Case Study:
Quantitative determination of steviol glycosides

Introduction

HPTLC is a powerful technique with regard to high sample throughput capacity, short analysis time, and low running costs. Our case study demonstrates a rapid characterization of Stevia formulations by a selective derivatization of steviol glycosides and additionally, it shows the concept of confirming identity of the different steviol glycosides by coupling with mass spectrometry.

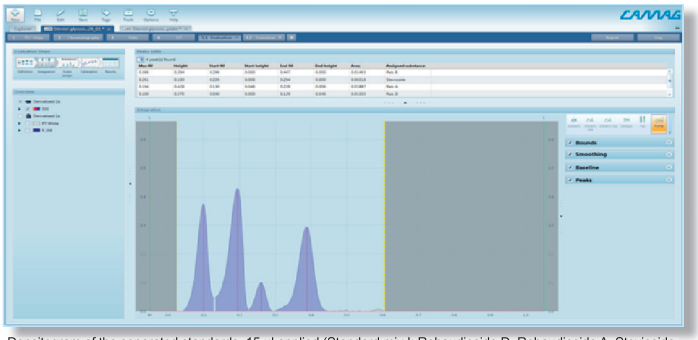
Challenge

Often chromatographic techniques require a time and cost intensive sample preparation step. In many cases HPTLC allows the analysis of samples after a minimal sample preparation (e.g. dissolving) in combination with the separation of several samples side by side. Quantification of the separated compounds is possible by densitometry using CAMAG's TLC Scanner. In addition, the identity can be proven by eluting the spot of interest using CAMAG's TLC-MS Interface and coupling it to any mass spectrometer.

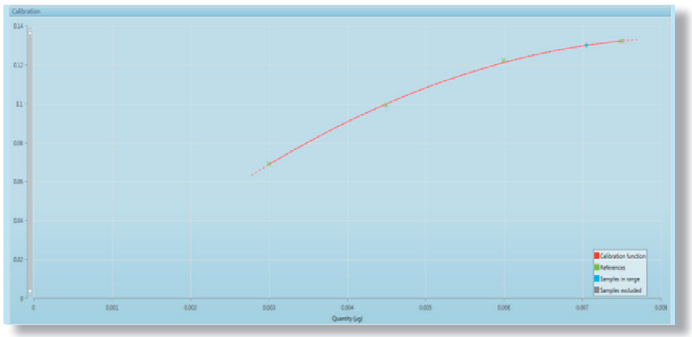
Description of the procedure & documentation

Sample and standard preparation are performed according to Morlock et al. (Journal of Chromatography A, 1350 (2014) 102-111 and CBS 109, p.10-12). All parameters (conditions for sample application, chromatogram development, and evaluation) are logged into the visionCATS 2.0 software, which controls all major CAMAG instruments needed for the HPTLC analysis. The samples and standards are applied band-wise on the HPTLC plate using the ATS 4, which guarantees precise and reliable application. Then the HPTLC plate is developed using the ADC 2. For the analysis of steviol glycosides the developed plate is dipped into β-naphtol reagent, heated at 120°C for 5 minutes, and documented by the TLC Visualizer under white light and UV 366 nm. For quantification each track is scanned in absorption mode at 500 nm with the TLC Scanner and evaluated with visionCATS software. If further investigations of separated sample components are of interest, their eluted zones can be analyzed with other techniques like MS, NIR, and NMR (off-line hyphenation).

The following images show the results: densitogramm, calibration curve and image comparison of Stevia formulation samples, standards and leaves after derivatization, and HPTLC-MS full scan mass spectrum of Rebaudioside A.



Densitogram of the separated standards, 15 µl applied (Standard mix I: Rebaudioside D, Rebaudioside A, Stevioside, Rebaudioside B)



Calibration curve of Rebaudioside A

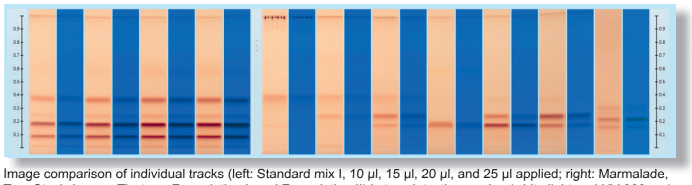
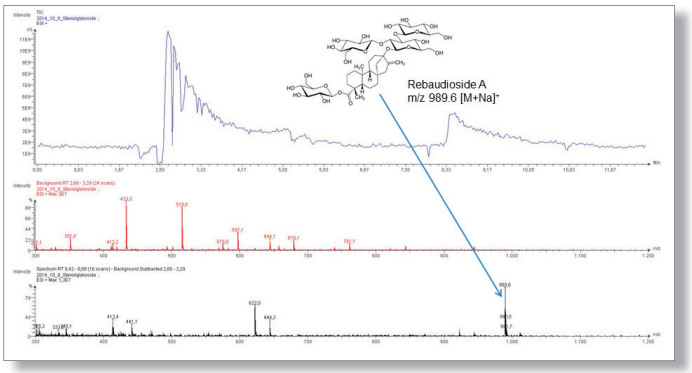


Image comparison of individual tracks (left: Standard mix I, 10 µl, 15 µl, 20 µl, and 25 µl applied; right: Marmalade, Tea, *Stevia* leaves, Tincture, Formulation I, and Formulation II) in two detection modes (white light and UV 366 nm)



HPTLC-MS full scan mass spectrum of Rebaudioside A zone showing most pronounced signals at m/z 989.6 [M+Na]+

Benefits of using modern HPTLC for the analysis of food samples

- HPTLC visualizes at the first glance the similarities and differences between samples and references.
- By using internal or external chemical reference standards compounds can be quantified precisely with the TLC Scanner.
- HPTLC is also used for assays (potency), determination of purity (adulteration/fraud), and stability studies (shelf life).
- The entire sample is detectable on the plate and allows evaluation of all components even if some of them remain at the application zone or in the solvent front.
- HPTLC can analyze samples with high matrix content because of the disposable stationary phase.
- HPTLC can be used as high throughput and low-cost technique for the analysis of food samples.
- Due to the non-destructive nature of the chromatographic method, analytes can be eluted after separation from the plates using CAMAG's TLC-MS Interface and analyzed by hyphenated techniques (e.g. MS, NIR, NMR).