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Flame and Furnace AAS as an efficient tool for clinical diagnostic – Determination of Cu, Zn and Se traces in human serum

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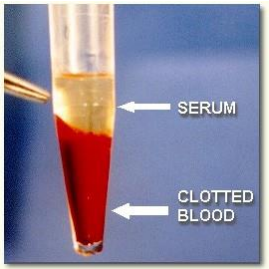
Shimadzu Europa GmbH

What is Serum (I)

- **Serum is a part of the human blood**
  - Take a blood sample
  - Let it coagulate 15-30 min
  - Centrifugation 5-10 min @ 2500 rpm
  - Collect liquid supernatant



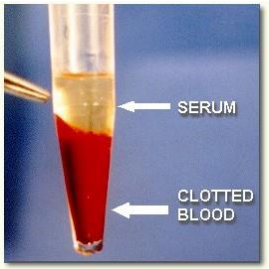
http://medimoon.com/2012/07/difference-between-plasma-and-serum/

- **Serum is not the blood plasma!**
  - Plasma is supernatant of anti-coagulated blood
  - Serum = Plasma – Clotting Factors (clotting proteins)

- **Serum is free of**
  - Blood cells (no white or red blood cells)
  - Clotting factors

What is Serum (II)

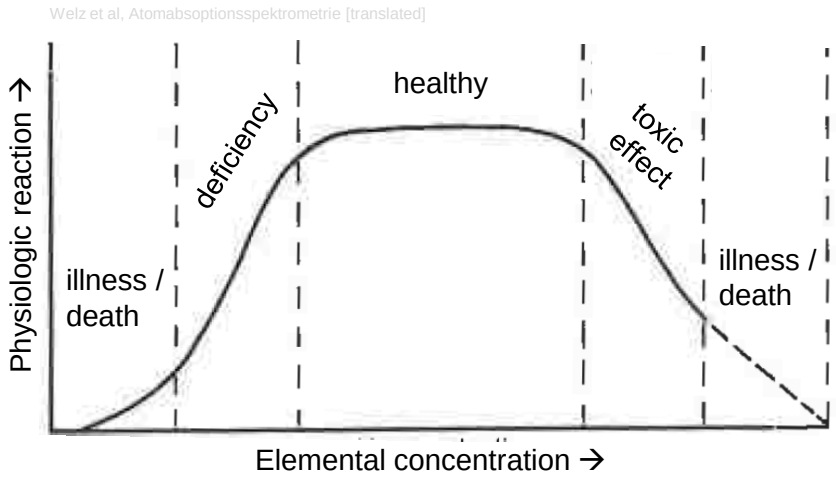
- **Serum is the most preferred specimen in Clinical Chemistry**
  - Basically less interferences can occur, because
  - No need to add anti-coagulants (like for plasma)
- **But it still contains:**
  - electrolytes,
  - antibodies,
  - antigens,
  - hormones, ….
- **Basically water and proteins**
  - 91% water
  - 7% proteins
  - 2% electrolytes, nutrients, waste products (urea,…) and hormones
  - any exogenous substances (e.g. drugs and microorganisms)

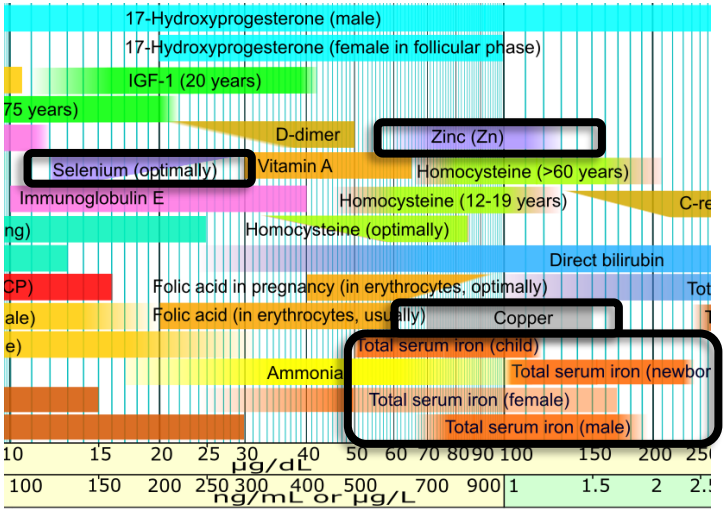


http://medimoon.com/2012/07/difference-between-plasma-and-serum/

Clinical Chemistry / Diagnostic

- Physological properties of essential trace elements
- **The more the better? – No !**





https://en.wikipedia.org/wiki/Blood_plasma

- Concentration ranges are of interest

Choice of elemental analysis technique

The question is what you need!

