analis R&D

Counterion Analysis

Phosphate as an example for Drug stoichiometry and impurity determination using Capillary Electrophoresis

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Analis R&D

2nd Joint CE user Meeting

KNCV SAC
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Aldhem Hotel, Grobbendonk
More information and registration at:
analytische.kvcv.be/#ce
Sectie Analytische kvcv

Outlook

- Pharmaceutical Counterions
- Analysis of anions, organic acids and cations with indirect UV detection
- Analysis of PO_4 as counterion
- Analysis of PO_4 as impurity

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Use of CE for pharmaceutical analysis

1. Chiral analysis
2. Impurity (CE-MS)
3. **Counterions**
4. pKa determination
5. Cleaning control
6. Protein/peptides

HPCE Stockholm 2002

Pharmaceutical Counterions

- Most drugs are weak bases or acids
- Most drugs are produced as a salt
- Regulatory agencies (FDA, European Pharmacopeia…):

….pharmaceutical products should be tested for composition of their identity, strength, quality and purity of the active as well as for the inactive ingredients….

Pharmaceutical Counterions

Counterion quantification needed for P (purity)

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

Example: Sufentanyl citraat
total MW 578.69 ⇨ 386.56 + 192.13

Organic Anions		
Acetate C2H3O2		
Benzoate (-1) C7H5O2		
Benzoate C7H6O2		
Citrate (-1) C6H7O7		
Citrate (-2) C6H6O7		
Citrate (-3) C6H5O7		
Citrate C6H8O7		
(+/-)-Camphorsulfonate C10H16O4S	(1R)-(-)-10-Camphorsulfonate	(1S)-(+)-10-Camphorsulfonate
Dibenzoyl-L-tartrate (-1) C18H13O8	Dibenzoyl-D-tartrate (-1)	
Dibenzoyl-L-tartrate (-2) C18H12O8	Dibenzoyl-D-tartrate (-2)	
Dibenzoyl-L-tartrate C18H14O8	Dibenzoyl-DL-tartrate	
Dibenzoyl-L-tartrate C18H14O8	Dibenzoyl-D-tartrate	
Di-p-Toluoyl-D-tartrate C20H18O8	Di-p-Toluoyl-L-tartrate	
Fumarate (-1) C4H3O4		
Fumarate (-2) C4H2O4		
Fumarate C4H4O4 (E)--2-Butenedioic acid	Maleate Z-2-butenedioic acid	
2-Hydroxybenzoate (-1) C7H5O3		
Hydroxybutanedioate C4H6O5	(R)-Hydroxybutanedioate	(S)-Hydroxybutanedioate
(+/-)-2-Hydroxypropanoate (-1) C3H5O3		
(+/-)-2-Hydroxypropanoate C3H6O3	(S)-(+)-2-Hydroxypropanoate	

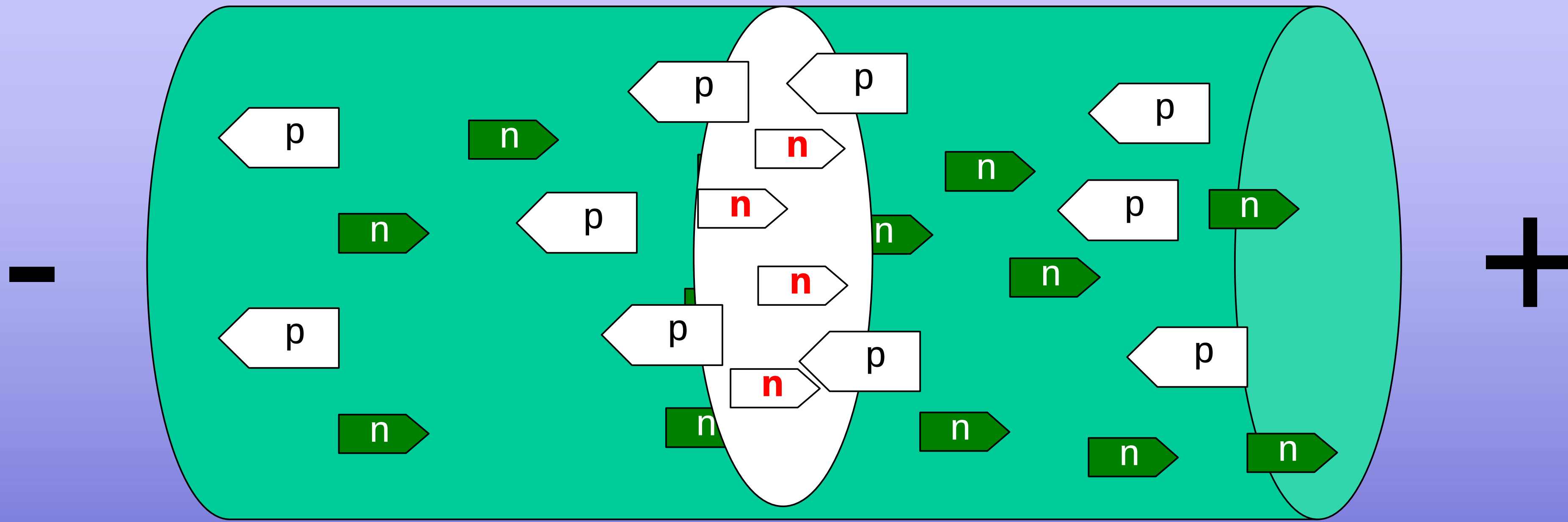
Maleate (-1) C4H3O4		
Maleate (-2) C4H2O4		
Malonate (-1) C3H3O4		
Malonate (-2) C3H2O4		
Malonate C3H4O4		
(R) Mandelate C8H8O3	(S)-mandelate	
Mandelate (-1) C8H7O3		
Methaansulfonate (-1) CH3O3S		
Methaansulfonate CH4O3S		
4-Methylbenzenesulfonate C7H8O3S		
Oxalate (-1) C2HO4		
Oxalate (-2) C2O4		
Oxalate C2H2O4		
Propanoate C3H6O2		
Succinate (-1) C4H5O4		
Succinate (-2) C4H4O4		
Succinate C4H6O4		
Tartrate C4H6O6	(-)-D-Tartrate	(+)-L-Tartrate
(-)-D-Tartate (-1) C4H5O6	(+)-L-Tartrate (-1)	
(-)-D-Tartrate (-2) C4H4O6	(+)-L-Tartrate (-2)	
(+/-)-DL-Tartrate C4H6O6		

Inorganic Anions
Nitrate HNO3
Phosphate H3PO4
Sulfate H2SO4
Sulfate (-1) HSO4
Sulfate (-2) SO4
HCl
HBr

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

Buffers for Indirect UV detection

- principle
- UV-absorbing probe
- Inverse the EOF
- pH – molarity of the buffer



Buffer:

n co-ion chromophore

= ***probe***

p counterion

Sample

n

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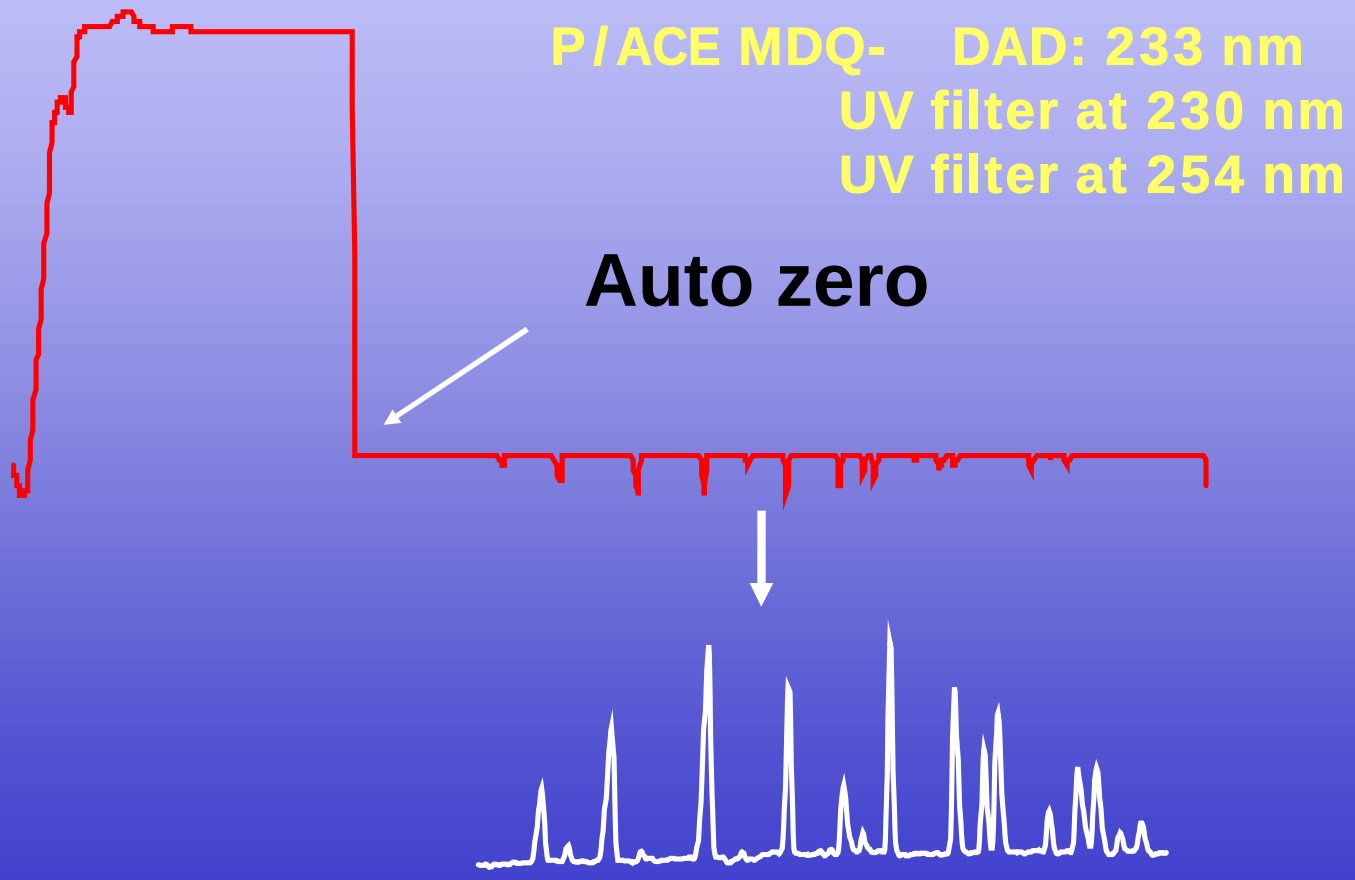
Detection: indirect-UV



