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Determination of chlorophacinone and diphacinone in commercial rodenticides by liquid chromatography–UV detection and liquid chromatography–electrospray ionization mass spectrometry

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Abstract

This work describes a simple method for the determination and mass-spectrometric confirmation of the indanediones in commercial rodenticides. The sample is sonicated in methanol containing 2% formic acid and analyzed by liquid chromatography–UV detection. Once retention time and UV–Vis spectrum provide tentative identification, mass-spectrometric confirmation is obtained by analyzing a second aliquot by LC and electrospray ionization mass spectrometry (LC–ESI–MS). The extensive fragmentation of the indanedione molecule under MS/MS conditions provides sufficient structural information for positive identification on analyte levels as low as 20 ng on column. © 2000 Published by Elsevier Science B.V.

Keywords: Rodenticides; Chlorophacinone; Diphacinone; Indanediones

1. Introduction

The indanediones, diphacinone (2-[diphenylacetyl]-1*H*-indene-1,3[2*H*]-dione) and chlorophacinone (2-[(chlorophenyl)phenylacetyl]-1*H*-indene-1,3[2*H*]-dione), are commonly used anticoagulant rodenticides for the control of rats and mice, and other rodents such as pocket gophers, belding ground squirrels and California ground squirrels. These compounds are class-6 poisons (extremely toxic) with an LD_{50} value of 2 mg/kg for rats. There have been many reports of the misuse and accidental ingestion of commercial rodenticides by humans and other vertebrates [1,2]. Consumer samples, i.e., foods and drugs, contaminated with small amounts of green particles are frequently submitted to the Forensic Chemistry Center for analysis. Based on the green color of the contaminant and history of consumer-complaint samples, it is suspected that the particles are due to commercially available rodenticides. In such tampering cases, it is absolutely essential that adulterants be unambiguously identified when present, or that adulteration can be ruled out with certainty when a suspected tampering has not, in fact, occurred. Because a false negative or a false positive could have costly consequences, mass-spectrometric confirmation of identity is generally required.

Methods of analysis for bait formulations typically involve extensive extractions, clean-up, concentration and large sample sizes [3–5]. In cases submitted to the Forensic Chemistry Center, the amount of

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sample available for analysis is often limited. Gas-chromatographic methods require lengthy derivatization procedures [6]. At the Forensic Chemistry Center, we have previously reported a method for the analysis of brodifacoum in commercial rodenticides [7].

Reversed-phase liquid chromatographic (LC) separation of indanediones results in poor separation and often requires ion-pairing agents that are not compatible with the mass spectrometric detector [8–10]. This work describes a simple method for the detection and mass-spectrometric confirmation of the indanediones chlorophacinone and diphacinone in commercial rodenticides.

2. Experimental

2.1. Reagents

HPLC-grade methanol, acetonitrile and 88% formic acid were obtained from Fisher Scientific (Pittsburgh, PA, USA). LC-grade water was prepared using a Milli-Q Gradient A10 water-purification system, from Millipore (Bedford, MA, USA). Ammonium acetate buffer was prepared by mixing equimolar amounts of ammonium hydroxide and acetic acid. The extraction solvent was 2% formic acid in methanol.

2.2. Indanedione standards and control bait

Chlorophacinone was obtained from the EPA Pesticide Repository, Research Triangle Park, NC, USA. Diphacinone was obtained from AccuStandard (New Heaven, CT, USA). Control steam-rolled oat bait, referred to as SRO in this paper, consisted of steam-rolled oats, Alcolec S as a binder and Dupont A as a marker dye. Control wax steam-rolled oat bait, referred to as WSRO in this paper, consisted of steam-rolled oats, paraffin wax, Alcolec S as a binder and Dupont A as a marker dye.

2.3. Apparatus

The LC–UV system consisted of a Model 1090 liquid chromatograph, equipped with a photodiode array detector, an autosampler and a Rheodyne injector, all from Hewlett Packard (Palo Alto, CA, USA). The sonicator is a Model 2510R-DTH, from Branson (Danbury, CT, USA). The vortex shaker is a model G-560 from Scientific Industries (Bohemia, NY, USA).

The LC/APESI–MS system consisted of a Model 1100 liquid chromatograph, equipped with an autosampler and a Rheodyne injector, from Hewlett Packard and a Finnigan LCQ mass spectrometer equipped with APCI and ESI sources, from Finnigan Corporation (San Jose, CA, USA).

2.4. Preparation of standard

Stock standards (500 μg/ml) were prepared in methanol. Working standards were prepared to match the expected concentration of analytes in the samples, by making dilutions in the LC mobile phase. Fortification standards were prepared in ethyl acetate.

