

# ENHANCED SELECTIVITY OF CAPILLARY ZONE ELECTROPHORESIS BY ON-LINE ISOTACHOPHORETIC SAMPLE PRETREATMENT

J. Hins<sup>1</sup>, M. Danková<sup>2</sup>, S. Strašák<sup>2</sup>, C. Dietrich<sup>1</sup>, J. Mannhardt<sup>1</sup>, D. Kaniansky<sup>2</sup>

<sup>1</sup>J&M Analytische Mess-und Regeltechnik GmbH, Robert-Bosch-Strasse 83, D-73431 Aalen, Germany

<sup>2</sup>Department of Analytical Chemistry, Faculty of Sciences, Comenius University in Bratislava, Mlynská Dolina CH-2, SK-84215 Bratislava, Slovakia

## CZE with on-line ITP sample pretreatment in the separation system with coupled columns

### Equipment

### ITP sample injection into CZE

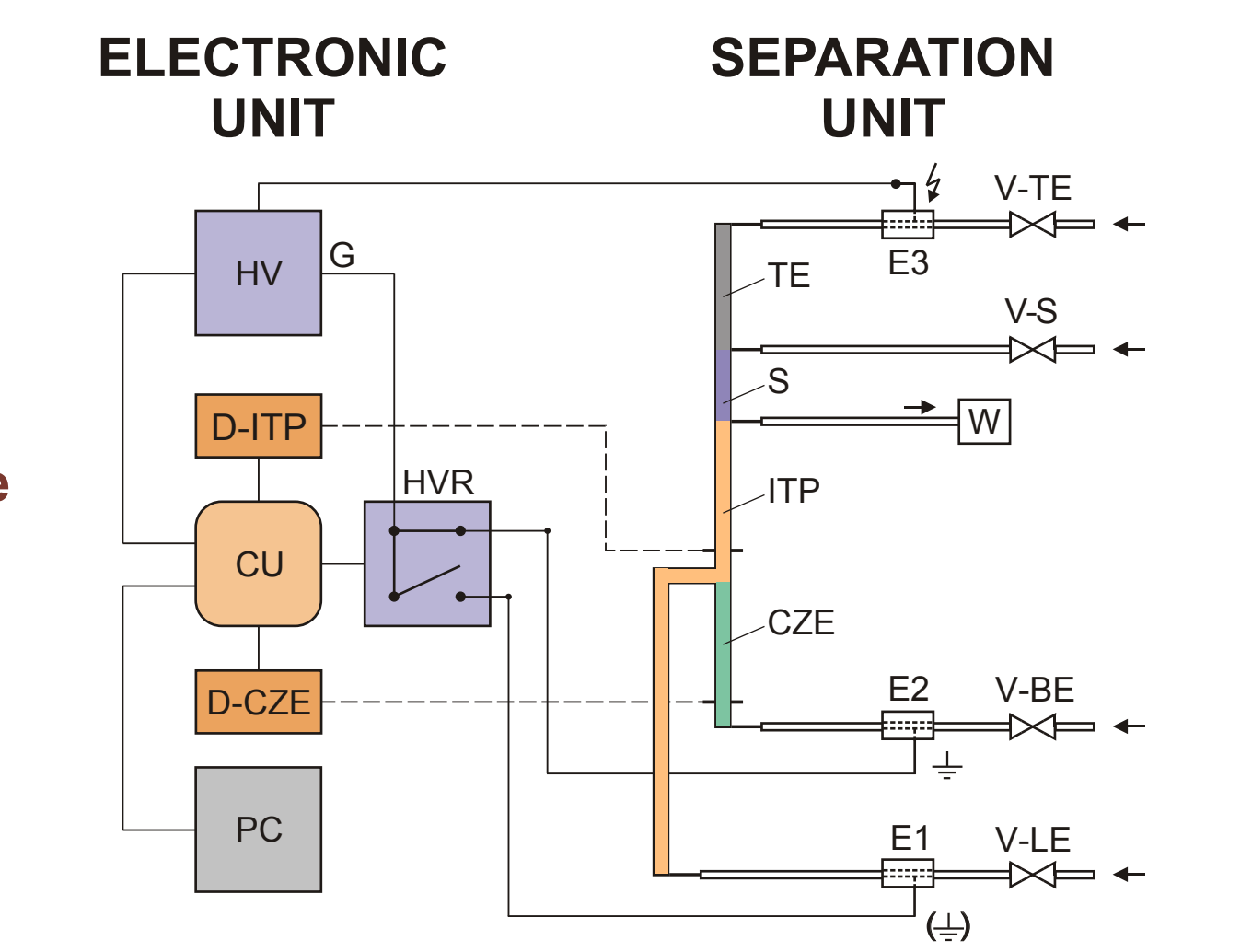
### ITP sample clean-up for CZE

#### ITP stage provides:

1. Well defined sample clean-up
2. Concentration of the trace and ultratrace analytes ( $10^2$ - $10^5$  times, in dependence on the sample volume)
3. Enhanced sample loadability to the separation system (by a factor of  $10^2$ - $10^4$  in comparison to a current single column CZE)

