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Analysis of enantiomers in biological matrices by charged cyclodextrin-mediated capillary zone electrophoresis in column-coupling arrangement with capillary isotachophoresis

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Abstract

The possibility to apply charged chiral selector as buffer additive in capillary zone electrophoresis (CZE) on-line coupled with capillary isotachophoresis (CITP) was studied. Enantioseparations and determinations of trace (ng/ml) antihistaminic drugs [pheniramine (PHM), dimethindene (DIM), dioxopromethazine (DIO)] present in samples of complex ionic matrices (urine) served as model examples. A negatively charged carboxyethyl-β-cyclodextrin (CE-β-CD) was used as a chiral selector in analytical CZE stage following upon a sample pretreatment by CITP (preconcentration of the analytes from 5 to 20-times diluted urine samples, partial sample clean up removing macroconstituents from the sample matrices). A high recognition capability of the oppositely charged CE-β-CD was demonstrated by enantioselective retardation of the drugs in presence of micro-and semi-macroconstituents migrating in CZE stage and detectable by UV detector. In this way, enantiomers of the drugs could be easily separated and determined. Due to lack of interferences between the drugs and sample-matrix constituents in presence of charged CE-β-CD, demands on both spacers in CITP step and multiple column-switching were minimized. CITP-CZE method with charged selector appeared to be a useful analytical approach for the trace enantiomers in complex ionic matrices as it combined enhanced separation selectivity and sample loadabitlity with high separation efficiency and provided favorable performance parameters including sensitivity, linearity, precision, accuracy/recovery and robustness with minimal demands on sample preparation. Analysis of urine sample taken from a patient treated by PHM, showing concentration profile of PHM enantiomers and their metabolites, illustrated potentialities of the method in clinical research.

Keywords: Capillary isotachophoresis; Capillary zone electrophoresis; Column-coupling electrophoresis; Pheniramine; Dimethindene; Dioxopromethazine; Drug enantiomers; Charged cyclodextrin; Chiral analysis; Urine

1. Introduction

In pharmaceutical research, including chiral purity control, pharmacokinetic studies etc., analytical methods are requested to provide high resolution power, high separation efficiency and high sensitivity. The separation of enantiomers is currently carried out by using high-performance liquid chromatography (HPLC), gas chromatography (GC), capillary electrophoresis (CE) and capillary electrochromatography (CEC) [1–3].

CE is a very rapidly growing microseparation technique and this is mainly due to the following advantages: (i) high separation efficiency, (ii) short analysis time, (iii) very small buffer and sample volumes are required, (iv) environmentally friendly technique due to minor organic solvent consumption, (v) CE techniques are easily on-line combinable, (vi) diverse application range. It is well known that separations in CE are predominantly driven by efficiency while in HPLC by selectivity. A common approach to enhance separation selectivity in CE, approved in many cases, is based on the use of selector migrating in opposite direction towards analytes. In this way, separation window is significantly spread and resolution can be increased [1].

Cyclodextrins (CDs) have been used extensively in separation science because they have been shown to discriminate between positional isomers, functional groups, homologues and enantiomers [4]. This property as well as transparency of CDs in UV region of optical spectrum makes them one of the most

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useful agents for a wide variety of separations and therefore this group of chiral selectors was also used in our present work. Charged CD derivatives have been used as chiral selectors in CE for the first time by Terabe [5] and separation effect of charged CDs in CE has been explained in detail by Chankvetadze et al. [6]. Very recently we demonstrated high effectivity of negatively charged carboxyethyl-β-cyclodextrin (CE-β-CD) for the separation of pheniramine and dioxopromethazine enantiomers comparing separation effect of the charged and native form of β-cyclodextrin (β-CD) [7,8]. However, CZE in a single column-configuration has several limitations so that its application for more complex samples is usually depend on a sample preparation.

Main limitation of CZE with a UV absorbance detector is the relatively low sample concentration detection limit so that many trace components in real samples cannot be analyzed by this technique directly. The limit of detection (LOD) can be improved by several orders of magnitude when more sensitive detectors such as laser-induced fluorescence are used; as only a small number of analytes exhibit native fluorescence, derivatization has to be carried out in most cases. Another way to increase the separation capability, sensitivity and detectability of CZE is the use of on-line pre-concentration techniques, e.g. by performing the CITP-CZE combination in two coupled capillaries [9–14]. The coupled column arrangement is characterized by isotachophoresis in the first capillary, serving as an efficient pre-separation and concentration stage, followed by the on-line transfer of the sample cut into the second capillary where analytical zone electrophoresis proceeds as the second stage. Besides the pre-concentration of the analytes, the CITP step has several other specific features that are advantageous for CZE, such as the high sample load (depend on the internal diameter of the pre-separation capillary), transfer of a well-defined fraction of the sample into CZE, and an ideal sample injection for CZE (injection of a short stack of sharp zones of interest).

