10-27-95

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

Analytical Chemistry Section
Building 306, BARC-East
Beltsville, Maryland 20705

OCT 27 1995

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: PP# 4F04407. Sulfentrazone in/on Soybeans, Method Validation Report. (ACL #B95-33)

FROM: Mark W. Law, Chemist
Analytical Chemistry Section

THRU: Harvey K. Hundley, Head
Analytical Chemistry Section

THRU Donald A. Marlow, Chief
Analytical Chemistry Branch

TO: R.B. Perfetti, Acting Section Head
Tolerance Petition Section III
Chemistry Branch I-Tolerance Support
Health Effects Division (7509C)

INTRODUCTION:

The Analytical Chemistry Section was requested to run a method validation for the analysis of the herbicide sulfentrazone (N-[2,4-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]phenyl]methanesulfonamide) and the major metabolite hydroxymethyl sulfentrazone (N-[2,4-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-hydroxymethyl-5-oxo-1h-1,2,4-triazol-1-yl]phenyl]methanesulfonamide) in/on soybeans. The requested fortification levels were 0.025, 0.05, and 0.1 PPM. The validation was requested for method P-2811M, "Residue Analytical Method for the Determination of FMC 97285 and FMC 106091 in/on Soybeans Treated with F6285 4F", 6/29/93, by A. Chen, FMC Co., MRID #429321-09.

METHOD SUMMARY:

Sulfentrazone and its hydroxy metabolite were extracted from 5 g. samples by refluxing with 75/25 Acetone/0.25n HCl. After filtering to remove solids, the acetone was removed by rotary evaporation and the samples were again filtered prior to clean up on a C-8 SPC cartridge. The C-8 cartridge was completely dried with a Nitrogen flow at 30 psi for 30 minutes. The samples were then eluted with 20/80 ethyl acetate/hexane and further cleaned up on a Silica SPE cartridge. The final eluant was evaporated to dryness, taken up in acetonitrile, and derivitized with BSTFA. The final determination is by GLC using a HP-50+ megabore capillary column with detection by electron capture detector..

COMMENTS

1. The analytical standard was not available from the EPA standards repository in RTP. The standards used in this method trial were provided by the petitioner.

2. A set of eight samples can be extracted and cleaned up in one working day, but the final GLC analyses required an overnight instrument sequence of 14 hours..

3. A copy of ACL's method validation pre-review is attached as additional information for this study and should be referenced for additional minor modifications that should be made to the proposed enforcement method.

4. Chromatograms of control samples showed peaks at or very near the retention times for both the parent and metabolite compounds. The largest peak found in a control calculated only to 0.012 ppm for the parent, and less than 0.002 ppm for the metabolite. This indicates that the 0.025 ppm limit of quantitation for this method is appropriate even though the limit of detection is much lower.

5. Low recoveries of the 0.1 ppm sulfentrazone spikes resulted from sticking valves in our equipment causing high flow rates in the final SPE clean-up steps. We found this problem is minimized if only 5" Hg vacuum is used instead of the 15-20" normally used in this procedure.

6. We agree with the ILV that it is very critical for the samples to be completely dried on the C-8 SPE column. The method needs to be modified to specify a 30 psi nitrogen pressure with at least 30 minutes of drying time.

7. The method meets the requirements in the Pesticide Assessment Guidelines, Subdivision O, Section 171-4(b) for all five commodities tested provided the above comments and those provided in the pre-review memo are incorporated.

METHOD: Residue Analytical Method for the Determination of FMC 97285 and FMC 106091 in/on Soybeans Treated with F6285. 6/29/93. By A. Chen. FMC Co. MRID# 429321-09.

| Commodity | Chemical Added | PPM ADDED | PPM FOUND | PERCENT RECOVERY |
|---|---|---|---|---|
| SOYBEANS | Sulfentrazone (FMC 97285) | 0 | N.D.* | |
| | | 0 | N.D. | |
| | | 0.0255 | 0.0269 | 106 |
| | | 0.0255 | 0.0240 | 94.1 |
| | | 0.0510 | 0.0523 | 103 |
| | | 0.0510 | 0.0543 | 106 |
| | | 0.1020 | 0.0713 | 69.9 |
| | | 0.1020 | 0.0655 | 64.2 |
| SOYBEANS | Hydroxymethyl Sulfentrazone (FMC 106091) | 0 | N.D. | |
| | | 0 | N.D. | |
| | | 0.0252 | 0.0264 | 105 |
| | | 0.0252 | 0.0260 | 103 |
| | | 0.0505 | 0.0437 | 86.6 |
| | | 0.0505 | 0.0513 | 102 |
| | | 0.1009 | 0.1081 | 107 |
| | | 0.1009 | 0.0979 | 97.0 |
| *N.D. = Not Detected at sensitivity of 0.015 ppm. | | | | |

Modifications to method (major or minor):

None

Special precautions to be taken:

None

Source of analytical reference standards:

Standards were not available from RTP, and were provided by petitioner.

If derivatized standards used, give source:

None--Standards are derivitized along with samples.

Instrument for Confirmation:

None

If instrument parameters differ from method as written, list parameters actually used:

Commercial source for any special chemicals or apparatus:

Sources for all equipment and reagents given in method.

Comments:

Attached

Chromatograms:

Representative chromatograms attached.

