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Determination of histamine by high-pH anion-exchange chromatography with electrochemical detection

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Abstract

A simple, sensitive and rapid method is described for determining histamine in alkaline media by anion-exchange chromatography with amperometric detection at a copper-based modified glassy carbon electrode. The influence of copper loading, applied potential and flow rate on the amperometric signal was evaluated. Under optimal chromatographic conditions, the detection limit for histamine (S/N = 3) was 0.1 μM (corresponding to 5 pmol injected by a 50-μl sample loop). The linear range spanned from 5 to 500 μM (correlation coefficient = 0.998). The proposed method allows a rapid and convenient quantitation of histamine in real matrices, such as canned tuna and red wine, without time-consuming clean-up or derivatisation procedures. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Histamine; Electrochemical detection; Copper electrode; Tuna; Wine

1. Introduction

Enzymatic decarboxylation of free amino acids and other metabolic processes can lead to the presence of biogenic amines in foods. Although low levels of metabolites such as histamine and tyramine are not considered a serious risk, they can be of health concern in the case of large food consumption. As a matter of fact, food intoxications such as scombrotoxicosis and cheese syndrome (Arnold & Brown, 1978; Taylor, Hui & Lyons, 1984; Taylor, Keefe, Windham & Howell, 1982) have been related to histamine levels exceeding 1 g/kg. Other disturbance from dietary biogenic amines include hypotension, hypertension, headache, nausea and emesis. Moreover, several other compounds, such as putrescine and cadaverine, can enhance the biological activity of histamine, thus reducing the histamine concentration causing toxic effects.

Several methods exist for isolating, identifying and determining histamine and other biogenic amines in foods and biological systems (Matsumoto, Tsuda & Suzuki, 1990; Shalaby, 1999; Stratton, Hutkins & Taylor, 1991; Vale & Gloria, 1997; Veciana-Nogues, Hernandez-Jover, Marine-Font & Vidal-Carou, 1995).

Aliphatic amines do not show pronounced absorption bands in the UV-Vis region, so that usual spectrometric detectors cannot be used. In order to increase sensitivity, derivatisation procedures with benzoyl chloride, benzene-sulphonyl chloride, dansyl chloride, fluorescamine, *ortho*-phthaldialdehyde, and 4-chloro-7-nitrobenzofurazene have been developed for UV-Vis detection. Of course, these methods are usually costly, require an extensive sample clean up, and have a low sample throughput. In addition, derivatisation efficiencies depend on actual experimental conditions and sample matrices. Alternatively, immunochemical methods for histamine determination based on antibodies against N-amino derivatives have been developed (Chevrier, Guesdon, Mazie & Avrameas 1986; Hammar, Bergland, Hedin, Rustas, Ytterström & Àkerblon, 1990; Rauch, Rychestsky, Hochel, Bilek & Guedson, 1992). However, because the antibodies tests are usually not reactive with the parent compound but with a histamine adduct, in this case also chemical derivatisation of histamine is necessary. In order to improve analytical measurements, it is desirable to develop a simple and

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direct detection method. Derivatization procedures generally increase analysis time and risk for indeterminate errors, thus direct determination of analytes, when available with sufficient sensitivity is preferred.

Direct determination of biogenic amines by electrochemical methods has been obtained by using pulsed waveforms at gold electrodes (Dobberpuhl, Hoekstra & Johnson, 1996; Hoekstra & Johnson, 1998) or chemically modified electrodes (CMEs) at constant applied potential (Casella, Rosa & Desimoni, 1998; Donten, Hyk, Gszkowska & Stojek, 1997; Koppang, Witek, Blau & Swain 1999) in alkaline media. Potentiometric stripping procedures using a DNA-modified electrode were also sucessfully employed for the determination of aromatic amines (Wang, Rivas, Luo, Cai, Valera, & Dontha, 1996).

In our laboratory, several chemically modified electrodes were prepared by anodic electrodeposition of various transition metals on glassy carbon or noble metal substrates which exhibited a promising electrochemical activity towards the electrooxidation of different organic compounds. In particular, a copper oxide/hydroxide film was anodically electrodeposited onto a glassy carbon (GC) substrate from alkaline solutions containing cuprous cyanide ions, and characterised by electrochemical and X-ray photoelectron spectroscopy (XPS) (Casella & Gatta, 2000). The Cu/GC modified electrode showed a powerful catalytic activity towards the electrooxidation of alditols, carbohydrates, amines, amino acids, etc. and can also be used for histamine detection.

