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Development of an analytical method for the determination of *tert*-butylhydroquinone in soybean biodiesel

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HIGHLIGHTS

- Analysis of the antioxidant TBHQ in soybean biodiesel by voltammetry.
- The samples were submitted to liquid–liquid extraction using acetonitrile and ethanol.
- The extracts with TBHQ were analysed by voltammetry and HPLC–UV.
- The method can be an alternative for the quality control in soybean biodiesel samples.

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ABSTRACT

In this paper we present a novel method for the analysis of the antioxidant *tert*-butylhydroquinone (TBHQ) in soybean biodiesel by differential pulse voltammetry (DPV) on a glassy carbon (GC) electrode. This method was developed by submitting biodiesel samples to liquid–liquid extraction using two different extraction solvents: acetonitrile (ACN) and ethanol (EtOH). The effects of type and volume of solvent, supporting electrolyte and pH were investigated and optimised. The parameters of the DPV technique were optimised and analytical curves were obtained by the standard addition method. Under optimum conditions, this method shows good linear response with correlation coefficient (r) values above 0.99. The limits of detection (LOD) and quantification (LOQ) were below 2.00 mg L^{-1}. The percentages of recovery of TBHQ when added to biodiesel were 96.7–100.8% for both extraction solvents, with relative standard deviation (RSD) lower than 3%. This method was applied to commercial samples of soybean biodiesel containing 877.00 ppm TBHQ, with recovery of 110.1% and 99.4% achieved when ACN and EtOH were used as the extraction solvents, respectively. These results were similar to those obtained using high-performance liquid chromatography with ultraviolet detection (HPLC–UV), demonstrating the applicability of this method for the determination of TBHQ in soybean biodiesel samples.

1. Introduction

Biodiesel is a renewable and biodegradable fuel that consists of long-chains of mono-alkyl esters of fatty acids obtained from the transesterification of vegetable oils or animal fats. Biodiesel has certain advantages over petroleum-based fuels, such as being virtually free of sulphur and aromatics, high cetane number, average content of oxygen, a higher flash point and lower particulate emissions of HC, CO and CO_2 [1–6]. However, despite its advantages, biodiesel is more susceptible to auto-oxidation than fossil fuels [7,8]. Exposure to heat, light, humidity, oxygen, and metal contaminants may accelerate oxidation processes which can play an important role in the formation of undesired compounds, and corrode engines or clog their filters and injection systems [7–10]. Oxidative stability is therefore a quality parameter to be evaluated in biodiesel, according to the standard methods EN 14114 and ANP 05/2012 [10–12].

The addition of antioxidants is one way to increase the resistance of biodiesel to oxidation, thereby allowing a longer storage time [5,9]. Several inhibitors are reported in the literature; however *tert*-butylhydroquinone (TBHQ) has shown the best results in the preservation of biodiesel samples [2,9,11,13]. Although the current quality control specifications for biodiesel do not require the determination of TBHQ, the quality control requirements for this fuel may evolve to include the monitoring of compulsory additives and other added substances. Therefore, it is necessary that the scientific community demonstrates the feasibility of measuring such additives.

A variety of analytical methods for determining TBHQ, generally used in oil and food samples, have been reported to date. Among

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these are UV spectrophotometry [14], high-performance liquid chromatography (HPLC) combined with different detection systems [15–21], gas chromatography [22–25], capillary electrophoresis [26], micellar electrokinetic capillary chromatography with electrochemical detection [27] and Fourier transform infrared (FTIR) spectroscopy [28]. However, these methods have negative aspects such as expense, complicated and lengthy procedures, and inappropriateness for field use.

Electrochemical techniques, such as voltammetric-based methods represent an alternative, because they combine the advantages of low operating cost, speed, simplicity and sensitivity [29]. Various voltammetric techniques have been applied for the determination of TBHQ in different samples [30–35], but there are few studies on the analysis of TBHQ in biodiesel samples [34,35]. Furthermore, these studies quantify the TBHQ in biodiesel samples without pretreatment of the sample and are based on high fortification of TBHQ with determinations made by diluting the sample. However, the concentrations of TBHQ added to biodiesel in the industry to preserve it against oxidation processes are of the order ppm hundreds of the antioxidant.