TMV Pre-review of Sulfentrazone
in Soybeans

Reviewed by: Everett S. Greer, Jr. ESG

Date: 3-21-95

Laboratory assignment number: B95-33

Analytes: Sulfentrazone (FMC 97285), Hydroxymethyl sulfentrazone (FMC 106091)

Commodities: Soybeans

Petitioner: FMC Corporation

Method: Residue Analytical Method for the Determination of FMC 97285 and FMC 106091 in/on Soybeans Treated with F6285 4F

IV. Materials and study design

E. Equipment

The specifications (length, film thickness, diameter, etc.) for the GC column should be given rather than simply stating a reference to a "HP-50+ (Hewlett Packard)" column. In the method summary, the column is referred to as a Megabore® with a 50/50 phenyl silicon coating. A complete description of the column is given in Appendix A.

V. Analytical procedure

A. Residue method

1. Acetone/0.025N HCl reflux

After each of the two filtering steps in this section, the flasks are rinsed with additional solvent, but no directions are given for filtering these additional rinses.

A Turbo Vap concentrator is used to evaporate the first filtrate. As ACL does not have this type of apparatus, a rotary evaporator will have to be substituted for this step and subsequent steps that require this equipment.

3. SI (silica gel) cleanup cartridge

A statement should be made as to whether the silica cartridge is allowed to go dry between additions of eluents. The temperature of the N-Evap is not given.

5

4. Derivatization

The temperature of the N-Evap is not given in this step.

E. Confirmatory techniques

The procedure uses a mass selective detector to confirm residues of the TMS derivative of the hydroxymethyl metabolite (FMC 106091). Only one ion is monitored and according to the method, this ion results from the loss of methyl from the TMS molecular ion. Most GC/MS procedures monitor at least three ions for positive confirmation.

H. Modifications and potential problems

6.

This section states that interference peaks could be observed when the soybeans were from different locations. It is stated that "switching the derivatization and the silica gel cleanup steps was found to be an effective means to eliminate such interferences." This would require an analyst to rerun an entire set of samples if these interfering peaks were noted. Such a practice should be precluded in a tolerance enforcement procedure. The method should be able to analyze for residues of the compounds in question regardless of the origin of the commodity without resorting to an alternate procedure. The modified procedure is described in Appendix B.

Additional reviewer's comments

1. The method states that the standards are derivatized at the time of the analysis, but no specific instructions are given for preparing the standards for derivatization. The parent compound is not affected by the TMS reagent, but the hydroxymethyl is converted to a trimethylsilyl derivative.

2. Recovery data is included with the method for soybeans spiked at 0.025 ppm. The recoveries range between 67% and 113% for the GC/ECD analysis. Recovery data for the GC/MSD determination of the hydroxymethyl metabolite range between 99& and 132% and include only three analyses.

3. Two sets of chromatograms are given for controls and 0.025 ppm spikes. The two analytes are separated by approximately 7 minutes, but the standards show some peak tailing for both compounds. The spiked sample chromatograms have small peaks appearing as shoulders on the trailing edge of the analyte peaks for both parent compound and its metabolite.

4. An independent laboratory report is included with

recovery data for soybeans spiked at 0.025 ppm and 0.125 ppm with recoveries ranging between 93.8% and 129.4%. The ILV laboratory had a problem drying the C8 cartridges in the first set of samples. The method states that the cartridge is to be dried for approximately 30 min. prior to the addition of the ethyl acetate/hexane elution solvent, but no back pressure is given. The validation laboratory used 5 psi for approximately 45 min., but the method failed. After consultation with the sponsor, it was determined that the back pressure should be 30 psi. for 30 min. This back pressure should be stated in the method.

The ILV chromatograms appear to be considerably sharper with less tailing than the sponsors's chromatograms. The ILV used slightly different GC parameters than the sponsor.

ANALYTICAL CHEMISTRY BRANCH
SCREEN FOR RESIDUE METHODS FOR TMV

1. LABORATORY ASSIGNMENT NUMBER: B95-33

2. PP#: 4F04407

3. TECHNICAL REVIEWER: Everett S. Green, Jr.

4. DATE: 3-21-95

5. ANALYTES/LEVEL: Sulfentrazone (FMC 97285), Hydroxy Sulfentrazone (FMC 106091) 0.025, 0.050, 0.1 PPM

6. COMMODITIES: Soybeans

7. METHOD: Residue Analytical Method for the Determination of FMC 97285 on FMC 106091 in/on Soybeans Treated with F6285

The Analytical Chemistry Section has been asked to screen the residue chemistry methods submitted by the registrant in order to determine if they contain the essential requirements identified in the Residue Chemistry Guidelines. Full scientific review and laboratory evaluation of those methods will take place after the initial screen. The following items need to be resolved before the analytical method can be evaluated.

