Using the iD*Quant*™ Standards Kit for Pesticide Analysis to Analyze Residues in Fruits and Vegetable Samples

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Overview

The iD*Quant*™ Standards Kit for Pesticide Analysis of certified reference material (ISP Guide 34, IOS/IEC 17025 and ISO 9001:2008) includes 204 pesticides. This kit has been designed to eliminate the need to source pesticides individually, and measure each one manually. This allows to setup, verify, and validate the performance of your pesticide screening method faster.

Here we present example data where we used the iD*Quant*™ Kit to screen for, quantify, and identify pesticides in extracts of fruits and vegetables using Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS) with an AB SCIEX QTRAP® 5500 system.

Introduction

Many recent developments such as generic extraction procedures, (like QuEChERS), generic LC separation methods, and highly selective and sensitive MS/MS detectors have established LC-MS/MS as a standard technique for the analysis of pesticide residues in food. Hundreds of targeted analytes can be detected in a single analytical run. Software designed for ease-of-use like Cliquid® software in conjunction with iMethod™ applications made the fast adaptation of LC-MS/MS in new routine testing laboratories possible. For a while, data processing was the bottleneck for most laboratories, but the development of fast and automatic data processing and reporting tools sped up the delivery of analytical results.

A major hurdle for each laboratory is method setup, verification, and validation. The newly available iD*Quant*™ kit can be used for the following tasks:

- Tune or verify MRM transitions for best selectivity and sensitivity of MS/MS detection
- Measure retention times to quickly update acquisition methods using the *Scheduled* MRM™ algorithm
- Verify the performance of LC methods
- Investigate recovery and reproducibility of sample preparation procedures
- Validate the performance of the complete method procedure

Description of the iD*Quant*™ Standards Kit for Pesticide Analysis

Content

The iD*Quant*™ Kit contains a total of 204 pesticides in 10 mixes at a concentration of 100 µg/mL and a certificate of analysis for each mixture. The pesticides are listed in Table 1.

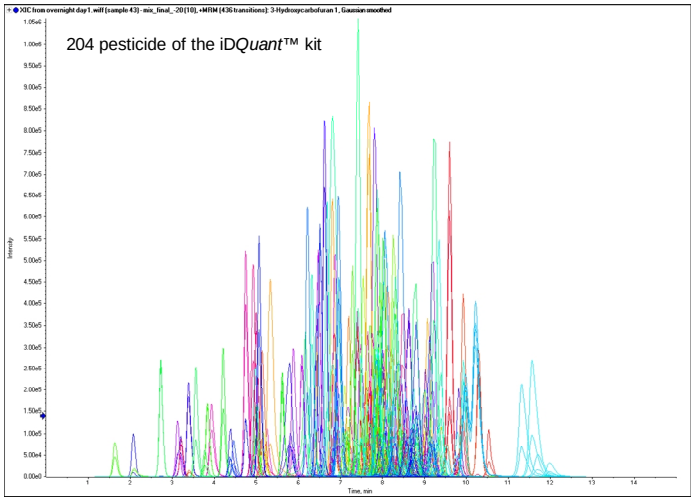


Figure 1. Detection of 204 pesticides of the iD*Quant*™ Kit using a 4000 QTRAP® system and 2 MRM transitions per pesticide

