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BASF CORPORATION
Agricultural Products
P.O. Box 13528
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Study Title:
Validation of BASF Method 343/1 for the Determination of BAS 9075 I (Hexythiazox) in Dried Hops

Data Requirement:
EPA Guideline Numbers 171-4C

Author:
Dr. N. Tilting

Study Completed On:
December 1995

Performing Laboratory:
BASF Aktiengesellschaft
Ecology and Environmental Analytics
Environmental Chemistry
Postfach 120
D-67114 Limburgerhof

Registration Document No. BASF:
95/11149

This Report Consists of 49 Pages

95/11149 0001

PR 86-5 DATA CONFIDENTIALITY CLAIM

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA 10 (d) (1) (A), (B) or (C).

Company: BASF Corporation, Agricultural Products

Company Agent: Rodney C. Akers Date: Feb 6, 1996

Title: Registration Scientist Signature: Rodney C. Akers

PR 86-5 GOOD LABORATORY PRACTICE CERTIFICATION

This study does not meet the requirements for 40 CFR 160, Good laboratory Practice Standards and differs in the following way:

1. This study was performed in accordance with the GLP-Regulation of Appendix 1 to § 19a, Section 1, Chemikaliengesetz of 25-July-1994 (Official Bulletin/Federal Republic of Germany, I 1994, p. 1703).

Study Director: See page 6

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Sponsor/ Submitter

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Validation of BASF method 343/1 for the determination of BAS 9075 I (hexythiazox) in dried hops

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Author

Dr. N. Tilting

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Testing facility

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Mainz, 20. Dezember 1994

G L P - B E S C H E I N I G U N G

Bescheinigung

Hiermit wird bestätigt, daß die Prüfeinrichtung

APS/U
der Firma BASF AG
- Landwirtschaftliche Versuchsstation -

67114 Limburgerhof

Bundesrepublik Deutschland

am 01. und 14. September 1993 und vom 15.-16. Dezember 1993 von der für die Überwachung zuständigen Behörde über die Einhaltung der Grundsätze der Guten Laborpraxis inspiziert worden ist.

Es wird hiermit bestätigt, daß die Prüfungen in dieser Prüfeinrichtung nach den Grundsätzen der Guten Laborpraxis durchgeführt werden.

Im Auftrag

Dr. Fresenius

Certificate

It is hereby certified that the test facility

APS/U
der Firma BASF AG
- Landwirtschaftliche Versuchsstation -

67114 Limburgerhof

Federal Republik of Germany

at 01. and 14. September 1993 and from 15.-16. December 1993 was inspected by the competent authority regarding compliance with the principles of Good Laboratory Practice.

It is hereby certified that studies in this test facility is conducted in compliance with den principles of Good Laboratory Practice.

Anlage zur GLP-Bescheinigung für die Prüfeinrichtung APS/U

Die Prüfeinrichtung führt in Übereinstimmung mit dem GLP-Grundsätzen Prüfungen in folgenden Prüfkategorien durch:

1. Prüfungen auf physikalisch-chemische Eigenschaften und Gehaltsbestimmungen
4. Umwelttoxikologische Prüfungen zu Auswirkungen auf aquatische und terrestrische Organismen
5. Prüfungen zum Verhalten im Boden, Wasser und in der Luft; Bioakkumulation; Metabolismus
6. Prüfungen auf Rückstände
7. Prüfungen zu Auswirkungen auf Mesokosmen und natürliche Ökosysteme
8. Analytische Untersuchungen an biologischen Materialien

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE

The study was conducted in compliance with Good Laboratory Practice Regulations; Appendix 1 to § 19 a, Section 1, Chemikaliengesetz of 25th July 1994 (Official Bulletin/Federal Republic of Germany, I 1994, p. 1703).

Study Director:

Date: 22.12.95 Signature: Tilking

B A S F Aktiengesellschaft

BASF Landw. Versuchsstation - Postfach 120 - 67114 Limburgerhof

STATEMENT OF THE QUALITY ASSURANCE UNIT

Study Code: 30796

The quality assurance unit of the testing facility inspected the study plan, study and audited the final report. Findings were reported to the study director and to management.

Date of inspections	Report to study director and to management
11-Jan-1995	
02-Feb-1995	
18-Dec-1995	

67114 Limburgerhof

Scheper 19.12.95

Signature QAU

Quality Assurance Statement

I hereby attest to the authenticity of the study and guarantee that the data are correct and accurate to the best of our knowledge and that the study was performed by the procedures described.

Study Director and Author:

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APS/UT
BASF Aktiengesellschaft
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Postfach 120
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Date: 20.12.95

Signature: Tilting

SUMMARY

The residue analytical method 343 for the gas chromatographic determination of hexythiazox was modified to improve performance of dry hops analysis. A GPC clean-up step was added to remove extracted plant material. It is possible to quantify residues of hexythiazox and its major metabolites. The analytes were extracted with methanol and cleaned-up by partitioning and column chromatography on florisil/charcoal. On injection into the hot injection port of the gas chromatograph the metabolite PT 1-3 was formed (T = 300 °C) in almost quantitative yield which was quantified against a PT 1-3 calibration standard.

The limit of quantitation of the method was 0.5 mg/kg for dried hops. The mean recovery at the limit of quantitation was 102.8 % with a coefficient of variation of 19%.

Samples fortified with hexythiazox metabolites PT 1-3, PT 1-8 and PT 1-9 at the 2 mg/kg level were also analysed according to the modified method. Mean recoveries ranged from 80.7 % to 103.1% with coefficients of variation from 2.2 to 5.1 (n = 4).

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GENERAL

Performing Laboratory

BASF Aktiengesellschaft
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Guidelines covered

EPA Guidelines Subdivision O, 171-4c.
GLP Consensus Document: The Application of the GLP Principles to Field Studies, Env. Monograph No. 50, OECD, Paris 1992
For Good Laboratory Practice: Appendix 1 to § 19a, Section 1, Chemikaliengesetz of 25 July 1994 (Official Bulletin, Federal Republic of Germany, I 1994, p 1703)

Location of raw data
Archive belonging to APS/U, building Li 444

Filing of reports, raw data, samples and specimen

Study plan, raw data, original report or certified duplicates, samples and specimen, will be stored in an archive at BASF Aktiengesellschaft, Landwirtschaftliche Versuchsstation, D-67117 Limburgerhof, at least for the period of time specified in the GLP regulations.

Schedule

Protocol signed:	January 1995
Start of experimental part:	January 1995
Completion of experim. part:	June 1995
Final report:	December 1995

Technical staff Manfred Metzger

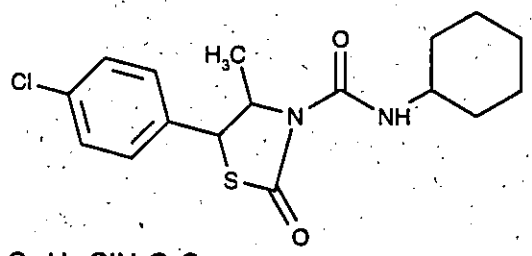
Test system Dried hops

1. TEST SUBSTANCES

1.1 Test Substances Used for Fortification

Hexythiazox

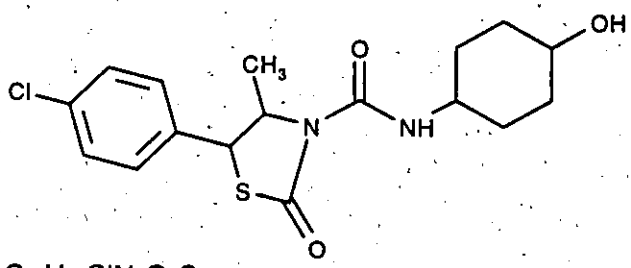
Common name: Hexythiazox
Code name: Reg. No. 282502
Chemical name: (4RS,5RS)-5-(4-chlorophenyl)-N-cyclohexyl-4-methyl-2-oxo-1,3-thiazolidine-3-carboxamide
Structural formula:



Empirical formula $C_{17}H_{21}ClN_2O_2S$
Molecular weight: 352.89
Solubility: Soluble in n-hexane, methanol, dichloromethane

PT 1-8

Code name: PT 1-8, Reg. No. 248650
Chemical name: (4RS,5RS)-5-(4-chlorophenyl)-N-4-hydroxycyclohexyl-4-methyl-2-oxo-1,3-thiazolidine-3-carboxamide
Structural formula:

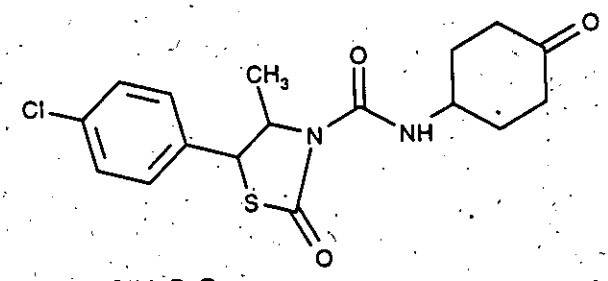


Empirical formula: $C_{17}H_{21}ClN_2O_3S$
Molecular weight: 368.99

PT 1-9

Code name : PT 1-9, Reg. No. 248667
Chemical name: 5-(4-chlorophenyl)-N-4-oxo-cyclohexyl-4-methyl-2-oxo-1,3-thiazolidine-3-carboxamide

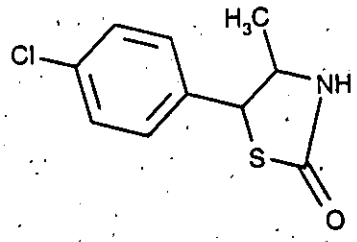
Structural formula:



Empirical formula: $C_{17}H_{19}ClN_2O_3S$
Molecular weight: 366.87

1.2 Test and Reference Substance (PT 1-3)

Code name: PT 1-3, Reg. No. 248649
Chemical name: trans-5-(4-chlorophenyl)-4-methyl-2-oxothiazolidone
Structural formula:



Molecular formula: $C_{10}H_{10}ClNOS$
Molecular weight: 227.7
Solubility: Soluble in acetone, dichloromethane, methanol
slightly soluble in n-hexane

1.3 Principle of the Method

Samples are extracted with methanol. After n-hexane partition, GPC and Florisil column clean-up, the residue of hexythiazox is determined by GC using NPD-detection. On injection, hexythiazox is pyrolized to PT 1-3, which is used as reference compound for the determination.

2. MATERIALS AND METHODS

Equipment and reagents in the following lists are examples and can be replaced by equivalent ones. (Suppliers, batch no., etc describe products that were in use at BASF's laboratories during method development.)

2.1 Equipment

Glass bottle (wide neck)	500 ml
Round bottom flasks with standard ground joint 29	100, 250 and 500 ml
Büchner funnels	90 mm i.d.
Separatory funnels	500 ml and 1000 ml
Funnels	glass, 90 mm i.d., 30 mm
Chromatography columns	glass, 20 mm i.d.
Tapered flasks with standard ground joint 14.5	10 ml
Volumetric pipettes	
Volumetric flasks	10 ml, 50 ml, 100 ml
Glass syringe 10 ml luer-fitting	
Round filters	90 mm i.d. , Art. No. 300 009, Schleicher & Schüll, Dassel, FRG
Rotary evaporator with water bath	e.g. Rotavapor, Büchi, Flawil, CH
Vacuum controller and pump	e.g. MZZC, Vacuubrand GmbH & Co, Wertheim, FRG
Instrumentation for GPC	
GPC Autoprep 1002A	Analytical Bio-Chemistry Laboratories Columbia M.O. 65205, USA
Column	Analytical Bio-Chemistry
Stationary phase	Bio Bead, S-X3, Bio Rad Laboratories, Richmond, CA 9404, USA 8
Filter: Millex-HV 0.45 µm	Millipore, SLHV025NB, Millipore Corp., Bedford M.A. 01730, USA

2.2 Reagents and Standards

It is recommended to use pesticide grade solvents.

Acetone dist.	
Cyclohexane	
Dichloromethane dist.	
Ethylacetate	
n-Hexane	
Methanol dist.	
Celite	Art. No. 16391, Serva, Heidelberg, FRG
Florisil, 0.15 - 0.25 mm	Art. No. 81534, Macherey & Nagel, Düren,FRG
Sodium sulfate	
Cotton wool	
Activated charcoal (powdered)	Merck, Darmstadt, FRG

Solutions and Solvent Mixtures

Dichloromethane + acetone 83 + 17 (v/v)
Cyclohexane + ethylacetate 1 + 1 (v/v)

Standard substance for fortification

Hexythiazox (structural formula see 1.1)

Reg. No.	282 502	282 502
Purity	> 99.9%	98.9%
Lot No.	L43-251	2EI 72D

PT 1-8 (structural formula see 1.1)

Reg. No.	248 650
Purity	99.9%
Lot No.	L43-265

PT 1-9 (structural formula see 1.1)

Reg. No.	248 667
Purity	99.9%
Lot No.	L43-267

Standards supplied by Dr. Roschger, BASF Aktiengesellschaft, APS/UD, 67114 Limburgerhof, Phone 0621/60-27103.

Preparation of standard solutions for fortification (example other dilution procedures can also be used):
Dissolve 10.0 mg hexythiazox in 100 ml methanol to yield a solution of 100 µg/ml. Dilute 1 ml of this solution with 99 ml of methanol to get a solution of 1 µg/ml. Use appropriate dilution for spiking, depending on sample weight and spiking level. Spike samples for fortification purposes prior to the extraction step (3.2.1)

Standard substance for Calibration

PT 1-3 (structural formula see 1.2)

Reg. No.	248 649
Purity	99.9 %
Lot No.	L43-255

Standards supplied by Dr. Roschger, BASF Aktiengesellschaft, APS/UD, 67114 Limburgerhof, Phone 0621/60-27103.

Preparation of standard solutions for GC calibration (recommended procedure)

Dissolve 10 mg of PT 1-3 in 10 ml of acetone to yield a 1 mg/ml solution. Dilute 1 ml of this 1 mg/ml solution to 100 ml to yield a 10 µg/ml solution.

To prepare calibration standards in the range of 0.25 µg/ml to 2.0 µg/ml proceed as follows (other concentrations can also be used):

Dilute 2.0 ml of the 10 µg/ml solution to 10 ml = 2.5 µg/ml ← should be 2.0 µg/l
Dilute 1 ml of the 10 µg/ml solution to 10 ml = 1.0 µg/ml
Dilute 0.5 ml of the 10 µg/ml solution to 10 ml = 0.5 µg/ml
Dilute 1 ml of the 2.5 µg/ml solution to 10 ml = 0.25 µg/ml
All dilutions with ~~hexane + acetone~~ (9 + 1 v/v)

The solutions must be stored in a refrigerator when not in use. At 4°C in the dark Hexythiazox and PT 1-3 solutions are stable for 30 days (ref. 2 and ref. 3). PT 1-8 and PT 1-9 solutions were prepared fresh before spiking.

3. ANALYTICAL PROCEDURE

Note:
The symbols in brackets are identical with the ones used in the formula for the calculation of the analytical results (see 4.2.2). The analytical procedure is described for one sample.

3.1 Preparation of Samples

Dried hops must be powdered before taking the laboratory sample.
The samples have to be stored at appr. -20° C until analysis.

3.2 Clean-up Procedure

3.2.1 Extraction

Sample weight: hops (dried, powdered): 5 g
Weigh sample into a wide mouth glass bottle, add 200 ml methanol and macerate with an Ultra Turrax for 3 minutes. Place a filter paper covered with a 0.5 cm layer

of Celite into a Büchner funnel and filter the suspension. Wash the filter cake with 50 ml methanol.

3.2.2 Partition

Transfer the extract from 3.2.1 into a 1000 ml separatory funnel. Add 200 ml of sodium chloride solution (200 g/l) and extract twice with 50 ml n-hexane each. Filter ~~Filtrate~~ the n-hexane phase through a glass funnel plugged with cotton wool and sodium sulfate. Repeat the extraction with dichloromethane (2 times 50 ml), filter over sodium sulfate, wash the sodium sulfate with 50 ml dichloromethane and combine the organic phases. Evaporate the organic phase to dryness, discard the water phase.

