

Fat- and Water-Soluble Vitamin Analysis in Foods and Supplements

The body requires 13 vitamins to function properly: A, C, D, E, K, and the B vitamins (thiamine, riboflavin, niacin, pantothenic acid, biotin, vitamin B-6, vitamin B-12, and folate). These essential vitamins can be either water soluble or fat soluble.



Fat-soluble vitamins

Fat-soluble vitamins—A, D (D2 and D3), E, and K—naturally occur in foods, such as liver, fish, mushrooms, eggs, seeds, and nuts. To ensure adequate vitamin intake, many people consume supplements like tablets, capsules, gummies, soft gels, and fortified drinks that contain fat-soluble vitamins. These vitamins may also be added to infant formula.



Water-soluble vitamins

Vitamin C and all the B vitamins (thiamine, riboflavin, niacin, vitamin B-6, folate, vitamin B-12, biotin, and pantothenic acid) are water soluble.



Regulatory guidance

To help ensure the identity, purity, strength, and composition of dietary supplements, the FDA has established good manufacturing practices (GMPs) that companies must follow. These GMPs can prevent the wrong ingredient (or wrong amounts of an ingredient) from being added as well as reduce the risk of contamination or improper packaging/labeling.

Challenges of analyzing fat-soluble vitamins

Method sensitivity

Quantifying all fat-soluble vitamins and carotenoids in complex food matrices often requires both LC-UV and LC/MS/MS methods. The high abundance (low ppm) of vitamins A and E in food samples can cause MS detector saturation, which is why analysis of these vitamins is widely conducted using HPLC coupled with a diode-array detector (DAD).

In contrast, food samples contain only trace levels (low to mid ppb) of vitamin D, which makes HPLC-DAD detection challenging. LC/MS/MS provides the required detection sensitivity for vitamins D2 and D3 without the need for complete baseline separation.

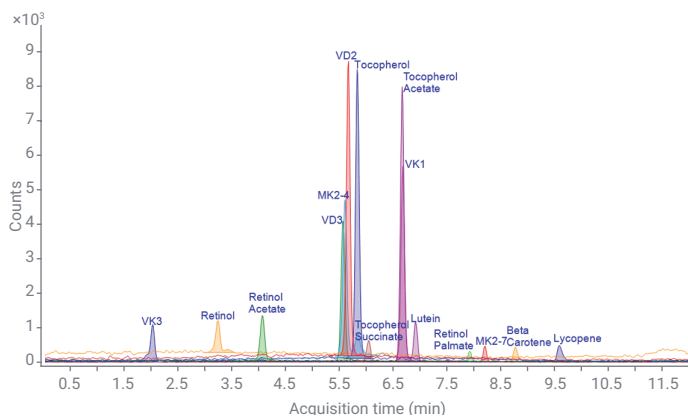
Vitamin A and E levels in multivitamin supplements are significantly lower than in food matrix. Triple quadrupole LC/MS/MS detection provides the required sensitivity, selectivity, and accuracy for simultaneous analysis of these vitamins as well as carotenoids.

Agilent has developed a highly sensitive LC/TQ method² for accurate identification and quantification of 14 fat-soluble vitamins and selected carotenoids in a single run from a multivitamin tablet matrix. An Agilent InfinityLab Poroshell 120 SB-AQ column provides good baseline separation between vitamins A, K, and E along with the vitamin D isomers D2 and D3.

Since vitamin D2 is much less effective than vitamin D3 in humans, any method that measures this vitamin in fortified foods should separate both isomers. Agilent ZORBAX Eclipse PAH columns separate compounds with similar structures, like Vitamin D2 and D3, and can resolve the four fat-soluble vitamins simultaneously in three minutes.³

