A Simple Sample Preparation Method for Pesticide Residue Analysis Using a Multilayer Solid-Phase Extraction Cartridge for GC-MS and LC-MS/MS

Takumi Kunieda, Shigenori Ota, Manabu Takayanagi
GL Sciences Inc., Tokyo, Japan

Introduction

Simultaneous analysis of multiple pesticide residues in food matrices requires efficient and robust sample preparation methods. The QuEChERS extraction technique has become widely adopted due to its simplicity and applicability to a broad range of analytes. However, the dispersive solid-phase extraction (d-SPE) step in conventional QuEChERS may not adequately remove interfering substances, potentially causing anomalous recoveries and contamination of analytical instruments. To address these issues, a multifunctional solid-phase extraction column, InertSep AL-N/VRA-PR, was developed for use in combination with QuEChERS extracts. This SPE column is packed with AL-N at the top and layered with three cleanup sorbents—C18, SAX, and PSA—providing comprehensive matrix removal. The column is designed to support both GC-MS and LC-MS/MS workflows. In this study, we evaluated the performance of InertSep AL-N/VRA-PR for pesticide residue analysis using both techniques. Agricultural crop samples were analyzed for recovery performance, and the versatility of the SPE column was examined under various loading and elution conditions.

Methods

Sample Preparation

Spinach and barley were used as representative agricultural matrices. The extraction procedure followed the QuEChERS (EN 15662) method. A total of 4 mL of the acetonitrile extract—equivalent to 4 g of spinach and 2 g of barley—was loaded onto the InertSep AL-N/VRA-PR column (400 mg/1600 mg/6 mL). The eluate was analyzed by GC/MS in scan mode.
To evaluate the purification performance, a comparison was made with the Japanese official method involving liquid-liquid extraction followed by cleanup using a GC/NH_2 SPE column.

Recovery Experiments

LC-MS/MS

For LC-MS/MS, the spinach extract was spiked with 137 pesticide compounds at 0.1 µg/mL (n = 3). The extract was passed through the SPE column, and two eluates were collected:

- **Elution 1**: Using acetonitrile.
- **Elution 2**: Using 2% formic acid in acetonitrile/water (9:1).

The combination of both eluates was analyzed to assess total recovery.

GC-MS

For GC-MS, extracts of spinach and barley were spiked with 169 pesticide compounds at 0.1 µg/mL (n = 3). Three tests were conducted:

- **Test 1**: Standard sample load (4 g spinach, 2 g barley).
- **Test 2**: Half sample load (2 g spinach, 1 g barley).
- **Test 3**: SPE column with an additional 1 g layer of anhydrous magnesium sulfate.

Recovery rates were evaluated in each case using GC/MS.

