

Phytos



**Your reliable partner for customized
analytical solutions**

www.phytos.de

Phytos | Experts for Analytical Solutions



Environmental Contaminants – Heavy Metals

Analytical Methods

Points to Consider



Contents

1. Heavy Metals – Insertion into the Environment, Uptake by Plants & Toxicology
2. Specifications set by Ph. Eur. and USP
3. Analytical Methods
4. Validation of Methods
5. Ways to Prevent Contamination in Pharmaceutical Herbal Drugs



1. Heavy Metals – Insertion into the Environment, Uptake by Plants, Toxicology

Environmental Relevant Heavy Metals:

Lead (Pb), Cadmium (Cd), Mercury (Hg)

Arsenic (As), Copper (Cu), Nickel (Ni), Zinc (Zn)

Caesium (Cs), Uranium (U), Plutonium (Pu).



Insertion into environment:

- Geogenic input via soil (Ni, As, Pb)
- Airborne input (traffic, industry, incineration plants etc.; mainly Pb, Cd, Hg, As, Cu, Ni, Zn)
- Agricultural insertion (pesticides, fertilizers; mainly Zn, Cu, U)



Agricultural Input:

A) Via Fertilizers:

Inorganic fertilizers are produced from natural minerals by chemical processes (Haber-Bosch method, chemical extraction of phosphates). Especially fertilizing with inorganic phosphates leads to accumulation of Uran and Cadmium. Uran and phosphate are chemically bonded in Apatite. Raw phosphates contain 10 to 200 mg/kg Uran! This problem will increase in the future because raw phosphates with low concentrations of metals are high in price and of limited source on earth.

Another problem for the input of heavy metals is the use of sewage sludge (Cd, Zn, Ni).



Agricultural Input:

B) via Pesticides:

Zinc (Zn) via Dithiocarbamates (Mancozeb, Methyl-metiram, Metiram, Propineb, Zineb, Ziram) used as pesticides and fungicides.

Zineb = $C_4H_6N_2S_4Zn$

Copper (Cu) via copper vitriol ($CuSO_4$) used in fungicides.



Uptake by Plants:

Plants incorporate the heavy metals like the essential mineral salts by uptake of aqueous solutions as ions with their roots. Poorly available metals like Zn or Cu build chelates with organic substances of the soil and are incorporated in this form by the plants.

Some plants even accumulate specific heavy metals like Cd:

Willow (up to 0.5 % of dried matter; Zn, Cd, Pb)

Silene spec. (campion)

The plants accumulate the heavy metals mostly in rhizomes (Valerian root; Pb), leaves (Birch leaves; Pb), barks (Willow bark; Cu, Pb) but not in fruits.



Toxicology:

- Lead: Dysfunction of Hemoglobine synthesis, disorders of central nervous system
- Cadmium: damage of kidneys and bone structure
- Mercury: chronic damages of nervous system



2. Specifications by Ph. Eur.

Ph. Eur. Monograph #1433:

„Heavy metals (2.4.27). Unless otherwise stated in an individual monograph or unless otherwise justified and authorised:

- *Cadmium*: maximum 1.0 ppm;
- *Lead*: maximum 5.0 ppm;
- *Mercury*: maximum 0.1 ppm.

Where necessary, limits for other heavy metals may be required.“



2. Specifications by USP

In USP chapter 851 AAS is described as a method. The chapter 561 „Articles of Botanical Origin“ does not mention any limit for heavy metals.

Most botanical drugs are monographed in the dietary supplement of the USP with specific limits for total heavy metals with reference to the spectrometric method 231 „metallic impurities coloured by sulfide ion...“



3. Analytical Methods

Actually the Ph. Eur. chapter 2.4.27 allows the use of atomic absorption spectrometry (AAS). In future also inductively coupled plasma-atomic emission spectrometry (ICP-OES) and inductively coupled plasma-mass spectrometry (ICP-MS) will be allowed.



