Analyzing Pesticide Residues in Lettuce by the EN 15662 Method using Phenomenex roQ[™] QuEChERS Kits Followed by LC/MS/MS and GC/MS Analysis

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The European Standard EN 15662 method for pesticide residues in foods is described for the analysis of 25 pesticides and 2 internal standards from lettuce using a Phenomenex roQ QuEChERS EN 15662 Extraction Kit and roQ dSPE Kit for sample preparation. Analysis was performed by LC/MS/MS using a Luna® C18(2) HPLC column and GC/MS using a Zebron[™] ZB-5MSi column. The roQ dSPE Kit effectively removed matrix interferences from the lettuce matrix and featured ease of use aspects as compared to other QuEChERS kits including leak-free tubes and user friendly extraction salt packets. Recoveries of all 25 pesticides were between 70 to 120 % with sample-to-sample variability <15 %. The lower limit of quantification (LLOQ) was well below the maximum residue limit reported in the International Maximum Residue Level (MRL) Database, demonstrating excellent performance of the roQ QuEChERS product for food safety testing.

Introduction

In the past, sample preparation for multiresidue pesticide screening from complex matrices entailed long and laborious extraction procedures using various analyte specific techniques. In recent years sample preparation has been consolidated with the introduction of the QuEChERS method (Quick, Easy, Cheap, Effective, Rugged, and Safe) which has provided useful and somewhat universal protocols for the cleanup of food samples, especially when analyzing multiple classes of compounds.

Following the same principles as the AOAC official method 2007.01 for pesticide residues in foods, the EN 15662 QuECh-ERS method begins with the extraction of pesticides from food samples using acetonitrile. Addition of magnesium sulfate induces phase separation of acetonitrile and water for liquid-liquid partitioning to occur. Buffering salts such as sodium chloride, sodium citrate and disodium citrate hydrogenate sesquihydrate can then be added to control the pH, maintaining a pH level between 4 and 6, for stability of base sensitive pesticides. After extraction, the top organic layer is then treated with a dispersive blend of anhydrous $MgSO_4$ to remove moisture from the acetonitrile and weak anion-exchange SPE sorbent (PSA) to remove fatty acids from the extract while leaving the target analytes in solution.

Different blends of SPE sorbents are used depending upon the nature of the sample to achieve sufficient cleanup. Magnesium sulfate aids in removing excess water while reversed phase C18 bonded silica (C18-E) and primary and secondary amine bonded silica (PSA) remove fats and organic acids, respectively. For pigmented samples, graphitized carbon black (GCB) can be added to remove matrix interferences due to pigments. In this study, the roQ brand of QuEChERS kits was selected to cleanup lettuce samples for pesticide analysis. Lettuce is one of the most consumed leafy greens. Besides having a high water content, lettuce contains vitamins, minerals, plus small amounts of sterols, proteins and sugars.1 When analyzing our lettuce samples, 25 pesticides and 2 internal standards were extracted using a roQ dSPE kit containing MgSO₄ and PSA (p/n KS0-8928). The split final extracts were further analyzed using LC/MS/MS as well as GC/MS.

Experimental Conditions

Reagents and Chemicals

Pesticide standards and triphenylphosphate (TPP) were obtained from Accustandard (New Haven, CT), Ultra Scientific (N. Kingstown, RI), and Supelco (Bellefonte, PA). HPLC grade water (Milli-Q, Millipore, Billerica, MA) was used to prepare HPLC mobile phase and for sample preparation. Methanol and acetonitrile (ACN) were obtained from Honeywell Burdick & Jackson (Muskegon, MI). Toluene was obtained from Fisher Scientific (Waltham, MA). Acetic acid and formic acid were obtained from Sigma-Aldrich (St. Louis, MO).

Solutions and Standards

Standard pesticide mix (80 μ g/g) stock solutions were prepared in 0.1 % Formic acid in acetonitrile. A QC spiking and stock solution of 40 μ g/mL was prepared by dilution from the standard pesticide mix in acetonitrile containing 0.1 % Acetic acid. Calibration curve standard solutions (5, 10, 50, 100, 250, and 1000 ng/g) were prepared from 2, 10, and 40 μ g/mL solutions in acetonitrile

containing 0.1% Acetic acid by serial dilution. A 2 % TPP solution in acetonitrile (1 % Acetic acid) was used to determine system suitability. d10-Parathion and d6- α -HCH were used as internal standards.

