Foods, Flavors & Fragrances Applications

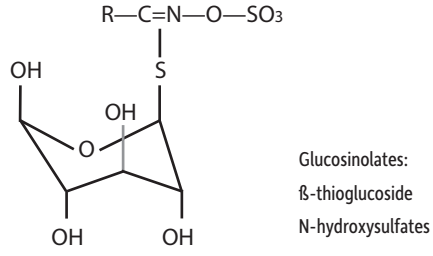
HPLC Analysis of Glucosinolates in Vegetable Extracts Without Ion Pairing Using an Ultra Aqueous C18 Column

Glucosinolates are a naturally occurring set of compounds found in a variety of edible plants, most notably in broccoli, radish, and cabbage. Agriculturally, the degradation compounds of glucosinolates have been shown to act as natural pesticides and fungicides. This breakdown occurs in the soil. These toxic compounds then further degrade into harmless compounds. Research on glucosinolates is continuing in hopes of bringing a more environmentally friendly approach to pest control.

Nutritionally, human consumption of these compounds is associated with a significantly reduced risk for a variety of malignant cancers along the alimentary canal. They also have been shown to suppress existing tumor growth. Glucosinolates are precursors to isothiocyanates, such as sulforaphane (4-methylsulfinylbutyl isothiocyanate), which regulates mammalian enzymes of xenobiotic metabolism.

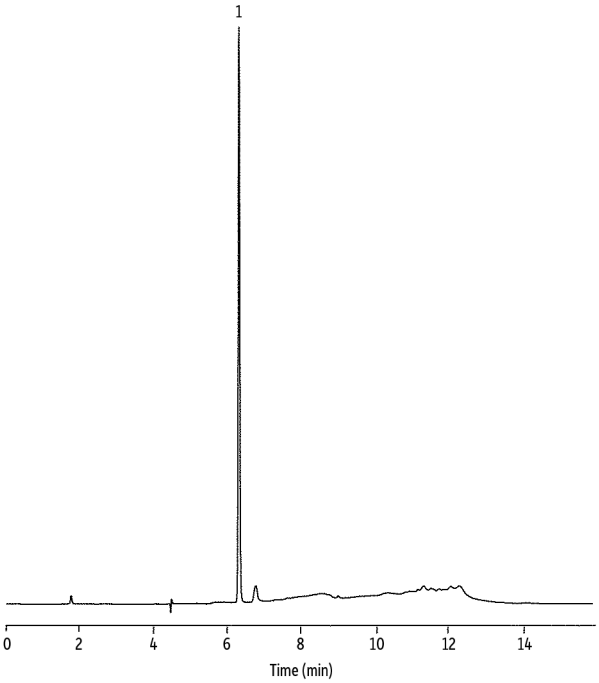
Phenethyl glucosinolate (gluconasturtiin) is one of the glucosinolates widely found in cruciferous vegetables. It is one of the least polar glucosinolates, making it relatively easy to retain by reversed-phase, high-performance liquid chromatography (HPLC). However, there are a number of glucosinolates with hydrophilic "R-" groups, such as 3-methylsulfinylpropyl glucosinolate, that are very difficult to retain by conventional reversed-phase HPLC. Additionally, the "R-" group of glucosinolates can vary greatly, resulting in a large number of glucosinolates with widely differing polarities (Figure 1). Thus, many analysts resort to reversed-phase ion-pairing methods to analyze glucosinolates. The addition of ion-pairing reagents is less convenient, and makes the analyses inherently less reproducible. Ion-pairing reagents also make gradient elution very impractical, due to long equilibration times.

Figure 1: Chemical Structure of Glucosinolates With Variable "R-" Group.



The analysis of a phenethyl glucosinolate standard using an Ultra Aqueous C18 column shows good peak shape without the use of ion-pairing reagents (Figure 2). Extracts of cabbage and watercress were analyzed using the same conditions (Figures 3 and 4). Gradient elution from 0 to 75% acetonitrile was used to retain and elute analytes having a wide range of polarities. The Ultra Aqueous C18 column allows the use of simple reversed-phase conditions for the analyses of glucosinolates, saving time as compared to reversed-phase ion-pairing methods.

Figure 2: Phenethyl Glucosinolate on Ultra Aqueous C18



LC_0166

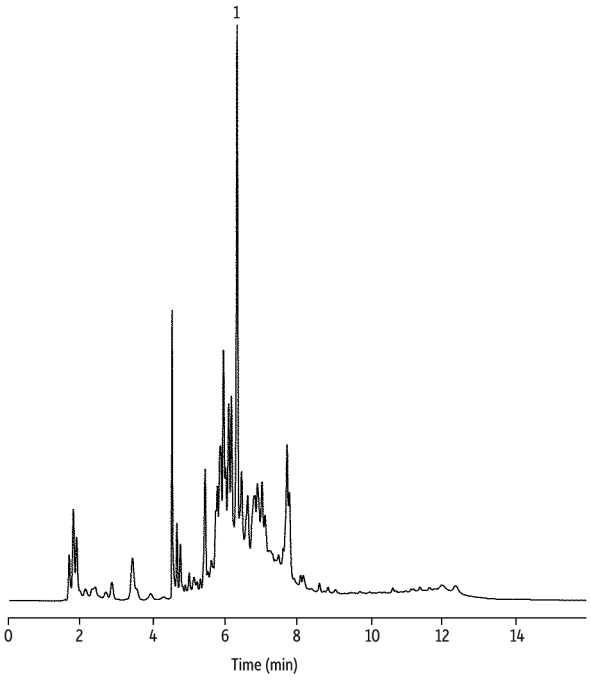
Peak
1. Phenethyl glucosinolate

Column	Ultra Aqueous C18 (cat.# 9178565)
Dimensions:	150 mm x 4.6 mm ID
Particle Size:	5 µm
Pore Size:	100 Å
Temp.:	ambient
Sample	
Diluent:	water
Conc.:	1,000 µg/mL
Inj. Vol.:	10 µL
Mobile Phase	
A:	50 mM potassium phosphate, pH 2.5
B:	acetonitrile

