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Method Development and Validation of Carbendazim Fungicide in Cucumber

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ABSTRACT

Evaluation of pesticide residues was carried out in cucumber to determine the residues of carbendazim which collected from different local markets in Egypt. During a 14-day, treated cucumbers samples were gathered and analyzed to determine the carbendazim residue quantity. A QuEChERS sample preparation has been applied with liquid chromatography provided with mass spectrometry (LC/MS-MS) to evaluate residual of tested fungicide. Two transitions ions was obtained from multiple reaction monitoring (MRM) after that higher sensitivity transition used for quatification but the lower sensitivity transitions used to confirmation analysis of carbendazin. The method was validated by setting performance parameters such as linearity, precision, recovery, limits of detection (LOD) (0.45 µg Kg^1) and limits of quantification (LOQ) (0.9 µg Kg^1). Good correlation coefficient R^2 0,9992 in pure solvent curve and R^2 1.000 for matrix-matched curve, which given the elevated calibration curve quality. The carbendazin recoveries founded in fresh cucumber samples of 10 replicates were found to be in the range between 119.11 to 124.45% (% RSD < 3.5) for two fortification levels (0.010 and 0.070 $mgKg^1$). The intra-day repeatability RSD value was 3.26%, while the inter-day repeatability RSD value was 5.27%. Matrix effect of carbendazim in cucumber was evaluated and the results showed that carbendazim had a soft matrix effect (6.53).Samples of tested fungicides showed distinct contamination levels up to a certain time period (7 days), after which no residues were identified between 11-14 days.

Keywords: Pesticide residue; Ultra Performance Liquid Chromatography; MS Detector; Carbendazim.

INTRODUCTION

In agriculture, pesticides are widely used to improve quality and extend food crop storage life[1] They are commonly used around the world to safeguard food from inf estation of pest. Residues of pesticides which stay on foods for human consumption after treatment with pest may pose a significant threat to food safety and may even have negative environmental effects such as soil, water, and air which lead to ecosystem imbalance.[2] It's also poisonous nature; their ongoing exposure can lead to their build up in body tissues with possibly severe negative health impacts.[3] Therefore. Monitoring the persistence of pesticide residues in foods produced for human consumption and global trad is essential. Routine analysis for pesticide residues assessment take time and consumption of solvents due to steps of samples preparation prior to chromatographic performance. Multi-class, multi-residue (MRMs) techniques are the most effective method to pesticide residues evaluation. The first significant MRM method based on acetonitrile extraction performed by Mills technique for pesticides in non-fatty foods established in the 1960s.[4]. Anastassiades, *et al.* [5] in 2003 was developed a QuEChERS method to overcome critical faults and practical constraints of current techniques. In a subsequent research, Lehotay, *et al.*[6] used liquid chromatography-tandem mass spectrometry (LC-MS / MS) and gas chromatography-mass spectrometry (GC-MS) to analyze > 200 pesticides in l several matrixes. Validation of the method is an important element of the measures that a laboratory should integrate into its pesticides residue testing to show that it can generate accurate analytical information.[7] The open literature contains several papers reporting methods validation for determining the residue concentrations of pesticides in vegetables and fruit. In QuEChERS technique Kaewsuya, *et al.* established extraction tips for GC-MS assessment for pesticides residues in vegetables and fruits[8]. Fenoll, *et al.* established and MRMin 2007 to simultaneously determine different pesticid e groups in tomato and pepper vegetable. GC separated the extracted parts and identified them using a detector of nitrogen– phosphorus [9]. Camino Sanchez, *et al.* used GCMS-MS and QuECHERS technique to quantify and evaluate the residues of 121 pesticides in different vegetables samples [10]. the aim of this research was to develop and validate a method for the detrmination of carbendazim residue in cucumbers based on QuEChERS rechnique

MATERIALS AND METHODS

Chemicals and reagents

Pesticide standard was obtained from Dr. Ehrenstorfer GmbH (Germany) with purity 99%. Methanol and acetonitrile (pesticide grade) were obtained from Fischer company, USA. Ultra-purifications deionized water obtained from (ELGA, UK). MgSO4 (magnesium sulfate), NaCl (sodium chloride), Sodium Citrate, disodium citrate sesquihydrate and PSA (primary secondary amine).

Preparation of intermediate, working solutions and calibration curves

By dissolving a corrected weight of pesticide standard (according to its purity) into 10 ml of acetonitrile, standard stock solutions were prepared at 1000 $mg.kg^{-1}$. An intermediate mix of standards with a concentration of 5 mg $L^{-1}$was then prepared. The working standard solutions were used to prepare matrix-matched calibrations between 10 and 100 µg L^{-1}.

