

SOME NEW APPLICATIONS of ITP-CZE and ITP-MS

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Electrophoretic techniques

$$m = \frac{v}{E}$$

m - mobility ($\text{cm}^2/\text{V.s}$)

E - electric field strength (V/cm)

v - velocity (cm/s)

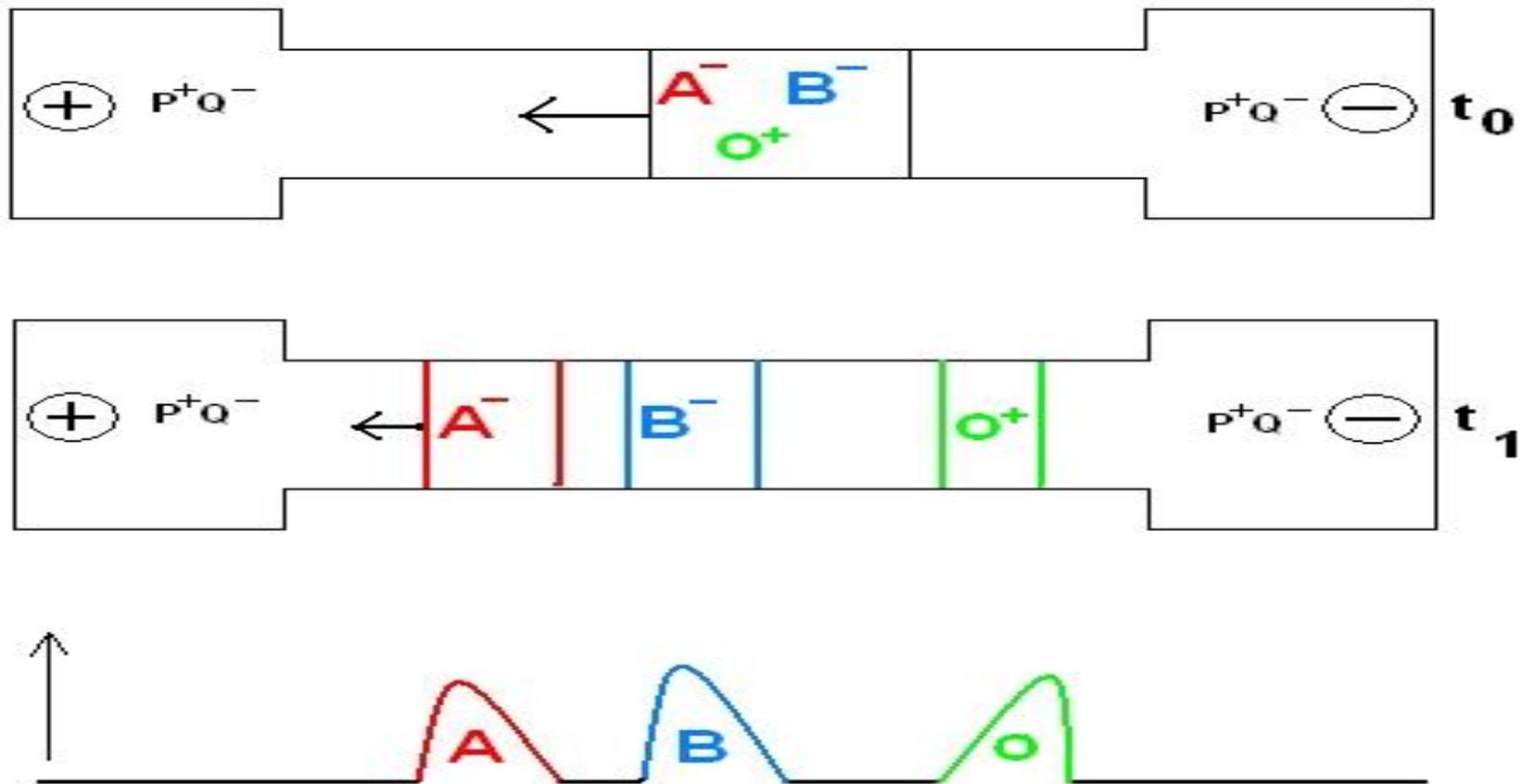
$$m_{\text{ef}} = \alpha \cdot \beta \cdot \gamma \cdot m_{\text{abs}}$$

m_{eff} = effective mobility

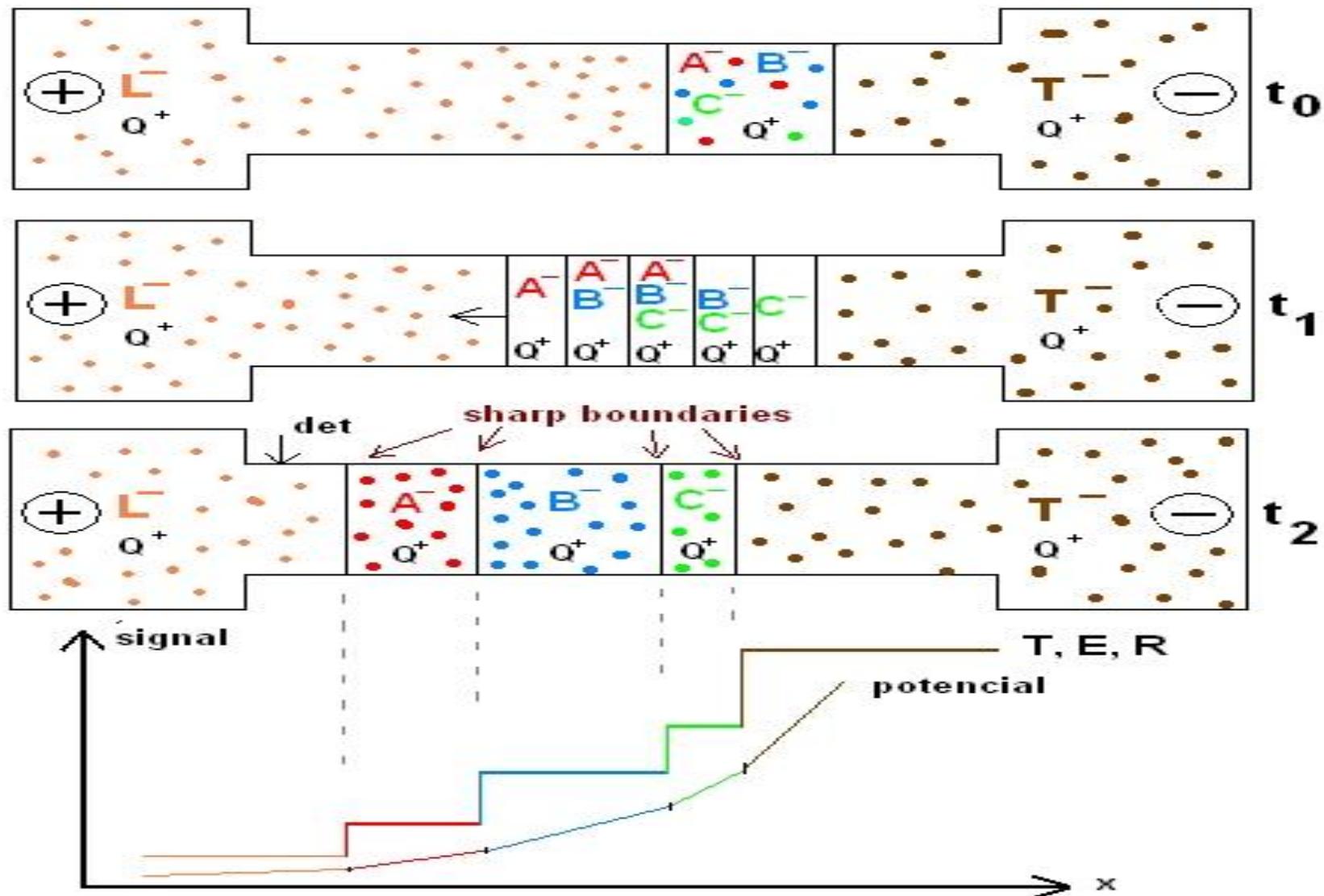
m_{abs} = absolute ionic mobility



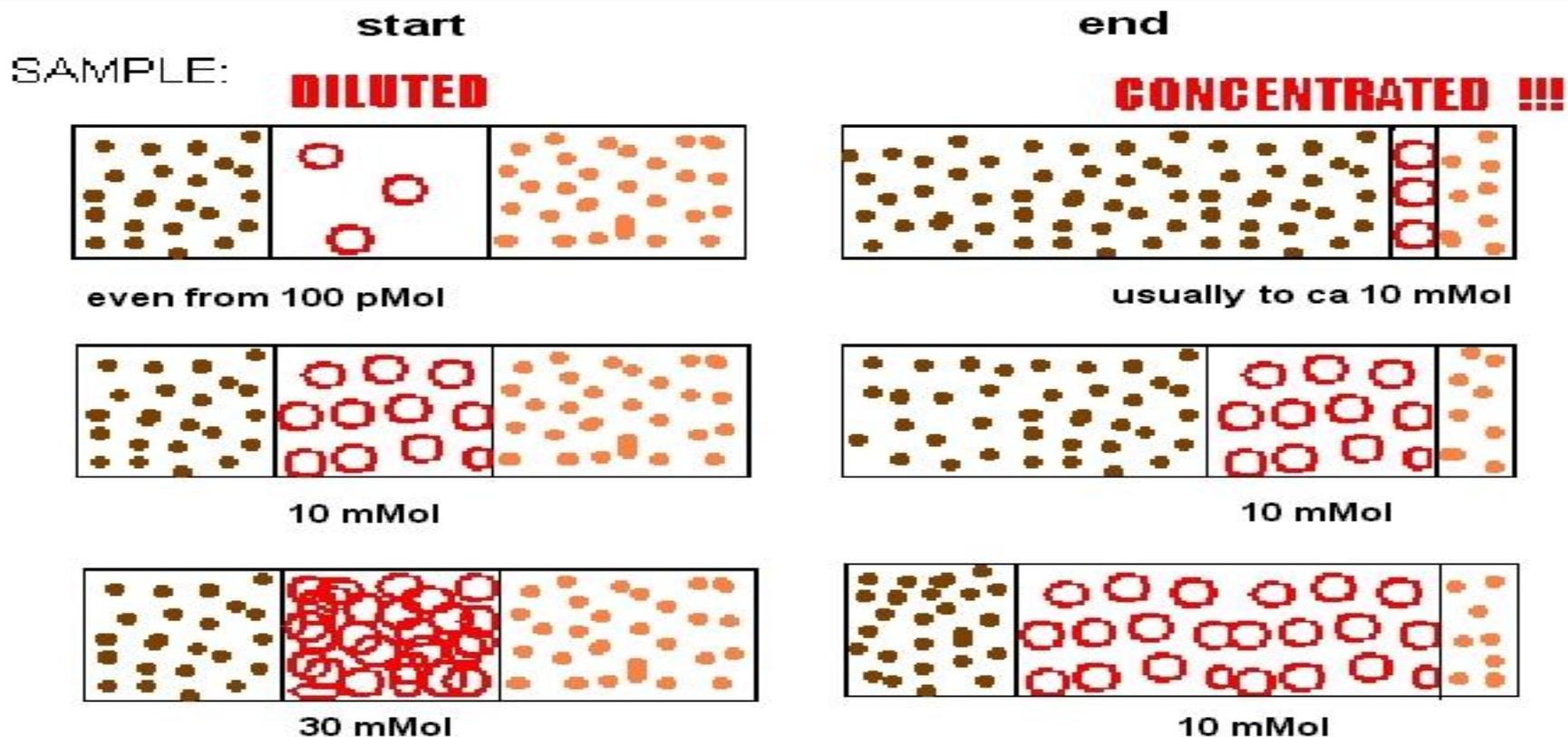
Principles of zone electrophoresis



Principles of isotachopheresis



Concentration effect in ITP



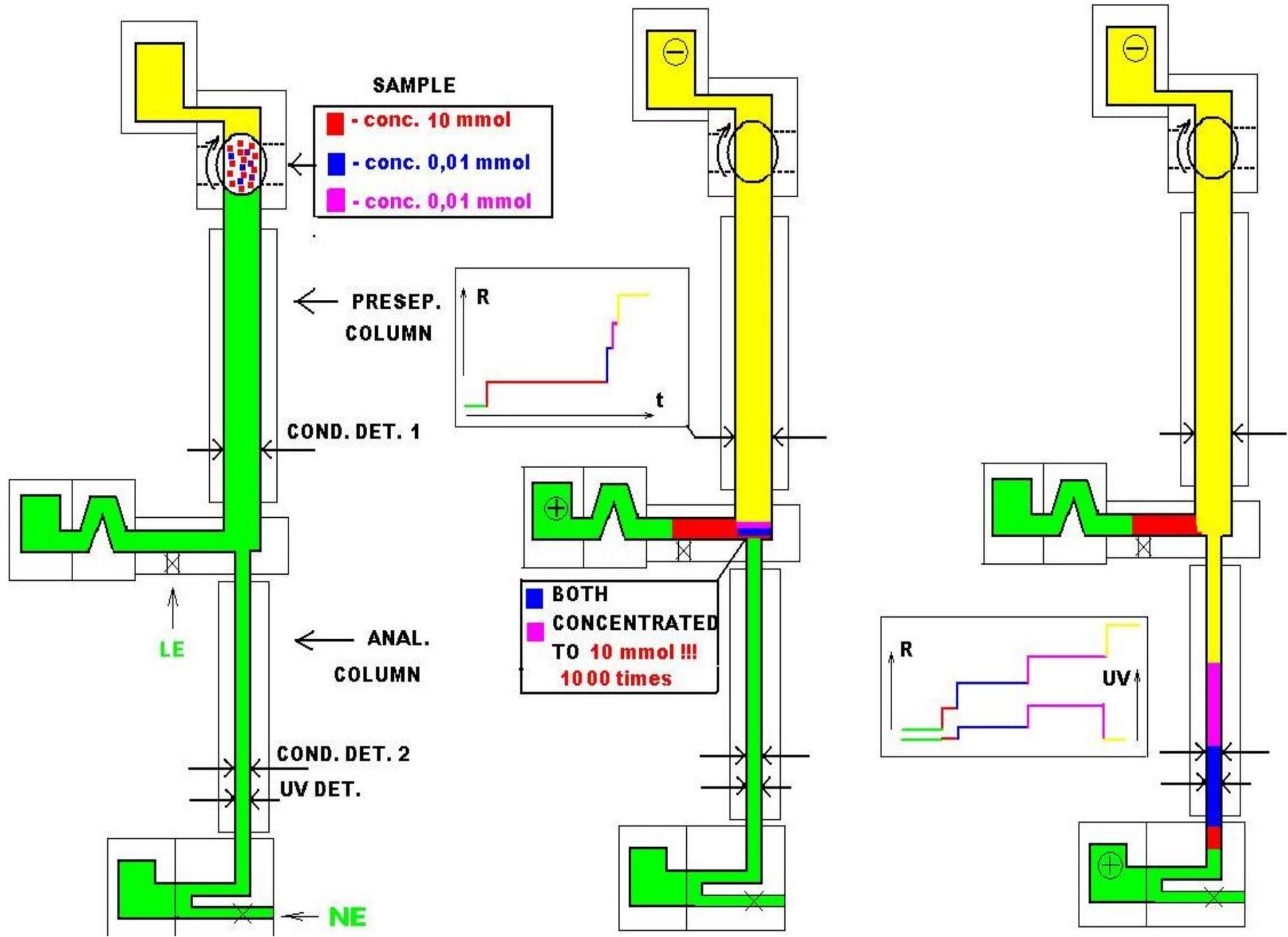
ADVANTAGE:

