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MERCK RESEARCH LABORATORIES
Division of Merck & Co., Inc.
Hillsborough Road, Three Bridges, NJ 08887-0450

Attachment 2
From MRSD No. 433791-01

METHOD OF ANALYSIS
ENFORCEMENT METHOD

**M-036: LIQUID CHROMATOGRAPHIC METHOD FOR THE
QUANTITATION OF TOTAL AVERMECTIN B1 AND 8,9-Z-
AVERMECTIN B1 IN DRIED HOPS USING FLUORESCENCE
DETECTION**

DATE

6/15/94

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FIGURE 1 TYPICAL HPLC CHROMATOGRAM FOR DERIVATIZED AVERMECTINS IN DRIED HOPS	16

Precautions:

Since the long term toxicological effects of avermectins are not known, skin contact and inhalation should be avoided when handling avermectins

A. PRINCIPLE

The total amount of avermectin B1a, B1b and 8,9-Z avermectin B1a and B1b in dried hops is determined by HPLC using the external standardization technique. The dried hops are rehydrated and subsequently extracted with a methanol-water mixture. The avermectins are partitioned into hexane and the hexane extract is concentrated/purified on an aminopropyl solid phase extraction (SPE) column. The purified extract is derivatized with trifluoroacetic anhydride and the derivatized avermectins analyzed by reversed phase HPLC using fluorescence detection. The limit of quantitation is 5 ppb avermectin B1/8,9-Z avermectin B1.

B. APPARATUS

Equipment from manufacturers other than those listed below may be substituted provided they are shown to be functionally equivalent.

- 1 *Analytical Balance:*
Mettler AT261 or a balance accurate to 0.1 mg is recommended.
- 2 *Cuisinart Food Processor*
Model DLC-X Plus
3. *Centrifuge*
IEC model HN-SII (International Equipment Company, Needham Heights, MA, USA).
- 4 *Vacuum Manifold (for SPE columns)*
(Supelco Inc., Bellefonte, PA, USA)
5. *Solid Phase Extraction (SPE) Column*
Mega Bond Elut (aminopropyl), 1000 mg/6 mL, Cat # 122560-12,
75 mL reservoirs, Cat # 121310-12,
Reservoir adaptors, Cat.# 121310-01,
(Varian Sample Preparation Products, Harbor City, CA, USA).

6. *Glass Bottles*

Narrow mouth clear glass, 8 oz and 16 oz (Cat # B7451-9, Cat # B7507-28) with Poly-Seal caps (Cat # B7507-24, Cat # B7507-28)
(Scientific Products, McGaw Park, IL)

7. *Pipets, volumetric:*

25, 10 and 5 mL

8. *Rainin EDP2 Motorized Pipette (1000 uL & 250 uL)*

(Rainin, Cat # E2-1000, E2-250)

9. *Conical Tubes, polypropylene, 50 mL*

(Cat # 2070, Becton Dickinson Labware)

10. *Shaker*

Reciprocating type A, speed setting, approximately 100-120 oscillations/min
(Eberbach Corp)

C. REAGENTS

1. *Solvents:*

Hexane, HR-GC grade	(Cat # HX0297)
Methanol, glass distilled	(Cat # MX0488)
Ethyl Acetate, glass distilled	(Cat # EX0241)
Acetonitrile, distilled	(Cat # AX0142)

(EM Science, Gibbstown, NJ)

2. *Chemicals*

Trifluoroacetic anhydride	(Cat # 67363, Pierce Chemical)
1-Methylimidazole	(Cat.# M5,083-4, Aldrich Chemical)
Triethylamine	(Cat # 13,206-3; Aldrich Chemical)
Calcium chloride dihydrate	(Cat # 22,350-6; Aldrich Chemical)
Sodium sulfate, anhydrous	(Cat.# S-421, Fisher Scientific)

3. *Deionized Water:*

Purified using a Millipore Milli-Q water purification system (Millipore Corp.)

4. *HPLC Mobile Phase:*

To a 1000 mL volumetric flask add 80 mL of water and 0.5 mL of triethylamine. Dilute to volume with methanol.

5. *Ethyl Acetate/Methanol Solution:*

To a 100 mL volumetric flask add 25 mL of methanol and dilute to the 100 mL mark with ethyl acetate.

6. *Calcium Chloride Solution (2%)*

Transfer 10 g of calcium chloride dihydrate to a 500 mL volumetric flask and dilute to the 500 mL mark with water.

D. HPLC APPARATUS

Equipment from manufacturers other than those listed below may be substituted provided they are shown to be functionally equivalent.

1. *HPLC Pump:*

Beckman model 110

2. *Automatic Sample Injector:*

Waters Wisp Model 710B

3. *Detector:*

Spectrovision FD100 fluorescence detector (Groton Technologies) with a xenon flash light source, a 365 nm excitation filter and a 470 nm emission cutoff filter. The unit contains a 24 μ L flow cell assembly.

Note! The optimum excitation and emission wavelengths for the derivatized avermectins are 365 nm and 470 nm respectively.

4. *Analytical Column:*

Chromagabond MC18 column, 15 cm x 4.6 mm, 3 micron particle size (ES Industries)

5. *Guard Column:*

Brownlee Newguard 15 x 3.2 mm, 7 micron RP-18 or equivalent, (Rainin Cat #G18-013) contained in a NewGuard Holder (Rainin Cat #140-601).

6. *Recorder*

Chromjet model 4400 integrator (Thermo Separations Products)

7. *Column Temperature Controller*

Goldenfoil CH-1530 basic model column heater (Systec, Inc , Minneapolis, MN,)

E. ANALYTICAL STANDARD

1. *Avermectin B1*

Analytical standard of avermectin B1 of known purity (Merck & Co , Rahway, NJ)
Analytical standard L-676,863-038A003 in glycerol formal solution containing 0.893
w/w% B1a component and 0.044 w/w% B1b component is acceptable.

F. PREPARATION OF AVERMECTIN B1 STANDARD SOLUTIONS

1. *Avermectin B1 Stock Solution*

- a Weigh accurately, about 225 mg of the avermectin B1 analytical standard into a 100 mL volumetric flask. Dilute the flask to the 100 mL mark with acetonitrile. Label the flask, "Avermectin B1 Standard Stock Solution". The stock solution contains approximately 20 ug/mL of the avermectin B1a component and 1.0 ug/mL of the B1b component.

Note! The weight of analytical standard can be adjusted depending on the concentration of avermectin B1 in the working standards used for the analysis of avermectin in the samples.

2. *Avermectin B1 Intermediate Stock Standard Solution*

- a Transfer a 5.0 mL aliquot of the "Avermectin B1 Standard Stock Solution" to a 50 mL volumetric flask. Dilute the flask to the 50 mL mark with acetonitrile. Label the flask "Avermectin B1 Intermediate Standard Stock Solution". The intermediate standard stock solution contains approximately 2.0 ug/mL of the avermectin B1a component and 0.10 ug/mL of the B1b component.

3. *Avermectin B1a Working Standards (HPLC Standards)*

- a Transfer a 5.0 mL aliquot of the "Avermectin B1 Intermediate Standard Stock Solution" to a 100 mL volumetric flask. Dilute the flask to the 100 mL mark with acetonitrile. Label the flask "Standard E". The solution contains approximately 100 ng/mL of the avermectin B1a component.

- b Transfer 2.5 mL, 5.0 mL, 10.0 mL and 25.0 mL aliquots of the "Standard E" solution to separate 50 mL volumetric flasks. Dilute each flask to the 50 mL mark with acetonitrile. Label the flasks "Standard A", "Standard B", "Standard C" and "Standard D" respectively. The Standards "A", "B", "C" and "D" solutions contain approximately 5.0 ng/mL, 10 ng/mL, 20 ng/mL and 50 ng/mL of avermectin B1a respectively.

Note! The concentration of avermectin B1a in the working standards can be adjusted depending on the concentration of avermectin B1 in the samples. The Standard A, 5 ng/mL B1a, is optional and is only used if actual field samples contain < 5 ppb of avermectin B1/8,9-Z avermectin B1.

G. SAMPLE PROCESSING

1. Dried Hop Cones

- a Transfer approximately 100-200 g of dried hop cones to a Cuisinart food processor.
Note! Dry ice may be added to the dried hop cones to achieve better processing.
- b Process the sample until a homogeneous blend is achieved and transfer to a suitable container or containers.
- c Store the sample at -20° C or colder prior to analysis.

H. SAMPLE ANALYSIS

1. Hydration and Extraction

- a Transfer 5 grams of homogenized dried hop cones to a 16 ounce glass bottle.
Note! Control dried hop samples should be fortified with avermectin B1 at this time for method recoveries.
- b Add 50 mL of water, cap and shake on a reciprocating shaker for 1 hour.
- c Add 75 mL of methanol and shake for an additional 30 minutes.
- d Allow the fines to settle. Transfer a 35-40 mL aliquot of the extract to a 50 mL conical centrifuge tube and centrifuge at 4000 rpm for 10 minutes.

2. Liquid-Liquid Partitioning

- a Transfer a 25 mL aliquot of the extract (equivalent to 1g of sample) to a clean 50 mL conical centrifuge tube. Add 20 mL of hexane and shake vigorously for approximately 30 seconds. Allow the layers to separate.
Note! If necessary, the sample should be centrifuged for approximately 3 minutes at 3000 rpm.
- b Remove the hexane layer with a capillary pipet. Discard the hexane.
- c Transfer the aqueous extract to an 8 oz glass bottle. Rinse the centrifuge tube with 20 mL of hexane and combine with the aqueous extract.
- d Add 25 mL of 2% calcium chloride solution to the extract and shake vigorously for approximately 30 seconds. Allow the layers to separate.
- e Transfer the hexane layer to a 125 mL erlenmeyer flask with a capillary pipet.
- f Extract the aqueous solution twice (2x) more with 20 mL portions of hexane. Combine the hexane extracts.
- g Add approximately 0.5-1g of anhydrous sodium sulfate to the combined hexane extracts. Mix well.

3. Clean-up of Final Extract on SPE Column

- a Attach a 1000 mg/ 6 mL aminopropyl Mega Bond Elut Solid Phase Extraction (SPE) cartridge, fitted with an adaptor and a 75 mL reservoir, to the vacuum manifold.
- b Apply a mild vacuum and condition the SPE column with 10 mL of methanol followed by 10 mL of hexane. Discard the rinse solutions.
Note! The SPE column should not be allowed to run dry during the clean-up procedure.
- c Transfer the entire hexane extract to the SPE column. Apply a mild vacuum and allow the hexane solution to drain from the column.
Note! The flow rate through the column should not exceed 3-4 drops per second.
- d Rinse the flask and sodium sulfate with 10 mL of hexane. Add the rinse solution to the SPE column. Apply a mild vacuum and allow the hexane solution to drain from the column.
- e Rinse the SPE column with 5 mL of ethyl acetate using a mild vacuum.

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- f Elute the avermectins from the column with 3 mL of ethyl acetate:methanol (75:25) solution using mild vacuum. Collect the eluant in a test tube.
Note! Residual ethyl acetate:methanol remaining in the SPE column should be drained and collected using moderate to high vacuum.
- g Evaporate the ethyl acetate:methanol solution to dryness under a stream of nitrogen using a heat lamp or water bath at a temperature not to exceed 35° C
- h Reconstitute the sample in 0.5 mL of acetonitrile

4. Derivatization of Samples and Standards

- a To 0.5 mL of each sample (step H-3h) and working standard (step F-3) add 0.5 mL of acetonitrile:triethylamine (95:5 v/v) solution
- b Add 50 µL of 1-methylimidazole to each sample and standard tube. Mix well
- c Add 50 µL of trifluoroacetic anhydride to each sample and standard tube. Mix well after each addition. Allow the solution to stand for approximately 3 minutes.
Note! The reaction should be performed under a hood.
- d To each tube add 1 mL of HPLC mobile phase (methanol:water:triethylamine, 92:8:0.05). Mix well.
Note! The derivatized avermectins are stable for at least 24 hours when stored in the refrigerator.

