Method validation and monitoring of emamectin benzoate in raw banana fruit with pulp, peel and pulp through LC-MS/MS

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Abstract

An efficient analytical approach for detecting and quantifying emamectin benzoate insecticide residues in raw banana peel and pulp samples was developed and validated using a modified QuEChERS technology and liquid chromatography mass spectrometry/mass spectroscopy (LC-MS/MS). Linearity, detection limits, quantification limits, precision, accuracy, and uncertainty were used to validate the approach. The detection and quantification limits for raw banana pulp and raw banana peel and pulp samples were 0.005 and 0.01 µg g^{-1}, 0.001 and 0.003 µg g^{-1}, respectively. The recovery investigation was conducted at five different fortification levels ranging from 0.01 to 0.1 g mL^{-1}. Emamectin benzoate recovery ranged from 90.02 to 113.06 percent and 99.67 to 111.70 percent. The validated approach was utilised to

analyse raw banana peel and pulp fruit samples obtained from emamectin benzoate treated fields, as well as banana samples purchased from the local market.

Keywords: Emamectin benzoate, QuEChERS, residues, recovery, raw banana, LC-MS/MS

1. Introduction

In tropical and subtropical regions, banana is one of the most important fruit crops grown for its edible fruits. Banana is an important medicinal food that provides carbohydrates as well as critical micronutrients such as potassium, phosphorus, calcium, iron, and vitamin A, B, C, and D (Haslinda *et al*., 2009). It is grown in over 130 countries with its origins in South-East Asia. In terms of global trade, it is the sixth agricultural food crop after coffee, cereals, sugar, and cacao; and, aside from grapes, citrus fruits, and apples, it is an important fruit crop in the globe (Auore *et al*., 2009). The average fruit weight is 125 grams, with roughly 75% water and 25% dry matter content. When ripe, banana fruits vary in size and colour, ranging from yellow to purple to red. However, practically all culinary bananas have seeds-free fruits, but wild kinds have many huge and hard seeds (Hikal *et al*., 2022). Banana crop is associated with a number of pests and non-pests that cause severe damage to the fruit, leaves, pseudostems, and rhizomes. Pests also transmit plant infections (viral, bacterial, and fungal) that cause plant mortality or reduce fruit yield. However, two weevil pests, *Odoiporus longicollis* and *Cosmopolites sordidus*, severely reduce banana production (Padmanaban *et al*., 2020). The Banana pseudostem weevil (BPW), also known as *Odoiporus longicollis* (Coleoptera: Curculionidae), is an oligophagous pest. It causes significant infestation and yield loss in Asian Pacific banana-growing countries such as South-east Asia (Justin *et al*., 2008).

Banana crop have been subjected to pseudostem injections of monocrotophos, phorate, carbofuran, quinalphos, cypermethrin, dimethoate, and triazophos for decades (Kannan *et al*., 2021). Insecticides are injected into pseudostems monthly from the fifth to seventh month of crop age (Reddy *et al*., 2020). Pesticides previously used in banana, such as monocrotophos and triazophos, have been restricted/banned in India (CIB & RC, 2023), and farmers are now using different insecticides to control the pseudostem weevil. Emamectin benzoate, a green insecticide compound effective against pseudostem borer and is now utilised by farmers in major banana-growing districts of Tamil Nadu in India. Emamectin benzoate has grown in favour in agricultural production because to its low application dosage and broad-spectrum efficacy. Emamectin benzoate trunk injection was proven to be effective against the emerald ash borer and the Pinewood nematode in forest ecosystems (Ouyang *et al*., 2023). Market banana samples from Tamil Nadu, India, were found to have carbendazim residues (Paranthaman *et al*., 2012). Chlorpyriphos residues were observed in the peels and pulp of Canary Island bananas (Hernandez-Borges *et al*., 2009). Zhou *et al*. (2016) and Deng *et al*. (2020) developed ultra-high-performance liquid chromatography-tandem mass spectrometry (UPLC/MS/MS) to investigate the residue and dissipation of benzoate in tea and rice, respectively, and Liu *et al*. (2012) and Wang *et al*. (2012) used LC-MS to determine the residues of emamectin benzoate in food and water. In light of this, the current work was done to 1) develop simplified extraction and clean-up processes for assessing pesticide residues in raw banana with pulp, peel and pulp; and 2) monitor insecticide residues in market samples of raw banana fruit with peel and pulp.

