Method of analysis of milk contaminants

Soma Maji

Need for analysis

- Residues of antibiotics and pesticides have adverse affect on the health of the consumers as well as affect the quality of the dairy products.
- Presence of these contaminants in the milk and milk products is through the indiscriminate use of antibiotics in the health management of cattle and excessive use of pesticides in the fields.
- Heavy metals also have health hazards.
- In the present era of globalization the quality is the top most considered factor in running the business.
- These contaminants are present in very small amounts, which are difficult to detect by the routine or old methods so sensitive methods are to be used.
- Sensitive methods help in ensuring the presence or absence of these contaminants in the food stuffs.

Methods for Pesticides

- Isolation of organochloro pesticide residues (OCPR) in milk and milk products essentially is based upon adsorption clean-up (IDF standard *75* C: 27 method F, 1991).
- This involves mixing of test portion in presence of water with florisil until a homogenous powder is obtained
- Transfer of this mixture to florisil column
- Selective elution of pesticides and concentration of the eluate followed by HPLC analysis.
- The sample isolates are analysed by standardised **HPLC** conditions on octadecylsilyl (ODS) column, with solvent system methanol: water (80:20) at flow rate of 1.0 ml/min and at wavelength of 254 nm.
- Gas liquid chromatography (GLC) with electron capture detector (ECD) can also be used for the analysis of OCPR.

Florisil powder

Florisil column

Detection of multi- residue pesticide

- Isolation of multi-residue of pesticides in milk is based upon solid phase extraction (SPE) over C18 cartridges.
- This involves blending of milk sample with acetonitrile followed by collection of supernatant over anhydrous sodium sulphate.
- The supernatant is concentrated to small volume, passed through SPE cartridges, eluant evaporated and again dissolved in known volume of acetonitrile followed by High pressure liquid chromatography (HPLC) analysis using binary gradient programming of acetonitrile and water solvent system.
- The sample isolates of multi-residue of pesticides are analysed by standardised HPLC conditions on ODS column, with solvent system acetonitrile: water (75:25) at flow rate of 0.5 ml/min and at wavelength of 200 nm.
- GLC with nitrogen phosphorus detector (NPD) can also be used for the analysis of organophosphates and carbamates.

Methods of detection of Antibiotics in milk

1. Routine test methods for detection of antibiotic residues in milk

Various rapid antibiotic detection methods have been commercialized in last two decades.

Currently, seven types of detection methods are commonly used for detection of antibiotic residues in milk i.e.

- Microbial growth inhibitor assay,
- Microbial receptor assay,
- Enzyme-colorimetric assay,
- Receptor binding assay,
- Spectrophotometric assay,
- Chromatographic methods and
- Immunoassay.

These methods are qualitative, quantitative or semi-quantitative.

