Application Note

Food, Beverage,

Health care

Hydrogen Peroxide

The finest LC-EC Applications for Food & Beverage analysis

Phenols
Bisphenol A
Catechins
Flavonoids
Phenols
Antioxidants
Resveratrol
Epicatechin
Quercetin
Other polyphenols

Carbohydrates
Monosaccharides
Lactose
Mono- and Disaccharides
Other oligo- and Polysaccharides

Vitamins, minerals etc.
A, C, D, E, and K
Iodide
Q10, ubiquinols

Hydrogen Peroxide

- **'Green' method using narrow-bore SweetSep™ AEX20**
- **Fast and sensitive HPAEC-PAD analysis**
- **SenCell™ with Au working electrode**
- **Hydrogen peroxide in health care products (toothpaste)**

Summary

Hydrogen peroxide is a strong oxidizing agent and disinfectant used in a wide variety of applications, such as chemical synthesis, propellant (space rockets), water treatment, food processing, cosmetics, personal and health care products [1].

In this application note an analytical method is presented for the measurement of hydrogen peroxide in liquid samples using the ALEXYS Carbohydrate Analyzer in combination with the new narrow-bore SweetSep™ AEX20 column. The method is based on separation by High Performance Anion Exchange Chromatography followed by Pulsed Amperometric Detection (HPAE-PAD) on a gold working electrode. The use of a 2.1 mm HPAEC column with 5 µm particle size, allowed the separation of hydrogen peroxide in less than 4 min in combination with a four-fold reduction of mobile phase usage compared to the use of a more traditional column. A commercially available whitening toothpaste, containing peroxide as bleaching agent, was analyzed as an example to demonstrate the applicability of the method to real samples.

Introduction

Hydrogen peroxide (H_2O_2) is a small molecule consisting of two hydroxy groups joined by a covalent oxygen-oxygen single bond. Pure hydrogen peroxide is a transparent liquid with a slightly pale blue color. Hydrogen peroxide is unstable and slowly decomposes into oxygen gas and water with the evolution of heat. The decomposition is accelerated in the presence of UV light. Although non-flammable, at higher concentrations it can cause spontaneous combustion when it comes in contact with organic material. Hydrogen peroxide is a strong oxidizing agent and disinfectant used in a wide variety of applications, such as chemical synthesis, propellant (space rockets), explosives, water treatment, food processing, paper industry, cosmetics, personal and health care products.

A range of different LC methods are available to measure hydrogen peroxide in various matrices. These methods are based on derivatization followed by either conductivity [2], UV [3] and fluorescence detection [4-6]. Derivatization is necessary due to the lack of a chromophore in hydrogen peroxide.

Ion chromatography in combination with electrochemical detection is the method of choice: it combines good selectivity with sensitive detection [6-8]. Moreover, hydrogen peroxide can be directly detected using electrochemical detection without the need of derivatization in both DC and PAD mode. Several HPAEC methods for quantification of hydrogen peroxide are reported in literature based on conventional anion-exchange columns with particle sizes in the range of 6.5 - 10 µm [7,8].

Figure 1. ALEXYS Carbohydrate Analyzer—isocratic, consisting of the ET 210 eluent tray (for N2 blanketing), a P 6.1L pump with solvent selection valve and dual channel degasser, AS 6.1L autosampler and DECADE Elite electrochemical detector.

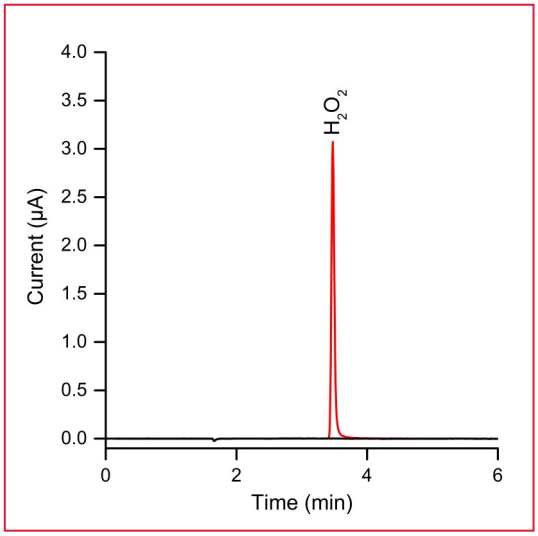

Figure 2. Chromatogram obtained from an 2 µL injection of a 10 mg/L (10 ppm) standard of hydrogen peroxide in water (red curve). Black curve: blank (DI water).

