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The Quantitative Analysis of Desoxyephedrine Hydrochloride in Pharmaceutical Preparations

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THE QUANTITATIVE ANALYSIS OF DESOXYEPHEDRINE HYDROCHLORIDE IN PHARMACEUTICAL PREPARATIONS

By

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PREFACE

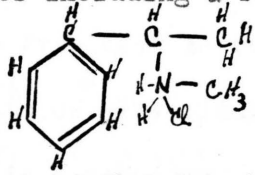
In choosing a subject for my thesis, I took into consideration my interest, place of employment and background in chemistry. As to the former, the pharmaceutical manufacturing industry has been a part of my work for the past twelve years. From Mr. Floyd H. Eggert, Director of Laboratories, U. S. Standard Products Co., I have received permission to utilize the analytical laboratory for all of my work including necessary materials, provided the work I chose is of interest to them.

Having worked for one and one-half years in the analytical laboratory of the above company I became interested in several analytical procedures which were troublesome. The particular problem I attempted to consider, with the consent and assistance of Dr. John R. Koch, of the Chemistry department of Marquette University, was the quantitative analysis of d-desoxyephedrine hydrochloride in pharmaceutical preparations. The intention of this work is, therefore, the formulation of an accurate assay either through the elimination of present analytical difficulties or by determination of a new specific method or possibly a combination of methods.

TABLE OF CONTENTS

INTRODUCTION

Before discussion any procedures for analysis it would seem wise to consider the compound in all of its known physical and chemical properties including a representation of its structural formula



(48)

Desoxyephedrine hydrochloride
(N,α-dimethyl phenethylamine hydrochloride
(methamphetamine hydrochloride)
(1-phenyl 2-methylamino propane)

Color	White
Form	crystalline salt
Molecular wgt.	185.69
Melting range	171-175°C
Specific rotation	+16 - +21
Solubility (one gram)	2 ml. water
	3 ml. alcohol
	5 ml. chloroform
	slightly soluble in ether
Chloride content	18.8 - 19.3%
	19.1% theoretical
Nitrogen content	7.3 - 7.7%
	7.55% theoretical

(48) U.S.Pharmacopeia XIV, Easton, Pa., Mack Printing Co., 1950, 359, 360.

An identification test for this compound consists in the addition of trinitrophenol test solution to a one percent solution of desoxyephedrine hydrochloride with the subsequent production of yellow needle-like crystals (48) with a melting point of 144°C. (18).

The free base is readily liberated by alkali as in the case with many salts of alkaloidal substances. Desoxyephedrine is a liquid.

It is a secondary amine and as such forms salts readily with acids. It can be titrated to a methyl red end point.

The product to be analyzed is in tablet form and contains either five or ten milligrams of d-desoxyephedrine hydrochloride. The remaining material consists of the usual excipients present in tablets of this nature. Specifically the desoxyephedrine hydrochloride is mixed with a prepared granulation consisting of 88.5% lactose, 9.86% powdered sucrose, 0.62% gum arabic, 1% magnesium stearate and 0.02% tartrazine.

One other product under consideration (containing five milligrams of d-desoxyephedrine hydrochloride) is coated in its final form. In addition it contains 35 milligrams of ascorbic acid and 130 milligrams of ferrous gluconate.

In the analysis used the free base is extracted from aqueous alkaline medium with ether. The base is removed from the ether with an excess of standard acid. The excess acid is determined by titration with standard alkali. This procedure proved successful on desoxy-

(18) Haley, Thomas J., J.Am.Pharm.Assoc.,Sci.Ed.,36, 301-4 (1947)

(48) U.S.Pharmacopeia XIV, Easton,Pa.,Mack Printing Co.,1950,356,360.

ephedrine hydrochloride alone. Several difficulties in analyzing the product arose which caused variations in the results. The specific procedure follows:

> A sample of 20 tablets are weighed accurately, then reduced to a fine powder with mortar and pestle. An aliquot representing 50 milligrams of desoxyephedrine hydrochloride is accurately weighed and transferred to a separatory funnel containing ten milliliters of distilled water. The flask is agitated to effect solution. The solution is saturated with sodium chloride and made alkaline by the addition of five milliliters normal sodium hydroxide. The liberated desoxyephedrine, which appears as an oil layer is extracted with 15 milliliters of diethyl ether six times. The combined ether extracts are washed with ten and five milliliter portions of saturated aqueous sodium chloride solutions. These combined washes are extracted with two ten milliliter portions of ether which are added to the previous ether extractions. To these combined ether extracts is added an accurately measured volume (ten milliliters)of 0.1 normal sulfuric acid, this is in excess of that required for combination with the theoretical amount of desoxyephedrine present. After shaking thoroughly the extract is withdrawn into a 250 milliliter beaker. The ether extract is washed with ten and then five milliliters of distilled water and added to the beaker. The beaker is then heated gently on a steam bath to drive off any ether that may be present. The excess acid is back-titrated with 0.02 normal sodium hydroxide. The weight in grams of desoxyephedrine hydrochloride present is calculated as follows:
>
> (ml. 0.1 normal acid--ml. 0.1 normal alkali) 0.01857

This method had several serious drawbacks. As always in laboratory work, ether is to be avoided when possible because of its obvious danger as a fire hazard. Because of the numerous time-consuming separations which are open to error because of faulty separatory funnels and the like, this method does not lend itself to great accuracy readily.

In the analysis magnesium stearate, one of the excipients, was carried with the ether phase causing high results because of its combining with the sulfuric acid. This difficulty led to a change in our

manufacturing process. Talc, which did not interfere analytically but still maintained the desirable properties of the product, was substituted for magnesium stearate.

The formation of occasional emulsions caused by the high concentration of sugars proved to be very troublesome. This is especially true in alkaline medium. To overcome this difficulty the powdered accurately weighed sample was triturated with a small volume of 0.1 normal sulfuric acid to effect solution of the desoxyephedrine hydrochloride and lessen the quantity of sugar when extracting. After frequent agitation for a period of approximately an hour the clear solution was decanted through a paper filter. The powdered sample was washed several times with small portions of 0.1 normal sulfuric acid and transferred to the separatory funnel through the paper filter.(47) The procedure was then carried on as previously described. Even though the volume of 0.1 normal sulfuric acid used was kept at a minimum the final volume was quite large for the relatively small amount of desoxyephedrine hydrochloride present so as to necessitate concentrating which ultimately greatly increased the operations of the entire procedure.

A procedure was obtained from the manufacturer who supplied the desoxyephedrine hydrochloride. This analysis is based upon a titration of the chloride content of the compound with 0.1 normal mercuric nitrate solution using diphenyl carbazone (0.1 per cent in methanol) as the indicator. This was regarded as unsatisfactory because of the base content is the therapeutically active portion of the molecule and of interest analytically.

