APPLICATION NOTE

QSight™ LC-MS/MS

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Estimation of 136 pesticide residue in Black Pepper using QuEChERS extraction technique and QSight™ LC-MS/MS.

Introduction

India is one of the largest producers of Spices in the world (40% of world production) with major exports being black pepper, cardamom, cumin, turmeric, etc. Kerala in the southern India is the major spice producers in the country and aptly know as 'The Land of Spices'. Amongst all spices, Black pepper is the most prominent one and is the third largest commodity with respect to production and export in the world. Due to its specific pungent aroma and flavor, Black pepper is used in various food preparations. Nowadays, black pepper cultivators are under threat due to the infestation of various diseases and pests.

Major diseases in Black pepper include foot rot, anthracnose, leaf rot, blight and basal wilt. *Lophobaris piperis, Diconocoris hewetti* and *Dasynus piperis* are some of the major pests[5-7]. Considering production of black pepper in India and import-export regulation of each country, existence of pesticide residue above maximum residue limit (MRLs) will create major effect on export trade market. More than 2.5 million tons of pesticides are used worldwide per year, which includes insecticides, herbicides and fungicides for better production of black pepper[8]. The uncontrolled use of pesticides has become a concern and poses potential risk to human health and environment. Nowadays, people are very conscious and aware about its harmful effect on health while the same awareness is comparatively

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less in producers in agriculture field. Considering the aforementioned concerns and current requirements, Analytical method for pesticide residues using QSight™ LC-MS/MS was developed and optimized in Black pepper. It is very well known that Black pepper is a complex matrix that requires a proper extraction and clean-up method for interferences. In this study, a fast, sensitive and selective multi-residue method has been developed for analysis of over 136 pesticides in Pepper samples by coupling a modified QuEChERS extraction method with LC/MS/MS. Using time-managed- MRM™ in the QSight™ triple quadrupole mass spectrometer, the optimum dwell time of multiple MRM transitions can be generated automatically for the targeted analytes. This not only saves time in method development but also improves data quality and analytical performance of instrument.

Experimental

Hardware/Software

The chromatographic separation was conducted by a PerkinElmer LX-50 UHPLC system and detection was achieved using a PerkinElmer QSight™ 220 triple quadrupole mass spectrometer, equipped with both ESI and APCI ionization sources. All instrument control, data acquisition and data processing were performed using single window software Simplicity 3Q™.

Method Parameters:

LC Parameters: LC parameters including column and mobile phase gradient program are given in the table 1.

Table 1. UHPLC Parameters

LC Column	Quasar AQ, (P/N N9308845)
Mobile Phase A	5 mM Ammonium Formate + 0.1% Formic acid in water
Mobile Phase B	5 mM Ammonium Formate + 0.1% Formic acid in Methanol
Mobile Phase Gradient	

Sr. No	Time	%A	%B
1	0.00	90	10
2	1.00	90	10
3	15.00	5	95
4	17.00	5	95
5	17.5.0	90	10
6	20.0	90	10

Column Oven Temperature	45 °C
Auto sampler Temperature	15 °C
Injection Volume	10 µL
Flow	0.6 mL/Min
Run Time	20 minutes

MS Parameters:

Table 2. MS parameters

ESI Voltage (Positive & Negative)	5500 &-5000
Drying Gas	150
Nebulizer Gas	200
Source Temperature	320 °C

***Note: Above parameters will vary instrument to instrument.*

Table 3. Selected MRM Transitions and Retention time of analytes

Sr. No	Compound Name	Polarity	Q1 Mass	Q2 Mass	RT	CE
1	Acephate-1	Positive	184.1	143	3.5	-12
2	Acephate-2	Positive	184.1	125	3.5	-28
3	Acetamiprid-1	Positive	223.2	126.1	5.7	-36
4	Acetamiprid-2	Positive	223.2	99.1	5.7	-58
5	Ametoctradin-1	Positive	277	150	11.9	-35
6	Ametoctradin-2	Positive	277	177	11.9	-28
7	Bifenazate-1	Positive	301.1	198	9.1	-16
8	Bifenazate-2	Positive	301.1	170	9.1	-36
9	Bifenthrin-1	Positive	440.1	166.1	16.2	-76
10	Bifenthrin-2	Positive	440.1	181.1	16.2	-28
11	Carbendazim-1	Positive	192.1	132.1	5.0	-40
12	Carbendazim-2	Positive	192.1	160.09	5.0	-24
13	Carbofuran 3 Hydroxy-1	Positive	238.3	163.06	5.6	-24
14	Carbofuran 3 Hydroxy-2	Positive	238.3	181.08	5.6	-18
15	Carbofuran-1	Positive	222.13	165.2	7.0	-18
16	Carbofuran-2	Positive	222.13	123.1	7.0	-60
17	Chlorpyriphos-methyl-1	Positive	322	125.1	11.9	-15
18	Chlorpyriphos-methyl-2	Positive	322	290	11.9	-22
19	Cyazofamid 1	Positive	325	261	9.6	-13
20	Cyazofamid 2	Positive	325	108	9.6	-21
21	Dementon-S-Methyl Sulphone 1	Positive	263	169	4.5	-22
22	Dementon-S-Methyl Sulphone 2	Positive	263	121	4.5	-22
23	Dichlorvos-1	Positive	221	109.1	7.3	-22
24	Dichlorvos-2	Positive	221	79	7.3	-38
25	Dodine 1	Positive	228	57	10.8	-20
26	Dodine 2	Positive	228	71	10.8	-20
27	Emamectin Benzoate 1	Positive	886.5	158	12.5	-52
28	Emamectin Benzoate 2	Positive	886.5	82	12.5	-56
29	Ethiprole 1	Positive	397.1	228	7.4	-64
30	Ethiprole 2	Positive	397.1	254.2	8.4	-46
31	Etrimphos 1	Positive	293.1	265	8.0	-24

