High-Performance Liquid Chromatographic Method for the Determination of Tiflamizole in Plasma

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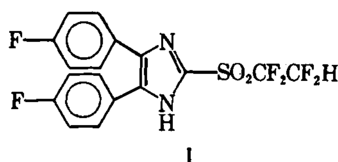
Received October 27, 1983, from *E. I. duPont de Nemours and Company, Inc., Pharmaceuticals Research and Development Division, DuPont Pharmaceuticals, Newark, DE 19714.* Accepted for publication February 24, 1984.

Abstract □ A sensitive, specific, high-performance liquid chromatographic procedure was developed for the measurement of plasma tiflamizole levels. Acidic plasma samples were extracted with three volumes of ether. The ether extracts were combined and evaporated to dryness. The residue was dissolved in acetonitrile, washed with hexane, and the acetonitrile was evaporated to dryness. The residue was dissolved in 0.5 mL of mobile phase consisting of acetonitrile and 0.007 M pH 3 sodium phosphate buffer (70:30, v/v) and then chromatographed on a octadecylsilane bonded microparticulate silica column. The assay is specific, precise, accurate, and can measure 10 ng of tiflamizole in 5 mL of plasma. The method was applied to human pharmacokinetic studies.

Keyphrases □ HPLC—tiflamizole, human plasma □ Tiflamizole—HPLC, human plasma

Tiflamizole, 4,5-bis(4-fluorophenyl)-2-[(1,1,2,2-tetrafluoroethyl)sulfonyl]-1*H*-imidazole (I), is a potent nonsteroidal antiarthritic drug that both prevents and treats adjuvant-induced arthritis in rats (1, 2). Tiflamizole is presently being evaluated in clinical studies.

The chemistry, pharmacology, and pharmacokinetics of tiflamizole were recently discussed (1-4). In the initial human studies low doses of tiflamizole were used. This required a specific and highly sensitive GC method[1] for tiflamizole analysis. However for the larger doses currently in use, the high sensitivity is not required and a simpler, more rapid method was developed. This paper describes a specific high-performance liquid chromatographic (HPLC) plasma assay for tiflamizole. The sensitivity of the method is 10 ng/mL.



EXPERIMENTAL SECTION

Reagents and Apparatus—Tiflamizole[2], 1 M pH 5.3, and 0.007 M pH 3.0 sodium phosphate (monobasic), acetonitrile[3], ether[4], and hexane[3] were used without further purification. The liquid chromatograph consisted of a variable-wavelength UV detector[5] set at 280 nm, a solvent pump[6], a reporting integrator[7], a valve-loop injector[8] with a 100-μL loop, and a 0.46 cm i.d., × 25 cm octadecylsilane bonded microparticulate column[9]. The mobile phase was acetonitrile-0.007 M pH 3 sodium phosphate buffer (70:30). The flow rate was 1.5 mL/min, the column temperature was ambient, and the column back pressure was ~1000 psi. The approximate retention times of tiflamizole and naphthalene were 4.5 and 5.8 min, respectively.

[1] D. C. Rakestraw and C. C. Whitney, unpublished results.
[2] E. I. duPont de Nemours and Co., Inc., Wilmington, Del.
[3] Burdick and Jackson Laboratories, Inc., Muskegon, Mich.
[4] J. T. Baker Chemical Co., Phillipsburg, N.J.
[5] Model 852; E. I. duPont de Nemours and Co.
[6] Model 870; E. I. duPont de Nemours and Co.
[7] Model 3390 A; Hewlett-Packard, Avondale, Pa.
[8] Model SVOV-6-1XC20 Sample Injection Valve; Glenco Scientific Inc., Houston, Tex.
[9] Zorbax ODS, Part Number 880952; E. I. duPont de Nemours and Co.

