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RAPID SEPARATION OF PESTICIDES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH 3-μm COLUMNS

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SUMMARY

A high-performance liquid chromatographic procedure was developed to quantitate the active ingredient in dipping vat samples containing coumaphos (Co-Ral), phosmet (Prolate), crotoxyphos (Ciodrin), synthetic permethrin (Atroban), amitraz (Taktic) or chlorpyrifos (Dursban). The procedure, based on chromatography with a short column (30 × 4.6 mm I.D.) packed with 3-μm particles, allows three times as many samples to be analyzed per unit time as chromatography with a conventional column (100 × 5.0 mm I.D.) with 5-μm particles. This increase in productivity was accomplished with normal operating conditions and standard equipment. There was also a corresponding reduction in solvent volume. Precision, calculated from peak areas, was calculated to be less than 1% relative standard deviation while the minimum detectable amount of amitraz decreased from 700 to 300 pg.

INTRODUCTION

Present economic conditions have placed increased pressure on service organizations, particularly analytical laboratories, to increase productivity. The trend continues toward decreasing personnel and operating costs while increasing the number of samples to be analyzed.

A high-performance liquid chromatographic (HPLC) procedure based on a short column (30 × 4.6 mm I.D.) and standard equipment has met this challenge. This paper describes the HPLC procedure developed to analyze the pesticides currently in use, or being tested for use, in the Tick and Scabies Eradication Programs of USDA.

A brief survey of the literature revealed HPLC methods of analysis for coumaphos[1] and permethrin[2]. Several of the manufacturers of these pesticides have developed HPLC procedures for quality control of their products. However, the procedures that we found could not be modified to be applicable to all of the pesticides of interest and to handle the large number of samples received by our laboratory.

Because the number of vat samples received is large (about 1500 every month), an accurate, rapid and easily automated method of analysis, suitable for several pesticides was needed. An HPLC procedure based on UV detection was developed

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for all of these pesticides because they contain a component that absorbs in the UV.

The 30 × 4.6 mm I.C. column has the advantage of greatly decreased analysis time without requiring changes in the liquid chromatographic (LC) instrument, as is often necessary for columns of low internal volume[3].

Short columns, packed with 3-μm particles, generate sharp, fast-moving peaks. The resolution of these columns and the integrity of the peaks are greatly affected by the peak broadening caused by extra-column or instrumental effects. While standard liquid chromatographic equipment typically has instrumental band widths of 50–150 μl[4], the integrity of small, eluted fast from a short 3-μm particle column, can be maintained by the use of larger k' values. A k' of 100 can be achieved in just 10 min with backpressures of less than 2000 p.s.i. Short columns containing 3-μm particles are ideal for high-speed LC because they are inherently more efficient and show higher efficiencies over a wider flow-rate than long columns, as can be seen from a flatter van Deemter plot[5].

EXPERIMENTAL

The HPLC system consisted of a Waters M6000 pump and Model 440 dual channel detector with an Extended Wavelength Module in one channel. The detection wavelength was 313 nm for coumaphos (Co-Ral, Bayvet Division of Cutter Laboratories), amitraz (Taktic, NOR-AM Chemical Co.) and chlorpyrifos (Dursban, DOW Chemical) or 229 nm for phosmet (Prolate, Zoecon Corporation), crotoxyphos (Ciodrin, Burroughs Wellcome Company) and permethrin (Atroban, Burroughs Wellcome Company). Samples were injected with a Perkin-Elmer 420B Autosampler. Samples were tested at a flow-rate of 2 ml/min of acetonitrile–water (85:15). The columns were 100 × 5.0 mm I.D., 5-μm (Radial-Pak C_{18}, Waters, Milford, MA, U.S.A.) and 30 × 4.6 mm I.D., C_{18}, 3-μm (P-E 3 × 3 C_{18}, Perkin-Elmer, Norwalk, CT, U.S.A.). Data reduction was performed with a Sigma 10 data station (Perkin-Elmer).

In addition to the active ingredient, pesticide formulations may also contain proprietary emulsifiers (see Fig. 2). Most vat samples also contain much filth (up to 10%). This filth was effectively removed by dilution and centrifugation. Samples were diluted 1 to 100 with absolute methanol and centrifuged at 1250 g for 10 min. After this, the samples needed no further filtering or preparation and were injected directly into the column. The injection volume was 10 μl in all cases.

An external standard was used for calibration. A reference standard for each pesticide was obtained from the Environmental Protection Agency (Research Triangle Park, NC, U.S.A.) or the manufacturer. Purity of all reference standards was >97%. The primary standard was prepared freshly each month, except for amitraz which was freshly prepared before use, by accurately weighing the reference standard into a 100-ml volumetric flask and diluting to volume with 100% acetonitrile. The primary standard is then treated like other samples and the results are used to check on instrument calibration at least once a month. By the external standard method, the primary standard is used to calculate the concentration of each pesticide.

In addition to this primary standard, a secondary standard was used for routine quality control. The secondary standard was made by diluting the manufacturer's product to specifications with water. This standard was used as every tenth

sample to determine that the instrument is functioning properly. If the value of this standard varied by more than ±2% of the predetermined value, all ten samples, including the secondary standard, were reanalyzed.

