

Supercritical Fluid Extraction of Strychnine Using Two Different Sequential Organic Modifiers

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Carbon dioxide supercritical fluid extraction (SFE) of strychnine from oat grain bait was accomplished using a methanol modifier during static extraction and a chloroform modifier during dynamic extraction. Extraction efficiency was better than with the use of either modifier alone. Analysis time, strychnine recoveries (82%), and reproducibility (SD = 7.8%) were not significantly different from the 85% recovery and 5.4% SD obtained with a routinely used organic solvent/solid extraction method. The supercritical fluid extraction method generated no hazardous waste as compared to the solvent/solid extraction method, which produced 50 mL of hazardous waste per sample. This two solvent modifier SFE method resulted in better precision and recovery of strychnine from oat bait than other reported SFE methods for the recovery of pesticides from treated grains.

Keywords: *Supercritical fluid extraction; SFE; modifier; strychnine; oat bait*

INTRODUCTION

Strychnine (Figure 1) is a toxicant which is extracted from the seeds of *Strychnos nux vomica* (*Farm Chemicals Handbook*, 1989). Strychnine grain baits are widely used for the management and control of rodent pests such as rats and ground squirrels (*The Merck Index*, 1983). For wildlife pest control situations, strychnine use is restricted to below ground applications. Analyses of strychnine grain baits are routinely conducted by our laboratory as well as other contract laboratories. Such analyses are required for U.S. Environmental Protection Agency reregistration studies, strychnine grain bait production quality control, and forensic studies stemming from the poisoning of non-target species.

Strychnine, like many other popular pesticides, is soluble in a variety of organic solvents. Solvents are traditionally used to extract the strychnine toxicant from strychnine-fortified grain baits as well as other biological matrices. The extracted strychnine is then quantified by high-performance liquid chromatography (HPLC) (Hoogenboom and Rammell, 1985; Hunter and Creekmur, 1984; Dennis, 1983; Alliot et al., 1982;), gas chromatography (Sharp, 1986; Bogusz et al., 1983; Miller et al., 1982), or spectrophotometry (Wapensky, 1969). Currently, organic solvents are coming under increasing scrutiny due to their cost and the environmental impact associated with their disposal (Snyder et al., 1993). Supercritical fluid extraction (SFE) is often a viable alternative to the traditional organic solvent based extraction methods used for the analyses of pesticides (King et al., 1993; King and Hopper, 1992; McNally and Wheeler, 1988; Capriel et al., 1986). Also, SFE has been used as an extraction technique for other pesticides in grain matrices (Thomson and Chesney, 1992). The potential of SFE for reducing our laboratory's output of hazardous waste was evaluated by comparing the SFE recovery of strychnine from strychnine-treated grain bait with the recovery obtained by an organic solvent based extraction method.

MATERIALS AND METHODS

Materials. HPLC grade methanol and acetonitrile were obtained from Fisher Scientific (Fair Lawn, NJ). The acetonitrile

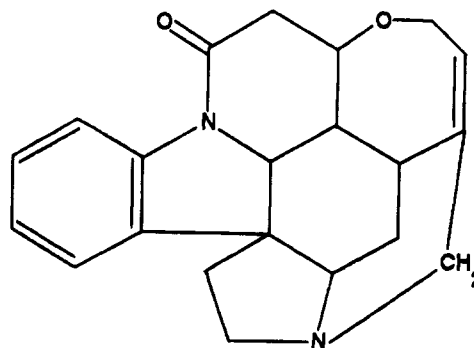


Figure 1. Structure of strychnine.

trile was filtered (0.45 μm) once before use. Burdick and Jackson high-purity chromatographic grade chloroform was purchased from Baxter Scientific (Muskegon, MI). A preformulated solution of heptanesulfonic acid, IPC B7 (Alltech, Deerfield, IL), was used as the ion pairing reagent. Hydro-matrix was obtained from Varian (Harbor City, CA). Water was purified using the Milli-Q+ purification system (0.22 μm , Millipore, Milford, MA). Analytical grade strychnine was purchased from Aldrich (Milwaukee, WI).

