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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF DIPHENHYDRAMINE IN LIQUID AND SOLID DRUG DOSAGE FORMS AND ITS APPLICATION TO STABILITY TESTING

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ABSTRACT

A simple, precise, rugged, stability-indicating method for diphenhydramine in liquid and solid drug preparations based on reversed phase ion-pair HPLC is described. The eluent used with the C8-bonded phase column, was acetonitrile/0.005 M aq. hexanesulfonic acid/acetic acid (70/30/1,v/v). The liquid product was simply dissolved in acetonitrile/water/acetic acid (70/30/1,v/v) and filtered; the solid product was briefly ground, dissolved in the same solvent, and filtered. The precision (rsd) of the method for diphenhydramine in the liquid sample is 0.52%, and

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0.50% for the solid sample. The method is linear up to 200 μg/mL. Based on spiking studies, the recoveries are 100.1% for the liquid sample, and 99.2% for the solid sample. The method was applied to the study of the thermal stability of the drug by following the degradation of diphenhydramine in the two products at temperatures in the 42°C to 62°C range for up to 16 weeks. Shelf lives of the products were determined assuming zero and first order kinetics of decomposition, and are at least 163 days for the liquid product, and at least 255 days for the solid product.

INTRODUCTION

Diphenhydramine [2-(diphenyl-methoxy)-N,N-dimethyl ethanamine] is an antihistamine widely used in over-the-counter (OTC) and prescription cough and cold preparations. A variety of methods for its determination has been published. The USP Official Monographs (1) use non-aqueous titration and UV-spectrophotometric procedures. Various techniques such as atomic absorption spectrophotometry (2), polarography (3), ion-selective electrodes (4), flow injection analysis with turbidimetric detection (5), TLC-densitometry (6), and gas chromatography (7) have been applied. However, the most useful procedures, i.e. those that are precise, accurate, rugged, and stability-indicating, are based on high performance liquid chromatography (HPLC) . These include high performance ion-exchange (8) and ion-pair reversed phase HPLC (9-11). Various ion-pairing reagents have been evaluated. Mahato et al. (9) used sodium dioctylsulfosuccinate at pH 2.5 to separate diphenhydramine from chlorpheniramine; the chromatographic efficiency was poor. The diphenhydramine eluted just

between solvent peak and the chlorpheniramine peak. Surmann and Glienke (10) evaluated various quaternary ammonium trifluoromethyl sulfonates for the best separation of diphenhydramine, dequalinium chloride, and dexamethacon; no quantitative results were reported. Lau et al. (11) found sodium dioctylsulfosuccinate at pH 4.6 in an aqueous methanol/THF eluent best for separating 8 ingredients, including diphenhydramine, in cold-cough mixtures. Thus none of these reported methods is truly specific for stability analysis of diphenhydramine alone in drug products. We report here a method for diphenhydramine in liquid and solid dosage forms, which is rapid, accurate, precise, simple, and suitable for stability testing. The method is applied to the study of the thermal stability of the drug, and the shelf-lives of two OTC products determined.

MATERIALS AND METHODS

Chemicals and Reagents - Diphenhydramine hydrochloride USP reference standard was purchased from the USP (Rockville, MD). OTC samples of a liquid product (Benadryl Elixer; label claim, 12.5 mg diphenhydramine/ 5 mL) and a solid product (Sominex; label claim, 25 mg/tablet) were purchased locally. The ion-pairing reagent, hexanesulfonic acid sodium salt, was obtained from Eastman Kodak (Rochester, NY). HPLC grade acetonitrile (B&J Chrompure) was purchased from B&J (Albany, NY). Glacial acetic acid was ACS reagent grade and was obtained from Fisher Scientific (Pittsburgh, PA).

Instrumentation - The HPLC components included a Waters 6000A pump, a Waters 710B WISP autoinjector, and a Waters 490 multiple channel variable wavelength UV-visible detector (Waters Associates, Milford, MA).

