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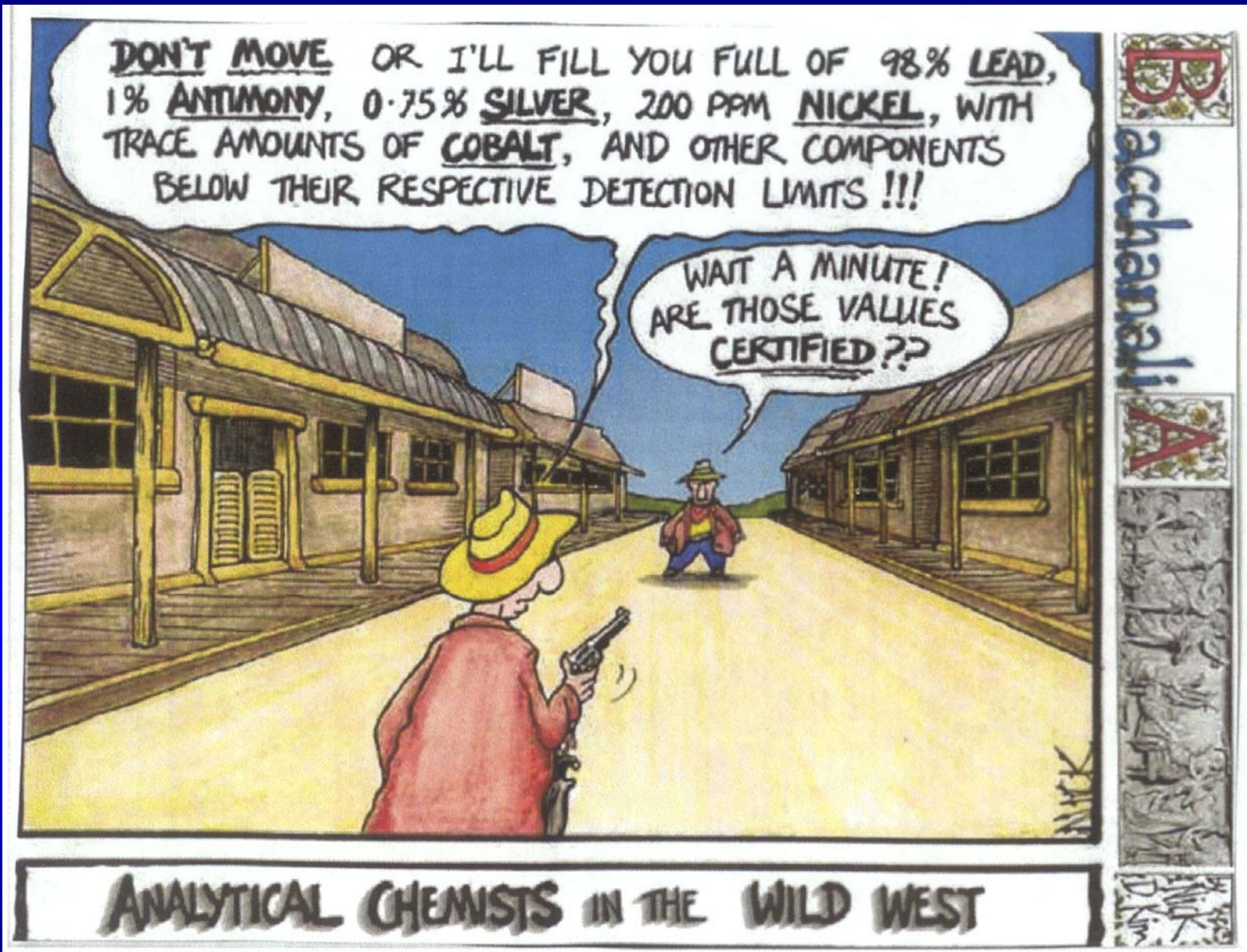
AN OVERVIEW OF CHEMICAL AND ANALYTICAL METHODS FOR THE STUDY OF TRACE ELEMENTS, METALS AND FOREIGN MATERIALS IN TISSUES


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??
Bacchanalia
ANALYTICAL CHEMISTS IN THE WILD WEST

