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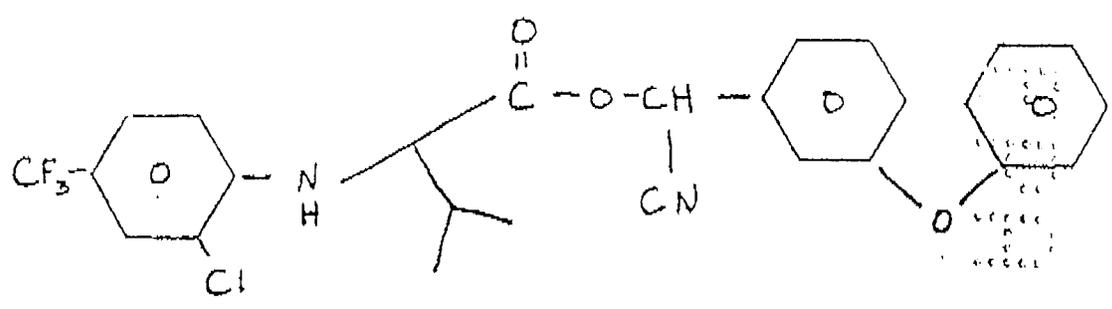
TITLE: Analytical Method for Fluvallinate in Honey by Gas Chromatography
Using a Wide Bore 0.53mm Column

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INTRODUCTION:

Fluvallinate, ((RS)- α -cyano-3-phenoxy benzyl-(R)-2-(2-chloro-4-(trifluoromethyl) anilino)-3-methyl-butanoate) is a Zoecon compound with substantial insecticidal activity.

The impregnated fluvallinate strip is a successful product form which show excellent control of varroa mites without an adverse effect on the honey bee. The chemical structure of fluvallinate is shown below.



The described method was designed to analyze fluvalinate residue in honey. A gas chromatograph with a wide bore silica capillary column is used to separate the components. Detection is accomplished by electron capture. The lowest quantifiable level is 4.50 part per billion.

METHOD OUTLINE:

A. Analytical Apparatus

1. Gas-Liquid Chromatograph: Hewlett-Packard 5890A fitted with a ^{63}Ni electron capture detector.
2. G.C. Column: Wide Bore fused silica column
DB-5, 0.53mm x 12m from J&W Scientific
3. Bond Elute Solid Phase Extractor: Analytichem International
Vac-Elut Extractor, S1 cartridge 500 mg/2.8 ml

B. Reagents

1. Fluvalinate Standard: 96.2% fluvalinate, available from SANDOZ
Crop Protection, Chicago, IL.
2. All Solvents: All solvents used for this assay were residue -
analyzed grade from Burdick & Jackson.

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1. Fluvalinate Stock Solution: (I) (45 ug/ml) Dissolve 23.388 mg
of 96.2% fluvalinate standard in 500 ml hexane.
 2. Fluvalinate Stock Solution: (II) (0.450 ug/ml) Dilute 1 ml
of the stock solution (I) to 100 ml of hexane.
 3. Fluvalinate Standard Solutions: (0.90 ng/ml - 45.0 ng/ml)
Dilute stock solution with appropriate volume of hexane.

PROCEDURE:

A. Instrument Parameters

Gas Chromatograph Conditions

Column: 12m x 0.53mm ID, DB-5 column from J&W Scientific Co.

Detector: Electron Capture

Injector Temp.: 220°C

Oven Temp.: 230°C

Detector Temp.: 350°C

Column Flow: Ar/CH₄, about 29 ml/min. or to achieve a relative retention time of 8 to 9 minutes for fluvialinate.

Attenuation: 2↑4

Makeup Gas: Ar/CH₄, 35 ml/min.

Instrument: Hewlett-Packard Gas Chromatograph 5890A

B. Sample Preparation

Weigh 5g of honey sample into a 500 ml beaker, add 70 ml water and stir to dissolve the honey completely. Transfer the solution into a 500 ml separatory funnel, extract the fluvialinate from the matrix with 75 ml of hexane. Transfer the aqueous portion to another separatory funnel and extract it with 30 ml of hexane. Discard the aqueous portion combine the hexane layers and wash it with an additional 70 ml of water. Pass the hexane solution through Na₂SO₄ (Na₂SO₄ was prewashed by 70 ml of hexane) then rinsed with 3 x 15

ml of hexane. Evaporate the hexane to dryness under vacuum rotary evaporator and reconstitute in 10 ml of hexane.

The samples of honey were fortified with 0.1 ml, 0.5 ml and 1 ml of 0.450 ug/ml fluvalinate solution (in hexane). The fortified honey was then processed by the described procedure.

