CEofix™ buffers voor robuste ionen analyse met Capillaire Electrophorese

Analis R&D s.a.

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Studiedag Ionenanalyse KVCV 24 november 2004

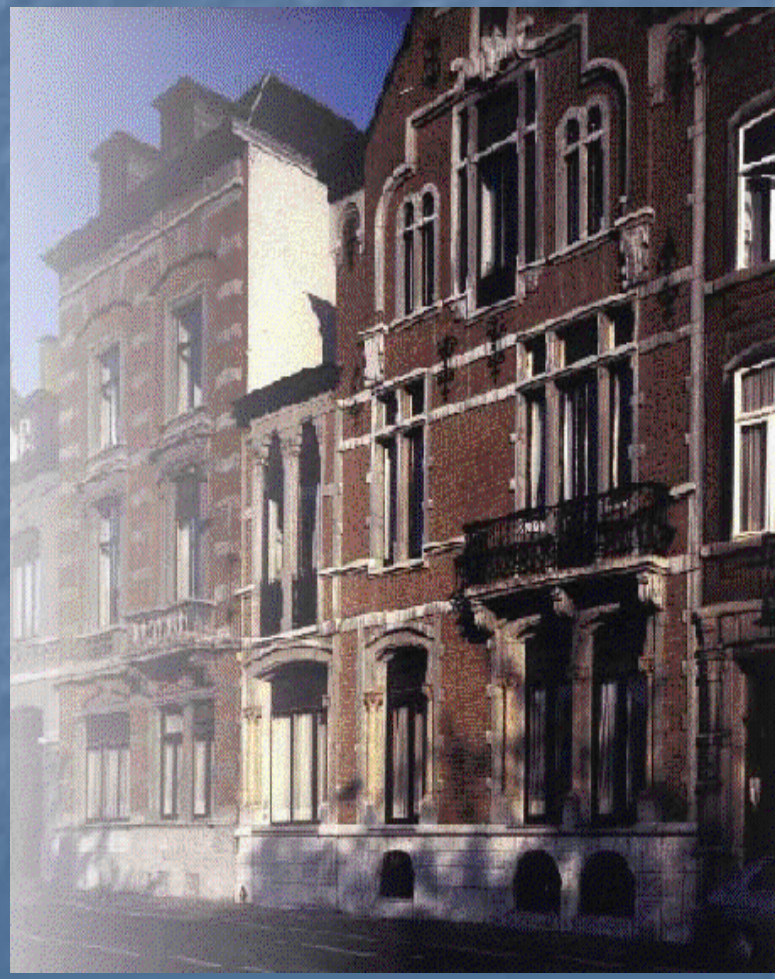
Outline

- Analis R&D and products
- CEofix™ buffers for CE
- CEofix™ Anions / Cations
- CEofix™ Applications

Analis R&D laboratory.

For more than 20 years, the R & D (established in Namur) specialized in electrophoresis has developed in vitro diagnosis kits which have been distributed throughout the world.

As a result of our long experience in the development of electrophoresis kits using agarose gels, Analis has now patented and marketed kits using new automated methods by means of capillary electrophoresis

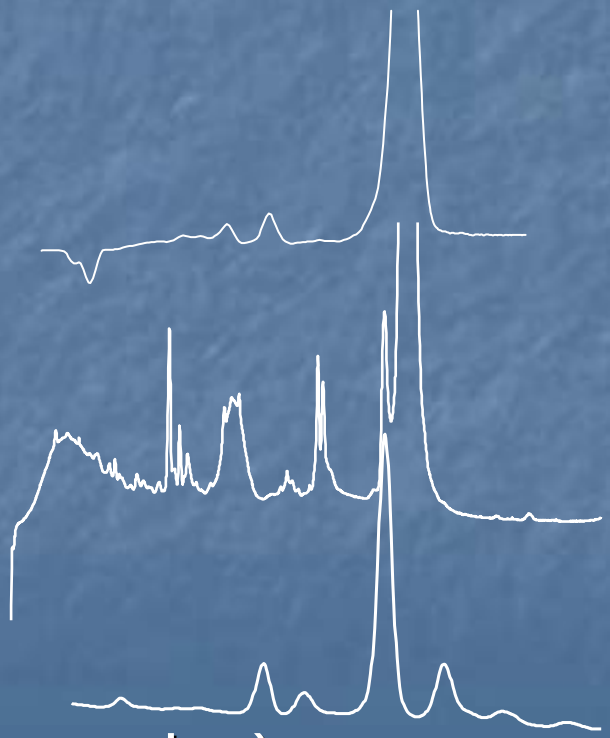


analis

Analis R&D

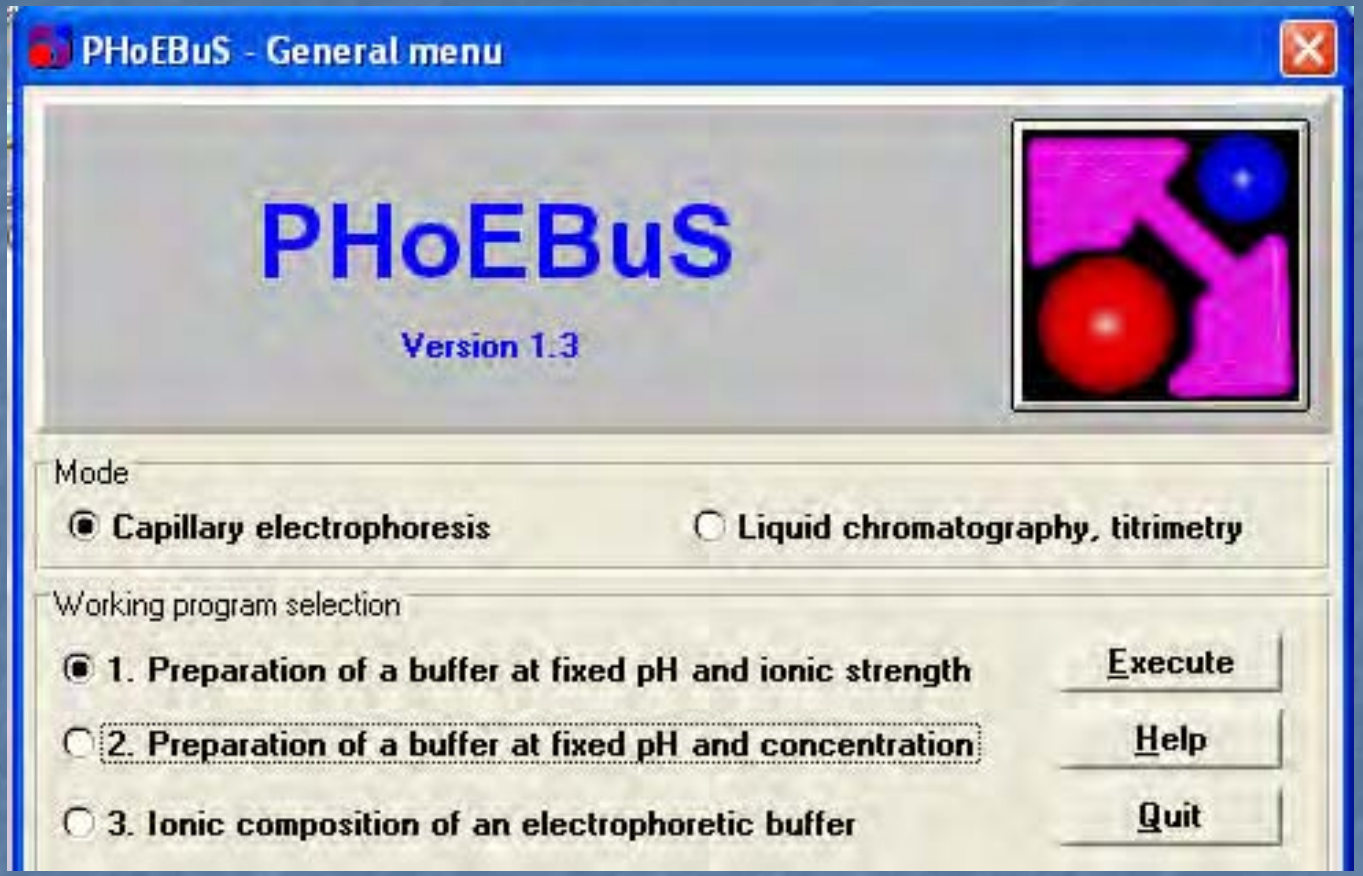
Bio-clinical Applications

- Electrophoresis : ISOPAL, ISOAMYL, PROTUR PLUS, MICROPROTUR, PROTUR B.J., DIATRAC
- Capillary Electrophoresis
  (P/ACE5000 and P/ACE MDQ)
  - HbA1c/HbA2
    hemoglobins and variants
  - SPE/HRE
    serum proteins
  - CDT
    transferrin isoforms (alcohol abuse marker)