2. Materials and methods

The reference standard of emamectin benzoate was purchased from Dr.Ehrenstorfer (Augsburg, Germany). Formic acid and acetonitrile of MS grade were purchased from Sigma Aldrich (Mumbai. India). The magnesium sulphate and anhydrous sodium chloride were provided by Merck India Ltd. (Mumbai, India), and heated at 650°C for four hours before to use. The graphitized carbon black (GCB) and primary secondary amine (PSA), which were purchased, were from M/s. Agilent Technologies (Agilent Technologies India Pvt Ltd, Chennai, India). Emamectin benzoate stock solution (400 g mL^{-1}) was made by dissolving the technical-grade material in acetonitrile (v/v). This was labelled and stored in a freezer at -20°C. The stock solution was used to create the intermediate stock solution and the working standard solution.

The Waters Alliance 2695 Separation system, which includes an autosampler, quaternary pump, and membrane degasser, was used for the liquid chromatographic (LC) analysis. An acquity TQD was linked for mass spectrometry using an ESI interface. For the analyte separation, a 5 m (4.8 x 250 mm) X Terra analytical C18 column was employed. The temperature of the column was fixed to 30°C. Following an isocratic flow of 30% A + 70% B at 0.5 mL min^{-1}. An injection volume of 10 µL and a 5 minute run were used to elute the analyte.

The temperatures of the source block and desolvation were set to 150 and 500^{o}C, respectively, and the desolvation (drying gas) and cone gas flows to 1100 and 80 L h^{-1} and 0.20 ml min^{-1}, respectively. Argon served as the collision gas, while nitrogen served as the desolvation and cone gases. To optimise the voltages on the lenses and the flow rate of the 20 µL min^{-1} syringe pump infusion, Tune Master (Mass Lynx software) was employed. The initial tweaking was accomplished by infusing a working standard solution. Chromatograms were

captured with the ESI interface in both positive and full scan mode. By arranging the chromatographic run in MRM mode (Multiple reaction monitoring), the analyte was found. The raw banana fruit samples were collected from organic banana fields in Coimbatore, Tamil Nadu, India.

The QuEChERS technique (Anastassiades *et al*., 2003) was used with a few minor modifications for the extraction and cleanup of samples. A 50 mL centrifuge tube was filled with 10 g of raw banana fruit. After adding 10 ml of acetonitrile, the centrifuge tube was agitated in a vortexer for one minute. To this, 4 g of anhydrous magnesium sulphate and 1 g of sodium chloride were added, and the sample was once more thoroughly mixed by vortex shaking for a minute. The samples were centrifuged at 6000 rpm for 10 min. Of this, 6 mL of supernanant was taken into a centrifuge tube together with 10 mg of GCB, 100 mg of PSA and 600 mg of anhydrous magnesium sulphate. These materials were thoroughly homogenized by vortexing for one minute and centrifuging for ten minutes at a speed of 3000 rpm.

The analytical method was used for assessing pesticide residues in raw banana fruit was validated in the lab by characterizing the method's performance in compliance with SANTE, 2021) standards. The current study's analysis method was validated by calculating and assessing a number of performance characteristics, such as linearity, limit of detection (LOD), limit of quantification (LOQ), matrix effect, recovery, precision, and uncertainty. The identical control samples of raw banana fruit were free of pesticide residues used for all tests. The linearity of the detector response for the test analyte was assessed by injecting five calibration working standard solutions in LC-MS/MS at concentrations of 0.01, 0.025, 0.05, 0.075, and 0.1g mL^{-1}, with three replicate injections per concentration. Following seven replications, the LOD and LOQ were calculated by injecting samples of spiked raw banana fruit at a spiking dose of 0.01 g mL^{-1}. The

LOD and LOQ were calculated using the standard deviation, standard error, and x variable (SANTE, 2021). The matrix effect was measured by using the method ME (%).

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

Before being spiked with standard emamectin benzoate solution, untreated raw banana fruit samples were homogenised. After being spiked with the analyte at concentrations of 0.01, 0.025, 0.05, 0.075, and 0.1g mL^{-1}, the samples were extracted and cleaned. Each spiking test was performed seven times. The relative standard deviation (RSD %) based on analyte value calculations produced during recovery analyses were used to measure the accuracy of the procedure.

