

GC-MS/MS – Automation – Food Safety

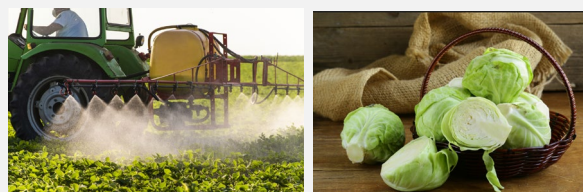
Analysis of Multi-Residue Pesticides in Cabbage with QuEChERS Extraction and Automated μ SPE Clean-up Using the AOC-6000 Plus

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Abstract

Gas chromatography/mass spectrometry (GC/MS) is an essential technique used for the analysis of residual contaminants in food products. To improve the signal quality, sample preparation is mandatory prior to introduction into these highly sensitive instruments. Conventionally, however, the sample preparation requires a long time and multistep efforts in which skilled chemists are required. In this Technology Brief, the sample preparation steps are greatly simplified by the means of automation. While initial extraction like liquid-liquid extraction typically requires careful selection of solvents with the right polarity, QuEChERS can reduce these steps significantly. Further steps such as SPE clean-up and preparation of calibration curves can be automated using the multifunctional autosampler, AOC-6000 Plus. This will allow the samples to be prepared freshly, right before injection to the GC/MS for analysis.



Rapid, accurate, and straightforward analysis is needed to monitor residual pesticides in food products

*Keywords:
Automated, QuEChERS, Clean-up,
Residual Pesticides*

Highlights

- *QuEChERS enables quick extraction of residual pesticides for quantitative GC-MS/MS analysis without the need for tedious sample preparation steps*
- *Clean-up of the QuEChERS extracts with solid-phase extraction can be automated using AOC-6000 Plus to greatly reduce manual workload*
- *It is also possible to automate the creation of calibration curves*
- *Smart Pesticides Database ver.2 further eases the requirement for method development when performing residual pesticide analysis because all parameters have been pre-optimized*

Technologies Featured

**Automated Sample Prep
with AOC-6000 Plus**



GCMS-TQ8040 NX



**Smart Pesticides
Database ver.2**



1. INTRODUCTION

Gas chromatograph-mass spectrometer (GC-MS) is an indispensable equipment for the analysis of residual pesticides. A solvent extraction is commonly used for pretreatment of these residual compounds in complex matrix such as foods and soils. As the residues tend to be present at a very low concentration, proper extraction and clean-up steps is required to ensure the removal of non-specific interferences while providing good recovery to the analytes of interest. As a result, the signals produced by the target analytes could be enhanced for easier identification and quantitation.

QuEChERS has been a popular extraction cum cleanup method for various chemical residues. The name is an acronym to its features: Quick, Easy, Cheap, Effective, Rugged, and Simple. While the resultant extracts are amenable to dispersive SPE clean-up, they tend to be dirty and less ideal for GC-based analysis. Therefore, a good SPE would be critical to effectively raise the concentration for sensitivity reasons against common co-extractants such as proteins, sugars, fats, and colourings. This will also prolong the lifetime of consumables (e.g. glass inserts, analytical columns) and in turn improve the instrument and method robustness.

In this work, we used the GC-MS/MS with a QuEChERS extraction and automated μ SPE clean-up to rapidly measure pesticides in cabbage with high performance. A ready-to-use method package was used for it comes with pre-optimized analytical parameters, recommended certified standard substances, pretreatment protocol, and various consumables.

2. EXPERIMENT

2.1 QuEChERS Extraction

QuEChERS kit and pesticide standard mixtures were purchased from Restek.

10 g of homogenised sample was first weighed into a 50 mL centrifuge tube. Then, 10 mL of acetonitrile was added and mixed well with the sample. A mixture of the European EN 15662 QuEChERS salt containing 4 g anhydrous magnesium sulphate, 1 g sodium chloride was added, 1 g trisodium citrate dihydrate and 0.5 g disodium hydrogen citrate sesquihydrate was added, and the tube was shaken upside down vigorously by hand for about 1 min, followed by another minute of vortex. Finally, after centrifuging at 5000 rpm for 3 minutes, 1 mL of the acetonitrile extract (top layer) was taken and subjected to automated μ SPE clean-up and for building a matrix-matched calibration curve.