| | YES | NO | |
|---|---|---|---|
| 1. Does the method use exotic equipment and/or supplies that are not commercially available in the U.S.? | ___ | X | |
| 2. Does the method require any new equipment before the laboratory work begins? | ___ | X | |
| 3. Are chromatograms included? | X | ___ | |
| a. Is (are) peak(s) of interest sufficiently resolved from other peaks? | X | ___ | See narrative comments |
| b. Has registrant included chromatograms of analyses at or below tolerance on all crop types for which tolerance is requested by HED? | X | ___ | |
| c. Do the control samples have reasonably low levels of the analyte in relation to the proposed tolerance? | X | ___ | |
| d. Is the method sufficiently sensitive and specific to measure and identify the residues at levels specified by HED in the TMV request? | X | ___ | |

| | YES | NO |
|---|---|---|
| 4. Has recovery data been provided to ACL for the residues that are specified in the TMV request? | X | |
| 5. Are recovery values between 70% and 120% at all levels and for all commodity types? | X | One 672 recovery |
| 6. Are all procedures clearly written with no ambiguities so that the method can be run without communication with the registrant? | See narrative review | |
| 7. Does the method require correction for a sample of the untreated commodities or a blank? | | X |
| 8. Does the method require the use of an internal or procedural standard to compensate for lost analyte during analysis? | | X |
| 9. Are 2nd laboratory validation data provided with the method? | X | |
| 10. Are there any deficiencies other than those covered above that would prevent ACS from conducting a method trial? | See narrative review | |
| 11. Is this method suitable for validation testing? | X | |

Any deficiencies/problems noted for any above items should be addressed in the full scientific review of this method to be attached as an addendum.

Egbert S. Herb — Signature

3-21-95 — Date

The following is to be completed by the analyst performing the TMV.

| | | |
|---|---|---|
| 12. Can a set of 6 samples be run within 24 hours? | X | |
| 13. a. Are standards available at RTP repository? | | X |
| b. Are derivatized analytical reference standards available? | Not USES | |

# LINEARITY OF SULFENTRAZONE AND 3-HYDROXY SULFENTRAZONE

Project Number: B95-33
Project Name: Sulfentrazone TMV
Instrument: HP 5890 GC W/ EC Detector
Column: HP-50+, 50% phenylmethylsilicone, 30M x 0.53mm x 1.0 um film thickness.
Injection Volume: 2 ul
Date: 10/5/95
Analyst: Mark W. Law

## SULFENTRAZONE

| Std. Conc. Ng/Ul | Area 1 | Area 2 | Area 3 | Average Area | Calculated Areas |
|---|---|---|---|---|---|
| 0.0510 | 54403 | 43261 | 42532 | 46732 | |
| 0.1020 | 138430 | 137746 | 131242 | 135806 | |
| 0.2550 | 338318 | 363779 | 388693 | 363597 | |
| 0.5099 | 1006535 | 954706 | 1019703 | 993648 | |
| 1.0198 | 1479794 | 1454383 | 1407709 | 1447295 | |
| 0.0000 | | | | | 20266 |
| 1.1000 | | | | | 1658526 |

**Regression Output:**

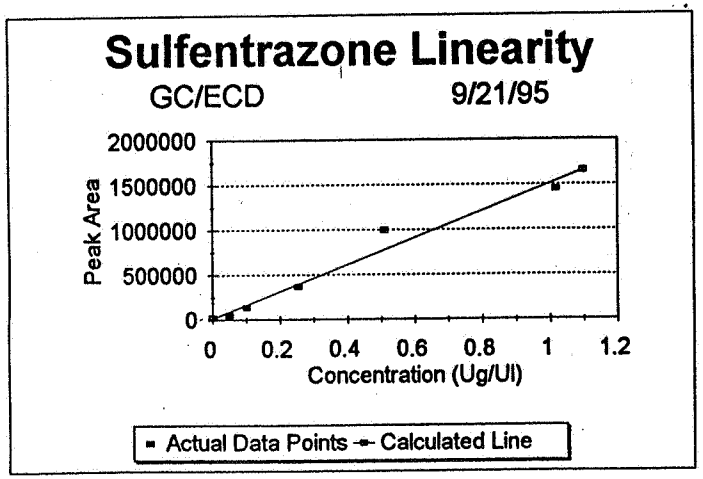
| | |
|---|---|
| Constant | 20265.51 |
| Std Err of Y Est | 140595.3 |
| R Squared | 0.959111 |
| No. of Observations | 5 |
| Degrees of Freedom | 3 |
| X Coefficient(s) | 1489327 |
| Std Err of Coef. | 177540.4 |

