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Determination of copper with 5,5-dimethylcyclohexane-1,2,3-trione 1,2-dioxime 3-thiosemicarbazone in olive oils by adsorptive stripping square wave voltammetry

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Abstract

In the present paper a method for the determination of Cu in olive oil samples by adsorptive stripping square wave voltammetry (Ad-SSWV) is presented. It has been proven that Cu reacts with 5,5-dimethylcyclohexane-1,2,3-trione 1,2-dioxime 3-thiosemicarbazone, DCDT, in strongly acid media giving rise to a complex. In Ad-SSWV the complex Cu–DCDT experiments an adsorptive reductive process which promotes the appearance of a peak at -0.570 V. The extraction process of Cu from olive oil is carried out with hot concentrated HCl. Calibration graph has been constructed from 0 to 35 ng mL^{-1} and the detection limit was 0.49 ng mL^{-1}. The method has been applied to commercial olive oils samples and the amounts of Cu found are very similar to those obtained when samples are analysed by AAS.

Keywords: Copper; Olive oil; Stripping voltammetry

1. Introduction

There are many factors that influence in the obtaining of quality olive oils. Especially it is very important that olive fruits are healthy and at the right stage of maturity. However, harvesting process, transportation and storage of the olive fruits as well as extraction process and storage of the olive oil are other factors that affect olive oil quality. During some of those practices, transition metals, such as iron and copper mainly, could reach olive oil. These metals may come from the soils and fertilizers, or from materials used in the processing and storage of the olive oil. Transition metals are promoters of the oxidation processes because they can generate free radicals.

In the oxidation process unsaturated fatty acids are oxidised giving rise to hydroperoxides. These compounds can also react producing secondary oxidation products such as hydrocarbons, esters, ethers, aldehydes and ketones and these substances give to the olive oil rancid flavor. This degradation process of the olive oil is a natural and irreversible process although it is possible to retard it by taking a set of precautions. Thus, it is important to determine the presence of metals in olive oil with the aim of knowing if they may be the cause of the deterioration, to find the origin of the contamination and to avoid this.

In the literature, they have been found many procedures reported for the determination of copper in olive oil. Most of them are developed in atomic absorption spectrometry and a pre-treatment of the olive oil samples, such as an extraction process in aqueous acidic solutions (De Leonardis, Macciola, & de Felice, 2000; Saleh, Jab, Rahman, & Norasiah, 1991) or the ashing (Garrido, Frias, Diaz, & Hardisson, 1994; Saleh, Murray, & Chin, 1988), is required. In some methods, sample is just diluted in a suitable solvent (Martin-Polvillo,

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Albi, & Guinda, 1994). Other techniques frequently used are ICP AES (Murillo et al., 1999; Fischer & Rademeyer, 1994) and spectrophotometry (Hussain Reddy, Prasad, & Sreenivasulu Reddy, 2003). They are scarce references related to voltammetric (http://www.metrohm.com) and potentiometric techniques (LoCoco, Monotti, Rizzotti, & Ceccon, 2000).

The development of a stripping square wave voltammetric method for the determination of copper in olive oil samples is described in this paper. The metal is extracted from olive oil samples by using HCl (http://www.metrohm.com; Lo Coco et al., 2000). Afterwards copper reacts with the DCDT reagent giving rise to a complex Cu–DCDT. This complex is reduced at the surface of the mercury electrode after being adsorbed on it.

5,5-Dimethylcyclohexane-1,2,3-trione 1,2-dioxime 3-thiosemicarbazone, DCDT, is a compound which belongs to the thiosemicarbazones group and also it possesses two oxime groups as complexing groups. DCDT reacts in acid medium with some inorganics to form complexes (Salinas, Jiménez Sánchez, & Galeano Díaz, 1986), being copper ion between them. In previous papers, it is described the use of the DCDT reagent to determine iron in wines, vegetables, and minerals (Salinas et al., 1986), and in acids (Lemus Gallego, Galeano Díaz, & Jiménez Sánchez, 1988) by spectrophotometry, and also in acids, waters, fruit juices and wines by polarography (Vázquez Díaz, Jiménez Sánchez, Callejón Monchón, & Guiraum Pérez, 1994) and in olive oil by spectrophotometry and voltammetry (Galeano Díaz, Guiberteau, López Soto, & Ortiz, 2003).

