Limit test for Iron

→ It is a chemical test for iron which determine the maximum amount of iron in a sample.

→ It is used to control small amount of impurities in a drug.

principle

→ This test is based on the reaction of iron & thioglycolic acid in an alkaline solution to form iron thioglycolate & is a reddish purple colour.

$$Fe^{++} + 2HSCH_2\text{-}COOH \rightarrow Fe(HSCH_2COO)_2 + 2H^+$$

(Thioglycolic acid) (Ferous thioglycolate)

function of thioglycolic acid

(1) If iron impurity present in trivalent feric form (Fe^{3+}), it redues to Fe^{2+}.

$$Fe^{3+} + \underset{\underset{COOH}{|}}{CH_2SH} \xrightarrow{\text{Ammonium citrate}} Fe^{2+} + 2\underset{\underset{COO^-}{|}}{CH_2SH} + 2H^+$$

(2) It produce purple colour & ferous (Fe^{2+}) iron

(3)

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citric acid: Reat ē ammonia solution, giving ammonium citrate, which act as buffer.

→ citrate ion also prevent the precipitation of iron by ammonia by forming a complex ē it.

$$2Fe + 10NH_3 \longrightarrow 2Fe(NH_2)_5 + 5H_2$$

Procedure (L.T. for iron in Mad I.P.)

S.No.	Test	standard
1)	Dissolve specify quantity (1gm, Mad) in Nesseler cylinder, in 40 ml D.W.	1) Place 2 ml of stand" iron (20 ppm fe) in a Nesseler cylinder in 40 ml D.W.
2)	Add 2 ml, 20% solution of iron-free citric acid	— do —
3)	Add 0.1 ml of thioglycolic acid	— do —
4)	make solution alkaline ē iron free amonia sol"	— do —
5)	Dilute to 50 ml mark ē distilled H_2O	— do —
6)	Stir & Stand for 5 minute	— do

Limit test for Lead

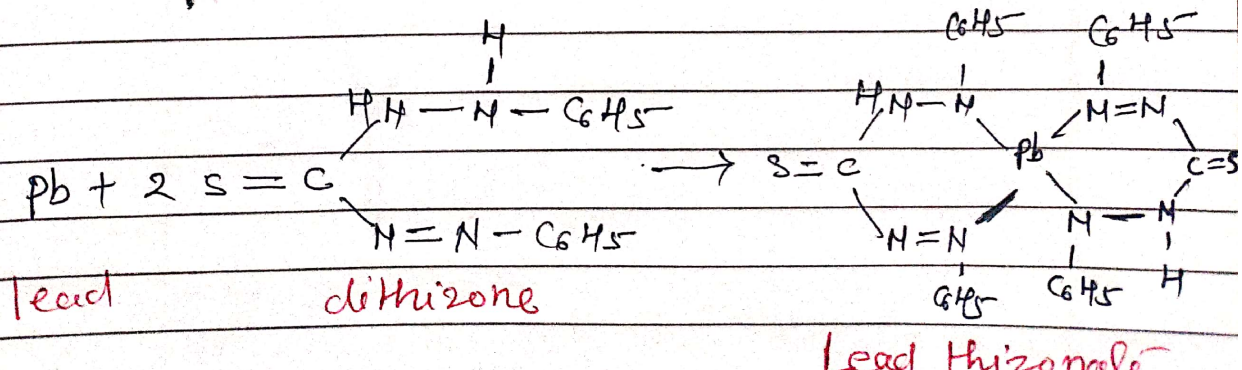
→ It is a chemical test that determine the amount of lead in a substance by reacting lead ⊂ diphenyl-thio-carbazone (dithizone) in an alkaline solution.

→ Lead is most undesirable impurity in medical compound and comes through use of sulphuric acid, lead lined apparatus and glass bottles used for storage of chemicals.

→ Common source of impurities of lead
1) equipment used for manufacturing
2) Storage container
3) from packaging material.

Principle

It is based on the reaction b/w lead & diphenyl thiocarbazone (dithizone) in alkaline solution to form lead dithizonate complex ⊆ is violet in color



