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# IC METHOD FOR DETERMINATION OF MEPIQUAT CHLORIDE (BAS 083 W) RESIDUES IN SOIL

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ABSTRACT:

Analytical Method No. A9105 was developed to determine the residues of mepiquat chloride, the active ingredient in Pix® Plant Growth Regulator, in soil. Method development and validation were carried out at BASF Corporation, Research Triangle Park, N.C., using representative soil from a soil dissipation study site collected prior to treatment with the plant regulator. Mepiquat Chloride can be extracted from soil by refluxing in a basic aqueous solution. Clean-up of the extract is achieved by precipitation of contaminants in acidic medium, complexing and extracting with dipicrylamine-containing dichloromethane, decomplexing and extracting with an acidic aqueous solution and subsequent column chromatography on aluminum oxide. An ion chromatography system with conductivity detection is used for the final determination.

Experimental Dates:
Start: August, 1991
Termination: September, 1991

# 1. INTRODUCTION AND SUMMARY

## 1.1 Scope and Source of the Method

### 1.1.1 Scope

The method is used to determine the residue of mepiquat chloride in soil.

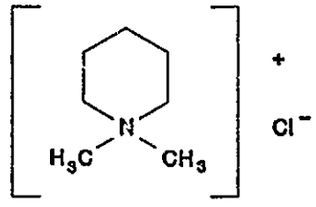
Metabolism investigations (see Reference 1) have shown that mepiquat chloride residues in soil consist almost exclusively of the parent compound. These investigations have also demonstrated the nearly quantitative extraction of the residues by refluxing in an alkaline solution. This method was therefore based on the determination of the active ingredient using the same type of extraction and final quantitation by conductivity detection using an ion chromatograph.

### 1.1.2 Source

This method was developed at the BASF Agricultural Research Center in Research Triangle Park, North Carolina.

## 1.2 Substance

| | |
|---|---|
| Common Name: | Mepiquat Chloride |
| BAS Number: | BAS 083 00 W |
| Chemical Name: | 1,1-Dimethylpiperidinium Chloride |
| CAS Number: | 24307-26-4 |
| Structural Formula: | |




| | |
|---|---|
| Empirical Formula: | $C_7H_{16}ClN$ |
| Molecular Weight: | 149.7 g/mole |
| Melting Point: | 285°C with decomposition |
| Boiling Point: | NA |
| Vapor Pressure: | $<7.6 \times 10^{-5}$ mbar at 20°C |
| Appearance: | White Crystals |
| Odor: | Odorless |

Solubility: (g substance in 100 g solvent at 20°C)

| | | | |
|---|---|---|---|
| Water | 202 | Dichloromethane | <0.1 |
| Methanol | 25.0 | Ethylacetate | <0.1 |
| Ethanol | 16.2 | Diethylether | <0.1 |
| Lutrol | approx. 3.8 | Cyclohexane | <0.1 |
| Chloroform | 1.1 | n-Hexane | <0.1 |
| Acetone | <0.1 | Olive Oil | <0.1 |

1.3 Principle of the Method

The sample is extracted with refluxing 0.5 N sodium hydroxide. After a precipitation of contaminants in acidic medium, the active ingredient is isolated in the form of a dipicrylamine-complex which is partitioned into dichloromethane. Mepiquat chloride is then decomplexed and extracted with an acidic solution and further purified by alumina column chromatography. For the final determination, ion pair chromatography with conductivity detection and a suppressor system is used.

Limit of quantitation: 0.01 mg/kg.

