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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF CARBARYL AND 1-NAPHTHOL IN BIOLOGICAL FLUIDS

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SUMMARY

A simple and sensitive method for the simultaneous analysis of carbaryl and 1-naphthol in whole blood by reversed-phase high-performance liquid chromatography and fluorescence detection is described. Spiked blood (heparinized) containing an internal standard was hemolyzed and extracted with ethyl acetate. After centrifugation the extractant was removed and taken to dryness. Reconstitution and subsequent high-performance liquid chromatography–fluorescence analysis yielded linear standard curves for carbaryl and 1-naphthol. Linear response *vs.* concentration profiles were obtained for carbaryl and 1-naphthol extracted from buffer solutions as well. A simple chemical hydrolysis study of carbaryl is included to illustrate the effectiveness of the extraction procedure and assay.

INTRODUCTION

Carbaryl (Sevin®) is an anticholinesterase insecticide that is widely used as an agricultural[1] and forest[2,3] spray in addition to home lawn and garden applications. Gas–liquid chromatography (GLC) has been widely used for the determination of carbaryl[4,5] and seems to be the method of choice for the analysis of plant material[6,7], soil[8], and agriculturally produced foods[9]. ^{14}C-labeled carbaryl has been extensively employed for the quantitative determination of the insecticide in experiments involving laboratory animals[10–21]. The use of direct fluorescence spectroscopy for the specific and sensitive determination of carbaryl and/or the 1-naphthol hydrolysis product would be ideal but this alternative is problematic when interfering substances from biological extracts are introduced[22]. Normal-phase high-performance liquid chromatography (HPLC) has successfully been used to measure carbaryl levels at 220–254 nm[23–25] and for the separation of carbaryl in analytical stock solutions[26]. Fluorescence detection HPLC has been used to measure carbaryl directly or as a Dns derivative[23]. Reversed-phase HPLC has been used to quantitate carbaryl as 1-naphthol in honey bees[27].

The preceding techniques enable the determination of carbaryl or 1-naphthol from various media. Since the extrahepatic metabolism of carbaryl *in vivo*, in large part, consists of the enzymatic and chemical hydrolysis of carbaryl to 1-naphthol it is of interest to quantitate the levels of each.

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This paper describes a simple and sensitive method for the simultaneous extraction and analysis of carbaryl and 1-naphthol from whole blood for use in metabolic and or pharmacokinetic studies. The utility of the reversed-phase HPLC assay will be demonstrated by studying the kinetics of the chemical hydrolysis of carbaryl in a buffer system.

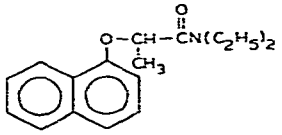
MATERIAL AND METHODS

HPLC and fluorescence detection

Reversed-phase HPLC was found to be ideal for the successful separation of carbaryl from its metabolite (hydrolysis product) 1-naphthol. A Micromeritics 750 Solvent Delivery System was employed to pump the mobile phase (acetonitrile–0.13 *M* phosphate buffer pH 6.2, 2:3) through the Alltech column. Separation was attained with a 25 cm × 4.6 mm column packed with a C_8 stationary phase bonded to particles averaging 5 μm in size. Mobile phase was pumped at a rate of 1.42 ml/min. The column effluent was exposed to an excitation wavelength of 285 nm and emission intensity measured above 340 nm (340 cutoff filter) at room temperature using a Schoeffel FS 970 fluorescence detector.

Internal standard

Napropamide was chosen as the internal standard since its fluorescence and chromatographic properties were similar to each of the compounds of interest.



The maximum emission and excitation wavelengths were elucidated by scanning a solution of each in mobile phase using an Aminco-Bowman spectrophotofluorometer (No. 4-8202). The excitation and emission maxima are; carbaryl 288, 342; 1-naphthol 306, 460; and napropamide: 296, 346 nm, respectively.

Whole blood standard curves

A carbaryl standard curve was constructed by adding carbaryl (10–500 ng) and 1-naphthol (52 ng) to 0.25 ml heparinized whole blood samples. The 1-naphthol standard curve was produced in an analogous fashion by adding 1-naphthol (12.5–525 ng) and carbaryl (50 ng) to the 0.25 ml blood samples.

