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

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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 crops and processed foods were analyzed to evaluate 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, comparisons were made with both the Japanese official method, which involves liquid-liquid extraction followed by cleanup using a GC/NH_2 SPE column, and the d-SPE procedure commonly used in the QuEChERS method.

Recovery Experiments

LC-MS/MS

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

- **Elution 1**: acetonitrile.
- **Elution 2**: 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 cartridge with an additional 1 g layer of anhydrous magnesium sulfate.

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

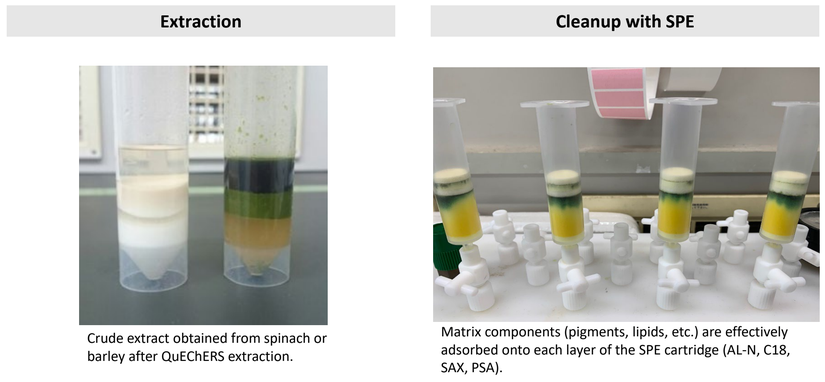


Fig. 1 Sample Preparation Procedure

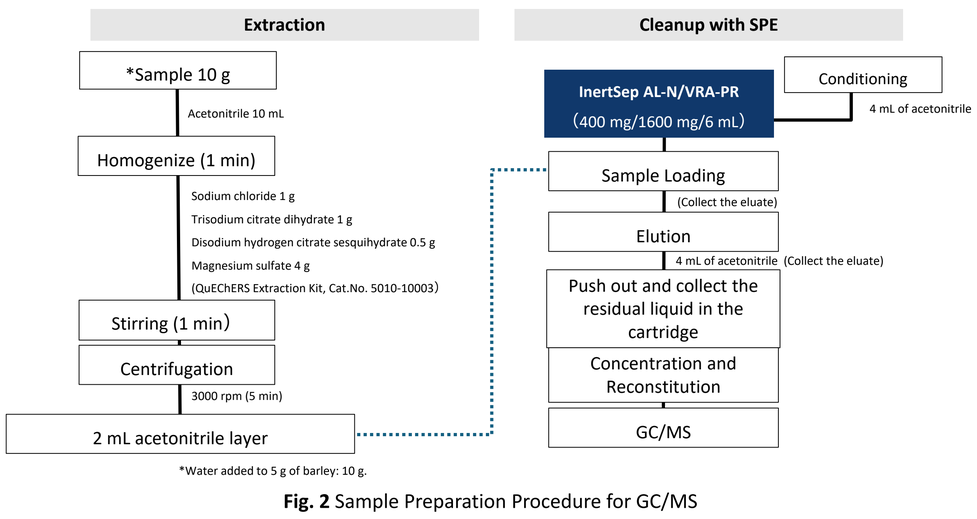


Fig. 2 Sample Preparation Procedure for GC/MS

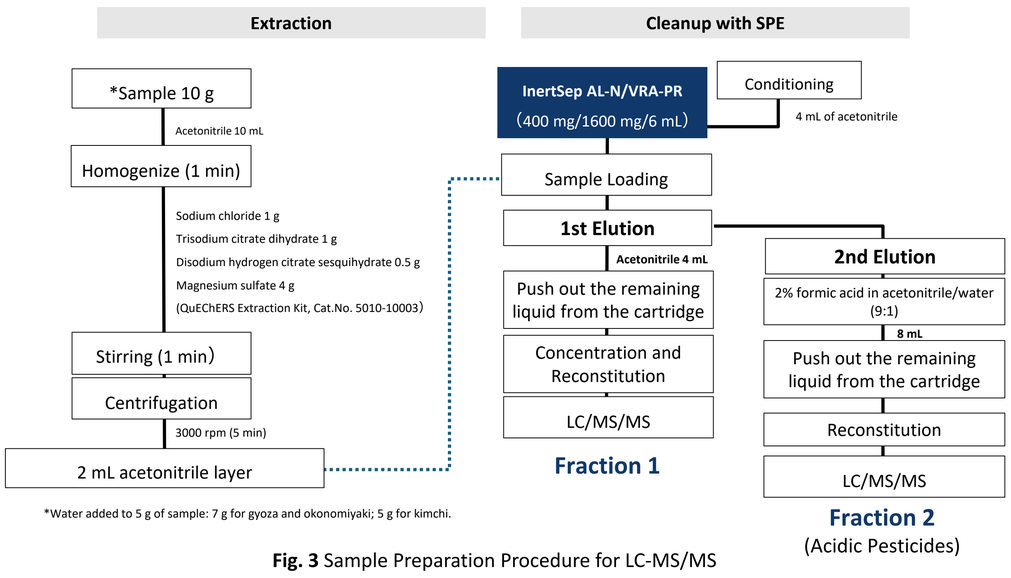


Fig. 3 Sample Preparation Procedure for LC-MS/MS

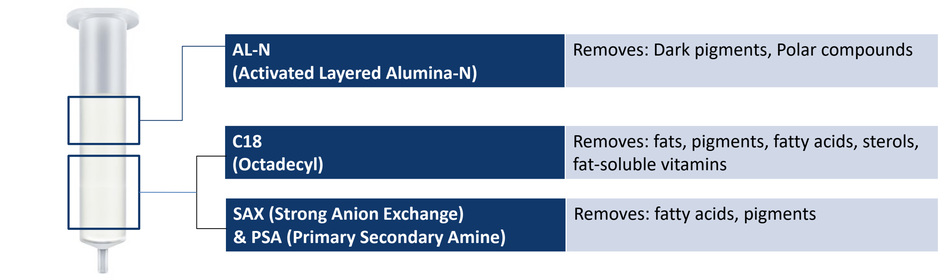


Fig. 4 Multilayer SPE Cartridge InertSep AL-N/VRA-PR

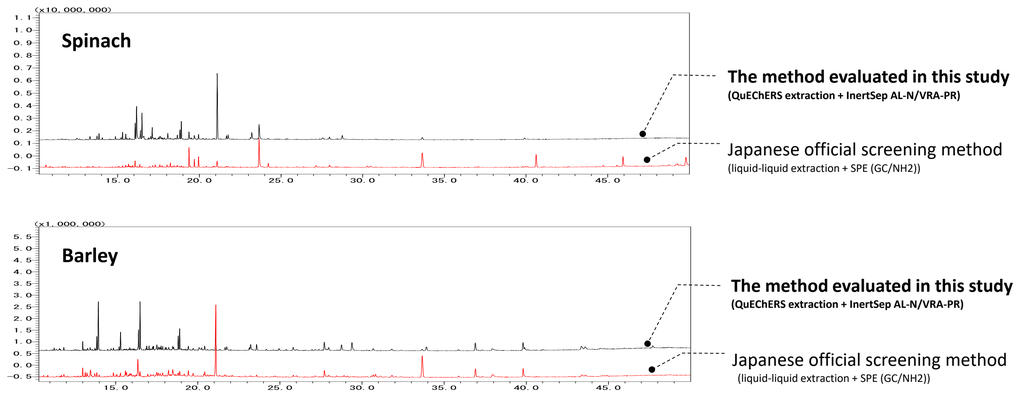


Fig. 5 Chromatographic Comparison of Cleanup Performance between the Method Evaluated in This Study and the Japanese Official Screening Method