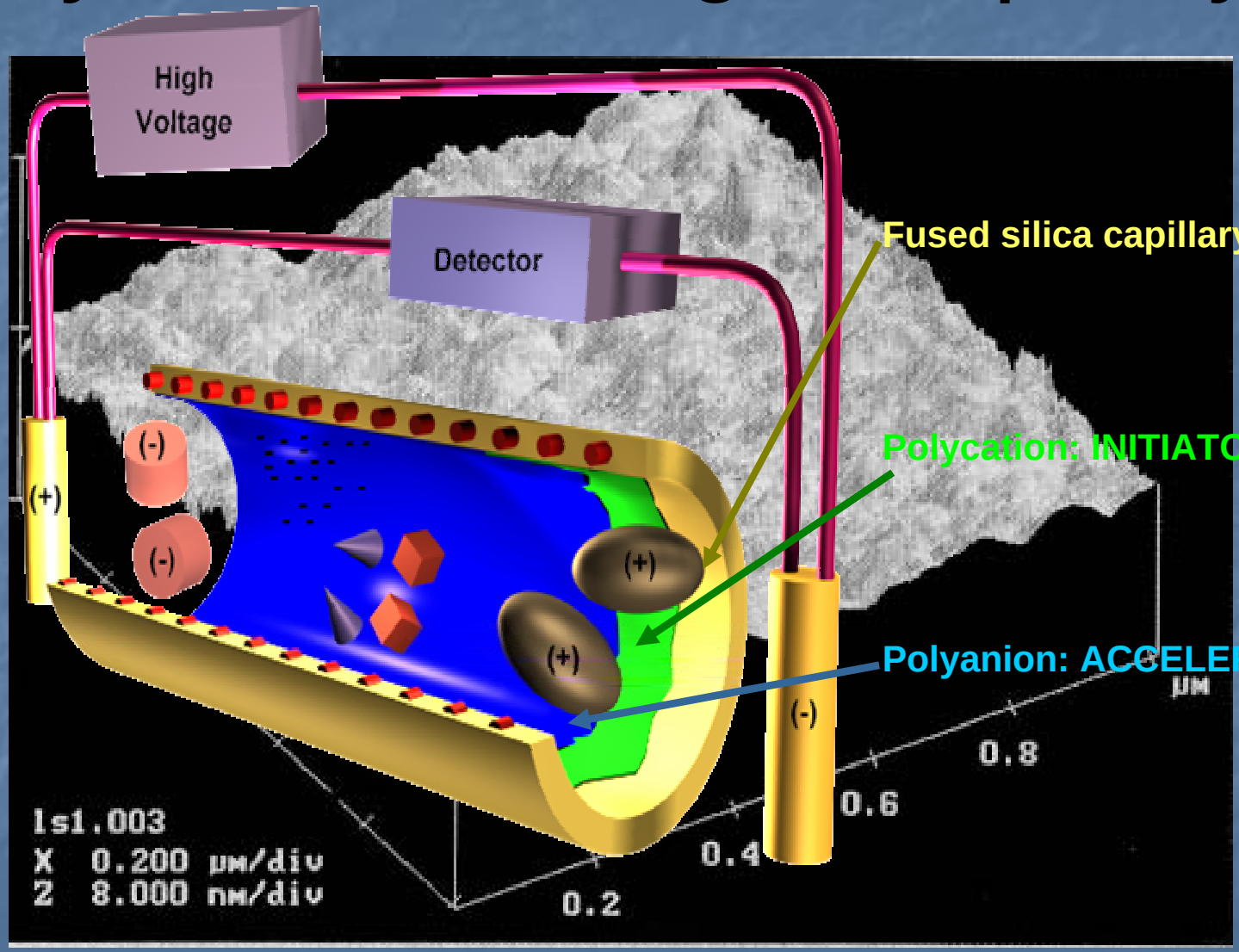


Analytical kits

- Capillary electrophoresis
  - CEofix™ pH
  - CEofix™ MS
  - CEofix™ MEKC
  - CEofix™ Anions
  - CEofix™ Cations
- Editor of PHoEBuS software
  (program help for electrophoresis buffer studies)


PHoEBuS - General menu
PHoEBuS
Version 1.3
Mode
Capillary electrophoresis
Liquid chromatography, titrimetry
Working program selection
1. Preparation of a buffer at fixed pH and ionic strength
2. Preparation of a buffer at fixed pH and concentration
3. Ionic composition of an electrophoretic buffer
Execute
Help
Quit

Dynamic Coating of capillary

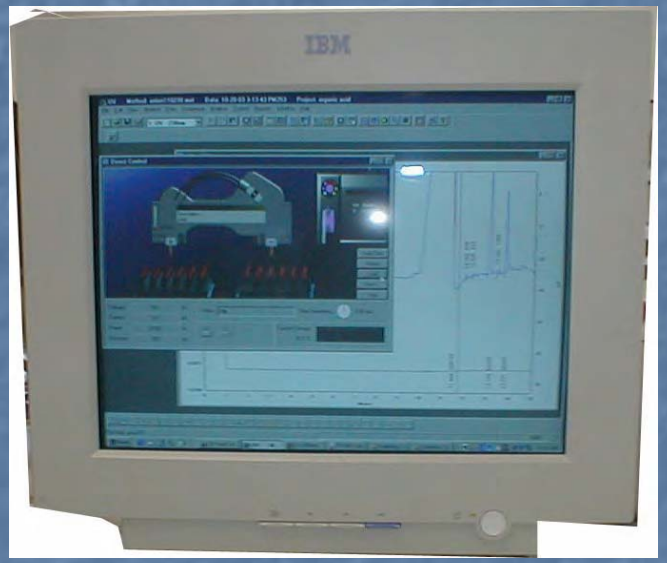


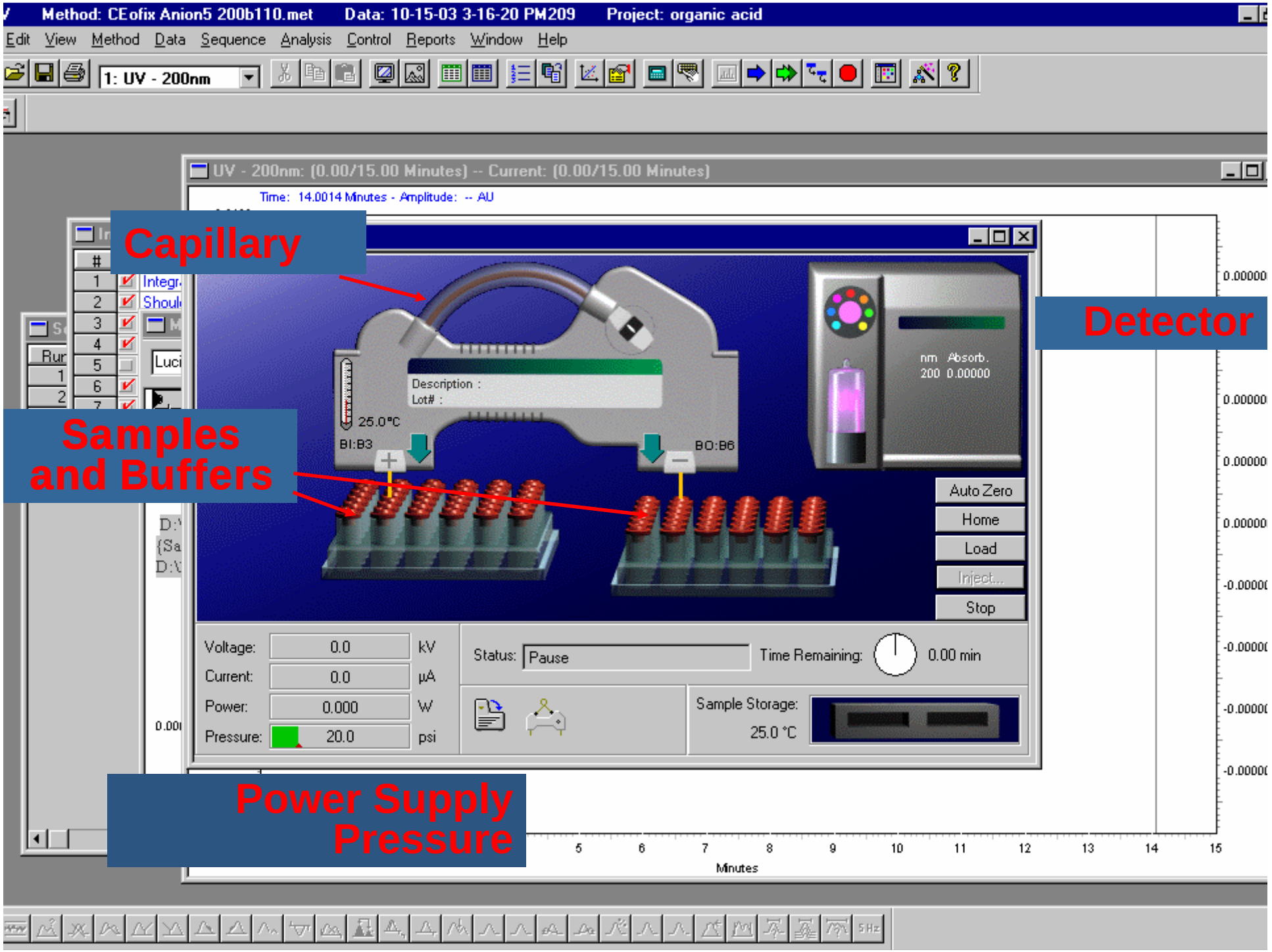
S. Kaupp, et al., Electrophoresis 20 (1999) 2566