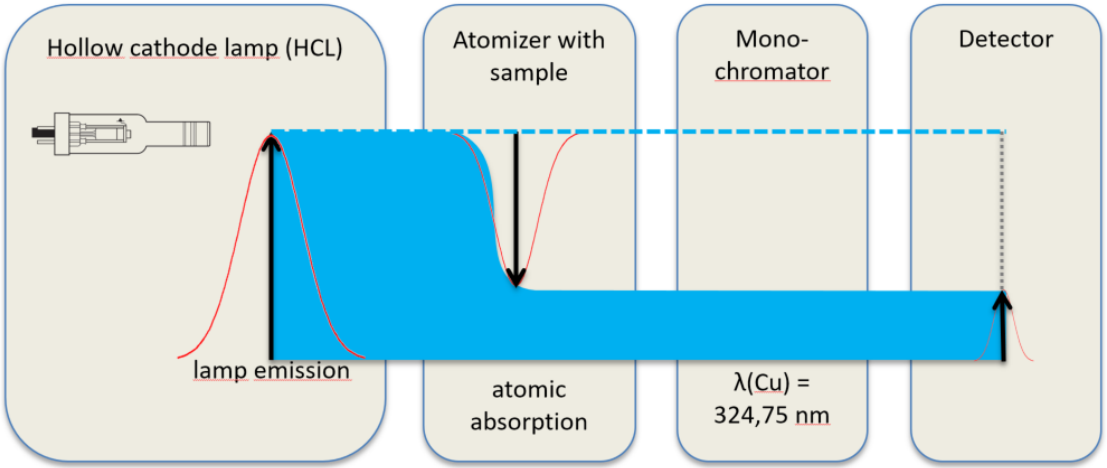
- **ICP-MS** (Shimdzu ICPMS-2030)
  - Ultra traces to higher concentrations
  - Fast (one single measurement generates all results in 1-3 mins)

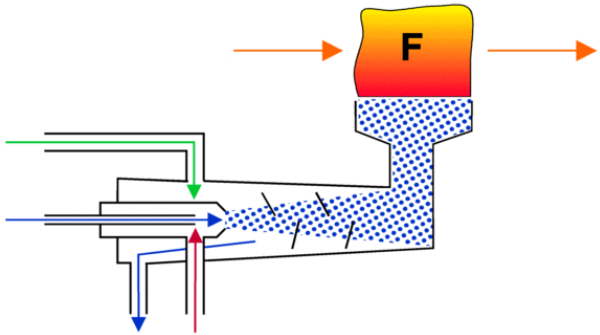
- **ICP-OES** (Shimadzu ICPE-9820)
  - Traces to higher concentrations (very sufficient for electrolytes)
  - Difficult e.g. for direct determination of selenium traces
  - For ultra traces:
    Accessories like hydride system or ultrasonic nebulizer might help

- **AAS** (Shimadzu AA-7000)
  - Focusses each element separately
  - Flame for electrolytes and Furnace for traces
  - Easy handling / lowest prize segment

Serum Analysis by Flame AAS (I)

- Most easy handling and robust
- Simple dilution, followed by direct sample aspiration



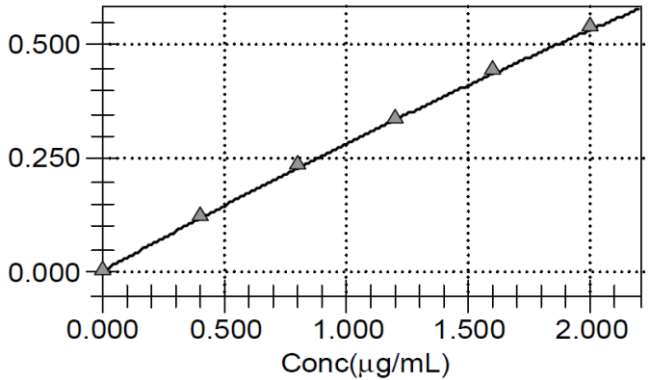


Example: Shimadzu AA-7000F

Serum Analysis by Flame AAS (II)

- **Lithium**
  - Li-compounds: therapeutic agents (psychiatric disorders)
  - Control necessary to avoid side effects

  - 0.2 ml sample + 0.2ml Cs buffer + 1.6ml acidified water
  - $V_{min,flame}$= ~2ml (question of reproducibility)



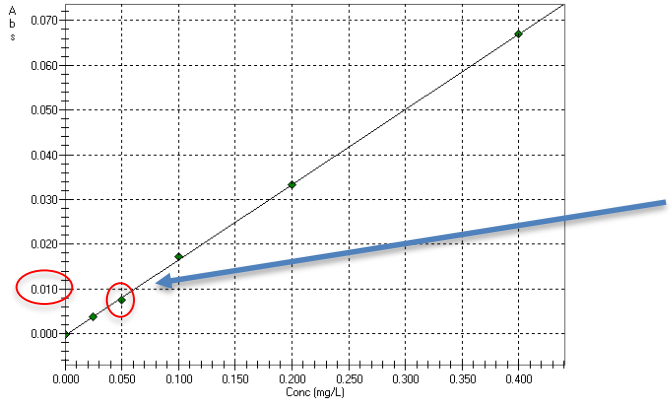
Sample Name	Analysis Value(µg/mL)	Certified Value(µg/mL)
NIST 909b Level Ⅰ	4.20	4.265 ±0.0034
NIST 909b Level Ⅱ	17.6	18.04 ±0.016