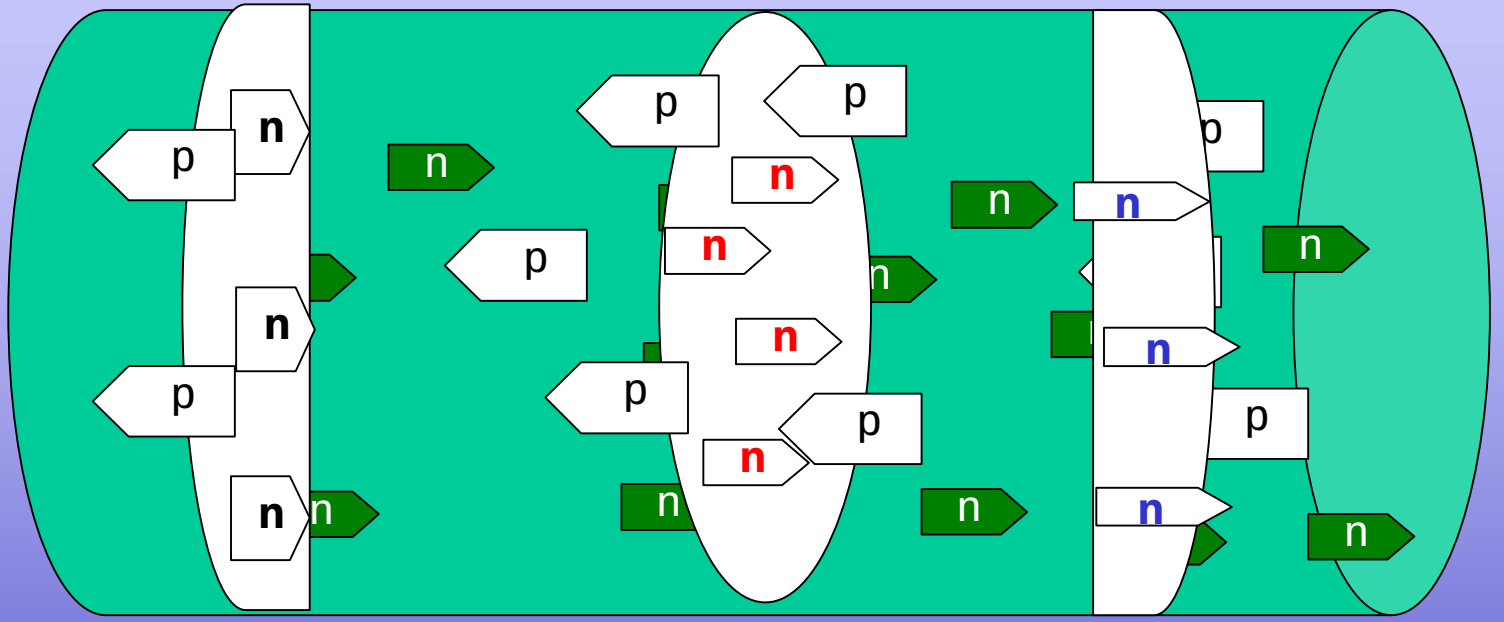
- Transfert Ratio

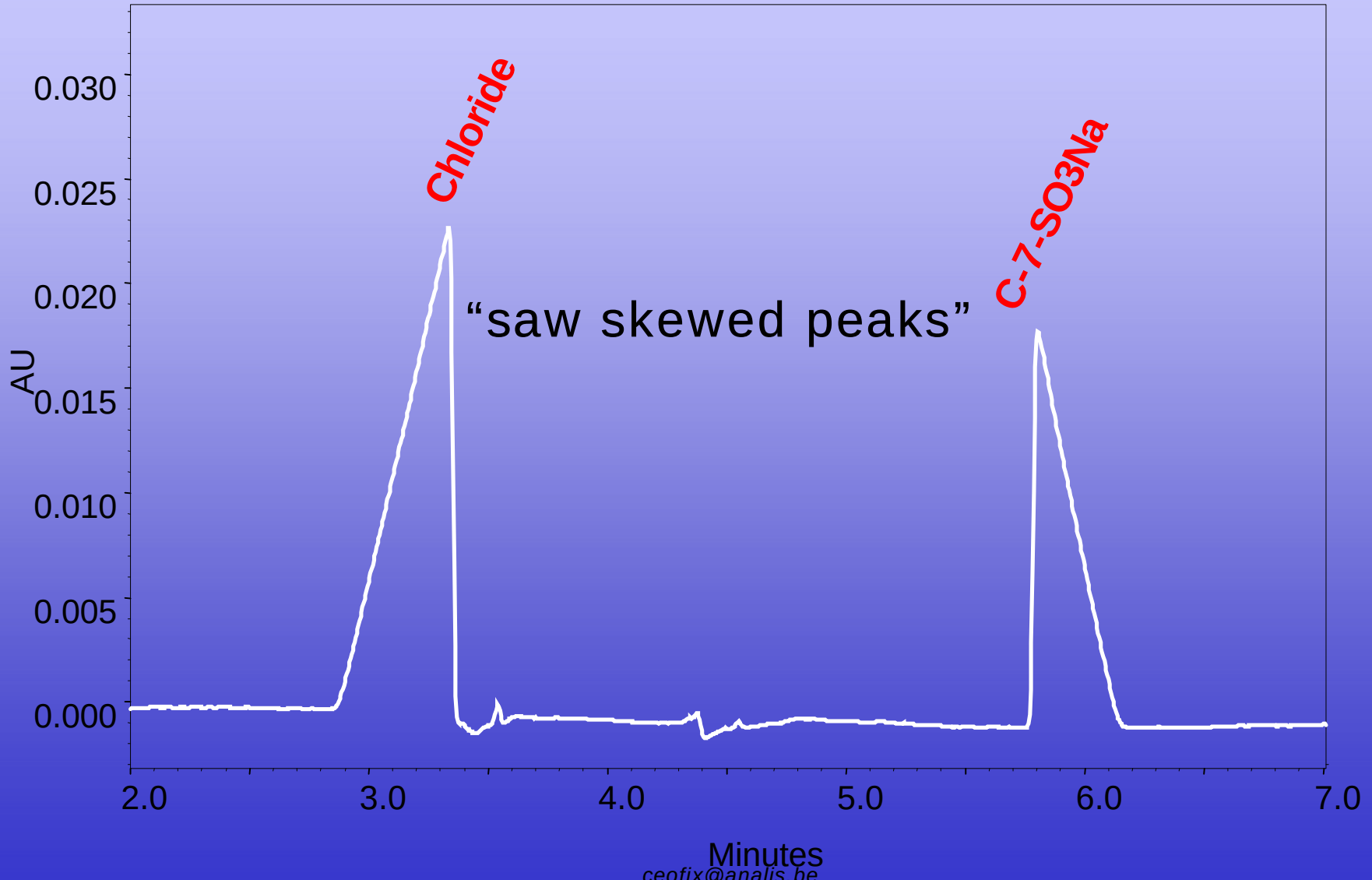
R = *number of moles of the probe displaced by one mole of analyte ion*