High concentration in detector

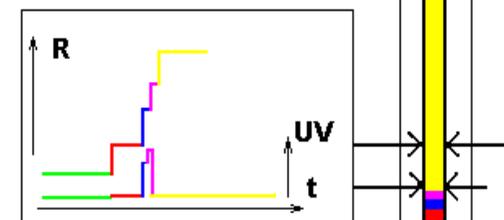
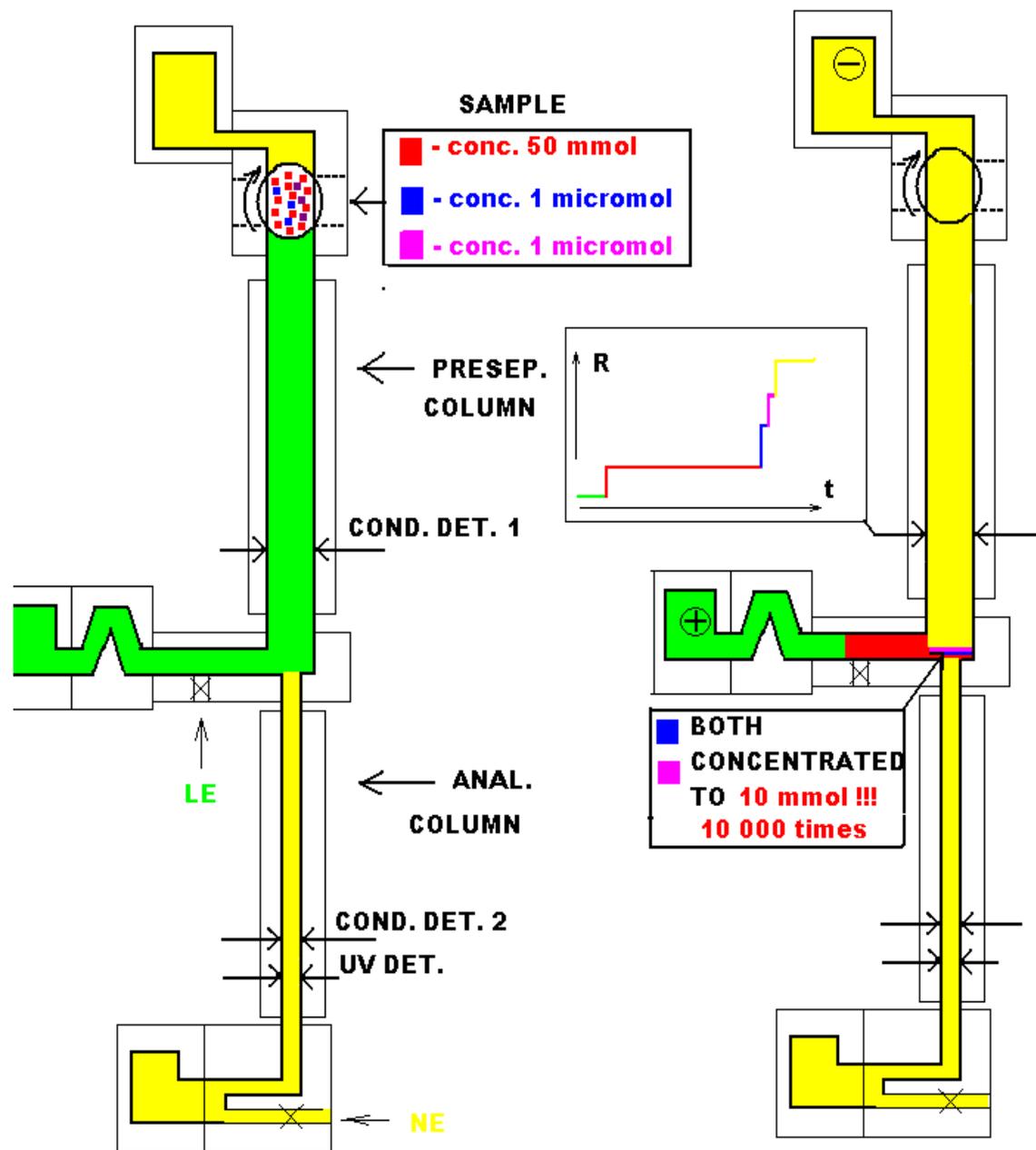
DISADVANTAGE:

At very low concentration, zone in ITP analysis is too short and too close to another zones to be detectable-
-solution: to use CZE in second column.

ITP - ITP

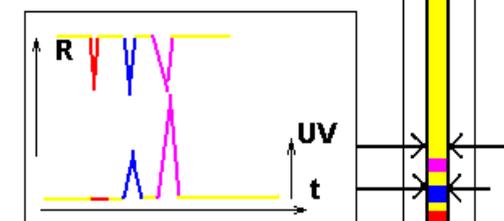
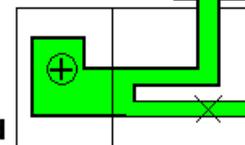


ITP - CZE



ITP - ITP

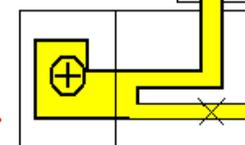
- zones too short
- peaks not separated each other



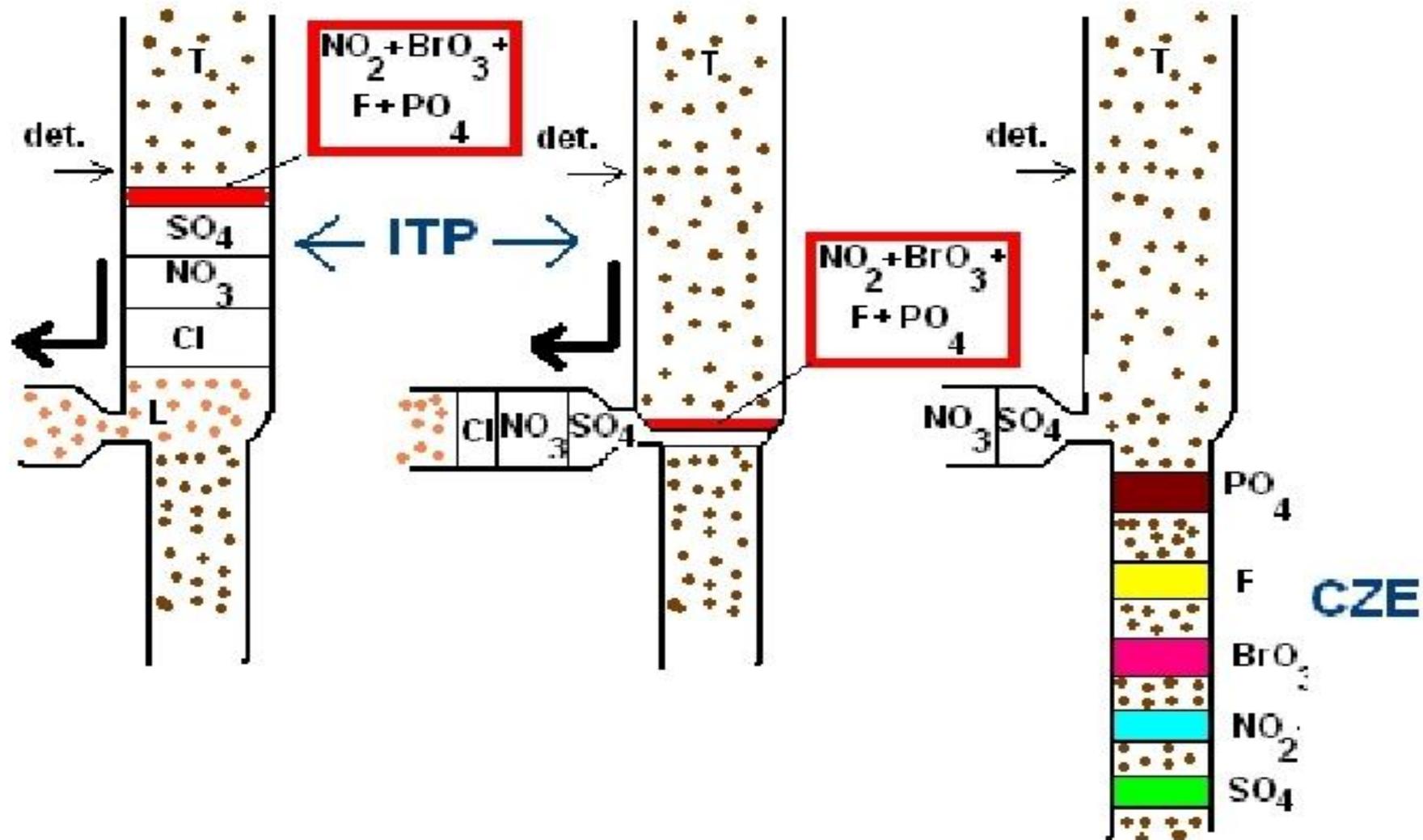
ITP - CZE

sensitivity 100 x higher

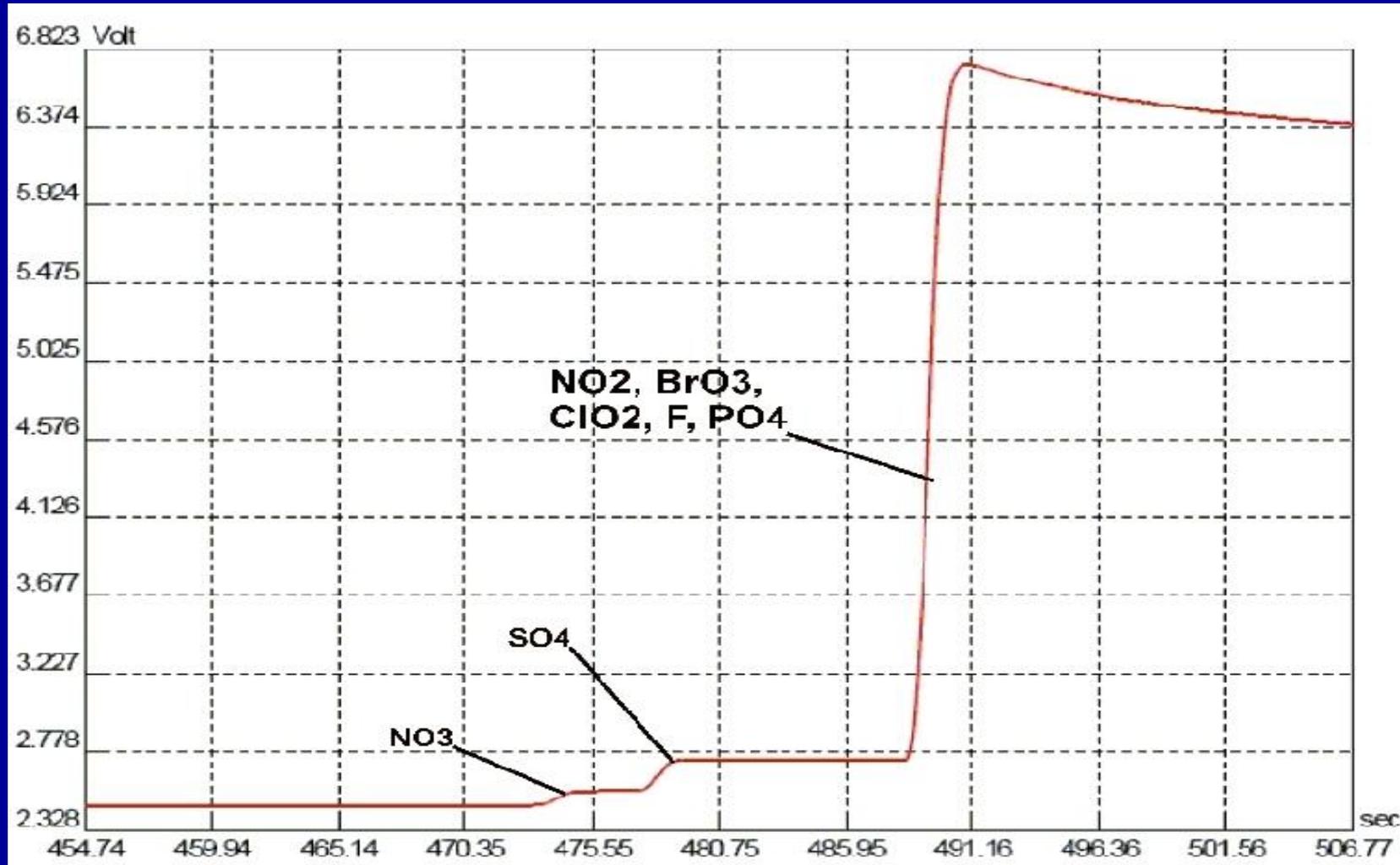
- 1 ppb for cond. det.
- 0.01 nM for UV det.



