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Note

High-performance liquid chromatographic analysis of aldicarb in the stomach contents of birds of prey

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Aldicarb [2-methyl-2-(methylthio)propionaldehyde O-(methylcarbamoyl)oxime] is the active principle of the pesticide Temik®. It is a compound with a systemic action and it has insecticidal, nematicidal and acaricidal properties. Temik is formulated as small granules for direct application on soil. It is one of the most toxic pesticides: the acute oral LD_{50} for rats is 0.93 mg/kg body weight[1].

In recent years, deliberate aldicarb poisoning of certain kinds of animals has been increasing in The Netherlands. Determination by semi-quantitative thin-layer chromatography, based on detection by acetylcholinesterase inhibition[2–4], did not allow sufficiently low detection limits for aldicarb. This method also lacked specificity with respect to paraoxon, a metabolite of the likewise often illegally used pesticide parathion.

The present method was devised as a rapid screening test for aldicarb in gizzard or stomach contents or as a confirmatory method in combination with thin-layer chromatographic results, thus permitting the unequivocal diagnosis of aldicarb intoxication.

EXPERIMENTAL

A 5-g amount of accurately weighed gizzard or stomach contents was ground in a mortar with anhydrous sodium sulphate to a free flowing powder. The powder was extracted with 30 ml of chloroform in a glass-stoppered erlenmeyer flask by mechanical shaking for 30 min. The extract was filtered through Whatman 41 paper and the residue was extracted twice with 30 ml of chloroform. The combined chloroform filtrates were transferred into a glass clean-up column (15 × 1 cm I.D.) filled successively with 2 g of anhydrous sodium sulphate, 10 g of freshly prepared neutral alumina with a 7.5% water content and 2 g of anhydrous sodium sulphate. The column was pre-washed with 10 ml chloroform. After transfer of the chloroform extract, the column was rinsed with 100 ml of chloroform. The eluate was collected, 2 ml of water were added and chloroform was distilled off in a rotary evaporator at 28°C. The residue was transferred with 10 ml of water into a disposable Baker-10 SPE octadecyl column, which was pre-treated with methanol and water. The disposable column was washed with two 5-ml volumes of water and aspirated dry during 5 min under vacuum. Aldicarb was eluted with 1 ml of methanol and 50 μl of the

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TABLE I

MEAN RECOVERIES OF ALDICARB IN GIZZARD AND STOMACH CONTENTS OF BIRDS OF PREY

Sample	*Aldicarb (mg/kg)*	*No. of samples*	*Mean recovery ± S.D. (%)*
Gizzard contents	5	4	104 ± 10
Gizzard contents	10	4	89 ± 11
Stomach contents	5	5	97 ± 25
Stomach contents	10	5	75 ± 4

eluate were injected into a Spectra-Physics SP 8700 high-performance liquid chromatograph equipped with an SP 4100 computing integrator and an SP 8400 UV detector at 247 nm, 0.01 a.u.f.s. The columns used were LiChrosorb 10 RP-18 (Chrompack, Middelburg, The Netherlands) or CPSpher C_{18} (Chrompack) (250 mm × 4.6 mm I.D.) with a guard column (75 mm × 2.1 mm I.D.) containing a pellicular RP-18 packing. The mobile phase was acetonitrile–water (27:73) at a flow-rate of 1.4 ml/min. Quantitation of aldicarb was based on standard solutions of aldicarb using external standard calibration.

RESULTS

Table I summarizes the results of some recovery experiments. Gizzard and stomach contents of birds of prey were spiked with known amounts of aldicarb. Fig. 1 shows a typical chromatogram representing a cleaned extract of the gizzard contents of an aldicarb-intoxicated buzzard.

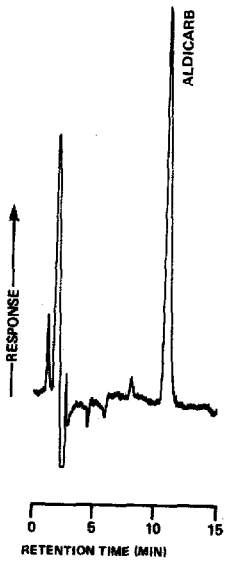

Fig. 1. Chromatograam of a cleaned extract of crop contents of an aldicarb-intoxicated buzzard.

The detection limit for standard solutions of aldicarb (signal-to-noise ratio = 3) is 0.25 ng. From a practical point of view, the detection limit for real samples was estimated to be 60 μg/kg. Although the retention behaviour of paraoxon and aldicarb on silica thin-layer plates is similar, the present method achieves a good separation of these compounds. Aldicarb sulphone and aldicarb sulphoxide, two toxic metabolites of aldicarb, did not interfere with the aldicarb peak.

DISCUSSION

Several other specific and sensitive methods for the detection of aldicarb have been published. High-performance liquid chromatographic (HPLC) methods use post-column fluorimetric labelling procedures[5–8], UV detection at 190–220 nm[9,10] or 254 nm[11]. Matrices encountered are vegetable crops[5,6] and water and soil[7,10,11]. Complex matrices such as liver and meat require oxidation to aldicarb sulphone, which can be analysed by gas–liquid chromatography with flame photometric detection[12]. In order to avoid this derivatization and in the absence of a flame photometric detector or post-column derivatization unit, an efficient clean-up procedure had to be developed for complex matrices, thus permitting HPLC analysis with UV detection.

The UV spectrum of aldicarb shows an absorption maximum at 207 nm. It appeared, that it was impossible to use this wavelength because of many interfering peaks caused by matrix components. Another maximum at 247 nm proved to be satisfactory with respect to the clean-up procedure and interfering peaks (see Fig. 1). Table I shows acceptable recoveries for this method. The method has proved valuable in daily routine analysis, especially in those instances where thin-layer chromatographic results were doubtful. Several aldicarb intoxications have been confirmed involving pet animals, foxes and birds of prey. In summary, this method is a valuable tool for screening purposes and for supplying confirmatory evidence in diagnosing cases of suspected aldicarb intoxication.

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