

Constituents and Additives

Food products are analyzed for a variety of reasons, e.g., compliance with legal and labeling requirements, assessment of product quality, determination of nutritive value, and detection of adulteration, etc. According to the Codex Alimentarius Commission – “Food Additive” means any substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value. The term “Food additive” does not include contaminants or substances added to food for maintaining or improving its nutritive value. “Food additives” do not include use of vitamins, minerals, herbs, salt, spices, yeast, hops, starter cultures, malt extract, etc. “Food additives” are intentionally added to food and must be safe for a lifetime of consumption based on current toxicological evaluation.

“Food additives” are classified on the basis of their functional use and are grouped as:

Colors	Preservatives	Acidity Regulators
Antioxidants	Anti-caking agents	Antifoaming Agents
Artificial sweeteners	Enzymes	Emulsifiers
Emulsifying agents	Flavors	Flavor enhancers
Modified Starches	Phosphates	Stabilizers
Thickening and jelling agents.		

Flavonoids and Isoflavones

Flavonoids (from the Latin word flavus meaning yellow, their color in nature) are a class of plant secondary metabolites. Flavonoids are all ketone-containing compounds and the IUPAC nomenclature classify flavonoids as:

Flavones from 2-phenylchromen-4-one (2-phenyl-1,4-benzopyrone) structure (examples: quercetin, rutin).

Isoflavonoids from 3-phenylchromen-4-one (3-phenyl-1,4-benzopyrone) structure
Neoflavonoids, from 4-phenylcoumarine (4-phenyl-1,2-benzopyrone) structure.

Food manufacturers have become interested in flavonoids for their possible medicinal properties. Although no physiological evidence is yet established, the beneficial effects of fruits, vegetables, tea, and red wine have sometimes been attributed to flavonoid compounds. Good sources of flavonoids include all citrus fruits, berries, ginkgo biloba, onions, parsley, pulses, tea (especially white and green), red wine, seabuckthorn, and dark chocolate (with > 70% cocoa content).

Quercetin, a flavonoid and more specifically a flavonol, is the aglycone (non-sugar) form of other flavonoid glycosides, found in citrus fruit, buckwheat and onions. The glycosides quercitrin and rutin are formed from quercetin reacting with rhamnose and rutinose, respectively. Epicatechin may improve blood flow. Cocoa, the major ingredient of dark chocolate, contains relatively high amounts of epicatechin. Daidzein can be found in food such as soybeans and soy products like tofu and textured vegetable protein.

To analyze flavonoids in fruit, vegetables and other flavonoid rich solid samples problems may or will arise from sample matrix. The presence and concentration levels among different interesting marker molecules (analytes) differ from high to low concentrations, and their presence differ among sample types (i.e. cocoa bean and lemon). The analysis of flavonoids thus constitute problems with finding suitable sample preparation, to resolve all interesting compounds from each other chromatographically, and to detect them accordingly.

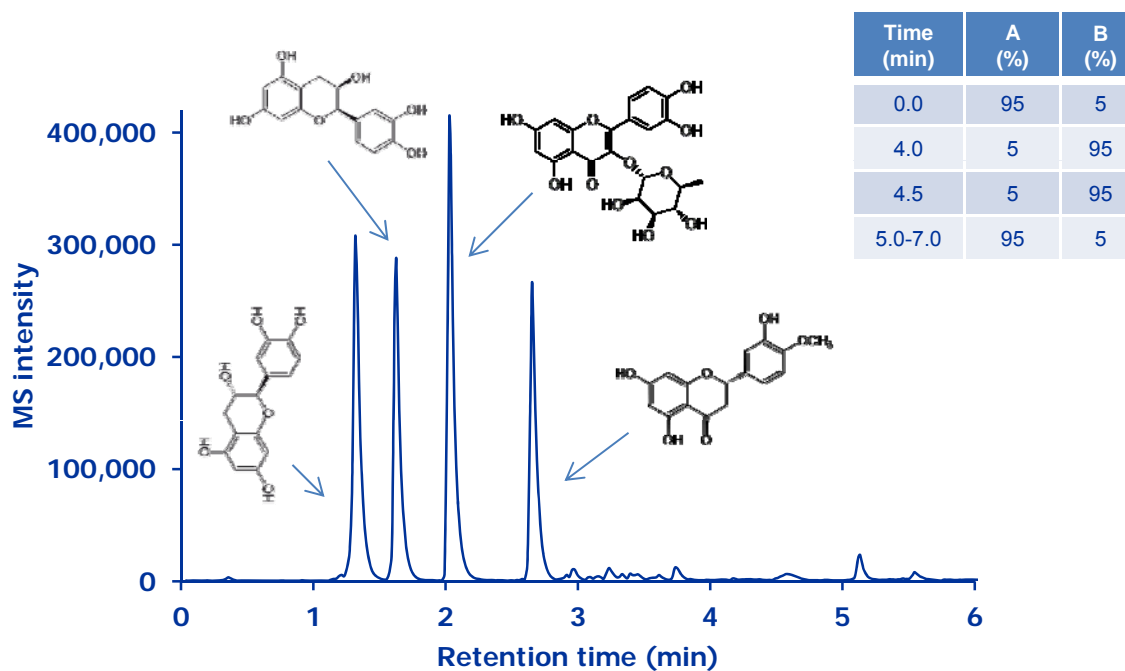
A few examples are presented herein to illustrate that Chromolith® columns are ideal tools for this purpose, and combined with MS detection it is possible to develop sensitive methods for monitoring flavonoids and isoflavones in food and beverage samples.