3.2.3 Preparation of GPC-column

A mixture of cylcohexane + ethylacetate is used as mobile phase in GPC. To prepare the column, weigh 40 g of SX3 Bio-Bead into a flask and add the solvent mixture that the beads are completely covered by solvent. Allow to swell over night. Pour the gel into the column and adjust the column adapters so that they touch the gel surface. Connect column to GPC system. Remove air from the system according to operating instructions from the manufacturer. Readjust column adaptors. As the retention volume of the analyte is dependent on column performance test the elution parameters (collect time) for the compound of interest (see 6).

3.2.4 GPC column clean-up

Dissolve extract from 3.2.2 in 5 ml ethylacetate. Sonicate if necessary. Add 5 ml cyclohexane. To remove undissolved particles, place a small piece of cotton wool into a funnel (i.d. 3 cm) and filter the solution into a 10 ml volumetric flask. Rinse cotton wool with some drops of cyclohexane-ethylacetate mixture and make up to the mark.

Fill filtrate into a 10 ml glass syringe, place a 0,45 µm filter disk onto the luer lock fitting and fill the injection loop of the GPC instrument. Make sure not to inject air into the injection loop. Start chromatographic run according to the instructions of the instrument manufacturer. The instrument injects only 5 ml (50%) of the sample. With a GPC instrument prepared according to 3.2.3, the first 125 ml (25 min) must be dumped, the active ingredient elutes within the next 60 ml (12 min). The column should be washed for 10 min (50 ml) before injection of the next sample. Evaporate eluent to dryness.

3.2.5 Florisil column clean-up

Place a cotton wool plug at the lower end of a chromatographic column and fill the column with dichloromethane + acetone 83 + 17 (v/v). Pour 5 g Florisil into the column. After settling of the Florisil lower the solvent meniscus to the Florisil surface.

Mix 15 g of Florisil with 1 g of charcoal and suspend in 35 ml of the dichloromethane acetone mixture. Transfer the slurry to the column and allow to settle. Lower the solvent meniscus to the Florisil surface. Cover the Florisil surface with a small amount of cotton wool. Quantitatively transfer the residue from 3.2.4 onto the column using 3 times 5 ml dichloromethane. Lower the solvent meniscus to the florisil surface and elute with 100 ml dichloromethane + acetone 83 + 17 (v/v). Collect the eluate and evaporate to dryness. Dissolve the residue in 1 ml or other appropriate volume (= V_E) hexane + acetone (9 + 1 v/v).

3.3 Instrumentation and Operating Conditions

Gas chromatograph	Perkin Elmer 8420 ← NPD
Detector	(N)PD
Column	l = 30 m, i.d. = 0.32 mm, film = 0.25 µm
Stationary phase	DB 1701
Injection port temp.	300 ° C
Detector temperature	300 ° C
Oven program	Start: 160 ° C, 0 minutes rate: 6 ° / minute final temperature 250 ° C/ 10 minutes
Injection volume	1 µl
Carrier gas	Helium 1000 mbar
Retention time	
PT 1-3	14 - 17 min

3.4 Calibration Procedures

Calculation of results is based on peak height measurements using calibration curves. To obtain this standard curve inject 1 µl of standard solutions containing 0.25 to 2.0 µg/ml of PT 1-3 into the gas chromatograph.
For routine analysis at least every third injection should be a standard. Plot peak heights versus amount (ng) of injected standard. Hexythiazox and its metabolites are derivatized in the injection port to PT 1-3 so that calibration against PT 1-3 includes the derivatization efficiency.

3.5 Interferences

Interferences from the matrix, other pesticides, solvents or labware are not known to date.

3.6 Confirmatory Technique

To confirm the identity of the PT 1-3 peak in the chromatogram, GC-MS can be used. An example of a GC- MS run with NH_3 chemical ionization can be found in

figure 31. (non-GLP, not produced under this study code).

3.7 Potential Problems

There can be variations in the properties of the Florisil from different lots. Elution volume for column clean-up should be checked (see 6.).

It is recommended to condition the GC column by several injections of standard substance or control extract before starting the analytical series. The derivatization reaction in the injection port of the GC must also be checked, a dependence on injector geometry and filling with glass wool could be observed.

4. METHOD OF CALCULATION

4.1 Calibration

For establishing the calibration curves see 3.4.

4.2 Analyte in Samples

4.2.1 Principle

Calculation of results is based on peak height measurements. Determine the amount of substance measured in the injected volume from the calibration curves and use the equation described in 4.2.2. Calculation can also be achieved by a suitable computer program.

4.2.2 Calculation of Residues

The residues R in mg/kg are calculated as follows:

$$R = \frac{V_E \cdot W_A \cdot D}{G \cdot V_I \cdot 0.5}$$

V_E = Final volume (ml) after all dilution steps
G = Weight (g) of sample extracted
W_A = Amount of analyte (ng) read from calibration curve
V_I = Volume (µl) injected into GC
D = Derivatization factor
To convert PT 1-3 to hexythiazox the derivatization factor is 1.548. $\frac{353}{228}$
For the conversion of PT 1-3 to PT 1-8 the factor is 1.62 and for the $\frac{369}{228}$
conversion PT 1-3 to PT 1-9 it is 1.611 $\frac{367}{228}$
0.5 = Aliquotation factor from GPC injection

4.2.3 Calculation of Recoveries

Determine the recovery factor F from fortification experiments

$$\% \text{ Recovery} = \frac{(\text{mg/kg in fortified sample} - \text{mg/kg in control}) \cdot 100}{\text{mg/kg analyte added}}$$

$$F = \frac{100\%}{\% \text{ Recovery}}$$

To correct results for recoveries, multiply the result from 4.2.2 with the recovery factor. The formula for corrected residues is:

$$\text{Residue corrected for 100\% recovery} = \frac{V_E \cdot W_A \cdot D}{G \cdot V_I \cdot 0.5} \cdot F$$

5. RECOVERIES, ACCURACY, AND PRECISION

5.1 Limit of Quantitation

The limit of quantitation which is defined to be the lowest concentration for which accuracy and precision of the method was tested by recovery trials, is 0.5 mg/kg for dry hops. For hexythiazox two analytical series of two controls and two samples at 3 different spiking levels were run at different times. For the metabolites series of one control and four spiked samples were analysed. The results of the trials was listed in table 1. Details of the analytical series were listed in the raw data spread sheets of tables 2 to 7.

Table 1: Recoveries, accuracy and precision of the method

Compound added	Fort.level (mg/kg)	Recoveries (%)	Mean (%)	Std. Dev.	Coeff. of Variation
Hexythiazox	0.5	96.7; 83.1; 101.3; 130.0	102.8	19.7	19.2
	2.0	99.8; 90.1; 97.0; 92.6	94.9	4.3	4.6
	5.0	99.4; 93.2; 77.6; 84.1	88.6	9.6	10.9
PT 1-3	2.0	98.6; 108.5; 106.6; 98.5	103.1	5.3	5.1
PT 1-8*	2.0	81.9; 81.2; 81.6; 78.1	80.7	1.8	2.2
PT 1-9*	2.0	90.8; 85.7; 94.7; 86.5	89.4	4.2	4.7

* new GPC column

6. ADDITIONAL INFORMATION

During analytical work, the GPC column had to be refilled. The new column had a slightly different elution behaviour. The collect time had to be readjusted. The first 150 ml of the eluate (30 min run time) were dumped, the next 75 ml (15 min run time) were collected and the wash phase remained at 10 minutes.
Due to the varying activity of florisil the elution volume of the column described in 3.2.5 was changed to 150 ml (DCM+acetone 83 + 17).

6.1 Ruggedness Testing

Not performed.

6.2 Limitations

Not known to date.

7. REFERENCES

1. Determination of Hexythiazox and its Metabolites as PT 1-3, Institut Fresenius Method 0012, Januar 1988, BASF Fremdmethode 925 (Mai 1988)

2. Stability of Hexythiazox in Methanol, Acetonitrile and Acetonitrile/Water (1+1, v/v), BASF Report No. 3803, Dr. Ch. Mackenroth, April 1994, Reg. Doc. No. 94/10250

3. Stability of PT 1-3 in Acetone and n-Hexane, Dr. N. Tilting, December 1994, Reg. Doc. No. 94/11071

4. Determination of Hexythiazox and its Metabolites in Soil, Apples, Strawberries, Hops, Beer, Yeast and Brewing Wastes by GC with NPD (Method 343), BASF Reg. No. 3944, Dr. N. Tilting, April 1994, Reg. Doc. No. 94/10226

Appendix

Figure 1: Flow Chart of Analytical Procedure

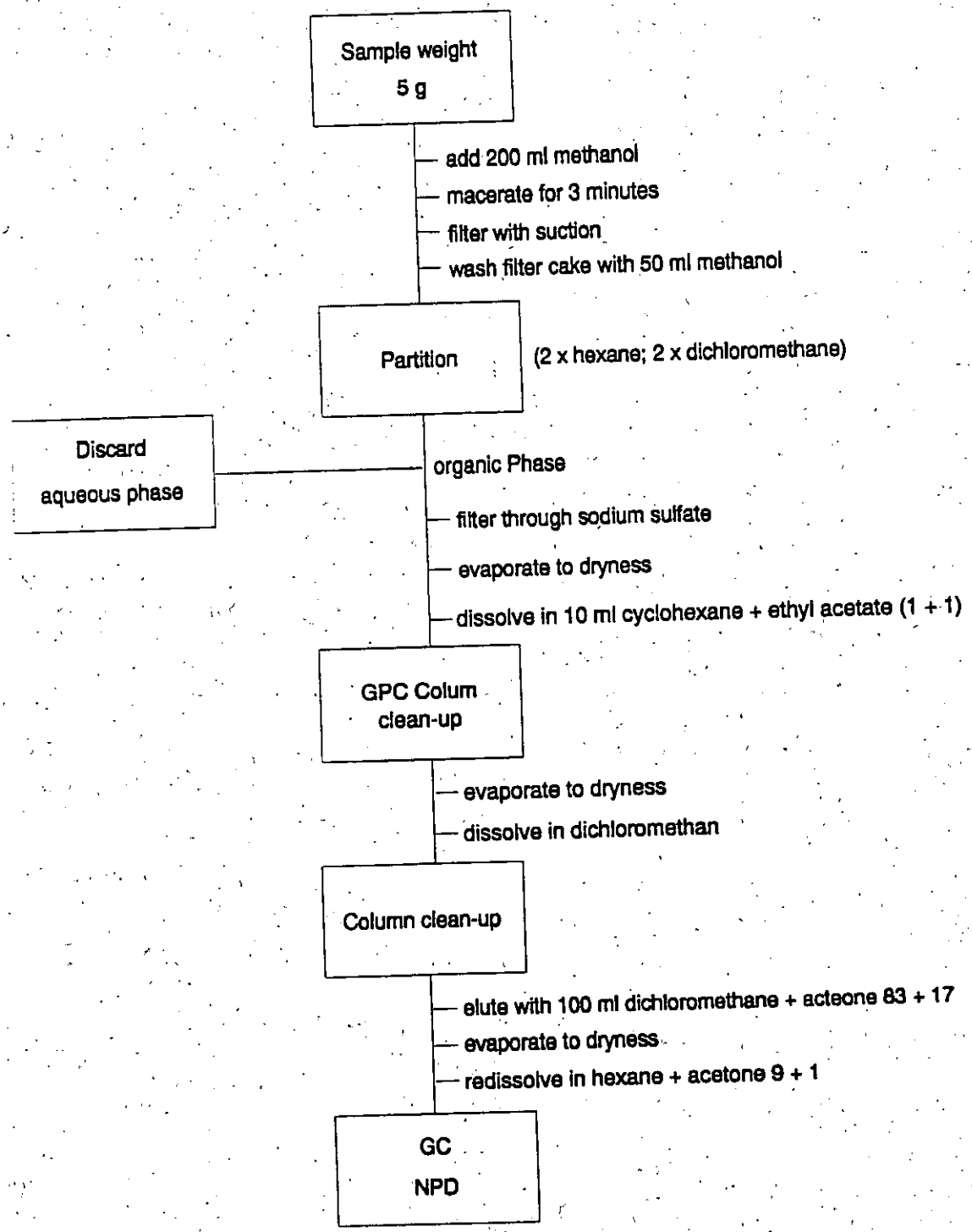


Table 2: Analytical raw data of analytical series ME95006

Study Code: 30796
Method validation with hexythiazox

Queuefile name: ME95006
Matrix:Dried hops

Lab Journal no.00842

Sample number	Type	Sample weight (g)	Final dilution(ml)	Sample injected (mg)	Peak height	Std. Conc. (µg/ml)	Substance injected (ng)	Residue (mg/kg)	Spiking level (µg)	Recovery %	Date of extraction	Date of measurem.
9500054	C*	5	1	2.5	159		0.07996	< 0.5			31.1.95	31.1.95
ME038	S				581	0.25	0.23956					31.1.95
ME041	S				1208	0.50	0.47668					31.1.95
9500055	C	5	1	2.5	148		0.07580	< 0.5			31.1.95	31.1.95
ME044	S				2640	1.00	1.01826					31.1.95
9500058	F	5	1	2.5	2321		0.89761		2.5	101.3	31.1.95	31.1.95
ME039	S				712	0.25	0.28910					31.1.95
9500059	F	5	1	2.5	2934		1.12944		2.5	130	31.1.95	31.1.95
ME042	S				1331	0.50	0.52301					31.1.95
9500063	F	5	4	0.625	2072		0.80344		10	97	31.1.95	31.1.95
ME045	S			-	2510	1.00	0.96909		-			31.1.95
9500064	F	5	4	0.625	1977		0.76751		10	92.6	31.1.95	31.1.95
ME040	S				612	0.25	0.25128					31.1.95
9500067	F	5	10	0.25	1626		0.63477		25	77.6	31.1.95	31.1.95
ME043	S				1180	0.50	0.46609					31.1.95
9500068	F	5	10	0.25	1765		0.68734		25	84.1	31.1.95	31.1.95
ME046	S				2636	1.00	1.01674					31.1.95
9500062	R		1	2.5	313		0.1382003				31.1.95	31.1.95

C = Control R = Reagent blank F = Fortified sample S = Standard

Injection volume: 1.0 µl
Calibration: linear
Coeff. of correlation: 0.9938

Aliquotation 50%
Intercept: 0.1983e-1
SD+/-: 0.2788e-1

Deriv. factor: 1.548
Slope: 0.3782e-3

* This blank was subtracted from recoveries

Table 3: Analytical raw data of analytical series ME95009

Study Code: 30796 Queuefile name: ME95009

Method validation with hexythiazox Matrix: Dried hops Lab Journal no.00842

Sample number	Type	Sample weight (g)	Final dilution(ml)	Sample injected (mg)	Peak height	Std. Conc. (µg/ml)	Substance injected (ng)	Residue (mg/kg)	Spiking level (µg)	Recovery %	Date of extraction	Date of measurem.
9500056	C	5	2	1.25	0		0.00000	< 0.5			13.2.95	16.2.95
ME050	S				672	0.50	0.50343					16.2.95
9500057	C	5	2	1.25	0		0.00000	< 0.5			13.2.95	16.2.95
ME053	S				1436	1.00	1.00624					16.2.95
9500060	F	5	2	1.25	500		0.39024		2.5	96.7	13.2.95	16.2.95
ME048	S				312	0.25	0.26651					16.2.95
9500061	F	5	1	2.5	927		0.67125		2.5	83.1	13.2.95	16.2.95
ME067	S				1161	1.00	1.03536					16.2.95
ME061	S				284	0.25	0.26847					16.2.95
ME051	S				507	0.50	0.46347					16.2.95
9500065	F	5	4	0.625	899		0.80625		10	99.8	13.2.95	16.2.95
ME054	S				1160	1.00	1.03448					16.2.95
ME062	S				293	0.25	0.27634					16.2.95
9500066	F	5	4	0.625	809		0.72755		10	90.1	13.2.95	16.2.95
ME065	S				535	0.50	0.48796					16.2.95
ME068	S				1045	1.00	0.93392					16.2.95
ME049	S				216	0.25	0.24299					16.2.95
ME063	S				249	0.25	0.27483					16.2.95
9500069	F	5	10	0.25	796		0.80246		25	99.4	13.2.95	16.2.95
ME052	S				476	0.50	0.49379					16.2.95
ME066	S				469	0.50	0.48704					16.2.95
9500070	F	5	10	0.25	744		0.75230		25	93.2	13.2.95	17.2.95
ME055	S				1041	1.00	1.03878					17.2.95
ME069	S				962	1	0.96258					17.2.95

