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Research Article

Pharmacopoeia reflects on permissible pharmaceutical impurities in parts per million limits

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ABSTRACT

PAT (Process Analytical Technology) produces enormous amount of inorganic impurities during unit operation. Inorganic impurities are mostly ionic matters which are water soluble cations & anions. Cations are iron (Fe^{2+}/Fe^{3+}), arsenic (As^{3+}/As^{5+}), lead (Pb^{2+}) and anions are chloride (Cl^{-}), sulfate (SO_4^{2-}). All heavy metal impurities are cumulative poison which has affinity to bind with lipid layer to produce health hazards. Pharmacopoeia says about permissible limit of each ion in parts per million (ppm) level which is acceptable by the living body with no harmful effect. This is checked by limit test which is quantitative or semi-quantitative analysis to identify the permissible impurity which is below the adverse effect. The test is generally performed by turbidity/opalescence comparison test, color comparison test and stain comparison test between test sample and standard sample. If the intensity of turbidity/colour/stain of test sample is equal to or less than standard sample then the sample passes the limit test, if the intensity of turbidity/color/stain of test sample is greater than standard sample then the sample fails to pass the limit test.

Keywords: ppm, ppb, ppt, ppq, cations, anions, heavy metals, test solution, standard solution, turbidity, color, stain, organic impurities, inorganic impurities, residual solvents, class-I/II/III solvents, ICH guidelines, API, USFDA

INTRODUCTION

In science and engineering, the **parts-per notation** is a set of pseudo units to describe small values of miscellaneous dimensionless quantities, e.g. mole fraction or mass fraction. Since these fractions are quantity-per-quantity measures, they are pure numbers with no associated units of measurement.[1]

Commonly used are **ppm** (parts-per-million, 10^{-6}), **ppb** (parts-per-billion, 10^{-9}), **ppt** (parts-per-trillion, 10^{-12}) and **ppq** (parts-per-quadrillion, 10^{-15}). One part per million (ppm) denotes one part per 1,000,000parts, one part in 10^6, 1/1,000,000×100% = 0.0001% (or 1% = 10,000ppm) and a value of 1×10^{-6}. This is equivalent to one drop of water diluted into 50liters (roughly the fuel tank capacity of a compact car) or about 32seconds out of a year.

PPM Definition: One PPM means one (defect or event) in a million or 1/1,000,000

How to convert ppm to percent

1% = 1/100

1ppm = 1/1000000

So

1ppm = 0.0001%

So to convert from ppm to percent, divide the ppm by 10000:

$x_{(\%)} = x_{(ppm)} / 10000$

Example: find how many percent are in 300ppm.

$x_{(\%)}$ = 300ppm / 10000 = 0.03%

ppm to percent conversion table is given below:

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Table 1: ppm vs percent

ppm	Percent (%)	ppm	Percent (%)	Ppm	Percent (%)	Ppm	Percent (%)
0ppm	0.0000%	10ppm	0.0010%	200ppm	0.0200%	3000ppm	0.3000%
1ppm	0.0001%	20ppm	0.0020%	300ppm	0.0300%	4000ppm	0.4000%
2ppm	0.0002%	30ppm	0.0030%	400ppm	0.0400%	5000ppm	0.5000%
3ppm	0.0003%	40ppm	0.0040%	500ppm	0.0500%	6000ppm	0.6000%
4ppm	0.0004%	50ppm	0.0050%	600ppm	0.0600%	7000ppm	0.7000%
5ppm	0.0005%	60ppm	0.0060%	700ppm	0.0700%	8000ppm	0.8000%
6ppm	0.0006%	70ppm	0.0070%	800ppm	0.0800%	9000ppm	0.9000%
7ppm	0.0007%	80ppm	0.0080%	900ppm	0.0900%	10000ppm	1.0000%
8ppm	0.0008%	90ppm	0.0090%	1000ppm	0.1000%	100000ppm	10.0000%
9ppm	0.0009%	100ppm	0.0100%	2000ppm	0.2000%	1000000ppm	100.0000%

