APPLICATION

Rapid and Simple Extraction and Analysis of Vitamin D2 and D3 from Dietary Supplements Using QuEChERS and HPLC-UV

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Introduction

Dietary supplements consumption is a growing trend worldwide. Many individuals are looking to supplements for additional vitamins, minerals or nutrients that are not available in their daily diet. The need for label claim accuracy is both a quality and a safety concern, as these supplements are not regulated by the FDA. The use of Chromatography via HPLC-UV analysis is a great tool to accurately identify and quantitate the amounts of specific nutrients to ensure that what is consumed is what is reported on the label.

Vitamins accounts for a large percentage of all dietary/nutritional supplements purchased and consumed. Vitamin D specifically is a fat-soluble vitamin that has been known for its ability to enhance intestinal absorption of calcium, iron, magnesium, phosphate, and zinc. The most important vitamin D compounds are ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3). Typically dietary supplements manufacturers add only the vitamin D3 form. As part of the Quality Assurance (QA) process, dietary supplement manufacturers must quantify the components on the label claim. In order to analyze vitamin D3 the compound must be extracted from the matrix before it can be analyzed using RP-HPLC. Presented here is a rapid and simple extraction procedure using QuEChERS salts and HPLC analysis using a Kinetex 2.6 µm Biphenyl Core-shell HPLC column.

Materials and Methods

Standards:
Triphenylene (Sigma-Aldrich®, 45804-100MG)
Vitamin D3 (Sigma-Aldrich, PHR1237-500MG)
Vitamin D2 (Sigma-Aldrich PHR1238-500MG)

Sample Preparation:
roQ™ QuEChERS Salt packets (AH0-9044)
roQ QuEChERS dSPE kit – 2 mL (KS0-8916)
5 mL Microtube centrifuge tube (Argos Technologies®
Cat. No. T2081A)
D.I. water (Sartorius® arium® comfort II)

HPLC:
Kinetex® 2.6 µm Biphenyl, 150 x 4.6 mm column (00F-4622-E0)
Verex™ Qsert Autosampler vial (AR0-9973-13)

Mobile Phase:
Acetonitrile (Honeywell Burdick & Jackson®)
0.1% TFA (Sigma-Aldrich, T6508-1L)
D.I. water (Sartorius arium comfort II)

Samples:
up & up™ Calcium vitamin D3 tablet (600 mg calcium, 800 IU D3, weight 1.77g)
Nature's Bounty® Oystercal-D™ Calcium with vitamin D3 tablet (500 mg calcium, 400 IU D3, weight 1.48 g)
Nature Made Vitamin D3 tablet (2000 IU D3, weight 0.343 g)

Experimental Conditions

Sample Preparation: small tablets (< 400 mg)

1. Grind into powder and transfer to a 2 mL roQ dSPE tube (KS0-8916)
2. Spike 20 µL of triphenylene internal standard (1 mg/mL) and standard additions (if applicable)
3. Add 800 µL of water
4. Sonicate until dissolved (approx. 5 min)
5. Add 800 µL of acetonitrile
6. Shake for 10 min using mechanical shaker
7. Centrifuge at 15000 rpm for 3 min
8. Aspirate 100 µL of supernatant and transfer to an autosampler vial for analysis (AR0-9973-13)

Sample Preparation: large tablets (> 400 mg)

1. Grind into powder and transfer to a 5 mL centrifuge tube
2. Spike 20 µL of triphenylene internal standard (1 mg/mL) and standard additions (if applicable)
3. Add 2 mL of water
4. Sonicate until dissolved (approx. 5 min)
5. Add 400 mg of roQ QuEChERS salt (AH0-9044)
6. Add 2 mL of acetonitrile
7. Shake for 10 min using mechanical shaker
8. Centrifuge at 6000 rpm for 3 min
9. Aspirate 100 µL of supernatant and transfer to an autosampler vial for analysis (AR0-9973-13)

Figure 1. Standard mix: (1) triphenylene at 20 ppm (2) vitamin D3 at 70 ppm, (3) vitamin D2 at 10 ppm, in acetonitrile.

