ASMS 2015

TP 354

Study on Carbosulfan Metabolites in Vegetable by UHPLC Q-TOF MS

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Introduction

Extensive use of agrochemicals at various stages of cultivation for improving agricultural productivity has increased the concern of consumers about the possibility of contamination in food. Many of these pesticides are harmful to humans and the environment owing to their short and long term toxic effects. The ensuing problem of pesticide application can be aggravated by the formation of highly toxic degradation products. Both the United States and the European Union, have created different documents to remark the necessity to assess the environmental impact of relevant degradation products and their identification and routine analysis., since some of those degradation products can be rather persistent and as hazardous as their parent compound.

A typical example is carbosulfan, which is broad-spectrum insecticide, extensively used across the world including US, EU and Asia for pest control in a wide range of crops, mainly citrus, tomato, and rice. In the environment, the metabolism of carbosulfan involves hydroxylation or oxidation reactions or both metabolizing first to carbofuran, which is actually more toxic than the carbosulfan itself. As recently developed technology, quadrupole-time-of-flight (Q-TOF) mass spectrometry with ultra-high mass resolution and MS/MS capability plays an essential role in unknown metabolites because the structural information including the accurate mass of both precursor and product ions can be provided. In this study, UHPLC/Q-TOF has been successfully applied to identify and confirm carbosulfan and its major degradation products and metabolites in tomato with excellent sensitivity and reliable confirmation.

Experimental

Sample extraction

A 10g(±0.05g) previously homogenized tomato was placed into a 50mL centrifuge tube. 100μL appropriate spiking solution was added to sample with concentration of 1μg/g. Tubes were capped and vortexed for 1min. 20mL ACN were added to each tube using the dispenser. To each tube, an Agilent AOAC buffered Extraction packet from the kit (p/n 5982-5755) containing 6g of anhydrous MgSO₄ and 1.5g of anhydrous NaOAc, was added directly to the tubes. Shaken vigorously for 5min, and centrifuged 4000rpm for 5min. 500μL of extract were transferred into an autosampler vial, then 500μL of water were added and ready for UHPLC/HRMS analysis.

Instrumentation

UHPLC/Q-TOF system consists of the Agilent 1290 Infinity, binary pump, autosampler, TCC, and 6540 Q-TOF ultra-high resolution mass spectrometry. The UHPLC/Q-TOF experimental condition are summarized in Table1.

The chemical formulas were calculated based on accuracy mass and isotope ratio calculation by MassHunter Qualitative(B.07). With the help of Mass Profiler(B.07) for statistical analysis and MetaboliteID(B.04) Software for the metabolite screening, the Target MS/MS data with the Molecular Structural Correlator (MSC,B.07) software for MSMS fragmentation elucidation were further applied to confirm the metabolite.

Table1 UHPLC/Q-TOF Conditions

| Column | Zorbax SB-Aq C18 2.1 x 100 mm, 1.8 μm |
| Mobile phase | A=2mM Ammonium formate in water <br/>B=Methanol |
| Flow rate | 0.4mL/min |
| Column temperature | 35°C |
| Injection volume | 2μL |
| Gradient Program | Time(min) | B(%) |
| | 0.00 | 5 |
| | 0.50 | 5 |
| | 3.00 | 20 |
| | 8.00 | 70 |
| | 10.0 | 95 |
| | 12.0 | 95 |
| Post Time | 3 min |
| AJS Dual ESI source | -Ion mode:positive <br/>-Capillary voltage: 3500V <br/>-Nozzle voltage: 300V <br/>-Drying gas: 7L/min@300°C <br/>-Sheath gas: 11L/min@350°C <br/>-Nebulizer: 40psi <br/>-Fragmentor: 130V <br/>-MS scan:50-1500 m/z |
Results and Discussion

Determination by UHPLC/ESI/Q-TOF and identification by Library

The accurate mass spectra of carbofuran and its degradation products were studied in positive and negative ionization mode for each compound. The compounds that have the intact carbamic ester group generated high intensity products ions in positive ion mode, but they did not respond in negative ion mode. The main instrumental parameters for each compound (collision energy, declustering potential, and focusing potential) were optimized to obtain the best sensitivity. From full scan in positive ion mode, we can find significant changes between spike sample and blank sample. The resulting chromatogram is shown in Figure1.

Figure1: Overlay of full scan chromatograms obtained by UHPLC-QTOF in MassHunter Qualitative Analysis software

As is known to all, carbofuran is one of the most important degradation and metabolite product for carbofuran. An accurate mass and all product ions could completely indentify the carbofuran in tomato sample. Using Molecular Formula Generate (MFG) function with spectral library search function (PCDL) by accurate mass and isotopic compounds matching to confirm the structure of carbofuran. It are shown in Figure2 and Figure3.

Figure2: Carbofuran accurate mass and product ions spectrum of mainly degradation product and metabolite of carbofuran

Figure3: Mirror compare sample spectrum with library spectrum of carbofuran

Find more degradation products and metabolites based on significant analysis by mass profiler

Molecular Feature Extraction (MFE) was used to extract all of the ion features from both blank and spike sample and saved as .cfe file. Both files are import to the different analysis software of Mass Profiler (MP) based on statistical analysis. It could found and identified more difference ions between blank and spike sample. It is shown in Figure4 include information of accurate mass and retention time etc.

Figure4: Using MP statistical software to compare differences between the blank sample and spike sample

Figure5: Accurate mass and product ions spectrum of unknown metabolite compound of carbofuran
Results and Discussion

Identification of unknown degradation products by MSC and Metabolite ID

The increased signal compared with blank sample was selected (m/z 130.1585, RT 3.58 min). Accurate mass and product ions are shown in Figure 6. Acquisition MS/MS product ions data were imported to Molecular Structure Correlator (MSC) software, connected with a public chemical database (Chemsider or local database) for search and structural identification of the unknown compound (Figure 6). The unknown degradation compound was identified as dibutylamine. The identification results are very agreement with original drug of carbosulfan. Using Metabolite ID, allowed for the discovery and identification of more potential degradation products and metabolites (Figure).

Figure 6: Unknown metabolite compound of carbosulfan identification by Molecular Structure Correlator (MSC)

Quantitation of carbosulfan, its degradation products and metabolites

Quantitation can be done by using UHPLC/Q-TOF or UHPLC-QQQ. In this study, QQQ (6400 system with Agilent JetStream ion source) have been used for quantitation of carbosulfan and its degradation products (Carbofuran and Dibutylamine) by high sensitivity MRM acquisition mode. (Figure 8).

Figure 8: UHPLC/QQQ Chromatogram of a spiked sample acquired with MRM mode

Conclusions

The study has demonstrated that the hybrid quadrupole time-of-flight mass spectrometer is a valuable tool for the identification and quantitation of transformation products of pesticides. In this work, the widely used insecticide carbosulfan was taken as the model compound for demonstrating the identification of carbosulfan by-products and investigating their appearance in matrix is possible. The study demonstrated that carbofuran and dibutylamine are the major metabolites of carbosulfan in tomato and its metabolic pathway was outlined. It was found that carbosulfan in tomato samples easily changed to dibutylamine, especially in the acidic condition, which can be regarded as the marker of using carbosulfan in vegetables. The methodology followed, based on a combination of UHPLC/Q-TOF-MS/MS and data analysis software (MassHunter Qual, MFE, MSC, Metabolite ID) can easily be applied to other groups of pesticides in real field studies to investigate their degradation and the relative toxicity of their transformation products with respect to the parent pesticides.