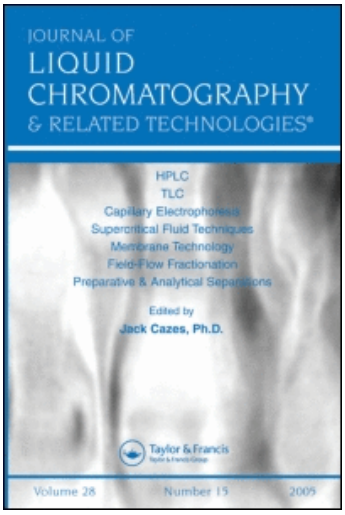

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REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC DETERMINATION OF NICOTINE IN PESTICIDE FORMULATIONS

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ABSTRACT

A simple and rapid reversed-phase high-performance liquid chromatographic procedure is described for the determination of nicotine in liquid formulations. Samples are diluted with methanol, and naphthalene is added as the internal standard. Peak height ratios obtained from injections of standard and sample filtrates are used for quantitation. An eluting solvent of 0.05*M* $(NH_4)_2 HPO_4$ (pH 7.5) - methanol (40/60, v/v) at a flow of 2 mL/min gives retention times of 3.13 and 6.88 min respectively for nicotine and naphthalene. Sample analysis can be completed in approximately one hour by the method described as compared to 1.5 days required by the Official AOAC gravimetric method (6.176-6.177).

INTRODUCTION

Nicotine, (S)-3-(1-Methyl-2-pyrrolidinyl) pyridine, is a botanical insecticide derived from tobacco. Although nicotine was used extensively a number of years ago, it has largely been replaced by newer insecticides, particularly the organo-

phosphates. Currently little or no nicotine is produced in the United States, however, limited supplies are imported. Nicotine is registered for insect control on numerous fruits and vegetables, and it is still employed to a limited extent, primarily in home gardens and greenhouses. Thus, State and Federal control laboratories must occasionally analyze nicotine formulations.

Since our laboratory infrequently encounters nicotine products, it is difficult to maintain the analytical skills generated through frequent application of the Official AOAC gravimetric method, 6.176-6.177 (1). In addition, the official method is lengthy, taking approximately 1.5 days for completion. To reduce the effort required for nicotine analysis, a simple, rapid method was sought.

The evolutionary, if not revolutionary, developments in gas-liquid chromatography (GLC) during the past quarter century and high-performance liquid chromatography (HPLC) in the current decade, have greatly changed the approach to pesticide analysis in most laboratories. In adopting the philosophy of employing chromatographic methods when possible, both GLC and HPLC were considered for development of a nicotine method. GLC analysis of nicotine is possible, but either derivatization (2) or a column packing not commonly used in pesticide analysis is needed (3). A report of normal phase HPLC on silica gel (4) encouraged us to explore this technique. To avoid the problems of maintaining silica gel activity and the lengthy equilibration typical of silica gel, an HPLC method using a reversed-phase column for nicotine analysis was developed.

MATERIALS AND METHOD

Apparatus and Reagents

(a) High performance liquid chromatograph.-Waters Model ALC-202 equipped with Model U6K septumless injector (Waters Associates, Inc., Milford, MA) in conjuction with a Laboratory Data Control Spectromonitor III detector (Laboratory Data Control, Riviera Beach, FL) operating at 1.0 absorbance units full scale and 254 nm. An Omni-Scribe recorder (Houston Instruments, Corp., Austin, TX) was set at 10 mV with a chart speed of 0.2 in/min. A flow rate of 2.0 mL/min. and a sample injection of 20 µL was used throughout.

(b) Chromatographic columns.-30 cm x 4 mm i.d. µBondapak C_{18} (Waters Associates) and 25 cm x 4.1 mm i.d. stainless steel column slurry packed in the laboratory with LiChrosorb RP-18 (10µm,Alltech Associates, Arlington Heights, IL). The latter column was packed using 2-propanol (Burdick & Jackson, Inc., Muskegon, MI) as the slurry and packing solvent. Packing was done with a Model 705 stirred slurry column packer (Micromeritics, Norcross, GA) and a Tracor Model 950 pump (Tracor Corp., Austin, TX) operated in the constant speed mode at 9.99 mL/min with maximum pressure limit set at 5,000 p.s.i. Each analytical column was preceded by a 10 cm x 2 mm stainless precolumn (packed with Vydac RP, Alltech Associates).

(c) Chromatographic eluting solvent.-Methanol (Burdick & Jackson)-0.05 M $(NH_4)_2 HPO_4$, pH7.5 (60/40, v/v). Filter after mixing and degas under light vacuum.

(d) Internal standard solution.-10 mg/mL in methanol. Weight 1.0 g naphthalene (Eastmen Kodak Co., Rochester, NY) into a 100 mL volumetric flask, dissolve and dilute to volume with methanol.

