

APPENDIX I

EN-CAS Method No. ENC-14/93 As Applied to Leafy Vegetables

Analytical Method for the Determination of Pyrethrins I Residues in
Various Agricultural Commodities

(reduced to 95%)



2359 Farrington Point Dr. • Winston-Salem, N.C. 27107
Phone (910) 785-3252-3

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Various Agricultural Commodities

EN-CAS Analytical Laboratories
2359 Farrington Point Drive
Winston-Salem, North Carolina 27107
Phone: (910) 785-3252

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1.0 INTRODUCTION AND SUMMARY

1.1 Scope

Natural pyrethrins are non-persistent insecticides with low mammalian toxicity extracted from chrysanthemum plants. The pyrethrum extract used for quantitation in this method contains a variety of compounds with about 50% being classified as Pyrethrins I and II. For this study, the pyrethrum extract is used as an analytical standard to quantitate the Pyrethrins I group only (the extract is 28.17% Pyrethrins I) as a summation of the three main components cinerin 1, jasmolin 1 and pyrethrin 1. A limit of quantitation (LOQ) of 0.02 ppm Pyrethrins I was achieved for leaf lettuce, head lettuce, spinach and celery.

Method validation results are included in this report in Tables I - IV.

See Figure 1 for a flowchart of the method.

1.2 Principle

A 10.0-g sample is extracted by blending in 1:1 hexane:acetone. After vacuum filtration, the sample is added to a separatory funnel where a two phase separation occurs and the lower (water/acetone) layer is discarded. The remaining organic layer is poured through anhydrous Na_2SO_4 and the sample is concentrated to incipient dryness using rotary evaporation. Following reconstitution with ethyl acetate and hexane, the sample is loaded onto a 5.0-g silica gel column and eluted using 20:80 ethyl acetate:hexane. The eluate is then brought to an exact final volume and analyzed by GC/ECD.

2.0 APPARATUS

NOTE: Apparatus and equipment that is considered "standard" in most laboratories may not be listed in this section. All equipment, apparatus and reagents may be replaced by equivalent items from alternate sources. Each substitution must be evaluated for functional equivalency.

- 2.1 French square bottles, 16-oz
- 2.2 Brinkman Polytron (Brinkman Instruments Model #PCU-11)
- 2.3 Buchner Funnels, 9-cm
- 2.4 Vacuum flasks, 500-mL
- 2.5 Whatman GF/C filter paper, 9-cm
- 2.6 Separatory funnels, 500-mL
- 2.7 Aluminum powder funnels, 9-cm
- 2.8 Erlenmeyer flasks, 250-mL with 24/40 ground glass stoppers
- 2.9 Rotary evaporator (Brinkman Rotavapor, Buchi)
- 2.10 Econo-Column (Bio-Rad Cat. #7371521)
- 2.11 Graduated centrifuge tubes, 15 mL (Kimax Cat. #45165)
- 2.12 Glass wool, non-silanized
- 2.13 Gas chromatograph with an ECD and a 15-m x 0.32-mm DB-1701 column connected to a 5-m x 0.32-mm DB-1 pre-column (see Sections 7.1-7.2)

3.0 REAGENTS

- 3.1 Hexane, pesticide grade
- 3.2 Acetone, pesticide grade
- 3.3 Water, deionized using Milli-Q system

3.0 REAGENTS (continued)

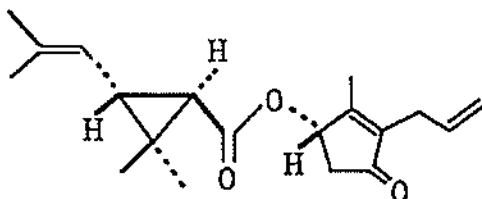
- 3.4 Ethyl acetate, pesticide grade
- 3.5 Sodium sulfate, anhydrous, ACS certified, oven baked at 600 °F for 2 hours and cooled to room temperature in a desiccator
- 3.6 Silica gel 60, 70-230 mesh (EM Science # 7734-3), 4% Deactivated

To prepare: Bake > 100 g (uncapped) in a jar at ≥ 130 °C for ≥ 6 hours, tightly cap and allow to cool. Weigh 100.0 g into a clean bottle, add 4.0 mL of deionized water, tightly cap and mix for at least 1 hour before using. Prepare weekly.