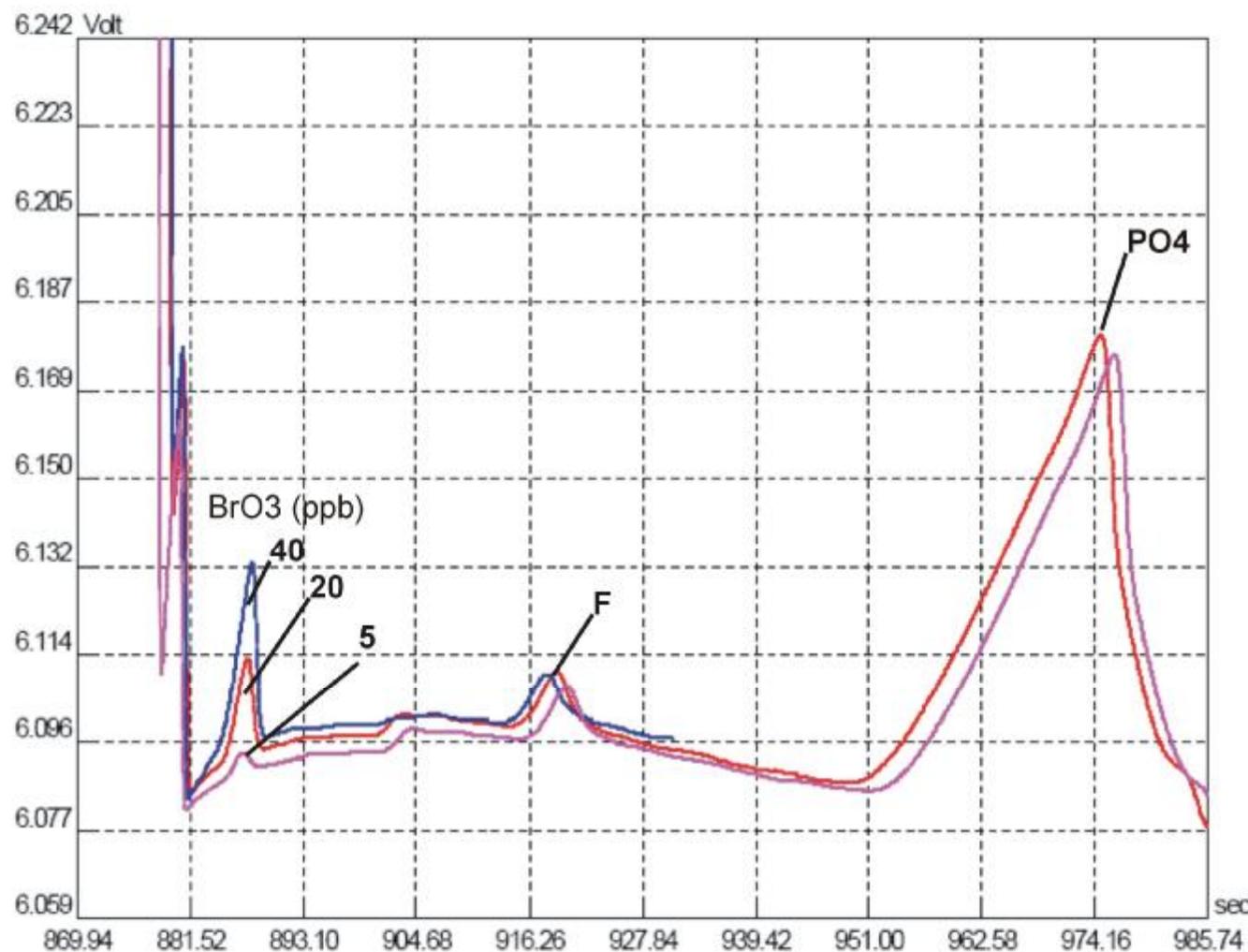
Practical example of ITP-CZE analysis – drinking water



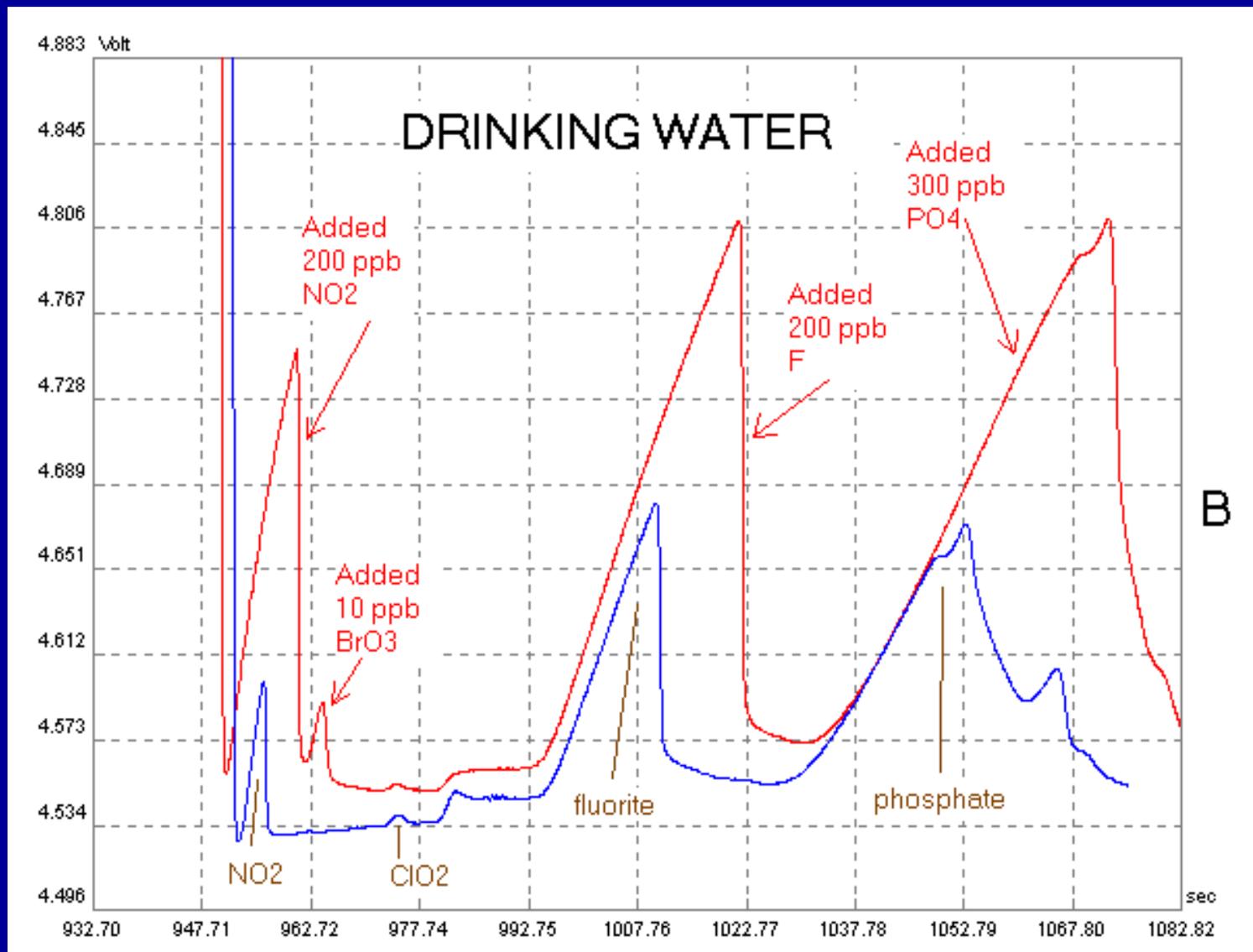
First column – ITP of drinking water



Drinking water – separation of different added concentrations of BrO_3



Analysis of drinking water and added: NO_2 , BrO_3 , F, PO_4

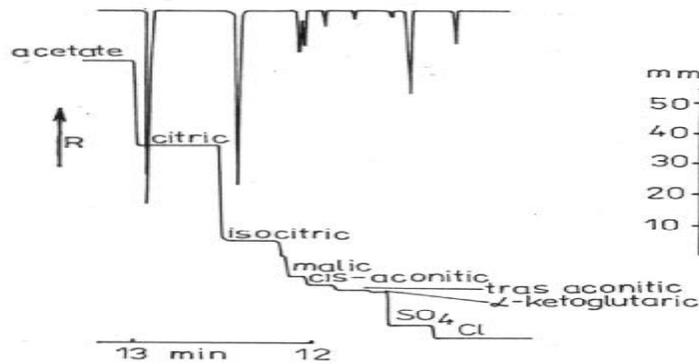


Comparison of different electroforetic techniques on analysis of hippuric acid in serum

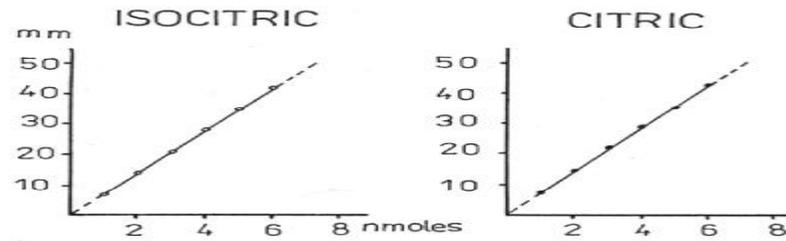
Method	Capillary	LOD [μmol]	
		Model sample	Serum
ITP	FEP 90 x 0.3 mm I.D.	40	60
CZE Closed	FEP 300 x 0.2 mm I.D.	50	60
CZE Open	silica 700 x 0,1 mm I.D.	0,04	10
ITP-CZE	FEP 1 - 160 x 0,8 mm I.D. 2 - 300 x 0,2 mm I.D.	0,4	0,7

TYPICAL ITP ANALYSIS – organic acids

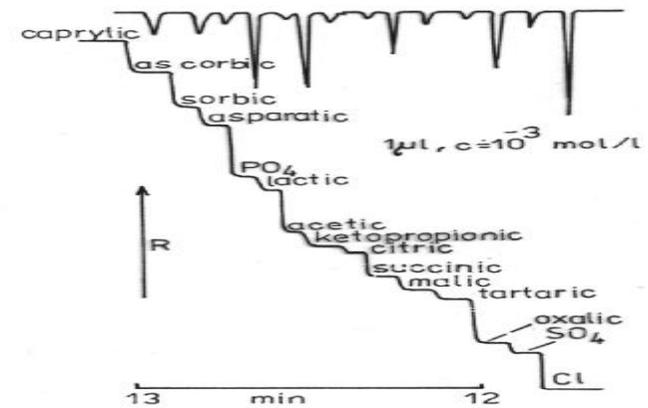
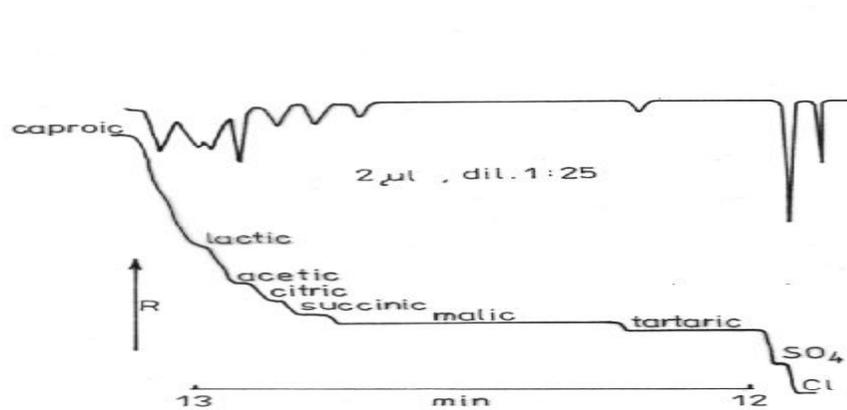
ANALYSIS OF FERMENTATION BROTH