In this paper, a simple, sensitive and accurate method is described for the amperometric determination of histamine at the Cu/GC electrode by anion-exchange chromatography in alkaline solutions without derivatisation or extensive sample clean-up procedures. The method was applied to the direct determination of histamine in some foods and beverages.

2. Experimental

2.1. Reagents

Solutions were prepared from analytical-reagent grade chemicals without further purification and by using doubly distilled deionized water. Sodium hydroxide pellets (99%), cyanocuprate (I), histamine and other biogenic amines, alditols and carbohydrates (Aldrich-Chemie) were prepared daily in distilled water. Unless otherwise specified, experiments were performed by using 0.2 or 0.4 M NaOH as background electrolyte. All experiments were carried out at ambient temperature.

2.2. Apparatus

A Model 273 Princeton Applied Research (PAR EG&G) potentiostat/galvanostat was used for electrochemical measurements. Cyclic voltammetry (CV) was carried out in a three-electrode cell using the Cu/GC working electrode, a SCE (4 M KCl) reference electrode and a platinum foil counter-electrode. The glassy carbon electrode (geometric area: 0.125 cm^2) used in CV experiments was purchased from PAR. All current densities are quoted in terms of mA/cm^2 of apparent geometric area of the electrode.

Amperometric measurements in flowing streams were performed by using a PAR Model 400 Electrochemical Detector and a flow-through thin-layer electrochemical cell consisting of the Cu/GC as working electrode, an Ag/AgCl (4 M KCl) reference electrode and a stainless steel counter electrode. A personal computer equipped with a Kontron PC Integration Pack Software allowed acquisition and processing of chromatograms. Flow injection experiments were carried out using a Varian 2510 pump equipped with a Model 7125 Rheodyne injector with a 50-μl sample loop.

Chromatographic separations were performed by using a CarboPac MA1 (Dionex) anion-exchange column (250×4 mm I.D. with particle size of 7.5 μm).

2.3. Sample treatment

Tuna canned in brine (Rio Mare, Italy, 1999) and red wine (Aglianico, Italy, 1998) were purchased from a local store. The can of tuna and the bottle of wine were opened just before analytical determinations. A precisely weighted 56-g tuna plus 24 g of brine were finely homogenised using a domestic blender and sonicated with 200 ml of 0.6 M $HClO_4$ for 30 min. The resulting suspension was filtered through filter paper and diluted 1:25 with pure water before injection in the column.

Samples of wine were syringe filtered through a 0.45-μm cellulose acetate membrane, diluted 1:25 with pure water and injected in the column.

2.4. Electrode preparation

Prior to each electrode modification, previous traces of copper species were removed from the electrode surface by soaking in hydrochloric acid (37% w/w) for several minutes. Successively, the electrode was polished with 0.05 μm α-alumina powder on a polishing microcloth, for improving the uniformity and adherence of the copper film, and washed with doubly distilled water. Films of copper oxide were deposited by potentiostatic conditions at 0.55 V vs. SCE or by voltage cycling (50 mV/s) between 0.1 and 0.65 V. All depositions were performed in 0.2M NaOH solutions that were not deaerated, containing 0.5 g/l of $KCu(CN)_2 + K_3Cu(CN)_4$.

The surface concentration of copper sites (Γ_{Cu}) was estimated by CV in alkaline medium (0.2 M NaOH) by integrating the cathodic wave between 0.15 and 0.65 V, originated by the $Cu^{III} \rightarrow Cu^{II}$ reduction process. Unless otherwise specified, a copper surface concentration of about 2.5÷4.0 $\mu g/cm^2$ was used.

3. Results and discussion

3.1. Voltammetry

Fig. 1 shows some cyclic voltammograms acquired during the growth of copper oxide on the glassy carbon surface under potential cycling between 0.1 and 0.65 V vs. SCE in 0.2 M NaOH solution containing 0.5 g/l of $KCu(CN)_2 + K_3Cu(CN)_4$. Due to the increase of deposited Cu material on the electrode surface, anodic and cathodic currents in the 0.20÷0.65 V potential range increase on repeated scanning. The wave visible in the anodic scan is due to the $Cu^{I} \rightarrow Cu^{II}$ redox couple. After 15–20 scans (i.e. when the thickness of the deposit is about 0.1 - 0.2 μm), the glassy carbon surface is covered by a yellowish, uniform and well adherent deposit. Similar results were obtained when the copper film was deposited on the platinum substrate electrode. Thus, the film deposition of copper oxide/hydroxide is easily accomplished in the same experimental conditions (i.e. composition of the electrolytic solution, pH and applied potentials) necessary for the copper mediator species performing their powerful catalytic activity towards the electro-oxidation of organic compounds.

