Journal of Chromatography, 428 (1988) 156-159
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4166

Note

Gas chromatographic–mass spectrometric method for the determination of dimethylacetamide and metabolites in whole blood

BJORN LINDSTRÖM*, PER SJÖBERG and SUSANNE FLOBERG

Department of Drugs, National Board of Health and Welfare, Box 607, S-751 25 Uppsala (Sweden)

(First received November 23rd, 1987; revised manuscript received February 9th, 1988)

Dimethylacetamide (DMA) is a liquid with excellent solvent properties. As such it has a variety of applications, including use as a solvent for intravenously administered drugs. At present there is a need to assess the toxicity of DMA exposure [1]. Monitoring of DMA and potential toxic metabolites in biological fluids is an important part of such an assessment.

This paper describes a gas chromatographic–mass spectrometric (GC–MS) method for the determination of DMA and its metabolites monomethylacetamide (MMA) and acetamide in whole blood.

EXPERIMENTAL

Instrumental

The measurements were performed on a Finnigan 4500 gas chromatograph–mass spectrometer equipped with a multiple-ion monitoring device (Finnigan, Sunnyvale, CA, U.S.A.). The instrument was operated in the electron-impact mode. The column was a DB Wax (60 m × 0.32 mm I.D.) with a stationary phase thickness of 0.5 μm (J.W. Scientific, Folsom, CA, U.S.A.). Between the column and the injector (SGE unijector/direct mode, Scientific Glass Engineering, Victoria, Australia) ca. 1 m of empty capillary tubing was mounted to protect the analytical column. The oven was operated at 160°C, and a helium pressure of 150 kPa (22 p.s.i.) was used to generate the carrier gas flow.

Internal standard

Deuterium-labelled DMA was prepared by treating [$^{2}H_{3}$]acetyl chloride with an excess of dimethylamine in dry diethyl ether. The DMA was purified by dis-

0378-4347/88/$03 50 © 1988 Elsevier Science Publishers B V.

tillation. The purity of the product was checked by MS and found to be adequate for use as internal standard in the quantitation of both MMA and DMA.

Extraction and analysis

To a sample of 100 μl of blood were added 130 μl of distilled water and 10–20 μl of internal standard aqueous solution (2.2 mg/ml). After addition of 250–500 μl of acetonitrile–ethanol (4:1), followed by saturation of the solution with sodium chloride, the sample was shaken on a Vortex mixer and then centrifuged (500 *g*). An aliquot (1 μl) from the organic phase was injected on the GC instrument. The molecular ions *m/z* 59, *m/z* 73 and *m/z* 87 of acetamide, MMA and DMA, respectively, were monitored on the mass spectrometer.

RESULTS AND DISCUSSION

The extraction with acetonitrile–ethanol gave mean recoveries of 90% for both MMA and DMA; the recovery of acetamide was ca. 70%. Sample extractions with solvents used by other investigators, i.e. chloroform [2] or dichloromethane [3], gave considerably lower recoveries.

The detection limits (peak heights twice the background noise level) of MMA and DMA were 1 and 0.5 μg/ml of blood, respectively. Acetamide could be quantitated down to 5 μg/ml (S.D. < 20%). The sensitivities are relatively low because the analysis is done in the low mass region, *m/z* 73 and 87 for MMA and DMA, respectively. In this region there is always considerable background noise due to fragments generated from endogenous compounds in the blood sample.

Several shorter columns (25 m) with different coatings were tested for the separation of the sample. Finally a 60-m DB Wax column with a coating of 0.5 μm was found to give good peak symmetry and sufficient separation from endogenous compounds.

A typical chromatogram obtained by analysing a blood sample containing DMA (216 μg/ml), MMA (36 μg/ml) and internal standard is shown in Fig. 1.