LC-UV Conditions

Column:	Kinetex® 2.6 µm Biphenyl
Dimensions:	150 x 4.6 mm
Part No.:	00F-4622-E0
Mobile Phase:	0.1% TFA/Acetonitrile (35:65)
Flow Rate:	1.75 mL/min
Injection:	10 µL
Temperature:	40 °C
Detection:	UV @ 280 nm (Ambient)
Analytes:	1. Triphenylene (IS) 2. Vitamin D3 3. Vitamin D2

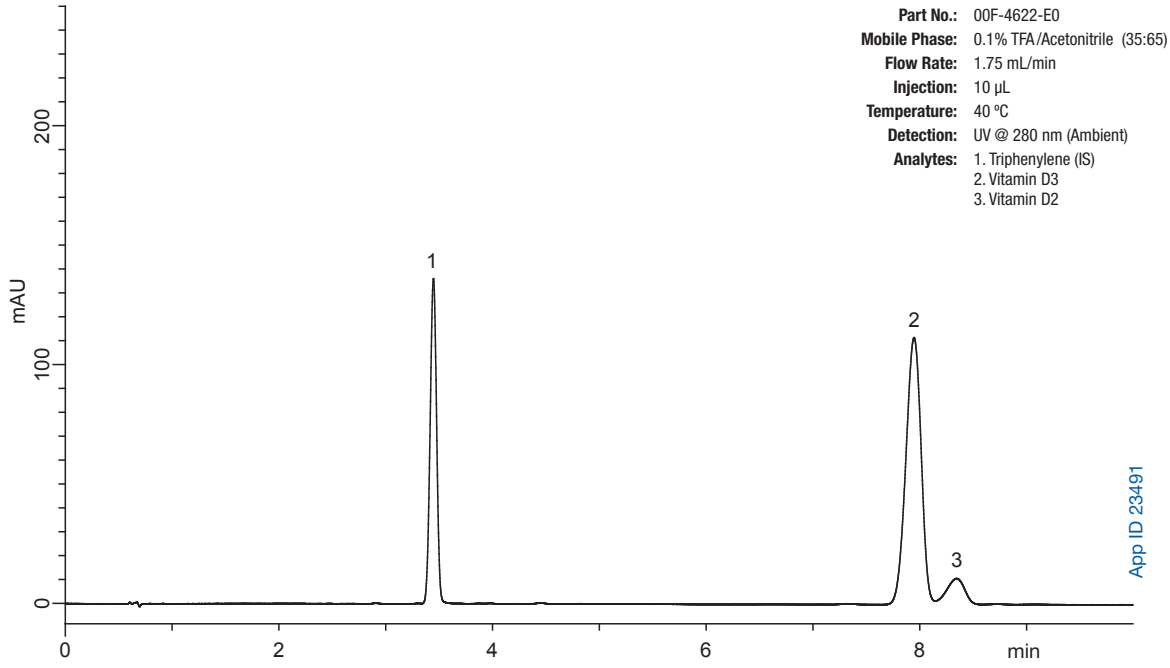


Figure 2. Neat standard calibration curve 0.5 ppm to 100 ppm of vitamin D3 (shows linearity of the analytical method).