2. MATERIALS AND METHODS

2.1 Equipment-Suggested Sizes/Manufacturer

| | |
|---|---|
| Glass Buchner funnel | 90 mm diameter |
| Centrifuge Bottle | 250 mL |
| Filter Paper | Whatman No. 5, 9 cm i.d. |
| Filter Flask | 1L |
| Flat bottom flask, 24/40: | 500 mL, 1 L |
| Rotary Evaporator | Buchi or equivalent |
| Temperature Bath | Buchler or equivalent |
| Separatory Funnel | 500 mL, 1 L |
| Glass Wool | e.g. sterile |
| Chromatographic Column | 300 x 14.5 mm glass column equipped with a Teflon stopcock and at least a 200 mL reservoir. |
| Ultrasonic Bath: | Branson 1200 or equivalent |
| Pyrex centrifuge tube w/screw cap: | 15 mL, 50 mL |
| Disposable syringes (Luer): | Becton Dickinson |
| Luer-tip Syringe Filter: | Pore size 0.45 µm |
| Autosampler Vials: | Dionex 0.5 mL vials |
| Vial Caps: | Dionex 0.5 mL filtercaps |
| Volumetric Flask: | 100 mL, 500 mL, 2 L, 5 L |
| Centrifuge: | Beckman or equivalent |
| Nitrogen Stream Evaporator: | N-EVAP Organomation Associates Inc., or equivalent |
| Volumetric Pipette: | 0.5-10 mL |
| Stirring Hot Plate: | Corning or equivalent |
| Reflux Condenser 24/40: | 200-500 mm |
| Mechanical Soil Homogenizer: | Fitz® Mill Model J Homoloid, Humboldt Model H-4199 Soil Grinder, Hobart Table Top Bowl Cutter, Stephans Floor Chopper, or equivalent |
| Balance (with at least: tenth of a gram capability) | Mettler or equivalent |

2.2 Reagents and Chemicals - Source/Preparation

| Reagents and Chemicals | Source/Preparation |
|---|---|
| Acetone | Distilled, high purity |
| Methanol | Distilled, high purity |
| Hydrochloric acid, Conc. | Reagent grade |
| Sodium Hydroxide | Reagent grade |
| Celite filter aid Type 545, | Fisher or equivalent |
| Deionized water | |
| Dichloromethane | Distilled, high purity |
| Alumina, acidic, ICN Alumina A, activity level I, particle size 50-200 um (70-290 mesh) | Reagent grade (Recommend Fisher, Cat. No. A948-500) |
| Ultra pure water (18 Megohm·cm resistivity) | Millipore water purification system, or Fluka, Cat. No. 95305 |
| Hexane sulfonic acid sodium salt anhydrous or monohydrate used for ion chromatography, puriss. p.a. | Mallinckrodt |
| Acetonitrile | Distilled, high purity |
| Tetrabutylammonium hydroxide 30-Hydrate, crystalline, purum; >98% | Fluka |
| Dipicrylamine solution: 0.1 mg dipicrylamine/mL of dichloromethane or 0.2 mg dipicrylamine (50% by weight)/mL of dichloro-methane | (See NOTE below) |
| Dowex Cation Exchange Resin: | 50W - x4, 50-100 mesh, hydrogen-form, Bio-Rad Laboratories |

NOTE: During the development of this method, the 50% by weight dipicrylamine material was available through Aldrich. Since that time, Aldrich has discontinued distribution of this chemical. However, the material may be synthesized by a two-step procedure (see Reference 2).

2.2.1 Activity Adjustment of Alumina

In order to adjust the standardized activity according to Brockmann (see Ref. 3), alumina of activity I is shaken for 60 seconds in a capped flask with the necessary amount of water, until no lumps are left. The alumina is then allowed to stand for two hours with the flask tightly sealed.

Alumina activity level II:
97g alumina activity level I + 3g deionized water

2.2.1 Activity Adjustment of Alumina (Continued)

Alumina activity level IV:
90g alumina activity level I + 10g deionized water

Keep the flask tightly sealed. Before use, shake the flask vigorously. Do not store the adjusted alumina for more than 3 days before use.

2.2.2 Standard and Standard Stability

| | |
|---|---|
| Standard Substance Used: | 1,1-Dimethylpiperidinium chloride |
| Purity: | >95% |
| Source: | Dr. Pawliczek, APS/UP<br>BASF Aktiengesellschaft<br>Landwirtschaftliche Versuchsstation<br>D6703 Limburgerhof<br>West Germany<br>Telephone: 06236/68-2422 |

The standard stability was determined using a concentration of 3 µg/mL of mepiquat chloride in "ultrapure" water. According to the results, the standard solutions are stable for at least 119 days at either 4°C or room temperature (see Reference 4). Table I contains a summary of the stability data.

2.2.3 Standard Solutions for Fortifications

1,1-Dimethylpiperidinium chloride (mepiquat chloride, BAS 083 W): 1000, 100, 10, 1, 0.1 µg/mL in "ultrapure" water.

Prepare a 1000 µg/mL (1.00 mg/mL) BAS 083 W stock solution by weighing an appropriate amount into a volumetric flask. Dissolve with "ultrapure" water and dilute to the mark. For example to prepare 100 mL stock solution, dissolve 100 mg BAS 083 W in a 100 mL volumetric flask with "ultrapure" water. Dilute to the mark with "ultrapure" water.