I. HPLC CHROMATOGRAPHIC CONDITIONS

The HPLC conditions cited below are provided as a guide in establishing operating conditions. Conditions should be adjusted as required to obtain chromatographic peak shape, resolution and sensitivity equivalent to or better than that shown in the attached chromatographic figure (Figure 1)

1. Mobile Phase

Methanol:water:triethylamine (92:8:0.05 v/v).

Note! The mobile phase composition (methanol content) can be adjusted in order to give an adequate separation and retention of derivatized avermectin B1a and B1b.

2. Flow Rate:

0.60 mL/min

100

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3. *Column Temperature:*
Approximately 35° C
4. *Sample Injection Volume:*
30 uL
5. *Detector:*
Fluorescence, Excitation max for derivatized avermectin B1a/B1b derivative = 365 nm,
Emission max = 470 nm
6. *Approximate Retention Times for Avermectin Derivatives*

Avermectin B1b/8,9-Z avermectin B1b -	12 minutes
Avermectin B1a/8,9-Z avermectin B1a -	14 minutes

J. HPLC ANALYSIS OF SAMPLES AND STANDARDS

Before beginning analysis of derivatized samples and standards, inject a derivatized avermectin standard and note the retention time and separation of the avermectin B1a and B1b peaks. If reproducibility of the autosampler is not known, make two or three injections of the derivatized standard to determine the precision of the injection system.

The following injection sequence is recommended for the analysis of sample and standard solutions:

1. Inject aliquots of the derivatized avermectin working standards. Inject aliquots of the derivatized sample solutions followed by injection of aliquots of the derivatized avermectin working standards.
2. The sample set should include a derivatized control sample and at least one avermectin B1a method recovery sample. The method recovery sample should yield a value between 70-110% of the theoretical fortification level.

K. CALCULATIONS

The ng/g (ppb) of avermectin B1a/8,9-Z avermectin B1a and avermectin B1b/8,9-Z avermectin B1b in the sample is determined from the avermectin B1a standard response curve

1. At the completion of the HPLC run, determine the linear regression coefficients (slope, y-intercept) for the avermectin B1a standard response curve by plotting the ng/mL of avermectin B1a working standards vs the response (B1a peak area) of the standards. Determine the coefficient of determination (r^2) for the standard response curve. The r^2 value should be greater than 0.97.
2. Use the following equation to calculate the ng/g of avermectin B1a / 8,9-Z avermectin B1a and avermectin B1b / 8,9-Z avermectin B1b in the sample.

$$\text{(Equation 1)} \quad C = \frac{(R - I) \times V \times DF}{S \times W}$$

C	=	ng/g of avermectin B1a or B1b in the sample
R	=	Response (peak area) of avermectin B1a or B1b in the sample
I	=	y-Intercept of the avermectin B1a standard response curve
S	=	Slope of the avermectin B1a standard response curve
V	=	Volume (mL) of sample before derivatization (0.5 mL)
DF	=	Sample dilution factor. The dilution factor is equal to 1 if no further dilution of the sample is made.
W	=	Weight (g) of sample processed through the method (1 g)

Note! Derivatized sample solutions exceeding 100 ng/mL of avermectin B1a or B1b should be diluted to approximately 25-50 ng/mL B1a or B1b with HPLC mobile phase prior to analysis.

L. REPORTING RESULTS

1. Reporting of residue values of avermectin B1a/8,9-Z avermectin B1a and avermectin B1b/8,9-Z avermectin B1b in actual field samples should follow the guidelines shown below:
 - a. Residue values less than 2 ppb (LOD) should be reported as ND (not detected).
 - b. Residue values between 2 ppb and 4.9 ppb (LOQ equals 5 ppb) should be reported as NQ (not quantitated).

M. VALIDATION

1. Introduction

Analytical method M-036 titled "*Liquid Chromatographic Method for the Quantitation of Total Avermectin B1 and 8,9-Z-Avermectin B1 in Dried Hops using Fluorescence Detection*" was validated by Merck Research Laboratories, Three Bridges, NJ. The method validation included the determination of the accuracy, precision, linearity, specificity and limit of detection (LOD) and quantitation (LOQ).

2. Preparation of 8,9-Z Avermectin B1a Fortification Solutions.

Note! The 8,9-Z avermectin B1a of known purity in glycerol formal solution (Merck & Co., Rahway, NJ) should be used to prepare the fortification solutions used for the determination of the method recoveries.

- a Weigh accurately, about 260 mg of the 8,9-Z avermectin B1a glycerol formal solution (0.38 w/w% 8,9-Z avermectin B1a) into a 50 mL volumetric flask. Dilute the flask to the 50 mL mark with acetonitrile. Label the flask, "8,9-Z Avermectin B1a Stock Solution". The stock solution contains approximately 20 ug/mL of the 8,9-Z avermectin B1a component.
- b Transfer a 5.0 mL aliquot of the "8,9-Z Avermectin B1a Stock Solution" to a 50 mL volumetric flask. Dilute the flask to the 50 mL mark with acetonitrile. Label the flask "8,9-Z Avermectin B1a- 2 ug/mL Solution". The solution contains approximately 2.0 ug/mL of the 8,9-Z avermectin B1a component.
- c Transfer a 5.0 mL aliquot of the "8,9-Z Avermectin B1a- 2 ug/mL Solution" to a 100 mL volumetric flask. Dilute the flask to the 100 mL mark with acetonitrile. Label the flask "8,9-Z Avermectin B1a- 100 ng/mL Solution". The solution contains approximately 100 ng/mL of the 8,9-Z avermectin B1a component.

3. Determination of the Accuracy of the Method

- a The accuracy of the analytical method is the statistical agreement of the test results obtained by the analytical procedure to the theoretical value. Duplicate five (5) gram aliquots of ground, dried, control hops were each fortified with approximately 5 ppb avermectin B1a, 5 ppb avermectin B1b, 5 ppb 8,9-Z avermectin B1a, 50 ppb avermectin B1b, 100 ppb avermectin B1a, 100 ppb 8,9-Z avermectin B1a, 1000 ppb of avermectin B1a and 1000 ppb 8,9-Z avermectin B1a.

Note! Each aliquot contained a single analyte at the specified fortification level.

Each sample was analyzed according to analytical method M-036 and the accuracy (% recovery) for each analyte at each fortification level was calculated using the following equation

$$\% \text{ recovery} = \frac{\text{ppb analyte added}}{\text{ppb analyte found}} \times 100$$

The individual assay results are tabulated in Table I

4. *Determination of the Precision of the Method*

- a The precision of the analytical method is the statistical agreement among individual test results on multiple samplings of a homogeneous sample. The precision of the analytical method is expressed as the relative standard deviation of the test results. The overall precision for each analyte was calculated from the standard deviation and the mean recovery value using the following equation

$$\text{precision (\%)} = \frac{\text{Standard Deviation}}{\text{Mean}} \times 100$$

The overall precision for recoveries of avermectin B1a, avermectin B1b and 8,9-Z avermectin B1a from dried hops was 2.6%, 2.1% and 10.1% respectively.

5. *Determination of the Linearity of the Method*

- a The standard response curve was shown to be linear over the concentration range of 5.4 ng/mL to 108 ng/mL or approximately 0.16 - 3.2 ng of avermectin B1a injected on the column (30 µL injection). Linear regression analysis of the plot of avermectin B1a area vs. avermectin B1a concentration (ng/mL) yielded a coefficient of determination (r^2) greater than 0.99 in all cases.

6. *Determination of the Specificity of the Method*

- a Analysis of control dried hops (hops not treated with avermectin) contained < 2 ppb (LOD) apparent residues of avermectin B1a/8,9-Z avermectin B1a or avermectin B1b/8,9-Z avermectin B1b (see Figure 1)

7. *Determination of the Limit of Detection (LOD) and Limit of Quantitation (LOQ) of the Method*

- a The limit of detection of the method is defined as the lowest concentration of avermectin that the analytical method can reliably detect ($S/N > 3$). Based on the signal to noise ratio (S/N) for the 5 ppb avermectin B1a fortified sample, we estimate the LOD of the method to be 2 ppb for each analyte.
- b The limit of quantitation of the method is defined as the lowest concentration of avermectin that the analytical method can quantitate with acceptable recovery ($S/N > 10$). Since the lowest avermectin fortification level used in the validation study was 5 ppb, we define the LOQ to be 5 ppb for each analyte.

TABLE I
SUMMARY OF RESULTS

<i>Analyte</i>	<i>Replicate #</i>	<i>ppb Added</i>	<i>ppb Found</i>	<i>% Recovery</i>	
avermectin B1a	1	50	54	108	
	2	50	53	106	
	1	100	104	104	
	2	100	101	101	
	1	1000	1055	106	
	2	1000	1022	102	
			average	105	
			std dev	2.7	
avermectin B1b	1	49	44	90	
	2	49	45	92	
	1	49	46	94	
	2	49	46	94	
				average	92.5
				std dev	1.9
8,9-Z avermectin B1a	1	5.0	4.6	92	
	2	5.0	4.8	96	
	1	100	88	88	
	2	100	80	80	
	1	1000	726	73	
	2	1000	801	80	
			average	84.8	
			std dev.	8.6	

FIGURE 1

Typical HPLC Chromatogram for Derivatized
Avermectins in Dried Hops

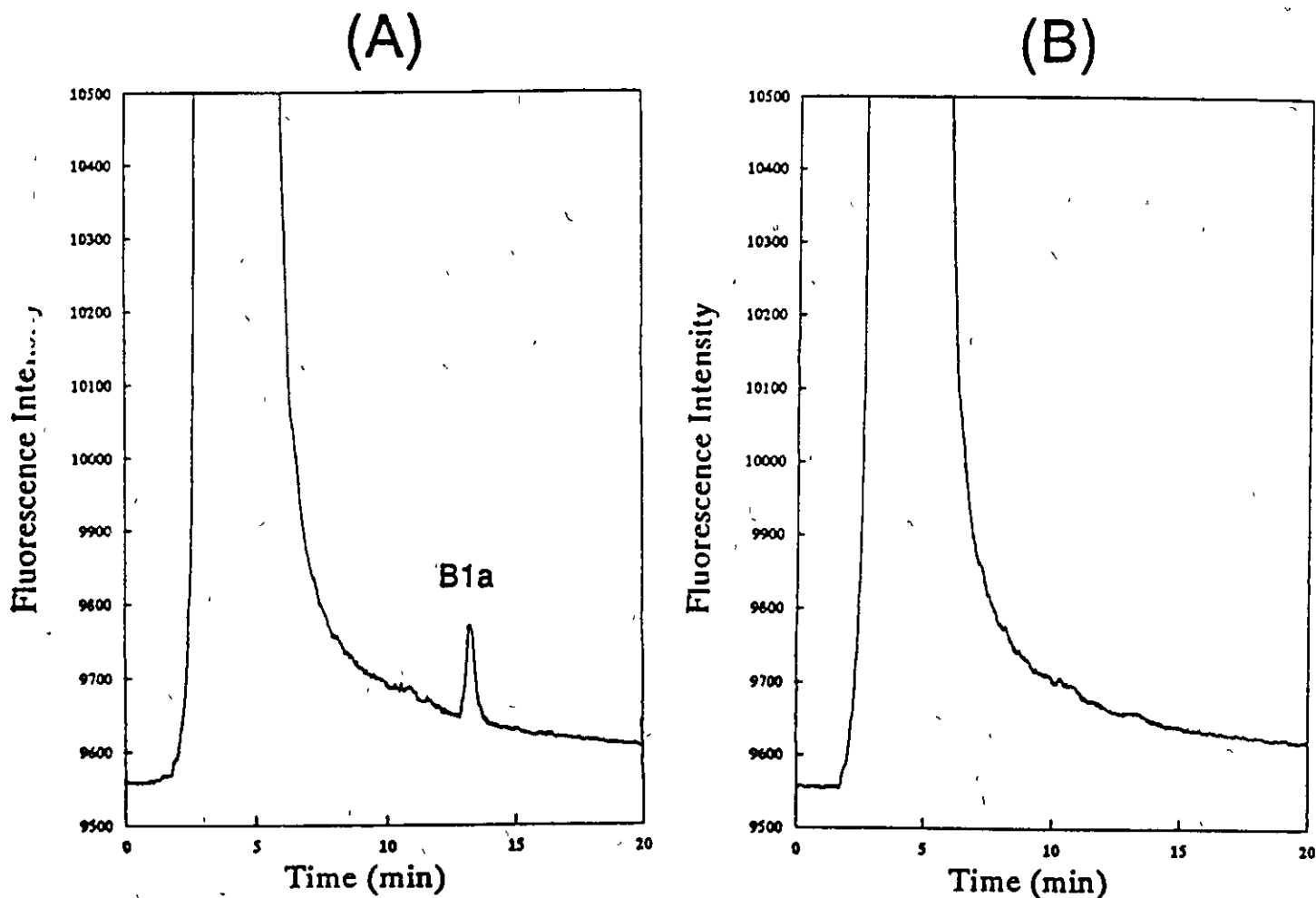


Fig 1: (A) Chromatogram for control dried hops fortified with 5 ppb of avermectin B1a;
(B) control dried hops

