Author's Accepted Manuscript

www.elsevier.com/locate/talanta

Copper nanoparticles applied to the preconcentration and electrochemical determination of β-adrenergic agonist: An efficient tool for the control of meat production

Matías Regiart, Luis A. Escudero, Pedro Aranda, Noelia A. Martinez, Franco A. Bertolino, Julio Raba

PII: S0039-9140(14)01009-1
DOI: http://dx.doi.org/10.1016/j.talanta.2014.12.026
Reference: TAL15299

To appear in: *Talanta*

Received date: 12 September 2014
Revised date: 18 December 2014
Accepted date: 19 December 2014

Cite this article as: Matías Regiart, Luis A. Escudero, Pedro Aranda, Noelia A. Martinez, Franco A. Bertolino, Julio Raba, Copper nanoparticles applied to the preconcentration and electrochemical determination of β-adrenergic agonist: An efficient tool for the control of meat production, *Talanta,* http://dx.doi.org/10.1016/j.talanta.2014.12.026

Copper nanoparticles applied to the preconcentration and electrochemical determination of β-adrenergic agonist: An efficient tool for the control of meat production

Matías Regiart, Luis A. Escudero, Pedro Aranda, Noelia A. Martinez,

Franco A. Bertolino*, Julio Raba*

[a] *Instituto de Química de San Luis (INQUISAL) – Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) – Universidad Nacional de San Luis (UNSL), Chacabuco 917, D5700BWS, San Luis, Argentina.*

* Authors to whom correspondence should be addressed: *Instituto de Química de San Luis (INQUISAL) – Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) – Universidad Nacional de San Luis (UNSL), Chacabuco 917, D5700BWS, San Luis, Argentina. (Tel.) +54 (0266) 452 0300-Int. 6815/16/17; (Fax) +54 (0266) 443 0224. E-mail address*: bertolin@unsl.edu.ar (F.A. Bertolino), jraba@unsl.edu.ar (J. Raba).

Abstract

A novel method for preconcentration and electrochemical detection of zinterol in bovine urine samples was developed. In order to improve the limit of detection, the surface of a screen-printed carbon electrode was modified with electrodeposited metal copper nanoparticles. The experimental electrodeposition optimization was performed using a central composite design (CCD), involving the variables: precursor concentration, potential and time applied. Copper nanoparticles were characterized by transmission electron microscopy, scanning electron microscopy, cyclic voltammetry, and energy dispersive X-ray spectroscopy. Mesoporous

shuttle-like copper oxide nanoparticles were used for the preconcentration step to avoid interferences with many compounds present in the sample matrix. The optimal working conditions for the preconcentration approach were found by means of both two-level fractional factorial and CCD designs. The obtained enhancement factor for a sample volume of 30 mL was 35 fold. The calibration curve showed linearity between 0.5-45 ng mL^{-1} and the limit of detection was 0.16 ng mL^{-1}. The intra and inter assay coefficients of variability were below 4% and 5%; respectively.

Keywords: Zinterol; Electrochemical detection; Screen-printed carbon electrode; Copper nanoparticles; Preconcentration; Central composite design.

1. Introduction

β-adrenergic agonists, such as zinterol (ZIN), improve livestock production because they control the efficiency of food use, affecting carcass characteristics and chemical composition of meat, by increasing lean meat deposition and/or improving the lean meat to fat ratio in animals [1]. However, the presence of these compounds in food represents a risk to human health due to its long half-life and stability [2]. Nowadays, symptoms of β-agonists residue-induced have been reported [3]. It is known zinterol residues are accumulated in animal tissues and could cause acute poisoning in humans [4]. Additionally, these residues may negatively impact the food poisoning export trade of edible animal products and result in nearly incalculable economic loss [5]. The use of ZIN in animal production has been banned in Argentina as well as in the European Union countries [6, 7].

To date, different analytical methods have been reported for the determination of β-agonists in many biological samples, including ultra high-performance liquid chromatography-mass spectrometry [8], gas chromatography-mass spectrometry [9, 10], conventional liquid

chromatography-mass spectrometry [11-13], surface-enhanced Raman scattering [14], capillary electrophoresis [15], enzyme-linked immunosorbent assay [16], and immunosensors [17]. Although the determination of β-agonists by these methods is promising because of their high selectivity and sensitivity, most of them have been associated with several shortcomings, including high cost, complex sample pre-treatments, and long analysis time. In this context, it becomes necessary to develop a simple and fast method for ZIN analysis.

Electrochemical methods have attracted increasing attention because of their excellent characteristics of high sensitivity, response speed, simple and less expensive instrumental, almost no-sample pretreatment, and may be incorporated into portable devices. In addition, due to their electroactive group, most of the β-agonists could be oxidized onto the electrode surface. Among the electrochemical techniques, square-wave voltammetry (SWV) combined with the use of screen-printed carbon electrodes (SPCE) as the detection system represents an attractive alternative [18, 19]. The main advantages of the SPCE include simplicity, versatility, low cost, portability, reliability, small size, mass production capabilities, and minimum sample volume required (20–40 μL) [20, 21].

Moreover, SPCE surface can be modified by electrodeposition of metal nanoparticles (NPs) such as copper, gold, platinum, palladium, and silver, which provides an increased active surface, high conductivity, and electrocatalytic characteristics to enhance the limit of detection.

Additionally, NPs have been widely and efficiently applied to electrochemical sensors development [22, 23], leading to the development of more sensitive and selective (bio) assays. In this context, copper nanoparticles (CuNPs) are cheaper and make significant improvements to the method's selectivity when compared with noble metal NPs [24-26].

ZIN is present at very low levels in complex matrices thus, preconcentration/separation approaches are necessary to improve sensitivity and selectivity. Solid-phase extraction is one of

the most common techniques used for sample cleanup and analyte's preconcentration in environmental and biological matrices [27-29]. The NPs enrich the performance of the analytical method, in addition increasing miniaturization in the whole process [30]. For this purpose, NPs have been used in sample preparation, as sorbents, pseudo-stationary phases or filters [31, 32].

On the other hand, the traditional optimization procedure varying "one variable-at-a-time", is a strategy "based on experience, educated guesswork and luck" that does not guarantee the attainment of a true optimum working condition [33]. Conversely, the chemometric approach relies on a rational experimental design, which allows the simultaneous variation of all experimental factors, saving time and materials [34]. The central composite design (CCD) is an experimental design useful in response surface methodology. The CCD is probably the most widely used experimental strategy for fitting a second-order response surface [35, 36]. The CCD uses quantitative experimental data to fit regression model equations and operating conditions as variables [37]. It contains an embedded factorial design with centre points and is augmented with a group of axial points which make the design rotatable. In addition, CCD can provide high quality predictions in studying linear, quadratic and interaction effect factors which influence a system; whereas interactions are unobserved in the normal orthogonal design and single factor tests [38].

