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Efficient technique for quantification of chlorantraniliprole residue in/on vegetables and soil using GC-MS/MS

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ABSTRACT

An analytical method for the determination of chlorantraniliprole residue in brinjal, capsicum, chilli, cucumber, tomato and soil samples using GC-MS/MS was developed and validated to fulfil the requirements of the SANTE/11813/2017 to support compliance with ISO/IEC 17025. The objective of the validation was to evaluate all required parameters, such as linearity of analytical curves, instrument and method limits of detection and quantification, matrix effects, accuracy (trueness and precision) using modified QuEChERS method. The overall recoveries of the method ranged between 84-98% for the vegetable and soil samples, spiked at 0. 005, 0. 01, 0. 05, 0. 1 and 0. 2 µg / ml. GC-MS/MS parameters were tuned up to optimize limits of quantification (0. 005 µg /mL for vegetables and 0. 01 µg /mL for soil). Repeatability and reproducibility of method was excellent (RSD>10%) for all evaluated matrices. Hence, a fast and efficient gas chromatography-tandem mass spectrometry method with acceptable performance was achieved for routine monitoring and surveillance programme for chlorantraniliprole in soil and vegetable matrices.

Key words: Chlorantraniliprole, GC-MS/MS, Pesticide residue, Standardization,

Chlorantraniliprole (CAP) {3-bromo-N-[4-chloro-2-methyl-6[(methylamino) carbonyl] phenyl]-1-(3-chloro-2-pyridinyl)-1H-pyrazole-5-carboxamide}, is an insecticide belonging to anthranilicdiamide group. CAP has a novel mode of action as an activator of insect Ryanodine Receptor (RyR) which results in rapid muscle dysfunction and toxicity leading to paralysis (Cordova *et al.* 2007). Because of high efficacy on most of the pest of lepidoptera species and some species of coleoptera, diptera and hemiptera (Kuhar *et al.* 2007, Malhat *et al.* 2012) this insecticide is an active ingredient of many formulations (Palumbo 2008). Various researches on CAP show its efficient toxic nature against bollworm (*Helicoverpa zea),* tobacco budworm (*Heliothis virescens)* and rice water weevil adults (*Lissorhoptrus oryzophilus)* (Bernhardt 2008, Hannig *et al.* 2007). CAP exhibits high selectivity and safety with its 350-fold lower activity on mammals than that on insects due to structural variation between insect and mammalian RyR (Lahm *et al.* 2007). Due to no insecticidal effect on helpful arthropods, pollinators, honeybees and non-target organisms as per norms of International Organization of Biological Control Classification (<30% effects), its safety profile is admirable (Dinter *et al.* 2007). The application of pesticides in agriculture usually leads to a residual amount of these pesticides on food products such as fruit and vegetables. To protect consumers, national authorities have established maximum limits for pesticide residues in foods. These limits can only be enforced if there are methods available to detect and monitor their concentrations in the applicable food products. To support the enforcement of this legislation, we have developed a multi-residue method in present work. Although there is some published literature regarding analytical determination of CAP in various substrates using liquid chromatography only (Caboni *et al.* 2008, Grant *et al.* 2010), no methodology particularly on gas chromatography tandem mass spectrometry (GC-MS/MS) is available. Therefore, this study describes the standardization of QuEChERS method with slight modification for the determination of CAP residues in brinjal, capsicum, chilli, cucumber, tomato and soil samples using GC-MS/MS.

MATERIAL AND METHODS

A standard stock solution of chlorantraniliprole (100 µg/ml) was prepared by dissolving 1 mg CAP in 100 ml acetonitrile. Further, sub-stock solution of 1µg/ml and working standard solutions 0.001, 0.005, 0.01, 0.05, 0.10, 0.20 and 0.50 µg/ml were prepared from stock solution by consecutive dilutions with acetonitrile. Matrix match linearity curve was drawn for calibration.

