Measuring Fruit Juice Adulteration by Changes in Flavonoid Content Using MEPS™ and HPLC

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Abstract

Cranberry and blueberry juice are notable example of functional foods that may be eroded in value by dilution or adulteration with lower value products. The cranberry is known as a source of polyphenolic antioxidants (including anthocyanidin flavonoids, cyanidin, peonidin and quercetin) and is the subject of investigation for potential anti-cancer properties and its effects on the cardiovascular and immune systems. The tannins are reputed to reduce urinary tract infections, exhibit anti-clotting properties and reduce gingivitis. Other fruits are also known or reputed to have functional characteristics and therefore of high value.

A rapid Micro-Extraction Packet Sorbent (MEPS™) method is described for extracting and concentrating the phenolic components from a variety of commercial fruit juices. The juice was passed through a C8 or C18 MEPS™ cartridge and the retained fraction eluted with methanol for direct injection into a HPLC and analysis on a ProteCo™l C18 GP125 column using a 0.1 % v/v aqueous trifluoroacetic acid – methanol mobile phase. Detection of the phenolic fraction at 350 nm was used to generate a characteristic profile for each species of fruit.

The method allowed profiling of fruit juice and the detection of diluents or juice mixtures. Because the solid-phase step is flowrate dependant, the small sample and elution volumes of MEPS™ allow rapid sample extraction that may be completed in realtime with the HPLC analysis.

The MEPS™ Principle

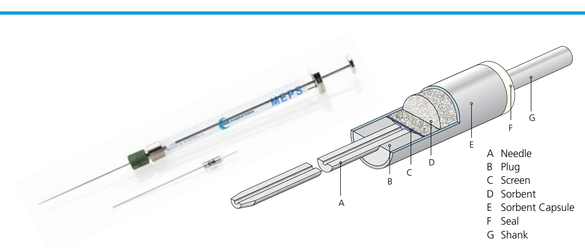

Figure 1: The MEPS™ cartridge

The MEPS™ consists of a small (~7 µl) compartment: "Barrel Insert and Needle Assembly (BIN), (Patent Pending)" that contains the stationary phase, and is built into the syringe needle. The packing material is 40-50 µm silica with 60 Å pore size and a range of common surface modifications.

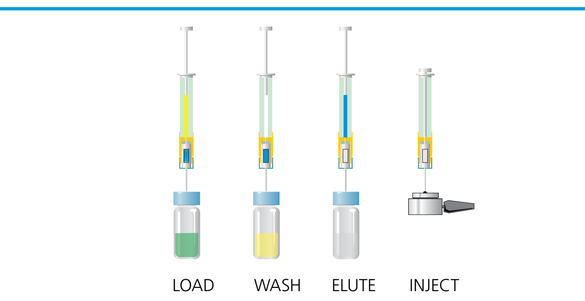

Figure 2: Standard operation of a MEPS™ extraction

MEPS™ works like other sample preparation tools with the common steps being sampling, washing and elution with the difference that the glass syringe design allows these steps to be performed by a robotic system (such as an autosampler) with the needle being robust enough to penetrate standard septa.

Advantages of MEPS™

Sample Size and Sensitivity:	Sample volumes may be as little as 10 µL, or by taking multiple aliquots of 100 µL or 250 µL, samples of 1mL or larger may be concentrated.
Robustness:	Samples can be drawn and dispensed through septa.
Automation:	The capability to extract samples and make injections on-line using a single device reduces both sample processing times and the need for operator intervention.
Sorbent Life:	Typical BIN life for extraction of whole plasma sample is conservatively about 40 to 100 samples. This significantly increases for cleaner samples.
Carry Over:	The small quantity of phase in the MEPS BIN can be easily and effectively washed between samples to reduce the possibility of carryover. This washing process is simply not practical with off-line SPE devices. With automation of MEPS washing can occur while the previous sample is running.
Flexible and easy to use:	The dimensions of the sorbent bed ensure that the performance remains identical to conventional SPE devices when used for extraction of similar samples.

Polyphenols in Plants

Polyphenols are highly abundant in a variety of forms in plants. The number of identified compounds found naturally exceeds 6000 (Harborne et al., Phytochem. 55 (1992) 481.).