2. Experimental

2.1. Apparatus

The Crison basic 20 pH-meter had a combined SCE-glass electrode

An Autolab AUT 12.v PSTAT10 (Ecochemie, The Netherlands) was used in combination with a Metrohm VA-663 polarographic stand, with a three electrode system with hanging mercury drop electrode as working electrode, an Ag/AgCl saturated KCl reference electrode and a Pt wire auxiliary electrode. The system is monitored by the General Purpose Electrochemical System (GPES4) version 4.0, software package (Ecochemie, Ultrecht, The Netherlands). Golden Software (Grapher, v. 1.32) (Golden, CO) was used for the transformation of initial signals.

2.2. Reagents

To obtain DCDT, 5,5-dimethyl-1,2,3-cyclohexanetrione 1,2-dioxime was previously obtained by the Haas's procedure (Haas, 1907), modified as described later (Salinas et al., 1986). Two grams of powdered 5,5-dimethyl-1,3-cyclohexanedione was dissolved in a solution of 10 g of potassium hydroxide in 30 mL of water, 30 mL of ethanol was then added and, while the solution was carefully cooled and stirred, 1.2 g of potassium nitrite was added, neutralizing later the solution with concentrated HCl until slight excess. Maintaining cold the solution, a solution of 1.0 g of hydroxylamine hydrochloride in water, and sodium acetate until reach a pH value near 5, were added. The white precipitate obtained was well washed with water and dried. To synthesize the DCDT, a solution of 1.24 g of thiosemicarbacide, in 50 mL of water plus 100 mL of ethanol, were added to a solution of 5,5-dimethyl-1,2,3-cyclohexanetrione 1,2-dioxime in ethanol. After 3 or 4 days the solvent was removed at 30 °C in a rota-evaporator, separating a yellow solid.

Stock standard Cu(II) solution (1000 mg L^{-1}) was from Panreac (Barcelona, Spain). Organometallic IPC-XRF standard copper (5000 μg g^{-1}) was from Accu-Standard, Inc (New Haven, CT). All subsequent copper solutions were prepared by dilution.

All other chemical were of analytical-reagent grade or better.

2.3. Recommended procedure for determination of copper in olive oil by Ad-SSWV

A suitable amount of olive oil (~30 g) sample containing between 3.2 and 224 ng g^{-1} of copper and 10 mL of concentrated HCl are introduced into a baker. The mixture is heated at 50 °C approximately and shaken on a magnetic heater plate until a fine emulsion is formed and it becomes boiling. This process takes about 5 min. Then the emulsion is transferred into an extraction flask and allowed to separate in two phases, the acidic layer being collected in a 25 mL volumetric flask. The organic layer is treated again as it has been described above and the extracts are joined. Five milliliters are taken from the obtained extracts and transferred into a 50 mL volumetric flask, diluting to the mark. The solution is passed through an Supelclean ENVI-Carb SPE Tubes activated carbon cartridge, 0.5 g, 6 mL (Supelco, Bellefonte, PA, USA) to eliminate the organic compounds that have been extracted from the olive oil together with copper and which interfere in the determination of the metal. The first 3 or 4 mL of the eluate are eliminated and 25 mL of the solution are collected into a 25 mL volumetric flask. 0.4 mL of 0.05% (1.95×10^{-4} M) DCDT in ethanol are added to the filtered solution. The sample is stirred while accumulation takes place. After the accumulation period, the stirring is stopped and, after 15 s of equilibrating time, the voltammogram of the sample is recorded. The instrumental conditions are as follow: $t_{ac} = 60$ s, $E_{ac} = E_i = -0.350$ V, frequency = 200 Hz, step potential =

5 mV, amplitude = 60 mV, stirring rate = 500 rpm. From the analytical signal, peak current (I_p) the concentration is calculated by using a suitably prepared calibration graph.

