Gas Chromatographic Determination and Mass Spectrometric Confirmation of Malathion in Milk and Blood

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A rapid and sensitive method for the determination of malathion and malaoxon in milk and plasma has been developed. Following a simple extraction and cleanup procedure malathion and malaoxon were analyzed by gas–liquid chromatography (GLC) equipped with a phosphorus-specific detector and a glass column (2.5 m × 3 mm i.d.) packed with 3% SE-30 on 80–100-mesh Chromosorb W AW DMCS. For quantification the internal standard technique was used with bromophos as the internal standard. All compounds eluted within 5 min. Confirmation of the identities was obtained by gas–liquid chromatography–mass spectrometry (GLC–MS) with a flexible quartz capillary column (25 m × 0.20–0.21 mm i.d.) coated with SE-54. Recoveries ranged from 85 to 98%. The detection limits of malathion and malaoxon in milk were 0.002 and 0.02 ppm, respectively, and in plasma 0.004 and 0.04 ppm, respectively. Linearity of the response was observed in the concentration range studied (linear regression coefficient, r = 0.99994, in the concentration range 0.002–0.2 ppm).

The organic phosphorus insecticide malathion, *O,O*-dimethyl *S*-(1,2-dicarbethoxyethyl)phosphorodithioate, is a selective, broad-spectrum insecticide that has a low acute mammalian toxicity. Thus, it has a widespread use in agriculture and as an ectoparasiticide in various species of livestock (Hayes, 1982).

Many of the insecticides in present use on dairy cattle in Norway are unsatisfactory with regard to some toxicological aspects and residues in edible products. A noncommercial formulation of malathion was developed, and for the investigation of some pharmacokinetic properties of malathion in lactating cows following dermal application, a sensitive and specific analytical method was required to determine malathion and malaoxon residues in milk and plasma. This method should also make possible the determination of malaoxon, which is a toxic metabolite of malathion.

Several methods for the determination of malathion in biological material have been reported [Desphande and Bhende (1982), Hladká and Kovác (1973), Hladká et al. (1975), Norris et al. (1958), Oehler et al. (1969), Ruzicka et al. (1967)]. Common drawbacks to most of these methods are that they are time consuming and that the sensitivity is low. Therefore, a rapid method was developed, which fulfilled our demands for sensitivity, specificity, and reproducibility.

EXPERIMENTAL SECTION

Chemicals. Standard bromophos and malathion, 95% quality grade, were obtained from Poly Science Corp. (Niles, England), and malaoxon reference standard was obtained from the U.S. Environmental Protection Agency (Research Triangle Park, NC). Three percent SE-30 on 80/100 mesh Gas Chrom Q was procured from Supelco, Inc. (Bellefonte, PA). Methanol and *n*-hexane were of high-performance liquid chromatographic quality grade.

Procedure. Samples of 15 mL of milk or 10 mL of plasma were transferred to 250-mL Erlenmeyer flasks. Suitable amounts of internal standard, 0.1–10 μg of bromophos in *n*-hexane, and 100 mL of methanol were added. The mixtures were shaken for 30 min and filtered through a Schwarzband No. 589 filter. Fifty-milliliter aliquots of the filtrates and 100 mL of distilled water were extracted for 4 min with 100 mL of *n*-hexane. The water was added to favor the phasic distribution with *n*-hexane. The *n*-hexane phases were dried over anhydrous sodium sulfate (heated at 600 °C for 4 h) and concentrated to 1–10 mL in a rotary vacuum evaporator. Aliquots of 2–5 μL of the *n*-hexane extracts were analyzed by GLC using a phosphorus-specific detector.

Malathion and malaoxon in milk and plasma samples were quantified by the internal standard technique. The standard curve used was prepared by plotting the ratio of the peak heights of malathion or malaoxon and the internal standard against the concentration of malathion or malaoxon. Recovery studies were performed by addition of malathion, malaoxon, and the internal standard to milk and plasma in concentrations of 0.01, 0.05, and 0.1 ppm, with six parallels at each concentration. The identities of malathion and malaoxon in milk and plasma were confirmed by examining volumes of 1 μL of the *n*-hexane extracts by GLC–MS.

Instrumentation. Quantitative measurements of malathion were carried out with a Carlo Erba gas chromatograph Model FTV 2150 (Carlo Erba Instruments, Milano, Italy), equipped with a nitrogen/phosphorus-specific flame ionization detector and a 2.5 m × 3 mm i.d. glass column packed with 3% SE-30 on 80–100-mesh Chromosorb W AW DMCS. The operating temperatures were as follows: column, 205 °C; injector, 250 °C; detector, 205 °C. Helium at a flow rate of 40 mL/min was used as the carrier gas, and the flow rates of hydrogen and air were adjusted for optimal conditions, approximately 44 and 600 mL/min, respectively.

