

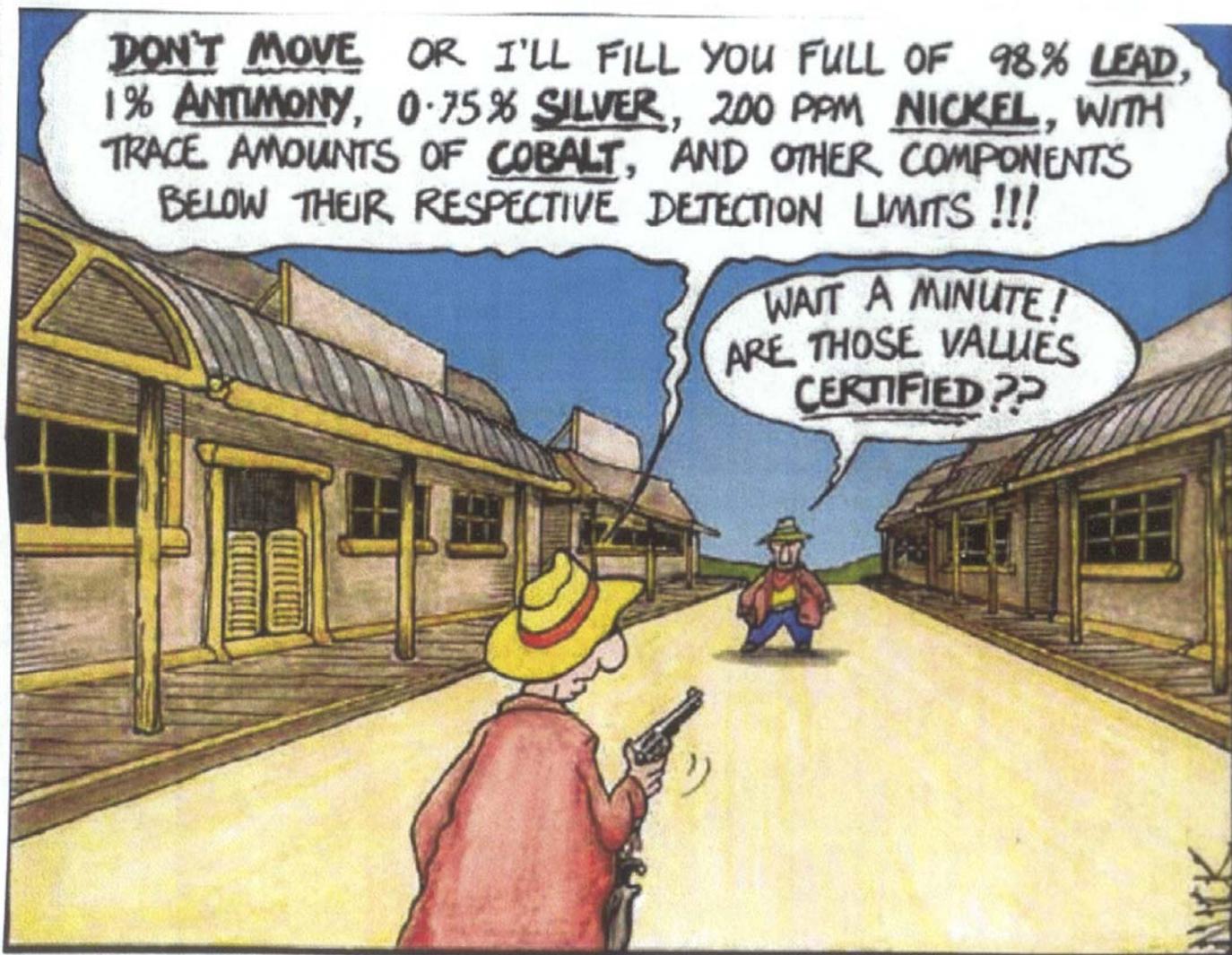


Armed Forces Institute
of Pathology

AN OVERVIEW OF CHEMICAL AND ANALYTICAL METHODS FOR THE STUDY OF TRACE ELEMENTS, METALS AND FOREIGN MATERIALS IN TISSUES

DON'T MOVE OR I'LL FILL YOU FULL OF **98% LEAD**,
1% ANTIMONY, **0.75% SILVER**, **200 PPM NICKEL**, WITH
TRACE AMOUNTS OF **COBALT**, AND OTHER COMPONENTS
BELOW THEIR RESPECTIVE DETECTION LIMITS !!!

WAIT A MINUTE!
ARE THOSE VALUES
CERTIFIED??



