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Title: QUANTITATIVE GAS CHROMATOGRAPHIC ANALYSIS OF EPHEDRINE HYDROCHLORIDE, PHENOBARBITAL, AND THEOPHYLLINE SUSPENSION

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A gas chromatographic method for the analysis of ephedrine hydrochloride, phenobarbital, and theophylline in suspension was developed. The method involved extraction with chloroform for the separation of ephedrine into one fraction and phenobarbital and theophylline into a second fraction. An internal standard, α-naphthylamine, was added to the ephedrine solution and this was gas chromatographed onto a packed column of OV-17 on Gas-Chrom Q at a temperature of 150° C. Hexobarbital was used as an internal standard for the solution containing phenobarbital and theophylline and this solution was gas chromatographed onto the same packed column at a temperature of 200° C. The recovery and precision of the procedure were determined by analyzing six replicate synthetic mixtures. The recoveries were found to be 99.2% for ephedrine hydrochloride, 97.5% for phenobarbital, and 100.3% for theophylline. The coefficients of

variation, which are measurements of precision, were found to be ±0.6, ±1.0, and ±0.1% respectively.

The synthetic mixtures were also subjected to an ultraviolet spectrophotometric assay method. Through extraction, ephedrine, phenobarbital, and theophylline were separated from each other into three fractions. The ultraviolet spectrophotometric absorbance values of the aqueous extracts of phenobarbital and theophylline were each directly determined. Ephedrine was converted to benzaldehyde prior to the determination of its absorbance value. The recoveries were found to be 101.7% for ephedrine hydrochloride, 110.0% for phenobarbital, and 104.8% for theophylline. The coefficients of variation were ± 1.1, ± 1.8, and ± 0.2% respectively.

Tedral, a commercial suspension, was assayed using both the gas chromatographic and ultraviolet spectrophotometric methods. The coefficients of variation found using the chromatographic method were ± 0.8, ± 1.4, and ±0.3%, while those obtained using the spectrophotometric method were ± 0.9, ± 1.3, and ± 0.4% respectively.

The gas chromatographic method, when compared to the ultraviolet spectrophotometric method, was found to be superior in the recovery of the three drugs. Also, the gas chromatographic method gave more precise results than the ultraviolet spectrophotometric method for two drugs, ephedrine hydrochloride and theophylline. Overall, the gas chromatographic method appears to be an accurate

and convenient method for the assay of ephedrine hydrochloride, phenobarbital, and theophylline suspension. With slight modification, it would be expected to give an acceptable method for the assay of the tablet dosage form of these drugs.

Quantitative Gas Chromatographic Analysis of Ephedrine Hydrochloride, Phenobarbital, and Theophylline Suspension

by

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QUANTITATIVE GAS CHROMATOGRAPHIC ANALYSIS OF EPHEDRINE HYDROCHLORIDE, PHENOBARBITAL, AND THEOPHYLLINE SUSPENSION

INTRODUCTION

Ephedrine hydrochloride, phenobarbital, and theophylline is a combination of drugs frequently used for their action of sedation and bronchodilation. Although two dosage forms, suspension and tablet, are widely used, only the tablet form is official in N. F. XIII (3). The official monograph of this preparation uses column chromatography and ultraviolet spectroscopy for the procedures of identification and assay. This requires several time-consuming steps. Furthermore, the method is not adaptable to the suspension form. At present, the N. F. has not found an acceptable analytical procedure for the assay of the suspension.[1] It is for this reason that the suspension form has not been included in N. F. XIII.

The use of gas chromatography in pharmaceutical analysis has proven highly successful in the past decade. It has become a powerful tool for both research and routine analytical work and is the most extensively employed of all separation methods involving instruments.

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[1] Personal communication from Dr. John V. Bergen, Director of the National Formulary (N. F.), to Dr. H. Wayne Schultz, School of Pharmacy, Oregon State University.

Undoubtedly, gas chromatographic methods will be increasingly recognized by the official compendia in the future.

The purpose of this investigation was to develop a gas chromatographic assay which could be acceptable for use as an official method for suspensions containing ephedrine hydrochloride, phenobarbital, and theophylline. A variety of conditions were investigated to find those which would be accurate and relatively easy to perform.

REVIEW OF LITERATURE

Gas Chromatography

Gas chromatography is an analytical method for separating mixtures of volatile substances. A component passes through the column at a rate dependent on its volatility and interaction with the stationary phase. The component will partially dissolve in the liquid and/or will adsorb onto the solid surface where it may again be vaporized into the gas stream. Molecules with the greater solubility and interaction with the stationary phase will stay longer in the column. The separation of two or more components is achieved when they have different times of removal from the column.

The two major functions of gas chromatography are: (1) the separation of the sample components by the column, and (2) the detection and measurement of these components by the detector.

Column

The column used in gas liquid chromatography may be either a packed or capillary type. The former is packed with an inert solid support which is coated with a nonvolatile liquid. In a capillary column the wall of the column acts as a solid support and a liquid is used to coat the inner walls of the column.

The column may be made of glass, copper, or stainless steel in the shape of a spiral, straight "U", or multiple "U". The diameter of the packed column may range from 4 to 12 mm. with length up to 50 ft. (22). The capillary column may be as long as 50 m. with the internal diameter as small as 0.25 mm (71). The advantage of the capillary column is that it provides high efficiency in terms of the large number of theoretical plates (22).

Solid Support

The solid support provides a surface area for holding the liquid film in place. Dal Nogare and Juvet (22) described the features of a good solid support to be: (1) chemically inert, (2) thermostable, (3) mechanically strong, and (4) having a high surface area. The materials commonly used are metal, glass, Teflon, firebricks, and diatomaceous earth (22). Metal, glass, and Teflon possess inert properties and cause no tailing, but they are very difficult to coat. Diatomaceous earth is most frequently used because it possesses most of the desired properties. In some systems the support will adsorb the solute and cause tailing of the peak. Pretreatment of the solid support may be necessary in order to reduce its surface activity. This may be done by silanizing the active site (18) or by removing the active sites through washing with acid (18) or alkali (10).

Gas-Chrom (Applied Science Laboratories) is a commercial solid support prepared by flux-calcining diatomaceous earth. The flux-calcined diatomaceous earth is called Celatom (Eagle-Picher Industries, Inc.). Gas-Chrom S is prepared by carefully screening Celatom. Gas-Chrom A is prepared by washing Gas-Chrom S with acid. The acid washing neutralizes Celatom and removes iron and other acid soluble minerals from the support surface. Gas-Chrom P is Gas-Chrom A which has been washed with base to remove organic contaminants. Gas-Chrom Q is Gas-Chrom P which has been silanized. This renders Celatom hydrophobic and reduces the adsorption of a polar solute. It is an especially useful commercial solid support because the pretreatment minimizes both the tailing of the peak and the catalytic decomposition of sensitive samples.

Liquid Phase

The liquid phase should have the features of being nonvolatile, stable at column temperature, and possess selectivity toward the sample components (22). A practical rule regarding the choice of a liquid phase is "like dissolves like." A nonpolar liquid phase separates nonpolar compounds, while a polar liquid phase separates polar compounds. The choice of a stationary phase includes: paraffinic hydrocarbons, such as squalane, which has specificity for nonpolar solutes; polyglycols, which are preferable for polar compounds; polyesters,

which are selective for fatty acid esters; and silicones, which are non-selective (39). In pharmaceutical analysis silicones are widely used because of their nonselective nature, low volatility, and high thermal stability. A popular commercial silicone is OV-17 (Applied Science Laboratories), a phenyl methyl silicone which may be used up to the temperature of 350° C (33, 40).

Column Performance

The separation of sample components by gas chromatograph is dependent upon the performance of the column. Martin and Synge (50) and later, Mayer and Tompkins (52) and Klinkenberg and Sjenitzer (41) developed the "Plate Theory" which provided a theoretical basis for column performance. According to this theory, the column is pictured as a series of plates with the solute entering the first plate in equilibrium with the gas phase. Some of the solute in the gas phase is swept onto the second plate where again it equilibrates with the stationary phase. The solute that remains in the first plate will equilibrate with the entering fresh gas. This process is repeated as solute moves continually to another plate. The concentration of solute in the first plate becomes less and less until virtually none remains. The separation of two solutes is achieved when one moves faster than the other. In the case of two very similar solutes, the separation may be

made by using a very long column or a column with a large number of plates.

It is more convenient to express the column performance in terms of "Number of Plates" or "Column Efficiency." As the number of plates increases, the column efficiency is increased. The number of plates or column efficiency can be calculated from the formula

$$N = 16\ (t_R/y)^2$$

where N = column efficiency, t_R = the retention time or distance, and y = the width of the peak base in the same unit as t_R.

Van Deemter, Zuiderweg, and Klinkenberg (78) related column packing, gaseous diffusion, and resistance to mass transfer to the column efficiency. This is now referred to as the van Deemter Equation,

$$H = A + \frac{B}{U} + CU,$$

where H = HETP or height equivalent to a theoretical plate.

A = The Eddy diffusion term, which involves the size and uniformity of solid support.

B = The molecular diffusion of the solute in the gas phase.

C = The resistance to mass transfer of the solute in the liquid and gas phases.

U = The carrier gas flow rate.

Using the equation, when the A term is minimized (for example, by the reduction of the solid support particle size), the plate height

will be minimized. Bohemen and Purnell (14) showed that uniformity of the particle size was also of great importance. With high carrier gas flow rate (U), the $\frac{B}{U}$ term is lowered, but the CU term becomes larger. When HETP is at its minimum, the column will be at its highest efficiency because $N = \frac{L}{H}$, where N = column efficiency, L = length of column, and H = HETP. It may be seen from the plot shown in Figure 1 that the optimum gas velocity occurs at the minimum HETP.

Detectors

The requirements for a good detector are the sum of many individual properties. Included are versatility, simplicity, safety, low cost, rapid response, good stability, and high sensitivity (22, 39).

Detectors may be classified into two general types, integral and differential. Integral detectors measure the total amount of the sample in the effluent gas. Examples of this type include the recording titration buret used by James and Martin (37) for the estimation of organic acids and bases, and the nitrometer used by Janák (38) for the estimation of gases. The integral detectors have low sensitivity and find limited application.

Differential detectors measure the instantaneous concentration of the effluent gas. There have been many types of these detectors developed. Examples of this type include the thermal conductivity cell,

the hydrogen flame detector, the argon ionization detector, the flame ionization detector, and the electron capture detector (22). Among these, the three most widely used detectors are thermal conductivity, flame ionization, and electron capture.

Thermal conductivity cells (TC) are the most widely used of the various detectors (22). They consist of an electrically heated metal filament which is situated in the gas stream. The resistance of the filament is measured by a Wheatstone bridge. When the pure carrier gas passes through the cell a constant heat, depending on the thermal conductivity of the carrier gas, is lost from the heated filament. When the carrier gas containing the solute vapor passes through the detector, the thermal conductivity of the carrier gas is changed by the presence of the solute and either more or less heat from the filament will be lost. This change in temperature will cause a change in the Wheatstone bridge, which in turn causes the balance of the Wheatstone bridge to change and a recording is made. Although thermal conductivity cells are popular detectors, their sensitivity is low. Dimbat, Porter, and Stross (23) reported a sensitivity of only 312 ml mv/mg with a background noise level of 0.005 mv. These detectors are of general applicability and will respond only to gases that conduct heat. They are most extensively employed in the analysis of permanent gases (45).

The hydrogen flam ionization detector was developed by McWilliam and Dewar in 1958 (56). This detector is based upon the

formation of ions when the effluent from the column is pyrolyzed in a flame. These ions, when collected at charged electrodes, will produce an ionization current, which is amplified and recorded. This detector offers the advantages of simplicity of design, linearity of response, low cost, and high sensitivity. McWilliam and Dewar reported the sensitivity value of their dual flame ionization detector to be 1×10^9 ml mv/mg. This was obtained from the injection of 0.01 ml. of a solution of 1 part of diethyl ether in 10^7 part of ethyl acetate. The detector responds to all organic compounds containing CH groups, but gives no response to inorganic compounds, such as water or carbon dioxide (45). This lack of response is useful because these materials do not interfere in the results when present as contaminants. This detector, due to its high sensitivity, may detect slight bleeding of the stationary phase which will result in background noise. A successful technique to overcome this difficulty is known as dual column compensation (56). In this modification, two similar columns are filled with the same packing and the two signals are placed in opposition so that the net signal is zero which will give a flat baseline.

