

Capillary Isotachopheresis with Spectral Diode-Array Detection and Chemometry Data Processing

S. Strašík, M. Danková, M. Molnárová, M. Lučaníková, D. Kaniansky

Department of Analytical Chemistry, Faculty of Natural Sciences, Comenius University, Mlynská Dolina CH-2, SK-84215 Bratislava, Slovakia

AIMS OF THE WORK

This work investigated potentialities of the ITP-DAD combination with chemometry processing of the DAD spectral data. It was focused on a zone purity assessment and identity confirmations of the analytes in situations typical for the ITP separations of samples characterized by complex ionic matrices.

Migrations of the analytes in fully developed ITP zones and in boundary layers between the zones of the corresponding effective mobilities were investigated. The latter migration alternative is of a general interest for trace ITP analysis as it very likely operates with the smallest volumes into which the analyte(s) can be focused by electrophoresis separation process.

INSTRUMENTATION

An ITAChrom EA-101 capillary electrophoresis analyzer (J&M, Aalen, Germany) was employed in ITP experiments carried out in this work.

A TIDAS, multiwavelength photometric absorbance DAD detector (J&M) was connected to an on-column detection cell via optical fibers. The DAD detector operated with the following settings: (1) a 200–350 nm scanned wavelength range; (2) a 15 ms integration time; (3) a 0.2 s scan interval; (4) 1 accumulation per 1 scan interval.

ITP ELECTROLYTE SYSTEM

Parameter	LE*	TE*
Solvent	water	water
Leading/terminating anion	chloride	MES*
Concentration (mmol/l)	5	10
Counterion	ϵ -ACA*	ϵ -ACA*
Concentration (mmol/l)	38	146
EOF suppressor	m-HEC*	-
Concentration (% w/v)	0.2	-
pH	5.2	6

*LE = leading electrolyte; TE = terminating electrolyte; MES = 2-[N-morpholino]ethanesul-fonic acid; ϵ -ACA = 6-amino-n-caproic acid; m-HEC = methylhydroxyethylcellulose.

CHEMOMETRY

In chemometry data processing **Orthogonal Projection Approach (OPA)**, **Needle Search (NS)** and **Singular Value Decomposition (SVD)** gave initial information on the number of constituents present within an investigated time interval and on their approximate locations along the separation coordinate.

Alternating Least Squares (ALS) and **Factor Rotation (FR)** served for estimations of concentration profiles and spectra of the detected constituents.

Target Transformation Factor Analysis (TTFA), providing matches of the standard spectra (acquired from the ITP runs with individual analytes) with the spectral data along the ITP stack, served for identity confirmations of the analytes.

Data processing procedures were programmed in our laboratory using Mathematica for Windows (version 4.2, Wolfram Research, Champaign, IL, USA).