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REPRESENTATIVE CHROMATOGRAMS

Method Validation Data for Avermectin B1a and B1b in Dried Hops

M-036 Liquid Chromatographic Method for the Quantitation of Total Avermectin B1 and 8,9-Z-Avermectin B1 in Dried Hops Using Fluorescence Detection

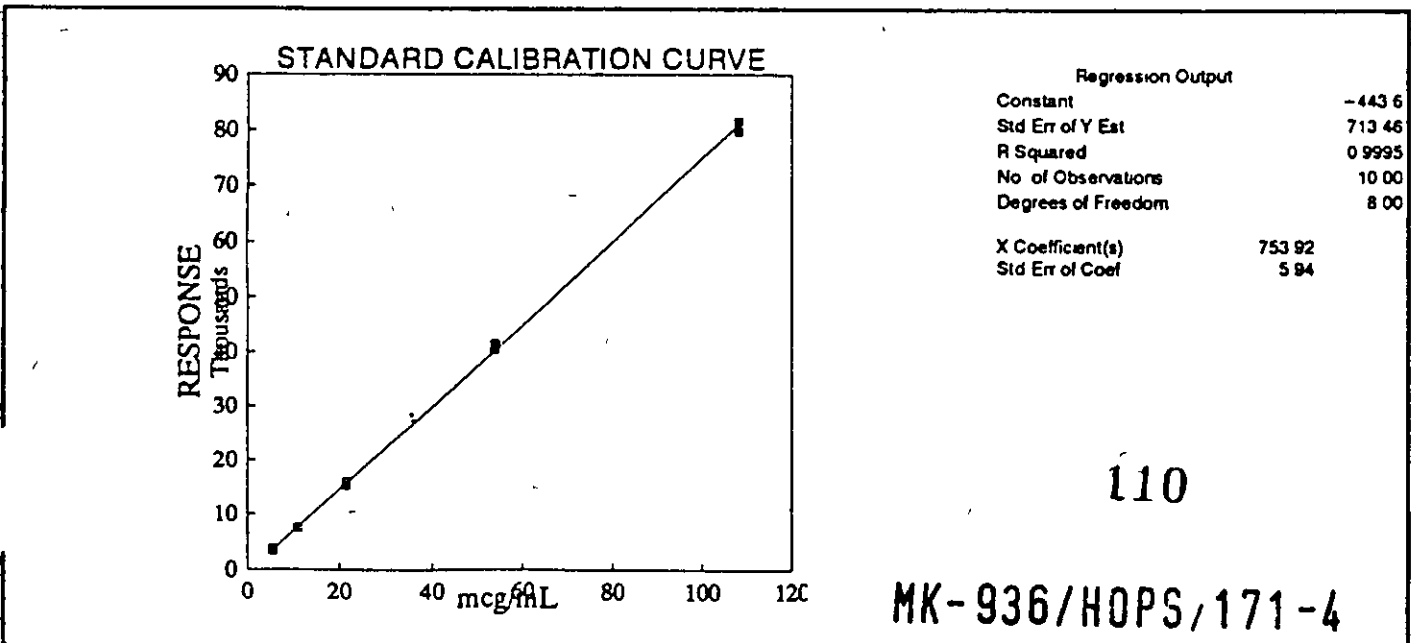
109

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STANDARD REGRESSION DATA – ABAMECTIN HOPS

STUDY PROTOCOL NO	N/A	ASSAY DATE	5/25/94
TYPE OF STUDY	Method Validation	STD PREP DATE	4/7/94
TEST MATRIX	Dry Hops	STOCK PREP DATE	4/7/94
TEST SUBSTANCE	Avermectin B1 (L-676,863)	REF NOTEBOOK	90471
ANALYT METHOD #	M-036		
TYPE OF EXPERIMENT	Avermectin B1 Analysis in Dry Hops		
ANALYT STD LOT NO	L-676,863-038A003		
ANALYT STD PURITY	0.893 w/w% B1a, 0.044 w/w% B1b		

SAMPLE ID	BIN #	B1a Area		STD B1a CONC (ng/mL)		COMMENTS SECTION
		ACTUAL	CALC	ACTUAL	CALC	
STD A	1	3387	3628	5.40	5.08	
STD A	17	3761	3628	5.40	5.58	
STD B	2	7519	7699	10.8	10.6	
STD B	18	7423	7699	10.8	10.4	
STD C	3	16046	15841	21.6	21.9	
STD C	19	15336	15841	21.6	20.9	
STD D	4	41513	40268	54.0	55.7	
STD D	20	40527	40268	54.0	54.3	
STD E	5	81600	80980	108	109	
STD E	21	79719	80980	108	106	



SAMPLE DATA - ABAMECTIN HOPS

STUDY PROTOCOL NO
 TYPE OF STUDY:
 TEST MATRIX:
 TEST SUBSTANCE:

N/A
 Method Validation
 Dry Hops
 Avermectin B1 (L-676,863)

ANALYTICAL METHOD # M-036
 TYPE OF EXPERIMENT: Avermectin B1 Analysis in Dry Hops
 ANALYTICAL STD LOT NO: L-676,863-038A003
 ANALYTICAL STD PURITY: 0.893 w/w% B1a, 0.044 w/w% B1b
 ASSAY DATE May 25, 1994

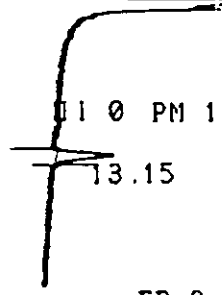
SAMPLE DESCRIPTION	SAMPLE ID	GRAMS SAMPLE	BIN #	B1b	PEAK AREA B1a	DILUTION FACTOR	ng/mL B1a FOUND	ng/mL B1b FOUND	LABEL PPB	ppb B1a SAMPLE	ppb B1b SAMPLE	ppb B1a or B1b AVG % NOMINAL
5.0 ppb B1a Fortification	WT A	5.00	6		7714	1.000	10.82		5.0	5.41		107.4%
5.0 ppb B1a Fortification	WT B	5.00	7		7571	1.000	10.63		5.0	5.32		
100 ppb B1a Fortification	WT A	5.00	8	1206	30913	0.200	41.59		100	104.0		102.7%
100 ppb B1a Fortification	WT B	5.00	9	1283	30097	0.200	40.51		100	101.3		
1002 ppb B1a Fortification	WT A	5.00	10	2478	63202	0.040	84.42		1002	1055		103.6%
1002 ppb B1a Fortification	WT B	5.00	11	2643	61181	0.040	81.74		1002	1022		
Control	WT A	5.00	12	Not Detected		1.000						
4.9 ppb B1b Fortification	WT A	5.00	13	6182	144414	1.000		8.79	4.9	4.40	4.43	90.4%
4.9 ppb B1b Fortification	WT B	5.00	14	6256	143603	1.000		8.89	4.9	4.45	4.43	90.4%
49 ppb B1b Fortification	WT A	5.00	15	34491	620095	0.500		46.34	49.0	46.3	46.3	94.5%
49 ppb B1b Fortification	WT B	5.00	16	34483	614764	0.500		46.33	49.0	46.3	46.3	94.5%

ILEC 1123936hops15-25-94

111

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CHANNEL A INJECT 05/25/94 17 33 47 STORED TO BIN # 1
11 1



ER 0
DATA SAVED TO BIN # 1

ABAMECTIN HOPS 05/25/94 17 33 47 CH= "A" PS= 1

FILE 1 METHOD 5. RUN 1 INDEX 1 BIN 1
 Column (Dim-Temp) CHROMEGABOND MC18, 15 X 4.6 mm, 3 um, #03294-7-98-27421
 HPLC Mobile Phase Methanol Water Triethylamine (92 8 0.05)
 Column Temp 35 deg C
 Inj Vol/Flow 30ul/0.6 ml/min
 Attn/PW/PT 2/25/200 (SP4400 Settings)
 EX/EH 365nm/470nm
 Lamp/Resp/Range 100/1/50 (SpectroVision)
 Component-Number Pump-111, Det-214 AutoInj-311, Integ-421 Col Heat -635
 Study Method Validation
 Method No M-036
 Notebook Ref Notebook No 90471

ANALYST N JOHNSON

SAMPLE 1 STD A BIN 1 NAME ARUN1582

NAME	CONC	RT	AREA BC	RF
B1a	0.	13.15	3387.01	
TOTALS	0.		3387	

CHANNEL A INJECT 05/25/94 17:54:40 STORED TO BIN # 2
11 1

11 0 PM 1

13 15

ER 0
DATA SAVED TO BIN # 2

ABAMECTIN HOPS 05/25/94 17:54 40 CH= "A" PS= 1.

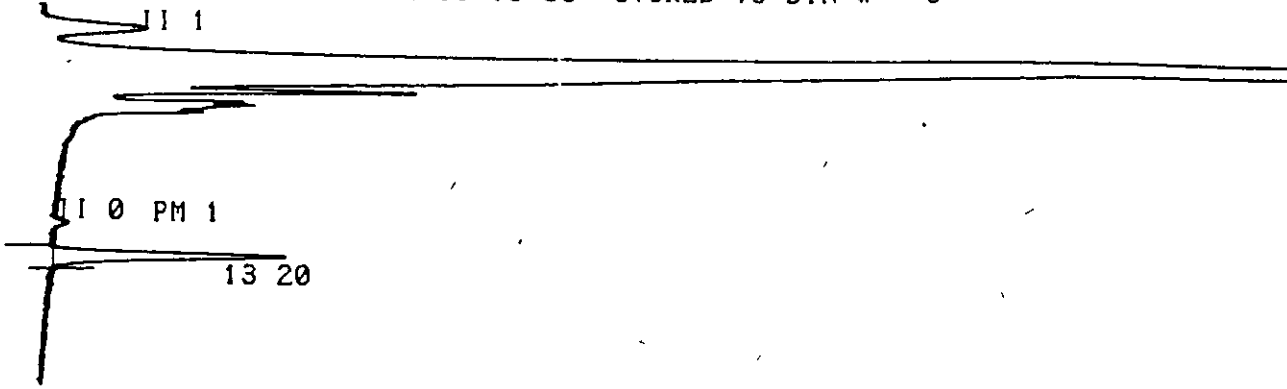
FILE 1 METHOD 5 RUN 2 INDEX 1 BIN 2
Column (Dim-Temp) . CHROMEGABOND MC18, 15 X 4.6 mm, 3 um, #03294-7-98-27421
HPLC Mobile Phase . Methanol.Water.Triethylamine (92:8:0 05)
Column Temp. 35 deg C
Inj. Vol/Flow 30ul/0.6 ml/min
Attn/PW/PT 2/25/200 (SP4400 Settings)
EX/EM 365nm/470nm
Lamp/Resp/Range 100/1/50 (SpectroVision)
Component-Number Pump-111, Det-214, AutoInj-311, Integ-421, Col.Heat -635
Study Method Validation
Method No M-036
Notebook Ref Notebook No 90471

