RECENT CONTRIBUTIONS OF CAPILLARY ELECTROPHORESIS

TO NEUROSCIENCE

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Abstract

1   Contributions to neuroscience are necessary to understand the behavior of the brain.

2   This requires using powerful analytical techniques to monitor neuroactive molecules and

3   their concentrations in biological samples (fluids, cells, and brain tissues). Capillary

4   Electrophoresis (CE) is well known for its high resolution power, short analysis times,

5   and low consumption of reagents and samples. It presents analytical advantages for the

6   determination of neuroactive molecules not easy determined by other analytical

7   techniques. CE also offers the possibility of controlling more than one neuroactive

8   molecule at a time which is interesting to detect changes as a result of a stimulus. CE is

9   well-established to accomplish enantioseparations, contributing with a better

10   understanding of the properties of a neuroactive chiral molecule. This review is focused

11   on the most relevant articles published from January 2008 to July 2014, based on the

12   determination in biological samples of potentially interesting molecules in neuroscience

13   using CE and microchip-CE.

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15   **Keywords:** amino acid, biogenic amine, capillary electrophoresis, neuroactive

16   compound, neurological disorder, neuroscience.

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Abbreviations

3-MT: 3-methoxytyramine; 5-HIAA: 5-hydroxyindole-3-acetic acid; 5-HT: serotonin; μ-TAS: micro-total analysis system; ABEI: N-(4-aminobutyl)-N-ethylisoluminol; ACh: acetylcholine; AD: amperometric detection ; Ala: alanine; Arg: arginine; Asn: asparagine; Asp: aspartate; BGE: background electrolyte; BPA: bisphenol A; Br-BQCA: 3-(4-bromobenzoyl)-2-quinolinecarboxaldehyde; CD: cyclodextrin; CEC: Capillary Electro-Chromatography; C^4D: capacitive coupled contactless conductivity detection; CE: Capillary Electrophoresis; CFSE: 5-carboxyfluorescein N-succinimidyl ester; Cit: citruline; CL: chemiluminiscence; CNS: central nervous system; CSF: cerebrospinal fluid; CSP: chiral stationary phase; Cys: cysteine; CZE: Capillary Zone Electrophoresis; DA: dopamine; DM-β-CD: dimethyl-β-CD; DOPA: 3,4-dihydroxyphenylalanine; DOPAC, 3,4-dihydroxyphenylaceticacid; DTAF: 5-(4,6-dichloro-s-triazin-2-ylamino) fluorescein; EC: electrochemical; EKC: Electrokinetic Chromatography; EP: epinephrine; FASS: field-amplified sample stacking; FITC: fluorescein isothiocyanate; FSCV: fast-scan cyclic voltammetry ; GABA: γ-aminobutyric acid; Gln: glutamine; Glu: glutamate; Gly: glycine; Him: histamine; His: histidine; HPA-β-CD: 6-monodeoxy-6-mono(3-hydroxy)-propylamino-β-cyclodextrin; HPLC: High Performance Liquid Chromatography; HP-β-CD: hydroxypropyl-β-CD; HVA: homovanillic acid; Ile: isoleucine; IT: ion trap; IXS: 3-indoxyl sulfate; LED: light-emitting electrodes; Leu: leucine; LIF: laser-induced fluorescence; LINF: laser-induced native fluorescence; LVSS: large volume sample stacking; Lys: lysine; MCE: microchip capillary electrophoresis; Met: methionine; MEKC: Micellar Electrokinetic Chromatography; MEEKC: Micro-Emulsion Electrokinetic Chromatography; MIP: molecular imprinted polymer; MISPE: molecular imprinted solid phase extraction; MMIP: magnetic

42 molecular imprinted polymer; NBD-F: 7-nitrobenzo-2-oxa-1,3-diazole; NDA:

43 naphthalene-2,3-dicarboxyaldehyde; NE: norepinephrine; NM: normetanephrine; OP:

44 octopamine; OPA: *o*-phthalaldehyde; Orn: ornithine; PBS: phosphate buffer saline;

45 PDDAC: poly(diallyldimethylammonium) chloride; PDMS: polydimethylsiloxane; Phe:

46 phenylalanine; Pro: proline; Q: quadrupole; SDS: sodium dodecilsulfate; Ser: serine;

47 SPE: solid phase extraction; SPME: solid phase micro-extraction; TA: tryptamine; Tau:

48 taurine; TCA: trichloroacetic acid; Thr: threonine; TOF: time-of-flight; Trp: tryptophan;

49 Tym: tyramine; Tyr: tyrosine; Val: valine; VMA: vanillomandelic acid.

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51 **Contents**

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1. Introduction

Neuroscience, the study of the nervous system, has been increasingly investigated over the last century. Neurons play the most important role in the nervous system since they are the cells in charge to transmit the information by electrical and chemical signals. Signal transmission can be driven by chemical messengers, also known as neuroactive compounds (neurotransmitters and/or neuromodulators), which are involved in plenty of signal transmissions occurring in the neuron's synapse. In this synapse, the neuroactive compounds are released to bind to the receptors in the membrane of a target cell [1]. Neurotransmission has been proven to be related to the behavioral, cognitive, and emotional state of an organism and also to be associated to conditions such as depression, drug dependence, schizophrenia, and degenerative diseases [2]. Most of the Central Nervous System (CNS) diseases are caused and can be accentuated by complex and abnormal disturbances or disruptions of regulatory mechanisms, protein expression profiles or in some metabolic pathways [3].

A large variety of molecules can be neurologically active ranging from gases as nitric oxide, carbon monoxide and hydrogen sulfide, to small molecules such as amino acids (both protein and non-protein) and biogenic amines (monoamines, histamine and acetylcholine), and to larger molecules as neuropeptides or hormones [1, 4]. Glycine (Gly), taurine (Tau) and γ-aminobutyric acid (GABA) are the main inhibitory amino acids [2], on the contrary to glutamate (Glu) and aspartate (Asp) which are the most widespread excitatory neuroactive compounds in the CNS and influence numerous neuronal networks [1]. Serotonin (5-HT), a monoamine derived from tryptophan (Trp), is implied in physiological functions such as memory, learning, feeding, sleep and body temperature regulation and it is also involved in pathologies such as depression, Alzheimer's disease, autism, schizophrenia, and bipolar disorder [1]. Other monoamines such as the

89   catecholamines dopamine (DA), epinephrine (EP), and norepinephrine (NE), all derived

90   from tyrosine (Tyr) and phenylalanine (Phe), are other important molecules in

91   neurotransmission. These catecholamines play an important role in the diagnosis of many

92   disorders such as Alzheimer's [5] and Parkinson's diseases [6], cocaine addiction,

93   pheochromocytoma and a variety of mental diseases [7]. Other important neuroactive

94   molecules are the biogenic amines histamine (Him), which is involved in a high variety

95   of physiological responses (the regulation of sleep, the secretion of hormones, and the

96   formation of cognition) [8, 9]; and acetylcholine (ACh), which was the first neuroactive

97   molecule discovered and it is known that dysfunction in the cholinergic system is related

98   with Alzheimer's and Parkinson's diseases [1].

99   The potential role of D-amino acids in aging and neurodegenerative processes such

100   as Alzheimer's disease was revealed years ago by Fisher and D'Aniello [10, 11] and this

101   role has been studied ever since [12]. D-serine (D-Ser), D-alanine (D-Ala), and D-Asp

102   have all been found in relatively significant levels in the CNS [13]. D-Ser plays an

103   important role in neuroplasticity, memory, and learning [12], and the amounts of D-Ser

104   and D-Ala in mammals have been related with schizophrenia and depression [13, 14]. D-

105   Asp has been related with some neuromodulator functions and it is involved in

106   developmental and endocrine functions [12]. In the case of catecholamines, it is known

107   that interaction of L/D-EP and L/D-NE with their receptors is stereoselective, exhibiting

108   their enantiomers different activity and selectivity [15], although no clear relation

109   between neurotransmission function and enantiomers has been established yet.

110   The above-mentioned information points out the importance of the development of

111   analytical tools to determine these small molecules to study neurological disorders to

112   measure and evaluate the progress of a disease or a process that occurs in the CNS, and/or

113   to find the possible response to a specific treatment [3]. As well, multiple analyte

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114   detection is an attractive characteristic from the point of view of neuroscience due to the

115   fact that the alteration of more than one neuroactive substance at a time can be studied as

116   a result of a stimulus. Thus, analytical techniques frequently employed for monitoring

117   neuroactive molecules are related to rapid, sensitive, and efficient analysis, and should

118   offer the possibility to perform it in samples with significantly low volumes (some of

119   them just few microliters) and with concentrations of the compounds of interest very low

120   (sometimes even below the nM level).

121   Some review articles [4, 16] have been focused on the application of different

122   analytical techniques for neuroactive compounds determination being High Performance

123   Liquid Chromatography (HPLC), and Capillary Electrophoresis (CE), or enzyme assays

124   and biosensors microelectrodes, the most widely used approaches. Among all these

125   techniques, CE can be considered as a suitable and reliable technique to study potentially

126   interesting molecules in neuroscience because of its high resolution power, fast-analysis

127   and the use of small sample volumes (nanoliters or even less) what makes it ideal for *in*

128   *vitro* or *in vivo* analysis of neurological samples. Moreover, as described before, chiral

129   analysis represents a promising topic in neuroactive compounds determination because

130   of the differences in the biological activities of a pair of enantiomers. Therefore, an

131   analytical technique capable of resolving enantiomers should facilitate the understanding

132   of the activity of the enantiomeric neuroactive compounds. In this aspect, CE is

133   considered as one of the most powerful and useful techniques in chiral separations due to

134   its wide possibilities [17, 18].

135   Contributions of CE to neuroscience have been summarized in different review

136   articles [2, 19].Taking into account that different works have been published afterwards,

137   our aim is to cover recent articles, published from January 2008 to July 2014, looking at

138   the review published in 2008 as a starting point. To the best of our knowledge, all relevant

139 articles published within the mentioned period of time which were focused on the

140 determination by CE and microchip CE of neuroactive compounds in biological samples

141 of interest in neuroscience have been considered. Our work sorts the CE methods by the

142 detector system employed highlighting the differences among them and indicating the

143 instrumentation needed. As a consequence, methods with different detection systems can

144 be easily followed and compared. We also discuss the importance and possibilities of the

145 sample preparation, pre-concentration techniques, and chirality in the determination of

146 potentially interesting molecules in neuroscience, whose contributions have been

147 increasing since 2008.

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149 **2.   Capillary Electrophoresis for the determination of neuroactive compounds**

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151 **2.1. Sample preparation**

152   Analysis of biological samples provides very useful information when studying

153 neuroactive molecules of interest in neuroscience research. The most widely analyzed

154 samples are different biological fluids, cells, and brain tissues. Biological fluids such as

155 urine, blood (plasma, serum, and whole blood), cerebrospinal fluid (CSF), and

156 extracellular fluid (ECF) of certain regions of the brain are the fluids analyzed to

157 determine neuroactive molecules. For instance, determination of catecholamines in urine

158 is a well-known tool in the diagnosis of pheochromocytoma [20], plasma levels of

159 neuroactive amino acids can be used in the diagnosis of several disorders as bipolar

160 disorder [21], CSF is very useful in the diagnosis of Alzheimer's disease [22], and ECF

161 can be useful to study the effects in the brain during ischemia and reperfusion periods

162 [23]. The analysis of single mammalian cells such as PC-12 nerve cells [24] or nerve cells

163 from invertebrates as the sea slug *Aplysia californica* [25] is carried out to increase the

knowledge regarding the CNS behavior. Finally, analysis of certain regions of the brain (white matter, gray matter, frontal cortex, hippocampus, parietal, temporal cortex, amygdale, or cerebellum) can be useful for the determination of neuroactive molecules. In this aspect, analysis of brain tissue provides useful information regarding neurodegenerative diseases, such as Alzheimer's [26].