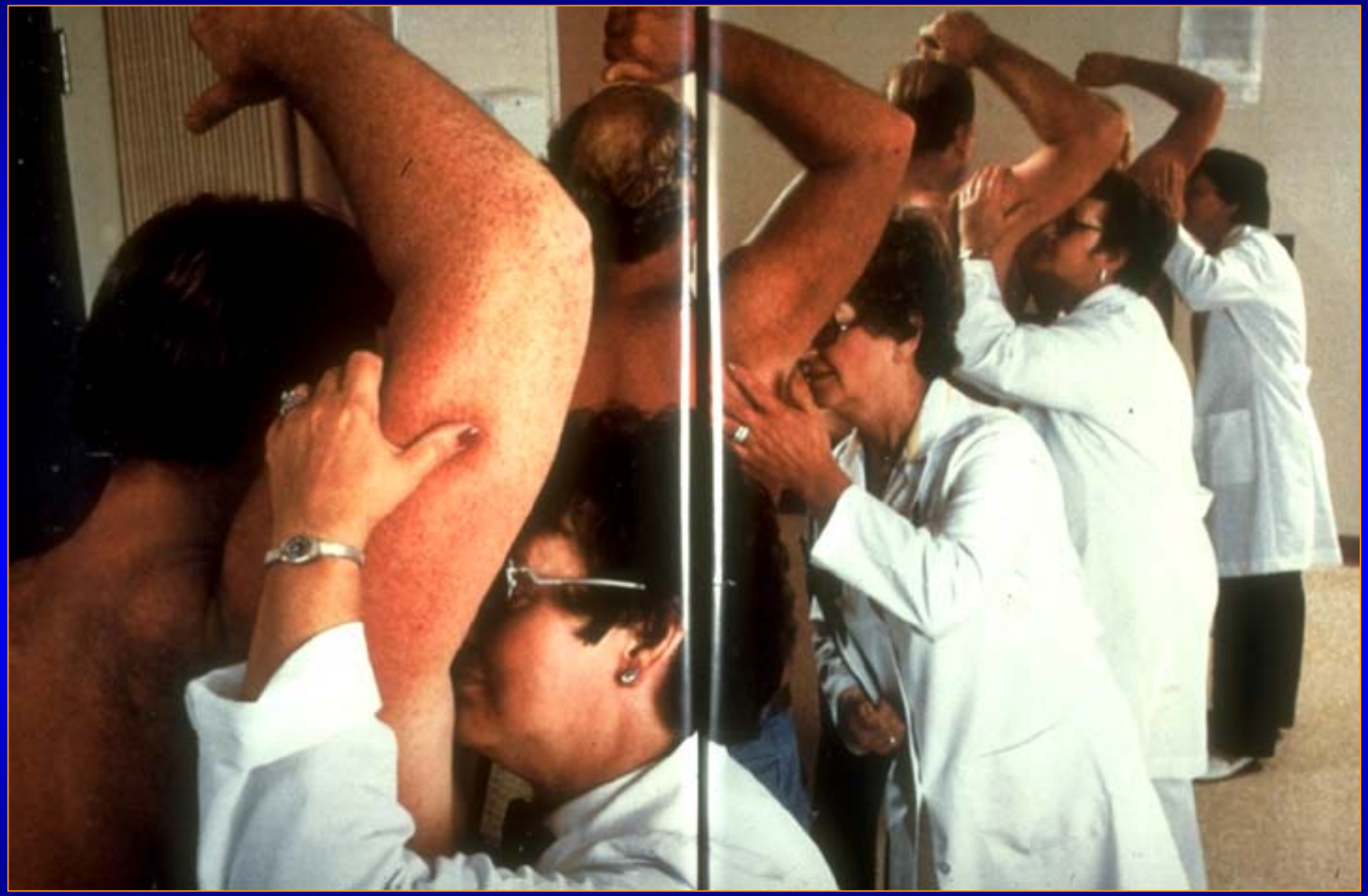


Photo : ***National Geographic, 1997***

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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

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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

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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.**