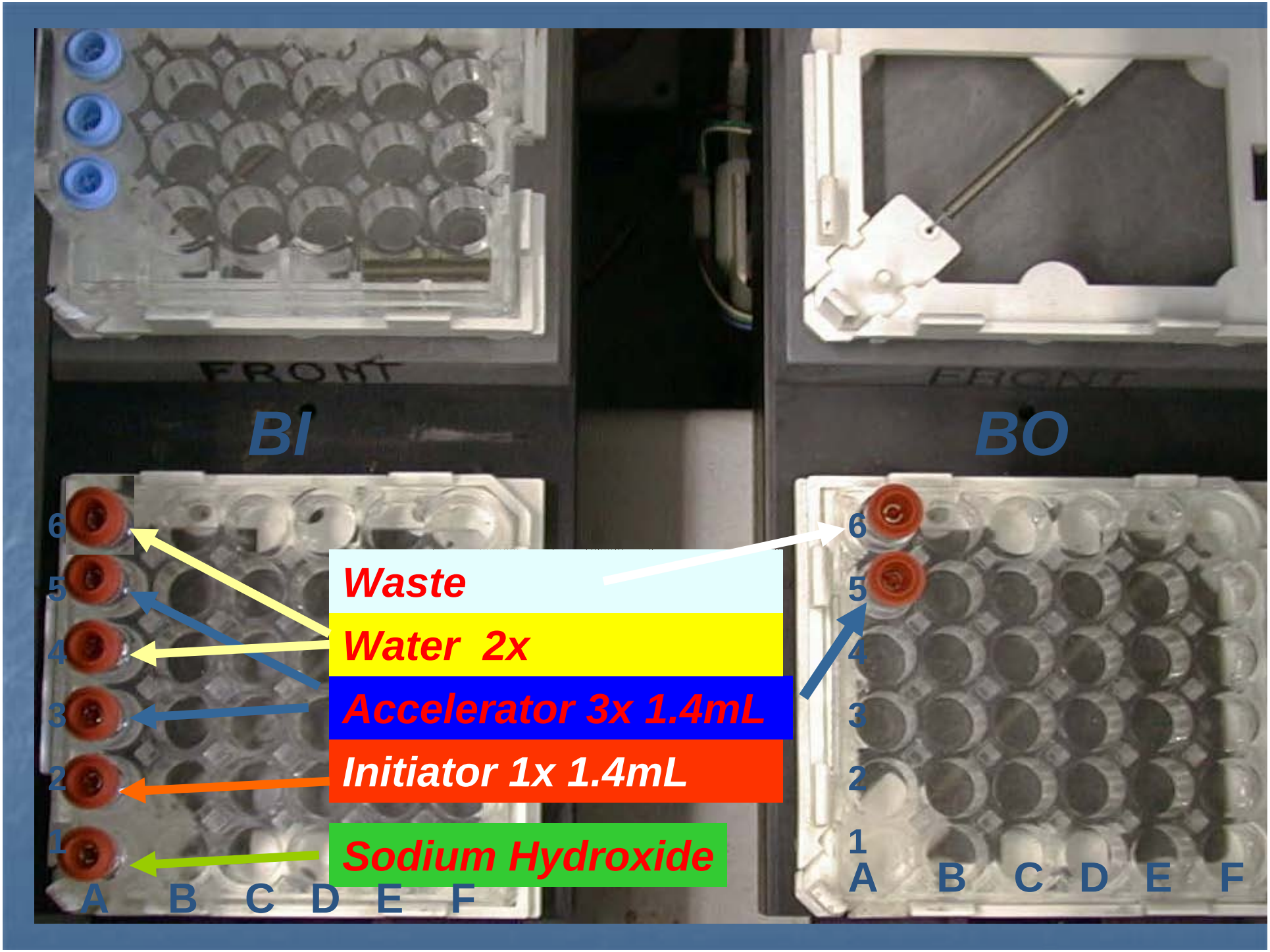
Features of CEofix™

- Control of EOF
- Reproducibility
  - No-memory

Beckman Coulter
P/ACE MDQ with 32K software



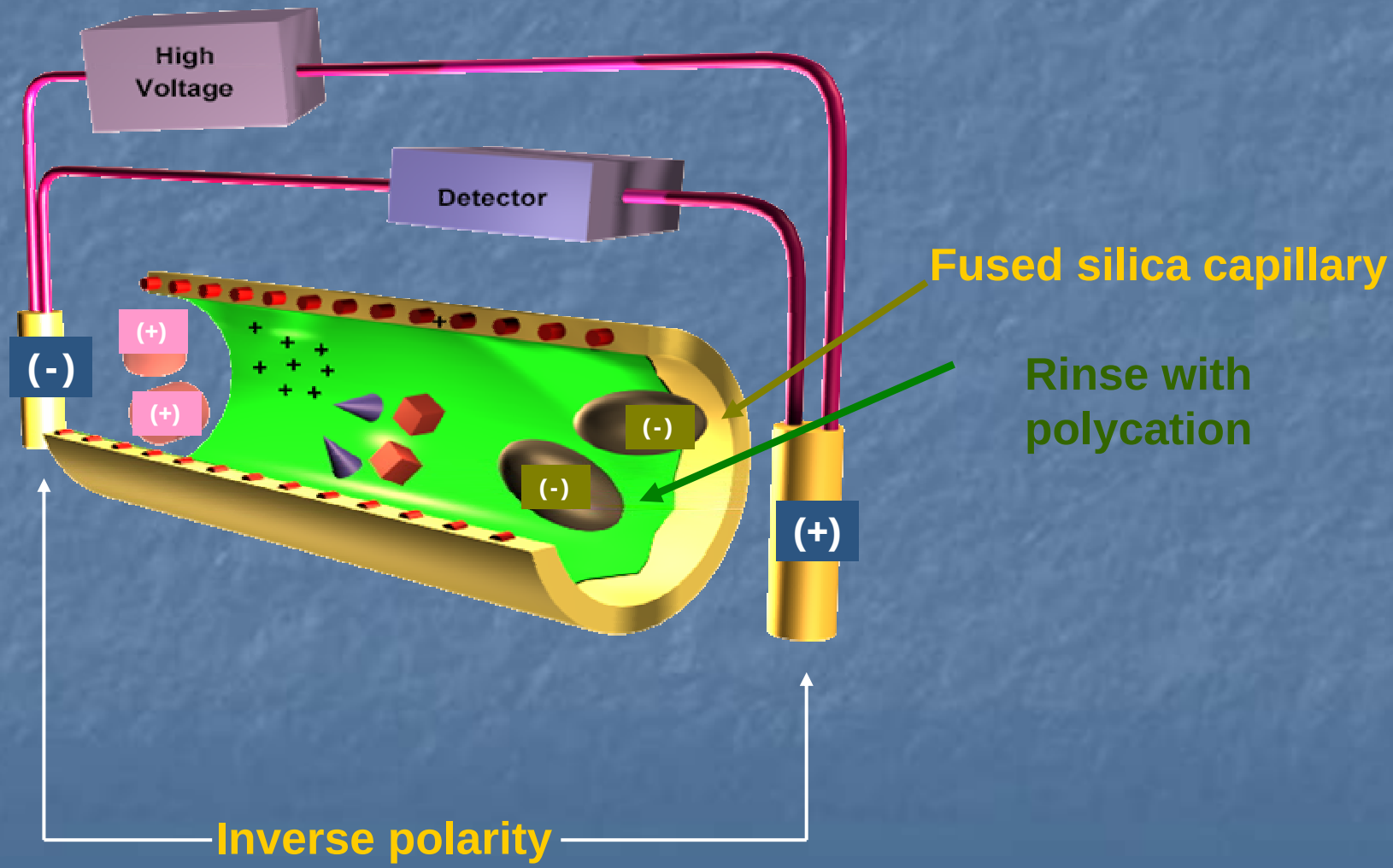

Method: CEofix Anion5 200b110.met
Data: 10-15-03 3-16-20 PM209
Project: organic acid
Edit View Method Data Sequence Analysis Control Reports Window Help
1: UV - 200nm
UV - 200nm: (0.00/15.00 Minutes) -- Current: (0.00/15.00 Minutes)
Time: 14.0014 Minutes - Amplitude: -- AU
Capillary
Samples and Buffers
Detector
Power Supply Pressure
Description :
Lot# :
25.0°C
BI:B3
BO:B6
nm Absorb.
200 0.00000
Auto Zero
Home
Load
Inject...
Stop
Voltage: 0.0 kV
Current: 0.0 µA
Power: 0.000 W
Pressure: 20.0 psi
Status: Pause
Time Remaining: 0.00 min
Sample Storage:
25.0 °C
Minutes


FRONT
BI
BO
6
5
4
3
2
1
A B C D E F
Waste
Water 2x
Accelerator 3x 1.4mL
Initiator 1x 1.4mL
Sodium Hydroxide

CEofix™ Anions and Organic Acids

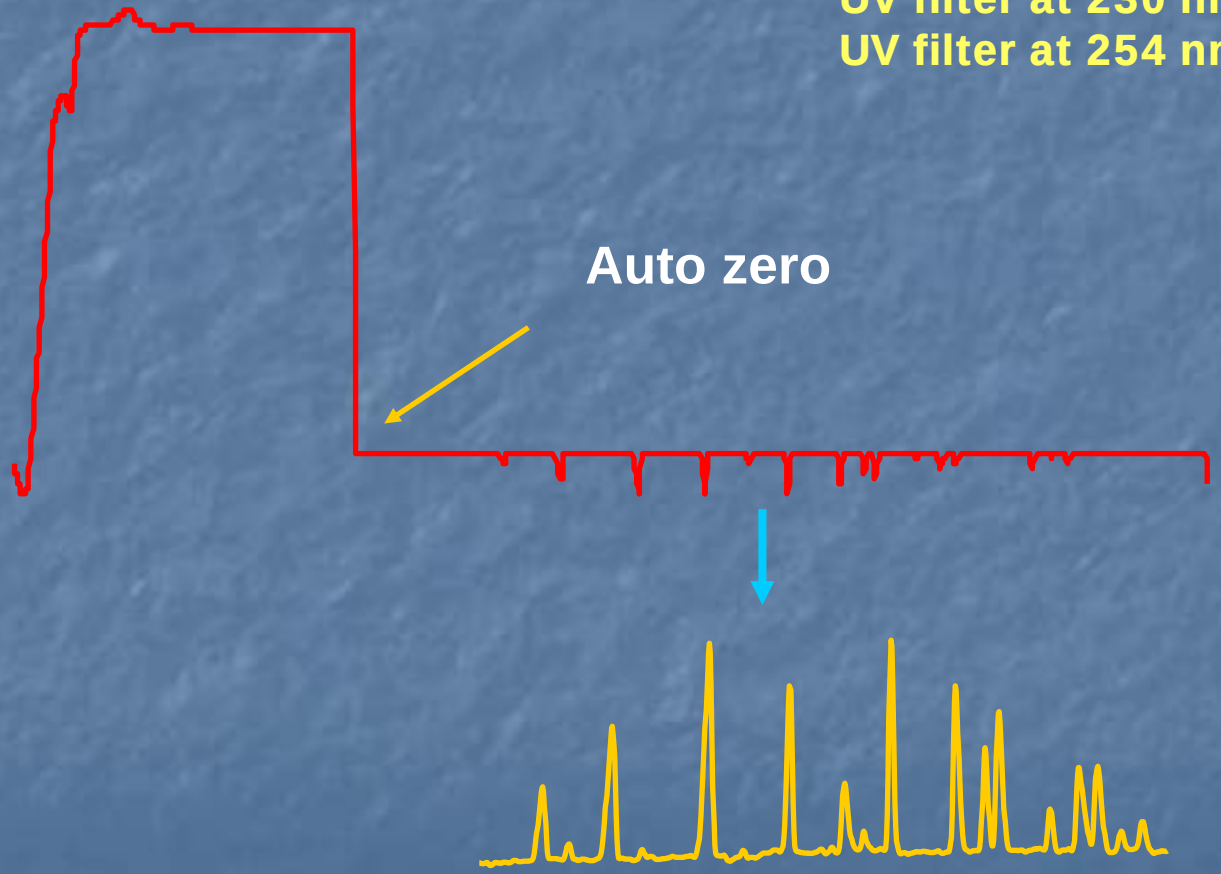
- Negative charge
  - CE: Reverse polarity
- Most are UV transparent
  - Buffer: indirect detection

