

LC-MS/MSによる野菜・果実中クロラントラニリプロールの 実態調査

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Report

Surveillance of Chlorantraniliprole Residues in Vegetables and Fruits Using LC-MS/MS

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We measured the residual amounts of chlorantraniliprole in various vegetables and fruits. Sample solutions were prepared according to our routine procedure based on the QuEChERS method and analyzed by LC-MS/MS. Performance characteristics were evaluated for 8 kinds of food samples by means of recovery tests of 5 replicates at the concentration of 10 ng/g. Recoveries and RSDs (%) ranged from 50.2 to 93.4% and from 2.1 to 9.7%, respectively. Application of this method to survey 207 vegetables and 163 fruits gave detection rates of 8.2 and 1.2%, respectively. In vegetables, detection rates were high in okra (4 out of 10 samples), paprika (4 out of 23 samples) and tomato (2 out of 6 samples), and leaf vegetables such as lettuce, mizuna, spinach and wrinkled greens also contained high concentrations of chlorantraniliprole. The highest residual concentration was 571 ng/g in mizuna. The samples containing chlorantraniliprole seemed to be mainly from Asian countries, including samples of domestic Japanese origin. However, none of them contained more than the MRL, which suggests that the use of chlorantraniliprole has been properly controlled.

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Key words: chlorantraniliprole; insecticide; LC-MS/MS; detection rate

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Introduction

Chlorantraniliprole, 3-bromo-*N*-(4-chloro-2-methyl-6-((methylamino)carbonyl)phenyl)-1-(3-chloro-2-pyridinyl)-1*H*-pyrazole-5-carboxamide, is an anthranilic diamide-class pesticide (Fig. 1) that can be delivered by spraying or irrigation for long-lasting control of insects, especially lepidoptera and diptera. Chlorantraniliprole binds to the ryanodine receptor (a calcium ion channel) of insects, inducing rapid muscle dysfunction, paralysis and eventual death¹⁾. Because its effects are limited to insects, and it does not affect warm-blooded animals or fishes, it is becoming widely used across the world as a next-generation neonicotinoid pesticide. In Japan, chlorantraniliprole was first registered for use as a pesticide in 2009, and since then the range of approved applications has increased. As of April 2018, maximum residual limits (MRLs) are set for 151 kinds of foods, including vegetables, fruits, meats and fishes^{*1)}. However,

there is no report describing the actual residual amounts of chlorantraniliprole in commercial fruits and vegetables available in Tokyo, so it is not clear whether it is currently being used properly.

Several reports have described analytical methods for chlorantraniliprole²⁻⁶⁾. Some of them adopted QuEChERS methodologies, which can deal with multi-

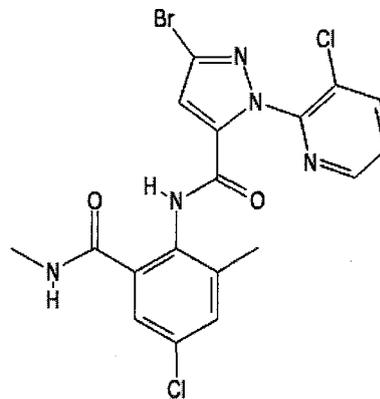


Fig. 1. Structure of chlorantraniliprole

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*1) The Japan Food Chemical Research Foundation, http://db.ffcr.or.jp/front/pesticide_detail?id=21050. (31 Mar 2018 accessed)

Table 1. LC-MS/MS parameters for chlorantraniliprole

LC conditions						
Column	YMC-UltraHT Hydrosphere C18 (2.0 mm i.d. × 100 mm, 2 μm, YMC CO., LTD.)					
Column temperature	40°C					
Flow rate	0.40 mL/min					
Mobile phase	(A) 5 mmol/L ammonium acetate (B) 5 mmol/L ammonium acetate in methanol (A% : B%) = 0 min (95 : 5) → 1 min (95 : 5) → 6 min (5 : 95) → 9 min (5 : 95) → 9.1 min (95 : 5) → 15 min (95 : 5)					
Injection volume	2 μL					
MS conditions						
Ionization	ESI positive					
Analysis mode	Multiple reaction monitoring (MRM)					
Ion-spray voltage	5,500 V					
Vaporizer temperature	300°C					
	Precursor ions (<i>m/z</i>)	Quantitative product ions (<i>m/z</i>)	DP* (V)	EP** (V)	CE*** (V)	CXP**** (V)
Quantitative ion	481.9	283.9	56	10	19	20
Qualitative ion	481.9	450.7	56	10	21	95

* Declustering Potential

** Entrance Potential

*** Collision Energy

**** Collision Cell Exit Potential

ple samples in a short time. Our laboratory has been employing a QuEChERS-based procedure in routine tests, and therefore, in this study, we monitored chlorantraniliprole in vegetables and fruits at the same time as routine tests.