Prepare a 100.0 µg/mL BAS 083 W standard solution by transferring an appropriate amount of the 1000 µg/mL standard solution with a volumetric pipet to a volumetric flask (typically 10 mL of the 1000 µg/mL standard solution into a 100 mL volumetric flask). Dilute to the mark with "ultrapure" water.

Prepare a 10.0 µg/mL BAS 083 W standard solution by transferring an appropriate amount of the 100.0 µg/mL standard solution with a volumetric pipet to a volumetric flask (typically 10 mL of the 100.0 µg/mL standard solution into a 100 mL volumetric flask). Dilute to the mark with "ultrapure" water.

2.2.3 Standard Solutions for Fortifications (Continued)

Prepare 1.00 µg/mL BAS 083 W standard solution by transferring an appropriate amount of the 10.0 µg/mL standard solution with a volumetric pipet to a volumetric flask (typically 10 mL of the 10.0 µg/mL standard solution into a 100 mL volumetric flask). Dilute to the mark with "ultrapure" water.

Prepare a 0.10 µg/mL BAS 083 W standard solution by transferring an appropriate amount of the 1.0 µg/mL standard solution with a volumetric pipet to a volumetric flask (typically 10 mL of the 1.00 µg/mL standard solution into a 100 mL volumetric flask). Dilute to the mark with "ultrapure" water

2.2.4 Standard Solutions for IC Analysis

1,1-Dimethylpiperidinium chloride (mepiquat chloride, BAS 083 W): Prepare 1000 µg/mL, 100 µg/mL, 10 µg/mL, and 1 µg/mL standards as defined in 2.2.3 or the fortification standards may be used for IC analysis.

Prepare 0.5 µg/mL BAS 083 W standard solution by transferring an appropriate amount of the 10.0 µg/mL standard solution with a volumetric pipet to a volumetric flask (typically 5 mL of the 10.0 µg/mL standard solution into a 100 mL volumetric flask). Dilute to the mark with "ultrapure" water.

Prepare 0.25 µg/mL BAS 083 W standard solution by transferring an appropriate amount of the 10.0 µg/mL standard solution with a volumetric pipet to a volumetric flask (typically 2.5 mL of the 10.0 µg/mL standard solution into a 100 mL volumetric flask). Dilute to the mark with "ultrapure" water.

2.2.5 Eluent and Regenerant Preparation

Eluent

Swell approximately 500 g of Dowex cation exchange resin for at least 24 hours in deionized water. Keep the water level above the resin during the swelling process. This quantity of cation exchange material can be used for the preparation of several columns. Place a glass wool plug just below the stopcock and add the resin into a glass column to a height of approximately 8-10 cm. Wash the exchange resin with 500 mL of ultrapure water.

2.2.5 Eluent and Regenerant Preparation (Continued)

Slowly add to the column hexane sulfonic acid sodium salt (2.06 g for the monohydrate, 1.88 g for the anhydrous) dissolved in 100 mL of ultrapure water. Elute the solution drop-wise into a 500 mL volumetric flask. Rinse the column further with 200 mL of ultrapure water and combine the eluate and the rinsing water. Dilute to the mark with "ultra pure" water. This solution is hereby referred to as the "eluent concentrate". Before use, the ready-to-use eluent is generated by transferring the "eluent concentrate" into a 5 L volumetric flask, adding the appropriate amount (250 mL for 5%) of acetonitrile, and diluting to the mark with "ultrapure" water. The final concentration of the hexane sulfonic acid should be approximately 2 mM.

Regenerant

Dissolve 20 g Tetrabutylammonium hydroxide 30-hydrate, (purum; >98%) in ultrapure water in a 1 L volumetric flask and dilute to the mark with ultrapure water. Any portion of the solution not being used with the IC is refrigerated.

2.3 Analytical Procedure

A flow chart of the analytical procedure is presented in Figure 1.

2.3.1 Preparation of Sample

Homogenize soil samples thoroughly before subsampling and weighing. Remove any extraneous material. Depending upon the soil consistency, the samples may be homogenized manually or mechanically. Dry ice may be used to aid in this process, but it must be completely sublimed before any sampling is done.