The nature of these biological samples require a cleanup step or a procedure to extract or isolate neuroactive molecules. Hence, in order to ensure the adequate determination of the compounds of interest, a reliable sample treatment is of great importance. The treatment of all biological fluids is usually very similar, this consisting of the elimination of interfering proteins by precipitation (with solvents as acetonitrile, or acids as trichloroacetic and perchloric), followed by a centrifugation step and finally a filtration of the supernatant, or directly by ultrafiltration with cut-off membranes. From the two procedures, ultrafiltration has the advantage of, not only being simpler and faster, but also enables working with smaller sample volumes and reducing sample dilution. However, the high cost of these cut-off membranes is the main drawback of this procedure. In the case of urine analysis, desalting procedures with liquid-liquid extraction and solid phase extraction (SPE) have also been reported.

SPE can be considered as an interesting technique for selective sample preparation and purification prior CE analysis of liquid biological samples. Additionally, molecular imprinted polymers (MIPs) have been applied as sorbents in several SPE cartridges for being high selective to the template molecules and for their easy preparation and long lifetime. This technique is known as molecularly imprinted solid-phase extraction (MISPE). Related procedures include MIP with solid phase micro-extraction (SPME) and magnetic molecular imprinted polymer (MMIP) with SPE.

188   Regarding cell analysis, a sample treatment based on washing with phosphate

189   buffer saline (PBS) solution, cell lysis by sonication, and elimination of interfering

190   proteins as described above are the most common procedures [27].

191   Finally, the main sample treatment used in the analysis of brain tissue is its

192   homogenization in a solution (buffer, EDTA, or acids such as formic, ascorbic or

193   perchloric) with or without sonication, and subsequent removal of proteins as described

194   above [26]. Freeze-drying is a novel sample preparation technique in which the sample is

195   frozen and dehydrated under reduced pressure, forcing the water within the sample to

196   sublimate directly into the gas phase. Berglund et al. [28] demonstrated that freeze-drying

197   is a faster process in sample preparation in comparison with a conventional dissection,

198   which allowed concentrating the sample by increasing the number of brains in a fixed

199   homogenate volume. As a complementary sampling, microdialysis offers the possibility

200   to analyze ECF in order to perform the *in vivo* determination of neuroactive molecules

201   present in certain regions of the brain. The microdialysis involves implanting a small

202   probe containing a semipermeable membrane that will allow molecules from a specific

203   tissue to diffuse across [29]. Thus, tiny volumes containing only small molecules will be

204   trapped and collected for a perfectly suitable analysis by CE [30]. When dialysate samples

205   are used, generally no other sample treatments are used.

206   Note that it is also possible to couple the microdialysis sampling on-line to CE

207   which will potentially increase the sampling frequency allowing to monitor rapid changes

208   correlated with behavioral episodes or after a stimulus [31, 32]. Thus, the *in vivo*

209   monitoring in rat brain tissues by on-line microdialysis of several amino acids (Asp,

210   GABA, Glu, Gly, Ser, and Tau), important neuroactive compounds, enabled to observe

211   changes in their levels after ethanol self-administration [31] or depending on the sleep

212   stage [32]. In this last work authors pointed out the importance of on-line microdialysis

213 to measure these neuroactive compounds in the sleep episodes of the rodents (during non-

214 rapid eye movement sleep and rapid eye movement sleep), since they are relatively short.

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216 **2.2. CE with UV absorption detection**

217 Many works have been described employing UV absorption detection in the

218 determination of neuroactive and related compounds (see **Table 1**). Amino acids,

219 catecholamines, and other biogenic amines in urine samples have been the most studied

220 compounds. Capillary Zone Electrophoresis (CZE) has been the CE mode employed in

221 all works except in one of them which employed Capillary Electro-Chromatography

222 (CEC) to carry out a chiral separation. LODs in the nanomolar range (between $2 \cdot 10^{-9}$ M

223 and $300 \cdot 10^{-9}$ M) have been achieved usually employing low UV wavelengths (< 220 nm)

224 and mostly used in-capillary sampling pre-concentration techniques during sample

225 injection. It is worth highlighting that in most of the cases the determined concentration

226 of these analytes was above the LOD [33-35, 37, 39, 41, 42], whereas in other works

227 spiking of the samples had to be performed [36, 38-40].

228 Simultaneous separation of catecholamines, indolamines and metanephrines in

229 urine sample from a healthy volunteer has been reported using an in-capillary sample pre-

230 concentration technique [33]. Based on the different mobility of the analytes in presence

231 and in absence of poly(diallyldimethylammonium) chloride (PDDAC) in the background

232 electrolyte (BGE), analytes could be easily stacked between the boundary of the sample

233 zone and the BGE containing PDDAC due to a raise in the viscosity of the BGE above

234 the sample zone. This pre-concentration strategy, named large volume sample stacking

235 (LVSS), enhanced the sensitivity up to 100 times (in the nM range). The same pre-

236 concentration methodology, using glycerol instead of PDDCA to increase the viscosity

237 of the BGE above the sample zone, was employed for the simultaneous determination of

different cationic and anionic neuroactive compounds in urine samples from a healthy

volunteer [34]. Nanoparticles have been demonstrated to play an important role to reduce

sample matrix due to their high specific surface area, demonstrating another promising

alternative in the sample treatment for the extraction of catecholamines [35]. This

methodology along with a pre-concentration technique by LVSS was applied to

determine NE and DA in human urine from healthy volunteers obtaining LODs in the nM

range. On the other hand, a dispersive microextraction such as ultrasound-assisted

emulsification microextraction in combination with an in-capillary pre-concentration

system named pH-mediated sample stacking was investigated as a sample treatment prior

CE-UV analysis for simultaneous determination of 5-HT in spiked urine from a healthy

volunteer [36].

Another pre-concentration strategy known as field-amplified sample stacking

(FASS) has been employed to enhance the sensitivity by means of injecting a sample with

lower conductivity than the BGE [37, 38]. Note that due to the fact that the presence of

salts from the sample of urine disrupt the stacking strategy, MISPE was used to retain the

target analytes and discard not only salts but also other components present in the samples

of spiked urine [38]. This methodology allowed the analysis of DA, 3-methoxytyramine

(3-MT) and 5-HT at nanomolar concentrations in less than 8 min using hydroxypropyl-

β-CD (HP-β-CD) to improve the selectivity of the separation.

On the one hand, MIP combined with SPME demonstrated to be a powerful tool

for the sensitive quantitative determination of catecholamines without pre-concentration

step in non-spiked urine and spiked-serum from healthy volunteers by CE-UV [39]. In

this work, the MIP fiber was developed *in-situ* obtaining a flexible, homogeneous, highly

cross-linked, and porous fiber. By means of a MMIP combined with SPE, catecholamines

DA, 3-MT, normetanephrine (NM), NE, and EP could be rapidly analyzed in spiked urine

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263   with acceptable sensitivity without a pre-concentration step [40]. Ultra-fast determination

264   (in only 3 min) of 5-HT was, for the first time, carried out in human whole blood by

265   means of the so-called reverse injection, in which the injection is performed by the short-

266   end of the capillary [41].

267   Finally, a chiral stationary phase (CSP) in a monolithic MIP column using (-)-NE

268   as a template was employed to the enantioseparation of (±)-EP, (±)-NE, (±)-octopamine

269   and (±)-synephrine along with the separation of DA and (-)-isoproterenol in urine from a

270   healthy volunteer [42]. Authors found a loss in the chiral separation power of some of the

271   studied compounds caused by the saltiness of the urine sample studied due to the use of

272   filtration as the only sample treatment.

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274   **2.3. CE with fluorescence detection**

275   Laser Induced Fluorescence (LIF) is the detection system of choice when

276   determining neuroactive compounds in biological samples by CE due to its excellent

277   sensitivity [43]. As seen in **Table 2**, LODs up to 10^{-13} M and in most of the cases below

278   10^{-9} M are obtained, even without the need to employ pre-concentration techniques.

279   Generally, a derivatization step is necessary since most of neuroactive compounds lack

280   of fluorescence moieties. However, depending on the excitation wavelengths used in LIF

281   detection, some groups of analytes such as indoleamines, catecholamines, or aromatic-

282   containing compounds can be selectively detected without a labeling procedure. Thus,

283   indoleamines, catecholamines, other biogenic amines, and amino acids as Trp and Tyr

284   were detected with high sensitivity (between 10^{-12} and 10^{-8} M) in human urine and serum

285   [44, 45, 47] and in rat brain tissue [46]. Interestingly, Li et al. used citrate-capped gold

286   nanoparticles to capture and extract thiol-containing molecules and nucleobases in human

287   urine and serum from a healthy volunteer [44]. Huang et al. developed a dynamic coating

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based on poly(L-lysine) and silica nanoparticles to obtain coated capillaries with higher efficiencies and a longer durability in the separation of DA, EP, 5-HT, other biogenic amines, and amino acids in human urine samples to study changes in the levels of different neuroactive compounds after drinking tea [45].

Derivatization reagents need to fulfill several requirements such as stability, minimal hydrolysis products, low reaction times and fitting in the excitation wavelengths of argon-ion laser (351 and 488 nm among others) because this is the most common used laser (see **Table 3**). A comparison between derivatization reagents (CFSE, FITC, and NBD-F) was performed by Wagner et al. for the determination of the primary excitatory neuroactive compounds Glu and Asp [48]. Although the best LOD was obtained with NBD-F, FITC was chosen based on the high stability obtained. In a later work, same authors applied the method to the determination of Asp and Glu in brain ECF of chicks. Authors found higher levels of these two amino acids when stimulated with potassium and also in stress conditions compared to basal levels [49].

FITC has been one of the most employed derivatization reagents, although several hydrolysis products are obtained and this derivatization procedure implies to be carried out overnight, in the dark and at room temperature. However, high temperatures during sample derivatization [67] or microwave-assisted methods [50] have been found to produce a drastic reduction in the reaction times of this derivatization reagent (a few minutes). Thus, the derivatization of some amino acids (Ala, Asp, Glu, Gly, Ser and Tau) with FITC at high temperatures in several samples (plasma, red blood cells, urine, cultured cells, CSF, saliva, and vitreous humor) allowed reducing derivatization reaction times down to 20 min [67]. On the other hand, a microwave-assisted derivatization method was described by Liu et al. [50] for the determination of FITC derivatized catecholamines, and a significant reduction in the derivatization time to only 2 min was

313 achieved. In addition, an in-capillary pre-concentration technique (FASS) was employed

314 to achieve higher sensitivities (in the picomolar range). **Figure 1** shows the

315 electropherograms of rat brain samples obtained in normal injection and FASS mode,

316 demonstrating the ability of the CE-LIF method to successfully determine concentrations

317 as low as 100 ngL^{-1} of catecholamines in rat brain. This CE–LIF method provided better

318 detectability when compared to those reported on catecholamines determination. On the

319 other hand, Diao et al. showed the possibility of using light-emitting diodes (LEDs) as a

320 radiation source alternative to other common lasers to determine FITC derivatized

321 catecholamines in urine from healthy volunteers. LEDs have the advantages of having a

322 small size, long lifetime, stable output with less energy consumption, broad range of

323 emission wavelengths (280–1300 nm) and to be easy to operate with [47], although

324 applications in CE have been very scarce.

325 Another strategy that can be used to improve the derivatization step is conducting

326 the derivatization reaction directly in the capillary before separation. An in-capillary

327 derivatization strategy with OPA was employed for the *in vivo* monitoring in rat brain

328 tissues by on-line microdialysis of several amino acids (Asp, GABA, Glu, Gly, Ser, and

329 Tau) as important neuroactive compounds, observing changes in their levels after ethanol

330 administration [31] or depending on the sleep stage [32]. Denoroy et al. [51] developed a

331 rapid in-capillary NDA derivatization strategy to determine amino acids in extracellular

332 fluid samples. Although it requires the use of the toxic reagent cyanide to react, NDA has

333 been commonly used in neuroactive compounds determination due to the fact that is not

334 fluorescent itself and reacts rapidly to give stable fluorescent derivatives that can be

335 excited using low-cost visible lasers. A NDA-based method was employed to derivatize

336 Glu, GABA and carbamathione, a metabolite of the drug disulfiram used in alcohol abuse

337 and cocaine addiction treatments. This method was interestingly applied to monitor in

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338 ECF from rats the *in vivo* changes induced by this metabolite in GABA and Glu levels

339 after the administration of disulfiram [52]. Although half-life of carbamathione in brain

340 is around 5 min, changes in basal concentrations of GABA and Glu after drug

341 administration persisted for more than 2 h. Authors state that carbamathione could

342 produce more long-lived metabolites producing the changes in GABA and Glu levels.