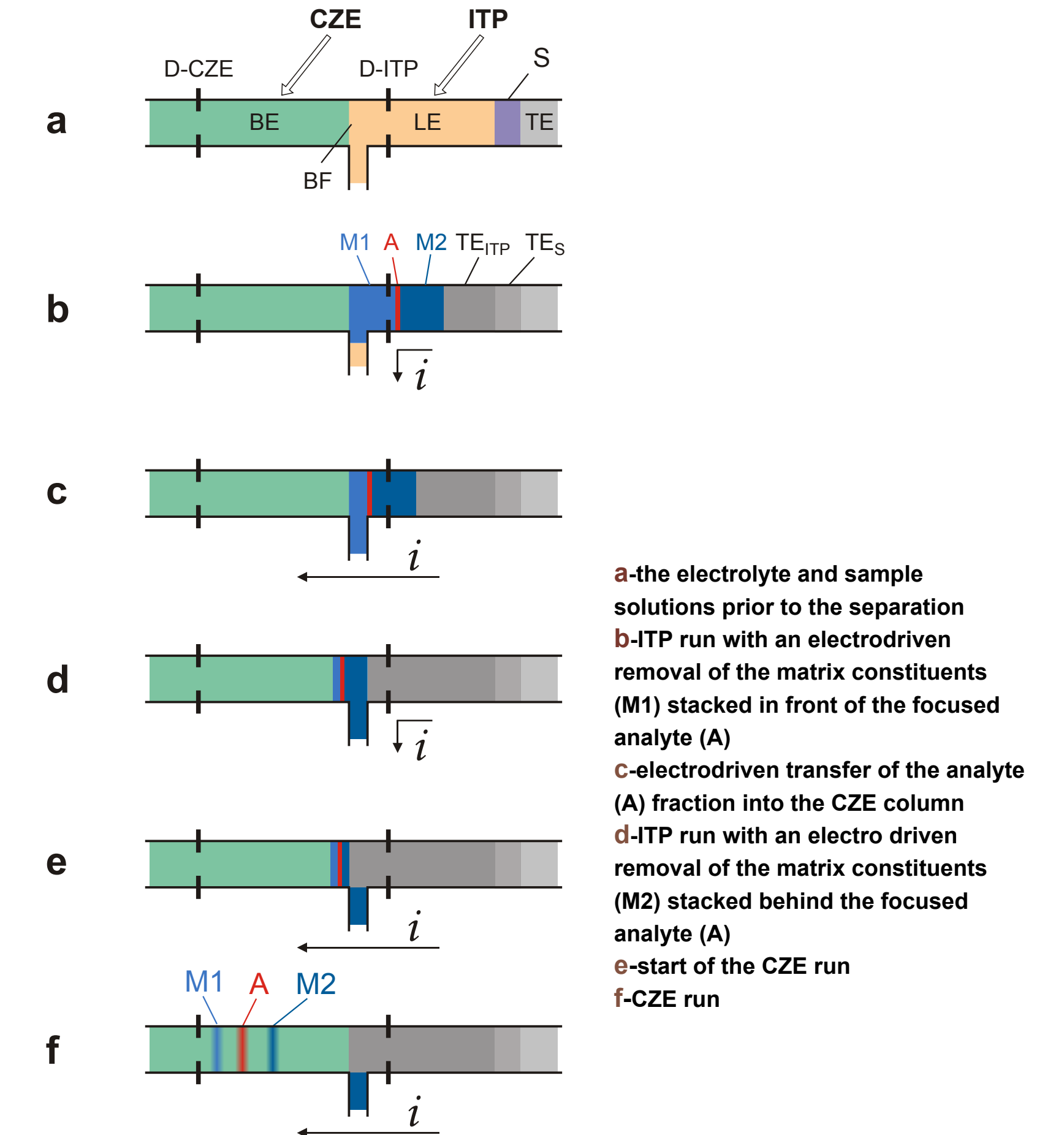
