1987

PRELIMINARY INVESTIGATION INTO THE SOLUBILITY OF AZQ (NSC - 182986), AN ANTI CANCER AGENT

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MASTER OF SCIENCE THESIS

of

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University of Rhode Island

1987

PRELIMINARY INVESTIGATION INTO THE SOLUBILITY OF AZQ (NSC - 182986), AN ANTI CANCER AGENT.

BY

LEKUNUTU CASALIS MATIMA

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IN

PHARMACY

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ABSTRACT

A number of potential anticancer compounds exhibit solubility and stability problems (1,2). This study examined the solubility of AZQ (NSC-182986) in water and various solvent systems, with the following aims:

1. To expand knowledge of the physical and chemical properties of AZQ, and
2. To serve as a valuable preformulation compilation of data to identify solvents which enhance the solubility of AZQ, and nave potential for human use.

In this study, a rapid, simple, selective, and sensitive stability-indicating high-performance liquid chromatographic (HPLC) assay was developed for the simultaneous identification and quantification of AZQ.

Of all the solvents tested, the solubility of AZQ in dimethylacetamide (DMA) was the best, followed by ethanol 95% and other lipid solvents. Phosphate buffer, at pH = 6.5, was a good solvent as compared to plain distilled water in that degradation was reduced. The effects of temperature were also studied. It was very clear from the results obtained that raising the incubation temperature from 25°C to 40°C led to the decline in the solubility of AZQ in phosphate buffer, water and peanut oil as solvents. Optimum temperature for maximum solubility was found to be 35°C.

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[1] Developmental Therapeutic Program, National Cancer Institute, National Institutes of Health, Bethesda, Md.

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I am grateful to Mr. J. Paul Davignon, Chief of Pharmaceutical Resources Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md., for providing the AZQ sample and technical data used in this study.

The data presented in this thesis are given in terms of peak height which is acknowledged as being less accurate than peak area. Because of the very limited quantity of AZQ available for the study (3 grams), there was little opportunity to replicate the samples to the extent desired. Consequently, the results are preliminary in nature and could be considered semi-quantitative.

1. INTRODUCTION

1.1 Chemotherapeutic agents for the treatment of Cancer

A basic goal of cancer chemotherapy is to discover drugs that will control the growth of cancer cells or destroy them without serious damage to the normal tissues of the host. Although the search for drugs with a selective action against cancer has occupied the scientific and medical community throughout the world for a long time, this objective has yet to be substantially achieved.

The model of the selective therapeutic activity of the sulfonamides and antibiotics on bacterial infections in man continues to spur investigators in cancer chemotherapy. Researchers envision that substances possibly exist or ultimately will be synthesized which have a high therapeutic index against cancer (3).

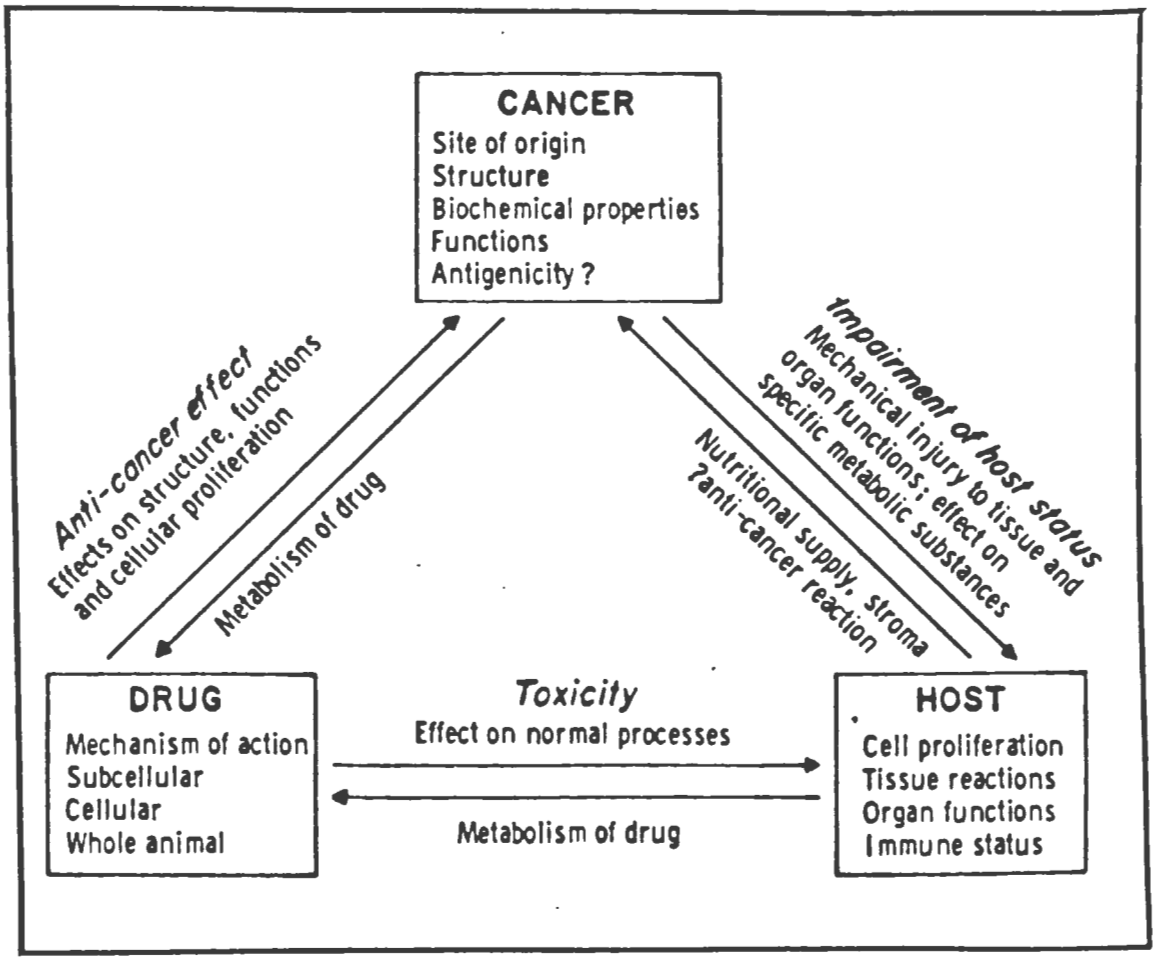
Screening programs, principally based on the use of laboratory animals inoculated with transplantable tumors or leukemias are presently employed to test a vast array of chemicals, antibiotics, and plant tissue extracts for anti-cancer activity (3).

Screening programs have discovered drugs that exhibit therapeutic activity in man, but only in rare patients do they cause a sustained response. In cancer, unlike in bacterial infections, there is no well-defined immunological or cellular defense against residual cancer cells.

In view of these facts, the proper use of the available anti-cancer drugs involves appreciation of the disease in the individual cancer patient and his reaction to his cancer, the host metabolism

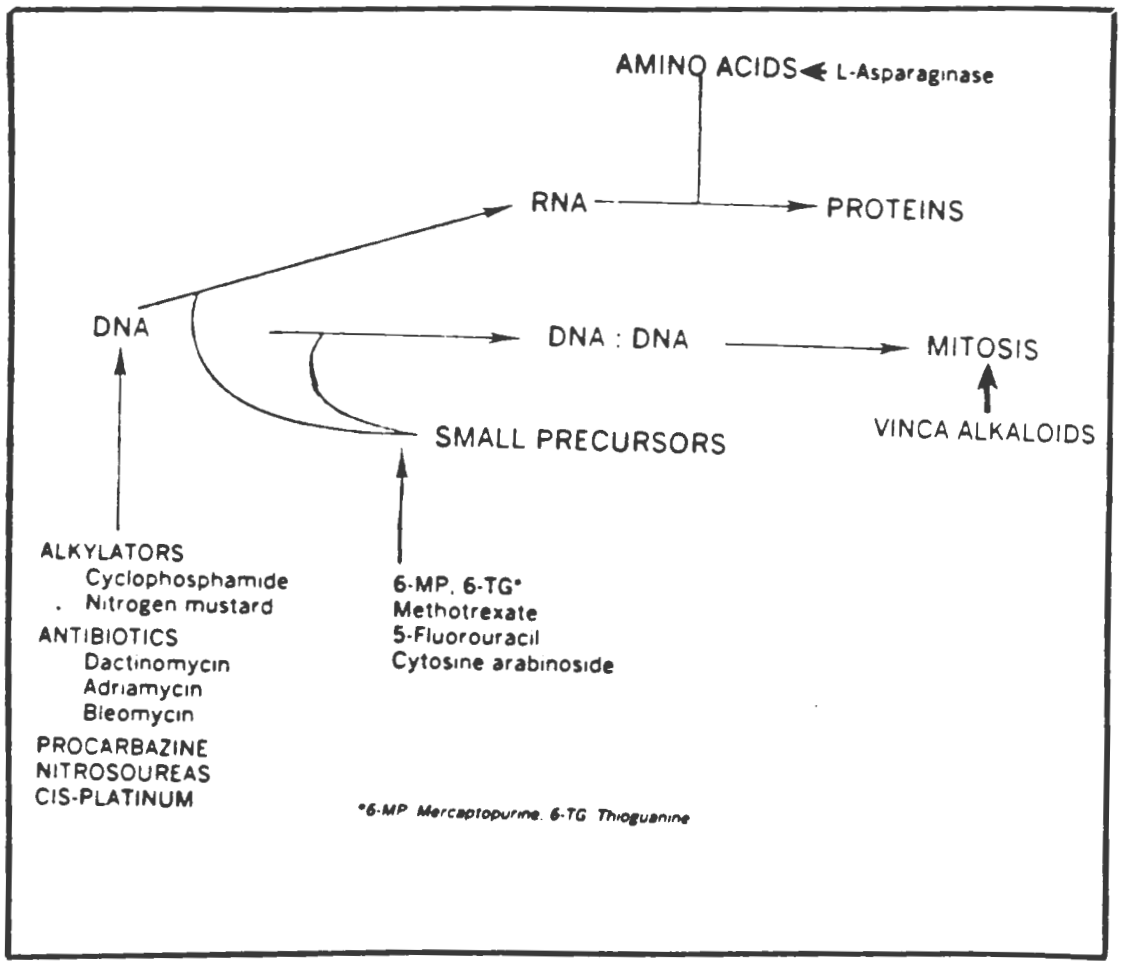
of the drug and the effects of the drugs on host tissues and functions, as well as their direct effects on the neoplastic process (3) (Figure 1).

FIGURE 1: INTERRELATION OF HOST, CANCER AND ANTI-CANCER DRUG



Interrelations between drug, host, and cancer in vivo in the analysis of the mechanism of action of an anit-cancer drug. The separate entities are the properties of the drug, the status of the host, and the specific properties of the cancer. These factors must be evaluated separately and in their complex interrelationship interpreting the therapeutic and toxicologic actions of a drug in specific clinical situations (4). Cole, W 1970.

FIGURE 2: SITE OF ACTION OF CHEMOTHERAPEUTIC AGENTS



(5) Cole, W. 1970.

1.2 Properties of AZQ and related compounds:

The National Cancer Institutes (NCI) is a branch of the National Institute of Health in Bethesda, Maryland. The NCI has been charged with the responsibility of developing and manufacturing pharmaceutical dosage forms of potential anti-tumor agents for subsequent evaluation in preclinical toxicology studies and in clinical studies.

The NCI's drug development activity has utilized the intravenous route as the primary mode of administration for most new anti-tumor compounds because these compounds have simple formulations and this route provides maximal bioavailability.

While some drugs exhibit adequate aqueous solubility and stability for intravenous delivery by formation of either a water-soluble salt or by the addition of water-miscible solvents, a significant number of potential anti-cancer agents cannot be formulated in this manner because of solubility and/or stability problems.

The aziridinylbenzoquinones constitute a class of quinones that exhibit significant anti-tumor activity against i.p. implanted lymphoid leukemia (6,7). (See Figure 2). AZQ or Diaziquone was determined to be the superior analog of the 31 aziridinylbenzoquinones tested (8). AZQ or Diaziquone was rationally synthesized to produce a compound possessing the three properties necessary to penetrate the Central Nervous System (CNS) (6):

i. Good lipid solubility;

ii. Low ionization, and

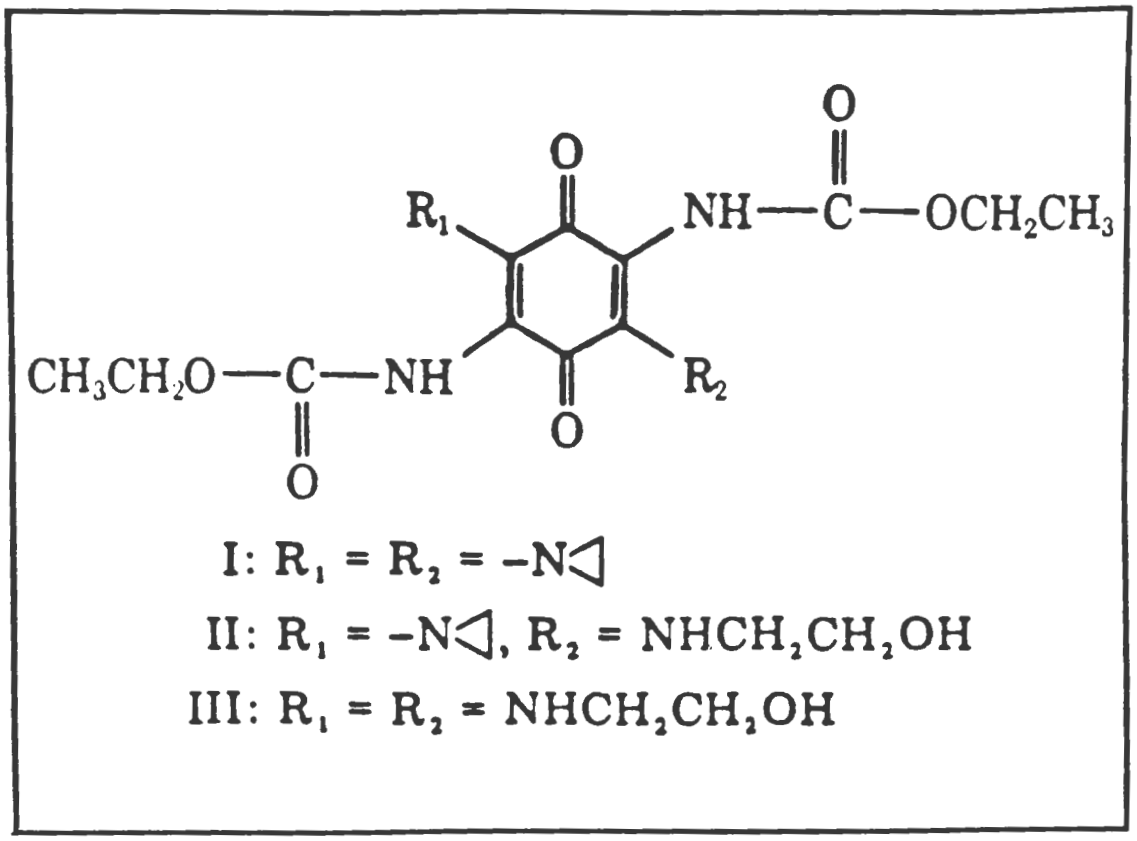
iii. Adequate aqueous solubility (9,10).

