JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Collaborative Validation of the QuEChERS Procedure for the Determination of Pesticides in Food by LC−MS/MS

Chris Sack,[†] Michael Smoker,[†] Narong Chamkasem,[‡] Richard Thompson,[§] Greg Satterfield,[§] Claude Masse,[∥] Greg Mercer,[⊥] Barbara Neuhaus,[⊥] Irene Cassias,[#] Eugene Chang,[#] Yi Lin,[#] Shaun MacMahon,[▽] Jon Wong,[▽] Kai Zhang,[▽] and Robert E. Smith*[,†]

[†]Total Diet and Pesticide Research Center, Kansas District Lab, KAN, U.S. Food and Drug Administration, 11510 W 80th Street, Lenexa, Kansas 66214, United States

[‡]Southeast Regional Lab, SRL, U.S. Food and Drug Administration, Atlanta, Georgia 30309, United States

[§]Arkansas Regional Lab, ARL, U.S. Food and Drug Administration, Jefferson, Alaska 72079, United States

[∥]Northeast Regional Lab, NERL, U.S. Food and Drug Administration, Jamaica, New York 11433, United States

[⊥]Pacific Regional Lab-Northwest, PRL-NW, U.S. Food and Drug Administration, Bothwell, Washington 22201, United States

[#]Pacific Regional Lab-Southwest, PRL-SW, U.S. Food and Drug Administration, Irvine, California 90015, United States

[▽]Center for Food Safety and Nutrition, CFSAN, U.S. Food and Drug Administration, College Park, Maryland 20740, United States

Supporting Information

ABSTRACT: Seven FDA pesticide laboratories collaborated to develop and validate an LC−MS/MS method to determine 173 pesticides in <20 min. The average determination coefficient (r^2) was >0.99 for all but two compounds tested. The limits of detection were <20 ng/mL for all compounds and <10 ng/mL for 363 of the 368 transitions reported. The method was used to determine pesticides in two AOAC sponsored proficiency samples. The LC−MS/MS determination was used for the analysis of oranges, carrots and spinach using the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) method. Each matrix was fortified at 20, 100, 400, and 1000 ng/g. No false positive responses were detected in controls of the three matrices. 165 pesticides had recoveries between 70 and 130%, and 161 had minimum detection levels less than 10 ng/g. Recoveries of 169 compounds for the 1000 ng/g spikes were within 50−150%. A matrix effect study indicated all three matrices caused a small net suppressing effect, the most pronounced attributable to the citrus matrix. The procedure proved to be accurate, precise, linear, sensitive and rugged, and adds 100 pesticides to the scope of the FDA pesticide program.

KEYWORDS: pesticides, QuEChERS, HPLC, LC−MS/MS

INTRODUCTION

In 2003, Anastassiades et al. introduced a new approach[1] for the extraction of pesticides from fresh fruits and vegetables with acetonitrile, called QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe). Since then, many modifications and studies of the procedure have been published.[2−16] In all the studies cited, recovery data were determined against standards prepared in the matching matrix, and usually against a 3−7 point standard curve. While this provides for excellent study data by removing matrix effects and enhancing the accuracy of the procedure, it is not practical in a regulatory laboratory analyzing up to 30−50 samples per day in as many matrices.

The matrix effect on several pesticides was studied, and it was concluded that matrix-matched calibration with one fruit sample does not automatically correct results for other samples of the same fruit, therefore only by standard additions can assurance of correction for the matrix effect be guaranteed.[17,18] However, this is not practical for routine analysis. That is, it would take longer to analyze each sample, so fewer samples could be analyzed per day. So, in this study results of spikes into sample matrices were calculated using a single level calibration standard in solvent.

The major international testing programs in Europe and Asia use liquid chromatography−tandem mass spectrometry (LC−MS/MS) in their pesticide monitoring programs, including Australia, Canada, China, Denmark, Germany, Japan, The Netherlands, and the United Kingdom.[19−22] Many US federal and state agencies are also employing LC−MS/MS in their pesticide programs; and the agrochemical industry uses LC−MS/MS almost exclusively for its analytical methods to detect pesticide residues.

To effectively monitor pesticides and chemical contaminants in food, the FDA purchased seven LC−MS/MS instruments and began the process to develop, validate and implement a pesticide method. A team of LC−MS/MS analysts was formed to devise a strategy to steer the process. Instruments were purchased for each lab, and the LC−MS/MS method they developed and validated is reported here.

MATERIALS AND METHODS

The FDA laboratories involved in the validation include Kansas District Lab, KAN; Southeast Regional Lab, SRL; Arkansas Regional

Received: April 22, 2011
Accepted: April 26, 2011
Published: April 26, 2011

Table 1. MS Transition Parameters[a]

	transition 1					transition 2				
compound	Q1	Q3	DP	CE	EXP	Q1	Q3	DP	CE	EXP
3-Hydroxycarbofuran	238.1	163	66	21	15	238.1	181	66	16	11
Acephate	184.1	143	61	13	5	184.1	49	61	33	6
Acetamiprid	223	126	60	29	10	223	99	60	51	14
Acibenzolar-S-methyl	211	136	46	39	8	211	140	46	31	8
Alanycarb	400.1	238.2	35	14	5	400.1	91.1	35	40	5
Aldicarb + NH_4	208.1	116	35	11	10	208.1	89	35	23	16
AldicarbSulfoxide	207.1	132.1	30	10	8	207.1	89.1	30	19	6
Aldoxycarb	223.1	86.1	52	21	5	223.1	148	52	13	9
Aminocarb	209.1	152	71	21	8	209.1	137.1	71	35	10
Amitraz	294.2	163.2	46	21	4	294.2	107.1	46	57	4
AvermectinB1a+NH_4	890.9	567.7	75	23	18	890.9	305.4	72	35	22
AvermectinB1b+Na	876.5	291	41	35	4	876.5	145	41	43	4
Azoxystrobin	404.1	372.1	51	19	5	404.1	344.1	51	27	5
BDMC	260	122	52	34	5	260	107	52	54	5
Benalaxyl	326.2	148.1	71	31	8	326.2	294.1	71	17	10
Bendiocarb	224.1	109	61	27	20	224.1	167.1	61	15	12
Benfuracarb	411.2	195.1	50	30	5	411.2	252.1	50	19	5
Bentazon	241	199	76	19	8	241	107	76	39	8
Benzoximate	364	199	51	13	13	364	105	51	35	4
Bifenazate	301.1	170.1	59	30	9	301.1	198.1	59	21	10
Bitertanol	338.2	70	51	31	12	338.2	269.2	48	13	14
Boscalid	343	307	90	27	7	343	140	90	27	6
BromuconazoleA	378	159	61	39	12	378	70	61	43	12
BromuconazoleB	378.1	159.1	61	39	12	378.1	70.1	61	43	12
Bupirimate	317	166.1	86	33	12	317	108	86	37	10
Buprofezin	306.2	201.1	46	17	5	306.2	116.2	46	21	5
Butafenacil+NH_4	492.1	331	58	33	16	492.1	349	61	21	12
Butocarboxim+Na	213.1	75	50	21	6	213.1	116	50	13	6
Butoxycarboxim	223.1	106	45	15	8	223.1	166	45	11	5
Carbaryl	202.1	145	57	15	9	202.1	127	54	41	8
Carbendazim	192.2	160.2	80	24	10	192.2	132.1	80	41	7
Carbetamide	237.1	192	55	13	10	237.1	118.1	56	19	10
Carbofuran	222.1	123	66	31	19	222.1	165.1	66	19	11
Chlorantraniliprole	484	452.9	66	23	14	484	285.9	66	19	16
Chlorfluazuron	540	158	91	27	4	540	383	91	28	4
Chlorotoluron	213.1	72.2	61	31	5	213.1	46.2	61	27	5
Chloroxuron	291.1	72.4	65	34	5	291.1	218.1	65	30	5
Clethodim	360.1	164	61	28	9	360.1	268.1	61	17	8
Clofentezine	303	138	65	22	8	303	102	65	51	14
Clothianidin	250	169	51	17	4	250	132	51	21	10
Cyazofamid	325	108	60	20	9	325	261.1	60	15	13
Cycluron	199.1	89.1	50	21	5	199.1	72.2	50	21	4
Cyflufenamid	413.1	295.1	56	23	8	413.1	223.1	56	33	14
Cymoxanil	199	128	60	13	5	199	111	60	25	5
CyproconazoleA	292	70	63	37	10	292	125	63	43	8
CyproconazoleB	292.1	70.1	63	37	10	292.1	125.1	63	43	8
Cyprodinil	226	93	95	49	13	226	77	95	64	12
Cyromazine	167.1	85.1	62	27	15	167.1	125.1	62	27	8
Desmedipham+NH_4	318.1	182	42	19	10	318.1	136	39	34	9
Diclobutrazol	328.1	70	81	49	12	328.1	158.9	81	49	10
Dicrotophos	238.1	112.1	66	19	8	238.1	193	66	15	13
Diethofencarb	268.1	226.1	60	15	12	268.1	124	61	45	8

Table 1. Continued

	transition 1					transition 2				
compound	Q1	Q3	DP	CE	EXP	Q1	Q3	DP	CE	EXP
Difenoconazole	406.1	251.1	80	37	13	408.2	253.1	76	33	5
Diflubenzuron	311	158.2	71	23	10	311	141.1	71	45	10
Dimethoate	230	199	49	16	12	230	125	50	27	8
DimethomorphA	388.1	301	66	25	5	388.1	165.1	66	45	5
DimethomorphB	388.2	301.1	66	25	5	388.2	165.2	66	45	5
Dimoxystrobin	327.1	205	40	15	5	327.1	116	40	35	5
Dinotefuran	203.1	129.2	51	19	8	203.1	157.2	51	13	14
Dioxacarb	224.1	167	51	13	10	224.1	123	51	23	21
Diuron	233.1	72	56	33	5	235.1	72.1	56	38	10
Doramectin+NH_4	916.9	593.6	68	20	16	916.9	331.5	65	33	22
Emamectin	886.5	158.1	111	51	10	886.5	82.1	111	127	13
Eprinomectin	914.5	186.2	77	27	12	914.5	154.2	77	58	10
Ethaboxam	321	183.1	86	33	12	321	200.1	86	39	12
Ethiofencarb	226.1	106.9	41	21	5	226.1	164.1	41	11	5
Ethiprole	397.3	350.9	81	29	24	397.3	255.2	81	49	16
Ethirimol	210.2	140.1	81	31	8	210.2	98.1	81	39	18
Etoxazole	360.1	141	76	45	5	360.1	57.2	76	45	5
Famoxadone+NH_4	392	331	32	15	6	392	238	37	23	6
Fenamidone	312.1	92	66	39	16	312.1	236.1	66	21	14
Fenazaquin	307.1	161.1	68	27	10	307.1	147	68	28	9
Fenbuconazole	337	124.9	81	41	8	337	70	81	39	12
Fenhexamid	302	97	75	34	14	302	55	75	67	9
Fenobucarb	208.1	95.1	61	21	18	208.1	152.1	61	13	10
Fenoxycarb	302.1	88	65	30	6	302.1	116.1	65	17	7
Fenpyroximate	422	366.1	56	23	5	422	135.1	56	43	5
Fenuron	165.1	72.1	56	25	5	165.1	46	56	29	5
Flonicamid	230.1	203.1	55	35	4	230.1	174	55	35	4
Flubendiamide	683	408	56	17	12	683	274	56	43	16
Fludioxinil+NH_4	266	229	41	23	14	266	227.1	41	13	14
Flufenoxuron	489	158	86	29	10	489	141.1	86	63	8
Fluometuron	233.1	72.1	71	37	12	233.1	46	71	35	4
Fluoxastrobin	459.2	427.2	55	28	5	459.2	188	55	35	5
Flusilazole	316.1	247.1	78	27	14	316.1	165.1	78	38	9
Flutolanil	324.1	262.1	74	26	14	324.1	242.1	74	34	12
Flutolanil+NH_4	341.1	242.1	61	35	4	341.1	262.1	61	35	4
Flutriafol	302.1	70.1	66	37	12	302.1	123	66	41	8
Forchlorfenuron	248	129.1	52	25	5	248	93.1	52	48	5
Formetanate	222.1	165	71	22	9	222.1	93	76	53	14
Fuberidazole	185	157	81	33	13	185	65	81	67	11
Furathiocarb	383.1	195.1	74	26	10	383.1	252.1	74	19	14
Halofenozide	331.1	275	41	11	16	331.1	105.1	41	25	8
Hexaflumuron	461.1	158.2	56	25	5	461.1	141.1	56	65	5
Hexythiazox	353.1	228	63	23	12	353.1	168	63	36	9
Hydramethylnon	495.2	323.2	146	45	18	495.2	151.1	146	95	8
Imazalil	297	159	65	34	12	297	201	65	29	10
Imidacloprid	256	209.1	61	23	10	256	175.1	61	28	10
Indoxacarb	528	203	89	54	10	528	218	86	33	14
Ipconazole	334.2	70	74	52	10	334.2	125	74	50	17
Iprovalicarb	321.2	119	66	29	8	321.2	203.1	66	13	13
Isoprocarb	194.1	95	60	23	13	194.1	137	60	13	10
Isoproturon	207.2	72.1	66	29	5	207.2	46.1	66	31	5
Isoxaflutole	360.1	251.1	62	24	9	360.1	220.1	62	50	9
Isoxaflutole+NH_4	377	251.1	56	29	14	377	69	56	35	12

dx.doi.org/10.1021/jf201618q |*J. Agric. Food Chem.* 2011, 59, 6383–6411

Table 1. Continued

	transition 1					transition 2				
compound	Q1	Q3	DP	CE	EXP	Q1	Q3	DP	CE	EXP
Ivermectin+NH_4	892.8	569.7	70	21	20	892.8	713.8	71	15	24
Kresoxim:methyl	314	116	51	21	4	314	206	51	13	4
Linuron	249.1	160	60	23	5	249.1	182.1	60	21	5
Lufenuron	511.1	158.1	61	27	5	511.1	141.2	61	67	5
Malathion	331	127	71	19	8	331	285	71	11	16
Mandipropamide	412.1	328.1	81	21	10	412.1	356.1	81	17	10
Mepanipyrim	224	106	86	37	8	224	77	86	59	14
Metaflumizone	507.1	178.1	101	39	12	507.1	287.1	101	37	16
Metalaxyl	280.1	220.2	60	20	12	280.1	192.2	60	26	10
Metconazole	320.1	70	81	51	12	320.1	125	81	59	10
Methamidophos	142	94	54	20	5	142	125	54	19	7
Methiocarb	226.1	169.1	61	13	11	226.1	121.1	61	27	8
Methomyl	163.1	88.1	35	12	6	163.1	106	35	13	6
Methoxyfenozide	369.1	149.1	56	24	9	369.1	313.2	56	13	10
Metobromuron	259	170.2	56	23	4	259	148.2	56	21	4
Mevinphos-E	225.1	127.1	51	20	7	225.1	193.2	51	10	10
Mevinphos-Z	225	127	51	20	7	225	193.1	51	10	10
Mexacarbate	223.2	166.1	64	23	10	223.2	151	64	35	9
Monocrotophos	224.1	127.1	53	23	10	224.1	98	53	17	5
Monolinuron	215.1	126.1	51	23	5	215.1	99	51	41	5
Moxidectin	640.5	528.5	61	12	16	640.5	498.5	61	17	16
Myclobutanil	289	70	71	37	12	289	125	71	47	8
Novaluron	493	158.1	71	27	5	493	141.1	71	69	5
Nuarimol	315	252.1	75	31	13	315	81	75	44	12
Omethoate	214	124.9	46	29	5	214	182.8	46	17	5
Oxadixyl	279.1	219.1	61	17	13	279.1	132.1	61	43	21
Oxamyl+NH_4	237.1	72.1	36	25	5	237.1	90.1	36	12	6
Paclobutrazol	294	70	62	46	10	294	125	58	49	8
Pencycuron	329.1	125	76	37	22	329.1	218.1	76	25	14
Phenmedipham	301.1	136	50	26	5	301.1	168.1	50	14	4
PhorateSulfone	293.1	97.1	36	41	5	293.1	171.1	36	17	5
Picoxystrobin	368	145	56	27	4	368	205	56	15	4
PiperonylButox+NH_4	356.2	177.2	49	22	9	356.2	119.1	49	46	8
Pirimicarb	239.2	72.1	64	35	10	239.2	182.1	64	23	10
Prochloraz	376	308	45	17	10	376	70	45	44	12
Promecarb	208.1	109	37	23	8	208.1	151	37	13	10
Propamocarb	189.2	102	60	25	8	189.2	144	61	19	13
Propargite+NH_4	368.2	231.1	46	15	13	368.2	175.1	46	23	12
Propiconazole	342.1	159	62	40	9	342.1	69	62	36	10
Propoxur	210.1	111	39	19	6	210.1	168.1	39	11	10
Pymetrozine	218	105	71	27	5	218	78	71	47	5
Pyracarbolid	218.1	125	59	27	8	218.1	97	59	40	14
Pyraclostrobin	388	194	31	19	5	388	163	31	29	5
Pyridaben	365	147	46	31	5	365	309	46	19	5
Pyrimethanil	200	107	71	33	5	200	82	71	35	5
Pyriproxyfen	322	96	45	21	5	322	185	45	29	5
Rotenone	395.1	213.1	90	32	12	395.1	192.1	90	34	10
Siduron	233.3	137.2	66	21	5	233.3	94	66	31	5
SpinetoramA	748.5	142.2	86	45	8	748.5	98.1	86	109	18
SpinetoramB	760.5	142.2	96	41	10	760.5	98.1	96	101	18
SpinosynA	732.5	142.2	111	43	10	732.5	98.1	111	103	16
Spirodiclofen	411.3	313.3	72	23	8	411.3	71.3	71	33	10
Spiromesifen	371.2	273.2	73	16	6	371.2	255.2	74	33	4

dx.doi.org/10.1021/jf201618q |*J. Agric. Food Chem.* 2011, 59, 6383–6411

Table 1. Continued

	transition 1					transition 2				
compound	Q1	Q3	DP	CE	EXP	Q1	Q3	DP	CE	EXP
Spiromesifen+NH_4	388.2	273.2	41	19	12	388.2	255.2	41	39	16
Spirotetramat	374.2	330.2	66	23	8	374.2	302.2	66	25	20
Spiroxamine	298.2	144.2	72	28	10	298.2	100.1	72	46	14
Sulfentrazone	387	307.1	81	27	5	387	146	81	57	5
Tebuconazole	308.2	70	81	49	11	308.2	125	81	51	8
Tebufenozide	353.2	133	54	24	9	353.2	297.2	54	14	9
Tebuthiuron	229.1	172.4	46	21	5	229.1	116.1	46	35	5
Teflubenzuron	381.1	141.2	66	52	5	381.1	158.2	66	23	5
Temephos	467	419.1	101	29	12	467	405	101	23	12
Thiabendazole	202.1	175.1	84	35	10	202.1	131.2	84	45	8
Thiacloprid	253	126	68	30	9	253	99	68	60	14
Thiamethoxam	292	211	64	18	10	292	181	64	32	10
Thidiazuron	221.1	102.1	57	28	6	221.1	128.2	57	22	7
Thiophanate-methyl	343	151.1	61	29	14	343	311	61	17	10
Triadimefon	294	197.1	63	22	12	294	225	63	19	8
Triadimenol	296.1	70	46	31	12	296.1	227.1	46	19	14
Trichlorfon	256.9	109.1	66	25	20	256.9	127	66	25	8
Tricyclazole	190	163	81	33	10	190	136	81	41	11
Trifloxystrobin	409	186	31	23	5	409	206	31	21	5
Triflumizole	346.1	278.1	51	15	8	346.1	73	51	27	6
Triflumuron	359.1	156.2	52	23	6	359.1	139	52	44	6
Triticonazole	318.1	70	63	42	10	318.1	125	63	41	8
Vamidothion	288	146	61	19	10	288	118	61	33	10
Zoxamide	336.1	187	55	33	11	336.1	159	53	39	12

[a] Q1 and Q3 are the m/z of the parent and transition ions, respectively. DP is the declustering potential, CE is the collision energy and EXP is exit potential.