The peak-height ratios between MMA, DMA and the internal standard were used to calculate the concentrations of the acetamides. The calibration graphs were constructed in high and low concentration intervals. Typical intervals were 2–60 and 50–250 μg/ml for MMA and 2–100 and 100–1000 μg/ml for DMA. The curves were linear and passed through the origin. At concentrations of 285 μg/ml for DMA and 61 μg/ml for MMA, the relative standard deviation (R.S.D.) was 2 and 4.5%, respectively ($n=8$ in both cases). The R.S.D. for acetamide was not determined since no measurable levels could be obtained even after very high doses. The concentrations studied were chosen from the mean levels obtained after continuous monitoring of DMA and MMA in rats given an intravenous infusion of 250 mg DMA per kg body mass.

The usefulness of the method was investigated by analysing blood samples of rats given intravenous infusions of 250 or 1000 mg of DMA per kg. These doses are ca. four and twelve times higher than those given to patients during treatment with the chemotherapeutic agent amsacrin [4]. Blood samples were drawn through a jugular vein catheter at the following intervals: 1, 2, 4, 6, 8, 12, 24, 30, 36 and 48

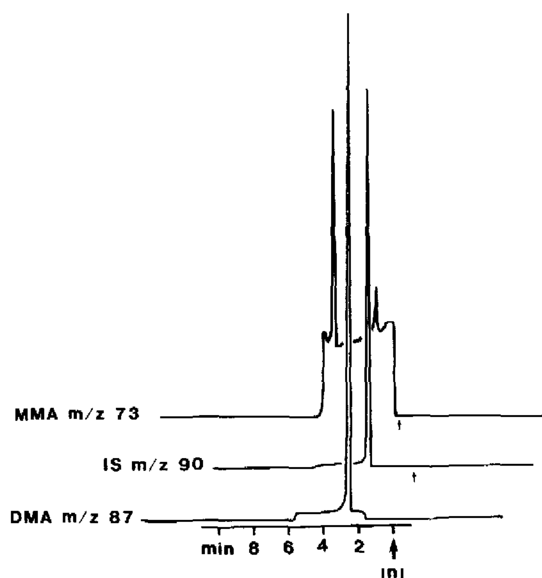

Fig. 1. Chromatogram showing the molecular ions of DMA, MMA and the internal standard (IS). The sample analysed was obtained 6 h after the intravenous administration of 250 mg DMA per kg.

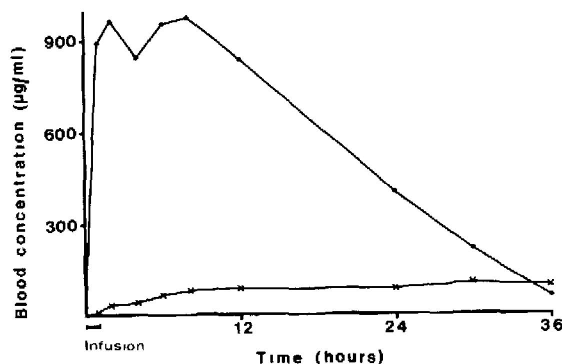

Fig. 2. Blood concentration–time curves of DMA and MMA in a rat given a 1-h infusion of 1000 mg DMA per kg: (●) DMA; (×) MMA.

h. The samples were extracted and analysed for MMA, DMA and acetamide according to the described method. Blood concentration–time curves of DMA and MMA from an animal given 1000 mg DMA per kg are given in Fig. 2. DMA and MMA could be detected up to 36 and 48 h, respectively. The presence of acetamide could only be confirmed in the 24-h sample. The concentration was just above the detection limit and consequently no proper quantitation could be made.

ACKNOWLEDGEMENT

We wish to honour the memory of the late Susanne Floberg, who took a major part in this work.

REFERENCES

1 G.L. Kennedy, Jr., CRC Crit. Rev. Toxicol., 17 (1987) 129-182.
2 V.S. Gladkov, S.M. Sakembaeva and T.A. Solokhina, Gig. Sanit., 2 (1980) 52-54.
3 J.R. Barnes and N.W. Henry, Am. Ind. Hyg. Assoc. J., 35 (1974) 84-87.
4 J. Hornedo and D.A. Van Echo, Pharmacotherapy, 5 (1985) 78-90.