Figure 1: Nessler cylinder

Limit test of chloride

Principle:

Limit test of chloride is based on the reaction of soluble chloride with silver nitrate in presence of dilute nitric acid to form silver chloride, which appears as solid particles (Opalescence) in the solution.

$$Cl^- + AgNO_3 \xrightarrow{HNO_3} AgCl\downarrow + NO_3^-$$

White turbidity/opalescence

Procedure:

Table 2: Chloride limit test

Test sample	Standard compound
Specific weight of compound is dissolved in water or solution is prepared as directed in the pharmacopoeia and transferred in Nessler cylinder	Take 1ml of 0.05845%W/V solution of sodium chloride in Nessler cylinder
Add 1ml of nitric acid	Add 1ml of nitric acid
Dilute to 50ml in Nessler cylinder	Dilute to 50ml in Nessler cylinder
Add 1ml of $AgNO_3$ solution	Add 1ml of $AgNO_3$ solution
Keep aside for 5min	Keep aside for 5min
Observe the Opalescence/Turbidity	Observe the Opalescence/Turbidity

Observation:

The opalescence produce in sample solution should not be greater than standard solution. If opalescence produces in sample solution is less than the standard solution, the sample will pass the limit test of chloride and vice versa.

Reasons:

Nitric acid is added in the limit test of chloride to make solution acidic and helps silver chloride precipitate to make solution turbid at the end of process. **[Official standard for chloride=50ppm]**

Limit Test of Sulphate

Principle:

Limit test of sulphate is based on the reaction of soluble sulphate with barium chloride in presence of dilute hydrochloric acid to form barium sulphate which appears as solid particles (turbidity) in the solution.

Procedure:

$$SO_4^{--} + BaCl_2 \xrightarrow{HCl} BaSO_4\downarrow + Cl^-$$

White turbidity/opalescence

Table 3: Sulfate limit test

Test sample	Standard compound
Specific weight of compound is dissolved in water or solution is prepared as directed in the pharmacopoeia and transferred in Nessler cylinder	Take 1ml of 0.1089% W/V solution of potassium sulphate in Nessler cylinder
Add 2ml of dilute hydrochloric acid	Add 2ml of dilute hydrochloric acid
Dilute to 45ml in Nessler cylinder	Dilute to 45ml in Nessler cylinder
Add 5ml of barium sulphate reagent	Add 5ml of barium sulphate reagent
Keep aside for 5min	Keep aside for 5min
Observe the Turbidity	Observe the Turbidity

Barium sulphate reagent contains barium chloride, sulphate free alcohol and small amount of potassium sulphate.

Figure 2: Turbidity/opalescence comparison test

Observation:

The turbidity produce in sample solution should not be greater than standard solution. If turbidity produces in sample solution is less than the standard solution, the sample will pass the limit test of sulphate and vice versa.

Reasons:

Hydrochloric acid helps to make solution acidic.

Potassium sulphate is used to increase the sensitivity of the test by giving ionic concentration in the reagent

Alcohol helps to prevent super saturation. **[Official standard for sulfate=50ppm]**

Limit test of Arsenic

Principle:

Limit test of Arsenic is based on the reaction of arsenic gas with hydrogen ion to form yellow stain on mercuric chloride paper in presence of reducing agents like potassium iodide. It is also called as Gutzeit test and requires special apparatus. Arsenic, present as arsenic acid in the sample is reduced to arsenious acid by reducing agents like potassium iodide, stannous acid, zinc, hydrochloric acid, etc. Arsenious acid is further reduced to arsine (gas) by hydrogen and reacts with mercuric chloride paper to give a yellow stain.[2]

