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Reverse-phase liquid chromatographic determination of α-lipoic acid in dietary supplements using a boron-doped diamond electrode

Weena Siangproh [a,*], Poomrat Rattanarat [b], Orawon Chailapakul [b,c]

[a] Department of Chemistry, Faculty of Science, Srinakharinwirot University, Sukumvit 23 Rd., Wattana, Bangkok 10110, Thailand
[b] Department of Chemistry, Faculty of Science, Chulalongkorn University, Patumwan, Bangkok 10330, Thailand
[c] Center for Excellence Petroleum, Petrochemicals and Advanced Materials, Chulalongkorn University, Patumwan, Bangkok 10330, Thailand

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ABSTRACT

A fast liquid chromatographic separation, coupled with sensitive and straightforward detection using a boron-doped diamond (BDD) electrode, was developed and validated for the determination of α-lipoic acid in dietary supplement samples. The analysis was carried out using a reversed phase C18 (150 mm × 4.6 mm, 5 μm) column with a mobile phase consisting of a 1:1 (v/v) ratio of 0.05 M phosphate solution (pH 2.5):acetonitrile, at a flow rate of 1.0 mL/min. The detection potential obtained from hydrodynamic voltammetry was 1.05 V vs. Ag/AgCl. Under optimized conditions, the chromatographic separation was performed in less than 5 min, a good linear relationship was obtained between the current and the α-lipoic concentration within the range of 0.01–60 μg/mL (correlation coefficient of 0.9971), and a detection limit of 3.0 ng/mL was determined. Furthermore, this method was successfully applied to determine α-lipoic acid concentrations in selected commercial dietary supplement samples. The recovery of α-lipoic acid in spiked samples at 0.5, 5.0 and 30 μg/mL ranged from 94.4% to 103.6% with a relative standard deviation (RSD) of between 1.2% and 3.7%. In real samples, this developed methodology produced results that were highly correlated with the standard HPLC-UV approach. Therefore, the present method can be used for fast, selective and sensitive quantification of α-lipoic acid in dietary supplements.

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1. Introduction

α-Lipoic acid (1,2-dithiolane-3-pentanoic acid, 1,2-dithiolane-3-valeric acid), or thioctic acid, is a natural sulfur-containing cofactor, which for example, occurs in four essential mitochondrial enzyme complexes. It is widely found in both prokaryotic and eukaryotic microorganisms as well as in animals and plants. It plays an essential role in various biological systems, such as improving mitochondrial function, increasing metabolic rate and decreasing oxidative damage [1–3]. Recently, α-lipoic acid has gained considerable attention as an 'ideal' or 'universal antioxidant' [4] and has been shown to provide protection against free radical attack both *in vitro* and *in vivo*. Furthermore, α-lipoic acid has been employed as therapy for many diseases associated with impaired energy utilization, such as type II diabetes, diabetic polyneuropathies [2], Alzheimer-type dementia [5], neurodegenerative disorders, acquired immunodeficiency syndrome [6] and heavy metal poisoning [7] and was recently shown to display positive effects in the treatment of age-associated neurodegenerative disease and HIV infections [8].

In addition to its use as a drug, α-lipoic acid has become one of the most recognized and in-demand health supplements. With the medical importance of α-lipoic acid in both toxicological and acceptance criteria, and its potential to treat multiple diseases in the future, a fast, accurate, selective and sensitive method for quantifying the concentration of α-lipoic acid in dietary supplements, among other types of samples, is needed.

Various analytical methodologies have been developed for the determination of α-lipoic acid in a diverse variety of biological, drug and food samples. Chromatographic methods are widely used, and the substantiated approaches for the determination of α-lipoic acid have been reviewed by Kataoka [9]. Gas chromatography coupled with detection by flame photometry [10,11] and mass spectrometry [12–14] are among the most regularly utilized techniques. However, these techniques are time-consuming because of the use of the derivatization approach.

Use of thin-layer chromatography has also been reported for the separation and determination of α-lipoic acid [15]. For densitometric analysis, derivatization with a palladium(II) chloride immersion reagent is necessary to overcome the poor absorbtivity of α-lipoic acid. A reversed phase HPLC [16,17] or capillary electrophoresis

* Corresponding author. Tel.: +66 2 649 5000x8208; fax: +66 2 259 2097.
E-mail addresses: weena@swu.ac.th, weenasi@hotmail.com (W. Siangproh).

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(CE) coupled with ultraviolet detection [18,19] is another technique that is used in the determination of α-lipoic acid, but it typically provides a high minimum detection limit due to lack of a strong chromophore. In addition, the main hindrance of using CE is the adsorption of α-lipoic acid to the capillary wall, such that a washing step is required for further use after only a few injections. HPLC combined with other detection modes, such as fluorescence [20–23], mass spectrometry [24–26] or electrochemical detection [27–32], have also been developed to determine α-lipoic acid levels. Among these detectors, fluorescence-based methods have demonstrated low detection limits, though this depends on the fluorogenic labeling reagent. The detection limits obtained by fluorescent labels are typically low enough to detect α-lipoic acid in biological samples; however, these assays require laborious sample preparation steps and also have a high reagent cost. HPLC with mass spectrometry usually offers very low sensitivity and high selectivity, but there is high equipment cost and a significant amount of labor and analytical resources required, which can potentially cause substantial delays in obtaining results.