Table 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

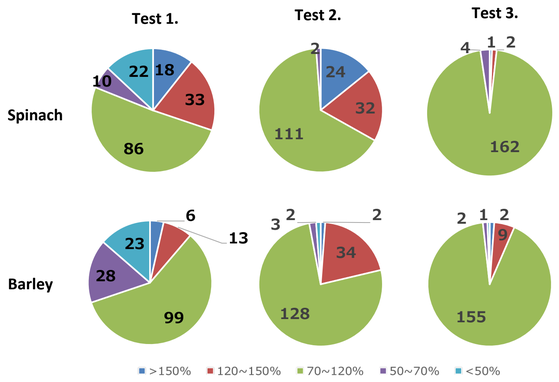


Fig. 6 Compound Recovery Distribution by SPE Condition in GC/MS Analysis

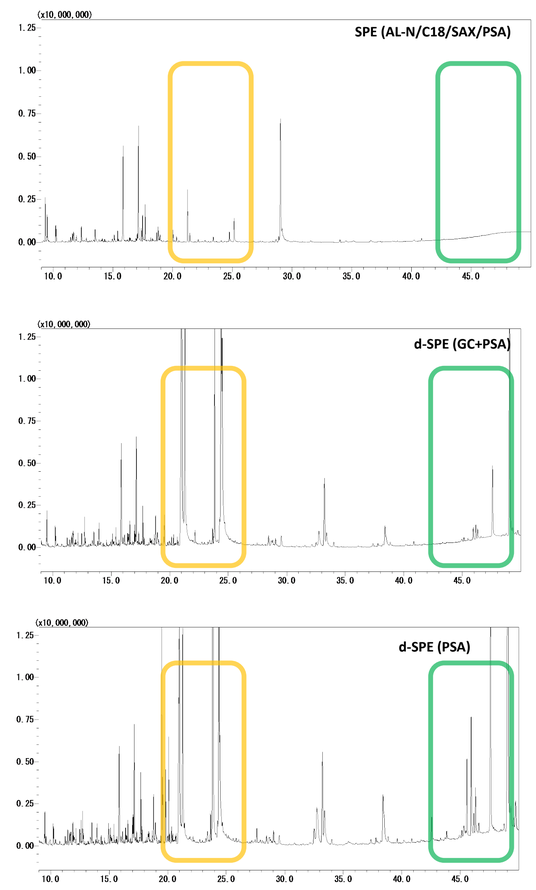


Fig. 7 Chromatographic Comparison of Cleanup Performance between the SPE and d-SPE Methods

Results and Discussion

Comparison with Official Methods

The cleanup performance of InertSep AL-N/VRA-PR was slightly lower than that of the Japanese official method but was considered sufficient given the simplicity and speed of the protocol.

Recovery Rates by LC-MS/MS

For LC-MS/MS analysis of spinach, 112 of the 137 pesticides showed acceptable recoveries with Elution 1 alone, increasing to 126 after combining Elutions 1 and 2. Similar results were obtained for the processed foods: 107–112 pesticides showed acceptable recoveries with Elution 1 alone, increasing to 123–127 pesticides after combining both eluates. These results demonstrate that the method is also applicable to acidic compounds and expands analyte coverage using a single SPE column.

Recovery Rates by GC-MS

In Test 1, 86 pesticides in spinach and 99 pesticides in barley showed acceptable recoveries of 70–120%. In Test 2, recoveries improved to 111 and 128 compounds, respectively, with lower sample loading. In Test 3, the addition of anhydrous magnesium sulfate further improved recoveries to 162 compounds (spinach) and 155 compounds (barley), suggesting improved moisture control and phase separation.
These results confirm that the InertSep AL-N/VRA-PR column provides effective cleanup and high recovery for both GC-MS and LC-MS/MS, under varied sample preparation 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

Ministry of Health, Labour and Welfare (MHLW), Japan. Test Methods for Substances Used as Pesticides, Feed Additives or Veterinary Drugs Residues in Foods. Available at: https://www.mhlw.go.jp/stf/seisakunitsuite/bunya/kenkou_iryou/shokuhin/zanryu/zanryu3/siken.html (Accessed: April 10, 2024)