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VALIDATION OF METHOD AG-325 FOR THE
DETERMINATION OF CGA-48988 RESIDUES IN TOBACCO

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A B S T R A C T

Analytical Method AG-325 is used for the determination of CGA-48988 [N-(2,6-dimethylphenyl)-N-(2'-methoxyacetyl)alanine methyl ester] residues in crop samples. This method was validated for the analysis of tobacco samples using fortification experiments and ϕ - 14 C-CGA-48988-treated crop. Samples were analyzed before and after fortification with CGA-48988 in the range of 1.0 to 60 ppm. An average recovery of $86 \pm 7\%$ was observed in these experiments. Analysis of ϕ - 14 C-CGA-48988-treated tobacco (both green and cured) by AG-325 determined 28-35% of the total radioactive residue in the crop as the parent. The lower detection limit for the method is 1.0 ppm.

INTRODUCTION

Analytical Method AG-325 was developed to determine the residues of CGA-48988 [N-(2,6-dimethylphenyl)-N-(2'-methoxyacetyl)alanine methyl ester] in crop samples. This method involves an initial extraction of crop samples by blending with 80% methanol. An aliquot of the extract is acidified and partitioned with dichloromethane. The dichloromethane solution is evaporated and the residue is passed through a Grade V alumina column. The sample is analyzed using a gas chromatograph equipped with an alkali flame ionization detector. The validity of this method is demonstrated in this report by recoveries using untreated tobacco samples fortified with CGA-48988, and by application of the method to green and cured tobacco leaves treated with ϕ - ^{14}C -CGA-48988.

EXPERIMENTAL

Fortification Studies: Untreated samples of green and cured tobacco from upper, middle and lower portions of the plant were analyzed by method AG-325. These samples were also fortified before extraction with CGA-48988 at residue levels ranging from 1.0 to 60.0 ppm.

Analysis of ϕ - ^{14}C -CGA-48988 Treated Tobacco Leaves: Samples of 12-week green bright tobacco treated with ϕ - ^{14}C -CGA-48988 at 0.25 and 0.50 lb. a.i./A, 12-week green burley tobacco treated with ϕ - ^{14}C -CGA-48988 at 6.0 lb. a.i./A and cured burley tobacco treated with ϕ - ^{14}C -CGA-48988 at 0.5 lb. a.i./A were analyzed by AG-325. These samples are more completely described in ABR-78036 (2). The radioactive tobacco was diluted with nonradioactive control tobacco before analysis by AG-325.

RESULTS AND DISCUSSIONS

Recovery Studies: Using untreated tobacco samples (both green and cured) fortified with CGA-48988 in the range of 1.0 to 60 ppm, the recoveries ranged from 71 to 95% with an average of $86 \pm 7\%$ (N = 13) (Table I). Analysis of untreated samples showed residues of <1.0 ppm. Results from these studies show that satisfactory (>70%) recoveries are obtained by using method AG-325.

Analysis of ϕ - ^{14}C -CGA-48988 Treated Tobacco Leaves: The results of the analysis of 12-week green and cured tobacco treated with ϕ - ^{14}C -CGA-48988 are shown in Table II.

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Data previously reported in ABR-78036 for these samples are given along with results of analyses by Analytical Method AG-325. The samples used for the latter analyses were diluted with nonradioactive control tobacco, and the total ppm values are given for diluted and undiluted material.

The extraction of total radioactivity by the procedure of AG-325 is seen to be very similar to the biphasic extraction reported in ABR-78036. From 87 to 104% of the total residues were extracted from these tobacco samples.

The residues determined by gas chromatography, expressed as a percentage of the total radioactive residue, range from 28 to 35%. These values are in good agreement with the percentages found by radioactivity measurements on the solutions prior to GC injection. They are also in agreement with the percentages of CGA-48988 previously determined and reported in ABR-78036.

Thus, Analytical Method AG-325 is shown to determine all of the residues of parent CGA-48988 in aged tobacco samples.

CONCLUSIONS

The validity of method AG-325 for the determination of residues of CGA-48988 in tobacco is demonstrated by satisfactory recovery values of untreated samples fortified with the parent. The method also has been shown to be valid on ϕ -¹⁴C-CGA-48988-treated tobacco accounting for 28-35% of the total residue and all of the parent fungicide remaining as a residue.

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REFERENCES

1. AG-325, "Gas Chromatographic Residue Determination of CGA-48988 in Crop Samples."
2. ABR-78036, "Uptake and Balance of ϕ - ^{14}C -CGA-48988 and its Metabolites in Greenhouse Grown Bright and Burley Tobacco."

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TABLE I. RECOVERY DATA FOR RESIDUE METHOD AG-325 FOR TOBACCO SAMPLES FORTIFIED WITH CGA-48988^a

AG-A No.	Tobacco Type	ppm, CGA-48988		% Recovery	
		Added	Found ^b		
4789	Cured	1.0	0.89	89	
	Cured	5.0	3.6	71	
	Cured	10.0	9.5	95	
	Green	5.0	4.4	88	
	4732	Green	5.0	4.4	88
		Cured	10.0	9.1	91
		Cured	5.0	4.4	88
		Cured	1.0	0.93	93
		Cured	60.0	55	92
4799	Cured	1.0	0.79	79	
4778	Green	1.0	0.82	82	
	Cured	5.0	3.9	77	
4785	Cured	2.0	1.8	90	
				Mean 86 ± 7%	

^a Data from AG-A Reports.

^b Found after fortification. Corrected for control.

TABLE II: COMPARISON OF RADIOACTIVITY VALUES VERSUS GAS CHROMATOGRAPHY VALUES FOR THE DETERMINATION OF CGA-48988 IN ϕ - ^{14}C -CGA-48988-TREATED TOBACCO

^{14}C -Tobacco Samples					
Bright Green 12-week 0.25 lb/A	Bright Green 12-week 0.5 lb/A	Bright Green 12-week 0.5 lb/A	Bright Green 12-week 0.5 lb/A	Burley Green 12-week 6 lb/A	
7.8 ppm	14.1 ppm	147.7 ppm	15.0 ppm		
94%	103%	81%	94%		
38%	35%	27%	33%		
Total ppm ^a					
Percent extractable ^a					
Percent as CGA-48988 ^a					
<u>Analysis by AG-325</u>					
Total ppm (diluted)					
Percent extracted ^b					
Percent ^{14}C in solution used for GC determination					
CGA-48988 residues found (ppm)					
Percent of total ppm					

^aReported in ABR-78036.

^bObtained by determination of radioactivity using a liquid scintillation counter.