Application Note 1111

Detection of Lipoic Acid and Dihydrolipoic Acid in Food Supplements Using HPLC-ECD

Ian Acworth and Bruce Bailey, Thermo Fisher Scientific, Chelmsford, MA, USA

Key Words

α-Tocopherol HPLC-ECD, Ascorbic Acid, Dihydrolipoic Acid (DHLA), Glutathione, Lipoic Acid, Ubiquinol

Goal

To develop a rapid and selective HPLC-electrochemical detection (ECD) method for the analysis of α-lipoic acid in complex nutritional supplements

Introduction

α-Lipoic acid (6,8-thioctic acid, 1,2-dithiolane-3-pentanoic acid, or 1,2-dithiolane-3-valeric acid) is a water insoluble compound found in vivo, and is consumed in the form of a supplement as an antioxidant. Lipoic acid was first isolated in the 1950's in the form of lipoamide it is an essential cofactor in the mitochondrial multienzyme complexes that catalyze oxidative decarboxylation of α-ketoacids. It is not essential and can be synthesized by both animals and man, although the exact biochemical pathway remains obscure. Lipoic acid is readily absorbed from the diet and taken up by cells. It is then reduced to DHLA by mitochondrial lipoamide dehydrogenase DHLA is then capable of passing through lipid bilayers.

α-Lipoic acid and DHLA (Figure 1) have received some attention recently for their possible role as antioxidants and regulators of cell function.[1,2] With an E°' = -0.29V, the DHLA/lipoic acid couple can readily regenerate a number of antioxidants from their oxidized forms including glutathione (from glutathione disulfide), ascorbic acid (from dehydroascorbic acid), ubiquinol (from ubiquinone) and indirectly, α-tocopherol (from the α-tocopheroxyl radical).

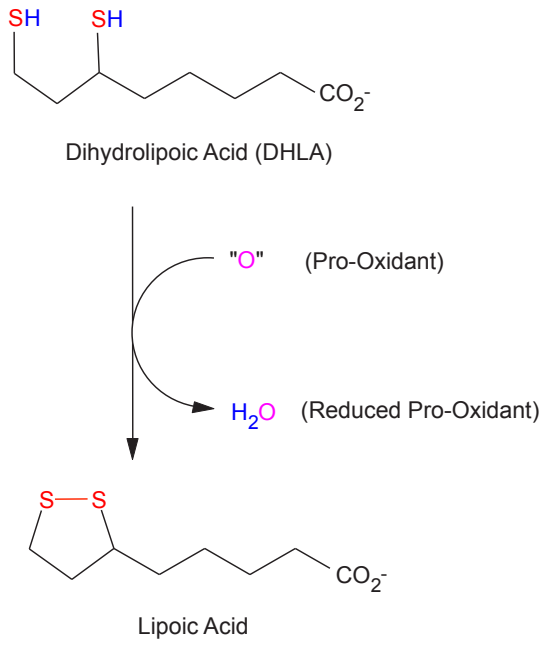

Figure 1. The antioxidant activity of dihydrolipoic acid.

Thermo SCIENTIFIC

α-Lipoic acid supplementation is being advocated in the treatment of AIDS, Chaga, diabetes, heavy-metal poisoning, ischemia-reperfusion injury, liver diseases (e.g., mushroom poisoning and alcoholic liver disease), neurodegenerative disorders, radiation injury, Wilson's disease and the effects of cigarette smoking.[1,3]

This Application Note describes the use of HPLC with coulometric array electrochemical detection for quality control analysis of α-lipoic acid in two nutritional supplement products. One product was in capsule form and contained only one other nutritional component. The other product was in tablet form and was a complex formulation of vitamins and antioxidants. This approach was also extended to the detection DHLA.

Materials and Methods

The isocratic analytical system consisted of a pump, an autosampler, a thermostatic chamber and an eight-channel Thermo Scientific™ Dionex™ CoulArray™ Coulometric Array Detector.

LC Conditions	
Column:	ODS 2, 4.6 × 150 mm, 5 µm
Mobile Phase A:	Water – Acetonitrile – 1.0 M Sodium Phosphate pH 3.5; 60:35:5 (v/v/v)
Flow Rate:	1.0 mL/min
Temperature:	35 °C
Injection Volume:	20 µL
Detector and Conditions	
Detector:	Model 5600A, CoulArray
Applied Potentials:	400, 460, 520, 580, 700, 720 and 820 (mV vs. Pd reference)

Standards

α-Lipoic acid and DHLA were obtained from Calbiochem® (San Diego, CA) and Sigma-Aldrich® (St. Louis, MO), respectively. Stock solutions were made by dissolving 10 mg standard in 10 mL methanol and were stored at –20 °C. Further dilutions were made in methanol.

