Electronic supplementary Material

Innovative Electrochemical Sensor for Brinzolamide Detection in Athletes' Urine Using Mercury-Phen complex: A Step Forward in Anti-Doping

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2.1. Materials and reagents

Brinzolamide (BRZ, 98%) was kindly supplied by Orchidia Pharmaceutical Industry (Al-Obour city, Egypt) and checked for purity by TLC. AZOPT® eye drops (10 mg BRZ/ml by Orchidia Pharmaceutical Industry (Al-Obour city, Egypt)) were purchased from local stores. 1,10-phenanthroline monohydrate (99.5%), mercuric (II) acetate (98.5%), Citric acid monohydrate (99%), methanol (>99.8%), NaOH (>98%), HCl (37%), HNO_3 (69%), KCl (98.5%), potassium ferricyanide (99.9%), potassium ferrocyanide (99.95%), and graphite powder (1–2 μm) were purchased from Sigma-Aldrich chemicals. Alfa Aesar provided us with paraffin oil. The supporting electrolyte is Britton Robinson Buffer. Water used is double-distilled and all chemicals used were of analytical grade.

2.2. Instrumentation

SWV, CV, and EIS were carried out using the Princeton Versa STAT MC (Versa STAT 3, Model RE-1). A system composed of three electrodes make up the electrochemical cell: A Silver/Silver Chloride (saturated KCl) (reference electrode), a platinum wire (auxiliary electrode) and a bare or modified carbon paste (working electrode). A pH meter (Hanna Instrument) was employed for pH measurement. The manufactured electrode was characterized with scanning electron microscopy (SEM) (JEOL JSM-7500F), x-ray diffraction (XRD) (An anode of Cu Kα (λ= 1.54180 Å) and a diffractometer of the Philips 1700 version with H. T. P. W 1730/104 KVA), Fourier Transform

Infra-Red (FTIR) (Shimadzu IR-470 spectrophotometer), and elemental analysis (FLASH 2000 CHNS/O analyzer). Melting points were measured using Stuart melting point analyzer.

2.6. Preparation of the stock solutions

To prepare a stock solution of 1.0 mmol L−1 of BRZ, 3.80 mg was dissolved in 10 mL of methanol. The stock solution was then diluted with methanol to obtain the working solutions.

2.8. Electrochemical impedance measurements

EIS has been employed to investigate the resistance to electron transfer at the electrode-solution interface. EIS of different electrodes was studied at open circuit potential in the frequency range of 1 Hz to 100 kHz. This analysis was conducted in a solution of 1.0 mmol L^{-1} $[Fe(CN)_6]^{-3}$/ $[Fe(CN)_6]^{-4}$ (1:1 mixture) dissolved in KCl (0.5 mol L^{-1}) and adjusted to pH 2.5 using HCl (1.0 mol L^{-1}). Nyquist plots were used to compare the EIS results between bare and modified electrodes.

2.9. Preparation of real samples

2.9.1. Application to pharmaceutical eye drops.

In order to prepare a 1.0 mmol L^{-1} BRZ, 0.38 mL of a 5-milliliter bottle of AZOPT® eye drops (10 mg BRZ/mL by Orchidia Pharmaceutical Industry, Al-Obour city, Egypt) was accurately transferred into a 10 ml volumetric flask. Subsequently, 8 mL of methanol was added, followed by sonication for 10 minutes and filtration through a 0.22 microfilter. Finally, methanol was used to complete the volume to 10 mL.[12] Working solutions were obtained by diluting the stock solutions with methanol. Then, the developed SWV method was applied as in *section 2.7.*

2.9.2. Application to spiked human urine samples.

The study protocol was approved by the "Institutional Review Board" as well as the research ethics committee of the Faculty of Medicine at Assiut University, Assiut, Egypt (**IRB approval number 04-2023-200631**). Urine samples were collected from healthy human volunteers. Prior to participation, all sampled subjects were duly informed, and their consent was obtained. Clean, sterile, and standardized containers were used for sample collection. BRZ was extracted from urine using a simplified extraction method: 500 µL of the urine sample was spiked with 1.0 mmol L^{-1} of BRZ stock standard solution and then diluted to 10.0 mL with Britton Robinson buffer (pH 7.5) in a centrifuge tube. The solution was vortexed and centrifuged at 5000 rpm for 30 minutes. An adequate volume of the clear supernatant was transferred into the electrochemical cell to obtain the final concentrations, and the SWV voltammograms for BRZ were recorded using $HgCl_2$-Phen

complex/CPE electrode under the optimal experimental conditions. A blank experiment was made in the same manner without the drug.

2.9.3. Application to real human urine samples.

The study protocol was approved by the "Institutional Review Board" as well as the research ethics committee of the Faculty of Medicine at Assiut University, Assiut, Egypt (**IRB approval number 04-2023-200631**). Eight healthy human volunteers (of both sexes) undergo topical ocular administration of AZOPT® eye drops three times daily (one drop/eye ~ 30µL) for 11 days. Urine samples were collected from them every day after application of the morning dose in clean, sterile containers and were frozen for further analysis. At the time of measurement, the urine samples were thawed and treated as mentioned in *section 2.9.2.*

