Quantification of acetamiprid and prochloraz in black pepper using the SCIEX Triple Quad™ 3500 LC-MS/MS System

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Today, several pesticides are available in the market, which are used to protect against various pests that damage plant food products such as spices, cereals, pulses, fruits and vegetables, etc. Due to the adverse effects of pesticide residues on human health and to the environment, the use of pesticides must be controlled and monitored. Therefore, maximum residue levels (MRLs) for pepper have been fixed by regulations (European Commission, FSSAI and other regulatory bodies) to assess food safety.[1,2] Monitoring of food quality and safety requires suitable analytical methods for pesticides and herbicide residues.

Pepper is a common food ingredient with the highest production and export values. The presence of pesticide residues more than the MRL concentration in pepper samples need to be analyzed to ensure quality of the product and protect the public.[3,4]

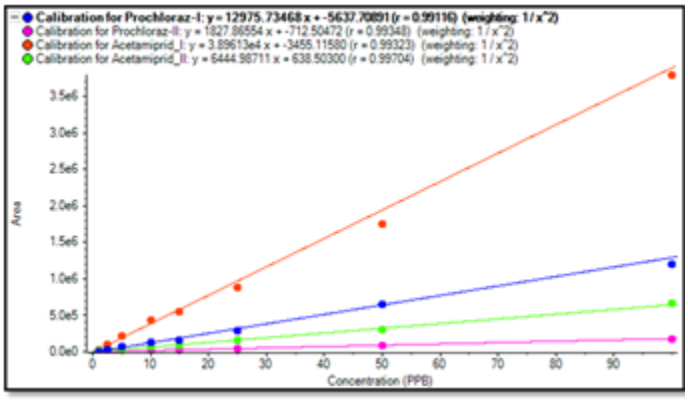


Figure 1: Representative calibration curves. Good linearity was observed for acetamiprid (red and green) and prochloraz (blue and pink) range for the concentration range of 1 to 100 ppb. Lower limits of quantification was 1 ppb for both pesticides in black pepper matrix.

Here, a sensitive, rugged and robust method was developed for the quantification of pesticide residues in pepper matrix using the SCIEX Triple Quad 3500 System.

Key features of targeted quantification method for pesticide residues in pepper

- A targeted quantitative method has been developed on the SCIEX Triple Quad 3500 System using two MRM transitions
- A modified QuEChERS method was developed for preparation of the black pepper samples
- The method achieved performance below the MRLs of acetamiprid and prochloraz residues in pepper as mentioned in the regulatory guidelines
- Experiments were performed with the five different concentration levels (1.0, 2.5, 5.0, 10.0 and 15.0 ppb) which meet the validation parameters as per the accuracy % requirements set by the global regulations (80%-120%).

Methods

Sample preparation: Pesticides standards were purchased from Sigma Aldrich and had ≥ 99% purity. All other chemicals used were of LC-MS grade, and were commercially available. Black pepper samples were procured from the local markets of Delhi and Gurgaon, India and were stored at room temperature until analysis.

1. Add 2 g of sample to a 50 mL Tarson tube and then add 0.5 g of disodium hydrogen citrate sesquihydrate, 1 g sodium chloride, 1 g trisodium citrate dihydrate, and 2 g magnesium sulphate (anhydrous)
2. Add 10 mL of acetonitrile, and then vortex for 10 minutes. Centrifuge at 4500 rpm for 10 minutes
3. Collect the organic layer and evaporate with N_2 to dryness.
4. Reconstitute the samples with 1 mL of water/acetonitrile/formic acid (90:10:0.2% v/v/v) and vortex well, then transfer into 2 mL centrifuge tubes. Centrifuge at 12000 rpm for 10 minutes
5. After centrifugation, collect the upper layer and transfer into autosampler vial for LC-MS/MS analysis.

Chromatography: LC separation was performed with a Phenomenex Kinetex® C18 LC column (100 Å, 100×4.6 mm, 2.6 µm) using the gradient outlined in Table 1. A 20 µL injection volume was used and the column oven temperature was set to 40°C.

Table 1. Gradient profile and mobile phase composition.

