Structure elucidation of a novel analogue of pesticide from express post parcel by HRMS

Mingchih Fang¹ · Chia-Fen Tsai² · Der-Yuan Wang² · Hwei-Fang Cheng²

Received: 26 August 2019 / Accepted: 7 November 2019 / Published online: 12 November 2019
© Springer Nature Switzerland AG 2019

Abstract

An express post parcel was confiscated due to the stuff was not equal to the named stuff. HPLC coupled with high resolution mass spectrometry (HRMS) was applied to separate and elucidate the unknown by the combination of the accurate molecular weight, isotope ratio, and fragments. Nuclear magnetic resonance spectroscopy was performed for further confirmation. A new derivative of pesticide chlorantraniliprole was found and first reported. The pesticide analogue named 3-Bromo-1-(3-chloro-2-pyridinyl)-N-[2,4-dichloro-6-(isopropylcarbamoyl)henyl]-1H-pyrazole-5-carboxamide. The derivative of chlorantraniliprole was not able to be detected by any routine pesticide residue analysis. It could be illegally used in claimed organic food or any natural vegetable. Besides, the dishonest people import illegal chemical through express post parcel without registering to any authority, made hazardous chemical transportation management very hard, as well the prevention of the entry of chemicals into food chain.

Keywords Unknown compound · High resolution mass spectrometry · Chlorantraniliprole derivative · Orbitrap

1 Introduction

An express post parcel was confiscated by customs due to the stuff was in fact not the equal of the named stuff and not met the actual needs of the regulation. The parcel was identified as unknown powder and previously sent to analytical labs for routine pesticide and drug screening. However, the reports all answered not detected. In this study, Orbitrap MS coupled with liquid chromatography was applied for the analysis of unknown compound in matrix with purification. The unknown compound structural elucidation was done by the combination of the accurate molecular weight, isotope ratio, and fragments of the unknown. A rare pesticide was identified and reported in this paper. The “new” pesticide was actually a derivative of a registered pesticide.

2 Materials and methods

2.1 Sample

Express post parcel was confiscated by Taipei customs. A simple extraction procedure described in Fig. 1 was necessary in order to remove the unwanted salt. The unknown powder was found not dissolved in water, but a clear solution could be obtained by adjusting the pH to alkali by 0.1 N NaOH. The solution was extracted with diethyl ether. The residue solution was saturated with sodium sulfate and then extracted with acetonitrile. The extract was diluted prior to analysis.

2.2 Separation and detection

An UltiMate 3000 Standard LC system (Thermo Fisher Scientific Inc. Waltham, MA, USA), an Acquity UPLC HSS T3 column

✉ Mingchih Fang, mcfang@mail.ntou.edu.tw | ¹Department of Food Science, National Taiwan Ocean University, Beining Road, No. 2, Keelung City 20224, Taiwan. ²Division of Research and Analysis, Taiwan Food and Drug Administration, Taipei, Taiwan.

Fig. 1 Common sample preparation procedure for an unknown sample prior to liquid chromatography and mass spectrometry analysis

(2.1 × 100 mm, 1.8 μm), and the eluent consisted of 0.1% formic acid water solution (A) and acetonitrile with 0.1% formic acid (B) was utilized. The linear gradient programed as following: 0–1 min, 10% B; 1–11 min, 10–100% B; 11–19 min, 100% B; 19–20 min, 100–10% B; 20–25 min, 10% B. The flow rate was 250 μL/min. Orbitrap MS was Q Exactive Plus Hybrid Quadrupole-Orbitrap Mass Spectrometer (Thermo Fisher Scientific Inc.). The heater and capillary temperature was set to 250 °C and 240 °C, respectively. The capillary of the ESI interface was set to 3.8 kV. Sheath gas and auxiliary gas were set to 50 and 5 units, respectively. Scan range was m/z = 100–1000 and resolution was 140 k (defined at m/z = 200 and was set at full width at half maximum). C-trap was set to allow 1×10^6 charges and the maximum injection time was 100 ms. For product ion scan, the HCD (higher-energy collisional dissociation) was set to 30 eV and 60 eV with precursor ion window 1.7 Da. The resolution was set to be 35 k.

2.2.1 $^1\text{H-NMR}$

The purified unknown powder was prepared in deuterated chloroform in a Wilman 528-PP 5 mm NMR tube. NMR spectra were acquired on a Bruker FT-NMR (400 MHz, Bruker, Billerica, MA, USA), equipped with a 5 mm PABBO BB probe. Chemical shift δ values were cited in parts per million (ppm) in reference to tetramethylsilane as zero ppm. The multiplicity abbreviations used to describe NMR signals are s=singlet, d=doublet, m=multiplet, and j=j coupling.

