SHIMADZU

Application News

Spectroscopy – AAS

**No.** SCA-120-031

# Species-Analysis of Organo-Magnesium-Compounds in case of Chlorophyll and its Derivates by using HPLC-FAAS

Chlorophylls were analyzed for a long time. But still it is an uncertain field for reliable analysis. Chlorophylls can be found in a wide variety of structures and concentrations. Because of the structure the chlorophylls are active for UV/Vis-radiation. Therefore the high performance liquid chromatography (HPLC) with a photodiode array detector (PDA) is used in the most experiments, as this technique can detect different chlorophylls with a good sensitivity.

However, accurate quantification just can be ensured if reference standard is used for calibration. Those substances cannot be purchased for all chlorophyll derivatives. Chlorophyll a and b are the only available chlorophyll standards.

The objective of this study was to develop a method to separate various chlorophyll derivatives and to quantificate them with high assurance.

This application segment describes how to analyze chlorophylls in extracted leaf samples. The research was performed with the coupling of HPLC Prominence Series with the Atomic Absorption Spectrophotometer AA-7000 in flame mode.

Keywords such as sample preparation, system and method parameters outline user expectations in this application segment.

## ■ Sample preparation

At first the samples have to be dried. The best way to ensure species trueness is the lyophilization. After this the homogenization of the sample has to be done, using mortar or laboratory mills. The dry matter is measured and can be extracted with methanol as extracting agent. The extraction time was 20 hours with exclusion of light. The methanol extract is not very stable [1] and should be measured with HPLC-FAAS, immediately. For more stable standards Dimethylformamide can be used[2]. The problem with this agent is the production of $NO_X$ in the flame, which can also absorb light in the measurement range. As a consequence, the measurement has worse resolution.

Tab 1: Standard System Parameters

| Parameter | Setting |
|---|---|
| **AAS** | |
| Flame | Air/Acetylene |
| Air | 15 L/min |
| Acetylene | 2.7 L/min |
| Burner height | 6.5 mm |
| Wavelength | 285.2 nm |
| Slit width | 0.7 nm |
| Background Correction | $D_2$-BGC |
| **HPLC** | |
| Column<br>- Particle Size<br>- Length | C18<br>4,5 µm<br>150 mm |
| Isocratic Eluent | Methanol: Ethylacetate: ddWater (64,6:30,4:5; v:v:v) |

## ■ Instrumental parameters

Before coupling the AAS, it has to be optimized for magnesium and the eluent. After this the coupling of HPLC with FAAS can be done. Therefore the analog output has to be connected with the HPLC Controller. The next step is the connection between the columns ending with the nebulizer capillary. Before measurement the flame has to be

ignited and the eluent needs to be equilibrated.

## ■ Calibration and method characterization

With the assumption that the atomization in the flame is species independent thus species true, an external calibration can be done. Therefore a conventional magnesium standard can be used. Because of a wide variety of expected peak areas, a non-equidistant calibration was done.

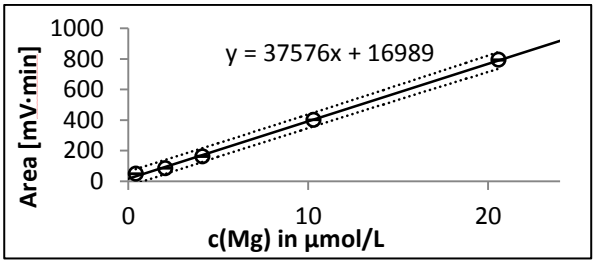


Fig. 1: Non-equidistant calibration

Additionally an external calibration was aimed because the resonance line for magnesium is free of spectral inter-ferences in a wide range.
With the adjusted parameters the method was characterized by sensitivity (slope), process standard deviation, relative standard deviation of 30 times repetition of a 100 µg/L magnesium standard and the limit of detection (LOD).

Tab 2: Method Perameters

| Parameter | Result |
|---|---|
| Sensitivity [µV · L · mol-1] | 37576 |
| Process standard deviation [µV · min] | 11767 |
| Relative standard deviation [%] | 3,4 |
| Limit of detection [µmol/L] | 0,178 |

## ■ Results and Conclusion

Using the HPLC-FAAS coupling to examine chlorophylls, the HPLC prominence series and AA-7000 can be used. With the described parameters all chlorophyll derivatives could be determined.

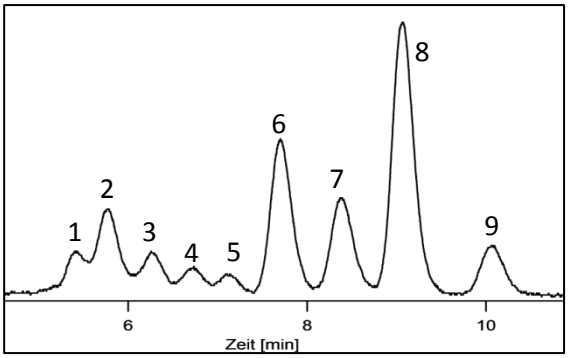


Fig. 2: Chlorophyll chromatogram

Tab 3: Quantification results

| # | Chlorophyll | Concentration [µmol/L] |
|---|---|---|
| 1 | HO-Chl b | 8,5 |
| 2 | Chl b | 19,7 |
| 3 | Chl b‘ | 9,2 |
| 4 | Unknown | 5,6 |
| 5 | Unknown | 3,7 |
| 6 | HO-Chl a | 35,1 |
| 7 | HO-Chl a‘ | 23,2 |
| 8 | Chl a | 63,8 |
| 9 | Chl a‘ | 12,0 |

[1] R. Mantoura, C.Llewellyn, Analytica Chimica Acta, Bd. 151, pp. 297-314, 1983.

[2] S. W. Jeffrey, R. F. C. Mantoura, S. W. Wright, Bd. 10, Paris: UNESCO Pub., 1997, p. 661.