343 Another NDA-based method was employed to derivatize amino acids, catecholamines,

344 and other biogenic amines and to monitor in rat brain ECF the *in vivo* changes induced

345 after 3-mercaptopropionic acid administrations, a convulsing drug which is used to induce

346 seizures what produces ischemic events [53].

347 Other derivatization reagents are CSFE, employed for monitoring the changes of

348 Glu and Asp in the periaqueductal gray matter of rats after been stimulated by formalin

349 injection [54], DTAF employed for the detection of DA and NE in spiked human serum

350 [55], and NBD used for the labelling of several amino acids in different samples [56, 57].

351 All works with LIF detection described so far have used the CZE mode for carrying

352 out the separation of the compounds of interest. However, in some cases, CZE does not

353 provide enough resolving power for the simultaneous separation of several compounds

354 or they cannot be separated from the matrix interfering compounds. For this reason, in

355 order to improve the separation selectivity of these approaches, some additives are

356 employed in the separation medium such as dextran [44], surfactants (below its critical

357 micelle concentration), sodium dodecyl sulfate (SDS) [48,49,56,57] or lithium dodecyl

358 sulfate (LDS) [53], as well as cyclodextrins (CDs) [31,32,56,57]. CDs are a group of

359 chiral oligosaccharides known for their high separation power and variability allowing

360 them to be, not only the most employed chiral selectors, but also to be useful additives

361 employed to improve separation and resolution. An example of the capability of CDs to

362 improve electrophoretic separations was shown by Shi et al. with the combination of β-

363 CD and SDS as additives in the BGE to determine NBD-GABA [56]. They determined

364 this labeled compound in a complex mixture of several amino acids from the head of

365 houseflies and diamondback moths. The same combination of SDS and β-CD was

366 selected by Casado et al. to avoid the overlapping of the signals corresponding to the

367 much higher concentration of NBD-Gln compared to the levels of NBD-GABA in CSF

368 samples (CSF Gln/GABA ratio=5000:1) [57].

369   Although CZE has been the mode of choice for LIF detection, the MEKC mode has

370 been also widely used with LIF detection, unlike in UV detection. MEKC is an approach

371 very promising and interesting to simultaneous monitoring multiple neuroactive

372 compounds in different biological samples. Thus, a clear example of the excellent

373 performance of the MEKC mode was showed by Zhang et al. who developed a MEKC

374 methodology to separate 19 amino acids and 2 catecholamines labeled with BQCA in

375 different samples (human plasma and rabbit vitreous humor [58], or HEK293 and PC12

376 cells [27]) using SDS together with acetonitrile to increase the hydrophobicity of the BGE

377 and prevent comigration of all amine derivatives formed in CZE. Note that, an increase

378 in several amino acids levels in plasma was found when compared diabetic to healthy

379 patients (**Figure 2**) [58]. On the other hand, hyperlipidemic patients showed higher levels

380 of Glu, Ala, Leu, Phe, and Lys, and lower levels of Gly in plasma compared to healthy

381 patients. In addition, rabbit vitreous humor samples helped to conclude that amino acids

382 levels increased under intraocular hypertension, whereas that Tau levels decreased. In this

383 way, authors demonstrated that Tau plays a role in the regulation of osmotic pressure in

384 eyes.

385   Other MEKC works include an article in which authors used methanol to modify

386 the selectivity in the determination of SIFA-labeled amino acids in saliva and in CSF

387 from healthy volunteers [59]. Besides adding an organic solvent, some authors have also

modified the nature of the salt of the surfactant and buffer (lithium instead of sodium) to

monitor GABA and Gly, derivatized with NDA, in rat brain ECF after administration of

the drug disulfiram [60]. Moreover, in another work authors changed the nature of the

surfactant completely using the cationic surfactant cetyltrimethyl ammonium bromide

(CTAB) to separate seven TMBB-Su-labeled amino acids and to monitor their levels to

establish a comparison between normal mice and Alzheimer's disease transgenic model

mice [26]. As in CZE, the use of CDs together with organic solvents can be useful in

MEKC to modify the selectivity of complex separations. Following this strategy, Li et al.

employed a mixture of HP-β-CD and dimethyl-β-CD (DM-β-CD) to investigate the

changes of fourteen DTAF-labeled amino acids in microdialysates during cerebral

ischemia/reperfusion period [23].

Another strategy devoted to perform the determination of neuroactive compounds

with LIF detection is the CE mode named Micro-Emulsion Electro-Kinetic

Chromatography (MEEKC). Lin et al. [61] demonstrated the suitability of this

methodology for hydrophobic FITC-labeled amino acid that easily entered into the oil

phase of the MEEKC system. Using an optimized microwave-assisted derivatization

protocol with FITC, the method was applied to different samples, including rat brain.

In other sense and as above mentioned, chirality plays an important role in

neuroscience and thus, the enantiomeric separation of neuroactive molecules has been

one of the goals of some studies applying CE with LIF detection. D/L-Ser has been the

most studied chiral amino acid due to its demonstrated enantioselectivity in neuronal

functions [21,62-64]. Lorenzo et al. [21, 62] developed two similar methodologies using

the most widely used mode in chiral CE, the Electrokinetic Chromatography (EKC). This

mode utilizes the experimental technique of CZE in combination with the principle of

chromatography, so that its separation principle relies on the different partition of

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enantiomers between the bulk solution and the chiral pseudo-phase (chiral selector). These methodologies used β-CD as chiral selector to enantioseparate several NBD-labeled amino acids (including Ser enantiomers) in mice rat tissue and in diabetic children urine [62] or in plasma from bipolar disorder patients [21]. In the latter work, the amino acids levels between patients with bipolar disorder and depressed patients' are discussed, and authors found around 2 µM of D-Ser in bipolar disease patients. Unlike the previous two studies, Li et al. used a new methodology in which, besides the chiral pseudo-phase (i.e., CDs), employed a second charged pseudo-phase non-chiral capable of interacting with analites such as a charged micelle in MEKC [63, 64]. By this strategy, several FITC-labeled amino acids, including only the chiral separation of D/L-Ser, were studied in ECF of rat brain. The enantioseparation of Ser could be used to study the importance of its D-enantiomer as a key neuromodulator after ischemia and reperfusion periods [63, 64]. In addition, chronic treatment with resveratrol decreases the release of Glu, Asp, and D-Ser in ischemia and reperfusion events, whereas increase levels of GABA, Gly and Tau [63].

D/L-Asp and D/L-Glu have also been studied by EKC, using a mixture of two CDs as chiral pseudo-phase to enantioseparate these analytes derivatized with NBD [65]. This method was applied in chicks brain tissues observing the presence of 1-2 % of D-Asp of the total Asp content, in all the studied brain areas.

Finally, Samakashvili et al. developed an EKC strategy based on β-CD as chiral pseudo-phase and SDS as second non-chiral charged pseudo-phase to study the levels of several FITC-labeled amino acids (GABA, Gly, and 8 D/L-amino acids) in CSF samples related with different stages of Alzheimer's disease [66]. They found that Alzheimer's disease subjects had lower amounts of L-Arg, L-Lys, L-Glu and L-Asp, but GABA levels increased. However, no significant differences were found in the rest of the studied amino

437 acids, including D-Ser. Once again, the importance of changes in amino acids levels is

438 highlighted as they can be used as biomarkers for diagnosing diseases.

439

440 **2.4. CE with other detection systems**

441   Other detection systems such as mass spectrometry (MS), electrochemical,

442 conductometric and chemiluminiscence have also been employed in CE determination of

443 neuroactive molecules (see **Table 4**).

444   CE-MS systems have been very scarcely used in the determination of neuroactive

445 compounds despite the fact that they facilitate their unequivocal identification in complex

446 biological samples. The most employed analyzer has been time of flight (TOF) followed

447 by the ion trap (IT) and a quadrupole (Q) coupled to a TOF (hybrid system called QTOF)

448 while in all cases the ionization source was ESI coupled with a coaxial sheath-flow

449 interface, as seen in **Table 4**. The determination of ACh, Him, DA, and 5-HT in the nM

450 range was performed for the metabolomics profiling of R2 neuron and metacerebral

451 single cells from *Aplysia californica* [25]. A CE-MS method with a QTOF analyzer has

452 been recently developed to simultaneously determine possible changes in GABA, some

453 biogenic amines, and their metabolites after administration of the psychostimulant drug

454 methylphenidate to *Drosophila* fruit fly [68]. In this work, using MS/MS experiments,

455 the selected neuroactive compounds and their metabolites were unequivocally identified,

456 observing that tyramine (Tym), OP, and DA levels changed when methylphenidate was

457 administrated. By means of urine metabolic profiles obtained by a developed CE-MS

458 methodology, Zeng et al. investigated the effect of the worldwide known plastic

459 manufacturer Bisphenol A (BPA) administrated to rats for 45 days [69]. Due to its

460 hormone-like properties, BPA dosage produced alterations of Glu, GABA, NE,

461 neuroactive compounds-related metabolites (Tyr, Him, Val, and Tau) and several

pathways (D-glutamine (Gln) and D-Glu metabolism, histidine (His) metabolism, (Phe), Tyr and Trp biosynthesis, among others).

Although just five CE-MS works were described, two of them were focused on chiral separations, both using an IT as analyzer in the MS/MS mode. The simultaneous enantioseparation of chiral precursors of DA as L/D-Phe, L/D-Tyr, and L/D-DOPA was described employing sulfated β-CD as chiral pseudo-phase in EKC and a partial filling technique (PFT) to prevent the entry of non-volatile chiral selector to the ionization source [24]. As **Figure 3** shows, this method was applied to their quantification in PC-12 cells, demonstrating the enantiospecific metabolism of DOPA in this neuronal model as these cells only metabolized L-enantiomer leaving the D-DOPA intact. Recently, a new EKC methodology has shown, for the first time, not only the simultaneous enantioseparation of chiral precursors of DA (L/D-Phe, L/D-Tyr, and L/D-DOPA), but also DA and its chiral metabolites (L/D-NE and L/D-EP), facilitating the study of the entire Phe-Tyr metabolic pathway in plasma and brain tissue of rat [70].

The amperometric detection (AD) is the electrochemical detection mode most used in CE both in CZE and MEKC. Using a standard electrode of Cu, six amino acids were detected by AD in serum samples from healthy volunteers with LODs in the range of μM [71]. Interestingly, Zhou et al. developed a FASS methodology along with a boron doped diamond electrode to detect the metabolites of catecholamines IXS, homovanillic acid (HVA) and VMA in the presence of Trp, other catecholamines and indoleamines with a good detection sensitivity (in the nM range) [72]. In this work, a fused-silica capillary was coated with gold nanoparticles embedded in PDDAC allowing the migration of catecholamines and indoleamines against the EOF not interfering with the determination of IXS, VMA, HVA, and Trp. Authors state that this method could be used to study the ratio HVA/MVA in urine samples, which is employed in the diagnosis of Menkes disease.

21

Another CZE methodology was developed by Liu et al. [73] to study the effect of noise in the levels of catecholamines and 5-HT present in rat brain tissue. A high sensitivity was obtained (in the nM range) using multiwalled carbon nanotubes as electrode.

A MEKC methodology with AD detection was developed to determine 24 biogenic amines and their metabolites in flies (*Drosophila melanogaster*) brain tissue [75], demonstrating the high-resolution capabilities of this CE mode. Only 6 of the 24 studied compounds are believed to be related to alcohol consumption, and these 6 compounds (DA, OP, salsolinol, norsalsolinol, N-acetyldopamine, and N-acetyloctopamine) were the only ones quantified in the analyzed samples. Same authors applied this methodology to micro-dissected brain regions of *Drosophila melanogaster* finding less levels of DA and DOPA (see **Figure 4**), among others, in white mutant flies compared to red-eyes types [74]. Some years after same authors compared the conventional dissection procedure with a novel treatment named drying-freeze, what makes it a faster process [28].

