Application Note

Food & Beverage

The finest LC-EC applications for Food & Beverage analysis

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

**Carbohydrates**
Monosaccharides
Lactose
Other oligo- and polysaccharides

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

# Iodide in Milk

- **FlexCell with exchangeable working electrode**
- **Diamond working electrode**
- **Robust and reproducible analysis**

## Summary

In this application note a sensitive and reliable LC-ECD method is presented for the analysis of iodide based on DC amperometry using a flow cell with a diamond working electrode. Conductive diamond has several advantages over conventional electrode materials such as a wide potential window in aqueous solutions, excellent chemical inertness and stability.

## Introduction

Iodine is an essential trace element for humans. It is a component of the thyroid hormone thyroxin, which regulates the body's metabolic state and the growth and development in children. Iodine-deficiency is an important health problem throughout much of the world. The dietary uptake of sufficient trace amounts of iodide is necessary for mental and physical development. Important sources of iodide are seafood, diary products like milk, iodized table salt and processed foods like iodized bread [1].

HPLC in combination with electrochemical detection is commonly used for the analysis of iodide in urine [2,3] and food products [4,5]. Those methods used a silver working electrode for detection of iodide. In thiss note we show the analysis of iodide in dairy products (with similar separation conditions), but with detection on a conductive boron doped diamond (BDD) electrode.

## Method

### Separation

The mobile phase and similar separation conditions were used as described in literature [4,5].

### Detection

Various working potential settings (Ecell) were tested with a 10 µM KI solution in water to find the optimal value. The so-called hydrodynamic voltammogram was constructed for the potential range between 900 and 2400 mV. The onset of the curve plateau (limiting current) was observed at 2000 mV, but a lower potential of 1500 mV was chosen for detection. At 1.5 V a low background current (<30 nA) and low noise level were attained in combination with a good iodide detection sensitivity.

### Online electrochemical regeneration/ activation procedure

It has been observed that online electrochemical regeneration/ activation is an effective method to restore the detection sensitivity in case the response of the BDD electrode attenuates over time, or when the electrode has not been used for a while. The flow cell does not have to be disassembled for on-line electrochemical regeneration. The online regeneration procedure consists of setting the detector to SCAN-mode and the slowly sweeping the working potential between – 3 Volts and + 3 Volts at a scan rate of 50 mV/s while running mobile phase through the cell.

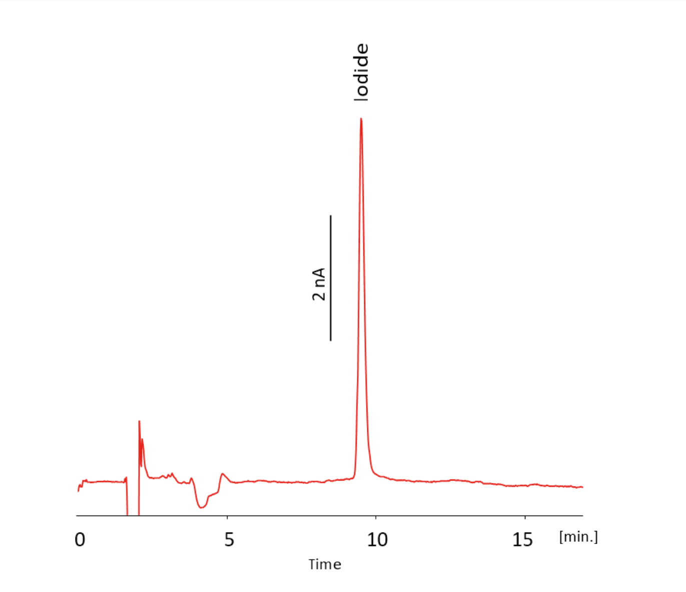

**Figure 1:** Chromatogram of a standard of 400 nM of potassium iodide (KI) in water (50.8 µg/L Iodide).

**Table 1**

| Conditions | |
|---|---|
| LC system | ALEXYS Iodide Analyzer |
| Columns | Thermo Scientific™ Hypersil™ ODS-2 C18, 150 x 3.0 mm ID, 3 µm |
| Mobile phase (MP) | 10 mM di-natrium-hydrogenphosphate dodecahydrate, pH set to 6.80 with $H_3PO_4$, 1 mM hexadecyltrimethylammonium hydroxide, 32% acetonitrile |
| Flow rate | 0.4 mL/min |
| Injection | 20 µL |
| Temperature | 35 °C (separation and detection) |
| Flow cell | FlexCell™ with boron doped diamond (BDD) working electrode and HyREF™ |
| Ecell | 1.5 V |
| I-cell | about 10 - 30 nA |
| ADF | 0.5 Hz |
| Range | 2 µA/V |

## Results

In Figure 1 an example chromatogram is shown of 50.8 µg/L potassium iodide in water. The peak efficiency for the iodide peak was 69.000 theoretical plates/meter.

