

APPLICATIONS

Determination of Pesticide Residues in Kale and Grapes using QuEChERS and LC-MS/MS

Scott Krepich, Matt Trass, and Laura Snow
Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA



Scott Krepich
Senior Field Application
Scientist

Scott enjoys surfing and eating. He is crazy about chromatography, because his mom is really into CSI and thinks that is what he does.

Introduction

Kale and grape consumption is increasing globally and is included in the USDA's national pesticide residue monitoring program for produce. They are also commonly listed annually on the Environmental Working Group (EWG) report of the most popular pesticide contaminated produce. The use of a QuEChERS technique followed by LC-MS/MS has proven to be an effective approach for screening multiresidue pesticides from various produce items including kale and grapes. Following the EN 15662 pesticide residue method using roQ[™] QuEChERS extraction and clean-up dSPE kits, along with the use of a highly efficient and broad polarity Kinetex[®] 5 μ m Biphenyl Core-Shell HPLC column, a pesticide screening method was developed using LC-MS/MS in under 16 minutes. Over 200 pesticides were screened at concentration ranges between 0.01 ppm to 1 ppm with the majority of analytes having a recovery range of 70-130 %. The method produced excellent selectivity and reproducibility for the earlier eluting polar pesticides owing to the use of QuEChERS for removing sample matrix interferences and the biphenyl bonded-phase chemistry of the Kinetex Biphenyl column for the chromatographic separation across the entire range of pesticides.

Materials and Methods

Materials and Equipment

Shimadzu Nexera[®] LC30 pumps and autosampler were used along with a SCIEX 4000 QTRAP[®] mass spectrometer in electrospray ionization (ESI) positive polarity using scheduled MRM mode. SCIEX iDQuant[™] Standards kits for pesticide analysis were used to prepare calibrators and to spike samples. A Sartorius[®] arium[®] comfort II was used for ASTM Type 1 ultrapure water. All other solvents were purchased from Honeywell. Kale and grapes were purchased from local grocers.

Sample Preparation

A calibration curve was prepared in purified water at concentrations of 0.01, 0.05, 0.1, 0.5 and 1 ppm. Kale and grapes samples were prepared at four concentrations: no spike (0.0 ppm), low spike (0.1 ppm), medium spike (0.5 ppm), and high spike (1 ppm)

QuEChERS Procedure (EN 15662 Method)

Pretreatment: Kale and grapes were frozen at $\sim -80^{\circ}\text{C}$ overnight

Step 1: Extraction

QuEChERS Kit: roQ QuEChERS EN 15662 Method Extraction kit
Description: 4.0 g MgSO_4 , 1.0 g NaCl, 1.0 g SCTD, 0.5 g SCDS
Part No.: KS0-8909

Procedure:

1. **HOMOGENIZE** sample using a blender or similar apparatus.
2. **WEIGH** 10 g of homogenized sample into a clean 50 mL tube (provided in roQ Extraction Kits).
3. **ADD** 10 mL of Acetonitrile containing internal standard.
4. **DISPENSE** contents of the included QuEChERS salt packet into the 50 mL tube containing homogenized sample.
5. **SHAKE** vigorously by hand for 1 minute.
6. **CENTRIFUGE** for 5 minutes @ 4000 rpm, making sure that the solid material is at the bottom of the tube and a liquid layer forms on top of the solid material.

Step 2: Dispersive SPE (dSPE)

