Cover Sheet for

ENVIRONMENTAL CHEMISTRY METHOD

Pestcide Name: Diflufenzopyr (SAN 835H)

MRID #: 443074-20

Matrix: Soil

Analysis: HPLC/UV-GC/MS

This method is provided to you by the Environmental Protection Agency's (EPA) Environmental Chemistry Laboratory (ECL). This method *is not* an EPA method but one which was submitted to EPA by the pesticide manufacturer to support product registration. EPA recognizes that the methods may be of some utility to state, tribal, and local authorities, but makes no claim of validity by posting these methods. Although the Agency reviews *all* Environmental Chemistry Methods submitted in support of pesticide registration, the ECL evaluates only about 30% of the currently available methods. Most methods perform satisfactorily but some, particularly the older methods, have deficiencies. Moreover, the print quality of the methods varies considerably because the methods originate from different sources. Therefore, the methods offered represent the best available copies.

If you have difficulties in downloading the method, or further questions concerning the methods, you may contact Elizabeth Flynt at 228-688-2410 or via e-mail at flynt.elizabeth@epa.gov.

VALIDATION OF THE ANALYTICAL METHOD FOR THE DETERMINATION OF SAN 835 H AND ITS PHTHALAZINONE RESIDUES (M1 AND M5 METABOLITES) IN SOIL, 1995

1. SUMMARY

An analytical method has been developed by Sandoz Agro LTD, Market Area 1, ACES , Huningue for the determination of residues of SAN 835 H in soil. The method allows determination of the parent molecule and of the metabolites containing the common moiety phthalazinone (M1 and M5 metabolites), those metabolites being quantitated as phthalazinone.
The method consists of an extraction of the parent molecule and its M1 and M5 metabolites with acetone/ sodium bicarbonate solution. Then the extract is split in 2 aliquots and the acetone is evaporated. The aliquot to be analyzed for SAN 835 H is acidified then partitioned on an Extrelut column, followed by a SPE clean-up step on C18 column and HPLC analysis. The aliquot to be analyzed for phthalazinone is partitioned on an Extrelut column, cleaned on a Envi-Carb SPE column before analysis on GC/MS.
This method successfully passed a in-house validation .
The validation was performed by fortifying untreated soil samples with SAN 835 H and phthalazinone (M1 metabolite) at levels equivalent to the limit of determination of the described method (0.01 µg/g) and to ten times that level (0.1 µg/g). Additionally, 5 untreated soil samples were fortified with carbamoyl phthalazinone (M5 metabolite) at 10 times the limit of determination of the method. 5 untreated soil samples were fortified with SAN 835 H only at ten times the limit of determination of the method.
For each compound and each fortification level, 5 replicated samples were analyzed under repeatability conditions.

The overall recoveries for both fortification levels averaged 88.6 ± 10.6 % for SAN 835 H (n=14) and 83.3 ± 15.9 % for phthalazinone (n=10).
The overall recovery of Carbamoyl phthalazinone as phthalazinone was 99 ± 6.6 % (n=5).
No significant conversion of SAN 835H to phthalazinone was observed during the analysis.

It is concluded that the method described in this report is capable to quantify SAN 835 H and its phthalazinone metabolites (M1 and M5) in soil with the accuracy, precision and repeatability required for a residue method.

2. INTRODUCTION AND STUDY OBJECTIVES

SAN 835 H is an experimental herbicide being developed by Sandoz Agro Ltd for postemergence use in corn. SAN 835 H is a growth inhibitor that acts by inhibiting the auxin transport. From the results of a soil route and rate of degradation study, phthalazinone residues as the sum of phthalazinone (or metabolite M1) and carbamoyl phthalazinone (or metabolite M5) account for more than 10 % of the initial concentration of SAN 835 H applied. As a consequence, they have to be included in the residue definition.

An analytical method, described in this report, was developed to quantify the parent compound of SAN 835 H and the metabolites containing the phthalazinone common moiety in field soil samples.

The objective of the study reported here was to validate this analytical method developed in the testing facility. The accuracy, precision and repeatability of the method was to be determined.

This objective included to demonstrate that carbamoyl phthalazinone (M5 metabolite) can be quantitatively quantified as phthalazinone (M1 metabolite) in soil and that no significant amount of phthalazinone coming from degradation of SAN 835 H during the sample analysis procedure was observed.

3. DATES/ DEADLINES

Protocol signature :	26-Sep-95
First amendment:	31-Oct-95
Start of analytical work:	02-Oct-95
End of analytical work:	07-Nov-95
Final report:	07-May-96

4. PROJECT STAFF

Test facility site:	Name: Sandoz Agro LTD, Market Area 1, ACES Address: Sandoz Agro Europe, c/o Clariant, F-68330 Huningue FRANCE
Technician:	G. Golling
Study director:	Dr. MN. Carrier
Sponsor:	Dr. P. Hertl

5. ARCHIVING

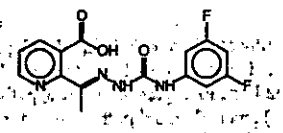
All analytical raw data including chromatograms, additional information, the study plans, the amendments and the final report are archived under the project number R 95-034 for a period of at least ten years in the archive of the test facility site.

6. TEST SUBSTANCE

SAN 835 H

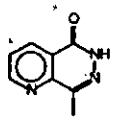
Systematic name: 2-[methyl((3,4-difluorophenylamino(carbonyl)hydrazono)methyl-a-pyridine carboxylic acid

Empirical formula: $C_{14}H_{12}F_2N_4O_3$



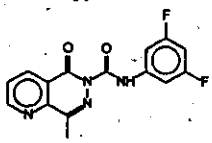
Molecular weight:	322 g/mol
Batch number :	5904-4-3
Purity:	98.1%
Expiry date:	11/97

phthalazinone (M1 metabolite of SAN 835H)
Systematic name: 8-methylpyrido(2,3-D)pyridazin-5(6H)-one
Empirical formula: $C_8H_7N_3O$



Molecular weight: 161 g/mol
Identification number: RS-M1-012095
Purity: 98.1%
Expiry date: 01/02/2000

Carbamoyl phthalazinone (M5 metabolite of SAN 835 H)
Systematic name: 6-((3,5-Difluorophenyl-carbamoyl-8-methyl-pyrido(2,3-d)-5-pyridazinone



Empirical formula: $C_{14}H_{10}F_2N_4O_2$
Molecular weight: 304 g/mol
Identification number: RS-M5-041395-1
Purity: 94.5 %
Expiry date: 09/05/2000

7. TEST SYSTEM

This study was performed using untreated field soil from a plot located at Leuggern , Switzerland. The sample was taken on June 6, 1995 on the control plot of site A of study R95-006 at day 0 just before the application to the treated sub-plot. The soil sample was received deep-frozen in good conditions on June 14, 1995, cut, sub-sampled, homogenized on June 21 and 22, 1995 and stored at a temperature below -20°C. The layer 0-10cm was used in this study. The soil parameters determined by Agrolab AG, Root, Switzerland are reported in Table 1 below.

Table 1: ***physico-chemical parameters of the test system***

% clay	19.6
% silt	55.2
% sand	25.2
pH (H_2O)	5.55
pH ($CaCl_2$)	5.05
CaCO3 content (%)	0.0
Organic Carbone content (%)	1.2
Cation exchange capacity (meq/100g)	8.0

8. EQUIPMENT

8.1 REAGENTS

Acetonitril gradient grade for chromatography,Lichrosolv , Merck art. 1.0030
Acetone GR, pro analysi , Merck art. 1.00014
Methanol for organic trace analysis, Suprasolv , Merck art. 1.06011
Ethyl acetate for organic residue analysis "Ultra-Resi analyzed" , JT Baker art. 9260-03
Dichloromethane for pesticides analysis, Pesti-pur , SDS art. 02922E21
Toluene for organic residue analysis "Baker Resi-analyzed" , JT Baker art. 9336-03
Sodium hydrogenocarbonate GR , Merck art. 1.06329
Filtration agent Clarcel , CECA
Diatomaceus earth Extrelut® 20 , Merck art. 11378
Supelclean™ Envi™-Carb SPE cartridges 0.25g, 3ml , Supelco, L.P.C.R. art. 5-7088
Bakerbond SPE C18 cartridges, 1 g, 6 ml, Baker art.7020-07

8.2 APPARATUS

Shaker , Edmund Bühler SM 25 and SM 25A
Rotary evaporator, Rotavapor R-124V, Büchi AG , CH
1 L bottle with screw cap, Verlabo 2000 , Strasbourg
13 cm diameter Büchner funnels , Store Sandoz Basel
11cm diam. round filters MN 640 M , L.P.C.R , Macherey-Nagel art. 203.011
various volumes graduated cylinders , Store Sandoz Basel
50 ml dropping funnels , Store Sandoz Basel
various volumes round bottom flask , Store Sandoz Basel
5 ml graduated tubes , Glasmechanic , CH
various volumes graduated pipettes , Store Sandoz Basel
Vacuum station for SPE columns, Vac Elut SPS 24 , Varian AG, Basel
Columns for Diatomaceous earth Extrelut® 20 , Merck art. 11737
Gas chromatography instrument with MS detector , Hewlett-Packard, HP5890/MSD 5972
HPLC instrument , Hewlett Packard, HP 1090 with DAD detector

9. STANDARD SOLUTIONS

All the stock solutions were stored at temperature equivalent to or below -20°C.
All the working solutions were stored at temperature below 5°C when not in use.