2.2 AOC-6000 Plus Setup and Parameters for QuEChERS μ SPE Clean-Up Automation

The extract produced in Section 2.1 was then subjected to the automated clean-up workflow. Here, the μ SPE tubes with the universal packing (CTC Analytics) [P/N: ARACTC-uSPEGCQuE145100] were used. The basic version of sorbent mix was selected to remove excess water, organic acids, fatty acids, and sugars from the cabbage sample matrix. Each cartridge was packed with 45 mg of sorbent materials. The AOC-6000 Plus modules required for this automated clean-up workflow are shown in Figure 1A.

An optional protocol was added to the sample preparation workflow to automatically build a calibration curve (auto-calibration). In this case, a stock standard solution of 5000 ppb was readied in the autosampler, and dilution factors ranging from 1:2 to 1:1000 were selected to prepare a series of calibration standards. In addition, post-spiking of internal standard or analyte protectants were also performed. The additional AOC-6000 Plus modules required to achieve these capabilities are shown in Figure 1B.

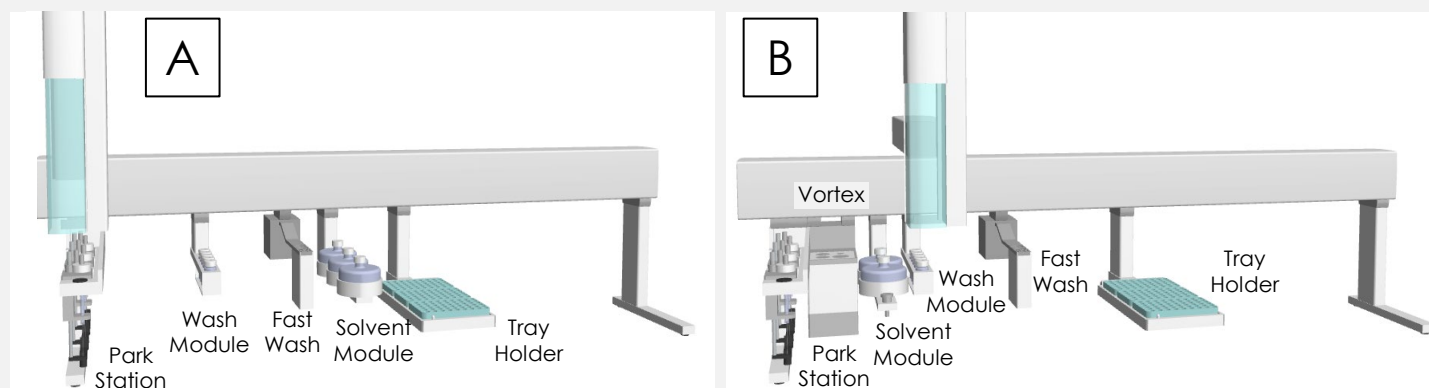


Figure 1: AOC-6000 Plus set-up for (A) the automated μ SPE workflow and (B) the optional auto-calibration building workflow. The setup can be combined into a single multifunctional autosampler with a minimal rail length of 120 cm

2.3 GC-MS Experimental Condition

Shimadzu GCMS-TQ8040 NX incorporates the patented ultra-fast technologies and can operate at a fast data acquisition rate of 50 Hz (20000 amu/s) without compromising sensitivities. Such fast-scanning speed in a mass spectrometer is perfect for multi-residue analysis in routine laboratories. Used in conjunction with the Smart Pesticides Database, up to 530 residual pesticides can be targeted specifically and quantified within 40 minutes. With the use of Smart Pesticides Database, all analytical parameters (including MRM transitions for individual compounds) have been pre-set. Method 3 of the database was chosen in this technology brief. The column of choice was SH-I-5MS 30m 0.25 mm ID 0.25 μm df, Shimadzu Corp) [P/N: 221-75954-30]. Other critical consumables of choice included the use of Topaz glass insert [P/N: APARES-23336].

