

## Analysis of Preservatives Using HPLC

### Background

Preservatives are chemical compounds that are used in a wide range of applications to maintain overall product quality.<sup>1</sup> For example, preservatives are used in foods, beverages, pharmaceuticals, and personal care products. Some preservatives act as antimicrobial agents, some act as antioxidants, and some can perform both functions. To some extent, the ability of the chemical to act as a preservative depends on the environment, so factors such as the type of product, water content, pH, and storage conditions all need to be considered when selecting a preservative.

Of the chemical compounds commonly used as preservatives, many of them can be effectively analyzed by high performance liquid chromatography (HPLC).<sup>2</sup> Chromatographic techniques such as HPLC separate preservative compounds from the rest of the sample matrix, providing more accurate results compared to traditional techniques such as spectrophotometry. Because preservatives include a number of different compound types, there are a variety of HPLC stationary phases, mobile phases, and detectors that can be used.

### Microbial Growth Inhibitors

Microbes such as molds, yeasts, and bacteria need to have certain conditions (e.g., water, pH, temperature) in order to flourish. Chemical preservatives can be used to kill or prevent the growth of these microbes by either changing their environment or reacting directly with the microbes.<sup>3</sup> Selecting the best preservative for a given product can be an important part of product development. In addition, other chemical compounds known as synergists can increase the effectiveness of some preservatives. Examples of synergists include: citric acid, isopropyl citrate, phosphoric acid, ascorbic acid, ascorbyl palmitate, tartaric acid, and lecithin.

Antimicrobial compounds include organic acids, benzoate and sorbate salts, sulfur dioxide and sulfites, nitrites, propionates, and parabens. Some antimicrobials act on yeasts, molds, and bacteria, while some specifically target certain classes of microbes. Sulfites can inhibit bacteria, but are not effective against yeasts. For this reason, they often are used as preservatives in wines. Nitrites can inhibit botulism (bacterial spores) in meats. Sorbates and benzoates are specific inhibitors of bacteria, while propionates act on molds and rope bacteria, but not on yeasts. Therefore propionates can be used in yeasted bread products. The parabens have both antimicrobial activity and antioxidant activity.

Organic acids, such as acetic acid and citric acid, can be used to control the pH of a product. For example, in food products these acidulants can lower the pH out of the optimum pH range for bacteria, yeast, and/or molds. Organic acids such as malic acid and citric acid can be found naturally in fruits; oxalic acid can be found in spinach and rhubarb; and tartaric acid can be found in grapes.

Using HPLC, concentrations of these preservatives can be monitored. However, analyzing polar organic acids can be difficult on conventional reversed phase columns, even when using highly aqueous mobile phases. The Ultra Aqueous C18 column provides enhanced retention and selectivity for challenging applications such as this. The novel bonding chemistry used for this phase allows the alkyl groups to remain extended, even in highly aqueous mobile phase, preventing the chain folding that occurs with conventional C18 phases. Therefore, stable and reproducible retention is possible even with 100% aqueous mobile phases. Notice the excellent retention for a series of organic acids using the Ultra Aqueous C18 column and UV detection (Figure 1).

Benzoate and sorbate salts also can be used as preservatives in a range of consumer products. These salts interact with the bacteria itself, limiting the viability of the microorganism. These compounds can be analyzed in their acid form (i.e. as benzoic acid and

Figure 1

*Analysis of a series of organic acids typically found in items such as foods, beverages, and personal care products, using an Ultra Aqueous C18 column shows excellent retention of organic acids, even with a highly aqueous mobile phase.*