Ripened fruits of brinjal, capsicum, chilli, cucumber,

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tomato procured from local market and soil samples collected from untreated plot of reasearch farm, CCS HAU, India were used as substrates for the method validation of CAP residue at different fortification levels. Out of 500 g representative sample, 15 g of chopped and macerated vegetable/fruit was mixed with 30 ml acetonitrile. The sample was homogenized for 2–3 min. at 14000-15000 rpm. Anhydrous sodium chloride 3 ± 0.1 g was added and shaken vigorously (1-2 min). For cleaning, dispersive solid phase extraction (DSPE) technique was used. Took an aliquot of 6 ml acetonitrile in a test tube containing 0.15 ± 0.01 g PSA sorbent, 0. 90 ± 0. 01 g anhydrous $MgSO_4$ and the mixtures were centrifuged at 2500-3000 rpm for 1 min. Subsequently, 4 ml aliquot of extract was taken and 1µl was injected on GC-MS/MS for analysis of CAP *via* auto injector. For soil samples, initially QuEChERS method was tried, but the recoveries were observed to be very poor. Afterward a modified method was practiced with a better yield of recoveries, in which a representative 20 g of the soil sample was shaken mechanically with 100 ml of acetonitrile for 1 h. Filtrate was evaporated to dryness and redissolved with 3 ml acetonitrile as solvent. For clean-up 0.3 g activated charcoal and 0.3 g florisil packed compactly in a glass column (60 cm × 22 mm i.d.) in between two layers of anhydrous sodium sulphate. Residues were eluted with 125 ml solution of acetonitrile at flow rate of 2-3 ml/min. Elute was further concentrated to 3 ml for GC-MS/MS analysis.

The GC separation was performed by column (SH-Rxi-5Sil MS; 30 m × 0.25 mm × 0.25 µm film thickness) composed of 5% diphenyl and 95% dimethyl polysiloxane. Argon (99.9999%) was used as a carrier gas at an initial flow of 1.46 ml/min. Oven temperature was programmed as 80°C for 2 min; 20°C/ min to 180°C for 0 min; 5°C/ min to 300°C for 10 min; injector port temperature 250°C; ion source temperature 200°C; interface temperature 300°C and loop time 0. 4 sec. The flow rate of gas was 1.46 ml/ min through the column with split ratio 1:10. Two transitions were specified; one for quantization and the other for confirmation. Smart MRM was used to create a measurement program.

Reagents and solvents used are of technical grade procured from high profile manufacturing firms. Certified Reference Material (CRM) of chlorantraniliprole with ≥ 99% purity were procured from Sigma Aldrich, India. Sodium chloride,anhydrous magnesium sulphate ($MgSO_4$) and the sorbent primary secondary amine (PSA) was procured from Merck (Germany), ACROS Organic, New Jersey(USA) and Agilent Technologies India Pvt. Ltd respectively. Acetonitrile and n-hexane were purchased from Suprasolv (Merck) Germany. Instruments used are listed below:

(a) Gas liquid tandem mass spectrometer model (GCM-STQ-8040) manufactured by M/s Shimadzu Corporation, Kyoto, Japan was used. GCMS Solution software was used. SIM segments were established containing a specific ion mass-to-charge ratio (m/z) for testing compound,viz. CAP, followed by the MS/MS characterization. Precursor ions were then subjected to different collision energy voltages to generate the subsequent product and the NIST mass spectral library was used to evaluate the ion products.

(b) Rotary vacuum film evaporator (Model BuchiRotavapor R-210) manufactured by Switzerland, Germany was used for evaporation of extracts.

(c) Low volume homogenizer (Model- Heidolph) supplied by Heidolph, Germany was used for homogenization.