Peak areas were determined and chromatograms recorded using a Hewlett-Packard 3390A recording integrator (Hewlett-Packard, Avondale, PA). Based on the UV spectrum of diphenhydramine taken on a Hewlett-Packard 8450 UV-Vis spectrophotometer, the UV detector was set at 258 nm, and for the ratioplot method of verifying peak purity (12, 13), absorbances at 252 nm and 264 nm were used in addition to that at 258 nm.

HPLC Conditions - The column was 25 cm x 4.6 mm id 10 μm Lichrosorb RP8 (Alltech Associates, Deerfield, IL). The mobile phase was composed of acetonitrile/0.005 M aq. sodium hexanesulfonate/acetic acid (70/30/1 v/v), degassed under vacuum and filtered through a 0.45 μm filter. The eluent flow rate was 1.8 mL/min. The auto-injected sample size was 15 μL. The diluent (simulated mobile phase) used to dissolve samples was acetonitrile/distilled water/acetic acid (70/30/1 v/v). The retention time of diphenhydramine under these conditions was 7.4 min, i.e. k' = 3.3.

Standards - For quantitation, a solution containing 100μg/mL diphenhydramine in diluent was prepared from accurately weighed USP reference standard material. To test the linearity of the method, 8 solutions of the reference standard in diluent were prepared over the range of concentrations from 25 to 450 μg/mL.

Sample Preparation - The compound of interest must be completely dissolved and free of particulate matter before injection. The liquid sample was simply diluted 1:25 in diluent in a 50 mL volumetric flask; based on the package label this solution should contain about 100 μg/mL. For the solid sample, 24 tablets were accurately weighed, placed in a Krupp electric coffee grinder, and ground for 3-20 sec pulses. About 90 mg of powder (equivalent to about 1/5th of a tablet) was accurately weighed and diluted with diluent to volume in a 50 mL volumetic flask; based on the package

label the concentration should be about 100 µg/mL. The solution was mixed well, filtered through a 0.45 µm filter, and placed into the WISP autosampler.

The solid sample must be ground to a fine powder to enable complete extraction of the drug. However, if ground excessively, surface charges are generated on the particles. Such static charges cause clumping of the particles, which can lead to a non-homogeneous distribution of the drug in the powder. To test the effect of grinding time, 24 accurately weighed tablets were placed in the grinder. The grinder was pulsed on for three 20 sec periods; half of the powder was removed and labeled Composite I. The remaining sample was ground for two additional 20 sec periods, removed, and labeled Composite II. For the determination, 7 portions of about 90 mg of each composite powder were accurately weighed and treated as above.

Quantitation - The weight of diphenhydramine in a sample was calculated from direct comparison of peak areas of the sample and the standard, and the known weight of the drug in the standard.

Ratioplot - For reliable stability testing, it is necessary to verify that the chromatographic peak of interest is that of the active drug ingredient alone, uncontaminated with degradation products or impurities. The "ratioplot" method (12, 13) is often useful for this verification. The chromatogram is monitored at two or more wavelengths and the ratio of absorbances at each wavelength is recorded. If the peak is pure diphenhydramine, the ratio of absorbances will be constant and independent of the amount of drug eluting, i.e. the ratio will be the same at the start, top, and end of the peak. This produces a ratioplot in the form of a square wave, the middle of which occurs at a time equal to the retention time of the symmetrical chromatographic peak. If the active drug peak is contaminated with another

substance whose UV spectrum is not identical to that of the drug, the ratio of absorbances will not be constant over the peak. In this case, the ratioplot will be a distorted square wave . Two separate ratioplots were obtained here using the Waters 490 detector, one corresponding to the ratio of the absorbance at 258 nm to that at 252 nm, A_{258}/A_{252}, and the other to A_{258}/A_{264}.

