Separation and Purification Technology 158 (2016) 24–30

Contents lists available at ScienceDirect

Separation and Purification Technology

journal homepage: www.elsevier.com/locate/seppur

Direct CE and HPLC methods for enantioseparation of tryptophan and its unnatural derivatives

Martina Riesová, Radim Geryk, Květa Kalíková *, Tereza Šlechtová, Markéta Voborná, Monika Martínková, Adéla Bydžovská, Eva Tesařová

Charles University in Prague, Faculty of Science, Department of Physical and Macromolecular Chemistry, Prague, Czech Republic

ARTICLE INFO

Article history:
Received 31 August 2015
Received in revised form 27 November 2015
Accepted 7 December 2015
Available online 8 December 2015

Keywords:
Enantioseparation
Tryptophan
Unnatural amino acids
HPLC
CE

ABSTRACT

Tryptophan and its eight derivatives considered in this work are biologically important compounds. Since their enantiomers can exhibit different behavior, efficient enantioselective separation methods are needed for both analytical and preparative purposes. In capillary electrophoresis cyclodextrins and their derivatives were proved to be suitable chiral selectors. Two pH values of background electrolytes were tested in order to affect ionization of the analytes and consequently their enantioseparation. Enantiomers of all analytes in this study were baseline separated within 8 min using capillary electrophoresis. However, different separation systems/conditions were required. In HPLC various separation modes and columns (based on derivatized polysaccharides, cyclofructan, cyclodextrin and teicoplanin) were used. The best results of enantioseparation of tryptophan and its amphoteric derivatives were achieved with teicoplanin based chiral stationary phases and methanol as a mobile phase. Proposed conditions were suitable even for purification purposes. This study can serve as a tool for simplifying the method development for enantioseparation of tryptophan and its derivatives.

1. Introduction

In a large number of biochemical processes the essential amino acid (AA) tryptophan (Trp) plays a crucial role [1]. The indolic side chain of tryptophan residue has unique structural and chemical properties. Trp and its unnatural (unusual) derivatives are important building blocks for the total synthesis of various products and development of new drugs, biological probes, and chiral small molecule catalysts [2–4]. Introduction of the unusual AAs in peptide chains has attracted a considerable interest to overcome the pharmacological limitations of bioactive peptides [5,6]. Various specific applications of Trp derivatives can be found in the literature. For example, 5-hydroxy Trp can serve as an effective antioxidant and radioprotector [7]; L-enantiomer of tryptophanol is used as a chiral synthon to assemble indolo[2,3-*a*]quinolizidine alkaloids [8]; L-Trp methyl ester was used as starting compound for synthesis of compounds with antimicrobial activity [9], various esters of tryptophan can be used for synthesis of antimicrobial peptides [10]; *N*-BOC-Trp (*N*-(tert-butoxy)carbonyl-Trp) is used in peptide synthesis [11,12].

Unnatural AAs, as many other pharmaceutical substances, can be chiral. They are often synthesized as racemates if prepared by non-asymmetric synthesis. For this reason, enantioseparation and purification are essential before further application. CE and HPLC techniques represent good choices for solving these tasks [13–22].

The aim of this work was to screen possibilities for the development of fast and effective separation environments of CE and HPLC for Trp and its structurally related but different derivatives. The analytes of our interest were D,L-Trp, D,L-Trp methyl ester (MET), D,L-Trp butyl ester (BUT), D,L-Trp benzyl ester (BEN), D,L-Trp octyl ester (OCT), 5-F-D,L-Trp (5FL), 5-OH-D,L-Trp (5OH), *N*-BOC-D,L-Trp (BTrp) and D,L-tryptophanol (OL). The structures are shown in Supplementary material, Fig. S1. Development and optimization of CE methods were focused especially on design of fast analyses for the enantiomeric purity screening. Developed HPLC methods are proposed for enantioseparation and further purification of Trp and its derivatives for semipreparative purposes. Widely used

Abbreviations: AcOH, acetic acid; ACN, acetonitrile; BEN, D,L-Trp benzyl ester; BOC, *N*-(tert-butoxy)carbonyl; BTrp, *N*-BOC-D,L-Trp; BUT, D,L-Trp butyl ester; CF, cyclofructan; CS, chiral selector; CSP, chiral stationary phase; DS, degree of substitution; EA, ethanolamine; HEX, n-hexane; IPA, propane-2-ol; MeOH, methanol; MET, D,L-Trp methyl ester; OCT, D,L-Trp octyl ester; OL, D,L-tryptophanol; 5FL, 5-F-D,L-Trp; 5-OH, 5-OH-D,L-Trp; TEA, triethylamine.