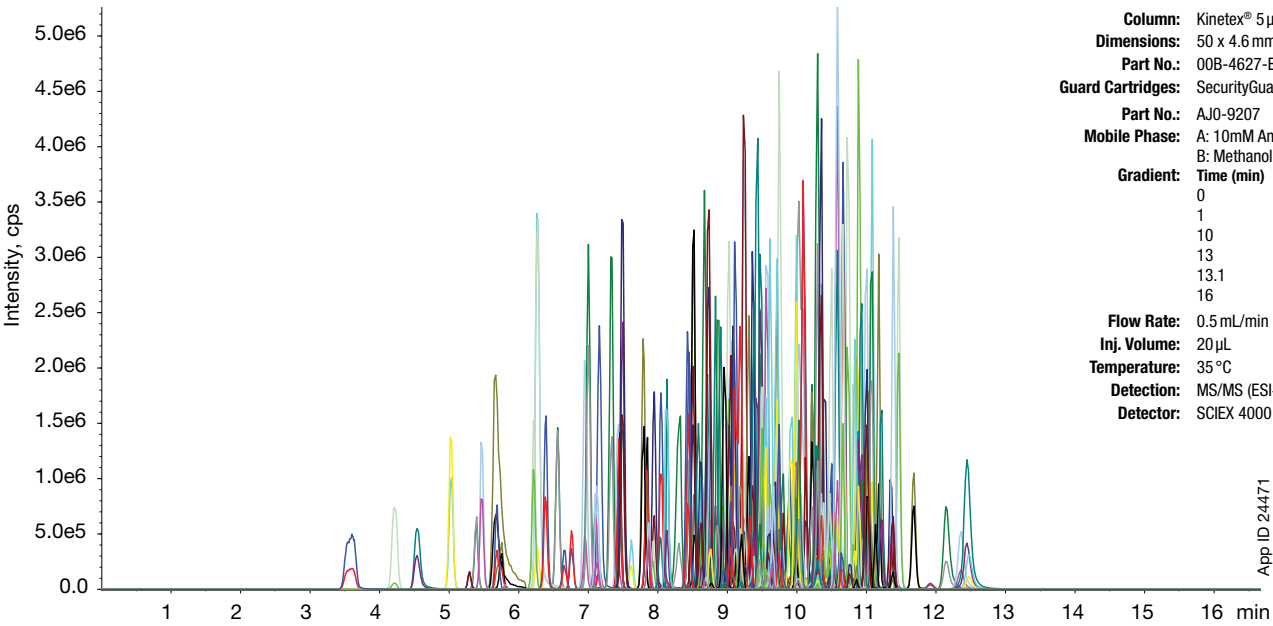
QuEChERS Kit: roQ QuEChERS EN 15662 15 mL DSPE Kit
Description: 15 mL CT, 900 mg MgSO_4 , 150 mg PSA, 15 mg GCB
Part No.: KS0-8922

Procedure:

1. **TRANSFER** 6 mL of supernatant from Step 6 of the extraction process into the 15 mL tube containing the QuEChERS dSPE sorbents.
2. **SHAKE** vigorously by hand for 30 seconds.
3. **CENTRIFUGE** for 5 minutes @ 4000 rpm to separate solid material from the liquid layer.
4. **TRANSFER** desired supernatant to an autosampler vial.
5. **DILUTE** samples 1:10 in mobile phase A prior to injection or LC-MS analysis.



Figure 1.
Standard Chromatogram (0.5 ppm standard)



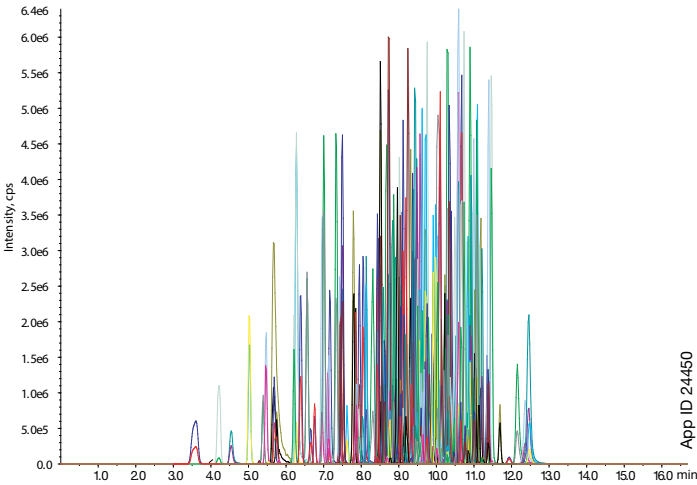
LC-MS/MS Method Parameters

Column: Kinetex[®] 5 μ m Biphenyl
Dimensions: 50 x 4.6 mm
Part No.: 00B-4627-E0
Guard Cartridges: SecurityGuard[™] ULTRA Cartridges
Part No.: AJ0-9207
Mobile Phase: A: 10mM Ammonium Formate in Water
B: Methanol
Gradient:

Time (min)	% B
0	2
1	2
10	100
13	100
13.1	2
16	2

Flow Rate: 0.5 mL/min
Inj. Volume: 20 μ L
Temperature: 35[°]C
Detection: MS/MS (ESI+), scheduled MRM
Detector: SCIEX 4000 QTRAP[®]

Kale Sample (1 ppm spike)



Grapes Sample (0.5 ppm spike)

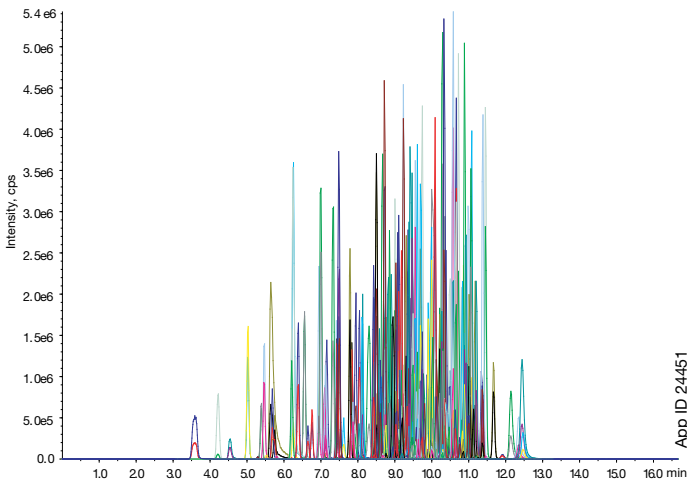


Table 1.
Mass Spectrometer Conditions

Analyte	RT	Q1	Q3	Analyte	RT	Q1	Q3
Methamidophos	3.58	142	94	Aldicarb	7.94	208.2	116.1
Acephate	4.24	184.1	143	Fuberidazole	7.96	185	157
Cyromazine	4.56	167.2	85.1	Thiabendazole	8.06	202.1	175.1
Omethoate	5.05	214	124.9	Thidiazuron	8.06	221.2	102.1
Mesotrione	5.34	357.1	228	Fluometuron	8.14	233.1	46
Dinotefuran	5.41	203.1	114.1	Acetamiprid	8.34	223.2	126.1
Aldicarb-sulfoxide	5.48	207.1	132.1	Clethodim E	8.37	360	164
Propamocarb	5.59	189.2	102.2	Tebuthiuron	8.44	229.2	172.4
Butoxycarboxim	5.71	223.1	106	Propoxur	8.45	210.1	111
Aldicarb-sulfone	5.77	223.1	86.1	Ethirimol	8.46	210.2	140.1
Flonicamid	5.82	230.1	203.1	Aminocarb	8.51	209.1	137.1
Formetanate	6.15	222	165.1	Bendiocarb	8.52	224.2	109.2
Methabenzthiazuron	6.16	222.1	165.2	Monolinuron	8.54	215.1	126.1
Monocrotophos	6.24	224	127	Propham	8.57	180	138
Oxamyl	6.41	237.1	72.1	Chlortoluron	8.59	213.1	72.2
Methomyl	6.58	163.1	88.1	Metribuzin	8.61	215.1	187.2
Nitenpyram	6.68	271.2	126.1	Forchlorfenuron	8.63	248	129.1
Clothianidin	6.79	250	132	Diuron	8.64	233.1	72
Fenuron	6.97	165.1	72.1	Pyracarbolid	8.68	218.2	125
Dicrotophos	7.02	238	112	Sulfentrazone	8.69	387	307.1
3-Hydroxycarbofuran	7.12	238.1	181	Isoprocab	8.71	194.1	95
Thiamethoxam	7.15	292	211	Carbofuran	8.73	222.2	123.1
Acibenzolar-S-methyl	7.16	211	181.1	Carbaryl	8.76	202.1	145
Pymetrozine	7.19	218	105	Thiofanox	8.76	219.1	145.1
Dimethoate	7.35	230	199.1	Metobromuron	8.76	259	170.2
Carbendazim	7.44	192.2	160.2	Isoproturon	8.83	207.2	72.1
Mevinphos E	7.46	225	127	Thiacloprid	8.88	253.1	126.1
Dioxacarb	7.51	224	123	Ethiofencarb	8.91	226.1	106.9
Vamidothion	7.51	288	146	Simetryn	8.92	214	124
Cymoxanil	7.64	199	128	Thiophanate-methyl	8.96	343	151
Carbetamide	7.81	237	192	Prometon	9.01	226.1	142.1
Mevinphos Z	7.86	225.1	127	Cycluron	9.02	199.1	89.1
Imidacloprid	7.89	256.2	209	Secbumeton	9.02	226.2	100
				Ethiprole	9.04	397	350.9



