PHA 389 PHARMACEUTICAL TECHNOLOGY- I

3rd week

Pharmaceutical Quality Water

Water is an important ingredient for pharmaceutical products.

- It is the most common vehicle for drugs,
- and first choice for solving materials.

Besides,

- Water is used in every step of pharmaceutical production
- It is physiologically inert

Water can be used as,

- solvent and vehicle in preparing pharmaceutical dosage forms
- for synthesize drugs and excipients
- in different steps of production such as cleaning.

In all these steps type/quality of water needed is different. For example,

- for preparing an injectable dosage form, water must be sterile and injectable
- if the formulation is orally given purified water can be used

Water for pharmaceutical purposes must be prepared from potable (drinking) water by using suitable methods.

Resources for potable water can be

- Groundwater
- Surface water

Surface water or ground water can contain components of earth, rock and air.

- Solubilized minerals and salts
- Suspended and colloidal particles
- Dissolved gases
- Living and dead organisms and their metabolic products (E.Coli)
- Organic substances (humic acid, tannin, lignin)

Also surface water can exhibit variations in quality according to the changes in seasons, location, pesticides, fertilizers and animal excretions. Especially total organic carbon (TOC) amount is affected by seasonal temperature.

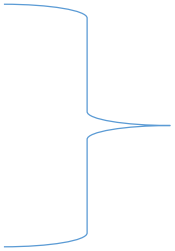
Potable water:

- is the water suitable for drinking.
- is controlled by EPA (Environmental Protection Agency)
- EPA concerns about microbiological purity rather than mineral contents.
- E. Coli is the most important bacterial content as it is the indicator of coliform microorganisms which comes from fecal contamination in water.
- Potable water must not involve E. Coli more than 5% of the total monthly samples.
- Total aerobic microbial count for potable water must be under 500 cfu/mL.

(cfu: colony forming unit)

Water for pharmaceutical purpose requires unchangeable properties and high quality.
Thus, potable water must be clarified before use.

