Journal of Chromatography, 89 (1974) 110–112

CHROM. 7095

Note

Indomethacin estimation in plasma and serum by electron capture gas chromatography

D. G. FERRY, D. M. FERRY, P. W. MOLLER and E. G. McQUEEN

Toxicology Research Unit, Medical Research Council of New Zealand and Department of Medicine, University of Otago Medical School, P.O. Box 913, Dunedin (New Zealand)

(Received September 27th, 1973)

Investigations involving estimation of indomethacin concentrations in biological fluids have formerly utilized spectrofluorimetric or radioisotope techniques. Metabolites and co-extractives may interfere with estimations utilizing these methods.

The method described below utilizes electron capture gas chromatography of a derivative of indomethacin. Its freedom from interference has enabled it to be successfully applied to a study of the interaction of indomethacin and aspirin[1].

EXPERIMENTAL

One milliliter of plasma or serum was placed in a 40-ml glass-stoppered centrifuge tube. Two milliliter of Sorensen's buffer, 0.1 *M*, pH 5.0, and 10 ml of 10% amyl alcohol in hexane were added and the tubes shaken for 15 min on a vortex mixer. After centrifuging to separate the phases, 9 ml of the organic layer were transferred to a graduated tube. The extract was concentrated to approximately 0.5 ml on a 52° water-bath under a gentle stream of dry air. Approximately 0.3–0.5 ml of a hexane solution of diazoethane (prepared from N-ethyl-N'-nitro-N-nitrosoguanidine) was added, the samples were mixed on a vortex mixer and after a few minutes the excess diazoethane was removed and the solution concentrated under an air stream on the water-bath as above. After dilution to an appropriate volume with hexane, the sample was analysed by gas–liquid chromatography. The indomethacin was quantitated by comparison of the peak heights for samples and standards. Stock standard solutions were prepared in ethanol and stored under refrigeration. Dilute standards were prepared daily from these stock solutions. As the ethylated derivatives may not be stable when exposed to sunlight, it is considered advisable to protect extracts and dilute standard solutions as much as possible.

The gas chromatograph used was a Varian Model 2100 one (Varian, Palo Alto, Calif., U.S.A.) fitted with tritium electron capture detectors. The Pyrex column was U-shaped, 1 m × 2.5 mm I.D. packed with 2% OV-1 on 100–120 mesh Chromosorb W AW-DMCS, prepared by evaporation–fluidisation and conditioned for 48 h at 225°. Operating conditions were: oven temperature, 190°; injector port temperature, 225°; detector block temperature, 275°; nitrogen carrier gas flow-rate 60 ml/min. The retention time of the ethylated derivative was 9 min.

RESULTS AND DISCUSSION

The recoveries of known amounts of indomethacin (0.1–5 μg/ml) added to serum are shown in Table. I The increase obtained by using two extractions instead of a single one was not considered sufficient to warrant the additional labour. The use of 10% amyl alcohol in hexane was found to be particularly important when extracting samples which had been stored deep-frozen for more than a few days. For example, a mixture of 1.5% amyl alcohol in hexane gave recoveries up to 98% when the extraction was carried out immediately after addition of the indomethacin to

TABLE I
RECOVERIES OF INDOMETHACIN

% amyl alcohol in hexane	*Number of extractions*	*Recovery (% ± S.D.)*	*Number of determinations*
5	1	71 ± 3	10
10	1	90 ± 3	22
5	2	92 ± 4	36
10	2	96 ± 3	24

plasma. When the samples were stored deep-frozen and subsequently analysed, the recovery decreased with time. After three weeks the recovery had fallen to 10%. Increasing the amyl alcohol concentration to 10% resulted in complete recovery of the indomethacin even after six weeks storage. The practice of analysing the samples as soon as possible after they are obtained is considered advisable as the effect of prolonged storage on indomethacin levels in experimental or clinical samples is not known. Other methods have used either 3% or 5% amyl alcohol in heptane. Hexane was used in this method as it is more readily removed during evaporation on the water-bath than heptane. It offers the additional advantage that amyl alcohol and hexane form an azeotropic mixture and by repeated addition of hexane the amyl alcohol can be completely removed permitting application of cleanup procedures or other derivative techniques if required.