The structure of AZQ suggests that it has alkylating activity. However, when a modified chemical test was used (11), no alkylating activity was detected under physiological conditions (i.e. incubating at 37°C). When the incubation temperature was raised to 100°C, alkylating activity equal to three-fourths the alkylating activity of nitrogen mustard was shown.

Although the exact mechanism of action of AZQ is unknown, members of this class of compounds are known to cross-link DNA (12).

AZQ (NSC-182986), (13, 14, 15), an aziridinylbenzoquinone, which was developed by the NCI, has been shown to be active against brain tumors, but its solubility in water and other aqueous systems is quite poor. while adequate levels in the blood can be achieved with dimethylacetamide (DMA), (16,17), an aqueous system is preferred or desirable for safety.

FIGURE 3: STRUCTURE OF AZQ AND ITS RELATED COMPOUNDS



Key: I = AZQ (NSC-182986) 2,5 diaziridinyl - 3,6 - bis (carboethoxyamino)-1,4-benzoquinone
II = 2-Aziridinyl-5- (2'-hydroxyethylamino)-3,6-bis (carboethoxyamino)-1,4-benzoquinone
III = 2,5-di (2'-hydroxyethylamino)-3,6-bis (carboethoxyamino)-1,4-benzoquinone.

II and III are hydrolytic degradation products of AZQ (I) which were isolated by semi-preparative HPLC from I. (ref. 18).

1.3 Utility of Preformulation and its relevance to therapy, with special reference to solubility:

"Preformulation involves the application of biopharmaceutical principles to the physico-chemical parameters of a drug with the goal of designing an optimum drug delivery system" (19).

The cost to develop new drugs or dosage forms is very high and preformulation studies can help to minimize this expense if they are initiated at the time of discovery of new compounds with promising pharmacological activity. It is very useful to make this study prior to either formulation or clinical formulation, to minimize the cost and effort of drug development. The parameters to be studied should include the following: chemical stability, aqueous solubility, pKa and dissolution rate.

Priority must be given to the effect of various diluents, pH and temperature on the chemical stability of the drug. These investigations should precede any attempt to design new dosage forms. In addition to the above parameters, preliminary, in vivo, animal studies of drug absorption, metabolism, protein-binding, distribution, and elimination are also part of preformulation.

The major goal of preformulation studies is to gather data which will permit a rational development of safe and useful dose forms. The importance of chemical stability of the drug cannot be overemphasized because the administration of degraded products might result in either sub-therapeutic blood-levels of the drug, or possibly, toxic effects. So, in order to formulate stable dosage

forms, the effects of pH, humidity, temperature and other factors must therefore be known (19).

Solubility is a very important aspect of preformulation. Orally administered drugs must dissolve in the fluids of the gastrointestinal tract (GIT) before the drug can be absorbed. These drugs must have sufficient aqueous solubility to achieve the desired dose level. It has generally been found by Kaplan that potential bioavailability or absorption problems are often present when the aqueous solubility of a drug is less than 1 gram per 100ml., in a pH range of 1 to 8 (20). In preformulation studies, solubility studies are performed by the addition of excess of drug to buffer solutions having a pH in the range of 1 to 8. Vials containing the drug and buffer and/or solvent, are often agitated at 37°C, with samples being removed at timed intervals, filtered, and assayed for drug content (21). The experiment is completed when the measured drug content or concentration is constant.

Results from this type of experiments yield data on solubility of the drug, that is, saturation solubility. Care should be taken if the study is done at elevated temperatures that sampling be done at the same temperature to avoid supersaturation. Also enough time should be allowed for equilibration, and it should be remembered that if not detected, degradation may give false readings. So, it is essential that a stability-indicating assay be a requisite part of this study.

Preformulation data is a useful guideline in formulation in that it points out which solvents are good for the drug. But the problem of dilution still exists. How much solvent or, what level of solvent is acceptable at the time of use?

Buffer solutions may be used to increase the solubility of various drugs, the enhanced solubility due to salt formation. The undissolved phase in equilibrium with the saturated solution, comprises unreacted acid. By applying the correct pH in a formulation of a dosage form, maximum solubility and stability may be achieved, but this pH must be acceptable for the particular route of administration. Many buffer systems are possible, but toxic effects, such as generation of harmful or inactive degradation products, when administered orally and parenterally, severely limit the number of buffer systems that may be used in pharmaceuticals.

The physical and chemical stability of a dosage form must be known to ensure that the patient receives the prescribed dose of a drug and not a therapeutically inactive degradation product. Chemical stability is important since many medicinal compounds are esters, amides, or lactams. Hydrolysis is the most frequently encountered type of degradation in pharmaceuticals. Hydrogen and hydroxyl ions are the most common catalysts of hydrolytic degradation in solutions (22). The correct use of buffer solutions will help prevent this problem.

Photolysis is another source of chemical instability. Molecules absorb the proper wavelength of light and acquire sufficient energy to undergo reactions. Photolytic degradation usually occurs upon

exposure to light of wavelength less than 400 mμ. Use of amber glass bottles or an opaque container will act as a barrier to this light.

Autoxidation is the spontaneous reaction under ordinary conditions of a drug with atmospheric or molecular oxygen. It is believed that most oxidation reactions involve a free radical and chain reactions (23) . Traces of metallic ions originating in the drug, solvent, or container may initiate oxidation reactions. As a precaution, chelating agents such as ethylenediaminetetraacetic acid (EDTA), may be added to bind any metal present.

Physical and chemical stability are very important aspects of formulation. Injections must be able to withstand sterilization by means of chemical, mechanical and physical processes. Color changes are also a result of degradation. Solutions may undergo a change in consistency and odor as a result of degradation. Emulsions may break or sediment due to degradation. These factors must all be considered in the development of a new formulation drug product.

In general, "substances dissolve faster if the system is warmed. If a substance absorbs heat in the dissolution process, its solubility is increased by an increase in temperature," (24). It is known that this increase in solubility provides an increased concentration gradient, which leads to an increased dissolution rate. The increased temperature of the system, increases kinetic motion and diffusion of the solute through the diffusion layer into the bulk solution, which increases the dissolution rate (24).

Arrhenius expressed this relationship between temperature and reaction rate as:

$$k = A \exp(-Ea/RT)$$

where: Ea = molar activation energy

R = Gas Constant (1.987 cal. $mole^{-1}$ deg^{-1}).

k = reaction -rate constant

T = absolute temperature (°Kelvin).

A = frequency factor; A embraces the number of collisions of the molecules and the probability that the orientation of the colliding molecules is such that a reaction may occur (25).

This equation has found general use in accelerated stability tests in order to predict the rate of decomposition at room temperature.

Care should be taken that no adverse interactions occur between the drug, the excipients and the container of the new formulation. Also no interaction should occur between the excipients themselves, as this might impair the integrity of the product. Incompatibilities within a formulation might lead to untoward physical and chemical changes in the formulation, with the result that the product of this kind might have limited or even undesirable therapeutic and/or toxic effects, e.g. precipitation of an injection product; breaking of an emulsion; hardening of tablets, etc.

Solubility data is just one aspect of formulation because of the need for parenterally acceptable and compatible excipients. This

solubility of the formulation is also a major issue to be investigated. Other limitations include the degradation of the drug in solution and, in the case of a suspended solid (suspension), the rate of dissolution must be known.

1.4 Problems associated with NCI drugs:

Many compounds are synthesized with the intent of producing selective cytotoxic activity in a variety of cancers. Typically, the solubility in water or blood serum of these compounds is a major concern and their stability in solution, are quite limited. Examples of NCI drugs as well as their associated formulation problems, include (26):

a) Taxol brevifolia, is currently formulated in castor oil/ethanol at concentrations of 6 mg/ml and is diluted to 0.6 mg/ml. before use. In spite of having excellent solubility in 75% PEG, the antitumor activity is reduced in this formulation. The cause of this reduction was not known but is not due to chemical degradation. The NCI prefers a formulation in the CremophorR, in a deliverable concentration of 20-25 mg/ml.

b) AZQ (NSC-182986), an aziridinyl benzoquinone which has shown activity in brain tumors. However, its solubility in water and other aqueous systems is quite poor. A system other than dimethylacetamide (DMA) as a solvent is desirable. The desirable concentration is 10-20 mg/ml. since intrathecal administration is being considered. (See Table I).

c. Mitindomide (NSC-284356), a diimide active in several tumor model systems. The disodium salt is soluble at high pH (>10.5), but

decomposes rapidly. Mitindomide is essentially insoluble in water and most common solvents. The NCI's solubility goal is 20 mg/ml.

d. Hexamethylmelamine (NSC-13875), has been used as an oral product in combination with other drugs, to treat ovarian cancers. The daily dose requirement is approximately 350 mg/m^2 (approx. 700 mg per dose). This oral product causes gastrointestinal toxicity. An aqueous intravenous delivery system is desirable, which would provide a deliverable dose of approximately 700 mg per dose.

The four products mentioned above illustrate some of the most important formulation problems presented by potentially useful anti-tumor drugs.

To overcome solubility problems, various techniques have been employed. A sampling of some of these methods is discussed below.

Colloidal dispersions of micelles have been used in pharmacy to solubilize water insoluble materials. Oil-soluble vitamins are dispensed in aqueous multivitamin liquids as a result of solubilization by nonionic surface-active agents. The solubilization of a substance may improve its chemical stability by protecting it in micelles. Solubilized Vitamin A is more stable to autoxidation than its oily solution.(29)

TABLE I: RELATIVE SOLUBILITY OF AZQ IN DIFFERENT SOLVENTS

SOLVENT	SOLUBILITY (MG/ML)
Water	0.72
pH4 Acetate Buffer	Less than 1
pH9 Borate Buffer	Less than 1
10% Ethanol	Less than 1
95% Ethanol	Less than 1
Methanol	Less than 1
Chloroform	Between 5 and 7
Dimethylacetamide (DMA)	Between 20 and 25
5% DMA	1.5
Dimethylsulfoxide (DMSO)	Between 25 and 30

NOTE: Data provided by National Cancer Institute (NCI) (27,28)

Approximate solubility found by visual observation after one hour's shaking on a Labquake® Shaker.

Aqueous solutions of AZQ decompose readily in 5% DMA, at room-temp 18% lost in 8 hours.

An oral suspension is an advantageous dosage form for administration to patients who cannot swallow tablets and capsules. Generally, a solid content of a suspension may range from 250 to 500 mg per teaspoonful. The shelf-life of a suspension of a drug is often greater than that of its solution, because of the extremely small amount of drug which is dissolved and thus subjected to degradation. Parenteral suspensions are injected subcutaneously and intramuscularly but not intravenously nor into the spinal canal (30). This type of dosage form can provide a means of prolonged release of drug.

The purpose of emulsification is to administer a uniform blend of immiscible liquids in a single preparation. Oral emulsions are generally the oil-in-water type where the emulsifying agent surrounds the oil so that the oily feel and taste are minimized. Only a few parenteral emulsions have been used successfully in the administration of phytonadione (Vitamin K) and in the intravenous feeding of rats. Unwanted physiological effects, e.g. febrile reaction and hemolysis, have limited the use of these intravenous emulsions (31). These systems are also subject to instability, primarily by coalescence.

pH is an important aspect of the stability of drugs in solution. The pH-rate profile of AZQ was shown to have a degradation minimum between pH 6.0 and 6.5 (32). The route of administration determines the pH range to which the drug can be formulated, e.g. with small-volume injections, a fairly large deviation from physiological pH

is tolerated because the blood buffers can readily adjust and maintain the blood at normal pH.

Since the National Cancer Institute (NCI) is only able to provide a limited amount of test drug (1-3 grams), there exists a need to develop a micro-scale technique to generate and evaluate the required data. A procedure as described below, can produce the sensitivity required when working with very small quantities of a compound:

1. development of a micro-assay method using ultraviolet (UV) spectroscopy or High-Performance Liquid Chromatography (HPLC) method (stability indicating) to indicate that decomposition has not occured over a time period or that decomposition products do not interfere with identification of the compound;
2. validation of this method using FDA guidelines;
3. use of this method on the test drug to generate solubility data for comparison with literature values. This will enable the establishment of the miniaturization assay as valid in terms of accuracy and reproducibility.

4. screening other selected solvents such as propylene glycol, ethanol, water, buffers, DMA and aqueous mixtures of these solvents to maximize solubility and stability of the drug.

The values that are generated in this manner may aid in some future investigation into improved solubility and consequently, improved formulation of particular anti-cancer drugs.

This project will entail the handling[1]. of this antitumor agent and should be considered a potent substance, requiring proper and safe handling in all aspects of the study.

2. Objectives of this Study:

The focus of this project is to determine the solubility of AZQ in parenterally acceptable solvents. It is expected that a preformulation study of this compound will provide information to produce significant solubility enhancement within these solvent limits.

Concurrently, it is our intent to ascertain the relative stability of AZQ in these solvents and to study the effect of temperature on solubility and stability.

The results of this study should contribute to the generation of an essential data base for AZQ, and should also give practical guidelines to improve the parenteral administration of this antitumor compound.

---

1. Recommendations for Handling Cytotoxic Agents", National Study Commission on Cytotoxic Exposure, September 1984. Sponsored by Bristol Laboratories.

3. EXPERIMENTAL

3.1 Materials

A. Equipment:

Waters detectors (UV), Models 441 and 480[1].

Waters HPLC Pump, Model 510[1].

HPLC Syringe (250 ml Capacity)[1].

C_{18} column (μ-Bondapak)[1] [P/N 27324S/N]

Cole-Parmer (Linear) Recorder[2].

Mettler H8 Balance[3].

Sartorius Balance [4].

Rotating Sample Holder with Motor[5].

Millipore Suction Pump and Micro-filters (0.45u)[6].

Wrist-Arm Shaker[7].

Eberbach Shaker[8].

Fisher Magnetic (Thermix) Stirrer[9].

Glassware, i.e. volumetric flasks, Pipettes, Screw cap bottles, etc.[5].

Millipore Swiney Adapters[6].

Hypodermic Syringes and Needles[10].

Orion Model 811-pH meter[11].

O'Dell Fish tank (25 Gallons) - Used as a water bath[12].

Beckman [illegible] 43 PH - meter[13].

Hitachi Model - 200 Spectrophotometer[14].

Perkin - Elmer Chart Recorder (UV-Vis)[15].

UV-Cuvettes (sizes 5cc, 1cc and 0.5cc)[16].

Cole-Parmer 1252-00 Circulator (Thermostat)[2].

Texas Instruments (TI55) hand Calculator[17].