4.0 DESCRIPTION OF REFERENCE SUBSTANCES

4.1 Cinerin 1

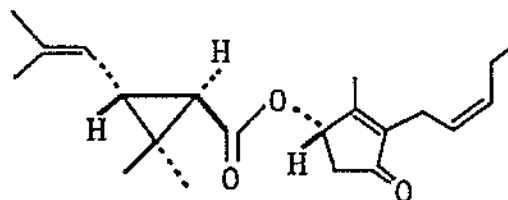
Chemical Name: $C_{20}H_{28}O_3$, 2,2-dimethyl-3-(2-methyl-1-propenyl)cyclopropanecarboxylic acid 3-(2-butenyl)-2-methyl-4-oxo-2-cyclopenten-1-yl ester



MW: 316 g/mole

4.2 Jasmolin 1

Chemical Name: $C_{21}H_{30}O_3$, 2,2-dimethyl-3-(2-methyl-1-propenyl)-cyclopropanecarboxylic acid 2-methyl-4-oxo-3-(2-pentenyl)-2-cyclopenten-1-yl ester, 4', 5'-dihydropyrethrin I

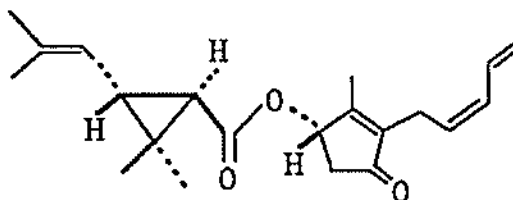


MW: 330 g/mole

4.0 DESCRIPTION OF REFERENCE SUBSTANCES (continued)

4.3 Pyrethrin 1

Chemical Name: $C_{21}H_{28}O_3$, 2,2-dimethyl-3-(2-methyl-1-propenyl) cyclopropanecarboxylic acid 2-methyl-4-oxo-3-(2,4-pentadienyl)-2-cyclopenten-1-yl ester, chrysanthemummono-carboxylic acid pyrethrolone ester



MW: 328 g/mole

5.0 PREPARATION OF ANALYTICAL STANDARDS

5.1 Stock Solutions

[NOTE: The Pyrethrins I analytical standard (pyrethrum extract) is a waxy solid that should be warmed to 60 °C using a water bath and mixed by manual inversion prior to weighing. Due to potential photodegradation, always minimize exposure of the pyrethrins to sunlight and some UV sources. Standards should be stored in amber bottles (see Section 12.1).]

Attain a 100.00 ± 0.01 mg active ingredient of Pyrethrins I standard (which is a combination of cinerin 1, jasmolin 1, and pyrethrin 1) by weighing 354.99 total mg pyrethrum extract. If volumetric flasks calibrated to contain 100 mL to 110 mL are available, any mass between 355 mg and 390 mg would be acceptable with compensation made in the volume. [NOTE: Pyrethrin 1 represent only 28.17% of the total pyrethrum extract and subsequently requires a substantial correction factor.]

Dissolve and dilute the Pyrethrins I combined analytical standard to a 100 mL volume with isooctane to prepare a 1000 $\mu\text{g/mL}$ stock solution. Typically, stock solutions should be prepared fresh every six months, or as determined from in-lab standard response comparison studies. [NOTE: Store all standard solutions in a freezer at a temperature of -10 °C to -17 °C].

5.0 PREPARATION OF ANALYTICAL STANDARDS (continued)

5.2 Fortification Standards

Serially dilute the 1000 µg/mL Pyrethrins I stock solution (Section 5.1) in 1:1 acetone:hexane to prepare 100 µg/mL, 10 µg/mL and 1.0 µg/mL standard solutions. Typically, fortification standards should be made every 6 months from the 1000 µg/mL stock solutions or as determined from in-lab standard response comparison studies. [NOTE: Store fortification standards in a freezer at a temperature of -10 °C to -17 °C.]

5.3 Gas Chromatographic Calibration Standards

Serially dilute the 1.0 µg/mL fortification standard solution (Section 5.2) to prepare the necessary GC calibration standards. Prepare these calibration standards, typically ranging from 0.01 µg/mL to 0.2 µg/mL, in 20:80 ethyl acetate:hexane. Typically, a minimum of five standards should be included within each gas chromatographic run. Typically, calibration standards should be prepared fresh every six months, or as determined from in-lab standard response comparison studies. [NOTE: Store all GC calibration standards in a freezer at a temperature of -10 °C to -17 °C.]

6.0 ANALYTICAL PROCEDURE

[NOTE: Natural pyrethrins are susceptible to degradation and precautions should be taken to minimize exposure to sunlight and some UV sources (see Section 12.1). This procedure should be run quickly and with no stopping points to minimize potential degradation. If the method must be stopped, it is recommended that it be after rotary evaporation (step 6.4.1) and before reconstitution (step 6.4.2).]