This paper describes a novel method for the determination of TBHQ in soybean biodiesel using liquid–liquid extraction and detection by DPV. The suitability of this method was demonstrated by the quantifying TBHQ in commercial samples of biodiesel and comparing the results with those of HPLC.

2. Experimental methods

2.1. Chemicals and solutions

All reagents were of analytical grade and employed without further purification. The standard stock solutions of TBHQ (97.0%, Sigma) were prepared in ACN (Merck).

The supporting electrolyte solutions were aqueous ACN (30% ACN, v/v) 10.0 mmol L^{-1} KNO_3 (Dinâmica), and aqueous EtOH (Vetec) (30% EtOH v/v) 10.0 mmol L^{-1} KNO_3, both pH_{cond} 1.5 adjusted with 1.0 mol L^{-1} HNO_3 (J.T. Baker®). HPLC grade ACN (J.T. Baker®) was used in the preparation of the mobile phase for HPLC analyses. All solutions were prepared using ultra-purified water (18.2 MΩ cm) supplied by a Milli-Q purification system.

2.2. Instrumentation

DPV experiments were performed using an Autolab PGSTAT 302 Potentiostat/Galvanostat (Eco Chemie, The Netherlands) operating with data processing software (GPES, software version 4.9.006, Eco Chemie). All experiments were carried out using a conventional three-electrode cell, using a GC electrode (0.07 cm^2) and a boron-doped diamond (BDD) electrode (~0.69 cm^2) as the working electrodes (WE). An auxiliary electrode of platinum (1.0 cm^2) and an Ag/AgCl (3.0 mol L^{-1} KCl) reference electrode were also used. Subsequent to each scan, the GC electrode was polished with felt moistened with a 0.3 μm alumina suspension followed by rinsing in ultra-pure water. The BDD electrode was subjected to a surface treatment (cathodic polarisation) to activate the diamond surface. The cathodic polarisation treatment consisted of applying −3.0 V vs. Ag/AgCl (3.0 mol L^{-1} KCl) for 2 min in an acid medium (H_2SO_4 0.5 mol L^{-1}) [29].

All electrochemical measurements were carried out in the presence of dissolved oxygen and at room temperature.

The HPLC analyses were performed on a chromatographic system with a pump model Series 200 Pump (Perkin Elmer), equipped with a 20 μL Rheodyne injector, a UV–Vis 50 Scan detector (Varian) set at 292 nm and CarywinUV Software. A Luna C18 (2) 100 mm (250 mm × 4.6 mm, 5 μm) (Phenomenex) column was used. The mobile phase was aqueous ACN (70%, v/v) at a flow rate of 1.0 mL min^{-1} at room temperature.

A vortex mixer, model AP56 (Phoenix), an ultrasonic bath, model Ultra Cleaner 1400 (Unique), a centrifuge (model TDL80-2B (Centribio) and an analytical balance, model AX200 (Shimadzu) were used for sample preparation. The pH measurements were performed on a Metrohm 827 pH meter.

2.3. Sample preparation

Samples of additive-free soybean biodiesel (Usina Barrálcool S/A, Mato Grosso, Brazil) were spiked with TBHQ to a final concentration of 250, 500 and 1000 mg L^{-1} (250, 500 and 1000 ppm). One commercial soybean biodiesel spiked with TBHQ was analysed. All samples were kept refrigerated before use. The TBHQ determination in biodiesel samples by DPV was based on the liquid–liquid extraction methods proposed by Hao et al. [21], Medeiros et al. [29], and Raymundo et al. [33], with modifications.

2.3.1. TBHQ extraction

A 200.0 μL aliquot of soybean biodiesel spiked with TBHQ was added to a centrifuge tube. Then 2.0 mL of ACN was added and the mixture was shaken for 2 min using the vortex mixer. After shaking, 1.0 mL of supporting electrolyte (30% ACN, v/v, 10.0 mmol L^{-1} KNO_3, $pH_{cond.}$ 1.5) was added to the mixture and the mixture was then stirred for 2 min. The sample was placed in an ultrasonic bath for 2 min, and was then centrifuged at 4000 rpm for 5 min. The extraction procedure was repeated once and both extracts were collected. The same extraction procedure was performed on antioxidant-free biodiesel, which was used as a control. When EtOH was employed as the extraction solvent 4.0 mL of the electrolyte was required to promote phase separation of the biodiesel-aqueous EtOH mixture.