The third widely used detector, the electron capture detector, was developed by Lovelock and Lipsky in 1959 (47). A carrier gas, such as hydrogen or nitrogen, is ionized by a radioactive source and free electrons are formed. When a solute vapor enters the detector some of the free electrons will be captured, resulting in a decrease

in the cell current which is recorded. The electron capture detector responds only to molecules containing electronegative elements, such as halogens, oxygen, and nitrogen. They are useful in the analysis of fluorocarbons and insecticides of the chlorinated hydrocarbon type.

Gas Chromatography as an Analytical Tool

Gas chromatographic analysis is used qualitatively in the identification of the sample components and quantitatively in the estimations of the amount of selected components.

Qualitative

The qualitative use of gas chromatographic analysis is based upon the retention behavior of the solute (22). When the solute is known with some certainty, identification may be established by comparing its retention time or retention volume to that of a pure known sample. Identical retention values are found for identical materials. Identification may also be made by the addition of a pure constituent to the unknown. If the materials are identical, the height of the peak will be increased (39). Additionally, gas chromatography may be used for separating components which are trapped and identified by mass spectrum (2) or infrared spectrum (5).

Quantitative

The quantitative determination of a component is based upon the relationship of peak area or peak height to concentration. The area of peaks may be determined by several different means. These include: (1) using a planimeter, (2) cutting the peak from the paper and weighing it, (3) employing an automatic integrating device, or (4) making a geometrical approximation, such as the product of peak height and width at half-height (22). The height of peaks is determined by the distance from the peak maximum to the baseline of the peak. It is easier and more accurate to measure the peak height of a completely resolved peak that rises from a perfectly flat baseline. In practice, these conditions do not always occur. Sometimes sloping baselines appear because of the tailing of another peak. The peak height in this case may be determined by the method of Hawkes and Russel (34). In this method, a straight line is drawn across the base of the peak and another line is drawn parallel to the sloping baseline and tangent to the peak top. The peak height is measured as the vertical height from the sloping baseline to the point on the peak which is tangent to the second line.

Both the peak height and the peak area are influenced by temperature and gas flow rate. When these conditions are changed, the response or sensitivity of the detector will change, and this results in a change of the peak height and peak area. Thus, in order to achieve

consistent and accurate results, the conditions of temperature and gas flow rate must be kept constant. An internal standard may be used to reduce these variables. Changes in the operating conditions affect, in the same manner, both the sample peak and the internal standard peak. The ratio of the sample peak to the standard peak remains the same, regardless of the operating condition. In addition, the absolute sample size need not be accurately measured. The requirements for the selection of an internal standard include the following: (1) its peak should be completely resolved from the peaks of the other components of the mixture, (2) it should be eluted close to the components of interest, and (3) the ratio of the internal standard peak to that of the solute should be close to unity (22).

A calibration curve showing the relationship between peak height and sample concentration may be determined by chromatographing a series of mixtures containing various known amounts of solute with a known amount of internal standard. The sample/internal standard peak height ratio is plotted against the amount of solute in the mixture. A similar procedure is utilized for an unknown sample. The concentration of the unknown may be determined upon comparison of the unknown/internal standard peak height ratio to the values found in the calibration curve. When the calibration curve is a straight line, the concentration of the unknown sample may be calculated directly from the formula:

$$\frac{\text{Peak height ratio of standard}}{\text{Concentration of standard}} = \frac{\text{Peak height ratio of unknown}}{\text{Concentration of unknown}}$$

History of Gas Chromatography in Analytical Methods

The first concept of gas chromatography resulted in 1941 from the suggestion of Martin and Synge (50) that volatile substances might be separated by using gas as a mobile phase. Eleven years later, James and Martin (37) described the first gas chromatographic apparatus which used a recording titration buret detector for the separation and estimation of organic acids. Ray (63, 64) reported in 1954 that gas chromatography was not restricted to the analyses of acids and bases. Using a thermal conductivity detector, hydrocarbons, ethers, and alcohols were analyzed. He also introduced such improvements as a rubber septum at the injection port and the use of an internal standard for quantitative determinations.

Although thermal conductivity cells are popular detectors, their sensitivity is low. The first major increase in sensitivity was made by Lovelock in 1958 with his invention of the argon ionization detector (46). This detector is widely used for gas chromatography of biological amines. Another high sensitivity detector is the flame ionization detector developed by McWilliam and Dewar in 1958 (56). In addition to its high sensitivity, flame ionization detector shows specificity toward organic compounds. Later, in 1959, Lovelock and Lipsky (47)

developed the electron capture detector which shows selectivity toward electronegative elements such as halogens, oxygen, and nitrogen.

An important development in columns was reported by Golay in 1958 (31). He proposed that capillary columns would provide a very high efficiency, with plate numbers approaching a million theoretical plates. The capillary column has since become popular for the separation of closely related compounds, such as mixtures occurring in the petrochemical field.

Tailing of peaks is a major problem in gas chromatography because it distorts the symmetry of peaks and makes the quantitation difficult. Tailing results from the adsorption of solutes on the solid support. This is particularly noticeable in the chromatography of polar compounds when the stationary liquid is nonpolar. Many methods have been developed to reduce this tailing. Silanization was first used by Horning, Moscatelli, and Sweeley in 1959 to render the column hydrophobic (35). Rader and Aranda (62) added a more polar liquid phase to a nonpolar column in order to saturate the active site on the solid support. Stevenson (69) converted phenobarbital into methylphenobarbital, a less polar derivative.

History of Gas Chromatography in Pharmaceutical Analysis

The first pharmaceutical application of gas chromatography was in 1959 when Brealey, Elvidge, and Proctor (16) reported the analysis

of chloroform in a variety of aqueous preparations. Other investigations which soon followed were concerned with such volatile materials as chloroform, ether, and essential oils (7, 12). Gas chromatography was later applied to other pharmaceutical substances, such as barbiturates (60), steroids (57), narcotic analgesics (58), and antihistamines (53). Throughout the years, gas chromatography has been mainly used for the separation and identification of active ingredients and impurities. It was not until 1960, when Wesselman (80) reported on the determination of alcohol in liquid formulations, that quantitative gas chromatography was introduced into pharmaceutical analysis. Using gas chromatography, he was able to assay ethanol in less than half an hour as compared with at least one hour for the then official U. S. P. and N. F. distillation method. The method of Wesselman, upon refinement, became the N. F. XIII method for the ethanol assay of such preparations as Thimerosal Tincture, Nitromersol Tincture, Acetaminophen Elixir, and Aprobarbital Elixir (3).

The use of gas chromatography for the quality control of a drug during manufacturing was reported by Parker, Fontan, and Kirk in 1962 (59). Following the storage of tranquilizers, they found in the gas chromatogram, new small secondary peaks which increased in height with the passage of time. This would suggest that drug decomposition products and rates could be determined through the use of gas chromatography.

Gas chromatography is finding increasing use in the field of drug detection. Beckett and Rowland (9) reported that colorimetric and spectrophotometric methods for the determination of amphetamine in urine and blood were nonspecific (55, 65). They developed a sensitive and specific gas chromatographic method which gave a recovery of 100 ±5%. Their method has been refined and improved by many workers. As an example, Lebish, Finkle, and Brackett (44) were able to obtain a retention time of less than four minutes for amphetamine. The determination of morphine and heroin in biological materials requires 12-24 hours by paper chromatography and 4-5 hours by thin layer chromatography (49). A gas chromatographic technique providing quantitative results within 15 minutes was developed for these materials (58). Farmilo, McConnell, and Davis (29) found that due to difference in climatic conditions, the proportion of the resin components of marihuana varied with the area in which the plant was grown. Using gas chromatography, information could be obtained for determining the origin of seized marihuana. A rapid gas chromatographic method for the determination of trace amounts of lysergic acid diethylamide (LSD) was developed following the discovery of illegally sold LSD in sugar cubes (61). In this method, results were obtained within four minutes.

Survey of Available Quantitative Methods

The existing procedures for the assay of ephedrine hydrochloride,

phenobarbital, and theophylline individually and in combinations with each other may be classified according to the following methods:

1. Volumetry
2. Argentimetry
3. Gravimetry
4. Spectrophotometry
5. Gas chromatography

Volumetric Assay of Ephedrine Hydrochloride

Although ephedrine is a basic amine, it is not sufficiently basic to allow aqueous titration. A widely used method involves the use of a nonaqueous solvent, such as chloroform. In the N. F. XIII (3) and U. S. P. XVIII (77) assay for the various ephedrine dosage forms, the sample is dissolved in chloroform and titrated with perchloric acid using methyl red as the indicator.

Gravimetric Assay of Ephedrine Hydrochloride

The usual method of gravimetric analysis involves dissolving the sample in water, adding base, and extracting with an organic solvent. The extract is treated with hydrochloric acid to form the hydrochloride salt, and evaporated. The residue is dried and weighed (20).

Spectrophotometric Assay of Ephedrine Hydrochloride

The first ultraviolet spectrophotometric determination of ephedrine was performed by Elvidge in 1940 (26). This method showed a low order of molar absorptivity and was nonspecific for ephedrine. These characteristics make the direct determination of samples containing a low concentration of ephedrine difficult. Increased values in absorptivity and sensitivity may be obtained by converting the ephedrine into derivatives (19, 72, 79). Procedures of this type are useful for the determination of ephedrine in dosage forms as well as in biological specimens.

Gas Chromatographic Assay of Ephedrine Hydrochloride

The first significant gas chromatographic study of ephedrine was by Fales and Pisano (28). Using a SE-30 (General Electric) silicone rubber column, it was found that ephedrine gave peak tailing due to its adsorption on the solid support of Gas-Chrom P. A procedure using a polar Carbowax column containing potassium hydroxide was reported by Beckett and Wilkinson in 1965 (10) to eliminate peak tailing. Rader and Aranda (62) reduced peak tailing by saturating the active site of the nonpolar SE-52 silicone rubber gum column with the polar polyester liquid phase of HI-EFF-8BP (Applied Science). They found that phenobarbital and theophylline could also be eluted from this mixed polar-nonpolar column with a 96% recovery of phenobarbital, 100% of

theophylline, and 97% of ephedrine. As another means, peak tailing may be minimized by converting ephedrine into an acetyl derivative (18, 44).

Volumetric Assay of Phenobarbital

Simple titration procedures are frequently utilized for the assay of phenobarbital. The various dosage forms in the U. S. P. XVIII (77) containing phenobarbital involve this method. Nonaqueous media are also used for the analysis of phenobarbital. Autian and Allen (6) developed a nonaqueous potentiometric method using chloroform and polyethylene glycol as the solvent and sodium methoxide as the titrant. This method could not be performed visually because of the unavailability of a suitable indicator for the end-point determination. Leavitt and Autian (43) later showed that, by changing the solvent to benzene-propanol-2 mixture and the titrant to tetra-n-butylammonium hydroxide, both potentiometric and visual titration could be performed with rapidity and high accuracy.

Argentimetric Assay of Phenobarbital

Mattocks and Voshall (51) developed an argentimetric method which gave greater accuracy than the official gravimetric method found in the U. S. P. XIII for phenobarbital elixir (75). They potentiometrically titrated phenobarbital, which was in a sodium carbonate solution,

with silver nitrate. Later, in 1956, Bodin (13) found that a more reproducible end point could be achieved by including a blank determination for each sample.

Gravimetric Assay of Phenobarbital

Phenobarbital may be extracted from an aqueous acidified solution with an organic solvent such as chloroform. Upon evaporation of the solvent, the residue is dried and weighed. A gravimetric method of this type was the assay given in the U. S. P. XIII for phenobarbital elixir (75).