This is the first report to describe the detection rates of chlorantraniliprole in vegetables and fruits. We found surprisingly high levels in some vegetables.

Materials and Methods

Chemicals and Reagents

Powdered standard chlorantraniliprole for testing residual pesticide (purity > 98.0%) was purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). A stock standard solution was prepared by dissolving approximately 20 mg of the powder in acetonitrile, and diluted with up to 50 mL of acetonitrile (400 μg/mL). This solution was stored at -20°C before use.

Acetonitrile (for extraction; pesticide residue and polychlorinated biphenyl analysis grade, for LC; LC/MS grade), methanol (LC/MS grade), toluene (pesticide residue and polychlorinated biphenyl analysis grade), ammonium acetate (LC/MS grade), anhydrous magnesium sulfate (analytical grade), trisodium citrate (analytical grade), disodium hydrogen citrate (analytical grade), sodium carbonate (analytical grade) and sodium chloride (analytical grade) were purchased from FUJIFILM Wako Pure Chemical Corporation. Ultrapure water was obtained from a Milli-Q water system.

A mini-cartridge of C18 (60 mg), graphite carbon (GC, 30 mg) and primary secondary amine (PSA, 60 mg) was custom-ordered from GL Science (Tokyo, Japan). It was used after conditioning with acetone and acetonitrile-

toluene (3 : 1).

Samples

Samples for recovery tests were purchased at local supermarkets and collected from warehouses in Tokyo, which were confirmed to be free of the targeted analyte. Samples for surveillance were also purchased from local supermarkets and collecting-by-order warehouses. These samples were pre-treated in accordance with Japanese Standards and Criteria for Food and Food Additives etc.*², then minced with an electric food processor and stored at -20°C before use.

LC-MS/MS conditions

Analysis was performed using LC-MS/MS instrumentation consisting of a Triple Quad 5500 (SCIEX, Framingham, USA) and Acquity UPLC series (Waters Corp., Milford, USA). The LC and MS conditions were those used in our routine laboratory tests, basically as described in the previous study⁷⁾ (Table 1).

Optimization of MS/MS parameters were performed by direct infusion of 0.1 μg/mL of standard solution in 1 : 1 mobile phase. The optimized conditions for multiple reaction monitoring (MRM) transitions are also presented in Table 1.

Sample preparation

Sample preparation followed our routine procedure, as described previously.⁷⁾ Briefly, 10 g of each sample was placed in a 100 mL polypropylene centrifuge tube and

*² The Japan Food Chemical Research Foundation, <http://www.ffcr.or.jp/zaidan/FFCRHOME.nsf/pages/MRLs-kentai-n>. (31 Mar 2018 accessed)

30 mL of acetonitrile was added. The sample was homogenized thoroughly for 1 min, then the reagents were added: 4 g of anhydrous magnesium sulfate, 1 g of trisodium citrate, 0.5 g of disodium hydrogen citrate and 1 g of sodium chloride. The tube was shaken by hand for 1 min, then the mixture was centrifuged at $1,800\times g$ for 10 min at 4°C and the supernatant was transferred into a 40 mL volumetric flask. After dilution to volume with extraction solvent, exactly 2 mL of the solution was passed through a C18/GC/PSA mini-cartridge. The analyte was eluted with 5 mL of acetonitrile–toluene (3 : 1). The eluate was evaporated to dryness under a gentle nitrogen stream at 37°C. The residue was reconstituted with 1 mL of acetonitrile, and a portion of the solution together with an equal volume of water was transferred to a 1.5 mL microtube, and centrifuged at $16,000\times g$ for 5 min. Two microliters of supernatant was injected into the LC-MS/MS system.

To analyze citrus fruits, chicories and onions, the amounts of reagents were changed as follows: (grapefruit, orange, lemon, chicory and Italian chicory) 4 g of anhydrous magnesium sulfate, 5 g of sodium carbonate and 2 g of sodium chloride; (onion, green onion, leek and scallion) 4 g of anhydrous magnesium sulfate, 1 g of trisodium citrate, 0.5 g of disodium hydrogen citrate and 2 g of sodium chloride.

