Journal of Chromatography, 181 (1980) 282—285
Biomedical Applications
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CHROMBIO. 447

Note

Estimation of serum indomethacin at therapeutic levels by means of thin-layer chromatography and spectrophotometry

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(First received April 10th, 1979; revised manuscript received July 26th, 1979)

The need for an accurate, sensitive and rapid method for the estimation of indomethacin arose from studies in this department on the bioavailability of various indomethacin preparations in normal human volunteers. Previously published methods utilized radioactively labelled indomethacin [1], spectrofluorimetry [2], electron-capture gas chromatography [3—7], radioimmunoassay [8] and high-speed liquid chromatography [9]. This paper describes a procedure for such estimations, which is simple enough to permit about eighty assays to be performed in a working day by one analyst.

EXPERIMENTAL

Materials

Anaesthetic diethyl ether (Natal Cane Byproducts, Durban, Republic of South Africa) was redistilled before use. All other reagents were Merck (Darmstadt, G.F.R.) reagent grade and were used without further purification. The internal standard, piretanide (HOE-118), a new diuretic (structure shown in Fig. 1)

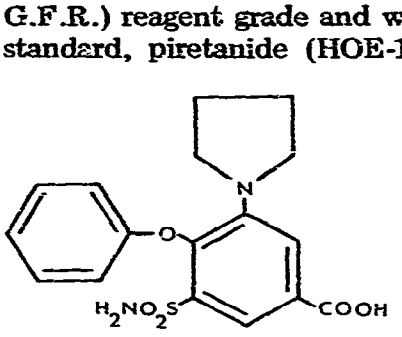

Fig. 1. Structure of piretanide (HOE-118).

was a gift from Hoechst (Frankfurt am Main, G.F.R.). High-performance thin-layer chromatographic (HPTLC) plates (silica gel 60 without fluorescence indicator) were obtained from Merck.

Apparatus

Serum was pipetted by means of an Oxford Laboratories sampler, and standards and phosphoric acid by means of a Gilford 6065 automatic pipettor-diluter. Extracts were spotted onto TLC plates by means of an EVA Chrom TLC applicator. Spectrophotometric measurements were made in the reflectance mode in a Zeiss KM 3 chromatogram spectrophotometer at 254 nm.

Method

Serum (1 ml) was pipetted into a glass-stoppered centrifuge tube (B14 Quickfit) and 20 μl of internal standard (100 mg per 100 ml acetone) was added, together with 500 μl of 1 *M* phosphoric acid. The sample was mixed for a few seconds on a Vortex mixer and 6 ml diethyl ether was then added. The contents were mixed on a horizontal shaker (100 strokes/min) for 15 min. After centrifugation (800 *g*), the diethyl ether layer was transfered to a 5-ml glass ampoule and evaporated to dryness under nitrogen. The sides of the ampoule were washed with 100 μl of acetone, and again evaporated to dryness under nitrogen. The residue was redissolved in 30 μl of acetone and 2.5 μl were spotted onto 10 × 10 cm HPTLC plates. Ten unknown and five standard extracts were spotted on each plate. The mobile phase dioxane—methanol—ammonia (7:2:1), was allowed to ascend to the 9 cm mark on the TLC plate in a Shandon N-chamber (non-saturated). The plates were then dried in a stream of cold air and scanned at 254 nm. The ratio of the peak heights of indomethacin to internal standard were calculated and plotted against indomethacin serum concentration. An equation of the calibration line was obtained by linear regression analysis and used to calculate unknowns from their peak height ratios.

RESULTS AND DISCUSSION

Fig. 2 depicts chromatograms obtained from (a) indomethacin-free serum, (b) serum spiked with indomethacin and HOE-118, and (c) serum from a

TABLE I

PRECISION AND ACCURACY OF THE TLC METHOD FOR ASSAYING INDOMETHACIN IN SPIKED SERUM

$n = 10$.

Spiked value (μg/ml)	Assayed value	
	Mean ± S.D. (μg/ml)	Coefficient of variation (%)
0.5	0.50 ± 0.03	6.0
1.0	1.02 ± 0.05	4.5
1.5	1.49 ± 0.03	2.2
2.0	2.00 ± 0.05	2.5
2.5	2.49 ± 0.03	1.2
5.0	4.97 ± 0.18	3.7
10.0	9.96 ± 0.08	0.8