3. Results and discussion

In preliminary studies, sampled DC, DPV, CV and SWV techniques have been used to examine the electrochemical behavior of strongly acidic solutions of copper and DCDT at the surface of the HMDE electrode. A solution of 500 ng mL^{-1} of Cu, 7.86×10^{-5} M DCDT, 2.4% ethanol, and 1 M HCl, was prepared into a 25 mL volumetric flask. The voltammogram of the solution was registered in DPV, CV and SWV. Since sampled DC technique is less sensitive than the other techniques, it was registered the DC voltammogram of a solution containing 3.0 mg mL^{-1} of Cu and 4.72×10^{-4} M DCDT, 2.4% ethanol, and 1 M HCl. On the other hand, voltammograms of a solution of DCDT reagent were registered in the identical conditions to those of the Cu–DCDT solutions. Looking at Figs. 1–4, it is observed that DCDT reagent originates a cathodic wave very close to that due to Cu–DCDT complex. With the aim of determining whether the second wave is really originated by Cu–DCDT complex, voltammograms of a solution of 7.86×10^{-5} M DCDT with different concentrations of Cu have been registered in CV (Fig. 5). Fig. 5 shows that the second wave increased as the Cu increased and thus this wave must be produced by the reduction of the complex. Therefore, the presence of the first wave is due to the reagent.

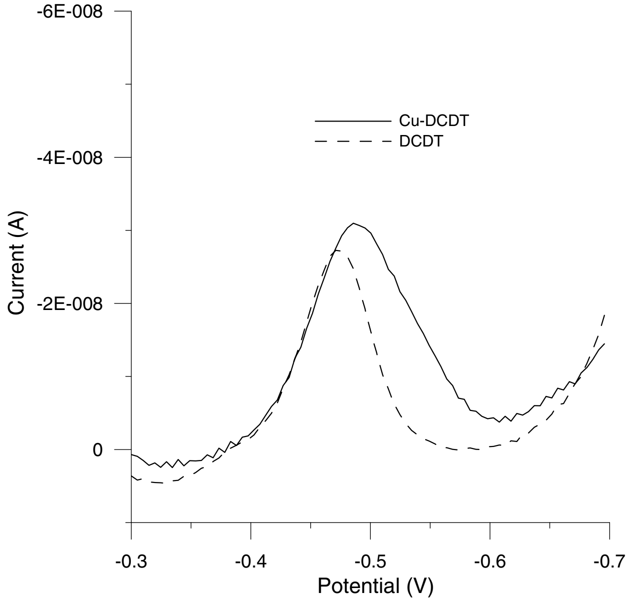

Fig. 1. Differential pulse voltammogram of Cu–DCDT (500 ng mL^{-1} of Cu, 7.86×10^{-5} M DCDT, 2.4% ethanol, and 1 M HCl).

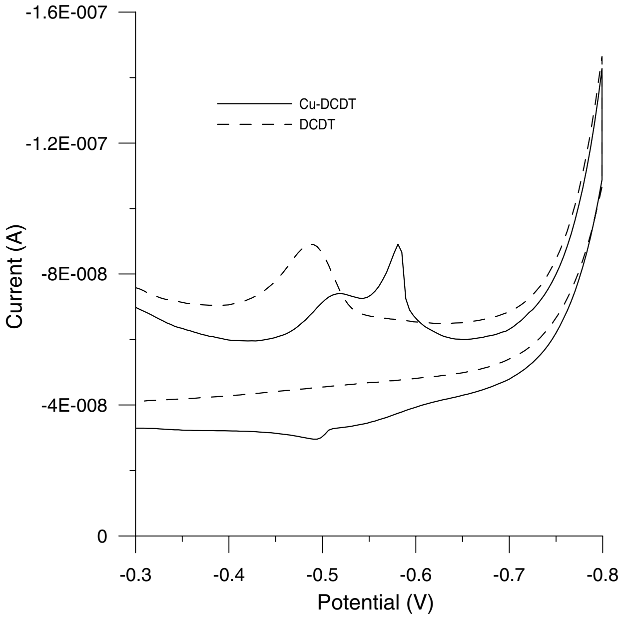

Fig. 2. Cyclic voltammogram of Cu–DCDT (500 ng mL^{-1} of Cu, 7.86×10^{-5} M DCDT, 2.4% ethanol, and 1 M HCl).

