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# VOLTAMMETRIC DETERMINATION OF CLOZAPINE FROMITS DRUG FORM

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***ABSTRACT***

*A voltammetry method was developed for the direct quantitative determination of clozapine in tablet dosage forms based on its oxidation behavior. The electrochemical determination of clozapine was easily carried out on glassy carbon electrode (GCE) using a variety of voltammetry techniques. The electrochemical measurements were carried out on GCE in various buffer solutions in the pH range from 3.00 to 12.00 by cyclic voltammetry (CV) and differential pulse voltammetry (DPV) techniques. The dependence of pH on the anodic peak current and peak potential was investigated. Acetate buffer (pH 5.50) was selected for analytical purposes. The diffusion-controlled nature of the peak was established. A linear calibration curve for DPV analysis was constructed in the clozapine concentration range from $3x10^{-6}$mol $L^{-1}$ to $1x10^{-5}$mol $L^{-1}$. Limit of detection (LOD) and limit of quantification (LOQ) were obtained as $4.082x10^{-7}$mol $L^{-1}$and $1.361x10^{-6}$mol $L^{-1}$respectively. The applied voltammetric method was validated.*

***Keywords**: clozapine, determination, voltammetry, GCE, drug forms.*

## 1. INTRODUCTION

Schizophrenia is a serious, chronic, and debilitating disease characterized by positive symptoms (hallucinations), negative symptoms (withdrawn behaviors, emotional expressions) and cognitive disorders. Clozapine (Figure 1) is a dibenzodiazepine derivative used in treatment-resistant schizophrenia patients (Centorrino*et al*., 2002;Baldessarini*et al*., 2001). Clozapine treatment suppresses the abnormal movements of tardive dyskinesia as well as TAPs, and may treat clozapine movement disorders differently. Clozapine alone or in

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combination with other psychotropic; are used in psychoactive disorders such as seizure disorders, severe bipolar disorder, early periods of schizophrenia, borderline personality disorder and Parkinson's disease (Lieberman et al., 2005; Fitton et al., 1990).

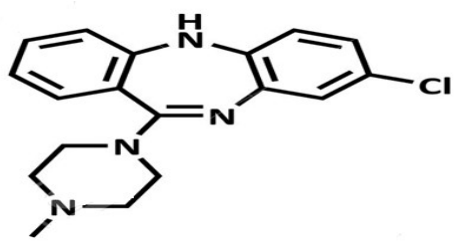


**Figure 1.** Chemical structure of clozapine

The purpose of this study is to investigate the electrochemical properties of clozapine using a glassy carbon electrode and to quantitate rapidly and precisely the amount of drug dosage forms by voltammetry technique.

## 2. MATERIAL ANDMETHOD

### *2.1. Apparatus*

A Model Metrohm 757 VA Trace Analyzer (Herisau, Switzerland) was used to the voltammetric measurements, with a three-electrode system consisting of glassy carbon working electrode (GCE; φ = 3 mm, Metrohm), a platinum wire auxiliary electrode and Ag/AgCl (KCl 3 M, Metrohm) reference electrode. The glassy carbon working electrode was polished with alumina (prepared from φ = 0.01µm aluminum oxide) on alumina polish pad before each experiment and then, rinsed with ultra-pure deionized water and ethanol. The firstly, the deoxygenation process of the supporting electrolyte solutions were carried out with argon gas for 5 min before all experiments. Then, the argon gas was also passed from the solutions for 60 s after the addition of each sample solution in the experiments. In each new experiment, a new bare electrode surface was used. All pH measurements were made with Model Metrohm 744 pH meter (Herisau, Switzerland. All measurements were carried out at ambient temperature of the laboratory (15-20°C) For the analytical application, the following parameters were employed: pulse amplitude 50 mV; pulse time 0.04 s, voltage step 0.009 V, voltage step time 0.04, potential step 10 mV (DPV); the scan rate in the range 10-1000 $mVs^{-1}$ (CV).

