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The stability method and analysis of diphenhydramine hydrochloride in liquid and solid drug dosage forms

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THE STABILITY METHOD AND ANALYSIS OF DIPHENHYDRAMINE HYDROCHLORIDE IN LIQUID AND SOLID DRUG DOSAGE FORMS

By

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Abstract

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By

Hai-Ping Yuan

Advisor: Professor David C. Locke

Diphenhydramine hydrochloride in two different drug dosage forms--one in liquid form and the other in solid form--was chosen as the active for the demonstration of this study. One can obtain these Over-The-Counter (OTC) drugs without a prescription. The study is comprised of two parts. PART ONE is the development and suitability check of the analytical method for accurately quantitating diphenhydramine hrdrochloride component in both pharmaceutical products. A reversed-phase ion-pair liquid chromatographic method was developed. The precision, linearity, specificity and recovery were checked, and

proved to be suitable for stability analysis of diphenhydramine hydrochloride in these pharmaceutical products. PART TWO is the thermal kinetic study of the active compound. Drug samples were stored at 42, 52 and 62 ^{o}C for times up to about 110 days. The method used for quantitation of the active compound in untreated and thermally treated samples was the reversed-phase ion-pair HPLC method developed in PART ONE. The decomposition rate constant of the compound was determined first through the time-based kinetic approaches by plotting the active levels vs. time for each storage temperature. Then the decomposition rate at room temperature can be predicted by the extrapolation of the Arrhenius plot, hence the shelf life of the drug compound at 25 ^{o}C can be calculated.

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PART ONE
ANALYTICAL METHODOLOGY FOR QUANTITATION OF DIPHENHYDRAMINE HYDROCHLORIDE IN DRUGS: REVERSED-PHASE ION-PAIR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

I. SUMMARY

An accurate and precise high performance liquid chromatographic (HPLC) method is developed for quantitating diphenhydramine hydrochloride compound in two pharmaceutical dosage forms. One drug dosage form is liquid ("L") and the other is solid ("S"). Description of the analytical instrumentation and methodology for the compound of interest will be presented below.

Diphenhydramine hydrochloride is well resolved from all other product ingredients or impurity peaks on an Alltech 10 micron RP-8 column using an ion-pairing technique. The detection wavelength, 258 nm, was determined from the UV spectrum of the compound. The response is linear over a wide concentration range, with an intercept close to zero. Precision studies of the HPLC system and the overall method both show low relative standard deviations. The accuracy was checked through a recovery test by spiking USP diphenhydramine hydrochloride standard into each drug

preparation. Furthermore, the so-called ratio plot of the diphenhydramine hydrochloride peak was performed using the Waters multiple-channel detector to insure that no hidden peak interferes with the analysis of the desired component.

The method is suitable for measuring small changes in diphenhydramine hydrochloride levels in the two pharmaceutical products. It is simple, fast, accurate, and precise. The stability of the drug active will be analyzed by measuring its rate of change through the kinetic study described in PART TWO.

II. INTRODUCTION

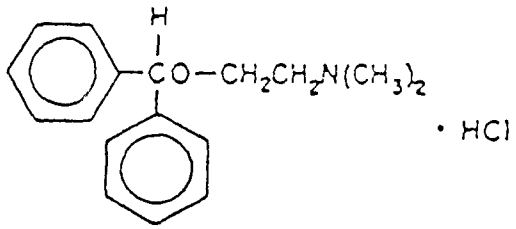
A. Diphenhydramine Hydrochloride

Diphenhydramine hydrochloride is a widely-used drug active for antihistamines (1, 2) that appear to compete with histamine for cell receptor sites on effector cells. It possesses drying, antitussive, and sedative effects, and has anti-emetic and local anesthetic properties. It is used in the treatment of allergies, Parkinsonism, motion sickness, and insomnia. It also serves as a cough suppressant for the control of coughs due to colds or allergy, because of its antitussive activity (3 - 6). The usual dose for adults is 25 to 50 mg, while for children aged 1 to 5 years it is 12.5 mg. In addition to being contained in prescription drugs (4), diphenhydramine hydrochloride is also found as a drug active in non-prescription drugs (7) that are commercially available on the drug counter and can be obtained without a physician's prescription.

The chemical name of diphenhydramine is 2-diphenyl methoxy-N,N-dimethylethylamine; and has other names such as 2-(benzhydroxy)-N,N-dimethylethylamine, β-dimethylaminoethyl benzhydryl ether,

O-benzhydryl ether, O-benzhydryldimethylaminoethanol, β-dimethylaminoethanol diphenylmethylether, α-(2-dimethylaminoethoxy) diphenylmethane, benzhydramine, Alledryl, Allergin, Amidryl, Bagodryl, Benodin, Benylan, Dibendrin, Dibondrin, Dihidral, Dimedrol, Diphantine, and Syntedril.

Its empirical formula is $C_{17}H_{21}NO$. Diphenhydramine hydrochloride $C_{17}H_{21}NO \cdot HCl$ is a white, odorless, crystalline powder. Its melting range is 167° to 172° C. It is very soluble in water - 1 gram dissolves in 1 mL of water. Its structure is: (8)



B. Methods of analysis

The USP Official Monographs (9) employ non-aqueous titration and UV-spectrophotometric methods for assaying diphenhydramine hydrochloride substance itself and drugs. The USP UV method uses ether to extract

diphenhydramine hydrochloride from the drug product. The extracts are diluted with 0.1 N sulfuric acid. These solutions are run concomitantly with a USP diphenhydramine hydrochloride reference standard of known concentration (400 to 500ug/mL) in 0.1 N sulfuric acid on a spectrophotometer at 258nm. The quantity of diphenhydramine hydrochloride is determined by comparison of these two absorbances. As indicated in the paper entitled " Determination of Diphenhydramine Hydrochloride in Elixir " by Woo et. al. (10), the USP UV-assay was found to be inapplicable to certain samples undergoing stability tests. With increasing storage temperatures, consistently higher results were obtained. The non-aqueous titration method would be even worse if it were used for assaying the drug sample. This method used glacial acetic acid to dissolve diphenhydramine hydrochloride drug substance and the solution is titrated with perchloric acid. The limitation of both methods is the interferences from other species in drug samples. Because of their separation power, chromatographic methods normally have a better chance to be stability-indicative (11). HPLC is the state-of-the-art method for analysis of drug actives that exhibit UV absorbance

and suffer from interferences by other UV-absorbing species present in the drug as matrix excipients and/or generated by degradation (decomposition products). Chromatographic methods such as paper chromatography (12), TLC (13 - 14), GC (15 - 34), column chromatography and HPLC (35 - 44) have been found to be useful in the identification and/or quantitation of diphenhydramine hydrochloride in drug products. Paper chromatography and TLC are lacking an easy method of obtaining accurate and precise quantitative results. A precision better than 5% is seldom achievied by these methods. Even with the so called HPTLC, which was developed as an instrumentalized high-performance TLC recently, the best precision achievied is only 2%. The HPLC is the modern form of column chromatography and has a high sophisticated analytical technique results from the need to have a separation system in the liquid phase whic is complementary to GC, i.e., a method which is capable of rapidly separating complex chemical mixtures and providing simultaneously a continuous record of the separation from which the quality of the separation and a quantitative assessment of the composition of the

original sample may be deduced. A precision of better than 1% has been reported by independent workers in the field of HPLC, suggesting that the technique is directly suitable for many assays of commercial importance. Unlike GC, the precision of the method does not normally vary a great deal from sample to sample, presumably since vaporization of the sample, with attendant possibilities of decomposition or variations in the rate of evaporation, is absent in the liquid phase of HPLC. Because the mobile phase is a liquid, HPLC has great power for high resolution. Not only there are many parameters that can be varied to achieve the desired separation, but solute-solvent interactions, as well as solute-stationary phase interactions, can be used to achieve optimal selectivity.

Among the HPLC methods reported in the literature so far, none is really specific for stability analysis of diphenhydramine hydrochloride itself or contained in drug products. To develop a simple, accurate, and precise HPLC method specific for detecting the small changes in diphenhydramine hydrchloride levels in drugs "L" and "S" is our first objective. Reversed phase HPLC with the ion-pair technique is the basis for this methodology.

C. Progress in LC to Ion-Pair RPLC

In 1906 Michael Tswett published what is considered to be the first paper on liquid chromatography in which the nature of the process and its potential was clearly explained. This open-column chromatography was not really widely adopted until many years later. In 1941, Martin and Synge developed a liquid partition chromatographic process. They used a packed column containing water-saturated silica gel and a mobile phase of butanol-chloroform. They perfected the experimental techniques and explained the theoretical aspects of the procedure so thoroughly that they were awarded the Nobel Prize in 1952 for this work. After classical LC, modern LC had its beginnings in the late 1950's with the introduction of automated amino acid analysis by Stein and co-workers (45).

Modern LC has been referred to by other names: high-performance or high pressure LC (HPLC), high-speed LC, and simply liquid chromatography (LC). By the early 1960's the fundamentals of chromatographic theory were extended to LC, and this led to the development of the first high performance column packings for LC and the design of the first modern LC units. The pioneering

work on the fundamental theory of HPLC in columns was done by Hamilton (46) and Giddings (47) in the mid 1960's.

The reversed-phase mode of HPLC (RPLC), which was suggested by Boscott (48) and originated from the fundamental work of Howard and Martin (49), uses a nonpolar stationary phase and a polar mobile phase. The development of RPLC was hampered by the lack of suitable supports until 1969. The combined efforts of a number of scientists led to the 1969 breakthrough. At the present time, microparticulate silica is the most popular support material of the several supports available (50 - 53). The idea of chemically bonding organic group to the silica surface for RP chromatography was pioneered by Halasz (54), Kirkland (55), and Locke (56).

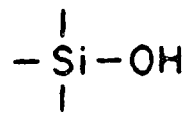
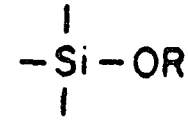
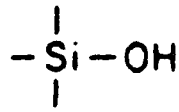
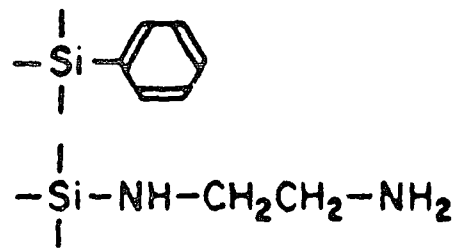
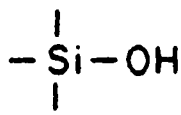
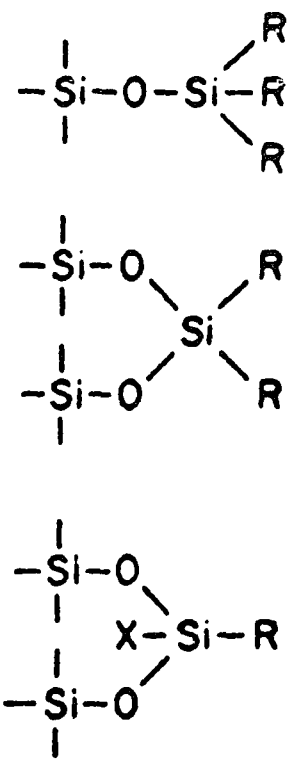
The latter chemically bonded microparticular packings offer significant improvements in column efficiency and resolution, and led to the rapid ascent and tremendous popularity of RPLC. The advantages of RPLC, summarized in Table 1, are described and discussed fully in several books (e.g. 57). Since the 1970's, there has been a very rapid increase in the use of RPLC in drug analysis.

Table 1. Advantages of RPLC

1. Operational simplicity.
2. Availability of columns with high efficiency and selectivity.
3. Wide range of solvent systems.
4. Compatibility of solvent systems with many samples, particularly those containing biological molecules.
5. Rapid analysis and fast equilibration.
6. Possible use of secondary chemical equilibria (ionization, ion-pair and liquid exchange).
7. Compatibility with detection devices necessitating aqueous media (electrochemical, post-column derivatization).
8. Large number of commercially available reversed-phase packings.
9. The use of reversed-phase as a tool for physio-chemical measurements (ionization constants, complex formation constants, hydrophobicity and purity determinations, study of structure-activity relationships for pharmaceutical compounds and antimetabolites).

The most widely used bonded phases in HPLC are nonpolar in nature, with an alkyl chain (e.g. octadecyl) bonded through the silicon atom of the alkylsilane. Today columns with octadecylsilyl bonded-phase are probably used more than any other type. However, these may be eventually replaced by bonded-phase columns which have a shorter, more flexible alkyl chain. Such a material is C-8 or RP-8 containing octylsilyl groups. Table 2 shows the structure of the various bonded-phase types. These are prepared by the reaction of the surface Si-OH groups of the support particles with various reagents.
With its high surface population of hydroxyl groups, silica gel provides an excellent medium onto which various substances can be bonded using substituted silylating agents to form a stable, non-polar stationary phase. Because of steric effects, not all of the hydroxyl groups of the silica gel are derivatized by the silylating agents, so the remainder are then reacted with trimethylsilyl chloride in a process called end-capping to reduce adsorption effect. Bonded phases are advantageous in that they can be made reproducibly from batch to batch and the surface does not change during the chromatography. They have the disadvantage being only effective over the pH range within which the backbone of silica gel is stable, usually pH 2 to 7.

Table 2. Structures of Bonded-phases

ORIGINAL GROUPS ON THE SUPPORT PARTICLES	FINAL BONDED-PHASE GROUPS
	
	
	

Generally a silica support is used to which a stationary phase of a long-chain alkane is bonded for reversed phase. It was predicted that greater efficiency could be obtained with support of smaller diameter. Much effort has been devoted to the developement of columns packed with smaller particles of support, from the previous pellicular or porous type, approximately in the size range of 10 to 50 um, to the high performance columns packed with 10 um or even finer (3-5 um) particles, and then to the recent microbore columns. The term " microbore " HPLC is generally used to refer to the packed stainless steel colums of between 0.5 and 2.0 mm i.d. bore. Packed microbore columns represent only one of three general miniaturized column types currently in use. The other two are packed capillary columns, with diameters on the order of 50 to 200 um, such as those developed by Novotny et. al., and the true open tubular columns, on the order of 10 to 30 um in i.d. as pioneered by Ishii, Tsuda et. al. This miniaturized HPLC is still very much at the specialist or developement stage and should not be considered for general routine separation. Many practical difficulties arise from the ability to pack very narrow columns and

as extra-column band broadening in the injector, column connections and detector can cause a severe loss in separation efficiency. This degradation in performance can be very severe if microbore columns are used with a standard liquid chromatograph. Columns of this type require highly specialized equipment which is not widely available commercially. For most practical purposes, taking into account the use of packing, ability to perform rapid analyses and compatibility with commercially available apparatus, packings with diameters in the range 5-10 um probably represent a realistic comprise. At present, the majority of the columns which are commercially available range in length from 5 to 30 cm and in diamter 2.0 to 4.6 mm. If the value is placed on the efficiency of separation more than the speed of separation, then a 25 cm column with 10 um particle size is still suitable for the anlysis of most pharmaceuticals.

In 1975 Wahlund (58) applied the ion-pair technique to the RPLC and since then the application of ion-pair RPLC has continued to grow. This technique has become widely adopted and has been comprehensively reviewed (57).

The mobile phase is altered by adding an ion-pairing reagent, which dissociates to give ions opposite in charge to those of the solutes, for the retention of the ionized solute in reverse phase material which is bonded onto the silica gel solid support. The ion-pair formation results from the attraction between the positively and negative charged ions to form an overall neutral species. The sample ion, S^{+n} or S^{-n}, plus the counter-ion part of the ion-pair reagent, C^{-n} or C^{+n}, combine to form an ion pair (S--C) which has sufficient affinity for the reverse phase to retain. As illustrated in Figure 1 the counter ion combines with the sample ion in solution and participates in the chromatographic process. Once the pair is formed, it will act like an organic molecule and not like an ionic compound at all. That is why that ion-pairing chromatography is the technique of choice for compounds which remain stable throughout the PH range (2 to 7) where silica is stable. It can be used as well for the samples containing both ionic and nonionic compounds. Desired retention time of ionized compound can be obtained by controlling the alkyl length of these counter ions. The counter ions are often sulfonic acid salts or quaternary amines. Some counter ions and their applications are also shown in Figure 1.

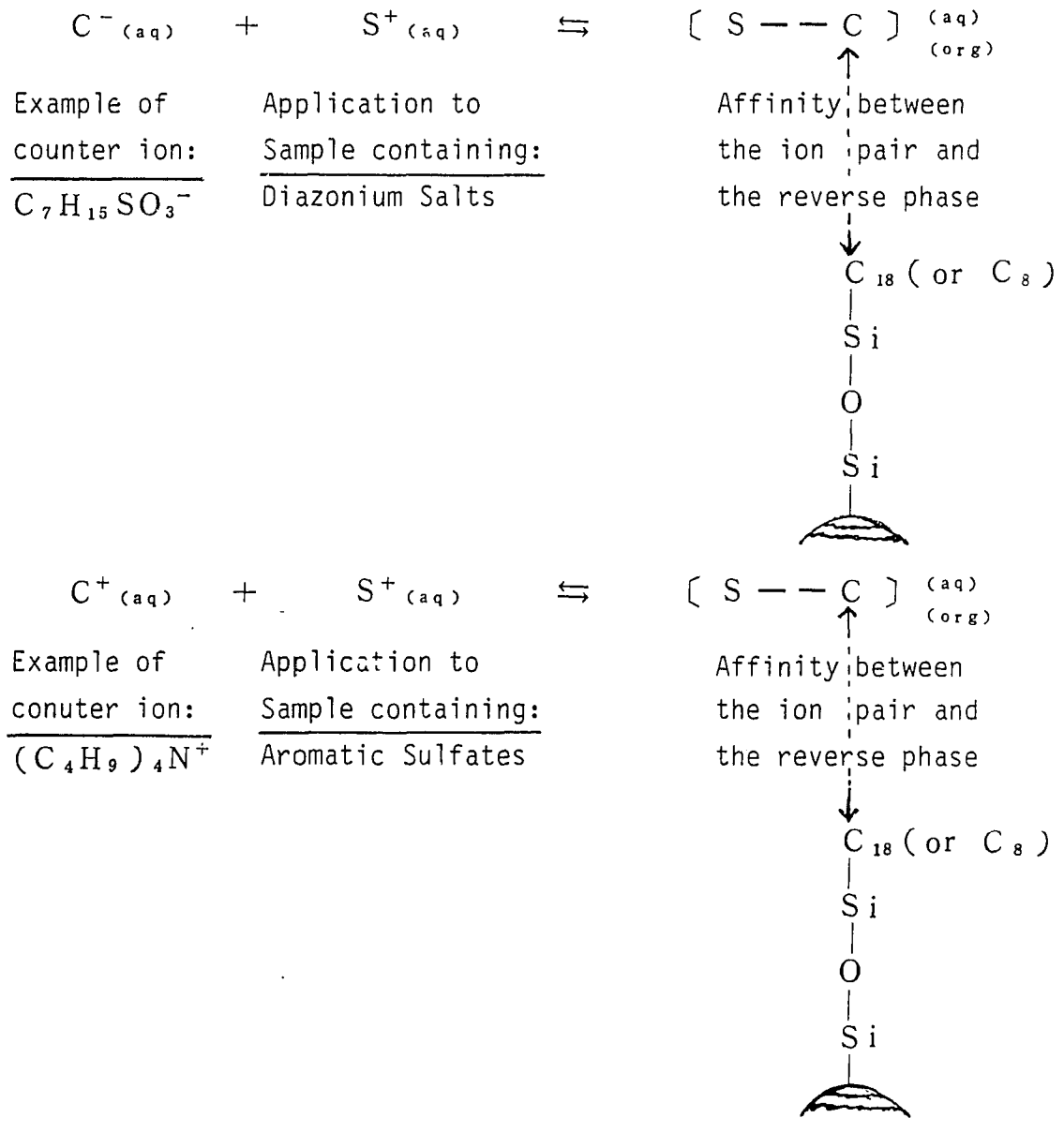


Figure 1. Ion-Pairing for RPLC

The extensive improvement in both instrumentation and columns (59) have made HPLC a technique with operational simplicity, high efficiency, column stability, and the ability to analyze simultaneously a broad spectrum of both closely related and widely different compounds.

D. HPLC SYSTEM

A typical HPLC system is composed of five modules, each with its own function. Figure 2 shows the schematic of basic modules. Sample solution is introduced into the system via an injector or a sampling valve (A) from which it is forced by a flowing stream of mobile phase through a short 1/16" o.d. stainless steel tube to the column (B). The column is typically 25cm long x 4.6mm id. It contains the stationary phase where the sample mixture separates into various zones of components as the mobile phase is forced through the column and elutes the component zones out of the column. The effluent then passes through the detector (C). The signal is fed into the recording device (D). A high

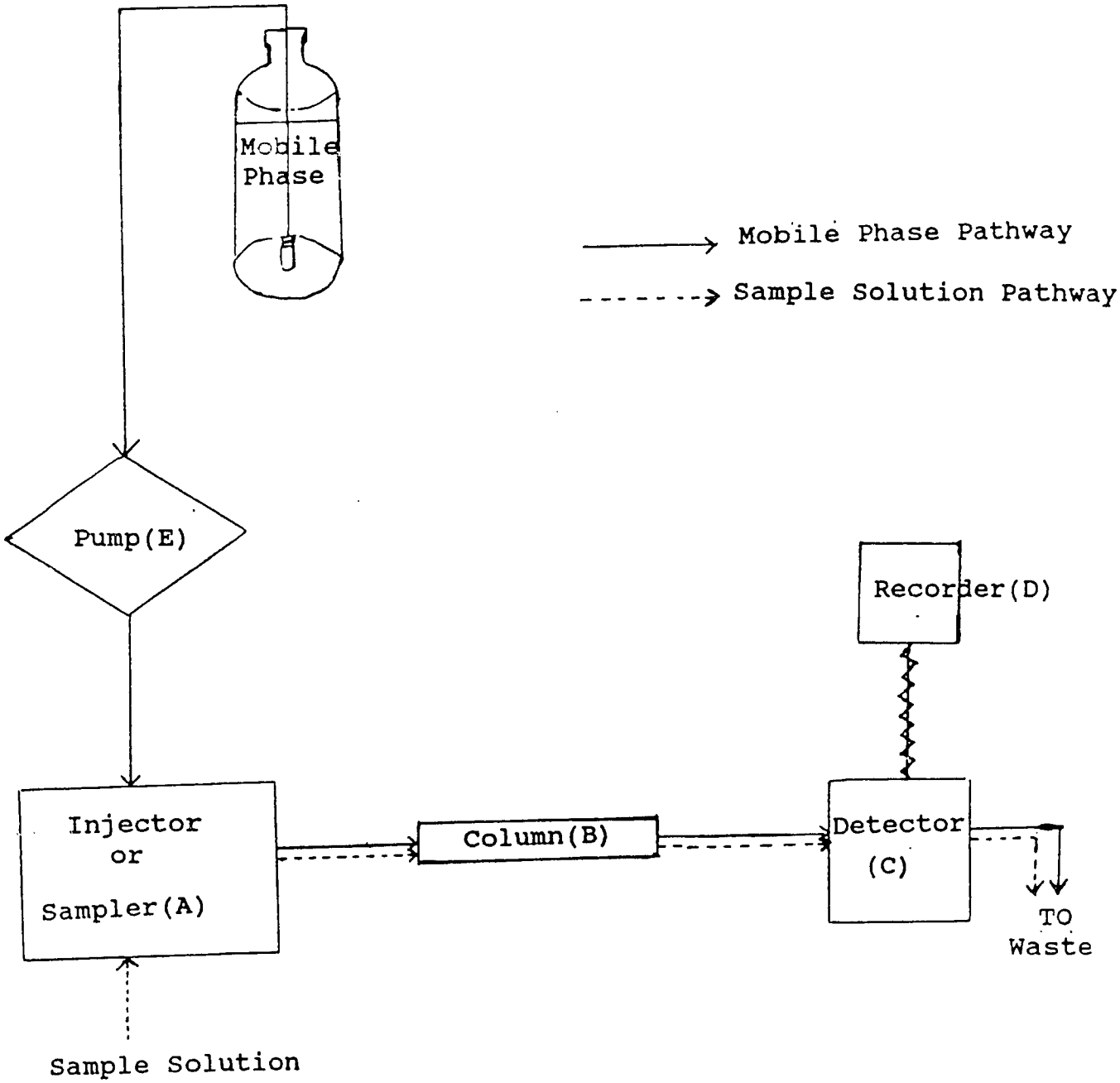


FIGURE 2. Schematic of Basic HPLC Modules

pressure pump (E) is needed for delivering solvent from an outside reservoir through the system at a constant and precise flow rate. From 1973 to 1987 McNair has published a series of papers (60 - 67) that update and describe the commercially available HPLC equipment.

In most HPLC columns, silica particle diameter is 10 um or less, and as a result, columns are packed tightly and develop high back pressures, approximate 2000psi, which necessitate pumping the mobile phase through the column. The mobile phase for RPLC is generally aqueous methanol or acetonitrile. For diphenhydramine Hydrochloride analysis, the ion-pairing reagent, sodium hexanesulfonate, is added to the mobile phase to give the optimum retention of diphenhydramine in the reverse phase column.

III. EXPERIMENTAL SECTION

A. Materials and Chemical

Diphenhydramine hydrochloride Reference standard, USP grade, was purchased from USP, Rockville, MD. OTC drugs " Benadryl Elixir " ("L" drug) containing 12.5mg of diphenhydramine hydrochloride per 5mL; and " Sominex Tablets " ("S" drug) containing 25mg of diphenhydramine hydrochloride per each tablet, were purchased from Shop-Rite supermaket, Springfield, NJ.
Hexanesulfonic Acid Sodium Salt was purchased from Eastman Kodak, Rochester, NY. Acetonitrile, B&J Chrompure (G.C. purity = 99.9%+) was purchased from B&J, Albany, OR. Glacial Acetic Acid, ACS reagent, was purchased from Fischer Scientific Products, Pittsburgh, PA.

B. Instumentation

1. UV-Vis Spectrophotometer. A Hewlett-Packard (HP) model 8450 UV-Vis spectrophotometer is a microprocessor-controlled, photodiode array instrument providing a spectrum over its full 200nm to 800nm wavelength range in one second (68,69). It uses advanced optical and electronic means to achieve fully processed analytical

results in 1/100 of the time needed by mechanically scanned instruments. A block diagram of the HP8450 is shown in Figure 3.

The optical system of the HP8450 shown in Figure 4 uses deuterium and tungsten-halogen common-axis radiation sources, a servo-controlled beam director instead of chopper, a holographic grating and a pair of photodiode arrays for simultaneous detection at all wavelengths. This instrument can be connected to many kinds of peripheral devices through its interface ports. A HP model 7470A plotter was interfaced to it to record spectra. This setup was not used for the quantitation of the active substance in drug products, but it was used for the determination of the UV absorption maximum wavelength(s) by measuring the spectrum of a solution of the compound. Based on this, the UV detection wavelength for the HPLC quantitation and two wavelengths for the ratioplot (see the description in III.F) were selected (70).

The UV spectrum of diphenhydramine hydrochloride (Figure 5) shows a maxima absorbance at 258nm which was selected as the HPLC detection wavelength (operating wavelength). Neighboring wavelengths (252 or 264nm), that show some absorbance, along with the operating wavelength (258nm) were used in the ratio plot (71).