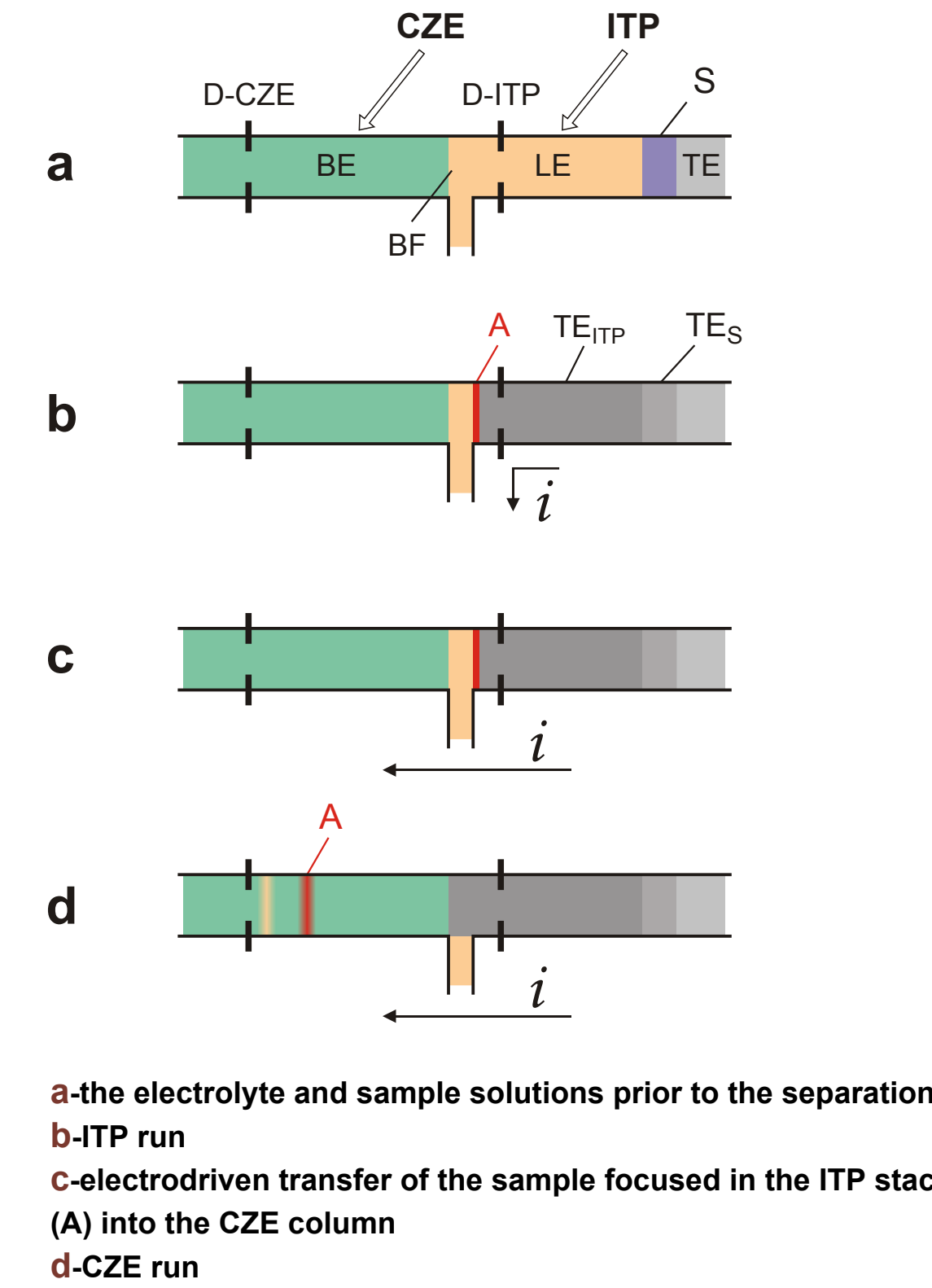
#### CZE stage provides:

4. Final separation of the analyte from the sample constituents transferred into this stage with the focused analyte
5. Detection of the analyte



HV-high voltage power supply  
HVR-high voltage relay for the column-switching  
D-ITP-conductivity detector in the ITP stage  
D-CZE-detectors in the CZE stage (conductivity, UV absorbance, fluorescence, DAD)  
CU-control unit

ITP- ITP column  
CZE-CZE column  
S- sample injector  
TE- the terminating electrolyte  
E1,E2,E3-driving electrodes  
V-TE,V-S,V-BE,V-LE- valves closing the separation system



## Examples of the use to the analysis of biosamples

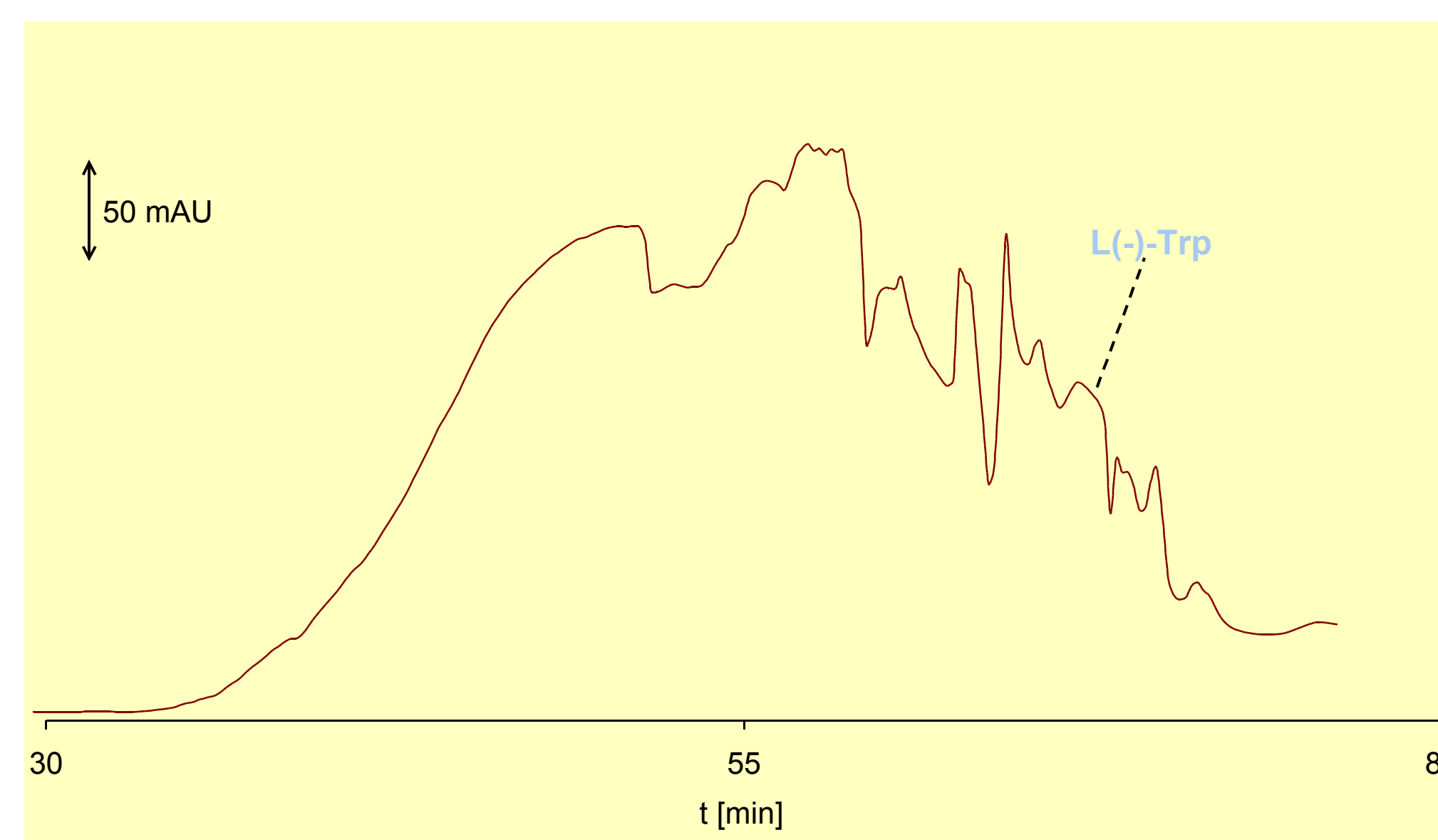
### Separation and detection of optical isomers in a urine matrix by ITP-CZE