- **Settling**
- **Filtration**
- **Ion Exchange**



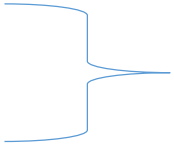
removal of insoluble material

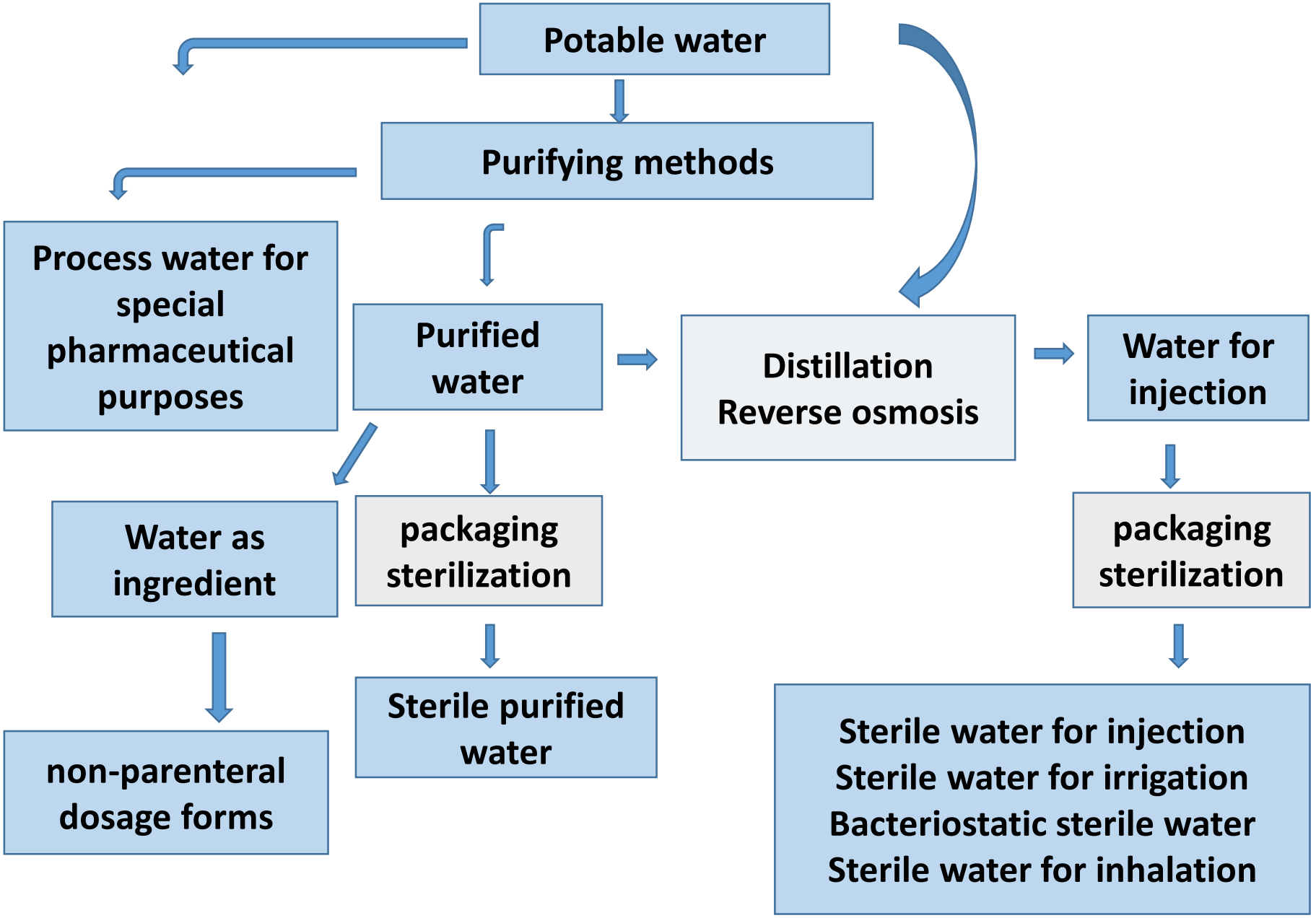
- **Aeration**
- **Chlorination**

palatability improvement

saving from pathogenic microorganisms

- **Activated charcoal**




Potable water
Purifying methods
Process water for special pharmaceutical purposes
Purified water
Distillation
Reverse osmosis
Water for injection
Water as ingredient
packaging sterilization
packaging sterilization
non-parenteral dosage forms
Sterile purified water
Sterile water for injection
Sterile water for irrigation
Bacteriostatic sterile water
Sterile water for inhalation

American Pharmacopoeia (USP)

- Purified water
- Sterile purified water
- Water for injection
- Sterile water for injection
- Bacteriostatic water for injection
- Sterile water for irrigation
- Sterile water for inhalation

European Pharmacopoeia (EP)

- Water, Purified
  - *a.* Purified water in bulk
  - *b.* Purified water in containers
- Water , highly purified
- Water for injection
  - *a.* Water for injection in bulk
  - *b.* Sterilized water for injection

Purified water (USP)

- It is prepared from drinking water by distillation, ion-exchange, reverse osmosis, filtration or other suitable process.
- Purified water is ingredient of pharmaceutical formulations, can be used for cleaning of equipment or in tests and assays, some bulk pharmaceutical chemicals can be prepared.
- Used is the source of sterile purified water for non-parenteral dosage forms
- Purified water must contain less than 100 cfu/mL aerobic microorganism

Purified water (EP)

Purified water in bulk

✓**Total viable aerobic microorganism limit** is important.
✓**TOC value** must be less than 0,5 mg/L.
✓**Conductivity** must be less than 4.3 µS/cm (20°C)

Purified water in containers

✓Packaged after purification, do not consist any additive.
✓Must be kept away from microbiologic contamination .
✓Sutable for preparing dialysis solutions.

Sterile Purified Water (USP)

✓This is the purified water that is **sterilized and suitably packaged.**

✓It contains no antimicrobial agent.

✓It is used in the preparation of non-parenteral dosage forms where sterile form of purified water is required.

✓**Do not use this type of water for parenterally administered dosage forms**

Highly purified water (EP)

- Combination of **double pass reverse osmosis and ultrafiltration** can be used for preparing this water.
- This is the water type which injectable water is not needed but a high quality of water must be used.
- It must be stored in suitable conditions to avoid contamination .

✓TOC value must be less than 0,5 mg/L.

✓**Conductivity value is: 1.1 µS/cm (20°C)**

Water for Injection (USP)

- Must carry the same properties with purified water but but **is also pyrogen-free**
- Intended to be used in the manufacture of injectable products which are to be sterilized after their preparation.
- Intended to be used for 24-hours after its collection. It must be protected from microbial contamination.
- **Maximum endotoxin limit is 0.25 IU/mL**
- **Re-distillation or reverse osmosis** methods should be applied for final purification.