9.1 *For SAN 835H*

A stock solution of 10 mg/ml of SAN 835 H was prepared in acetone and working solutions of concentrations ranging from 0.05 µg/ml to 50 µg/ml in DMSO/ acetonitrile (5:95) were prepared by successive dilutions of the stock solution. The maximum dilution ratio used was 1 to 100. These working solutions were used for fortifications of untreated samples. They were stored at below 5°C for not more than 2 months.

These solutions were further diluted 5 times with water to give the standard solutions (in water/acetonitrile (8:2)) which were used as external standard calibration for HPLC analysis. These HPLC solutions were prepared just before use and were not stored.

9.2 *For phthalazinone (M1 metabolite)*

A stock solution of 1 mg/ml of phthalazinone in acetone and working solutions (concentration range: 10 µg/ml to 0.01 µg/ml) in toluene prepared by successive dilutions of the stock solution were used. The maximum dilution ratio used was 1 to 100.
These working solutions were used as standard solutions for external standard calibration for GC/MS analysis and also for fortification of untreated samples.

9.3 For carbamoyl phthalazinone (M5 metabolite)

A stock solution of 10 mg/ml in DMSO/ acetone (2:8) of carbamoyl phthalazinone was prepared. A working solution in acetone at a concentration of 5 µg/ml was prepared by successive dilutions of the stock solution and was used for fortification of untreated samples within 10 days of preparation. The maximum dilution ratio used was 1 to 100.

10. ANALYTICAL PROCEDURE

10.1 SAMPLE PREPARATION

The homogenized laboratory sample was prepared and used. Stones larger than 1 cm diameter were removed. 100 g of soil were let to dry overnight and weighed again to determine the weight of dry soil. This weight divided by five (to correspond to the amount analyzed in each aliquot) was used as soil weight (see point 13.2.3) for the calculation of the amount of residues.

10.2 FORTIFICATION

The untreated soil sample (50g) was fortified by direct addition of 1 ml of the appropriate solution of SAN 835 H (in DMSO/ acetonitrile= 5:95) , and /or phthalazinone (in toluene) or carbamoyl phthalazinone (in acetone) to the undried soil before extraction.

10.3 EXTRACTION

50 g of undried soil and about 10 g of Clarcel were weighed in a 1L screw-cap bottle. 50 ml of a 0.5% sodium hydrogenocarbonate solution and then 150 ml of acetone were added. The mixture was shaken for 15 minutes and filtered into a 1L round bottom flask under vacuum on a Büchner funnel containing a round filter moistened with acetone. The filter was rinsed with about 50 ml of acetone. The filtration cake was extracted again for 15 minutes with 200 ml acetone and then filtered in the same round bottom flask using another moistened filter. The cake was rinsed with about 50 ml of acetone.

The extract was adjusted to a definite volume with acetone and two aliquots each corresponding to 20 g of initial sample were withdrawn. The remaining solution was discarded. One aliquot was analyzed for residues of SAN 835H parent compound, the other one for phthalazinone residues.

10.4 ANALYSIS FOR SAN 835 H

The acetone was evaporated using the rotavapor under maximum vacuum and at a temperature of 20-30°C until about 20 ml of aqueous residue remained.

10.4.1 Clean-up on Extrelut column

An Extrelut column was filled with one dose of Extrelut® 20 material. 1 ml of 5N HCl was added to the aqueous residue , the mixture was swirled and transferred to a dropping funnel set over the Extrelut column. After checking that the volume was below 20 ml, the acidified solution was poured on the Extrelut silica, allowed to stand for 10 minutes and then eluted with 100 ml of ethyl acetate. The ethyl acetate was used to rinse the round bottom flask and the dropping funnel. The eluat was collected in a 250 ml round bottom flask, evaporated to dryness using the rotavapor under maximum vacuum and at a temperature of 20-30°C.

10.4.2 Clean-up on C18 column

10.4.2.1 Conditioning of the C18 column

A C18 column (1g /6 ml) was rinsed first with 2 volumes (=12 ml) of methanol and then with 2 volumes (=12 ml) of 0.5% sodium hydrogenocarbonate solution. The column was not allowed to dry.

10.4.2.2 Reversed Phase-Chromatography with C18

The residue of point 10.4.1 was reconstituted in 5 ml of the 0.5 % sodium hydrogenocarbonate solution. This solution was passed through the conditioned column using vacuum (the column was not allowed to dry). The round bottom flask was rinsed with 5 ml of the 0.5 % sodium hydrogenocarbonate solution , the rinse was added on the column. This rinse was repeated with 5 ml water. The column was then dried by applying vacuum and the eluate was discarded.
The column was eluted successively with 2 x 1 ml of the acetonitril/water mixture (2:8) bringing the column to dryness between each elution. The eluate was collected in a 5 ml graduated tube and the final volume was recorded. This solution was injected in HPLC.

10.5 ANALYSIS FOR PHTHALAZINONE

The acetone was evaporated using the rotavapor under maximum vacuum and at a temperature of 20-30°C until about 20 ml of aqueous residue remained.

10.5.1 Clean-up on Extrelut column

An Extrelut column was filled with one dose of Extrelut® 20 material. The aqueous residue was transferred to a dropping funnel set over the Extrelut column. After checking that the volume was below 20 ml, the solution was poured on the Extrelut silica, allowed to stand for 10 minutes and then eluted with 100 ml of ethyl acetate. The ethyl acetate was used to rinse the round bottom flask and the dropping funnel. The eluat was collected in a 250 ml round bottom flask, evaporate to dryness using the rotavapor under maximum vacuum and at a temperature of 40-50°C.

10.5.2 *Clean-up on Envi-Carb column*

10.5.2.1 Conditioning of the Envi-Carb column

An Envi-Carb column (0.25 g/3 ml) was rinsed first with 2 volumes (=6 ml) of methanol/ dichloromethane (2:8) and then with 2 volumes (= 6ml) of water. The column was not allowed to dry.

10.5.2.2 *Adsorption on Envi-Carb column*

The residue from point 10.5.1 was reconstituted in 3 ml water and passed through the conditioned column using vacuum (the column was not allowed to dry). The round bottom flask was rinsed with 2 x 3 ml water and the rinses were added on the column. The column was dried by applying vacuum and the eluate was discarded.
The column was eluted with 2 successive volumes (= 2 x 3 ml) of the mixture methanol/ dichloromethane (2:8) drying the column between each elution. The eluate was collected in a 50 ml round bottom flask. The solvent was evaporated to dryness using the rotavapor under maximum vacuum and at a temperature of 40-50°C. The residue was reconstituted in 2 ml of toluene.
The toluene solution was transferred to the GC vial using a Pasteur pipette with the tip covered with cotton in order to filter the carbon particles eluted from the SPE column. This solution was injected in GC/MS.

10.6 IMPORTANT REMARKS

The stability of SAN 835H is limited in solution and at high temperature.
As a consequence all the extracts containing SAN 835 H were stored at below 5°C overnight. It was found that a good timing to stop the analysis overnight was just after the clean-up step on Extrelut column when the sample is in solution in ethyl acetate. For stability reasons, final extracts ready for HPLC (in solution in acetonitrile/water (2:8)) need to be kept at a temperature below -18°C, if storage for more than 1 day is necessary.
Also when using the rotavapor with samples containing SAN 835 H, the temperature of the water bath was not set above 30°C and the temperature of the cooling fluid was set to -10°C. In order to reduce the time needed for the analysis of SAN 835 H, the analysis were performed in the following order:

a) Extrelut clean-up of the aliquot analyzed for SAN 835H (point 10.4) and of the aliquot analyzed for phthalazinone (point 10.5).
b) finalization of the analysis for SAN 835 H
c) Finalization of the analysis for phthalazinone

11. CHROMATOGRAPHIC DETERMINATION

11.1 Chromatographic determination using HPLC for SAN 835H

11.1.1 Clean-up of the required water

Only ultra-pure water was used (deionised water purified through Millipore purification system (Milli Q)). This water was used for the preparation of a 0.5 % trifluoroacetic acid solution and then this solution was filtered on a 10 g C18 SPE column before being used for HPLC.