- Kohlrausch Regulating Function(KRF) = constant

$$? = \sum_i \frac{c_i z_i}{\mu_i}$$

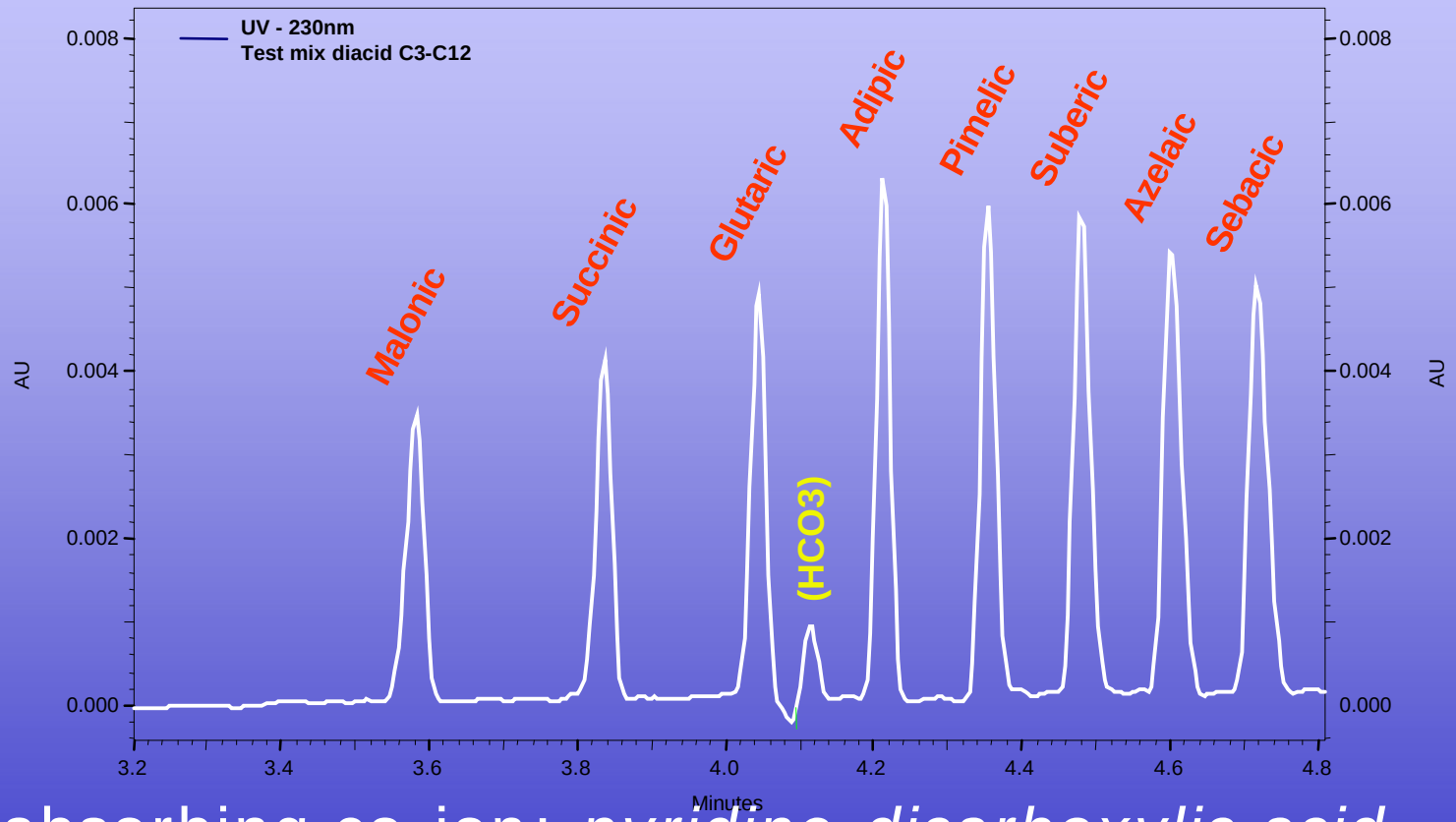
C = ionic concentration
z = value of the charge
μ = effective mobility


-
+
p
n

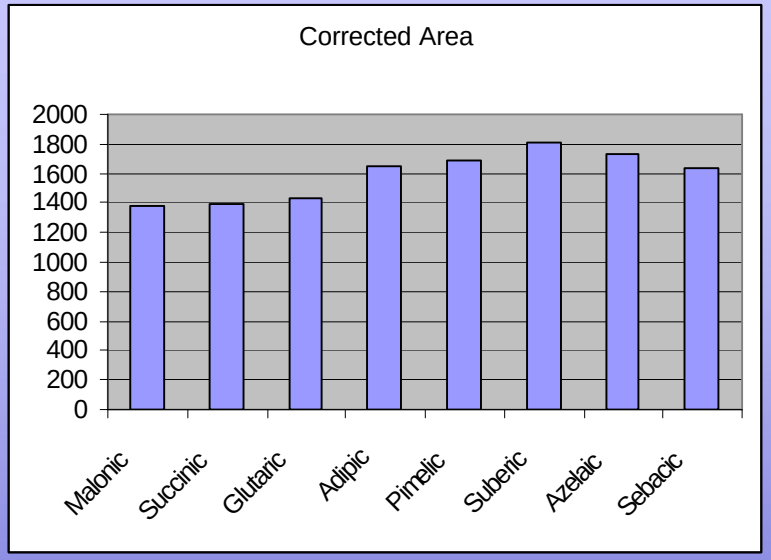

Chloride
C-7-SO3Na
"saw skewed peaks"
AU
0.030
0.025
0.020
0.015
0.010
0.005
0.000
2.0
3.0
4.0
5.0
6.0
7.0
Minutes

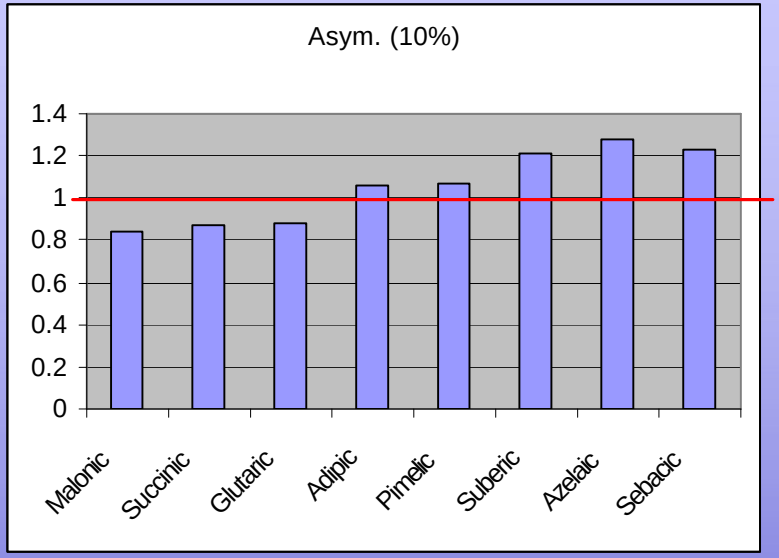
Probe absorbing co-ion

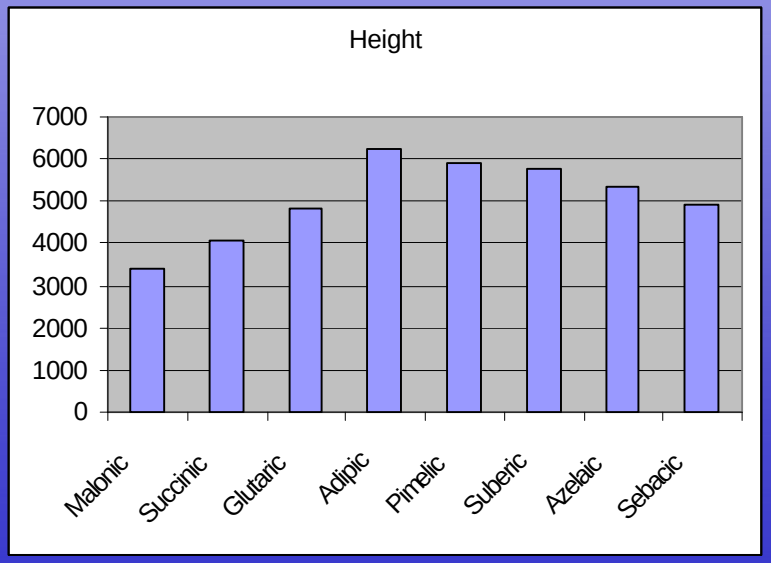
CEofix™ Anions8

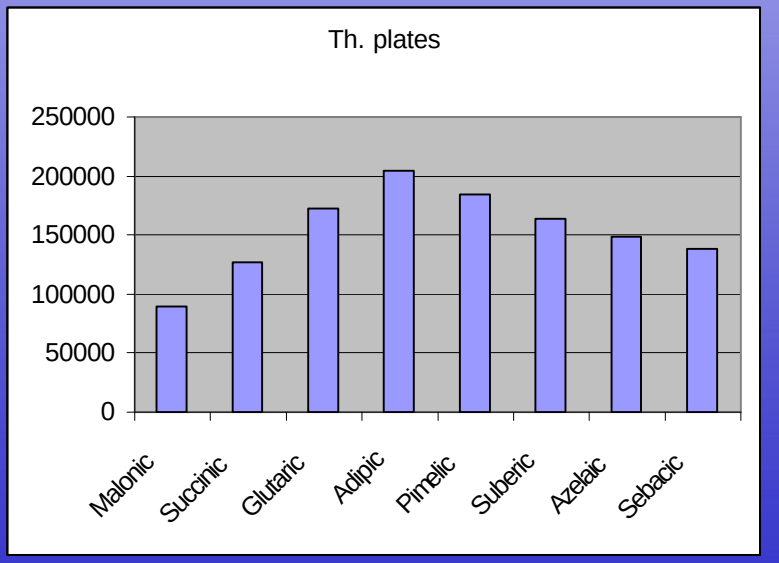


- absorbing co-ion: *pyridine-dicarboxylic acid*
- pH 8.2


Corrected Area
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Malonic
Succinic
Glutaric
Adipic
Pimelic
Suberic
Azelaic
Sebacic