2.5. Sample preparation

Pellets from two brands of commercial rodenticides from two different manufacturers, referred to as brands B1 and B2 in this paper, were ground using a Krups model 408 coffee grinder. One brand contained chlorophacinone, and the other contained diphacinone. Labels on the boxes declared that the active ingredients were present at levels of 50 μg/g.

Preparation of fortified control bait: For steam-rolled oats bait, 100 μl of 100 μg/μl fortification standard was added to 200 mg of sample, and the ethyl acetate was evaporated under a stream of dry nitrogen. For wax bait, the evaporation of ethyl acetate was done under nitrogen and at 70°C, to help melt the wax and encapsulate the analytes [8]. It was necessary to add the extraction solvent while the wax was in the liquid form. The level of fortification corresponded to 50 μg/g in bait.

Extraction of commercial rodenticides and fortified control baits: Approximately 200 mg of sample was mixed with 2 ml of extraction solvent in a vial (4 ml vials from National Scientific, Lawrenceville, GA, USA). The mixture was then sonicated for 10 min and vortex-mixed for 1 min, and shaken for 5 min. This process of sonicating/vortex-mixing/shaking was repeated three times. The sample was then

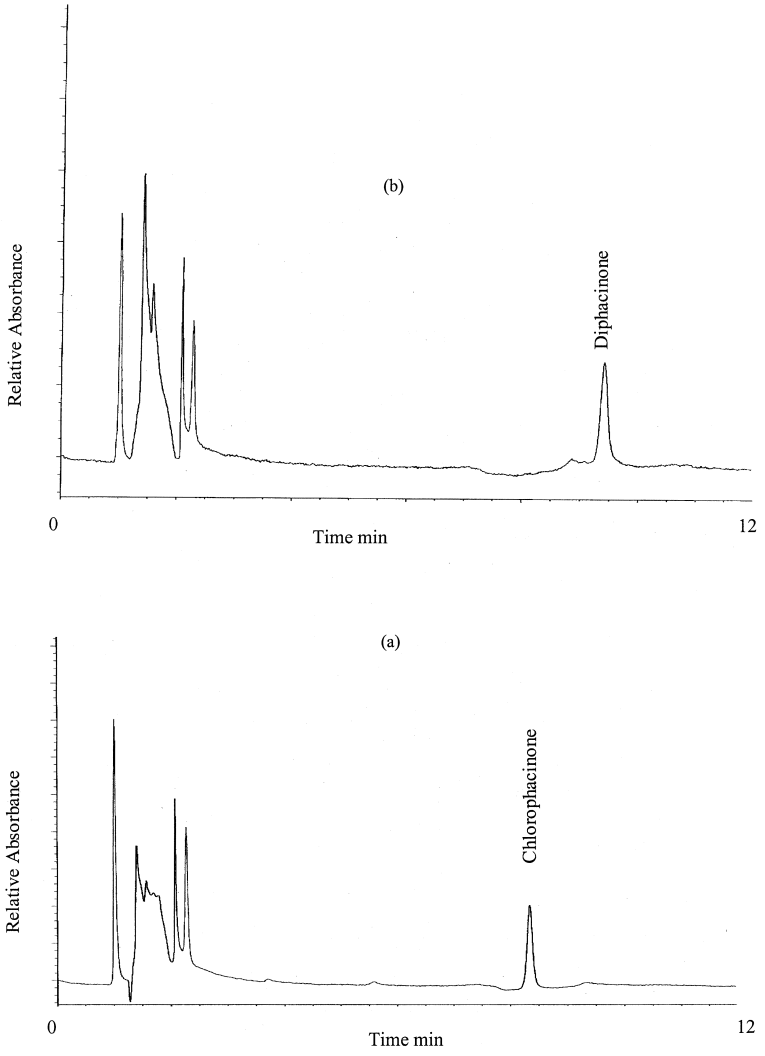

Fig. 1. LC–UV trace at 324 nm of a 5-μl injection of the extract of (a) chlorophacinone-containing brand and (b) diphacinone-containing brand.

centrifuged for 3 min in a bench-top centrifuge. An aliquot of the supernatant liquid, i.e., the extract, was filtered through a 0.2-μm Nylon filter.

2.6. Chromatographic conditions

The column used was a 15 cm×4.6 mm, 5 μm particle size, Econosphere-NH_2 column, from Alltech (Waukeegan, WI, USA). The mobile phase consisted of a ternary gradient as follows: acetonitrile (A), water containing 1% acetic acid (B) and water containing 10 m*M* ammonium acetate (C). Injections (5 or 10 μl) were made in all cases. UV–Vis spectra were stored from 210 to 450 nm, while the UV signal was recorded at 324 nm. The flow-rate was 1.0 ml/min. The ramped 5-min gradient was as follows: initial composition was 70% A and 30% B, at 5 min 70% A and 30% C. An equilibration time of 7 min was used at the end of each run.

