CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Ibuprofen In Serum

Purpose

Determination of ibuprofen [2-(4-isobutylphenyl) propionic acid, F1) in serum.

Ibuprofen is an anti-inflammatory analgesic available by prescription (e.g., Motrin®) and over-the-counter (e.g., Advil®, Nuprin®). It is used for the treatment of arthritis, pain and fever [1]. Therapeutic doses range from 1-40 µg/mL blood. Adverse effects include gastro-intestinal disturbances (including ulceration), dizziness and tinnitus. The drug is contra-indicated in cases of gastro-intestinal disorders, renal dysfunction and sensitivity to anti-inflammatory drugs.

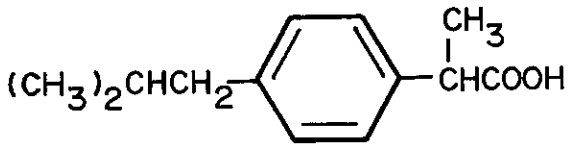

Figure 1. Structure of ibuprofen.

An interesting aspect of ibuprofen metabolism is that the drug is supplied as a racemic mixture, but only the S-(+) form is active [2]. Moreover, the inactive R-(-) enantiomer undergoes metabolic conversion to the active form. These enantiomers may be separated by LC [2].

The major metabolites of ibuprofen are 2-[4-(2-hydroxy-2-methylpropyl)phenyl]propionic acid (OH-ibuprofen) and 2-[4-(2-carboxyproxyl)phenyl]propionic acid (COOH-ibuprofen). These may be separated by LC with gradient elution [3].

Existing Methods

Gas chromatography and LC. GC typically requires a 1-mL serum sample, while some LC analyses require 500 µL. The procedure below requires only 50 µL [1].

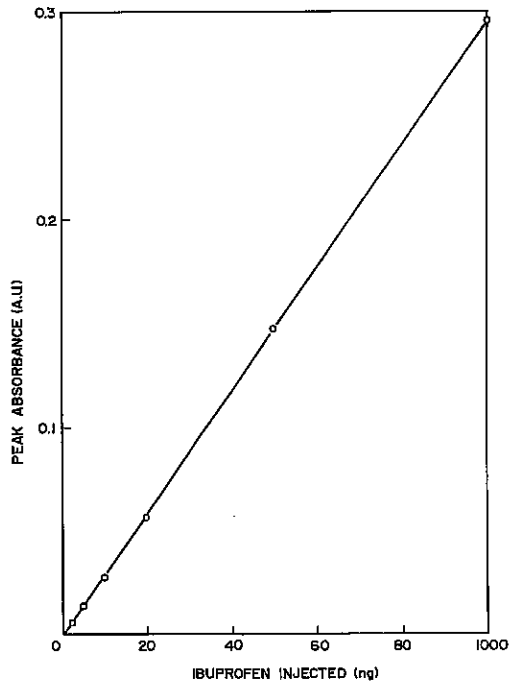

Figure 2. Calibration curve for injected standards. Each symbol represents the mean of two determinations.

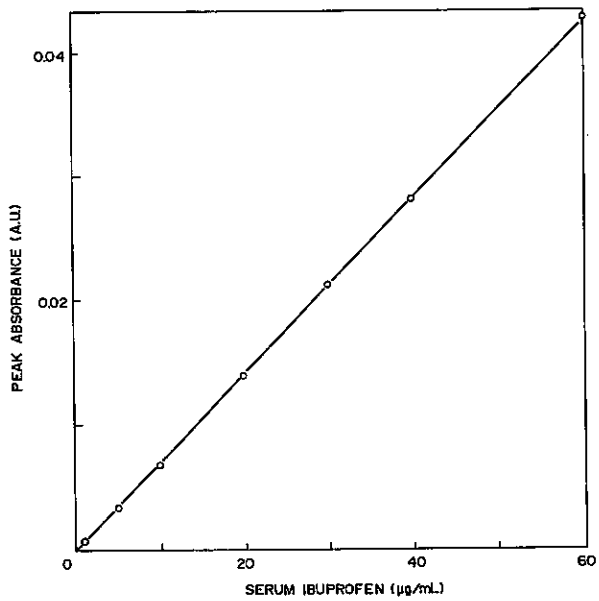

Figure 3. Calibration curve for spiked serum samples. Each symbol represents the mean of two determinations.