11.1.2 Chromatographic determination

Column: Hypersil BDS-C18 , 5 µm, 250 x 4 mm
Hewlett-Packard , ref: 79926 OB 584
Temperature : 40°C

Mobile phase: Solvent A: Acetonitril
Solvent B: 0.5 % trifluoroacetic acid solution

Flow: 1 ml/minute

Gradient:

time	Acetonitril	0.5% TFA
Min. 0	10 %	90 %
Min. 2	10 %	90 %
Min. 22	50 %	50 %
Min. 25	50 %	50 %
Min. 27	80 %	20 %
Min. 29	80 %	20 %

Injection volume: 250 µl

Equilibration time: 10 minutes

Analysis time: 25 minutes

Retention time: about 17.8 minutes

Detector: Diode-Array detector or UV detector with variable wave length ,
wave length : 240 nm

11.2 Chromatographic determination using GC/MS for phthalazinone

Column: HP-5 MS , film thickness: 0.25 µm, lenght: 30 m, ID: 0.250 mm
Hewlett-Packard , ref: 19091S-433

Carrier gas: Helium

Temperature program: 2 min at 100° C
+ 15° C / min to 220° C,
220° C for 3 min.

Injector: 250° C

Detector: 280° C

Injected volume: 1 µl

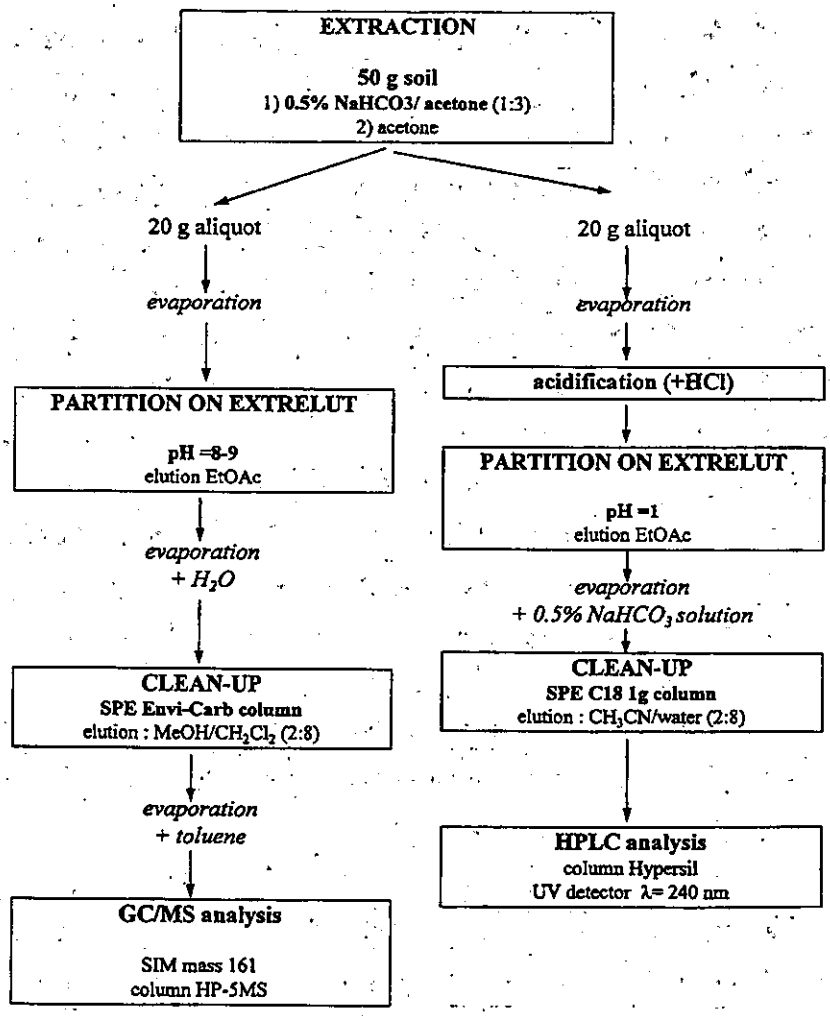
Analysis time: 13 minutes

Retention time: about 9 minutes

Detector: MSD

Single Ion Monitoring mass 161 (M^+)

12. FLOW CHART OF THE METHOD



13. EVALUATION OF RESULTS

13.1 Analysis sequence

Each analysis sequence injected was composed of the extracts from the samples spaced out with the external standards of the appropriate compound at selected concentrations. Each sequence started with one standard solution. The concentration of the standard solutions covered the expected working range.

13.2 Calculation of results

Concentrations in the sample extracts are quantified by their detector response with reference to an external standard calibration curve determined within the respective analytical sequence. The calibration curve is obtained by correlation of the detector responses of the external analytical standards with their corresponding concentrations (concentration range 0.005 to 10 µg/ml). A complete external standard calibration, consisting of at least ten different concentrations, was performed for each analytical sequence.

13.2.1 calculation of calibration curves

The data pairs are correlated by using the first or second order least square fitting functions (see equations 1 and 2).

$$y = a_0 + a_1 * x \qquad (1)$$

or

$$y = a_0 + a_1 * x + a_2 * x^2 \qquad (2)$$

where
x = concentration of analyte in the injected standard solution
y = detector response (in counts or mm)
a_0 = y-axis intercept
a_1 = first order correlation coefficient
a_2 = second order correlation coefficient.

Standard criteria for acceptance of the calibration functions was that the correlation coefficient R exceeded 0.99 and the coefficient of variation was smaller than 16 %.
The coefficient of variation was calculated by the following formula:

$$\text{Variation coefficient of calibration} = \frac{RSS}{S * X_{mean}}$$

where RSS: Residual Sum of Squares of the external standard calibration curve
S: Sensitivity a_1 (linear) or $a_1+2a_2*X_{mean}$ (quadratic) of the calibration function
X_{mean}: Mean of the external standard concentrations

13.2.2 Calculation of residue concentrations in sample extracts

The residue concentration x_u of the analyte was calculated using equations (3) or (4):

First order calibration function: $$x_u = \frac{y_u - a_0}{a_1} \quad (3)$$

Second order calibration function:

$$x_u = -\frac{a_1}{2a_2} \pm \sqrt{\left(\frac{a_1}{2a_2}\right)^2 - \left(\frac{a_0 - y_u}{a_2}\right)} \quad (4)$$

where
x_u = residue concentration in the sample extract [µg/ml]
y_u = detector response [counts or mm]

13.2.3 Calculation of residue concentrations in samples

The residue concentration C_u of the analyte in the sample was calculated using equation (5):

$$C_u = x_u * \frac{V_f}{W} \quad (5)$$

where
C_u = residual concentration of the analyte [in µg/g]
x_u = residue concentration in the sample extract [µg/ml]
V_f = final volume including all dilution steps [in ml]
W = dry weight of the sample analyzed [in g] (corresponding to a wet aliquot of 20g)

13.2.4 Calculation of recoveries

The recovery R is calculated using equation (6):

$$R = 100 * \frac{Cu}{Cs}\% \quad (6)$$

where
C_u = residual concentration of the analyte found in the fortified sample [in µg/g]
C_s = concentration of the analyte added to the fortified control sample [in µg/g]

13.3 Limit of determination/ detection

The upper limit of the working range of the external calibration function is defined by the maximum concentration used for calibration. The lower limit is the limit of determination of the actual analytical sequence calculated for each sequence as the lowest concentration being different from zero with a 95 % confidence level.
A limit of detection is also calculated as the lowest concentration being different from zero with a 50% confidence level.
The actual limit of detection (ng) and limit of determination (bg) in µg/g are calculated from these limits using equation (5).
Results are considered valid when they are within the working range of the calibration function.
All results from the fortified samples were within the acceptable range.

13.4 Limit of quantitation

The limit of quantitation is defined as the lowest concentration added to the samples and giving acceptable recovery rates. The analytical method was validated at a limit of quantitation of 0.01 mg/kg for SAN 835 H and for phthalazinone.

14. METHOD VALIDATION

14.1 General procedure

The validation was performed as an independent GLP compliant study.

Samples were fortified by addition of appropriate volumes of standard solutions of SAN 835 H and/or of phthalazinone or of carbamoyl phthalazinone directly on the soil as described in point 10.2 .
To test the repeatability of the analytical method, the 5 fortified samples prepared for each level and each compound were analyzed by the same technician within the same analytical sequence.
The corresponding control sample was analyzed with each set of fortified samples.

2 sets of samples were fortified with both test substances SAN 835 H and phthalazinone at the same level: 5 fortified samples were prepared at the limit of quantitation of the analytical method (0.01 mg/kg) and 5 other samples were prepared at 10 times the limit of quantitation (0.1 mg/kg).
5 samples were fortified with phthalazinone at 10 times the limit of quantitation of the analytical method (0.1 mg/kg). Those samples were analyzed for SAN 835 H and also for phthalazinone residues.
5 samples were fortified with carbamoyl phthalazinone at 10 times the limit of quantitation of the analytical method (0.1 mg/kg). Those samples were analyzed for residues of phthalazinone only.