CEofix™ Anions Organic Acids



Detection: indirect-UV

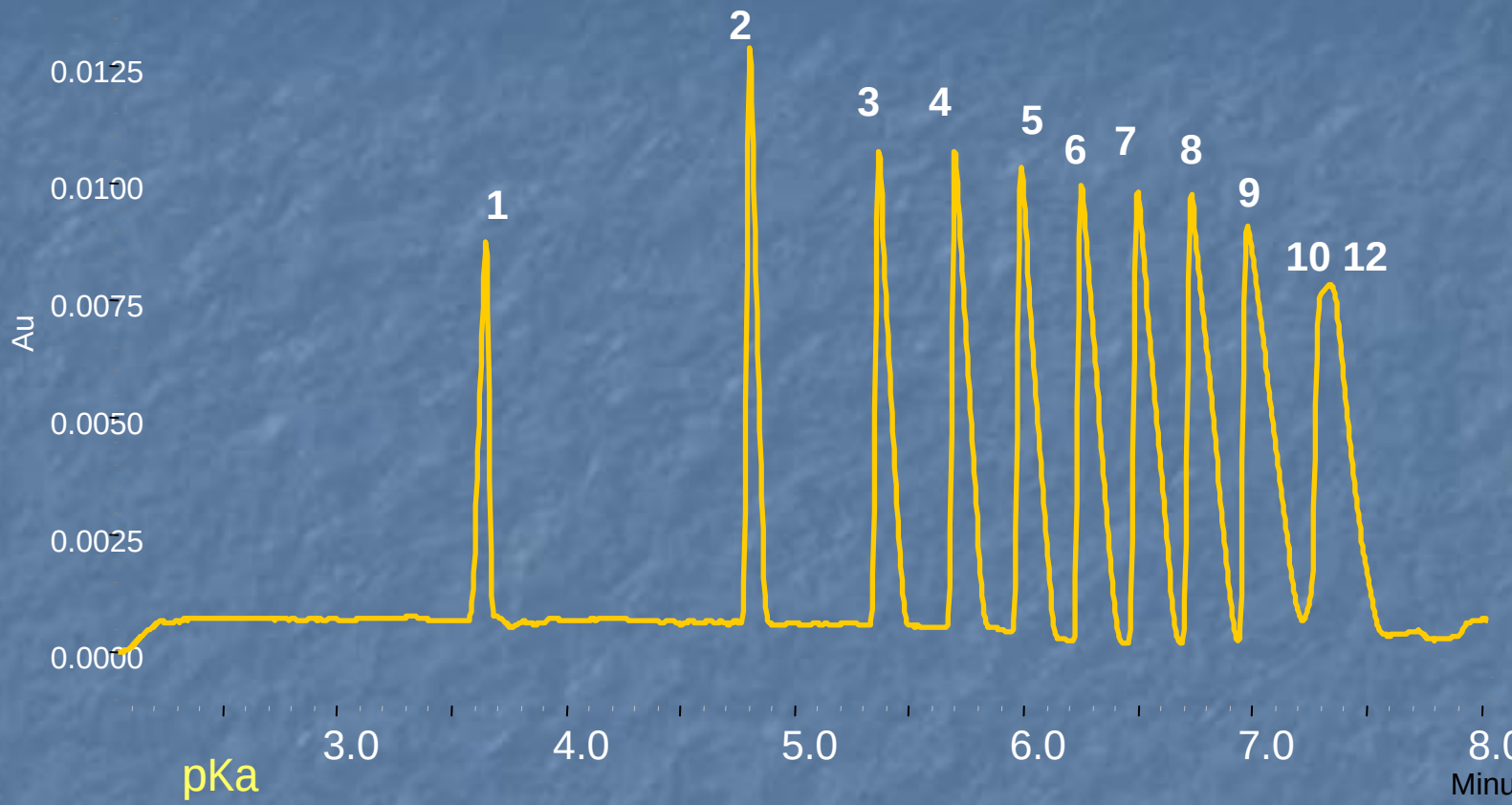
P/ACE MDQ- DAD: 233 nm
UV filter at 230 nm
UV filter at 254 nm



Composition

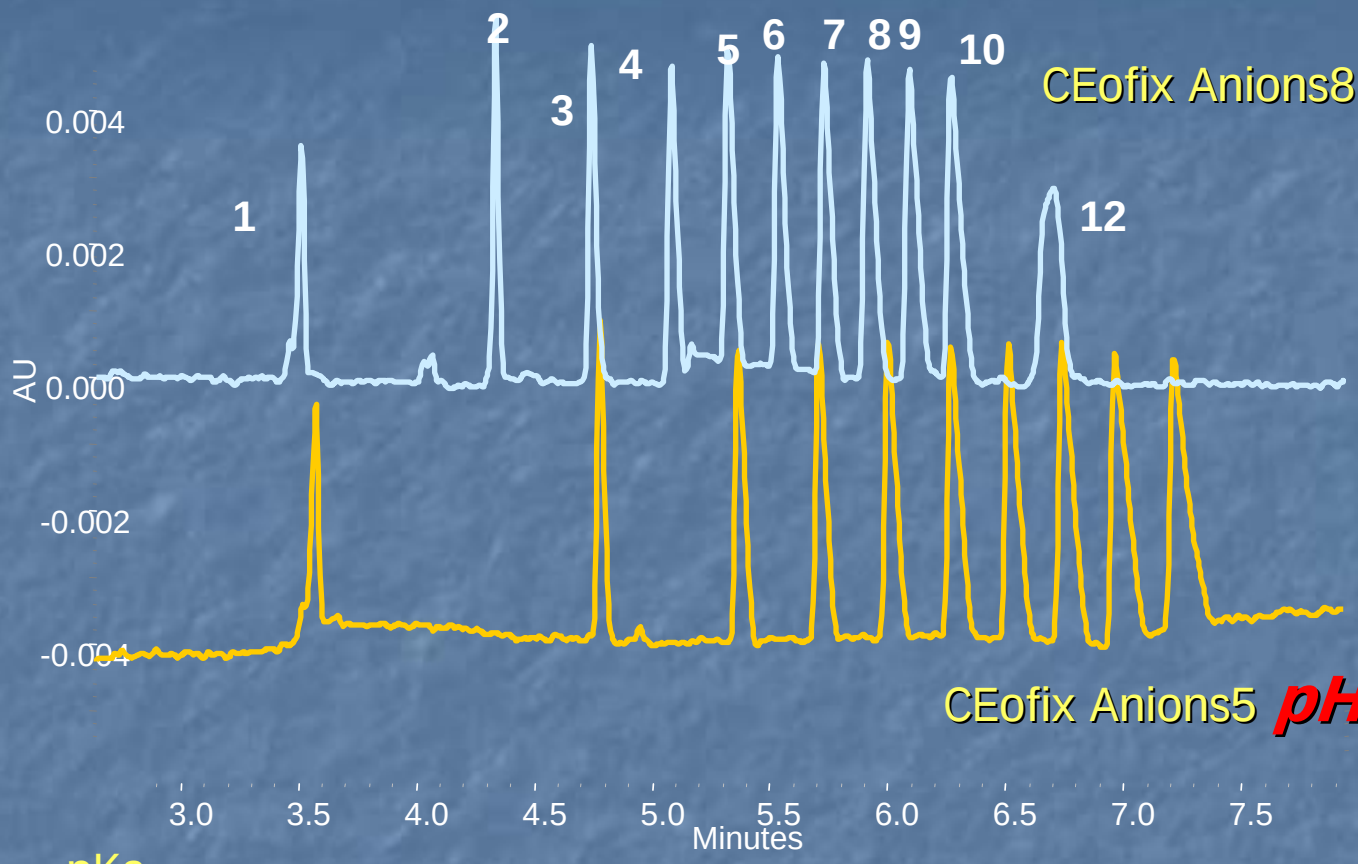
- **CEofix™ Anions5:**
  pyridine-dicarboxylic pH 5.6
  Reverse polarity, indirect detection (230 / 254 nm)
- **CEofix™ Anions8:**
  pyridine-dicarboxylic pH 8.2
  Reverse polarity, indirect detection (230 / 254 nm)
- **CEofix™ Anions2:**
  phosphate pH 2.6
  Reverse polarity and direct absorbance (200nm):
  nitrate, nitrite, oxalate

Organic acids (CEofix Anions5) *pH 5.6*



		pKa								
1	Formic	3.74	4	Butyric	4.87	7	Heptanoic		10	Capric
2	Acetic	4.76	5	Valeric	4.81	8	Caprylic		11	-
3	Propionic	4.87	6	Caproic	...	9	Pelargonic		12	Lauric

Organic acids



		pKa			pKa					
1	Formic	3.74	4	Butyric	4.87	7	Heptanoic		10	Capric
2	Acetic	4.76	5	Valeric	4.81	8	Caprylic		11	-
3	Propionic	4.87	6	Caproic	...	9	Pelargonic		12	Lauric