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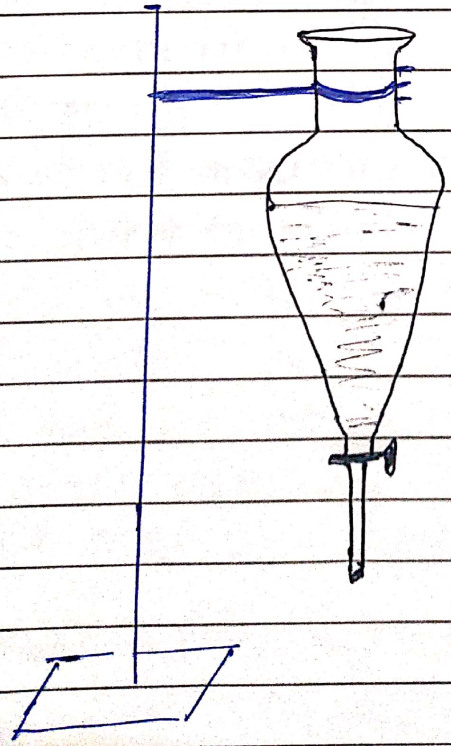
* Dithizone is green in colour in $CHCl_3$. and lead-dithizone complex is violet in color. so

* Dithizone in chloroform extract lead from alkaline aqueous solution as lead thizone complex (violet)

* The intensity of colour (violet) of complex depends on the quantity of lead present in solution, compared to standard colour produced by treating standard solution containing definit amount of pb in similar manner.

Apparatus used



separating funnel

Procedure for Pb

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SN.	Test	Standard (1ppm Pb)
1.	F/n quantity of Sample is transfered in separating funnel or Prepare a solution as per I.P.	Take lead Standard sol" (1ppm) equivalent to the amount of lead permitted in sample under examination in separating funnel
2.	Add 6 ml Ammonium citrate	— do —
3.	Add 2ml, hydroxylamine hydrochloride	— do —
4.	Add 2 ml of phenol red	— do —
5.	Make sol" just-alkaline by adding strong Ammonia sol"	— do —
6.	cool the solution if necessary & add 2ml KCN sol"	— do —
7.	extract ⊂ several quantities each 5 ml of dithizone extraction solution (DES), ↓ Drain of each extract into another separating funnel untill DES retain it's green colour.	— do —
8.	Combined dithizone extract are shaked for 30 ~~min~~ sec ⊂ 30 ml HNO_3 (1% V/V) & discard $CHCl_3$ layer	— do —

9. To the acid solution add 5 ml of dithizone stand. solution (DSS)	— do —
10. Add 4 ml of ammonium citrate ~~hydroxide~~ if necessary, Shake for 30 sec and observe colour.	— do —
11.	

OBSERVATION

1) If the colour intensity < standard = sample passed
2) If the colour intensity > standard = sample fail

① * Preparation of dithizone extraction sol" (DES)

Dissolve 30 mg of dithizone in 1000 ml of $CHCl_3$ and add 5 ml of C_2H_5OH (95%), Store the sol" in refrigerator.

→ B/F use Shake the suitable volume of sol" & about half it's volume of 1% v/v Solution of HNO_3 acid ↓ and ↓ discard the acid layer (it will remove if any impurity there) Shake then

② Prep" of dithizone stand" sol" (DSS)

Dissolve 10 mg of dithizone in 1000 ml of $CHCl_3$

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(3) Lead Standard Solution (1 PPM Pb)

By two steps

(1) Lead standard sol^{n} (0.1 %. or 1000 ppm. Pb)

