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An improved microbore UHPLC method with electrochemical detection for the simultaneous determination of low monoamine levels in *in vivo* brain microdialysis samples

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ABSTRACT

The simultaneous determination of the monoamines dopamine (DA), noradrenaline (NA) and serotonin (5-HT) in *in vivo* microdialysis samples remains challenging because of the low extracellular neurotransmitter levels in different brain regions, specific sample characteristics, and the quest for high temporal resolution and a multi-target strategy in neuropharmacological research. A fast and sensitive microbore (1.0 mm i.d. column) UHPLC method coupled to electrochemical detection (ECD) is developed by means of design of experiments with the emphasis on sufficient retention of NA within an acceptable total analysis time. Indeed, NA is the earliest eluting compound and often interferes with the broad solvent front originating from the sample matrix. The sensitive UHPLC-ECD assay (LLOQ of 100 pM for NA and 150 pM for DA and 5-HT) with an analysis time of 8 min for standard solutions and 20 min for *in vivo* microdialysis samples originating from rat hippocampus, prefrontal cortex and striatum, is validated applying accuracy profiles. The combination of *in vivo* microdialysis and microbore UHPLC-ECD has shown to be particularly suitable for future contributions to neuropharmacological research on the monoaminergic system.

1. Introduction

The well-described monoaminergic neurotransmitter system is still topic of various neuropharmacological studies because of its widespread involvement in neurological disorders [1], such as Parkinson's disease [2–4], epilepsy [5,6], Alzheimer's disease [7–9] and neuropsychiatric disorders [10,11]. For the monitoring of neurotransmitter levels in a specific brain area of freely moving animals following a pharmacological stimulus, the *in vivo* microdialysis technique is particularly suitable. The persistent interest in this neurochemical research is reflected in the number of recent research papers reporting analytical methods for the quantitative determination of the monoamine neurotransmitters such as dopamine (DA), noradrenaline (NA) and/or serotonin (5-HT) in *in vivo* brain microdialysis samples since the publication of the book chapter of Sarre and Michotte [12] in 2007 and the review of Perry et al. [13] in 2009 until the first half of 2015, as listed in Table 1. Indeed, chromatographic advances emerged very fast in recent years and have reached the end users to overcome specific problems related to former bioanalytical methods. Generally, it can be stated that detection of monoamine neurotransmitters using mass spectrometry is extremely sensitive on the condition of a preceding derivatization step to improve the fragmentation patterns and the ionization efficiencies [14]. Besides, the widespread use of electrochemical detection (ECD), which was introduced by Kissinger et al. in 1973 [15] and is less complex and expensive, demonstrates to be well suited for monoamine analysis in *in vivo* microdialysis samples originating from diverse brain areas [16,17]. Moreover, earlier work has shown that the coupling of ECD to a

Abbreviations: 5-HT, serotonin; DA, dopamine; DoE, design of experiments; ECD, electrochemical detection; LLOQ, lower limit of quantification; LOD, limit of detection; LOF, lack of fit; MS/MS, tandem mass spectrometry; NA, noradrenaline; POISe, performance optimizing injection sequence; RSD, relative standard deviation; S/N, signal-to-noise ratio; UHPLC, ultra-high performance liquid chromatography.

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Table 1
Literature overview of analytical methods for the determination of monoamine neurotransmitters (sometimes combined with other neurotransmitters) in *in vivo* brain microdialysis samples, reported between 2009 and the first half of 2015.