Considering the above mentioned, optimization of the electrodeposition process was performed using a CCD, involving precursor concentration, potential and time applied as variables. Additionally, for the preconcentration step mesoporous shuttle-like copper oxide nanoparticles (CuONPs) were employed [39], and the experimental optimization was carried out by means of two-level fractional factorial design and a central composite design. The ZIN was

released with a desorption buffer and measured by SWV on CuNPs/SPCE. The current response was directly proportional to the amount of ZIN present in the sample.

To the best of our knowledge, there have been no records regarding to the use of electrochemical detection for the quantitative analysis of ZIN in biological samples. Thus, in this report, a novel, accurate, sensitive and selective method for the preconcentration and determination of ZIN in bovine urine samples is presented for first time. The obtained results showed that the proposed methodology could have promising analytical applications for the direct determination of ZIN at trace levels ensuring the food safety, as well as, consumer's health.

2. Experimental

2.1. Reagents and solutions

Analytical grade reagents and high purity solvents were used. ZIN, mercaptopropionic acid (MPA) and methanol (CH_3OH) were purchased from Sigma Aldrich (St. Louis, MO, USA). Sodium hydroxide (NaOH), cetyltrimethylammonium bromide (CTAB), and copper (II) chloride ($CuCl_2$) were obtained from Merck (Darmstadt, Germany). Syringe filters with controlled porosity (13 mm, 0.2 µm) were purchased from Millipore (Billerica, MA. USA). Solutions were prepared with ultra-pure water obtained from a Barnstead Easy pure RF compact system (Barnstead, Dubuque, IA, USA).

2.2. Instruments

Electrochemical measurements were performed using BAS 100 B/W (electrochemical analyzer Bioanalytical System, West Lafayette, IN, USA). Cyclic and square wave voltammograms were obtained by employing a SPCE, which was made up of a graphite circular

working electrode (Ø = 3 mm). Silver (Ag) and graphite electrodes were used as the pseudo-reference and the auxiliary electrode, respectively. The temperature for the electrochemical experiments was set at 25 ± 1 ºC.

All pH measurements were made with an Orion expandable ion analyzer (Orion Research Inc., Cambridge, MA, USA) Model EA 940 equipped with a glass combination electrode (Orion Research Inc.).

The morphology of the NPs was studied by JEOL JEM-FS2200 HRP transmission electron microscope (TEM) operated at 100 kV. The morphology of CuNPs was studied by LEO 1450VP scanning electron microscope (SEM). The composition of NPs was examined by energy dispersive X-ray spectroscopy (EDS) with a LEO 1450VP scanning electron microscope.

2.3. Modification of SPCE

For the electrodeposition procedure of CuNPs, the SPCE was immersed into a 0.1 mmol L^{-1} $CuCl_2$ solution containing 0.01 mol L^{-1} KCl as supporting electrolyte. After that, a constant potential value of -300 mV (vs. Ag-SPCE) was applied for 61.69 s. The study and optimization of these variables was carried out using chemometric tools, as described later in Section 3.3. Then, the modified electrode (CuNPs/SPCE) was rinsed by mechanically stirring for 30 s with ultra-pure water and dried carefully with pure nitrogen gas. The CuNPs were characterized by TEM, SEM, CV and EDS.

2.4. Synthesis of mesoporous shuttle-like CuONPs

Firstly, 10 mL of 1 mmol L^{-1} $CuCl_2$ with 1% CTAB solution was mixed with 2 mL of 0.01 mol L^{-1} NaOH at 80 ºC under stirred conditions. The solution was adjusted to pH 8.00, so the Cu^{2+} present in the solution passed to $Cu(OH)_2$, the formed precipitate was washed several

times with ultra-pure water and centrifuged at 1500 rpm. Later, it was dried at 60 ºC for 2 h and calcined at 450 ºC for another 2 h. The solid obtained was washed several times with CH_3OH and then dispersed with the assistance of ultrasonic stirring for 30 min. The surface area of CuONPs was 41.5 m^2 g^{-1} [40]. Nitrogen adsorption isotherms of CuONPs indicated type IV isotherms with H1 hysteresis loop, which it is due to the existence of channel-like pores within the examined material. These observations were consistent with reported literature [41]. Such mesoporous structure provided an efficient transport pathway to their interior voids, which is critical for preconcentration procedures and other applications [42]. As mentioned above, CuONPs characterization studies were performed by TEM and EDS. The CuONPs stock suspension was stored at 4 ºC.

2.5. Preconcentration procedure

The method described below was applied to ZIN determination in bovine urine samples (Scheme 1). Initially, 5 mL of 0.04 mol L^{-1} in EtOH/H_2O (75/25, v/v) MPA was put in contact with the CuONPs for 15 h at room temperature [43]. In this step, the -SH group of MPA reacted with the CuONPs surface as can be seen in the literature [44, 45], thus leaving free -COOH groups and a negative charge on the oxide surface when the pH value was between 5.00-8.00, increasing in this way, the selectivity of the PC. After that, 5 mL of mesoporous shuttle-like CuONPs were packaged in a syringe filter containing controlled porosity, in order to obtain the preconcentration column (PC) [27].

A sample volume of 30 mL, previously filtered and pH adjusted (pH 6.00), was passed through the PC. Consequently, the ZIN present in the sample was adsorbed onto the mesoporous shuttle-like CuONPs surface through physical [46] and chemical adsorption [47] due to any positive charge in the sample was adsorbed from the negative charge of $-COO^-$ on the CuONPs

surface via electrostatic effect (Scheme 1A). After several washes with ultra-pure water, 2 mL of 0.1 mol L^{-1} glycine (pH 2.04) was used as desorption buffer to elute the retained ZIN (at pH 2.04 the charge on the CuONPs surface changed to positive breaking the electrostatic interaction) (Scheme 1B). Finally, the analyte present in the solution was oxidized by SWV at +700 mV. Therefore, the current response was directly proportional to the amount of ZIN present in the sample. The blank solution was prepared in the same way except that 30 mL of ultra-pure water was used instead of sample solution. The difference between the current of the blank and the sample solution was then calculated.

3. Results and discussion

3.1. Characterization of CuNPs and CuONPs

Regarding to the NPs characterization, Fig. 1A shows the TEM and SEM images of the CuNPs. The CuNPs size distribution histogram and the fitted normal distribution curve were observed in Fig. 1B. The diameters ranged from 9 + 2 nm. The elemental composition was determined by EDS as can be seen in Fig. 1C. The Fig. 1D shows the TEM images of the CuONPs. The CuONPs size distribution histogram and the fitted normal distribution curve were performed in Fig. 1E. The length ranged from 265 + 85 nm. The elemental composition was determined by EDS as can be seen in Fig. 1F. Histogram plots were made by measuring 200 randomly selected particles in enlarged TEM images. To determine the average particle size, the data were fitted with the normal distribution function.

