



## Determination of Stevioside and Rebaudioside A in beverages and sweetener using TLC-MS

### Abstract

HPTLC-MS (High Performance Thin Layer Chromatography – Mass spectrometry) is a very useful analytical technique with low-level sample preparation shown in the example of determination of stevioside and rebaudioside A in beverages and food additives. Rebaudioside A and stevioside are the main ingredients of the Stevia plant extract. Rebaudioside A is the sweetest, least bitter glycoside of Stevia plants. Therefore, in Stevia products, a high proportion of the material is desirable. As of today a purity of 98 % is the highest possible concentration of rebaudioside A in Stevia products.

The stevia leaves were extracted in water and filtered with a 0.45 syringe filter. Coke® and the isotonic drink were decarbonized by ultrasonic and filtered with a 0.45 µm syringe filter. The sweetener was solved in water. After chromatographic separation the analyte was eluted from the TLC plate with a TLC-MS interface, transferred and finally detected with the mass spectrometer. A second plate was derivatized with anisaldehyde-sulfuric acid reagent.

### Results

Stevioside (hRf = 64, m/z = 828.5, Fig. 1 and 2) and Rebaudioside A (hRf = 56, Fig. 1) were detected under UV light (366nm) and identified by TLC-MS.

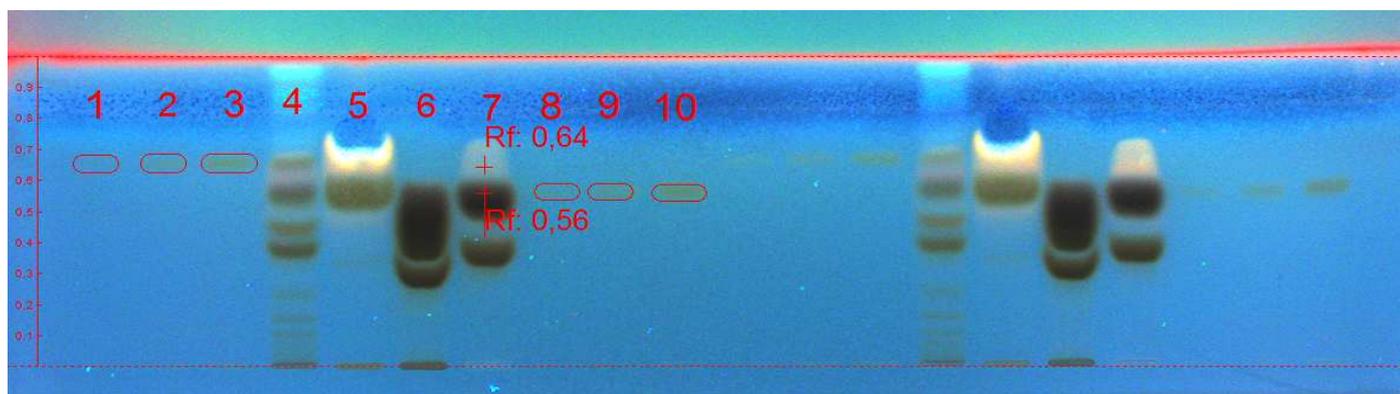


Fig. 1: Developed and derivatized plate at 366 nm. Chromatographic data shown on next page.

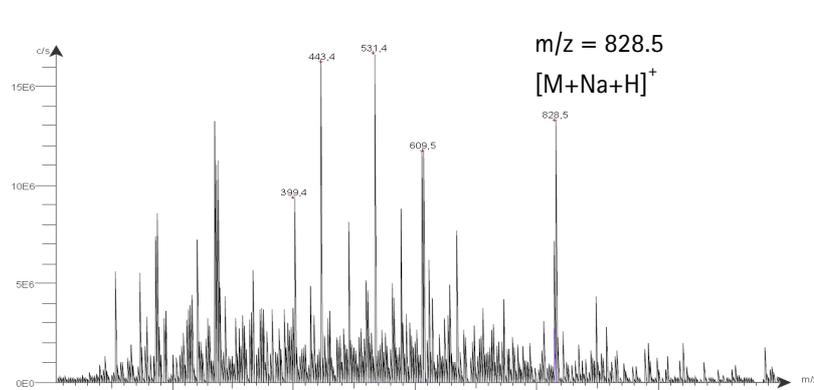
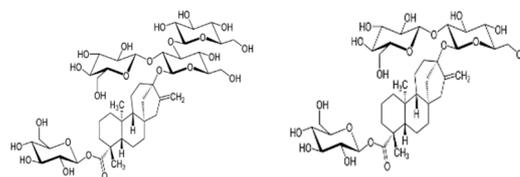


