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Determination of Chlormequat and Mepiquat Residues in Tomato Plants Using Accelerated Solvent Extraction-Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry

ZHANG Hongxia[a],*; LI Fengge[a], [b]; LI Xiaoyan[b]; LI Xuewen[a]; YAO Weiqin[b]

[a] Institute of Food Science and Medicine, Xinjiang Agriculture University, Urumqi 830052, China.
[b] Technical Center of Xinjiang Entry-Exit Inspection and Quarantine Bureau, Urumqi 830063, China.
*Corresponding author.

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Abstract

An Accelerated-Solvent Extraction-Ultra performance Liquid Chromatography-Tandem Mass Spectrometry (ASE-UPLC-MS/MS) method using purified water as extraction solvent for quantitative analysis of chromequat (CQ) and mepiquat (MQ) in samples of tomato plants with higher sensibility and shorter extraction time was developed. The CQ and MQ residues and their dissipation rate were both covered in this paper. The limits of detection (S/N>3) and limits of quantitation (S/N>10) for CQ and MQ were 0.02 μg/kg and 0.1 μg/kg respectively. The linear range was 0.2~10 μg/kg and the correlation coefficients (r^2) was no less than 0.9990, The average recoveries of CQ and MQ from tomato root, stem and leaf in the three spiked range of 1.0, 2.0 and 5.0 μg/kg were in the range of 100.0%~118.8% and 93.2%~110.7% respectively. The dissipation experiment showed that, on average, 98.8% of CQ residues and 99.7% of MQ residues had dissipated after 33 days, with a half-life of 3.67d and 3.66d, which can provide with guideline for using CQ and MQ on tomato in safe range.

Key words: Tomato plants; Accelerated solvent extraction; Ultra-performance liquid chromatography-tandem mass spectrometry; Chlormequat; Mepiquat

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INTRODUCTION

Chlormequat Chloride (CQ), known as 2-Chloro-N, N, N-trimethylethylammonium salts, is a plant growth regulator that can promote flower formation and prevent premature fruits drop of. It is widely used for growth control in wheat, rice, tobacco, cotton and tomato (ZHU, *et al.*, 2006). Mepiquat-Chlovide (MQ) is also a plant growth regulator that can accelerate plant premature and increase yield. However, CQ and MQ can easily pollute groundwater due to high water solubility (Jim, *et al.*, 2004). Therefore their residues are received great interests worldwide. Nowadays many countries has set up the the maximum residue limits (MRL) of CQ and MQ residues in some fruits, vegetables and plants. In European Union the CQ MRL is 0.05 mg/kg in fruits such as apple and pear, while in Japan the MRL of CQ and MQ is 0.05 mg/kg and 0.01 mg/kg in tomato respectively. In China CQ MRL is 5 mg/kg in wheat and maize (FENG & WANG, 2001).

Recent yeas gas Chromatography (Allender, 1992) and Liquid Chromatography-Mass Spectrometry (Castro, *et al.*, 2001 & 2002) are the widely-used methods for the detection of CQ and MQ. In view of the polar and non-volatile nature of the molecule of CQ and MQ, Liquid Chromatography or capillary electrophoresis combined

with Mass Spectrometry (MS) has been regarded the most suitable strategies for confirmatory analysis and quantitation at low level. To date, the occurrence of CQ and MQ in food, vegetables, fruits, soil and water has been investigated in several studies, but their residues and dissipation in Tomato root, stem and leaf and possible pollution they pose to tomato fruits have not previously been researched.

Accelerated solvent extraction (ASE), firstly proposed by Richter *et al.* in 1995, has developed as a mature detection technology in modern analysis and testing. It is very suitable for extraction solid or semi-solid under certain temperature and certain pressure conditions. Moreover it has the unique advantages of high degree of automation, saving time and little solvent consumption and small pollution to environment (ZHAO, *et al.*, 2009).

