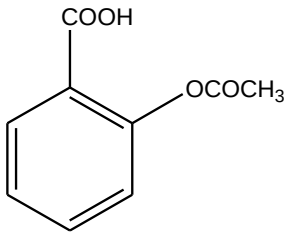
Acetyl salicylic acid (Anti pyretic – Analgesic)



Salicylic acid is a derivative of mono hydroxy benzoic acid. Mono hydroxy benzoic acid exists as O,P,meta isomers. O,P isomers of salicylic acid and it's derivatives are most important pharmaceuticals because of their physiological and Antiseptic properties.

Ex:- For Salicylic acid derivatives are

Sodium salicylate

Methyl salicylate

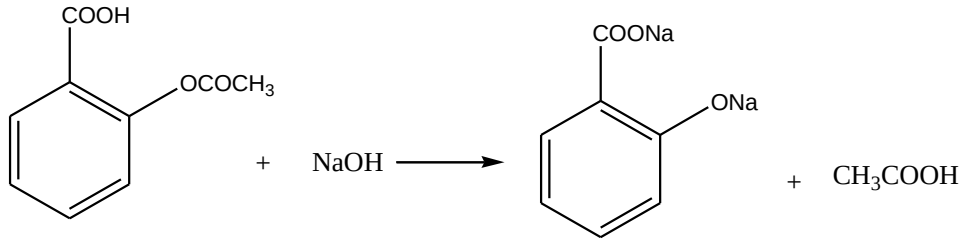
Acetyl salicylate

Determination of Acetyl Salicylic acid:-

Esters of the above in which hydroxyl group is esterified can be determined by titrimetric method

Principle:-

Known strength of standard alkaline is added to acetyl salicylic acid (known weight). The contents are boiled for 10 min and the excess alkali is back titrated with acid. Acetyl salicylic acid is readily dissolved in dil. NaOH and is completely hydrolysed by boiling.



Procedure:- Approximately 1.5 – 2.0 gms acetyl salicylic acid is accurately weighed and Transferred into a flask and the compound is dried previously over H_2SO_4 for 5 hours. Exactly.50 ml of 0.5N NaOH is added and the mixture is boiled for 10 min. The amount of 0.5N NaOH is sufficient to neutralize, the salicylic acid and acetic acid formed in the Hydrolysis. Solution is then cooled and the excess of NaOH is titrated with 0.5N H_2SO_4 using phenolphthalein indicator .A blank determination also made using the same conditions.

$$\% \text{ of Aspirin} = \frac{(A-B) \times N \times Eq.Wt \times 100}{\text{Wt of substance}}$$

Other methods also used for the determination of salicylic acid, acetyl salicylic

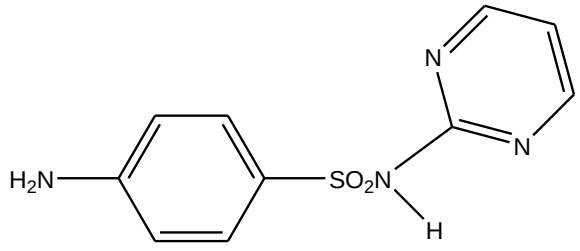
acid are

1 Non-aqueous titrimetry

2 Polarography

3 Ion exchange

Sulphadiazine:-



The sulpha drugs used for the therapeutic interest are sulphanamides. The general formula is $R_1NHC_6H_4SO_2NR_2R_3$

In many of the compounds, the amino group & the sulphanamide groups are in para position.

Determination:-

Principle:- Diazotisation is the reaction whereby an aromatic amine reacts with nitrous acid to form a diazonium salt.

$$Ar\text{-}NH_2 + HNO_2 + HCl \longrightarrow Ar\text{-}N_2Cl + 2H_2O$$

When diazotization is used analytically, the sample is dissolved in excess of strong mineral acid and titrated with standard sodium nitrate. The end point detected can be known by adding starch indicator.