CZE separations of enantiomers with on-line CITP sample pretreatment have been carried out using neutral chiral selectors in separation electrolytes so far. As examples are given the separations of tryptophan enantiomers spiked into complex ionic matrices (90-component model mixture, urine) using native α-cyclodextrin [15,16]. The results from the runs with urine samples showed that only the CITP-CZE combination with a post-column on-line coupled CITP sample clean-up (responsible for a removal of more than 99% of the sample anionic constituents migrating in the on-line coupled CITP stack and detectable in the CZE stage) provided a universal alternative for the detection and quantitation of the model analyte [15].

The aim of the present work was to demonstrate potentialities of on-line coupled CITP-CZE method including charged chiral selector as buffer additive in analytical (CZE) stage. An enhancement of separation selectivity in comparison with a neutral selector-modified CE was expected maintaining other benefits of coupled CE methods (enhanced sensitivity, minimization of sample pretreatment, etc.). A simplification of working conditions (use of CITP spacers, multiple column switching) due to minimization of interferences between sample matrix constituents and the analytes transferred from CITP into CZE was expected too. An influence of separating conditions (pH, concentration of chiral selector, sample matrix concentration) on the enantioresolution of various drugs (pheniramine, PHM, dimethindene, DIM, dioxopromethazine, DIO), serving as model analytes, in urine, serving as complex ionic matrices, was investigated. Performance parameters of the proposed CITP-CZE method were evaluated and application example was shown.

2. Experimental

2.1. Instrumentation

A CS isotachophoretic analyzer (Villa-Labeco, Spišská Nová Ves, Slovak Republic), assembled in the column-coupling configuration of the separation unit, was used in this work. The separation unit consisted of the following modules: (i) an injection valve with a 30 μl internal sample loop; (ii) an CITP column provided with a 800 μm I.D. capillary tube made of fluorinated ethylene–propylene copolymer (FEP) and an on-column conductivity sensor; its total length was 90 mm; (iii) a CZE column provided with a 300 μm I.D. capillary tube made of FEP of 210 mm total length (160 mm to the photometric detector); (iv) a bifurcation block for an on-line coupling of the CITP and CZE columns; (v) a counter-electrode compartment with a hydrodynamically (membrane) closed connecting channel to the separation compartment. The columns were assembled in plexiglass cartridges for better dissipation of Joule heat.

The CZE column was provided with a LCD 2083 on-column photometric detector with variable wavelengths, 190–600 nm (Ecom, Praha, Czech Republic). In this work the photometric detector was set at 265 nm (PHM, DIM) or 240 nm (DIO) detection wavelengths. The signals from the detectors were led to a PC via a Unilab data acquisition unit (Villa-Labeco). ITP Pro32 Win software (version 1.0) obtained from KasComp (Bratislava, Slovak Republic) was used for data acquisition and processing.

Prior to the use, the capillary was not particularly treated to suppress an electroosmotic flow (EOF). A dynamic coating of the capillary wall by means of a 0.2% methylhydroxyethylcellulose (m-HEC 30 000; Serva, Heidelberg, Germany) in leading and background electrolyte solutions served for this purpose [17]. CITP and CZE analyses were carried out in cationic regime of the separation with direct injections of the samples. The experiments were performed in constant current mode [18] at 20 °C. The driving currents applied were 250 μA (CITP) and 120 μA (CZE) and the corresponding driving voltages were 1–2 kV (CITP) and 5–6 kV (CZE).

2.2. Chemicals and samples

The electrolyte solutions were prepared from chemicals obtained from Merck (Darmstadt, Germany), Aldrich (Steinheim, Germany), and Fluka (Buchs, Switzerland) in water demineralized by a Rowapure-Ultrapure water purification system (Premier, Phoenix, Arizona, USA). All chemicals used were of analytical grade or additionally purified by the usual methods.