CE of organic acids in cerebrospinal fluid using direct sample injection and a triple-layer coating

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outline

- Background
- Aim of the study
- Overview results
- Conclusions and future developments

Organic acids in cerebrospinal fluid

•Organic acids => key metabolites => quantitative analysis in body fluids yields information on physiological and pathophysiological state.

•Analysis in cerebrospinal fluid (CSF) important for early diagnosis of metabolic disorders and neurological diseases:

Disease	*Organic acid(s)*
Bacterial meningitis	Lactic acid ↑
Respiratory chain disorder	Lactic acid ↑
L-2-hydroxyglutaric acidurias	2- and 3-hydroxybutyric acid ↑ Glycolic acid ↑

Analysis of organic acids

GC-MS:

- Long separation times
- Complex sample pretreatment and derivatization

CE:

- High selectivity and resolution
- Low sample consumption => ideal for CSF samples
- Fast separation times (27 organic acids < 15 min)[1]

[1]Barbas C (1998) Clin Chem 44(9):1905-1912

CE of organic acids

In general, organic acids are analyzed by CE-UV using a suppressed or reversed electro-osmotic flow system, which can be achieved by:

- polyacrylamide-coated capillaries (EOF suppression)
- Use of cationic surfactants (EOF reversal)

Aim of the study

To develop a CE method:

- for the direct analysis, i.e. without any sample pretreatment,

of organic acids in CSF

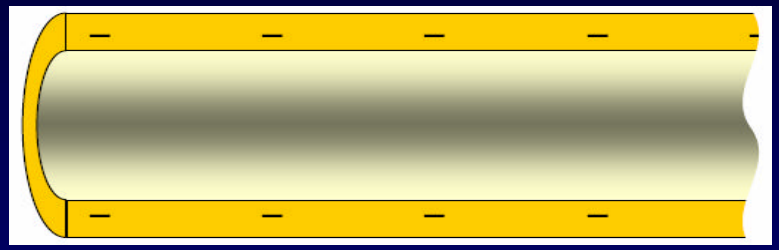
- using a multiple ionic polymer coating

Study performed with 6 *clinically relevant* organic acids:

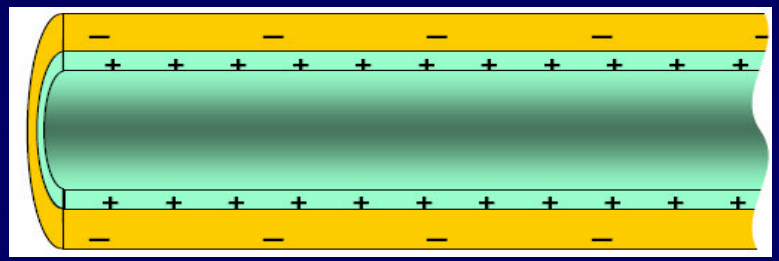
oxalic acid, citric acid, glycolic acid, lactic acid, 2-hydroxybutyric acid

and 3-hydroxybutyric acid

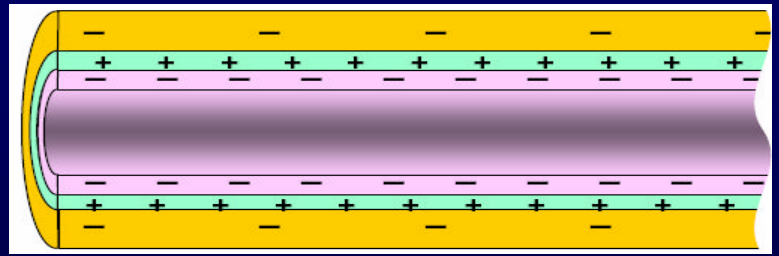
Capillary coating



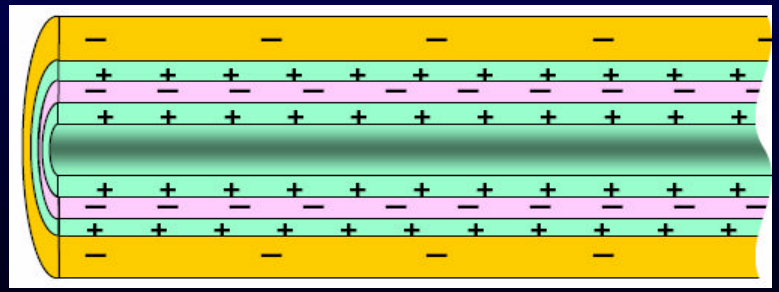
•Deprotonation of SiOH groups using NaOH