ANALYTICAL CHEMISTS IN THE WILD WEST

acchanali
A



Photo : National Geographic, 1997



Biomedical Trace Element Analysis

- **What to Analyze?**
 - *Urine*: Homeostasis view of metal metabolism in the human system. Picture of overall exposure.
 - *Serum/Whole Blood*: Immediate status in the human system.
 - *Tissue*: Gauge of chronic exposure or cumulative exposure. Almost incidental to treatment. **Historical.**
 - Others samples: hair, nails, adipose tissue



Four Steps to Success in Trace Element Analysis

- **Sample collection**
- **Sample storage and preparation**
- **Method development (corrections and calibration)**
- **Contamination control**
- **Stability of the instrument**



Trace Metal Analyses in the Toxicologic & Medical Laboratory

- **A metal analysis lab shall demonstrate:**
 - that its analytical systems are under statistical control;
 - that it uses validated analytical methods;
 - that it participates in proficiency testing programs.



Current Practices in Trace Metal Analyses

- **Internal Quality Controls**
- **External Quality Controls**
- **Proficiency Testing**
- **Control and Spiked Materials**
- **Certified Reference Materials (NIST)**
- **Method Validation (i.e., sample collection, analysis, reporting results, etc)**

Analytical Techniques Used for Metals Analysis

- Flame Atomic Absorption Spectroscopy (FAAS)
- Graphite Furnace Atomic Absorption Spectroscopy (GFAAS)
- Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)
- Inductively Coupled Plasma Mass Spectroscopy (ICP-MS)
 - Electrothermal Vaporization (ETV)
 - Flow Injection Analysis (FIAS)
 - Dynamic Reaction Collision Cell (DRC)
 - Laser ablation microprobe

Electric Sector Analyzer (ESA):

Focuses ions with diverging angles of motion on the exit slit. It is dispersive with respect to ion kinetic energy ($\frac{1}{2}mv^2$). When the energy dispersion of the magnet and ESA are equal in magnitude but of opposite direction, the magnet and ESA focus both ion angles and ion energies (double focusing) while being dispersive for m/z : a mass spectrometer!

The order of fields (magnet first and then ESA) in the **ELEMENT2** is called reverse geometry.

Magnetic Sector Field:

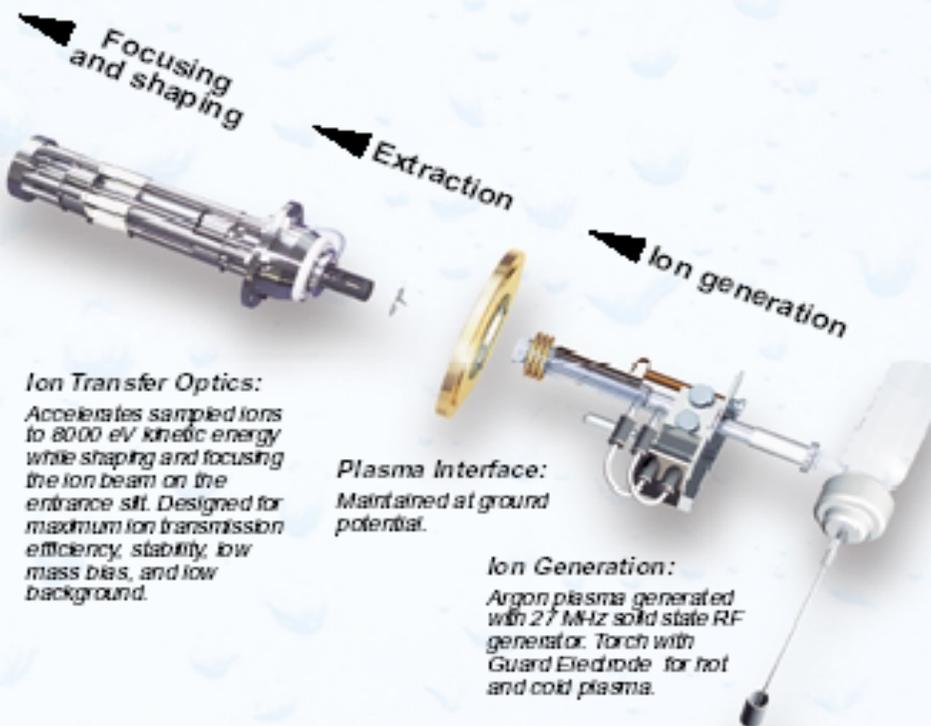
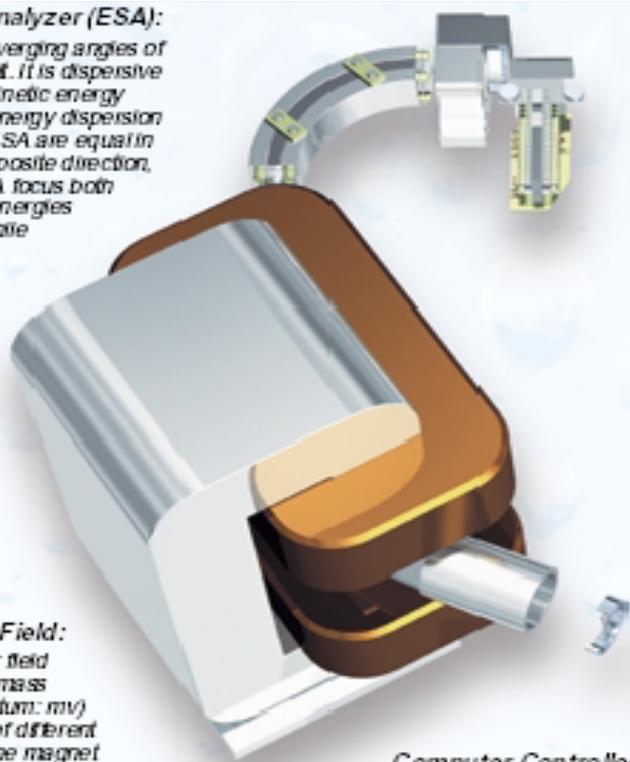
The magnetic sector field is dispersive for ion mass and energy (momentum: mv) while focusing ions of different angles of motion. The magnet of the ELEMENT2 is designed for maximum speed. Less than 150 ms are required for a jump from ^7Li - ^{238}U - ^{238}U . The magnet coils are water cooled for maximum mass stability.

