Multipesticides Residue Determination in Fresh Okra Using QuEchERS Sample Preparation and Gas Chromatography Tandem Mass Spectrometry

Application Note

Food safety

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Abstract

This application note describes the use of a quick, easy, cheap, effective, rugged, and safe (QuEChERS) AOAC sample preparation approach for the extraction and cleanup of multipesticide residues in fresh okra. The AOAC method used involves:

- Initial extraction in a buffered aqueous/acetonitrile system
- An extraction/partitioning step by the addition of salts
- A cleanup step using dispersive solid-phase extraction (dispersive SPE).

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The pesticides to be quantified were taken from the Agricultural Product Export Promotion Council (APEDA) procedure for the export of okra through control of agrochemicals [1]. APEDA is an Indian regulatory authority for monitoring residues and export promotion activity which had described compounds to be monitored for okra export and its MRLs. Gas chromatography amenable compounds were segregated for GC-MS/MS analysis. The spiking levels for the recovery experiments were 10, 25, and 50 ng/g.

Introduction

Okra (*Abelmoschus esculentus*), also known as ladyfinger or gumbo, is a flowering plant belonging to the Malvaceae family. It is valued for its edible green seed pods. The plant is cultivated in tropical, subtropical, and warm temperate regions around the world. Okra is an important vegetable of tropical countries, and is popular among consumers in Cameroon, Ghana, India, Iraq, Nigeria, Pakistan, Switzerland, UAE, and USA. It is believed to have originated in Africa and is grown in most subtropical and tropical regions of the world. India, Pakistan, Iraq and so forth, are major okra growing countries. India ranks first in the production of okra (73% of world production). It is mainly used as a food supplement in India. In many areas of West Africa, mucilaginous foods are commonly used to impart a desired slimy consistency to local soups and stews.

This application note describes how to set up a sample preparation to optimize pesticide recovery for the quantification of okra while minimizing liner contamination, and therefore streamline routine analysis with high throughput.

Table 1. Okra Edible Portion Content in 100 g

Item	Quantity
Water	90.17 g
Energy	31 kcal (129 kJ)
Protein	2.00 g
Total Lipids	0.10 g
Ash	0.70 g
Carbohydrate	7.03 g
Total dietary fiber	3.2 g
Total sugars	1.2 g
Sucrose	0.40 g
Glucose	0.13 g
Fructose	0.21 g
Starch	0.34 g

Sample preparation

Extraction

Homogenization of fresh okra was carried out in a blender after removing the peduncle (the small portion attached to the plant). Two grams of samples were weighed in a 50-mL centrifuge tube. A piece of ceramic homogenizer (p/n 5982-9313) was added for effective shaking. A modified QuEChERS technique was used for sample preparation [2], 15 mL of distilled water was added, and the sample was allowed to sit for 30 minutes for hydration. Ten mililiters of acetonitrile with 1% acetic acid was added, and the tube was vigorously agitated for 1 minute. Magnesium sulphate (6 g) and sodium acetate (1.5 g) were added and vigorously agitated for one minute. The tube was centrifuged at 6,000 rpm for 5 minutes.

Dispersive

A 1 mL aliquot was transferred to a 2-mL dispersive tube containing 50 mg of primary secondary amine (PSA primary secondary amine), 50 mg of C18, 7.5 mg of graphitized carbon black (GCB), and 150 mg of magnesium sulphate (p/n 5982-0028CH). The tube was vigorously shaken for 1 minute, and then centrifuged at 9,000 rpm for 10 minutes.

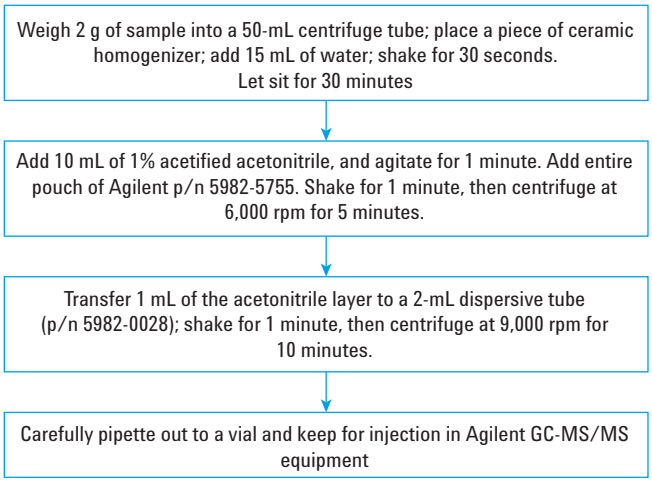

Figure 1. Sample preparation flow chart.

