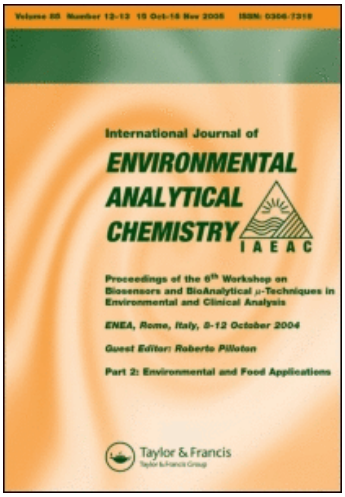

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G. Mariani[a]; E. Benfenati[a]; R. Fanelli[a]
[a] Istituto di Ricerche Farmacologiche "Mario Negri", Milano, Italy

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A NICI-GC-MS METHOD TO ANALYZE ENDOSULFAN IN BIOLOGICAL SAMPLES

G. MARIANI, E. BENFENATI and R. FANELLI

Istituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea 62, 20157 Milano, Italy

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A mass spectrometric method in the negative ion chemical ionization mode was used to analyze α- and β-endosulfan in biological samples. The selected ion recording technique was employed, monitoring the ions at m/z 35 and 37. Biological samples of animals treated with endosulfan were analyzed. The method is selective and sensitive enough to analyze crude extracts of mouse brain and plasma directly without purification, since the chromatograms were clean.

KEY WORDS: Endosulfan, mass spectrometry, negative ions, mouse.

INTRODUCTION

Endosulfan is a chloro-organic insecticide containing two isomers, called α- and β-endosulfan or endosulfan I and II. It is widely used in agriculture and may therefore enter human and animal systems. The most common procedure for the analysis of endosulfan and the other chloro-organic pesticides is gas chromatography using the electron-capture detector (ECD). However, for the analysis of biological samples, a purification step is generally added, with a column filled with a phase such as Florisil, alumina or other materials.[1-3]

Gas chromatography-mass spectrometry (GC-MS) is a useful confirmatory technique for pesticide analysis[4] and the negative ion chemical ionization (NICI) mode is increasingly recommended. One study considered a NICI method for acidic herbicides.[5] We measured DDT, DDE, DDD, hexachlorocyclohexane isomers, hexachlorobenzene, heptachlor and aldrin in pine needles with an NICI method.[6]

Described here is a method to measure α- and β-endosulfan using GC-MS in the NICI technique. This method will be used for toxicological studies on endosulfan.

EXPERIMENTAL PART

Materials

Endosulfan (α:β ratio 2:1) was obtained from Riedel-de Haen, Seelze, Germany. Solvents were pesticide grade.

Animal Treatment

Mice were treated with 1–10 mg of endosulfan i.p., sacrified at selected times and the brain was removed. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and policies.

Sample pretreatment

Brain samples (0.2–0.25g) were homogenized with ethanol (5 ml). The samples were cooled with dry ice and acetone and centrifuged for 5 min at 4000 RPM. The ethanol was drawn off and the samples taken to dryness in a stream of nitrogen. The residue was redissolved in 250 µl of ethanol and 50 ng of DDE were added as internal standard.

Plasma samples were extracted with hexane, and processed like the brain samples.

Instrumental analysis

A mass spectrometer VG TS-250 coupled with a gas chromatograph HP 5890 was used for the analysis in the NICI mode, and an HP 5971 mass selective detector, coupled to an HP 5890 gas chromatograph, for the analysis in the electron impact (EI) mode.

The chromatographic column was a CPSil8 CB, 15 m X 0.32 mm internal diameter, with a film thickness of 0.12 µm. The head pressure was 40 kPa. The injector was at 240°C. The chromatographic program was: 80°C for 1 min, then programmed temperature steps (20°C/min) to 280°C.

In the NICI mode the mass spectrometer was operated using methane as ionizing gas. The recorded ions were at m/z 35 and 37, corresponding to the isotopic chlorine ions. In the EI mode the recorded ions were at m/z 195 and 246.

Calibration curve

The calibration curve was constructed using a standard solution of endosulfan at different concentrations (0, 50, 250, 1000 ng in 250 µl of absolute ethanol). We also spiked brain samples (0.2 g) of untreated mice with endosulfan (0, 250, 500 and 1000 ng) and processed the samples as described.

