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Application News

High Performance Liquid Chromatography

# ELSD and UV - Complementary Detectors for the HPLC Analysis of Commercial Stevia Sweeteners

## Introduction

Stevia, *stevia rebaudiana bertoni,* is a plant of the Compositae family native to Paraguay whose leaves have been used for centuries as a sweetener. Recently, it has been introduced as a crop in the United States and Canada in response to increased interest in natural foods. In its leaves, Stevia produces several sweet diterpene glycosides, which are non-glycemic, yet range in sweetness from 30 to 320 times that of sucrose.[1] Four major Stevia diterpene glycosides are recognized in the literature: stevioside, rebaudioside A, rebaudioside C and dulcoside A. In addition to these, previously identified leaf constituents include volatile oil components, sterols, triterpenes, flavonoids, coumarins, and non-glycosidic diterpenes (sterebins). Research interest in Stevia has been oriented toward developing genetic lines in which levels of sweet-tasting glycosides are maximized and non-glycosidic diterpenes are minimized.[1,2]

Reversed phase HPLC with UV detection at 210nm has been used to determine constituents in commercial Stevia products, but the methodology is limited in its ability to separate all components of interest, especially when higher order oligosaccharides are present. Another problem is that any single UV wavelength is a detection compromise as the constituents exhibit a wide range of absorbance maxima (193, 204, 236, 238, 284nm). Some are only weakly chromophoric.[3]

The Evaporative Light Scattering Detector (ELSD) is a valuable complement to spectroscopic detectors for HPLC. The ELSD makes a measurement of photons scattered from semi- and non-volatile particles that have been dried of mobile phase through evaporation. Because its response is independent of the light absorbing properties of molecules, it can reveal sample components that UV detectors miss and provide a more accurate profile of relative component abundance than is possible with a spectroscopic detector.[4]

For this study, a normal-phase methodology with complementary detectors, ELSD and multi-wavelength UV (PDA), reveals a wide range of constituents in commercial Stevia products in a single run.

## Sample Preparation and Method

Commercial Stevia product A, a water solution, was prepared by using a 2:1 dilution with water. Commercial Stevia product B, a crystalline solid, was prepared by weighing 50mg into a 10mL volumetric flask and diluting to the mark with water. The mixture was sonicated for 2 minutes. Stevioside was identified by authentic injection of the standard. Experiments using ELSD-LT evaporation temperatures from 28 - 40°C showed no variation in detector response to the Stevia constituents, indicating that precise adherence to drift tube temperature is not critical to detection accuracy and reproducibility.

## Analytical Conditions

| | |
|---|---|
| Mobile Phase: | A: acetonitrile, B: 0.04% ammonium hydroxide |
| Gradient: | (Time, B)(0,15)(60,65) |
| Column: | Shimadzu Premier Carbohydrate, 5μm, 250x4.6mm |
| Injection Volume: | 5μL |
| Flow Rate: | 1mL/min. |
| Column Temp: | 30°C |
| Detector Settings | Gain = 6; Temp = 40°C; Press. = 350kPa |