Sample collection

According to 2002/63/EC (11) regulation, cucumber samples were collected from big supermarkets in Egypt. These samples were transported under cold conditions to the laboratory and kept at 4°C. Shortly after their arrival, they were analyzed to detect pesticide residue carbendazim following the QuEChERS method described below.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis

LC-MS/MS analysis was conducted using a liquid chromatography (Thermo ultimate 3000, DionexSoftron GmbH, Germany) combined with a triple quadruple mass detector with heated electrospray ionization (HESI) source (Thermo, TSQ Quantum Access Max, San Jose, CA, USA) and Thermo Scientific Hypersil GOLD aQ column (100 x 2.1 mm, 1.9 µm particle). Time-specific SRM (t-SRM) windows were used at target compounds retention

times to maximize the performance of the mass spectrometer. The sheath gas flow rate was 55 unit, while the AUX gas flow rate was 15 unit, the capillary temperature and the heater temperature were 280ºc and 295ºc respectively, the spray voltage was 3500v and the cycle time was 0.2s. Water with 4 mM ammonium formate and 0.1% formic acid (mobile phase A), and Methanol with 4 mM ammonium formate and 0.1% formic acid (mobile phase B) were used for the gradient program, which started with 2% B then sharply increased to 30% B over 0.25 min, after that linearly increased to 100%B over 19.75 min, finally the gradient 100% B maintained for 6 min. The column was then reconditioned to 2% B for 4 minutes. Temperature of the column was kept at 40 ^{o}C and the injectionvolume was 10µL at a flow rate 0.3 ml/min. Two multi-reaction monitoring (MRM) transitions have been monitored for carbendazim.

Extraction procedure

The QuEChERS acetatebuffered sample preparation method used to determine pesticides (AOAC 133 Official Method 2007.01) (12). Homogenization for more than 1min. was carried out using a blender (Waring, DCA, CT, USA) to obtain thoroughly mixed homogenates. A 15 g was weighed from the homogenized sample in a 50 ml PTFE tube and added 15 ml of acetonitrile containing 1% acetic acid.Then 6g of $MgSO_4$and 2.5g of sodium acetate trihydrate were added and the sample was shaken for 4 min and centrifuged for 5 min at 4000 rpm (Eppendorf 5804 R, Hamburg, Germany) after that 5 mL of the supernatant was transferred to a 15 mL PTFE tube containing 250 mg PSA and 750mg MgSO4. The extract was shaken for 20s using a vortex mixer and centrifuged at 4000 rpm again for 5 min.A 3 ml of supernatant approximately was filtered using a 0.45 µm PTFE filter (13 mm diameter).

Quality control

Recovery tests were done using blank cucumber samples free from targeted pesticide. Subsamples of those blanks from the different studied commodities were spiked at 2 levels 0.010 and 0.070 mg $kg^{-1}$with carbendazim working solution. Then they were extracted in accordance with QuEChERS pre-described procedure. Recovery and precision (expressed as relative standard deviation) were measured.

RESULTS AND DISCUSSION

LC-MS/MS method optimization

Experiments carried out to determine the ideal conditions of instrumental to identify the analyte clearly at minimum concentration levels in samples. In positive mod the Full-scanning mode was used to determine parent ions in the range from m/z 50 to 500.Based on the detection of quantitative mass spectrometric guideline for the European Commission SANTE (2017) [10], three ions have been chosen to satisfy the performance criteria for identification. Different ratios of mobile phase from methanol, water, acetonitrile and ammonium format without and with formic acid were used to optimize chromatographic separation. Acetonitrile with water as mobile phase was not an appropriate due to bad poor of peak shape.When formic acid was added a highly sensitivity for instrument separation occurred and observed. Different parameters were optimized such as column temperature, flow rate and injection volume and the optimum parameters which selected were column temperature was set at 40 °C, flow rate of 0.3 mL / min and injection volume was 5µL. Carbendazim retention time under this conditions was 4.96 min and values of RSD was <0.8%.

Validation

Calibration curves and linearity

The evaluation of method validity was based on Guideline for both EURACHEM (1998) [13] and European Commission criteria SANTE (2017) [10]. The calibration curve linearity was assessed with injecting five levels of carbendazim standard concentration (10, 30, 50, 70 and 100µg / kg) which prepared in A: B (7:3, v / v) as mobile phase and in blank of cucumber extracts. Three times for each level of concentration was injected. Evaluation of linearity carried out by a linear regression analysis which calculated by a least squared method. Responses of the detector were linear for various concentration levels with good correlation coefficient R2= 0.9992 in pure solvent curve and R2 = 1.000 for matrix-matched curve, which observed excellent calibration curves quality (Table1).

Table 1. Linearity and matrix effect of carbendazim in cucumber.

Analyte	Calibration curve range (µg/kg)	Parameter	Solvent	Matrix	ME%	Level
Carbendazim	10-100	Slope	7746.66	8252.6	6.53	Soft
		R^2	0.9992	1		

LODs and LOQs

The detection limits (LODs) and quantitation limits (LOQs) presented in (Table2) calculated by multiplying standard deviation by factor 3 for LOD and 6 for LOQ [14] and they were 0.45 and 0.9 µg/kg, respectively. The practical LOQ value of carbendazim using spiked sample was 30µg/kg which corresponded to guideline of SANTE (2017) [10] which reported that value of LOQ must be equal to or below maximum residue limit (MRL) established for each analyte in matrix.

Table 2. LOD, LOQ, Recovery, Accuracy and Precision validation parameters.