SHIMADZU Application News No. A316A

Serum Analysis by Flame AAS (III)

- **Copper**
  - Cu is present in many enzymes
  - E.g. important for resorption of iron in the gastrointestinal tract

- **Challenge: Flame Sensitivity still sufficient?**
  - Typically present in low concentrations: 750 to 1400 µg/l
  - But: limited volume + serum viscosity requires factor 10 dilution
  - Cu-deficiency → concentrations far below 75 µg/l needs to be determined



Client request:
Stable analysis of 5 µg/l Cu
(50 µg/l Cu in Serum)

Serum Analysis by Flame AAS (IV)

- **Copper - Challange: Stability?**
  - Example – lowest QC is 50 µg/l Cu in serum
  - → Real concentration is 5 µg/l (x10 dilution)
  - Detected absorption signal is 0.008 Abs (peak height)
  - → 0.001 Abs change = change of +/- 12.5 %

- **Solution:**
  - Precise lamp current control
  - Real double beam optics for real time drift correction

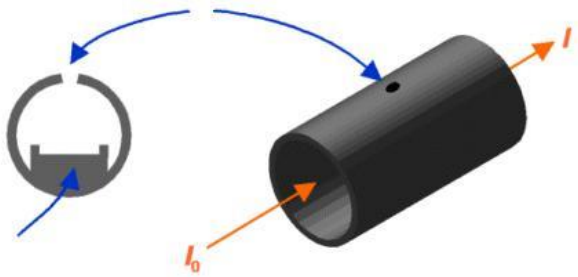
  - Over 25 minutes (more than 20 samples)
    - the recovery of QC 50 is within the range of +/- 10% !
    - The drift is below 0.001 Abs !

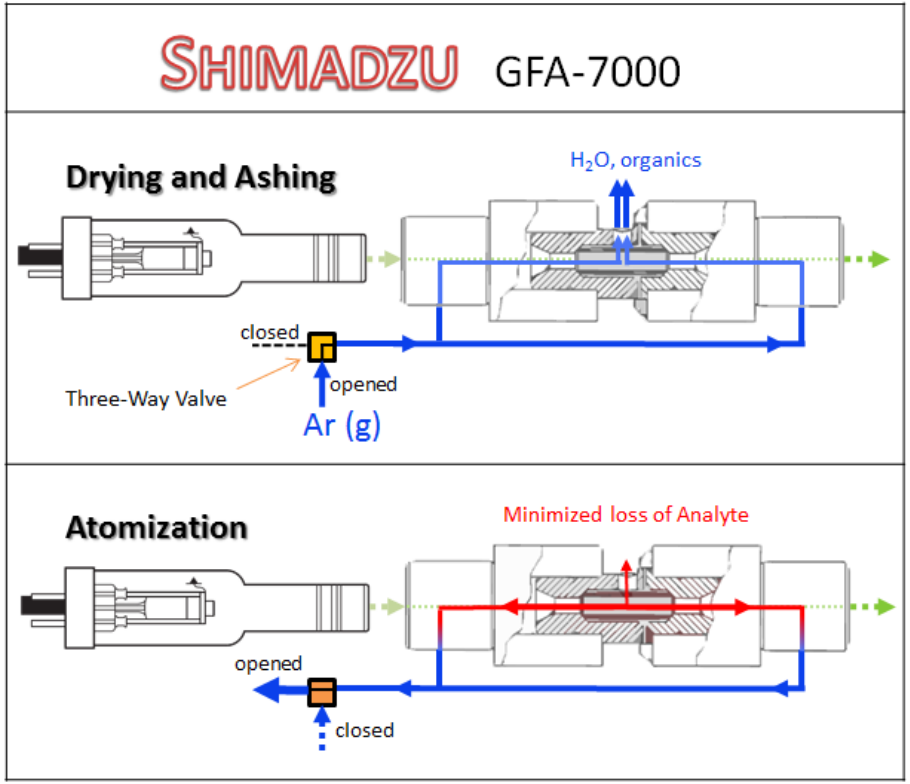
Sample ID	Conc. (mg/L)	Abs.	Date	Time
etalon 0.025	0.0244	0.0038	08.11.2017	15:10:18
etalon 0.05	0.0470	0.0076	08.11.2017	15:11:08
etalon 0.1	0.1040	0.0172	08.11.2017	15:11:58
etalon 0.2	0.1991	0.0332	08.11.2017	15:12:49
etalon 0.4	0.3999	0.0670	08.11.2017	15:13:38
qc 200	0.2026	0.0338	08.11.2017	15:14:29
qc 50	0.0476	0.0077	08.11.2017	15:15:19
Serum L1	0.1836	0.0306	08.11.2017	15:16:10
qc 200	0.1997	0.0333	08.11.2017	15:17:02
qc 50	0.0517	0.0084	08.11.2017	15:17:53
qc 200	0.2074	0.0346	08.11.2017	15:18:43
qc 50	0.0494	0.0080	08.11.2017	15:19:35
Serum L1	0.1801	0.0300	08.11.2017	15:20:25
qc 200	0.2050	0.0342	08.11.2017	15:21:18
qc 50	0.0505	0.0082	08.11.2017	15:22:08
qc 200	0.2026	0.0338	08.11.2017	15:22:59
qc 50	0.0523	0.0085	08.11.2017	15:23:49
Serum L1	0.1812	0.0302	08.11.2017	15:24:41
qc 200	0.2026	0.0338	08.11.2017	15:25:32
qc 50	0.0547	0.0089	08.11.2017	15:26:23
qc 200	0.2050	0.0342	08.11.2017	15:27:14
qc 50	0.0500	0.0081	08.11.2017	15:28:05
Serum L1	0.1836	0.0306	08.11.2017	15:28:55
qc 200	0.2044	0.0341	08.11.2017	15:29:47
qc 50	0.0482	0.0078	08.11.2017	15:30:38
qc 200	0.1991	0.0332	08.11.2017	15:31:29
qc 50	0.0494	0.0080	08.11.2017	15:32:19
Serum L1	0.1812	0.0302	08.11.2017	15:33:10
qc 200	0.2044	0.0341	08.11.2017	15:34:02
qc 50	0.0494	0.0080	08.11.2017	15:34:53

