

**THE B.D.H BOOK OF
ORGANIC REAGENTS**

The B.D.H. Book of Organic Reagents

THE BRITISH DRUG HOUSES LTD.
B. D. H. LABORATORY CHEMICALS DIVISION
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PREFACE

The considerable advances in the use of organic reagents in analytical chemistry that have taken place since the last edition of "The B.D.H. Book of Organic Reagents" appeared twelve years ago have made it necessary to rewrite this tenth edition completely. Only the title remains unchanged.

Forty-eight reagents are described, of which seven are included for the first time. This represents a considerable reduction on the seventy-one in the last edition. The reasons for this are twofold. Firstly, a number of reagents which have been found in our laboratories to be somewhat unsatisfactory or difficult to manipulate have been omitted. Secondly, to avoid unnecessary duplication with "The B.D.H. Spot Test Outfit Handbook", the present volume deals in particular with the quantitative uses of the reagents. This has led to the omission of the majority of those which were recommended for qualitative use only. However, the amount of practical detail given for those reagents which remain has been increased.

Each monograph follows a set pattern. An introductory section gives a brief description of the reagent and its general uses. Then follow sections on qualitative tests (if any) and quantitative tests. These are subdivided as follows: firstly, a description of the test together with details of interferences and how to eliminate them; secondly, a list of reagents and solutions required; thirdly, the method of performing the test. The text is liberally provided with references, which are listed at the end of each monograph.

All methods described have given satisfactory results in our laboratories. Many absorptiometric and instrumental colorimetric methods are given, on account of the rapid rise to popularity of spectrophotometry. Nevertheless, it has not been forgotten that many laboratories are still not equipped for these techniques and that comparison with standards very often still provides the simplest and most satisfactory procedure.

It is hoped that "The B.D.H. Book of Organic Reagents" in its new form will prove even more valuable as a practical guide than its

predecessors. It is intended to be of use, not only to regular analytical chemists, but also to those who may occasionally require to conduct analyses and have not the opportunity of maintaining a close acquaintance with modern developments in the subject.

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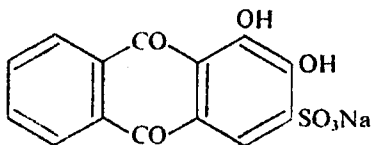
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ALIZARIN RED S

Sodium Alizarin Sulphonate



M. Wt. = 342.25

A Reagent for the Determination of Fluorides and the Colorimetric Determination of Boron

Alizarin red S occurs as a water-soluble orange-yellow powder. Under suitable conditions it forms coloured lakes with a number of metals including aluminium, chromium, manganese, thorium and zirconium.¹ It was the first of the large number of dyestuffs having this property to be proposed for the colorimetric determination of aluminium,² but it has now been almost completely superseded by aurine tricarboxylic acid. Alizarin red S can also be used for the determination of boron; the method makes use of the fact that a solution of the reagent in sulphuric acid is intensified in colour by the presence of boric acid.

The most important application of alizarin red S is in the determination of fluorides. Lakes formed with thorium or zirconium are decolorized in the presence of fluoride,¹ and this reaction has formed the basis of many volumetric^{3,4,5,6} and colorimetric⁷ procedures for its determination.

DETERMINATION OF FLUORIDES

The reaction between fluoride and the alizarin red S lakes of thorium and zirconium is disturbed by a number of ions and, except for certain samples of water, it is necessary to isolate the fluoride before applying the test under closely controlled conditions. The following method was developed for the examination of foods;⁸ it may easily be adapted for use on other materials.

- Reagents:* 0.01 per cent aqueous alizarin red S.
0.025 per cent aqueous thorium nitrate.
Standard solution of sodium fluoride containing 0.0221 g of NaF per litre (1 ml = 10 μ g of F).
0.5N sodium hydroxide.
0.05N hydrochloric acid.
60 per cent perchloric acid.
Calcium carbonate (low in fluorine)
Silver sulphate.