In this application note a 'green' HPAEC-PAD method is presented using the new narrow-bore AEX20 anion-exchange column with 5 µm particles for the fast and sensitive analysis of hydrogen peroxide. A commercially available whitening toothpaste, containing peroxide as bleaching agent, was analyzed as an example to demonstrate the applicability of the method to real samples.

Table 1.

Conditions	
LC system	ALEXYS Carbohydrate Analyzer
Detector	DECADE Elite electrochemical detector
Columns	SweetSep™ AEX20, 2.1 x 200 mm column, 5 µm SweetSep™ AEX20, 2.1 x 50 mm precolumn, 5 µm Borate ion trap, 4 x 50 mm column, 10 µm (Antec Scientific)
Mobile phase (MP)	100 mM KOH prepared and blanketed with Nitrogen 5.0 gas
Flow rate	0.2 mL/min
Back pressure	About 165 bar
Injection	2 µL (partial loop fill)
Temperature	35 °C for separation and detection
Flow cell	SenCell with Au WE, stainless steel AE and HyREF, AST 2
Potential waveform (4-step)	E1, E2, E3, E4: +0.1, -2.0, +0.6, -0.1 V ts, t1, t2, t3, t4: 0.2, 0.4, 0.02, 0.01, 0.07 s
I-cell	about 0.2 - 0.4 µA
ADF	0.1 Hz
Range	0.05, 5 and 50 µA/V

Method

The analysis was performed using an ALEXYS Carbohydrate Analyzer –isocratic (Fig 1.). The pump in this system has the option to switch between two mobile phases during a run, thus enabling step-gradient profiles. For detection, a DECADE Elite electrochemical detector with SenCell flow cell was used. The system was controlled via a PC using Clarity Data System software. The LC-EC conditions are listed in Table 1. Figure 2 shows an example chromatogram of a hydrogen peroxide standard. The ET210 is a dedicated eluent tray with integrated pneumatic components for sparging and blanketing of mobile phase with an inert gas to keep it free from dissolved CO_2 and the formation of carbonate ions (CO_3^{-2}). Carbonate ions bind strongly to the anion exchange groups on the stationary phase and interferes with anion retention, causing shortened retention times, decrease in column selectivity and loss in resolution.

Separation

The narrow-bore SweetSep™ AEX20 column and precolumn with an internal diameter of 2.1 mm were chosen for the separation of hydrogen peroxide. The innovative new stationary phase of this column consists of a highly monodisperse 5 µm ethylvinylbenzene-divinylbenzene copolymer (80% crosslinked) coated with functionalized nanoparticles with dual ion exchange sites (quaternary amine + tertiary amine). These features result in high-resolution separation and low consumption of mobile phase. The fact that the separation is performed at 35°C allows the column to be placed in the DECADE Elite oven compartment, eliminating the need of an external column thermostat.

The analysis is based on isocratic elution using 100 mM KOH as mobile phase. To minimize the introduction of carbonate ions in the mobile phase, the eluents were carefully prepared manually using a 45% w/w KOH solution (commercially available). The diluent was DI water (resistivity >18 MΩcm) which was sonicated and sparged with nitrogen 5.0 (purity >99.999%) prior to use. The mobile phase should be prepared in plastic bottles instead of glass, as KOH is a strong etching agent. Otherwise, the KOH will react with the inner glass wall resulting in the release of silicates and borates.

