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# Residue and Dissipation of Cyprodinil in Apple Using RRLC-QQQ-MS/MS Combined with QuEChERS Method

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A simple, quick and efficient approach for quantitative analysis of cyprodinil residues in apple and soil was established and validated with rapid resolution liquid chromatography tandem mass spectrometry (RRLC-MS/MS) combined with QuEChERS preparation method. The limits of quantification in soil and apple were 0.005 and 0.01 mg/kg. The mean recoveries (n = 5) in apple varied from 88.05 to 92.45 % with the relative standard deviation of 3.74-6.16 %. The recoveries in soil were 84.12-105.70 % with the RSDs of 3.99-8.83 %. The dissipation kinetics followed the pseudo-first-order model and the half-lives in apple ranged from 7.1 to 14.1 days and that in soil were 9.4-15.4 days. The terminal residue in apple found 28 days after application were much lower than the maximum residue limit (MRLs) by America, European, Korea and Japan. These findings will be contribution to provide scientific basis for Chinese government about establishing the MRLs of cyprodinil in apple.

**Keywords: Cyprodinil, Dissipation, Apple, QuEChERS, RRLC-QQQ-MS/MS.**

## INTRODUCTION

Cyprodinil [4-cyclopropyl-6-methyl-N-phenylpyrimidin-2-amine], as a kind of absorption-type and broad-spectrum fungicide, has been considered as one of the most popular fungicide used in fruit fields and widely used frequently in many countries to control the various diseases and insect pests in the process of planting with a long growth cycle[1]. The chemical structure of cyprodinil was shown in Fig. 1.

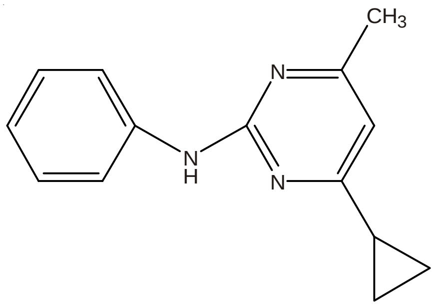

Fig. 1. Chemical structure of cyprodinil

The apple was susceptible to alternaria Mali Roberts which seriously affect the quality and yield of apple. To avoid this situation, the application of fungicides such as cyprodinil is a common practice in the open fields. The fungicide can effectively lead to death of the pathogen in apple by inhibiting the biosynthesis of methionine and the biological activity of hydrolytic enzymes.

To date, intensive studies of cyprodinil have focused on the residues in lettuce, in grapes, must and wine[2,3], in blueberries[1], bottled wines[4], in milk[5], in peas, limes and apple-blueberry sauce[6], in agricultural water and soil[7], in lemon, raisin, tomato, avocado and various vegetable matrices[8]. Meanwhile, the activity of cyprodinil had been reported by Knauf-Beiter *et al.*[9]. The determinations in a variety of matrices were carried out *via* different techniques including gas chromatograph[1,5,10,11], GC-MS[4,5,12,13,15-21]. In addition, LC-MS technique has also employed in detecting the fungicide residue in different samples including fresh fruits, vegetables, wines, incurred water and soil[7,8,22-24]. However, no previous references with regard to the dissipation of cyprodinil residues in apple were observed in view of an extensive and thorough analysis of the available literatures. There is no doubt that it will be bound to pose a risk to human health and increase an insurmountable hurdle for trade export of apple for China. Hence, further in depth investigation should be performed for detailed data associated with cyprodinil in apple matrix.

As is well known, the typical QuEChERS method has its superiorities in dimensions of quick, easy, cheap, effective, rugged and safe. In view of the references cited in literature, the QuEChERS methodology has proven successful for the extraction of pesticides from a variety of foods, fruits and vegetables[25,26]. There is no reason to believe that the sample preparation method could not be been applied for extractions of cyprodinil residues in apple and soil. There are two official methods available: AOAC Official Method 2007. 01 and European Committee for Standardization (CEN) Standard Method EN 15662[6,27].

The main objectives of the study exhibited in paper were to develop a simple, rapid and efficient QuEChERS pretreatment method combined with RRLC-MS/MS for determination of cyprodinil residue in apple and soil. Meanwhile, the degradation dynamics of this fungicide in apple and soil from Beijing, Shandong and Anhui province in 2014 were also investigated to obtain the data of half-lives. The work performed in this project would be a contribution towards providing scientific information for Chinese government about establishing the maximum residue limits of cyprodinil in apple.