Sterile Water for Injection (USP)

- **sterile, non-pyrogenic** preparation of water for injection which contains no bacteriostatic, antimicrobial agent or added buffer
- is supplied only in **single-dose containers** with **less than 1L**
- It is used to dilute or dissolve drugs for injection.
- **Maximum endotoxin limit is 0.25 IU/mL**

Precautions:

✓For I.V. injection, add sufficient solute to make an approximately isotonic solution,

✓pH 5.0 to 7.0

✓Do not use unless water is clear, seal is intact and container is undamaged.

Bacteriostatic Water for Injection (USP)

- is sterile water **containing suitable antimicrobic preservatives**
- **Bacterial endotoxin limit must not exceed 0.5 IU/mL**
- can be used in diluting drugs that can subsequently be administered by intravenous, intramuscular, or subcutaneous injection.
- İt is supplied in plastic containers and not pressurized.

  Single dose or 30 mL multiple-dose
- Their container can be re-entered multiple times (usually by a sterile needle)
- It is not used for neonatal medications because of possible blood pressure changes and toxicity of preservatives
- If it is injected i.v. without any diluted compound, it may cause some red blood cell lysis because it is not isotonic.

Sterile Water for Irrigation (USP)

- is a **sterile, distilled, non-pyrogenic water** for injection **intended only for sterile irrigation**, washing, rinsing and dilution purposes.
- pH 5.5 (5.0 to 7.0).
- It contains no bacteriostatic, antimicrobial agent or added buffer
- is intended for use only as a **single-dose (containers larger than1litre)**or short procedure irrigation.
- When smaller volumes are required the unused portion should be discarded.
- Sterile Water for Irrigation may be classified as a sterile irrigant, wash, rinse, diluent and pharmaceutical vehicle.
- NOT FOR INJECTION BY USUAL PARENTERAL ROUTES
- Do not heat container over 66 C (150 F).

Sterile Water for Inhalation (USP)

- is Water for Injection that is packaged and rendered sterile and is intended for use in inhalators and in the preparation of inhalation solutions.
- It carries a less stringent specification for bacterial endotoxins than Sterile Water for Injection, and therefore, is not suitable for parenteral applications.
- Bacterial endotoxin limit must not exceed 0.5 IU/mL

Water for injection (EP)

In EP water for injection is divided into 2 subgroups;

- **Water for injection in bulk**
- **Sterilized water for injection**

✓**Bakterial endotoxin limit: 0.25 IU/mL**

✓TOC value: 0,5 mg/L

✓**Conductivity value: 1.1 µS/cm (20°C)**

Water for injection in bulk is used as vehicle in parenteral dosage forms

Sterilized water for injection is used for dissolving or diluting of parenteral dosage forms. It is injectable water and is sterilized after sealing the container.

Conductivity:
✓25 $\mu S \cdot cm^{-1}$ for volume less than 10mL
✓5 $\mu S \cdot cm^{-1}$ for volume higher than 10 mL

Total Organic Carbon (TOC) (EP 5)

TOC determination is an indirect measure of organic substances present in water for pharmaceutical use. This test also can monitor the performance of various operations in preparation of medicines.

Humic acid (high MW; colloid structure) and Fulvic acid (low MW) are the main reasons of TOC in water. These organic colloidal complexes cause poor negative electric flow in water and thus resulted with an increase in conductivity which is the indicator of poor purification in water. Cl^- ions in water can react with humic acid and this resulted with trihalomethane .

TOC water must comply with the following specifications,

- ✓ TOC value must be less than 0,5 mg/L in purified water.
- ✓ **Conductivity: not greater than 1.0 µS/cm at 25ºC in injectable water**
- ✓ **Total organic carbon: not greater than 0.1 mg/L injectable water**

The objective of TOC test is, completely oxidizing the organic substances in sample water to produce carbon dioxide followed by measuring the amount of CO_2 produced.Amount of CO_2 gives the carbon concentration in water which is indicator of organic substances.

Endotoxin:

- Endotoxin is a toxin that is found on the outer membrane of various **Gram negative bacteria and are lipopolysaccharide (LPS)** structured.
- LPS consists of a polysaccharide (sugar) chain and a lipid moiety, which is responsible for the toxic effects
- water is the main source for endotoxins
- **They are heat stable** to 250ºC and and cannot be inactivated by autoclaving.