C = Control F = Fortified sample S = Standard

Injection volume: 1µl Aliquotation: 50% Deriv. factor: 1.548

Calibration: linear Intercept: 0.6118e-1/0.2013e-1/0.3464e-1 Slope: 0.6581e-3/0.8744e-3/0.9646e-3

Coeff. of correlation: 0.9966/0.9873/0.9935 SD+/-: 0.2246e-1/0.4317e-1/0.3073e-1

Table 4: Analytical raw data of analytical series ME95015

Study Code: 30796
Method validation with PT 1-3

Queuefile name: ME95015
Matrix:Dried hops

Lab Journal no.00842

Sample number	Type	Sample weight (g)	Final dilution(ml)	Sample injected (mg)	Peak height	Std. Conc. (µg/ml)	Substance injected (ng)	Residue (mg/kg)	Spiking level (µg)	Recovery %	Date of extraction	Date of measurem.
9500053	C	5	1	* 2.5	317		0.14171	< 0.5			7.3.95	10.3.95
ME142	S				1184	0.50	0.47788					10.3.95
ME140	S				650	0.25	0.27083					10.3.95
ME141	S				624	0.25	0.26075					10.3.95
9500356	F	5	5	0.5	2567		1.01412		10	98.6	7.3.95	10.3.95
ME143	S				1291	0.50	0.51937					10.3.95
ME145	S				2582	1.00	1.01994					10.3.95
9500357	F	5	6	0.417	2345		0.92805		10	108.5	7.3.95	10.3.95
ME158	S				697	0.25	0.28905					10.3.95
ME160	S				1133	0.50	0.45811					10.3.95
9500358	F	5	6	0.417	2303		0.91176		10	106.6	7.3.95	10.3.95
ME162	S				2602	1.00	1.02769					10.3.95
ME159	S				596	0.25	0.24989					10.3.95
9500359	F	5	6	0.417	2130		0.84468		10	98.5	7.3.95	10.3.95
ME161	S				1128	0.50	0.45617					10.3.95
ME163	S				2454	1.00	0.97031					10.3.95

C = Control T = Treated F = Fortified sample S = Standard

Injection volume: 1.0 µl
Calibration: linear
Coeff. of correlation: 0.9911

Aliquotation: 50%
Intercept: 0.1880e-1
SD+/-: 0.3102e-1

Deriv. factor: 1
Slope: 0.3877e-3

95/11149 0027

Table 5: Analytical raw data of analytical series ME95026 (PT 1-8)

Study Code: 30976
Method validation PT 1-8

Queuefile name:ME95026
Matrix: Dried hops

Lab Journal no. 00842

Sample number	Type	Sample weight (g)	Final dilution(ml)	Sample injected (mg)	Peak height	Std. Conc. (µg/ml)	Substance injected (ng)	Residue (mg/kg)	Spiking level (µg)	Recovery %	Date of extraction	Date of measurem.
9500354	C	5	1	2.5	215		0.06696	< 0.5			29.5.95	31.5.95
ME146	S				629	0.25	0.22602					31.5.95
ME148	S				1449	0.50	0.54107					31.5.95
9500360	F	5	3	0.833	2293		0.86534		10	81.9	29.5.95	31.5.95
ME150	S				2599	1.00	0.98290					31.5.95
9500361	F	5	3	0.833	2272		0.85727		10	81.2	29.5.95	31.5.95
ME147	S				598	0.25	0.24468					31.5.95
9500362	F	5	3	0.833	2070		0.86728		10	81.6	29.5.95	31.5.95
ME149	S				1221	0.50	0.50819					31.5.95
9500363	F	5	3	0.833	1985		0.83133		10	78.1	29.5.95	31.5.95
ME151	S				2377	1.00	0.99713					31.5.95

C = Control F = Fortified sample S = Standard

Injection volume: 1.0 µl
Calibration: linear
Coeff. of correlation: 0.9912/0.9996

Aliquotation 50%
Intercept: -0.1564e-1/-0.8252e-2
SD+/-: 0.5054e-1/0.1018e-1

Deriv. factor: 1.62
Slope: 0.3842e-3/0.4230e-3

Table 6: Analytical raw data of analytical series ME95026 (PT 1-9)

Study Code: 30976
Method validation with PT 1-9

Queuefile name: ME95026
Matrix:Dried hops

Lab Journal no. 00842

Sample number	Type	Sample weight (g)	Final dilution(ml)	Sample injected (mg)	Peak height	Std. Conc. (µg/ml)	Substance injected (ng)	Residue (mg/kg)	Spiking level (µg)	Recovery %	Date of extraction	Date of measurem.
9500355	C	5	1	2.5	165		0.10618	< 0.5			29.5.95	31.5.95
ME164	S				500	0.25	0.23952					31.5.95
ME168	S				1135	0.50	0.49225					31.5.95
9500364	F	5	3	0.833	2348		0.97505		10	90.8	29.5.95	31.5.95
ME172	S				2377	1.00	0.98659					1.6.95
9500365	F	5	3	0.833	2215		0.92211		10	85.7	29.5.95	1.6.95
ME165	S				594	0.25	0.27693					1.6.95
9500366	F	5	3	0.833	2449		1.01525		10	94.7	29.5.95	1.6.95
ME169	S				1120	0.50	0.48628					1.6.95
9500367	F	5	3	0.833	2235		0.93007		10	86.5	29.5.95	1.6.95
ME173	S				2457	1.00	1.01843					1.6.95

C = Control F = Fortified sample S = Standard

Injection volume: 1.0 µl
Calibration: linear
Coeff. of correlation: 0.9973

Aliquotation 50%
Intercept: 0.4051e-1
SD+/-: 0.2002e-1

Deriv. factor: 1.611
Slope: 0.3980e-3

Figure 2: PT 1-3 Standard 0.25 µg/ml

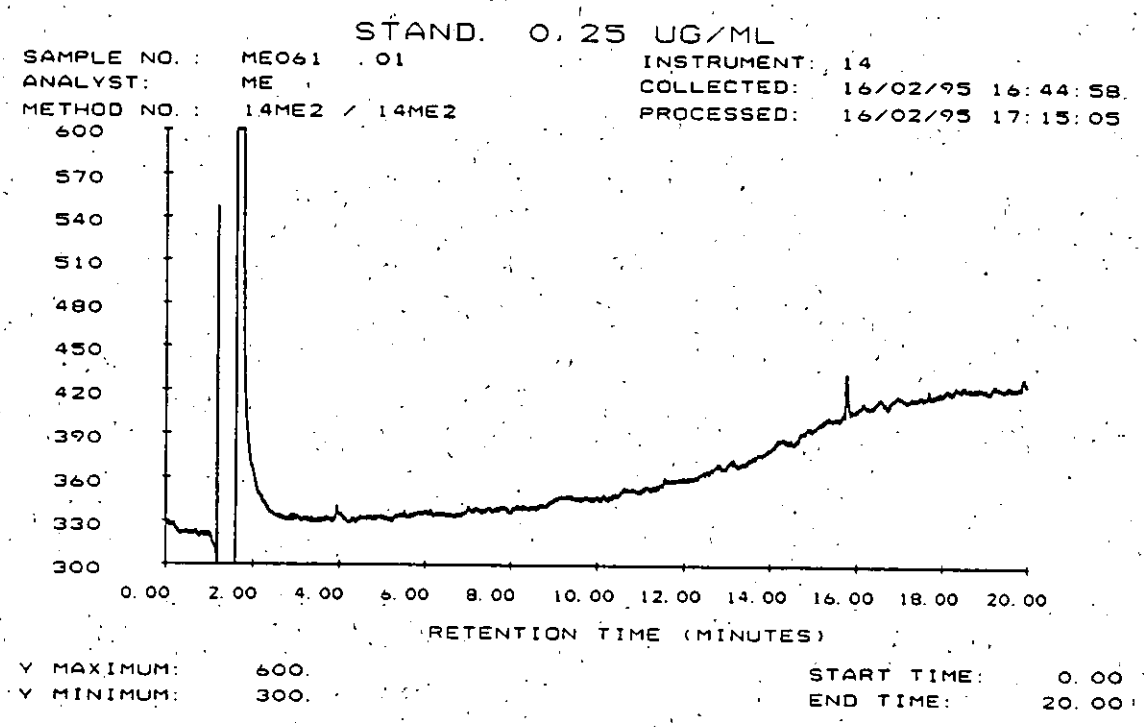


Figure 3: PT 1-3 Standard 0.5 µg/ml

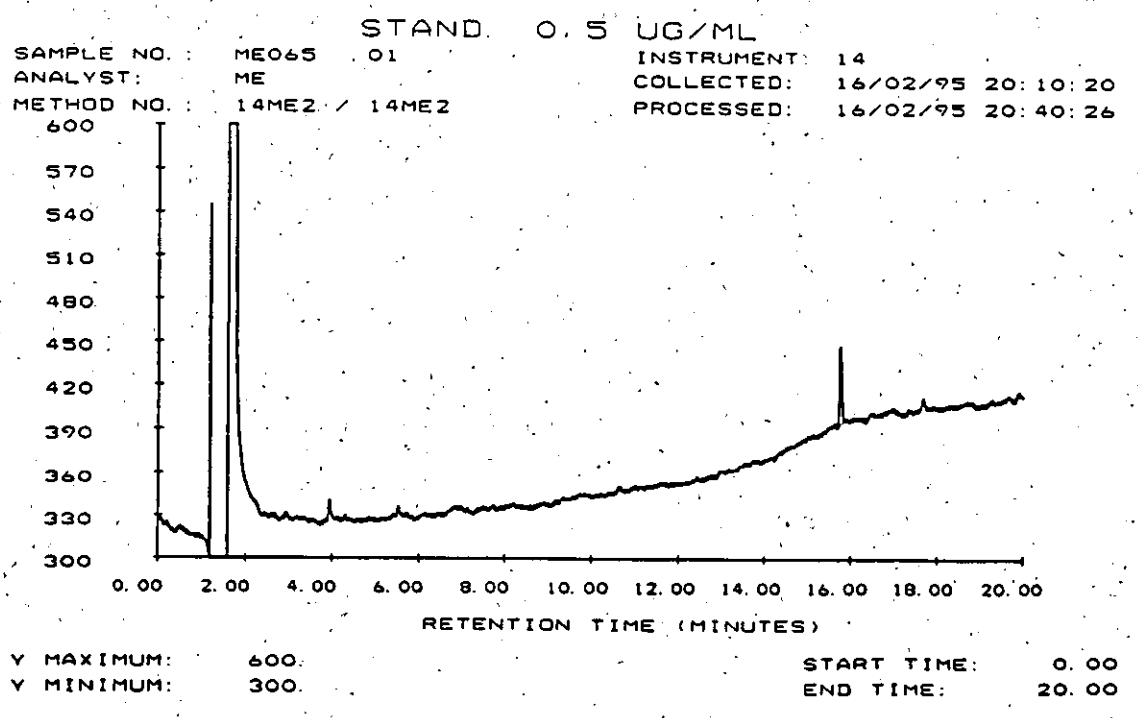


Figure 4: PT 1-3 Standard 1.0 µg/ml

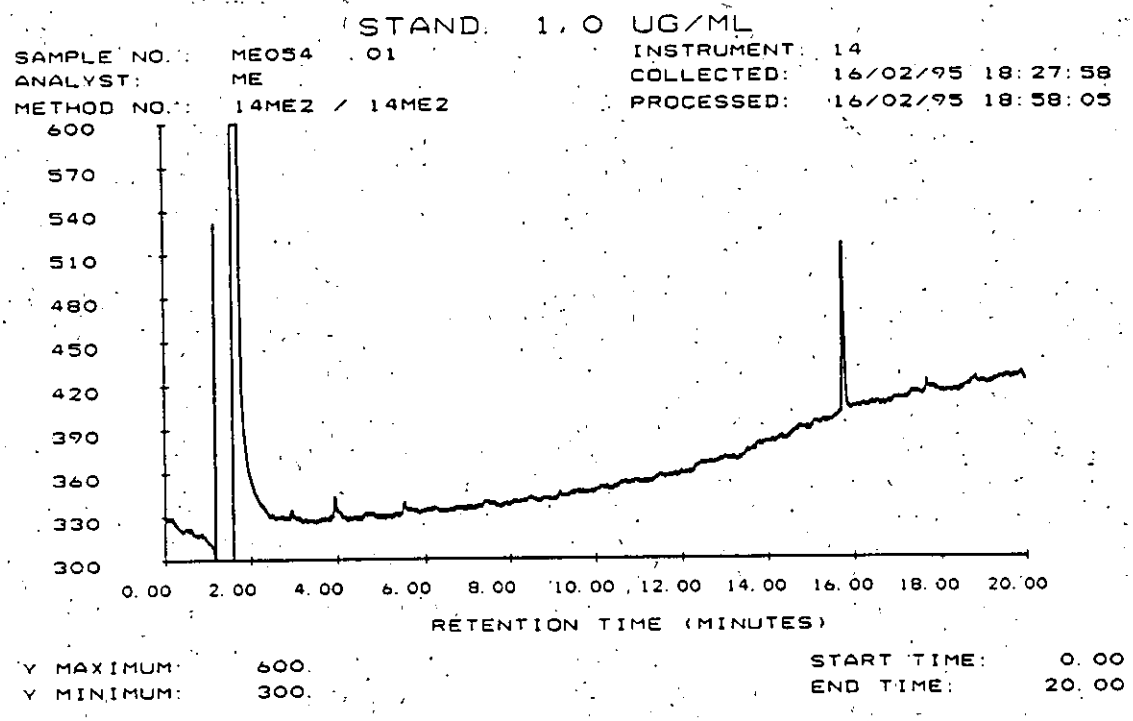


Figure 5: Example of reagent blank (no.: 9500062)