Stability Testing - Federal regulations (14, 15) require expiration dating and stability information on pharmaceutical packaging and information sheets. A study of the stability of drug substances towards degradation by heat, light, humidity, and/or other conditions must be carried out by the manufacturer to determine the product shelf life, i.e. the time required for the potency of the drug to degrade to 90% of its original activity, $t_{0.90}$ (16, 17). For efficiency of testing, the tests are usually carried out under accelerated conditions, and the results extrapolated to ambient conditions. Straightforward chemical kinetics are used to carry out the extrapolation. One has to measure or assume the order of the reaction. If the rate-determining step in the decomposition does not involve the active drug substance, the concentration of the drug, [A], at time t, relative to its initial concentration, $[A]_o$, is given by

$$[A]/[A]_o = -k_o t \quad (1)$$

where k_o is the rate constant for a zero order reaction. In this case a plot of drug concentration vs. time should be linear with a negative slope. If the decomposition reaction rate is proportional to the concentration of the drug, then

$$\ln [A] = \ln [A]_o - k_1 t \quad (2)$$

where k_1 is the rate constant for a first order reaction, and a plot of the ln[A] vs. time should be linear. In the case of thermal degradation, tests are conducted at temperatures above ambient. Rate constants, k, vary with temperature according to the Arrhenius equation (14, 15),

$$\ln k = \ln s - E_a / RT \quad (3)$$

where s is a constant, E_a is the energy of activation of the degradation reaction, T is the absolute temperature, and R is the gas constant, 1.987 cal/mol-deg. Thus rate constants measured at higher temperatures can be extrapolated to ambient temperature, 25°C, at which drug products are assumed to be stored in practice.

To evaluate the HPLC method described above for this application, the two products were subjected to thermal stability tests in which they were stored for up to 16 weeks at 42°C, 52°C, and 62°C in Lab Line model 3400 ovens capable of temperature control to ±1.0°C. There is a potential pitfall in testing stability of these products. The liquid product contains ethanol in the solvent. Thus if one batch each of the liquid product was stored in the three ovens, and samples removed periodically from these batches, the resulting weight loss from evaporation of ethanol over time would obfuscate the quantitative analysis of the active ingredient. In order to circumvent this problem, initially 2.0 mL of liquid product was pipetted into each of twenty-three 50 mL volumetric flasks. Seven of these flasks were placed in the 42°C oven, and 8 flasks each were placed in the ovens at the two higher temperatures studied. Flasks were removed at 7- to 26-day intervals, starting at the 15th day for the 42°C oven, the 21st day for the 52°C oven, and the 7th day for the warmest oven. The contents were diluted to volume

with diluent, and analyzed directly using the method described to determine the rate of decomposition, and thus the shelf life.

The potential problems for the solid sample are its heterogeneity, and the loss of water on heating. To overcome these problems, 24 tablets were briefly ground into powder, mixed well, and twenty-three 90 mg portions accurately weighed into volumetric flasks. Again, 7 flasks were placed into the 43°C oven and 8 each into the other two ovens. Flasks were removed at 7- to 21-day intervals, starting on the 14th day for the 42°C oven, the 27th day for the 52°C oven, and the 15th day for the 62°C oven. The contents were treated as above for quantitative analysis. Weight change by loss of water through evaporation is not a problem because it was determined that drying is complete by the 14th day at 42°C.

RESULTS AND DISCUSSION

HPLC - Typical chromatograms are shown in Figure 1 for the standard and the two commercial product extracts. The diphenhydramine is well-separated from any interfering peaks.

Solid sample preparation - Seven preparations and HPLC analyses of each Composite were carried out. The recoveries of the two were identical, but the rsd (n=7) of the results for Composite I, which was ground for a total of 60 sec, was 0.56%, whereas that for Composite II (100 sec total grinding) was 2.1%. Thus overgrinding is disadvantageous, presumably because of static charge-induced sample heterogeneity. Thus three-20 sec pulses of the grinder were used for the determinations.

