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Short Communication

Determination of aldicarb, aldicarb sulfoxide and aldicarb sulfone in tobacco using high-performance liquid chromatography with dual post-column reaction and fluorescence detection

S.S. Yang*, I. Smetena

Research Center, Philip Morris USA, P.O. Box 26583, Richmond, VA 23261, USA

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Abstract

A screening method for the determination of aldicarb (AS) and its sulfoxide (ASX) and sulfone (ASN) metabolites in tobacco at low ppm levels is described. Tobacco samples are extracted using methanol with the aid of sonication at ambient conditions. The extract is filtered and then injected into a high-performance liquid chromatograph equipped with a dual post-column reaction system and a fluorescence detector. Chromatographic separation is performed on a C_{18} column with a mixture of methanol–acetonitrile–water containing 0.1% of triethanolamine as the mobile phase. Triethanolamine is added to improve peak shape of AS residues and to reduce the undesired interaction between residual silanols and interferences, mainly amino acids and other amines. The average recoveries for AS residues spiked in tobacco are higher than 95% for AS, 91% for ASN and 85% for ASX at levels of 0.5–10 ppm (w/w). The detection limit is 0.5 ppm for each of the target compounds.

1. Introduction

Aldicarb (AS) is an N-methylcarbamate pesticide for the control of insects, mites and nematodes in the farming of fruits, vegetables, tobacco and other agriculture products. After its application, AS gradually turns into the metabolites, aldicarb sulfoxide (ASX) and aldicarb sulfone (ASN), and can be further degraded to oxime and nitrile forms. The wide use of AS has led to increasing demand for monitoring of its residues in both crops and the environment (*i.e.*, farm soil and ground water) [1–11]. Both gas chromatographic (GC) and high-performance liquid chromatographic (HPLC) methods have been used to measure AS and its degradation products. Because of their thermal instability, GC analysis usually requires conversion of AS residues to nitriles by pyrolysis on the GC injection port at a temperature above 300°C [12–15] or conversion to sulfone by precolumn oxidation. This latter method provides no information for the individual quantities of AS, ASX and ASN. Because of these problems with the GC methods, most of the reported analyses have been based on HPLC with dual post-column reaction and fluorescence detection (Fig. 1) [1–3,7–9,11,16–18]. In the first reactor, aldicarb

* Corresponding author.

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residues in the HPLC eluate are hydrolyzed with aqueous NaOH solution at an elevated temperature to yield methylamine, which is subsequently derivatized in the second reactor with *o*-phthaladehyde (OPA) prior to fluorescence detection.

To determine AS residues at sub-ppm or ppb levels, the GC or HPLC procedures usually involved an extensive sample cleanup procedure using liquid–liquid or solid-phase extraction. These cleanup procedures are necessary, especially for crop samples in which severe matrix interferences exist. During the prolonged cleanup steps, sample loss leading to low recovery is a common problem. Recoveries for polar compounds such as ASX usually range from 20 to 50% [3,5,16]. However, it should be noted that the low recoveries of these polar compounds, normally seen in the methylene chloride extraction of water or high water-content samples, is for a large part due to the unfavorable partitioning [16]. For water samples containing little organic matter, these extraction and cleanup steps are eliminated and direct large volume injection (up to 500 μL) is employed [7,8]. Although providing satisfactory recovery, this approach is restricted by the loss of column efficiency, shorter column life and impracticality when dealing with complex samples.

In this study, a screening method using HPLC with post-column reaction and fluorescence detection for the determination of AS, ASX and ASN in tobacco at low ppm levels was developed. Tobacco samples were extracted using methanol with the aid of sonication. No sample cleanup other than filtration was involved. Chromatographic separation was performed on a C_{18} column. Triethanolamine (TEOA) was added to the mobile phase to mask the residual silanols on the surface of HPLC packing (*e.g.*, chemically modified silica particles). The use of TEOA as the mobile phase modifier improved peak shape of AS residues and, more importantly, reduced the undesired interaction between residual silanols and interferences, mainly amino acids and other amines. Most of these amines were eluted from the HPLC column immediately after the solvent peak, thus eliminating their interference with peaks for aldicarb residues. The elution order of AS and its metabolites, ASX and ASN, on C_{18} columns with different carbon loading and surface bonding chemistry was also studied.