The use of an electrochemical detector is an alternative method for α-lipoic acid determination and has the benefits of simplicity, speed, sensitivity and low cost. A dual Hg–Au electrode in pulsed mode was used for the determination of nanomolar levels of α-lipoic acid, but loss of sensitivity over repeated or long analysis times and the necessity of renewal of the electrode are considerable drawbacks that offset the advantage of its sensitivity [33]. Alternative working electrodes that can be employed for the detection of α-lipoic acid are glassy carbon (GC) [30,31,33], gold [29] and modified electrodes, including carbon paste electrodes modified with nickel(II)-cyclohexylbutyrate [34], lipoate-selective membranes [35] and GC electrodes modified with carbon nanotubes [36]. From the work with sensors in the literature, it can be deduced that a GC electrode suffers from a lack of sensitivity due to poisoning of the electrode surface at high oxidation potentials, whereas the gold electrode requires a pulsed amperometric mode. Lastly, the modified electrodes have disadvantages due to the different tedious preparation steps and lack of reproducibility of signal.

Thus, efforts to extend electrochemical detection to α-lipoic acid have been challenging. Recently, boron-doped diamond (BDD) electrodes have been examined extensively in the field of electrochemical analysis. Compared to conventional electrodes, the BDD electrode offers many unique benefits, such as a low background, a wide working potential window, long-term response, high mechanical strength and a lack of adsorption [37,38]. With this excellent electrode material and ongoing research interest, the work reported here is an extension of our efforts in the determination of α-lipoic acid in dietary supplements with a BDD electrode following chromatographic resolution from matrixes.

Strangely, there is currently very little information about the determination of α-lipoic acid in health or dietary supplements and, to the best of our knowledge, there is no information about the determination of α-lipoic acid levels using a BDD electrode as the detector in chromatography. Therefore, this report is the first to use a BDD electrode for the quantification of α-lipoic acid following its chromatographic separation. The ultimate goal of this study is to investigate the feasibility of using the BDD electrode to detect α-lipoic acid and to compare the BDD sensor with the conventional HPLC-UV based method.

Strong analytical figures with limits of detection in the low ng/mL range, good sensitivity, excellent response precision and stability were observed with the BDD electrode. Therefore, this innovative concept could contribute to the development of a practical, rapid, highly sensitive and accurate method for assaying α-lipoic acid levels in food or food supplements. In addition, this work also presented the possibility to use the proposed method for the simultaneous determination of α-lipoic acid and lipoamide. Currently, even though lipoamide is not found in the supplements but lipoamide has been described as antioxidant [39] and has been used for many years as a drug for the treatment of the varieties diseases [9]. In future, it maybe uses both α-lipoic acid and lipoamide for the treatment to increase the drug potential. Thus, the development of a HPLC-EC method for the determination of α-lipoic acid and lipoamide is important.

2. Materials and methods

2.1. Reagents and solutions

Chemicals were of analytical grade unless indicated otherwise and were used as received. HPLC-grade acetonitrile, ethanol and methanol were obtained from Merck (Darmstadt, Germany). Milli-Q water from Millipore ($R \geq 18.2\,\mathrm{M\Omega\,cm^{-1}}$) was used throughout this research. Potassium dihydrogen orthophosphate (KH_2PO_4) was purchased from BDH (VWR International Ltd., England). Disodium hydrogen phosphate dehydrate (Na_2HPO_4) was purchased from Merck (Darmstadt, Germany) and *o*-phosphoric acid was obtained from Fluka (Buchs, Switzerland). Capsules or tablets of α-lipoic acid (15, 50, 105 and 200 mg of α-lipoic acid) were purchased from local drugstores in Japan. Standards of α-lipoic acid, lipoamide, carnitine and coenzyme Q10 were purchased from Sigma–Aldrich (St. Louis, MO, USA).

A stock standard solution of α-lipoic acid (500 μg/mL) was prepared by accurately weighing 5 mg of analyte and dissolving it in a 1:1 (v/v) ratio of acetonitrile: Milli-Q water to 10 mL in a volumetric flask and storing it at 4 °C. The working solutions were prepared by suitable dilution of the stock standard solutions with the mobile phase.

2.2. Boron-doped diamond (BDD) electrode

The experimental conditions and the apparatus used for the diamond film growth have been described in detail elsewhere [40]. The films were prepared by deposition of the BDD thin films on highly conductive n-Si (1 1 1) substrates by microwave plasma-assisted chemical vapor deposition. Deposition was usually carried out for 10 h to achieve a film thickness of approximately 30 μm. The nominal B/C atomic ratio in the gas phase was 1:100, and the typical boron-doping level in the film was ca. $10^{21}\,\mathrm{cm^{-3}}$. The BDD electrodes were rinsed with ultra-pure water prior to use.

2.3. Cyclic voltammetry

Electrochemical measurements were recorded using an Autolab Potentiostat 30 (Metrohm, Switzerland) with a standard three-electrode configuration. The working electrode was either a BDD electrode or a glassy carbon (GC) electrode. The auxiliary and reference electrodes were platinum wire and Ag/AgCl, respectively. Cyclic voltammetry was used to probe the electrochemical reaction. The electrochemical measurements were housed in a Faradaic cage to reduce electronic noise. All experiments were done at room temperature.