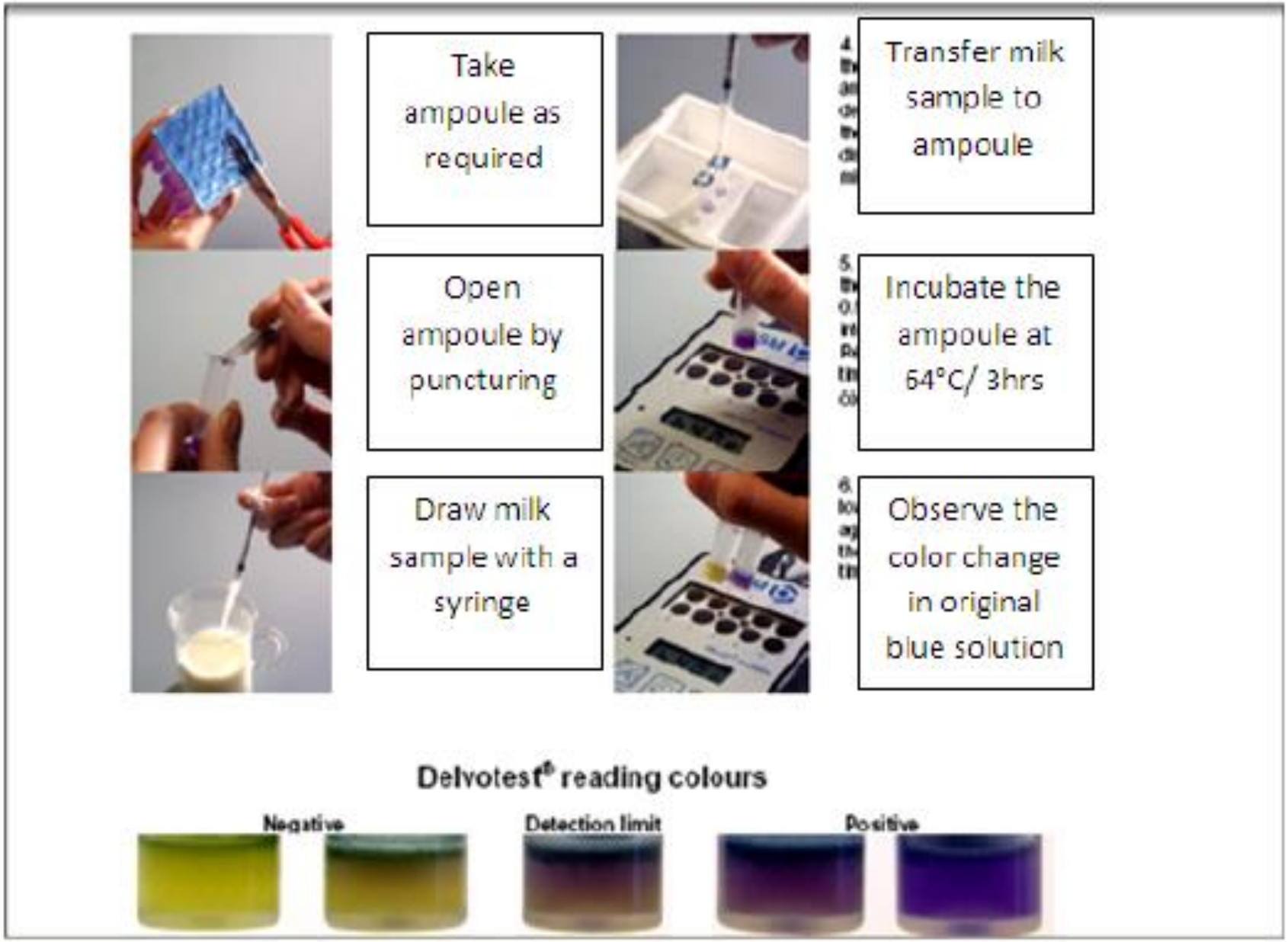
Currently, microbial inhibitor & immuno-receptor tests have gained most popularity in the dairy industry at international level.

1.1 *Reference method*

- The reference method for the determination of antibiotic residues in raw milk and in heat-treated milk is the International Dairy Federation microbial inhibition test.
- It has relatively high sensitivity to inhibitory substances.
- The method uses *B. stearothermophilus* as the test organism.
- It involves the continual growth of large quantities of *B. stearothermophilus* spores. That's why it is lengthy and complex process
- The test involves a color change, which is dependent on the growth of *B. stearothermophilus*, if the organism fails to grow then a false negative result may occur.
- Due to the aforementioned technical difficulties in carrying out the reference method, microbial inhibitor test kit assays based on the IDF method, using *B. stearothermophilus* are the routine methods used for the determination of antibiotic residues in milk.

1.2 *Microbial Inhibitor test*

- The 'traditional' tests for antibiotics in milk, known as 'microbial inhibitor' tests, involve incubating a susceptible organism in the presence of the milk sample.
- In the absence of an antibiotic, the organism grows and can be detected visually either by opacity of the agar growth medium or by a color change resulting from acid production.
- In the presence of an antibiotic, or any other inhibitor, the organism fails to grow and a zone of inhibition or lack of a color change is observed.
- Such tests are exceptionally sensitive to ß-lactam antibiotics.
- They are generally reliable and cost-effective but require incubation for several hours before the result can be visualized.



