

## VARIABLE COLUMN LENGTH UHPLC FOR THE SEPARATION OF STEVIOL GLYCOSIDES

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Steviol glycosides were recently approved as food additives in the EU. Steviol glycosides (SVGly's) are extracts from the shrub *Stevia Rebaudiana Bertoni* and are a very large family of more than 30 compounds, differing from each other in the number and identity of sugar moieties attached to the diterpene steviol. The main SVGly's, Stevioside (Ste) and Rebaudioside A (RebA) are about 300 times sweeter than common table sugar. This very large group of similar compounds poses an interesting challenge to the analytical chemist.

The automatic column coupler, described by Cabooter et al. (2010) was used to optimize the resolution and separation of SVGly's. Several Acquity BEH C18 columns ( $d_p = 1.7 \mu\text{m}$ ) were coupled in series. The total column length ranged between 5 and 25 cm. Various gradients of aqueous formic or phosphoric acids (0.1%) – ACN were run on the shortest column (5 cm) allowing a rapid choice of the best gradient conditions. The best gradient was repeated on longer column combinations, allowing a separation of 26 peaks on 20 cm support.

An attempt was made to identify some of the unknown peaks using MS/MS.

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### Reference:

Cabooter, D., Decrop, W., Eeltink, S., Swart, R., Ursem, M., Lestremau, F., & Desmet, G. (2010). Automatic Column Coupling System To Operate Chromatographic Supports Closer To Their Kinetic Performance Limit and To Enhance Method Development. *Analytical Chemistry*, 82(3), 1054-1065.