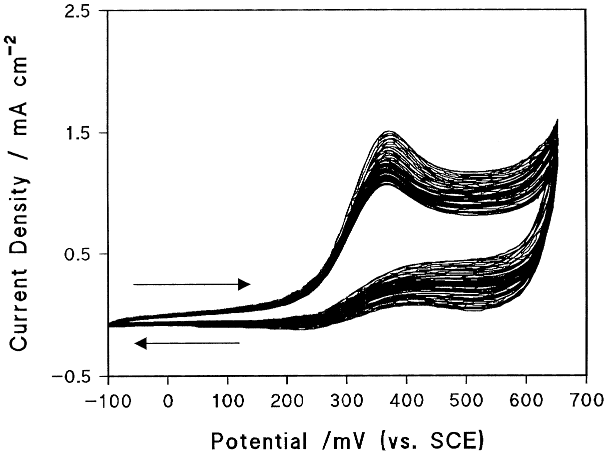

Fig. 1. Cyclic voltammograms recorded at a glassy carbon electrode (0.125 cm^2) in 0.2 M NaOH solution containing 0.5 g/l of $KCu(CN)_2 + K_3Cu(CN)_4$. Scan rate, 50 mV/s.

The dashed and continuous voltammetric profiles in Fig. 2, obtained at the Cu/GC electrode in a 0.2 M NaOH medium, correspond to the blank supporting electrolyte and, respectively, to 3.0 mM histamine. A large oxidation wave of histamine is visible at potentials higher than 0.45 V vs. SCE. The relevant anodic current, measured at 0.55 V, increased linearly on increasing histamine concentration up to 5 mM. The absence of any appreciable oxidation current in voltammograms obtained under the same experimental conditions, but at unmodified glassy carbon electrodes, confirmed that copper microparticles dispersed on the electrode surface act as catalytic sites of histamine electrooxidation. According to literature information (Marioli & Kuwana, 1992; Xie & Huber, 1991) the oxidation mechanism of polar aliphatic compounds (such as carbohydrates, amines, etc.) at copper-based electrodes involves, before the oxidation step, a weak association of the reacting molecule with CuO and CuOOH species present at the electrode surface. It is believed that active CuOOH species formed at high applied potentials (i.e. 0.45–0.6 V) have catalytic properties toward the oxidation of several electroactive compounds, and involve the transfer of oxygen from water to the oxidation products. The influence of copper loading on catalytic activity toward histamine oxidation was also examined by CV experiments. Fig. 3, obtained in 0.2 M NaOH

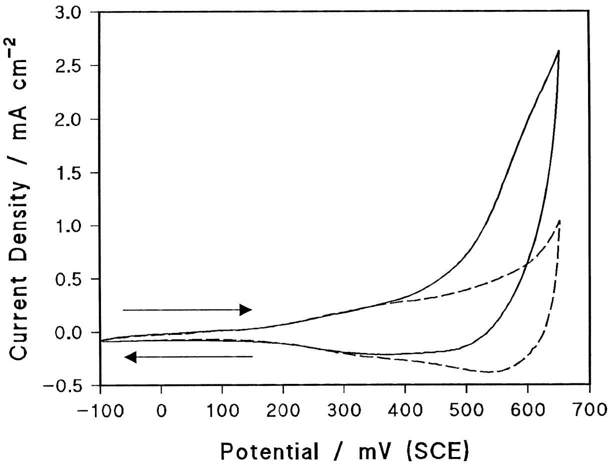

Fig. 2. Cyclic voltammogram (5th cycle) of 3.0 mM histamine in 0.2 M NaOH solution at the Cu/GC electrode (solid curve) and in blank 0.2 M NaOH (dashed curve). Scan rate, 50 mV/s.