ANALYTE MIGRATING IN A FULLY DEVELOPED ITP ZONE

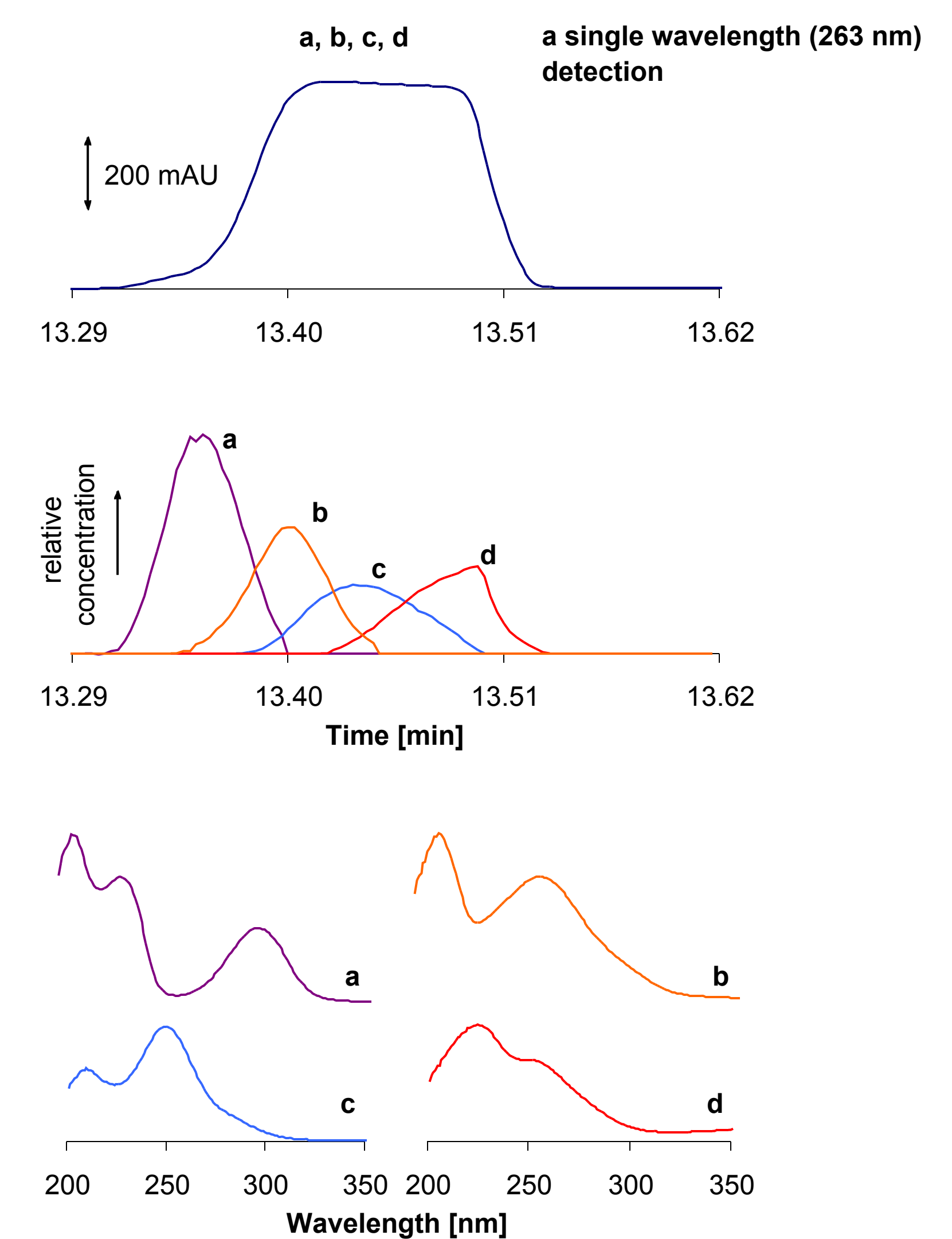
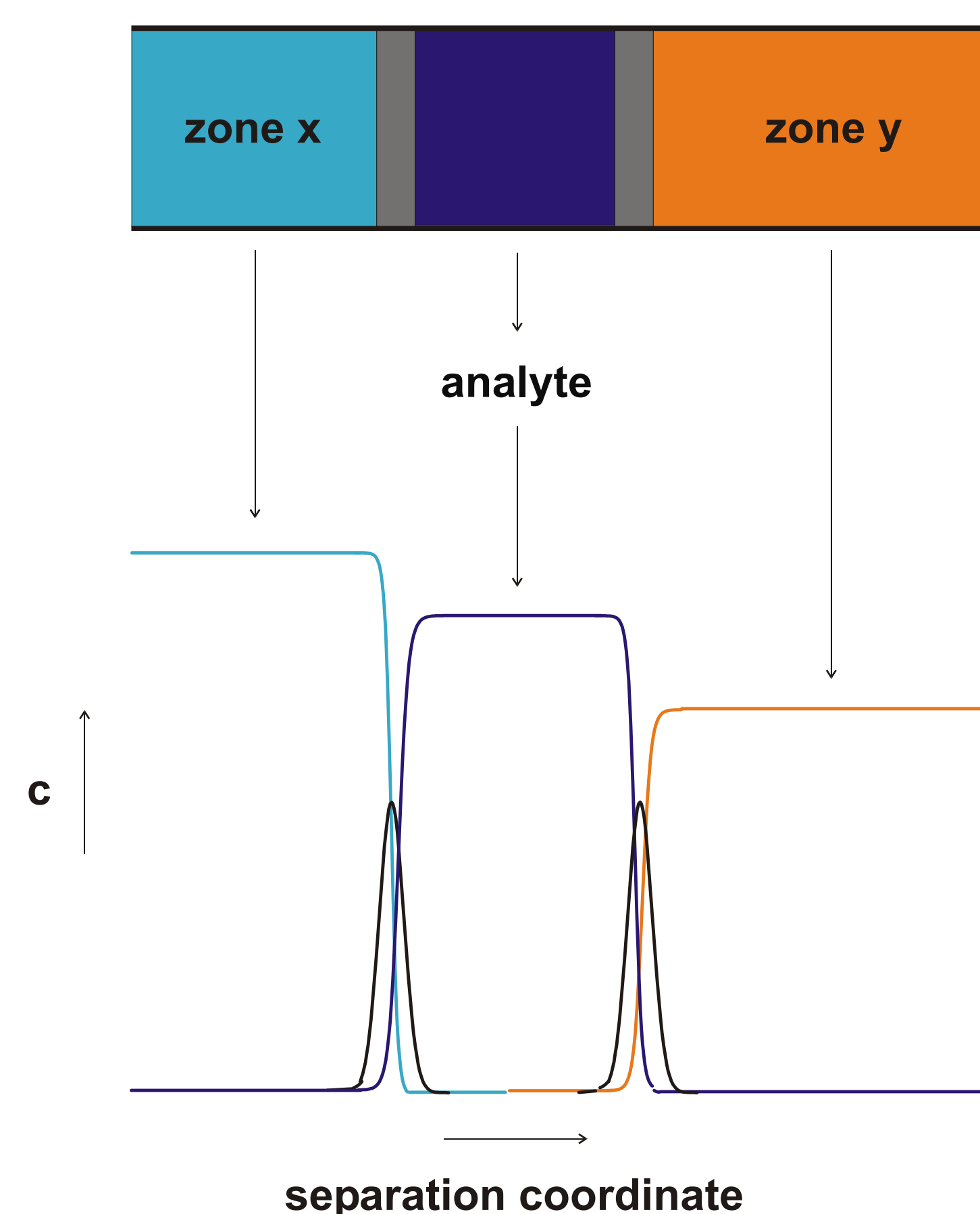