Asym. (10%)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Malonic
Succinic
Glutaric
Adipic
Pimelic
Suberic
Azelaic
Sebacic


Height
7000
6000
5000
4000
3000
2000
1000
0
Malonic
Succinic
Glutaric
Adipic
Pimelic
Suberic
Azelaic
Sebacic


Th. plates
250000
200000
150000
100000
50000
0
Malonic
Succinic
Glutaric
Adipic
Pimelic
Suberic
Azelaic
Sebacic

Probe

- Limit of detection (C_{LOD})

$$C_{LOD} = \frac{N_{BL}}{Re\,I}$$

N_{BL} = baseline noice
e = molar absortivity of the probe
I = detection path length
R = transfer ratio

- Dynamic Reserve (D_r)

$$D_r = \frac{S}{s}$$

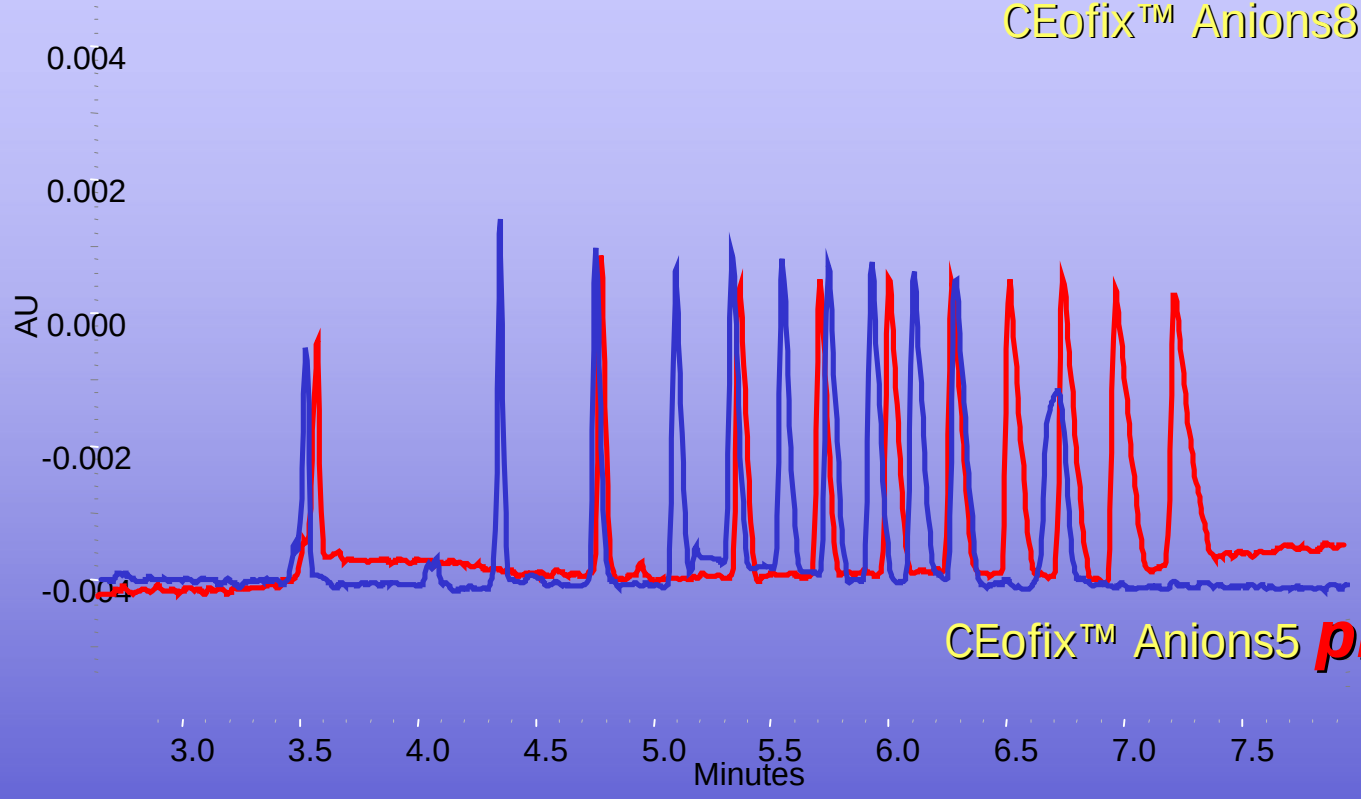
S = signal of detector
s = standard deviation of the signal

Absorbing Probe

- mobility close to the mobility of the analyte
- co-ion of the buffer: *phtalate, pyridinedycarboxylic acid….*
- specific co-ion: *chromate, molybdate…*
- high molar absorptivity
- soluble in the buffer

BGE effect of pH

CEofix™ Anions8 **pH 8.2**

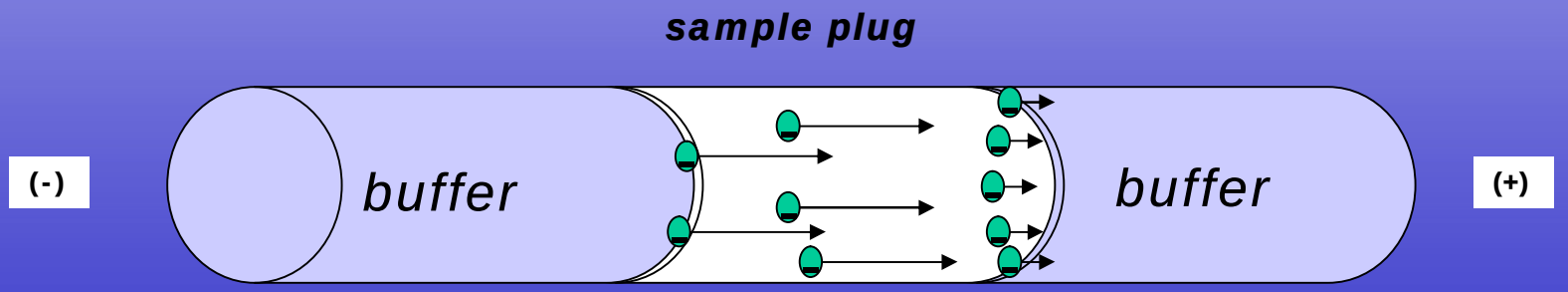


CEofix™ Anions5 **pH 5.6**

1	Formic 3.74	4	Butyric 4.87	7	Heptanoic 4.89	10	Capric 4.9
2	Acetic 4.76	5	Valeric 4.81	8	Caprylic 4.89	11	-
3	Propionic 4.87	6	Caproic 4.80	9	Pelargonic 4.96	12	Lauric 4.9

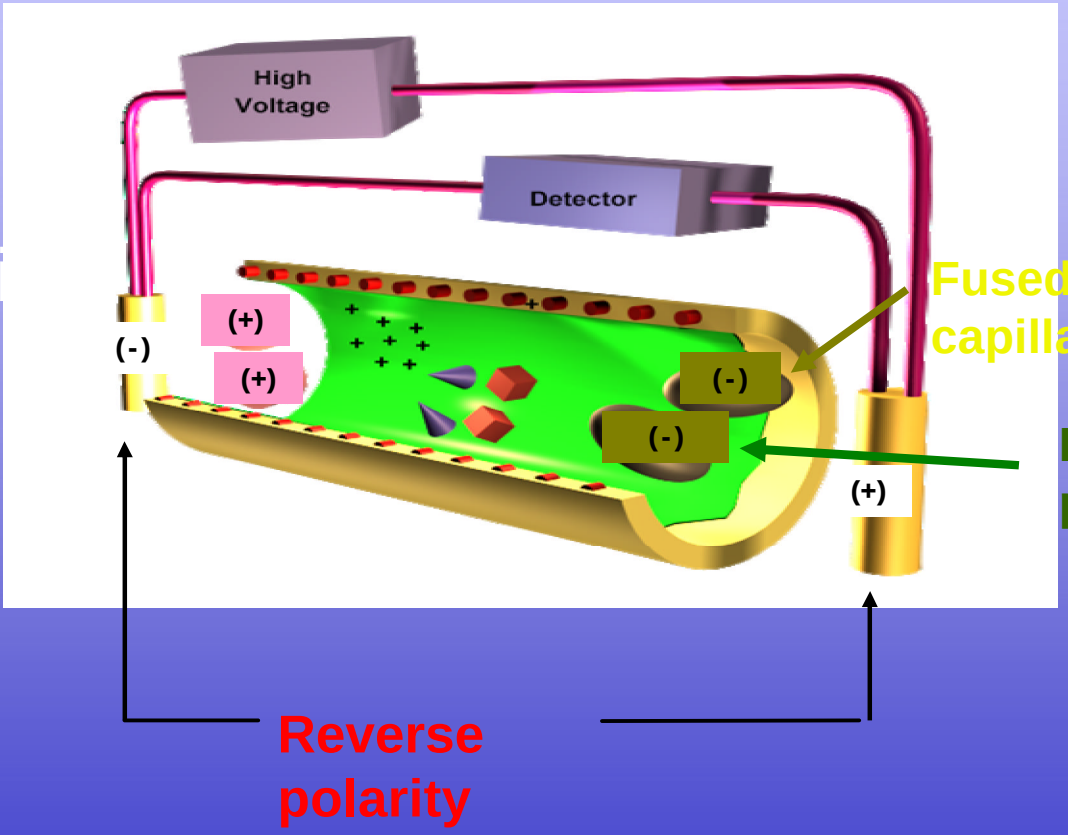
Background Electrolyte

- BGE: pH, ionic strength, viscosity
  - buffer
  - pKa of the analyte
  - 10 x higher as the ionic strength of sample: FASS



Co-electro Osmotic mode

- reverse polari
- EOF modifier (surfactant)



EOF flow modifiers

- To inverse the EOF flow
- Cationic surfactants:

  CTAB, CTAH, TTAB…
- Polyanion:

  P*olybrene…*

Buffers for Indirect UV detection which system to chose?