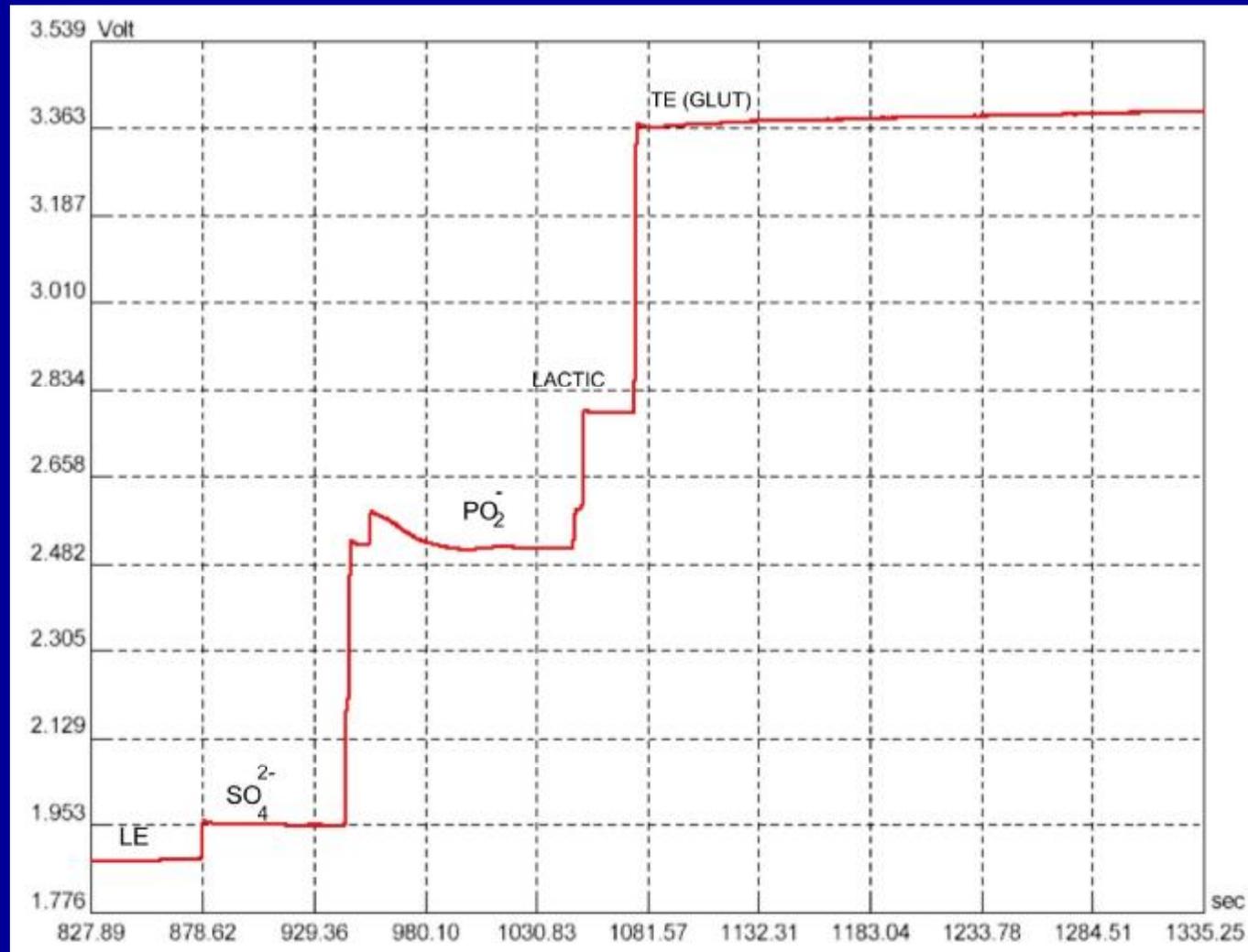
CALIBRATION CURVES



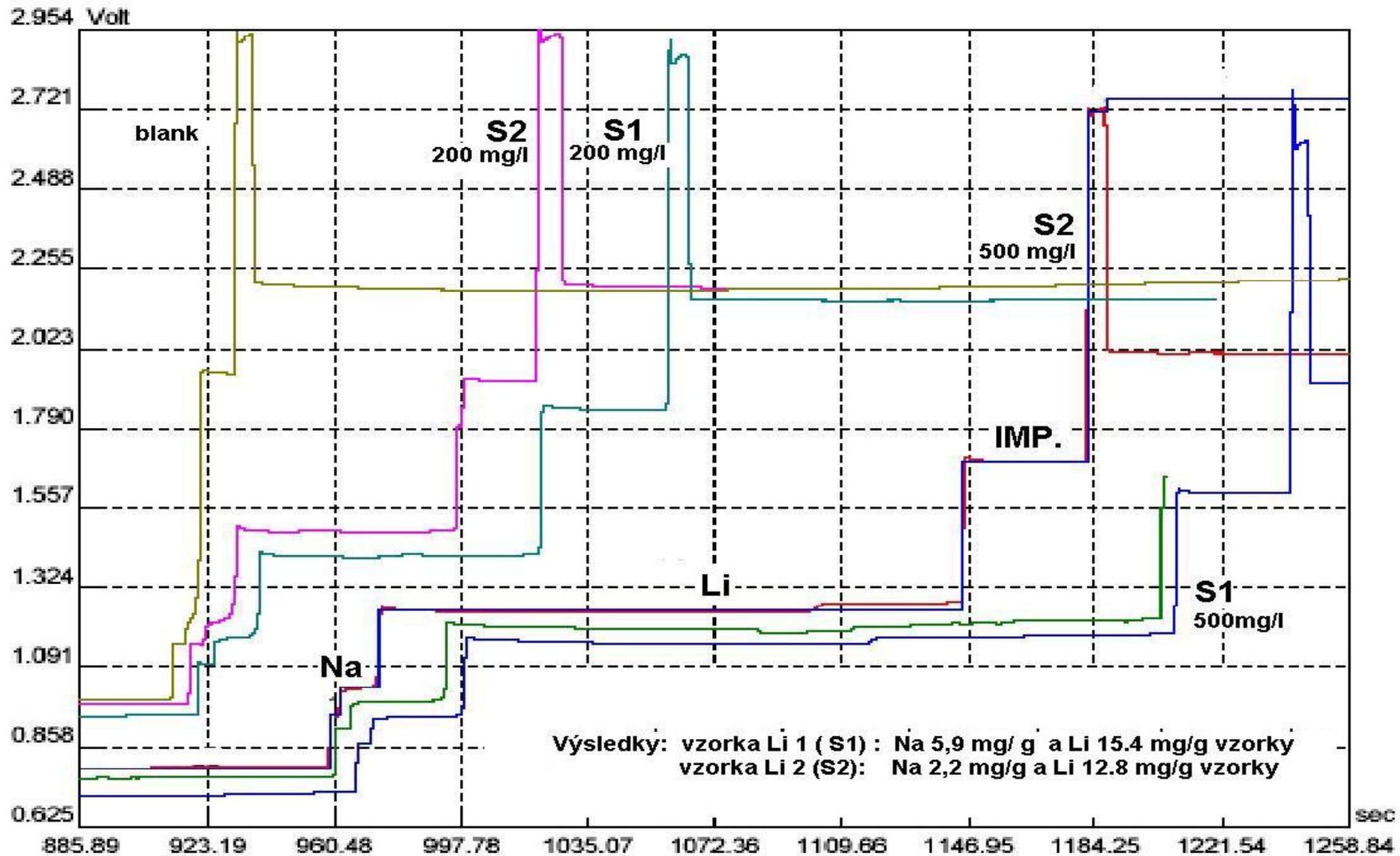
ANALYSIS OF WINE



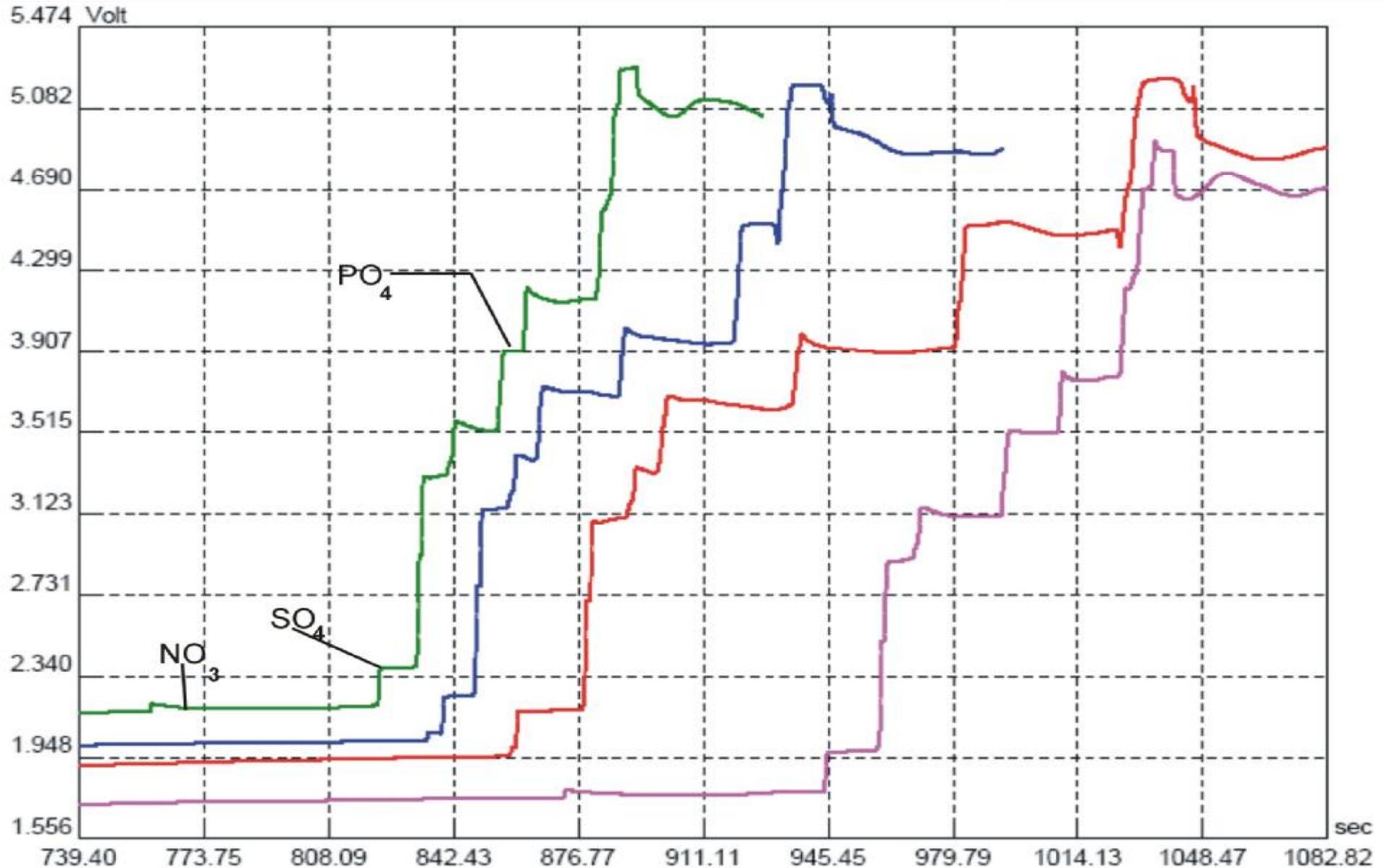
Quality Control Of Ni – Galvanic Path (dil. 1000 x) t = 4 min.