In this study, we aim to develop a simple, rapid and sensible method, free of clean procedure, for analysis of CQ and MQ residues and its dissipation dynamics in tomato plants (root, stem and leaf). For first time, we use ASE-UPLC-MS/MS to determine CQ and MQ residue levels in tomato plants, which can be used as guideline for using the growth regulators of CQ and MQ on tomato plant in safe range.

1. EXPERIMENTAL

1.1 Materials and Reagents

Tandem Mass Spectrometry apparatus with the brand of ACQUITY UPLC-Quattro premier XETM was from Waters Company (America). Electrospary Ionisation (ESI) connector. High speed refrigerated centrifuge was bought from Kontron Company (Italy). Wrist type oscillator was from Burrell Company (America). Accelerated solvent extraction (ASE) instrument was from Diane company. Vortex mixer (MSC) was purchased from IKA Company (German).

All solvents were of catalytical grade. Acetonitrile with HPLC grade was purchased from Fisher Company (America). Ammonium acetate and formic acid with HPLC grade were brought from Waters Company (America).Water was purified in-house using a Millipore Milli-Q water purification systems (Millipore, Volketswil, Switzerland). Screw sample bottle with 1.5 mL was also brought from Waters Company (America). Extraction tank with 22 mL was from Diane company. Glass tube, regenerated cellulose and Nylon filter membrane (0.2 μm, 26 mm) was from Agilent Company (America).

CQ and MQ were purchased from Dr. Ehrenstorfer Company (German) with the purity >97.0% respectively. The Standard stock solutions with 1000 mg/mL was prepared with the two analytes, then the working standard solution was obtained by diluting the standard stock solution in 2% formic acid and methanol-water (70:30, V/V) and was stored at +4 °C in amber glass bottle, which was stable for one month.

1.2 Sample Treatments

Samples of tomato root, stem and leaf were collected from experimental base located in Hutubi, Xinjiang (China). 10g of the above mentioned samples were milled homogeneously with 2g of diatomites, and then the mixture was put in extraction tank of 22 mL. After filling with marine sand, two cycles of 15 min static extraction using purified water were carried out at heating temperature of 100°C and pressure of 1000psi, the extraction solvent was flushed into 60 mL-glass tube after inflowing nitrogen for 60 sec and then fixed to the volume of 50mL with purified water. 1 mL of supernatant was taken out and filtered by nylon filter membrane (0.2 μm, 26 mm), and stored in sample bottle (1 mL) for analysis by UPLC-MS/MS.

1.3 Chlormequat and Mepiquat Dissipation

Experiment was conducted in 2011 at experimental base in Hutubi, Xinjiang, China. Each experimental plot was no less than 30 m^2, and each treatment replicated three plots. A buffer area was used to separate the plots with different treatments. To investigate the dissipation of CQ and MQ in tomato stem and leaf, CQ (WP, 98%) and MQ (WP, 97%) were dissolved into water and sprayed onto tomato plants, then, at 1, 5, 7, 14, 21, 28, 33 days after spraying, samples of tomato root, stems and leaves were randomly collected from several points in each plots in seedling stage and florescence respectively, and were minced into samples for analysis.

1.4 Accelerated Solvent Extraction (ASE)

The extraction efficiency under accelerated solvents extraction (ASE) varied greatly under different temperature, pressure as well as extraction time, so the temperature, pressure and time of static extraction should be optimized. Temperature in the range of 80-170°C, pressure in the range of 1000-2000psi, and the static extraction time was set at 5min, 10min, 15min and 20min were investigated in order respectively.

1.5 Liquid Chromatography

LC separation was preformed by ion exchange chromatography on a SeQuant ZLC-HILIC MEKCK (150 mm×2.1 mm, 5 μm). All runs were performed under isocratic condition at a flow of 0.3 mL/min, using 20 mM ammonium acetate +0.1% formic acid and Acetonitrile and 0.1% formic acid in 20 mmol/L ammonium acetate (6:4,V/V). The column temperature and ambient temperature were set to 35°C and 7°C respectively and the injection volume was 5 μL.