Time (min)	**%B**
0	0
10	75
11	0
16	0

Flow:	1.0 mL/min
Detector	UV/Vis @ 210 nm

Figure 3: Cabbage Extract on Ultra Aqueous C18



LC_0165

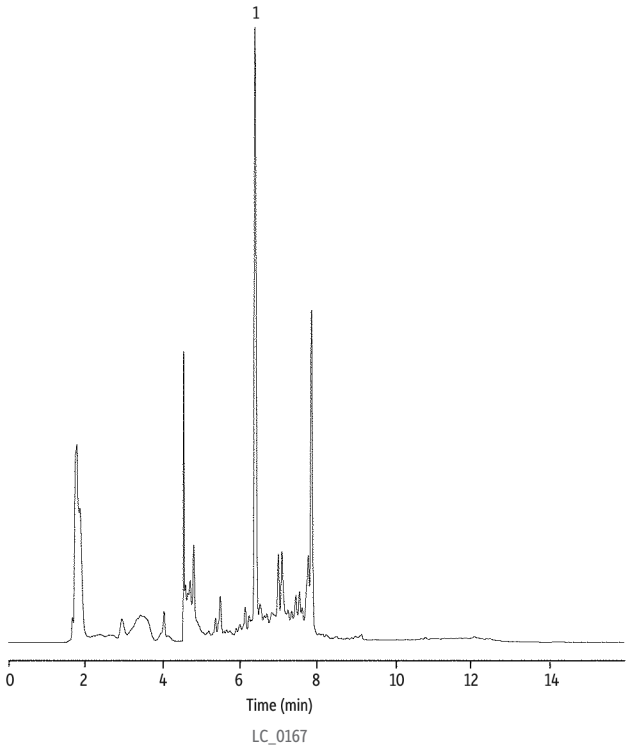
Peak
1. Phenethyl glucosinolate

Column	Ultra Aqueous C18 (cat.# 9178565)
Dimensions:	150 mm x 4.6 mm ID
Particle Size:	5 µm
Pore Size:	100 Å
Temp.:	Ambient
Sample	
Diluent:	Water
Inj. Vol.:	20 µL
Mobile Phase	
A:	50 mM Potassium phosphate, pH 2.5
B:	Acetonitrile

Time (min)	**%B**
0.00	0
10	75
11	0
16	0

Flow:	1.0 mL/min
Detector	UV/Vis @ 210 nm

Figure 4: Watercress Extract on Ultra Aqueous C18



Peak
1. Phenethyl glucosinolate

Column	Ultra Aqueous C18 (cat.# 9178565)
Dimensions:	150 mm x 4.6 mm ID
Particle Size:	5 µm
Pore Size:	100 Å
Temp.:	Ambient
Sample	Watercress extract
Diluent:	Water
Inj. Vol.:	100 µL
Mobile Phase	
A:	50 mM Potassium phosphate, pH 2.5
B:	Acetonitrile

Time (min)	**%B**
0.00	0
10	75
11	0
16	0

Flow:	1.0 mL/min
Detector	UV/Vis @ 210 nm

Acknowledgement: The phenyl glucosinolate standard and extracts of cabbage and watercress were generously provided by Dr. Gerard Engelen-Eigles, University of Minnesota, Horticulture Department.

Ultra Aqueous C18 Columns (USP L1)

Chromatographic Properties

The Restek® Aqueous C18 is a rugged, reversed-phase column with a well-balanced retention profile. It can effectively retain more types of solutes than a conventional C18 and is ideal for multi-component LC-MS analyses. The general-purpose Aqueous C18 boasts high reproducibility and compatibility with many mobile phase conditions—even 100% aqueous and acidic. And when used with a gradient, it eliminates the all-too-common issue of multiple compounds eluting near the column void time.

Column Characteristics:

particle size:	3 µm or 5 µm, spherical
pore size:	100 Å
carbon load:	15%
end-cap:	no
pH range:	2.5 to 8
temperature limit:	80 °C
USP phase code:	L1
phase category:	modified C18
ligand type:	proprietary polar modified and functionally bonded C18

Length	1.0 mm ID cat.#	2.1 mm ID cat.#	3.0 mm ID cat.#	4.6 mm ID cat.#
3 µm Columns				
30 mm	9178331	9178332	917833E	9178335
50 mm	9178351	9178352	917835E	9178355
100 mm	9178311	9178312	917831E	9178315
150 mm	9178361	9178362	917836E	9178365
5 µm Columns				
30 mm	9178531	9178532	917853E	9178535
50 mm	9178551	9178552	917855E	9178555
100 mm	9178511	9178512	917851E	9178515
150 mm	9178561	9178562	917856E	9178565
200 mm	9178521	9178522	917852E	9178525
250 mm	9178571	9178572	917857E	9178575

Ultra Aqueous C18 Guard Cartridges

Guard Cartridges	3-pk. (10 x 2.1 mm)	3-pk. (10 x 4.0 mm)
Ultra Aqueous C18 Guard Cartridge	917850212	917850210

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