2. Experimental

2.1. Chemicals

Methanol and acetonitrile were HPLC grade from Baxter (McGaw Park, IL, USA). Distilled and deionized water was obtained from a Milli-Q system. AS, ASX and ASN were manufactured by Riedel-de Haen (Germany). OPA and N,N-dimethyl-2-mercaptoethylamine hydrochloride (tradename Thiofluor) were purchased from Pickering Lab. (Mountain View, CA, USA). Other chemicals were purchased from various sources.

Sodium hydroxide solution (0.1 *M*) was prepared by dissolving 4 g of NaOH in 1 l of water. Tetraborate buffer (pH 9.1, 50 m*M*) was prepared by dissolving sodium tetraborate decahydrate in water. Both tetraborate buffer and NaOH solution were thoroughly degassed by sparging with helium. OPA solution was prepared by dissolving 100 mg of OPA in 10 ml of methanol, then added to a premixed solution of 2 g Thiofluor in 1 l of tetraborate buffer.

2.2. Preparation of standard solution

Stock solutions of AS, ASX and ASN were prepared by dissolving approximately 10 mg of each compound (weighed to 0.1 mg) into 250 ml of methanol (nominally 40 μg/ml). Working standards were prepared by pipetting 0.5, 1.0, 3.0 and 10 ml of the stock solution separately into four 100-ml volumetric flasks (nominally 0.2, 0.4, 1.2 and 4 μg/ml of each compound) and diluting to volume with methanol.

2.3. Sample preparation

A portion of ground tobacco (1.0 g) was placed in a 30-ml glass vial with a PTFE-lined cap. After adding 6 ml of methanol, the vial was

capped, placed in an ultrasonic bath, and sonicated for 30 min. A 1-ml aliquot of the extract was filtered through a Gelman filter (0.45 μm) into an 1.5-ml autosampler vial and capped for HPLC analysis.

2.4. Instrument and procedure

A Hewlett-Packard Model 1090L HPLC system equipped with an autosampler, a Model 1046A programmable fluorescence detector and a Hewlett-Packard 9000 LC workstation was used. A dual post-column reaction system (Pickering Lab) was connected to the HPLC system. The post-column reaction unit consisted of two reagent pumps (flow-rate fixed at 0.3 ml/min), an HPLC column thermostat controlled at 42°C and two reaction coils. The first rection coil was heated to 100°C for NaOH hydrolysis and the second one was kept at ambient temperature for OPA derivatization.

The analytical column selected for routine analysis a was a Hewlettt-Packard Hypersil C_{18} column (200 mm × 4.6 mm I.D.). The other columns tested in this study included a Waters Novapak C_{18} column (150 mm × 3.9 mm I.D., 4 μm packing), a Phenomenex Ultracarb C_{18} column (150 mm × 4.6 mm I.D.) and two Pickering C_{18} columns for carbamate analysis 150 mm × 4.6 mm I.D. and 250 mm × 4.6 mm I.D.). For each test, HPLC column was placed in the thermostat of the post-column reaction unit and maintained at 42°C.

The mobile phase consisted of two solutions: solvent A was 0.1% of TEOA in a mixture of acetonitrile–methanol (20:80, v/v); solvent B was 0.1% of TEOA in water. The initial composition of 10% solvent A was maintained for a 4-min hold period, after which a 7-min gradient program to 30% of solvent A was begun. Mobile phase composition was then changed over a 5-min period to 90% solvent A, and finally, 90% solvent A was held for 10 min to provide column cleanup before returning to the initial conditions. The flow-rate was 1 ml/min. The HPLC run was stopped at 30 min. Both the NaOH solution and the OPA solution in the post-column reaction unit were constantly pumped at a flow-rate of 0.3 ml/min during the whole sequential cycle. The injection volume of tobacco extract was 10 μl. Excitation and emission wavelengths of the fluorescence detector were set at 330 and 465 nm, respectively. Calculation was based on the external standard procedure.