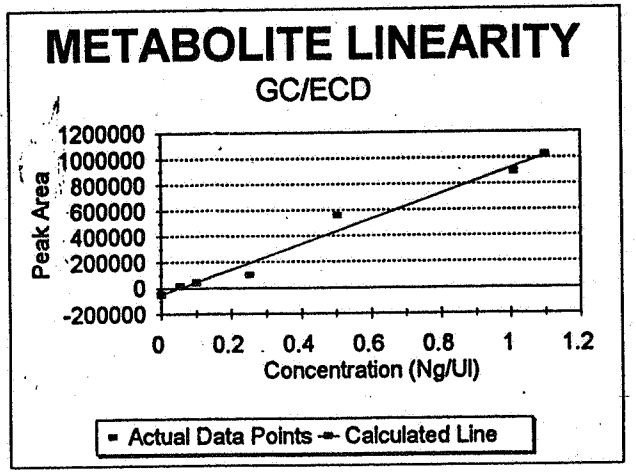
## 3-HYDROXY SULFENTRAZONE

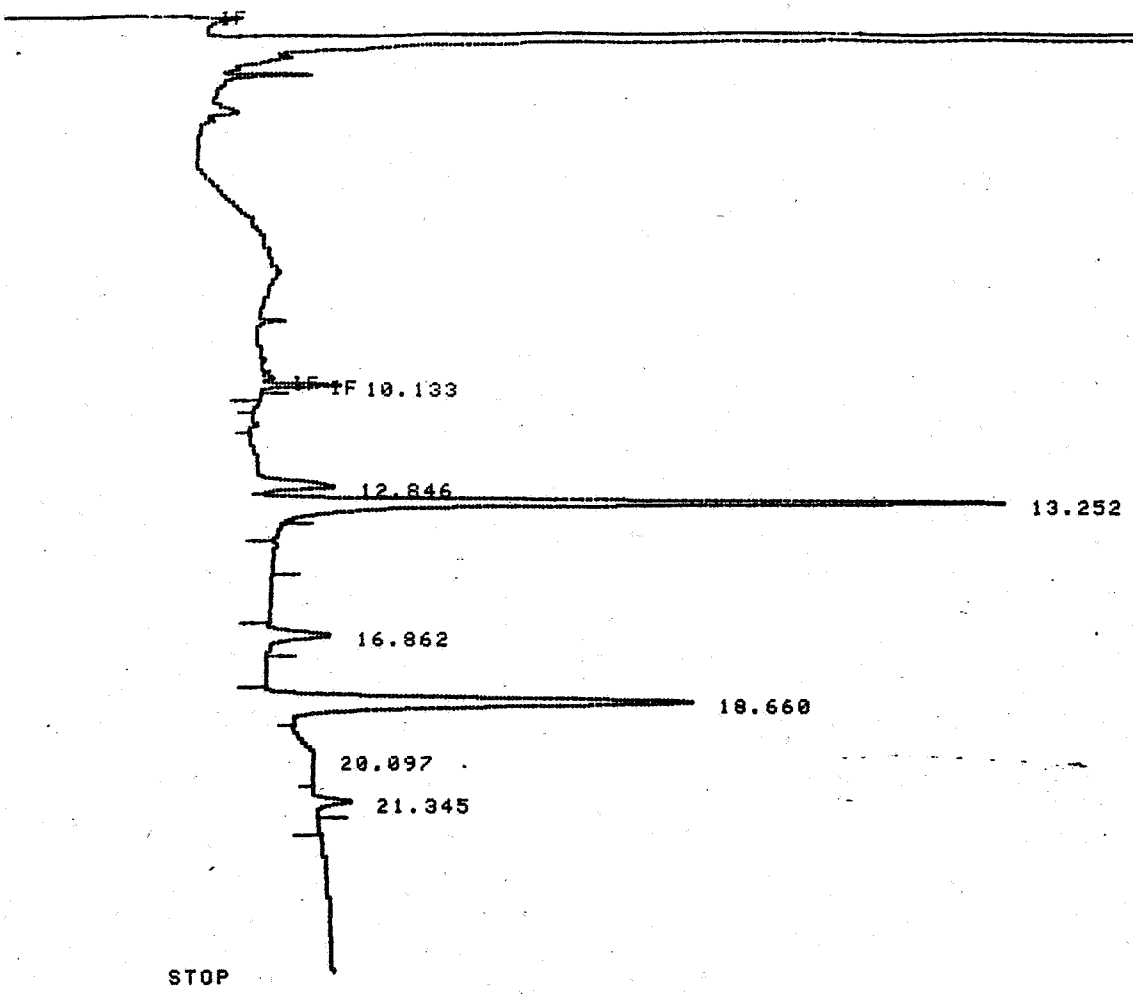
| Std. Conc. Ng/Ul | Area 1 | Area 2 | Area 3 | Average Area | Calculated Areas |
|---|---|---|---|---|---|
| 0.0547 | 15464 | | | 15464 | |
| 0.1009 | 41329 | 42722 | 54579 | 46210 | |
| 0.2524 | 90593 | 98225 | 119688 | 102835 | |
| 0.5047 | 655686 | 407270 | 617327 | 560094 | |
| 1.0094 | 930565 | 919386 | 837854 | 895935 | |
| 0.0000 | | | | | -50220 |
| 1.1000 | | | | | 1020907 |

**Regression Output:**

| | |
|---|---|
| Constant | -50220 |
| Std Err of Y Est | 89859.72 |
| R Squared | 0.959902 |
| No. of Observations | 5 |
| Degrees of Freedom | 3 |
| X Coefficient(s) | 973751.9 |
| Std Err of Coef. | 114904.3 |



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RUN# 707 OCT 17, 1995 08:24:10

SAMPLE NAME: STANDARD SAMPLE# 10
METHOD NAME: B*CYCLO.MET
2 UL INJ OF 0.25 NG/UL MIXED STANDARDS

SIGNAL FILE: A:Q18560AA.BNC

B95-33 SULFENTRAZONE TMV (SOYEANS)

