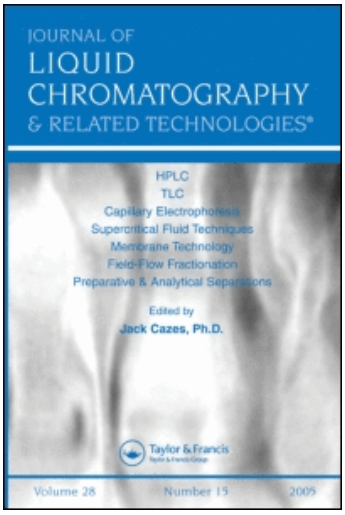

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High Performance Liquid Chromatographic Method for the Assay of Tebufenozide Insecticide in Suspension Formulations

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE ASSAY OF TEBUFENOZIDE INSECTICIDE IN SUSPENSION FORMULATIONS

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ABSTRACT

A simple, reliable, and robust liquid chromatographic method is described to separate and quantify tebufenozide insecticide in suspension concentrates. The analyte was extracted with acetonitrile and partitioned with hexane to remove lipoid and other nonpolar additives in the formulation to improve selectivity and specificity. The concentration of the insecticide in the polar phase was adjusted volumetrically and analysed using an RP-8, 10-μm, 200 × 4.6 mm i.d. bonded phase column, with diode-array detection at 236 nm and 50% water/50% acetonitrile-dioxane (4:1, v/v), as the mobile phase. The linear concentration range, limit of detection, and limit of quantification for the insecticide were 5 to 500 ng (in 40-μL injection volume), 1.0 ng and 5.0 ng, respectively. Triplicate analysis of 3 lots of commercial suspension concentrates gave values that were agreeable with those given on the labels. The method, with necessary modifications, could be extended and used routinely as a quality control method in the analysis of different types of tebufenozide formulations.

INTRODUCTION

With the current public concern about the widespread use of broad-spectrum neurotoxic insecticides in forest insect control programs and their perceived risk to human health and the environment,[1] considerable interest has been shown in recent years to develop and test promising new chemicals which are environmentally benign and have a narrow-spectrum of activity. Tebufenozide [N,N'-*t*-butyl-N.-(3,5-dimethylbenzoyl)-N-(4-ethylbenzoyl) hydrazine], also known as MIMIC® or RH-5992, developed and marketed by Rohm and Haas Co. (Spring House, PA, USA), is a hormonal insecticide acting as an insect growth regulator interfering with the molting process of lepidopteran insects.[2] It is a nonsteroidal ecdysone agonist causing premature and incomplete ecdysis, and eventual death of the exposed insects. The material is found to have low mammalian and aquatic toxicities.[3] Because of these desirable properties, it is field tested in Canada to control the insect pest, spruce budworm (*Choristoneura fumiferana* Clemens), a destructive defoliator and killer of spruce and fir forests of the New England states in USA and the Maritime provinces in Canada.

Aerial application of insecticides is an economical method used in forestry to cover large areas of infested forests. In the past few years, different aqueous and oil-based formulations of tebufenozide have been field tested to evaluate their stability, sprayability, target coverage, and biological performance. Few analytical methods have been reported in open literature to quantify the active ingredient (AI) in the formulations.[3] In 1994, Rohm and Haas introduced a low volume suspension concentrate (SC) for forestry use to enhance its deposition characteristics on conifer needles and its biological effectiveness, and, also to improve its dispersion, suspension, and shelf-life. The new formulation consisted of a suspension of finely ground particles (3 to 5 μm) of tebufenozide mixed in a fluid medium containing oils, water, emulsifiers, stickers, surfactants, and microgranular solid matrices. The chemical identity of additives and their composition in the SC are the proprietary information of the company. The formulation was mixed with water at the spray site to form an emulsion which was then sprayed aerially over the budworm-infested forests. Although a high performance liquid chromatographic (HPLC) method has been reported in literature to monitor the tebufenozide content in experimental formulations,[4] the method did not specifically address the analysis of the new SC which contained a host of additives such as oils, emulsifiers, surfactants, solid matrices, etc., which could cause interference in the analysis. This paper describes a reliable, reversed-phase HPLC method to quantify the AI in the SC formulations, and examines its suitability as a quality control method to monitor the AI content in other tebufenozide formulations that will be used routinely in forestry spray operations to control the spruce budworm populations.