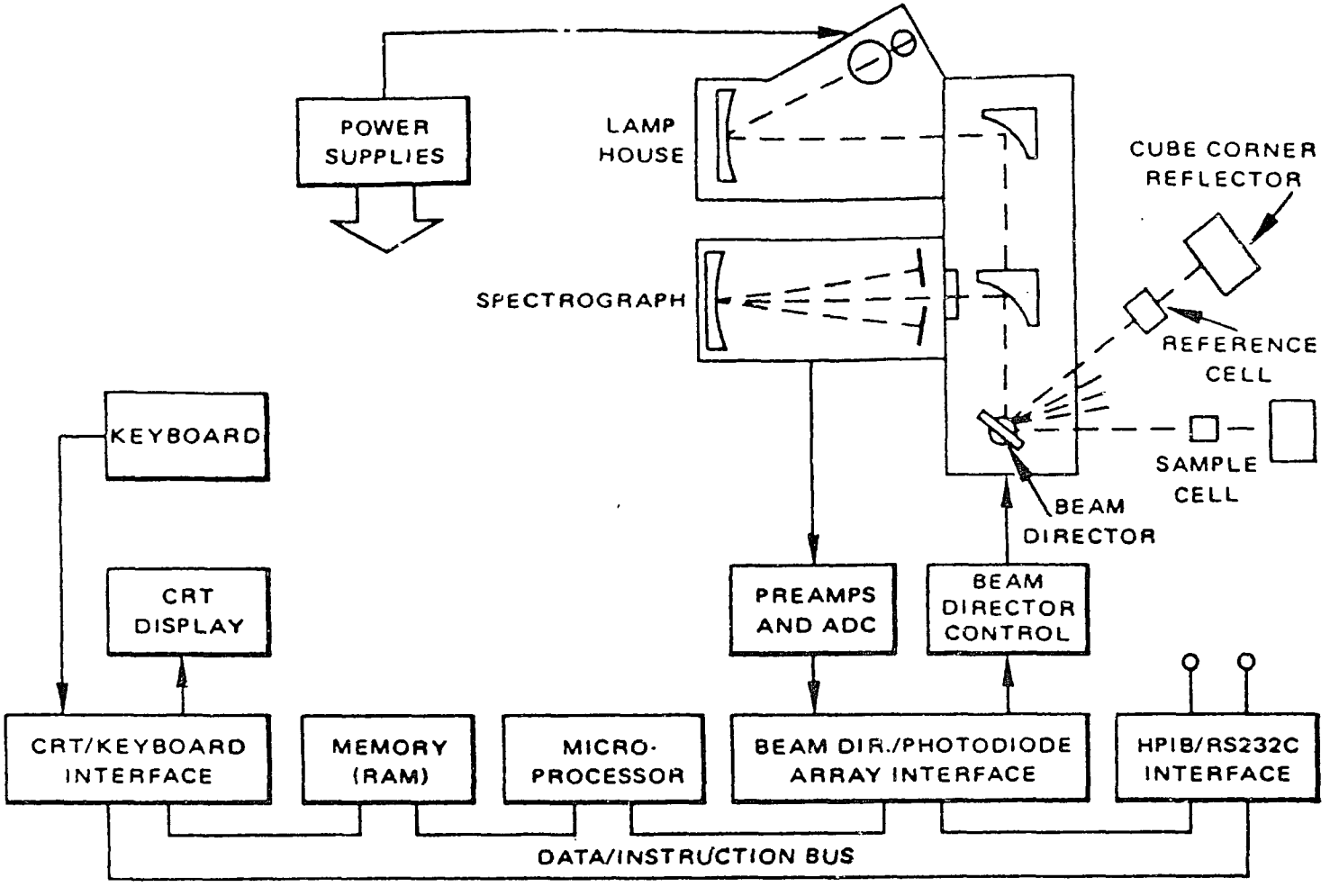


Figure 3. Block Diagram of HP8450

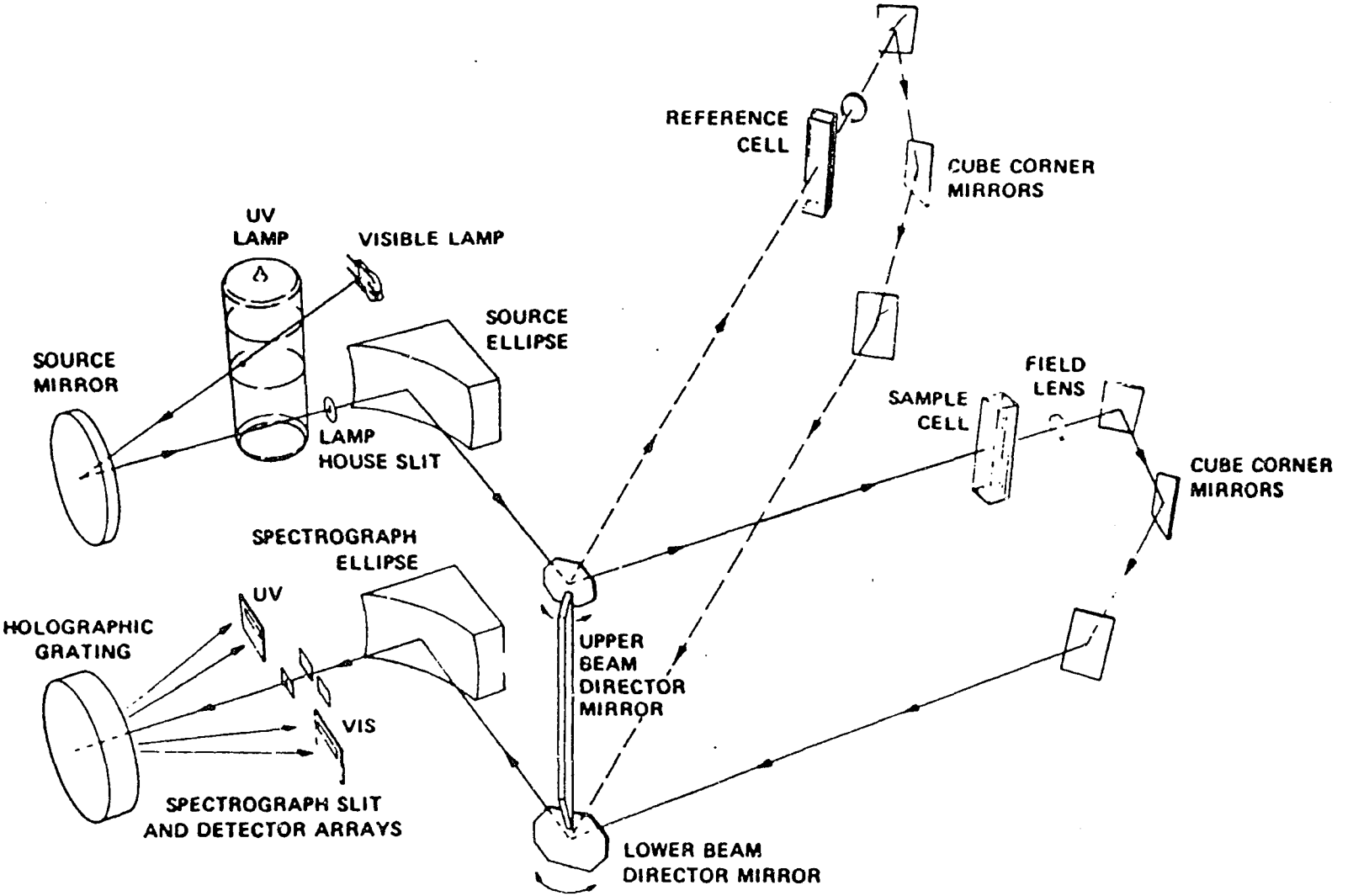


Figure 4. Optical System of HP8450

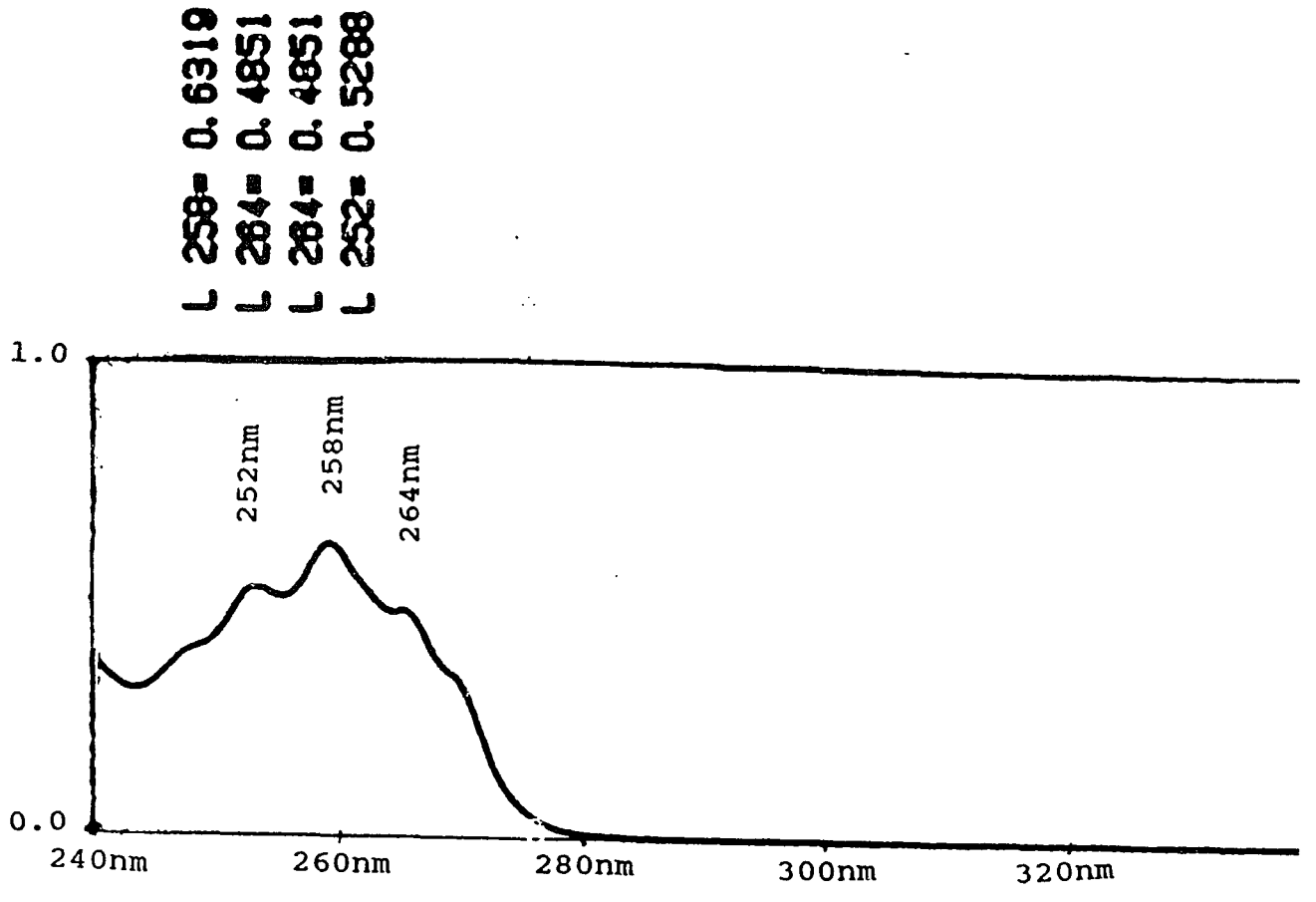
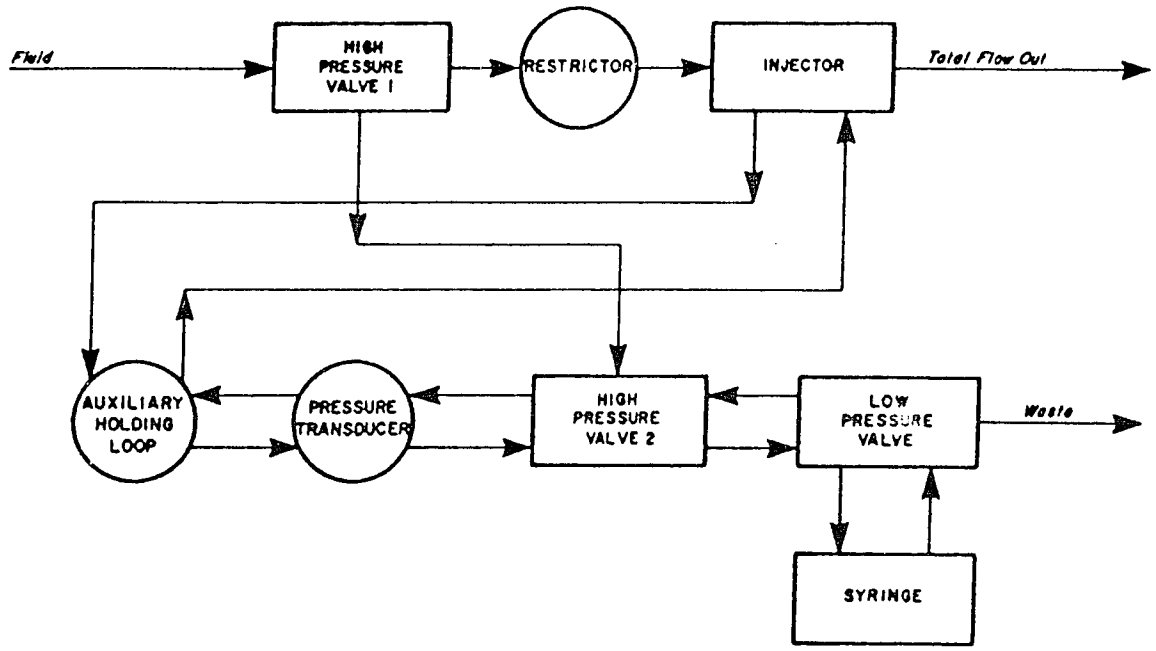


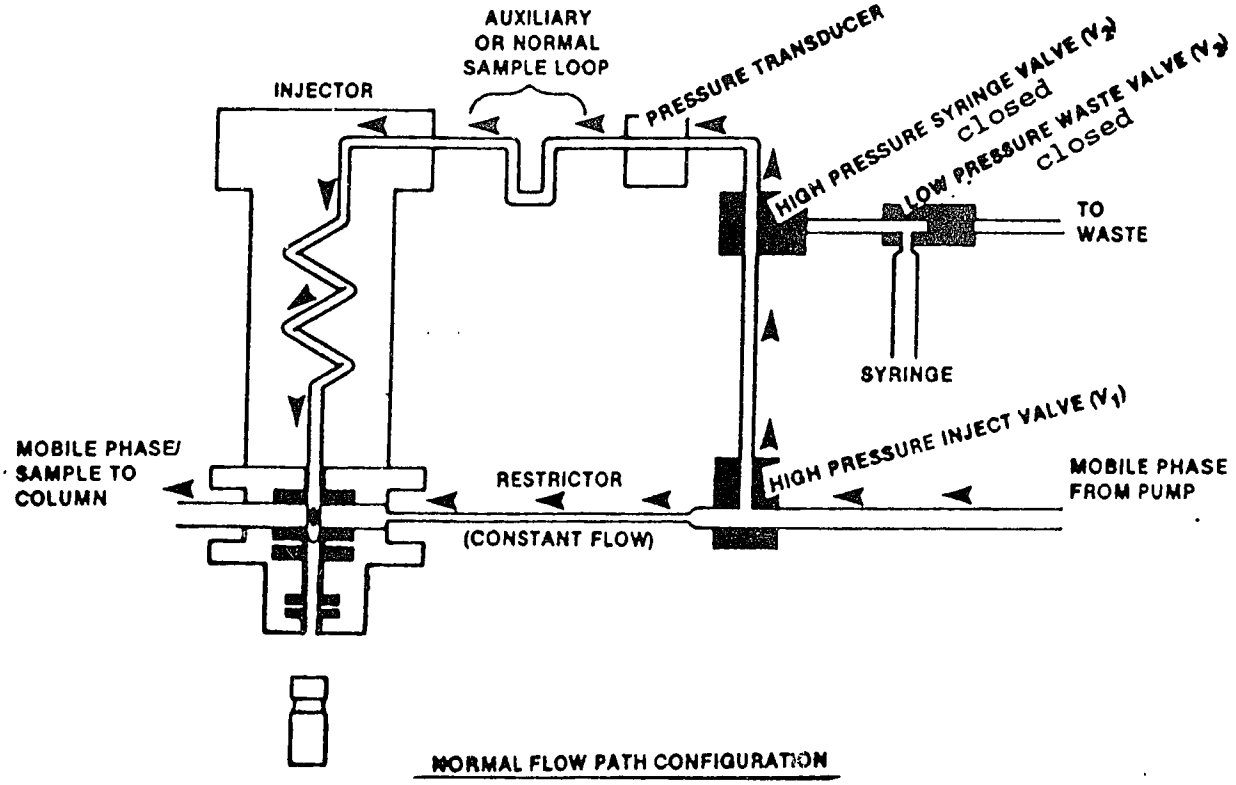
Figure 5. UV Spectrum of Diphenhydramine Hydrochloride

2. HPLC. The HPLC setup consisted of a Waters model 710B intelligent sample processor (WISP) as an autoinjector, Waters 6000A solvent delivery pump, and a Hewlett-Packard 3390A recording integrator (HP 3390A). A Waters model 490 multiple channel variable wavelength detector was used because of the higher detection sensitivity of diphenhydramine hydrochloride at 258nm than 254nm. The multiple channel variable wavelength detector can also perform the ratioplot (see III.F) that is often useful for detecting a hidden chromatographic peak underneath the peak of interest. This is important for the verification of the specificity of a stability method (72, 73). Descriptions and functions of these HPLC components are given below.

The Waters Intelligent Sample Processor (WISP) is an automatic sample injection module. Measured aliquots (1 to 2000uL) can be programmed for injection onto a column while maintaining a continuous flowing mobile phase. The WISP can be operated to perform single injections or programmed for up to 48 vials set on a carousel for the unattended operation combining various samples and standards. Fifteen uL was the amount used for the injections of diphenhydramine hydrchloride standard and sample solutions. Figure 6 shows the fluid system diagram and normal flow path configuration of WISP 710B.



FLUID SYSTEM BLOCK DIAGRAM



NORMAL FLOW PATH CONFIGURATION

Figure 6. Diagram of WISP 710B

The normal flow state is the passive state set initially by the system (reset). After any function (e.g. injection) is complete the WISP always returns to the "reset" configuration and normal flow state. At the normal flow state only the mobile phase is flowing into the column and at the sample injection state both the mobile phase and the sample solution are passed to the column. The injection cycle is a preprogrammed route to remove the sample aliquot and introduce it into the mobile phase flowing to the column. Between the normal flow and the injection stages there is a sample withdrawal stage. Figure 7 shows the sample withdrawal and the sample injection configurations.

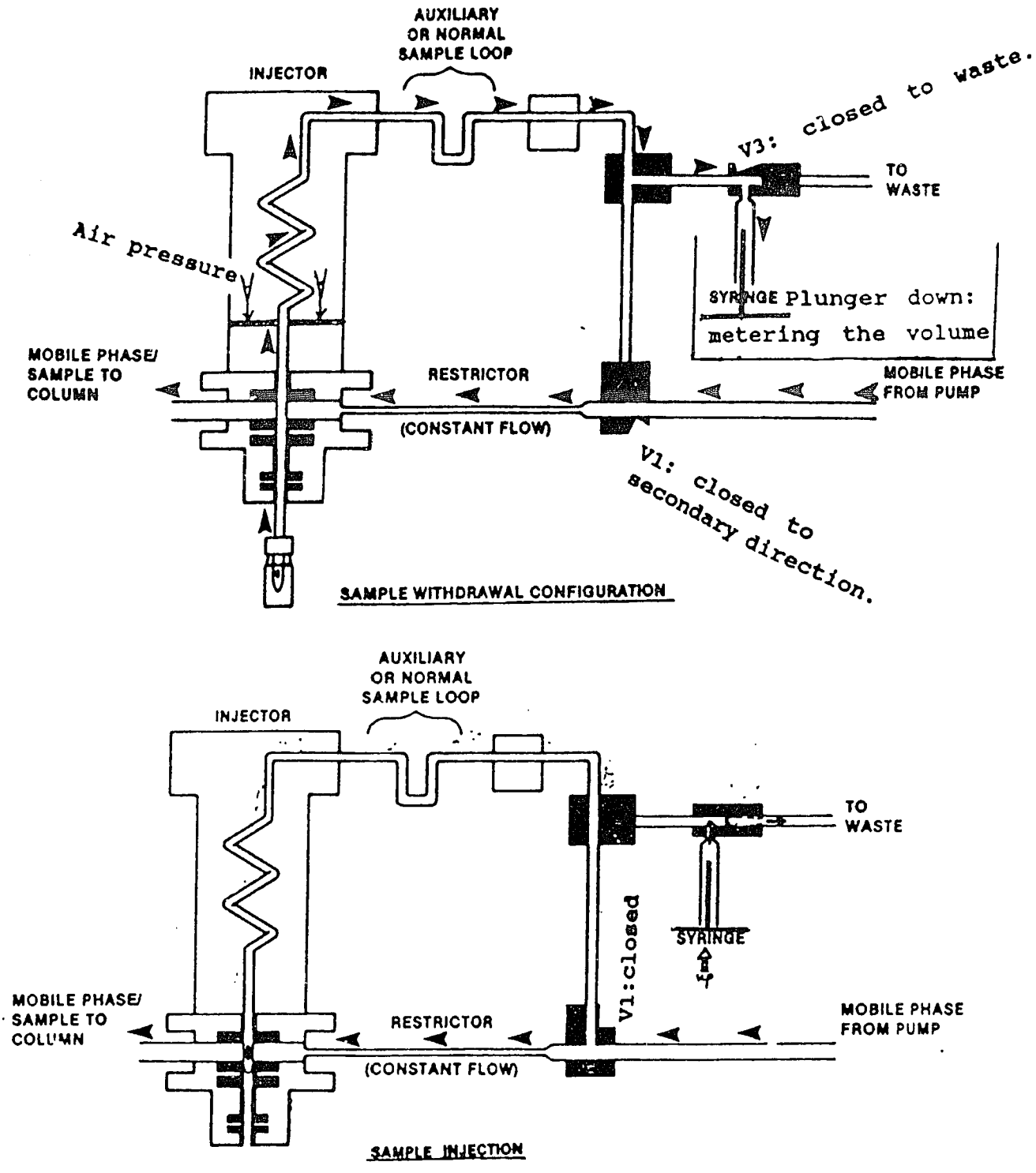


Figure 7. Flow path of sample withdrawal/injection

Constant flow of the solvent is achieved with a Waters model 6000A solvent delivery system. This pump uses a pair of specially-driven positive-displacement pumping chambers as shown in Figure 8. Flow rate is manually selectable in 0.1 mL/min increments from 0.1 to 9.9 mL/min at a working pressure up to 6000psig. In each pumping chamber there is pump head cavity in which a small, accurately machined plunger slides back and forth. The two pistons are driven such that their reciprocating directions are opposing and result in one plunger drawing in solvent while the other is expelling solvent. In this way the pumping chambers alternately supply the solvent. Figure 9 illustrates how the flow from each pumping chamber may be combined to obtain a steady composite flow. This steady flow is achieved by complex piston displacements. Noncircular gears are utilized to drive the pistons so that their forward motion in expelling solvent takes more time than their reverse motion in drawing solvent. The forward motion of the right piston accelerates as it starts in interval "A". During this time the flow rate is increasing. During interval "B" the flow rates are constant, with all the flow provided by the right piston. The chamber of the left pump head fills during this period. In interval "C", the forward motion of the right piston

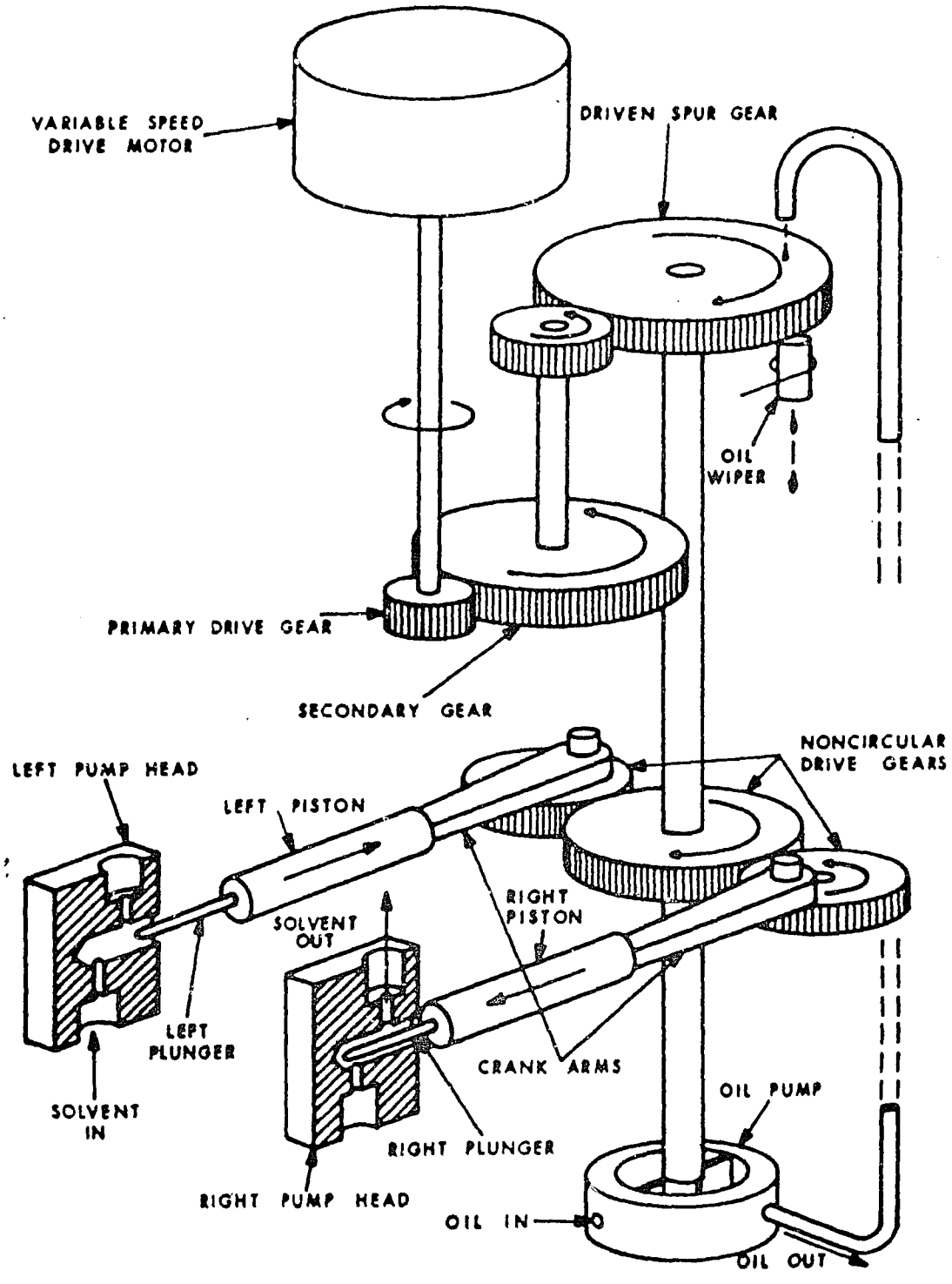


Figure 8. Pump Unit Mechanical Drive

decelerates and the left piston begins to accelerates in its forward motion. The decreasing flow of the right pump head adds to the increasing flow of the left pump head to produce a combined delivery with a steady flow throughout the entire pumping cycle as shown in Figure 9.