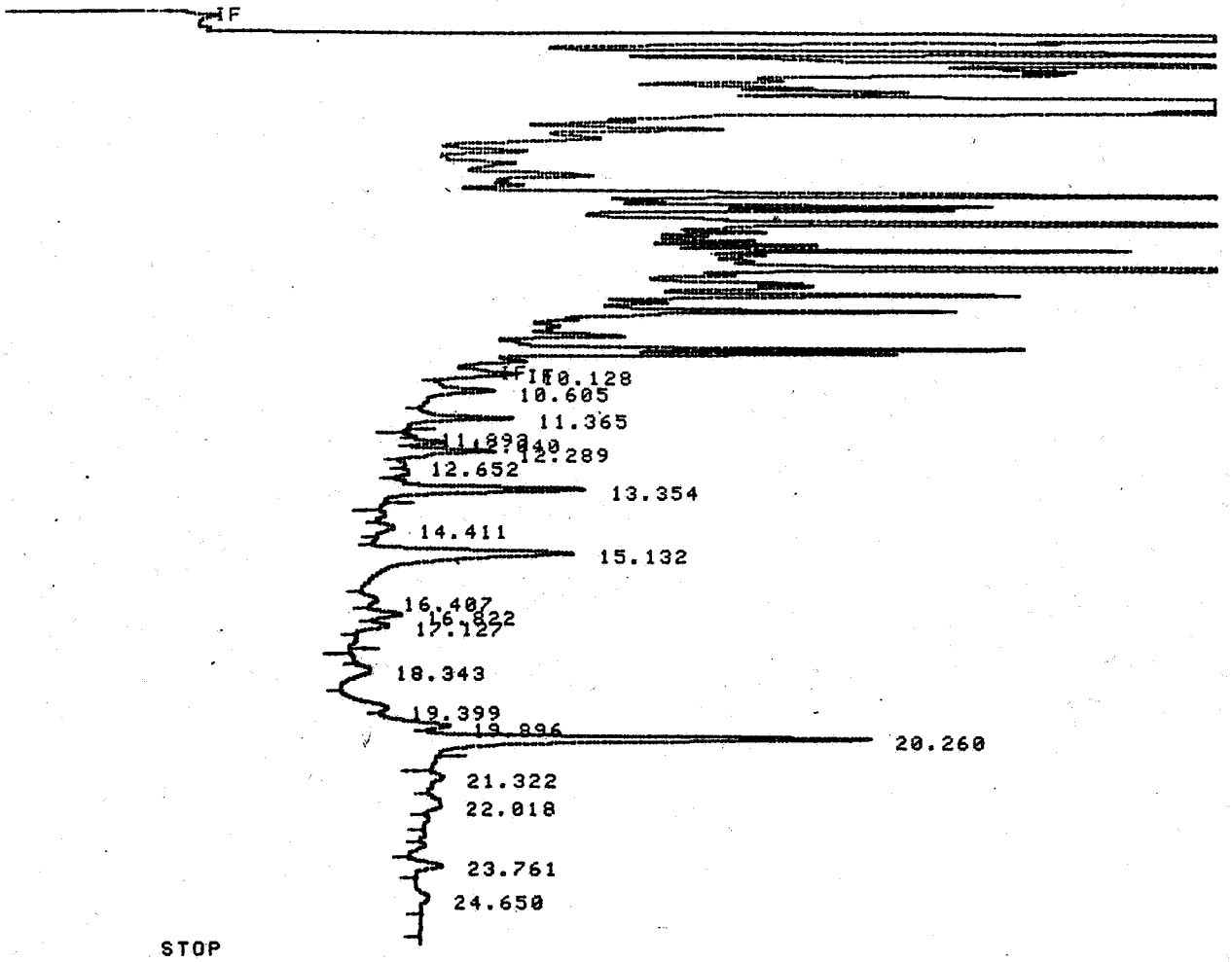
AREA%

| RT | AREA | TYPE | WIDTH | AREA% |
|---|---|---|---|---|
| 10.133 | 25010 | BB | .092 | 2.15859 |
| 12.846 | 56914 | PP | .229 | 4.91219 |
| 13.252 | 477073 | PB | .187 | 41.17570 |
| 16.862 | 52173 | BB | .237 | 4.50300 |
| 18.660 | 427553 | PV | .295 | 36.90168 |
| 20.097 | 77594 | VV | 1.011 | 6.69706 |
| 21.345 | 42311 | VB | .309 | 3.65182 |