### Linearity and detection limits

Calibration curves were recorded with Iodide standards in the concentration range between 6 µg/L – 1.3 mg/L. Within this concentration range a linear detector response was observed with correlation coefficients better than 0.999 (Fig. 2).

A concentration detection limit (cLOD) of approximately 3 nM iodide (0.4 µg/L) was found, demonstrating the excellent detection sensitivity achieved with the BDD electrode. The cLOD is based on an injection volume of 20 µL and defined as the concentration that gives a signal that is three times the peak- to-peak noise.

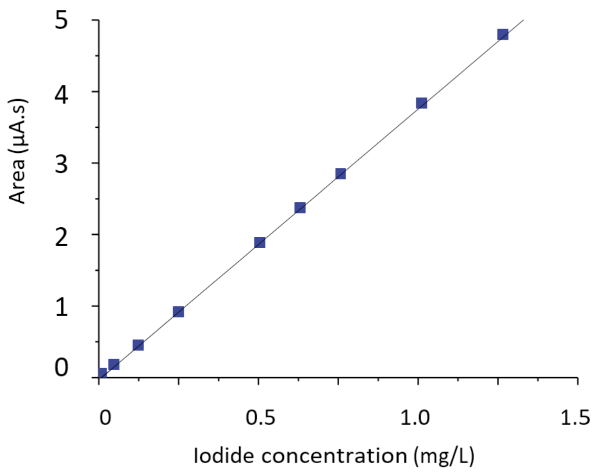

**Figure 2:** Calibration plot for Iodide in water.
Concentration range 6 µg/L - 1.3 mg/L. R = 0.9999. For conditions see Table 1

### Repeatability

The repeatability of the method was evaluated by executing 10 repetitive injections of a 0.13 mg/L and 1.23 mg/L iodide solution. The relative standard deviation (RSD%) for retention time, peak area and height are given in Table 2.

The relative standard deviation for both peak height and peak area are 1% or better at a concentration of 0.13 mg/L and 0.2-0.3% at 1.26 mg/L.

The long-term repeatability was assessed with a standard with a higher iodide concentration of 13.7 mg/L (Fig. 3). 225 repetitive injections were performed in a time period of 22

**Table 2**

Relative Standard Deviations (n=10)

| *Parameter* | *0.13 mg/L iodide* | *1.26 mg/L iodide* |
|---|---|---|
| Retention time | 0.1% | 0.1% |
| Peak area | 0.9% | 0.2% |
| Peak height | 1.0% | 0.3% |

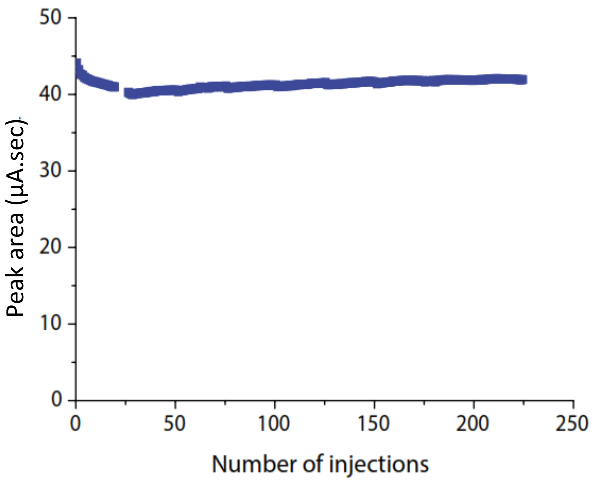

**Figure 3:** Long-term repeatability of DC amperometric detection of iodide on a BDD electrode. Sample concentration 13.7 mg/L iodide in water. After the 20th injection a gap is visible due to 6 analysis from an empty vial. For conditions see Table 1

hours (Fig 5). For this experiment the flow rate was increased with a factor 2 (0.8 mL/min, system backpressure approximately 180 bar) to reduce the analysis time to only 6 minutes). The relative standard deviations for the peak area was only 1.4%. This value demonstrates the good response stability for BDD electrodes, even at high iodide concentrations.

## Iodide in milk products

In Figure 4 an example chromatogram of a low-fat (1.5%) milk sample is shown.