Performance characteristics

Performance characteristics of this method for analyzing chlorantraniliprole were evaluated by means of recovery tests of 5 replicates at 10 ng/g, and the recovery rates and RSDs were calculated.

A calibration curve was obtained by the external absolute calibration curve method, using calibration points of 0, 1, 2.5, 5, 10 ng/mL in acetonitrile–water (1 : 1).

Matrix effects were also checked by measuring 2.5 ng/mL of matrix-matched standard, which was prepared by mixing 900 μ L of blank sample and 100 μ L of 25 ng/mL standard solution.

The limits of quantification (LOQs) and detection (LODs) were estimated by analyzing blank samples fortified at the levels of 1, 2.5, 5 and 10 ng/mL. They were defined as the lowest concentration of an analyte for which the signal-to-noise ratios were over 10 and 3, respectively.

Quantification of real samples

A calibration curve was obtained as described above. When the estimated residue concentration was outside the range of the calibration curve, sample solution was diluted with acetonitrile–water (1 : 1) to bring it into the calibration range.

Results and Discussion

Performance characteristics

Chlorantraniliprole was monitored using our standard methods, alongside our routine tests of residual pesticides. Therefore, first, we evaluated the performance characteristics of our routine tests for analyzing chlorantraniliprole by means of recovery tests from fortified samples. It should be noted that the reason for changing the combination of buffers and salts for citrus fruits and onions was that the original combination gave low recoveries of other pesticides such as thiabendazole, imazalil, acephate and methamidophos, and the change was irrelevant to the determination of chlorantraniliprole.

The recoveries of chlorantraniliprole from vegetables and fruits were over 74.2% except for grapefruit, for which marked ion suppression was observed (Table 2). However, we found no grapefruit samples containing residual chlorantraniliprole.

Selectivity was good, because no interfering peaks were observed.

Considering the low RSDs, good selectivity, and low LOQs and LODs (much lower than the MRLs), our routine sample preparation procedure was judged suitable for analyzing chlorantraniliprole in various kinds of food samples.

Surveillance of real samples

The total number of samples tested was 370, consisting of 207 vegetables and 163 fruits. The kinds and origins of those samples are listed in Tables S1 and S2.

The overall detection rate in all 370 samples was 5.1%, but was much higher in vegetables (8.2%) than in fruits (1.2%). The detection rate of chlorantraniliprole in vegetables was almost equal to that of boscalid and dinotefran (both 7.9%), which were evaluated in our routine laboratory tests in 2016^(8,9). However, as shown in Figure 2, the residue concentrations of chlorantraniliprole were much higher than those of boscalid and dinotefran especially in leaf vegetable such as lettuce

Table 2. Performance characteristics for chlorantraniliprole

	Recovery (%)	RSD (%)	LOQ (ng/g)	LOD (ng/g)	Matrix effect (%)
Cabbage	88.9	9.0	10	4	82
Grapefruit	50.2	7.9	20	10	43
Japanese mustard spinach	92.1	6.5	10	4	98
Okra	93.4	4.9	10	4	113
Paprika	85.8	2.1	10	4	88
Potato	79.2	5.8	10	4	96
Strawberry	74.2	8.4	10	4	80
Kiwifruit	85.0	9.7	10	4	85