ANALYST N JOHNSON

SAMPLE 2 STD B BIN 2 NAME ARUN1583

NAME	CONC	RT	AREA	BC	RF
B1a	0.	13.15	7519	01	
TOTALS	0.		7519		

CHANNEL A INJECT 05/25/94 18:15 33 STORED TO BIN # 3



ER 0
DATA SAVED TO BIN # 3

ABAMECTIN HOPS 05/25/94 18.15 33 CH= "A" PS= 1

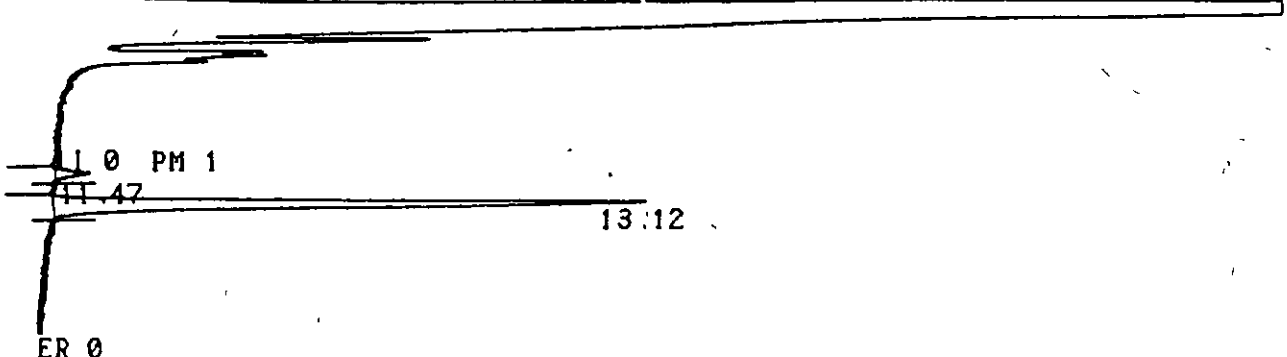
FILE 1 METHOD 5 RUN 3 INDEX 1 BIN 3
 Column (Dim-Temp) CHROMEGABOND MC18. 15 X 4 6 mm. 3 um. #03294-7-98-27421
 HPLC Mobile Phase Methanol:Water:Triethylamine (92:8:0:05)
 Column Temp 35 deg C
 Inj Vol/Flow 30ul/0.6 ml/min
 Attn/PW/PT 2/25/200 (SP4400 Settings)
 EX/EM 365nm/470nm
 Lamp/Resp/Range 100/1/50 (SpectroVision)
 Component-Number Pump-111, Det-214, AutoInj-311, Integ-421, Col.Heat.-635
 Study Method Validation
 Method No M-036
 Notebook Ref Notebook No 90471

ANALYST N JOHNSON

SAMPLE 3 STD C BIN 3 NAME ARUN1584

NAME	CONC	RT	AREA	BC	RF
B1a	0.	13.2	16046	01	
TOTALS	0.		16046		

CHANNEL A INJECT 05/25/94 18:36:27 STORED TO BIN # 4
11 1



DATA SAVED TO BIN # 4

ABAMECTIN HOPS 05/25/94 18:36.27 CH= "A" PS= 1.

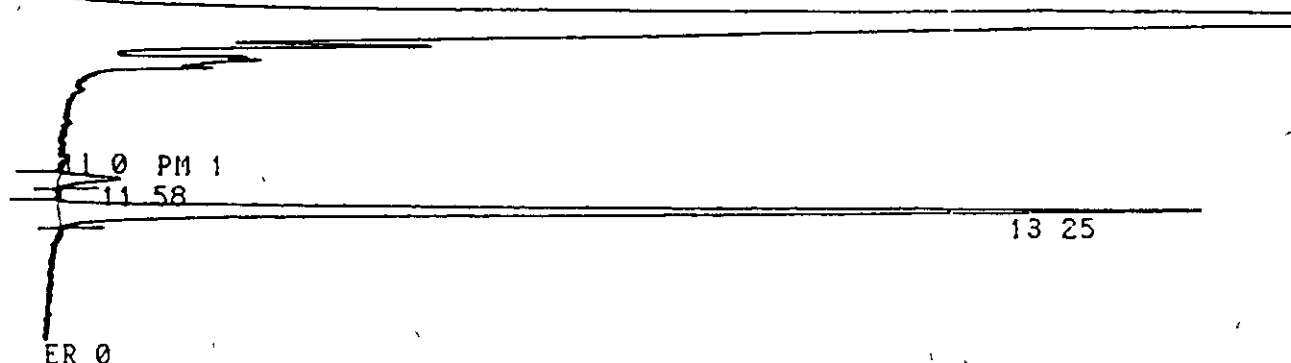
FILE 1. METHOD 5 RUN 4 INDEX 1 BIN 4
Column (Dim-Temp) : CHROMECABOND MC18, 15 X 4.6 mm, 3 um. #03294-7-98-27421
HPLC Mobile Phase : Methanol:Water:Triethylamine (92.8:0.05).
Column Temp. : 35 deg C.
Inj Vol/Flow : 30ul/0.6 ml/min
Attn/PW/PT : 2/25/200 (SP4400 Settings)
EX/EM : 365nm/470nm
Lamp/Resp/Range : 100/1/50 (SpectroVision)
Component-Number : Pump-111, Det-214, AutoInj-311, Integ-421, Col.Heat -635
Study : Method Validation
Method No : M-036
Notebook Ref. : Notebook No 90471

ANALYST. N JOHNSON

SAMPLE 4 STD D BIN 4 NAME ARUN1585

NAME	CONC	RT	AREA BC	RF
B1b	0.	11.47	1919 01	
B1a	0	13.12	41513 01	
TOTALS	0.		43432	

CHANNEL A INJECT 05/25/94 18.57.20 STORED TO BIN # 5
11 1



DATA SAVED TO BIN # 5

ABAMECTIN HOPS 05/25/94 18.57.20 CH= "A" PS= 1

FILE 1 METHOD 5 RUN 5 INDEX 1 BIN 5
 Column (Dim-Temp) CHROMEGABOND MC18, 15 X 4.6 mm, 3 um, #03294-7-98-27421
 HPLC Mobile Phase Methanol:Water:Triethylamine (92:8.0:0.5)
 Column Temp 35 deg C.
 Inj Vol/Flow 30ul/0.6 ml/min
 Inj/PW/PT 2/25/200 (SP4400 Settings)
 X/EI 365nm/470nm
 Lamp/Resp/Range 100/1/50 (SpectroVision)
 Component-Number Pump-111, Det-214, AutoInj-311, Integ-421, Col Heat -635
 Study Method Validation
 Method No M-036
 Notebook Ref Notebook No. 90471

ANALYST: N JOHNSON

SAMPLE 5 STD E BIN 5 NAME ARUN1586

NAME	CONC	RT	AREA	BC	RF
B1b	0.	11.58	3827	01	
B1a	0.	13.25	81600	01	
TOTALS	0.		85427		

CHANNEL A INJECT 05/25/94 19:18:13 STORED TO BIN # 6
11 1

11 0 PM 1

13.22

ER 0
DATA SAVED TO BIN # 6

ABAMECTIN HOPS 05/25/94 19.18.13 CH= "A" PS= 1.

FILE 1 METHOD 5 RUN 6 INDEX 1 BIN 6
Column (Dim-Temp) : CHROMEGABOND MC18, 15 X 4 6 mm. 3 um, #03294-7-98-27421
HPLC Mobile Phase : Methanol:Water:Triethylamine (92.8:0.05)
Column Temp : 35 deg C
Inj Vol/Flow : 30ul/0.6 ml/min
Attn/PW/PT : 2/25/200 (SP4400 Settings)
EX/EM : 365nm/470nm
Lamp/Resp/Range : 100/1/50 (SpectroVision)
Component-Number : Pump-111, Det-214, AutoInj-311, Integ-421, Col.Heat -635
Study : Method Validation
Method No : H-036
Notebook Ref : Notebook No. 90471

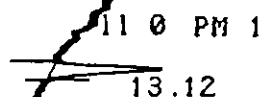
ANALYST N. JOHNSON

SAMPLE 6 5ppb-1 BIN 6 NAME ARUN1587

NAME	CONC	RT	AREA BC	RF
B1a	0.	13.22	7714 01	
TOTALS	0		7714	

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CHANNEL A INJECT 05/25/94 19:39 07 STORED TO BIN # 7
11 1



ER 0
DATA SAVED TO BIN # 7

ABAMECTIN HOPS 05/25/94 19.39.07 CH= "A" PS= 1.

FILE 1 METHOD 5 RUN 7 INDEX 1 BIN 7
 Column (Dim-Temp) CHROMECABOND MC18, 15 X 4 6 mm, 3 um. #03294-7-98-27421
 HPLC Mobile Phase Methanol Water Triethylamine (92.8 0 05)
 Column Temp 35 deg C.
 Inj Vol/Flow 30ul/0.6 ml/min
 Attn/PW/PT 2/25/200 (SP4400 Settings)
 EX/EM 365nm/470nm
 Lamp/Resp/Range 100/1/50 (SpectroVision)
 Component-Number Pump-111, Det-214, AutoInj-311 integ-421, Col Heat -635
 Study Method Validation
 Method No M-036
 Notebook Ref Notebook No 90471

ANALYST N JOHNSON

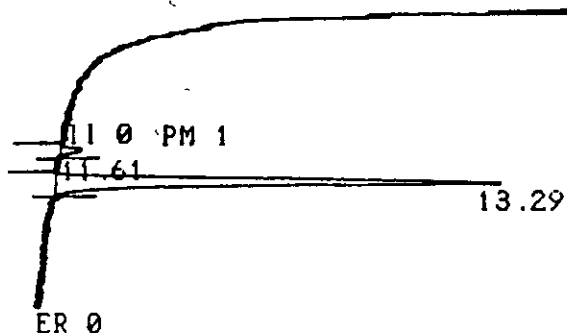
SAMPLE 7 5ppb-2 BIN 7 NAME ARUN1588

NAME	CONC	RT	AREA	BC	RF
B1a	0.	13.12	7571	01	
TOTALS	0.		7571		

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MK-936/HOPS/171-4

CHANNEL A INJECT 05/25/94 20:00:00 STORED TO BIN # 8
11 1



DATA SAVED TO BIN # 8

ABAMECTIN HOPS 05/25/94 20:00.00 CH= "A" PS= 1.

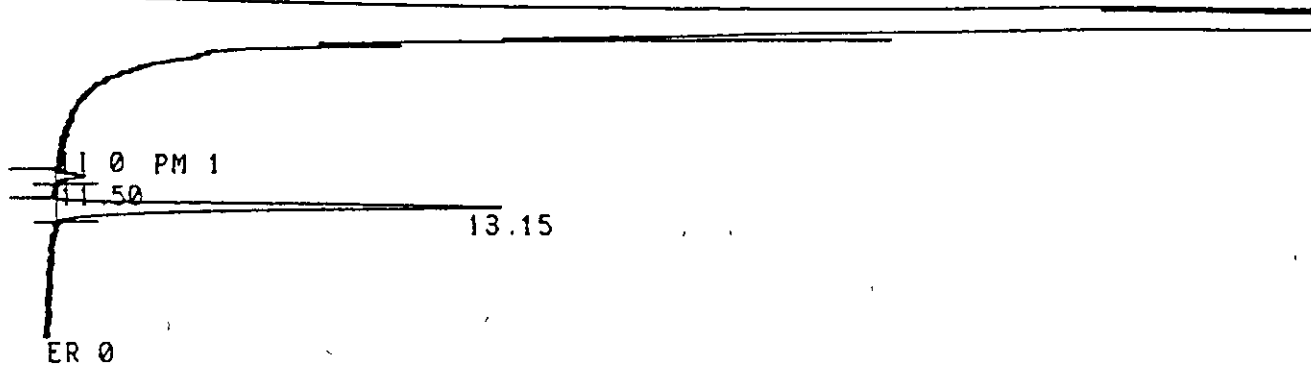
FILE 1 METHOD 5 RUN 8 INDEX 1 BIN 8
Column (Dim-Temp) : CHROMEGABOND MC18, 15 X 4.6 mm, 3 um, #03294-7-98-27421
HPLC Mobile Phase : Methanol.Water.Triethylamine (92.8:0 05)
Column Temp : 35 deg C.
Inj Vol/Flow : 30ul/0.6 ml/min
Attn/PW/PT : 2/25/200 (SP4400 Settings)
EX/EM : 365nm/470nm
Lamp/Resp/Range : 100/1/50 (SpectroVision)
Component-Number : Pump-111, Det-214, AutoInj-311, Integ-421, Col Heat -635
Study : Method Validation
Method No. : M-036
Notebook Ref : Notebook No. 90471

ANALYST. N JOHNSON

SAMPLE 8 100ppb-1 BIN 8 NAME ARUN1589

NAME	CONC	RT	AREA	BC	RF
B1b	0.	11 61	1206	01	
B1a	0.	13.29	30913	01	
TOTALS	0		32119		

CHANNEL A INJECT 05/25/94 20.20.53 STORED TO BIN # 9
11 1



DATA SAVED TO BIN # 9

ABAMECTIN HOPS 05/25/94 20.20 53 CH= "A" PS= 1.