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(47) U.S.Pharmacopoeia XIII, Easton,Pa.,Mack Printing Co.,(1947),196.

As the compound contains one atom of nitrogen in its molecule, the possibility of a Kjeldahl determination (21)(31) seems likely, although it has been noted that certain alkaloids do not give up all of their nitrogen quantitatively to digestion with sulfuric acid.(47)

A diligent search of the literature was made with the possibility of finding some more specific method of analysis.

Alkaloids are recovered from plants (20)(30) and cadaveric material (11) by steam distillation. Subsequent to this separation one might titrate the free base thus completing the determination. Titration of organic bases have been reported in non-aqueous media. (13) (45)

As titrations of some secondary amines are feasible and acceptable according to methods of the U.S.Pharmacopoeia, a search was made for effective methods of quantitative separation. Several methods seemed applicable. Three of these are rather new in so far as utilization for quantitatively separating various constituents of mixtures is concerned.

The use of ion exchange resins for the production of de-ionized water of great purity has been commercially used for some time. Now by selective use of specific resins charged with the proper ion one can

(11) Dultz, G.,Z.Anal.Chem.,120,84-88 (1940), J.Amerc.Pharm.Assoc.36,168(1947)
(13) Faleo, Federico, Rev. Facultad Qui. Ind. Y. Agr.,15/16 No. 26,48-50 (1946/47), cf C.A. 43,1529d(1949).
(20) Henry, T.A.,The Plant Alkaloids,4th Ed.,Toronto, The Blakiston Co., (1949), 634-45.
(21) Hilty, W.W.,J. Am. Pharm.,Assoc., 33,28-9 (1944).
(30) Manske and Holmes, The Alkaloids, Vol. 1, New York, Academic Press, Inc.,(1950), 8-11.
(31) Marquis, Ignacia Ribas, Anales Real Soc.Espan. Fis. Y. Quim.Ser.B,44 483-92(1948) cf C.A. 42,8207(1948).
(45) Trautner, E.M. and Shaw, F.H., Australian Chem.Inst.J.& Proc. 12, 405-12 (1945) cf C.A. 40,1281-9 (1946)
(47) U.S.Pharmacopoeia XIII, Easton,Pa.,Mack Printing Co.,(1947),196.

remove ions under consideration from a mixture. The remaining undesirable ions and molecules pass through. The deposited material can be recovered by washing the resinous material with a solution of the appropriate ions (28). A similar method that can be employed is based on adsorption of the active principle on various gels (16), or on colloidal silver iodide (3).

Although chromotography has been known for nearly one hundred and fifty years, it is only recently that it has become a great tool to the researcher and analyst. The principle is based on the difference of velocity of solutions of various substances when passed through an appropriate adsorption column composed of some material as kaolin, calcium carbonate, powdered aluminum hydroxide and many others. If the substances to be separated are colored, rings or bands are formed at different levels in the column. The substance with the greatest velocity appears closest to the bottom of the column. These areas can be physically separated and passed through a new column and again separated until only the pure substance remains (52). This general method, however, does not lend itself readily to quantitative determinations, but rather qualitative separations.

During the literature search about six specific reagents were found which could be used as identification reactions for this class of amines. However, none gave crystalline precipitates but only colored solutions

(3) Bodforss, Sven, Kgl. Fysiograf Sallskap, Lund Handl, 54,No. 12,20 pp.,(1943), cf. C.A. 37,3560-1 (1943)
(16) Gyani, B.P. and Ganguly, P.B., J. Indian Chem. Soc.,19,453-60(1942), cf. C.A. 37,6990-4 (1943)
(28) Jindra, A.,J.Pharm.Pharmacol,1,87-94(1949).
(52) Zechmeister, L. and Cholvoky, L., Principles and Practice of Chromotography, New York, John Wiley and Sons, Inc.,(1941),1-89.

of variable compositions. (19) Three reagents which specifically reacted with desoxyephedrine giving colored crystalline precipitates are as follows:

(1) chloroauric acid ---- yellow needles
melting point 126°C.

(2) chloroplatinic acid---orange needles
decomposing at 215°C.

(3) picric acid ---- yellow needles
melting point 144°C. (18)

Some work has been done on the estimation of amines in human fluids on a micro basis. Picric acid in chloroform was the reagent used. The colored solution formed was compared with standard solutions. (37) In this work a spectrophotometer is to be used to determine the depth of color.

(18) Haley, Thomas J., J. Am. Pharm.Assoc.Sci.Ed. 36,301-4 (1947)
(19) Haley, Thomas J., J. Am. Pharm.Assoc.Sci.Ed. 37, 378-9(1948)
(37) Richter, D., Biochem. J.32, 1763-69 (1938).

EXPERIMENTAL WORK

In the Kjeldahl analysis of desoxyephedrine hydrochloride good results were obtained, showing 97.2% purity. None of the excipients gave trouble in this analysis of the product as they were reasonably free of nitrogen. However, a blank of the excipients alone was run under similar conditions with each analysis as a precaution against the introduction of error.

The presence of tartrazine as coloring agent in the product formulation does not interfere appreciably with the accuracy of the determination as only 19 micrograms are present in the analysis sample. This represents a maximum error of 0.8%.

When the problem of analysis of the coated product was encountered a new difficulty arose giving high results. On further examining the product it was learned that gelatine was used in the coating procedure and thus gave rise to the added source of nitrogen. This product could be analyzed before being coated but it is the usual procedure to analyze the finished product. It is quite likely, also, that at some future date the product would be checked for stability. Obviously a nitrogen determination is of no value for products that contain nitrogen in a coating material.

In attempting separation by steam distillation a few difficulties were encountered, some of which involved technique. It was found that if the solution in the flask to which steam was being supplied were not alkaline enough (below 20-25% sodium hydroxide) low variable results would be obtained in successive analyses. This great difference was

noted even though identical quantities of material and reagents were used and other factors, such as rate and time of distillation, were maintained as closely as possible. According to Remington sufficient alkali is necessary for quantitative extraction of alkaloids from their salts. (8).

In work on the compound alone good results were obtained (98.1%) when compared to that obtained by nitrogen analysis, provided the high alkalinity of the distilling solution was maintained. Only a few minutes were required for all of the desoxyephedrine to volatalize and be collected with the steam in the receiving vessel of an accurately measured volume of 0.1 normal sulfuric acid. However, the distillation was carried out until 100 milliliters were collected.