Cyclic voltammetry detection (CVD), is another electrochemical detection mode used in CE which was reported by Fang et al. [76]. Interestingly, the quantification of neuroactive compounds in a single *Drosophila* larva brain was carried out by implanting a microelectrode in their intact CNS prior analysis. LODs in the nM range were achieved by means of FASS as in-capillary pre-concentration technique.

Regarding the conductometric detection, the main advantage is that in this detection mode the detector response does not depend on the presence of chromophores, fluorophores or electroactive groups and, although the conductometric detection is usually associated with poor sensitivities, in some cases LODs values are comparable to those obtained by UV, LIF, MS, AD or CVD detection, as **Table 4** shows. Moreover, the sensitivity of these methodologies can be enhanced using pre-concentration strategies. Thus, LVSS was employed to increase the sensitivity a capacitive coupled contactless

conductivity detection (C^4D) system for the analysis of GABA, Gly, and Glu in micro-dialysates of periaqueductal gray matter obtaining LODs in the range of nM, values totally comparable with those obtained with a CE-LIF system [77]. An increase in Gly and Glu levels and a decrease in GABA after carrageenan-induced hyperalgesia was observed using the developed methodology. After perioral administration of paracetamol in this treatment, a reduction of Gly levels, without affecting Glu and GABA, was produced.

Chemiluminiscence detection (CLD) has been another system employed for neuroactive molecules determination, principally in the case of the catecholamines DA, EP, and NE, as it is shown in **Table 4**. The luminol-based chemiluminiscence is the system used in CZE methods with CLD [20,78-80]. Catecholamines generally enhance the chemiluminiscence resulted from the reaction between luminol and some metal complexes. Thus, in the case of Ag (III)-luminol complex, Xiangdong et al. developed a methodology which enabled to obtain LODs from 10^{-8} to 10^{-7} M for EP, NE and DA determination in urine from pheochromocytoma patients (patients who showed abnormal levels of catecholamines in urine) [20]. Lower LODs were obtained in the determination of DA and EP levels in human urine from healthy volunteers using, in this case, nanocrystals quantum dots in the CE buffer to catalyze the chemiluminiscence reaction between luminol and hydrogen peroxide [78]. Finally, diperiodatocuprate (III) was employed as a transition metal chelate at an unstable high oxidation state, to react with luminol in a basic media allowing to obtain comparable LODs in the determination of DA and EP levels in human urine [79, 80]. In this system, EP enhanced the chemiluminiscence reaction to produce a very strong signal and enabling the application of this methodology to study differences in EP levels in urine of smoker and nonsmoker groups [80].

2.5. Microchip Capillary Electrophoresis

Microfluidic devices or microchip have been gaining broad interest as separation platforms in CE. As **Table 5** shows, different detection systems have been chosen for the determination of neuroactive compounds. Shi et al. [81] made a comparison between the derivatization reagents FITC and OPA and discussed the possibility of using an Hg-lamp excitation for LIF detection which, although it lowered the sensitivity, it offered a broad spectral excitation source. LODs in the µM range were obtained for several OPA-labeled amino acids determined in single human vascular endothelial (ECV-304) cells using a CZE mode where β-CD and a certain percentage of acetonitrile were necessary to improve the separation efficiency, obtaining an acceptable resolution in just 200 seconds.

On the other hand, Li and Martin developed a strategy with LIF detection to quantify the amount of catecholamines released from PC-12 cells immobilized within the same microfluidic device [82]. This strategy allowed immobilized cells to be stimulated on-chip, and then, the released compounds were injected into the microchip where they were separated and then post-column derivatizated with NDA to be finally detected by LIF. LODs for the analytes of interest, DA and NE, were 70, and 250 µM, respectively.

Amperometric detection (AD) has also been applied in microchip CE [83-85]. Wu et al. developed a dual-asymmetry electrokinetic flow focusing applied in-microchip for the pre-concentration of DA in spiked PC-12 cells obtaining LODs in the nM level [83]. This strategy was also applied by the same authors for the determination of DA and NE, in PC-12 cells of cerebral infarction and intracranial infection patients [84]. Zhao et al. [85] developed another application with AD to determine 5-HT, DA, and EP in spiked CSF from healthy human using a microfluidic PDMS device with its microchannel coated

561   with polystyrene nanosphere/polystyrene sulphonate to increase the separation efficiency

562   and to stabilize the EOF.

563   CLD has been used in microfluidic devices for the determination of biogenic

564   amines including EP, DA, His, Tym, and agmatine in human urine samples from healthy

565   volunteers [86]. In order to achieve a high sensitivity, a derivatization of the analytes with

566   N-(4-aminobutyl)-N-ethylisoluminol (ABEI) previous the separation was carried out.

567   LODs in the nM range were obtained using chemiluminiscence emission produced by the

568   ABEI-tagged analytes, which reacted with hydrogen peroxide in the presence of the

569   enzyme horseradish peroxidase.

570   Recently, Li et al. employed a microchip platform coupled with MS detection using

571   a nano-ESI interface and a IT analyzer for neuroactive molecules determination [87, 88].

572   In the first work, several amino acids were baseline separated in a glass/PDMS hybrid

573   microchip in less than 120 seconds [87]. The developed systems obtained interesting

574   LODs in the μM range and allowed to demonstrate the cellular release of these excitatory

575   amino acids from PC-12 nerve cells incubated with ethanol. Likewise, the second paper

576   describes the enantioselective metabolism of SH-SY5Y cells (cells often employed to

577   study Parkinson's disease) [88]. Authors observed how human SH-SY5Y neuronal cells

578   only metabolized L-DOPA and left the D-enantiomer intact as it was observed in a

579   previous work for PC-12 cells [24].

580   One of the advantages of microfluidic devices is its ease to be coupled to several

581   sampling systems. Thus, a PDMS based microfluidic system for on-line coupling of

582   microdialysis sampling to microchip in CE-LIF was described for *in vivo* determination

583   of amino acids as neuroactive compounds in rat brain samples [89]. Although further

584   studies and some modification should be considered, the described system shows a great

585   potential in the deal of continuously monitoring the levels of neuroactive molecules. In

586 this sense it is necessary to consider that the development of a microchip system may also

587 offer the possibility of creating a micro-total analysis system (μ-TAS). Cakal et al. [90]

588 developed the first μ-TAS application for the determination of the catecholamines DA,

589 EP, and NE where pre-concentration, separation, and detection steps were carried out on

590 a single microchip. Monolithic disks were employed as SPE to purificate and pre-

591 concentrate the analytes which were detected in a standard solution after the elution and

592 the electrophoretic separation by LIF.

593

594 **3. Conclusions and future trends**

595   Research in neuroscience requires the determination of potential neuroactive

596 molecules in biological matrices related with the nervous system. When analyzing

597 biological samples with effective methodologies the knowledge of the CNS can be further

598 explored. Among all the biological samples related to neuroscience, CSF, ECF, and brain

599 tissue are the most straightforward samples for studying biochemical changes in the CNS

600 due to the fact that they are in direct contact with the extracellular space of the brain.

601 Nevertheless, the invasiveness of these samples, especially in the case of brain tissue, is

602 remarkable. CSF and ECF are obtained by lumbar puncture or microdialysis, whereas

603 brain tissue sampling can be only performed in post-mortem brain tissue from human or

604 from model animals. These reasons make translational research a future trend, understood

605 as the transferring of information obtained in the laboratory to the patients [91]. In this

606 aspect, information obtained through analytical methodologies by the analysis of blood

607 or urine can be easily extrapolated from model animals to human as these are translational

608 samples.

609   From all the CE methodologies described in this review it is worth highlighting the

610 use of MEKC with LIF detection. In recent years, MEKC has demonstrated to be capable

of simultaneously monitor a great number of neuroactive substances offering a higher

resolving power than the most CE mode employed, CZE. Meanwhile, LIF detection has

proven to be the most sensitive of all the ones herein described, reaching LODs in the

picomolar range, i.e. about 1000 times lower than other detection systems which

generally achieve values in the nanomolar range. However, it is well known that LIF is a

detection system with many drawbacks derived from the necessary derivatization

reaction. Some of these drawbacks involve tortuous derivatization processes, high-cost

derivatization reagents, and large fluorescence backgrounds when detecting low

concentrated analytes. Therefore, avoiding the derivatization reaction is desirable to avoid

the problems derived, situation that could be accomplished when the analytes present

native fluorescence. An alternative to LIF detection is MS detection due to its ability to

provide the unambiguous identification of analytes as well as for its capability to avoid

interfering compounds present in biological samples. Still, one of the main limitations of

the CE-MS system is the inconvenient raised from the coupling with a MEKC system,

given the non-volatility of the surfactants commonly used in this separation mode. This

is reflected by the fact that none of the applications described in the studied period of time

used MEKC as separation mode.

Note that the conclusions outlined above are perfectly reflected in a very recent

research article published in the journal *Nature*, in which three different CE methods, CE-

LIF for labeled-molecules, CE-LIF for molecules with native fluorescence, and CE-MS

were applied to detect and determine the concentration of several neuroactive compounds

in ctenophores, a marine invertebrate with complex nervous and muscular systems, with

the aim to demonstrate how ctenophore neural systems have evolved independently from

those in other animals [92]. The methods described were used to study a broad range of

635 amino acids and other neuroactive molecules, including the L- and D- enantiomers of Glu

636 and Asp, and enabled to obtain LODs in the range of nanomolar.

637   Indeed, the importance of multiple and simultaneous detection of several analytes

638 is an interesting feature in neuroscience since the alteration of more than one neuroactive

639 substance at a time can be studied as a result of a stimulus or a disruption in a metabolomic

640 pathway. Understanding chirality of neuroactive molecules is essential to increase the

641 knowledge of the enantioselectivity of neurobiological and neurotransmission processes.

642 However, information about D-amino acids in biological samples is not yet completed

643 and thus, more work is required on this research aspect. Likewise, on-line coupling of

644 sampling techniques followed by CE separation is one of the main promising trends in

645 the analysis of biological samples, acquiring high relevance the microdialysis sampling.

646 In another sense, further advances in methodologies to better harmonize the coupling of

647 MS to the CE, especially in its MEKC mode as well as in applications of chiral separations

648 for neuroscience studies are still required. Finally, building a comprehensive map of

649 neuroactive compounds distribution and elucidating better models of neurotransmission

650 is of remarkable importance. Therefore, a better understanding of the regulatory functions

651 of neuroactive molecules will provide an increase of the knowledge of neurodegenerative

652 diseases, or even their premature diagnosis.

653

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Acknowledgements

945 **Figure 1**. Electropherograms showing the determination of catecholamines in rat brain

946 sample spiked or non-spiked in normal injection mode (A) or FASS mode (B) by CZE-

947 LIF. Peaks: E: epinephrine; NE: norepinephrine; DA: dopamine. Experimental

948 conditions: BGE: 50 mM boric acid with 40 mM borax (pH 8.9); voltage: 20 kV;

949 temperature: 25 ºC; 40 x 50 µm i.d. uncoated fused-silica capillary; in (A) hydrodynamic

950 injection: 0.5 psi for 3 s; in (B) electrokinetic injection: -5 kV for 25 s. Reproduced with

951 permission from [50].

952 **Figure 2**. Electropherograms showing the separation of several amino acids and

953 catecholamines by a MEKC-LIF method in plasma of a healthy person (A), plasma of a

954 diabetes patient (B), plasma of a hyperlipidemia patient (C) and plasma of a

955 hyperlipidemia patient spiked with 2 x 10^{-7} M of standard amino acids (except for Leu

956 $1x10^{-7}$ M, NE, and DA $2x10^{-6}$ M). Peaks: 1, Asn; 2, His, Met, and Gln; 3, Ser; 4,Thr; 5

957 Tyr; 6, Gly; 7, Glu; 8, Asp; 9, Ala; 10, Tau; 11, GABA; 12, NE; 13, Val; 14, DA; 15, Ile;

958 16, Leu; 17, Phe; 18, Arg; 19, Lys. Experimental conditions: BGE: 120 mM boric acid

959 (pH 9.1), 38.5 mM SDS, 19 % (v/v) ACN; voltage: 22.5 kV; temperature: 25 ºC; 60.2 cm

960 x 75 µm i.d. uncoated fused-silica capillary; hydrodynamic injection: 0.5 psi for 5 s.

