Journal of Chromatography B, 993-994 (2015) 26–35

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Analysis of drugs in plasma samples from schizophrenic patients by column-switching liquid chromatography-tandem mass spectrometry with organic–inorganic hybrid cyanopropyl monolithic column

Diego Soares Domingues, Israel Donizeti de Souza, Maria Eugênia Costa Queiroz*

Departamento de Química, Faculdade de Filosofia Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Brazil

ARTICLE INFO

Article history:
Received 6 March 2015
Received in revised form 24 April 2015
Accepted 26 April 2015
Available online 5 May 2015

Keywords:
Column-switching
Organic–inorganic hybrid monolith
Liquid chromatography-tandem mass spectrometry
Plasma samples
Schizophrenia

ABSTRACT

This study reports on the development of a rapid, selective, and sensitive column-switching liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to analyze sixteen drugs (antidepressants, anticonvulsants, anxiolytics, and antipsychotics) in plasma samples from schizophrenic patients. The developed organic-inorganic hybrid monolithic column with cyanopropyl groups was used for the first dimension of the column-switching arrangement. This arrangement enabled online pre-concentration of the drugs (monolithic column) and their subsequent analytical separation on an XSelect SCH C_{18} column. The drugs were detected on a triple quadrupole tandem mass spectrometer (multiple reactions monitoring mode) with an electrospray ionization source in the positive ion mode. The developed method afforded adequate linearity for the sixteen target drugs; the coefficients of determination (R^2) lay above 0.9932, the interassay precision had coefficients of variation lower than 6.5%, and the relative standard error values of the accuracy ranged from −14.0 to 11.8%. The lower limits of quantification in plasma samples ranged from 63 to 1250 pg mL^{-1}. The developed method successfully analyzed the target drugs in plasma samples from schizophrenic patients for therapeutic drug monitoring (TDM).

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Schizophrenia is a severe, chronic, and debilitating neuropsychiatric disorder that affects one percent of the general world population. This disorder generally appears in late adolescence or early adulthood, and involves cognitive, emotional, and behavioral abnormalities [1–3]. The reintroduction of clozapine in the market in the 1990s and the advent of the second-generation or atypical antipsychotics (olanzapine, quetiapine, risperidone, and ziprasidone) have benefitted patients with schizophrenia [4].

Physicians frequently recommend that patients switch from one antipsychotic to another before exploring full dose ranges [5,6]. Another common strategy in management of these difficult-to-treat patients is the combination of psychoactive drugs [7]. Apart from antipsychotics, most schizophrenic patients use other drug classes such as antidepressants, anxiolytics, and anticonvulsants, to lessen the symptoms associated with the disease [8]. Therefore, therapeutic drug monitoring of schizophrenic patients is important to adjust doses, minimize adverse effects, and check adherence to therapy.

Column-switching LC-MS/MS has become a powerful technique to determine drugs in biological samples. It enables direct injection of the samples into the analytical system or sample injection into the system after a simple treatment. This system uses two columns with different stationary phases; fractions from one column are selectively transferred to a secondary column for further separation. Direct on-line injection methods reduce the sample preparation steps, effectively pre-concentrate the target drugs, and remove endogenous compounds from biological samples. Thereby, these methods minimize the exposition to biological fluids. Moreover, the column-switching technique can improve the sensitivity, the resolution of complex samples, and the sample throughput by means of heart-cutting, front- or end-cutting, and straight- or back-flushing modes [9–13].

The monolithic column has been developed as an alternative to conventional packed columns. It displays a continuously porous structure characterized by macropores and mesopores that results in a good column permeability and high mass transfer efficiency [14–17]. In recent years, organic–silica hybrid monolithic columns have attracted great attention, because they are biocompatible with biological samples, do not shrink or swell upon exposure

* Corresponding author. Tel.:/fax: +55 16 36034465/+55 16 36024838.
E-mail address: mariaeqn@ffclrp.usp.br (M.E.C. Queiroz).

http://dx.doi.org/10.1016/j.jchromb.2015.04.040
1570-0232/© 2015 Elsevier B.V. All rights reserved.

to organic solvents, and exhibit good mechanical and chemical stability [18].