Computer Controlled Fixed Slits:

Three resolution settings of $R = 300, 4000 \text{ \& } 10000$ guarantee unambiguous elemental spectra.

Detection System consisting of:

- 8 kV Conversion Dynode for uniform mass response. Ions from the exit slit impact the dynode which, in turn, emits electrons. The release of secondary electrons is mass-independent at 8 kV ion energy, making cross calibration between the analog and counting detector circuits independent of ion mass.
- Discrete Dynode Secondary Electron Multiplier (SEM). Simultaneous, automatically calibrated, dual mode detector for analog and counting measurements. A linear dynamic range of $> 10^8$ from dark noise $< 0.2 \text{ cps}$ to $> 2.5 \times 10^8 \text{ cps}$ enables quantification from sub-ppq to ppm concentrations.



Ion Transfer Optics:

Accelerates sampled ions to 8000 eV kinetic energy while shaping and focusing the ion beam on the entrance slit. Designed for maximum ion transmission efficiency, stability, low mass bias, and low background.

Plasma Interface:
Maintained at ground potential.

Ion Generation:

Argon plasma generated with 27 MHz solid state RF generator. Torch with Guard Electrode for hot and cold plasma.

Sample Introduction:

Easy coupling to laser ablation and chromatographic techniques, for example: HPLC, GC, CE, FFF.

High-Resolution Inductively Coupled Plasma Mass Spectrometer



Instrumental Approaches to Trace Metal Analysis

- **FAAS** - Moderate detection limits restrict application of the technique to elements present at part-per-million (ppm) to part-per-billion (ppb) concentrations
- **GFAAS** - Parts-per-billion detection limits, microliter sample volumes, and ability for direct solids analysis make the technique well suited
 - **Disadvantages: speed, single element determinations**



Instrumental Approaches to Trace Metal Analysis

- **ICP-OES** - most useful for the determination of elements at parts-per-million (ppm) and high parts-per-billion (ppb) concentrations
- **ICP-OES** - will determine those elements faster (multielement) using less sample volume than FAAS
- **ICP-OES** - detection limits typically not adequate for the determination of elements typically present at the low parts-per-billion level (Al, As, Se, Cr, Pb).



Instrumental Approaches to Trace Metal Analysis

- **ICP-MS** - the most versatile tool for clinical analysis
- **ICP-MS** provides part-per-trillion detection limits, wide linear range, and moderate sample volume requirements
- **ICP-MS** may be combined with numerous sample introduction accessories to provide for microliter sample volumes
- **ICP-MS** provides the ability to measure individual isotopes, hence the capability of conducting metabolism studies