Tryptophan enantiomers served as test analytes in experiments performed in the anionic mode of the separation (pH = 9). ITP, under achiral separating conditions, provided the sample pretreatment while CZE, under chiral separating conditions (alpha-cyclodextrin), served for the resolution of tryptophan enantiomers and their detection.

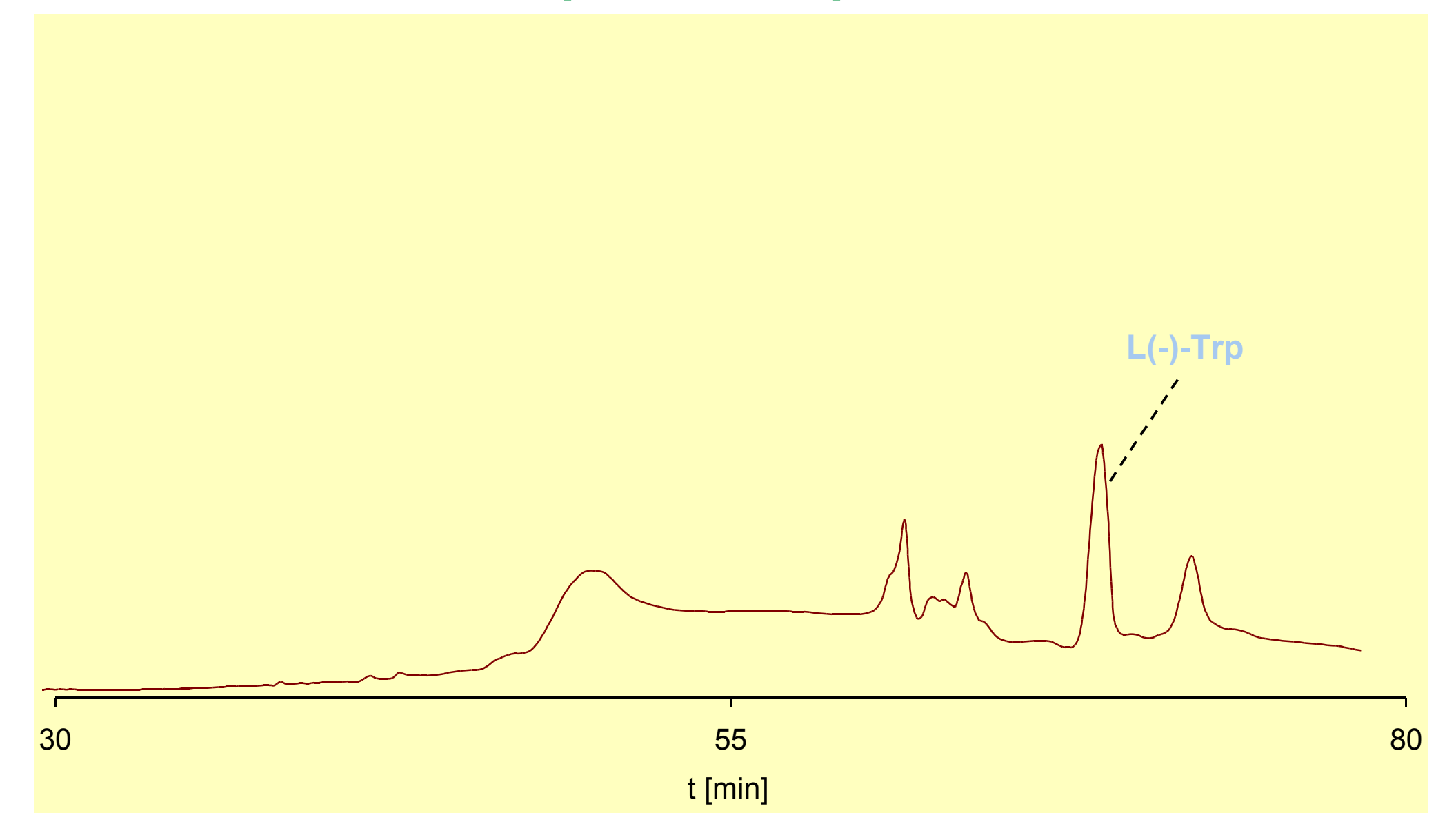
#### Main Results

- The concentration limits of detection for tryptophan enantiomers were at ~10 nmol/l concentrations (UV detector set at 220 nm and a 30 ul sample load).
- The two were baseline resolved in the CZE stage of the combination also when their concentration ratio in the sample was 1:200.