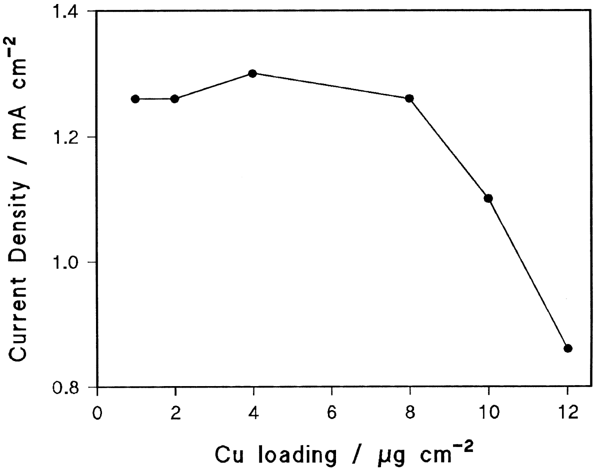

Fig. 3. Dependence of current density of 3.0 mM histamine on copper loading. Current densities are evaluated at 0.55 V vs. SCE during the forward scan (5th scan). Experimental conditions as in Fig. 2.

solution containing 3.0 mM histamine, shows the dependence of current density at 0.55 V on copper loading. As can be seen, the catalytic activity remains practically constant on changing the copper loading from 1 to 8 μg/cm^2 and decreases at a loading higher than 8 μg/cm^2. The reason for the decreased response for higher copper loading can be ascribed to an alteration of surface electrochemical properties (i.e. diminution of the conductivity of the film and/or formation of passive oxide species). Noticeably, the increase of copper loading from 1 to 8 μg/cm^2 produced only a 10–15% variation of the oxidation current. The limited effects of catalytic activity on copper loading variations is responsible for the appreciable reproducibility of electrode performance notwithstanding possible anodic corrosion and random variations of copper surface concentration in the course of working operations.

3.2. Amperometric flow injection and liquid chromatographic measurements

To optimize the applied potential, the hydrodynamic voltammogram for the oxidation of 0.5 mM histamine at the Cu/GC electrode was evaluated at 50 mV step in the 0.350–0.650 V potential range. The maximum current was observed at 0.55–0.60 V. At potential values higher than 0.60 V, competition by excess hydroxide ions and analyte for sites on the electrode surface apparently limited the currents for the histamine oxidation.

The effect of flow rate on detector response was also tested in FIA at 0.2 mL/min steps between 0.1 and 4.0 ml/min (applied potential: 0.55 V). Peak areas relevant to repetitive injections of 0.5 mM histamine sharply decreased on increasing the flow rate over the entire explored range, while peak heights remained practically constant from 0.5 ml/min to 1.8 ml/min. Depending on experimental needs, relatively low flow rates (i.e. 0.4–0.5 ml/min) should be used under chromatographic conditions in order to obtain lower detection limits and higher column efficiencies. Representative responses of 10.0 μM (a), 20.1 μM (b) and 41.0 μM (c) histamine solutions, obtained by using a 0.4 M NaOH mobile phase and applying 0.55 V at the Cu/GC electrode, are shown in Fig. 4. As can be seen, the fast peak current decay and the good reproducibility of the peak height indicate a rapid withdrawal of the reaction products from the electrode surface, under constant applied potential, and a fast restoring of the optimum working conditions at the electrode surface. The repeatability of the amperometric response, expressed as relative standard deviation (RSD) of peak height for a set of 30 consecutive injections of 0.1 mM histamine, was about 1.1%, indicating a good short-term stability of the Cu/GC electrode.

The catalytic response, evaluated in FIA, is relatively stable: a maximum 5.2% variation in electrode response was observed over 6 h of operation time.

On considering that copper-based electrodes can catalyse the electro-oxidation of organic molecules in strong alkaline solutions, and that a 7.5 μm, fully functionalised (with quaternary ammonium groups) macroporous resin is able to separate polyhydric aliphatic compounds

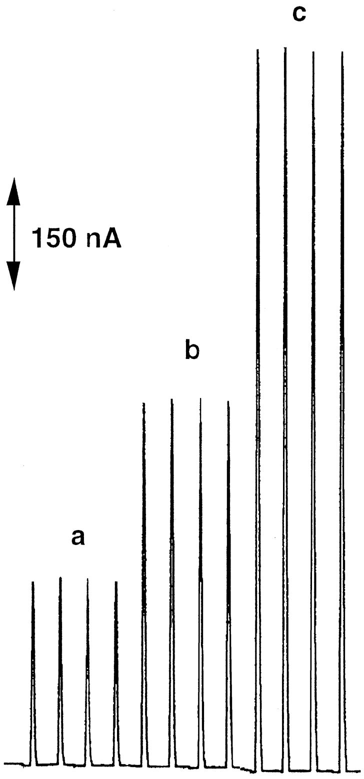

Fig. 4. Multiple flow-injection peaks of (a) 10.0 μM, (b) 20.1 μM and (c) 41.0 μM of histamine at a Cu/GC electrode. Applied potential: 0.55 V vs. Ag/AgCl; carrier, 0.4 M NaOH; flow rate, 1.0 ml/min; sample loop, 50 μl.

