

1.0 INTRODUCTION

1.1 SCOPE

This method is used to determine the residues of BAS 490 F and BF 490-1 in aquatic media. An aquatic medium is extracted through a C18 solid phase extraction cartridge (SPE). The cartridge is extracted with methanol (MeOH). A sample of the extract is injected onto a GC/ECD. This gives the amount of BAS 490 F in the sample. An aliquot of the extract is then methylated and injected onto the GC/ECD. From this, a total amount of parent and hydrolysis product is determined. By subtracting the non-methylated result from the methylated one, an amount of, BF 490-1, can also be determined. BF 490-1 is a known aqueous hydrolysis product⁽¹⁾ of BAS 490 F.

1.2 SOURCE OF METHOD

The basic method for determining residues of BAS 490 F and BF 490-1 in water media was developed by BASF Corporation and modified by ADPEN Laboratories, Inc.

1.3 PRINCIPLE OF THE METHOD

An aliquot of aqueous medium is passed through a pre-conditioned C18 solid phase extraction column. BAS 490 F, the parent compound (a methyl ester) and its hydrolysis product, BF 490-1 (the corresponding acid), are eluted with 30 ml of methanol. The eluate is concentrated in a rotary evaporator to less than 10 ml at a water bath temperature in the range of 30 to 35°C. The sample is allowed to sit in the refrigerator prior to filtering with an Acrodisc syringe filter. An aliquot of the extract is injected onto a GC capillary column and analyzed for the parent compound using an electron capture detector. A 1 ml aliquot of the extract containing both the parent and hydrolysis product is methylated with TMS-diazomethane to convert the hydrolysis product to the parent. The resulting sample contains the "total" contribution of both the parent and derivatized hydrolysis product. It is then injected onto a GC capillary column and analyzed for the parent compound using an electron capture detector. The BF 490-1 contribution is determined by difference. A flow diagram of the method is shown in Figure 1. The structures of BAS 490 F and BF 490-1 are shown in Figure 2. Description of the reference substances can be found in Table I.

3.0 EQUIPMENT

Names of equipment manufacturers and brands are suggested. These may be substituted and equivalent equipment may be used.

- 3.1 Concentration tube, 50-ml, standard tapered joints, Corning or equivalent
- 3.2 Flasks, 24/40 standard tapered joints, 125 ml, flat bottoms
- 3.3 General laboratory glassware
- 3.4 N-Evap (Nitrogen Stream Evaporator): Organomation Assoc.
- 3.5 Rotary evaporator, Buchi Model RE 121 or equivalent, equipped with a heated water bath
- 3.6 Screw cap vials, 6 dram or 30 ml equivalent
- 3.7 Solid Phase Extraction, vacuum manifold, 24-port
- 3.8 Syringe Filter, Acrodisc[®] LC13 PVDF 0.45 μ , Gelman
- 3.9 Syringe, gas tight, 5 ml, #1005, Hamilton, or 20-ml syringe
- 3.10 Volumetric flasks, miscellaneous sizes
- 3.11 Volumetric pipettes, type A, various sizes
- 3.12 Solid Phase Extraction (SPE) column, 500 mg, Octadecyl (C18), Varian
- 3.13 Reservoir, 20-ml, with connector for SPE column, Varian
- 3.14 Glass wool, silanized

4.0 SAFETY

All analysts must be familiar with the potential hazards of each of the reagents, solvents, and products used in this method before any laboratory work is done. Material Safety Data Sheets (MSDS), laboratory Safety Manual, product information, and other related materials should be consulted. Exposure to all chemicals should be reduced to the lowest possible level. Analysts should also be aware of OSHA regulations regarding the safe handling of the chemicals specified in this method. Disposal of all chemicals must be in compliance with local, state, and federal laws and regulations.

- 4.1 Trimethylsilyl (TMS) -diazomethane is a safer reagent and a substitute for diazomethane. Diazomethane is a very toxic and explosive yellow gas. Concentrated solutions may explode violently especially in the presence of impurities. Gaseous diazomethane may explode on heating above 90°C or on rough surfaces. Although TMS-diazomethane is safer, handle it with care, as it is an irritant and a highly flammable liquid. Avoid the use of glass stirrers or disposable pipettes. Keep refrigerated.
- 4.2 Acetone, acetonitrile, methanol, and n-propanol are flammable. Care should be taken to use these solvents in well ventilated areas away from ignition sources.
- 4.3 All open flask evaporations with an N-Evap should be done inside a hood.

