



Extended-Target Multiresidue Pesticide Method for Investigating Off-Label Residues in Commonly Consumed Leafy Vegetables.

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Abstract

As pesticide resistance increases and some highly toxic and persistent pesticides are gradually banned, producers often seek alternative pesticides or resort to unconventional practices, resulting in illegal pesticide use. Currently, routine pesticide residue monitoring of agricultural products in Taiwan mainly relies on the Multi-residue Analysis (5). However, as producers become familiar with the fixed monitoring list, which covers only 410 pesticides, they may select pesticides outside the list to circumvent routine testing, creating gaps in the monitoring system.

To clarify this monitoring gap, this study further expanded the Multiresidue Analysis (5) by adding 23 pesticides and analyzed a total of 20 commercially available leafy vegetable samples to investigate whether illegal pesticide use occurs in practice.

The results showed that one sample contained the unapproved pesticide broflanilide. Isocycloseram was detected in four samples, with three exceeding the MRLs and one remaining below the MRLs.

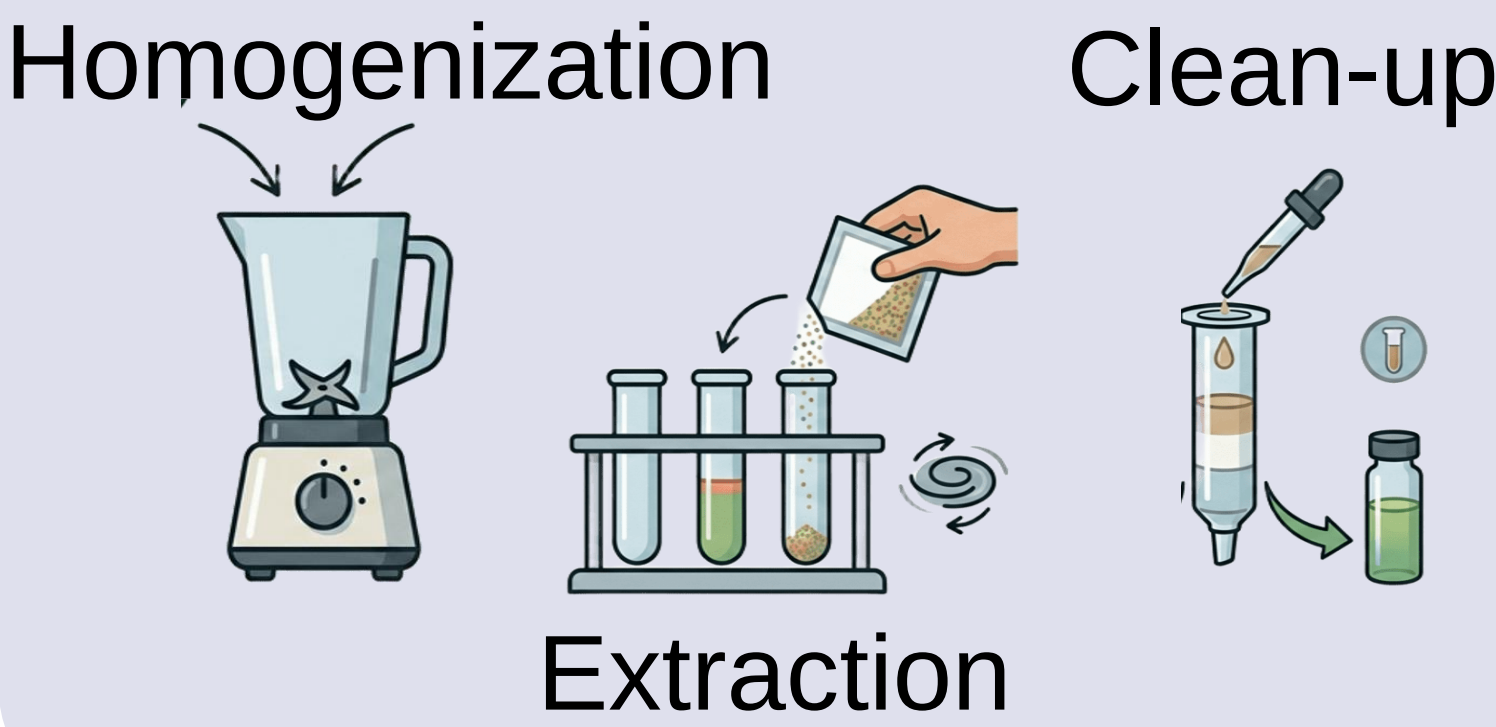
Materials & Method

Materials



- 20 samples
- leafy vegetables

Sample preparation (QuEChERS)



Instrumental Analysis

LC System: Agilent 1290 Infinity LC
Column: CORTECS UPLC C18
Column Temperature & Flow Rate: 40° C, 0.3 mL/min
Mobile Phase A: 0.04% (w/v) Ammonium acetate in water
Mobile Phase B: 0.04% (w/v) Ammonium acetate in methanol
MS System: Agilent 6470 Triple Quadrupole LC/MS

GC/MS System: Agilent 7000D Triple Quadrupole GC/MS System
Column: DB-5MS UI Capillary Column (0.25mm.i.d. × 30m, 0.25µm)
Carrier Gas & Flow Rate: Helium, 1.3mL/min
Inlet Temperature: 280° C

Result

How to Evaluation?

