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Note

High-performance liquid column chromatography of fenoprofen in serum

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Fenoprofen is a nonsteroidal anti-inflammatory drug which has been shown to be effective in reducing inflammation in osteoarthritis [1, 2] and rheumatoid arthritis [3, 4] in adults.

Recently Nash et al. [5] elucidated the pharmacokinetics of oral fenoprofen in adults, but no such information is available for children. Factors contributing to this lack are the large sample size required for gas chromatographic analysis by the procedure of Nash et al. [6] and the fact that the gas–liquid chromatographic procedure is tedious and time-consuming. The purpose of this paper is to report a high-performance liquid chromatographic (HPLC) method which requires as little as 50 μl of serum, making it particularly well suited for pediatric studies and therapeutic monitoring. The method is fast, simple and reliable.

MATERIALS AND METHODS

All reagents were Baker reagent grade (J.T. Baker, Phillipsburg, NJ, U.S.A.). Chloroform, methanol and acetonitrile were distilled in glass (Burdick and Jackson Labs., Muskegon, MI, U.S.A.). No additional purification was carried out. Sodium fenoprofen and valeric acid were supplied by the Eli Lilly Company (Indianapolis, IN, U.S.A.).

A Perkin-Elmer Model 601 high-performance liquid chromatograph equipped

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with an LC55 UV/VIS variable-wavelength detector and interfaced to a Perkin-Elmer Sigma 10 data system was used for the chromatography and data analysis.

The samples were chromatographed on a 25 cm × 0.26 cm HC-ODS-Sil-X column. This is a high-efficiency, high-capacity octadecyl silane (10 μm) packing. The column was maintained at 40°C during the chromatography and the compounds were detected at 272 nm. The mobile phase was acetonitrile—distilled water—glacial acetic acid (50:50:2); the flow-rate was 1.5 ml/min.

A 50- or 100-μl volume of serum is placed in a 1.5-ml Eppendorf centrifuge tube and 100 μl 1 *N* HCl are added; the tube is then vortexed. A 500-μl aliquot of chloroform, containing 20 μg/ml valeric acid (internal standard) is added, vortexed vigorously for 2 min and then centrifuged for 5 min in a Brinkmann table-top centrifuge.

The chloroform layer is placed in a clean glass tube and evaporated to dryness with nitrogen (40°C). The sample is reconstituted with either 20 or 50 μl of methanol, vortexed vigorously and 5 μl are injected into the HPLC column.

RESULTS AND DISCUSSION

Under these conditions, fenoprofen and valeric acid have retention times of 1.50 and 2.45 min, respectively. Fig. 1 shows typical chromatograms obtained from blank serum, control serum and patient's serum. Concentration is determined by the integrated area under the peak, relative to the internal standard (valeric acid). The two early peaks are unidentified artifacts which do not interfere with the analysis. The peak heights and areas remain relatively constant and are not appreciably influenced by increasing concentrations of fenoprofen.

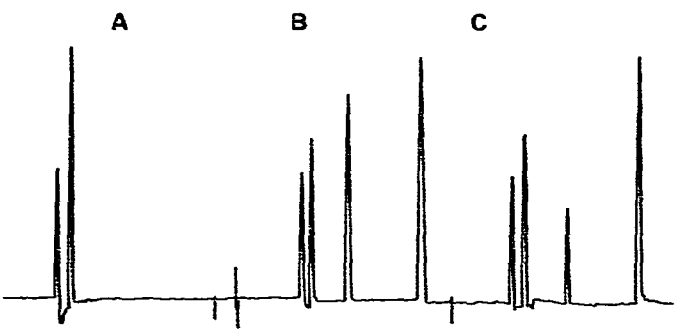

Fig. 1. Typical serum chromatograms: (A) blank, drug-free serum; (B) drug-free serum fortified with 10 μg/ml fenoprofen; (C) patient's serum determined as 4.7 μg/ml. The first two peaks in the chromatogram are unidentified artifacts; the third peak is fenoprofen; the last peak is the internal standard (valeric acid).

As mentioned in the Methods section, either 50 or 100 μl of serum can be used, with a corresponding reduction of the methanol reconstitution step from 50 to 20 μl when 50 μl of serum is used. The 100-μl sample size is preferred because it allows greater accuracy and reproducibility.

Reproducibility and day-to-day variation studies were carried out by

preparing fresh samples of 10 and 20 μg of fenoprofen per ml in drug-free serum. The results are shown in Table I. All results presented are averages of at least duplicate analyses.