Spectrophotometric Assay of Phenobarbital

Early ultraviolet spectrophotometric procedures for the determination of phenobarbital lacked specificity and sensitivity. Goldbaum in 1952 (32) reported a method that was accurate and specific for the quantitative determination of small amounts of phenobarbital, such as may be found in blood. The absorbance of phenobarbital in a solution having pH 10 was measured at 260 mμ and a second measurement was made of phenobarbital in a strong alkaline solution. The concentration of phenobarbital was calculated from the difference that occurred in the two absorbances.

Gas Chromatographic Assay of Phenobarbital

In recent years gas chromatography has been extensively investigated for the analysis of phenobarbital. Direct methods of this type have utilized low concentration of such liquid phases as DC-200 silicone fluid (4), SE-52 silicone rubber gum (70), and OV-17 silicone polymer (40). In these methods the resulting peaks showed some tailing because of the partial adsorption of phenobarbital onto the solid support. Allen (1) and Leach and Toseland (42) obtained good separation with symmetrical peaks upon using a higher load of the liquid phase. Peak tailing may also be eliminated by silanizing the support material (42, 70) or by converting the sample into a less polar derivative. The methyl derivative (8, 30) of phenobarbital is more volatile and may be gas chromatographed with less adsorption than phenobarbital. The first on-column methylation of phenobarbital was reported by Stevenson in 1966 (69). In this method, the sample was methylated by pyrolysis of its tetramethylammonium salt. Brochmann-Hanssen and Oke (17) applied Stevenson's method to other salts and found the tetramethylanilinium salt to be superior. This method may be applied quantitatively to theophylline as well.

Volumetric Assay of Theophylline

Nonaqueous titration methods may be used for the analysis of theophylline. These methods overcome the low solubility and weak

acidity of theophylline which occur in aqueous solutions. McEniry (54) reported a sharp end point and 99.8% recovery by a nonaqueous titration method involving dimethylformamide as the solvent and sodium methoxide as the titrant.

Argentimetric Assay of Theophylline

An argentimetric method for the assay of theophylline was developed by Stevens and Wilson in 1937 (68). This later became official in the U. S. P. XI (74) and has been continued through the U. S. P. XVIII (77). In this method, theophylline is dissolved in ammonium hydroxide and an excess of silver nitrate is added to form a precipitate of silver theophylline. The excess silver nitrate is titrated with ammonium thiocyanate.

Gravimetric Assay of Theophylline

Theophylline may be easily methylated quantitatively with dimethyl sulfate. The product, caffeine, is extracted, the solvent evaporated, the residue dried and weighed (67). Schmitt (66) reported a method involving the precipitation of theophylline with silver ammonium chloride. The excess silver was determined by acidifying the filtrate and weighing the precipitated silver chloride. This method showed specificity for the determination of theophylline in ethylenediamine theophylline.

Spectrophotometric Assay of Theophylline

A spectrophotometric method was developed by Comer and Hilty in 1954 (21). This method was based on the absorption of theophylline in 0.1 N hydrochloric acid at 270 mμ. The method was found to be more precise than the argentimetric method and required only one-fourth of the time.

Gas Chromatographic Assay of Theophylline

Theophylline, because of its polar and acidic nature, tends to be adsorbed on a solid support. This results in tailing of the peak and a partial loss of material. The problem may be overcome by converting theophylline into a suitable nonpolar derivative, such as caffeine. On-column methylation of theophylline into caffeine was reported by MacGee (48), who pyrolyzed the theophylline tetramethylammonium salt on a carbowax column. Brochmann-Hanssen and Oke (17) showed better results upon using a SE-30 silicone rubber gum column and the trimethylanilinium salt of theophylline.

Gas Chromatographic Assay of Ephedrine Hydrochloride, Phenobarbital, and Theophylline Tablets

Elefant, Chafetz, and Talmage (25) used gas chromatography for the direct assay of phenobarbital and theophylline contained in tablets of ephedrine hydrochloride, phenobarbital, and theophylline. An

indirect method was used for the assay of ephedrine hydrochloride. They applied the method of Tompsett (72) for the periodate oxidation of ephedrine into benzaldehyde prior to chromatographing. Based upon ten assays, each ingredient was determined with a relative standard deviation of about 1%.

EXPERIMENTAL

Apparatus

The gas chromatograph used for this investigation was a Hewlett Packard Model 402 equipped with a dual hydrogen flame ionization detector. Attached to this instrument was a Hewlett Packard recorder which performed at 4×10^{-12} ampere for a 1 mv. full scale output.

Column Preparation

Gas-Chrom Q, mesh size 100-120, coated with 3% OV-17 was obtained from Applied Science Laboratories Incorporated. The material was in the form of white granular, free flowing beads. The column used was a 6 ft. x 6mm. OD. (4mm. ID.) borosilicate glass U-tube. The column was thoroughly cleaned with toluene and dried before packing. The coated support was packed with the aid of a vibrator. Each end of the tube was plugged with silanized glass wool.

Column Conditioning

The column was conditioned in the oven for one hour at 100° C and the temperature was increased at a rate of 1° C per minute until a final temperature of 250° C was reached. The column was held at this temperature for 18 hours. The detector end of the column was not connected during the conditioning.

Determination of Optimum Nitrogen Flow Rate

The objective of this phase of study was to determine the optimum flow rate of the nitrogen carrier gas which would allow the column to operate at its highest efficiency.

Ephedrine Hydrochloride

Preliminary investigations showed that the injection of 4 μl. of ephedrine solution, equivalent to about 2 μg. of ephedrine hydrochloride, was the appropriate volume and concentration which would give a peak with a height of about one-half the chart width.

A solution containing 12 mg. of ephedrine hydrochloride in 5 ml. distilled water was placed into a separatory funnel, the pH was adjusted to 11 with 20% sodium hydroxide solution, and the solution was extracted with 2 x 12 ml. of chloroform (reagent grade). The extract was placed into a 25 ml. volumetric flask and made up to volume with chloroform. This solution was equivalent to about 2 μg. ephedrine hydrochloride in 4 μl.

The nitrogen flow rate was adjusted to 50 ml. per minute with the nitrogen rotameter knob of the machine. After the flow rate had stabilized, 4 μl. of the prepared solution was chromatographed. The flow rate was then increased by 4-8 ml. per minute and the injection was repeated. In this manner a series of injections were made over a

range of carrier gas flow rates. Three injections were performed for each flow rate and the average value was determined. The retention time (t_R) and base width (y) of each peak were measured. For each of the nitrogen flow rates, the number of theoretical plates, N, was calculated using $N = 16 (t_R/y)^2$. The height equivalent to a theoretical plate, H, was obtained by dividing the column length in centimeters by the number of plates. A curve of H values was plotted against the corresponding flow rates. The flow rate having the minimum H value was considered as the optimum rate. The results are shown in Figure 1.

Phenobarbital and Theophylline

The optimum nitrogen flow rates for phenobarbital and theophylline were each determined in the same manner as for ephedrine. About 4 μl. of phenobarbital solution containing about 0.7 μg. phenobarbital were injected each time. About 4 μl. of theophylline solution containing about 12 μg. theophylline were injected each time. For each of the solutions, an equal volume of chloroform and methanol was used as the solvent. The results obtained are shown in Figure 1.

Operating Conditions

Nitrogen flow	75 ml. per minute
Hydrogen flow	35 ml. per minute

Air flow	280 ml. per minute
Nitrogen pressure	40 psi.
Hydrogen pressure	10 psi.
Air pressure	20 psi.
Electrometer	
Range	10^2
Attenuation	from 1 to 4
Chart speed	0.25 inch per minute
Temperature for ephedrine assay	
Flash heater	200° C
Column	150° C
Detector	200° C
Temperature for phenobarbital and theophylline assay	
Flash heater	250° C
Column	200° C
Detector	250° C

The apparatus was allowed to stabilize for one hour before the first sample was injected.

Ephedrine Hydrochloride Standard Curve

α-Naphthylamine Internal Standard Solution

α-Naphthylamine was selected as an internal standard for

ephedrine. A chloroform solution containing 12.5 mg. per ml. α-naphthylamine was used.

Ephedrine Hydrochloride Standard Solution

Exactly 500.0 mg. Ephedrine hydrochloride U. S. P. was dissolved in 10 ml. distilled water contained in a 125 ml. separatory funnel. The pH was adjusted to 11 with 20% sodium hydroxide solution and the solution was extracted with 4 x 25 ml. chloroform (reagent grade). The extract was placed into a 100 ml. volumetric flask and made up to volume with chloroform. The concentration of the solution was 5 mg. ephedrine hydrochloride per ml. In order to minimize the effects of decomposition, ephedrine solution was either freshly prepared or stored in a refrigerator.

Gas Chromatographic Procedure

The following quantities of ephedrine standard solution were pipetted into six 10 ml. volumetric flasks: 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 ml. There was added to each flask 0.3 ml. of α-naphthylamine internal standard solution and sufficient chloroform to bring to volume. The concentrations prepared were: 0.25, 0.5, 1.0, 1.5, 2.0, and 2.5 mg. ephedrine hydrochloride per ml.

A 25 µl. syringe was used to inject 4 µl. of each of the prepared solutions into the gas chromatograph. This injected volume

represented the following quantities of ephedrine hydrochloride: 1.0, 2.0, 4.0, 6.0, 8.0, and 10.0 μg. Each of the various concentrations was injected four times. The peak heights were measured and the average value for each concentration was determined. A standard curve was obtained by plotting the peak height ratio of ephedrine hydrochloride to α-naphthylamine against the amount of ephedrine hydrochloride in μg. The standard curve and the chromatogram for these determinations are shown in Figure 2 and 5 respectively.

Phenobarbital Standard Curve

Hexobarbital Internal Standard Solution

Hexobarbital was selected as an internal standard for both phenobarbital and theophylline. The standard solution contained 0.2 mg. hexobarbital per ml. An equal volume of reagent chloroform and reagent methanol was used as the solvent.

Phenobarbital Standard Solution

A series of phenobarbital standard solutions containing the following concentrations of phenobarbital U. S. P. were prepared: 0.095, 0.18, 0.48, 0.96, 1.92, and 3.84 mg. per ml. The above hexobarbital internal standard solution was used as the solvent.

Gas Chromatographic Procedure

Data for the standard curve of phenobarbital was obtained using the same procedure as described for the ephedrine standard curve. The quantities of phenobarbital injected were 0.38, 0.72, 1.92, 3.84, 7.68, and 15.36 μg. The standard curve is shown in Figure 3 and the chromatogram in Figure 7.

Theophylline Standard Curve

Hexobarbital Internal Standard Solution

Hexobarbital was used as the internal standard for theophylline. The same solution as used for the phenobarbital standard curve was used.

Theophylline Standard Solution

A series of theophylline standard solutions containing the following concentrations of theophylline N. F. was prepared: 0.78, 1.56, 6.24, 15.6, 19.5, and 21.06 mg. per ml. The above hexobarbital internal standard solution was used as the solvent.

Gas Chromatographic Procedure

Data for the standard curve of theophylline was obtained using the same procedure as described for the ephedrine standard curve.

The quantities of theophylline injected were 3.12, 6.24, 24.96, 62.4, 78.0, and 84.24 μg. The standard curve is shown in Figure 4 and the chromatogram in Figure 7.

Gas Chromatographic Assay of a Synthetic Mixture

A synthetic mixture containing the same proportion of ephedrine hydrochloride, phenobarbital, and theophylline as contained in Tedral (Warner-Chilcott Laboratories), a commercial suspension, was formulated. This mixture was extracted and injected into the gas chromatograph in the same manner as that which was later utilized for the analysis of the commercial suspension.

Ephedrine Hydrochloride Assay

A 5 ml. mixture containing 4.0 mg. phenobarbital, 12.0 mg. ephedrine hydrochloride, and 65.0 mg. theophylline in distilled water was prepared. To this, 5 ml. distilled water was added, the pH adjusted to 11 with 20% sodium hydroxide solution, and the solution extracted with 2 x 12 ml. chloroform. The chloroform extract was placed into a 25 ml. volumetric flask containing 0.75 ml. of α-naphthylamine internal standard solution and made up to volume with chloroform. Four μl. of the solution was injected into the gas chromatograph. The injection represented 1.92 μg. of ephedrine hydrochloride. The results are given in Table 2.