Sample Preparation

Lettuce was chopped into 2-4 cm pieces, placed into a zip-lock bag, and stored in a -80 $^{\circ}$ C freezer for at least 24 hours prior to further processing. The lettuce was first immersed in liquid nitrogen and homogenized in a blender to generate a powdery consistency.

QuEChERS Extraction

Liquid- Liquid Partitioning

10 g of pretreated sample was directly weighed into a stand alone 50 mL centrifuge tube (provided in the roQ[™] Extraction Kit, p/n KS0-8911). The stand alone centrifuge tube provided a benefit over other QuEChERS kits which require that a beaker be used to hold a conical bottom centrifuge tube upright during sample addition. Spiking was done to two sets of QC samples at 80 ng/g and 200 ng/g. After fortification, 10 mL of ACN was added to the samples and 75 µL of internal standards d10-Parathion and d6- α -HCH were added. A pre-weighed salt packet containing a blend of 4 g MgSO, 1 g NaCl, 1 g trisodium citrate and 0.5 g disodium hydrogenate sesquihydrate (provided in the roQ Extraction Kit, p/n KS0-8909) was dispensed into each tube. The tubes were first shaken by hand for 1 min and then centrifuged at 3500 rpm for 2 min. 6 mL of supernatant was transferred into a roQ dSPE tube containing 900 mg of anhydrous MgSO, and 150 mg PSA (provided in the roQ dSPE Kit, p/n KS0-8924).

roQ dSPE Cleanup

The roQ dSPE tubes were sealed carefully and shaken by hand for 30 seconds. Then the samples were centrifuged at 3500 rpm for 1 min. An aliquot of 250 μ L of supernatant was transferred into a Verex[™] vial for LC/MS/MS solvent exchange and 2.25 mL of supernatant was transferred into a 15 mL centrifuge tube for GC/ MS solvent exchange.

LC/MS/MS Sample Preparation

Appropriate standard solutions were added to the samples. Then, the extracts were evaporated to dryness under a slow stream of nitrogen and reconstituted in 5 mM Formic acid in 20 % methanol solution and transferred into Verex vials with formed low volume inserts.

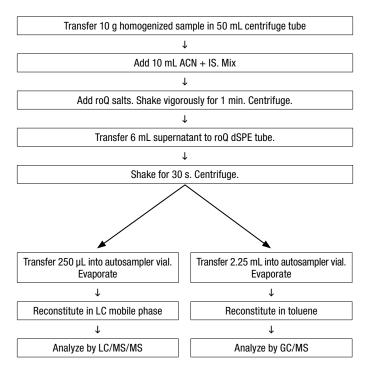
GC/MS Solvent Exchange

The samples were evaporated under a slow stream of nitrogen at 50 °C until approximately 100 μL of volume was left. The appropriate standard solutions were added and then toluene was added to reach the 0.5 mL mark. The samples were transferred to Verex amber autosampler vials containing inserts for GC/MS analysis.

QuEChERS Procedure Outline

Figure 1.

Flow chart summary for European Standard EN 15662 method for pesticide residues in foods. The final extracts were split for LC/MS/MS and GC/MS analyses.



Chromatographic Conditions

LC/MS/MS was performed using a Luna[®] 3 µm C18(2) 150 x 3.0mm column (Phenomenex, Torrance, CA, USA, p/n 00F-4251-Y0) on an Agilent[®] 1200 LC system (Agilent Technologies, Palo Alto, CA, USA), equipped with a binary pump, autosampler and interfaced with an API 4000[™] triple quadrupole mass spectrometer (AB SCIEX, Framingham, MA, USA), and analyzed by ESI in positive ion mode.

GC/MS analysis was performed using a Zebron[™] ZB-5MSi Guardian[™] 30 m x 0.25 µm GC column which had an integrated 5 m guard column (Phenomenex, Torrance, CA, USA, p/n 7HG-G018-11-GGA) on an Agilent 6890 GC coupled with a 5973 mass spectrometer.

TN-0052

Table 1.