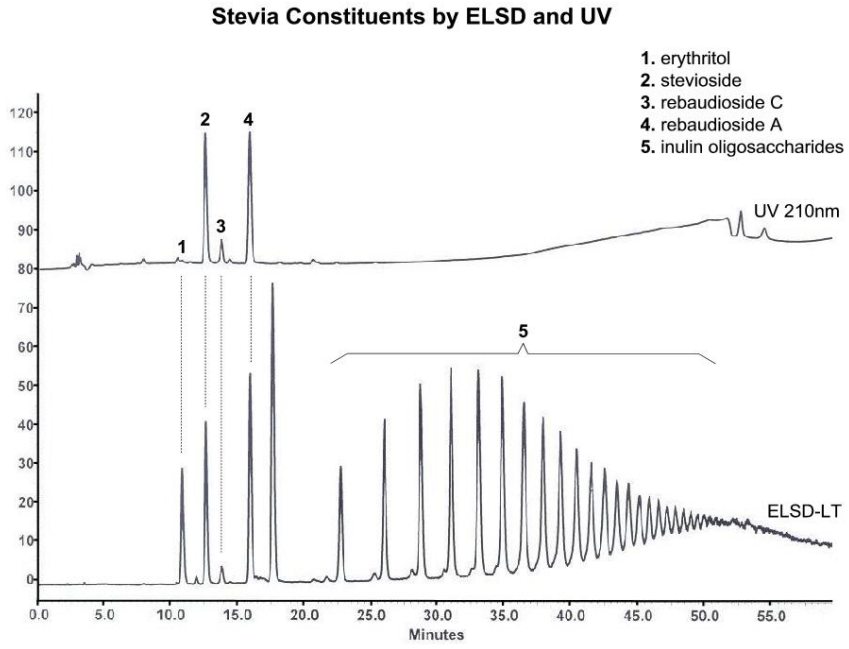


**Figure 1:** Simultaneous UV (210nm) and ELSD-LT chromatograms obtained for commercial Stevia product B containing erythritol, Steviol glycosides and inulin oligosaccharides.

