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Application Brief 134

LC-MS Analysis of Anthocyanins in Bilberry Extract

INTRODUCTION

Anthocyanins are water-soluble plant pigments widely present in fruits, vegetables, and flowers. Anthocyanins have gained considerable interest in the scientific community and consumer market due to their anti-inflammatory action and strong antioxidant and radiation-protection properties. Bilberries are known to have a high anthocyanin content and are, therefore, one of the most expensive botanical ingredients in the health food industry. The high price of the extract makes bilberries more susceptible to adulteration. Analytical characterization and quantification methods for anthocyanins specific to bilberries are therefore required. This study demonstrates a rapid separation liquid chromatography-mass spectrometry (RSLC-MS) method using the Thermo Scientific MSQ Plus™ detector for the determination of anthocyanins in bilberry extract and pomegranate juice based on previously developed LC separations.[1,2]

ANALYSIS

The experiment was performed on a Thermo Scientific Dionex UltiMate® 3000 RSLC system with chromatographic separation achieved on a Thermo Scientific Acclaim® RSLC 120 C18 reversed-phase column (2.1 × 100 mm, 2.2 µm) operated at 40 °C with gradient elution at 0.5 mL/min.

The mobile phase consisted of three components: A) acetonitrile, B) deionized (DI) water, and C) 20% formic acid. Mobile phase C was held constant at 10% to provide 2% total formic acid in the mobile phase throughout the run. Mobile phase A was ramped from 0% to 8% from 11 to 42 min, then held for 13 min before returning to the initial composition from 55 to 60 min.

Electrospray ionization (ESI) was used as the interface and was operated in positive selected ion monitoring (SIM) mode. The probe temperature was set at 500 °C and needle voltage was set at 2000 V. The cone voltage was set at 50 V for all SIM scans with a span of 0.3 amu for each SIM.

RESULTS

As shown in Figure 1, 15 anthocyanins were separated on the Acclaim RSLC 120 C18 column, and selectively detected by MS in SIM mode. Mass spectrometry offers molecular ion information for identity confirmation and SIM acquisition provides excellent detection sensitivity for quantitation.

REFERENCES

1. Dionex Corporation. *Fast Determination of Anthocyanins in Pomegranate Juice.* Application Note 264, LPN 2647-01, 2010, Sunnyvale, CA.
2. Dionex Corporation. *Determination of Anthocyanins in Bilberries.* Application Note 281, LPN 2848, 2011, Sunnyvale, CA.

Chromatographic Conditions

System:	UltiMate 3000 RSLC System
Column:	Acclaim RSLC 120 C18 (2.1 × 100 mm, 2.2 µm)
Mobile Phase:	A) CH_3CN, B) DI water, C) 20% formic acid
Gradient:	C held constant at 10%, A from 0% to 8% from 11 to 42 min, held for 13 min, return to 0% in 5 min
Flow Rate:	0.5 mL/min
Inj. Volume:	5 µL
Temperature:	40 °C
Sample:	NIST Bilberry Extract Standard Reference Material

Mass Spectrometric Conditions

System:	MSQ Plus single quadrupole mass spectrometer
Ionization interface:	ESI
Probe Temperature:	500 °C
Needle Voltage:	2000 V
Nebulizer Gas:	Nitrogen at 80 psi
Detection Mode:	SIM
	Refer to chromatogram for SIM Acquisitions

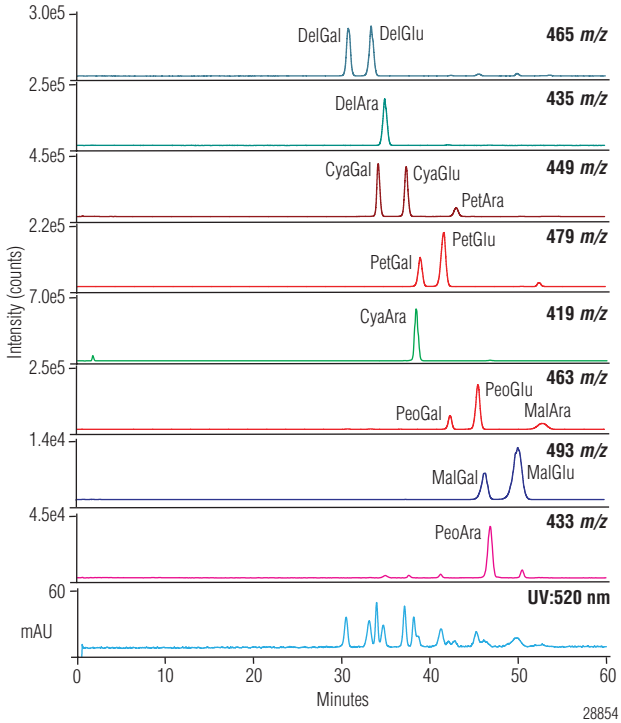

Figure 1. Determination of 15 anthocyanins in bilberry extract by LC-MS.

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