5.0 REAGENTS

Names of chemical manufacturers and brands are suggested. These may be substituted and equivalent equipment may be used.

- 5.1 Acetonitrile, Burdick & Jackson, pesticide residue grade
- 5.2 Acetone, Burdick & Jackson, pesticide residue grade
- 5.3 Diazomethane, trimethylsilyl (TMS), 2.0M solution in hexane is recommended, Fluka, # 92738, or Aldrich Chemical Co. #36283-2
- 5.4 0.02M TMS-diazomethane in hexane, prepared from 2.0M solution of the reagent by taking a 1.0 ml aliquot and diluting to 100.0 ml with hexane. TMS-diazomethane is a safer reagent and a substitute for diazomethane. Handle with care due to the irritant and highly flammable nature of the liquid. See Section 4.0.
- 5.5 Methanol, Burdick & Jackson, pesticide residue grade
- 5.6 n-Propanol, Burdick & Jackson, pesticide residue grade
- 5.7 Water, deionized

6.0 INSTRUMENTATION

Names of instrument manufacturers are suggested, equivalent brands may be substituted.

- 6.1 Gas Chromatograph, Hewlett Packard 5890, Series II, or equivalent, equipped with electron capture detector operated according to conditions outlined in Table I.
- 6.2 Gas Chromatography Capillary Column, DB[®]-17, 30 meter column, 0.25um film thickness, 0.32 mm ID. J & W.
- 6.3 Inlet liner, 4mm ID., deactivated, tapered on one side, 5181-3316, Hewlett Packard.

7.0 PREPARATION OF STANDARD SOLUTIONS

All analytical standards shall be kept in the freezer. All standard solutions shall be kept in amber bottles and stored in a refrigerator. The details given for making dilutions are suggested. Concentrations and method of dilution may be modified if needed.

7.1 STOCK SOLUTIONS

Prepare a 0.10 mg/ml BAS 490 F stock solution by accurately weighing 0.0250 g of BAS 490 F standard into a 250 ml volumetric flask. Dissolve in acetonitrile and dilute to the mark. Prepare a 0.10 mg/ml BF 490-1 stock solution by accurately weighing 0.0250 g of BF 490-1 standard into a 250 ml volumetric flask. Dissolve in acetonitrile and dilute to the mark.

7.2 STANDARD FORTIFICATION SOLUTIONS

Prepare a 4.5 ng/ul mixed stock solution of BAS 490 F and the hydrolysis product, BF 490-1 from the 0.10 mg/ml stock solutions and dilute in deionized water. Make further dilutions in deionized water as needed. These solutions are used for fortification and shall be prepared fresh every two days.

7.3 STANDARD SOLUTIONS FOR GAS CHROMATOGRAPHY

These standards may be prepared either by serial dilution or from direct dilution of the BAS 490 F stock solution into methanol. The following concentrations are suggested: 1.0 ng/ul, 0.75 ng/ul, 0.375 ng/ul, 0.30 ng/ul, 0.15 ng/ul, 0.075 ng/ul, and 0.0375 ng/ul. Different preparation schemes may be used and additional standard concentrations may be prepared and used as needed.

8.0 SAMPLE WORKUP

8.1 RECOVERY TEST

The validity of the procedure should be demonstrated by recovery tests before analysis of unknown samples is attempted. An untreated sample (control) and two or three fortified samples shall also be processed with each set of samples analyzed. Typically, one of the fortification samples is run at the limit of quantitation. For each fortified sample, an appropriate volume of BAS 490 F and BF 490-1 mixed standard solution is added to a control medium sample. Fortifications are made into the sample after placing the sample on the C18 column.

8.2 PREPARATION OF SAMPLES FOR EXTRACTION

Each lot of SPE columns should be profiled to determine if the lot being used will provide adequate recovery of BAS 490 F and BF 490-1. Elution parameters may be modified if needed.

8.2.1 Keep all samples frozen until ready for analysis, thaw out sample and shake well to make homogeneous.

8.2.2 Connect a 20-ml reservoir to the C18 SPE column and condition by pulling 20 ml of n-propanol through the column using a vacuum of about 5 in. of mercury (Hg), followed by 20 ml of deionized water. Do not allow column to go dry at this step. The use of a one way stopcock to stop the flow is recommended.

