

LC Application Note



ENVIRONMENTAL



FOOD SAFETY

**Automated SPE clean-up
of QuEChERS extracts for
multi-residue pesticide
analysis**





Automated SPE clean-up of QuEChERS extracts for multi-residue pesticide analysis

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Introduction

The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe, s. ref. 1) method for extraction of pesticides from fruits and vegetables has become widely used by commercial testing laboratories worldwide, due to its simplicity and applicability to a wide range of sample-types and pesticide chemistries. Typically, dispersive solid-phase extraction (dSPE) is used to clean up acetonitrile extracts prior to LC-MS/MS analysis, using mixtures of primary-secondary amine and C-18 functionalized silicas, with graphitized carbon black ($MgSO_4/PSA/C-18/GCB$), to remove matrix sugars, oils and pigments. Column SPE (cSPE) is generally accepted as giving improved removal of sample matrix, however it is more time consuming, and costly. This note covers the development and use of miniaturized cSPE for QuEChERS, using Instrument Top Sample Prep (ITSP, <http://www.itspolutions.com/>) cartridges (Figure 1), automated on a PAL System autosampler.

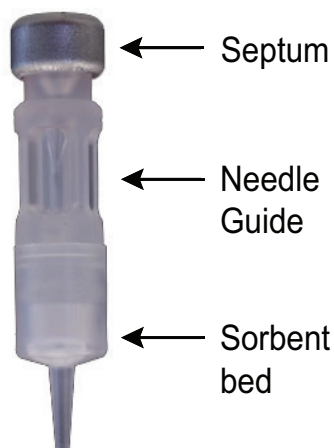


Figure 1: ITSP SPE cartridge

This allows high-throughput cSPE clean-up on an LC-MS/MS instrument, in place of dSPE in the laboratory, reducing time, cost and solvent usage.

Oily extracts are often poorly cleaned up by dSPE using $MgSO_4/PSA/C-18/GCB$, giving rise to cloudy extracts when aqueous buffer is added for LC-MS/MS injection, contamination of the LC system and possible losses of non-polar pesticides into oil droplets. Therefore, ITSP sorbent mixtures and unique elution solvents were developed to allow effective removal of oils and some pigments, with acceptable recoveries for a suite of 274 pesticide analytes.

QuEChERS-ITSP Automation

SPE clean-up of QuEChERS extracts is undertaken in scavenging mode, where unwanted matrix is retained by the sorbent, and pesticide analytes are recovered. To enable this on a PAL System autosampler, extracts are loaded onto and eluted from cartridges sitting directly on 2-mL vials with split-septa caps, and both load and elution volumes collected. The cartridge tips are able to pass through the split-septa into the vials, while still providing a seal against solvent evaporation. An aluminum cover for the 54-well tray holds the cartridges vertically in place between load and elution steps, and allows the cartridges to be removed from the vials at the end of the SPE cycle, without lifting the vials from the tray (Figures 2 and 3).

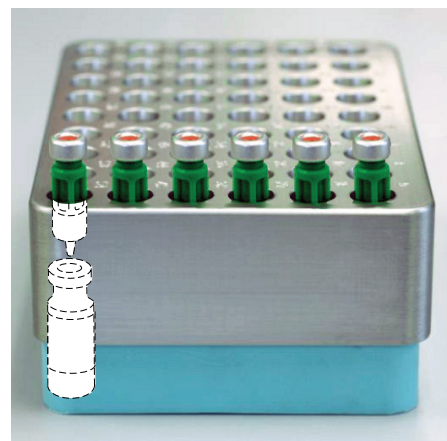


Figure 2: Aluminum cover for 54-well vial tray, with ITSP cartridge positioned over vial.



Figure 3: Elution of QuEChERS extract through an ITSP cartridge, using the aluminum tray cover.

The PAL System unit provides the ability to use low flow-rates (2 $\mu L/min$) which avoids high back-pressures, and gives some degree of chromatography on the cartridge sorbent bed.