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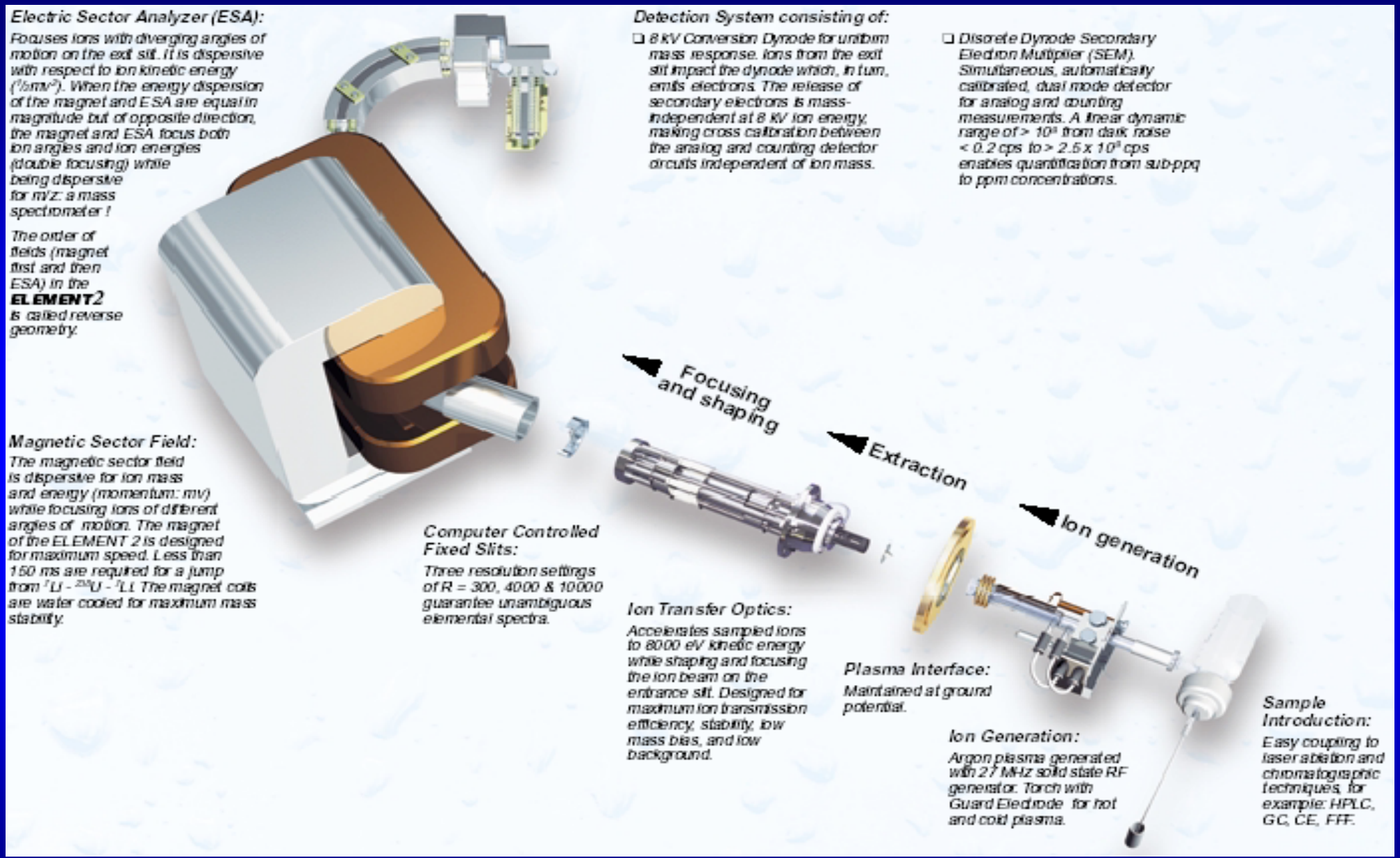
Current Practices in Trace Metal Analyses

- 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



High-Resolution Inductively Coupled Plasma Mass Spectrometer

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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**

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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).