EXPERIMENTAL

Materials

HPLC-grade acetonitrile, dioxane, hexane, and methanol were purchased from Caledon Laboratories Ltd. (Georgetown, ON, Canada). Ultrapure water was drawn from a Milli-Q water purification system (Millipore Corp., Bedford, MA). Analytical grade tebufenozide (99.6% purity, m.p. 186-1880C) was kindly donated by Rohm and Haas Co., USA. Among the three lots of commercial SC formulations used in the analysis, one (SC-1) was from the 1994 field study, the second (SC-2) was from the scientist in charge of the Formulation Project at the local CFS research centre, and the third (SC-3) was supplied courtesy of Rohm and Haas Canada Inc. (West Hill, ON). However, all three SC formulations, containing 24.0 g AI/100 mL as per the label claim, were initially manufactured and distributed by the parent Rohm and Haas Co. (Spring House, PA).

Chromatography

The instrument used was a Hewlett-Packard (HP) (Palo Alto, CA) model 1090M HPLC, fitted with an autosampler, variable volume auto-injector, a binary solvent delivery system with He degassing, and two dual-syringe metering pumps giving stable and reproducible flows. The instrument was connected to a UV-visible diode-array detector (DAD) with a wavelength range of 190 to 600 nm. The HPLC system was monitored by a HP-9000/310 computer work station operated by HP-7995 R software. An HP LiChrosorb RP-8, 10-μm, 200 × 4.6 mm i.d. analytical column, preceded by a MOS-Hypersil C-8, 5-μm, 20 × 4.6 mm i.d. guard column, both thermostated at 40°C, were used. The elution was achieved isocratically using a mobile phase consisting of 50% water/50% acetonitrile-dioxane (4:1, v/v) with a flow-rate of 0.8 mL/min. Prior to analysis, the mobile phase was filtered using a 0.20-μm Nylaflo nylon membrane filter (Gelman Sciences, Rexdale, ON) and degassed. The sample and reference wavelengths were set at 236 and 430 nm, respectively, and the injection volume used was 40-μL. The system was equilibrated for 30 min before making an injection.

Stock and Working Solutions

A stock solution of tebufenozide was prepared by weighing 100.0 mg of the analytical material into a volumetric flask, dissolving it in acetonitrile, and adjusting

the volume to 100 mL. The flask was covered with aluminum foil to prevent photolytic loss of the analyte and stored in darkness at 0°C. Five working solutions in the concentration range of 0.125 to 12.5 μg/mL were prepared by serial dilution of the stock solution to calibrate the HPLC. Forty-μL of each working solution was injected into the HPLC six times and the DAD response (mAU) was recorded.

A calibration curve was prepared by plotting the average mAUs against concentration. The detector response was linear in the concentration range of 5 to 500 ng. The slope and intercept of the straight line, calculated using the least-square regression analysis, were 2.026 and 0.602, respectively, with a coefficient of determination of 0.998.

The SC formulations had the tendency to separate into two phases and the solid ingredients in the mixture sedimented at the bottom of the container on prolonged storage. Each container was first inverted several times to break-up the lumps and then agitated vigorously on a mechanical shaker for 2 h. About 1 mL of each formulation was pipetted into a weighing bottle and its exact mass was determined on an analytical balance. The formulation was then transferred, quantitatively, to a 50-mL Teflon centrifuge tube and its volume was adjusted to 30 mL with acetonitrile. The tube was agitated for 1 h for dissolution of the AI and then centrifuged at 6000 rpm for 20 min to coagulate and sediment some of the additives in the formulation.

A 20-mL aliquot of the supernatant was transferred to a 50-mL separatory funnel, 7 mL of hexane was added and shaken for 10 min. The bottom acetonitrile layer, containing the AI and other polar additives, was transferred to a 100-mL volumetric flask. The hexane layer containing the oil and other nonpolar materials was partitioned two more times, with 15 mL of acetonitrile each time, to remove the trace levels of AI in the hexane phase. The acetonitrile layers were added to the initial sample in the volumetric flask and the hexane layer was then discarded. The volume of the pooled acetonitrile was adjusted to 100 mL with further addition of the solvent.

A measured volume of the filtered acetonitrile extract was then taken in a graduated centrifuge tube and its volume adjusted by either concentration under N_2 (Meyer N-Evap®) or dilution with acetonitrile, so that the concentration of AI in the formulation extract was within the concentration range of the calibration curve prepared for the tebufenozide standard. A 40-μL aliquot of each extract was injected several times (n = 6) into the HPLC and the average DAD response was calculated. The concentration of the AI in the extract was computed from the calibration curve and expressed as g AI/100 mL of the SC.