QuEChERS-ITSP Method and Instrumentation

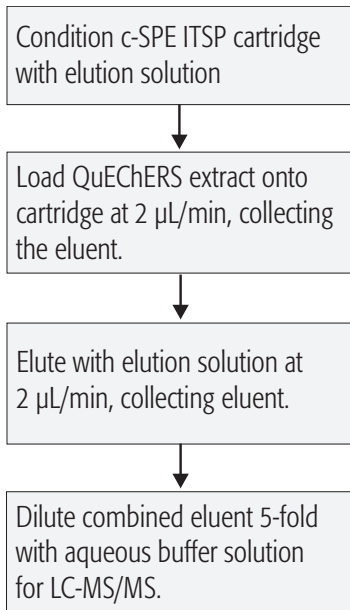


Figure 4: QuEChERS-ITSP steps.

Autosampler:	PAL RTC (or PAL-xt), fitted with HW-KITQ hardware kit.			
LC-MS/MS:	Sciex Triple Quad 5500, positive ESI, with an Agilent 1200 pump.			
HPLC column:	Ascentis Express C-18, 10 cm × 2.1 mm, 2.7µm particles (Supelco).			
Injection volume:	10 µL			
Column Temp.:	50 °C			
Solvent A:	10mM ammonium formate (pH = 4)/MeOH (9:1).			
Solvent B:	MeOH			
Gradient:	Total Time (min)	Flow Rate (µl/min)	A (%)	B (%)
	0.0	400	87.5	12.5
	0.5	400	87.5	12.5
	2.0	400	60.0	40.0
	8.0	400	37.3	62.7
	14.0	400	5.0	95.0
	16.0	400	5.0	95.0
	16.5	400	87.5	12.5
	18.5	400	87.5	12.5

Table 1: LC-MS/MS instrument parameters.

