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DETERMINATION OF CHLOROPHACINONE IN FORMULATIONS BY REVERSED-PHASE ION-PAIR CHROMATOGRAPHY

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SUMMARY

The retention behaviour of chlorophacinone [2-(*p*-chlorophenyl)phenylacetyl-1,3-indandione] has been studied using an octadecylsilica column and different eluents, *e.g.*, methanol–water, methanol–aqueous phosphoric acid, methanol–aqueous McIlvaine buffer–tetramethylammonium bromide and tetrahydrofuran–aqueous McIlvaine buffer–tetrabutylammonium bromide. The dependence of log k' on the organic modifier content and the apparent pH of the eluents was determined. The retention behaviour could be interpreted by assuming that chlorophacinone existed mostly as a weakly acidic enol that could dissociate depending on the pH of the eluent. Chlorophacinone could be separated and determined quantitatively in 0.25–25% (w/w) oily formulations and in 10–100 ppm crushed grain bait using diphacinone [2-(diphenylacetyl)-1,3-indandione] as an internal standard.

INTRODUCTION

Chlorophacinone [2-(*p*-chlorophenyl)-phenylacetyl-1,3-indandione] is a powerful anticoagulant rodenticide and most formulations contain up to 5% (w/w) of active ingredient. A Hungarian patent[1] described the preparation of concentrates containing up to 25% (w/w) of active ingredient by adding long-chain trialkylamines in vaseline oil to chlorophacinone. On the other hand, certain formulations, especially crushed corn baits, contain chlorophacinone in concentrations as low as 75 ppm. Therefore, a flexible analytical method is required for the determination of chlorophacinone in a number of sample matrices at widely varying concentration levels.

Existing photometric[2] and electroanalytical[3,4] methods are not sufficiently selective and sensitive. Known thin-layer chromatographic methods[5–7] were not designed for low-level formulations. Two gas chromatographic (GC) methods have been published[8–10], based on the oxidation by chromic acid in acidic medium of chlorophacinone to chlorobenzophenone, separation and detection with an

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electron-capture detector[8,9], and the GC separation of brominated chlorophacinone[10]. Both are tedious and require chemical modification of chlorophacinone. Grant and Pike[11] published a reversed-phase (RP) high-performance liquid chromatographic (HPLC) method using a pellicular C_{18} packing and methanol–water containing 0.75% of ammonia as the eluent. The separation between chlorophacinone and matrix peaks arising from the corn-based rodenticide bait was not complete, but they excluded the possibility of using microparticulate octadecylsilica on account of its instability in the alkaline eluent used.

It was felt that with a more judicious choice of the composition of the mobile phase microparticulate reversed-phase packings could perform better in the analysis of various rodenticide formulations with widely varying concentration levels.

EXPERIMENTAL

Chemicals

Chlorophacinone, diphacinone, Redentin samples, methanol, tetrahydrofuran and acetone (all analytical-reagent grade) were obtained from Reanal (Budapest, Hungary). Tetramethylammonium chloride and tetrabutylammonium bromide were purchased from BDH (Poole, Great Britain) and Fluka (Buchs, Switzerland), respectively.

Apparatus and procedure

A Varian LC 5020 liquid chromatograph (Varian Aerograph, Walnut Creek, CA, U.S.A.) equipped with a 10-μl loop injector was used, connected to a Type LC-55 variable-wavelength detector (Perkin-Elmer, Norwalk, CT, U.S.A.) set at 285 nm and a Model 9176 dual-channel recorder (Varian). Separations were carried out on a 250 × 4.0 mm I.D. stainless-steel column (Labor MIM, Budapest, Hungary) packed[12] with 10-μm RP-18 material (Merck, Darmstadt, G.F.R.). The column was jacketed[13] and thermostated at 30.0°C by a Type U10 circulating water bath (MLW, Medingen, G.D.R.).

Redentin samples were dissolved in a mixture of tetrahydrofuran (THF) and eluent. Crushed corn bait samples were extracted for 3 h with 100 ml of acetone in a Soxhlet apparatus, the acetone was evaporated and the residue was dissolved in THF and made up to volume with eluent.