2.4. Voltammetric and chromatographic analysis

An aliquot of the sample extract was transferred to the electrochemical cell, then diluted to 10.0 mL with the supporting electrolyte. DPV analysis was performed under previously optimised conditions, and quantification of TBHQ was made using the standard addition method. All experiments were performed in triplicate.

For the chromatographic analysis, following the extraction procedures, extracts with a concentration above 10.0 mg L^{-1} were diluted in ACN before measurements were performed. A 20 μL aliquot was injected into the HPLC system under the operating conditions described in Section 2.2. Quantification was based on a calibration curve constructed by plotting the measured peak areas versus concentration.

3. Results and discussion

3.1. Investigation of the electrochemical behaviour of TBHQ

Cyclic voltammetry was initially used to investigate the electrochemical behaviour of 15.0 mg L^{-1} TBHQ at the GC and BDD electrode surfaces, and in various supporting electrolytes: EtOH with 0.1 mol L^{-1} $LiClO_4$; 0.1 mol L^{-1} Britton–Robinson buffer, pH 1.5; 0.1 mol L^{-1} Britton-Robinson buffer (30% EtOH, v/v), pH_{cond}1.5; 10.0 mmol L^{-1} KNO_3 solution (30% methanol, v/v), pH_{cond} 1.5; 10.0 mmol L^{-1} KNO_3 solution (30% ACN, v/v), pH_{cond} 1.5; and 10.0 mmol L^{-1} KNO_3 solution (30% EtOH, v/v), pH_{cond} 1.5. The best results were obtained with solutions of 10.0 mmol L^{-1} KNO_3 (30% ACN, v/v) and (30% EtOH, v/v), pH_{cond} 1.5 adjusted with 1.0 mol L^{-1} HNO_3 [29].

Cyclic voltammograms for TBHQ recorded for both supporting electrolytes at the GC and BDD electrodes are shown in Fig. 1.

Cyclic voltammograms using the GC electrode showed well-defined oxidation and reduction peaks in both electrolytes (Fig. 1a and c). For the electrolyte in ACN the anodic peak potential (Epa) and cathodic peak potential (Epc) corresponded to +0.44 V and +0.11 V, respectively. For the electrolyte in EtOH the Epa was +0.53 V and the Epc was +0.04 V. The ΔEp for both electrolytes was greater than 300 mV, however, the oxidation process is considered reversible in other media described in the literature [36]. The reversibility of the oxidation process involves two electrons and two protons, based on the oxidation of TBHQ to its corresponding quinone [36,37].

In the cyclic voltammograms using the BDD electrode were only observed oxidation peaks in +0.81 V and +0.65 V for electrolytes in ACN and EtOH respectively. In this electrode the peak potentials shifted to more positive potentials compared with the GC electrode.

3.2. Influence of the WE

Differential pulse voltammograms for TBHQ were recorded in the optimised conditions in the supporting electrolytes KNO_3 (30% ACN, v/v) and KNO_3 (EtOH 30% v/v), pH_{cond} 1.5, using GC and BDD as the WE. In Fig. 2, the voltammograms are presented as a function of current density versus potential.

The oxidation peak of TBHQ at the GC electrode is shifted to lower potentials than for the BDD electrode, that is,+0.44 compared with +0.68 V for the ACN electrolyte, and +0.46 V compared with +0.60 V for EtOH supporting electrolyte. It was further found that the current density for the TBHQ on the GC electrode surface was greater than that observed with the BDD electrode, whereas a well-defined and symmetric oxidation peak at the GC electrode was observed for both electrolytes. Therefore, further studies were conducted uniquely with the GC electrode.

3.3. Optimisation of the extraction procedures

For the extraction of TBHQ in biodiesel samples, solvents and solvent mixtures such as those frequently applied in the extraction of antioxidants in food samples were tested. These included EtOH, methanol (MeOH), ACN, ACN/EtOH (50%/50%, v/v), ACN/MeOH (50%/50%, v/v), ACN/MeOH (70%/30%, v/v) and EtOH/ether (50%/50%, v/v) [15,17,21,23,29,32,33]. Various ratios of biodiesel, solvent and supporting electrolyte were tested, however, the optimal ratio for extraction with ACN was 1/10/5 (biodiesel/ACN/electrolyte, v/v/v) and 1/10/20 (biodiesel/EtOH/electrolyte, v/v/v) for extractions with EtOH. Also optimised was the duration of mechanical agitation, ultrasonication and centrifugation. During optimisation testing, the mixture of biodiesel, extraction solvent and supporting electrolyte was vortexed for 5, 4 and 2 min, ultrasonicated for 2 and 1 min, and centrifuged for 10 and 4 min. It was found that 2 min of agitation by vortex with the solvent followed by 2 min of agitation with the electrolyte and 2 min of ultrasonication, were sufficient for the extraction of TBHQ. For the separation of the biodiesel/aqueous-organic phases, it took only 4 min of centrifugation at 4000 rpm.