Fig. 1. Concentration distributions of the constituents along the ITP mixed zone as obtained by chemometry processing of the DAD spectral data.

a = salicylic acid (10^{-3} mol/l), b = 3-nitrobenzenesulfonic acid (10^{-3} mol/l), c = sulfanilic acid ($2 \cdot 10^{-3}$ mol/l), d = 2,6-dinitrophenol (10^{-3} mol/l). The sample containing these analytes was loaded by a 200 nl loop of the injection valve. The mixed zone was spaced from the leading and terminating zones by adipic and propionic acids, respectively. The driving current was stabilized at 15 iA and the DAD settings were: 0.2 s scan interval and 1 s accumulation. The distribution curves a–d (below the isotachopherogram), based on a chemometry resolution of the mixed zone, belong to the following analytes: salicylic acid (a), 3-nitrobenzenesulfonic acid (b), sulfanilic acid (c), 2,6-dinitrophenol (d). The spectra of the analytes as obtained by a chemometry procedure consisting of SVD and FR are below the distribution curves.

ANALYTE MIGRATING IN A BOUNDARY LAYER

A single wavelength detection (210 nm) provided an isotachopherogram in which the detected peak integrated signals of the light absorbing constituents migrating in the boundary layer.

The curves w–z (below the isotachopherogram) in Fig. 2, obtained by a chemometry resolution of the constituents present in the boundary layer, describe distributions of the anionic impurities along the boundary layer. The spectra of these impurities (below the distribution curves) were obtained by a chemometry procedure consisting of OPA and the multivariate curve resolution technique ALS.

The use of adipate and propionate as discrete spacers provided a spatial resolution of the anionic electrolyte impurities into different boundary layers. Their spectra, obtained directly (Fig. 3), matched the ones as acquired by chemometry processing of the spectral data (Fig. 2). Under such conditions analytes could be detected and identified by TTFA (Fig. 4).