1.6 Mass Spectrometry

Electrospray ionization mass spectrometry was in positive ion mode (ESI+) with multiple reaction monitoring (MRM). The electrospary capillary was at 3.0 kV. The extraction voltage was at 30 V. The six polar voltage was at 0.20 V. The ion source temperature and desolvation

temperature were 110°C and 380°C respectively. The nebulizer and desolvation gas were 701 L/h and 68 L/h respectively. Moreover, the mobile phrase was separately cut into and out the mass spectrometry at 2 min and 3.95 min. Other analytic parameters were illustrated in Table 1.

Table 1
Tandem Mass Spectrometric Parameters for CQ and MQ. Linearity Equation, Correlation Coefficient

Compound	Parent ion(*m/z*)	Daughter ions(*m/z*)	Cone voltage/V	Collision energy/eV	Linearity equation	Correlation coefficient
Chlornequat	121.9	57.8*, 62.8	42	24，20	y=2106.9x+83.5039	0.9998
Mpiquat	113.8	57.9, 97.85	30	20，20	y=2811.79x+106.086	0.9993

2. RESULTS AND DISCUSSION

2.1 Selection for ASE Extraction Solvents

CQ and MQ not only can dissolve into water, but also into many organic solutions such as methanol, ethanol, but they were stable in neutral and weak acid solution (ZHU, *et al.*, 2006). So the solution of methanol-water and methanol-ammonium acetate were widely used as extraction solvents to separate CQ and MQ residues in fruits, soil and vegetables (WANG, *et al.*, 2007; Nunez, & Galceran, 2002; Vahl, *et al.*, 1998). In our experiment, in order to find the most optimized extraction solvents as separating CQ and MQ in the tomato root, stem and leaf, we tried a range of methanol-water, solvent of methanol-ammonium acetate and purified water respectively, the results indicated that the recoveries for CQ and MQ were much lower as the methanol-water and solvent of methanol-ammonium acetate were used. When the purified water was used, however, the recoveries were much better with the recoveries of CQ and MQ higher than 60% respectively. Therefore purified water was finally selected as the optimal extraction solvent.

2.2 Validation of ASE Extraction Temperature, Pressure and Time

Based on changing trends of residues of CQ and MQ under preset extraction temperatures, pressures and times in Figure 1, 2 and 3 respectively, the optimal conditions for extraction CQ and MQ in Tomato root, stem and leaf were carefully selected, the static extraction temperature, pressure and time was 100°C,1500 psi and 15 min respectively.