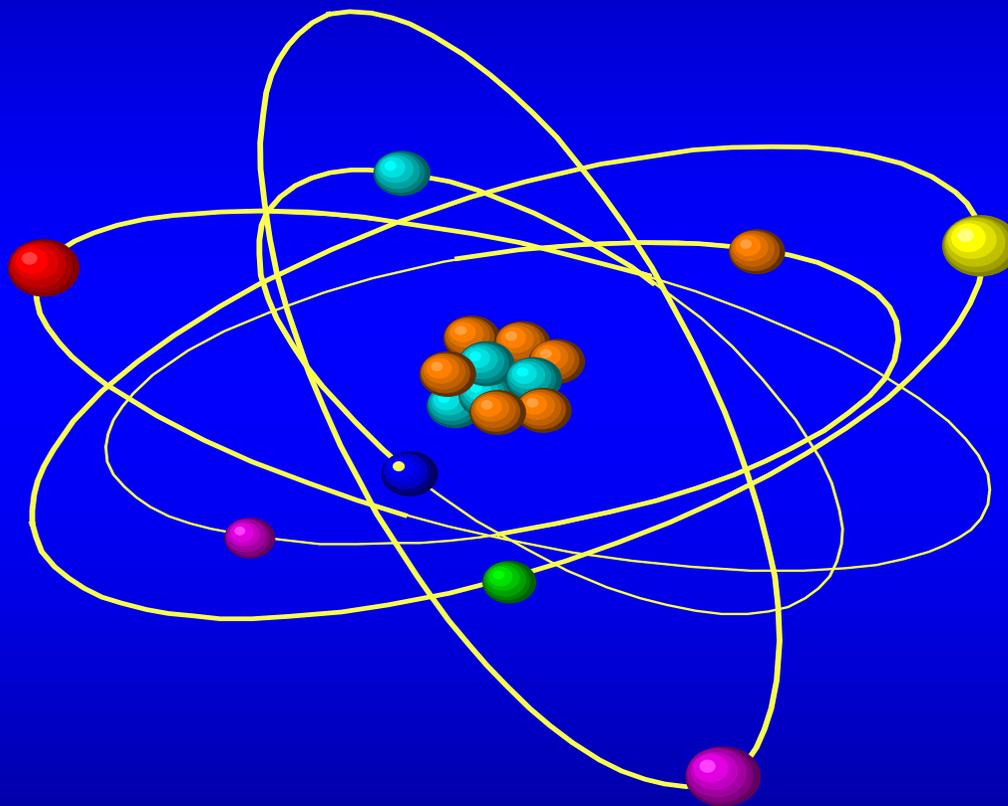
What is Inductively Coupled Plasma Mass Spectrometry?

A technique for the determination of

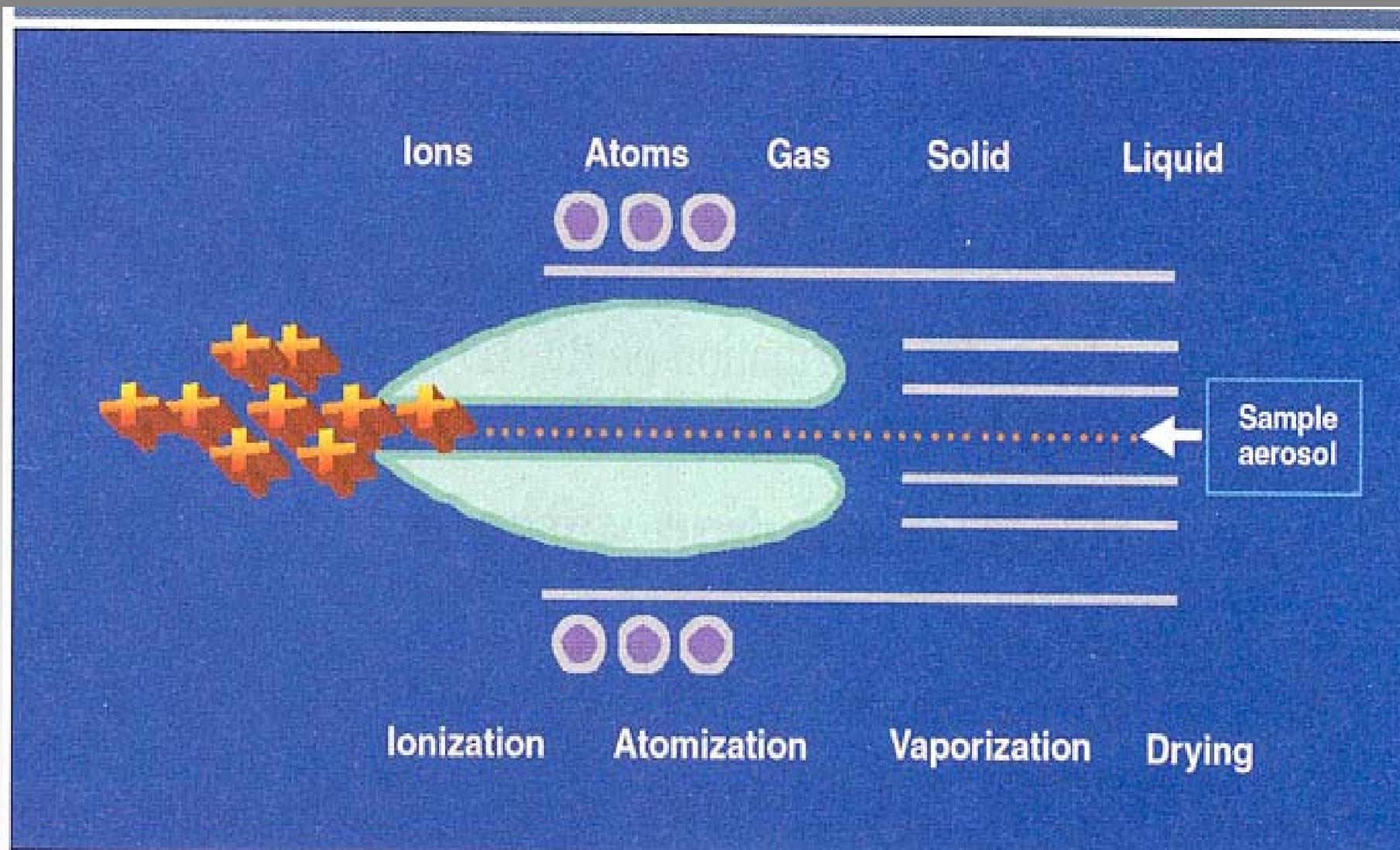
- Elements using
- Mass Spectrometry of
- Ions generated by an
- Inductively Coupled Plasma

