DOCTORAL THESIS

Novel applications of comprehensive two-dimensional gas chromatography and capillary electrophoresis for the chiral discrimination

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Date of Award:
2007

Novel Applications of Comprehensive Two-dimensional Gas Chromatography and Capillary Electrophoresis for the Chiral Discrimination

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A thesis submitted in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

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Hong Kong Baptist University

July 2007

Abstract

The development of new and effective analytical tools for the sensitive and selective determination of enantiomers has become an important research topic in recent years.

In the thesis, the feasibility of employing two-dimensional gas chromatography (GC×GC) for the chiral discrimination of trace amounts of enantiomers present in complex herb matrices was demonstrated. The fundamental understanding of on-line preconcentration methods in capillary electrophoresis (CE) for chiral separations was obtained by studying the effects of the sample matrix and/or the running buffer on the stacking and the separation performance. Furthermore, a novel approach for the biological sample clean-up and concentration in CE, based on the coupling of acetonitrile deproteinization and salting-out extraction with acetonitrile stacking, was demonstrated for the separation and determination of chiral drugs present in urine with high enrichment factors and good precision.

In Chapter 2, the separation of ephedrine-type alkaloids and their enantiomers in raw herbs and commercial herbal products was investigated by carrying out enantioselective separation using GC×GC. Naturally occurring ephedrine-type alkaloids and their enantiomeric counterparts were adequately resolved from each other, as well as from potential interference in the sample matrices (raw herb materials and commercial herbal products), whereas single column GC analysis was unable to separate all the alkaloids of interest. Detection limits of 0.1 – 1.3 μg/mL and linearity of calibration with $R^2 \geq$

0.999 over the range of 0.5 – 100 μg/mL for the quantitative determination were obtained. The present GC×GC method is effective and useful for the determination of the principle ephedrine-type alkaloids in commercial health supplements and complex raw herb formulations, and for the differentiation of ephedrine-containing products that were derived from natural plant or synthetic sources.

In Chapter 3, large volume sample stacking using the electroosmotic flow pump (LVSEP), field-amplified sample injection with sample matrix removal using the EOF pump (FAEP) and acetonitrile stacking for the on-line concentration of cationic propranolol enantiomers have been developed and compared. With methanol as the sample solvent, FASP provided more than 10000-fold enhancements in sensitivity, together with a better reproducibility. The presence of salt in sample matrix had a deleterious effect on LVSEP and FAEP, whereas it benefited acetonitrile stacking. The acetonitrile stacking of cationic propranolol was achieved in the presence of mixture of acetonitrile and salts in the sample matrix by using triethanolamine buffer.

In Chapter 4, concurrent sample clean-up and enhancement in detection sensitivity for chiral capillary electrophoresis was demonstrated based on the coupling of salting-out extraction with acetonitrile stacking and the use of dimethyl-beta-cyclodextrin as the chiral selector for the sensitive and enantioselective separation of warfarin enantiomers. In this novel approach, acetonitrile was added into the biological samples for protein precipitation (acetonitrile deproteinization), followed by the addition of an appropriate

salting-out agent to effect phase separation via salting-out extraction. Additional sample enrichment was achieved by coupling (off-line) salting-out extraction with an (on-line) CE sample enrichment technique known as “acetonitrile stacking”. By optimizing the experimental condition, warfarin enantiomers can be efficiently extracted from the aqueous sample solution and a combined enrichment factor of higher than 1000 can be obtained.

In Chapter 5, the feasibility of using high concentration of salts in sample matrix to induce stacking of APTS-monosaccharide in CZE-LIF was demonstrated. Further improvement in stacking efficiency was obtained by adding acetonitrile in the sample matrix. More than 40 fold increase in the peak heights was obtained. Furthermore, an effective chiral MEKC-LIF procedure using mixed selectors has been developed for the analysis FITC-amino acids enantiomers. The on-line concentration of FITC-amino acids enantiomers in MEKC was achieved in the presence of either salts or acetonitrile-salts mixture in the sample matrix. However, the best stacking occurred when the sample matrix contained only salts, for which more than 10-fold increase in peak height was obtained. Possible mechanisms being responsible for the stacking of APTS-monosaccharides and FITC-amino acids were briefly discussed.

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