2.3.2 Extraction

a. Place 50 ± 0.2 g of the sample material, weighed to the nearest tenth of a gram, into a 1 L flat-bottom flask, and add 200 mL of 0.5 N NaOH. For fortified samples, the fortifications are added at this time.

b. Reflux the material for 30 minutes. Allow the material to return to room temperature (may be aided with an ice bath), and rinse the condenser with 5-10 mL of distilled water before removing the sample.

c. Transfer the material quantitatively to a 250 mL centrifuge bottle with the aid of several rinses of distilled water.

2.3.2 Extraction (Continued)

d. Centrifuge the material until the particulate matter is pelletized enabling the supernatant liquid to be decanted back into the 1 L flask.

e. Extract the soil three more times by adding 100 mL of 0.5 N NaOH to the centrifuge bottle, shaking the bottle to loosen the soil, and centrifuging. For each re-extraction, add the supernatant to the 1 L flask.

2.3.3 Precipitation

a. Adjust the pH of the sample to 1-2 using concentrated HCl. Let the sample stand for 10 minutes to allow precipitation of contaminants.

b. Filter the solution through a Buchner funnel containing a sheet of Whatman #5 filter paper with a layer of Celite (1-2 cm height) into a 1 L filter flask. Rinse the flat bottom flask twice with 25 mL of distilled water and filter the rinses through the filter cake and collect into the 1 L filter flask.

2.3.4 Liquid-Liquid Partition with Dipicrylamine

a. Adjust the pH of the acidic solution from 2.3.3 to pH 10 with 12 N NaOH. Quantitatively transfer the basic solution to a 1 L separatory funnel with the aid of several rinses of distilled water.

b. Extract the basic aqueous phase twice with 100 mL of dipicrylamine solution (see preparation). Collect the lower organic phase, and the emulsion layer if present, from both partitions in a 500 mL flat bottom flask. Discard the alkaline water phase.

NOTE: If there is poor phase separation due to the formation of an emulsion, centrifuge the phases until separation occurs. Transfer with a pipette the aqueous phase from the first dipicrylamine partition from the centrifuge bottle back to the separatory funnel. Save the organic phase and the emulsion layer. After the second partition and centrifugation step, save the organic phase and emulsion layer and remove and discard the aqueous phase from the centrifuge bottle with a pipette.

c. Transfer the organic phases to a clean 500 mL separatory funnel. Rinse the flat bottom flask with 100 mL of 2M HCl. Add the rinse to the separatory funnel.

2.3.4 Liquid-Liquid Partition with Dipicrylamine (Continued)

d. Extract the mepiquat chloride into the acidic aqueous phase. Discard the organic phase, but if present save the emulsion layer along with the aqueous phase.

NOTE: When extracting the mepiquat chloride into the acidic aqueous phase, shake the separatory funnel until the organic layer has returned to a color similar to the dipycrylamine solution.

e. Wash the aqueous layer with 50 mL of dichloromethane. Discard the lower dichloromethane layer and save the emulsion layer with the aqueous phase.

f. Transfer the acidic aqueous phase from the 500 mL separatory funnel into a 500 mL flat bottom flask and rinse the separatory funnel with 20 mL deionized water and add to the flat bottom flask. Rotary evaporate the solution to dryness at a water bath temperature of 60±5°C.

NOTE: If traces of hydrogen chloride are thought to be present (from odor or poor chromatography on the IC), remove by adding 10 mL of deionized water followed by sonication and rotary evaporation to dryness.

2.3.5 Alumina Column Separation

NOTE: Each time a new lot of alumina used in the method, a column profile needs to be performed to ensure the elution pattern has not changed. The profile may be run using only standard material.

a. Add 10g alumina with activity level IV (see 2.2.1) to the dry residue from 2.3.4. Swirl the flask to ensure contact with all of the residue adhered to the flask.

b. Add 20 mL of acetone/methanol (95/5, v/v) hereby referred to as the eluting solvent. Swirl the flask and sonicate for at least 60 seconds and until no particles adhere to the inside surface of the flask.

c. After settling of the alumina residue, decant the solvent into a 100 mL beaker. Repeat the procedure by adding another 20 mL of eluating solvent to the flask decanting the solvent into same the 100 mL beaker. Save the decanted solvent for eventual addition to the alumina column.

d. Dry the alumina residue by applying a gentle stream of nitrogen into the flask.