Total Time (min)	Flow Rate (µL/min)	A%	B%
0.01	*800*	*90*	*10*
0.50	*800*	*90*	*10*
3.00	*800*	*40*	*60*
10.00	*800*	*10*	*90*
12.00	*800*	*10*	*90*
13.00	*800*	*90*	*10*
15.00	*800*	*Controller*	*Stop*

Mobile phase A: 5mM ammonium formate in water + 0.1% formic acid
Mobile phase B: 5mM ammonium formate in methanol + 0.1% formic acid

Mass spectrometry: The SCIEX Triple Quad 3500 System was operated in multiple reaction monitoring (MRM) mode. The Turbo V™ Ion Source was used with an electrospray ionization (ESI) probe in positive ion mode. Two selective MRM transitions were monitored (Table 2). Analyst® Software 1.7 was used for method development and data acquisition. Ionization voltage was 5500 V, source temperature was 550 ºC, GS1 was 55 and GS2 was 50.

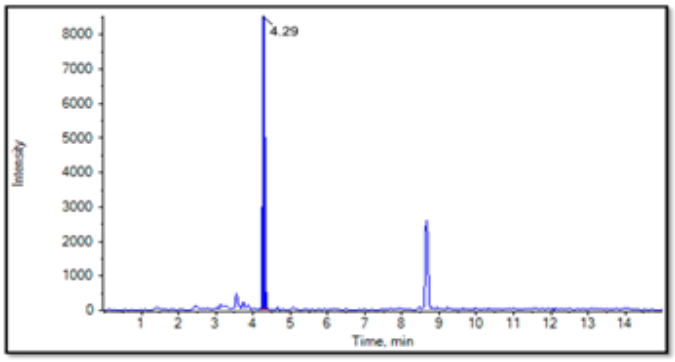
Table 2. MRM transitions of Acetamiprid and Prochloraz.

Compound	Precursor Ion	Product Ion (Quantifier)	Product Ion (Qualifier)
Acetamiprid	*223.0*	*126.0*	*56.0*
Prochloraz	*376.1*	*308.1*	*266.1*

Data processing: LC-MS/MS data was processed using the MultiQuant™ Software 3.0.2. Ion ratio is calculated automatically by the software for compound identification.

Results

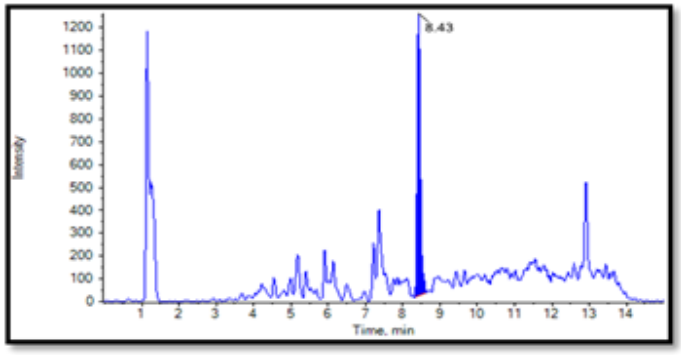
Due to the complexity of the pepper matrix, a modified QuEChERS method was used in this study to reduce the matrix effects and improve the assay sensitivity for the quantification of pesticide residues. The matrix matched calibration curve showed excellent linearity (1.0 to 100.0 ppb), with a correlation coefficient r≥0.99 for pesticide residues in pepper using linear regression and weighing factor $1/X^2$ (Figure 1). The lowest calibration point for both pesticide residues was 1 ppb in matrix (Figure 2 and 3).



Sample Name	Sample Type	Component Name	Mass Info	Actual Concentration	Area	Calculated Concentration	Accuracy
RCS	Blank	Acetamiprid-I	223.0 / 126.0	N/A	N/A	N/A	N/A
SAMPLE BLANK	Blank	Acetamiprid-I	223.0 / 126.0	N/A	2650	0.11	N/A
EXT_STD CC_01	Standard	Acetamiprid-I	223.0 / 126.0	1.00	34792	0.94	93.91
EXT_STD CC_02	Standard	Acetamiprid-I	223.0 / 126.0	2.50	103256	2.71	108.29
EXT_STD CC_03	Standard	Acetamiprid-I	223.0 / 126.0	5.00	215657	5.61	112.20
EXT_STD CC_04	Standard	Acetamiprid-I	223.0 / 126.0	10.00	438309	11.36	113.60
EXT_STD CC_05	Standard	Acetamiprid-I	223.0 / 126.0	15.00	538477	13.95	92.98
EXT_STD CC_06	Standard	Acetamiprid-I	223.0 / 126.0	25.00	888125	22.98	91.91
EXT_STD CC_07	Standard	Acetamiprid-I	223.0 / 126.0	50.00	1723967	44.56	89.12
EXT_STD CC_08	Standard	Acetamiprid-I	223.0 / 126.0	100.00	3792892	97.99	97.99

Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV
Acetamiprid-I	1.00	6 of 6	3.554e4	5.714e2	1.61

Figure 2. Acetamiprid results. (Top) Representative chromatogram at LLOQ level of 1 ppb acetamiprid in matrix. (Middle) Statistical data on calibration curve for acetamiprid from 1 to 100 ppb. The correlation coefficient r ≥ 0.99 for both the transitions (quantifier and qualifier Ions). (Bottom) High reproducibility was observed at the lowest concentration, 1.61%CV.