Microbial inhibitor test

1.3 Commercially available microbial inhibitor test

- Based on the microbial inhibitor test principle several commercially available kits are popularly used.
- The **Delvotest** (Gist-brocades BV, The Netherlands) is the best known microbial inhibitor test.
- The first version developed, in the 1970s, was the Delvotest P, designed to detect ß-lactams.
- The target organism, *B. stearothermophilus*, is encapsulated in an agar medium containing a pH indicator, a nutrient tablet and the substantial excretion of these residues into milk sample both being dispensed onto the agar surface.
- The 'ampoule version' is designed for individual tests or small-scale testing whilst a micro-tire plate version is designed for mass testing where 96 tests can be undertaken simultaneously.
- A negative result is indicated by a color change from purple to yellow, due to acid development during incubation at 64°C for 2½ hours.

- The Delvotest P has sensitivity to penicillin G of 0.005 IU/ml. is capable of detecting a wider spectrum of substances, notably sulphonamides, but also has increased sensitivity to erythromycin, neomycin, gentamicin, trimethoprim and other antimicrobials.
- The Delvotest SP appears identical to the Delvotest P, the only difference being the need to incubate the Delvotest SP for 2¾ hours.
- The Delvotest SP has sensitivity to penicillin G of 0.003-0.004 IU/ml.

- The Charm AIM-96 test is a micro-tire plate test, similar to the Delvo test and capable of detecting ß-lactams, sulphonamides, tetracycline, macrolides and amino glycosides in 96 samples simultaneously
- End point is a blue-yellow color change which indicates that a sample is negative.
- The Charm Farm test is a 'test-tube' version of the AIM-96 test, designed for on-farm use and employs the same microbial inhibitor principle with a color change.

2. Rapid test kits for the detection of antibiotics in milk

- Microbial inhibition tests are lengthy and test for a broad spectrum of antibiotics
- Whereas rapid test kits generally detect a specific family of antibiotics.
- To avoid delays at milk intake points, rapid antibiotic screening tests are often used on raw milk prior to completion of the Delvo® SP test.

2.1 Immuno-receptor test

- A more rapid and reliable method and this method is a variation of the well-established enzyme-linked immunosorbent assay (ELISA).

- A specific target antibiotic group is captured by immobilized antibodies, or by a broader-spectrum receptor such as a bacterial cell.

- Most tests involve a competitive principle in which antibiotic in the sample competes with an internal antibiotic standard for the immune receptor.

- The antibody-antibiotic complex is then usually linked to an enzyme that catalyses a color or fluorescence reaction and a comparison of the intensity of the 'test' reaction with that of a 'control' determines whether the sample is positive or negative.

- A low intensity usually means 'positive' and a high intensity is regarded as 'negative'.