2.3.5 Alumina Column Separation (Continued)

e. Prepare a column by plugging the end with glass wool and filling with 10 g of alumina activity level II (see 2.2.1) slurried in 20 mL of eluting solvent. After the alumina has settled, drain the solvent until the solvent level is within 8-10 cm of the alumina. The 10g of alumina containing the residue is slowly poured onto the column. For better introduction of the solid residue, a dry long stemmed glass funnel can be used. Gently tap the sides of the glass column to release any trapped air bubbles. Drain the solvent to within 1-2 cm of the alumina.

f. Add a glass wool plug to the column about 2 cm above the solvent. Quantitatively transfer the rinses from the 100 mL beaker to the column with the aid of several rinses of the eluting solvent. Collect the eluate in a 500 mL flat bottom flask until the solvent is within 0.5-1 cm of the alumina. Add 50 mL of the eluting solvent to the residues remaining in the flask which contained the alumina and transfer onto the column. Collect the eluate in the same 500 mL flask.

g. When the upper level of the liquid is within 0.5-1.0 cm of the surface of the column packing, add 150 mL of the eluting solvent onto the column and allow the column to run dry. Evaporate the eluate to dryness using a rotary evaporator at 45±5°C.

h. Rinse the flask 3 times with 5 mL of MeOH and transfer the rinses to a pyrex centrifuge tube which has a size appropriate to handle the final dilution volume. Evaporate to dryness using a gentle stream of nitrogen with a nitrogen evaporator. Set the water bath to approximately 50°C.

2.3.6 Preparation for Final Determination by Ion Chromatography

Dissolve the dry residue of 2.3.5 in an appropriate amount of "ultrapure" water so that the residue will be within the standard curve. If a 10 ng standard is the lowest standard used, the final volume for controls and samples fortified at the quantitation limit is 2.5 mL. If a 25 ng standard is the lowest, the final volume is 1 mL for these types of samples. The solution is sonicated and vortexed.

Draw up approximately 0.7 mL into a 1 mL disposable syringe and filter through a 0.45 µm syringe filter into a 0.5 mL Dionex vial. Seal the vial with the special Dionex filter caps. Inject 100 µL of this solution for ion chromatographic analysis.

Instrument and conditions for the ion chromatographic analysis are presented in 2.4. It is important to strictly follow the footnotes for the precolumn and regenerant (see 2.4 and 2.4.1).

2.4 Instrumentation

Equipment and operating conditions in the following list are examples and may be changed if necessary.

| | |
|---|---|
| Instrument: | Dionex Bio LC 4000 I (Basic Chromatography Module, Conductivity Detector, Gradient Pump, Eluant Degas Module, Automated Sampler) |
| Precolumn: | Ionpac-Column, NG-1, 5 µm (See note below) |
| Main column: | Ionpac-Column, NS-1, 10 µm |
| Suppressor: | Cation Micro Membrane Suppressor, Model CMMS-II |
| Additional pump for column switching: | Dionex Model DQP-1 |
| Restrictor: | Back pressure regulators equalling the back pressure caused by the separator column and suppressor are used. Typical back pressure equals approximately 750 psi. Several regulators in series may be used (Upchurch Scientific, Inc.). |

NOTE: Before running a set of samples, check the retention time of mepiquat chloride on the precolumn and adjust the switching times. if necessary. The highest standard of the calibration curve is used to determine the exact switching times.

2.4.1 Principle of Operation

The ion chromatography separation is based on an ion-pairing technique, where mepiquat chloride is paired with hexane sulfonic acid. The chromatography of the ion pair is conducted on a neutral, hydrophobic column with an aqueous/acetonitrile mobile phase.

A pre-column and a separator column are connected in series by an 8-port valve and a 4-port valve . The system is configured such that the pre-column can be channeled in 3 directions:

1. directly to the suppressor column and detector, by-passing the separator column,

2. to waste, by-passing the separator column and detector,

3. to the separator column, suppressor column and detector.

2.4.1 Principle of Operation (Continued)

In order to produce a clean sample and prolong the life of the separator column, samples and standards are always injected onto the pre-column. The pre-column effluent is initially channeled to waste. At the retention time of mepiquat chloride, the effluent is switched onto the separator column, through the suppressor column and to the detector. Once mepiquat chloride has completely eluted from the pre-column, the pre-column effluent is again switched to waste. A second pump maintains the flow through the separator column. The correct retention time of mepiquat chloride through the pre-column is determined by connecting the pre-column directly to the suppressor column and detector and injecting standards. This should be done on a daily basis, as the "switching window" changes periodically. The window should be made long enough to allow all of the mepiquat chloride to be eluted onto the separator column.