The appropriate amount of 45% w/w KOH solution was carefully pipetted into the diluent under gentle stirring and Nitrogen sparging when preparing the mobile phase. The bottles with mobile phase and column clean-up solution were blanketed with nitrogen (0.2-0.4 bar overpressure) on the ET210 eluent tray during the analysis, to minimize the build-up of carbonate ions in the mobile phase and to assure a reproducible analysis.

A column clean -up /regeneration step with 200 mM KOH can be applied in case of loss of retention to remove bound carbonate ions or late eluting compounds like oligo- and polysaccharides, which might be present in real samples. See reference [9] for an example of step-gradient elution in HPAEC -PAD.

Detection

For the pulsed amperometric detection of hydrogen peroxide the Antec SenCell electrochemical flow cell is used. This novel flow cell [10] has a confined wall-jet design and consists of an Au working electrode (WE), HyREF (Pd/ H_2) reference electrode (RE) and stainless steel auxiliary electrode (AE). A 4-step potential waveform was applied as shown in figure 3. The

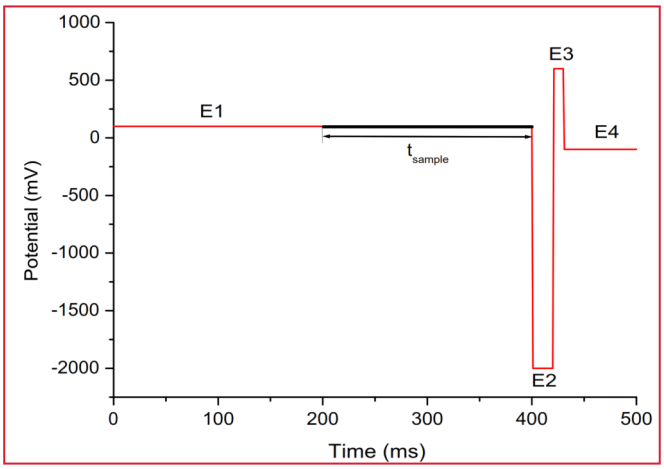

Figure 3. 4-step PAD potential waveform for the detection mono-saccharides and other carbohydrates.

temperature for detection was set to 35 °C. The cell current was typically about 0.2 - 0.4 µA with these PAD settings under the specified conditions. This particular 4-step waveform with a pulse duration of 500 ms has been claimed to have as benefits: (1) a consistent long-term peak area response and (2) minimal electrode wear [11], resulting in less flow cell maintenance and system down time.

Preparation of standards and samples

Standards: a 1000 mg/L H_2O_2 stock solution was prepared by pipetting 283 µL of a 31.6 wt% H_2O_2 solution (Acros Organics, Germany) into a 100 mL volumetric flask, which was brought to volume with DI water. Working standards in the range of 0.1 mg/L to 100 mg/L were made in the same manner using 10 mL volumetric flasks using the appropriate dilution factors.

Sample preparation: Two commercial tooth paste samples were analyzed. The following sample prep procedure was used for the analysis:

Procedure:

- 100 mg of toothpaste was weighted and transferred into a 10 mL volumetric flask.
- The volumetric flash was filled with approximately 5 mL of DI water and gently mixed to dissolve the toothpaste.
- Subsequently, the flask was brought to volume with water.
- A part of the solution was transferred into a 5 mL plastic syringe and filtered over a 0.20 µm PES (Polyethersulfone) syringe filter (GVS life sciences, Sanford, USA.
- The filtered solution was 10 or 100 times diluted with water and transferred into a 1.5 mL plastic polypropylene vial, from which 2µL was injected into the LC system and analyzed.

Results

In figure 2 an overlay is shown of two chromatograms obtained with a 2 µL injection of a 10 ppm H_2O_2 standard in water (red curve and a water blank injection (black curve). H_2O_2 is eluting within four minutes without coeluting interferences. The H_2O_2 peak has a plate number of more than 100.000 plates/meter and a tailing factor of 1.2.