- The ITP stage quantitatively concentrated the analytes from 3-6 ul volumes of undiluted urine.
- Electropherograms show a key role of the ITP sample clean-up (a 99 % of the matrix removed by ITP before the analyte fraction was loaded into CZE).

#### ITP sample injection into CZE



#### ITP sample clean-up included



### Spectral identification of orotic acid in urine by ITP-CZE-DAD

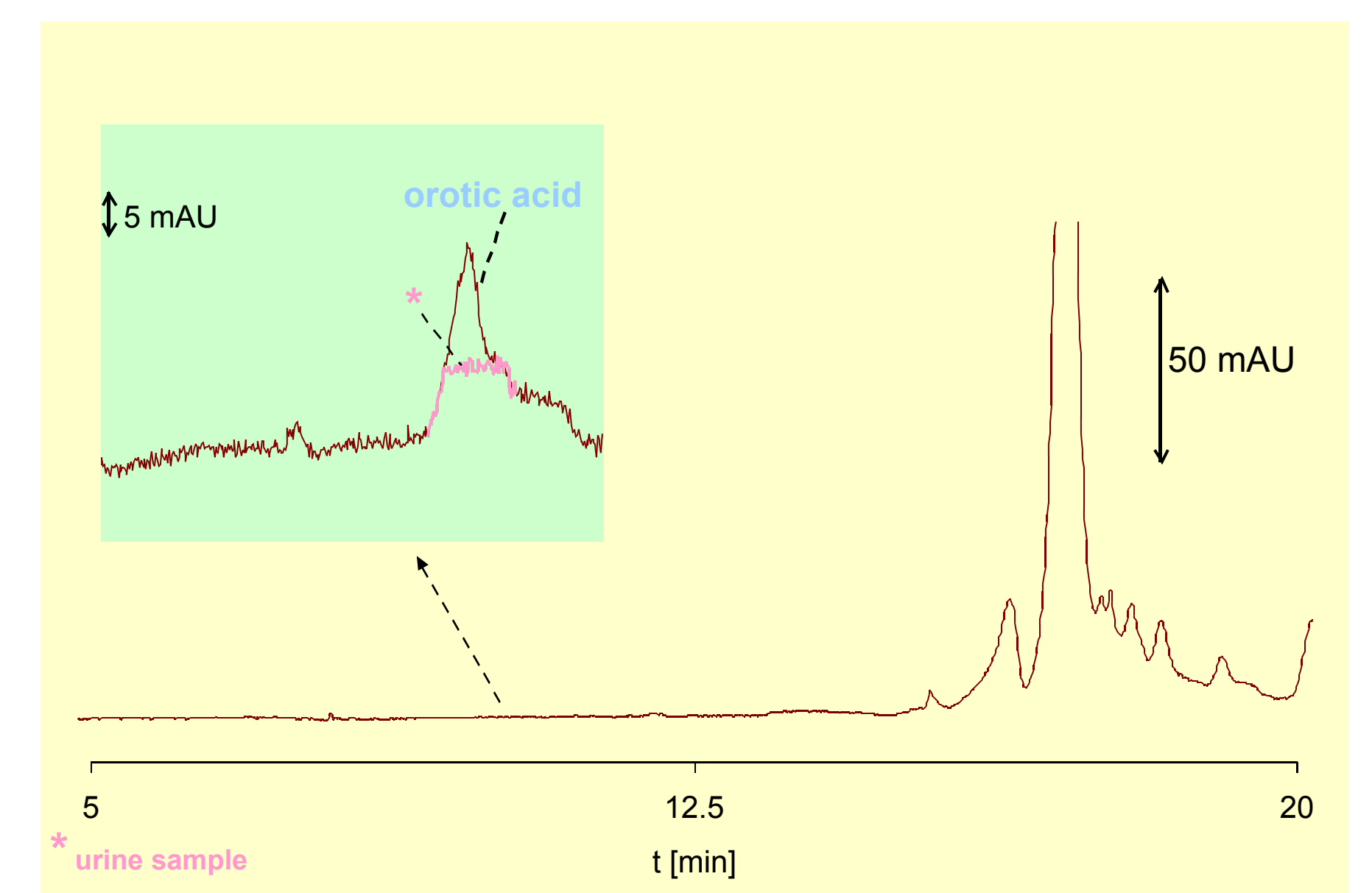
Urine served as a multicomponent, variable and high ionic strength matrix while orotic acid was used as a test analyte of a practical clinical relevance. The experiments were carried out at low pH (ITP at pH = 3.2, CZE at pH = 3.5) with diode array detection (DAD) to the detection and identification of orotic acid.