Sr. No	Compound Name	Polarity	Q1 Mass	Q2 Mass	RT	CE
32	Etrimphos 2	Positive	293.1	125	8.0	-42
33	Fenthion Sulphoxide 1	Positive	295	279.9	7.0	-26
34	Fenthion Sulphoxide 2	Positive	295	47	7.0	-68
35	Fipronil-1	Negative	435	250	10.1	36
36	Fipronil-2	Negative	435	330	10.1	20
37	Flufenzine-1	Positive	307.4	131.07	15.8	-78
38	Flufenzine-2	Positive	307.4	161.11	15.8	-28
39	Fluopicolide 1	Positive	383	173	8.8	-64
40	Fluopicolide 2	Positive	383	145	8.8	-64
41	Iprobenphos 1	Positive	289.1	204.9	10.3	-14
42	Iprobenphos 2	Positive	289.1	91	10.3	-62
43	Iprovalicarb-1	Positive	321.2	119	9.4	-60
44	Iprovalicarb-2	Positive	321.2	203.2	9.4	-12
45	Kresoxim-methyl-1	Positive	314.1	235	10.5	-30
46	Kresoxim-methyl-2	Positive	314.1	222	10.5	-32
47	Nitenpyram 1	Positive	271	237	4.3	-28
48	Nitenpyram 2	Positive	271	126	4.3	-60
49	Oxydementon Methyl-1	Positive	247.2	109	10.7	-46
50	Oxydementon Methyl-2	Positive	247.2	169.04	10.7	-20
51	Paraxon Methyl-1	Positive	248.1	202	6.8	-22
52	Paraxon Methyl-2	Positive	248.1	90	6.8	-38
53	Phoxim-1	Positive	299.1	129.1	11.3	-15
54	Phoxim-2	Positive	299.1	77.1	11.3	-60
55	Propargite 1	Positive	368	231	13.7	-12
56	Propargite 2	Positive	368	175	13.7	-20
57	Pyridaben 1	Positive	365	309.1	14.9	-16
58	Pyridaben 2	Positive	365	147	14.9	-36
59	Spirotetramate Enol 1	Positive	302	269.9	7.3	-28
60	Spirotetramate Enol 2	Positive	302	215.9	7.3	-40
61	Tridemorph-1	Positive	298.1	57	10.3	-38
62	Tridemorph-2	Positive	298.1	98	10.5	-40

***Note: Above parameters will vary from instrument to instrument.*

Stock Solutions and Calibration Standard

Stock solution of 10 mg/L of 10 mL standard mixture of all analytes were prepared by adding appropriate volume from mother stock to 10 mL volumetric flask and finally made up with methanol. The all standard solutions were stored at -20 °C. The calibration standards (1, 2, 5, 10, 20, 50, 100 ng/ml) were prepared from stock standard solutions of the stock solution using 0.1% formic acid water:methanol mixture (20:80, v/v).

Extraction Protocol:

- Weighed 2 g ± 0.1 g of sample in 50 mL PTFE centrifuge tube.
- Added 8 mL of ultra-pure water, shook well and vortexed for 30 s and left for 30 minutes.
- Added 15 mL of acidified acetonitrile and shook/vortexed properly for 4-5min for proper interaction of analytes and solvent.
- Added 6 g of magnesium sulphate, 1.5 g of sodium chloride.
- Shook/vortexed well in the centrifuge tube for 4-5 min.
- Centrifuged for 10 min at 4000 rpm.
- Transferred 6 mL of supernatant in to 15 mL centrifuge tube containing 150 mg PSA + 150 mg Calcium chloride + 20 mg GCB and vortexed it for 2 min.
- Centrifuged for 10 min at 4000 rpm.
- Took 2 mL of supernatant in evaporating tube and evaporated it up to dryness under nitrogen evaporator at 40° C.
- Reconstituted with 2 mL of Methanol: water (80:20) and vortexed it properly.
- Filtered the sample through 0.2 µm filter paper and vial was ready for LC-MS/MS analysis.

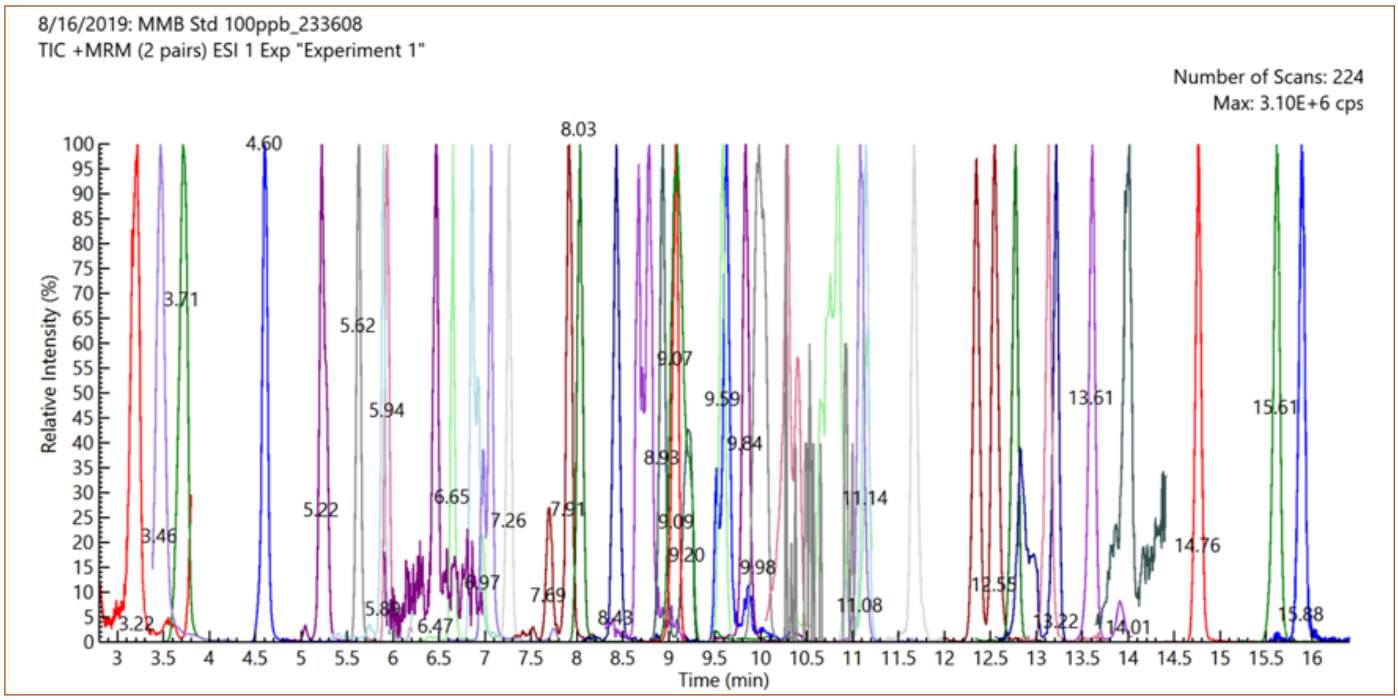
Results and Discussion:

Pesticides tested in this study contains both polar and non-polar analytes, so acidified acetonitrile solution was used to extract all these analytes from the sample matrix. However, reverse phase HPLC method was used with aqueous mobile phase at beginning to retain the polar compounds on the column. Chromatographic separation of all pesticide MRM transitions obtained within 20 min run time which is well separated and well resolved. The detection of each peak at 0.002 mg/kg concentration level and it is well below the MRL of most of regulatory requirement for black pepper.

Traditional MRM method development is not suitable for analysis of large number of analytes such as hundreds of pesticide residues in a single run. It is both time consuming and labor intensive to input all the mass transitions to a method manually. In addition, the dwell time for each transition cannot be optimized easily by traditional methods. Therefore, to resolve or come out with the Solutions

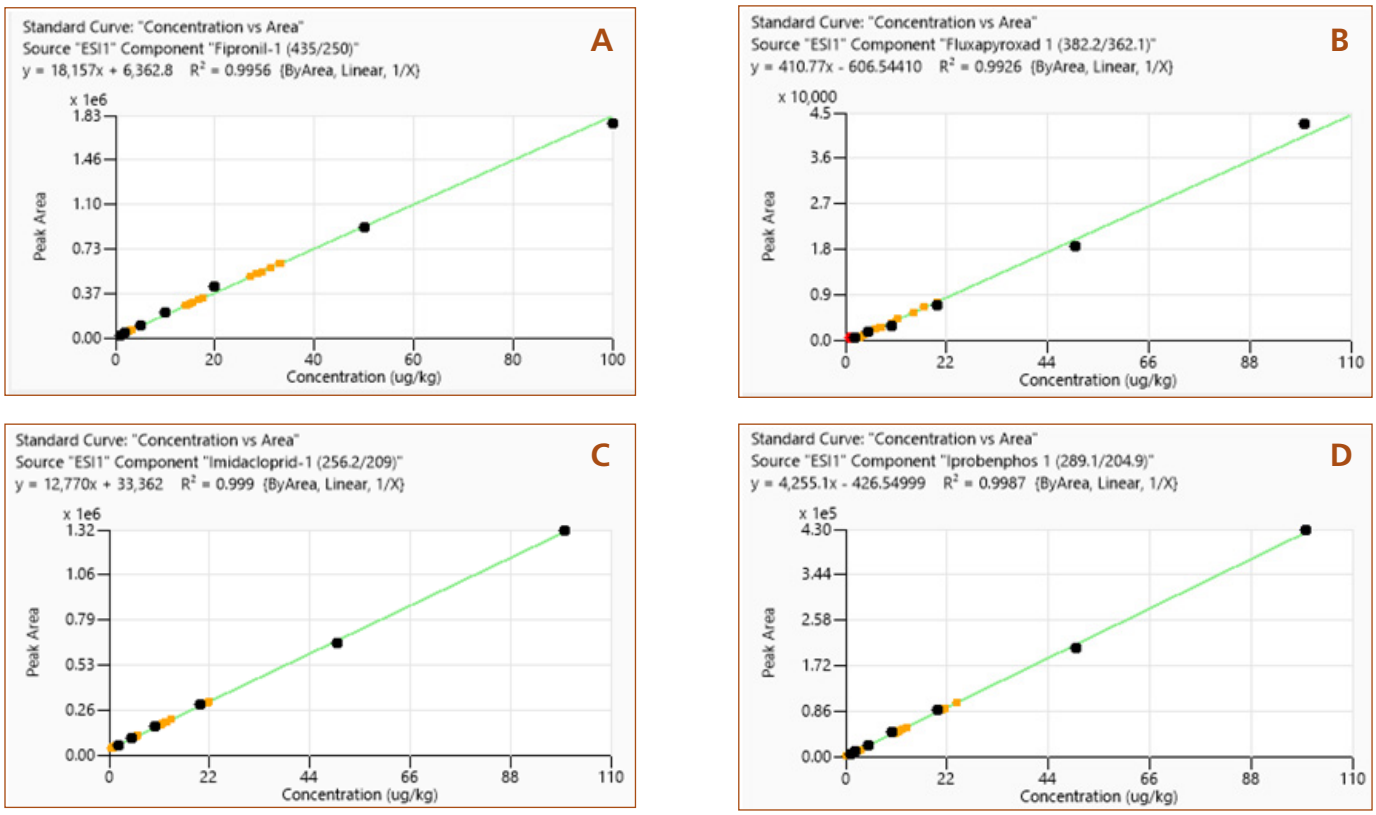
for all above problems, a time-managed-MRM™ was applied for method development in this study to improve efficiency, data quality and method performance. Figure 1 represent the TIC of QSight™ chromatograph shows peak separation for all targeted compounds within 20 minutes.