Table 1. Pesticides in the iD*Quant*™ Standard Kit for Pesticide Analysis

Pesticide	CAS	Formula
Standard A1		
3-Hydroxycarbofuran	16655-82-6	C12H15NO4
Acephate	30560-19-1	C4H10NO3PS
Aldicarb sulfone	1646-88-4	C7H14N2O4S
Aldicarb sulfoxide	1646-87-3	C7H14N2O3S
Aminocarb	2032-59-9	C11H16N2O2
Butocarboxim	34681-10-2	C7H14N2O2 S
Butoxycarboxim	34681-23-7	C7H14N2O4S
Carbendazim	10605-21-7	C9H9N3O2
Cymoxanil	57966-95-7	C7H10N4O3
Dicrotophos	141-66-2	C8H16NO5P
Dimethoate	60-51-5	C5H12NO3PS2
Dioxacarb	6988-21-2	C11H13NO4
Formetanate HCL	23422-53-9	C11H15N3O2 . HCl
Fuberidazole	3878-19-1	C11H8N2O
Methamidophos	10265-92-6	C2H8NO2PS
Methomyl	16752-77-5	C5H10N2O2S
Mevinphos	7786-34-7	C7H13O6P
Monocrotophos	6923-22-4	C7H14NO5P
Omethoate	1113-02-6	C5H12NO4PS
Oxamyl	23135-22-0	C7H13N3O3S
Propamocarb	24579-73-5	C9H20N2O2
Thiabendazole	148-79-8	C10H7N3S
Tricyclazole	41814-78-2	C9H7N3S
Vamidothion	2275-23-2	C8H18NO4PS2
Standard B1		
Aldicarb	116-06-3	C7H14N2O2S
Bendiocarb	22781-23-3	C11H13NO4
Carbaryl	63-25-2	C12H11NO2
Carbetamide	16118-49-3	C12H16N2O3
Carbofuran	1563-66-2	C12H15NO3
Carboxin	5234-68-4	C12H13NO2S
Clethodim	99129-21-2	C17H26ClNO3S
Desmedipham	13684-56-5	C16H16N2O4
Diethofencarb	87130-20-9	C14H21NO4
Ethiofencarb	29973-13-5	C11H15NO2S
Furalaxyl	57646-30-7	C17H19NO4
Iprovalicarb	140923-17-7	C18H28N2O3
Isoprocarb	2631-40-5	C11H15NO2
Metalaxyl	57837-19-1	C15H21NO4
Methiocarb	2032-65-7	C11H15NO2S
Mexacarbate	315-18-4	C12H18N2O2
Oxadixyl	77732-09-3	C14H18N2O4
Pirimicarb	23103-98-2	C11H18N4O2
Promecarb	2631-37-0	C12H17NO2
Propham	122-42-9	C10H13NO2
Propoxur	114-26-1	C11H15NO3
Pyracarbolid	24691-76-7	C13H15NO2
Thiofanox	39196-18-4	C9H18N2O2S
Thiophanate-methyl	23564-05-8	C12H14N4O4S2
Standard C1		
Alanycarb	83130-01-2	C17H25N3O4S2
Amitraz	33089-61-1	C19H23N3
Benalaxyl	71626-11-4	C20H23NO3
Benfuracarb	82560-54-1	C20H30N2O5S
Benzoximate	29104-30-1	C18H18ClNO5
Bifenazate	149877-41-8	C17H20N2O3
Cyazofamid	120116-88-3	C13H13ClN4O2S
Fenamidone	161326-34-7	C17H17N3OS
Fenazaquin	120928-09-8	C20H22N2O
Fenhexamid	126833-17-8	C14H17Cl2NO2
Fenoxycarb	79127-80-3	C17H19NO4
Flufenacet	142459-58-3	C14H13F4N3O2S
Furathiocarb	65907-30-4	C18H26N2O5S
Indoxacarb	144171-61-9	C22H17ClF3N3O7
Mefenacet	73250-68-7	C16H14N2O2S
Mepronil	55814-41-0	C17H19NO2
Piperonyl butoxide	51-03-6	C19H30O5
Quinoxyfen	124495-18-7	C15H8Cl2FNO
Spiroxamine	118134-30-8	C18H35NO2
Thiobencarb	28249-77-6	C12H16ClNOS
Zoxamide	156052-68-5	C14H16Cl3NO2