In addition, Scheme 2A shows the electrochemical characterization of CuNPs/SPCE. The CV of 1 mmol L^{-1} $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ in 0.1 mol L^{-1} KCl (pH 6.50) solution was a convenient and valuable tool to examine the characteristics of the modified surface. The mentioned figure shows the CVs of (a) unmodified SPCE and (b) modified with CuNPs. The

potential sweep was varied from -100 to +800 mV at a scan rate of 75 mV s^{-1}. CVs well defined and characteristics of a diffusion controlled redox process were observed at the CuNPs/SPCE surface. The electrodes employed in this characterization step were optimized as mentioned previously in Section 2.3.

3.2. Electrochemical behaviour of ZIN at the CuNPs/SPCE

The use of mesoporous shuttle-like CuONPs was an efficient method for the preconcentration of very low levels of ZIN in real samples. The bound analyte could be desorbed and directly measured in an acidic solution. In a first step occurred the hydrolysis of sulfoxide group in aqueous media thus, leaving free the electroactive groups of the ortho-aminophenol substituted, which was oxidized to give the corresponding ortho-benzoquinonimine substituted [48]. The progress of this reaction was monitored measuring the current response of ZIN using CuNPs/SPCE. A typical cyclic voltammogram of 1 mmol L^{-1} of ZIN in 0.1 mol L^{-1} glycine (pH 2.04) solution as supporting electrolyte (scan rate: 75 mV s^{-1}; T = 25±1 ºC) is shown in Scheme 2B. The cyclic voltammogram showed a single anodic peak at Epa = +700 mV for the potential from +350 to +1000 mV, which corresponded to the one-electron transfer of ZIN. In this irreversible process, the oxidation current was decreasing with successive scans, due to ZIN adsorption on the CuNPs/SPCE surface is very strong in acid electrolytes, blocking in this way the electrode surface.

3.3. Study of variables

In order to optimize the working conditions for ZIN voltammetric determinations in bovine urine samples, many variables that affect the preconcentration and electrochemical response and, as a consequence, the obtained results must be evaluated. The fixed

electrochemical parameters to carry out the optimization of some relevant variables were: step E = 10 mV, S.W. amplitude = 50 mV, S.W. frequency = 10 Hz, samples per point = 256, studied potential range = 0-1000 mV, sensitivity = 1×10^{-5} A V^{-1}, using CuNPs/SPCE as working electrode. Regarding to the ZIN concentration, a standard solution of 32.5 ng mL^{-1} was employed in all studies.

On the other hand, the optimization of the electrodeposition procedure of CuNPs was carried out using a CCD design involving three factors: concentration of $CuCl_2$, potential, and time applied. The experimental data were processed by using the MINITAB® 15 computer software (2003, Minitab Inc, USA).

The experiment was cube central with six axial points, eight point and six central points in cube. The experiment design and the relative current response can be observed in Table 1. All experiments were performed in random order. The $CuCl_2$ concentration ranged from 0.01 mmol L^{-1} to 0.18 mmol L^{-1}, containing 0.01 mol L^{-1} KCl as supporting electrolyte. Potential and time applied were ranged from -131,8 mV to -468.1 mV and 9.5 s to 110.4 s; respectively.

The main and interaction effects were optimized and evaluated for the CCD design. To find the most important effects and interactions, analysis of variance (ANOVA) was calculated using MINITAB 15. A probability (p)-value less than 0.05 in the ANOVA indicates the statistical significance of an effect at 95% confidence level. With the information obtained from the presented CCD, a response surface model and contour graphs were created as shown in Fig 2.

Data analysis gave a semi-empirical expression of a quadratic equation (Eq.(1)), where A represents the [$CuCl_2$], B the potential, C the time and the terms A*A, B*B, C*C, A*B, A*C and B*C their interactions. The obtained expression was as follows:

$$Y = 53.92 + 567.59 * A + 0.50* b + 1.05 *C – 2206.60 (A * A) – 0.001 * (B * B) – 0.01 * (C * C) +0.05 * (A*B) + 0.50 * (A* C) + 0.001 * (B*C)$$

Finally, the multiple response criteria on using the desirability function were successfully used to optimize the controlling factors on the electrodeposition procedure of CuNPs. The experimental conditions corresponding to desirability function (D = 1) were: $CuCl_2$ concentration: 0,1 mmol L^{-1}, potential and time applied: -300 mV and 61.69; respectively. In addition, the suggested values during the optimization procedure were experimentally corroborated.

As mentioned before, the ZIN bound to the CuONPs was desorbed in an acidic solution. Preliminary tests were performed to investigate the factors that could influence on the ZIN desorption. The ones that showed to be important were pH value, sample volume, and concentration and volume of the desorption buffer. Then, the mentioned variables were chosen as factors in a 2^{4-1} fractional factorial design (Table 2). Minimum and maximum, levels of each factor were chosen according to the data from previous experiences.

The pH value and the concentration of desorption buffer was evaluated from 1.5 to 2.5 and 0.05 to 0.15 mol L^{-1}; respectively. The volume of the buffer used for ZIN desorption was evaluated between 1 to 3 mL. Additionally, the sample volume was evaluated from 20 to 40 mL. Analysis of variance (ANOVA) and *p*-value were also used to evaluate the significance of the effects, main effects and their interactions on the electrochemical determination as depicted in the Pareto chart shown in Fig. 3.

From the obtained results, it was demonstrated that sample volume, eluent volume, and pH value of desorption buffer (A, B, and D; respectively) were the variables controlling the system and required a final optimization. Thus, a CCD design involving them was developed for such purpose. The experiment design and relative current response can be observed in Table 3.

The pH value and the volume of desorption buffer ranged from 1.15 to 2.84 and 0.31 to 3.68 mL; respectively. On the other hand, the volume sample ranged from 13.18 to 46.81 mL. With the information obtained by the presented CCD, surface responses were created and they are depicted in Fig. 4.

The analytical response for the analyte and the considering factor under study were fitted to polynomial models and validated by the ANOVA test. Solving this system of multiple responses by overlaying the surface and the contour plots for every combination of factors and responses is a very difficult task and, therefore, the use of a simultaneous optimization method was required. For this reason, the multiple response criteria based on using the desirability function were successfully used to optimize the controlling factors on the ZIN determination. The results after applied the desirability function criteria, showed that the optimal experimental conditions were a 30 mL of sample volume, a 2 mL of desorption buffer volume, and a desorption buffer with a pH adjusted to 2.04. These conditions were experimentally corroborated with closely agreement.