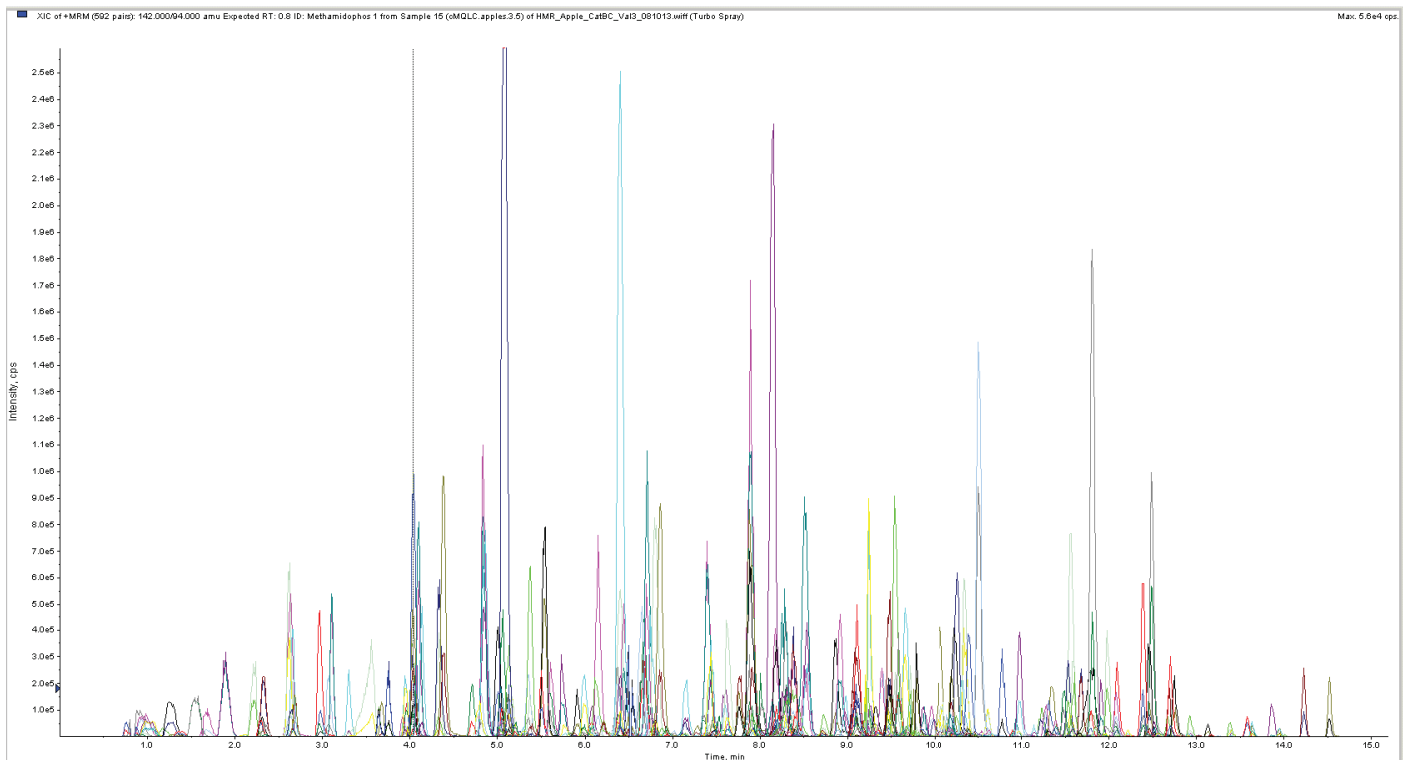


Figure 5: LC-MS/MS chromatogram for a 0.02 mg/kg spike of pesticides, cleaned up by ITSP.

Effectiveness of extract clean-up

Avocado was chosen to represent samples containing high levels of lipids, with up to 30% by weight of mono-, di- and triacylglycerides, free fatty acids, sterols and tocopherols. An extract was obtained using the European Committee for Standardization (CEN) QuEChERS method, with citrate buffering, and a 1:2 ratio of blended fruit to acetonitrile (5 g of fruit, 10 mL of acetonitrile).

After dilution with aqueous buffer (10mM ammonium formate/formic acid, pH = 4), the crude extract was visually cloudy, whereas that cleaned up by ITSP was clear, indicating effective oil removal (Figure 6).



Figure 6: Avocado extract without clean-up, after ITSP clean-up and after dSPE clean-up ($\text{MgSO}_4/\text{PSA}/\text{C}-18$). Each had the same final dilution.

Use of dSPE clean-up ($\text{MgSO}_4/\text{PSA}/\text{C}-18$), using the same extract:sorbent ratio as ITSP, resulted in an extract less cloudy than the crude, but cloudier than that from ITSP, likely requiring filtration before LC-MS/MS (Figure 6).

Avocado extract was analysed for di- and triacylglyceride content by high resolution LC-MS, before and after ITSP cSPE (Figure 7). The first group of peaks (10.93 – 11.54 min) includes diacylglycerides, the second group (13.1 – 13.65 min) had accurate masses identifying them as triacylglycerides. This

indicated that the clean-up method was effectively removing triacylglycerides, which are the major component of avocado oils (Fig. 7).

Pesticide recoveries

A spike recovery experiment was undertaken on avocado, to give data for 274 pesticides, covering a wide range of classes, polarities and pK_a s. Spikes (100 $\mu\text{g}/\text{kg}$) were added to 5 g of blended fruit, along with a system monitoring compound (SMC, triphenylphosphate), before extraction by CEN QuEChERS using 10 mL of acetonitrile. Following appropriate dilution and ITSP, extracts were diluted 5-fold with aqueous buffer and analysed by LC-MS/MS. Of note is that solvent-only standards were used to build the calibration curves.

Ten replicates were performed to give mean recoveries and standard deviations. Of the 274 analytes, 257 were within 70 - 120% and 40% standard deviation, with the majority (216) between 80 - 100% and with less than 20% standard deviation, indicating excellent reproducibility of ITSP recoveries. Low recoveries were observed for analytes with typically poor QuEChERS recoveries, such as pymetrozine, forchlorfenuron and anilazine, while there was some retention of the benzimidazole fungicides carbendazim and thiabendazole on the ITSP cartridges (thiabendazole also exhibited poor extraction recoveries). This data has been represented graphically in Figure 8, showing a tight cluster of peaks between 80 and 100% recoveries.

To illustrate the loss of non-polar pesticides that is possible without effective oil removal, a spiked avocado extract was diluted 2-fold and cleaned up by dSPE ($\text{MgSO}_4/\text{PSA}/\text{C}-18$), then the cloudy solution (as in Figure 6), filtered before LC-MS/MS injection. Figure 9 shows low recoveries for many non-polar pesticides (those with late retention times) due to partitioning into the oil droplets.