14.2 Results

The results from samples fortified with both SAN 835 H and phthalazinone are reported in Table 2.
The results from samples fortified with SAN 835 H only and with carbamoyl phthalazinone respectively are reported in Table 3 and Table 4.
Results are summarized in Table 5.

Table 2: *Overall results for the quantitation of SAN 835 H and phthalazinone in control soil samples fortified with SAN 835H and phthalazinone.*

Fortification level [1]	Extraction date	Concentration added [µg/g] [2]		GC acquisition date	Concentration of phthalazinone found [µg/g] [3]		Recovery of phthalazinone [%]		HPLC acquisition date	Concentration of SAN 835 H found [µg/g] [3]		Recovery of SAN 835 H [%]	
		phthalazinone	SAN 835H		Conc.	95% Conf.int	R	95 % confidence		Conc.	95% Conf.int	R	95 % conf. int
	17-Oct-95			18-Oct-95	0.003	0.012			18-Oct-95	0	0.009		
0.1 ppm	17-Oct-95	0.12	0.12	18-Oct-95	0.116	0.012	96.6	9.7	18-Oct-95	0.088	0.009	73.7	7.7
0.1 ppm	17-Oct-95	0.12	0.12	18-Oct-95	0.118	0.012	98.1	9.7	18-Oct-95	0.103	0.01	86.4	8.2
0.1 ppm	17-Oct-95	0.12	0.12	18-Oct-95	0.126	0.012	105.5	9.9	18-Oct-95	0.095	0.009	79.1	7.8
0.1 ppm	17-Oct-95	0.12	0.12	18-Oct-95	0.11	0.011	92	9.5	[4]				
0.1 ppm	17-Oct-95	0.12	0.12	18-Oct-95	0.1	0.011	83.4	9.4	18-Oct-95	0.1	0.009	83.6	7.8
	19-Oct-95			30-Oct-95	0	0.004			20-Oct-95	0.001	0.003		
0.01 ppm	19-Oct-95	0.012	0.012	30-Oct-95	0.01	0.004	80	29.5	20-Oct-95	0.013	0.003	104.5	28.1
0.01 ppm	19-Oct-95	0.012	0.012	30-Oct-95	0.01	0.004	82.7	29.4	20-Oct-95	0.013	0.003	107.8	28.1
0.01 ppm	19-Oct-95	0.012	0.012	30-Oct-95	0.006	0.004	52.5	29.7	20-Oct-95	0.012	0.003	97.2	28.1
0.01 ppm	19-Oct-95	0.012	0.012	30-Oct-95	0.009	0.004	75.1	29.5	20-Oct-95	0.012	0.003	102.5	28.1
0.01 ppm	19-Oct-95	0.012	0.012	30-Oct-95	0.008	0.004	66.8	29.6	20-Oct-95	0.011	0.003	89.4	28.2

50 g undried soil => 41.75 g dry soil
1 mg/kg in undried soil => 0.12 mg/kg in dry soil
1) fortification level on undried soil
2) calculated on dry soil
3) Concentration calculated per weight of dry soil
4) aliquot of sample to be analyzed for SAN 835H accidently lost during work-up

Table 3: ***Overall results for the quantitation of phthalazinone M1 in control soil samples fortified with carbamoyl phthalazinone.***

Fortification level [1]	Extraction date	GC acquisition date	Concentration of carbamoyl phthalazinone M5 added [µg/g] [2]	Concentration of phthalazinone(M1) found [µg/g] [3]		Recovery of phthalazinone [4] [%]	
				Conc.	95% Conf.int	R	95 % Conf.int
	06-Oct-95	09-Oct-95		0.002	0.006		
0.1 ppm	06-Oct-95	09-Oct-95	0.12	0.063	0.005	98.6	8.7
0.1 ppm	06-Oct-95	09-Oct-95	0.12	0.068	0.006	107.5	8.7
0.1 ppm	06-Oct-95	09-Oct-95	0.12	0.064	0.005	101.1	8.7
0.1 ppm	06-Oct-95	09-Oct-95	0.12	0.063	0.005	98.7	8.7
0.1 ppm	06-Oct-95	09-Oct-95	0.12	0.057	0.005	89.2	8.6

50 g undried soil => 41.75 g dry soil
1 mg/kg in undried soil => 0.12 mg/kg in dry soil

1) fortification level on undried soil
2) calculated on dry soil
3) Concentration calculated per weight of dry soil
4)Calculated as follow:

$$\text{Recovery of phthalazinone} = \frac{\text{concentration phthalazinone} \cdot 304}{161 \cdot 0.12}$$

304 = molecular weight of carbamoyl phthalazinone
161 = molecular weight of phthalazinone
0.12 = concentration of carbamoyl phthalazinone added (calculated on dry soil)

Table 4: *Overall results for the quantitation of SAN 835 H and phthalazinone in control soil samples fortified with SAN 835 H.*

Fortification level [1]	Extraction date	Acquisition date	Concentration of SAN 835 H added [ng/g] [2]	Concentration of SAN 835 H found [µg/g] [3]		Recovery of SAN 835 H [%]		Concentration of phthalazinone found [µg/g] [3]		% SAN 835 H converted to phthalazinone [4]	
(SAN 835 H)				Conc.	95% Conf.Int	R	95 % confidence	Conc.	95% Conf.int	%	95% Conf.int
0.1 ppm	06-Nov-95	07-Nov-95	0.12	0.099	0.003	82.3	2.5	0.0015	0.0063	2.5	10.5
0.1 ppm	06-Nov-95	07-Nov-95	0.12	0.108	0.003	89.9	2.6	0.0017	0.0066	2.8	11.0
0.1 ppm	06-Nov-95	07-Nov-95	0.12	0.101	0.003	84.4	2.5	0.0021	0.0071	3.5	11.9
0.1 ppm	06-Nov-95	07-Nov-95	0.12	0.099	0.003	82.8	2.7	0.0030	0.0084	5.0	14.0
0.1 ppm	06-Nov-95	07-Nov-95	0.12	0.092	0.003	76.8	2.4	0.0039	0.0098	6.5	16.4
								mean [5]: 0.0024 ± 0.0035		mean [5]: 4.1 ± 5.8 %	

50 g undried soil => 41.75 g dry soil
1 mg/kg in undried soil => 0.12 mg/kg in dry soil

1) fortification level on undried soil
2) fortification calculated on dry soil
3) Concentration calculated per weight of dry soil
4) Calculated as follow: % SAN 835 H converted = $\frac{\text{phthalazinone concentration (mg/kg) x 41.75 x 322}}{161 \times 5}$

41.75 = dry weight of 50 g wet soil
322 = molecular weight of SAN 835 H
161 = molecular weight of phthalazinone
5 = amount of SAN 835 H added to 50 g undried soil (µg)

5) since all results were below the limit of determination,the standard deviation was calculated by error propagation formula:
std dev= $\frac{\sqrt{\sum s_{xi}^2}}{N}$ where s_{xi} is the value for a 95 % Confidence Interval

Table 5: ***Summarized recovery rates for SAN 835 II, phthalazinone and carbamoyl phthalazinone***

Compound	fortification level on undried soil (µg/g)	fortification level on dry soil (µg/g)	number of analysis	average concentration found* (µg/g)	standard deviation* (µg/g)	average recovery (%)	Standard deviation (%)	Relative standard deviation (%)
M1	0.1	0.12	5	0.1140	0.0097	95.1	8.2	8.6
M5	0.1	0.12	5	0.0630	0.0039	99.0	6.6	6.6
SAN 835 H	0.1	0.12	9	0.0983	0.0060	82.1	4.9	6.0
M1	0.01	0.012	5	0.0086	0.0017	71.4	12.2	17.1
SAN 835 H	0.01	0.012	5	0.0122	0.0008	100.3	7.2	7.2
M1	0.01 + 0.1	0.012 + 0.12	10			83.3	15.9	19.0
SAN 835 H	0.01 + 0.1	0.012 + 0.12	14			88.6	10.6	12.0

50 g undried soil => 41.75 g dry soil
1 mg/kg in undried soil => 0.12 mg/kg in dry soil

* calculated per weight of dry soil

15. DISCUSSION

15.1 control sample

No detectable residues[1] of SAN 835 H or of phthalazinone were found in the control sample.
Therefore no correction from the amount of analyte found in the corresponding control sample was made on the fortified sample results.

15.2 Accuracy

The accuracy of an analytical method is defined as the difference encountered between the measured mean result and the true or correct value. The requirement for the accuracy of a residue method is that the measured mean value falls within the range of 70-110 % of nominal with a relative standard deviation of less than 20 %[2].

15.2.1 SAN 835 H

The mean recovery rate for SAN 835 H is 82.1 % with a relative standard deviation of 6% (n=9) when added to the control samples at the level of 0.1 mg/kg and 100.3 % with a relative standard deviation of 7.2% (n=5) when added to the control samples at the limit of quantitation of the analytical method (0.01 mg/kg). The average recovery rate for both level of fortification is 88.6 % with a relative standard deviation of 12 %. No individual result was outside the recovery range 70-110%.
The required criteria for accuracy are met for SAN 835 H at each level of fortification and on the overall level.