3.4. Analytical performance

A calibration curve for the proposed method was created after analyzing five different (n = 5) standard solutions containing 0.5-80 ng mL^{-1} of ZIN. The calibration curve was obtained by plotting current variation (ΔI, nA) versus ZIN concentration (ng mL^{-1}), which showed a linear range between 0.5-45 ng mL^{-1}. The data were analyzed by linear regression least-squares fit method. The calibration graph was described by the calibration equation ΔI (nA) = 120.74 + 3.14 C_{ZIN} with a correlation coefficient for this plot of 0.994, where ΔI is the difference between current of the blank and the sample (Fig. 5). The coefficient of variation for 32.5 ng mL^{-1} ZIN determination was 3.95% (five replicates). The limit of detection (LOD) and quantification

(LOQ) were determined according to the IUPAC recommendations [49], achieving values of 0.16 and 0.5 ng mL $^{-1}$; respectively. The within-assay precision was tested with five measurements in the same run for each control. These series of analyses were repeated for three consecutive days in order to estimate the between-assay precision. The assay showed good precision; intra and inter-assay coefficients of variability were 3.87% and 4.91% respectively. The obtained enhancement factor for a sample volume of 30 mL was 35 fold. The enrichment factor was obtained as the ratio of the slopes of the calibration curves for ZIN with and without the preconcentration step. The total assay time for ZIN determination was 15 min.

3.5. Applicability

Since a recovery study can be considered as a validation alternative [50], this procedure was applied to the developed methodology. Thus, five samples consisting of 300 mL of bovine urine from different animals were divided in ten aliquots of 30 mL each. The methodology described above was applied to five portions for each sample and the average quantity of ZIN obtained was taken as a base value. Then, increasing quantities of ZIN were added to the other aliquots of sample. After that, ZIN was determined by the same method. The results are given in Table 4.

The use of CuONPs for the preconcentration step in the developed electroanalytical method for ZIN determination was relevant, because it helped to avoid interferences with many compounds present in the sample matrix, since many of these substances have electrochemical properties. The optimum experimental conditions were used to study the interfering effect of some ions and organic compounds in the preconcentration and determination of ZIN. The maximum acceptable error was + 5%. The obtained results showed that the investigated ions and organic compounds do not interfere during the preconcentration and determination of ZIN in

bovine urine samples. As a result of this assay, oxidation current was not observed in the same potential range.

4. Conclusions

This study showed that ZIN, a beta adrenergic agonist which causes symptoms of acute poisoning in humans when used as animal feed additives, could be monitored using a SPCE modified with CuNPs by SWV.

The development of a voltammetric technique combined with the use of NPs was suitable for the determination of ZIN in bovine urine samples after a preconcentration step based on CuONPs-modified PC. This method showed many advantages such as the low cost, wide linear range, reproducibility, selectivity, and accuracy with excellent sensitivity.

The analytical utility of multivariate chemometric techniques was used to obtain the optimum conditions for ZIN determination. In this work, a CCD was performed to study the electrodeposition of CuNPs on SPCE as first approach. In addition, a fractional factorial design and CCD were used to investigate the best conditions for ZIN preconcentration and determination. Desirability function criteria were performed to identify the factors optimum levels for the methodology.

The proposed method could be considered as a very promising veterinary analytical tool for the direct determination of ZIN in real samples and it could help to assure food quality and safety as well as consumer's health.

Acknowledgements

The authors wish to thank the financial support obtained from Universidad Nacional de San Luis (UNSL), Instituto de Química de San Luis (INQUISAL), Consejo Nacional de

Investigaciones Científicas y Técnicas (CONICET), and Servicio Nacional de Sanidad y Calidad Agroalimentaria (SENASA).

References

[1] H.J. Mersmann, Overview of the effects of beta-adrenergic receptor agonists on animal growth including mechanisms of action, J. Anim. Sci. 76 (1998) 160–172.

[2] R.L. Avendaño, R.V. Torres, M.F.J. Meraz, L.C. Pérez, S.F. Figueroa, P.H. Robinson, Effects of two β-adrenergic agonists on finishing performance, carcass characteristics, and meat quality of feedlot steers, J. Anim. Sci. 84 (2006) 3259–3265.

[3] J.M. O'Donnell, Effects of the beta-2 adrenergic agonist zinterol on DRL behavior and locomotor activity, Psychopharmacology. 113 (1993) 89–94.

[4] A.J. Kaumann, L. Sanders, J.A. Lynham, S. Bartel, M. Kuschel, P. Karczewski, E.G. Krause, β_2-Adrenoceptor activation by zinterol causes protein phosphorylation, contractile effects and relaxant effects through a cAMP pathway in human atrium, Mol. Cell. Biochem. 163 (1996) 113–123.

[5] D.J. Smith, The pharmacokinetics, metabolism, and tissue residues of beta-adrenergic agonists in livestock, J. Anim. Sci. 76 (1998) 173–194.

[6] G.A. Mitchell, G. Dunnavan, Illegal use of beta-adrenergic agonists in the United States, J. Anim. Sci. 76 (1998) 208–211.

[7] M. Regiart, S.V. Pereira, V.G. Spotorno, F.A. Bertolino, J. Raba, Nanostructured voltammetric sensor for ultra-trace anabolic drug determination in food safety field, Sens. Actuators B 188 (2013) 1241–1249.

[8] R. Nicoli, M. Petrou, F. Badoud, J. Dvorak, M. Saugy, N. Baume, Quantification of clenbuterol at trace level in human urine by ultra-high pressure liquid chromatography-tandem mass spectrometry, J. Chromatogr. A. 1292 (2013) 142–150.

[9] S. Yang, X. Liu, Y. Xing, D. Zhang, S. Wang, X. Wang, Y. Xu, M. Wu, Z. He, J. Zhao, Detection of clenbuterol at trace levels in doping analysis using different gas chromatographic-mass spectrometric techniques, J. Chromatogr. Sci. 51 (2013) 436–445.

[10] L. Amendola, C. Colamonici, F. Rossi, F. Botrè, Determination of clenbuterol in human urine by GC-MS-MS-MS: confirmation analysis in antidoping control, J. Chromatogr. B. 773 (2002) 7–16.

[11] C. Crescenzi, S. Bayoudh, P.A.G. Cormack, T. Klein, K. Ensing, Determination of Clenbuterol in Bovine Liver by Combining Matrix Solid-Phase Dispersion and Molecularly Imprinted Solid-Phase Extraction Followed by Liquid Chromatography/Electrospray Ion Trap Multiple-Stage Mass Spectrometry, Anal. Chem. 73 (2001) 2171–2177.

[12] O.J. Pozo, K. Deventer, P. Van Eenoo, F.T. Delbeke, Efficient Approach for the Comprehensive Detection of Unknown Anabolic Steroids and Metabolites in Human Urine by Liquid Chromatography-Electrospray-Tandem Mass Spectrometry, Anal. Chem. 80 (2008) 1709–1720.

[13] J. Lu, G. He, X. Wang, Y. Xu, S. Yang, Y. Dong, Y. Wu, Z. Yang, M. Wu, G. Ouyang, An improved LC-MS-MS method for the determination of Clenbuterol in human urine, LC–GC N. Am. 31 (2013) 240–247.