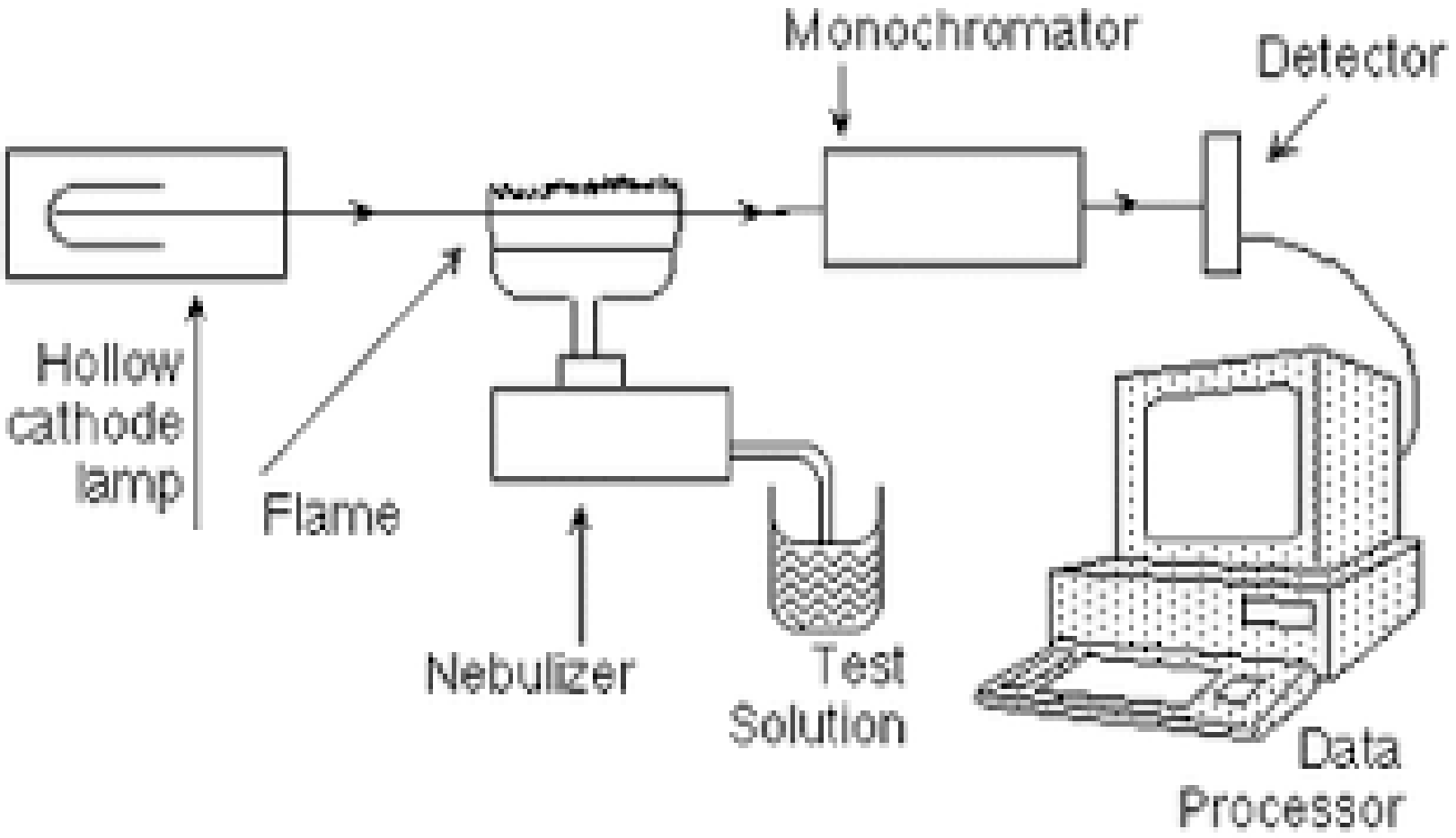
- Immune receptor tests can be made quantitative but are generally used to provide a 'pass/fail' result.

- More expensive than microbial inhibitor tests but only detect substances that react immunologically with the immobilized receptor and they provide a result in less than 10 minutes.