LC/MS/MS MRM Transitions and Parameters

MRM Transitions and Parameters	
Curtain Gas (CUR): 20.00	
Ion Spray Voltage (IS): 4000.00	
Temperature (TEM): 550.00	
Collision Gas (CAD): 5.00	

	MRM P	air				
Analyte	Q1	Q3	Dwell Time (sec)	DP (V)	CE (V)	CXP (V)
Atrazine	216	173	50	66	25	18
Azoxystrobin	404	372	50	51	19	10
Carbaryl	202	145	50	46	15	14
Chlorpyrifos	352	200	50	56	31	12
Chlorpyrifos-methyl	322	125	50	46	29	24
Cyprodinil	226	108	50	46	35	10
Ethion	385	199	50	41	15	14
d10-Parathion	302	238	50	51	23	8
Imazalil	297	159	50	66	29	16
Imidacloprid	256	209	50	46	21	20
Kresoxim-methyl	314	222	50	41	17	8
Linuron	249	160	50	56	35	16
Methamidophos	142	94	50	61	21	8
Methomyl	163	88	50	46	13	8
Pymetrozine	218	105	50	56	29	10
Tebuconazole	308	70	50	36	35	6
Thiabendazole	202	175	50	51	35	18
Tolyfluanid	347	238	50	46	13	8
Triphenylphosphate	327	77	50	106	47	8

Figure 2. MRM chromatogram of lettuce extract spiked at 200 ng/g

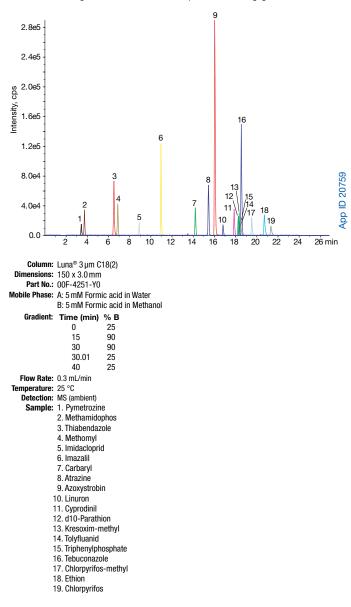


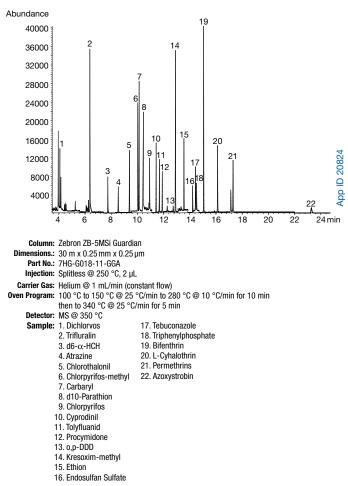
Table 2.

GC/MS analyte retention and detection parameters using a Zebron™ ZB-5MSi Guardian[™] GC column

Analyte	Base Peak	MS/MS Transition	RT
Atrazine	200.0	173, 217,202	8.181
Azoxystrobin	344.0	372, 388, 403	22.201
Bifenthrine	181	165, 166, 182	15.062
Carbaryl	144.0	115, 116, 201	9.751
Chlorothalonil	265.9	194, 264, 268	9.393
Chlorpyrifos	97.0	197, 199, 314, 316	10.545
Chlorpyrifos-methyl	125.0	286,288,197,109	9.643
Cyprodinil	224.0	225, 210, 208	11.048
Dichlorvos	109.0	185, 145, 79	4.054
d10-Parathion	301.0	156, 187, 237, 269	10.494
d6-α-HCH	224.0	222, 226, 185, 189	7.766
Endosulfan Sulfate	272.0	274, 387, 229, 239	13.802
Ethion	97.0	153,231, 125	13.155
Kresoxim-methyl	116.0	131, 206, 89	12.514
L-Cyhalothrin	181.0	208, 197, 449	15.672
o,p-DDD	235.0	237, 165, 199	12.508
Permethrins	183.0	163, 165, 184	16.756
Procymidone	96.0	283, 285, 255	11.507
Tebuconazole	250.0	163, 125	14.045
Tolyfluanid	137	181, 238, 240	11.681
Trifluralin	306.0	264, 290, 248	7.39
Triphenylphosphate	325.0	233, 215, 169, 170	14.125

Figure 3.

GC/MS chromatogram of lettuce extract spiked at 200 ng/g



Results and Discussion

The QuEChERS method offers a relatively simple solution for the determination of a wide range and extensive list of pesticide compounds from many different matrices. The method development time is significantly faster than other sample preparation/ extraction techniques. Matrix effects are successfully removed to extend column lifetime, reduce system maintenance costs and reduce matrix interferences. The pre-weighed salt packets included in the roQ[™] extraction kits added convenience while boosting throughput and consistency for the QuEChERS method. The roQ extraction kits also contain stand alone flat bottom 50 mL centrifuge tubes that make sample addition and weighing easy as well as leak-free caps, easy to pour salt packets, and low extractable centrifuge tubes.