3.4. Analytical characteristics of the method

Analytical curves were constructed by plotting the measured peak oxidation current (Ipa) versus TBHQ concentration for the

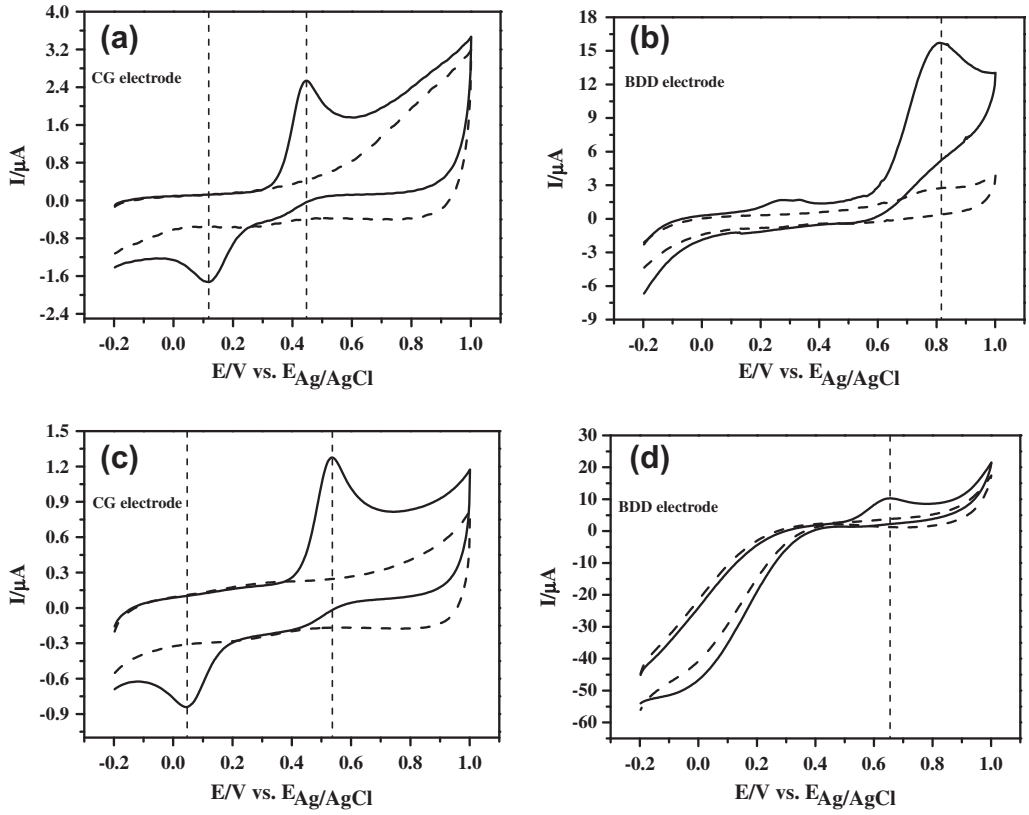

Fig. 1. Cyclic voltammograms for 15.0 mg L^{-1} TBHQ. (a) (—) TBHQ in a supporting electrolyte solution (KNO_3 solution (30% ACN, v/v) pH_{cond} 1.5), (—) supporting electrolyte, GC WE; (b) (—) TBHQ 15.0 mg L^{-1} in the supporting electrolyte (KNO_3 (30% ACN, v/v) pH_{cond} 1.5), (—) supporting electrolyte, BDD WE; (c) (—) TBHQ 15.0 mg L^{-1} in supporting electrolyte (KNO_3 solution (30% EtOH, v/v) pH_{cond} 1.5), (—) supporting electrolyte, GC WE; (d) (—) TBHQ 15.0 mg L^{-1} in supporting electrolyte (KNO_3 solution (30% EtOH, v/v) pH_{cond} 1.5); (—) supporting electrolyte, BDDE WE. v = 50 mV s^{-1}.

