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Note

Assay of methoxyacetic acid in body fluids and tissues by gas chromatography–mass spectrometry following *tert.*-butyldimethylsilylation

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2-Methoxyethanol is a glycol ether widely used in surface coatings and removers, as an anti-icing additive in aviation fuels and in many consumer products. Occupational exposure to this chemical has recently become of great concern because of its teratogenicity and reproductive toxicity in mice, rats and rabbits[1–6]. Evidence has accumulated that the tetragenicity of 2-methoxyethanol is mediated via its metabolite 2-methoxyacetic acid (MAA)[6–9]. Detailed pharmacokinetic studies are necessary for an evaluation of the experimental findings and their significance with regard to the teratogenic risk of exposure of humans to 2-methoxyethanol.

Smallwood *et al.*[10] described a gas chromatographic (GC) method for the assay of MAA using dichloromethane extraction, perfluorobenzylation and flame ionization detection. Although large samples were used (1 ml), the detection limit was 11 μg/ml, which is not sufficiently low for measurements in small samples or for the kinetic analysis of lower concentrations. The GC method by Groeseneken *et al.*[11] is more sensitive, but poor recoveries were obtained.

We have developed an assay method for MAA which employs ethyl acetate extraction of the samples, *tert.*-butyldimethylsilylation of the concentrated extracts and capillary gas chromatographic–mass spectrometric (GC–MS) analysis. The high sensitivity and selectivity of the assay allowed measurements of MAA in small samples of body fluids and tissues.

EXPERIMENTAL

Chemicals and reagents

MAA and the internal standard (*n*-valeric acid) were obtained from EGA-Chemie (Steinheim, F.R.G.), acetonitrile and ethyl acetate (Nanograde) from Promochem (Weser, F.R.G.) and N-methyl-N-(*tert.*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) from Regis (Günther Karl OHG, Geisenheim, F.R.G.).

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Sample preparation

Serum samples (20–50 μl, depending on the concentrations or sample amounts available) were pipetted into 1.5-ml disposable microtubes. Embryo and other tissue homogenates were prepared by adding 200 μl of distilled water and four small glass

