Fully Automated QuEChERS Extraction and Clean-up of Organophosphate Pesticides in Orange Juice

Chiew Mei Chong[1], Hans-Joachim Hübschmann[2*]

[1]*CTC Analytics Pte. Ltd., Singapore.*

[2]*HANS Analytical Solutions, Osaka, Japan.*

*Correspondence author
Hans-Joachim Hübschmann,
HANS Analytical Solutions
Osaka
Japan.

Abstract
A fully automated QuEChERS extraction and extract clean-up method for GC-MS and LC-MS analysis is presented by using an industry standard robotic x,y,z-sampling system. The application describes the analysis of organophosphate pesticides from homogeneous liquid samples, as an example orange juice was used. The automated workflow includes the extraction with acetonitrile, salting out and using a µSPE cartridge for matrix clean-up prior to injection into a GC-MS/MS system. The method validation techniques such as pre-spike and post-spike were fully integrated into the automated workflow as well. Calibration linearities of the applied organophosphate pesticides in orange juice matrix range from 1 to 100 ng/mL with a precision achieved better than 0.995 for all compounds. By spiking 10 ng/mL of pesticides into the orange juice samples, recoveries were obtained in the of range 70% - 115%, while the precision from pre-spike (n=7) and post-spikes (n=6) under the same concentration was less than 10% RSD. The calculated method detection limits (MDLs) of the monitored pesticides were in the range of 1.8 ng/mL to 4.1 ng/mL which are well below the regulated maximum residue limits (MRLs) of 10 ng/g for these pesticides.

Introduction

QuEChERS is the well-established quick, easy, cheap effective, rugged, and safe pesticide extraction procedure, developed by Anastassiades et al. (2003). Since then, this technique has become a widely used sample preparation approach in pesticides residue analyses. According to the QuEChERS website, about 45 minutes are needed to manually prepare eight samples in the laboratory for subsequent GC-MS or LC-MS analysis (Anastassiades et al., 2003). In the traditional QuEChERS method, acetonitrile is used as the extraction solvent for an aqueous sample of approx. 10 g, followed by adding buffer salts for phase-separation and pH-adjustment, and an intense shaking of the mixture. After centrifugation the clean-up of the raw extracts is manually achieved via a dispersive solid phase extraction (dSPE) using a dedicated combination of sorbent materials for different food comodities. Clean-up sorbents like primary/secondary amine (PSA, mainly removing sugars, organic acids and pigments), GCB (graphitized carbon black, removes pigments, chlorophyll, and non-polar interferences), C18 (octadecylsilane, removing lipids and other non-polar interferences) or other specific sorbent materials are used in varying ratios to suit different matrix conditions. Anhydrous magnesium sulphate is added for increasing the ionic strength and water removal in case of subsequent GC-MS analysis.

The steps for pesticide residue analysis start with the representative sampling and comminution pre-treatment, the necessary manual steps to provide a homogeneous subsample for processing. Solid samples like plant materials, food or soil require individually optimized homogenization of a larger amount of a representative sample by cutting, grinding, or milling, including cryo-milling for potentially volatile pesticides (QuEChERS, 2021; Lehotay et al., 2020). This comminution of raw sample materials to achieve a representative aliquot as a test portion for analysis is typically done manually using suitable blenders. Vegetable and fruit juices are considered homogenous after thorough shaking the commercial packaging, the bottles or carton packages, before transferring an aliquot to analysis vials.

This report describes for the first time a fully automated QuEChERS extraction and clean-up workflow for homogeneous matrices like fruit juices, in this case, demonstrated for orange juice, using an industry standard robotic x,y,z-sampling system for online or offline GC-MS and LC-MS pesticide analysis. Only 0.5 mL of homogenized juice are required, transferred into a regular 2 mL autosampler vial for the automated extraction, clean-up and online analysis. The raw extract clean-up and removal of the high matrix load are achieved by using micro-SPE cartridges (µSPE). The advantage of µSPE is the straightforward separation of the pesticide fraction from the matrix by elution of the pesticide fraction through a small sorbent bed keeping the matrix behind.

Extract dilution and solvent evaporation are avoided, keeping the initial concentration level of the pesticides, providing high recoveries and short processing times of a few minutes only being compatible with the chromatographic runtimes. A prep-ahead mode of the x,y,z robot allows the processing of a next sample during the chromatographic run.