Methods for detection of Heavy Metals in milk

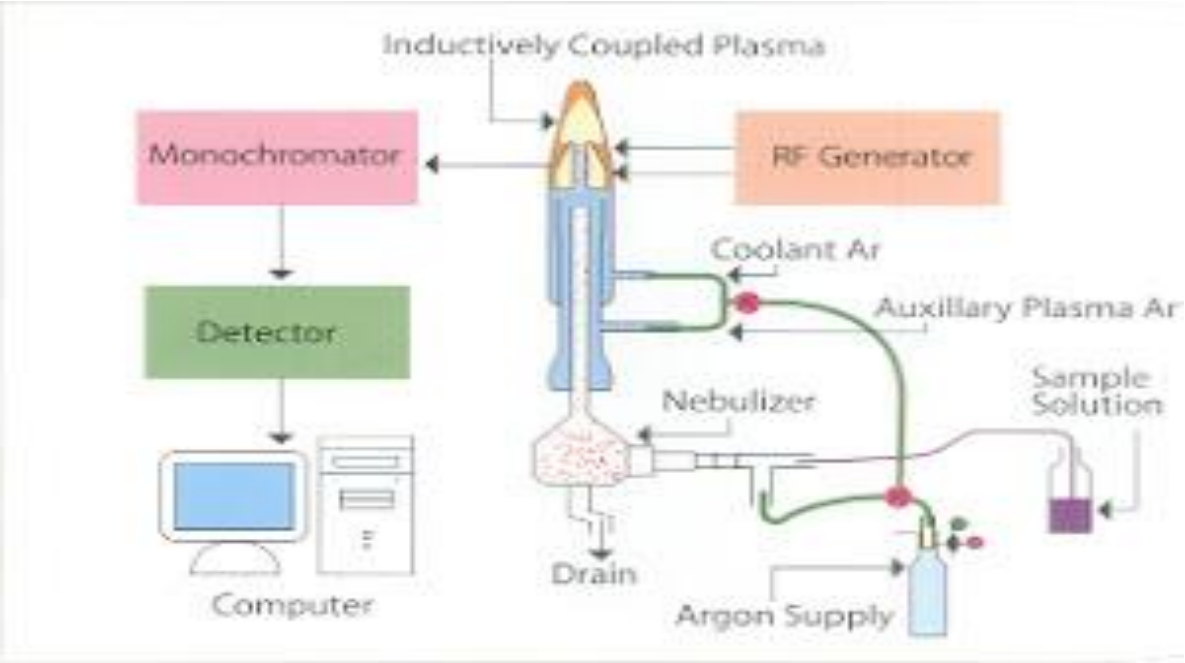
Atomic absorption spectrometery

- For routine analysis of heavy metals the method of choice is the atomic absorption spectrometry (AAS).
- The main approaches in this technique are either the wet acidic digestion or the dry ashing to yield the final inorganic extract, suitable for flameless determination in the AAS apparatus.
- Most preparation procedures lead to undesirable high backgrounds levels from chemicals us and may therefore lead to false results.
- For the determination of arsenic, selenium, and mercury, special AAS techniques with the thermal decomposition of arsenic gas, selenium hydride or the cold absorption of mercury vapors are frequently used and yield reliable results.
- Neutron activation analysis (NAA) is based on element specific γ radiation of irradiated elements, but is not suitable for routine.
- Other procedures are spectrophotometric methods, voltametric and isotop dilution mass spectrometry.


Monochromator
Detector
Hollow cathode lamp
Flame
Nebulizer
Test Solution
Data Processor

Inductively coupled plasma atomic emission spectroscopy (ICP-AES)

- ICP-AES, also referred to as inductively coupled plasma optical emission spectrometry (ICP-OES), is an analytical technique used for the detection of trace metals. It is a type of emission spectroscopy that uses the inductively coupled plasma to produce excited atoms and ions that emit electromagnetic radiation at wavelengths characteristic of a particular element.



Inductively coupled plasma-mass spectrometry (ICP-MS)

- ICPMS is a relatively new technique for the determination of trace elements in solution.
- It offers better sensitivity than graphite furnace AA with the multi-element speed.
- It is a type of mass spectrometry that is highly sensitive and capable of the determination of a range of metals and several non-metals at concentrations below one part in 1012.
- It is based on coupling together a high-temperature ICP (inductively coupled plasma) source with a mass spectrometer.
- The ICP source converts the atoms of the elements in the sample to ions.
- These ions are then separated and detected by the mass spectrometer.
- In a typical application, metals are placed in solution by acid digestion.
- The solution is sprayed into flowing argon and passed into a torch which is inductively heated to approximately 10,000^{o}C.

- At this temperature, the gas and almost everything in it is atomized and ionized, forming a plasma which provides a rich source of both excited and ionized atoms.