ICP-MS = the use of high-temperature (~6000-7000K) plasma discharge to generate positively charged ions.

How does ICP-MS Work?



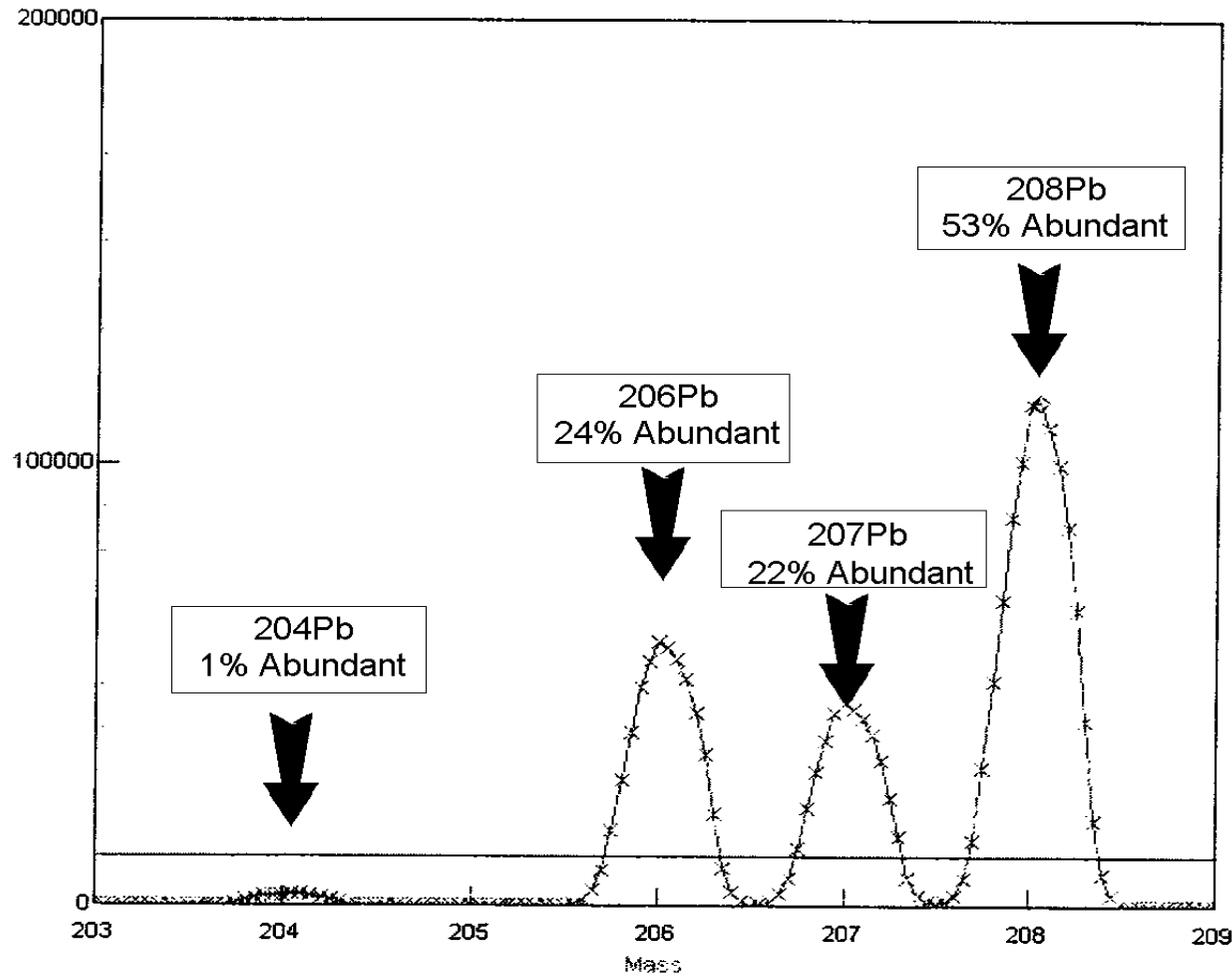
ICP-MS : Generation of positively charged ions in the plasma