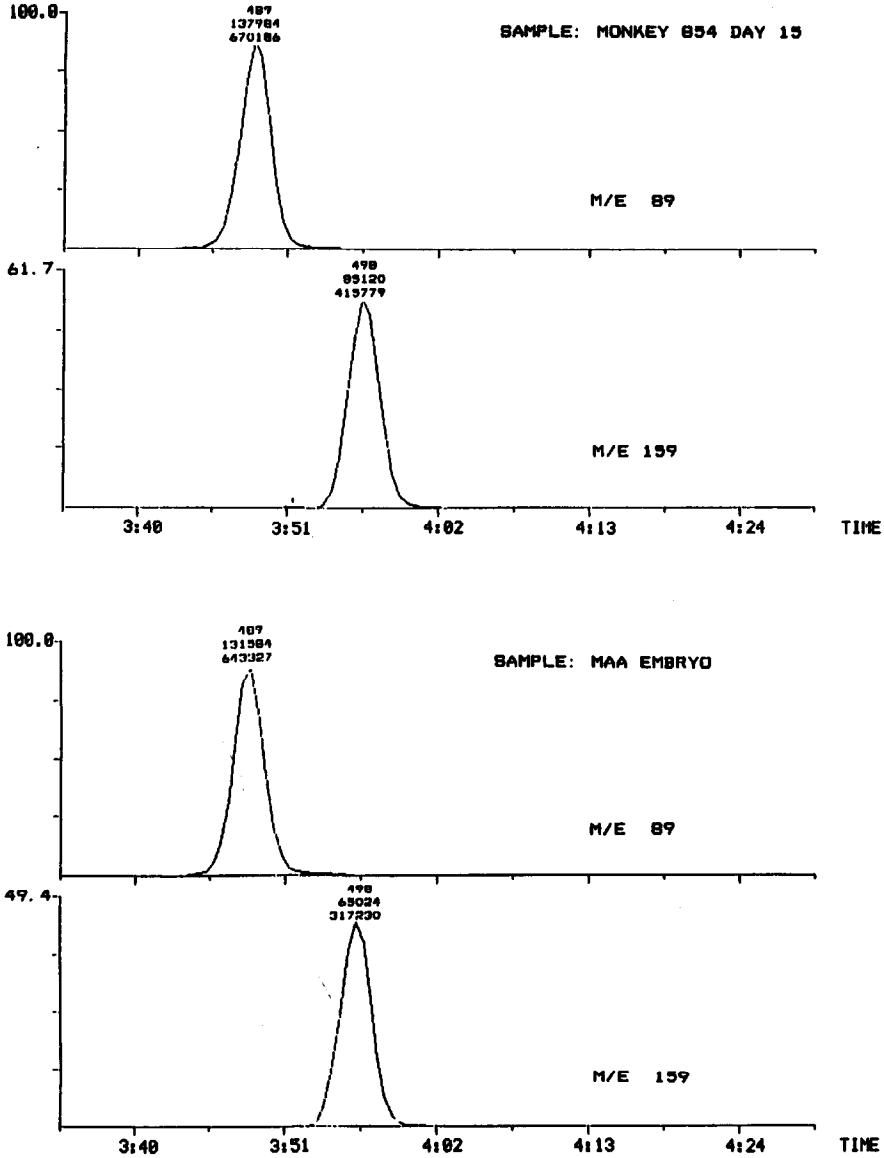

Fig. 1. Selected ion monitoring (*m/z* 89 for MAA; *m/z* 159 for the internal standard, *n*-valeric acid) of extracted and tert.-butyldimethylsilylated samples from (A) monkey treated orally with 0.47 mmol of MAA per kg body weight (50 μl of plasma taken 2 h after drug application containing 115.6 μg·ml/of MAA); (B) monkey embryo following maternal treatment with 0.47 mmol MAA per kg body weight (43 mg wet weight taken 4 h after drug application containing 156 μg MAA per gram of embryo).

pearls (2 mm diameter) to the microtube containing one embryo or a small amount of tissues. The samples were ultrasonicated for 15–30 min at 4°C in an ultrasonication bath to obtain an opaque suspension.

To the serum or homogenates, 50 μl of 1 *N* NaH_2PO_4 buffer (adjusted to pH 5.0), 10 μl of 1 *N* hydrochloric acid and 1.0 ml of ethyl acetate (via a Dispensette from Brand) containing the internal standard (*n*-valeric acid) were added. The tubes were shaken for 20 min and then centrifuged for 2 min at 500 *g* in a Model 5012 Eppendorf centrifuge. An 800-μl portion of the supernatant organic phase was transferred into a 1-ml glass reaction vial and preconcentrated to about 100 μl with a stream of nitrogen, followed by the addition of 100 μl of acetonitrile. The extraction was repeated using 1 ml of ethyl acetate. The combined extracts were evaporated at 30°C under a stream of nitrogen to a final volume of 10–20 μl. The samples were *tert.*-butyldimethylsilylated by adding 20 μl of acetonitrile and 30 μl of MTBSTFA. The samples were allowed to react at room temperature for 20 min or longer prior to analysis by GC–MS. The derivatized compounds were stable for several days in the capped glass vials.

GC–MS analysis

Samples of 1 μl were injected splitless into the GC–MS system (Finnigan-MAT 4600 operated by a 2100D Superincos). The GC separations were achieved using a 30 m × 0.25 mm I.D. bonded phase DB 17 (0.25 μm film thickness) fused-silica capillary column (from ict Handelsgesellschaft, Frankfurt, F.R.G.) with helium as carrier gas (40 cm/s). The initial temperature of 80°C was held for 1 min, then raised at 5°C min^{-1} to 130°C. The injector temperature was 200°C.

The quadrupole mass spectrometer (electron impact, 80 eV electron energy) was operated in the multi-ion detection mode, switching between *m/z* 89 (MAA) and *m/z* 159 (internal standard).

Quantification

Standard samples were prepared by spiking serum from untreated animals directly with MAA at the highest concentration (100 μg/ml). Lower concentrations were prepared by diluting this highest concentration sample with serum. The concentration range was 0.5–100 μg/ml. Calibration graphs were constructed from the data system by plotting the ratios of the peak area of MAA to that of the internal standard.

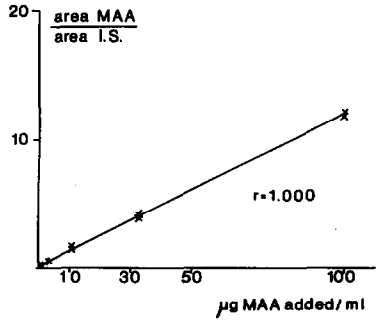

Fig. 2. Calibration graph for determination of MAA.