A concentrated standard solution of 3920 $\mu\text{g/mL}$ strychnine was prepared in chloroform. For quantification of strychnine, a working standard of 100.4 $\mu\text{g/mL}$ of strychnine in chloroform was prepared from the concentrated standard solution.

The HPLC aqueous eluent was prepared by adding 12.5 mL of IPC B7 to 1 L of water to yield a concentration of approximately 2.4 mM heptanesulfonic acid. This solution was filtered once with a 0.45 μm nylon membrane filter.

Industrial grade nitrogen and carbon dioxide and SFC/SFE grade carbon dioxide were obtained from Air Products (Allentown, PA).

Rolled oats were fortified in our laboratory with strychnine to achieve an approximate concentration of 0.4% w/w. Molasses and glycerine were used as adhesives and mixed with strychnine prior to addition to the oats. The mixture was mixed uniformly and allowed to air-dry. Steamed rolled oats treated with molasses and glycerine (no strychnine) were used as the control. The baits used for this study were prepared during 1992. To ensure homogeneity, a Wiley mill with a 1 mm sieve was used to grind the oat bait and the control bait into a fine grain powder.

High-Performance Liquid Chromatography. A Hewlett-Packard 1090M chromatograph (Hewlett-Packard, Palo Alto,

CA) equipped with a diode array detector was used for the analysis of strychnine. The mobile phase consisted of a 70% aqueous solution of IPC B7 and 30% acetonitrile. The analytical column was a 250 × 4.6 mm i.d. Econosil C₁₈ (10 μm), coupled with a 10 × 4 mm guard column containing the same packing (Alltech). The mobile phase flow rate was 1 mL/min. Injection volumes were 10 μL. Strychnine content was determined using a wavelength of 254 nm. All mobile phase solvents were purged with helium.

Supercritical Fluid Extraction. Supercritical fluid extraction was performed using a Suprex Prepmaster supercritical fluid extraction system (Suprex, Pittsburgh, PA). The collection of the extract was facilitated by an AccuTrap unit, consisting of a variable flow restrictor, a cryogenic trap filled with glass beads, a liquid pump for the desorption solvent rinse to recover the analytes from the glass beads, and a fraction collector. A modifier pump was used for the addition of either methanol or chloroform to the carbon dioxide.

Prior to extraction of any samples, the efficiency of recovery of strychnine from the cryogenic trap was evaluated by spiking the trap with 40 ng of strychnine in 10 μL of chloroform. The contents of the trap were eluted with two subsequent 1.5 mL chloroform rinses. Recoveries were quantified by HPLC and compared with the HPLC response of a solution containing 10 μL of the strychnine solution in 1.5 mL of chloroform.

Bait samples were prepared for SFE by adding approximately 0.050 g of oat bait to a 1 mL stainless steel extraction cell. To ensure an even flow of mobile phase through the extraction cell, Hydromatrix (diatomaceous earth) was used to completely fill the void volume of the cell at both ends. Prior to extraction, 1.0 mL of either chloroform or methanol was spiked into the cell. To evaluate whether the recovery of strychnine was affected by storage time, SFE was also performed on control bait which was fortified by the addition of strychnine to the extraction cell immediately prior to extraction.

The extractions were carried out with supercritical fluid carbon dioxide modified with either chloroform or methanol. The extractions were performed in two steps: an 8 min static extraction followed by a 12 min dynamic extraction, at a flow rate of 2.5 mL/min. The pressure and temperature in the extraction cell were maintained at 450 atm and 85 °C at all times.

The temperature of the trap was maintained at 25 °C during the extraction and at 40 °C during the desorption rinse. Chloroform (1.5 mL) was used to rinse the trap at a flow rate of 1 mL/min. A second rinse was performed to test for the completeness of strychnine recovery from the trap.

Solvent Extraction. To permit a direct comparison of SFE with traditional solvent-based extraction methodology, solvent extraction of strychnine grain bait was performed according to the Denver Wildlife Research Center (DWRC) validated method 24B, "Strychnine Alkaloid Technical and Bait Assay". Replicate 1.0 g samples of fortified strychnine oat bait were extracted with 7.0 mL of 70% aqueous IPC B7/30% acetonitrile by vortex mixing, followed by horizontal mechanical shaking for 10 min. Each sample was then centrifuged and the supernatant decanted and saved. The extraction was repeated two more times; the supernatants were combined for each sample and diluted to a final volume of 50 mL with 70% aqueous IPC B7/30% acetonitrile.