*Sample preparation procedure:*

1. 1 mL 3% acetic acid was mixed with 5 mL of sample for deproteination of the milk solution. The acidified solution was centrifuged and the supernatant collected.
2. The supernatant was passed over a RP SPE column (Alltech C18-fast column) for further clean-up of the milk matrix (removal of fat).
3. The first 2 mL of the eluate were discarded
4. 20 µL of the eluate was injected in the LC system for analysis.

*It should be stated that this sample preparation procedure is not validated and is only used to obtain a series of example chromatogram for this application note.*

It is evident from Figure 4 that the iodide peak is well separated from the matrix. The iodide peak in the chromatogram was confirmed to be identified correctly by analyzing an additional spiked milk-sample. The concentration of iodide in the milk sample was estimated to be 45 µg/L.

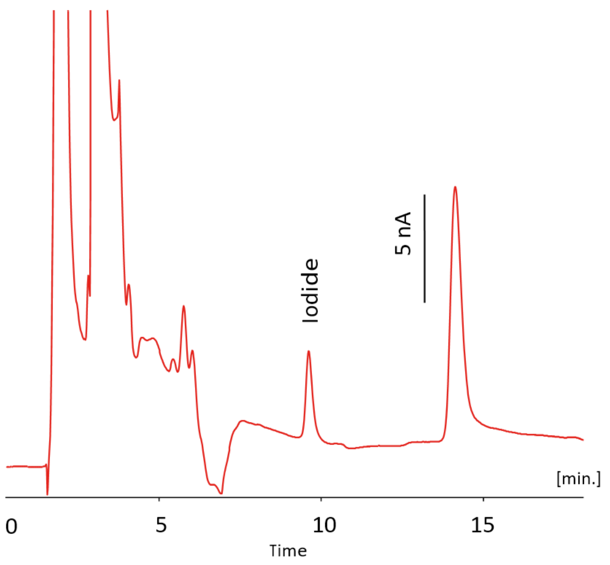

**Figure 4:** Chromatogram of Iodide in low-fat (1.5%) milk sample. For conditions see Table 1

## References

1. John T. Dunn, "Editorial: What's happening to our Iodine?", J Clin Endocrinol Metab., 83(10), 1998, 3398-400
2. K.Han, W. Koch, K.W. Pratt, "Improved procedure for the determination of Iodide by Ion Chromatography with Electrochemical detection", Anal. Chem., 59, 1987, 731-736
3. J. Rendl, S. Seybold, W. Borner," Urinary Iodide determined by Paired-Ion Reversed Phase HPLC with Electrochemical detection, Clin. Chem., 40/6, 1994, 908-913
4. "Determination of iodide content in milk and dried milk by HPLC", International IDF standard, 167:1994
5. "Milk and dried milk – Determination of iodide contents – method using HPLC", International ISO standard, 14378:2000
6. W.R. LaCourse, "Pulsed Electrochemical Detection in High Performance Liquid Chromatography", John Wiley & Sons, New York, 1ed,1997.
7. Alexander Kraft, "Doped diamond: A compact review on a new, versatile electrode material", Int. J. Electrochem. Sci., 2, 2007, 355 - 385

## Conclusion

The ALEXYS Iodide Analyzer provides a user-friendly and reliable solution for the determination of iodide by means of DC amperometry. With the use of the BDD electrode, iodide can be detected with excellent reproducibility and sensitivity.

**Figure 6**: Recommended instrument configuration for this application: the ALEXYS Iodide Analyzer.
The system consists of a P6.1L pump with integrated degasser, an AS6.1L autosampler, and the DECADE Elite electrochemical detector. The ALEXYS Iodide Analyzer can be operated under different Chromatography Data System (CDS) software: DataApex™ Clarity™ CDS (version 8.3 and up) or Thermo Scientific™ Chromeleon™ CDS (version 7.2 SR 5 and up).

## Ordering information

| | |
|---|---|
| ***Detector only*** | |
| 176.0035A | DECADE Elite SCC electrochemical detector |
| 102.4305M | FlexCell BDD HyREF |
| ***Recommended ALEXYS analyzer*** | |
| 180.0095W | ALEXYS Iodide Analyzer |
| 102.4305M | FlexCell BDD HyREF |
| ***Software*** | |
| 195.0035[#] | Clarity CDS single instr. incl LC, AS module |

#) optional: Antec ECD drivers for use with Chromeleon CDS , OpenLAB CDS or OpenLAB Chemstation CDS are available.

***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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