HPLC^{1,2} and DAD¹ conditions for vitamin A and E analysis

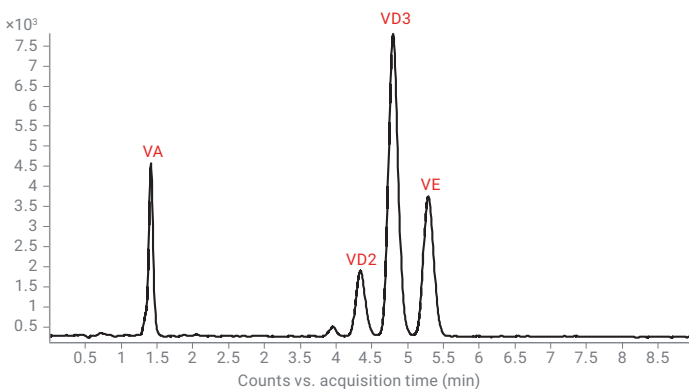
Parameter	Setting
Analytical Column	Agilent Poroshell 120 SB-AQ, 2.1 × 150 mm, 2.7 μm (p/n 68377 5-914)
Column Oven	45 ± 2 °C
Injection Volume	2 μL
Run Time	10.5 minutes
Mobile Phase	A) 0.1% formic acid in water B) 0.1% formic acid in methanol
DAD Wavelength	Retinol (VA): 326 nm; α-tocopherol (VE): 295 nm
Needle Wash	0.1% formic acid in acetonitrile
Gradient	Time (min) %A %B Flow rate (mL/min)
	0 20 80 0.25
	7.0 0 100 0.25
	8.0 0 100 0.25
	8.5 20 80 0.25
	10.5 20 80 0.25



Elution profile of all fat-soluble vitamins on an InfinityLab Poroshell 120 SB-AQ, 2.1 x 150 mm, 2.7 μm column.²

MS conditions^{1,2} for vitamin D2 and D3 detection

Parameter	Setting
MS Acquisition	dMRM
Stop Time	7 minutes
Ion Source Type	Agilent Jet Stream electrospray ionization (AJS ESI positive)
Drying Gas Temperature	250 °C
Drying Gas Flow	11 L/min
Nebulizer	40 psi
Sheath Gas Heater	350 °C
Sheath Gas Flow	12 L/min
Capillary	4,000 V
Nozzle Voltage	1,000 V
Precursor Ion and Production Ion Resolution	Unit
Compound-specific Conditions	See Table 1

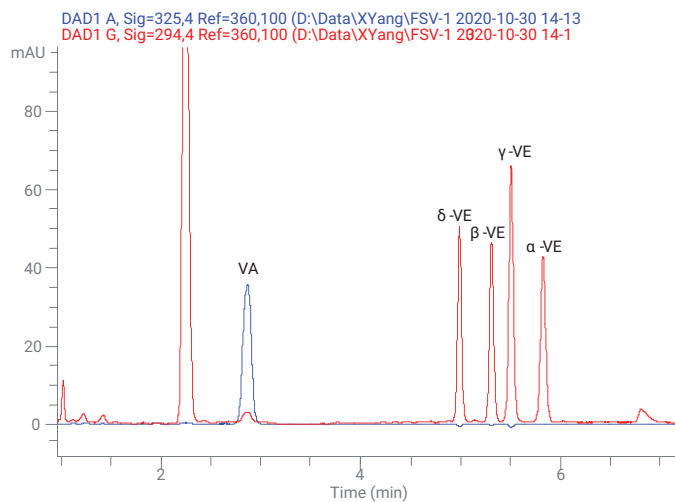


TIC of four fat-soluble vitamins on an Agilent ZORBAX RRHT Eclipse PAH column.³

Sometimes, separation of α , β , γ , and δ forms of vitamin E is required. InfinityLab Poroshell 120 PFP columns provide excellent peak shape and baseline separation of vitamin A and the four forms of vitamin E on HPLC-DAD.⁴

HPLC-DAD conditions for separating vitamin A and four isomers of vitamin E

Parameter	Setting																		
Column	Agilent InfinityLab Poroshell 120 PFP, 2.1 × 100 mm, 2.7 μ m (p/n 695775-408)																		
Column Temperature	40 °C																		
Autosampler Temperature	15 °C																		
Injection Volume	5 μ L																		
Mobile Phase	A) Water B) MeOH																		
Gradient	<table border="1"> <thead> <tr> <th>Time (min)</th> <th>%B</th> <th>Flow rate (mL/min)</th> </tr> </thead> <tbody> <tr> <td>0</td> <td>80</td> <td>0.5</td> </tr> <tr> <td>2</td> <td>80</td> <td>0.5</td> </tr> <tr> <td>3</td> <td>90</td> <td>0.5</td> </tr> <tr> <td>6</td> <td>90</td> <td>0.5</td> </tr> <tr> <td>7</td> <td>100</td> <td>0.5</td> </tr> </tbody> </table>	Time (min)	%B	Flow rate (mL/min)	0	80	0.5	2	80	0.5	3	90	0.5	6	90	0.5	7	100	0.5
Time (min)	%B	Flow rate (mL/min)																	
0	80	0.5																	
2	80	0.5																	
3	90	0.5																	
6	90	0.5																	
7	100	0.5																	
Stop Time	9 min																		
DAD Detection	294 nm for Vitamin E and 325 nm for Vitamin A																		