Standard D1

Acetamiprid	135410-20-7	C10H11ClN4
Acibenzolar-S-methyl	135158-54-2	C8H6N2OS2
Bromucanozole	116255-48-2	C13H12BrCl2N3O
Clothianidin	210880-92-5	C6H8ClN5O2S
Cyproconazole	113096-99-4	C15H18ClN3O
Epoxiconazole	135319-73-2	C17H13ClFN3O
Etaconazole	60207-93-4	C14H15Cl2N3O2
Fenarimol	60168-88-9	C17H12Cl2N2O
Flutriafol	76674-21-0	C16H13F2N3O
Imazalil	35554-44-0	C14H14Cl2N2O
Imidacloprid	138261-41-3	C9H10ClN5O2
Metribuzin	21087-64-9	C8H14N4OS
Myclobutanil	88671-89-0	C15H17ClN4
Nitenpyram	120738-89-8	C11H15ClN4O2
Nuarimol	63284-71-9	C17H12ClFN2O
Paclobutrazol	76738-62-0	C30H40Cl2N6O2
Pyrimethanil	53112-28-0	C12H13N3
Thiacloprid	111988-49-9	C10H9ClN4S
Thiamethoxam	153719-23-4	C8H10ClN5O3S
Triadimenol	55219-65-3	C14H18ClN3O2
Triticonazole	131983-72-7	C17H20ClN3O

Standard E1

Abamectin	71751-41-2	C48H72O14; C47H70O14
Bitertanol	55179-31-2	C20H23N3O2
Bupirimate	41483-43-6	C13H24N4O3S
Butafenacil	134605-64-4	C20H18ClF3N2O6
Clofentezine	74115-24-5	C14H8Cl2N4
Cyprodinil	121552-61-2	C14H15N3
Diclobutrazol	75736-33-3	C15H19Cl2N3O
Difenoconazole	119446-68-3	C19H17Cl2N3O3
Diniconazole	83657-24-3	C15H17Cl2N3O
Ethofumesate	26225-79-6	C13H18O5S
Fenbuconazole	114369-43-6	C19H17ClN4
Flusilazole	85509-19-9	C16H15F2N3Si
Flutolanil	66332-96-5	C17H16F3NO2
Hexaconazole	79983-71-4	C14H17Cl2N3O
Mepanipyrim	110235-47-7	C14H13N3
Metconazole	125116-23-6	C17H22ClN3O
Methoxyfenozide	161050-58-4	C22H28N2O3
Penconazole	66246-88-6	C13H15Cl2N3
Prochloraz	67747-09-5	C15H16Cl3N3O2
Propiconazole	60207-90-1	C15H17Cl2N3O2
Rotenone	83-79-4	C23H22O6
Tebufenozide	112410-23-8	C22H28N2O2
Triflumizole	68694-11-1	C15H15ClF3N3O

Standard F1

Carfentrazone-ethyl	128639-02-1	C15H14Cl2F3N3O3
Doramectin	117704-25-3	C50H74O14
Emamectin-benzoate	155569-91-8	C49H75NO13; C48H73NO13
Eprinomectin	123997-26-2	C50H75NO14; C49H73NO14
Fluquinconazole	136426-54-5	C16H8Cl2FN5O
Hexythiazox	78587-05-0	C17H21ClN2O2S
Hydramethylnon	67485-29-4	C25H24F6N4
Ipconazole	125225-28-7	C18H24ClN3O
Ivermectin	70288-86-7	C48H74O14
Mesotrione	104206-82-8	C14H13NO7S
Moxidectin	113507-06-5	C37H53NO8
Propargite	2312-35-8	C19H26O4 S
Spinosad	168316-95-8	C41H65NO10
Spirodiclofen	148477-71-8	C21H24Cl2O4
Spiromesifen	283594-90-1	C23H30O4
Tebuconazole	107534-96-3	C16H22ClN3O
Tetraconazole	112281-77-3	C13H11Cl2F4N3O

Standard G1

Chlortoluron	15545-48-9	C10H13ClN2O
Cycluron	2163-69-1	C11H22N2O
Diuron	330-54-1	C9H10Cl2N2O
Fenuron	101-42-8	C9H12N2O
Fluometuron	2164-17-2	C10H11F3N2O
Forchlorfenuron	68157-60-8	C12H10ClN3O
Isoproturon	34123-59-6	C12H18N2O