### 2.2. Reagents and materials

Clozapine and leponex were kindly supplied by (Novartis, Istanbul, Turkey). A stock solution of $1.0x10^{-2}$ M of clozapine was prepared by dissolving an accurate mass of the drug in an appropriate volume of ethanol kept in the refrigerator. The working solutions for the voltammetric investigations were prepared by dilution of the stock solution. All solutions were protected from light and were used within 24 h to avoid decomposition. 0.067 mol $L^{-1}$ phosphate buffer; pH:4.50-8.00 (sodium hydrogen phosphate ($Na_2HPO_4$, Riedel, Seelze, Germany, and sodiumdihydrogen phosphate $NaH_2PO_4$, Riedel, Seelze, Germany). 0.2 mol $L^{-1}$ acetate buffer; pH:3.50-5.50 (acetic acid: Riedel, Seelze, Germany, 100 m/m % and sodium

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hydroxide: Riedel, Seelze, Germany) and 0.04 mol $L^{-1}$ Britton Robinson buffer; pH:2.02-12.00 (acetic acid: Riedel, Seelze, Germany, 100 m/m %; boric acid; Merck, Darmstadt, Germany, and phosphoric acid, Carlo Erba, Rodeno, France, 85 m/m %) were used to the supporting electrolyte solutions. Ultra-pure-deionized (0.055 µS/cm) water obtained from TKA Smart 2 model was used to prepare supporting electrolytes. Other chemicals, all of the analytical-reagent grade (Merck) were used.

### 2.3. Calibration graph for quantitative determination

The stock solution of clozapine was diluted with ethanol to obtain different clozapine concentrations. Using the optimum conditions described in the experimental section, a linear calibration curve was constructed in the clozapine concentration range $3x10^{-6}$-$1x10^{-5}$mol $L^{-1.}$ The repeatability, accuracy, and precision were checked.

### 2.4. Working voltammetric procedure of spiked tablet dosage forms

Ten tablets were weighed and ground to a fine powder. An adequate amount of this powder, corresponding to a stock solution of concentration $1x10^{-2}$ M was weighed and transferred 10 mL calibrated flask and the volume was adjusted with ethanol. The content of the flask was centrifuged for 20 min at 4000 rpm to affect complete dissolution and then diluted to volume with the same solvent. Appropriate solutions were prepared by taking suitable aliquots of the clear supernatant liquor and diluting with selected supporting electrolyte solutions. Each solution was transferred to the voltammetric cell. The nominal content of the corresponding regression equations was compared with previously platted calibration plats.

## 3. RESULTS AND DISCUSSION

### 3.1. Electrochemical oxidation behavior of clozapine

The electrochemical oxidation process and the determination using this electrode were firstly carried out by CV and DPV techniques. CV measurements performed with clozapine $1x10^{-4}$ M at scan rates between 10 – 1000 $mVs^{-1}$ on GCE in 0.2 mol $L^{-1}$ acetate buffer (pH 5.50) are given in Figure 2.

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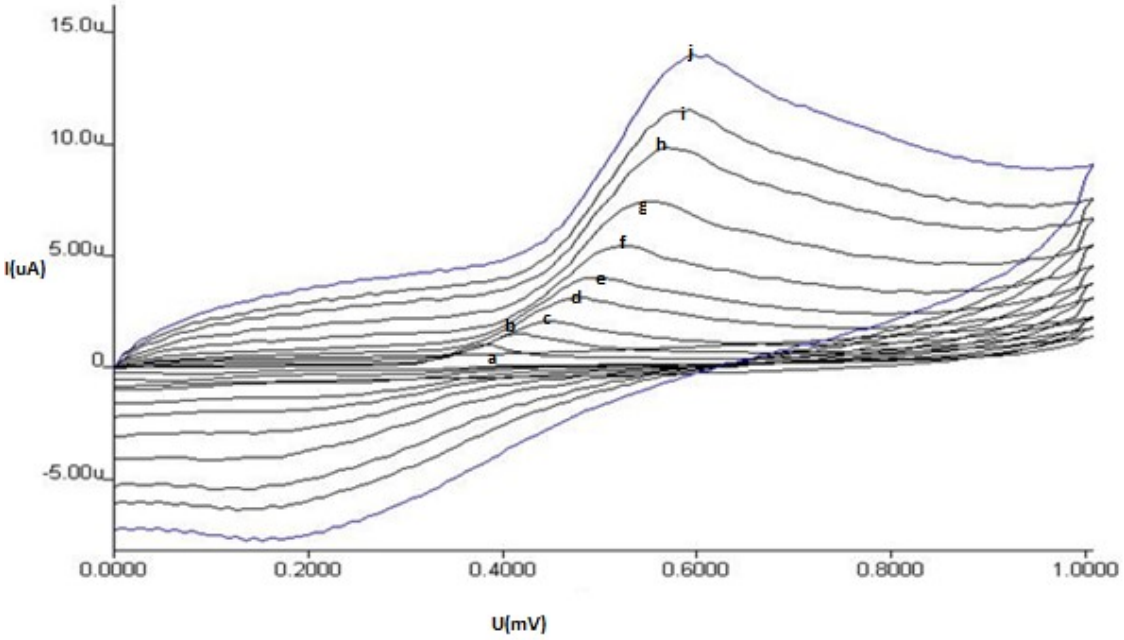


