ANALYTICAL METHOD FOR THE DETERMINATION OF E9636 AND V9360 IN WATER BY HPLC

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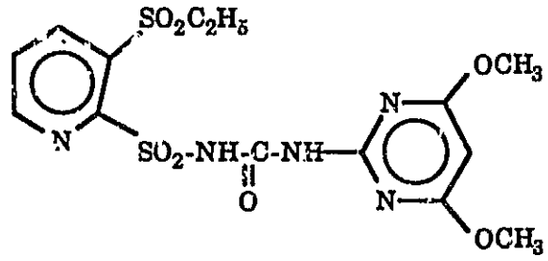
I. INTRODUCTION

An analytical method based on the use of a liquid chromatograph and a programmable multi-wavelength, UV absorbance detector is described for the simultaneous determination of E9636 and V9360 in water. By means of a straightforward isolation step, it is possible to measure these two compounds at a level of 0.1 ppb.

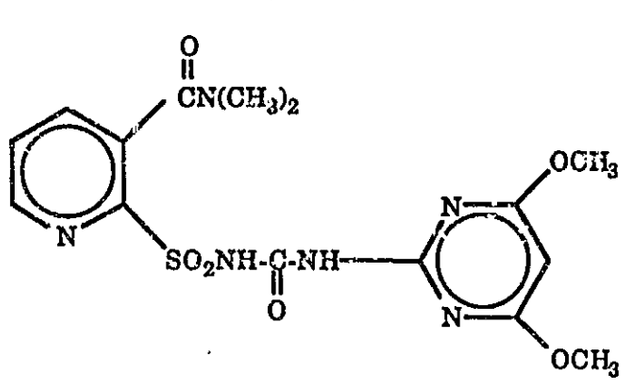
Recoveries of 70% or greater have been obtained with E9636 from the fortification level of 0.1 ppb to a fortification level 100 times greater of 10 ppb, whereas with V9360 the recoveries were 82% or greater over the same concentration range.

The structures of the compounds are shown below:

E9636



V9360



II. EXPERIMENTAL SECTION

A. Apparatus and Reagents

Liquid Chromatograph - Waters 590 Programmable HPLC Pump (Waters Chromatography Division, Millipore Corporation)

Column Compartment - Waters TCM and Controlled Column Oven (Waters Chromatography Division, Millipore Corporation)

Detector - Waters 490 Programmable Multi-Wavelength Detector (Waters Chromatography Division, Millipore Corporation)

Injection System - Waters WISP 710B Auto-Sampler (Waters Chromatography Division, Millipore Corporation)

Chromatographic Column - Waters Nova-Pak C-18, 3.9 mm i.d. x 150 mm Column (Waters Chromatography Division, Millipore Corporation)

Data Acquisition - VAX Multichrom Data Acquisition System (VG Laboratory Systems, Ltd.)

Nitrogen Evaporator - N-Evap® (Organomation Associates)

Sample Mixer - Vortex-Genie® test tube mixer (Fisher Scientific)

Filtration Equipment - Type XX10 04720 and Type XX15 04700 apparatus; Type HAWP 04700 filters; Type HVLP filters; Millex® - LCR filter units (Millipore Corporation)

Storage Bottles - amber, glass, with Teflon®-lined closures, #349-0500 (I-Chem Research, New Castle, Delaware)

Storage Bottles - translucent polyethylene with polypropyiene linerless screw closures, #2092-9016 (Nalge Company, a subsidiary of Sybron Corp., Rochester, NY)

Waters PIC® A Low UV Reagent - Part No. 84189 - (Waters Chromatography Division, Millipore Corporation)

Acetone - Omnisolv® HPLC Grade, Part No. AX0116-1 - (EM Science)

Acetonitrile - Omnisolv® HPLC Grade, Part No. AX0142-1 - (EM Science)

C-18 Bond Elut® - Part #1210-2028 - (Varian)

E9636 - Analytical standard grade (Du Pont Agricultural Products, Research and Development Division, Du Pont Company)

V9360 - Analytical standard grade (Du Pont Agricultural Products, Research and Development Division, Du Pont Company)

With the exception of the two analytical standards listed above, equivalent items may be substituted for any of the apparatus or reagents.

B. Preliminary Treatment

Samples should be taken directly into amber, glass bottles (suitably cleaned) fitted with Teflon®-lined caps. Precleaned bottles, liners and caps are available from I-Chem Research.

If glass bottles, liners and caps from others sources are used, they should be cleaned as follows:

(1) Wash in hot tap water with laboratory grade, non-phosphate detergent.
(2) Rinse three times with tap water.
(3) Rinse with 1:1 nitric acid.
(4) Rinse three times with ASTM-Type I deionized water.
(5) Rinse with pesticide grade methylene chloride.
(6) Rinse once with water as in (4).
(7) Oven dry at 110°C.
(8) After the bottles, liners, and caps have been removed from the oven and cooled, place liners in caps and caps on bottles as soon as possible. Anyone assembling the containers should wear gloves.

Once taken, the samples should be kept in crushed ice until they can be refrigerated at 3-4°C.

While plastic bottles are not as acceptable as glass, there may be instances where they are the containers of choice, e.g. when samples must be frozen. If plastic containers are used, one should keep in mind the possibility of analytical interferences which leach from the plastic or which might diffuse through the walls of the containers. Analyte losses by adsorption on the plastic surface should also be evaluated.

C. Processing Procedure

Wash a C-18 Bond Elut® with 5 mL of acetonitrile followed by 5 mL of Milli-Q® water (obtained from Milli-Q® Water System, Millipore Corporation). The flow rate should be such that discrete drops can be seen forming.