Configuration of the x,y,z-robotic sampler

For the described experiments an industry standard robotic x,y,z-sampling system with automated tool change was employed. Different syringes sizes for extraction, clean-up, standard addition and GC injection are used in the automated workflow. The system configuration as shown in Figure 1 further comprises a vortex mixer, solvent and wash modules as well as a trayholder with the vial racks for the sample and extract vials, and the micro-SPE clean-up cartridges. A system park station holds the tools with different syringes for use in the programmed workflow.
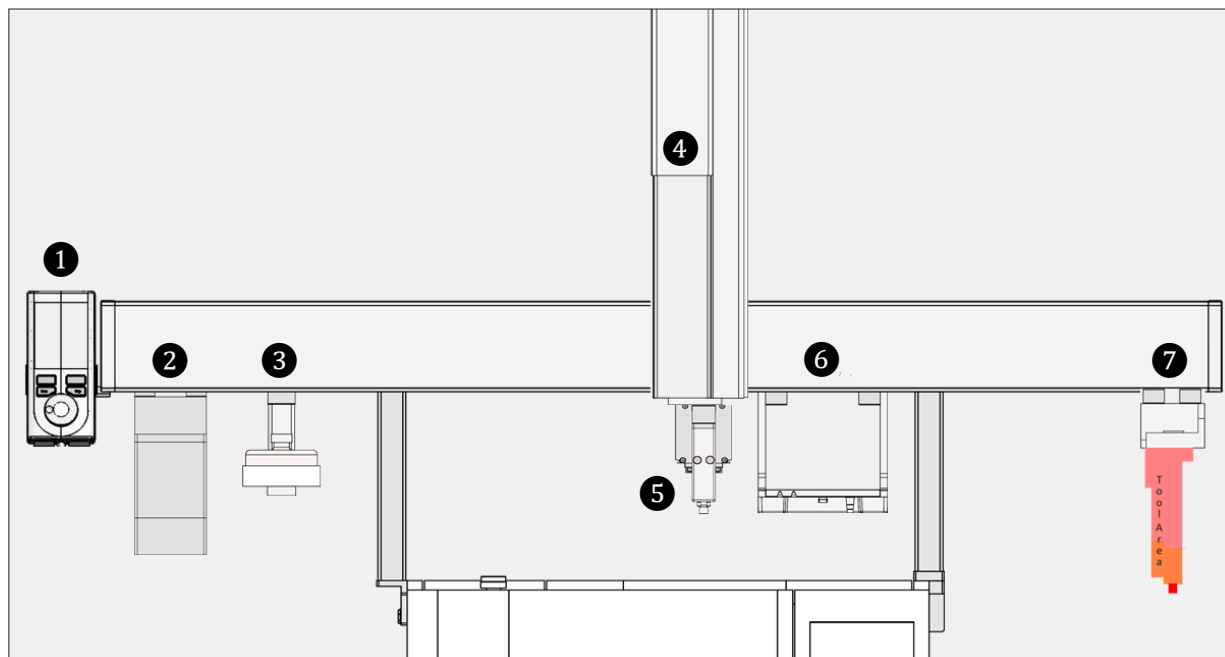

Figure 1: Configuration of the robotic x,y,z-sampler for the automated QuEChERS extraction and clean-up of juice samples. 1. Handheld Terminal, 2. Vortex Mixer, 3. Solvent Module, 4. Head of robotic sampler, 5. Fast Wash Module, 6. Tray Holder for vial racks and µSPE cartridges, 7. Tool Park Station with 3 syringes.

For the automated workflow, a saturated NaCl solution is provided in one of the large solvent modules. Acetonitrile is used for the QuEChERS extraction. Ethylacetate can be used as well for the so-called Swedish ethylacetate extraction method (SweEt) (Ekroth 2017). The syringe cleaning solvent is provided with a fast wash module from an external reservoir. The fast wash module includes a pump for solvent delivery, active only when the syringe needle enters the sink-shaped port.

The workflow includes the automated dilution of pesticide standards to build a calibration curve as well. A working stock solution is placed in rack 1 with a row of empty vials for in-time preparation of the calibration for quantitation, shown in Figure 2. The same vial rack carries the sample vials, empty vials to collect the cleaned extract, as well the necessary µSPE cartridges for the clean-up.
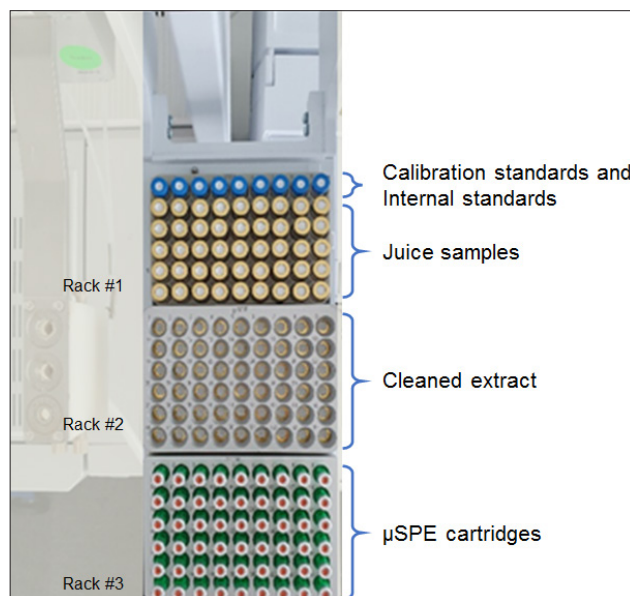