Sample analysis

The GC method was retention time (RT) locked to chloropyrifos methyl at 9.14 minutes, and the backflush pre-set on post run, mid column.

The mucilage and gummy material in okra are polysaccharides in nature. They are water-soluble [3], and were removed by adding 15 mL of water. Okra contains chlorophyll, carotenoids, and xanthophyll pigments, which are not volatile, and stuck to the hot liner when injected. The dispersive cleanup, having graphitized carbon black, adsorbed these pigments (step 3 in the extraction procedure).

Instrumental parameters such as oven programming, backflushing, inlet temperature, and selective reaction monitoring (SRM) transitions were taken from the Agilent pesticides and environmental database [4].

To select the method, all the available transitions were analyzed, and two matrix free transitions were chosen, based on peak shape, abundance, and ion ratio. Matrix-free transition was also considered as a method of transitions from accuracy experiments. A 50 ppb matrix matched standard chloropyrifos methyl with all transitions was shown. Q4 and 5 were selected as Quantification ion and Qualification ion, respectively.

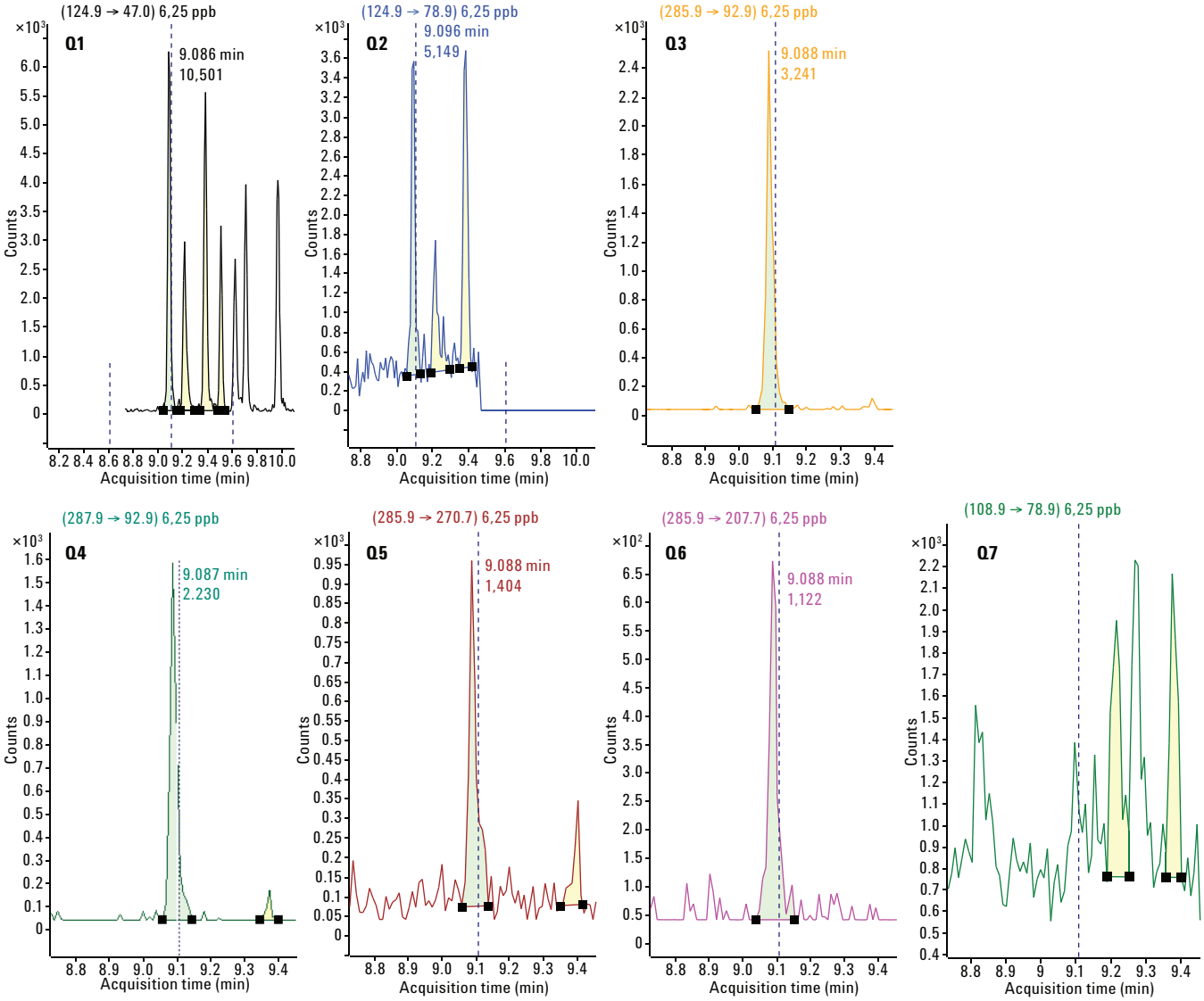

Figure 2. All the available SRM in the database for Chlorpyrifos methyl.

GC instrumental parameters

Column 1	Agilent HP-5MS, 15 m × 0.25 mm, 0.25 µm (p/n 19091s-431)
Column 2	Agilent HP-5MS, 15 m × 0.25 mm, 0.25 µm (p/n 19091s-431)
Capillary flow device	Pressure controlled Tee (PCT) with Electronic Pneumatic control (EPC)
Autosampler	Agilent 7693A