3. Analytical Methods

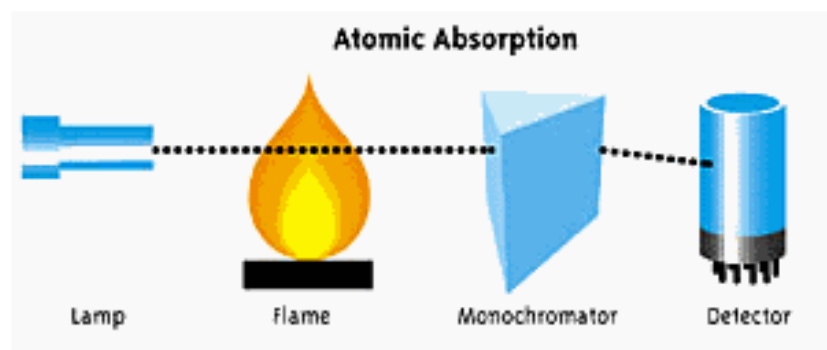
Main Principle:

Atomization of the analytes from a liquid sample and detection via absorption of light of a specific wavelength (AAS), emission of light (ICP-OES) or via specific mass of the ions (ICP-MS).



3. Analytical Methods

AAS:



F-AAS: Flame atomic absorption spectrometry

Atomization in a flame (usually air/acetylene, approx. 2,300 to 2,800 °C); an aliquote of the sample is sprayed as an aerosol into the flame



3. Analytical Methods

AAS:

GF-AAS:

Graphite furnace atomic absorption spectrometry

Atomization in a graphite tube by electric heating

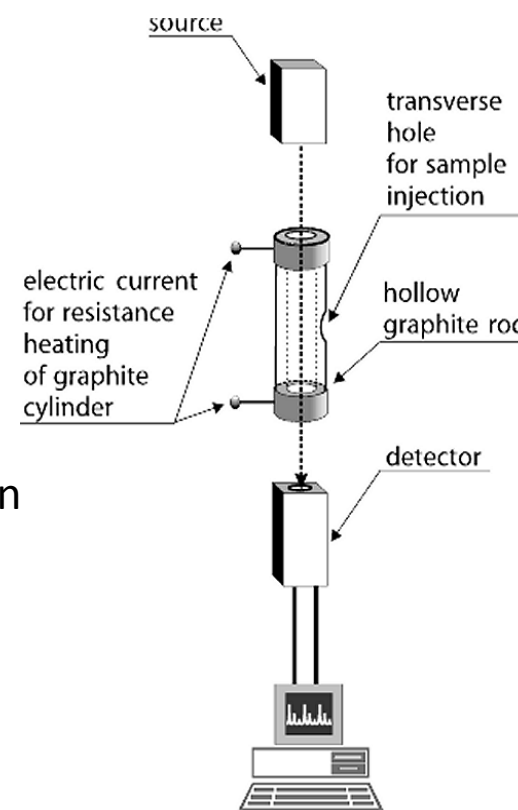
(graphite heats by electric current on its own

electrical resistance); the sample gets quantitatively in

the tube, remains longer there (7 sec.) and therefore

limit of detection is 3 decimal powers more sensitive

than by F-AAS or ICP-OES.





3. Analytical Methods

AAS:

HG-AAS: Hydride-generation atomic absorption spectrometry

(e.g. Arsenic); reaction of the analyte with nascent

Hydrogen (e.g. AsH_3) and heating in a quartz-glass-cuvette

(up to 1,000 °C) until decay of the hydride; also suitable for

ICP-OES; comparable LODs as Graphite-furnace technique.



