Application Note
Food & Beverage

The finest LC-EC Applications for food & beverage analysis fver processed

- **Bisphenol A**
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- **Phenols**
- **Antioxidants**
- **Polyphenols**
  - Resveratrol
  - Epicatechin
  - Quercetin
  - other polyphenols
- **Carbohydrates**
- **Iodide**
- **Vitamins A, C, D, E, and K**
- **Q10**
- **ubiquinols**

Iodide in milk

- **Flexcell with echangeable working electrode**
- **Diamond working electrode**
- **Robust & reproducible Analysis**

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 therefore necessary for mental and physical development. Important sources of Iodide are seafood, diary products, 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].

Summary

In this application note a sensitive and reliable LC-EC 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.

Figure 1: ALEXYS Iodide Analyzer.

Method

The ALEXYS Iodide analyzer is equipped with a DECADE Elite electrochemical detector and Flexcell with replaceable diamond electrode disc. The LC conditions used with the ALEXYS iodide analyzer were selected with the focus on the analysis of iodide in dairy products. Therefore, the same mobile phase and similar separation conditions were used as described in reference [4,5].

Table 1

LC-EC Conditions	
HPLC	ALEXYS Iodide analyzer (part no 180.0095E)
Flow rate	0.4 mL/min
Flow cell	Flexcell™ with MD™ WE and HyREF™
Temperature	35°C for separation and detection
Range	2 µA/V
ADF	0.5 Hz
I-cell	10 - 30 nA

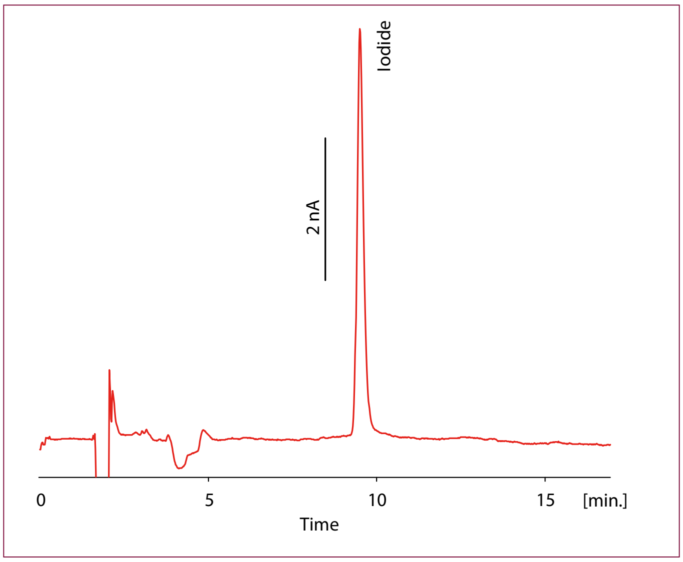

Figure 2: Chromatogram of a 400 nM of potassium iodide (KI) in water (50.8 µg/L Iodide).Injection volume 20 µL.

A hydrodynamic voltammogram was recorded in the potential range between 900 and 2400 mV (vs. HyREF) to determine the optimum detection potential, using a 10 µM KI solution in water. Although the onset of the limiting current was observed at 2000 mV, a lower potential of 1500 mV was chosen for detection. At this potential a low background current (< 30 nA) and noise level are attained in combination with a good detection sensitivity for Iodide. In figure 2 an example chromatogram is shown of 50.8 µg/L Potassium Iodide in water (injection volume 20 µL). The peak efficiency for the iodide peak was 69.000 theoretical plates/meter.

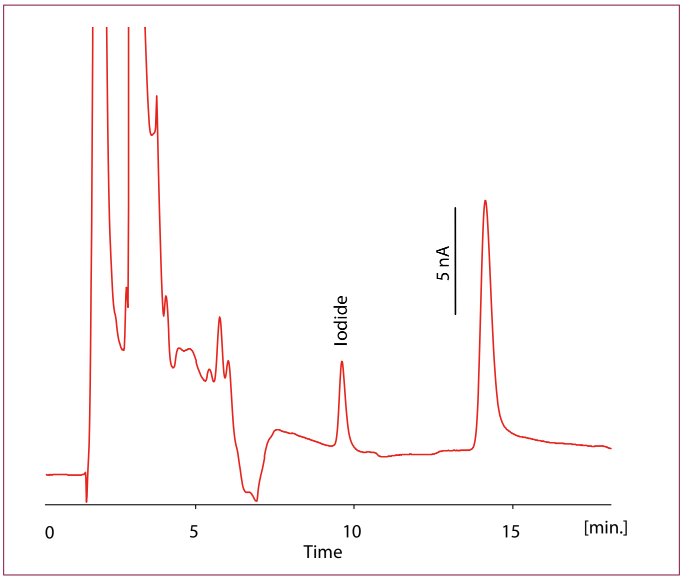

Figure 3: Chromatogram of Iodide in low-fat (1.5%) milk sample. Injection volume 20 µL.

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.