Figure 2: Trayholder top view showing the rack placement of standards, samples, cleaned extracts and the µSPE cartridge reservoir.
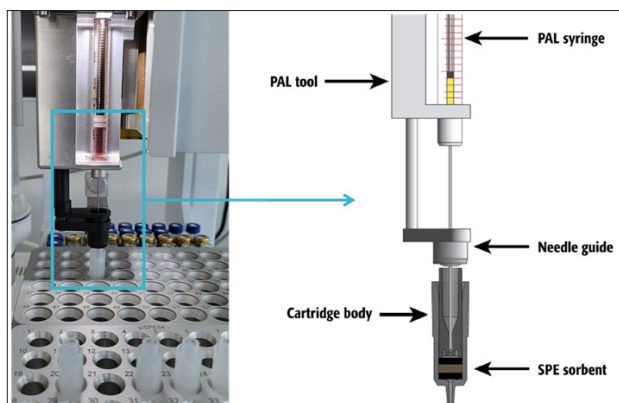
Figure 3: Principle of the automated µSPE operation

Automated Workflow

The workflow for the automated analysis of pesticides from juices comprises several stages:

- Preparation of the calibration standards
- Standards addition
- Extraction with acetonitrile
- Extract clean-up
- GC-MS and/or LC-MS injection and analyses

The first part with a fresh preparation of the calibration curve can be used optionally, as well as the addition of internal standards to the sample. Typically, commercial multi-residue pesticide standards are applied for building the quantitative calibration. The dilution of standards can be achieved in routine by entering the required dilution factors.

The automated workflow describing the sections extraction, clean-up and a GC-MS injection with the steps performed is illustrated in Figure 4. The QuEChERS extraction step is performed here with the original high NaCl salting-out conditions (Anastassiades et al., 2003). A pH adjustment as of AOAC 2007.01 or EN 15662 methods can be achieved by providing the required buffer salts in accordingly prepared 2 mL vials before adding a juice sample. Extraction solvent is dispensed to the vial followed by intense vortexing.

After phase separation the extract clean-up is achieved by applying the raw extract from the 2 mL vial to µSPE cartridges, as shown in Figure 3. Here the syringe works like an LC pump and pushes the extract in constant slow flow through the cartridge. The pesticides fraction elutes first leaving the sample matrix behind on the cartridge. The cleaned extract is collected in empty vials on the same trayholder, as shown in Figure 2. The sorbent material mix of the µSPE cartridge is optimized for GC-MS and LC-MS analysis (QuEChERS, 2021). Both cartridge types contain C18 and activated carbon material, but only for GC-MS primary/secondary amine (PSA) and anhydrous $MgSO_4$. Silica coated ZrO_2 sorbent material is applied for LC-MS analysis for lipid scavenging. A big benefit of the optimized sorbent material mix for laboratory logistics is the wide versatility of the cartridges for any kind of food samples. This also includes high fat content and spice containing samples making any further modification of the sorbent material mix for different kind of sample matrices unnecessary (Lehotay et al., 2020; Directorate-General for Health and Food Safety, 2017).

In the online configuration to GC-MS and LC-MS, every sample is processed on an identical time axis within 5 to 7 minutes, depending on the chosen tasks and processed volumes. A so-called 'prep-ahead' mode allows the processing of the next same during analysis of the previous one, as shown in Figure 3. A built-in scheduler of the robotic system starts the processing of a next sample just in time to be ready for injection at the expected ready signal from the mass spectrometer connected. The 'prep-ahead' mode increases sample throughput significantly and maximizes the duty cycle of the connected analysis system. All samples are treated on the same timeline "just in time". No sample that is processed is waiting for injection, providing high reproducibility within even large sample series.

The described workflow integrates into the chromatography data systems of the leading instrument manufacturer for GC-MS and LC-MS. Also, the automated process can be executed off-line, and the cleaned extracts directed to different instruments.