A finished tablet of desoxyephedrine hydrochloride will contain almost 98.5% sugar. As previously stated, this causes troublesome frothing. This was lessened satisfactorily by controlling the rate of distillation. Frothing can be controlled also by the introduction of paraffin in the flask with the alkali. Bumping can be eliminated by the use of a special steam-distillation apparatus consisting of a steam heated inner jacket containing the alkaline solution.(4)

The acid solution in the receiving vessel was back-titrated with 0.1 normal sodium hydroxide using methyl red as indicator. The weight in grams of desoxyephedrine hydrochloride present was then calculated as follows:

$$\underline{\text{(ml. 0.1 normal acid-ml. 0.1 normal alkali) (0.01857)}}$$

(4) Bowen, C.V., and Barthel, W.F., Ind.Eng.Chem., Anal.Ed. 15,596(1943).
(8) Cook, E.F., and Martin,E.W., Remington's Practice of Pharmacy, Ed.9, Mack Publishing Co., Easton,Pa.,991-8.

The question of the use of this method for the product containing gelatine was raised. There could be no volatilization of the free amino acids on breakdown of the gelatine for the distillation was made from an alkaline medium in which all of the amino acids would be present as their sodium salts.

As mentioned previously, this analysis worked quite well on the pure substance and on the product giving accurate reproducible results. But to have enough substance present to give good results requires a sample of 40-50 tablets containing five milligrams of desoxyephedrine hydrochloride and 20-25 tablets containing ten milligrams. A smaller sample would lower the accuracy for a difference in titration of 0.1 milliliter of two equal samples using 0.1 normal acid means a difference of 0.8%. The total titration would be approximately 12.5 milliliters.

The size of the sample would introduce approximately five to ten grams of sugars, requiring approximately 30 milliliters of water for solution. Added to this would be 30 milliliters of 40% sodium hydroxide to attain the desired alkalinity of at least 20% (as discussed previously). This volume is beyond that which is desired for use in the distillation apparatus described previously (4).

A search was made to locate a specific reagent that would react quantitatively with desoxyephedrine hydrochloride forming a colored compound. The reagent should be sensitive to minute quantities. This latter requirement would thereby reduce greatly the size of the sample needed. Then too, the colored compound formed, when in a suitable solvent, might possibly be accurately determined spectrophotometrically.

(4) Bowen, C.V. and Barthel, W.F., Ind.Eng.Chem., Anal.Ed. 15,596,(1943)

None of the three specific reagents listed were immediately available. However, with Richter's work as a background, (37) picric acid was the reagent chosen. It was synthesized from phenol by sulfonation and subsequent nitration and purified by repeated crystallizations from hot aqueous solutions until the melting point remained constant at 122°C.

In the work cited amine concentrations as low as 0.5 microgram per milliliter were claimed to be easily estimated. As aqueous picric acid solutions are colored deep yellow, a different medium is necessary. Free picric acid in a 50% chloroform-toluene solution is almost colorless. Desoxyephedrine picrate has a yellow color in this solvent. In the procedure of Richter, toluene was used to extract the secondary amine from its aqueous alkaline medium. The extract was centrifuged and an aliquot removed and combined with an equal amount of chloroform. It was then reacted with 0.1 milliliter of 2% picric acid in chloroform. The clear amine picrate solution was then compared colorimetrically with standard amine solutions prepared in a similar manner.

In the analysis of desoxyephedrine hydrochloride a few significant changes were made in the preparation and analysis as follows:

> As desoxyephedrine hydrochloride is soluble in chloroform a standard solution was made containing one gram per 100 milliliters. From this solution various concentrations were prepared according to graph II to determine the range and limitations of accuracy.
>
> In the final dilution one-half of the solvent is toluene. To a six milliliter aliquot is added 0.1 milliliter of 2% picric acid solution. This is agitated to affect complete mixing and is then allowed to stand in the corked tube for twelve hours to allow the color to develop.

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(37) Richter, D., Biochem. J. 32, 1763-69 (1938)

The color was read in a Coleman No. 11 Spectrophotometer at 420 millimicrons as determined by graph I using a No. Pc-4 filter. The machine was set with a blank prepared as the above solutions with the exclusion of the desoxyephedrine hydrochloride. The log of the density was plotted versus the concentrations. It was determined that the reaction obeyed Beer's Law within the range of one of six micrograms, and that the best straight line was obtained when the concentrations were between three and six micrograms per milliliter.

In a sample containing six micrograms of desoxyephedrine hydrochloride per milliliter there is 40 times the theoretical amount of picric acid present, so as to complete the reaction to the right.

As the readings obtained varied (for the same concentrations) with time, a standard of necessity, had to be run with the analysis. The time allowed for color development may vary between 12 and 24 hours. In analyzing the product:

> A finely divided one tablet aliquot (five milligrams desoxyephedrine hydrochloride) was treated with chloroform and diluted to volume (50 milliliters).
>
> After frequent shaking for a few minutes the insolubles are allowed to settle. The supernatant liquid is decanted through filter paper. The aliquot should not be taken from the first ten milliliters of filtrate. A five milliliter aliquot of the filtrate is diluted to 50 milliliters with chloroform. This solution contains ten micrograms per milliliter. To a three milliliter aliquot of this solution is added three milliliters of toluene and 0.1 milliliter of 2% picric acid reagent into a stoppered test tube. The tube is agitated to affect solution and then allowed to stand for at least twelve hours. A standard is prepared containing four, five and six micrograms each. The unknown solution contains theoretically five micrograms per milliliter. Three tubes of the unknown and of each concentration of standard should be prepared along with a blank.

It has been determined that it is sufficient to prepare only one set of standards as long as the concentration is the same as is theoretically present in the unknown. The lines obtained graphically are parallel, or nearly so, and the variance seems to be due to the difference of reaction time. Only if the unknown readings differ considerably is there any great chance of error.

In the product containing gelatine, good results can be obtained by eluting the desoxyephedrine hydrochloride with chloroform provided the sample is finely divided, as the gelatine is not intimately mixed with the product but is present only as a coating. However, refluxing with chloroform or the use of an automatic extraction apparatus (30) may be necessary to insure complete extraction.