RESULTS AND DISCUSSION

Method Development

The objective of this work was directed towards the development of a simple, rapid, and accurate HPLC method for the routine analysis of tebufenozide in SC formulations which, are nowadays, viewed favourably for forestry use. Using the previous work[4] as a guideline, suitable mobile and stationary phases, detection mode, column temperature, etc. were selected. Quantitative recovery of the analyte from each of the three SCs was obtained initially by repetitive experimentation using different solvents for extraction. Acetonitrile dissolution followed by hexane partition gave good analyte recovery from the formulations. Hexane partition, although somewhat time consuming, helped to remove the lipoid components and other nonpolar additives in the formulation, enhancing optimum analyte resolution and separation with good peak purity.

The use of RP-8 bonded phase column (10-μm, 200 × 4.6 mm i.d.) and the mobile phase consisting of 50% water/50% acetonitrile-dioxane (4:1, v/v), produced good elution pattern of the analyte and its separation from the impurity peaks in the formulation. The necessity of dioxane, its amount and effect on the peak separation were investigated, and 10% v/v of the solvent in the mobile phase was found to be optimum, producing good peak separation. However, slight variations (± 2%) in the composition did not unduly affect the analysis, indicating the ruggedness of the method. The DAD detection mode chosen (sample λ set at 236 nm) responded linearly to the analyte in the concentration range of 5 to 500 ng when 40-μL aliquots of the standard solutions were injected into the HPLC. The selection of column temperature (40°C) and the mobile phase flow rate of 0.8 mL/min gave optimum run time and good peak separation of the analyte. With ambient temperature or increased flow rate, the run time decreased but the peak resolution and peak quality were below marginal.

Method Validation

Figure 1 shows typical chromatograms obtained for the reagent blank and tebufenozide standard, after injecting 40-μL volumes of each into the RP-8 column and eluting with the selected mobile phase. It can be seen that the analyte was eluted and detected, and its peak was well resolved with good baseline separation. The retention time (RT) was 18.2 min. The precision of the method was determined by replicate injection (n = 6) of the same standard solution and measuring the corresponding RTs and peak areas. The relative standard deviations (RSD) in RTs and peak areas from the mean were 1.2 and 1.6%, respectively. This exercise was

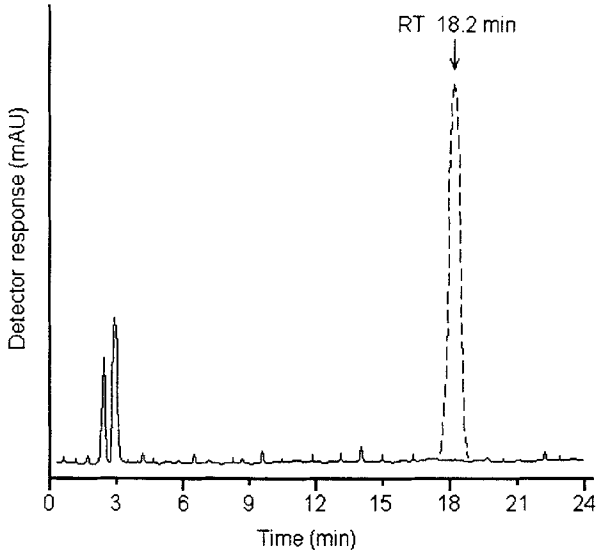

Figure 1. Liquid chromatogram of a blank solution (——) and of a 2 μg/mL tebufenozide standard (- - - -) after a 40-μL injection.

repeated for the other standard solutions and similar results were obtained, indicating the precision of the chromatographic response. Repeat injections on different days indicated a good degree of reproducibility of the results. The linearity of the detector response was confirmed by plotting peak area counts against the analyte concentration range studied (5 to 500 ng) and a linear relationship passing through the origin was obtained with a coefficient of determination of 0.998.

The calibration of the HPLC system reported above was carried out before and during the analysis of SC formulations to check that the instrument was performing within the limits set during validation.

The limit of detection (LOD) was reported in this paper as the concentration which gave a signal to noise ratio of 2:1. The ratio was determined by measuring the peak area of the analyte and dividing it by the absolute value of the largest noise fluctuation from the baseline of the chromatogram of a blank solution.[5] The limit of quantification (LOQ) of the analyte, determined with acceptable precision and accuracy, was expressed arbitrarily as five times the LOD value. The LOD and LOQ values obtained in this study were 1.0 and 5.0 ng, respectively.