Sample Name	Sample Type	Component Name	Mass Info	Actual Concentration	Area	Calculated Concentration	Accuracy
RCS	Blank	Prochloraz-I	376.1 / 308.1	N/A	N/A	N/A	N/A
SAMPLE BLANK	Blank	Prochloraz-I	376.1 / 308.1	N/A	N/A	N/A	N/A
EXT_STD CC_01	Standard	Prochloraz-I	376.1 / 308.1	1.00	6463	0.94	94.10
EXT_STD CC_02	Standard	Prochloraz-I	376.1 / 308.1	2.50	29498	2.74	109.57
EXT_STD CC_03	Standard	Prochloraz-I	376.1 / 308.1	5.00	65651	5.56	111.23
EXT_STD CC_04	Standard	Prochloraz-I	376.1 / 308.1	10.00	136229	11.07	110.71
EXT_STD CC_05	Standard	Prochloraz-I	376.1 / 308.1	15.00	161273	13.03	86.84
EXT_STD CC_06	Standard	Prochloraz-I	376.1 / 308.1	25.00	285966	22.76	91.04
EXT_STD CC_07	Standard	Prochloraz-I	376.1 / 308.1	50.00	650147	51.19	102.38
EXT_STD CC_08	Standard	Prochloraz-I	376.1 / 308.1	100.00	1200160	94.13	94.13

Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV
Prochloraz-I	1.00	6 of 6	9.726e3	1.040e2	1.07

Figure 3. Prochloraz results. (Top) Representative chromatogram at LLOQ level of 1 ppb prochloraz in matrix. (Middle) Statistical data on calibration curve for prochloraz from 1 to 100 ppb. The correlation coefficient r ≥ 0.99 for both the transitions (quantifier and qualifier Ions). (Bottom) High reproducibility was observed at the lowest concentration, 1.07%CV.

Using the developed method, pepper samples from local markets were tested for the presence of these pesticide residues (Figure 4).

Conclusions

The method and data acquired demonstrate the assay provides sensitive and accurate results for the quantification and confirmation of pesticide residues in pepper samples using the SCIEX Triple Quad 3500 System. For both the analytes, a LLOQ of 1 ppb was achieved, which was easily below the MRL level as mentioned by FSSAI (MRL – 10 ppb) and European Union (MRL – 100 ppb) regulatory bodies. The developed method achieved acceptable accuracy (80-120%) for the calibration curves in matrix without any significant matrix interferences.

Sample Name	Sample Type	Component Name	Mass Info	Area	Calculated Concentration
Sample_01	Unknown	Acetamiprid-I	223.0 / 126.0	107438	2.82
Sample_02	Unknown	Acetamiprid-I	223.0 / 126.0	59777	1.58
Sample_03	Unknown	Acetamiprid-I	223.0 / 126.0	11250	0.33
Sample_04	Unknown	Acetamiprid-I	223.0 / 126.0	3452	0.13

Sample Name	Sample Type	Component Name	Mass Info	Area	Calculated Concentration
Sample_01	Unknown	Prochloraz-I	376.1 / 308.1	3521	0.71
Sample_02	Unknown	Prochloraz-I	376.1 / 308.1	3881	0.73
Sample_03	Unknown	Prochloraz-I	376.1 / 308.1	4200	0.76
Sample_04	Unknown	Prochloraz-I	376.1 / 308.1	3814	0.73

Figure 4. Analysis of unknown pepper samples from local markets. Results for acetamiprid and prochloraz from four unknown samples showed detection of some pesticide residue but all were below the MRL level of 10 ppb.

The method performed as per commission decision SANTE/11813/2017 directive recommendations and fulfilled regulatory requirements for of sensitivity, precision, and accuracy. Overall, the high-throughput, accurate, and sensitive method on the SCIEX Triple Quad 3500 System has demonstrated required ability to monitor pesticide residues in pepper samples.

References

1. Food safety and standard authority of India. A Statutory authority established under the food safety standard act, 2006. File No. 1-1605/FSSAI/Imports/2016 (pt-2).
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