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Letter to the Editor

Simple and rapid determination of fenoprofen in plasma using high-performance liquid chromatography

Sir,

Fenoprofen calcium has been shown to be a useful anti-inflammatory agent in osteoarthritics and rheumatoid arthritics.

The concentration of this drug in plasma has been measured by gas—liquid chromatography (GLC) [1, 2], but the GLC methods require somewhat large sample size, lengthy clean-up procedures and derivatisation. Recently, high-performance liquid chromatography (HPLC) has been utilized for the quantitation of fenoprofen in human plasma or serum [3—5]. Although these methods are accurate and reproducible, they have the disadvantages of time-consuming extractions and/or large sample requirements. Therefore, a rapid and simple HPLC assay with a small plasma sample volume was developed for the measurement of fenoprofen in plasma that did not require a complex prior extraction. The method described here involves a simple protein precipitation technique using 0.1 ml of plasma and estimating the amount of drug present with HPLC. The minimum detectable concentration of fenoprofen in plasma was 2.5 μg/ml using 0.1 ml of plasma.

The plasma sample (0.1 ml) was deproteinized by the addition of 0.5 ml of methanol which contained diphenylamine (1.5 μg) as the internal standard. After shaking for 1 min, the mixture was centrifuged at 1500 *g* for 10 min. The upper layer (100 μl) was injected into the HPLC column. The HPLC system consisted of a solvent pump (Model 6000A, Waters Assoc.), a reversed-phase HPLC column (Nucleosil $5C_{18}$, 15 cm × 4 mm I.D., Macherey-Nagel, Düren, F.R.G.), and a variable-wavelength UV detector set at 240 nm (Model UVIDEC-100-II, Japan Spectroscopic Co.). The mobile phase was acetonitrile—0.35 *M* acetic acid solution (60:40, v/v) and was passed at a flow-rate of 1.0 ml/min.

Separation of fenoprofen and internal standard was satisfactory since retention times were about 4 and 6 min, respectively, under the experimental conditions proposed. No interference by normal-plasma components was noted.

Linear regression indicated excellent linearity with a correlation coefficient

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of 0.9999, a slope of 0.0572, and an intercept of −0.0387 in the range 2.5—100.0 μg/ml of human plasma.

The precision of the assay, as determined by the coefficient of variation, was ⩽2.0%. In addition, the assay was quite accurate even at plasma concentrations as low as 5.0 μg/ml (bias = 2.0%) and was ⩽1.6% for all other concentrations. The recovery of fenoprofen was ⩾91.5%. Table I shows the precision and accuracy for assay of fenoprofen assessed at five concentrations.

TABLE I

VARIABILITY OF FENOPROFEN CONCENTRATION IN HUMAN PLASMA SAMPLES

n = 5 in all cases.

Concentration added (μg/ml)	Concentration measured (μg/ml)	C.V.* (%)	Bias** (%)	Recovery*** (%)
5.0	5.1 ± 0.1†	2.0	2.0	91.5
10.0	9.6 ± 0.1	1.0	−4.0	96.3
25.0	24.3 ± 0.4	1.6	−2.8	97.4
50.0	50.8 ± 0.7	1.4	1.6	97.1
100.0	99.8 ± 0.7	0.7	−0.2	98.2

*Coefficient of variation (%).
**100 × (measured concentration − added concentration)/added concentration.
***Plasma versus water.
†Mean ± standard deviation.

Fenoprofen is extensively metabolized to fenoprofen glucuronide and 4-hydroxyfenoprofen glucuronide and is excreted in the urine within 24 h [6, 7]. The elution time of the major metabolite, 4-hydroxyfenoprofen (2 min), was sufficiently different from fenoprofen, but since the human plasma components interfered with the peak of 4-hydroxyfenoprofen, this metabolite could not be determined by this method.

The simplicity and short extraction procedure are advantageous in the analysis of a large number of samples often encountered in pharmacokinetics and bioavailability studies. The small plasma volume needed for the analysis is also an important consideration in such studies. Moreover, this HPLC method is superior to others, since it allows higher precision and accuracy with a small volume of plasma.

The analytical method is suitable for bioavailability and pharmacokinetic studies in human subjects.

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