Experimental

The only manual step in the project was transferring orange juice from the well-shaken bottle into sample vials. The subsequent QuEChERS extraction steps, such as adding acetonitrile, adding saturated sodium chloride salt, clean-up and injection into the GC-MS/MS are all carried out by the x,y,z-robotic sampler with the aid of a method composer software provided by the manufacturer to build the automation workflow. The instrumentation used were an AOC-6000Plus robot (Shimadzu Corp., Kyoto, Japan), GCMS-TQ8040 with GCMSSolutions software (Shimadzu Corp., Kyoto, Japan), and PAL Method Composer software (CTC Analytics, Zwingen, Switzerland). As pesticide standards the GC multi-residue standards #8 and #9, and as internal standard (ISTD) tris (1, 3-dichloroisopropyl) phosphate, were applied (Restek, Bellefonte PA, USA). Solvents and reagents were acetonitrile p.a. as well as sodium chloride reagent grade (Merck KG, Darmstadt, Germany) and water in HPLC grade (ACRO, Singapore). The µSPE clean-up cartridge contained a sorbent material mixture 45mg of $MgSO_4$, PSA, C18EC, and CarbonX (CTC Analytics, Zwingen, Switzerland). Orange juice was sourced from a local grocery store.

Analysis Parameter

x,y,z Robotic sampler

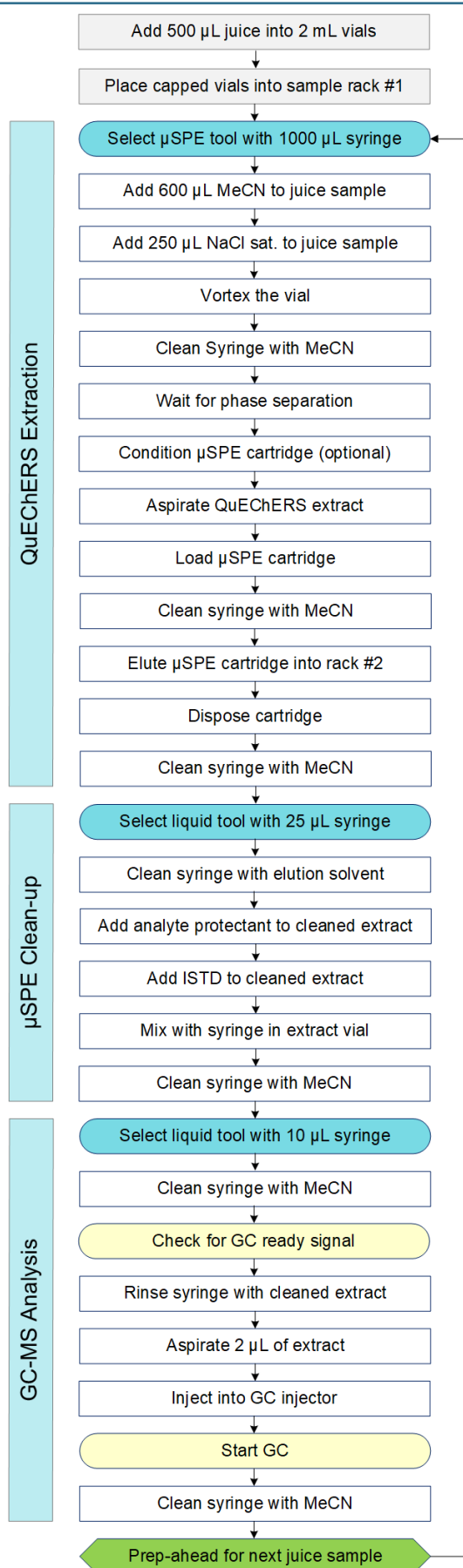|	
Sample volume	400 µL
Standard volumes	50 µL each, for calibration and ISTD
MeCN volume	3x 200 µL (extraction solvent)
Salting-out	200 µL (NaCl sat.)
Vortexing speed, time	1500 rpm, 60 s
Extract clean-up	250 µL raw extract (applied to µSPE)
Extract load flow	2 µL/s

Figure 4: Automated workflow for juice extraction, extract clean-up and GC injection

Gas chromatograph

Inlet Temperature	250°C
Inlet Mode	Splitless
Injection volume	3 µL
Flow	1.15 mL/min
Pressure	64.7kPa
Column	SH-Rxi-5Sil MS, 30 m x 0.25 µm x 0.25 mm
Oven Temperature	50°C (2 min), 30°C /min to 75°C (1 min), 4°C/min to 250°C (1 min), 20°C/min to 300°C (0.92 min)

Mass spectrometer

Ion source temp.	250°C
Solvent cut time	3 min
Detector Voltage	+0.3 kV relative to tuning result
Detection	MRM mode, as of manufacturer pesticide database

Results and Discussion

For the automated extraction the sample size of the homogeneous juice sample is scaled down from the usual sample amount of 10 g to only 400 to 500 µL because usually less than 10 µL of sample extract is typically injected into the GC-MS to obtain good signal and recovery.