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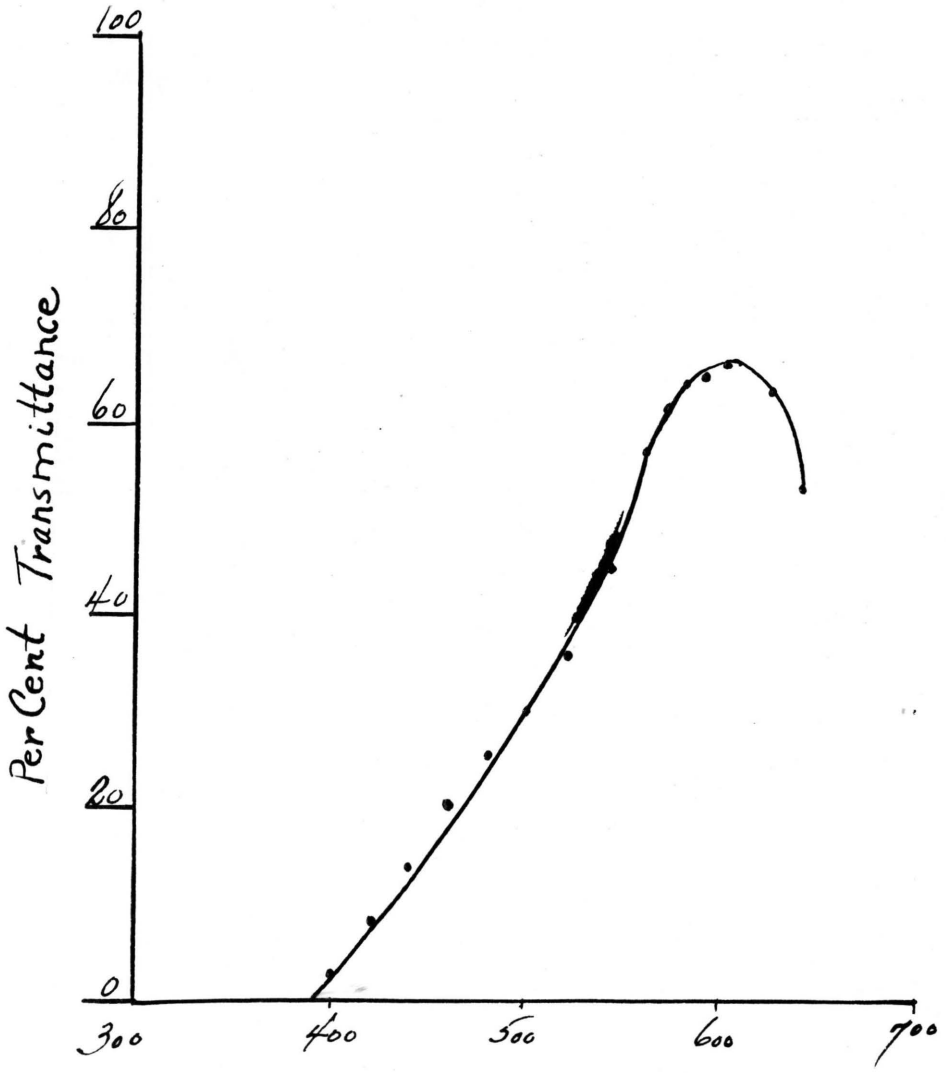
(30) Manske and Holmes, The Alkaloids, Vol.I, New York, Academic Press, Inc.(1950) 8-11.

Table I - Comparison of Analysis Methods

Method of Analysis	Results	
	d-desoxyephedrine hydrochloride	product
(1) Ether extraction --titration	96.3% , 96.9%	101.8%, 103.8%
(2) Chloride	100.2% , 101.0%	-------
(3) Kjeldahl	99.1% , 98.1%	99.5% , 99.6% , 100.0% , 101.1%
(4) Steam distillation	97.2%	-------
(5) Colorimetric	used as standard	99.6% , 100% , 102.0% , 102.5%

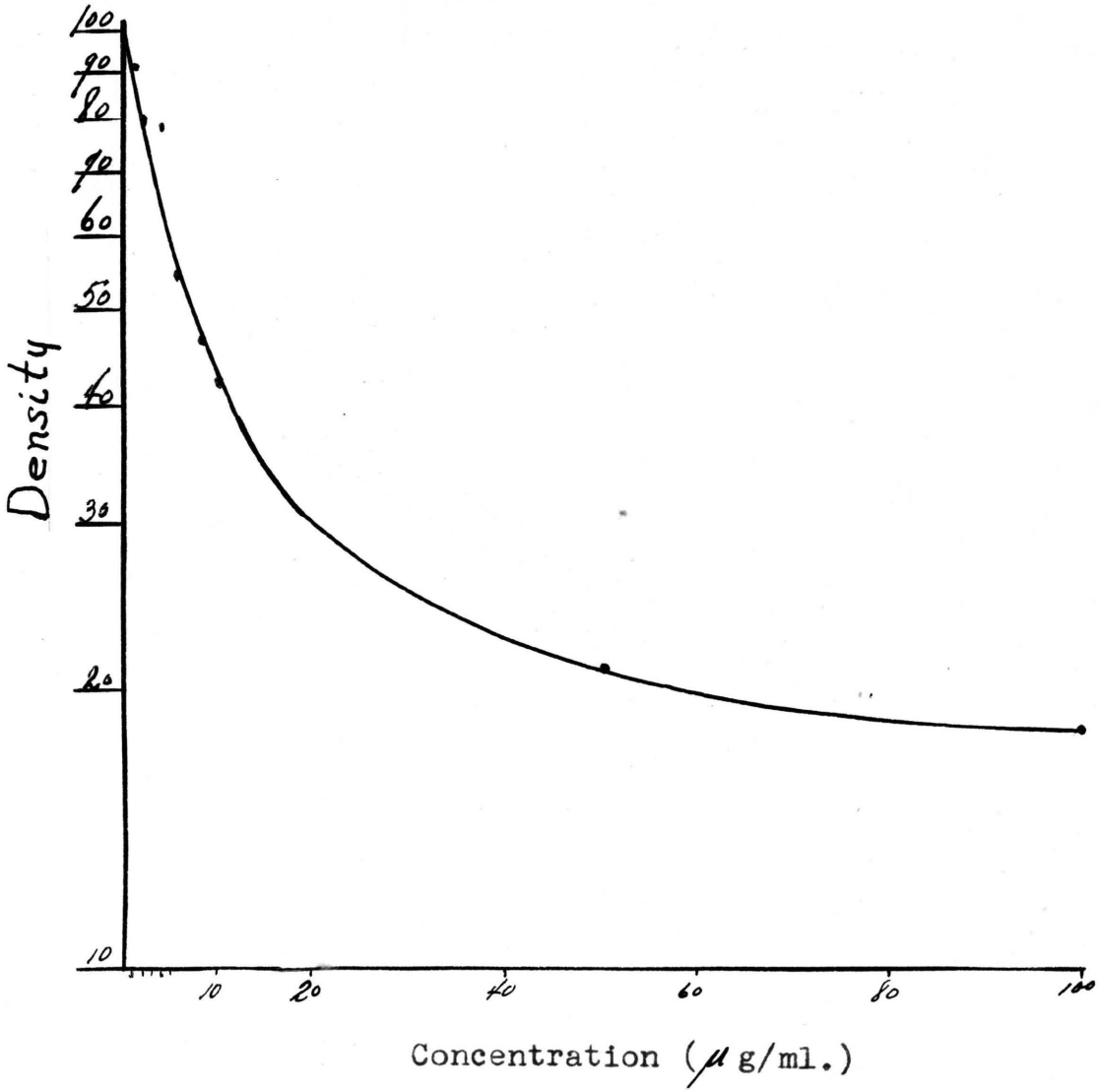
The results obtained are based upon a theoretical 100% for comparison, as varying amounts were used in the different analyses.