Component	Analytical value	Analytical uncertainty U	Analytical uncertainty 95 % Confidence interval	Method	Acceptable range
Copper Cu	1883 µg/L 29,6 µmol/L	91 1,4	1792 - 1974 µg/L 28,2 - 31,0 µmol/L	ICP-AES	1701 - 2065 µg/L 1) 26,7 - 32,5 µmol/L

Serum Analysis by Furnace AAS (I)

- Highly sensitive
- Low volumes
- More skills necessary

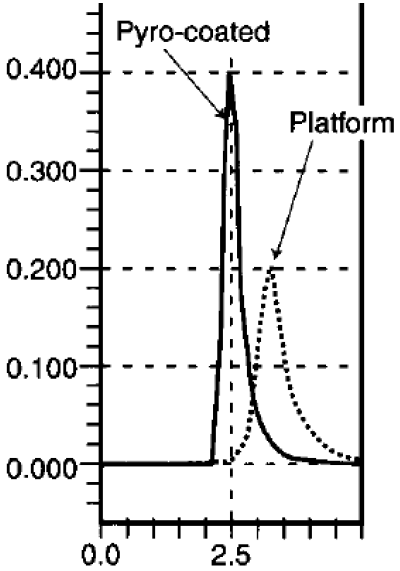


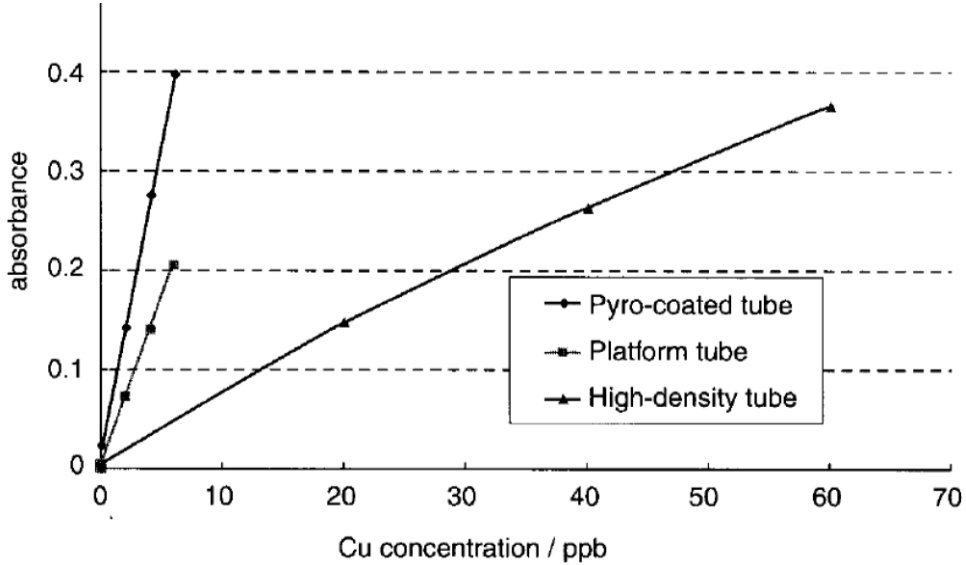


Example: Shimadzu AA-7000G

How to achieve highest sensitivity

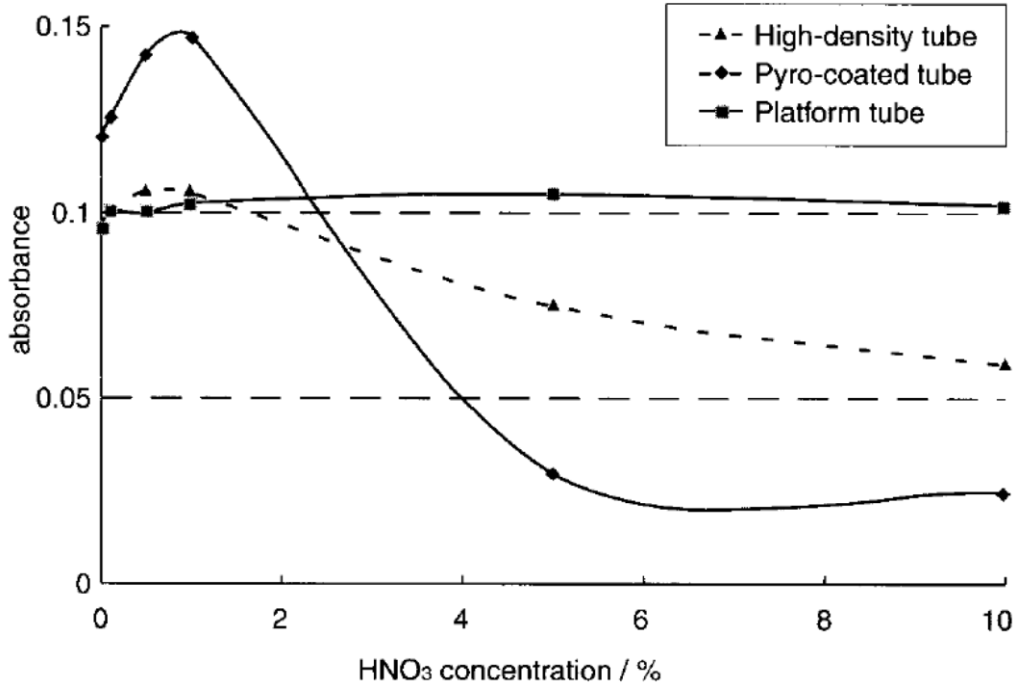