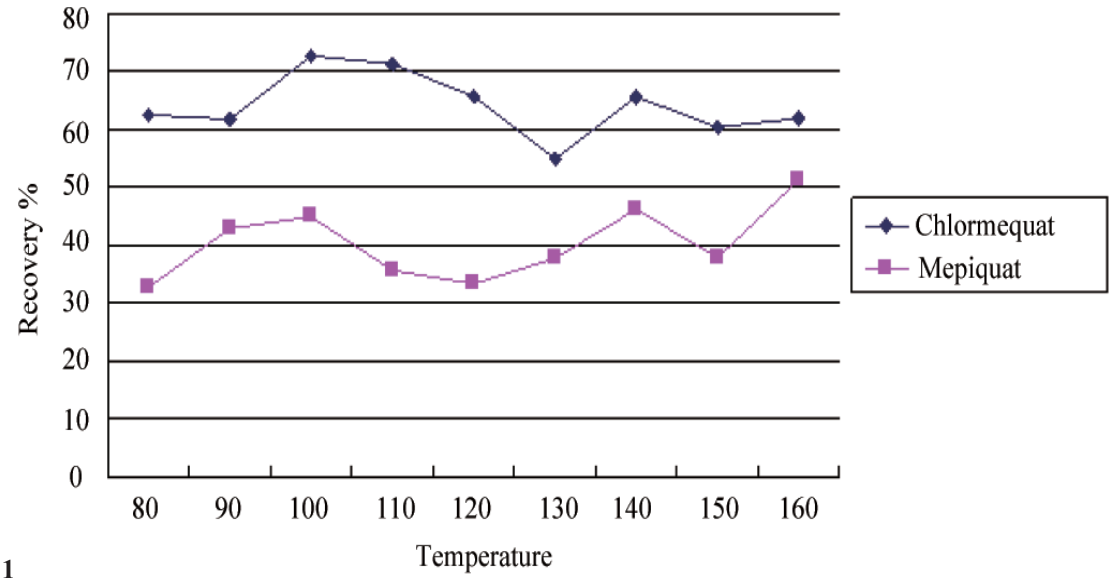

Figure 1
Changing Trends in Residue of CQ and MQ Under Extraction Temperature

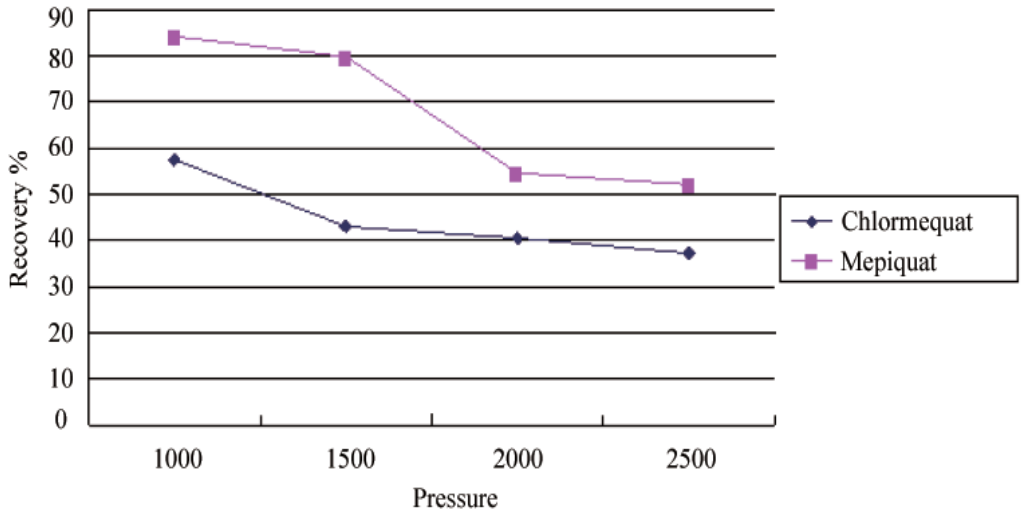

Figure 2
Changing Trends of Residue of Cq and Mq Under Extraction Pressure

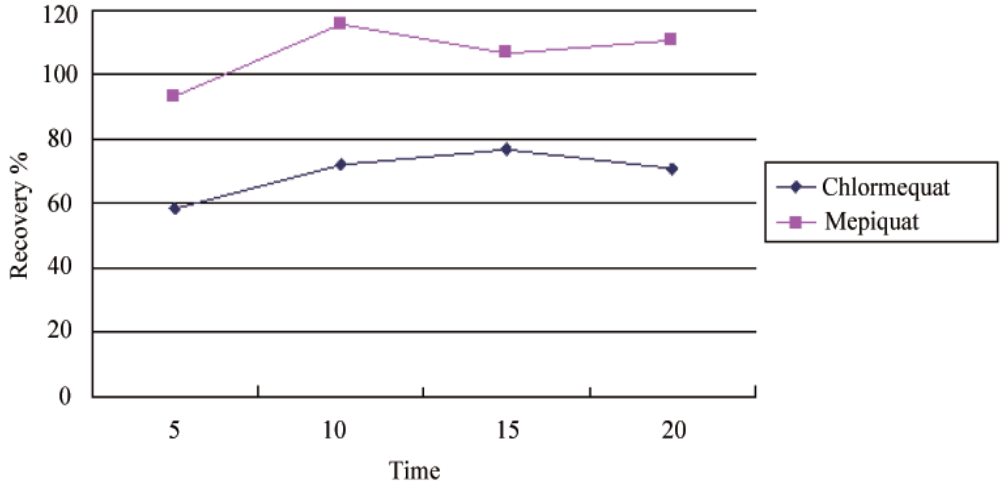

Figure 3
Changing Trends in Residue of CQ and MQ Under Extraction Time

2.3 Optimization and Validation of LC

After investigating the performance of mass spectrometry for C18 reverse phase column (Kromasil Etermity), ACQUITY UPLC BEH HILIC columnand mobile phase such as acetonitrilem, methanol, 20 mM ammonium acetate and 0.1% formic acid, we found that interference of sodium salts can be eliminated by adding 20 mM ammonium acetate. Moreover, the peak of [M-CL] for two analytes of CQ and MQ can be easily produced by using 0.1% formic acids. The acetonitrile and 20 mmol/L ammonium acetate containing 0.1% formic acid (6:4, V/V) was selected as a mobile phase in this experiment as to meet necessary sensitivity. The results illustrated that, whether the methanol or acetonitrilem solvent was used, the target analytes were not separated. However, better separation and peak shape can be obtained when HILIC column and acetonitrile and 20 mmol/L ammonium acetate containing 0.1% formic acid (6: 4, V/V) were used.