•Rinsing with polybrene



•Rinsing with poly-dextran sulfate

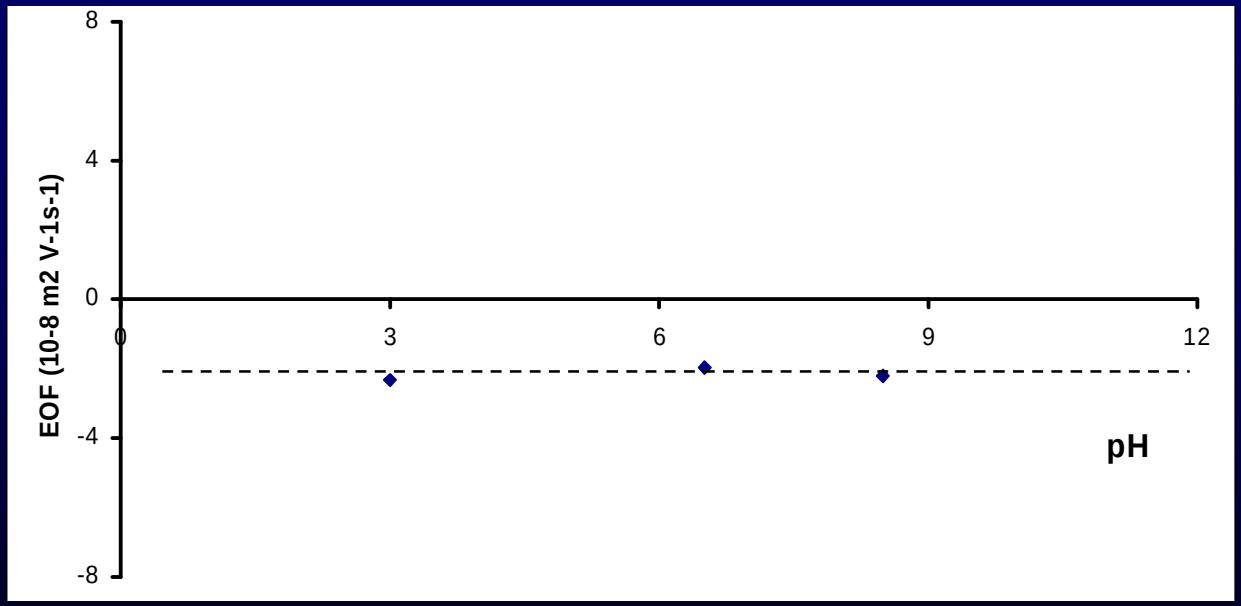


•Rinsing with polybrene

Coating stability

•Stability of produced coating determined by measuring mobility of formamide at different pH values