961 Reproduced with permission from [58].

962 **Figure 3.** CE-MS/MS electropherograms obtained in the study of the DOPA metabolism

963 in PC-12 cells: (A) racemic DOPA standard solution (50 µM for each enantiomer); (B)

964 500 µM racemic DOPA incubated with the culture medium for 2 h; (C) 500 µM racemic

965 DOPA incubated with PC-12 cells (2 x 106 cells/ml) for 2 h. Experimental conditions:

966 BGE: 200 mM formic acid; PFT: 100 mbar for 50 s of 5 mM sulfated β-CD in BGE;

967   voltage: 30 kV; temperature: 20 ºC; 80 cm x 75 µm i.d. uncoated fused-silica capillary;

968   hydrodynamic injection: 50 mbar for 12 s. Reproduced with permission from [24].

969   **Figure 4.** MEKC-AD electropherograms obtained from dissected *Drosophila* brain

970   homogenates for (A) wild-type, (B) *white* mutant and (C) TH-GFP mutant flies. Peaks

971   correspond to DA (1), salsolinol (2), OP (3), N-acetyltyramine (4), N-acetylserotonin (5),

972   N-acetyldopamine (6), L-DOPA (7), and the internal standard catechol (8). Experimental

973   conditions: BGE: 25 mM borate (pH 9.7), 50 mM SDS, 2 % isopropranol; voltage: 15

974   kV; temperature: not specified; 45 cm x 14 µm i.d. uncoated fused-silica capillary;

975   electrokinetic injection: 5 kV for 5 s. Reproduced with permission from [74].

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Tables

991   **Table 1.** Applications of CE to the determination of neuroactive molecules in biological samples using UV detection.

Analytes	Sample	Sample treatment	Separation mode and conditions	Detection λ	In-capillary pre-concentration technique	LOD (M)	Ref.
5-HIAA, DA, IXS, TA, VMA	Human urine	Protein precipitation with acetonitrile	**CZE** BGE: 5 mM formic acid (pH 4.0) and 1.2 % PDDAC Hydrostatic injection: 20 cm for 180 s 60 cm x 75 µm, i.d. PDDAC coated capillary Temperature: not specified. Voltage: -15 kV	220 nm	LVSS	$10 - 120$ x 10^{-9}	[33]
3-MT, IXS, 4-hydroxy-3-methoxybenzylamine, 5-HIAA, 5-HT, catechol, DA, DOPA, TA, Trp, VMA	Human urine	Protein precipitation with acetonitrile	**CZE** BGE: 500 mM Tris-borate (pH 9) and 10 % glycerol Hydrostatic injection: 25 cm for 180 s 60 cm x 75 µm, i.d. PEO coated capillary Temperature: not specified. Voltage: 18 kV	220 nm	LVSS	$10 - 300$ x 10^{-9}	[34]
DA, EP, NE	Human urine	Extraction with nanoparticles (Fe_3O_4)	**CZE** BGE: 5 mM formic acid (pH 4.0) with 1.2 % PDDAC Hydrostatic injection: 20 cm x 120 s 60 cm x 75 µm i.d. PDDAC coated capillary Temperature: not specified. Voltage: -15 kV	200 nm	LVSS	8 x 10^{-9}	[35]
5-HT	Spiked Human urine	Ultrasound-assisted emulsification microextraction	**CZE** BGE: 80 mM Tris-phosphate (pH 2.85) Hydrodynamic injection: 0.5 psi for 20 s 58 cm x 75 µm i.d. uncoated fused-silica capillary Temperature: 25 °C. Voltage: 20 kV	214 nm	pH-mediated sample stacking	8 x 10^{-9}	[36]

Analytes	Sample	Sample treatment	Separation mode and conditions	Detection λ	In-capillary pre-concentration technique	LOD (M)	Ref.
5-HT, DA, EP,TA, Tym	Human urine	Liquid-liquid extraction	**CZE** BGE: a pH 6.5 mixture of 20 mM MES and 30 mM phosphate buffer, 0.05 % hydroxypropylcellulose and 10% (v/v) methanol Electrokinetic injection: 12 kV for 30 s 100 cm x 75 µm i.d. cellulose coated fused-silica capillary Temperature: 25 ℃. Voltage: 30 kV	210 nm	FASS	10 – 120 x 10^{-9}	[37]
3-MT, 5-HT, DA	Spiked human urine	MISPE	**CZE** BGE: 10 mM of HP-β-CD in 80 mM phosphate buffer (pH 4.0) Electrokinetic injection: 3 kV for 60 s 60.2 cm x 50 µm i.d. uncoated fused-silica capillary Temperature: 25 ℃. Voltage: 30 kV	214 nm	FASS	2 x 10^{-9}	[38]
DA, EP, NE	Human urine and spiked serum	Protein precipitation with acetonitrile and MIP-SPME	**CZE** BGE: 20 mM borate buffer (pH 9.0) Hydrostatic injection: 15 cm for 10 s 55 cm x 75 µm i.d uncoated fused-silica capillary Temperature: not specified. Voltage: 14 kV	210 nm	-	5 x 10^{-9}	[39]
3-MT, DA, EP, NE, NM	Spiked human urine	MMIP-SPE	**CZE** BGE: 20 mM phosphate-borate (pH 5.5) Hydrodynamic injection: 50 mbar for 5 s 64 cm x 50 µm i.d. uncoated fused-silica capillary Temperature: 25 ℃. Voltage: 20 kV	205 nm	-	40 – 60 x 10^{-9}	[40]
5-HT	Human whole blood	Protein precipitation with acetonitrile	**CZE** BGE: 400 mM Tris phosphate (pH 3.25) Reverse hydrodynamic injection: 0.5 psi for 10 s 40 cm x 75 µm i.d. uncoated fused-silica capillary Temperature: 20 ℃. Voltage: -14 kV	220 nm	-	30 x 10^{-9}	[41]

Analytes	Sample	Sample treatment	Separation mode and conditions	Detection λ	In-capillary pre-concentration technique	LOD (M)	Ref.
DA, (±)-**EP**, (-)-isoproterenol, (±)-**NE**, (±)-**OP**, (±)-**synephrine**	Spiked human urine	Filtration	**Chiral CEC** BGE: 10 mM citrate buffer (pH 3.0), 40 mM SDS, and acetonitrile (2:2:1, v:v:v) Hydrostatic injection: 10 cm for 5 s Temperature: 30 ºC. Voltage: 10 kV 70 cm x 75 µm i.d chiral monolithic MIP column	210 nm	-	Not specified	[42]

992
993 Abbreviations: MES: 2-(morpholino)ethanesulfonic acid; PEO: poly(ethylene oxide); PDDAC: poly-diallyldimethylammonium chloride; HP-β-CD: hydroxypropyl-beta-cyclodextrin; SDS:
994 sodium dodecyl sulfate; 3-MT: 3-methoxytyramine. 5-HIAA: 5-hydroxyindole-3acetic acid. 5-HT: serotonin. DA: dopamine. DOPA: 3,4-dihydroxyphenylalanine. EP: epinephrine. IXS: 3-indoxyl
995 sulfate. NE: norepinephrine. NM: normetanephrine. OP: octopamine. TA: tryptamine. Trp: tryptophan. Tym: tyramine. VMA: vanillomandelic acid.
996

997 **Table 2**. Applications of CE to the determination of neuroactive molecules in biological samples using LIF detection.

Analytes	Sample	Sample treatment	Separation mode and conditions	Derivatization reagent	Detection λ	LOD (M)	Ref.
5-HIAA, 5-HT, 5-hydroxytryptophan, TA, Trp	Human urine and serum	Extraction with citrate-capped gold nanoparticles	**CZE** BGE: 2 % (w/v) dextran sulfate 100 mM in tris-borate buffer (pH 9.0) Hydrostatic injection: 20 cm for 20 s Temperature: not specified. Voltage: 15 kV 90 cm x 75 µm i.d. uncoated fused-silica capillary	No derivatization needed	λ_{exc}=266 nm	4 – 400 x 10^{-12}	[44]
5-HIAA, 5-HT, DA, DOPA, EP, HVA, TA, Trp, VMA	Human urine	Centrifugation	**CZE** BGE: 10 mM formic acid (pH 3.7) Electrokinetic injection: 1 kV for 10 s Temperature: room temperature. Voltage: 15 kV 40 cm x 75 µm i.d. coated capillary with poly(L-lysine) and silica nanoparticles	No derivatization needed	λ_{exc}=266 nm	2 – 15 x 10^{-10}	[45]

Analytes	Sample	Sample treatment	Separation mode and conditions	Derivatization reagent	Detection λ	LOD (M)	Ref.
5-HT, DA, EP, NE, TA, Trp, Tyr, Tym	Rat brain tissue	Homogenization Ultrafiltration	**CZE** BGE: 25 mM citric acid (pH 2.5) Hydrodynamic injection: 0.5 psi for 20 s Temperature: not specified. Voltage: 30 kV 48 cm x 50 µm i.d. uncoated fused-silica capillary	No derivatization needed	λ_{exc}=224 nm λ_{em}=variable	$4 - 30 \times 10^{-9}$	[46]
DA, EP, NE	Human urine	No sample treatment was employed	**CZE** BGE: 20 mM β-CD in 20 mM borate (pH 9.5) with 10 % (v/v) acetonitrile Hydrostatic injection: 20 cm for 10 s Temperature: room temperature. Voltage: 13 kV 47 cm x 75 µm i.d. uncoated fused-silica capillary	FITC	LED	$3\text{-}10 \times 10^{-10}$	[47]
Asp, Glu	ECF of rat brain	Off-line microdialysis	**CZE** BGE: 20 mM SDS in 100 mM borate buffer (pH 8.5) Hydrodynamic injection: 3447 Pa for 5 s Temperature: 25 ℃. Voltage: -9 kV 50 cm x 75 µm i.d. polyacrylamide coated capillary	FITC	λ_{exc}=488 nm λ_{em}=520 nm	3×10^{-9}	[48, 49]
DA, EP, NE	Rat brain tissue	Not reported	**CZE** BGE: 50 mM boric acid with 40 mM borax (pH 8.9) Electrokinetic injection: -5 kV for 25 s Temperature: not specified. Voltage: 20 kV 40 cm x 50 µm i.d. uncoated fused-silica capillary	FITC	λ_{exc}=488 nm λ_{em}=520 nm	$1.4 - 1.8 \times 10^{-13}$	[50]
Asp, Gly, Glu, Leu	ECF of rat brain	Off-line microdialysis	**CZE** BGE: 75 mM sodium borate (pH 9.2) Electrokinetic injection: 5 kV for 2.5 s Temperature: 30 ℃. Voltage: 25 kV 70 cm x 75 µm i.d. uncoated fused-silica capillary	NDA (In-capillary)	λ_{exc}=410 nm λ_{em}=490 nm	$1 - 9 \times 10^{-8}$	[51]

