Fully Automated QuEChERS in Analyzing Organophosphates Pesticides in Orange Juice

Chiew Mei Chong, Gwen Lim, Jianxia Lv, Benson Zhu, Hans-Joachim Huebschmann

CTC Analytics Asia Ple. Ltd., 117610 Singapore

Contact: CCHONG@CTC.CH

PAL SYSTEM Ingenious sample handling

Overview

A fully automated QuEChERS is developed by using the PAL Robotic Tool Change (RTC) system in analyzing organophosphate pesticide residuals in orange juices. The automated workflows included the extraction with acetonitrile, salting out and using a µSPE cartridge for sample matrix clean-up prior to injection into the GC-MS/MS. The common method validation techniques such as pre spike and post spike were fully integrated into the PAL RTC automated workflows as well. With the complete automated method validation, the organophosphate based pesticides calibration linearities in orange juice from 1ng/mL – 100ng/mL were at least 0.995. By spiking 10ng/mL of pesticides into the orange juice samples, recoveries were obtained in the of range 70% - 115%, while the precision (%RSDs) from pre-spike (n=7) and post spikes (n=6) under the same concentration were mostly less than 10%. The calculated Method Detection Limits (MDLs) of all the monitoring pesticides were in the range of 1.8ng/mL – 4.1ng/mL which were well below the general Maximum Residual Limits (MRLs) of 10ng/g.

Introduction

QuEChERS is a quick, easy, cheap effective, rugged and safe measure, developed by M. Anastassiades and S.J. Lehotay in 2003. Since then, this technique has become widely used sample preparation approach in pesticide residuals analyses. According to the QuEChERS website, about 45 minutes were needed to manually prepare 8 samples in the laboratory[1]. In the traditional QuEChERS method, the acetonitrile was added, followed by adding salt that was suitable to the sample matrices, cleaning the matrices with the dispersive Solid Phase Extraction before injecting the cleaned extract into GC-MS or LC-MS for analyses. Matrix effect which could enhance or suppress extraction recoveries was one of the major challenges in the QuEChERS pesticides analyses that required to be corrected with the matrix match calibration standards.

Analytical Strategy

A cartridge based miniaturize solid phase extraction, known as µSPE, was fully automated by using the PAL RTC system coupled with the newly released PAL Method Composer to generate the automated sample preparation workflow.

PAL Method Compose – Create Customized Automation Workflow

PAL Method Composer is a newly released software by CTC that enables users to control all the PAL RTC capabilities by using “Drag&Drop” feature to build customizable workflows. A simple matrix blank clean-up with QuEChERS workflow is illustrate in the Figure 1.

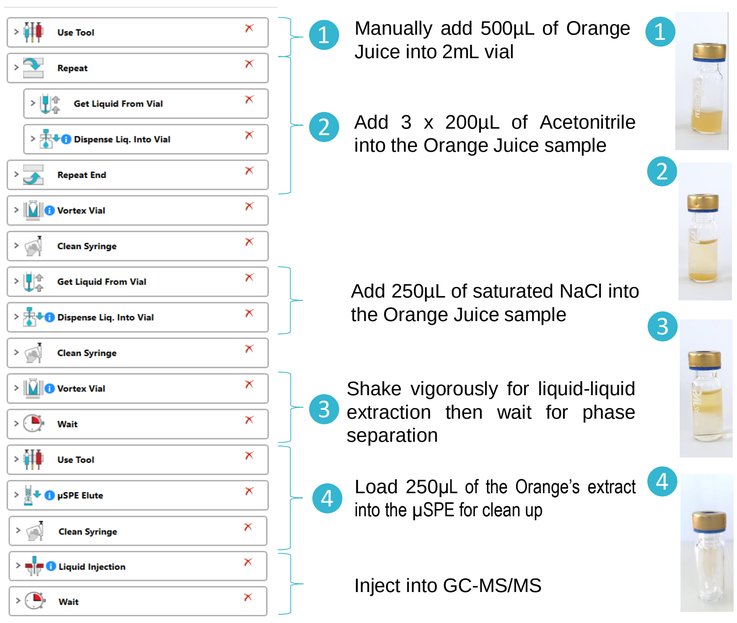
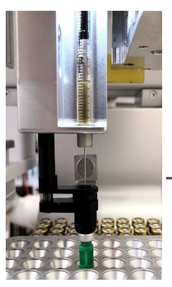


Figure 1: QuEChERS automation workflow by PAL Method Composer

Using µSPE in Matrix Clean-Up



The µSPE cartridge is an optimized sorbent bed for GC QuEChERS analysis, containing a mixture of 45mg of MgSO4, PSA, C18EC and CarbonX. It serves as a filter to retain the samples matrices inside the cartridge, and the pesticide compounds would be eluted into the sample vials beneath of the cartridges.

