Analytica Chimica Acta 846 (2014) 51–59

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Preparation and evaluation of open tubular C18-silica monolithic microcartridges for preconcentration of peptides by on-line solid phase extraction capillary electrophoresis

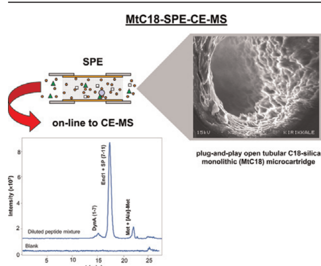
Elena Ortiz-Villanueva [a], Fernando Benavente [a,*], Estela Giménez [a], Fatma Yilmaz [b], Victoria Sanz-Nebot [a]

[a] *Department of Analytical Chemistry, University of Barcelona, Diagonal 645, 08028 Barcelona, Spain*
[b] *Department of Chemical Technology, Abant Izzet Baysal University, 14900 Bolu, Turkey*

HIGHLIGHTS

- C18 monolithic (MtC18) microcartridges were prepared for the analysis of neuropeptides by SPE-CE-UV and -MS.
- MtC18 sorbents were especially selective against endomorphin 1 and substance P (7–11).
- LODs were 50 times better than by CE-MS.
- The methodology was used to analyse plasma samples.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 23 March 2014
Received in revised form 28 May 2014
Accepted 26 June 2014
Available online 28 June 2014

Keywords:
Mass spectrometry
Neuropeptides
On-line preconcentration
Open tubular column
Silica monolith

ABSTRACT

In this study, C18-silica monoliths were synthesized as a porous layer in open tubular capillary columns, to be cut later into microcartridges for the analysis of neuropeptides by on-line solid-phase extraction capillary electrophoresis with UV and MS detection (SPE-CE-UV and SPE-CE-MS). First, several types of C18-silica monolithic (MtC18) microcartridges were used to analyse standard solutions of five neuropeptides (i.e. dynorphin A (1–7), substance P (7–11), endomorphin 1, methionine enkephalin and [Ala]-methionine enkephalin). The MtC18 sorbents were especially selective against endomorphin 1 and substance P (7–11)). The best results in terms of sensitivity and inter-microcartridge reproducibility were achieved with the microcartridges obtained from a 10-cm open tubular capillary column with a thin monolithic coating with large through-pores (1–5 μm). Run-to-run repeatability, microcartridge durability, linearity ranges and LODs were studied by MtC18-SPE-CE-MS. As expected due to their greater selectivity, the best LOD enhancement was obtained for End1 and SP (7–11) (50 times with regard to CE-MS). Finally, the suitability of the methodology for analysing biological fluids was tested with plasma samples spiked with End1 and SP (7–11). Results obtained were promising because both neuropeptides could be detected at 0.05 $\mu g\,mL^{-1}$, which was almost the same concentration level as for the standard solutions (0.01 $\mu g\,mL^{-1}$).

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Capillary electrophoresis (CE), like many other microanalytical techniques, has poor concentration sensitivity due to the limited

* Corresponding author at: University of Barcelona, Department of Analytical Chemistry, Diagonal 645, 3rd floor, 08028 Barcelona, Spain. Tel.: +34 934039123; fax: +34 934021233.
E-mail address: fbenavente@ub.edu (F. Benavente).

http://dx.doi.org/10.1016/j.aca.2014.06.046
0003-2670/© 2014 Elsevier B.V. All rights reserved.

volume of a diluted sample that can be loaded onto the capillary [1–6]. Several strategies to improve CE sensitivity have been investigated [1–13]. Today, the use of on-line solid phase extraction capillary electrophoresis (SPE-CE) is widely recognized as a powerful approach to overcoming this major drawback [8–14]. In SPE-CE, an extraction microcartridge or analyte concentrator placed near the inlet of the separation capillary contains a sorbent which retains the target analyte, enabling a large volume of sample to be introduced. After rinsing to eliminate non-retained molecules, the retained analyte is eluted in a smaller volume of appropriate solution, resulting in sample clean-up and concentration enhancement, before its electrophoretic separation [8–14].

In the most characteristic configuration for SPE-CE, the microcartridge is packed with particles of sorbent [8–14]. To avoid bleeding, these sorbent particles are usually retained in the microcartridge body with two frits [8–17]. Microcartridges with frits containing silica-based sorbents normally used in off-line SPE (e.g. C18) are widely used in preconcentration of peptides, proteins and drugs [10–17]. Nevertheless, frits may promote column backpressure, electrosmotic flow (EOF) disturbance, bubble formation and current drops or breakdowns. For these reasons and for simplicity of preparation, some authors prefer fritless microcartridges [11–14]. A fritless microcartridge can be easily packed when the sorbent particle size is slightly greater than the inner diameter of the separation capillary to prevent bleeding [11–14]. However, other alternatives, which use as a sorbent magnetic particles, membranes, filters, molecularly imprinted polymers or monoliths functionalized in various ways, have also been explored [1,11–14,18–26].