A diagram of the suggested configuration for this system is presented in Figure 2.

Suggested Valve Configurations

Valve 1 is the injection port. It is ON during injections, and OFF after injections.

The diagram in Figure 2 as shown is in the configuration described in Situation 2.

Situation 1 - Pre-column to waste, separator column to detector.
Use: To be used during a run except for during the switching window.

Valve 2: OFF Valve 3: ON Valve 4: OFF

Situation 2 - Pre-column to separator column to suppressor to detector.
Use: Used only during the switching window, to transfer mepiquat chloride from the pre-column to the separator column.

Valve 2: ON Valve 3: ON Valve 4: ON

Situation 3 - Pre-column to detector, separator column to waste.
Use: Used to determine the retention time of mepiquat chloride through the pre-column (switching window).

Valve 2: OFF Valve 3: OFF Valve 4: OFF

2.4.2 Operating Conditions

Mobile Phase: 2 mM hexane sulfonic acid with 5% acetonitrile, in "ultrapure" water. The 5% may be adjusted depending on the chromatography. Less acetonitrile causes longer retention times.

NOTE: Preferably, both eluent reservoir lines for Pumps 1 and 2 should draw eluent from the same reservoir.

Regenerant: ca. 25 mM tetrabutylammonium hydroxide in "ultrapure" water.

NOTE: Do not use the Dionex autoregenerant cation cartridge. The cartridge causes severe baseline fluctuations that inhibit chromatographic analysis.

He-pressure above eluent and regenerant: (If regenerate is pumped through the suppressor with pressure) 0.2 bar (= 2.9 psi)

Temperature: Room temperature

Flow: 1 mL/min

Injection volume: 100 µL (sample loop)

Range: 0.3 µS → 1 µS

Retention times (approx.):

Precolumn only: 1.2-2.0 min (switching times) (the actual switching times need to be determined daily)

Precolumn + main column: 17 min.

NOTE: If retention times start to drift, it is possible to regenerate the columns by flushing them with a 90:10 (v/v) acetonitrile/water solution.

2.5 Interferences

2.5.1 Sample Matrices

None observed to date.

2.5.2 Other Sources

| | |
|---|---|
| Other Pesticides: | None observed to date. |
| Solvents: | None observed to date. |
| Lab Ware: | None observed to date. |

2.6 Confirmatory Techniques

None used to date.

2.7 Time Required for Analysis

For a set of 7 treated samples, 2 fortifications and one control approximately 23 man hours including the final determination and data reduction are required provided that no special problems arise.

2.8 Potential Problems

Only reagents with a purity as stated should be used. Reagents from different manufacturers should be checked by analyzing a reagent blank. Any new lot of alumina should be checked by running a column profile using a standard of the active ingredient.

If instrument sensitivity causes problems in quantitating samples at the quantiation limit, the amount of acetonitrile in the mobile phase may be increased which will cause a shorter retention time for mepiquat chloride and a greater peak height.

3. METHODS OF CALCULATION

3.1 Calibration

Calibration Procedures. Calculation of results is based on peak height measurements using a calibration curve. To obtain a standard curve, 100 µL of at least three different standard concentrations (e.g. 0.1 µg/mL, 0.5 µg/mL and 1.0 µg/mL) of mepiquat chloride are injected. This corresponds to 10, 50 and 100 ng of mepiquat chloride injected. The peak height (signal counts) is plotted versus amount of injected standard (ng).

3.2 Analyte in Sample

3.2.1 Principle

Calculation of results is based on peak height measurements. The amount of mepiquat chloride in injected samples ($W_A$) is determined from the calibration curve and the equation described in 3.2.3 is utilized for the determination of residue (R). Calculation can also be made by a suitable computer program.

At least one fortification and one untreated sample (= control) are run with each set of samples. The amount of mepiquat chloride for fortification trials should be on the order of magnitude of the expected residue. The recovery (= B) is determined from the fortification experiments (see 3.2.2).

