Determination of Hydroperoxides Using High Performance Liquid Chromatography with Reductive Electrochemical Detection

Jun Cheng[1], Yan Liu[1] and Khalil Divan[2]
[1]Thermo Fisher Scientific, Sunnyvale, CA, USA
[2]Thermo Fisher Scientific, Hemel Hempstead, Hertfordshire, GB

Poster Note 71984

Overview

Purpose: To develop a new liquid chromatography (LC) method using the Thermo Scientific™ Dionex™ Acclaim™ 120 C18 Reversed-Phase LC Column and electrochemical detection (ED) for analysis of hydroperoxides.

Methods: A Thermo Scientific Dionex ICS-5000 system and reductive electrochemical direct detection with a disposable carbon working electrode was utilized in this application.

Results: The new and improved method provides excellent linearity calibration and limits of detection for analysis of butyl hydroperoxide and cumene hydroperoxide.

Introduction

Determination of hydroperoxides is very important in many research areas range from biochemistry [1], drug development [2], atmospheric chemistry [3] to plastic industrial products [4]. Numerous methods currently exist for the analysis of hydroperoxides [2, 4-6]. Various chromatographic methods have been adapted for analysis of hydroperoxides. High performance liquid chromatography (HPLC) is one of the most prevalent separation techniques utilized for analysis of hydroperoxides [2]. Electrochemical detection is becoming a more and more widely used technique due to its high selectivity and sensitivity, simple direct detection and easy microfabrication [7]. The approach of combining HPLC separation and electrochemical detection technique offers the attractive analytical technique for the determination of hydroperoxides.

Girotti *et al.* reported analysis of hydroperoxides with HPLC and a reductive electrochemical detection. This was a simpler analytical method since it was a direct injection of the sample without any derivertization involved [6, 8]. However, this reductive electrochemical detection had employed a mercury drop electrochemical detection. This type of working electrode is poisonous so care should be taken in its handling. In addition, Surface area of a drop of mercury is never constant. Carbon electrode is an alternative working electrode for reductive electrochemical detection [9-10]. But the electrode can suffer from surface fouling due to progressive adsorption of reduced analytes. In addition, the linear calibration is poor. In this report, we try to optimize the reductive potential and to improve analyte stability and linear calibration with a disposable thin-film carbon working electrode.

Methods

High Performance Liquid Chromatography and Reductive Electrochemical Detection

The HPLC and reductive electrochemical detection was performed using a with a Thermo Scientific Dionex ICS-5000 system. The system consisted of a DP dual gradient pump with on-line eluent degassing, and an AS autosampler with a sample tray set at 15°C (injection loop volume: 20 µl), and an Acclaim 120 C18 column (2.1 x 150 mm) unless otherwise specified. The separation column and ED electrochemical detection cell were placed in two separate chambers of a Column-Detector Module. The column and detection temperatures were set both at 30°C. The titanium cell body of the electrochemical detector was used as the counter electrode across the 50-µm thin-layer channel defined by a gasket cutout (flow channel length and width: 8.91 x 1.27 mm). A combination reference electrode of pH and Ag/AgCl (4 M KCl) was placed downstream from the thin-layer channel.

A disposable thin-film carbon working electrode was mounted against the detector cell body using a holder block and spring-loaded knob. The cell gasket dimensions defining the flow path of the detection cell were as follows: thickness: 0.051 mm; flow channel: 1.2 x 8 mm.

The chromatographic system control, data acquisition and analysis were accomplished using Thermo Scientific Dionex Chromeleon® 7.2 Software.

Standard Preparation

The concentrates of hydroperoxides were prepared by dissolving the hydroperoxides into DI water and diluted to the desired concentrations with DI water for injections.

Results and Discussion

Analysis of two organic hydroperoxides, t-butyl hydroperoxide (BHP) and cumene hydroperoxide (CHP) was pursued without any derivatization reactions. The reductive ED detection was performed with a disposable carbon electrode by applying a negative constant potential.

Figure 1 shows the overlaid chromatograms obtained with various citrate buffers for eluent optimization with detection potential -1.4 V and 1 mM BHP and CHP standard.

Methanol concentration was optimized to be 55% (vol.%) with the best separation from oxygen peak and solubility of BHP, CHP and citrate buffer before the optimization of citrate buffer composition (data are not shown).

Eluent A: 100 mM Citric acid in water/methanol (45/55, v/v); Eluent B: 100 mM trisodium citrate in water/methanol (45/55, v/v).