(e) Mixed standard solution.-2 mg/mL nicotine and 2 mg/mL naphthalene in methanol. Weigh 0.1 g nicotine (Eastman) into a 50 mL volumetric flask and add by pipet 10 mL internal standard solution (d). Mix, dissolve and dilute to volume with methanol.

Preparation of Sample

Weigh portion of well mixed liquid formulation equivalent to 0.1 g nicotine into 50 mL volumetric flask and add by pipet 10 mL internal standard solution (d). Mix and dilute to volume with methanol. Filter portion through 0.5 µm Millipore filter (type FHLP, Millipore Corp., Bedford, MA) into small vial and cap. Inject filtrate into HPLC unit for quantitation.

Quantitation

Inject 20 µL aliquots of mixed standard into HPLC until variation in peak height ratios of nicotine to naphthalene is ca. 1%. Adjust detector sensitivity as needed to give peak heights ca.60-80% full scale. Inject mixed standard, inject sample twice, and repeat injection of mixed standard. Calculate peak height ratios (nicotine : naphthalene) for the 2 mixed standard and sample injections. Average peak height ratios and calculate percent nicotine:

$$\text{Nicotine, \%} = (R / R') \times (W' / W) \times P$$

where R' and R = average peak height ratios for mixed standard and sample, respectively; W' = g nicotine standard / 50 mL mixed standard solution (0.1 g for method described); W = g sample weighed for analysis; and P = percent purity of nicotine standard.

RESULTS AND DISCUSSION

Linearity of response was checked for 20 µL injections containing from 8 to 100 µg of both nicotine and naphthalene.

With the detector employed, the response, as measured by peak height, was linear from 8 to 50 μg injected for both compounds. An injection of 40 μg in 20 μL was selected for use throughout the remainder of the study. This injection mass and volume permit reasonable sample weights and easily measured injection volumes. The average peak height ratio (nicotine : naphthalene) for 6 consecutive injections was 0.915 with a coefficient of variation of 0.111, demonstrating good precision.

Since the solvent system selected as giving acceptable chromatography has a pH of 7.5, continuous use of the solvent could cause some column deterioration; however, periodic use of the solvent for 6 months did not significantly reduce the efficiency of the column. This was probably due, at least in part, to protection provided by the precolumn. Additionally, the precisions of analysis with the laboratory and commercially packed columns were comparable. Thus, with a minor sacrifice in analysis time, the laboratory packed column was preferentially used and the commercial column reserved for more difficult separations. The chromatography of nicotine and naphthalene on the two columns is shown in Figures 1 and 2.

Using the established chromatographic conditions, three commercial nicotine formulations were analyzed for nicotine content. These samples were specified by their labels to contain 40% nicotine alkaloid. The results of analysis for the samples are given in Table 1. Nicotine levels in all samples were found to be between 2 and 5% above the label claims. An average coefficient of variation of 0.45 was obtained for the three samples analyzed using the laboratory packed column. Although more analyses would be necessary before a good comparison can be made, the limited data obtained with the commercial column indicates that the greater efficiency of this column is not necessary in routine analysis. Similarly, the laboratory packed column has been found to be

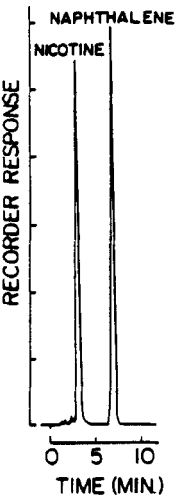

Figure 1. Nicotine and naphthalene, μBondapak C_{18} column

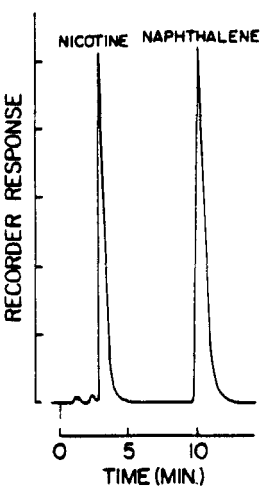

Figure 2. Nicotine and naphthalene, LiChrosorb RP column

Table 1. Assay of Commercial Nicotine Formulations

Sample	Label claim, %	Column	Nicotine found[a] %
#355	40.0	LiChrosorb RP	42.94 ± .28
#355	40.0	μBondapak C_{18}	42.70 ± .42
#285	40.0	LiChrosorb RP	42.84 ± .42
#679	40.0	LiChrosorb RP	41.14 ± .66

[a]Mean of 3 determinations and coefficients of variation.

adequate for HPLC determination of the majority of pesticides analyzed in our laboratory. A notable exception is the HPLC analysis of rotenone.

One of the samples in Table 1, #679, gave a result of 41.34% nicotine when analyzed by the AOAC gravimetric method (1). This value is not significantly different from the values obtained by HPLC. Thus, the method reported herein appears to offer a rapid procedure for scanning formulations for nicotine content.

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