8.3 EXTRACTION OF RESIDUE

8.3.1 Using a volumetric pipette, transfer 15 ml of the aqueous sample onto the reservoir on top of the conditioned C18 solid phase extraction column.

- 8.3.2 Using a vacuum of at least 3 in. of Hg., pull the sample through the cartridge. Do not save the eluant.
- 8.3.3 Dry the cartridge for 5 to 8 minutes with a vacuum of 5 in. Hg. to get rid of as much water as possible. Increase vacuum pressure several times during this time to force droplets of water through.
- 8.3.4 After removing the water from the cartridge, elute the sample with 30 ml of methanol and collect the eluate in an 6 dram or 30-ml vial.
- 8.3.5 Transfer the eluate quantitatively from the 6-dram vial into a 125 ml flat-bottom flask rinsing the vial with three small portions of methanol and transferring each to the flat-bottom flask.
- 8.3.6 Concentrate the eluate to approximately 7 ml, using a rotary evaporator at a water bath temperature in the range of 30 to 35°C.
- 8.3.7 Transfer quantitatively with methanol to a 10 ml volumetric flask or calibrated concentration tube and bring the extract to exactly 10.0 ml and mix. Refrigeration of the extract prior to filtration may be required, see Section 4.0. /S. 0.
- 8.3.8 Allow to come to room temperature if sample was refrigerated. Fit a 0.45µm Acrodisc syringe filter to a 5.0 ml gastight syringe and transfer quantitatively 5.0 ml of the extract to the syringe. Filter the extract through the syringe filter and into a 5.0 ml volumetric flask. Rinse the syringe with about 0.5 ml of methanol and pass through the filter and into the volumetric flask to dilute sample to the 5.0 ml mark.
- 8.3.9 Transfer approximately 1 ml of the extract into a GC vial and cap it. Save vial for injection together with the methylated samples on Section 8.3.13.
- 8.3.10 Take 1.0 ml of the extract from step 8.3.8 and place it in a 50-ml concentration tube. Add 10 ml of 0.02M TMS-diazomethane in hexane, add 5 ml of acetone, and mix well by swirling. Allow to sit for 30 minutes under a hood and then add 3 ml of MeOH. TMS-diazomethane is a safer reagent and a substitute for diazomethane, see Section 4.0. The sample size may be doubled in this step as long as the amount of reagent and solvents are doubled.
- 8.3.11 Take the methylated extract and blow it down with a gentle stream of nitrogen to just dryness using an N-Evap with a water bath temperature of approximately 45°C.
- 8.3.12 Add 3 ml of MeOH and repeat step 8.3.11 to remove traces of diazomethane. The sample should be colorless. If not, gently repeat step 8.3.11 only one more time. Dilute sample accurately to 1.0 ml or an appropriate volume with methanol.

8.3.13 Inject samples from 8.3.9 and 8.3.12 into a gas chromatograph equipped with an electron capture detector (ECD) as described in Section 9.0 below.

9.0 CHROMATOGRAPHY

- 9.1 The suggested chromatographic conditions are given in Table II. The chromatography should be checked for response whenever a new injection port liner, column, or instrument is used. Approximately 5 cm. of the inlet end of the GC column should be cut off if the peak shape or sensitivity deteriorates. The GC column should be conditioned with several injections of sample extracts and standards prior to injecting samples to be quantitated. See Figures 4 and 5 for typical chromatograms.
- 9.2 Change the deactivated glass injection port liner daily. The liner should contain a very small wad of silanized glass wool. Replace the septum daily or after approximately 100 injections have been made if HP 5181-1263 low-bleed red septa or equivalent is used.
- 9.3 Inject 1- μ l aliquots of the standards and samples. A small injection size is suggested to protect the capillary column, but 2- μ l injection size may be used if necessary. Calibrate the detector response and retention times by injections of the standard solutions throughout a set of analyses. Standards shall also be injected at the beginning and at the end of a set of analyses.
- 9.4 Analytical recoveries are determined as described in Section 13.5. Sample residues are determined as described in Section 13.1 through 13.4.

10.0 TIME REQUIRED FOR ANALYSIS

Analysis of a set of 10 media water samples requires about 7 man hours. GC analysis may be done overnight by autosampler. Data entry, integration and data report may take up to an additional 3 man hours.

11.0 INTERFERENCES

11.1 Sample Matrices

Baseline resolution was attained for BAS 490 F when using the DB-17 column. If interfering peaks from the matrix occur in the chromatogram, change the GC operating conditions or use an alternate GC column such as DB-1701 or DB-5 of the same length.