Methabenzthiazuron	18691-97-9	C10H11N3OS
Methoprotryne	841-06-5	C11H21N5OS
Metobromuron	3060-89-7	C9H11BrN2O2
Monolinuron	1746-81-2	C9H11ClN2O2
Prometon	1610-18-0	C10H19N5O
Pymetrozine	123312-89-0	C10H11N5O
Secbumeton	26259-45-0	C10H19N5O
Simetryn	1014-70-6	C8H15N5S
Sulfentrazone	122836-35-5	C11H10Cl2F2N4O3S
Tebuthiuron	34014-18-1	C9H16N4OS
Terbumeton	33693-04-8	C10H19N5O
Thidiazuron	51707-55-2	C9H8N4OS
Triadimefon	43121-43-3	C14H16ClN3O2

Standard H1

Ametryn	834-12-8	C9H17N5S
Azoxystrobin	131860-33-8	C22H17N3O5
Boscalid	188425-85-6	C18H12Cl2N2O
Chloroxuron	1982-47-4	C15H15ClN2O2
Diflubenzuron	35367-38-5	C14H9ClF2N2O2
Dimethomorph	110488-70-5	C21H22ClNO4
Dimoxystrobin	149961-52-4	C19H22N2O3
Famoxadon	131807-57-3	C22H18N2O4
Fipronil	120068-37-3	C12H4Cl2F6N4OS
Fludioxonil	131341-86-1	C12H6F2N2O2
Hexaflumuron	86479-06-3	C16H8Cl2F6N2O3
Kresoxim-methyl	143390-89-0	C18H19NO4
Linuron	330-55-2	C9H10Cl2N2O2
Neburon	555-37-3	C12H16Cl2N2O
Phenmedipham	13684-63-4	C16H16N2O4
Picoxystrobin	117428-22-5	C18H16F3NO4
Prometryne	7287-19-6	C10H19N5S
Siduron	1982-49-6	C14H20N2O
Terbutryne	886-50-0	C10H19N5S
Triflumuron	64628-44-0	C15H10ClF3N2O3

Standard J1

Buprofezin	69327-76-0	C16H23N3OS
Chlorfluazuron	71422-67-8	C20H9Cl3F5N3O3
Ethiprole	181587-01-9	C13H9Cl2F3N4OS
Etoxazole	153233-91-1	C21H23F2NO2
Fenpropimorph	67564-91-4	C20H33NO
Fenpyroximate	111812-58-9	C24H27N3O4
Fluazinam	79622-59-6	C13H4Cl2F6N4O4
Flufenoxuron	101463-69-8	C21H11ClF6N2O3
Fluoxastrobin	361377-29-9	C21H16ClFN4O5
Lufenuron	103055-07-8	C17H8Cl2F8N2O3
Novaluron	116714-46-6	C17H9ClF8N2O4
Prothioconazole	178928-70-6	C14H15Cl2N3OS
Pyraclostrobin	175013-18-0	C19H18ClN3O4
Pyridaben	96489-71-3	C19H25ClN2OS
Pyriproxyfen	95737-68-1	C20H19NO3
Tebufenpyrad	119168-77-3	C18H24ClN3O
Teflubenzuron	83121-18-0	C14H6Cl2F4N2O2
Trifloxystrobin	141517-21-7	C20H19F3N2O4

Standard K1

Chlorantraniliprole	500008-45-7	C18H14BrCl2N5O2
Cyromazine	66215-27-8	C6H10N6
Dinotefuran	165252-70-0	C7H14N4O3
Ethirimol	23947-60-6	C11H19N3O
Fenobucarb	3766-81-2	C12H17NO2
Flonicamid	158062-67-0	C9H6F3N3O
Flubendimide	272451-65-7	C23H22F7IN2O4S
Halofenozide	112226-61-6	C18H19ClN2O2
Isocarbophos	24353-61-5	C11H16NO4PS
Mandipropamid	374726-62-2	C23H22ClNO4
Metaflumizone	139968-49-3	C24H16F6N4O2
Pencycuron	66063-05-6	C19H21ClN2O
Spinetoram	187166-40-1	C42H69NO10
Spirotetramat	203313-25-1	C21H27NO5
Temephos	3383-96-8	C16H20O6P2S3

Storage and Stability

The pesticides of the iD*Quant*™ Kit are stable in the sealed ampoules for one year at -20°C. An example of stability test results is shown in Figure 2.