Test mix

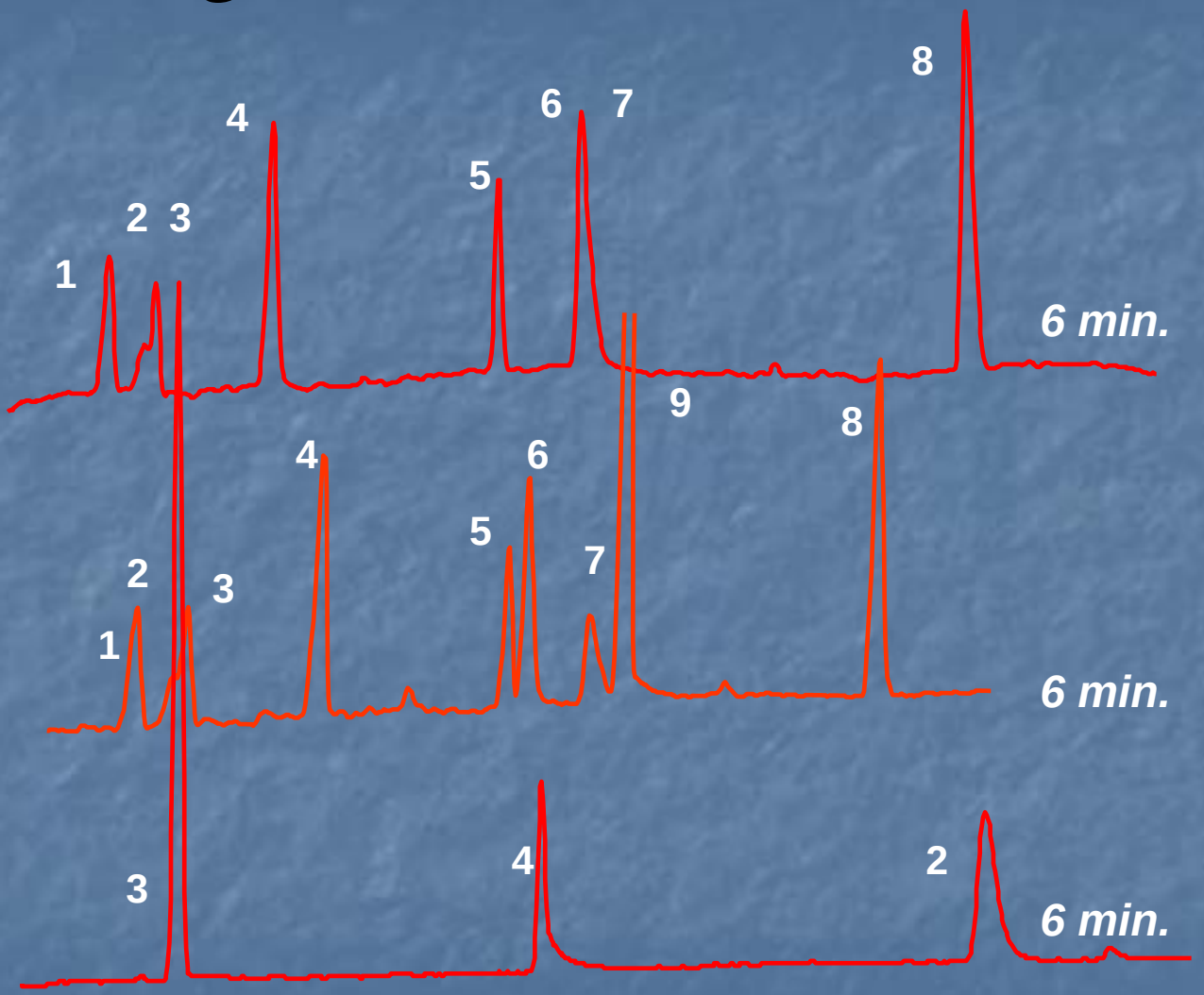
- Anions:
  - Chloride
  - Nitrite
  - Nitrate
- Organic Acid
  - Propionic pKa 4.87
  - Oxalic pKa: 1.25 – 3.67
  - Malic pKa: 3.46 – 5.10
  - Isocitric pKa: 3.29 – 4.71 – 6.40
  - Citric: pKa: 3.13 – 4.76 – 6.40

Anions and Organic Acids: testmix

CEofix™ Anions 5
pyridine-dicarboxylic acid
(pH 5.6)

CEofix™ Anions 8
pyridine-dicarboxylic acid
(pH 8.2)

CEofix™ Anions 2
phosphoric acid
(pH 2.6)
direct UV



1. Chloride, 2. Nitrite, 3.Nitrate, 4. Oxalic, 5. Malic, 6. Iso-citric, 7. Citric, 8. Propionic, (9 Carbonate)

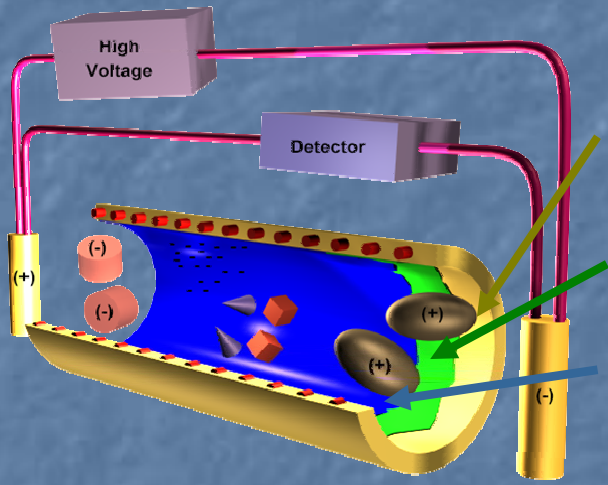
CEofix™ Cations

- Positive charge
  - CE: Normal polarity
- Some are UV transparent
  - Buffer: indirect detection