**Figure 2.** The cyclic voltammograms of $1x10^{-4}$ M clozapine in 0.2 M acetate buffer (pH5.50) on GCE . Scan rate,mV $s^{-1}$ a) 10, b) 25, c) 50 , d) 100, e) 150, f) 250, g) 400, h) 600, i) 750, j) 1000

The linear relationship existing between peak current and the square root of the scan rate between 10-1000 mV s-1 (Ip(µA) = 0.2426v1/2 -0.2578, correlation coefficient 0.9993) were observed. The correlation coefficient is closed to 1 indicated that the oxidation process is predominantly diffusion-controlled. In addition, a plot of the logarithm of peak current versus the logarithm of scan rate gave a straight line (correlation coefficient 0.9993) with a slope of 0.2426, which is the expected value for an ideal reaction of solution species (Çıtak*et al.,* 2007; Skrzypek*et al*., 2005;Yılmaz*et al*., 2013).

The cyclic voltammogram of clozapine exhibited only one anodic peak, with no peak on the reverse scan, indicating the totally irreversible nature of the electrode reaction. In addition, for an irreversible oxidation process, the peak potential Ep shifts to less negative values with the increasing of scan rate. Therefore, the oxidation process of clozapine was proved to be irreversible(Çıtak*et al*., 2007; Skrzypek*et al*., 2005; Yılmaz*et al*., 2013).

### 3.2. Effect of pH on peak current and peak potential of clozapine

The voltammetric response was strongly pH dependent. The DPV peak current of the oxidation shifted with increasing pH Fig. (3a). The effect of pH on the peak potential was shown in Figure (3b). The maximum current was observed at the 0.2 mol $L^{-1}$ acetate buffer (pH 5.50). Therefore, this pH value and supporting electrolyte were chosen to carry out the electroanalytical determination of clozapine.

