RESTEK HPLC

# Applicationsnote

## Analysis of Preservatives Using HPLC

### Background

Preservatives are chemical compounds that are used in a wide range of applications to maintain overall product quality.[1] 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).[2] 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.[3] 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.

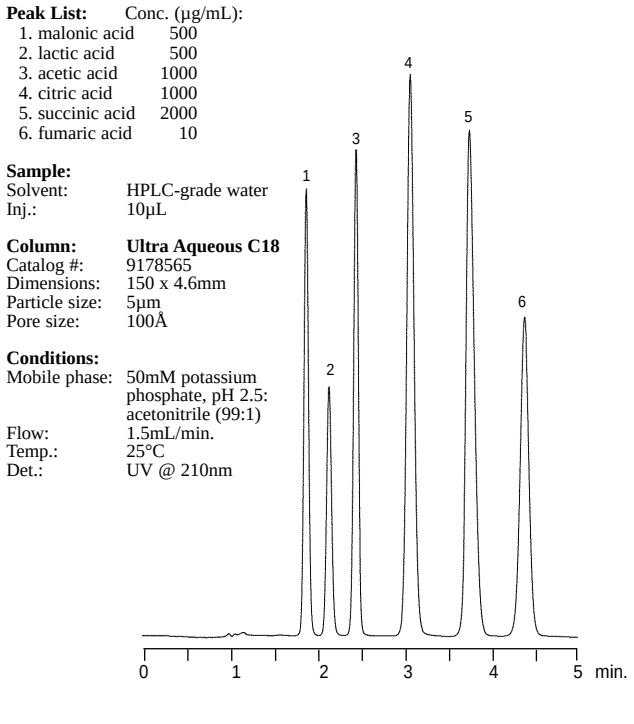
Organic acids, such as acetic acid and citric acid, can be used to control the pH of a product. For example, in food products these acidulants can lower the pH out of the optimum pH range for bacteria, yeast, and/or molds. Organic acids such as malic acid and citric acid can be found naturally in fruits;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




HPLC

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

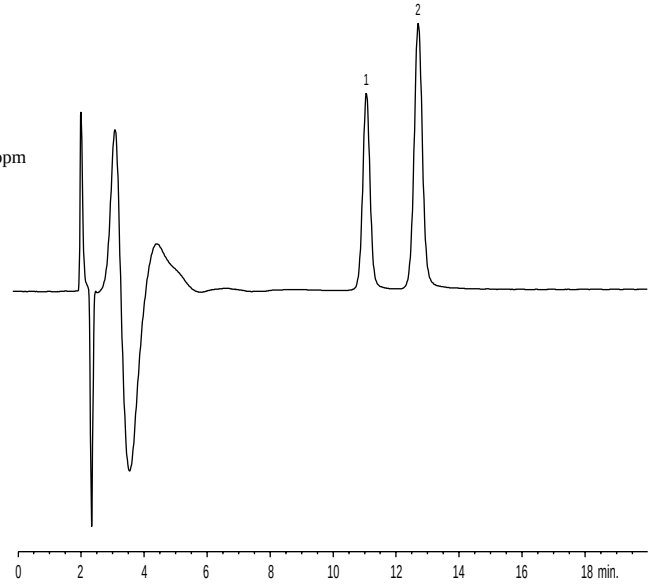
Products containing fats and oils are prone to lipid oxidation, which can promote off-flavors, off-odors, and color changes as well as limiting shelf life. To inhibit lipid oxidation, antioxidants