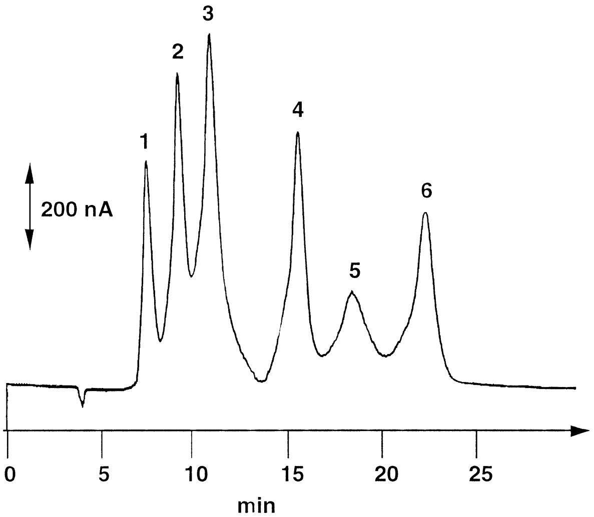

Fig. 5. Anion-exchange chromatogram with electrochemical detection of a mixed standard solution containing 0.125 mM of: (1) glycerol, (2) meso-erythritol, (3) xylitol, (4) adonitol, (5) histamine, (6) glucose. Flow Rate, 0.4 ml/min. Isocratic elution with 0.4 M NaOH. Other experimental conditions as in Fig. 4.

in alkaline media, analytical separations of histamine from other electroactive molecules were performed by using 0.4 M NaOH as the mobile phase with a Carbopac MA1 anion-exchange column. Fig. 5 shows a typical chromatogram relevant to a standard mixture of carbohydrates and hystamine. Unfortunately, other amines such as: cadaverine, spermine, spermidine, putrescine, histidine, methylamine, ethylamine and propylamine are co-eluted close to the solvent front (when using a 0.5 ml/min flow, they are eluted after about 5 min). Nevertheless, the presence of a relatively high concentration of other biogenic amines (about 1 mM) did not produce visible effects on the chromatographic peak of the histamine.

A 0.998 correlation coefficient was evaluated by analysing a series of 10 standard solutions in the 5–500 μM concentration range. The detection limit, evaluated as a signal-to-noise ratio of 3 at the lowest injected concentration, was 0.1 μM (corresponding at 5 pmol injected). At last the precision, expressed as percent relative standard deviation (RSD%) and evaluated over eight consecutive injections of 0.1 mM histamine samples (spanning over 2 h of operation time) was 6.4%.

3.3. Histamine determination in fish and red wine

The proposed analytical method was applied to the analysis of commercially available products such as canned tuna and red wine. The samples were chosen as examples of complex real matrices containing high concentrations of salts, proteins, carbohydrates, vitamins and other reducing compounds and because histamine has been proposed as an indicator of poor hygienic quality of the relevant raw materials and of poor manufacturing conditions.

The concentration of histamine was obtained by ordinary, linear, least-square regression by using the method of standard additions. Peak identification was based on the retention time of histamine and was confirmed by spiking the sample with known amounts of histamine. The peak purity of the histamine in the real samples, was checked by measuring the degree of peak asymmetry factor (Snyder, Glajch & Kirkland, 1988) as a function of the added histamine. Generally, asymmetry factor values of 0.98±0.05 were observed and these values are practically independent of the histamine amount added in the extracts solutions. The previous result represents a good indication of the purity of the peak assigned to histamine compound in the analysed samples. Recoveries were evaluated by spiking the extraction solutions with pure histamine solution at the level of 37 and 91% of the measured content for tuna and wine, respectively.