2.4 Optimization and Validation of MS

Under ESI condition, one-level mass sepectrometry scanning was carried out in relation to the 0.5 mgL^{-1} standard solution of CQ and MQ, then took the obtained molecular ions as mother ion, and scanned the daughter ions under MRM Modes The ions monitored in MRM modes were m/z 121.9 and m/z 113.8 for CQ and MQ respectively, the daughter ions of the CQ were m/z 57.8 and m/z 62.8 respectively, then ion with m/z 57.8 was selected as quantitative ion; at the same time the daughter ions of MQ were m/z 57.9 and m/z 97.85

respectively, similarly, ion with m/z 97.85 was selected as quantitative ion. Then cone voltage and collision energy were optimized to obtain the optimal ion intensity. The optimized MS conditions illustrated in Table 1, and the MRM chromatograms obtained from samples of Tomato root, stem and leaf containing 10μg/kg of CQ and MQ presented in Figure 4.

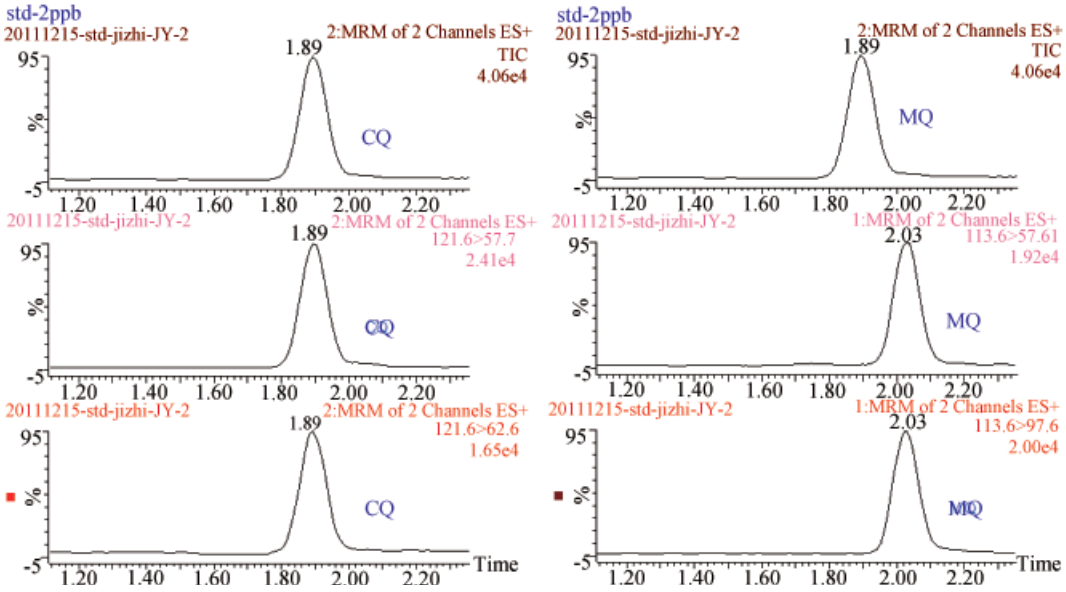

Figure 4
MRM Chromatograms Obtained from Sample of Tomato Root, Stem and Leaf Containing 10 μg/kg of CQ and MQ