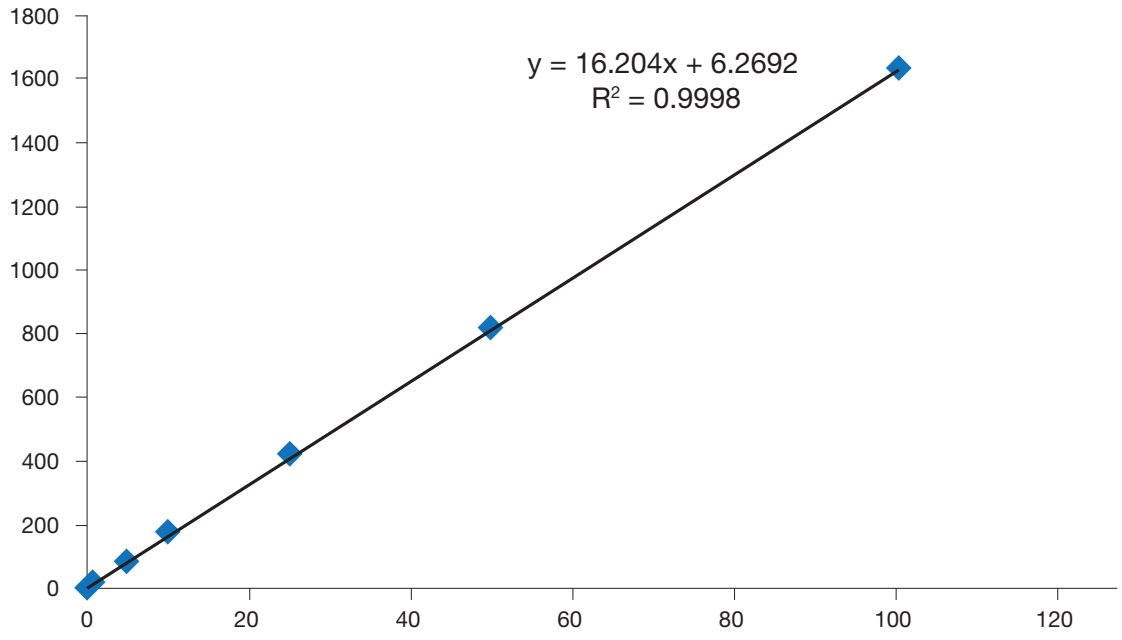
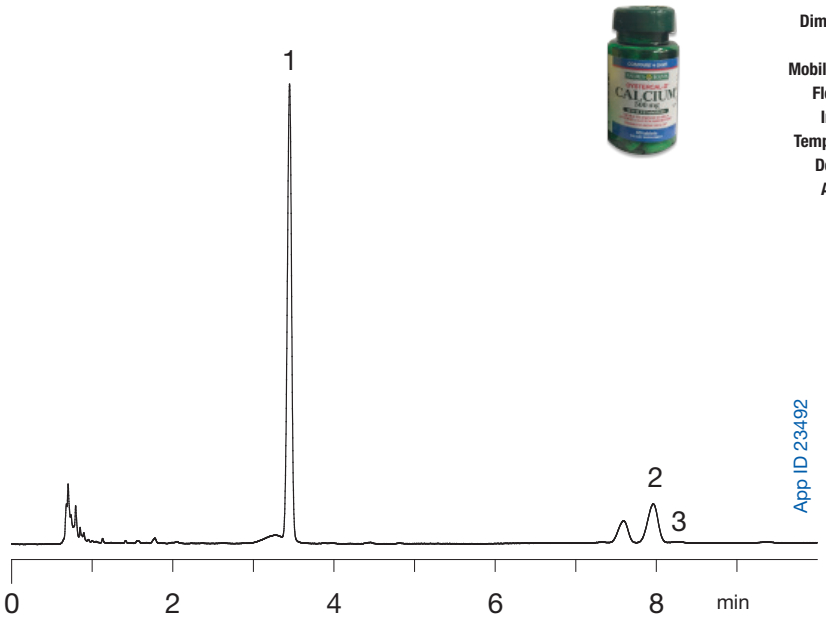


Figure 3. Nature's Bounty® Oystercal-D™ 400 IU vitamin D supplement extract (no addition).



LC-UV Conditions

Column:	Kinetex® 2.6 µm Biphenyl
Dimensions:	150 x 4.6 mm
Part No.:	00F-4622-E0
Mobile Phase:	0.1% TFA/Acetonitrile (35:65)
Flow Rate:	1.75 mL/min
Injection:	10 µL
Temperature:	40 °C
Detection:	UV @ 280 nm (Ambient)
Analytes:	1. Triphenylene (IS) 2. Vitamin D3 3. Vitamin D2

Figure 4. Nature's Bounty Oystercal-D 400 IU vitamin D supplement extract (30 µg addition).