Representative chromatograms of unspiked samples of tuna and red wine are reported in Figs. 6 and 7, respectively. The relevant quantitative results are listed in Table 1. Notwithstanding the low pristine level of

Table 1
Analysis of histamine in canned tuna and red wine[a]

Sample	Added	Found	Recovery (%)	r^2
Tuna	–	51.2 mg/kg	–	0.998
	27.8 mg/kg	75.3 mg/kg	95.0	0.997
Red wine	–	3.9 mg/l	–	0.999
	27.8 mg/l	30.6 mg/l	96.5	0.998

[a] Column; Carbopac MA1; eluent, 0.4 M NaOH; flow rate; 0.5 ml/min; applied potential, 0.55 V vs. Ag/AgCl. Concentrations were evaluated by the standard additions method (four additions).

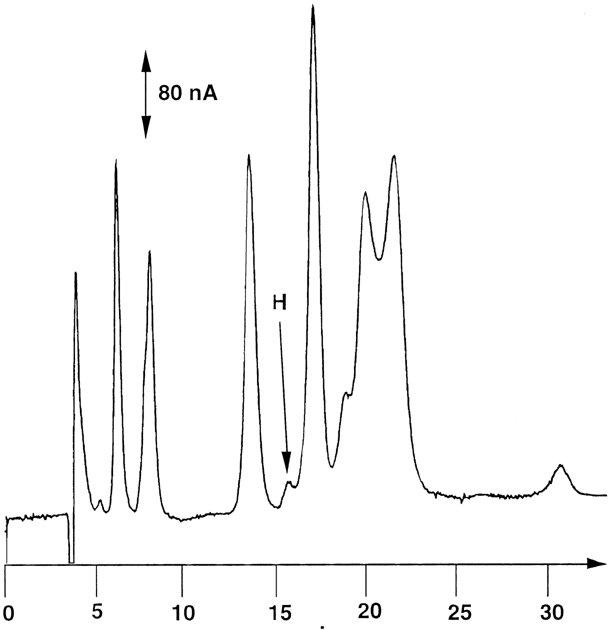

Fig. 6. Liquid chromatogram of unspiked canned tuna. (H) histamine. Column; Carbopac MA1 (250×4 mm I.D.);applied potential, 0.55 V vs. Ag/AgCl; flow rate, 0.5 ml/min. Other experimental conditions as in Fig. 5.

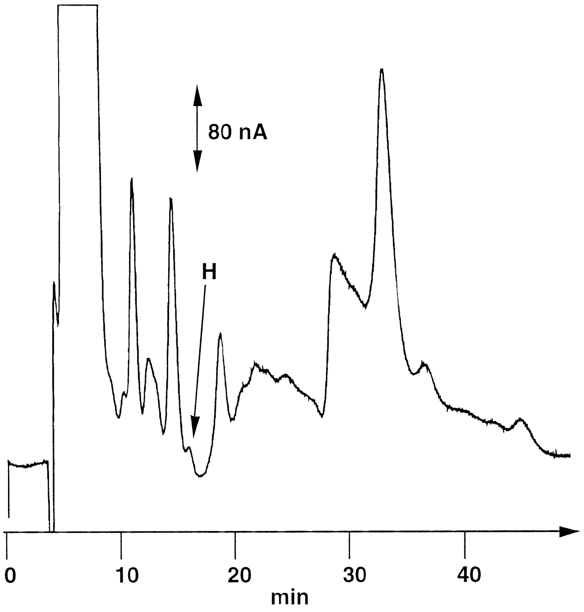

Fig. 7. Liquid chromatogram of unspiked red wine. (H) histamine. Other experimental conditions as in Fig. 6.

histamine in these samples, repetitive chromatographic injections led to an acceptable reproducibility without apparent deterioration of the catalytic activity. The good correlation coefficients ($\geqslant 0.997$) of the calibration curves likely suggest the absence of fouling effects on the catalytic performances of the Cu/GC electrode. In addition, the good mean recoveries (two replicates) of each spiked sample (i.e. 95 and 96.5% for tuna and wine, respectively) confirm the absence of interfering electroactive compounds and/or time-dependent variations of the electrode activity.

4. Conclusions

A Cu/GC modified electrode, obtained by anodic deposition of cuprous cyanide ions in alkaline medium and coupled with an anion-exchange chromatography, was developed for the quantitative analysis of histamine. Electrochemical detection at a constant applied potential of 0.55 V vs. Ag/AgCl allowed the rapid and direct determination of traces of histamine in real matrices at pmol level without any tedious and time-consuming pre- or post-column chemical derivatisation. From the aforementioned findings, the proposed method appears characterised by appreciable analytical and practical performances (recovery, precision, sensitivity and simplicity). Work is in progress to evaluate the applicability of the proposed method to other real samples such as cheese, beer and biological samples.

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