3 Results and discussion

3.1 Unknown powder structural elucidation

The unknown powder was concrete like in appearance, and impossible to dissolve in water. Our first guess was

that powder may contain high minerals content. The powder was tested to dissolve in acid or basic water solution, and found it well dissolved in basic water solution to obtain a clear liquid by adjusting pH to over 11 utilizing 0.1 N NaOH. The solution was extracted with diethyl ether (the extract was discarded). The residue solution was saturated with excess sodium sulfate and extracted with acetonitrile to give an extract without minerals to prevent damages to LC and mass spectrometer system. The separation was done by HPLC, the chromatogram was showed as Fig. 1. A main peak was found at retention time (RT) 6.85 min. The suspicious peak was selected to give a mass spectrum of the unknown showed in Fig. 2 at ESI positive. The accurate molecular weight of the unknown compound was 529.9550 (monoisotope, $M + H^+$). The highest isotope was $M + 2$ isotope (m/z 531.9524). Because of the significant $M + 2$ and $M + 4$ isotopes (Fig. 3), bromide and chloride were assigned to the element composition of the unknown according to their isotopic mass (^{35}Cl – ^{37}Cl , ^{79}Br – ^{81}Br). Bromide or chloride only contributed to $M + 2n$ isotopes, and the percentages of M , $M + 2$, $M + 4$, and $M + 6$ in Fig. 3 were 50%, 100%, 65%, and 20%, the relative abundances of M , $M + 2$, $M + 4$, and $M + 6$ were calculated to be 21%, 42.5%, 27.6%, and 8.5%, respectively (for example, $M\% = 50/(50 + 100 + 65 + 20) \times 100\% = 21\%$; $M + 8$ was ignored) [1, 2]. To calculate the possible number of bromide and chloride in the element formula of the unknown, one bromide and two chlorides was our first guess. If the unknown contained one bromide and two chlorides, the abundance of the $M + 2$ will be 46.9% (possibility of $^{81}\text{Br}^{35}\text{Cl}_2 + ^{79}\text{Br}^{37}\text{Cl}^{35}\text{Cl}$, Algorithm: $C_1^1 \times 0.493 \times 0.758^2 + 0.507 \times C_2^1 \times 0.242 \times 0.758 = 0.469$) which is not close to the experimental data 42.5%.

The most likely numbers of bromide and chloride were 1 and 3 in the elemental composition of the unknown. For one bromide and three chlorides the abundance of $M + 2$ isotope was calculated through the algorithm $C_1^1 \times 0.493 \times 0.758^3 + 0.507 \times C_2^3 \times 0.242 \times 0.758^2 = 0.426$ (possibility of $^{81}\text{Br}^{35}\text{Cl}_3 + ^{79}\text{Br}^{37}\text{Cl}^{35}\text{Cl}_2$) which closely matched the experimental data 42.5%. The $M + 4$ was calculated to be 27.3% (possibility of $^{81}\text{Br}^{37}\text{Cl}^{35}\text{Cl}_2 + ^{79}\text{Br}^{37}\text{Cl}_2^{35}\text{Cl}$, Algorithm: $C_1^1 \times 0.493 \times C_2^3 \times 0.242 \times 0.758^2 + 0.507 \times C_2^3 \times 0.242^2 \times 0.758 = 0.273$) which closed to experimental value 27.6%. The number of carbon in the unknown was calculated to be 19 (the abundance of $M + 1$ was approximately 20% to monoisotope, $20/1.1 \approx 19$, 1.1 is the natural abundance of ^{13}C in percentage). The combination of monoisotope 529.955, one bromide, 3 chlorides, and 19 carbons gave seven possible formulas in Table 1 with mass tolerance 50 mmu to the theoretical value. The unknown compound was collected from HPLC and repeated for 5 times to obtain around 1 mg

RT: 0.00 - 15.00

Fig. 2 Chromatograms **a** TIC, **b** UV 225 nm of the unknown sample and the spectrum **c** of peak at 6.85 min