---

1. Waters Products Division, Millipore Corp., Mipford, MA 01757.
2. Cole-Parmer Instrument Company, Chicago. Illinois, 60648.
3. Mettler Instrument Corp., Hightstown, N.J. 08520.
4. Brinkmann (Sartorius) Instruments Westbury, N.Y., 11590.
5. Department of Pharmaceutics, College of Pharmacy, URI.
6. Millipore Corp., Bedford, MA 01730.
7. Burrell Corp., Pittsburg, PA.
8. Eberbach Corporation, Ann Arbor, Michigan, 48106.
9. Fisher Scientific Corp., Fair Lawn, N.J. 07410.
10. Becton Dickenson and Co, Rutherford, N.J., 07070.
11. Orion Instruments, Cambridge, MA, 02139.
12. The Aquarium, N. Main Street, Providence, RI 02906.
13. Beckman Instruments Inc., Palo Alto, CA 94304.
14. Hitachi Limited, UV Spectrophotometer, Tokyo, Japan.
15. Perkin-Elmer Corp., Norwalk, CT, 06856.
16. Markson Science, Phoenix, Az, 85076.
17. Texas Instruments, Model TI55 Calculator

B. Facilities:

URI Library,

URI Computers,

Department of Pharmaceutics' Laboratory Workspace.

C. Chemicals

DMA, lot AM686[18]

AZQ (NSC-182986), lot AJ-58-5[19]

Acetonitrile, Lot #5210[20]

Methanol, Lot 5347[20] and lot 8644449.

Sodium Phosphate (Dibasic), lot 7723749.

Potassium Phosphate (Monobasic), lot 7229649.

Propylene glycol, lot 94F-0209[21].

Ethyl alcohol 95%, lot CTN 86F08[22].

Distilled Water[5].

Peanut Oil (Arachis Oil) USP, lot C618635[23].

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18. Burdick and Jackson, Muskegon, Michigan, 49442.

19. Developmental Therapeutics Program, National Cancer Institute, National Institutes of Health, Bethesda, MD.

20. E-M Science, Cherry Hill, NJ 08034.

21. Sigma Chemical Company, St. Louis, Mo., 63178.

22. U.S. Industrial Chemicals Co., Tuscola, Ill. 61953.

23. Amend Drug and Chemical Co. Inc., East 24th Street, N.Y., NY.

3.2 Methods:

An initial objective of this study was to develop a simple, reproducible and sensitive miniaturized HPLC assay for AZQ. The method method.of Poochikian and Cradock (32) was used as the basis for assay development.

3.3 Assay Conditions:

The mobile phase used initially was acetonitrile (CH_3CN), 20% (V/V), in phosphate buffer (pH of approximately 6.5). A modular HPLC, equipped with a constant-flow pump was used. This chromatograph delivered eluent at the rate of 1 ml. per minute to a C_{18} column. Column temperature was ambient. Injections were made with a rotary valve injector, equipped with either a 20 or 50 micro-liter injection loop. A single-wavelength or a variable-wavelength detector, set at 254 nm and 0.05 AUFS deflection, was used to detect eluted material. A strip-chart recorder was used to record output signals. The chart speed was 30 cm per hour. Column-pressure was 1.0-1.5 psi (x 1,000).

Chromatographic separations were effected isocratically at room-temperature. A standard curve was generated by dissolving 25 mg. AZQ in 500 ml. phosphate buffer, thereafter making dilutions of this solution, with buffer, to yield concentrations of 50, 40, 30, 25, 20 and 10 micro-grams per ml.

3.4 Miniaturization of HPLC Assay for AZQ:

Due to the small amount of AZQ provided for this study by the NCI, a semi-micro-assay method was imperative. Work done by Poochikian and Cradock (1), indicated that a useful range of concentration for the HPLC analysis of AZQ was 5-50 micro-grams per ml.

Initially, 80 mg. of AZQ was dissolved in 100 ml. of solvent, after dilution, the concentration was 40 micro-grams per ml. and the sensitivity of the assay was determined. The test sample was then reduced to the equivalent of 1 mg. of AZQ in 1 ml. of solvent. Appropriate dilution produced a concentration of 32 micro-grams per ml., and this concentration proved to be reproducible. This 3 ml. sample was then used as the working amount for the solubility study.

3.5 Validation of HPLC Assay:

The assay method developed for AZQ was validated for precision, sensitivity and reproducibility using Food and Drug Administration (FDA) guidelines for the validation of analytical data (33). The following data is required for the validation of the assay method:

A. Data demonstrating that fresh or degraded placebos do not significantly interfere with the assay. For drugs that decompose during the assay procedure, the method should be stability indicating.

B. Data should demonstrate sufficient precision, linearity and accuracy over the useful range, (approximately 90 to 110% of the label claim).

C. To demonstrate reproducbility or "ruggedness", data should be presented which shows day-to-day, lab-to-lab, technician-to-technician, and/or column-to-column variability.

D. Data should be provided which demonstrates the recovery from the actual sample matrix where the nature of the products so indicates, e.g. time-release or polymer matrices.

E. Where necessary, data which demonstrates the specificity of the method, and the limits of sensitivity for degradation products or impurities, should be provided.

3.6 Stability:

The solubility of AZQ in different media was determined by the following method:

Each weighed sample, in excess of literature values, was placed in a teflon-lined, screw-cap glass vial (20cc) and the appropriate amount of solvent was added. The vials were then capped and sealed with Parafilm[1]., and rotated at approximately 28 R.P.M. in a constant-temperature water bath (± 0.1°C), at the temperature of 25°C for a period of 4 hours to reach equilibrium.

After the incubation period, the vials were removed for HPLC assay. A portion of the sample was then filtered through a Millipore filter (0.45 micro-meter) by means of a hypodermic syringe (attached to a swiney adapter) and a needle, into a 10 ml. volumetric

---

1. American Can Company, Greenwich, CT 06830

flask, and made up to volume with phosphate buffer diluent (pH approx. 6.5).

The drug concentration was then determined by HPLC.

For the solubility of AZQ in peanut oil, an extraction method was developed. Methanol (100%) was found to be a suitable immiscible solvent for extracting AZQ from the Peanut Oil solutions. The saturated oil samples were shaken against methanol in three volumes to effect complete removal of the drug from the oil. The resulting methanol solutions were pooled and drug content was determined as previously described.

The pH of the solutions was determined at the time of the assay. For each solvent system studied, the stability of AZQ was ascertained by monitoring the appearance of adegradation peak as a function of time in these solutions.

Samples of the drug at a concentration of 30-40 micro-gram per ml. were rotated in a water-bath for varying lengths of time, and the concentration of active component was determined by HPLC.

3.7 Effect of Temperature:

The degree to which elevated temperatures could improve the solubility was determined by completing a solubility study of AZQ in distilled water, buffer and peanut oil at 25, 30 and 40°c.

The data was used to estimate the relative energy of activation (Ea) for the compound.

4. RESULTS AND DISCUSSION

4.1 Development of the Assay:

AZQ has a structure amenable to UV detection. Unfortunately, the stability of the compound in aqueous solution is poor, and the decomposition products interfere with UV analysis. An HPLC method has been described in the literature (1,2,14,15,28).

In order to optimize separation of active compound from degradation peaks, a number of solvents and solvent mixtures were evaluated for suitability as a mobile phase (Table II). As seen in this table, a mobile phase of 20:80 Acetonitrile (CH_3CN): phosphate buffer produced the greatest sensitivity and reproducibility. After the assay conditions were established, the assay was validated as stability-indicating by injection of degraded AZQ to freshly prepared solution. No change in peak height was observed in the main peak. Injections of degraded samples showed complete loss of the main peak with rapid loss of degradation peaks (Figures 4,5,6) in the aged samples.

TABLE II: EFFECT OF MOBILE PHASE ON THE DETECTION OF AZQ

Mobile Phase	Mobile Phase Apparent pH	Peak Height (cm)	Retention Time (min.)
50:50 Methanol: Water(dist.)	7.25	1.5 cm	3 minutes
10:90 acetonitrile (CH_3CN): Phosphate buffer (PO_4 buffer)	6.99	6.1 cm.	14 minutes
15:85 " "	6.99	12.3 cm.	9.4 minutes
20:80 " "	7.00	18.7 cm.	6.0 minutes

a) Calculation of Retention Time:

Chart Speed = 30 cm/hr.
= 30 cm/60 min.
= 5 mm/min.

Retention time = distance from injection - time (0 mm) to peak maximum (in mm).
÷ chart speed (5 mm/min.)

Example: from Figure 9, page 37.

Distance to peak = 3 cm (= 30 mm).
Retention time = 30 mm ÷ 5 mm/min. = 6 minutes.

FIGURE 4: CHROMATOGRAM OF 'FRESH' AZQ SOLUTION

Solvent = phosphate buffer (pH = 6.5)
M.P. = 20% CH_3CN in buffer.
AUF = 0.05; $Lamba_{max}$ = 254 nm; Loop = 50 micro liter;
Pressure = 1.5 (x1000) psi; Flow-rate = 1 ml./min;
Chart Speed = 30 cm/hour; Retention time = 6 min.

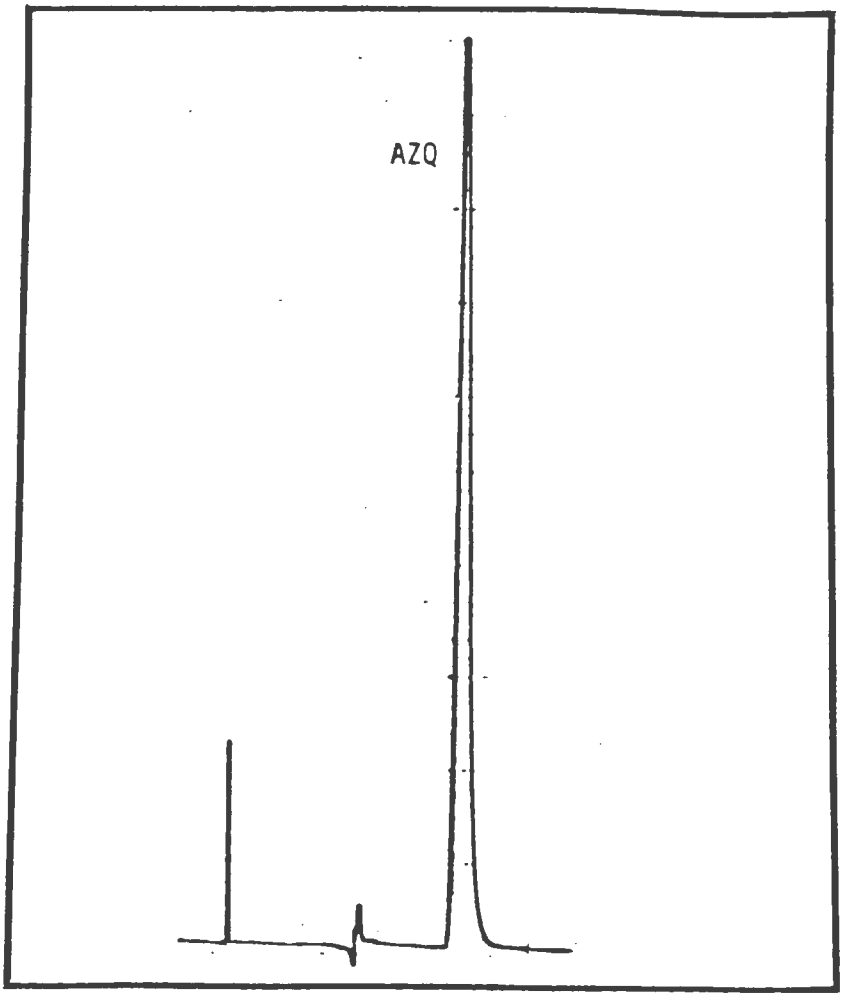


FIGURE 5: A 50:50 MIXTURE OF FRESH AND DEGRADED AZQ SOLUTIONS

AUF = 0.05;
Flow = 1 ml./min.
Pressure = 1.5 - 2.0 (x 1,000) psi;
$Lamba_{max}$ = 254 nm.
AZQ solution concentration = 40 micrograms per liter for both 'fresh' and degraded solutions.

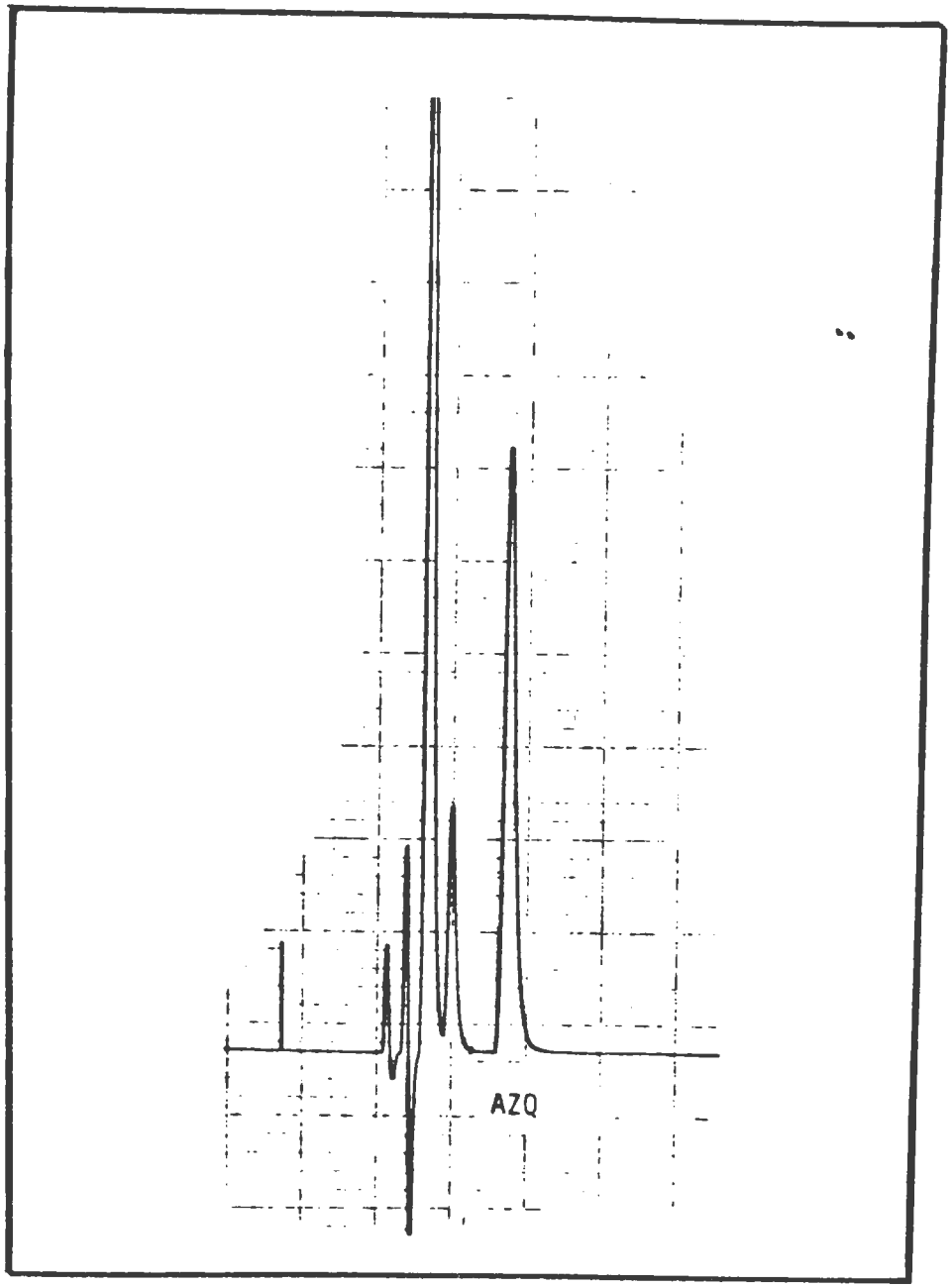


FIGURE 6: MOBILE PHASE IS 50:50 METHANOL: DISTILLED WATER

AUF = 0.01
Chart-Speed = 30 cm./hr.
Flow-rate = 1.0 ml./min.
Pressure = 2.5 (x 1,000) psi
Loop volume = 50 micro-liter