Isotopes and Mass Spectra

Lead





Molecular Interferences

Element	Interference	Correction
Al 27	CN	X
V 51	OCl	X
Ni 60	CaO	X
Cr 52	ArC, OClH	X
Cu 63	ArNa	
As 75	ArCl	X



Typical ICP-MS Detection Limits (*PE-ELAN 6000*)

		<u>µg/L (ppb)</u>
Li	7	0.0035
Be	9	0.004
Mg	24	0.0087
Co	59	0.00068
Y	89	0.0001
In	115	0.00024
Pb	208	0.00046
U	238	0.000059

multielement, 3-sigma, 3-sec integrations, n=10

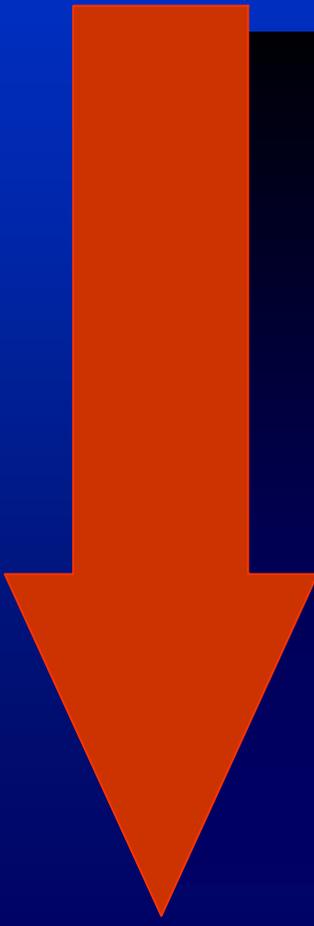
Comparison of Detection Limits (ug/L)

<u>Element</u>	<u>FAAS</u>	<u>GFAAS</u>	<u>ICP-OES</u>	<u>ICP-MS</u>
Al	45	0.1	3	0.006
As	150	0.2	50	0.006
Cd	0.8	0.008	1	0.003
Cr	3	0.03	2	0.02
Cu	1.5	0.1	0.4	0.003
Hg	300	0.6	1	0.004
K	3	0.008	20	0.015
Mn	1.5	0.035	0.4	0.002
Ni	6	0.3	5	0.005
Pb	15	0.06	10	0.001
Sb	45	0.15	10	0.001
Se	100	0.3	50	0.06
Tl	15	0.15	30	0.0005



Arsenic Species

Decreasing
Toxicity



AsH_3 - arsine (gas)

As(III) - inorganic arsenite

As(V) - inorganic arsenate

MMAA – monomethylarsonic (As^{3+})

DMAA – dimethylarsinic acid (As^{3+})

MMAA - monomethylarsonic acid

DMAA - dimethylarsinic acid

TMAO - trimethylarsine oxide

AsB - arsenobetaine (marine) *

AsC - arsenocholine (marine) *

Thus, arsenic speciation studies are critical for accurate toxicological evaluation, bioaccessibility studies, and risk assessment.

As speciation studies

Environmental Analysis:

- As speciation in water (numerous reports)

Biological Analysis:

- Urine analysis (numerous reports)
- Body fluids – blood, bile, plasma (Suzuki)
- Hair and nail samples (Suzuki)
- Marine animal samples (several reports, review by McSheehy)
- Tissues?

Overview of As speciation techniques

- **Separation:**
 - Liquid chromatography – most common
 - Reverse phase, ion pair, ion exchange
 - Gas chromatography
 - Capillary electrophoresis
 - Supercritical fluid chromatography
- **Detection:**
 - **ICP-MS (element-specific)**
 - **Hydride generation AA**
 - **Mass spectrometry**
 - **Voltametry**

