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Analytical Methods

Rapid profiling and identification of anthocyanins in fruits with Hadamard transform ion mobility mass spectrometry

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ABSTRACT

The use of Hadamard transform ion mobility mass spectrometry (HT-IMMS) in the profiling of anthocyanins from different fruits is presented. Samples extracted with acidic methanol and purified with solid phase extraction were analyzed with direct IMMS infusion. The separation of various anthocyanins was achieved within 30 s with resolving powers up to 110. The ion mobility drift times correlated with their mass-to-charge ratios with a correlation coefficient of 0.979 to produce a trend line that was characteristic for anthocyanins. Isomers with the same anthocyanidin but different hexoses were differentiated by ion mobility spectrometry. Furthermore, mobility separated ions underwent collision induced dissociation at the IMMS interface to provide MS/MS spectra. These fragmentation spectra aided in the identification of anthocyanidins via the loss of the saccharide groups. IMMS appears to be a rapid and efficient approach for profiling and identifying anthocyanins.

1. Introduction

Anthocyanins are a group of glycosylated flavonoid compounds that are water-soluble vacuolar pigments of plant origin. Found in most fruits and flowers, they may appear red, purple, or blue depending on the pH (Holton & Cornish, 1995) of their environment and may play an important role in protecting plants from strong UV irradiation (Mol, Grotewold, & Koes, 1998). They may be used as natural colorants instead of synthetic pigments in the food industry (Castaneda-Ovando, de Lourdes Pacheco-Hernandez, Elena Paez-Hernandez, Rodriguez, & Andres Galan-Vidal, 2009), and recent research showed that anthocyanidins may have anticarcinogen (Boivin, Blanchette, Barrette, Moghrabi, & Beliveau, 2007; Gerhauser, 2008), antiaging (Chen, Mueller, Richling, & Wink, 2013), antiinflammation (Hassellund et al., 2013), and antibacterial activities.

Due to their strong antioxidant properties (Wang, Cao, & Prior, 1997), anthocyanins are of considerable interest to the scientific community and consumer market for both nutraceutical and medicinal reasons. Anthocyanins have electron deficient chemical structures that make them highly reactive with free radicals and, consequently, powerful natural antioxidants for food supplements (Duthie, Duthie, & Kyle, 2000; Wang & Jiao, 2000). Awareness of their health benefits has led to a growing interest in determining anthocyanins in fruits, nutraceuticals, and other natural supplements. Blueberries, raspberries, blackberries, strawberries and other fruits such as pomegranates represent the major sources of anthocyanins and are often called "super fruits" for their high concentration of anthocyanins.

Many analytical techniques have been developed to characterize this class of compounds in fruits: these include high performance liquid chromatography (HPLC) with UV or DAD detection (Hong & Wrolstad, 1990; Merken & Beecher, 2000), HPLC-tandem mass (Wu, Gu, Prior, & McKay, 2004; Wu & Prior, 2005), capillary electrophoresis (CE) (da Costa, Horton, & Margolis, 2000), capillary electrophoresis mass spectrometry (CE-MS) (Segura-Carretero et al., 2008), and ion exchange chromatography (Asenstorfer, Hayasaka, & Jones, 2001). More recently, mass spectrometry has been used as a standalone technique for rapid profiling of anthocyanins from different fruits (Flamini et al., 2012). Because of the similarity in their structures, especially for isomeric and isobaric anthocyanins, however, tedious sample preparation is often required to produce sufficient separation. Novel methods of analyses are needed to improve the ability to rapidly separate and detect isomers in complex samples.

Ion mobility mass spectrometry (IMMS) is a novel analytical method that has been used for the determination of isomers and isobars in complex samples (Inutan, Wager-Miller, Narayan, Mackie, & Trimpin, 2013). It is a high throughput and sensitive analytical method based on a unique separation mechanism in which gaseous ions are separated by their size, shape and charge (Kaplan

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et al., 2013; Lapthorn, Pullen, & Chowdhry, 2013). Generally, IMMS provides six times the peak capacity of mass spectrometry alone (Dwivedi, Puzon, et al., 2010).

IMMS has been applied for profiling metabolites of cancer tissue (Jurneczko et al., 2013; Takebayashi, Hirose, Izumi, Bamba, & Fukusaki, 2013), blood, microbes, brain tissue and the differentiation of oligosaccharides (Li, Bendiak, Siems, Gang, & Hill, 2013a, 2013b; Li et al., 2012; Yamagaki & Sato, 2009). The aim of this study is to investigate the potential of IMMS for the rapidly differentiation and profiling of anthocyanins in various fruits with ion mobility mass spectrometry, especially for those anthocyanins with the same molecular weight and similar structures.

2. Materials and methods

2.1. Chemicals

All solvents and water were HPLC grade purchased and used as obtained from Fisher (Pittsburgh, PA). 2,6-di-tert butylpyridinen (2,6-DtBP), acetic acid, ethyl acetate and hydrochloric acid were purchased from Sigma–Aldrich (St. Louis, MO).