Table 1

Tebufenozide Concentrations in Three Lots of Suspension Concentrate (SC) Formulations

Sample Lot	Expected or Label Conc. (% w/v)	Measured Conc. (% w/v) (mean ± SD)	RSD (%)	Abs. Error (%)
SC-1	24.0	22.3 ± 1.6	7.2	7.1
SC-2	24.0	24.2 ± 0.9	3.8	0.8
SC-3	24.0	22.7 ± 1.1	4.8	5.4

Formulation Analysis

The acetonitrile extracts of the three SC formulations after hexane partition, were injected into the HPLC, and the average (n = 6) peak areas were computed. The corresponding concentrations were read from the calibration curve and the %AI in the formulations (w/v) were calculated and compared with the data on the label. The results are recorded in Table 1.

Figures 2 and 3 show typical chromatograms obtained for the acetonitrile extract of SC-1 formulation before and after hexane partition, respectively. Comparison of the sample chromatograms shows noticeable impurity peaks in Figure 2, probably from oil and other nonpolar components present in the extract. Although the analyte peak was somewhat resolved, the baseline separation and peak purity were unsatisfactory. In addition, drift in RT was appreciable from sample to sample (range, 18.0 to 18.8 min). A minor peak near the up-slope and a shoulder at the down-slope of the analyte peak in Figure 2 introduced considerable error in quantification, primarily due to selectivity and specificity in assaying the AI in the formulation. However, the hexane partition, although time consuming, rectified the problem by completely removing the minor peak and the shoulder in the chromatographic trace (Figure 3), as well as the drift in RT.

The AI measured in the three sample lots of SC formulations (Table 1) agreed reasonably well (22.3 to 24.2%, w/v) with the expected or label value (24.0%, w/v) of each sample. The absolute error (deviation of the measured value from the expected or theoretical value in absolute terms of percentage) ranged from 0.8 to 7.1%, whereas the range in RSD was 3.8 to 7.2%. The sample lot, SC-2, received from the local Formulation Project had the lowest RSD (3.8%) and absolute error (0.8%), whereas the field sample (SC-1) had the highest values for both.

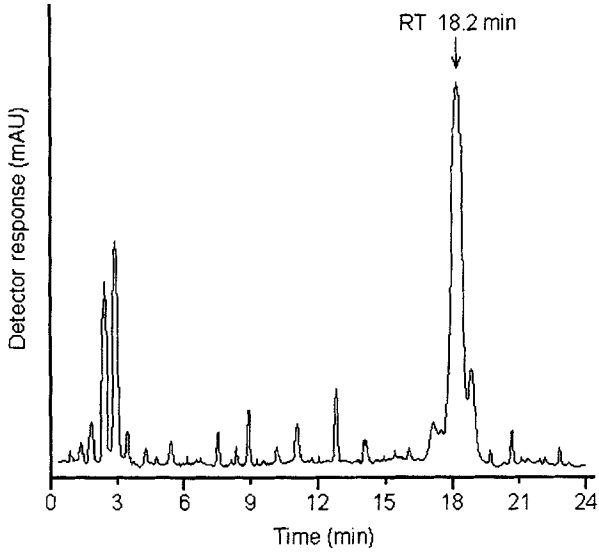

Figure 2. Liquid chromatogram of a suspension concentrate in acetonitrile (40-μL injection); interference from formulation matrices observed.

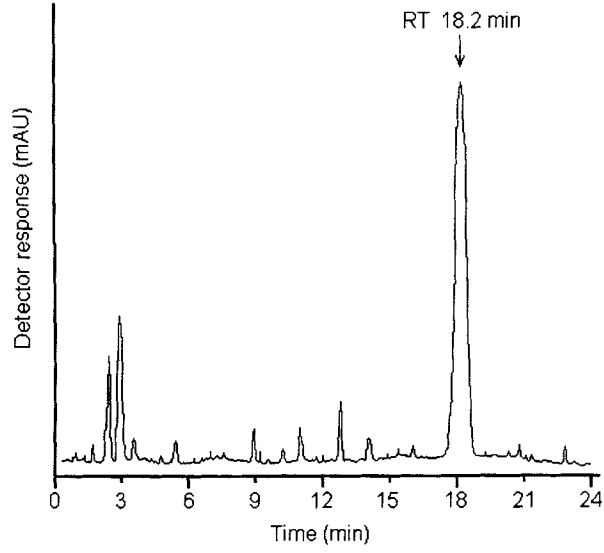

Figure 3. Liquid chromatogram of a suspension concentrate after acetonitrile extraction and hexane partition (40-μL injection).

CONCLUSIONS

The HPLC method described in this paper, provides an easy, accurate, and rugged technique for the extraction and quantification of tebufenozide in commercial SC formulations. This method could be extended easily to routinely analyse the AI in aqueous and emulsifiable concentrates, granular, and wettable powder formulations of tebufenozide, and could become a standard method for quality control operations.

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