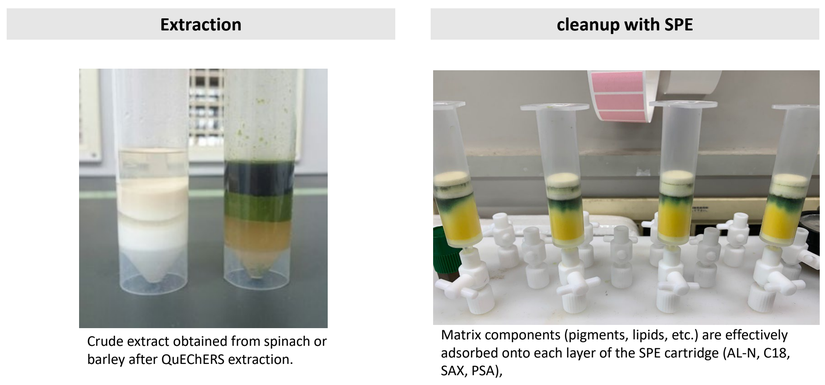

Fig. 1 Sample Preparation Procedure for GC/MS

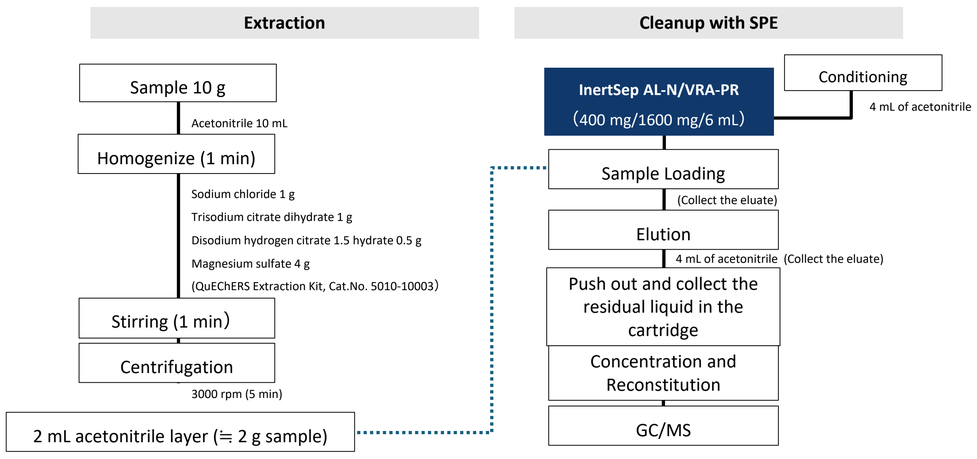

Fig. 2 Sample Preparation Procedure for GC/MS

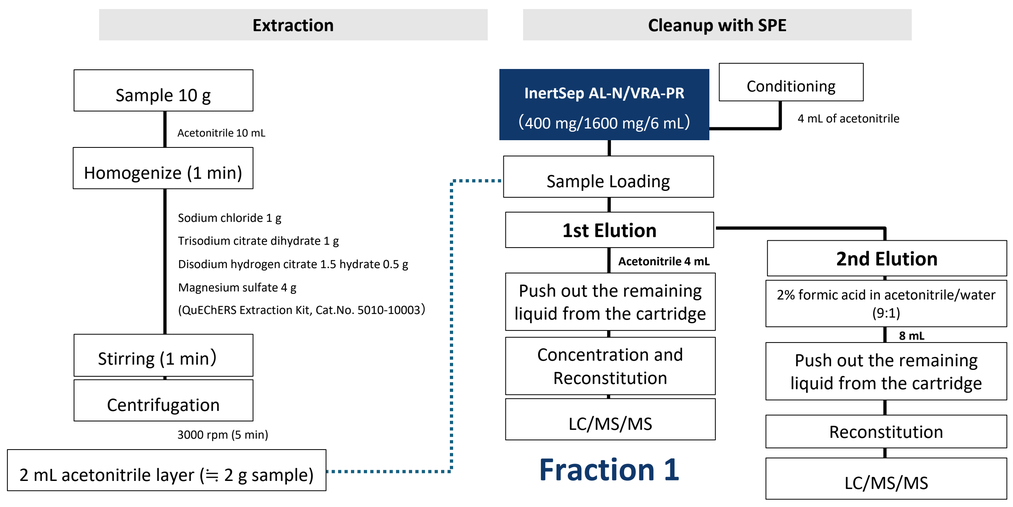

Fig. 3 Sample Preparation Procedure for LC-MS/MS

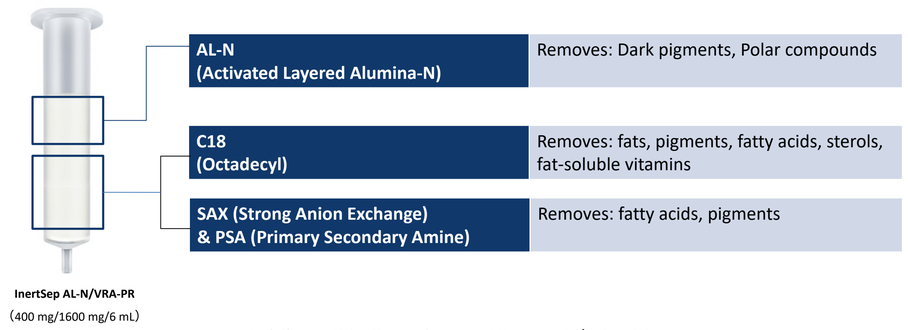

Fig. 4 Multilayer SPE Cartridge InertSep AL-N/VRA-PR

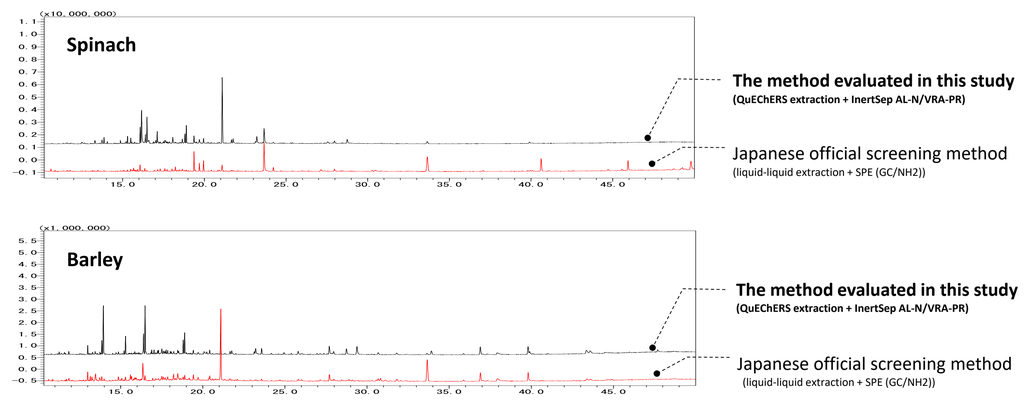

Fig. 5 Chromatographic Comparison of cleanup performance between the method in this study and Japanese official screening method