Phenobarbital and Theophylline Assay

To the remaining aqueous phase from the above Ephedrine Hydrochloride Assay, 4 gm. of sodium chloride was added, the pH adjusted to 4.8 with dilute hydrochloric acid, and the mixture extracted with 4 x 50 ml. chloroform. The chloroform extract was placed into a 200 ml. volumetric flask and made up to volume with chloroform. Using a pipette, 9.0 ml. of this solution was placed in a test tube and the solution evaporated to dryness on a 65°C water bath. One ml. of the hexobarbital internal standard solution was pipetted into the tube to redissolve the residue. Four µl. of the solution was injected into the gas chromatograph. The injection represented 0.72 µg. of phenobarbital and 11.7 µg. theophylline. The results for phenobarbital are given in Table 4 and for theophylline in Table 6.

Ultraviolet Spectrophotometric Assay of a Synthetic Mixture

A synthetic mixture having the same composition as that previously described under Gas Chromatographic Assay of a Synthetic Mixture was assayed for each component by an ultraviolet spectrophotometric method provided by Warner-Chilcott Laboratories, the manufacturer of Tedral suspension (24). The procedure is shown in Appendix F.

Gas Chromatographic Assay of Tedral Suspension

Tedral suspension[2] was selected as the sample material. Using a pycnometer, the specific gravity of the suspension was determined to be 1.2783. Approximately 6.4 gm., or about 5 ml., of the sample was accurately weighed into a separatory funnel. The exact volume was determined by converting the weight into volume using the specific gravity value. Five ml. distilled water was added to the sample. The resulting mixture was extracted and gas chromatographed by the same procedure used for the analysis of the synthetic mixture. The results for ephedrine hydrochloride are given in Table 3 and Figure 6; for phenobarbital in Table 5 and Figure 8; and for theophylline in Table 7 and Figure 8.

Ultraviolet Spectrophotometric Assay of Tedral Suspension

Tedral suspension[2] was assayed for its components of ephedrine hydrochloride, phenobarbital, and theophylline. The procedure was the same as that used for the previously described Ultraviolet Spectrophotometric Assay of a Synthetic Mixture. The results for ephedrine hydrochloride are given in Table 3; for phenobarbital in Table 5; and for theophylline in Table 7.

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[2]Quality Control Number 5987042 A.

RESULTS AND DISCUSSION

Preliminary Considerations

Various stationary phases have been reported for the gas chromatographic analyses of ephedrine hydrochloride, phenobarbital, and theophylline. An alkaline-treated column appears to be generally useful for amines, such as ephedrine, because it prevents adsorption and allows a symmetrical peak to be obtained (11). However, it is unsuitable for the analysis of phenobarbital and theophylline which are acidic. Similarly, a column which has been acid-treated is not suitable for ephedrine analysis.

Liquid phases consisting of silicone materials have been considered to be nonselective (39). The silicone OV-17 has found widespread use in the analysis of barbiturates (40), and xanthines (33). Preliminary investigations (Appendix A) with OV-17 gave sharp resolved peaks for ephedrine, phenobarbital and theophylline. Elution times of less than 12 minutes were obtained. In addition to these characteristics, OV-17 has low volatility and may be employed at temperatures up to 350^{o} C. These properties are necessary in order to achieve a steady base line when a high sensitivity detector, such as flame ionization, is used.

Gas-Chrom Q was selected as a solid support because of its commercial availability in a pure form. Also, this material has been

silanized to reduce adsorptive effects which cause peak tailings.

Determination of Optimum Nitrogen Flow Rate

Nitrogen was used as the carrier gas in this investigation. The optimum velocity of nitrogen was determined from the data obtained upon comparing flow rates with HETP (Figure 1). The results, as shown in Figure 1, indicate that 73 ml. per minute was the optimum flow rate for ephedrine and phenobarbital; and 75 ml. per minute for theophylline. The optimum velocity should be selected for the component which is most difficult to separate (27). Also the analysis time is decreased when the velocity is increased. Therefore, the flow rate of 75 ml. per minute was selected for use in this experiment.

Determination of Optimum Temperature

A column temperature of 150° C was found to be most suitable for the ephedrine hydrochloride assay (Appendix B). A temperature lower than 150° C could be used, but the time of analysis would be increased unnecessarily. As shown in Figure 6, there were several components which were extracted in the solution containing ephedrine. As none of the peaks for these substances occurred in the ephedrine or α-naphyhylamine peaks, they were of no further interest.

A column temperature of 200° C was found to be most suitable for the separation of phenobarbital and theophylline (Appendix C).

The elution of these peaks was completed in about 12 minutes. At a higher temperature, theophylline could be eluted earlier as a sharper peak, but this peak was not completely resolved from that of phenobarbital.

Preparation of Standard Curves

Preliminary investigations showed that several substances (possibly flavoring or coloring agents), in addition to ephedrine, were extracted from the suspension. As their elution times were found to be shorter than that of ephedrine, the choice of an internal standard was restricted to a substance that would be eluted after ephedrine in a complete resolved manner.

In order to obtain maximum accuracy from an internal standard, it is necessary that the internal standard: (1) not be present in the sample, (2) be resolved from the sample peak, (3) be eluted close to the sample peak, (4) be similar in chemical nature to the sample. and (5) be available in a pure form. Several chemicals were investigated as potential internal standards (Appendix D). Included were the following: aniline, chloroaniline, methylaniline, ethylaniline, diphenylamine, diallylamine, α-naphthylamine, nicotine, and methyl laurate. It was found that aniline, chloroaniline, methylaniline, and ethylaniline gave peaks which appeared before ephedrine. These chemicals were eliminated from further consideration because their

peaks interfered with peaks occurring in the sample prior to the appearance of ephedrine. Nicotine and methyl laurate were not used because their peaks could not be resolved from the ephedrine peak. Diphenylamine, diallylamine, and α-naphthylamine were found to be eluted after ephedrine. However, the first two compounds were eluted from the column too slowly -- about ten minutes to one hour after ephedrine. The remaining chemical, α-naphthylamine, was selected as the internal standard. It was found to be eluted from the column in about four minutes after ephedrine. The peak was sharp and completely resolved from the ephedrine peak.

At 200° C, phenobarbital and theophylline were eluted from the column within 12 minutes and the peaks were completely resolved from each other. In selecting the internal standard, a substance was sought which would elute close to the sample peaks. As the phenobarbital and theophylline peaks were close to each other, it was necessary to select an internal standard which eluted either before phenobarbital or after theophylline. A material that would elute before phenobarbital was sought because the time of analysis would be reduced.

Several chemicals were investigated for use as an internal standard. Included were the following: pentobarbital, secobarbital, hexobarbital, anthracene, phenacetin, and nicotine (Appendix E). It was found that all of these substances gave peaks which were eluted from the column before phenobarbital and theophylline. Pentobarbital,

secobarbital, anthracene, phenacetin, and nicotine were eluted too close to the solvent peak for complete resolution. The remaining chemical, hexobarbital, was selected as the internal standard. It was found to give a sharp peak close to the phenobarbital and theophylline peaks and yet completely resolved from them (Figure 7).

There was found consistent linear detector response for ephedrine hydrochloride from 1.0 μg. to 10.0 μg; for phenobarbital from 0.0 μg. to 15.36 μg.; and for theophylline from 6.24 μg. to 78.0 μg. These may be seen from the standard curves of ephedrine hydrochloride, phenobarbital, and theophylline as shown in Figure 2, 3, and 4 respectively.

The chromatogram of standard ephedrine is shown in Figure 5. The chromatogram of phenobarbital and theophylline is shown in Figure 7. Retention data for these standards and the internal standards is shown in Table 1.

Table 1. Retention Times for Standards and Internal Standards.

	t_R (minutes)	t_R/t_R [a]
Ephedrine	2.0	0.363[b]
α-Naphthylamine	5.5	1.000
Hexobarbital	3.5	1.000
Phenobarbital	6.4	1.828[c]
Theophylline	9.4	2.685[c]

[a] Relative Retention Time
[b] Based on α-Naphthylamine = 1.000
[c] Based on Hexobarbital = 1.000

