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STABILITY AND ACCOUNTABILITY OF RESIDUES OF METALAXYL AND SELECTED METABOLIC MOIETIES USING ANALYTICAL METHOD AG-395

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A B S T R A C T

A freezer storage stability study was conducted to determine the stability of metalaxyl and its metabolites, CGA-62826, CGA-67869, CGA-107955, CGA-37734, and CGA-94689 in fruit and vegetable crops. Strawberries, apples, cabbage, lettuce, and potatoes were fortified at 1.0 ppm of each moiety and stored in a freezer at -15°C. These samples were analyzed for "total" metalaxyl residues over a one-year period at six-month intervals. No loss of metalaxyl nor its plant metabolites due to storage was observed. Thus, residues of metalaxyl and its metabolites are stable for at least one year under freezer storage conditions.

The method accountability of metalaxyl and metabolites was also determined using the data obtained from the storage stability study and statistically determining the mean and standard deviation of the results. The accountability using analytical method AG-395 for metalaxyl and the metabolites ranged from 30 percent for CGA-94689 to 77 percent for CGA-67869 indicating that the analytical method (AG-395) is a valid method for determining total metalaxyl residues in crops.

INTRODUCTION

The stability of pesticide residues in stored samples must be determined to ensure the validity of the analytical data. The stability of metalaxyl and its plant metabolites, CGA-62826, CGA-67869, CGA-107955, CGA-37734, and CGA-94689 (See Figure 1), in stored samples was, therefore, investigated. Homogeneous samples of strawberries, apples, cabbage, lettuce, and potatoes each fortified in the laboratory were stored under freezer conditions (-15°C) and analyzed over a one-year period. Another important factor in determining the validity of the data is the accountability of the residues of metalaxyl and metabolites using analytical method AG-395. This was determined by statistically determining the mean and standard deviation of the uncorrected results for each compound obtained during the storage stability study.

EXPERIMENTAL

Samples of strawberries, apples, cabbage, lettuce, and potatoes were individually weighed into square, wide-mouth, amber jars. Samples of each substrate were fortified separately with 1.0 ppm of metalaxyl, CGA-62862, CGA-67869, CGA-107955, CGA-37734, and CGA-94689 using acetone stock solutions. The solvent was allowed to evaporate and the jars were capped and stored in the freezer at -15°C. Detailed experimental conditions are described in Biochemistry Protocol 4-85.

A control, a freshly fortified control and duplicate stored fortified samples of each substrate were analyzed at 0-day and after 6 and 12 months of storage using analytical method AG-395.

Residues of metalaxyl and its metabolic moieties were determined according to analytical method AG-395. According to this method, residues are extracted by blending with a Polytron Homogenizer for one minute using 80% (v/v) methanol/water. An aliquot of the sample is evaporated to dryness. One ml of water is added to dissolve the residues and the sample is refluxed for 15 minutes after addition of 10 ml of methanesulfonic acid. The extract is basified and the 2,6-dimethylaniline formed in the reaction is steam distilled using a steam distillation apparatus. The steam distilled product is cleaned up with a silica Sep-Pak® cartridge prior to analysis by capillary gas

chromatography using a nitrogen/phosphorus detector (NPD) operating in the nitrogen-specific mode.

The mean and standard deviation were determined by entering all the results from the 0, 6, and 12-month intervals for all substrates into a Hewlett-Packard HP-11C calculator for metalaxyl and each moiety. The following formulas were used to determine the results:

$$\text{Mean} = m = \frac{\Sigma x}{N}$$

$$\text{Standard Deviation} = Sx = \frac{N\Sigma x^2 - (\Sigma x)^2}{N(N-1)}$$

Where x = Individual results not corrected for procedural recoveries.
N = Number of measurements.

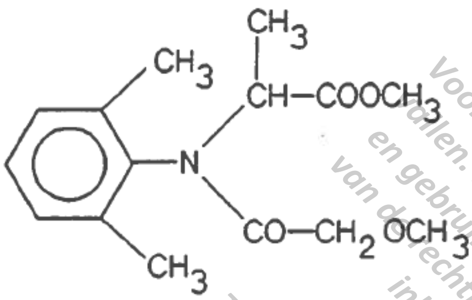
RESULTS

The data shows that no loss of metalaxyl, CGA-62826, CGA-67869, CGA-107955 or CGA-37734 occurred during the 12-month time period in any of the substrates. Any variation in results could be attributed to experimental deviation. An apparent decline in residues of CGA-94689 was observed in three (cabbage, lettuce, and potatoes) out of five substrates. However, CGA-94689 residues in strawberries and apples remained stable for the 12-month period. Also, residues did not decline from the 6-month to the 12-month intervals. This, along with the low accountability of CGA-94689, indicates that experimental deviation can be attributed for the decline in residues. Results are shown in Table I.

