Development and Validation of an Anodic Stripping Voltammetric Method for Determination of Zn$^{2+}$ Ions in Brain Microdialysate Samples

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Abstract An easy, rapid, and sensitive anodic stripping voltammetric method with a controlled growth mercury drop electrode has been developed and validated for the determination of Zn$^{2+}$ ions in brain microdialysate samples obtained from rats. The considered level of the zinc concentration in the dialysate was 0.5–6 ppb. In the investigated method, the stripping step was carried out by using a differential pulse potential-time voltammetric excitation signal. The optimal experimental conditions as well as the instrumental and accumulation parameters and supporting electrolyte composition were investigated. The optimized method was validated for precision, linearity, and accuracy. Mean recovery 82–110% was achieved, the precision expressed by CV not greater than 7.6% and the linearity given by correlation coefficient not lower than 0.9988. The limit of detection was 0.1 ppb. No interferences were observed. Due to high linearity, precision, and sensitivity, the developed method may be successfully applied in the determination of zinc ions in microdialysate brain samples. The results obtained for the first time demonstrate detailed characteristics of the determination of zinc in the brain microdialysate fluid by the ASV method. It may be applied in a wide range of physiological and pharmacological studies which focus on very low zinc concentration/alteration in various compartments of the organisms.

Keywords Brain microdialysate samples · Zinc · Anodic stripping voltammetry · Hanging mercury drop electrode

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Introduction

Zinc is a key component of many proteins and a co-factor of many enzymes that take part in the important role of the brain’s function [1–3]. Zinc in the brain is mainly located in the hippocampus, amygdala, and cerebral cortex. The majority of brain zinc is bound to zinc metalloproteins, while the rest is present in presynaptic vesicles [4]. Neurons containing these vesicles are termed zinc-enriched neurons (ZEN) and in the above-mentioned brain areas, the ZEN terminals are glutamatergic [5]. By inhibiting both the GABA and glutamatergic receptors, zinc seems to modulate neuronal excitability [3, 6] and is also thought to play an important role in synaptic plasticity [7]. Recent data points to zinc as a signaling molecule modulating protein function [8–11]. Zinc deficiency (e.g., dietary deprivation) influences zinc homeostasis in the brain and leads to behavioral disturbances, such as anorexia, dysphoria, impaired learning and cognitive function [4], and some neurological disorders [4, 12]. The studies of the neuromodulatory role of zinc in the synapse are based on the determination of zinc concentration in the extracellular fluid using the microdialysis technique in different brain areas. Brain microdialysis is the in vivo method to evaluate physiological concentration and the effect of various factors (e.g., drugs, stress) on extracellular concentration of a variety of endogenous and/or exogenous compounds [13, 14]. The concentration of zinc in the brain dialysates was determined by the following methods: flame atomic absorption spectrometry [15, 16], fluorescence spectrometry [17], and ligand-trapping technique [18]. The main limitation of these methods is the low volume of the microdialysate probe (5–20 μl) and low zinc concentration (μg/l level), and moreover, the necessity to separate and concentrate the zinc ions during the preparation of the probe.

Stripping voltammetry (SV) analysis is a powerful and simple tool to determine trace target metal species [19]. SV methods are widely applied in biomedical and pharmacological analysis [20–23] as well as in the analysis of metals [23–25]. The major advantage of SV compared with a direct voltammetric measurement is the preconcentration factor [26, 27]. In trace analysis of metal ions, anodic stripping voltammetry (ASV) is the most popular SV technique. It is very important that ASV enables in situ zinc preconcentration in a measured dialysate, which is not possible with the other analytical techniques. In ASV, only free and hydrated Zn ions are accumulated, and this information is useful, for example, in the diagnostics and pharmacotherapy of depression. Sowa-Kucma et al. observed the increase in the concentration of zinc ions in the brain microdialysate after chronic administration of the antidepressant drugs [28]. Likewise, Opoka et al. demonstrated that single intraperitoneal administration of zinc induces an increase in extracellular zinc concentration in the rat prefrontal cortex, which indicates the fast penetration of zinc through the blood/brain barrier [29].