- EOF-flow modifier ?
- Buffer ?
- Absorbing Co-ion with matched mobility ?
- What about system peaks?

In most cases a validated buffer system will provide a practical solution!

Instrument considerations

- Capillary
  - length (60 cm total length)
  - diameter (50 – 75 µ)
- Instrument
  - temperature
    - pH of the buffer
    - viscosity of the buffer
  - voltage
- Method

Method

	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	
1		Rinse - Pressure	20.0 psi	0.50 min	BI:B1	BO:A1	forward	Initiator
2		Rinse - Pressure	20.0 psi	0.50 min	BI:C1	BO:A1	forward	Accelerator
3		Inject - Pressure	0.5 psi	8.0 sec	SI:A1	BO:C1	No override, forward, In vial inc 1	Sample-H2O
4		Inject - Pressure	0.1 psi	10.0 sec	BI:D1	BO:C1	No override, forward	H2O-H2O
5	0.00	Separate - Voltage	30.0 KV	8.00 min	BI:E1	BO:B1	1.00 Min ramp, reverse polarity	Buffer-Buffer
6	1.25	Autozero						
7	8.00	Stop data						
8	8.00	Rinse - Pressure	20.0 psi	0.50 min	BI:A1	BO:A1	forward	NaOH 0.1M-Waste
9	8.50	Rinse - Pressure	20.0 psi	0.50 min	BI:F1	BO:A1	forward	H2O-Waste
10	9.00	End						

- rinse step
- H_20 post-injection ? H_20 rinse step
- buffer rinse ? buffer separation step
- 1 min. ramping time
- increment every 20 analysis

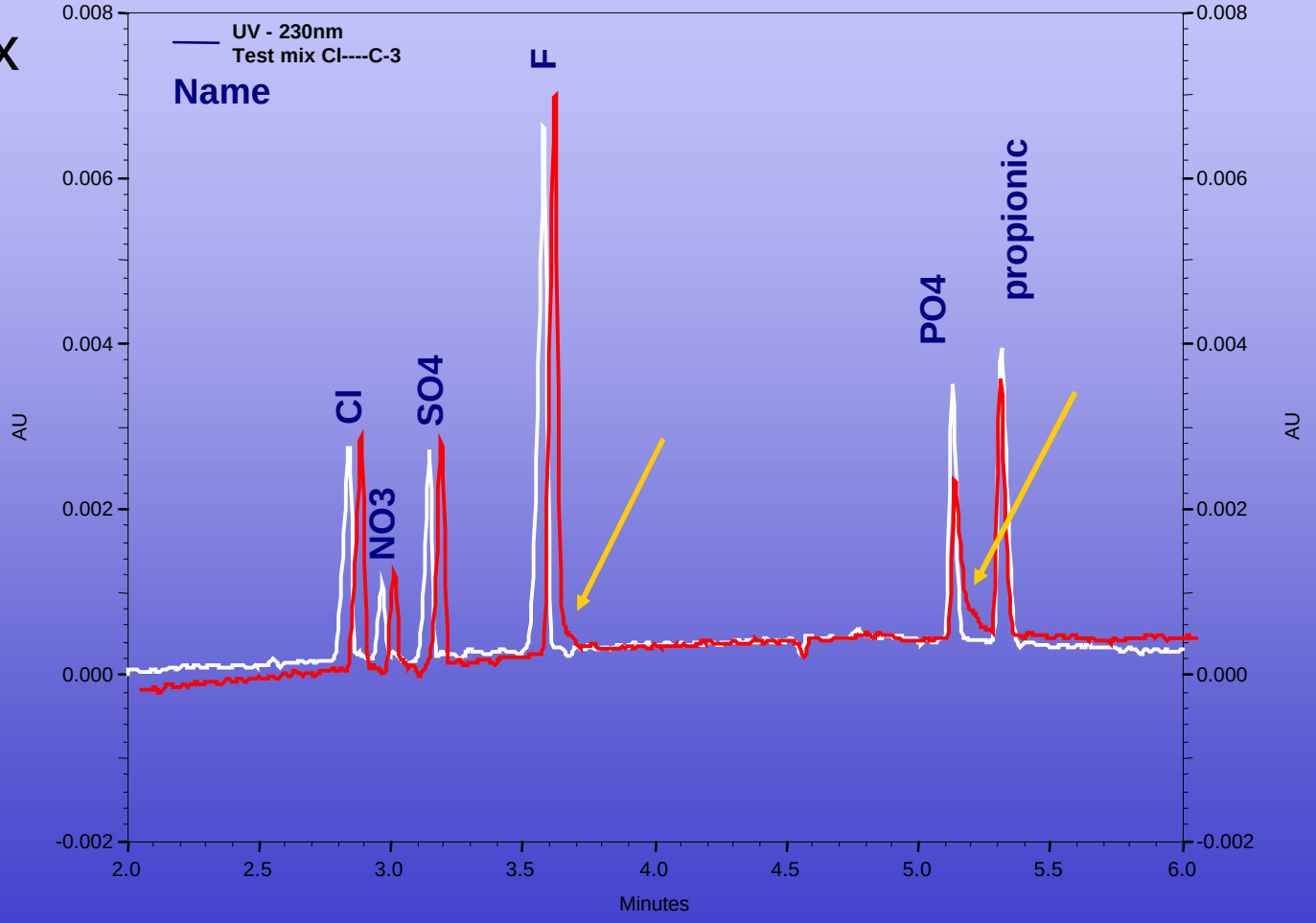
Analysis of Primaquine diphosphate (PQ)

- Sample: 200 mg/L PO_4
- Standards at 70%, 100% and 130%
- Octanoic Acid as I.S.
- Sequence: 3 x alternating standards and samples
- CEofix™ Anions5