2.7. Mass spectrometric conditions

LCQ conditions were set as follows: API Source

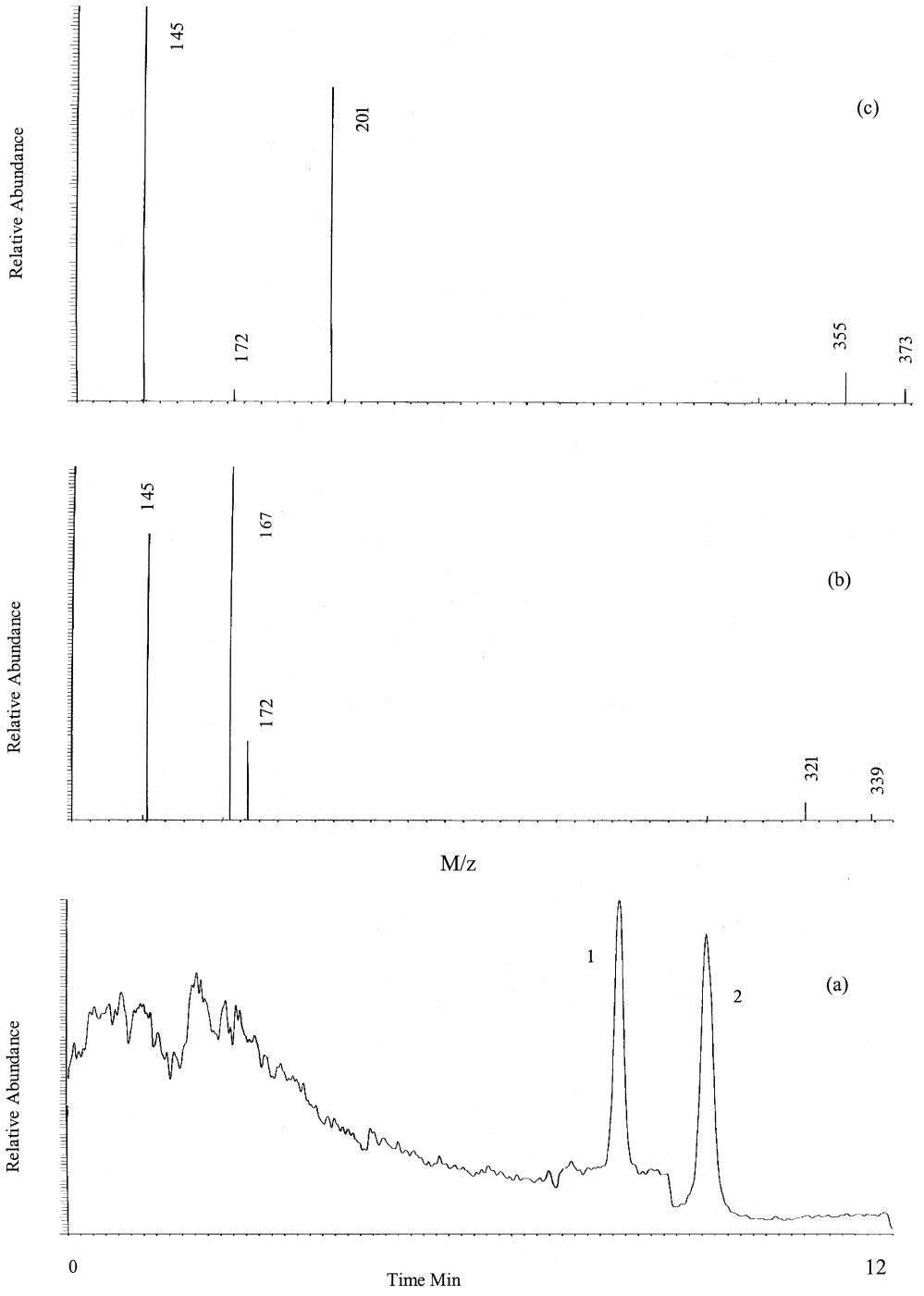

Fig. 2. (a) Total ion chromatogram of a 5-μl injection of a standard containing 5 ng/μl each of chlorophacinone (1) and diphacinone (2). Product ion mass spectrum of $[M-H]^-$ ion for diphacinone at m/z 339 (b) and chlorophacinone at m/z 373 (c).

in ESI mode; source polarity, negative; capillary temperature, 250°C; capillary voltage, −5 V; tube lens offset, 0 V; mass ranges, 365–380 for chlorophacinone and 335–345 for diphacinone. For MS/MS experiments, the isolation width was set to 1 amu and the collision energy was 25% of full scale.