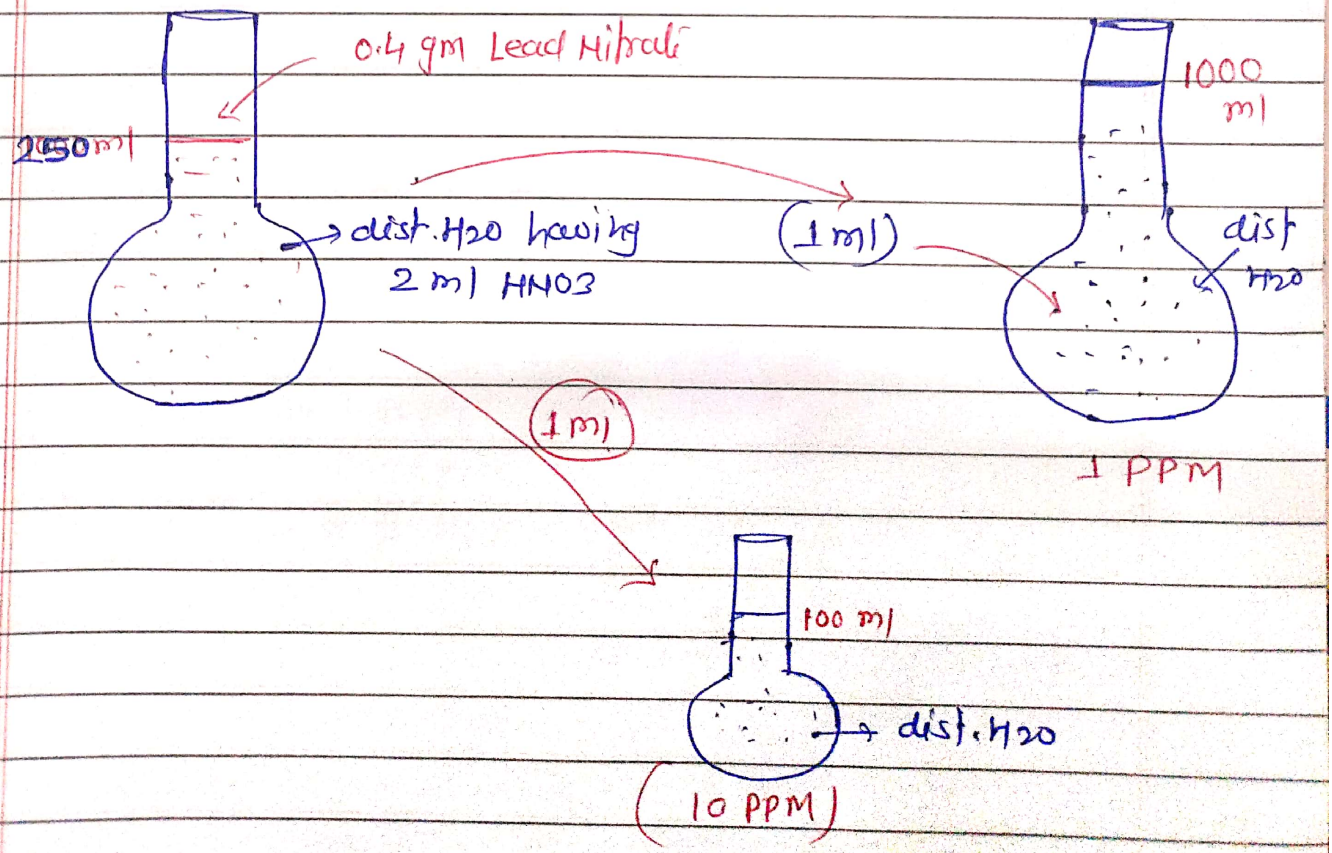
Sol A → Dissolve 0.4 g of Lead nitrate in H_2O containing 2 ml of HNO_3

↓

Add sufficient H_2O to produce 250 ml.

(2) Lead Standard solution (1 PPM)

Dilute 1 ml of solution 'A' (0.1 %. ~~DBS~~) to 1000 ml with distilled H_2O



* Ammonium citrate, KCN and hydroxylamino hydrochloride is used to make pH optimum so influence of other impurities have been eliminated.

* Phenol red is used as indicator to develop the colour at the end of process.

* Dithizone extraction solution help to extract the lead from sample as lead present in sample in minute quantity & not easy to be extract out.

Limit Test for Arsenic (Gutzeit test)

→ It is test that determine the concentration of As in a sample wether it is below or above the limits.

→ It is important to test as As is toxic in nature.

→ This test is also k/as Gutzeit test and require speical Appacatus.

Principle of Arsenic Limit test

→ It is based on the reaction of "arsenic gas" & Hydrogen ion in Gutzeit appacatus to form a yellow stain on mercuric chloride paper in presence of reducing agents.

→ Depth of colour depends on arsenic content of sample.

→ sample stain colour is compared & standard one to find the limits.

Steps

(1) Arsenic impurity may be present as trivalent or pentavalent form, it is first converted to arsenious acid by reducing agent like Zn/HCl, KI etc.

(2) arsenious acid then reduced to arsine by action of nacent Hydrogen produced by reaction b/w Zn & HCl.

(3) The liberated arsine gas then react ē mercuric chloride paper to form a yellow stain.

Reaction

Step - I

As^{3+} (Trivalent (90%)) $\xrightarrow{HCl}$ H_3AsO_3 or $As(OH)_3$ (Arsenious acid)

As^{5+} $\xrightarrow{HCl}$ H_3AsO_4 or $O=As(OH)_3$ (Arsenic Acid)

Step - II

Red. $SnCl_2$

H_3AsO_3 (Arsenious acid)