Graph I - Determination of Wave Length

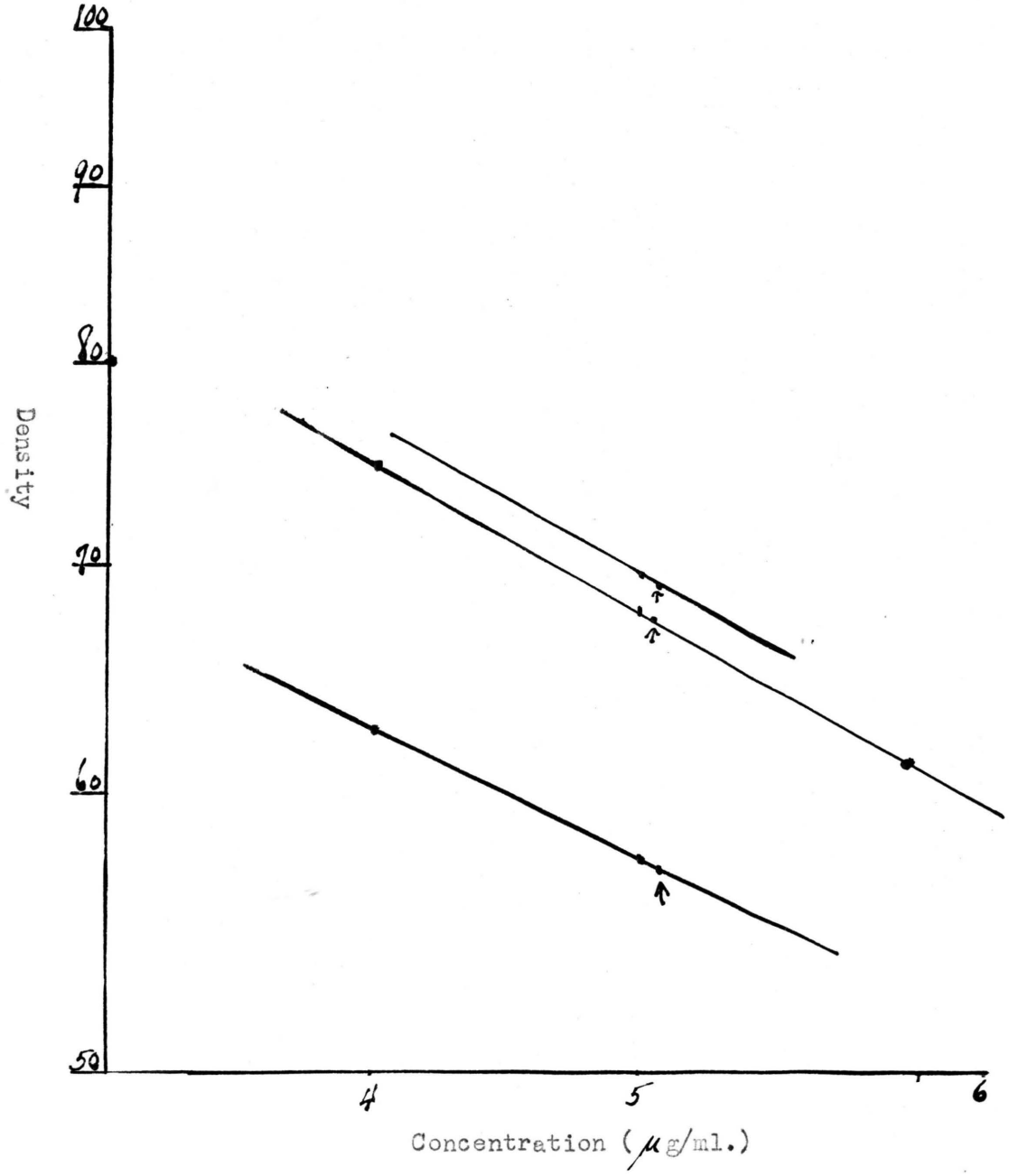


The solution used contained six micrograms of d-desoxy-ephedrine hydrochloride per milliliter of 50% chloroform-toluene To five milliliters of this solution was added 0.1 milliliter of 2% picric acid in chloroform. A Coleman Model 11 spectrophotometer employing a Pc-4 filter was used.

Graph II - Determination of Limiting Range of Concentration



Graph III - Typical Analyses



The arrows indicate the concentrations of the unknown curves. All unknowns are of the same product; the only variation is time. The uppermost curve was run using only one concentration of standard, corresponding to the theoretical concentration of unknown.

DISCUSSION

In discussing the relative merits of the various possible procedures for quantitative estimation of desoxyephedrine hydrochloride the following factors seem to be pertinent:

1. Methods based upon the estimation of chlorine in desoxyephedrine hydrochloride are not reasonable when used alone. The physiologically active portion of the molecule, as previously stated, is the base, desoxyephedrine.

2. Kjeldahl nitrogen determinations have the advantage of analyzing for an element that is a part of the desoxyephedrine molecule. These analyses were found to be very satisfactory for determinations made upon the raw material and the finished tablets, except those having gelatine coatings.

3. When other materials containing nitrogen, such as gelatine, are present the desoxyephedrine hydrochloride must first be separated.

a) Ether extraction from aqueous alkaline solutions was satisfactory on the raw material. If gelatine or sugars were present troublesome emulsions were formed. Magnesium stearate, if present, was found to be carried along in the ether extract. This absorbed some of the standard acid used for the final determination and caused results apparently over 100%.

b) Steam distillation separated the free base, desoxyephedrine, from other nitrogen containing materials, such as gelatine. Excessive foaming was controlled by careful slow distillation or by the addition of paraffin. The chief difficulty with this type of separation, as with other separ-

ations is the size of sample required. From 25-50 tablets are necessary to obtain the desired 200-250 milligrams of desoxyephedrine hydrochloride.