The data obtained from the chromatogram was used for calculating the relative standard deviation, matrix effect, recovery percentage, and residue concentration. The following equation was used to perform the residue quantification process.

$$\text{Residues } (\mu g\ g^{-1}) = \frac{As}{Astd} \times \frac{Wstd}{Ws} \times Vs$$

As - Peak area of Sample

Astd - Peak area of Standard

Wstd - Wt of standard ($\mu g\ mL^{-1}$)

Ws - Wt of the sample (g)

Vs - Volume of extract in ml

Raw banana fruit with peel and pulp (20 nos.) sold for fruit and vegetable purposes were obtained from several marketplaces in Tamil Nadu, India, for this study. The samples (1–2 kg

each) were packed in polythene bags, labelled, and delivered to the laboratory for analysis. The samples were homogenised and kept at 40°C in glass containers. The samples were analysed using the above validated method.

3. Results and discussion

The LC-MS/MS was tuned to determine the best instrument settings for identifying target analytes. It was able to achieve increased parent ion fragmentation at collision energies ranging from 2 to 80V. The quantifier and qualifier ions (m/z) of 158.17 and 82.103 were utilised to confirm emamectin benzoate residues in samples, respectively, while the most enhanced parent ion (m/z) of 886.935 was used for quantification. The first chromatographic separation studies were conducted using matrix-fortified standards. Peak sharpness and separation effectiveness were investigated for acetonitrile and water mobile phase mixtures at varied ratios. The ideal mixture of 0.1% HCOOH in water and 0.1% HCOOH in acetonitrile with isocratic run was discovered to be 30:70 v/v. The analyte examined under the predefined chromatographic conditions demonstrated good peak shape and separations over the course of 5 minutes (Figure 1). Two MRM transitions were discovered for the analyte, allowing for simultaneous quantification and identification.

The linearity of the calibration curve for the emamectin benzoate standard was found to be acceptable (0.9963). There were no background peaks above a signal-to-noise ratio of 3 for the retention duration of the examined analyte, showing that the selective ion monitoring approach was interference-free. The LOD and LOQ for raw banana pulp and raw banana peel and pulp were 0.005 and 0.015 µg g^{-1}, 0.001 and 0.003 µg g^{-1}, respectively. Malhat *et al*. (2013) reported LOD and LOQ of emamectin benzoate for tomato were 0.005 and 0.01 µg kg^{-1} using

high performance liquid chromatography with fluorescence detector. Suganthi *et al*. (2018) observed lower limits of detection and quantification of thiamethoxam in mature banana fruit with pulp of 0.002 and 0.008 $\mu g\ g^{-1}$, respectively, which are consistent with the current findings. Using a high-performance liquid chromatography-fluorescence detector, the LOQ of emamectin benzoate in whole longan and pulp was 0.001 mg kg^{-1} (Liu *et al*., 2023).

The sample matrix may have a considerable impact on the accuracy of pesticide analysis results (Santilio *et al*., 2014). Interferences in the matrix can suppress or amplify analytical data, resulting in poor or high analyte recoveries (Zhang *et al*., 2011). The analyte in the raw banana pulp and raw banana with peel and pulp samples was measured using matrix match standards to reduce the matrix effect. In this investigation, the matrix effect for emamectin benzoate residues in raw banana pulp and raw banana with peel and pulp samples ranged from - 10.75 to 17.20 and 0.37 – 13.69 percent (Table 1). Emamectin benzoate showed ion enhancement and inhibition of ion responsiveness in raw banana matrices.

In the present study, the recovery of raw banana with peel and pulp ranged from 99.67 to 111.70%, whereas the recovery of pulp alone ranged from 90.02 to 113.06% (Table 2, 3). According to Wang *et al*. (2021), the average recoveries of emamectin benzoate in cowpeas ranged from 83.55-103.69%, with relative standard deviations ranging from 1.55% to 3.20%. Similarly, Reddy *et al*. (2021) reported typical recoveries of emamectin benzoate in grapes ranged from 99.77 to 102.44%, with a relative standard deviation of less than 5%. Mean recoveries and relative standard deviations (RSDs) for emamectin benzoate in cabbage samples ranged from 87.8 to 100.0% and 3.6 to 12.6%, respectively (Dong *et al*., 2015). For emamectin benzoate, recoveries varied from 75.9 to 97.0 percent with a relative standard deviation (RSD) of 4.4-19.0 percent at fortification levels of 0.001, 0.01 and 0.1 mg/kg in cabbage, apple, and soil

(Wang *et al*., 2012). In tea leaves, the average recoveries of emamectin benzoate varied from 85.3 to 101.3%, with a relative standard deviation (RSD) of less than 15% (Zhou *et al*., 2016).

