Determination of Metaldehyde in Suspected Cases of Animal Poisoning Using Gas Chromatography−Ion Trap Mass Spectrometry

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A method was developed to detect the molluscicide metaldehyde in samples of stomach contents for forensic toxicology investigations. Gas chromatography−ion trap mass spectrometry in full-scan mode was used to identify and quantify metaldehyde. The limit of detection based on mass chromatograms for the *m*/*z* 89 ion was 3 μg/g. Mean recoveries from six different spiked samples were 74% at 25 μg/g and 94% at 500 μg/g. The relative standard deviation of six replicate determinations of a sample containing 632 μg/g metaldehyde was 7.3%.

Keywords: *Metaldehyde; molluscicide; stomach contents; toxicology; GC−MS*

INTRODUCTION

Metaldehyde is a cyclic tetramer of acetaldehyde and is extensively used as a molluscicide throughout Europe and North America for the control of slugs and snails. It is of moderate toxicity to mammals with reported acute oral LD 50 values of 227−690 mg/kg (body weight) for rats, 290−1250 mg/kg (body weight) for rabbits, and 100−1000 mg/kg (body weight) for dogs (Booze and Oehme, 1985a). Despite its relatively low toxicity, it has been a regular cause of poisonings and deaths of animals, with 30 cases of animal poisoning reported in the U.K. alone in 1996 (Fletcher et al., 1997). This laboratory investigates suspected cases of poisoning of nontarget animals by pesticides, including metaldehyde, through the Ministry of Agriculture, Fisheries and Food's Wildlife Incident Investigation Scheme. As an aid in diagnosing poisoning, we require a reliable method for the determination of metaldehyde.

The time from ingestion of metaldehyde to death varies from a few hours to a day or more. In many cases large residues remain in the stomach, and this is the preferred tissue for chemical analysis to diagnose poisoning.

Most published procedures for the analysis of metaldehyde in biological material involve conversion of metaldehyde into acetaldehyde, which is then determined by techniques such as headspace gas chromatography (GC) (Griffiths, 1984) or by GC after conversion to a derivative (Selim and Seiber, 1973). In the procedure previously used in this laboratory, acetaldehyde was determined by high-performance liquid chromatography (HPLC) with fluorescence detection after reaction with 1,3-cyclohexanedione in ammonium acetate solution to form a fluorescent derivative (Brown et al., 1996). As well as being time consuming, the method lacked specificity for metaldehyde, and although it was possible to perform a separate acetaldehyde analysis to determine background levels, this added to the complexity of the procedure. In addition, high background acetaldehyde levels could give rise to poor sensitivity since the total acetaldehyde level determined (metaldehyde-derived + background) needed to be substantially above the background level determined to identify the presence of metaldehyde with any certainty.

Few methods have been published for the determination of metaldehyde without prior conversion to acetaldehyde. A thin-layer chromatography method (TLC) for metaldehyde in plant material has been described (Mays et al., 1968). TLC lacks the specificity required for our needs, and the procedure is troublesome to perform, involving spraying with hot sulfuric acid. Plasma and urine have been analyzed directly by GC with flame ionization detection (FID) without any sample preparation steps (Booze and Oehme, 1985b). In most cases of pesticide poisoning, plasma or urine is not available and solid material must be used. In addition, GC−FID determination may be subject to chromatographic interferences and would not offer the unequivocal identification needed for our investigations, which can sometimes lead to criminal prosecution for the misuse of metaldehyde.

The aim of this work was therefore to develop an analytical method capable of specific and quantitative determination of metaldehyde in animal stomach contents at concentrations of toxicological significance.

MATERIALS AND METHODS

Materials. Metaldehyde standard was purchased from ChemService/Greyhound (Birkenhead, U.K.). Sodium sulfate (analytical grade) and chloroform (HPLC grade) were from Fisher Scientific (Loughborough, U.K.).

Gas Chromatography−Ion Trap Mass Spectrometry (GC−ITMS). Analyses were performed with a Finnigan MAT GCQ ion trap mass spectrometer fitted with an A200S autosampler (Finnigan MAT, Hemel Hempstead, U.K.) with electronic pressure control (EPC). The column was directly coupled to a Restek 20336 model, 4 mm internal diameter (i.d.) injection liner (Thames Restek, Windsor, U.K.). Injections of 1 μL were made into the injector operated at 80 °C. The initial oven temperature was 35 °C, which was held for 1 min, then linearly increased at 25 °C/min to 280 °C, and held for 10 min. The column was 30 m × 0.25 mm i.d. coated with BPX5 at 0.25 μm film thickness (SGE Europe, Milton Keynes, U.K.). The EPC was used to provide a constant linear velocity of helium carrier gas at 40 cm/s. To ensure fast initial injection flow conditions, the carrier gas was programmed for an initial