4. A satisfactory colorimetric method can be carried out with a much smaller sample of finished material. Sugars and gelatine did not interfere with the determination. In this analysis it is assumed that the only color-producing component is the physiologically active desoxyephedrine.

5. In the colorimetric analysis a dilution of material in chloroform is made so that the final concentration of desoxyephedrine hydrochloride is ten micrograms per milliliter. An aliquot is removed and added to an equal volume of toluene. The 2% picric acid solution in chloroform is added and allowed to react for at least twelve hours. A blank and a series of standards are treated similarly. The density of the yellow color is determined by the use of a Coleman spectrophotometer. By graphing the log of the density versus the concentration of the known standards one obtains a straight line. The concentration of the unknown sample can be determined by using the density of color found for it and comparing to the graph.

6. In a rare case of deliberate adulteration with a color producing material, the separation methods previously described would immediately show that an adulterant was present. On a given sample of raw material, the results varied less than 1% percent (97.2-98.1%) regardless of the methods of separation or analysis used.

7. For most cases of checking the purity of the raw material and analyzing the product of reliable firms the method under 5 above has proven to be very satisfactory.

SUGGESTIONS FOR FURTHER STUDY

Of great advantage for further consideration is the determination of the equilebrium constant, or specific rate constant. It seems likely that time is a factor in the reaction. It is quite probable that by applying heat the determination could be hastened. A suggestion would be to react the materials in sealed glass ampoules. Thus they could be submerged completely in hot or boiling water without chance of leakage, while the temperature and volume would remain the same and constant in all of the samples.

Of interest is the report on the assay of ephedrine colorimetrically using picryl chloride as reagent. The reaction is carried on in a water bath at 75-77° C. to develop the color. The color is measured in a photo-electric colorimeter. (5)

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(5) Chatten,L.G. and Prigsley,L.I.,Food and Drugs Laboratory Department of National Health and Welfare, Ottawa, Canada. Presented at the Convention of the American Pharmaceutical Association Scientific Section,August 29, 1951.

B I B L I O G R A P H Y

(1) Allport, Noel L. and Jones, Norman R.,"Colorimetric data for assay of certain alkaloidal hypodermic tablets and injections.", Quart.J.Pharm. Pharmacol.,15, 238-50(1942).

Conditions for the quantitative application of color tests for alkaloids including ephedrine are given. Rapid colorimetric assays using the Lovibond tintometer in control testing are described.

(2) Bersin,Th., and Meyer, Hans George, "Exchange adsorptions in non-aqueous solutions," Die Chemie, 57, 117-118 (1944), cf. C.A. 40, 4581-4 (1946).

Detailed data and procedure are presented for the exchange reaction of alkaloid bases followed by means of displacement with p-dimethylamino-azobenzene on bolus adsorbate.

(3) Bodforss, Sven, "Adsorption of amine ions on colloidal silver iodide", Kgl. Fysiograf Sallskap, Lund Handl, 54, No. 12, 20 pp.,(1943), cf. C.A. 41, 2958f (1947).

Metaniline yellow colors an acid silver iodide hydrosol blue by adsorption on the deformed micelles. Organic amines (as hydrochlorides) displace metaniline yellow giving a red color.

(4) Bowen, C.V. and Barthel,"Improved steam - distilling apparatus", Ind. Eng.Chem.,Anal. Ed. 15, 596 (1943).

An all glass apparatus is described for steam-distillation of volatile alkaloids on the macro scale. Bumping and foaming is avoided and a high degree of efficiency is realized.

(5) Chatten, L.G. and Prigsley, L.I.,"The assay of ephedrine colorimetrically in pharmaceutical preparation", Food and Drugs Laboratory, Department of National Health and Welfare, Ottawa, Canada.

A method of assay for ephedrine and its salts based on the coupling of the alkaloid base in benzene and picryl chloride. The yellow color is developed by heating the reaction mixture for twenty minutes in a water bath.

(6) Chen, James L. et al.,"Formation of fluorescing substances from amino acids, also ephedrine", J.Am.Chem. Soc.,70,3145-6 (1948).

Denige's reagent (formaldehyde in concentrated sulfuric acid (1-5)) reacts with ephedrine dissolved in sulfuric acid forming a fluorescing compound. The relation of intensity of fluorescence to concentration is practically linear over the range from two to eight micrograms per milliliter.

(7) Christensen, V.A.,"Alumina for chromatographic analysis of alkaloid salts", Dansk Tids Farm. 19, 129-48 (1945), cf. C.A.39,5405-6 (1945)

Preparation of aluminum oxide to be used in chromatographic analysis of alkaloid salts is described. The free alkaloid is titrated with 0.1 normal hydrochloric acid using bromophenol blue as indicator.

(8) Cook, E.F., and Martin, E.W., Remington's Practice of Pharmacy, ed. 9, Mack Publ. Co., Easton, Pa.

Alkaloidal assays of galenicals. Separation accomplished by automatic extraction apparatus. Volatile alkaloids may be separated by steam distillation then titrated with standard acid using methyl red as indicator. Sufficient alkali is necessary (not below pH 9) to extract completely the alkaloid. Ephedrine salts are alkalinized with sodium hydroxide solution.

(9) Degering, Univ.,"Methods of extraction and separation. Formation of crystalline ppts. --reagents picric acid, etc.", Lithoprinters, Ypsilanti, Mich.(1945), An Outline of Organic Nitrogen Compounds, Chapter 37, p.580.

Methods of extraction and separation of alkaloids outlined. Picric acid used as a reagent in the formation of crystalline precipitates with alkaloids.

(10) de la Vega, F. Alvarez, "Chromatography in galenical pharmaceuticals", Galenica Acta, Ano 2, 85-118 (1949) cf. C.A. 43, 8094 f (1949).

A theoretical introduction, apparatus, reagents and chromatographic methods in general are described for use in the determination of alkaloids and other substances.

(11) Dultz, G.,"Determination of Alkaloids in cadaveric material", Z.Anal. Chem.,120, 84-88 (1940), cf J. Amer. Pharm. Assoc. 36, 168, (1947).

Volatile alkaloids were steam-distilled from cadaveric material in alkaline medium and subsequently analyzed.

(12) Fialkov, Ya. A., "Adsorption properties of kaolin", J. Applied Chem., 18, 221-9 (1945) cf. C.A. 40, 3035-9 (1946).

The treatment of kaolins with acids or alkalies changes the adsorptive properties quantitatively and qualitatively. Basic treatment leads to improved adsorption of basic substances, such as alkaloidal salts.