Measure a 200-g sample of water (filtered through a Type HAWP filter using Type XX10 04720 and Type XX15 04700 apparatus), and pass this through the Bond Elut®. Rinse the sample container with 5 mL of Milli-Q® water, and pass this through the Bond Elut® also. Discard all liquids up to this point.

Draw air through the Bond Elut® for 60 minutes before proceeding to the next step.

Begin to put 5 mL of acetone through the Bond Elut®, collecting the liquid in a small centrifuge tube. After 1-2 mL have eluted, stop the flow. Wait 5 minutes; then put the remainder of the acetone through, and collect it in the same centrifuge tube. Evaporate this liquid to dryness at 25°C using a gentle nitrogen stream.

If the sample is to be analyzed immediately, dissolve the residue in 1 mL of HPLC mobile phase, using a vortex mixer or ultrasonic bath. Then filter the solution through a Millex®-LCR filter unit attached to a 1-mL hypodermic syringe.

If the sample cannot be analyzed immediately, it should be stored dry in a freezer.

D. Analysis Procedure

HPLC is used for analysis of prepared samples, and the conditions are as follows:

Chromatograph - See Section IIA

Column - Waters Nova-Pak C-18

Temperature - 35°C

Detector - Waters 490 Multi-Wavelength Detector (set at 240 nm/0.05 AUFS)

Sample Loop - 100 µL

Mobile Phase - 15% acetonitrile/85% Milli-Q® water with 1 vial of PIC® A added to each liter of solution

Flow Rate - 1.5 mL/min

Under these conditions, the retention time of V9360 is approximately 5.0 minutes, and that of E9636 is approximately 15 minutes. When peak heights are measured, the response factors for V9360 and E9636 are in the ranges of 2.0 - 2.8 x 10^5 µV mL/µg and 0.7 - 1.2 x 10^5 µV mL/µg, respectively, normalized to 0.05 AUFS using the VAX Multichrom Data Acquisition System.

E. Standards

Standard stock solutions are prepared by accurately weighing out 10.0 mg of E9636 and V9360 into separate, 100-mL volumetric flasks. The compounds are dissolved in methylene chloride, following which the solutions are diluted to the mark with methylene chloride and mixed thoroughly.

Working standards, also used for fortifications, are prepared by pipetting 2-mL aliquots of the stock solutions into separate, 100-mL volumetric flasks, and evaporating the solvent with a gentle stream of nitrogen. Mobile phase is then added to the flasks, and the flasks are swirled and/or put in an ultrasonic bath for a few minutes. The solutions are then diluted to the mark with mobile phase and mixed thoroughly. Standard solutions of 1.0, 0.50, 0.20, 0.10, 0.05, and 0.02 µg/mL are prepared from the 2.0 µg/mL solutions by appropriate volumetric dilution. Mixed standards of E9636 and V9360 can be prepared in the same manner.

When not in use, all standard solutions must be stored in a refrigerator. The solid E9636 and V9360 standards must be kept frozen.

The stock solutions are generally stable for a few months, but the working standards should be freshly prepared weekly.

F. HPLC Mobile Phase

This solution contains an ion-pairing reagent (tetrabutylammonium phosphate) and is buffered to a pH of about 7.5. The directions for preparation and use which come with the PIC® A concentrate should be followed to assure the best performance and maximum column life.

It is particularly important that all particles be filtered from the solution, and that solutions not in use are refrigerated to retard the growth of micro-organisms which eventually leads to the development of turbidity and particulates in the solution. Also, when the chromatograph is not in use, the mobile phase should be completely flushed out with a 1:1 methanol-water mixture.

III. RESULTS AND DISCUSSION

In general, it is advisable to analyze a complete set of standards, which covers the anticipated range of values in the samples, at the beginning and end of each analysis set. Average response factors are used for quantitation of E9636 and V9360 in the samples, and typical sets of values are shown in Table I. Usually the relative standard deviations (RSD) are less than 5%, and if they exceed 10%, it is advisable to check all operations before proceeding further.

V. CALCULATIONS

Quantitation is based on measurements of peak height, and the following equation is used to calculate the concentrations of E9636 and V9360 in samples.

$$\text{ng/g (ppb)} = H \times \frac{1}{RF} \times V \times \frac{1000}{W}$$

(a) H is the peak height in μV.

(b) RF is the response factor in μV mL/μg.

(c) V is the total volume of the sample extract in mL.

(d) W is the sample weight in g.

If the quantities described in this procedure are substituted in the equation, the result is:

$$\text{ng/g (ppb)} = \frac{5H}{RF}$$

It is important to be certain that the peak height measurement is normalized to the same detector sensitivity as is used for the response factor calculation.

If samples are to be analyzed which contain E9636 and V9360 at concentrations above 10 ppb, it is necessary to do one of the following:

(1) Use a sample smaller than 200 grams.

(2) Prepare standards of concentrations greater than 2.0 μg/mL and check the response linearity.

(3) Dilute the final extract for HPLC analysis to a volume greater than 1 mL.

In addition, it is necessary to demonstrate recovery at the higher level by analysis of fortified samples.

VII. CERTIFICATION

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We, the undersigned, declare that the work described in this report was performed under our supervision, and that this report, to the best of our knowledge, provides an accurate record of the procedures and results.

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Research Supervisor — Date

Date Study Initiated: 4/4/91

Date Study Completed: 9/11/91

Storage Location of Raw Data, Samples, and Final Report:
E. I. du Pont de Nemours and Company
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Experimental Station
Wilmington, Delaware 19880-0402
and/or
Du Pont Records Management Center
Wilmington, Delaware 19880-0870

Sponsor:
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