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Evaluation of fused-core and monolithic versus porous silica-based C18 columns and porous graphitic carbon for ion-pairing liquid chromatography analysis of catecholamines and related compounds

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ABSTRACT

This paper evaluates the performances of reversed-phase (RPLC) and ion-pairing chromatography (IPLC) coupled with UV detection for the analysis of a set of 12 catecholamines and related compounds. Different chromatographic columns (porous C18-silica, perfluorinated C18-silica, porous graphitic carbon, monolithic and fused-core silica-based C18 columns) were tested using semi-long perfluorinated carboxylic acids as volatile ion-pairing reagents. Much more promising results were obtained by IPLC than by RPLC and important improvements in analytes peak symmetry and separation resolution were observed when using the "fast chromatography" columns (monolithic and fused-core C18) under IPLC conditions. For UV detection, a satisfactory separation of the 12 selected analytes was achieved in less than 20 min by using a fused-core particles column (Halo C18) and a mobile phase composed of a 1.25 mM nonafluoropentanoic acid aqueous solution and methanol under gradient elution mode. The chromatographic method developed can be directly coupled with electrospray ionization tandem mass spectrometry (ESI–MS/MS) in positive ionization mode and 10 solutes among those selected can be observed. The presence of the acidic ion-pairing reagent in the mobile phase makes this system incompatible with negative ionization mode and thus unable to detect the two acidic compounds that only responded in negative mode. In terms of MS detection, Monolithic C18 column proved to be the best one to reach the lowest detection limits (LODs) (from 0.5 ng mL^{-1} to 10 ng mL^{-1} depending on the neurotransmitter). The applicability of the optimized LC–MS/MS method to a "real world" sample was finally evaluated. The presence of the matrix leads to signal suppression for several solutes and thus to higher LODs.

1. Introduction

Catecholamines and indolamines play a significant role in the nervous system as central and peripheral neurotransmitters. The concentration level of these compounds in different biological fluids or tissues can offer important information about the state of health of the person. Among the biological amines there are three catecholamines known to occur in vivo: adrenaline (A) (epinephrine), noradrenaline (NA) (norepinephrine) and dopamine (DA) [1]. Their physiological precursors are tyrosine (Tyr) and 3,4-dihydroxy-phenylalanine (DOPA), while homovanillic acid (HVA), 3-methoxytyramine (3-MT) and 3,4-dihydroxy-phenylacetic acid (DOPAC) are some of their metabolites present in the organism. Serotonin (S) is an indolamine that is present in many tissues (blood platelets, lining of the digestive tract, brain). It is produced in the body from tryptophan (Trp) and metabolized into 5-hydroxyindole-3-acetic acid (5HIAA). These compounds are markers for the diagnosis and treatment of different diseases like: asthma, myocardial infarction, Parkinson's disease [2], pheochromocytoma [3] or neuroblastoma [4].

Concerning separation techniques for neurotransmitter analysis, liquid chromatography (LC) is widely used but capillary electrophoresis has been also reported [5–7]. Different LC systems have been described using UV [8] or fluorescence [9] detection however today, the most widespread technique for the investigation of these molecules in biological samples is ion-pairing chromatography (IPLC) associated with an electrochemical detection (ECD) [10–14]. Lately the mass spectrometry (MS) detection has been extensively used as this mode of detection has the advantage of providing additional structural information about the eluted compounds [15–22]. ECD is still the most sensitive detection mode with detection limits that can reach 0.01 ng L^{-1} [23] however, its specificity is limited to distinguishing only a family of compounds from other ones in relation to differences into their reduction or oxidation potential values and this is not sufficient when a new metabolite is formed. Thus, in view of a structural identification for

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any new metabolite detected in biological samples, the complementary information obtained by MS detection is very important. This explains the interest for a chromatographic method that would be compatible with both mass spectrometry and electrochemical detection.

Reversed-phase liquid chromatography (RPLC) either on octadecyl [24] or porous graphitic carbon (PGC) [20,25] columns and highly aqueous mobile phases using formic or acetic acid as additive have been used for catecholamine analysis. These polar compounds are often derivatized in order to obtain less polar compounds more easily retained on non-polar supports [26–29]. In order to effectively increase the retention of ionizable polar compounds, an alternative approach to RPLC is IPLC [30]. Regarding catecholamine separation, IPLC system using sodium octyl/dodecyl sulfonate or related compounds as ion-pairing agent is probably the most popular method [13,31–34] associated with ECD. Unfortunately, these ion-pairing reagents are not volatiles and then not compatibles with an MS detection. In order to overcome this inconvenient, volatile ion-pairing agents such as perfluorinated carboxylic acids [9,35–37] have been tested showing encouraging results. The use of hydrophilic interaction liquid chromatography (HILIC) has also been reported [38,39].

The aim of our project is to study the feasibility of replacing the actual IPLC–ECD method by one compatible with both ECD and MS detection for the analysis of catecholamines and related compounds either presenting a biological interest or can be physiologically or therapeutically present in biological samples. This includes a preliminary study of the performances of different RPLC and IPLC systems satisfying two major criteria: (i) volatile mobile phase with a minimum of 5% organic modifier for more favorable electrospray MS ionization conditions and (ii) baseline resolution between the target solutes to be compatible with an electrochemical detection. This preliminary study will be further followed by the coupling of the optimized chromatographic systems with MS detection and electrochemical detector, in order to offer a versatile chromatographic system. Comparison of different analytical instruments or chromatographic supports is very useful as it help scientists to choose among them, in relation to their analytical requirements (high sensitivity or more specificity).