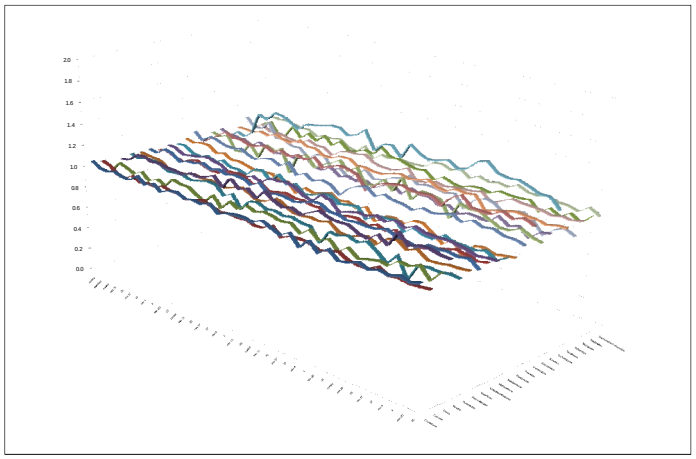


Figure 2. Results of the stability testing indicating stability of all pesticides over a period of one year at -20°C

Stock solutions of the 204 pesticides at 1 µg/kg can be simply prepared by mixing 10 µL of each mix and diluting with 900 µL of acetonitrile. It is recommended to store aliquots of this stock solution in amber glass vials at -20°C, for future use.

Calibration standards can be prepared by serial dilution of the prepared stock solutions using water or the aqueous mobile phase of the LC system. It is recommended to prepare calibration standards daily to avoid degradation of pesticides.

LC-MS/MS Method Setup

The iMethod™ application for pesticide screening contains a ready-to-use method to screen for all 204 pesticides of the iD*Quant*™ kit.

The kit can also be used to fine tune MRM conditions or to adjust retention times of existing methods to update the *Scheduled* MRM™ algorithm for best performance (Figure 3).

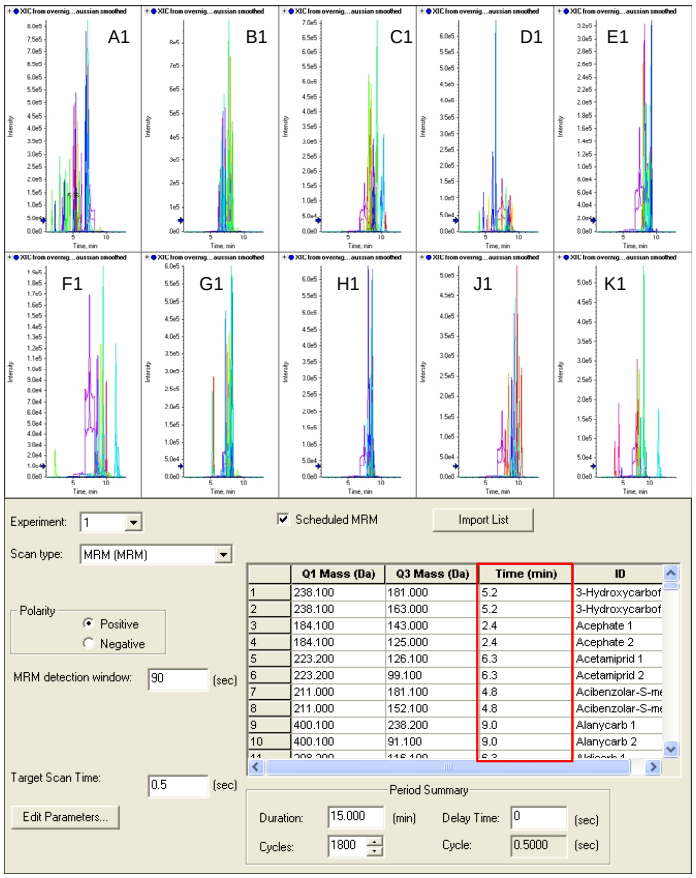


Figure 3. The injection of each mix and processing in MultiQuant™ software can be used to quickly update retention times in the *Scheduled* MRM™ method for best performance