[14] I. Izquierdo-Lorenzo, S. Sanchez-Cortes, J.V. Garcia-Ramos, Adsorption of beta-adrenergic agonists used in sport doping on metal nanoparticles: a detection study based on surface-enhanced Raman scattering, Langmuir 26 (2010) 14663–14670.

[15] W. Wang, Y. Zhang, J. Wang, X. Shi, J. Ye, Determination of β-agonists in pig feed, pig urine and pig liver using capillary electrophoresis with electrochemical detection, Meat Sci. 85 (2010) 302–305.

[16] J. Pleadin, A. Vulic, N. Persi, S. Terzic, M. Andrisic, I. Zarkovic, Rapid immunoassay method for the determination of clenbuterol and salbutamol in blood, J. Anal. Toxicol. 37 (2013) 241–245.

[17] W. Tan, Y. Huang, T. Nan, C. Xue, Z. Li, Q. Zhang, B. Wang, Development of protein a functionalized microcantilever immunosensors for the analyses of small molecules at parts per trillion levels, Anal. Chem. 82 (2010) 615–620.

[18] A. Avramescu, S. Andreescu, T. Noguer, C. Bala, D. Andreescu, J.L. Marty, Biosensors designed for environmental and food quality control based on screen-printed graphite electrodes with different configurations, Anal. Bioanal. Chem. 374 (2002) 25–32.

[19] R.M. Pemberton, J.P. Hart, Electrochemical behaviour of triclosan at a screen-printed carbon electrode and its voltammetric determination in toothpaste and mouth rinse products, Anal. Chim. Acta 390 (1999) 107–115.

[20] J. Metters, R. Kadara, C. Banks, New directions in screen printed electroanalytical sensors: an overview of recent developments, Analyst 136 (2011) 1067–1076.

[21] S.V. Pereira, F.A. Bertolino, M.A. Fernández-Baldo, G.A. Messina, E. Salinas, M.I. Sanz, J. Raba, A microfluidic device based on a screen-printed carbon electrode with electrodeposited gold nanoparticles for the detection of IgG anti-Trypanosoma cruzi antibodies, Analyst 136 (2011) 4745–4751.

[22] W. Siangproh, W. Dungchai, P. Rattanarat, O. Chailapakul, Nanoparticle-based electrochemical detection in conventional and miniaturized systems and their bioanalytical applications: a review, Anal. Chim. Acta 690 (2011) 10–25.

[23] A. Sánchez Arribas, E. Bermejo, M. Chicharro, A. Zapardiel, G. Luque, N. Ferreyra, G. Rivas, Analytical applications of glassy carbon electrodes modified with multi-wall carbon nanotubes dispersed in polyethylenimine as detectors in flow systems, Anal. Chim. Acta 596 (2007) 183–194.

[24] J. Luo, S. Jiang, H. Zhang, J. Jiang, X. Liu, A novel non-enzymatic glucose sensor based on Cu nanoparticle modified graphene sheets electrode, Anal. Chim. Acta 709 (2012) 47–53.

[25] M.L. Mena, P. Yánez-Sedeno, J.M. Pingarrón, A comparison of different strategies for the construction of amperometric enzyme biosensors using gold nanoparticle-modified electrodes, Anal. Biochem. 336 (2005) 20–27.

[26] Y. Oztekin, M. Tok, E. Bilici, L. Mikoliunaite, Z. Yazicigil, A. Ramanaviciene, A. Ramanavicius, Copper nanoparticle modified carbon electrode for determination of dopamine, Electrochim. Acta 76 (2012) 201–207.

[27] C.C. Acebal, B.M. Simonet, M. Valcárcel, Nanoparticles and continuous-flow systems combine synergistically for preconcentration, Trends in Anal. Chem. 43 (2013) 109–120.

[28] P.R. Aranda, S. Moyano, L.D. Martinez, I.E. De Vito, Determination of trace chromium (VI) in drinking water using X-ray fluorescence spectrometry after solid-phase extraction, Anal. Bioanal. Chem. 398 (2010) 1043–1048.

[29] P.R. Aranda, L. Colombo, E. Perino, J. Raba, I.E. De Vito, Solid-phase preconcentration and determination of mercury(II) using activated carbon in drinking water by X-ray fluorescence spectrometry, X-Ray Spectrom. 42 (2013) 100–104.

[30] M. Valcárcel, B.M. Simonet, S. Cárdenas, Analytical nanoscience and nanotechnology today and tomorrow, Anal. Bioanal. Chem. 391 (2008) 1881–1887.

[31] H. Parham, B. Zargar, M. Rezazadeh, Removal, preconcentration and spectrophotometric determination of picric acid in water samples using modified magnetic iron oxide nanoparticles as an efficient adsorbent, Mat. Sci. Eng. C. 32 (2012) 2109–2114.

[32] J.P. Kumar, G.K. Prasad, P.V.R.K. Ramacharyulu, P. Garg, K. Ganesan, Mesoporous CuO-ZnO binary metal oxide nanocomposite for decontamination of sulfur mustard, Mater. Chem. Phys. 142 (2013) 484–490.

[33] K.K. Hockman, D. Berengut, Design of Experiments, Chem. Eng. New York, 102 (1995) 142–148.

[34] N. Motohashi, H. Nagashima, C. Párkányi, Supercritical fluid extraction for the analysis of pesticide residues in miscellaneous samples, J. Biochem. Biophys. Methods. 43 (2000) 313–328.

[35] D.C. Montgomery, Design and Analysis of Experiments, John Wiley and Sons, New York, (1991) 521.

[36] S. Bolton, Pharmaceutical Statistics: Practical and Clinical Applications, Marcel Dekker, New York, (1984) 435.

[37] H. Xu, L. He, S. Nie, J. Guan, X. Zhang, X. Yang, W. Pan, Optimized preparation of vinpocetine proliposomes by a novel method and in vivo evaluation of its pharmacokinetics in New Zealand rabbits, J. Control. Release. 140 (2009) 61–68.

[38] D.C. Montgomery, Design and Analysis of Experiments, (fifth ed.) John Wiley, New York (2001).

[39] D. Chen, G. Shen, K. Tang, Y. Qian, Large-scale synthesis of CuO shuttle-like crystals via a convenient hydrothermal decomposition route, J. Cryst. Growth 254 (2003) 225–228.

[40] Y. Zhang, S. Wang, X. Li, L. Chen, Y. Qian, Z. Zhang, CuO shuttle-like nanocrystals synthesized by oriented attachment, J. Cryst. Growth 291 (2006) 196–201.

[41] S.J. Gregg, K.S.W. Sing, Adsorption, Surface Area and Porosity. Academic press, London (1982) 111–190.