RESULTS

The calibration curves for standard solutions were linear from 0 to 1000 ng of endosulfan injected; the equation for α-endosulfan was $y = 0.0108x - 0.041$ ($r = 0.993$) and $y = 0.000184x - 0.0001$ ($r = 0.994$) for β-endosulfan. For the spiked brain samples we checked

the linearity from 0 to 1000 ng added; the equation for α-endosulfan was $y = 0.0158x - 0.370$ ($r = 0.997$) and $y = 0.0044x + 0.007$ ($r = 0.999$) for β-endosulfan. The limit of detection was 10 pg injected and was the same for α- and β-endosulfan. The limit of concentration detectable was 5 ng/ml for plasma samples and 5 ng/g for brain samples. These limits were the same for α- and β-endosulfan. The recovery of endosulfan from brain and plasma samples was between 85 and 93%. The analysis of samples from four animals given the same dose (10 mg/kg) gave reproducible results: in the four brain samples α-endosulfan was 816.4 ± 68.0 ng/g, and β-endosulfan was 325.4 ± 100.2 ng/g. In plasma the levels were 149.5 ± 32.6 ng/g for α-endosulfan and 221.2 ± 43.0 ng/g for β-endosulfan; these data include the variability in absorption.

Figure 1 shows the ion traces of a brain extract sample from a mouse injected with endosulfan using GC-MS in the NICI technique. The trace in the upper box corresponds to the chlorine-35 ion, and the trace in Figure 1B to the chlorine-37 ion. The intensity ratio of the areas of the peaks in the two traces is 3 to 1, as expected. This ratio has to be checked in all analyses, particularly of biological samples, to assess correct identification.

The injected solution has not been purified. The chromatogram is clean, with no interfering peaks, even though it is a crude extract. We obtained the same result with plasma samples.

This method allows the direct analysis of biological samples, which simplifies the whole procedure, while also improving its accuracy and reproducibility, since the variability of the purification column recovery is avoided.

Considering all the parameters measured, there are four elements of selectivity: the retention time, the presence of the peak in the first ion trade, the corresponding peak in the second ion trace, and the ratio between the areas of the peaks in the two traces. Analysis with the ECD relies on two parameters for the identification of a compound: the retention time and the answer to the detector, that may be due to several atoms.

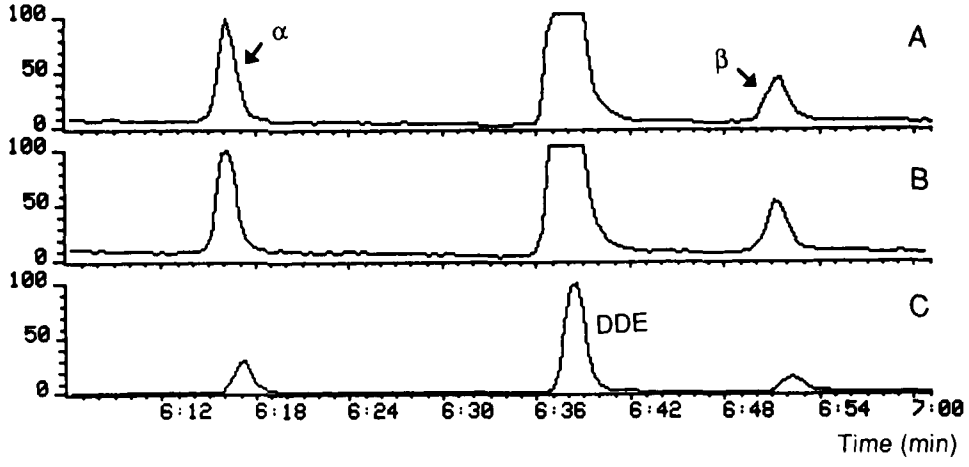

Figure 1 NICI-GC-MS ion traces at m/z 35 (A) and 37 (B) showing the two endosulfan isomers (peaks α and β) and the DDE (internal standard) in a crude brain extract from a mouse given 0.5 mg/kg of endosulfan. The concentration of the α isomer was 47 ng/g; the β isomer was 48 ng/g. Figure 1C is the same as 1A, but normalized to the DDE peak.