HPLC chromatogram of vitamin A (325 nm) with 25 μ g/g spiking, and alpha-, beta-, gamma-, and delta-vitamin E (294 nm) with 250 μ g/g spiking in infant formula. An InfinityLab Poroshell 120 PFP column was used.⁴

LC/MS/MS separation of vitamins D2 and D3 can be achieved with an InfinityLab Poroshell 120 EC-C18 column.⁴

LC/MS/MS conditions for separating vitamins D2 and D3

Parameter	Setting																								
Column	Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 μm (p/n 695775-902)																								
Column Temperature	40 °C																								
Autosampler Temperature	15 °C																								
Injection Volume	5 μL																								
Mobile Phase	A) H ₂ O with 0.1% FA, 4.5 mM ammonium formate and 0.5 mM ammonium fluoride B) MeOH with 0.1% FA, 4.5 mM ammonium formate and 0.5 mM ammonium fluoride																								
Gradient	<table border="1"> <thead> <tr> <th>Time (min)</th> <th>%B</th> <th>Flow rate (mL/min)</th> </tr> </thead> <tbody> <tr><td>0</td><td>88</td><td>0.4</td></tr> <tr><td>1</td><td>88</td><td>0.4</td></tr> <tr><td>4</td><td>90</td><td>0.4</td></tr> <tr><td>5</td><td>93</td><td>0.4</td></tr> <tr><td>5.1</td><td>94</td><td>0.4</td></tr> <tr><td>5.8</td><td>94</td><td>0.4</td></tr> <tr><td>6</td><td>100</td><td>0.4</td></tr> </tbody> </table>	Time (min)	%B	Flow rate (mL/min)	0	88	0.4	1	88	0.4	4	90	0.4	5	93	0.4	5.1	94	0.4	5.8	94	0.4	6	100	0.4
Time (min)	%B	Flow rate (mL/min)																							
0	88	0.4																							
1	88	0.4																							
4	90	0.4																							
5	93	0.4																							
5.1	94	0.4																							
5.8	94	0.4																							
6	100	0.4																							
Stop Time	17 min																								
Ionization Mode	Positive																								
Cell Accelerator Voltage	4																								
Gas Temperature	300 °C																								
Gas Flow	5 L/min																								
Nebulizer	45 psi																								
Sheath Gas Temperature	250 °C																								
Sheath Gas Flow	11 L/min																								
Capillary	4,000 V																								

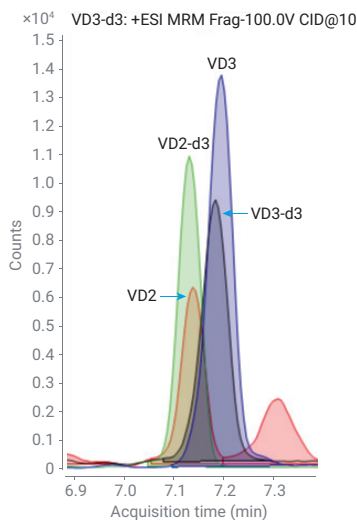
Sample preparation

Fat-soluble vitamins coexist with other lipid contents (triglycerides, sterols, phospholipids, etc.) in the lipid fraction of foods, making their isolation and measurement challenging. Traditional sample preparation for fat-soluble vitamin analysis consists mainly of saponification and liquid-liquid extraction (LLE), which are time consuming, labor intensive, and error prone. Supported liquid extraction (SLE) is an excellent alternative that makes sample preparation up to 50% faster and easier, while increasing reproducibility.

Agilent Chem Elut S uses a synthetic sorbent that improves batch to-batch reproducibility, analyte recovery, and product performance consistency. It has been used¹ for extraction and clean up of fat-soluble vitamins in infant formula and other complex food matrices, such as eggs, canned tuna, and mushrooms.

SPE-based methods also provide a sample preparation solution for fat-soluble vitamin analysis in food. Agilent Bond Elut Plexa has successfully been used to demonstrate⁴ its applicability for fat-soluble vitamin analysis in infant formula.

Sample preparation for supplements is much simpler and typically involves dissolution, dilution, digestion, and filtration. Agilent Captiva regenerated cellulose syringe filters used with Captiva disposable syringes provide efficient sample filtration with excellent target recoveries for water- and fat-soluble vitamins. For faster and easier filtration requiring fewer sample transfer steps, consider Agilent Captiva filter vials. These disposable units replace the combination of syringe filter, syringe, vial, cap, and septa.