C. Clean Up and Analysis

Pass 1 ml of the sample solution (or spiked solution) through silica Bond Elute column (precondition the column with two column volumes of hexane), wash the column with 0.5 ml of hexane and then 0.5 ml of 10% ethyl ether/hexane. Elute the fluvalinate from column with 3 ml of 20% ethyl ether/hexane. Evaporate the collected solution to dryness at 40°C by vacuum rotary evaporator. Reconstitute the residue in 1 ml of hexane and proceed with GC analysis.

Validation &

Discussion: In this procedure a direct injection technique on wide bore fused silica column (0.53 mm) was used instead of a 0.25 mm capillary column to simplify the instrument operation. The widebore column also produced better resolution and reproducibility in comparison to a packed column. The recoveries of the fortified samples from 8.91-89.11 ppb are shown on Table 1. The standard solutions from 2.25-45 ng/ml were run under the same described condition to establish the calibration curve. The regression coefficient of the calibration curve is 0.99649. A plot of concentration of standards vs. response along with representative chromatograms of placebo, standard and fortified sample are attached.

The limit of detection is based on the following procedure: (1) Prepare a series of standard solution with the peak area count of at least 2000 to 3000 for the lowest concentration inject each standard 3 times. Calculate the % RSD from the area counts for each standard solution, as shown below (Table 2).

Table 1. Recoveries of the Fortified Sample

<u>Fortified Level (PPb)</u>	<u>Recovery %</u>
8.91	46.15 ± 5.47
44.73	77.36 ± 5.29
89.11	116.56 ± 2.02

Table 2. Linear Dynamic Range

<u>Conc. of STD (ng/ml)</u>	<u>Area Counts</u>	<u>% RSD = $\frac{SD}{PA} \times 100$</u>
0.90	7849 ± 3508	44.69
2.25	23345 ± 1417	6.07
4.50	72912 ± 857	1.16
9.0	91853 ± 7175	7.81
22.5	237945 ± 7327	3.08
45.0	508555 ± 18209	3.58

(2) Plot the concentration vs. % RSD of area counts as shown on Figure 1. We observe that the % RSD remains fairly constant varying from 1.18% to 7.81% (for 45.0 to 2.25 ng/ml) which is an acceptable range. The concentration below 2.25 ng/ml shows high % RSD for replicate injections. Based on the % RSD distribution of area counts and the signal/noise ratio (≈ 5 , based on peak height), the lowest quantifiable level for the sample that can be calculated based on the following equation.

$$\begin{aligned}\text{Lowest quantifiable level} &= 2.25 \text{ ng/ml} \times V \times f/\text{Wt (g)} \\ &= 4.50 \text{ ng/g} = 4.50 \text{ PPb}\end{aligned}$$

$$f = \text{dilution factor} = \frac{100 \text{ ml}}{100 \text{ ml}} = 10$$

$$V = \text{volume of final sample solution} = 1 \text{ ml}$$

$$\text{Wt (g)} = \text{sample weight} = 5 \text{ g}$$

CALCULATION:

The concentration of fluvalinate is calculated below based on external standard.

$$\text{Fluvalinate (ppm) in honey} = \frac{\text{Sample area} \times \text{STD in mcg/ml} \times \text{extract volume (ml)}}{\text{Sample wt (g)} \times \text{STD area}} \times f'$$

The extraction volume in ml is the final volume of the sample solution and f' is the dilution factor.

Attachments:

- Figure 1. Lowest quantifiable level determination
- Figure 2. Linear dynamic range of fluvalinate (2.25 to 45.0 ng/ml)
- Figure 3. Typical chromatograms for honey placebo and spiked sample and standard