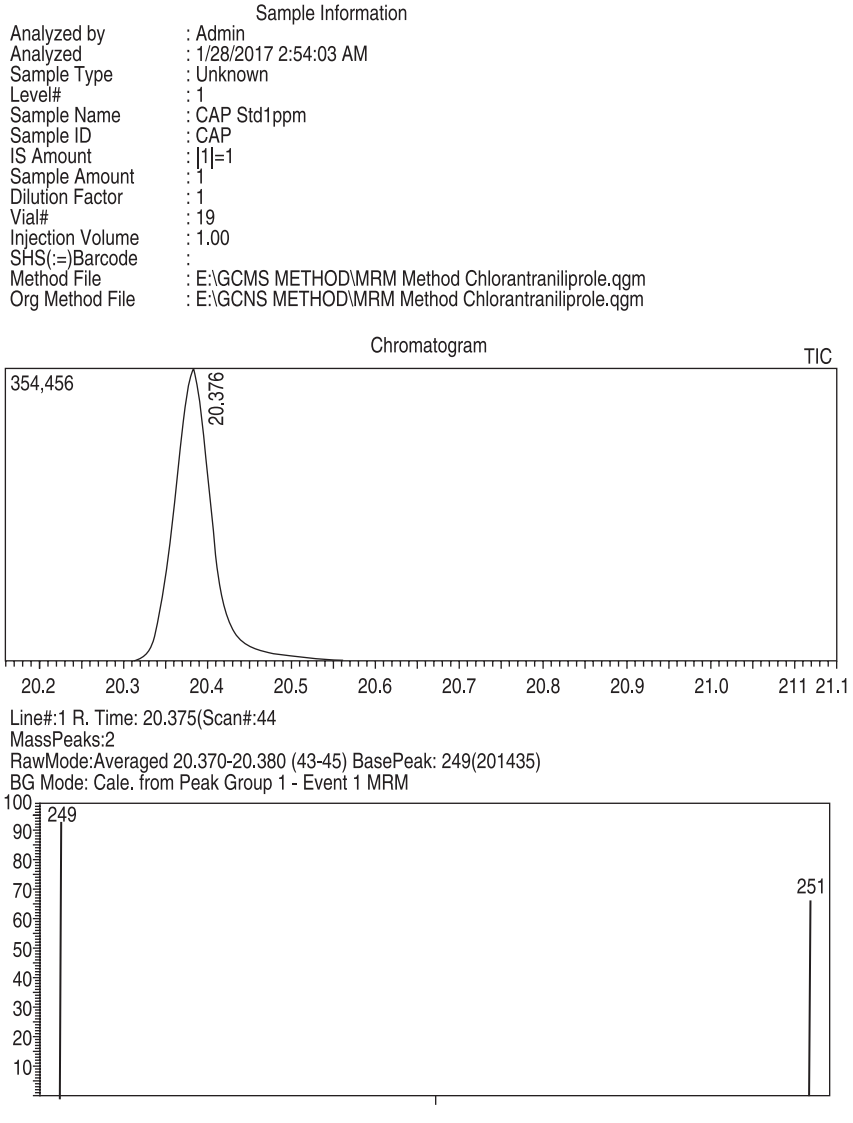

Fig 1 GC-MS/MS chromatograms of CAP standard (1µg/ml) showing daughter ions.

RESULTS AND DISCUSSION

Under the conditions selected, two MS–MS transitions were used in MRM mode 278>249 (quantification transition, Q) and 280>251 (confirmation transition, q). Under these conditions, CAP shows a retention time of 20.4 ± 0.3 min. allowing complete separation of its signal from those of foreign substance present in the sample (Fig 1). Operation of the triple quadruple GC-MS/MS in the multiple reactions monitoring (MRM) mode facilitates matchless sensitivity and selectivity for analytical detection of objective pesticides in traces along with interfering matrices. Quantitative determination of CAP is straight correlated to the assessment of data in diverse biological substrate like vegetables and soil samples. The method was fully validated according to recommendations described in SANTE/11813/2017 guidelines in term of linearity, selectivity, precision (repeatability), precision (reproducibility) and accuracy for both substrates under detection.

Using the matrix-matched calibration approach, calibration standards were prepared over the range of 0.005-0.5 µg/ml along with blank and accessed for selectivity of the method using the instrument conditions outlined above. Due to high sensitivity of GC-MS/MS, matrix effects got nullify and no interference was observed consequently consistent retention time obtained. The detector voltage was attuned for the remarkable sensitivity at lower calibration level. CAP produces a linear connection between detector response(y) and concentration (µg/ml) (x). The criterion for the acceptance of the linearity is a correlation coefficient (r^2) equal or higher than 0.95. In this case, coefficient of determination (r^2) was higher than 0.99.

The limit of detection (LOD) was determined based on the sample concentration that produces a peak with a height three times the level of the baseline noise and the limit of quantification (LOQ) was calculated as the sample concentration that produces a peak with a height 10 times the ratio of signal to noise. It was decided based on response to sample injected as well as sample weight so that base line of the instrument remains stable without any interference. Moreover, LOD and LOQ were calculated by using following formulas:

$$LOD = \frac{3.3 \times \sigma}{S}$$

$$LOQ = \frac{10 \times \sigma}{S}$$

Table 1 Area responses at lowest spiked level i. e. limit of quantification

Matrix	LOQ (µg/ml)	Response Area
Brinjal	0.0050	5131
Capsicum	0.0050	4931
Chilli	0.0050	4946
Cucumber	0.0049	4602
Tomato	0.0052	5596
Soil	0.010	11203

Where "σ" standard deviation of analyte and "S" is the slope of calibrative curve.