Fig. 3 Isotope pattern and isotope ratio of the unknown

stuff. The solvent was removed and the residue was dissolved in deuterated chloroform for NMR analysis. Spectrum was showed in Fig. 4. ^1H NMR (400 MHz, CDCl_3): d, ppm 9.65 (1H, s), 8.48 (1H, d, $J = 3.9$ Hz), 7.87 (1H, d, $J = 7.8$ Hz), 7.45 (1H, d), 7.40 (1H, m), 7.28 (1H, s), 7.17 (1H, s), 5.98 (1H, d, $J = 6.4$ Hz), 4.16 (1H, m), 1.21 (6H, d, $J = 6.4$ Hz). The proton NMR indicated that there were 15 hydrogens in the unknown. The shift of 7.28 ppm was

interfered by chloroform due to the hydrogen in position 6 in Fig. 4 was very close to chlorine. The possible formula was assigned to be $\text{C}_{19}\text{H}_{16}\text{O}_2\text{N}_5\text{BrCl}_3$. The possible structure was elucidated by the fragment spectrum in Fig. 5 (procurer m/z 529.9 at $\text{HCD} = 30$ eV) and NMR spectrum. The structures of the fragments were assumed by matched molecular weight within 10 ppm. The result turned out of a single compound named

Table 1 Possible element composition of the unknown (M + H)

No.	Formula	RDBE	Delta mmu
1	C ₁₉ H ₂₄ O ₅ NBrCl ₃	6.5	5.2
2	C ₁₉ H ₂₀ N ₇ BrCl ₃	11.5	-7.3
3	C ₁₉ H ₁₈ ON ₆ BrCl ₃	12.0	16.4
4	C ₁₉ H ₂₆ O ₄ N ₂ BrCl ₃	6.0	-18.6
5	C ₁₉ H ₂₂ O ₆ BrCl ₃	7.0	29.0
6	C ₁₉ H ₁₆ O ₂ N ₅ BrCl ₃	12.5	40.3
7	C ₁₉ H ₂₈ O ₃ N ₃ BrCl ₃	5.5	-42.4

3-Bromo-1-(3-chloro-2-pyridinyl)-N-[2,4-dichloro-6-(isopropylcarbamoyl)phenyl]-1H-pyrazole-5-carboxamide via searching in ChemSpider website. This compound was actually a derivative of a legal insecticide chlorantraniliprole. The structure comparison of the two compounds was shown in Fig. 6. The compound was identified as an insecticide and it was illegally imported through express post parcel. This was the first report of the chemical C₁₉H₁₅BrCl₃N₅O₂ imported from border. Due to this compound is rare, artificial synthesized, and

not listed in any list for routine pesticide residue testing. It will not be detected, if it was used in agricultural produce, especially in claimed organic food.

4 Conclusion

3-Bromo-1-(3-chloro-2-pyridinyl)-N-[2,4-dichloro-6-(isopropylcarbamoyl)phenyl]-1H-pyrazole-5-carboxamide is an pesticide analogue and discovered in illegal import. The newly synthesized pesticide derivative is not possible to be detected by any routine pesticide residue analysis. The illegal import of harmful chemical to use in food production sure will harm people and the environment. There have been reports that more and more unapproved analogues found in foods, such as structurally modified analogues of phosphodiesterase type-5 (PDE-5) inhibitor drugs and new psychoactive substances (NPS) [3, 4]. Our finding of a novel pesticide analogue should get the governmental authorities to pay attention to chemical and pesticide managements. The provided mass spectrum is a good tool for scientists on the compound

Fig. 4 The ¹H NMR (400 MHz, CDCl₃) spectrum of the unknown

Fig. 5 The fragments (precursor m/z 529.9 at HCD=30 eV) and structure elucidation of the unknown sample

Fig. 6 Structure of chlorantraniliprole (a) and its derivative (b) found in the unknown powder

detection and the data library establishment. High resolution Orbitrap mass spectrometry is successfully applied in the structure elucidation of unknowns assisted with HPLC

offers fast detection with simple purification and other instrumentation.

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

References

1. Kaufmann A (2012) The current role of high-resolution mass spectrometry in food analysis. *Anal Bioanal Chem* 403(5):1233–1249
2. Ruf J, Walter P, Kandler H, Kaufmann A (2012) Discovery and structural elucidation of the illegal azo dye basic red 46 in sumac spice. *Food Addit Contam Part A* 29:897–907
3. Singh S, Prasad B, Savaliya AA, Shah RP, Gohil VM, Kaur A (2009) Strategies for characterizing sildenafil, vardenafil, tadalafil and analogues in herbal dietary supplements, and detecting counterfeit products containing these drugs. *Trac-Trends Anal Chem* 28:13–28
4. Elliott S, Evans J (2014) A 3-year review of new psychoactive substance in casework. *Forensic Sci Int* 243:55–60

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.