Problem of phosphate: peak tailing

CEofix™ Anions5

testmix



rinse H_20 ? rinse HCl 0.1M

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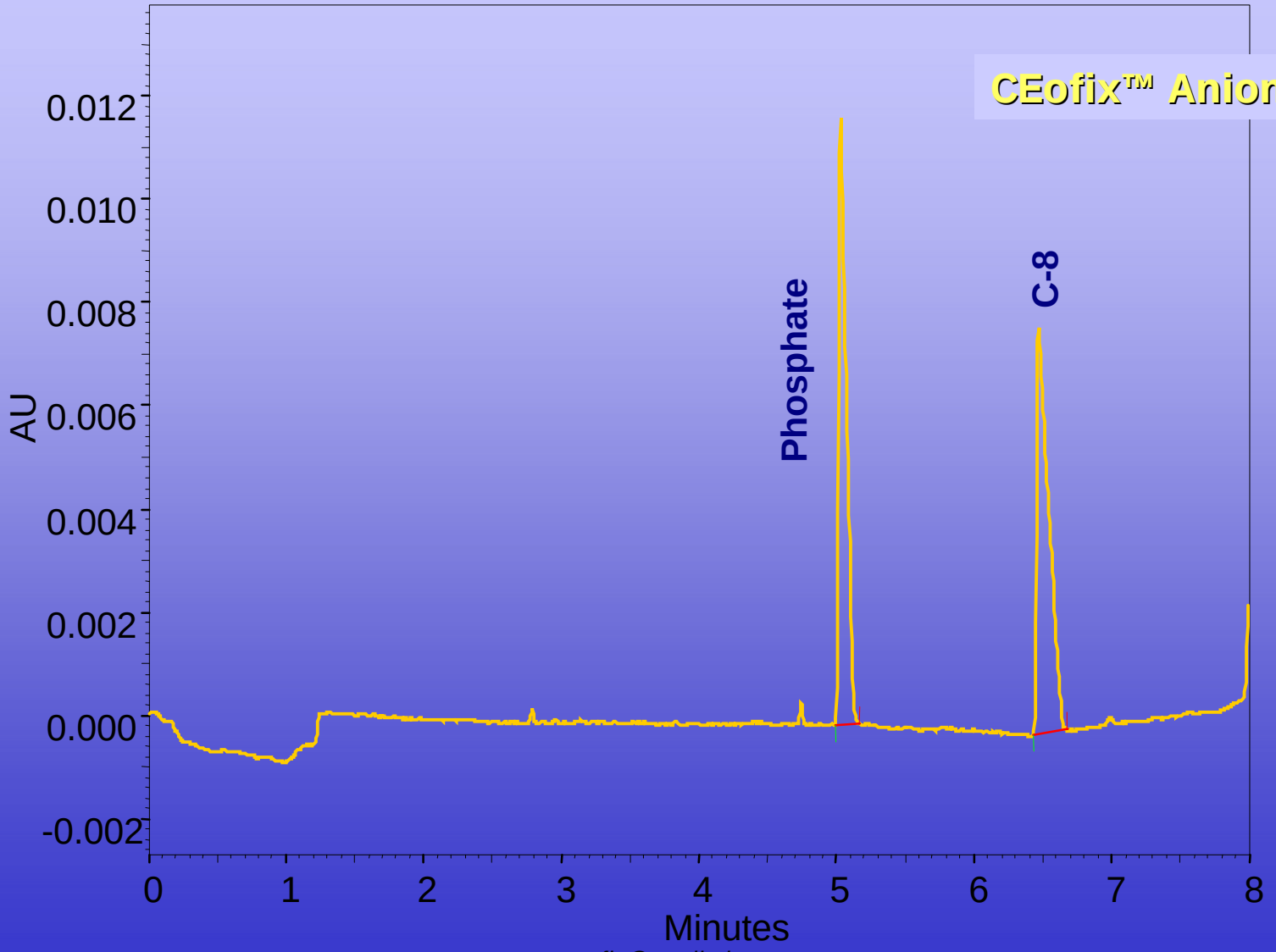
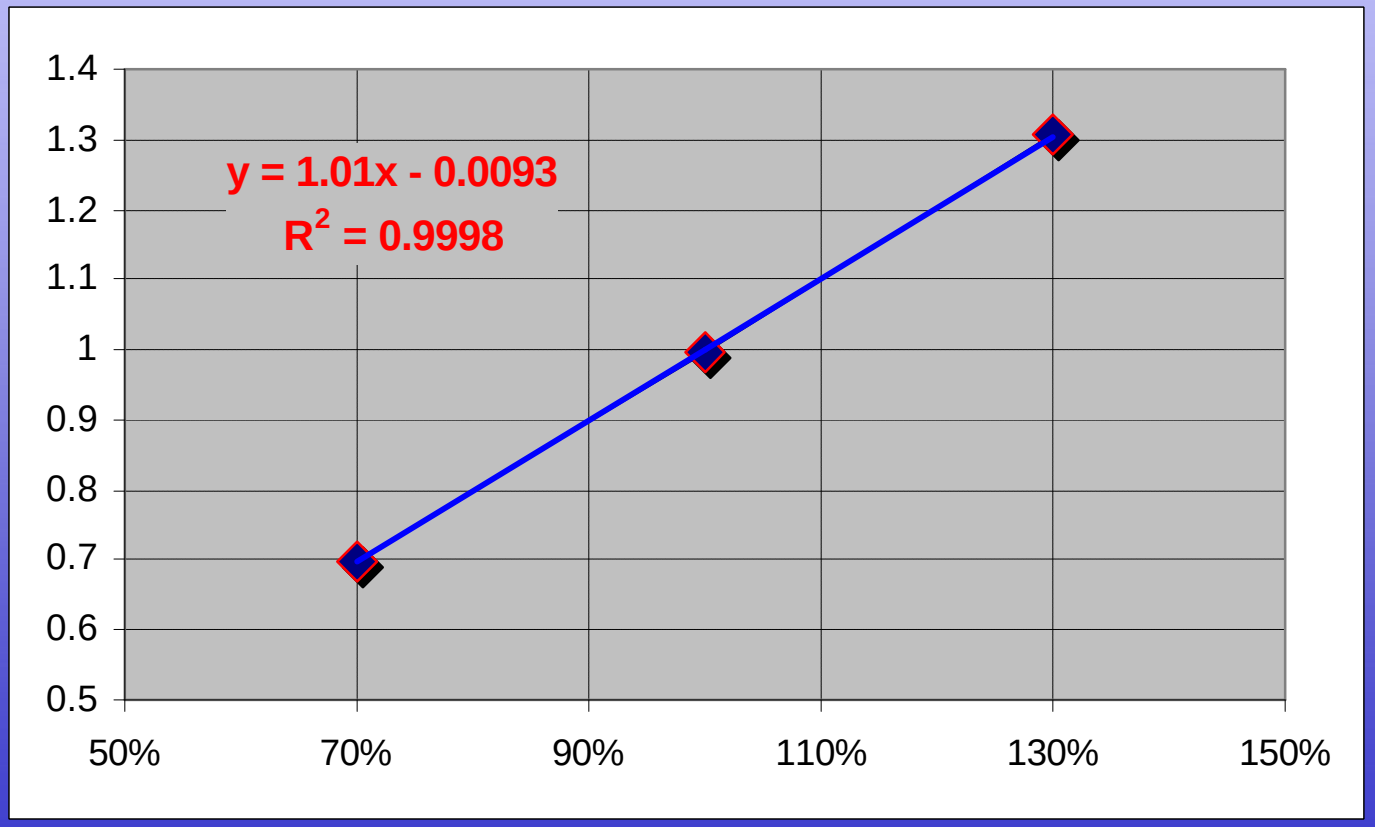

CEofix™ Anions5
Phosphate
C-8
AU
0.012
0.010
0.008
0.006
0.004
0.002
0.000
-0.002
0
1
2
3
4
5
6
7
8
Minutes

Linearity


y = 1.01x - 0.0093
R2 = 0.9998
1.4
1.3
1.2
1.1
1
0.9
0.8
0.7
0.6
0.5
50%
70%
90%
110%
130%
150%

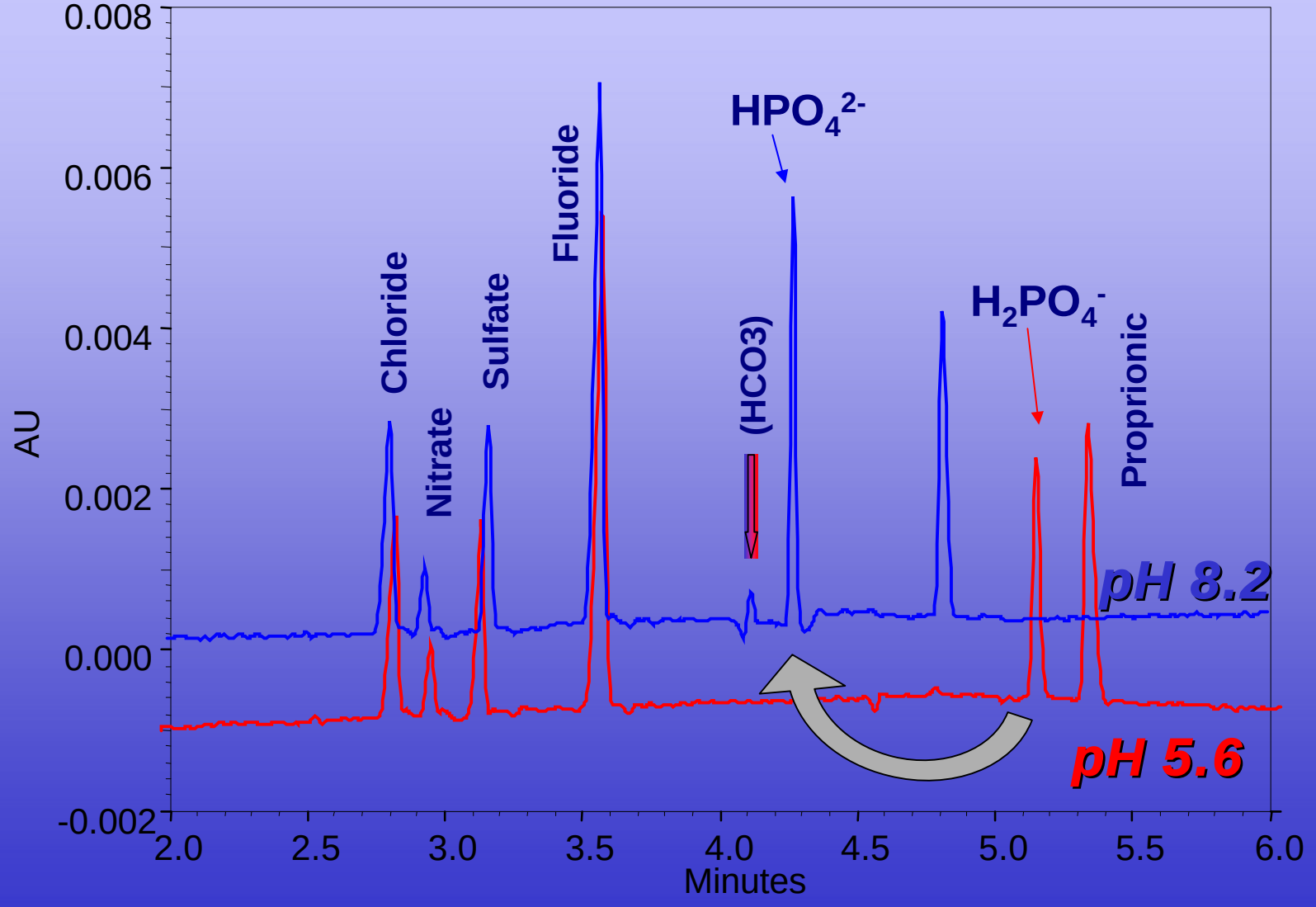
Results

	Migration Time		Area
	Phosphate	I.S.	I.S.
Mean	5.03	6.468	49749
%SD	0.25	0.21	2.79