RESULTS AND DISCUSSION

Prior to optimization of the SFE conditions for the extraction of strychnine from grain bait, the recovery of strychnine from the glass bead SFE trap was evaluated by spiking 40 ng of strychnine in 10 μL of chloroform onto the trap. A recovery of 97% was achieved by rinsing with 1.5 mL of chloroform. A subsequent 1.5 mL chloroform rinse yielded an additional 0.6%.

The effect of varying both the static and dynamic supercritical fluid compositions on the SFE recovery of strychnine from fortified oat bait are presented in Table

Table 1. Effect of Mobile Phase Modifier on Recovery of Strychnine

static modifier	dynamic modifier	% recovery
none	chloroform	15
chloroform	chloroform	6
methanol	chloroform	77
methanol	methanol/chloroform	24
methanol	methanol	32

1. Recoveries were calculated on the basis of the initial 1.5 mL chloroform trap rinse. The strychnine recovered in the second trap rinse was always less than 1% of the strychnine recovered in the initial rinse. Figure 2 is a typical chromatogram resulting from HPLC analysis of the SFE strychnine grain bait extract. The retention time for strychnine was 5.86 min.

Due to the high solubility of strychnine in chloroform, chloroform was initially evaluated as the mobile phase modifier. Addition of chloroform to the carbon dioxide mobile phase at a level of 7.5% by volume resulted in a strychnine recovery of 15%. In an attempt to increase the recovery of strychnine during the static phase of the extraction, 1.0 mL of chloroform was added to the extraction cell. Strychnine recovery was reduced to 6%. In contrast, the addition of 1 mL of methanol to the extraction cell increased recovery to 77%. We believe the addition of methanol to the extraction cell resulted in a swelling of the oat bait and subsequent increased penetration of the supercritical fluid extraction solvent into the oat matrix. The net result was to render the strychnine more available for extraction. This demonstration of increased recoveries associated with the use of two different organic solvents during the static and dynamic portions of an SFE method has not been previously reported.

Due to the increased strychnine recovery associated with the addition of methanol to the extraction cell, we changed the modifier from 7.5% chloroform in carbon dioxide to 7.5% methanol/chloroform (25:75) in carbon dioxide. Extraction of strychnine bait with the addition of 1 mL of methanol to the extraction cell and the methanol/chloroform-modified carbon dioxide yielded a recovery of 24%. The lack of effectiveness of the methanol/chloroform modifier to extract strychnine from the grain bait could be due to H-bonding between the two organic modifiers. Thus, the addition of methanol modifier to the carbon dioxide decreased recovery, while the addition of methanol to the extraction cell increased recovery. Changing the modifier to 10% methanol resulted in a strychnine recovery of 32%, less than half of the maximum recovery achieved with the chloroform mobile phase modifier.

As the addition of chloroform to the carbon dioxide phase increased the recovery of strychnine from the grain bait, we performed multiple ($n = 4$) supercritical extractions of the grain baits with a supercritical fluid containing 7.5% chloroform in carbon dioxide and 1 mL of methanol added to the extraction cell to facilitate static extraction. Quantification of the recovered strychnine by HPLC yielded a recovery of 82% with a standard deviation of 7.8%. Analysis of four strychnine bait samples required 2.5 analyst hours. SFE/HPLC analysis of the control grain baits which were fortified immediately preceding SFE gave a recovery of 84.1% with a standard deviation of 0.7%. As the strychnine oat bait used for the initial analyses was stored in a light-proof container at room temperature for over 2 years, these results indicate that aging of the strychnine was not of concern to analysis by this SFE method.