Sample Preparation

1. Following incision the contents of 10 CoQuinone® capsules were mixed with 250 mL methanol and stirred until dissolved. A working solution was made by diluting the stock solution 1:10 in methanol (v/v). This was passed through a 0.45 µm filter prior to analysis.
2. Ten Mega Antioxidant™ tablets were ground in a coffee grinder. A 300 mg weight of this powder was added to 50 mL chloroform-methanol (1:1, v/v). Following sonication (5 minutes) and shaking (20 minutes) the solution was passed through a 0.45 µm filter prior to analysis.

Results and Discussion

Multi-component analysis using ECD has previously been α-lipoic acid eluted at approximately 5.8 minutes and responded across channels 3–8 with dominant response on sensor 4 (Figure 2). Intra-assay response variability (RSD) for standard was 1.25% (20 µL of 6.6 µg/mL, n = 6). Standard response was linear (r^2 = 0.9964, by least squares regression) over the range studied (1.6–6.9 µg/mL).

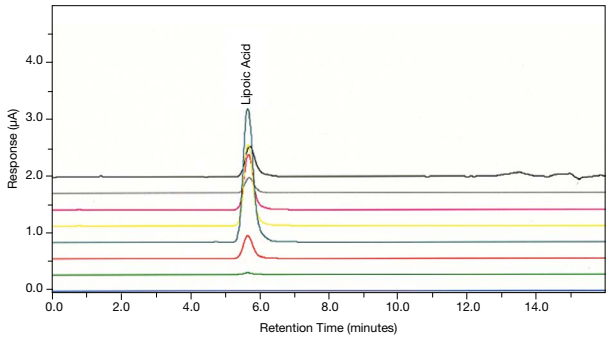

Figure 2. Eight channel chromatogram obtained for α-lipoic acid external standard (20 µL of 2 mg/mL).

A chloroform:methanol extract of Mega Antioxidant tablets is shown in Figure 3. A peak identified as α-lipoic acid responded on the same dominant channel and at the expected retention time. Another major peak was also present in the extract eluting at an earlier retention time. As can be seen this analyte showed a very different electrochemical response profile across the array than α-lipoic acid.

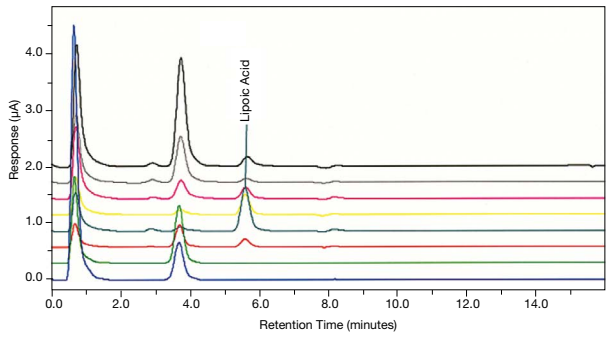

Figure 3. Chloroform-methanol extract of Mega Antioxidant tablets.

A more detailed comparison of the response profiles of the standard and sample peak is shown in Figures 4A and B, respectively. The response ratios calculated between adjacent sensors allowed further confirmation of peak identity and purity.

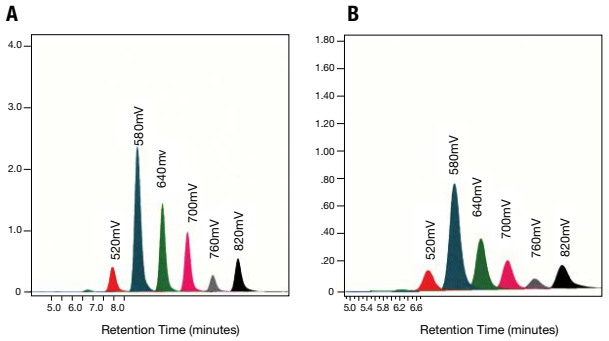

Figure 4. Examination of the response profiles across the coulometric array show that the voltammertric behavior of the sample peak (B) matches that of the standards (A).