The Waters 490 programmable multiwavelength UV-Vis detector monitors four analog output channels simultaneously. Each analog output channel may be connected to an external plotter or integrator. This detector can thus monitor a peak at two (or more) different wavelengths and plot the ratio of the absorbances at the two wavelengths. This RATIO PLOT is performed in real time as the sample passes through the analytical cell and can be used to determine the purity of a sample (70 - 73). The analytical cell employs a refractive index correction for detection of true absorbance. Comparison of a conventional HPLC UV flow cell and this detector's analytical cell is shown in Figure 10. The refractive index change in the solvent when a peak elutes causes light rays to bend within the cell. In the conventional cell the light then strikes the stainless steel cell wall and does not reach the detector. The Waters 490 employs a tapered cell which eliminates the refractive index effect and gives true absorbance

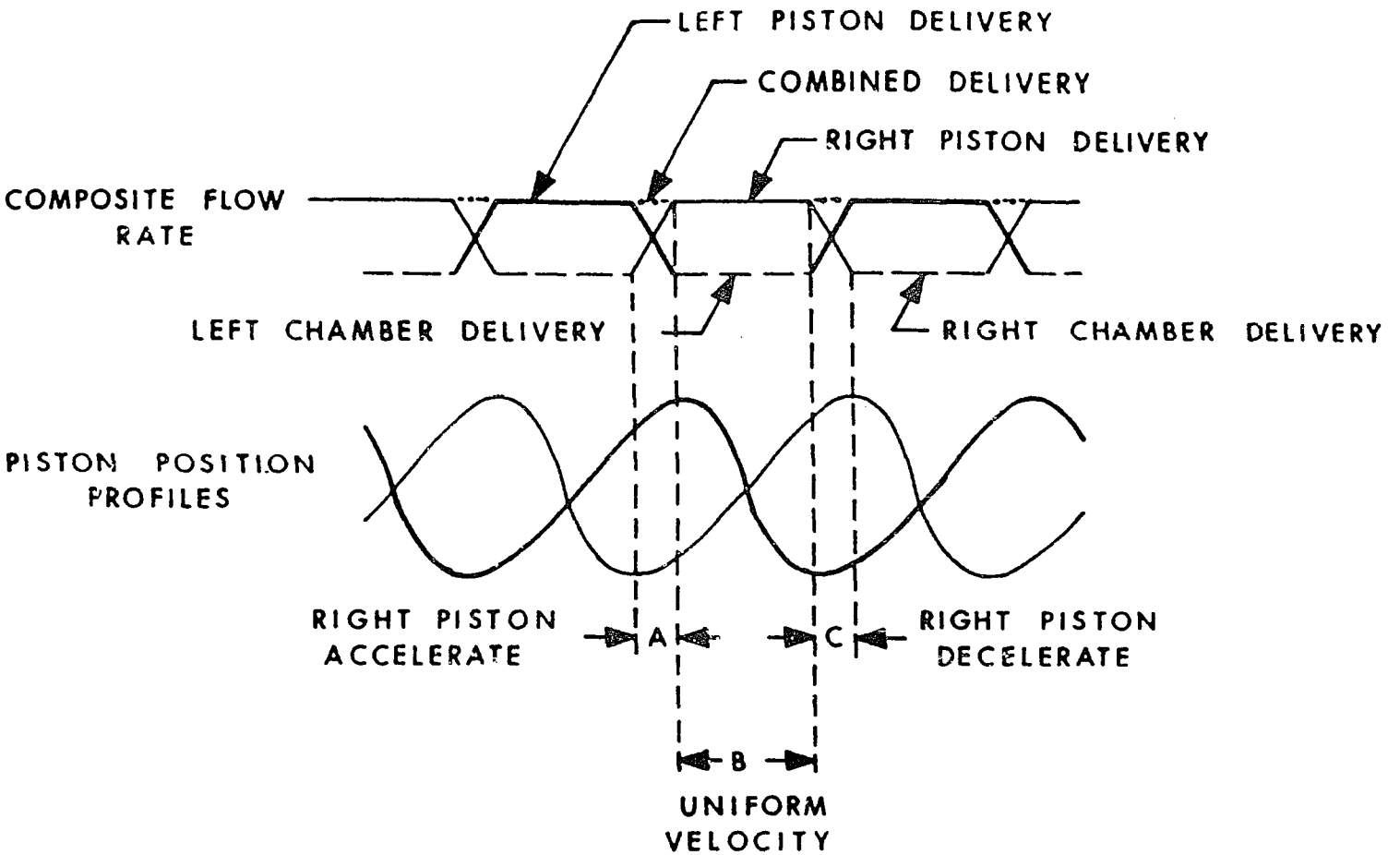


Figure 9. Pump 6000A and Its Combined Delivery

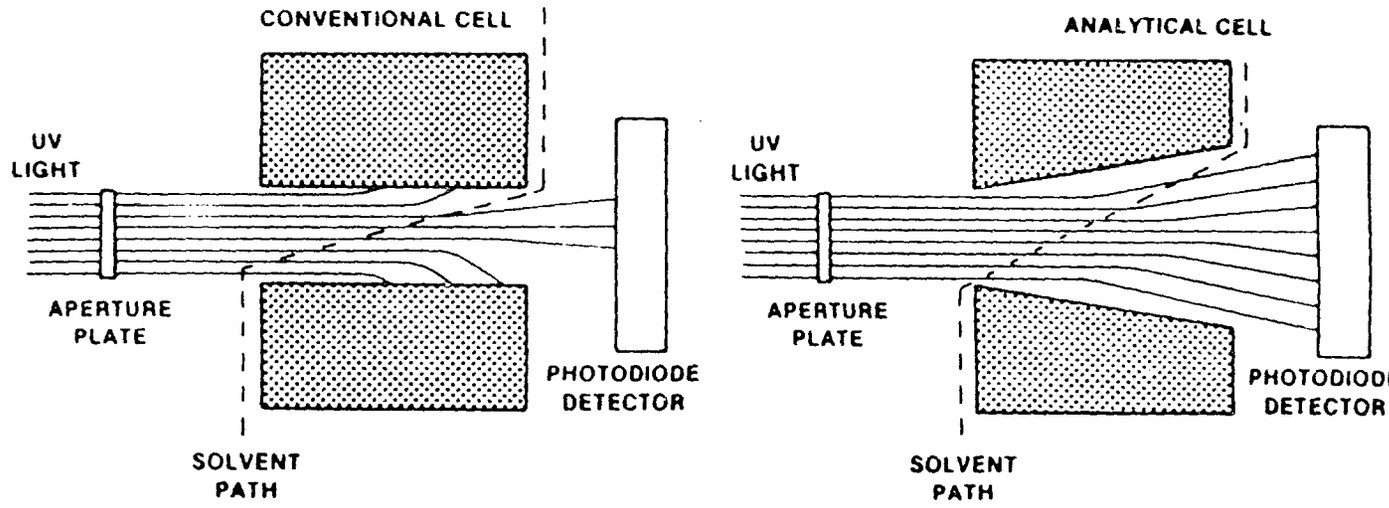


Figure10. Comparison of conventional and analytical cells

readings. The cell design ensures that all the light entering the cell will leave it if no true absorbance takes place. The cell wall diverges to a greater extent than the most excessive case of light refraction. This permits more light to reach the photodiode, thus increasing the sensitivity of the detector. Figure 11 illustrates the path of the light beam as it passes through the components in the optics system. The light is divided into two identical beams by a beam splitter, and sent through the sample and reference sides of the analytical cell. Two photodiodes then convert the light into an electrical signal that can be recorded by the recording device.

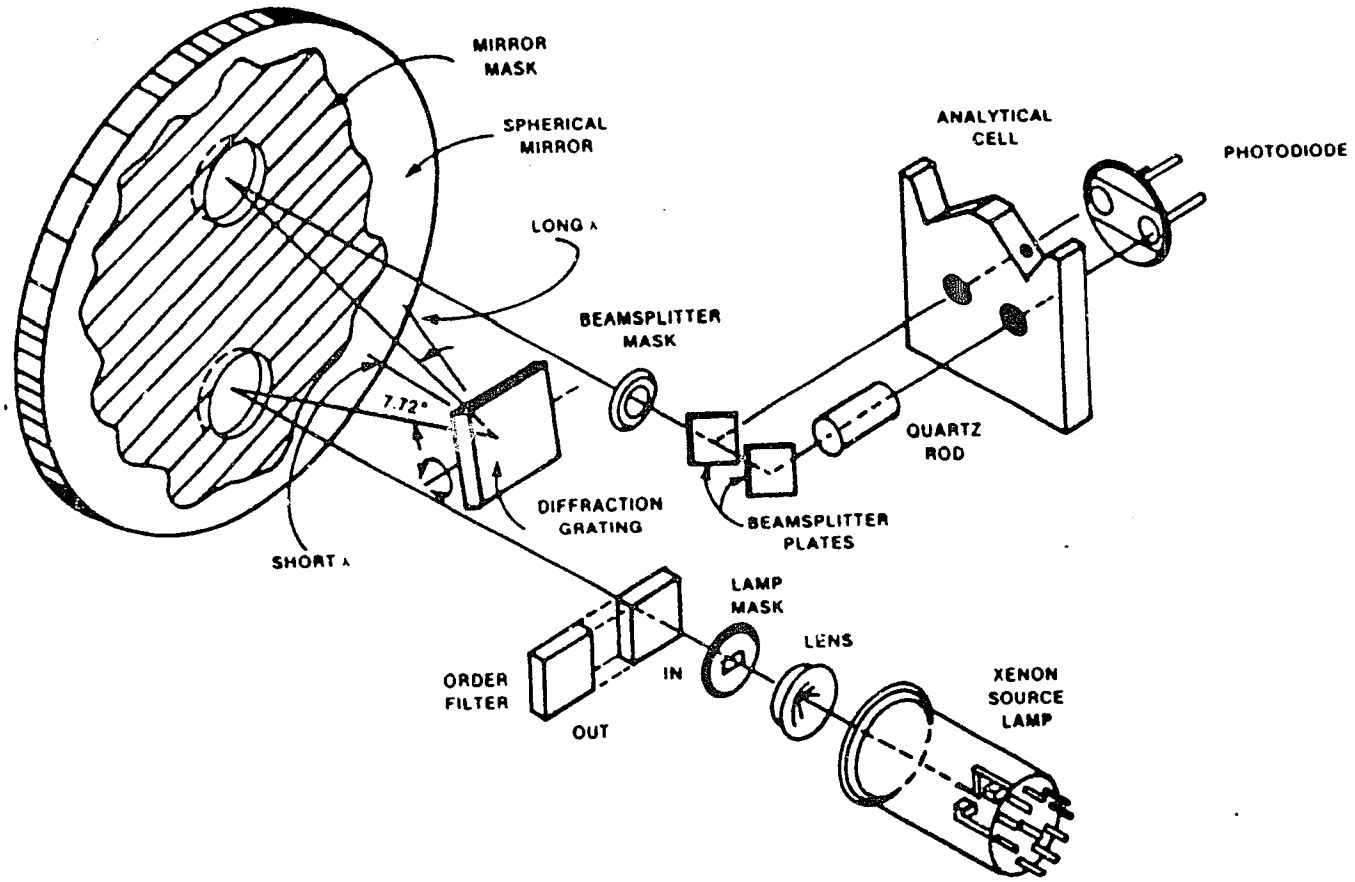


Figure 11. **Optical System of Detector 490**

The HP 3390A reporting integrator was used to plot the chromatograms and integrate the peaks. It consists of a single main PC board, keyboard, printer/plotter and an optional external I/O board. The main board contains five functional sections: Power Supply, CPU-Memory, A/D converter, I/O Interrupt-Keyboard, and Printer/Plotter control. The input is entered through the keyboard, chromatographic input is fed directly to the A/D converter, and the plots, reports are produced via the plotter/printer. Its block diagram is shown in Figure 12.

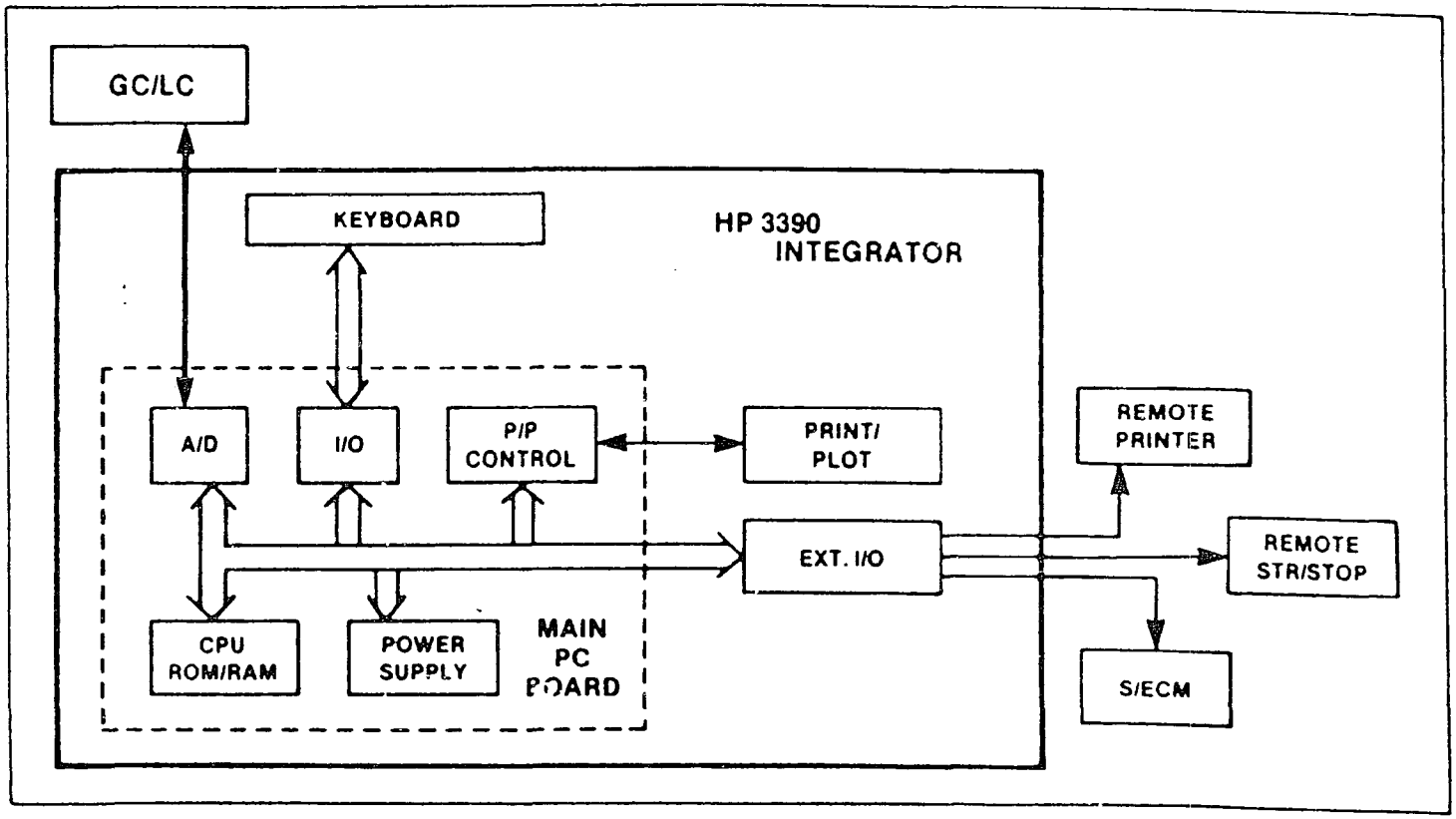


Figure 12. Integrator/Plotter HP 3390A block diagram

C. HPLC parameters:

The column was 250mm x 4.6mm i.d. packed with Lichrosorb RP8, 10 micron; purchased from Alltech. The composition of the mobile phase was acetonitrile /distilled water/acetic Acid (70/30/1, v/v), with 0.005M hexanesulfonic acid sodium salt.
The flow rate was 1.8mL/min. The injection volume was 15uL. The detection wavelength was 258nm.
The diluent (simulated mobile phase) was acetonitrile/ distilled water/acetic Acid (70/30/, v/v).
Both the mobile phase and diluent were degassed in vacuo and filtered through a 0.45 micron filter.

D. Sample Preparation and Quantitation Procedures:

Compound of interest must be completely dissolved and free of particulate matter before injection. Diphenhydramine hydrochloride is readily soluble in the diluent containing 30% water. For the preparation of standard solution, approximately 50mg of USP reference standard was accurately weighed into a 500mL volumetric flask and diluted with the diluent to volume.
The concentration of diphenhydramine hydrochloride in solution was 0.1 mg/mL.

The liquid drug sample lends itself well to analysis by HPLC because of the active ingredient is already in solution. Exactly 2.0mL of "L" sample was pipetted into a 50mL volumetric flask and simply diluted with diluent to volume. The expected active concentration was approximately 0.1mg/mL.

The solid dosage form has to be ground into a fine powder for the complete extraction of the active compound into the diluent, but it should not be overground due to the possible segregation effect. During grinding, the particulate solids, once mixed, have a tency to segregate because the mixing of particles whose surfaces are nonconducting often results in the generation of surface charges, as evidenced by a tendency of the power to clump following a period of agitation. Unfortunately, these surface charges of particles during grinding are not measurable. This effect will cause a nonuniform sample composite. An experiment to determine the optimum grinding time by comparing the precision of two grindings with two different length of grinding time was carried out. It shows overgrinding is not beneficial. Twenty-four tablets of drug "S" were weighed to obtain the average tablet weight (A.T.W.), and then put into a grinder. The grinder was pulsed on for 20 seconds. After the third 20 second pulse, half of the

ground powder was taken as " Composite I " and the other half remaining in the grinder was ground further with two more pulses. The second half was taken as " Composite II ". Seven preparations and HPLC injections of each composite were carried out. Comparison of the results of I and II, 3-20 second pulses and 5-20 second pulses, are shown in Table 3. It demonstrates that overgrinding of these tablets is not preferable. "Composite II" yields a larger relative standard deviation than "Composite I" but improves the recovery very little. Therefore the 3-20 second pulses' grinding is better and " composite I " was used in this study. About 90mg of the solid drug composite powder, which is equivalent to approximately 1/5 of one " S " tablet weight, was weighed accurately and diluted with the diluent to volume in a 50mL volumetric flask. Again the expected concentration of the active component was around 0.1mg/mL. All the prepared solutions were mixed well, filtered, and injected. The total amount of diphenhydramine hydrochloride per injection was approximately 1.5ug. The dihpenhydramine hydrochloride peak was recorded as shown in Figure 13 - the typical chromatograms of standard, sample "L" and sample "S".

TABLE 3. GRINDING EXPERIMENT FOR "S" TABLETS

WEIGHT OF 24 TABLETS = 10.94317 g

A.T.W. (AVERAGE TABLET WEIGHT) = 0.456 g/tablet

COMPOSITE I (20 Sec. X 3 pulses)			COMPOSITE II (20 Sec. X 5 pulses)		
WEIGHT (g)	RESPONSE (mv)	RATIO (mv/g)	WEIGHT (g)	RESPONSE (mv)	RATIO (mv/g)
0.09203 g	$2.480X10^5$	$2.705X10^6$	0.09148 g	$2.500X10^5$	$2.732X10^6$
0.09213 g	$2.520X10^5$	$2.735X10^6$	0.09150 g	$2.576X10^5$	$2.815X10^6$
0.09217 g	$2.519X10^5$	$2.734X10^6$	0.09171 g	$2.485X10^5$	$2.725X10^6$
0.09168 g	$2.477X10^5$	$2.702X10^6$	0.09190 g	$2.513X10^5$	$2.735X10^6$
0.09103 g	$2.474X10^5$	$2.718X10^6$	0.09218 g	$2.462X10^5$	$2.662X10^6$
0.09164 g	$2.484X10^5$	$2.711X10^6$	0.09184 g	$2.452X10^5$	$2.670X10^6$
0.09204 g	$2.489X10^5$	$2.704X10^6$	0.09283 g	$2.486X10^5$	$2.678X10^6$
	MEAN =	$2.715X10^6$		MEAN =	$2.717X10^6$
	S.D. =	$1.37 X10^4$		S.D. =	5.33 [illegible]
	R.S.D. =	0.56 %		R.S.D. =	2.1 %

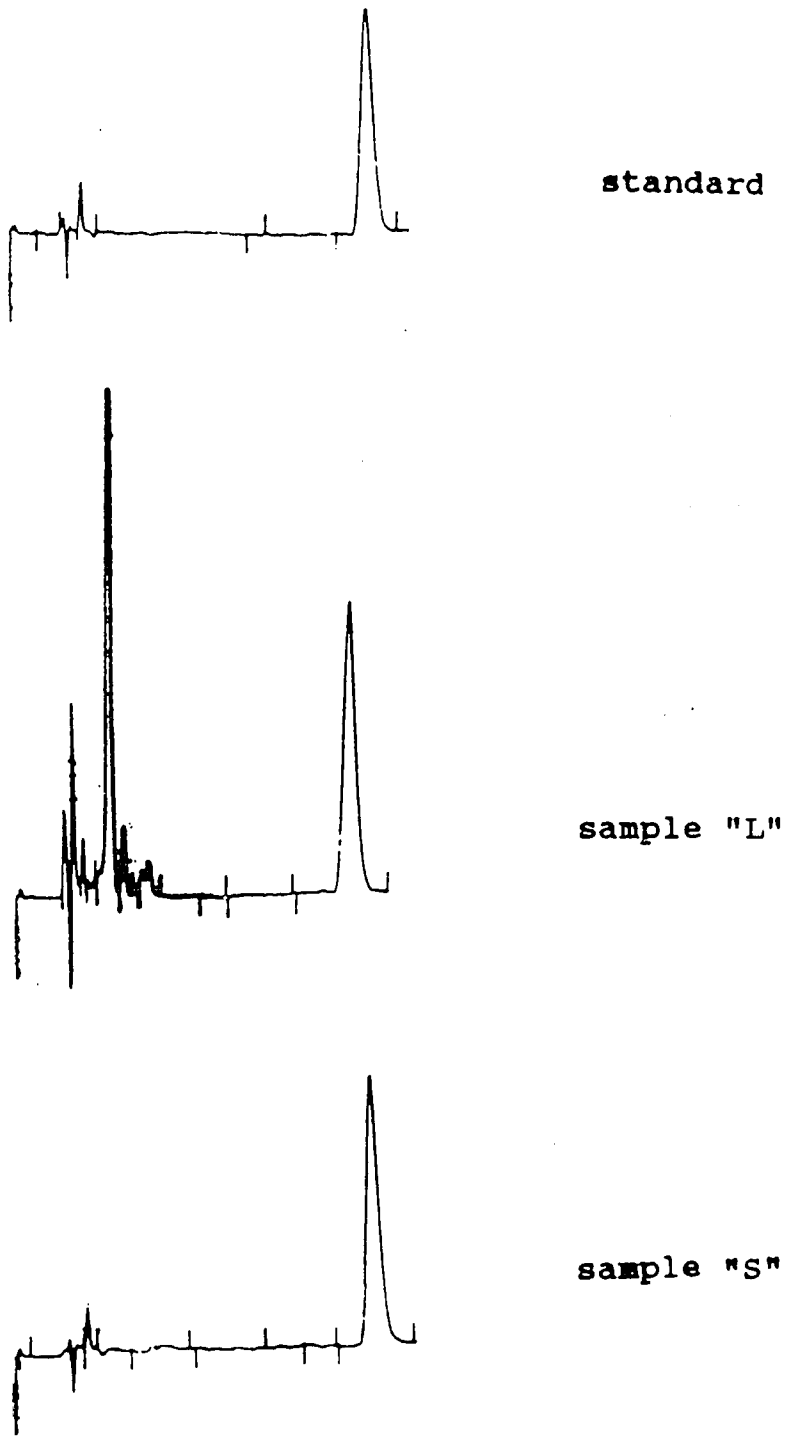


Figure 13. The typical chromatograms

E. Calculations:

The weight (in mg) of diphenhydramine hydrochloride in 5mL of "L" is calculated from:

$$\frac{\text{Peak Area of "L" x Conc. of Std. x 50 x 5}}{\text{Peak area of standard x 2}}$$

The weight (in mg) of diphenhydramine hydrochloride in one tablet of "S" is calculated from:

$$\frac{\text{Peak Area of "S" x conc. of Std. x 50 x A.T.W.}}{\text{Peak Area of Std. x "S" weight}}$$

F. Ratioplot:

Since the HPLC method is to be used for stability testing, it is important to verify the peak purity. The interferences from degradation products and/or from other sources can often be minimized or eliminated by using the ratioplot method (73 - 77). The Waters 490 is capable of dividing absorbance at two selected wavelengths and plotting the resulting ratios (ratiogram) on any of four channels to help detect components that might be hidden under desired peaks. Two wavelengths are used for the calculation of the ratio A(O)/A(M). A(O) is the absorbance of the compound at the operating wavelength (i.e. detection wavelength) and A(M) is the absorbance of the same at the master wavelength which is the neighboring wavelength showing some absorbance. A quick way of determining the operating and master wavelengths using detector 490 was suggested by Waters. The general picture of this kind of scan as shown in Figure 14 is not as accurate as a regular UV spectrum. In this study the UV-Vis specrtrophotometer instead of detector 490 was used for this purpose. The actual operating wavelength and two master wavelengths of diphenhydramine hydrochloride determined by the UV spectrum (Figure 5) was described in III.B.1.

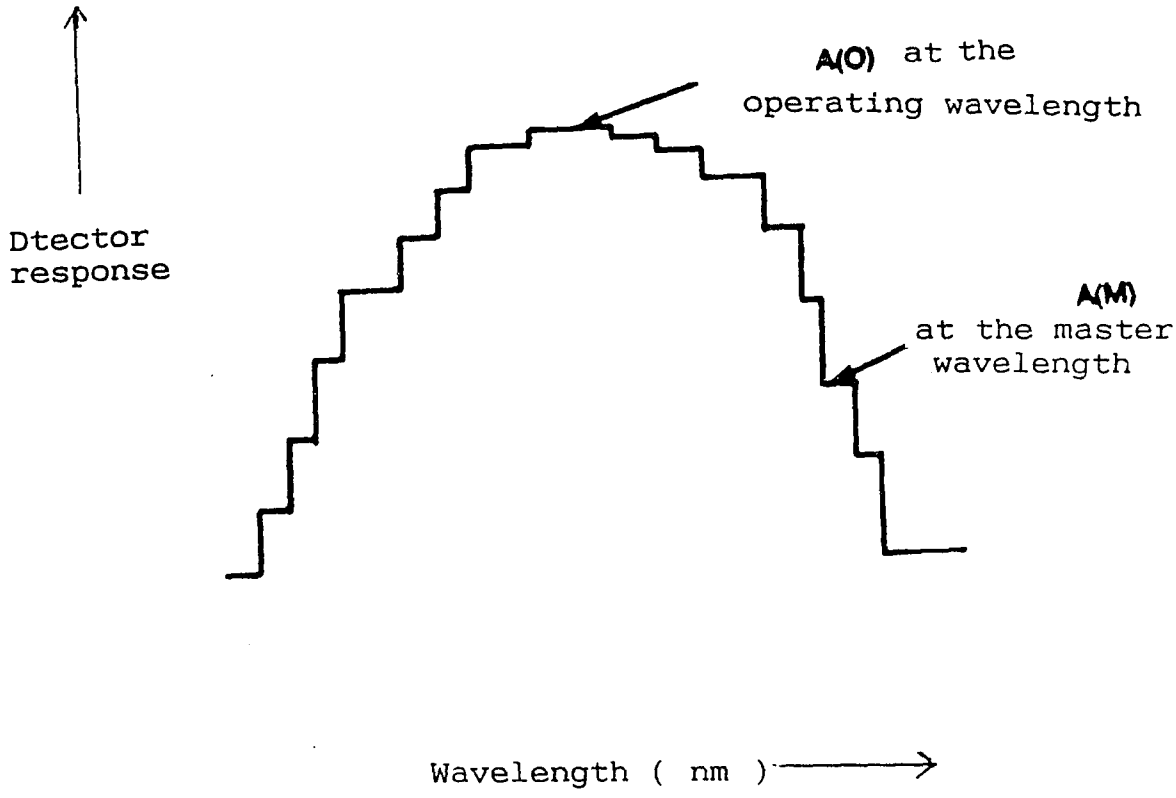


Figure 14. Scan by Waters Detector 490

Figure 15 illustrates the ratioplot method for pure vs. impure peaks. The absorbance ratio of a pure compound should remain constant in the same mobile phase regardless the amount injected. Pure peak yields a "square wave", because the ratio of two absorbances at any point of the same component peak remains constant, while the impurity coeluting with the peak of interest distorts the square top. The ratio is calculated as absorbance of a compound at the operating wavelength divided by the one at the master wavelength. It is better to check more than one ratioplot to increase the chance of detecting hidden peak underneath the desired peak. Based on the UV spectrum of diphenhydramine hydrochloride (Figure 5), two master wavelengths 252nm and 264nm can be used. This means two ratioplots of A(258nm)/A(252nm) and A(258nm)/A(264nm) can be checked for diphenhydramine peak generated each time.