FILE 1. METHOD 5 RUN 9 INDEX 1 BIN 9
 Column (Dim-Temp) CHROMECABOND MC18, 15 X 4 6 mm. 3 um #03294-7-98-27421
 HPLC Mobile Phase Methanol:Water Triethylamine (92:8:0:05)
 Column Temp 35 deg C
 Inj Vol/Flow 30ul/0.6 ml/min
 Att/PW/PT 2/25/200 (SP4400 Settings)
 EX/EM 365nm/470nm
 Lamp/Resp/Range 100/1/50 (SpectroVision)
 Component-Number Pump-111, Det-214, AutoInj-311, Integ-421, Col Heat -635
 Study Method Validation
 Method No H-036
 Notebook Ref Notebook No 90471

ANALYST N JOHNSON

SAMPLE 9 100ppb-2 BIN 9 NAME ARUN1590

NAME	CONC	RT	AREA BC	RF
B1b	0.	11.5	1293 01	
B1a	0	13.15	30097 01	
TOTALS	0.		31390	

CHANNEL A INJECT 05/25/94 20:41:47 STORED TO BIN # 10
11 1

11 0 PM 1
11 55

13.19

ER 0

DATA SAVED TO BIN # 10

ABAMECTIN HOPS 05/25/94 20.41:47 CH= "A" PS= 1.

FILE 1. METHOD 5. RUN 10 INDEX 1 BIN 10
Column (Dim-Temp) : CHROMEGABOND MC18, 15 X 4 6 mm, 3 um, #03294-7-98-27421
HPLC Mobile Phase : Methanol:Water:Triethylamine (92 8:0.05)
Column Temp : 35 deg C.
Inj Vol/Flow : 30ul/0.6 ml/min
Attn/PW/PT : 2/25/200 (SP4400 Settings)
EX/EM : 365nm/470nm
Lamp/Resp/Range : 100/1/50 (SpectroVision)
Component-Number : Pump-111, Det-214, AutoInj-311, Integ-421, Col.Heat.-635
Study : Method Validation
Method No : M-036
Notebook Ref : Notebook No. 90471

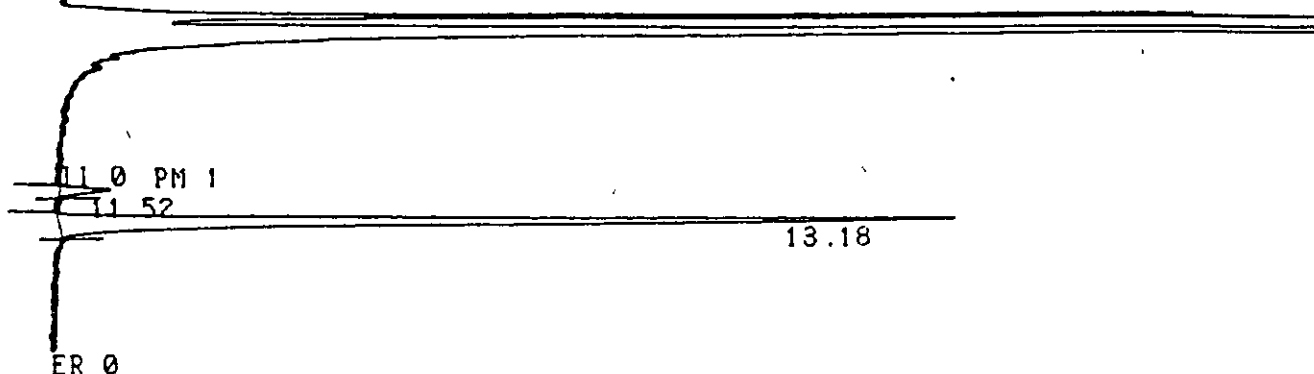
ANALYST: N JOHNSON

SAMPLE 10 1000ppb-1 BIN 10 NAME ARUN1591

NAME	CONC	RT	AREA BC	RF
B1b	0.	11.55	2478 01	
B1a	0.	13.19	63202 01	
TOTALS	0.		65680	

CHANNEL A INJECT 05/25/94 21 02 40 STORED TO BIN # 11

11 1



DATA SAVED TO BIN # 11

ABAMECTIN HOPS 05/25/94 21:02:40 CH= "A" PS= 1

FILE 1 METHOD 5. RUN 11 INDEX 1 BIN 11
 Column (Dim-Temp) . CHROMEGABOND MC18, 15 X 4 6 mm, 3 um. #03294-7-98-27421
 HPLC Mobile Phase . Methanol Water.Triethylamine (92 8 0 05)
 Column Temp. 35 deg C
 Inj Vol/Flow 30ul/0.6 ml/min
 Attn/PW/PT 2/25/200 (SP4400 Settings)
 EX/EM 365nm/470nm
 Lamp/Resp/Range 100/1/50 (SpectroVision)
 Component-Number . Pump-111.Det-214.AutoInj-311,Integ-421, Col Heat -635
 Study Method Validation
 Method No . H-036
 Notebook Ref . Notebook No 90471

ANALYST N JOHNSON

SAMPLE 11 1000ppb-2 BIN 11 NAME ARUN1592

NAME	CONC	RT	AREA BC	RF
B1b	0	11.52	2643 01	
B1a	0.	13.18	61181 01	
TOTALS	0.		63824	

CHANNEL A. INJECT 05/25/94 21:23:32 STORED TO BIN # 12
11 1

11 0 PM 1

ER 0
DATA SAVED TO BIN # 12

ABAMECTIN HOPS 05/25/94 21:23 32 CH= "A" PS= 1

FILE 1 METHOD 5 RUN 12 INDEX 1 BIN 12
Column (Dim-Temp) CHROMEGABOND MC18. 15 X 4 6 mm, 3 um, #03294-7-98-27421
HPLC Mobile Phase : Methanol:Water:Triethylamine (92 8 0.05)
Column Temp : 35 deg C.
Inj Vol/Flow : 30ul/0.6 ml/min
Attn/PW/PT : 2/25/200 (SP4400 Settings)
EX/EH : 365nm/470nm
Lamp/Resp/Range : 100/1/50 (SpectroVision)
Component-Number : Pump-111, Det-214 AutoInj-311, Integ-421, Col.Heat -635
Study : Method Validation
Method No : M-036
Notebook Ref : Notebook No. 90471

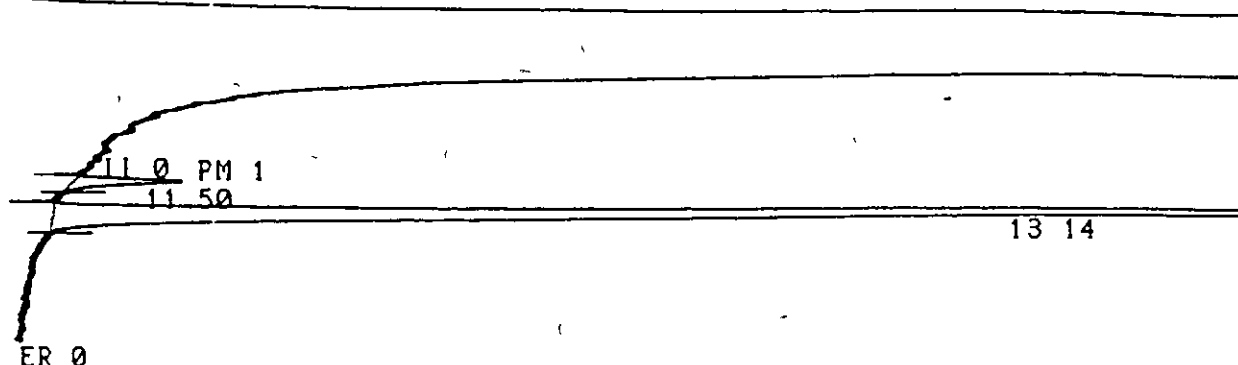
ANALYST. N JOHNSON

SAMPLE 12 CONTROL BIN 12 NAME ARUN1593

NAME CONC RT AREA BC RF

TOTALS 0.

CHANNEL A INJECT 05/25/94 21:44:25 STORED TO BIN # 13
11 1



DATA SAVED TO BIN # 13

ABAMECTIN HOPS 05/25/94 21:44:25 - CH= "A" PS= 1.

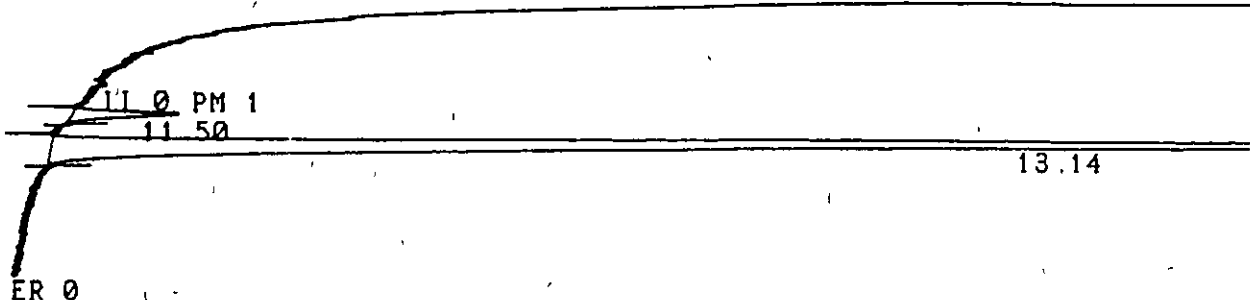
FILE 1 METHOD 5. RUN 13 INDEX 1 BIN 13
 Column (Dim-Temp) CHROMEGABOND MC18, 15 X 4.6 mm, 3 um. #03294-7-98-27421
 HPLC Mobile Phase Methanol:Water:Triethylamine (92.8:0.05)
 Column Temp 35 deg C
 Inj Vol/Flow 30ul/0.6 ml/min
 Attn/PW/PT 2/25/200 (SP4400 Settings)
 EX/EM 365nm/470nm
 Lamp/Resp/Range 100/1/50 (SpectroVision)
 Component-Number Pump-111, Det-214, AutoInj-311, Integ-421. Col Heat -635
 Study Method Validation
 Method No M-036
 Notebook Ref Notebook No. 90471

ANALYST. N JOHNSON

SAMPLE 13 100-1A BIN 13 NAME ARUN1594

NAME	CONC	RT	AREA BC	RF
B1b	0.	11.5	6182 01	
B1a	0	13.14	144414 01	
TOTALS	0.		150596	

CHANNEL A INJECT 05/25/94 22:05:19 STORED TO BIN # 14
11 1



DATA SAVED TO BIN # 14

ABAMECTIN HOPS 05/25/94 22 05.19 CH= "A" PS= 1.