The accountability of residues of metalaxyl and its metabolites in crops using method AG-395 is shown in Table II. These results show that the accountability for metalaxyl and its metabolites ranged from 30 to 77 percent.

CONCLUSION

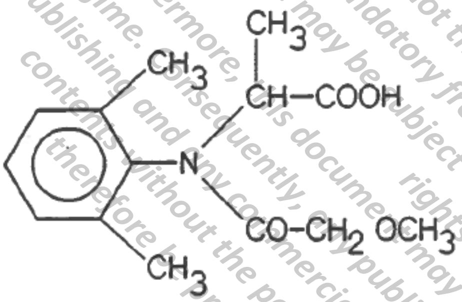
Residues of metalaxyl and its metabolites, CGA-62826, CGA-67869, CGA-107955, CGA-37734, and CGA-94689 are stable for at least 12-months under freezer storage conditions. Method AG-395 is a valid method for determining metalaxyl and its plant metabolites.



Metalaxyl (CGA-48988)

N-(2,6-Dimethylphenyl)-N-(methoxyacetyl)alanine methyl ester

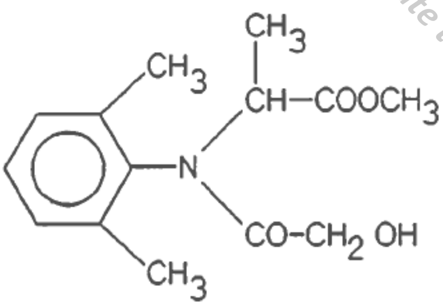
$C_{15}H_{21}NO_4$



CGA-62826

N-(2,6-Dimethylphenyl)-N-(methoxyacetyl)alanine

$C_{14}H_{19}NO_4$

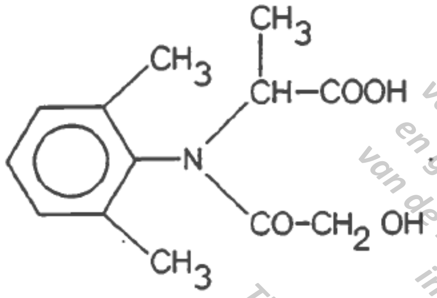


CGA-67869

N-(2,6-Dimethylphenyl)-N-(hydroxyacetyl)alanine methyl ester

$C_{14}H_{19}NO_4$

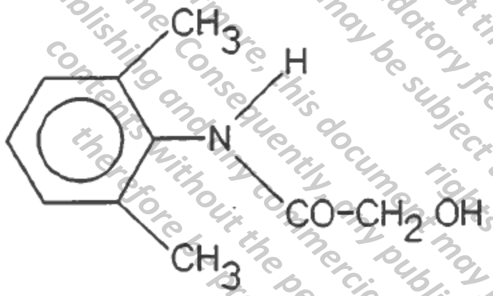
Figure 1: Chemical Names, Structures and Nomenclatures for Metalaxyl and Related Compounds



CGA-107955

N-(2,6-Dimethylphenyl)-N-(hydroxyacetyl) alanine

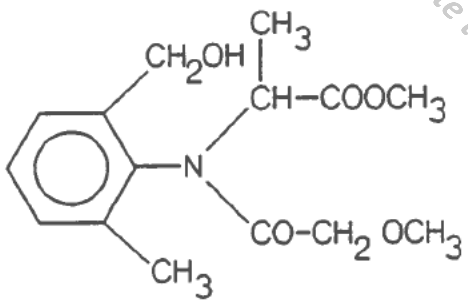
$C_{13}H_{17}NO_4$



CGA-37734

N-(2,6-Dimethylphenyl)-2-hydroxyacetamide

$C_{10}H_{13}NO_2$



CGA-94689

N-[2-(Hydroxymethyl)-6-methylphenyl]-N-(methoxyacetyl) alanine methyl ester

$C_{15}H_{21}NO_5$

Figure 1: continued

TABLE I. RESIDUE RESULTS OF METALAXYL AND ITS METABOLITES IN STRAWBERRIES, APPLES, CABBAGE, LETTUCE, AND POTATOES FORTIFIED AT 1.0 PPM FOLLOWING FREEZER STORAGE CONDITIONS