RESOLVING TRACE IMPURITIES BY CHEMOMETRY

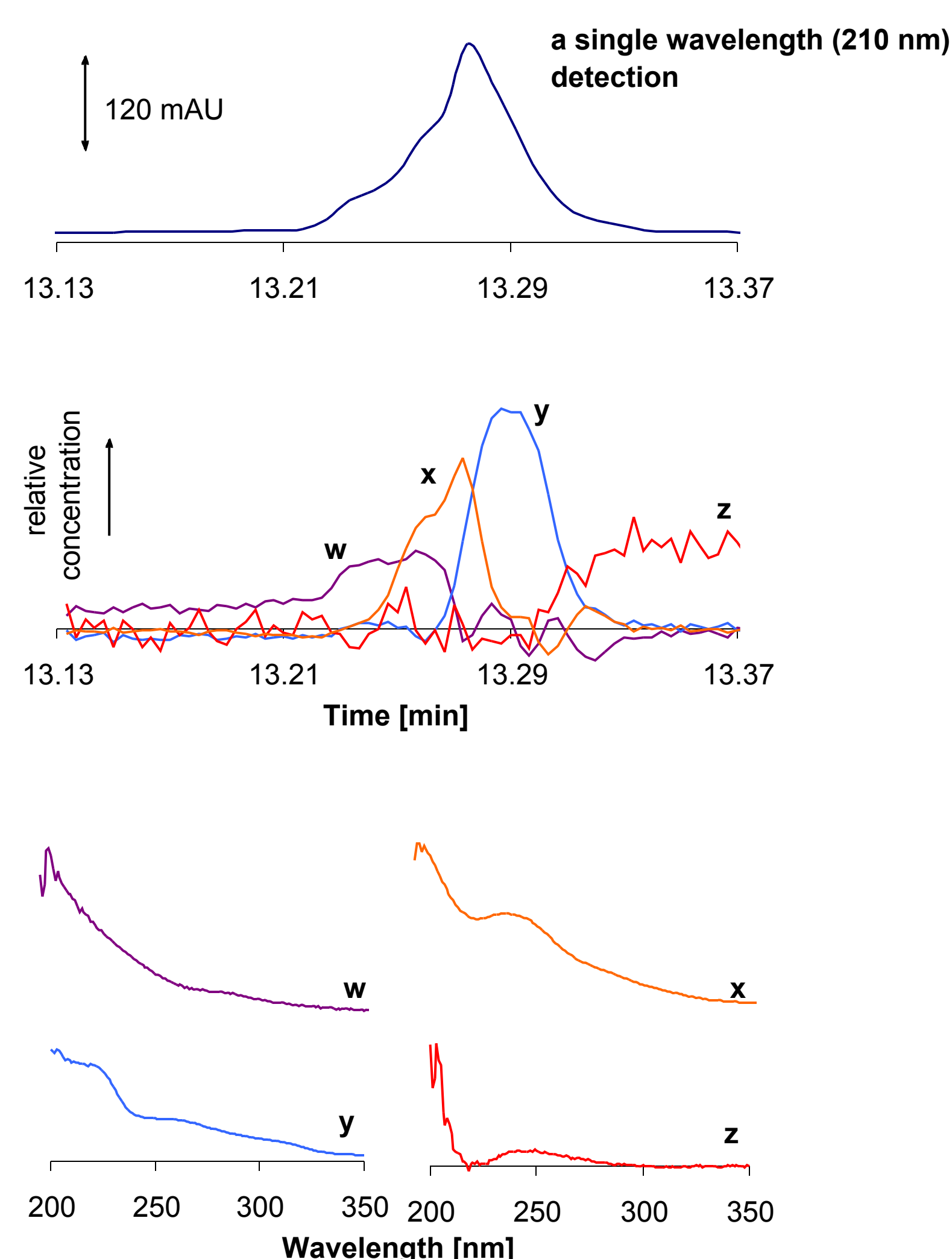
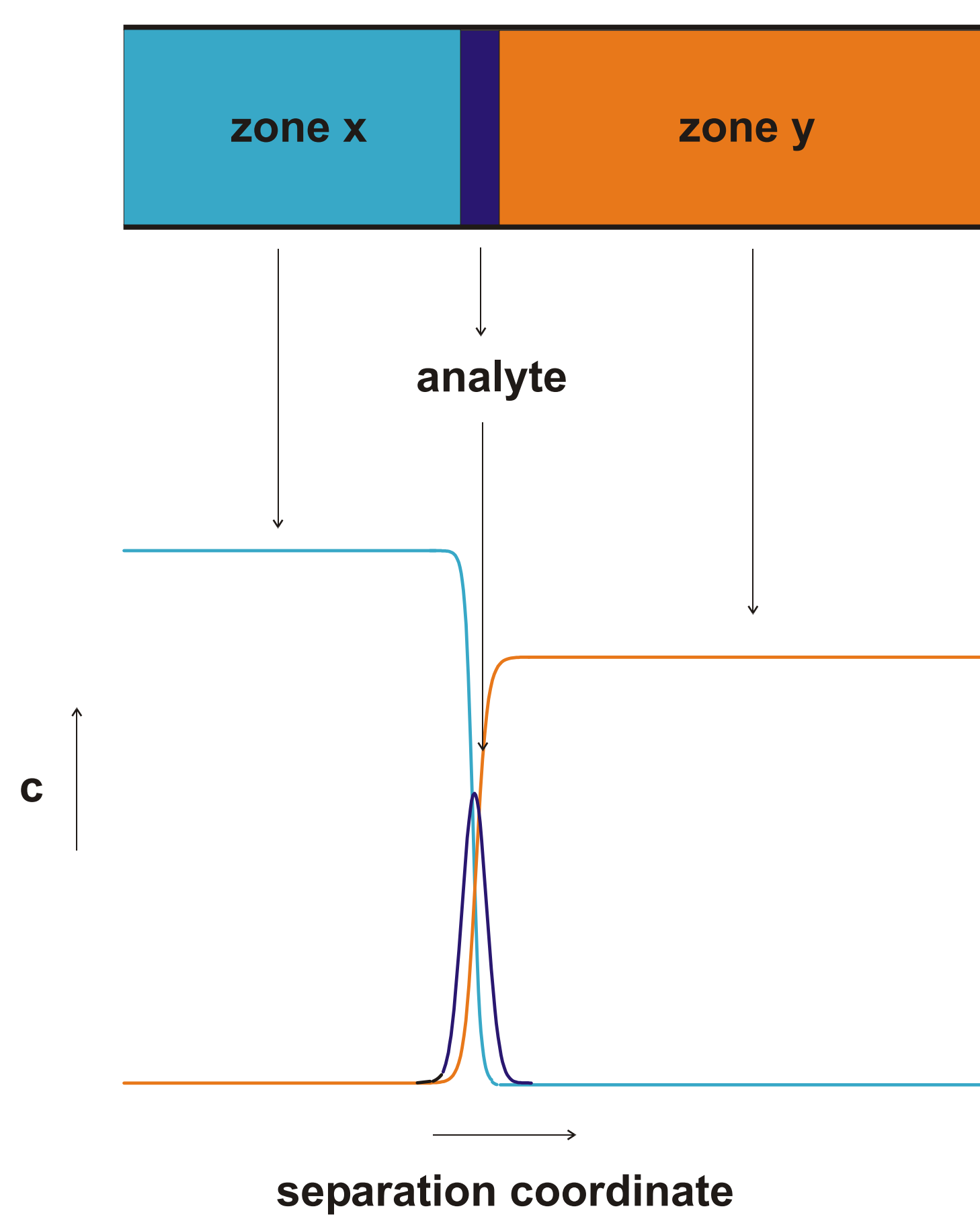