Step - III

$[Zn + 2HCl \longrightarrow ZnCl_2 + (H_2)]$

$H_3AsO_3 + 3H_2 \xrightarrow[\text{Reduction}]{Zn/HCl} AsH_3 + 3H_2O$

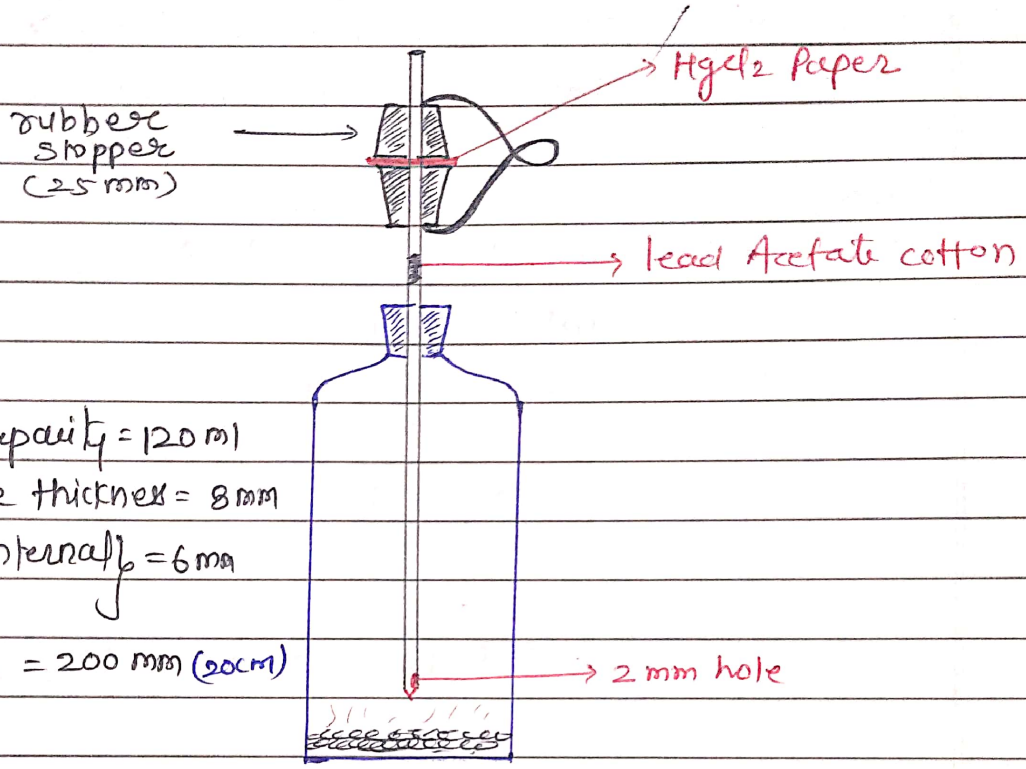
(Arsenious acid) (Arsine gas)

Step III

$2AsH_3 + HgCl_2 \longrightarrow Hg(AsH_2)_2 + 2HCl$

(Arsine gas) (Mercuric chloride)

Apparatus (Gutzeit Apparatus)



* bottle capacity = 120 ml
* Glass tube thickness = 8 mm
* Glass tube internal radius = 6 mm
* tube length = 200 mm (20cm)

Lead Acetat cotton :

It is used to avoid H_2S gas (if synthesized) during reaction. Lead Acetate react with H_2S and prevent to go up & react with $Hgcl_2$. if it react to $Hgcl_2$, paper turn black

Procedure

(1) Prep^{n} of test

1) Accurately measure the b/n amount of sample as directed by pharmacoepia & dissolve it in 50 ml dist. H_2O.

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(2) Now Add 5 ml, Arsenic free 1.0 M KI, ~~10 mg mt Stannated Hydrochloric~~ 10 ml stannated hydrochloric acid AST and 10 gm granulated zinc AST

(3) Immediatly assemble apparatus and immerse the flask in a water bath at temperature such the constant gas evolution is maintained for 40 minute.

(4) Observe stain on $HgCl_2$ paper.

(II) Preparation of standard

(1) Prepare 1 ml standard Arsenic solution and add 50 ml dist. H_2O.

(2) Add 05 ml ~~Stan~~ Arsenic free 1M KI + 10 ml stannated HCl + 10 gm granulated Zn

(3) Allow for 40 minute to react & release of arsenic gees.

(4) Observe the colour on paper.

Procedure

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S.H.	Test	standard
1)	Specify quantity of test sample (As per I.P.), dissolved in 50 ml dist H_2O	1. take K/n quantity of arsenic in 50 ml. distilled H_2O.
2)	Add 5 ml (1M) KI (AsT) + 10 ml stanated HCl (AsT) + 10 gm granulated Zn (AsT)	—— do ——
3)	Allow for 40 minute to reaction formation of Arsenic gas.	—— do ——
4)	Observe the spot color on $HgCl_2$ paper	—— do ——

Observation

* check the yellow spot on $HgCl_2$ paper.

(i) if intensity is less than standard → sample pass

(ii) if intensity is more than standard → sample fail.

* Important

(1) Zn granule → Provide surface area for releasing [H]

(2) $SnCl_2$ → Reduction of Arsenic acid → Arsenious acid.

(3) Lead Acetate cotton → to trap H_2S gas.