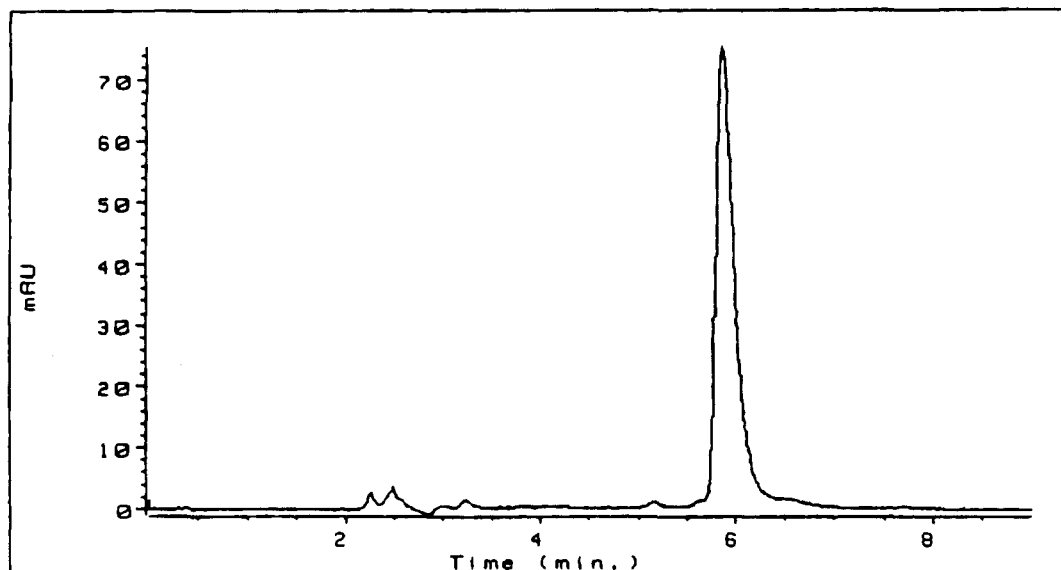


Figure 2. HPLC chromatogram of strychnine recovery from supercritical fluid extraction of strychnine oat bait. Retention time of strychnine is 5.86 min.

The recovery and precision of strychnine from oat bait with the two solvent modifier SFE technique are superior to reported SFE recoveries for other pesticides from treated grains. To improve the low SFE recoveries of dichlorophenol from treated barley and triticale seeds, Thompson and Chesney (1992) evaluated the effects of sample pretreatment with a number of solvents, as well as acid and base hydrolysis on SFE extraction efficiency. Their optimal pretreatment, a 4 h 17% phosphoric acid hydrolysis followed by SFE, gave dichlorophenol recoveries of 22 and 18% (SD = 10%) from barley and triticale seeds, respectively.

To compare our two solvent modifier SFE technique with traditional solvent extraction methodology, four 0.6 g samples of strychnine grain bait were analyzed by DWRC method 24B. These traditional analyses generated a strychnine recovery of 85% with a standard deviation of 5.4%. Analysis of these four strychnine bait samples also required 2.5 analyst hours. However, the solvent extraction procedure generated 200 mL of strychnine-contaminated aqueous acetonitrile hazardous waste.

When compared by a Student's *t* test ($\alpha \leq 0.01$), the recoveries resulting from the two extraction methods were not significantly different. When analyzed by an *F* test (Anderson, 1987), the variances associated with each method were also not significantly different ($F \leq 0.05$). With respect to analysis time, recovery, and precision, SFE gave results comparable to those of traditional solvent-based extractions of strychnine grain baits used for pest control.

However, SFE methodology generated practically no hazardous waste (1 mL of extract and 0.6 g of post-extracted bait). Thus, SFE offers an environmentally responsible alternative to traditional solvent-based extraction methodology for the analysis of strychnine bait samples. Furthermore, utilizing an SFE instrument with an autosampler and collector would dramatically decrease the required analyst time per sample. The use of two different solvent modifiers for SFE extraction of pesticides from seeds offers an improved approach over previously reported methodologies.

SAFETY

Strychnine is toxic, and protective laboratory gear such as latex gloves, lab coat, and goggles should be worn when this compound is handled. Carbon dioxide can cause suffocation. Chloroform and methanol should be kept in sealed containers or used in a fume hood. These organic solvents should be disposed of in a legal and environmentally safe manner.

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