All operations and acquisition were controlled with Shimadzu GCMSsolution software suite. Data analysis was performed using Shimadzu LabSolutions Insight™ software.

3. RESULTS AND DISCUSSION

3.1 Automated μSPE Cleanup

Cabbage was chosen as the representative food matrix in this Technology Brief to demonstrate the utility of the universal sorbent material in the μSPE cartridges. The method and results could be further extended to various types of fruits and vegetables which have minimal amounts of fats, waxes, or pigments.

The μSPE clean-up workflow can be extended to other types of sorbent materials as well. Figure 2 summarizes the sequences performed by the AOC-6000 Plus autosampler for the μSPE clean-up workflow. The use of analyte protectants is included as an optional step in the automation workflow. Analyte protectants, such as polyethylene glycol (PEG) 300 or a mixture of ethylglycerol, sorbitol, and gulonolactone, have been demonstrated to improve recovery rates of different pesticides in various food matrices. [1] While the automated clean-up workflow saves time and labour, the biggest advantage lies in the consistency of the results. With high-precision instrumentations and the minimization of human errors, the quality of routine analysis is improved significantly.

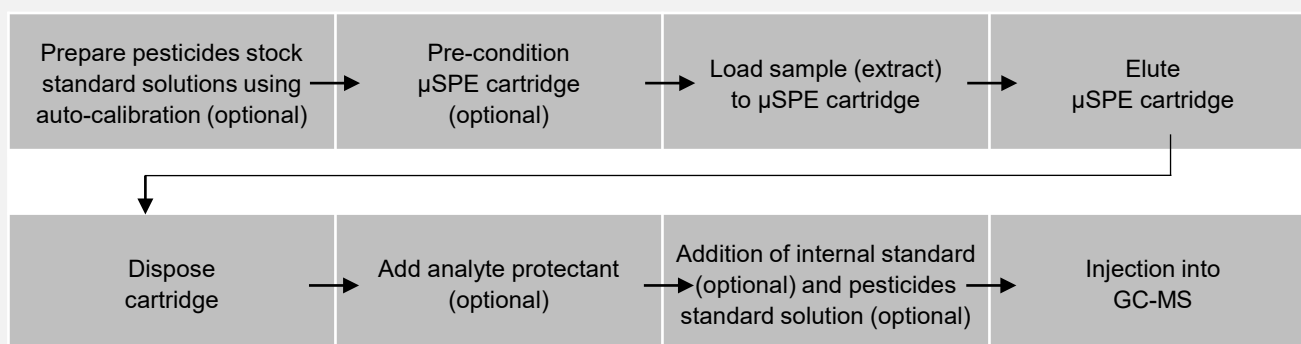


Figure 2: A general sequence of the automated μSPE clean-up workflow after QuEChERS extraction using AOC-6000 Plus. A complete Standard Operating Procedure (SOP) is available separately to explain how to operate this workflow in detail

Upon the completion of automated μSPE clean-up, the resultant eluate could be injected directly into the GC-MS for tandem MS analysis. This enables the building of pre-spike calibration curves, in which pesticide standards of varying concentrations were spiked before QuEChERS extraction. To build a post-spike calibration curve (such as in this Technology Brief), a subsequent automated calibration curve building protocol (auto-calibration) should be employed. The combination of both automated workflows can help to further increase productivity. Notably, to accommodate both sets of modules depicted in Figure 2, a 120 cm autosampler rail is required. The advantage, in such a case, is that the user only needs to manually perform QuEChERS extraction and prepare pesticide stock solutions. The remaining sample preparation and analysis will be executed by the instrument.