In this report, we present the separation of a set of 12 catecholamines and related compounds (A, NA DA, Tyr, DOPA, HVA, 3-MT, DOPAC, S, Trp, 5HIAA and DHBA) on different chromatographic columns: conventional C18-silica, perfluorinated (PFP)-silica, porous graphitic carbon (PGC), monolithic C18-silica and fused-core C18 columns. Then, the most outstanding chromatographic systems were coupled to MS/MS detection and compared in terms of detection limits. The applicability of LC–MS/MS method to "real world" samples was finally tested.

2. Experimental

2.1. Chemicals and reagents

HPLC-grade acetonitrile (MeCN) and methanol (MeOH) were purchased from J.T. Baker (Noisy-le-Sec, France) and perchloric acid from VWR Prolabo (Darmstadt, Germany). Ammonium acetate and ammonium formate, acetic acid, formic acid and trifluoroacetic acid (TFA) were purchased from Fluka (St.-Quentin-Fallavier, France). Heptafluorobutyric acid (HFBA), nonafluoropentanoic acid (NFPA) and pentadecafluorooctanoic (PDFOA) were purchased from Sigma–Aldrich (Saint-Quentin-Fallavier, France). Adrenaline (A), noradrenaline (NA), dopamine (DA), tyrosine (Tyr), 3,4-dihydroxy-phenylalanine (DOPA), homovanillic acid (HVA), 3-methoxytyramine (3-MT), 3,4-dihydroxyphenylacetic acid (DOPAC), serotonin (S), tryptophan (Trp), 5-hydroxyindole-3-acetic acid (5HIAA) were purchased from Sigma–Aldrich (Saint-Quentin-Fallavier, France). 3,4-dihydroxybenzylamine (DHBA) was purchased from Fluka (Saint-Quentin-Fallavier, France)

Deionised (18 MΩ) water, purified using an Elgastat UHQ II system (Elga, Antony, France) was used for preparation of analyte and mobile phase solution.

Marvin 4.1.11 software (ChemAxon, Budapest, Hungary) was used to calculate the analyte pK_a and log D values

2.2. Standards and solutions

Stock standard solutions of each catecholamine, indolamine, and metabolite prepared at a concentration of 1000 μg mL^{-1} were obtained by dissolving the adequate weighed amount of each compound with 0.2 M perchloric acid. The use of perchloric acid is dictated by the fact that the neurotransmitter analysis was inscribed in a larger study aiming at analyzing these molecules in brain extracts, which are prepared in perchloric acid. Thus it was found important to maintain identical conditions in the whole method development process. All stock solutions were stored at −80 °C. The injected solutions were obtained by diluting the corresponding stock standard solutions in the mobile phase in order to obtain a final analyte concentration about 5–10 μg mL^{-1}.

For the brain extract preparation, the sheep encephalon was dissected out of the skull and was separated in different regions that were weighed and then immersed in cold 0.2 mol L^{-1} perchloric acid at the ratio of 5 mL g^{-1} tissue. The brain tissue was homogenized by sonication or using a Potter apparatus. The tissue homogenate was centrifuged at 20,000 × g for 1 h at 4 °C. The supernatant was utilized as the brain extract and stored at −80 °C. Just before analysis, the brain extract was filtered through a 0.45 μm syringe filter (Millipore) and an aliquot (500 μL) of the filtrate was mixed in 500 μL of an aqueous solution of NFPA 1.25 mM. 20 μL of the so prepared sample were injected in the HPLC system.

2.3. Instrumentation

The chromatographic systems consisted of a Merck-Hitachi quaternary pump model Lachrom L-7100 (Darmstadt, Germany), a Rheodyne (Cotati, CA, USA) model 7725 injection valve fitted with a 20 μL loop, column oven Jet Stream 2 Plus and a 785A UV-visible HPLC Detector (Applied Biosystems, Courtaboeuf, France). The UV detection was carried out at 280 nm in order to obtain maximal absorbance for all the compounds. Physicochemical parameters of the different columns studied are reported in Table 1.

MS detection was realized with Perkin-Elmer Sciex (Forster City, CA, USA) API 300 or API 3000 mass spectrometers with triple-quadrupole and Turbo Ionspray as ion source. The mass spectrometers were operated in positive ionization mode. The optimized MS parameters were the following: ion spray voltage 5800 V, nebulizer gas was compressed air at a flow rate of 1.2 L min^{-1}, curtain gas was nitrogen at a flow rate of 0.9 L min^{-1}, source temperature 300 °C and focusing potential (FP) 100 V. The values for the declustering potential (DP), the entrance potential (EP) and the collision energy (CE) are different for each selected transition and they are presented in Table 2. For the LC–MS/MS coupling, a split was necessary at the mass spectrometer entry, this split was of 1/3 for the columns operating at a 1 mL min^{-1} flow rate (PGC, porous silica-based C18 and monolithic C18) and 1/5 for the fused-core column that had a 1.5 mL min^{-1} operating flow rate.

The chromatographic data handling was accomplished using EZChrom Server software (Merck, Darmstadt, Germany) for the UV detection and Analyst (Applied Biosystem MDS Sciex) for the MS detection.