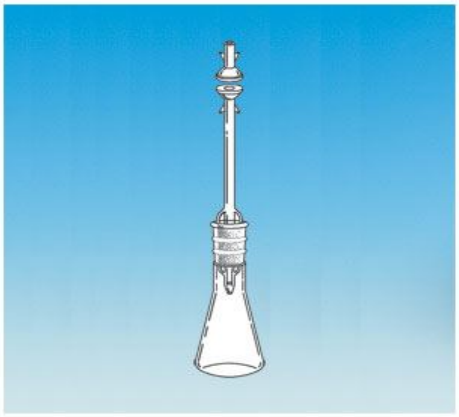


Figure 3: Gutzeit apparatus

$H_3AsO_4 + H_2SnO_2 \rightarrow H_3AsO_3 + H_2SnO_3$

Arsenic acid Arsenious acid

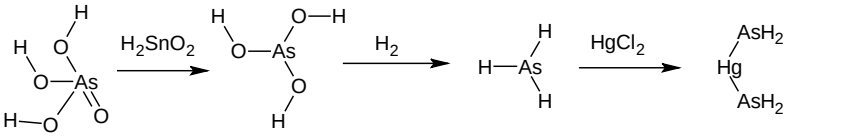
$H_3AsO_3 + 3H_2 \rightarrow AsH_3 + 3H_2O$

Arsenious acid Arsine

$Hg(AsH_2)_2$

Mercuric arsenide (Yellow stain)

Pentavalent arsenic (arsenic acid) is reduced into trivalent state (arsenious acid) by the action of stannic chloride by reduction which is further reduced into arsine gas. This arsine produces yellow stain by reaction with mercuric chloride to mercuric arsenide.



The depth of yellow stain on mercuric chloride paper will depend upon the quality of arsenic present in the sample.

Procedure:

Test solution:

The test solution is prepared by dissolving specific amount in water and stannated HCl (arsenic free) and kept in a wide mouthed bottle. To this solution 1gm of KI, 5ml of stannous chloride acid solution and 10gm of zinc is added (all these reagents must be arsenic free). Keep the solution aside for 40min and stain obtained on mercuric chloride paper is compared with standard solution.

Standard solution:

A known quantity of dilute arsenic solution is kept in wide mouthed bottle and rest procedure is followed as described in test solution. Approximately 60ml generator bottle with 40ml indicating line. Glass tube with 6.5mm inner diameter. A ground joint glass tube with 6.5mm inner diameter and 18mm outer diameter at the joint. Inner joint and the outer joint form a concentric circle. Rubber stopper. Narrow part of the glass tube. Glass wool is inserted up to this part. Rubber board (Lead acetate cotton plug). Clamp

Reasons:

Stannous chloride is used for complete evolution of arsine. Zinc, potassium iodide and stannous chloride are used as a reducing agent. Zinc reacts with stannous chloride in acidic medium to produce zinc-tin couple which slowly reacts with acid to generate hydrogen gas and this gas acts as reducing agent *in-situ*. Pentavalent arsenic (As^{5+}) is reduced into trivalent arsenic (As^{3+}) and this is now converted arsine which makes yellow stain by the reaction with mercuric chloride paper. Hydrochloric acid is used to make the solution acidic. Lead acetate pledger or papers are used to trap any hydrogen sulphide which may be evolved along with arsine. **[Official standard for arsenic=2ppm]**

$H_2S + (CH_3COO)_2Pb \rightarrow PbS\downarrow + 2CH_3COOH$

Black

Limit test of Lead

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

Principle:

Limit test of lead is based on the reaction of lead and diphenyl thiocabazone (dithizone) in alkaline solution to form lead dithizone complex which is red in color. Dithizone is green in color in chloroform and lead-dithizone complex is violet in color, so the resulting color at the end of process is red.