Fig. 2. A chemometry resolution of trace anionic impurities in the boundary layer.

The curves w–z (below the isotachopherogram), obtained by a chemometry resolution of the constituents present in the boundary layer between the leading and terminating zones, describe distributions of the anionic impurities along the boundary layer. The spectra of these impurities (below the distribution curves) were obtained by a chemometry procedure consisting of OPA and ALS.

RESOLVING TRACE IMPURITIES BY SPACERS

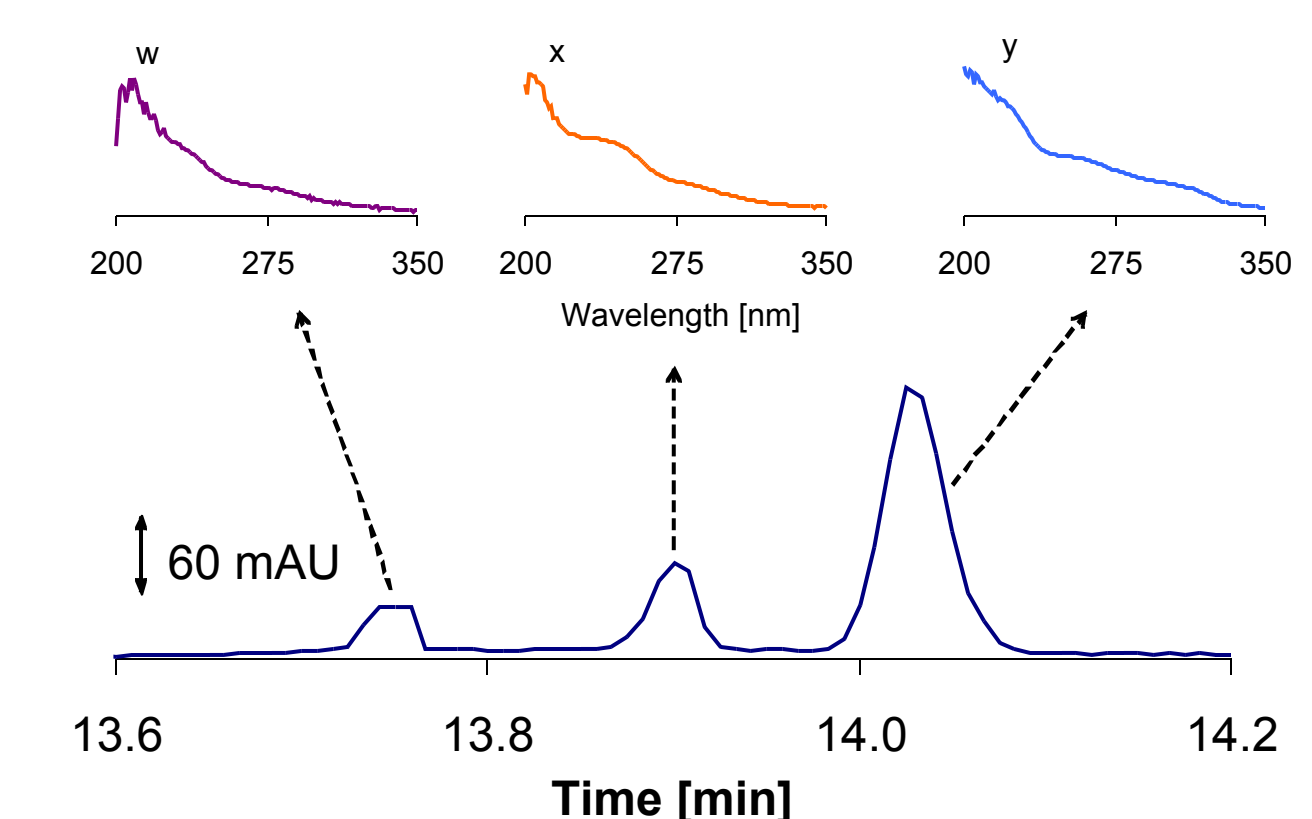


Fig. 3. ITP separation of adipate ($2 \cdot 10^{-3}$ mol/l) and propionate ($3 \cdot 10^{-3}$ mol/l) and identification of the anionic electrolyte impurities.

The sample containing these discrete spacers was loaded by a 200 nl loop of the injection valve. The driving current was stabilized at 15 iA, while the DAD settings were: a 0.2 s scan interval and 1 accumulation per 1 scan interval. The spacers provided three boundary layers along the ITP stack and these were proven to be homogeneous by chemometry means (OPA, SVD and NS). The spectra of the anionic electrolyte impurities (taken from the peak apexes) are shown in the upper part (w–y).