15.2.2 Phthalazinone

The mean recovery rate for phthalazinone is 95.1 % with a relative standard deviation of 8.6 % (n=5) at the level of fortification of 0.1 mg/kg. At the limit of quantitation of the analytical method (0.01 mg/kg) the average recovery rate was 71.4 % with a relative standard deviation of 17.1% (n=5). Two individual results were outside the 70-110 % range (52.5% and 66.8% respectively).
The average recovery rate for both level of fortification is 83.3 % with a relative standard deviation of 19 %.
The analytical method for the quantitation of phthalazinone met the required criteria for accuracy.

15.2.3 Carbamoyl phthalazinone

Phthalazinone (M1 metabolite of SAN 835 H) was recovered from the samples fortified at the level of 0.1 mg/kg with carbamoyl phthalazinone with a mean rate of 99 % and a relative standard deviation of 6.6 %.
This proves that carbamoyl phthalazinone is quantitatively converted to phthalazinone during the analytical procedure.
The analytical method described in this report allows the determination of all phthalazinone residues (M1 and M5 metabolites) as phthalazinone.

---

[1] detections different from 0 on a 50 % confidence level.
[2] EU Guideline 94/43/EC (Uniform principles) Annex VI to 91/414/EEC

15.2.4 Conversion of SAN 835 H to phthalazinone residues

An average amount of 0.0024 ± 0.0035 mg/kg (n=5) of phthalazinone was found in the samples fortified with SAN 835 H only at the level of 10 time the limit of determination or 0.1 mg/kg. It accounts for 4.1 ± 5.8 % of the level of SAN 835 H added. But this amount is far below the actual limit of detection of the analytical sequence [1] (0.0063 mg/kg in this case) and therefore is not significant.

The recovery rates for SAN 835 H and for phthalazinone in the samples fortified with both compounds were both in the acceptable range of 70-110%.

It can be concluded from this that the amount of SAN 835 H that may be converted to phthalazinone during the analytical procedure described in the report is marginal and do not affect the accuracy of the analytical method.

15.3 Repeatability

The repeatability is defined as the value below which the absolute difference between two single results obtained under repeatability conditions may be expected to lie with a probability of 90 % [3].

The acceptance criteria for a residue analytical method are an absolute difference of 0.005 mg/kg (50%) at the 0.01 mg/kg level and an absolute difference of 0.025 mg/kg (25%) at the 0.1 mg/kg level [2].

Table 6: *Repeatability data of the analytical method*

		SAN 835 H			phthalazinone		
Fortification level on unidried soil (mg/kg)	Concentration added to dry soil (mg/kg)	standard deviation [1] (mg/kg)	repeatability absolute [1] [2] (mg/kg)	repeatability relative [3] (%)	standard deviation [1] (mg/kg)	repeatability absolute [1] [2] (mg/kg)	repeatability relative [3] (%)
0.1	0.12	0.0066 (n=4) 0.0057 (n=5) mean:	0.0151 0.0131 0.0141	13 11 12	0.0097	0.0223	19
		Trigger value [2]	≤ 0.025	≤ 25		≤ 0.025	≤ 25
0.01	0.012	0.0008 (n=5)	0.0019	16	0.0017	0.0038	32
		Trigger value [2]	≤ 0.005	≤ 50		≤ 0.005	≤ 50

1) calculated for dry soil — 0.1 mg/kg wet soil → 0.12 mg/kg dry soil; 50 g wet soil → 41.75 g dry soil)

2) calculated according to [4]

$$\text{repeatability} = 0.82 \cdot \sqrt{2} \cdot f \cdot \sigma \cong 2.296 \cdot \sigma$$

σ = standard deviation under repeatability conditions
f = factor depending on the number of tests and shape of the distribution (f ≅ 2)
√2 = multiplying factor to take into account that the difference between two single results is considered
0.82 = multiplying factor for a 90% probability

3) calculated by reference to nominal value (0.12 or 0.012 mg/kg)added per dry soil

$$\text{repeatability relative} = \frac{\text{repeatability absolute}}{0.12 \text{ (or } 0.012)} \times 100$$

[3] EU directive 91/414/EEC, Annexes II and III Commission document 4701/VI/94-EN, Rev:1-Feb-95

The repeatability of the analytical method was estimated [4] by comparison of the individual results of the analysis of 4 or 5 replicated samples under identical conditions (samples analyzed with the same equipment within the same analytical sequence by the same operator). The results are summarized in Table 6.

At the level of concentration of 0.1 mg/kg, there is a 90 % probability that the difference between 2 individual results will be 0.0141 mg/kg or less for SAN835 H and 0.0097 mg/kg or less for phthalazinone. This is below the trigger value of 0.025 mg/kg for the absolute repeatability (25% relative repeatability).
At the level of concentration of 0.01 mg/kg, the probability is 90 % for 2 individual results to differ by 0.0019 mg/kg or less for SAN 835 H and 0.0017 mg/kg or less for phthalazinone. This is below the trigger value of 0.005 mg/kg for the absolute repeatability (50% relative repeatability).

For both compounds and at both levels, the repeatability falls well within the range required for a residue analytical method.

15.4 Specificity

15.4.1 SAN 835 H

The specificity of the analytical method for the determination of SAN 835 H is achieved by the retention time on the HPLC column and the UV spectrum.

The retention times of SAN 835 H on the HPLC column varied for less than 0.035 min (0.2%) during an analytical sequence of about 20 injections of samples extracts spaced out with external standards.

Additionaly, the UV spectrum in the wavelength range of 220-400 nm can be used for identification of the compound. The UV spectrum of SAN 835 H is displayed in Figure 8.
The UV spectra obtained on the apex of the SAN835H peak on the HPLC chromatograms from sample extracts acquired using a DAD detector were compared with the UV spectrum of SAN 835 H obtained from a standard at 0.1 µg/ml (equivalent to a concentration of 0.01 mg/kg in sample extract). Results are listed in Table 7.
Positive identification could be achieved at the level 0.1 mg/kg : the agreement between sample and standard UV-spectra was above 99 %. At the level 0.01 mg/kg, the agreement between sample and standard UV-spectra ranged from 66.2 % to 90.7 %.
At both levels, the agreements of the spectra of fortified samples with the UV spectrum of SAN835H were superior to those of the control samples.

The specificity of the method may depend on the soil matrix. The trigger percentage for accepting or excluding a result at the limit of determination by virtue of the agreement of its UV spectrum with the reference UV-spectrum of SAN835 H must be established for each matrix. This can be achieved by comparing with the reference UV spectrum of SAN 835 H, UV-spectra of control samples and fortified control samples at the limit of determination.

---

[4] International Standard ISO 5725
Precision of test methods. Determination of repeatability and reproducibility for a standard test method by inter-laboratory tests. Second Edition 1986.

Table 7 : ***Agreement of peak apex UV spectrum in sample HPLC chromatogram with SAN 835 H UV spectrum***

fortification level (mg SAN 835 H/kg)	Agreement of UV spectra (%)
	44.1
0.1	99.8
0.1	99.8
0.1	99.8
0.1	99.9
	33.5/ 39.6
0.01	90.0
0.01	90.7
0.01	88.3
0.01	66.2
0.01	79.4

15.4.2 Phthalazinone

Retention time on the GC column and if needed acquisition of the mass spectrum of the compound ensures specificity of the analytical method for the determination of phthalazinone.

The retention times of phthalazinone on the GC column varied for less than 0.02 min (0.22%) during an analytical sequence of about 20 injections of samples extracts spaced out with external standards.

With this analytical method, quantitation of phthalazinone was performed using the Single Ion Monitoring acquisition mode on the GC/MS instrument. The ion at a mass of 161 g/mol, molecular ion for phthalazinone, was monitored. This ion is specific for phthalazinone. Additionaly, if needed, positive identification of the compound can be achieved using a scan acquisition mode on the GC/MS in the same conditions in order to get the full mass spectrum. The mass spectrum of a standard of phthalazinone is displayed in Figure 7.

The specificity of the method may depend on the soil matrix. The trigger percentage for accepting or excluding a result at the limit of determination by virtue of the agreement of its mass spectrum with the reference mass spectrum of phthalazinone must be established for each matrix. This can be achieved by comparing with the reference mass spectrum of phthalazinone, mass spectrum of control samples and control samples fortified at the limit of determination.

16. CONCLUSION

It is concluded that the residue method described in this report has been demonstrated to determine residues of SAN 835 H and of phthalazinone in soil with the sensitivity, accuracy, selectivity and repeatability required.

The limit of quantitation has been validated to be 0.01 mg/kg for SAN 835 H and 0.01 mg/kg for phthalazinone.