3. Results and discussion

3.1. LC–UV

Fig. 1 shows the chromatograms obtained for the extracts of two commercial rodenticides, one containing chlorophacinone and the other diphacinone. The peaks of interest are well resolved under the conditions used and are free of any interferences. Starting with acidic conditions (1% acetic acid) and going to 10 m*M* ammonium acetate drastically improved the separation. When the aqueous fraction of the mobile phase was changed to 100% ammonium acetate, i.e., a significantly higher pH value, the analytes were mostly in the ionized form and were quickly eluted. That the analytes elute mostly in the ionic form is further evidenced by the strong negative ion ESI/MS signal obtained, as discussed below. The indanediones have a characteristic UV–Vis spectrum that provides useful qualitative information. To determine the validity of the method, five sets each of control SRO and WSRO, all spiked at 50 μg/g levels, and three sets of each of two brands of commercial rodenticides, were extracted. The recoveries for the fortified control SROs and WSROs were between 94 and 104%, while the relative standard deviations (RSDs) ranged between 3.4 and 7.6%. Determination of indanediones in commercial rodenticide samples shows similar RSDs for replicate analyses. The levels of indanediones found in the rodenticides were off by as much as 37% from declared values, possibly indicating a lack of strict quality control on the part of manufacturers. Linear dynamic ranges were established by injecting five standards that ranged in concentration from 1 to 300 ng/μl. The correlation coefficients for the calibration curve were 0.9999 in each case. The estimated method detection limit was 2 ng, defined

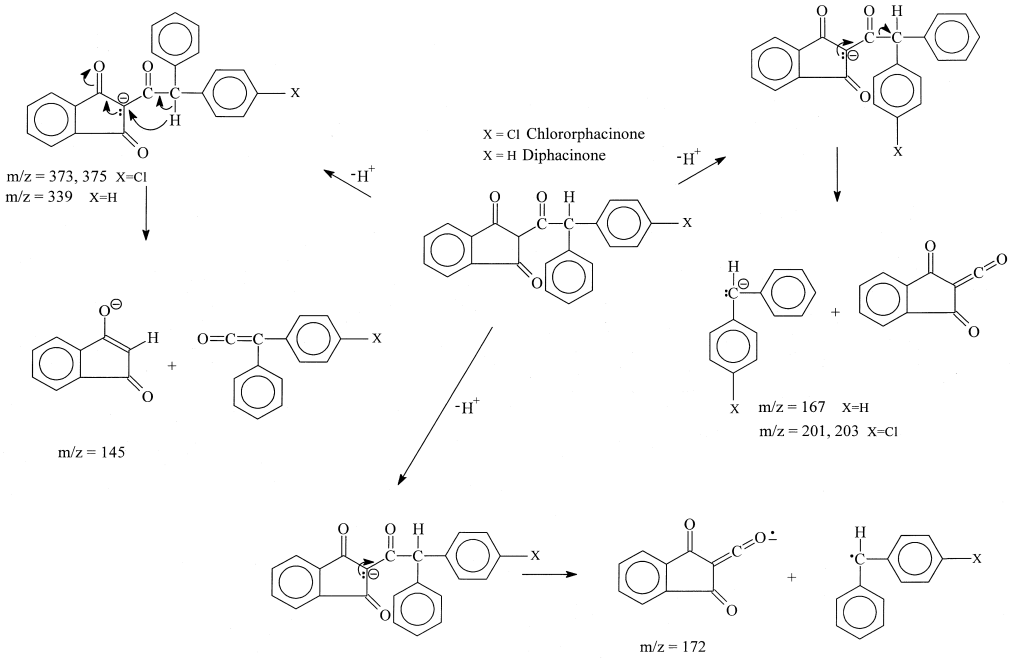

Fig. 3. Proposed structures for the ions observed in product ion mass spectra of chlorophacinone and diphacinone.

as the amount of analyte in the sample that resulted in a signal that was 2.5 times the peak-to-peak noise in the baseline.