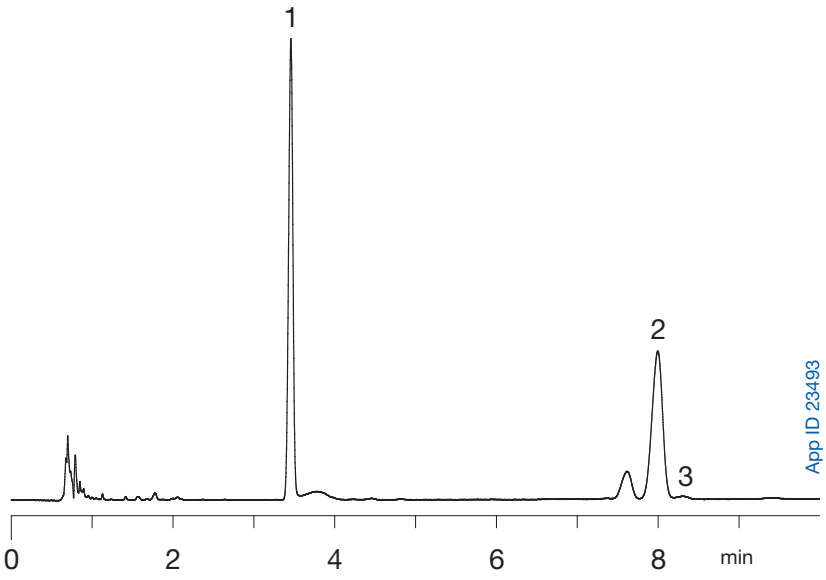


Figure 5. up & up™ 800 IU vitamin D tablet extract (no addition).

LC-UV Conditions

Column:	Kinetex® 2.6 µm Biphenyl
Dimensions:	150 x 4.6 mm
Part No.:	00F-4622-E0
Mobile Phase:	0.1% TFA/Acetonitrile (35:65)
Flow Rate:	1.75 mL/min
Injection:	10 µL
Temperature:	40 °C
Detection:	UV @ 280 nm (Ambient)
Analytes:	1. Triphenylene (IS) 2. Vitamin D3 3. Vitamin D2

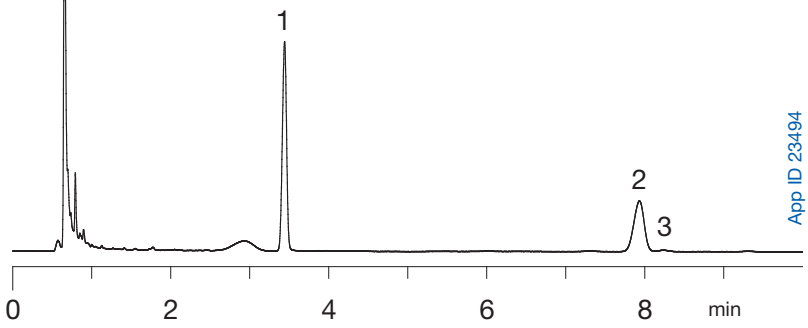


Figure 6. up & up 800 IU vitamin D tablet extract (75 µg addition).

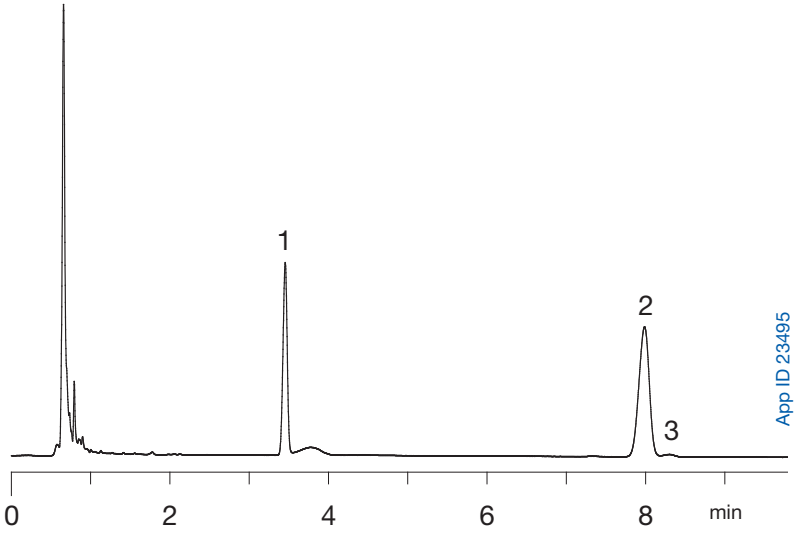
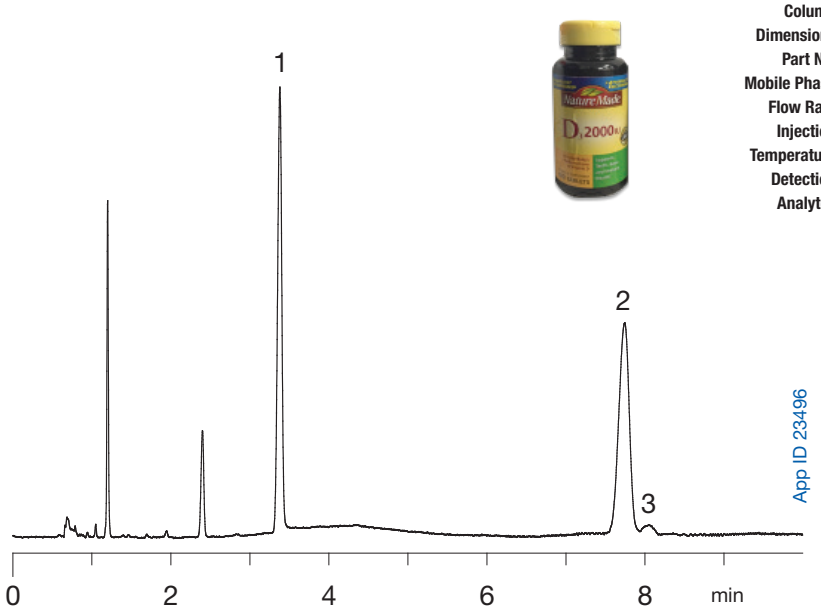


