Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography A, 1119 (2006) 285–293
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Limitations of porous graphitic carbon as stationary phase material in the determination of catecholamines

Sandra Rinne *, Anders Holm, Elsa Lundanes, Tyge Greibrokk

Department of Chemistry, University of Oslo, Pb.1033 Blindern, 0315 Oslo, Norway

Available online 20 March 2006

Abstract

A fast and sensitive capillary liquid chromatography (cLC) column-switching method with electrospray ionization time-of-flight mass spectrometry (ESI–TOF–MS) detection for the simultaneous determination of dopamine (D), epinephrine (E), norepinephrine (NE) and serotonin (SE) was pursued. A sample volume of 100 μl was loaded with a mobile phase containing 0.1% pentafluoropropionic acid (PFPA) as ion-pairing agent on a 25 mm × 0.32 mm (i.d.) 5 μm Hypercarb column. A water–acetonitrile (AcN) gradient with 0.1% acetic acid (AcOH) backflushed the compounds onto a 34 mm × 0.32 mm (i.d.) 5 μm Hypercarb analytical column. However, during a series of analyses, oxidation of the catecholamines (CAs) was observed. This was suspected to be due to the loading mobile phase composition and precluded the usefulness of this method even though the achievable detection limit was in the range of 0.75–3.0 ng/ml. The combination of the porous graphitic carbon (PGC) material and the fluorinated strong acids which were required to get enough retention for preconcentration of large volumes cannot be used for easily oxidized compounds as the CAs.

Keywords: Catecholamines; Porous graphitic carbon; Oxidation; Fluorinated carboxylic acids; Large volume injection; LC–MS

1. Introduction

Catecholamines (CAs) play an important role as neurotransmitters in the central nervous system [1]. Accurate and selective measurements of the CAs dopamine (D), epinephrine (E), norepinephrine (NE) and serotonin (SE) in biological samples are important both for the clinical diagnosis and the pathological study of certain diseases as Parkinson or Alzheimer's disease, depression, schizophrenia and other mental illnesses [2]. Additionally, CAs are involved in a variety of regulatory systems such as stress and learning, as well as in the control of many processes of metabolism and the immune system [1]. Sensitive and selective methods are required for the quantification of low concentration of these analytes in a complex matrix. The sample preparation, separation, and detection methods used for determination of CAs in tissue and biological fluids have recently been reviewed [1,3].

The purpose of this study was to develop a LC–MS method for quantification of low concentrations of CAs in small samples requiring capillary liquid chromatography (cLC) with large volume injection [4–7].

In cLC, large volumes can be injected using column-switching methods based on on-column focusing on a precolumn while still maintaining the chromatographic efficiency [8,9]. To obtain concentration on the precolumn, a rather hydrophobic stationary phase has to be used for CAs, which are highly polar. C_{18} materials and polystyrene-divinyl benzene (PS-DVB) did not provide enough retention of the CAs, even with the use of hydrophobic ion-pairing agents. Thus, porous carbon materials, like Hypercarb, which is known to provide higher retention, was a natural choice.

Porous graphitic carbon (PGC) stationary phases offer different retention mechanisms from C_{18} stationary phases [10]. Analytes are retained by complimentary mechanisms, e.g. hydrophobic and electronic interactions. Charge induced interactions, e.g. electron-pair donor–acceptor and dipole–dipole induced types of interaction of the polarizable or polarized functional groups of the analyte with the polarizable stationary phase are believed to be the most important electronic interactions. The delocalization of the π-electrons in the graphite is responsible for dispersive interactions between the analyte and the stationary phase. PGC consists of sheets of hexagonally arranged carbon atoms with sp^2 hybridization, which results in strong π–π interactions with analytes containing aromatic rings. Thus, analytes containing aromatic rings like the CAs are better retained than on a C_{18}-material.

* Corresponding author. Tel.: +47 22857431; fax: +47 228 55441.
E-mail address: sandrari@kjemi.uio.no (S. Rinne).

0021-9673/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2006.03.003

Due to the reported physical and chemical stability of graphitic carbons a variety of applications has been published, especially for the determination of polar compounds, which have little retention on both silica-based and organic polymer-based packing materials [11]. The separation of polar phenolic compounds [12], underivatized protein amino acids [13,14], CAs [15] and aniline [16] are just some of the applications that have been demonstrated. Water–acetonitrile (AcN) or water–methanol (MeOH) mixtures with addition of formic acid/ammonium formate [12,15,16] or perfluoroalkyl carboxylic acids [13,14,17] have been applied as mobile phases.

However, there are some problems with the use of the PGC materials, which can be oxidized by the treatment with oxidizing-agents or dissolved oxygen in the mobile phase [18,19], and by the backward current from an ESI-spray capillary [20].

In the present study a cLC column-switching method with electrospray ionization time-of-flight (ESI–TOF) MS detection for the simultaneous determination of dopamine, epinephrine, norepinephrine and serotonin was set up. A sample volume of 100 μl was loaded on a Hypercarb column. The loading mobile phase contained 99.9% H_2O and 0.1% pentafluoropropionic acid (PFPA) as ion-pairing agent. A water–acetonitrile gradient containing 0.1% acetic acid (AcOH) in both reservoirs backflushed the compounds onto a Hypercarb analytical column. The analytes were detected in positive ion mode. Severe oxidation of the CAs was observed and this will be discussed in this paper.