6.1 Extraction

- 6.1.1 Weigh 10.00 g (\pm 0.02 g) of chilled homogenized sample (EN-CAS SOP III-5.2) into a 16-oz French square bottle.
- 6.1.2 For laboratory fortifications add an appropriate aliquot (typically 0.1 to 2.0 mL) of fortification standard solution (section 5.2) to chilled control (non-treated) sample. Leave the fortified samples open to the air (e.g., in a fume hood) for 5 to 10 minutes before adding the extraction solvent.

6.1 Extraction (continued)

- 6.1.3 Add 100 mL of 1:1 hexane:acetone to the sample in the French square bottle and blend at a medium speed (typically, a setting of 5 out of 10) for 1 minute using a Brinkman Polytron (or equivalent).

6.2 Filtration

- 6.2.1 Vacuum-filter the sample using a Whatman GF/C filter paper in a 9-cm Buchner funnel, collecting the filtrate in a 500-mL side-arm vacuum flask. Rinse the bottle with 50 mL of 1:1 hexane:acetone and pour the rinsate over the filter cake, combining the rinse with the extract filtrate.

6.3 Partition

- 6.3.1 Pour the sample filtrate from the vacuum flask into a 500-mL separatory funnel and allow phase separation to occur. [NOTE: The lower layer separates due to water extracted from the crops.] For reagent blanks that do not yield a phase separation, add 15 mL d.i. water and shake ~20 seconds. Allow the layers to separate and drain the lower (water/acetone) layer to waste.
- 6.3.2 Pour the upper layer (hexane/acetone) through 30-40 g of sodium sulfate (in an aluminum powder funnel with a glass wool plug in the neck of the funnel) into a 250-mL Erlenmeyer flask. Rinse the separatory funnel with 10 mL of 1:1 hexane:acetone, then pour the rinsate over the sodium sulfate and add to the Erlenmeyer flask containing the sample.

6.4 Concentration and Reconstitution

- 6.4.1 Concentrate the sample in the Erlenmeyer flask to incipient dryness using rotary evaporation under vacuum in a ~30 °C water bath. After removing the sample from the rotary evaporator, ensure that all residual organic solvent is removed by blowing the flask dry with an air or nitrogen stream. [NOTE: Some residual water may be present which will not easily evaporate by this procedure. If the amount of water is less than 200 uL, then the water can be disregarded. If the amount of water is greater than 200 uL, more sodium sulfate (up to 2 cm) should be added to the top of the column. See Section 6.5.1 for more details.]

6.4 Concentration and Reconstitution (continued)

- 6.4.2 Reconstitute the sample by adding 1.0 mL of 1:1 ethyl acetate:hexane, stoppering the flask, and swirling in an ultrasonic bath for approximately 1 minute (or as long as needed to remove residue from the walls of the flask). All visible residue should go into solution. Add 4.0 mL of hexane and swirl to mix.

6.5 Silica Gel Clean-up

- 6.5.1 Prepare a 5.0-g silica gel column (See Section 3.6) in a 1.5-cm i.d. glass column (Bio-Rad, see section 2.10). Plug the bottom of the column (with the supplied plug) and add 5 mL of 5:95 ethyl acetate:hexane. Weigh 5.0 g of silica gel into a small glass beaker or bottle and add 10 mL of 5:95 ethyl acetate:hexane to the silica to create a slurry. Swirl the container to thoroughly wet the silica and suspend it somewhat in the solvent and then quickly pour most of the silica slurry into the column. Use three 5 mL aliquots of 5:95 ethyl acetate:hexane to transfer as much of the remaining silica slurry as possible to the column. After the silica gel has settled, agitate the column as needed to level the top of the packing and then add ~1 cm of anhydrous sodium sulfate.
- 6.5.2 If any of the samples, after rotary evaporation, contains more than 200 μ L of water then add ~2 cm of sodium sulfate to the columns of all of the samples analyzed in the set. Release the plug from the bottom of the column and allow the solvent to drain until it stops flowing. [NOTE: Solvents flow by gravity through the column and will stop flowing as the top of the solvent reaches the sodium sulfate. Each solvent addition to the column should be allowed to drain to the top of the sodium sulfate before the next solvent is added (with a typical flow rate of 5 ml/min).]
- 6.5.3 Load the sample from Section 6.4.2 (to waste).
- 6.5.4 Rinse the sample flask with 5 ml of 10:90 ethyl acetate:hexane and then pour the rinsate onto the column (to waste).

6.5 Silica Gel Clean-up (continued)

6.5.5 Rinse the column with 10 mL of 10:90 ethyl acetate:hexane (to waste).

6.5.6 Elute the Pyrethrins I from the column using 10 mL of 20:80 ethyl acetate:hexane. Collect in a 15-mL graduated tube.