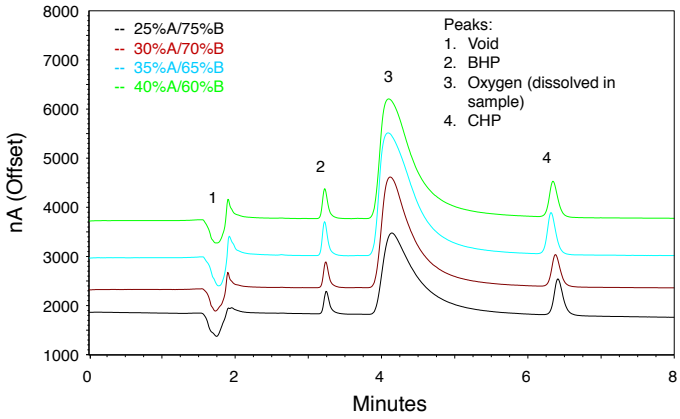


FIGURE 1. Overlaid Chromatograms Obtained with Various Eluents Detection Potential: -1.4 V; Standard Concentration: 1 mM

Thermo SCIENTIFIC

The results shown in Table 1 show that eluent was optimized to be 35 mM citric acid/65 mM trisodium citrate with the best response. The retention time and noise were not affected significantly by the variation of citrate buffer.

Table 1. Summary Results from Overlaid Chromatograms in Figure 1

Eluent	Area (nA.min)		Height (nA)	
	BHP	CHP	BHP	CHP
25%A/75%B	40.81	459.27	104.74	734.43
30%A/70%B	45.48	523.25	88.89	647.52
35%A/65%B	60.20	688.71	113.28	843.52
40%A/60%B	52.68	606.32	97.98	723.30

Table 2. Calibration Results from Detection Potential Optimization

Det. Pot. (V)	Response / Conc., BHP (nA.min/mM)					Response / Conc., CHP (nA.min/mM)				
	0.01	0.1	1	10	Max/ Min	0.01	0.1	1	10	Max/ Min
-1.0	N.D.	3.71	3.77	3.84	1.04	N.D.	4.53	6.24	6.69	1.48
-1.1	N.D.	6.48	6.97	7.24	1.12	N.D.	8.65	11.13	12.11	1.40
-1.2	14.26	11.49	12.52	11.60	1.24	16.27	17.48	19.94	20.18	1.24
-1.3	35.79	16.70	19.04	17.07	2.14	36.24	26.57	30.08	30.40	1.36

From the results in Table 2, detection potential was optimized to be -1.2 V with the best linear calibration results and good limits of detection for both BHP and CHP.

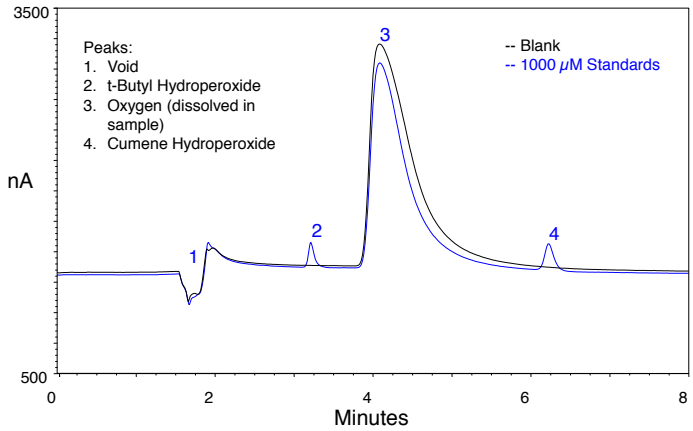


FIGURE 2. Overlaid Chromatograms of Blank and 1000 µM t-Butyl Hydroperoxide and Cumene Hydroperoxide.

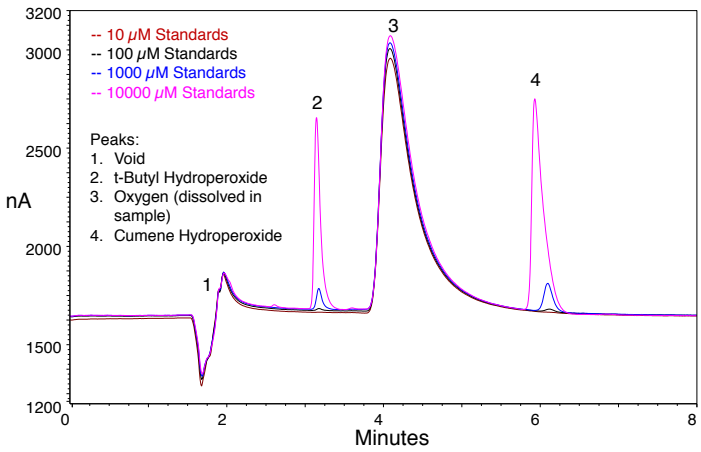
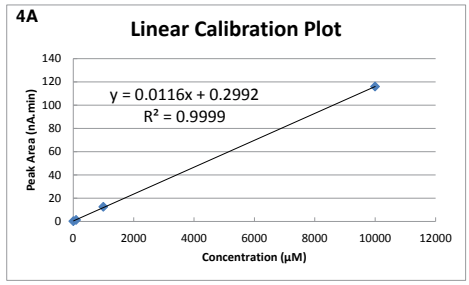


FIGURE 3. Overlaid Calibration Chromatograms of t-Butyl Hydroperoxide and Cumene Hydroperoxide.