## EXPERIMENTAL

Rapid resolution liquid chromatography tandem triple quadrupled mass spectrometer (Agilent 6420, USA) equipped with an reversed phase C18 column (3.0 mm × 50 mm I.D., 2.7 μm) which was employed for separation at 30 °C and an electro spray ionization interface which was operating in the positive ion mode (ESI+). Sample injection volume was 5 μL. The mobile phase was the mixture of acetonitrile (A) and 0.2 % formic acid aqueous (B) in volume ratio 85:15 (v:v) and the flow rate was 0.5 mL/min. The acquisition time was 2 min. The parameters of MS detection were as follows: gas temperature of 300 °C; gas flow rate of 10 L/min; nebulizer gas pressure of 35 psi; the heater temperature of MS1 and MS2 was controlled at 100 °C; capillary voltage 4000 v. The multiple reaction monitoring (MRM) model was selected and the residues of cyprodinil were calculated based on quantitative fragment ions (*m/z* 93).

Cyprodinil standard (98.2 % purity) was provided by Chengdu Keli Long Biochemical Co., Ltd. (Chengdu, China). All of the analytical-grade reagents and the HPLC-grade acetonitrile and formic acid were purchased from Dikma Limited (China). PSA powder (40-60 μm in size) was supplied by Angela Technologies Venusil Technology Co., Ltd. (Tianjin, China). Syringe filter (nylon, 0.22 μm) was provided by Peak Sharp Company, P.R. China. Analytical-grade anhydrous sodium chloride and magnesium sulfate were purchased from Sinopharm Chemical Reagent Co., Ltd., P.R. China. Acetic acid in acetonitrile (1.0 %, v:v) and formic acid water (0.2 %, v:v) were prepared before experiments.

**Field trials:** The field trials including the degradation dynamics and final residues experiments were conducted at three representative locations: Beijing (116.46°E, 39.92°N, warm temperate and semi-humid continental monsoon climate, north of China), Laiyang of Shandong province (120.99°E, 36.97°N, warm temperate regions, semiarid continental monsoon climate, east of China), Anhui (116.58°E, 33.38°N, continental monsoon climate area of temperate zone, mid-eastern of China), from April to September in 2014. The field trials were designed according to NY/T 788-2004 (Guideline on Pesticide Residue Trials) issued by Ministry of Agriculture, P.R. China. There were five experimental treatments with 30 $m^2$ and each treatment had three replications. A buffer zone was used to separate the plots with different treatments.

The average daily temperatures during the whole trials from July to September in 2014 were 33.2/22.6 °C (Beijing), 30.9/19.8 °C (Shandong) and 32.1/21.3 °C (Anhui), respectively. The characteristics for the soil used for the field trials at the three locations were as follows: the type of soil from Beijing belongs to sandy brown soil, with an organic matter of 2.70 %, pH value of 6.73 and cation exchange capacity (CEC) of 29.7 cmol/kg. The soil of Shandong was clay loam and the parameters of it were 3.89 % (organic matter), 7.32 (pH), 16.7 cmol/kg (CEC). The parameters of soil (sandy loam) from Anhui included organic matter (1.71 %), pH (6.8) and CEC (36.4 cmol/kg).

To study the dissipation of cyprodinil residue in apple and soil, 40 % cyprodinil suspension diluted with water was sprayed on the bare soil with no plants and the apple surface at a dosage of 133.3 mg active ingredient per kilogram (a.i./kg) (1.5 times of recommended high dosage). A plot with the same size and no pesticide application was compared simultaneously. The representative apple and soil samples (2 kg) were collected randomly from each plot in different parts at the different time intervals (0, 1, 3, 5, 7, 10, 14, 21, 28 d). And then the soil from experimental field was sieved through a 40-mesh sieve and the apples were mashed by food cutter. After that, both apple and soil were stored at -20 °C until analyzed.

To investigate the terminal residue of cyprodinil, a low-dose level of 133.3 mg a.i./kg (recommended high dosage) and a high-dose level of 200 mg a.i./kg (1.5 times of recommended high dosage) were selected applying the final residual experimental plots in the early stages of leaf spot disease by a portable sprayer for 3 times or 4 times. Both apple (2.0 kg) and soil (1.0 kg) samples were collected at the harvest time and were stored in a deep freezer at -20 °C before further analysis to achieve the final residue and detection.

