Journal of Chromatography, 161 (1978) 340–342

CHROM. 11,256

Note

Gas–liquid chromatographic determination of mefenamic acid in human serum

L. J. DUSCI and L. P. HACKETT

State Health Laboratories, Toxicology Section, G.P.O. Box F312, Perth, Western Australia 6001 (Australia)

(Received May 16th, 1978)

Mefenamic acid (Ponstan) is used as an analgesic and anti-inflammatory agent in the management of rheumatoid arthritis[1–4]. Several analytical methods have been published for its analysis including thin-layer chromatography[5], fluorimetry[6,7], colorimetry[8] and electron-capture gas–liquid chromatography[9].

The method described in this paper was devised for the estimation of the drug at therapeutic levels and in cases of overdosage. The procedure is rapid, the acid being butylated with iodobutane, and allows the drug concentrations to be analysed down to a level of 1 mg/l using 2 ml of serum.

EXPERIMENTAL AND RESULTS

Reagents

The following reagents were used: 1 *M* HCl; dichloromethane (nanograde; Mallinckrodt, St. Louis, Mo., U.S.A.); N,N-dimethylacetamide (spectrophotometric grade; Aldrich, Milwaukee, Wisc., U.S.A.); 1-iodobutane (Aldrich); 20% tetramethylammonium hydroxide in methanol (Aldrich).

The internal standard was a 1000 mg/l solution of 5-(*p*-tolyl)-5-phenylhydantoin in ethanol.

Gas–liquid chromatography

A Pye GCD gas chromatograph equipped with a flame ionization detector was used. The column was a 1.5 m × 4 mm I.D. glass tube packed with 3% SP 2250 DA on Supelcoport 100–200 mesh (Supelco, Bellefonte, Pa., U.S.A.). The instrument settings were as follows; injection temperature 285°; detector temperature 290°; carrier gas flow-rate, 60 ml/min; hydrogen flow-rate 60 ml/min; air flow-rate, 240 ml/min. Under these conditions the retention times of mefenamic acid and the internal standard were 1.8 min and 4.1 min, respectively.

Extraction procedure

A 2.0-ml sample of serum was acidified with 0.5 ml of 1 *M* hydrochloric acid and extracted with 15 ml of dichloromethane by shaking for 5 min in a glass tube. The tube was centrifuged and the aqueous phase aspirated from the surface. The organic layer was filtered through a solvent-washed Whatman No. 54 filter paper.

50 μl of the internal standard were added to a 12-ml aliquot of the solvent and the mixture taken to dryness at 50° under a stream of nitrogen. To the residue were added 80 μl N,N-dimethylacetamide, 10 μl tetramethylammonium hydroxide and 20 μl 1-iodobutane. The contents of the tube were mixed after each addition and left to stand for 5 min. The sample was centrifuged and an aliquot of 2.0 μl of the supernatant injected into the gas chromatograph.

Quantitation

To known amounts of standard mefenamic acid in pointed glass tubes was added 50 μl of the internal standard solution. Each sample was taken to dryness, butylated and subjected to gas–liquid chromatography as described above. The ratio of the peak height of mefenamic acid to that of the internal standard was linear over a range of 0.05–0.8 μg of the drug.

Recovery studies

Amounts of mefenamic acid ranging from 2 to 50 μg were added to 2 ml of blank serum in order to examine the efficiency of the extraction procedure. The mean recovery on ten spiked samples (1–25 mg/l) was 82 ± 5%.

Specificity

In all the serum samples examined the gas chromatograms have been free

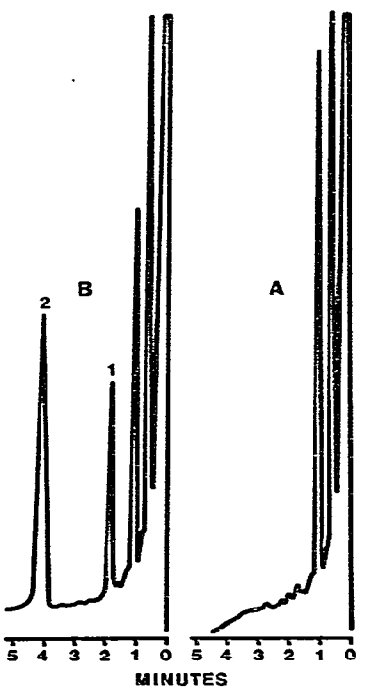

Fig. 1. (A) Gas chromatogram of a blank serum extract. (B) Gas chromatogram of a serum extract containing added mefenamic acid at a concentration of 10 mg/l. Peaks: 1, mefenamic acid (butylated); 2, 5-(*p*-tolyl)-5-phenylhydantoin (butylated).

from any significant interfering peaks. In addition, none of the other common anti-inflammatory drugs such as flufenamic acid, phenylbutazone, indomethacin and ibuprofen were found to have a similar retention time to either butylated mefenamic acid or the internal standard (Fig. 1).

DISCUSSION

Mefenamic acid is usually prescribed in relatively large doses, up to 1.5 g/day, and a plasma level of the free drug in the order of 10 mg/l has been obtained[6].

The method outlined in this paper is specific and allows for the rapid determination of the drug at therapeutic and overdose levels.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the Commissioner of Public Health, Western Australia, for allowing this work to be published.

REFERENCES

1 D. E. Barnado, *Brit. Med. J.*, 2 (1966) 342.
2 W. J. Cahill, *Ann. Phys. Med.*, 8 (1965) 26.
3 E. Buchmann, *Ann. Phys. Med.*, Suppl. 9 (1967) 119.
4 P. Young, *Arthritis Rheum.*, 6 (1963) 307.
5 B. Demetriou and B. G. Osborne, *J. Chromatogr.*, 90 (1974) 405.
6 A. J. Glazko, *Ann. Phys. Med.*, Suppl. 9 (1967) 23.
7 R. A. Buchanan, C. J. Eaton, S. T. Koeff and A. W. Kinkel, *Curr. Ther. Res., Clin. Exp.*, 10 (1968) 592.
8 G. Deveaux, P. Mesnard and A. M. Brisson, *Ann. Pharm. Fr.*, 27 (1969) 239.
9 S. A. Bland, J. W. Blake and R. S. Ray, *J. Chromatogr. Sci.*, 14 (1976) 201.