A combined glass electrode, calibrated with aqueous buffers (pH 4 and 7), and a Type OP-204/1 pH meter (Radelkisz, Budapest, Hungary) was used to measure the pH of the buffer solutions and the eluents. The latter uncorrected (apparent) values as measured in the hydro-organic medium are reported here.

RESULTS AND DISCUSSION

Aqueous methanol eluents

Chlorophacinone was strongly retained in pure methanol ($k' = 10$) and produced a poor peak shape. It is known that chlorophacinone in its enolic form behaves like a weak acid, so this large retention and unfavourable peak shape can be attributed to strong interactions between chlorophacinone and the residual hydroxyl groups of the packing. Addition of 1% concentrated (85%) phosphoric acid to estab-

lish an apparent pH of 2.2 decreased the retention dramatically and improved the peak shape. By decreasing the methanol concentration of the eluent, the capacity factor (k') of chlorophacinone increased, as shown in Fig. 1.

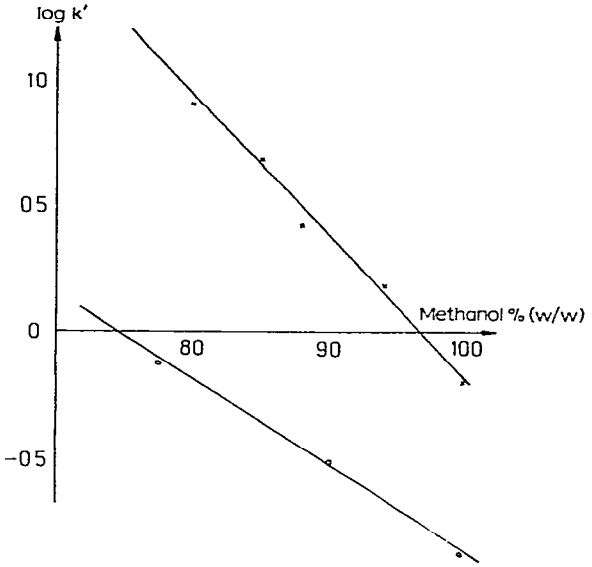


Fig. 1. k' of chlorophacinone (×) as a function of the methanol concentration in the eluent. Column: RP-18 (250 × 4.0 mm I.D.), thermostated at 30.0°C. Eluent: pH 2.2 (apparent) aqueous methanol. O, Nitrohexane.

For comparison purposes the behaviour of a simple compound, nitrohexane, is also shown. As the width of the chlorophacinone peak was still wider than that of a simple compound of comparable retention, the measured pH of the eluent was adjusted to about 6.3 by mixing varying amounts of methanol and aqueous McIlvaine buffer (0.2 *M* disodium hydrogen orthophosphate and 0.1 *M* citric acid). The k' values obtained are given in Table I. Compared with the values obtained in eluents of low pH, the k' values are significantly lower and the peak shape was also improved.

TABLE I

k' VALUES OF CHLOROPHACINONE AS A FUNCTION OF THE METHANOL CONCENTRATION IN THE ELUENT

Column: RP-18 (250 × 4.0 mm I.D.), thermostated at 30.0°C. Eluent: pH 6.3 (apparent) methanol–McIlvaine buffer–water.

Methanol concentration (%, w/v)	*Retention volume (ml)*	*k'*
67	2.6	0.24
62.2	3.22	0.53
53.8	5.08	1.42
53.8*	5.80	1.76

* Plus tetramethylammonium chloride (0.05 *M*).

This behaviour can be rationalized by assuming that chlorophacinone exists mostly in the enolic form. This weakly acidic enolic form can dissociate at high pH, resulting in an ionic species that is only slightly retained by the column, while at low pH the enol form is believed to stabilize by intermolecular hydrogen bonding, forming a chelate ring that is better retained by the RP-18 column. To substantiate this assumption, tetramethylammonium chloride (TMA) was added to the previous 53.8% (w/v) methanol–water eluent. As shown in Table I, the addition of TMA increased the k' of chlorophacinone, *i.e.*, ion-pair formation could be postulated.