(13) Fritz, James,"Titration of organic bases in non-aqueous solvents", Anal. Chem. 22, 1028-9 (1950)

Organic bases can be accurately titrated when dissolved in acetic acid, benzene, phenyl chloride, nitrobenzene, chloroform, ether or methyl cyanide. The titrant used is perchloric acid in acetic acid using methyl violet as indicator.

(14) Genevois, L., "Microchemical quantitative analysis by direct and indirect titration with titanous chloride", Chem. Anal. 29, 101-6(1947), cf. C.A. 41, 4400b (1947).

The formation of nitro compounds of alkaloids is described of which many can be analyzed by the Ti Cl_3 reaction.

(15) Gupta, H.N.,"Utilization of diastase in the extraction of alkaloids from crude drugs", J. Indian Chem. Soc., Ind. & News Ed.,9, 124-5 (1946), cf. C.A. 42, 6059b (1948).

Digestive action of diastase frees alkaloids from extraneous materials, liberating them for easier extraction.

(16) Gyani, B.P. and Ganguly, P.B.,"Adsorption of alkaloids by silica gel.", J.Indian Chem. Soc., 19, 453-60 (1942), cf. C.A. 37, 3990-4 (1943).

Alcoholic solutions of alkaloids are shaken with two to five grams of silica gel, left until equilibrium is attained, and then analyzed by direct titration to determine the amount of adsorption.

(17) Haley, Thomas J.,"Desoxyephedrine-a review of the literature", J. Am. Pharm. Assoc. Sci. Ed.,36, 161-9(1947).

A review of the literature on desoxyephedrine including its chemistry and several attempts at methods of analysis.

(18) Haley, Thomas J.,"Chemical differentiation of desoxyephedrine from other sympathomimetic amines", ibid, 301-4 (1947)

A rapid method describing the reaction of desoxyephedrine hydrochloride with ten alkaloidal reagents. New derivatives, chloraurate, chloroplatinate and picrate are described.

(19) Haley, Thomas J., "Color reactions of sympathomimetic amines", J. Am. Pharm. Assoc. Sci. Ed. 37, 378-9 (1948)

Six reagents used for color identification of 15 amines or their isomers.

(20) Henry, T.A.,The Plant Alkaloids, 4th ed.,Toronto,The Blakiston Co. (1949)

For the assay of ephedrine a colorimetric method based on the biuret reaction has been used. The estimation of ephedrine in its salts or simple solutions, titration methods and Kjeldahl estimation have been used. The formation of iodoform from ephedrine has been proposed by Sanchez.

(21) Hilty, W.F.,"Determination of ephedrine by Kjeldahl distillation", J. Am. Pharm. Assoc., 33, 28-9 (1944).

Fluctuating results are obtained on synthetic ephedrine when method of U.S.Pharmacopeia XI 2nd supplement is followed. More constant results are obtained by refluxing sample one and one-half hours with hydrochloric acid, alkalinizing with sodium hydroxide, distilling into a measured volume of 0.05 normal hydrochloric acid and titrating with 0.02 normal sodium hydroxide using methyl red as indicator.

(22) Hilty, W.F.,"Determination of ephedrine in various medicinal combinations", J. Am. Pharm. Assoc. Sci. Ed., 37, 227-31 (1948).

For the determination of ephedrine and its salts in various preparations containing no other nitrogenous substances, the proposed method is based on an acid digestion, followed by an alkaline distillation into 0.05 normal sulfuric acid and titration of excess acid with 0.02 normal sodium hydroxide.

(23) Huyck, "Absorption and liberation of ephedrine from ion - exchange resins", Am. J. Pharm. 122, 228-30 (1950).

Amberlite IRC 50 is a good absorbing medium for a weak base such as ephedrine. Some other ion-exchange resins investigated proved unsatisfactory.

(24) Ivanov-Emin, B.N.,"The Chemistry of germanium", J. Gen. Chem. U.S.S.R.,17, 430-5 (1947), cf. C.A. 42, 475g (1948).

Sparingly soluble salts are formed with heavy metals pyridine, guanidine and alkaloids

of 1-vanadium - 10 tungsten germanic acid.

(25) Jackerott, Knud A.,"Determination of ephedrine in sprays", Dansk, Tids. Farm.,16, 134-8 (1942), cf. C.A. 38, 3086-6 (1944).

An ether solution of ephedrine is extracted with 2% sulfuric acid. This is made alkaline with sodium hydroxide and extracted with chloroform, then converted to the hydrochloride.

(26) Jenkins & Hartung, The Chemistry of Organic Medicinal Products, Third Ed.,New York, John Wiley & Sons, Inc.,(1950), Ch. VIII, Amines and amine derivatives.

Discussion of properties of ephedrine, amphetamine and desoxyephedrine.

(27) Jenkins,Glenn L.,et al, Quantitative Pharmaceutical Chemistry,New York, McGraw-Hill Book Company, Inc.,1949.

Ephedrine is soluble in water (1 in 20); therefore a saturated solution of sodium chloride is used for dissolving the salt. Ether is used instead of chloroform in extracting ephedrine from the aqueous solution, as this alkaloid is a sufficiently strong base to decompose chloroform and form ephedrine hydrochloride.

(28) Jindra, A.,"Determination of alkaloids by exchange of ions", J. Pharm. Pharmacol, 1, 87-94 (1949.

The alkaloidal salt in alcohol is passed through a prepared resin column. The flask and column is rinsed with alcohol. The resulting solution is titrated with 0.1 normal hydrochloric acid using a mixture of methyl red and methylene blue (5-1) as indicator.

(29) Lynch, G. Roche et al., "Assay of ephedra and ephedrine in nasal sprays", Analyst 73, 309-10 (1948).

Steam distill ephedrine from alkaline medium containing sodium chloride and glass beads into 0.05 normal sulfuric acid. Titrate the excess acid with 0.05 normal sodium hydroxide using methyl red indicator.

(30) Manske and Holmes, The Alkaloids, Vol. I, New York, Academic Press, Inc.,(1950)

Isolation and separation of alkaloids. Uses of low boiling solvents suggested for extraction, chloroform being preferred but benzene and ethylene dichloride may also be used.

(31) Marquis, Ignacia Ribas, "The use of selenium and perchloric acid in the determination of nitrogen by the Kjeldahl method", Anales Real Soc. Espan. Fis. Y Quim. Ser. B,44,483-92 (1948), cf. C.A. 42, 8707h (1948)

The selenium-perchloric acid method gave good results with aniline hydrochloride and ephedrine hydrochloride.

(32) Novelli, A. and Tainter, M.L.,"Stimulant power of secondary and tertiary phenylisopropyl amines", J. Pharmacol, 77, 324-31 (1943).

Desoxyephedrine hydrochloride was determined by the standard chloride procedure.