- CEofix™ Cation HR

  4-Aminopyridine pH 4.2

CEofix™ Cations

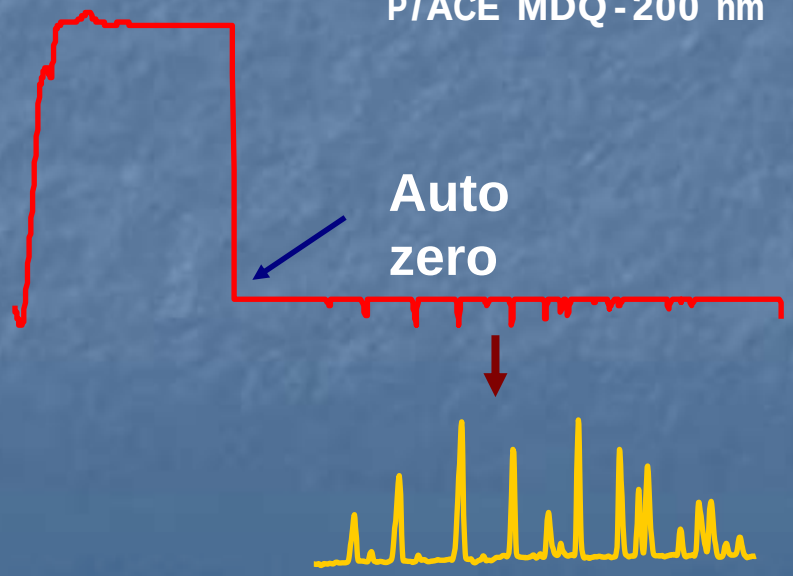


Fused silica capillary

Polycation: INITIATOR

Polyanion: ACCELERATOR

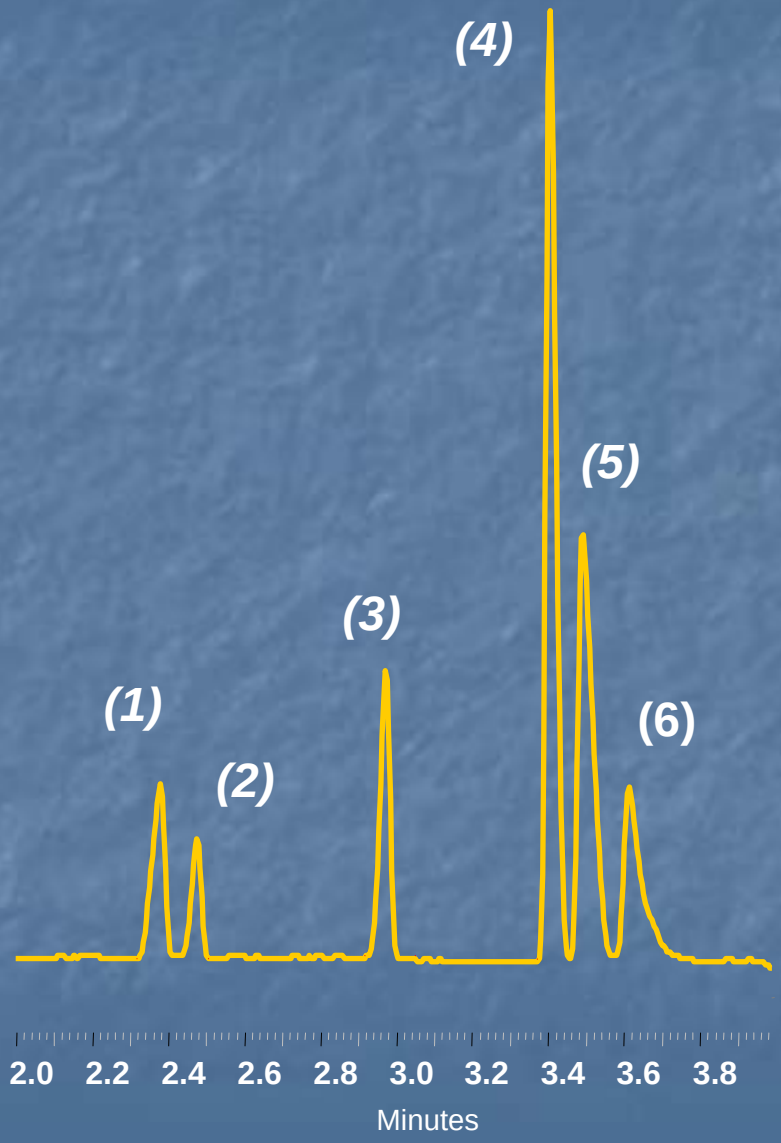
P/ACE MDQ-200 nm



CEofix™ Cations

- Cations: 10 mg/L

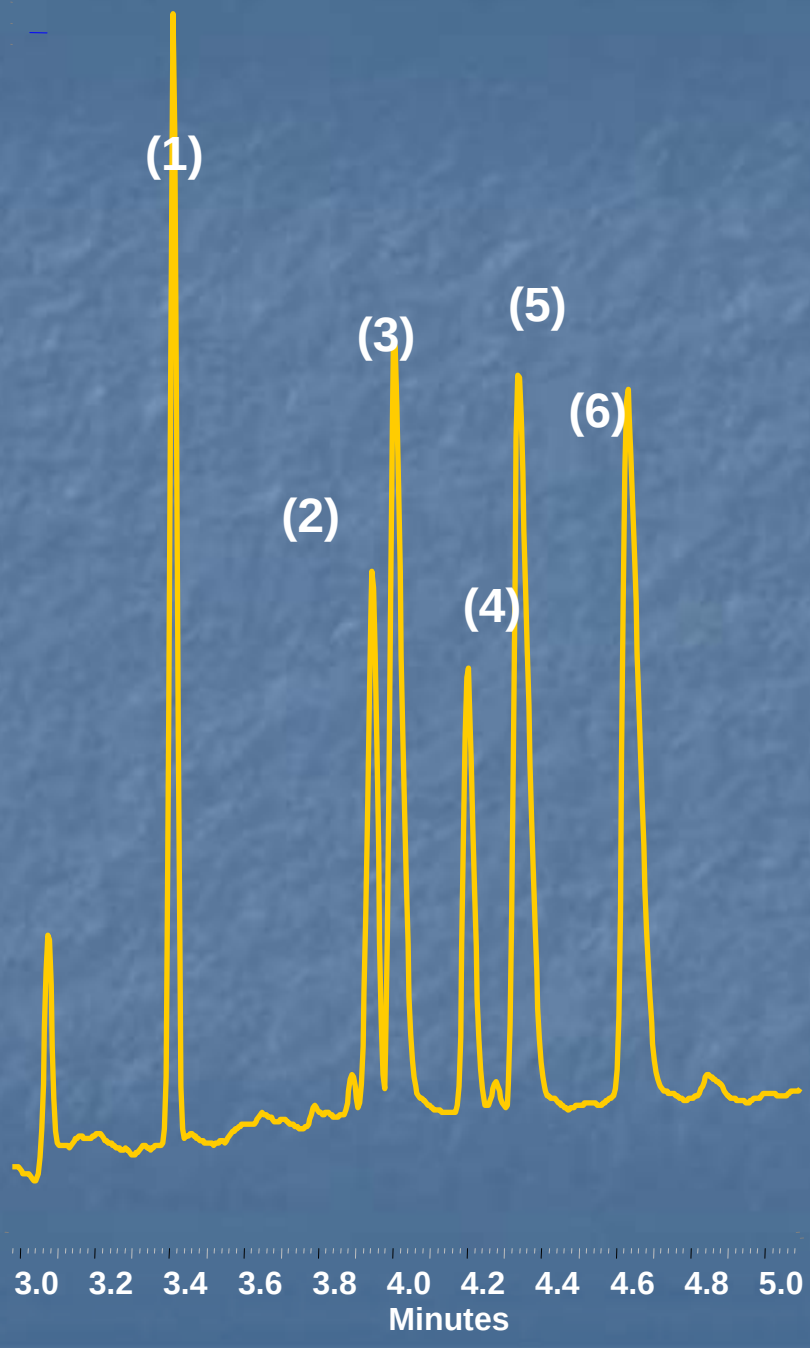
  (1) NH_4^+, (2) K^+, (3) Na^+,
  (4) Li^+, (5) Mg^{++}, (6) Ca^{++}
- Reproducibility on MT:
  % RSD: < 0.5%
- Linearity: 1 to 25 mg/L
  R^2 > 0.996



CEofix™ Cations

- Amines:

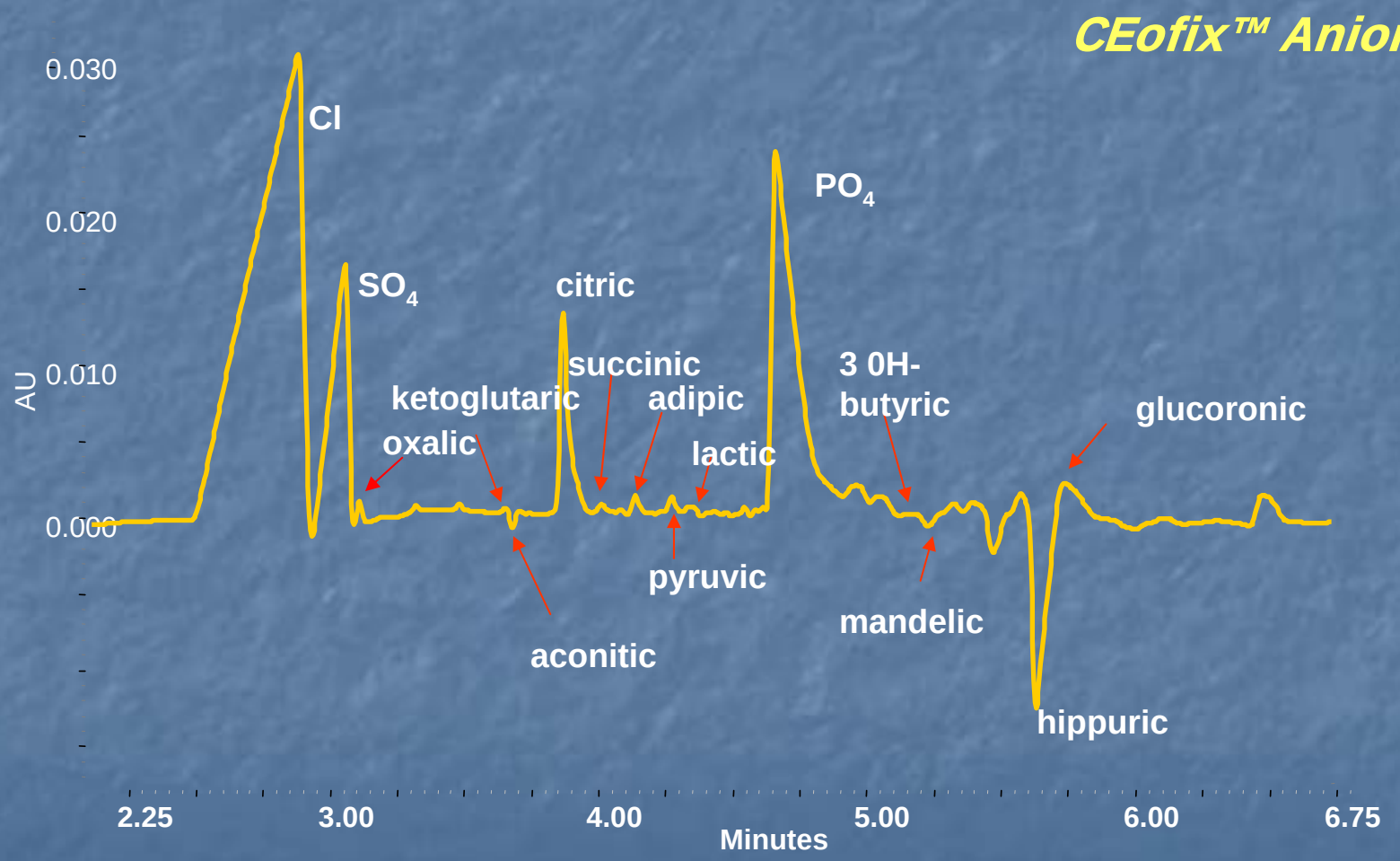
  (1) mono-ethanolamine, (2) di-ethanolamine, (3) N-benzyl-dimethylamine, (4) tri-ethanolamine, (5) 3-amino 1 phenylbutane, (6) dibenzylamine



Applications

- General conditions
  - Beckman Coulter P/ACE MDQ
  - Capillary 75µ x 60 cm (50cm to the detector)
  - Injection: 0.5psi during 5 sec.
  - Separation at 30 kV
  - Analysis time < 7 min
  - Detection UV filter or DAD

Application: urine



24 hrs - diluted ½ with H_20

Application: Pharmaceutical Counter ions

- Drug: weak acid or base function
- Are produced as a salt
  - Counter-ion quantification needed for P (purity)

P=100% - (m/M% impurities + m/M% water + m/M% remaining solvents + m/M% inorganic material + m/M% Counter-ion)

For example: Sufentanyl citraat
total MW 578.69 -> 386.56 + 192.13
factor acid/base = 578.69/386.56= 1.497

Table 1

ORGANIC ANIONS MT in min.	Anions 5	Anions 2
Acetate	4.71	
Benzoate		10.36
Citrate	4.21	
Camphorsulfonate	5.89	
Di-benzoyl-tartrate		5.07
Di-p-Toluoyl-tartrate		5.38
Fumarate		6.71
2-Hydroxybenzoate		6.43
Hydroxybutanedioate	3.93	
2-Hydroxypropanoate	4.85	
Maleate		3.99
Malonate	3.89	
Mandelate		8.40
Methanesulfonate	3.99	
4-methylbenzenesulfonate		4.27
Oxalate	3.28	3.80
Propanoate	5.23	
Succinate	4.28	
Tartrate	3.73	