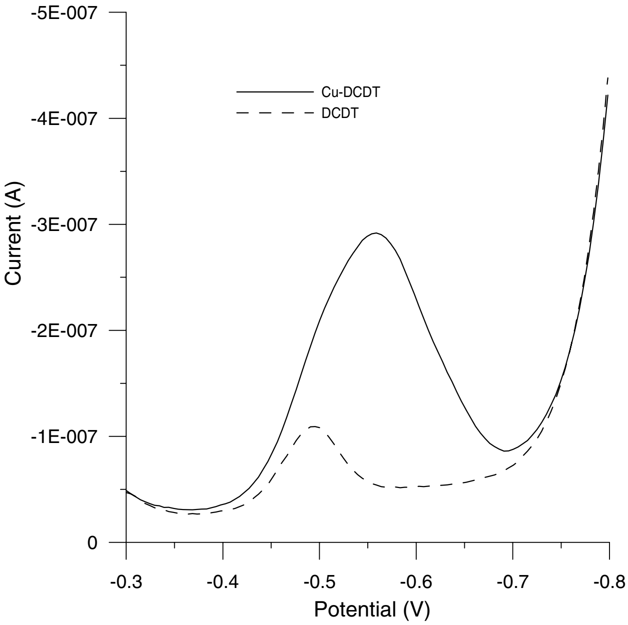

Fig. 3. Square wave voltammogram of Cu–DCDT (500 ng mL^{-1} of Cu, 7.86×10^{-5} M DCDT, 2.4% ethanol, and 1 M HCl).

From these studies, we can say that Cu–DCDT complex is reduced irreversibly on the electrode, producing a cathodic wave as it is shown in the cyclic voltammogram (Fig. 2). The non-reversibility of the process has been also confirmed by applying the Birke‘s criterium. On the other hand, it has been recorded the cyclic voltammograms of the Cu–DCDT complex with different scan rates (from 20 to 200 mV s^{-1}) but because of the proximity of the two waves it has not been possible to

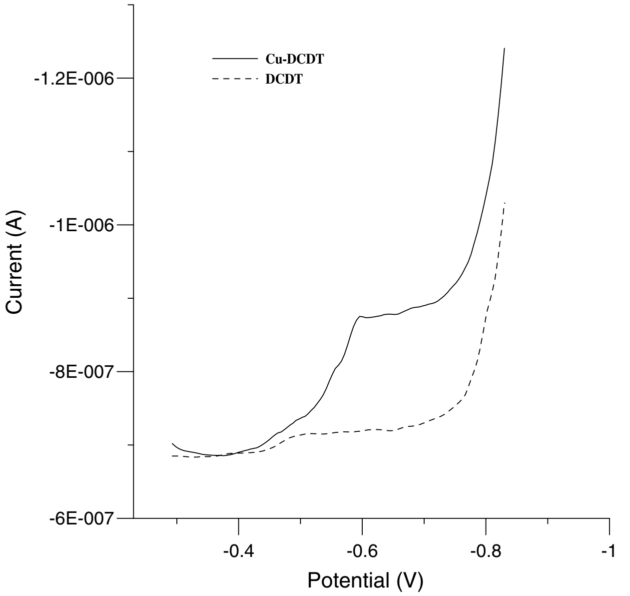

Fig. 4. Sampled DC voltammogram of Cu–DCDT (3.0 μg mL^{-1} of Cu, and 4.72×10^{-4} M DCDT, 2.4% ethanol, and 1 M HCl).

