Analysis of flavonoids in plant extracts by CE-MS

Application Note

Foods and Flavors

Authors
Martin Greiner,
Gordon Ross
Agilent Technologies,
Waldbronn, Germany

Abstract

Complex extracts of plant compounds often need a very effective separation mechanism for a clear compound identification. MS detection can provide a specific identification of the analyte in such complex and difficult matrices. Here we show the analysis of flavonoids from extracts of oranges, illustrating the specificity and sensitivity of an electrospray mass spectrometer (ESI-MS) which can add a new dimension to capillary electrophoresis separations.

Hesperidine, a flavone glucoside, is present in the peel of green oranges while naringin, a rhamnoglucoside flavanone, is a bitter component of the orange peel. If not controlled properly, the high squeeze pressure applied to oranges during production of orange juice yields a high content of these flavonoids which gives it a bitter taste. Monitoring the presence of these in orange juice provides a way of monitoring the quality of the product.

CE was the separation technique of choice to allow a fast and efficient separation and additionally to reduce sample preparation to centrifugation and filtering of the juice.

Agilent Technologies

Experimental

All experiments were performed using an Agilent Capillary Electrophoresis system equipped with DAD detection and controlled via Agilent ChemStation software. The Agilent capillaries for CE-MS were 50 µm id with a total length of 75 cm. These capillaries have a UV detection window at 22 cm. The buffer used was 50 mM borate, pH 9.3 diluted tenfold by Agilent ultra pure water (all Agilent Technologies, Waldbronn, Germany). Sheath flow (4 µL/min) was delivered by an Agilent isocratic HPLC pump running at 400 µL/min split 1:100 by an Agilent splitting device. Sheath liquid was 1 % formic acid in 50 % methanol for positive ion detection.

Figure 1 shows hesperetin (a glycon of the hesperidine), found at m/z value 325 (the Na adduct mass). Naringin was found under these conditions as the protonated adduct at m/z 581. Besides the qualitative aspects of identifying compounds by mass spectra CE-MS can also provide quantitative information. The calibration curve shown in figure 2 resulted from a naringin standard diluted in a range from 200 to 2 µg/mL. The lowest amount used here still gave a reasonable signal larger than 5:1 s/n. Figure 3 shows the MS trace of 2 µg/mL naringin.

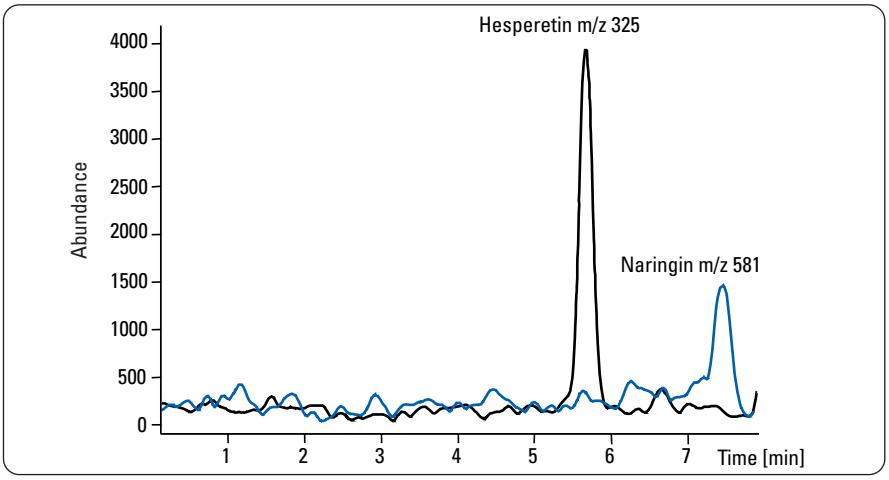

Figure 1
Analysis of hesperetin and naringin.

Chromatographic conditions

Sample:	spiked orange juice
Injection;	1500 mbar sec
Capillary:	75 cm (22 cm UV) x 50 µm id
Buffer:	5 mM borate pH 9.3
Voltage:	30 kV
Temperature:	25 °C
Sheath liquid:	4 µL/min, 50 % MeOH/H_20, 1 % formic acid
Nebulizing gas:	20 psi
Drying gas:	6.0 L/min
Acquisition:	positive
SIM:	325 m/z, 581 m/z

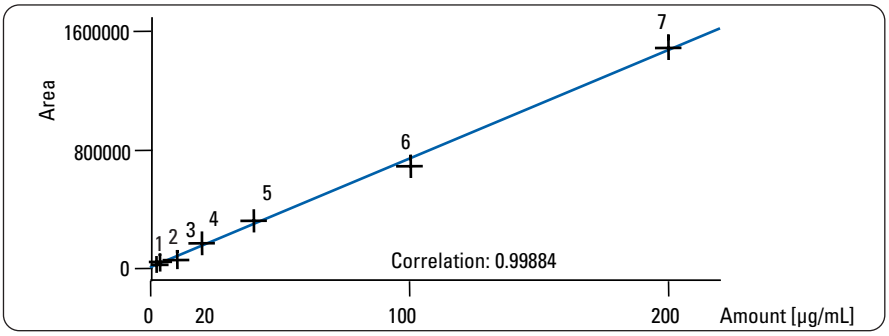

Figure 2
Linearity of naringin detection with MS.

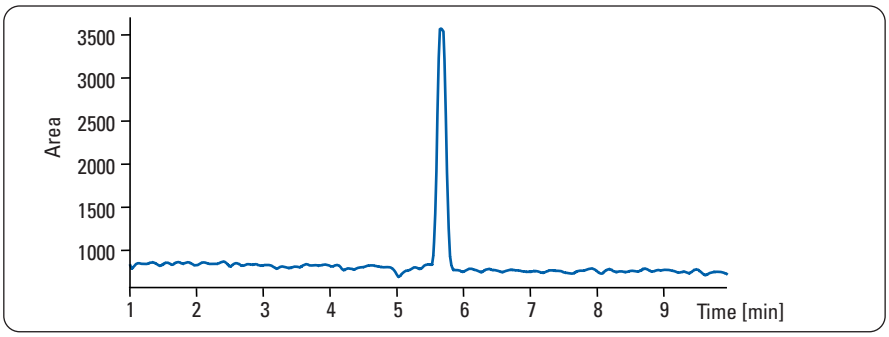

Figure 3
Analysis of 2 µg/mL naringin.

www.agilent.com/chem/ce

Published March 1, 2009
Publication Number 5990-3388EN

Agilent Technologies