Analysis of Artificial Sweeteners by HILIC–MS Method

Jonas Henschel[1], Alexander Schriewer[1], Heiko Hayen[1], and Wen Jiang[2], [1]Institute of Inorganic and Analytical Chemistry, University of Münster, Münster, Germany, [2]HILICON AB

In recent decades, the use of artificial sweeteners greatly increased. As sugar substitutes they have become popular in today's calorie-conscious society. The most commonly used artificial sweeteners in beverages and food are acesulfame, aspartame, cyclamate, neohesperidin dihydrochalcone (NHDC), neotame, saccharin, and sucralose. Some of them show resistance to wastewater treatment and distribute in the aquatic environment as emerging contaminants. In addition, concerns about health risks from the artificial sweeteners have been raised as well (1–3).

The analytical challenge is to simultaneously determine the artificial sweeteners with diverse physicochemical properties. Considering the polar and hydrophilic nature of these compounds, hydrophilic interaction liquid chromatography (HILIC) should be considered as an alternative to commonly used reversed-phase liquid chromatography. In this application, we used one iHILIC-Fusion(+) column packed with charge modulated hydroxyethyl amide silica, which combines hydrophilic partitioning, weak electrostatic interactions, and hydrogen bonding in HILIC separation.

Experimental

LC–MS–MS system: Advance UHPLC with a CTC PAL autosampler and Bruker EVOQ™ Elite triple quadrupole system with electrospray ionization; multiple reaction monitoring mode (MRM) in ESI(+) and ESI(-) was used for selective and sensitive detection, see details in Table 1.
Column: 150 × 2.1 mm, 3.5-µm 100Å iHILIC-Fusion(+) (P/N 100.152.0310, HILICON AB, Sweden)
Gradient elution: A) acetonitrile; B) 10 mM ammonium formate, pH = 3.5; gradient elution from (95/5) A/B to (84/16) A/B in 8.5 min.
Flow rate: 0.3 mL/min
Column temperature: 40 °C
Injection volume: 5 µL
Sample preparation: details on request

Results and Conclusion

Seven artificial sweetener standards can be simultaneously determined by the combination of iHILIC-Fusion(+) HILIC column and EVOQ™ Elite triple quadrupole MS–MS in a 12 min run, as shown in Figure 1. The developed method was verified to detect artificial sweeteners in the diet cola of a well-known brand. After a simple and fast sample preparation, we can identify acesulfame, cyclamate, and aspartame in the tested diet cola (Figure 2).

Table 1: Precursor ions, product ions, and corresponding collision energy parameters for MS–MS detection of artificial sweeteners.

Analyte	Precursor ions *m/z* [M-H]⁻	Product ions (P1/P2/P3) *m/z*	Collision energy [V]
Acesulfame	162.2	82.2/78.1/64.2	-11/-27/-46
Cyclamate	178.2	80.1	-24
Saccharin	182.2	42.3/106.0	-22/-17
NHDC	611.2	303.0/166.0/125.0	-43/-59/-45
Sucralose	442.6 (+formate)	396.9/361.0/359.0	-6/-9/-9
	[M-H]⁺		
Aspartame	295.3	120.1/180.0/235.0	+23/+12/+11
Neotame	379.5	172.1/319.1/120.1	+21/+14/+31

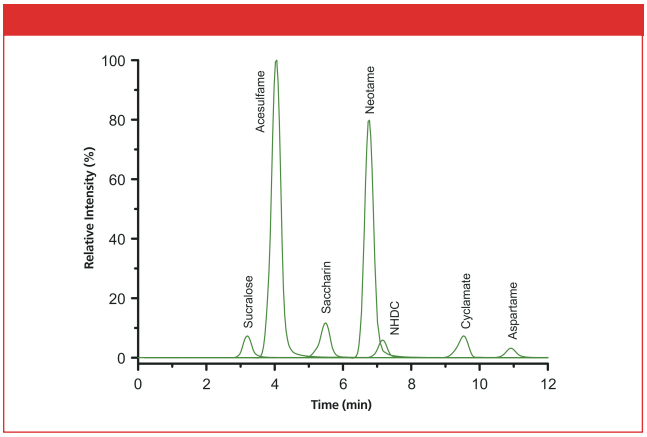

Figure 1: LC–MS–MS chromatograms of seven artificial sweetener standards with iHILIC-Fusion(+).

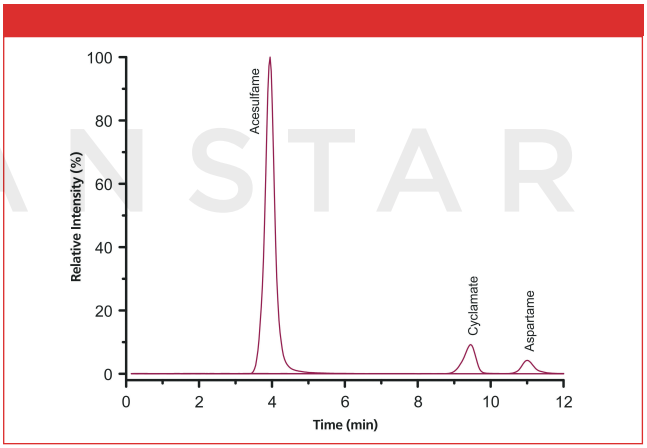

Figure 2: LC–MS–MS chromatograms of diluted and degassed diet coke with iHILIC-Fusion(+).

This work describes an efficient HILIC–MS method for separation and detection of artificial sweeteners. It can be further developed to a routine analysis method with a combination of HILIC and aerosol-based detectors (ELSD, NQAD, and CAD) and used in different application areas.

References

(1) M. Scheurer, H.J. Brauch, and F.T. Lange, *Anal. Bioanal. Chem.* **394**, 1585–1594 (2009).
(2) F.T. Lange, M. Scheurer, and H.J. Brauch, *Anal. Bioanal. Chem.* **403**, 2503–2518 (2012).
(3) R.A. Lorenzo, M.T. Pena, P. Fernandez, P. Gonzalez, and A.M. Carro, *Food Control* **47**, 43–52 (2015).