Analytes	Sample	Sample treatment	Separation mode and conditions	Derivatization reagent	Detection λ	LOD (M)	Ref.
Carbamathione, GABA, Glu,	ECF of rat brain	Off-line microdialysis	**CZE** BGE: 50 mM borate buffer (pH 9.6) Hydrodynamic injection: 0.5 psi for 5 s Temperature: not specified. Voltage: 27.5 kV 75 cm x 50 µm i.d. uncoated fused-silica capillary	NDA	λ_{exc}=442 nm	$6 - 15 \times 10^{-9}$	[52]
Glu, GABA, NE, DA, DOPAC, 5-HIAA, HVA, 5-HT	ECF of rat brain	Off-line microdialysis	**CZE** BGE: 20 mM LDS in 22.5 mM lithium tetraborate buffer (pH 9.2) Hydrodynamic injection: not specified Temperature: not specified. Voltage: 21 kV 75 cm x 50 µm i.d. uncoated fused-silica capillary	NDA	λ_{exc}=442 nm λ_{em}=490 nm	Not specified	[53]
Asp, Glu	ECF of rat brain	Off-line microdialysis	**CZE** BGE: 25 mM borate with 120 mM boric acid (pH 8.5) Hydrodynamic injection: 0.5 psi for 5 s Temperature: 25 ºC. Voltage: 25 kV 60.2 cm x 50 µm i.d. uncoated fused-silica capillary	CFSE	λ_{exc}=488 nm λ_{em}=520 nm	$6.9 - 8.1 \times 10^{-10}$	[54]
DA, NE	Spiked human serum	Protein precipitation with MeOH	**CZE** BGE: 20 mM borate buffer (pH 9.8) Hydrodynamic injection: 0.5 psi for 3 s Temperature: 25 ºC. Voltage: 15 kV 37 cm x 75 µm i.d. uncoated fused-silica capillary	DTAF	λ_{exc}=488 nm λ_{em}=520 nm	$3 – 6 \times 10^{-9}$	[55]
Ala, Arg, Asp, GABA, Glu, Gly, Leu, Phe, Pro, Thr, Tyr	Heads of houseflies and diamondback moths	Homogenization with derivatization buffer and acetonitrile	**CZE** BGE: 5.3 mM β-CD and 10.4 mM SDS in 32 mM borate buffer (pH 9.2) Hydrodynamic injection: 0.5 psi for 3 s Temperature: 25 ºC. Voltage: 15 kV 57 cm x 75 µm i.d. uncoated fused-silica capillary	NBD-F	λ_{exc}=488 nm λ_{em}=520 nm	1.6×10^{-8}	[56]

Analytes	Sample	Sample treatment	Separation mode and conditions	Derivatization reagent	Detection λ	LOD (M)	Ref.
GABA, Gln	Human CSF	Not reported	**CZE** BGE: 8.5 mM β-CD and 10 mM SDS in 200 mM borate buffer (pH 10.0) Hydrodynamic injection: 0.7 psi for 12 s Temperature: 25 °C. Voltage: 20 kV 57 cm x 75 µm i.d. uncoated fused-silica capillary	NBD-F	λ_{exc}=488 nm λ_{em}=520 nm	1.0×10^{-9}	[57]
Ala, Arg, Asn, Asp, DA, GABA, Glu, Gln, Gly, His, Ile, Leu, Lys, Met, NE, Phe, Ser, Tau, Thr, Tyr, Val	Human plasma	Protein precipitation with acetonitrile	**MEKC** BGE: 38.5 mM SDS in 120 mM sodium borate (pH 9.1) with 19% (v/v) acetonitrile Hydrodynamic injection: 0.5 psi for 5 s Temperature: 25 °C. Voltage: 22.5 kV 60.2 cm x 75 µm i.d. uncoated fused-silica capillary	Br-BQCA	λ_{exc}=488 nm λ_{em}=520 nm	$6.5 - 14 \times 10^{-10}$	[58]
	Rabbit vitreous humor	Homogenization on ice					
	HEK293 and PC12 cells	Wash with PBS, cell lysed by sonication Protein precipitation with chloroform					[27]
Asp, GABA, Gln, Glu, Gly, Tau	Human CSF and saliva	Protein precipitation with acetonitrile	**MEKC** BGE: 100 mM SDS in 100 mM boric acid (pH 9.6) with 8 % (v/v) methanol Hydrodynamic injection: 0.5 psi for 5 s Temperature: 25 °C. Voltage: 20 kV 60.2 cm x 75 µm i.d. uncoated fused-silica capillary	SIFA	λ_{exc}=488 nm λ_{em}=520 nm	$6 - 10 \times 10^{-11}$	[59]

Analytes	Sample	Sample treatment	Separation mode and conditions	Derivatization reagent	Detection λ	LOD (M)	Ref.
DA, GABA, Glu	ECF of rat brain	Off-line microdialysis	**MEKC** BGE: 25 mM LDS in 22.5 mM lithium tetraborate with 10% (v/v) methanol Hydrodynamic injection: 5 psi for 2 s Temperature: room temperature. Voltage: 15 kV 50 cm x 50 µm i.d. uncoated fused-silica capillary	NDA	λ_{exc}=442 nm λ_{em}= ---	$5\text{-}10 \times 10^{-9}$	[60]
Ala, Asp, GABA, Gln, Glu, Gly, Tau	Mice brain tissue	Homogenization with borate buffer and Protein precipitation with chloroform	**MEKC** BGE: 5.5 mM CTAB in 70 mM phosphate buffer (pH 4.0) with 20 % (v/v) acetonitrile Hydrodynamic injection: 3447 Pa for 5 s Temperature: 25 ºC. Voltage: -22.5 kV 60 cm x 75 µm i.d. uncoated fused-silica capillary	TMBB-Su	λ_{exc}=488 nm λ_{em}=520 nm	$2 - 14 \times 10^{-10}$	[26]
Ala, Arg, Asn, Asp, GABA, Glu, Gln, Gly, Lys, Phe, Pro Ser, Trp, Tau	ECF of rat brain	Off-line microdialysis	**MEKC** BGE: 100 mM SDS, 5 mM HP-β-CD, 5 mM DM-β-CD in 15 mM borate (pH 9.0) with 4 % (v/v) isopropanol Injection not specified Temperature: 25 ºC. Voltage: 17.5 kV 57 cm x 75 µm i.d. uncoated fused-silica capillary	DTAF	λ_{exc}=488 nm λ_{em}=520 nm	$9 - 54 \times 10^{-11}$	[23]
His, Arg, Gln, Pro, Tyr, Asn, Thr, Ser, Ala, Val, Met, Gly, Ile, Leu, Phe, Trp, Cys, Glu, Lys, Asp	Rat brain tissue	Not reported	**MEEKC** BGE: 2.16% SDS, 6% 1-butanol, 0.6% cyclohexane, and 87.4% 30 mM phosphate buffer (pH 6.0) Hydrodynamic injection: 0.5 psi for 3 s Temperature: not specified. Voltage: 20 kV 50 cm x 50 µm i.d. uncoated fused-silica capillary	FITC	λ_{exc}=488 nm λ_{em}=520 nm	$0.3\text{-}2.2 \times 10^{-9}$	[61]

Analytes	Sample	Sample treatment	Separation mode and conditions	Derivatization reagent	Detection λ	LOD (M)	Ref.
D/L-Ser, **D/L-Ala**, Asp, GABA, **D/L-Gln, D/L-Glu**, Gly, His, **D/L-Ile, D/L-Leu,** Met **D/L-Orn, D/L-Phe, D/L-Pro, Tau, D/L-Thr**, Val, 4-hydroxyproline	Human urine and mice brain tissue	Protein precipitation with MeOH	**Chiral EKC** BGE: 12.5 mM β-CD in 90 mM borate buffer (pH 10.2) Hydrodynamic injection: 0.5 psi for 10 s Temperature: 17 ºC. Voltage: 21 kV 60 cm x 75 µm i.d. uncoated fused-silica capillary	NBD-F	λ_{exc}=488 nm λ_{em}=522 nm	$10 - 26 \times 10^{-8}$	[62]
D/L-Ser, D/L-Ala, D/L-Gln, D/L-Glu, Gly, **D/L-Ile, D/L-Leu, D/L-Orn, D/L-Phe, D/L-Pro, Tau, D/L-Thr**	Human plasma	Ultrafiltration				$4 – 40 \times 10^{-8}$	[21]
D/L-Ser, Ala, Arg, Asp, DA, GABA, Gln, Glu, Gly, Leu, Lys, Phe, Tau, Trp	ECF of rat brain	Off-line microdialysis	**Chiral EKC** BGE: 17.5 mM HP-β-CD, 5 mM DM-β-CD and 70 mM SDS in 15 mM borate (pH 10.2) with 5 % (v/v) methanol Hydrodynamic injection: 0.5 psi for 5 s Temperature: 25 ºC. Voltage: 22.5 kV 57 cm x 75 µm i.d. uncoated fused-silica capillary	FITC	λ_{exc}=488 nm λ_{em}=520 nm	$1.0-0.1 \times 10^{-9}$	[63,64]
D/L-Asp, D/L-Glu	Chicken brain tissue	Homogeneization and protein precipitation with acetonitrile	**Chiral EKC** BGE: 8 mM DM-β-CD and 5 mM HPA-β-CD in 100 mM borate buffer (pH 8.0) Voltage: -24 kV Temperature: 25 ºC 60 cm x 75 µm i.d. polyacrylamide-coated fused-silica capillary Hydrodynamic injection: 1 psi for 20 s	Pre-column derivatization with NBD-F	λ_{exc}=488 nm λ_{em}=520 nm	$9 - 17 \times 10^{-9}$	[65]

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Analytes	Sample	Sample treatment	Separation mode and conditions	Derivatization reagent	Detection λ	LOD (M)	Ref.
D/L-Ala, D/L-Arg, D/L-Asp, D/L-Glu, D/L-Gln, D/L-Leu, D/L-Lys, D,L-Ser, GABA, Gly	Human CSF	Ultrafiltration	**Chiral EKC** BGE: 20 mM β-CD and 80 mM SDS in 100 mM tetraborate buffer (pH 10.0) Hydrodynamic injection: 0.5 psi for 3 s Temperature: 30 ℃. Voltage: 20 kV 57 cm x 50 µm i.d. uncoated fused-silica capillary	FITC	λ_{exc}=488 nm λ_{em}=520 nm	$1 - 16 \times 10^{-9}$	[66]

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999   Abbreviations: SDS: sodium dodecilsulfate; LDS, lithium dodecilsulfate; CTAB, cetyltrimethyl ammonium bromide; 5-HIAA: 5-hydroxyindole-3-acetic acid. 5-HT: serotonin. Ala: alanine.

1000   Arg: arginine. Asn: asparagine. Asp: aspartate. Cit: citruline. Cys: cysteine. DA: dopamine. DOPA: 3,4-dihydroxyphenylalanine. DOPAC, 3,4-dihydroxyphenylaceticacid. EP: epinephrine.

1001   GABA: γ-aminobutyric acid. Gln: glutamine. Glu: glutamate. Gly: glycine. Him: histamine. His: histidine. HVA: homovanillic acid. Ile: isoleucine. IXS: 3-indoxyl sulfate. Leu: leucine. Lys:

1002   lysine. Met: methionine. NE: norepinephrine. NM: normetanephrine. OP: octopamine. Orn: ornithine. Phe: phenylalanine. Pro: proline. Ser: serine. TA: tryptamine. Tau: taurine. Thr: threonine.

1003   Trp: tryptophan. Tym: tyramine. Tyr: tyrosine. Val: valine. VMA: vanillomandelic acid.

1004 **Table 3.** Derivatizing reagents (sorted in alphabetical order of abbreviations) used in the determination of neuroactive compounds by CE with LIF
1005 detection.

Abbreviation	Full name	Most commonly selected wavelengths		Selected laser
		Excitation (nm)	Emission (nm)	
Br-BQCA	3-(4-bromobenzoyl)-2-quinolinecarboxaldehyde	488	520	Argon
CFSE	5-carboxyfluorescein N-succinimidyl ester	488	520	Argon
DTAF	5-(4,6-dichloro-s-triazin-2-ylamino) fluorescein	488	520	Argon
FITC	fluorescein isothiocyanate	488	520	Argon
NBD-F	4-fluoro-7-nitro-2,1,3-benzoxadiazole	488	520	Argon
NDA	Naphthalene-2,3-dicarboxyaldehyde	410	490	Diode
		442	490	Helium-Cadmium or diode
		455	465-495	Diode
OPA	*o*-phthalaldehyde	351	Not specified	Argon
SIFA	N-hydroxysuccinimidyl fluorescein-O-acetate	488	520	Argon
TMBB-Su	1,3,5,7-tetramethyl-8-(N-hydroxysuccinimidyl butyric	488	520	Argon

			ester)difluoroboradiaza-S indacene			

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1008   **Table 4.** Applications of CE to the determination of neuroactive molecules in biological samples using other detection systems.