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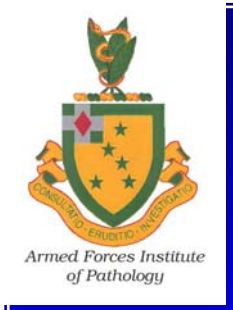
Instrumental Approaches to Trace Metal Analysis

- **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



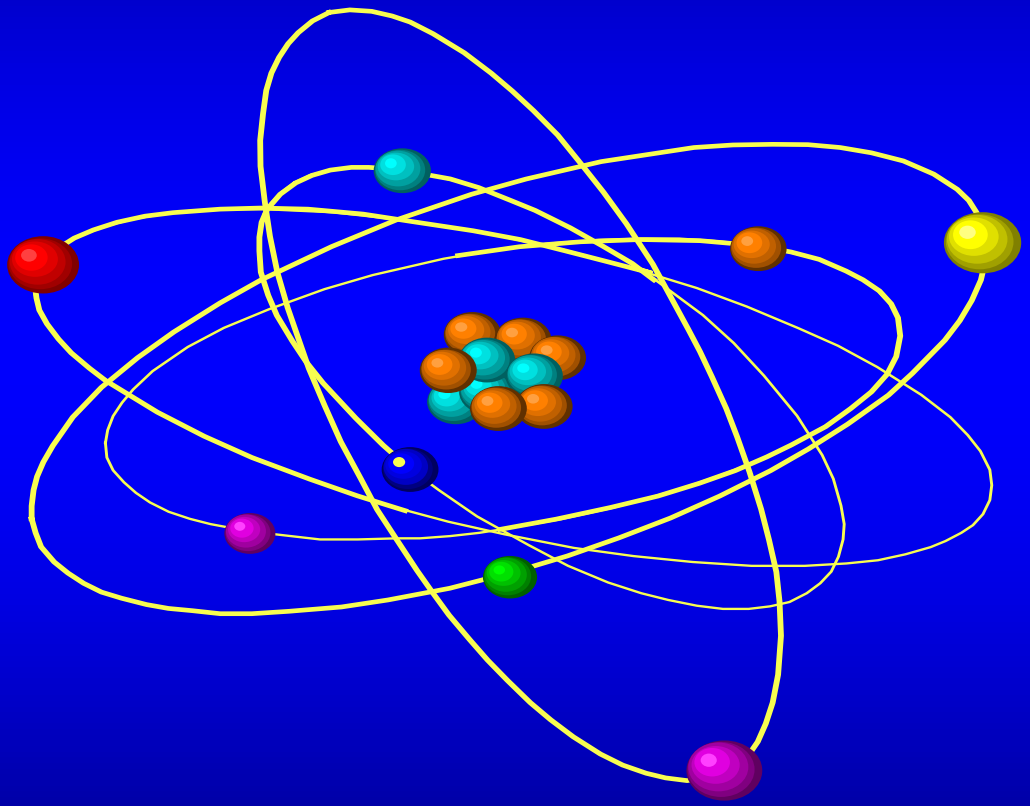
What is Inductively Coupled Plasma Mass Spectrometry?

