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Application Note

Reversed-Phase Liquid Chromatography of Steviol Glycosides - Benefits of MaxPeak™ High Performance Surfaces

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

Steviol glycosides are often used as natural non-caloric sweeteners in food and beverages. This group of compounds shares the same steviol aglycone structure, but with different numbers and types of glycoside units (*e.g.,* glucose, rhamnose, or xylose). The FAO/WHO JECFA Monograph 26 (2021) includes the most up to date international standard for steviol glycosides, in which reversed-phase liquid chromatography (RPLC) is recommended for the determination of major and minor steviol glycosides. Recently, we improved the JECFA RPLC method by optimizing the gradient elution conditions and achieved better resolutions for the steviol glycosides. Besides the fine-tuned elution program, the MaxPeak High Performance Surfaces (HPS) also contributed to the improved resolution. In this application brief, the improved performance in resolution and efficiency obtained on the MaxPeak HPS incorporated column, XSelect™ Premier HSS T3 Column, are highlighted.

Benefits

- Higher chromatographic resolution and higher chromatographic efficiency were achieved on XSelect Premier

HSS T3 Column than the XSelect HSS T3 Column

- MaxPeak HPS technology helped to achieve better chromatography for the determination of Steviol Glycosides

Introduction

Steviol glycosides (SG) are constituents of the leaves of the plant, *Stevia rebaudiana* Bertoni (stevia) and have a sweet taste that is 100 to 300 times sweeter than sucrose. They are often used as non-caloric sweeteners in foods and beverages. More than 40 SG have been identified.[1] The Food and Agriculture Organization of the United Nations, and the World Health Organization (FAO/WHO) Joint Expert Committee on Food Additives (JECFA) has published a series of monographs on SG since 2006. The latest monograph published in the FAO/WHO JECFA Monographs 26 (2021), recommended RPLC methods for the analysis of the major and the minor SG.[1] However, the chromatographic resolution obtained in these JECFA methods was not adequate. We recently improved the JECFA methods by optimizing the gradient elution conditions and adopting the MaxPeak High Performance Surfaces (HPS).[2] MaxPeak HPS was developed by Waters™ to mitigate analyte adsorption in LC.[3] It has already been demonstrated that significant improvements can be achieved by using the MaxPeak HPS in the analyses of a wide range of compounds.[4–7] Here, during the method development, we found for the first time that the MaxPeak HPS also helped the RPLC of SG. Here the key benefits of the MaxPeak HPS for the RPLC of SG are highlighted.

Results and Discussion

Higher chromatographic resolution and higher separation efficiency (apparent number of theoretical plate) were achieved on the MaxPeak HPS incorporated column (XSelect Premier HSS T3 Column) as compared to the conventional column (XSelect HSS T3 Column) under the sample LC conditions. The details of the LC conditions are reported elsewhere.[2] Figure 1 shows a comparison of the chromatograms obtained on the XSelect Premier HSS T3 Column and the XSelect HSS T3 Column under the same experimental conditions. Better resolution, especially for the critical pair (rebaudioside A/stevioside), were achieved on the XSelect Premier HSS T3 Column.

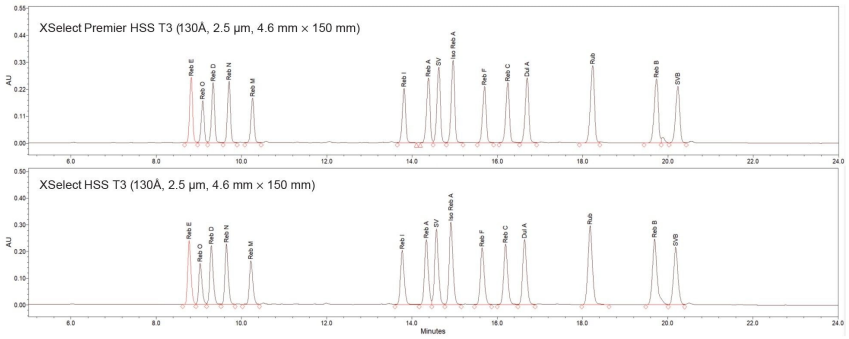

Figure 1. Comparison of chromatograms obtained on XSelect Premier HSS T3 Column and XSelect HSS T3 Column of the same column dimensions and the same particle size under the same LC conditions. Peak ID: Reb E, rebaudioside E; Reb O, rebaudioside O; Reb D, rebaudioside D; Reb N, rebaudioside N; Reb M, rebaudioside M; Reb I, rebaudioside I; Reb A, rebaudioside A; SV, stevioside; Iso Reb A, isorebaudioside A; Reb F, rebaudioside F; Reb C, rebaudioside C; Dul A, dulcoside A; Rub, rebusoside; Reb B, rebaudioside B; SVB, steviobioside.