Fig. 2: mass-spectrum of stevioside



Rebaudioside A

Stevioside

In the mass spectra of rebaudioside A and stevioside there is no difference visible by measurement with a single-quadrupole MS

m/z = 828.5  
[M+Na+H]<sup>+</sup>

## Conclusion

These results demonstrate that stevioside and rebaudioside A can easily be analyzed by HPTLC-MS with a minimum of sample preparation even in a complex matrix like stevia plant, coke® and isotonic drinks.

| Chromatographic data |                         |               |                         |     |                   |
|----------------------|-------------------------|---------------|-------------------------|-----|-------------------|
| Track                | Compound                | Conc. [mg/mL] | Application volume [μL] | hRf | Detected mass m/z |
| 1, 2, 3, 11, 12, 13  | Stevioside Standard     | 1.0           | 0.5, 1.0, 2.0           | 64  | 828.5             |
| 4, 14                | Stevia plant            |               | 2                       |     | 828.5             |
| 5, 15                | Sweetener               |               | 2                       |     | 828.5             |
| 6, 16                | Coke®                   |               | 2                       |     | 828.5             |
| 7, 17                | Isotonic drink          |               | 2                       |     | 828.5             |
| 8, 9, 10, 18, 19, 20 | Rebaudioside A Standard | 1.0           | 0.5, 1.0, 2.0           | 56  |                   |

## Application data

|                |                      |                                                                                                                                                                                                           |
|----------------|----------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Chromatography | Plate                | HPTLC Silica gel 60 F <sub>254</sub> S, 20x10cm                                                                                                                                                           |
|                | Sample preparation   | Stevia leaves: extracted in water and filtered with an 0.45μm syringe filter;<br>Sweetener: dissolved in water;<br>Coke® and isotonic drink were decarbonized and filtered with an 0.45 μm syringe filter |
|                | Sample application   | ATS 4 sample applicator (Camag), 6 mm bandwise                                                                                                                                                            |
|                | Application volume   | 0.5, 1.0 and 2.0 μL                                                                                                                                                                                       |
|                | Mobile phase         | Ethylacetate / Glacial acid / Formic acid / Water 100/11/11/27                                                                                                                                            |
|                | Migration distance   | 5 cm                                                                                                                                                                                                      |
|                | Migration time       | 29 min                                                                                                                                                                                                    |
| Extraction     | Extraction equipment | Plate Express (Advion)                                                                                                                                                                                    |
|                | Extraction solvent   | Acetonitrile / Water 95/5 (v/v) + 0.1% Formic acid                                                                                                                                                        |
|                | Extraction flow      | 0.2 mL/min                                                                                                                                                                                                |
| Detection      | Wavelength           | UV light at 366 nm                                                                                                                                                                                        |
|                | Staining             | Anisaldehyde-sulfuric acid reagent                                                                                                                                                                        |
|                | MS equipment         | Single-quadrupole mass spectrometer expression CMS (Advion)                                                                                                                                               |
|                | MS detection         | ESI (+) mode (m/z 50 - 1200)                                                                                                                                                                              |

## Ordering Information

|                                                                   |           |
|-------------------------------------------------------------------|-----------|
| HPTLC Si60 F <sub>254</sub> S, 20x10 cm                           | 1.15696   |
| Ethylacetate gradient grade for liquid chromatography LiChrosolv® | 1.00868   |
| Glacial acid EMSURE®                                              | 1.00063   |
| Formic acid LiChrosolv®                                           | 1.00264   |
| Water for chromatography (LC-MS Grade) LiChrosolv®                | 1.15333   |
| Acetonitrile hypergrade for LC-MS LiChrosolv®                     | 1.00029   |
| Formic acid for analysis EMSURE                                   | 1.00264   |
| Millex®-FH filter, 0.45 μm hydrophobic PTFE, 25 mm, non-sterile   | SLFH025NS |