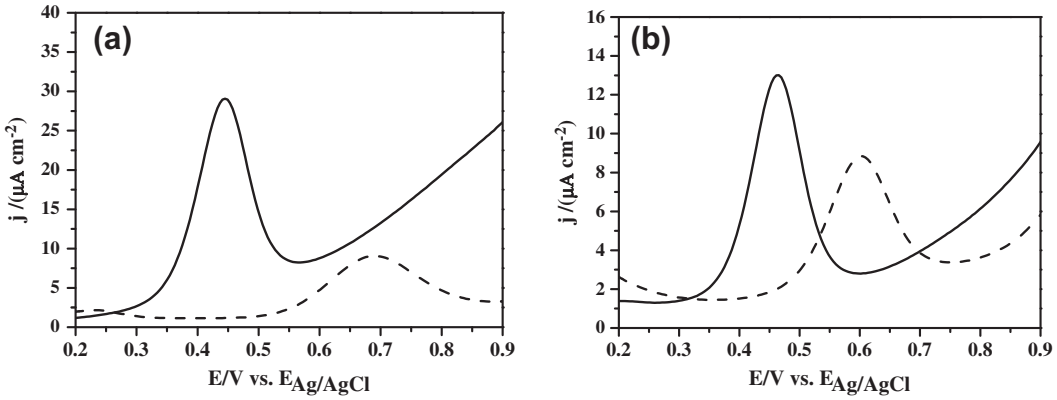

Fig. 2. Differential pulse voltammograms for 15.0 mg L^{-1} TBHQ. (a) (—) GC WE, (—) BDD WE in a supporting electrolyte solution of 10.0 mmol L^{-1} KNO_3 with 30% ACN, pH_{cond} 1.5 (b) (—) GC WE, (—) BDD WE in a supporting electrolyte solution of 10.0 mmol L^{-1} KNO_3 with 30% EtOH, pH_{cond} 1.5. v = 10 mV s^{-1}, ΔE = 50 mV and t = 0.5 s.

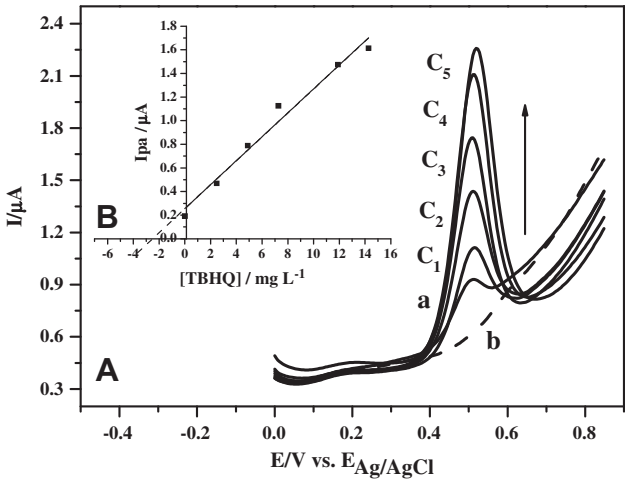

Fig. 3. (A) Differential pulse voltammograms recorded for the standard addition of TBHQ to biodiesel samples; (a) biodiesel sample not spiked with TBHQ; (b) biodiesel sample spiked with TBHQ (250 mg L^{-1}); (C_1–C_5) standard additions of TBHQ. (B) Inset - Dependence of Ip as a function of TBHQ concentration in a sample of soybean biodiesel spiked with TBHQ (250 mg L^{-1}). All samples were analysed in a supporting electrolyte solution of 10.0 mmoL^{-1} KNO_3 30% ACN.

two electrolytes studied. Excellent linearity was obtained within the concentration range of 0.2–100.0 mg L^{-1}, with the corresponding calibration equations providing correlation coefficients of 0.99 in both supporting electrolytes. The LOD and LOQ were calculated using the relationship $3s_b/b$ and $10s_b/b$, respectively, where s_b is the standard deviation of ten values of peak currents reported for the blank, and b is the slope of the analytical curve. The LOD and LOQ were 0.55 mg L^{-1} and 1.83 mg L^{-1}, respectively, for the analyses performed in ACN. For analyses performed in the EtOH electrolyte the LOD and LOQ were 0.57 mg L^{-1} and 1.92 mg L^{-1}, respectively.