Figure 1: ***Representative calibration curves for SAN 835 H***

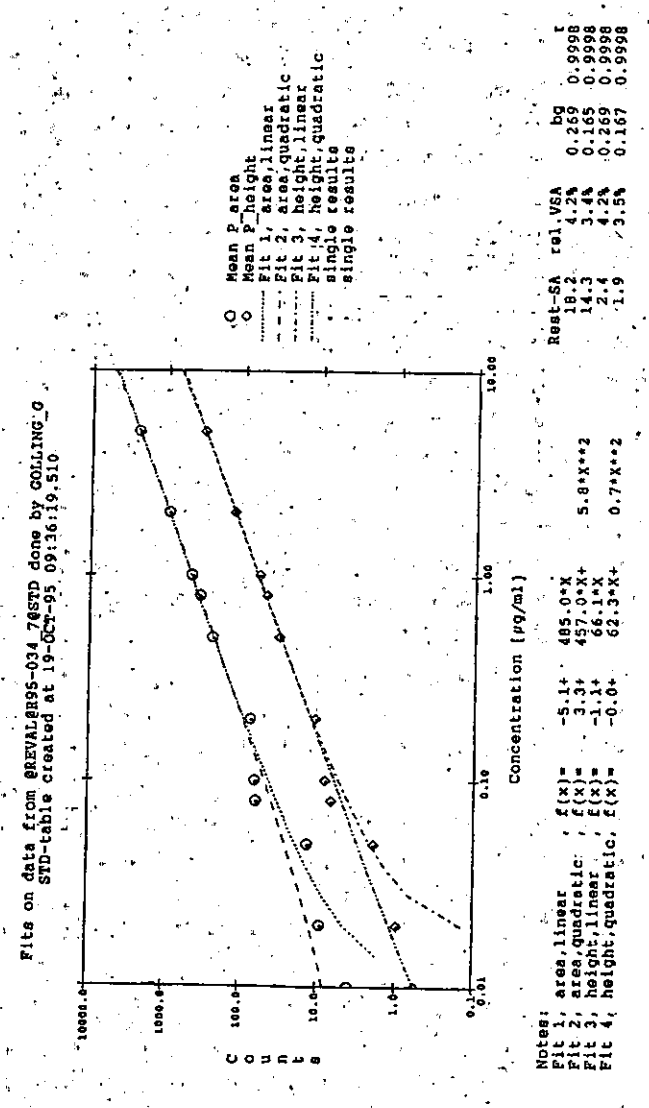


Figure 2: ***Representative calibration curves for phthalazinone.***

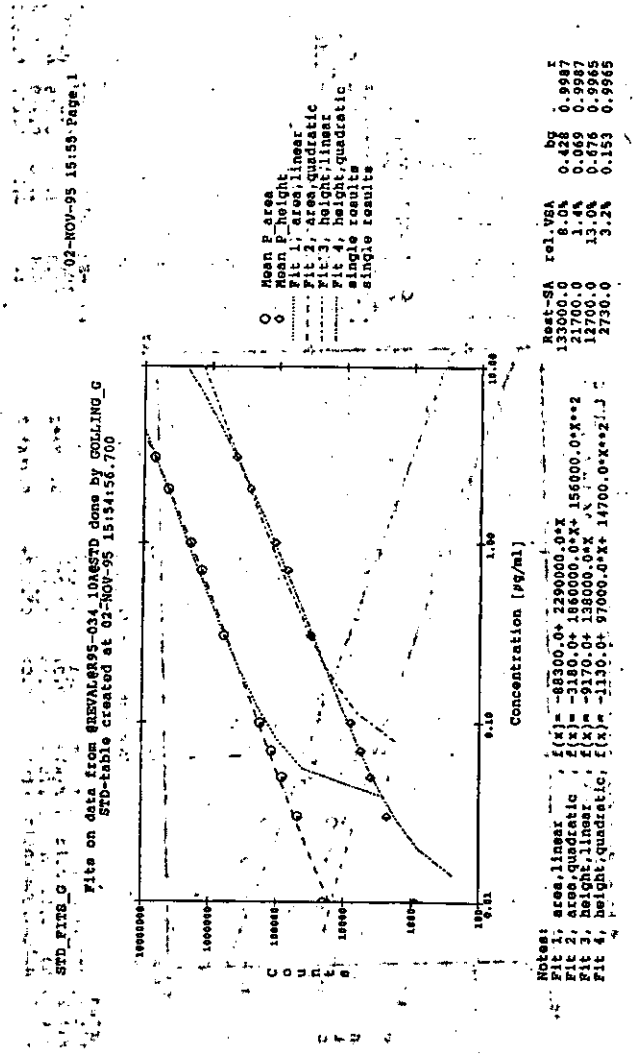


Figure 3: ***Representative chromatograms of a standard solution of concentration 0.1 µg/ml (equivalent to concentration in final extract of a sample containing 0.01mg/kg)***

a) HPLC chromatogram of SAN835 H standard solution

```
Data File c:\HPCHEM\1\DATA\R95-034.11\008-0801.D   Sample Name: STD 0.1 UG/ML

   STD 0.1 UG/ML

   ------------------------------------------------------------------------
   Injection Date  : 11/7/95 5:40:18 PM          Seq. Line :   8
   Sample Name     : STD 0.1 UG/ML                  Vial :   8
   Acq. Operator   : GOLLING_G                      Inj :   1
                                                Inj Volume : 250 µl

   Sequence File   : X:\R95-034.11\S.S
   Acq. Method     : C:\HPCHEM\1\METHODS\SAN835B.M
   Last changed    : 11/7/95 1:27:59 PM by GOLLING_G
   Analysis Method : C:\HPCHEM\1\METHODS\SAN835B.M
   Last changed    : 11/8/95 8:19:57 AM by GOLLING_G
   Solvent A: 0.2% TFA IN WATER.                                  Solvent
   B: acetonitril.                                              Method for
   SAN 835H  -  column BDS hypersil C18 - # 291
```

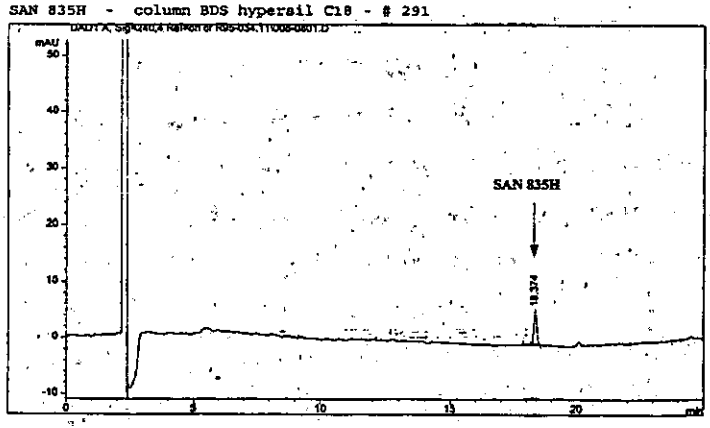


Figure 3: Representative chromatograms of a standard solution of concentration 0.1 µg/ml (equivalent to concentration in final extract of a sample containing 0.01mg/kg)
b) GC/MS chromatogram (SIM, m/z = 161) of phthalazinone standard solution

```
Information from Data File:
File          : C:\HPCHEM\1\DATA\9503410A\1101016.D
Operator      : GOLLING_G
Acquired      : 30 Oct 95   11:23 pm using AcqMethod MSD_M1
Sample Name:  STD 0.1 ug/ml
Misc Info     :
Vial Number:  11
CurrentMeth:  C:\HPCHEM\1\METHODS\MSD_M1.M
```

#	Compound	Ret Time	Signal	Response
1	PHTHALAZINONE (height)	8.93	161.0	8020
2	PHTHALAZINONE (area)	8.93	161.0	174459

END OF REPORT

Mon Oct 30 23:36:34 1995

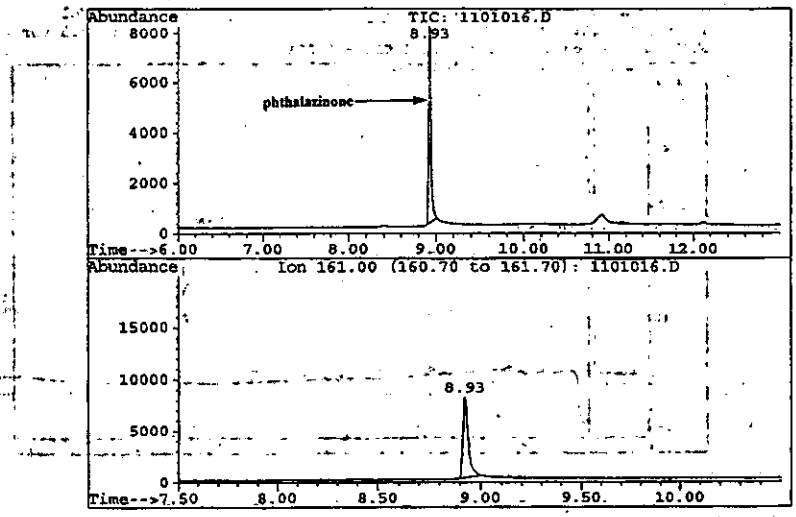


Figure 4: ***Representative chromatograms of untreated soil***

a) HPLC chromatogram; analysis for SAN 835 H

```
a File c:\HPCHEM\1\DATA\R95-034.7\002-0201.D  Sample Name: ANR.9079 UA01/0

ANR.9079 UA01/0B/1,0-10CM,M=41.75,A=16.7,E=2,V=1

=================================================================
Injection Date  : 10/18/95 5:01:35 PM          Seq. Line :  2
Sample Name     : ANR.9079 UA01/0                  Vial :  2
Acq. Operator   : GOLLING_G                        Inj :  1
                                              Inj Volume : 250 µl

Sequence File   : X:\R95-034.7\S.S
Method          : C:\HPCHEM\1\METHODS\SAN835B.M
Last changed    : 10/18/95 4:21:33 PM by GOLLING_G
Solvent A: 0.5% TFA IN WATER.                                  Solvent
B: acetonitril.                                              Method for
SAN 835H  -  column BDS hypersil C18 - # 291
```