While low in fat and rich in water content, lettuce is a good source of fiber, vitamin A and vitamin C. Lettuce is the base ingredient of salads and also complements a variety of foodstuffs. This

leafy green matrix is a relatively simple sample matrix to analyze compared to other vegetables however, dark green lettuce is rich in vitamin A and pigment which necessitates the use of a dSPE cleanup that incorporates GCB for pigment removal.

When both LC/MS/MS and GC/MS are used for analyses of samples prepared using the roQ[™] QuEChERS Kits, excellent results were achieved for most analytes. For some analytes, good results were obtained by only one of the analysis methods: LC/MS/MS or GC/MS.² This is particularly true for non-polar and polar pesticides. In multiresidue analysis, LC/MS/MS running conditions may not be applicable to all non-polar compounds. Similarly, polar compounds may not display optimal results as compared to other analytes when analyzed by GC/MS. Compounds with medium polarity can be analyzed by either LC/MS/MS or GC/MS methodologies. Comparing results from both analytical techniques can provide complete analytical coverage.

The Luna[®] 3 µm C18(2) HPLC column provided separation by LC/ MS/MS for a wide range of pesticides with excellent resolution. The pesticide mix consisted of pesticides with a large variety of properties, including polar and semi-polar compounds such as methamidophos and kresoxim-methyl, respectively. Of particular note, separation was achieved for difficult to resolve semi-polar analytes; kresoxim-methyl, tolyfluanid, and tebuconazole between retention times 18 to 19 minutes. Baseline resolution and high sensitivity were also achieved by GC/MS using a Zebron[™] ZB-5MSi Guardian[™] GC column. Sharp symmetric peaks were observed, even for early eluting polar pesticides, which have tendencies to show fronting and asymmetrical peaks.

Recoveries for all pesticides except for pymetrozine were between 76-115 % and reproducibility (RSD) was below 15 % using the roQ dSPE Kit according to the EN 15662 method criteria (**Table 3**). Excellent results were obtained even for analytes known to be light sensitive and degrade in acetonitrile, such as chlorothalonil and tolyfluanid. Low recoveries were obtained for pymetrozine however the European Standard EN 15662 method also reports low recoveries for this compound. The low recovery most likely occurs because pymetrozine is an acid labile, basic compound and is also matrix dependent, making extraction from acidic matrices such as citrus fruits difficult.

Table 3.

LC/MS/MS & GC/MS recovery data

	80 ng/g					200 ng/g				
Analyte	LC/ MS/MS Recovery (%)	RSD (%)	GC/MS Recovery (%)	RSD (%)	LC/ MS/MS Recovery (%)	RSD (%)	GC/MS Recovery (%)	RSD (%)		
Atrazine	86	10	90	6	90	4	102	4		
Azoxystrobin	92	9	110	5	90	4	104	4		
Bifenthrine	n/a	-	104	3	n/a	-	106	5		
Carbaryl	87	10	81	10	82	5	99	5		
Chlorothalonil	n/a	-	73	8	n/a	-	92	4		
Chlorpyrifos	97	10	98	8	92	5	104	3		
Chlorpyrifos-methyl	102	11	89	7	88	5	101	3		
Cyprodinil	85	9	88	6	83	4	105	12		
Endosulfan Sulfate	n/a	-	108	6	n/a	-	115	4		
Ethion	92	9	103	4	78	6	110	3		
Imazalil	94	8	n/a	-	92	4	n/a	-		
Imidacloprid	90	9	n/a	-	84	3	n/a	-		
Kresoxim-methyl	96	10	97	4	84	4	104	3		
L-Cyhalothrin	n/a	-	102	4	n/a	-	107	4		
Linuron	86	11	n/a	-	84	4	n/a	-		
Methamidophos	81	10	n/a	-	68	5	n/a	-		
Methomyl	89	9	n/a	-	98	4	n/a	-		
o,p-DDD	n/a	-	90	4	n/a	-	99	3		
Permethrins	n/a	-	107	3	n/a	-	106	4		
Procymidone	n/a	-	96	4	n/a	-	104	3		
Pymetrozine	48	11	n/a	-	68	6	n/a	-		
Tebuconazole	95	9	95	6	100	3	101	6		
Thiabendazole	76	9	n/a	-	92	4	n/a	-		
Tolyfluanid	82	11	65	13	81	7	82	7		
Trifluralin	n/a	-	79	18	n/a	-	100	3		