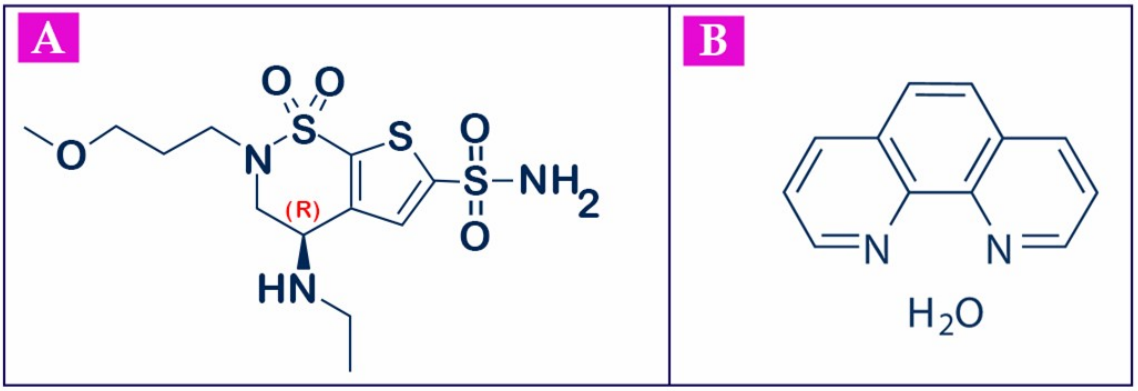

Fig. S1. Chemical structure of (A) brinzolamide, and (B) 1,10-phenanthroline monohydrate.

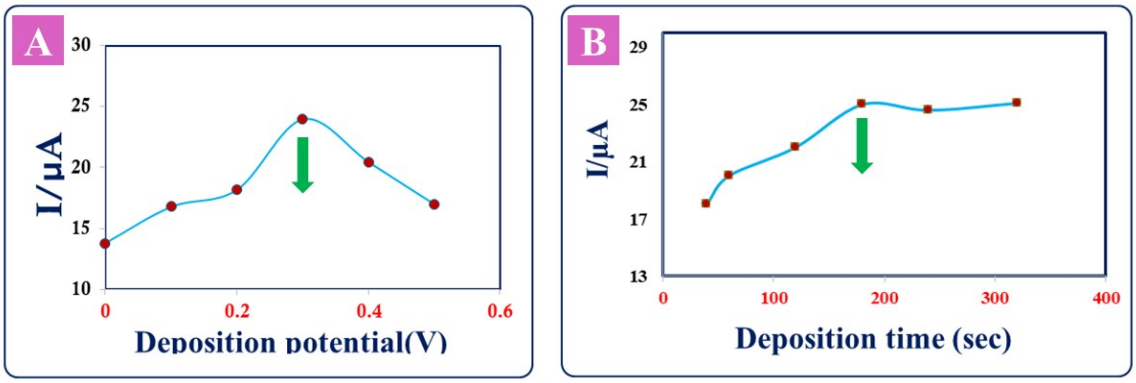

Fig. S2 Peak current plot of (BRZ 2.0 µmol L^{-1}) *vs* (**A**) deposition potential and (**B**) deposition time at $HgCl_2$-Phen complex/CPE. Each point is the average of three measurements.

Table S1. Accuracy and precision of the developed SWV method.

Drug	Concentration (μmol L^{-1})	Accuracy		Intra-day precision		Inter-day precision	
		% Recovery ± SD	RSD %	% Recovery ± SD	RSD %	% Recovery ± SD	RSD %
BRZ	**0.5**	99.46 ± 2.70	2.71	99.97±2.07	2.08	99.96±2.80	2.80
	2.0	100.93±2.57	2.55	99.74 ± 1.64	1.64	100.72±2.8	2.78
	5.0	100.07±2.73	2.73	99.43±1.36	1.37	100.33±2.47	2.46

Variable[a]	% Recovery ± SD[b] (n = 3)
pH of the medium (7.5 ± 0.2)	
7.7	100.6 ± 2.1
7.5	99.8 ± 0.89
7.3	98.9 ± 1.36
Initial potential (0.30 ± 0.01 V)	
0.31	101.8 ± 1.4
0.30	101.9 ± 0.077
0.29	100.1 ± 2.7
Scan rate (0.1 ± 0.01 Vs^{-1})	
0.11	102.03 ± 1.38
0.10	101.85 ± 1.4
0.09	102.4 ± 1.81

Table. S2. Robustness of the proposed voltametric method for BRZ (1.0 μmol L^{-1}).

[a] Other experimental parameters were kept fixed (accumulation time, 180 s; step height, 10 mV; frequency, 150 Hz; and pulse height, 9 mV).

[b] Average of three determinations

Table. S3. Statistical analysis of the results obtained by applying the proposed method and the reported TLC-densitometric method for the determination of BRZ in pharmaceutical dosage form.

Value	Proposed method	Reported method***
Mean	99.35	99.7
SD	1.034	0.697
n*	6	6
Variance	1.069	0.485
t ** (2.23)	1.89	-
F (5.05)**	2.2	-

* No. of experimental

** The value in parenthesis are tabulated values of t and F at (P=0.05)

*** TLC method (on silica gel 60 F_{254} plates using chloroform: methanol: ammonia (9:0.5:0.1 by volume) as a mobile phase and detection was done at 254 nm.[12]

Table S4 The Blue Applicability Grade Index (BAGI) 10 factors utilized in evaluation of the practicality of the proposed method.

	Parameter	**Rating**	**Remarks**
1	Type of Analysis	Blue	Quantitative
2	Multi- or single determination	White	Single analyte
3	Analytical technique	Blue	Electrochemical device which is available in most labs
4	Simultaneous sample preparation	Blue	Sample preparation is time saving
5	Sample preparation	Blue	Sample preparation is very simple
6	Samples/ hour	Blue	More than five samples can be analyzed per hour
7	Materials and reagents used	Light blue	Materials were easily synthetized in lab
8	The need for preconcentration	Dark blue	No need for a preconcentration step
9	Automation degree	White	Analysis was done and treated manually
10	Sample's amount	Blue	500 µL urine