3. Results and discussion

Shown in Fig. 1 are the representative chromatograms from an AS-free, mixed tobacco

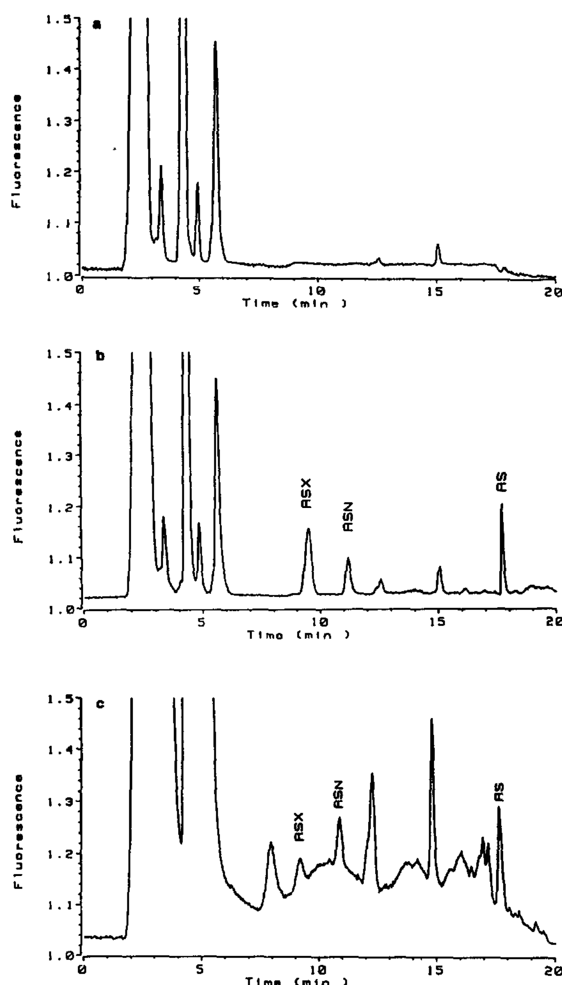

Fig. 1. Representative chromatograms for tobacco sample spiked with aldicarb residues (the sum of ASX, ASN and AS) at (a) 0 ppm, (b) 15 ppm and (c) 3 ppm levels. Details and chromatographic conditions are described in the text.

sample (burley–bright–oriental, 1:1:1) and tobacco samples spiked with aldicarb residues at levels of 15 and 3 ppm (the sum of AS, ASN and ASX). Tobacco samples were extracted by methanol with sonication and, after filtration, an aliquot of 10 μl was injected into the HPLC system. Chromatographic separation was performed on a Hypersil C_{18} column. The mobile phase was a mixture of acetonitrile–methanol (20:80, v/v) and water under a gradient elution mode. TEOA was added to the mobile phase at 0.1% level as the modifier. Relative standard deviation (2σ) for peak area of the target compounds in 10 replicate injections was 7%.

Satisfactory recoveries were obtained using methanol as the extraction solvent. For the spiking levels of 0.5–10 ppm of each target compound, the average recoveries were 98% for AS, 91% for ASN and 85% for ASX (Table 1). Other organic solvents, such as methylene chloride, were also tested for extraction efficiency. Methylene chloride has been frequently used to extract AS residues and other carbamates from water samples [7–9,11,16]. However, the extraction efficiency for polar compounds, such as ASX, were low and improvement can only be made by time-consuming multiple extractions [16]. The poor recovery was explained as a result of unfavorable partitioning of ASX into the organic layer due to its polarity [16]. For tobacco samples, methylene chloride demonstrated the advantage of extracting less polar interferences but, as in the cases of water samples, it failed to provide sufficient extraction of aldicarb residues, particularly ASX.

In the published reports for the determination of aldicarb residues [1–11,17], chromatographic separations were performed on columns of various types such as C_{18}, C_8, cyano, phenyl and silica. Although reasonable separation was accomplished in each case, C_{18} columns were the most commonly used. In this study, silica-based C_{18} columns evaluated included Hypersil C_{18} (1), NovaPak C_{18} (2), Phenomenex Ultracarb C_{18} (3) and two Pickering C_{18} columns of different lengths (150 mm, 4; 250 mm, 5). The first three columns gave the elution order of ASX-ASN-AS, which is consistent with most of the reported reversed-phase separations for aldicarb residues [1–11,17]. Under the same chromato-