In this work, differential pulse anodic stripping voltammetry (DPASV) with the control growth mercury drop electrode (CGMDE) is applied for the quantitative determinations of zinc(II) ions in the brain microdialysate samples (matrix). DPASV is considered a convenient method of voltammograms registration because of the wide range of linearity, excellent reproducibility, low experimental cost, and the attainment of a low detection limit. Also the CGMDE is the electrode of preference due to its high sensitivity, reproducibility, and linearity [30]. No sample preparation is required except the pretreatment intended to destroy organic compounds (complexants and organic surfactants). To control the proposed method of determination, the standard addition method has been chosen. The presentation of the optimized methodology and validation of the voltammetric method of Zn(II) determination were the main purpose of this paper. The method was fully validated.
according to ICH guidelines [31–33] and may be successfully applied for the determination of Zn in brain microdialysate samples.

**Experimental**

**Instrumentation and Software**

An Electrochemical Analyzer M161 with the electrode stand M164 (both MTM-ANKO, Poland) were used for all voltammetric measurements. The classical three-electrode quartz cell, volume 5 ml, consisting of the CGMDE type M164 (MTM-ANKO, Poland) with the surface area of 1.8 mm$^2$ as a working electrode, used in the hanging mercury drop electrode mode, a double junction reference electrode Ag/AgCl/3M KCl with replaceable outer junction (2 M KNO$_3$) and a platinum wire as an auxiliary electrode. pH measurements were performed with a laboratory pH-meter. All solutions used for analyses were purged with argon. A magnetic Teflon-coated bar was used for stirring (approx. 600 rpm.) during the accumulation period. Experiments were carried out at room temperature. The MTM-ANKO EAGRAPH software enabled electrochemical measurements, data acquisition, and advanced processing of the results.

**Reagents and Solutions**

All solutions and the sample preparation were realized with quadruply distilled water (last two stages from quartz). HNO$_3$ 65% and KNO$_3$ (Merck, Suprapur®) were used for the preparation of samples and supporting electrolyte. Also Zn(II) standard stock solution (1,000 mg l$^{-1}$, Merck) was applied. Solutions with lower zinc concentrations were prepared just before the measurements. Brain microdialysate samples were supplied by the Department of Neurobiology, Institute of Pharmacology, Polish Academy of Sciences, Poland. Prior to use, all glassware and, if necessary, the electrode’s body were cleaned by immersion in a 6 M nitric acid, followed by copious rinsing in distilled water to avoid contamination.

**In Vivo Microdialysis**

The experiments were carried out on male rats (280–320 g). All procedures were conducted in compliance with the National Institutes of Health Animal Care and Use Committee guidelines and were approved by the Ethics Committee of the Institute of Pharmacology Polish Academy of Sciences (Cracow, Poland).

The samples were obtained from the rat’s brain using in vivo microdialysis technology. One day before the microdialysis experiment, the rats were anesthetized with ketamine (75 mg/kg i.m.) and xylazine (10 mg/kg i.m.) and placed into a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA). The scalp was retracted and holes drilled through the skull for insertion of a vertical microdialysis probe into the examined brain region (e.g., prefrontal cortex). Microdialysis probes were constructed by inserting two fused silica tubes (30 and 35 mm long, 150 μm outer diameter (o.d.); Polymicro Technologies Inc., Phoenix, AZ) into a Microdialysis fiber (220 μm o.d.; AN69, Hospal, Bologna, Italy). The tube assembly was placed in a stainless steel cannula (22 gauge, 10 mm), forming the shaft of the probe. Portions of the inlet and outlet tubes were individually placed inside polyethylene PE-10 tubing and were glued for protection.

One day after the surgery and probe implantation, the inlet of the dialysis probes was connected to a syringe pump (BAS, IN, USA) which delivered an artificial cerebrospinal
fluid composed of [in mM]: NaCl 145, KCl 2.7, MgCl₂ 1.0, CaCl₂ 1.2; pH=7.4 at a flow 1.5 μl min⁻¹. After 3 h of a washing period, when the extracellular level of zinc became stable, samples were collected every 40 min from freely moving animals.

Sample Preparation

Brain microdialysate samples were transferred, immediately after they were collected, directly into a miniature quartz tube and were acidified with nitric acid by the addition of 2 μl HNO₃ (conc.) to each 20 μl of sample. The quartz tubes were kept at room temperature, and the microdialysate samples were measured at a minimum of 24 h intervals after acidification. Of the microdialysate, 0.05 ml was introduced into the electrochemical cell containing the supporting electrolyte. The intention of the pretreatment was to destroy organic compounds (complexants and organic surfactants).