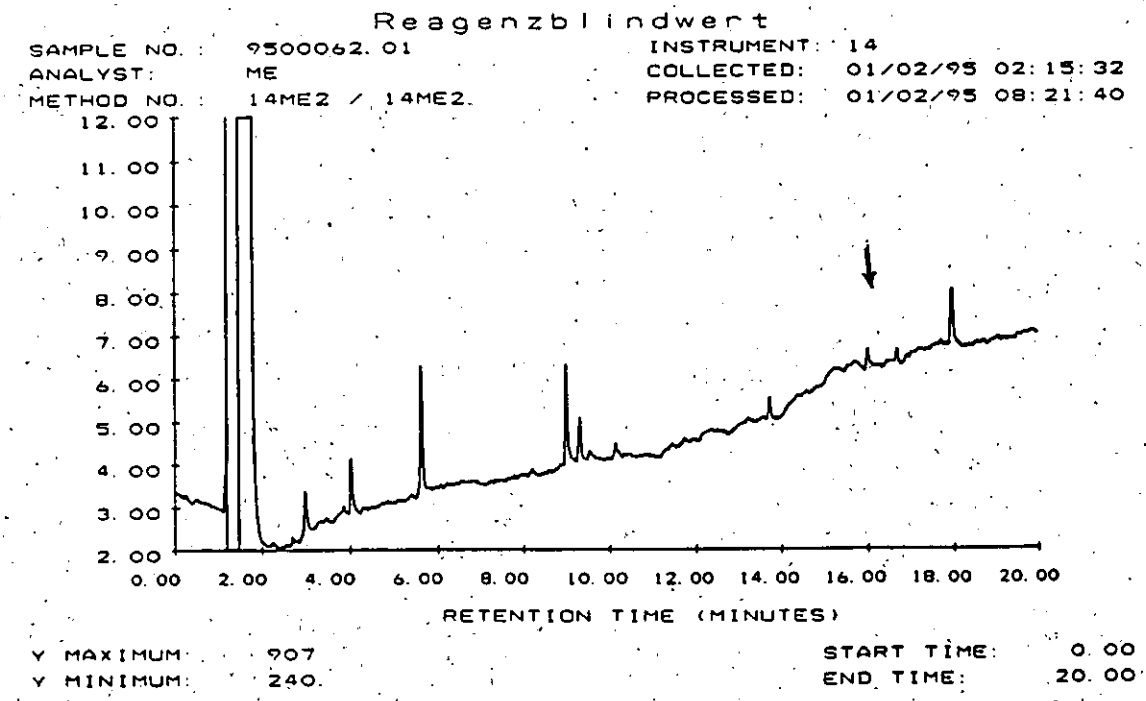


Figure 6: PT 1-3 Example of calibration curve

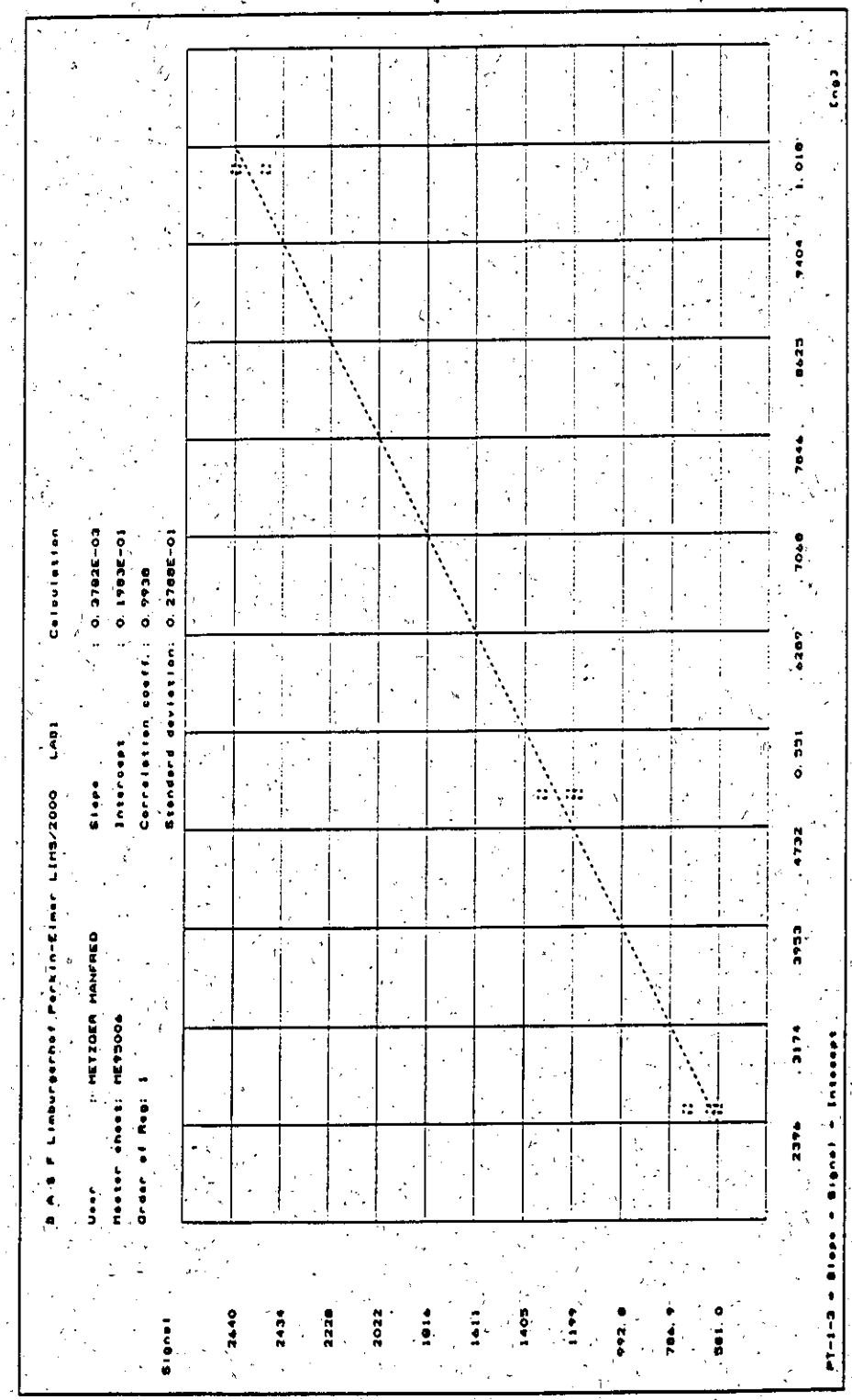


Figure 7: Dried hops control sample no.: 9500054

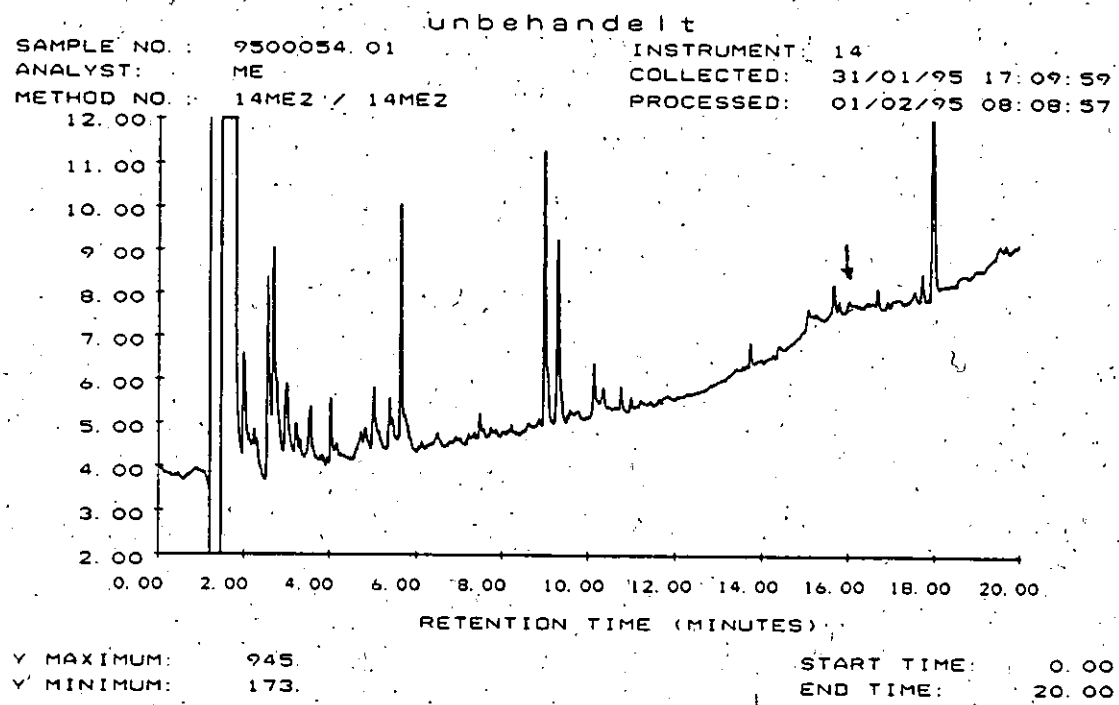


Figure 8: Dried hops control sample no.: 9500055

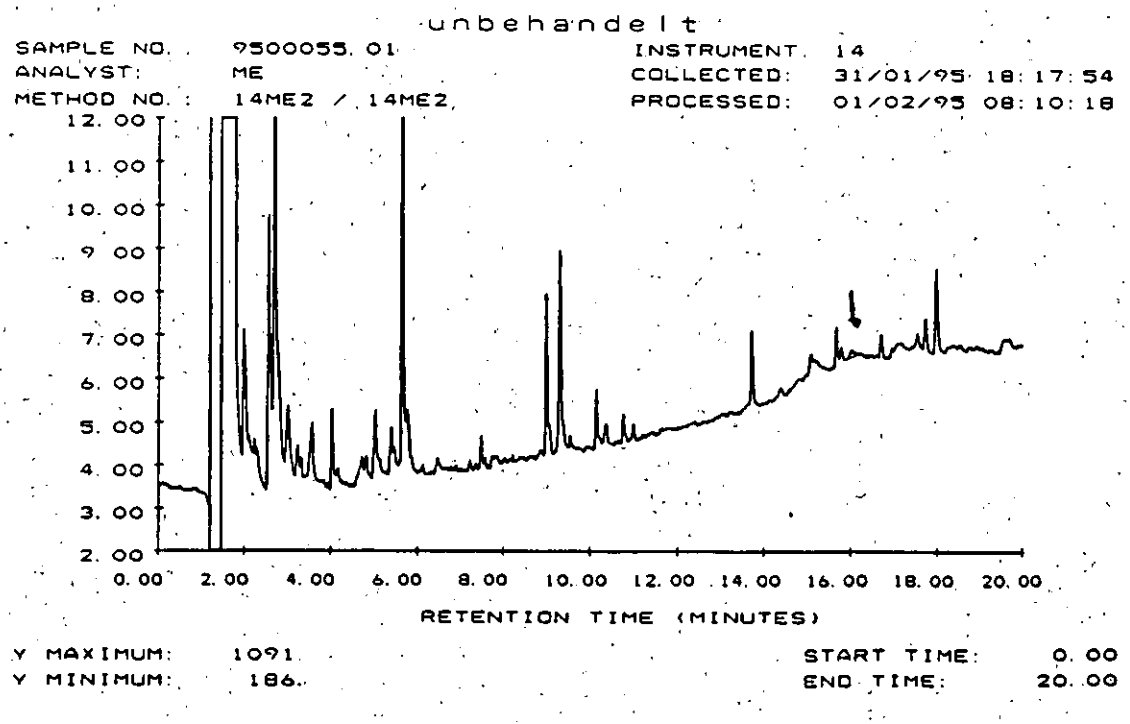


Figure 9: Dried hops control sample no.: 9500056

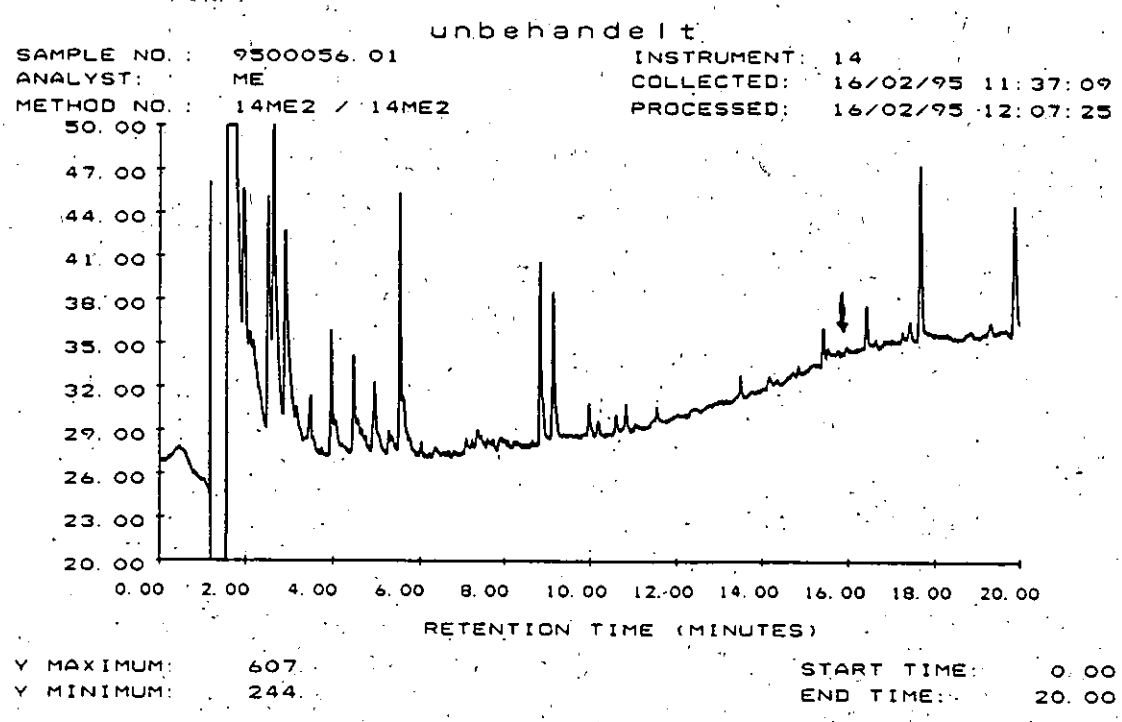


Figure 10: Dried hops control sample no.: 9500057

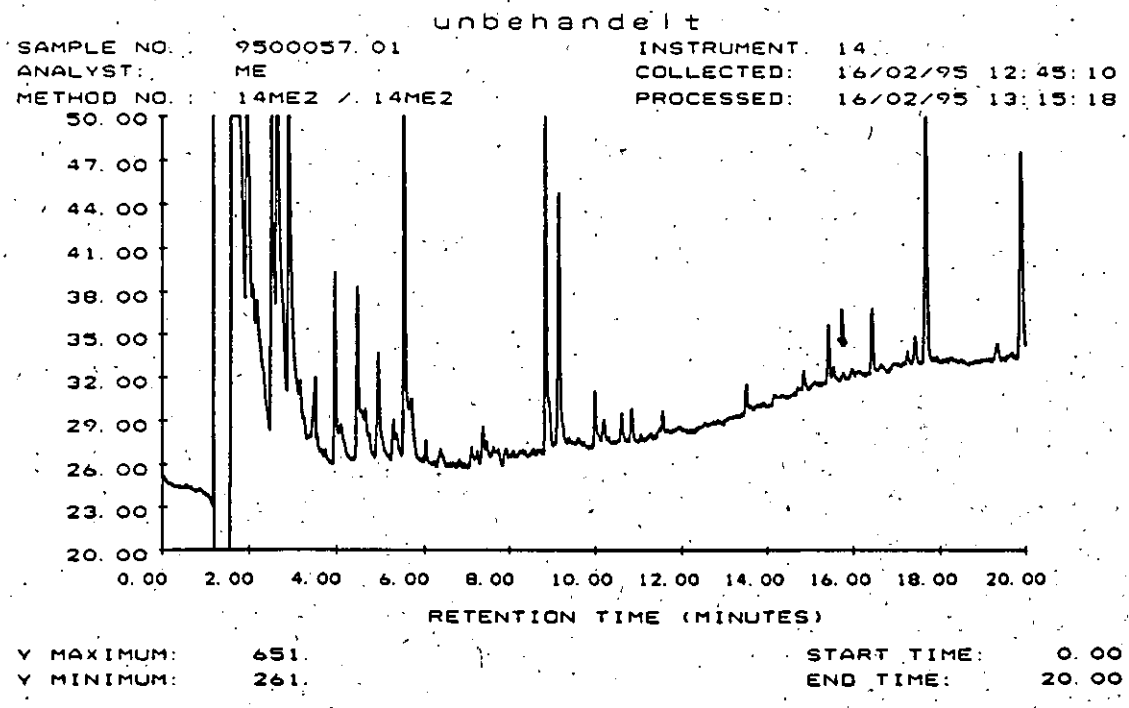


Figure 11: Dried hops spiked with 0.5 mg/kg hexythiazox (no.: 9500058)