2.5 Qualitative and Quantitative Analysis

When the samples were tested by UPLC-MS/MS, If the retention time of peak chromatograph in samples was in line with that in standard solution (tolerance within ± 2.5%), and the maximum permitted relative tolerances of selected ion intensity obtained from sample solution than that in that in standard solution are less than the stipulation of EU Directive Council (Commission of the European Communities, 2002) listed in Table 2, then we can verified that target compounds were existed. In our experiment, the method of calibration with external label was used for quantitation, calibration standard solutions were used to obtain calibration curves and quantitation was performed.

Table 2
Maximum Permitted Relative Tolerances for Relative Ion Intensity

Relative intensity(% of base peak)	>50%	>20%-50%	>10%-20%	≤10%
maximum permitted relative tolerances	±20%	±25%	±30%	±50%

2.6 Calibration and Method Performance

The method linearity was evaluated by standard solution spiked with the two analytes of CQ and MQ at the concentration of 0.2-10.0 ng/mL together with a fixed amount of mixture solution of internal standard (2.0 ng/m). Linear calibration graphs were constructed by least squares regression of concentration versus peak area and peak height of the calibration standards. Good linearity was found in the tested concentration range, with determination coefficients (r2) higher than 0.999. Linearity equation, correlation coefficient and internal standards were illustrated in table 1.Instrumental limit of detection (LOD) based on a signal-to-noise ratio (S/N) of 3:1 and limit of quantitation (LOQ) based on a signal-to-noise ratio (S/N) of 10:1 were calculated, the results of LODs and LOQs detained in our experiment were 0.05 μg/kg and 1.0 μg/kg respectively.

2.7 Recovery and Accuracy

Run-to-run precision was evaluated as per the condition in 1.5 by analyzing six replicated standard solutions at three concentrations: 1 μg/kg, 2 μg/kg and 5 μg/kg. The average recoveries for CQ and MQ ranging from100.0%~118.8% and 93.2%~110.7% with relative standard deviations in the range of 3.1%~14.04% and 4.1%~17.2% respectively (Table 3), which can meet the requirement of quantitative analysis.

Table 3
Recovery and Relative Standard Deviations(RSD) of CQ and MQ Spiked in Blank Seeding Cultivation (N=6)

Drug	1μg/kg		2μg/kg		5μg/kg	
	Recovery(%)	RSD(%)	Recovery(%)	RSD(%)	Recovery(%)	RSD(%)
Chlormequat	111.6	5.6	105.1	9.2	110.7	4.0
Mepiquat	110.2	7.7	103.9	9.3	72	12.5

2.8 Analysis of Samples of Tomato Root, Stem and Leaf

4 groups of tomato root, stem and leaf were randomly collected from Tomato planting base located in Hutubi, Xinjiang, China, the samples were saved in low temperature with 24h after picking up and then were tested based on the established method in this experiment. The results indicated that CQ and MQ residues were detected from all the tested samples, and their concentrations ranging from 0.18 mg/kg to 29.99 mg/kg (Table 4).

Table 4
Results of 4 Samples from Experimental Base

Compound	Concentration of two compounds(mg/kg)				compound	Concentration of two compounds(mg/kg)			
	Tomato roots, leaves1	Tomato roots leaves2	Tomato roots leaves3	Tomato roots leaves4		Tomato roots leaves1	Tomato roots leaves2	Tomato roots leaves3	Tomato roots leaves4
Chlormequat	5.49	2.18	1.69	29.99	Mepiquat	0.26	0.22	0.39	0.18