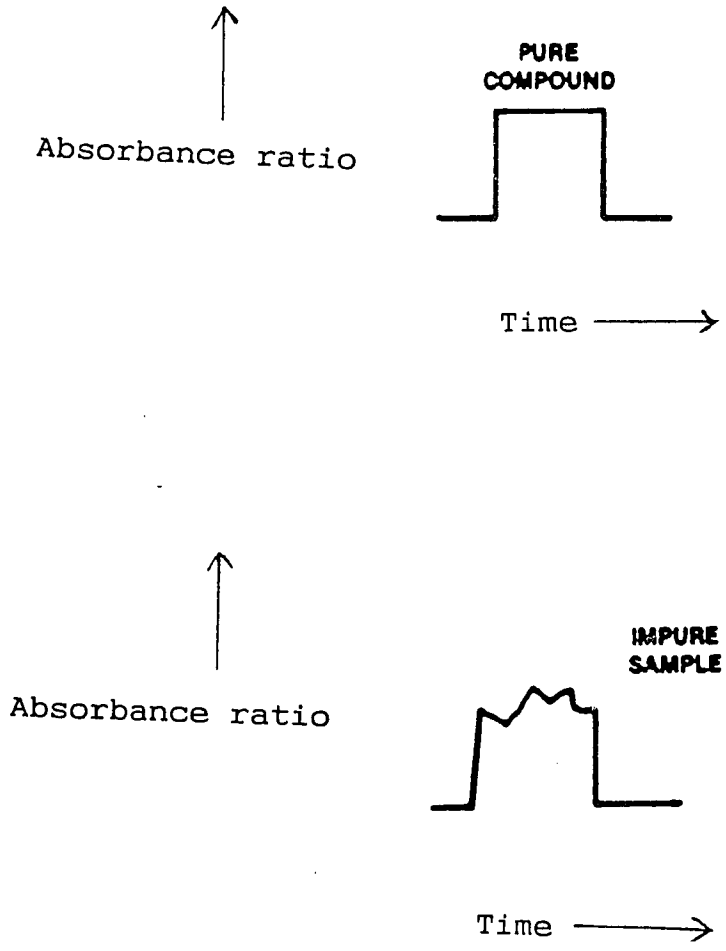


Figure 15. Ratio Plot

IV. RESULTS

A. Typical Chromatograms and Measurements:

The typical chromatograms of standard and two samples are shown in Figure 13. The retention time of diphenhydramine hydrochloride is about 7.5 min., and well separated from other peaks. The major one of Other peak(s) present in the "L" sample chromatogram is pseudoephrine peak which is the other active of "L" drug. It is not the peak of interest in this study. Chromatogram of "S" which is a single active drug does not show any other major peak as in "L" chromatogram. The blips in the chromatograms are from the intergration. The tailing factor (T) and the capacity factor (k') were determined as shown in the followings and Figure 16 (78).

$$T_{0.1} = W_{bc} / W_{ab} = 7mm/5mm = 1.4$$

where:

W_{ab} = peak width from the leading edge to the peak midpoint at 10% full peak height

W_{bc} = peak width from the peak midpoint to the tailing edge at 10% full peak height

T is greater than 1.0. It means the peak is not totally symmetrical and suggests peak area quantitation will be

more suitable than peak height quantitation. This point was proved by the study in III.C., and the integrated peak area was used in the quantitation of diphenhydramine hydrochloride for this whole study.

The change in the water to organic solvent ratio changes the capacity factor k'. The change in k' can tell the HPLC parameter change of the aqueous mobile phase. The HPLC conditions can be controlled more pricisely by checking k' from one run to another. k' of the diphenhydramine peak was measured as shown in the followings and Figure 16.

$$k' = (t_r - t_0) / t_0 = (7.42-1.73)/1.73 = 3.29$$

where:

t_r = time from injection to peak maximum

t_0 = time from injection to the disturbance right before the solvent front (= an unretained peak)

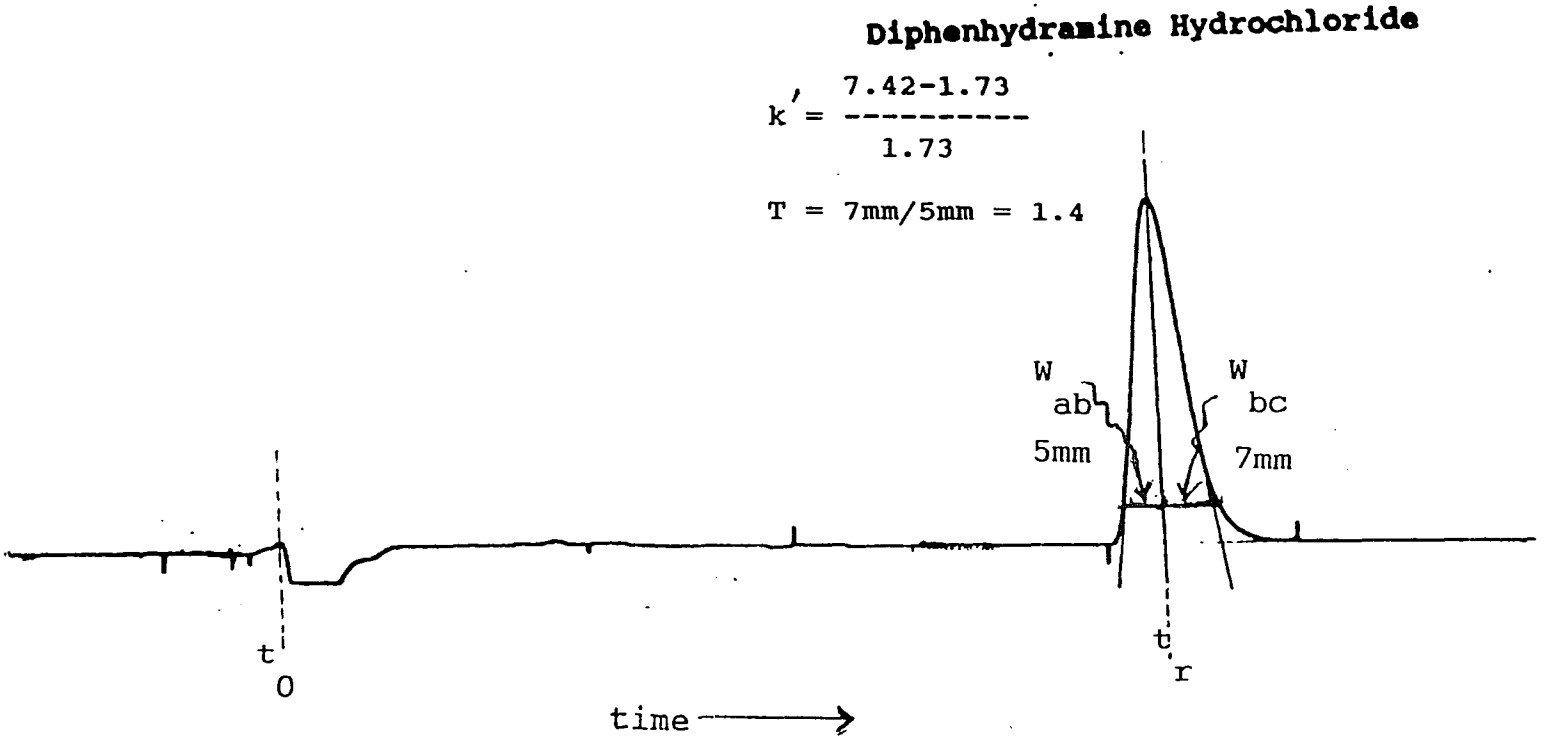


Figure 16. Measurements of k′ and T

B. Linearity

The linearity of response to diphenhydramine hydrochloride was initially measured at 254nm using the Waters fixed wavelength detector and at 258nm using the variable wavelength detector.

The results in the concentration ranges from 50 to 450ug were plotted using an equi-weighted least square fit of a computer "MPCAL" (multi-point calibration) program written by CIS (Computer Inquiry System, Inc.). The description of this program is in the APPENDIX.

The linearity plots of diphenhydramine hydrochloride with the concentration up to approximately 400ug/mL at 254nm and 258nm are shown in Figures 17 and 18. The slope at 258nm is slightly larger than that at 254nm. A UV detection wavelength of 258nm was used instead of 254nm for better sensitivity which is needed for dealing with the small concentration change in this study.

The deviation from Beer's law starting approximately 200ug/mL is also demonstrated by the dotted line in these Figures. Hence the solution concentrations ranging from 25 to 180ug/mL were reinjected and resulted in an excellent linearity as shown in Figure 19. This provides the basis for using the sample solution preparations around 0.1mg/mL (about middle of the linear range) as the working concentration.

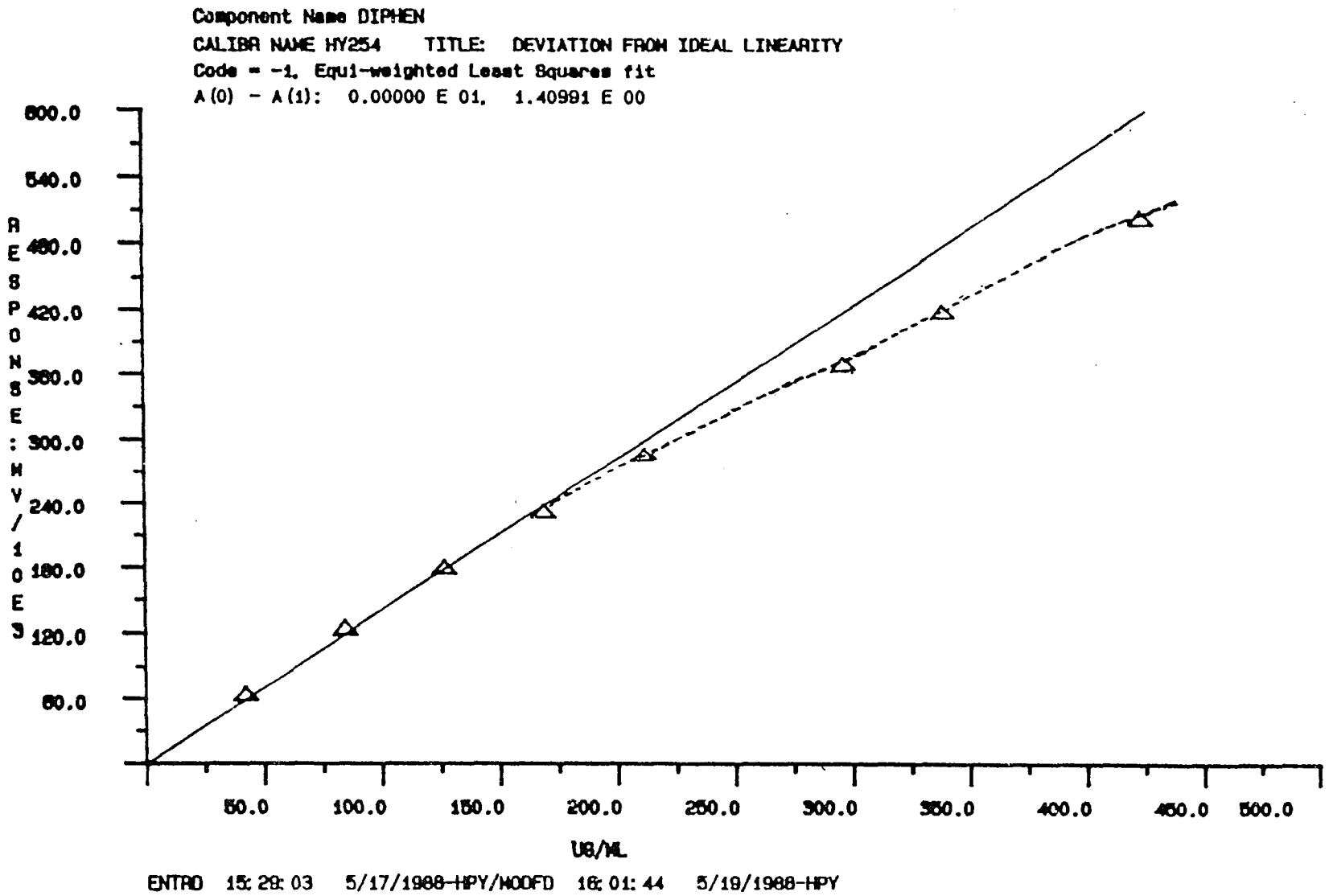


Figure 17. Deviation from Beer's Law at 254nm

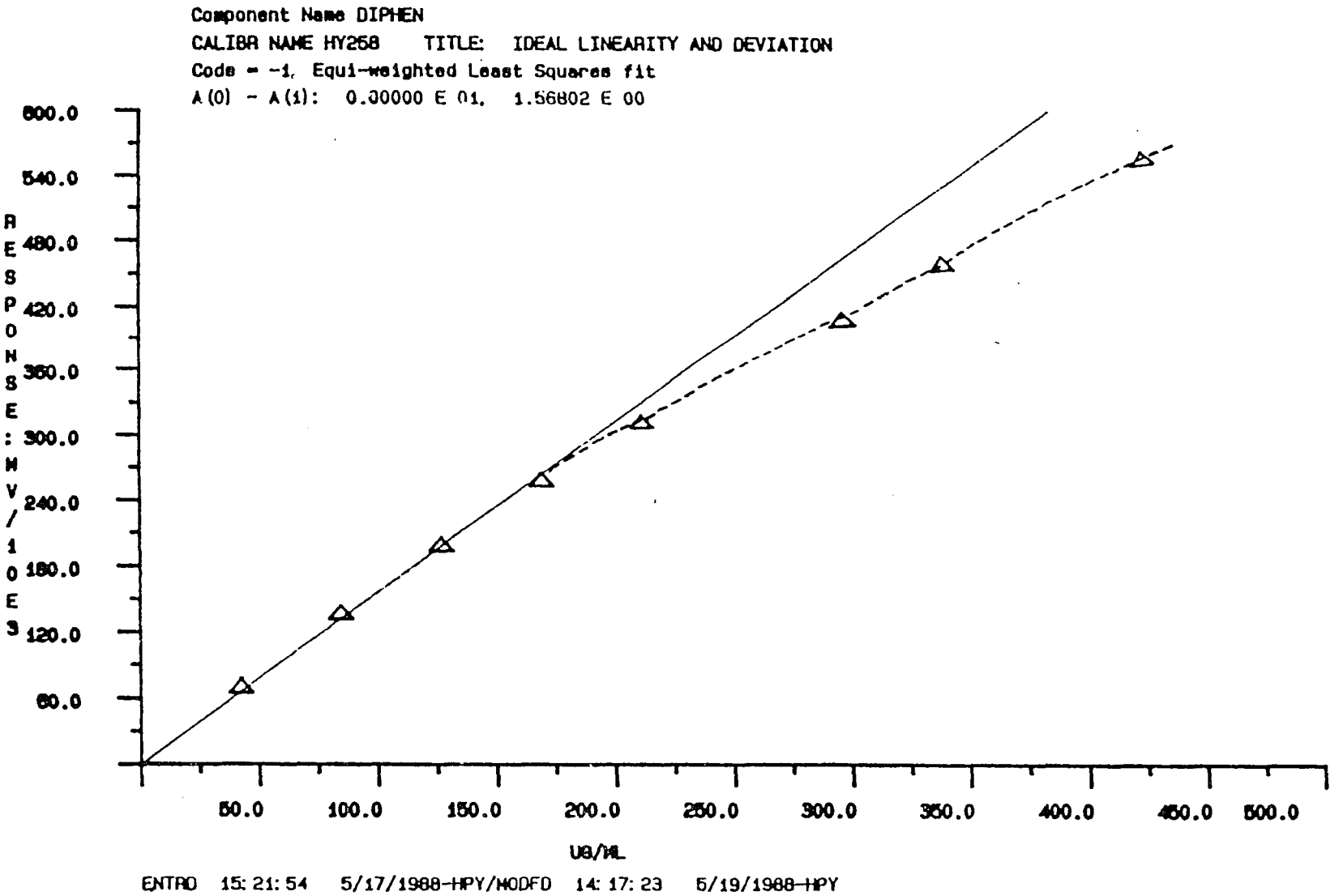


Figure 18. Deviation from Beer's Law at 258nm

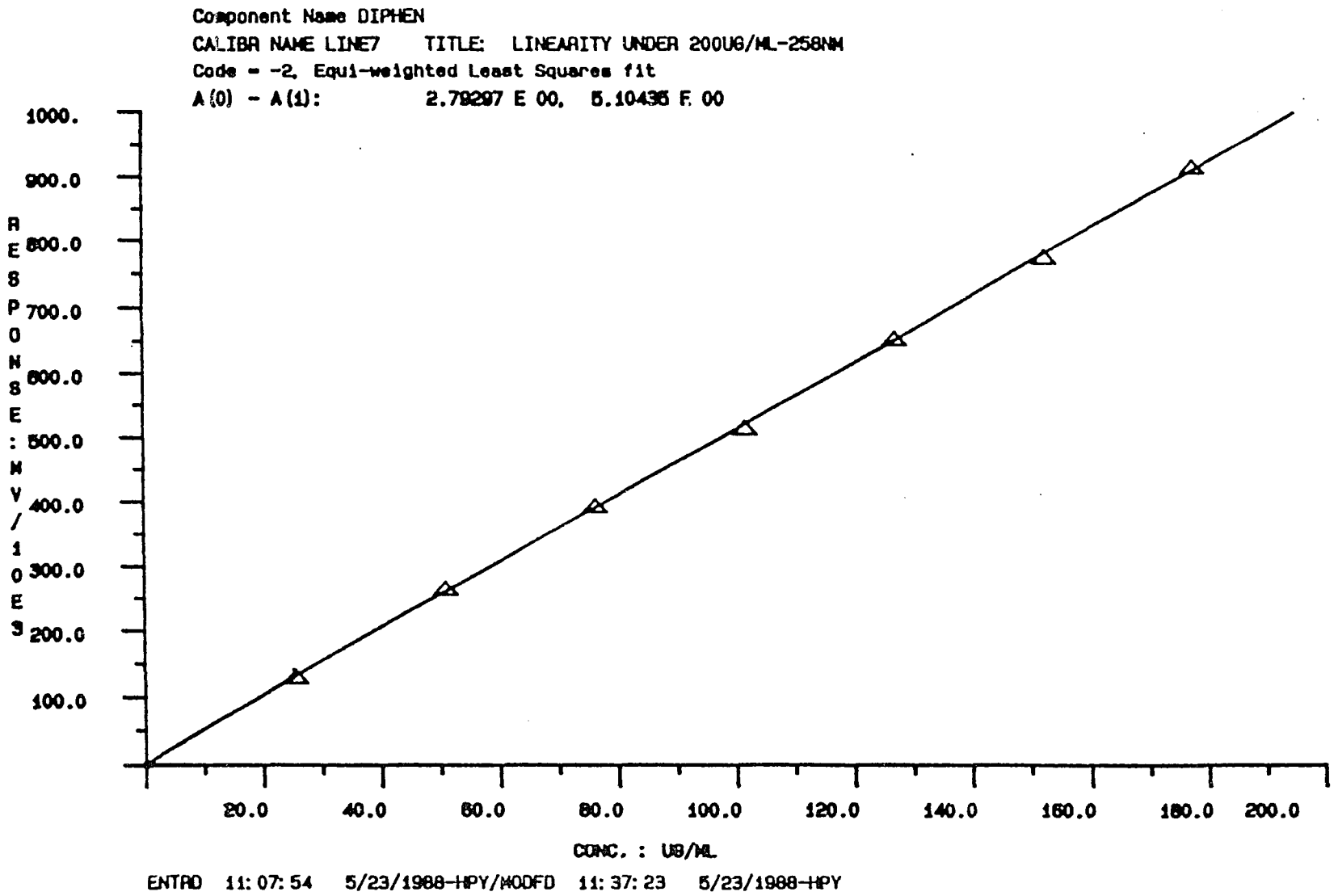


Figure 19. Linearity of Proper Concentration Range

C. Precision Study

Both the precisions of the HPLC system and for the whole method (whole procedure including HPLC system) were checked.

To determine the precision of the HPLC system, only One solution of standard, "L" sample or "S" sample was injected into the HPLC repeatedly. The resulting R.S.D.s are shown in 1. of Table 4. The values are all small and show no sinificant difference between the injections of "L" or "S" sample and standard.

To determine the method precision, seven different sample solutions ("L" or "S" sample) were prepared, and underwent the whole procedures. The resulting R.S.D.s were low, and show little difference between the runs of "L" and "S" samples as shown in 2. of Table 4.

It proves the precision of the HPLC equipment and reproducibility of the whole method are good enough for the quantitative analysis of the subject active in drugs "L" and "S".

Table 4. Results of Precision Study

1. HPLC system precision:

Sample	R.S.D., %
Standard	0.78
"L"	0.86
"S"	0.68

2. Method precision:

Sample	R.S.D., %
"L"	0.52
"S"	0.50

D. Peak Area vs. Peak Height

Peak height measurements for quantitative purposes require that the peak of interest must be symmetrical (tailing factor = 1). In this study quantitation was carried out by electronic integration of peak areas which should be better than peak height measurements, since the tailing factor calculated for the diphenhydramine peak was 1.4 (IV. A.).

An actual comparision of quantitation by peak area and peak height for this method was also studied. From six repetitive injections of a 0.1mg/mL diphenhydramine hydrochloride solution, the R.S.D.s for the peak area and peak height were 0.68% and 1.27% respectively. It proves that measurement of peak area is more precise than peak height for this analysis. Similar finding were obtained by Pauls et. al. (80) in 1986.

E. Accuracy (Recovery) Study

A recovery study is normally carried out by spiking a placebo with a known amount of reference standard. The spiked sample is prepared and quantitated according to the test method. The recovery is the amount determined divided by the known amount. In pharmaceutical laboratories the placebo is defined as a mixture of ingredients without the active(s), i.e. without the analyte of interest. The formulation chemist can prepare the placebo according to the known formulations or compositions of drug(s) (81).

However, the compositions of the drugs "L" and "S", are proprietary and are not labeled in detail. Thus for our recovery study we could only spike the drugs with an increment of USP diphenhydramine hydrochloride standard. The incremental amount was the same as the active amount in each drug and it made the further dilution easier. This sample was diluted twice as much with the HPLC diluent to make the final working concentration around 0.1mg/mL, and injected into HPLC.
The recovery was obtained by dividing the incremental amount of diphenhydramine hydrochloride determined with the known amount added to the drug samples.

If the recovery is much above 100%, there are probably interference(s) present. If the recovery is low, the sample matrix may have a binding effect on the analyte of interest (e.g. the diluent is not strong enough to extract 100% of analyte from the sample matrix). The recoveries of diphenhydramine hydrochloride in "L" and "S" were 100.1% and 99.2% respectively. They did not indicate any of the abovementioned problems.

F. Ratioplot

This technique helps sustantiate the specificity of this HPLC method and was used to check the purity of the diphenhydramine hydrochloride peak of the thermally treated samples in PART TWO. It served as a useful tool for testing interference in quantitation of the peak of interest. In stability testing there is always the possibility that interfering compound(s) might be generated by the heat treatment and might be hidden underneath the peak of interest (82).

Ratioplot was performed on each diphenhydramine peak of all the untreated and treated samples in PART TWO as well as the standard. A ratiogram for the standard is shown in Figure 20. No distoration of the square top was noted for any of the samples in this study.

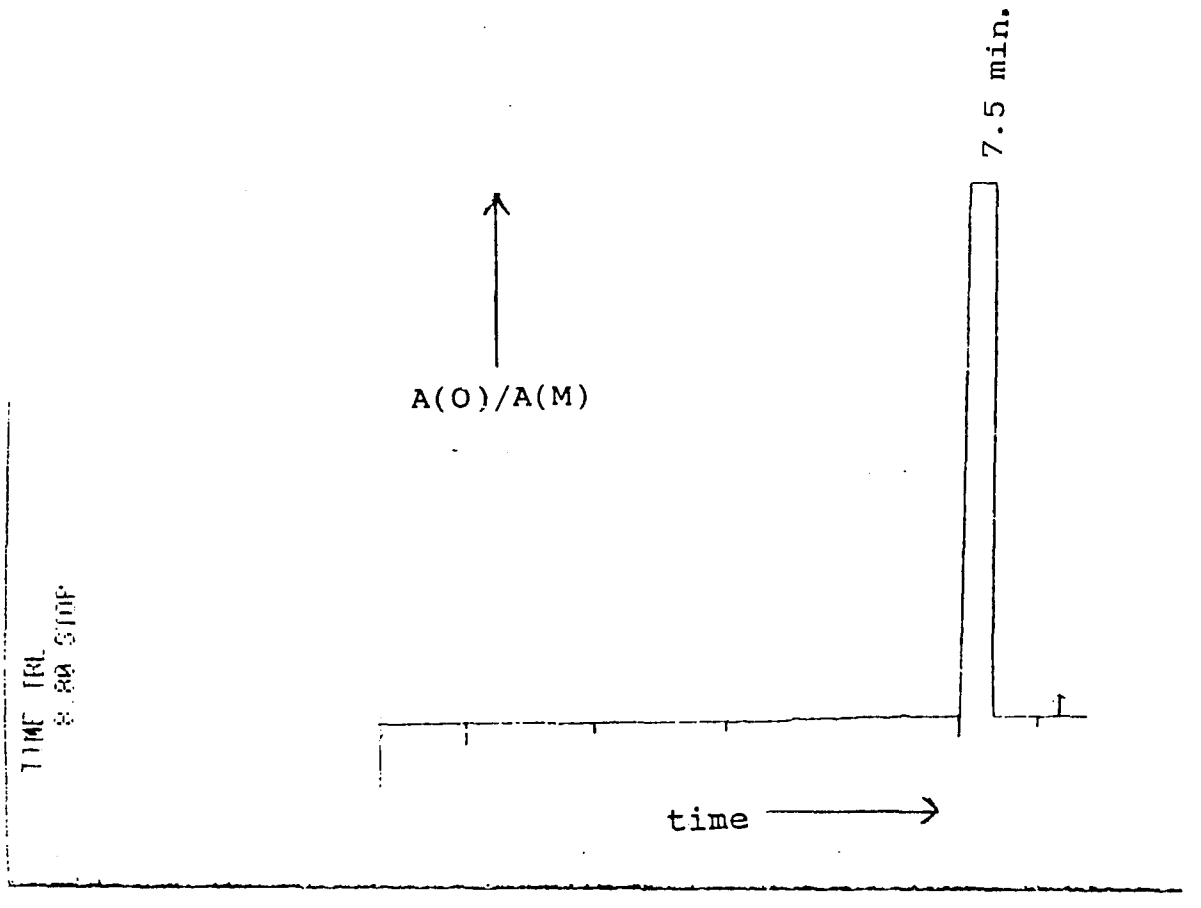


Figure 20. Ratioplot of Diphenhydramine Hydrochloride

V. DISCUSSION

HPLC becomes the most important method of pharmaceutical analysis prensently available. Most separation problems can be solved by reversed-phase HPLC. Appropriate mobile phases are based on either acetonitrile-water or metanol-water mixtures that are beneficial for the dissolutions of the pharmaceuticals. During the chromatography of pharmaceuticals containing different active components and different classes of active, information provided by the UV spectrum largely confirms the identity of the separated peak, and thereby satisfies the requirements of pharmaceutical analysis. The unique value of HPLC analysis lies in the fact that a pharmaceutical must be analyzed not oly before but also after incorporation into various matrices (e.g. elixir, tablets, creams, etc.), often in mixtures with other compounds. The ion-pairing techque which can be used for ionized as well as unionized compounds, makes the applications of the reversed-phase HPLC even broader. This reversed-phase ion-pair high performance liquid chomatographic method has been established as a useful analytical method for stability analysis of diphenhydramine hydrochloride in liquid and solid drug

mixtures. The method procedures, starting from the sample preparation, detection, chromatographic separation to the final quantitation, were all optimized and well thought about. Its validity and suitability for stability analysis were demonstrated with the results obtained from the equipment precision study, method precision study, accuracy test and ratioplot method. Even the comparison of peak area vs. peak height quantitation was performed to confirm the peak area should be used for this quantitative analysis. At present the chromatographic quantitation is usually carried out by electronic integration as in our HPLC system. Electronic integrators are commercially available, relatively inexpensive, and easy to operate. If an electronic integrator were not available, measurement of peak area would not be preferable because the peaks resulting using current instrumentation are too narrow for accurate area measurements. Thus there could be great deviations in peak areas because of the variation in peak width.

Method validation insures that the separation which has been developed will adequately meet all criteria for the particular application. Recent work with reversed-phase ion-pair HPLC for the determination of diphenhydramine in

cough syrup (44) lacked of validation for the diphenhydramine analysis. It used UV detection at 254nm. This wavelength is not specific for diphenhydramine. Futhermore the resolution of the diphenhydramine peak from other peaks was not good. Our method is more specific by using 258nm as a detection wavelength, and the peak purity was ensured further using the ratioplot method. The desired peak was well resolved from other component peaks that had much smaller k′ as shown in the typical chromatogram of "L" sample (Figure 3). Usually the more polar degradation products also elute near the solvent front in reversed-phase HPLC, therefore they will be away from the diphenhydramine peak. It was worthwhile to investigate the method using "real" samples as well as standards in a sample matrix, and to spend enough time in validating this sensitive yet rugged method to eliminate the problems we could forsee for the subsquent analysis of the thermally treated samples (83 - 85). This provides a good foundation for the drug active kinetic study in which very small changes of active concentration and possible generation of interference(s) or degradation product(s) have to be dealt with.

PART TWO
STABILITY ANALYSIS OF LIQUID AND SOLID DRUGS CONTAINING DIPHENHYDRAMINE HYDROCHLORIDE

I. SUMMARY

For the study of the stability of liquid and solid drug formulations containing diphenhydramine hydrochloride, drugs "L" and "S" were purposely subjected to elevated temperatures for about three and a half months. The three elevated temperatures were $42^{0}C$, $52^{0}C$ and $62^{0}C$. At each temperature, samples were withdrawn at seven or eight times and then analyzed to determine the rate of decomposition. The potency levels (i.e. the concentrations of diphenhydramine hydrochloride) in the treated samples and untreated control samples were determined using the reversed-phase ion-pair high performance liquid chromatographic method established in PART ONE. The active concentration in the untreated sample was considered as the initial concentration and the potency level as 100%. The active concentration of each thermally treated sample was divided by the initial concentration to calculate the "percentage of initial".