3.2 GC-MS/MS Data Acquisition

Out of the three methods provided in the Smart Pesticides Database ver.2, Method 3 was chosen because it is the most suitable with acetonitrile as the extraction solvent. The choice of GCMS-TQ8040 NX was also sufficient for this analysis as it fulfills the requirements by the regulatory for monitoring of pesticide residues in foods, in which the LOQ should be less than 10 ppb. [2,3] The injection uses 2 μL at high-pressure injection (splitless mode) to ensure the maximum introduction of the sample into the GC column, thus improving the signal of each target analyte.

The calibration results presented in this Technology Brief were obtained based on the post-spike protocol as aforementioned. Using auto-calibration, the required volumes of diluent and primary stock solution were automatically calculated for preparation of up to 9 calibration levels, according to a pre-defined dilution factor (i.e., 1:2 to 1:1000). The diluent (acetonitrile) and primary stock solution (5000 ppb pesticide mixture) were added into empty sample vials in series to prepare eight vials of diluted standard solutions with concentrations ranging from 12.5 to 2500 ppb. Subsequently, a 10 μ L aliquot of the diluted standard solution and internal standard solution were each added to the μ SPE eluate. However, pre-conditioning step was intentionally left out in this study as the μ SPE cartridge should ideally adsorb only co-extracted matrix components and not analytes. [2] As a result, the amount of dead volume had to be taken into consideration. The final total volume of the μ SPE eluate mixture was an underestimation and the calibration levels were corrected from 0.4-100 ppb to 0.37-92.6 ppb, as summarized in Table 1.

Table 1: Corrected concentration levels using the automated μ SPE cleanup workflow

Standard solution concentration (after auto-calibration)	Intended Concentration (in cleaned extract)	Corrected Concentration	Standard solution concentration (after auto-calibration)	Intended Concentration (in cleaned extract)	Corrected Concentration
12.5 ppb	0.4 ppb	0.37 ppb	250 ppb	10 ppb	9.26 ppb
25 ppb	1 ppb	0.93 ppb	500 ppb	20 ppb	18.50 ppb
50 ppb	2 ppb	1.85 ppb	1250 ppb	40 ppb	37.00 ppb
125 ppb	4 ppb	3.70 ppb	2500 ppb	100 ppb	92.60 ppb

The evaluation results are summarized in Table 2. Out of the 92 compounds analyzed, 91% achieved a linearity (R^2) of more than 0.99. This indicates that the calibration curves were well corrected and could be useful in meeting the major regulatory requirement of 10 ppb. The method suffices a quantitation down to 25 times lower for 63% of the compounds, with a limit of quantitation (LOQ) around 0.4 ppb. The calibration curves were plotted using the quadratic model, as illustrated by some of the calibration curves in Figure 3.

The method recovery and μ SPE efficiency were also evaluated. To test for method recovery, appropriate amounts of pesticides standards and internal standard (TPP) were spiked into the cabbage matrix before the addition of QuEChERS extraction salt. Meanwhile, for μ SPE efficiency evaluation, the pesticides standards and TPP were spiked into the QuEChERS extract instead, before it was subjected to the μ SPE automated clean-up. In this Technology Brief, the concentration of pesticides spiked for both method recovery test and μ SPE efficiency was 10 ppb. The method recovery and μ SPE efficiency of all the target pesticides were found to be within $\pm 30\%$. Up to 88% of the compounds achieved recovery of $\pm 20\%$. This indicated that the effectiveness of the automated μ SPE clean-up workflow is highly reliable.