2.4. HPLC separation and apparatus

The HPLC-electrochemical measurement using a BDD electrode as an amperometric detector was carried out in the mobile phase (1:1 (v/v) ratio of acetonitrile:0.05 M phosphate solution, which was adjusted to pH 2.5 with phosphoric acid) with an applied potential of 1.05 V vs. Ag/AgCl and a flow rate of 1.0 mL/min. The HPLC system consisted of an HPLC compact pump model

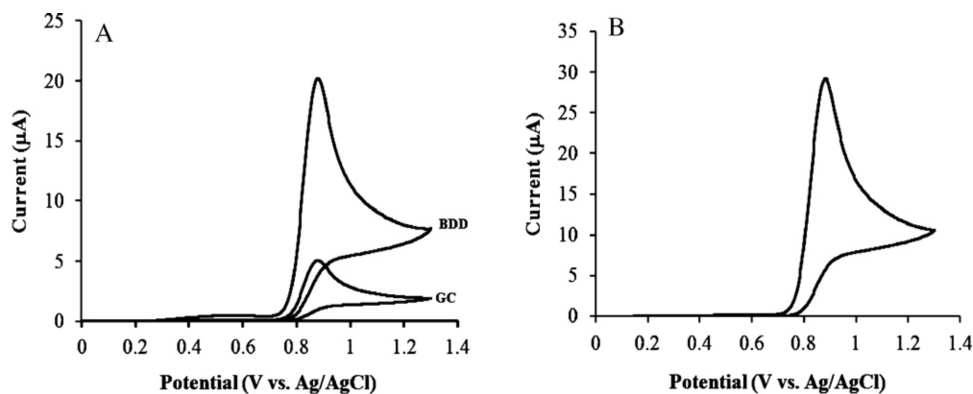

Fig. 1. Cyclic voltammograms for the BDD electrode vs. Ag/AgCl of (A) 1.0 mM α-lipoic acid at BDD and GC electrode and (B) 1.0 mM lipoamide in a 1:1 (v/v) ratio of 0.05 M phosphate solution (pH 2.5):acetonitrile. Sweep rate 100 mV/s, electrode area 0.07 cm^2. Voltammograms shown are representative of at least five independent repetitions.

2250 (Bischoff, Germany), a 20 μL sample loop injection (Rheodyne No. 7125, USA), a thin-layer flow cell (GL Sciences, Inc.), an amperometric detector and a data acquisition system (Ecochemie Netherland). The chromatographic column was a Luna 5u C18 column (150 mm × 4.6 mm i.d.) from Phenomenex and all chromatographic separations were performed at room temperature (∼25 °C). The amperometric determination was performed after obtaining the hydrodynamic voltammogram. The peak current after each injection was recorded along with the corresponding background current. These data were plotted as a function of applied potential to obtain the hydrodynamic voltammograms. The amperometric measurements were carried out at the potential that provided the maximum signal-to-background (S/B) ratio in the hydrodynamic voltammograms.

2.5. Electrochemical measurement

The thin-layer flow cell consisted of three electrodes: a BDD working electrode, a Ag/AgCl reference electrode (Bioanalytical System Inc., USA) and a stainless steel tube counter electrode. The geometric area of the BDD electrode in the flow cell was estimated to be 0.37 cm^2 using a 1-mm thick silicon rubber gasket as a spacer. The measurement step was performed in a copper Faradaic cage to reduce the electronic noise. An Autolab Potentiostat 30 (Ecochemie Netherland) was used for amperometric control and signal processing.

2.6. Sample preparation

The average weight per capsule or tablet was calculated from the weight of 10 capsules or tablets. Five capsules or tablets were finely ground and homogenized in an agate mortar. The amount of the powdered mass analyte corresponding to one capsule or tablet was dissolved in the mobile phase, and the solution was diluted appropriately so that the concentration of α-lipoic acid in the final test solution was within the linear dynamic range (0.01–60 μg/mL). The solution was filtered through a 0.45-μm nylon membrane filter before injection into the HPLC-electrochemical system.

2.7. Method validation

To obtain the validation parameters, evaluation of the peak area was employed to determine the level of α-lipoic acid. A calibration curve was constructed and treated with linear least square regression analysis. The slope, intercept and correlation coefficient data were also calculated. The limit of detection (LOD) and the limit of quantitation (LOQ) were determined from $3S_{bl}/S$ to $10S_{bl}/S$, respectively, where S_{bl} is the standard deviation of the blank measurement ($n = 10$) and S is the sensitivity of the method, evaluated as the slope of the linearity. To determine the intra-day and inter-day precision of the analytical process, three concentrations (0.5, 5.0 and 30 μg/mL) were studied five times on the same day and on three different days, respectively. In addition, the selectivity of the proposed methodology was investigated by observing any interference encountered from excipients presented in the formulations.

3. Results and discussion

3.1. Electrooxidation of α-lipoic acid at the BDD electrode

The cyclic voltammograms of 1 mM α-lipoic acid and lipoamide at the BDD electrode in the mobile phase revealed a well-resolved oxidation wave for both compounds during the scan of potential towards the positive direction at the BDD electrode (Fig. 1). However, compared to those obtained with the GC electrode (Fig. 1A), the charging currents at the BDD electrode were much lower, leading to a much larger signal-to-background ratio. Interestingly, it was found that a slight fouling of the product occurred during consecutive scans with the BDD electrode in α-lipoic acid solutions. However, in contrast to the GC electrode, the background current was almost the same after rinsing with distilled water and immerging in the blank solution as the background current before exposure to the α-lipoic acid solution (data not shown). These results indicate that the BDD electrode offers greatly improved performance and substantially higher sensitivity than the GC electrode. Therefore, the electrochemical behavior of α-lipoic acid can be improved by this electrode.

