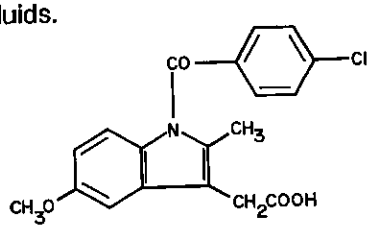
CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

Indomethacin In Urine

Purpose

Determination of indomethacin in urine and other body fluids.



INDOMETHACIN

Figure 1. Structure of indomethacin.

Indomethacin (F1,1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid) is an anti-inflammatory, antipyretic analgesic. It is used to relieve the symptoms of ankylosing spondylosis, osteoarthritis, rheumatoid arthritis and gout. Therapeutic concentrations may range from 0.5 - 3 µg/mL blood. Levels of the unmetabolized drug in urine are reported to range from 0.25 - 1.25 µg/mL [1].

Indomethacin is metabolized into two main derivatives and their glucuronide conjugates: *o*-desmethylindomethacin and *o*-desmethyl-*n*-deschlorobenzoylindomethacin. These derivatives have no anti-inflammatory activity, however.

In addition to its analgesic uses, indomethacin promotes the constriction of patent ductus arteriosus (the opening that allows fetal blood to bypass the lungs) in newborns.

Existing Methods

TLC, GC-ECD (with derivatization), radioimmunoassay and fluorometry. These may be non-specific, time-consuming, or unsuitable for routine use.

Conditions

System: BAS 400 Liquid Chromatograph

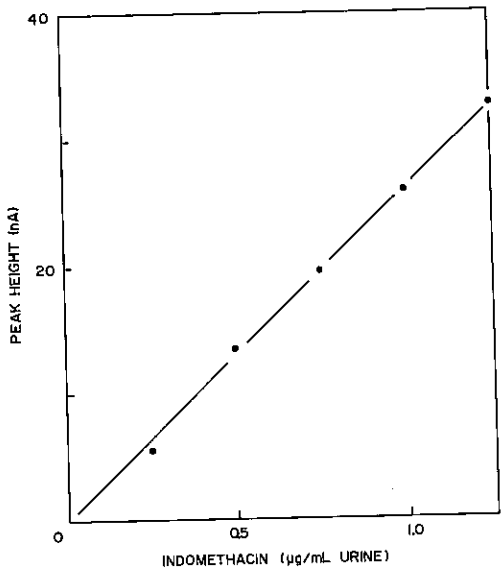


Figure 2. EC calibration curve for spiked urine samples. The UV curve was similar.

EC Detector: BAS LC-4B amperometric detector
Electrode: BAS glassy carbon
Potential: + 0.925 V vs Ag/AgCl
UV Detector: BAS UV-108 variable wavelength (220 nm)
Column: BAS 3 µm Phase II Octyl reverse-phase (100 x 3.2 mm) (PN MF-6214)
Mobile Phase: 65% (v:v) 0.1 M sodium acetate, adjusted to pH 5.0 with acetic acid, 35% acetonitrile. Flow rate was 1 mL/min.
Detection Limit: EC: 30 pg injected standard (S/N = 3), 2 ng/mL urine (S/N = 3). UV: 1 ng injected standard (S/N = 5), 30 ng/mL urine (S/N = 3).
Linear Range: EC: 100 pg - 100 ng injected standards, 0.25 - 1.25 µg/mL urine. UV: 1 - 100 ng injected standards, 0.25 - 1.25 µg/mL urine.

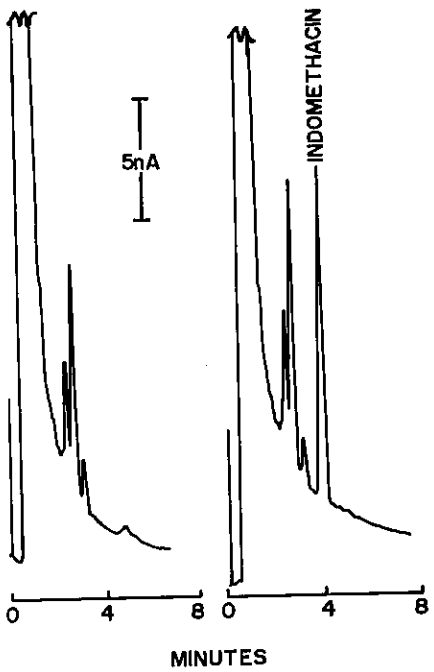


Figure 3. Sample EC chromatograms of unspiked (left) and spiked (0.50 µg/mL) urine

Sample Preparation

1. Combine the following in a 10 mL screw-capped test tube: 0.5 mL urine, internal standards (if desired), and 5 mL methylene chloride.

2. Shake the tubes vigorously for 5 minutes.

3. Centrifuge for 10 minutes at 10,000 x g.

4. Remove the lower (organic) layer to clean tubes and evaporate to dryness in a vacuum desiccator or a stream of nitrogen.

5. Redissolve the samples in 0.350 mL mobile phase. Filter through 0.45 µm membranes (PN MF-5645) by centrifuging at 1600 x g in MF-1 microfiltration tubes (PN MF-5500).

6. Inject 20 µL aliquots into the chromatograph.

Notes

The EC calibration curve for spiked urine samples is presented in F2. Recovery of standard indomethacin from spiked samples was 65%. Sample chromatograms are presented in F3. The minimum detectable concentration of indomethacin was 10 times greater with UV than with EC detection. Nevertheless, UV detection was suitable for the expected range of sample concentrations.

Indole-3-propionic acid [1], phenylbutazone [2], mefanamic acid [3] and glutethimide [4] have been used as internal standards for the determination of indomethacin.

Extraction methods for the determination of indomethacin in 200 µL of plasma [3] and 50 µL blood [4] have been described.

A 3 µm C 18 reverse-phase column (100 x 3.2 mm, PN MF-6213) also would be suitable for this analysis.

Glucuronide conjugates of indomethacin can be separated and detected after pre-treatment of samples with β-glucuronidase [1]. The *o* -desmethyl metabolites are electrochemically oxidized at very low potentials, a fact which may be useful in enhancing selectivity for these hydroxyindoles in biological samples.

The determination of indomethacin also can be performed on the BAS 200 Problem Solver.

References

1. Smith, P.W. and L.Z. Benet, J. Chromatogr. 306 (1984): 315-321.

2. Astier, A. and B. Renat, J. Chromatogr. 233 (1982): 279- 288.

3. Cooper, J.K., G. McKay, E.M. Hawes and K.K. Midha, J. Chromatogr., 233 (1982) 289-296.

4. Greizerstein, H.B. and I. G. McLaughlin, J. Liq. Chromatogr. 5 (1982) 337-343.