### 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




**Column:** **Pinnacle II™** Phenyl
Catalog #: 9215565
Dimensions: 150 x 4.6mm
Particle Size: 5µm
Pore Size: 110Å

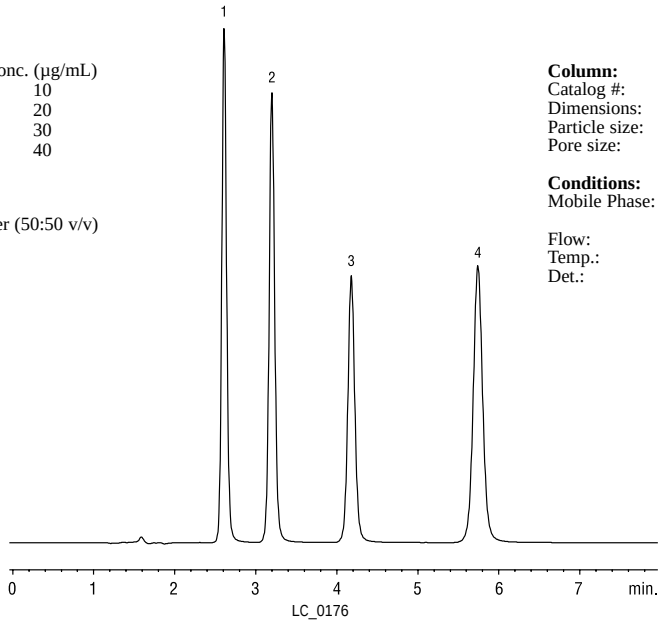
**Conditions:**
Mobile Phase: 1% acetic acid:methanol (80:20)
Flow: 1.0 mL/min
Temp.: 25°C
Det.: UV @ 230nm

### 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)



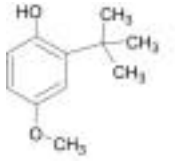

**Column:** **Pinnacle II™ C8**
Catalog #: 9213565
Dimensions: 150 x 4.6mm
Particle size: 5µm
Pore size: 110Å

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

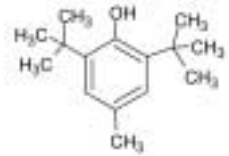
## Figure 4

*Structures of three commonly-used phenolic antioxidants.*

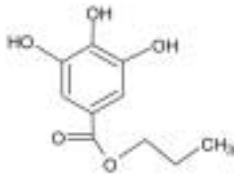
BHA



BHT



PG



## Figure 5

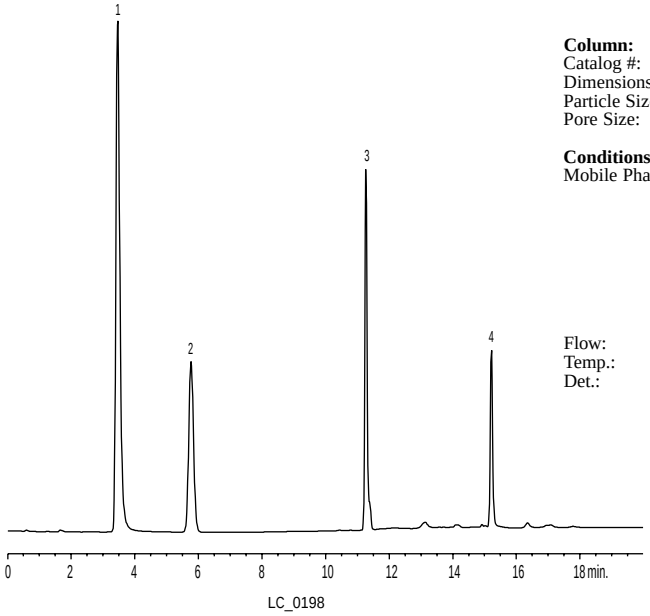
*Phenolic antioxidants can be quantitated easily using a Pinnacle II™ C18 column and UV detection at 280nm.*

**Peak List:** **conc.:** (ppm)

| | |
|---|---|
| 1. propyl gallate | 168 |
| 2. TBHQ | 182 |
| 3. 2-BHA + 3-BHA | 197 |
| 4. BHT | 193 |

**Sample:**

| | |
|---|---|
| Inj.: | 10µL |
| Conc.: | see peak list |
| Solvent: | methanol |




**Column:** **Pinnacle II™ C18**

| | |
|---|---|
| Catalog #: | 9214565 |
| Dimensions: | 150 x 4.6mm |
| Particle Size: | 5µm |
| Pore Size: | 110Å |