[42] Q. Zhang, K. Zhang, D. Xu, G.Yang, H. Huang, F. Nie, C. Liu, S. Yang, CuO nanostructures: Synthesis, characterization, growth mechanisms, fundamental properties, and applications, Prog. Mat. Sci. 60 (2014) 208–337.

[43] S.V. Pereira, F.A. Bertolino, G.A. Messina, J. Raba. Microfluidic immunosensor with gold nanoparticle platform for the determination of immunoglobulin G anti-Echinococcus granulosus antibodies. Anal. Biochem. 409 (2011) 98–104.

[44] H. Parham, F. Khoshnam, Solid phase extraction–preconcentration and high performance liquid chromatographic determination of 2-mercapto-(benzothiazole, benzoxazole and benzimidazole) using copper oxide nanoparticles, Talanta 114 (2013) 90–94.

[45] O. Fiehn, T. Reemtsma, M. Jekel, Extraction and analysis of various benzothiazoles from industrial wastewater, Anal. Chim. Acta 295 (1994) 297–305.

[46] Y. Ren, Z. Ma, P.G. Bruce, Ordered mesoporous metal oxides: synthesis and applications, Chem. Soc. Rev. 41 (2012) 4909–4927.

[47] A. El-Trass, H. El-Shamy, I. El-Mehasseb, M. El-Kemary, CuO nanoparticles: Synthesis, characterization, optical properties and interaction with amino acids, Appl. Surf. Sci. 258 (2012) 2997–3001.

[48] D. Gonçalves, R.C. Faria, M. Yonashiro, L.O.S. Bulhões, Electrochemical oxidation of o-aminophenol in aqueous acidic medium: formation of film and soluble products, J. Electroanal. Chem. 487 (2000) 90–99.

[49] L.A. Currie, Nomenclature in evaluation of analytical methods including detection and quantification capabilities (IUPAC Recommendations 1995), Pure Appl. Chem. 67 (1995) 1699–1723.

[50] E. Prichard, G.M. Mackay, In: Points J (ed) Trace analysis: a structured approach to obtaining reliable results. Royal Society of Chemistry, London (1996) 120–141.

Figure captions

Scheme 1. Schematic representation of A) Adsorption process at pH: 6.00 and B) Desorption process at pH: 2.04 of ZIN in bovine urine sample.

Scheme 2. A) Electrochemical characterization by cyclic voltammogram was performed in 1 mmol L^{-1} $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ in 0.1 mol L^{-1} KCl. Cyclic voltammograms on a) Unmodified SPCE and b) CuNPs/SPCE. B) Electrochemical behavior of 1 mmol L^{-1} ZIN in 0.1 mol L^{-1} glycine (scan rate: 75 mV s^{-1}; T = 25±1 ºC).

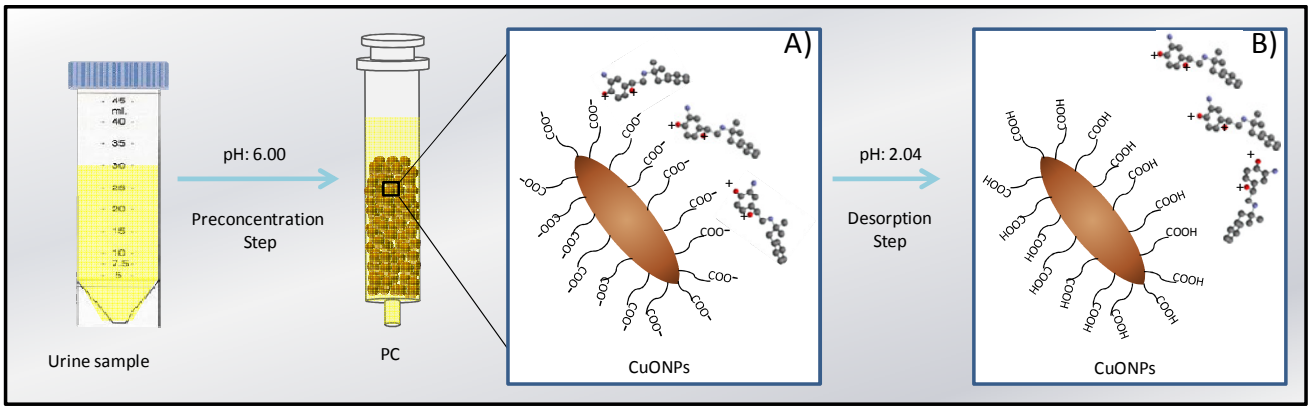
Figure 1. Characterization of CuNPs and CuONPs. A) Morphology of CuNPs was investigated by TEM and SEM, B) CuNPs size distribution histogram and the fitted normal distribution curve, C) Elemental composition of CuNPs was determined by EDS, D) Morphology of CuONPs was investigated by TEM, E) CuONPs size distribution histogram and the fitted normal distribution curve and F) Elemental composition of CuONPs was determined by EDS.

Figure 2. Contour plots of variables involving in CCD used for optimization of electrodeposition procedure of CuNPs.

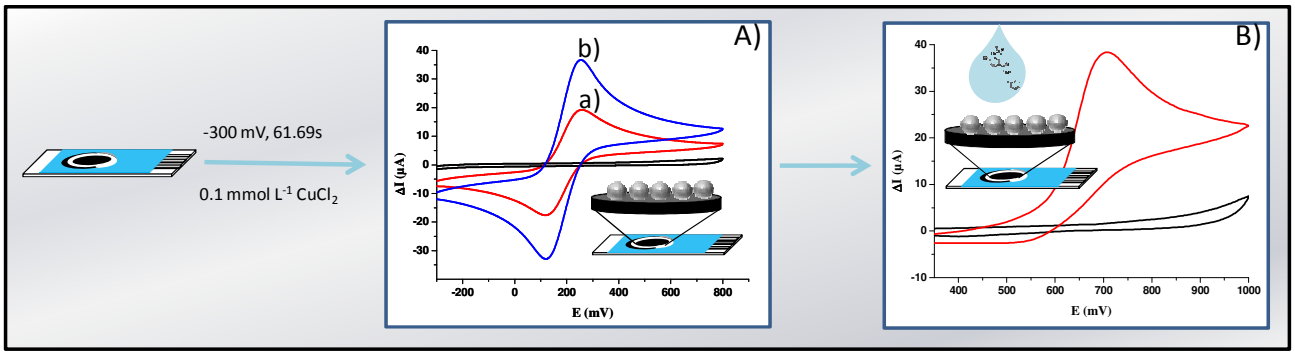
Figure 3. Pareto chart obtained from 2^{4-1} fractional factorial design used as first optimization approach for variables related to preconcetration/elution of ZIN. A) Sample volume, B) Eluent volume, C) Eluent concentration, and D) pH of eluent.

Figure 4. Response surface obtained using central composite design for final optimization of variables related to preconcetration/elution of ZIN.