The instrument LOD was worked out to be 0.001 µg/ml and LOQ of CAP in samples was found to be 0.005 µg/ml for vegetables and 0.01 µg/ml for soil samples respectively with high detection ability. Area responses regarding LOQ for respective matrix are demonstrated in Table 1.

Accuracy is usually conveyed as the recovery by the method standardized with known spiking concentration of analyte (Francotte *et al.* 1996). Accuracy in presented method was assessed by spiking CAP at four fortification levels in different vegetables (0.005, 0.010, 0.050, 0.10 µg/ml) and soilsubstrates (0.010, 0.050, 0.10, 0.20 µg/ml). Six replicates of each recovery level were analyzed to review the accuracy of the method mainly near the lower concentration of the calibration range. The results were highly suitable for all the fortification levels under study as the recovery obtained at all concentrations and conditions investigated were more than 84% in all the samples under study. At each level of studies, % RSD values of replicates (Table 2, 3) provided the precision in terms of repeatability (RSD_r) and reproducibility (RSD_R). When spiked sample analysis was done by different analysts on different days, reproducibility for those samples was achieved. Results summarized reproducibility range of different substrates for

Table 2 Recovery of CAP in different vegetable and soil samples

Sample	Level of fortification (µg/ml)	CAP Recovery (%)	SD	RSD_r (%)
Brinjal	0.005	84.51	1.7	2.0
	0.01	89.32	2.2	2.4
	0.05	86.64	1.9	2.2
	0.1	93.71	2.5	2.6
Capsicum	0.005	85.45	2.8	3.2
	0.01	88.90	3.1	3.4
	0.05	88.20	2.6	2.9
	0.1	91.33	1.8	1.9
Chilli	0.005	87.25	3.5	4.0
	0.01	86.94	3.1	3.5
	0.05	92.40	2.0	2.1
	0.1	93.65	2.4	2.5
Cucumber	0.005	89.26	3.6	4.0
	0.01	92.37	2.3	2.4
	0.05	93.20	2.7	2.8
	0.1	93.69	3.3	3.5
Tomato	0.005	85.13	2.6	3.0
	0.01	92.52	3.6	3.8
	0.05	90.44	3.0	3.3
	0.1	90.73	3.2	3.5
Soil	0.01	84.49	2.9	3.4
	0.05	89.18	1.8	2.0
	0.1	88.94	2.7	3.0
	0.2	94.39	2.3	2.4

RSD_r: relative standard deviation for repeatability; RSD_R: relative standard deviation or reproducibility

Table 3 Recovery and RSD values calculated from analyses of samples spiked with CAP at LOQ level

Sample	Day	CAP recovery (%)	RSD_r (%)	RSD_R (%)
Brinjal	1	84.51	2.0	2.2
	2	88.33	3.1	
	3	86.47	2.9	
Capsicum	1	85.45	3.2	2.4
	2	89.25	3.7	
	3	89.13	2.7	
Chilli	1	87.25	4.0	4.1
	2	92.05	3.5	
	3	94.67	3.1	
Cucumber	1	89.26	4.0	3.0
	2	84.66	1.7	
	3	89.31	2.5	
Tomato	1	85.13	3.0	4.0
	2	92.05	3.8	
	3	90.23	3.2	
Soil	1	84.49	3.4	2.7
	2	88.92	2.9	
	3	88.04	2.5	

SD: standard deviation; RSDr: relative standard deviation for repeatability; RSD_R: relative standard deviation or reproducibility.

CAP between 2.2-4.1%. To identify and quantify trace-level pesticides in food matrices; the most significant challenges have been matrix interferences, even after QuEChERS extraction and cleanup. Triple quadruple GC-MS/MS, a highly sophisticated instrument, has become prominent as a vital practice for the analysis of barely discernible residues in food commodities. In presented study of CAP, GC-MS/MS in the multiple reaction monitoring (MRM) mode provided precise sense for detection and quantitation even at 0.005 µg/ml. The detection limit of this method was analogous with or better than reported LC-MS/MS or HPLC methodologies.

Modified QuEChERS technique used in this method along with GC-MS/MS instrumentation is suitable for determining CAP in brinjal, capsicum, chilli, cucumber and tomato which accounts for its practical nature. Therefore, similar matrices can also be tried for analyzing CAP using same approach. Moreover, due to good linearity, precision and recoveries of significant range, high sensitivity of GC-MS/MS, this method is a confirmatory substitute for other methodologies like LC-MS/MS for monitoring CAP.

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