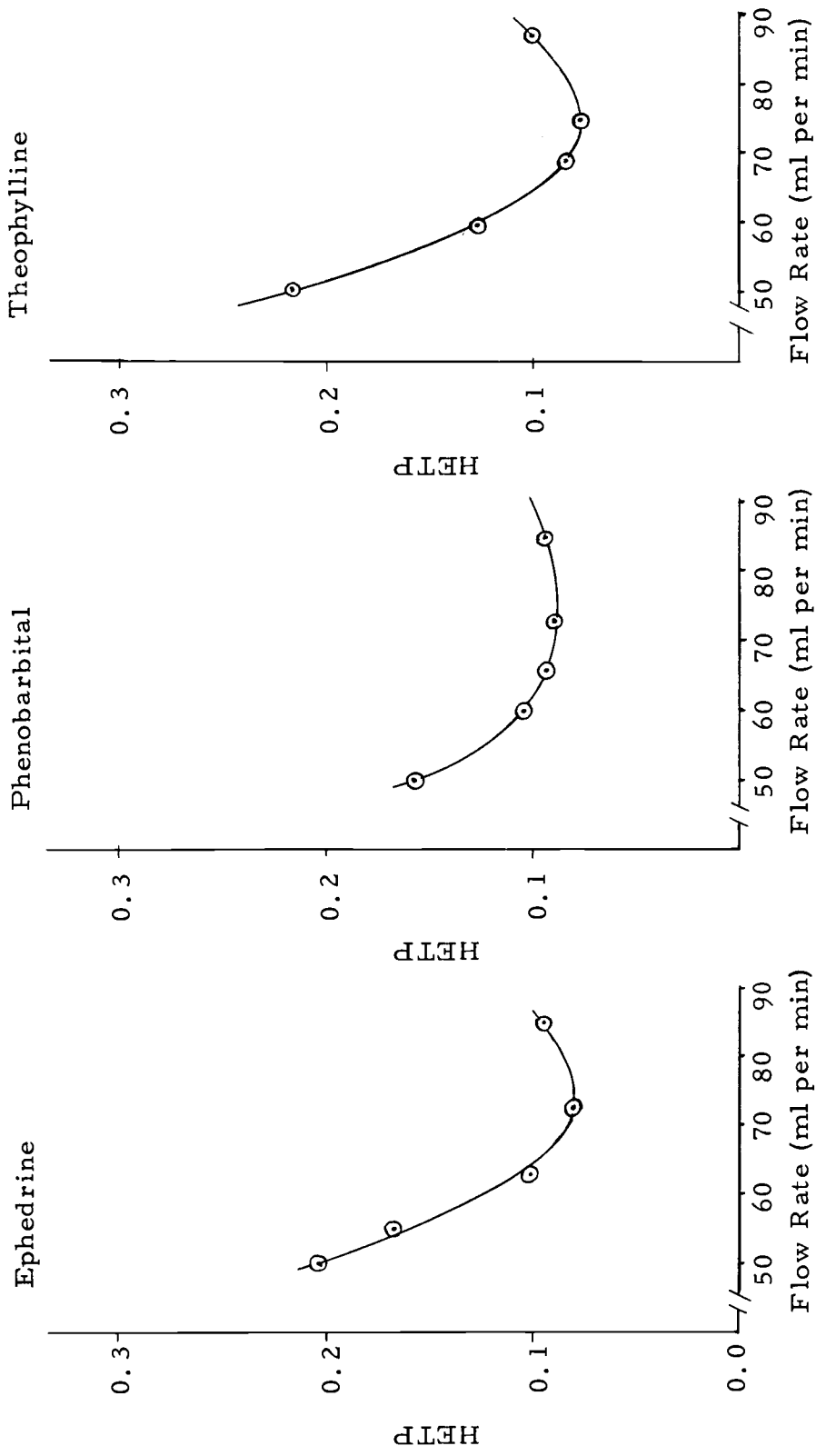

Figure 1. Effect of Nitrogen Flow Rates on HETP

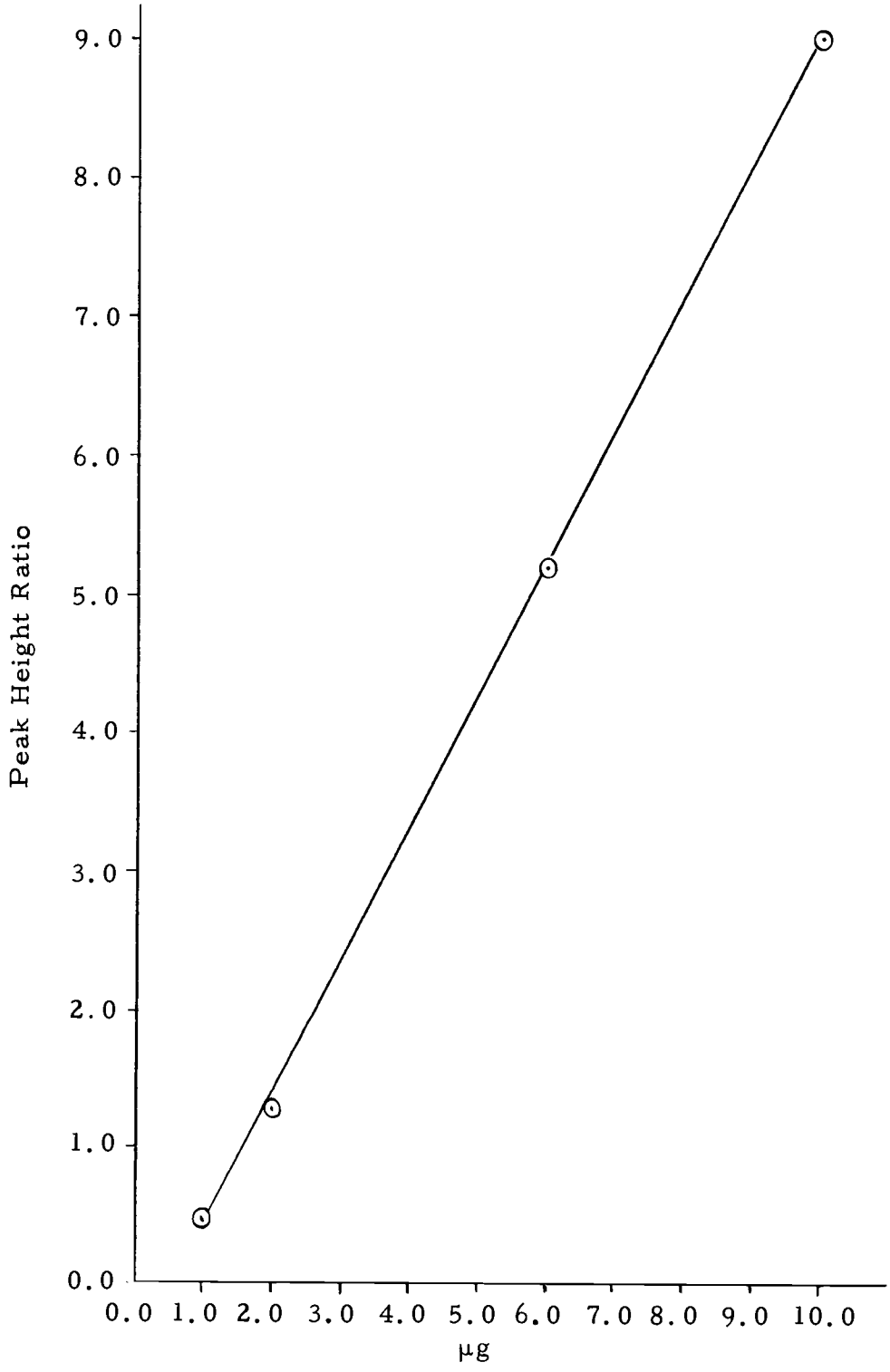

Figure 2. Standard Curve for Ephedrine Hydrochloride

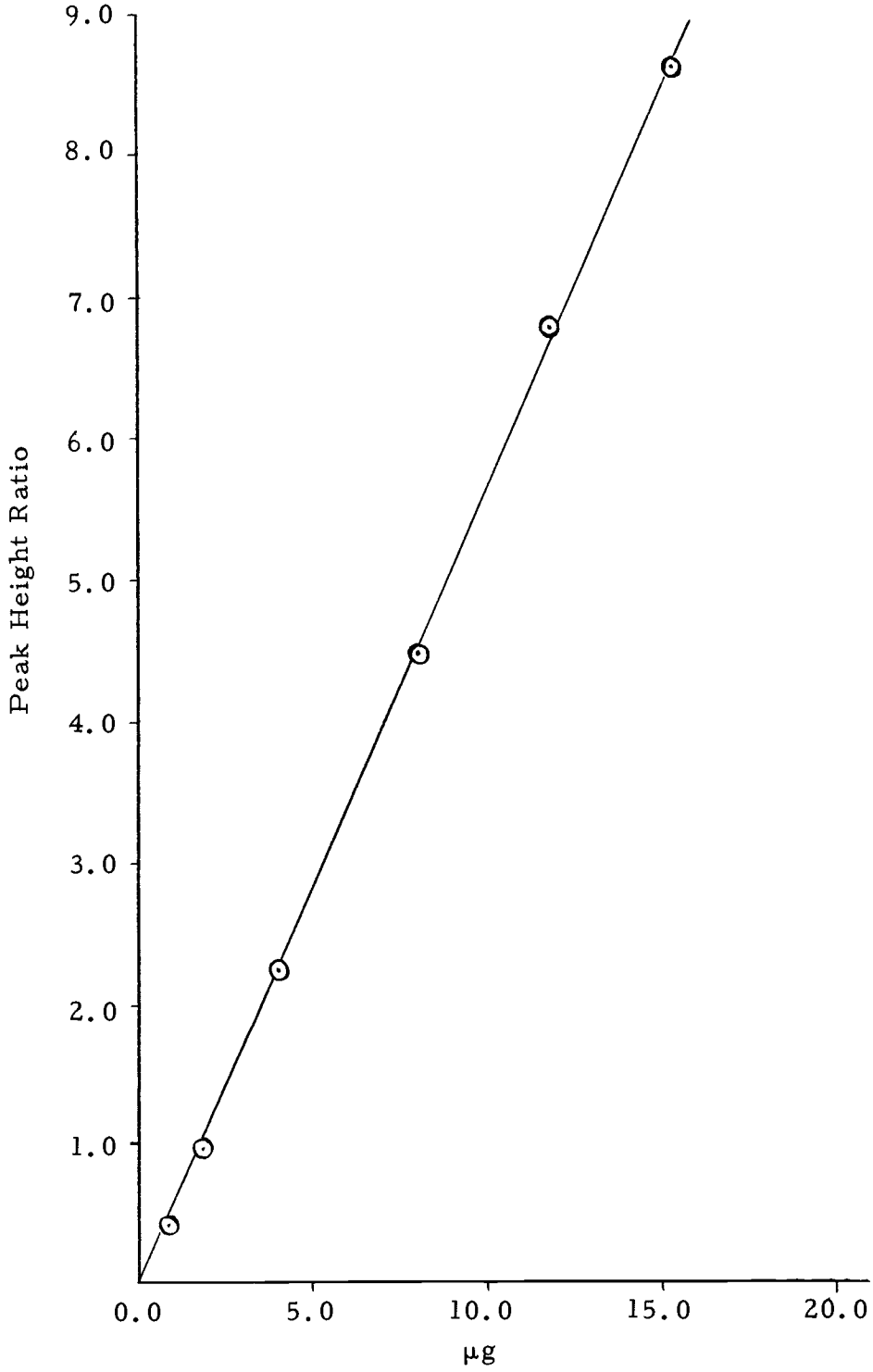

Figure 3. Standard Curve for Phenobarbital

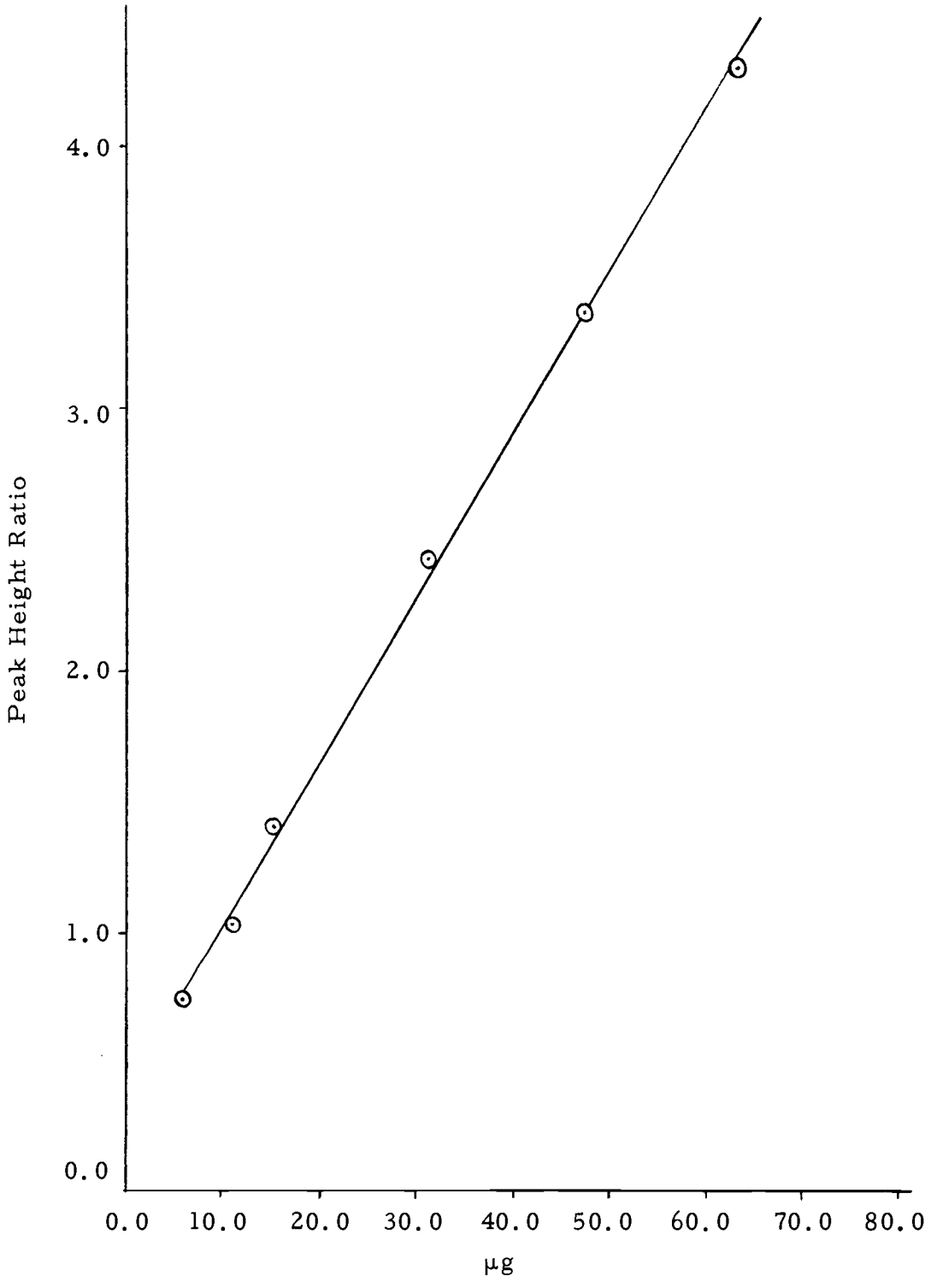

Figure 4. Standard Curve for Theophylline

Gas Chromatographic Assay

It was felt that a sample of ephedrine hydrochloride, phenobarbital, and theophylline suspension, because of its varied components, could not be analyzed by a single direct injection into the gas chromatograph. Therefore, an extraction procedure was incorporated into the assay. The extraction was based upon the acid-base properties of the drugs in the mixture. As ephedrine is basic and both phenobarbital and theophylline are acidic, it was necessary to use two different extractions. The volatility of the three drugs on the gas chromatographic column was an additional concern. They could not be injected simultaneously as a mixture because of the relatively high volatility of ephedrine. At a column temperature of 150° C, which was optimum for ephedrine, the theophylline peak did not appear within one hour. At a column temperature of 200° C, which was optimum for phenobarbital and theophylline, the ephedrine peak appeared in the solvent peak. Thus, it was necessary to include in the assay procedure two extractions and one gas chromatographic injection of each extract.