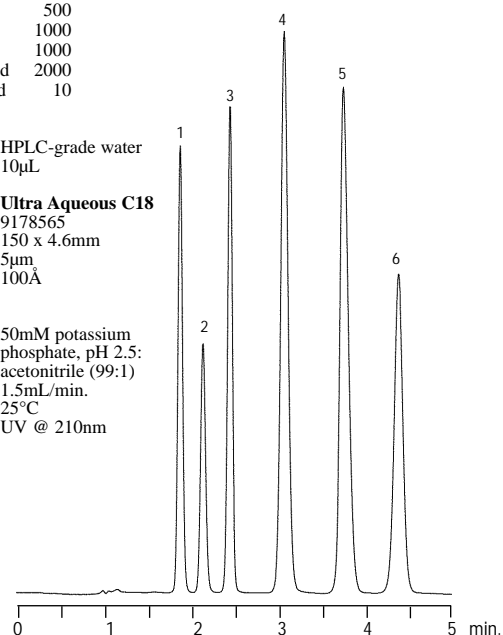
Peak List:	Conc. (µg/mL):
1. malonic acid	500
2. lactic acid	500
3. acetic acid	1000
4. citric acid	1000
5. succinic acid	2000
6. fumaric acid	10

**Sample:**  
Solvent: HPLC-grade water  
Inj.: 10µL

**Column:** Ultra Aqueous C18  
Catalog #: 9178565  
Dimensions: 150 x 4.6mm  
Particle size: 5µm  
Pore size: 100Å

**Conditions:**  
Mobile phase: 50mM potassium phosphate, pH 2.5: acetonitrile (99:1)

Flow: 1.5mL/min.  
Temp.: 25°C  
Det.: UV @ 210nm



LC\_0140

sorbic acid) by reversed phase HPLC using a Pinnacle II™ Phenyl column and acidified water:methanol as the mobile phase (Figure 2). By monitoring the UV absorbance at 245nm, sensitive detection of both benzoic and sorbic acids can be achieved. For optimum sensitivity, benzoic acid can be monitored at 228nm and sorbic acid at 259nm.

Parabens, such as propyl paraben, can be used as antimicrobial agents and as antioxidants. As antimicrobials, they act on yeasts and molds. Although they tend to be somewhat higher in cost, they still are used in a range of applications. The maximum allowable

concentration in most products is 0.1%. A series of parabens analyzed using a Pinnacle II™ C8 column is shown in Figure 3. The separation was effected using an acidified mobile phase to suppress ionization of the analytes. Because these compounds are strong UV absorbers, very sensitive detection can be achieved by monitoring the UV at 254nm.

### Antioxidants

Products containing fats and oils are prone to lipid oxidation, which can promote off-flavors, off-odors, and color changes as well as limiting shelf life. To inhibit lipid oxidation, antioxidants

Figure 2

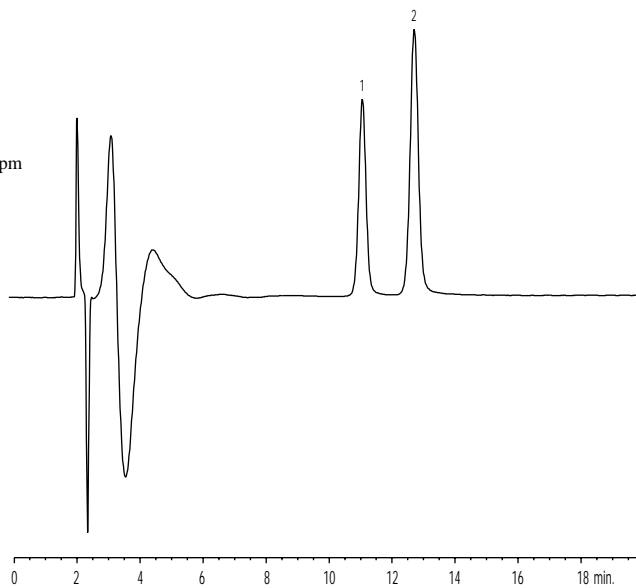
The Pinnacle II™ phenyl column is an excellent choice to analyze medium polarity compounds such as sorbic and benzoic acids.