Injection	2 µL cold splitless in multimode inlet (MMI)
Liner	4 mm Single tapered ultra-inert with glass wool (p/n 5180-2293)
Inlet temperature program	70 °C (0.1 minute) 450 °C/ min to 325 °C
Purge flow to inlet	50 mL/min at 1 minute
RTL compound	Chloropyrifos methyl locked at 9.14 RT
Carrier gas	Helium
Oven program	60 °C, 1 minute hold 40 °C/ min raise to 170 °C 10 °C/min to 310 °C, 3 minutes hold
Post run time	3.3 minutes
Post run temperature	310 °C
MS transfer line temperature	310 °C

The Agilent 7000 Triple Quadrupole GC/MS was operated in MS/MS electron ionization (EI) mode and quantified by using SRM mode.

MS parameters

Ionization mode	Electron Ionization
Electron energy	–70 eV
Tune	EI Autotune
EM gain	50
Dwell time	10 msec
Transitions	Refer to Table 1
Collision cell gas	Nitrogen at 1.5 mL/min, helium at 2.25 mL/min
Flows	
MS temperatures	Ion source 300 °C, Q1 150 °C, Q2 150 °C

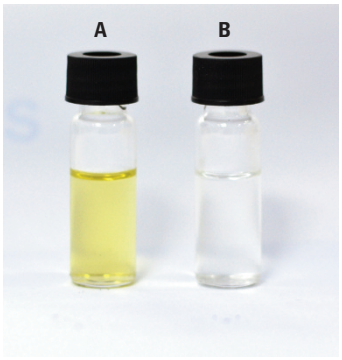

Figure 3. Acetonitrile extract of an okra sample before cleanup (A) and after dispersive cleanup (B).

Instrument calibration

Okra matrix matched calibration standard of pesticides were prepared using okra control at concentrations of 0.09 0.19, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50, 100, and 200 ng/mL. Each matrix matched standard also contained Mirex as an internal standard (IS) at 25 ng/mL concentration.

Method performance

The sample preparation method described in this application note was evaluated in a validation study involving the analysis of six replicates of each at three different levels: 10, 25, and 50 ng/g.