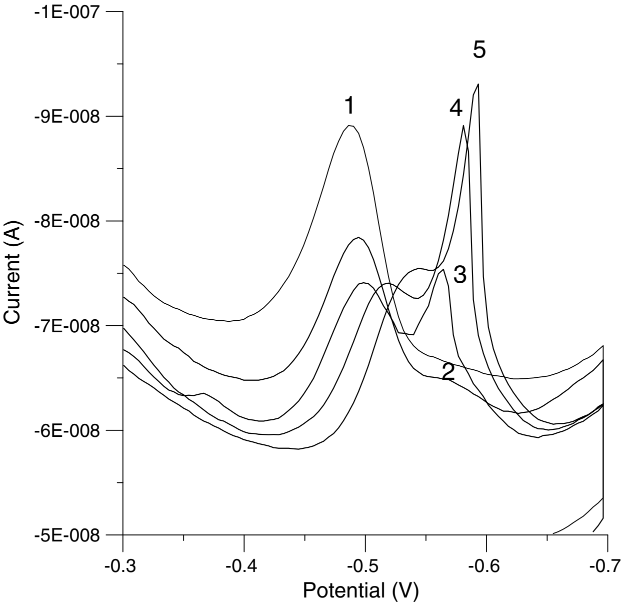

Fig. 5. Cyclic voltammograms of Cu–DCDT with different Cu concentrations and 7.86×10^{-5} M DCDT, 2.4% ethanol, and 1 M HCl (1, DCDT; 2, 300 ng mL^{-1} Cu; 3, 500 ng mL^{-1} Cu; 4, 700 ng mL^{-1} Cu; 5, 1000 ng mL^{-1} Cu).

measure accurately the Cu–DCDT peak intensities. However, taking into account that the shape of the wave is acute and symmetrical, it is deduced that this process is due to adsorption.

We have studied the influence of chemical and instrumental variables over the peak current (I_p) in Ad-SSWV, for the complex Cu–DCDT, selecting 20 ng mL^{-1} of copper, since preliminary studies showed that the shape of the reduction peak changed when the amount of adsorbed product was higher than a certain value (i.e., when the concentration of copper or DCDT or the accumulation time increased excessively).

3.1. Influence of HCl concentration

The hydrochloric acid concentration was varied between 0.2 and 2 M HCl. Peak current of the complex showed maximum values between 0.5 and 1 M HCl and the peak potential (E_p) displaced towards more negative potentials as the HCl concentration increased. From this study the HCl concentration of 1 M was selected as the most suitable for the determination of copper by Ad-SSWV.

3.2. Influence of DCDT and ethanol concentration

The DCDT concentration was varied from 1.0×10^{-4} to 1.6×10^{-4} g L^{-1}and it was found that the peak current remained constant with concentrations of reagent above 7.5 fold molar excess. A 8×10^{-4} g L^{-1} concentration of DCDT (0.4 mL of 0.05 g L^{-1} solution in ethanol in 25 mL volumetric flasks) was selected for subsequent experiments.

Taking into account that the presence of ethanol in the samples is necessary as it is the solvent used to prepare the solutions of DCDT, different concentrations of ethanol ranged from 1.6% to 9.6% were also tested and it was found that peak current did not vary over this range of ethanol concentration. A 1.6% of ethanol was used thereafter.

3.3. Effect of instrumental variables

With regard to the instrumental variables, the relationship between peak current and accumulation time (t_{ac}) was studied for solutions of 20 and 50 ng mL^{-1} of copper, and t_{ac} was varied from 20 to 200 s. It was found that for 20 ng mL^{-1} there is linearity up to 160 s (Fig. 6). The peak shape changes when the t_{ac} increases above this value. For 50 ng mL^{-1} the linearity is only up to 120 s.

The accumulation potential (E_{ac}) was varied from 0 to −0.500 V. The maximum values of peak current were obtained between −0.300 and −0.450 V. For subsequent experiments −0.350 V was selected.