To authenticate the adsorption of α-lipoic acid on the BDD electrode surface, the scan rate dependence was carried out within the range of 0.01–0.3 V/s (data not shown). The anodic peak currents increased linearly with the square root of the scan rate over the examined range (linear regression $r^2 > 0.999$), supporting the theories that α-lipoic acid is slightly adsorbed on the electrode surface and that the reaction is controlled by the diffusion process.

3.2. Liquid chromatographic separation

α-Lipoic acid and related compounds, such as lipoamide, were separated using a C18 packed column and an isocratic system. Initially, the mobile phase was investigated using different (v/v) ratios of 0.05 M phosphate solution (pH 2.5):acetonitrile at a flow rate of 1.0 mL/min, with optimization based upon obtaining a shorter run time without loss of purity (Fig. 2A). The 0.05 M phosphate solution (pH 2.5) was chosen as the most suitable solution for

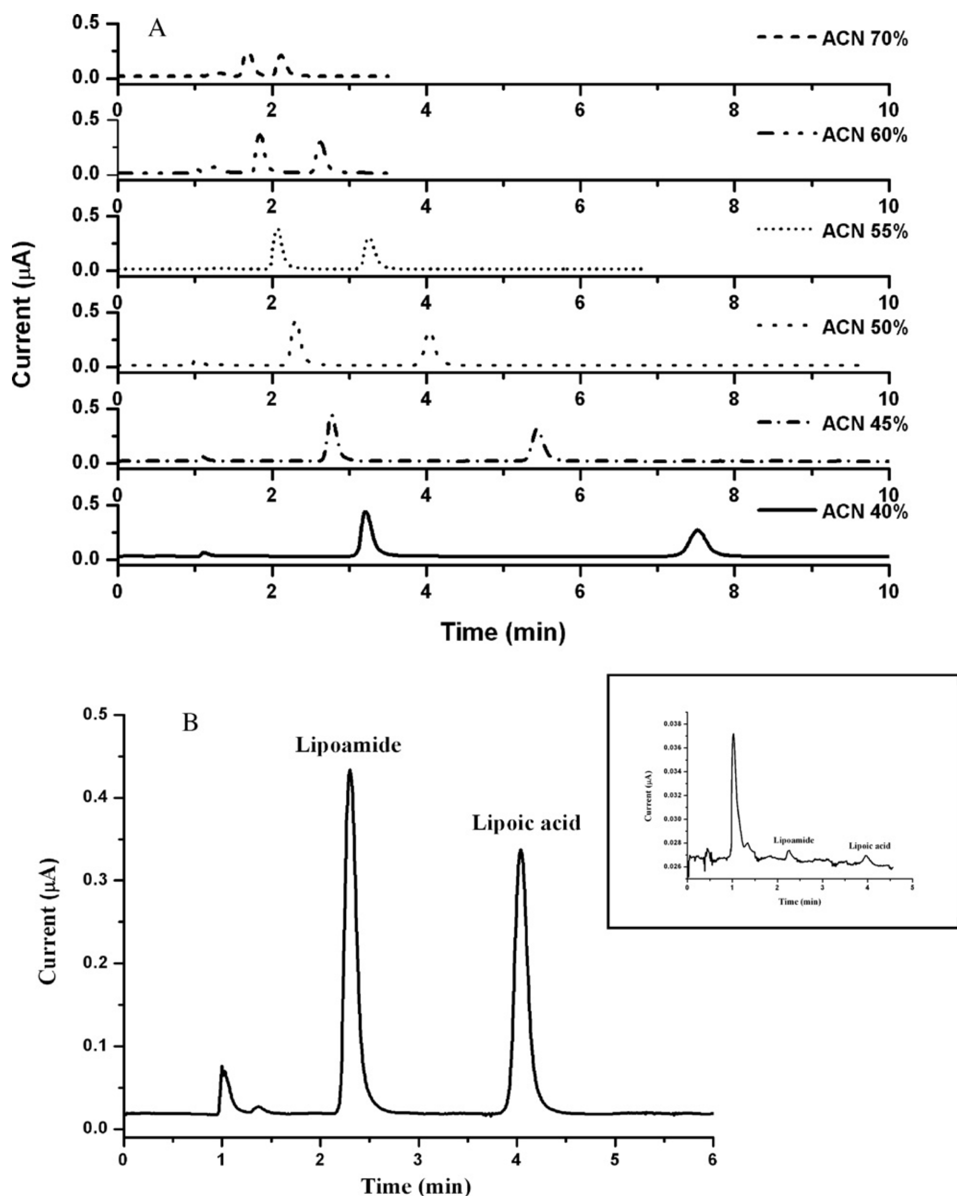

Fig. 2. Representative HPLC-EC chromatograms of 10 μg/mL lipoamide and α-lipoic acid in a mobile phase (0.05 M phosphate solution (pH 2.5):acteonitrile) comprised of (A) six different acetonitrile proportions and (B) a 1:1 (v/v). The detection potential was 1.05 V vs. Ag/AgCl using a BDD electrode. The injection volume was 20 μL, and the flow rate was 1.0 mL/min. Chromatograms shown are representative of at least five independent repetitions. Inset (B) is chromatogram of the LOD concentration.