An overlay of chromatograms of two organic hydroperpoxides and of a blank (water) is shown in Figure 2. At the optimized detection potential of -1.2 V, in order to obtain the linear calibration results of the two hydroperoxides, we had run the 10, 100, 1000 and 10000 µM standards and plotted the obtained chromatograms in Figure 3.

4A

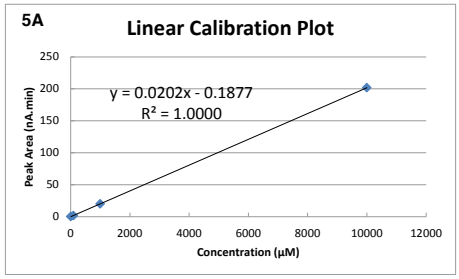


4B

Conc. (µM)	Area (nA.min)
10	0.14
100	1.15
1000	12.52
10000	116.00

FIGURE 4A and 4B. Calibration Plot and Calibration Data of Butyl Hydroperoxide Obtained with the Optimized Experimental Conditions and Disposable Carbon Electrodes

5A



5B

Conc. (µM)	Area (nA.min)
10	0.16
100	1.75
1000	19.94
10000	201.80

FIGURE 5A and 5B. Calibration Plot and Calibration Data of Cumene Hydroperoxide Obtained with the Optimized Experimental Conditions and Disposable Carbon Electrodes

Analytical performance obtained with disposable carbon electrodes is summarized in Table 3. The calibration results are from the data shown in Figures 4 and 5. The limits of detection (LOD) are calculated as 3x the noise level in concentration units for 20 µL injections. The detection limits of BHP and CHP are 8.5 and 9.5 µM, respectively.

Table 3. Calibration Results and Limits of Detection of Butyl Hydroperoxide and Cumene Hydroperoxide Obtained with the Optimized Experimental Conditions and Disposable Carbon Electrodes

Analyte	Linear Calibration Range (µM)	Correlation Coefficient	LOD (µM)
Butyl Hydroperoxide	10-10000	0.9999	8.5
Cumene Hydroperoxide	10-10000	1.0000	9.5

Conclusion

- Liquid chromatography with reductive electrochemical detection is a highly sensitive and selective analytical technique for determination of butyl hydroperoxide and cumene hydroperoxide
- The method based on the Acclaim 120 C18 column and optimized eluent conditions improves the determination of butyl hydroperoxide and cumene hydroperoxide significantly
- The new and improved method provides excellent linear calibration and limits of detection for determination of butyl hydroperoxide and cumene hydroperoxide
- Detection potential requires more optimization for further improvement on the response reproducibility

References

1. H. W. Gardner, *J. Agric. Food Chem.*, **1979**, *27 (2)*, 220-229 and references cited therein.
2. D. P. Elder, D. Snodin and A. Teasdale, *J. Pharm.Biomed. Anal.,* **2010**, *51*, 1015-1023 and references cited therein.
3. A. V. Jackson and C. N. Hewitt, *Crit. Rev. Env. Sci. Tec.*, **1999**, *29(2)*, 175-238 and references cited therein.
4. J. Scheirs, D. J. Carlsson and S. W. Bigger, Polym.-Plast. Technol. Eng., 1995, 34(1), 97-116 and references cited therein.
5. D. E. Henderson, A. M. Slickman and S. K. Henderson, *J. Agric. Food Chem.*, **1999**, *47 (7)*, 2563-2570.
6. W. Korytowski, P. G. Geiger and A. W. Girotti, *J. Chromatogr. B*, **1995**, *670*, 189-197.
7. J. Wang, *Talanta*, **2002**, *56(2)*, 223-231 and references cited therein.
8. W. Korytowski, G. J. Bachowski and A. W. Girotti, *Anal. Biochem.*, **1993**, *213*, 111-119.
9. W. Korytowski, G. J. Bachowski and A. W. Girotti, *Anal. Biochem.*, **1991**, *197*, 149-156.
10. K. Yamada, J. Terao and S. Matsushita, *Lipids*, **1987**, *22(2),* 125-128.
11. J. Cheng and P. Jandik, *J. Chromatogra. A*, **2008**, *1198-1199*, 148-153.

www.thermofisher.com

Thermo Fisher Scientific, Sunnyvale, CA USA is ISO 9001 Certified.

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 2730 3006
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 10 3292 200
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

PN71984-EN 0216S

Thermo SCIENTIFIC
A Thermo Fisher Scientific Brand