Linearity, repeatability and LOD

The linearity of the H_2O_2 response was investigated in the concentration range of 0.1—10 mg/L and 10—100 mg/L, which corresponds with a molar concentration range of 2.9 µmol/L - 2.9 mmol/L (figure 4). In both the low and high concentration ranges the linearity was excellent with correlation coefficients better than 0.999. In fact the response was linear over the complete concentration range between 0.1 and 100 mg/L, which is significantly better than reported in ref [8]. When measuring larger concentrations, it is advised to decrease injection volume or use AST setting 3 to extend the linear range.

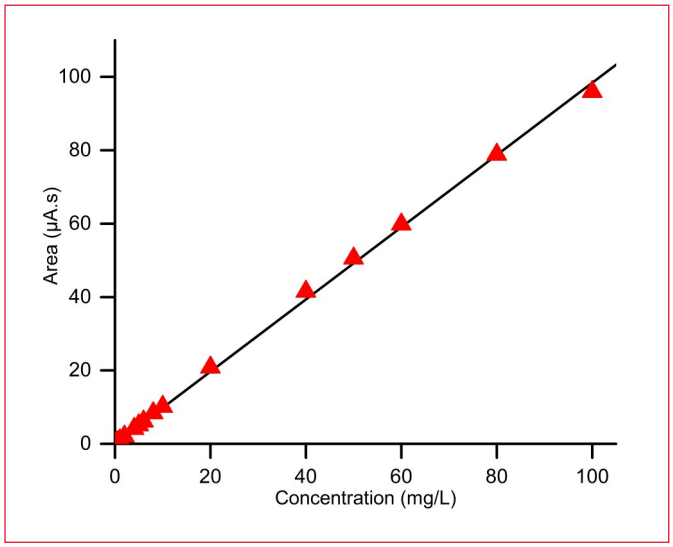

Figure 4. Calibration curve of hydrogen peroxide in the concentration range of 0.1 - 100 mg/L. (R = 0.9996).

The repeatability of the method and system was evaluated by ten repetitive injections with a 0.1, 1 and 10 mg/L H_2O_2 standard. The relative standard deviations (RSD) for peak area were 2.1, 0.7 and 0.7 %, respectively, demonstrating the good repeatability of the method. It is evident that the RSD for the lowest concentration was higher, but still close to 2%.

The Limit of Detection (LOD) was determined based on the response of the 0.1 mg/L H_2O_2 standard with the detector set at the 50 nA/V range. The LOD was calculated as the analyte response corresponding to 3x the ASTM noise (average peak-to-peak baseline noise of 12 segments of 0.5 min). The noise was calculated based on a 6 minute section of the baseline during a run (after elution of H_2O_2 starting at t = 4 min). A LOD of 9 µg/L (260 nmol/L, 0.5 pmol on-column) was found, demonstrating the outstanding sensitivity of the method.

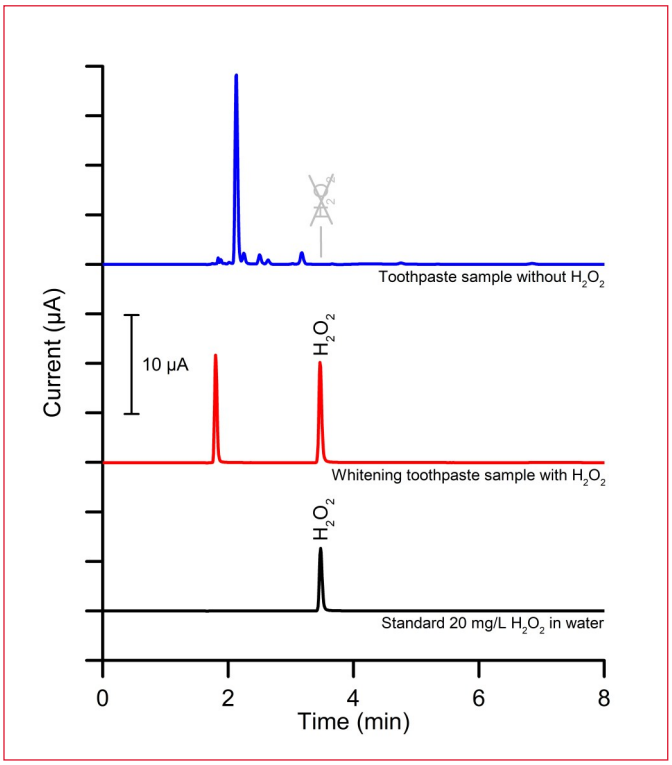

Figure 5. Top: chromatogram of a 2 µL injection of the toothpaste sample without peroxide (blue), middle: whitening toothpaste with peroxide (red), and bottom: 20 mg/L standard.