Lab, ARL; Northeast Regional Lab, NERL, Jamaica, NY; Pacific Regional Lab-Northwest, PRL-NW; Pacific Regional Lab-Southwest, PRL-SW; and the Center for Food Safety and Nutrition, CFSAN.

Each lab obtained an LC−MS/MS and participated in the process to develop, validate, and implement a pesticide method. An LC−MS/MS determination procedure was developed and optimized; and then validated for accuracy, reproducibility, linearity, instrument detection limit, and extended range. A matrix effect study was conducted demonstrating that ion suppression from sample matrices was minimal. The validated LC−MS/MS procedure was then used for the determination of pesticides in fortified sample matrices extracted using the QuEChERS method, and recoveries were measured.

Chemical Reagents. Pesticide standard mixes were purchased from AccuStandards (New Haven, CT) consisting of 9 mixtures of 20−25 analytes (total of 196 compounds) at 100 μg/mL in methanol (CH_3OH). PRL-NW prepared an additional mix of 20 μg/mL each of 17 compounds in CH_3OH from neat solids supplied by the National Pesticide Standard Repository (EPA, Fort Meade, MD). The Center for Food Safety and Nutrition (CFSAN) prepared a stock (100 μg/mL) flonicamide standard from neat also supplied by the National Pesticide Standard Repository. The following injection and spiking standards were prepared from the 3.0 μg/mL mixture of all standards: 1000, 500, 200, 100, 50.0, 20.0, 10.0, 5.00, and 2.00 μg/mL.

QuEChERS Sample Extraction. QuEChERS prefilled centrifuge tubes were from UCT Enviro-Clean (Bristol, PA). They contained 6 g of anhydrous magnesium sulfate ($MgSO_4$) plus 1.5 g of NaCl (UCT Enviro-Clean #ECMSSC50CTFS), 1200 mg of anhydrous $MgSO_4$, plus 400 mg of primary and secondary amine (PSA) sorbent (UCT Enviro-Clean #ECMS12CPSA415CT), and 150 mg of anhydrous $MgSO_4$ plus 50 mg of PSA (UCT Enviro-Clean #CUMPS2CT). The QuEChERS procedure was done as follows. For most samples, 15 g of sample was added with 15 mL of acetonitrile (CH_3CN) to an empty 50 mL centrifuge tube, but in some samples, 10 g of sample was added with 10 mL of CH_3CN. The volume was adjusted to maintain ratio of 1 g of sample per mL of CH_3CN. That is, for a 5 mL spike volume add 10 mL of CH_3CN to 15 g of sample. This was shaken for 1 min at 1000 strokes/min. Then, the anhydrous $MgSO_4$ plus NaCl was added. For 15 g samples, 6 g of $MgSO_4$ + 1.5 g of NaCl was added, and for 10 g samples, 4 g of $MgSO_4$ + 1 g of NaCl was added. This was spiked to obtain 2−1000 μg/mL concentrations of analytes. This was shaken for 1 min at 1000 strokes/min and then centrifuged at about 4500 rpm for 5 min. The supernatant was cleaned up using the PSA sorbent. That is, 1.0 mL of the extract was transferred to a 2 mL centrifuge tube containing 50 mg of PSA + 150 mg of $MgSO_4$, or the entire extract was transferred to a 15 mL centrifuge tube containing 300 mg of PSA + 900 mg of $MgSO_4$. Then, 0.5 mL of the extract was diluted to 1.0 mL with LC−MS aqueous buffer (0.5 g sample/mL), filtered through a nylon filter and analyzed by LC−MS.

LC−MS/MS System. The LC−MS/MS was an AB Sciex (Foster City, CA) 4000 QTrap: *Scheduled* MRM in the positive ionization mode. The transition parameters are in Table 1 MS parameters are in Table 2.

The HPLC was from Shimadzu (Kyoto, Japan). It had a LC-20AD Pump, Sil-20AC Autosampler and CTO-20AC Column oven. An Ultra Aqueous C18 column (Restek, Bellefonte, PA) 3 μm, 100 × 2.1 mm with 10 × 2.1 mm guard column (Restek) was used by Arkansas Regional Lab (ARL), Pacific Regional Lab-Northwest (PRL-NW), Pacific

Table 2. Mass Spectrometer Parameters[a]

	SRL	PRL-NW	NRL	PRL-SW	KAN	ARL	CFSAN
MRM det window (s)	80	60	40	90	60	60	60
target scan time (s):	0.5	1.2	0.5	1	1	1	0.5
resolution Q1	unit	unit	unit	unit	unit	unit	unit
resolution Q3	unit	unit	low	unit	unit	unit	unit
MR pause (ms)	5	5	5	5	5	5	5
collision gas	high	med	med	med	med	high	med
curtain gas (mL/min)	30	30	20	20	20	20	30
exit potential (V)	10	10	10	10	10	10	10
ion source gas 1 (mL/min)	60	60	70	50	50	50	50
ion source gas 2 (mL/min)	60	50	70	60	60	60	50
interface heater:	on	on	on	on	on	on	on
ion spray voltage (V)	5000	5000	1500	5000	5500	5500	5000
turbo spray temp (°C)	350	400	400	600	400	400	400

[a] Acronyms for the different FDA Labs: Southeast Regional Lab (SRL), Pacific Regional Lab Northwest (PRL-NW), Northeast Regional Lab (NRL), Pacific Regional Lab Southwest (PRL-SW), Kansas District Lab (KAN), Arkansas Regional Lab (ARL), Center for Food Safety and Nutrition (CFSAN).

Table 3. HPLC Parameters

	SRL	PRL-NW	NRL	PRL-SW	KAN	ARL	CFSAN
equilibration time (min)	0.1	1.0	0.0	0.0	0.0	0.1	1.5
tnjection vol (μL)	2.0	2.0	2.0	2.0	2.0	2.0	20
total flow (mL/min)	0.4	0.4	0.5	0.5	0.5	0.4	0.5
rinsing vol (μL)	500	200	200	200	200	200	200
rinsing speed (μL/s)	35	35	35	35	35	35	35
sampling speed (μL/s)	15	15	15	15	15	15	15
cooler temp (°C)	15	15	15	15	15	15	15
column oven temp (°C)	50	40	40	40	40	50	40

Regional Lab-Southwest (PRL-SW), Southeast Regional Lab (SRL) and CFSAN. An Atlantis T3 C18, 3 μm, 100 × 3 mm with guard column (Waters, Milford, Mass) was used by KAN. A Zorbax Eclipse Plus C18, 1.8 μm, 50 × 4.6 mm (Agilent, Santa Clara, CA) was used by Northeast Regional Lab (NRL). The HPLC instrument parameters are in Tables 3 and 4.

LC−MS/MS Method Validation. The validation protocol was as follows. Participating laboratories prepared the standard mixes in solvent at 1000, 500, 200, 100, 50.0, 20.0, 10.0, 5.00, and 2.00 μg/mL. Each level was analyzed in duplicate. All data was combined in a Microsoft Access database for analysis. Data analysis and calculations were conducted for each individual transition analyzed. Average responses of the calibration standard (200 ng/mL) for each analytical run were used to calculate the concentrations and percentage of nominal concentration of the analytes in the run using the external standard calibration method.

From the data six elements of instrument validation were evaluated: accuracy, reproducibility, linearity, limits of detection (LOD), extended range, and ruggedness. For accuracy and reproducibility, the average and relative standard deviation (RSD) for all laboratories of the percentage of nominal concentrations for the 50, 100, and 200 ng/mL standards were calculated. For linearity the determination coefficients (r^2) were calculated for each laboratory from the responses of the 2, 5, 10, 20, 50, 100, and 200 ng/mL standards. The average r^2 among the reporting laboratories is reported. For limits of detection (LODs), an average response factor for the 5, 10, and 20 ng/mL standards was calculated for each laboratory, and then used to calculate the concentration of the 10 ng/mL standards. The LODs were calculated by multiplying the standard deviation of the 10 ng/mL standard concentrations by 3. To evaluate the extended range, the average, minimum, and maximum for all laboratories of the percentage of nominal concentrations of the 500 and 1000 ng/mL standards, i.e. 250% and 500% of the calibration level respectively, were calculated. For ruggedness, PRL-NW used the LC−MS/MS method as a determinative step for proficiency samples from AOAC. Water was removed from the samples as described previously[23] and reconstituted in CH_3OH/H_2O. Quantitation was performed using matrix matched standards.

Matrix Effect Study. QuEChERS extracts of orange, spinach, and carrots were used to prepare 200 ng/mL standards with matrix concentrations of 0.5 and 0.2 g/mL. The response of the matrix standards was compared to the standard prepared in CH_3OH. Matrix effects were calculated for all laboratories per matrix/transition/sample concentration combination. The matrix effects were also averaged for all transitions to summarize the effect of each matrix.

QuEChERS Validation Protocol. Participating laboratories received from CFSAN 250−300 g each of frozen carrot, orange and spinach composites. Each matrix was fortified at 20, 100, 400, and 1000 ng/g and analyzed. The 200 ng/mL calibration standard in CH_3OH was analyzed at the beginning and end of the analytical runs; some laboratories analyzed the standard additionally during the analysis of samples. All data were submitted to the study director for analysis and evaluation.

From the data the method uncertainty (MU), method detection limit (MDL) and average recovery for each matrix with standard deviation was calculated for each transition. The MU was calculated at the 95% confidence level using the relative standard deviation (RSD) of the recoveries from all matrices and laboratories, i.e. 2*RSD. The MDL was calculated at the 99% confidence level by multiplying the standard deviation of the calculated concentrations of the 20 ng/g spikes by 3.

Table 4. HPLC Mobile Phase Composition[a]

	parameter			parameter			parameter			parameter	
time			time			time			time		
	ARL			PRL-SW			PRL-NW			CFSAN	
0.0	% B	20	0.0	% B	20	0.0	% B	11	0.0	% B	5
0.1	% B	20	8.0	% B	90	8.0	% B	90	1.0	% B	5
8.0	% B	90	12.0	% B	100	12.0	% B	90	9.0	% B	95
12.0	% B	100	15.0	% B	100	12.1	% B	100	11.3	% B	95
13.0	% B	20	15.1	% B	20	14.0	% B	100	12.0	% B	5
15.0	% B	20	19.9	% B	20	14.1	% B	11	13.4	% B	5
15.1		stop	20.0		stop	16.0		stop	13.5		stop
	KAN			SRL			NRL				
0.0	% B	0	0.0	% B	20	0.0	% B	5			
5.0	% B	70	0.1	% B	20	1.0	% B	5			
6.0	% B	70	8.0	% B	90	5.0	% B	65			
6.0	curve	3	12.0	% B	95	10.5	% B	95			
8.0	% B	90	12.0	flow	0.4	12.1	% B	95			
12.0	% B	90	12.1	flow	0.6	12.5	% B	5			
12.2	% B	0	14.0	% B	95	15.0	% B	5			
15.0	% B	0	14.1	% B	20	15.1		stop			
15.1		stop	17.0	% B	20						
			17.1	flow	0.4						
			17.2		stop						

[a] Pump A: water with 4 mM ammonium formate and 0.1% formic acid. Pump B: Methanol with 4 mM ammonium formate and 0.1% formic acid.

RESULTS AND DISCUSSION

Method Development of the LC−MS/MS Determination. Initial LC−MS/MS parameters were provided by AB Sciex. They also provided invaluable and extensive onsite and offsite help and guidance for the development of the LC−MS/MS procedure and operation of the instrument.

For the development of the LC−MS/MS determination three studies were conducted using 63 analytes (126 transitions) in standard mixtures to determine the optimal mobile phase composition, column parameters, and the effect of solvent composition of the standards on the chromatography. For the mobile phase (MP) composition study two different mobile phases were compared, an acidic and a neutral. The acidic MP contained 4 mM ammonium formate and 0.1% formic acid in methanol and water. The neutral MP contained 10 mM ammonium acetate in methanol and water. Each lab was instructed to make 5 injections of the 10.0 and 100 ng/mL standards using one or both of the mobile phases. As expected, results from the MP comparison indicated the average signal-to-noise (S/N) levels of all standard levels analyzed for the acidic formate MP exceeded the neutral acetate MP. The average responses using the acidic MP were almost 300% greater than for the neutral MP. Only 12 of the 126 transitions analyzed exhibited a reduced S/N when using the acidic MP, making it the clear choice. The acidic MP enhances the ionization process when using the electrospray ionization in the positive mode.

In the HPLC column comparison study six different octadecylsilane (C18) reverse phase columns of various dimensions, particle sizes, pore sizes, and vendors were evaluated: the three listed in Materials and Methods plus three others: Restek Ultra Aqueous C18, 1.9 μm, 50 × 2.1 mm, Phenomenex Synergi C18, 3 μm, 150 × 3 mm, and a Phenomenex Synergi C18, 2.5 μm, 100 A, 50 × 4.6 mm with guard column. Results of the column comparison clearly demonstrated the Ultra Aqueous C18, 1.9 μm, 50 × 2.1 mm provided 200−300% higher responses, best resolution and S/N. However, during the column study three uHPLC columns became plugged when only standard solutions were injected. Also, back pressures were high, exceeding 6000 lb/in.^{2}. Alternatively, the Agilent Zorbax Eclipse Plus C18, 1.8 μm, 50 × 4.6 mm performed extremely well providing excellent resolution with very low back pressures. Among the non-uHPLC columns 10 cm columns performed comparably and the longer 15 cm column exhibited a nominal loss of sensitivity. The data indicated C18 columns of ≤10 cm length by 2−3 mm interior diameter with particle sizes of 2−3 μm provided sufficient resolution and sensitivity for the analysis of 200 pesticides in 15 min, or less, when the mobile phase composition was programmed appropriately.

In the third study the effect of solvent composition of the standard solutions was studied. Standard solutions in CH_3OH, CH_3OH/H_2O (1:1), and CH_3CN were injected and examined for distortion of the chromatographic peak shape. Very early eluting polar compounds were slightly affected by injections in pure solvents, but the effect was not significant for injection volumes of 5 μL or less. When more than 5 μL of standard solution in CH_3OH or CH_3CN was injected, the peak shape of the early eluting compounds was skewed. Later eluting compounds were not affected by the standard solvent composition or injection volumes. Also, preliminary investigation into the effect of increasing total mass injected indicated instrument response for many compounds began to drop when over 0.5 ng was injected. This effect was further explored in the linearity and range study.

Table 5. Results of LC−MS/MS Validation Study of Standards in Methanol[a]

	accuracy		linearity	LOD	extended range	
transition	av	(RSD)	av r^2	ng/mL	500 ng/mL	1000 ng/mL
3-Hydroxycarbofuran.1	111	(11)	0.9957	1.7	85	65
3-Hydroxycarbofuran.2	110	(10)	0.9956	1.7	84	66
Acephate.1	103	(8)	0.998	1.7	89	79
Acephate.2	105	(8)	0.9973	4.9	95	83
Acetamiprid.1	111	(12)	0.9961	2	84	63
Acetamiprid.2	110	(12)	0.9961	1.9	85	65
Acibenzolar-S-methyl.1	102	(6)	0.9977	4.5	98	87
Acibenzolar-S-methyl.2	105	(14)	0.9914	9.9	92	83
Alanycarb.1	102	(10)	0.9928	5.2	89	79
Alanycarb.2	104	(13)	0.9952	4	91	76
Aldicarb+NH_4.1	100	(8)	0.9975	2.2	92	82
Aldicarb+NH_4.2	103	(7)	0.9968	2.7	95	82
AldicarbSulfoxide.1	105	(9)	0.9976	4.8	86	72
AldicarbSulfoxide.2	106	(7)	0.9984	3.7	86	72
Aldoxycarb.1	106	(13)	0.9961	2.5	89	75
Aldoxycarb.2	105	(8)	0.9971	4.6	88	74
Aminocarb.1	108	(13)	0.9942	2.5	79	62
Aminocarb.2	109	(13)	0.9955	2.3	84	65
AvermectinB1a+Na.3	103	(16)	0.9951	4.5	79	69
AvermectinB1a+Na.4	103	(13)	0.9939	7.3	83	78
AvermectinB1a+NH_4.1	102	(5)	0.9983	11.4	106	107
AvermectinB1a+NH_4.2	100	(14)	0.9932	9.6	111	116
Azoxystrobin.1	112	(12)	0.9914	2.9	76	57
Azoxystrobin.2	105	(11)	0.9967	4.1	84	68
Benalaxyl.1	108	(12)	0.9963	2	84	66
Benalaxyl.2	105	(11)	0.9968	1.6	85	69
Bendiocarb.1	107	(9)	0.9974	2.1	85	70
Bendiocarb.2	112	(12)	0.9943	1.3	84	66
Benfuracarb.1	103	(9)	0.9978	3.5	83	69
Benfuracarb.2	105	(9)	0.9968	4.1	87	74
Benzoximate.1	106	(12)	0.9925	2.6	82	65
Benzoximate.2	105	(8)	0.9976	3	82	66
Bifenazate.1	106	(11)	0.9974	2.6	83	65
Bifenazate.2	106	(13)	0.9955	4	82	65
Bitertanol.1	101	(8)	0.998	2.9	97	90
Bitertanol.2	98	(9)	0.9974	4.4	94	90
Boscalid.1	105	(8)	0.9978	2.3	87	72
Boscalid.2	108	(13)	0.9954	2.9	98	81
BromuconazoleA.1	103	(8)	0.9958	3	91	79
BromuconazoleA.2	104	(10)	0.9967	4.5	87	76
BromuconazoleB.1	107	(9)	0.9973	3.4	87	71
BromuconazoleB.2	103	(6)	0.9979	3.8	91	80
Bupirimate.1	107	(10)	0.995	2.6	88	76
Bupirimate.2	105	(9)	0.9978	4.1	89	74
Buprofezin.1	112	(15)	0.9906	2.7	83	65
Buprofezin.2	108	(8)	0.9975	1.7	85	68
Butafenacil+NH_4.1	106	(8)	0.9973	3.5	85	71
Butafenacil+NH_4.2	106	(13)	0.9948	3.6	92	76
Butocarboxim+Na.1	98	(13)	0.9973	4.9	97	85
Butocarboxim+Na.2	98	(20)	0.9904	4.2	80	68
Butoxycarboxim.1	107	(8)	0.9971	2	86	67
Butoxycarboxim.2	106	(11)	0.996	5.7	85	73
Carbaryl.1	109	(10)	0.9971	1.6	84	67
Carbaryl.2	105	(9)	0.9986	2.2	86	69