DETECTION LIMIT DETERMINATION

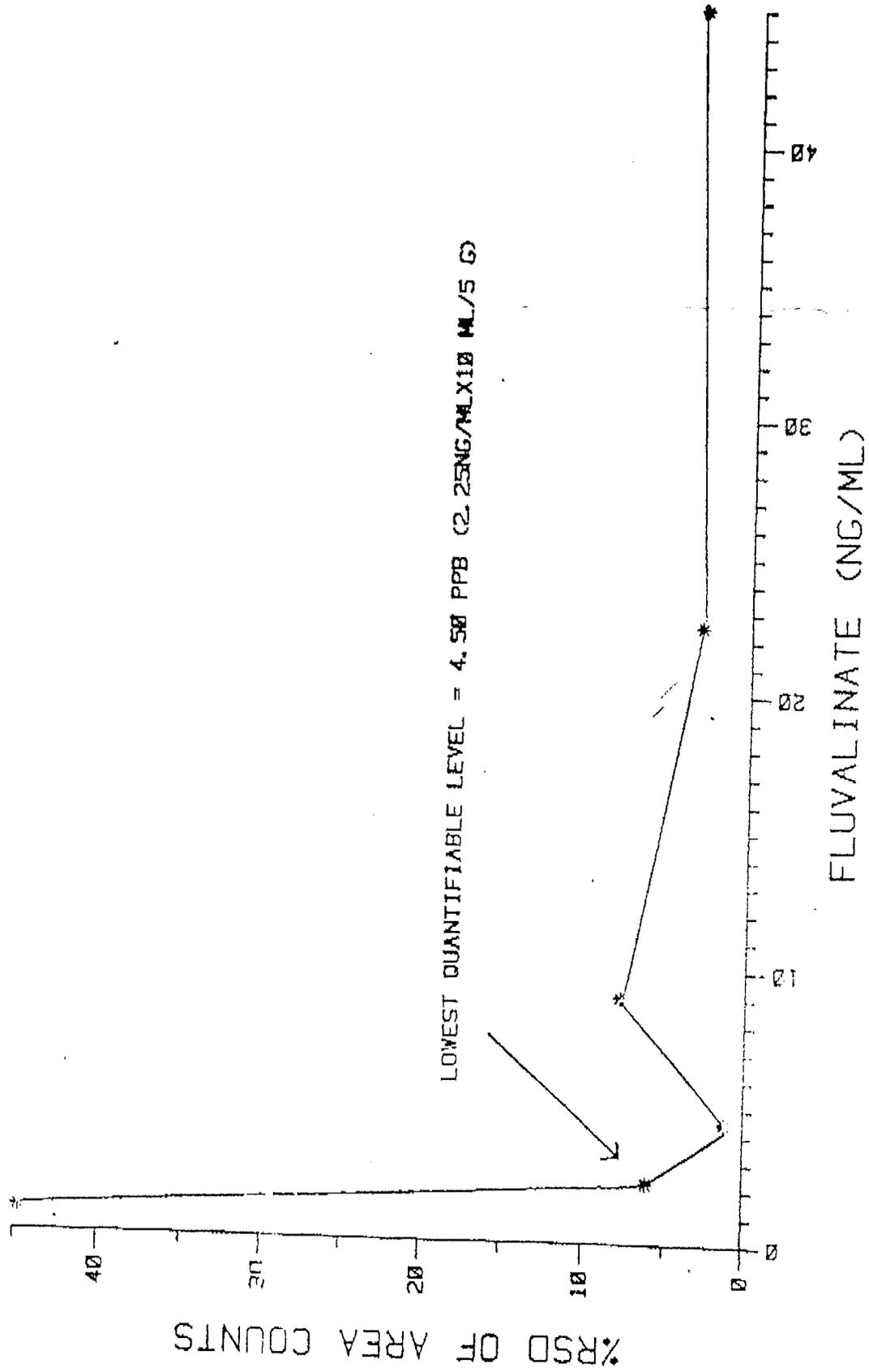


Figure 1

EQUATION NUMBER 1 WITHOUT SD

$y = A + Bx$

X	Y	Yc	-Yc	+Yc
2.25	22820.0	27154.8	15295.9	39013.7
2.25	22265.0	27154.8	15295.9	39013.7
2.25	24950.0	27154.8	15295.9	39013.7
4.50	73668.0	52118.4	41060.6	63176.3
4.50	71980.0	52118.4	41060.6	63176.3
4.50	73089.0	52118.4	41060.6	63176.3
9.00	92113.0	102045.7	92304.2	111787.2
9.00	84552.0	102045.7	92304.2	111787.2
9.00	98895.0	102045.7	92304.2	111787.2
22.50	238438.0	251827.6	242477.3	261178.0
22.50	230384.0	251827.6	242477.3	261178.0
22.50	245013.0	251827.6	242477.3	261178.0
45.00	487733.0	501464.1	483467.1	519461.1
45.00	516432.0	501464.1	483467.1	519461.1
45.00	521500.0	501464.1	483467.1	519461.1

INTERCEPT (A) = 2191.12972
 SD OF A = 22990.94594
 CONFIDENCE LEVEL OF A = 12731.93151

SLOPE (B) = 11094.95517
 SD OF B = 258.46089
 CONFIDENCE LEVEL OF B = 554.34217

SD ABOUT REGRESSION LINE = 15836.60964
 STANDARD DEVIATION OF Y = 182328.8761

REGRESSION COEFFICIENT = .99649