Flavonoids – LC-MS

Chromolith® RP-18 endcapped

Chromatographic Conditions

Column: Chromolith® FastGradient RP-18 endcapped, 50x2.0 mm (1.52007.0001)
 Injection: 1 µL
 Detection: Positive ESI-MS (m/z range 100-455)
 Flow Rate: 0.4 mL/min
 Mobile Phase : A: water + 0.1 % formic acid
 B: acetonitrile + 0.1 % formic acid
 Gradient: See table
 Temperature: 25° C
 Diluent: water
 Sample: 317 ppb Catechin, 270 ppb epicatechin, 198 ppb quercetin and 177 ppb hesperetin in diluent.
 Pressure Drop: 47-17 Bar (677-245 psi)



Chromatographic Data

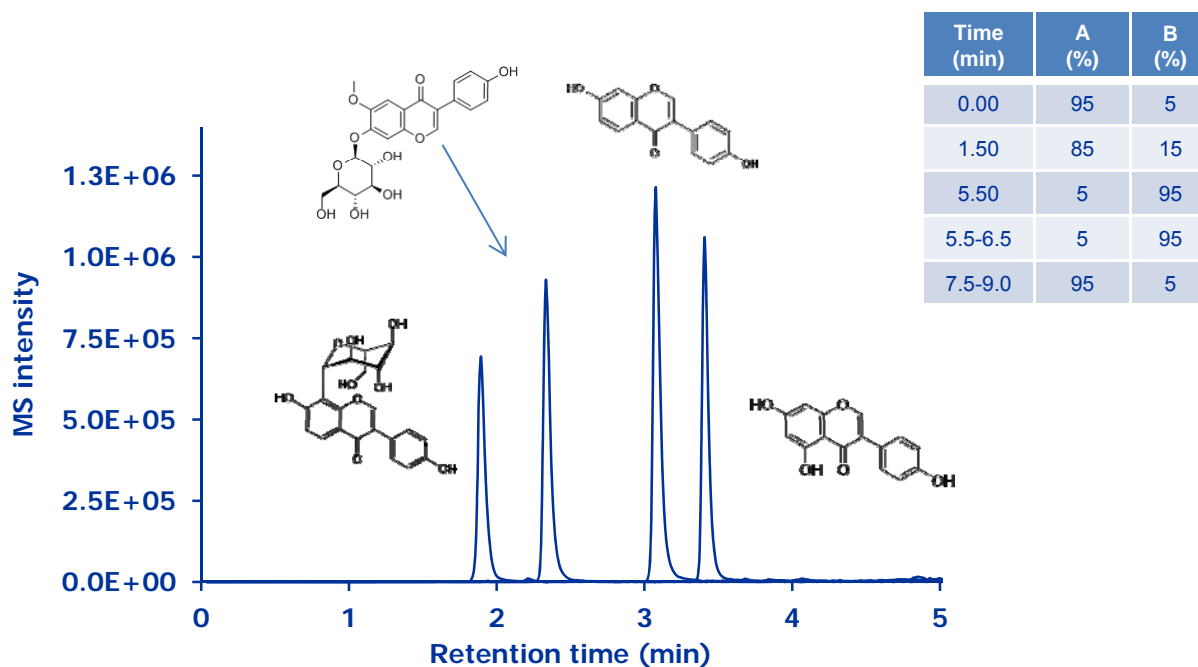
No.	Compound	Retention Time (min)	[M+H] ⁺ (m/z)
1	(+)-Catechin	1.3	291.1
2	(-)-Epicatechin	1.6	291.1
3	Quercetin	2.0	449.1
4	Hesperetin	2.7	303.1