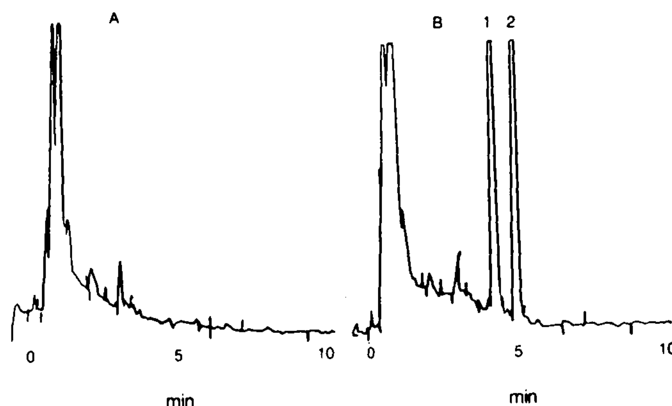


Figure 1—*Chromatograms of extracts from a plasma blank (A) and spiked (0.5 μg) plasma sample (B). Key: (1) tiflamizole; (2) naphthalene.*

Table I—Stability of Tiflamizole in Human Plasma [a]

Day	Amount Spiked, μg[b]	Amount Found, μg	*CV*, %
1	0.48	0.49	4.7
11	0.48	0.50	1.2
21	0.48	0.49	2.0
117	0.48	0.49	1.2
530	0.48	0.48	2.1

[a] Stored at −20°C. [b] $n = 3$.

Standards—Standard curves were prepared from unextracted tiflamizole standards. These standards (0.1, 0.2, 0.5, and 1 μg/mL) were prepared in a mobile phase containing 1 μg/mL of the internal standard, naphthalene, and 100 μL of each was injected into the column and chromatographed.

The peak areas of tiflamizole and naphthalene were measured and the ratio of tiflamizole/naphthalene was calculated. Linear regression least-squares fit was used to calculate the standard curve. Concentration of tiflamizole in unknown samples was determined from the peak area ratio of tiflamizole/naphthalene and the standard curve.

Extraction—A measured volume of plasma (0.5-10 mL) was transferred to a 15-mL polytef-lined screw-cap test tube[10] containing 5 mL of 1 M pH 5.3 sodium phosphate buffer. The mixture was extracted with one 10-mL and two 5-mL portions of ether. After shaking the extract for 30 min, the tube was centrifuged to separate the ether and the aqueous layers.

The organic layers were combined in another 15-mL tube and evaporated to dryness using a gentle stream of air. The residue was dissolved in 5 mL of acetonitrile and washed with three 5-mL volumes of hexane. After shaking for 20 min, the tube was centrifuged to separate the acetonitrile and hexane layers. The hexane layer was discarded by aspiration, then the acetonitrile was evaporated to dryness. The residue was dissolved in 0.5 mL of mobile phase containing 1 μg/mL of naphthalene; 100 μL was then injected into the liquid chromatograph.

Recovery Study—Control plasma samples (1 mL) spiked with 0.1, 0.2, 0.5, and 1.0 μg of tiflamizole were used to determine the extraction recovery. Control plasma samples (1 mL) spiked with 0.5 μg of tiflamizole were run concomitantly with the plasma of each subject to monitor daily recovery of tiflamizole.

Plasma Level Study—Tiflamizole (5 mg) was administered orally with ~237 mL (8 oz.) of water to 3 healthy human males, 64-76 kg, who had fasted

[10] VWR Scientific, Philadelphia, Pa.

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Table II—Recovery of Tiflamizole from Spiked Plasma

Tiflamizole, μg/mL	Recovery, %	Mean ± *SD*
0.1	90, 92, 89	90.3 ± 1.5
0.2	103, 97, 100	100.0 ± 3.0
0.5	97, 98, 99	98.0 ± 1.0
1.0	98, 100, 100	99.3 ± 1.2