The response to an injection of 10 μl of a sample containing 5 ppm pesticide ranged from 0.143 absorbance units (a.u.) for phosmet to 0.021 a.u. for permethrin with the detector set at 2.0 absorbance units full scale (a.u.f.s.). The detector gave a linear response to each pesticide over a range from 0.5 to 100 ppm. The detection limit ranged from 400 pg for permethrin to 80 pg for phosmet at a signal-to-noise ratio of 4 (S/N = 4).

All samples were tested in duplicate, and a computer program calculated the concentration of the active ingredient in each pair and the mean of each pair, and then compared the mean with the individual answers to flag reruns that deviated from the mean by more than 2%.

RESULTS AND DISCUSSION

Fig. 1 compares a chromatogram on the 100 × 4.6 mm I.D. column (a) and on the 30 × 4.6 mm I.D. column (b), and shows the benefits of the short column packed with 3-μm particles. Fig. 1 also shows an increase in sensitivity for the short column. Peak heights have increased from 5.3 to 11.5 mm for coumaphos and 5.3 to 12.5 mm for amitraz. The minimum detectable amount of amitraz decreased from 700 to 300 pg.

Table I compares resolution and efficiency of both columns and demonstrates a large increase in resolution per unit time. The short, 3-μm particle column has increased this resolution per unit time 2.4 fold. The compounds used for column comparison were coumaphos and amitraz.

Fig. 2 demonstrates the application of the HPLC system to the different pesticides used in the two eradication programs. The use of the short, 3-μm particle columns effected a potential saving of over 100 machine operating hours for the first month of use. When the saving in solvents is added to the saving in operating costs, the increase in productivity provides dramatic benefits. The use of less solvent also decreases the cost of disposal.

The precision of the system was determined by using an amitraz sample containing 25.500 ppm active ingredient. The sample was then run through the entire system twenty times with a mean of 25.522 ppm and a standard deviation of 0.117. The relative standard deviation (R.S.D.) was calculated to be 0.46%. Amitraz was

TABLE I

COMPARISON OF COLUMNS AND PARTICLE SIZE

Conditions the same as in Fig. 1.

Column size	*Particle size*	*Resolution* R_s	*Time (coumaphos)*	*Efficiency HETP*	R_s/*time*
100 × 5.0 mm	5 μm	7.4	1.07	0.064	6.9
30 × 4.6 mm	3 μm	5.1	0.31	0.048	16.5

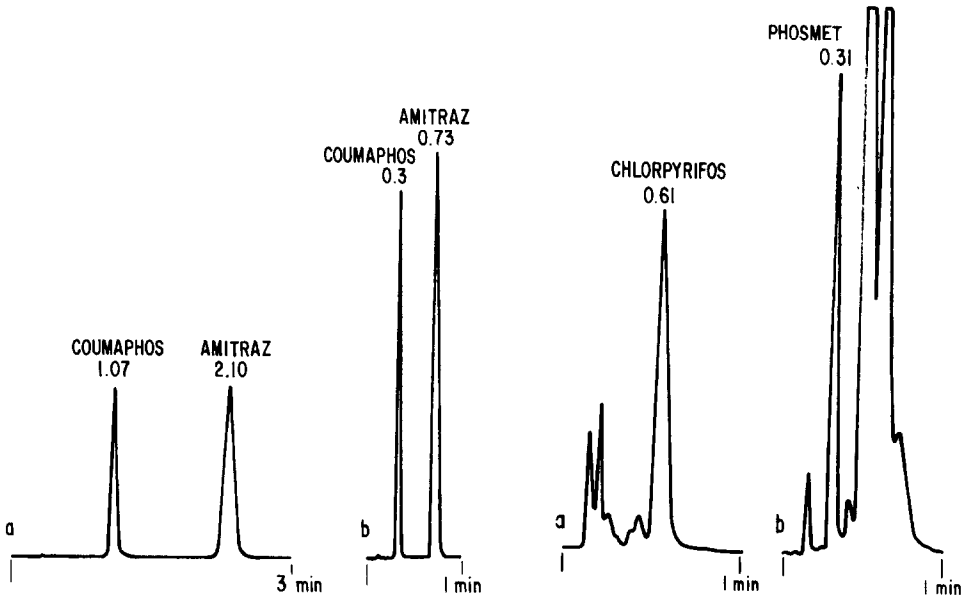

Fig. 1. Comparison of retention time and sensitivity for coumaphos and amitraz on (a) 100 × 5.0 mm I.D. 5-μm particle size column and (b) 30 × 4.6 mm I.D. 3-μm particle size column. Mobile phase: acetonitrile–water (85:15); flow-rate: 2.0 ml/min; UV detection: 313 nm. Injection at the mark below a and b.

Fig. 2. Chromatograms of dipping vat samples of (a) Dursban and (b) Prolate. Conditions the same as in Fig. 1.

chosen for use in precision tests because of its instability, particularly at low pH values and in organic solvents, such as methanol. Use of these columns is not limited to isocratic separations[6]. Gradients as short as one minute have been used[7].

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