The percentages obtained at each time at each temperature were plotted vs. storage time. The values for the rate constants were obtained from the slopes of these plots. The natural logarithm of these values (k) were plotted against the reciprocials of the absolute temperatures according to the Arrhenius equation. The time required for the drug to decrease in potency to certain level at room temperature ($25^{0}C$ is used in this study) can then be predicted from the extrapolation of the Arrhenius plot. The maximum level that a drug is allowed to drop at expiration date is 90% of the active compound. The so called drug shelf life, $t_{0.90}$, is the time for the active potency dropping to 90% level. Although the decompositions of most drugs are likely as zero- or first-order reactions, their shelf lives are calculated based only on the zero-order rate equation without concerning the exact mechanisms. Primarily because there is little difference in plotting data of 75% to 100% range vs. time using zero- or first-order as illustrated in Figure 21. There are also advantages of simple data treatment and conservative prediction for the shelf life. In this study, the first-order rate equation was also used in addition to the zero-order rate equation to treat the data. It confirms the

insignificant difference in predicting the shelf life and obtaining the Ea values based on these two rate equations.

II. INTRODUCTION:

A. Pharmaceutical Stability:

Before 1950 concepts of kinetics and half-lives were known and used for the stability of food but not for pharmaceuticals (86). In the 1950's more papers were seen (87-92) concerning pharmaceutical stability but they were qualitative or semiquantitative stability evaluations rather than academic research. In 1972 and 1973 Carstensen and Litner (93 , 94) published more comprehensive treatments of pharmaceutical product stability. Federal regulations placed more restrictive requirements on pharmaceutical stability in 1980. These were covered in " current Good Manufacturing Practice" (CGMP) and " United States Pharmacopeia " (USP) (95 , 96). Nowadays expiration dating and stability information on pharmaceutical products may be seen on the package insert, label copy and in monographs, and books such as the Physicians' Desk Reference (PDR) of prescription and nonprescription drugs. The pharmaceutical industry often sets up stability testing programs and treats the data according to certain rules of thumb (94 , 97) based on estimates for the decision-making used in establishing the expiration dates for

commercial products. The perdiction of stability and expiration is a direct application and interpretation of chemical kinetics. On the application of predicting shelf life of pharmaceutical product (How long a drug will survive being stored on a shelf without losing a significant part of its effectiveness as a drug.), assumed zero-order rate equation is used to treat the data without deducing mechanisms. The distinction between orders was not clearly observable due to the small drug concentration change. The drugs should drop less than 10% before expiration date at shelf temperature (usually 25^{0}C) or 25% even for an accerlerated study like this. Figure 21 shows the illustration of treating data from 100% to 75% of initial (on y axis) makes little difference for zero- and first-order reactions that overall drug decomposition can be characterized into. This fact along with the benefit of simple data treatment makes that zero-order rate equation being used in the pharmaceutical stability prediction. In this study, data treatment using first-order rate equation was also done in parallel with zero-order for comparison purpose.

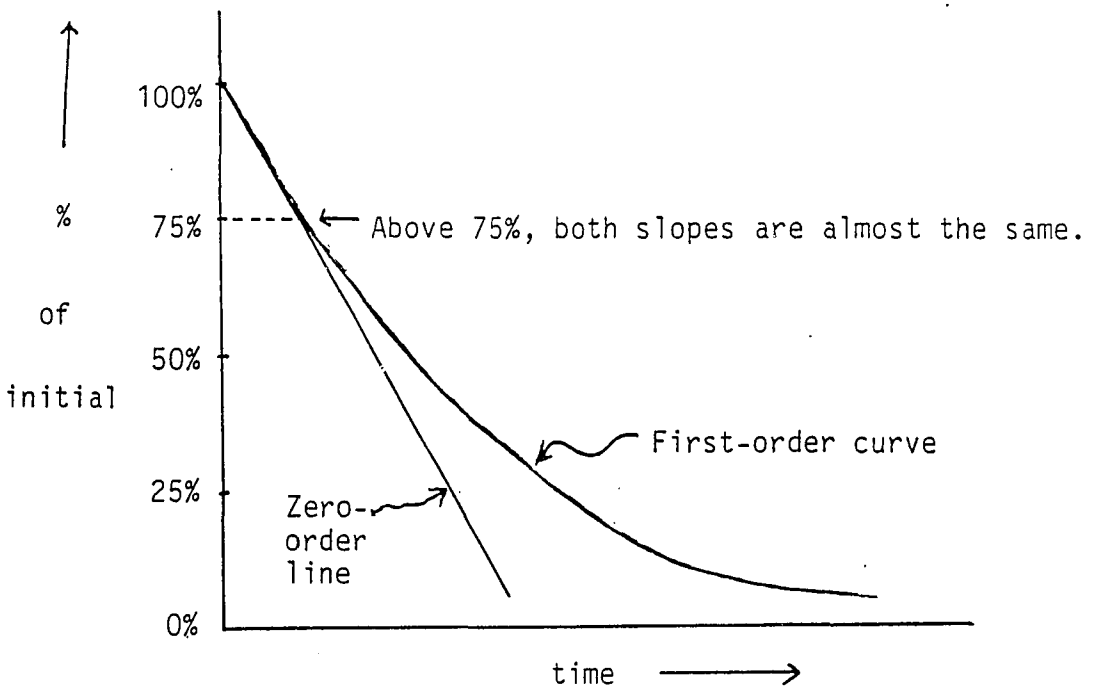


Figure 21. Simple treatment of data above 75% potency

B.Reaction Kinetics

Reaction kinetics is the study of how changes in enviroment change the rate of a reaction. The reaction rate is usually defined as the rate of change of concentration of a substance involved in the reaction, and is obtained by observing the concentration as a function of time under certain condition (e.g. heat, light or humidity).

The study of the kinetics begins with the experimental determination of the variation of the concentration of reactant with time. Having gathered concentration vs. time data, the next step is to fit the data to a rate equation, which gives the mathmatical relationship between the reaction rate and the concentration of reactant. In most kinetic experiments and in the determination of rate reactions, it is the overall result of the reaction that is under study. Practically only one reactant is studied at a time. In a first-order reaction, the rate of the reaction is proportional to the first order concentration of the reactant.

$$rate=K[A]$$

This equation is transformed into an integrated rate

equation,

$$\ln [A] = -kt + \ln[A]_0$$

$$\text{or, } \log[A] = -\frac{kt}{2.303} + \log[A]_0$$

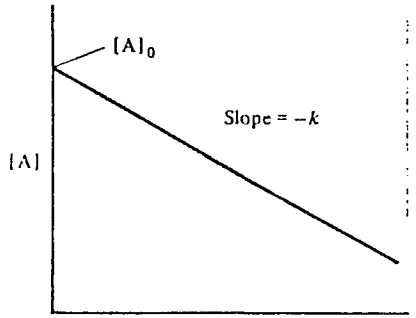
$[A]_0$ is the concentration of the reactant under test at the begining of the reaction when t=0. To test data for first-order reaction the logarithemic form of concentration plotted vs. time should be linear with a slope of -k. The reaction rate is dependent on the first power of concentration of this single reactant. It means if $[A]_0$ is doubled, the rate is doubled.

The rate of a zero-order reaction is not dependent on the reactant concentration and therefore is a constant.

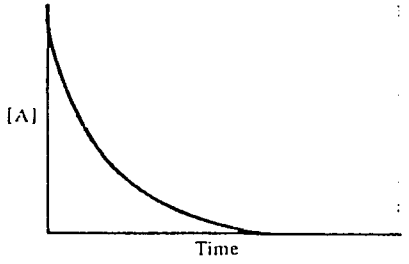
$$\text{Rate} = k, \quad \text{or,} \quad [A]/[A]_0 = -kt$$

The rate is independent of concentration of the reactant. For a zero-order reaction the plot of concentration vs. time should be linear with a negative slope.

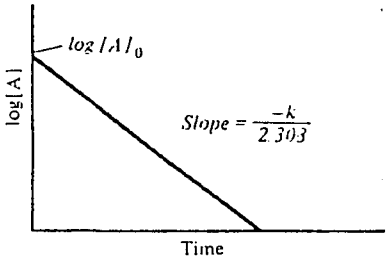
Graphically, the straight-line plots of zero- and first-order reactions are shown in Figure 22.



Plot for a Zero-Order Reaction



(a) Concentration vs. time for a first-order reaction



(b) Plot of the equation $\log [A] = -\frac{kt}{2.303} + \log [A]_0$ for a first-order reaction

Plots for a First-Order Reaction

Figure 22. Zero- and first-order graphs

In the prediction of shelf life (or stability) of drug active, the straight line of zero-order reaction is used before the Arrhenius plot to treat the data obtained, because the data generated covers only the upper 25 % range of the reaction curve. As illustrated in Figure 21 the slopes of the zero-order and first-order curves above 75% make little difference.

The inactive ingredients (i.e. excipients) in drug products are very stable, there is no significant change over time in the concentration or nature of excipients. When the active component concentration compared to the drug matrix is small, and the change in concentration is even smaller, the distinction between orders is not obvious.

The drug shelf life can be predicted in a shorter period of time by so called accelerated study. The drug shelf life is the time it takes for 10% of the drug to disappear and is expressed as $t_{0.90}$.

Experimentally the temperature effect is used in the drug accelerated study, because the number of collision increases as the temperature increases and the reaction rate is expected to increase with the higher temperature so that the rate at the lower temperature (e.g. room temperature $25^{0}C$) can be predicted in a shorter period of time by applying the Arrhenius equation.

C. Arrhenius Equation and Applications

The number of molecules with sufficient energy to react increases with temperature. The activation energy, Ea, is defined as the minumum energy that reactant(s) must have for reaction to occur. As the temperature is increased, the molecules move more rapidly, so collisions are more frequent. Arrhenius observed that there is a mathmatical relationship that connects activation energy, temperature and the rate constant. It's now known as the Arrhenius equation which is

$$k = se^{-Ea/RT}$$

$$\text{or, } \ln k = \ln s - Ea/RT$$

Ea is the Arrhenius activation energy, s is the frequency factor, a constant; R is the gas constant (1.987 cal/k mol) and T is the absolute temperature. Effective activation energy can be found even though the reaction mechanisms are unknown. The value of Ea can be calculated from Ea = R x (slope). The slope is obtained from the plot of lnk versus 1/T. The value of the rate constant at lower temperature can also be obtained by the extrapolation of this line as illustrated in Figure 23. This is the base of predicting the drug shelf (or stability) at room temperature (i.e. drug shelf temperature, 25^0C).

A term, $t_{0.90}$, used in studies of drug concentration is the time required for loss of 10% of the original concentration. This is comparable to the term for half-life, $t_{1/2}$, used in kinetic studies. Drug industrial practice used the reasonable standard, that is, the drug potency should fall less than 10% from its initial or label claim before the expiration date when the product is stored at 25°C. The shelf life of the drug, which corresponds to $t_{0.90}$, can be calculated from the k at 25 °C obtained from the Arrhenius plot. therefore the stability, i.e. the expiration date or shelf life can be predicted from these equations (102 - 104).

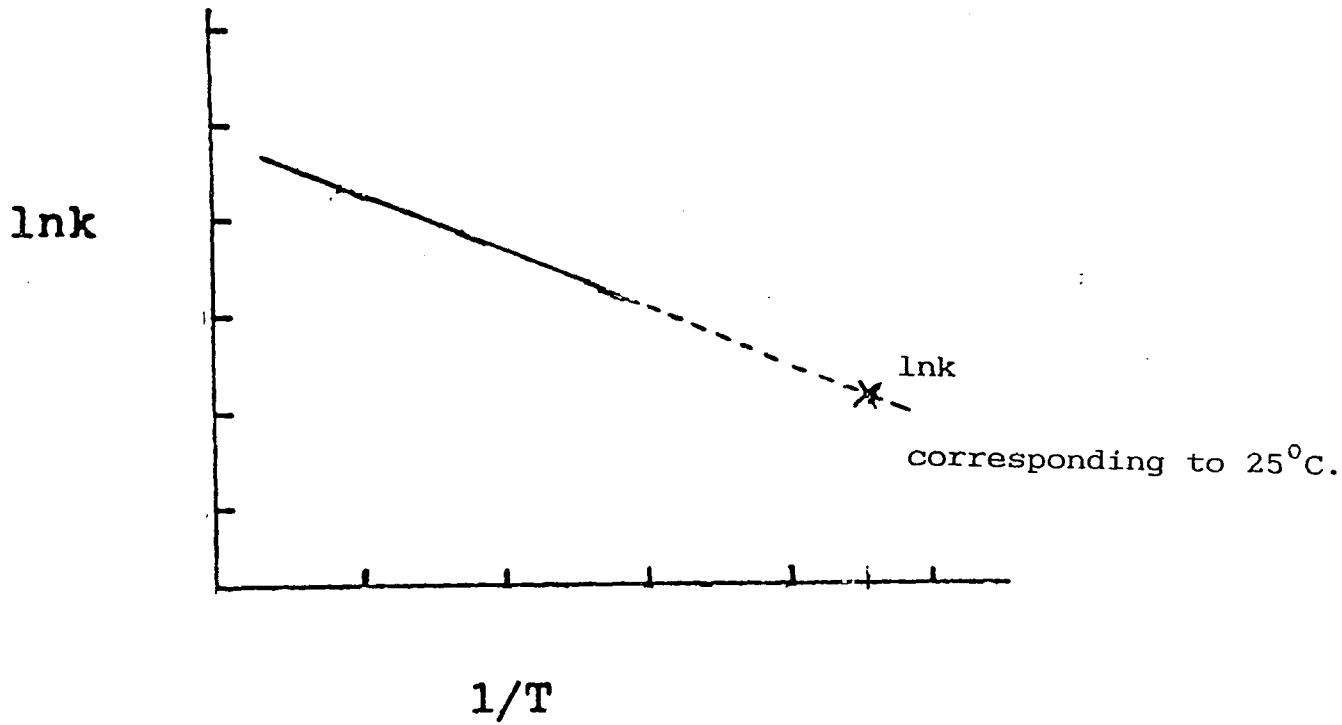


Figure 23. Arrhenius plot and extrapolation

III. EXPERIMENTAL SECTION

A. Materials and Equipment:

The materials used for the stability analysis are Drug "S" containing 25mg of diphenhydramine hydrochloride per tablet, and Drug "L" containing 12.5mg of diphenhydramine hydrochloride per 5mL. A Lab Line oven model 3400 which is capable of maintaining $\pm 1.0^{0}C$ was used for the accelerated storage conditions ($42^{0}C$, $52^{0}C$ and $62^{0}C$) for this experiment. The HPLC system described in PART ONE was used for the quantitation of diphenhydramine hydrchloride in the treated and untreated samples.

B. Sampling:

Preliminary studies showed that sampling and sample preparation are important considerations. The liquid sample contains ethanol which evaporates readily, causing changes in the concentration of the active. The solid sample picks up moisture, and has a non-uniform distribution of the active from one tablet to another.

The sampling was initially performed at different times. Table 5 shows the results obtained from this preliminary study in which the sample homogeneity and consistency were not well considered. As shown the results of "L" sample tend to increase instead of decrease at elevated temperatures untill the completion of the 3 week treatment at $67^{o}C$, in which the extra peaks were observed. The results for "S" sample does not have such a trend. The extra peaks were observed at $67^{o}C$/4week, a little later than "L" sample, which indicated the "S" sample might be more stable than "L" sample.

The evaporation of ethanol (one of the "L" drug ingredients) from "L" sample was probably the cause of the abovementioned problem, judging from the increasing active concentration trend. It would not be a problem if all the "L" samples had the same amount of active initially since they had to be quantitively diluted after each heat treatment. Evaporation of any component other than diphenhydramine hydrochloride during the treatment does not effect the quantitaive results, but the evaporation between the different sampling time will make difference on the initial active concentration because of the increasing active concentration in the "L" sample which is pipetted each time for each condition.

Table 5. Results of the preliminary study

Condition		"L" sample	"S" sample	
week	°C	mg/mL	mg/tab.	mg/g
initial	25	12.22	24.17	53.36
1 wk.	25	12.84	24.43	53.90
1	47	12.50	24.20	54.67
	57	12.60	24.73	55.39
	67	12.67	24.63	55.85
2	47	12.44	24.14	54.25
	57	13.25	24.91	57.19
	67	13.40	25.51	58.70
3	47	12.48	26.80	60.37
	57	13.48	24.78	55.80
	67	11.89(*extra	26.37	58.97
		peaks started showing)	26.37	58.97
4	47	12.59	26.66	60.27
	57	13.39	25.27	56.51
	67	10.25(*)	23.87(*)	53.83(*)

This problem was solved by pipetting the "L" sample at same time for every experimental condition (i.e. every storage temperature/time).

For "S" sample different tablets with different degree of moisture aborption were taken for treatment at different time. There was also the inhomogeneity problem with the "S" tablets, which had a wide range of content uniformity specification, minmum of 10% (105, 106). This means the active content in some tablets could be as low as 95% of label claim, while some other tablets could be as high as 105% of label claim. These cause the abovementioned inconsist results. Grinding and sampling the "S" samples at the same time eleminated these problems of non-uniform active contents in the initial (to-be-treated) samples.

The experiment was stopped and the method of sampling was modified. The samplings were carried out at the same time instead of different time to insure that the amount of the active was the same for the initial and the samples that would be treated with three elevated temperatures for different lengths of time. Sampling performed at the same time from the same composite eliminated the sampling inconsistency problem so that

each sample placed under different conditions and stored for different lengths of time actually had same active amount to start with. The "L" sample only had to be pipetted into each flask, while th "S" sample had to be ground and weighed for sampling.

C. Storage Conditions and Time:

Table 6 shows the temperatures used to treat the samples and the length of storage time. There were minimum of seven or eight time stations that samples were removed from the oven so that meaningful rate constants could be obtained.

Table 6. Storage condition and Time

	"L" sample			"S" sample		
Temperature(^{0}C)	42	52	62	42	52	62
	15	21	7	14	27	15
Time	20	28	42	21	42	25
Stations	40	42	42	42	52	30
in	66	56	49	49	63	40
Days	77	63	60	55	70	49
	84	75	70	65	75	70
	112	80	80	110	84	80
	-	102	120	-	110	98

D. Quantitation

The procedures established in PART ONE were followed for quantitation of diphenhydramine hydrochloride in "L" and "S" drugs. The standard solution (working concentration approximately 0.1 mg/mL) was always run with the treated as well as the untreated (initial) samples. The % of diphenhydramine hydrochloride remaining in each treated sample was calculated and used to obtain the rate constants.

IV. RESULTS

A. "L" sample:

The results for the "L" sample treated at the three temperatures are listed in Tables 7, 8 and 9.
The %initial was vs. time plotted based on the zero order equation for all three temperatures as shown in Figures 24, 25 and 26. The -k at these three temperatures obtained from these plots were -1.06×10^{-1}, -1.40×10^{-1} and -1.88×10^{-1} for 42, 52 and 62^{0}C.

The ln(%initial) vs. time were also plotted for all three temperatures based on the first order equation as shown on Figures 27, 28 and 29. These first order -k obtained for 42, 52 and 62 C were -1.13×10^{-3}, -1.51×10^{-3} and -2.12×10^{-3}.

The Arrhenius plots using the logarithmical forms of these two sets of rate constants (k) vs. (1/T x10E3) are shown in Figures 30 and 31.

Table 7. "L" - Results at 42°C

Time in Days	Conc. in ug/mL*	%initial	ln(%initial)
15	105.1	98.57	4.591
20	104.8	98.28	4.588
40	101.3	95.01	4.554
66	98.1	92.03	4.522
77	97.7	91.57	4.517
84	97.6	91.49	4.516
112	94.3	88.45	4.482

* : concentration of the working solution calculated

Table 8. "L" - Results at $52^{o}C$

Time in Days	Conc. in ug/mL*	%initial	ln(%initial)
21	103.9	97.46	4.579
28	103.1	96.67	4.571
42	100.0	93.73	4.540
56	98.4	92.2	4.524
63	97.4	91.29	4.514
75	95.8	89.85	4.498
80	94.9	89.02	4.489
102	91.5	85.82	4.452

* concentration of the working solution calculated

Table 9. "L" - Results at $62^{O}C$

Time in Days	conc. in ug/mL*	%initial	ln(%initial)
7	106.1	99.47	4.600
42	97.8	91.74	4.519
49	97.2	91.14	4.125
60	95.3	89.40	4.493
70	93.8	87.94	4.471
80	91.8	86.10	4.456
120	82.3	77.19	4.346