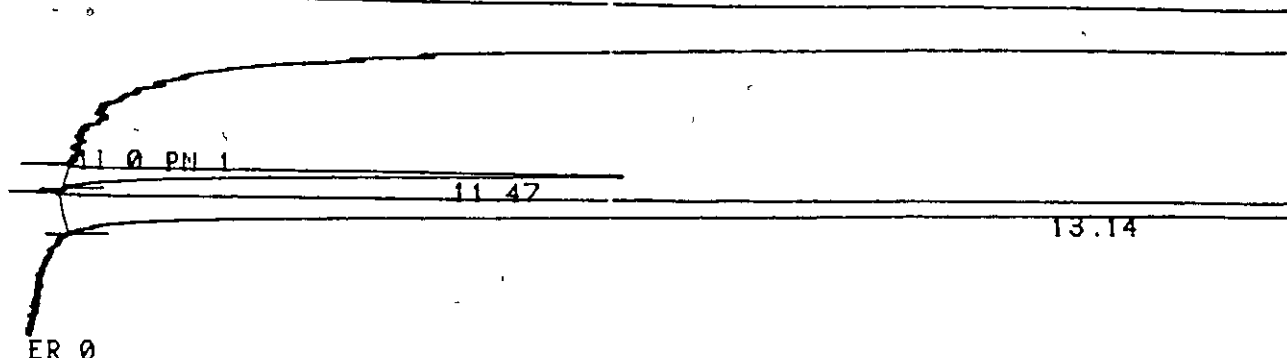
FILE 1 METHOD 5 RUN 14 INDEX 1 BIN 14
Column (Dim-Temp) : CHROMEGABOND MC18, 15 X 4 6 mm, 3 um, #03294-7-98-27421
HPLC Mobile Phase : Methanol:Water:Triethylamine (92:8:0.05)
Column Temp : 35 deg C.
Inj Vol/Flow : 30ul/0.6 ml/min
Attn/PW/PT : 2/25/200 (SP4400 Settings)
EX/EM : 365nm/470nm
Lamp/Resp/Range : 100/1/50 (SpectroVision)
Component-Number : Pump-111, Det-214, AutoInj-311 Integ-421, Col Heat -635
Study : Method Validation
Method No. : M-036
Notebook Ref : Notebook No. 90471

ANALYST N JOHNSON

SAMPLE 14 100-1B BIN 14 NAME ARUN1595

NAME	CONC	RT	AREA	BC	RF
B1b	0.	11.5	6256	01	
B1a	0.	13.14	143603	01	
TOTALS	0.		149859		

CHANNEL A INJECT 05/25/94 22:26:12 STORED TO BIN # 15
11 1



DATA SAVED TO BIN # 15

ABAMECTIN HOPS 05/25/94 22:26:12 CH= "A" PS= 1.

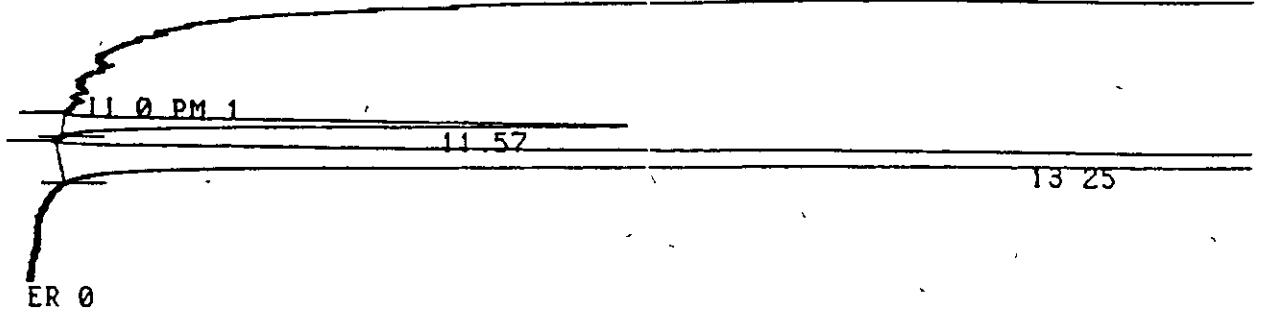
FILE 1 METHOD 5. RUN 15 INDEX 1 BIN 15
 Column (Dim-Temp) CHROMEGABOND MC18, 15 X 4 6 mm. 3 um, #03294-7-98-27421
 HPLC Mobile Phase Methanol Water.Trietnylamine (92.8:0.05)
 Column Temp. 35 deg C.
 Inj Vol/Flow 30ul/0.6 ml/min
 Attn/PW/PT 2/25/200 (SP4400, Settings)
 EX/EM 365nm/470nm
 Lamp/Resp./Range 100/1/50 (SpectroVision)
 Component-Number Pump-111, Det-214, AutoInj-311, Integ-421, Col Heat -635
 Study Method Validation
 Method No M-036
 Notebook Ref Notebook No 90471

ANALYST N JOHNSON

SAMPLE 15 1000-1A BIN 15 NAME ARUN1596

NAME	CONC	RT	AREA BC	RF
B1b	0.	11.47	34491.01	
B1a	0.	13.14	620095.01	
TOTALS	0.		654586	

CHANNEL A INJECT 05/25/94 22:47:05 STORED TO BIN # 16
11 1



DATA SAVED TO BIN # 16

ABAMECTIN HOPS 05/25/94 22:47 05 CH= "A" PS= 1.

FILE 1 METHOD 5. RUN 16 INDEX 1 BIN 16
Column (Dim-Temp) : CHROMEGABOND MC18, 15 X 4.6 mm, 3 um, #03294-7-98-27421
HPLC Mobile Phase : Methanol:Water:Triethylamine (92:8:0:05)
Column Temp : 35 deg C.
Inj Vol/Flow : 30ul/0.6 ml/min
Attn/PW/PT : 2/25/200 (SP4400 Settings)
EX/EN : 365nm/470nm
Lamp/Resp/Range : 100/1/50 (SpectroVision)
Component-Number : Pump-111, Det-214, AutoInj-311, Integ-421, Col.Heat -635
Study : Method Validation
Method No : M-036
Notebook Ref. : Notebook No. 90471

ANALYST. N JOHNSON

SAMPLE 16 1000-1B BIN 16 NAME ARUN1597

NAME	CONC	RT	AREA	BC	RF
B1b	0.	11.57	34483	01	
B1a	0.	13.25	614764	01	
TOTALS	0.		649247		

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MK-936/HOPS/171-4

REPRESENTATIVE CHROMATOGRAMS

Method Validation Data for 8,9-Z Avermectin B1a in Dried Hops

M-036 Liquid Chromatographic Method for the Quantitation of Total Avermectin B1 and 8,9-Z-Avermectin B1 in Dried Hops Using Fluorescence Detection

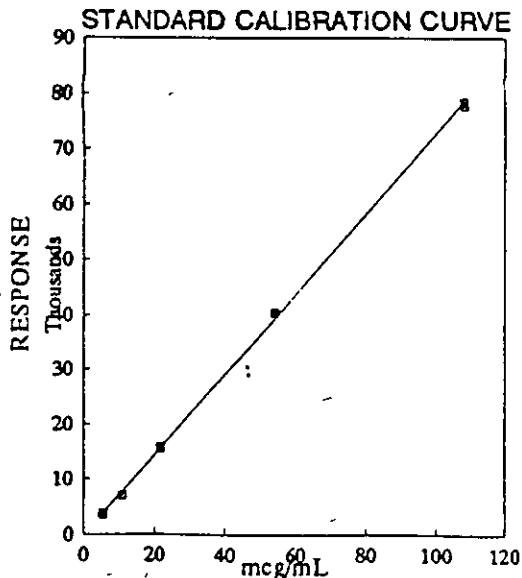
STANDARD REGRESSION DATA - 8,9-Z AVERMECTIN B1a HOPS

TYPE OF STUDY: Method Validation
 TEST MATRIX: Dry Hops
 TEST SUBSTANCE: 8,9-Z avermectin B1a (L-652,280)

ASSAY DATE: 6/1/94
 STD PREP DATE: 4/7/94
 STOCK PREP DATE: 4/7/94
 REF NOTEBOOK 90471 p 1-3

ANAL METHOD #: M-036
 TYPE OF EXPERIMENT: 8,9-Z avermectin B1a Analysis in Dry Hops
 ANAL STD LOT NO: L-676,863-038A003 (avermectin B1)
 ANAL STD PURITY: 0.893 w/w% B1a, 0.044 w/w% B1b

SAMPLE ID	BIN #	B1a Area		STD B1a CONC (ng/mL)		COMMENTS SECTION
		ACTUAL	CALC	ACTUAL	CALC	
STD A	63	3484	3764	5.40	5.02	
STD A	75	3796	3764	5.40	5.44	
STD B	64	7066	7708	10.8	9.92	
STD B	76	6998	7708	10.8	9.83	
STD C	65	15607	15596	21.6	21.6	
STD C	77	15854	15596	21.6	22.0	
STD D	66	40581	39261	54.0	55.8	
STD D	78	40415	39261	54.0	55.6	
STD E	67	78426	78702	108	108	
STD E	79	77835	78702	108	107	



Regression Output

Constant	-180.3
Std Err of Y Est	787.78
R Squared	0.9994
No. of Observations	10.00
Degrees of Freedom	8.00
X Coefficient(s)	730.39
Std Err of Coef	6.56

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MK-936/HOPS/171-4

SAMPLE ANALYSIS DATA - 8,9-Z AVERMECTIN B1a HOPS

TYPE OF STUDY:
TEST MATRIX:
TEST SUBSTANCE:

Method Validation
Dry Hops
8,9-Z avermectin B1a (L-652,280)

ANAL. METHOD #
TYPE OF EXPERIMENT:
ANALYTICAL STD LOT NO:
ANALYTICAL STD PURITY:

M-036
8,9-Z avermectin B1a Analysis in Dry Hops
L-676,863-038A003 (avermectin B1)
0.893 w/w% B1a, 0.044 w/w% B1b

ASSAY DATE: June 1, 1994

SAMPLE DESCRIPTION	SAMPLE ID	GRAMS SAMPLE	BIN #	B1b	PEAK AREA B1a	DILUTION FACTOR	ng/mL B1a FOUND	LABEL ppb	ppb B1a SAMPLE	8,9-Z B1a % RECOVERY	AVG
4.99 ppb 8,9-Z B1a Fortification	WT A	5.00	68		6484	1.00	9.12	4.99	4.56	91.4%	93.4%
4.99 ppb 8,9-Z B1a Fortification	WT B	5.00	69		6773	1.00	9.52	4.99	4.76	95.4%	
99.7 ppb 8,9-Z B1a Fortification	WT A	5.00	70		25605	0.200	35.3	99.7	88.3	88.6%	84.4%
99.7 ppb 8,9-Z B1a Fortification	WT B	5.00	71		23210	0.200	32.0	99.7	80.0	80.2%	
997 ppb 8,9-Z B1a Fortification	WT A	5.00	72		42234	0.040	58.1	997	726	72.8%	76.6%
997 ppb 8,9-Z B1a Fortification	WT B	5.00	73		46661	0.040	64.1	997	801	80.3%	
Control	WT A	5.00	74		Not Detected	1.00					

FILE C:\123\936hops\6-1-94

MK-936/HOPS/171-4

CHANNEL A INJECT 06/01/94 11:24:40. STORED TO BIN # 63
II 1

11 0 PM 1

13.43

ER 0
DATA SAVED TO BIN # 63

8,9-Z DRY HOPS 06/01/94 11 24 40 CH= "A" PS= 1

FILE 1. METHOD 5. RUN 1 INDEX 1 BIN 63
Column (Dim-Temp) . CHROMEGABOND MC18 15 X 4 6 mm, 3 um, #03294-7-98-27421
HPLC Mobile Phase . Methanol Water Triethylamine (92 8.0 05)
Column Temp. . 35 deg C
Inj Vol/Flow . 30ul/0.6 ml/min
Attn/PW/PT . 2/25/200 (SP4400 Settings)
EX/EM . 365nm/470nm
Lamp/Resp/Range . 100/1/50 (SpectroVision)
Component-Number . Pump-111, Det-214 AutoInj-311, Integ-421, Col Heat -c35
Study . Method Validation
Method No . M-036
Notebook Ref . Notebook No 90471

ANALYST N JOHNSON

SAMPLE 1 STD A BIN 63 NAME ARUN1642

NAME	CONC	RT	AREA	BC	RF
B1a	3484	13.43	3484	01	1
TOTALS	3484.		3484		

06/01/94 11:45:33 STORED TO BIN # 64



06/01/94 11 45:33 CH= "A" PS= 1

8. 12- 4861

5 RUN 2 INDEX 1 BIN 64

FILE: CHROMECABOND MC18 15 X 4.6 mm, 3 um. #03294-7-98-27421

Column: Methanol Water Triethylamine (92:8 0.05)

HPLC: 35 deg C

Column: 10ul/0.6 ml/min

Inj: 2/25/200 (SP4400 Settings)

Attr: 365nm/470nm

X/EH: 100/1/50 (Spectrovision)

Lamp: Pump-111, Det-214, AutoInj-311, Integ-421, Col Heat -635

Component: Method Validation

Study: M-036

Method No: Notebook No 90471

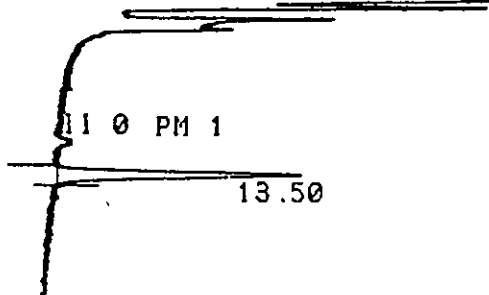
Notebook Ref.