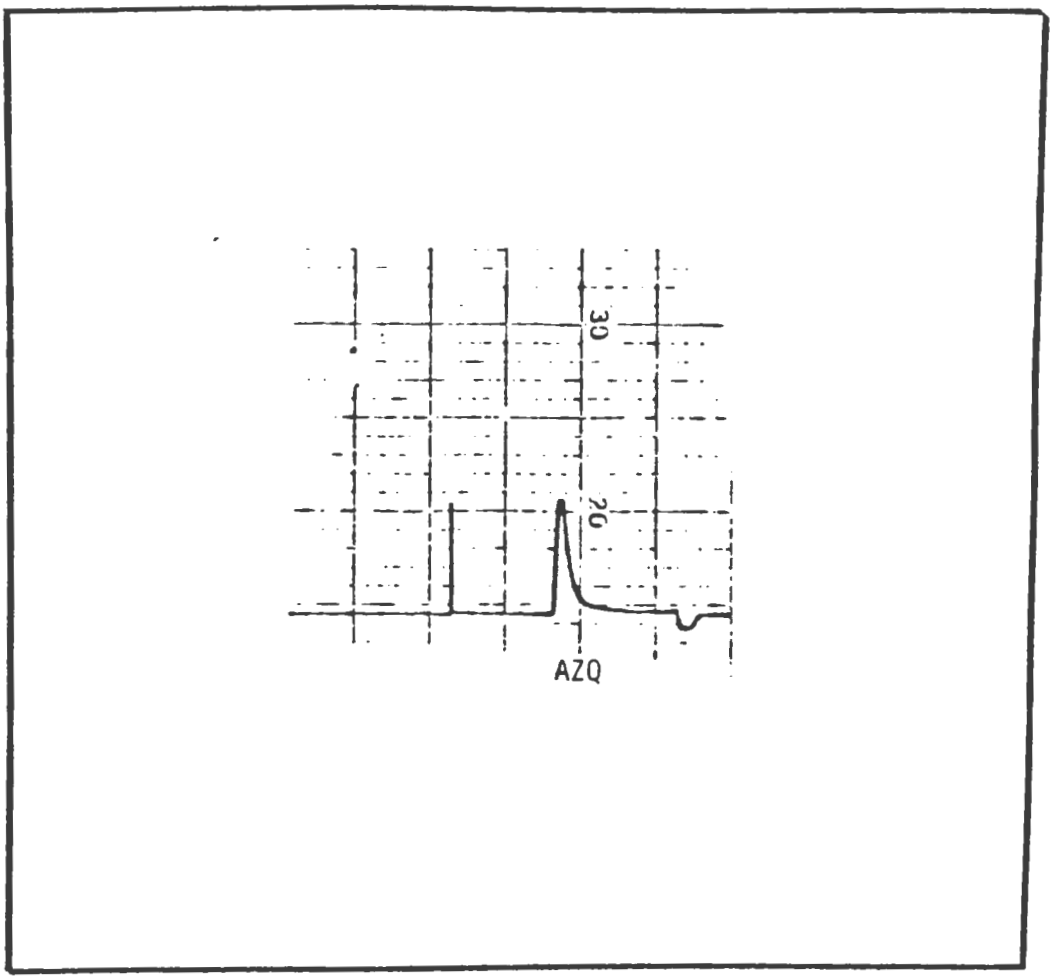


FIGURE 7: MOBILE PHASE IS 10% CH_3CN IN PHOSPHATE BUFFER

AUF = 0.10
Flow rate = 1.0 ml/min.
Pressure = 2.5psi (x1000)
Loop vol. = 50 micro liter

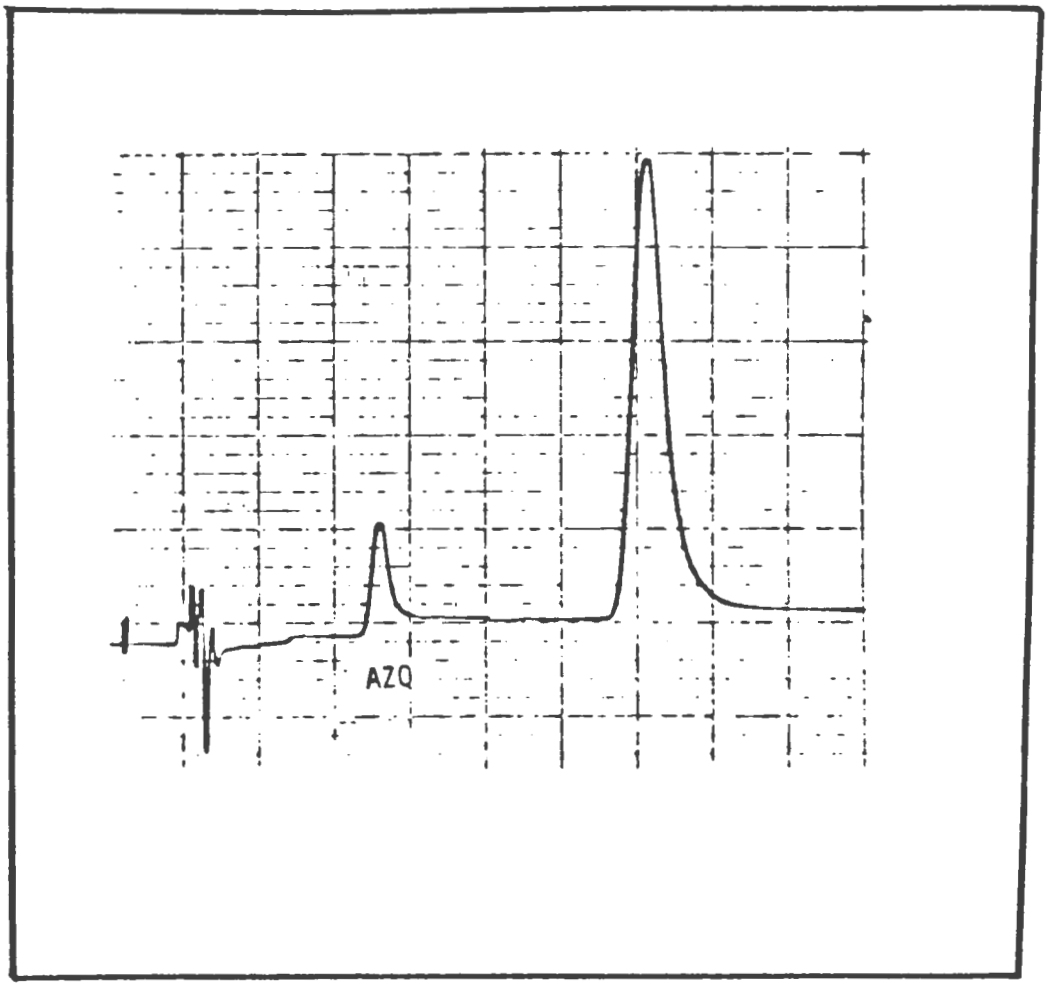


FIGURE 8: MOBILE PHASE IS 15% CH_3CN IN BUFFER

AUF = 0.10
Flow rate = 1.0 ml/min.
$Lambda_{max}$ = 254 nm

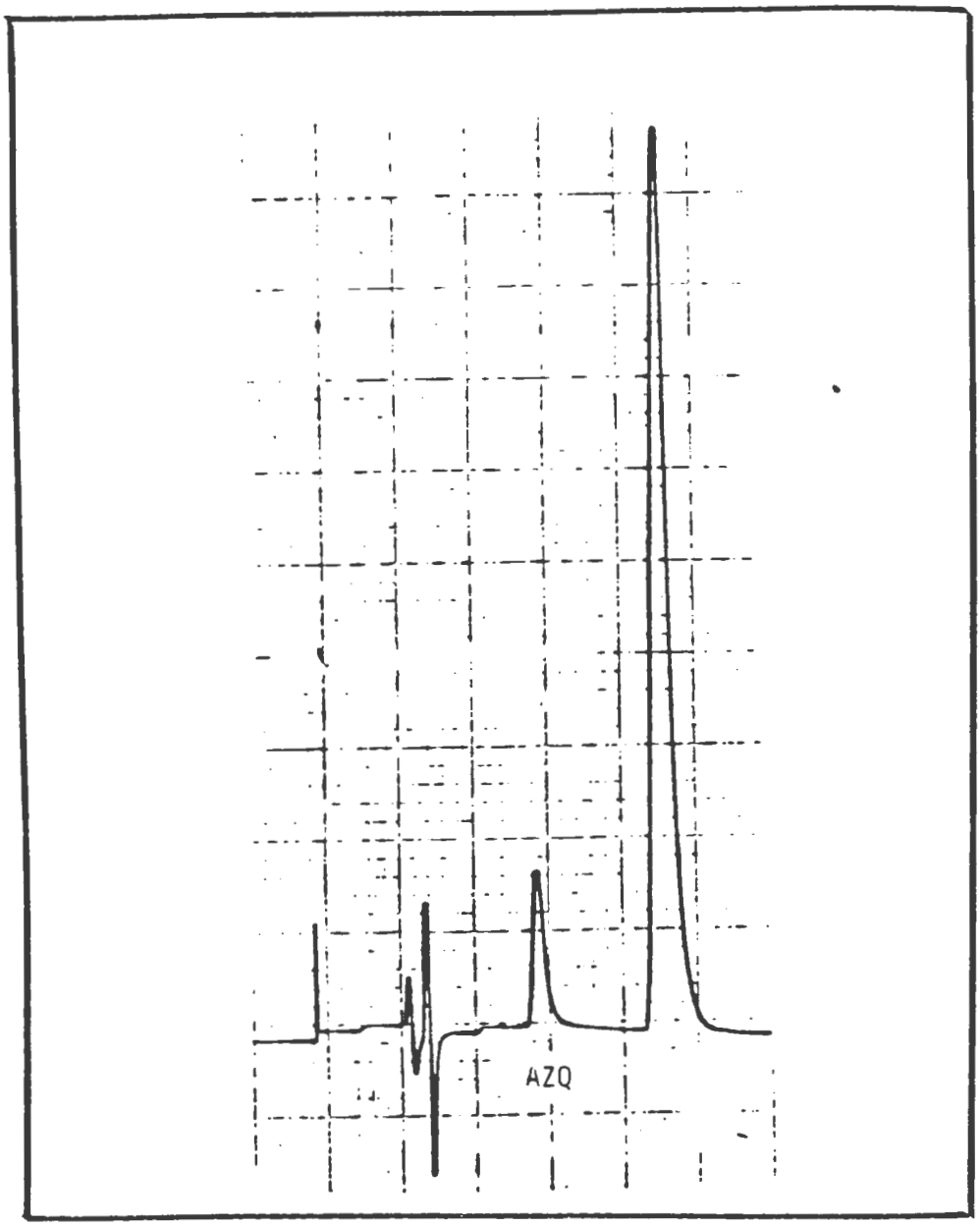
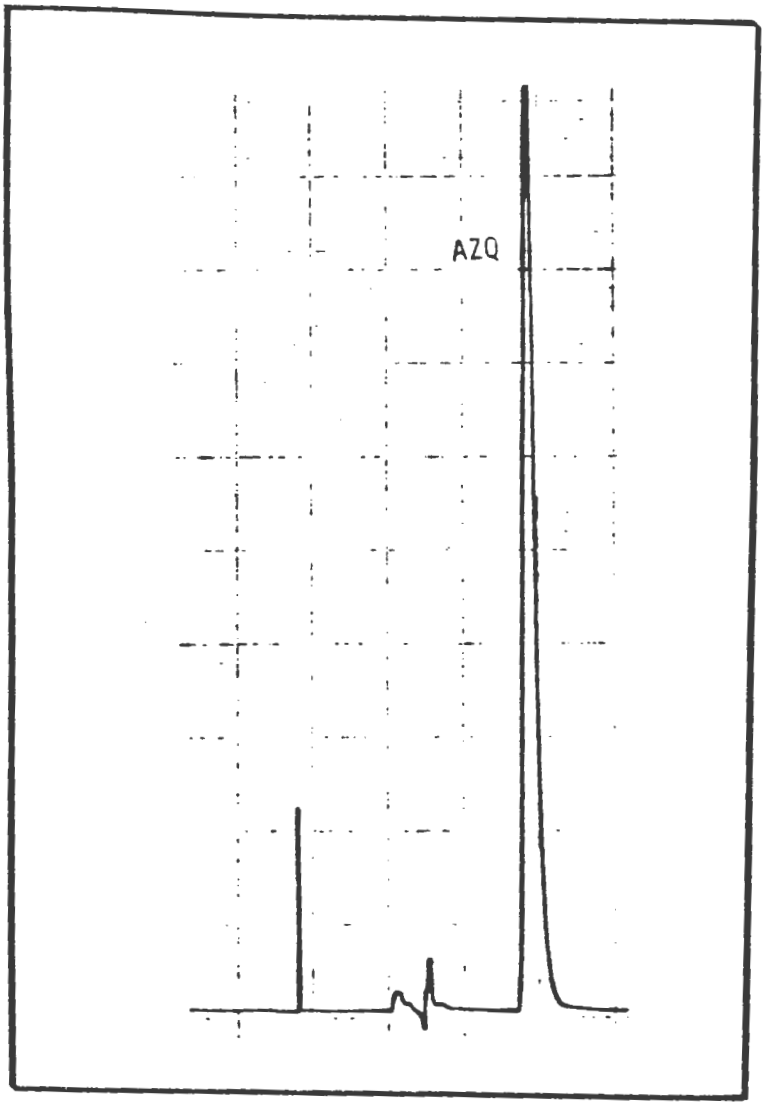


FIGURE 9: MOBILE PHASE IS 20% CH_3CN IN BUFFER

AUF = 0.10
Flow rate = 1 ml/min.
Loop = 50 micro liter
Lambda$_{max}$ = 254 nm
Chart speed = 30 cm./hr.



b) The Standard Curve:

Distilled Water as the solvent for the standard curve was ruled out early in this study because of the very rapid hydrolysis it causes in AZQ (34) and the low aqueous solubility of the drug, (Table I). Phosphate buffer (pH = 6.5) was subsequently used in all dilutions of AZQ stock solutions because it provided sufficient stability of AZQ in solution.

The standard solution was prepared by dissolving approximately 25 mg. of AZQ in 500 ml. of buffer (pH = 6.5). The solution was stirred with a magnetic stirrer and the resulting solution was then filtered through a micro-pore filter (0.45 microns), and degassed.

The solution was diluted serially with buffer solution, from 50 to 10 micro-grams per ml. (Table III). The standards were assayed and peak heights were measured and used to determine the concentrations of the unknown solutions of AZQ. In retrospect, peak area would have been more accurate. Since this is only a preliminary study, peak height was easier to determine as automated equipment was not available.