	Sample PQ % Target Value
Run 4	99.82
Run 8	99.62
Run 12	99.81
Mean	99.75
%SD	0.11

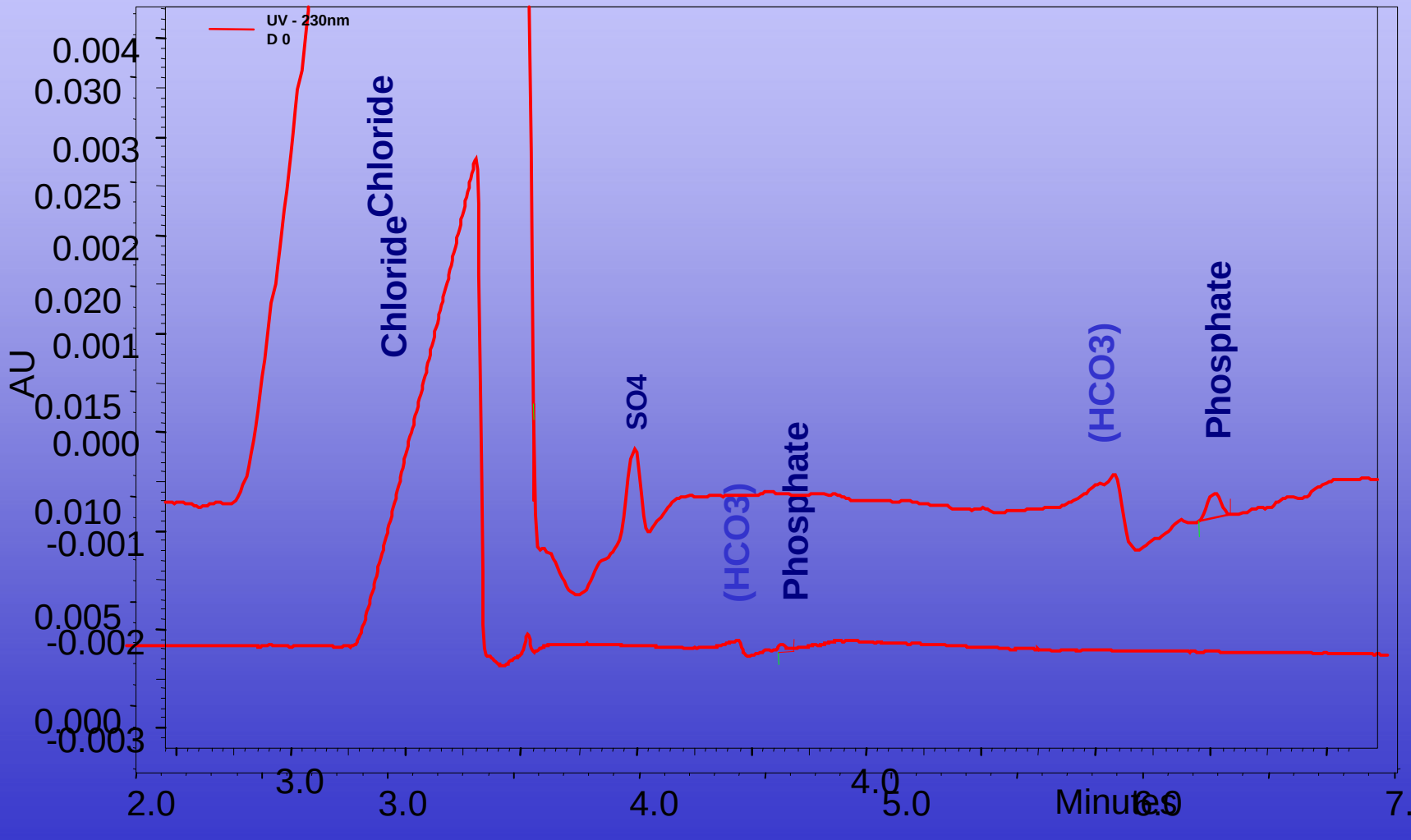
Impurity Analysis

- Phosphate impurity of a drug HCl salt
- Low level of impurity
- High level of Chloride
- CEofix™ Anions8


0.008
0.006
0.004
0.002
0.000
-0.002
AU
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
Minutes
Chloride
Nitrate
Sulfate
Fluoride
(HCO3)
HPO4 2-
H2PO4 -
Proprionic
pH 8.2
pH 5.6

