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Direct quantitative determination of amlodipine enantiomers in urine samples for pharmacokinetic study using on-line coupled isotachophoresis-capillary zone electrophoresis separation method with diode array detection☆

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ABSTRACT

The present work illustrates possibilities of column-coupling capillary electrophoresis (CE-CE) combined with chiral selector (2-hydroxypropyl-β-cyclodextrin, HP-β-CD) and fiber-based diode array detection (DAD) for the direct quantitative enantioselective determination of trace drug (amlodipine, AML) in biological multicomponent ionic matrices (human urine). Capillary isotachophoresis (ITP) served as an ideal injection technique in CE-CE. Moreover, the ITP provided an effective on-line sample pretreatment prior to the capillary zone electrophoresis (CZE) separation. Enhanced separation selectivity due to the combination of different separation mechanisms (ITP vs. CZE–HP-β-CD) enabled to obtain pure zones of the analytes, suitable for their detection and quantitation. The DAD, unlike single wavelength UV detection, enabled to characterize the purity (i.e. spectral homogeneity) of the analytes zones. A processing of the raw DAD spectra (the background correction and smoothing procedure) was essential when a trace analyte signal was evaluated. Obtained results indicated pure (i.e. spectrally homogeneous) zones of interest confirming effective ITP–CZE separation process. The proposed ITP–CZE–DAD method was characterized by favorable performance parameters (sensitivity, linearity, precision, recovery, accuracy, robustness, selectivity) and successfully applied to an enantioselective pharmacokinetic study of AML.

1. Introduction

Calcium ions are needed for electrical activity for the contraction of cardiac and smooth muscle and conduction of nerve cell. Calcium channel blocker is a drug which inhibits the entry of excess calcium into cells and/or prevents calcium from the mobilization from intracellular stores, resulting in relaxation of blood vessel walls and cardiac muscle for blood to flow more freely, lowering blood pressure thereby reducing oxygen demand in the heart and relieving anginal pain. Amlodipine besylate (AML) is used in the treatment of hypertension and chronic stable and vasospastic angina. The chemical name is (RS)-3-ethyl-5-methyl-2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylate benzenesulphonate [1].

Enantioselective drug absorption, distribution, metabolism, elimination, or liberation studies are included among the most advanced problems being solved in pharmaceutical and clinical research [2–5]. It is due to a multicomponent character of biological matrices, a very low concentration of the analyte(s) among the matrix constituents, and identical physicochemical properties of enantiomers in an achiral environment. Among high performance separation techniques, capillary electromigration methods provide the best solution for the analytical enantioseparations of ionic compounds [6–8]. Here, a high-resolution power is given by (i) an extremely high peak efficiency and (ii) wide scale of various electromigration effects producing/enhancing (enantio)selectivity.

Capillary isotachophoresis (ITP) coupled on-line with capillary zone electrophoresis (CZE) provides very significant CE tool applicable for a complex ionic matrices [9,10]. Main benefits of ITP–CZE combination are: (i) compatible separation mechanisms providing different selectivity, (ii) ITP sample treatment (preconcentration, clean-up) and (iii) ITP enhancing a sample load capacity [11–14]. These factors considerably reduce (i) the concentration limits of detection (cLOD) when compared to current (single column) CZE, and (ii) external sample preparation.

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Spectral detection is useful when analyzed complex ionic mixtures. In many cases, diode array detection (DAD) has appeared to be a simple solution in a preliminary characterization of electropherograms in *z* axis (absorbance vs. migration time vs. wave length, *x*–*y*–*z*), see e.g. analysis of drugs in body fluids [15,16]. In this way DAD is expected to enhance reliability of results reflected in validation parameters (the ICH guideline [17]).

Some general concepts for CE enantioseparations of dihydropyridine derivatives by means of cyclodextrins (CDs) have been reported in refs. [18,19]. AML enantiomers have been successfully separated using CE and HPLC techniques with charged and uncharged CDs as chiral selectors or chiral stationary phases [20–23]. AML was enatiomerically resolved in ca. 22 min using phosphate buffer with pH 3 and 5 mmol/l hydroxypropyl-β-cyclodextrin (HP-β-CD), 50 mmol/l tetraethylammonium-chloride and 2 mol/l urea as buffer additives [20]. Another work [21] compared CE enantioseparation of AML using 20 mmol/l HP-β-CD and the anionic CDs, sulphobutylether-β-CD (1 mmol/l) and carboxymethyl-β-CD (2.5 mmol/l). The anionic CDs were shown to offer an enhanced enantioselectivity over the neutral CD in CE systems with electroosmotic flow (EOF). α-CD was shown to be enantioselective towards AML too [22]. However, there is only one paper dealing with an enantioselective CE determination of AML in physiological samples (human serum) [23]. The AML enantiomers were separated in ca. 10 min using phosphate buffer (75 mmol/l, pH 2.5) containing 15 mmol/l HP-β-CD. The range of quantitation for both enantiomers was 2.0–16.0 μg/ml. Intra-day and inter-day relative standard deviation (RSD; $n = 5$) was <10%. The limits of detection (LOD) and quantification (LOQ) of the AML enantiomers, at 214 nm, were approximately 0.5 and 0.7 μg/ml, respectively (S/N = 3 and 10, respectively; 5-s injection). Recovery was always >85%. The samples have been analyzed after their pretreatment (extraction procedure).