Application of the iD*Quant*™ Standards Kit for Pesticide Analysis

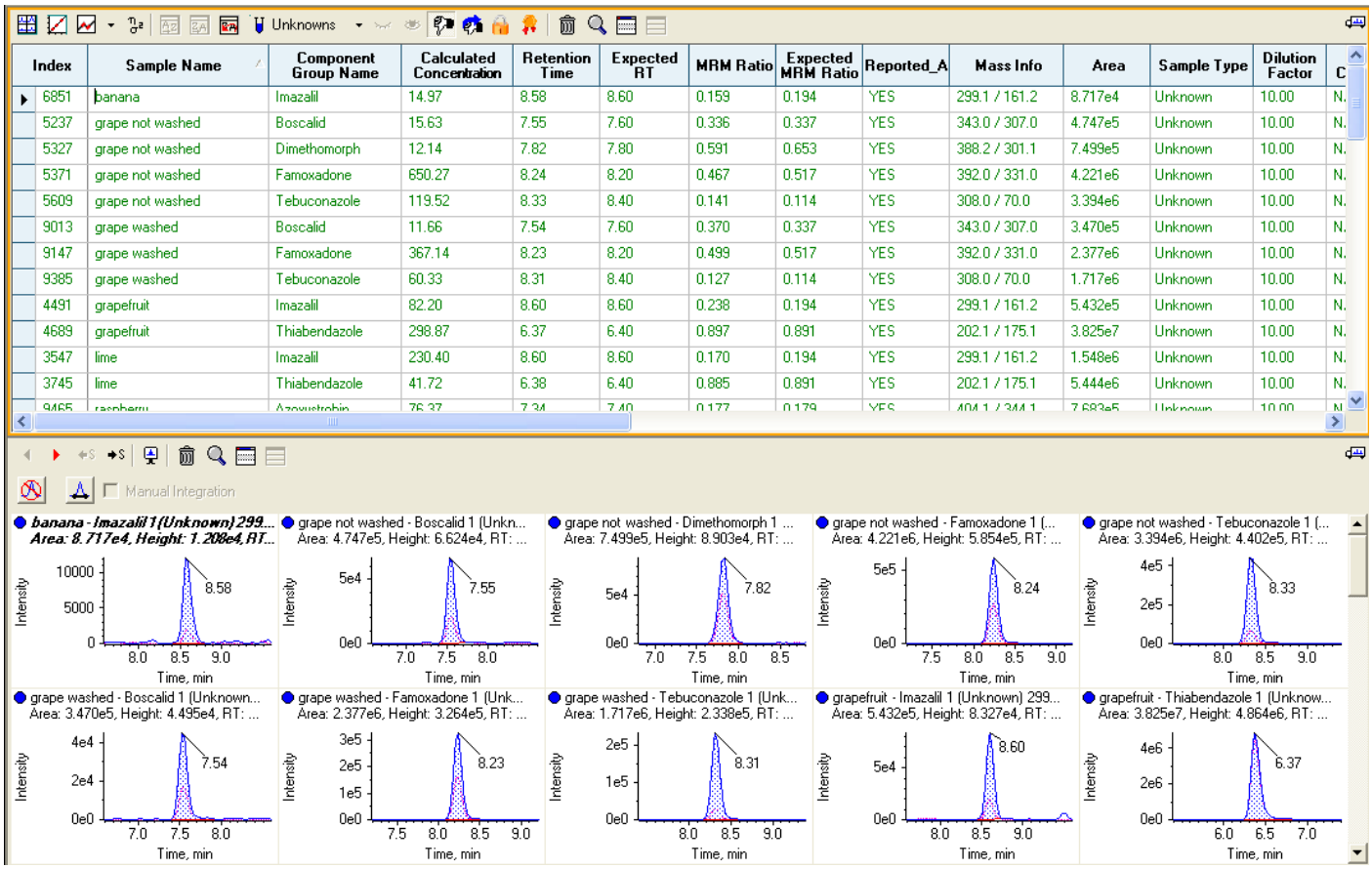
Experimental

- The iD*Quant*™ standards kit for pesticide analysis was used for method setup and preparation of calibration standards.
- Fruit and vegetable samples were extracted using a QuEChERS procedure and diluted 10x with water to optimize chromatographic peak shape and minimize possible matrix effects and interferences.[1-2]
- LC separation was achieved on a Shimadzu UFLC$_{XR}$ system with a Restek Ultra Aqueous C18 3 µm (100x2.1 mm) column and a 15 min gradient of water and methanol with ammonium formate buffer at a flow rate of 0.5 mL/min. The injection volume was set to 10 µL.
- The AB SCIEX QTRAP® 5500 system was operated with Turbo V™ source and Electrospray Ionization (ESI) probe. 436 MRM transitions were monitored using the *Scheduled* MRM™ algorithm with a detection window of 120 s and a target scan time of 0.7 s.
- MultiQuant™ 2.0.2 software with the 'Multicomponent' query was used for quantitative data processing (Figure 4).

Table 2. Pesticides quantified in fruit and vegetable samples above 10 µg/kg and positively identified using their retention time and MRM ratio

Sample	Pesticide	Concentration (µg/kg)	Retention time (min)	Expected retention time (min)	MRM ratio	Expected MRM ratio
Tomato (grape)	Imidacloprid	16.1	4.44	4.50	1.02	0.98