α-lipoic acid detection because it provided the lowest stable background current. As the percentage of acetonitrile in the mobile phase increased, a shorter elution time was obtained; however, peak broadening increased, and lower resolution and sensitivity were observed, especially at and above 60% (v/v) acetonitrile. Thus, a 1:1 (v/v) ratio of 0.05 M phosphate solution (pH 2.5):acetonitrile was selected as the mobile phase, resulting in α-lipoic acid and lipoamide being well resolved as separate sharp peaks with the high signal. The chromatogram for the separation of a standard solution of lipoamide and α-lipoic acid under these conditions is presented in Fig. 2B, where the retention times were 2.3 min and 4.1 min for lipoamide and α-lipoic acid, respectively. To our knowledge, this is the fastest reported separation time of α-lipoic acid and lipoamide with clear separation and indicates that α-lipoic acid and lipoamide can be determined very quickly by validated HPLC with BDD amperometric detection.

3.3. Hydrodynamic voltammetry

Hydrodynamic voltammetry was employed to optimize the detection potential of the α-lipoic acid from within a detection potential range of 0.8–1.3 V vs. Ag/AgCl. Analysis of the hydrodynamic voltammetric *i–E* curves of α-lipoic acid and lipoamide and the background current at each potential reveals that the oxidation current of α-lipoic acid and lipoamide, as well as the background current, were significantly affected by the detection potentials (Fig. 3A). Therefore, the net current after background subtraction (S/B) was considered and the S/B ratios were plotted against the potential curves (Fig. 3B), revealing that the signals increased when the potential increased up to 1.05 V vs. Ag/AgCl for both analytes. Thus, a detection potential at 1.05 V vs. Ag/AgCl was selected as the optimal potential for the amperometric detection of α-lipoic acid and lipoamide, following their HPLC separation.

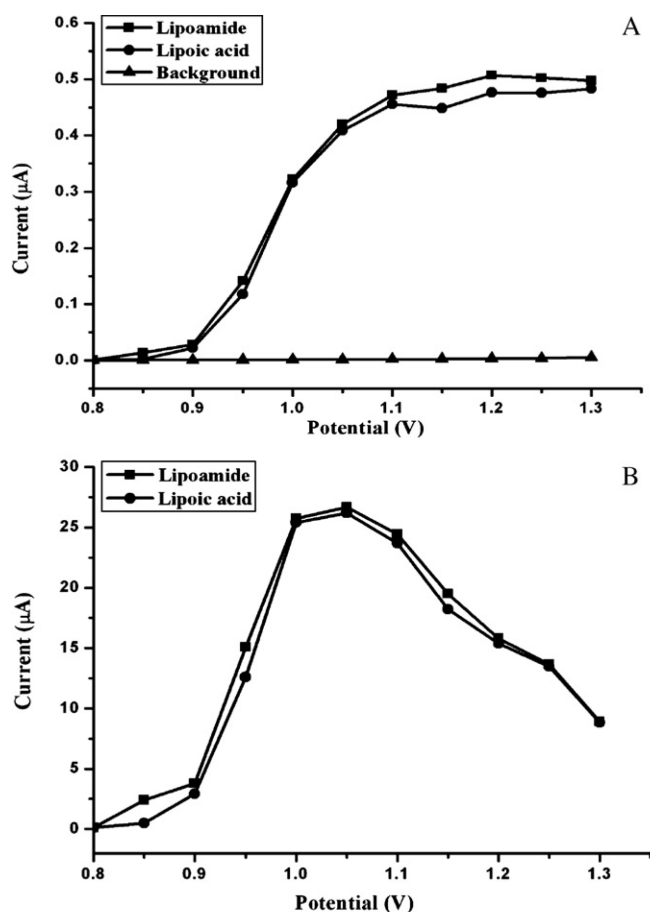

Fig. 3. Hydrodynamic voltammetric results at the BDD electrode for a 10 μg/mL each mixture (20 μL total) of α-lipoic acid and lipoamide. (A) (■) lipoamide, (●) α-lipoic acid and (▲) background; (B) hydrodynamic voltammogram of signal-to-background ratios. The other conditions are the same as in Fig. 2B. Data are shown as the mean ± 1 SD derived from three independent repetitions.

3.4. Method validation

3.4.1. Analytical performance

The calibration of the peak areas against concentrations generated linear functions for both of the analytes within the range of 0.01 and 120 μg/mL, and the coefficients of determination (r^2) were all higher than 0.99 (n = 5). Typical calibration curves had the regression equations of $y = 0.4029x + 0.2887$ for α-lipoic acid and $y = 0.3858x + 0.3452$ for lipoamide, respectively. The LOD and LOQ were calculated from $3S_{bl}/S$ to $10S_{bl}/S$, as described in the methods section, and are summarized in Table 1. When sensitivity was compared to that of previous studies, particularly in the electroanalysis field, it was found that the detection of α-lipoic acid with the BDD electrode was 1.6-, 6- and 380-fold more sensitive than the detection by HPLC-CEAD [28], lipoate-selective membrane sensors [35] and the GC electrodes [33], respectively. Though there are a few methods reported that provide a lower detection limit than the present method, those methods are tedious and complicated, requiring additional stages that cause a several-fold increase in the total analysis time, such as a derivatization step or modification of the electrode surface.