Q.Are compounds detected?

Detected

Or

Not Detected

Pass!!

↓ Q.Are MRLs established?

MRLs Established

Or

No MRLs established

Failure

↓ Q.Does it exceed MRLs?

Exceeded MRLs

Or

Within MRLs

Pass!!

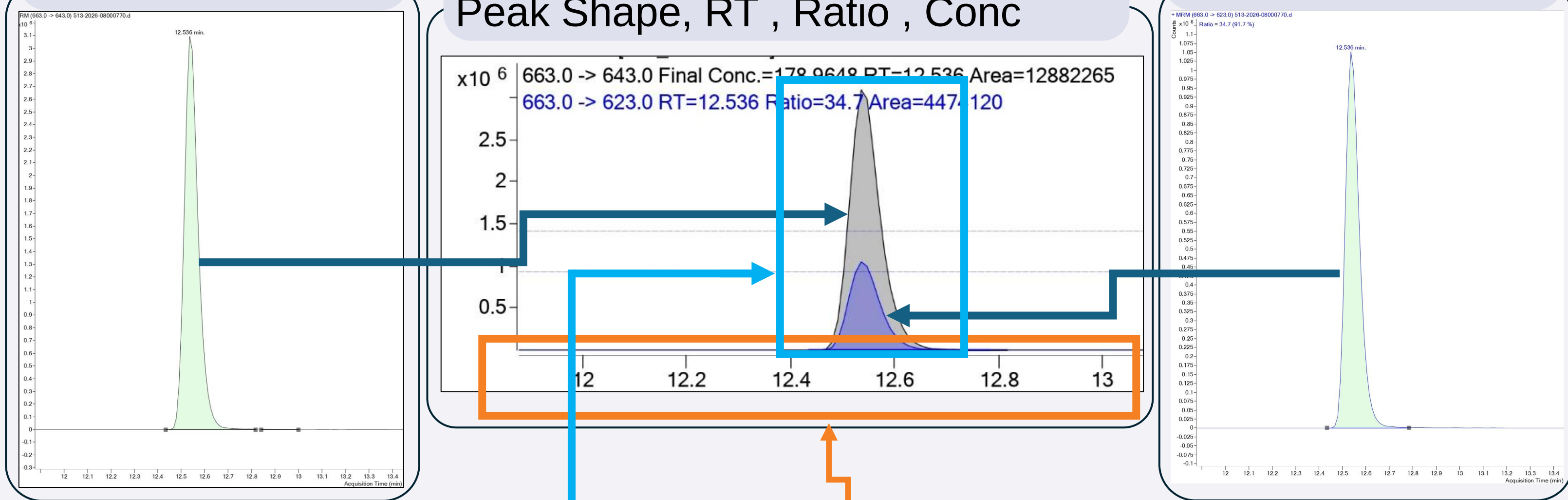
Failure

How to Read the Charts ?

Quantitation : Measure chemical amount

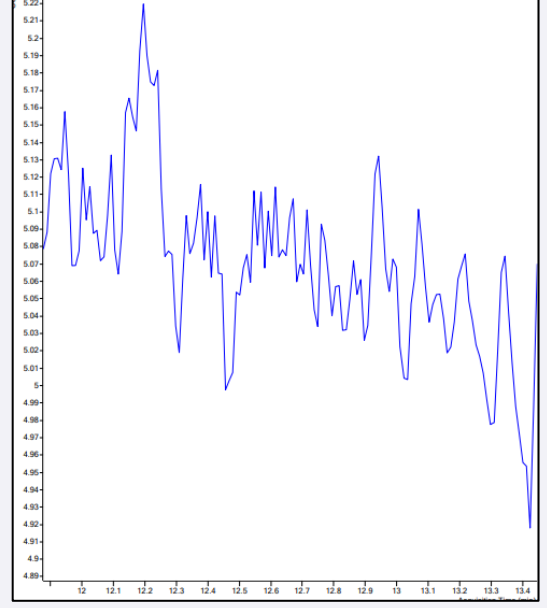
Overview Charts: Quantitation, Qualification, Peak Shape, RT , Ratio , Conc