**Sample pretreatment:** The above samples including soil (5 g) and apple (10 g) were extracted respectively with acid-acetonitrile-water (10 mL, 0.05:5:2) and pure acetonitrile (15 mL) *via* vortexing process for 2 min and then NaCl (1 g) and NaCl (1.5 g) since this will facilitate cyprodinil transferring to the organic phase. $MgSO_4$ (4 g) and $MgSO_4$ (6 g) were separately added to the soil and the apple samples in order to remove a small amount of water dissolved in organic phase. The above vortex procedure was carried out in triplicate. The extract-solutions of soil and the apple with a volume of 2 mL were transferred to a centrifuge tube equipped with 100 mg PSA and 300 mg $MgSO_4$ and another centrifuge tube equipped with 200 mg PSA and 300 mg $MgSO_4$, respectively and then they were vigorously shaken for 1 min until the supernatant is almost colourless. After that, the centrifugal procedure with a speed of 10000 rpm was carried out for 3 min in order to stratify completely. The supernatant was filtered into an auto-sampler vial with 0.22 μm syringe filter and then analyzed by RRLC-MS/MS.

**Recovery assay:** The cyprodinil standard solutions were added to the untreated soil samples and the apple to achieve the followed fortified concentration (soil: 0.005, 0.1, 1.0 mg/kg; apple: 0.01, 0.1, 1.0 mg/kg). The samples were processed and analyzed according to the procedure as mentioned previously. Five parallel treatments for each fortified level were exhibited. Simultaneously, blank sample was also performed for the objective of check interference from the matrix.

## RESULTS AND DISCUSSION

**Method validation:** The matrix-matched calibration curves, as a common external standardization approach, were selected to eliminate the interference resulting from matrix effects which can induce signal enhancement or suppression. The calibration curves were obtained from plotting concentration against peak area and they give an expression in good linearity and correlation degree ranging from 0.005 to 5 mg/L. The regression equations for cyprodinil in soil and apple were as followed.

$$\text{Soil: } y = 43984.587x + 1379.471 \ (r = 0.9998)$$

$$\text{Apple: } y = 97529.296x + 3525.687 \ (r = 0.9999)$$

where, x and y were defined as the absolute injection volume and the average peak area of quantitative ion, respectively.

The limits of quantifications (LOQs) of the method were defined as the lowest spiked concentrations of the analyte in matrix that can be determined with acceptable precision and accuracy. The LOQs of cyprodinil in soil and apple matrix exist 0.005 and 0.01 mg/kg, respectively. The LODs of method matrix show respectively $1.54 \times 10^{-3}$ ng in soil and $5.0 \times 10^{-3}$ ng in apple which were calculated at a signal-to-noise ratio of 3/1. The fortified recoveries of cyprodinil in apple samples were 88.05 to 92.45 % and the relative standard deviation (RSDs) ranged from 3.74 to 6.16 % according to the recovery experiments carrying out five consecutive extractions (n = 5) at three different levels. Simultaneously, the recovery values (n = 5) in soil varied from 84.12-105.7 % with a RSDs of 3.99 to 8.83 %.

The above findings demonstrated adequately the predominant performance in accuracy, reproducibility and reliability to the proposed approach. Further stated, the LOQs of 0.005 mg/kg and 0.01 mg/kg were suitable for quantitative analysis and detection of cyprodinil residues in the soil and apple.

**Dissipation of cyprodinil in soil and apple:** The residues of cyprodinil were determined in apple collected from the evaluated fields at the harvest time in year 2014 and these samples were analyzed triplicates. The dissipation curves and dynamic parameters were presented in Fig. 2(a, b) and Table-1.