- A great help to achieve high sensitivity and reproducibility is the correct graphite tube selection!





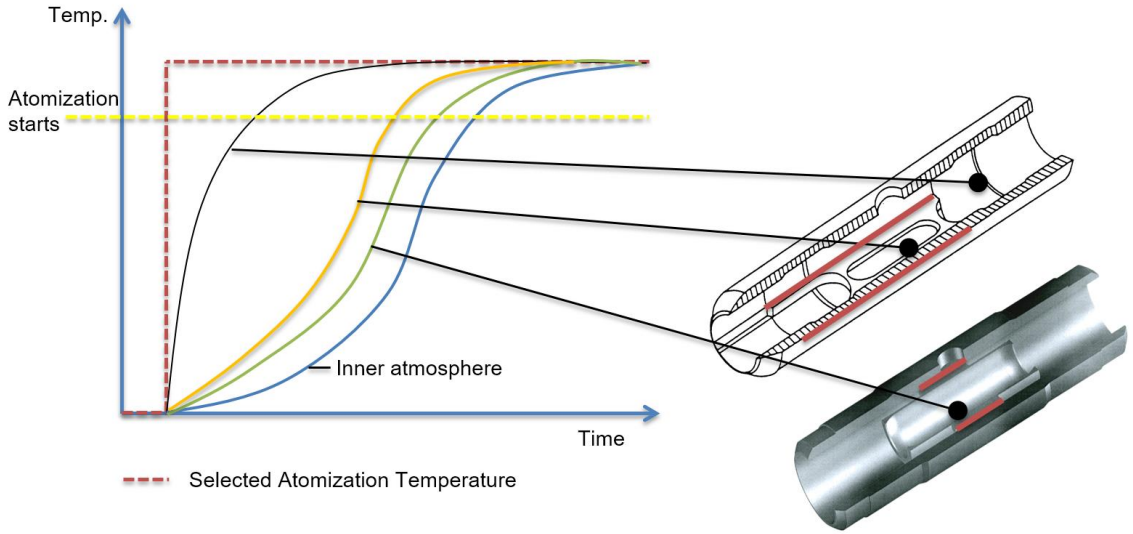
Excellent Matrix Tolerance

- Matrix tolerance important for clinical samples



How to achieve maximum performance

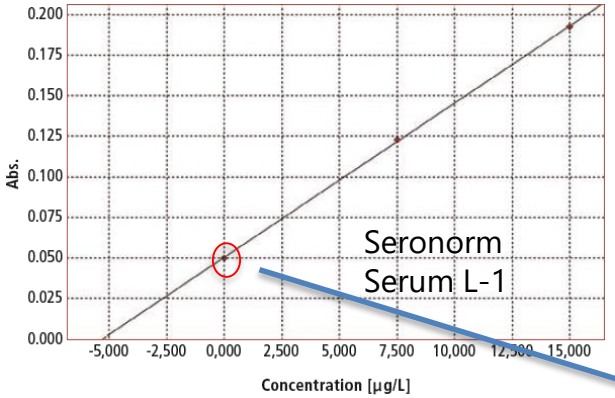
- Not every platform tube is the same…

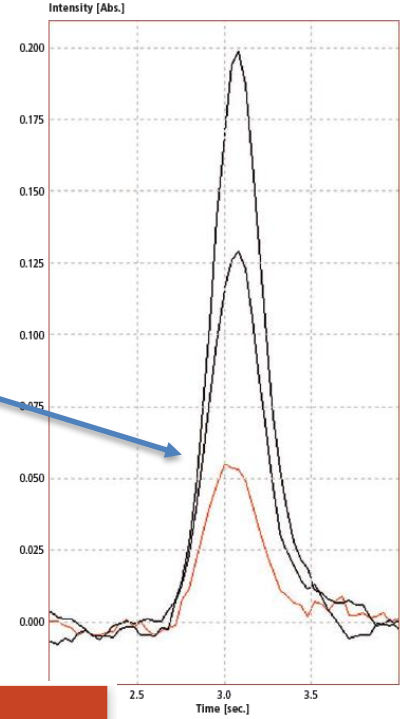


As less contact area between tube and platform, as higher is the time-delayed heating and as higher is the sensitivity/ reproducibility enhancement.