Figure 5 shows the orange juice sample in undergoing QuEChERS sample preparation and clean-up steps in the used 2 mL vials. The orange juice first is completely miscible with acetonitrile. Only after adding the saturated sodium chloride, two layers of liquid are formed, in which, after vortexing and sedimentation, the upper layer is the raw pesticide extract in acetonitrile. The bottom layer is the remaining aqueous layer. The colourful extract is typically not suitable to be directly analysed due to its high matrix co-extracts from the juice sample. An aliquot of this raw extract is transferred to the µSPE cartridge for clean-up. The clean-up effect can already be noticed visually be in Figure 5 on colourants removed by the µSPE cartridge after the clean-up procedure.
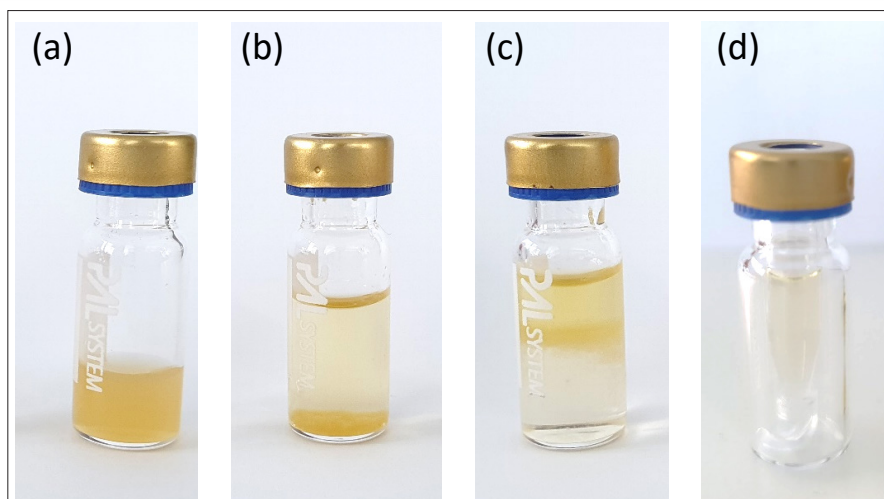
Figure 5: Workflow steps visualized in the 2 mL vials of the automated juice extraction and clean-up
(a) Orange juice from juice box
(b) Orange juice + acetonitrile, vortexed
(c) Orange juice + acetonitrile + NaCl sat. phase separation
(d) Cleaned extract after µSPE step, injected

A group of organophosphate pesticides was evaluated based on pre-spike and post-spike of pesticide standards into the juice samples. By using the PAL Method Composer software, the pre-spike and post-spike steps can be integrated optionally into the automation workflow. In the pre-spike procedure, the pesticides and internal standards were added into the orange juice sample prior to the extraction with acetonitrile. A post-spike procedure starts with extracting the juice sample followed by µSPE clean-up, then adding the pesticide and internal standards into the cleaned extracts before injecting into the GC-MS/MS.