The aim of the present work was to develop an enantioselective ITP–CZE–DAD method for a highly sensitive determination of trace AML enantiomers in complex ionic matrices (human urine), useful for the enantioselective pharmacokinetic study. One of the main benefits of the ITP–CZE–DAD method should also be a possibility to perform direct analyses of samples avoiding any sample preparation procedure and, by that, enhancing reliability of analyses.

2. Experimental

2.1. Instrumentation

A capillary electrophoresis analyzer EA-101 (Villa-Labeco, Spišská Nová Ves, Slovakia), assembled in the column-coupling configuration of the separation unit, was used in this work for performing the ITP–CZE runs. The samples were injected by a 30 μl internal sample loop of the injection valve of the analyzer. An ITP column was provided with an 800 μm I.D. fused silica capillary tube of a 90 mm total length and a contactless conductivity detector. A CZE column was the same as the ITP one except for a 320 μm I.D. and a 160 mm total length.

A multiwavelength photometric absorbance diode array detector Smartline PDA Detector 2800 (Knauer, Germany) was connected to an on-column photometric detection cell, mounted on the CZE column, via optical fibers. The detector operated under the following conditions: (1) scanned wavelength range 200–800 nm; (2) integration time 6 ms; (3) scan interval 0.2 s; (4) number of accumulations 1.

Prior to the use, the capillaries were not treated by any rinsing procedures to suppress an electroosmotic flow. A dynamic coating of the capillary wall by means of hydroxyethylcellulose (HEC 30 000; Serva, Heidelberg, Germany) in leading and background electrolyte solutions served for this purpose. The separating electrolytes in the capillaries were replaced by the fresh ones between each run. ITP and CZE analyses were carried out in the cationic regime of the separation (i.e. cathodic movement of the analytes) with direct injections of the samples. The experiments were performed in constant current mode at 20 °C. The driving currents applied were 250 μA (ITP) and 150 μA (CZE).

2.2. Data evaluation and performance parameters

The absorption maximum wavelength (238 nm) of AML was used for the evaluation of analytical parameters of the optimized method. Performance parameters of the method were evaluated according to the ICH guideline [17]. Peak area of AML was corrected for the migration time [24].

Parameters of calibration lines for AML enantiomers were calculated by using QCExpert ver.2.5 statistical software (Trilobyte, Prague, Czech Republic).

Limit of detection (LOD) and limit of quantification (LOQ) were calculated as the ratio of standard deviation of y-intercept of regression line (s_a) and the slope of the regression line (b) multiplied by factor 3.3 (LOD) or 10 (LOQ). Concentrations of AML taken for the calibration lines are given in Section 2.5.1.

Precision was evaluated as the repeatability which is expressed via relative standard deviation of (i) peak areas measured within the concentration range of calibration line and (ii) migration times of AML.

Recovery was evaluated by spiking of blank urine and water samples with AML at three different concentration levels (see Section 2.5.1) and comparing peak areas of AML obtained in different matrices. Accuracy (expressed via relative error, RE) was evaluated through the recovery test.

Robustness test examined the effect that deliberate variations in operational parameters (concentration of the complexing agent (48–52 mg/ml), leading (19–21 mmol/l) and carrier (48–52 mmol/l) cation, pH ± 0.1) had on the analysis results (enantioresolution, *R*).

2.3. Processing and comparing of DAD spectra

The migration and spectral data were acquired and processed by a EuroChrom program (version 3.05, Knauer).

The background correction (subtraction of background spectrum from the raw spectrum of the analyte) [25] was carried out to minimize the impact of the electrolyte system on AML spectrum. Such corrected spectrum was further smoothed by the procedure of Savitzky–Golay [26] (implemented in EuroChrom software) with a 5-point window.

Homogeneity of spectra of AML enantiomers was expressed via Pearson's correlation coefficients (PCCs) [27]. The value of PCC higher than 0.99 is assumed to provide an acceptable certainty in a confirmation of the identity of the analyte [25], i.e. a match of the tested (AML in urine) and reference (AML in water) spectrum.

2.4. 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, U.S.A.). All chemicals used were of analytical grade or additionally purified by the usual methods. The solutions of the electrolytes were filtered before use through disposable membrane filters of a 1.2 μm pore size (Millipore, Molsheim, France).