Analytes	Technique	Analysis time	Sample volume	Sensitivity level (pM)	Sample origin	Reference
Arg, Lys, Trp, GABA, l-Ser, Ala, Tau, Gly, Glu, Asp, DA, d-Ser, PEA	Derivatization with FITC, CE-LIF	20 min	6 μL	DA: 100[a]	Rat hypothalamus	[57]
5-HT	Capillary UHPLC-ECD	1 min	500 nL	5-HT: 70[a]	Mouse hippocampus	[49]
5-HT	Capillary UHPLC-PFET	1 min	500 nL	5-HT: 200[a]	Mouse hippocampus	[49]
DA, HVA, 3-MT, DOPAC	Lyophilization, HPLC-MS/MS	20 min	80 μL	DA: 3.71[a], 4.24[b]	Rat nucleus accumbens	[50]
5-HT, 3-MT	On-line capillary UHPLC-ECD	36 s	500 nL	5-HT: 300[a]	Rat striatum	[46]
ACh, Tau, Hist, Ser, Asp, Gly, Glu, GABA, Ado, 5-HIAA, HVA, NM, 5-HT, 3-MT, DOPAC, NA, DA	Derivatization with benzoyl chloride, microbore UHPLC-MS/MS	8 min	5 μL	NA: 200[a] DA: 30[a] 5-HT: 100[a]	Rat ventral tegmental area, nucleus accumbens, prefrontal cortex	[51]
Ado, DA, ACh, 5-HT	HPLC-MS/MS	7 min	20 μL	DA: 10[a], 50[c] 5-HT: 10[a], 50[c]	Mouse nucleus accumbens	[52]
DA, NA	Derivatization with dansyl chloride, HPLC-MS/MS	5 min	20 μL	DA: 68[c] NA: 59[c]	Rat prefrontal cortex	[53]
DA, NA, 5-HT	Derivatization with SPTPP, HPLC–MS/MS	3 min	30 μL	NA: 13[a], 30[c] DA: 7[a], 30[c] 5-HT: 1[a], 30[c]	Rat striatum	[54]
5-HT, DA, 5-HIAA, DOPAC, HVA	UHPLC–MS/MS	25 min	15 μL	DA: 200[a], 500[b] 5-HT: 200[a], 1000[b]	Human cortex	[55]
5-HT	On-line capillary UHPLC-ECD	30 s	500 nL	5-HT: 160[a]	Rat striatum	[47]
NA, DA, 5-HT, DOPAC, HVA, 5-HIAA	Microbore UHPLC-ECD	12 min	5 μL	NA: 32[a] DA: 42[a] 5-HT: 83[a]	Rat prefrontal cortex	[45]
NA, AD, DA, 3-MT, 5-HT	Capillary UHPLC-ECD	21 min	1 μL	NA: 750[a], 2500[b] DA: 750[a], 2500[b] 5-HT: 1500[a], 5000[b]	Rat striatum, amygdala, hippocampus	[44]
DA	On-line capillary UHPLC-ECD	1 min	500 nL	DA: 150[a]	Rat striatum	[48]
NA, DA, 5-HT	Microbore UHPLC-ECD	8 min	5 μL	NA: 83[a], 250[c] DA: 58[a], 100[c] 5-HT: 60[a], 100[c]	Rat hippocampus	[24]
DA	UHPLC–MS/MS	2.5 min	4 μL	DA: 130[a], 1100[c]	Rat striatum	[56]

3-MT, 3-methoxytyramine; 5-HIAA, 5-hydroxyindole-3-acetic acid; 5-HT, serotonin; 5-HTP, 5-hydroxytryptofaan; ACh, acetylcholine; AD, adrenaline; Ado, adenosine; Ala, alanine; Arg, arginine; Asp, aspartate; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; ECD, electrochemical detection; FITC, fluoresceine-5-isothiocyanate; GABA, γ-aminobutyric acid; Glu, glutamate; Gly, glycine; Hist, histamine; HVA, homovanillic acid; LIF, laser-induced fluorescence detection; Lys, lysine; NA, noradrenaline; NM, normetanephrine; PEA, *O*-phosphoethanolamine; PFET, photoluminescence following electron transfer; S/N, signal-to-noise ratio; Ser, serine; SPTPP, 5-*N*-succinimidoxy-5-oxopentyl triphenylphosphonium bromide; Tau, taurine; Trp, tryptophan; Tyr, tyrosine.

[a] LOD calculated as S/N = 3.
[b] LOQ calculated as S/N = 10.
[c] LLOQ.

microbore (1.0 mm i.d. column) UHPLC device is highly appropriate for sensitive neurochemical analysis [18].

The main challenges for the simultaneous monoamine determination using (U)HPLC with ECD are defined by the characteristics of the sample [12,17]. When using membranes with low cut-off values (≤20 kDa), large biomolecules, such as proteins and enzymes, cannot cross the probe membrane, thereby obtaining a protein and cell free aqueous solution consisting of a complex mixture of small molecules. This allows direct injection of the sample onto a chromatographic device without a sample pretreatment step [19]. Typically, low concentrations of neurotransmitters (pM range) in low sample volumes (1–50 μL) are collected, depending on the perfusion rate and the temporal resolution of the experiment [20]. In this way, many samples per animal or per probe are obtained and the analysis is often the time limiting step in the process of a microdialysis experiment. Obviously, a fast analysis is pursued in order to allow high throughput of samples.

The basic monoamines are typically retained on a reversed-phase column with the use of an ion-pair reagent and isocratic elution. Most reported assays include the determination of their acidic metabolites, using a mobile phase at pH ~3. When the researcher is interested in the monitoring of the three monoamine neurotransmitters DA, NA and 5-HT solely, a pH ~6 is however preferred. In this way, the relatively high metabolite peaks which may interfere with the neurotransmitter peaks at low pH, are not retained on the column and elute in the solvent front [12]. Nevertheless, special attention should be paid to the occurrence of peak-splitting in ion-pair UHPLC at high flow rates [21].

Because of the relative complexity of the sample, high selectivity of the separation technique is required. Generally, method