dx.doi.org/10.1021/jf201618q |J. Agric. Food Chem. 2011, 59, 6383–6411

Table 5. Continued

	accuracy		linearity	LOD	extended range	
transition	av	(RSD)	av r^2	ng/mL	500 ng/mL	1000 ng/mL
Carbendazim.1	108	(10)	0.9973	2.7	92	78
Carbendazim.2	102	(7)	0.9989	2.1	92	80
Carbetamide.1	106	(9)	0.9977	2.7	86	70
Carbetamide.2	106	(10)	0.9976	2.1	87	70
Carbofuran.1	106	(11)	0.9965	1.6	81	62
Carbofuran.2	108	(12)	0.9945	1.7	80	61
Chlorantraniliprole.1	105	(7)	0.9983	3.5	89	75
Chlorantraniliprole.2	105	(8)	0.9948	1.8	85	76
Chlorfluazuron.1	98	(7)	0.9994	3.4	95	99
Chlorfluazuron.2	98	(6)	0.9989	3	97	95
Chlorotoluron.1	107	(11)	0.9972	2.1	86	70
Chlorotoluron.2	106	(10)	0.9958	3.1	83	64
Chloroxuron.1	106	(14)	0.9951	3.9	88	74
Chloroxuron.2	107	(12)	0.9963	4.3	82	66
Clethodim.1	100	(9)	0.9974	2.1	94	83
Clethodim.2	101	(11)	0.9972	2.9	94	84
Clofentezine.1	101	(8)	0.9981	1.6	93	81
Clofentezine.2	101	(7)	0.9989	3.6	92	85
Clothianidin.1	104	(10)	0.9978	2	90	76
Clothianidin.2	106	(9)	0.9978	2.5	93	74
Cyazofamid.1	112	(12)	0.9954	2.1	80	62
Cyazofamid.2	109	(13)	0.994	5	81	65
Cycluron.1	109	(12)	0.9968	2.3	83	66
Cycluron.2	110	(10)	0.9955	2.5	77	62
Cyflufenamid.1	106	(7)	0.9989	1.9	86	69
Cyflufenamid.2	106	(6)	0.9992	1.4	85	70
Cymoxanil.1	107	(9)	0.9948	2.9	88	79
Cymoxanil.2	103	(8)	0.9982	2.5	86	78
CyproconazoleA.1	104	(11)	0.9962	2.5	92	77
CyproconazoleA.2	101	(9)	0.9966	4	107	97
CyproconazoleB.1	105	(7)	0.9972	2.7	90	85
CyproconazoleB.2	104	(10)	0.9969	5.5	94	77
Cyprodinil.1	101	(6)	0.9987	3.7	95	81
Cyprodinil.2	106	(10)	0.9953	2.9	94	76
Cyromazine.1	106	(7)	0.9982	1.8	88	69
Cyromazine.2	105	(7)	0.9985	1.5	89	71
Desmedipham+NH_4.1	113	(12)	0.9933	3.1	91	72
Desmedipham+NH_4.2	107	(10)	0.9974	2.2	83	65
Diclobutrazol.1	105	(7)	0.9986	2.5	90	78
Diclobutrazol.2	104	(11)	0.9963	6.6	90	82
Dicrotophos.1	108	(12)	0.9954	2.2	82	63
Dicrotophos.2	105	(8)	0.9985	0.9	87	70
Diethofencarb.1	109	(12)	0.9953	2.3	83	64
Diethofencarb.2	105	(11)	0.9943	2	83	64
Difenoconazole.1	104	(8)	0.9968	1.6	94	85
Difenoconazole.2	102	(6)	0.9991	2.4	91	83
Diflubenzuron.1	107	(10)	0.9975	2.1	86	75
Diflubenzuron.2	106	(9)	0.9975	3.5	86	73
Dimethoate.1	108	(9)	0.9977	1.3	81	62
Dimethoate.2	110	(13)	0.9961	2.3	80	61
DimethomorphA.1	101	(11)	0.9921	1.8	95	85
DimethomorphA.2	101	(8)	0.9963	3.1	97	88
DimethomorphB.1	101	(7)	0.9976	2.9	98	87
DimethomorphB.2	99	(12)	0.9918	3.5	90	85
Dimoxystrobin.1	105	(12)	0.9957	1.9	80	65

Table 5. Continued

	accuracy		linearity	LOD	extended range	
transition	av	(RSD)	av r^2	ng/mL	500 ng/mL	1000 ng/mL
Dimoxystrobin.2	108	(10)	0.9966	1.8	82	67
Dinotefuran.1	108	(9)	0.9959	2.2	80	62
Dinotefuran.2	110	(10)	0.9943	3.9	81	62
Dioxacarb.1	110	(9)	0.9973	1.9	81	62
Dioxacarb.2	109	(11)	0.9972	1.5	80	62
Diuron.1	109	(10)	0.9971	1.4	84	66
Diuron-Cl37.2	109	(9)	0.9977	2.7	81	61
Doramectin+Na.3	103	(7)	0.9979	4.9	87	78
Doramectin+Na.4	104	(10)	0.9964	6.5	85	74
Doramectin+NH_4.1	97	(19)	0.9846	13.8	104	112
Doramectin+NH_4.2	99	(15)	0.993	13.4	103	109
Emamectin.1	105	(11)	0.9956	3.2	92	83
Emamectin.2	103	(6)	0.9989	1.8	95	87
Eprinomectin.1	103	(12)	0.9955	5.4	105	100
Eprinomectin.2	101	(11)	0.9946	9.6	99	100
Eprinomectin+Na.3	111	(13)	0.9915	2.2	92	76
Eprinomectin+Na.4	109	(12)	0.9934	7.7	85	70
Ethaboxam.1	106	(9)	0.9966	3	84	68
Ethaboxam.2	104	(11)	0.9962	2.1	88	73
Ethiofencarb.1	106	(11)	0.997	1.5	89	75
Ethiofencarb.2	103	(7)	0.9987	2	90	76
Ethiprole.1	108	(13)	0.9941	3.4	85	69
Ethiprole.2	109	(17)	0.9891	4.7	85	74
Ethirimol.1	104	(6)	0.9986	1.4	89	72
Ethirimol.2	105	(7)	0.9985	2.8	90	75
Etoxazole.1	104	(7)	0.9984	1.7	89	81
Etoxazole.2	106	(8)	0.9983	1.7	89	67
Famoxadone+NH_4.1	98	(9)	0.9974	2.5	99	88
Famoxadone+NH_4.2	98	(11)	0.9939	4.2	97	91
Fenamidone.1	108	(10)	0.9958	3	88	75
Fenamidone.2	105	(9)	0.997	3.5	84	73
Fenazaquin.1	104	(8)	0.9987	1.3	82	70
Fenazaquin.2	102	(6)	0.9994	1.7	84	74
Fenbuconazole.1	104	(9)	0.9979	4.3	89	81
Fenbuconazole.2	103	(7)	0.9984	3.3	89	80
Fenhexamid.1	105	(9)	0.9976	3.8	89	76
Fenhexamid.2	104	(13)	0.9949	5.9	84	72
Fenobucarb.1	103	(8)	0.9976	1.9	85	71
Fenobucarb.2	105	(8)	0.9963	3.6	85	68
Fenoxycarb.1	104	(9)	0.9975	2.7	83	69
Fenoxycarb.2	109	(8)	0.9978	2.5	84	67
Fenpyroximate.1	100	(10)	0.9983	1.5	93	88
Fenpyroximate.2	101	(8)	0.9981	1.8	98	92
Fenuron.1	107	(10)	0.9975	1.8	85	68
Fenuron.2	106	(8)	0.9984	1.8	85	69
Flonicamid.1	106	(10)	0.9961	2.2	90	79
Flonicamid.2	103	(8)	0.9948	6.3	90	77
Flubendiamide.1	103	(17)	0.9916	4.7	86	78
Flubendiamide.2	96	(19)	0.9903	5.8	86	78
Fludioxinil+NH_4.1	109	(10)	0.996	4.4	79	60
Fludioxinil+NH_4.2	114	(13)	0.9956	4.4	77	57
Flufenoxuron.1	102	(6)	0.9989	2.4	94	91
Flufenoxuron.2	101	(8)	0.9977	2.5	97	90
Fluometuron.1	109	(10)	0.9963	2.4	81	63
Fluometuron.2	108	(8)	0.9974	2	82	66

dx.doi.org/10.1021/jf201618q |*J. Agric. Food Chem.* 2011, 59, 6383–6411

Table 5. Continued

	accuracy		linearity	LOD	extended range	
transition	av	(RSD)	av r^2	ng/mL	500 ng/mL	1000 ng/mL
Fluoxastrobin.1	103	(13)	0.9963	2.6	81	65
Fluoxastrobin.2	104	(11)	0.9929	4.4	89	72
Flusilazole.1	104	(8)	0.9982	4.8	88	72
Flusilazole.2	105	(8)	0.9974	2.1	87	73
Flutolanil.1	110	(12)	0.9951	2.9	81	65
Flutolanil.2	109	(11)	0.9955	3.6	84	65
Flutriafol.1	104	(10)	0.9973	1.8	91	79
Flutriafol.2	103	(10)	0.9968	4.5	92	84
Forchlorfenuron.1	107	(12)	0.9969	1.9	86	69
Forchlorfenuron.2	105	(12)	0.9957	1.8	86	70
Formetanate.1	108	(14)	0.9963	1.9	84	71
Formetanate.2	104	(16)	0.9916	4.9	84	76
Fuberidazole.1	107	(11)	0.9969	2.4	85	67
Fuberidazole.2	104	(9)	0.9976	2.7	87	73
Furathiocarb.1	107	(10)	0.9971	2.1	86	66
Furathiocarb.2	106	(9)	0.9963	1.8	86	71
Halofenozide.1	108	(8)	0.9958	3.2	84	64
Halofenozide.2	105	(12)	0.9914	3.7	84	63
Hexaflumuron.1	99	(12)	0.9937	5.6	95	91
Hexaflumuron.2	102	(10)	0.9968	7.7	95	89
Hexythiazox.1	101	(5)	0.9988	1.5	91	80
Hexythiazox.2	103	(5)	0.9991	1.6	91	82
Hydramethylnon.1	106	(8)	0.9981	2.3	89	76
Hydramethylnon.2	105	(9)	0.9978	2.2	93	81
Imazalil.1	103	(8)	0.9975	1.6	93	79
Imazalil.2	103	(8)	0.9985	3.3	92	79
Imidacloprid.1	108	(10)	0.9949	2.7	90	77
Imidacloprid.2	105	(10)	0.9971	1.9	86	72
Indoxacarb.1	107	(12)	0.9949	6.4	93	83
Indoxacarb.2	101	(8)	0.997	3.6	90	82
Ipconazole.1	101	(8)	0.9962	3.2	93	84
Ipconazole.2	103	(10)	0.9965	3.1	93	84
Iprovalicarb.1	107	(10)	0.9963	1.8	80	66
Iprovalicarb.2	106	(8)	0.9975	2.9	89	73
Isoprocarb.1	101	(9)	0.9976	2.5	92	79
Isoprocarb.2	102	(7)	0.9993	1.9	89	74
Isoproturon.1	111	(11)	0.9958	3	84	67
Isoproturon.2	105	(10)	0.9975	2.7	85	70
Isoxaflutole.1	97	(4)	0.9994	11	100	105
Isoxaflutole.2	112	(15)	0.9921	nr[b]	125	113
Isoxaflutole+NH_4.1	104	(16)	0.9929	7.2	100	95
Isoxaflutole+NH_4.2	109	(25)	0.9513	5.3	115	88
Ivermectin+Na.3	103	(6)	0.9978	2	95	89
Ivermectin+Na.4	101	(7)	0.9987	5.5	88	83
Ivermectin+NH_4.1	100	(5)	0.9982	4.4	96	103
Ivermectin+NH_4.2	98	(12)	0.9966	7.5	94	90
Kresoxim:methyl.1	106	(11)	0.9947	4.1	86	71
Kresoxim:methyl.2	105	(9)	0.9968	4.4	86	68
Linuron.1	107	(9)	0.9969	3.2	77	60
Linuron.2	110	(11)	0.9969	2.7	80	61
Lufenuron.1	103	(8)	0.9953	3.1	94	88
Lufenuron.2	103	(8)	0.9967	2.9	94	86
Malathion.1	115	(14)	0.9962	4.0	75	55
Malathion.2	112	(12)	0.9972	2.1	74	57
Mandipropamide.1	107	(10)	0.9964	2.8	84	67

dx.doi.org/10.1021/jf201618q |*J. Agric. Food Chem.* 2011, 59, 6383–6411

Table 5. Continued

	accuracy		linearity	LOD	extended range	
transition	av	(RSD)	av r^2	ng/mL	500 ng/mL	1000 ng/mL
Mandipropamide.2	105	(8)	0.9974	2.1	84	69
Mepanipyrim.1	106	(10)	0.9982	2.2	83	67
Mepanipyrim.2	109	(10)	0.996	2.6	85	67
Metaflumizone.1	102	(7)	0.9983	3.5	98	89
Metaflumizone.2	101	(7)	0.997	3.3	98	94
Metalaxyl.1	108	(11)	0.9968	2.4	82	63
Metalaxyl.2	108	(9)	0.9976	2.6	86	66
Metconazole.1	105	(8)	0.9983	2.7	89	76
Metconazole.2	104	(10)	0.9959	4.3	93	76
Methamidophos.1	105	(6)	0.9986	1.4	89	78
Methamidophos.2	104	(6)	0.9988	1.5	88	78
Methiocarb.1	107	(14)	0.9951	2.1	83	68
Methiocarb.2	111	(11)	0.9959	3.8	82	64
Methomyl.1	103	(7)	0.9984	1.2	92	74
Methomyl.2	103	(9)	0.9977	2.6	89	72
Methoxyfenozide.1	106	(8)	0.9965	2.9	84	69
Methoxyfenozide.2	108	(10)	0.9957	2.5	89	73
Metobromuron.1	103	(8)	0.9987	1.8	83	65
Metobromuron.2	105	(11)	0.9968	2.6	82	65
Mevinphos-E.1	105	(8)	0.9974	1.6	89	74
Mevinphos-E.2	103	(6)	0.998	1.2	90	76
Mevinphos-Z.1	105	(11)	0.9954	2.8	86	72
Mevinphos-Z.2	105	(7)	0.9981	2.2	89	74
Mexacarbate.1	107	(10)	0.9976	1.6	84	67
Mexacarbate.2	110	(12)	0.997	3.5	84	68
Monocrotophos.1	105	(7)	0.9987	1.7	87	72
Monocrotophos.2	107	(9)	0.997	1.5	91	78
Monolinuron.1	106	(10)	0.997	2.3	87	73
Monolinuron.2	103	(8)	0.9985	2.7	89	73
Moxidectin.1	102	(9)	0.9969	2.4	92	97
Moxidectin.2	101	(12)	0.9903	5.3	97	93
Moxidectin+Na.3	103	(10)	0.9968	4.2	85	75
Moxidectin+Na.4	103	(12)	0.9946	4.6	87	73
Myclobutanil.1	107	(10)	0.9962	1.8	90	77
Myclobutanil.2	106	(10)	0.9967	3	90	79
Neburon.1	111	(10)	0.9967	1.9	81	63
Neburon.2	111	(12)	0.9954	2.1	79	62
Nitenpyram.1	104	(9)	0.9975	2.6	92	77
Nitenpyram.2	107	(8)	0.997	5.1	90	76
Novaluron.1	101	(7)	0.9978	2.6	93	90
Novaluron.2	98	(10)	0.995	5.8	93	84
Nuarimol.1	105	(10)	0.9953	3.1	92	83
Nuarimol.2	104	(7)	0.9982	5.6	94	81
Omethoate.1	105	(8)	0.998	1.2	87	71
Omethoate.2	103	(9)	0.9978	1.9	87	70
Oxadixyl.1	102	(6)	0.9992	1.5	92	78
Oxadixyl.2	101	(8)	0.9987	1.5	94	85
Oxamyl+NH_4.1	105	(9)	0.9967	2.8	87	72
Oxamyl+NH_4.2	106	(8)	0.9969	3.1	90	76
Paclobutrazol.1	105	(8)	0.9974	2.1	89	74
Paclobutrazol.2	105	(8)	0.9961	3.9	92	78
Pencycuron.1	109	(11)	0.9962	1.6	81	60
Pencycuron.2	107	(9)	0.9973	2.9	88	71
Phenmedipham.1	110	(15)	0.9928	2.3	89	76
Phenmedipham.2	105	(8)	0.998	2.5	90	74