Contents of Na nad Li in salts of hyaluronic acid

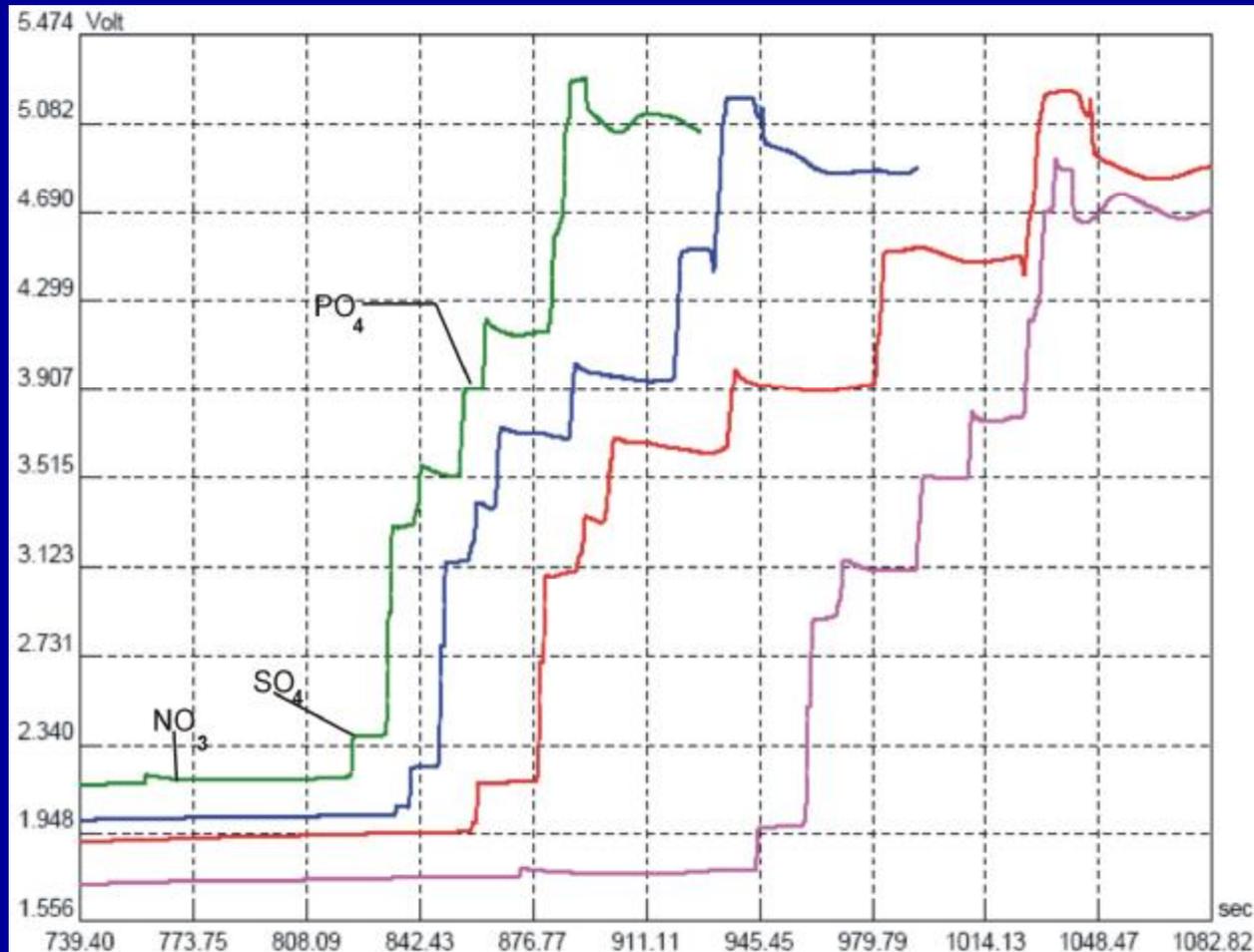


ANALYSIS OF ANIONS in root extract-total amount of sample-30 ul

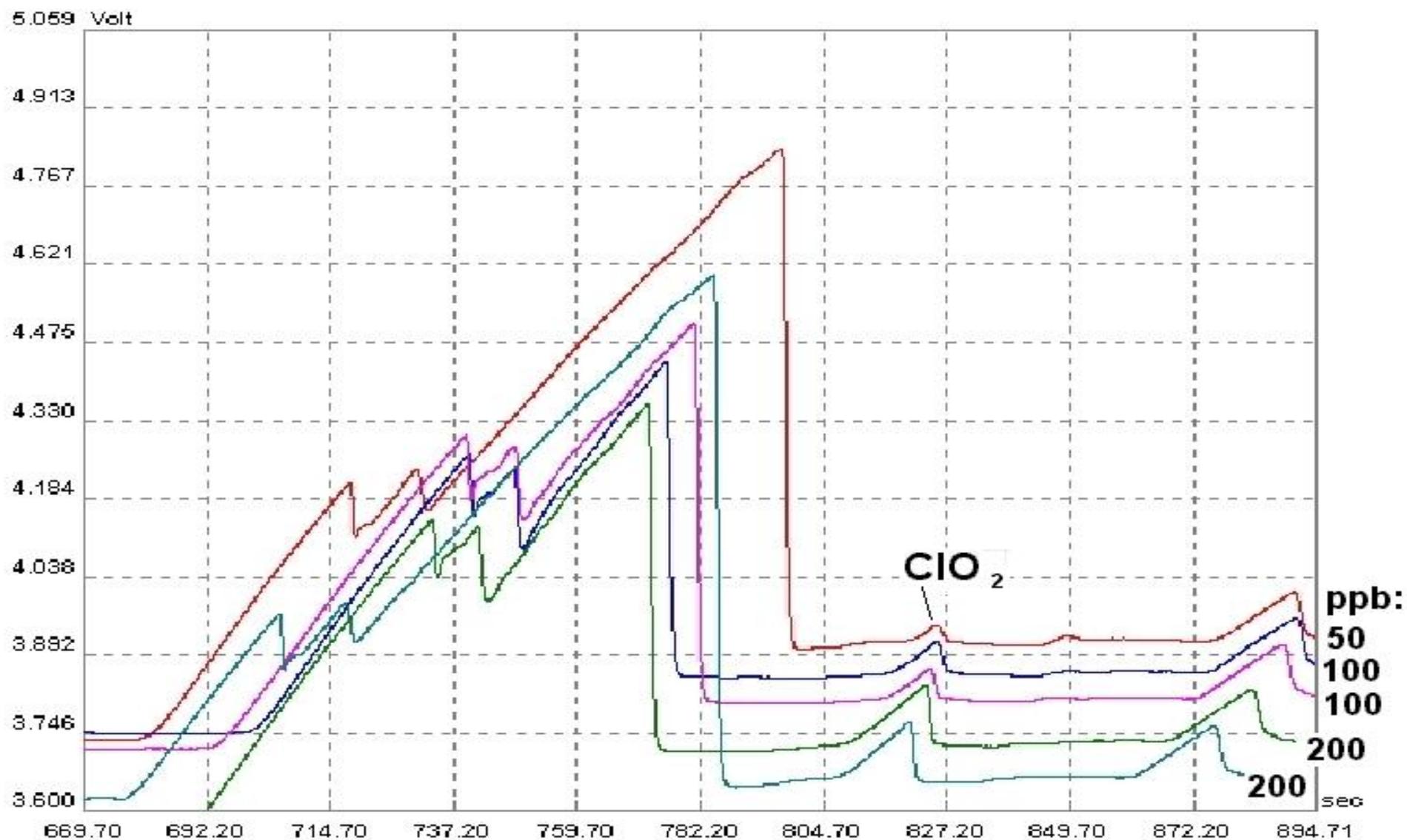


Anionic Profile of Different Parts of Leaf (Vein)

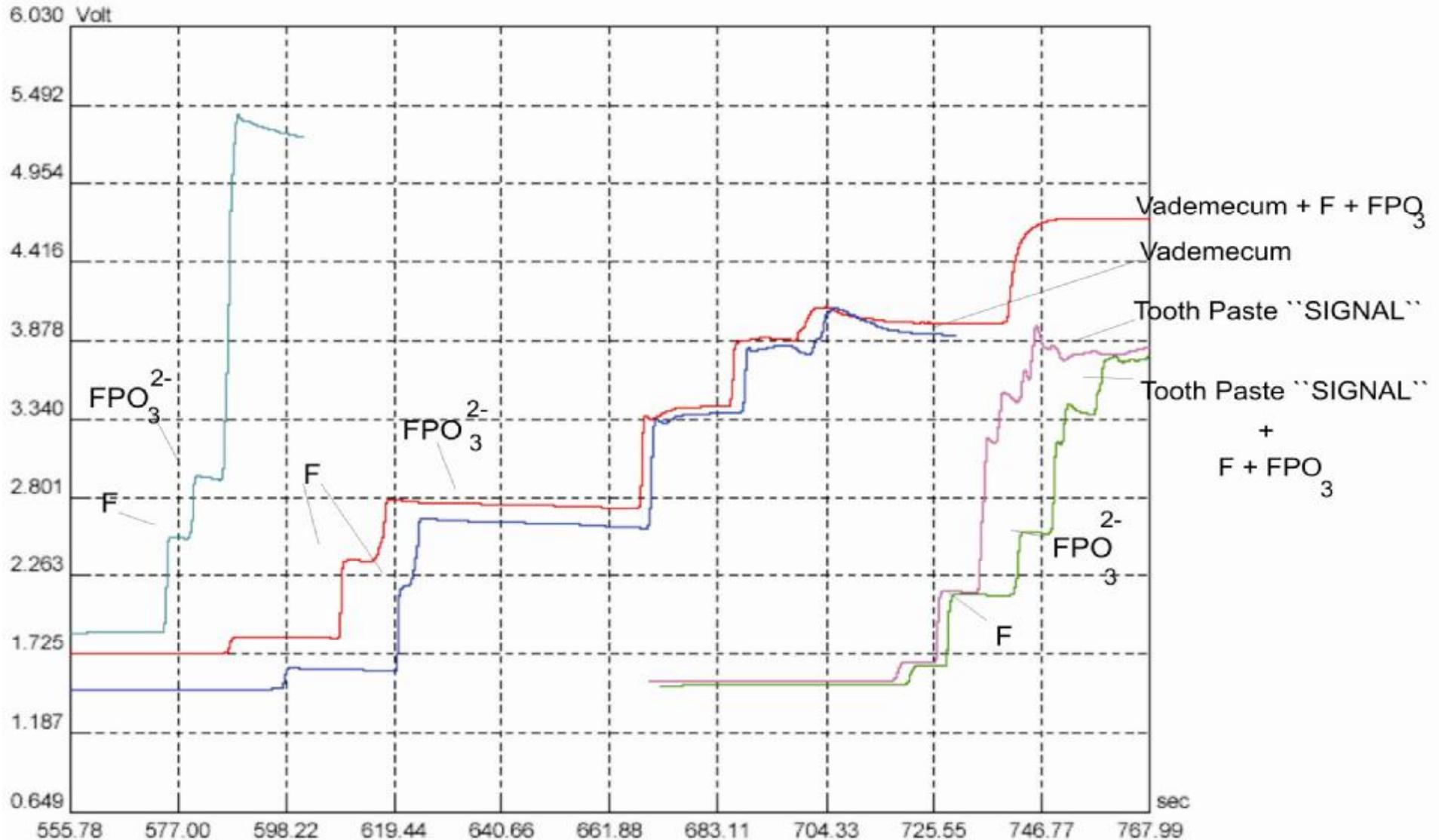
(Total Amount of Sample From 2 to 20 Nanograms)



Reproducibility of ITP-CZE analysis of ppb level

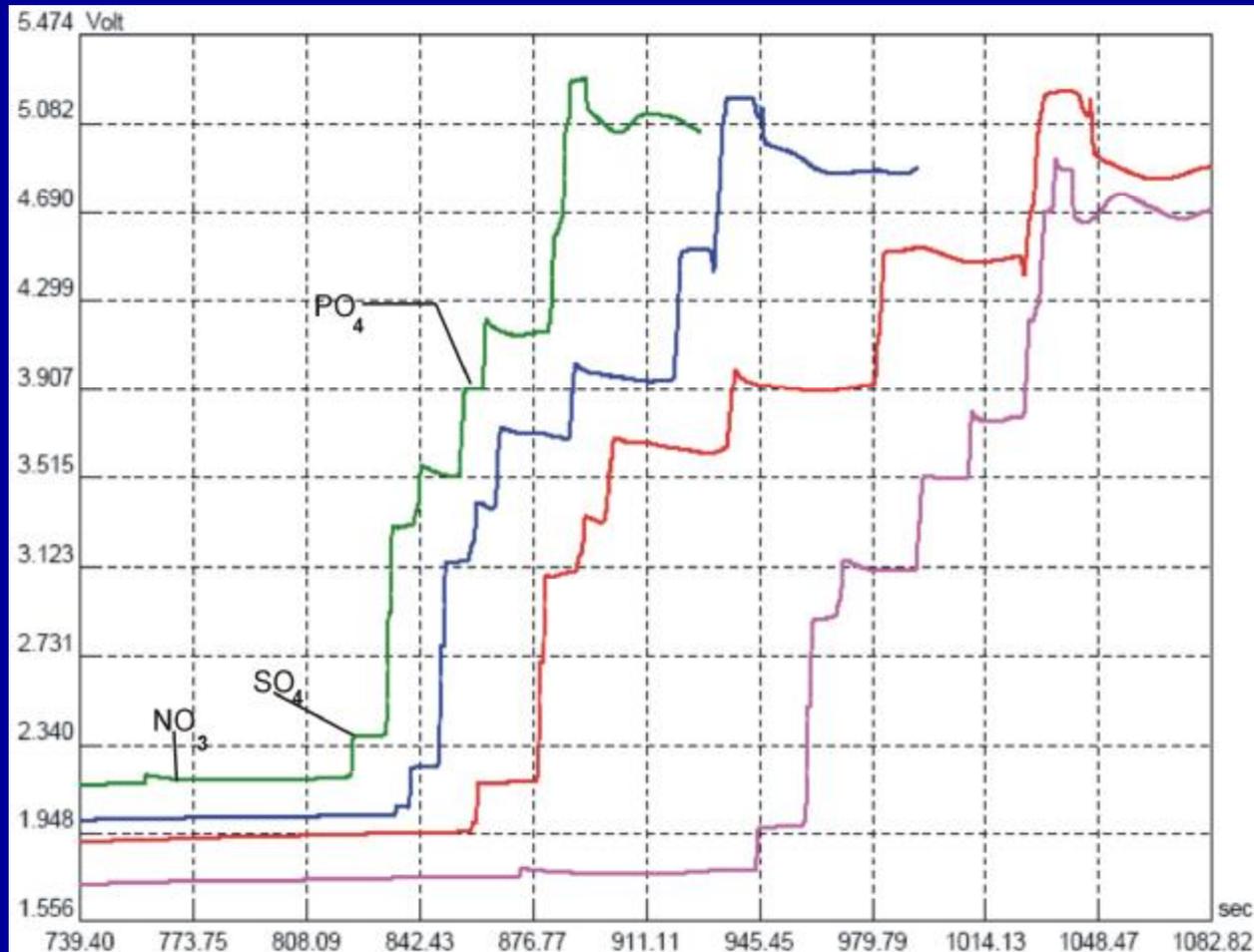


Analysis of fluor in tooth paste

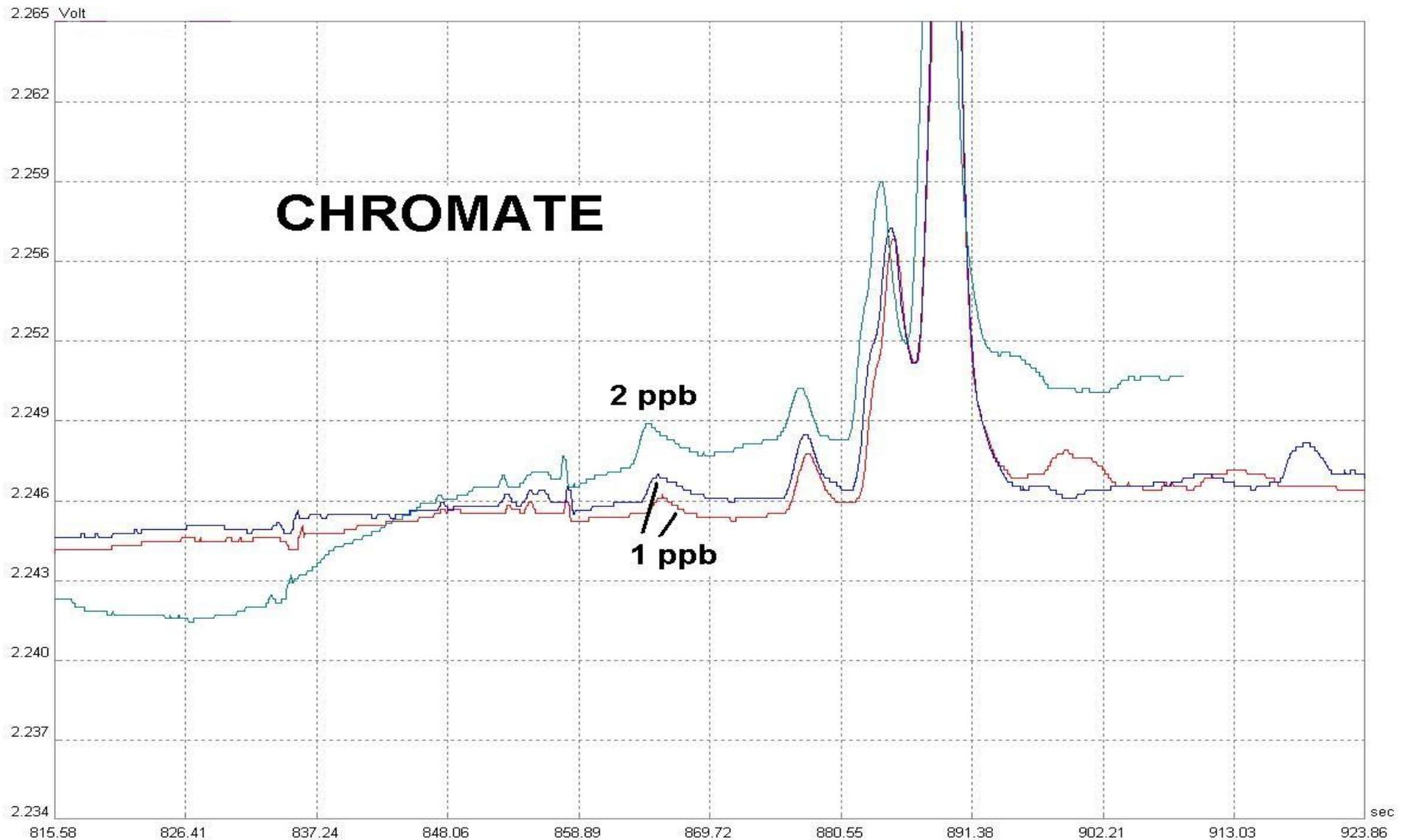


Anionic Profile of Different Parts of Leaf (Vein)