PTFE-lined screw capped Kahn (centrifuge) tubes containing the blood samples were prepared for analysis by adding 162 ng of napropamide (residue from 25 μl of a 6.5 μg/ml stock solution) and 0.25 ml distilled water. After red blood cell hemolysis occurred (5–10 min), 2.5 ml ethyl acetate (HPLC grade) was introduced and the extraction performed by shaking the capped tubes for 10 min. The mixture was centrifuged at 1200 *g* for 8 min and approximately 2 ml of the clear supernatant solution transferred to a clean 15-ml conical centrifuge tube. The sample was evaporated to dryness under a gentle stream of nitrogen and reconstituted in 0.25 ml of ethanol–mobile phase (50:50) for analysis. A 50- to 100-μl portion of this solution

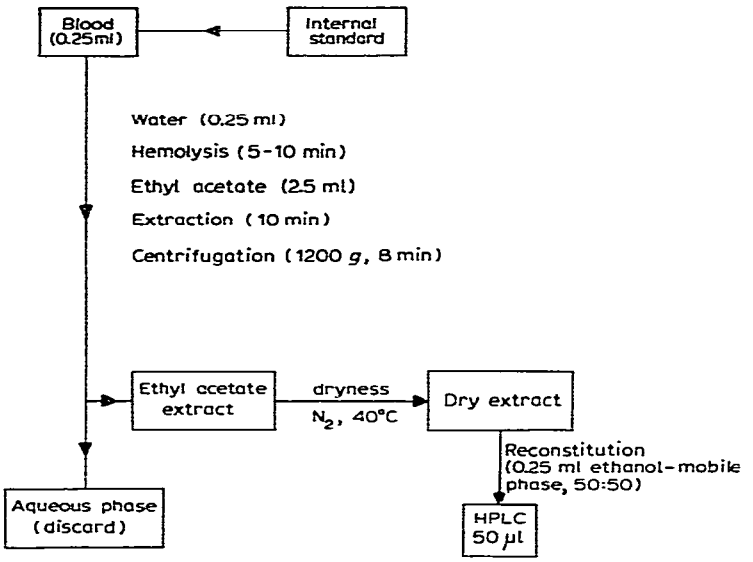


Fig. 1. Flow chart for the extraction and analysis of carbaryl and 1-naphthol from whole blood.

was injected into the HPLC system. The blood extraction procedure is illustrated schematically in Fig. 1.

Buffer standard curves

Aliquots of buffer solution were added to tubes containing carbaryl (12.5–400 ng). The 1-naphthol buffer standard curve involved the addition of 12.5–525 ng of 1-naphthol to the 0.25-ml buffer aliquots. Buffer solutions were prepared for analysis by first adding 0.25 ml of an ethanolic napropamide solution (650 ng/ml) to the buffer solution. The mixture was vortexed for 20 sec (or until a solution resulted) and 50–100 µl injected into the HPLC system.

Chemical hydrolysis of carbaryl in vitro

A pH 7.4 isotonic phosphate buffer was prepared with a capacity of 0.008 equiv./l (0.78 g KH_2PO_4, 1.26 g Na_2HPO_4, 8.0 g NaCl per l) and ionic strength of 0.17. A rubber stoppered glass vial containing approximately 12.3 µg carbaryl and 7 ml buffer (warmed to 37°C) was stirred for 5 min to allow for complete dissolution. The reaction solution was incubated at 37°C and 0.25 ml aliquots were obtained at appropriate time intervals and added to tubes containing 0.25 ml of a 650 ng/ml ethanolic solution of napropamide (162 ng). After vortexing 50 to 100 µl was injected into the HPLC system for analysis.

RESULTS AND DISCUSSION

Chromatograms and standard curves

Sample chromatograms from blank blood and blood fortified with carbaryl and 1-naphthol are presented in Fig. 2. The carbaryl and 1-naphthol peaks are well separated and the endogenous compounds that are coextracted do not interfere with

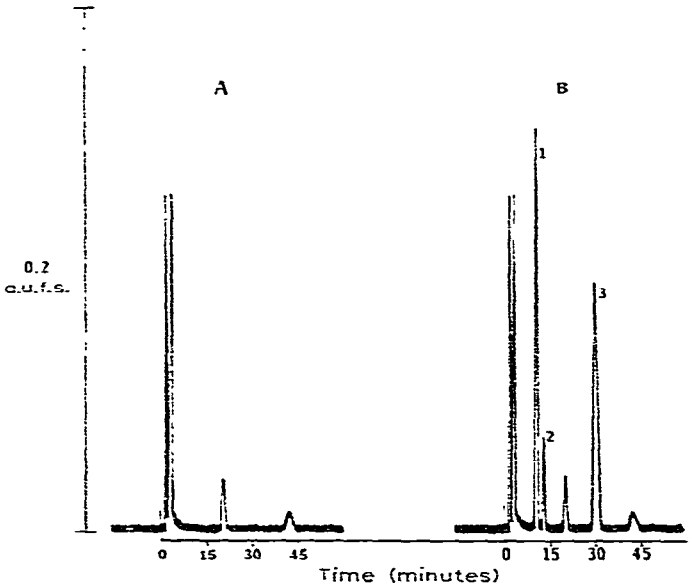


Fig. 2. HPLC–fluorescence chromatograms of: A, blank blood and B, blood fortified with (1) carbaryl, 400 ng ml; (2) 1-naphthol, 52 ng ml; and (3) napropramide, 650 ng ml.