dx.doi.org/10.1021/jf201618q |*J. Agric. Food Chem.* 2011, 59, 6383–6411

Table 5. Continued

	accuracy		linearity	LOD	extended range	
transition	av	(RSD)	av r^2	ng/mL	500 ng/mL	1000 ng/mL
PhorateSulfone.1	107	(7)	0.9988	2.3	87	64
PhorateSulfone.2	106	(7)	0.9983	2.4	87	67
Picoxystrobin.1	104	(10)	0.9962	3.5	87	75
Picoxystrobin.2	105	(15)	0.997	2.6	83	65
PiperonylButox+NH_4.1	107	(8)	0.9972	1.8	86	68
PiperonylButox+NH_4.2	104	(7)	0.9987	2	89	76
Pirimicarb.1	111	(13)	0.996	1.9	84	65
Pirimicarb.2	105	(8)	0.9985	1.7	89	72
Prochloraz.1	105	(8)	0.9981	2.7	88	75
Prochloraz.2	104	(8)	0.998	2.1	90	76
Promecarb.1	104	(8)	0.9982	1.5	84	65
Promecarb.2	108	(10)	0.9967	1.5	83	68
Propamocarb.1	105	(11)	0.9974	2.6	83	68
Propamocarb.2	102	(8)	0.9988	1.7	91	76
Propargite+NH_4.1	108	(12)	0.9973	1.6	81	65
Propargite+NH_4.2	108	(9)	0.9975	2.4	87	72
Propiconazole.1	105	(8)	0.9977	1.8	90	76
Propiconazole.2	107	(8)	0.9972	3.9	90	75
Propoxur.1	109	(12)	0.9968	3.1	85	70
Propoxur.2	109	(11)	0.9962	2.2	85	71
Pymetrozine.1	107	(11)	0.9971	2.8	87	70
Pymetrozine.2	101	(8)	0.9977	3.5	90	75
Pyracarbolid.1	107	(6)	0.9984	1.6	89	72
Pyracarbolid.2	105	(8)	0.9987	2	90	76
Pyraclostrobin.1	107	(13)	0.9949	3	81	63
Pyraclostrobin.2	105	(9)	0.9979	3.1	81	67
Pyridaben.1	108	(12)	0.9975	2	88	77
Pyridaben.2	103	(5)	0.9991	1.7	90	73
Pyrimethanil.1	100	(11)	0.9961	2.7	94	85
Pyrimethanil.2	104	(8)	0.996	4.5	101	92
Pyriproxyfen.1	108	(9)	0.9973	1.6	80	63
Pyriproxyfen.2	107	(8)	0.9972	2.4	86	70
Rotenone.1	103	(6)	0.9984	2.6	91	81
Rotenone.2	103	(5)	0.999	3.7	92	81
Siduron.1	108	(10)	0.9969	3	87	74
Siduron.2	109	(11)	0.9939	1.9	86	70
SpinetoramA.1	105	(9)	0.9971	2	91	81
SpinetoramA.2	100	(7)	0.9965	1.7	92	83
SpinetoramB.1	101	(8)	0.9981	2.9	96	90
SpinetoramB.2	98	(7)	0.9987	3.6	98	94
SpinosynA.1	107	(11)	0.9952	3.2	89	78
SpinosynA.2	101	(9)	0.9973	3.9	91	80
Spirodiclofen.1	97	(9)	0.9982	2.6	96	93
Spirodiclofen.2	101	(9)	0.9962	3.3	97	85
Spiromesifen.1	101	(15)	0.9952	3	87	79
Spiromesifen.2	106	(13)	0.9943	3.3	93	80
Spiromesifen+NH_4.1	105	(15)	0.9952	2.3	102	89
Spiromesifen+NH_4.2	109	(17)	0.993	5.3	98	84
Spirotetramat.1	103	(8)	0.9969	3.6	96	88
Spirotetramat.2	103	(9)	0.9966	2	99	89
Spiroxamine.1	107	(9)	0.9975	2.4	83	73
Spiroxamine.2	106	(8)	0.9986	1.7	86	79
Sulfentrazone.1	101	(12)	0.9907	8.2	97	88
Sulfentrazone.2	100	(10)	0.9955	8.2	95	93
Tebuconazole.1	101	(6)	0.9988	2.2	96	79

dx.doi.org/10.1021/jf201618q |*J. Agric. Food Chem.* 2011, 59, 6383–6411

Table 5. Continued

	accuracy		linearity	LOD	extended range	
transition	av	(RSD)	av r^2	ng/mL	500 ng/mL	1000 ng/mL
Tebuconazole.2	102	(11)	0.9937	3.9	95	83
Tebufenozide.1	105	(10)	0.9951	3.5	91	74
Tebufenozide.2	104	(8)	0.9981	3.1	87	68
Tebuthiuron.1	109	(12)	0.9958	2.1	83	65
Tebuthiuron.2	110	(11)	0.9961	2.3	88	68
Teflubenzuron.1	103	(10)	0.9966	7	95	85
Teflubenzuron.2	107	(11)	0.995	3.9	92	85
Temephos.1	102	(7)	0.9969	2.1	95	81
Temephos.2	103	(8)	0.9988	2	91	84
Thiabendazole.1	104	(9)	0.9973	1.4	83	69
Thiabendazole.2	106	(9)	0.997	2.1	89	73
Thiacloprid.1	109	(11)	0.997	1.6	83	65
Thiacloprid.2	103	(7)	0.9975	2.7	88	72
Thiamethoxam.1	104	(7)	0.9983	2.8	91	75
Thiamethoxam.2	107	(10)	0.9965	2.7	92	80
Thidiazuron.1	102	(10)	0.9969	1.5	90	80
Thidiazuron.2	108	(13)	0.9928	6.9	95	85
Thiophanate-methyl.1	110	(13)	0.9958	1.9	80	63
Thiophanate-methyl.2	107	(11)	0.9961	2.4	86	70
Triadimefon.1	109	(12)	0.9945	3.2	100	90
Triadimefon.2	105	(11)	0.9981	2.4	92	76
Triadimenol.1	104	(9)	0.9968	4.4	96	83
Triadimenol.2	106	(21)	0.9885	5.2	97	88
Trichlorfon.1	106	(11)	0.9965	2.1	90	81
Trichlorfon.2	104	(8)	0.9982	1.5	90	80
Tricyclazole.1	104	(8)	0.9981	2.1	88	70
Tricyclazole.2	104	(8)	0.998	1.3	85	67
Trifloxystrobin.1	104	(9)	0.9958	2.7	83	66
Trifloxystrobin.2	105	(11)	0.9968	2.9	91	71
Triflumizole.1	110	(10)	0.9962	2.3	86	64
Triflumizole.2	108	(9)	0.9973	2.8	91	74
Triflumuron.1	105	(10)	0.9952	3.9	88	70
Triflumuron.2	105	(9)	0.9969	4.4	83	71
Triticonazole.1	103	(10)	0.9953	2.6	92	80
Triticonazole.2	101	(10)	0.9968	9.1	97	84
Vamidothion.1	110	(10)	0.9966	2.6	78	62
Vamidothion.2	106	(8)	0.9976	2.8	84	68
Zoxamide.1	105	(13)	0.9945	2.9	82	66
Zoxamide.2	108	(10)	0.9964	6.4	87	71

[a] Accuracy: average accuracies and RSDs of standards at 50, 100, and 200 ng/mL levels. Linearity: average determination coefficients (r^2) from linear regression analyses of standards at concentrations 2, 5, 10, 20, 50, 100, and 200 ng/mL. LOD: average limit of detection statistically calculated from standard responses for the 2, 5, and 10 ng/mL levels. Extended range: average accuracies of levels exceeding the calibration level of 200 ng/mL. [b] Not reported due to insufficient data.

As a result of the MP evaluation, column comparison, and standard composition studies, the acidic formate MP and the columns listed in Materials and Methods were selected for validation, and standards were prepared in CH_3OH. Each lab was instructed to modify their MP composition programs to optimize chromatographic separation and resolution of the full standard mix of over 200 compounds.

LC−MS/MS Method Validation Using Standards in Solvent. Once the LC−MS/MS determination method was developed, it was validated in this first part of the project. All seven laboratories collaborated in the validation contributing over 40,000 records for evaluation. Summary results are in Table 5. Figure 1 contains extracted ion chromatograms of all analytes from one of the laboratories. Extracted ion chromatograms from the other laboratories are in the Supporting Information. So, 175 analytes are reported. They consist of 372 transitions, 11 with multiple adducts (avermectin, doramectin, eprinomectin, ivermectin, moxidectin, and spiromesifen) or multiple components (cyproconazole, bromuconazole, dimethomorph, and spinetoram).

Overall, the data indicate that the LC−MS/MS procedure is accurate, precise, linear, sensitive, and rugged. The performances of two compounds, bentazon and amitraz, were sporadic and

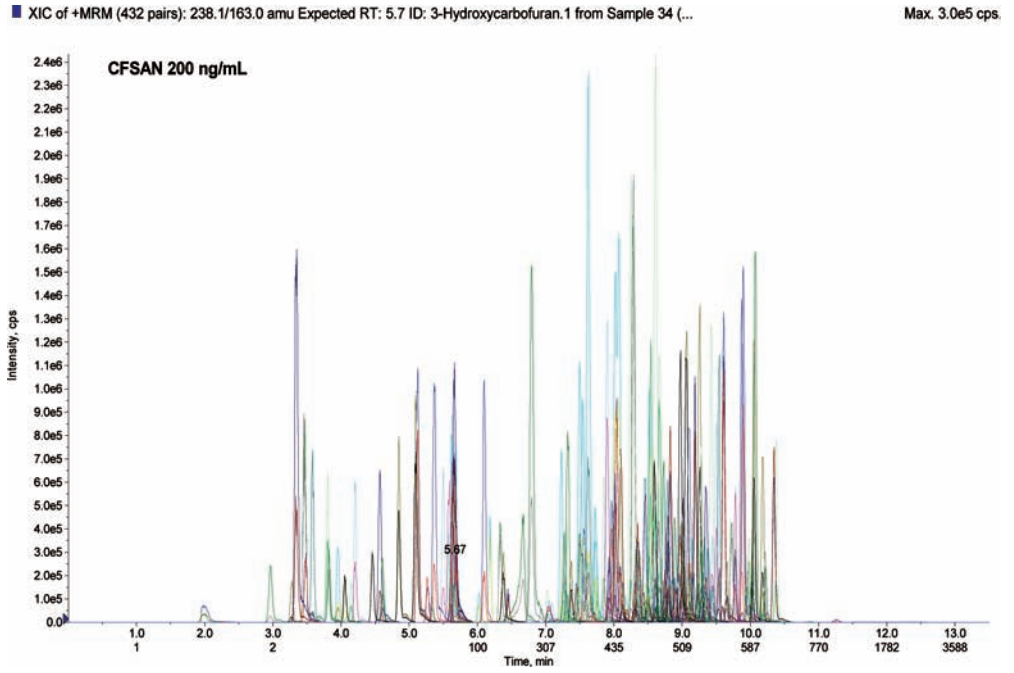

Figure 1. Extracted ion chromatogram of the mixture of standards (200 ng/mL) in methanol.

unreliable. Bentazon would be better suited using negative ionization, and amitraz is simply too unstable in solution. For the remainder of the report the results for these compounds will be excluded, and the summary statistical analyses refer to the 173 remaining analytes, or 368 transitions.

The performances of some weaker transitions were less desirable, as expected. Many of the weaker transitions were sodium and ammonium adducts that could be used as an alternative if they produce sufficient response in the individual laboratory. Some of the weak transitions can be analyzed separately in negative ion mode, a procedure that has not been finalized.

The overall average accuracy for all transitions reported as a percentage of the nominal standard concentration was 105% with an RSD of 3%. All were within 95−115% and only 17 of 368 transitions evaluated were above 110%. The reproducibility of the method was measured by the RSD of the accuracy data for each transition. 357 of 368 transitions reported had RSDs ≤ 15%, i.e. only 11 exceeded 15%; and all RSDs were ≤25%.

Linearity was determined for each transition in each laboratory by calculating the determination coefficients (r^2) for the standard curve responses. The average r^2 exceeded 0.95 for all transitions and 0.99 for 364 of 368 transitions reported. Only one transition, the weaker transition of isoxaflutole ammonium adduct, had a minimum r^2 of less than 0.95. The weaker transition for isoxaflutole faired poorly both as the molecular ion and the ammonium adduct, however the stronger transition performed acceptably.

The LODs for 363 of 368 transitions were <10 ng/mL and <5 ng/mL for 331 transitions; none exceeded 14 ng/mL. The LOD of the weaker transition of isoxaflutole was not determined because insufficient data was available. As expected, the three transitions with LODs >10 ng/mL were all mectins, i.e. the ammonium adducts of avermectin B1 and both doramectins. As a group, the mectins proved to be more sensitive than expected, partially because some laboratories were able to increase sensitivity by analyzing the corresponding sodium adduct transition ions.

The extended range was determined by analyzing the 500 and 1000 ng/mL standards, corresponding to 250 and 500% of the calibration level. The overall average accuracies for the 500 and 1000 ng/mL levels were excellent at 89% (range 74−125%) and 76% (range 55−122%), respectively. The data indicates the procedure is linear at 250% of the calibration level of 200 ng/mL; and it provides a good estimate at concentrations of 500% of the calibration level.

The ruggedness of the LC−MS/MS determination, slightly modified to include calibration by matrix matched standards, was demonstrated by the results of two proficiency potato and cucumber samples from AOAC that were analyzed by the PRL-NW lab (Table 6).

Matrix Effect Study. The suppression effects of matrix on ionization when using LC−MS/MS determination is well-known. One strategy to minimize the matrix effects is to dilute the sample to reduce the total matrix in the ionization chamber. Of course, the analyte is also diluted, and for trace level residue analysis the resulting loss of analyte response can become a factor. Prior to validation, the effect of the three matrices was evaluated by comparing the response of standards prepared in CH_3OH to those prepared in QuEChERS matrix extracts at 0.2 and 0.5 g of sample per mL. Results for 362 transitions are reported in Table 7. In this table the response ratios (R) represent the response of transitions in matrix calculated as a percentage of the response in solvent, therefore R_{500} is the response of the standard in matrix extract at 500 mg sample/mL; R_{200} is the standard response at 200 mg sample/mL. Δ_{Eff} is the difference ($R_{500} - R_{200}$) calculated for each transition/matrix combination. A negative Δ_{Eff} is indicative of the matrix suppression associated with the greater sample concentration. The overall difference (Δ_{Eff}) of −4% clearly indicates the advantage of minimizing matrix effects by diluting the matrix from 500 to 200 mg/mL does not overcome the loss of sensitivity associated

Table 6. Ruggedness of the LC−MS/MS Determination in CH_3OH, Slightly Modified To Include Calibration by Matrix Matched Standards, as Demonstrated by the Results of Two Proficiency Samples Analyzed by the PRL-NW Lab

	concentration (ng/g)			
pesticide	found	assigned	median	*Z* score
	AOAC Potato Sample, February 2010			
3-Hydroxycarbofuran	215	175	198	1.14
Aldicarb Sulfoxide	251	300	289	−0.82
Propoxur	221	215	159	0.14
	AOAC Cucumber Sample, October 2009			
Methamidophos	197	221	173	−0.53
Mevinphos	160	195	170	−0.9
3-Hydroxycarbofuran	374	289	307	1.47
Carbaryl	276	231	233	0.97
Oxamyl	265	245	236	0.42

Table 7. Matrix Effect Study: Percent Recoveries of Standards Spiked into Sample Extracts at Two Matrix Levels Determined by Calibration Using Standards in Methanol[a]

	orange			carrot			spinach		
transition	R_{500}	R_{200}	Δ_{Eff}	R_{500}	R_{200}	Δ_{Eff}	R_{500}	R_{200}	Δ_{Eff}
3-Hydroxycarbofuran.1	91	102	−11	101	103	−1	105	106	−1
3-Hydroxycarbofuran.2	91	101	−10	101	102	−1	105	106	−1
Acephate.1	102	105	−3	102	106	−4	106	108	−1
Acephate.2	99	106	−7	101	103	−2	105	109	−4
Acetamiprid.1	90	102	−12	102	104	−2	106	109	−2
Acetamiprid.2	90	103	−12	100	103	−3	106	108	−2
Acibenzolar-S-methyl.1	88	96	−8	105	114	−9	113	115	−3
Acibenzolar-S-methyl.2	85	100	−15	102	105	−3	107	112	−4
Alanycarb.1	75	96	−21	90	107	−17	91	103	−11
Alanycarb.2	68	97	−29	91	103	−13	91	105	−14
Aldicarb+NH_4.1	102	102	0	101	102	−1	103	108	−5
Aldicarb+NH_4.2	97	101	−5	102	106	−4	99	104	−5
AldicarbSulfoxide.1	93	101	−8	101	106	−6	110	108	2
AldicarbSulfoxide.2	93	104	−11	102	105	−2	108	108	0
Aldoxycarb.1	99	113	−14	112	109	3	109	113	−4
Aldoxycarb.2	100	103	−4	108	105	3	111	115	−4
Aminocarb.1	101	105	−4	104	105	0	107	108	−1
Aminocarb.2	101	104	−4	103	107	−3	108	108	0
AvermectinB1a+Na.3	70	84	−14	77	79	−3	87	88	−1
AvermectinB1a+Na.4	76	85	−9	73	79	−5	79	90	−11
AvermectinB1a+NH_4.1	103	109	−6	112	93	19	128	124	4
AvermectinB1a+NH_4.2	108	110	−2	101	101	−1	115	116	−1
Azoxystrobin.1	83	91	−8	102	103	−2	106	106	−1
Azoxystrobin.2	82	93	−11	104	106	−1	109	110	−1
BDMC.1	71	103	−32	114	104	10	124	131	−7
BDMC.2	69	85	−16	90	108	−18	100	109	−9
Benalaxyl.1	91	100	−9	99	105	−5	108	107	2
Benalaxyl.2	88	98	−10	98	102	−5	109	108	0
Bendiocarb.1	99	103	−4	105	106	−2	119	112	7
Bendiocarb.2	90	101	−10	101	104	−3	105	106	−1
Benfuracarb.1	127	107	19	129	137	−8	129	120	9
Benfuracarb.2	126	110	16	128	132	−3	129	121	7
Benzoximate.1	88	91	−3	92	101	−9	105	107	−2

