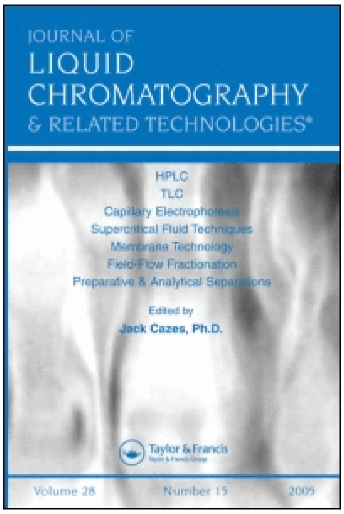

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AN INTERNAL STANDARD HPLC METHOD FOR THE ANALYSIS OF AZINPHOS-METHYL USING A BONDED AMINE STATIONARY PHASE

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ABSTRACT

Azinphos-Methyl technical and formulations have been successfully analyzed using high performance liquid chromatography. Rapid reproducible results have been realized by utilizing a bonded amine stationary phase. Flow programming can be employed to further increase the speed of analysis without significantly affecting data precision and accuracy.

INTRODUCTION

Organic Phosphates, as a class, form one of the most widely used" groups of pesticides in the world today. In light of this fact, their rapid and accurate analysis is of prime importance. Azinphos-Methyl {O,O-dimethyl S-(4-oxo-1,2,3-benzotriazin-3(4H)-yl)methylphosphorodithioate/Guthion}, first described for use on cotton by Ivy[1] et al. in 1955, is one of the most widely used organic phosphates. This compound originated in Germany with Bayer AG[2] in 1955.

Being a rather persistent material, the analysis of azinphos-methyl has occupied a great deal of varied effort[3]. Recently reported methodology has concentrated on the use of either gas chromatography (4-8) or thin layer chromatography (9-15). The U. S. Environmental Protection Agency has published an infrared method[16]. Szalontai[17] has published a study of the retention behavior of organic phosphates using normal phase HPLC.

This paper reports an internal standard HPLC method for the analysis of azinphos-methyl utilizing a bonded amine stationary phase. This method has been found suitable for the analysis of technical and formulated azinphos-methyl samples.

EXPERIMENTAL

The instrument employed in this study consisted of components obtained from Waters Associates (Milford, Mass.). The pumping system consisted of a 6000A pump controlled by a model 660 solvent programmer. Injections were made via a model U6K sample injection valve onto a homemade 30 cm L X 2.1 mm I.D. 10 um bonded amine column. All work was performed at ambient temperatures. A model 440 absorbance detector monitoring at 280 nm was employed for detection at 0.05 AUFS. The mobile phase consisted of 30% ethylene dichloride, 69.78% heptane, 0.11% methanol and 0.11% acetonitrile. All solvents, obtained from Burdick and Jackson (Muskegon, Mich.), were filtered and degassed before use. Data were collected on an Omniscribe dual-pen recorder (10 mv full scale, chart speed 0.5 in/min). In order to speed up the analysis, a step-flow program (Curve 11) going from 1 ml/min to 2 ml/min was employed 8 minutes into the run. Pressures were 1,100 psi at 1 ml/min and 2,400 psi at 2 ml/min.

The bonded amine stationary phase was prepared by reacting 3.7 g of pretreated Partisil 10 (Whatman, Inc.) with 10 g of γ-aminopropyltrimethoxysilane (Silar Laboratories, Inc., Scotia, N.Y.) in 50 ml of benzene at ambient temperature for 3 hours with constant shaking. Pretreatment of the silica consisted of a 2 hour washing in a 75% H_2SO_4/25% HNO_3 solution. The silica was then washed with 3x100 ml of distilled deionized water and 3x50 ml of methanol, chloroform and dry benzene each. Finally the silica was dried for 24 hours at 120°C in a vacuum oven ($P \approx 0.5$-1 mmHg). The packing was washed 3 times each with 50 ml of benzene, chloroform and methanol and subsequently air dried. A subsample of the packing was dried at 40°C for 3 hours under vacuum and submitted for carbon and nitrogen analysis yielding 5.03% carbon and 1.96% nitrogen. Packing of the column was achieved with a system described elsewhere[18] from a 50% ethylene glycol/50% methanol slurry.

Azinphos-methyl standard (96%) was obtained from Chem Service (West Chester, Pa.). Carbofuran 99.5% was employed as the internal standard. Standard weights were obtained to the nearest microgram on a Mettler Model M5 microbalance. The standard solution consisted of 1.702 mg of azinphos-methyl and 2.718 mg of carbofuran in 10 ml of mobile phase + 2 drops of methanol via a Pasteur-Pipette to facilitate total dissolution. When analyzing formulated samples the sample weight is adjusted upward so that the azinphos-methyl content roughly approximates that in the standard solution. After obtaining the proper weight of formulated azinphos-methyl sample, weigh in carbofuran so the content is similar to the standard solution and dilute in a like manner. The standard injection volume employed in this study was 6 μl via a Glenco 10 ul syringe into the U6K valve.