From the data, linearity was good ($r > 0.99$, $df = 5$) and the assay method was sensitive to the extend of 5 mg./ml. (Table III).

TABLE III: TYPICAL STANDARD CURVE DATA FOR AZQ

Concentration (μg./ml)	Peak Height (cm)
10	2.90
20	5.90
25	7.85
30	9.55
40	12.70
50	15.15

Slope = 0.312

X-intercept = -0.1

Correlation Coefficient = 0.997

4.2 Miniaturization of the Method:

In order to have confidence in the values generated with small samples, the assay was miniaturized, using progressively smaller volumes of AZQ solutions. For the final set of samples, amounts of AZQ equivalent to 80 mg. in 100 ml. were prepared in water and phosphate buffer. one ml. volumes were taken and diluted to 25 ml., the drug content of the solutions was determined against a standard curve and results agreed well with previous determinations. Test sample volume was then reduced to 3 ml. Aliquots of 0.5 ml. were withdrawn and diluted to 10 ml. The concentration of AZQ was determined and it compared well with initial data. In addition, the reproducibility at this level was good. Having decided that a 3 ml. working volume was satisfactory, it was necessary to validate the assay at this micro level.

4.3 Validation of Assay Method:

According to the Food and Drug Administration (FDA): Guidelines for Submission of Supportive Analytical Data for Methods Validation in NDA's (New Drug Applications), (33), the following steps are necessary to validate the newly developed HPLC assay for AZQ, reproducibility, linearity and accuracy. Slopes of 4 (four) HPLC runs, done on different days, were used to show the variability of the assay method. From the data below, it is very clear that the assay method is reproducible, as required by the FDA (33).

Example 1: Reproducibility

Day #	Slope	Date of Run
1	0.3120	5-6-87
2	0.3273	6-2-87
3	0.3325	6-4-87
4	0.3386	6-9-87

Example 2: Linearity

Day #	Correlation Coefficient (r)	Date of Run
1	0.9970	5-6-87
2	0.9998	6-2-87
3	0.9997	6-4-87
4	0.9997	6-9-87

df = 5

In all the determinations made, the method always exhibited good linear responses.

Example 3: Accuracy

Run #	Date	Theoretical Peak-Height (cm) of 10 micro-grams/ml.	Actual Peak-(cm) Height Measured
1	5/6/87	3.03	2.90
2	6/2/87	3.28	3.25
3	6/4/87	3.30	3.35
4	6/9/87	3.30	3.35
TOTALS		12.91	12.85

i) Average = 12.91 cm. ÷ 4 = 3.2275 cm.

ii) Average of Measured Peak-heights = 12.85 cm ÷ 4
= 3.2121 cm.

Degree of error = i. - ii.

= (3.2275 - 3.2125) cm.

= 0.0150 cm.

$$\% \text{ error} = \frac{0.015}{3.2275} \times 100$$

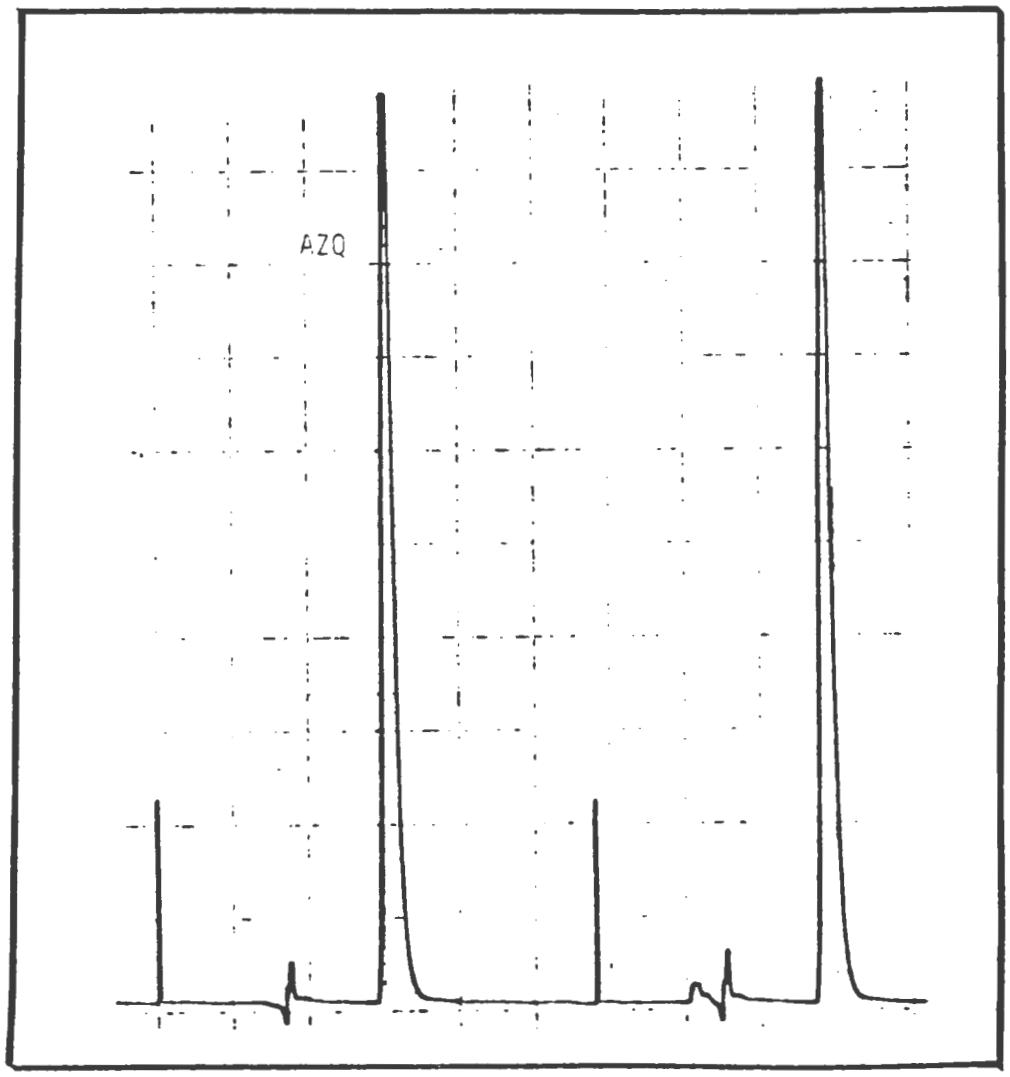
= 0.45%

The value obtained above, approximately 0.5%, falls well within the confidence level of 100 ± 10% required by the FDA.

See next page for Typical Chromatograms of AZQ solutions, Figure 10.

FIGURE 10: TYPICAL CHROMATOGRAMS OF AZQ SOLUTIONS

Solvent = Phosphate buffer (pH = 6.5).
Mobile Phase = 20% CH_3CN in phosphate buffer.
Loop = 50 micro-liters; Flow-rate = 1 ml./min.;
Pressure = 1.5 x 1,000 psi; AUF = 0.05; $Lambda_{max}$ = 254nm;
Chart Speed = 30 cm./hr.; Retention Time = 6 minutes



Concentration of AZQ solution = 40 micro-grams/ml.

4.4 The Stability of AZQ Solutions:

Aqueous solutions of AZQ (at a concentration of 50 micro-grams/ml.) degraded approximately 58% in a 12 hour period. During this time period, the pH changed from 4.15 to 5.0 (Figure 11), and the chromatogram showed evidence of decomposition of the main peak. A similar effect was seen in a sample aged 60 (sixty) days, in a dark room, at 25°c.

FIGURE 11: 'FRESH' AND 12 HOURS-OLD SOLUTIONS

Mobile Phase = 20% CH_3CN in buffer;
Solvent = Distilled Water;
AUF = 0.05; $Lambda_{max}$ = 254 nm; Loop = 20 microliters;
Flow = 1.0 ml./min.;
Pressure = 1.2 x 1,000 psi.;
Column = C_{18};
Concentration = 50 micro-grams/ml.

FIGURE 11: 'FRESH' AND 12 HOURS-OLD SOLUTIONS

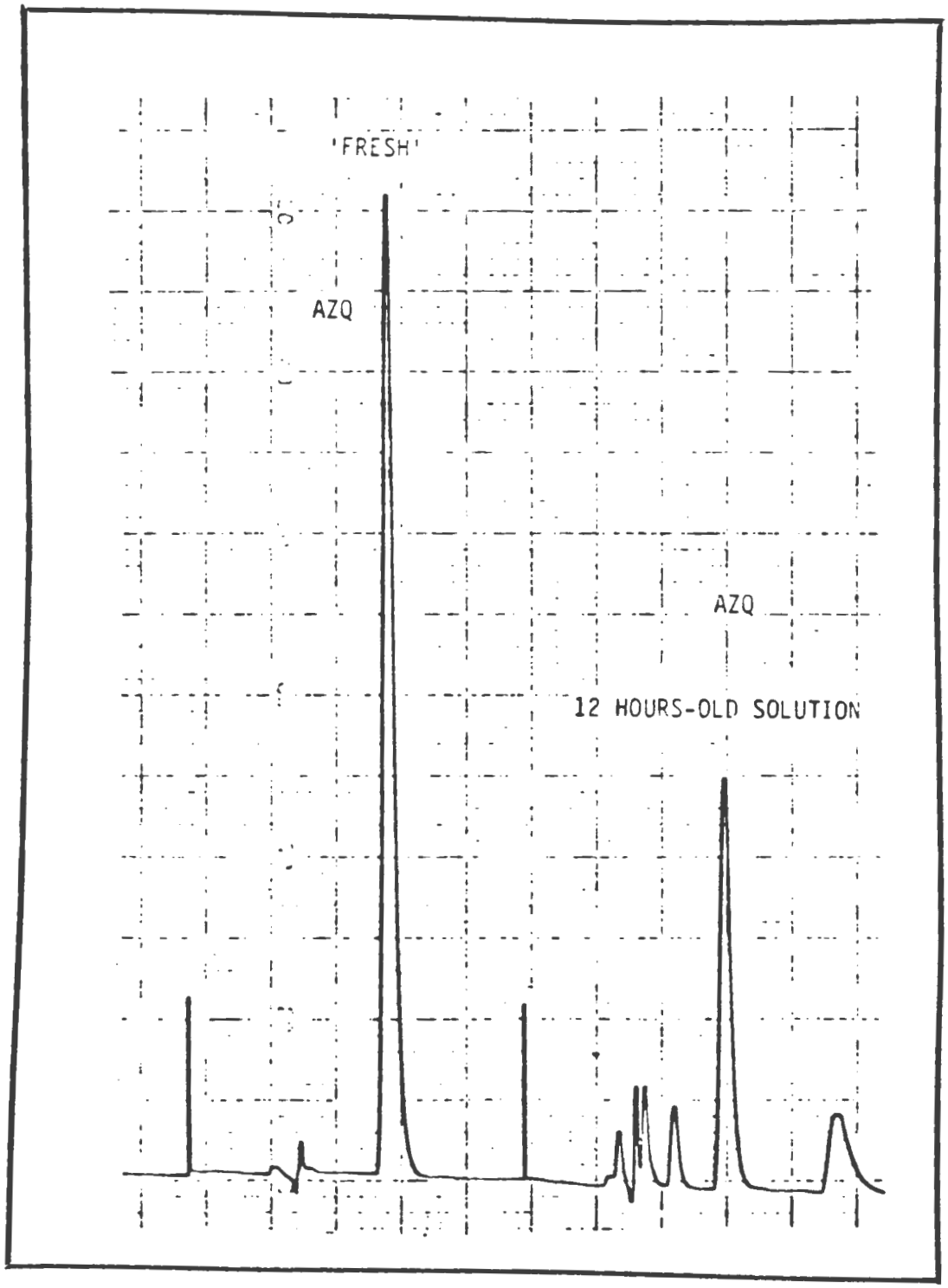
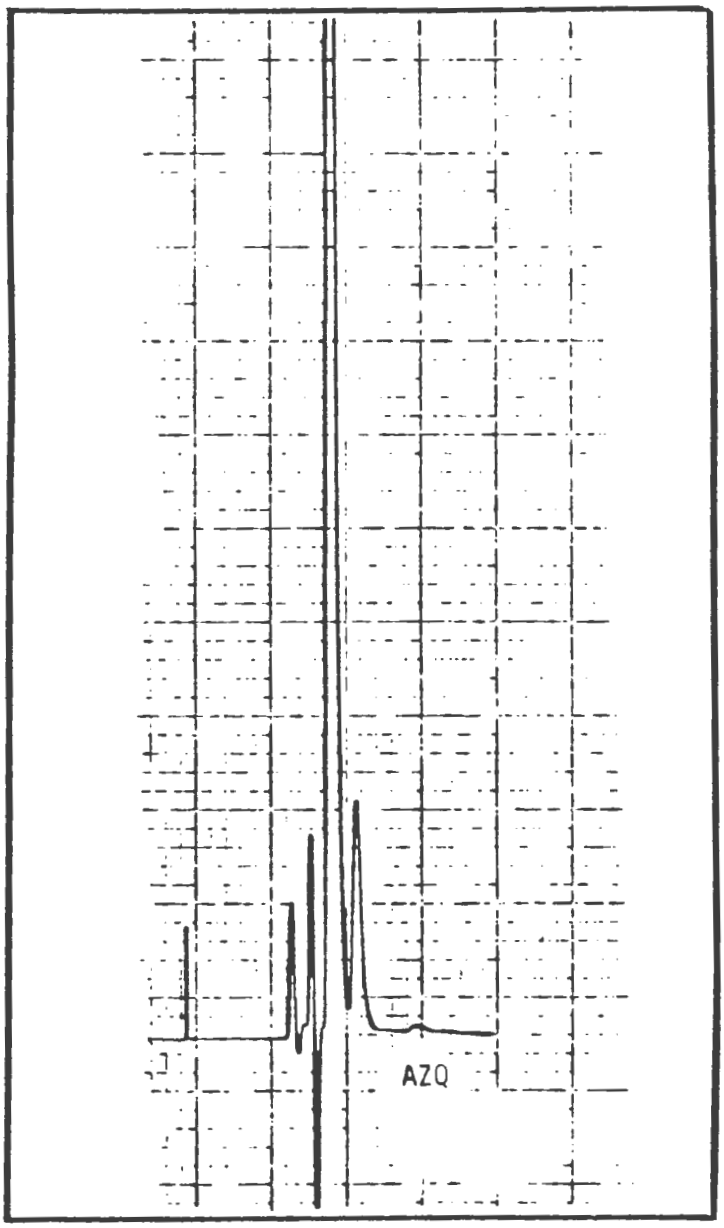


FIGURE 12: DEGRADATION PEAKS OF AZQ SOLUTION, SHOWING TOTAL LOSS OF AZQ PEAKS AFTER 60-DAYS

Solvent = Distilled Water;
Flow = 1 ml./min.;
AUF = 0.1;
Pressure = 2 x 1,000 psi.;
$Lambda_{max}$ = 254 nm;
Concentration of AZQ solution =
40 micrograms/ml.



When other solvents were investigated, the results were considerably different. Compared to water, these solutions showed very little apparent degradation in the same time period, (See Table IV, Figure 11, Figure 15).