Solutions required:-

(1) Sodium nitrite:- The solution is standardized by pure sulphanilic acid.

(2) Starch indicator:- 750 mg of pot. Iodide and 2 gms of $ZnCl_2$ are dissolved in 100 ml water. The solution is heated to boiling and 5 gm of starch (smooth suspension) is added with continuous stirring.

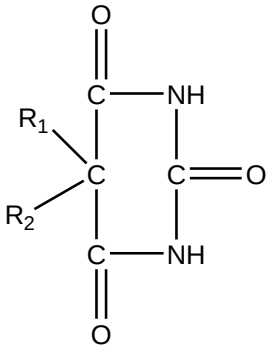
Procedure:- 1 gm of dried sulphadiazine sample is taken in a beaker and dissolved in 40 ml conc. HCl and 10 ml distilled water. The solution is cooled and titrated with 0.1 gm $NaNO_2$ with the tip of burette well under the surface $NaNO_2$ is added initially at the rate of 4-5 ml per minute. As the end point is reached, it should be added very slowly. Then the end point detection can be obtained after adding 1 drop of the solution on filter paper which contains starch iodine paste. The end point detection is the indication of blue colour. A blank titration should be performed on Hydrochloric acid.

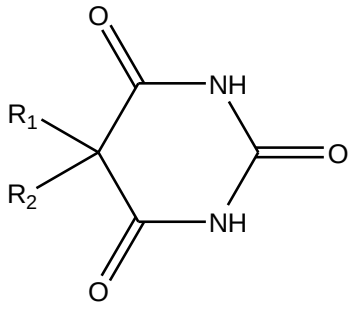
$$\% \text{ Sulphadiazene} = \frac{V \times M \times \text{eq. wt} \times 100}{\text{wt of sample}}$$

V = vol. of total nitrite solution
M = molarity of $NaNO_2$

Note:- Excess HNO_2 oxidises the iodide in the indicator to iodine which gives the blue colour with the starch.

Phenobarbitone (Barbituric acid & Derivatives): Barbituric acid is often referred to as Barbiturates.





barbituric acid

$R_1 = R_2$ = Ethyl then Barbitone

R_1 = Ethyl, R_2 = Phenyl then Phenyl Barbitone

Derivatives of Barbituric acid have been identified based on M.P.

Methods of estimation:-

(1) U.V Spectrophotometric method:- Because of its sensitivity and specificity spectroscopy has been widely applied to barbiturate analysis in body fluids & tissues. This technique is also suited for pharmaceutical preparation.

Procedure:-

An accurately weighed powdered sample equivalent to 25 mg of phenobarbitone is transferred into a 250 ml volumetric flask and appropriate

solvent is added to dissolve the sample. A solution is diluted to the mark with the solvent and is filtered through whatmann filter paper (41) and the first 25 ml of the solution is discarded. 10.0 ml of aliquot is diluted to 100.0 ml with the solvent and absorbance is determined at 480 mμ.

(2) Titrimetric method: All the barbituric acids can be titrated as monobasic acids. The titration in water is hindered by their insolubility and weekly acidic nature. Hence, the titrations usually performed in alcoholic or hydro alcoholic medium.

Procedure:

The titrimetric procedure is carried out by making use of the mixture of two indicators. The two indicators that are being used for the titration process or thymolphthalein or alizarin yellow. 0.1 to 0.2 gm of barbituric acid is dissolved in 10 ml of neutralized methanol and 10ml of freshly boiled water. Six drops of mixed indicator solution is added and the solution is titrated with 0.1N NaOH.

1 ml of 0.1 N NaOH = mol. Wt/10mg of barbituric acid.

Note: Thyomolphthalein is used for Phenobarbital titration while alizarin yellow is used for barbital.

Non aqueous titrimetric method: The most satisfactory titrations of the barbituric acid is performed in non aqueous media.

Reagents: Thymol blue indicator – 0.5 % in anhydrous methanol.