Method: A steam distillation apparatus, consisting of a Claisen flask of about 250 ml capacity, a large flask for generating steam and an efficient condenser, is required. The main neck of the Claisen flask is

fitted with a two-holed rubber stopper through which pass a thermometer and a glass tube for connecting to the steam supply. These should reach almost to the bottom of the flask. The side neck is closed with a solid rubber stopper and the side arm is connected to the condenser. Local overheating during distillation is avoided either by the use of an asbestos board with a hole in it, which must fit closely to the lower surface of the flask, or by the use of an asbestos gauze.

Place into the Claisen flask a number of fragments of Pyrex glass, together with 0.2 g of silver sulphate (or sufficient to precipitate all the chloride in the sample taken), 7 ml of water and 15 ml of perchloric acid solution. Heat the flask until the temperature of the contents reaches 120–125°C and connect the steam supply, which should be derived from water rendered alkaline with sodium hydroxide. Adjust the gas and steam supplies so that distillation proceeds at 137–140°C and at such a rate that 150 ml distils in 25–35 minutes. Steam out the condenser towards the end of the distillation. Discard this distillate. Collect a further 150 ml under the same conditions and titrate an aliquot part by the method given below. The amount of fluorine in the whole of this second distillate, which should not exceed about 1.5 µg, may be termed the apparatus blank and should be approximately constant for any further 150 ml fractions distilled.

Cool the flask and decant the contents, which are required later, into a beaker, rinse the flask and glass fragments with distilled water and reject the washings.

Weigh a suitable quantity of the sample, 10 g or less, depending upon the expected fluorine content, into a platinum basin and mix with about 1 g of calcium carbonate and 50 ml of water. Evaporate on a water-bath and char at a temperature below red heat over a small flame. Transfer the basin to a muffle and ignite at dull red heat (600°C) for two hours, taking care to avoid contamination of the sample with dust from the furnace. Transfer the bulk of the ash to the Claisen flask of the distillation apparatus and wash in the remainder with about 5 ml of water containing a few drops of the acid liquor saved from the blank determination. Cool and add the remainder of the liquor, washing the neck of the flask with about 2 ml of water. Reassemble the still and distil exactly 150 ml as before.

Determine the acidity of this distillate by titrating 50 ml in a Nessler cylinder with 0.5N sodium hydroxide, using methyl orange as indicator, until the colour matches that of a similar cylinder containing 50 ml of distilled water and the same amount of indicator.

Transfer the remaining 100 ml of the distillate to another Nessler cylinder and add sufficient 0.05N hydrochloric acid to render the total acidity equivalent to 5.0 ml of 0.05N acid. Prepare a control cylinder containing 100 ml of distilled water and 5.0 ml of 0.05N hydrochloric acid. Add to the contents of both cylinders 2 ml of alizarin red S solution. From a burette, calibrated in 0.02 ml divisions, run into the test

cylinder thorium nitrate solution until a slight pink colour persists as compared with the yellow colour of the control. Then add exactly the same amount of thorium nitrate solution to the control cylinder, the colour of which will then become more pink than the test solution. Run in slowly from a microburette standard solution of sodium fluoride until the tints of the two solutions match exactly. The volume of standard fluoride solution added corresponds to the amount of fluoride present in the test portion of the distillate. Calculate the fluorine content of the sample after deducting the apparatus blank.

If more than 0.5 g of phosphorus, calculated as P_2O_5 , is present in the sample taken for this determination, high results may be obtained due to traces of phosphoric acid passing into the distillate.⁹ When phosphates, or substances containing a high proportion of phosphorus, are examined, it is recommended that two successive 150 ml portions be distilled and, if the second portion shows an appreciable apparent fluorine content, the first be evaporated to dryness in the presence of 1 g of calcium carbonate and redistilled.