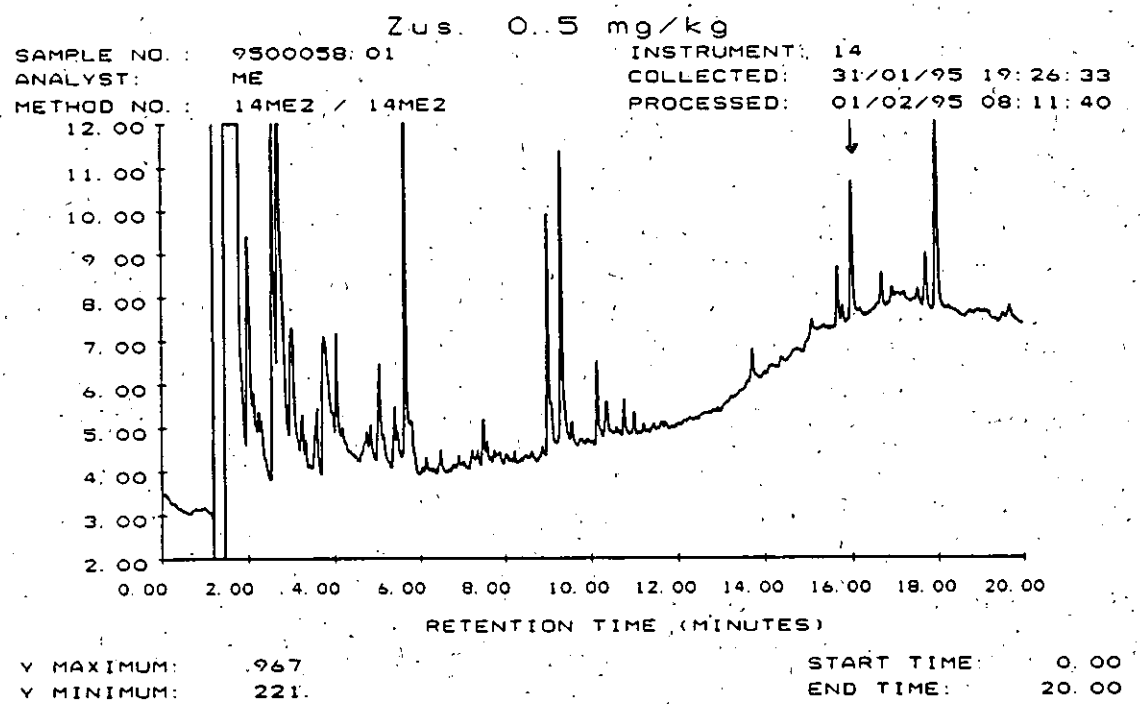


Figure 12: Dried hops spiked with 0.5 mg/kg hexythiazox (no.: 9500059)

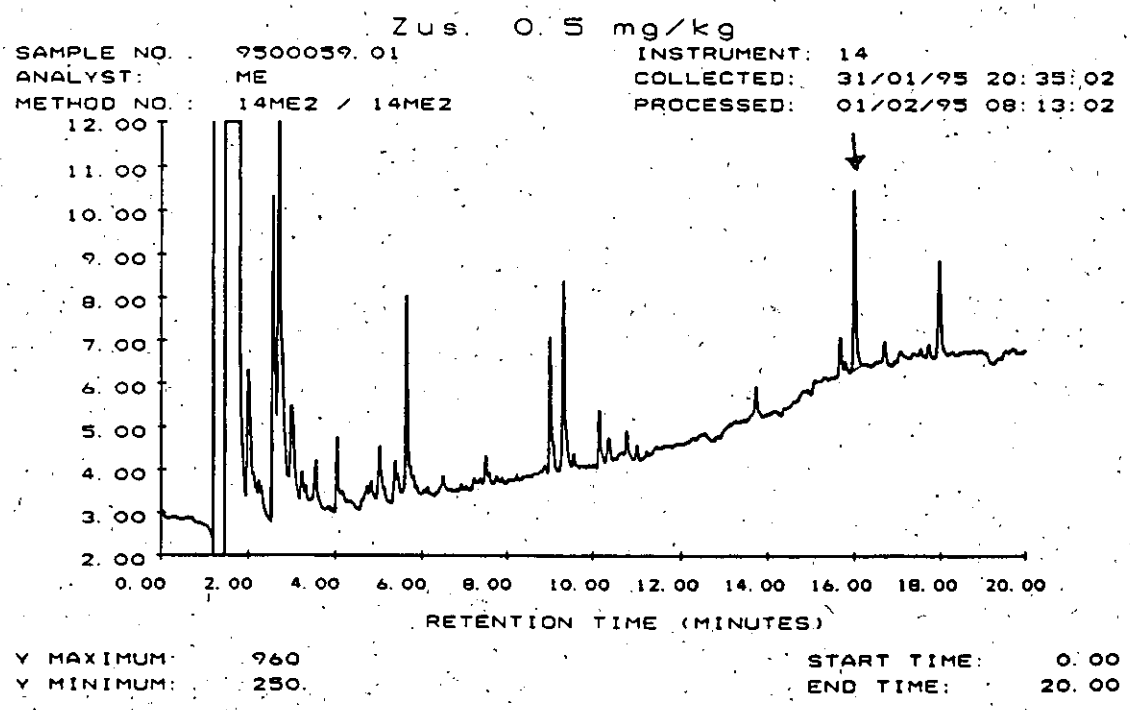


Figure 13: Dried hops spiked with 0.5 mg/kg hexythiazox (no.: 9500060)

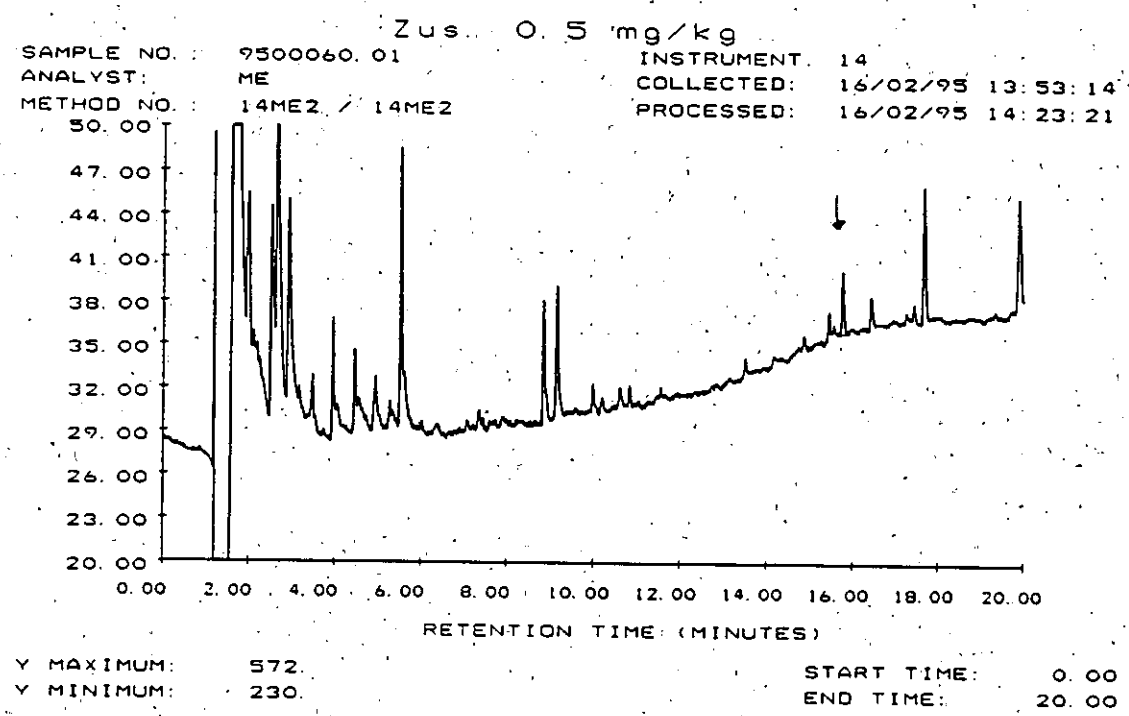


Figure 14: Dried hops spiked with 0.5 mg/kg hexythiazox (no.: 9500061)

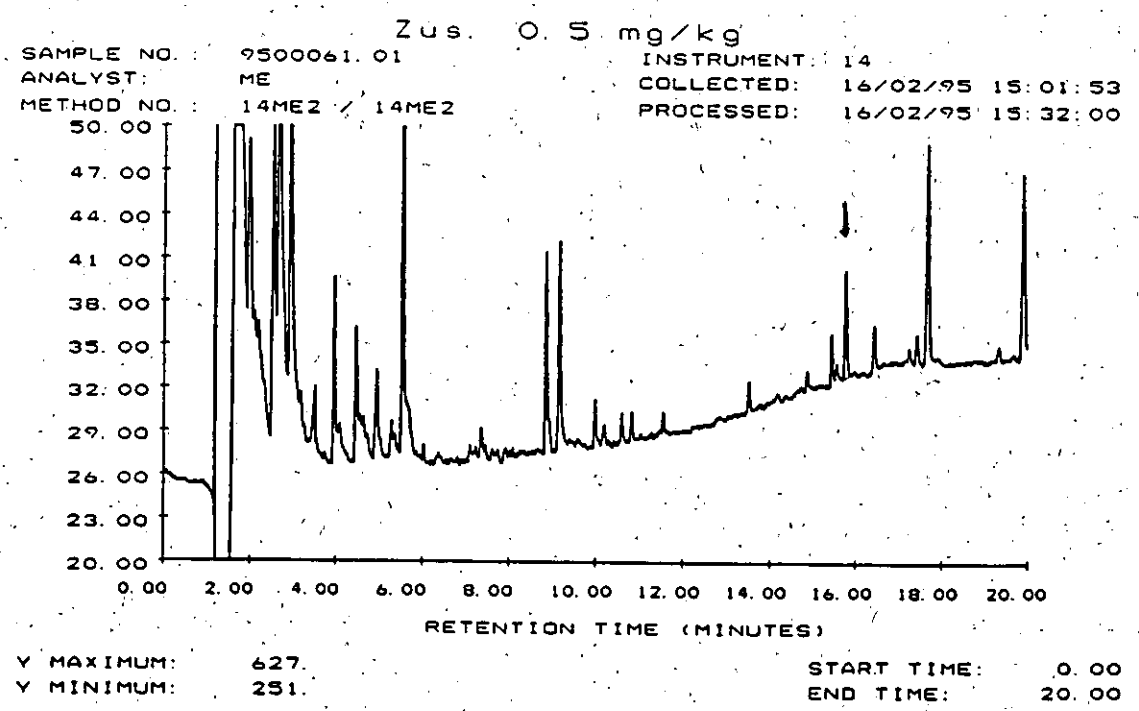


Figure 15: Dried hops spiked with 2.0 mg/kg hexythiazox (no.: 9500063)

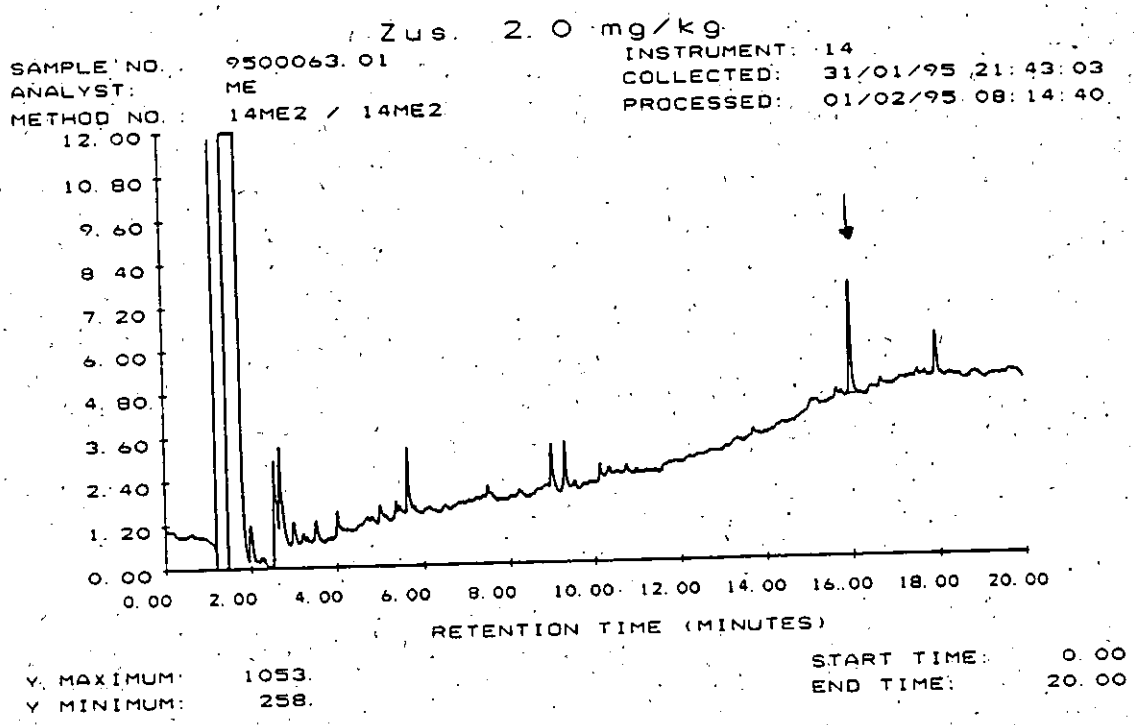


Figure 16: Dried hops spiked with 2.0 mg/kg hexythiazox (no.: 9500064)

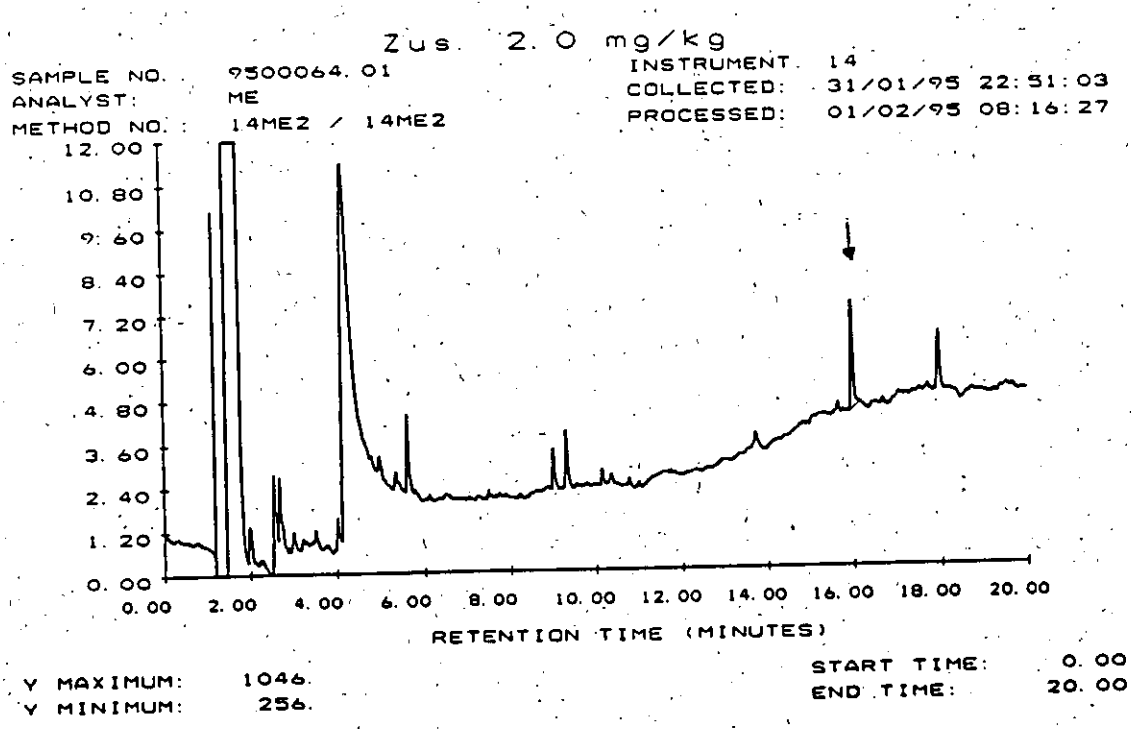


Figure 17: Dried hops spiked with 2.0 mg/kg hexythiazox (no.: 9500065)