Pyrogen:

- Any substance which on injection causes a rise of temperature can be called as pyrogen.
- Endotoxins are pyrogenic materials. They can easily pass through the normal bacterial filters. Special filters which act by adsorption and ion exchange can be used rather than simple filtration.
- Official method for preparing non- pyrogenic water is distillation rather than ion exchange resins, because the pyrogenic materials are non-volatile.

Tests for pyrogen

1- rabbit test → **in vivo**

2- bacterial endotoxin test (LAL test) → **in vitro**

Rabbit test (USP 27, EP 2002)

The test involves measurement of the rise in body temperature of rabbits following the intravenous injection of a sterile solution of the substance being examined.

Advantages

- It measures the fever by pyrogenic level
- Detects all kinds of injectable pyrogen unlike LAL test.

Disadvantages

- Time consuming, Expensive , cannot be used to test certain drugs that depresses the fever, Tolerance to certain class of drugs can develop in rabbits & also biological variations are observed

Bacterial endotoxin test (LAL)

is used to detect or quantify endotoxin of gram negative bacterial origin using amoebocyte lysate from horseshoe crab (Limulus polyphemus).

- The test is based on the observation that when a endotoxin contacts clot protein from circulating amoebocyte of horse shoe crab (Limulus) a gel clot forms.

- There are three general technique for this test

Gel clot technique: based on gel formation.

Turbidimetric method: based on development of turbidity after cleavage of an endogenous substrate.

Chromogenic method: based on the development of color after cleavage of a synthetic peptide-chromo-gen complex

Bacterial endotoxin test is 5-10 times more sensitive than rabbit test and because of the serial dilutions used in this test, it is considered as semiquantitative.

However it has some limitations,

- Only gram(-) bacteria sourced pyrogen can be examined
- Sensitivity affected from source of microorganism
- This is an in vitro test; you cannot determine the potential of fever for the tested endotoxin

Methods for water purification given in pharmacopeias.

- **Pre-purification** → Particules, visible substances, organic and inorganic materials and ions can be removed in this step.
  - Microporous filters
  - Filtering from activated carbon
  - Softening
  - Organic scavengers
  - Addition of chemical additives

  can be used
- **Distillation**
- **Deionisation**
- **Reverse osmosis**
- **Ultrafiltration**
- **Electrodialysis**
- **Electrodeionisation**

Distillation

Distillation is a process in which a liquid or vapour mixture of two or more substances is separated into its component fractions of desired purity, by the application and removal of heat.

- Distillation is done on the basis of differences in their volatilities in a boiling liquid mixture.
- Distillation is a physical separation process, and not a chemical reaction.

1. Non-volatile residue such as;

*particulates

*inorganic substances

*high MW substances

*microorganisms

2. Organic substances having different boiling points than water

can be removed by this method.

BASIC DISTILLATION EQUIPMENTS

Simple distillation is the process of converting a liquid into its vapour, transferring the vapour to another place and recovering the liquid by condensing the vapour.

- ✓ **Distiller is the place volatile material is vaporized**
- ✓ **Condanser is the heat exchanger (electricity, gas, water vapour ..)**
- ✓ **Receiver is the place distillate is collected.**

In distilation,

- Feed water must be drinking/potable water. However, deionised water is more useful as feed water as it avoids stalactite formation. Storage of the prepared water is also as important as production.
- If not used immediately, it is sterilized for 1 hour at 120 °C.
- In large scale productions it is stored at 80 °C and above at constant temperature or under UV irradiation

Deionization

Ion exchange resins are used for removing ions from the water.

Deionized water can be used for,

- ✓glassware cleaning (especially high volume bottles)
- ✓non-parenteral liquid dosage form preparation
- ✓Suitable as feed water for distillation
- ✓Can not be used as parenteral (involves pyrogen)

There are 2 types of ion exchange resins;

- Cation exchange resins which carry acid groups on their surface and traps cations from water
- Anion exchange resins which carry alkaline groups on their surface and traps anions from water

✓Resins used in deionization do not dissolve in water or solvents, resistant to heat, acid and alkaline, mechanically stable, porous structured and do not swell highly.

Problems of ion exchange resins,

- Microorganisms can easily grow on the surface of ion exchange resins if the system is not continuously used (in stagnant water)and irregularly regenerated

- Cation exchange resins can be regenerated by mineral acids and anion exchange resins can be regenerated by alkaline medium.