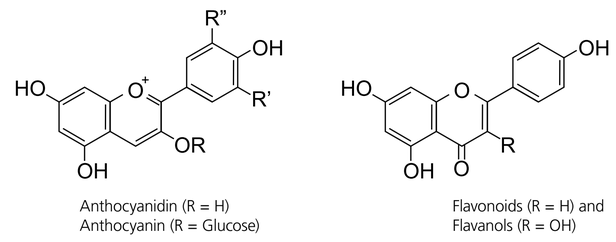

Figure 3: Classes of Polyphenols in Plants

Anthocyanins and anthocyanidins are commonly found in plant pigmentation while other polyphenols such as flavones, isoflavones, flavanols and flavonoids are distributed across a number of plant organs. All polyphenols are attributed to a number of health benefits such as antimicrobial functions, antioxidant properties and anti cancer activity.

Even though polyphenols are wide spread the concentration of these compounds in different plant species can vary significantly, making some sources more valuable than others. It is common practice in the fruit juice industry for example to add low value juice (such as apple) to high value juice (such as cranberry). Aim of this research was to develop a fast and simple sample preparation to characterise the polyphenol content of fruit juice and determine possible adulterations of high value juices with inferior sources.

Experimental

MEPS™ extraction conditions

Fruit juices were purchased from a local supermarket. Contents were labelled as cranberry (water, fruit juice (from concentrate) (cranberry 25 %, sugar, vitamin C (ascorbic acid)), raspberry and cranberry (water, fruit juice (from concentrate) (raspberry 11 %, cranberry 8 %, grape 3 %, apple 3 %, sugar, natural flavours, acidity regulator (citric acid), vitamin C (ascorbic acid)) and blackcurrant and cranberry (water, fruit juice (from concentrate) (cranberry 11 %, blackcurrant 10 %, apple 4 %, sugar, natural flavours, vitamin C (ascorbic acid)) were Ocean Spray brand (Ocean Spray International Inc., MA, USA). Apple juice (reconstituted apple juice, food acid 330, flavour, vitamin C) was from P&N beverages Australia Pty. Ltd. (NSW, Australia) and blackcurrant fruit juice syrup 20.8 % v/v was Ribena brand (GlaxoSmithKline, NSW, Australia).

A M21-C18, C8 or C2 MEPS™ BIN was fitted on a 100 µL syringe and conditioned with methanol (50 µL) and water (50 µL) at 10 µL/sec. A sample of each fruit juice (100 µL) was loaded to waste at 10 µL/sec. The sorbent was washed with water (20 µL) and dried with air (3 x 80 µL) at 80 µL/sec. The cartridge was eluted with methanol (5 x 10 µL) into a vial and diluted with mobile phase A. Samples were analysed directly. Processing time was less than 2 minutes per sample. Each sorbent was recycled by washing with methanol (3 x 30 µL) at 10 µL/sec.

Column Design

Polyphenols have the potential to chelate iron (P. Sestili et al, J.Biochem., 364(1)(2002)121-128.). Therefore a column was employed which eliminates all contact of the sample with metal surfaces. The frit of a ProteCol-P column is made of PEEK™ and the inner wall of the column body is coated with a PEEK™ film, thus providing a completely metal-free flowpath.

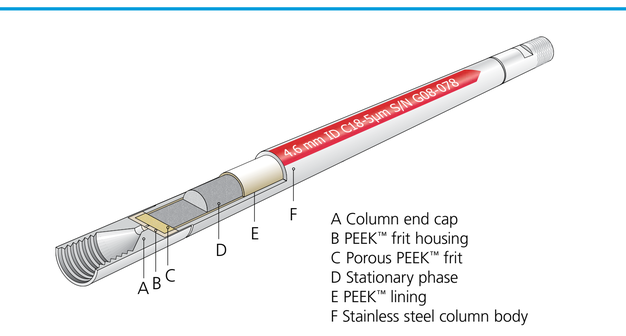

Figure 4: Schematic view of the PEEK™-lined columns

Chromatographic Conditions

LC system:	Shimadzu Prominance LC20
Column:	ProteCol™ HQ105 150 x 5.6 mm ID
Mobile Phase A:	0.1 % TFA in water
Mobile Phase B:	0.1 % TFA in 80 % methanol
Flow rate:	1.0 ml/min
Gradient profile:	0 min - 0 % B 20 min - 50 % B 30 min - 100 % B 40 min - 100 % B 41 min - 0 % B 60 min - 0 % B
Column temperature:	40 °C
Detection:	254, 350 and 550 nm

The detection wavelengths were chose as a general selectivity wavelength (254 nm) a wavelength to detect flavonoids (330 nm) and a wavelength specific for anthocyanidins (550 nm).