Serum Analysis by Furnace AAS (II)

- **Selenium**
  - e.g. protection of cell membranes from oxidative destruction





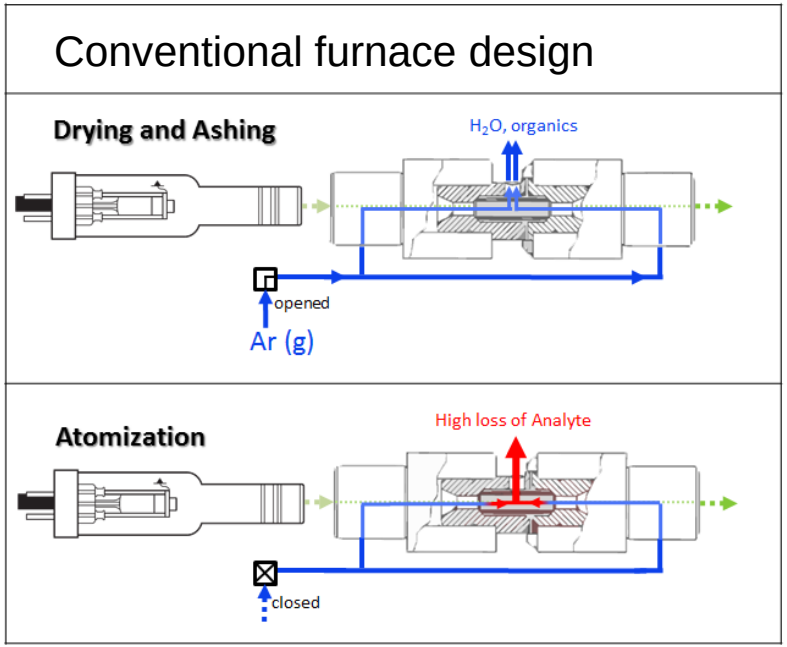
Sample volume	Water added	Standard added (Selenium concentration 30 µg/L)	Palladium modifier added	Resulting selenium addition
10 µL	10 µL	0 µL	5 µL	0 µg/L
10 µL	5 µL	5 µL	5 µL	7,5 µg/L
10 µL	0 µL	10 µL	5 µL	15 µg/L

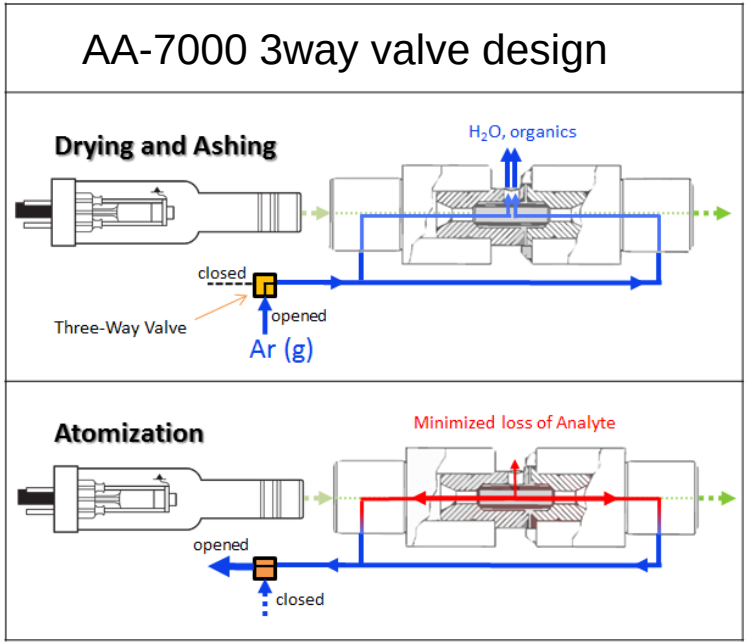
Serum	Specifications in accordance with analysis certificate			Results Shimadzu AA-7000G			
Sample	Measured values	Analyt. uncertainty*	Acceptable range	Measured values	RSD [%]	SD [µg/L]	Final result
Serum L-1	107 µg/L	100 - 114 µg/L	93 - 121 µg/L	107	3.92 %	4	107 ± 4 µg/L
Serum L-2	157 µg/L	150 - 164 µg/L	143-171 µg/L	158	4.00 %	6	158 ± 6 µg/L

