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Note

High-performance liquid chromatographic separation of indandione rodenticides

JOEL E. HOUGLUM*, RICHARD D. LARSON and ROSE M. NEAL
*Chemistry Department and *College of Pharmacy, South Dakota State University, Brookings, SD 57007 (U.S.A.)*
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Chlorophacinone {2-[(*p*-chlorophenyl)phenylacetyl]-1,3-indandione}, diphacinone [2-(diphenylacetyl)-1,3-indandione], pindone (2-pivaloyl-1,3-indandione) and valone (2-isovaleryl-1,3-indandione) are used as anticoagulant rodenticides. These indandiones are available as concentrates, tracking powders or as ready-to-use baits on whole or crushed grain. There are published reports of high-performance liquid chromatographic (HPLC) separations of two[1–3] or three[4] of these indandiones, but no reports of an HPLC method that separates all four of these compounds. After attempting modifications of these reported procedures, we eventually developed a new procedure which utilizes a CN column with phosphoric acid in the mobile phase.

The purpose of this research, as a result of investigating the identity of a mislabelled sample, was to develop an HPLC method that could be used to separate all four of these indandione rodenticides so as to be able to identify which one is present in a sample of unknown or questionable content.

EXPERIMENTAL

Reagents and materials

Diphacinone was obtained from Beltsville Agricultural Research Center (Beltsville, MD, U.S.A.), chlorophacinone from EPA (Research Triangle Park, NC, U.S.A.) and pindone and valone from Bell Labs. (Madison, WI, U.S.A.).

Standard stock solutions were prepared in methanol at 500 μg/ml. Intermediate standard solutions were prepared at 5 μg/ml by dilution of 1 ml of stock solution to 100 ml with acetonitrile for chlorophacinone and diphacinone or bringing to volume with methanol–acetonitrile (1:3, v/v) for pindone and valone. Working standard solutions of 2.5 μg/ml were prepared from 5 ml of intermediate solution diluted to 10 ml with 0.2% (v/v) phosphoric acid.

The solvent to extract chlorophacinone and diphacinone was acetonitrile, whereas the extraction solvent for pindone and valone was methanol–acetonitrile (1:3, v/v).

Apparatus

The HPLC system consisted of an Alltech (5 μm) cartridge CN column (150

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mm × 4.6 mm I.D.) with a direct-connect CN guard column (10 mm × 4.6 mm), a Waters 6000 pump at a flow-rate of 1.5 ml/min, a Valco C6U injector with a 25-μl loop, an Isco V^4 variable-wavelength detector at 280 nm and a Varian Model 9176 chart recorder. The mobile phase consisted of acetonitrile–phosphoric acid solution (45:55, v/v). The phosphoric acid solution was 0.2% (v/v) in water. For samples injected at 2.5 μg/ml, the detector sensitivity was set at 0.01 absorbance units full scale for pindone and valone and at 0.005 absorbance units full scale for chlorophacinone and diphacinone.

Procedure

To prepare spiked samples, 1 ml of standard stock solution was added to 10 g ground corn to give 0.005% chlorophacinone or diphacinone, and 1 ml to 2 g ground corn to give 0.025% pindone or valone. The spiked bait was dried under a gentle stream of nitrogen and extracted with 100 ml of extraction solvent for 1 h on a mechanical shaker. After centrifugation for 5 min at 1500 rpm (500 *g*), 5 ml of the extract were diluted to 10 ml with 0.2% phosphoric acid, mixed and injected onto the HPLC system. Samples were injected in duplicate and bracketed with injections of the standard. The results were calculated based upon the relative average peak height of the sample and the standard.

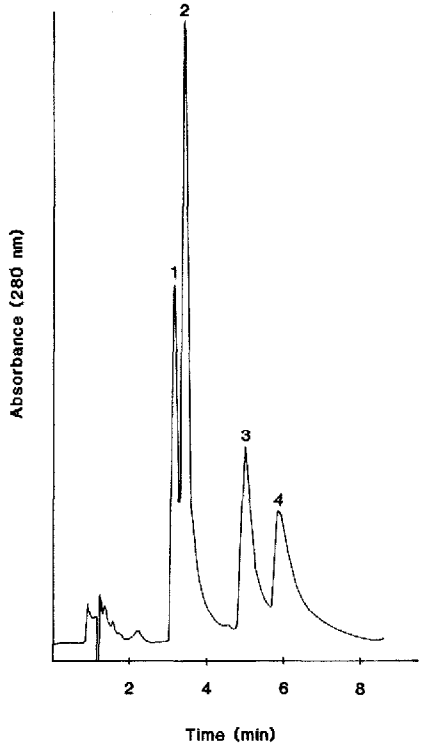

Fig. 1. High-performance liquid chromatogram of valone (1), pindone (2), diphacinone (3) and chlorophacinone (4) standards at a concentration range of 2–2.6 μg/ml on a 5 μm CN column (150 mm × 4.6 mm I.D.). Mobile phase, acetonitrile–0.2% phosphoric acid (45:55, v/v); flow-rate, 1.5 ml/min; UV detection, 280 nm.

TABLE I

MEAN RECOVERY OF INDANDIONES FROM SPIKED GROUND CORN

Results are from samples spiked in triplicate.

Compound	*Spiked level (%)*	*Mean recovery (%)*	*Coefficient of variation (%)*
Chlorophacinone	0.005	83.3	2.5
Diphacinone	0.005	96.3	3.8
Pindone	0.025	96.2	1.1
Valone	0.025	96.9	0.6

RESULTS AND DISCUSSION

Fig. 1 is a chromatogram of a mixture of valone, pindone, diphacinone and chlorophacinone standards with capacity factors of 2.5, 2.8, 4.6 and 5.6, respectively. Use of less than 0.2% phosphoric acid in the mobile phase caused a more pronounced broadening and tailing of the indandione peaks. Also, peaks were broader if the extracts and standards were not diluted with the phosphoric acid solution. Use of 0.5, 1.0 and 1.5% phosphoric acid did not improve the separation of the indandiones or change the retention times significantly and would likely decrease the longevity of the column.

The response was linear through the concentration range tested: 0.2–20 μg/ml. Least-squares linear regression analyses of the data gave correlation coefficients which ranged from 0.997 to 0.999 for the four indandiones.

As shown in Table I, the average recovery from spiked samples of ground corn ranged from 83.3% for chlorophacinone to 96.9% for valone. Even though acetonitrile has been reported by others to extract indandiones from baits[4,5], we found it necessary to add methanol to the extraction solvent to obtain good recovery of pindone and valone from spiked ground corn samples. The addition of methanol to the extraction solvent did not improve the percentage recovery of chlorophacinone nor did the use of a 2-h extraction. A similar recovery was obtained from the addition of chlorophacinone to wheat bait. Unspiked ground corn and wheat samples did not contain interfering peaks when extracted with either of the extraction solvents.

This method provides a convenient, isocratic HPLC system for the separation of these four indandione rodenticides and thus a means to identify the indandione in samples that are mislabelled or of unknown indandione content. Warfarin, another rodenticide, also separates from these indandiones and gives a sharp peak with a capacity factor of 1.9 (data not shown).

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