The efficiency of the standardised approach in assessing trace levels of emamectin benzoate was evaluated using commercially available raw banana pulp and pulp (20 No's) samples taken from different retail marketplaces in Tamil Nadu, India. The fresh banana peel and pulp fruit contained no emamectin benzoate residues. There is no MRL for emmectin benzoate in raw banana fruit.

4. Conclusions

An effective, sensitive, and quick LC-MS/MS technique was designed and validated satisfactorily (recovery of 70 to 120% and RSD 20%) to determine emamectin benzoate residues in raw banana fruit. The method used in the current study was validated in accordance with European Union guidelines and also suitable for monitoring emamectin residues in raw banana fruit with peel and pulp.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Anastassiades, M., Lehotay, S. J., Štajnbaher, D., & Schenck, F. J. (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *Journal of AOAC international*, 86(2), 412-431.

Aurore, G., Parfait, B., & Fahrasmane, L. (2009). Bananas, raw materials for making processed food products. *Trends in Food Science & Technology*, 20(2), 78-91.

Central insecticide board and registration committee (2023). Major uses of insecticides. Retrieved from https://ppqs.gov.in/divisions/cib-rc/major-uses-of-pesticides.

Deng, L., Chen, L., Guan, S., Liu, J., Liang, J., Li, X., & Li, Z. (2020). Dissipation of emamectin benzoate residues in rice and rice-growing environments. *Molecules*, 25(3), 483.

Dong, B., Zhao, Q., & Hu, J. (2015). Dissipation kinetics of emamectin benzoate and lufenuron residues in cabbage grown under field conditions. *Environmental monitoring and assessment*, 187, 1-11.

Falcomer, A. L., Riquette, R. F. R., de Lima, B. R., Ginani, V. C., & Zandonadi, R. P. (2019). Health benefits of green banana consumption: A systematic review. *Nutrients*, 11(6), 1222.

Haslinda, W. H., Cheng, L. H., Chong, L. C., & Aziah, A. N. (2009). Chemical composition and physicochemical properties of green banana (Musa acuminata× balbisiana Colla cv. Awak) flour. *International journal of food sciences and nutrition*, 60(sup4), 232-239.

Hernández-Borges, J., Cabrera, J. C., Rodríguez-Delgado, M. Á., Hernández-Suárez, E. M., & Saúco, V. G. (2009). Analysis of pesticide residues in bananas harvested in the Canary Islands (Spain). *Food Chemistry*, 113(1), 313-319.

Hikal, W. M., Ahl, S. A., Hussein, A. H., Bratovcic, A., Tkachenko, K. G., Sharifi-Rad, J., & Atanassova, M. (2022). Banana peels: A waste treasure for human being. *Evidence-Based Complementary and Alternative Medicine*, 2022.

Justin, C., Leelamathi, M., & Nirmaljohson, S. B. (2008). Bionomics and management of the pseudostem weevil *Odoiporus longicollis* Oliver (Coleoptera: Curculionidae) in banana–A review. *Agricultural Reviews*, 29(3), 185-192.

Liu, S., Zhang, F., Wang, L., & Pan, C. (2012). Dissipation and residues of emamectin benzoate in cabbage. *Bulletin of environmental contamination and toxicology*, 89, 654-657.

Malhat, F., Fayz, A. E. S., Loutfy, N. M., & Ahmed, M. T. (2013). Residues and dissipation of the pesticide emamectin benzoate under Egyptian field condition: a case study. *Toxicological & Environmental Chemistry*, 95(7), 1099-1107.

Ouyang, X., Fan, Q., Chen, A., & Huang, J. (2023). Effects of trunk injection with emamectin benzoate on arthropod diversity. *Pest Management Science*, 79(3), 935-946.

Padmanaban, B., Kannan, M., Thangavelu, R., Uma, S., Backiyarani, S., & Ashif, K. K. (2020). Identification of banana corm weevil Cosmopolites sordidus Germar resistance in Musa germplasm.

Paranthaman, R., Sudha, A., & Kumaravel, S. (2012). Determination of pesticide residues in banana by using high performance liquid chromatography and gas chromatography-mass spectrometry. *American journal of biochemistry and biotechnology*, 8(1), 1-6.

Reddy, B. K. K., Bhuvaneswari, K., Suganthi, A., Paramasivam, M., & Geetha, P. (2021). Dissipation kinetics and dietary risk of emamectin benzoate in grapes using liquid chromatograph-mass spectrometry. *ANGRAU*, 1.