A linear increase of peak current of the complex was observed when frequency was varied from 25 to 200 Hz. Two hundred Hz was adopted as optimum. Peak current increased linearly with variations of step potential from 2 to 10 mV. However, this increment of the I_p was accompanied with a loss of the peak definition, therefore 5 mV was chosen to carry out subsequent experiments because it offered good values of peak current and good peak definition. Regarding to the influence of the square wave amplitude, the maximum

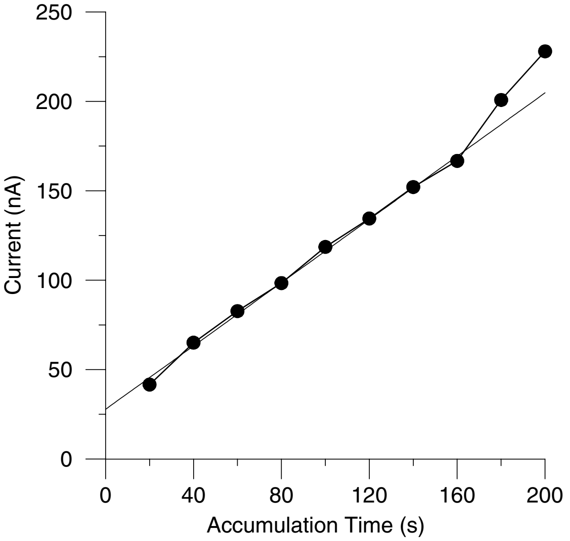

Fig. 6. Influence of the accumulation time on the Cu–DCDT peak current (20 ng mL^{-1} of Cu, 4×10^{-5}% DCDT, 0.8% ethanol and 1-M HCl). E_{ac} = 0 V; frequency = 50 Hz; step potential = 5 mV; amplitude = 50 mV.

value for I_p is produced at 60 mV when amplitude was increased from 20 to 100 mV.

3.4. Calibration and precision

A study of the influence of copper concentration was performed in aqueous solutions, under the optimal conditions mentioned, and by using different accumulation times. It is important to remark that DCDT reagent produces a signal at a potential very close to that of the Cu–DCDT complex, and the voltammograms of the complex have been obtained by subtracting the corresponding blank signal. The stripping current of Cu–DCDT complex after being preconcentrated during 120 s yielded a linear dependence in a concentration range 0–15 ng mL^{-1} of copper. For 100 s, peak current increased linearly with copper concentration from 0 to 20 ng mL^{-1}. Finally for 60 s the response is linear from 0 to 35 ng mL^{-1}. To construct the calibration plot we decided to use 60 s as accumulation time. This time allowed us the copper determination in a wider the range of concentration and we ensured that the electrode is not going to be saturated and that the nature of the process is not going to change.

On the other hand a calibration plot was also constructed with olive oil samples (free of copper) fortified with organometallic copper standard, applying the proposed method for the determination of copper that we described above. Voltammograms of these samples are shown in Fig. 7. The equations of the calibration plots were $I_p = 31.22\,C + 103.9$ in aqueous solutions and $I_p = 28.95\,C + 146.9$ in olive oil extracts (I_p = peak current in nA; C = copper concentration in ng mL^{-1}, independent term = current peak for a blank of DCDT or for a blank of DCDT and olive oil free of copper).