COLORIMETRIC DETERMINATION OF FLUORIDES IN WATERS

In clear colourless waters fluorine may be determined by the following colorimetric procedure without distillation, provided that the water contains less than 500 p.p.m. of chloride (as Cl⁻), 200 p.p.m. of sulphate (as SO_4^{2-}), 200 p.p.m. alkalinity (as $CaCO_3$), 2 p.p.m. total iron, 1 p.p.m. of phosphate (as PO_4^{3-}) and 0.5 p.p.m. of aluminium.

Reagents: 0.03 per cent zirconium oxychloride + 0.007 per cent alizarin red S in dilute sulphuric acid, prepared as follows: Dissolve 0.3 g of zirconium oxychloride in 50 ml of water, and 0.07 g of alizarin red S in a further 50 ml. Mix the two solutions. Dilute the mixture to 1 litre with an acid solution prepared by making 112 ml of concentrated sulphuric acid up to 1 litre with water. Allow to stand for at least one hour before use. Store in an amber glass bottle in a refrigerator.

Standard solution of sodium fluoride containing 0.0221 g of NaF per litre (1 ml = 10 μ g of F).

Method: Before starting the determination make sure that the sample, the reagent and the distilled water which will be used do not vary in temperature by more than 1°C. Transfer 100 ml of the sample to a Nessler cylinder and into a series of similar cylinders measure 1.0, 2.0, 3.0 ml etc., up to 10.0 ml of the standard sodium fluoride solution. Dilute each of the standards to 100 ml with water. To each cylinder add 5 ml of the zirconium-alizarin red S reagent, mix well and compare the colours after allowing to stand for one hour.

DETERMINATION OF BORON

Alizarin red S, although reported to be a less sensitive reagent for

boron than quinalizarin,¹⁰ has been used for the determination of this element in the ash from plant material. The test is based on the fact that, in sulphuric acid solution, the colour of the reagent is intensified by the addition of boric acid.

Reagents: 0.04 per cent alizarin red S, in concentrated sulphuric acid (not less than 98 per cent by weight H_2SO_4)

0.4N sulphuric acid.

0.02 per cent methyl orange in dilute alcohol, prepared as follows: Dissolve 0.10 g of methyl orange in a mixture of 100 ml of 95 per cent alcohol and 100 ml of water. Dilute this solution to 500 ml with water.

Standard solution of boric acid containing 0.0572 g of H_3BO_3 per litre (1 ml = 10 μ g of B).

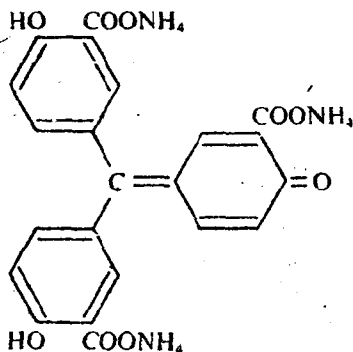
Method:¹¹ Incinerate 0.5 to 1.0 g of the dry sample at about 500°C in a porcelain crucible which has previously been boiled in dilute hydrochloric acid. Triturate the ash with exactly 5 ml of 0.4N sulphuric acid and allow to settle. Pipette 1 ml of the clear supernatant liquid into a flat-bottomed, glass-stoppered test tube and add 9 ml of the alizarin red S reagent from a burette. Allow to stand for 30 ± 2 minutes. Run methyl orange solution into an exactly similar tube until the colour, when viewed through the whole depth of the liquid, matches that of the test solution. Note the volume required. Calculate the boron content of the sample by reference to a calibration curve constructed from the results obtained by treating varying aliquots of the standard boron in a similar manner.

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ALUMINON

Aurine-tricarboxylic Acid, Ammonium Salt



M.Wt. = 473.45

A Reagent for the Determination of Aluminium and Beryllium

Aluminon is a red-brown powder which is freely soluble in water. It is adsorbed from aqueous solution by freshly formed aluminium hydroxide to give a colloidally dispersed red lake.¹ Many other metals give colours or turbidities, including beryllium, chromium, iron, titanium, bismuth, lead, tin, mercury, zirconium, the rare earths and the alkaline earths.