Conditions

System: BAS 400 Liquid Chromatograph
Detector: BAS UV-108 (variable wavelength)
Wavelength: 220 nm
Column: BAS 3 µm Phase II ODS (100 x 3.2 mm) (PN-6213)
Mobile Phase: 75% (v:v) 0.1 M sodium acetate pH 6.4, 25% acetonitrile
Detection Limit: 0.4 ng injected standard (S/N = 3); 0.2 µg/mL serum
Linear Range: 0.4 ng to at least 1 µg injected standard; 0.2 to at least 60 µg/mL serum

Sample Preparation

1. Prepare 100 mL extraction buffer (1 M sodium acetate adjusted to pH 4.6 with glacial acetic acid).

2. Add 50 µL serum, 100 µL extraction buffer and 1 mL ethyl acetate to a 1.5 mL microcentrifuge tube for each sample.

3. Vortex each tube for 1 minute.

4. Centrifuge the samples for 3 minutes at 10,000 x g.

5. Transfer the upper (organic) layer to glass tubes and dry under a stream of nitrogen or in a vacuum evaporator.

6. Redissolve the samples in 200 µL mobile phase. Filter through 0.45 µm membranes (PN MF-5655) by centrifuging at 1600 x g in microfiltration tubes (PN MF-5500).

7. Inject 20 µL into the chromatograph.

Plasma samples were spiked with appropriate amounts of standard ibuprofen for the determinations below.

Notes

Calibration curves for ibuprofen standards (F2) and spiked plasma samples (F3), and a sample chromatogram (F4) are presented.

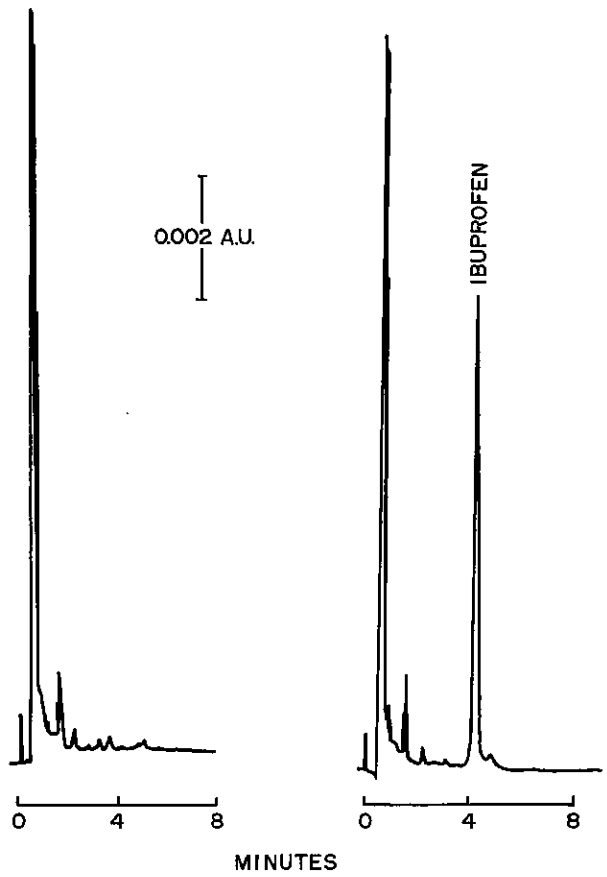

Figure 4. Chromatograms of unspiked (left) and spiked (10 µg ibuprofen/mL) serum.

The small amount of serum required (50 µL) makes this an appropriate technique for pediatric patients.

ETBA (5-ethyl-5-*p*-tolylbarbituric acid) [1], *p*-chlorophenoxyacetic acid [2], tolmetin [3], phenylbutazone [4], and mefenamic acid [5] have been used as internal standards.

A number of commonly used drugs (gentamycin, tobramicin, chloramphenicol, salicylates and acetaminophen) were tested and found not to interfere with this ibuprofen assay [1].

Caution is always advised when using organic extraction solvents and mobile phases with plasticware and filters. Samples and blanks run at sensitive detector ranges should be examined for peaks associated with the use of these products.

The determination of ibuprofen detailed above also can be performed on the BAS 200 Problem Solver, which is available with an integral variable-wavelength UV detector.

References

1. *Determination of Ibuprofen by High-Performance Liquid Chromatography, M.K. Aravind, J.N. Miceli and R.E. Kauffman, J. Chromatogr. 308 (1984) 350-353.*

2. *Determination of the Enantiomeric Composition of Ibuprofen in Human Plasma by High-Performance Liquid Chromatography, A. Avgerinos and A.J. Hutt, J. Chromatogr. 415 (1987) 75-83.*

3. *High-Performance Liquid Chromatographic Determination of Ibuprofen and its Major Metabolites in Biological Fluids, G.F. Lockwood and J.G. Wagner, J. Chromatogr. 232 (1982) 335-343.*

4. *Rapid High-Performance Liquid Chromatographic Determination of Ibuprofen in Human Plasma, M. Lalande, D.L. Wilson and I.J. McGilveray, J. Chromatogr. 377 (1986) 410-414.*

5. *Improved High-Performance Liquid Chromatographic Assay of Ibuprofen in Plasma, A. Shah and D. Jung, J. Chromatogr. 344(1985) 408-411.*