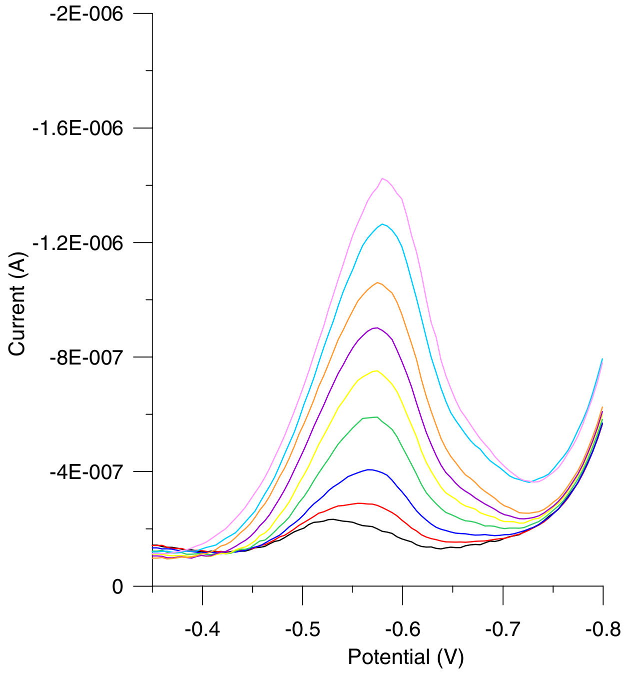

Fig. 7. Ad-SSWV voltammograms of Cu–DCDT obtained for Cu concentrations between 0 to 35 ng mL^{-1} (8×10^{-5}%DCDT, 1.6% ethanol and 1-M HCl). E_{ac} = −0.350 V; t_{ac} = 60 s; frequency = 200 Hz; step potential = 5 mV; amplitude = 60 mV.

Slopes of both plots were compared and no significant differences were observed. Taking into account that there are no differences between results obtained from aqueous solutions and from olive oil extracts, determination of copper in olive oil was carried out by the external standards method. The correlation coefficient of the calibration plot was 0.9991 and detection limit, calculated by the method of Winefordner y Long (Long & Winefordner, 1983) was 0.49 ng mL^{-1}. The precision was determined by analysing 8 samples of olive oil by the proposed method. The average concentrations was 20.88 ng g^{-1} and the relative standard deviation was 9.53%.

On the other hand, several metals such as iron, copper, chromium, manganese or lead, have been reported to be present in olive oil samples, but iron is the most abundant one (between 0.5 and 3 μg g^{-1}). Therefore, we have studied the effect that this metal produces over the copper determination. To detect the possible interference from iron, samples of 10 ng mL^{-1} of Cu and with different amounts of iron were prepared. Ratio (w/w) Cu:Fe was varied from 1:1 to 1:100. The voltammetric signal obtained for Cu was compared with the corresponding to a sample of this ion, without iron. Signals was considered affected by the iron if the difference was 5% at least, and it was found that Fe did not interfere in the concentration range assayed.

Table 1
Determination of copper in olive oil samples

Olive oil sample	Cooper found (ng g^{-1}) ± standard deviation	
	AAS	Ad-SSWV
1	20.33 ± 0.86	19.44 ± 0.37
2	27.33 ± 3.22	29.56 ± 3.92
3	8.58 ± 0.39	8.74 ± 1.51
4	8.07 ± 0.55	8.99 ± 0.84
5	4.76 ± 0.25	4.66 ± 0.28

We have performed the determination of copper in five different olive oils, following the method we propose in this paper. On the other hand olive oil samples were also analysed by atomic absorption spectrophotometry (De Leonardis et al., 2000).

Results obtained from both methods have been compared (Table 1) and it can be appreciated that there is not significant differences between them.

In conclusion, the method of copper determination by Ad-SSWV that we propose in this paper is a very low-cost method because reagents and technique are not expensive. Other advantage is that the time required to carry out the analysis is shorter than in the other polarographic methods in which a determination of cooper in edible olive oils is made (http://www.metrohm.com; Lo Coco et al., 2000). We can also point out that with this method it is possible to determine very low quantities of copper because detection limit is 0.49 ng mL^{-1}.

Besides, DCDT reacts in acid medium with the two main metallic ions of olive oils, copper and iron, and therefore its use can be considered an interesting alternative to other methods used for these samples. It must be pointed out that the extraction of ions from the matrix, using a strong acid medium is compatible with the later used voltammetric procedure. On the other hand, conditions used for iron and copper are not the same and both ions can be determined in the proportions usually found in olive oils, without interference between them. The main drawback of the method is that reagent is not commercially available. However, the synthesis procedure is not very difficult, being the control of the temperature in each one of the stages the more decisive aspect to get good recovery. Even when this step is time-consuming, it could be easily adapted to a greater scale, avoiding this way to frequently make it.

Explaining legend

Ad-SSWV	adsorptive stripping square wave voltammetry
AAS	atomic absorption spectrometry
ICP AES	inductively coupled plasma-atomic emission spectrometry
t_{ac}	accumulation time
E_{ac}	accumulation potential
E_i	initial potential
sampled DC	sampled direct current
DPV	differential pulse voltammetry
CV	cyclic voltammetry
SWV	square wave voltammetry

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