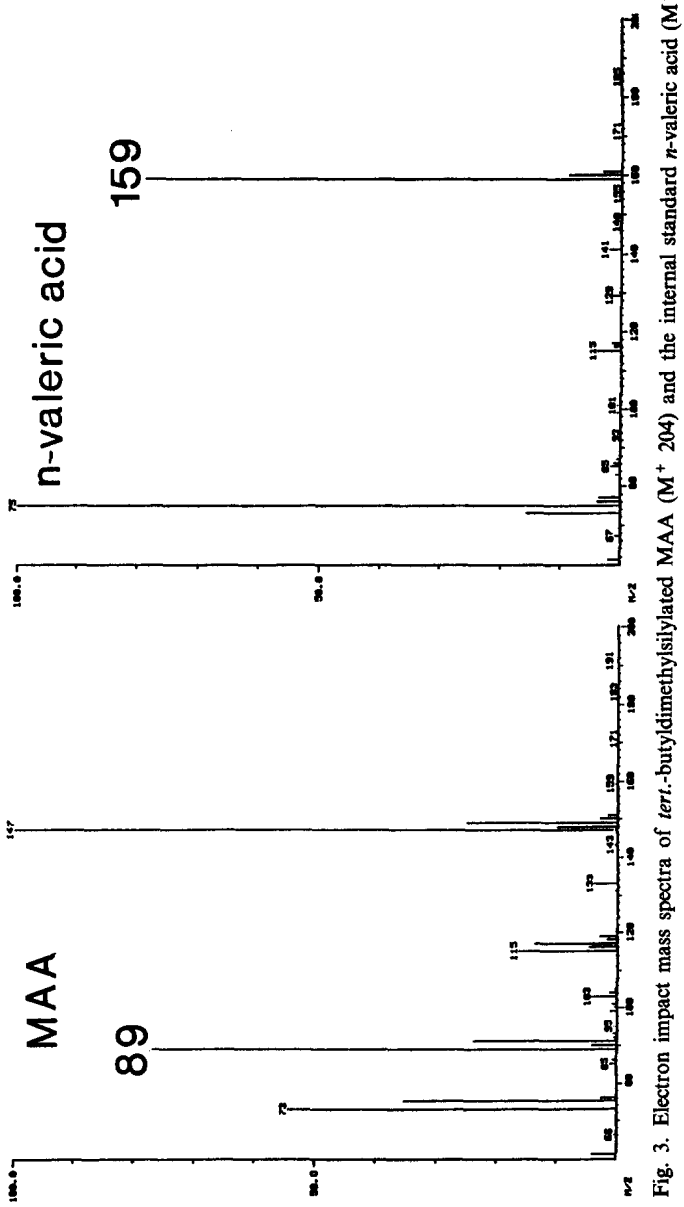

Fig. 3. Electron impact mass spectra of *tert.*-butyldimethylsilylated MAA (M^+ 204) and the internal standard *n*-valeric acid (M^+ 216).

RESULTS

Representative ion chromatograms obtained from the analysis of a monkey serum sample and a monkey embryo homogenate are shown in Fig. 1. The precision of the determination was established by repeated analysis ($n = 10$) of one extracted serum samples all of which were processed through the extraction, derivatization and analysis procedure (<4.3%). The slope of the plot (peak-area ratio *vs.* amount of MAA added) indicated a linear dependence in the concentration range 0.5–100 μg/ml (Fig. 2). The detection limit, using 50-μl samples, was 50 ng/ml. The recovery of the extraction procedure was 90% for serum samples.

DISCUSSION

The derivatives of both MAA and the internal standard exhibited simple mass spectra with few but intense ions, including the M − 57 ions (elimination of *tert.*-butyl group) on electron impact (*m/z* 147 for MAA; *m/z* 159 for *n*-valeric acid) (Fig. 3). We used this M − 57 ion for the measurement of the internal standard (*m/z* 159), but not for MAA, because the ion of *m/z* 147 did not yield an acceptable baseline. Therefore, the ion of *m/z* 89 was chosen for the measurement of MAA because of the very low and stable base values.

The method proved simple to perform, the derivatized samples were very stable and the reproducibility was good. Of great importance is the high sensitivity of the assay: concentrations of 50 ng/ml could be measured with relatively small sample sizes (50 μl).

This assay was applied to the study of the pharmacokinetics of MAA following administration of 2-methoxyethanol to the cynomolgus monkey[12]. Surprisingly, the half-life of the drug was relatively long in this species (around 18–25 h); daily

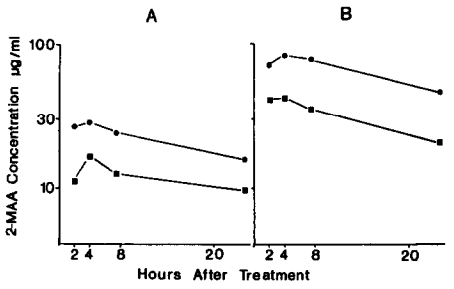

Fig. 4. MAA concentrations in monkey plasma following the first application (■) and after eight daily treatments (●) at two dose levels: (A) 0.16 mmol/kg; (B) 0.32 mmol/kh.

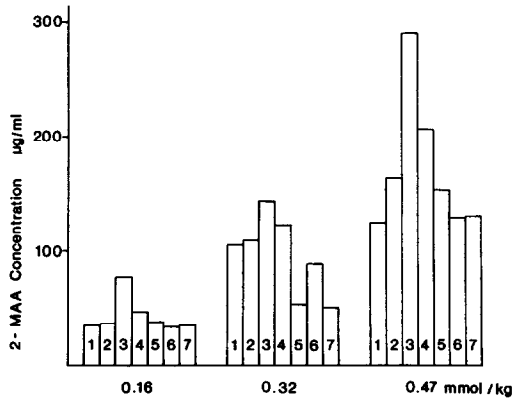

Fig. 5. MAA concentrations in maternal plasma and various gestational tissues and fluids following different doses. Key: 1 = serum maternal; 2 = embryo; 3 = yolk sac; 4 = amniotic fluid; 5 = chorionoc fluid; 6 = placenta secondary; 7 = placenta primary.

administration therefore resulted in considerable drug accumulation (Fig. 4). This finding may be important with regard to human occupational exposure. MAA was also found to cross the placenta in the monkey (Fig. 5). The concentrations in most of the gestational compartments, including the embryo, were similar to those in maternal plasma; the yolk sac exhibited higher concentrations (Fig. 5).

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