TOTAL AREA=1158628
MUL FACTOR=1.0000E+00

RUN # 708 OCT 17, 1995 08:55:31
START

RUN # 713 OCT 17, 1995 11:32:19
START




STOP

Closing signal file A:Q1858CC4.BNC

RUN# 713 OCT 17, 1995 11:32:19

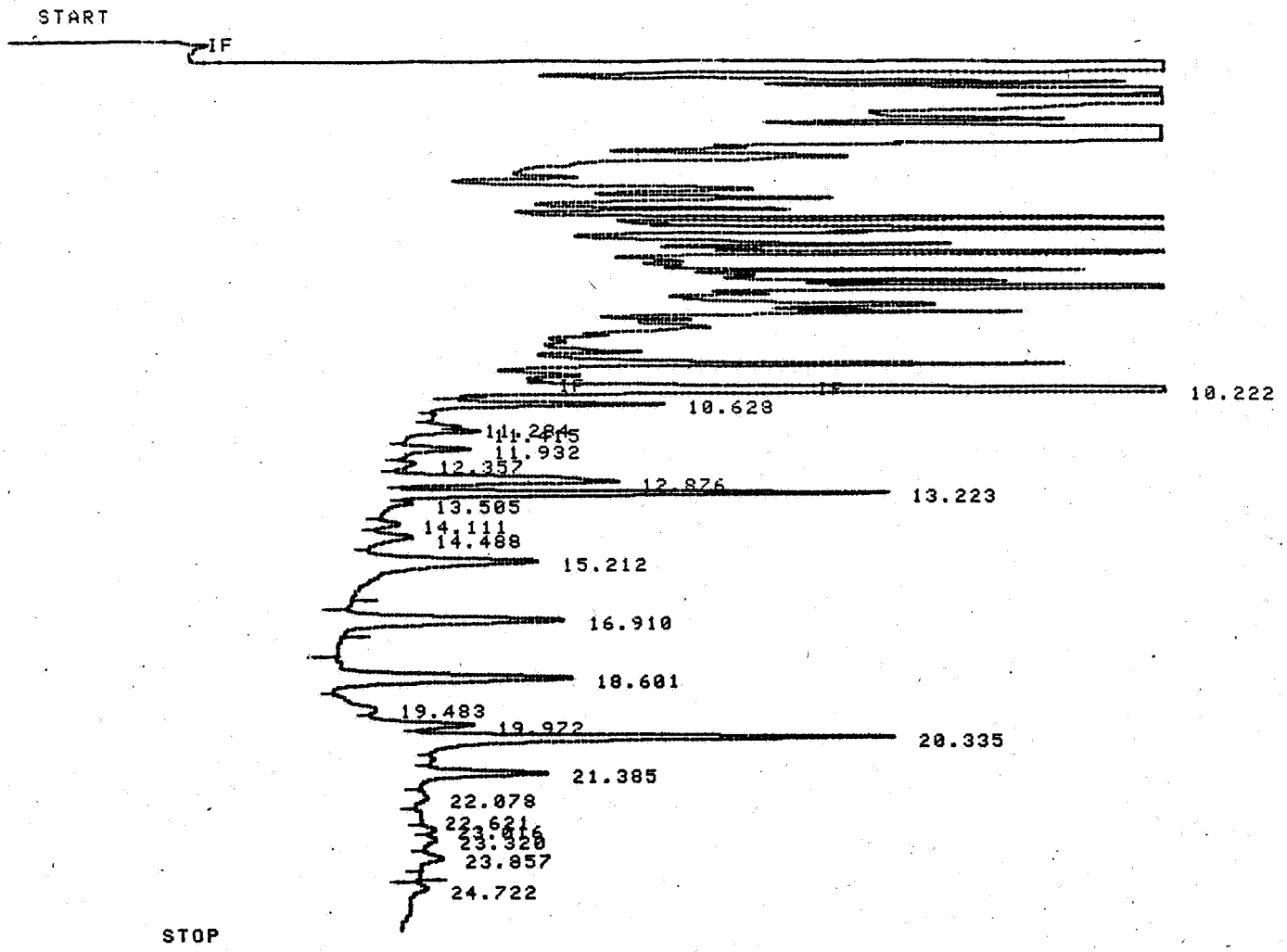
SAMPLE NAME: CONTROL 2 SAMPLE# 12
METHOD NAME: B*CYCLO.MET
5 G. SOYBEANS, 1 ML FINAL, 2 UL INJ.

SIGNAL FILE: A:Q1858CC4.BNC

B95-33 SULFENTRAZONE TMV (SOYEANS)

AREA%

| RT | AREA | TYPE | WIDTH | AREA% |
|---|---|---|---|---|
| 10.128 | 20475 | BP | .119 | 1.79292 |
| 10.605 | 43574 | PV | .187 | 3.81561 |
| 11.365 | 49115 | VB | .135 | 4.30082 |
| 11.893 | 4475 | BV | .094 | .39186 |
| 12.040 | 20514 | VV | .129 | 1.79633 |
| 12.289 | 52382 | VV | .157 | 4.58689 |
| 12.652 | 6033 | VV | .150 | .52829 |
| 13.354 | 130506 | VB | .187 | 11.42792 |
| 14.411 | 16787 | PV | .231 | 1.46997 |
| 15.132 | 218440 | VP | .301 | 19.12797 |
| 16.407 | 18359 | PV | .271 | 1.60763 |
| 16.822 | 34829 | VV | .222 | 3.04984 |

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RUN# 720 OCT 17, 1995 15:11:33