(33) Opfer-Schaum, R. and Pisisti, M.,"Microchemical procedure for the detection of volatile alkaloids and bases", Mikrochemie Ver Mikrochim. Acta 32, 148-54 (1944), cf. C.A. 41, 4410b (1947)

Qualitative tests on pervitin (desoxyephedrine) and other organic bases, by determining the eutectic temperature of the reaction product using styphnic acid as the reagent, is outlined.

(34) Panini, Francesco, "Preliminary assays on some powdered pharmaceutical drugs", Riv.Ital.Essenze, Profumi, Piante Offic.,Olii Vegetali, Saponi 29, 238-40 (1947), cf. C.A. 41, 7663f (1947).

Simple qualitative reactions for identification of alkaloids in drugs are described.

(35) Portnoy, E.O., "Identification of alkaloids by microcrystallization", Anales Quim Farm. 1945 in Rev. Quim. Farm. No. 35, 21-4 (1945), cf. C. A. 40, 29309 (1946).

By use of picric acid and other reagents separately ephedrine showed useful reactions.

(36) Reimers, F. et al., "Chromatographic analysis of alkaloidal salts using tested aluminum oxide", Quart J. Pharm. Pharmacol, 20, 99-109(1947).

Individual procedures for salts of ephedrine and other alkaloids are discussed. The chromatographic column is washed with alcohol. This solution, with water added, is titrated with 0.1 normal hydrochloric acid using bromphenol blue as indicator.

(37) Richter, D.,"Micro-estimation of amines", Biochem. J. 32, 1763-69(1938)

A colorimetric analysis of p-phenylethylamine using picric acid as the reagent. The reaction is carried out in 50% chloroform - 50% toluene solution. Concentrations as low as 0.5 microgram per milliliter can be easily estimated.

(38) Schoen, Karl, "Rapid and simple method for the determination of ephedrine", J. Am. Pharm. Assoc. 33, 116-18 (1944).

Ephedrine and amphetamine can be quantitatively determined by steam-distilling from aqueous alkaline medium into a slight excess of standard sulfuric acid and back titrating with 0.02 normal sodium hydroxide in the presence of methyl red. The average error appears to be less than 0.5%. The original sample should preferably be 20-400 milligrams.

(39) Schoorl, N. et al.,"Titration of Alkaloids and alkaloid salts", Pharm. Weekblad 78, 4-8 (1941), cf. C.A. 37, 2513-8 (1943).

The alkaloid salt is titrated with 0.1 normal sodium hydroxide using phenolphalein as indicator. The free alkaloid can then be titrated using bromophenol blue as indicator. In both titrations the alkaloid must remain in solution.

(40) Shmuk, A.A.,"Identification of alkaloids by the method of methylation of their picrates", J. Applied Chem. U.S.S.R.,14 864-6 (1941), cf. C.A. 37, 2135-6 (1943).

The picrate of the secondary base is methylated without isolation of the pure base first from its solution. It is effective with two to ten milligrams of the alkaloid. The methylated picrate is crystallized from water.

(41) Smith, Frank B. et al.,"Amine salts of dinitrophenols", U.S. 2,385, 848, Oct. 2, 1945, cf. C.A. 40, 361-4 (1946)

Amine salts are formed by the addition of dinitrophenol to an amine of the structure -NHR where R may be alkyl. They are generally yellow to orange crystalline solids, difficultly soluble in water, and stable to light and air.

(42) Snell & Snell, Colorimetric Analysis, Vol. I, Third Ed.,New York, D. Van Nostrund Co., Inc.

A study of colorimetric methods, apparatus and standards used in this type of analysis, and a study of limitations of methods and evaluation of data obtained.

(43) Sussman, S. et al., "Recovery of alkaloids by ion exchange", Chem. Industries 57, 455-549 (1945)

The recovery of plant alkaloids have been effected by bringing the alkaloid containing extract into contact with a cation exchanger, then treating the cation exchanger with aqueous alkali and solvent.

(44) Toffoli, Cesco, "Riptographic analysis", Gazz. Chim. Ital. 74,207-18 (1944), cf. C.A. 40, 3360,3 (1946).

In riptographic (fractional precipitation) analysis picric acid may be used to determine alkaloids colorimetrically.

(45) Trautner, E.M. and Shaw, F.H.,"A short assay of alkaloids by titration in chloroform solution", Australian Chem. Inst. J. & Proc. 12, 405-12 (1945) cf. C.A. 40, 1281-9 (1946).

A detailed account of the application of this method in relation to pharmaceutical preparations is given.

(46) Uffelie, O.F.,"Dilituric acid as a micro-chemical reagent for alkaloids", Chem. Weekblad, 41, 101-3 (1945) cf. C.A. 40, 2038-7 (1946).

Dilituric acid (5-nitrobarbituric acid) is a specific reagent for several alkaloids including pervitine, (desoxyephedrine).

(47) U.S.Pharmacopeia,XIII,Easton,Pa.,Mack Printing Co. (1947)

A powdered aliquot of ephedrine sulfate tablets is macerated with ten milliliters of water and one milliliter of normal sulfuric acid for two hours. It is then decanted through filter paper and the residue washed with small portions of water. Evaporate the combined filtrate and washings to seven milliliters and transfer completely to a separator with the aid of a few milliliters of water.

(48) U.S.Pharmacopeia,XIV,Easton,Pa.,Mack Printing Co.,1950.

Description, solubility, physical constants, identification and other pertinent information on methamphetamine hydrochloride (d-desoxyephedrine hydrochloride) is presented.

(49) Welsh, L.H., "Determination of ephedrine", J. Am. Pharm. Assoc. 33, 96 (1944)

Report on volatility of ephedrine with steam from alkaline medium as method of analysis described by Hilty (21) rather than Kjeldahl determination as reported.

(50) Welsh, L.H.,"Report on the determination of ephedrine", J. Assoc. Offic. Agr. Chemists, 30, 467-73 (1947).

Combined ephedrine may be liberated by hot dilute acid. The acid solution is alkalinized with sodium hydroxide and extracted with chloroform. The solvent is evaporated after addition of sufficient concentrated hydrochloric acid. The ephedrine hydrochloride formed is weighed. Several necessary precautions are described.

(51) Willard, Instrument Methods of Analysis, New York, D. Van Nostrund Co. Inc., 1948.

Principles and discussion of use of the spectrophotometer. Use and interpretation of data. Preparation and care of absorption cells.

(52) Zechmeister, L. and Cholvoky, L., Principles and Practice of Chromatography, New York, John Wiley and Sons, Inc., 1941.

Outline of principles and methods of chromatography.

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