Titrant solution: 0.1 N KOH in anhydrous methanol, KOH solution is standardized against benzoic acid which is dissolved in chloroform and methanol.

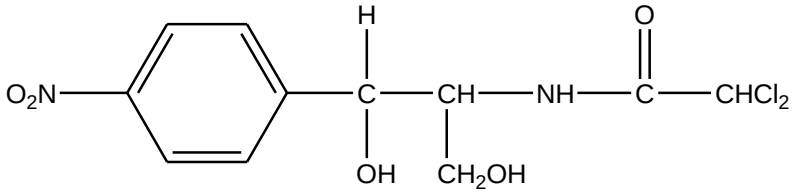
Procedure: 40 to 50 mg barbituric acid sample is dissolved in 50ml chloroform in 250 ml beaker. 1 ml of methanol and 4 drops of thymol blue indicator are added to the contents in the beaker. The solution is then titrated with 0.1 N KOH. The end point colour should be in violet.

1 ml of 0.1 N KOH = mol. Wt/10 mg of barbituric acid

Note: Methanol is added to the titrated solvent to prevent the precipitation of the salt.

3) Antibiotics: Antibiotics can be defined as substances capable of inhibiting the growth of micro organisms and which are themselves elaborated by micro organisms.

1. Chloramphenicol: It is also known as chloromycetin has a chemical formula
   $C_{11}H_{12}N_2O_5Cl_2$



In general esterification of 1^o alcohol with palmitic acid is chloramphenicol palmate.

Determination:

Diazotisation of colorimetric method:

Principle: A colorimetric method for chloramphenicol was reported in which reduction of of nitro group with metallic zinc or titanium chloride …followed by diazotization and coupling with N-1 naphthyl ethylene diamine. In this method first of all chloramphenicol was separated from interfering substances

by employing a preliminary solvent extraction process. Chloroform-ethylacetate solution is used in the solvent extraction method.

Reagents required:

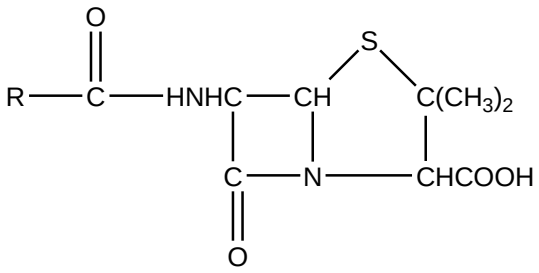
1. 0.2 M buffer (p^H =6)
2. Coloring agents: 0.5 % N – 1.naphthyl ethylene diamine hydrochloride
3. Chloroform – ethyl acetate mixture 2:1 by volume

Procedure: A suitable quantity of sample is dissolved in water, 2 to 5 ml of aliquot of sample containing 0 to 5 mg of chloramphenicol is taken in a separating funnel. The contents are extracted with 25 ml portion of chloroform-ethylacetate. The contents are mixed gently for 2 minutes and allow the layers to separate. The organic layer is filtered through a dry filter paper into a porcelain container. The contents are evaporated to dryness on a steam bath.

The dried residue is dissolved in 3ml 0.1 N NaOH and 25mg of sodium hydrosulphite is added. The contents are allowed to standard room temperature for 15 minutes. Add 0.5 ml of 5% sodium nitrite and 5 to 10 drops of HCl. After 5 minutes add 1 ml of 5% sulphanilic acid followed by 0.5 ml of colouring agent. After 2 hours the absorbance is noted at 558 mμ. A series of standards are run to establish a standard curve.

Volumetric method: In this method a known amount of periodate is added to the sample, which hydrolyses the amide group to amine group. The excess periodate is determined by Arsenite – iodine titration.