**Conditions:**

Mobile Phase: A = 1% acetic acid
B = methanol

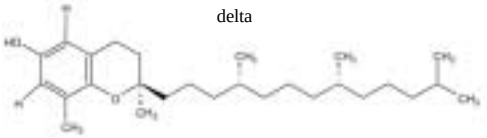
| Time (min.) | A (%) | B (%) |
|---|---|---|
| 0 | 50 | 50 |
| 4 | 50 | 50 |
| 10 | 10 | 90 |
| 25 | 10 | 90 |
| 26 | 50 | 50 |

| | |
|---|---|
| Flow: | 1.0mL/min |
| Temp.: | 30°C |
| Det.: | UV @ 280nm |

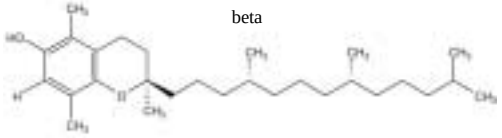
## Figure 6

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

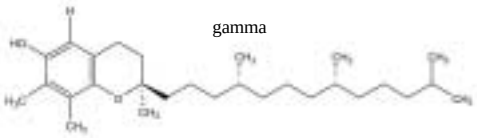
delta



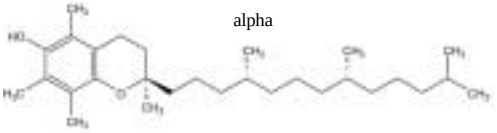
beta



gamma



alpha



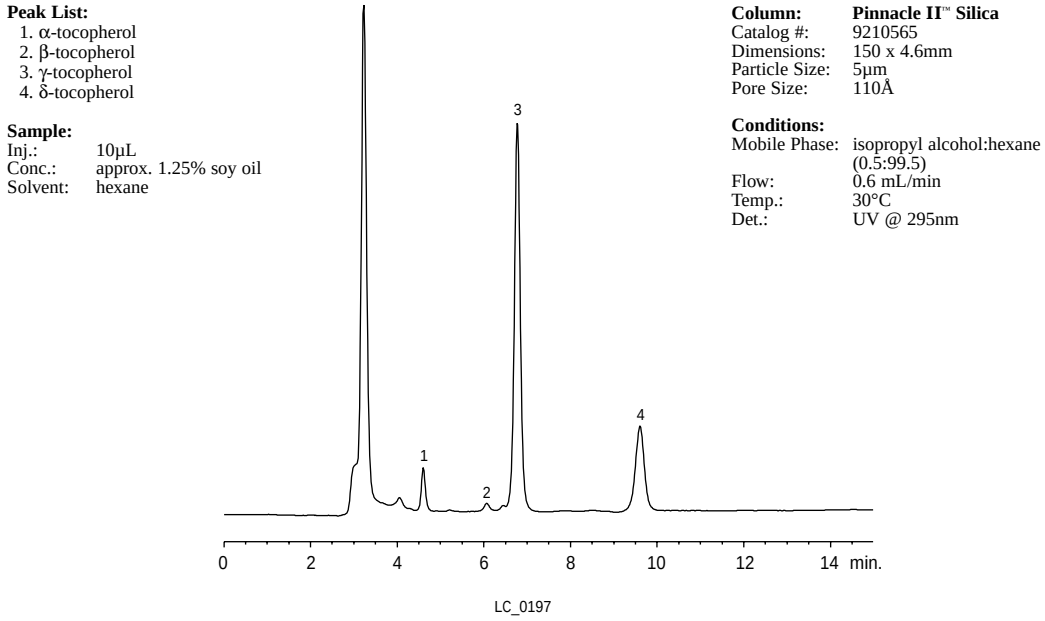
can be added to the product. Phenolic antioxidants include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), and tert-butyl hydroquinone (TBHQ). These four, plus the tocopherols, act as the primary antioxidants found in foods and beverages produced in the US. The structures 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.*




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.

## 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

**Trident™ Direct high-pressure filter**
Protection against particulate matter.
**cat.# 25082**

**Trident™ Direct 1cm guard cartridge holder with filter**
Moderate protection against particulate matter and irreversibly adsorbed compounds.
**cat.# 25084**

**Trident™ Direct 2cm guard cartridge holder with filter**
Maximum protection against particulate matter and irreversibly adsorbed compounds.
**cat.# 25086**

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