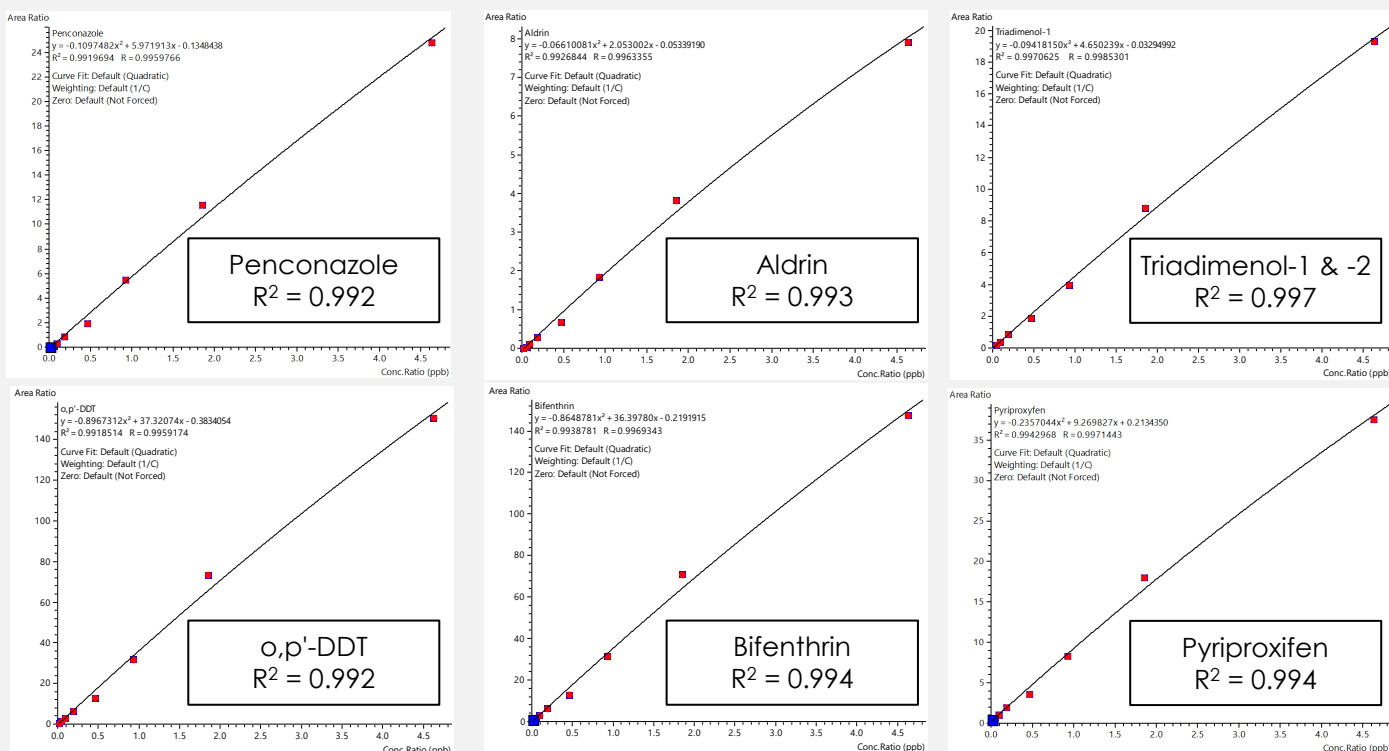


Figure 3: Calibration curves of selected pesticides, from the range of 0.37 to 92.6 ppb. The linearity (R^2) of most pesticides was found to be more than 0.99 (see individual pesticide data in Table 2)

Table 2: A summary of performance for the pesticides analyzed, showcasing the calibration linearity (R^2), method recovery and μ SPE clean-up efficiency of individual compounds