Reddy, D. S., Madhumati, C., & Nagaraju, R. (2020). Evaluation of insecticdes and non-edible oils against Banana stem weevil. *Indian Journal of Entomology*, 82(2), 393-396.

SANTE (11312/2021) – Guidance document on analytical quality control and method validation procedures for pesticide residue analysis in food and feed. European Commission Directorate – General for Health and Safety.

Santilio, A., Girolimetti, S. and Barbini, D.A. 2014. Estimation of the validation parameters for a fast analysis of herbicide residues by LC-MS/MS. *Food Addit. Contam. Part A Chem.,* 31(5): 845-851.

Sidhu, J. S., & Zafar, T. A. (2020). Chemical Composition and Nutritional Profile of Raw and Processed Banana Products. *Handbook of Banana Production, Postharvest Science, Processing Technology, and Nutrition*, 207-225.

Suganthi, A., Nikita, S. A., Kousika, J., Bhuvaneswari, K., & Sridharan, S. (2018). Determination of thiamethoxam residues in banana stem and fruit through LC-MS/MS. *Environmental monitoring and assessment*, 190, 1-8.

Wang, L., Zhao, P., Zhang, F., Li, Y., Du, F., & Pan, C. (2012). Dissipation and residue behavior of emamectin benzoate on apple and cabbage field application. *Ecotoxicology and environmental safety*, 78, 260-264.

Wang, R., Liu, B., Zheng, Q., Qin, D., Luo, P., Zhao, W., & Zhang, Z. (2021). Residue and dissipation of two formulations of emamectin benzoate in tender cowpea and old cowpea and a risk assessment of dietary intake. *Food Chemistry*, 361, 130043.

Zhang, K., Wong, J. W., Yang, P., Tech, K., DiBenedetto, A. L., Lee, N. S., & Schreiber, A. (2011). Multiresidue pesticide analysis of agricultural commodities using acetonitrile salt-out extraction, dispersive solid-phase sample clean-up, and high-performance liquid chromatography–tandem mass spectrometry. *Journal of Agricultural and Food Chemistry*, 59(14), 7636-7646.

Zhou, L., Luo, F., Zhang, X., Jiang, Y., Lou, Z., & Chen, Z. (2016). Dissipation, transfer and safety evaluation of emamectin benzoate in tea. *Food chemistry*, 202, 199-20

Table 1. Linearity, LOD, LOQ and matrix effect of raw banana fruit with pulp, peel and pulp on of emamectin benzoate

Matrix	Calibration range (mg L^{-1})	Regression equation	Correlation coefficient (R^2)	Matrix effect (%)	LOD	LOQ
Raw banana fruit with peel and pulp	0.01-0.1	y = 2092.36x + (-4118.18)	0.997	0.37 – 13.69	0.001	0.003
Raw banana fruit with pulp	0.01-0.1	y = 813.04x + (-398.397)	0.992	- 10.75 – 17.20	0.005	0.015

Table 2. Recovery of emamectin benzoate from raw banana fruit with peel and pulp

Spiking level (ug/g)	Recovery (%)									
	R1	R2	R3	R4	R5	R6	R7	Mean	SD	RSD
0.01	100.55	103.74	95.53	98.00	100.23	96.26	103.39	99.67	3.24	3.25
0.025	111.89	114.06	112.42	109.90	109.57	108.86	115.16	111.70	2.38	2.13
0.05	112.12	107.74	105.90	101.84	99.54	110.74	101.69	105.65	4.83	4.57
0.075	105.88	107.72	100.39	107.53	102.07	101.95	104.81	104.34	2.91	2.79
0.1	103.00	102.03	101.09	100.35	103.38	101.86	101.57	101.90	1.05	1.03

Table 3. Recovery of emamectin benzoate from raw banana fruit with pulp

Spiking level (ug/g)	Recovery (%)									
	R1	R2	R3	R4	R5	R6	R7	Mean	SD	RSD
0.01	93.37	98.72	92.97	93.35	97.90	96.17	96.55	95.58	2.35	2.46
0.025	90.64	92.20	83.00	88.36	91.23	93.91	90.82	90.02	3.52	3.91
0.05	108.42	94.56	97.00	93.88	94.34	90.97	88.33	95.36	6.40	6.71
0.075	91.46	84.93	89.57	91.74	101.89	102.38	98.57	94.36	6.66	7.05
0.1	111.20	117.04	119.44	115.97	116.56	105.56	105.62	113.06	5.66	5.01

Fig 1. LC-MS/MS Standard chromatogram of emamectin benzoate (0.01 µg g^{-1})