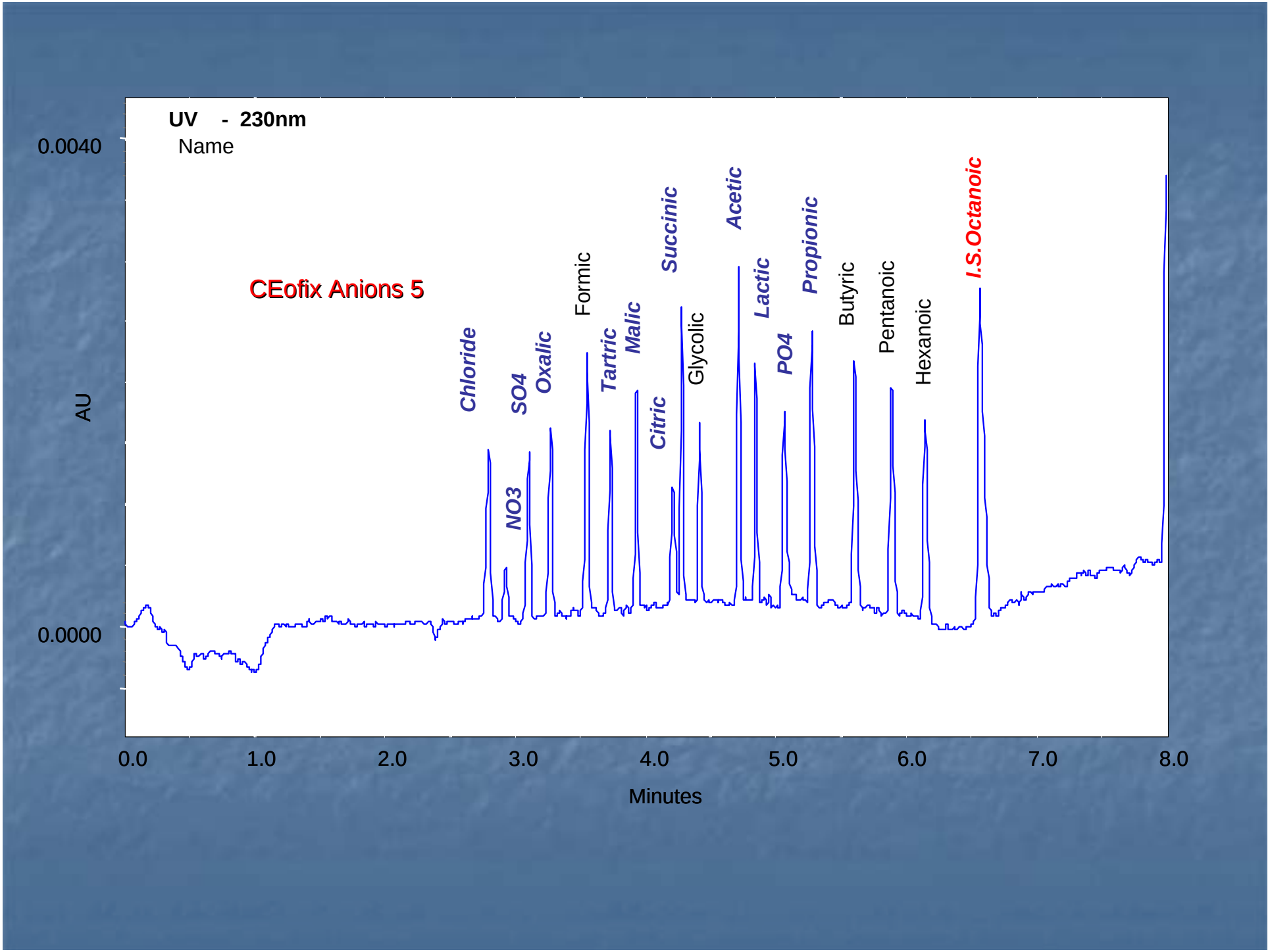
Table 2

INORGANIC ANIONS MT in min.	Anions 5	Anions 2
Nitrate HNO_3	2.93	2.55
Phosphate H_3PO_4	5.10	
Sulfate H_2SO_4	3.11	
Chloride HCl	2.80	
Bromide HBr	2.77	2.40

Table 1 and 2 : Recorded migration time in minutes for the different anions, analysed with CEofix™ Anions5 and/or CEofix™ Anions2.

Table 3

Cations: Cations HR
Ammonium (+1)
Calcium (+2)
Potassium (+1)
Magnesium (+2)
Sodium (+1)


UV - 230nm
Name
CEofix Anions 5
Chloride
NO3
SO4
Oxalic
Formic
Tartric
Malic
Citric
Succinic
Glycolic
Acetic
Lactic
PO4
Propionic
Butyric
Pentanoic
Hexanoic
I.S.Octanoic
0.0040
0.0000
AU
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
Minutes

Succinic acid (-1) MT 4.13 / Succinate (-2) MT 4.11

Repeatability on migration time (octanoic acid as I.S.)

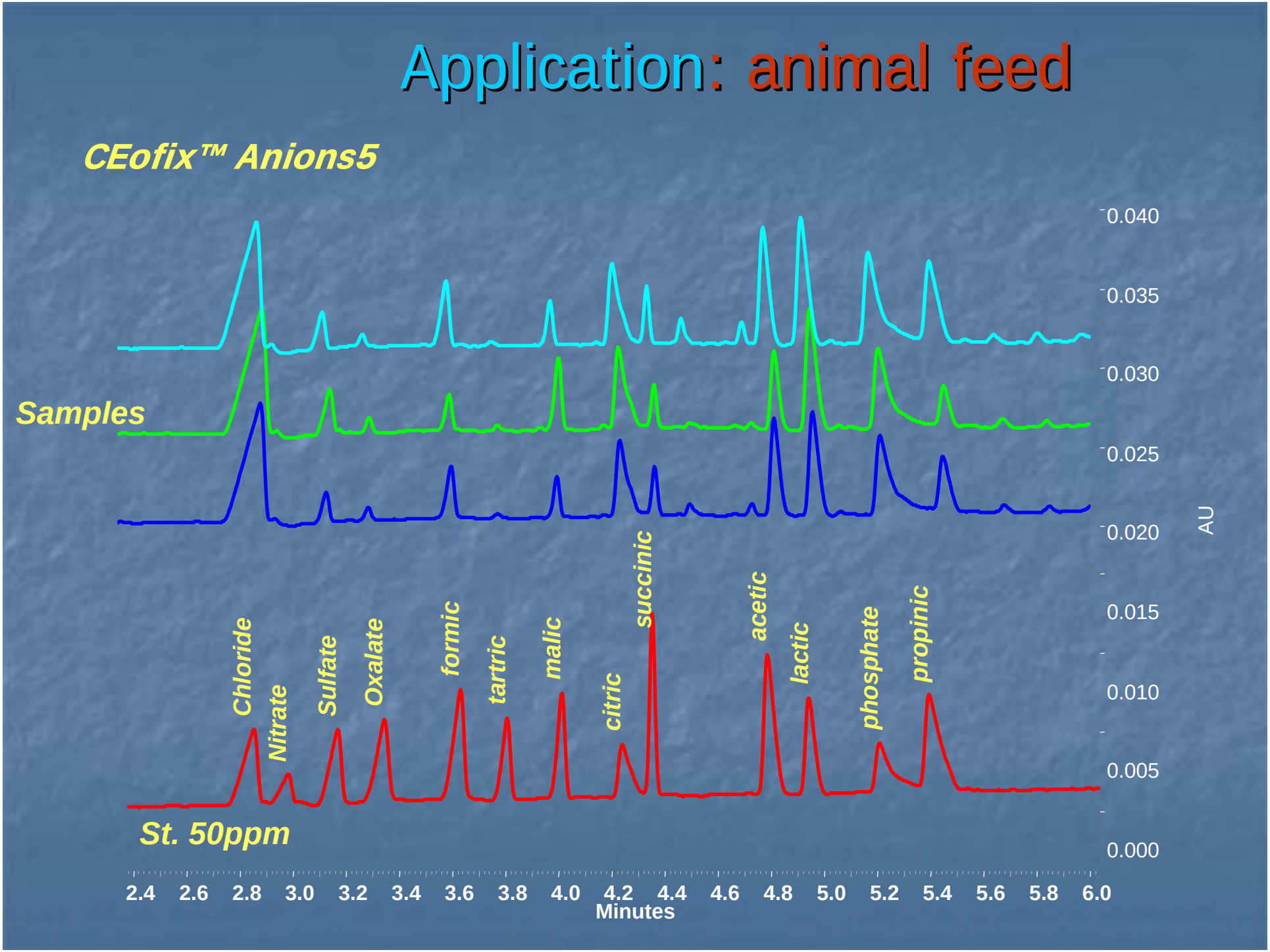
(N=10)	MT HCl	MT succinic acid	MT IS	Rel MT HCl	Rel MT succinic acid
	2.722	4.080	6.230	0.437	0.655
%SD	0.12%	0.07%	0.07%	0.09%	0.06%

Repeatability on area (octanoic acid as I.S.)

(N=10)	Area HCl	Area succinic acid	Area IS	Rel Area HCl	Rel Area succinic acid
	5523	3920	2218	2.490	1.768
%SD	2.91%	3.00%	3.22%	1.16%	1.06%

Silvie Verschakelen – Ivan Somers – Ivo Beyns – Rudy Sneyers – Achille Pluym
Johnson & Johnson Pharmaceutical Research & Development Drug Evaluation - Analytical Development