A technique for the determination of

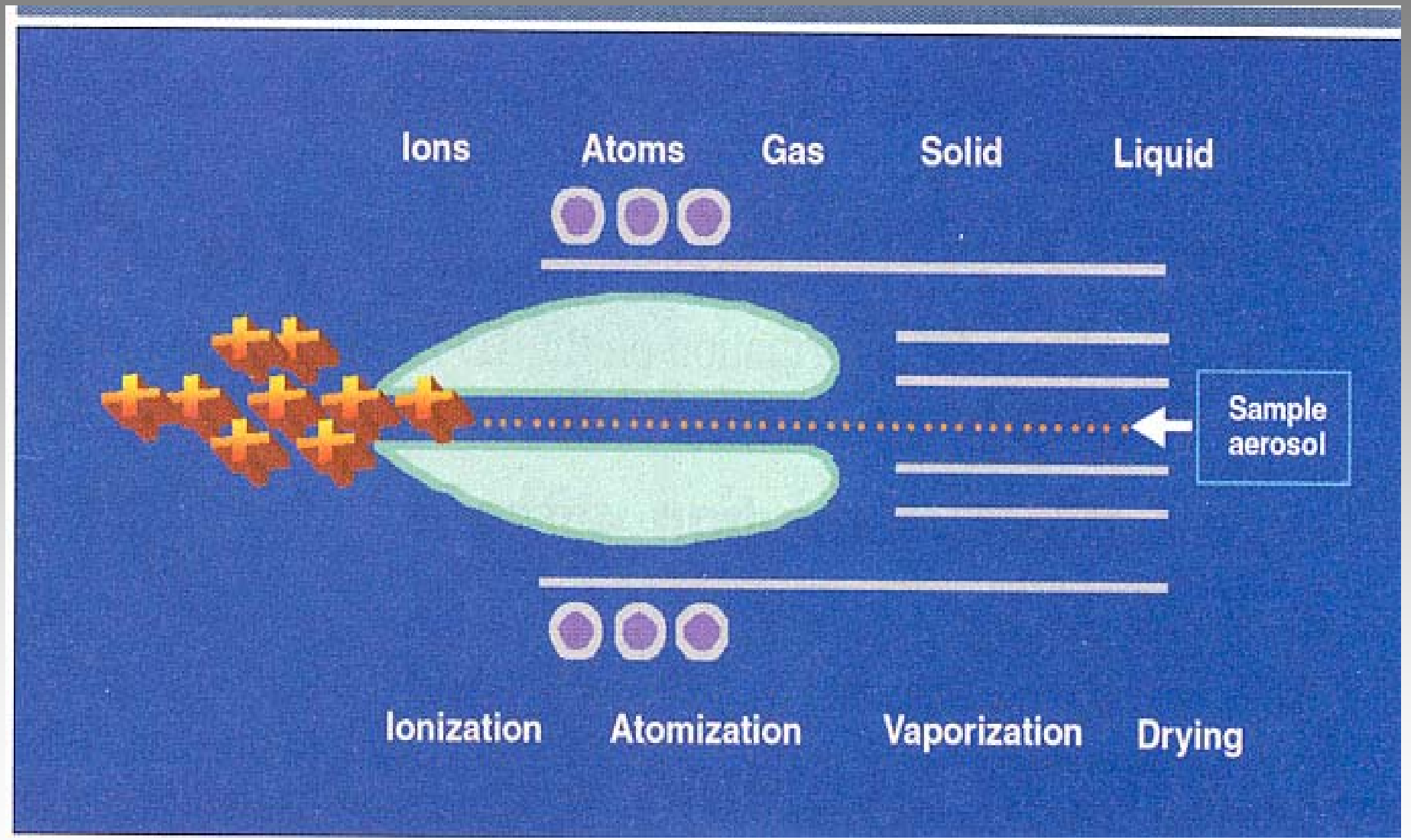
- Elements using
- Mass Spectrometry of
- Ions generated by an
- Inductively Coupled Plasma

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



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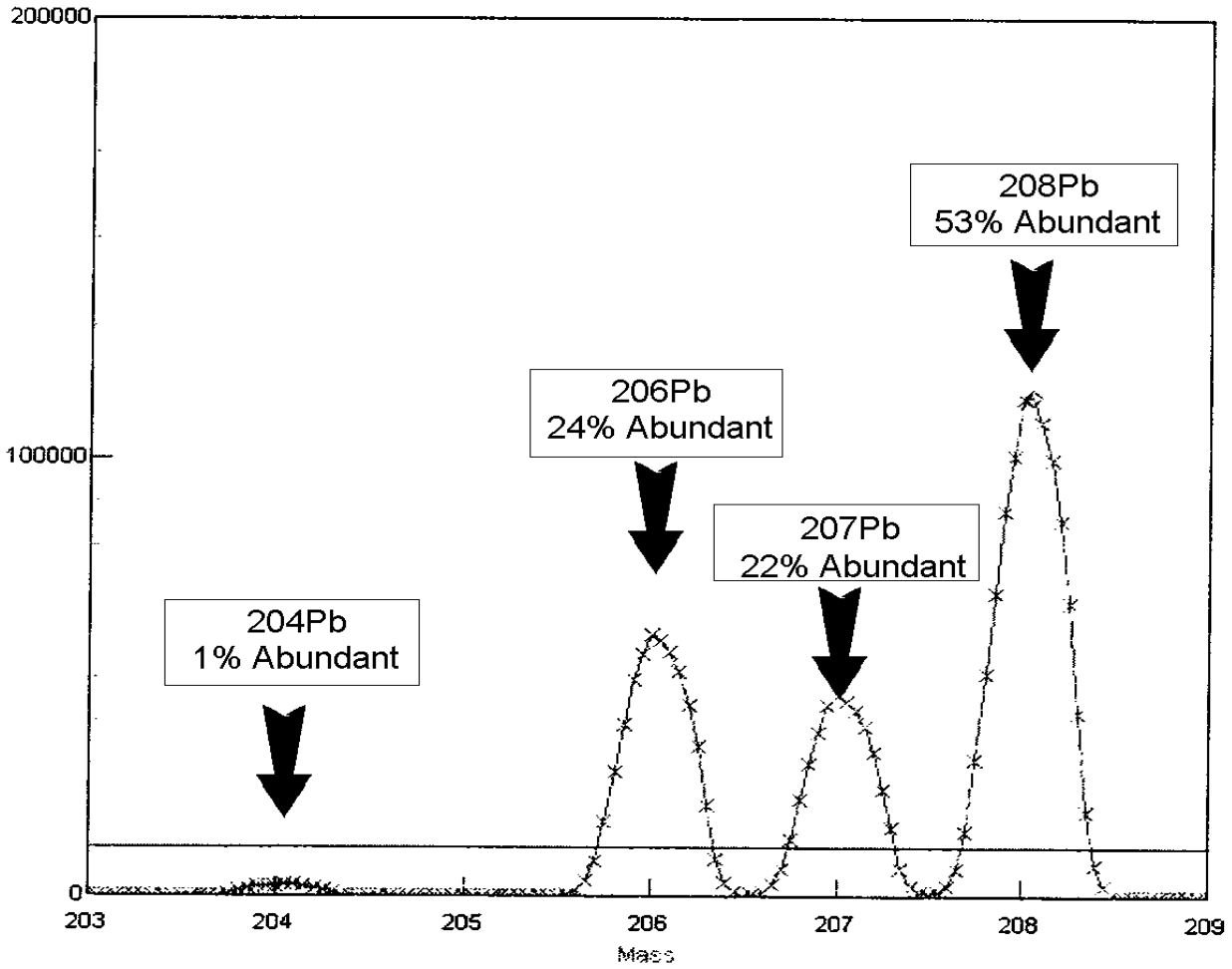
Isotopes and Mass Spectra