ANALYST: B. ARUN

SAMPLE 2 SUB B BIN 64 NAME ARUN1643

NAME	CONC	RT	AREA BC	RF
B1a	7066.	13.54	7066 01	1.
TOTALS	7066.		7066	

CHANNEL A INJECT 06/01/94 12:06:26 STORED TO BIN # 65
11 1



ER 0
DATA SAVED TO BIN # 65

8,9-Z DRY HOPS 06/01/94 12 06 26 CH= "A" PS= 1

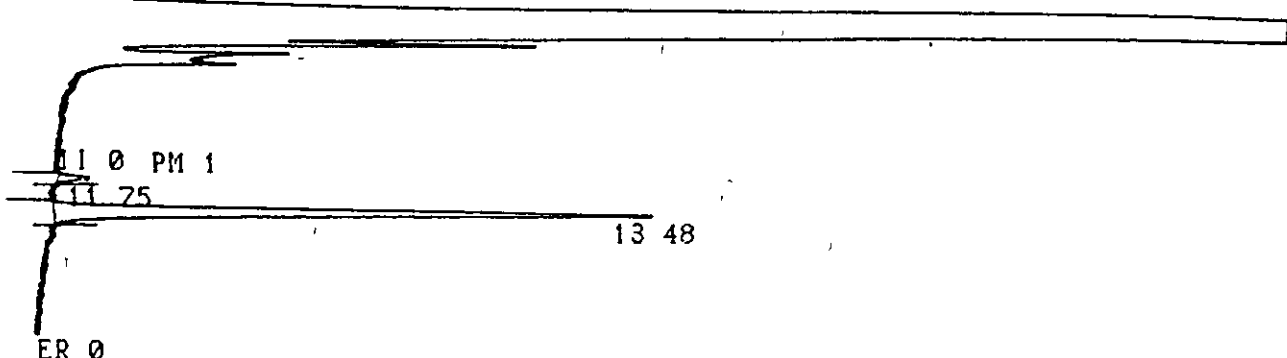
FILE 1 METHOD 5. RUN 3 INDEX 1 BIN 65
Column (Dim-Temp) . CHROMEGABOND MC18, 15 X 4 6 mm. 3 um, #03294-7-98-27421
HPLC Mobile Phase Methanol:Water:Triethylamine (92 8.0 05)
Column Temp. 35 deg C.
Inj Vol/Flow 30ul/0.6 ml/min
Affn/PW/PT . 2/25/200 (SP4400 Settings)
EX/EM . 365nm/470nm
Lamp/Resp/Pange 100/1/50 (SpectroVision)
Component-Number . Pump-111, Det-214, AutoInj-311, Integ-421, Col Heat -c35
Study Method Validation
Method No . H-036
Notebook Ref : Notebook No 90471

ANALYST N JOHNSON

SAMPLE 3 STD C BIN 65 NAME ARUN1644

NAME	CONC	RT	AREA BC	RF
B1a	15607	13.5	15607 01	1
TOTALS	15607.		15607	

CHANNEL A INJECT 06/01/94 12 27 20 STORED TO BIN # 66
11 1



DATA SAVED TO BIN # 66

8.9-Z DRY HOPS 06/01/94 12 27.20 CH= "A" PS= 1

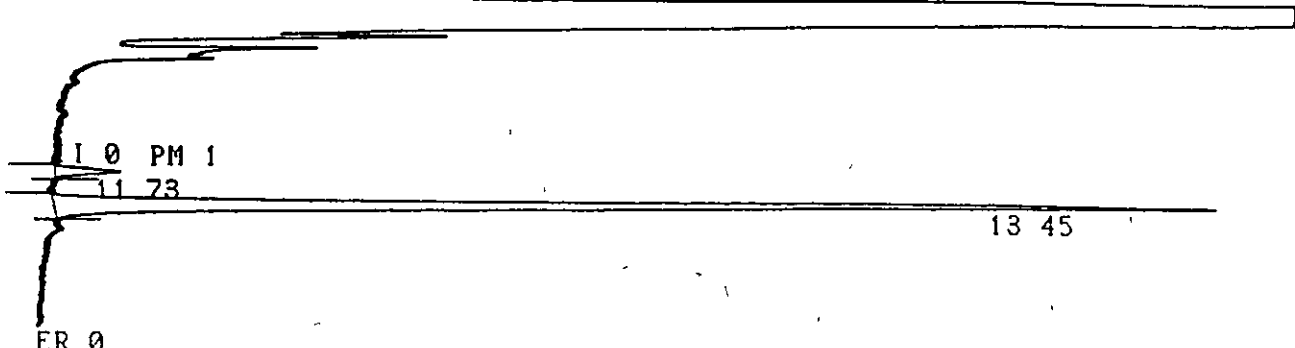
FILE 1. METHOD 5. RUN 4 INDEX 1 BIN 66
 Column (Dim-Temp) CHROMEGABOND MC18, 15 X 4.6 mm, 3 um. #03294-7-98-27421
 HPLC Mobile Phase Methanol:Water Triethylamine (92:8.0 05)
 Column Temp 35 deg C
 Inj Vol/Flow 30ul/0.6 ml/min
 Attn/PW/PT 2/25/200 (SP4400 Settings)
 EX/EM 365nm/470nm
 Lamp/Resp/Range 100/1/50 (SpectroVision)
 Component-Number Pump-111, Det-214, AutoInj-311, Integ-421, Col Heat -635
 Study Method Validation
 Method No M-036
 Notebook Ref Notebook No 90471

ANALYST. N JOHNSON

SAMPLE 4 STD D BIN 66 NAME ARUN1645

NAME	CONC	RT	AREA	BC	RF
1	0.	11.75	1497	01	
B1a	40581.	13.48	40581	01	1.
TOTALS	40581.		42078		

CHANNEL A INJECT 06/01/94 12:48:13 STORED TO BIN # 67
11 1



DATA SAVED TO BIN # 67

8,9-Z DRY HOPS 06/01/94 12 48.13 CH= "A" PS= 1

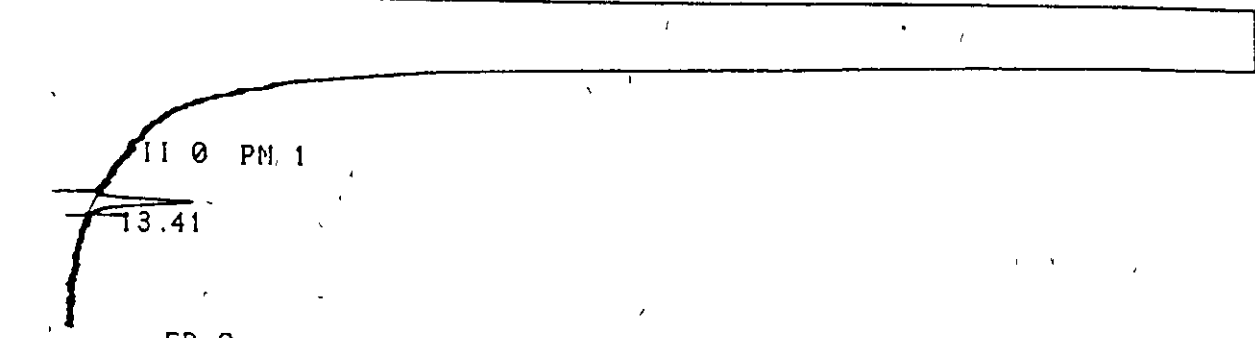
FILE 1 METHOD 5 RUN 5 INDEX 1 BIN 67
Column (Dim-Temp) CHROMEGABOND HC18. 15 X 4 6 mm, 3 um. #03294-7-98-27401
HPLC Mobile Phase Methanol Water Triethylamine (92 8.0 05)
Column Temp 35 deg C.
Inj Vol/Flow 30ul/0.6 ml/min
Attn/PW/PT 2/25/200 (SP4400 Settings)
EX/EM 365nm/470nm
Lamp/Resp/Range 100/1/50 (SpectroVision)
Component-Number Pump-111. Det-214. AutoInj-311 Integ-421. Col Heat -635
Study Method Validation
Method No M-036
Notebook Ref Notebook No 90471

ANALYST N JOHNSON

SAMPLE 5 STD E BIN 67 NAME ARUN1646

NAME	CONC	RT	AREA BC	RF
1	0.	11.73	3587 01	
Bla	78426.	13.45	78426 01	1.
TOTALS	78426.		82013	

CHANNEL A INJECT 06/01/94 13 09.07 STORED TO BIN # 68
11 1



ER 0
DATA SAVED TO BIN # 68

8.9-Z DRY HOPS 06/01/94 13 09:07 CH= "A" PS= 1.

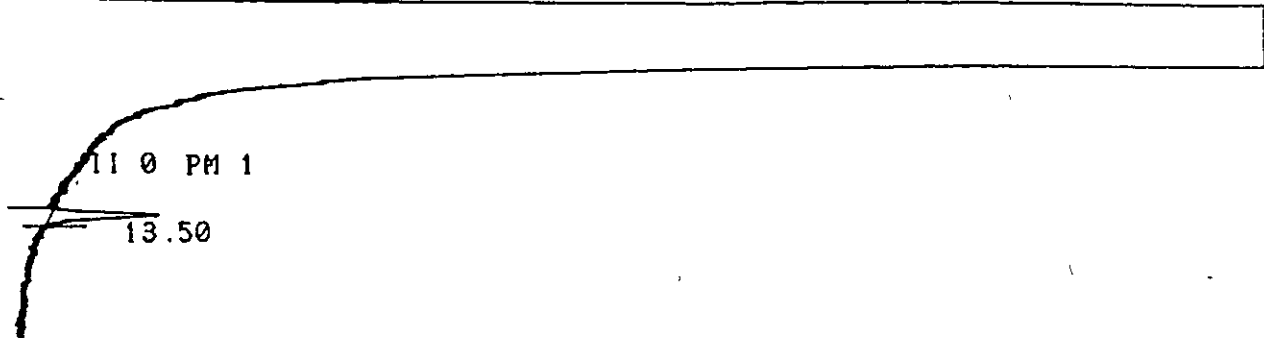
FILE 1 METHOD 5. RUN 6 INDEX 1 BIN 68
 Column (Dim-Temp) CHROMEGABOND NC18, 15 X 4.6 mm, 3 um, #03294-7-98-27421
 HPLC Mobile Phase Methanol Water Triethylamine (92 8.0.05)
 Column Temp 35 deg C
 Inj Vol/Flow 30ul/0.6 ml/min
 Attn/PW/PT 2/25/200 (SP4400 Settings)
 EX/EM 365nm/470nm
 Lamp/Resp/Range 100/1/50 (SpectroVision)
 Component-Number Pump-111, Det-214, AutoInj-311, Integ-421, Col Heat -635
 Study Method Validation
 Method No M-036
 Notebook Ref Notebook No 90471

ANALYST N JOHNSON

SAMPLE 6 5ppb-1 BIN 68 NAME ARUN1647

NAME	CONC	RT	AREA BC	RF
B1a	6484.	13.41	6484 01	1.
TOTALS	6484.		6484	

CHANNEL A INJECT 06/01/94 13.30:00 STORED TO BIN # 69
11 1



ER 0
DATA SAVED TO BIN # 69

8,9-Z DRY HOPS 06/01/94 13 30 00 CH= "A" PS= 1

FILE 1 METHOD 5. RUN 7 INDEX 1 BIN 69
Column (Dim-Temp) . CHROMECABOND MC18 15 X 4 6 mm 3 um, #03294-7-98-27421
HPLC Mobile Phase . Methanol:Water Triethylamine (92 8 0 05)
Column Temp : 35 deg C.
Inj Vol/Flow : 30ul/0.6 ml/min
Attn/PW/PT 2/25/200 (SP4400 Settings)
EX/EM . 365nm/470nm
Lamp/Resp/Range . 100/1/50 (SpectroVision)
Component-Number Pump-111, Det-214 AutoInj-311 Integ-421. Col Heat -635
Study Method Validation
Method No . M-036
Notebook Ref . Notebook No 90471