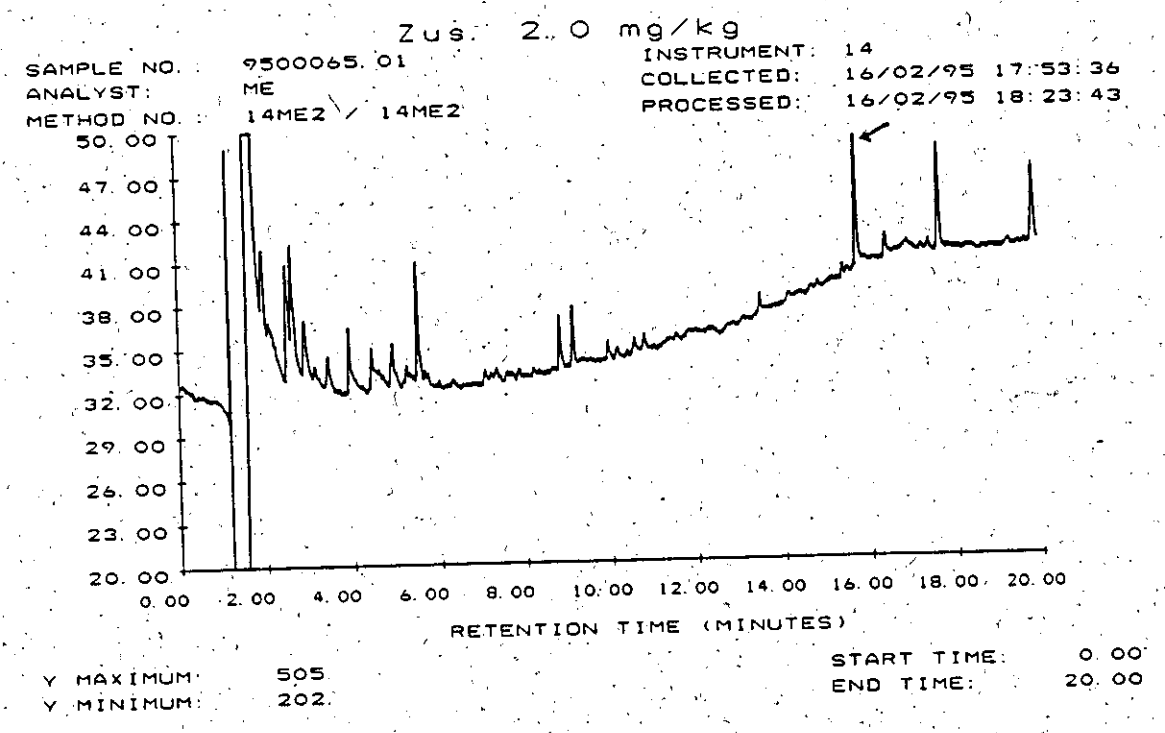


Figure 18: Dried hops spiked with 2.0 mg/kg hexythiazox (no.: 9500066)

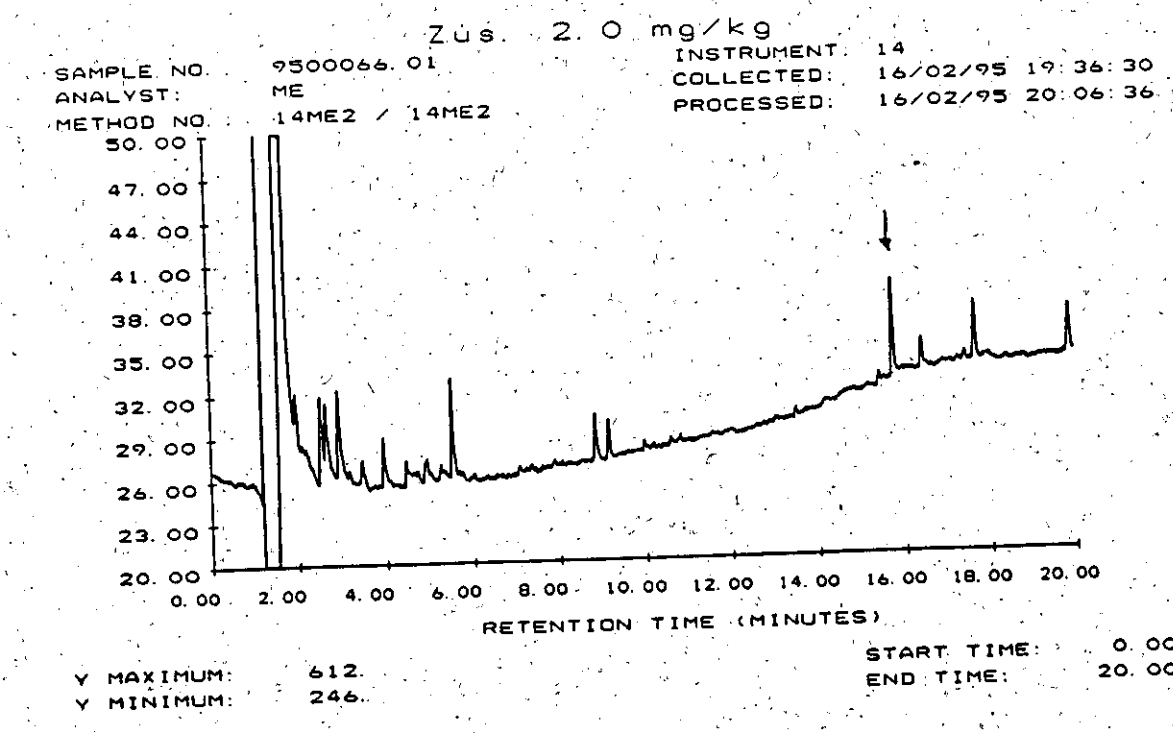


Figure 19: Dried hops spiked with 2.0 mg/kg PT 1-3 (no.: 9500356)

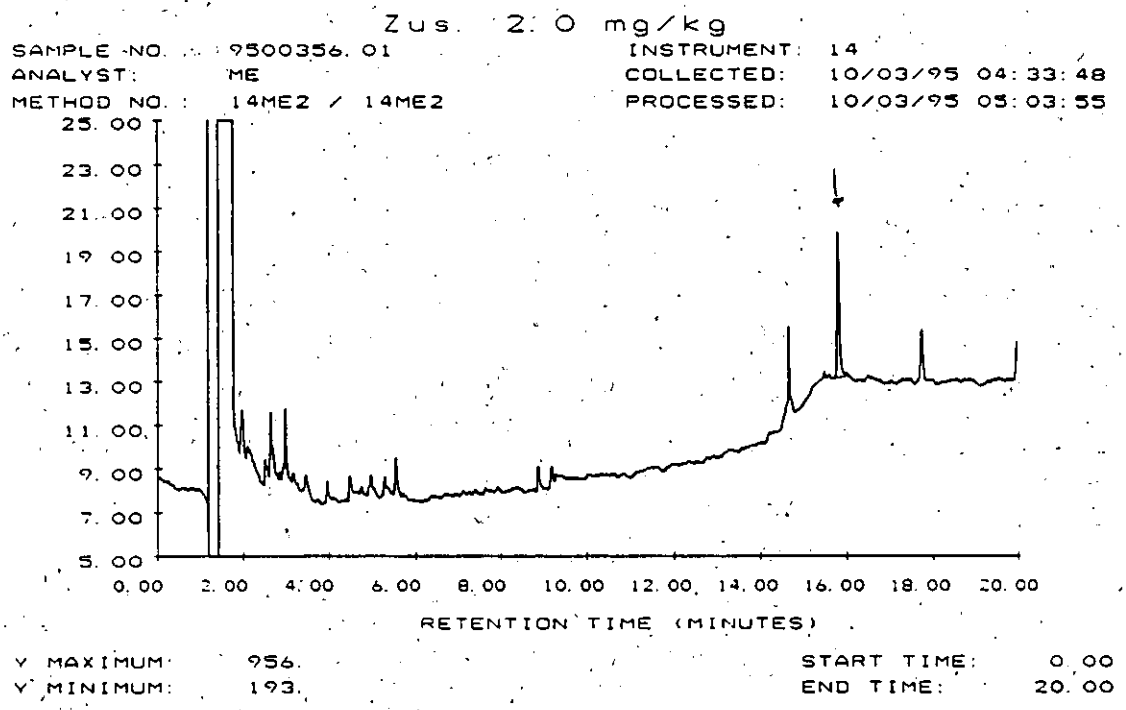


Figure 20: Dried hops spiked with 2.0 mg/kg PT 1-3 (no.: 9500357)

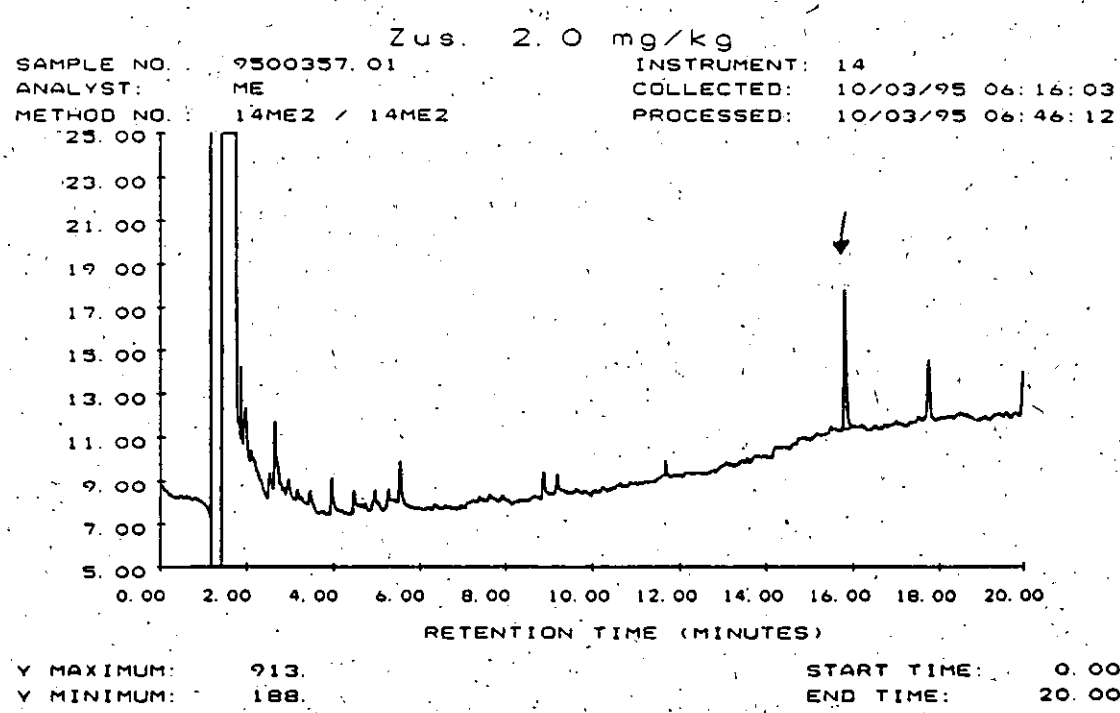


Figure 21: Dried hops spiked with 2.0 mg/kg PT 1-3 (no.: 9500358)

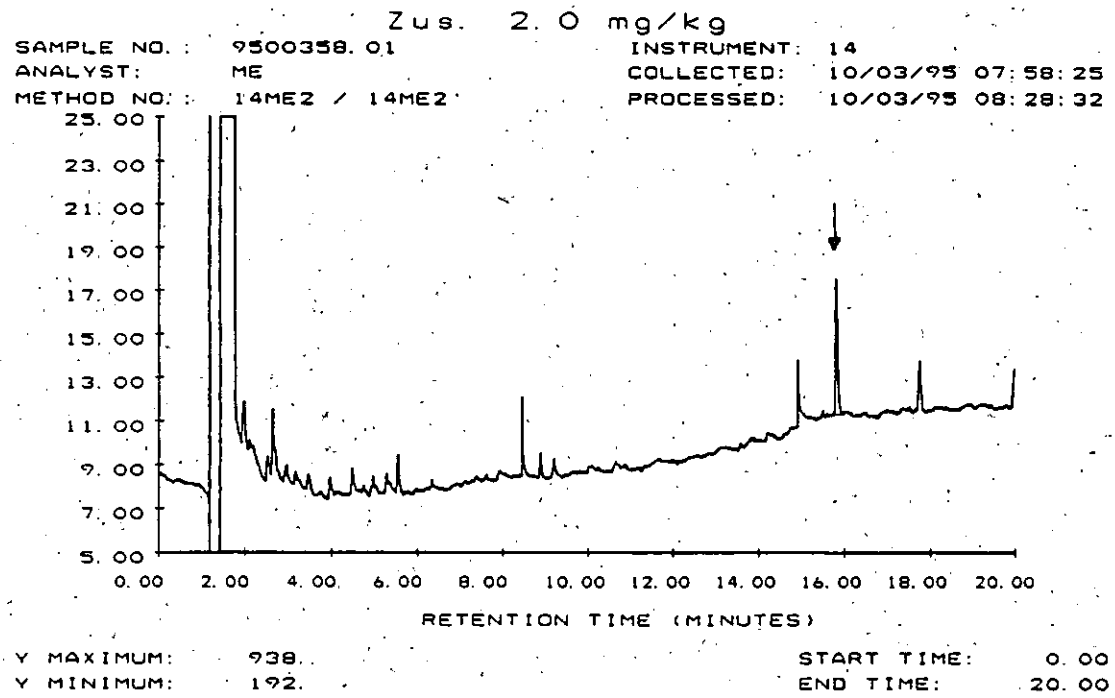


Figure 22: Dried hops spiked with 2.0 mg/kg PT 1-3 (no.: 9500359)

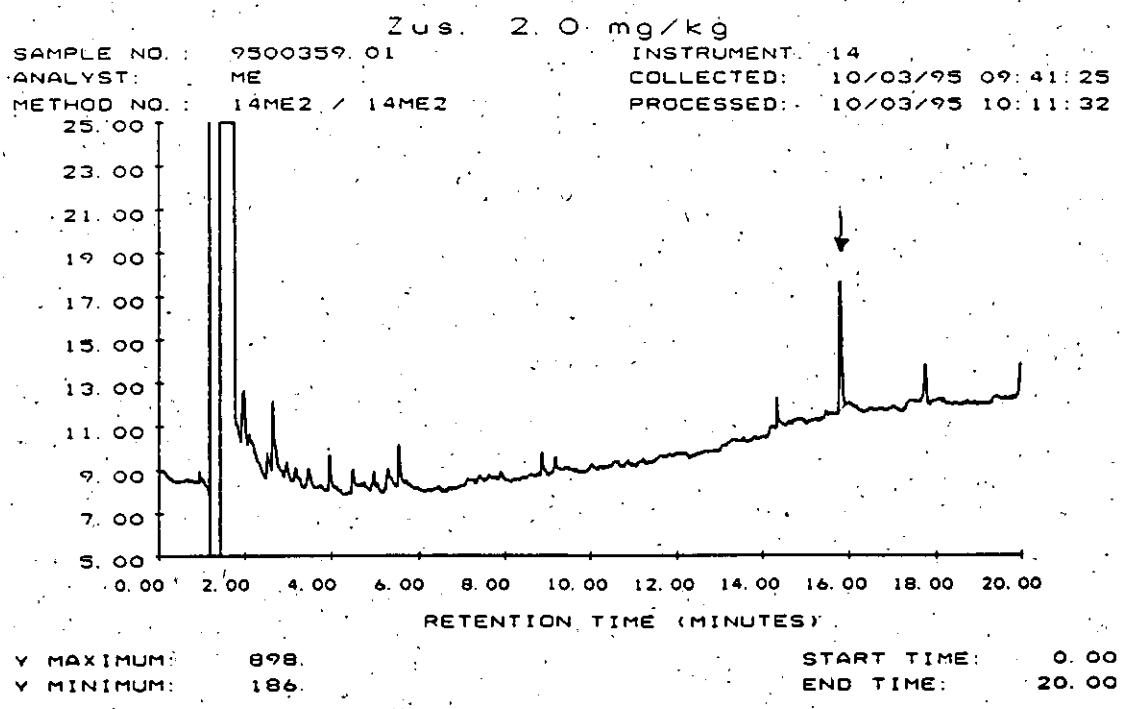


Figure 23: Dried hops spiked with 2.0 mg/kg PT 1-8 (no.: 9500360)