11.2 Other Sources

BAS 490 F is resolved from a background unknown peak with the DB-17 column. No interfering peaks from pesticides, solvents, or labware known to date.

12.0 CONFIRMATORY TECHNIQUES

No problems with interferences or questionable peak identity have been encountered to date. A GC column with different polarity, such as a DB-5, 30 meter column, may be used for confirmation if necessary.

13.0 METHODS OF CALCULATION

13.1 STANDARD CALIBRATION CURVE

Each standard should be injected at least twice in the analysis set. Standards are injected at the beginning, after every 1 to 3 samples and at the conclusion of the analysis. The peak height or peak area for each injected standard, for at least four concentration levels, is determined by manual measurement or computer integration using a chromatography data system. Regression analysis of peak height or peak area versus nanograms injected may be performed by a scientific calculator or a computer chromatography data system. This regression analysis gives an equation for a standard curve for calculation of sample concentration. The standard curve should have a correlation coefficient (r) of 0.9800 or better. A scientific calculator or a computer chromatography data system is used to calculate nanograms injected from the slope and intercept of the standard curve and the chromatographic peak height or area of each sample injection.

13.2 CALCULATION OF EQUIVALENT SAMPLE WEIGHT

The milligrams of sample injected must be determined to calculate ppb (Section 13.4). The equivalent sample weight in the final solution is calculated as follows:

$$\text{mg inj.} = \frac{(W)(V_s)}{(V_e)} \times \frac{(V_i) \times 1000}{(V_f)}$$

- W = weight of sample extracted (g) (1 ml=1 g for clear water only)
- 1000 = conversion factors (mg/g)
- V_s = aliquot volume (ml) (section 8.3.9 for parent or 8.3.10 for BF 490-1)
- V_e = volume of the extract (ml), prior to sample aliquoting (section 8.3.7)
- V_f = total volume of final injection soln. (ul)
- V_i = injection volume (ul)

13.3 DETERMINATION OF SAMPLE RESIDUES (NANOGRAMS)

The peak height or area from a sample injection (Section 13.1) and the slope and intercept of the standard curve (Section 13.1) are used to determine the nanograms of residue in each sample injection. This can be done by a chromatography data system, calculator or by graphing a standard curve of nanograms injected versus detector response. The next section shows how to calculate sample residues in parts-per-billion.

13.4 DETERMINATION OF SAMPLE RESIDUES (PPB)

Calculate the sample residue for each sample expressed in terms of parts-per-billion (ppb) using the following equation:

$$\text{ppb of BAS 490 F found} = \frac{(\text{ng of BAS 490 F found } ^1) \times 1000^2}{(\text{mg of sample injected } ^2)}$$

¹ Section 13.3

² Section 13.2

³ Conversion factor (ppb/ppm)

To calculate results for BF490-1, the BAS 490 F ppb found in the derivatized extract (total BAS 490 F) is subtracted from the BAS 490 F ppb found in the non-derivatized extract.

$$\text{ppb BF490-1} = (\text{ppb of BAS 490 F, total} - \text{ppb of BAS 490 F}) \times 0.9553$$

Where 0.9553 = the molecular weight correction factor for the conversion of BAS490F to BF490-1 analytes.

$$= \frac{\text{Gram Molecular Weight of BF 490-1 (289.34)}}{\text{Gram Molecular Weight of BAS 490 F (313.34)}}$$

13.5 FORTIFICATION RECOVERIES

The ppb of compound found in the final solution (Section 13.4) is divided by the amount of compound added to the control sample. This ratio times 100 is the percent recovery of the method at that level of fortification.

$$\% \text{ Recovery} = \frac{\text{ppb analyte found in injected solution}}{\text{ppb analyte added to control sample}} (100)$$

If the control sample shows a chromatographic response corresponding to the analyte(s) of interest, the ppb value corresponding to this control sample response should be subtracted from the ppb residues found in the fortified samples before the percent recovery calculation is made, i.e.:

$$\text{ppb found in recovery} = \text{ppb in fortified sample} - \text{ppb in control sample}$$

Because the residue determination of the hydrolysis product is calculated from the difference between parent residues and total residues, a lower or higher recovery of parent than 100% will affect the percent recovery of the hydrolysis product by increasing or decreasing the percent recovery value. An average recovery value between 70 and 120% for each set of analysis should be acceptable for this type of procedure.