DETECTION OF ALUMINIUM

The formation of the bright red lake by aluminium with aluminon forms the basis of a test which will detect less than 10 μ g of the metal. Interference by other metals may be minimized by carefully controlling the conditions under which the test is carried out.

Reagents: 0.1 per cent aluminon in water.

Hydrochloric acid.

3N ammonium acetate solution.

Ammonia solution containing ammonium carbonate.

Method: Acidify the solution under test with hydrochloric acid to give a final acidity of about 1N. Add an equal volume of ammonium acetate solution followed by the same volume of aluminon solution. Mix well and allow to stand for a few minutes while the lake forms, then make alkaline with the ammonia-ammonium carbonate solution. A bright red coloration or precipitate which persists after making alkaline indicates the presence of aluminium.

DETERMINATION OF ALUMINIUM

Under closely controlled conditions the intensity of the red colour produced by aluminium with aluminon is proportional to the amount of the metal present, thus providing a method for its determination.

Beryllium, chromium, titanium, bismuth, lead, tin, mercury, zirconium, the rare earths and the alkaline earths interfere. The disturbance due to heavy metals may be overcome by removing them by electrolysis,² and control of the pH at which the reaction is carried out will eliminate most of the other interfering metals. Iron must be removed from the solution either by chelation³ or by the extraction⁴ of its chloride or thiocyanate with an immiscible solvent. If beryllium is present special procedures are necessary.^{5,6}

Reagents: 0.2 per cent aluminon in water.

5N hydrochloric acid.

2 per cent thioglycollic acid in water, freshly prepared.

Ammonium acetate buffer, prepared as follows: Mix

210 g of glacial acetic acid with 596 ml of an exactly

10 per cent aqueous solution of ammonia and dilute to 1 litre.

1 per cent starch mucilage (filtered).

Standard solution of potassium aluminium sulphate con-

taining 1.757 g of $K_2SO_4 \cdot Al_2(SO_4)_3 \cdot 24H_2O$ and 50 ml

of 5N hydrochloric acid in 1 litre (1 ml = 0.1 mg of Al).

Method: Transfer a volume of the sample, which should contain about 20 μ g of aluminium, to a 50 ml graduated flask, add 2.0 ml of 5N hydrochloric acid, 2.0 ml of thioglycollic acid, 3.0 ml of starch mucilage, 5.0 ml of ammonium acetate buffer and dilute the mixture to about 45 ml. Add 3.0 ml of the aluminon solution and dilute to 50 ml with water. Immerse the flask in a boiling water-bath for exactly 4 minutes, remove and allow to cool gradually for 1 hour. Adjust the temperature to exactly 20°C and maintain the mixture at this temperature for a further 20 minutes. Prepare a series of standards by treating suitable aliquots of the potassium alum solution in exactly the same manner. Determine the aluminium content of the sample by comparing the red colours produced. Alternatively, the optical density of the coloured solution may be measured in a 4 cm cell at a wavelength of about 550 $m\mu$, the aluminium content being deduced by reference to a calibration curve prepared using the standard potassium alum solution.

Hexametaphosphate interferes and must be converted to orthophosphate by adding 5 ml of 5N hydrochloric acid, evaporating to dryness and dissolving the residue in dilute hydrochloric acid. The resulting solution should be digested on a steam bath for 20 minutes before proceeding with the test. Allowance should be made for the acid added at this stage when conducting the determination. The final concentration of phosphate should not exceed 20 mg per mg of aluminium.

DETERMINATION OF ALUMINIUM IN ORGANIC MATERIALS⁷

Reagents: Concentrated sulphuric acid.

Concentrated nitric acid.

Ammonia solution.
2N hydrochloric acid.
5N sodium hydroxide.