Figure 5. Square wave voltammetry for ZIN detection. Calibration curve showed a linear range between 0.5-45 ng mL^{-1} of ZIN with a LOD of 0.16 ng mL^{-1}.



Scheme 1



Scheme 2

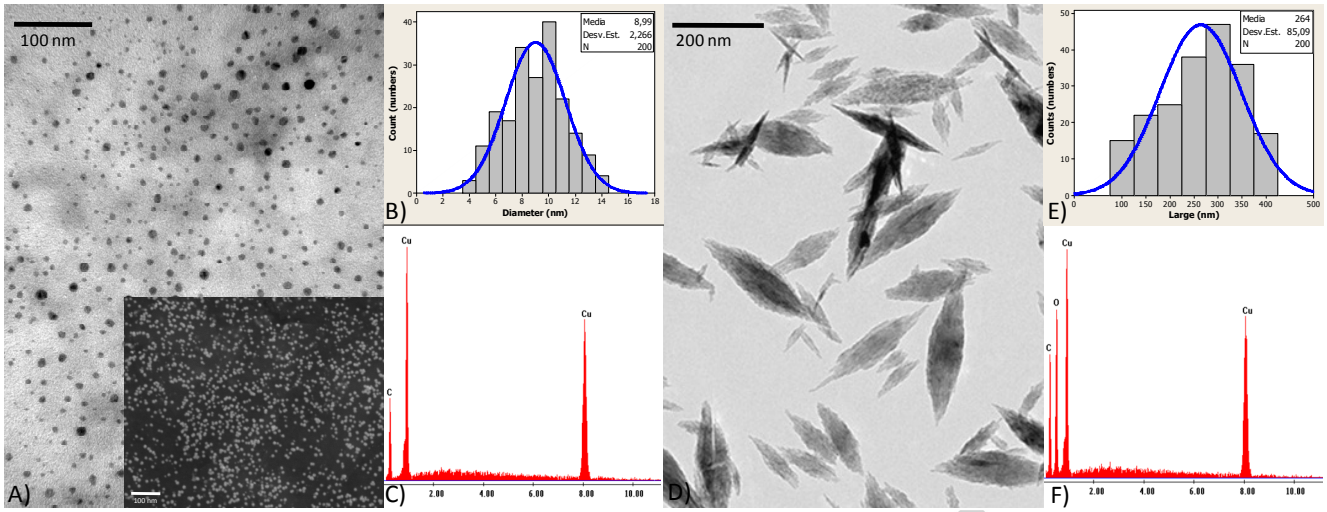


Figure 1

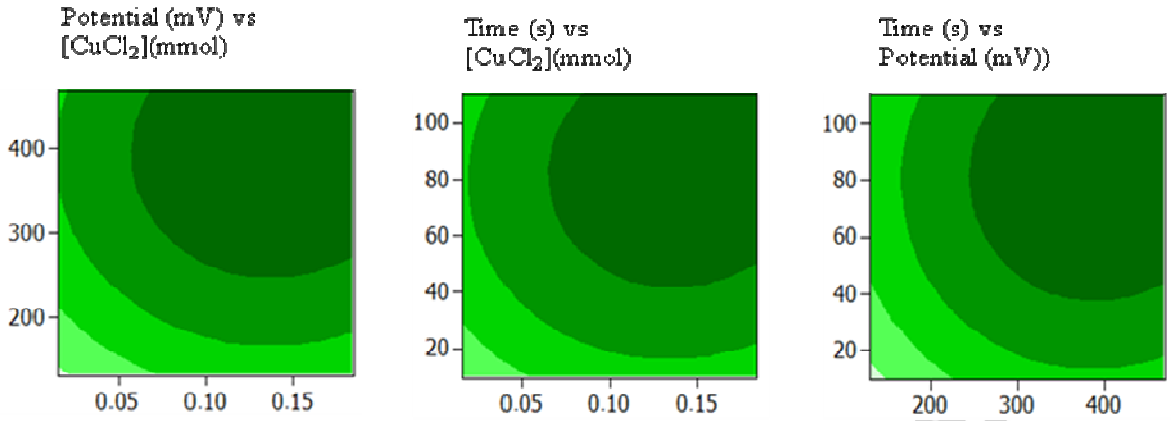


Figure 2

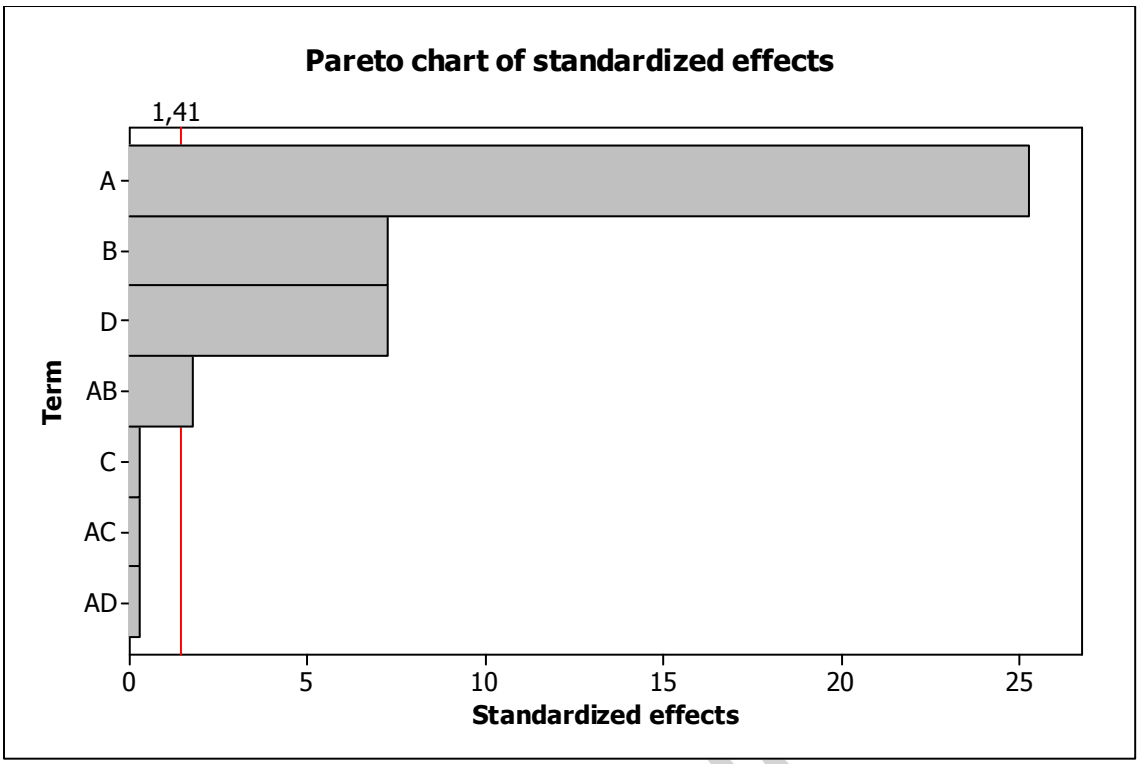


Figure 3

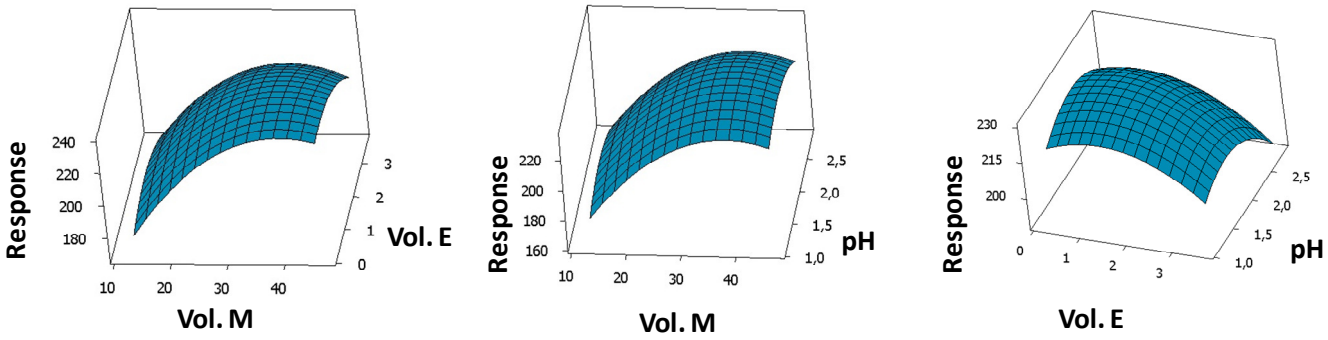


Figure 4

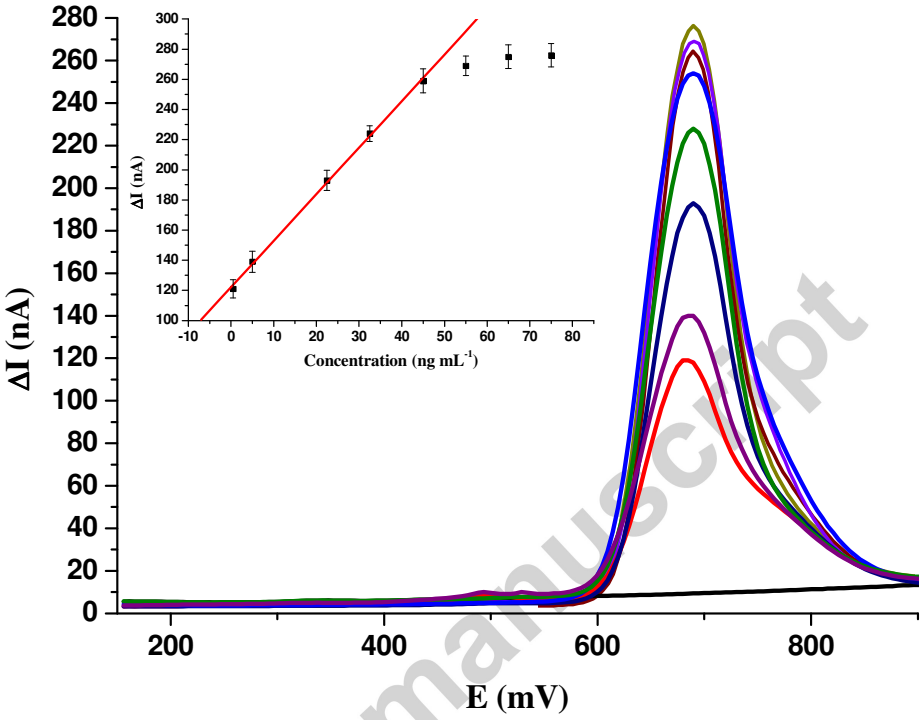


Figure 5

Table 1. Matrix and obtained the results of the central composite design used for CuNPs electrodeposition procedure optimization.

Experiment	[$CuCl_2$](mmol)	Potential (mV)	Time (s)	Response
1	-1	-1	-1	184
2	1	-1	-1	195
3	-1	1	-1	205
4	1	1	-1	215
5	-1	-1	1	197
6	1	-1	1	209
7	-1	1	1	219
8	1	1	1	234
9	-α	0	0	189
10	α	0	0	230
11	0	-α	0	182
12	0	α	0	231
13	0	0	-α	186
14	0	0	α	228
15	0	0	0	224
16	0	0	0	228
17	0	0	0	227
18	0	0	0	226
19	0	0	0	223
20	0	0	0	225

Table 2. Matrix and the results of the fractional factorial 2^{4-1} design, used as first optimization approach for the variables related to the preconcentration/elution of ZIN.

Experiment	vol. M	vol. E	Conc. E	pH E	Response
1	-1	-1	-1	-1	205
2	1	-1	-1	1	221
3	-1	1	-1	1	189
4	1	1	-1	-1	223
5	-1	-1	1	1	198
6	1	-1	1	-1	229
7	-1	1	1	-1	196
8	1	1	1	1	216