Linearity - The peak areas of the standards at concentrations from 50 to 450 µg/mL were plotted using a least squares program. Negative deviations

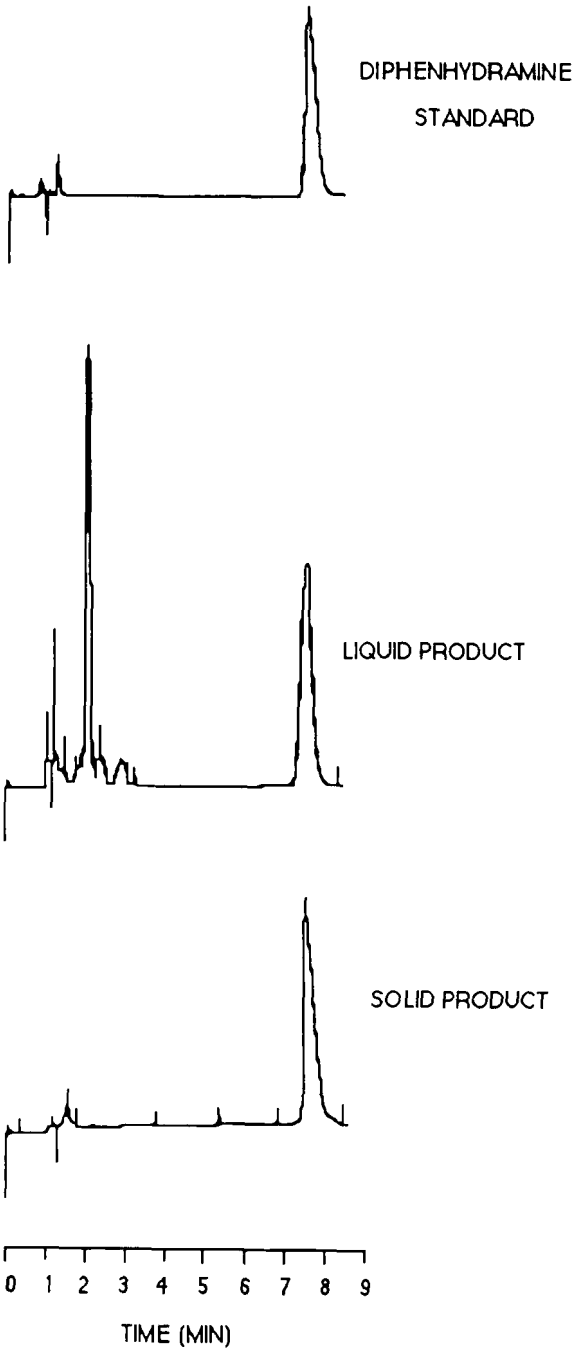

FIGURE 1

HPLC chromatograms of the diphenhydramine standard, a commercial liquid product, and a commercial solid product. The diphenhydramine elutes at about 7.4 min.