TABLE IV: STABILITY OF AZQ IN DIFFERENT SOLVENTS

Solvent	Time (HR)	Apparent pH	Retention Time(min.)	Peak-Height (cm)	Peak/Height Ratio
95% Ethanol	0	9.87	6.2	8.15	4.29
	12	8.63	6.2	8.10	4.05
Phosphate buffer	0	6.58	6.0	15.40	39.50
	12	6.58	6.0	15.80	39.50
Propylene glycol	0	5.82	6.3	5.40	2.70
	12	5.22	6.3	5.30	2.65
DMA, 100%	0	12.04	5.6	12.20	2.30
	12	11.21	5.6	12.20	2.30
DMA in buffer, 50%	0	8.09	5.8	10.20	1.90
	12	7.43	5.8	10.00	1.85
Peanut Oil	0	-	6.0	1.00	1.45
	12	-	6.0	0.65	0.93
Distilled Water	0	4.48	5.8	15.20	30.40
	12	5.80	5.8	6.40	5.30

It can be noted that the phosphate buffer provides a useful aqueous diluent for the solubility study. Therefore, when aliquots of AZQ were taken from sample vials, they were diluted to volume

with phosphate buffer. This insured stability of the drug during the assay period (Figure 13 demonstrates the stability of AZQ in phosphate buffer solutions).

DMA was the only system which showed appreciable change in retention. This was expected since the literature indicates that the UV cut-off point for this solvent is 267 nm. In the chromatogram of this compound, (Figure 14), the main peak of AZQ is much lower than degradation peaks in unsaturated solutions. An injection of aged samples in this solvent showed no interference of the main peak due to decomposition products. Saturated solutions of AZQ in DMA show familiar peaks and retention, after proper dilution.

FIGURE 13: THE EFFECT OF AGEING (TIME) ON AZQ SOLUTIONS:

M.P. = 20% CH_3CN in Buffer; Flow = 1.0 ml/min.; Loop = 20 micro liter
Pressure = 1.5 psi (x1000); $Lambda_{max}$ = 254nm; Chart speed = 30cm/hr
Solvent = Buffer (pH = 6.5); AZQ conc. = 50 micro gram/ml;
Retention time = 6 min.

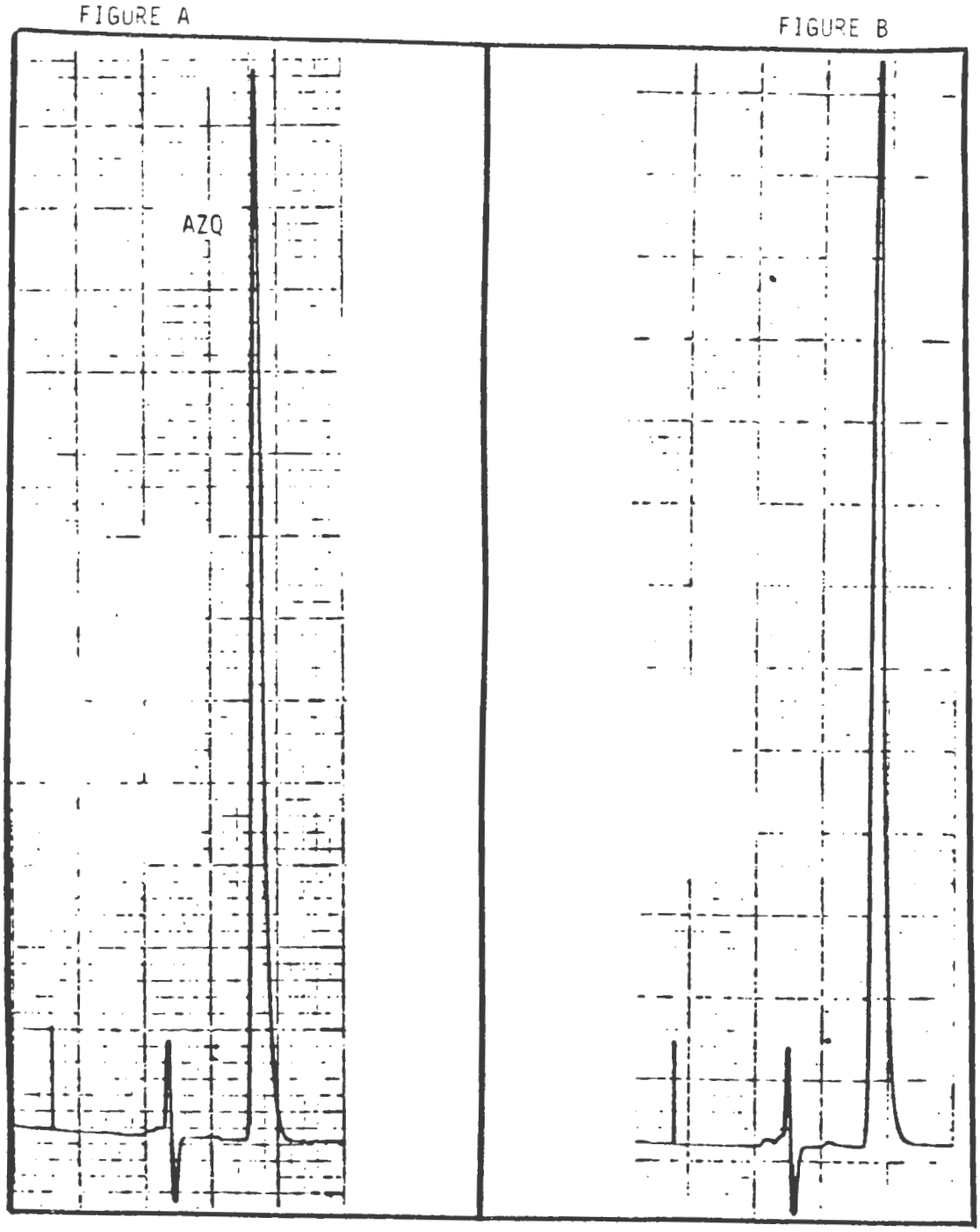


FIGURE 14: CHROMATOGRAM OF AZQ IN DMA

Solvent = DMA, 100%;
Mobile Phase = 20% CH_3CN in buffer;
Loop = 20 micro-liters; Flow = 1 ml./min; Pressure = 1.5 x 1,000 psi;
AUF = 0.05; $Lambda_{max}$ = 254 nm;
Chart Speed = 30 cm/hr.; Retention Time = 6 minutes

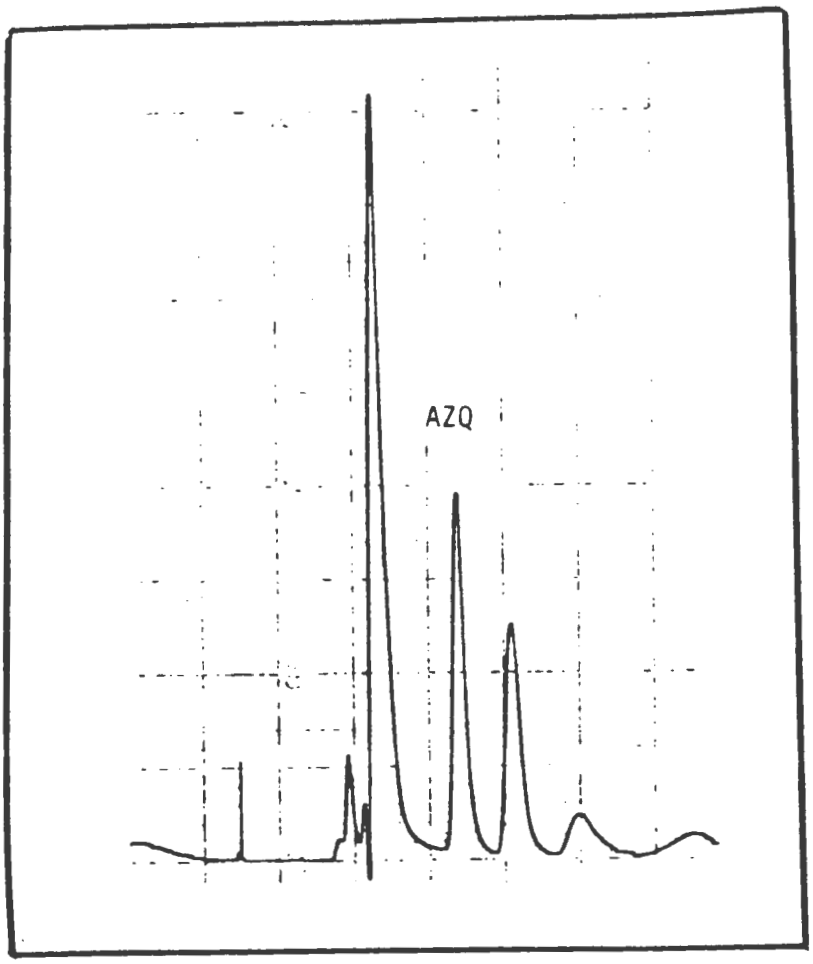
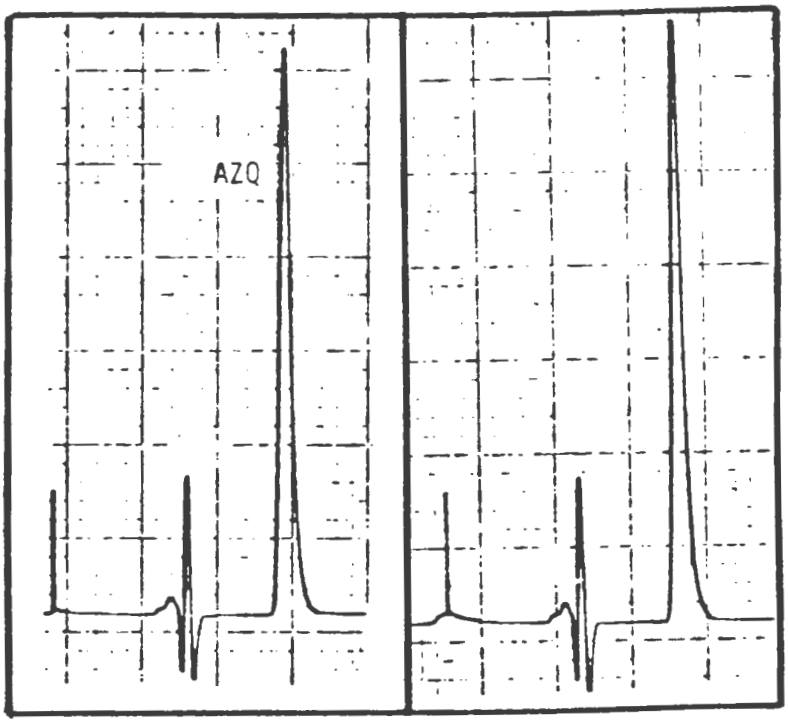


FIGURE 15: EFFECT OF TIME ON pH, PEAK-HEIGHT AND RETENTION-TIME OF AZQ IN DIFFERENT SOLVENTS

Zero Time | After 12 hours

15(a) 95% Ethanol



In all determinations, HPLC conditions were similar, ie.

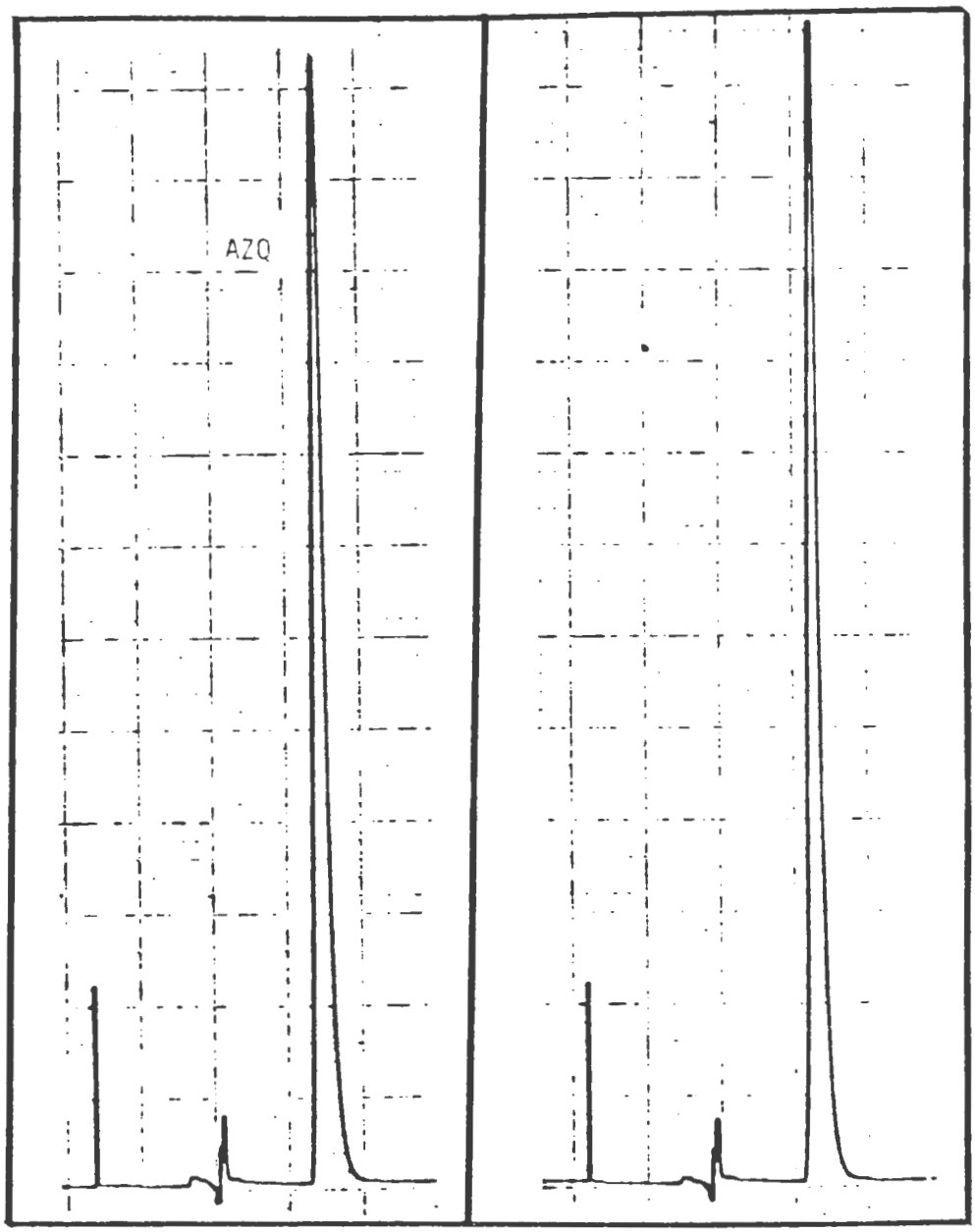
Auf = 0.05;
$Lambda_{max}$ = 254 nm;
Loop volume = 50 micro=liters;
Pressure = 1.0 - 1.5 (x1,000) psi;
Flow rate = 1 ml./min.;
Chart-speed = 30 cm/hr.

FIGURE 15: EFFECT OF TIME ON pH, PEAK-HEIGHT AND RETENTION-TIME OF AZQ IN DIFFERENT SOLVENTS (cont.)