6.6 Final Volume

6.6.1 Bring the eluted sample to exactly 10.0 mL by adding 20:80 ethyl acetate:hexane or, if the volume is over 10.0 mL, use a gentle stream of air or nitrogen to evaporate the volume down to 10.0 mL.

7.0 GAS CHROMATOGRAPHIC DETERMINATION

Leafy vegetable matrices are quantitated using the gas chromatographic conditions listed in Section 7.1. GC conditions may be optimized for individual matrices providing all parameters are documented in the raw data.

7.1 Description and Typical Operating Conditions

Inject the sample and calibration standards on a gas chromatographic system configured as follows:

Gas Chromatograph: Hewlett Packard 5890

Column: DB-1701 (J&W Scientific): 15-m x 0.32-mm i.d. x 0.25- μ m film thickness
+
DB-1 (J&W Scientific) pre-column: 5-m x 0.32-mm i.d. x 0.25- μ m film thickness

[NOTE: columns connected via a glass column connector, Supelco Cat # 2-0479]

Oven Program: 80 °C for 1 minute, ramp 40 °C/minute to 180 °C for 17 minutes, ramp 40 °C/minute to 300 °C for 5 minutes

Injection port: 200 °C, using a 4-mm i.d. double gooseneck liner (Restek, Cat. # 5181 3315)

7.1 Description and Typical Operating Conditions (continued)

Detector:	300 °C, argon/methane auxiliary gas at 40 mL/minute
Head Pressure:	16 psi, helium carrier gas
Injection volume:	1 µL
Retention times:	~ 13 min for cinerin 1 ~ 16 min for jasmolin 1 ~ 19 min for pyrethrin 1

7.2 Typical Integration Parameters

Integrator: Hewlett Packard 3396A

<u>Run Parameters</u>	<u>Integrator Definitions</u>
ZERO = 5	0. Set baseline now
ATT ^2 = -1	1. Set baseline next valley
CHT SP = 0.0	2. Set baseline all valleys
AR REJ = 0	3. Skim from next peak
THRSH = -2	4. Disable autotangent skimming
PK WD = 0.2	5. Extend baseline horizontally
	6. Measure and update threshold
	7. Turn off retention time labeling
	8. Turn on start/stop marks
	9. Turn off integration
	10. Increment threshold
	11. Invert negative peaks
	12. Clamp negative peaks
	13. Show IF11, IF12
	14. Start peak sum window
<u>Timetable events</u>	
0.00 INTG # = 8	
0.00 INTG # = 9	
0.00 INTG # = 2	
12.00 CHT SP = 0.5	
12.00 ZERO = 30	
12.00 INTG # = -9	
19.5 ATT ^2 = 10	
19.5 CHT SP = 0.0	
19.5 INTG # = 9	
28.5 STOP	

7.3 Representative Chromatograms

A typical calibration curve for the sum of the three compound responses vs ng injected is shown in Figure 2. Typical chromatograms illustrating a GC calibration standard as well as a control and recovery for each matrix are shown in Figures 3 to 14.

7.4 Calibration

Standards for gas chromatography are prepared in 20:80 ethyl acetate:hexane and typically range from 0.01 µg/mL to 0.20 µg/mL. The standards are injected after every two to four samples throughout the GC run, always beginning and ending a set with a standard.

7.4 Calibration (continued)

A linear regression function is generated using the sum of the resulting peak heights (obtained from an integrator) vs nanograms injected. The response curve should be linear, having a correlation coefficient ≥ 0.990 . In a sample, the nanograms found of Pyrethrins I (Section 8.4) are determined by inserting the sample peak height value (sum of cinerin 1, jasmolin 1 and pyrethrin 1 peak heights), into the standard curve linear regression equation that follows:

Linear Curve Regression Equation:

$$y = mx + b$$

where: y = GC detector response
 x = nanograms injected
 m = slope of the response curve
 b = response axis intercept

8.0 CALCULATION OF RESULTS

8.1 Calculation of mg-Equivalent Injected

$$\text{mg-equiv. injected} = \frac{\text{g-sample extracted} \times (1000 \text{ mg/g}) \times \mu\text{L injected}}{\mu\text{L final volume} \times \text{dilution factor}}$$

8.2 Calculation for Peak Height Summation (Pyrethrins I)

$$\begin{aligned} \text{peak height summation} &= \text{cinerin 1 peak height (counts)} \\ \text{(Pyrethrins I)} &+ \text{jasmolin 1 peak height (counts)} \\ &+ \text{pyrethrin 1 peak height (counts)} \end{aligned}$$

8.0 CALCULATION OF RESULTS (continued)

8.3 Calculation for Peak Height Subtraction

NOTE: Laboratory fortifications are corrected for the average peak height contribution, if any, from the corresponding controls (adjusted for the differences in final volumes and dilutions) by using the following equation:

$$\text{net peak height (corrected)} = \text{peak height (found)} - \left[\frac{\text{average peak height (control)}}{\text{mg-equiv. injected (control)}} \right] \times \text{mg-equiv. injected (fort.)}$$

NOTE: The mg-equiv. injected (fort.)/mg-equiv. injected (control) adjusts for any dilution factor.

