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Nota applicativa

Determination of Flavonoids in Fruit Juice

Waters Corporation

Abstract

This application note describes the use of HPLC-UV to identify flavonoids in a citrus juice sample. Separations were performed utilizing XBridge Shield RP_{18} and XBridge C_8 columns.

Introduction

Flavonoids, a group of related polyphenols derived from flavone, are widely distributed in the plant kingdom. There are more than 4000 naturally occurring flavonoids that have been identified and characterized. Among these, citrus fruit derived flavonoids and their metabolites have been shown to have significant biological activities such as anti-carcinogenic effects, anti inflammatory properties and inhibitory activities against histamine release. Figure 1 highlights a number of flavonoids and their identified properties.

Flavonoid	Source	Potential Health Benefit
Tangeritine	Tangerine and other citrus peels	• cholesterol lowering agent • protective effects against Parkinson's disease • anti-cancer agent
Ferulic acid	Brown rice, whole wheat, oats, coffee, apple, artichoke, peanuts, orange and pineapple	• antioxidant (may neutralize free radicals involved with DNA damage and accelerated cell aging) • antitumor activity against breast and liver cancer
Isosakuranetin	Citrus fruits	• cytotoxic and fungicide properties
Nobiletin	Citrus fruits	• anticancer, antiviral and anti-inflammatory activities
p-Coumaric acid	Peanuts, tomatoes, carrots, garlic and wide variety of edible plants	• believed to reduce the risk of stomach cancer by reducing the formation of carcinogenic nitrosamines
Mangiferin	Widely distributed in higher plants	• one of the constituents of many folk medicines • anti-oxidant and anti-viral agent • chemopreventive agent • antiinflammatory, diuretic, chloretic and cardiotonic activities • antibacterial activity against gram postive bacteria • has been recommended as a drug in preventing dental plaques

Figure 1. Potential health benefits of selected flavonoids as documented in scientific literature.

This report will describe the use of HPLC-UV to identify flavonoids in a citrus juice sample. Separations were performed utilizing XBridge Shield RP_{18} and XBridge C_8 columns.

Experimental

Chromatographic Conditions			
Columns	XBridge Shield RP18, 4.6 x 150 mm, 5 µm		
	Part Number 186003009		
	XBridge C_8, 4.6 x 150 mm, 5 µm		
	Part Number 186003017		
Mobile Phase A	2% Acetic acid		
Mobile Phase B	Acetonitrile		
Gradient	Time	Profile	
	(min)	%A	%B
	0.0	90	10
	15.0	86	14
	20.0	82	18
	30.0	75	25
	55.0	45	55
	67.0	5	95
	80.0	5	95
	85.0	90	10
Flow Rate	0.75 mL/min		
Injection	20 µL		
Temperature	Ambient		
Detection	UV @ 310 nm		
System	Alliance® 2695 with a 996 PDA detector		

Results and Discussion

Figure 2 illustrates the reversed-phase HPLC chromatograms of flavonoids utilizing both the XBridge Shield RP_{18} and XBridge C_8.

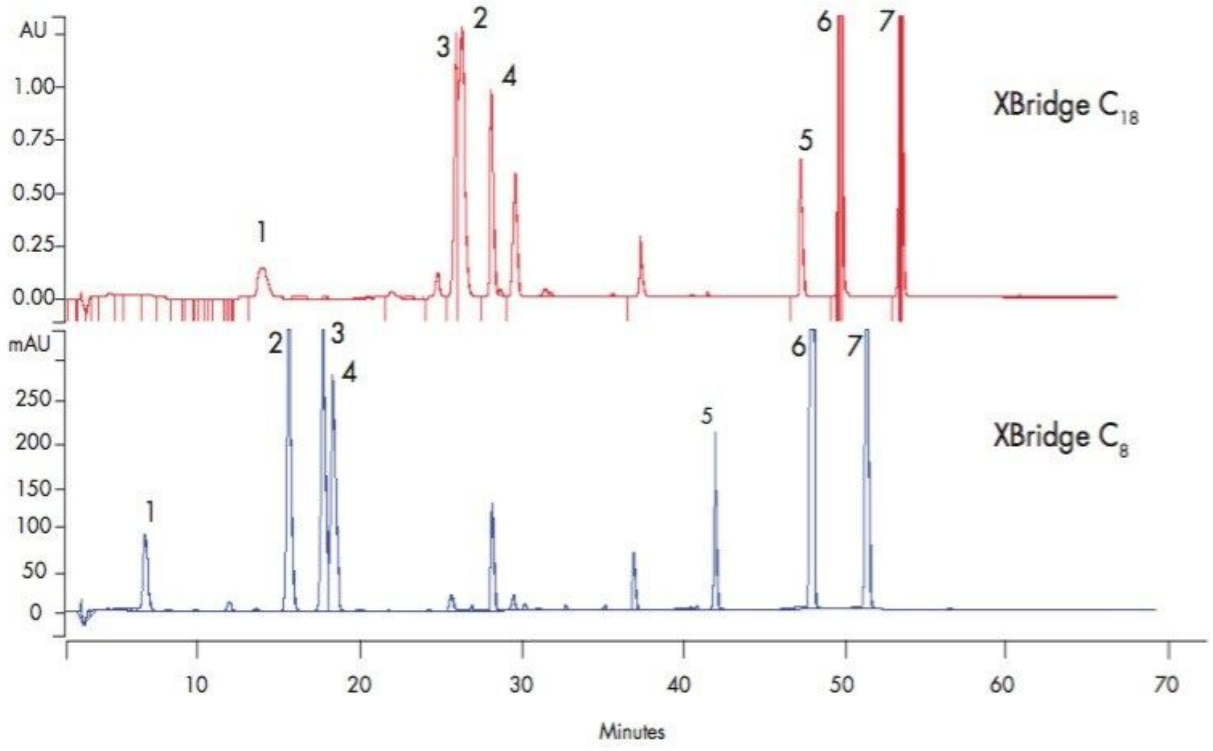

Figure 2. HPLC chromatograms of flavonoids in fruit juice. Compounds: (1) mangiferin; (2) p-coumaric acid; (3) ferulic acid; (4) apigenin glucoside; (5) isosakuranetin; (6) nobiletin; (7) tangeretin

Conclusion

Currently there is much biomedical interest in flavonoids because of their apparent health benefits. In this study HPLC analysis was utilized to identify flavonoids in grapefruit juice. This accurate identification is a critical first step towards preparative-isolation of these compounds for further analysis.

Acknowledgement

Chromatograms courtesy of Dr. John Manthey, USDA, Agricultural Research Service.

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Alliance HPLC System <https://www.waters.com/534293>

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