3.2.2 Calculation of Recoveries

The fortification recovery for mepiquat chloride (B) in % is calculated from the recovery trials as follows (example calculation given in Figure 3):

$$\% \text{ Recovery} = \frac{W_F \times V_{EF}}{V_{IF}} - \frac{W_C \times V_{EC}}{V_{IC}} \times \frac{100\%}{F}$$

$W_F$ = Amount of active ingredient (in ng) determined from the calibration curve (fortified sample)

$W_C$ = Amount of active ingredient (in ng) determined from the calibration curve (control sample)

F = Amount of active ingredient (in µg) fortified

$V_{EF}$ = Final volume of the fortified extract before injection (in mL)

$V_{IF}$ = Actual injected volume (in µL) of fortified extracted

$V_{EC}$ = Final volume of the control extract before injection (in mL)

$V_{IC}$ = Actual injected volume (in mL) of control extract

3.2.3 Calculation of Residues

The residue of mepiquat chloride (R) in ppm is calculated as follows (example calculation given in Figure 4):

$$R = \frac{W_A}{G} \times \frac{V_E}{V_I}$$

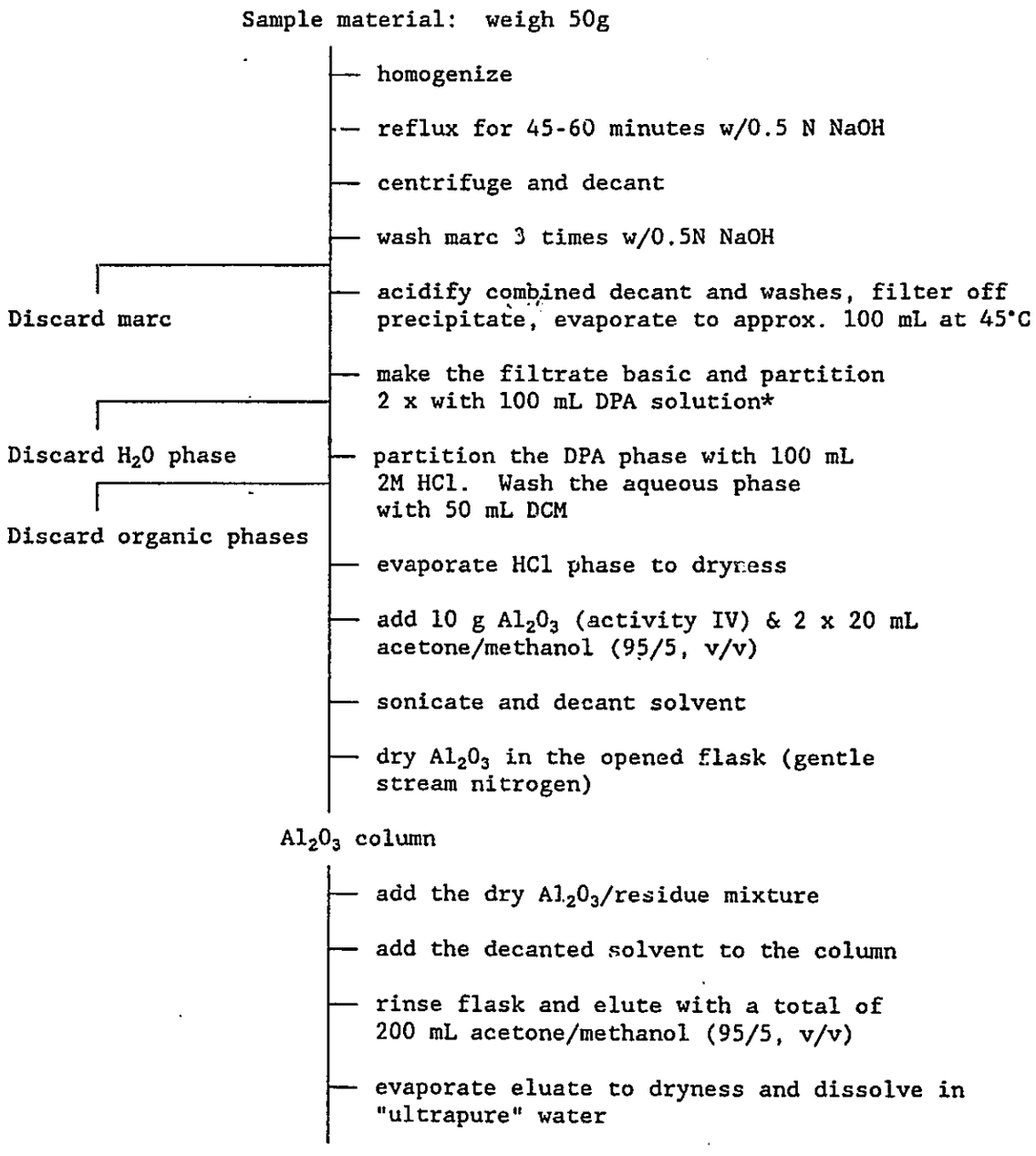
R = Residue in mg/kg

$W_A$ = Amount of mepiquat chloride (in ng) determined from the calibration curve

G = Sample weight in g

$V_E$ = Final volume of the extract before injection (in mL)

$V_I$ = Actual injected volume (in $\mu$L) from final volume $V_E$.