The literature brings a wide variety of reports on organic-inorganic hybrid monoliths with different organic moieties and distinct macro-mesoporous structures applied as stationary phases in chromatographic separations [19–21], electrophoreses [22], solid-phase extraction (SPE) [23,24], stir bar sorptive extraction (SBSE) [25], solid-phase microextraction (SPME) [26–28], and in-tube SPME-LC [29].

The present work describes the preparation of an organic-inorganic hybrid cyanopropyl monolithic capillary column functionalized with cyanopropyl groups for the first dimension of the column-switching LC-MS/MS system. This method was successfully applied to determine sixteen drugs (antidepressants, anticonvulsants, anxiolytics, and antipsychotics) in plasma samples from schizophrenic patients for therapeutic drug monitoring.

2. Materials and methods

2.1. Standards and reagents

Haloperidol, olanzapine, clonazepam, mirtazapine, paroxetine, citalopram, sertraline, chlorpromazine, imipramine, clomipramine, quetiapine, diazepam, fluoxetine, clozapine, carbamazepine, and lamotrigine standards were purchased from Cerilliant (Round Rock, TX, USA). Stable labeled (internal standards) haloperidol-d4, clonazepam-d4, paroxetine-d6, citalopram-d6, sertraline-d3, imipramine-d3, clomipramine-d3, quetiapine-d8, diazepam-d5, fluoxetine-d6, clozapine-d4, and carbamazepine-d10 were also acquired from Cerilliant (Round Rock, TX, USA). Tetraethyl orthosilicate (TEOS) (98%), 3-cyanopropyltriethoxysilane (CN-TEOS) (98%), (3-aminopropyl) triethoxysilane (APTES) (98%), and N-dodecylamine (99%) were obtained from Sigma Aldrich (St. Louis, USA). Acetonitrile, ethanol, and methanol (HPLC grade) as well as ammonium acetate and formic acid were supplied by JT Baker (Phillipsburg, USA). Cetyltrimethylammonium bromide (CTAB, 95%), sodium hydroxide (NaOH), and hydrochloric acid (HCl) were purchased from Sigma–Aldrich (St. Louis, USA). Aqueous solutions were prepared by using ultrapure water obtained from a Milli-Q (18 Ω) system (Millipore, São Paulo, Brazil).

2.2. Preparation of the organic-silica hybrid cyanopropyl monolithic capillary

The fused-silica capillary (530 μm × 4.5 cm), purchased from Ohio Valley (Ohio, USA), was activated with NaOH (1 mol L^{-1}), followed by HCl (1 mol L^{-1}). After rinsing with ultrapure water, the capillary was dried under a N_2 stream at 160 °C, for 8 h. The hybrid monolithic capillary was synthesized by hydrolysis (at low pH value) and polycondensation (higher pH value) of precursors via a two-step catalytic sol-gel process, according to previously described procedures [29], with some modifications. The precursors, 110 μL of CN-TEOS and 110 μL of TEOS, were added to a solution consisting of 180 μL of ethanol and 25 μL of acetic acid (2 mol L^{-1}) in an Eppendorf vial (1.5 mL). Hydrolysis was performed at 60 °C, for 5 h. After that, 10 mg of N-dodecylamine was added to the solution, at room temperature. This mixture was manually injected into a pre-treated capillary of an appropriate length with the aid of a syringe. Both ends of the capillary were sealed with two pieces of rubbers. Then, the capillary was incubated at 40 °C, for 15 h, for condensation and polymerization. Subsequently, the capillary was extensively rinsed with ethanol, to remove N-dodecylamine and soluble hydrolysis products, and dried at 60 °C, for 30 h.