Compound Name	Ret. Time (min)	R^2	Method Recovery (%)	μ SPE Clean-up Efficiency (%)	Area Ratio RSD (%) (n = 6)
Etridiazole	6.610	0.9952	115.14	116.09	9.07
Chloroneb	7.765	0.9965	117.85	106.50	7.91
Pentachlorobenzene	7.801	0.9966	79.53	76.00	10.46
alpha-BHC	11.616	0.9918	101.86	94.35	6.72
Pentachloroanisole	12.007	0.9954	88.60	79.84	6.50
beta-BHC	12.885	0.9918	70.19	94.70	7.07
gamma-BHC (Lindane)	13.053	0.9942	124.78	111.69	5.47
Atrazine	13.111	0.9894	108.39	107.64	7.54
Terbutylazine	13.692	0.9922	108.07	111.56	7.30
Pyrimethanil	14.034	0.9935	89.48	90.13	5.85
delta-BHC	14.449	0.9913	103.30	96.29	8.27
Terbacil	14.456	0.9916	110.84	110.88	5.16
Endosulfan ether	14.962	0.9901	111.28	100.78	5.87
Tefluthrin	14.988	0.9921	105.51	105.50	5.34
Vinclozolin	16.158	0.9906	114.06	111.55	5.02
Heptachlor	16.206	0.9912	99.12	98.39	7.63
Transfluthrin	16.833	0.9934	105.6	106.35	5.80
Aldrin	17.729	0.9927	101.27	92.33	7.07
4,4'-Dichlorobenzophenone	18.593	0.9933	98.48	93.72	5.35
Triadimefon	18.632	0.9955	102.53	107.99	5.44
Fenson	18.762	0.9902	104.32	97.08	4.55
Isodrin	18.971	0.9943	105.62	98.01	5.50
MGK 264-1	19.179	0.9915	123.50	125.28	4.74
Heptachlor-exo-epoxide*	19.562	0.9898	103.66	96.57	8.60
Cyprodinil*	19.606	0.9913	71.87	70.64	3.69
MGK 264-2	19.724	0.9913	114.18	110.55	6.03
Penconazole*	19.839	0.9920	100.85	108.07	5.70
Fipronil	20.142	0.9909	111.09	112.22	6.94
Procymidone	20.429	0.9900	109.88	108.08	4.84
Triadimenol-1 & -2	20.518	0.9971	106.75	102.98	8.96
trans-Chlordane	20.639	0.9891	98.62	99.13	6.54
Chlorbenside	20.714	0.9910	90.36	88.89	5.33
Triflumizole	20.840	0.9932	106.17	110.52	5.97
o,p'-DDE	20.999	0.9920	99.08	94.55	4.89
alpha-Endosulfan*	21.142	0.9929	96.15	101.36	4.80
Paclobutrazol	21.168	0.9922	106.07	115.52	8.11
cis-Chlordane	21.218	0.9911	103.99	93.21	4.46
trans-Nonachlor*	21.387	0.9887	100.36	97.05	6.18
Flutriafol	21.698	0.9900	116.93	121.05	5.12
Chlorfenson	21.958	0.9908	100.12	98.40	5.40
Fludioxonil*	22.397	0.9969	107.34	111.65	6.45
Dieldrin*	22.364	0.9897	108.67	99.14	8.73
p,p'-DDE	22.524	0.9922	97.97	95.55	4.78
o,p'-DDD	22.749	0.9916	100.34	98.61	5.49
Myclobutanil	22.880	0.9920	106.22	112.84	6.41
Flusilazole*	23.033	0.9914	104.53	114.48	8.64
Bupirimate	23.192	0.9925	109.26	106.85	4.79
Endrin*	23.212	0.9890	100.48	93.41	8.99

Table 2 (cont'd): A summary of performance for the pesticides analyzed, showcasing the calibration linearity (R^2), method recovery and μ SPE clean-up efficiency of individual compounds