* Corresponding author at: Faculty of Science, Department of Physical and Macromolecular Chemistry, Charles University in Prague, Albertov 2030, CZ-128 43 Prague 2, Czech Republic.

E-mail address: kveta.kalikova@natur.cuni.cz (K. Kalíková).

http://dx.doi.org/10.1016/j.seppur.2015.12.012
1383-5866/

cyclodextrin (CD) and its derivatives [23–25] were used as chiral selectors (CSs) in CE experiments. For HPLC analyses, application of different types of chiral stationary phases (CSPs) and separation modes was essential for successful enantioseparation due to significant structural differences of tested analytes.

2. Experimental

2.1. Chemicals and reagents

Methanol (MeOH, Chromasolv®, gradient grade, for HPLC, ⩾99.9%), acetonitrile (ACN, Chromasolv®, gradient grade, for HPLC, ⩾99.9%), *n*-hexane (HEX, Chromasolv® for HPLC, ⩾97%), propane-2-ol (IPA, Chromasolv® for HPLC, ⩾99.8%), ammonium acetate (purity ⩾ 99%), acetic acid (AcOH, purity > 99.8%), ammonium hydroxide solution (ACS reagent, 28–30%), ethanolamine (EA, ACS reagent ⩾ 99.0%), triethylamine (TEA, minimum 99%), trifluoroacetic acid (TFA, 99%), dimethylsulfoxide (DMSO, ACS reagent, 99.9%), lithium hydroxide monohydrate, (2-hydroxypropyl)-β-cyclodextrin (HP-β-CD) of 0.8 M substitution and average M_r = 1460, heptakis(2,6-di-O-methyl)-β-cyclodextrin (DM-β-CD), β-cyclodextrin (β-CD), 6-O-α-maltosyl-β-cyclodextrin hydrate (Malt-β-CD) and sulfated β-cyclodextrin (S-β-CD) sodium salt (12–15 mol per mol β-CD) were supplied by Sigma Aldrich (St. Louis, USA). Orthophosphoric acid 85% was purchased from Lachema (Neratovice, Czech Republic) and 0.1 M sodium hydroxide solution was product of Agilent Technologies (Waldbronn, Germany). Sulfated α-cyclodextrin (S-α-CD) sodium salt (DS (degree of substitution) ∼12), sulfated γ-cyclodextrin (S-γ-CD) sodium salt (DS∼14) and heptakis(6-amino-6-deoxy)-β-cyclodextrin (A-β-CD) heptahydrochloride were purchased from CycloLab (Budapest, Hungary). Water for solution preparation was deionized by the Watrex Rowapur and Ultrapur system (Prague, Czech Republic). Tryptophan and its derivatives were obtained as follows: D-tryptophan methyl ester hydrochloride, L-tryptophan methyl ester hydrochloride, D-tryptophan benzyl ester, L-tryptophan benzyl ester, D-tryptophanol, 97%, L-tryptophanol, 97%, D,L-tryptophan, 99%, 5-fluoro-D,L-tryptophan were purchased from Sigma–Aldrich (St. Louis, USA). D,L-tryptophan butyl ester hydrochloride was obtained from Pfaltz&Bauer (Waterbury, USA) and D,L-tryptophan octyl ester from Santa Cruz Biotechnology (Heidelberg, Germany). 5-hydroxy-D,L-tryptophan was purchased from Molekula (München, Germany) and *N*-α-BOC-L-tryptophan, *N*-α-BOC-D-tryptophan from Fluka (Buchs, Switzerland).

2.2. Instrumentation

CE experiments were carried out using Agilent 3D^{CE} capillary electrophoresis instrument operated by ChemStation software from Agilent Technologies (Waldbronn, Germany). Detection was performed with a built-in diode array detector at a wavelength of 217 nm. Fused silica capillary with i.d. of 50 μm and o.d. of 375 μm (Polymicro Technologies, Phoenix, AZ) was used. The total and effective lengths of the capillary were 49.5 cm and 41.0 cm, respectively.