The precision of the analytical process was calculated by determining the RSD for the repeated injection of solutions containing the complete set of standard compounds. To evaluate the repeatability of the analytical process, three concentrations (0.5, 5.0 and 30.0 μg/mL) were studied. These spiked concentration levels were chosen in order to check the results obtained from low, medium and high concentrations with respect to the probable range of interest in food and dietary supplement samples.

The intra- and inter-day precision and recovery obtained from the proposed method are summarized in Table 2. The intra-day RSDs and recoveries of α-lipoic acid were found to vary over the ranges of 1.2–3.7% and 94.4–103.6%, respectively, while the inter-day RSDs and recovery of α-lipoic acid varied over the range of 1.4–4.1% and 93.0–102.7%, respectively.

Table 1
Linearity, limit of detection (LOD) and limit of quantitation (LOQ) of the HPLC-EC method using a BDD electrode (n = 3).

Analyte	Linear range (μg/mL)	Slope (peak area) (units/μg/mL)	Intercept (μA)	r^2	LOD (ng/mL)	LOQ (ng/mL)
Lipoamide	0.01–60	0.3858	0.3452	0.9961	3.62	12.07
Lipoic acid	0.01–60	0.4029	0.2887	0.9971	3.00	10.00

Table 2
The intra- and inter-precisions and recoveries of the HPLC-BDD method (n = 3).

Samples	Spiked level (μg/mL)	Intra-day		Inter-day	
		Mean of % recovery (x ± SD)	RSD (%)	Mean of % recovery (x ± SD)	RSD (%)
	0.5	97.5 ± 1.7	1.7	95.3 ± 3.6	3.5
Sample 1	5	97.5 ± 3.6	3.7	98.3 ± 1.3	1.4
	30	100.4 ± 1.6	1.6	99.8 ± 1.8	1.8
	0.5	100.4 ± 1.8	1.7	100.2 ± 0.7	1.7
Sample 2	5	99.4 ± 1.6	1.6	101.5 ± 3.2	3.1
	30	100.8 ± 1.2	1.2	99.7 ± 1.8	1.8
	0.5	94.4 ± 3.4	3.6	93.0 ± 2.4	2.6
Sample 3	5	103.6 ± 2.7	2.7	100.0 ± 4.1	4.1
	30	99.8 ± 1.6	1.6	100.7 ± 1.4	1.4
	0.5	97.4 ± 3.3	3.4	99.8 ± 2.7	2.7
Sample 4	5	101.9 ± 2.1	2.1	100.8 ± 1.9	1.9
	30	100.3 ± 2.0	2.0	102.7 ± 3.4	3.2
	0.5	99.8 ± 2.7	2.7	100.4 ± 1.8	3.6
Sample 5	5	100.8 ± 1.9	1.9	99.4 ± 1.6	2.7
	30	102.7 ± 3.4	3.2	100.8 ± 1.2	2.3

Table 3
Determination of α-lipoic acid levels in different food supplement samples ($n = 5$) by the traditional HPLC-UV method and the developed HPLC-BDD electrode method reported here.

Sample	Amount drug label (mg/capsule)	Amount found (mg/capsule) ($x \pm$ SD)		% Recovery	
		HPLC-BDD[a]	HPLC-UV[b]	HPLC-BDD[a]	HPLC-UV[b]
Sample 1	15	14.9 ± 0.2	14.6 ± 0.5	99.6 ± 1.3	97.3 ± 1.5
Sample 2	50	49.4 ± 0.8	49.1 ± 1.7	98.8 ± 1.7	98.2 ± 1.3
Sample 3	50	50.4 ± 1.7	51.7 ± 1.5	100.7 ± 3.5	103.4 ± 2.3
Sample 4	105	105.4 ± 2.5	104.4 ± 2.4	100.4 ± 2.4	99.4 ± 1.9
Sample 5	200	200.9 ± 1.4	201.9 ± 1.3	100.5 ± 2.7	101.0 ± 2.3

[a] This developed method.
[b] Traditional method (Ref. [17]).

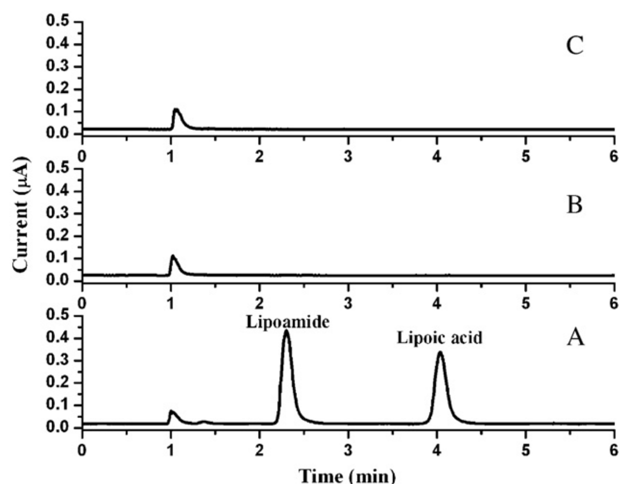

Fig. 4. Representative HPLC-EC chromatograms of 10 μg/mL standard solutions (20 μL) of (A) lipoamide, α-lipoic acid, carnitine and coenzyme Q, (B) 10 μg/mL carnitine and (C) 10 μg/mL coenzyme Q. The mobile phase was 1:1 (v/v) 0.05 M phosphate solution (pH 2.5):acetonitrile. The detection potential was 1.05 V vs. Ag/AgCl using a BDD electrode and a flow rate of 1.0 mL/min. Chromatograms shown are representative of at least five independent repetitions.