8.4 Calculation of ng Found

Residue samples are not corrected for either procedural recovery or control sample background.

$$\text{ng found} = \frac{\text{net peak height (counts)} - \text{standard curve y intercept (counts)}}{\text{standard curve slope (counts/ng)}}$$

8.5 Calculation of ppm Pyrethrins I Found

$$\text{ppm found} = \frac{\text{ng found}}{\text{mg-equivalent injected}}$$

8.6 Calculation of Percent Procedural Recovery

$$\% \text{ Recovery} = \frac{\text{ppm found}^*}{\text{fortification level (ppm)}} \times 100\%$$

* Laboratory fortifications were corrected for any control contribution by the equation in Section 8.4.

8.0 CALCULATION OF RESULTS (continued)8.9 Example Calculation

EN-CAS Sample ID # EL8150-S1, Set NMV3, GC run # 48675, Spinach, see Figure 11	
Sample wt.	= 10 grams
Injection volume	= 1 μ L
Final volume	= 10.0 mL (10,000 μ L)
Dilution factor	= 1
Sum of peak heights (sample)	= 981 counts (Pyrethrins I)*
Sum of peak heights (control)	= 0 counts
y-intercept	= -91.95 counts
Slope	= 55111.07 counts/ng
Fortification level	= 0.02 ppm
$\text{mg-equiv. injected (control and sample)} = \frac{10 \text{ g} \times (1000 \text{ mg/g}) \times 1.0 \text{ } \mu\text{L}}{10,000 \text{ } \mu\text{L} \times 1} = 1.0 \text{ mg}$	
$\text{peak height (corrected)} = 981 \text{ counts (net)} - [0 \text{ counts} \times 1.0 \text{ mg}/1.0 \text{ mg}] = 981 \text{ counts}$	
$\text{ng found} = \frac{981 \text{ counts (net)} - (-91.95 \text{ counts})}{55111.07 \text{ counts/ng}} = 0.01946 \text{ ng}$	
$\text{ppm found} = \frac{0.01946 \text{ ng}}{1.0 \text{ mg}} = 0.01946 \text{ ppm}$	
$\% \text{ Recovery} = \frac{0.01946 \text{ ppm}}{0.02 \text{ ppm}} \times 100 = 97\% \text{ Pyrethrins I}$	
* 981 counts (Pyrethrins I) = 289 counts cinerin I + 138 counts jasmolin I + 554 counts pyrethrin I.	

9.0 SAFETY PRECAUTIONS

Use normal safety precautions, including wearing gloves and safety glasses, and use of a fume hood to minimize exposure to the analytes and organic solvents used in this procedure.

10.0 TIME REQUIRED FOR ANALYSIS

A residue chemist/technician can analyze a set of 12 samples (including controls and recoveries) and prepare them for injection on the gas chromatograph in approximately one 8-hour day. An additional one half day is required for annotating and calculating the data.

11.0 LIMIT OF QUANTITATION

For leafy vegetables validated herein, this method is proven effective to a LOQ of 0.02 ppm as the sum of the Pyrethrins I (cinerin 1, jasmolin 1 and pyrethrin 1). Adjust the instrument sensitivity, GC calibration standards, and final sample volumes to permit detection at 50% of the LOQ (typically signal/noise ≥ 3).

12.0 METHOD DISCUSSION

12.1 Photodegradation

Natural pyrethrins are sensitive to photodegradation by sunlight and some types of fluorescent lighting. EN-CAS Laboratories covered windows exposed to sunlight and used fluorescent bulbs with a color temperature rating of 4100 and a color index (CRI) of 70 (Phillips, ordering code F40/SPEC41 through Grainger) in the areas where samples were processed, analyses were performed and standards were prepared. Amber tinted bottles were used for storage of all standard solutions and amber tinted vials were used for gas chromatographic analysis.

12.2 Method Stopping Point Recommendation

Due to potential degradation of the Pyrethrins I when exposed to sample matrix, it is recommended that the analyst proceed through the analytical method efficiently and with no stopping points. If the method must be stopped, it should be stopped only after the rotary evaporation step (Section 6.4.1) and before reconstitution with ethyl acetate:hexane (Section 6.4.2). Stopper and store the samples at $\leq 0^\circ\text{C}$.

12.3 Silica Column Profile

A silica column profile is recommended at the onset of a project and when the lot number of the silica changes or when recoveries fall outside of the expected 70% to 120% range.