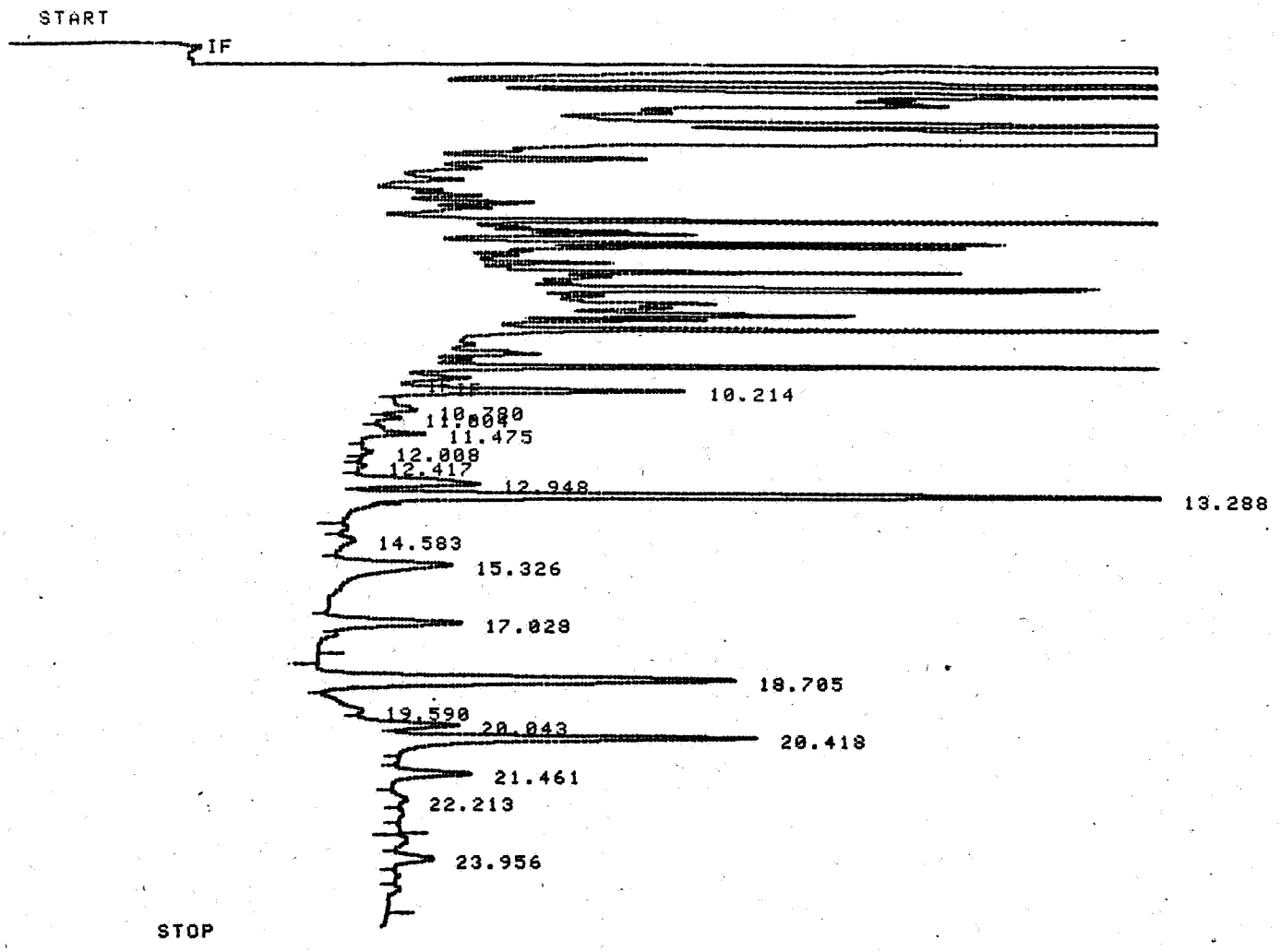
SAMPLE NAME: 0.025-1 SAMPLE# 14
METHOD NAME: B*CYCLO.MET
5 G. SOYBEANS, 0.025 PPM SPIKE, 1 ML FINAL, 2 UL INJ.

SIGNAL FILE: A:Q185C026.BNC

B95-33 SULFENTRAZONE TMV (SOYEANS)

AREA%

| RT | AREA | TYPE | WIDTH | AREA% |
|---|---|---|---|---|
| 10.222 | 1038561 | BB | .117 | 29.26956 |
| 10.628 | 89410 | BP | .111 | 2.51982 |
| 11.284 | 20981 | VV | .131 | .59130 |
| 11.415 | 29461 | VP | .119 | .83029 |
| 11.932 | 40519 | PV | .158 | 1.14194 |
| 12.357 | 12086 | VV | .169 | .34062 |
| 12.876 | 203123 | VV | .240 | 5.72458 |
| 13.223 | 260017 | VV | .139 | 7.32801 |
| 13.505 | 20657 | VV | .186 | .58217 |
| 14.111 | 15121 | VV | .172 | .42615 |
| 14.488 | 34578 | VP | .226 | .97450 |
| 15.212 | 204492 | PB | .317 | 5.76316 |
| 16.910 | 191439 | BB | .237 | 5.39529 |
| 18.601 | 227054 | BP | .257 | 6.39902 |
| 19.483 | 47017 | PV | .345 | 1.32507 |
| 19.972 | 122070 | VV | .260 | 3.44028 |
| 20.335 | 487834 | VV | .242 | 13.74853 |

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RUN# 731 OCT 17, 1995 20:56:01