Procedure:

Table 4: Lead limit test

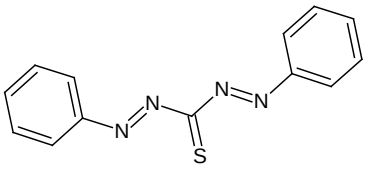
Test sample	Standard compound
A known quantity of sample solution is transferred in a separating funnel	A standard lead solution is prepared equivalent to the amount of lead permitted in the sample under examination
Add 6ml of ammonium citrate	Add 6ml of ammonium citrate
Add 2ml of potassium cyanide and 2ml of hydroxylamine hydrochloride	Add 2ml of potassium cyanide and 2ml of hydroxylamine hydrochloride
Add 2drops of phenol red	Add 2drops of phenol red
Make solution alkaline by adding ammonia solution.	Make solution alkaline by adding ammonia solution.
Extract with 5ml of dithizone until it becomes green	Extract with 5ml of dithizone until it becomes green
Combine dithizone extracts are shaken for 30mins with 30ml of nitric acid and the chloroform layer is discarded	Combine dithizone extracts are shaken for 30mins with 30ml of nitric acid and the chloroform layer is discarded
To the acid solution add 5ml of standard dithizone solution	To the acid solution add 5ml of standard dithizone solution
Add 4ml of ammonium cyanide	Add 4ml of ammonium cyanide
Shake for 30mins	Shake for 30mins
Observe the color	Observe the color

Observation:

The intensity of the color of complex, is depends on the amount of lead in the solution. The color produce in sample solution should not be greater than standard solution. If color produces in sample solution is less than the standard solution, the sample will pass the limit test of lead and vice versa.

Reasons:

Ammonium citrate, potassium cyanide, hydroxylamine hydrochloride is used to make pH optimum so interference and influence of other impurities have been eliminated. Phenol red is used as indicator to develop the color at the end of process. Lead present as an impurities in the substance, gets separated bye extracting an alkaline solution with a dithizone extraction solution.



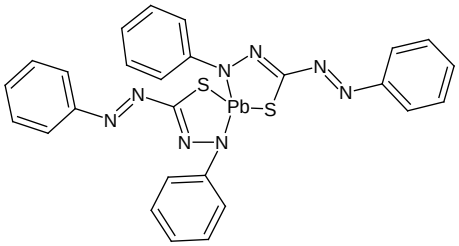
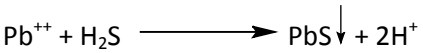


Figure 4: Dithizone and lead dithizone complex

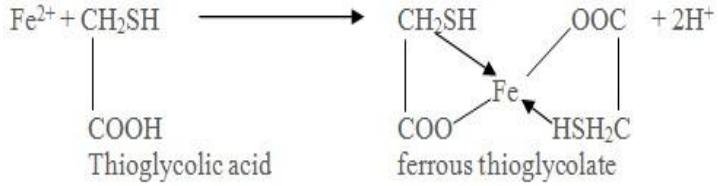
Lead limit test is also done by turbidity comparison test where lead solution is treated with sodium sulfide in acidic media. Acid reacts with sodium sulfide to produce hydrogen sulfide which makes black turbidity and that is compared between test and standard samples. **[Official standard for lead=10ppm]**



Limit test of Iron

Principle:

Limit test of Iron is based on the reaction of iron in ammoniacal solution with thioglycolic acid (sulfanyl acetic acid) in presence of citric acid to form iron thioglycolate which is pale pink to deep reddish purple in color.



Procedure:

Table 5: Iron limit test

Test sample	Standard compound
Sample is dissolved in specific amount of water and then volume is made up to 40ml	2ml of standard solution of iron diluted with water upto 40ml
Add 2ml of 20% w/v of citric acid (iron free)	Add 2ml of 20% w/v of citric acid (iron free)
Add 2drops of thioglycollic acid	Add 2drops of thioglycollic acid
Add ammonia to make the solution alkaline and adjust the volume to 50ml	Add ammonia to make the solution alkaline and adjust the volume to 50ml
Keep aside for 5min	Keep aside for 5min
Color developed is viewed vertically and compared with standard solution	Color developed is viewed vertically and compared with standard solution

Earlier ammonium thiocyanate reagent was used for the limit test of iron. Since thioglycolic acid is more sensitive reagent, it has replaced ammonium thiocyanate in the test.

Observation: The purple color produce in sample solution should not be greater than standard solution. If purple color produces in sample solution is less than the standard solution, the sample will pass the limit test of iron and vice versa.