ANALYST N. JOHNSON

SAMPLE 7 5ppb-2 BIN 69 NAME ARUN1648

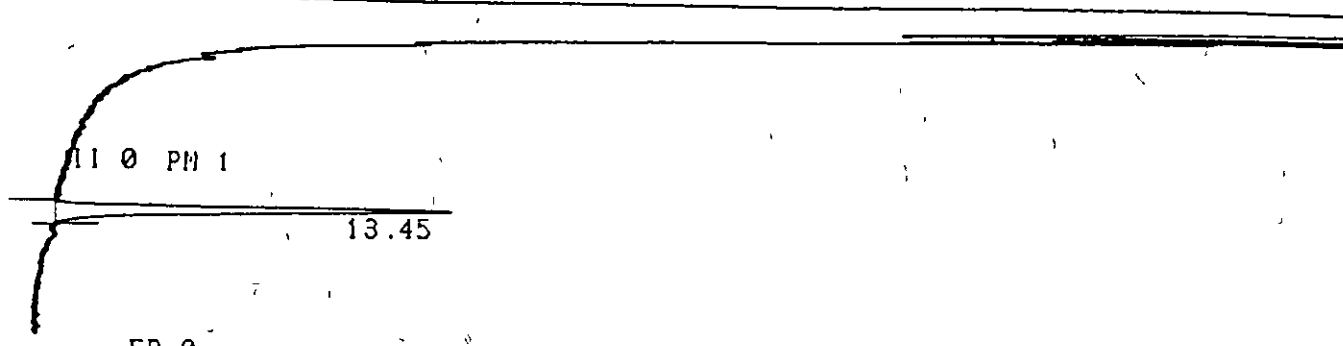
NAME	CONC	RT	AREA	BC	RF
B1a	6773.	13.5	6773	01	1
TOTALS	6773.		6773		

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CHANNEL A INJECT 06/01/94 13.50.53 STORED TO BIN # 70

11 1



ER 0

DATA SAVED TO BIN # 70

8,9-Z DRY HOPS

06/01/94 13.50.53

CH= "A" PS= 1

FILE 1 METHOD 5 RUN 8 INDEX 1 BIN 70
 Column (Dim-Temp) CHROMEGABOND MC18. 15 X 4 6 mm. 3 um, #03294-7-98-27421
 HPLC Mobile Phase Methanol:Water:Triethylamine (92 8.0 05)
 Column Temp 35 deg C.
 Inj Vol/Flow 30ul/0.6 ml/min
 Attn/PW/PT 2/25/200 (SP4400 Settings)
 EX/EM 365nm/470nm
 Lamp/Resp/Range 100/1/50 (SpectroVision)
 Component-Number Pump-111, Det-214 AutoInj-311, Integ-421, Col.Heat -635
 Study Method Validation
 Method No H-036
 Notebook Ref. Notebook No. 90471

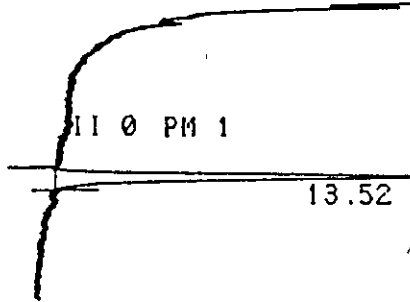
ANALYST, N JOHNSON

SAMPLE 8 100ppb-1 BIN 70 NAME ARUN1649

NAME	CONC	RT	AREA	BC	RF
B1a	25605.	13.45	25605	01	1
TOTALS	25605.		25605		

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CHANNEL A INJECT 06/01/94 14:11:46 STORED TO BIN # 71
11 1



ER 0
DATA SAVED TO BIN # 71

8,9-Z DRY HOPS 06/01/94 14:11:46 CH= "A" PS= 1.

FILE 1 METHOD 5 RUN 9 INDEX 1 BIN 71
Column (Dim-Temp) CHROMECABOND MC18 15 X 4 6 mm. 3 um. #03294-7-98-27421
HPLC Mobile Phase Methanol Water Triethylamine (92 8 0 05)
Column Temp 35 deg C.
Inj Vol/Flow 30ul/0 6 ml/min
Attr/PW/PT 2/25/200 (SP4400 Settings)
EX/EH 365nm/470nm
Lamp/Resp/Range 100/1/50 (SpectroVision)
Component-Number Pump-111, Det-214, AutoInj-311, Integ-421, Col Heat -635
Study Method Validation
Method No H-036
Notebook Ref Notebook No 90471

ANALYST: N JOHNSON

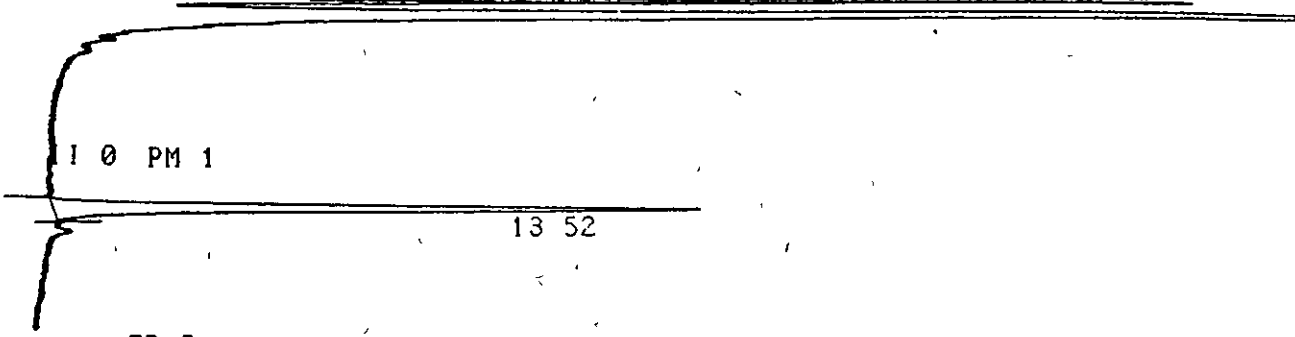
SAMPLE 9 100ppb-2 BIN 71 NAME ARUN1650

NAME	CONC	RT	AREA BC	RF
B1a	23210	13.52	23210 01	1
TOTALS	23210		23210	

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CHANNEL A INJECT 06/01/94 14.32 39 STORED TO BIN # 72
11 1



ER 0
DATA SAVED TO BIN # 72

8,9-Z DRY HOPS 06/01/94 14 32 39 CH= "A" PS= 1

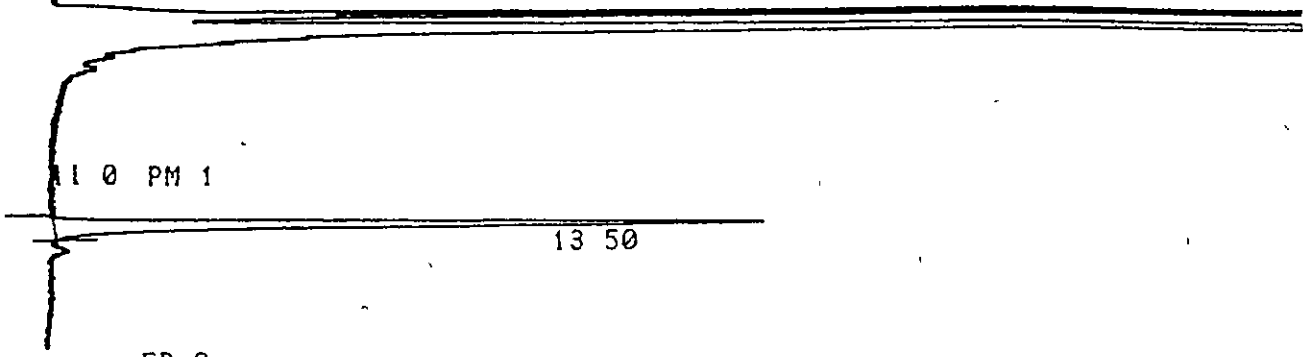
FILE 1 METHOD 5. RUN 10 INDEX 1 BIN 72
 Column (Dim-Temp) CHROMEGABOND MC18, 15 X 4 6 mm, 3 um, #03274-7-98-27421
 HPLC Mobile Phase Methanol Water Triethylamine (92 8 0.05)
 Column Temp : 35 deg C.
 Inj Vol/Flow : 30ul/0.6 ml/min
 Attn/PW/PT : 2/25/200 (SP4400 Settings)
 EX/EM : 365nm/470nm
 Lamp/Resp/Range : 100/1/50 (SpectroVision)
 Component-Number Pump-111, Det-214 AutoInj-311, Integ-421, Col Heat -635
 Study Method Validation
 Method No M-036
 Notebook Ref Notebook No. 90471

ANALYST N JOHNSON

SAMPLE 10 1000ppb-1 BIN 72 NAME ARUN1651

NAME	CONC	RT	AREA BC	RF
B1a	42234.	13.52	42234 01	1.
TOTALS	42234		42234	

CHANNEL A INJECT 06/01/94 14:53:32 STORED TO BIN # 73
11 1



ER 0
DATA SAVED TO BIN # 73

8,9-Z DRY HOPS 06/01/94 14 53 32 CH= "A" PS= 1

FILE 1. METHOD 5 RUN 11 INDEX 1 BIN 73
Column (Dim-Temp) CHROMEGABOND MC18, 15 X 4 6 mm, 3 um, #03294-7-98-27421
HPLC Mobile Phase Methanol Water Triethylamine (92:8:0.05)
Column Temp 35 deg C
Inj Vol/Flow 30ul/0.6 ml/min
Attn/PW/PT 2/25/200 (SP4400 Settings)
EX/EH 365nm/470nm
Lamp/Resp/Range 100/1/50 (SpectroVision)
Component-Number Pump-111, Det-214 AutoInj-311, Integ-421, Col.Heat -635
Study Method Validation
Method No M-036
Notebook Ref Notebook No. 90471

ANALYST N JOHNSON

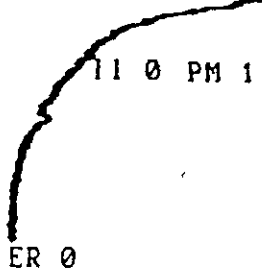
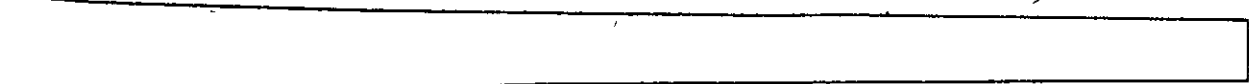
SAMPLE 11 1000ppb-2 BIN 73 NAME ARUN1652

NAME	CONC	RT	AREA BC	RF
B1a	46661.	13 5	46661 01	1.
TOTALS	46661.		46661	

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CHANNEL A INJECT 06/01/94 15:14:26 STORED TO BIN # 74
11 1



DATA SAVED TO BIN # 74

8.9-Z DRY HOPS 06/01/94 15:14:26 CH= "A" PS= 1

FILE 1 METHOD 5. RUN 12 INDEX 1 BIN 74
 Column (Dim-Temp) CHROMEGABOND MC18, 15 X 4.6 mm, 3 um, #03294-7-98-27421
 HPLC Mobile Phase Methanol:Water Triethylamine (92 8 0 05)
 Column Temp 35 deg C
 Inj Vol/Flow 30ul/0.6 ml/min
 Attn/PW/PT 2/25/200 (SP4400 Settings)
 EX/EM 365nm/470nm
 Lamp/Resp/Range 100/1/50 (SpectroVision)
 Component-Number Pump-111, Det-214, AutoInj-311, Integ-421, Col Heat -635
 Study Method Validation
 Method No M-036
 Notebook Ref Notebook No. 90471

ANALYST N JOHNSON

SAMPLE 12 CONTROL BIN 74 NAME ARUN1653

NAME	CONC	RT	AREA	BC	RF
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TOTALS	0.				
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