All chromatographic measurements were performed on Waters Alliance System (Waters, Milford, USA) composed of Waters 2695 Separation Module, Waters 2996 Photodiode Array Detector, an autosampler 717 Plus, and Waters Alliance Series column heater, controlled by Empower software, which was used for data acquisition and analyses. In this study, following columns were used: two teicoplanin-based columns Astec CHIROBIOTIC™ T (T) and Astec CHIROBIOTIC™ T2 (T2); a cyclodextrin-based CSP containing *R,S*-hydroxypropylether derivatized β-CD - Astec CYCLOBOND™ I 2000 HP-RSP column (HP CD) (SUPELCO®, Bellefonte, USA); two cyclofructan (CF) -based columns Larihc CF7-DMP and Larihc CF6-RN (AZYP, Arlington, TX, USA) containing 3,5-dimethylphenylcarbamate functionalized cyclofructan 7 (CF7) and *R*-naphthylethyl carbamate modified CF6, respectively; two polysaccharide-based columns with guard columns, CHIRALPAK AD-RH (AD RH) and CHIRALPAK IB (IB) obtained from Chiral Technologies Europe (Illkirch, France), containing tris(3,5-dimethylphenylcarbamate) of amylose and tris(3,5-dimethylphenylcarbamate) of cellulose, respectively. All tested columns and guard columns were sized 250 × 4.6 mm i.d. and 10 × 4.6 mm i.d, respectively, both particle size 5 μm.

2.3. Software

Computer program PeakMaster 5.3 [26,27] was used to design and optimize the composition of buffers for CE and HPLC measurements and for calculation of their ionic strength and the theoretical pH values. For CE data evaluation programs Origin 8.1 (OriginLab Corporation, Northampton, MA) and Microsoft Office Excel 2010 were used. MarvinSketch online calculator (ChemAxon Kft., Budapest, Hungary) was utilized for acid dissociation constants estimations. Resolution *R* was calculated by the means of the operating software of CE and HPLC instruments.

2.4. Procedures

All CE and HPLC experiments were carried out in triplicates. The temperature of capillary or column was maintained at 25 °C. All buffers employed in CE and HPLC experiments were filtered with Minisart syringe filters (Sartorius Stedim Biotech, Goettingen, Germany), pore size 0.45 μm. Stock solutions of samples for both CE and HPLC experiments were prepared in concentration of 1 mg/mL using MeOH for MET, BUT, OCT, BEN, BTrp, OL or MeOH/water 80/20 (*v*/*v*) for Trp, 5FL, 5OH as solvents.

2.4.1. CE measurements

A new capillary was conditioned with deionized water for 20 min, with 0.1 M NaOH for 10 min and again with water for 5 min. Prior to each run, the capillary was flushed with separation buffer for 3 min. Samples were injected hydrodynamically at 10 mbar × 9 s. Separation voltage was −15 kV (anode at the detector side) or +25 kV (cathode at the detector side) depending on the charge of the particular analyte. When indicated, additional pressure of 30 or 40 mbar was applied to shorten the analysis time. Two acidic background electrolytes (BGEs) with a 10 mM ionic strength were employed for CE measurements: the acetate buffer composed of 10 mM LiOH and 20 mM acetic acid with theoretical pH 4.72 and the phosphate buffer composed of 20 mM phosphoric acid with theoretical pH 2.07. Experimental pH values of acetate and phosphate buffers were 4.74 and 2.09, respectively. Appropriate amount of particular CS was dissolved directly in BGEs and filtered. The pH of the BGE was verified again after addition of CSs as addition of even neutral CS to BGE can substantially change the buffer pH [28,29]. No significant pH changes were observed. Stock solutions of analytes were diluted by BGE to inject sample containing 0.05 mg/mL of individual enantiomers. When electrophoretic mobilities were calculated, 0.1% DMSO was present as electroosmotic flow marker in the sample injected. Migration times of analytes were determined as a time of the peak apex. Only for the electrophoretic mobility calculations, migration times were obtained by fitting the peak by Haarhoff-Van der Linde function [30,31].

2.4.2. HPLC measurements

Chromatographic measurements were carried out at a flow rate 1.0 mL/min and UV detection at wavelengths of 220 and 280 nm.