Reasons:

Citric acid helps precipitation of iron by ammonia by forming a complex with it. Thioglycolic acid helps to oxidize iron (II) to iron (III). Ammonia to make solution alkaline. **[Official standard for iron=40ppm]**

Limit Test of Heavy Metals

Principle: Limit test of heavy metals is based on the reaction of metallic impurities with hydrogen sulfide in acidic medium to form brownish colour solution. Metals that response to this test are lead, mercury, bismuth, arsenic, antimony, tin, cadmium, silver, copper and molybdenum. The metallic impurities in substances are expressed as parts of lead per million parts of the substance. The usual limit as per Indian Pharmacopoeia is 20ppm.[3,4]

Procedure:

The Indian Pharmacopoeia has adopted three methods for the limit test of heavy metals.

Method I: Use for the substance which gives clear colorless solution under the specific condition.

Table 6: Heavy metal limit test (Method I)

Test sample	Standard compound
Solution is prepared as per the monograph and 25ml is transferred in Nessler's cylinder	Take 2ml of standard lead solution and dilute to 25ml with water
Adjust the pH between 3 to 4 by adding dilute acetic acid 'Sp' or dilute ammonia solution 'Sp'	Adjust the pH between 3-4 by adding dilute acetic acid 'Sp' or dilute ammonia solution 'Sp'
Dilute with water to 35ml	Dilute with water to 35ml
Add freshly prepared 10ml of hydrogen sulphide solution	Add freshly prepared 10ml of hydrogen sulphide solution
Dilute with water to 50ml	Dilute with water to 50ml
Allow to stand for five minutes	Allow to stand for five minutes
View downwards over a white surface	View downwards over a white surface

Observation:

The color produce in sample solution should not be greater than standard solution. If color produces in sample solution is less than the standard solution, the sample will pass the limit test of heavy metals and vice versa.

Method II: Use for the substance which do not give clear colorless solution under the specific condition.

Table 7: Heavy metal limit test (Method II)

Test sample	Standard compound
Weigh specific quantity of test substance, moisten with sulphuric acid and ignite on a low flame till completely charred Add few drops of nitric acid and heat to 500°C Allow to cool and add 4ml of hydrochloric acid and evaporate to dryness Moisten the residue with 10ml of hydrochloric acid and digest for two minutes Neutralize with ammonia solution and make just acid with acetic acid	Take 2ml of standard lead solution and dilute to 25ml with water
Adjust the pH between 3 to 4 and filter if necessary	Adjust the pH between 3 to 4 by adding dilute acetic acid 'Sp' or dilute ammonia solution 'Sp'
Dilute with water to 35ml	Dilute with water to 35ml
Add freshly prepared 10ml of hydrogen sulphide solution	Add freshly prepared 10ml of hydrogen sulphide solution
Dilute with water to 50 ml	Dilute with water to 50ml
Allow to stand for five minutes	Allow to stand for five minutes
View downwards over a white surface	View downwards over a white surface

Observation:

The color produce in sample solution should not be greater than standard solution. If color produces in sample solution is less than the standard solution, the sample will pass the limit test of heavy metals and vice versa.

Method III: Use for the substance which gives clear colorless solution in sodium hydroxide solution.

Table 8: Heavy metal limit test (Method III)

Test sample	Standard compound
Solution is prepared as per the monograph and 25ml is transferred in Nessler's cylinder or weigh specific amount of substance and dissolve in 20ml of water and add 5ml of dilute sodium hydroxide solution	Take 2ml of standard lead solution
Make up the volume to 50ml with water	Add 5ml of dilute sodium hydroxide solution and make up the volume to 50ml with water
Add 5drops of sodium sulphide solution	Add 5drops of sodium sulphide solution
Mix and set aside for 5min	Mix and set aside for 5min
View downwards over a white surface	View downwards over a white surface

Observation:

The color produce in sample solution should not be greater than standard solution. If color produces in sample solution is less than the standard solution, the sample will pass the limit test of heavy metals and vice versa. **[Official standard for heavy metals=20ppm]**

Conclusion:

According to ICH guidelines, impurities related to drug substances can be classified into three main categories: organic impurities, inorganic impurities and residual solvents.