2.2. Ion mobility spectrometry

A stack ring drift tube ion mobility spectrometer was (IMS) interfaced to a time of flight mass spectrometer as shown in Fig. 1. This ion mobility mass spectrometer has previously been described (Crawford et al., 2013) but the basic components of the instrument will be briefly discussed here as well. The IMS tube consisted of an 8 cm desolvation region and a 23 cm long drift region, a Bradbury–Nielsen ion gate separated the drift region from the desolvation region. Ion gate was operated with a closure voltage of ±38 V and a 180 μs chip time in the Hadamard transform mode. The electric field across the IMS drift region was 429 V/cm. The IMS tube was maintained at 200 °C during the whole experiment. A 150 μm inner diameter silica capillary tube with polyimide coating was used to transfer the sample solution from the syringe pump to the ESI needle. A 3.2 kV bias above the drift voltage was applied to the ESI needle. A Harvard syringe pump with a 250 μL syringe was used for sample injection at a flow rate of 3 μL min^{-1}. Dried compressed nitrogen was passed through a molecular sieve gas filter and used as the drift gas (50 PSI, 900 mL/min^{-1}).

2.3. Hadamard transform time of flight mass spectrometer

A Tofwerk H-TOF Hadamard Transform time of flight mass spectrometer was provided by Tofwerk AG (Thun, Switzerland) and has been described elsewhere (Dwivedi, Schultz, & Hill, 2010). Briefly, the interface between the IMS and the TOF region of the MS consisted of a pinhole leak with a 300 μm diameter. The pressure inside the interface was stepped down in two stages, from atmospheric pressure (approximately 950 mbar) to 2–4 mbar within the interface. The ions traversing the pressure interface region were guided towards the ion focusing lenses by two segmented quadrupole ion guides. The TOF mass spectrometer extraction frequency was 16,667 Hz (or 60 μs TOF period) thus provided 1000 points for a 60 μs ion mobility spectrum.

2.4. Sample preparation

Blueberry, raspberry, blackberry, strawberry and pomegranate fruits were purchased from a local grocery store near Washington State University, Pullman, WA. Two grams of fruit were manually ground with a mortar and pestle with 10 mL precooled 50:50 methanol:water with 1% HCl. The mixture was removed to a 20 mL glass vial and extracted for 4 h under room temperature. Three mL of the mixture was centrifuged under 14,000*g* for

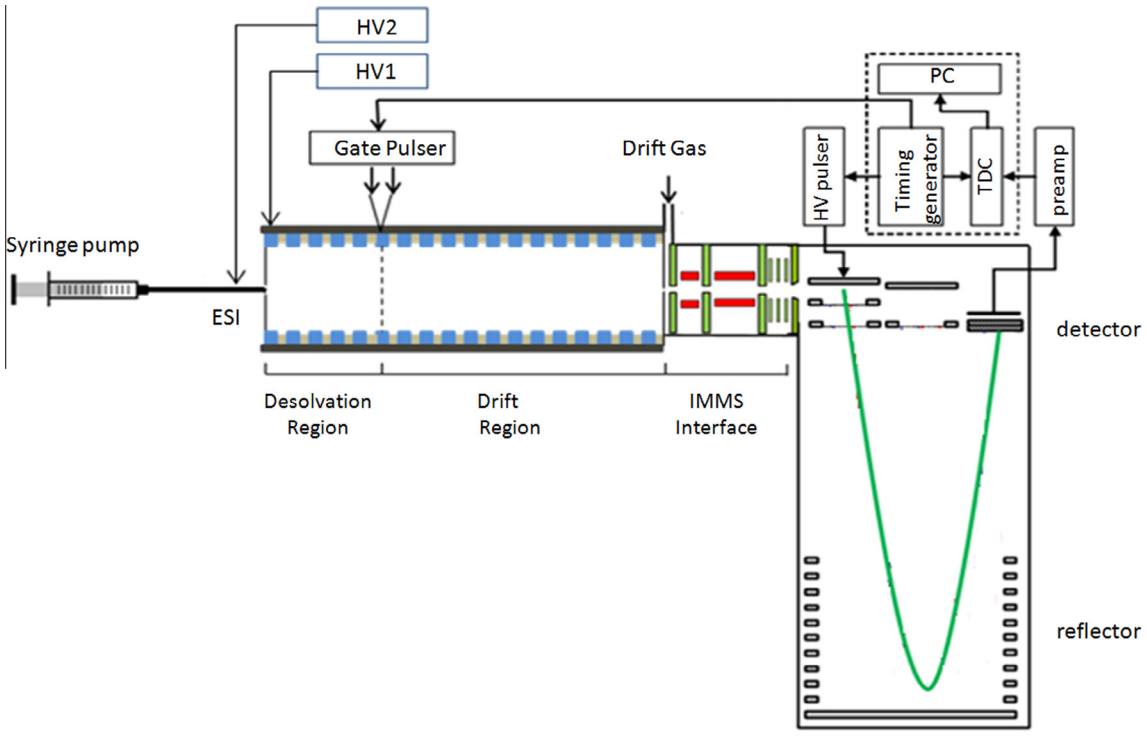

Fig. 1. Schematic diagram of the electrospray ionization Hadamard transform atmospheric pressure ion mobility time-of-flight mass spectrometer. HV1 and HV2: high voltage supplier; TDC: time to digit converter.