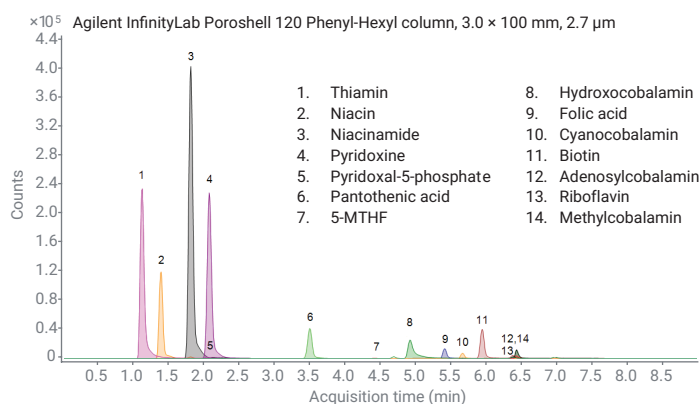


LC/MS/MS chromatogram of vitamins D2 and D3 (0.8 mg/g spiking) and their corresponding internal standards (0.4 mg/g spiking) in infant formula. An InfinityLab Poroshell 120 EC-C18 column was used.

Challenges of analyzing water-soluble vitamins

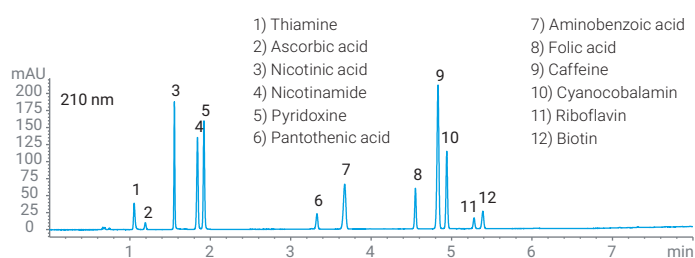
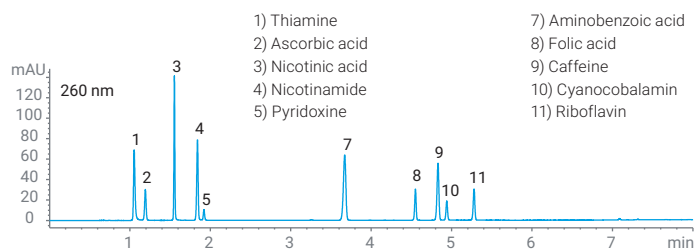
Water-soluble vitamins, like ascorbic acid and most vitamin B compounds, are polar in nature and are not retained well with conventional C18 columns. New InfinityLab Poroshell 120 Aq-C18 columns based on superficially porous particles (pore size of 120 Å) have optimized C18 ligands and proprietary endcapping surfaces. They provide stronger polar compound retention, including thiamine and ascorbic acid, and can be used with highly aqueous mobile phases.

Good resolution and peak shapes were achieved⁵ using this column to analyze 10 vitamin B compounds—plus ascorbic acid and caffeine—using phosphate buffer and acetonitrile gradient. Analysis was performed on an Agilent 1290 Infinity LC system equipped with a DAD.



Water-soluble vitamin separation with an Agilent InfinityLab Poroshell 120 Aq-C18 column under UV 260 and 210 nm.⁴

The InfinityLab Poroshell 120 Phenyl-Hexyl column also demonstrates good selectivity⁶ due to its balanced mixture of retention mechanisms. These mechanisms include hydrophobic interactions, π - π interactions of the phenyl group, and the hexyl linkage of the stationary phase. Methanol is a good choice when paired with phenyl-hexyl columns.



Water-soluble vitamin separation by LC/MS/MS using an Agilent InfinityLab Poroshell 120 Phenyl-Hexyl column.⁶

References

1. Determination of Fat-Soluble Vitamins in Foods using Agilent Chem Elut S Extraction with LC/DAD and LC/MS/MS Triple Quadrupole, [5994-5063EN](#)
2. Simultaneous Detection and Quantitation of 14 Fat-Soluble Vitamins and Carotenoids by LC/MS/MS Triple Quadrupole, [5994-5064](#)
3. Fat-Soluble Vitamins Analysis on an Agilent ZORBAX Eclipse PAH Polymeric C18 Bonded Column, [5990-5342EN](#)
4. Determination of Fat-Soluble Vitamins in Infant Formula Using Agilent Bond Elut Plexa Polymeric SPE with HPLC and LC/MS/MS, [5994-2948EN](#)
5. Separation of Water-Soluble Vitamins on the Agilent InfinityLab Poroshell 120 Aq-C18 Column, [5994-5535EN](#)
6. Simultaneous Detection and Quantitation of 14 Water-Soluble Vitamins in a Supplement by LC/MS/MS Triple Quadrupole, [5994-3016](#)