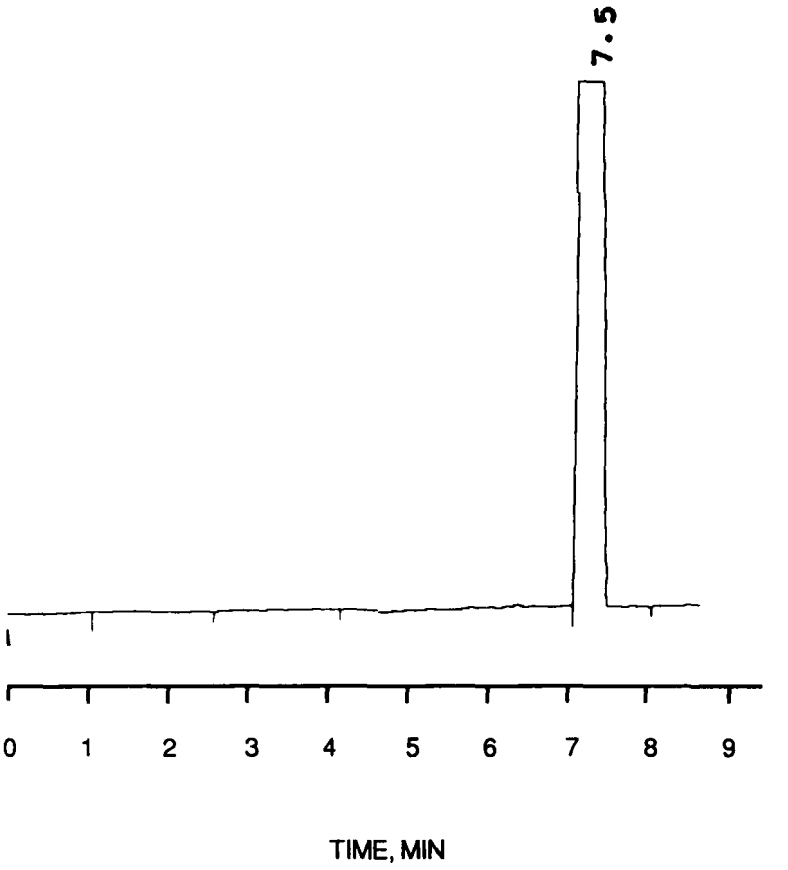

FIGURE 2

Ratioplot chromatogram of the commercial solid sample. The wavelengths used were 258 nm and 252 nm.

from Beer's Law occurred at concentrations exceeding about 200 μg/mL. Seven standard solutions in the concentration range of 25 to 180 μg/mL were reinjected. The calibration plot was linear with r = 0.987. Thus making sample solutions at concentrations near 100 μg/mL puts them into the middle of the linear calibration plot.

Precision - The precision of the method was determined by preparing 7 sample solutions each of the liquid and solid samples, and following the entire procedure. The rsd for the liquid sample was 0.52%, and that for the solid sample 0.50%.

Recovery study - Because no placebo (all the product ingredients except the active drug) was available for these commercial products, a spiking study was used instead. Three samples each of the liquid and solid samples were spiked with an increment of USP diphenhydramine equal to the amount of the drug in the product, and diluted with twice as much diluent to bring the concentration to about 100 µg/mL. Recoveries averaged 100.1% for the liquid sample and 99.2% for the solid.

Stability tests - It should first be noted that in all cases, the ratioplots were undistorted square waves, indicating purity of the diphenhydramine peak. An example is shown in Figure 2 for the solid sample. Analytical results at each temperature were plotted both as % of initial concentration of diphenhydramine vs. days of storage, corresponding to zero order kinetics, and as log (% of initial concentration) vs. time, corresponding to first order decomposition. It was not possible in the present case to distinguish between the reaction orders from the experimental plots because they were both sensibly linear, i.e. the change in concentration was small over the time interval studied. The rate constants at the 3 temperatures were calculated from the slopes of these lines. In turn, a plot of ln k vs. 1/T allowed extrapolation of k to 25°C. The shelf life at 25°C was then calculated: for zero order kinetics, from equation (1), $t_{0.90} = (90 - 100)/k_0$, which gives a shelf life of 163 days for the liquid sample, and 255 days for the solid tablets. If first order kinetics apply, then from equation (2), $t_{0.90} = (\ln 90 - \ln 100)/k_1$, which yields a shelf life of 173 days for the liquid and

268 days for the solid.The former leads to a more conservative estimate of shelf life, although in fact the differences in predicted shelf life are quite small. As expected, the solid product is more stable than the liquid product. If zero order kinetics are assumed, the activation energy E_a for the liquid sample is 6.0 kcal/mol, and 5.9 kcal/mol for the solid sample. For first order kinetics, E_a is 6.6 and 6.4 kcal/mol, respectively. Since E_a refers to the decomposition of the active ingredient alone, the values should agree between the two types of samples, as is observed.

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