DPASV Procedure

The stripping was performed in the differential pulse (DP) mode. Before the measurements, the voltammetric cell was conditioned in 0.1 M nitric acid, rinsed with distilled water, and shortly conditioned in supporting electrolyte. Of 0.05 M KNO₃, 5 ml was added in the electrochemical cell as a blank, and the solution was purged with the argon of 99.995% purity for at least 5–7 min. The preconcentration step was carried out from the stirred solution for a period of \( t_{\text{acc}}=60 \) s at \( E_{\text{acc}}=−1.15 \) V versus Ag/AgCl electrode, at a fresh mercury drop. After a rest period of 5 s, the differential pulse voltammogram was recorded in the anodic direction from −1,150 to −750 mV with a potential scan rate of 25 mV s⁻¹ and pulse amplitude of −30 mV. The voltammogram for the blank solution demonstrates the electrochemical cell and supporting electrolyte purity. Then, 20 or 50 μl of a sample solution (brain microdialysate) was added to the cell while maintaining an argon atmosphere over the solution before the differential pulse voltammograms were recorded. All measurements were performed in the same conditions.

Methodology for Validation

Validation of an analytical method is the process by which it is established, by laboratory studies, so that performance characteristics of the method meet the requirements for the intended analytical applications. The elements required for method validation are: linearity range, limit of detection, accuracy, precision, and selectivity. Validation was carried out according to available guidelines \([31–33]\) and refers to the following aspects.

Selectivity

There is no problem with selectivity because in the potential region used no other signal apart from Zn is observed. These remarks confirm the selectivity of the method.

Precision

Precision was evaluated through repeatability of the final determined concentration and repeatability of the signal (expressed by the Zn peak height) recorded for the sample. Four different microdialysate extracts were chosen for experiments which contain various
concentrations of Zn. Three to five replicates were realized for each sample. Determinations were made in double experimental sessions (within a day and repeated the successive day, delayed by a minimum 24 h interval). The sample was stored between sessions in a HNO₃ environment. This leads to evaluation of the influence of the organic matter which may be present in the solution in the trace concentration. The procedure evaluates together the variability of the sample preparation operations, storage, and the variability of the Zn determination.

Precision was expressed as standard deviations and coefficient of variability (CV) calculated for Zn peak heights and as standard deviations and coefficient of variability of the determined concentration of Zn.

Accuracy

Due to the fact that a brain’s microdialysate samples are not commercially available as a CRM, and taking into account the influence of the matrix on the recorded signal, the accuracy was tested by the calculation of the recovery of the added Zn standard to the real sample. Three different microdialysate extracts were chosen for experiments that contain various concentrations of Zn. The standard addition was 0.5 and 1 ppb of Zn. All such prepared samples (initial and those spiked by Zn) were analyzed according to the standard addition method.

Experimental values obtained from spiked samples were compared to the “true” values, corresponding to initial values with added known concentration values and expressed in terms of their recovery (%). Determinations were made in double experimental sessions (within a day and repeated the successive day, delayed by a minimum 24 h interval).

Linearity and Detection Limit

Linearity was studied for an extended concentration interval (up to 6 ppb) of Zn in the brain microdialysate samples by standard addition methodology. Up to 12 Zn standard additions were considered, each level being analyzed for three to five replicates. The linearity study was repeated on solutions with a different Zn concentration (with matrix) in two successive days. Before quantification the recorded voltammograms were transformed by a background subtraction. The baseline was simulated by the 3rd degree polynomial. The approximation intervals were chosen manually and were the same for all of the curves in the set for calibration. The simulated background was calculated separately for each voltammogram. This corrected data was then used to calculate linear regression parameters. Additionally, the correlation coefficient and detection limit were obtained and given.

Results and Discussion

All optimization and validation experiments were realized using the pharmacological samples which contain the matrix—brain microdialysate. It is very important that the volume of the microdialysate obtained from one rat is minimal (not more than 5–30 μl). The samples matched from successive rats are different, and the level of the zinc ions concentration may be determined by only using the methodology described in this work. Additionally, the sample may not be diluted because the zinc concentration is very low and such an operation may have an unfavorable influence over the level of the uncertainty. Therefore, there is no possibility to extend the volume of the sample. The brain
microdialysates are kept from their being gathered for the experiment in a frozen state. The defrosted samples may be preserved before their determination for only a short time. Both the small volume of the microdialysate samples and low zinc concentration cause the experiments to be very difficult.