Table 1.
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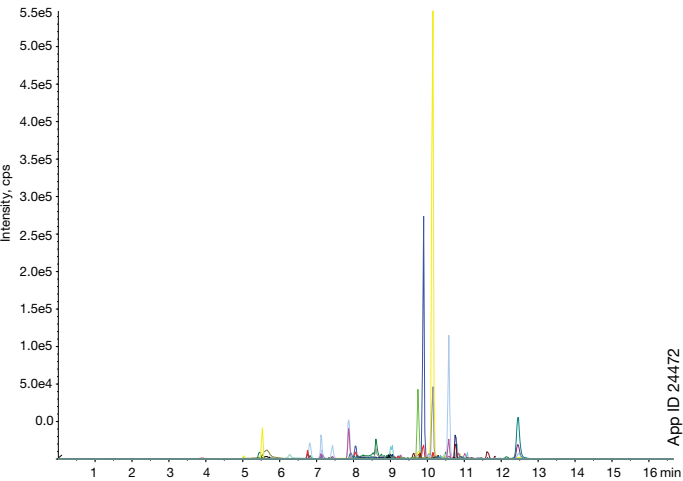
Analyte	RT	Q1	Q3	Analyte	RT	Q1	Q3
Carboxin	9.09	236	143	Methoxyfenozide	9.72	369	149
Linuron	9.09	249.1	160	Terbutryn	9.73	242.2	186.1
Desmedipham	9.11	318	182	Chloroxuron	9.73	291	72
Fludioxinil	9.12	266	229	Fenamidone	9.73	312.1	92.1
Isocarbophos	9.18	290	231	Myclobutanil	9.74	289	70
Phenmedipham	9.18	301.1	136	Tricyclazole	9.78	190	163.1
Halofenozide	9.18	331.1	105	Triticonazole	9.78	318	70
Fipronil	9.18	437	367.9	Boscalid	9.79	343	307
Paclobutrazol	9.23	294	70	Ethofumesate	9.8	304	121
Terbumeton	9.24	226	170	Hexaconazole	9.86	314	70
Ametryn	9.3	228.1	186.1	Tebuconazole	9.87	308	70
Triadimenol	9.32	296.1	70.1	Prothioconazole	9.9	344	189
Clethodim Z	9.34	360.1	164	Tebufenozide	9.9	353.1	133.1
Diethofencarb	9.36	268	226	Fenarimol	9.92	331	268
Promecarb	9.37	208.2	109.1	Diflubenzuron	9.94	311	158.2
Oxadixyl	9.39	279.2	219.2	Diniconazole	9.94	326	70
Pirimicarb	9.42	239.2	72.1	Spiroxamine	9.96	298.3	144.2
Methoprotryne	9.47	272.2	198	Zoxamide	9.97	336.1	186.9
Iprovalicarb	9.47	321.2	119	Triflumuron	9.97	359.1	156.2
Flutolanil	9.48	324.1	262	Flufenacet	9.98	364.1	194.2
Cyproconazole (isomer 2)	9.49	292.1	70.2	Novaluron	10	493	158.1
Cyproconazole (isomer 1)	9.49	292.2	70.2	Bifenazate	10.01	301.1	170.1
Methiocarb	9.53	226.1	169.2	Mandipropamid	10.01	412.1	328.1
Mexacarbate	9.55	223.2	166.2	Metconazole	10.04	320	70
Nuarimol	9.55	315	252	Penconazole	10.05	284	70
Neburon	9.57	275	88	Mepanipyrim	10.06	224	106
Fenhexamid	9.58	302	97	Furalaxyl	10.08	302.1	95.1
Prometryn	9.6	242.2	158.1	Flusilazole	10.12	316	165
Pyrimethanil	9.61	200	107	Ipconazole	10.19	334.2	70
Tetraconazole	9.63	372	159	Fenoxycarb	10.2	302.2	88.1
Diclobutrazol	9.66	328	70	Bupirimate	10.2	317	166
Etaconazole (isomer 1)	9.66	328.2	159	Cyprodinil	10.23	226	93
Triadimefon	9.68	294	197	Epoxiconazole	10.24	330	121
Mepronil	9.69	270.1	119.1	Hydramethylnon	10.24	495.2	323.1
Mepronil	9.69	270.1	119.1	Bitertanol	10.26	338	269