IDENTITY CONFIRMATION

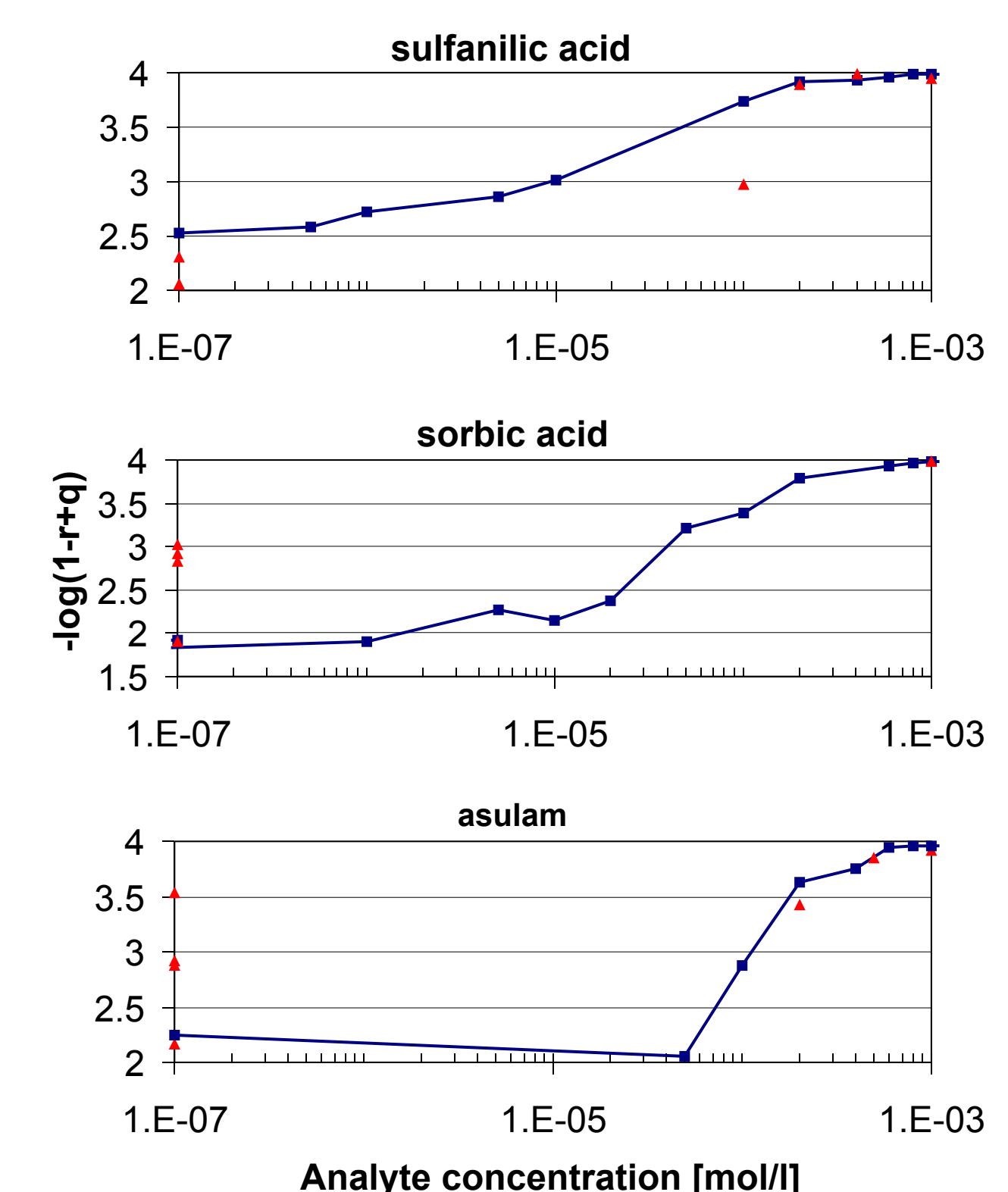


Fig. 4. Identity confirmation of the analyte migrating in the boundary layers using DAD with TTFA.

Dependences of the matching coefficient (logarithmic transformation of Pearson's correlation coefficient) between standard spectrum of the model analyte and its TTFA projection into the latent variables of investigated migration region on the analyte concentration.

CONCLUSIONS

Our experiments carried out with model analytes showed that DAD significantly enhances detectabilities of the light absorbing constituents in ITP.

The DAD detection can be beneficial in connections with three kinds of chemometry tools suitable to examining two-way data: multivariate curve resolution, identity confirmation and quantitation by multicalibration methods.

The use of multivariate curve resolution to fully developed ITP zones, for example, made possible to check a purity of the ITP zone and perform identity confirmation of the analytes with no additional experiments.

Unknown electrolyte impurities present in the boundary layer between the leading and terminating zones were shifted enough along the separation coordinate to obtain their concentration profiles and spectra in our experiments. Apparently, DAD combined with chemometry procedures provides a promising tool for investigations of the migrations of trace analytes focused into the ITP boundary layers.

The use of discrete spacers, intended to minimize spectral disturbances due to these electrolyte impurities, led to invincible restraints linked with reduced thicknesses of the boundary layers (multivariate resolution tools need not be effective in such instances). Nevertheless, confirmations of the identities of the analytes by TTFA were still possible. The multicalibration techniques were not examined in this work. Nevertheless, considering principles of the chemometry methods employed, their use seems applicable with no specific requirements.

ACKNOWLEDGMENTS

This work was supported by J&M (Aalen, Germany) under the project No. 720.830-1-21/9 and, in part, by a grant from the Slovak Grant Agency for Science under the project No. 1/0087/03. Two of us (S.S. and M.D.) thank for grants (UK/23/2003/, UK/220/2004 and UK/265/2004) awarded by the Rector of Comenius University.