Analytes	Sample	Sample treatment	Separation mode and conditions	Detection system	LOD (M)	Ref.
5-HT, ACh, DA, Him	*Aplysia californica* neurons	Wash with artificial seawater and isolation of neurons	**CZE** BGE: 1 % (v/v) formic acid Hydrostatic injection: 15 cm for 60 s Temperature: not specified. Voltage: 20 kV 100 cm x 40 µm i.d. uncoated fused-silica capillary	**MS** (TOF) ESI+ coaxial sheath-flow Sheath liquid: 50:50 (v/v) methanol/water with 0.1% (v/v) formic acid	5 - 35 x 10^{-9}	[25]
DA, GABA, N-acetyldopamine, N-acetyloctopamine, OP, Tym	Flies brain tissue	Homogenization in formic acid	**CZE** BGE: 50 mM citric acid (pH 2.1) Hydrodynamic injection: 10 psi for 5 s Temperature: not specified. Voltage: 20 kV 80 cm x 50 µm i.d. uncoated fused-silica capillary	**MS** (QTOF) ESI+ coaxial sheath-flow Sheath liquid: 70:30 (v/v) isopropanol/water	---	[68]
Asn, GABA, Glu, Him, NE, Tau, Tyr, Val	Rat urine	Centrifugation and ultrafiltration with 5-kDa cutoff filters	**CZE** BGE: not specified Injection: not specified Voltage: not specified. Temperature: 20 °C. 80 cm x 50 µm i.d. uncoated fused-silica capillary	**MS** (TOF) ESI+ coaxial sheath-flow Sheath liquid: 50:50 (v/v) methanol/water with 0.1 µM hexakis-(2,2-difluoroethoxy)-phosphazene	---	[69]

Analytes	Sample	Sample treatment	Separation mode and conditions	Detection system	LOD (M)	Ref.
D/L-DOPA, D/L-Phe, D/L-Tyr	PC12 cells	Protein precipitation with TCA	**Chiral EKC** BGE: 200 mM formic acid PFT: 5 mM sulfated β-CD in BGE at 100 mbar for 50 s Hydrodynamic injection: 50 mbar for 12 s Temperature: 20 ℃. Voltage: 30 kV. 80 cm x 75 µm i.d. uncoated fused-silica capillary	**MS (IT)** ESI+ coaxial sheath-flow Sheath liquid: 50:50 (v/v) methanol/water with 0.1 % (v/v) formic acid	5×10^{-7}	[24]
D/L-DOPA, D/L-Phe, D/L-Tyr, DA, D/L-NE, D/L-EP	Rat plasma	Protein precipitation with acetonitrile	**Chiral EKC** BGE: 2 M formic acid PFT: 180 mM M-β-CD and 40 Mm HP-β-CD in 2 M formic acid at 1 bar for 2.5 min Hydrodynamic injection: 50 mbar for 250 s Temperature: 15 ℃. Voltage: 30 kV. 120 cm x 50 µm i.d. uncoated fused-silica capillary	**MS (IT)** ESI+ coaxial sheath-flow Sheath liquid: 50:50 (v/v) methanol/water with 0.1 % (v/v) formic acid	$0.4 - 2 \times 10^{-7}$	[70]
	Rat brain tissue	Homogenization in perchloric and ascorbic acids and EDTA				
Ala, Asp, Glu, Gly, Ser, Tau	Human serum	Protein precipitation with acetonitrile	**CZE** BGE: 50 mM borate buffer (pH 9.2) Electrokinetic injection: 15 kV for 10 s Temperature: room temperature. Voltage: 15 kV 65 cm x 25 µm i.d. uncoated fused-silica capillary	AD	$5 – 28 \times 10^{-7}$	[71]

Analytes	Sample	Sample treatment	Separation mode and conditions	Detection system	LOD (M)	Ref.
HVA, IXS, Trp, VMA	Human urine	No sample treatment was employed	**CZE** BGE: 50 mM Tris-phosphate buffer (pH 3.0) Electrokinetic injection: -10 kV for 5 s Voltage: -10 kV. Temperature: 25 ºC 45 cm x 50 µm i.d. gold nanoparticles embedded in poly(diallyl dimethylammonium) chloride-coated fused-silica capillary	AD	7×10^{-8}	[72]
5-HT, DA, EP, NE	Rat brain tissue	Homogenization in phosphate buffer and protein precipitation with acetonitrile	**CZE** BGE: 180 mM phosphate buffer (pH 5.8) Electrokinetic injection: 15 kV for 10 s Voltage: 15 kV. Temperature: 20 ºC 70 cm x 25 µm i.d. uncoated fused-silica capillary	AD	9×10^{-10}	[73]
3,4-dihydroxymandelic acid, 3-MT, 5-HIAA, 5-HT, ascorbic acid, OP, DA, DOPA, DOPAC, EP, NE, guanine, HVA, salsolinol, norsalsolinol, p-hydroxymandelic acid, Tyr, Tym, VMA N-acetyldopamine, N-acetyloctopamine, N-acetylserotonin, N-acetyltyramine,	Flies brain tissue	Homogenization in PCA, centrifugation, and ultrafiltration with 3-kDa cutoff filters	**MEKC** BGE: 50 mM SDS in 25 mM borate buffer (pH 9.7) with 2 % isopropanol Electrokinetic injection: 5 kV for 5 s Temperature: not specified. Voltage: 15 kV 45 cm x 14 µm i.d. uncoated fused-silica capillary	AD	Not specified	[74, 75]

Analytes	Sample	Sample treatment	Separation mode and conditions	Detection system	LOD (M)	Ref.
DA, DOPA, OP, salsolinol, Tym, N-acetyldopamine, N-acetyloctopamine, N-acetylserotonin, N-acetyltyramine	Flies brain tissue	Freeze-drying, homogenization with PCA and centrifugation	**MEKC** BGE: 50 mM SDS in 25 mM borate buffer (pH 9.5) with 2 % isopropanol Electrokinetic injection: 5 kV for 5 s Temperature: not specified. Voltage: 15 kV 45 cm x 14 µm i.d. uncoated fused-silica capillary	AD	Not specified	[28]
5-HT, DA, OP, Tym	Flies brain tissue	Homogenization with PCA and centrifugation	**CZE** BGE: 200 mM phosphate buffer (pH 4.5) with 1 mM tetraborate Electrokinetic injection: 5 kV for 15 s Temperature: not specified. Voltage: 15 kV 39 cm x 11 µm i.d. uncoated fused-silica capillary	CVD	6.5×10^{-10}	[76]
GABA, Glu, Gly	Rat brain tissue	Off-microdialysis	**CZE** BGE: 4 M acetic acid (pH 1.9) Hydrodynamic injection: 50 mbar for 100 s Temperature: 25 ºC. Voltage: 25 kV 43 cm x 50 µm i.d. INST-coated fused-silica capillary	C^4D	$9 - 15 \times 10^{-9}$	[77]
DA, EP, NE	Human urine	Centrifugation, and filtration	**CZE** BGE: 0.2 mM Ag (III) with 1 mM luminol in 20 mM borate buffer (pH 9.5) Hydrostatic injection: 20 cm for 10 s Temperature: not specified. Voltage: 15 kV 54 cm x 50 µm i.d. uncoated fused-silica capillary	CLD	$7 – 10 \times 10^{-8}$	[20]

Analytes	Sample	Sample treatment	Separation mode and conditions	Detection system	LOD (M)	Ref.
DA, EP	Human urine	Centrifugation	**CZE** BGE: 0.3 mM luminol with 0.6 mM CdTe quantum dots in 25 mM borate buffer (pH 9.8) Hydrostatic injection: 20 cm or 10 s Voltage: 14 kV. Temperature: not specified. 50 cm x 75 µm i.d. uncoated fused-silica capillary	CLD	$9 - 23 \times 10^{-9}$	[78]
DA	Human urine	SPE	**CZE** BGE: 0.1 mM luminol with 0.1 mM diperiodatocuprate (III) in 2 mM phosphate buffer (pH 11.5) Hydrostatic injection: 12 cm for 15 s Temperature: not specified. Voltage: 12 kV 75 cm x 75 µm i.d. uncoated fused-silica capillary	CLD	4×10^{-8}	[79]
EP	Human urine	Protein precipitation with acetonitrile	**CZE** BGE: 0.1 mM luminol with 0.1 mM diperiodatocuprate (III) in 2.5 mM phosphate buffer (pH 6.0) Hydrostatic injection: 10 cm for 15 s Temperature: not specified. Voltage: 12 kV 50 cm x 75 µm i.d. uncoated fused-silica capillary	CLD	4.5×10^{-9}	[80]

Abbreviations: 3-MT: 3-methoxytyramine. 5-HIAA: 5-hydroxyindole-3-acetic acid. 5-HT: serotonin. Ala: alanine. Asp: aspartate. DA: dopamine. DOPA: 3,4-dihydroxyphenylalanine. DOPAC, 3,4-dihydroxyphenylaceticacid. EP: epinephrine. GABA: γ-aminobutyric acid. Glu: glutamate. Gly: glycine. HVA: homovanillic acid. IXS: 3-indoxyl sulfate. NE: norepinephrine. OP: octopamine. Ser: serine. Tau: taurine. Trp: tryptophan. Tym: tyramine. Tyr: tyrosine. VMA: vanillomandelic acid; 5-HT: serotonin. ACh: acetylcholine. Asn: asparagine. DA: dopamine. DOPA: 3,4-dihydroxyphenylalanine. GABA: γ-aminobutyric acid. Glu: glutamate. Him: histamine. NE: norepinephrine. OP: octopamine. Phe: phenylalanine. Tau: taurine. Tym: tyramine. Tyr: tyrosine. Val: valine.

1016   **Table 5.** Applications of microchip CE to the determination of neurotransmitters or related compounds in biological samples.

| Analytes	Sample	Sample treatment	Separation conditions	Detector system	LOD (M)	Ref.
Ala, Asp, Gly, Glu, Tau	ECV-304 cells	Wash with phosphate buffer, lysed by sonication, and protein precipitation with chloroform	**CZE** BGE: 10 mM β-CD and 20 % (v/v) ACN in 20 mM borate buffer (pH 9.0) Pyrex glass microchip Electrokinetic injection in unpinched mode: 1000 V for 20 s Separation channel: 76 mm long x 50 µm wide x 20 µm deep Voltage: 2500 V	LIF	$0.4 - 1 \times 10^{-6}$	[81]
DA, NE, Asp, Glu,	PC-12 cells	Cell culture, immobilization and stimulation	**CZE** BGE: 3 mM SDS in 25 mM borate (pH 9.5) PDMS microchip Injection not specified Separation channel: 12 mm long x 250 µm wide x 90 µm deep Voltage: 1500 V	LIF	$70 - 250 \times 10^{-6}$	[82]
DA	Spiked PC-12 cells	Cell culture, immobilization and stimulation	**CZE** BGE: 5 mM HEPES (pH 7.2) Pyrex glass microchip Electrokinetic injection in unpinched mode: 100 V for 60 s Separation channel: 30 mm long x 50 µm wide x 0.5 µm deep Voltage: 100 V	AD	$1 - 3 \times 10^{-9}$	[83]
DA, NE	PC-12 cells	Cell culture, immobilization and stimulation	**CZE** BGE: 10 mM HEPES (pH 7.5) PDMS microchip Electrokinetic injection in unpinched mode: 100 V for 60 s Separation channel: 30 mm long x 50 µm wide x 0.5 µm deep Voltage: 50 V	AD	$1 - 3 \times 10^{-9}$	[84]