	Methoxyfenozide	21.0	7.42	7.50	0.25	0.23
Tomato (vine)	Boscalid	33.8	7.55	7.60	0.30	0.34
	Pyraclostrobin	11.4	8.47	8.50	0.76	0.77
	Pyridaben	18.6	9.79	9.80	0.93	0.91
	Tebufenozide	42.4	7.90	7.90	0.40	0.43
Raspberry	Azoxystrobin	76.4	7.34	7.40	0.18	0.18
	Boscalid	42.0	7.52	7.60	0.31	0.34
	Myclobutanil	46.7	7.75	7.80	0.56	0.65
	Pyraclostrobin	10.4	8.44	8.50	0.80	0.77
Strawberry	Boscalid	23.0	7.54	7.60	0.30	0.34
	Cyprodinil	203	8.67	8.70	0.82	0.93
	Fenhexamid	141	7.59	7.60	0.94	0.87
	Fludioxinil	127	7.47	7.50	0.25	0.25
	Novaluron	27.8	8.82	8.80	0.70	0.84
	Pyrimethanil	24.2	7.81	7.90	0.21	0.22
Banana	Imazalil	15.0	8.58	8.60	0.16	0.19
Lime	Imazalil	230	8.60	8.60	0.17	0.19
	Thiabendazole	41.7	6.38	6.40	0.89	0.89
Grapefruit	Imazalil	82.2	8.60	8.60	0.24	0.19
	Thiabendazole	299	6.37	6.40	0.90	0.89
Red pepper	Azoxystrobin	12.4	7.37	7.40	0.16	0.18
	Clothianidin	18.4	4.15	4.20	1.05	1.14
	Imidacloprid	83.0	4.46	4.50	0.97	0.98
	Tebufenozide	41.1	7.90	7.90	0.36	0.43
	Thiamethoxam	64.0	3.61	3.60	0.84	0.96
Grapes not washed	Boscalid	15.6	7.55	7.60	0.34	0.34
	Dimethomorph	12.1	7.82	7.80	0.59	0.65
	Famoxadone	650	8.24	8.20	0.47	0.52
	Tebuconazole	130	8.32	8.4	0.14	0.11
Grapes washed	Boscalid	11.7	7.54	7.60	0.37	0.34
	Dimethomorph*	5.91	7.81	7.80	0.63	0.65
	Famoxadone	367	8.23	8.20	0.50	0.52
	Tebuconazole	60.3	8.31	8.40	0.13	0.11