Table 7. Continued

	orange			carrot			spinach		
transition	R_{500}	R_{200}	Δ_{Eff}	R_{500}	R_{200}	Δ_{Eff}	R_{500}	R_{200}	Δ_{Eff}
Benzoximate.2	88	96	−7	94	105	−11	108	110	−2
Bifenazate.1	57	72	−15	102	107	−5	104	106	−3
Bifenazate.2	65	79	−14	104	106	−3	105	107	−2
Bitertanol.1	97	105	−8	102	105	−3	108	104	4
Bitertanol.2	115	102	13	136	103	33	105	95	10
Boscalid.1	93	103	−10	101	104	−3	108	107	1
Boscalid.2	94	104	−10	104	105	−1	111	116	−5
BromuconazoleA.1	62	72	−10	98	101	−3	105	104	1
BromuconazoleA.2	67	78	−10	95	96	−1	106	107	0
BromuconazoleB.1	89	98	−9	95	105	−9	106	107	−1
BromuconazoleB.2	89	100	−11	95	102	−8	106	108	−1
Bupirimate.1	65	83	−19	100	105	−5	107	109	−2
Bupirimate.2	63	79	−16	98	103	−5	107	109	−2
Buprofezin.1	98	103	−6	97	102	−5	106	107	−1
Buprofezin.2	97	103	−7	96	99	−3	105	106	−1
Butafenacil+NH_4.1	81	92	−10	106	106	0	112	110	2
Butafenacil+NH_4.2	75	86	−12	104	105	−1	108	109	−1
Butocarboxim+Na.1	81	96	−16	98	101	−3	91	94	−3
Butocarboxim+Na.2	95	101	−6	110	102	8	97	111	−14
Butoxycarboxim.1	97	107	−9	104	105	−2	107	109	−2
Butoxycarboxim.2	90	112	−22	110	124	−14	101	110	−9
Carbaryl.1	94	102	−9	100	105	−5	106	107	−1
Carbaryl.2	92	102	−10	100	104	−4	104	108	−3
Carbendazim.1	81	85	−4	95	93	2	101	100	1
Carbendazim.2	78	84	−6	96	92	4	100	101	−1
Carbetamide.1	102	106	−4	100	103	−2	110	109	1
Carbetamide.2	103	108	−4	102	102	0	111	111	1
Carbofuran.1	88	95	−7	98	99	−1	101	103	−2
Carbofuran.2	88	97	−9	98	100	−2	102	103	−1
Chlorantraniliprole.1	80	89	−9	103	104	0	114	116	−2
Chlorantraniliprole.2	79	89	−10	104	102	2	115	117	−2
Chlorfluazuron.1	104	105	−2	96	100	−4	111	111	0
Chlorfluazuron.2	100	105	−5	96	103	−7	106	111	−5
Chlorotoluron.1	92	102	−10	103	106	−3	108	108	0
Chlorotoluron.2	88	100	−13	103	105	−3	106	108	−2
Chloroxuron.1	67	79	−13	102	105	−3	109	107	2
Chloroxuron.2	60	74	−13	101	101	1	108	108	1
Clethodim.1	105	104	1	102	100	2	116	107	9
Clethodim.2	105	104	0	98	100	−2	115	109	6
Clofentezine.1	101	108	−7	87	97	−10	112	111	1
Clofentezine.2	98	107	−9	85	97	−13	109	110	−1
Clothianidin.1	121	123	−1	103	106	−3	104	111	−6
Clothianidin.2	122	121	1	103	104	−1	107	110	−2
Cyazofamid.1	87	100	−12	104	104	0	110	110	0
Cyazofamid.2	116	129	−12	129	146	−17	142	143	−1
Cycluron.1	89	99	−10	98	103	−5	102	105	−3
Cycluron.2	90	100	−9	101	104	−4	103	107	−4
Cymoxanil.1	91	106	−15	106	111	−5	109	111	−2
Cymoxanil.2	89	107	−19	105	112	−7	108	113	−5
CyproconazoleA.1	76	86	−9	100	103	−2	107	109	−3
CyproconazoleA.2	77	104	−27	102	104	−3	105	108	−3
CyproconazoleB.1	59	75	−15	98	105	−7	107	109	−2
CyproconazoleB.2	62	86	−24	115	107	8	106	109	−3

dx.doi.org/10.1021/jf201618q |J. Agric. Food Chem. 2011, 59, 6383–6411

Table 7. Continued

	orange			carrot			spinach		
transition	R_{500}	R_{200}	Δ_{Eff}	R_{500}	R_{200}	Δ_{Eff}	R_{500}	R_{200}	Δ_{Eff}
Cyprodinil.1	86	99	−13	95	101	−6	108	107	0
Cyprodinil.2	85	99	−14	96	103	−7	108	106	2
Cyromazine.1	64	73	−9	73	92	−19	61	71	−9
Cyromazine.2	61	71	−10	69	88	−19	59	68	−9
Desmedipham+NH_4.1	95	103	−8	104	108	−4	110	110	1
Desmedipham+NH_4.2	97	103	−6	100	103	−3	103	106	−3
Diclobutrazol.1	78	87	−9	98	104	−7	108	107	1
Diclobutrazol.2	79	87	−8	95	102	−7	95	107	−11
Dicrotophos.1	95	102	−7	102	104	−2	106	109	−3
Dicrotophos.2	95	104	−9	101	104	−3	107	108	−1
Diethofencarb.1	94	102	−7	102	103	−2	107	107	0
Diethofencarb.2	94	102	−8	103	104	−1	109	109	0
Difenoconazole.1	103	104	−2	102	102	0	106	107	−1
Difenoconazole.2	102	105	−2	102	104	−2	108	108	0
Diflubenzuron.1	64	79	−15	103	107	−5	111	109	2
Diflubenzuron.2	63	79	−16	100	107	−7	109	110	−1
Dimethoate.1	87	99	−12	101	104	−3	106	107	−1
Dimethoate.2	86	97	−12	99	102	−3	103	104	−2
DimethomorphA.1	76	78	−2	96	99	−3	110	107	3
DimethomorphA.2	69	79	−10	98	98	0	108	109	−1
DimethomorphB.1	64	64	−1	89	101	−13	120	96	24
DimethomorphB.2	60	64	−4	88	98	−10	108	104	4
Dimoxystrobin.1	76	86	−10	98	103	−5	106	108	−2
Dimoxystrobin.2	74	85	−11	95	101	−6	105	107	−2
Dinotefuran.1	106	109	−3	104	105	−1	107	111	−4
Dinotefuran.2	104	107	−3	97	106	−9	90	122	−32
Dioxacarb.1	82	98	−17	99	106	−7	105	107	−2
Dioxacarb.2	82	99	−17	99	106	−7	104	107	−3
Diuron.1	95	103	−8	101	104	−2	105	107	−2
Diuron-Cl37.2	91	101	−9	99	103	−4	102	108	−6
Doramectin+Na.3	101	107	−6	80	106	−27	98	113	−14
Doramectin+Na.4	101	103	−1	76	104	−27	91	109	−18
Doramectin+NH_4.1	105	97	8	90	101	−11	106	115	−9
Doramectin+NH_4.2	104	106	−2	94	112	−18	110	120	−9
Emamectin.1	108	110	−2	107	109	−3	115	121	−6
Emamectin.2	116	116	0	110	112	−2	115	120	−5
Eprinomectin.1	97	106	−9	112	108	5	122	119	2
Eprinomectin.2	109	104	5	103	107	−4	118	107	11
Eprinomectin+Na.3	94	118	−24	95	120	−25	91	114	−24
Eprinomectin+Na.4	106	124	−18	102	121	−19	97	130	−32
Ethaboxam.1	108	113	−4	101	106	−4	106	108	−2
Ethaboxam.2	109	112	−3	100	104	−4	101	108	−7
Ethiofencarb.1	95	103	−9	102	105	−3	107	108	0
Ethiofencarb.2	94	104	−10	102	104	−2	107	106	1
Ethiprole.1	99	106	−7	106	105	0	110	106	4
Ethiprole.2	97	101	−4	106	104	2	112	108	4
Ethirimol.1	73	84	−11	101	101	−1	110	112	−2
Ethirimol.2	69	82	−13	97	100	−3	101	111	−9
Etoxazole.1	105	107	−2	102	105	−3	108	106	2
Etoxazole.2	102	105	−3	100	103	−3	107	106	2
Famoxadone+NH_4.1	104	107	−3	105	110	−4	110	111	−1
Famoxadone+NH_4.2	101	106	−5	99	110	−11	110	112	−3

Table 7. Continued

	orange			carrot			spinach		
transition	R_{500}	R_{200}	Δ_{Eff}	R_{500}	R_{200}	Δ_{Eff}	R_{500}	R_{200}	Δ_{Eff}
Fenamidone.1	81	92	−11	102	106	−4	107	106	1
Fenamidone.2	79	91	−12	102	105	−3	106	106	0
Fenazaquin.1	95	104	−9	98	104	−6	103	105	−2
Fenazaquin.2	93	106	−13	96	102	−6	101	104	−3
Fenbuconazole.1	84	93	−9	104	104	0	111	112	−1
Fenbuconazole.2	81	92	−11	100	105	−5	108	108	0
Fenhexamid.1	82	91	−9	102	104	−3	107	108	−1
Fenhexamid.2	84	90	−7	104	105	−1	111	110	1
Fenobucarb.1	80	90	−9	101	105	−4	104	107	−3
Fenobucarb.2	82	92	−9	100	104	−4	105	95	10
Fenoxycarb.1	68	83	−15	100	105	−5	106	108	−1
Fenoxycarb.2	67	82	−15	100	102	−2	107	107	0
Fenpyroximate.1	107	108	−1	109	103	6	117	109	8
Fenpyroximate.2	103	102	1	108	103	4	115	109	6
Fenuron.1	86	98	−12	100	104	−4	103	106	−3
Fenuron.2	84	97	−13	100	103	−3	103	105	−2
Flonicamid.1	108	111	−3	103	109	−6	108	110	−1
Flonicamid.2	108	115	−7	103	111	−8	108	110	−2
Flubendiamide.1	68	92	−23	91	90	0	114	111	4
Flubendiamide.2	78	78	0	92	102	−11	121	104	18
Fludioxinil+NH_4.1	83	100	−17	98	106	−8	102	106	−5
Fludioxinil+NH_4.2	84	97	−13	101	104	−3	108	109	−2
Flufenoxuron.1	105	100	4	104	95	9	119	106	12
Flufenoxuron.2	105	101	4	104	96	8	120	106	14
Fluometuron.1	88	98	−10	105	103	2	105	107	−1
Fluometuron.2	88	99	−11	100	104	−5	103	106	−3
Fluoxastrobin.1	62	78	−16	101	103	−3	111	109	2
Fluoxastrobin.2	61	77	−16	98	102	−4	108	109	−1
Flusilazole.1	61	78	−16	96	104	−8	104	108	−4
Flusilazole.2	61	76	−15	96	104	−7	105	107	−2
Flutolanil.1	81	93	−12	101	108	−8	109	104	5
Flutolanil.2	83	96	−13	104	101	3	111	107	3
Flutriafol.1	93	103	−10	99	104	−5	102	106	−4
Flutriafol.2	93	104	−11	100	106	−6	100	106	−6
Forchlorfenuron.1	94	104	−10	101	106	−5	102	110	−8
Forchlorfenuron.2	92	104	−12	97	107	−10	98	108	−10
Formetanate.1	96	104	−8	100	104	−3	105	110	−5
Formetanate.2	108	103	5	96	99	−3	96	115	−19
Fuberidazole.1	81	95	−15	99	103	−4	104	105	−1
Fuberidazole.2	77	93	−16	98	101	−3	103	104	−1
Furathiocarb.1	106	104	2	106	102	4	119	109	10
Furathiocarb.2	102	104	−1	103	102	1	110	107	3
Halofenozide.1	89	99	−9	101	105	−4	115	112	3
Halofenozide.2	87	96	−9	101	104	−4	108	110	−2
Hexaflumuron.1	113	111	2	97	99	−2	109	107	2
Hexaflumuron.2	102	107	−5	96	101	−5	110	99	11
Hexythiazox.1	101	102	−1	96	96	0	113	105	7
Hexythiazox.2	99	102	−3	94	98	−3	111	105	7
Hydramethylnon.1	94	102	−8	100	105	−5	111	113	−2
Hydramethylnon.2	97	102	−5	99	107	−8	108	110	−2
Imazalil.1	94	102	−8	100	105	−6	105	106	−1
Imazalil.2	94	103	−8	99	103	−4	106	106	−1

Table 7. Continued

	orange			carrot			spinach		
transition	R_{500}	R_{200}	Δ_{Eff}	R_{500}	R_{200}	Δ_{Eff}	R_{500}	R_{200}	Δ_{Eff}
Imidacloprid.1	139	126	13	101	104	−3	119	117	2
Imidacloprid.2	139	127	12	103	106	−3	119	117	2
Indoxacarb.1	98	109	−10	100	99	2	106	106	1
Indoxacarb.2	102	104	−3	102	100	2	110	109	0
Ipconazole.1	101	103	−3	99	98	1	106	105	2
Ipconazole.2	99	104	−5	101	102	−1	108	107	1
Iprovalicarb.1	85	96	−11	101	103	−2	103	107	−4
Iprovalicarb.2	86	95	−9	101	103	−3	107	106	1
Isoprocarb.1	99	104	−5	101	105	−4	107	108	−1
Isoprocarb.2	94	103	−8	102	105	−3	108	108	0
Isoproturon.1	95	103	−8	99	103	−4	102	104	−2
Isoproturon.2	95	103	−8	102	105	−4	104	104	0
Isoxaflutole.1	109	118	−9	116		116	115	111	4
Isoxaflutole.2	116	124	−8	137		137	114	112	2
Isoxaflutole+NH_4.1	114	117	−3	85	103	−18	94	108	−13
Isoxaflutole+NH_4.2	107	107	0	87	104	−17	96	101	−5
Ivermectin+Na.3	96	111	−15	78	99	−21	73	100	−27
Ivermectin+Na.4	106	110	−3	78	105	−26	74	103	−29
Ivermectin+NH_4.1	123	111	12	100	109	−9	115	124	−9
Ivermectin+NH_4.2	127	119	7	97	111	−14	120	124	−4
Kresoxim:methyl.1	77	87	−10	98	99	−1	97	108	−11
Kresoxim:methyl.2	80	90	−10	101	100	1	108	109	−1
Linuron.1	90	99	−10	100	103	−3	105	107	−2
Linuron.2	89	99	−10	98	104	−6	103	107	−4
Lufenuron.1	97	103	−6	100	98	1	108	105	3
Lufenuron.2	96	101	−5	102	96	6	112	104	8
Mandipropamide.1	92	103	−10	107	108	−1	108	108	0
Mandipropamide.2	90	100	−10	107	107	0	113	110	3
Mepanipyrim.1	47	67	−21	97	106	−9	106	107	−1
Mepanipyrim.2	45	65	−21	96	104	−8	105	107	−1
Metaflumizone.1	103	105	−1	104	105	−1	110	108	3
Metaflumizone.2	103	106	−3	102	104	−2	108	109	−1
Metalaxyl.1	95	101	−7	99	101	−2	104	105	−1
Metalaxyl.2	95	102	−7	99	101	−2	104	105	−1
Metconazole.1	92	100	−9	100	103	−3	108	110	−2
Metconazole.2	91	99	−8	99	105	−6	106	109	−3
Methamidophos.1	97	106	−9	99	107	−8	97	104	−8
Methamidophos.2	98	106	−8	100	106	−6	97	103	−6
Methiocarb.1	89	100	−11	102	103	−1	106	108	−2
Methiocarb.2	86	96	−11	99	101	−2	105	107	−2
Methomyl.1	90	99	−8	101	104	−4	105	105	0
Methomyl.2	89	98	−9	102	101	0	106	106	−1
Methoxyfenozide.1	70	80	−10	102	102	0	106	110	−5
Methoxyfenozide.2	69	78	−9	102	97	5	103	106	−3
Metobromuron.1	93	101	−9	100	105	−5	105	107	−2
Metobromuron.2	93	104	−10	103	105	−2	107	107	0
Mevinphos-E.1	79	93	−14	99	103	−4	105	106	−1
Mevinphos-E.2	80	94	−14	101	103	−2	105	107	−2
Mevinphos-Z.1	82	96	−14	99	102	−4	100	103	−4
Mevinphos-Z.2	82	96	−14	100	104	−4	105	107	−1
Mexacarbate.1	91	101	−10	99	102	−3	94	105	−10
Mexacarbate.2	90	100	−10	100	103	−3	100	104	−4

Table 7. Continued

	orange			carrot			spinach		
transition	R_{500}	R_{200}	Δ_{Eff}	R_{500}	R_{200}	Δ_{Eff}	R_{500}	R_{200}	Δ_{Eff}
Monocrotophos.1	95	105	−10	102	104	−2	107	108	−1
Monocrotophos.2	95	103	−8	101	104	−3	105	108	−3
Monolinuron.1	92	101	−9	101	104	−3	107	108	−1
Monolinuron.2	93	102	−10	101	104	−3	107	107	0
Moxidectin.1	107	111	−4	97	104	−7	101	112	−11
Moxidectin.2	97	105	−7	88	101	−13	96	100	−4
Moxidectin+Na.3	96	113	−17	88	116	−29	92	115	−24
Moxidectin+Na.4	97	112	−15	81	114	−33	87	111	−24
Myclobutanil.1	60	75	−15	101	103	−2	105	109	−4
Myclobutanil.2	61	75	−14	100	105	−5	109	109	1
Neburon.1	67	76	−10	94	105	−11	107	108	−1
Neburon.2	64	74	−10	93	102	−8	107	107	0
Nitenpyram.1	95	105	−9	101	104	−3	94	107	−13
Nitenpyram.2	98	107	−8	101	103	−2	94	105	−11
Novaluron.1	99	104	−5	94	97	−2	109	107	2
Novaluron.2	104	106	−1	100	98	2	111	110	1
Nuarimol.1	95	102	−6	99	105	−6	106	108	−2
Nuarimol.2	93	100	−7	101	105	−4	107	106	1
Omethoate.1	101	106	−5	104	106	−2	106	110	−4
Omethoate.2	98	105	−8	103	106	−3	107	108	−1
Oxadixyl.1	96	104	−8	99	103	−5	103	106	−3
Oxadixyl.2	97	104	−8	100	103	−3	106	108	−2
Oxamyl+NH_4.1	93	103	−10	104	106	−3	96	110	−14
Oxamyl+NH_4.2	89	103	−13	100	101	−1	95	111	−17
Paclobutrazol.1	82	92	−10	104	108	−3	109	111	−2
Paclobutrazol.2	85	94	−9	103	102	1	126	107	19
Pencycuron.1	94	94	0	101	103	−2	106	102	4
Pencycuron.2	84	93	−9	96	102	−6	108	106	2
Phenmedipham.1	89	101	−12	103	106	−4	94	106	−12
Phenmedipham.2	91	101	−10	104	106	−3	95	105	−10
Picoxystrobin.1	70	86	−16	102	105	−3	104	105	−2
Picoxystrobin.2	67	82	−16	101	103	−1	106	107	−1
PiperonylButox+NH_4.1	102	105	−3	103	104	−1	108	107	1
PiperonylButox+NH_4.2	102	105	−4	104	102	1	108	108	0
Pirimicarb.1	90	99	−9	99	103	−3	104	105	0
Pirimicarb.2	85	98	−13	100	104	−4	102	105	−3
Prochloraz.1	96	101	−6	97	102	−5	108	109	−1
Prochloraz.2	97	101	−5	96	102	−7	107	108	−1
Promecarb.1	81	93	−11	99	103	−4	104	105	−1
Promecarb.2	82	93	−11	100	104	−4	108	106	1
Propamocarb.1	102	109	−7	104	101	2	112	108	4
Propamocarb.2	105	104	0	101	102	−1	111	103	8
Propargite+NH_4.1	101	104	−3	98	101	−4	108	105	3
Propargite+NH_4.2	101	104	−3	96	100	−4	109	105	4
Propiconazole.1	94	101	−8	99	104	−4	108	108	−1
Propiconazole.2	96	102	−6	98	104	−5	109	109	0
Propoxur.1	89	101	−11	101	104	−3	104	107	−3
Propoxur.2	89	100	−11	100	103	−3	104	107	−3
Pymetrozine.1	111	113	−2	104	107	−3	107	108	0
Pymetrozine.2	111	115	−4	102	107	−4	106	109	−3
Pyracarbolid.1	90	99	−9	100	104	−3	105	105	0
Pyracarbolid.2	87	100	−12	100	104	−4	105	106	−1