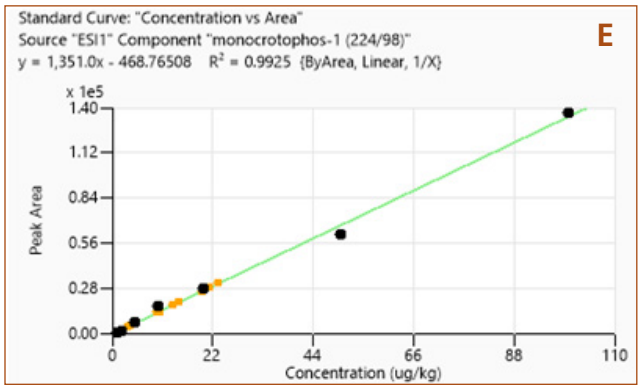
Figure 1. Total Ion Chromatograph (TIC) of pesticide residue standard

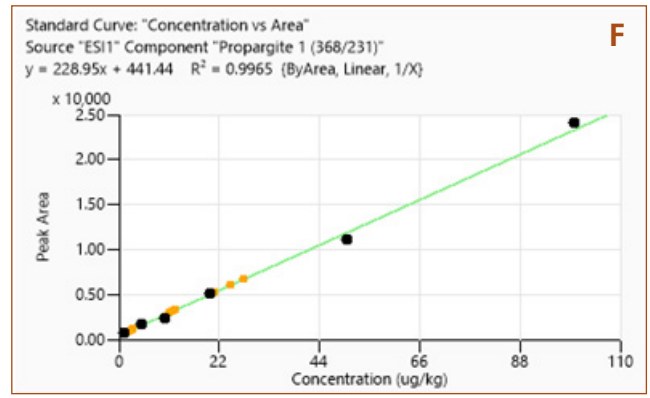


All calibration curves built from both the neat solution and Pepper sample matrix (matrix-matched calibration) showed good linearity with correlation coefficient (r2) larger than 0.99. Method covers linearity range from 0.001 mg/L to 0.100 mg/L concentration level with 7 calibration points. In figure 2 shows some representative calibration curves for pesticides.

Figure 2. Calibration curve for all detected compounds shows r^2 more than 0.99 or more.







Matrix Effect:

The responses of the matrix-matched standards (peak area of pre-extraction spike) were compared with the corresponding peak areas of standards in solvent in six replicates. When the percentage of the difference between the slopes of the two curves is positive, there is a signal enhancement effect, whereas a negative value indicates signal suppression effect. The matrix effect (ME) was quantified as the average percent suppression or enhancement in the peak area using the following equation:

$$\% \text{ ME} = \frac{\text{(Peak area of matrix standard – Peak area of solvent standard)}}{\text{Peak area of solvent standard}} \times 100$$

For example, some pesticides, such as Dazomate, Methamedophos, Paraoxon-methyl and methomyl, showed signal enhancement (positive values), while most of other compounds, such as Chlorpyriphos methyl and Acetamipride, Carbaryl, Etoxazole and other compounds showed signal suppression (negative values). As shown in Table 4, If matrix effect for most of analytes is less than 20%, then calibration curve can be build from neat solvent standard and can be use for quantitative analysis without significant error according to EU regulation. However, significant signal suppression effects were observed for fenarimol (-89%) and most of the LC analytes have signal suppression which is very known in liquid chromatography mass spectrometry analysis. Therefore, to overcome matrix effects and reduce variations in analytical results comparatively with solvent standards, matrix-matched calibrations were used in this study for quantification of all analytes.

Table 4. LOQ spike at 0.01 mg/L in Solvent and matrix match standard with its matrix effect

Sr. No	Compound Name	Std 10ppb	MM Std 10ppb	Matrix Effect (%)
1	Acetamiprid	188789	79925	-57.66
2	Bendiocarb	147227	27527	-81.30
3	Bitertanol	24399	6075	-75.10
4	Carbendazim	353597	231068	-34.65
5	Carbofuran 3 Hydroxy	103390	65318	-36.82
6	Carbryl	46856	8614	-81.62
7	Chlorantraniliprole	86134	25436	-70.47
8	Chlorpyriphos-methyl	37334	20316	-45.58
9	Dazomet	22637	29294	29.41
10	Dichlorvos	113119	22786	-79.86
11	Dodine	10748	5516	-48.68
12	Etoxazole	2759469	954778	-65.40
13	Etrimphos	17902	8153	-54.46
14	Fenarimol	79366	8046	-89.86
15	FENTHION	6939	5484	-20.97
16	Fluopyram	81773	9910	-87.88
17	Idosulfuron	105879	75226	-28.95
18	Indoxacarb	87690	23664	-73.01
19	Methamidophos	12403	15006	20.99
20	Methomyl	1941	2404	23.85
21	Monocrotophos	41332	29139	-29.50
22	Myclobutanil	53379	14279	-73.25
23	Paraxon Methyl	2581	4387	69.97
24	Phenthoate	428363	62506	-85.41
25	Picoxystrobin	1001828	270163	-73.03
26	Propamocarb	317747	337663	6.27
27	Tebuconazole	331943	64710	-80.51
28	Tetraconazole	75525	22073	-70.77
29	Thiacloprid	196776	98547	-49.92
30	Tolfenpyrad	26295	8153	-68.99
31	Triazophos	2705080	428299	-84.17

***Note: Matrix effect is shown for representative compounds of pepper matrix.*

Recovery Study:

In this pesticide recovery for all compounds were determined by spiking at 0.010, 0.025 and 0.050 mg/kg level in six replicates (all the samples were brought form local grocery stores). Most of the compound's recovery was between 70 to 120 % and % RSD for all compounds below 20 % which is well accepted and as per the regulatory requirements. Table – 5 represents the all three-level recovery with recovery percent and RSD. All samples tested in six replicates after calculating final dilution factor.

Table 5. Recovery at 10, 25 and 50 ug/kg spike with 7.5 times dilution at actual spike