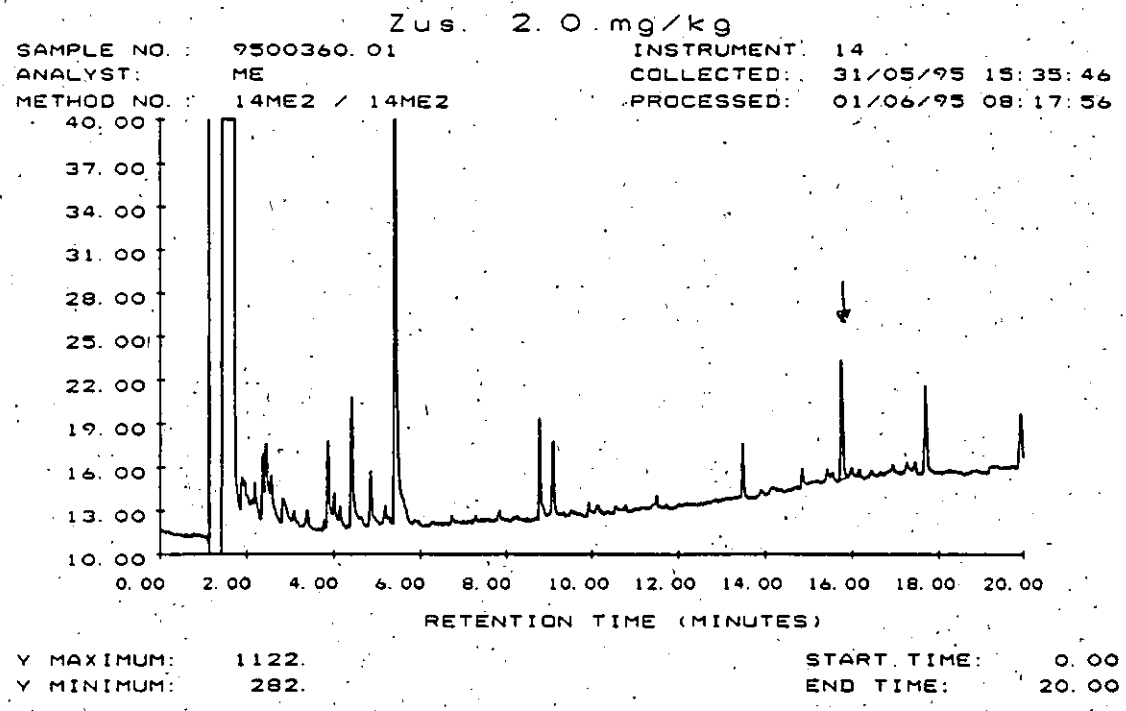


Figure 24: Dried hops spiked with 2.0 mg/kg PT 1-8 (no.: 9500361)

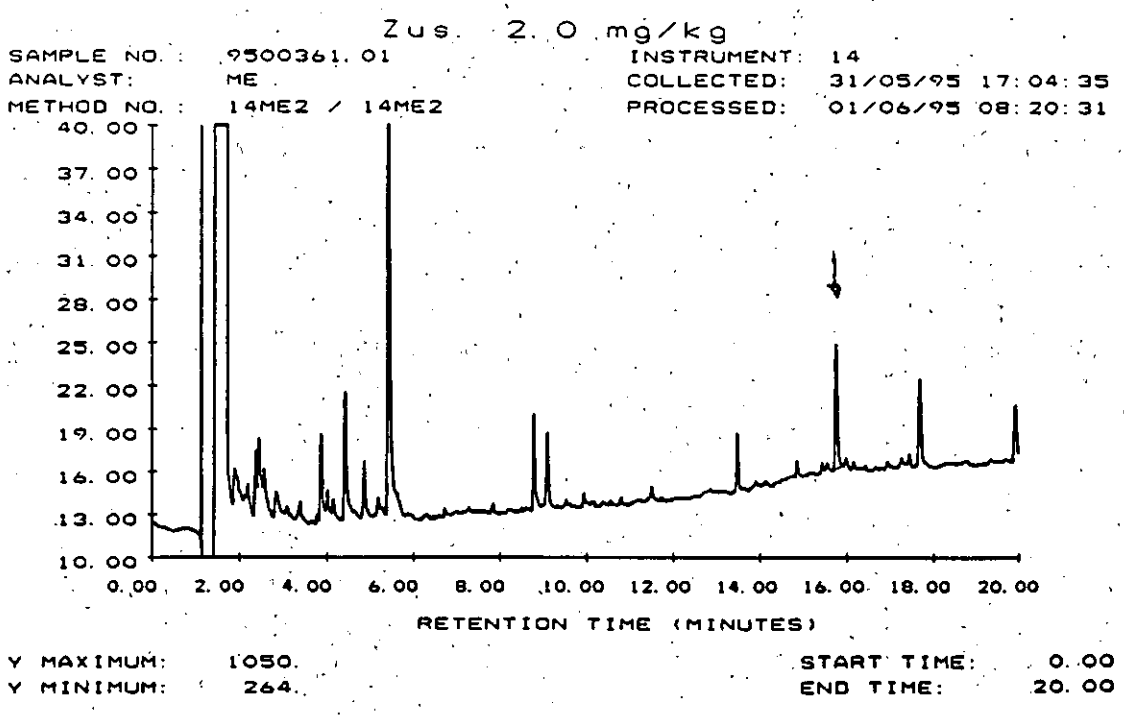


Figure 25: Dried hops spiked with 2.0 mg/kg PT 1-8 (no.: 9500362)

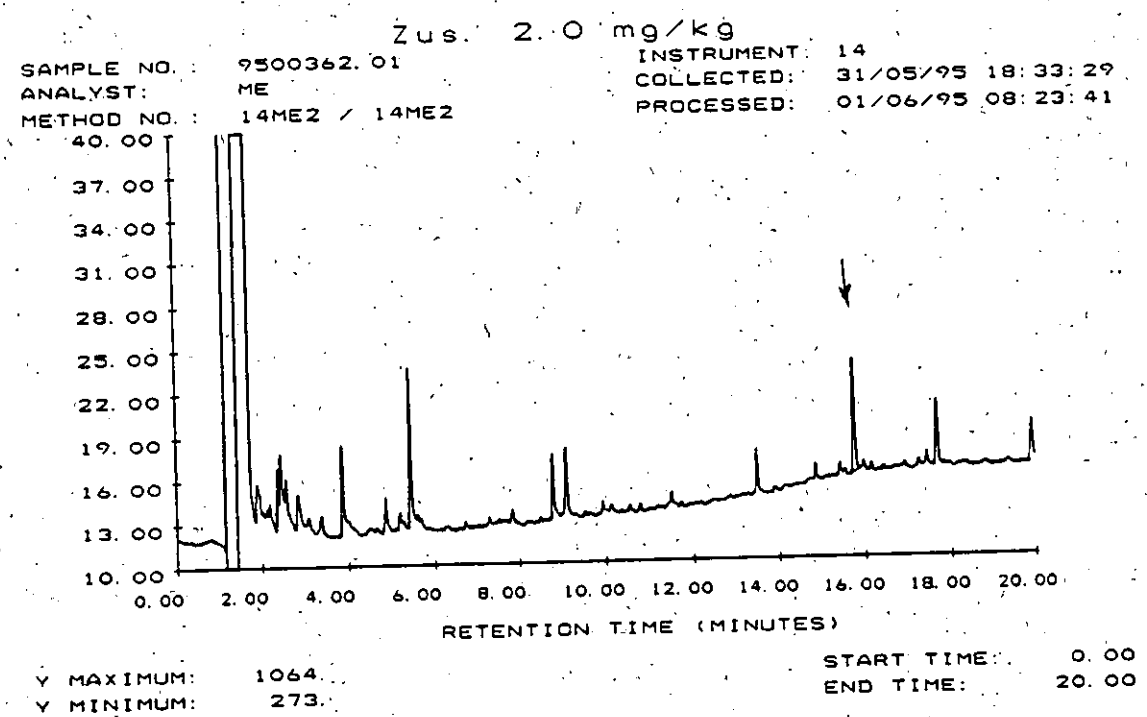


Figure 26: Dried hops spiked with 2.0 mg/kg PT 1-8 (no.: 9500363)

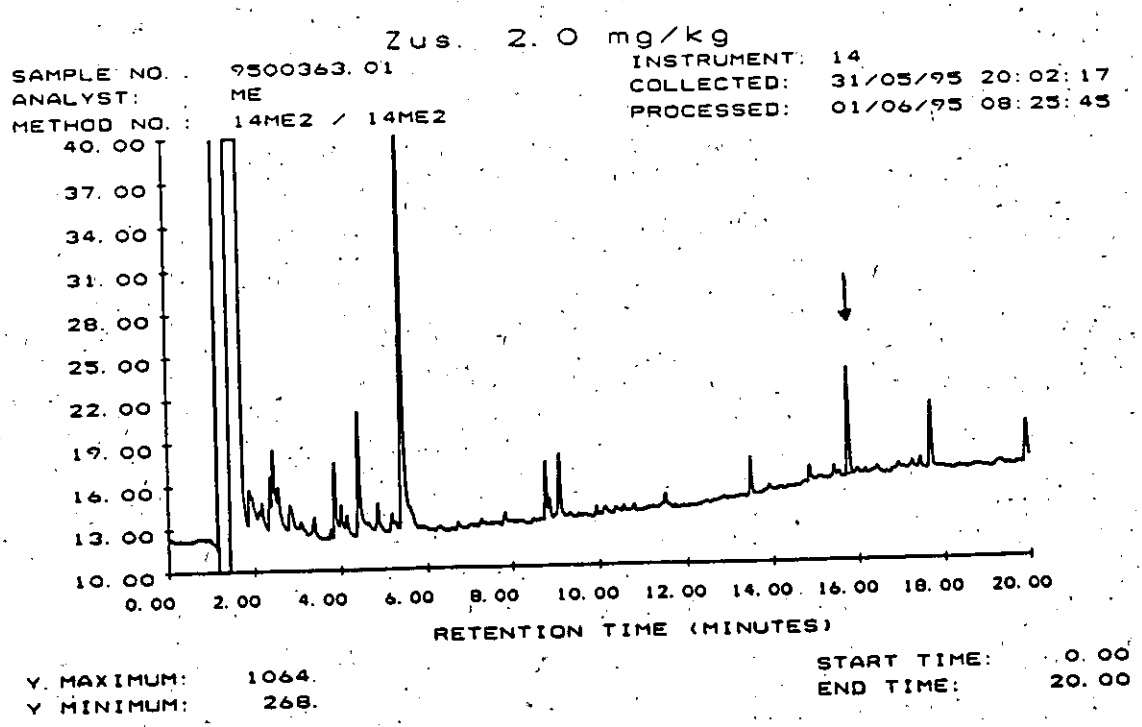


Figure 27: Dried hops spiked with 2.0 mg/kg PT 1-9 (no.: 9500364)

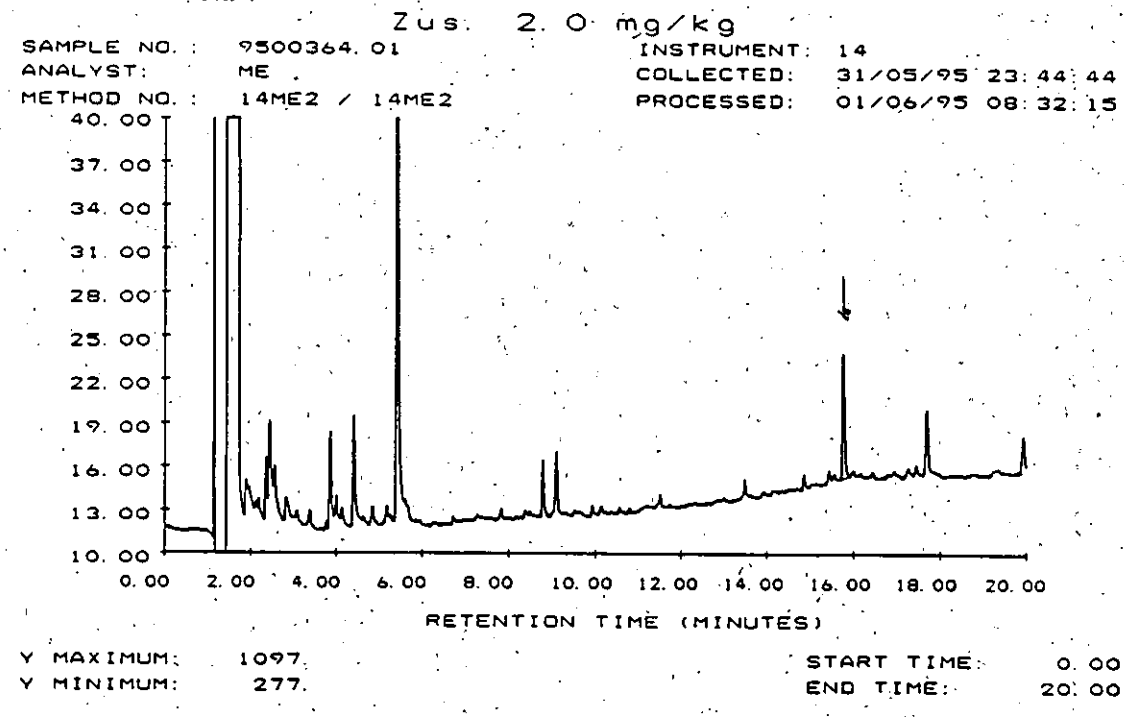


Figure 28: Dried hops spiked with 2.0 mg/kg PT 1-9 (no.: 9500365)

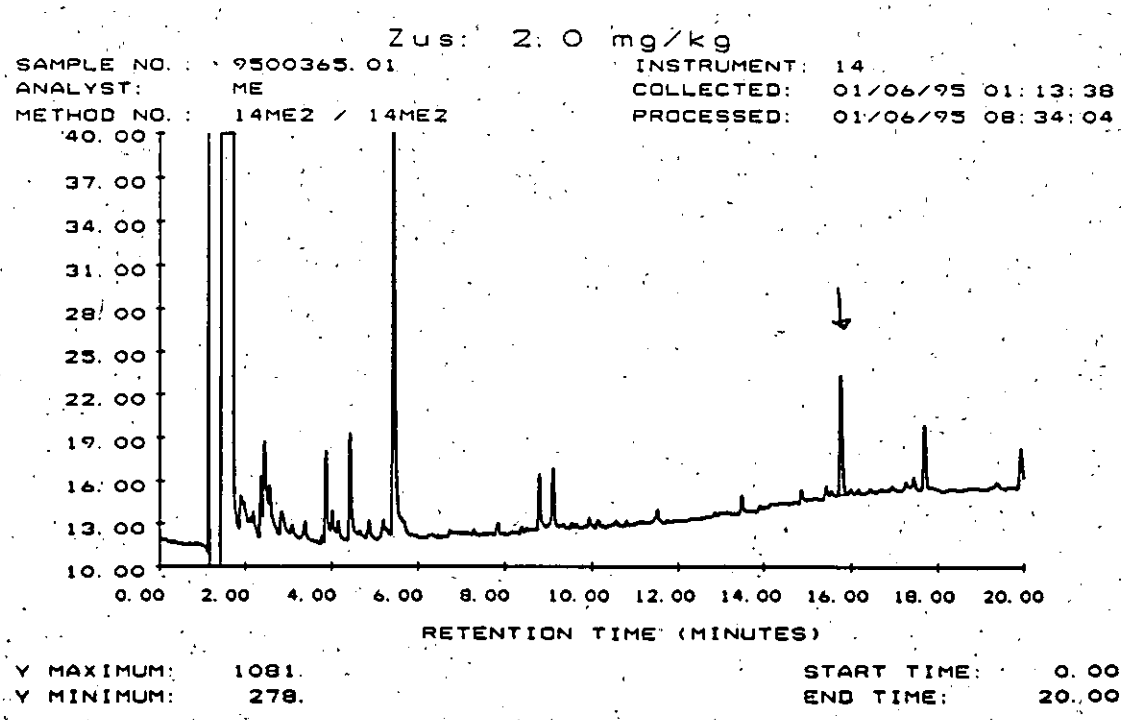


Figure 29: Dried hops spiked with 2.0 mg/kg PT 1-9 (no.: 9500366)