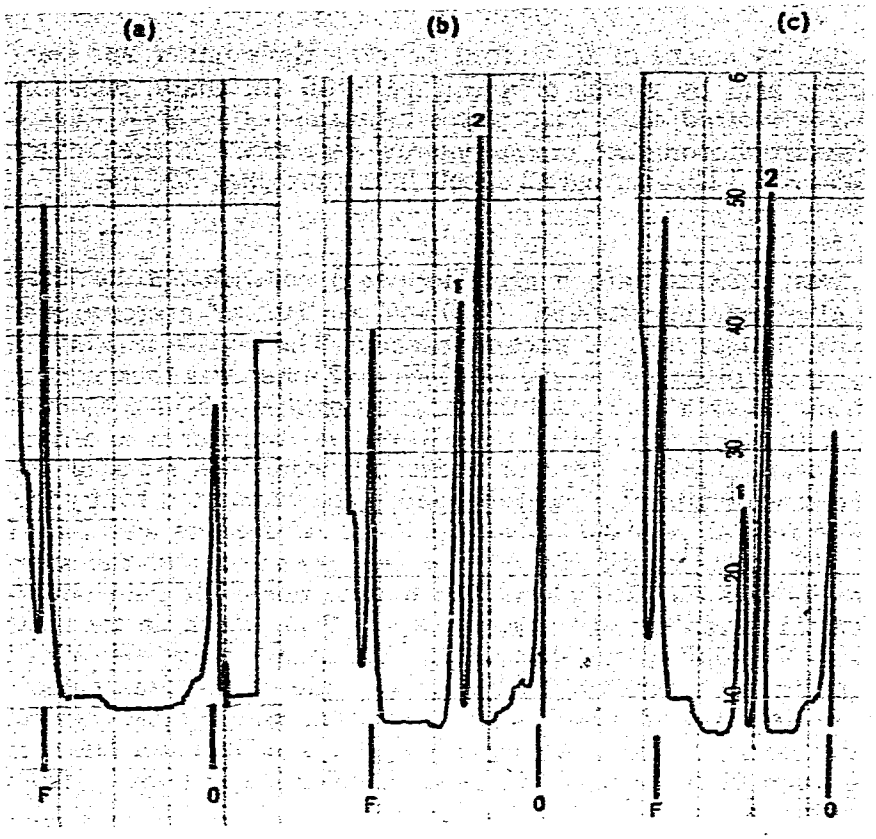

Fig. 2. Chromatograms of (a) indomethacin-free serum, (b) serum spiked with indomethacin and HOE-118, and (c) serum from volunteer after indomethacin ingestion. Peaks: 1 = indomethacin, 2 = piretanide (HOE 118).

volunteer during the trial. There are no interfering peaks from indomethacin-free serum.

The R_F value for piretanide is 0.37 and for indomethacin 0.48. Plots of the standard curves for indomethacin over the range 0.5—10 μg/ml were linear, and the mean of forty such plots is depicted in Fig. 3.

The results shown in Table I, summarize the data obtained from ten assays. The accuracy and precision of the method are excellent. The concentrations of indomethacin in the experimental samples compare favourably with the spiked values.

The method described for the quantitation of indomethacin in serum at therapeutic levels is relatively simple requiring a single extraction and separation on a TLC plate. Quantitation by means of absorption measurements is reproducible and accurate.

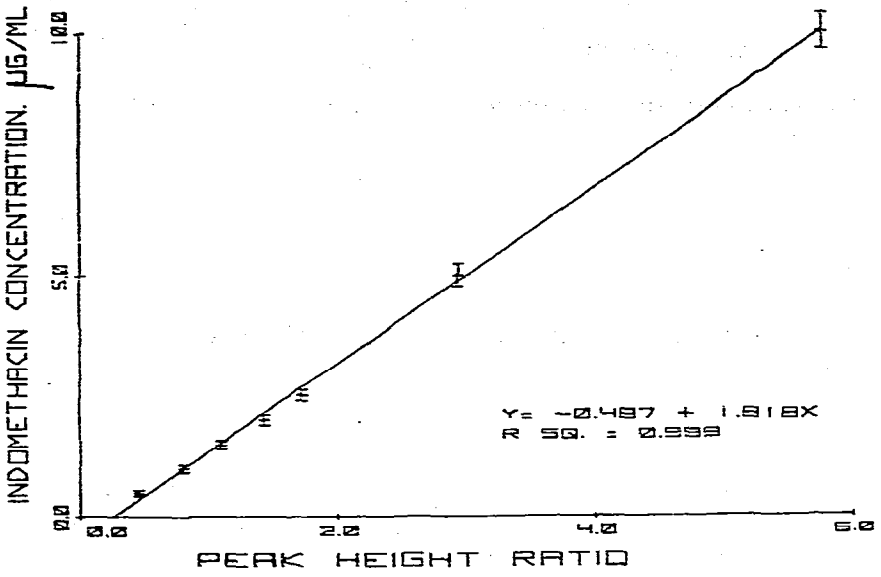

Fig. 3. Standard curve for indomethacin. Plot of indomethacin concentration versus peak height ratio of indomethacin and piretamide (n = 40).

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