		10 ppb recovery data			25 ppb recovery data			50 ppb recovery data			
Sr. No.	Name of pesticide	Avg.	Std. Dev	% Rec	Avg.	Std. Dev	% Rec	Avg.	Std. Dev	% Rec	Avg. Rec.
1	Acephate	11.38	0.99	113.83	23.25	4.52	93.00	42.20	4.56	84.40	97.07
2	Acetamiprid	8.32	1.21	83.17	21.27	1.96	85.08	43.25	1.35	86.50	84.91
3	Alachlor	6.71	1.38	67.06	21.85	1.81	87.40	36.61	2.55	73.21	75.89
4	Ametoctradin	7.74	1.69	77.37	18.55	1.95	74.18	37.42	5.65	74.85	75.47
5	Atrazine	10.04	1.32	100.39	24.34	1.92	97.37	39.59	4.52	79.18	92.31
6	Azoxystrobin	8.92	0.63	89.23	23.16	0.85	92.64	40.57	2.43	81.14	87.67
7	Benalaxyl	12.34	2.24	123.37	23.49	2.07	93.95	46.06	5.61	92.12	103.15
8	Bendiocarb	7.97	0.60	79.68	21.28	1.79	85.12	35.10	2.46	70.20	78.34
9	Bifenazate	8.96	1.75	89.61	23.36	2.34	93.45	36.95	4.75	73.90	85.65
10	Bifenthrin	9.18	2.98	91.78	24.57	5.81	98.29	45.97	13.65	91.94	94.00
11	Bitertanol	7.49	1.30	74.93	23.93	1.91	95.72	36.84	4.48	73.68	81.45
12	Boscalid	10.34	1.83	103.38	22.95	1.59	91.78	36.70	2.90	73.41	89.52
13	Buprofezin	8.22	0.24	82.18	23.21	1.56	92.84	43.02	2.93	86.04	87.02
14	Butachlor	16.95	3.19	169.51	24.44	1.60	97.77	48.98	3.69	97.97	121.75
15	Carbendazim	8.15	0.90	81.50	22.71	1.21	90.86	41.30	1.62	82.60	84.99
16	Carbofuran	9.17	2.46	91.68	24.50	6.30	97.98	38.30	3.55	76.59	88.75
17	Carbofuran 3 Hydroxy	8.56	1.14	85.55	22.32	2.63	89.28	42.46	3.15	84.93	86.59
18	Carboxin	8.66	0.66	86.63	22.49	2.06	89.94	41.19	3.00	82.38	86.32
19	Carbryl	9.35	0.67	93.51	22.27	2.05	89.09	39.08	2.78	78.16	86.92
20	Chlorantraniliprole	7.06	0.41	70.58	22.33	2.14	89.32	37.65	1.31	75.30	78.40
21	Chlorfenvinphos	8.20	2.68	81.99	21.53	2.50	86.10	40.56	3.45	81.12	83.07
22	Chlorfluazuron	7.49	1.96	74.87	20.98	7.15	83.93	36.70	2.95	73.40	77.40
23	Chlorpyriphos-methyl	6.10	0.95	60.99	20.59	1.55	82.34	42.35	3.21	84.71	76.01
24	Clothianidin	9.12	0.84	91.25	24.47	1.74	97.88	45.18	1.22	90.37	93.17
25	Cyantraniliprole	7.37	1.10	73.66	19.18	3.64	76.71	42.37	5.38	84.74	78.37
26	Cyflumetofen	20.53	3.74	205.30	20.04	1.96	80.18	48.32	8.67	96.64	127.37
27	Cymoxanil	8.88	0.57	88.76	18.34	0.97	73.36	41.15	0.53	82.30	81.47
28	Dementon-S- Methyl Sulphone	10.05	5.47	100.46	19.37	1.44	77.47	35.38	1.99	70.76	82.89
29	Diafenthiuron	10.87	0.91	108.75	25.53	1.71	102.13	37.63	2.55	75.26	95.38
30	Diazinon	9.96	2.22	99.58	20.48	2.40	81.90	43.85	5.84	87.70	89.73
31	Dichlorvos	14.40	7.95	143.96	20.76	2.07	83.03	40.29	4.08	80.58	102.52
32	Difenconazole	8.95	1.05	89.48	21.38	2.89	85.53	39.65	3.14	79.30	84.77
33	Diflubenzuron	10.34	0.84	103.44	13.35	5.64	53.41	40.89	4.62	81.77	79.54
34	Dimethoate	7.73	0.94	77.27	20.82	2.02	83.27	41.82	1.98	83.64	81.40
35	Dimethomorph	6.81	2.49	68.07	21.58	2.36	86.32	36.61	2.95	73.21	75.87
36	Dinotefuron	9.64	1.09	96.36	23.26	0.99	93.04	39.35	0.77	78.70	89.37
37	Dithianon	7.03	2.72	70.30	24.71	2.04	98.85	44.43	6.55	88.87	86.01
38	Diuron	8.05	1.35	80.46	21.44	1.68	85.75	40.79	3.88	81.59	82.60
39	Dodine	7.02	1.66	70.19	25.27	2.63	101.07	38.21	7.11	76.42	82.56