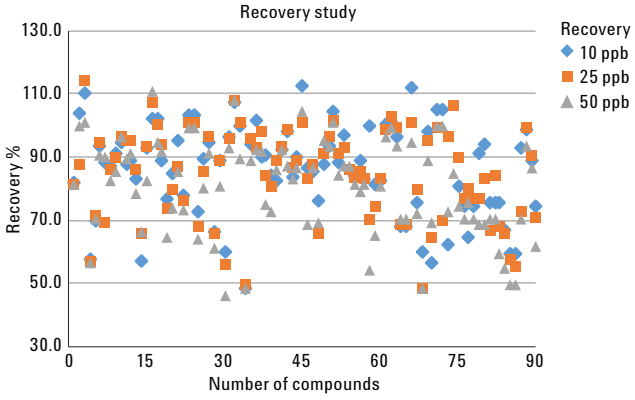

Figure 4. Recovery study at 10, 25, 50 ng/g level.

Table 2. Matrix Free Two SRM Transitions with Collision Energy

TS	Compound	Q1		CE	Q2		CE
4.5	4-Bromo 2-chlorophenol	207.8	172	20	207.8	142	20
	Dichlorvos	184.9	63	25	109	47	10
6.6	Atrazine	214.9	58.1	10	200	122.1	5
	BHC-*alpha*	218.9	183.1	5	181	145	15
	Hexachlorobenzene	283.8	213.9	30	281.8	211.9	30
	Phorate	260	75	5	230.9	128.9	25
	Propoxur	110	64	15	110	63	15
	Trifluralin	305.9	264	5	289.9	201.9	15
	Aldrin	262.9	227.7	30	262.9	192.3	30
	Atrazine	214.9	58.1	10	200	122.1	5
	BHC-*beta*	181	145	15	181	109	30
	BHC-*delta*	219	183.1	5	216.9	181.1	5
	BHC-*gamma*	216.9	181	5	181	145	15
	Chlorothalonil	265.8	133.1	40	263.8	168	25
	Chlorpyrifos-methyl	287.9	92.9	20	285.9	92.9	20
	Diazinon	137.1	84	10	137.1	54	20
	Dicofol	138.9	111.1	25	138.9	75	30
	Iprobenfos	203.9	121.1	35	203.9	91	5
	Propanil	163	101	30	161	99	30
	Propetamphos	138	110	10	138	64	15
9	Alachlor	237	188.2	5	237	160.1	5
	Aldrin	262.9	227.7	30	262.9	192.3	30
	Chlorpyrifos	198.9	171	15	196.9	169	15
	Chlorpyrifos-methyl	287.9	92.9	20	285.9	92.9	20
	Dicofol	138.9	111.1	25	138.9	75	30
	Fenitrothion	125.1	79	5	125.1	47	15
	Fenthion	278	125	15	278	109	15
	Fipronil	254.9	228	15	254.9	157	35
	Heptachlor	273.7	238.9	15	271.7	236.9	15
	Malathion	157.8	125	5	126.9	99	5
	Metalaxyl	234	174.1	10	220	192.1	5
	Metalaxyl M	234	174.1	10	220	192.1	5
	Metolachlor	240	162.2	10	238	162.2	10
	Parathion	290.9	109	10	290.9	80.9	25
	Parathion-methyl	232.9	109.0	10	125	47	10
	Propanil	163	101	30	161	99	30
	Tetraconazole	170.9	136	10	170.9	99	25
	Transfluthrin	335	163	10	163.1	143.1	20
10.25	Chlorfenvinphos I	268.9	161	15	266.9	159.1	15
	Chlorfenvinphos II	268.9	161	15	266.9	159.1	15
	Chlordane-*cis*	271.9	236.9	15	236.9	118.9	30
	Chlorpyrifos-methyl	287.9	92.9	20	285.9	92.9	20
	Dicofol	138.9	111.1	25	138.9	75	30

Table 2. Matrix Free Two SRM Transitions with Collision Energy (continued)