A monolithic sorbent can be seen as a continuous unitary porous structure without inter-particle voids that can be synthesized inside a capillary column ("in situ") as a layer of a certain thickness coating the inner wall (open tubular capillary columns) or filling completely the lumen [27–37]. The monolith's physical structure (e.g. thickness, porosity and pore size) and the number and type of active groups on the surface can be tailored to obtain a monolithic sorbent with no flow restriction and with the desired selectivity and extraction efficiency [27–37]. Monolithic sorbents can be classified in two main categories: silica-based and polymer-based monoliths. Silica-based monoliths are generally prepared with a sol–gel process, whereas polymer-based ones are made by "in situ" polymerization of monomers and cross-linkers [27–37]. Several types of polymer-based monoliths have been described for SPE-CE, in capillary or microchip format, probably because preparation is relatively simple and straightforward [18–25]. However, it is known that these columns shrink or swell, depending on the mobile phase composition, and may show a less uniform mesopore structure [24,36]. In contrast, only a few papers have reported silica-based monoliths [26], even though surface silanols are easier to derivatize and silica-based particles with C18 or immobilized antibodies have produced excellent results in SPE-CE [8–17]. The first uniform porous silica columns were reported by Tanaka and co-workers [34]. Later on, the sol–gel process described was adapted to create silica monoliths within fused-silica capillaries for capillary electrochromatography (CEC) [34,35], which were then derivatized for reversed-phase applications [34–37]. Although sol–gel silica-based monoliths have been used as an SPE sorbent and stationary phase in CEC to separate different analytes [27–37], they are still not widely used in SPE-CE [26].

In this study, the performance of several open tubular C18-silica monolithic (MtC18) microcartridges for preconcentration of peptides was explored by SPE-CE-UV and SPE-CE-MS, as an alternative to the frit and fritless C18 particle-packed microcartridges normally used in SPE-CE [8–17], as well as to the widely applied polymer-based monolithic microcartridges [18–25]. A group of neuropeptides of biomedical interest was selected as the main model peptides because they are usually found at very low concentrations in complex biological samples (between 0.01 ng/mL and 1 μg/mL in CSF, plasma, brain dialysates, etc.) [15,16,38,39]. Immunoassays (e.g. RIA), LC–MS or MALDI-MS are the most common analytical methods for neuropeptides [38,39], but SPE-CE-MS may be regarded as an excellent alternative because of the simplicity, sensitivity, low reagent and sample consumption, minimum sample handling, throughput, complementary separation mechanism and mass spectrometric confirmation [15,16]. Using MtC18-SPE-CE-MS, inter-microcartridge reproducibility and durability, run-to-run repeatability, linearity ranges and LODs were evaluated for the analysis of neuropeptides in standards and plasma samples. The results show the advantages and disadvantages of using MtC18 microcartridges in SPE-CE-MS for the detection and characterization of peptides at low concentrations in biological samples.

2. Materials and methods

2.1. Chemicals and reagents

All the chemicals used in the preparation of buffers and solutions were analytical reagent grade. Acetonitrile, ethanol, methanol, 2-propanol, formic acid (98–100%), acetic acid (glacial), ammonia (25%), hydrochloric acid (25%) and sodium hydroxide were purchased from Merck (Darmstadt, Germany). Tetramethoxysilane (TMOS), chlorodimethyloctadecylsilane, polyethylene glycol (PEG) (M 8 kDa and 10 kDa), acetone, tetrahydrofuran and xylene were provided by Sigma (St. Louis, MO, USA). Water with conductivity lower than 0.05 $\mu S\,cm^{-1}$ was obtained using a Milli-Q water purification system (Millipore, Molsheim, France).

Methionine enkephalin (Tyr-Gly-Gly-Phe-Met 573.23 Da, Met), methionine enkephalin-Arg-Phe acetate salt hydrate (Tyr-Gly-Gly-Phe-Met-Arg-Phe 876.39 Da, Meap), endomorphin 1 (Tyr-Pro-Trp-Phe-NH_2, 610.29 Da, End1), endomorphin 2 (Tyr-Pro-Phe-Phe-NH_2, 571.28 Da, End2), bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg, 1059.56 Da, Brk), eledoisin (Pyr-Pro-Ser-Lys-Asp-Ala-Phe-Ile-Gly-Leu-Met-NH_2, 1187.60 Da, Eled), oxytocin (Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2, 1006.44 Da, Oxy) and triptorelin (Pyr-Pro-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH_2, 1310.63 Da, Trip) were supplied by Sigma (St. Louis, MO, USA). Dynorphin A (1–7) (Tyr-Gly-Gly-Phe-Leu-Arg-Arg 867.47 Da, Dyn A (1–7)), substance P (7–11) (Phe-Phe-Gly-Leu-Met-NH_2, 612.31 Da, SP (7–11)), β-amyloid (1–15) (Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln 1825.78 Da, β(1–15)), β-amyloid (10–20) (Tyr-Glu-Val-His-His-Gln-Lys-Phe-Phe 1445.75 Da, β(10–20)) and histatine-8 (Lys-Phe-Glu-His-His-Ser-His-Arg-Gly-Tyr, 1561.77 Da, Hist(8)) were provided by Bachem (Bubendorff, Switzerland). [Ala]-methionine enkephalin (Tyr-Ala-Gly-Phe-Met 587.24 Da, [Ala]-Met) and methionine enkephalinamide (Tyr-Gly-Gly-Phe-Met-NH_2, 572.24 Da, Met-NH_2) were purchased from Neomps (Strasbourg, France). Des-Tyr-Leucine enkephalin (Gly-Gly-Phe-Leu 392.21 Da, Des) was supplied by Phoenix Pharmaceuticals (Belmont, CA, USA); and buserelin (Pyr-Pro-His-Trp-Ser-Tyr-D-Ser (^{t}Bu)-Leu-Arg-Pro-NHC_2H_5, 1322.68 Da, Bus), by Hoechst Ibérica (Barcelona, Spain).

2.2. Electrolyte, sheath liquid and standard solutions

An aqueous standard solution (2500 $\mu g\,mL^{-1}$) of each peptide was prepared and stored in a freezer at −20 °C when not in use. Working standard solutions were obtained by diluting with water. These mixtures were used to spike human plasma samples in order to obtain fortified samples. The background electrolyte (BGE) for the separation contained 50 mM of acetic acid and 50 mM of formic

Download English Version:

https://daneshyari.com/en/article/1164011

Download Persian Version:

https://daneshyari.com/article/1164011

Daneshyari.com