3.4.2. Inference study

In order to check whether the matrix compounds reported on the label (carnitine and coenzyme Q10) influence the measured α-lipoic acid content, standard carnitine and coenzyme Q10 were analyzed by the proposed method. From Fig. 4(B and C), it can be seen that no signal of standard carnitine and coenzyme Q10 were observed. Therefore, no evidence of any interference effects on the determination of α-lipoic acid within the same samples was found under these optimized conditions (Fig. 4A).

3.5. Analysis of α-lipoic acid in dietary supplements

To verify the applicability of the BDD electrode and the methodology developed in the present study, target compounds, in terms of supplement samples from local Japanese supermarkets, were investigated by standard addition. Representative chromatograms obtained from the analysis of α-lipoic acid in two such supplement samples, "1" and "2", are illustrated in Fig. 5(A) and (B), in which the peaks were identified by comparison with the retention times of reference compounds following injection of standard solutions. In terms of consistency, note that the RSD ($n = 3$) for the determined content of α-lipoic acid in all supplements was lower than 4%.

This quantitative evaluation revealed that the experimentally calculated values closely matched the manufacturer's claim in all five supplements examined (Table 3). These results were compared to those obtained by the HPLC-UV method [17] and statistically analyzed using the paired *t*-test at the 95% confidence interval. The paired two-tail test gave calculated *t*-values (1.896) below the critical *t*-value (2.306), therefore accepting the null hypothesis. The data, summarized in Table 3, reveal that the results of HPLC coupled with BDD amperometry are of comparable accuracy to, and not significantly different from, those values obtained by the traditional HPLC-UV method.

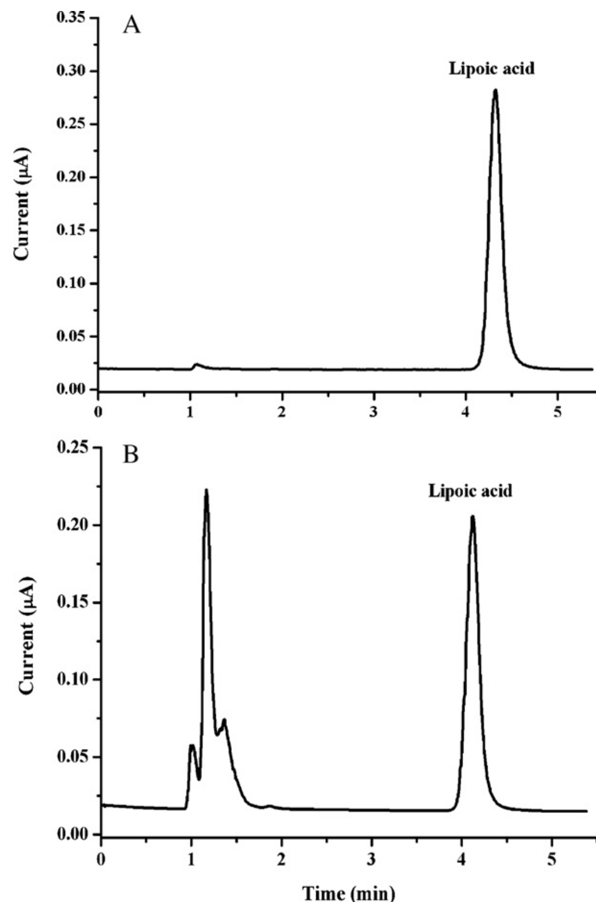

Fig. 5. Representative HPLC-EC chromatograms of dietary supplement samples (A) '1' and (B) '2'. The mobile phase, detection potential, injection volume and flow rate were as detailed in Fig. 4. Chromatograms shown are representative of at least five independent repetitions.

4. Conclusions

The innovative concept for coupling of HPLC to a BDD electrode for the determination of α-lipoic acid in dietary supplements is reported. Particular effort was focused on the use of the BDD electrode as an alternative detector to overcome the drawback of using metal, GC or modified electrodes. With the superior electrochemical properties of the BDD electrode, it not only allows highly sensitive detection but also simplicity and cost-effectiveness due

to the fact that α-lipoic acid can be detected amperometrically without derivatization or the use of a pulse waveform. Compared to other validated chromatographic methods, the total assay could be completed in a several-fold shorter time period and yet could successfully determine α-lipoic acid contents in dietary supplements without extraction or pre-concentration. Reproducible signals obtained with %RSD for intra- and inter-assay were in all cases below 5%. These results implied that a BDD detection exhibited a high reproducibility. Although, we have not tested BDD electrodes for long-term stability under continuous operation, they exhibited highly reproducible responses from day to day. Also, the response was reproducible after several days of exposure to the laboratory atmosphere. Indeed, the good agreement in the determination of α-lipoic acid concentrations in supplement samples by the developed method and the standard HPLC-UV method further vouches for the simplicity and straightforwardness of this technique. In conclusion, HPLC coupled with BDD amperometry provides an attractively alternative method for the determination of α-lipoic acid in food supplement samples, among others, and may also be useful for biomedical and clinical investigation of α-lipoic acid levels.