Quantitative inter-assay variability for duplicate samples prepared on each of three separate days was 1.1% RSD for CoQuinone and 2.36% RSD for Mega Antioxidant. Average recovery from sample augmented with 3 different levels of standard was within 97–103% of the expected value (r^2 = 0.9964, by least squares regression).

Figure 5 shows resolution of both α-lipoic acid and DHLA standards. DHLA, was more strongly retained under reversed phase conditions than α-lipoic acid. DHLA was oxidized at upstream sensors (not shown) to form a compound that exhibited the same voltammetric profile as that of α-lipoic acid. These data suggest that this technique may also be useful for examining the interconversion of these two substances.

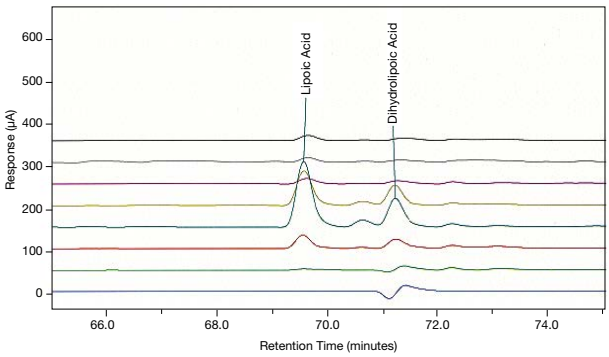

Figure 5. Chromatogram obtained for α-lipoic acid and DHLA standards (0.5 mg/mL). DHLA was oxidized at upstream sensors to form a compound that showed identical voltammetric behavior to α-lipoic acid.

Conclusion

The HPLC-coulometric array method described in this Application Note offers high selectivity, good precision and excellent linearity for the analysis of α-lipoic acid in complex nutritional supplements. Sample preparation and analysis is rapid and straightforward, and suitable for routine quality control analysis. Voltammetric data can be used to demonstrate peak purity and to study the interconversion of oxidized and reduced forms of this analyte.

References

1. Packer, L., Tritschler, H. J., and Wessel, K. (1997). Neuroprotection by the metabolic antioxidant α-lipoic acid. Free Radic. Biol. Med., **22**, 359-378.

2. Roy, S., and Packer, L. (1998). Redox regulation of cell functions by alpha-lipoate: Biochemical and molecular aspects. Biofactors, **7**, 263-267.

3. Packer, L., Witt, E. H., and Trischler, H. J. (1995). Alpha-lipoic acid as a biological antioxidant. Free Radic. Biol. Med., **19**, 227-250.

Acknowledgement

Thank you to Drs. A. B. Rabovsky and J. Cuomo, Research and Development, USANA, Inc., 3838 West Parkway Blvd., Salt Lake City, UT 84120, for their collaboration in this study.

Ordering Information

Description	Part Number
CoulArray, Model 5600A – 8 channel	70-4329
CoulArray Organizer with Temp. Control	70-4340T
Accessory Kit, CoulArray Detector to Accessory Kit, CoulArray Detector to Thermo Scientific™ Dionex™ UltiMate™ HPLC System	70-9191
HPG-3400RS Biocompatible Binary Rapid Separation Pump with two solvent selector valves	5040.0046
WPS-3000TBRS Biocompatible Rapid Separation Thermostatted Autosampler	5841.0020

www.thermosfisher.com/chromatography

Africa +43 1 333 50 34 0
Australia +61 3 9757 4300
Austria +43 810 282 206
Belgium +32 53 73 42 41
Brazil +55 11 3731 5140
Canada +1 800 530 8447
China 800 810 5118 (free call domestic) 400 650 5118
Denmark +45 70 23 62 60
Europe-Other +43 1 333 50 34 0
Finland +358 9 3291 0200
France +33 1 60 92 48 00
Germany +49 6103 408 1014
India +91 22 6742 9494
Italy +39 02 950 591
Japan +81 6 6885 1213
Korea +82 2 3420 8600
Latin America +1 561 688 8700
Middle East +43 1 333 50 34 0
Netherlands +31 76 579 55 55
New Zealand +64 9 980 6700
Norway +46 8 556 468 00
Russia/CIS +43 1 333 50 34 0
Singapore +65 6289 1190
Sweden +46 8 556 468 00
Switzerland +41 61 716 77 00
Taiwan +886 2 8751 6655
UK/Ireland +44 1442 233555
USA +1 800 532 4752

Thermo SCIENTIFIC
A Thermo Fisher Scientific Brand

AN71320-EN 09/16M