TS	Compound	Q1		CE	Q2		CE
	Fipronil	368.8	214.8	25	366.8	212.8	25
	Heptachlor endo-epoxide	352.7	288.7	10	352.7	252.7	20
	Heptachlor exo-epoxide	354.8	264.9	15	352.8	262.9	15
	Pendimethalin	251.8	162.2	10	251.8	161.1	15
	Quinalphos	298	155.9	10	192.9	101.9	30
	Tetraconazole	170.9	136	10	170.9	99	25
11	Butachlor	236.9	160.2	5	176.1	147.1	10
	Chlordane-*cis*	271.9	236.9	15	236.9	118.9	30
	Chlordane-*trans*	271.7	236.9	15	236.8	118.9	25
	Chlorfenapyr	362.8	246.8	25	246.9	227	15
	DDD-*o,p'*	237	165.2	20	235	165.2	20
	DDD-*p,p'*	236.9	165.2	20	234.9	165.1	20
	DDE-*o,p'*	248	176.2	30	246	176.2	30
	DDE-*p,p'*	317.8	248	15	246.1	176.2	30
	DDT-*o,p'*	237	165.2	20	235	165.2	20
	Dicofol	138.9	111.1	25	138.9	75	30
	Dieldrin	262.9	193	35	262.9	191	35
	Endosulfan I	262.8	192.9	30	236.8	142.9	30
	Endosulfan II	194.9	158.9	10	194.9	124.9	25
	Endrin	262.8	227.9	20	262.8	193	35
	Ethion	230.9	129	20	124.9	96.9	0
	Hexaconazole	256	159	15	231	175	10
	Iprodione	313.5	271.1	5	313.5	245.1	5
	Kresoxim-methyl	116	89	15	116	63	30
	Oxyfluorfen	252	196	20	252	146	30
	Profenofos	338.8	268.7	15	338.8	187.8	30
12.9	DDT-*p,p'*	237	165.2	20	235	165.2	20
	Endosulfan sulfate	273.8	238.9	15	271.9	237	15
	Propargite I	135	107.1	10	135	77.1	30
	Propargite II	149.9	107.1	20	135	107.1	10
	Propiconazole I	172.9	145	15	172.9	74	45
	Propiconazole II	172.9	109	30	172.9	74	45
	Tebuconazole	125	99	20	125	89	15
	Triphenyl phosphate	232.9	215.1	10	232.9	168.1	25
13.95	Bitertanol I	170.1	141.1	20	170.1	115	20
	Bitertanol II	170.1	141.1	20	170.1	115	20
	Cyfluthrin I	226.9	76.9	25	162.9	127	5
	Cyfluthrin II	162.9	127	5	162.9	90.9	15
	Cyfluthrin III	162.9	127	5	162.9	90.9	15
	Cyfluthrin IV	162.9	127	5	162.9	90.9	15
	Cyhalothrin (*lambda*)	208	181	5	208	152	35
	Cypermethrin I	163	127	5	163	91	10
	Cypermethrin II	163.1	127.1	5	163.1	91	15

Table 2. Matrix Free Two SRM Transitions with Collision Energy (continued)

TS	Compound	Q1		CE	Q2		CE
	Cypermethrin III	164.9	127	5	163	127	5
	Cypermethrin IV	163.1	127.1	5	163.1	91	15
	Fenpropathrin	264.9	210	10	181.1	152.1	25
	Mirex	273.8	238.8	15	273.8	236.8	15
	Permethrin I	163	127	5	163	91	15
	Permethrin II	162.9	127.1	5	162.9	91.1	15
	Phosalone	182	111	15	182	75.1	30
	Pyriproxyfen	136.1	96	15	136.1	78.1	20
16.8	Cypermethrin I	163	127	5	163	91	10
	Cypermethrin II	163.1	127.1	5	163.1	91	15
	Cypermethrin III	164.9	127	5	163	127	5
	Cypermethrin IV	163.1	127.1	5	163.1	91	15
	Difenoconazole I	322.8	264.8	15	322.8	201.9	40
	Difenoconazole II	322.8	264.8	15	322.8	201.9	40
	Esfenvalerate	225	119.1	20	167	125.1	10
	Etofenprox	163	135.1	10	163	107.1	20
	Fenvalerate I	224.9	147.1	10	167	88.9	40
	Fenvalerate II	224.9	119	15	167	88.9	40
	Fluvalinate-*tau* I	250	200	40	250	55	40
	Fluvalinate-*tau* II	250	200	40	250	55	40
17.85	Azoxystrobin	344.1	182.9	25	344.1	171.9	40
	Deltamethrin	252.9	174	5	250.7	172	5
	Difenoconazole I	322.8	264.8	15	322.8	201.9	40
	Difenoconazole II	322.8	264.8	15	322.8	201.9	40
	Difenoconazole I	322.8	264.8	15	322.8	201.9	40
	Difenoconazole II	322.8	264.8	15	322.8	201.9	40

TS = Time Segment; Q1 = Quantitative ion; Q2 = Qualitative ion; CE = Collision energy

After method development and validation, the method was checked for performance, suitability, and ruggedness using market samples collected from the Tamilnadu and Karnataka districts.