Application: animal feed

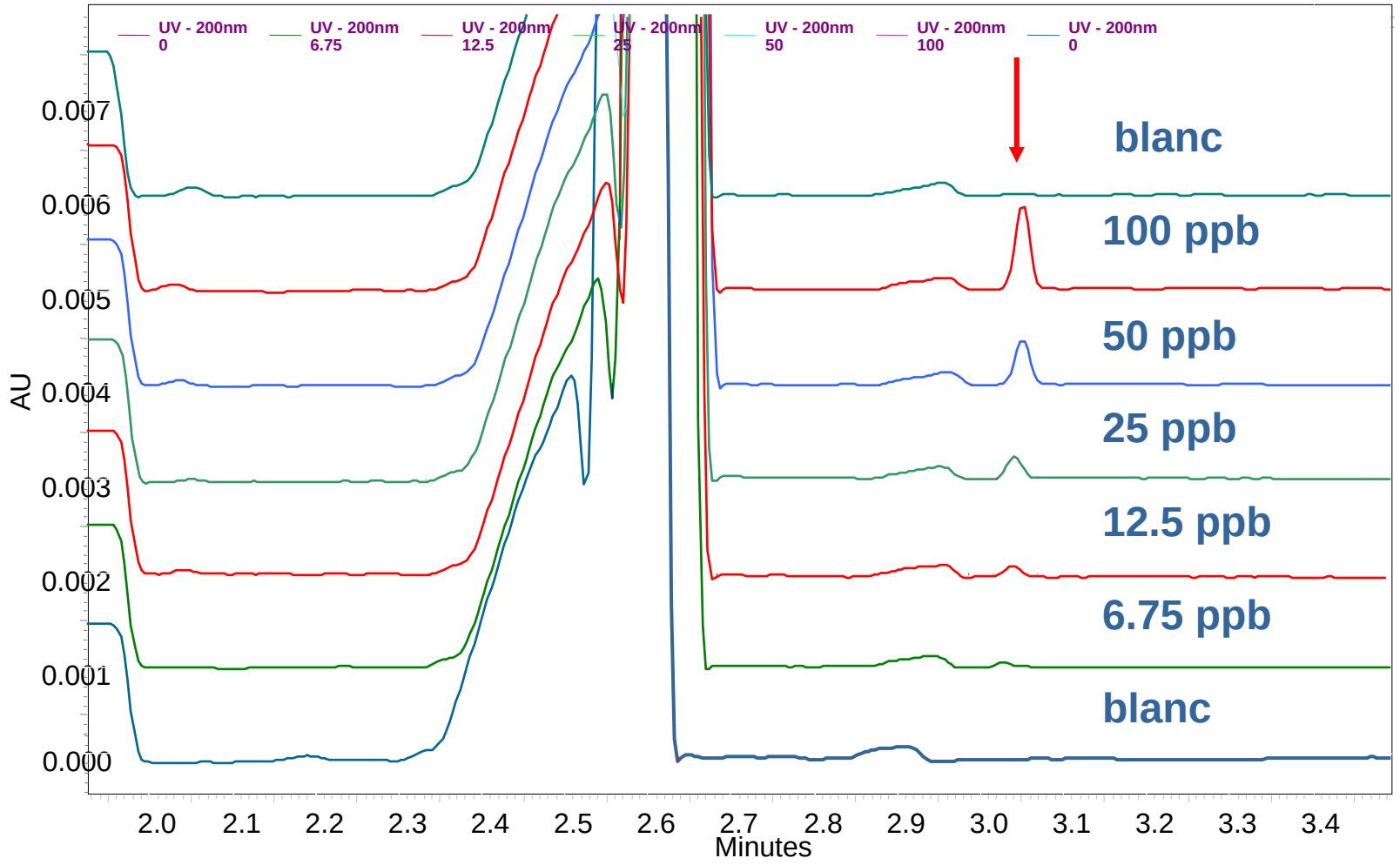


Application: Bromate in drinking water

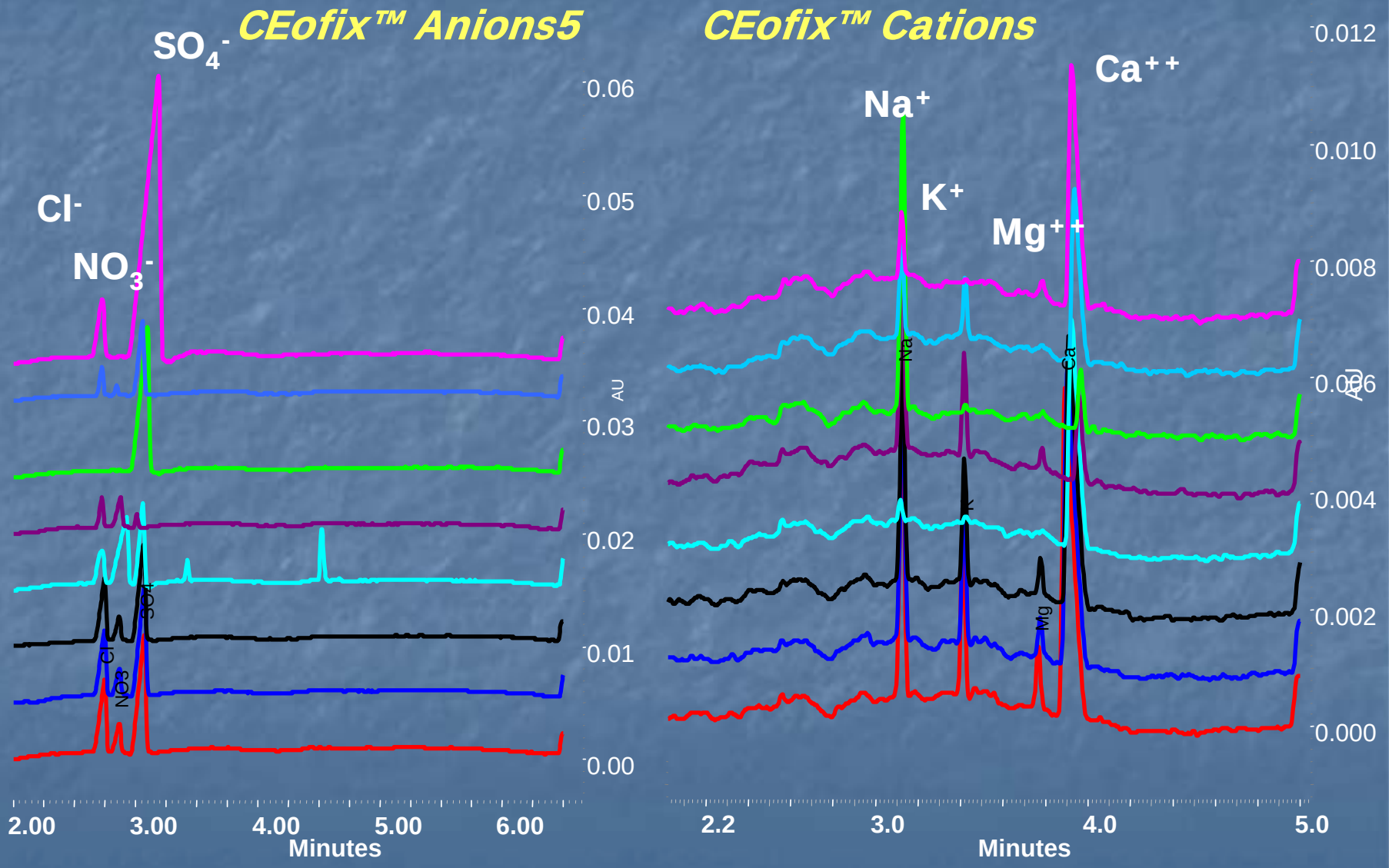
CEofix Anions2 @ 200nm

Electrokinetic injection:
20 sec. @ 3 kV

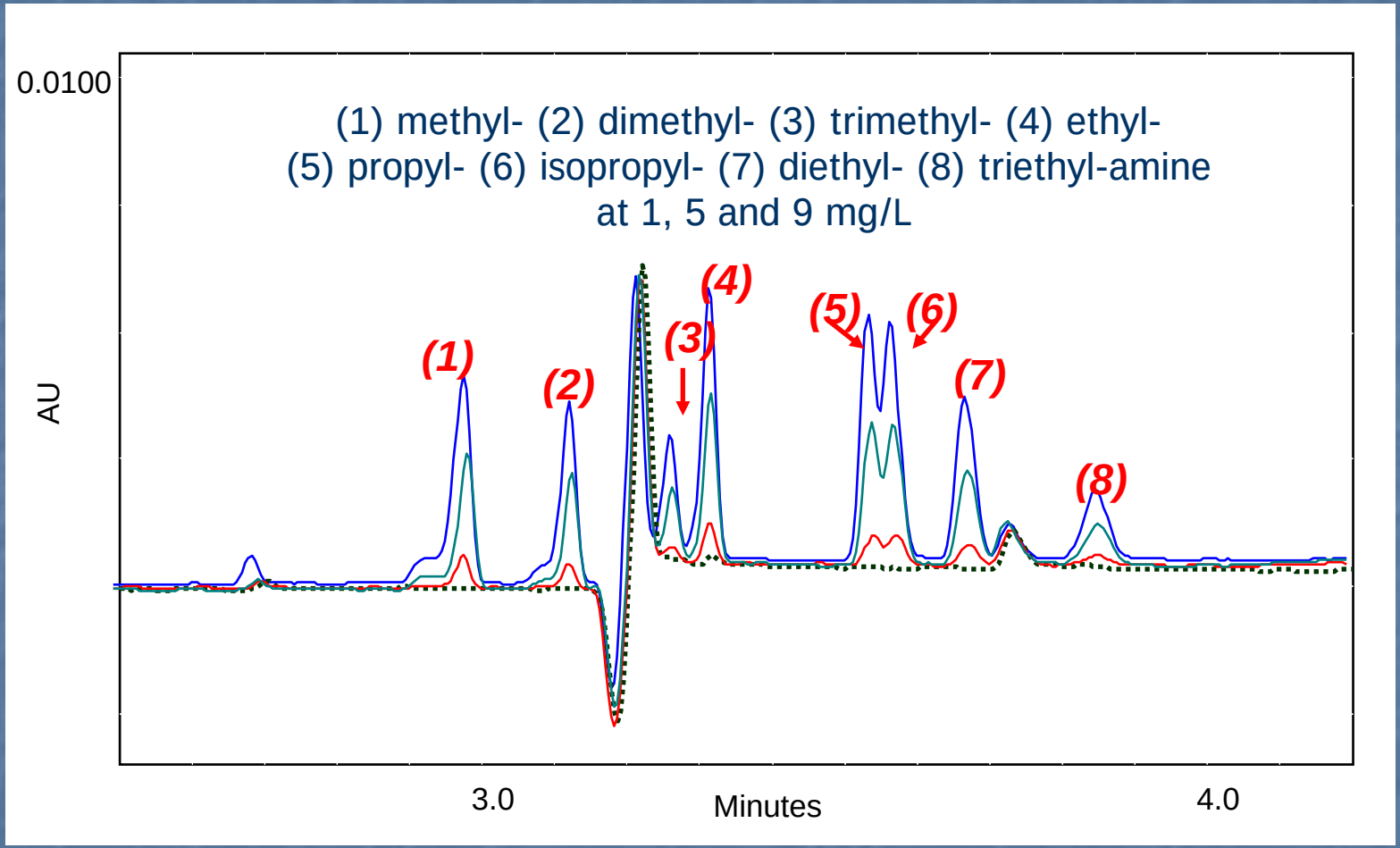
R2 = 0.998



Application: mortar



Applications: Amines in $MeSO_3H$ (3.8 g/L)



Buffer: diluted CEofix™ Cations HR – separation at 15°C

Points to consider when using CE

- No column but a capillary
- You work with ionised molecules
  - pH is very important
- Dilute your sample to be in the dynamic range
  - verify pH of dilution
- Be aware of matrix effects

Conclusion

- Fast method with CE
- Several applications on the same instrument
- CEofix™ buffers easy to use + support

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http://www.analis.be
http://ceofix.analis.be/