Table 1 shows a summary of the resolution results obtained in the comparison study. Higher resolution values were obtained on the column incorporated with the MaxPeak HPS over the conventional column (2–14% higher). Figure 2 shows a comparison plot of the apparent number of theoretical plates obtained on these two columns. Significantly higher separation efficiency was obtained on the XSelect Premier HSS T3 Column. The peak areas were also compared, however, no significant difference between the two columns was observed (results not shown).

	Reb E	Reb O	Reb D	Reb N	Reb M	Reb I	Reb A	SV	ISO Reb A	Reb F	Reb C	Dul A	Rub	Reb B	SVB
Column	**XSelect Premier HSS T3 Column (2.5 µm, 4.6 × 150 mm)**														
Resolution (USP HH)															
Mean (n=5)	–	2.17	1.94	2.93	4.26	25.77	3.89	1.63	2.28	4.94	3.56	2.91	9.24	8.53	*
RSD (%)	–	0.21	0.05	0.2	0.09	0.15	0.15	0.18	0.16	0.13	0.15	0.16	0.1	0.11	*
Column	**XSelect HSS T3 Column (2.5 µm, 4.6 × 150 mm)**														
Resolution (USP HH)															
Mean (n=5)	–	1.90	1.89	2.56	4.10	23.81	3.65	1.51	2.18	4.64	3.39	2.71	8.83	8.35	2.70
RSD (%)	–	0.44	0.51	0.32	0.37	0.2	0.07	0.18	0.16	0.1	0.19	0.09	0.1	0.08	0.23
Rel. resolution+		114%	103%	114%	104%	108%	107%	108%	104%	106%	105%	107%	105%	102%	

*: Resolution larger than 2.7 was obtained between Reb B and SVB. The resolution was not shown because the original number was calculated incorrectly (due to a small peak between Reb B and SVB). +: Relative resolution values of XSelect Premier HSS T3 Column over XSelect HSS T3 Column.

Table 1. Summary of chromatographic resolutions obtained on XSelect Premier HSS T3 Column and XSelect HSS T3 Column under the same conditions for 15 steviol glycosides. The peak IDs are the same as in Figure 1.

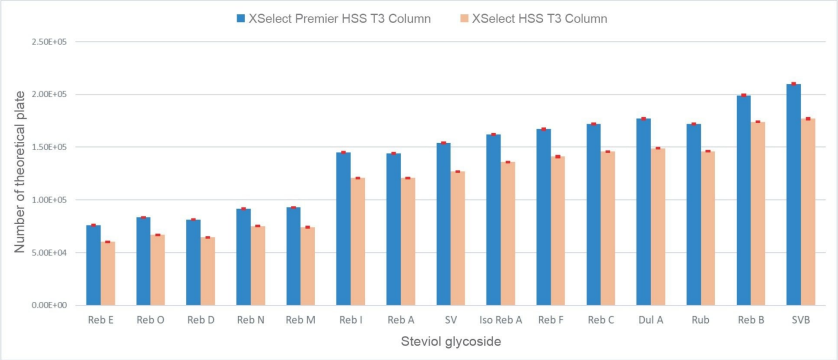

Figure 2. Comparison of separation efficiency (the apparent number of theoretical plate) of steviol glycosides obtained on XSelect Premier HSS T3 Column and on XSelect HSS T3 Column under the same experimental conditions. Results were obtained from replicated injections (n=5). The standard deviations were shown as error bars (±SD).

Conclusion

Higher chromatographic resolution and separation efficiency were achieved using the MaxPeak HPS

incorporated column in the RPLC-UV analysis of steviol glycoside. Combining the MaxPeak HPS incorporated Arc™ Premier System and the XSelect Premier HSS T3 Column, this premier LC system and column offers a better solution than the conventional LC system and column for the determination of steviol glycosides.

References

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