Influence of the MEPS™ Packing Material

Fruit juices contain a number of hydrophilic compounds such as sugars and fruit acids. In addition all fruit juices tested had a large amount of ascorbic acid added which gave by far the biggest signal at 2.3 minutes retention time. In this study three different modified silicas were used for the sample preparation step (C2, C8 and C18). The result of the analysis of the pre-treated samples compared to the neat juice is shown in Figure 5.

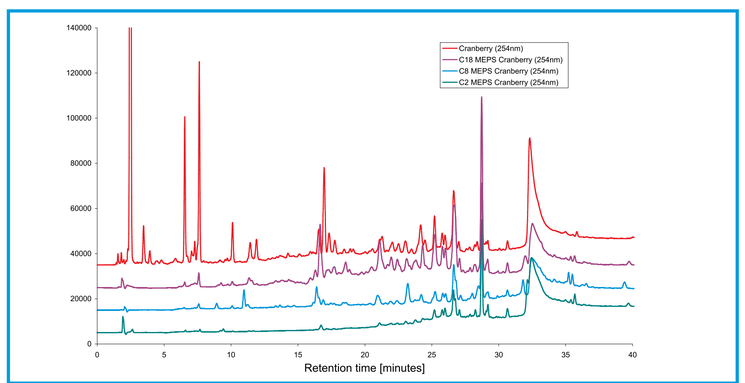

Figure 5: Chromatograms of cranberry juice prepared with different MEPS™ sorbents

As expected, the ability to retain hydrophilic compounds increases with the chain length of the ligand of the sorbent in the MEPS™ cartridge. All MEPS™ cartriges eliminate the early eluters (sugar and vitamin C).

Flavonoid Content of Different Fruit Juices

As an example the flavonoid content of Cranberry - Black Currant juice was analysed. According to the lable the juice contained cranberry, black currant and apple juice. In comparison the individual fruit juices were analysed under the same conditions.

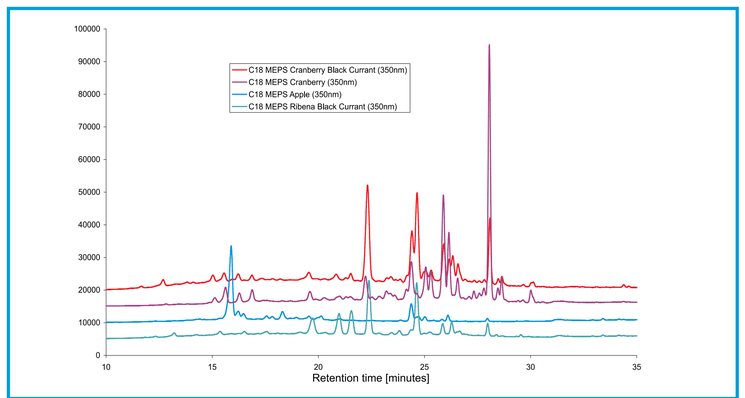

Figure 6: Flavonoid fingerprinting of various fruit juices

The sample preparation with the C18 MEPS™ and the use of a specific wavelength resulted in a chromatogram with significantly reduced complexity. This allows a much easier peak assignment. The different sources clearly show unique flavonoid contents and it is easy to assign the peaks of combined juice to their origin. Even though apple was mentioned to be part of the combined juice its contribution was minor.

Conclusions

MEPS™ was shown to provide a fast and powerful method for the sample preparation in fruit juice analysis. Looking specifically at the flavonoid content it was possible to fingerprint various fruit sources and assign the signals from a combined fruit juice to their origin. The use of PEEKsil™ (PEEK™ coated fused silica capillaries) in the LC system and a metal-free column hardware further enhanced the usefulness of the method by reducing nonspecific binding of the polyphenols to metal surfaces.

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