Journal of Chromatography B, 951–952 (2014) 52–57

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Analysis of microdialysate monoamines, including noradrenaline, dopamine and serotonin, using capillary ultra-high performance liquid chromatography and electrochemical detection

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ARTICLE INFO

Article history:
Received 2 September 2013
Accepted 16 January 2014
Available online 24 January 2014

Keywords:
Monoamine neurotransmitter
Ultra high performance liquid chromatography
Microdialysis
Electrochemical detection

ABSTRACT

Electrochemical methods are very often used to detect catecholamine and indolamine neurotransmitters separated by conventional reverse-phase high performance liquid chromatography (HPLC). The present paper presents the development of a chromatographic method to detect monoamines present in low-volume brain dialysis samples using a capillary column filled with sub-2 μm particles. Several parameters (repeatability, linearity, accuracy, limit of detection) for this new ultrahigh performance liquid chromatography (UHPLC) method with electrochemical detection were examined after optimization of the analytical conditions. Noradrenaline, adrenaline, serotonin, dopamine and its metabolite 3-methoxytyramine were separated in 1 μL of injected sample volume; they were detected above concentrations of 0.5–1 nmol/L, with 2.1–9.5% accuracy and intra-assay repeatability equal to or less than 6%. The final method was applied to very low volume dialysates from rat brain containing monoamine traces. The study demonstrates that capillary UHPLC with electrochemical detection is suitable for monitoring dialysate monoamines collected at high sampling rate.

1. Introduction

Chemical communication in the brain relies on very fast neurotransmitter release in extracellular space. One of the major classes of neurotransmitters involved in the central nervous system [1] are biogenic amines, including noradrenaline (NA), adrenaline (Ad), dopamine (DA), and serotonin (5-hydroxytryptamine, 5-HT). A classical technique used to monitor extracellular neurotransmitters such as monoamines *in vivo* is brain microdialysis [2]. This technique consists in implanting into the living brain a probe with a semi-permeable membrane continuously perfused with artificial cerebrospinal fluid (aCSF) at low flow-rate. The extracellular compounds can diffuse towards the probe according to their concentration gradient and the cut-off of the membrane (<6–100 kDa). The neurotransmitters of interest are then sampled in microdialysates, with a collection time which determines the temporal resolution of the microdialysis: the higher the temporal resolution, the more accurate the monitoring of rapid extracellular variations occurring during neurotransmission [3–5].

Monoamines contained in microdialysis samples are mainly detected by high performance liquid chromatography (HPLC) coupled to electrochemical detection (ED). Currently, classical ion-pairing HPLC (columns of 2–4.6 mm i.d.; 3–5 μm particle size) is mostly widely used, providing good retention of the polar amine moiety of monoamines [6–8]. One of the main limitations of classical HPLC-ED is the need for large sample volumes (5–20 μL) for efficient detection of monoamines present at trace concentrations in the microdialysate. This requires sampling every 5–20 min, which is far too low a temporal resolution compared to the speed of neurochemical events. Another problem with ion pairing chromatography is the difficulty of analyzing NA, Ad, DA and 5-HT in the same run, as their retentions differ: NA is retained the least by the

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http://dx.doi.org/10.1016/j.jchromb.2014.01.023

column and can be eluted in the solvent front of the chromatogram, whereas 5-HT has the longest retention and can be detected at retention times greater than 20–25 min; like NA, Ad is also poorly retained by the column, while DA shows moderate retention. In practice, neuroscientists may run two HPLC methods to quantify these neurotransmitters or else select just one or two compounds. Consequently, DA and 5-HT are often monitored simultaneously [9–11] or alone (5-HT: [12,13]), whereas NA, and also Ad, which has a lower concentration, are the monoamines least studied in brain microdialysis [6]. Beside the issue of retention, electrochemical detection is another important feature of the method described herein: NA is very often detected on coulometry, which provides a 100%-yield of oxidation, in contrast to amperometry for which the detection yield is considered to be only around 10%. The lack of available miniaturized coulometric cells also explains the use of large diameter HPLC columns for NA detection. To avoid the inconvenience of requiring large-volume samples, some analysts have tried alternative techniques such as capillary electrophoresis with laser-induced fluorescence detection (CE-LIFD) (NE: [14,15]), have coupled HPLC to mass spectrometry (MS) (DA and 5-HT: [16]; NE and DA: [17]), or have improved HPLC-ED by miniaturizing both the system and columns [13,18]. For instance, attomolar quantities of 5-HT can be detected using a recent UHPLC strategy by enhancing the eluting flow-rate and gaining in limits of detection (LOD) [12]. To date, there have been very few reports of the use of UHPLC columns for small volumes of microdialysate and methods are limited to one monoamine for routine use with 0.5 μL for 5-HT [12] or 9 μL for DA [19]. Indeed, UHPLC can be used with sub-2 μm or sub-3 μm particles only when the HPLC pumps are compatible with the high pressures generated by the small sized particles packed in the columns.