*: concentration of the working solution calculated

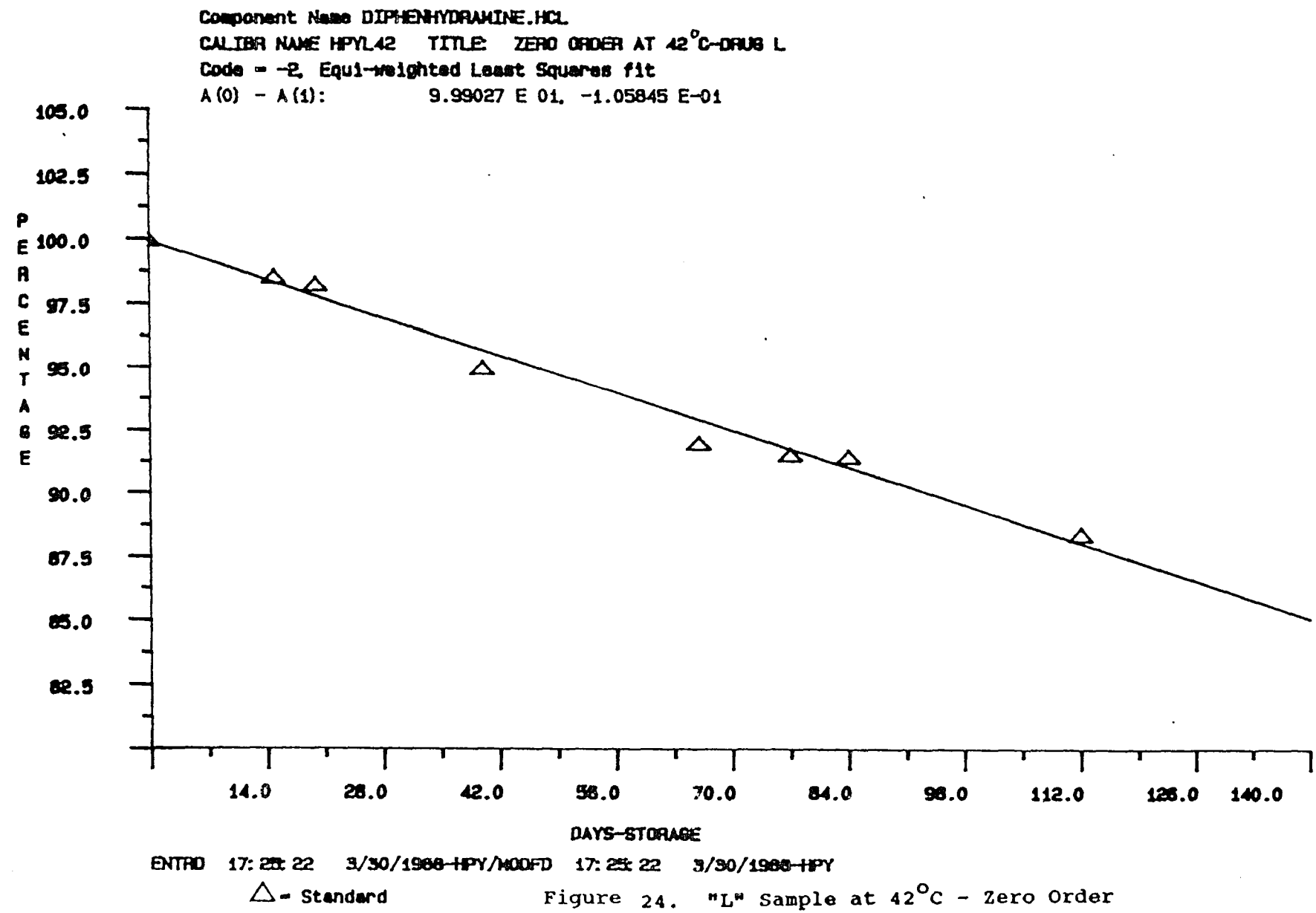


Figure 24. "L" Sample at 42°C - Zero Order

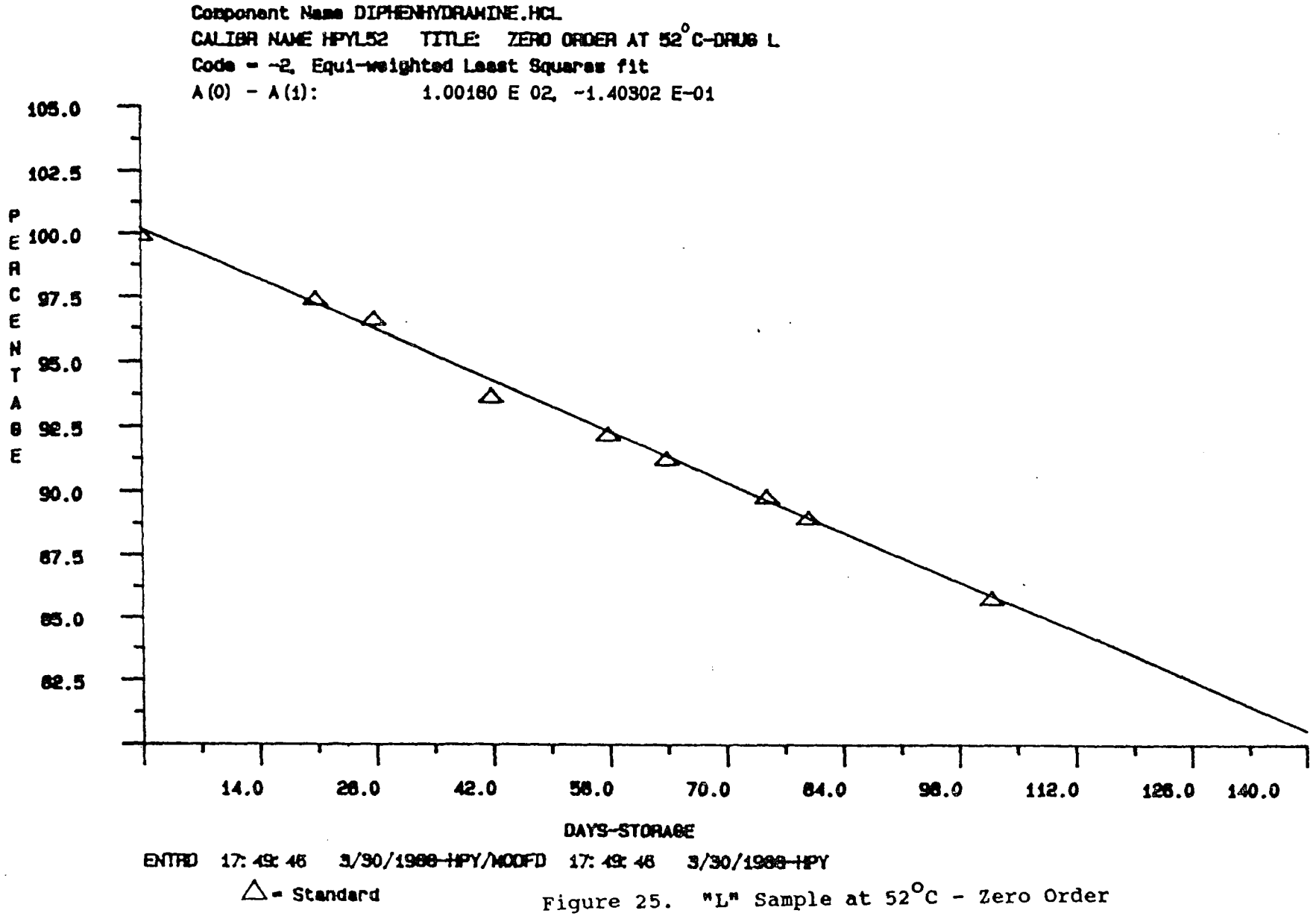


Figure 25. "L" Sample at 52°C - Zero Order

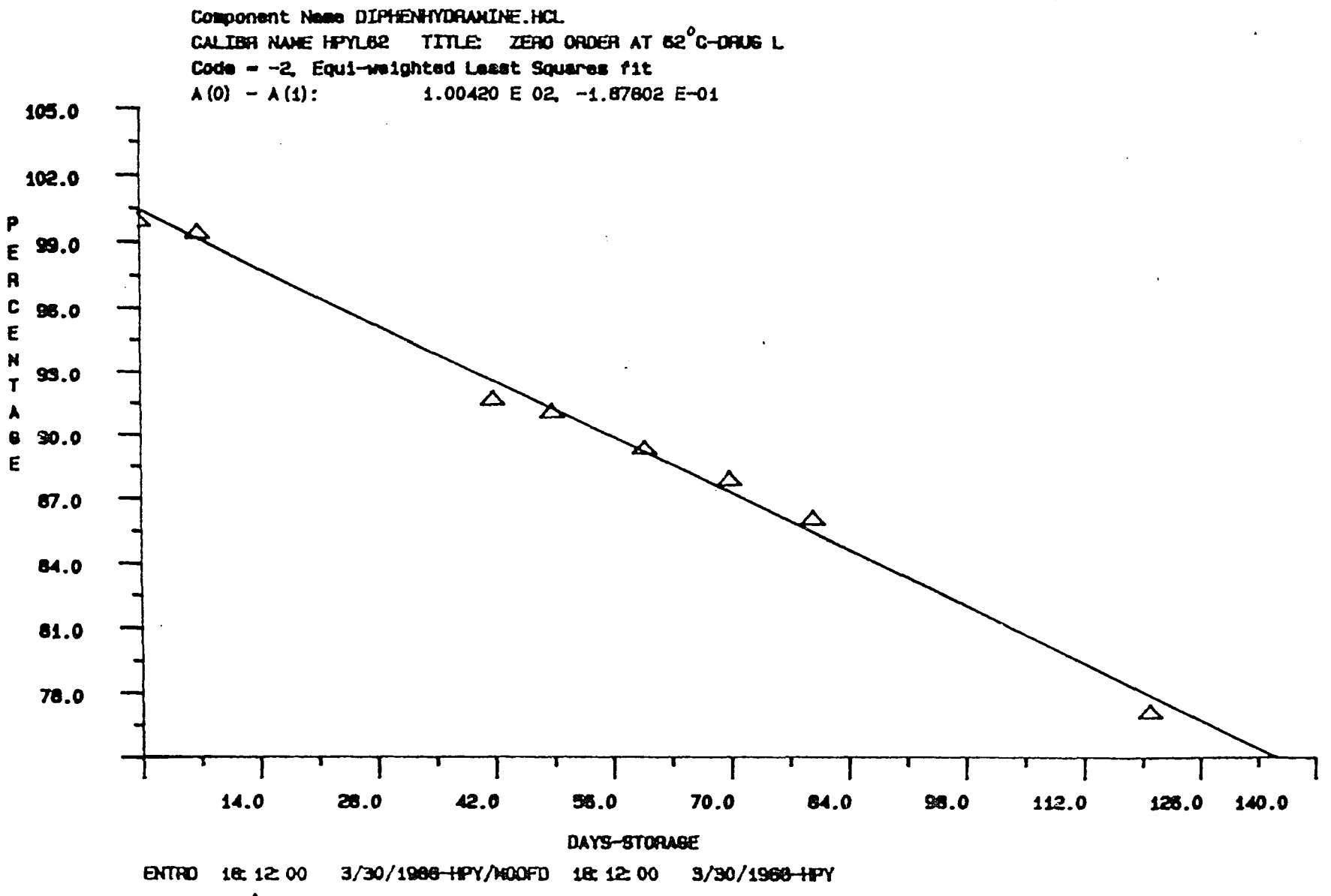


Figure 26. "L" Sample at 62°C - Zero Order

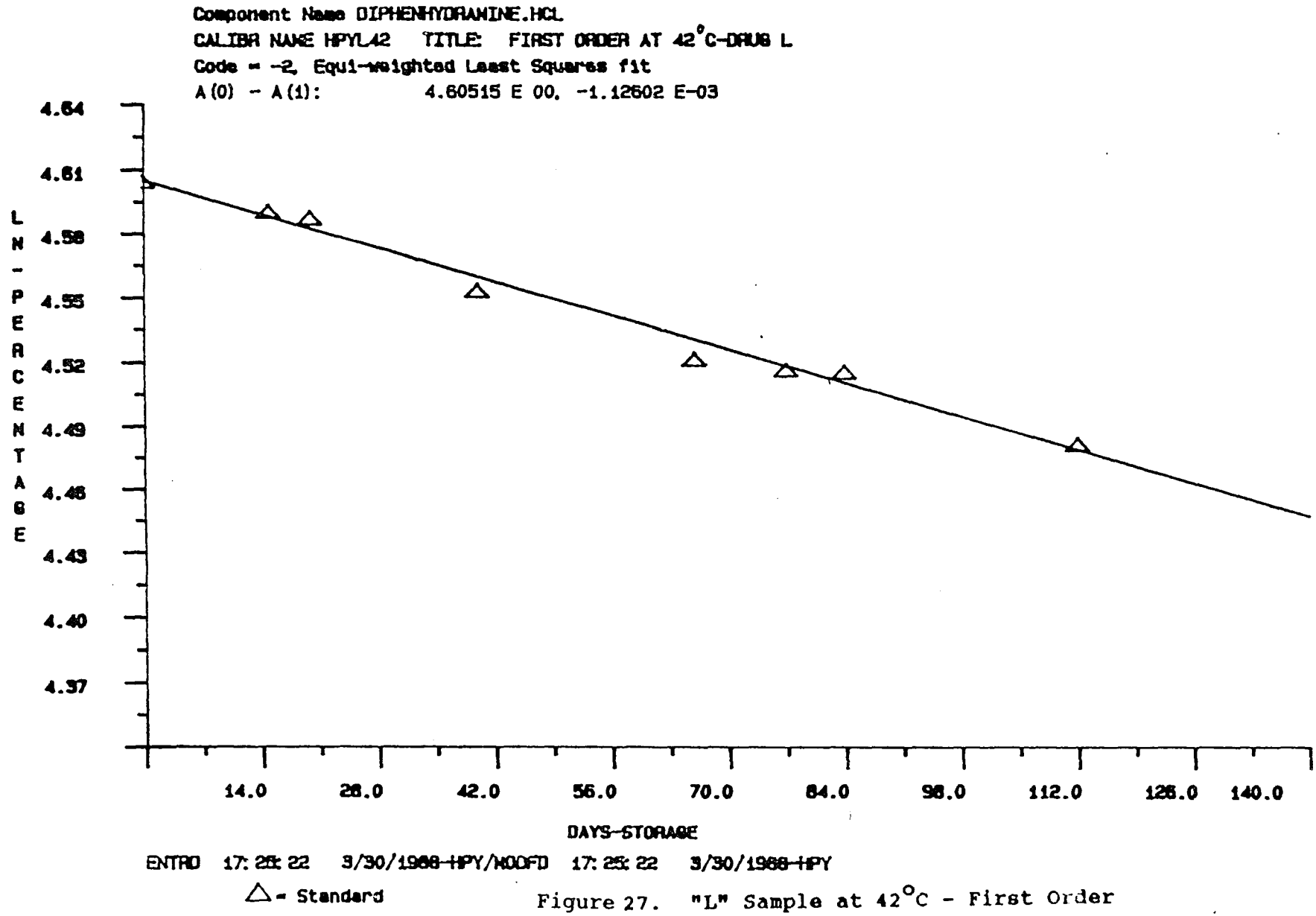


Figure 27. "L" Sample at 42°C - First Order

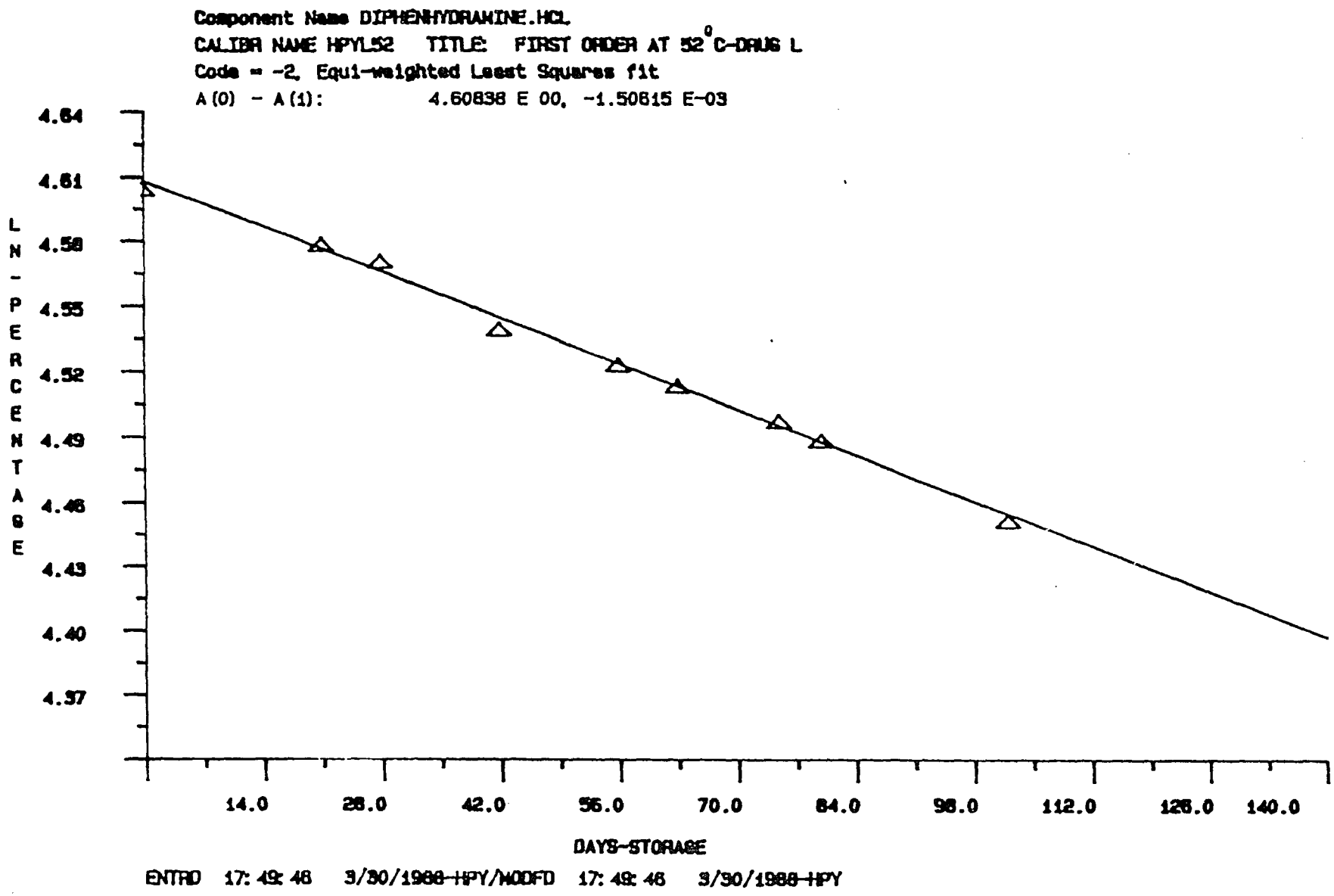


Figure 28. "L" Sample at 52°C - First Order

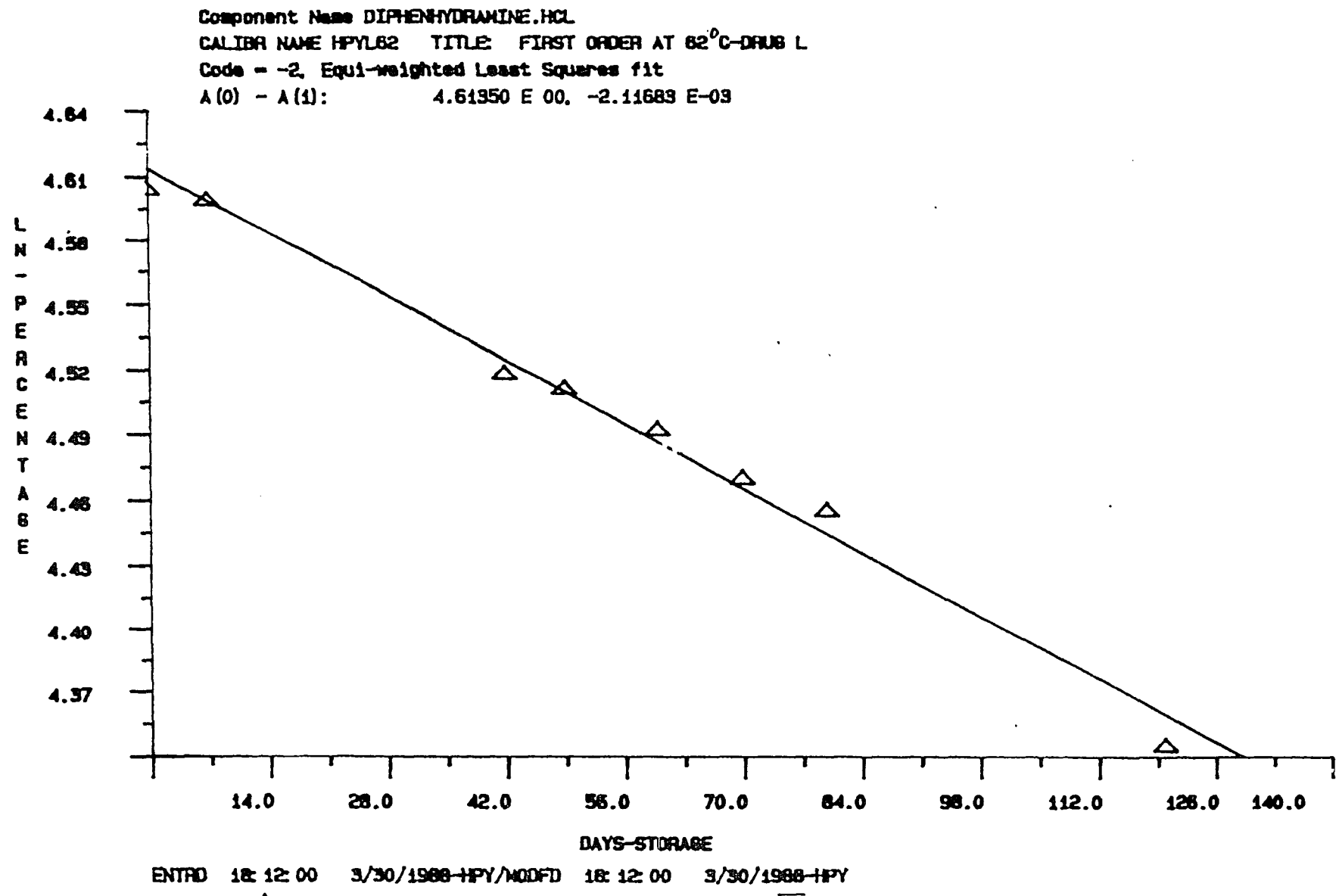


Figure 29. "L" Sample at 62°C - First Order

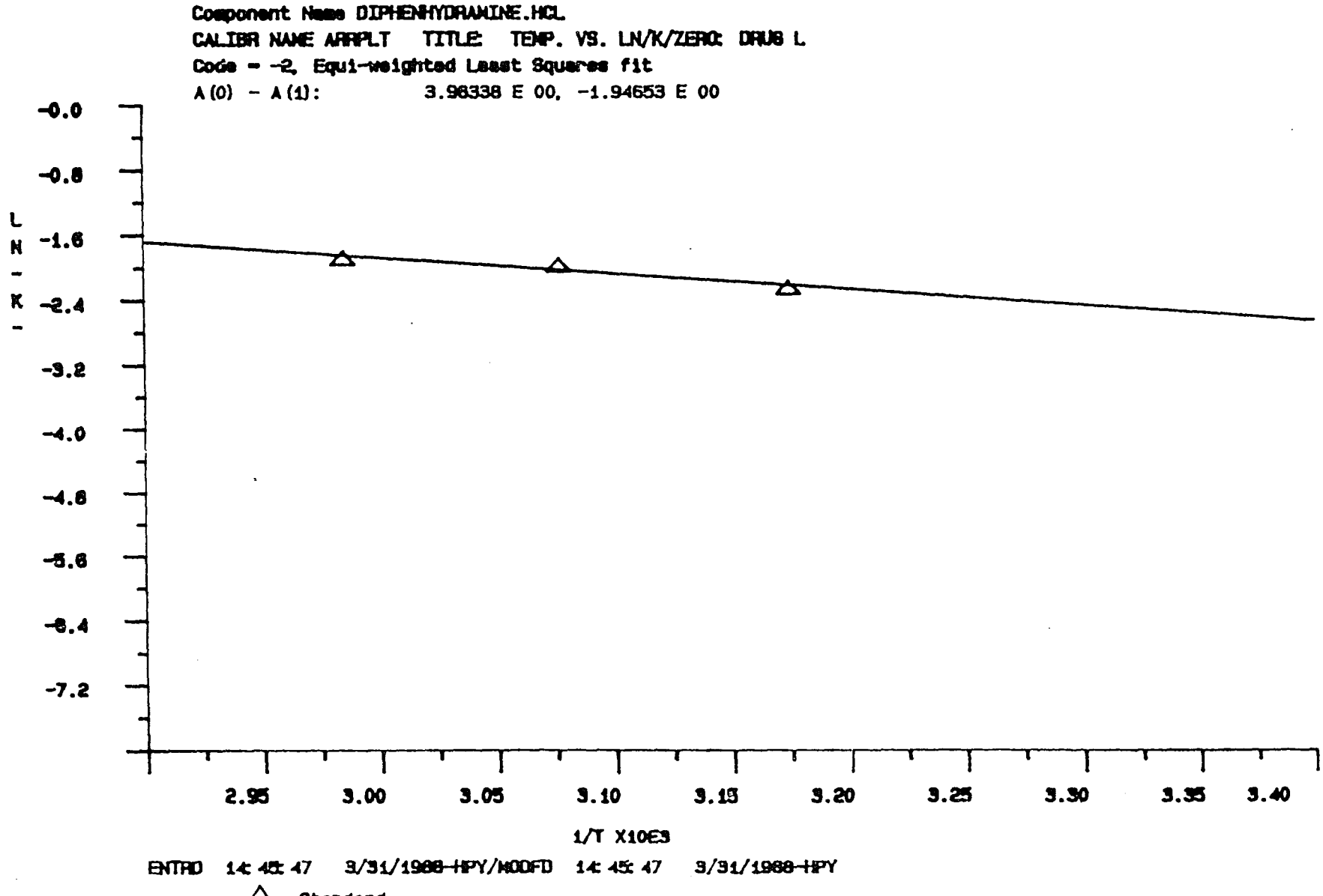


Figure 30. Arrhenius Plot - "L" Sample - Zero Order

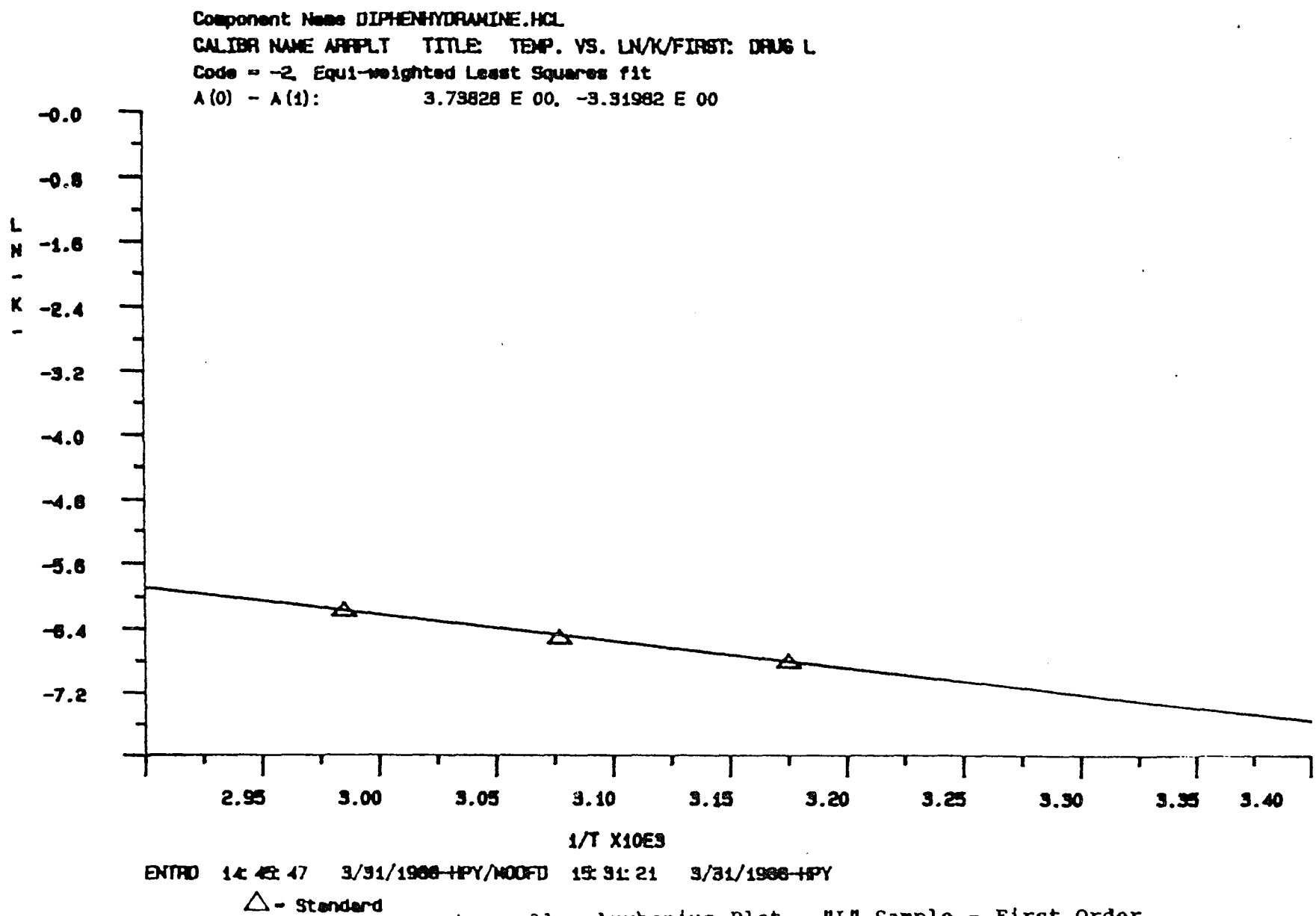


Figure 31. Arrhenius Plot - "L" Sample - First Order

B. "S" Sample:

The "S" samples had different weights as shown in following Tables 10 , 11 and 12. It was possible to pipet the same volume of "L" solution into flasks but not possible to weigh the exact same amount of "S" powder into flasks. The results obtained in mg of diphenhydramine hydrochloride per each sample weight could be converted into mg of diphenhydramine HCl per average tablet weight (A.T.W.).

The results in %initial and ln(%initial) were also plotted against time based on the zero order (Figures 32, 33 and 34.) and first order equations (Figures 35, 36 and 37.) at all three temperatures. The -k obtained from the zero order plots (slpoes of Figures 32, 33 and 34) were -6.68×10^{-2}, -9.12×10^{-2} and -1.18×10^{-1} for 42, 52 and 62 ^{0}C, and from the first order plots (Figures 35, 36 and 37) were -6.94×10^{-4}, -9.67×10^{-4} and -1.27×10^{-3} for the three elevated temperatures. Rate constants (k) obtained from these time-based plots were converted into the logarithmical forms and used in the Arrhenius plots as shown in Figures 38 and 39.

Tables 10, 11 and 12 shows the results for the "S" sampls treated at the three elevated temperatures.

Table 10. "S" - Results at 42°C

Spl.Wt.	Time-Days	Conc.- mg/mL*	%initial	ln(%initial)
0.09194g	14	0.1049	98.83	4.593
0.09226g	21	0.1048	98.45	4.590
0.09209g	42	0.1035	97.38	4.581
0.09272g	49	0.1034	96.68	4.571
0.09230g	55	0.1024	96.13	4.566
0.09240g	65	0.1018	95.48	4.559
0.09220	110	0.0985	92.59	4.528

* Concentration of the "S" working solution calculated

Table 11. "S" - Results at 52°C

Spl.Wt.	Time-Days	Conc.-mg/mL*	%initial	ln(%initial)
0.09256g	27	0.1015	94.98	4.554
0.09190g	42	0.0100	93.99	4.543
0.09246g	52	0.0100	93.37	4.537
0.09221g	63	0.0099	92.88	4.531
0.09208g	70	0.0099	92.65	4.529
0.09179g	75	0.0098	92.25	4.525
0.009183	84	0.0097	91.72	4.483
0.09288g	110	0.0092	88.50	4.483

* Concentration of the "S" working solution calculated

Table 12. "S" - Results at 62°C

Spl.Wt.	Time-Days	Conc.-mg/mL*	%initial	ln(%initial)
0.09184g	15	0.0103	96.65	4.571
0.09075g	25	0.0100	95.07	4.555
0.09123g	30	0.0097	94.93	4.553
0.09142g	40	0.0099	94.18	4.545
0.09135g	49	0.0096	93.71	4.540
0.09047g	70	0.0092	90.75	4.508
0.09185g	80	0.0095	89.51	4.494
0.09060g	98	0.0092	87.49	4.472

* Concentration of the "S" working solution calculated

All the time-based plots included the initial point on the y-axis which was 100(%) for the zero order plot (Figures 32, 33 and 34), and 4.605 ($\underline{\ln 100} = \underline{4.605}$) for the first order plot (Figures 35, 36 and 37). The Arrenhenius plots for both "L" and "S" samples are shown on Figures 38 and 39.

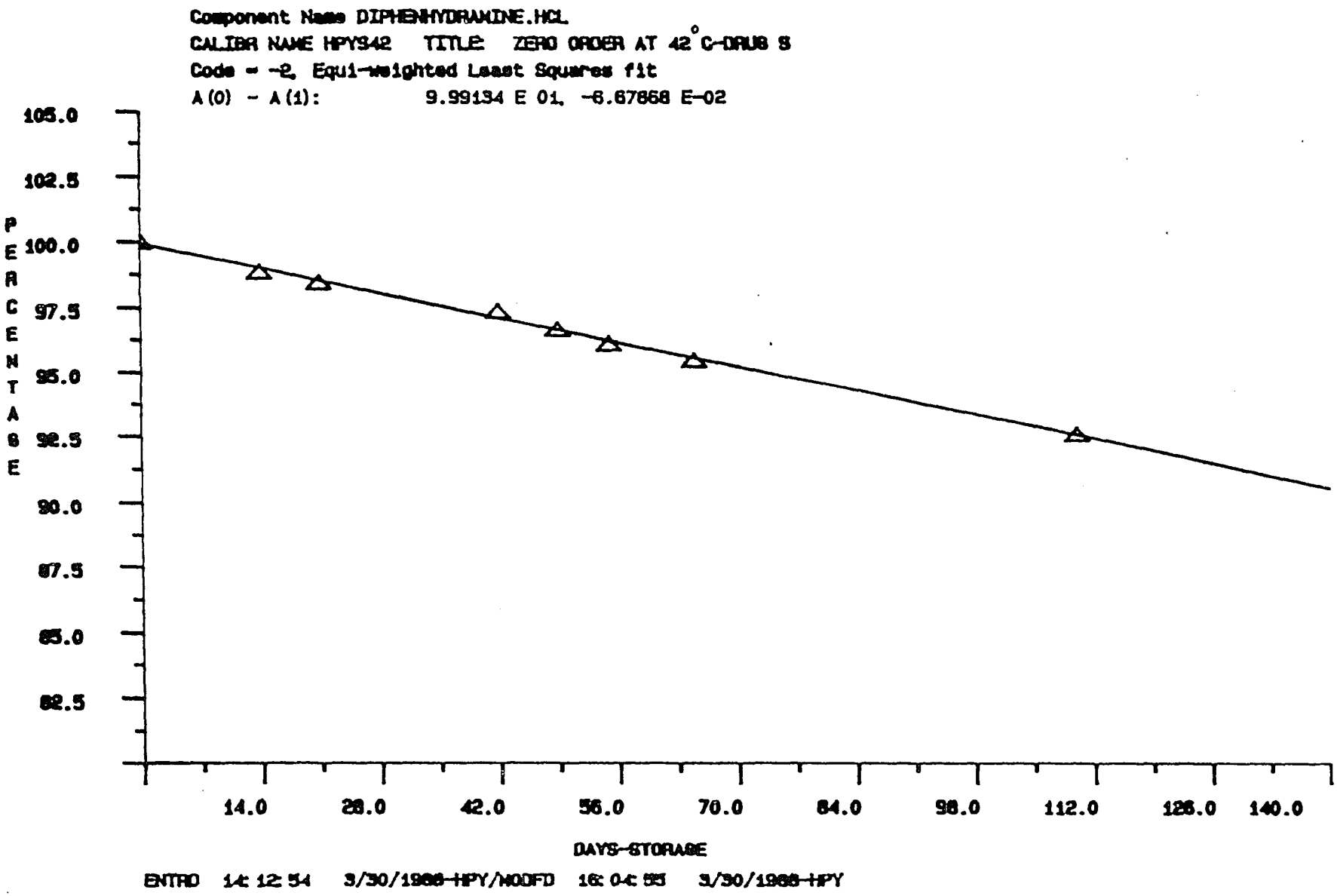


Figure 32. "S" Sample at 42°C - Zero Order.