		10 ppb recovery data			25 ppb recovery data			50 ppb recovery data			
Sr. No.	Name of pesticide	Avg.	Std. Dev	% Rec	Avg.	Std. Dev	% Rec	Avg.	Std. Dev	% Rec	Avg. Rec.
40	Edifenphos	8.76	0.58	87.59	22.82	0.96	91.29	38.82	1.53	77.64	85.51
41	Emamectin Benzoate	6.54	1.41	65.43	20.04	2.65	80.16	37.55	4.74	75.10	73.57
42	Epoxyconazole	9.54	0.71	95.44	21.73	1.64	86.93	36.49	1.73	72.97	85.11
43	Ethion	8.53	0.53	85.30	20.61	1.04	82.45	40.38	2.57	80.76	82.84
44	Ethiprole	6.54	1.48	65.38	21.68	2.18	86.71	39.41	3.43	78.81	76.97
45	Etoxazole	6.47	0.45	64.67	20.54	1.98	82.16	37.31	0.93	74.62	73.81
46	Famoxadone	9.00	3.22	90.01	20.65	2.87	82.59	40.77	9.16	81.55	84.72
47	Fenamidone	10.00	1.34	99.98	23.95	2.07	95.79	41.26	5.87	82.51	92.76
48	Fenarimol	10.60	0.69	106.03	23.88	2.31	95.52	38.86	1.32	77.72	93.09
49	Fenazaquin	7.35	1.23	73.51	20.41	1.30	81.62	39.95	4.33	79.89	78.34
50	Fenobucarb	7.21	1.11	72.06	20.61	1.39	82.46	39.37	3.93	78.74	77.75
51	Fenpyroximate	6.49	0.60	64.92	21.36	1.99	85.45	36.64	2.11	73.27	74.55
52	Fenthion	10.01	0.99	100.13	21.54	1.46	86.14	39.11	2.74	78.22	88.16
53	Fenthion Sulphoxide	7.25	1.13	72.52	21.18	2.73	84.74	40.57	4.24	81.15	79.47
54	Fipronil	9.76	0.87	97.61	24.61	1.50	98.42	44.76	1.18	89.53	95.19
55	Flonicamid	6.46	3.03	64.57	17.90	7.12	71.62	35.86	2.57	71.73	69.31
56	Flufenoxuron	8.99	0.88	89.94	23.63	2.93	94.51	44.72	5.89	89.45	91.30
57	Flufenzine	8.45	0.74	84.48	22.88	1.66	91.51	41.47	2.82	82.95	86.31
58	Fluopyram	12.54	1.83	125.41	26.95	6.50	107.78	38.65	3.42	77.29	103.49
59	Flusilazole	8.02	0.98	80.22	23.30	1.23	93.18	39.94	2.32	79.89	84.43
60	Fluxapyroxad	10.93	1.87	109.25	24.60	3.14	98.39	40.70	8.99	81.40	96.35
61	Furathiocarb	9.42	0.52	94.17	25.11	1.69	100.43	45.03	3.75	90.06	94.89
62	Hexaconazole	9.00	3.22	90.01	20.65	2.87	82.59	40.77	9.16	81.55	84.72
63	Hexythiazox	8.73	0.61	87.35	23.03	1.95	92.11	39.47	1.64	78.94	86.13
64	Idosulfuron	10.25	1.31	102.55	26.44	1.61	105.76	45.06	4.62	90.13	99.48
65	Imazalil	12.23	2.03	122.34	26.30	2.03	105.22	44.52	1.94	89.04	105.53
66	Imidacloprid	7.89	1.10	78.90	20.56	1.12	82.25	40.15	0.93	80.30	80.48
67	Indoxacarb	7.70	2.87	77.03	16.70	11.28	66.79	44.50	7.99	88.99	77.60
68	Indoxacarb	8.59	1.01	85.91	22.46	0.83	89.83	40.33	3.12	80.67	85.47
69	Iprobenphos	8.33	0.94	83.34	20.03	0.97	80.12	39.58	0.72	79.16	80.87
70	Isoprothiolane	9.41	0.95	94.07	24.16	1.43	96.65	42.40	2.25	84.80	91.84
71	Isoproturon	8.11	0.91	81.11	21.99	1.07	87.95	42.59	2.11	85.17	84.74
72	Kresoxim-methyl	8.04	1.02	80.44	20.76	6.17	83.03	37.24	2.45	74.48	79.32
73	Linuron	8.80	1.34	88.03	22.28	2.05	89.13	48.99	4.11	97.97	91.71
74	Lufenuron	4.36	3.85	43.60	19.86	2.21	79.44	36.90	3.95	73.80	65.61
75	Malaoxon	6.61	0.47	66.12	15.58	3.28	62.30	32.33	9.99	64.67	64.36
76	Malathion	8.28	1.01	82.76	23.86	2.47	95.43	53.16	4.56	106.31	94.83
77	mandipropamid	10.85	0.86	108.53	22.42	2.84	89.67	46.28	5.17	92.55	96.92
78	Metalaxyl	5.64	1.71	56.37	19.45	2.61	77.81	41.44	3.06	82.88	72.35
79	Metconazole	7.16	1.10	71.58	19.99	1.46	79.97	39.30	2.81	78.59	76.72
80	Methamidophos	10.67	0.73	106.71	20.38	2.18	81.53	35.99	1.36	71.98	86.74
81	Methomyl	6.76	0.19	67.58	19.65	0.77	78.60	41.69	1.76	83.39	76.52
82	Metolachlor	8.89	0.67	88.95	22.15	1.34	88.62	40.60	1.93	81.19	86.25
83	Monocrotophos	8.56	0.90	85.61	19.81	2.36	79.22	41.53	3.59	83.05	82.63
84	Myclobutanil	9.78	0.69	97.77	21.01	2.03	84.06	35.95	3.58	71.89	84.57
85	Novaluron	8.97	0.42	89.66	22.98	1.38	91.91	41.47	1.34	82.93	88.17