Figure 7. Nature Made® 2000 IU vitamin D tablet extract (no addition).



LC-UV Conditions

Column:	Kinetex® 2.6 µm Biphenyl
Dimensions:	150 x 4.6 mm
Part No.:	00F-4622-E0
Mobile Phase:	0.1% TFA/Acetonitrile (35:65)
Flow Rate:	1.75 mL/min
Injection:	10 µL
Temperature:	40 °C
Detection:	UV @ 280 nm (Ambient)
Analytes:	1. Triphenylene (IS) 2. Vitamin D3 3. Vitamin D2

Figure 8. Nature Made 2000 IU vitamin D tablet extract (100 µg addition).

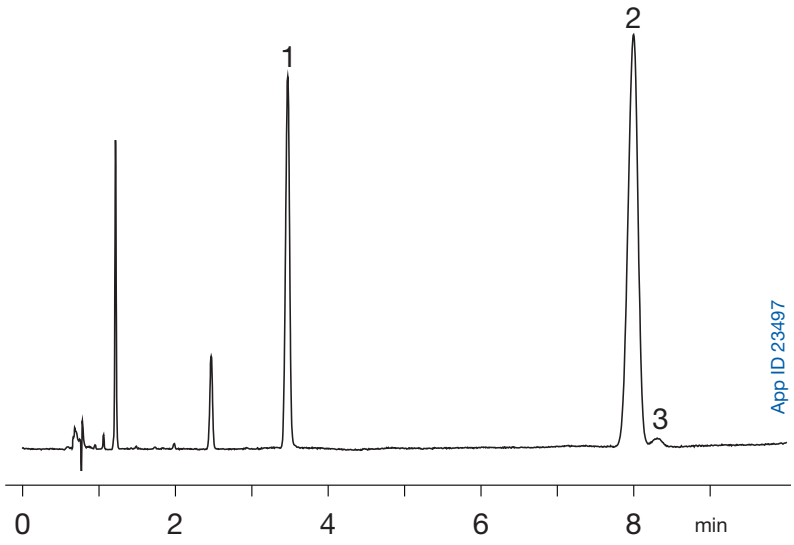


Table 1. Vitamin D3 from supplements results.

Supplement	Tablet Weight	Vitamin D3 (label claim)	Vitamin D3 (calculated)	RSD (%)
up & up™, Calcium and Vitamin D3 tablet	1.77 g	800 IU (20 µg)	1498 IU (37.5 µg)	4.4
Nature Made®, D3 2000 IU tablet	0.343 g	2000 IU (50 µg)	4666 IU (116.7 µg)	3.9
Nature's Bounty®, Oystercal-D™ Calcium with Vitamin D3 tablet	1.48 g	400 IU (10 µg)	499 IU (12.5 µg)	10.4

Figure 9. Nature Made 2000 IU (50 µg) tablet standard addition curve.