Zero Time | After 12 hours

15(b) Phosphate Buffer:

(pH = 6.5)



15(c) Propylene Glycol:

Zero Time

After 12 hours

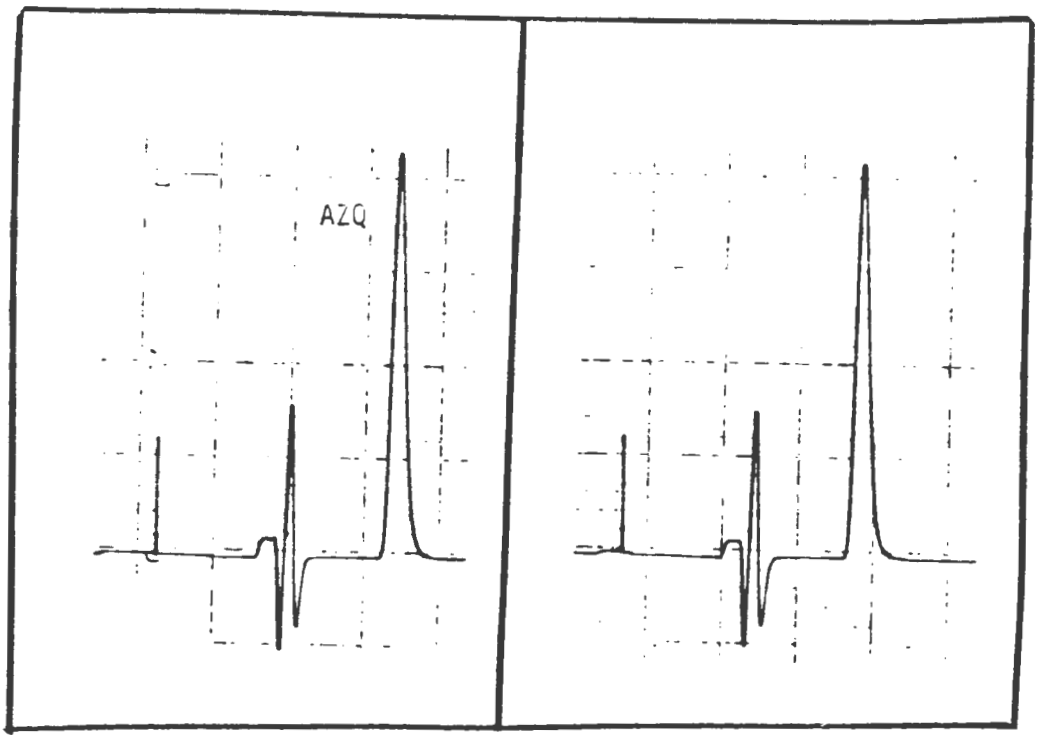


FIGURE 15: EFFECT OF TIME ON pH, PEAK-HEIGHT AND RETENTION-TIME OF AZQ IN DIFFERENT SOLVENTS (cont.)

Zero Time | After 12 hours

15(d) 50:50 DMA: Phosphate Buffer

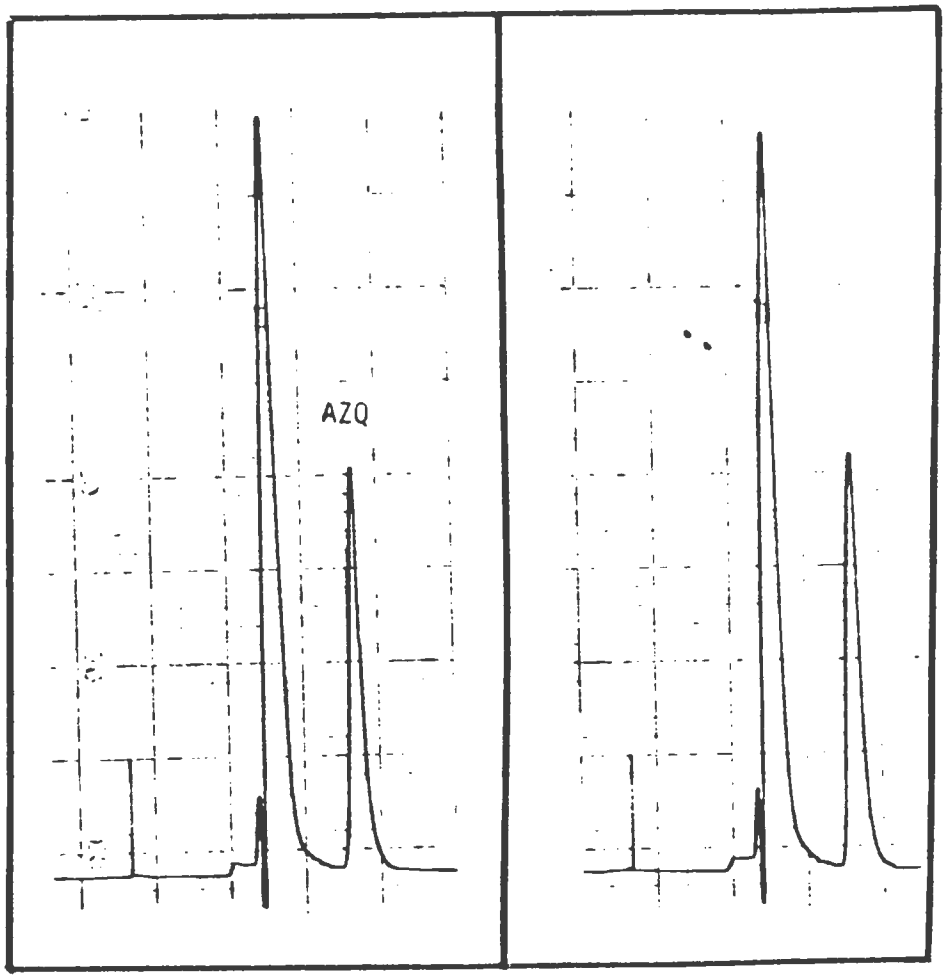
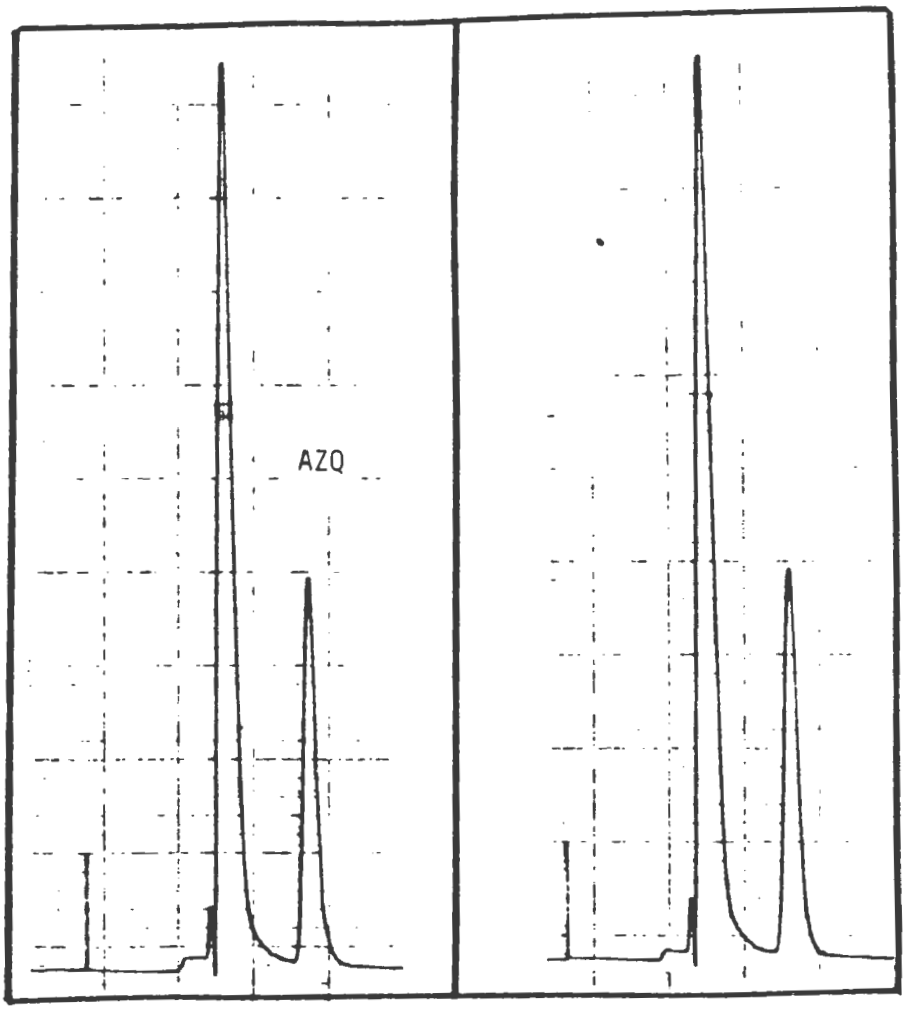


FIGURE 15: EFFECT OF TIME ON pH, PEAK-HEIGHT AND RETENTION-TIME OF AZQ IN DIFFERENT SOLVENTS (cont.)

Zero Time | After 12 hours

15(e) 100% DMA:



Depending on the solvent environment, AZQ can be expected to undergo degradation by a number of different pathways (35,36). When the pH is strongly acidic (pH = 1), AZQ degrades instantaneously to yield a dialcohol, whereas under alkaline conditions, AZQ undergoes simple stepwise and consecutive hydrolytic cleavage of the aziridine rings to produce the monoalcohol, then finally the dialcohol (37). Buffer systems have also been shown to have a mixed effect on the degradation (38). At acid pH and above pH 8, degradation increases significantly. Increasing the buffer concentration will generally also increase the rate of disappearance of AZQ from solution although examination of the chromatograms of degraded solutions has shown that this reaction is partially a function of phosphate ion. For our work, a phosphate buffer (pH 6.5) showed good stability over a 12-hour period (Figure 15(b) and Table V), and this has been shown to be the pH of optimal stability (39).

In this study, stability was monitored by measuring pH and peak-height ratio of the main peak to a major degradation peak as a function of time. The results are summarized in Table V for solutions at an initial concentration below saturation (40 ugm/ml). As can be seen, the compound was very unstable in water and decomposed 58% after 12-hours. This is in agreement with NCI data which showed 67% loss. Additionally, an aqueous sample stored at 25°C in the dark, showed very little AZQ concentration after 60 days (Figure 12). This was not unexpected after observing the 12-hour data.

TABLE V: STABILITY OF UNSATURATED AZQ SOLUTIONS

Solvent	Time (hr.)	App. pH	Ret. (Min.)	Pk.Ht (cm)	Pk.Ht. degr.(cm)	Pk.Ht. Ratio	AZQ. mg/ml	% Change
Distilled Water	0	4.48	5.8	15.20	30.40	0.50	9.80	
	12	5.80	5.8	6.40	5.30	1.21	4.16	57.5%
Phosphate 100% Buffer (pH = 6.5)	0	6.58	6.0	15.80	0.40	39.50	10.18	
	12	6.58	6.0	15.40	0.40	38.50	9.93	2.5%
DMA in Buffer, 5:95	0	6.03	6.0	5.80	5.52	1.05	3.45	
	12	6.83	6.0	5.60	5.44	1.03	3.33	3.4%
DMA in Buffer, 10:90	0	6.43	6.0	4.90	5.10	0.96	2.91	
	12	6.87	6.0	4.40	5.43	0.81	2.62	10.1%
DMA in Buffer, 25:75	0	6.88	6.0	5.40	7.83	0.69	3.21	
	12	7.10	6.0	5.30	7.79	0.63	3.15	1.9%
DMA in Buffer, 50:50	0	8.09	5.8	10.20	5.37	1.90	6.04	
	12	7.43	5.8	10.00	5.41	1.81	5.93	1.95%
DMA, 100%	0	12.0	5.6	12.00	5.22	2.30	7.11	
	12	11.21	5.6	12.00	5.22	2.30	7.11	0.0%
Ethanol, 95%	0	9.87	6.2	8.50	1.98	4.29	5.23	
	12	8.63	6.2	8.10	2.00	4.05	4.99	4.70%
Peanut Oil	0	-	6.0	1.00	0.69	1.45	2.28	
	12	-	6.0	0.65	0.70	0.93	1.98	13.10%
Propylene Glycol	0	5.82	6.3	5.40	2.00	2.70	3.19	
	12	5.22	6.3	5.30	2.00	2.65	3.13	1.90%

NCI data has also shown that aqueous solutions of this drug decompose readily at room-temperature in 5% DMA, losing 18% of active-ingredient in 8 hours (27, 28). In our investigation, a series of DMA solutions in pH 6.5 buffer was prepared to evaluate this effect. In fact, the same effect was noticed at 5% DMA, where a 1% increase in drug loss was observed. At 10% DMA, the degree of degradation had increased to 10%. Further increase in DMA concentration, reversed this effect such that at 100% DMA there was little change in the initial concentration. It is unclear at this time what the actual mechanism is for this behavior. However, it would be reasonable to assume that addition of DMA would alter the solubility of buffer ingredients, thus producing a shift in degradation rate as described previously (Figure 16).

Alcohol has also been suggested as a co-solvent for this compound. Solutions of AZQ in this vehicle showed a drop in pH of approximately 1 unit over the test period and an estimated loss of drug of 5%. this solvent would therefore appear to be useful as a solubility enhancer since the degree of change is relatively small, and alcohol is acceptable for intravenous use.

Propylene glycol should provide some improvement in solubility since it is often used as a co-solvent for semi-polar compounds. Unsaturated solutions of AZQ in propylene glycol showed little change in pH or drug content over 12-hours. Therefore, it could be considered as a solvent for this drug.