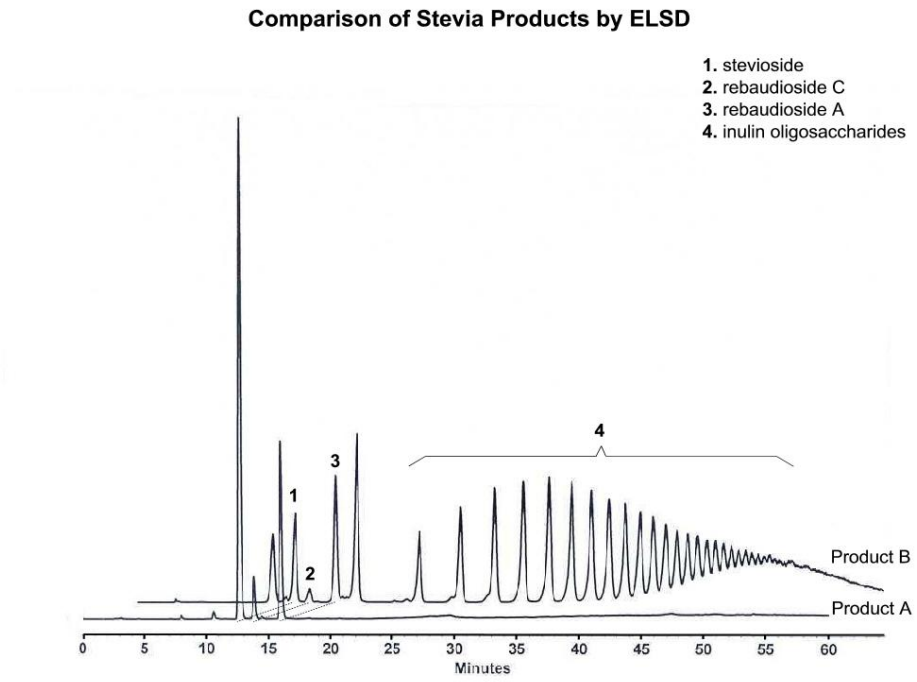


**Figure 2:** ELSD-LT chromatograms obtained for commercial Stevia products, A and B.

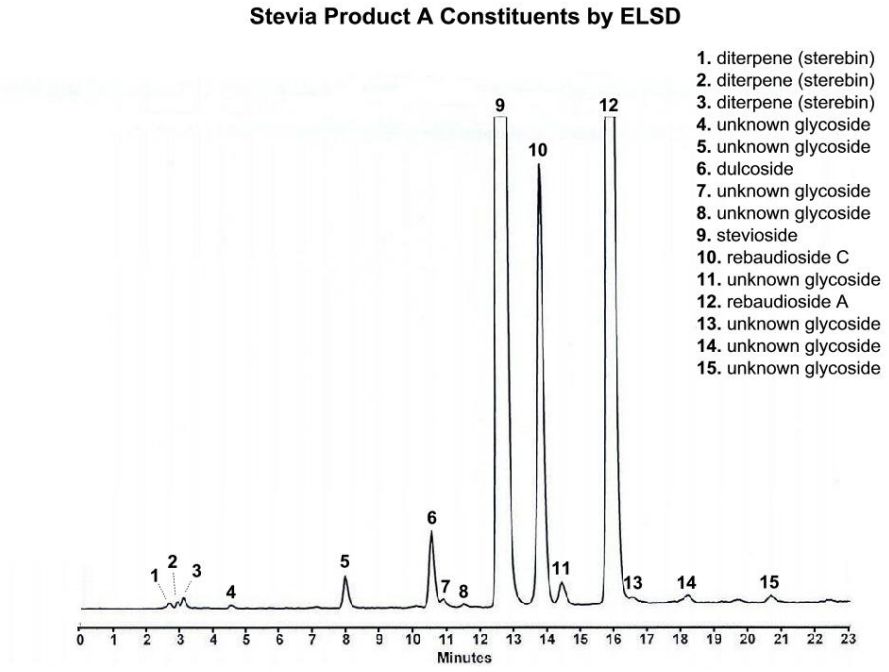


**Figure 3:** ELSD-LT chromatogram of diterpenes, diterpene glycosides and non-diterpene glycosides obtained for commercial Stevia product A.

## Results and Conclusion

As shown in Figure 1 and consistent with the literature, UV detection at 210nm reveals the principal Stevia diterpene glycosides for commercial Stevia product B. The ELSD-LT, on the other hand, reveals a complex mixture of chromophoric and non-chromophoric constituents in addition to the Stevia diterpene glycosides. The commercial additives erythritol and inulin fructo-oligosaccharides (FOS) are consistent with the label claim. Erythritol and Dulcoside are co-eluting under these chromatographic conditions, the erythritol being detected by ELSD alone. When compared to low UV wavelengths, it is clear that the ELSD-LT delivers better baseline response for the gradient elution. Figure 2 shows comparison ELSD-LT profiles for the principal Stevia constituents of products A and B.

The ELSD-LT reveals diterpenes, diterpene glycosides and non-terpenoid glycosides for Stevia product A in one run with a more accurate representation of constituent relative abundance. This is readily seen in Figure 3. Many of the minor constituents are weakly or non-chromophoric, making UV detection alone problematic when the analyst is interested in more than the Steviol glycosides alone. Table 1 shows peak assignment and area % data for the Steviol glycosides of product A according to ELSD response. The % relative abundance of Steviol glycosides as determined by ELSD data is consistent with the literature.[1,2]

**Table 1**

| Retention Time, ELSD (min.) | UV λmax, PDA (nm) | Constituent Class | Peak Assignment | Area % Steviol glycosides |
|---|---|---|---|---|
| 2.7 | 236, 282 | diterpene (sterebin) | | |
| 2.9 | 229 | diterpene (sterebin) | | |
| 3.1 | 238, 282 | diterpene (sterebin) | | |
| 4.5 | 193 | unknown glycoside | | |
| 8.0 | 193 | unknown glycoside | | |
| 10.6 | 193 | unknown glycoside | Dulcoside | 0.78 |
| 10.9 | 193 | unknown glycoside | | |
| 11.5 | 193 | unknown glycoside | | |
| 12.7 | 197 | diterpene glycoside | Stevioside | 68.64 |
| 13.8 | 193 | diterpene glycoside | Rebaudioside C | 5.54 |
| 14.4 | 193 | unknown glycoside | | |
| 15.9 | 195 | diterpene glycoside | Rebaudioside A | 25.04 |
| 16.5 | 193 | unknown glycoside | | |
| 18.2 | 193 | unknown glycoside | | |
| 20.7 | 193 | unknown glycoside | | |

From the data, ELSD and multi-wavelength UV detection complement one another for the determination of commercial Stevia product constituents. While the four Steviol glycosides may be determined by low-wavelength UV alone, the myriad of Stevia constituents are revealed by the evaporative light scattering detector.

## References


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