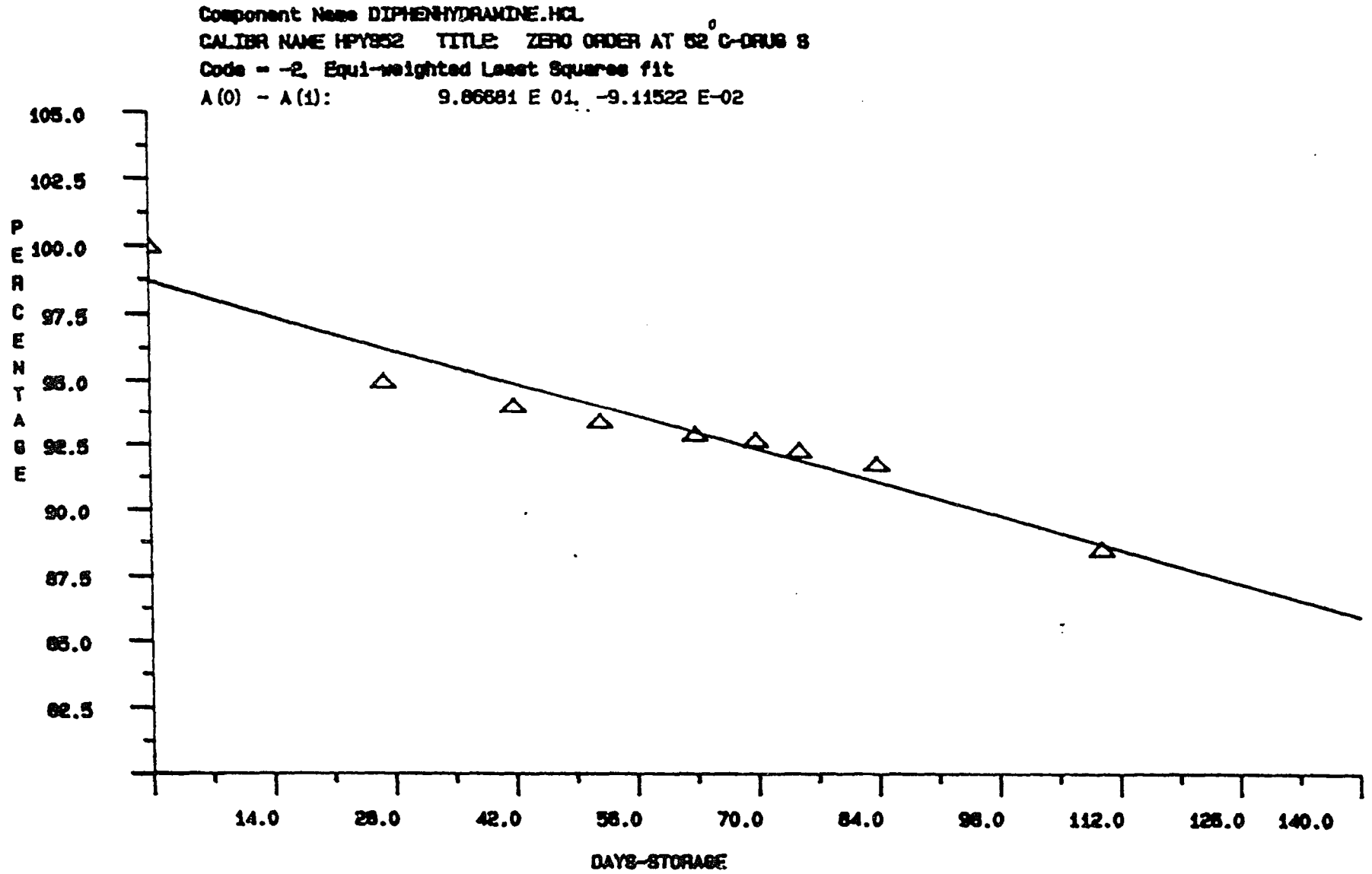


Figure 33. "S" Sample at 52°C - Zero Order

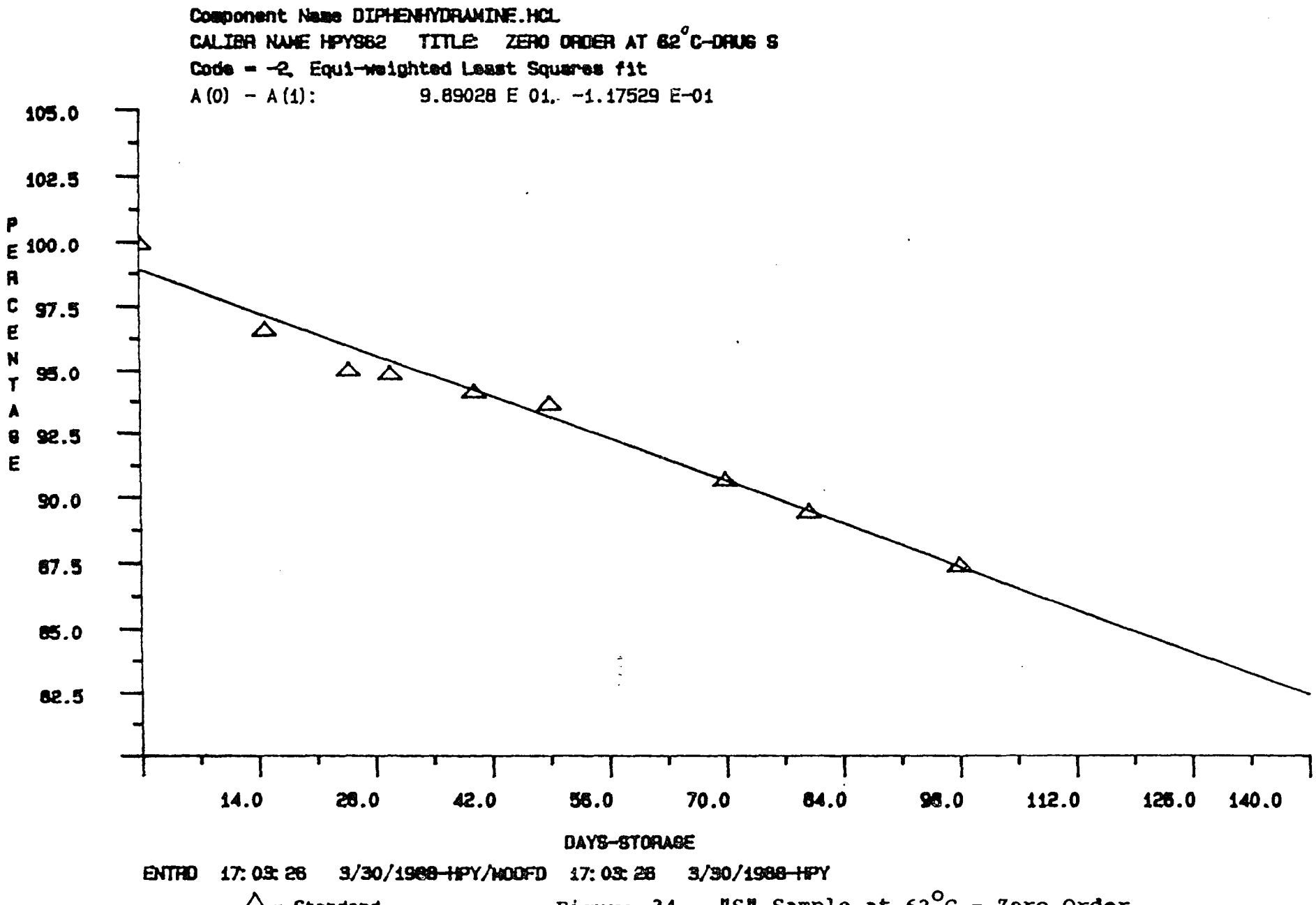


Figure 34. "S" Sample at 62°C - Zero Order

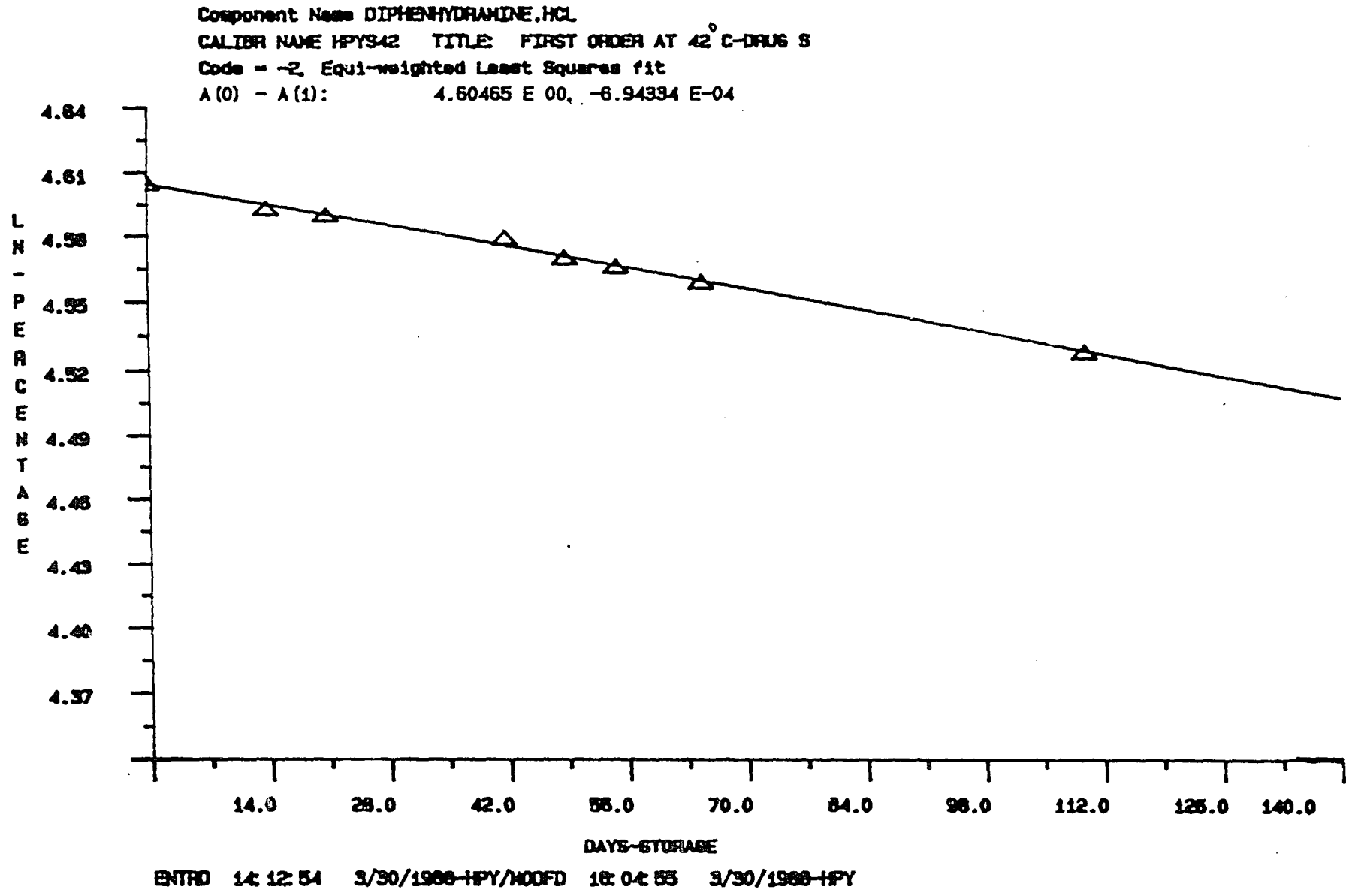


Figure 35. "S" Sample at 42°C - First Order

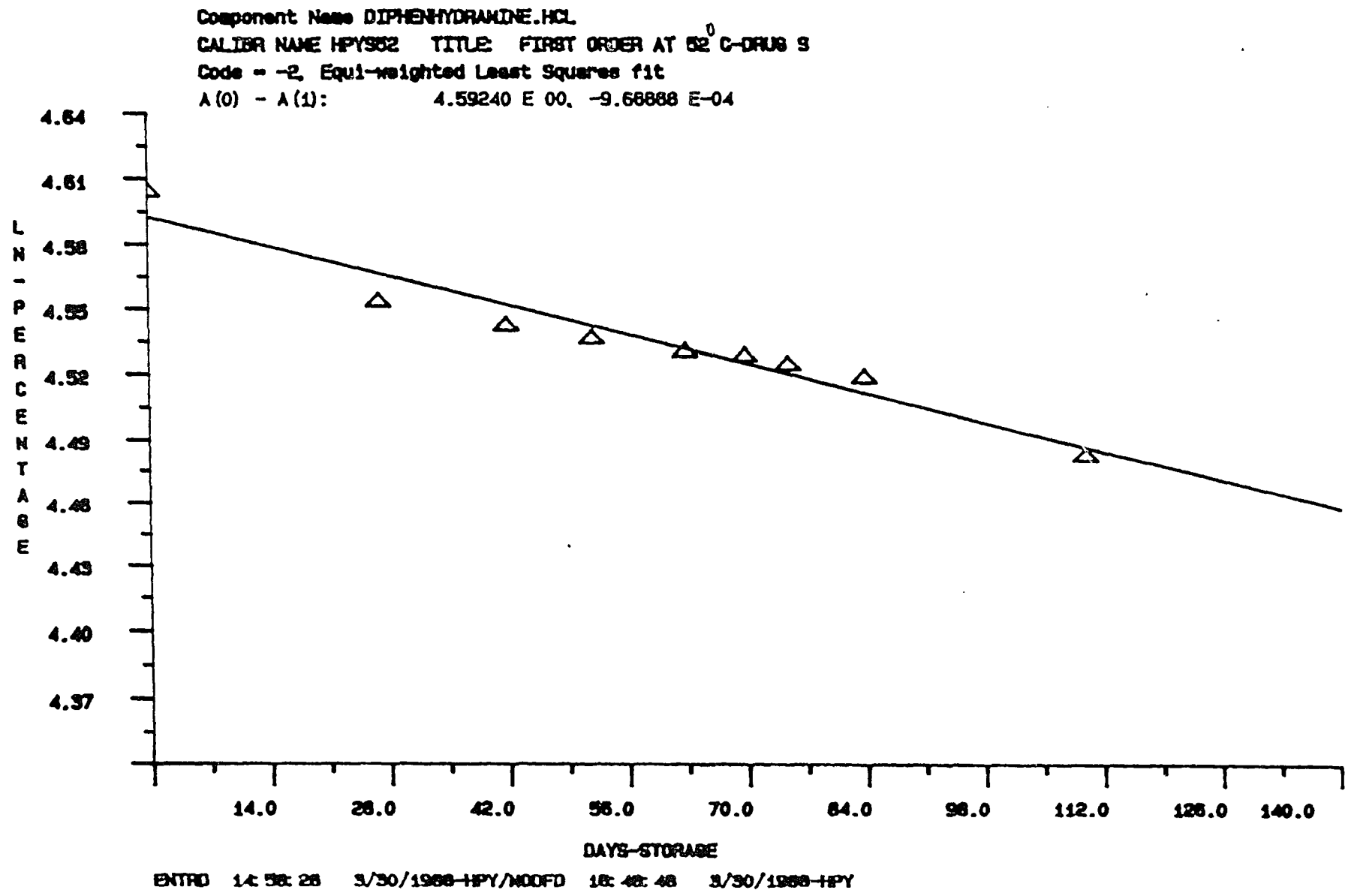


Figure 36. "S" Sample at 52°C - First Order

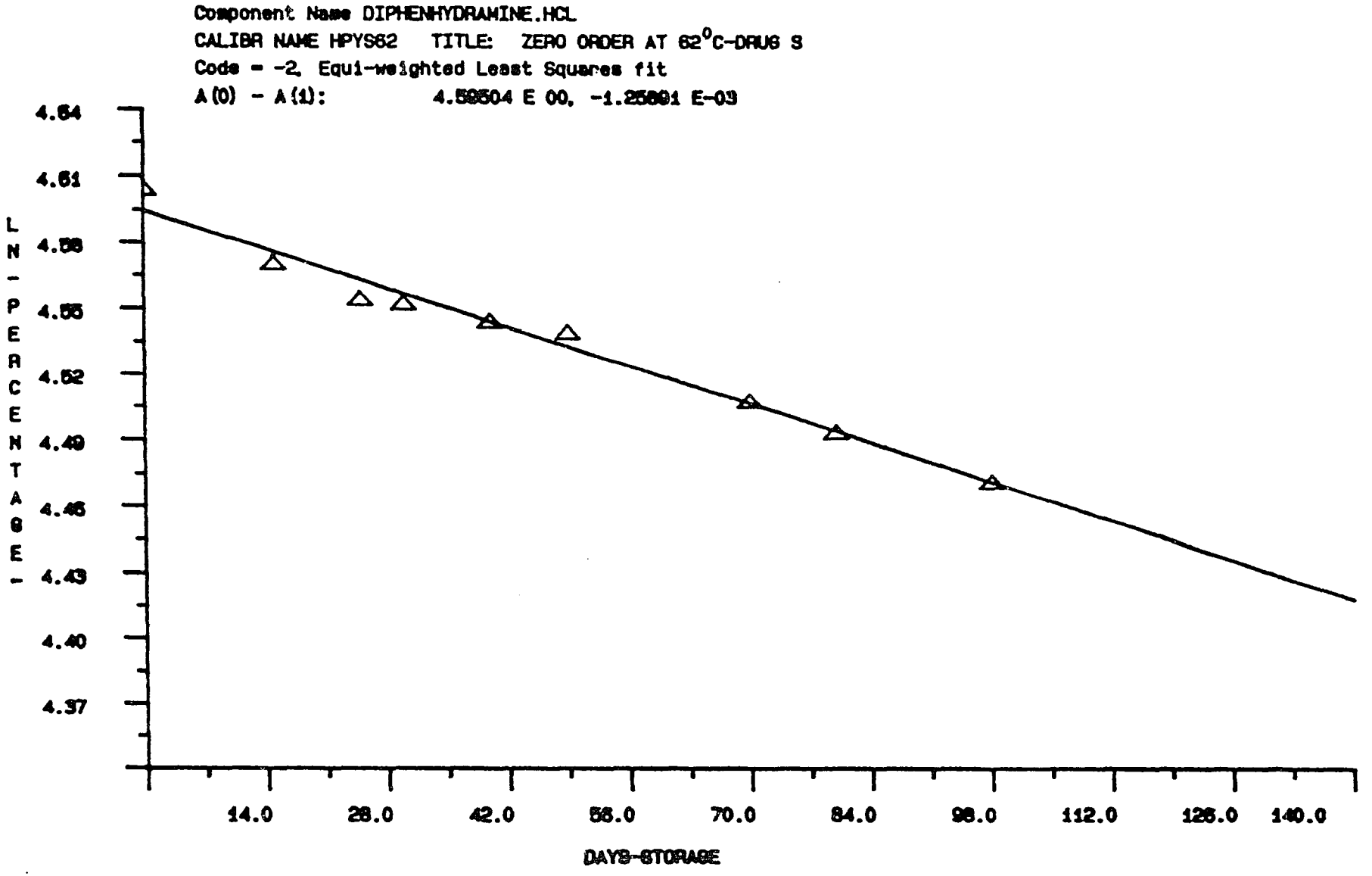


Figure 37. "S" Sample at 62°C - First Order

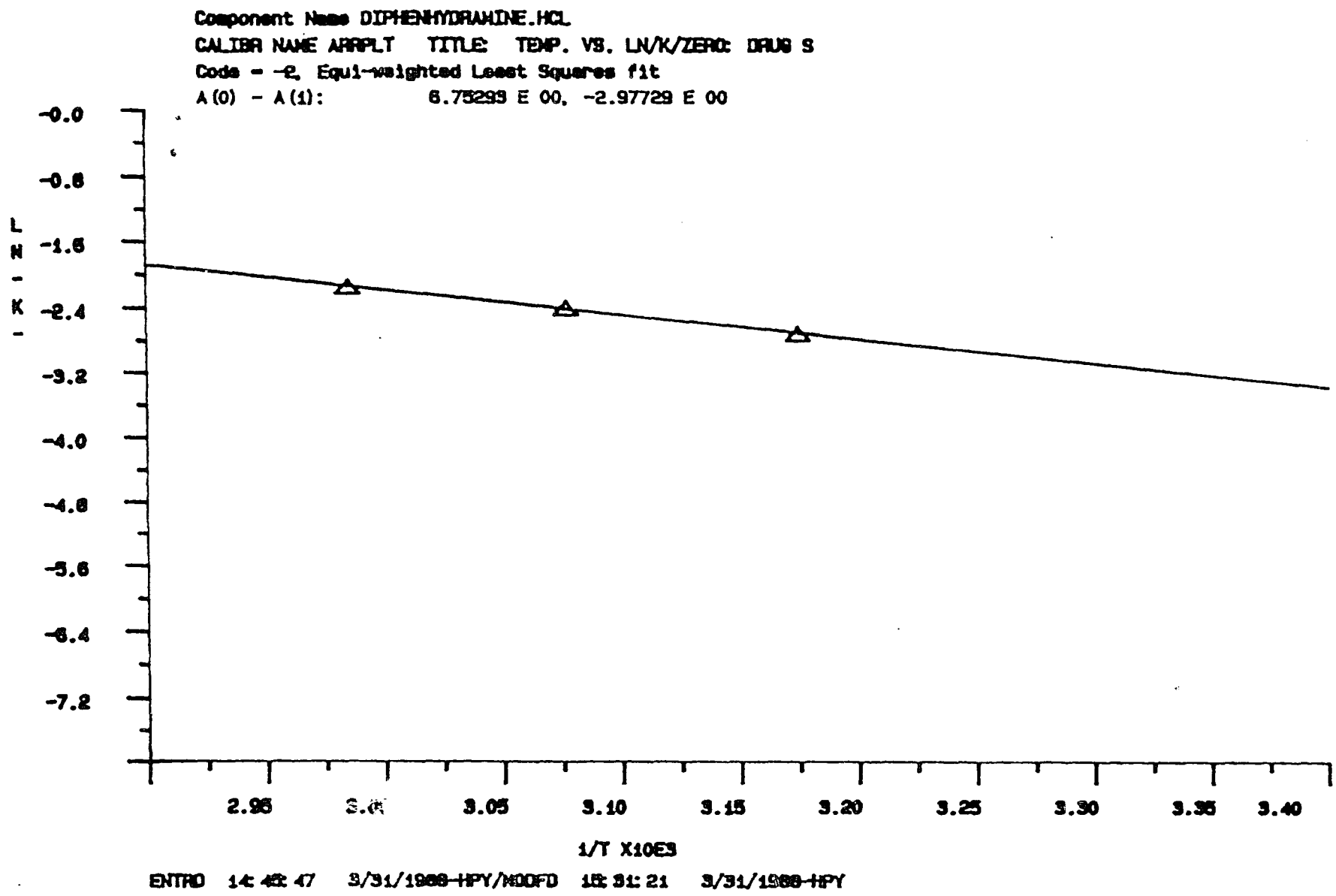


Figure 38. Arrhenius Plot - "S" Sample - Zero Order

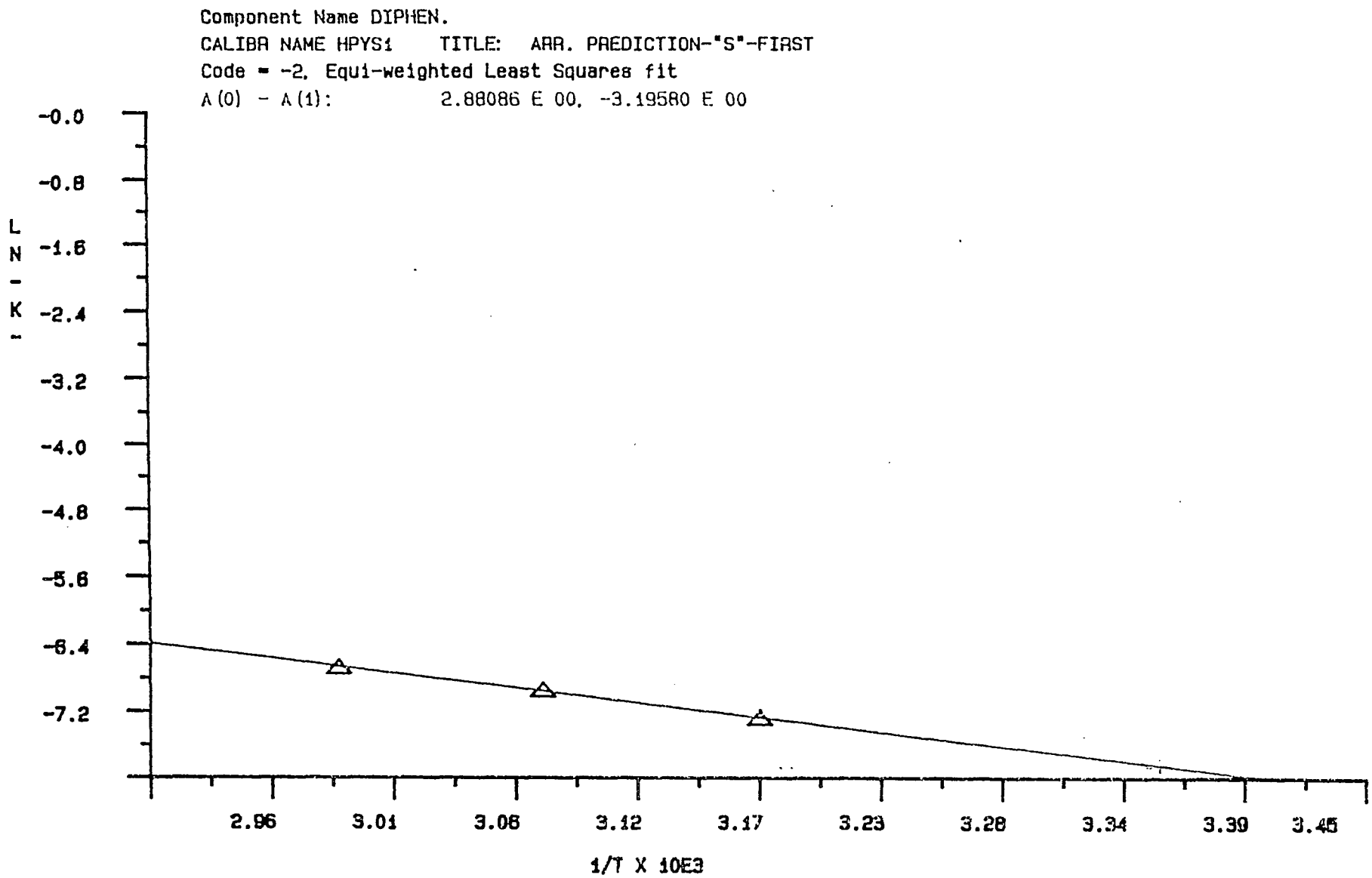


FIGURE 39. Arrhenius Plot - "S" Sample - First Order

C. Interpretation and Prediction:

The Arrhenius plot extrapolation can be made to predict the lnk at 25^0C. Figures 40 and 41 are the Arrhenius prediction for "L" sample and Figures 42 and 43 are for "S" sample. The + represents the point of lnk corresponding to 25^0C. The MPCAL program was used to calculate the exact value of lnk as a function of 1/T. The results are shown in Figures 40 to 43. Then $t_{0.90}$ was calculated by placing the value k (from lnk obtained by the extrapolation of Arrhenius plot at 25^0C) into the following zero or first order equation :

For zero order

$$100 = 90 - kt_{0.90} \quad \text{or,} \quad t_{0.90} = 10/k$$

For first order

$$\ln 100 = \ln 90 - kt_{0.90} \quad \text{or,} \quad t_{0.90} = 0.105/k$$

The results of $t_{0.90}$ in days obtained for "L" and "S" samples are listed below:

	"L"sample	"S"sample
Zero order	163 days	255 days
First order	173 days	268 days

The results do not show much difference significantly.