#### Main Results

- ITP enhanced the sample loadability of the separation system (a 30 ul sample injection volume), concentrated the analyte and provided an efficient sample clean-up (acid-base properties and host-guest complexation)
- CZE performed a final separation of orotic acid from residual matrix constituents and provided favorable conditions for its DAD detection and identification. Using current correction and smoothing procedures analytically relevant DAD spectra of orotic acid were obtained also in instances when this was loaded in a model sample at a 200 nmol/l concentration (an estimated limit of determination of orotic acid at 218 nm)
- DAD spectra of orotic acid (matching the reference spectrum) were acquired also in instances when the acid was present in urine matrices at 400-600 nmol/l concentrations. Here, residual trace matrix interferences prevented a closer approach to the value attained for the model samples.
- ITP-CZE-DAD may serve as an effective analytical tool for the identification and quantitation of orotic acid in clinically relevant samples (orotic aciduria).

#### Identity of orotic acid as confirmed by DAD

Concentration of orotic acid (mol/l)	Raw spectrum	Spectrum corrected for the background	Spectrum corrected for the background and smoothed
Urine sample	6.10 <sup>-7</sup>	873.9	993.4
		993.4	995.2



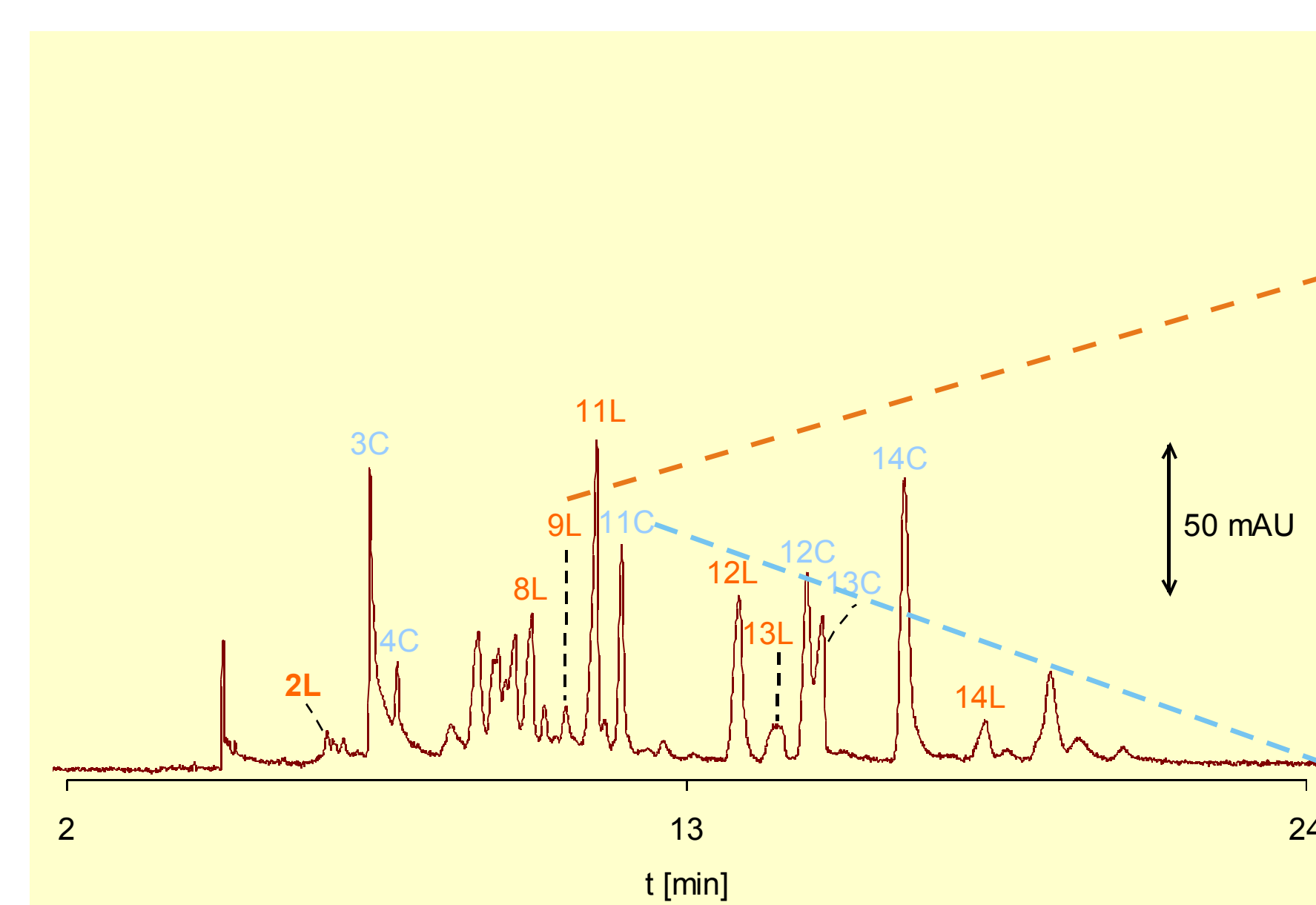