Tabel 1 Number of Compounds Recovered in Each Elution Fraction (Spinach, LC/MS/MS)

Recovery Rate(%)	1st Elution	2nd Elution	Total
>150	3	0	
120~150	1	0	
70~120	112	**14**	**126**
30~70	4	**2**	
<30	17	**1**	

Total 137 compounds

Table 2 Number of Compounds with 70–120% Recovery in Each Elution Fraction (Processed Foods, LC/MS/MS)

	Dumplings	Okonomiyaki	Kimchi
1st Elution	107	112	110
2nd Elution	16	15	15
Total	**123**	**127**	**125**

Total 137 compounds

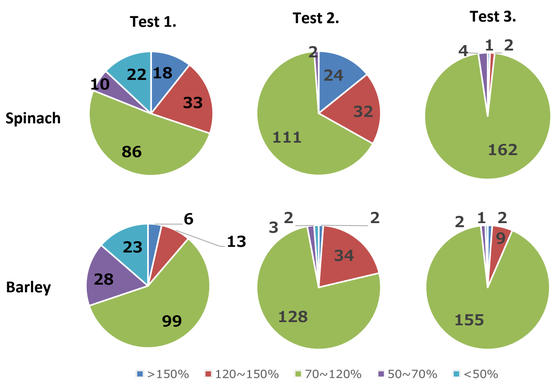

Fig. 6 Compound Recovery Distribution by SPE Condition in GC/MS Analysis

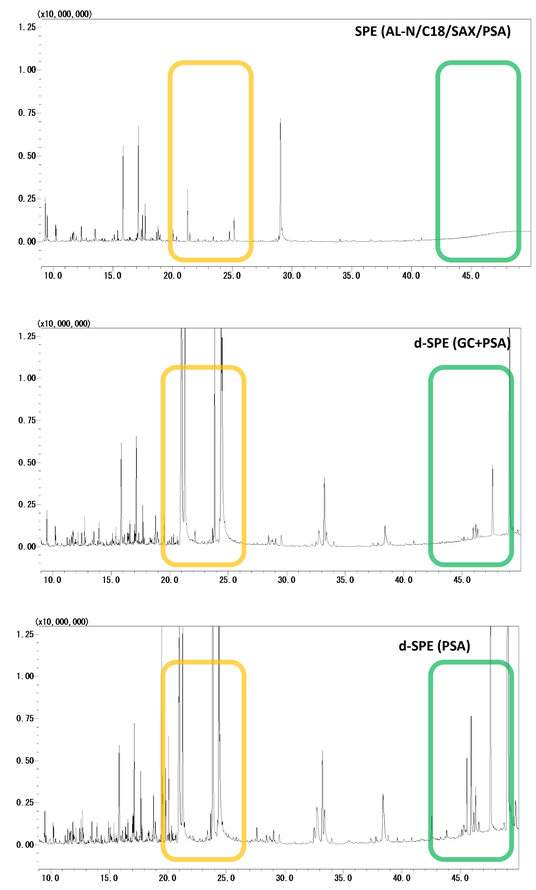

Fig.7 Chromatographic Comparison of cleanup performance between SPE and d-SPE method.

Results and Discussions

Comparison with Official Methods

The purification effect of InertSep AL-N/VRA-PR was slightly lower than that of the Japanese official method but deemed sufficient considering the simplicity and speed of the protocol.

Recovery Rates Using LC-MS/MS

Using a two-step elution process, pesticides with acidic functionalities that were not recovered in acetonitrile alone were successfully eluted using the acidified solvent. This demonstrates the applicability of the method to acidic analytes, expanding the range of detectable compounds with a single SPE column.

Recovery Rates Using GC-MS

In Test 1, 86 out of 169 pesticides in spinach and 99 out of 169 in barley were recovered within the acceptable range of 70–120%. In Test 2, these numbers increased to 111 (spinach) and 128 (barley), indicating improved efficiency with lower sample load. In Test 3, the addition of magnesium sulfate further enhanced recovery to 162 (spinach) and 155 (barley) compounds, suggesting improved moisture control and phase separation.
These results confirm that the InertSep AL-N/VRA-PR column provides adequate cleanup and high recovery for both GC-MS and LC-MS/MS, even under varied conditions.

Conclusions

The combination of QuEChERS extraction and the InertSep AL-N/VRA-PR SPE column enables streamlined sample preparation for both GC-MS and LC-MS/MS pesticide residue analysis. By using a single column with a unified protocol, laboratories can achieve efficient cleanup and broad analyte coverage across multiple analytical platforms.

References

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(Accessed: April 10, 2024)

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GL Sciences Inc.
June 3, 2025 Doc No.: SLPS0503E