dx.doi.org/10.1021/jf201618q |J. Agric. Food Chem. 2011, 59, 6383–6411

Table 7. Continued

	orange			carrot			spinach		
transition	R_{500}	R_{200}	Δ_{Eff}	R_{500}	R_{200}	Δ_{Eff}	R_{500}	R_{200}	Δ_{Eff}
Pyraclostrobin.1	88	97	−8	97	101	−4	107	105	2
Pyraclostrobin.2	89	97	−8	98	102	−4	108	107	1
Pyridaben.1	101	105	−5	105	103	2	111	107	4
Pyridaben.2	97	103	−5	101	101	0	108	104	3
Pyrimethanil.1	96	102	−7	98	103	−5	105	106	−1
Pyrimethanil.2	97	104	−7	101	105	−4	106	108	−2
Pyriproxyfen.1	95	102	−8	95	101	−5	105	106	0
Pyriproxyfen.2	95	104	−9	94	100	−6	106	108	−2
Rotenone.1	76	87	−11	100	109	−9	115	113	2
Rotenone.2	69	81	−12	98	109	−12	110	111	0
Siduron.1	88	105	−17	109	105	4	120	121	−2
Siduron.2	84	102	−18	103	108	−5	113	106	7
SpinetoramA.1	88	93	−6	96	95	1	105	106	−1
SpinetoramA.2	96	100	−4	97	100	−3	114	109	5
SpinetoramB.1	95	101	−6	94	101	−7	103	105	−3
SpinetoramB.2	97	104	−8	95	101	−5	103	106	−3
SpinosynA.1	83	92	−10	104	105	−1	113	113	0
SpinosynA.2	86	93	−7	106	103	3	112	111	1
Spirodiclofen.1	112	105	7	109	98	11	122	106	17
Spirodiclofen.2	105	100	5	105	99	6	119	103	16
Spiromesifen+NH_4.1	115	110	5	109	110	0	108	113	−4
Spiromesifen+NH_4.2	104	105	0	96	103	−6	102	106	−4
Spirotetramat.1	86	90	−4	107	108	−1	115	112	3
Spirotetramat.2	70	81	−10	105	108	−3	115	113	2
Spiroxamine.1	94	95	−2	97	104	−7	101	96	5
Spiroxamine.2	93	95	−1	102	105	−3	103	97	6
Sulfentrazone.1	131	123	8	101	112	−11	119	111	8
Sulfentrazone.2	134	115	19	103	101	2	117	112	5
Tebuconazole.1	85	93	−9	97	96	1	102	102	0
Tebuconazole.2	91	100	−9	101	104	−3	111	113	−2
Tebufenozide.1	96	105	−10	111	104	6	116	107	9
Tebufenozide.2	87	94	−8	101	101	0	107	105	2
Tebuthiuron.1	91	99	−8	100	104	−3	107	106	0
Tebuthiuron.2	88	98	−9	98	101	−3	104	105	−1
Teflubenzuron.1	98	105	−7	102	101	1	111	109	3
Teflubenzuron.2	90	101	−11	101	96	5	106	104	2
Temephos.1	116	114	3	106	105	1	113	109	4
Temephos.2	118	113	5	106	105	1	113	108	5
Thiabendazole.1	86	97	−11	100	103	−4	92	105	−13
Thiabendazole.2	85	98	−13	101	103	−2	92	107	−14
Thiacloprid.1	81	95	−14	100	104	−3	106	108	−2
Thiacloprid.2	81	94	−13	99	103	−4	106	108	−2
Thiamethoxam.1	110	117	−8	104	105	−2	95	109	−13
Thiamethoxam.2	108	119	−11	104	107	−3	97	110	−13
Thidiazuron.1	103	112	−9	99	105	−6	102	108	−6
Thidiazuron.2	98	110	−12	97	102	−5	99	105	−5
Thiophanate-methyl.1	96	108	−13	100	106	−6	105	109	−3
Thiophanate-methyl.2	93	107	−14	99	107	−8	104	108	−4
Triadimefon.1	86	92	−6	101	104	−3	108	107	1
Triadimefon.2	84	94	−10	99	105	−6	106	109	−3
Triadimenol.1	86	91	−5	103	104	−1	106	104	2
Triadimenol.2	85	96	−11	107	105	1	107	104	3

dx.doi.org/10.1021/jf201618q |J. Agric. Food Chem. 2011, 59, 6383–6411

Table 7. Continued

	orange			carrot			spinach		
transition	R_{500}	R_{200}	Δ_{Eff}	R_{500}	R_{200}	Δ_{Eff}	R_{500}	R_{200}	Δ_{Eff}
Trichlorfon.1	120	134	−14	113	122	−10	119	126	−6
Trichlorfon.2	116	118	−2	97	117	−20	120	115	5
Tricyclazole.1	91	100	−8	101	104	−3	101	104	−3
Tricyclazole.2	90	98	−8	100	103	−3	99	104	−5
Trifloxystrobin.1	96	102	−6	101	105	−4	105	106	−1
Trifloxystrobin.2	94	102	−8	99	103	−4	107	108	−1
Triflumizole.1	104	108	−4	102	109	−8	110	111	−1
Triflumizole.2	104	109	−5	101	110	−9	111	113	−1
Triflumuron.1	94	100	−6	96	101	−5	109	107	2
Triflumuron.2	94	102	−8	96	104	−7	110	107	3
Triticonazole.1	84	96	−13	103	106	−3	106	108	−2
Triticonazole.2	84	95	−12	103	107	−4	108	110	−2
Vamidothion.1	90	106	−15	98	98	0	101	103	−2
Vamidothion.2	93	103	−10	101	101	0	105	107	−3
Zoxamide.1	78	87	−8	94	102	−8	109	109	−1
Zoxamide.2	78	86	−8	91	101	−10	110	110	0

[a] R_{500} and R_{200} are percent recoveries for spikes fortified in matrix at 500 and 200 mg of sample/mL, respectively. Δ_{Eff} is the percent difference between R_{500} and R_{200}.

with injection of 2.5-fold less pesticide. As anticipated, the suppression effect was greater for the citrus matrix, however the average Δ_{Eff} for the orange matrix of −8% is very good for the worst case matrix. For this reason, the decision was made to validate the method using a final dilution of 500 mg of sample/mL, and the remaining discussion about the matrix effects pertains to that sample concentration only.

The matrix effects for individual transitions at the 500 mg sample/mL level were more pronounced than the averages but still acceptable. All but two, both mepanipyrim transitions in orange at 45 and 47%, were within 50−150%. Interestingly, mepanipyrim responses were very good in the other two matrices. Cyromazine responses were adversely affected in all matrices ranging from 59 to 73%. Apart from cyromazine, the matrix effects were between 70 and 130% for all transitions in the carrot and spinach matrices. While the matrix effects of carrots and spinach were minimal, the matrix effects in orange were significantly greater; 36 of the 362 transitions were below 70%. However, as noted earlier, only two of the transitions were below 50%.

QuEChERS Method Validation Results Using Spiked Samples. Results of the validation are summarized in Table 8. Six ORA laboratories (PRL-NW, PRL-SW, KAN-DO, ARL, SRL, and NRL) submitted over 150,000 records for the 174 analytes reported. 170 analytes were collaborated by at least three of the participating laboratories. SEA reported four additional analytes: carbosulfan, cyflufenamid, malathion and phorate sulfone. Data are reported by MS transitions, 370 transitions in all, i.e. two transitions per 163 analytes and 4 transitions for 11 analytes (7 analytes with multiple adducts: avermectin, doramectin, eprinomectin, isoxaflutole, ivermectin, moxidectin, and spiromesifen; and 4 analytes with multiple components: bromuconazole, cyproconazole, dimethomorph, and spinetoram).

Of the 174 analytes reported 169 met a majority of the minimum validation performance specifications. The five problematic compounds are carbosulfan, benfuracarb, bifenazate, alanycarb, and cyromazine. Of these, carbosulfan simply did not work because of its inherent instability in light and acidic media and will not be reported further. The other problematic compounds had a combination of high MDLs and/or erratic recoveries. Low recoveries are likely attributable to the QuEChERS extraction because the matrix effect study showed insignificant bias from the matrix in the LC−MS/MS determination. Bifenazate and alanycarb are easily hydrolyzed in acidic media and subject to photolysis; and benfuracarb is susceptible to thiolytic cleavage in acidic media. Cyromazine recoveries averaged less than 50% for all three matrices but were lowest (<30%) in the oranges indicating the acidic nature of the citrus fruit might have caused ionization of one of unsaturated tertiary amines. The resulting cyromazine ion would partitioned into the aqueous phase during the QuEChERS extraction. Most likely cyromazine would have benefited from the use of a buffered extraction. It is also possible that the unacceptable recoveries were also due to tight binding between the analytes and biopolymers (proteins, lipids, complex carbohydrates) that are present in the matrix.

77 of the 173 analytes reported are currently analyzed by GC-MSD in the selective ion mode (SIM), and the remaining 97 compounds have not been previously analyzed in the pesticide program.[23] Many of the 77 compounds analyzed by both techniques will be migrated to the LC−MS/MS procedure because they respond much better than GC-MSD SIM. The total targeted pesticide coverage of about 400 compounds will be fairly evenly distributed among the two technologies.

For the collaboration specificity, accuracy, reproducibility, method uncertainty (MU), method detection limit (MDL), linearity, and the extended range of the method were evaluated using a variety statistical tools (Table 9). Outliers were determined and omitted from the calculations of analyte concentrations and validation performance parameters.

Specificity Using Spiked Samples. For the controls, the concentrations of approximately 4500 transitions were determined. Of those, 56 transitions had levels above their respective

Table 8. Matrix Effect Study Summary: Overall Average, Minimum and Maximum Percent Recoveries of Standards Spiked into Sample Extracts at Two Matrix Levels Determined by Calibration Using Standards in Methanol[a]

	all matrices			oranges			carrots			spinach		
	R_{500}	R_{200}	Δ_{Eff}	R_{500}	R_{200}	Δ_{Eff}	R_{500}	R_{200}	Δ_{Eff}	R_{500}	R_{200}	Δ_{Eff}
av	99	104	−4	91	99	−8	100	104	−3	106	108	−2
min	45	64	−19	45	64		69	79		59	68	
max	142	146	−4	139	134		137	146		142	143	

[a] R_{500} and R_{200} are percent recoveries for spikes fortified in matrix at 0.5 and 0.2 mg sample/mL, respectively. Δ_{Eff} is the percent difference between R_{500} and R_{200}.

Table 9. Average Percent Recoveries of Spikes from three Sample Matrices (± Standard Deviation), Method Uncertainties (MU), and Minimum Detection Limits (MDL). Numbers in bold indicate unsatisfactory results

	av				
transition	carrots	oranges	spinach	MU	MDL
3-Hydroxycarbofuran.1	104 ± 6	95 ± 3	107 ± 5	17	5
3-Hydroxycarbofuran.2	103 ± 6	94 ± 4	107 ± 4	17	4
Acephate.1	91 ± 5	90 ± 2	92 ± 4	13	3
Acephate.2	91 ± 5	90 ± 3	91 ± 4	11	5
Acetamiprid.1	104 ± 6	93 ± 3	104 ± 6	22	4
Acetamiprid.2	103 ± 6	92 ± 4	106 ± 6	21	5
Acibenzolar-S-methyl.1	103 ± 8	80 ± 4	103 ± 7	17	7
Acibenzolar-S-methyl.2	105 ± 5	81 ± 8	102 ± 6	20	10
Alanycarb.1	**40** ± 13	**10** ± 7	**68** ± 21	**47**	8
Alanycarb.2	**40** ± 13	**10** ± 7	76 ± 15	**41**	9
Aldicarb+NH_4.1	103 ± 5	95 ± 7	103 ± 10	21	7
Aldicarb+NH_4.2	102 ± 7	91 ± 6	97 ± 10	22	5
AldicarbSulfoxide.1	96 ± 6	85 ± 3	100 ± 5	17	4
AldicarbSulfoxide.2	96 ± 7	85 ± 3	101 ± 6	17	4
Aldoxycarb.1	105 ± 7	98 ± 8	113 ± 8	21	6
Aldoxycarb.2	104 ± 8	94 ± 3	112 ± 6	19	4
Aminocarb.1	100 ± 7	94 ± 4	106 ± 4	20	3
Aminocarb.2	101 ± 7	93 ± 3	105 ± 4	21	4
AvermectinB1a+Na.3	73 ± 10	**35** ± 7	78 ± 12	**34**	6
AvermectinB1a+Na.4	79 ± 9	**42** ± 6	74 ± 13	30	**15**
AvermectinB1a+NH_4.1	108 ± 4	107 ± 4	114 ± 4	13	**12**
AvermectinB1a+NH_4.2	110 ± 7	100 ± 7	113 ± 8	19	**12**
Azoxystrobin.1	105 ± 3	102 ± 2	106 ± 4	25	4
Azoxystrobin.2	104 ± 4	99 ± 3	109 ± 5	12	5
Benalaxyl.1	100 ± 7	94 ± 3	104 ± 4	21	5
Benalaxyl.2	100 ± 7	93 ± 4	105 ± 4	18	5
Bendiocarb.1	101 ± 5	93 ± 3	102 ± 4	16	4
Bendiocarb.2	99 ± 6	92 ± 3	100 ± 5	18	3
Benfuracarb.1	**13** ± **27**	**6** ± **19**	**27** ± 7	**43**	3
Benfuracarb.2	**31** ± **17**	**26** ± 8	**26** ± 8	**243**	2
Benzoximate.1	95 ± 6	85 ± 7	101 ± 5	19	6
Benzoximate.2	94 ± 5	85 ± 7	101 ± 5	18	6
Bifenazate.1	**51** ± **27**	**38** ± **20**	**40** ± 29	**69**	4
Bifenazate.2	**43** ± **19**	**39** ± **20**	**35** ± 16	**71**	5
Bitertanol.1	107 ± 5	104 ± 4	110 ± 6	13	7
Bitertanol.2	110 ± 5	104 ± 5	106 ± 5	13	6
Boscalid.1	105 ± 6	96 ± 5	106 ± 4	16	6
Boscalid.2	105 ± 6	95 ± 6	109 ± 5	15	6
BromuconazoleA.1	102 ± 4	76 ± 4	102 ± 4	18	6
BromuconazoleA.2	99 ± 6	81 ± 3	101 ± 6	18	9
BromuconazoleB.1	96 ± 4	81 ± 4	102 ± 5	13	7
BromuconazoleB.2	97 ± 5	90 ± 4	99 ± 5	15	6
Bupirimate.1	103 ± 8	**67** ± 5	107 ± 6	18	7
Bupirimate.2	101 ± 6	**64** ± 3	106 ± 7	18	7
Buprofezin.1	96 ± 5	83 ± 5	101 ± 4	19	4
Buprofezin.2	95 ± 5	81 ± 5	101 ± 3	17	5
Butafenacil+NH_4.1	110 ± 5	93 ± 3	111 ± 7	17	5
Butafenacil+NH_4.2	112 ± 7	88 ± 2	111 ± 6	16	5
Butocarboxim+Na.1	101 ± 9	79 ± 4	86 ± 5	21	9
Butocarboxim+Na.2	100 ± 20	78 ± 21	102 ± 21	**48**	**16**
Butoxycarboxim.1	103 ± 7	95 ± 4	107 ± 6	17	6
Butoxycarboxim.2	101 ± 9	95 ± 5	106 ± 7	25	5
Carbaryl.1	101 ± 6	90 ± 4	108 ± 6	20	4
Carbaryl.2	101 ± 6	90 ± 4	107 ± 6	18	5
Carbendazim.1	113 ± 6	75 ± 6	111 ± 6	19	4
Carbendazim.2	103 ± 7	75 ± 6	110 ± 7	19	6
Carbetamide.1	105 ± 5	102 ± 2	108 ± 4	19	4
Carbetamide.2	104 ± 5	102 ± 3	107 ± 3	19	4
Carbofuran.1	109 ± 4	92 ± 3	100 ± 2	21	4
Carbofuran.2	111 ± 5	94 ± 4	102 ± 2	23	4
Carbosulfan.1	**2** ± **130**	**0** ± 11	**0** ± 147	**404**	**n/a**
Carbosulfan.2	**2** ± **126**	**0** ± 11	**0** ± 141	**387**	**n/a**
Chlorantraniliprole.1	109 ± 5	92 ± 3	114 ± 5	17	5
Chlorantraniliprole.2	111 ± 5	92 ± 3	116 ± 6	18	5
Chlorfluazuron.1	106 ± 5	93 ± 4	106 ± 4	13	6
Chlorfluazuron.2	103 ± 4	90 ± 3	102 ± 2	10	5
Chlorotoluron.1	107 ± 3	94 ± 3	102 ± 6	17	5
Chlorotoluron.2	101 ± 6	90 ± 3	99 ± 4	20	5
Chloroxuron.1	103 ± 5	73 ± 3	107 ± 6	20	5
Chloroxuron.2	106 ± 7	**66** ± 3	103 ± 6	21	8
Clethodim.1	**68** ± 8	79 ± 7	**64** ± 9	20	5
Clethodim.2	**67** ± 9	76 ± 6	**62** ± 7	20	6
Clofentezine.1	87 ± 7	95 ± 4	102 ± 3	13	5
Clofentezine.2	88 ± 7	93 ± 5	102 ± 4	13	6
Clothianidin.1	104 ± 4	113 ± 3	103 ± 4	17	7
Clothianidin.2	104 ± 5	116 ± 4	104 ± 6	17	6
Cyazofamid.1	108 ± 5	84 ± 3	109 ± 4	21	5
Cyazofamid.2	105 ± 5	82 ± 5	111 ± 5	21	8