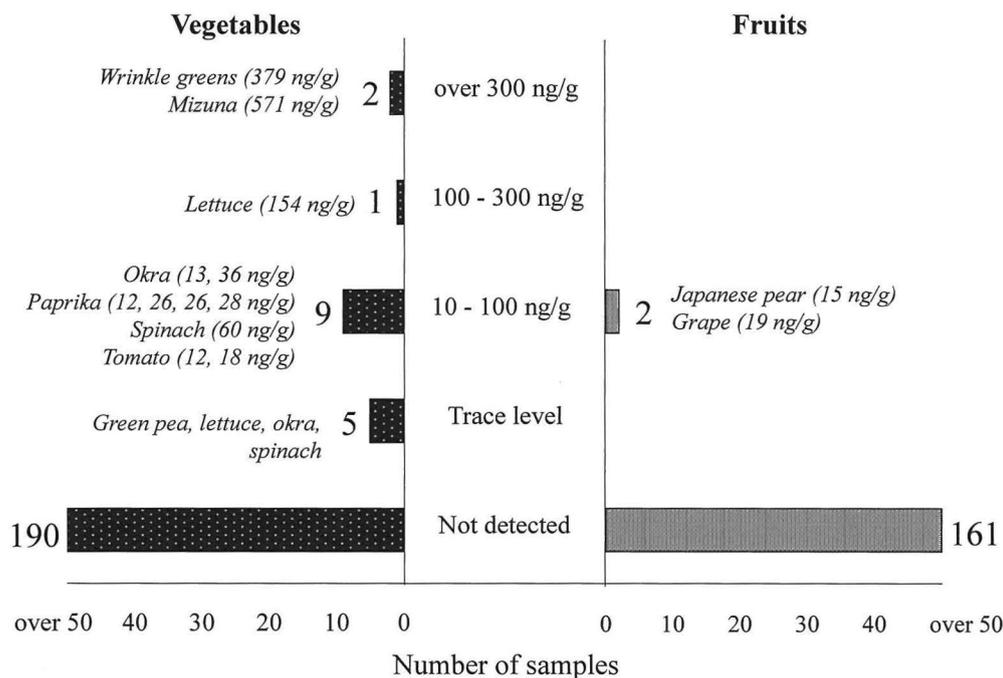


Fig. 2. Distribution of residual concentrations of chlorantraniliprole between vegetables and fruits

(154 ng/g), mizuna (571 ng/g), spinach (60 ng/g and trace level) and wrinkled greens (379 ng/g). Other than leaf vegetables, okra, paprika and tomato also frequently contained residues of chlorantraniliprole: 4 out of 10 okra samples, 4 out of 23 paprika samples and 2 out of 6 tomato samples. These kinds of vegetables are mainly imported from Asian countries such as the Philippines, the Republic of Korea and Thailand, but the 135 samples included 15 samples of domestic origin. The only other vegetables that contained chlorantraniliprole were from the Netherlands: 18 ng/g in tomato and 12 ng/g in paprika.

On the other hand, only two samples of fruits, grape and Japanese pear, contained chlorantraniliprole. These results are presented in Table S3.

Although the test part of Japanese pear is set as the whole fruit including the rind, we also examined the flesh part of a Japanese pear that contained residual chlorantraniliprole. Interestingly, there was no trace of chlorantraniliprole in the flesh part of the sample, suggesting that residual chlorantraniliprole only contaminated the skin of Japanese pear and did not penetrate into the flesh.

None of the samples tested contained more than about 1/20th of the MRL of chlorantraniliprole, suggesting that its use has been properly controlled.

Conclusion

We surveyed residual chlorantraniliprole in vegetables and fruits for the first time, using our routine laboratory tests. Performance was reasonable, with recoveries from 50.2 to 93.4% and RSDs below 10%. In 207 vegetables and 163 fruits, the detection rates were 8.2 and 1.2%, respectively. Detection rates were high in

okra, paprika and tomato, as well as also leaf vegetables such as lettuce, mizuna, spinach and wrinkled greens, but none of the samples contained more than the MRL, suggesting that use of chlorantraniliprole has been properly controlled.

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LC-MS/MSによる野菜・果実中クロラントラニプロールの実態調査 (調査・資料, 英文)

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食衛誌 59(5), 234~238(2018)

殺虫剤であるクロラントラニプロールの実態調査を行った。試験溶液は、QuEChERS法を応用した抽出とC18/GC/PSAによる精製により調製し、LC-MS/MSにより測定・定量を行った。8食品で分析法の性能評価 (n=5)を行ったところ、回収率は50.2~93.4%、RSDは9.7%以下であった。本分析法を用い、野菜207検体および果実163検体を分析したところ、検出限界 (4 ng/g) を超える検体数 (検出率) は野菜で17検体 (8.2%)、果実で2検体 (1.2%) であった。なかでもオクラ (10検体中4検体)、パプリカ (23検体中4検体) およびトマト (6検体中2検体) の検出率が高く、さらに葉菜類では高濃度に残留している検体があり、最も高濃度の残留が認められたのは国産のみず菜 (571 ng/g) であった。また、国産を含めてアジア圏の検体からの検出が大半を占めた。しかし、いずれの検体においても、MRLを超える残留は認められなかったことから、クロラントラニプロールの適切な使用が伺えた。