15.0 NOTES

- 15.1 The aquatic media used for this validation contains a relative large number of inorganic salts and metals that are not removed by the C18 extraction column. These inorganic components in the media coat the GC glass insert and the inlet side of the capillary column causing chromatographic problems related to sensitivity. These salts also appear to cause the autosampler syringe plunger to stick. To correct these problems a filtration step was included in the method. For the aquatic media substrates used in the validation of this procedure these inorganic components could be observed as a colloidal suspension and/or larger particulates in the sample extract when kept in a refrigerator for at least one day. It is recommended that the samples be refrigerated overnight or for a day, be brought to room temperature, and then filtered as noted in Section 8.3.8.

TABLE I. Description of Reference Substances

Reference Substances:

1. Common Name:	Not available
2. Chemical Name:	methyl-(E)-methoxyimino-(o-tolyl)oxy)-o-tolyl)acetate
3. Experimental Name:	BAS 490 F
4. CAS Number:	none
5. BASF Lot number:	CH397149-1
6. Purity:	99.6%
7. Date Received at ADPEN:	12/15/92
8. Storage Conditions at ADPEN:	Freezer, in the dark
9. ADPEN Code Number:	P-294
10. Expiration or Reassay Date: a/	12/94
11. Empirical formula:	C ₁₇ H ₁₉ NO ₄
12. Molecular weight:	313.34
13. Melting Point:	101°C
14. Appearance:	Colorless crystals
15. Odor:	None
16. Partitioning Coefficient: 1 g. Pow	3.4 (n-octanol/water)
17. Solubility (mg/liter at 20°C)	
Acetone	Not available
Acetonitrile	Not available
DMSO	Not available
Ethanol	Not available
n-Hexane	Not available
Methylene chloride	Not available
Water	2.0
Std. fat	17.2 g/kg

1. Common Name:	NA
2. Chemical Name:	(E)-methoxyimino-(o-tolyl)oxy)-o-tolyl)acid
3. Experimental Name:	BF490-1 or BF490-A
4. CAS Number:	none
5. BASF Lot number:	LS1/9
6. Purity:	99.9%
7. Date Received at ADPEN:	12/15/92
8. Storage Conditions at ADPEN:	Freezer, in the dark
9. ADPEN Code Number:	P-293
10. Expiration or Reassay Date: a/	12/94
11. Empirical formula:	C ₁₇ H ₁₇ NO ₄
12. Molecular weight:	299.34
13. Solubility (mg/liter, water, 20C)	Not available

Analytical standards supplied by:

Landwirtschaftliche
Versuchsstation der BASF
Produktsicherheit
Pflanzenschutz
Produktchemie
Gebäude LI 444/GLP-Archiv von APS/U
Postfach 220
D-6703 Limburgerhof, Germany

a/ The purity of these materials has been determined under GLP's by BASF. BASF has archived an aliquot of these standards and has access to documentation relating to the synthesis and characterization of these compounds, including expiration dates / reassay dates. If no expiration or reassay date is available upon receipt of standards, ADPEN Laboratories, Inc. assigns two years after receipt of standard for the expiration date.

Table II. Suggested Gas Chromatographic Conditions

Gas Chromatograph: HP 5890 Series II

Initial Oven Temperature: 50° C
 Initial Hold Time: 0.75 min.

Temperature Program:

	Rate:	Final Temp.:	Final Time:
Ramp 1	37° C/min.	280° C	2.0 min.
Ramp 2	40° C/min.	300° C	0.0 min.

Injector Temperature: 200° C
 Injection Type: Splitless
 Injection Volume: 1 or 2 µl, (1µl is preferred)
 Purge Valve: On Time: 1.0 min.
 Off Time: 8.5 min.
 Detector: Electron Capture

Detector Gases: Auxiliary: 5% Methane/Argon at 60 ml/min (Nitrogen may also be used)

GC Columns: DB®-17, 30 meters, 0.25 um film thickness, 0.32 mm ID.

Carrier Gas: Helium, at 10 psi column back pressure with a regulated carrier source pressure of 40 psi.

Septum Purge: Helium, at 1.1 ml/min

Total Flow at Split Vent: Helium, at 150.0 ml/min

Minimum Response: 0.0375 ng of BAS 490 F

Approximate Retention Time: 8.3 min.