**Peak List:**

- 1. sorbic acid
- 2. benzoic acid

**Sample:**

Inj.: 10µL  
 Conc.: each 100ppm  
 Solvent: methanol



LC\_0199

**Column:** Pinnacle II™ Phenyl

Catalog #: 9215565  
 Dimensions: 150 x 4.6mm  
 Particle Size: 5µm  
 Pore Size: 110Å

**Conditions:**

Mobile Phase: 1% acetic acid:methanol (80:20)  
 Flow: 1.0 mL/min  
 Temp.: 25°C  
 Det.: UV @ 230nm

Figure 3

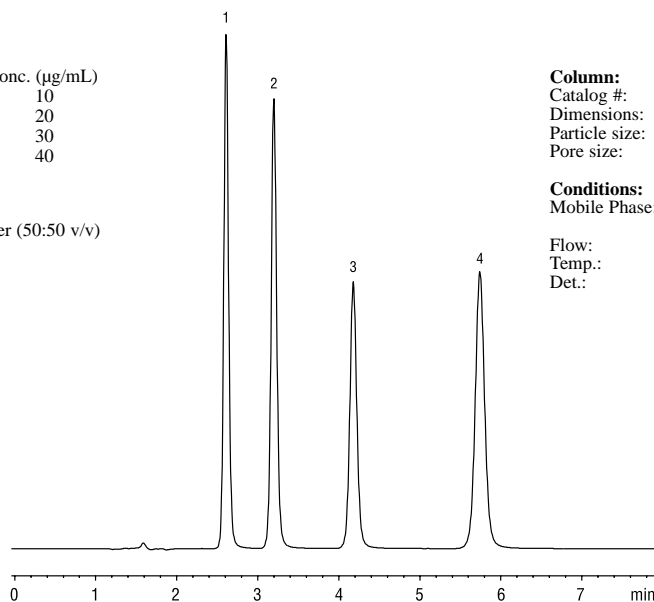
A series of parabens can be efficiently separated on a Pinnacle II™ C8 column, allowing for fast and accurate quantitation.

**Peak List:**

	conc. (µg/mL)
1. methyl paraben	10
2. ethyl paraben	20
3. propyl paraben	30
4. butyl paraben	40

**Sample:**

Inj.: 5µL  
 Solvent: methanol:water (50:50 v/v)



LC\_0176

**Column:** Pinnacle II™ C8

Catalog #: 9213565  
 Dimensions: 150 x 4.6mm  
 Particle size: 5µm  
 Pore size: 110Å

**Conditions:**

Mobile Phase: 0.1% acetic acid in water: acetonitrile (50:50, v/v)  
 Flow: 1.0mL/min.  
 Temp.: ambient  
 Det.: UV @ 254nm

Figure 4

Structures of three commonly-used phenolic antioxidants.

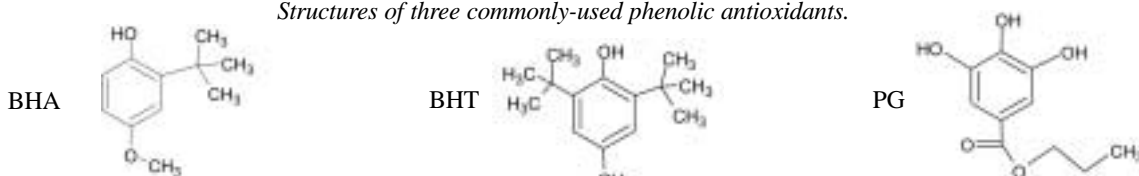


Figure 5

Phenolic antioxidants can be quantitated easily using a Pinnacle II™ C18 column and UV detection at 280nm.