Method: Transfer a suitable quantity of the sample, say 10 g, to a silica flask, add 7 ml of concentrated sulphuric acid and destroy the organic material by wet oxidation in the usual way with concentrated nitric acid. Cool the acid solution, add 50 ml of water and agitate until the aluminium sulphate has completely dissolved; particular care should be taken over this step, especially if more than 100 parts per million of aluminium are present. Add a slight excess of ammonia and boil until the vapours no longer smell of ammonia and the solution is neutral to methyl orange. Complete removal of ammonia is essential at this stage if loss of aluminium is to be avoided. On the other hand, unduly long boiling causes the solution to become definitely acid and the precipitated aluminium hydroxide is redissolved. Filter the neutral solution through a 9 cm Whatman No. 41 paper and wash with cold water, testing the filtrate with methyl orange for neutrality. Dissolve the precipitate from the paper by pouring 15 ml of hot 2N hydrochloric acid drop by drop round the upper edge and wash in the same manner with 10 ml of hot water, collecting the filtrate and washings in a silica flask. Add 10 ml of sodium hydroxide, boil, cool, filter through a 9 cm Whatman No. 54 paper, wash the flask and the filter with water, collect the filtrate and washings in a 50 ml graduated flask and dilute to the mark with water. Neutralize a suitable aliquot of this solution and determine the aluminium content by the general method described above.

DETERMINATION OF ALUMINIUM IN ALLOYS

The determination of aluminium in alloys presents special problems in view of the large number of metals which interfere with the aluminium-aluminon reaction. Most of these may be removed by electrolysis,² leaving vanadium, beryllium and the rare earth metals. Traces of manganese remain in solution, but the quantity is insufficient to affect the determination of the aluminium. It is stated⁶ that interference due to beryllium can be overcome by adding a quantity of zirconium about 40 times the weight of the expected aluminium, and co-precipitating both metals with 8-hydroxyquinoline. Under these conditions the beryllium remains in solution. After destroying the organic complex by wet oxidation the aluminium may be determined; zirconium in amounts up to 10 mg does not interfere with the accuracy of the test.

A number of alternative procedures have been developed for the routine analysis of alloys containing aluminium either as a major constituent or as a trace element, for example in iron,⁸ steel⁹ and non-ferrous alloys;¹⁰ the following method is applicable to the determination of from 0.1 to 12 per cent of aluminium in manganese bronze, zinc and magnesium alloys.¹¹

Reagents: Aluminon-buffer solution, prepared as follows: Dissolve 250 g of ammonium acetate in 500 ml of distilled water, add 40 ml of glacial acetic acid and filter. Add a solution of 0.5 g of aluminon in 25 ml of water followed by a solution of 1 g of benzoic acid in 10 ml of methanol. Dilute to 1 litre. Finally add a solution of 5 g of gelatin in 250 ml of water and dilute the mixture to 1500 ml. Store in a dark glass bottle.

Concentrated hydrochloric acid.

30 per cent hydrogen peroxide.

2.0 per cent thioglycollic acid in water.

Standard solution of potassium alum containing 0.1757 g of $K_2SO_4 \cdot Al_2(SO_4)_3 \cdot 24H_2O$ per litre (1 ml = 10 μ g of Al).

Method: Weigh between 0.1 and 0.2 g of the sample into a small conical flask, add 5 ml of concentrated hydrochloric acid and 5 ml of 30 per cent hydrogen peroxide, and cover with a watch glass, cooling if the reaction becomes violent. After the sample has dissolved evaporate the solution down to about 2 ml to destroy the excess of hydrogen peroxide, transfer to a suitable graduated flask (200 ml for alloys of low aluminium content or 500 ml for high) and dilute to the mark with water. Transfer a quantity of this solution, expected to contain between 20 and 60 μ g of aluminium, to a 100 ml graduated flask and add 2.0 ml of thioglycollic acid solution and 15 ml of aluminon-buffer solution. Immerse the flask in a boiling water-bath for exactly 5 minutes, remove, allow to stand for 1 minute, then cool to room temperature with running water. Dilute to 100 ml with water, allow to stand for 1 or 2 minutes, then measure the optical density of the solution at 525 m μ . Construct a calibration curve by treating suitable aliquots of the standard potassium alum solution, each containing 5 μ g of added copper, in exactly the same manner. Calculate the aluminium content of the sample by reference to this curve.