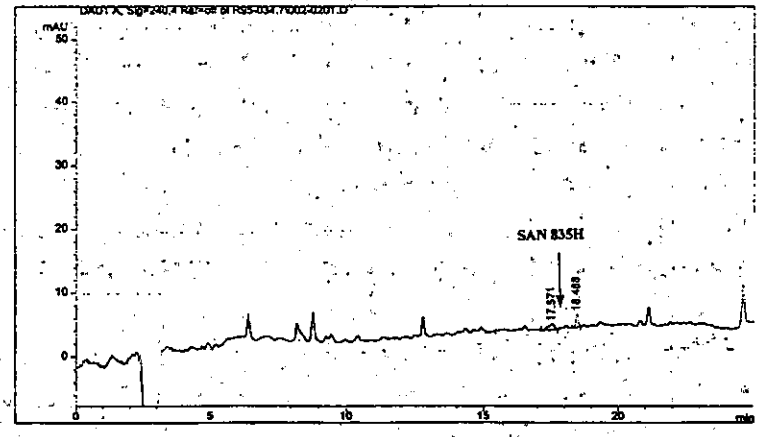


Figure 4: Representative chromatograms of untreated soil
b) GC/MS chromatogram (SIM m/z= 161) ; analysis for phthalazinone

```
Information from Data File:
File       : C:\HPCHEM\1\DATA\R95034.8\0201002.D
Operator   : G.GOLLING
Acquired   : 18 Oct 95   4:43 pm using AcqMethod MSD_M1
Sample Name: ANR.9079 UA01/0B/1,0-10CM
Misc Info  : UA01/0B/1, 0-10 cm
Vial Number: 2
CurrentMeth: C:\HPCHEM\1\METHODS\MSD_M1.M
```

#	Compound	Ret Time	Signal	Response
1	PHTHALAZINONE (height)	9.00	161.0	1144
2	PHTHALAZINONE (area)	9.00	161.0	44137

END OF REPORT

Wed Oct 18 16:56:57 1995

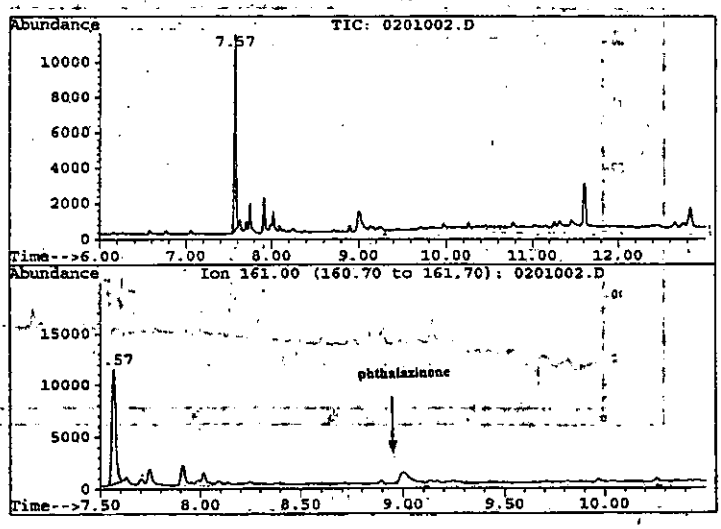


Figure 5: ***Representative chromatograms of soil sample spiked with 0.1 mg/kg SAN 835 H and 0.1 mg/kg phthalazinone***

a) HPLC chromatogram; analysis for SAN 835 H

```
File c:\HPCHEM\1\DATA\R95-034.7\006-0601.D  Sample Name: ANR.9081 UA01/0

ANR.9081 UA01/0B/1,0-10CM,M=41.75,A=16.7,Z=5,E=2.1,V=1

================================================================================
Injection Date  : 10/18/95 7:24:36 PM          Seq. Line :  6
Sample Name     : ANR.9081 UA01/0                  Vial :  6
Acq. Operator   : GOLLING_G                        Inj :  1
                                              Inj Volume : 250 µl

Sequence File   : X:\R95-034.7\S.S
Method          : C:\HPCHEM\1\METHODS\SAN835B.M
Last changed    : 10/18/95 4:21:33 PM by GOLLING_G
Solvent A: 0.5% TFA IN WATER.                                    Solvent
B: acetonitril.                                                  Method for
SAN 835H  ;  column BDS hypersil C18 - # 291
```

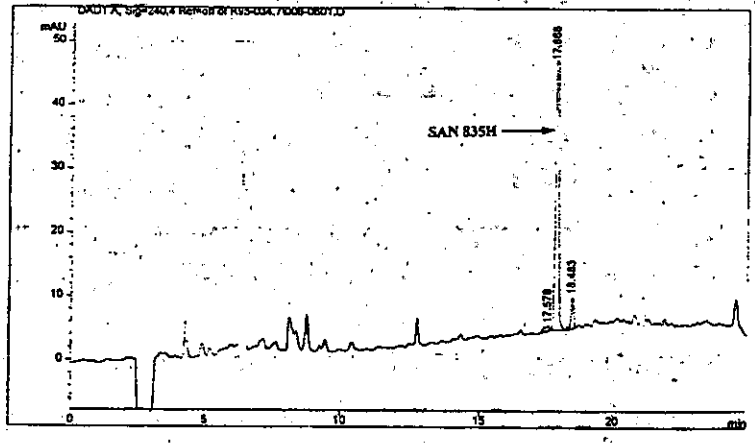


Figure 5: Representative chromatograms of soil sample spiked with 0.1 mg/kg SAN 835 H and 0.1 mg/kg phthalazinone
b) GC/MS chromatogram (SIM m/z= 161) , analysis for phthalazinone

```
Information from Data File:
File       : C:\HPCHEM\1\DATA\R95034.8\0401004.D
Operator   : G.GOLLING
Acquired   : 18 Oct 95   5:20 pm using AcqMethod MSD_M1
Sample Name: ANR:9080 UA01/0B/1,0-10CM
Misc Info  : UA01/0B/1, 0-10 cm
Vial Number: 4
CurrentMeth: C:\HPCHEM\1\METHODS\MSD_M1.M
```

#	Compound	Ret Time	Signal	Response
1	PHTHALAZINONE (height)	8.99	161.0	127204
2	PHTHALAZINONE (area)	8.99	161.0	2773692

END OF REPORT

Wed Oct 18 17:34:00 1995

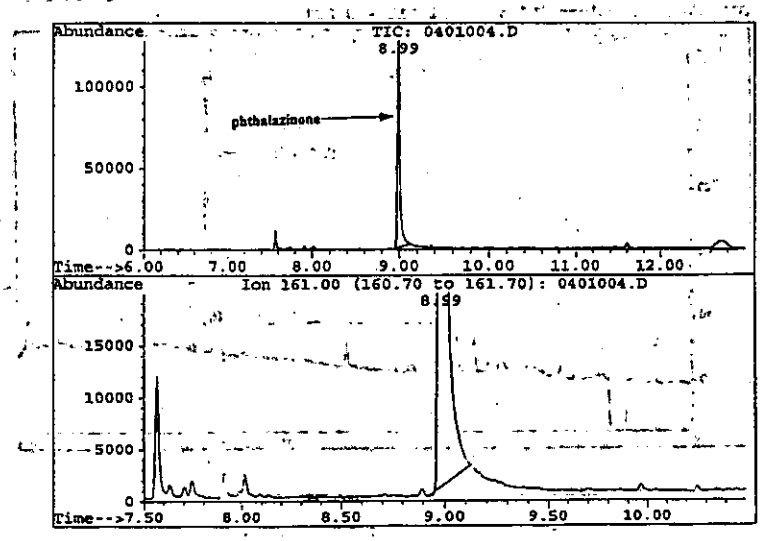


Figure 6: ***Representative chromatograms of soil sample spiked with 0.01 mg/kg SAN 835 H and 0.01 mg/kg phthalazinone.***

a) HPLC chromatogram; analysis for SAN 835 H

```
i File c:\HPCHEM\1\DATA\R95-034.9\004-0401.D  Sample Name: ANR.9178 UA01/0

ANR.9178_UA01/0B/1,0-10CM,M=41.75,A=16.7,Z=0.5,E=2,V=1


================================================================
Injection Date  : 10/20/95 5:58:45 PM          Seq. Line :  4
Sample Name     : ANR.9178 UA01/0                   Vial :  4
Acq. Operator   : GOLLING_G                         Inj :  1
                                              Inj Volume : 250 µl

Sequence File   : C:\HPCHEM\1\DATA\R95-034.9\S.S
Acq. Method     : C:\HPCHEM\1\METHODS\SAN835B.M
Last changed    : 10/20/95 4:02:20 PM by carrier mn
Analysis Method : C:\HPCHEM\1\METHODS\SAN835B.M
Last changed    : 12/28/95 10:32:53 AM by GOLLING_G
Solvent A: 0.2% TFA IN WATER.                          Solvent
B: acetonitril.                                       Method for
SAN 835H  -  column BDS hypersil C18 - # 291
```