Influence of pH on Zinc(II) Peak

During the measurements, organic matter (blood, serum, tissue, etc.) absorption takes place on the working electrode, and this process distorts the ASV signal. Therefore, their removal from the brain microdialysate samples analyzed by ASV is necessary. In this work, it was conducted through the use of nitric acid. However, application of the acid for the decomposition of the organic matter changes the pH of the measured solution and during this process the background current rapidly increases (Fig. 1a). This current, which is carried out by the reduction of the hydrogen ions, additionally reduces the signal-to-background current ratio and obstructs interpretation of the recorded signals. But the zinc peak potential and current is independent of the pH caused by the presence of nitric acid (Fig. 1b). It is because of this that the addition of 1 μl of HNO₃ (conc.) per 10 μl microdialysate sample was used in this work. Such an addition of this acid guaranteed the total degradation of the organic matter in all of the measured samples.

Optimization of DPASV Parameters

The optimization experiments were done in 5 ml of 0.05 M KNO₃ supporting electrolyte into which 50 μl of D1 microdialysate was added. This dialysate was prepared 24 h earlier by the addition of 5 μl nitric acid. The Zn²⁺ concentration in the D1 microdialysate was 3.6 ppb, while the pH of the solution was 2.

Fig. 1 Zn²⁺ voltammograms recorded with matrix in increasing concentration of HNO₃. a Experimental data for D1 sample and 0, 1.5, 3, 4.5, 6, and 7.5 mM addition of HNO₃. b The same curves after baseline correction. The arrow matches the increase of the HNO₃ concentration.
In order to adapt the DPASV method to nanomolar concentrations of Zn\(^{2+}\) three parameters were optimized: step potential \((E_s)\), pulse amplitude \((\Delta E)\), and pulse time \((t_p = t_w + t_s\) (waiting time + current sampling time)). Consequently, these parameters were investigated. To optimize the conditions for Zn\(^{2+}\) measurements, the following instrumental parameters were systematically verified: \(E_s\) in the range 1–5 mV, \(\Delta E\) in the range 10–60 mV, and \(t_p\) from 10 to 60 ms.

An increase of the potential step (in the given range) causes the increase on the peak’s current by 50% in relation to the value for the lowest step (Fig. 2a). However, increasing \(E_s\) is then accompanied by the background current rising. As such, it is not suggested that the \(E_s > 2\) mV is applied. The step potential of 2 mV was applied in further work.

Next, the different pulse time was tested (Fig. 2b), which in this apparatus is closely connected with the waiting time and sampling time. The best results (precision, reproducibility, and the signal-to-background current ratio) were obtained for pulse width 40 ms, and this was the value chosen for the further work. In each case, \(t_w = t_s = 20\) ms (waiting time = current sampling time). The height of the Zn peak strongly decreases with the increasing time \(t_p\) but also background current decreases.

In further tests, it was discovered that the peak current of Zn\(^{2+}\) was similar for both negative and positive amplitude values. The best results (signal-to-background current ratio) were obtained for the pulse amplitude \(-30\) mV. Higher pulse amplitude (absolute value greater than 30 mV) caused major growth of the background current. The peak potential of zinc was shifted to negative potentials with increased pulse amplitude (ca.

![Fig. 2 Optimization of measuring parameters, data obtained for the D1 sample: a potential step, b pulse time (waiting time + current sampling time), c pulse potential, and d accumulation time. The gray arrows point to the optimal value of the considered parameter, which was further applied in Zn determinations](image-url)
28 mV in the given range). For $\Delta E$ changing in the range 10–60 mV, the width of the peak at half-height for $\text{Pb}^{2+}$ was 44 mV. The pulse amplitude of $-30$ mV was chosen (Fig. 2c).

Optimization of Accumulation Time and Potential

The influence of preconcentration potential and time are always important factors in the sensitivity and detection limit of the ASV method. The influence of the accumulation potential was studied in the range from $-1.20$ to $-1.05$ V with the supporting electrolyte spiked with brain microdialysate. The repeatability and the magnitude of the analytical signal were found to be independent of the accumulation potential in the given range. The accumulation potential $-1.15$ V was chosen.

The accumulation time was changed from 1 to 150 s (Fig. 2d). The peak current for the supporting electrolyte containing brain microdialysate increased linearly with the accumulation times. For further study, the accumulation time of 60 s was chosen. The larger accumulation times are impractical in experiments.

Validation Parameters

Further experiments were carried out using four different microdialysate samples: D2, D3, D4, and D5, which are characterized by different zinc concentrations. The measurements were done in 5 ml of 0.05 M KNO$_3$ supporting electrolyte into which 20 $\mu$l of microdialysate was added. The pH of the solution was 2.4. This dialysate was prepared 24 h earlier by the addition of 2 $\mu$l nitric acid.