13.0 METHOD VALIDATION RESULTS

See Tables I to IV in this report.

13.1 Statistical Method

The mean recoveries and standard deviations are calculated from the validation data and appear in validation tables included in this method. Statistical methods used were limited to calculations of the mean (\bar{x}) and standard deviation (σ) using the following formula

When n is < 30

$$\sigma_{(n-1)} = \sqrt{\frac{\sum_{i=1}^n (x_i - \bar{x})^2}{n-1}}$$

where the sum of the squares of the individual deviations from the mean ($x_i - \bar{x}$) is divided by n-1 (when the total number of measurements is less than 30).

13.2 Discussion of Validation Results

Results of the method validation indicate that EN-CAS Method No. ENC-14/93 As Applied to Leafy Vegetables is an appropriate method for the analysis of Pyrethrins I in leafy vegetable crops. Validation levels were 0.02 ppm and 2.0 ppm for all matrices, with good recovery results at both levels. Recoveries from the leaf lettuce matrix ranged from 79% to 97%. Head lettuce recoveries ranged from 81% to 101%. Spinach recoveries ranged from 76% to 101% and celery recoveries from 81% to 96%. A reagent blank and a control were analyzed with each validation set and in all cases these samples yielded results of <0.02 ppm Pyrethrins I. The overall mean and standard deviation for Pyrethrins I recovery from leafy vegetables was 88% \pm 7.4 (n=24).

14.0 REFERENCES

1. Pharmaco-LSR Analytical Method entitled AN ANALYTICAL METHOD FOR DETERMINATION OF PYRETHRINS BY GAS CHROMATOGRAPHY AND PIPERONYL BUTOXIDE BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY IN VARIOUS MATRICES.
2. Landis International, Inc. Analytical Protocol #18009A003 entitled RAW AGRICULTURAL COMMODITY (RAC) RESIDUE EVALUATION OF PYRETHRUM + PIPERONYL BUTOXIDE APPLIED AS PYRENONE CROP SPRAY TO LEAFY VEGETABLES, issued 5/08/92.

TABLE I
Method Validation Results for Leaf Lettuce
(Set # NMV1)

Sample Number	EN-CAS Number	Sample Type	Date Fortified	Date Extracted	Date Analyzed	Pyrethrins I Concentration Added (ppm)	Pyrethrins I Concentration Found (ppm)	Percent Recovery
--	RBLK	Reagent Blank	--	23-Feb-94	24-Feb-94	--	<0.02	--
EC93-976	EM2821-C	Control	--	23-Feb-94	24-Feb-94	--	<0.02	--
EC93-976	EM2821-S1	Fortification	23-Feb-94	23-Feb-94	24-Feb-94	0.02	0.0180	90
EC93-976	EM2821-S2	Fortification	23-Feb-94	23-Feb-94	24-Feb-94	0.02	0.0178	89
EC93-976	EM2821-S3	Fortification	23-Feb-94	23-Feb-94	24-Feb-94	0.02	0.0194	97
EC93-976	EM2821-S4	Fortification	23-Feb-94	23-Feb-94	24-Feb-94	2.0	1.585	79
EC93-976	EM2821-S5	Fortification	23-Feb-94	23-Feb-94	24-Feb-94	2.0	1.572	79
EC93-976	EM2821-S6	Fortification	23-Feb-94	23-Feb-94	24-Feb-94	2.0	1.609	80

NOTE: The mean and standard deviation for leaf lettuce recoveries is 86% ± 7.5 (n=6).

f:\93enc14\93tab1.doc

TABLE II
Method Validation Results for Head Lettuce
(Set # NMV2)

Sample Number	EN-CAS Number	Sample Type	Date Fortified	Date Extracted	Date Analyzed	Pyrethrins I Concentration Added (ppm)	Pyrethrins I Concentration Found (ppm)	Percent Recovery
--	RBLK	Reagent Blank	--	16-Feb-94	17-Feb-94	--	<0.02	--
EC93-946	EM1269-C	Control	--	16-Feb-94	17-Feb-94	--	<0.02	--
EC93-946	EM1269-S1	Fortification	16-Feb-94	16-Feb-94	17-Feb-94	0.02	0.0177	89
EC93-946	EM1269-S2	Fortification	16-Feb-94	16-Feb-94	17-Feb-94	0.02	0.0202	101
EC93-946	EM1269-S3	Fortification	16-Feb-94	16-Feb-94	17-Feb-94	0.02	0.0163	81*
EC93-946	EM1269-S4	Fortification	16-Feb-94	16-Feb-94	17-Feb-94	2.0	1.718	86
EC93-946	EM1269-S5	Fortification	16-Feb-94	16-Feb-94	17-Feb-94	2.0	1.694	85
EC93-946	EM1269-S6	Fortification	16-Feb-94	16-Feb-94	17-Feb-94	2.0	1.644	82

NOTE: The mean and standard deviation for head lettuce recoveries is 87% ± 7.3 (n=6).