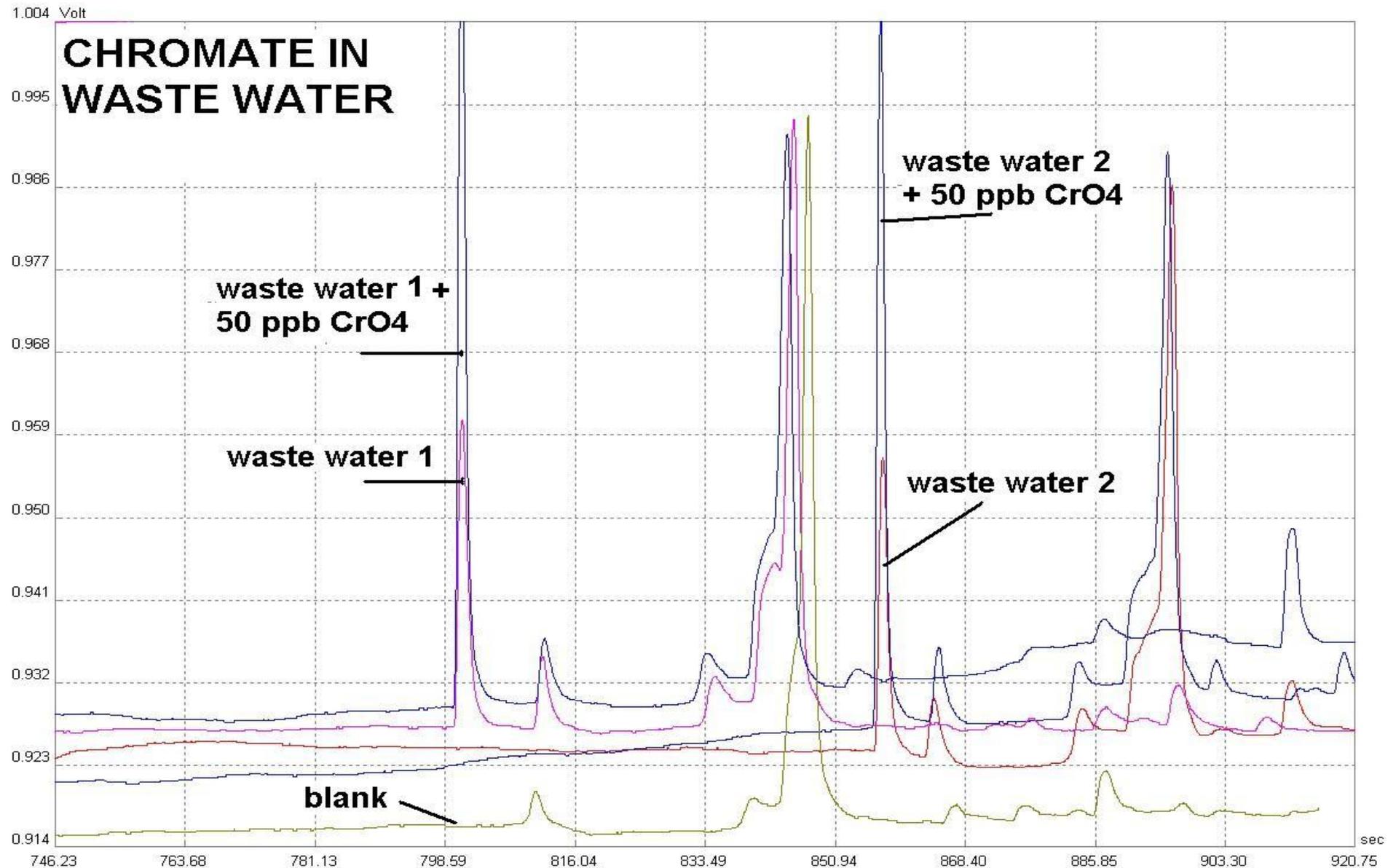
(Total Amount of Sample From 2 to 20 Nanograms)



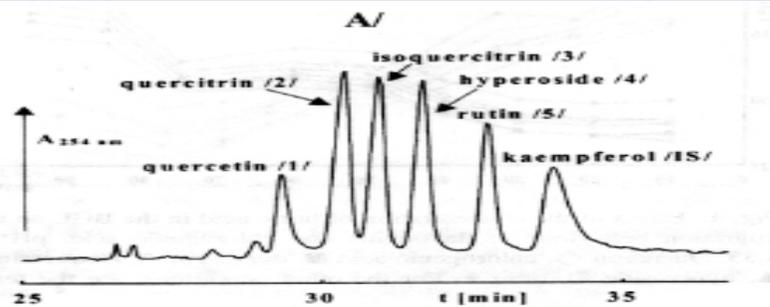
Chromate in drinking water/ det. limit



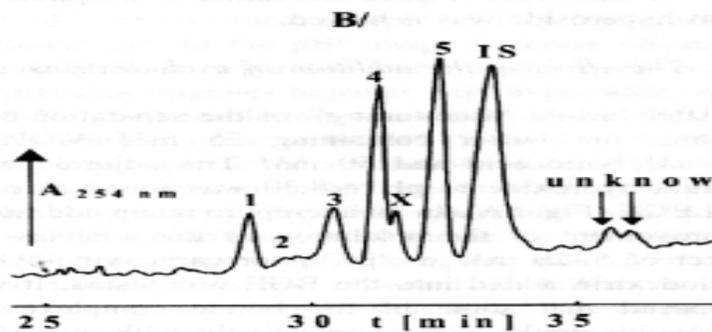
CHROMATE in waste water



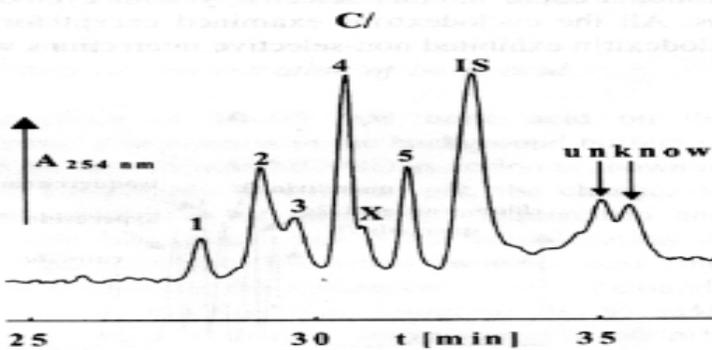
ITP – CZE analysis of extracts from flowers and leaves



MODEL MIXTURE

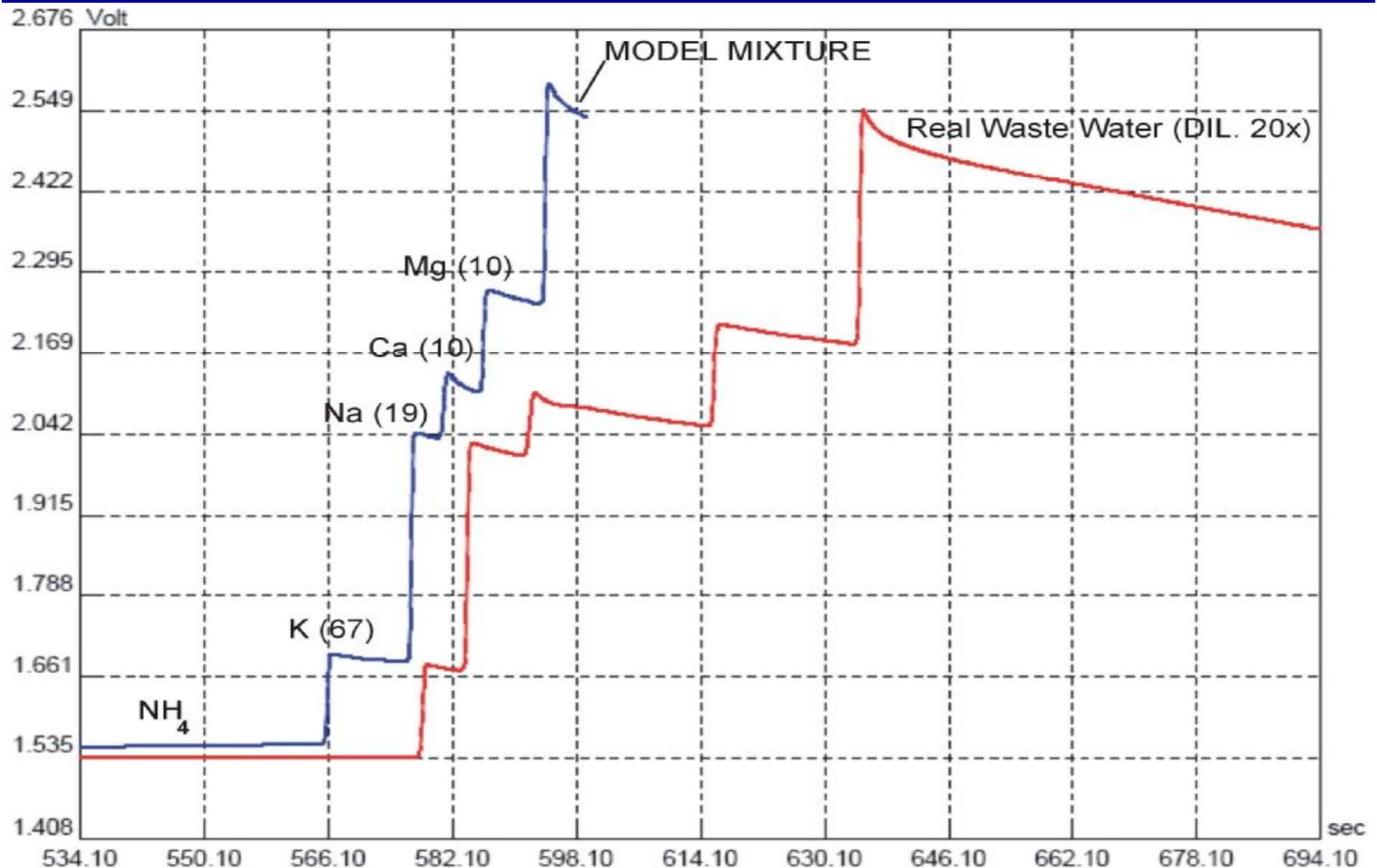


EXTRACT OF LEAVES
AND FLOWERS
(HYPERICUM)