2.3. Characterization of the organic–inorganic cyanopropyl hybrid silica monolithic capillary

The morphological structural aspects of the monolithic capillary were examined by Scanning Electron Microscopy. The samples were coated with gold in a Bal-Tec SCD050 Sputter coater instrument (Fürstentum Liechtenstein), for 180 s. Then, they were analyzed in a Zeiss EVO 50 scanning electron microscope (Cambridge UK). The chemical groups were identified by Fourier Transform Infrared Spectroscopy (FTIR), which was conducted on the Shimadzu- IRPrestige-21 equipment (using KBr pellets).

2.4. Plasma samples

To optimize and validate the developed LC-MS/MS method, drug-free plasma samples from volunteers that had not been exposed to any drug for at least 72 h (blank plasma) were used. These blank plasma samples as well as the plasma samples from schizophrenic patients were supplied by the Psychiatric Nursing staff of Hospital das Clínicas de Ribeirão Preto, University of São Paulo, Brazil. This study has been approved by Research Ethics Committee, process n. 591/2011-2011.1.1664.59 2 .from *Faculdade de Filosofia Ciências e Letras de Ribeirão Preto*–University of São Paulo.

Bench top and autosampler stability tests showed that plasma samples containing the target drugs were stable for 24 h at room temperature. Patients' plasma samples containing the target drugs were stable over three freeze-thaw cycles or when stored at −20 °C for six months [30–33].

2.5. Standard Solutions

The working standard solutions were prepared by diluting the stock standard solutions (200 μg mL^{-1}) to concentrations ranging from 0.075 to 40.5 ng mL^{-1} for haloperidol and olanzapine, from 0.625 to 155.0 ng mL^{-1} for clonazepam, from 0.125 to 155.0 ng mL^{-1} for mirtazapine, from 0.250 to 155.0 ng mL^{-1} for paroxetine, from 0.125 to 290.0 ng mL^{-1} for chlorpromazine and imipramine, from 0.250 to 510.0 ng mL^{-1} for clomipramine, from 0.125 to 510.0 ng mL^{-1} for quetiapine, from 1.250 to 405.0 ng mL^{-1} for citalopram, from 0.625 to 405.0 ng mL^{-1} for sertraline, from 0.313 to 1050.0 ng mL^{-1} for diazepam and fluoxetine, from 0.188 to 1550.0 ng mL^{-1} for clozapine, from 0.063 to 10500.0 ng mL^{-1} for carbamazepine, and from 1.250 to 10500.0 ng mL^{-1} for lamotrigine. These solutions were stable for two months, at −20 °C [34,35]. The internal standard solutions were prepared in methanol at the following concentrations: haloperidol-d4 (20.5 ng mL^{-1}), clonazepam-d4 and paroxetine-d6 (80.0 ng mL^{-1}), imipramine-d3 (185.0 ng mL^{-1}), citalopram-d6 and sertraline-d3 (205.0 ng mL^{-1}), clomipramine-d3 and quetiapine-d8 (260.0 ng mL^{-1}), diazepam-d5 and fluoxetine-d6 (6550.0 ng mL^{-1}), clozapine-d4 (850.0 ng mL^{-1}), and carbamazepine-d10 (5.5 μg mL^{-1}).

2.6. Pre-treatment of the plasma samples

The proteins of the plasma samples (200 μL) were precipitated with acetonitrile (400 μL). The supernatant (500 μL) was dried in a vacuum concentrator (Eppendorf, Brazil), and the dried extract were reconstituted (100 μL) with ammonium acetate (5 mmol L^{-1})/ammonium hydroxide (5 mmol L^{-1}) (1:1 v/v) solution. Then, 10.0 μL of this solution was injected into a two-dimensional system. Different pH values (3.0, 7.0, 10.0) of the ammonium solution were evaluated to establish the sorption capability of the monolith capillary.

Download English Version:

https://daneshyari.com/en/article/1212145

Download Persian Version:

https://daneshyari.com/article/1212145

Daneshyari.com