The aim of the present study was to optimize a UHPLC method with amperometry for simultaneous detection of the three major monoamines (NA, DA and 5-HT) in a single run, using sub-2 μm columns when processing low volumes of dialysates. Despite their low concentrations in extracellular space, Ad and 3-methoxytyramine (3-MT, a metabolite of dopamine) were also studied, as their structures are close to those of NA and DA, respectively. Eventually, the method will be applied to very low-volume microdialysis samples containing trace concentrations of monoamines.

2. Experimental

2.1. Chemicals

NA, Ad, DA,3,4-dihydroxybenzylamine (DHBA), 3-MT, 5-HT, 1-octanesulfonic acid (OSA), triethylamine (TEA), ethylene–diamine–tetra-acetic acid (EDTA) disodium salt, and, sodium hydroxide were purchased from Sigma (St. Louis, MO, USA), potassium dihydrogenphosphate and methanol U-HPLC gradient grade from Fisher Scientific (Loughborough, UK), citric acid from Merck (Darmstadt, Germany). Ultrapure water was produced using a Milli-Q system (Millipore, Bedford, MA, USA). Standard solutions of 1 mmol/L of NA, Ad, DA, DHBA, 3-MT, and 5-HT were stored at −30 °C as aliquots in water (5-HT), in 0.1 mol/L hydrochloric acid (NA, Ad, DA, DHBA) or in 0.1 mol/L perchloric acid (3-MT).

2.2. Ultra high-performance liquid chromatography

The UHPLC system consisted of a Prominence degasser, a LC-30 AD pump, a SIL-30AC autosampler, and a CTO-20AC column oven (Shimadzu). Detection was carried out at 40 °C using a Decade II electrochemical detector fitted with a 0.7 mm glass carbon working electrode, an *in situ* Ag/AgCl reference electrode, and a 25 μm spacer (cell volume 80 nL, Antec, Leyden, The Netherlands). Separations were performed at 40 °C (in detector oven) using a 100 × 0.32 mm Kappa Hypersil Gold 1.9 μm C18 column (Thermo Scientific). The mobile phase, which was pumped at 8.5 μL/min, consisted of 0.14 mol/L potassium phosphate, 0.1 mmol/L EDTA, 6 mmol/L OSA, 0.01% TEA (v/v), 8 mmol/L KCl, pH adjusted to 5 with 10 mmol/L sodium hydroxide, 6% methanol, and was filtered through a 0.22 μm cellulose acetate membrane before use. Analytes were detected at an oxidation potential of 450 mV versus the reference electrode. Chromatograms were acquired at a rate of 10 Hz using Lab Solutions software. The acquisition time was 22 min. On the day of analysis, the samples were placed in the autosampler and kept at +4 °C before injection. The injection volume was 1 μL.

2.3. Analysis of brain dialysates

Dialysis samples were obtained in dorsal hippocampus of freely-moving rats as well as in striatum or basolateral amygdala of anaesthetised rats. Samples were analyzed just after microdialysis collection, except the samples of the hippocampus that were kept at −40 °C until analyzes.

2.4. Quantification validation

The calibration standards for the quantification validation contained NA, Ad, DHBA, DA, 3-MT, and 5-HT (range: 5×10^{-10}–3×10^{-8} mol/L). Calibration plots were generated by plotting the peak area versus the concentration (at least six concentrations of each compound plus a blank). Regression equations were calculated using the least-squares linear regression method. Using the blanks and calibration plots, the LOD was calculated as the analyte concentration that gave a peak area with a signal-to-noise ratio of 3, the LOQ was calculated as the analyte concentration that gave a peak area with a signal-to-noise ratio of 10. Intra-day repeatability was determined using standard solutions and samples containing the six synthetic compounds at 5×10^{-8} and 10^{-9} mol/L. Intra-assay repeatability was determined using standard solutions containing NA, Ad, DA, 3-MT, and 5-HT at 5×10^{-9} mol/L. The accuracy of the method was calculated from the analysis of samples spiked with known quantities of standard (5×10^{-9} mol/L). Analyte concentrations in biological samples were expressed as mol/L.

2.5. Data analysis

The dead volumes of the columns were determined using the equation $\pi d_c^2 \varepsilon L/4$, where d_c is the internal diameter of the column, ε the percentage of the column volume occupied by the mobile phase (usually estimated at 70%), and L the length of the column. The dead time was determined using the equation Dead time = L/linear velocity, where L is the length of the column and the linear velocity is the optimal speed for the highest efficiency based on a value of 0.1 cm/s for 5 μm porous particles and calculated using the equation (linear velocity × diameter of particles) = constant. The theoretical optimal flow rate was calculated as the dead volume divided by the dead time. The retention factor for an analyte was expressed as $k = (t_r - t_m)/t_m$, where t_r is the retention time of the analyte and t_m the dead time of the chromatographic system. The resolution was calculated using the equation $Rs = 2(t_2 - t_1)/(w_1 + w_2)$, where t_1 and t_2 are the retention times for the two analytes and w_1 and w_2 their respective widths at baseline. The number of plates was calculated as $5.54 \times (\text{retention time}/2.35\sigma)^2$.