Isoflavones – LC-MS

Chromolith® RP-18 endcapped

Chromatographic Conditions

Column: Chromolith® FastGradient RP-18 endcapped, 50x2.0 mm (1.52007.0001)
 Injection: 1 µL
 Detection: Positive ESI-MS (m/z range 100-600), overlay of four EICs (m/z 271.0, 255.0, 447.1, 417.1)
 Flow Rate: 0.5 mL/min
 Mobile Phase : A: water + 0.1 % formic acid
 B: acetonitrile + 0.1 % formic acid
 Gradient: See table
 Temperature: 25° C
 Diluent Methanol and water (50:50 v/v)
 Sample: 10 ppb (10 µg/mL) of each Genistein, Daidzein, Glycitin, and 20 ppb of Puerarin in diluent
 Pressure Drop: 57-22 Bar (821-317 psi)



Chromatographic Data

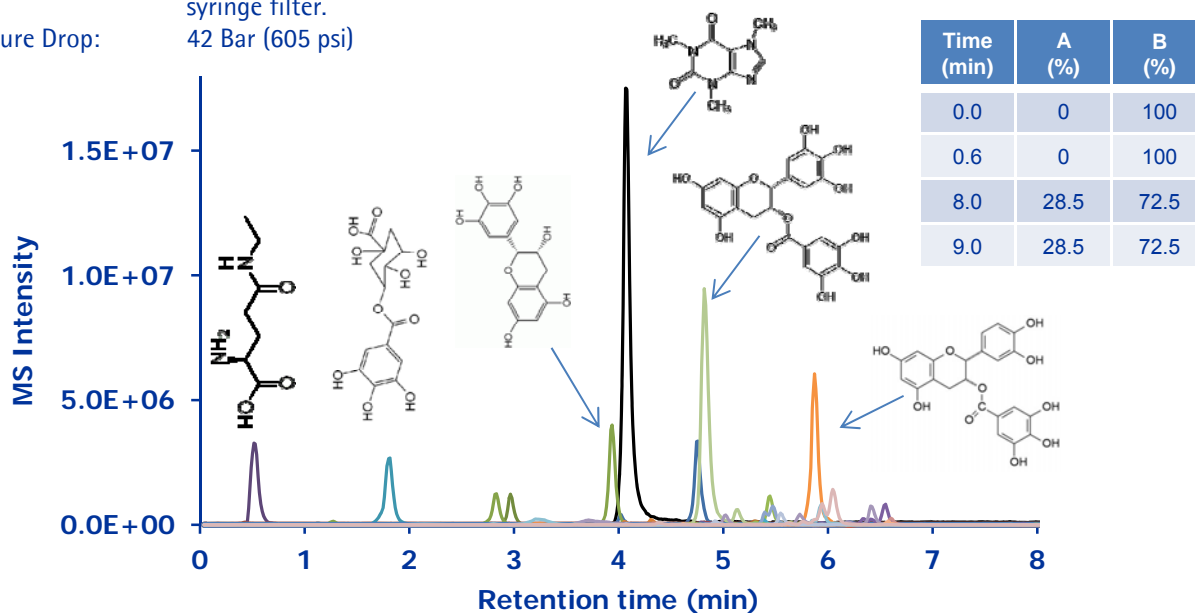
No.	Compound	Retention Time (min)	[M+H] ⁺ (m/z)
1	Puerarin	1.9	417.1
2	Glycitin	2.3	447.1
3	Daidzein	3.1	255.0
4	Genistein	3.4	271.0

Analysis of Green Tea – LC-MS

Chromolith® RP-18 endcapped

Chromatographic Conditions

Column:	Chromolith® FastGradient RP-18 endcapped, 50x2.0 mm	(1.52007.0001)
Injection:	1 µL	
Detection:	Positive ESI-MS (m/z range 150-950), BPC and overlay of EICs	
Flow Rate:	0.4 mL/min	
Mobile Phase :	A: acetonitrile + 0.1 % formic acid B: water + 0.1 % formic acid	
Gradient:	See table	
Temperature:	25° C	
Diluent	Water	
Sample:	1.75 g of green tea was extracted for three minutes using 100 mL of boiling water. Prior to injection both samples were centrifuged at 4500 rpm for 10 min and cleaned using a 0.45 µm syringe filter.	
Pressure Drop:	42 Bar (605 psi)	



Chromatographic Data

No.	Compound	Retention Time (min)	[M+H] ⁺ (m/z)
1	Theanine	0.5	175.1
2	Theogallin	1.8	345.1
3	Epigallocatechin	3.9	307.1
4	Caffeine	4.1	195.1
5	Epigallocatechin gallate	4.8	459.1
6	Epicatechin gallate	5.9	443.1