Table 1
Recoveries of aldicarb residues spiked in tobacco samples

Compound	Concentration (ppm)		Recovery (%)
	Added[a]	Found[b] (S.D.)	
Aldicarb	0.50	0.48 (0.06)	95
	2.0	1.98 (0.11)	99
	5.0	4.95 (0.26)	99
	10.0	9.80 (0.32)	98
Average			98
Aldicarb sulfone	0.5	0.44 (0.08)	88
	2.0	1.86 (0.20)	93
	5.0	4.55 (0.41)	91
	10.0	9.20 (0.88)	92
Average			91
Aldicarb sulfoxide	0.5	0.41 (0.08)	82
	2.0	1.72 (0.22)	86
	5.0	4.41 (0.50)	88
	10.0	8.60 (0.98)	86
Average			85

[a] Individual amount of aldicarb, aldicarb sulfone and aldicarb sulfoxide spiked in tobacco.
[b] Average value of five replicate analyses.

graphic conditions, the last two columns gave a different elution order, ASN-ASX-AS. Although detailed information for the manufacturing of each column is not available, it can be speculated that columns 4 and 5 are significantly different from the others in several aspects, including the nature of silica support and the chemical modification of the silica surface (*e.g.*, carbon loading, bonding chemistry, endcapping etc.) [19,20].

Without the addition of OPA reagent, chromatograms (not shown) obtained from a tobacco extract gave a clean baseline, indicating that most of the peaks detected under the given conditions were OPA derivatives, presumably from amino acids and other amines. Since tobacco abounds in amino acids [21], under the experimental conditions used in the current study, these compounds may be the major peaks on the chromatograms. This assumption was further confirmed by injecting a mixture of 18 amino acids commonly found in tobacco and obtaining a chromatographic pattern similar to that of a tobacco extract.

In this study, the mobile phase was a mixture of acetonitrile–methanol (20:80, v/v) and water containing 0.1% TEOA as the modifier. Initially, when methanol and water were used as the mobile phase without any modifier, the elution of the major peaks, *e.g.*, amino acids, shifted and became faster as the column aged. Peak shifting made the separation of target peaks from matrix a difficult job. A possible explanation for the peak shifting is that the amino acids strongly interact with residual silanols on the silica surface of HPLC packing. Eventually, when the residual silanols are "shielded" and hydrophobic interaction with the immobilized C_{18} ligands dominated the solute retention, these polar amino acids are quickly eluted from the column. By adding TEOA as the mobile phase modifier, the residual silanols can be masked and peak shifting, as described above, can be eliminated. As shown in Fig. 1a, most of these amines are eluted immediately following the solvent peak, leaving a big window in the chromatogram for the latter eluted aldicarb residues.

Triethylamine (TEA) also was tested as a modifier at 0.1% level for the same purpose. Theoretically TEA, a tertiary amine, should be retained strongly by silanols on the silica surface and, if eluted, should not react with OPA and become detectable by fluorescence. However, adding 0.1% TEA in the aqueous part of the mobile phase gave a drifting baseline with a broad peak on the second half of the chromatogram. It was possible that TEA molecules retained in the column initially were eluted when the gradient mobile phase became rich in organic solvent. These eluted TEA might be decomposed and could then be derivatized by OPA.

In the post-column reactions, 0.1 *M* NaOH solution and 0.05 *M* of tetraborate buffer were used. The concentrations of these two solutions are higher than the average of reported values, *e.g.*, 0.05 to 0.2 *M* for NaOH solution and 0.01 to 0.05 *M* for tetraborate buffer [1–3,7–9,11,16]. However, the factory preset flow-rate, 0.3 ml/minute, is lower than the 0.5 ml/min rate used in the other reported procedures. Excitation and emission wavelengths for the fluorescence detection were set at 330 and 465 nm, respectively. Excitation at 230 nm using a deuterium lamp was reported to increase the sensitivity by three-fold [3,7]; however, no significant improvement was observed from the fluorescence detector equipped with a xenon lamp used in this study.

4. Conclusions

A quick screening method for the determination of AS, ASX and ASN in tobacco at low ppm levels is described. An HPLC system equipped with a dual post-column reaction system and a fluorescence detector is used for the analysis. Sample preparation consists of a single extraction with methanol. Chromatographic separation is performed on a Hypersil C_{18} column. Mobile phase composition is optimized so that major interferences (amino acids and other amines) are eluted as early peaks and are well separated from the peaks of target compounds. With modification, this procedure could be extended to the determination of the other carbamates in tobacco.

5. References

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