Chromatograms and Calibration Curves

A full chromatogram of the extracted orange juice after undergoing the automated QuEChERS extraction and clean-up using the post-spike of standards with 100 ng/mL of the organophosphate pesticide compounds is shown in Figure 6.
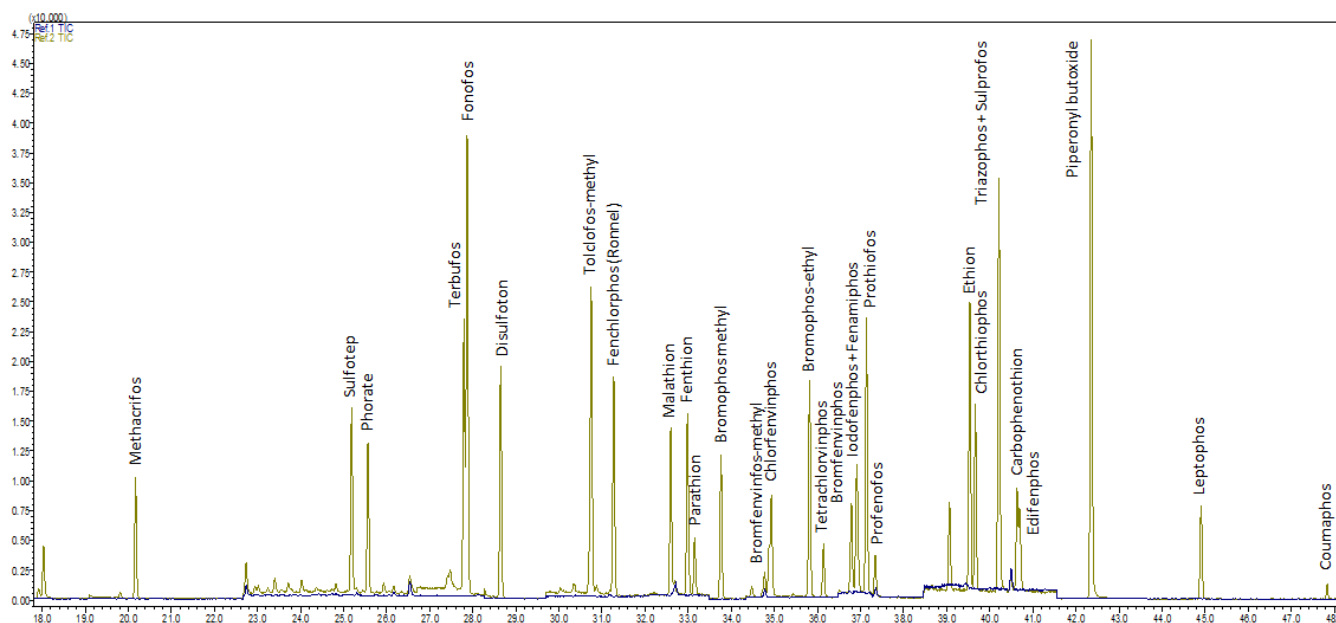

Figure 6: Chromatograms of post-spiked orange juice (100ng/mL) after undergoing the automated QuEChERS extraction and µSPE clean-up.

Quantification

The calibration curves were automatically prepared in a concentration range from 1.0 to 100.0 ng/mL with the standards post-spiked into a blank and μSPE cleaned orange juice extract. A very good linearity with correlation coefficients better than 0.995 for all the investigated organophosphorus pesticides could be achieved. The calibration curves of the late eluting compounds piperonyl butoxide, leptophos and coumaphos are shown representative for the group of compounds in Figure 7.
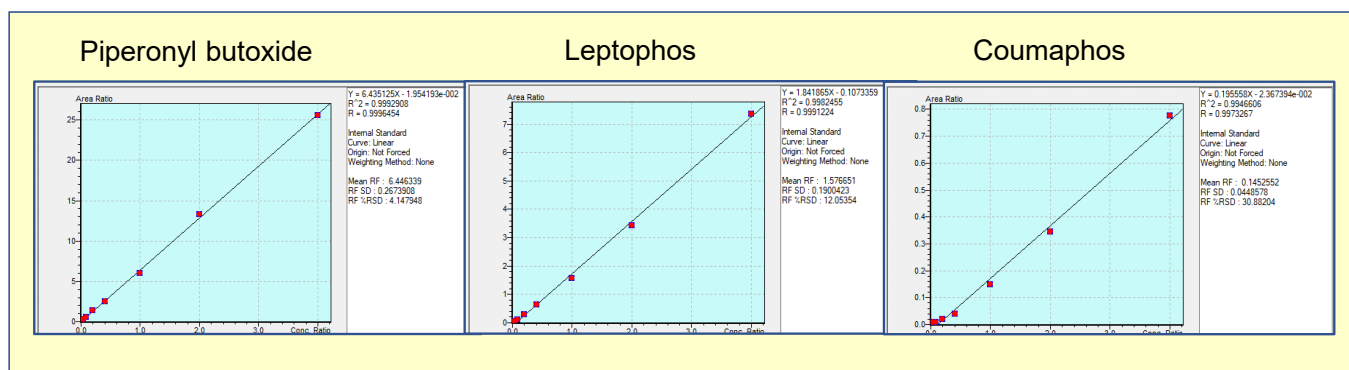

Figure 7: Linear calibration curves post-spiked into μSPE cleaned orange juice extracts of the late eluting compounds piperonyl butoxide, leptophos and coumaphos.