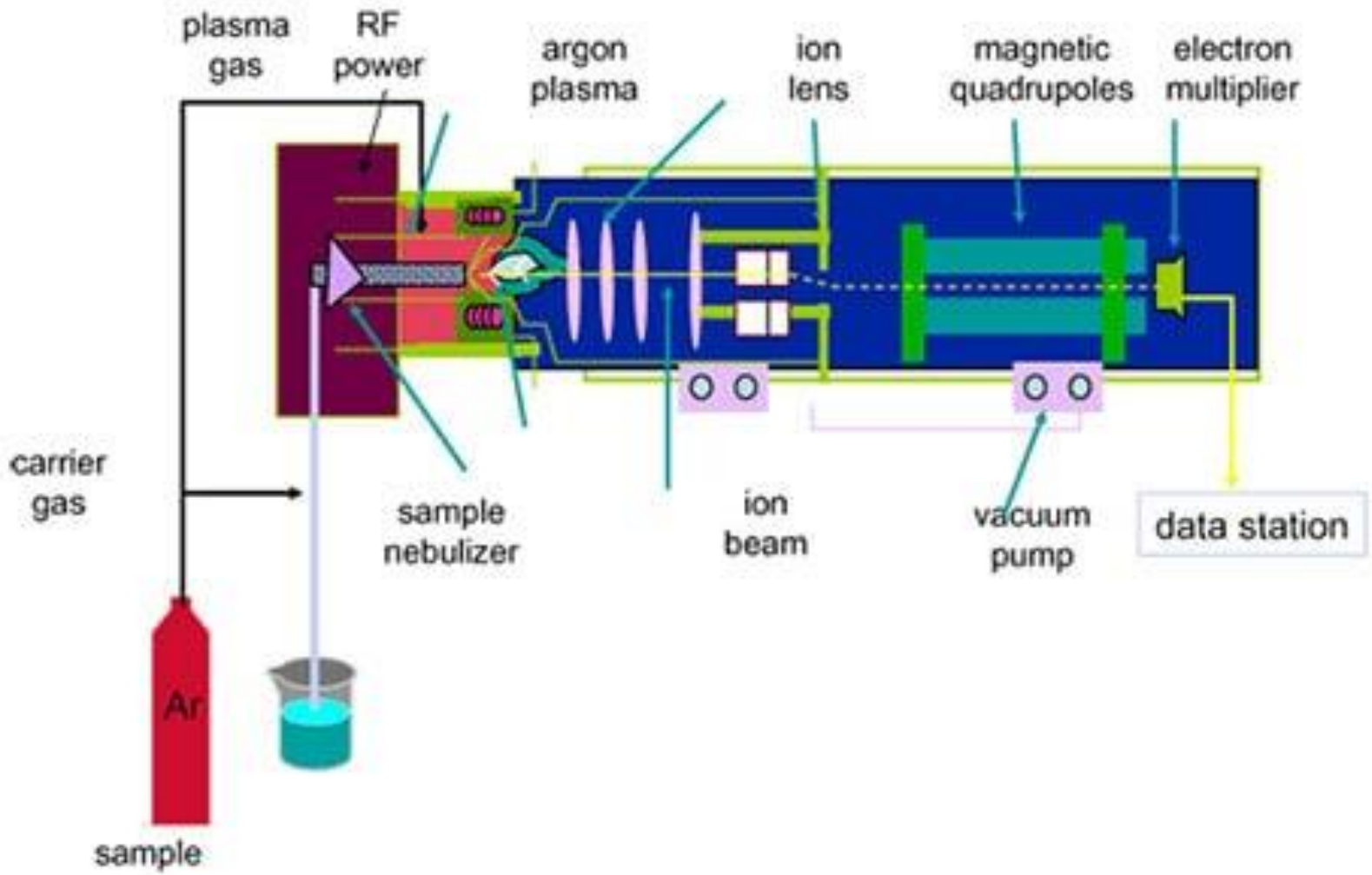
- In ICPMS, positive ions in the plasma are focused down a quadrapole mass spectrometer.

- By acquiring the mass spectrum of the plasma, data can be obtained for almost the entire periodic table in just minutes

- with detection limits vary from metal to metal and ranges from 0.1 – 100 ppb depending upon the type of elements.

- This method requires a very small amount of sample about 10 mg.

ICP - MS


plasma gas
RF power
argon plasma
ion lens
magnetic quadrupoles
electron multiplier
carrier gas
sample nebulizer
ion beam
vacuum pump
data station
Ar
sample

Advantages of ICP - MS

- Detection limits for most elements equal to or better than those obtained by Graphite Furnace Atomic Absorption Spectroscopy (GFAAS)
- Higher throughput than GFAAS
- The ability to handle both simple and complex matrices with a minimum of matrix interferences due to the high-temperature of the ICP source
- Superior detection capability to ICP-AES with the same sample throughput
- The ability to obtain isotopic information.