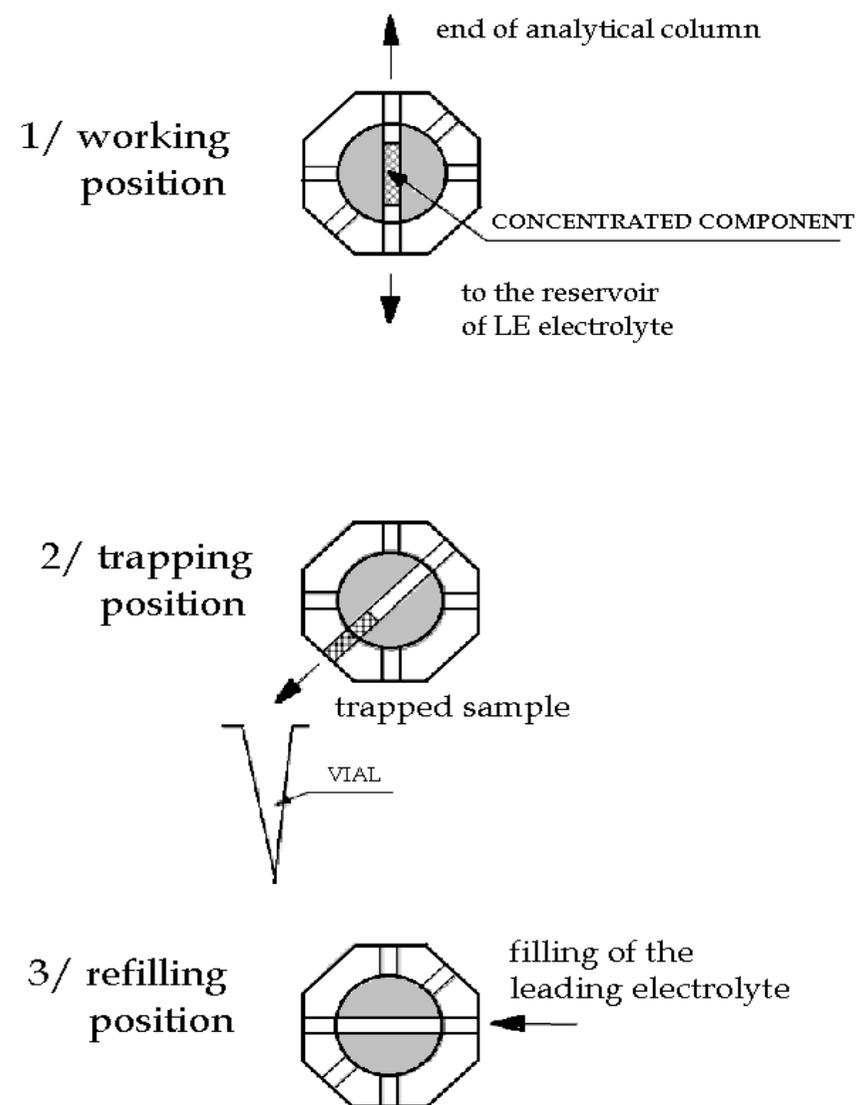
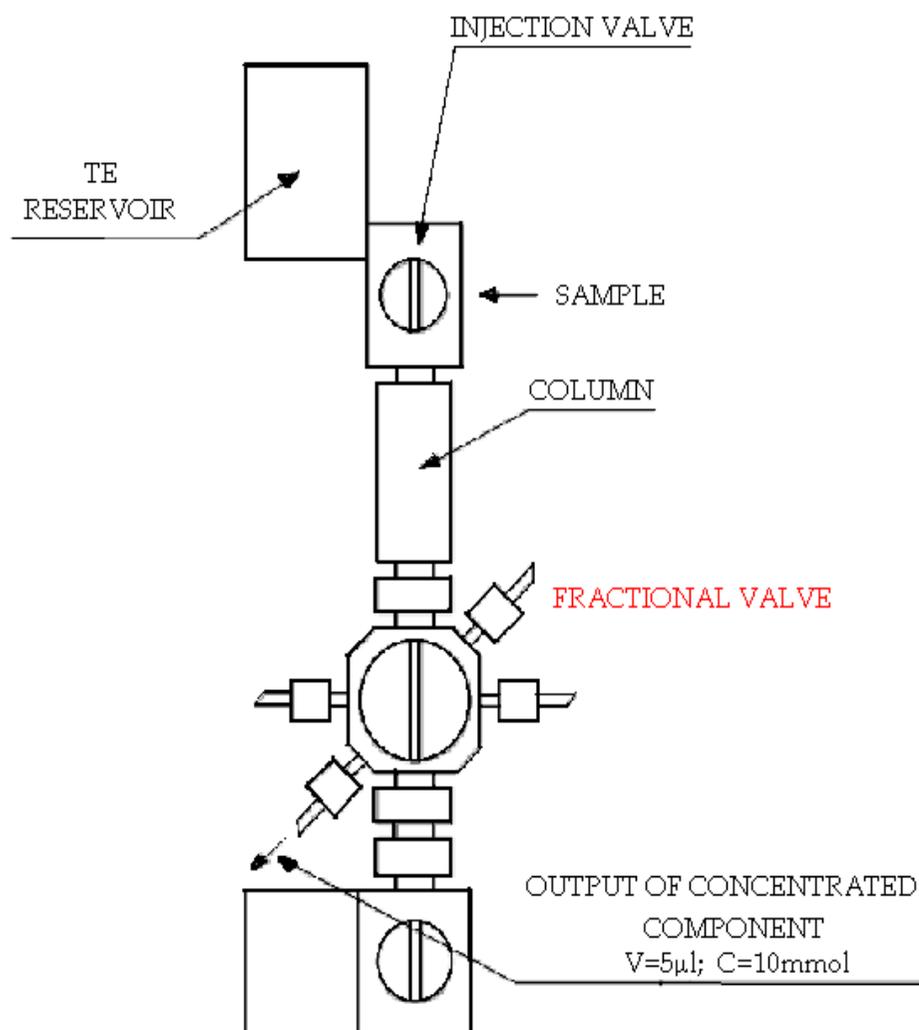


EXTRACT
OF FLOWERS

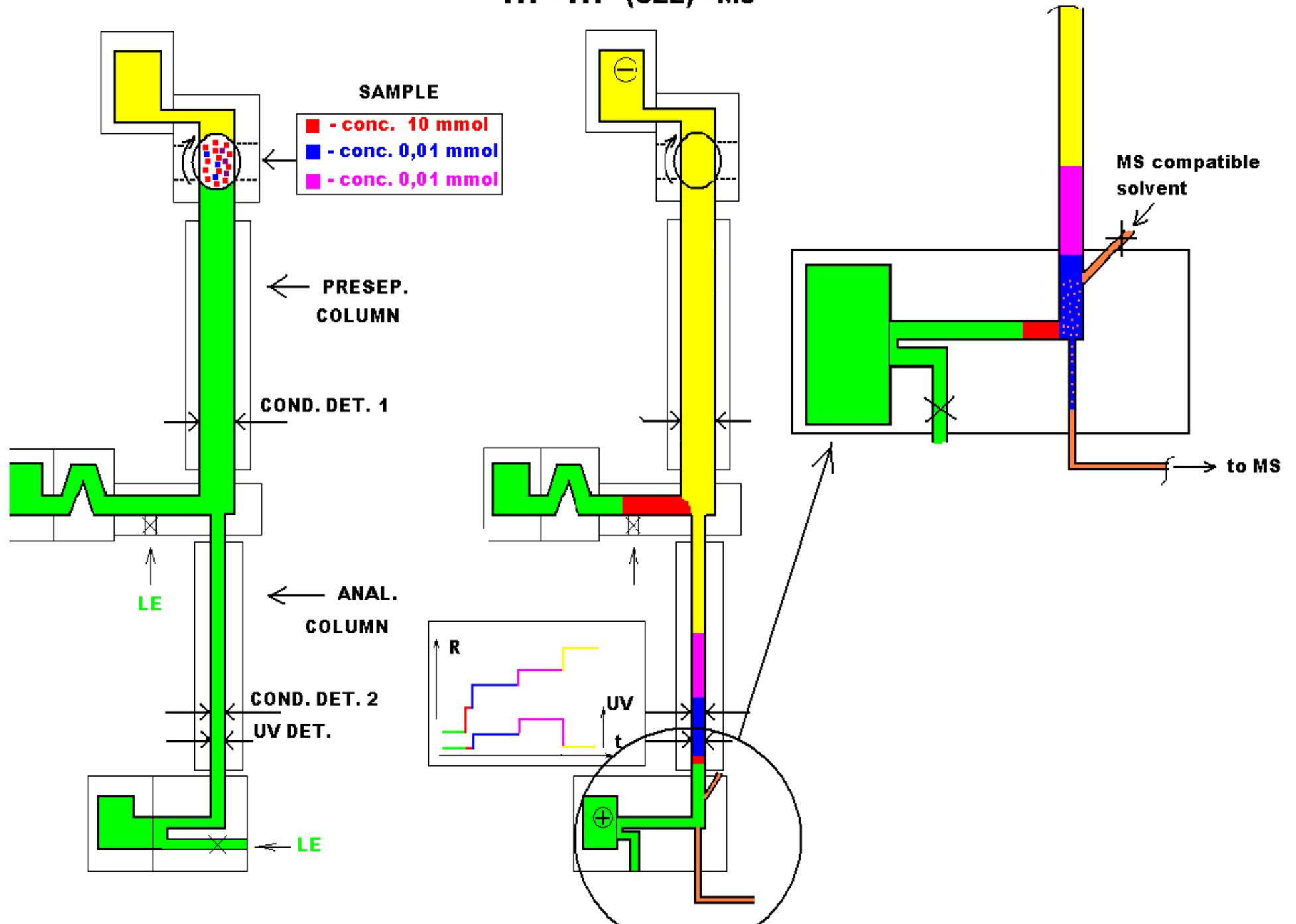
Analysis of cations in waste water



Principles of micropreparative valve

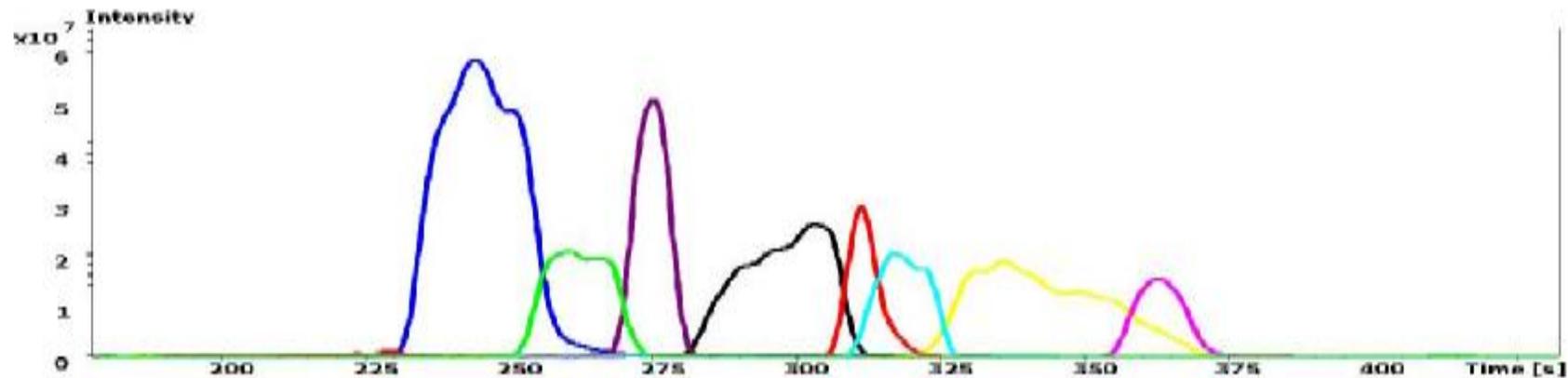
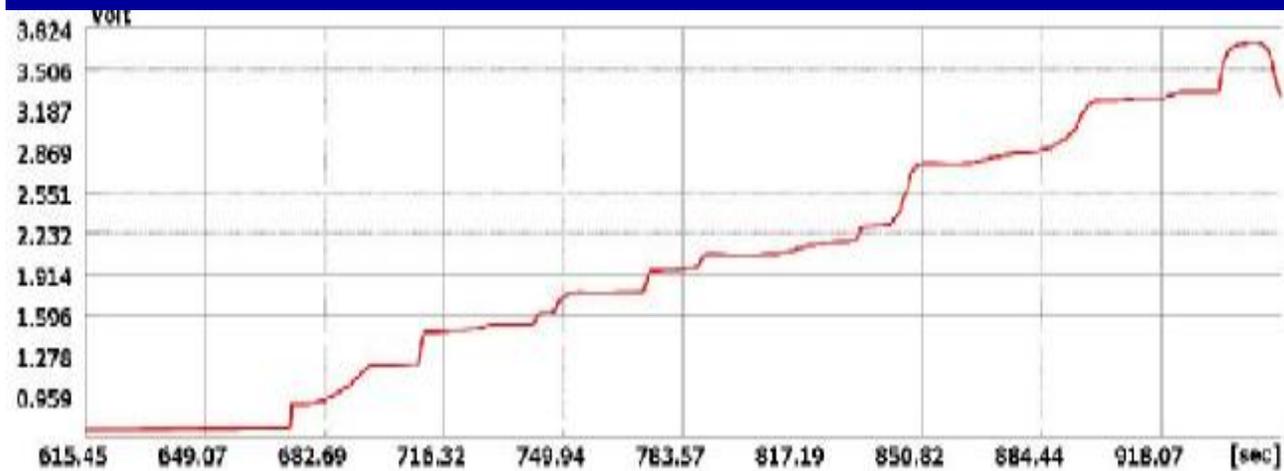


ITP - ITP (CZE) - MS



DIGEST OF CYTOCHROME C

ITP record and MS ion monitoring



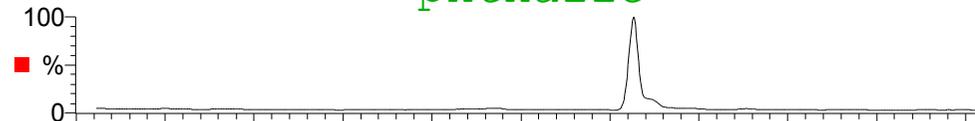
Mix of 9 acids, 5 uM, ITP-CZE, 100 um cap.

06-Dec-2006 13:22:03

61206-01 Sm (Mn, 2x3)

phthalic

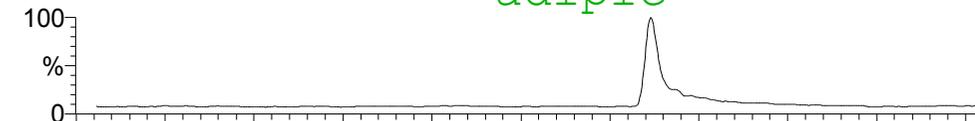
SIR of 8 Channels ES-
165.1
5.62e4



61206-01 Sm (Mn, 2x3)

adipic

SIR of 8 Channels ES-
145.1
4.96e4



61206-01 Sm (Mn, 2x3)

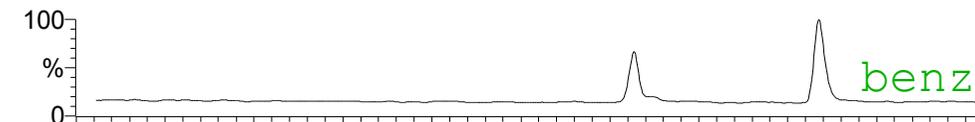
glutaric

SIR of 8 Channels ES-
131.1
4.54e4



61206-01 Sm (Mn, 2x3)

SIR of 8 Channels ES-
121.1
8.21e4



benzoic ?

61206-01 Sm (Mn, 2x3)

succinic

SIR of 8 Channels ES-
117.1
4.36e4



61206-01 Sm (Mn, 2x3)

malonic

SIR of 8 Channels ES-
103.1
1.81e4



?