1. Organic impurities: Organic impurities can arise in APIs or drug product formulations during the manufacturing process or during the storage of drug substances. They may be known, unknown, volatile, or non-volatile compounds with sources including starting materials, intermediates, unintended by-products and degradation products. They may also arise from racemization or contamination of one enantiomeric form with another. In all cases they can result in undesired biological activity. Recently, genotoxic pharmaceutical impurities, which may potentially increase cancer risks in patients, have received considerable attention from regulatory bodies and pharmaceutical manufacturers. In general, genotoxic impurities include DNA reactive substances that have the potential for direct DNA damage. Potential genotoxic impurities include process impurities or degradants, present at trace levels, which are generated during drug manufacturing and storage. As per FDA and EMA guidelines, potential genotoxic impurities are to be controlled at levels much lower than typical impurities. The recommended acceptable thresholds for genotoxic impurities in pharmaceuticals can be found in the guideline documents published by the USFDA and EMA. The ICH M7 guidance on genotoxic impurities is currently under 5 preparations with the working title "M7 Assessment and Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk".

2. Inorganic (elemental) impurities: Inorganic impurities can arise from raw materials, synthetic additives, excipients and production processes used when manufacturing drug products. Several potentially toxic elements may be naturally present in the ingredients and these elements must be measured in all drug products. A further group of ingredients may be added during production and must be monitored for elemental impurities once they are known to have been added. Sources of inorganic impurities include manufacturing process reagents such as ligands, catalysts (e.g., platinum group elements (PGE)), metals derived from other stages of production (e.g., process water and stainless steel reactor vessels), charcoal and elements derived from other materials used in filtration. The United States Pharmacopeia (USP) is in the process of developing a new test for inorganic impurities in pharmaceutical products and their ingredients. The current Heavy Metals Limit Test (USP) is widely acknowledged to be inadequate in terms of scope, accuracy, sensitivity and specificity and is due to be replaced with two new general chapters, Limits (USP) and Procedures for Elemental Impurities (USP), due to be implemented in 2013. In parallel with the development of USP and other pharmacopoeias the USP is also introducing a related method which is specific to dietary supplements. USP defines new, lower permitted daily exposure (PDE) limits for a wider range of inorganic elemental impurities: As, Cd, Hg, Pb, V, Cr, Ni, Mo, Mn, Cu, Pt, Pd, Ru, Rh, Os and Ir. USP further defines the sample preparation and method validation procedures that should be used for system suitability qualification of any instrumentation used for the analysis of elemental impurities in pharmaceutical materials. Validation of

analytical instruments that are used for the new USP and USP methods will be performance based. USP defines the analytical and validation procedures that laboratories must use to ensure that the analysis is specific, accurate and precise.

3. Residual solvents: Residual solvents are the volatile organic chemicals used during the manufacturing process or generated during drug production. A number of organic solvents used in synthesis of pharmaceutical products have toxic or environmentally hazardous properties and their complete removal can be very difficult. In addition, the final purification step in most pharmaceutical drug substance processes involves a crystallization step which can lead to the entrapment of a finite amount of solvent which can act as a residual impurity or can cause potential degradation of the drug. Residual solvent levels are controlled by the ICH, USP and EP. Depending on their potential risk to human health, residual solvents are categorized into three classes with their limits in pharmaceutical products set by ICH guidelines Q3C. The use of **Class I solvents**, including benzene, carbon tetrachloride, 1,1-dichloroethane, 1,2-dichloroethylene and 1,1,1–trichloroethane should be avoided. **Class II solvents**, such as methanol, pyridine, toluene, *N,N*-dimethyl formamide and acetonitrile have permitted daily exposure limits (PDEs). **Class III solvents**, such as acetic acid, acetone, isopropyl alcohol, butanol, ethanol and ethyl acetate should be limited by GMP or other quality-based requirements.

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