Linearity The linearity study was realized by the standard addition calibration and its results are presented in Table 1. In the case of dialysates D2, D3, and D4, the standard of concentration 1 ppb was added three times. Here, each calibration point was prepared in triplicate. In the case of the dialysate D5, the standard was added 12 times, and the concentration of one standard was 0.5 ppb. Here, each calibration point was measured five times. Each analysis was repeated on the next day. The results were calculated by linear regression. It may be observed that the sensitivity changed insignificantly from 5.0 to 6.8 nA ppb$^{-1}$ in relation to the sample and the day of analysis. The standard deviation of the slope is not greater than 0.1 nA. A good linearity is evident from the values of the correlation coefficients $r = 0.9988 - 0.9999$ (Table 1). The correlation coefficient was lower than 0.999 and obtained 0.9988 only in one case. This method presented linearity in the range of 1–6 ppb. Additionally it was observed that the zinc peak potential is independent of the concentration of zinc ions.

Detection Limit The achieved limit of detection (LOD) following from the Zn preconcentration at the time of 60 s was ca. 0.1–0.3 ppb. The achieved LOD revealed the high sensitivity of the described voltammetric method.

Precision The high sensitivity of an analytical method is usually accompanied by very good precision. The analytical performance of the described DPASV voltammetric method was evaluated as standard deviation and coefficient of variability. The experiments were realized in the presence of the matrix. The repeatability of the Zn peak height recorded for the dialysate (four different concentration) without standard addition was tested. For brain microdialysates D2, D3, and D4, three replicates were recorded, while five replicates were
recorded for D5. Each test was repeated on the next day. The achieved CV of no more than 4.2% confirmed the excellent precision of the described method (Table 2). The very good repeatability of the recorded signals was also observed after standard additions.

The validation of precision was continued by the realization of the whole Zn determination process which additionally was repeated on another day. The experiments were repeated three times for samples D2, D3, and D4 and five times for dialysate D5. The obtained CV in this situation was not greater than 7.6% (Table 3). The obtained variability of the whole determination process is not so low as in the repeatability of the Zn peak high. It is caused by the influence of the dispersion of the calibration points around the regression line. This lack of consistency introduced the variability of the final result of concentration.

**Accuracy** Samples for the recovery test were prepared as follows. Firstly, the whole calibration process was realized for the dialysate. Next, the sample was spiked by, initially, 0.5 ppb of Zn(II) and, next, 1.0 ppb. In each case, the standard addition calibration was

| Analyte | No. of standard additions | Mean slope/μA ppb⁻¹ | Standard deviation of b/μA ppb⁻¹ | Mean intercept/μA | Standard deviation of a/μA | Correlation coefficient | Detection limit/ppb |
|---------|--------------------------|---------------------|----------------------------------|------------------|--------------------------|----------------------|------------------|
| D2      | 3                        | 0.0050              | 0.00006                          | 0.0085           | 0.0001                   | 0.9994               | 0.1              |
| D2'     | 3                        | 0.0055              | 0.00006                          | 0.0083           | 0.0001                   | 0.9995               | 0.1              |
| D3      | 3                        | 0.0054              | 0.00004                          | 0.0070           | 0.00008                  | 0.9997               | 0.1              |
| D3'     | 3                        | 0.0050              | 0.00006                          | 0.0071           | 0.00001                  | 0.9993               | 0.1              |
| D4      | 3                        | 0.0061              | 0.0001                           | 0.0050           | 0.0002                   | 0.9988               | 0.2              |
| D4'     | 3                        | 0.0052              | 0.00005                          | 0.0059           | 0.00008                  | 0.9997               | 0.1              |
| D5      | 12                       | 0.0059              | 0.00003                          | 0.0052           | 0.0001                   | 0.9999               | 0.1              |
| D5'     | 12                       | 0.0068              | 0.00008                          | 0.0036           | 0.0003                   | 0.9994               | 0.3              |