- Regeneration also enhances the exchange capacity
- Microrganisms in the water cannot be removed by this method

Types of deionization

- **Conventional method**

  1st step is water passage from the column involving strong cationic resin

  2nd step is water passage from the column involving poor anionic resin

- **Reverse method**

  First anionic resin then cationic resin can be used

- **Mixed bed method**

  Both resin types are found in the same column in a mixed state.

Electrodeionization

- Contains a combination of mixed resin, selectively permeable membrane and electric charge to povide continuous flow (product and waste water)and continuous regeneration.
- Produces very high water purity
- Unlike other water treatment processes, electrodeionization does not require any chemical treatment. Instead , the process utilizes a mild electrical current from electrodes to deionize water.
- This process separates impurities and regenerates resin to produce ultra-pure water.
- EDI is often a supplemental step to reverse osmosis , which relies on semi-permeable membranes to filter impurities from water.

Electrodialysis

- This is a similar process that uses only electricity and selectively permeable membranes together.
- They can separate the removed ions, small organic molecules and some colloidal particles from water stream.
- It is less efficient than electrodeionization process as it does not contain a resin.

Reverse Osmosis

- is a process in which pressure greater than the natural osmotic pressure is applied on the high concentration side of the membrane, forcing the water to travel through the membrane from higher to lower chamber.
- Reverse osmosis membranes also hold back suspended impurities; such as, silt, colloidal particles and microorganisms by virtue of their ultra-fine pore size

➡ **Particles under 1 nm**
➡ **Organic molecules over 200 Da**
➡ **Microorganisms can be removed**

Chloride, ammonia, CO_2 can pass through reverse osmosis membranes; therefore, water must be prefiltrated from activated carbon.

Types of reverse osmosis membranes

- A reverse osmosis membrane must be freely permeable to water, highly impermeable to solutes, and able to withstand high operating pressures.
- It should ideally be tolerant of wide ranges of pH and temperature and should be resistant to attack by chemicals like free chlorine and by bacteria.
- Ideally, it should also be resistant to scaling and fouling by contaminants in the feed water.
- the pore size for R.O membrane is around 0.0001 microns

There are three major types of membranes:

- **Cellulosic**
- **Fully aromatic polyamide**
- **Thin film composite**

- Cellulose acetate membranes are inexpensive and easy to manufacture but suffer from several limitations such as to hydrolysis and can only be used over a limited pH range (low pH 3 to 5 and high pH 6 to 8)
- They have a high water permeability but reject low molecular weight contaminants poorly.

- Aromatic polyamide membranes have better resistance to hydrolysis and biological attack than cellulosic membranes.
- They have better salt rejection characteristics than cellulosic membranes.
- They can be operated over a pH range of 4 to 11.

- In the thin film composites the water flux and solute rejection characteristics are predominantly determined by the thin surface layer, whose thickness ranges from 0.01 to 0.1 micrometers

Advantages of reverse osmosis

- Energy requirement is low
- RO systems can be installed on very small household basis
- Very high salt rejection rate (>99%)
- Excellent rejection of microorganisms and organic compounds

Disadvantages of reverse osmosis

- Pre-treatment is always required before reverse osmosis
- Low chemical compatibility-highly sensitive to pH
- Not compatible with micro organisms which have acetate attacking enzymes

ULTRAfiltration

- ✓This is another technology using permeable membranes but unlike reverse osmosis it works by mechanical separation.
- ✓Due to the ability of membranes endotoxins can also be removed.
- ✓They can be appropriate for intermediate or final purification step.
- ✓Care should be takent o avoid stagnant water conditions that could promote microorganism growth

Quality controls on pharmaceutical water (EP 5)

Purified water in bulk

- Appearance,
  color, odor, taste
- Aluminium
- Nitrates
- Sulphates
- Bacterial endotoxins

Purified water in containers

- Acidity/Alkalinity
- Oxidisable substances
- Chloride
- Heavy metals
- Ammonium
- Calcium, magnesium

Microbial contamination

Highly purified water

- Appearance
- Aluminium
- Nitrates
- Heavy metals
- Bacterial endotoxins

Water for Injection (WFI)

WFI in bulk

- Appearance
- Nitrates
- Aluminium
- Heavy metals
- Bacterial endotoxins
- TOC
- Conductivity

Sterilised WFI

- Acidity/Alkalinity
- Conductivity
- Oxidisable substances
- Chlorides

Nitrates

- Sulphates
- Aluminium

- Ammonium
- Calcium, Magnesium
- Residue on evaporation
- Particulate contamination
- Sterility
- Bacterial endotoxins