Analytes	Sample	Sample treatment	Separation conditions	Detector system	LOD (M)	Ref.
5-HT, DA, EP	Spiked human CSF	Centrifugation and protein precipitation with acetonitrile	**CZE** BGE: acetate solution (pH 6.0) PDMS microchip Electrokinetic injection in unpinched mode: 200 V for 1 s Separation channel: 35 mm long x 50 µm wide x 18 µm deep Voltage: 1000 V	AD	$1 - 6 \times 10^{-6}$	[85]
Agmatine, EP, DA, His, Tym	Human urine	Protein precipitation with acetonitrile	**CZE** BGE: 10 µM horseradish peroxidase and 25 mM SDS in 20 mM phosphate buffer (pH 10.0), PDMS microchip Electrokinetic injection in pinched mode (see protocol) for 15 s Separation channel: 70 mm long x 70 µm wide x 25 µm deep Voltage: 1800 V	CLD	0.1×10^{-9}	[86]
Arg, Asp, Glu, Lys, Tyr, Val	PC-12 cells	PBS dilution, sonication, centrifugation and filtration	**CZE** BGE: 25 mM ammonium acetate/acetic acid buffer (pH 4.3) in methanol/water 1:1 Glass/PDMS microchip Electrokinetic injection in gated mode (see protocol) for 15 s Separation channel: 35 mm long x 60 µm wide x 20 µm deep Voltage: 1550 V	MS (IT)	3×10^{-7}	[87]
D,L-DOPA, D,L-Glu, D,L-Ser	SH-SY5Y cells	Cells harvested and suspended in PBS. Incubation with D,L-DOPA	**Chiral EKC** BGE: 15 mM ammonium acetate buffer (pH 5.5)/methanol (1:1) PFT: 15 mM sulfated-β-CD in BGE Glass/PDMS microchip Electrokinetic injection in unpinched mode: 600 V for 15 s Separation channel: 40 mm long x 60 µm wide x 20 µm deep Voltage: 3850 V	MS (IT)	4×10^{-8}	[88]

1017 Abbreviations: 5-HT: serotonin. Ala: alanine. Arg: arginine. Asp: aspartate. DA: dopamine. DOPA: 3,4-dihydroxyphenylalanine. GABA: γ-aminobutyric acid. Gln: glutamine. Glu: glutamate.
1018 Gly: glycine. Him: histamine. His: histidine. Lys: lysine. NE: norepinephrine. Phe: phenylalanine. Ser: serine. Tau: taurine. Trp: tryptophan. Tym: tyramine. Tyr: tyrosine. Val: valine.
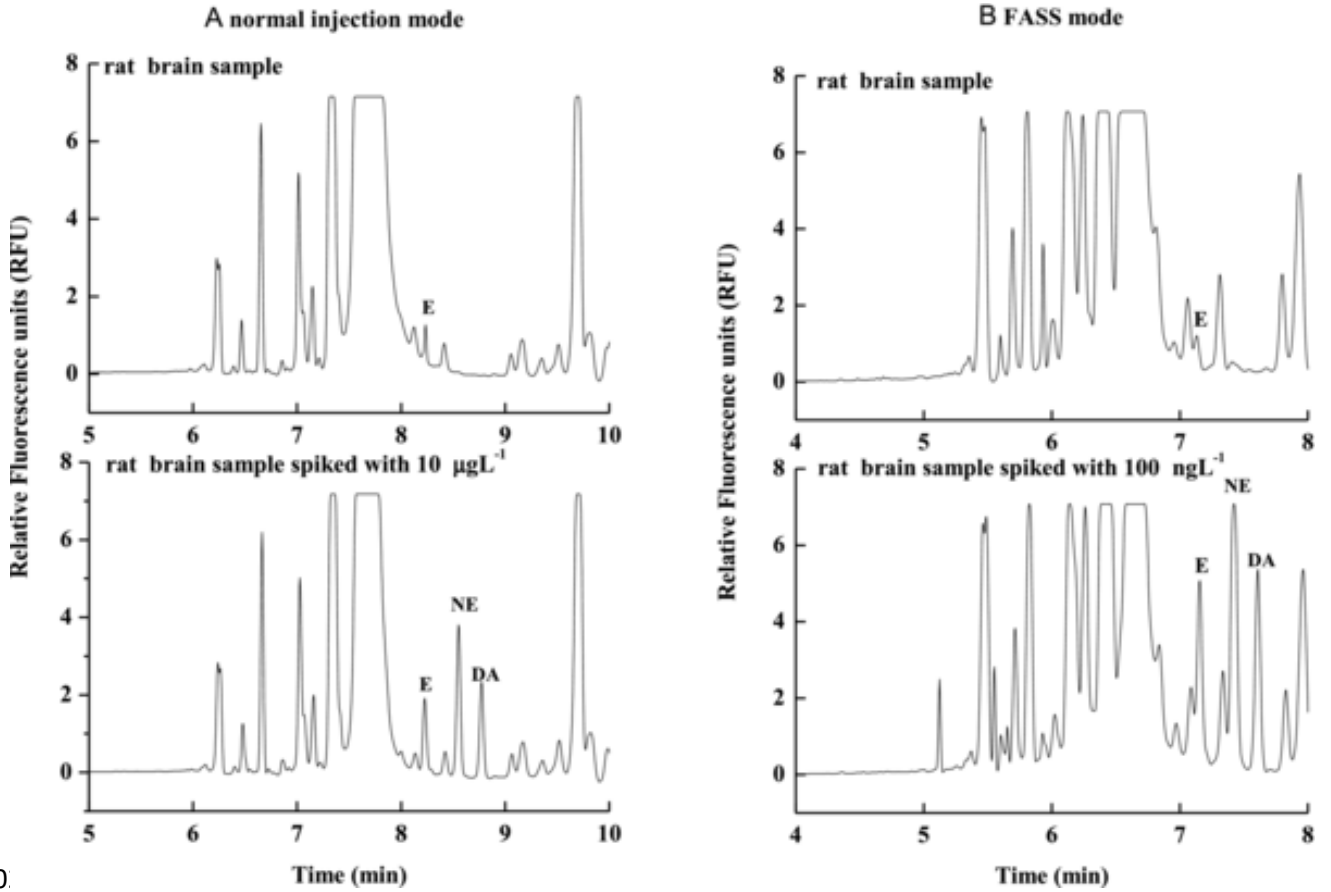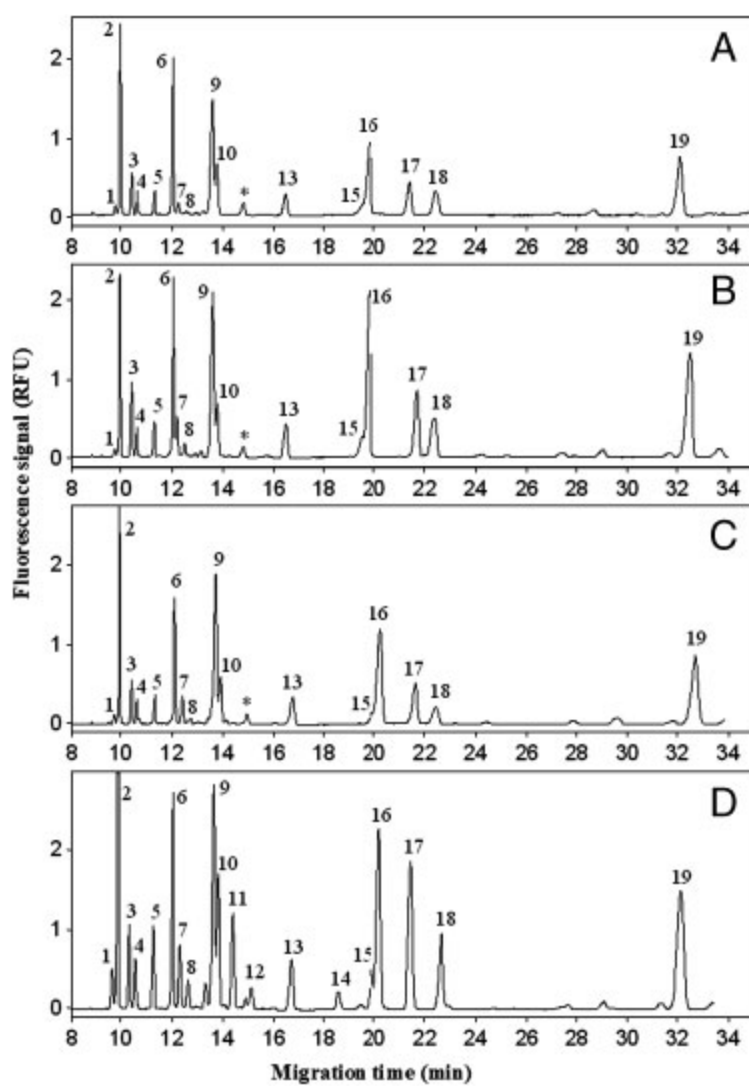
Figure 1.

Figure 2.

1051   **Figure 3.**

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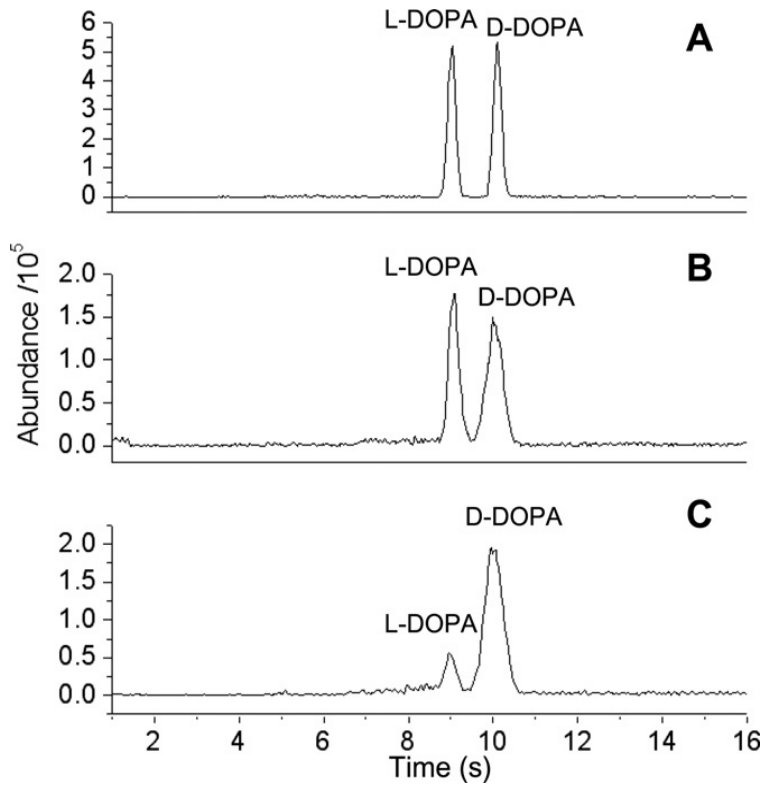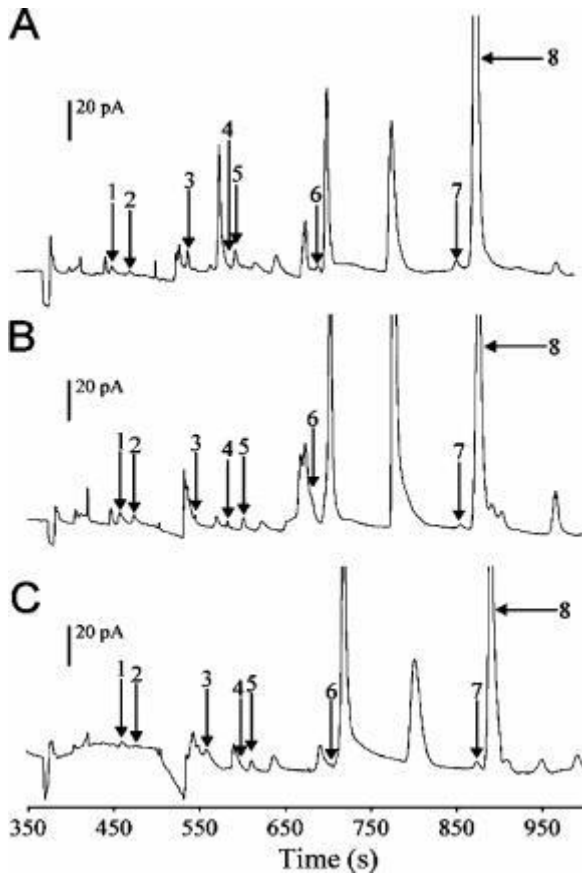
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1067   **Figure 4.**

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