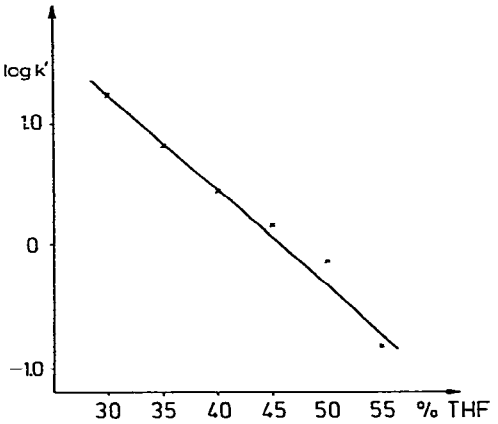
Although the retention of chlorophacinone could be easily controlled in this phase system, the eluent failed to dissolve the Redentin samples composed from vaseline oil, tridecylamine and chlorophacinone. Redentin samples could not be dissolved in aqueous acetonitrile either, only in THF. Therefore, the retention of chlorophacinone in aqueous THF eluents was investigated.

Aqueous tetrahydrofuran eluents

At first the k' of chlorophacinone in THF–McIlvaine buffer (1:1) (pH 6.3) as eluent was investigated without and with tetrabutylammonium bromide (TBA) as ion-pair reagent. Chlorophacinone eluted very close to the dead volume in the absence of TBA, whereas k' increased to 0.72 at a TBA concentration of 0.05 *M*, a behaviour similar to that observed with aqueous methanol eluents.

The k' values of chlorophacinone as a function of the THF concentration using 0.05 *M* TBA eluent of pH 6.3 are shown in Fig. 2. The retention can be easily controlled by changing the organic modifier content of the eluent.

The pH dependence of the k' of chlorophacinone in a 35.09% (w/v) THF eluent containing 0.05 *M* TBA is shown in Fig. 3. The shape of the curve is similar to



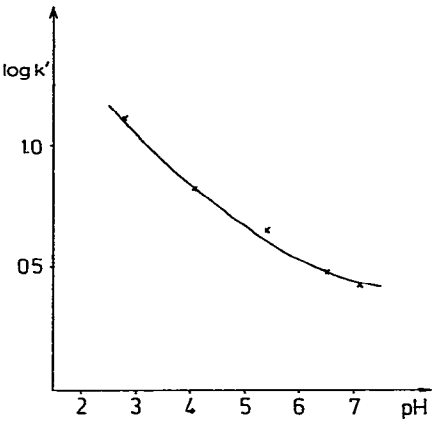


Fig. 2. k' of chlorophacinone as a function of the THF concentration in the eluent. Column: RP-18 (250 × 4.0 mm I.D.), thermostated at 30.0°C. Eluent: pH 6.3 (apparent) THF–McIlvaine buffer–water containing 0.05 *M* tetrabutylammonium bromide.

Fig. 3. k' of chlorophacinone as a function of the pH of the eluent. Column: RP-18 (250 × 4.0 mm I.D.), thermostated at 30.0°C. Eluent: 35.09% (w/v) THF–McIlvaine buffer–water containing 0.05 *M* tetrabutylammonium bromide.

that predicted by Terweij-Groen and Kraak[4] for weak acids. In the intermediate pH range (3.5–5) the peak of chlorophacinone became very wide and at some point a separate shoulder appeared.

As the peak shape was best at pH > 6, pH 6.5 was selected for the analysis of the Redentin samples.

It is believed that chlorophacinone forms an adduct with long-chain trialkylamines[1]. However, when the amine-chlorophacinone adduct and pure chlorophacinone were injected successively in the eluent of pH 6.5 they had identical k' values, indicating that the adduct decomposed in this eluent. This is an additional practical advantage of the eluent system developed, as the same eluent can be used for the analysis of both the free chlorophacinone and adduct-containing formulations.

Quantitative analysis

Oily Redentin formulations in the range 0.25–25% (w/w) could be readily analysed using a peak-height calibration graph measured at 285 nm. The equation for the calibration graph was

$$h = 1.103 + 6.794\ c\ (r^2 = 0.99926)$$

where h is the peak height (mm) and c is the concentration of chlorophacinone (μg/ml).