Pencillins: The common structure of Pencillin is



R-group varies with nature of Pencillin

Ex. R = Benzyl

R = P-hydroxy benzyl

R = Phenoxy methyl

R = n-heptyl

The pencillins are commonly used as potassium or sodium salts. In addition certain organic compounds are employed. These often aid in prolonging duration of action of the dose.

i. Pencillin – G (or) Benzyl pencillin:

In general ultra violet and infrared methods are normally used for the determination of different types of pencillins.

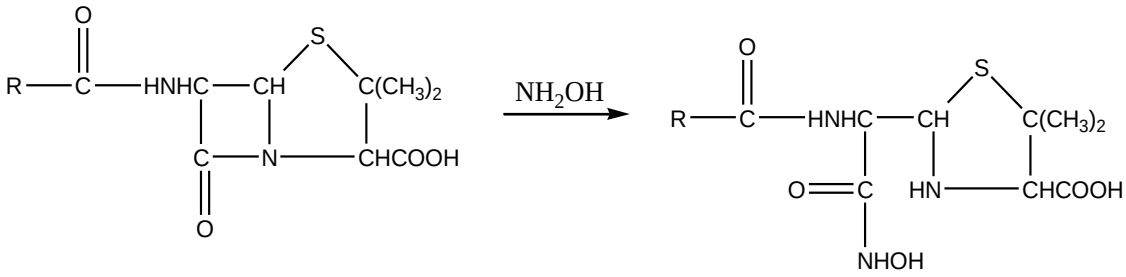
1. U.V method:

Pencillin-G exhibits max at about 257-263 mμ which may be attributed to the benzyl portion of molecule. The pencillin is extracted into chloroform and total phenyl compounds are determined spectrometrically.

Procedure: 40 to 50 mg of pencillin is weighed and transferred into 25 ml volumetric flask and dissolved in water and make up to the mark with distilled water. 5 ml of aliquot are pipetted out into each of two 25 ml glass stoppered tubes. In tube I add 1 ml of 2 N NaOH, 10 ml water saturated

with chloroform, add 1 ml of $H_3 PO_4$. In tube (2) add 2 ml 1:1 2 N NaOH and 3 ml $H_3 PO_4$. Both the stoppered tubes are placed in ice-bath for half an hour. The tubes are shaken for two minutes and centrifused. The chloroform layers withdrawn from tubes by making use of a syringe. It is filtered through cotton and measure the absorbance at 263 mμ. A standard curve is prepared using the above procedure.

2. Hydroxamic acid method: The ß-lactum ring of the pencillin molecule is split by reacting with hydroxyl amine. In this process hydroxamic acid being formed. The addition of ferric ion to the hydroxamic acid solution produces a colour which is suitable for quantitative measurement.



Reagents:

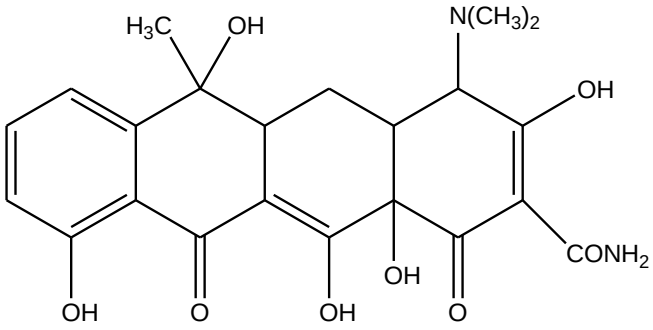
1. Hydroxyl amine hydrochloride 5%
2. Alkali buffer 86.5 gm NaOH + 10.5 gm CH_3COONa in 500 ml water
3. Ferric ammonium sulphate (100gm) + 46.5 ml concentrated H_2SO_4 diluted to 500 ml
4. Pencillin solution: Pencillnose – 100000 units is dissolved in 10 ml water

Procedure:

Suitable quantity of pencillin (3 to 4 mg/ml) sample is taken in a test tube add 3 ml of hydroxyl amine hydrochloride. After 5 min add 1 ml alkali buffer

followed by 1 ml of ferric ammonium sulphate. The absorbance is measured at 515nm after 15 min. In another test tube a blank should be possessed in the similar manner. A standard curve is plotted with pure pencillin-G.