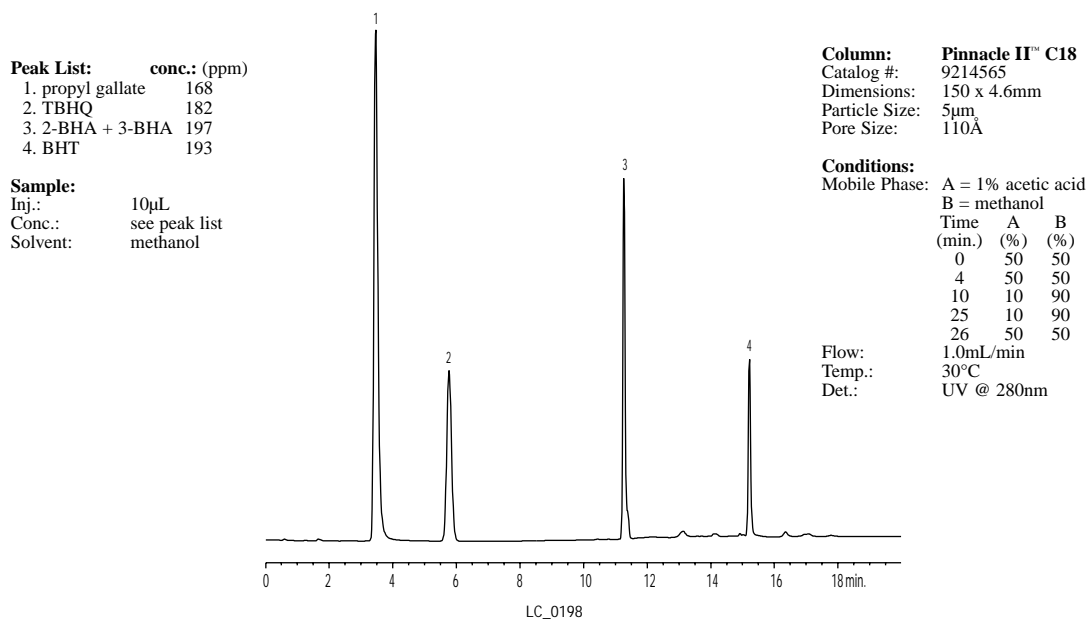
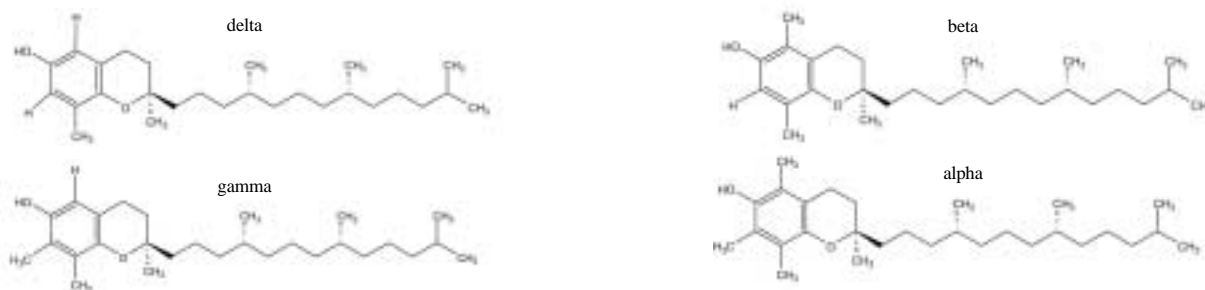


Figure 6

Structures of tocopherols—these compounds readily oxidize when exposed to light or oxygen.  
(the activity is as follows:  $\delta > \gamma > \beta > \alpha$ )



can be added to the product. Phenolic antioxidants include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butyl hydroquinone (TBHQ). These four, plus the tocopherols, act as the primary antioxidants found in foods and beverages produced in the US. The structures of BHA, BHT, and PG—the three most common phenolic antioxidants—are shown in Figure 4. Phenolic antioxidants, such as BHT, are regulated by the FDA, and can be added to many products at levels up to 200ppm based on the fat content.

Phenolic antioxidants can be analyzed by reversed phase HPLC using a Pinnacle II™ C18 column and an acidified mobile phase. As with the previous methods, an acidic mobile phase is used to

suppress ionization of the analytes. The HPLC separation of BHA, BHT, PG, and TBHQ using UV detection at 280nm shows how effectively these compounds can be separated using the Pinnacle II™ C18 column (Figure 5).