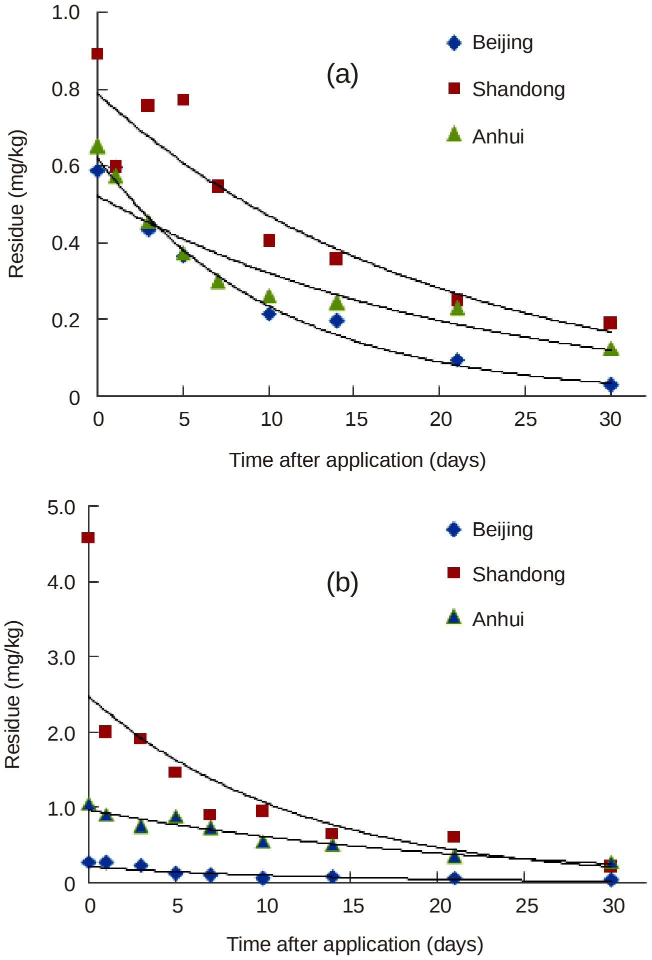

Fig. 2. Degradation dynamics of cyprodinil in apple (a) and soil (b)

As seen from Fig. 2, the loss in amplitude of cyprodinil in apple cultivars from Beijing, Shandong and Anhui remarkably followed pseudo-first-order kinetics model. And the same patterns were observed in soil samples. The residues of it in both apple and soil were reduced more than 50 % of the initial deposit amounts after 10 days in the three growing regions and declined quickly as time passes. The fitting degree and validity of the models can be demonstrated with the parameters of regression coefficients (r) summarized in Table-1.

It can be easily observed from Table-1 that the initial deposition amounts the fungicide in apple were 0.5901 mg/kg in Beijing, 0.8935 mg/kg in Shandong, 0.6516 mg/kg in Anhui, respectively. However, there were sharp differences to a larger extent in soil samples as a result of 0.2697 mg/kg (Beijing), 4.58 mg/kg (Shandong) and 1.05 mg/kg (Anhui). The dissipation half-lives ($DT_{50}$) for cyprodinil in apple were 7.1 days in Beijing, 13.4 days in Shandong and 14.1 days in Anhui, respectively. Obviously, the half-lives in soil samples

TABLE-1
DISSIPATION DYNAMICS PARAMETERS OF CYPRODINIL IN SOIL AND APPLE

| Matrix | Location | Dynamics equation | Half-life (d) | Rate constant ($d^{-1}$) | Initial concentration ($C_0$) | Regression coefficient (r) |
|---|---|---|---|---|---|---|
| Soil | Beijing | $C_t = 0.208e^{-0.074t}$ | 9.4 | 0.074 | 0.2697 | 0.891 |
| | Shandong | $C_t = 2.47^{e-0.084t}$ | 8.3 | 0.084 | 4.5800 | 0.939 |
| | Anhui | $C_t = 0.960e^{-0.045t}$ | 15.4 | 0.045 | 1.0500 | 0.981 |
| Apple | Beijing | $C_t = 0.533e^{-0.094t}$ | 7.1 | 0.094 | 0.5901 | 0.933 |
| | Shandong | $C_t = 0.792e^{-0.052t}$ | 13.3 | 0.052 | 0.8935 | 0.959 |
| | Anhui | $C_t = 0.523e^{-0.049t}$ | 14.1 | 0.049 | 0.6516 | 0.948 |