ICP-MS (with DRC) Instrument setup

Mobile phases

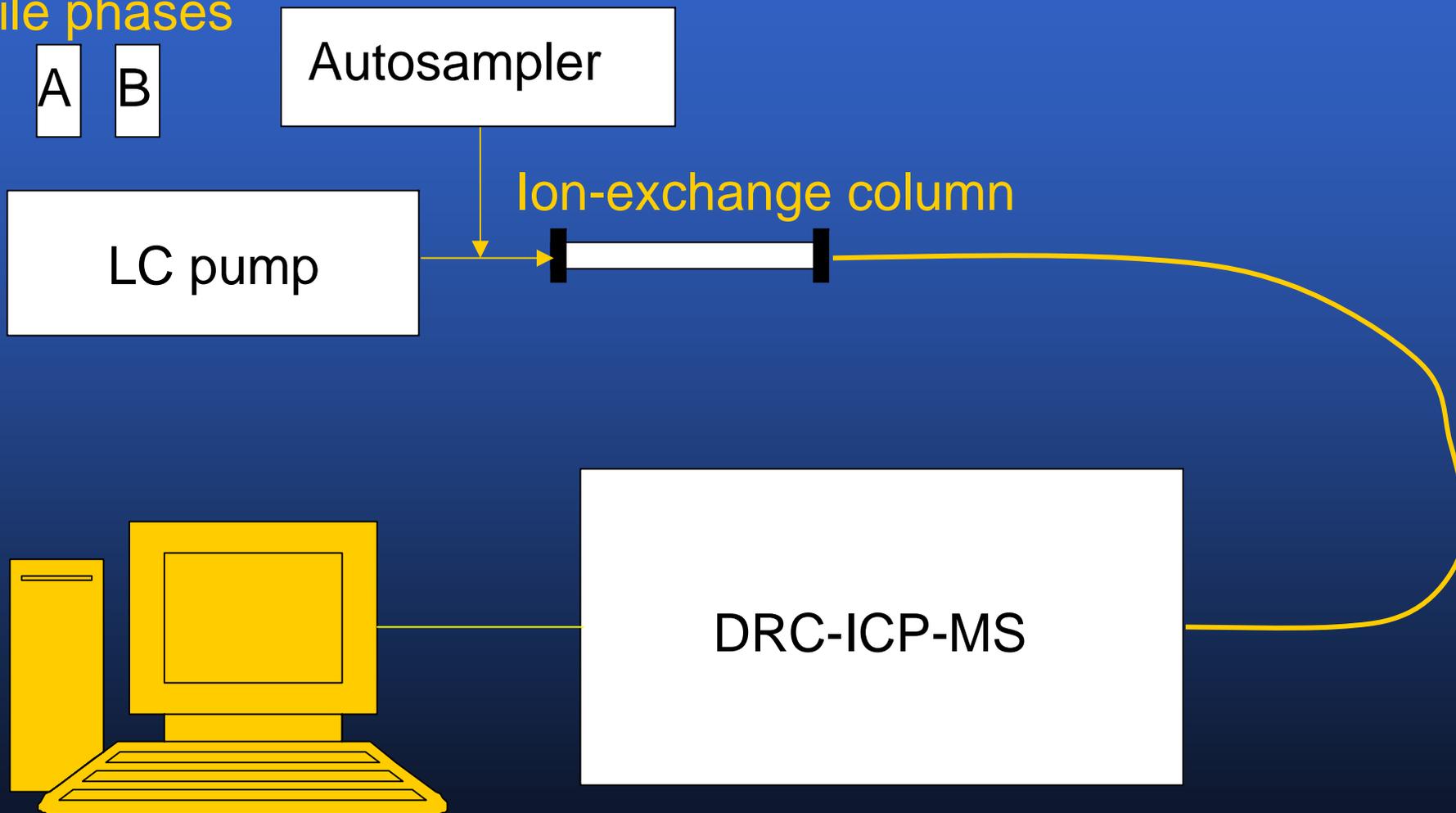
A B

Autosampler

LC pump

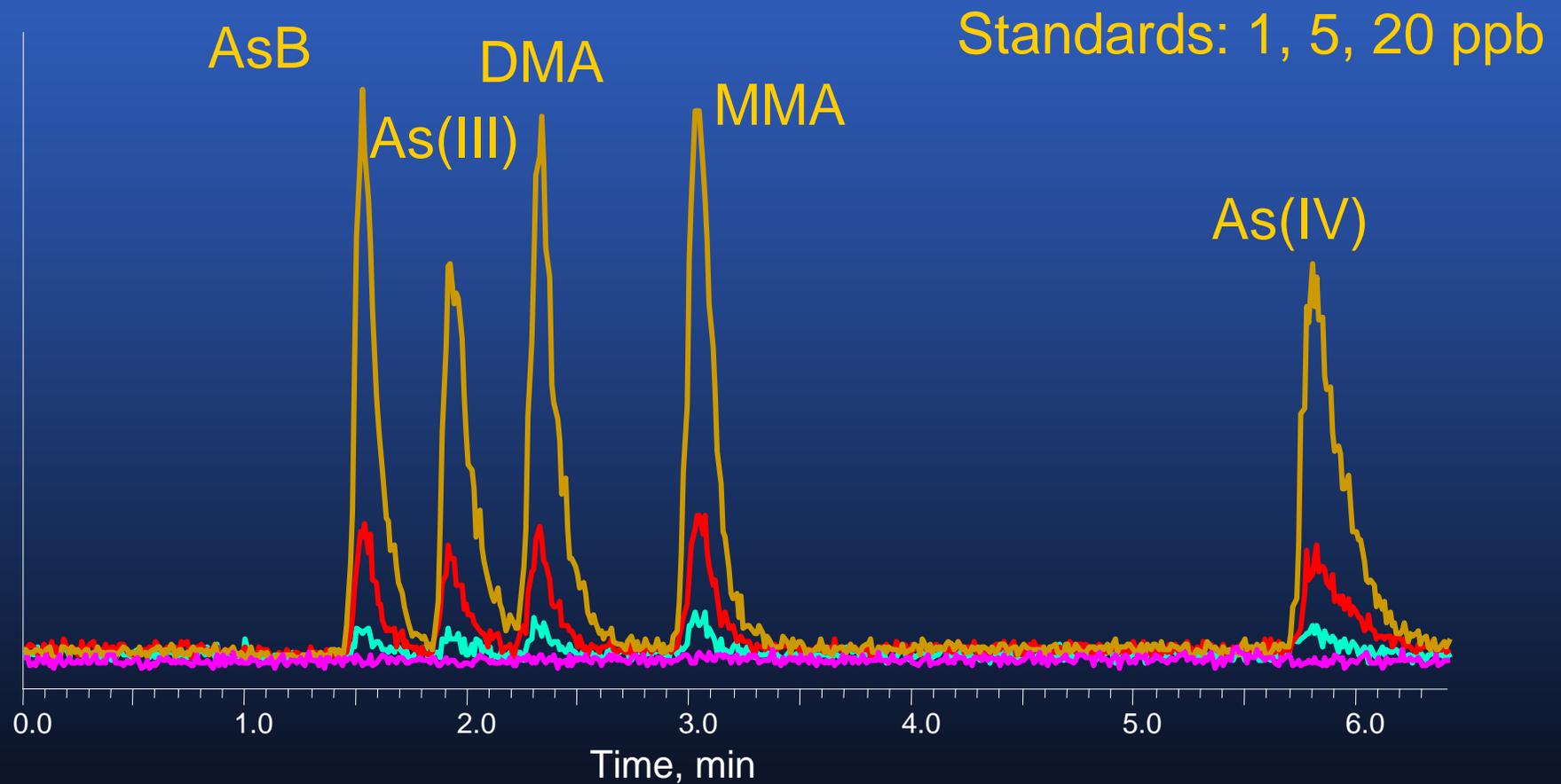
Ion-exchange column

DRC-ICP-MS

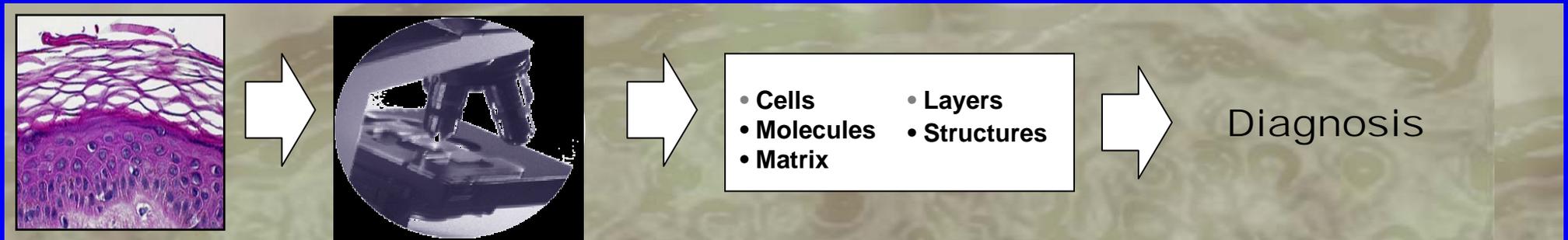




Isocratic HPLC-DRC-ICP-MS Calibration



Tissue Analysis As Presently Performed



Tissue

Pathologist Visual
Inspection

Cognition

Correlation
With Other Data

Microscopy Techniques in Chemical and Analytical Pathology

- Optical Microscopy
- Polarized Light Microscopy
- Electron Microscopy (TEM, SEM, STM)
- Fluorescence Microscopy
- Infrared Microscopy
- Laser Raman microprobe

Molecular Microanalysis

**A non-invasive, non-destructive
approach**

with 1 μm Spatial Resolution

**Providing Chemical Information on
Environmental and Pathological Specimens,
Avoiding Complicated Sample Preparation
Procedures**

Examples of Raman spectra with different functional silicate groups