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MyList 1: Sample preparation, HPLC columns, and supplies for analyzing fat-soluble vitamins

Product Category	Description	Part number
Sample preparation supplies	Agilent Chem Elut S, 12 mL cartridges, 3 mL sample, 12 mL tube	5610-2008
	Agilent Vac Elut SPS 24 manifold with collection rack for 16 x 100 mm test tubes	12234004
	16 x 100 mL glass tubes, 250/pack	17-5001
	Agilent Bond Elut Plexa polymeric SPE, 200 mg, 6 cc, 45 µm	12109206
	Captiva premium RC syringe filter, 15 mm, 0.2 µm pore size, 100/pk	5190-5108
	Captiva disposable syringe, 5 mL, 100/pk	9301-6476
	Captiva filter vial, 0.2 µm, RC, preslit septa, 100/pk (simpler option)	5610-2125
	Captiva filter vial, 0.2 µm, RC, 100/pk (simpler option)	5191-5940
HPLC columns for LC/MS/MS	Separates vitamin D2/D3 isomers, but not vitamin E isomers	
	InfinityLab Poroshell 120 SB-AQ, 2.1 x 150 mm, 2.7 µm	683775-914
	InfinityLab Poroshell 120 SB-AQ, 2.1 mm, 2.7 µm, UHPLC guard, 3/pk	821725-924
	Separates vitamin D2/D3 isomers and 3 vitamin E isomers (α, β, γ, and δ)	
	InfinityLab Poroshell 120 EC-C18, 2.1 x 100 mm, 2.7 µm	695775-902
	InfinityLab Poroshell 120 EC-C18, 2.1 mm, 2.7 µm, UHPLC guard, 3/pk	821725-911
HPLC columns for LC/DAD	Separates vitamin D2/D3 isomers, but not vitamin E isomers	
	ZORBAX RRHT Eclipse PAH, 4.6 mm x 50 mm, 1.8 µm, 600 bar pressure limit	959941-918
	ZORBAX RRHT Eclipse PAH, 3.0 mm x 50 mm, 1.8 µm, 1,200 bar pressure limit	959757-318
	ZORBAX RRHD Eclipse PAH, 3.0 mm, 1.8 µm, 1,200 bar pressure limit, UHPLC guard, 3/pk	823750-933
	Separates 4 vitamin E isomers but not vitamin D2/D3 isomers	
		InfinityLab Poroshell 120 PFP, 4.6 x 100 mm, 2.7 µm
	InfinityLab Poroshell 120 PFP, 4.6 mm, 2.7 µm, UHPLC guard, 3/pk	820750-915
DAD supplies	Max-Light cartridge cell (10 mm, V(s)) 1.0 µL*	G4212-60008
	Inline pressure relief valve kit	G4212-68001
	InfinityLab long-life deuterium lamp, 8 pin, with RFID tag for diode-array detectors	5190-0917
HPLC supplies	InfinityLab Quick Connect assembly, 0.12 x 105 mm, for column inlet connection on UHPLC	5067-5957
	InfinityLab Quick Connect assembly, 0.17 x 105 mm, for column inlet connection on HPLC	5067-6166
	Quick Turn capillary 0.12 x 280 mm, for connection from column to detector	5500-1191
	InfinityLab Quick Turn fitting, for connection between column outlet and detector	5067-5966
	InfinityLab Quick Change inline filter assembly for HPLC with 4.6 mm id, 0.5 µm filter discs	5067-1602
Solvents/additives	InfinityLab Ultrapure LC/MS methanol	5191-4497
	InfinityLab Ultrapure LC/MS water	5191-4498
	Formic acid, 5 mL	G2453-85060

* Use the Inline Pressure Relief Kit (G4212-68001) to protect the Max-Light cell from overpressure, especially at higher flow rates. The kit also reduces the risk of cell breaks when used with extra detectors.