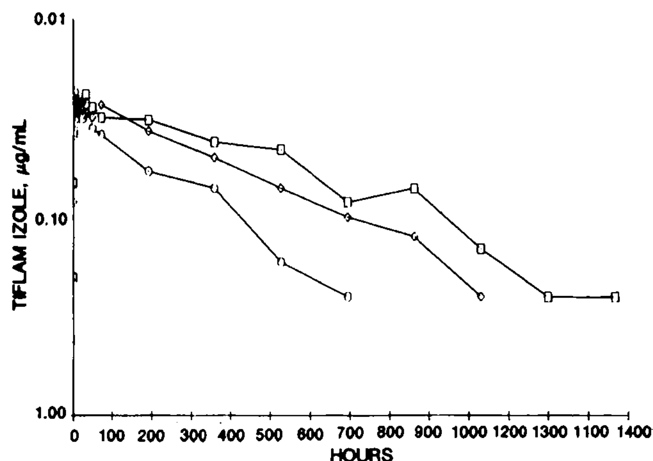

Figure 2—*Tiflamizole profiles in three healthy adult male subjects following a single oral 5-mg dose.*

overnight. Blood samples (10 mL) were withdrawn[11] prior to dosing and at specified times up to 57 d after dosing. The blood samples were centrifuged and the plasma was stored frozen in clean plastic tubes[10]. The elimination half-lives for these individuals were determined using linear regression analysis of the terminal plasma level-time data points.

[11] Heparinized vacutainers.

RESULTS AND DISCUSSION

Typical chromatograms of extracted control plasma and plasma spiked with tiflamizole are shown in Fig. 1. Tiflamizole/naphthalene peak area ratios were linear (from 0.1 to 1.0 μg/mL) and the correlation coefficients for the fit of experimental points were 0.99995. The intercepts were not significantly different from zero. There was no interference from constituents in control plasma or in the plasma from subjects or patients prior to administration of tiflamizole. Tiflamizole is stable in plasma stored at −20°C for at least 530 d. The results are shown in Table I.

Tiflamizole recoveries from spiked plasma (0.1–1.0 μg/mL) were 87.0–100% with *SD* values of 1.0–3.0% (Table II). Daily recoveries of 0.5 μg of tiflamizole spiked to 1 mL of control plasma were 99.7% ($n \simeq 131$) with a *SD* of 3.2%. Tiflamizole can be determined at levels as low as 0.01 μg/mL using 5 mL of plasma.

Figure 2 illustrates the plasma tiflamizole concentration profile in three healthy subjects given a single oral 5-mg dose. Peak concentrations of 0.36–0.61 μg/mL occurred between 4 and 12 h. The elimination half-lives were 9.9–18.0 d.

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Analysis of Tablets Containing Aspirin, Acetaminophen, and Ascorbic Acid by High-Performance Liquid Chromatography

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Abstract □ The high-performance liquid chromatographic method described enables the quantitation of the components and the main impurities of tablets containing aspirin, acetaminophen, and ascorbic acid. A C_8 reverse-phase column was used; the mobile phase was methanol–0.2 M phosphate buffer (pH 3.5)-water (20:10:70). Results obtained for a brand of effervescent tablets, normally aged for 5 years and stressed at 37°C, 50°C, or in 79% relative humidity at room temperature, are reported. Salicylic acid was the main product of decomposition. Diacetyl-*p*-aminophenol was observed to be formed by transacetylation.

Keyphrases □ Acetaminophen—HPLC with ascorbic acid and aspirin □ Aspirin—HPLC with acetaminophen and ascorbic acid □ Ascorbic acid—HPLC with acetaminophen and aspirin

During the last decade, phenacetin has been often replaced by acetaminophen in analgesic tablet formulations containing aspirin as the main component (1). Acetaminophen–aspirin mixtures have lower stability due to acetylation of the former by the latter, producing diacetyl-*p*-aminophenol (2, 3). This acetylation, however, was not evident in another study (4).

Tablets containing ascorbic acid were reported to be stable for over 5 years under normal storage conditions (5), but in combination with aspirin the eventual acetylation of ascorbic acid should be considered. Acetylation, in aqueous medium, of ascorbic acid by acetic anhydride has been described (6). Acetylation of other compounds present in tablet formulations,

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