TABLE-2
TERMINAL RESIDUES OF CYPRODINIL IN APPLE

| Dosage (g a.i./ha) | Applications | Days after spraying | Residues (mg/kg, mean ± SD) | | |
|---|---|---|---|---|---|
| | | | Beijing | Shandong | Anhui |
| 133.3 | 3 | 14 | 0.019 ± 0.082 | 0.677 ± 0.059 | 0.318 ± 0.018 |
| | | 21 | 0.029 ± 0.065 | 0.444 ± 0.041 | 0.253 ± 0.057 |
| | | 28 | 0.028 ± 0.042 | 0.335 ± 0.070 | 0.209 ± 0.061 |
| | 4 | 14 | 0.144 ± 0.009 | 0.786 ± 0.043 | 0.368 ± 0.019 |
| | | 21 | 0.046 ± 0.065 | 0.495 ± 0.035 | 0.314 ± 0.055 |
| | | 28 | 0.029 ± 0.021 | 0.398 ± 0.026 | 0.190 ± 0.119 |
| 200 | 3 | 14 | 0.055 ± 0.045 | 0.744 ± 0.041 | 0.543 ± 0.039 |
| | | 21 | 0.080 ± 0.019 | 0.459 ± 0.011 | 0.370 ± 0.011 |
| | | 28 | 0.060 ± 0.017 | 0.355 ± 0.017 | 0.311 ± 0.079 |
| | 4 | 14 | 0.323 ± 0.034 | 0.965 ± 0.056 | 0.799 ± 0.052 |
| | | 21 | 0.090 ± 0.019 | 0.744 ± 0.047 | 0.791 ± 0.051 |
| | | 28 | 0.065 ± 0.008 | 0.404 ± 0.019 | 0.530 ± 0.052 |

were 9.4 days, 8.3 days and 15.4 days in Beijing, Shandong and Anhui respectively. Thus, the conclusions drawn that cyprodinil degraded more rapidly in apple matrix from Beijing than that from Shandong and Anhui in the light of the data of half-lives and rate constants. Similarly, a faster degradation rate can reflect in soil matrix from Shandong compared with that from Beijing and Anhui. It is noteworthy that the half-life in soil matrix of Beijing is almost close to that of Shandong and Shandong was similar with Anhui about the half-life of cyprodinil in apple matrix, but no significant positive or negative correlation was observed between the dissipation rate and the initial concentration.

These distinctions were primarily result from the following factors. The pesticides residues in crops and soil disappeared through various ways, in which environmental factors including soil texture, organic matter content, moisture, light intensity, pH and temperature had a significant impact on dissipation kinetics[1,28,29]. Besides, the degradation rates of pesticides were closely related to the physical and chemical properties. Last but not least, microbial degradation could be the essential parameter governing the dissipation of cyprodinil but further investigations are required.

**Final residues of cyprodinil in apple and soil:** Although there have been no corresponding MRLs of cyprodinil in apple, 1 mg/kg as a result of the MRLs in pear has been recommended by the Institute of the Control of Agrochemicals, Ministry of Agriculture, P.R. China.

As seen from Table-2, it can be easily observed that the final residues levels of cyprodinil in apple with an interval of 14, 21 and 28 days after applying three times or four times ranged from 0.019 to 0.09 mg/kg in Beijing, from 0.335 to 0.965 mg/kg in Shandong, from 0.190 to 0.799 mg/kg in Anhui, individually, which were under the MRLs of cyprodinil in pear (1.0 mg/kg) in China belonging to the rose family maloideae like apple. Additionally, the ultimate concentrations of cyprodinil were much below the official MRLs in apple legislated by America (4.6 mg/kg), European (1.0 mg/kg), Korea (1.0 mg/kg) and Japan (5.0 mg/kg)[1]. The results suggested that it was safe to use with an interval of 28 days after both spraying three times and four times according to recommended dosage. It is also noteworthy that extending the harvest interval can reduce risk as much as possible.

## Conclusion

A simple, fast and sensitive methodology-based RRLC-MS/MS for determination of cyprodinil in apple and soil was developed and validated. The proposed approach exhibits satisfactory performance in dimensions of accuracy, linearity, correlation, reproducibility. Apart from this, the particular interest was paid to lower LOQs in the soil and apple which were 0.005 and 0.01 mg/kg, respectively. The dissipation results indicated the rapid degradation of cyprodinil in apple and soil under the field conditions. It is safe to use for consumer under the recom-mended dosage and the recommend safety interval should not be less than 28 days from the terminal residue data.

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