Pre- and post-spiked data from seven consecutive sample runs were used to calculate the method recovery values and method detection limits (MDL) listed in Table 1. The resulting data show a high recovery between 71% and 114% for all pesticides investigated. The MDLs confirm a very good and regulation-compliant sensitivity of the described method. Selected real-life mass chromatograms of the lower recovery and late eluting compounds at the 10 ng/mL decision level are shown in Figure 8.
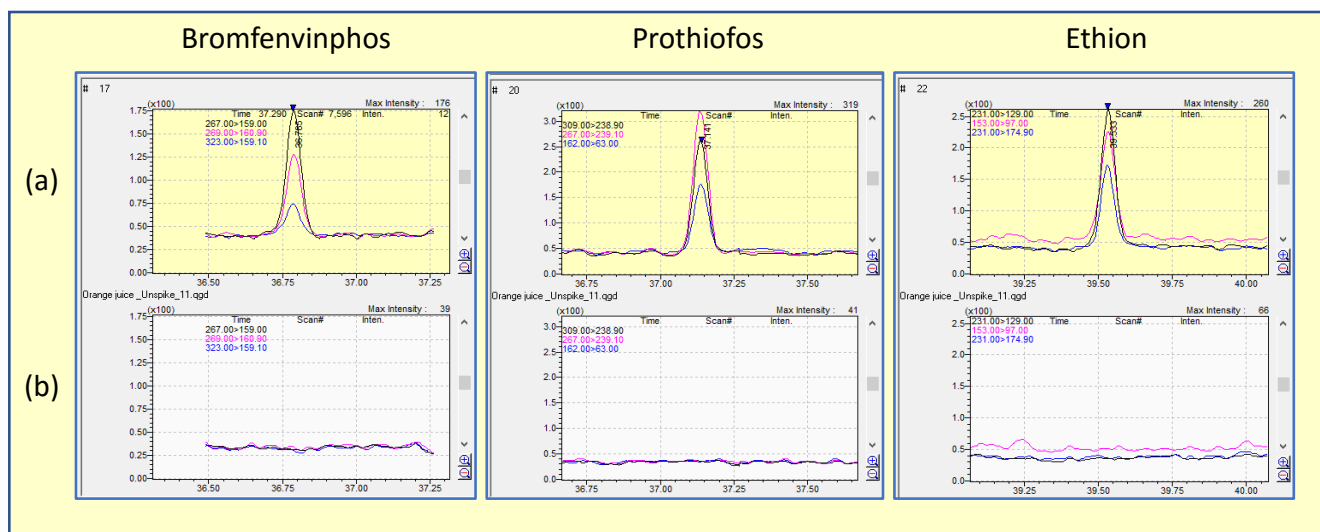

(a) spike 10 ng/mL, (b) blank run
Figure 8: Real-life mass chromatograms (3 MRM transitions each) at the 10 ng/mL decision level

Compound Name	Retention Time (min)	Linearity 1 ng/mL – 100 ng/mL	Pre-Spike at 10 ng/mL		Post-Spike at 10 ng/mL	MDL (ng/mL)
			%RSD (n=7)	Recovery	%RSD (n=6)	
Methacrifos	20.167	0.9985	8.7%	114%	7.8%	3.1
Sulfotep	25.200	0.9989	9.7%	106%	8.2%	3.2
Phorate	25.581	0.9988	10.9%	115%	8.8%	4.0
Terbufos	27.816	0.9972	7.0%	91%	6.9%	2.0
Fonofos	27.884	0.9979	8.4%	115%	10.7%	3.1
Disulfoton	28.662	0.9980	4.8%	110%	11.6%	1.7
Tolclofos-methyl	30.770	0.9982	5.9%	91%	6.3%	1.7
Fenchlorphos (Ronnel)	31.293	0.9966	7.2%	95%	6.0%	2.1
Malathion	32.620	0.9960	12%	108%	11%	4.1
Fenthion	33.014	0.9962	6.3%	91%	5.6%	1.8
Parathion	33.168	0.9974	10%	99%	8.1%	3.1
Bromophos methyl	33.789	0.9977	7.0%	90%	5.9%	2.0
Bromfenvinfos-methyl	34.888	0.9974	8.3%	82%	7.7%	2.1
Chlorfenvinphos	34.952	0.9977	7.8%	91%	2.9%	2.2
Bromophos-ethyl	35.847	0.9976	7.6%	81%	2.1%	1.9
Tetrachlorvinphos	36.167	0.9985	7.7%	86%	9.7%	2.1
Bromfenvinphos	36.814	0.9990	9.1%	88%	4.7%	2.5
Iodofenphos	36.938	0.9971	9.4%	76%	8.4%	2.3
Fenamiphos	36.951	0.9976	7.8%	85%	10%	2.1
Prothiofos	37.168	0.9962	9.2%	74%	5.8%	2.1
Profenofos	37.366	0.9989	10%	87%	6.7%	2.6
Ethion	39.571	0.9957	7.5%	76%	3.9%	1.8
Chlorthiophos	39.694	0.9950	7.9%	80%	1.7%	2.0
Triazophos	40.227	0.9982	7.9%	88%	8.4%	2.2
Sulprofos	40.252	0.9968	8.5%	84%	23%	2.2
Carbophenothion	40.675	0.9984	8.2%	74%	6.4%	2.0
Edifenphos	40.729	0.9960	9.9%	80%	8.8%	2.5
Piperonyl butoxide	42.393	0.9993	8.5%	86%	6.9%	2.3
Leptophos	44.947	0.9982	9.1%	71%	6.9%	2.0
Coumaphos	47.854	0.9947	9.7%	80%	8.3%	2.5

Table 1: Linearity, precision, recovery and method detection limits (MDL) of the organophosphates pesticides investigated automatically extracted from orange juice.