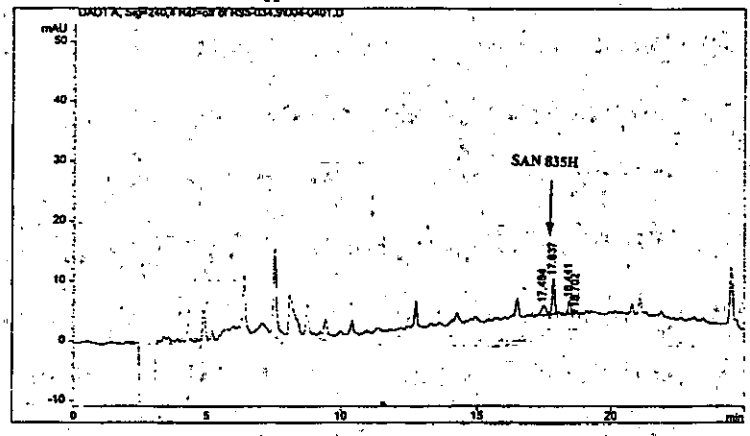


Figure 6: Representative chromatograms of soil sample spiked with 0.01 mg/kg SAN 835 H and 0.01 mg/kg phthalazinone

b) GC/MS chromatogram (SIM m/z= 161) , analysis for phthalazinone

```
Information from Data File:
File       : C:\HPCHEM\1\DATA\9503410A\1001015.D
Operator   : GOLLING_G
Acquired   : 30 Oct 95  11:04 pm using AcqMethod MSD_M1
Sample Name: ANR.9181 UA01/0B/1,0-10CM
Misc Info  : UA01/0B/1, 0-10 cm
Vial Number: 10
CurrentMeth: C:\HPCHEM\1\METHODS\MSD_M1.M
```

#	Compound	Ret Time	Signal	Response
1	PHTHALAZINONE (height)	8.93	161.0	6825
2	PHTHALAZINONE (area)	8.93	161.0	137662

END OF REPORT

Mon Oct 30 23:18:02 1995

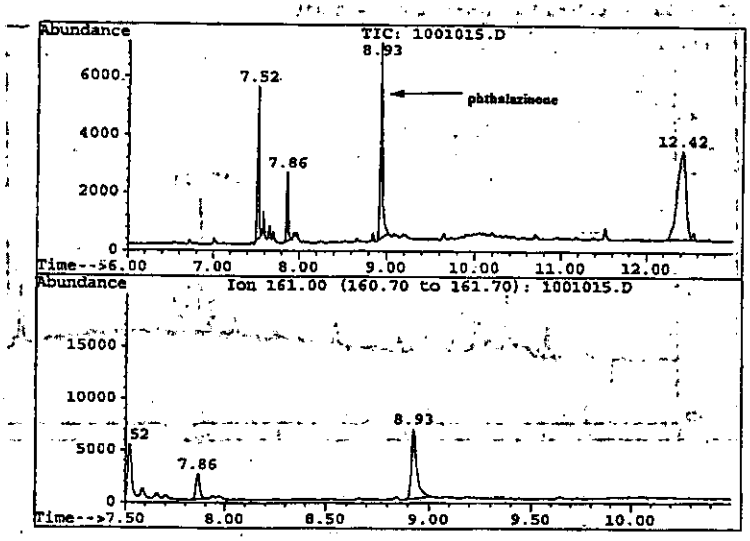
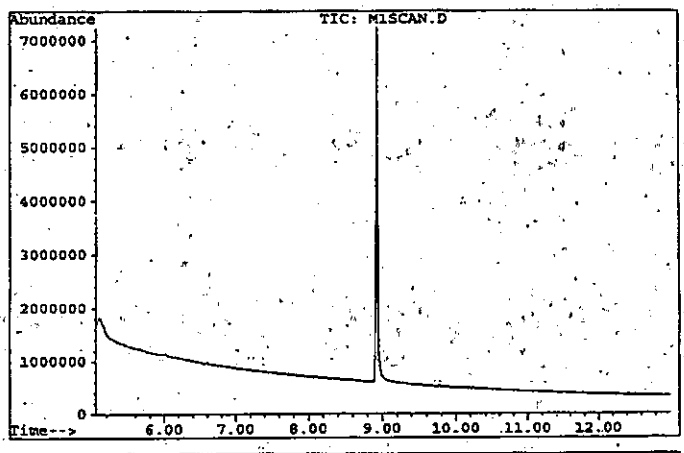


Figure 7: ***Mass spectrum of phthalazinone***

```
File       : C:\HPCHEM\1\DATA\M1\M1SCAN.D
Operator   : MN CARRIER
Acquired   : 6 Nov 95   1:00 pm using AcqMethod SCAN_M1
Instrument : 5972 - In
Sample Name: PHTHALAZINONE M1 10 UG/ML
Misc Info  :
Vial Number: 1
```



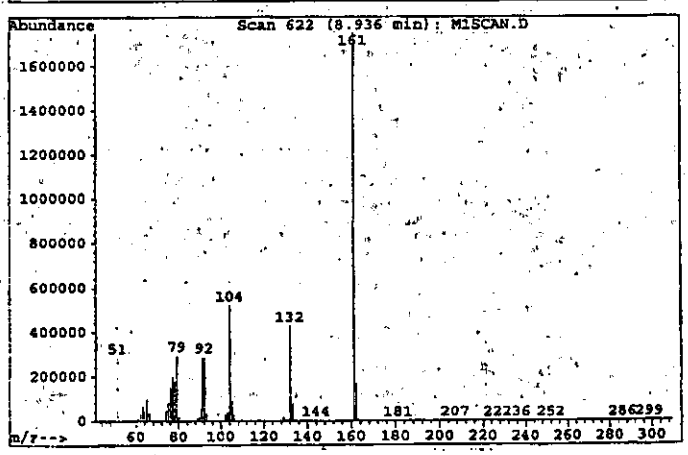
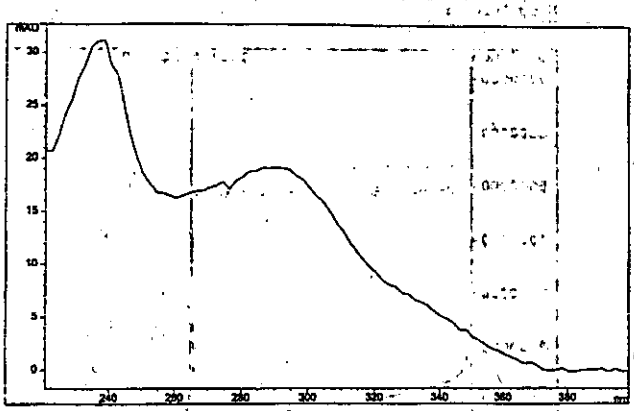


Figure 8: *UV spectrum of SAN 835 H*

Library :SAN835.uvl

:(1) STD 1 UG/ML



name : STD 1 UG/ML
Time [min] : 17.87
ID# : 0
ID Name : SAN 835 H
Info :
Sample : STD 1 UG/ML
Solvent :

created : 28-DEC-1995 created by : GOLLING_G
changed : 28-DEC-1995 changed by :

File : C:\HPCHEM\1\DATA\R95-034.7\014-1401.D
Time [min] : 17.872
scan# : 42
Range [nm] : 220.5 - 398.5 (step : 2.0)
[mUA] : -0.2 - 31.1

Instrument : DAD1 Instrument Revision :
Cell Length [mm] : 0 Software : LC-UVLibrary G130x
Volume [ul] : 0 Software Revision : Rev. A.01.00
Slit Width [nm] : 0

*** End of Report ***