LOD (µg/kg)	LOQ (µg/kg)	Practical LOQ (µg/kg)	Recovery		RSD%		inter-day repeatability RSD% At 50µg/kg	intra-day repeatability RSD% at 50µg/kg
			10µg/kg	70µg/kg	10µg/kg	70µg/kg		
0.44	0.9	30	119.11	124.45	1.95	3.48	5.27	3.26

Accuracy and precision

Accuracy of the method expressed by recovery of carbendazim fungicide from samples of spiked blank cucumber. The carbendazim recovery for two spiked levels, 10 and 70 µg/kg, was determined from ten replicates for every level. The recovery values ranged from 119.11to 124.45%. RSD values between each level's replicates were 1.95% and 3.48% for 10 µg/kg and 70 µg/kg, respectively. Method's precision expressed by RSD for the 50µg/kg spiked blank samples measured during the same day and during separate days and (repeatability intra-day and repeatability inter-day). The RSD value of intra-day repeatability was 3.26%, while the inter-day repeatability RSD value was 5.27%. Values of accuracy and precision conformity with SANTE (2017) criteria [10]. The RSD values and accuracy of intra-day and inter-day are showed in (Table 2).

Matrix effect

A calibration curve can be used to determine the linearity range (sensitivity) and estimate the effect of the cucumber matrix on the detector response (suppression or enhancement), which determines the quantitation calibration type used. Matrix effect of carbendazim fungicide determined by comparing slop of matrix-matched calibration curve (MMCC) and slop of solvent calibration curve (SCC), Which were built using five levels of concentration and the following formula (C. Ferr, *et al.*, 2011) [15]. ME (%) = (MMCC slope –SCC slop)/(SCC slope) × 100. The effect of the matrix was classified to three levels according to calculated ration as soft 0-20, medium 20-50 and strong >50% (B. Kmellar *et al.*, 2008) [16]. From results in (table 1) the carbendazim fungicide was a soft matrix effect.

CONCLUSION

The validated method is an efficient analytical method for analyzing cucumber based on using acetonitrile LC/+ESIMS/MS. Matrix effect test indicated that carbendazim had a soft matrix effect. The validation criteria, involving the linearity, accuracy (recoveries average), LOQ values and precision (RSD_R), given evidence that the technique is acceptable for the purpose of the research. The extracted cucumber samples have shown no residues after the analysis between 11-14 days. However, the elevated consumption of cucumbers in Egypt compared to other nations can lead to bioaccumulation of pesticide residues. MRL values, ADI and PHI for pesticides used in the management of cucumber pests in Egypt should be updated and a constant and rigorous surveillance program should be implemented to restrict these residual concentrations.

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تطوير واعتماد طريقة لتحليل المبيد الفطرى الكاربندازيم فى الخيار

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فى هذه الدراسة تم تحليل متبقيات المبيدات (مركب الكاربندازيم) فى عينات خيار تم جمعها من السوق المحلية فى مصر. تم تحليل عينات الخيار خلال ١٤ يوم وذلك للكشف عن المتبقى من مبيد الكاربندازيم. تم استخدام طريقة الكاتشر مع جهاز كروماتوجرافيا السوائل المقترن بمطياف الكتلة عالى الحساسية رباعى الأقطاب لاستخلاص و تعيين مبيد الكاربندازيم فى عينات الخيار. تم استخدام المسح متعدد التفاعلات من خلال اختيار ٢ تكسير للمركب و اختيار التكسير ذات الاعلى حساسية للتقدير الكمى و الأقل حساسية للتأكيد على تقدير مركب الكاربندازيم. الطريقة المقترحة تم اعتمادها عن طريق تعيين معايير الأداء مثل الخطية ونسبة الإسترجاع والدقة وحد التعيين(٠.٤٥ ميكروجرام / كيلو جرام) و الحد الكمى (٠.٩ ميكروجرام / كيلو جرام). تم الحصول على معامل خطية R^2= 0.9992 ومعامل خطية $R^2 = 1$ للمنحنى المعيارى داخل مستخلص الخيارمما يؤكد الجودة العالية للمنحنى المعيارى المستخدم.نسب الاسترجاع المتحصل عليها من مستويين للتلويث المعلوم (٠.٠١٠ و ٠.٠٧٠ ميكروجرام / كيلو جرام) لعينات الخيار الطازجة بين ١١٩.١١% - ١٢٤.٢٥ % (بنسبة انحراف معيارى ٣.٥ %). الانحراف المعيارى لمعيار التكرارية خلال اليوم الواحد ٣.٢٦% بينما الانحراف المعيارى لمعيار التكرارية خلال ايام مختلفة ٥.٢٧%. تم تعيين تأثير المستخلص على تقدير نسبة مبيد الكاربندازيم و كان التأثير ضعيف (٦.٥٣). تم رصد مستويات مختلفة من مبيد الكاربندازيم فى عينات الخيار المستخلصة خلال ٧ أيام فى حين لم يتم العثور على تركيزات من مبيد الكاربندازيم فى العينات المستخلصة خلال ١١ – ١٤ يوم.