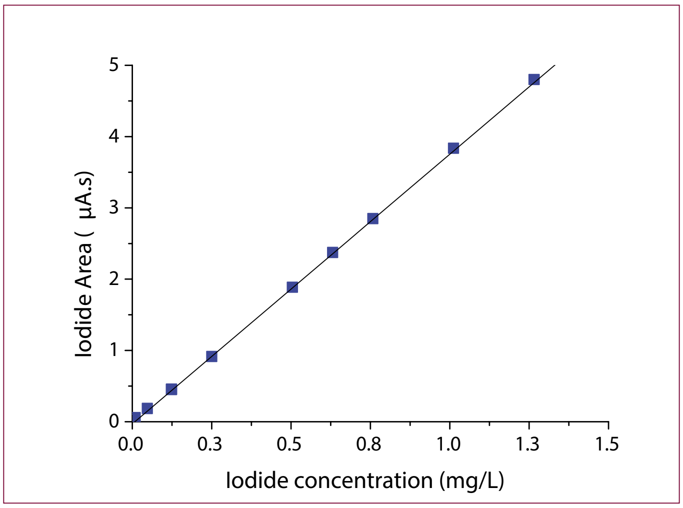

Figure 4: Calibration plot for Iodide in water. Concentration range 6 µg/L – 1.3 mg/L . R = 0.9999.

A concentration detection limit (C_{LOD}) of approximately 0.4 µg/L (3 nM) was found, demonstrating the excellent detection sensitivity achieved with the MD electrode. The C_{LOD} 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.

Repeatability

The repeatability of the method was evaluated by executing 10 repetitive injections (20 µL injection volume) 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 listed in table I for both concentrations.

Table 2

Repeatability (n=10)			
Iodide(mg/L)	tR	Area	Height
0.13	0.1	0.9	1.0
1.26	0.1	0.2	0.3

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 high Iodide concentration of 13.7 mg/L. 225 repetitive injections were performed in a time period of 22 hours, see figure 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 (6 minutes). After the 20th injection a gap of 6 injections is visible due to an empty vial.

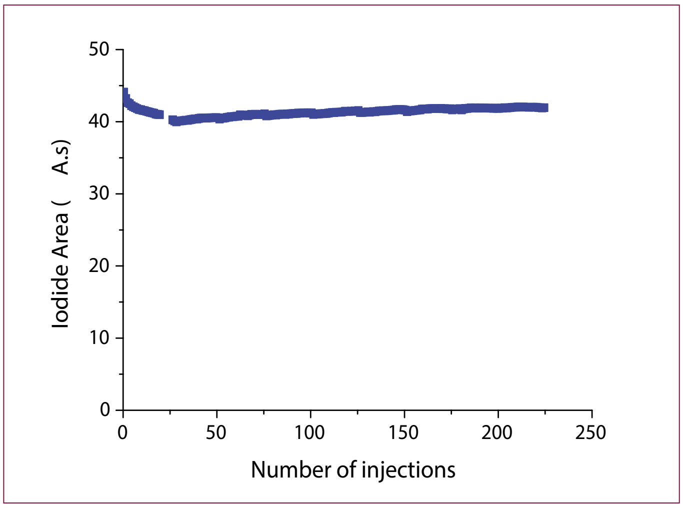

Figure 5: Long-term repeatability of DC amperometric detection of Iodide on a MD electrode. Sample concentration 13.7 mg/L, injection volume 20 µL.

The relative standard deviations (RSD%) for the peak area was 1.4%. It demonstrates the good response stability of MD, even at high iodide concentration.

It has been observed that online electrochemical regeneration/ activation is an effective method to restore the detection sensitivity in case the response of the Magic Diamond electrode attenuated over time, or the electrode has not been used for a while. The flow cell does not have to be disassembled in this case. During the online regeneration procedure the detector was operated in the SCAN mode. Typically, the electrode was scanned between – 3 Volts and + 3 Volts in mobile phase, with a scan rate of 50 mV/s..

Iodide in Milk Products

In figure 3 an example chromatogram of a low-fat (1.5%) milk sample is shown. The following sample preparation procedure was performed prior to injection

- 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.
- Subsequently, the supernatant was passed over a RP SPE column (Alltech C18-fast column) for further clean-up of the milk matrix (removal of fat).
- The eluate (first 2 mL discarded) was collected and 20 uL injected in the LC system.

It should be stated that the sample preparation procedure described above is not validated and is only used to obtain a series of example chromatograms.

The iodide peak in the chromatogram was identified by spiking a second milk sample prior to sample work-up with a known amount of Iodide, corresponding to a concentration of 317 µg/L. It is evident from figure 3 that the Iodide peak is well separated from the matrix. The concentration of Iodide in the milk sample was estimated to be 45 µg/L.

Conclusion

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

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
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4. "Determination of iodide content in milk and dried milk by HPLC", *International IDF standard*, 167:1994
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6. W.R. LaCourse, "Pulsed Electrochemical Detection in High Performance Liquid Chromatography", *John Wiley & Sons, New York,* **1**^{ed},1997.
7. Alexander Kraft, "Doped diamond: A compact review on a new, versatile electrode material", *Int. J. Electrochem. Sci.*, **2**, 2007, 355 - 385

Ordering number

180.0095E	ALEXYS Iodide analyzer
250.1104	ALE-315 column, 150x3.0mm, 3um C18