Compound Name	Ret. Time (min)	R^2	Method Recovery (%)	μ SPE Clean-up Efficiency (%)	Area Ratio RSD (%) (n = 6)
Chlorfenapyr*	23.550	0.9861	94.07	116.61	12.39
1,1-Dichloro-2,2-bis(4-ethylphenyl)ethane	23.774	0.9926	102.98	101.63	6.55
cis-Nonachlor	24.004	0.9884	98.15	95.52	6.20
o,p'-DDT & p,p'-DDD	24.271	0.9919	101.21	100.42	4.93
Endosulfan sulfate	25.211	0.9927	103.59	105.29	2.93
4,4'-methoxychlor olefin	25.380	0.9931	100.46	98.19	4.59
Lenacil*	25.487	0.9961	104.17	118.34	14.09
p,p'-DDT	25.575	0.9948	109.65	98.26	5.42
Hexazinone*	25.849	0.9979	115.33	124.54	8.09
2,4'-Methoxychlor	25.911	0.9922	107.95	102.32	5.14
Tebuconazole	26.048	0.9926	103.10	114.96	10.30
TPP (ISTD)	26.284				
Propargite-1 & -2*	26.335	0.9960	111.89	110.99	5.26
Endrin ketone*	26.622	0.9874	98.04	106.86	12.07
Iprodione*	27.054	0.9918	113.52	122.30	9.13
Tetramethrin-1*	27.172	0.9965	112.99	123.01	4.73
Bifenthrin	27.394	0.9939	97.10	100.72	5.49
Tetramethrin-2	27.447	0.9957	106.47	114.25	7.55
Tetradifon*	27.877	0.9941	105.78	105.66	8.89
Mirex	28.370	0.9920	88.02	88.12	4.94
Pyriproxyfen	28.466	0.9943	106.76	109.68	4.72
Fenarimol*	28.801	0.9955	117.84	121.18	11.26
Acrinathrin-1*	28.879	0.9955	102.65	114.35	10.35
Acrinathrin-2*	29.256	0.9932	94.09	112.33	15.38
cis-Permethrine	29.863	0.9943	97.70	106.58	5.79
trans-Permethrine	30.052	0.9936	99.24	106.86	6.50
Cyfluthrin-1*	30.650	0.9942	100.00	112.12	7.03
Cyfluthrin-2*	30.796	0.9944	100.42	110.84	12.12
Cyfluthrin-3*	30.862	0.9948	101.95	115.89	11.85
Cyfluthrin-4*	30.935	0.9913	101.46	121.11	10.31
Cypermethrin-1*	31.052	0.9915	117.03	130.08	13.15
Cypermethrin-2*	31.206	0.9961	107.21	110.84	8.81
Cypermethrin-3*	31.261	0.9906	115.58	126.69	11.13
Flucythrinate-1	31.295	0.9931	105.35	112.69	8.43
Cypermethrin-4*	31.330	0.9939	106.37	101.96	7.89
Etofenprox	31.442	0.9942	95.18	102.37	10.40
Flucythrinate-2	31.566	0.9924	102.73	108.78	7.44
Fenvalerate-1*	32.160	0.9938	95.45	104.90	7.16
tau-Fluvalinate-1	32.380	0.9913	96.09	106.96	11.47
Fenvalerate-2 (Esfenvalerate)*	32.434	0.9962	111.08	115.80	9.02
tau-Fluvalinate-2*	32.486	0.9935	100.78	101.89	10.89

* Compounds with an LOQ above 0.37 ppb but still lower than the common MRL of 10 ppb

4. CONCLUSION

Shimadzu offers an extremely efficient solution for the analysis of multi-residue pesticides in cabbage with QuEChERS extraction and automated μ SPE clean-up using the AOC-6000 Plus. The analysis of residual pesticides, alongside other food safety applications, are necessary for the well-being of humankind. Considering the extent of routine requirements in this area, automation comes in handy in improving the efficiency and throughput of all laboratories. Not only does it save time, errors associated with manual processes will also be minimized. Combined with the convenient plug-and-play method package, Smart Pesticides Database, users can basically skip from simple sample preparation straight to data analysis. The barrier of entry into such highly sophisticated analyses has never been lower. Even for data analysis, Shimadzu LabSolutions Insight™ is the perfect, user-friendly software that helps save time with automatic processing features.

5. REFERENCES

1. Shimadzu App News M279 - Study of a Method for Coping with Matrix Effects in Pesticide Residue Analysis Using GC/MS/MS
2. Lehotay, S. J., Han, L., & Sapozhnikova, Y. (2016). Automated mini-column solid-phase extraction cleanup for high-throughput analysis of chemical contaminants in foods by low-pressure gas chromatography — tandem mass spectrometry. *Chromatographia*, 79(17), 1113-1130.
3. EU Legislation on MRLs. Food Safety. (n.d.). Retrieved November 12, 2021, from https://ec.europa.eu/food/plants/pesticides/maximum-residue-levels/eu-legislation-mrls_en