dx.doi.org/10.1021/jf201618q |J. Agric. Food Chem. 2011, 59, 6383–6411

Table 9. Continued

transition	av carrots	av oranges	av spinach	MU	MDL
Cycluron.1	100 ± 7	93 ± 3	95 ± 4	22	5
Cycluron.2	102 ± 5	93 ± 4	96 ± 5	22	5
Cyflufenamid.1	78 ± 8	**35** ± 2	109 ± 4	15	4
Cyflufenamid.2	80 ± 6	**34** ± 2	109 ± 2	12	5
Cymoxanil.1	101 ± 7	93 ± 4	95 ± 3	17	4
Cymoxanil.2	100 ± 6	100 ± 4	95 ± 5	18	6
CyproconazoleA.1	102 ± 5	87 ± 2	104 ± 5	17	6
CyproconazoleA.2	102 ± 7	91 ± 19	104 ± 5	27	8
CyproconazoleB.1	100 ± 7	**58** ± 5	103 ± 7	20	6
CyproconazoleB.2	100 ± 6	**63** ± 4	104 ± 7	20	7
Cyprodinil.1	102 ± 4	76 ±	105 ± 6	13	6
Cyprodinil.2	100 ± 6	71 ± 5	100 ± 5	16	6
Cyromazine.1	**50** ± 12	**29** ± 6	**43** ± 4	22	4
Cyromazine.2	**48** ± 12	**26** ± 17	**39** ± 5	26	4
Desmedipham+NH_4.1	107 ± 4	94 ± 5	109 ± 3	16	4
Desmedipham+NH_4.2	120 ± 11	109 ± 10	103 ± 5	21	6
Diclobutrazol.1	102 ± 5	**67** ± 4	104 ± 5	19	7
Diclobutrazol.2	100 ± 7	**62** ± 7	105 ± 5	24	7
Dicrotophos.1	101 ± 5	93 ± 2	101 ± 3	16	4
Dicrotophos.2	101 ± 5	93 ± 3	100 ± 3	14	4
Diethofencarb.1	108 ± 4	99 ± 3	109 ± 4	19	4
Diethofencarb.2	107 ± 4	98 ± 2	109 ± 4	18	4
Difenoconazole.1	108 ± 3	108 ± 3	106 ± 3	11	4
Difenoconazole.2	106 ± 3	107 ± 3	105 ± 4	10	5
Diflubenzuron.1	103 ± 6	**59** ± 4	106 ± 4	20	5
Diflubenzuron.2	104 ± 6	**58** ± 3	105 ± 4	19	5
Dimethoate.1	98 ± 6	84 ± 3	102 ± 4	20	4
Dimethoate.2	98 ± 6	83 ± 3	101 ± 5	21	4
DimethomorphA.1	109 ± 7	75 ± 4	111 ± 6	13	6
DimethomorphA.2	109 ± 6	77 ± 4	109 ± 5	18	6
DimethomorphB.1	108 ± 4	**50** ± 3	110 ± 4	13	5
DimethomorphB.2	109 ± 4	**54** ± 6	112 ± 8	16	5
Dimoxystrobin.1	103 ± 5	74 ± 3	104 ± 4	20	5
Dimoxystrobin.2	104 ± 5	74 ± 4	105 ± 4	18	4
Dinotefuran.1	100 ± 6	102 ± 3	103 ± 5	22	4
Dinotefuran.2	99 ± 6	101 ± 3	101 ± 6	23	5
Dioxacarb.1	93 ± 7	74 ± 4	99 ± 5	21	6
Dioxacarb.2	93 ± 6	78 ± 2	98 ± 5	20	6
Diuron.1	100 ± 6	96 ± 3	95 ± 7	23	6
Diuron-Cl37.2	99 ± 6	93 ± 3	97 ± 4	22	7
Doramectin+Na.3	87 ± 10	83 ± 8	86 ± 7	23	**15**
Doramectin+Na.4	81 ± 9	83 ± 7	86 ± 10	23	**18**
Doramectin+NH_4.1	114 ± 8	116 ± 12	113 ± 8	23	**11**
Doramectin+NH_4.2	114 ± 10	116 ± 10	114 ± 8	23	**12**
Emamectin.1	98 ± 5	94 ± 4	98 ± 6	13	5
Emamectin.2	104 ± 6	101 ± 4	102 ± 4	12	6
Eprinomectin.1	102 ± 8	91 ± 6	99 ± 9	20	9
Eprinomectin.2	107 ± 7	101 ± 6	108 ± 7	20	9
Eprinomectin+Na.3	97 ± 7	**67** ± 7	96 ± 8	22	7
Eprinomectin+Na.4	95 ± 8	**67** ± 7	91 ± 7	20	9
Ethaboxam.1	102 ± 5	107 ± 3	106 ± 5y	20	5
Ethaboxam.2	99 ± 5	105 ± 3	104 ± 5	20	5

Table 9. Continued

transition	av carrots	av oranges	av spinach	MU	MDL
Ethiofencarb.1	101 ± 6	93 ± 3	87 ± 4	19	4
Ethiofencarb.2	98 ± 6	89 ± 4	89 ± 6	16	4
Ethiprole.1	112 ± 5	103 ± 4	112 ± 5	24	6
Ethiprole.2	116 ± 8	98 ± 6	115 ± 7	20	8
Ethirimol.1	89 ± 6	**65** ± 5	77 ± 7	16	3
Ethirimol.2	90 ± 6	**65** ± 5	78 ± 8	16	3
Etoxazole.1	98 ± 7	85 ± 5	101 ± 5	26	4
Etoxazole.2	95 ± 6	81 ± 7	100 ± 5	19	4
Famoxadone+NH_4.1	106 ± 5	94 ± 4	106 ± 4	11	5
Famoxadone+NH_4.2	105 ± 4	113 ± 5	110 ± 4	12	7
Fenamidone.1	108 ± 6	96 ± 2	107 ± 5	21	6
Fenamidone.2	107 ± 6	96 ± 3	108 ± 4	23	6
Fenazaquin.1	83 ± 5	73 ± 7	84 ± 4	16	6
Fenazaquin.2	81 ± 5	71 ± 7	82 ± 3	17	3
Fenbuconazole.1	105 ± 6	82 ± 4	107 ± 4	15	6
Fenbuconazole.2	106 ± 5	80 ± 4	108 ± 4	15	7
Fenhexamid.1	92 ± 7	77 ± 3	97 ± 7	17	5
Fenhexamid.2	92 ± 9	78 ± 4	98 ± 5	17	7
Fenobucarb.1	104 ± 6	99 ± 2	103 ± 2	16	4
Fenobucarb.2	100 ± 6	96 ± 3	101 ± 5	18	4
Fenoxycarb.1	106 ± 6	**66** ± 3	106 ± 3	16	4
Fenoxycarb.2	105 ± 6	**63** ± 3	106 ± 4	18	4
Fenpyroximate.1	104 ± 4	93 ± 5	106 ± 2	13	4
Fenpyroximate.2	102 ± 5	93 ± 5	112 ± 3	18	4
Fenuron.1	103 ± 5	88 ± 4	100 ± 4	24	5
Fenuron.2	102 ± 5	85 ± 3	99 ± 4	21	6
Flonicamid.1	101 ± 6	100 ± 4	105 ± 6	18	6
Flonicamid.2	103 ± 7	102 ± 4	102 ± 7	19	7
Flubendiamide.1	114 ± 11	**69** ± 13	116 ± 11	**30**	**12**
Flubendiamide.2	109 ± 10	70 ± 8	115 ± 9	22	7
Fludioxinil+NH_4.1	96 ± 6	87 ± 5	96 ± 7	29	8
Fludioxinil+NH_4.2	97 ± 6	83 ± 5	98 ± 6	27	**12**
Flufenoxuron.1	98 ± 7	96 ± 3	109 ± 4	11	5
Flufenoxuron.2	97 ± 5	96 ± 3	108 ± 3	10	5
Fluometuron.1	101 ± 5	92 ± 3	101 ± 7	20	4
Fluometuron.2	101 ± 7	96 ± 4	101 ± 5	18	5
Fluoxastrobin.1	104 ± 6	**69** ± 4	107 ± 4	17	4
Fluoxastrobin.2	105 ± 5	**61** ± 3	107 ± 4	14	6
Flusilazole.1	104 ± 6	**53** ± 4	106 ± 5	16	5
Flusilazole.2	101 ± 4	**53** ± 5	106 ± 4	16	5
Flutolanil.1	105 ± 5	98 ± 3	107 ± 5	20	6
Flutolanil.2	104 ± 9	97 ± 3	105 ± 7	22	6
Flutriafol.1	101 ± 5	100 ± 3	105 ± 5	16	5
Flutriafol.2	102 ± 6	99 ± 3	104 ± 6	19	6
Forchlorfenuron.1	96 ± 5	87 ± 3	95 ± 4	22	5
Forchlorfenuron.2	96 ± 5	90 ± 3	96 ± 5	21	7
Formetanate.1	94 ± 7	96 ± 4	98 ± 5	18	4
Formetanate.2	88 ± 12	92 ± 5	103 ± 5	22	6
Fuberidazole.1	98 ± 6	80 ± 4	92 ± 3	18	5
Fuberidazole.2	99 ± 7	80 ± 5	91 ± 5	17	3
Furathiocarb.1	108 ± 5	98 ± 3	102 ± 5	17	5
Furathiocarb.2	105 ± 4	97 ± 3	102 ± 3	13	4

dx.doi.org/10.1021/jf201618q |*J. Agric. Food Chem.* 2011, 59, 6383–6411

Table 9. Continued

transition	av carrots	av oranges	av spinach	MU	MDL
Halofenozide.1	106 ± 5	102 ± 4	107 ± 5	28	5
Halofenozide.2	106 ± 6	102 ± 4	108 ± 6	24	6
Hexaflumuron.1	91 ± 6	100 ± 6	103 ± 7	16	9
Hexaflumuron.2	93 ± 8	102 ± 8	106 ± 6	19	9
Hexythiazox.1	86 ± 7	8 ± 5	93 ± 4	14	4
Hexythiazox.2	84 ± 6	87 ± 6	94 ± 3	13	4
Hydramethylnon.1	98 ± 6	93 ± 4	86 ± 5	24	5
Hydramethylnon.2	100 ± 7	98 ± 6	85 ± 5	25	6
Imazalil.1	101 ± 5	87 ± 3	99 ± 4	14	4
Imazalil.2	102 ± 5	87 ± 3	99 ± 4	14	5
Imidacloprid.1	104 ± 4	**139** ± 4	114 ± 4	22	6
Imidacloprid.2	102 ± 4	**135** ± 4	113 ± 3	21	5
Indoxacarb.1	102 ± 5	98 ± 4	107 ± 5	14	8
Indoxacarb.2	106 ± 6	105 ± 4	106 ± 4	15	7
Ipconazole.1	105 ± 5	107 ± 4	107 ± 5	12	6
Ipconazole.2	97 ± 6	102 ± 5	103 ± 6	15	7
Iprovalicarb.1	107 ± 5	102 ± 2	104 ± 4	19	4
Iprovalicarb.2	105 ± 6	99 ± 2	106 ± 4	15	4
Isoprocarb.1	106 ± 5	97 ± 2	107 ± 4	16	3
Isoprocarb.2	105 ± 5	95 ± 3	107 ± 6	15	3
Isoproturon.1	104 ± 6	96 ± 3	99 ± 4	21	4
Isoproturon.2	104 ± 6	96 ± 2	99 ± 4	18	5
Isoxaflutole.1	**163** ± 3	120 ± 5	**157** ± 2	9	**10**
Isoxaflutole.2	**162** ± 4	115 ± 7	**153** ± 7	16	**49**
Isoxaflutole+NH_4.1	83 ± 8	94 ± 7	90 ± 9	**28**	8
Isoxaflutole+NH_4.2	79 ± **16**	97 ± 16	96 ± 8	**87**	**32**
Ivermectin+Na.3	95 ± 9	73 ± 5	**68** ± 5	26	**19**
Ivermectin+Na.4	97 ± 10	75 ± 5	72 ± 5	29	**15**
Ivermectin+NH_4.1	112 ± 8	116 ± 7	94 ± 7	21	8
Ivermectin+NH_4.2	105 ± 10	102 ±	96 ± 12	22	8
Kresoxim:methyl.1	100 ± 6	74 ± 7	103 ± 7	23	6
Kresoxim:methyl.2	104 ± 6	73 ± 7	106 ± 7	20	5
Linuron.1	101 ± 5	98 ± 2	103 ± 5	15	5
Linuron.2	102 ± 5	97 ± 2	102 ± 4	17	6
Lufenuron.1	94 ± 8	96 ± 4	104 ± 7	15	8
Lufenuron.2	93 ± 7	98 ± 4	106 ± 6	15	8
Malathion.1	98 ± 9	95 ± 2	110 ± 6	29	5
Malathion.2	99 ± 10	96 ± 2	111 ± 6	28	8
Mandipropamide.1	111 ± 4	100 ± 3	111 ± 4	16	5
Mandipropamide.2	109 ± 3	96 ± 4	111 ± 3	15	6
Mepanipyrim.1	100 ± 6	**47** ± 4	101 ± 4	20	5
Mepanipyrim.2	100 ± 6	**45** ± 5	100 ± 4	22	7
Metaflumizone.1	100 ± 5	106 ± 5	103 ± 3	13	8
Metaflumizone.2	101 ± 6	107 ± 5	101 ± 4	12	6
Metalaxyl.1	105 ± 6	98 ± 3	102 ± 4	19	4
Metalaxyl.2	105 ± 4	98 ± 2	101 ± 3	16	3
Metconazole.1	104 ± 7	82 ± 3	104 ± 4	15	5
Metconazole.2	102 ± 6	81 ± 4	104 ± 5	15	6
Methamidophos.1	86 ± 7	84 ± 3	84 ± 3	16	3
Methamidophos.2	86 ± 7	81 ± 6	83 ± 4	17	4
Methiocarb.1	106 ± 11	93 ± 3	106 ± 4	24	5
Methiocarb.2	103 ± 6	97 ± 2	106 ± 6	21	5

Table 9. Continued

transition	av carrots	av oranges	av spinach	MU	MDL
Methomyl.1	108 ± 6	88 ± 3	106 ± 5	17	5
Methomyl.2	107 ± 6	85 ± 4	104 ± 4	18	4
Methoxyfenozide.1	101 ± 6	100 ± 4	102 ± 5	20	6
Methoxyfenozide.2	100 ± 7	99 ± 3	105 ± 5	17	6
Metobromuron.1	104 ± 4	90 ± 3	104 ± 5	14	5
Metobromuron.2	103 ± 5	90 ± 3	102 ± 4	14	3
Mevinphos-E.1	96 ± 6	80 ± 4	99 ± 6	16	6
Mevinphos-E.2	98 ± 3	79 ± 3	99 ± 4	14	6
Mevinphos-Z.1	104 ± 6	84 ± 5	102 ± 5	18	5
Mevinphos-Z.2	102 ± 6	82 ± 5	105 ± 7	16	4
Mexacarbate.1	91 ± 6	89 ± 3	102 ± 4	22	5
Mexacarbate.2	94 ± 5	90 ± 4	104 ± 4	20	7
Monocrotophos.1	101 ± 7	92 ± 3	104 ± 4	15	3
Monocrotophos.2	100 ± 7	91 ± 3	103 ± 4	15	4
Monolinuron.1	103 ± 6	92 ± 3	104 ± 5	17	4
Monolinuron.2	102 ± 6	92 ± 3	104 ± 5	16	4
Moxidectin.1	105 ± 6	97 ± 9	101 ± 8	21	8
Moxidectin.2	107 ± 5	96 ± 8	97 ± 5	20	8
Moxidectin+Na.3	96 ± 8	92 ± 6	74 ± 9	**34**	10
Moxidectin+Na.4	96 ± 10	93 ± 10	76 ± 11	27	**19**
Myclobutanil.1	106 ± 6	**68** ± 4	107 ± 4	15	5
Myclobutanil.2	107 ± 6	**69** ± 3	108 ± 4	19	8
Neburon.1	100 ± 4	**44** ± 4	102 ± 3	22	4
Neburon.2	100 ± 5	**45** ± 5	103 ± 4	24	5
Nitenpyram.1	93 ± 8	92 ± 4	90 ± 4	17	5
Nitenpyram.2	89 ± 8	97 ± 3	91 ± 6	20	4
Novaluron.1	92 ± 9	102 ± 6	104 ± 5	14	6
Novaluron.2	94 ± 5	101 ± 4	103 ± 5	13	7
Nuarimol.1	99 ± 5	101 ± 4	104 ± 6	16	7
Nuarimol.2	99 ± 7	98 ± 4	107 ± 7	20	7
Omethoate.1	96 ± 6	94 ± 3	98 ± 4	15	4
Omethoate.2	96 ± 7	93 ± 4	99 ± 5	14	4
Oxadixyl.1	104 ± 5	96 ± 4	105 ± 5	12	5
Oxadixyl.2	103 ± 5	94 ± 3	104 ± 4	11	6
Oxamyl+NH_4.1	103 ± 6	93 ± 3	104 ± 4	18	4
Oxamyl+NH_4.2	103 ± 6	92 ± 2	105 ± 4	16	4
Paclobutrazol.1	109 ± 6	98 ± 6	106 ± 6	21	6
Paclobutrazol.2	103 ± 7	90 ± 6	106 ± 7	30	9
Pencycuron.1	100 ± 6	88 ± 3	101 ± 4	21	3
Pencycuron.2	99 ± 5	85 ± 4	102 ± 4	16	5
Phenmedipham.1	109 ± 4	91 ± 6	105 ± 4	15	5
Phenmedipham.2	108 ± 3	90 ± 4	88 ± 4	16	4
PhorateSulfone.1	101 ± 8	96 ± 2	115 ± 6	22	6
PhorateSulfone.2	102 ± 8	94 ± 2	117 ± 8	22	6
Picoxystrobin.1	105 ± 5	72 ± 4	103 ± 6	19	6
Picoxystrobin.2	103 ± 5	**66** ± 5	105 ± 4	21	5
PiperonylButox+NH_4.1	101 ± 5	100 ± 4	101 ± 4	24	6
PiperonylButox+NH_4.2	100 ± 5	93 ± 3	103 ± 3	15	4
Pirimicarb.1	101 ± 5	88 ± 3	102 ± 5	20	5
Pirimicarb.2	101 ± 5	87 ± 4	100 ± 4	15	5
Prochloraz.1	97 ± 6	99 ± 2	102 ± 4	14	4
Prochloraz.2	97 ± 5	100 ± 3	102 ± 3	12	4