*If necessary, centrifuge.

Figure 1. Flow Chart of the Analytical Procedure.

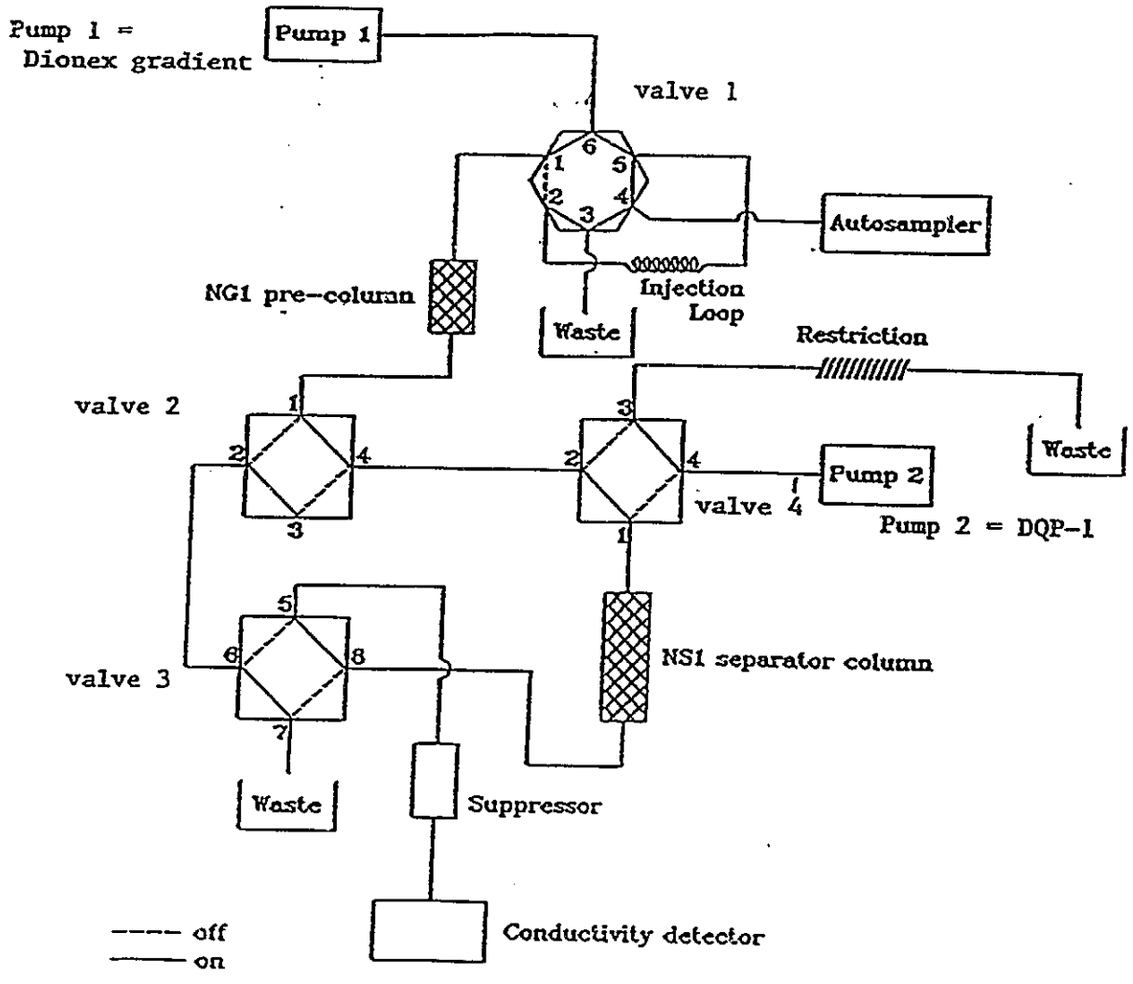



Figure 2. Suggested Configuration for the IC System.