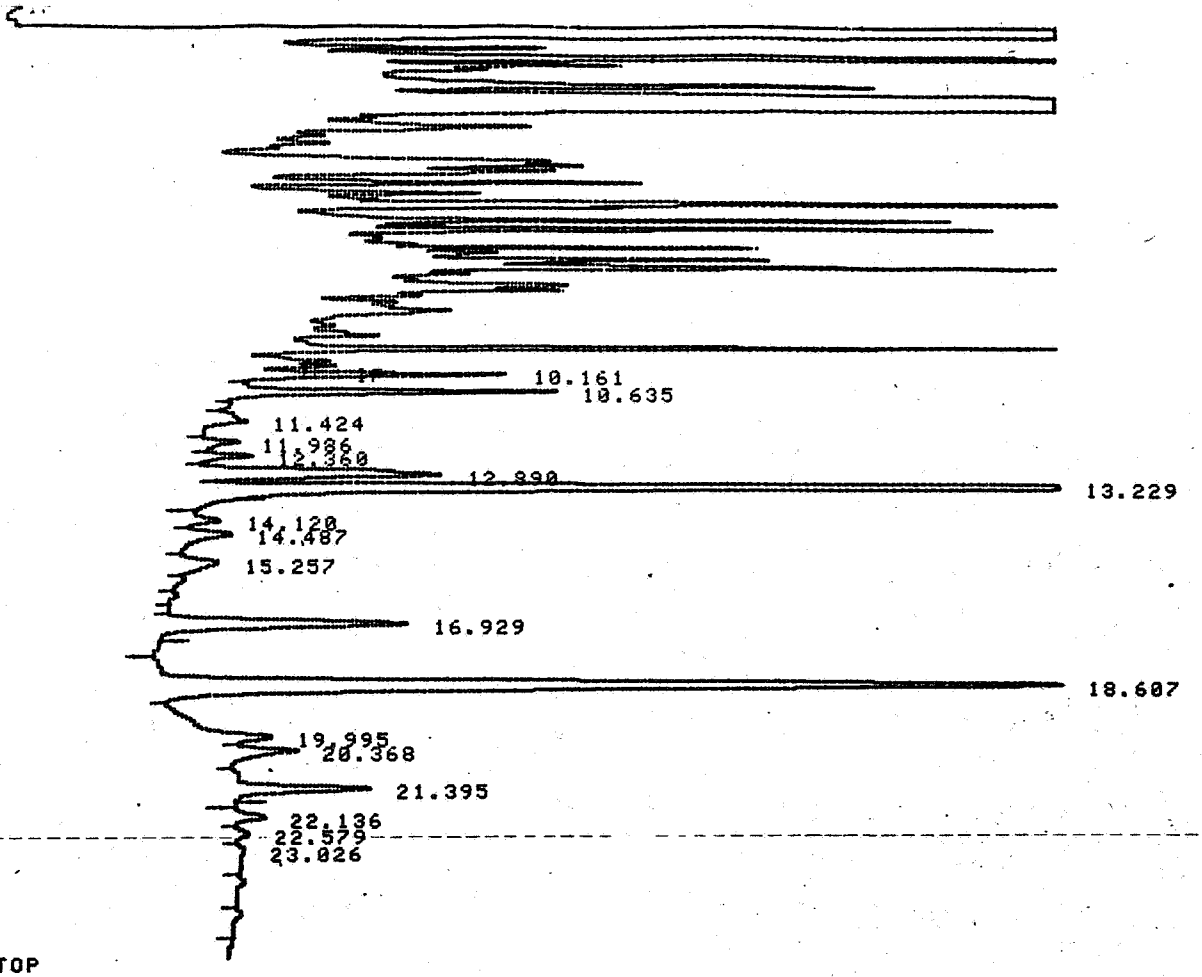
SAMPLE NAME: 0.05-2 SAMPLE# 18
METHOD NAME: B*CYCLO.MET
5 G. SOYBEANS, 0.05 PPM SPIKE, 1 ML FINAL, 2 UL INJ.

SIGNAL FILE: A:Q18610E2.BNC

B95-33 SULFENTRAZONE TMV (SOYEANS)

AREA%

| RT | AREA | TYPE | WIDTH | AREA% |
|---|---|---|---|---|
| 10.214 | 145812 | BP | .135 | 6.68249 |
| 10.780 | 27569 | PV | .218 | 1.26347 |
| 11.004 | 9767 | VV | .109 | .44762 |
| 11.475 | 34683 | VP | .154 | 1.58950 |
| 12.008 | 8039 | PV | .155 | .36842 |
| 12.417 | 4180 | PP | .116 | .19157 |
| 12.948 | 105374 | PP | .228 | 4.82924 |
| 13.288 | 455147 | PB | .139 | 20.85916 |
| 14.583 | 16009 | PP | .252 | .73368 |
| 15.326 | 163458 | PV | .367 | 7.49120 |
| 17.028 | 120542 | VV | .232 | 5.52438 |
| 18.705 | 388778 | BP | .251 | 17.81750 |
| 19.590 | 40067 | PV | .349 | 1.83625 |
| 20.043 | 114437 | VV | .258 | 5.24459 |
| 20.418 | 355826 | VV | .231 | 16.30733 |
| 21.461 | 120861 | VV | .309 | 5.53900 |
| 22.213 | 40441 | VV | .418 | 1.85339 |




STOP

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RUN# 738 OCT 18, 1995 00:35:09

SAMPLE NAME: 0.1-1 SAMPLE# 20
METHOD NAME: B*CYCLO.MET
5 G. SOYBEANS, 0.1 PPM SPIKE, 1 ML FINAL, 2UL INJ.

SIGNAL FILE: A:Q186443E.BNC

B95-33 SULFENTRAZONE TMV (SOYEANS)

AREA%

| RT | AREA | TYPE | WIDTH | AREA% |
|---|---|---|---|---|
| 10.161 | 95757 | BP | .113 | 3.28151 |
| 10.635 | 137786 | PP | .122 | 4.72181 |
| 11.424 | 24553 | VP | .205 | .84141 |
| 11.986 | 22240 | PV | .167 | .76215 |
| 12.360 | 25969 | VP | .145 | .88994 |
| 12.890 | 189718 | PV | .233 | 6.50148 |
| 13.229 | 912671 | VB | .148 | 31.27649 |
| 14.120 | 16728 | BP | .166 | .57325 |
| 14.487 | 34775 | PP | .224 | 1.19171 |
| 15.257 | 40484 | PV | .295 | 1.38735 |
| 16.929 | 198808 | VB | .237 | 6.81299 |
| 18.607 | 815928 | PP | .258 | 27.96118 |
| 19.995 | 100908 | PV | .353 | 3.45804 |
| 20.368 | 111049 | VV | .321 | 3.80556 |
| 21.395 | 132803 | VB | .263 | 4.55105 |
| 22.136 | 20870 | BV | .190 | .71520 |
| 22.579 | 11202 | VV | .211 | .38388 |
| 23.026 | 25825 | VV | .582 | .88500 |