2.9 Dissipation of Chlormequat and Mepiquat in Tomato Stem and Leaf

After the application of CQ and MQ on tomato plants, their residues dissipation in samples of tomato stem and leaf were investigated at different intervals. As shown in figure 5 and 6, after spraying 5 days, CQ and MQ residues in tomato stem and leaf planted dropped to 88% and 50.3% in the first experimental plot, dropped to 78.9% and 62.5% in the second experimental plot and dropped to 76.0% and 62.8% in the third experimental plot respectively. Similarly, after spraying 14 days, CQ and MQ residues decreased to 90.4% and 45.39% in the first experimental plot, decreased to 85.4% and 70.2% in the second experimental plot, and decreased to 85.6% and 70.0% in the third experimental plot respectively. On average, 98.8% of CQ residues and 99.7% of MQ residues had dissipated after 33 days, with a half-life of 3.67d and 3.66d. The reason for the gradual dissipation of CQ and MQ residues may be attributed to the higher metabolism in leaves (Ezzell, *et al.*, 1995).

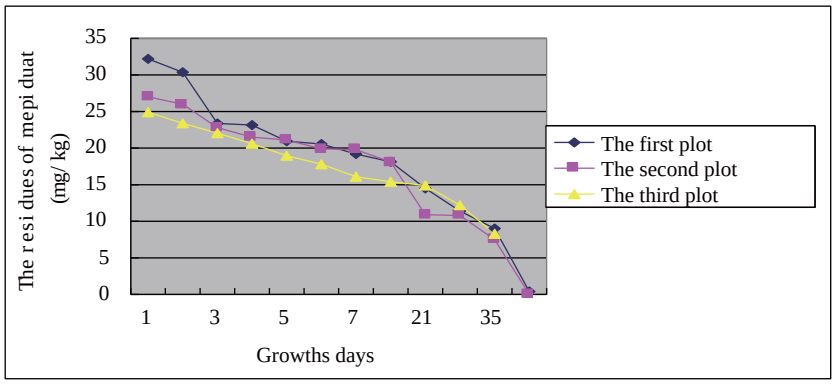

Figure 5
Dissipation of CQ in Tomato Stem and Leaf

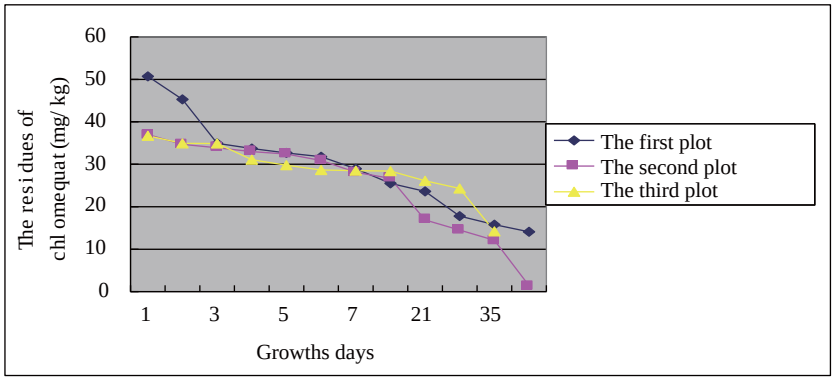

Figure 6
Dissipation of MQ in Tomato Stem and Leaf

CONCLUSIONS

An accelerated solvent extraction-liquid chromatography-tandem mass spectrometry (ASE-UPLC-MS/MS) method using purified water as extraction solvent for the quantitative determination of CQ and MQ residues in Tomato plants (root, stem and leaf) has been developed, and the LODs and LOQs detained were 0.05 μg/kg and 1.0 μg/kg respectively. The experiment procedure indicated that the established method is simple, rapid, sensitive and, and is suitable for the identification and quantification of CQ and MQ residues in tomato root, stem and leaf. Moreover, with the established method, the dissipation rate in Tomato stem and leaf of CQ and MQ was studied, showing that 98.8% of CQ residues and 99.7% of MQ residues had dissipated after 33 days, with a half-life of 3.67d and 3.66d, which can provide with guideline for using CQ and MQ on tomato plants in safe range.

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