No needle residence time is specified. A fast injection type autosampler was used for the method validation. These are suggested conditions and may be modified if needed if it provides similar or better chromatography. Setting of carrier source pressure to 40 psi and column back pressure to 10 psi with above column will produce a set linear velocity, this linear velocity was not measured.

Figure 1. FLOW CHART OF ANALYTICAL PROCEDURE

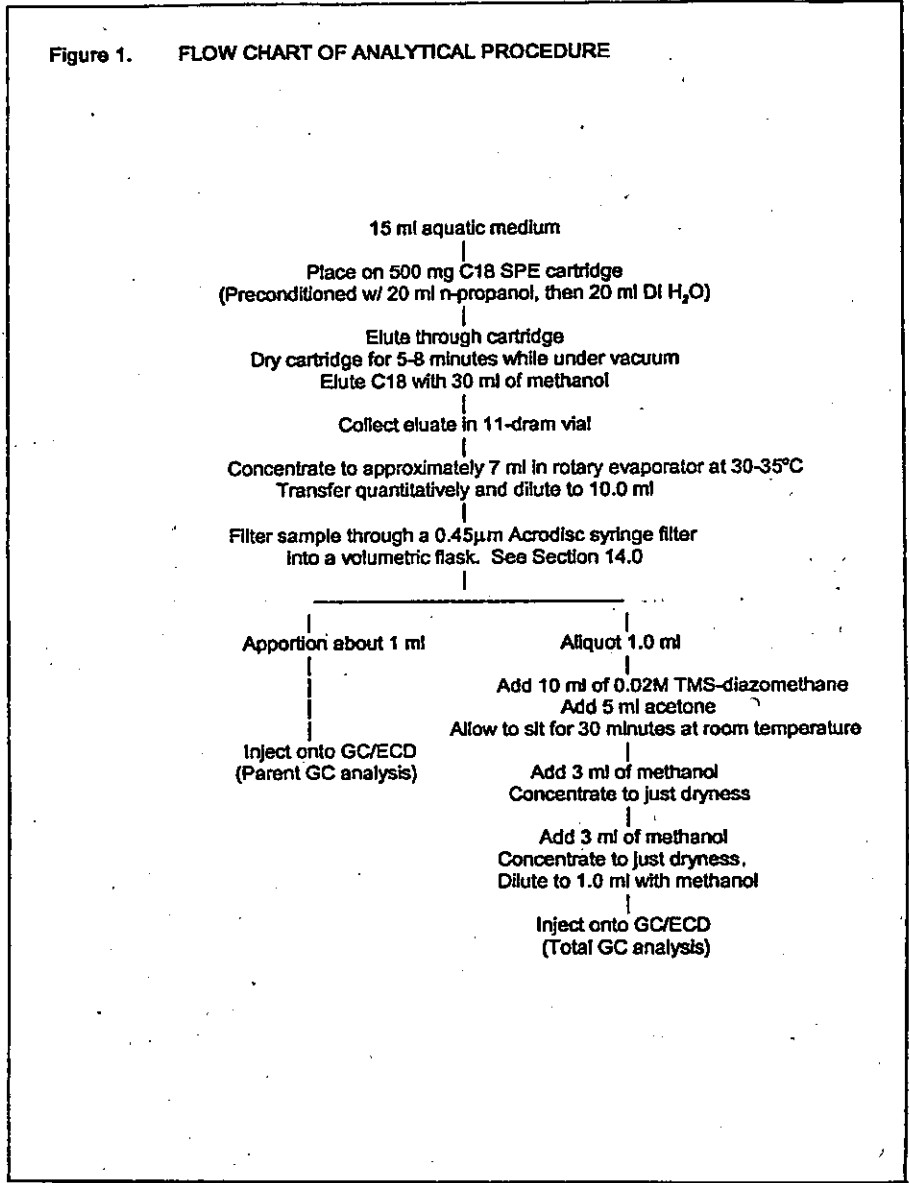
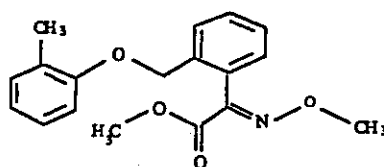
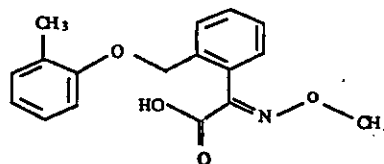


Figure 2. Structures Of The Test Substance And The Final Analytes



BAS 490 F



BF 490-1