* Dimethomorph in the grapes sample after washing was quantified and identified below 10 µg/kg



Index	Sample Name	Component Group Name	Calculated Concentration	Retention Time	Expected RT	MRM Ratio	Expected MRM Ratio	Reported_A	Mass Info	Area	Sample Type	Dilution Factor	C
6851	banana	Imazalil	14.97	8.58	8.60	0.159	0.194	YES	299.1 / 161.2	8.717e4	Unknown	10.00	N.
5237	grape not washed	Boscalid	15.63	7.55	7.60	0.336	0.337	YES	343.0 / 307.0	4.747e5	Unknown	10.00	N.
5327	grape not washed	Dimethomorph	12.14	7.82	7.80	0.591	0.653	YES	388.2 / 301.1	7.499e5	Unknown	10.00	N.
5371	grape not washed	Famoxadone	650.27	8.24	8.20	0.467	0.517	YES	392.0 / 331.0	4.221e6	Unknown	10.00	N.
5609	grape not washed	Tebuconazole	119.52	8.33	8.40	0.141	0.114	YES	308.0 / 70.0	3.394e6	Unknown	10.00	N.
9013	grape washed	Boscalid	11.66	7.54	7.60	0.370	0.337	YES	343.0 / 307.0	3.470e5	Unknown	10.00	N.
9147	grape washed	Famoxadone	367.14	8.23	8.20	0.499	0.517	YES	392.0 / 331.0	2.377e6	Unknown	10.00	N.
9385	grape washed	Tebuconazole	60.33	8.31	8.40	0.127	0.114	YES	308.0 / 70.0	1.717e6	Unknown	10.00	N.
4491	grapefruit	Imazalil	82.20	8.60	8.60	0.238	0.194	YES	299.1 / 161.2	5.432e5	Unknown	10.00	N.
4689	grapefruit	Thiabendazole	298.87	6.37	6.40	0.897	0.891	YES	202.1 / 175.1	3.825e7	Unknown	10.00	N.
3547	lime	Imazalil	230.40	8.60	8.60	0.170	0.194	YES	299.1 / 161.2	1.548e6	Unknown	10.00	N.
3745	lime	Thiabendazole	41.72	6.38	6.40	0.885	0.891	YES	202.1 / 175.1	5.444e6	Unknown	10.00	N.
9465	raspberry	Azoxystrobin	76.37	7.34	7.40	0.177	0.179	YES	404.1 / 344.1	7.693e5	Unknown	10.00	N.

Figure 4. Result display after applying the 'Multicomponent' query in MultiQuant™ software, only pesticides above 10 µg/kg and with a positive identification based on their MRM ratio are displayed

Results

All identified and quantified pesticides are listed in Table 2. The 'Multicomponent' query was used in MultiQuant™ software to automatically report concentrations above the threshold of 10 µg/kg and to compare MRM ratios for compound identification. The maximum permitted tolerances for MRM intensities of the SANCO guideline 'Method validation and quality control procedures for pesticide residue analysis in food and feed' were used for identification.[3]

As part of the samples set grapes were analyzed with and without washing before extraction. Half of the grapes were simply rinsed under running tap water for approximately 30 seconds.

The results in Table 2 show that this washing method is not sufficient to completely remove fungicide before consumption.

Summary

The iD*Quant*™ Standards Kit for Pesticide Analysis was used to successfully setup an LC-MS/MS method to identify and quantify pesticides in fruit and vegetable samples. This was accomplished in less time than it was previously possible when a standard kit was either unavailable or had to be created from individual standards.

References

1 M. Anastassiades, et al.: J. AOAC Int. 86 (2003) 412-431

2 J. Wong et al.: J. Agric. Food Chem. 58 (2010) 5897-5903

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