TABLE I

REPRODUCIBILITY AND DAY-TO-DAY VARIATION OF ANALYSIS

Day	Fenoprofen (μg/ml)					
	Calculated	Observed	Mean (± S.D.)	Calculated	Observed	Mean (± S.D.)
1	20	20.7, 20.2, 19.1, 20.0, 19.0	19.98 (0.58)	10	10.0, 10.0, 9.9, 8.9, 9.5	9.66 (0.47)
2	20	20.0, 20.0, 20.2, 19.6, 20.3	20.02 (0.27)	10	10.9, 10.2, 10.1, 9.9, 9.7	9.98 (0.19)
3	20	20.0, 20.4, 18.9, 19.9, 20.5	19.94 (0.63)	10	10.3, 10.4, 9.9, 9.8, 10.0	10.08 (0.26)
4	20	20.6, 19.8, 19.9, 20.1, 19.7	20.02 (0.36)	10	10.0, 9.7, 8.9, 10.0, 10.5	9.82 (0.59)
5	20	21.0, 20.5, 18.9, 19.9, 19.5	19.96 (0.82)	10	10.2, 10.3, 9.9, 10.0, 10.6	10.20 (0.27)
Total (n = 25)			19.98 (0.52)			9.95 (0.40)

Analyses of prepared serum samples containing fenoprofen concentrations of 5—100 μg/ml were performed. The results indicate that the relationship between the serum concentration and the peak area is essentially linear over this range with a greater variability at the upper concentrations (Fig. 2). For example, at 100 μg/ml the range for five samples was 92—107, mean 98 μg/ml. This should present no problem because the recent work by Nash et al. [5] indicates a maximum serum concentration of 20 μg/ml in adults under therapeutic dosing conditions.

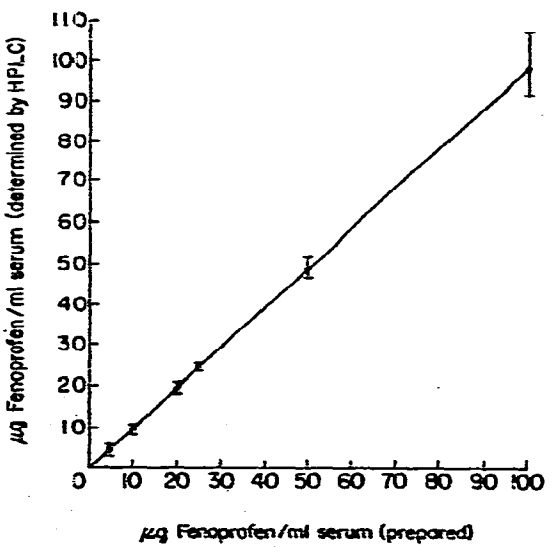

Fig 2. Fenoprofen standard curve. Mean (•) and range (n = 5 at each concentration).

Recovery studies were carried out by preparing a standard serum concentration of 20 μg/ml. An aliquot of this sample was analyzed as such. Another aliquot was fortified with an additional 20 μg/ml to yield a total concentration of 40 μg/ml. The results (Table II) indicate virtually 100% recovery (Table I also indicates 100% recovery of samples studied).

TABLE II

RECOVERY STUDY OF STANDARD SERUM SAMPLES

Theoretical amount	Amount measured	Range	Mean ± S.D.
40 μg/ml	38.0, 38.65, 39.43, 39.8, 40.6, 40.6, 41.6	38—41.6	39.81 ± 1.24

Refrigerator and freezer stability studies were carried out by preparing 20 μg/ml fenoprofen in serum which was analyzed on the day of preparation. Part of the sample was stored frozen and part placed in the refrigerator. Analysis of the refrigerated samples over the next four days indicated a gradual decline of assayable fenoprofen (Table III). Samples were removed from the freezer on days 7, 10, and 14 and analysis indicated essentially no loss of fenoprofen (Table III). It is not necessary, therefore, to have major concern about sample stability; if samples are to be analyzed within a week they can be stored in the refrigerator.

TABLE III

STORAGE STABILITY STUDY OF STANDARD SERUM SAMPLES

Refrigerator		Freezer	
Day	Measured value* (μg/ml)	Day	Measured value* (μg/ml)
1	20.0	7	19.8
2	20.7	10	20.1
3	19.6	14	19.9
4	19.0		
5	18.2		
Mean (±) S.D.	19.5 (0.95)		19.3 (0.15)
Range	20—18.2		19.8—20.1

*Theoretical amount 20 μg/ml.

The method presented here is simple, fast (50 samples can conveniently be analyzed per day), accurate and reliable. In addition, the use of small sample sizes (50 or 100 μl of serum) makes the assay ideal for pediatric studies and therapeutic monitoring.

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