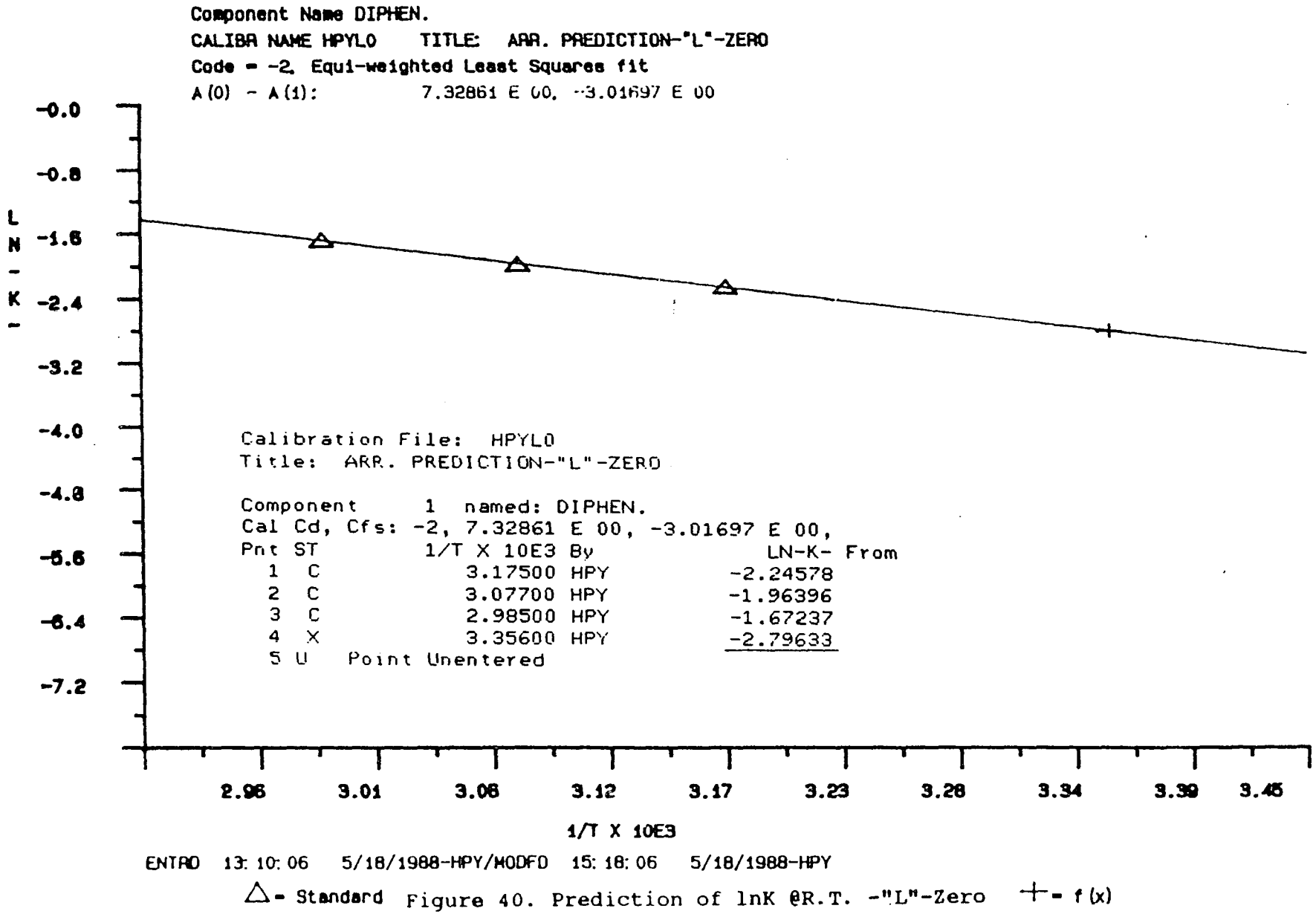
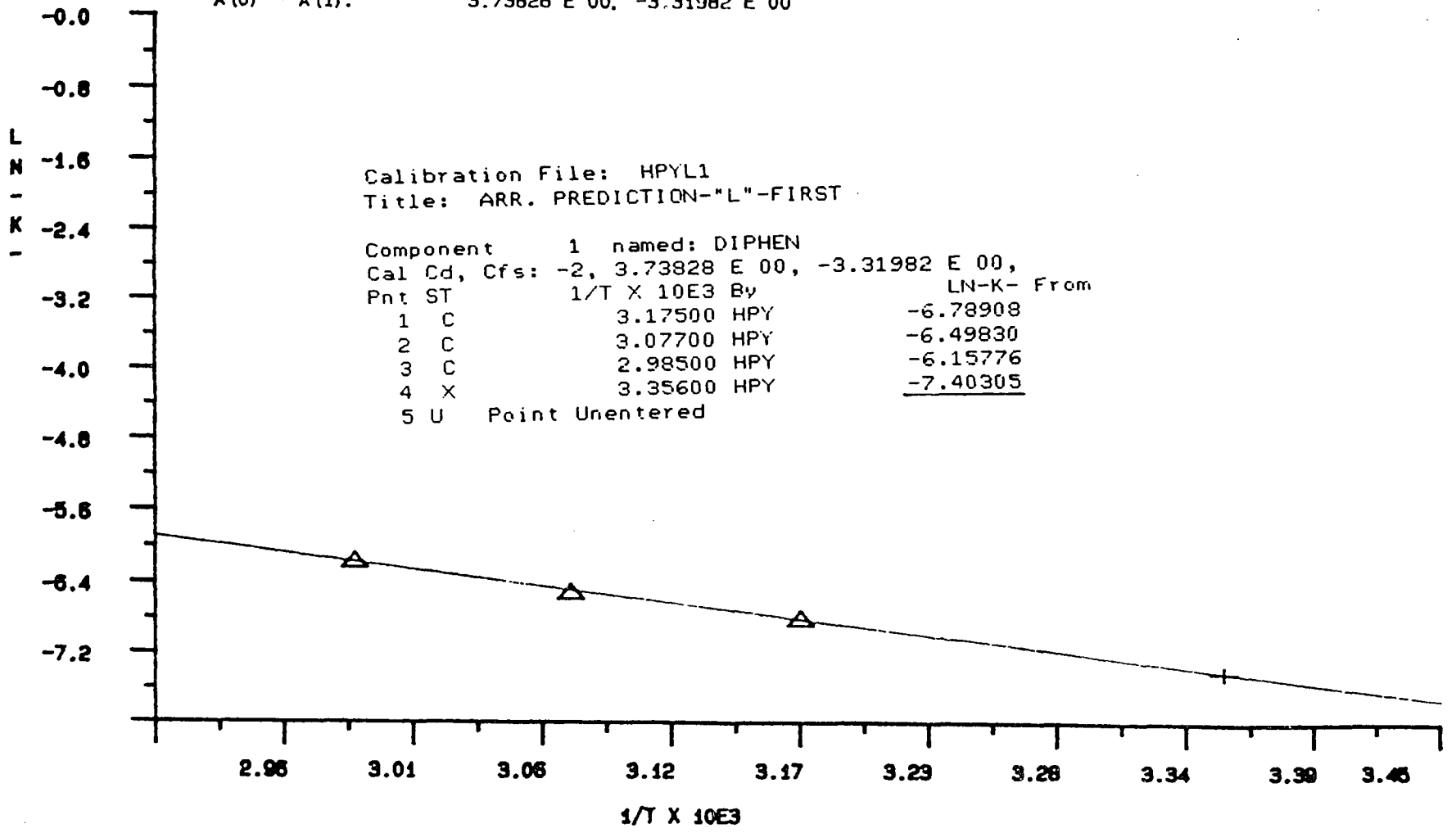


Figure 40. Prediction of lnK @R.T. -"L"-Zero

Component Name DIPHEN
CALIBR NAME HPYL1 TITLE: ARR. PREDICTION-"L"-FIRST
Code = -2, Equi-weighted Least Squares fit
A(0) - A(1): 3.73828 E 00, -3.31982 E 00



Calibration File: HPYL1
Title: ARR. PREDICTION-"L"-FIRST

Component 1 named: DIPHEN
Cal Cd, Cfs: -2, 3.73828 E 00, -3.31982 E 00,

Pnt	ST	1/T X 10E3 By	LN-K- From
1	C	3.17500 HPY	-6.78908
2	C	3.07700 HPY	-6.49830
3	C	2.98500 HPY	-6.15776
4	X	3.35600 HPY	-7.40305
5	U	Point Unentered	

ENTRD 14:45:29 5/18/1988-HPY/MODFD 15:08:32 5/18/1988-HPY

△= Standard Figure 41. Prediction of lnK @R.T. -"L"-First +- f(x)

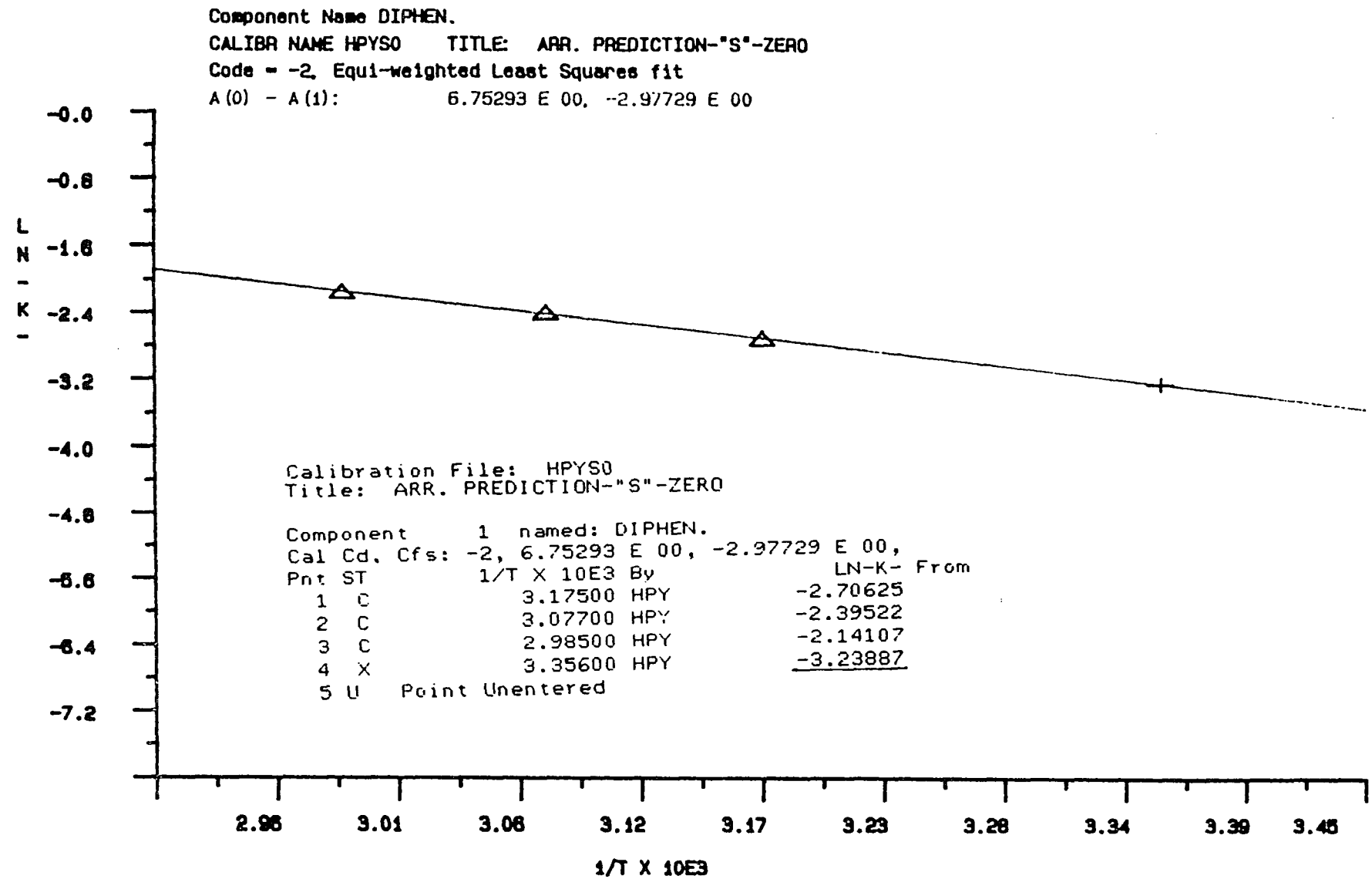


Figure 42. Prediction of lnK @R.T. -"S"-Zero

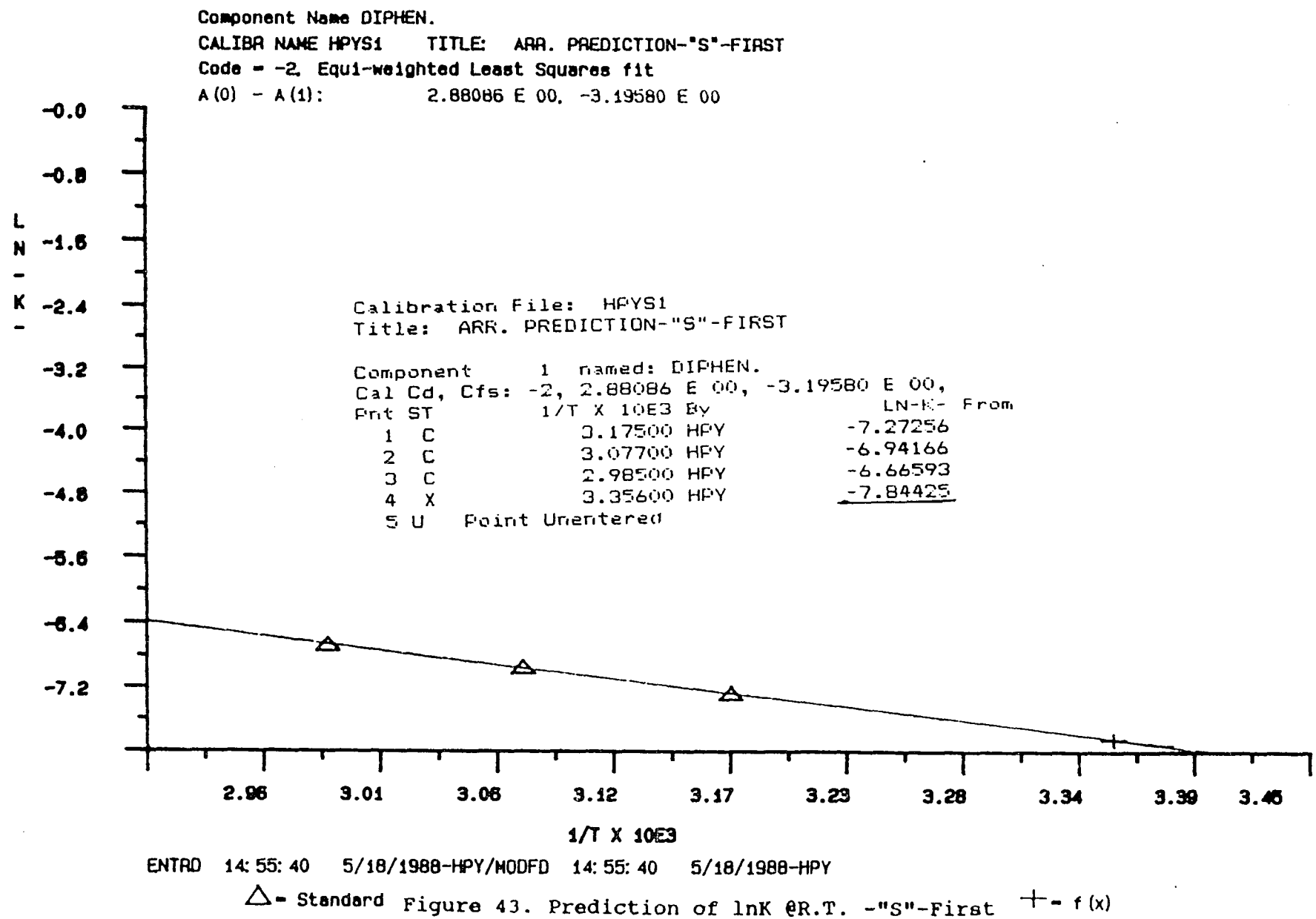


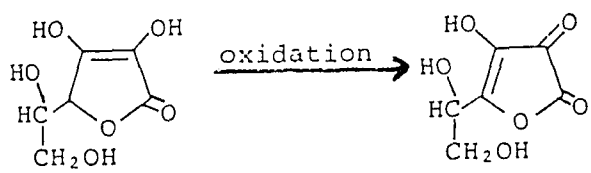
Figure 43. Prediction of lnK @R.T. -"S"-First

The results obtained from the data treatment through the zero order plot, which is more conservative, is used by the pharmaceutical industry to set the the tentative expiration date. In this study the data was also treated with the first-order plot. It only resulted in small difference (10 to 13 days more) for the prediction of shelf life. This was explained in II.A. and II.B. as well as in Figure 21.

Ea values were also calculated by treating the data with both order rate equations and Arrhenius equation. Again they show little difference. They are as follows:

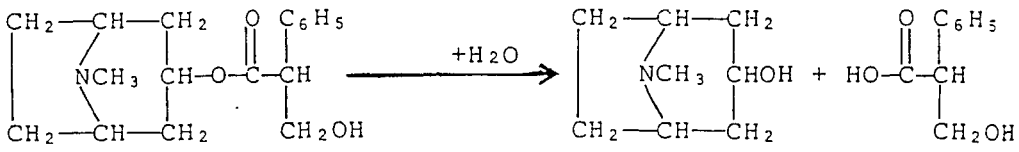
	"L" sample, kcal/mol	"S" sample, kcal/mol
zero-order	6.0	5.9
first-order	6.6	6.4

These values are similar to the values reported for the decomposition reactions of two drug actives, ascorbic acid and atropine. Ea of 7.8kcal/mol was obtained for the oxidation of ascorbic acid at PH 6.6 (110).



Ascorbic acid Dehydroascorbic acid

Ea of 7.7kcal/mol was obtained for the hydrolysis of atropine (111).



Atropine Tropine Tropic acid

Oxidation, hyrolysis and heat decompositions occur in drugs pretty often and are studied more. Although diphenhydramine hydrochloride was only treated with the moderate temperatures (42, 52, 62^{0}C) for this study, the possibility of oxidation or hydrolysis reaction should not be excluded since oxygen and water or moisture were still available to the samples. The possible mechanisms are proposed in V. DISCUSSION AND CONCLUSION.

V. DISCUSSION AND CONCLUSION

It is to be expected that the compound in solid dosage form is more stable than in liquid dosage form. The experiment bears this out. There is not much difference by using the zero order or first order predictions. For the drug industry it is safer to predict the drug shelf life or the tentative expiration date using the lower order estimate which is also easier and more convenient for them. They are allowed to monitor the real expiration time later on at the shelf temperature and possibly to extend the expiration date by showing the data obtained at shelf temperature. Their goal is to set initially a safe tentative shelf life so that the drugs can be markted without risk. To prolong the shelf life of drugs, they can use protection such as better package, container, or enclosures; add stabilizer and/or preservatives to increase the stability of the product. Sometimes they even add the overage to obtain a longer shelf life (107 - 109). The Overage is the additional active amount over the 100% label claim and it is added into the drug in manfacturing to extend the time for drug to reach 90% of label claim.

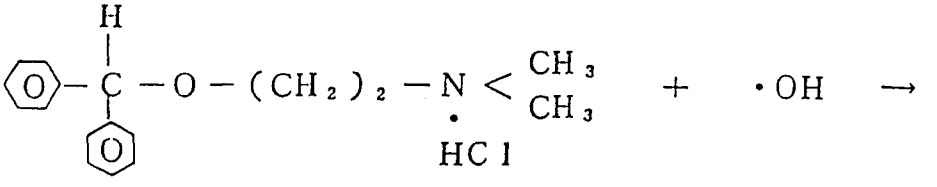
This experiment was carried out without any protection of the "S" and "L" samples. They were exposed to the heat directly without enclosure. This study was designed for measuring reliable reaction rate constants that can be used for the calculation of shelf life. It was a means of getting information about the stability of the drug per se. It was also an application of utilizing the knowledge and techniques of both analytical chemistry and chemical kinetics, even though the determination of the reaction order or mechanism was not within the scope of this study. To determine the order and mechanism of the decomposition reaction, the active should be decomposed more than than 25% (i.e. extend the reaction curve to much lower potency than 75%) using much more virgorous conditions instead of these elevated temperatures. Usually the oxidation, hydrolysis or even reduction at higher temperature is carried out purposely. If the decomposition products could be isolated and their structures could be elucidated (e.g. by GC/MS and/or NMR), then the mechanism could be possibly deduced.

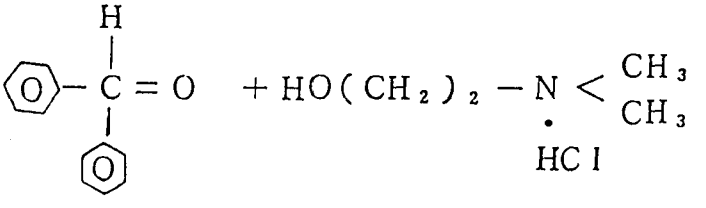
Different chemists may have different opinions in writing possible reaction mechanisms based only on the experimental rate equations. The rate of a zero-order reaction is independent of the concentration of the reactant, so that the rate must be controlled by

something other than collisions that involve reactant. The controlling factors might be the amount of water (moisture), oxygen, light available or other ingredients present and are invovled in the rate-determining step (slow step). If the rate reaction causing the decomposition of diphenhydramine hydrochloride were zero order, the mechanisms could be proposed as follows:

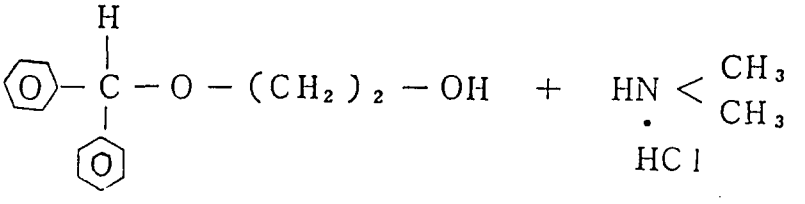
$$O_2 \xrightarrow[\text{step}]{\text{slow}} O\cdot + O\cdot \quad \text{or,} \quad O_3 \xrightarrow[\text{step}]{\text{slow}} O_2 + O\cdot$$

$$H_2O + O\cdot \rightarrow HO\cdot + \cdot OH$$



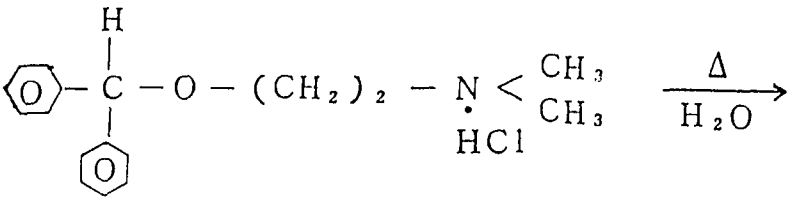


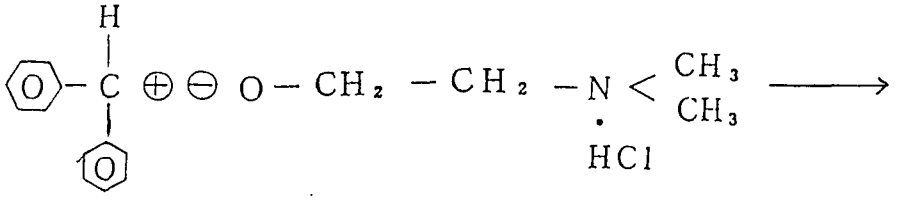
or,

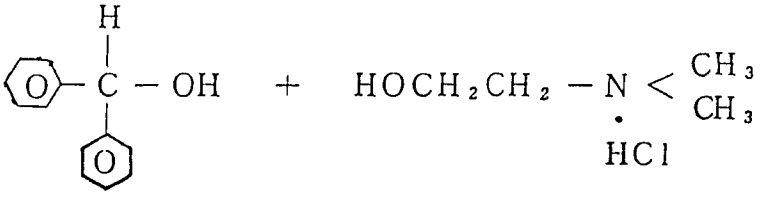


Diphenhydramine hydrochloride is not involved in the rate determining step, therefore it's a zero-order reaction with respect to the compound.

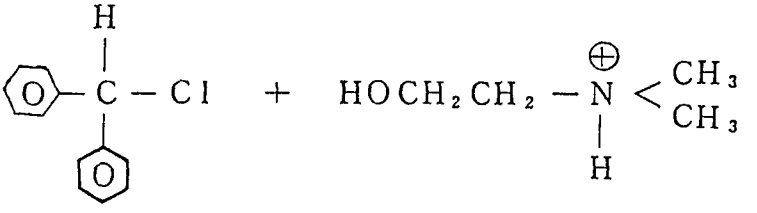
If the decomposition reaction of diphenhydramine were first-order, the following mechanism could be proposed:







or,



Since the purpose of this study was the prediction of shelf life which did not cover the whole range of rate curve as illustrated in Figure 21. We can not really conclude if the actual decomposition of diphenhydramine hydrochloride follows the zero- or first-order rate reaction. The mechanisms can only be proposed under the assumption of zero- or first-order reaction. Isolation and analysis of the decomposition products as mentioned before, or addition of initiator/scanvanger are the best ways to deduce the actual mechanisms. They are not within the scope of this study, but can be investigated in the future project titled such as " Decomposition mechanisms of diphenhydramine hydrochloride ".

To our knowledge, the stability prdiction of diphenhydramine hydrochloride has not been reported in the literature. The HPLC methods for analysis of this compound have been seen in the literature. However, none of them is really suitable for this kind of stability analysis as mentioned in II. INTRODUCTION of PART ONE.

For kinetic study of pharmaceutical that are relatively stable and changing on a small scale, the precision of the sample preparation and method are very important. The method precision as well as other validations (112) were performed in PART ONE.

The sampling precision was well considered and tested in PART TWO. The homogenous "L" solution and properly ground "S" powder were taken accurately in the beginning of this entire study so that the best initial sample consistency was obtained and the problem experienced in the preliminary study was avoided. Futhermore, the two ratioplots performed for every diphenhydramine peak as mentioned in IV.F. of PART ONE make this method more stability-indicative. With some or little modification(s) this whole method established will also be suitable for the stability analysis and shelf-life prediction of diphenhydramine hydrochloride contained in other drug matrices.

MULTI-POINT CALIBRATION (MPCAL)

What does MPCAL do? Fits a set of x,y pairs to a polynomial of varying degree

```
   Set-up and/or process multi-component calibration file
   Example: RU,MPCAL

 '/'(=More help), or KEY, or <cr> : /

   Prompt:                Response:
 CALIBR FILE NAME:        AAAAAA -- 6 character file name

 SECURITY CODE:           AAA.... -- 10 characters
 TITLE:                   AAAA... -- 30 characters
 NO COMP'S, NO POINTS:    [# of components],[  of points]
 COMPONENT 01 NAMED:      AAAA... -- 28 characters max ("/" first = blank
                                     init,)
            .
            .

 '/'(=More help), or KEY, or <cr> : /

 CALIBR CODE, COEF'S:  N, A0,A1,A2..... (N=calibration curve code 0 - 8)
            where:

                  N=0 -- piecewise linear; N=1 -- least-square thru orig.
                  N=2 thru 8 -- polynomial least-squares, degrees
 USER'S INITIALS:       ABC (ID code)

 '/'(=More help), or KEY, or <cr> :
 FUNCTION:       PR/PL/PC/CA/EN/MO,[P1],[P2],[P3],[P4]
                 |  |  |  |  |  |
                 |  |  |  |  |  | modify component calibration pts.
                 |  |  |  |  |  |    P1 = comp. name; P2,3 = point range
                 |  |  |  |  |  |    P4 = output LU; defs--all,1,NRC,LUcon.
                 |  |  |  |  |  |
                 |  |  |  |  |  |__ enter calibration pts.
                 |  |  |  |  |        def--all,1,NRC,none.
                 |  |  |  |  |
                 |  |  |  |  |___ compute calibration curve
                 |  |  |  |           def--all,1,NRC,LUcon.
                 |  |  |  |
                 |  |  |  |_____ plot inverse of calibration curve
                 |  |  |________ plot calibration curve
                 |  |                 def--none,1,NRC,LUcon.
                 |  |
                 |  |_____________ display calibration values
                 |                    def--all,1,NRC,LUcon.
```

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