(ii) Tetracyclines:



Tetracycline antibiotics are mainly of 3 types

i. Tetra cyclin – C_{22} H_{24} N_2O_8
ii. Chloro tetra cyclin – C_{22} H_{28} N_2 O_8 Cl
iii. Oxy tetra cyclin – C_{22} H_{24} N_2 O_9

All the above tetra cyclones are water insoluble

Tetracyclines: (Achromycin, Tetracyn, Polycyclin)

Tetracycline is a phenolic compound. It is determined by spectrophotometric method.

Method-1: Since tetracyclin is a phenolic compound gives colour when treated with ferric ion. The addition of Fe 3+ion gives orange brown coloured complex.

Reagent: 0.1 N of Hydrochloric acid

Ferric chloride solution – 0.05%

Procedure: Suitable quantity of sample is weighed and dissolved in 0.01 N HCl. The final concentration of sample should be 0.2 mg/ml. 5 ml aliquot is pipetted into a suitable volumetric flask and 5 ml of water is added followed by 10 ml 0.05% $FeCl_3$ solution. After 15 min the absorbance of orange brown colour complex being measured at 490 nm. A blank should also be possessed in the similar manner. The absorbance value of the sample is compared with standard samples of tetracycline.

Method-2: When Tetracycline is dissolved in NaOH, a yellow coloured solution is produced. The absorption max is at 380 mμ.

Procedure: 25 mg of Tetracycline hydrochloride is weighed and dissolved in 250 ml volumetric flask. After 15 minutes the aliquot is pipetted out into a 100 ml volumetric flask. 70 ml of water and 5 ml of 5 N NaOH are added and the solution is diluted with water. Exactly 6 min after the addition of NaOH the absorbance is determined at 380 mμ. A blank should also be possessed in the above manner.

Vitamins: vitamins are essential nutritional factors and organic compounds. Analytical procedures based on chemical, physical, biological and microbiological methods are used in their assay.

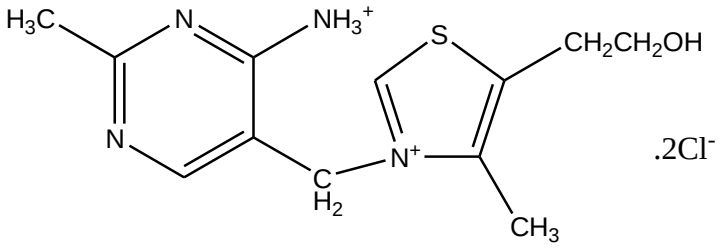
i. Thiamine (B1)
ii. Riboflavin (B2)
iii. Ascorbic acid (C)

1. Vitamin B1: (Thiamine) General formula of vitamin B_1

    1. $C_{12}\ H_{18}\ Cl_2\ N_4\ 0_2$ – Thiamine hydrochloric acid

2. $C_{12}\ H_{17}\ N_2\ 0_5$ - Thiamine mono nitrate

Thiamine hydrochloric acid is a white crystal solid. Different chemical methods are used in thiamine. Out of the different existing methods gravimetric & colorimetric methods are found to be extensive used in the analysis of thiamine.



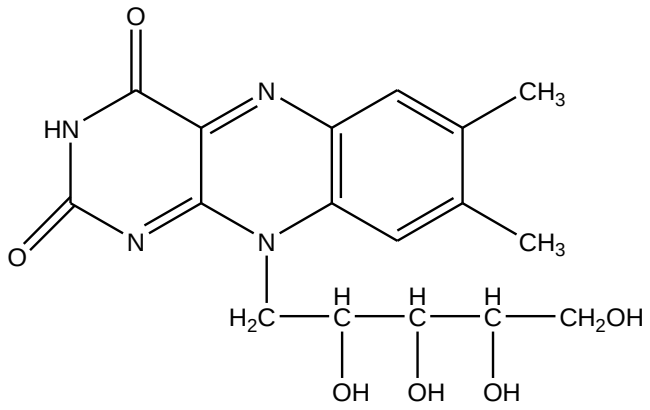
Colorimetric method: C6-aminothymol method)

The colour reaction between thiamine and diazotized 6-amino thymol is used for determination of vitamin B1.