The mixture was made alkaline in order to extract ephedrine with chloroform. The remaining aqueous phase, containing phenobarbital and theophylline, was treated with sodium chloride in order to obtain greater separation when the mixture was extracted with chloroform.

Assay of Synthetic Mixture

The results of the assays are shown in Tables 2, 4, and 6. The mean recoveries from the gas chromatographic method for ephedrine hydrochloride, phenobarbital, and theophylline were 99.2 ± 0.6%, 97.5 ± 1.0%, and 100.3 ± 0.1% respectively. Those from the ultraviolet spectrophotometric method were 101.7 ± 1.1%, 110.0 ± 1.8%, and 104.8 ± 0.2% respectively. This indicated that the results obtained by gas chromatography were closer to the known amounts than those obtained by ultraviolet spectrophotometry. Also, the precision from the gas chromatographic method was greater.

Assay of Tedral Suspension

The precision of the two analytical methods was determined by analyzing six replicate samples. For the gas chromatographic method, each solution was injected four times and the average result was found. For the ultraviolet method one absorbance reading of each solution was made. The results are shown in Tables 3, 5, and 7. The coefficients of variation obtained from the gas chromatographic method for ephedrine hydrochloride, phenobarbital, and theophylline were ± 0.8, ± 1.4, and ± 0.3% respectively. Those from the spectrophotometric method were ± 0.9, ± 1.3, and ± 0.4% respectively. With the exception of phenobarbital, the gas chromatographic method gave more

precise results than the ultraviolet spectrophotometric method. The large variation of phenobarbital for the gas chromatographic method may have resulted from the small sample of phenobarbital injected into the gas chromatograph. For small injected samples, the relative adsorptive effect by the solid support is greater than when a larger amount is injected. A more concentrated solution could have been used, but this would have increased the amount of theophylline present in the solution. Preliminary investigations indicated that overloading of the column occurred when theophylline was injected in quantities greater than 12 μg. (Appendix G). Column overloading results in a skewness of the peak and this is undesirable for quantitative determination.

The chromatograms obtained from the gas chromatographic analyses are shown in Figure 5, 6, 7, and 8. The numbered peaks are identified as follows:

1. Unknown
2. Unknown
3. Unknown
4. Unknown
5. Unknown
6. Ephedrine
7. α-Naphthylamine
8. Hexobarbital

9. Phenobarbital

10. Theophylline

In the chromatograms there were obtained well-resolved symmetrical peaks for each of the three components. Several additional components (peaks number 1 through 5), possibly flavoring or coloring agents, were extracted by chloroform into the ephedrine extract. Complete elimination of these impurities was not attempted because they did not give interferences with either the ephedrine or the internal standard peaks.

The specificity toward the sample components by the gas chromatographic method was greater than that by ultraviolet spectrophotometric method. In addition, the retention behavior offered a means of identification for each of the components. The retention behavior is constant for each drug and could be duplicated in other laboratories using the same column and operating conditions.

Upon comparison of the two methods, the precision obtained from the gas chromatographic method was greater and the mean recoveries were closer to the known amounts than those obtained from the ultraviolet spectrophotometric method. Also, the developed gas chromatographic method was less time consuming. The major difference in time occurred because of the fewer extractions used by the gas chromatographic method. As an additional advantage, the reagents

required for the gas chromatographic method were those commonly found in most laboratories.

Overall, the developed gas chromatographic method appeared to be more useful for the assay of Tedral suspension than the manufacturer's ultraviolet spectrophotometric method. It is possible that, with minor modification, the gas chromatographic method could be adapted to the assay of ephedrine hydrochloride, phenobarbital, and theophylline tablets.