Table 3. Central composite design used to obtain the response surface for the final optimization of variables related to the preconcentration/elution of ZIN.

Experiment	Sample volume	Eluent volume	pH	Response
1	-1	-1	-1	198
2	+1	-1	-1	232
3	-1	+1	-1	192
4	+1	+1	-1	219
5	-1	-1	+1	195
6	+1	-1	+1	228
7	-1	+1	+1	187
8	+1	+1	+1	211
9	-α	0	0	189
10	+α	0	0	231
11	0	-α	0	230
12	0	+α	0	216
13	0	0	-α	229
14	0	0	+α	213
15	0	0	0	229
16	0	0	0	227
17	0	0	0	226
18	0	0	0	227
19	0	0	0	225
20	0	0	0	228

Table 4. Recovery study of ZIN in urine bovine samples (n = 5).

Base (ng mL^{-1})	**ZIN added** (ng mL^{-1})	**ZIN found** (ng mL^{-1})	**Recovery (%)**[a]
2.0	0	2.0 + 0.1	-
2.0	9.5	11.9 + 0.4	104.2
2.0	17.4	19.3 + 0.7	99.4
2.0	28.3	29.4 + 0.9	96.8
2.0	39.7	42.4 + 1.2	101.7

[a] [(found-base)/added] × 100

Highlights

- This is the first report for preconcentration and electrochemical detection of zinterol.
- Metal copper nanoparticles on a screen-printed carbon electrode to improve the LOD.
- Mesoporous shuttle like oxide copper nanoparticles for the preconcentration step.
- The experimental optimization was performed using a central composite design.
- Low LOD, fast response time, wide linear range, good selectivity and reproducibility.

Graphical abstract