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References

[1] F. Navari-Izzo, M.F. Quartacci, C. Sgherri, Plant Physiol. Biochem. 40 (2002) 463.
[2] T.M. Hagen, R.T. Ingersoll, J. Lykkesfeldt, J. Liu, C.M. Wehr, V. Vinarsky, J.C. Bartholomew, B.N. Ames, FASEB J. 13 (1999) 411.
[3] L. Packer, E.H. Witt, H.J. Tritschler, Free Radic. Biol. Med. 19 (1995) 227.
[4] A.I. Haj-Yehia, P. Assaf, T. Nassar, J. Katzhendler, J. Chromatogr. A 870 (2000) 381.
[5] K. Hager, A. Marahrens, M. Kenklies, P. Riederer, G. Münch, Arch. Gerontol. Geriat. 32 (2001) 275.
[6] J. Fuchs, G. Packer, G. Zimmer, Lipoic Acid in Health and Disease, Marcel Dekker, New York, 1997.
[7] H. Gurer, H. Ozgunes, S. Oztezcan, N. Ercal, Free Radic. Biol. Med. 27 (1999) 75.
[8] L. Patrick, Altem. Med. Rev. 5 (2000) 290.
[9] H. Kataoka, J. Chromatogr. B 717 (1998) 247.
[10] H. Kataoka, N. Hirabayashi, M. Makita, J. Chromatogr. Biomed. Appl. 615 (1993) 197.
[11] H. Kataoka, N. Hirabayashi, M. Makita, Methods Enzymol. 279 (1997) 166.
[12] K.J. Pratt, C. Carles, T.J. Carne, M.J. Danson, K.J. Stevenson, Biochem. J. 258 (1989) 749.
[13] S.A. Jackman, D.W. Hough, M.J. Danson, K.J. Stevenson, F.R. Opperdoes, Eur. J. Biochem. 193 (1990) 91.
[14] A. Mattulat, W. Baltes, Z. Lebensm, Unters. Forsch. 194 (1992) 326.
[15] N. Ravanic, S. Filipic, K. Nikolic, G. Popovic, I. Vovk, B. Simonovska, D. Agbaba, Acta Chromatogr. 21 (2009) 433.
[16] E.I. Kozlov, I.A. Solunina, M.L. Lyubareva, M.A. Nadtochii, Pharm. Chem. J. 38 (2004) 642.
[17] H.Y. Aboul-Enein, H. Hoenen, J. Liq. Chromatogr. Relat. Technol. 27 (2004) 3029.
[18] A. Sitton, M.G. Schmid, G. Gübitz, H.Y. Aboul-Enein, J. Biochem. Biophys. Methods 61 (2004) 119.
[19] M. Trentin, T. Carofiglio, R. Fornasier, U. Tonellato, Electrophoresis 23 (2002) 4117.
[20] T. Inoue, M. Sudo, H. Yoshida, K. Todoroki, H. Nohta, M. Yamaguchi, J. Chromatogr. A 1216 (2009) 7564.
[21] H. Yoshida, M. Sudo, K. Todoroki, H. Nohta, M. Yamaguchi, Anal. Sci. 25 (2009) 829.
[22] S. Satoh, T. Toyo'oka, T. Fukushima, S. Inagaki, J. Chromatogr. B 854 (2007) 109.
[23] W. Witt, B. Rüstow, J. Chromatogr. B 705 (1998) 127.
[24] H.T. Chng, L.S. New, A.H. Neo, C.W. Goh, E.R. Browne, E.C.Y. Chan, J. Pharm. Biomed. Anal. 51 (2010) 754.
[25] J. Chen, W. Jiang, J. Cai, W. Tao, X. Gao, X. Jiang, J. Chromatogr. B 824 (2005) 249.
[26] R.K. Trivedi, R.R. Kallem, R.N.V.S. Mamidi, R. Mullangi, N.R. Srinivas, Biomed. Chromatogr. 18 (2004) 681.
[27] A.I. Durrani, H. Schwartz, M. Nagl, G. Sontag, Food. Chem. 4 (2010) 1143.
[28] A.I. Durrani, H. Schwartz, W. Schmid, G. Sontag, J. Pharm. Biomed. Anal. 45 (2007) 694.
[29] J. Teichert, R. Preiss, J. Chromatogr. B 769 (2002) 269.
[30] J. Teichert, R. Preiss, J. Chromatogr. B 672 (1995) 277.
[31] J. Teichert, R. Preiss, Methods Enzymol. 279 (1997) 159.
[32] C.K. Sen, S. Roy, S. Khanna, L. Packer, Methods Enzymol. 299 (1999) 239.
[33] G.K. Ziyatdinova, G.K. Budnikov, V.I. Pogorel'tsev, J. Anal. Chem. 59 (2004) 288.
[34] K. Sugawara, S. Tanaka, K. Hasebe, M. Taga, J. Electroanal. Chem. 347 (1993) 393.
[35] M.N. Abbas, A.A. Radwan, Talanta 74 (2008) 1113.
[36] G.K. Ziyatdinova, L.V. Grigor'eva, G.K. Budnikov, J. Anal. Chem. 64 (2009) 185.
[37] W. Siangproh, N. Wangfuengkanagul, O. Chailapakul, Anal. Chim. Acta 499 (2003) 183.
[38] W. Siangproh, P. Ngamukot, O. Chailapakul, Sens. Actuat. B 91 (2003) 60.
[39] H.L. Persson, A.I. Svensson, U.T. Brunk, Redox Rep. 6 (2001) 327.
[40] T. Yano, D.A. Tryk, K. Hashimoto, A. Fujishima, J. Electrochem. Soc. 145 (1998) 1870.