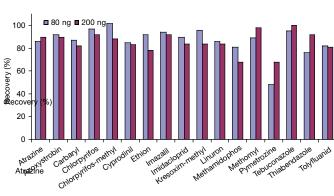
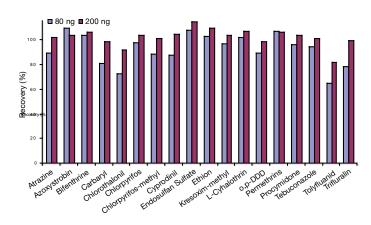


Figure 3. LC/MS/MS recovery data of samples spiked at 80 ng/g and 200 ng/g

Figure 4.

GC/MS recovery data of samples spiked at 80 ng/g and 200 ng/g



Conclusion

Phenomenex roQ[™] EN 15662 Method Extraction and dSPE QuEChERS Kits successfully extracted 25 pesticides of different classes from lettuce while providing benefits such as effective removal of pigment and other matrix interferences. The kits also incorporated ease of use features such as stand alone centrifuge tubes which made it easy to weigh sample, easy to pour salt packets, leak-free tubes, and low leachable tubes. This method produced acceptable recoveries and reproducibilities per the official EN 15662 method. The roQ QuEChERS dSPE MgSO₄/PSA Kit can also be used for other fruit and vegetable matrices that are not highly pigmented.

References

1. www.fda.gov

- Lehotay, S. J., de Kok, A., Hiemstra, M., van Bodegraven, P. Validation of a Fast and Easy Method for the Determination of Residues from 229 Pesticides in Fruits and Vegetables Using Gas and Liquid Chromatography and Mass Spectrometric Detection. JAOAC Int, **2005**, 88(2) 595- 614
- 3. http://www.ars.usda.gov/Services/docs.htm?docid=14147

Ordering Information

roQ Extraction Kits

Extraction kits contain fifty easy-pour salt packets and fifty 50 mL stand-alone centrifuge tubes

Description	UIIIL	Fall NO.						
EN 15662 Method Extraction Kits								
4.0 g MgSO_4 , 1.0 g NACI , 1.0 g SCTD , 0.5 g SCDS	50/pk	KS0-8909						
AOAC 2007.01 Method Extraction Kits								
6.0 g MgSO ₄ , 1.5 g NaOAc	50/pk	KS0-8911						
Original Non-buffered Method Extraction Kits								
4.0 g MgSO ₄ , 1.0 g NaCl	50/pk	KS0-8910						
6.0 g MgSO ₄ , 1.5 g NaCl	50/pk	KS0-8912						

roQ dSPE Kits

dSPE kits contain pre-weighed sorbents/salts inside 2 mL or 15 mL centrifuge tubes

Description	Unit	Part No.
2 mL dSPE Kits		
150 mg MgSO₄, 25 mg PSA, 25 mg C18-E	100/pk	KS0-8913
150 mg MgSO₄, 25 mg PSA, 2.5 mg GCB	100/pk	KS0-8914
150 mg MgSO₄, 25 mg PSA, 7.5 mg GCB	100/pk	KS0-8915
150 mg MgSO₄, 25 mg PSA	100/pk	KS0-8916
150 mg MgSO ₄ , 50 mg PSA, 50 mg C18-E, 50 mg GCB	100/pk	KS0-8917
150 mg MgSO₄, 50 mg PSA, 50 mg C18-E	100/pk	KS0-8918
150 mg MgSO₄, 50 mg PSA, 50 mg GCB	100/pk	KS0-8919
150 mg MgSO ₄ , 50 mg PSA	100/pk	KS0-8920

15 mL dSPE Kits

900 mg MgSO ₄ , 150 mg PSA, 150 mg C18-E	50/pk	KS0-8921
900 mg MgSO ₄ , 150 mg PSA, 15 mg GCB	50/pk	KS0-8922
900 mg MgSO ₄ , 150 mg PSA, 45 mg GCB	50/pk	KS0-8923
900 mg MgSO ₄ , 150 mg PSA	50/pk	KS0-8924
1200 mg MgSO ₄ , 400 mg PSA, 400 mg C18-E, 400 mg GCB	50/pk	KS0-8925
1200 mg MgSO₄, 400 mg PSA, 400 mg C18-E	50/pk	KS0-8926
1200 mg MgSO ₄ , 400 mg PSA, 400 mg GCB	50/pk	KS0-8927
1200 mg MgSO ₄ , 400 mg PSA	50/pk	KS0-8928