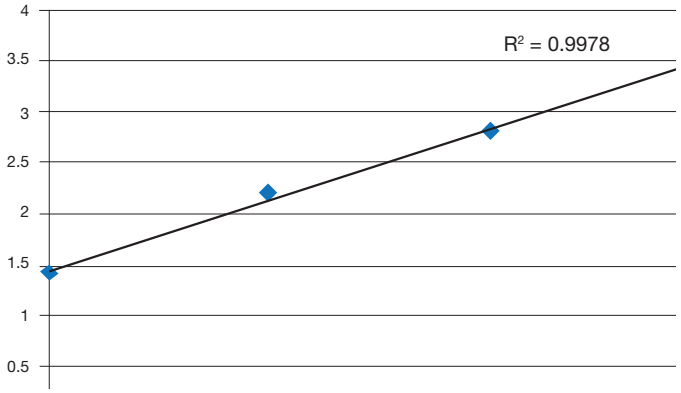


Figure 10. Oystercal-D 400 IU (10 µg) tablet standard addition curve.

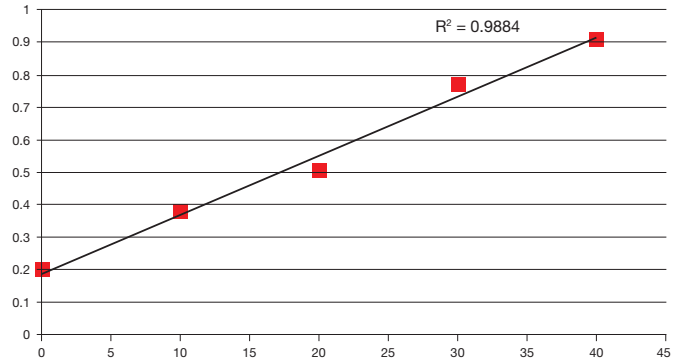
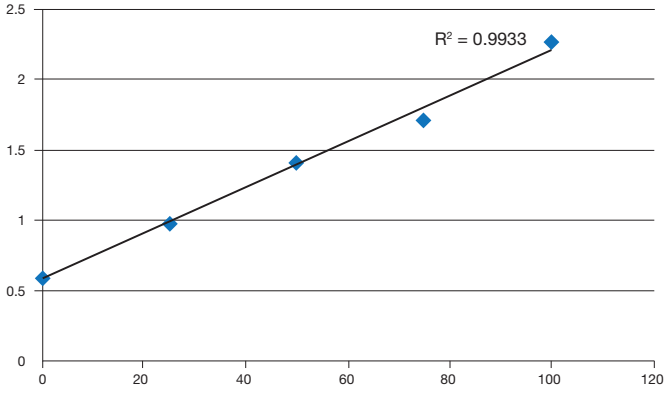


Figure 11. up & up 800 IU (20 µg) tablet standard addition curve.



The HPLC method uses a Kinetex® 2.6 µm Biphenyl Core-shell column under isocratic conditions. Vitamin D3 and Vitamin D2 are baseline resolved in less than 10 minutes with vitamin D3 eluting at 8 minutes. Triphenylene which elutes at 3.4 minutes was included as an internal standard. **Figure 2** shows the linearity of the analytical method using neat standards.

The standard addition method was used to quantify vitamin D3 levels in the tablets. The quantification results for the 3 supplements tested are displayed in **Table 1**. RSD values obtained were 10.4% or less and linearity of the standard addition curves was R_2 = 0.9884 or higher. For each tablet the reported vitamin D3 level was higher than the label claim. The reason for the higher than label claim values could be due to the manufacturer adding excess vitamin D3 to account for degradation of the active ingredient over time. By adding excess vitamin D3 the label claim would be valid for an extended length of time.

Conclusion

The presented method is an effective way of testing vitamin D3 from supplements. By using the roQ™ QuEChERS extraction salts, sample preparation time and complexity is significantly reduced. In comparison, previously reported sample preparation methods require additional steps such as dry-down and reconstitution resulting in extended sample preparation time and steps leading to lower laboratory productivity.

The Kinetex 2.6 µm, Biphenyl Core-shell HPLC column gives excellent separation of the Vitamin D forms, good peak symmetry and a fast analysis time. By combining this QuEChERS extraction approach and Kinetex Biphenyl analytical method, vitamin D analysis of dietary supplements is completed rapidly and efficiently.