Lead



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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

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

		µg/L (ppb)
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)

Element	FAAS	GFAAS	ICP-OES	ICP-MS
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

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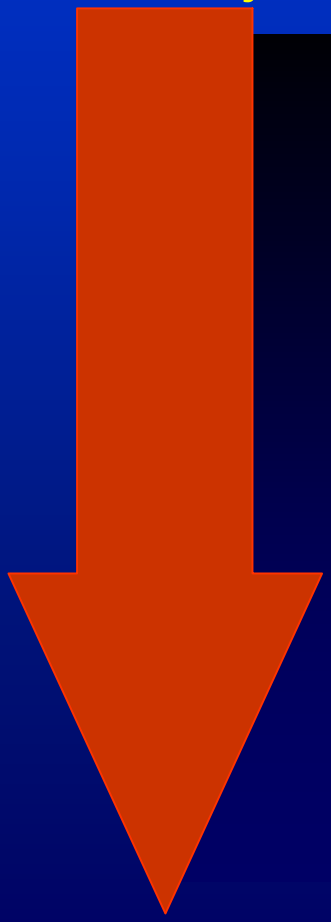
Arsenic Species

Decreasing Toxicity



AsH_3 - arsine (gas)

As(III) - inorganic arsenite

As(V) - inorganic arsenate

MMAA – monometylarsonic (As^{3+})

DMAA – dimetylarsinic 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:

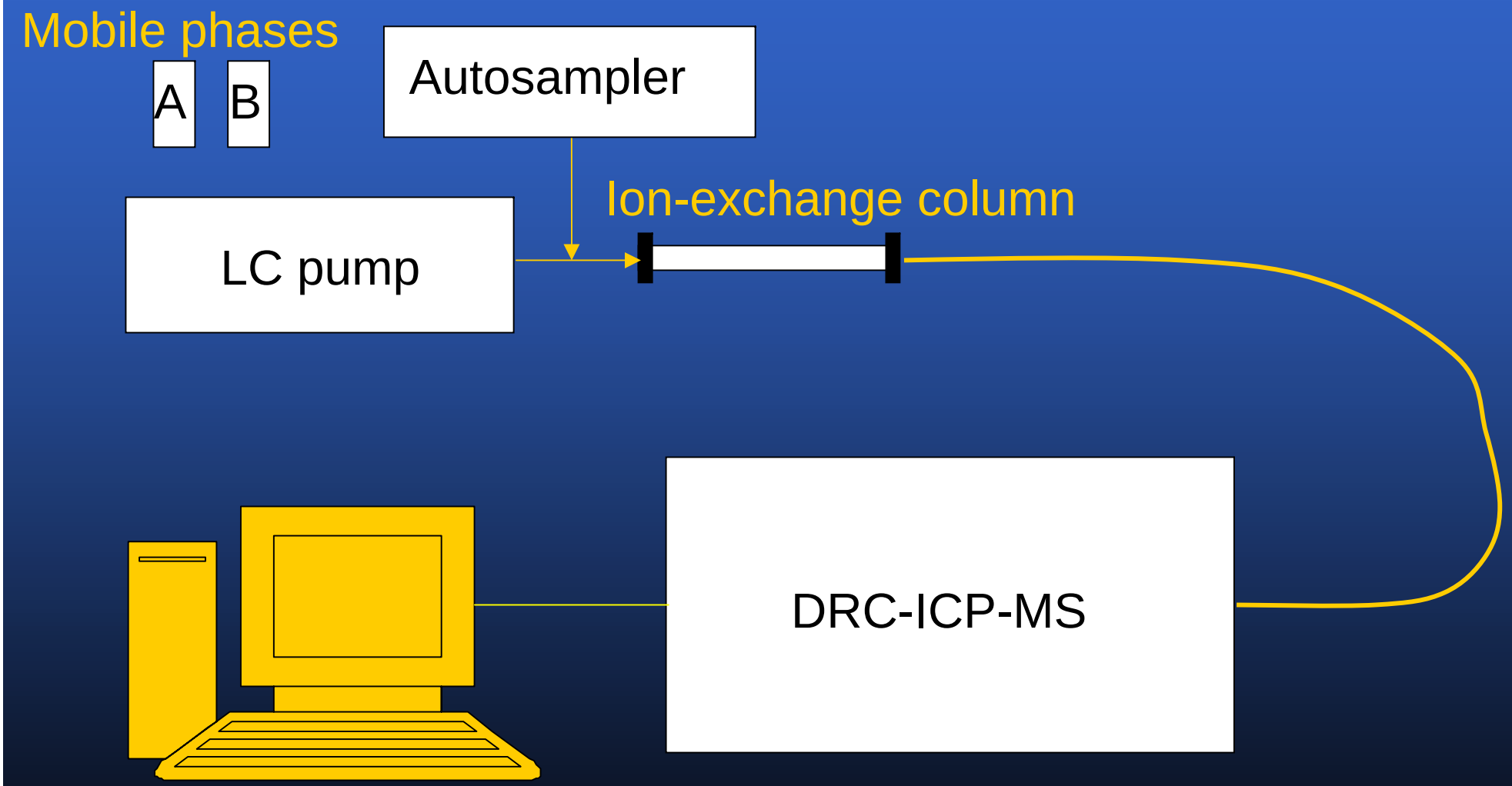
- Urine analysis (numerous reports)
- Body fluids – blood, bile, plasma (Suzuki)
- Hair and nail samples (Suzuki)
- Marine animal samples (several reports, review by McSheehy)
- Tissues?