Sample analysis

Two commercial toothpaste samples were analyzed using this method:

- Whitening toothpaste containing 4% peroxide
- Normal toothpaste without peroxide

The sample preparation of the toothpaste samples is simple and described in the previous section. The chromatograms of the toothpaste samples are shown in figure 4 together with a chromatogram of a 20 mg/L H_2O_2 standard for reference and identification. It is evident from figure 5 that the whitening toothpaste contained hydrogen peroxide.

The concentration of H_2O_2 in the worked-up sample was calculated using the 10-100 mg/L calibration curve and was 34 mg/L undiluted. This amount corresponds with 35 mg H_2O_2 / gram toothpaste (4 wt%) which matches the labeled 4% hydrogen peroxide and confirms the accuracy of the method. The control sample (normal toothpaste without peroxide) did not contain any measurable amount of peroxide. However, the chromatogram of the control sample showed several other unidentified compound peaks. These peaks/compounds did not interfere or coelute with H_2O_2. One of the ingredients mentioned on the content label of the control sample is sorbitol. Sorbitol is a sugar alcohol with a sweet taste which is often used as sweetener. Toothpaste manufacturers add it to toothpastes to create a sweet flavor without leading to tooth decay. Due to the fact that oral bacteria can not metabolize sorbitol, it is not converted to cavity-causing acids and, therefore, the teeth are protected. Sorbitol is a fast eluting sugar alcohol. Although no sorbitol standard was injected for identification, it is very likely that the large peak at 2.1 min is due to sorbitol.

References

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Conclusion

The ALEXYS HPAEC-PAD system based on the DECADE Elite detector, SenCell flow cell and a narrow-bore SweetSep™ AEX20 column, offers a simple and sensitive analysis solution for the quantification of hydrogen peroxide with minimal sample preparation. The presented HPAEC-PAD method allows fast separation, within 4 min, of hydrogen peroxide followed by direct PAD detection without the need for derivatization. A four-fold reduction of mobile phase usage was achieved by using a 2.1 mm ID column instead of a standard bore version. The method was successfully applied for the analysis of hydrogen peroxide in a commercial whitening toothpaste sample.

Ordering information

Detector only	
176.0035B*	DECADE Elite SCC electrochemical detector
116.4321	SenCell 2 mm Au HyREF
ALEXYS analyzer	
180.0055W	ALEXYS Carbohydrate Analyzer - isocratic
116.4321	SenCell 2 mm Au HyREF
195.0035#	Clarity CDS single instr. incl LC, AS module
Columns	
260.0011	SweetSep™ AEX200, 2.1x200mm column, 5µm
260.0016	SweetSep™ AEX200, 2.1x50mm precolumn, 5µm
260.0030	Borate ion trap, 4x50mm column, 10µm
260.0100**	Pre-column filter PEEK, 0.5 µm

*) For the DECADE Elite electrochemical detector control drivers are available in DataApex Clarity CDS, Thermo Fisher Scientific Chromeleon™ CDS, Waters Empower™, Agilent OpenLab CDS and Agilent OpenLab CDS Chemstation Edition. Please contact Antec for more details.

#) The ALEXYS Carbohydrates Analyzer and ALEXYS FDG analyzer can also be fully controlled under Thermo Fisher Scientific Chromeleon™ CDS. Please contact Antec for more details.

**) In case samples might contain particulate matter it is advised to use a pre-column filter.

For research purpose only. The information shown in this communication is solely to demonstrate the applicability of the ALEXYS system and DECADE Elite detector. The actual performance may be affected by factors beyond Antec's control. Specifications mentioned in this application note are subject to change without further notice.

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