RESULTS AND DISCUSSION

Figure 1 illustrates the 12 minute chromatographic scan of the standard solution specified in the previous section. The analytical conditions for all work discussed are identical to those specified in the experimental. At 8 minutes, the flow was step programmed to 2 ml/min from 1 ml/min. At this point the azinphos-methyl peak has completely eluted and enough time remains before the elution of the carbofuran peak to establish flow equilibrium. By using the flow program we were able to decrease the analysis time 25% without compromising precision or accuracy. Essentially no significant base line deviation was observed due to the flow program. This is most likely due to the flow-through cell design of the Waters 440 detector. Older detectors which do not utilize a taper cell may not exhibit this excellent base line stability. In any event, if the flow program is not practical for the user, one can always operate at a constant flow rate and allow for longer elution times.

The mobile phase composition is somewhat critical from the standpoint of elution of the internal standard peak. The critical point is in the content of the polar modifiers, methanol and acetonitrile. To maintain a reasonable retention time and to prevent coelution of low level impurities in carbofuran the 0.11% specified modifier levels should deviate no more than ±10%. This requirement can be relaxed if the error introduced by coelution of typically 0.1%-0.4% impurities is unimportant.

Figures 2 and 3 illustrate chromatographic scans for a technical and a 2 EC (emulsifiable concentrate) formulation of azinphos-methyl. Several additional peaks now appear in each chromatogram. Over the period of a year many EC's have been run without significant degradation in column performance. If irreversible on column adsorbtion of emulsifi components, contained in the EC, are suspected the use of a precolumn may be warranted. Wave lenght ratio studies (254 nm/280 nm) and