3. Analytical Methods

AAS:

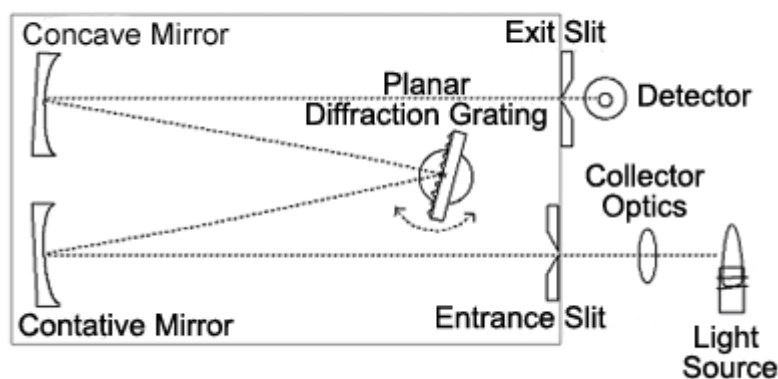
CV-AAS: Cold-vapour atomic absorption spectrometry

(e.g. Mercury); a different HG-AAS technique; with the help of a reducing agent (e.g. Stannum II chloride) atomic mercury is produced; quartz-glass-cuvette is only heated up to 100 °C (cold vapour).



3. Analytical Methods

ICP-OES:

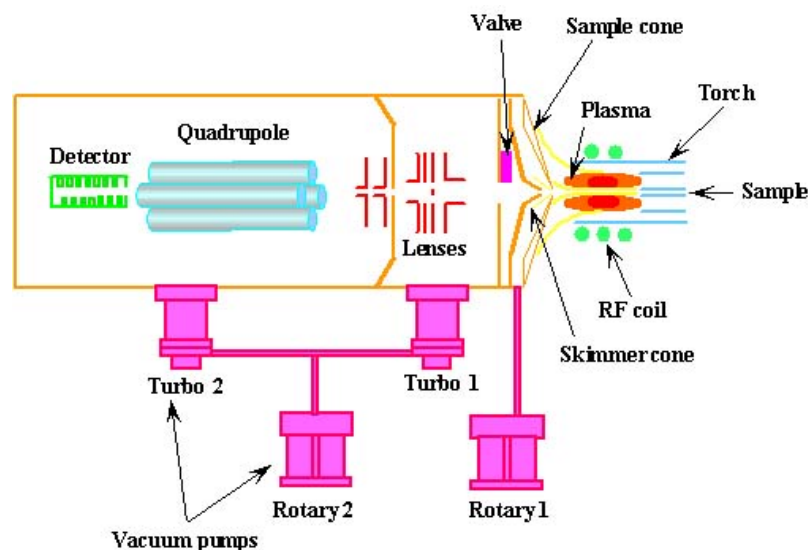


Plasma is an ionized gas, containing atoms, electrons and ions. Argon is used as plasma (approx. 10,000 °C) and produced by a high-frequency-generator. Sample aerosol is produced by an ultrasonic generator. On the contrary to AAS ion lines are measured.



3. Analytical Methods

ICP-MS:



Plasma is an ionized gas, containing atoms, electrons and ions. Argon is used as plasma (approx. 5,000 to 10,000 °C) and produced by a high-frequency-generator. Sample aerosol is produced by an ultrasonic generator. On the contrary to ICP-OES ions and their isotopes are detected by their masses.



3. Analytical Methods

Comparison of LODs of the different methods:

Method	LOD (ppt)
GF-AAS	> 0.1
ICP-OES (pneumatic der. aerosol)	> 30
ICP-OES (ultrasonic der. Aerosol)	> 10
ICP-MS	> 0.02

Under optimal conditions !!!!





4. Validation of Methods

Validation acc. to ICH guideline Q2 (R1) „Validation and Analytical Procedures“

Specific criteria:

- For ICP molecular ion interferences or isotope interferences of different metals consisting same masses should be considered.
- Recovery rate 80 to 120 %
- LOD in minimum 10 % of the limit of the metal detected
- Specified limits for RSD values for repeatability and reproducibility depending on the measured concentrations



5. Ways to Prevent Contamination in Pharmaceutical Herbal Drugs

- Strict production under GACP
- No use of heavy metal containing pesticides or fertilizers
- Pre-analysis of the soil and selection of non accumulating plants for critical soil types
- pH management of the soil



**Thank you very much for
your kind attention !**