Soybean biodiesel samples were spiked with three different concentrations of TBHQ: 250, 500 and 1000 mg L^{-1}. Samples were then subjected to extraction with ACN or EtOH and analysed. For each concentration three experiments were performed. The voltammetric technique was used to quantify TBHQ in biodiesel samples using the standard addition method. The differential pulse voltammograms for an unspiked sample (Fig. 3 voltammogram b), a sample spiked with 250.0 mg L^{-1} TBHQ, and five successive additions of aliquots of TBHQ stock solution are shown in Fig. 3. These results demonstrate that the analytical curves for TBHQ present good linearity. The mean values obtained for TBHQ agree with the three concentrations employed.

The recoveries of TBHQ were 100.2–101.5% with %RSD values below 2.0%, for extraction with ACN, and for extraction with EtOH the recoveries of TBHQ were 98.3–99.7% with %RSD values below 3.0% (Table 1). These results were also compared with chromatography as the reference method [38]. Application of the paired t-test to the results obtained by both methods and both extraction solvents results in t values that do not reach the 95% confidence level, indicating that there is no difference between the results obtained using either extraction solvent, and that no significant difference exists between the results obtained using DPV compared to those obtained using chromatography.

The values obtained experimentally by both methods are in good agreement with spiked values, with lower %RSD values for the voltammetric method, which has the advantage of being simpler and less expensive than chromatographic analysis. The recovery percents found are within the range of acceptable percent recovery as a function of concentration, according to Brito et al. [39]. The proposed method has accuracy and precision for the determination of TBHQ in samples of soybean biodiesel.

3.5. TBHQ detection in commercial samples of biodiesel

Real samples of biodiesel with added TBHQ were analysed using the method developed. The results obtained by DPV and HPLC are summarised in Table 2.

Table 1
Recovery of TBHQ in soybean biodiesel samples spiked with different levels of TBHQ by DPV and HPLC, extraction solvents ACN and EtOH, and the respective RSD (%) values.

Spiked level (mg L^{-1})	Spiked (mg L^{-1})	HPLC		DPV	
		ACN	EtOH	ACN	EtOH
250	242.50/252.20	98.9 ± 4.6	104.0 ± 6.9	100.1 ± 0.5	99.5 ± 1.0
500	485.00	99.8 ± 4.6	85.0 ± 6.5[a]	96.7 ± 1.4	98.7 ± 2.5
1000	990.00	94.6 ± 2.1	99.6 ± 1.5	100.8 ± 1.9	99.7 ± 1.4

[a] A value considered anomalous was discarded for the averaging.

Table 2
Recoveries of TBHQ in commercial biodiesel sample containing 877.00 ppm TBHQ.

	DPV			HPLC		
	Found[a] (mg L^{-1})	Recovery (%)	RSD (%)	Found[a] (mg L^{-1})	Recovery (%)	RSD (%)
ACN	878.40 ± 26.81	100.1	3.0	837.20 ± 35.81	95.4	4.2
EtOH	869.37 ± 6.66	99.4	0.7	742.30 ± 44.70	84.6	6.0

RSD: relative standard deviation.
[a] Mean ± SD (n = 3).

It was found that both extraction procedures were effective for the analysis of TBHQ present in the biodiesel commercial sample. Results of the TBHQ recovery were between 97.0–100.4% for the extraction with ACN, and 98.6–100.0% for the extraction with EtOH. The values obtained by the proposed method agree well with values added, industrially, as well as with the values obtained by the chromatographic method, with a %RSD of less than 4%. Furthermore, the method also has the advantage of using comparatively low quantities of organic solvent for the analysis of TBHQ in soybean biodiesel than the HPLC method.

4. Conclusions

The voltammetric determination of TBHQ in soybean biodiesel samples was possible using liquid–liquid extraction of the sample preparation. The accuracy and applicability of the method was demonstrated by analysis of a commercial biodiesel sample. The proposed method is accurate and reliable, and when compared with the chromatography method, simpler, cheaper, and also has the advantage of using small quantities of organic solvents, and generating less waste. Thus, this novel method represents a viable alternative for the quality control of soybean biodiesel samples.

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