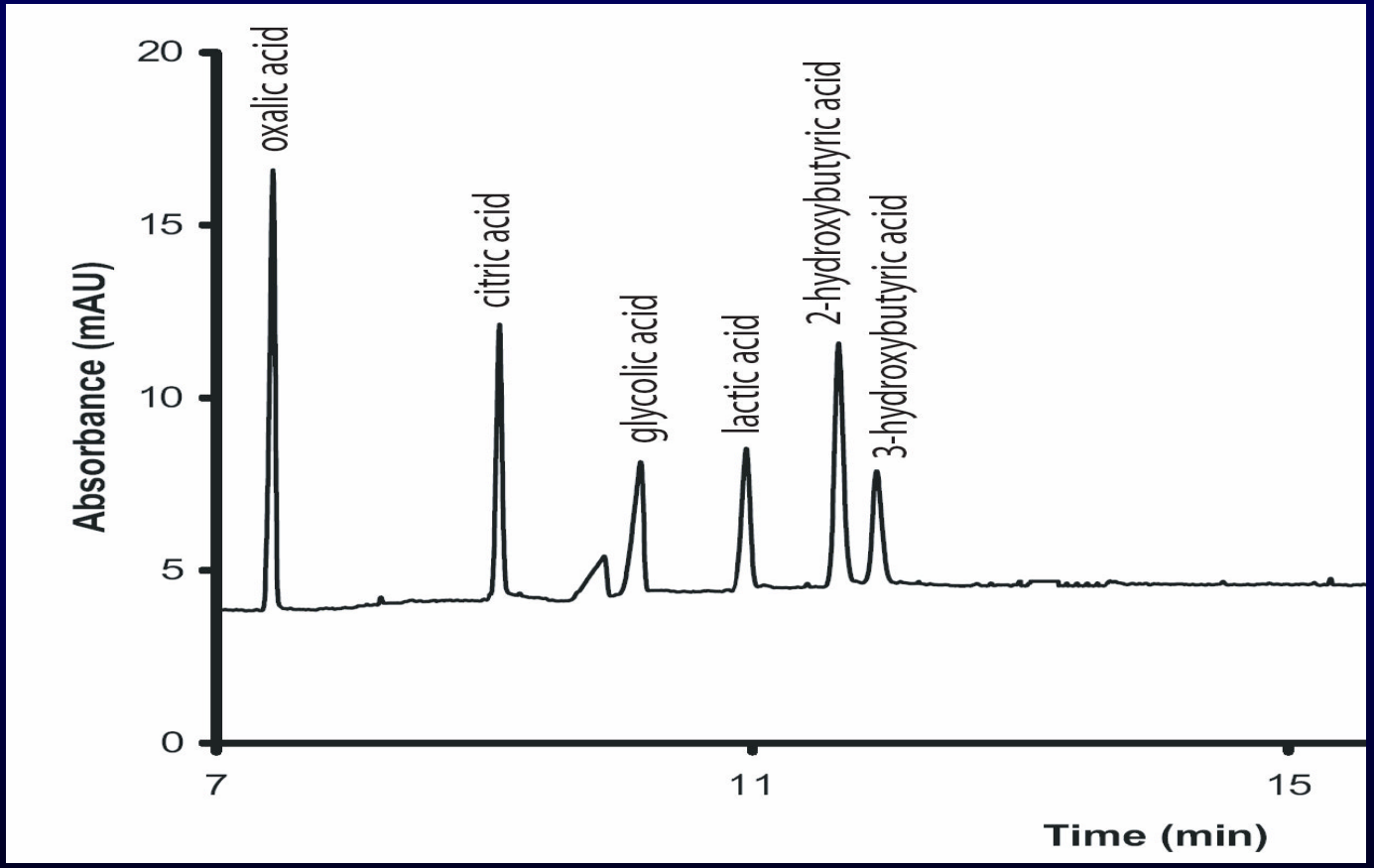
•At all pH values an anodic and virtually pH-independent EOF was found with a mobility of about -2.2×10- 8 m^2 V^{-1} s^{-1}.



Standard analysis using PB-DS-PB capillary

Optimal BGE: 200 mM phosphate (pH 6.0) => plate numbers 100,000-200,000

CE of six organic acids dissolved in water:



Influence of the sample matrix

CSF contains ca. 150 mM NaCl and 0.2 mg/mL albumin

•High NaCl concentrations cause the electrical conductivity of the samples to be high,