a Prim denotes experiments done on another day

**Table 1** The linearity study carried out on samples with a matrix through the standard addition method

| Analyte | Zn peak height found for replicate/μA | Mean peak height/μA | Standard deviation of mean/μA | CV/% |
|---------|--------------------------------------|---------------------|-------------------------------|------|
| 1st     | 2nd                                  | 3rd                 | 4th                           | 5th  |
| D2      | 0.0085                               | 0.0084              | 0.0088                        | –    | 0.0086 | 0.0002 | 2.3 |
| D2'     | 0.0085                               | 0.0085              | 0.0086                        | –    | –     | 0.0085 | 0.0001 | 1.1 |
| D3      | 0.0068                               | 0.0070              | 0.0071                        | –    | –     | 0.0070 | 0.0002 | 2.9 |
| D3'     | 0.0070                               | 0.0071              | 0.0075                        | –    | –     | 0.0072 | 0.0003 | 4.2 |
| D4      | 0.0047                               | 0.0046              | 0.0047                        | –    | –     | 0.0047 | 0.00006 | 1.2 |
| D4'     | 0.0053                               | 0.0054              | 0.0054                        | –    | –     | 0.0054 | 0.00006 | 1.1 |
| D5      | 0.0049                               | 0.0051              | 0.0050                        | 0.0053 | 0.0052 | 0.0051 | 0.00015 | 2.9 |
| D5'     | 0.0042                               | 0.0043              | 0.0043                        | 0.0043 | 0.0042 | 0.0043 | 0.00004 | 0.9 |

**Table 2** The study of the repeatability of Zn peak height carried out on samples with a matrix through the standard addition method
realized. The considered dialysates were: D2, D3, and D4. Recordings were done with three replicates. Each test was repeated on the next day. The results were analyzed by linear regression. Data recovery values from the accuracy study are presented in Table 4. The obtained values are localized in the interval ca. 82–110%. This result of the test may be accepted because of a very low concentration of the analyte (not greater than 2 ppb).

### Conclusions

The main goal of this work was the optimization and validation of the anodic stripping voltammetry method for the determination of Zn$^{2+}$ ions in brain microdialysate samples. The optimal experimental variables as well as accumulation parameters were investigated as: potential increment 2 mV, time of potential increment 40 ms, scan pulse amplitude $dE$ = −30 mV and an accumulation potential $E_{acc}$ 60 ms at $−1.15$ V using a 50 mM KNO$_3$ solution (pH 2.4) as a supporting electrolyte. The optimized method was validated and was found to be very sensitive, linear, precise, and accurate. Mean recovery 82–110% was achieved, precision expressed by CV not greater than 7.6%, and the linearity given by correlation coefficient not lower than 0.9988. The measured repeatability of the peak height was not greater than 4.2%. The LOD was 0.1 ppb.

### Table 3
The precision study of Zn determination carried out on samples with a matrix through the standard addition method

| Analyte | Zn concentration found for replicate/ppb | Mean Zn concentration/ppb | Standard deviation of mean/ppb | CV/% |
|---------|----------------------------------------|---------------------------|--------------------------------|------|
|         | 1st | 2nd | 3rd | 4th | 5th | | | | | |
| D2      | 1.70 | 1.65 | 1.75 | − | − | 1.70 | 0.05 | 2.9 |
| D2'     | 1.50 | 1.50 | 1.56 | − | − | 1.52 | 0.04 | 2.6 |
| D3      | 1.25 | 1.33 | 1.32 | − | − | 1.30 | 0.05 | 3.8 |
| D3'     | 1.33 | 1.44 | 1.54 | − | − | 1.44 | 0.11 | 7.6 |
| D4      | 0.82 | 0.77 | 0.80 | − | − | 0.80 | 0.03 | 3.8 |
| D4'     | 1.09 | 1.11 | 1.10 | − | − | 1.10 | 0.02 | 1.8 |
| D5      | 0.96 | 0.96 | 0.93 | 1.00 | 1.00 | 0.97 | 0.03 | 3.1 |
| D5'     | 0.72 | 0.75 | 0.73 | 0.72 | 0.72 | 0.73 | 0.02 | 2.7 |

### Table 4
The accuracy study of Zn determination carried out on samples with a matrix through the standard addition method

| Analyte | Initial Zn concentration/ppb | Recovery/% (first day) 0.5 ppb added | 1.0 ppb added | Recovery/% (another day) 0.5 ppb added | 1.0 ppb added |
|---------|-------------------------------|--------------------------------------|---------------|----------------------------------------|---------------|
| D2      | 1.70                          | 82.2                                 | 109.7         | 89.9                                   | 97.7          |
| D3      | 1.30                          | 84.1                                 | 92.2          | 83.1                                   | 94.0          |
| D4      | 0.80                          | 92.6                                 | 106.0         | 88.7                                   | 98.0          |
These results demonstrate, for the first time, detailed characteristics of the determination of zinc in the brain microdialysate fluid by the ASV method, which is an indication for using this method for future zinc determinations in biological fluids. This already validated method may be applied in a wide range of physiological and pharmacological studies which focus on very low zinc concentration/alteration in various compartments of the body.

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