Qualification : Identify target compounds in samples



Peak Shape

- Symmetrical peaks show low interference/noise
- Right chart indicates noise

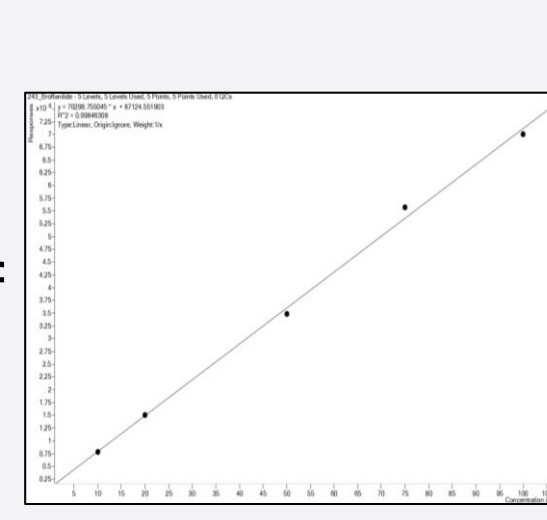


RT:Retention Time

- Product ion abundance ratio (Qualifier ion peak area ÷ Quantifier ion peak area) × 100%
- Confirms analyte identity and rules out false positives/matrix interferences

Calibration curve

- the relationship between the response value (area) of the standard solution and concentration



Ion Ratio

- Fragment mass ratio (Qualification ÷ Quantitation = Ion Ratio)
- Distinguishes similar peaks or background noise

Instrumental Analysis

- Instrumental analysis detected the following two pesticides in five samples.
- **Broflanilide** was detected in **kale sprouts (Kaklan vegetable)**.
- **Isocycloseram** was detected in **two Bok choy** samples, one **Chinese kale** sample, and one **cabbage** sample.

Broflanilide						
Name	Concentration(ng/mL)	RT(min)/Ratio(%)				
Broflanilide(Standard)		12.536/34.7				
Kale sprouts	178.96	12.536/34.7				
Cabbage(A)	0.71	12.547/28.6				
Cabbage(B)	ND	Fail				
Cabbage(C)	ND	Fail				
Cabbage(D)	ND	Fail				
Non-heading Chinese cabbage(A)	ND	Fail				
Non-heading Chinese cabbage(B)	ND	Fail				
Non-heading Chinese cabbage(C)	ND	Fail				
Non-heading Chinese cabbage(D)	ND	Fail				
Non-heading Chinese cabbage(E)	ND	Fail				
Bok choy(A)	ND	Fail				
Bok choy(B)	ND	Fail				
Bok choy(C)	ND	Fail				
Bok choy(D)	ND	Fail				
Amaranth	ND	Fail				
Water spinach	ND	Fail				
Thai basil	ND	Fail				
Green onion	ND	Fail				
Cauliflower	ND	Fail				
Chinese kale	ND	Fail				

Broflanilide	Insecticide: Highly stable & persistent; economical with a unique MoA effective against resistant pests.
Pesticide Residue Limit Standards	Prohibited pesticide by official announcement must not be detected (ND).

Isocycloseram						
Name	Concentration(ppb)	RT/Ratio				
Isocycloseram(Standard)		13.206/26.5				
Chinese kale	32.61	13.206/26.5				
Bok choy	43.84	13.206/26.5				
Bok choy	64.96	13.206/26.5				
Cabbage	7.46	13.217/23.6				
Kale sprouts	ND	Fail				
Cabbage	ND	Fail				
Cabbage	ND	Fail				
Non-heading Chinese cabbage	ND	Fail				
Non-heading Chinese cabbage	ND	Fail				
Non-heading Chinese cabbage	ND	Fail				
Non-heading Chinese cabbage	ND	Fail				
Non-heading Chinese cabbage	ND	Fail				
Bok choy	ND	Fail				
Bok choy	ND	Fail				
Amaranth	ND	Fail				
Water spinach	ND	Fail				
Thai basil	ND	Fail				
Green onion	ND	Fail				
Cauliflower	ND	Fail				

Isocycloseram	Insecticide: Highly stable & persistent; economical with a unique MoA effective against resistant pests.
Pesticide Residue Limit Standards	Other brassica leafy vegetables (excl. cabbage): 0.6 ppm Cabbage: 3.0 ppm Non-head-forming leafy vegetables: Must not be detected (ND)

Reference

- (i) Method of Test for Pesticide Residues in Foods - Multiresidue Analysis (5)
- (ii) Standard Operating Procedure for the Method of Test for Pesticide Residues in Foods – Multiresidue Analysis (5), Eurofins Food Testing Co., Ltd.

Conclusion

- kale sprouts (Kaklan vegetable) : A high concentration of **Broflanilide** was detected.
- Two Bok choy and one Chinese kale : **Isocycloseram** was detected. As no MRLs have been established for these three samples, the pesticide should not be detected.
- Cabbage : **Isocycloseram** was detected. The MRL is 3.0 ppm, while the detected concentration was 7.4573 ng/mL; therefore, the sample was within the acceptable limit.
- Monitoring based on a single method and a fixed pesticide list can create gaps in agricultural pesticide regulation. Expanding the scope of multi-residue testing can effectively identify the actual use of pesticides not included in the monitoring list, improve the accuracy of risk assessment, and provide scientific evidence for regulatory authorities to continuously update pesticide monitoring policies and analytical methods.