2. Experimental

2.1. Materials and reagents

Dopamine, epinephrine, norepinephrine, serotonin and heptafluorobutyric acid (HFBA) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Grade 1 water was obtained from a Milli-Q ultrapure water purification system (Millipore, Bedford, MA, USA). HPLC grade AcN from Rathburn (Walkerburn, UK), sds (Peypin, France) or LabScan (Dublin, Ireland) was used. MeOH (HPLC grade) was obtained from BDH Hipersolv (Poole, UK). Glacial AcOH, trifluoroacetic acid (TFA), ascorbic acid, sodium thiosulphate, hydrochloric acid (HCl, 37%) and benzoic acid were obtained from Merck (Darmstadt, Germany). PFPA, formic acid (FA) and ammonium formate (analytical grade for MS) were purchased from Fluka (Buchs, Switzerland). Nitrogen (99%) and helium (99%) were purchased from AGA (Oslo, Norway). Deuterium oxide (D_2O, 99.96% D), deuterated acetonitrile (AcN-d3, 99.8% D) and deuterated acetic acid (AcOH-d4, 99.96% D) were obtained from Cambridge isotope laboratories (Andover, MA, USA).

2.2. Columns and column preparation

Hypercarb capillary columns were slurry packed in-house using a downward high pressure liquid slurry method and the stationary phase material was 5 μm Hypercarb PGC (batch PGC 221, Thermo Hypersil-Keystone, Chesire, UK). The precolumn was 25 mm × 0.32 mm (i.d.) while the analytical column was 34 mm × 0.32 mm (i.d.). Valco (Houston, TX, USA) ZU1C unions in combination with Valco FS1.4 polyimide ferrules and Valco 2SR1 steel screens were used as column end fittings.

Additionally, a commercial Tracy × 5 mm, 1 mm (i.d.) 5 μm Hypercarb precolumn (G&T Septech, Kolbotn, Norway) and a × 50 mm, 0.32 mm (i.d.) 5 μm Hypercarb analytical column (Thermo Hypersil-Keystone, Chesire, UK) were used.

2.3. Chromatographic system

An Agilent 1100 capillary gradient pump (Palo Alto, CA, USA) with an incorporated on-line vacuum degasser was used to deliver the mobile phase providing back-flushed desorption from the pre-column and elution on to the analytical column, while a LC-10AD VP isocratic pump from Shimadzu Corporation (Kyoto, Japan) was used for sample loading. Elution of the analytes was conducted using a solvent gradient where solvent A consisted of H_2O and solvent B consisted of AcN, both containing 0.1% AcOH. The gradient started at 0% B, was linearly increased to 10% B in 2.5 min, followed by a step to 50% B which was held for 2.5 min, and finally a step to 100% B which was held for 5 min. The mobile phase was delivered at a constant flow rate of 5 μl/min. The solution used for sample loading consisted of H_2O containing 0.1% PFPA and was delivered at a flow rate of 25 μl/min. A Rheodyne (Cotati, CA, USA) 7010 6 ports injector (Cotati, CA, USA) (V I) was used for manual injections of sample volumes of 25–100 μL. A Valco Cheminert C2 six-port valve was used for column-switching (V II), and a schematic drawing of the applied system is presented in Fig. 1. For evaluation of the separation gradient and stability testing of the columns, a Valco Cheminert Model C4 injection valve with an internal loop of 50 nl was used. For UV detection, a Spectra System UV2000 (Thermo Separation Products, Herts, UK) with an on-column detection was used at a wavelength of 280 nm.

2.4. Mass spectrometric detection

The outlet of the analytical column was connected via a union, which served as grounding point, to a Micromass (Manchester, UK) LCT orthogonal accelerated TOF–MS. The TOF–MS was equipped with a Z-spray atmospheric pressure ionization source for ESI, which was modified with a spray capillary of 25 μm i.d. to handle flow rates in the low μl/min range. Electrospray ionization was performed in the positive mode with the molecular ion masses $[M+H]^+$ of 154.1, 184.1, 170.1 and 177.1 for D, E, NE and SE, respectively. Additionally fragments of masses $[M+H\text{-}17]^+$ for D and SE and $[M+H\text{-}18]^+$ for E and NE were used for detection. The voltages were set to 2800 V (spray capillary), 15 V (sample cone) and 3 V (extraction cone). The source temperature was 100 °C, and the desolvation gas flow rate was ~250 l/h. The TOF–MS instrument was controlled and data were acquired using MassLynx version 4.0 software (Micromass). Additional experiments were carried out using an Esquire 3000 + IT–MS instrument (Bruker Daltonics, Bremen, Germany) equipped with a low-flow nebulizer to accommodate flows in the low μl/min range. The IT–MS instrument was operated in the positive mode with a capillary voltage of 2800 V,