3.2. LC–ESI–MS

Fig. 2(a) shows the total ion chromatogram for a standard mixture that contained chlorophacinone and diphacinone. The mobile phase and the amino-bonded column used provided adequate selectivity for the indanediones; more importantly, the mobile phase was compatible with the ESI–MS detection. Full-scan mass spectra of chlorophacinone and diphacinone showed very intense $[M-H]^-$, with no fragmentation; this feature is very important if one opts to do single-ion monitoring for added sensitivity. It is believed that the $[M-H]^-$ results from the loss of the acidic proton on the α-carbon atom to all three carbonyl groups. Full-scan MS/MS on the $[M-H]^-$ ion yielded characteristic product ion mass spectra, as shown in Fig. 2 (b)–(c). For chlorophacinone, only MS/MS data corresponding to the ^{35}Cl-containing isotope are shown; it is to be noted that the presence of Cl with a significant ^{37}Cl isotope

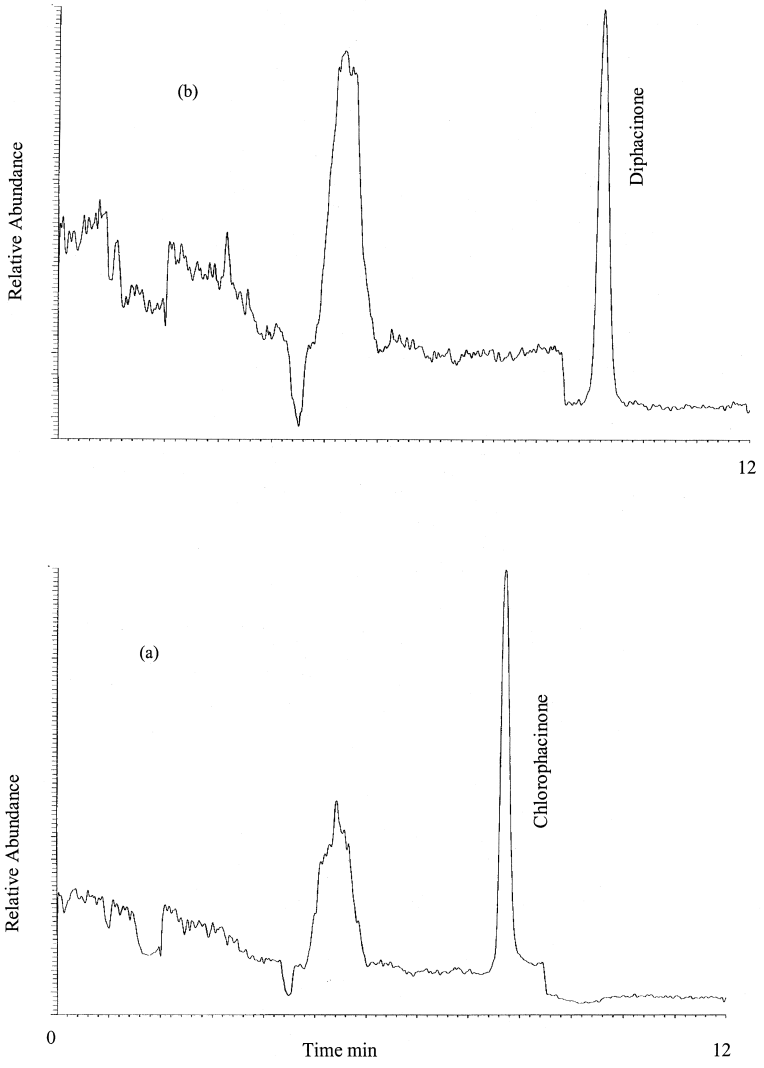

Fig. 4. Total ion chromatogram for commercial rodenticides containing (a) chlorophacinone and (b) diphacinone, using a 10-μl injection.

abundance provides additional qualitative information. Both chlorophacinone and diphacinone also showed a water-loss fragment. MS/MS spectra could be obtained from as low as 20 ng of analyte. The four characteristic ions in the MS/MS spectrum provide important qualitative information. The proposed structures for the various ions observed in the MS/MS spectra are given in Fig. 3. A much cleaner-looking chromatogram was obtained by restricting the scan range to 335–380 amu. The total ion chromatograms for two commercial rodenticides are shown in Fig. 4.

4. Conclusions

The present LC–ESI–MS and MS/MS method provides a simple and sensitive method for the identification and quantitation of indanedione-based active ingredients in commercial rodenticides. Useful MS/MS spectra could be obtained from as low as 20 ng of analyte. The method is simple, fast and adequate for the levels expected in the finished bait products. The selective and sensitive nature of the mass spectrometric detector makes it possible to determine this class of compounds in rat poison without extensive sample clean-up and preconcentration. At the Forensic Chemistry Center, we have found this technique to be very useful in the analysis of rodenticides that were accidentally or intentionally mixed with food products or drugs.

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