SHIMADZU News 03/2014
G.I.T. Labor-Fachzeitschrift 8/2014

How to achieve highest sensitivity (II)

- **Focus your atoms within the optical pathway!**

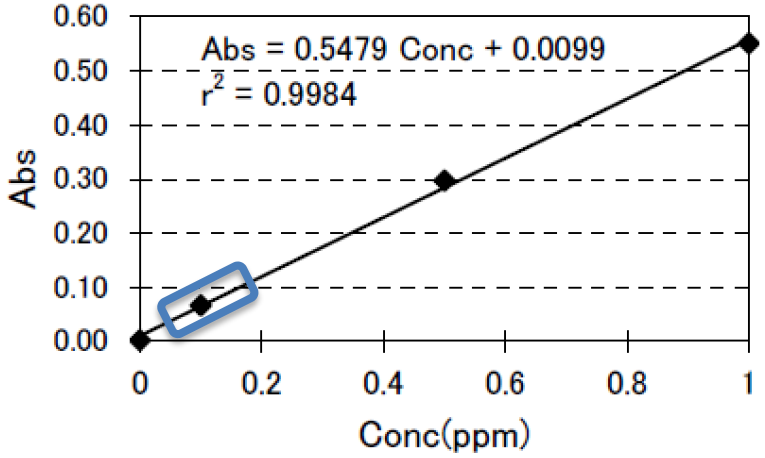




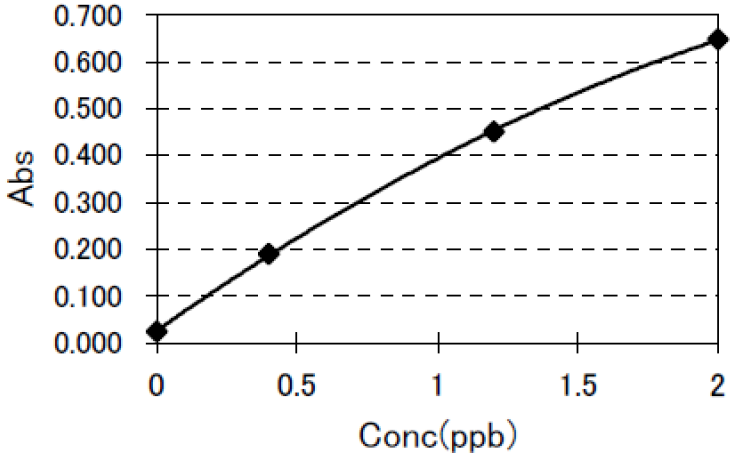
Serum Analysis by Furnace AAS (III)

- **Zink**
  - 2nd most abundant essential trace element
  - Typical range is 600-1300 ppb (60-130 ppb after dilution)
  - Flame AAS is the method of choice

Flame

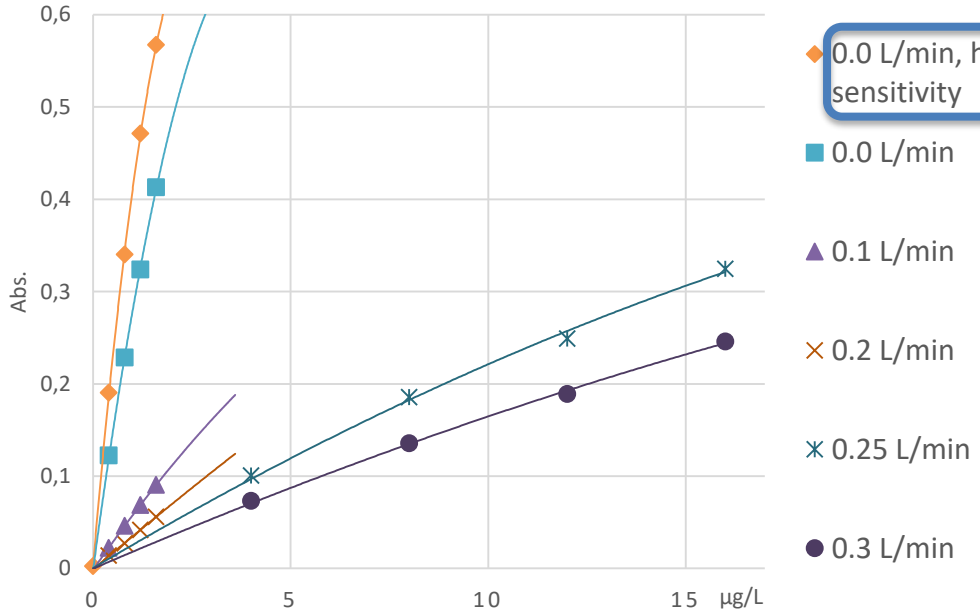


Furnace



Serum Analysis by Furnace AAS (IV)

- Design your furnace sensitiviy…



Not only helpful for serum!