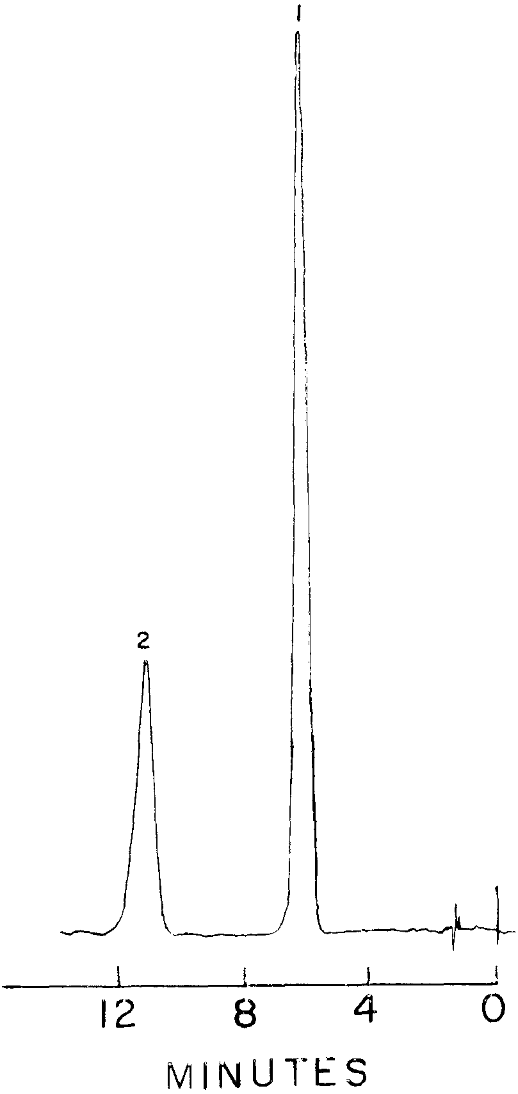

FIGURE 1

Azinphos-Methyl Standard Solution
1) Azinphos-Methyl
2) Carbofuran

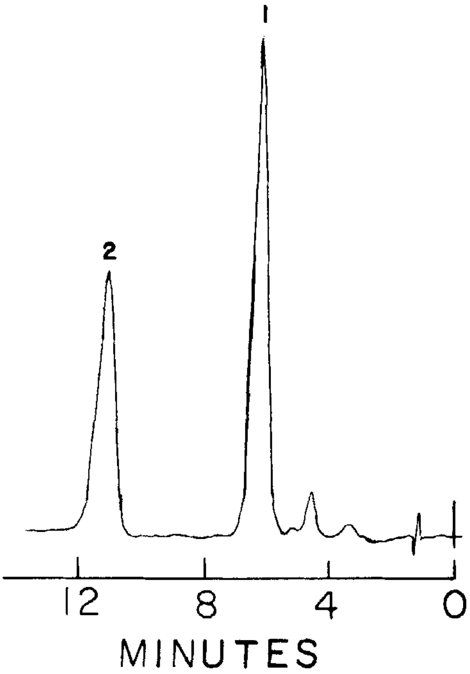

FIGURE 2

Azinphos-Methyl Technical
1) Azinphos-Methyl (68.5%)
2) Carbofuran

formulation blank studies showed that there was neither significant interference under the azinphos-methyl peak due either to impurities in the technical or formulation components. We did not include a blank chromatogram in this report since it would illustrate nothing. Azinphos-methyl gave a wave length ratio (254 nm/280 nm) of 0.82 while carbofuran gave a ratio of 0.13. As far as azinphos-methyl is concerned it matters little whether 254 nm or 280 nm is used since the peak height ratios vary by only 18%. We chose 280 nm in order to obtain sufficient response for the carbofuran internal standard peak while maintaining the

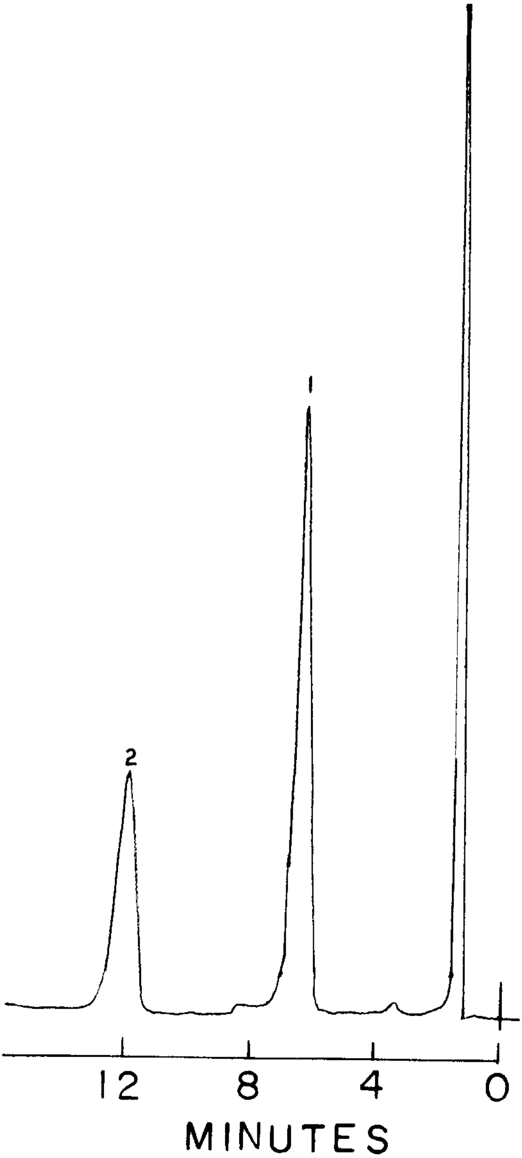

FIGURE 3

Azinphos-Methyl 2 EC
1) Azinphos-Methyl
23.6%
2) Carbofuran

weight of carbofuran in the sample roughly similar to that of azinphos-methyl.

In order to quantitate the data we first calculate a system response factor (F) from data obtained for the standard.

$$(1) \qquad F = \frac{A_{CF} \times W_{AM} \times P_{AM}}{A_{AM} \times W_{CF} \times P_{CF}}$$

where A_{CF} = area of carbofuran peak

A_{AM} = area of azinphos-methyl peak

W_{CF} = wt carbofuran in the sample

W_{AM} = wt of azinphos-methyl in the sample

P_{CF} = purity carbofuran

P_{AM} = purity azinphos-methyl

After getting an average F value from several runs, we can proceed to analyze samples. The results for an unknown sample are calculated thusly:

$$(2) \qquad \%\ \text{Azinphos-methyl} = \frac{A_{AM} \times W_{CF} \times P_{CF} \times 100 \times F}{A_{CF} \times W_{AM}}$$

It should be noted that if an integrator or data system is not available, peak heights can be substituted for areas with excellent results.

The bonded amine column used in this study was prepared in-house. From past experience, we consider this column fairly typical of many such columns commercially available. We feel that almost any good bonded amine column capable of generating at least 3000 plates per meter is suitable for this analysis.

CONCLUSION AND SUMMARY

Using a bonded amine column we were able to analyze both formulated and technical azinphos-methyl samples with good reproducibility. Since wavelength ratio studies showed no significant interference, for both formulated and technical samples, and results obtained for a 2 EC (figure 3) are reasonable, 2 EC's usually assay 20-25%, we have confidence in the accuracy of the method. Standard deviations of 1 were typical. If further precision is required, a temperature control system[19] would undoubtedly help. An optional step-flow program can be used to speed up the analysis without significantly compromising data quality.

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