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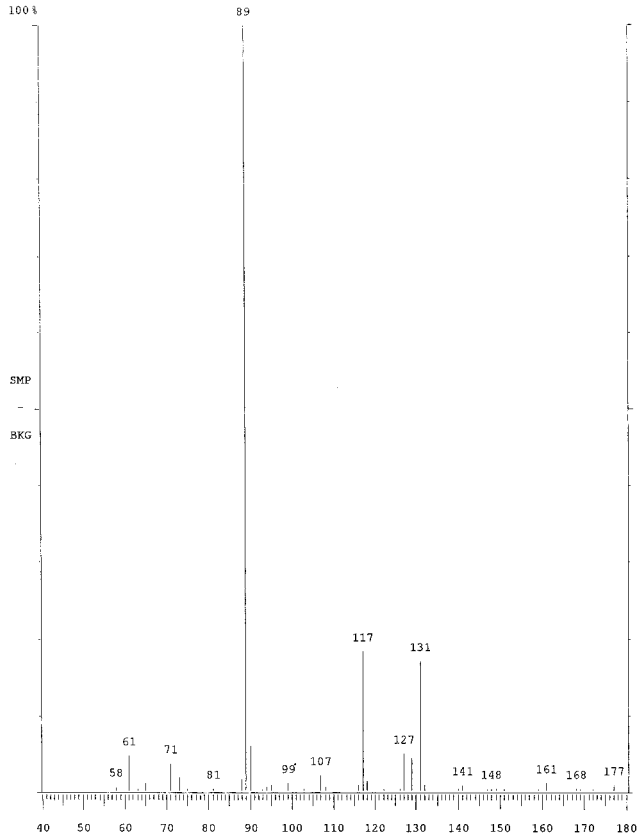

Figure 1. Mass spectrum of metaldehyde (MW = 176) showing characteristic fragment ions at *m/z* 89, 117, and 131.

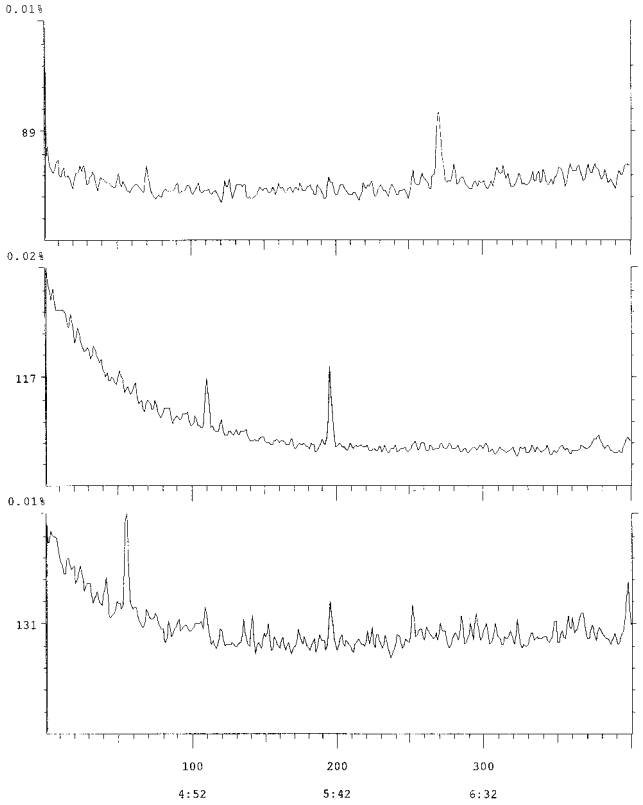

Figure 2. Mass chromatograms for ions at *m/z* 89, 117, and 131 from a blank control stomach contents extract.

pressure of 30 psi, which was held for 1 min. Typical mass spectrometer operating conditions were full-scan acquisition mode from *m/z* 50 to *m/z* 200 at 2 scans/s, ion source temperature 180 °C, electron impact (EI) ionization at 70 eV, and 1450 V multiplier tube voltage.

Sample Preparation. A 2 g subsample of stomach contents was thoroughly homogenized with 10 g of sodium sulfate by grinding in a glass pestle and mortar. After 20 min was allowed for all water to be absorbed, this mixture was transferred to a 250 mL screw-capped Erlenmeyer flask and 40 mL of chloroform added. The flasks were placed on a IKA HS250 reciprocal shaker (Janke and Kunkel, Staufen, Germany) operated at a speed of 300 oscillations/min for 1 h. The resulting extract was filtered through Whatman no. 1 filter paper. The flask was washed with a further 20 mL of chloroform, and this was also filtered. The combined filtrate was adjusted to a volume of 50 mL prior to analysis by GC−ITMS.

GC−ITMS Calibration and Quantitation. All standard solutions were prepared in chloroform. Stock standards were stored at −18 °C and used to prepare spiking standards and working calibration standards. Four-point calibration curves were constructed by comparing concentrations with peak areas from mass chromatograms of *m/z* 89.