(continued)

MyList 1: Sample preparation, HPLC columns, and supplies for analyzing fat-soluble vitamins (continued)

Product Category	Description	Part number
Sample containment	Agilent vial, screw top, amber, write-on spot, certified, 2 mL, 100/pk	5182-0716
	Agilent bonded screw cap, bonded blue, PTFE/red silicone septa, 100/pk	5182-0717
	Vial inserts, 250 µL, glass with polymer feet, 100/pk	5181-1270
Solvent handling supplies	Agilent InfinityLab solvent bottle, amber, 1,000 mL	9301-6526
	Stay Safe starter kit and purging bottle, includes InfinityLab Stay Safe purging bottle and Stay Safe caps starter kit	5043-1340
	InfinityLab Stay Safe purging bottle	5043-1339
	Stay Safe caps starter kit	5043-1222

MyList 2: Sample preparation, HPLC columns, and supplies for analyzing water-soluble vitamins

Product Category	Description	Part number
Sample preparation supplies	Agilent Captiva Econofilter, PTFE membrane, 13 mm diameter, 0.2 µm pore size, 1,000/pk	5190-5265
	Captiva disposable syringe, 5 mL, 100/pk	9301-6476
	Captiva filter vial, 0.2 µm, RC, preslit sept, 100/pk	5610-2125
	Captiva filter vial, 0.2 µm, RC, 100/pk	5191-5940
HPLC columns for LC/MS/MS	InfinityLab Poroshell 120 Phenyl-Hexyl, 3.0 × 100 mm, 2.7 µm	695975-312
	InfinityLab Poroshell 120 Phenyl-Hexyl, 3.0 mm, 2.7 µm, UHPLC guard, 3/pk	823750-914
	InfinityLab Poroshell 120 Aq-C18, 3.0 × 100 mm, 2.7 µm (recommended)	695675-742
	InfinityLab Poroshell 120 Aq-C18 3.0 mm 2.7 µm, UHPLC guard, 3/pk (recommended)	823750-953
HPLC columns for LC/DAD	InfinityLab Poroshell 120 Aq-C18, 4.6 × 100 mm, 2.7 µm	695975-742
	InfinityLab Poroshell 120 Aq-C18, 4.6 mm, 2.7 µm, UHPLC guard, 3/pk	820750-942
DAD supplies	Max-Light cartridge cell (10 mm, V(s)) 1.0 µL*	G4212-60008
	Inline pressure relief valve kit	G4212-68001
	InfinityLab long-life deuterium lamp, 8 pin, with RFID tag for diode-array detectors	5190-0917
HPLC supplies	InfinityLab Quick Connect assembly, 0.12 x 105 mm, for column inlet connection on UHPLC	5067-5957
	InfinityLab Quick Connect assembly, 0.17 x 105 mm, for column inlet connection on HPLC	5067-6166
	Quick Turn capillary, 0.12 x 280 mm, for connection from column to detector	5500-1191
	InfinityLab Quick Turn fitting, for connection between column outlet and detector	5067-5966
	InfinityLab Quick Change inline filter assembly for HPLC with 4.6 mm id, 0.5 µm filter discs	5067-1602
	InfinityLab Quick Change inline filter assembly for HPLC with 2.1 mm id, 0.2 µm filter discs	5067-1603
	InfinityLab Quick Change filter discs, 2.1 mm id, 0.2 µm, 5/pack	5067-1610
	InfinityLab Quick Change filter discs, 4.6 mm id 0.2 µm, 5/pack	5067-1612
Solvents/additives	InfinityLab Ultrapure LC/MS methanol	5191-4497
	InfinityLab Ultrapure LC/MS water	5191-4498
	InfinityLab Ultrapure LC/MS acetonitrile	5191-4496
	Formic acid, 5 mL	G2453-85060
	InfinityLab deactivator additive, 25 mL	5191 3940
	InfinityLab deactivator additive, 50 mL	5191-4506
Solvent handling supplies	Agilent InfinityLab solvent bottle, amber, 1,000 mL	9301-6526
	Stay Safe starter kit and purging bottle, includes InfinityLab Stay Safe purging bottle and Stay Safe caps starter kit	5043-1340
	InfinityLab Stay Safe purging bottle	5043-1339
	Stay Safe caps starter kit	5043-1222
Sample containment	Agilent vial, screw top, amber, write-on spot, certified, 2 mL, 100/pk	5182-0716
	Agilent bonded screw cap, bonded blue, PTFE/red silicone septa, 100/pk	5182-0717
	Vial inserts, 250 µL, glass with polymer feet, 100/pk	5181-1270

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