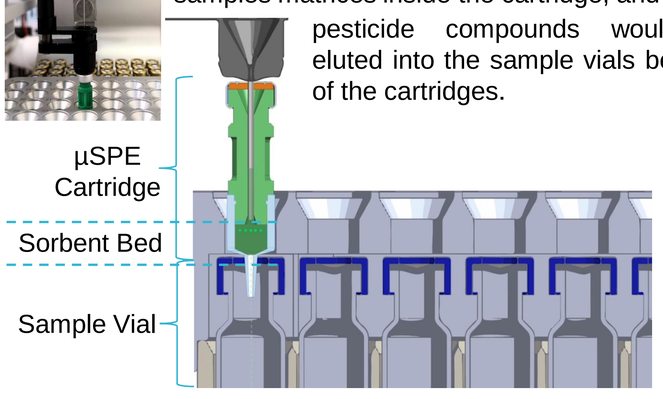


Figure 2: Principle of µSPE clean-up

Instrument Setup

The fully automated workflow, including sample blank, pre spike, and post spike with calibration and internal standards were carried out by the instrument setup as shown in Figure 3. The system setup included the main PAL RTC system with a vortexing module, solvent module to store up to 100mL of the acetonitrile and saturated sodium chloride solution, a wash module equipped with acetonitrile and water for active syringe wash. In the Tray Holder, as shown in Figure 4, the first row of the Rack 1 was used to store the internal standard and calibration standards then followed by the orange juice samples from row 2 to 6. The clean up cartridges were placed on the dedicated cartridge holder in Rack 3. Empty vials with slitted septa were placed at the middle rack to receive cleaned extract.