RESULTS AND DISCUSSION

Development of the Method. Metaldehyde is not an obvious candidate for analysis by GC since it is thermally unstable and depolymerizes at >112 °C. Nevertheless, the GC conditions used gave rise to sharp symmetrical peaks. In the injection mode used, the column and injector were directly coupled together with no purging. This gave much better sensitivity than a split/splitless injector operated in a typical manner with the purge switched off for 0.5−1 min. Sensitivity was actually greater when the injection temperature was increased to >80 °C but only at the expense of a reduced linear range. A temperature of 80 °C was chosen as the best compromise between sensitivity and linearity for our purposes. The full-scan mass spectrum obtained for metaldehyde is shown in Figure 1. A softer ionization technique such as chemical ionization might have resulted in more ions at higher mass and possible improved sentivity. However, it was possible to achieve the desired sensitivity and selectivity using EI, and this ionization mode was chosen due to its simplicity of operation. No previously published mass spectra of metaldehyde were found, but the spectrum obtained is in good agreement with the entry for metaldehyde in the NIST library. Figures 2 and 3 show that there were few or no chromatographic interferences from stomach contents samples for mass chromatograms at *m/z* 89, 117, and 131. Metaldehyde eluted with a retention time of approximately 5.2 min, but a temperature gradient up to 280 °C and a total run time of 20.8 min were employed to elute strongly retained coextractives from the column, preventing an excessive buildup of these coextractives.

Linearity and Limits of Detection. Four-point calibration curves were linear over the concentration range 0.16−7.53 μg/mL. Linear regression of the peak area against concentration typically gave correlation coefficients of 0.999. The limit of detection (LOD) is here defined as approximately 3 times the baseline noise in the matrix. There was considerable variation in the composition of the stomach contents samples used, resulting in some variation in baseline noise, and hence the LOD, between matrixes. For the samples investigated in this study the LODs, based on the mass chromatogram for *m/z* 89, were 3−4 μg/g.

Recoveries and Reproducibility. The recoveries given in Table 1 were assessed by spiking six different

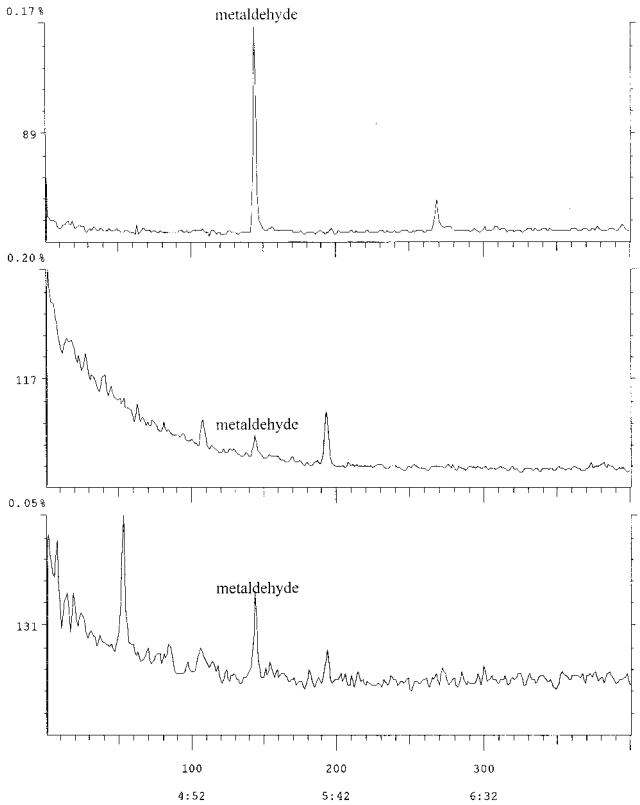

Figure 3. Mass chromatograms for ions at *m*/*z* 89, 117, and 131 from an extract of stomach contents spiked at 25 μg/g.

Table 1. Recoveries of Metaldehyde from Spiked Stomach Contents

spiking level (μg/g)	% recovery (mean ± % RSD)
25	74 ± 9.2, $n = 6$
500	94 ± 18.5, $n = 6$

stomach contents samples: two from cats, two from dogs, and one each from a fox and a cow. Each of the six samples was also analyzed without spiking. Metaldehyde was not detected above the limit of detection in any of the samples. The relative standard deviations are rather high, although it should be remembered that there was considerable variation in the composition of the samples used. The results therefore demonstrate that the method is capable of giving results of acceptable accuracy in a variety of matrixes, something that is very important in methods designed for analysis of stomach contents. The six samples were also analyzed without spiking, and no metaldehyde was detected above the LOD. For the determination of reproducibility, six subsamples from stomach contents samples known to contain metaldehyde were extracted and analyzed. The mean concentration was 632 μg/g with a relative standard deviation of 7.3%.

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