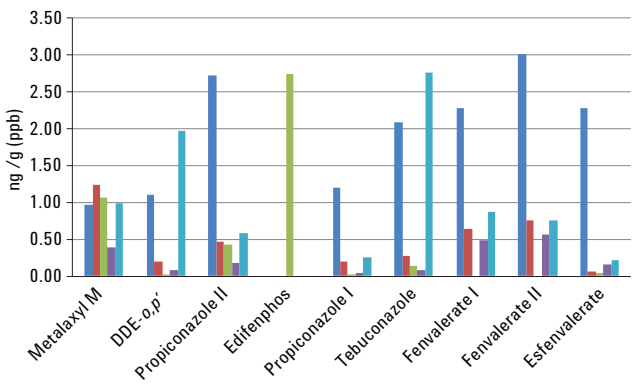

Figure 5. Number of pesticides quantified and pesticide Karnataka samples.

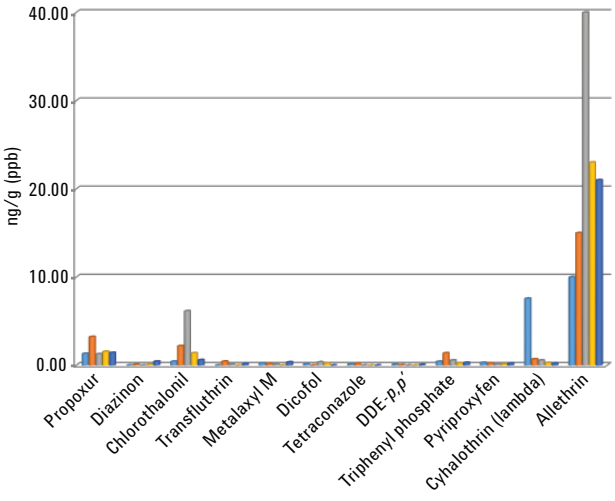

Figure 6. Pesticides found in market sample of Tamilnadu samples.

Conclusion

Due to okra's complex matrix, the quantification of pesticide residues in fresh okra sample preparation and analysis is challenging.

Effort was taken to remove pigments and gummy material to extend liner and column life for better recovery. A QuEChERS sample preparation technique was used to clean up. This involved extraction with acetonitrile followed by a dispersive step. The cleanup step improves targeted compound sensitivity through the reduction of pigments and gummy material, which also increases the column and liner life.

The post run midcolumn backflush was configured using capillary flow technology to remove high-boiling components between injections, and reduce contamination of the mass spectrometer ion source.

Most of the target compounds comply with the DG SANCO 12495/2013 [5] document in that recoveries were within the acceptable limit. That is, recoveries were 70–120%, and repeatability was < 20% at all three spiking levels (10, 25, and 50 ng/g). A few compounds, < 70%, obtained lower recoveries, but gave consistent RSD in all three levels of accuracy. Due to the consistency (< 20% RSD) of the results for these pesticides, the results could be corrected for the known recovery factor in the analysis. In terms of sensitivity, the majority of the compounds could be quantified at 1–3 ng/g level.

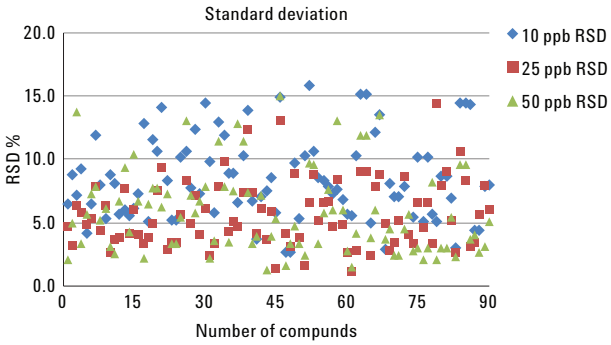

Figure 7. Relative standard deviation of all three levels recovery.

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