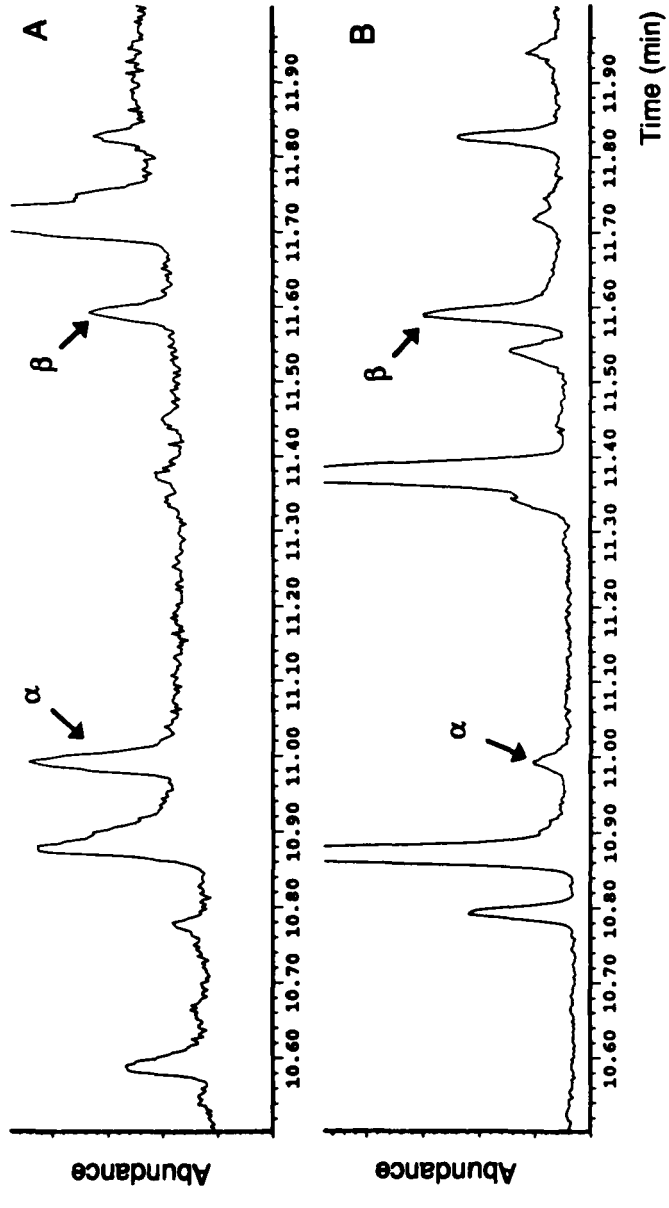

Figure 2 EI-GC-MS ion traces at m/z 195 (A) and 246 (B) showing the two endosulfan isomers (peaks α and β) and the DDE (internal standard) in a crude brain extract from a mouse given 10 mg/kg of endosulfan. Some interferences are present.

Recently another study using NICI-MS to confirm an ECD analysis of endosulfan in water samples was reported.[7] The monitored ions correspond to molecular ions and fragments in the high mass region of the spectrum. In our hands sensitivity to the chlorine ions was better, so we preferred to monitor these. Of course, if sensitivity is not a problem other ions can be added. In this case the method is more selective and the danger of interferences from other chlorinated compound is greatly reduced.

Many interfering peaks were present in the EI analysis of a crude brain extract from a mouse given 0.5 mg/kg of endosulfan (Figure 2).

We used this procedure to study the kinetics of endosulfan in mice. Figure 3 shows the time-course of brain concentrations after injection of 5 mg/kg of total endosulfan. Maximal concentration of endosulfan was achieved 20 min after administration of endosulfan.

CONCLUSIONS

We present here a fast and easy analytical procedure with very simple sample treatment. Analysis of the two endosulfan isomers showed good sensitivity. The use of MS gave the advantage of good selectivity, on account of the analysis of ion traces. With the NICI technique recording the chlorine ions at m/z 35 and 37, crude extracts of mouse plasma and brain could be analyzed without any purification. This results is a fast procedure, and avoids the danger of loss of analytes during the clean-up steps. This method has proved useful in toxicological studies of the kinetics of endosulfan in mice.

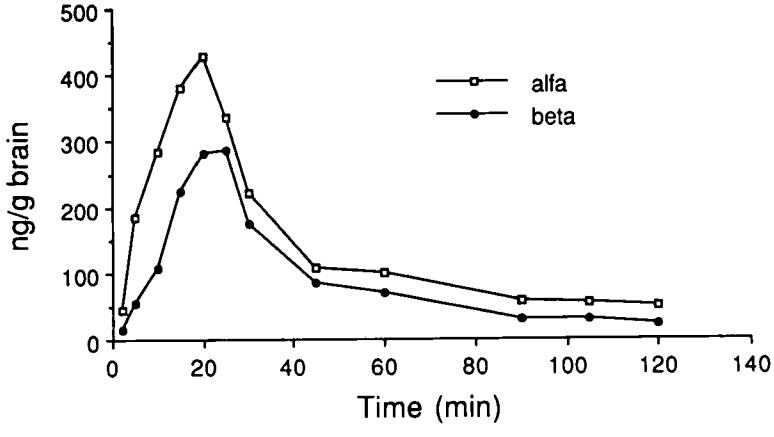

Figure 3 Time-course of brain endosulfan concentrations after injection of 10 mg/kg i.p..

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