DETERMINATION OF BERYLLIUM IN BERYLLIUM-COPPER

ALLOYS¹²

Beryllium forms a lake with aluminon similar to that of aluminium, and under similar conditions. This provides a useful means of determining beryllium, particularly in alloys such as beryllium-copper, the method for which is given. Interference from small amounts of copper, aluminium, zirconium, titanium, manganese, iron, cobalt, nickel and zinc can be prevented by adding a solution of diamino-ethane-tetraacetic acid.¹³ The colour of the beryllium-aluminon lake intensifies slowly with passage of time, and measurements should therefore always be made after a fixed time interval.

Reagents: Aluminon-buffer solution, as used for the determination of aluminium in alloys (see above).

Diamino-ethane-tetra-acetic acid solution, prepared as follows: Disperse 2.5 g of diamino-ethane-tetra-acetic acid in 30 ml of water. Add 1 drop of methyl red solution and add 50 per cent by volume ammonia solution until the indicator changes from red to yellow. Warm to dissolve the solid then dilute to 100 ml.

Concentrated hydrochloric acid.

30 per cent hydrogen peroxide.

Standard solution of cupric chloride containing 5.366 g of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ per litre (1 ml = 2 mg of Cu).

Standard solution of beryllium sulphate containing 0.1965 g of $\text{BeSO}_4 \cdot 4\text{H}_2\text{O}$ per litre (1 ml = 10 μg of Be).

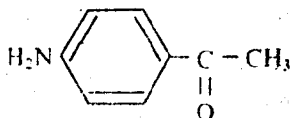
Method: Dissolve 0.200 g of the sample under test in 5 ml of concentrated hydrochloric acid and 5 ml of 30 per cent hydrogen peroxide, cooling if the reaction becomes too violent. After the sample has dissolved evaporate the solution down to about 2 ml to destroy the excess hydrogen peroxide, transfer to a 500 ml graduated flask and dilute to the mark. Introduce a quantity of this solution, expected to contain not more than 100 μg of beryllium, into a Nessler cylinder and dilute to about 75 ml. Add 2 ml of diamino-ethane-tetra-acetic acid solution followed by 15 ml of aluminon-buffer solution. Dilute to 100 ml, mix and allow to stand in the dark for exactly 20 minutes from the time of adding the aluminon-buffer solution. Prepare a series of standards by treating varying aliquots of the beryllium sulphate solution, each containing 1 ml of standard cupric chloride solution, in exactly the same manner. Compare the colours produced.

Alternatively, the optical density of the coloured solution may be measured at 515 $m\mu$. In this case calculate the beryllium content of the sample by reference to a calibration curve constructed from the results obtained by measuring the optical densities of the standards prepared as described above. A fresh curve should be prepared for each batch of aluminon-buffer solution.

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p-AMINO-ACETOPHENONE



M.Wt. = 135.17

A Reagent for the Determination of Palladium, and the Colorimetric Determination of Aneurine Hydrochloride

p-Amino-acetophenone is a white crystalline compound which melts at 105–6°C. It is soluble in alcohol, ether and hot water, but is only slightly soluble in cold water and benzene.

p-Amino-acetophenone forms a voluminous yellow precipitate with palladium salts in neutral or dilute acid solution, a reaction which has been proposed for the gravimetric^{1,2} and nephelometric¹ determination of palladium. The diazotized derivative of the reagent reacts with aneurine hydrochloride (vitamin B₁) to produce a highly coloured, water-insoluble compound which is soluble in xylene.^{3,4} This reaction has been used as a basis for the colorimetric determination of aneurine hydrochloride,^{5,6,7} which has been applied to the determination of vitamin B₁ in biological and pharmaceutical products.^{8,9}

