

High-performance electromigration separation methods, capillary zone electrophoresis (CZE) and capillary isoelectric focusing (CIEF), have been applied to physico-chemical characterization of new synthetic low-molecular mass markers of isoelectric points. Amphoteric compounds on the basis of aminomethylnitrophenols, their derivatives and other structurally related substances were analyzed by CZE in a series of background electrolytes in a wide pH range, 1.86 – 11.18. From the measured pH dependencies of effective electrophoretic mobilities of analytes (beforehand corrected to reference temperature of 25 °C), their isoelectric points ( $pI$ ) were determined. In addition, using the non-linear regression analysis of the above dependencies, acid-base dissociation constants ( $pK_a$ ) of ionogenic groups of selected analytes were calculated. Subsequently, the analytes with sharply defined isoelectric points were analyzed by CIEF. CIEF confirmed applicability of these compounds as markers of isoelectric points for calibration of pH gradient in CIEF in the determination of  $pI$  of amphoteric compounds, especially peptides and proteins. The determined  $pK_a$  values of ionogenic groups in particular compounds will be utilized in the development of new  $pI$  markers with desired  $pI$  values.