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



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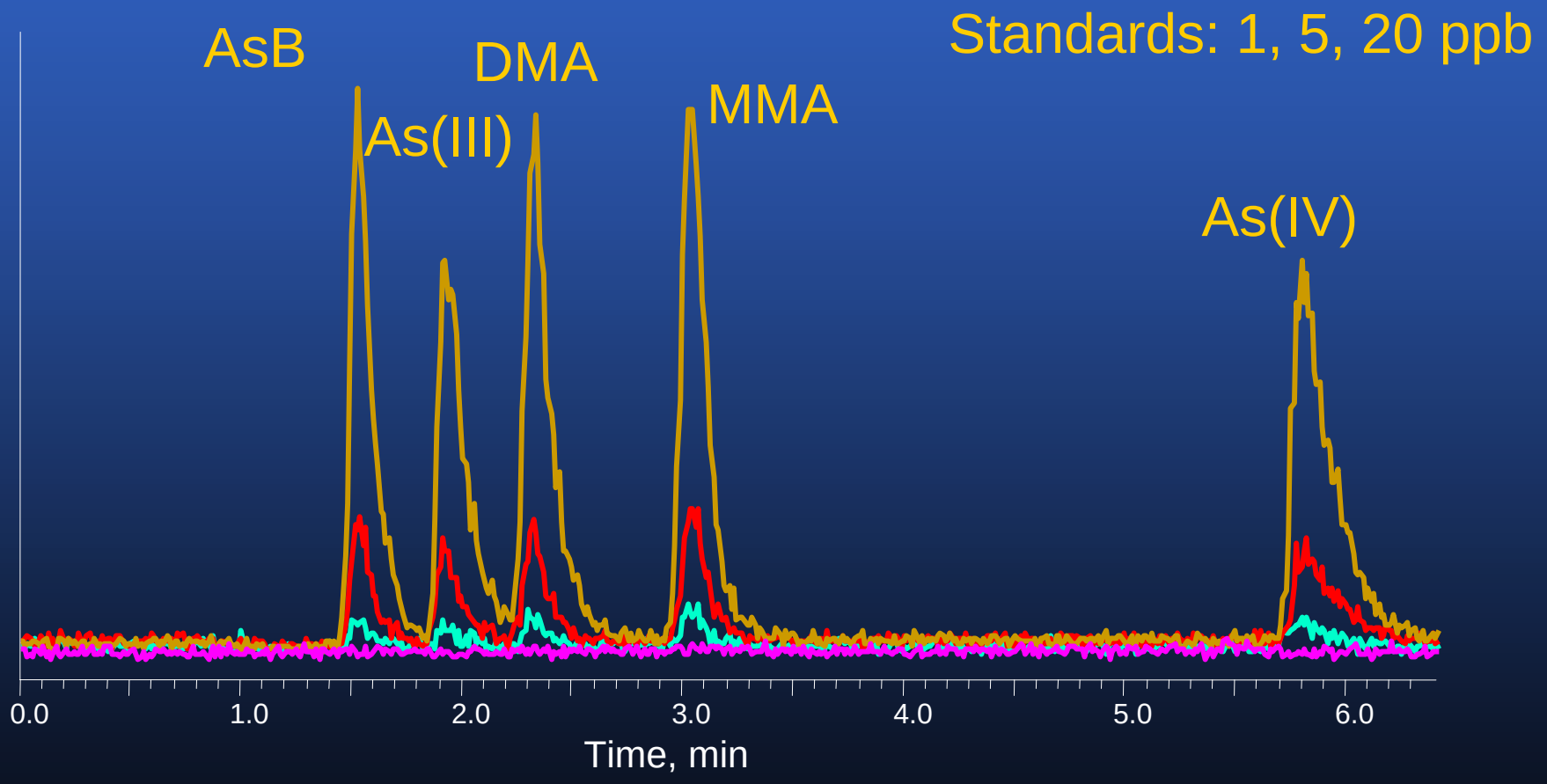
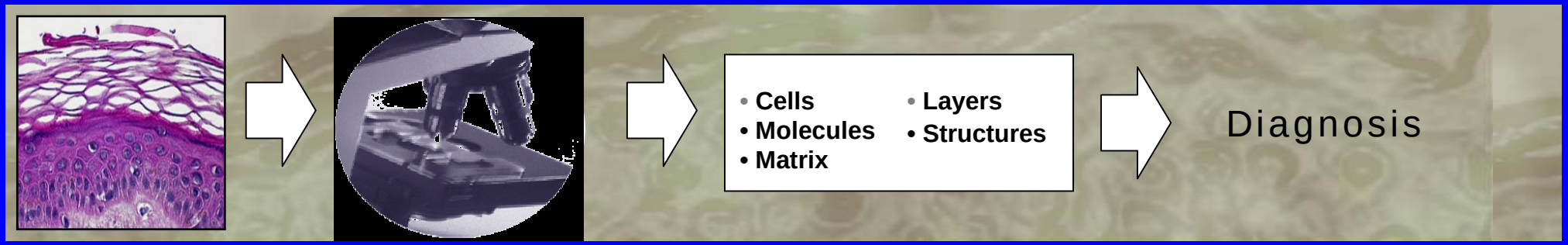
Isocratic HPLC-DRC-ICP-MS Calibration



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