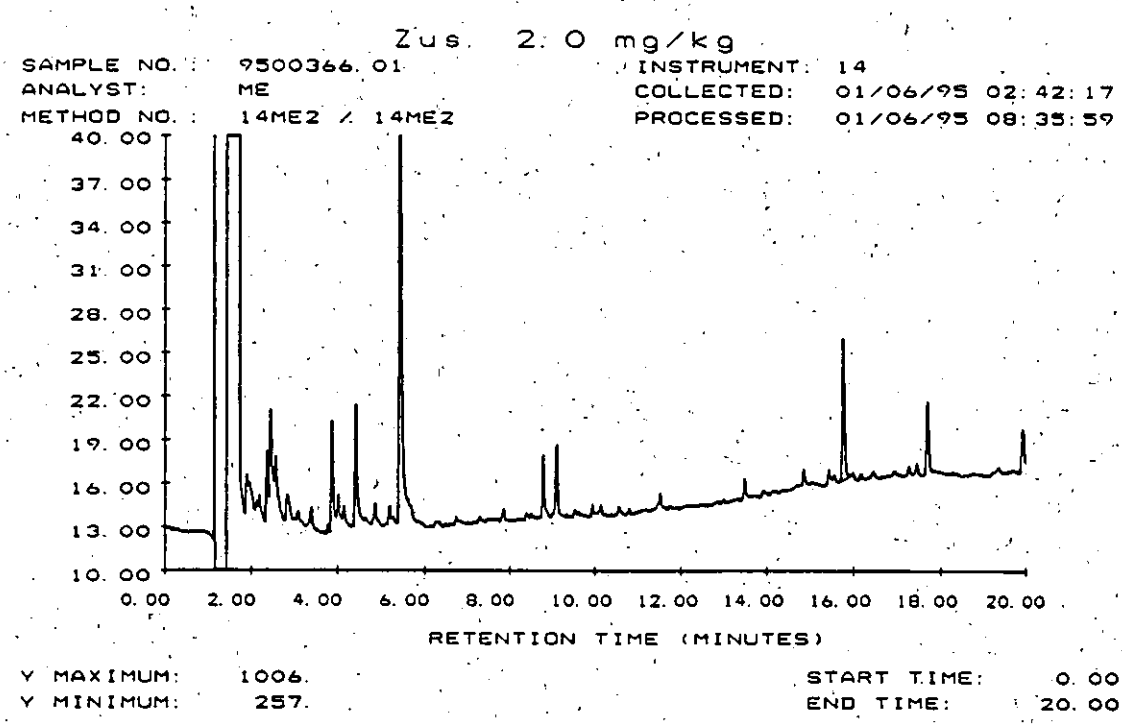


Figure 30: Dried hops spiked with 2.0 mg/kg PT 1-9 (no.: 9500367)

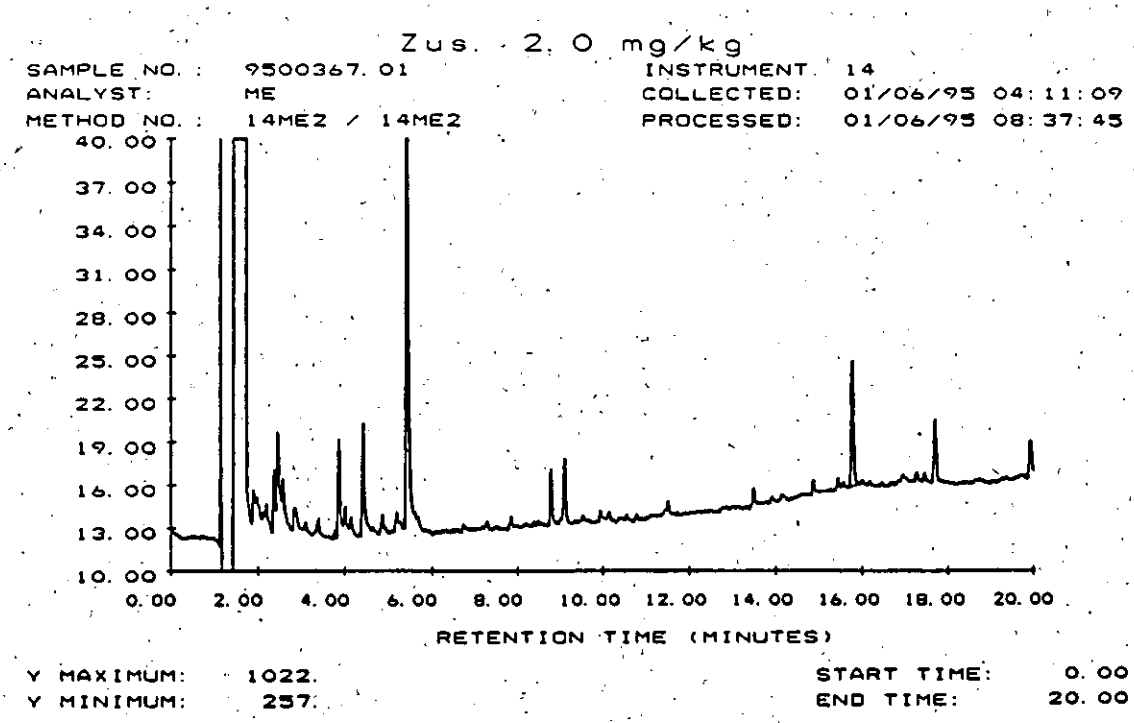


Figure 31: Example of GC-MS run of PT 1-3 with NH_3 CI

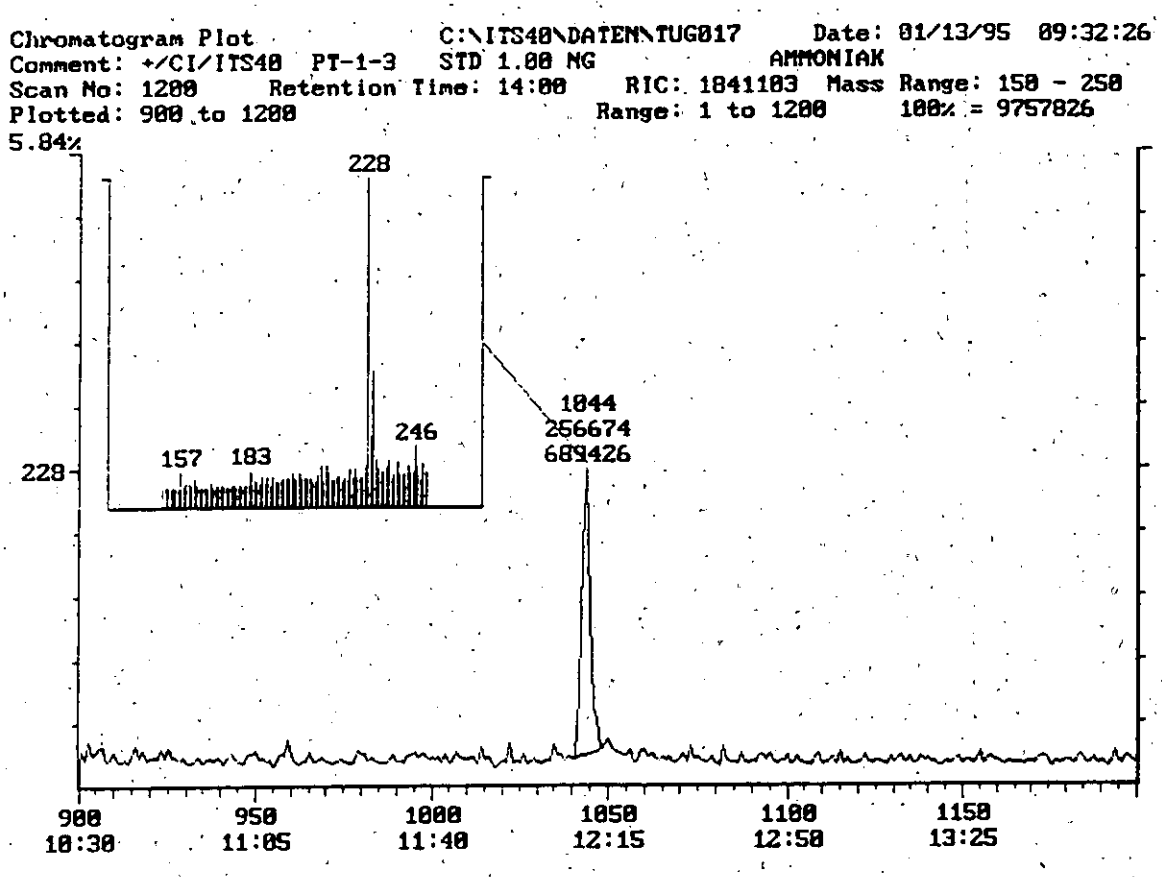


Figure 32: Analytical certificates of hexythiazox

BASF Aktiengesellschaft

BASF

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Purity Statement

- External Reference Standard -

APS/UP
Product Chemistry

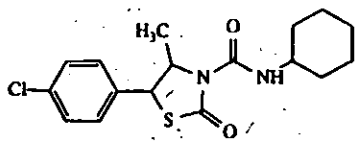
Reg.No.: 282502 **Lot-No.:** L43-251

Common name: Hexythiazox Supplier: Nippon Soda Co., Ldt.

Chemical name: (4RS, 5RS)-5-(4-chlorophenyl)-N-cyclohexyl-4-methyl-2-oxo-1,3-thiazolidine-3-carboxamide

Empirical formula: $C_{17}H_{21}ClN_2O_2S$
Molecular weight: 352.89

Structure:



Purity stated: >99.9%, homogeneous **Date:** 04.11.1993

Methods used for purity check:

HPLC:
Stationary Phase: Zorbax BP ODS, 250 x 4.6mm
Mobile Phase: $CH_3CN + H_2O = 75 + 25$
Wavelength: 240nm
Flowrate: 1.5ml/min

Handling advice:
Keep in refrigerator.
Before opening cooled sample vial must be warmed to room temperature in desiccator.

Stability:
Expected to be stable at least for 2.5 years at room temperature (PCP01404).

Purity Statement valid until: 04/96

Date: 21 SEP. 1994 Signature: R Lisicki

95/11149 0046

BASF Aktiengesellschaft

BASF

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Purity Statement

APS/UP
Product Chemistry

Reg.No.: 282502 **Lot-No.:** 2EI72D

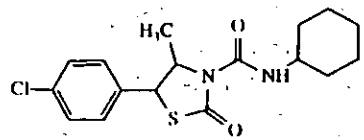
Study Code: PCP00526

Common name: Hexythiazox

Chemical name: (4RS, 5RS)-5-(4-chlorophenyl)-N-cyclohexyl-4-methyl-2-oxo-1,3-thiazolidine-3-carboxamide

Empirical formula: $C_{17}H_{21}ClN_2O_2S$
Molecular weight: 352.89

Structure:



Purity stated: 98.9%, homogeneous **Date:** 07.12.1988
Checked: 20.10.1994 (PCP03305)

Method of Purification: Recrystallization from diisopropylether

Methods used for purity check:
HPLC

Storage advice:
Keep in refrigerator.
Before opening cooled sample vial must be warmed to room temperature.

Stability:
Expected to be stable at least for 6 years at room temperature.

Purity Statement valid until: 11/2000

Date: 12 MAI 1995 Signature: R Lisicki

Figure 33: Analytical certificates of PT 1-3

BASF Aktiengesellschaft

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BASF

Purity Statement

- External Reference Substance -

APS/UP
Product Chemistry

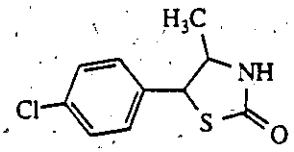
Reg.No.: 248649 **Lot-No.:** L43-255

Supplier: Nippon Soda Co., Ldt.

Chemical name: -/-

Empirical formula: $C_{10}H_{10}ClNOS$
Molecular weight: 227.71

Structure: PT-1-3



Purity stated: >99.9%, homogeneous **Date:** 27.12.1993

Methods used for purity check:

HPLC:
Stationary Phase: CAPCELL PAK C18, 250 x 4.6mm
Mobile Phase: $CH_3OH + H_2O = 7 + 3$
Wavelength: 223.235nm
Flowrate: 1.0ml/min

Result: >99.9%

Storage advice:
Keep in freezer.
Before opening cooled sample vial must be warmed to room temperature.

Stability:
Expected to be stable at least for 6 years stored in freezer (PCP03304).

Purity Statement valid until: 01/2000

Date: 12. MAI 1995 Signature: R. Resolper

95/11149 0047

Figure 34: Analytical certificates of PT 1-8

BASF Aktiengesellschaft

BASF

Landwirtschaftliche Versuchsstation der BASF - Postfach 120 - 67114 Limburgerhof

Purity Statement

- External Reference Substance -

APS/UP
Product Chemistry

Reg.No.: 248650 **Lot-No.:** L43-265

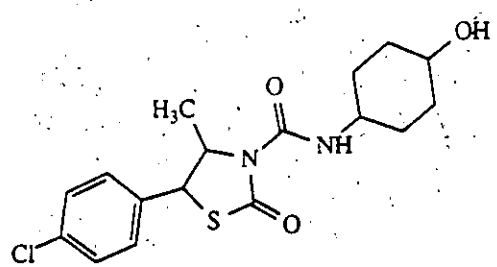
Supplier: Nippon Soda Co., Ldt.

Common name : -/-

Chemical name: -/-

Empirical formula: $C_{17}H_{21}ClN_2O_3S$
Molecular weight: 368.89

Structure: **Code:** PT-1-8-t



Purity stated: 99.9%, homogeneous **Date:** 27.12.1993

Methods used for purity check:

HPLC:
Stationary Phase: CAPCELL PAK C18, 250 x 4.6mm
Mobile Phase: $CH_3OH + H_2O = 75 + 25$
Wavelength: 225.240nm
Flowrate: 1.0ml/min

Result: 99.9%

The analysis is conducted in compliance with GLP.

Storage advice:
Keep in freezer.

Stability: -/-

Expiry Date: -/-

Date: 09 MAI 1995 Signature: R Pesige 95/11149 0048

Figure 35: Analytical certificates of PT 1-9

BASF Aktiengesellschaft

BASF

Landwirtschaftliche Versuchsstation der BASF - Postfach 120 - 67114 Limburgerhof

Purity Statement

- External Reference Substance -

APS/UP
Product Chemistry

Reg.No.: 248667 **Lot-No.:** L43-267

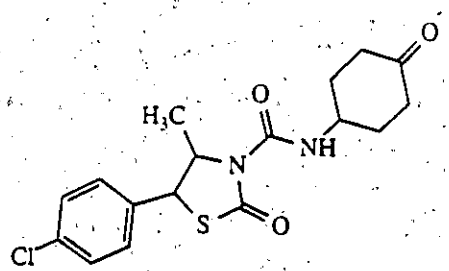
Supplier: Nippon Soda Co., Ldt.

Common name: -/-

Chemical name: -/-

Empirical formula: $C_{17}H_{19}ClN_2O_3S$
Molecular weight: 366.87

Structure: **Code:** PT-1-9



Purity stated: 99.9%, homogeneous **Date:** 27.12.1993

Methods used for purity check:

HPLC:
Stationary Phase: CAPCELL PAK C18, 250 x 4.6mm
Mobile Phase: $CH_3OH + H_2O = 7 + 3$
Wavelength: 225.240nm
Flowrate: 1.0ml/min

Result: 99.9%

The analysis is conducted in compliance with GLP.

Storage advice:
Keep in freezer.

Stability: -/-

Expiry Date: -/-

95/11149 0049

Date: 09 MAI 1995 Signature: R Rosi Lyel