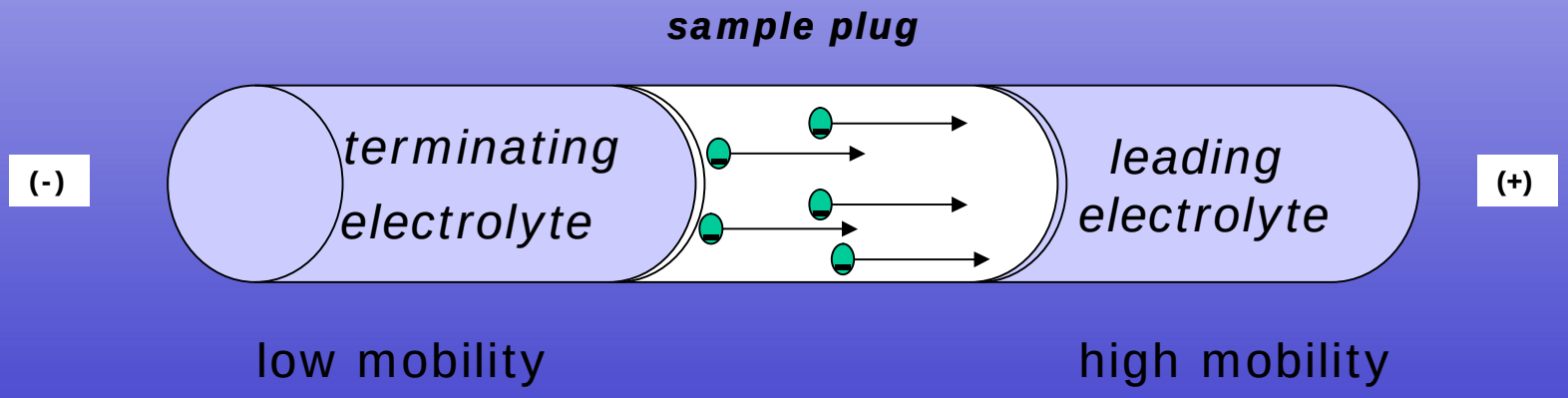
400 mg/L chloride and 0.1% phosphate

CEofix™ Anions8

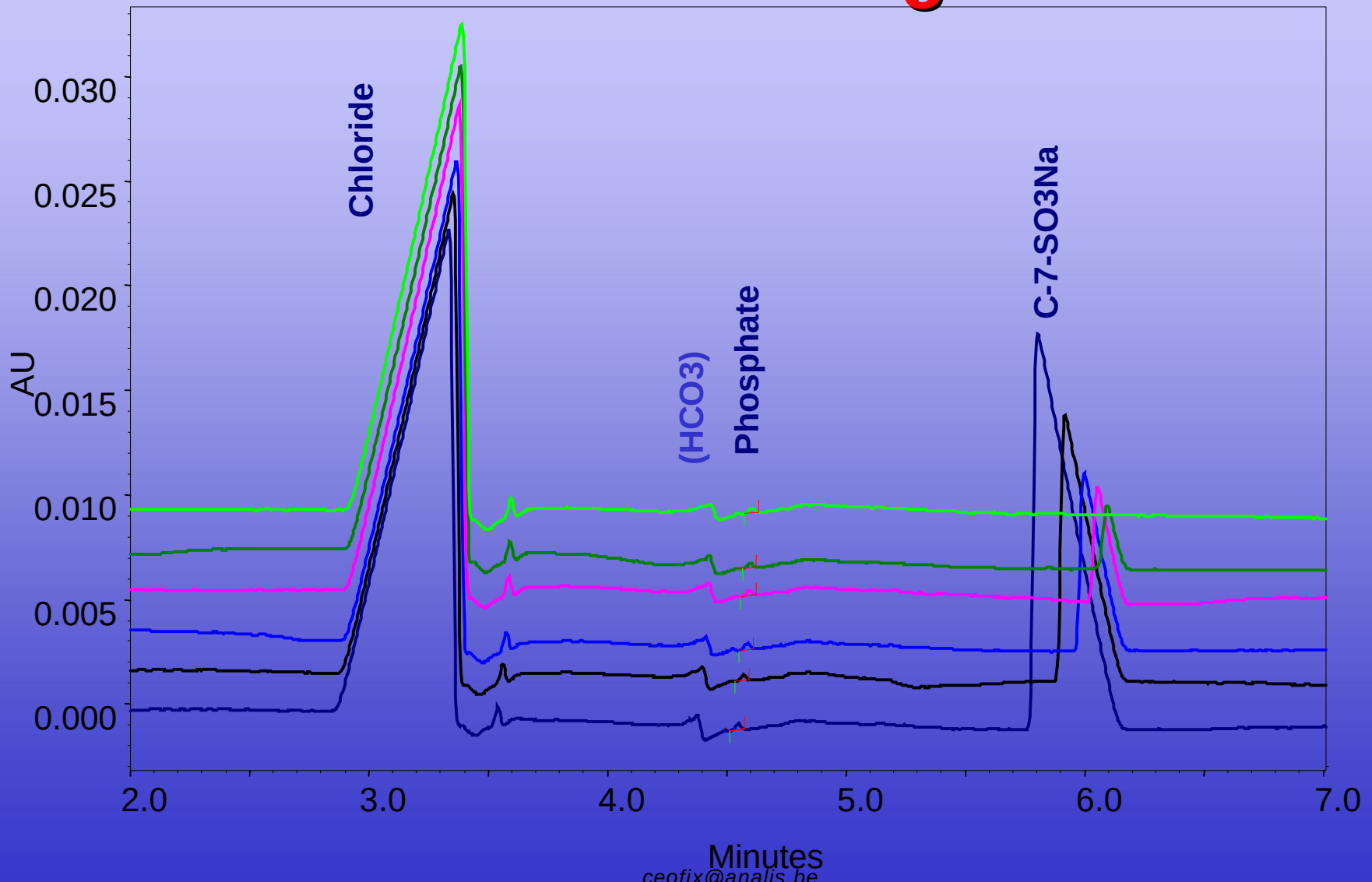


IsoTachoPhoretic Stacking (ITP)

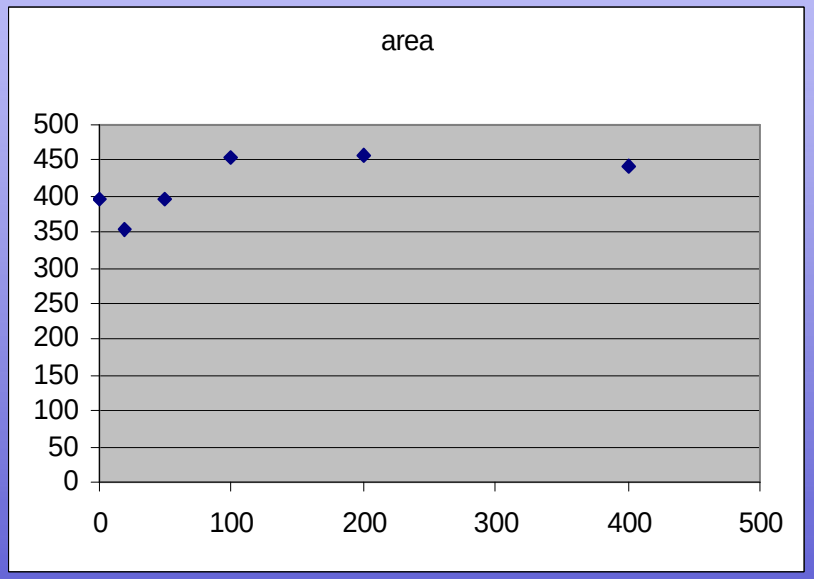
- Chloride high mobility ion -> de-stacking effect
- Addition of low mobility ion (in sample) -> re-stacking effect

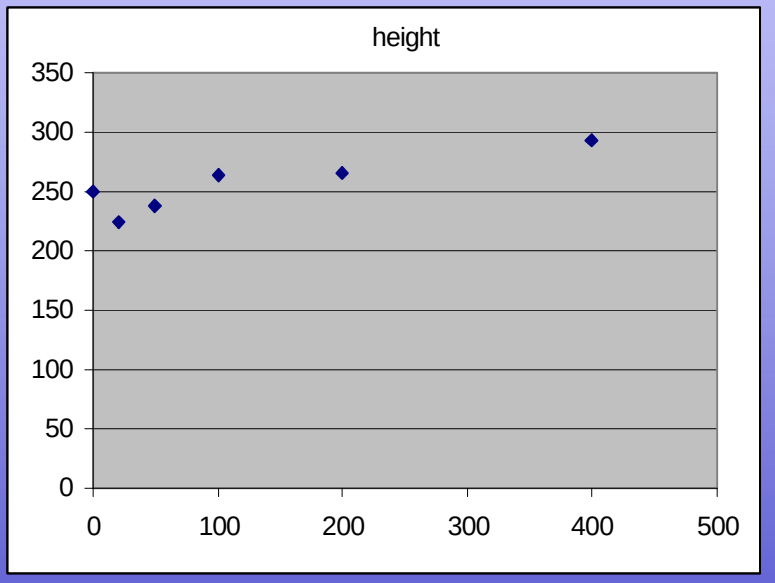


Heptane sulfonate as terminating ion

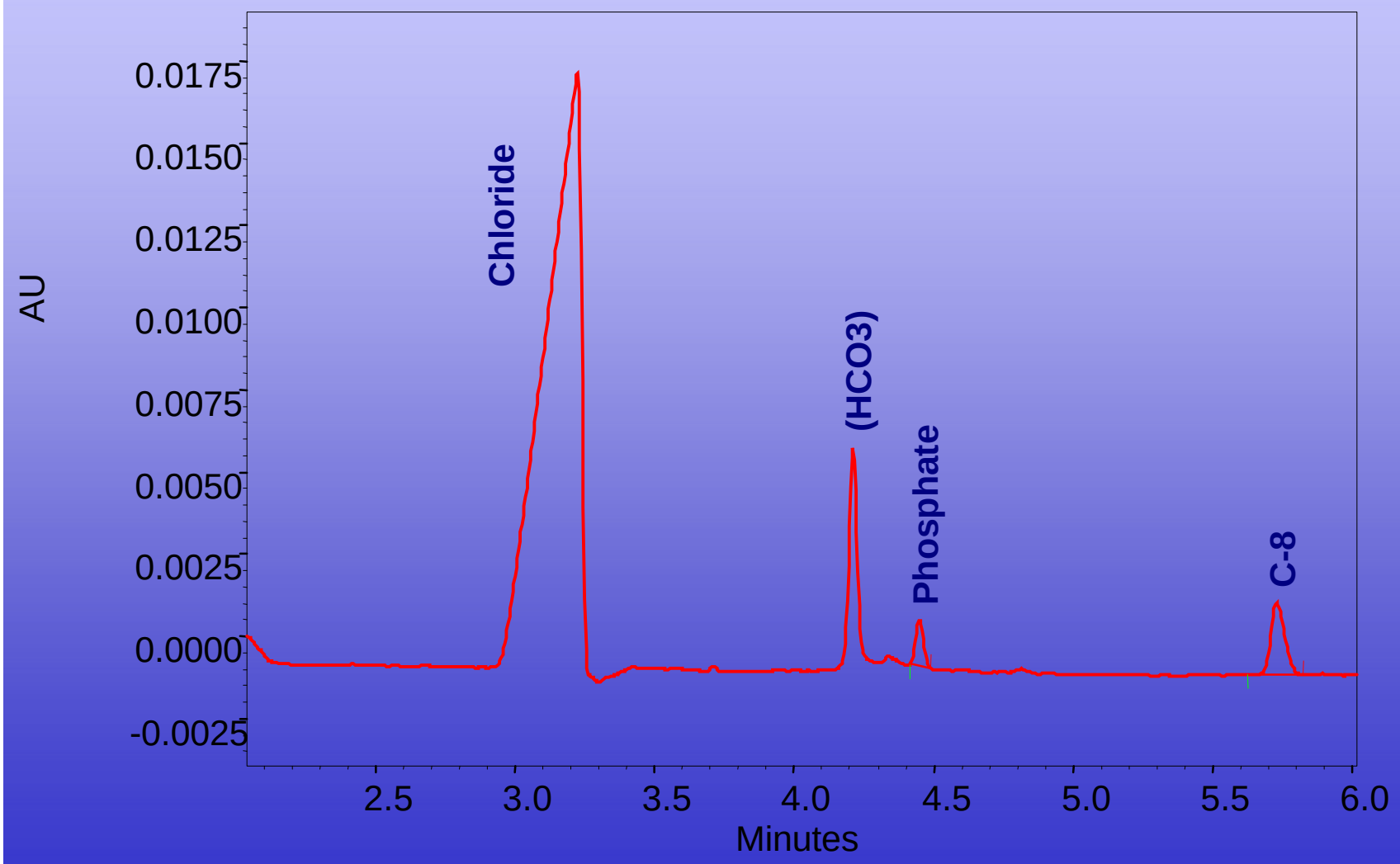


Effect of increasing amount of Heptanesulfonate as terminating ion





- SD on area: 9.82%
- no need for ITP re-stacking


AU
0.0175
0.0150
0.0125
0.0100
0.0075
0.0050
0.0025
0.0000
-0.0025
Chloride
(HCO3)
Phosphate
C-8
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
Minutes

Conclusion

- CE ion analysis with indirect UV detection is able to analyze
  - phosphate as counterion in a drug
  - phosphate as impurity in a drug
- Easy and fast method