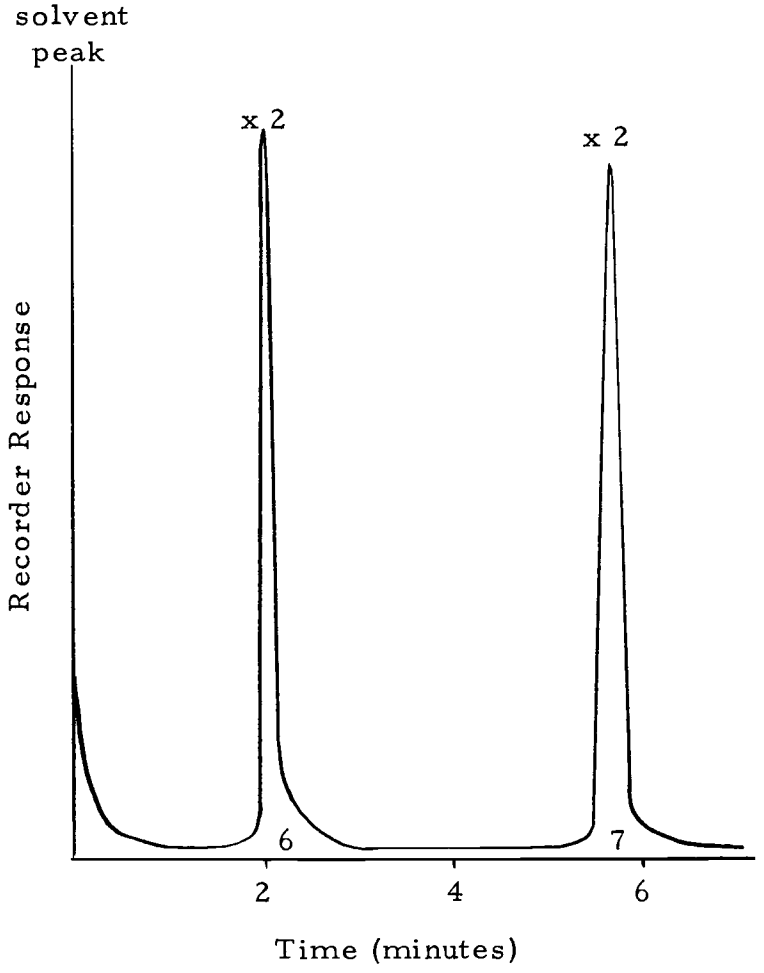

Figure 5. Gas Chromatogram for Standard Ephedrine Solution

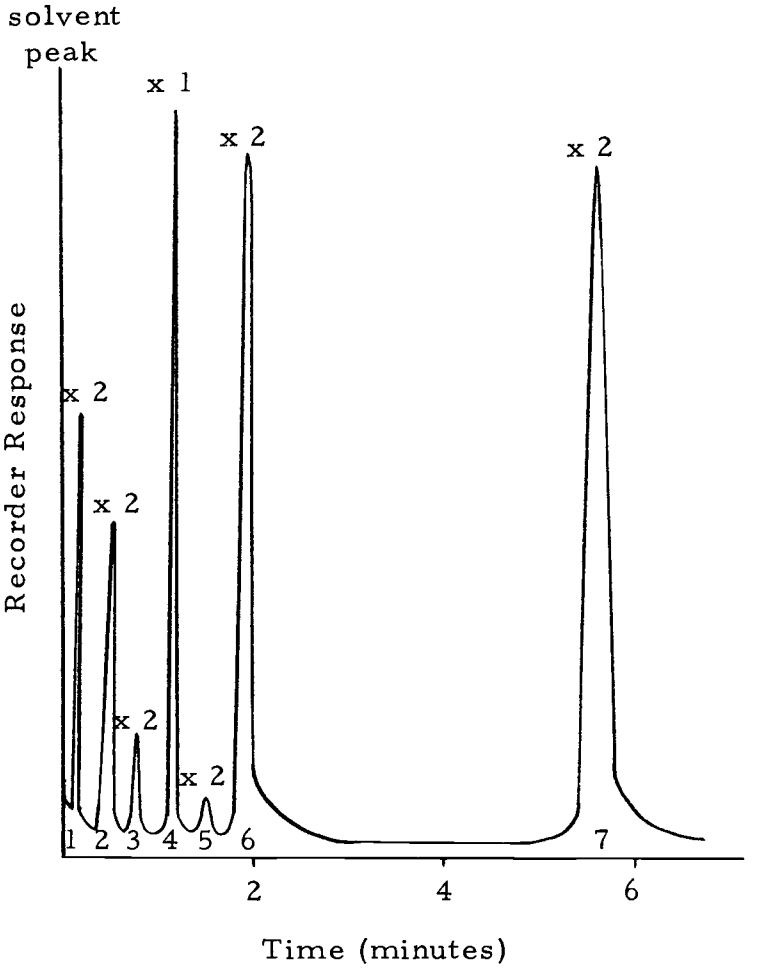

Figure 6. Gas Chromatogram for Ephedrine in Tedral Suspension

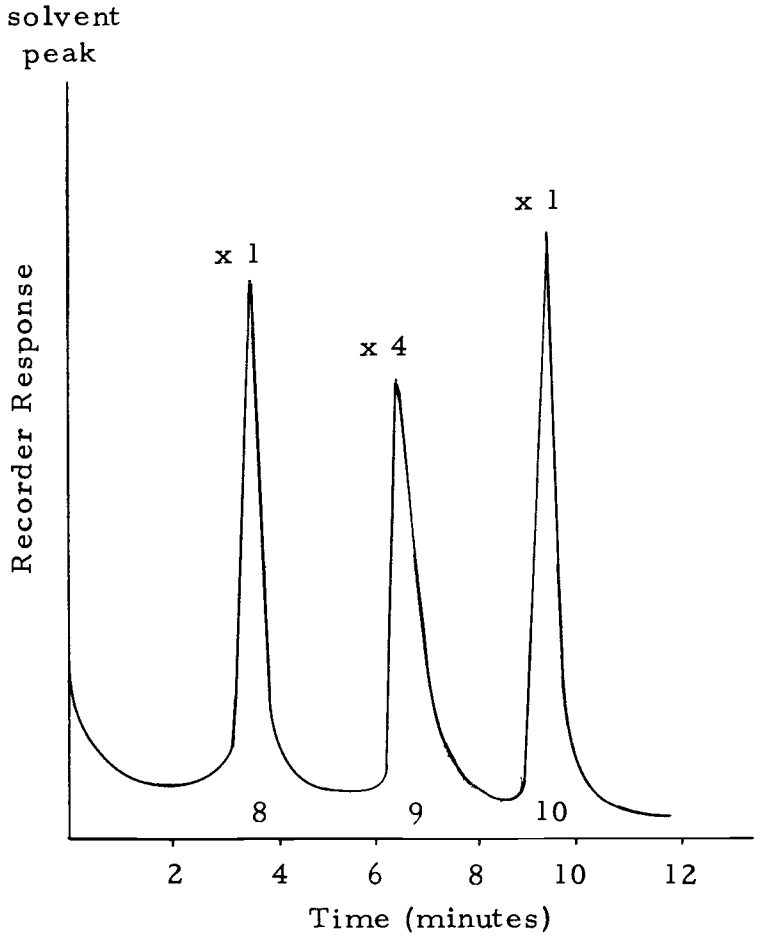

Figure 7. Gas Chromatogram for Standard Phenobarbital and Theophylline Solution

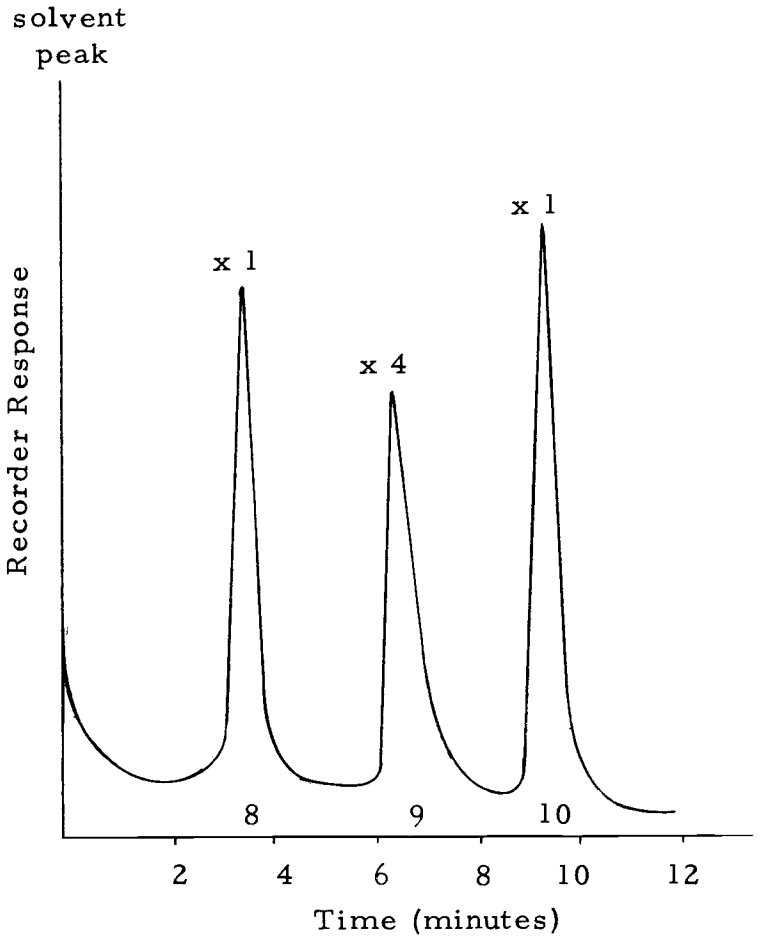

Figure 8. Gas Chromatogram for Phenobarbital and Theophylline in Tedral Suspension

Table 2. Analysis of Ephedrine Hydrochloride in Synthetic Mixtures by Ultraviolet Spectrophotometric and Gas Chromatographic Methods.

	UV Method		GC Method	
	Found (mg/5.0 ml)	Recovery[a] (%)	Found (mg/5.0 ml)	Recovery[a] (%)
Run 1	12.3		11.8	
2	12.3		11.8	
3	12.1		11.9	
4	12.2		11.9	
5	12.0		11.9	
6	12.1		11.9	
Mean	12.2	101.7	11.9	99.2
Standard deviation	±0.13		±0.07	
Coefficient of variation		±1.1		±0.6

[a]Amount of ephedrine hydrochloride added to 5.0 ml. mixture was 12.0 mg.

Table 3. Analysis of Ephedrine Hydrochloride in Tedral Suspension by Ultraviolet Spectrophotometric and Gas Chromatographic Methods.

	UV Method		GC Method	
	Found (mg/5.0 ml)	Recovery[a] (%)	Found (mg/5.0 ml)	Recovery[a] (%)
Run 1	11.8		11.4	
2	11.6		11.6	
3	11.9		11.6	
4	11.8		11.5	
5	11.7		11.5	
6	11.8		11.4	
Mean	11.8	98.3	11.5	95.8
Standard deviation	±0.11		±0.09	
Coefficient of variation		±0.9		±0.8

[a]Labeled amount of ephedrine hydrochloride in 5 ml. suspension was 12 mg.

Table 4. Analysis of Phenobarbital in Synthetic Mixtures by Ultraviolet Spectrophotometric and Gas Chromatographic Methods.

	UV Method		GC Method	
	Found (mg/5.0 ml)	Recovery[a] (%)	Found (mg/5.0 ml)	Recovery[a] (%)
Run 1	4.3		3.9	
2	4.3		3.9	
3	4.4		3.9	
4	4.3		3.9	
5	4.3		4.0	
6	4.5		3.9	
Mean	4.4	110.0	3.9	97.5
Standard deviation	±0.08		±0.04	
Coefficient of variation		±1.8		±1.0

[a]Amount of phenobarbital added to 5.0 ml mixture was 4.0 mg.

Table 5. Analysis of Phenobarbital in Tedral Suspension by Ultraviolet Spectrophotometric and Gas Chromatographic Methods.

	UV Method		GC Method	
	Found (mg/5.0 ml)	Recovery[a] (%)	Found (mg/5.0 ml)	Recovery[a] (%)
Run 1	4.5		4.4	
2	4.4		4.5	
3	4.5		4.4	
4	4.5		4.4	
5	4.4		4.3	
6	4.5		4.4	
Mean	4.5	112.5	4.4	110.0
Standard deviation	±0.06		±0.06	
Coefficient of variation		±1.3		±1.4

[a]Labeled amount of phenobarbital in 5 ml suspension was 4 mg.

Table 6. Analysis of Theophylline in Synthetic Mixtures by Ultraviolet Spectrophotometric and Gas Chromatographic Methods.

	UV Method		GC Method	
	Found (mg/5.0 ml)	Recovery[a] (%)	Found (mg/5.0 ml)	Recovery[a] (%)
Run 1	68.0		65.2	
2	68.0		65.3	
3	68.0		65.2	
4	68.2		65.1	
5	68.1		65.3	
6	68.1		65.1	
Mean	68.1	104.8	65.2	100.3
Standard deviation	±0.13		±0.09	
Coefficient of variation		±0.2		±0.1

[a]Amount of theophylline added to 5.0 ml mixture was 65.0 mg.

Table 7. Analysis of Theophylline in Tedral Suspension by Ultraviolet Spectrophotometric and Gas Chromatographic Methods.

	UV Method		GC Method	
	Found (mg/5.0 ml)	Recovery[a] (%)	Found (mg/5.0 ml)	Recovery[a] (%)
Run 1	65.9		64.8	
2	65.8		64.5	
3	65.9		64.5	
4	65.8		64.8	
5	65.3		64.6	
6	65.6		64.5	
Mean	65.7	101.1	64.6	99.4
Standard deviation	±0.25		±0.18	
Coefficient of variation		±0.4		±0.3

[a]Labeled amount of theophylline in 5 ml suspension was 65 mg.

SUMMARY AND CONCLUSIONS

The following conclusions were drawn from the results of the investigation:

1. A packed column containing OV-17 on Gas-Chrom Q 100-120 mesh size was found to give well resolved and symmetrical peaks for ephedrine, phenobarbital, and theophylline.

2. An analytical procedure involving two different extractions and one gas chromatographic injection of each extract into the gas chromatograph was developed. Ephedrine was extracted with chloroform from an alkaline solution of the sample. The remaining solution, containing phenobarbital and theophylline, was treated with sodium chloride, acidified, and extracted with chloroform.

3. Internal standards were used in order to minimize instrumental and injection errors. Various agents were investigated for use as internal standards. For the assay of ephedrine hydrochloride, α-naphthylamine was found to be the most useful; for the assay of phenobarbital and theophylline, hexobarbital was the most useful.

4. Using the described column packing, ephedrine and its internal standard (α-naphthylamine) were found to be completely eluted from the column within 8 minutes at a nitrogen flow rate of 75 ml. per minute and a column temperature of 150° C. Using the same column, phenobarbital, theophylline, and the internal standard (hexobarbital)

were completely eluted from the column within 12 minutes at the same nitrogen flow rate and a column temperature of 200° C.

5. Calibration curves for each of the three drugs were determined. Linear detector responses were obtained over the range of 1.0 - 10.0 μg. for ephedrine hydrochloride; 0.0 - 15.36 μg. for phenobarbital; and 6.24 - 78.0 μg. for theophylline.

6. Synthetic mixtures containing known amounts of each of the three drugs were analyzed by the described procedure. The recoveries were found to be 99.2% for ephedrine hydrochloride, 97.5% for phenobarbital, and 100.3% for theophylline. Upon analysis of these synthetic mixtures by an ultraviolet spectrophotometric method, the recoveries were found to be 101.7%, 110.0%, and 104.8% respectively. This would indicate that the gas chromatographic method gave results which were closer to the amounts known to be present.

7. The precision of the described procedure was determined using six replicate synthetic samples. The coefficient of variation obtained by the gas chromatographic method was within ±1.0%, while that by the ultraviolet spectrophotometric method was within ± 1.8%. This would indicate that the gas chromatographic method gave more precise results than the ultraviolet spectrophotometric method.

8. The described gas chromatographic method appears to be a useful method for the assay of ephedrine hydrochloride, phenobarbital,

and theophylline suspension. In addition, it would be expected that this method could be easily adapted to the assay of these drugs in tablet form.

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APPENDICES

Appendix A. Effect of Liquid Phase on the Separation of Ephedrine, Phenobarbital, and Theophylline.

Column	Column length (ft.)	Retention time (minutes)			Remarks
		Ephedrine[a]	Phenobarbital[b]	Theophylline[b]	
OV-1[c] 3% on Gas-Chrom Q 100-120 mesh size	2	0.7	1.8	3.0	Phenobarbital peak was not completely resolved from theophylline peak.
HI-EFF-1BP[d] 15% on Gas-Chrom P 100-120 mesh size	6	5.0	50.3	no peak within one hour	
OV-17[e] 3% on Gas-Chrom Q 100-120 mesh size	6	2.0	6.4	9.4	

[a] Column temperature, 150°C; flash heater and detector temperature, 200° C.

[b] Column temperature, 200°C; flash heater and detector temperature, 250° C.

[c] Registered trademark of Applied Science Laboratories for methyl silicone

[d] Registered trademark of Applied Science Laboratories for diethylene glycol succinate

[e] Registered trademark of Applied Science Laboratories for phenyl methyl silicone

Appendix B. Effect of Temperature on Retention Time of Ephedrine and α-Naphthylamine.

Flash heater and detector temperature (°C)	Column temperature (°C)	Retention time (minutes) Ephedrine	Retention time (minutes) α-Naphthylamine	Remarks
210	160	1.0	4.0	Incomplete resolution of the ephedrine peak from the solvent peak
200	150	2.0	5.5	
190	140	3.5	8.9	
170	120	6.0	15.2	

Appendix C. Effect of Temperature on Retention Time of Hexobarbital, Phenobarbital, and Theophylline.

Flash heater and detector temperature (°C)	Column temperature (°C)	Retention time (minutes) Hexobarbital	Retention time (minutes) Phenobarbital	Retention time (minutes) Theophylline	Remarks
260	210	1.8	5.1	7.0	Phenobarbital peak was not completely resolved from theophylline peak
250	200	3.5	6.4	9.4	
240	190	4.8	8.3	15.1	

Appendix D. Retention Time of Selected Chemicals for Use in the Determination of a Suitable Internal Standard for Ephedrine. [a]

Chemicals	Retention time (minutes)	Remarks
Ephedrine	2.0	
Aniline	0.5	Interference from peaks of unknown presented in the suspension which were extracted into the ephedrine extract
Chloroaniline	1.2	
Methylaniline	0.6	
Ethylaniline	0.8	
Diphenylamine	11.1	
Diallylamine	-	No peak within one hour
Nicotine	1.8	Incomplete resolution from the ephedrine peak
Methyl laurate	1.9	
α-Naphthylamine	5.5	

[a] Column temperature, 150°C; flash heater and detector temperature, 200° C.

Appendix E. Retention Time of Selected Chemicals for Use in the Determination of a Suitable Internal Standard for Phenobarbital and Theophylline. [a]

Chemicals	Retention time (minutes)	Remarks
Phenobarbital	6.4	
Theophylline	9.4	
Pentobarbital	1.7	
Secobarbital	1.8	Incomplete resolution from the solvent peak
Anthracene	0.3	
Phenacetin	1.9	
Nicotine	1.1	
Hexobarbital	3.5	

[a] Column temperature, 200°C; flash heater and detector temperature, 250° C.