Conclusion

The fully automated QuEChERS extraction and clean-up procedure frees up resources in the routine laboratory. The low solvent and consumables consumption marks the described method as a true contribution to the attempt for a Green Analytical Chemistry (Directorate-General for Health and Food Safety, 2017). The typical high amount of solvents, glassware and consumables required for pesticides analysis is significantly reduced providing a true green analytical method. The automated method avoids solvent evaporation steps, uses only one cartridge type for all matrices, and is fast to be executed online during a chromatographic run in 'prep-ahead' mode optimizing the sample throughput of the MS detection system in use.

The analytical data show an excellent sensitivity for the investigated organophosphates pesticides with MDLs in the range of 3 to 4 ng/mL. The quantitative calibration is linear in the range of 1 to 100 ng/L. The method precision at the decision level is excellent with less than 10% RSD for all compounds, making this automated method a well suitable solution for the pesticide analysis of homogeneous juice samples.

The industry standard x,y,z-robotic system provides a reliable method for pesticides analysis of homogeneous juice samples as shown for organophosphates pesticides from orange juice. The described automated extraction and clean-up workflow can be applied for unattended online GC-MS and LC-MS analysis.

References

1. Anastassiades, M., Lehotay, S. J., Stajnbaher, D., & Schenck, F. J. (2003). Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and 'Dispersive Solid-Phase Extraction' for the Determination of Pesticide Residues in Produce. *JAOAC Int, 86*(2), 412-31. http://dx.doi.org/10.1093/jaoac/86.2.412

2. QuEChERS, https://www.quechers.com/index.php (as of Jan 11, 2021)

3. Lehotay, S. J., Michlig, N., & Lightfield, A. R. (2020). Assessment of Test Portion Sizes after Sample Comminution with Liquid Nitrogen in an Improved High-Throughput Method for Analysis of Pesticide Residues in Fruits and Vegetables. *J. Agric. Food Chem. 68*(5), 1468-1479. https://doi.org/10.1021/acs.jafc.9b07685

4. Directorate-General for Health and Food Safety (2017). SANTE 11312/2021 Guidance Document on Analytical Quality Control and Validation Procedures for Pesticide Residues Analysis in Food and Feed. *European Commission Health & Consumer Protection Directorate General*.

5. Ekroth, S. (2017). The SweEt Method: An Efficient Alternative to Analyze Pesticide Residues in Food. National Food Administration (NFA) Sweden, presented at the *LAPRW Conference*, San Jose, Costa Rica, 2017.

6. Barceló, D. (2008). Food Contaminants and Residue Analysis. N.A., edited by Y. Picó, Vvol. 51, *Elsevier* B.V. Retrieved from https://shop.elsevier.com/books/food-contaminants-and-residue-analysis/pico/978-0-444-53019-6

7. Morris, B. D., & Schriner, R.B. (2025). Development of an Automated Column Solid-Phase Extraction Cleanup of QuEChERS Extracts, Using a Zirconia-Based Sorbent, for Pesticide Residue Analyses by LC-MS/MS. J. Agric. Food Chem, 63, 5107−5119. https://doi.org/10.1021/jf505539e

8. 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. https://doi.org/10.1007/s10337-016-3116-y

9. Goon, A., Shinde, R., Ghosh, B., & Banerjee, K. (2019). Application of Automated Mini–Solid-Phase Extraction Cleanup for the Analysis of Pesticides in Complex Spice Matrixes by GC-MS/MS. *J. AOAC Int, 103*(1), 40–45. http://dx.doi.org/10.5740/jaoacint.19-0202

10. PAL Method Composer User Manual, vers. 1.3. 2020. CTC Analytics AG, Zwingen, Switzerland. https://www.palsystem.com/index.php?id=850

11. Gionfriddo, E. (Ed.). (2022). Green Approaches for Chemical Analysis. Elsevier B.V. Retrieved from https://shop.elsevier.com/books/green-approaches-for-chemical-analysis/gionfriddo/978-0-12-822234-8.