*Manual calculation may differ due to computer rounding.

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TABLE III
Method Validation Results for Spinach
(Set # NMV3)

Sample Number	EN-CAS Number	Sample Type	Date Fortified	Date Extracted	Date Analyzed	Pyrethrins I Concentration Added (ppm)	Pyrethrins I Concentration Found (ppm)	Percent Recovery
--	RBLK	Reagent Blank	--	16-Feb-94	16-Feb-94	--	<0.02	--
EC92-3917	EL8150-C	Control	--	16-Feb-94	16-Feb-94	--	<0.02	--
EC92-3917	EL8150-S1	Fortification	16-Feb-94	16-Feb-94	16-Feb-94	0.02	0.0195	97*
EC92-3917	EL8150-S2	Fortification	16-Feb-94	16-Feb-94	16-Feb-94	0.02	0.0201	101
EC92-3917	EL8150-S3	Fortification	16-Feb-94	16-Feb-94	16-Feb-94	0.02	0.0180	90
EC92-3917	EL8150-S4	Fortification	16-Feb-94	16-Feb-94	16-Feb-94	2.0	1.556	78
EC92-3917	EL8150-S5	Fortification	16-Feb-94	16-Feb-94	16-Feb-94	2.0	1.644	82
EC92-3917	EL8150-S6	Fortification	16-Feb-94	16-Feb-94	16-Feb-94	2.0	1.512	76

NOTE: The mean and standard deviation for leaf lettuce recoveries is 87% ± 10 (n=6).

*Manual calculation may differ due to computer rounding.

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TABLE IV
Method Validation Results for Celery
(Set # NMV4)

Sample Number	EN-CAS Number	Sample Type	Date Fortified	Date Extracted	Date Analyzed	Pyrethrins I Concentration Added (ppm)	Pyrethrins I Concentration Found (ppm)	Percent Recovery
--	RBLK	Reagent Blank	--	22-Feb-94	22-Feb-94	--	<0.02	--
EC92-2327	EM2796-C	Control	--	22-Feb-94	22-Feb-94	--	<0.02	--
EC92-2327	EM2796-S1	Fortification	22-Feb-94	22-Feb-94	22-Feb-94	0.02	0.0189	94*
EC92-2327	EM2796-S2	Fortification	22-Feb-94	22-Feb-94	22-Feb-94	0.02	0.0162	81
EC92-2327	EM2796-S3	Fortification	22-Feb-94	22-Feb-94	22-Feb-94	0.02	0.0191	96
EC92-2327	EM2796-S4	Fortification	22-Feb-94	22-Feb-94	22-Feb-94	2.0	1.765	88
EC92-2327	EM2796-S5	Fortification	22-Feb-94	22-Feb-94	22-Feb-94	2.0	1.738	87
EC92-2327	EM2796-S6	Fortification	22-Feb-94	22-Feb-94	22-Feb-94	2.0	1.831	92

NOTE: The mean and standard deviation for celery recoveries is 90% ± 5.5 (n=6).

*Manual calculation may differ due to computer rounding.