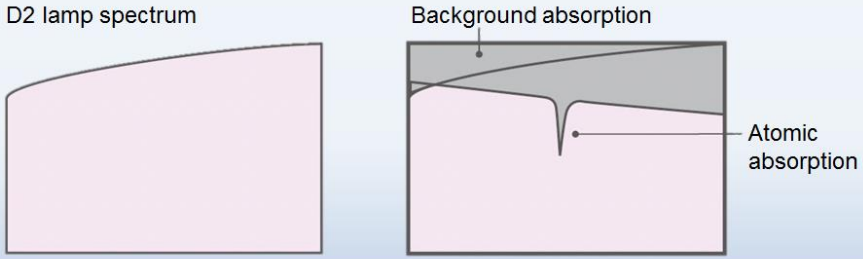
Reduce sensitivity to decrease blank interferences due to the overall occurrence of Zn.

Serum Analysis by Furnace AAS (V)

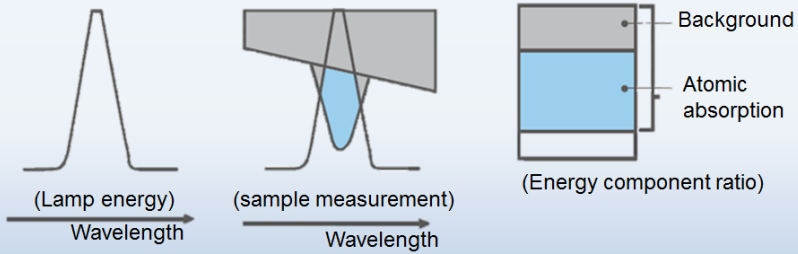
- **Chromium**
  - Physiological importance is unclear
  - Most likely the reason of analysis is because of it's toxicity
  - Can be released e.g. from metal implants

- **Too high results by D2-BG-correction**
  - The recovery of reference serum was too high!
  - Instead of 9.3 ppb the result was 22.8 ppb (245%)

→ Background information FIG 3

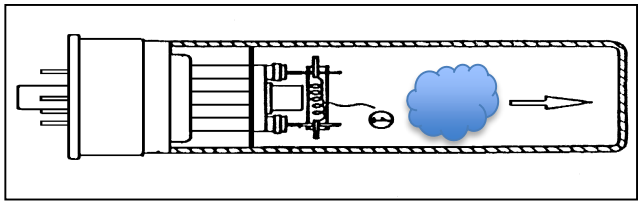


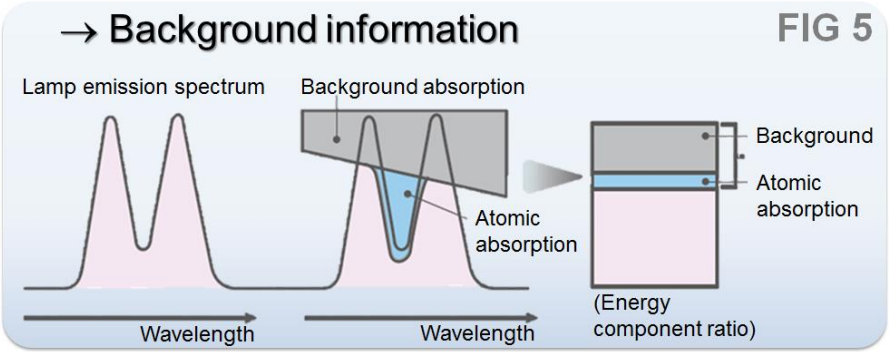
→ Analyte information (line absorption) + Background absorption FIG 4

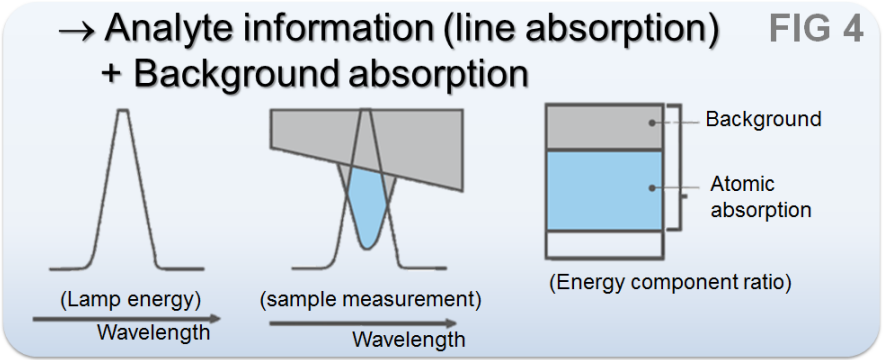


Serum Analysis by Furnace AAS (VI)

- **Self-Reversal Background Correction**
  - Corrects line overlapping
  - Corrects structured backgrounds







- With SR correction, the recovery is 108% (10.1 ppb instad of 9.3)
- Can be applied for flame and furnace

Summary

- **Flame**
  - Ideal for electrolytes and high concentrated elements (Lithium)
  - Double beam optics ensure high sensitivity for low Abs. (Copper)

- **Furnace**
  - The correct graphite tube selection is important
  - Platform tubes are most matrix tolerant
  - Latest developments (3way valve) ensure utmost sensitivity (Selenium)
  - Lower sensitive programs eliminate background issues (Zinc)
  - Highly sophisticated background technics should be considered (Chromium)

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