“Natural” antioxidants, such as tocopherols and tocotrienols, are used to inhibit lipid oxidation and to promote general health in the consumer. These compounds are found naturally in products such as fats and oils. When used as additives, however, they are regulated. Antioxidants such as tocopherols can be challenging to analyze, because they readily oxidize when exposed to light or oxygen. The structures of four different tocopherols are shown in Figure 6. The analysis of these tocopherols by normal phase

Figure 7

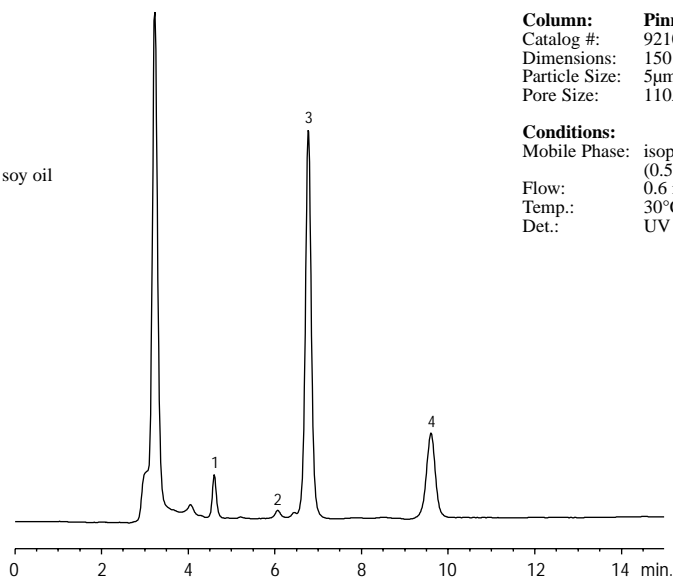
A Pinnacle II™ silica column effectively separates the positional isomers of tocopherol by normal phase HPLC.

**Peak List:**

1.  $\alpha$ -tocopherol
2.  $\beta$ -tocopherol
3.  $\gamma$ -tocopherol
4.  $\delta$ -tocopherol

**Sample:**

Inj.: 10 $\mu$ L  
Conc.: approx. 1.25% soy oil  
Solvent: hexane



LC\_0197

**Column:** Pinnacle II™ Silica

Catalog #: 9210565  
Dimensions: 150 x 4.6mm  
Particle Size: 5 $\mu$ m  
Pore Size: 110Å

**Conditions:**

Mobile Phase: isopropyl alcohol:hexane (0.5:99.5)  
Flow: 0.6 mL/min  
Temp.: 30°C  
Det.: UV @ 295nm

HPLC using a Pinnacle II™ silica column shows how effectively these positional isomers can be separated (Figure 7). These compounds can be quantified using either fluorescence or UV detection.

### Summary

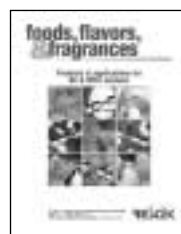
Preservatives are added to a wide range of products to help maintain the overall quality of the product as well as to extend its shelf life. The level of each preservative compound present needs to be monitored for several reasons. First, because many of these products are consumed, the preservative content needs to be measured to ensure a safe level. Second, the stated shelf-life is dependent upon a certain concentration of preservatives and incorrect concentrations can lead to premature spoilage of the product.

HPLC is a powerful tool for analyzing preservatives in a wide range of consumer products. One of its benefits is that many times only minimal sample preparation is required. Chromatographic techniques allow analysts to separate preservatives from other compounds in the sample matrix, improving the overall quality of the results. The Pinnacle II™ line of HPLC columns is an excellent choice for the analysis of preservative compounds such as parabens, benzoate and sorbate salts, phenolic antioxidants, and tocopherols. Pinnacle II™ columns are available in a range of stationary phases, including C18, C8, phenyl, amino, and silica. For analyzing organic acids, the Ultra Aqueous C18 column is the perfect choice, offering superior retention and reproducibility for polar compounds, even when using highly aqueous mobile phases.

### References

1. Fennema, Owen R. Food Chemistry (1996), Marcel Dekker, New York.
2. Nollet (ed.), Food Analysis by HPLC (2000), 2nd edition, Marcel Dekker, New York.
3. Foulke, Judith E. "A Fresh Look at Food Preservatives" in FDA Consumer (October 1993), US. Food & Drug Administration.