For confirmation of the identities of malathion and malaoxon, a Hewlett-Packard 5992 GLC–MS system (Hewlett-Packard, Palo Alto, CA), equipped with a 25 m × 0.20–0.21 mm i.d. flexible quartz capillary column coated with SE-54, was used. The operating temperatures were as follows: column, 150–250 °C, programmed at 16 °C/min; injector, 80 °C ("splitless injection") or 50 °C ("on-column injection"); ion source, 230–260 °C. Helium at a flow rate of 2 mL/min was used as the carrier gas. Electron energy was 80 eV.

RESULTS AND DISCUSSION

The present GLC system readily separates malathion, malaoxon, and the internal standard bromophos, and all of the substances are eluted within 5 min. Gas chromatograms of malathion, bromophos, and malaoxon stand-

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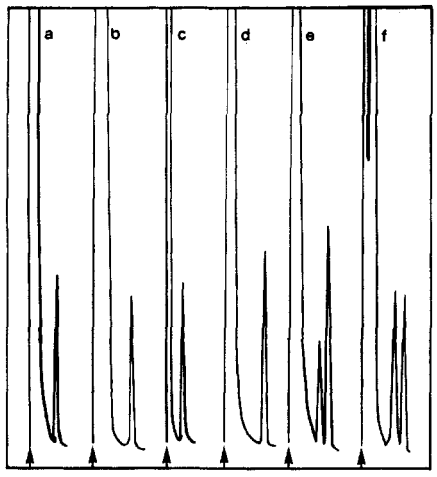

Figure 1. Gas chromatograms: (a) malathion, 0.1 μg/mL of hexane (retention time (t_R) 3.3 min; (b) the internal standard bromophos, 0.1 μg/mL of hexane (t_R 4.3 min); (c) malaoxon, 1.0 μg/mL of hexane (t_R 1.8 min); (d) *n*-hexane extract of a blank milk sample containing 0.3 ppm of the internal standard; (e) *n*-hexane extract of a milk sample containing 0.05 ppm malathion (To the milk sample, collected 6 h after dermal application to the cow of 5 g of malathion, was added 0.07 ppm internal standard); (f) *n*-hexane extract of a blood sample containing 0.04 ppm malathion (To the blood sample, drawn 40 min after intravenous administration to the cow of 2.5 g of malathion, was added 0.05 ppm internal standard). Attenuation was 10 × 64.

ards, an *n*-hexane extract of a blank milk sample containing the internal standard, and an *n*-hexane extract of milk and blood samples obtained from cows treated with malathion are shown in Figure 1. As can be seen, no coextracted compounds interfere with the GLC analysis when a phosphorus-specific detector is used.

The recoveries of malathion, malaoxon, and bromophos added to milk and plasma at levels of 0.01, 0.05, and 0.1 ppm were 85–110%, 20–38%, and 88–96%, respectively, when not evaporated. The reproducibility was satisfactory, the recoveries of six parallels at a concentration of 0.1 ppm of malathion and bromophos were 95.8 and 90.9%, and the standard deviations were 10.1 and 7.9, respectively. The ratio between the recoveries of malathion and bromophos was 1.30 ± 0.10. Even though the recovery of malaoxon was extremely low, the reproducibility of the recovery was acceptable and the variability of the ratios between the recoveries of malaoxon and bromophos was low (0.096–0.110 in the concentration range 0.01–0.1 ppm, concentrated up to 100-fold).

Evaporation up to a 100-fold increase in concentration led to a decrease in the recovery of malathion to 72–81% and to an increase in the recovery of bromophos to 95–105%. This tendency was most pronounced for the highest up-concentrated samples of 0.01 ppm of malathion and bromophos. The recovery of malaoxon was not altered by evaporation.

The detection limits of malathion and malaoxon in milk were 0.002 and 0.02 ppm and in plasma 0.004 and 0.04 ppm, respectively. The detection limits were determined from the lowest concentrations of the compounds present in 1.0 mL of final *n*-hexane extract that gave a GLC peak twice the noise of the base line.

Linearity of the detector response was observed in the concentration range studied (linear regression coefficient, $r = 0.99994$, in the concentration range 0.002–0.2 ppm). The identities of malathion and malaoxon were verified by GLC–MS analysis.

The method described is rapid and simple. The use of a phosphorus-specific detector allows a simple isolation and cleanup procedure. Bromophos is a suitable internal standard that is coextracted and cochromatographed together with malathion due to similar chemical and physical properties. The method fulfilled our demands for specificity, sensitivity, and reproducibility.

Registry No. Malathion, 121-75-5; malaoxon, 1634-78-2.

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