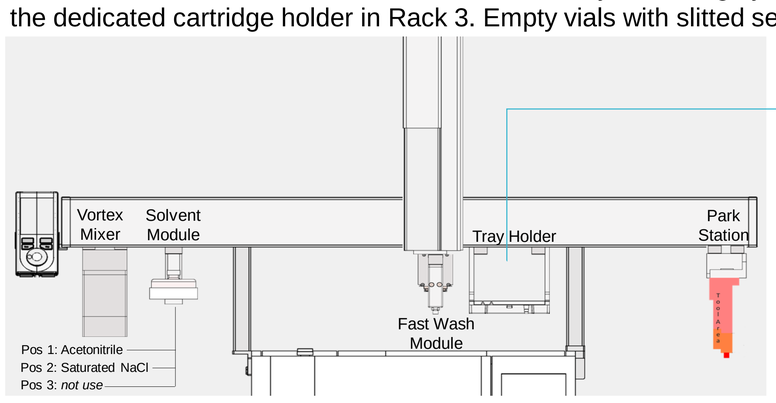


Figure 3. PAL RTC setup for the fully automated QuEChERS analysis

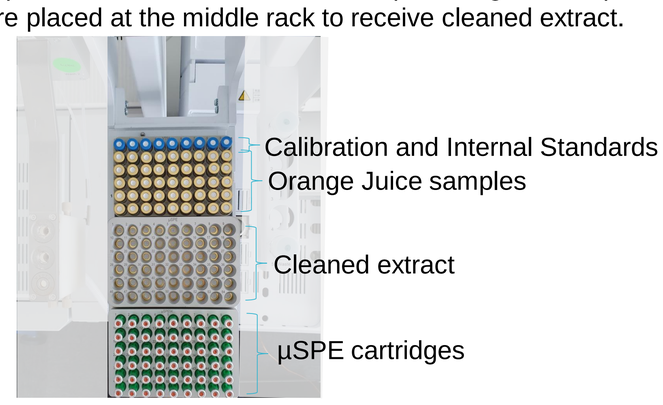


Figure 4. Vial arrangements on the PAL RTC

Automated Method Validation Workflows

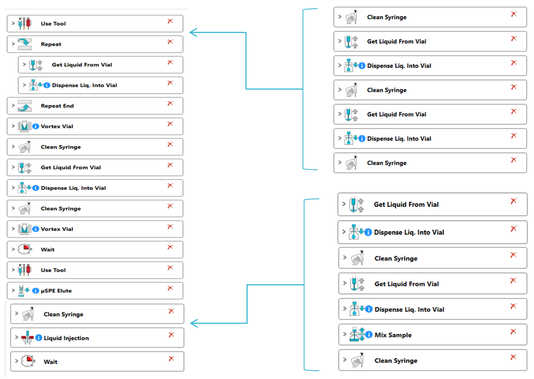


Figure 5. Modified PAL Method Composer workflow to include Pre Spike and Post Spike into the automated QuEChERS evaluations

Pre Spike

The pesticides standard and internal standard were added into the orange juice sample prior to the extraction with acetonitrile.

Post Spike

Pesticides standards and internal standard were added into the cleaned extract before injecting into the GC-MS/MS.

The QuEChERS validation procedures that required pre spike and post spike were fully automated by modifying the workflow shown in the Figure 1 with the PAL Method Composer. The steps “Get Liquid From Vial” and “Dispense Liq. Into Vial” were inserted into the appropriate positions of the clean-up workflow. The Pre Spike workflow was used to determine the µSPE clean-up recoveries, extraction precisions and the method detection limits, while the Post Spike was used to build matrix matched calibration curves and determine the GC-MS/MS instrument precision.

Results

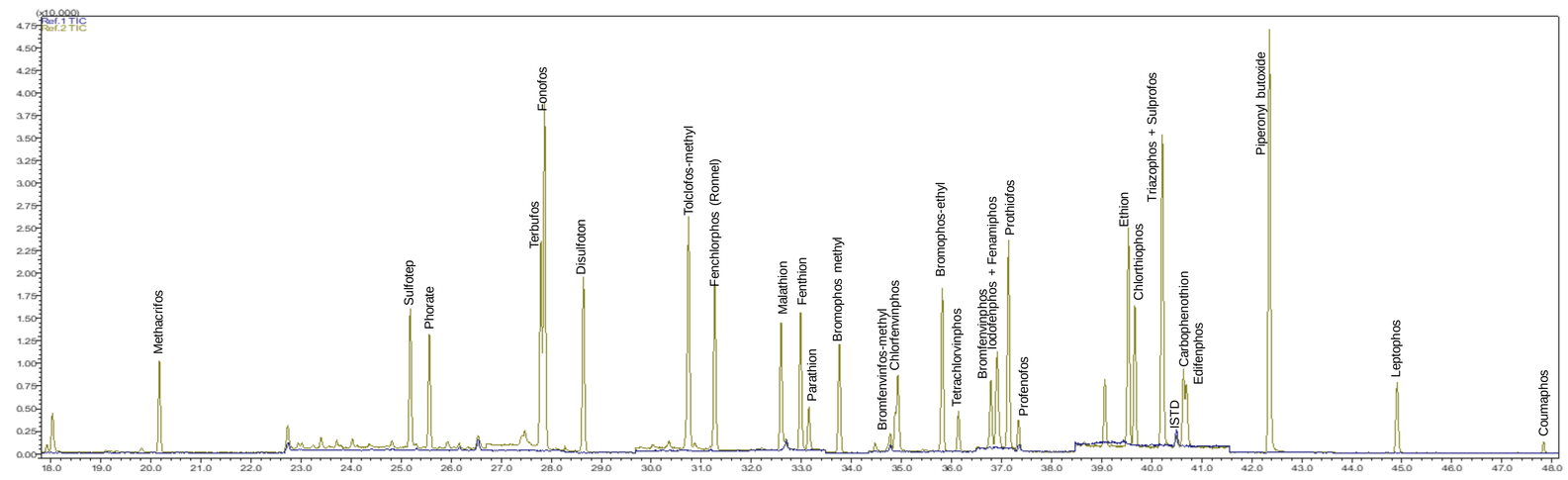


Figure 6. Total Ion Chromatograms (TICs) of unspiked and post spiked (100ng/mL) orange juice after the automated µSPE clean-up