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## Ultra Aqueous C18 5µm Columns



Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9178531	9178532	9178533	9178535
50mm	9178551	9178552	9178553	9178555
100mm	9178511	9178512	9178513	9178515
150mm	9178561	9178562	9178563	9178565
200mm	9178521	9178522	9178523	9178525
250mm	9178571	9178572	9178573	9178575



## Ultra Aqueous C18 Guard Cartridges

Dimensions	cat.#	qty.
10 x 2.1mm	917850212	3-pk.
10 x 4.0mm	917850210	3-pk.
20 x 2.1mm	917850222	2-pk.
20 x 4.0mm	917850220	2-pk.

## Pinnacle II™ C18 5µm Columns



Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9214531	9214532	9214533	9214535
50mm	9214551	9214552	9214553	9214555
100mm	9214511	9214512	9214513	9214515
150mm	9214561	9214562	9214563	9214565
200mm	9214521	9214522	9214523	9214525
250mm	9214571	9214572	9214573	9214575



## Pinnacle II™ C18 Guard Cartridges

Dimensions	cat.#	qty.
10 x 2.1mm	921450212	3-pk.
10 x 4.0mm	921450210	3-pk.
20 x 2.1mm	921450222	2-pk.
20 x 4.0mm	921450220	2-pk.

## Pinnacle II™ C8 5µm Columns



Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9213531	9213532	9213533	9213535
50mm	9213551	9213552	9213553	9213555
100mm	9213511	9213512	9213513	9213515
150mm	9213561	9213562	9213563	9213565
200mm	9213521	9213522	9213523	9213525
250mm	9213571	9213572	9213573	9213575



## Pinnacle II™ C8 Guard Cartridges

Dimensions	cat.#	qty.
10 x 2.1mm	921350212	3-pk.
10 x 4.0mm	921350210	3-pk.
20 x 2.1mm	921350222	2-pk.
20 x 4.0mm	921350220	2-pk.

## Pinnacle II™ Silica 5µm Columns



Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9210531	9210532	9210533	9210535
50mm	9210551	9210552	9210553	9210555
100mm	9210511	9210512	9210513	9210515
150mm	9210561	9210562	9210563	9210565
200mm	9210521	9210522	9210523	9210525
250mm	9210571	9210572	9210573	9210575



## Pinnacle II™ Silica Guard Cartridges

Dimensions	cat.#	qty.
10 x 2.1mm	921050212	3-pk.
10 x 4.0mm	921050210	3-pk.
20 x 2.1mm	921050222	2-pk.
20 x 4.0mm	921050220	2-pk.

## Pinnacle II™ Phenyl 5µm Columns



Length	1.0mm ID cat.#	2.1mm ID cat.#	3.2mm ID cat.#	4.6mm ID cat.#
30mm	9215531	9215532	9215533	9215535
50mm	9215551	9215552	9215553	9215555
100mm	9215511	9215512	9215513	9215515
150mm	9215561	9215562	9215563	9215565
200mm	9215521	9215522	9215523	9215525
250mm	9215571	9215572	9215573	9215575



## Pinnacle II™ Phenyl Guard Cartridges

Dimensions	cat.#	qty.
10 x 2.1mm	921550212	3-pk.
10 x 4.0mm	921550210	3-pk.
20 x 2.1mm	921550222	2-pk.
20 x 4.0mm	921550220	2-pk.

## Trident™ Direct Guard Column System

Unlike “one size fits all” guard systems, the Trident™ Direct system gives you the power to select the right level of protection for your analysis. The system offers three levels of protection and guard cartridges in four dimensions, with a variety of bonded phases to match your analytical column. The economical, leak-free cartridge design provides an unprecedented combination of convenience, economy, and reliability. The foundation of the Trident™ Direct system is a reusable direct connect holder that easily attaches to any HPLC column using CPI- or Waters®-style end fittings.\* The system is available in the following configurations to match different protection level needs: in-line filter, in-line filter with holder for 1cm guard cartridge, and in-line filter with holder for 2cm guard cartridge. The guard cartridges are available in 2.1 and 4.0mm ID and are interchangeable with the appropriate length holder.

### Trident™ Direct provides three levels of protection



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Protection against  
particulate matter.  
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**Trident™ Direct 1cm guard cartridge  
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Moderate protection against particulate matter  
and irreversibly adsorbed compounds.  
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