Table 1.
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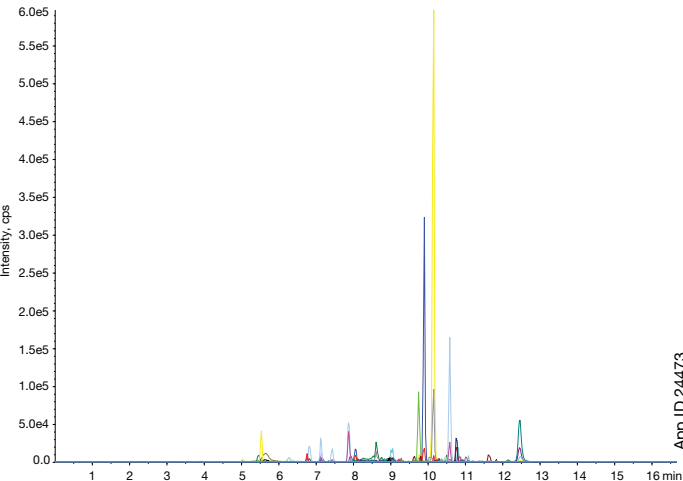
Analyte	RT	Q1	Q3	Analyte	RT	Q1	Q3
Spirotetramat	10.27	374.2	330.2	Chlorfluazuron	10.83	540	383
Lufenuron	10.27	511.1	158.1	Fenpropimorph	10.84	304	117
Dimoxystrobin	10.28	327.1	205	Piperonyl butoxide	10.87	356.2	177.2
Etaconazole (isomer 2)	10.28	328.1	159	Difenoconazole (isomer 2)	10.87	406.1	251.1
Metaflumizone	10.28	507.1	178.1	Difenoconazole (isomer 1)	10.87	406.2	251.1
Fenbuconazole	10.29	337	125	Pyraclostrobin	10.91	388	194
Hexaflumuron	10.29	461.1	141.1	Alanycarb	10.92	400.1	238.2
Butafenacil	10.29	492.2	331.1	Spiromesifen	10.94	371.3	273.3
Picoxystrobin	10.33	368	205	Benzoximate	10.95	364	199
Carfentrazone-ethyl	10.33	412	366	Ettoxazole	10.97	360.1	141
Triflumizole	10.34	346	278	Benfuracarb	10.98	411.2	195.1
Fluquinconazole	10.37	376	307	Propargite	11	368	231
Bromuconazole-cis	10.37	378	159.1	Quinoxifen	11.04	308	197
Dimethomorph (isomer 1)	10.37	388.2	301.1	Furathiocarb	11.05	383.2	195.2
Cyazofamid	10.38	325	108	Pyriproxyfen	11.06	322	96
Dimethomorph (isomer 2)	10.39	388.1	301.1	Spirodiclofen	11.11	411.3	313.3
Pencycuron	10.46	329.1	125.1	Avermectin B1b	11.15	876.5	291
Imazalil	10.48	297.1	159.2	Amitraz	11.16	294.1	163.2
Tebufenpyrad	10.49	334	117	Hexythiazox	11.19	353	228
Thiobencarb	10.5	258.1	125	Avermectin B1a	11.19	890.5	305
Propiconazole	10.51	342.1	159.1	Ivermectin B1b	11.23	878.7	186.2
Kresoxim-methyl	10.55	314	206	Fenazaquin	11.33	307	161
Mefenacet	10.56	299	148.1	Temephos	11.35	467	419.1
Azoxystrobin	10.58	404.1	372.1	Chlorantranilinprole	11.35	484	125
Famoxadone	10.6	392	331	Fenpyroximate	11.37	422	366.1
Flufenoxuron	10.63	489.1	158.2	Pyridaben	11.44	365	309
Benalaxyl	10.64	326.2	148.2	Ivermectin B1a	11.46	892.5	307.2
Trifloxystrobin	10.64	409	186	Moxidectin	11.5	662.4	467
Buprofezin	10.7	306.2	201.1	Doramectin	11.5	916.7	331.4
Bromuconazole-trans	10.71	378.1	159.1	Rotenone	11.68	395	213
Indoxacarb	10.75	528	203	Enamectin B1b	11.75	872.5	126.3
Prochloraz	10.8	376.1	308	Enamectin B1a	11.83	886.5	126.3
Clofentezine	10.81	303	138	Spinosyn A	12.12	732.6	142.1
Fluoxastrobin	10.82	459.2	427.2	Spinosyn D	12.34	746.6	142.1
				Spinetoram A	12.43	748.5	142.2
				Spinetoram B	12.47	760.5	142.2