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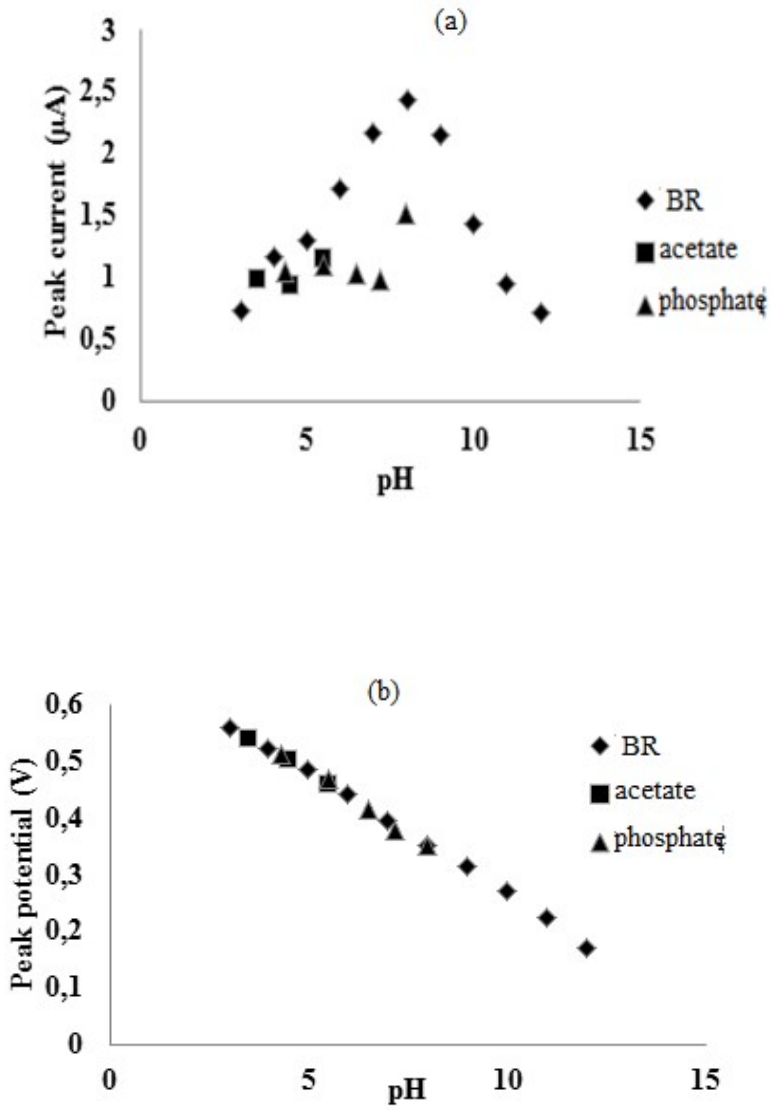


**Figure 3.** The changing of pH on the peak current (a) and peak potential (b) of $7x10^{-6}$ M clozapine in various supporting electrolyte by DPV voltammograms.

### 3.3. Determination of clozapine

DPV technique was used to develop a voltammetric methodology for determination of the drug in pharmaceutical. Under the optimized experimental conditions,linear relationship between the oxidation peak currentof clozapineat GCE and concentration can be established in the range of $3x10^{-6}$-$1x10^{-5}$mol $L^{-1}$ (Figure 4).

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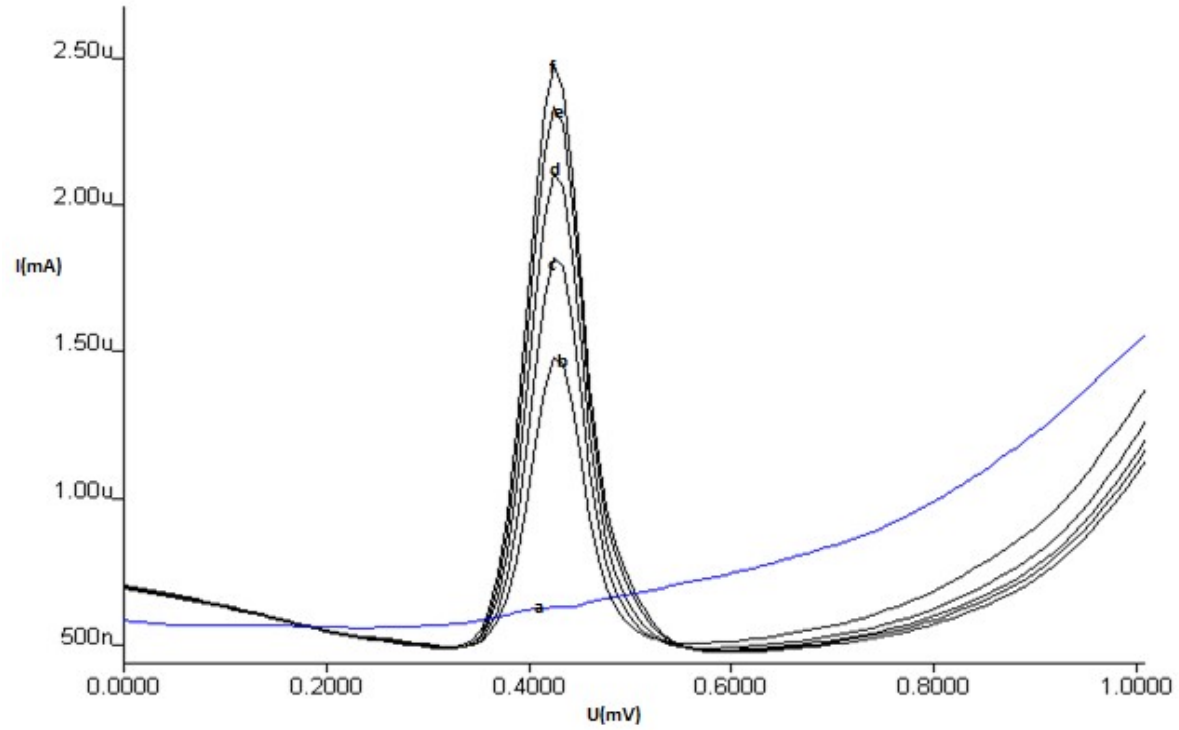