61206-01 Sm (Mn, 2x3)

lactic

SIR of 8 Channels ES-
89.1
2.30e5



oxalic

61206-01 Sm (Mn, 2x3)

pyruvic

SIR of 8 Channels ES-
87.1
1.77e5



0.00 5.00 10.00 15.00 20.00 25.00 Time

25 uA
10-9 kV

all 5 uM,
except
oxalic,
lactic,
pyruvic
are 25 uM

61206-01

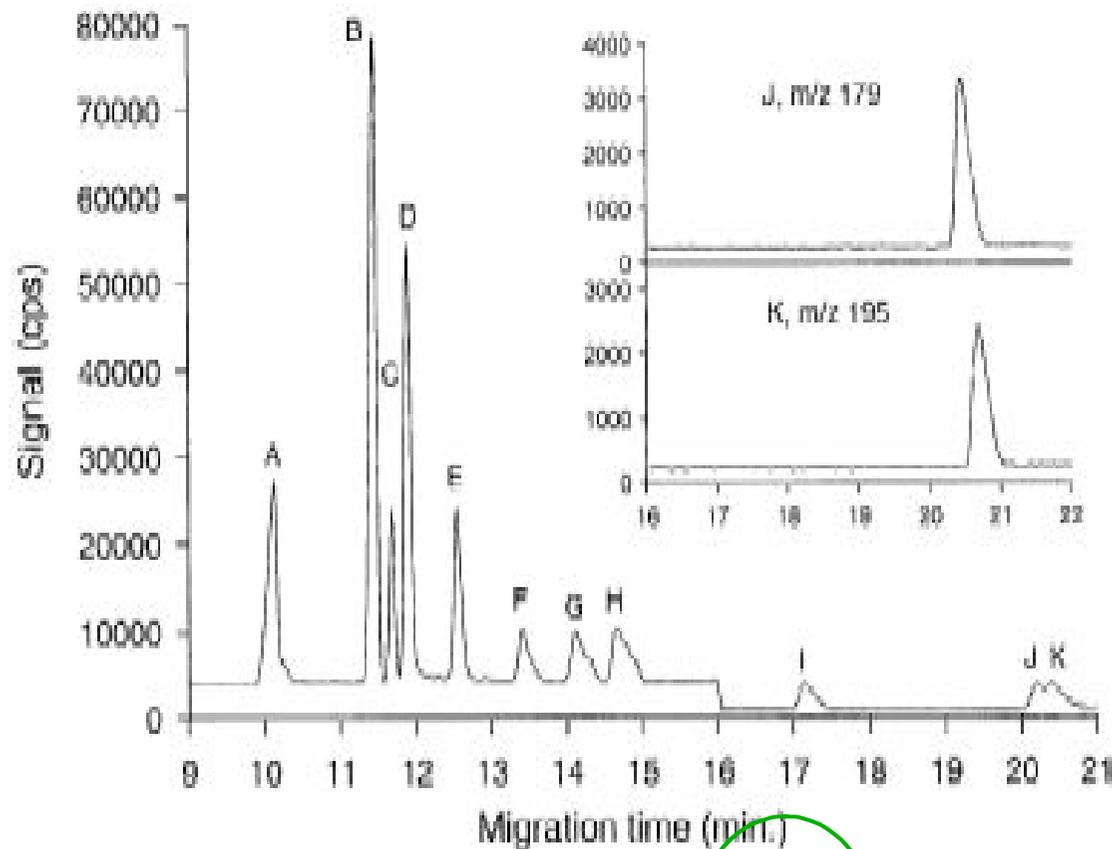


Fig. 1. Separation of LMW organic acids (20 mg/l) by CZE-ESI-MS with optimized parameters, see Table 1. Conditions: capillary: 103 cm \times 50 μ m I.D.; electrolyte composition: 2 mM TMA and 5 mM Tris, pH 8.5; voltage: -17 kV. Large diagram: total ion electropherogram, small diagrams: single ion electropherograms. (A) Oxalate, (B) malate, (C) malonate, (D) citrate, (E) tartrate, (F) succinate, (G) glutarate, (H) adipate, (I) lactate, (J) iso-saccharinate, (K) gluconate.

FOR COMPARISON:

Conventional single
CE – MS

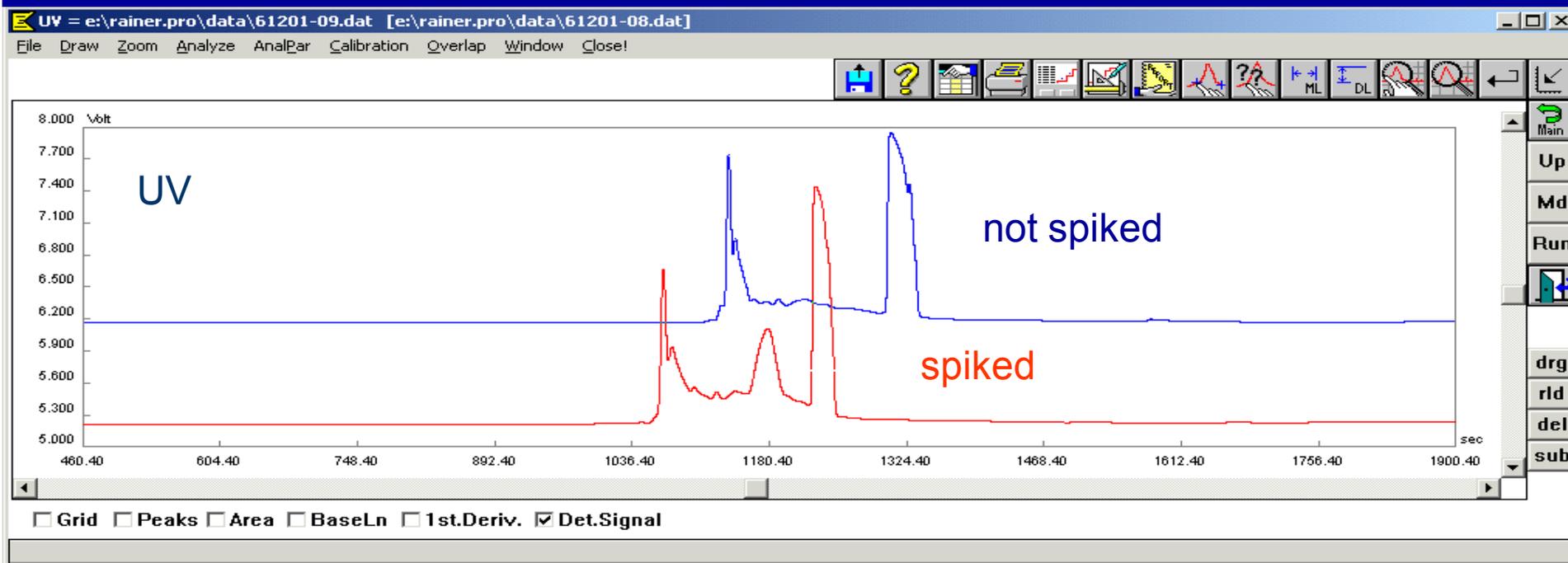
Malonate 20 mg/l ca
200 μ M

ITP-CZE-MS

Malonate **5 μ M**

Harberg, J., 2003, J.
Chromatogr., A 988 127-133

Analysis of flurbifen (5 uM) in urine

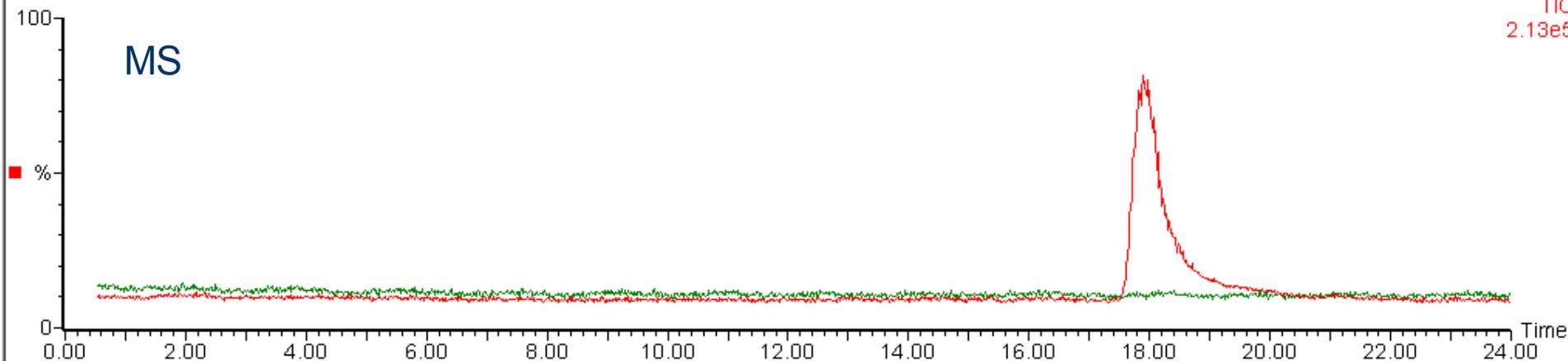


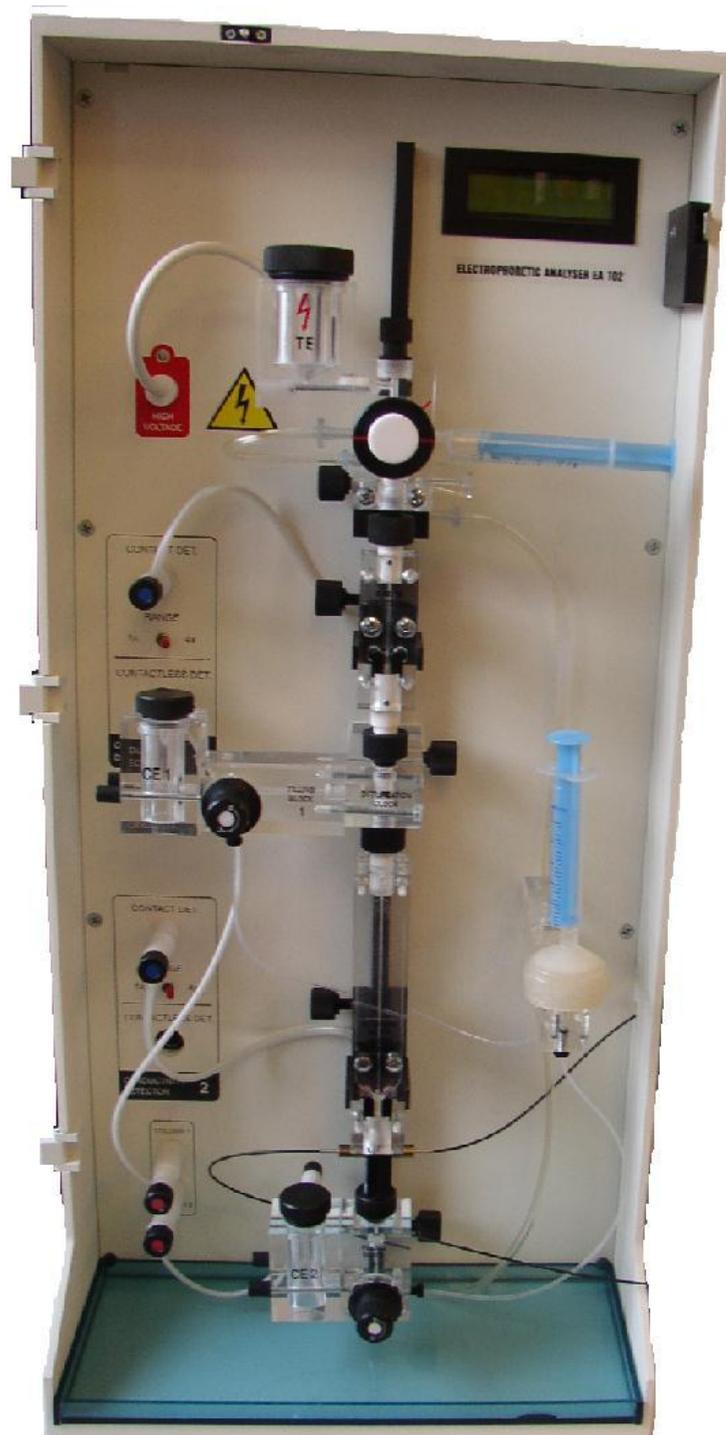
Urine 1:10, flurb. 5 uM, ITP-CZE, 100 um cap.

01-Dec-2006 20:15:33

61201-09

SIR of 1 Channel ES-
TIC
2.13e5





- Column- coupling
Electrophoretic
analyser -
manual version

Electrophoretic Analyser EA 202A (fully automatic version)



FIELDS OF APPLICATIONS OF ITP AND ITP-CZE

- **Water analysis** — Cl, SO₄, NO₃, NO₂, F, BrO₃, ClO₂
- **Food analysis** - additives, org. acids ...
- **Drink analysis** — org. acids, vit.C, sweeteners, bitrex....
- **Feed analysis** — lysine, amprolium, Ca, Na, Mg...
- **Blood, urine** — org. acids, lipoproteins
- **Industrial samples** — any ionogenic substances
- **Farmaceuticals** — purity, research, samples...

What is possible to analyse by CZE and ITP

- - inorganic anions and cations
- - organic acids
- - amines
- - aminoacids
- - peptides
- - proteins
- - generally ionic substances that moves in electric field

ADVANTAGES OF ITP and ITP-CZE

- Minimal or no sample pretreatment
- Concentration ratio up to $1:10^5$
- Detection limit : **ppb level** (cond. det.) **tenths ng/l** (UV det.)
- Simultaneous analysis of organic and inorganic ions
- Nonionogenic substances do not interfere
- Typical time of analysis: 10 – 25 min.

THANK YOU VERY MUCH FOR
YOUR ATTENTION