The oily samples were dissolved in 25 ml of THF, and the sample weight was varied in the range 0.05–2 g, depending on the concentration level to be determined. Portions of 200 μl of these solutions were added to 1800 μl of a 1:1 mixture of THF and eluent. Erroneous results were obtained when the injected samples were dissolved in pure THF only. The presence of oil caused no apparent problems in the quantitation procedure. The proposed sample amounts and the analytical results (the averages for three independent solutions, each injected twice) are given in Table II.

TABLE II

RESULTS FOR ANALYSIS OF REDENTIN FORMULATIONS

Nominal sample concentration (%, w/w)	*Amount added to 25 ml of THF (g)*	*Chlorophacinon concentration found (%, w/w)*
0.25	1.5–2.0	0.25 ± 0.03
1.0	0.4–0.5	1.01 ± 0.01
25	0.08–0.1	23.5 ± 0.2

Crushed corn bait Redentin contains 50–100 ppm of chlorophacinone. A 10-g amount of crushed untreated corn was extracted with 100 ml of acetone as described under Experimental. This solution was used as a blank to determine the presence or absence of interfering peaks and components originating from the sample matrix. No peak of significant intensity eluted at the retention volume of chlorophacinone and diphacinone. The latter was added to the crushed corn sample as an internal standard prior to extraction.

The equation of the calibration graph was

$$\frac{h_{CF}}{h_{DF}} = 0.002 + 0.6641 \cdot \frac{c_{CF}}{c_{DF}} \qquad (r^2 = 0.999783)$$

where h_{CF} and h_{DF} are the peak heights of chlorophacinone and diphacinone, respectively, and c_{CF} and c_{DF} are their concentrations.

The recovery for 0.5 μg of chlorophacinone per gram of crushed corn was 105 ± 10% (average of three separate extractions). The chromatograms of untreated crushed corn and a Redentin corn bait are shown in Figs. 4 and 5.

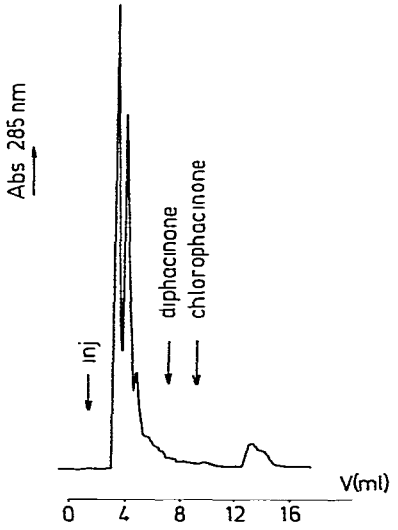


Fig. 4. Chromatogram of untreated crushed corn extract.

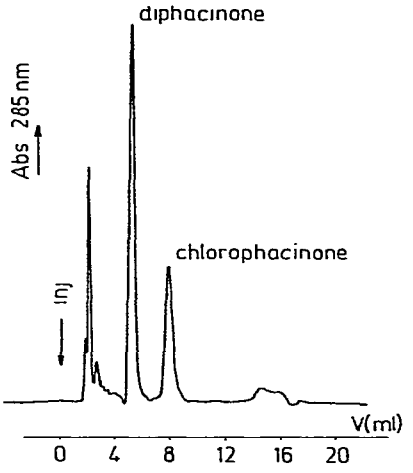


Fig. 5. Chromatogram of crushed Redentin 75 bait extract.

The same column could be used for over 6 months provided that the buffer-containing eluent was replaced with an aqueous THF eluent of identical THF concentration when the column was not being used. As another preventive measure, the column was washed free of contaminants with pure THF each week.

CONCLUSIONS

The retention of chlorophacinone as a function of the methanol and tetrahydrofuran content and pH of the eluent and the absence or presence of tetraalkylammonium cation ion-pair reagent was investigated. The observed behaviour could be readily interpreted by assuming that chlorophacinone existed mostly in a weakly acidic enolic form which, depending on the pH of the eluent, could dissociate and form ion pairs.

The tetrahydrofuran-aqueous McIlvaine buffer (pH 6.5) eluent system developed allows the quantitative determination of the concentration of the active ingredient in products prepared with free chlorophacinone, chlorophacinone–trialkylamine adducts and crushed corn bait.

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