TABLE I

REPRODUCIBILITY OF THE MEASUREMENT OF CARBARYL AT VARYING BLOOD CONCENTRATIONS

Spiked concentrations (*ng ml*)	*Number of observations*	*Area ratio* $\left(\frac{\text{carbaryl area}}{\text{int. st. area}}\right)$	*Standard deviation* (*ng ml*)	*Coefficient of variation* (%)
40	3	0.064	0.005	7.8
80	3	0.128	0.003	2.3
200	3	0.284	0.005	1.8
400	3	0.627	0.007	1.1
600	3	0.872	0.017	1.9
800	3	1.193	0.009	0.8
1200	3	1.731	0.019	1.1
1600	3	2.302	0.011	0.5
2000	3	2.919	0.027	0.9

any of the peaks of interest. The retention times are: carbaryl 10.6 min, 1-naphthol: 12.52 min. and napropamide: 30.0 min.

Standard curves were constructed using the peak area ratio method. The response of both compounds in blood and buffer was linear over the range of concentrations studied ($r^2 > 0.998$).

The precision of the method was determined by analyzing fortified blood samples of carbaryl. The results are tabulated in Table I.

The extraction and quantitation of the two compounds of interest from plasma

and urine using the same protocol has been accomplished in an equally effective manner.

The baseline separation of carbaryl and 1-naphthol with this C_8 column requires a mobile phase of at least 60% buffer (not more than 40% acetonitrile). However, the chromatographic analysis of carbaryl and 1-naphthol can be performed in less than one-half the time if complete baseline separation is not required. An acetonitrile–0.013 *M* phosphate buffer (pH 6.1) (50:50) mixture produces retention times of 6.94, 7.73 and 14.1 min for carbaryl, 1-naphthol, and napropamide, respectively, at a flow-rate of 1.2 ml/min. Standard curves under these conditions are also linear.

The chemical hydrolysis of carbaryl to 1-naphthol at 37°C under the conditions described previously appeared to be first order with an apparent rate constant of 0.0351 h^{-1} (half-life = 19.74 h). The logarithm of the concentration of carbaryl remaining *vs.* time is plotted in Fig. 3. The appearance of 1-naphthol, of course, could have been utilized as effectively to establish the degradation rate.

In conclusion, the simultaneous separation and subsequent analysis of carbaryl and 1-naphthol in whole blood or aqueous solution by reversed-phase HPLC can be achieved using fluorescence detection. The described assay offers several advantages over other methods of analysis. Since carbaryl and 1-naphthol are chromatographically separated before an attempt at detection is made both compounds can be measured independently. Colorimetric and GLC–electron capture detection assays require conversion of all carbaryl to 1-naphthol prior to complexation or derivatization so that the relative contributions of each are unknown. Direct UV determination measures carbaryl plus 1-naphthol as does ring-labeled ^{14}C analysis and, again, individual concentrations cannot be ascertained. GLC with nitrogen–phosphorus detection as well as methods using ^{14}C on the carbonyl group of the pesticide yield carbaryl levels only. The present analytical technique thus provides a specific and sensitive method which can be used in studying the pharmacokinetics and stability of carbaryl.

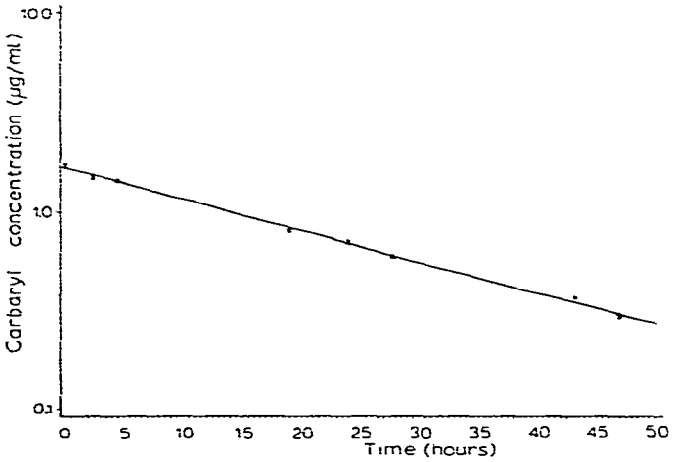


Fig. 3. Log linear decline of carbaryl concentration with time in pH 7.4 phosphate buffer (isotonic, ionic strength 0.17, capacity 0.008 equiv./l).

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