		10 ppb recovery data			25 ppb recovery data			50 ppb recovery data			
Sr. No.	Name of pesticide	Avg.	Std. Dev	% Rec	Avg.	Std. Dev	% Rec	Avg.	Std. Dev	% Rec	Avg. Rec.
86	Omethoate	10.33	0.16	103.31	24.04	2.21	96.18	43.10	1.85	86.19	95.23
87	Oxadiazon	7.47	0.77	74.72	21.08	1.01	84.30	40.10	2.24	80.19	79.74
88	Oxamyl	9.52	0.51	95.16	24.07	0.30	96.26	42.78	1.21	85.57	92.33
89	Oxycarboxin	7.97	1.96	79.75	23.78	1.75	95.12	49.14	4.89	98.27	91.05
90	Oxydementon Methyl	8.42	1.03	84.20	22.10	1.34	88.40	39.15	1.90	78.29	83.63
91	Paclobutrazol	-	-	-	20.93	1.81	83.73	40.53	1.48	81.06	82.39
92	Paraxon Methyl	7.44	0.35	74.40	20.56	1.31	82.26	43.22	3.36	86.44	81.03
93	Paraxon Methyl	8.72	1.42	87.20	20.11	1.50	80.43	44.33	7.01	88.66	85.43
94	Penconazole	10.47	1.35	104.72	21.37	3.52	85.50	38.38	3.34	76.75	88.99
95	pencycuron	6.65	0.67	66.45	20.04	1.16	80.16	37.72	1.02	75.45	74.02
96	Pendimethalin	7.25	1.63	72.51	19.21	1.27	76.84	39.18	2.04	78.36	75.91
97	Phenthoate	9.21	1.09	92.09	22.59	2.83	90.38	40.81	2.56	81.62	88.03
98	Phorate-Sulfoxide	8.22	1.64	82.20	24.55	4.24	98.21	44.43	5.60	88.85	89.76
99	phosalone	9.42	1.33	94.17	24.46	1.27	97.84	44.03	3.63	88.07	93.36
100	Phosphamidon	9.22	0.67	92.18	21.88	2.38	87.53	40.94	2.16	81.88	87.20
101	Phoxim	24.66	13.64	246.57	21.67	3.29	86.67	40.85	2.24	81.70	138.31
102	Picoxystrobin	8.25	1.54	82.46	23.31	1.40	93.25	44.31	4.20	88.62	88.11
103	Primiphos-methyl	8.23	0.54	82.27	22.59	0.79	90.35	41.49	1.34	82.97	85.20
104	Profenophos	7.39	0.96	73.87	21.28	2.55	85.13	38.72	3.61	77.45	78.81
105	Propamocarb	8.61	0.35	86.12	25.17	0.44	100.66	41.62	1.65	83.25	90.01
106	Propanil	8.88	1.00	88.83	24.19	1.80	96.76	42.64	3.07	85.27	90.28
107	Propargite	9.07	1.33	90.66	22.14	7.51	88.55	47.20	6.76	94.40	91.20
108	Pyraclostrobin	10.50	1.13	104.99	21.30	3.63	85.22	44.26	7.55	88.53	92.91
109	Pyridaben	9.06	0.45	90.60	23.19	1.77	92.75	39.12	1.50	78.24	87.20
110	Pyriproxifen	9.41	2.97	94.08	23.94	1.17	95.76	48.10	5.35	96.20	95.35
111	Quninalphos	8.77	1.70	87.73	23.26	1.28	93.05	52.20	4.90	104.40	95.06
112	Simazin	7.75	0.84	77.45	20.42	0.49	81.68	39.19	1.51	78.38	79.17
113	Spinetoram	8.91	2.53	89.05	21.58	2.53	86.31	37.08	11.03	74.15	83.17
114	Spinosad A	5.56	2.46	55.62	16.51	3.50	66.05	40.80	8.55	81.60	67.76
115	Spinosad D	7.68	1.81	76.83	22.03	1.56	88.13	36.97	3.86	73.93	79.63
116	Spirodiclofen	8.01	1.31	80.11	20.50	1.50	81.99	38.66	2.43	77.31	79.80
117	spiromesifen	8.65	1.05	86.47	23.59	0.96	94.35	41.38	2.59	82.77	87.86
118	Spirotetramat Mono	4.69	0.91	46.85	16.64	0.85	66.58	30.46	3.12	60.92	58.12
119	Spirotetramate	10.55	1.56	105.53	23.03	2.71	92.12	38.58	2.21	77.16	91.60
120	Spirotetramate_Enol	5.56	0.68	55.61	19.51	1.21	78.06	32.62	3.11	65.25	66.30
121	Tebuconazole	8.27	1.26	82.65	21.90	2.57	87.58	35.49	2.35	70.97	80.40
122	Temephos	7.81	1.10	78.08	19.12	1.29	76.48	38.67	1.68	77.35	77.30
123	Tetraconazole	10.22	0.32	102.15	13.34	5.68	53.37	33.26	2.76	66.53	74.02
124	Thiabendazole	9.58	0.97	95.77	24.65	2.35	98.62	42.15	2.86	84.31	92.90
125	Thiacloprid	9.17	1.13	91.73	23.40	2.14	93.62	45.24	2.67	90.47	91.94
126	Thiamethoxam	8.27	0.86	82.70	22.22	0.95	88.87	40.93	1.22	81.86	84.48
127	Thiodicarb	11.86	0.47	118.56	24.21	1.40	96.84	43.38	7.48	86.77	100.72
128	Thiophanata methyl	9.98	0.73	99.80	22.97	1.48	91.86	42.47	4.34	84.95	92.20
129	Tolfenpyrad	8.30	0.69	83.05	21.85	2.48	87.42	41.09	2.75	82.17	84.21
130	Triadimefon	10.05	1.02	100.54	24.39	2.14	97.55	41.99	2.45	83.98	94.02
131	Triazophos	8.02	0.96	80.19	20.99	1.45	83.97	36.24	1.97	72.49	78.88

		10 ppb recovery data			25 ppb recovery data			50 ppb recovery data			
Sr. No.	Name of pesticide	Avg.	Std. Dev	% Rec	Avg.	Std. Dev	% Rec	Avg.	Std. Dev	% Rec	Avg. Rec.
132	Tricyclazole	8.63	0.39	86.28	22.10	0.75	88.39	39.15	1.67	78.30	84.32
133	Trideminol	8.80	1.41	88.01	20.30	5.21	81.22	36.24	14.24	72.48	80.57
134	Tridemorph	11.02	2.50	110.17	23.03	2.71	92.12	38.58	2.21	77.16	93.15
135	Trifloxystrobin	7.88	0.25	78.77	19.80	0.92	79.19	38.23	1.66	76.46	78.14
136	Trychlorfon	7.70	0.39	77.01	24.01	1.37	96.04	43.30	2.12	86.61	86.55

Instrument Capacity Check:

In this study across 3 days, experiment started with 0.01 mg/L solvent standard and at the end of the experiment, 0.01 mg/L solvent standard was analyzed. Various complex matrices such as tea, black pepper and turmeric were analysed. All these complex matrices are well known for mass system contamination (source, spray chamber, capillary tubing etc.). The response of solvent standard solution before start of batch analysis and after batch analysis is given in table 6. Figure 3 shows sample cone with no contamination deposition at end of 3 days experiment. The long term stability data demonstrate that a system can be used for analysis without any maintenance downtime for running samples with dirty matrices.

Figure 3. Photo of sample cone before and after analysis of hevy matrix samples over three days.

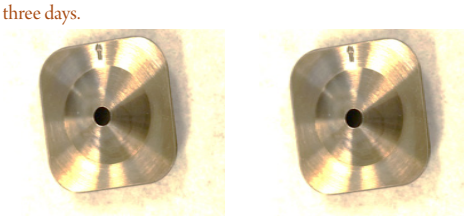


Table 6. Area comparison of 0.01 mg/L solvent standard before sample analysis and after three days sample analysis.