The ethylation of indomethacin was essentially instantaneous and considerably improved the chromatographic response of indomethacin (Fig. 1), which was linear over the range of 0.1–3.0 ng injected (Fig. 2). The ethyl derivative was used as it permits estimation of indomethacin in the presence of one of its principal metabolites, desmethylindomethacin, which can be extracted by the above procedure. Under the above operating conditions the peaks corresponding to the ethylated derivatives of indomethacin and desmethylindomethacin are not completely separated. The separation is sufficient, however (Fig. 1) to ensure that a peak height for desmethylindomethacin of up to 10% of that of indomethacin does not significantly interfere. If the amount of desmethylindomethacin did appear to interfere with the estimation of indomethacin, the column length and conditions could be altered accordingly. In studying the interaction of indomethacin and salicylate the levels of desmethylindomethacin in the blood of volunteers was below the limits of detection.

Some other reagents were examined for the preparation of derivatives but they did not offer any advantages over diazoethane. Methylation of indomethacin with diazomethane produced a derivative with a slightly greater response and shorter

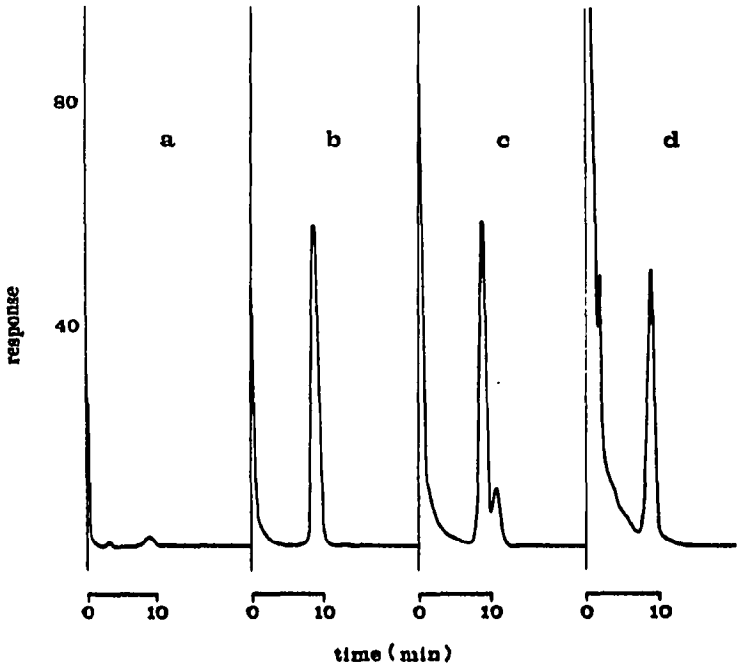

Fig. 1. Chromatograms of indomethacin. (a) 20 ng non-derivatised; (b) 2.5 ng ethylated indomethacin; (c) 2.5 ng ethylated indomethacin+0.25 ng ethylated desmethylindomethacin; (d) extract of human serum estimated to contain 0.5 μg/ml indomethacin.

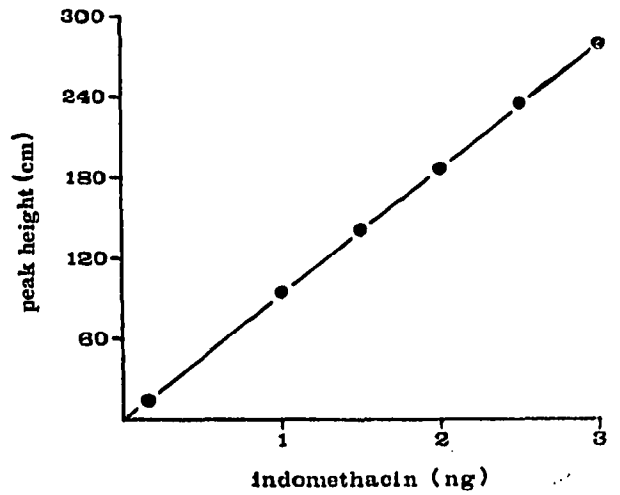

Fig. 2. Detector response of indomethacin.

retention time than the ethylated derivative but also converted desmethylindomethacin to indomethacin. N,O-Bis (trimethylsilyl)acetamide gave derivatives which could be distinguished by gas chromatography and BF_3–methanol formed a derivative with indomethacin only.

REFERENCES

1 P. W. Moller, E. G. McQueen, D. G. Ferry and D. M. Ferry, in preparation.