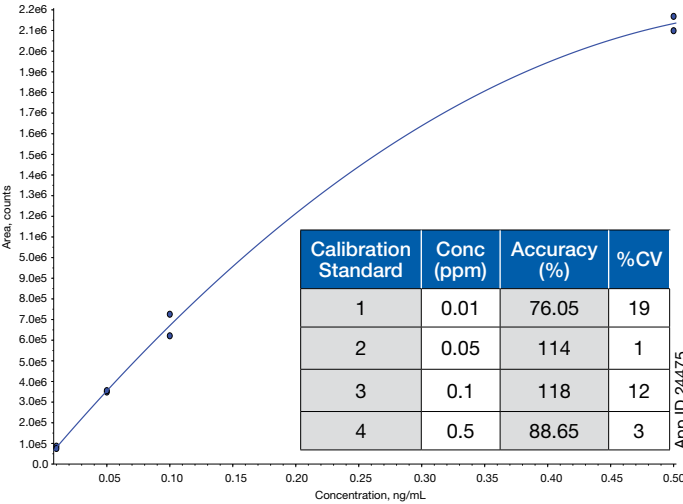
Kale (not spiked)



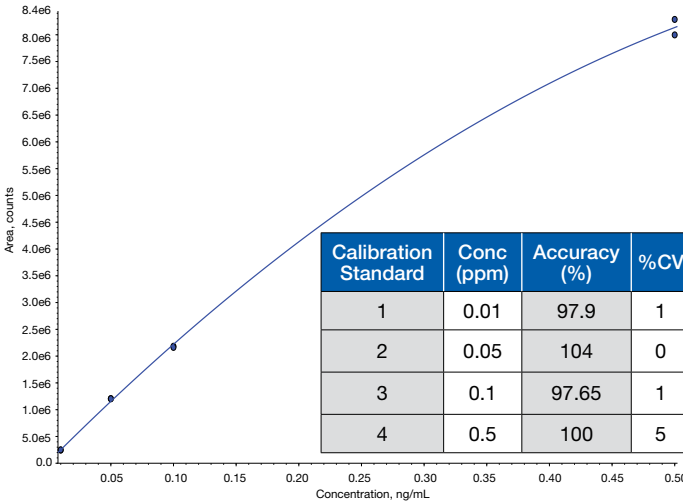
Grapes (not spiked)