**Figure 4.**The calibration voltammograms at different concentrations of clozapine in 0.2 mol $L^{-1}$ acetate buffer (pH5.50)on GCE by DPV. a) supportingelectrolyte, b) $3x10^{-6}$ c) $5x10^{-6}$d) $7x10^{-6}$e)$9x10^{-5}$f)$1x10^{-5}$(mol $L^{-1}$).

Validation of the procedure for the quantitative determination of the clozapine was examined via evaluation of the limit of detection (LOD), limit of quantification (LOQ), precision (repeatability and reproducibility)in Table 1, accuracy (bias) and recovery values in Table 2. LOD and LOQ were calculated on theoxidation peak current using the following equations: LOD = 3 s / m, LOQ = 10 s / m (s is the standard deviation of the peak currents (five runs, m is the slope of the calibration curve) (Çıtak*et al.,* 2007; Skrzypek*et al*., 2005;Yılmaz*et al*., 2013; Yagmur*et al*., 2017).

The LOD and LOQ were calculated as $4.082x10^{-7}$ and $1.361x10^{-6}$mol $L^{-1}$ respectively. A good repeatability andreproducibility of the peak current and potential were calculated from five independent measurements for 5 $x10^{-6}$mol $L^{-1}$clozapine (Skrzypek*et al*., 2005; Çıtak*et al.,* 2007;Yılmaz*et al*., 2013; Yagmur*et al*., 2017).

Repeatability of peak current and peak potential (R.S.D %) were found as 1.77 and 0.423 respectively. Reproducibility of peak current and peak potential (R.S.D %) were found as 1.79 and 0.423 respectively (Table 1).

The equation of the linear regression plots was Ip(µA)= $1.40x10^{4}$ C (mol $L^{-1}$) +0.558 correlation coefficient, r=0.993; n=5 repeat measurements. Standard deviations for intercept and slope of the calibration curve are given in Table 1.

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**Table 1**. Regression data of the calibration lines for the quantitative determination of clozapine. The calibration plots were obtanied in 0.2mol $L^{-1}$ acetate buffer (pH5.50)on GCEby DPV technique.

| Parameter | Results |
|---|---|
| Measured potential (V) | 0.418 |
| Linear concentration range (mol $L^{-1}$) | $3x10^{-6}$-$1x10^{-5}$ |
| Slope (µA mol $L^{-1}$) | $1.40x10^{4}$ |
| SD of slope | 698.0 |
| Intercept (nA) | 0.558 |
| SD of intercept | 0.071 |
| Correlation coefficient, r | 0.993 |
| Number of measurements, n | 5 |
| LOD (mol $L^{-1}$) | $4.08x10^{-7}$ |
| LOQ (mol $L^{-1}$) | $1.36x10^{-6}$ |
| Repeatability of peak current (R.S.D %) | 1.77 for $5x10^{-6}$mol $L^{-1}$ |
| Repeatability of peak potential (R.S.D %) | 0.423 for $5x10^{-6}$mol $L^{-1}$ |
| Reproducibility of peak current (R.S.D %) | 1.79 for $5x10^{-6}$mol $L^{-1}$ |
| Reproducibility of peak potential (R.S.D %) | 0.423 for $5x10^{-6}$mol $L^{-1}$ |

### 3.4. Determination of clozapine in leponex®tablets byvoltammery techniques

The amount of clozapine in lopenex commercial tablets was calculated by calibration plots. The results obtained are given in Table 2. To determine whether excipients in the tablets interfered with the analysis, the accuracy of the proposed methods were evaluated by recovery tests after the addition of a certain amount of pure drug to pre-analyzed formulations of clozapine (Table 2). The results showed the validity of the proposed techniques for the quantitative determination of clozapine in tablets.