Compound Name	Retention Time (min)	Linearity, from 1ng/mL – 100ng/mL	Pre-Spike at 10ng/mL		Post-Spike at 10ng/mL	MDL (ng/mL)
			%RSD (n=7)	Recovery	%RSD (n=6)	
Methacrifos	20.167	0.9985	8.7%	114(±15)%	7.8%	3.1
Sulfotep	25.200	0.9989	9.7%	106(±17)%	8.2%	3.2
Phorate	25.581	0.9988	10.9%	115(±18)%	8.8%	4.0
Terbufos	27.816	0.9972	7.0%	91(±10)%	6.9%	2.0
Fonofos	27.884	0.9979	8.4%	115(±14)%	10.7%	3.1
Disulfoton	28.662	0.9980	4.8%	110(±8)%	11.6%	1.7
Tolclofos-methyl	30.770	0.9982	5.9%	91(±11)%	6.3%	1.7
Fenchlorphos (Ronnel)	31.293	0.9966	7.2%	95(±9)%	6.0%	2.1
Malathion	32.620	0.9960	12%	108(±22)%	11%	4.1
Fenthion	33.014	0.9962	6.3%	91(±11)%	5.6%	1.8
Parathion	33.168	0.9974	10%	99(±14)%	8.1%	3.1
Bromophos methyl	33.789	0.9977	7.0%	90(±13)%	5.9%	2.0
Bromfenvinfos-methyl	34.888	0.9974	8.3%	82(±12)%	7.7%	2.1
Chlorfenvinphos	34.952	0.9977	7.8%	91(±9)%	2.9%	2.2
Bromophos-ethyl	35.847	0.9976	7.6%	81(±10)%	2.1%	1.9
Tetrachlorvinphos	36.167	0.9985	7.7%	86(±9)%	9.7%	2.1
Bromfenvinphos	36.814	0.9990	9.1%	88(±12)%	4.7%	2.5
Iodofenphos	36.938	0.9971	9.4%	76(±11)%	8.4%	2.3
Fenamiphos	36.951	0.9976	7.8%	85(±9)%	10%	2.1
Prothiofos	37.168	0.9962	9.2%	74(±13)%	5.8%	2.1
Profenofos	37.366	0.9989	10%	87(±10)%	6.7%	2.6
Ethion	39.571	0.9957	7.5%	76(±9)%	3.9%	1.8
Chlorthiophos	39.694	0.9950	7.9%	80(±13)%	1.7%	2.0
Triazophos	40.227	0.9982	7.9%	88(±10)%	8.4%	2.2
Sulprofos	40.252	0.9968	8.5%	84(±12)%	2.3%	2.2
Carbophenothion	40.675	0.9984	8.2%	74(±9)%	6.4%	2.0
Edifenphos	40.729	0.9960	9.9%	80(±10)%	8.8%	2.5
Piperonyl butoxide	42.393	0.9993	8.5%	86(±15)%	6.9%	2.3
Leptophos	44.947	0.9982	9.1%	71(±9)%	6.9%	2.0
Coumaphos	47.854	0.9947	9.7%	80(±11)%	8.3%	2.5

Table 1. Linearity, precision, recovery and detection limits of the organophosphates pesticides in orange juice based on the fully automated workflow with the Pre Spike and Post Spike features

Sample Evaluation

The organophosphate pesticide residuals were evaluated based on pre spike and post spike of the pesticide standards into the orange juice samples. Due to matrix effect, the pesticide analyte signals were enhanced in the GC-MS/MS. Therefore, the calibration curves were plotted based on spiking the pesticide standards into the cleaned orange juice extracts. Method Detection Limits (MDLs) were calculated based on 7 orange juice samples pre spiked with 10ng/mL of the pesticide standards then undergone the full QuEChERS workflows from extraction, salting out and clean-up with the µSPE.

Conclusion

- A fully automated QuEChERS based on the PAL RTC system is stable and reliable in the analysis of organophosphates pesticides residuals in orange juices.
- By using the newly released PAL Method Composer, a complete method validation including the Pre Spike and the Post Spike procedures can be easily integrated into the QuEChERS workflow for complete automation sample preparation and validation.

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https://www.palsystem.com/index.php?id=1061

References

[1] QuEChERS Home Page
https://www.quechers.com/index.php (accessed Sep 7, 2020)

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