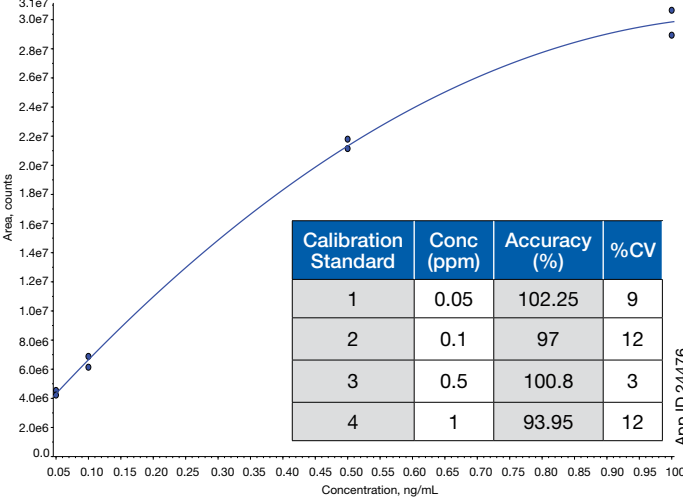
Imidacloprid



Acetamiprid



Azoxystrobin



Results & Discussion

Described is a dependable LC-MS/MS method covering 212 pesticides in the SCIEX iDQuantTM Standards Kit using a QuEChERS clean-up sample preparation and a Kinetex[®] Biphenyl LC Column Chemistry. The quick, easy, cheap, effective, rugged and safe (QuEChERS) method offers a relatively simple solution for the determination of a wide range and extensive list of pesticide compounds from many different matrices. To add method consistency and throughput, roQTM QuEChERS kits feature pre-weighed salt packets and pre-filled dispersive SPE sorbents in centrifuge tubes to increase throughput and improve consistency. Over 200 pesticides were screened at concentration ranges between 0.01 ppm to 1 ppm with the majority of analytes having a recovery range between 70-130 %.

In reviewing the top pesticides tested in kale and grapes, imidacloprid, azoxystrobin, and acetamiprid all demonstrated excellent linearity across the range with $r^2 > 0.997$. The highest and lowest calibrators were excluded for weak or strong ionizing compounds, respectively. Precision was good with CV < 20 %. Unspiked kale and grape samples were screened. The kale sample was found to have imidacloprid, tebuconazole, indoxacarb, Spinetoram A, and Spinetoram B. Acetamiprid, myclobutanil, fenhexamid, tebuconazole, and difenoconazole were present in the grape sample.

Conclusion

Kinetex Biphenyl has proven to be an excellent column chemistry for multiresidue pesticide LC-MS/MS screening. The Biphenyl stationary phase yields improved polar retention through dipole-dipole interactions with conjugated biphenyl rings, improved aromatic selectivity, and a large enough carbon skeleton for aliphatic selectivity suitable for the range of polarities and functionalities in multiresidue analyses.

Kale:

Pesticide	Concentration (ppm)	CV (%)
Imidacloprid	0.033	6 %
Tebuconazole	0.10	3 %
Indoxacarb	0.054	1 %
Spinetoram A	0.023	3 %
Spinetoram B	0.016	1 %

Grapes:

Pesticide	Concentration (ppm)	CV (%)
Acetamiprid	0.016	11 %
Myclobutanil	0.079	10 %
Fenhexamid	0.016	15 %
Tebuconazole	0.056	17 %
Difenoconazole	0.023	2 %



APPLICATIONS

Kinetex Core-Shell LC Column Ordering Information

Kinetex 5 µm Columns (mm)		SecurityGuard TM ULTRA Cartridges [†]		SecurityGuard TM ULTRA Cartridges [†]		SecurityGuard TM ULTRA Cartridges [†]		SecurityGuard TM ULTRA Cartridges [†]	
Phases	50 x 2.1	3/pk	50 x 3.0	3/pk	50 x 4.6	100 x 4.6	150 x 4.6	250 x 4.6	3/pk
Biphenyl	00B-4627-AN	AJO-9209 for 2.1 mm ID	00B-4627-YO	AJO-9208 for 3.0 mm ID	00B-4627-E0	00D-4627-E0	00F-4627-E0	00G-4627-E0	AJO-9207 for 4.6 mm ID