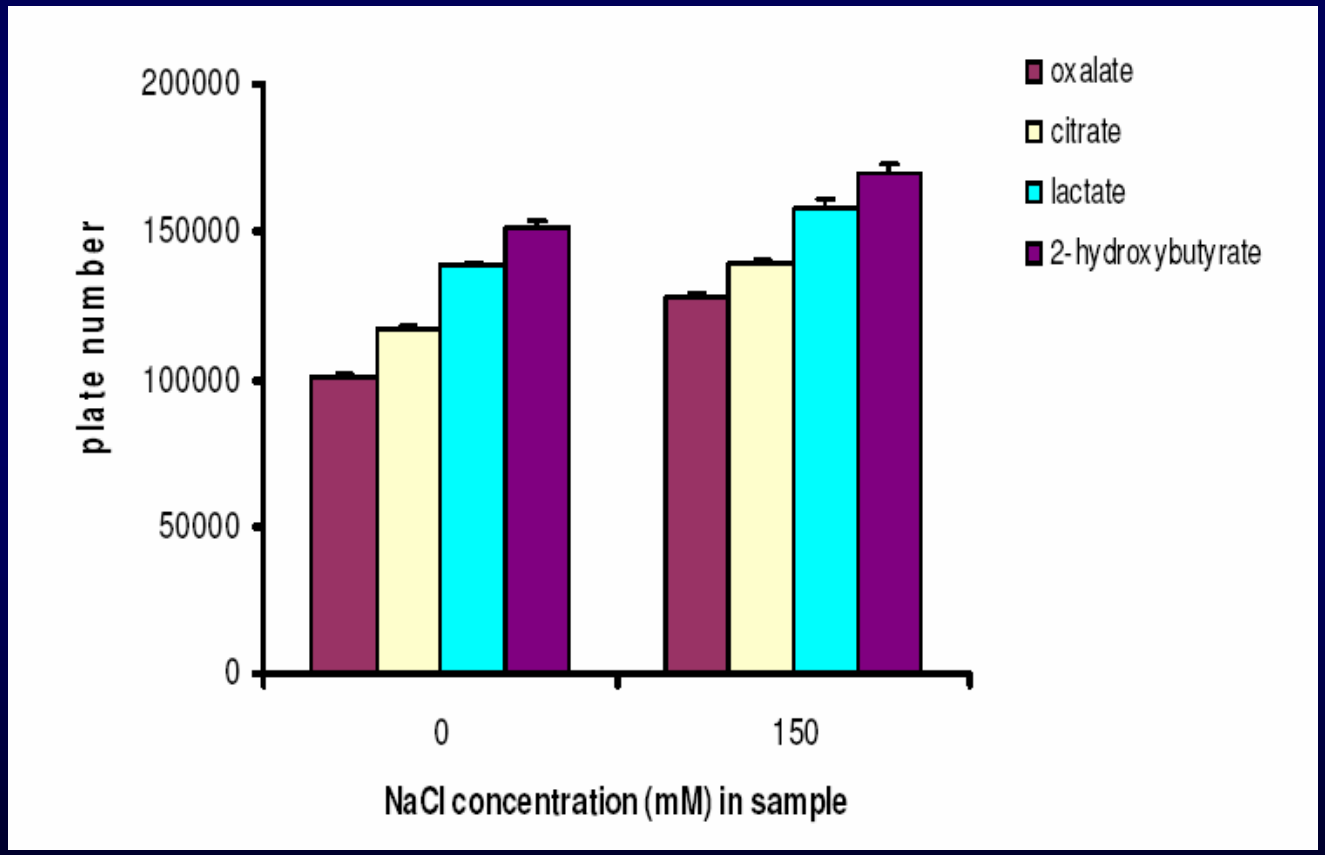
=> reduced separation efficiency

•Proteins can adsorb to the capillary wall/coating causing alterations of the EOF,