PPM

Months	Strawberries			Apples			Cabbage			Lettuce			Potatoes		
	0	6	12	0	6	12	0	6	12	0	6	12	0	6	12
Metalaxyl	1.0	0.98	1.3	1.0	0.96	1.1	1.0	0.93	1.1	1.0	1.1	0.98	1.0	0.97	1.0
CGA-62826	1.0	1.1	1.0	1.0	1.2	1.0	1.0	0.94	1.0	1.0	1.0	1.1	1.0	1.0	0.96
CGA-67869	1.0	0.86	0.96	1.0	1.1	1.1	1.0	0.94	0.96	1.0	1.2	1.2	1.0	0.91	0.90
CGA-107955	0.99	1.1	1.0	1.0	1.0	0.86	1.0	1.1	0.95	0.99	1.3	0.95	1.0	0.84	1.0
CGA-37734	1.0	1.4	0.98	1.0	1.1	1.3	1.0	0.97	0.96	0.99	1.0	1.2	1.0	0.77	0.91
CGA-94689	1.0	1.1	1.1	0.98	1.0	1.1	0.99	0.74	0.74	0.99	0.72	0.84	1.0	0.67	0.72

Results are the average of duplicate samples and corrected for procedural recoveries. Individual replicates are reported in AG-A 9559.

TABLE II. ACCOUNTABILITY OF METALAXYL AND METABOLITES FOR ALL SUBSTRATES FORTIFIED AT 1.0 PPM USING METHOD AG-395

PPM

Metalaxyl	CGA-62826	CGA-67869	CGA-107955	CGA-37734	CGA-94689
0.76	0.73	0.80	0.55	0.77	0.24
0.84	1.11	0.92	0.52	0.75	0.34
0.84	0.59	0.84	0.42	0.41	0.21
0.67	0.65	0.55	0.43	0.53	0.23
0.72	0.63	0.82	0.52	0.60	0.25
0.74	0.63	0.81	0.47	0.59	0.23
0.56	0.64	0.74	0.48	0.60	0.24
0.71	0.67	0.81	0.47	0.55	0.25
0.93	0.82	0.74	0.57	0.70	0.34
0.72	0.57	0.70	0.45	0.60	0.19
0.76	0.52	0.77	0.45	0.58	0.21
0.80	0.63	0.83	0.45	0.65	0.20
0.65	0.63	0.85	0.46	0.67	0.22
0.69	0.56	0.67	0.47	0.40	0.13
0.72	0.61	0.71	0.40	0.47	0.16
0.82	0.57	0.74	0.41	0.58	0.12
0.79	0.68	0.77	0.47	0.69	0.37
0.80	0.68	0.78	0.45	0.65	0.36
0.71	0.69	0.76	0.40	0.61	0.42
0.62	0.64	0.78	0.43	0.59	0.31
0.70	0.65	0.75	0.46	--	--
0.77	0.69	0.90	0.52	0.70	0.45
0.87	0.72	0.85	0.48	0.61	0.32
0.83	0.69	0.87	0.51	0.73	0.34
0.78	0.55	0.83	0.49	0.63	0.45
0.78	0.71	0.82	0.52	0.68	0.44
0.80	0.70	0.63	0.39	0.64	0.43
0.85	0.76	0.85	0.54	0.74	0.30
0.88	0.67	0.71	0.47	0.55	0.32
0.82	0.66	0.60	0.45	0.53	0.31
0.88	0.73	0.74	0.44	0.67	0.21
0.72	0.67	--	0.41	0.59	0.31
0.78	0.74	0.84	0.55	--	0.43

TABLE II. ACCOUNTABILITY OF METALAXYL AND METABOLITES FOR ALL SUBSTRATES FORTIFIED AT 1.0 PPM USING METHOD AG-395 (Continued)

PPM

	Metalaxyl	CGA-62826	CGA-67869	CGA-107955	CGA-37734	CGA-94689
	0.84	0.70	0.75	0.47	0.56	0.41
	0.75	0.59	0.95	0.62	0.84	0.61
	0.68	0.59	0.88	0.45	0.67	0.39
	0.77	0.63	0.84	0.59	0.62	0.42
	0.67	0.61	0.63	0.37	0.51	0.27
	0.72	0.54	0.47	0.37	0.44	0.22
	0.69	0.63	0.66	0.39	0.49	0.17
Mean	0.76	0.66	0.77	0.47	0.61	0.30
S.D.	0.08	0.10	0.10	0.06	0.10	0.11
N	40	40	39	40	38	39

These results were determined during the storage stability study reported in AG-A-9559. The results were not corrected for recoveries in order to represent the true accountability.