Kinetex 2.6 µm Columns (mm)		SecurityGuard TM ULTRA Cartridges [†]		SecurityGuard TM ULTRA Cartridges [†]		SecurityGuard TM ULTRA Cartridges [†]		SecurityGuard TM ULTRA Cartridges [†]	
Phases	50 x 2.1	100 x 2.1	3/pk	50 x 3.0	3/pk	50 x 4.6	100 x 4.6	150 x 4.6	3/pk
Biphenyl	00B-4622-AN	00D-4622-AN	AJO-9209 for 2.1 mm ID	00B-4622-YO	AJO-9208 for 3.0 mm ID	00B-4622-E0	00D-4622-E0	00F-4622-E0	AJO-9207 for 4.6 mm ID

Kinetex 1.7 µm Minibore Columns (mm)				SecurityGuard ULTRA Cartridges [‡]
Phases	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
Biphenyl	00B-4628-AN	00D-4628-AN	00F-4628-AN	AJO-9209

[‡] SecurityGuard ULTRA cartridges require holder, Part No. AJO-9000. for 2.1 mm ID

[†] SecurityGuard ULTRA cartridges require holder, Part No. AJO-9000.

Australia

t: +61 (0)2-9428-6444
f: +61 (0)2-9428-6445
auiinfo@phenomenex.com

Austria

t: +43 (0)1-319-1301
f: +43 (0)1-319-1300
anfrage@phenomenex.com

Belgium

t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
f: +31 (0)30-2383749
beinfo@phenomenex.com

Canada

t: +1 (800) 543-3681
f: +1 (310) 328-7768
info@phenomenex.com

China

t: +86 400-606-8099
f: +86 (0)22 2532-1033
phen@agela.com

Denmark

t: +45 4824 8048
f: +45 4810 6265
nordicinfo@phenomenex.com

Finland

t: +358 (0)9 4789 0063
f: +45 4810 6265
nordicinfo@phenomenex.com

France

t: +33 (0)1 30 09 21 10
f: +33 (0)1 30 09 21 11
franceinfo@phenomenex.com

Germany

t: +49 (0)6021-58830-0
f: +49 (0)6021-58830-11
anfrage@phenomenex.com

India

t: +91 (0)40-3012 2400
f: +91 (0)40-3012 2411
indiainfo@phenomenex.com

Ireland

t: +353 (0)1 247 5405
f: +44 1625-501796
eireinfo@phenomenex.com

Italy

t: +39 051 6327511
f: +39 051 6327555
italiainfo@phenomenex.com

www.phenomenex.com

Phenomenex products are available worldwide. For the distributor in your country, contact Phenomenex USA, International Department at international@phenomenex.com

Luxembourg

t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

Mexico

t: 01-800-844-5226
f: 001-310-328-7768
tecnicomx@phenomenex.com

The Netherlands

t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

New Zealand

t: +64 (0)9-4780951
f: +64 (0)9-4780952
nzinfo@phenomenex.com

Norway

t: +47 810 02 005
f: +45 4810 6265
nordicinfo@phenomenex.com

Puerto Rico

t: +1 (800) 541-HPLC
f: +1 (310) 328-7768
info@phenomenex.com

Spain

t: +34 91-413-8613
f: +34 91-413-2290
espinfo@phenomenex.com

Sweden

t: +46 (0)8 611 6950
f: +45 4810 6265
nordicinfo@phenomenex.com

United Kingdom

t: +44 (0)1625-501367
f: +44 (0)1625-501796
ukinfo@phenomenex.com

USA

t: +1 (310) 212-0555
f: +1 (310) 328-7768
info@phenomenex.com

All other countries Corporate Office USA

t: +1 (310) 212-0555
f: +1 (310) 328-7768
info@phenomenex.com

roQTM 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 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
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

Bulk roQ QuEChERS Sorbents

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



If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to other products of the same phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

Terms and Conditions

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