=> significantly changed migration times

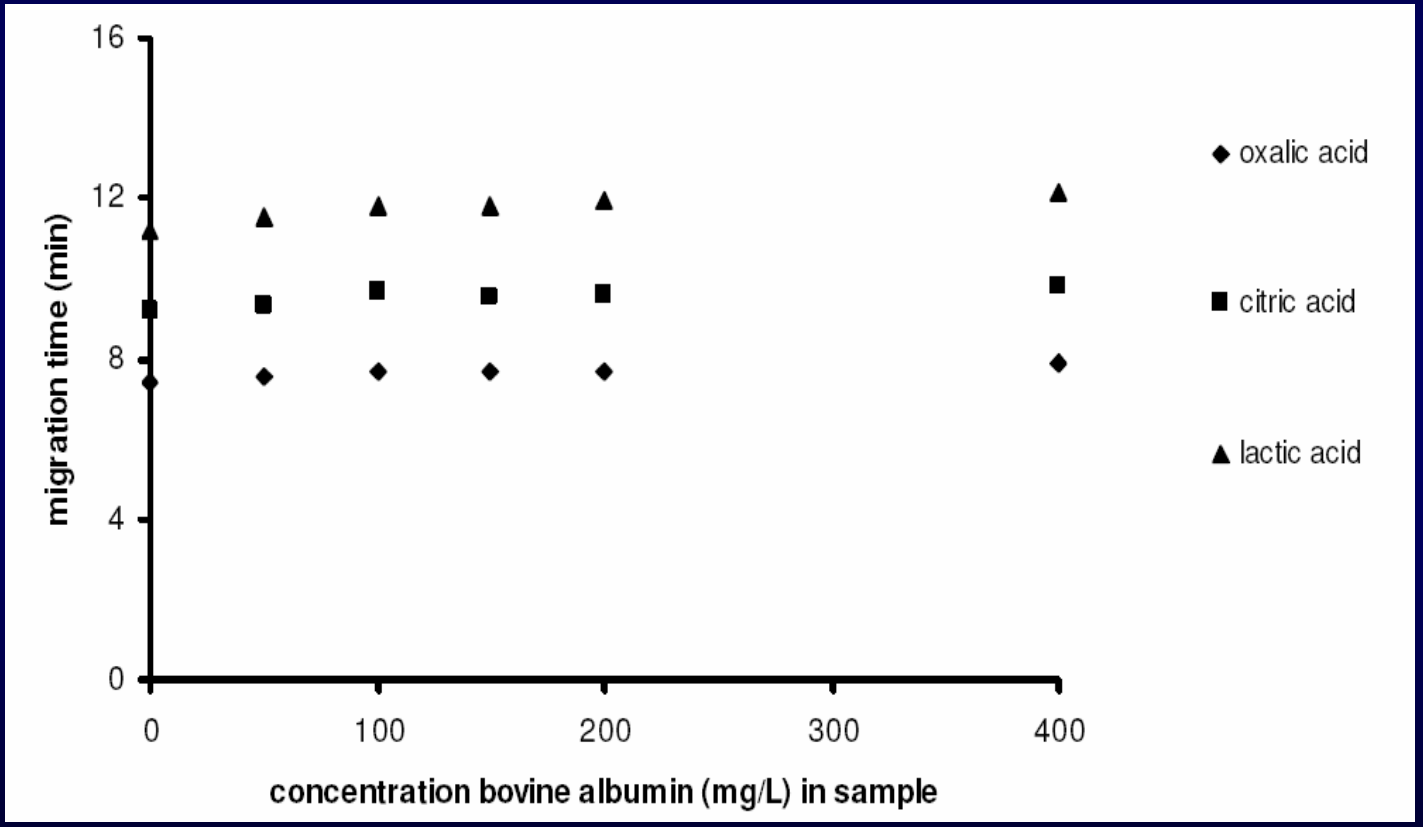
Influence of NaCl on plate numbers

Plate numbers slightly improved in presence of NaCl, which was most probably due to sample-induced transient-isotachophoresis



Influence of Albumin on migration times

Migration times of organic acids when different concentrations of albumin were included in the sample and HCl rinses were applied between runs:



Linearity and repeatability

- Linearity

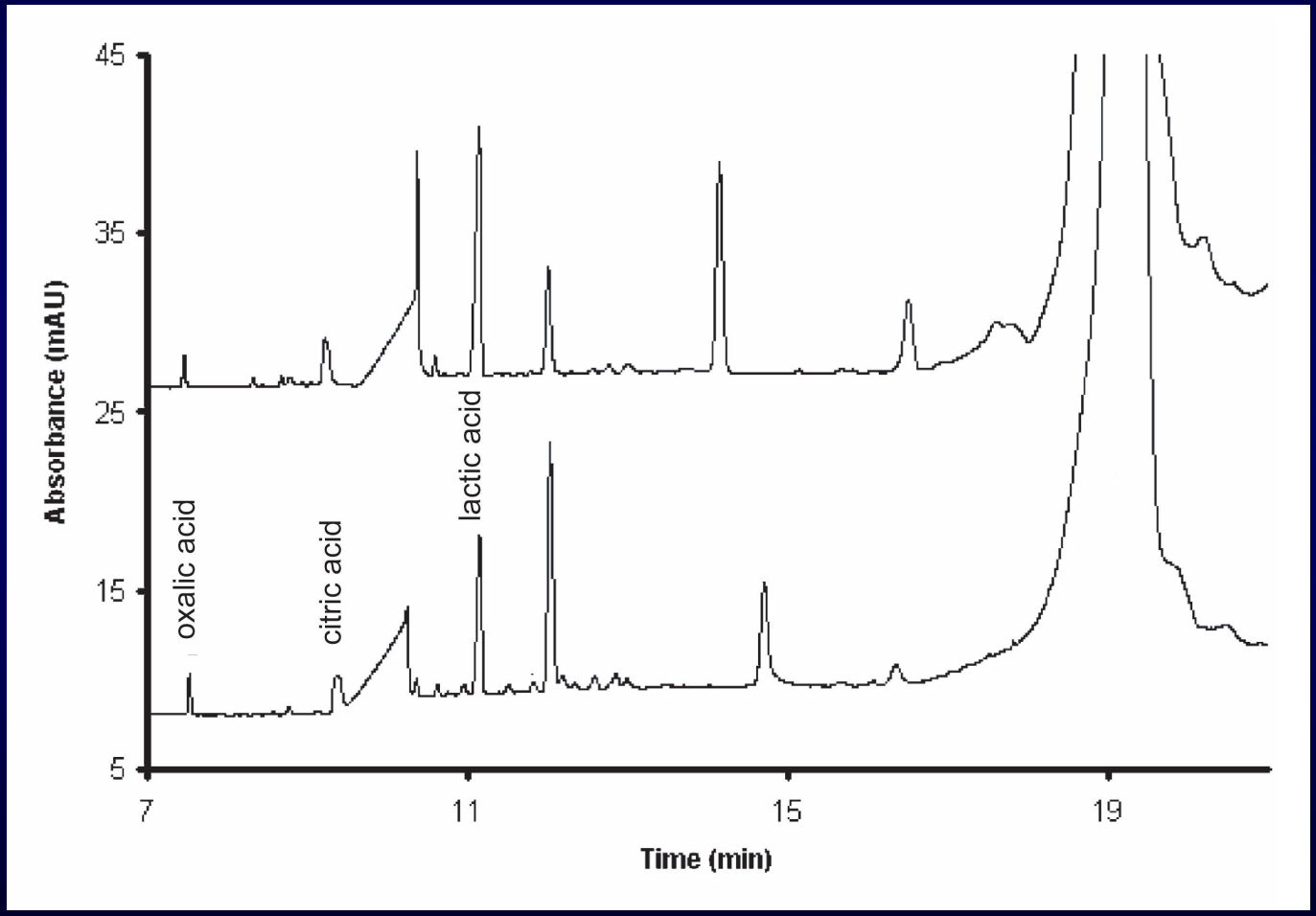
  For all organic acids: r > 0.99 (artificial CSF)

  LODs: 2-8 µg/mL

- Repeatability

Acid	Oxalic	Citric	Lactic
RSD for migration time			
Artificial CSF (n=5)	<1%	<1%	<1%
CSF (n=5)	<2%	<2%	<3%
RSD for peak area			
Artificial CSF (n=5)	<5%	<5%	<5%
CSF (n=5)	<5%	<5%	<7%

Applicability – Bacterial Meningitis



Bacterial meningitis CSF

Control CSF

Applicability – Bacterial Meningitis

Concentration of organic acids (µg/mL) in two CSF samples

Acid	Control CSF	Bacterial meninigitis CSF
Oxalic	9.9	8.4
Citric	112	112
Lactic	**222**	**384**

Indeed, an increased concentration of lactic acid was found in the bacterial meningitis CSF sample

Conclusions

- CE method developed for determination of organic acids using direct injection

- Organic acids can be analyzed rapidly of with high efficiency and good repeatability of migration times and peak areas

- These merits can be primarily attributed to the use of a triple layer capillary coating in combination with HCl rinsing procedures

- It can be used to distinguish control CSF samples from bacterial meningitis CSF samples on the basis of the selected organic acids (total analysis time < 30 min)

Future developments

- Stability and separation power of the present system offers high potential for profiling endogenous metabolites in biological samples

- Currently, we are developing a CE-ESI-MS method for the direct analysis of endogenous metabolites in CSF (more selectivity and characterization)

Questions?