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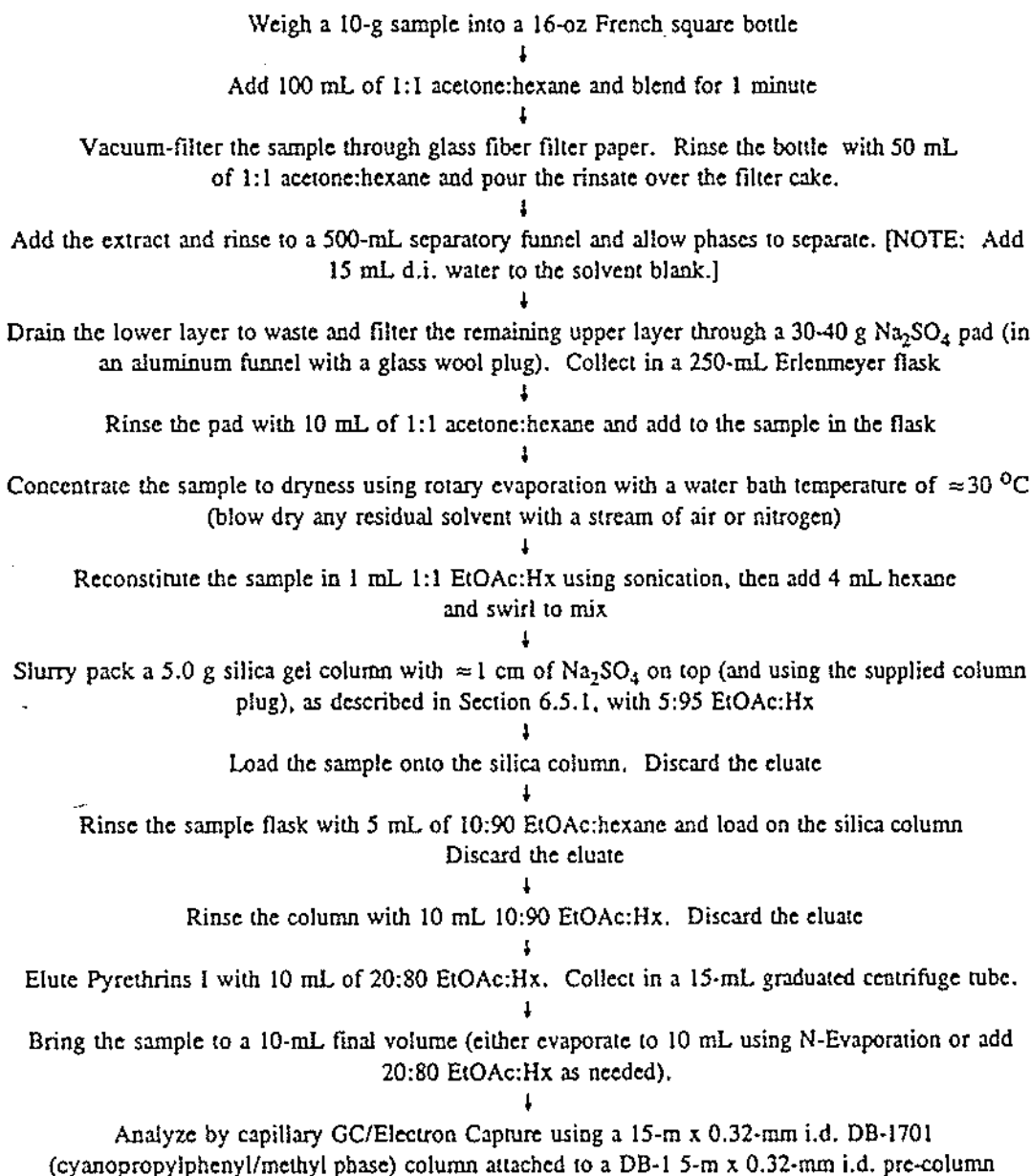
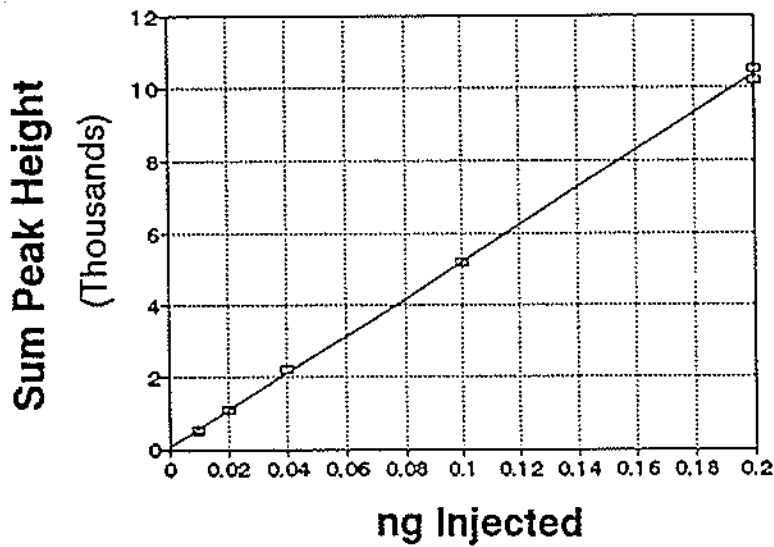
FIGURE 1**Flowchart of Analytical Method No. ENC-14/93 As Applied to Leafy Vegetables****The Determination of Pyrethrins in Various Crop Matrices**

FIGURE 2
Typical GC Calibration Curve (Celery)
Pyrethrins I



STATISTICS

Regression Output:

Constant	62.92492	(y-int)
Std Err of Y Est	109.2318	
R Squared	0.999491	(variance)
No. of Observations	7	
Degrees of Freedom	5	
X Coefficient(s)	51500.91	(slope)
Std Err of Coef.	519.899	
Corr. Coeff.	0.999745	

set # NMV4, run # 48723, dated 2/22/94