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**Table 2.** Application of the DPV technique for the assay of clozapine in leponex tablets and mean recoveries on GCE.

| Parameter | Results |
|---|---|
| Labeled clozapine (mg) | 25.00 |
| Amount Found (mg) | 25.50 |
| Relative Standard deviation, R.S.D.% | 0.98 |
| Bias % | 2.00 |
| clozapine (mg) | 5.00 |
| Found(mg) | 4.92 |
| Number of measurement, n | 5.00 |
| recovery (%) | 98.30 |
| Relative standard deviation of recovery, R.S.D. % | 0.20 |
| Bias % | 0.02 |

The detection limits reported fornon-electrochemical method and electrochemical methodsare given in Table 3.

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**Table 3.** Comparison of linear range and detection limits for clozapine to different known methods

| Linear range | Limit of detection *(LOD)* | Limit of quantification *(LOQ)* | Method | Reference |
|---|---|---|---|---|
| 0.5-45 µM | – | – | voltammetry | Marshhadizadeh andAfhsar (2013) |
| $1.0\times10^{-9}$-$1\times10^{7}$ mol $L^{-1}$ | $2.08 \times 10^{-10}$ mol $L^{-1}$ | $6.95 \times 10^{-10}$ mol $L^{-1}$ | voltammetry | ArvandandShiraz (2011) |
| 1-3.5 mmol $L^{-1}$ | – | – | voltammetry | Manjunatha*et al.* (2011) |
| 25-50 µg $mL^{-1}$ | – | – | voltammetry | Farhadi*et al.* (2007) |
| $1\times10^{-6}$- $1\times10^{-5}$mol $L^{-1}$ | $1.7 \times10^{-7}$ mol $L^{-1}$ | $5.6\times10^{-7}$ mol $L^{-1}$ | voltammetry | Blankert*et al.* (2007) |
| – | $4.5\times10^{-1}$_$1.5\times10^{-10}$mol $L^{-1}$ | – | voltammetry | Hammam*et al.*(2004) |
| $3x10^{-6}$-$1x10^{-5}$mol $L^{-1}$ | $4.082x10^{-7}$ mol $L^{-1}$ | $1.361x10^{-6}$ mol $L^{-1}$ | voltammetry | **Thisstudy** |
| 0.1-2 µM | 30 nM | - | Voltammetry | Fat'hiandAlmasifar. (2017) |
| 3-70 nM | 1.53 nM | - | Voltammetry | Tammari*et al.*(2017) |
| – | 23.6 µg $L^{-1}$ | – | chromatography | Dural *et al.* (2015) |
| 25-2000 ngm$L^{-1}$ | – | – | chromatography | Wongsinsup*et al.* (2010) |
| 0.1-0.5 µg | – | – | chromatography | Patil andGhosh (2009) |
| 4-200 µg | 1.12-1.76-2.22-0.95-13.26 µg $mL^{-1}$ | – | Spectrometry | Darwish*et al.* (2005) |

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## 4. CONCLUSIONS

A simple, sensitive, selective DPV technique for the quantitative determination of clozapine based on the electrochemical oxidation at GCE was established. From the CV and DPV measurements, it is understood that electrode reaction process is irreversible and pH dependent. Clozapine was successfully determined in 0.2 mol $L^{-1}$ acetate buffer in tablets dosage by DPV technique.

The principal advantage of the DPV technique over the other techniques is that it may be applied directly to the analysis of pharmaceutical dosage form without the need for extensive sample preparation since there was no interference from the excipients and endogenous substances. Another advantage is that the developed DPV technique is rapid, requiring about 5 min to run any sample and involves no sample preparing other than dissolving, diluting, precipitating, centrifuging and transferring an aliquot to the supporting electrolyte.

## Conflict of Interest Statement

The authors declare no conflict of financial, academic, commercial, political, or personal interests.

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