Appendix F. Ultraviolet Spectrophotometric Method for the Assay of Tedral Suspension[a]

Assay, Theophylline (Monohydrate)

Reagents:

1N Hydrochloric Acid

0.1N Hydrochloric Acid

pH 11.0 Phosphate Buffer: Weigh accurately 38 g of Sodium Phosphate, Tribasic, then transfer to a 1-L volumetric flask. Dissolve in about 800 ml of distilled water, adjust the pH to 11.0 with 1N hydrochloric acid and dilute to volume with distilled water. Store in refrigerator. Use within one week.

pH 6.5 Phosphate Buffer: Weigh accurately 14.2 g of Sodium Phosphate, Dibasic, then transfer to a 1-L volumetric flask. Dissolve in 800 ml distilled water, adjust the pH to 6.5 with 1N hydrochloric acid and dilute to volume with water. Store in refrigerator. Use within one week.

Saturated Sodium Carbonate Solution: Dissolve 30 g of anhydrous sodium carbonate in 100 ml of distilled water. Prepare fresh.

25% Sulfuric Acid: Slowly add 100 ml of concentrated sulfuric acid with stirring to 300 ml of distilled water. Caution: heat is produced. Allow to cool before using.

0.5% Sulfuric Acid: Dilute 2.0 ml of 25% Sulfuric Acid to 100 ml with distilled water.

Sodium Metaperiodate (2%): Accurately weigh about 2 g of sodium metaperiodate, transfer to a 100-ml volumetric flask, dissolve in and dilute to volume with water. Prepare fresh.

Preparation of Standard: Weigh accurately about 120 mg (S) of Theophylline Anhydrous Reference Standard into a 250-ml, glass stoppered,

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[a]Doane A. H. Manager, Professional Products, Group quality control. Warner-Lambert Company, New Jersey. Personal communication. Sept. 27, 1972.

conical flask. Add exactly 100.0 ml of 0.1N hydrochloric acid and shake vigorously until dissolved. Pipet 20 ml of this solution into a 200-ml volumetric flask, add about 100 ml of distilled water, 40 ml of 25% sulfuric acid, dilute to volume with water, and mix well. Pipet 10 ml of this solution into a 100-ml volumetric flask, dilute to volume with distilled water, and label as theophylline standard solution.

Preparation of Sample: Mix the suspension thoroughly, then by means of a calibrated syringe, transfer about 10 ml to a 100-ml volumetric flask. Dilute to volume with pH 11 phosphate buffer, then disperse the suspension evenly by mixing for 5 to 10 minutes. Pipet 20 ml of the diluted suspension into a 125-ml separator, add 10 ml of 1N hydrochloric acid and extract with two 25 ml portions of ethyl ether. Transfer the aqueous portion to a 200-ml volumetric flask. Combine the ether portions, rinsing together with a small amount of ether. Wash the combined ether with two 20-ml portions of 25% sulfuric acid, back washing each portion of sulfuric acid in turn with a 20-ml portion of ether. Transfer the sulfuric acid washes to the 200-ml volumetric flask containing the aqueous solution, dilute to volume with distilled water and mix well. Pipet 10 ml of this solution to a 100-ml volumetric flask, dilute to volume with distilled water and mix well. Filter, if necessary, through Whatman No. 40, or equivalent, filter paper. Label this solution as the "Theophylline Assay Sample." Save the remaining solution in the 200-ml volumetric flask for the ephedrine assay and label as "Ephedrine Sample Solution." Combine the ether, rinsing together with a small amount of ether. Save for the phenobarbital assay, and label as "Phenobarbital Sample Solution."

Note: Complete the phenobarbital assay before continuing with the theophylline and ephedrine assays.

Procedure: Concomitantly determine the absorbance of the Theophylline standard (As) and sample (Au) solutions, in a suitable spectrophotometer, at a wavelength of maximum absorbance, about 271 mμ, using matched 1-cm silica cells. Set the instrument to zero with 0.5% sulfuric acid. Where SV equals the volume of sample taken in ml and 1.1 equals the conversion factor for theophylline (anhydrous) to theophylline (hydrous),

Calculation:

mg of theophylline per 5 ml =

$$\frac{Au}{As} \times \frac{S}{100} \times \frac{20}{200} \times \frac{10}{100} \times \frac{100}{SV} \times \frac{200}{20} \times \frac{100}{10} \times 5 \times 1.1$$

or

mg of theophylline per 5 ml = $\frac{Au}{As} \times \frac{S}{Au} \times 5.5$

Assay, Ephedrine Hydrochloride

Reagents: Refer to Theophylline Assay.

Preparation of Standard: Weigh accurately about 120 mg (S) of Ephedrine Hydrochloride Reference Standard into a 125-ml glass stoppered conical flask. Add exactly 50.0 ml of distilled water and shake vigorously until dissolved. Pipet 10 ml of this into a 100-ml volumetric flask and dilute to volume with distilled water. Pipet 10 ml of this solution into a 100-ml volumetric flask and dilute to volume with 0.1N hydrochloric acid.

Preparation of Sample: Refer to Theophylline Assay.

Procedure: Pipet 5 ml of sample and 5 ml of standard solutions into separate 60-ml separators. To the sample solution slowly add 5 ml of saturated sodium carbonate solution and approximately 1 g of sodium metaperiodate. To the standard solution pipet 1 ml of saturated sodium carbonate and 2 ml of 2% sodium metaperiodate. Swirl both separators gently for 10 minutes. Into each separator, pipet 20 ml of n-hexane and shake for 30 seconds. Allow the phases to separate completely.

Concomitantly determine the absorbance of the sample (Au) and standard (As) hexane layers, in a suitable spectrophotometer, at a wavelength of maximum absorbance, about 242 mμ, using matched 1-cm silica cells. Set the instrument to zero with n-hexane. Where SV equals volume of suspension taken in ml,

Calculation:

mg ephedrine hydrochloride per 5 ml =

$$\frac{Au}{As} \times \frac{S}{50} \times \frac{10}{100} \times \frac{10}{100} \times \frac{5}{20} \times \frac{100}{SV} \times \frac{200}{20} \times \frac{20}{5} \times 5$$

or

mg ephedrine hydrochloride per 5 ml = $\frac{Au}{As} \times \frac{S}{Sv}$

Assay, Phenobarbital

Reagents: Refer to Theophylline Assay.

Preparation of Standard: Weigh accurately about 80 mg (S) of Phenobarbital Reference Standard into a 250-ml glass stoppered conical flask. Add exactly 100.0 ml of pH 11.0 phosphate buffer and shake vigorously until dissolved. Pipet 10 ml of this solution into a 100-ml volumetric flask and dilute to volume with pH 11.0 phosphate buffer. Pipet 20 ml into a 100-ml volumetric flask and dilute to volume with pH 11.0 phosphate buffer.

Preparation of Sample: Extract the ether solution retained in the theophylline assay (Preparation of Sample) with three 40-ml portions of pH 6.5 phosphate buffer, washing each buffer extract in turn with a 20 ml portion of ether. Discard the buffer. Combine the ether, rinsing together with a small amount of ether, and extract with two 20-ml portions of pH 11.0 phosphate buffer. Combine the buffer extracts in a 125-ml separator and wash with 50 ml of ether. Transfer the buffer to a 100-ml volumetric flask. Extract the ether with a third 20-ml portion of pH 11.0 phosphate buffer, add the buffer to the 100-ml volumetric flask, and dilute to volume with pH 11.0 phosphate buffer.

Procedure: Concomitantly determine the absorbance of the sample (Au) and standard (As) solutions in a suitable spectrophotometer, at a wavelength of maximum absorbance, about 242 mμ, using matched 1-cm silica cells. Set the instrument to zero with pH 11.0 buffer. Where SV equals the volume of suspension taken in ml,

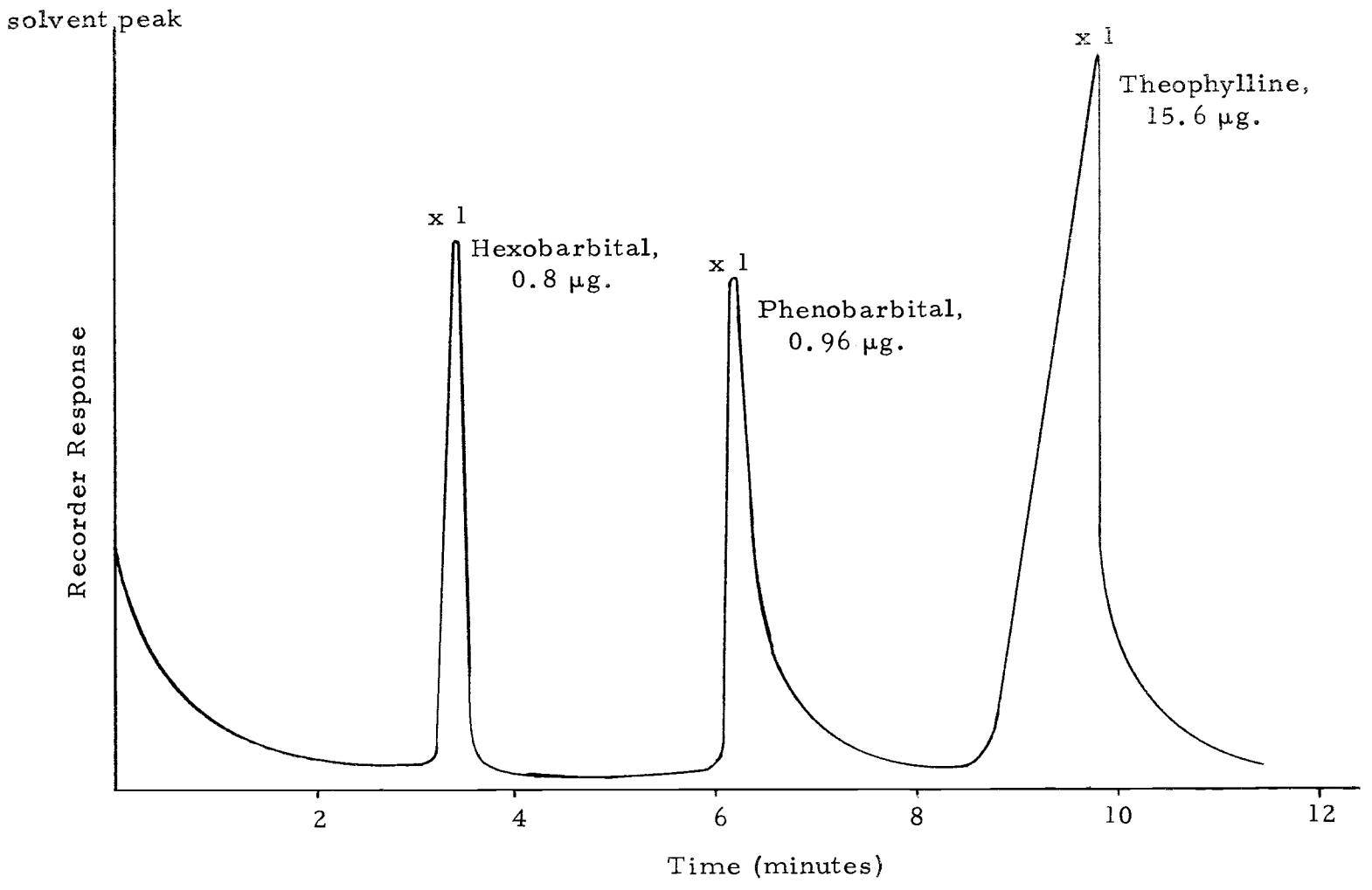
Calculation:

mg phenobarbital per 5 ml =

$$\frac{Au}{As} \times \frac{S}{100} \times \frac{10}{100} \times \frac{20}{100} \times \frac{100}{SV} \times \frac{100}{20} \times 5$$

or

$$\text{mg phenobarbital per 5 ml} = \frac{Au}{As} \times \frac{S}{SV} \times \frac{1}{2}$$



Appendix G. Gas Chromatogram Showing Skewed Theophylline Peak for Column Overloaded with Theophylline.