DETERMINATION OF PALLADIUM^{1,2}

The precipitate formed by *p*-amino-acetophenone with palladium has the composition $(\text{NH}_2 \cdot \text{C}_6\text{H}_4 \cdot \text{CO} \cdot \text{CH}_3)_2\text{PdCl}_2$, is insoluble in cold water, dilute acids, alcohol, ether and chloroform, and is decomposed by strong alkalis. The precipitate is readily filtered and is non-hygroscopic; it is therefore ideally suitable for the gravimetric determination of palladium. Only platinum and cerium interfere. Cerium must be absent from the test solution, but provided the solution is sufficiently diluted, interference from platinum is negligible.

Reagents: 1 per cent *p*-amino-acetophenone in water containing 2 per cent by volume hydrochloric acid.

Hydrochloric acid.

Method: Add sufficient hydrochloric acid to the test solution to bring the concentration to 0.1N, followed by an excess of the reagent solution. Stir vigorously for 15 minutes then filter through a tared sintered glass crucible. Wash the precipitate well with cold water and dry at 80°C to constant weight. Each gramme of precipitate contains 0.2382 g of Pd.

DETERMINATION OF ANEURINE HYDROCHLORIDE (VITAMIN B₁)

A solution in xylene of the diazotized *p*-amino-acetophenone-

aneurine hydrochloride compound possesses a colour whose intensity is proportional to the amount of aneurine present, up to a concentration of 0.25 mg. Phosphorylated vitamin B₁ has no reaction and must first be quantitatively hydrolysed by incubating with active yeast powder at 45°C for 12 hours, after the reaction of the solution has been adjusted to pH 4.5. Allowance must be made for the aneurine content of the yeast.⁵ Other biologically important compounds react with the diazotized reagent. These include histamine, histidine and inositol, which give orange colours, and tyrosine and adrenaline which form reddish compounds. However, none of these coloured derivatives is soluble in xylene.

Reagents: Diazotized *p*-amino-acetophenone solution, prepared as follows: Dissolve 3.18 g of *p*-amino-acetophenone in 45 ml of 10N hydrochloric acid and dilute to 500 ml with water. Mix a convenient volume of this solution with an equal volume of 4.5 per cent sodium nitrite in water and cool the mixture in an ice-bath. Add a further 4 volumes of sodium nitrite solution after 10 minutes and maintain the mixture at 0–5°C for 20 minutes. Add 20 ml of this solution to 275 ml of an aqueous solution containing 2 per cent sodium hydroxide and 2.88 per cent sodium bicarbonate. Stir for 10 minutes, then adjust the reaction to between pH 5 and pH 6.

25 per cent potassium chloride in water.

0.5 per cent phenol in 95 per cent ethyl alcohol.

N sodium hydroxide.

Xylene.

Standard solution of aneurine hydrochloride containing 0.1 g of C₁₂H₁₇ON₄SCl.HCl per litre (1 ml=0.1 mg of aneurine hydrochloride).

Method: The test solution, which may be a simple solution of the vitamin or an eluate from a zeolite column, should consist of the aneurine hydrochloride dissolved in 25 per cent potassium chloride solution, acidified to pH 2 with sulphuric acid. Pipette 3 ml of this solution, containing not more than 0.25 mg aneurine hydrochloride into a 15 ml centrifuge tube and add 3 ml of the phenol in alcohol solution. Pass a fine stream of nitrogen through the mixture and titrate dropwise with N sodium hydroxide, using thymol blue as indicator, until a faint blue colour appears. Add 6 ml of the diazotized reagent solution, close the tube with a cork and allow to stand overnight at room temperature. Finally add 2 ml of xylene, shake for 1½ minutes and centrifuge. Compare the colour of the xylene layer with standards prepared by treating quantities of 0 to 2.5 ml of the standard aneurine solution in a similar manner.