Bulk roQ QuEChERS Sorbents

Phases	10 g	100 g
C18-E	-	04G-4348
GCB (Graphitized Carbon Black)	04D-4615	04G-4615
PSA	-	04G-4610

Ordering Information

Luna® C18(2) HPLC Columns

3 µm Micro	bore and Minibore	Columns (mm)					SecurityGuard [™] * Cartridges (mm)
Phase	50 x 1.0	150 x 1.0	30 x 2.0	50 x 2.0	100 x 2.0	150 x 2.0	4 x 2.0*
							10/pk
C18(2)	00B-4251-A0	00F-4251-A0	00A-4251-B0	00B-4251-B0	00D-4251-B0	00F-4251-B0	AJ0-4286
							for ID: 2.0-3.0 mm

3μm Narrow Bore Columns (mm)									SecurityGuard Cartridges (mm)	SecurityGuard Cartridges (mm)
Phase	30 x 3.0	50 x 3.0	150 x 3.0	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	4 x 2.0*	4 x 3.0*
									10/pk	10/pk
C18(2)	00A-4251-Y0	00B-4251-Y0	00F-4251-Y0	00A-4251-E0	00B-4251-E0	00C-4251-E0	00D-4251-E0	00F-4251-E0	AJ0-4286	AJ0-4287
									for ID: 2.0-3.0 mm	for ID: 3.2-8.0 mm

5 µm Micr	SecurityGuard Cartridges (mm)							
Phase	50 x 1.0	150 x 1.0	250 x 1.0	30 x 2.0	50 x 2.0	150 x 2.0	250 x 2.0	4 x 2.0*
								10/pk
C18(2)	00B-4252-A0	00F-4252-A0	00G-4252-A0	00A-4252-B0	00B-4252-B0	00F-4252-B0	00G-4252-B0	AJ0-4286
								for ID: 2.0-3.0 mm

5 µm Narro	ow Bore Columns	(mm)			SecurityGua Cartridges (n		
Phase	30 x 3.0	50 x 3.0	150 x 3.0	250 x 3.0	4 x 2.0*		
					10/pk		
C18(2)	00A-4252-Y0	00B-4252-Y0	00F-4252-Y0	00G-4252-Y0	AJ0-4286		
					for ID: 2.0-3.0	mm	
5 µm Anal	ytical Columns (m	m)					SecurityGuard Cartridges (mm)
Phase	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	4 x 3.0*

							10/pk
C18(2)	00A-4252-E0	00B-4252-E0	00C-4252-E0	00D-4252-E0	00F-4252-E0	00G-4252-E0	AJ0-4287
							for ID: 3.2-8.0 mm

* SecurityGuard Analytical Cartridges require holder, Part No.: KJ0-4282

Zebron[™] ZB-5MSi

ID(mm)	df(µm)	Temp. Limits °C	Part No.
10-Meter			
0.18	0.18	-60 to 360/370	7CD-G018-08
12-Meter			
0.20	0.33	-60 to 360/370	7DE-G018-14
15-Meter			
0.25	0.25	-60 to 360/370	7EG-G018-11
30-Meter			
0.25	0.25	-60 to 360/370	7HG-G018-11
0.25	0.50	-60 to 360/370	7HG-G018-17
0.25	1.00	-60 to 360/370	7HG-G018-22
0.32	0.25	-60 to 360/370	7HM-G018-11
0.32	0.50	-60 to 360/370	7HM-G018-17
60-Meter			
0.25	0.25	-60 to 360/370	7KG-G018-11

Note: If you need a 5 in. cage, simply add a (-B) after the part number, e.g., 7HG-G018-11-B. Some exceptions may apply. Agilent 6850 and some SRI and process GC systems use only 5 in. cages.

Zebron ZB-5MSi Guardian[™] (integrated guard column)

Dimensions	Temp. Limits °C	Part No.
20 meter x 0.18 mm x 0.18 df(µm)	-60 to 360/370	7FD-G018-08-GGA
30 meter x 0.25 mm x 0.10 df(µm)	-60 to 360/370	7HG-G018-02-GGA
30 meter x 0.25 mm x 0.25 df(µm)	-60 to 360/370	7HG-G018-11-GGA



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