dyes on stability

Drug stability in solution can be affected adversely by the presence of a colorant (1). Since colors are used routinely to identify solid dosage forms, a study was initiated to determine whether such reactions could occur with compressed tablets. Although there are no compendial or regulatory procedures to evaluate the effect of light, previous reports (2) correlated accelerated light studies with ordinary room light exposure.

Reported interactions between light and substances in solid dosage forms usually were confined to the top layer(s) of the tablet (3–5) and occurred when a single component was photosensitive. Photo-induced drug interactions in solid form have not been reported.

This report presents results of studies on the stability of norethindrone and ethinyl estradiol in uncolored compressed tablets as well as in tablets containing selected FD&C colorants when exposed to accelerated light stress.

EXPERIMENTAL

Tablets—All tablets contained 0.5 mg of norethindrone and 0.035 mg of ethinyl estradiol and were prepared with the same excipients by classical granulation techniques. Three formulas were prepared: pink, containing 4.5 μg of erythrosine (FD&C Red No. 3)/tablet; white, containing no colorant; and orange, containing 30 μg of the disodium salt of 1-*p*-sulfophenylazo-2-naphthol-6-sulfonic acid (FD&C Yellow No. 6)/tablet.

The dyes were added in a methanolic solution to the basic excipient system. The uncolored tablets were processed with the same amount of methanol as used in the tablets containing a colorant. All tablets were then dried, screened, lubricated, blended, and compressed in similar fashions.

Analytical Methods—Single tablets were disintegrated and then dissolved in a homogenizer with methanol as the solvent. The solution containing the dissolved steroids was filtered and divided into two

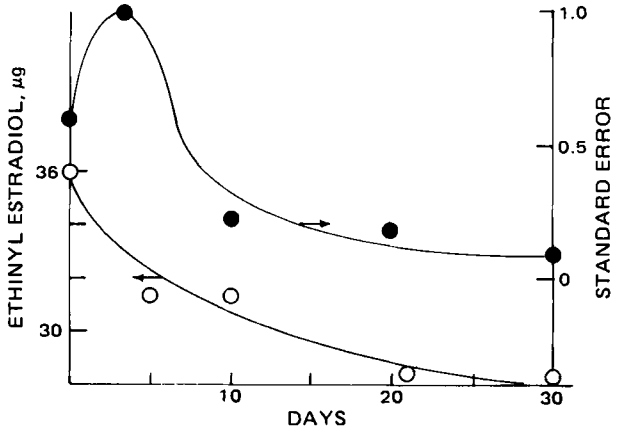

Figure 1—*Ethinyl estradiol assays as a function of time (left scale, open circles) and standard error of the assay mean as a function of time (right scale, closed circles).*

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