6. ORDERING GUIDE

Item Description	Part Number	Item Description	Part Number
QuEChERS extraction		AOC-6000 Plus auto-calibration building workflow	
Q-sep QuEChERS Extraction Kit, European EN 15662 (50/pk)	APARES-25850	Vortex Mixer for the intensive mixing, used for sample prep	PAL3-VortexMixer
AOC-6000 Plus automated μSPE clean-up workflow		Additional park station	PAL3-ParkStation-RTC
AOC-6000 Plus Standard Model, RTC model with 120 cm system	PAL3-RTC-LGH-X-SHIM2	Smart Syringe 250 μ L, for Tool D8/57, gauge 26, PST cone	227-35356-01
QuEChERS Clean up Modules	PAL3-HW-uSPE54-SHIM	1.5 mL Gold magnetic crimp vial cap, with PTFE/white Silicone Septa (100/pk)	226-54128-01
μ SPE Cartridges, sorbent mix 1, 45 mg (100/pk)	APACTC-uSPEGCQuE145100	GCMS analysis	
Smart Syringe 10 μ L, for Tool D7/57, gauge 23, PST cone	227-35353-02	Smart Pesticides Database ver.2	225-31946-92
Smart Syringe 25 μ L, for Tool D7/57, gauge 23, PST cone	227-35354-01	SH-I-5Sil MS capillary column (30 m x 0.25 mm I.D., df = 0.25 μ m)	221-75954-30
Smart Syringe 1000 μ L, for Tool D8/57, gauge 23, flat	226-88001-01	Topaz Liner Single Goose Neck Liner with Wool	APARES-23336
1.5 mL Clear, screw vial with write on spot (100/pk)	226-54110-11	Qualitative Retention Time Index Standard	APARES-31080
1.5 mL Clear screw vial cap, with PTFE/white Septa (100/pk)	226-54113-01	GC Low-bleed Septum, Green (25/pk)	221-76650-01
1.5 mL Clear screw vial cap, with Starburst Septa (100/pk)	226-84133-01	O Ring, 4D P5, (5 pcs/pkt)	036-11203-84
100 μ L Micro-Insert with plastic spring, (100/pk)	226-84201-11	Vespel ferrule for 0.25mmID capillary column (10/pk)	670-15003-03

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AOC-6000 Plus



The AOC-6000 Plus Multifunctional Autosampler enables various syringes injections, including liquid injection, headspace (HS) injection, and solid-phase microextraction (SPME) injection. The automatic syringe exchange RTC (Robotic Tool Changer) function enables continuous sample injection using different syringes. It is also the feature that allows sample preparation steps to be automated. By combining with the Twin Line MS system, different analytical methods such as SPME and HS can even be performed continuously without changing the column.

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GCMS-TQ8040 NX

The Shimadzu triple-quadrupole GCMS-TQ8040 NX features smart technologies to boost the efficiency of routine analytical work. For example, Smart MRM provides fully-automated MRM method development. It automatically determines all optimum transitions and collision energies, seamlessly incorporating them into the Smart Database Series. With features like this, the GCMS-TQ8040 NX GC-MS/MS is so smart it almost runs itself! The instrument is highly robust and is highly used by routine laboratories around the world. It is perfect for applications in fields such as environmental monitoring and food science.



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Smart Pesticides Database



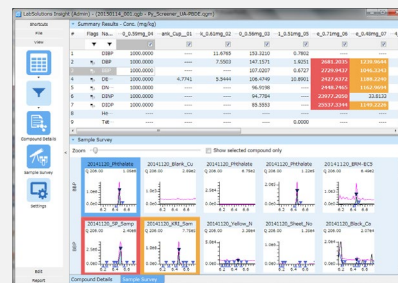
Smart Pesticides Database Ver. 2 is a method package which supports the simultaneous analysis of 530 residual pesticides in foods via GC-MS(/MS). It entails pre-optimized analytical parameters, certified standard substances, a pretreatment kit, pretreatment protocol, and various consumables. Simply put, it is the total solution for high-accuracy pesticide analysis.

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LabSolutions Insight

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