Reagents required:

1. 0.1% of $NaNO_2$
2. Sodium hydroxide – 20%
3. HCl
4. Mixed solvent (redistilled toluene is mixed with n-butanol)
5. 6-amino thymol reagent: 50 ml of 6-amino thymol hydrochloride is dissolved in 50 ml of HCl and diluted to 100 ml.

2. Vitamin B_2 (Riboflavin)



6, 7 dimethyl-9 (D, 1 – Sibityl iso alloxazine)

Riboflavin is a yellow crystallized compound. It is insoluble in ether, chloroform, benzene etc. It is soluble in water and dilute alkali.

Riboflavin can be determined by following methods:

1. Flourometric method
2. Spectrophotometric method
3. Polarographic method

Spectrophotometric method: Riboflavin has a characteristic UV spectrum in water at a maximum at 267 mμ. This property provides a basis for analysis of riboflavin of 90% purity.

Procedure: About 20 mg of riboflavin is weighed and transferred into 1000 ml flask. About 500 ml of water and 3 ml of 1 N NaOH are added. After shaking gently 2ml of 5N acetic acid is added and dilute the contents to 1000 ml. 20 ml of aliquot is taken and transferred into separating funnel. To it 25 ml of chloroform is added and the contents are mixed gently for one

minute. Allow the layers to separate and discard the chloroform layer. Again add small portions of chloroform and discard organic fractions. Collect the aq layer and determine the absorbance at 267 mill microns using 1 cm quartz cell. The procedure is repeated with reference sample.

% of Riboflavin = 100 x AS/AR X WR/WS

WS = weight of sample

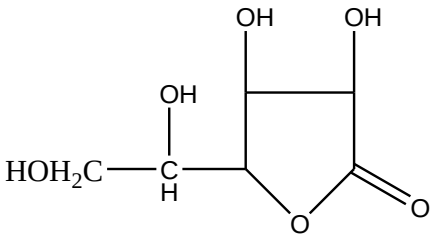
AS = Absorbance of sample

Wr = Weight of reference sample

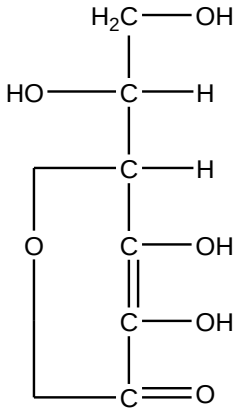
Ar = Absorbance of reference sample

Other methods: Polarographic methods is useful in determining Riboflavin, Partially purified Riboflavin, Vitamin mixture.

Vitamin-C (Ascorbic acid):



or



1. Ascorbic acid occurs as a white or slightly yellow crystalline – powder,
2. In dry state it is stable in air. But in solution it is readily oxidized in presence of air
3. In general determination of Ascorbic acid can be made by volumetric, colorimetric and polarographic techniques

4. the most widely used method for its determination is by using colorimetric technique

Colorimetric methods:

1. 2, 4 di nitro phenyl hydrazine method,
2. 4 – methoxy 2. nitro aniline method

4. Methoxy 2 – nitro aniline method:

Several method for determination of ascorbic acid is based upon coupling with diazonium compound. Ascorbic acid with diazotized 4-methoxy 2-Nitro and aniline to form a deep blue color compound. This method is specific for ascorbic acid. Other constituents like thiamine, riboflavin, pyridoxine, folic acid. Vitamins A, E etc., do not interfere during the determination.