Peanut Oil was also evaluated for improving the solubility and stability of AZQ. Unsaturated solutions using this solvent required

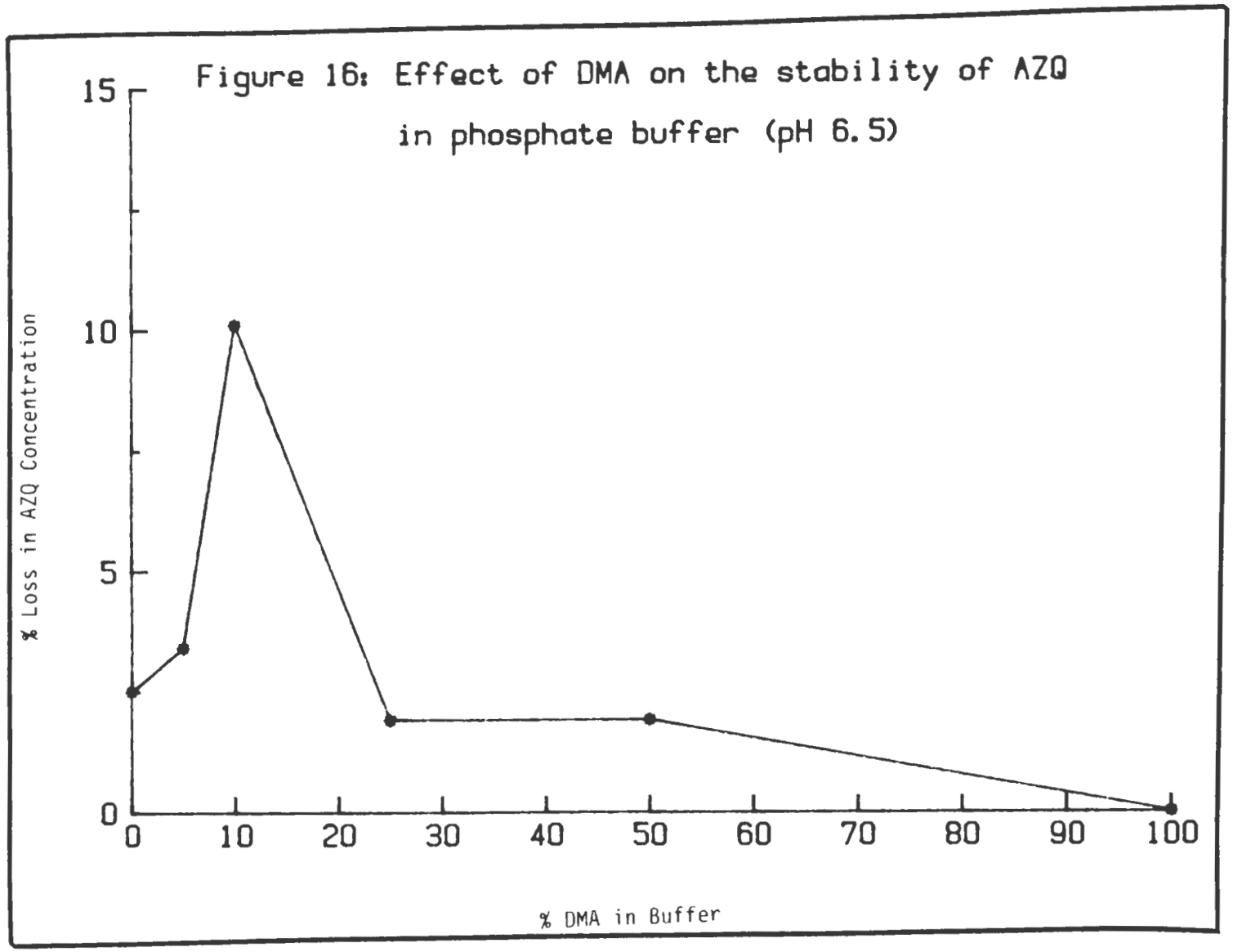


Figure 16: Effect of DMA on the stability of AZQ
in phosphate buffer (pH 6.5)
% Loss in AZQ Concentration
15
10
5
0
0
10
20
30
40
50
60
70
80
90
100
% DMA in Buffer

extraction by methanol before analysis by HPLC. Amounts of active drug in solution were small and therefore any results can be considered inconclusive.

In summary, the stability study indicated that AZQ was stable in three solvents (DMA, Ethanol 95% and Propylene glycol) for at least 12 hours. This degree of stability could potentially provide the opportunity to develop a reconstitutable product. Justification for this type of product would also depend on the degree of solubility enhancement which could be achieved with these solvents. Following are the results of a solubility study of AZQ.

4.5 Solubility of AZQ:

In order to study the solubilizing capacity of the various solvents, excess drug was exposed to solvents in vials as described previously. In one trial, samples were filtered and assayed after 24-hours, while another trial was completed at a 4 hour sampling time. This allowed evaluation of the stability of concentrated solutions as well as confirmation of solubility and time required for equilibrium. A previous study showed that 12 hours was more than sufficient for equilibrium. Since decomposition is occurring simultaneously, it was interesting to monitor development of degradation. Degradation peaks were evident in samples showing loss of AZQ at 24 hours.

Table VI summarizes the results of these investigations. In comparing results at 4 hours and 24 hours, a slight decrease in concentration (0.76 and 7.6%) can be seen in DMA and ethanol systems, respectively, when 24 hours was used for equilibrium. This was not

considered to be extensive. There was a slight increase in solubility over the same time period in the aqueous and buffered systems (33.3% and 25.9%). Four hours therefore appeared to be sufficient time to reach equilibrium. In the case of propylene glycol and peanut oil, four hour samples were not prepared because of the limited amount of AZQ available, and since the 24-hour samples gave a good indication of solubility.

The saturation solubility of AZQ in water was found to be approximately 0.49 mg./ml. (n=3). This is in the range of results reported by NCI, (Table 1). Phosphate buffer produced results similar to but slightly lower than water (0.35 mg./ml.). This should not be unreasonable if one considers competition for solubility with buffer ingredients. A distinct advantage of phosphate buffer was improved stability, particularly at sub-saturated levels of the drug.

In the case of ethanol, there was a ten-fold increase in solubility (3.7 mg./ml.) , and as discussed previously, the rate of decomposition in ethanol solutions was low. Thus ethanol appears to be useful in providing improved solubility of AZQ. As mentioned before, ethanol is approved for intravenous (IV) use and it would therefore appear to be a suitable solubilizer of this drug.

The effect of propylene glycol was disappointing. The solubility in this solvent was approximately the same as in water (0.46 mg./ml). This solvent is very viscous and made analysis difficult at the filtering step. Perhaps a repeat of this portion of the test would have shown slightly higher results as had been anticipated since it is often used to improve solubility. Peanut Oil showed similar

effects. AZQ was not easily wetted by the viscous oil and did not appear to be very soluble in this material (0.05 mg./ml). Extraction Extraction with methanol created an additional opportunity for the loss of product. The disappointingly short peak heights are really too small to draw any significant conclusions. Both these results would indicate that neither of these two solvents would be acceptable in an injectable dosage system. However it must be recognized that lack of samples prevented more extensive evaluation of these solvents.

DMA was by far the most effective solvent for AZQ. A saturation concentration of 44 mg./ml. was achieved with this solvent. These results are approximately double those reported by the NCI but the NCI data is a useful comparison. It should be noted that the NCI data are a rough estimate of solubility. While the DMA results are encouraging, the ratio of DMA required to achieve this level of solubility exceeds permissible concentrations for human use. DMA is an animal teratogen (40), and it is recommended that DMA concentrations not exceed 5%. Given the structural similarity of alkyl amides, one could expect the same trend in these solvents (40).

In summary, DMA can achieve a significant increase in solubility of AZQ. However, clinical application would be limited.

TABLE VI: SOLUBILITY OF AZQ AT 25°C

Solvent	Incubation Time (hrs)	Peak Height (cm)	Milligrams per ml.	% Change
Distilled Water	4	7.35	0.42	
	24	9.95	0.56	+33.28
95% Ethanol	4	7.00	3.89	
	24	6.63	3.59	-7.64
Propylene Glycol	4	-	-	
	24	8.48	0.46	-
DMA	4	8.15	44.38	
	24	7.60	44.04	-0.77
Peanut Oil	4	-	-	
	24	0.95	0.055	-
Phosphate Buffer (pH 6.5)	4	6.00	0.296	
	24	7.40	0.400	+25.98

4.6 Effect of Temperature on AZQ Solubility:

Standard Curve:

Flask #	Conc. AZQ (ug/ml.	Average Peak-Height(cm)
1	50	16.7
2	40	13.0
3	30	9.5
4	25	7.8

Calculations from Standard Plot:

Slope = 0.356
X-intercept = -1.1525
Correlations Coefficient = 0.999
Mean Value = 11.75

Results:

TABLE VII: THE EFFECT OF TEMPERATURE INCREASE ON SOLUBILITY

Temp. °C	Time (hours)	pH Change	Average Pk. Ht. (cm)	ug/ml. from Plot	Dilution Factor	AZQ mg/ml	% Change
30	0	6.76	3.6	13.35		0.267	
	4	6.52	2.5	10.26	20	0.206	-23.22
35	0	6.76	3.6	13.35		0.267	
	4	6.52	5.0	17.28	20	0.346	+29.59
40	0	6.76	3.6	13.35		0.267	
	4	6.51	3.8	13.91	20	0.278	+ 4.12

The effects of temperature on the solubility of AZQ were studied at 25,30,35 and 40°c. Solubility of AZQ at 25°C was studied and reported in a previous chapter. A previous study by Poochikian and Cradock (41) had shown the temperature dependence of the disappearance of AZQ from solution. In that study, the apparent Arrhenius parameters were calculated from the regression equation ($r > 0.99$) and were found to be as follows: the apparent activation energy (Ea) was 14.0 Kcal per mole, and the frequency factor (A) was 7.70×10^3 sec^{-1}.

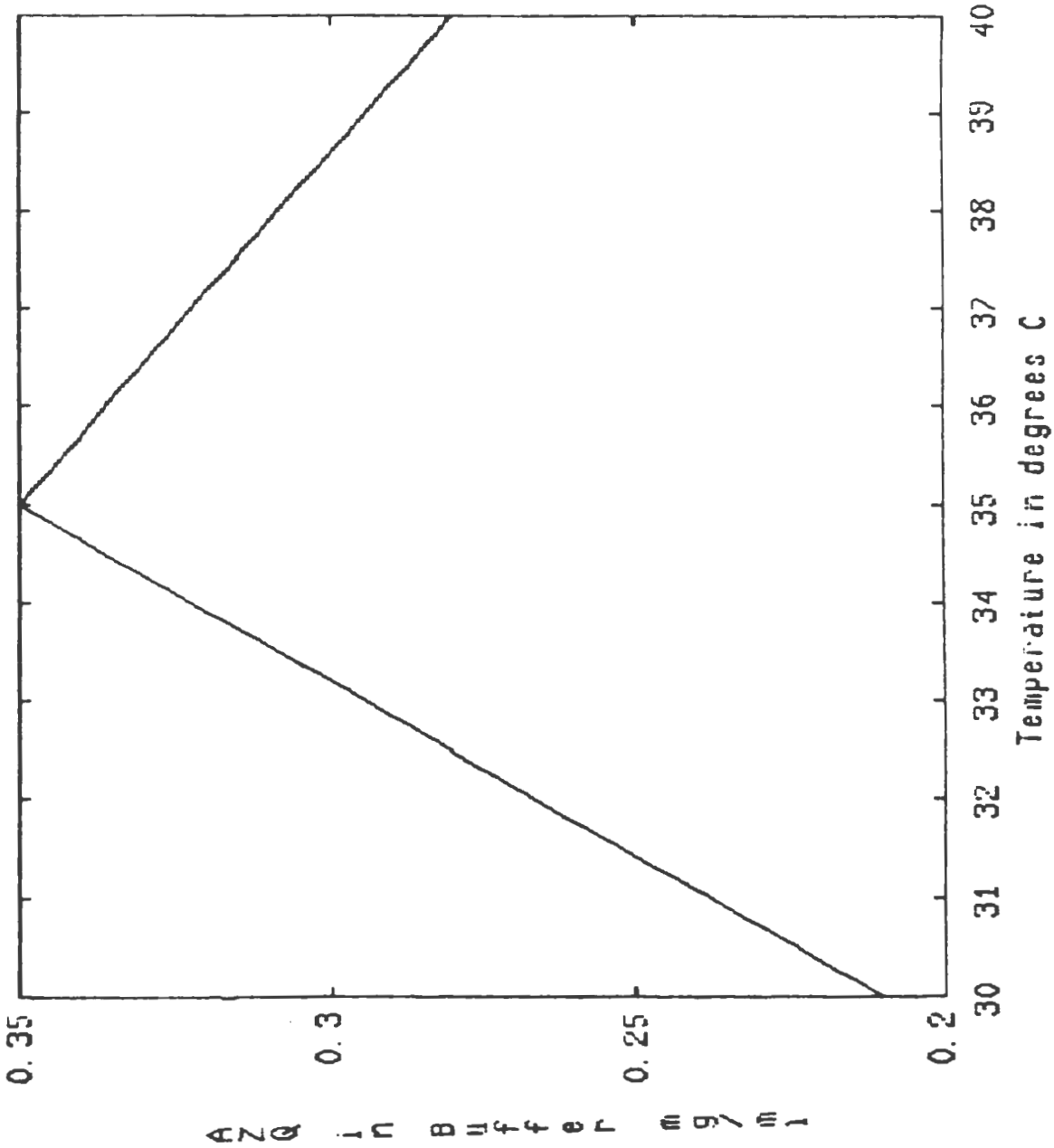
From the solubility versus temperature plot (Figure 17), it is apparent that raising the water-bath temperature from 25°C to 30°C and 35°C respectively, improved slightly the amount of AZQ dissolved in the buffer solution and incubation temperature of 35°C provided maximum solubility of the drug. This is not unexpected since evidence gathered by Poochikian and Cradock (41) showed that more degradation of AZQ occured at higher temperatures. Elevating the temperature to 40°C increased the loss of AZQ.

An apparent activation energy of 38.0 Kcal/mole and a frequency factor of 8.3×10^3 $sec.^{-1}$ can be estimated because of the limited amount of material available, this portion of the study could not be replicated more than twice and the results should only serve as a rough estimate of these parameters.

In summary, this study has generated data that can help to identify the solubility characteristics of AZQ. There were a number of frustrating problems arising from the work which limit the application of the results. Namely, the compound is unstable in an aqueous

FIGURE 17: THE SOLUBILITY OF AZQ IN PHOSPHATE BUFFER (pH 6.5)

AT VARIOUS TEMPERATURES



medium, solubility is relatively poor, and availability of the drug restricted the extent of experimentation. Developing a reliable method for identifying AZQ in small quantities was a major achievement. The results obtained with the micro-method agreed well with other data. Enhanced solubility and stability of this compound in solvents such as ethanol and DMA is noteworthy. However, acceptable use levels of these solvents must be of concern. Ethanol would obviously be more appropriate as a parenteral solvent since it is widely used (socially) in humans and would be expected to have less serious side-effects than the DMA which provided maximal solubility enhancement.

Future work in this area could focus on the use of sugars, albumins, ureas, and other natural substances for solubility improvement. Alternatively, unique delivery systems such as monoclonal antibodies, liposomes, etc. might provide sufficient blood levels of this drug.

While the results of this study are not overly profound, the work did provide an opportunity to learn solubility techniques, good laboratory procedures, and demonstrated the need for considering miniaturization studies for drugs undergoing preliminary preformulation and clinical studies.

5. CONCLUSIONS AND SUMMARY

1. A rapid, simple, sensitive and selective stability-indicating high-performance liquid chromatographic (HPLC) micro-assay method was developed and validated.
2. The solubility of AZQ was found to be quite low in water (0.49 mg/ml.). Phosphate buffer did not improve AZQ solubility which was even lower (0.35 mg/ml.) in this solvent. However, stability was much a distinct advantage in this aqueous system.
3. DMA and Ethanol improved the solubility of AZQ significantly. The use of DMA as a solvent is limited by safety considerations and it would appear that ethanol may be the most practical solvent at this point in time for AZQ.
4. Propylene glycol could be a useful co-solvent for AZQ but the degree of enhancement is not great.
5. Peanut oil was a disappointingly poor solvent. Increasing the time and temperature of agitation did not improve its performance as a solvent.
6. Raising the temperature of incubation during agitation of the solutions of AZQ in Phosphate buffer did not significantly improve the solubility. Maximal enhancement of solubility seemed to peak at 35°C, but raising the temperature of the solution only accelerated the rate of degradation as discussed earlier in this study.

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