Table 9. Continued

transition	av carrots	av oranges	av spinach	MU	MDL
Promecarb.1	104 ± 7	95 ± 3	104 ± 7	19	4
Promecarb.2	102 ± 5	92 ± 5	105 ± 6	20	5
Propamocarb.1	85 ± 5	81 ± 4	90 ± 4	12	5
Propamocarb.2	85 ± 4	80 ± 4	90 ± 3	11	4
Propargite+NH_4.1	93 ± 5	87 ± 5	102 ± 3	18	3
Propargite+NH_4.2	93 ± 5	86 ± 5	101 ± 3	16	4
Propiconazole.1	104 ± 5	88 ± 3	106 ± 3	13	5
Propiconazole.2	105 ± 5	89 ± 5	107 ± 3	14	6
Propoxur.1	100 ± 5	86 ± 3	98 ± 4	17	3
Propoxur.2	98 ± 7	89 ± 3	99 ± 6	18	3
Pymetrozine.1	75 ± 10	**44** ± 9	87 ± 6	20	5
Pymetrozine.2	75 ± 8	**43** ± 9	86 ± 5	18	6
Pyracarbolid.1	98 ± 7	88 ± 4	104 ± 5	25	3
Pyracarbolid.2	100 ± 6	86 ± 5	106 ± 5	18	4
Pyraclostrobin.1	102 ± 4	82 ± 3	105 ± 3	16	4
Pyraclostrobin.2	101 ± 4	81 ± 2	104 ± 3	15	4
Pyridaben.1	98 ± 4	82 ± 6	94 ± 5	19	5
Pyridaben.2	96 ± 4	81 ± 5	94 ± 3	20	4
Pyrimethanil.1	101 ± 6	94 ± 4	102 ± 6	15	6
Pyrimethanil.2	103 ± 6	93 ± 3	103 ± 6	15	5
Pyriproxyfen.1	93 ± 5	87 ± 4	97 ± 4	23	5
Pyriproxyfen.2	91 ± 7	87 ± 5	97 ± 3	15	5
Rotenone.1	106 ± 5	80 ± 4	110 ± 3	12	6
Rotenone.2	105 ± 5	77 ± 5	110 ± 3	13	6
Siduron.1	108 ± 15	92 ± 17	105 ± 11	34	10
Siduron.2	106 ± 9	99 ± 8	106 ± 6	25	7
SpinetoramA.1	95 ± 6	80 ± 6	99 ± 5	18	4
SpinetoramA.2	99 ± 6	87 ± 6	102 ± 5	13	4
SpinetoramB.1	98 ± 5	99 ± 4	101 ± 2	10	4
SpinetoramB.2	98 ± 5	100 ± 5	102 ± 4	13	6
SpinosynA.1	102 ± 7	101 ± 4	105 ± 5	14	4
SpinosynA.2	103 ± 6	96 ± 3	107 ± 5	13	5
Spirodiclofen.1	100 ± 4	78 ± 6	105 ± 4	14	7
Spirodiclofen.2	99 ± 3	79 ± 8	104 ± 3	14	27
Spiromesifen.1	107 ± 7	96 ± 17	112 ± 8	28	**13**
Spiromesifen.2	113 ± 12	101 ± 5	117 ± 8	20	**12**
Spiromesifen+NH_4.1	102 ± 5	85 ± 8	100 ± 6	18	7
Spiromesifen+NH_4.2	100 ± 4	83 ± 9	99 ± 5	16	6
Spirotetramat.1	99 ± 4	86 ± 8	105 ± 6	21	6
Spirotetramat.2	95 ± 5	**58** ± 2	97 ± 7	16	5
Spiroxamine.1	101 ± 6	96 ± 4	105 ± 3	26	9
Spiroxamine.2	98 ± 6	91 ± 6	101 ± 4	23	5
Sulfentrazone.1	99 ± 9	**177** ± 6	**136** ± 8	21	8
Sulfentrazone.2	95 ± 8	**166** ± 6	129 ± 8	19	**12**
Tebuconazole.1	110 ± 9	89 ± 4	107 ± 8	19	5
Tebuconazole.2	103 ± 5	90 ± 4	104 ± 6	19	8
Tebufenozide.1	107 ± 7	92 ± 6	108 ± 8	25	6
Tebufenozide.2	105 ± 7	88 ± 6	107 ± 7	24	5
Tebuthiuron.1	101 ± 7	85 ± 2	106 ± 6	24	4
Tebuthiuron.2	99 ± 6	82 ± 3	104 ± 5	22	4
Teflubenzuron.1	95 ± 8	91 ± 7	107 ± 8	18	11
Teflubenzuron.2	94 ± 8	92 ± 6	106 ± 6	16	8
Temephos.1	105 ± 6	105 ± 3	110 ± 5	16	5

Table 9. Continued

transition	av carrots	av oranges	av spinach	MU	MDL
Temephos.2	105 ± 5	114 ± 3	107 ± 5	13	6
Thiabendazole.1	98 ± 5	72 ± 5	92 ± 3	16	4
Thiabendazole.2	96 ± 7	71 ± 5	93 ± 8	17	5
Thiacloprid.1	101 ± 4	87 ± 4	103 ± 4	19	5
Thiacloprid.2	100 ± 5	99 ± 3	102 ± 5	15	5
Thiamethoxam.1	101 ± 4	107 ± 4	106 ± 3	12	5
Thiamethoxam.2	100 ± 5	109 ± 6	108 ± 6	15	6
Thidiazuron.1	92 ± 7	92 ± 4	91 ± 6	18	5
Thidiazuron.2	91 ± 8	92 ± 5	89 ± 6	21	8
Thiophanate-methyl.1	95 ± 6	101 ± 4	77 ± 7	24	4
Thiophanate-methyl.2	95 ± 7	100 ± 4	79 ± 7	23	5
Triadimefon.1	107 ± 6	101 ± 2	109 ± 6	14	6
Triadimefon.2	108 ± 6	105 ± 3	107 ± 6	16	6
Triadimenol.1	103 ± 6	98 ± 3	106 ± 6	20	6
Triadimenol.2	106 ± 8	97 ± 6	109 ± 7	29	**11**
Trichlorfon.1	96 ± 6	98 ± 4	103 ± 11	25	7
Trichlorfon.2	96 ± 6	94 ± 5	107 ± 4	18	6
Tricyclazole.1	101 ± 6	86 ± 3	95 ± 3	16	3
Tricyclazole.2	97 ± 5	85 ± 3	94 ± 5	16	3
Trifloxystrobin.1	103 ± 3	99 ± 3	105 ± 4	19	4
Trifloxystrobin.2	99 ± 4	96 ± 4	105 ± 3	12	4
Triflumizole.1	91 ± 6	96 ± 4	99 ± 4	19	5
Triflumizole.2	90 ± 7	95 ± 5	98 ± 4	16	5
Triflumuron.1	96 ± 6	89 ± 3	106 ± 4	14	5
Triflumuron.2	97 ± 5	89 ± 3	107 ± 4	15	6
Triticonazole.1	105 ± 6	88 ± 3	104 ± 4	15	6
Triticonazole.2	107 ± 7	85 ± 6	101 ± 9	20	8
Vamidothion.1	100 ± 5	96 ± 3	98 ± 6	19	5
Vamidothion.2	99 ± 4	92 ± 3	100 ± 7	17	5
Zoxamide.1	97 ± 6	**63** ± 3	107 ± 2	15	5
Zoxamide.2	96 ± 7	**67** ± 4	105 ± 4	25	8

MDL that were not confirmed by their secondary transition. Although matrix responses responded to slightly more than 1% of the transitions determined, no false positives were found in the three matrices analyzed.

Accuracy Using Spiked Samples. Over 6000 recoveries were determined. 169 of the 173 compounds analyzed had recoveries in the range 50−150%, and 165 compounds had recoveries in the range 70−130%. Four compounds exhibited unacceptable recoveries outside the range of 50−150%: alanycarb, benfuracarb, bifenazate, and cyromazine. Difficulties with these specific compounds were confirmed by a CFSAN study using matrix matched standard curves.

Matrix did affect the recoveries: recoveries from oranges were lower, i.e. more suppressed, than those from carrots and spinach. Still, 163 compounds were recovered from the orange matrix in the 50−150% range and 149 in the 70−130% range. These results correspond with those of the matrix effect study. When average recoveries were adjusted for the matrix effect, the number of recoveries from oranges in the 50−150% range was similar to those of spinach and carrots.

Reproducibility Using Spiked Samples. Recoveries of the 400 ng/g spikes for 170 of 173 compounds had RSD ≤15%, and

Table 10. Comparison of Results of Analysis of the Spinach Control Sample Containing Incurred Residue of Imidicloprid Using Two Different Mass Transitions

	imidicloprid residue level (ng/g)	
lab	transition 1	transition 2
ARL	16	16
ATL	22	20
KAN	17	16
SEA	22	23
av	19	19

167 compounds had RSDs ≤10%. Only the problematic compounds, alanycarb, benfuracarb, and bifenazate, had RSDs >15%.

The reproducibility of the method was also evaluated based upon an incurred residue of imidacloprid found in the spinach matrix controls by four of the six laboratories; the other two laboratories did not analyze spinach controls. Results shown in Table 10 demonstrate excellent agreement between transitions and laboratories.

Method Uncertainty (MU) Using Spiked Samples. 171 of the 173 analytes reported had MU ≤ 30. Only two analytes (benfuracarb, bifenazate), both problematic, had MU > 30%.

Method Detection Limit (MDL) Using Spiked Samples. 161 of the 173 compounds had an MDL of less than 10 ng/g. Ten compounds had MDLs of 10−20 ng/g (moxidectin, doramectin, butocarboxim, avermectin, fludioxinil, sulfentrazone, flubendiamide, triadimenol, teflubenzuron). Two compounds (isoxaflutole and spirodiclofen) had MDLs > 20 ng/g. As expected the mectins exhibited high MDLs, and 3 of the others (fludioxinil, sulfentrazone, teflubenzuron) would respond better using negative ionization.

Conclusion. The method was developed and validated using standards dissolved in solvent and shown to be rugged by using standards dissolved in the matrix of the AOAC samples (cucumber and potato). The analyses of oranges, spinach and carrots using a single level calibration with a standard prepared in solvent has been demonstrated to be an effective screening tool for the determination of pesticide residues. The procedure (QuEChERS and LC−MS/MS) was shown to be specific, accurate, reproducible, sensitive, and linear. Method uncertainties in all but two analytes were less than the international standard for pesticide residues of 30%.

Matrix effects, although marginally significant in oranges, do not warrant the use of matrix matched standards for screening of pesticide residues, which would increase the time required to analyze each sample. In carrots and spinach, only one compound exhibited recoveries outside 70−130%, and in oranges, only one compound was <50%.

The procedure adds almost 100 new analytes to the FDA scope of pesticide coverage and provides improved determination of an additional 77 analytes currently analyzed by GC.

This work should not be taken as reflecting FDA policy or regulations.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Extracted ion chromatograms. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: 913-752-2127. Fax: 913-752-2122. E-mail: robert.smith@fda.hhs.gov.

■ REFERENCES

(1) Anastassiades, M.; Lehotay, S. J.; Štajnbaher, D.; Schenck, F. J. Fast easy multiresidue method employing acetonitrile extraction partitioning and 'dispersive solid phase extraction' for determination of pesticide residues in produce. *J. AOAC Int.* **2003**, *86*, 412–431.

(2) Lehotay, S.; Mastovská, K; Lightfield, A. Use of buffering to improve results of problematic pesticides in a fast and easy method for residue analysis of fruits and vegetables. *J. AOAC Int.* **2005**, *88*, 615–629.

(3) Official Methods of Analysis of AOAC International, AOAC Int., Gaithersburg, MD, Method 2007.1

(4) Okihashi, M.; Kitagawa, Y.; Akutsu, K.; Obana, H.; Tanaka, Y. Rapid Method for the Determination of 180 Pesticide Residues in Foods by Gas Chromatography/Mass Spectrometry and Flame Photometric Detection. *J. Pestic. Sci.* **2005**, *30*, 368–377.

(5) Lehotay, S.; Mastovska, K. Evaluation of Two Fast and Easy Methods for Pesticide Residue Analysis in Fatty Food Matrices. *J. AOAC Int.* **2005**, *88*, 630–638.

(6) Pang, G. F.; Fan, C. L.; Liu, Y. M.; C., Y. Z.; Zhang, J. J.; Li, X. M.; Li, Z. Y.; Wu, Y. P.; Guo, T. T. Determination of 446 Pesticides in Fruits and Vegetables by Three- Cartridge Solid-Phase Extraction-Gas Chromatography-Mass Spectrometry and Liquid Chromatography-Tandem Mass Spectrometry. *J. AOAC Int.* **2006**, *89*, 740–771.

(7) Okihashi, M.; Takatori, S.; Kitagawa, Y.; Tanaka, Y. Simultaneous Analysis of 260 Pesticide Residues by Gas Chromatography/Triple Quadripole Mass Spectrometry. *J. AOAC Int.* **2007**, *90*, 1165–1179.

(8) Schenck, F.; Wong, J.; Holcomb, J.; Li, J.; Lu, C.; Mitchell, L. *Multiresidue Analysis of 102 Organophosphorus Pesticides in Produce at Parts-Per Billion Levels using a Modified QuEChERS Method and GC with Pulsed Flame Photometric Detection*; Laboratory Information Bulletin 4418,; U.S. Food and Drug Administration, Division of Field Science: HCC-140, Rockville, MD, 2008.

(9) Schenck, F. J.; Brown, A.; Podhorniak, L.; Parker, A.; Reliford, M.; Wong, J. A. Rapid Multiresidue Method for Determination of Pesticides in Fruits and Vegetables by Using Acetonitrile Extraction/Partitioning and Solid-Phase Extraction Column Cleanup. *J. AOAC Int.* **2008**, *91*, 422–438.

(10) Takatori, S.; Okihashi, M.; Okomata, Y.; Kitagawa, Y.; Kakimoto, S.; Murata, H.; Sumimoto, T.; Tanaka, Y. A Rapid and Easy Multiresidue Method for the Determination of Pesticide Residues in Vegetables, Fruits and Cereals Using Liquid Chromatography/Tandem Mass Spectrometry. *J. AOAC Int.* **2008**, *91*, 871–883.

(11) Chamkasem, N.; Schenk, F. J.; Armstead, H.; Parker, A.; Wong, J.; Dimandja, J. M.; Mitchell, L. Laboratory Information Bulletin 4378; U.S. Food and Drug Administration, Division of Field Science: HCC-140, Rockville MD, 2009.

(12) Schenck, F.; Wong, J; Zhang, D.; Krynitsky, A.; Makovi, C.; Garris, S.; Craft, C.; Bogner, M.; Mayers, D.; Stock, R.; Barry, E; Podhorniak, L.; Brown, A.; Pelt, J.; Stepp, J.; Johnson, Y.; Hansen, P.; Eide, D.; Sack, C.; Phillips, T.; Liu, X.; Abdelmesseh, H.; Katsoudas, E.; Grant, D.; Leung, D.; Mazurin, T.; Pequegnat, J.; Wang, J.; Mitchell, L.; Dimandja, J. M. *Two Modified QuEChERS Methods for the Multiresidue Determination of Pesticides in Produce Samples: An Interlaboratory Study*; Laboratory Information Bulletin 4442; U.S. FDA, Division of Field Science: HCC-140, Rockville MD, 2009.

(13) Wong, J.; Zhang, D.; Tech, K.; Hayward, D.; Krynitsky, A.; Cassias, I.; Schenck, F.; Banerjee, K.; Dasgupta, S.; Brown, D. *Multiresidue Pesticide Analysis of Ginseng Powders using Acetonitrile or Acetone-based Extraction, Solid-phase extraction cleanup and Gas Chromatography−Mass spectrometry/Selective Ion Monitoring (GC-MS/SIM) or −Tandem Mass Spectrometry (GC−MS/MS)*; Laboratory

Information Bulletin 4453; U.S. FDA, Division of Field Science: HCC-140, Rockville MD, 2010.

(14) Wong, J. W.; Zhang, K.; Tech, K.; Hayward, D.; Makovi, C.; Krynitsky, A. J.; Schenck, F.; Banerjee, K.; Dasgupta, S.; Brown, D. Multiresidue Pesticide Analysis in Fresh Produce by Capillary Gas Chromatography—Mass Spectrometry/Selective Ion Monitoring (GC-MS/SIM) and — Tandem Mass Spectrometry (GC-MS/MS). *J. Agric. Food Chem.* **2010**, *58*, 5868–5883.

(15) Wong, J. W.; Hao, C.; Zhang, K.; Yang, P.; Banerjee, K.; Hayward, D.; Iftakhar, I.; Schreiber, A.; Tech, K.; Sack, C.; Smoker, M.; Chen, X.; Utture, S.; Oulkar, D. Development and Interlaboratory Validation of a QuEChERS-Based Liquid Chromatography—Tandem Mass Spectrometry Method for Multiresidue Pesticide Analysis. *J. Agric. Food Chem.* **2010**, *58*, 5897–5903.

(16) Lehotay, S.; Son, K.; Kwon, H.; Koesukwiwat, U.; Fu, W.; Mastovska, K.; Hoh, E.; Leepipatpiboon, N. Comparison of QuEChERS Sample Preparation Methods for the Analysis of Pesticide Residues in Fruits and Vegetables. *J. Chromatogr., A* **2010**, *1217*, 2548–2560.

(17) Kruve, A.; Künnapas, A.; Herodes, K.; Leito, I. Matrix Effects in Pesticide Multi-residue Analysis by Liquid Chromatography-Mass Spectrometry. *J. Chromatogr., A* **2008**, *1187*, 58–66.

(18) Report of the ORA Science Peer Review Committee on FDA Pesticides Program. October 12, 2004.

(19) Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. Revision of the List of Methods for Pesticide Residue Analysis Including Methods of Determination for Dithiocarbamates. July, 2007.

(20) Analytical Methods for Residual Compositional Substances of Agricultural Chemicals, Feed Additives, and Veterinary Drugs in Food, Department of Food Safety, Ministry of Health, Labor and Welfare. Japan. May 26, 2006.

(21) Application Note 3200 QTRAP LC/MS/MS System. Simultaneous Quantitative Screening and Qualitative Confirmation of 300 Pesticides Using the New 3200 Q TRAP LC/MS/MS System.

(22) Alder, L.; Greulich, K.; Kempe, G.; Vieth, B. Residue analysis of 500 high priority pesticides: better by GC-MS or LC-MS/MS?. *Mass Spectrom. Rev.* **2006**, *25*, 838–865.

(23) Mercer, G. A. *A multi-residue pesticide monitoring procedure using gas chromatography with mass selective detection and selected ion monitoring for the determination of pesticides containing nitrogen, sulfur and/or oxygen*; Laboratory Information Bulletin 4308; 2003. Website: http://inside.fda.gov:9003/downloads/PolicyProcedures/Laboratories/LaboratoryInformationBulletins/UCM193060.pdf.