### ITP-CZE-DAD of tryptic digests of proteins

Analytical potentialities of the separative and detection tools of the ITP-CZE-DAD combination in peptide mapping were investigated. Experiments carried out in this context used the cationic mode of the separation (ITP at pH = 5.7, CZE at pH = 3.2).

#### Main Results

- ITP was found effective for an on-line desalting of the digest and, at the same time, to the concentration of the digest constituents before their CZE resolutions.
- CZE with on-column DAD detection provided maps at several detection wavelengths while the DAD data served for spectral identifications of the digest constituents.
- ITP-CZE runs with different digests, obtained for the same protein (Cytochrome C), revealed excellent agreements of the migration times of the digest constituents. Repeatabilities of the peak areas of these constituents assessed from the runs with one of the digests were significantly higher when related to those as obtained for the same constituents in different digests (digestion-to-digestion repeatability).
- The analyses of the digests of mixtures of Cytochrome C and beta-Lactoglobulin B indicate potentialities of the ITP-CZE-DAD combination in identifying proteins present in mixtures (see an accompanying electropherogram).

#### Tryptic digests of a Cytochrome C and beta-Lactoglobulin B mixture



C-Cytochrome C digest constituents  
L-beta-Lactoglobulin B digest constituents

#### Confirmations of the digest constituents identities