	0.01 mg/L Pesticide std area			
Sr. No	Name of pesticide	Befor analysis	After analysis	CV % Area
1	Acephate	43055	52370	13.81
2	Acetamiprid	182921	188789	2.233
3	Alachlor	87934	105224	12.66
4	Atrazine	120895	142622	11.66
5	Azoxystrobin	1627222	2397601	27.07
6	Benalaxyl	858573	998007	10.62
7	Bendiocarb	114502	147227	17.68
8	Bifenazate	249922	336253	20.83
9	Carbendazim	334468	353597	3.932
10	Carbofuran	63423	75303	12.11
11	Carbofuran 3 Hydroxy	115829	107692	5.148

	0.01 mg/L Pesticide std area			
Sr. No	Name of pesticide	Befor analysis	After analysis	CV % Area
12	Carboxin	177770	238059	20.5
13	Carbryl	81475	100388	14.71
14	Chlorantraniliprole	63723	86134	21.15
15	Chlorfenapyr	1281	1800	23.82
16	Chlorfenvinphos	303203	360622	12.23
17	Chlorpyrifos	883803	952114	5.262
18	chlorpyriphos-methyl	35204	37334	4.153
19	clothianidin	69103	78006	8.559
20	Cyflumetofen	2793	3859	22.66
21	Cymoxanil	46096	63856	22.84
22	Cymoxanil 3	81218	104719	17.88
23	Dazomet	21878	22637	2.411
24	Dementon-S- Methyl Sulphone	21000	24176	9.942
25	Diazinon	963838	1088161	8.568
26	Diazinon	488471	601600	14.68
27	Dichlorvos	108179	113119	3.157
28	Difenconazole	66249	87010	19.16
29	Diflubenzuron	110383	134372	13.86
30	Dimethoate	249301	234518	4.321
31	Dimethomorph	206852	258884	15.8
32	Dinotefuron	4444	6747	29.1
33	Diuron	49852	64703	18.33
34	Dodine	6301	10748	36.89
35	Edifenphos	800790	884964	7.062
36	Epoxyconazole	180460	296559	34.42
37	Ethion	123479	157705	17.21
38	Ethiprole	54700	70948	18.29
39	Ethiprole 3	332477	410179	14.8
40	Etrimphos	16960	17902	3.821
41	Fenamidone	166	113	26.87
42	Fenobucarb	154264	178363	10.25
43	Fenthion	247822	300786	13.65
44	Fenthion Sulphoxide	75769	85771	8.756
45	Fipronil	94452	136061	25.53
46	Flonicamid	5861	8041	22.18
47	Fluopicolide	17074	19287	8.607

	0.01 mg/L Pesticide std area			
Sr. No	Name of pesticide	Befor analysis	After analysis	CV % Area
48	Flusilazole	447377	521680	10.84
49	Fluxapyroxad	56568	63051	7.665
50	Hexythiazox	301604	407470	21.11
51	Idosulfuron	11528	11467	0.375
52	Imazalil	185864	182595	1.255
53	Imidacloprid	197070	205475	2.953
54	Indoxacarb	67220	87690	18.69
55	Iprobenphos	847443	988219	10.85
56	Isoprothiolane	1056222	1124667	4.438
57	Isoproturon	341273	358160	3.414
58	Kresoxim-methyl	8546	11032	17.96
59	Linuron	204395	197967	2.259
60	Malathion	180370	176151	1.674
61	Metalaxyl	75938	88971	11.18
62	Metconazole	300946	436708	26.03
63	Methamidophos	4367	3543	14.73
64	Methomyl	2221	2383	4.976
65	Metalochlor	619229	723228	10.96
66	Monocrotophos	16047	18997	11.91
67	Myclobutanil	61897	85173	22.38
68	Nitenpyram	5190	7599	26.64
69	Novaluron	140024	166619	12.27
70	Omethoate	323561	372850	10.01
71	Oxadiazon	102684	101014	1.159
72	Oxamyl	17712	18464	2.94
73	Oxycarboxin	6033	6644	6.816
74	Oxydementon Methyl	42101	48015	9.281
75	paclobutrazol	939	1248	19.98
76	Paraxon Methyl	3112	4680	28.46
77	Penconazole	174548	224112	17.58
78	pencycuron	1619371	1856312	9.641
79	Pendimethalin	32132	42787	20.11
80	Phenthoate	326025	428363	19.19
81	Phorate-Sulfoxide	27583	28563	2.468
82	phosalone	132834	187044	23.97
83	Phosphamidon	11604	16201	23.38
84	Phoxim	93947	95058	0.831
85	Picoxystrobin	931230	1001828	5.165
86	Primiphos-methyl	1015553	1191653	11.28
87	Propamocarb	754588	765102	0.978
88	Propanil	125665	152197	13.5
89	propoxur	706446	759804	5.146
90	Pymetrozine	39846	46381	10.72
91	Pyraclostrobin	458233	672703	26.82
92	Pyriproxifen	61000	80331	19.34

	0.01 mg/L Pesticide std area			
Sr. No	Name of pesticide	Befor analysis	After analysis	CV % Area
93	Quninalphos	960	1057	6.801
94	Simazin	194418	239996	14.84
95	spinosad A	267254	309056	10.26
96	spinosad A	27793	26840	2.467
97	Spinosad D	7648	8882	10.56
98	Spiromesifen	331407	414132	15.69
99	Temephos	36485	38817	4.38
100	Tetraconazole	52476	75525	25.47
101	Thiacloprid	146469	196776	20.73
102	Thiamethoxam	153084	170088	7.441
103	Thiobencarb	101652	97493	2.953
104	Thiodicarb	12203	16734	22.14
105	Thiophanata methyl	125917	146932	10.89
106	Thiophanate methyl	868513	787827	6.889
107	Tolfenpyrad	24774	26295	4.212
108	Triadimefon	237628	321434	21.2
109	Triazophos	431251	609037	24.17
110	Tricyclazole	144135	176701	14.36
111	Trideminol	5074	6601	18.5
112	Trychlorfon	19594	20434	2.968

Conclusion

The results obtained confirm the capability and applicability of PerkinElmer QSight™ LC-MS/MS for black pepper (complex matrix sample) analysis for routine analysis. The robust chromatographic and quantitative analysis of multiclass pesticides in black pepper is possible by QSight™ LC-MS/MS. The results showed excellent repeatability and sample analyte identities were positively confirmed via their qualifier/quantifier ion ratios. A quick and reliable UHPLC-MS/MS method was developed for the simultaneous estimation of pesticides in pepper matrix. The LOQ for all the analytes are 0.01 mg/kg or below. Linerity range is from 0.001 to 0.100 mg/kg with the regression coefficient > 0.99. The LOQs achieved using this method are well below that the permitted level, suggesting that PerkinElmer QSight™ 220 LC-MS/MS System provides a very sensitive and robust platform for the analysis of pesticides in heavy matrix sample like black pepper.

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