Ordering Information

roQ™ Extraction Kits

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

Description	Unit	Part No.
EN 15662 Method Extraction Kits		
4.0 g $MgSO_4$, 1.0 g NaCl, 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

*AOAC and EN Extraction Kits also available in traditional non-collared 50 mL centrifuge tubes, Part No.: KS0-8911-NC and KS0-8909-NC

roQ Extraction Salt Packets

Salt packets only. Centrifuge tubes not included.

Description	Unit	Part No.
AOAC 2007.01 Method Extraction Packets		
6.0 g $MgSO_4$, 1.5 g NaOAc	50/pk	AH0-9043
EN 15662 Method Extraction Packets		
4.0 g $MgSO_4$, 1.0 g NaCl, 1.0 g SCTD, 0.5 g SCDS	50/pk	AH0-9041
Original Non-Buffered Method Extraction Packets		
4.0 g $MgSO_4$, 1.0 g NaCl	50/pk	AH0-9042
6.0 g $MgSO_4$, 1.5 g NaCl	50/pk	AH0-9044

Bulk roQ QuEChERS Sorbents

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

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 $MgSO_4$, 25 mg PSA, 25 mg C18-E	100/pk	KS0-8913
150 mg $MgSO_4$, 25 mg PSA, 2.5 mg GCB	100/pk	KS0-8914
150 mg, $MgSO_4$, 25 mg PSA, 7.5 mg GCB	100/pk	KS0-8915
150 mg $MgSO_4$, 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 $MgSO_4$, 50 mg PSA, 50 mg C18-E	100/pk	KS0-8918
150 mg $MgSO_4$, 50 mg PSA, 50 mg GCB	100/pk	KS0-8919
150 mg $MgSO_4$, 50 mg PSA	100/pk	KS0-8920

roQ dSPE Kits (cont'd)

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

Description	Unit	Part No.
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 $MgSO_4$, 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

Kinetex Ordering Information

5 µm Minibore Columns (mm)

Phases	30 x 2.1	50 x 2.1	100 x 2.1	SecurityGuard™ ULTRA Cartridges‡ 3/pk
Biphenyl	00A-4627-AN	00B-4627-AN	00D-4627-AN	AJ0-9209 for 2.1 mm ID

5 µm MidBore™ Columns (mm)

Phases	50 x 3.0	100 x 3.0	150 x 3.0	SecurityGuard ULTRA Cartridges‡ 3/pk
Biphenyl	00B-4627-Y0	00D-4627-Y0	00F-4627-Y0	AJ0-9208 for 3.0 mm ID

5 µm Analytical Columns (mm)

Phases	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	SecurityGuard ULTRA Cartridges‡ 3/pk
Biphenyl	00B-4627-E0	00D-4627-E0	00F-4627-E0	00G-4627-E0	AJ0-9207 for 4.6 mm ID

2.6 µm Minibore Columns (mm)

Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	SecurityGuard ULTRA Cartridges‡ 3/pk
Biphenyl	00A-4622-AN	00B-4622-AN	00D-4622-AN	00F-4622-AN	AJ0-9209 for 2.1 mm ID

2.6 µm MidBore™ Columns (mm)

Phases	50 x 3.0	100 x 3.0	150 x 3.0	SecurityGuard ULTRA Cartridges‡ 3/pk
Biphenyl	00B-4622-Y0	00D-4622-Y0	00F-4622-Y0	AJ0-9208 for 3.0 mm ID

2.6 µm Analytical Columns (mm)

Phases	50 x 4.6	100 x 4.6	150 x 4.6	SecurityGuard ULTRA Cartridges‡ 3/pk
Biphenyl	00B-4622-E0	00D-4622-E0	00F-4622-E0	AJ0-9207 for 4.6 mm ID

1.7 µm Minibore Columns (mm)

Phases	50 x 2.1	100 x 2.1	150 x 2.1	SecurityGuard ULTRA Cartridges‡ 3/pk
Biphenyl	00B-4628-AN	00D-4628-AN	00F-4628-AN	AJ0-9209 for 2.1 mm ID

‡ SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000

APPLICATION

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