Conclusions

The QuEChERS-ITSP clean-up that was developed was effective at removing matrix oils from avocado extracts, resulting in clear extracts with dilution in aqueous buffer, compared with the cloudy extracts resulting from $\text{MgSO}_4/\text{PSA}/\text{C}-18$ dSPE clean-up. Removal of triacylglycerides from oily QuEChERS extracts has commonly been problematic, and is important for LC-MS/MS analysis, as they can accumulate on HPLC systems, or cause losses of non-polar pesticides if cloudy samples are filtered, or left on autosamplers for extended periods, allowing oil drop out.

The combination of automation on a PAL System autosampler and use of the elution solution developed, resulted in acceptable recoveries of 274 pesticides, with low relative standard deviations and minimal LC-MS/MS matrix suppression.

ITSP automation on a PAL System autosampler utilizes the idle time between injections, therefore LC-MS/MS run times are not affected. However, lab efficiency is improved by removing the need for manual clean-up steps. This ITSP method has proven to be reliable and robust in a commercial, high-throughput laboratory setting.

References:

- [1] Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce, Anastassiades, M., S. J. Lehotay, Štajnbaher, D. and Schenck, F. J. Journal of AOAC International 2003, 86(2): 412-431.

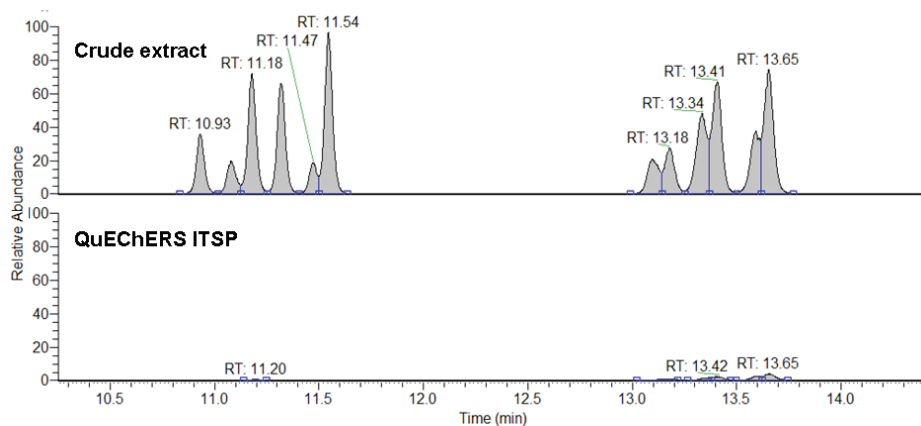


Figure 7: High resolution LC-MS chromatograms for crude avocado extract and the same extract diluted 2-fold then cleaned up by ITSP. Final dilutions were the same for both prior to injection. Chromatograms have the same y-axis scale.

Percent spike recoveries vs. percent standard deviations Avocado ITSP.

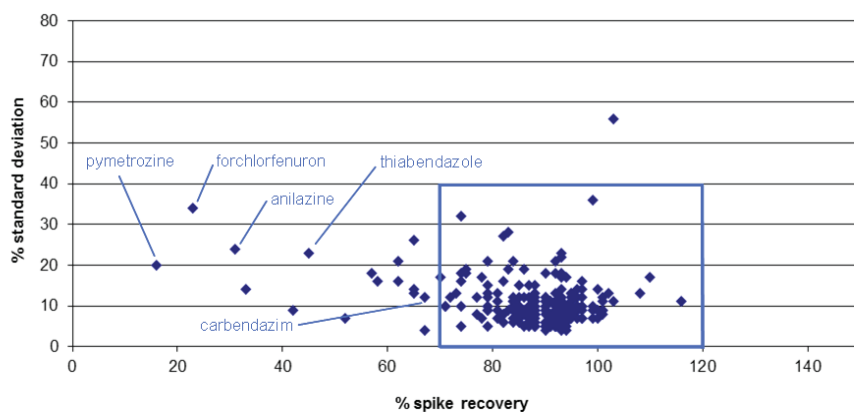


Figure 8: Percent recoveries versus percent standard deviations for 100 µg/kg spikes of 274 pesticide analytes on avocado, extracted by CEN QuEChERS, cleaned up by ITSP and analysed by LC-MS/MS. Calibrated against solvent-only standards, normalized to triphenylphosphate, N = 10.

Percent spike recoveries vs. LC-MS/MS retention times Avocado dSPE (PSA/C-18), plus filtration.

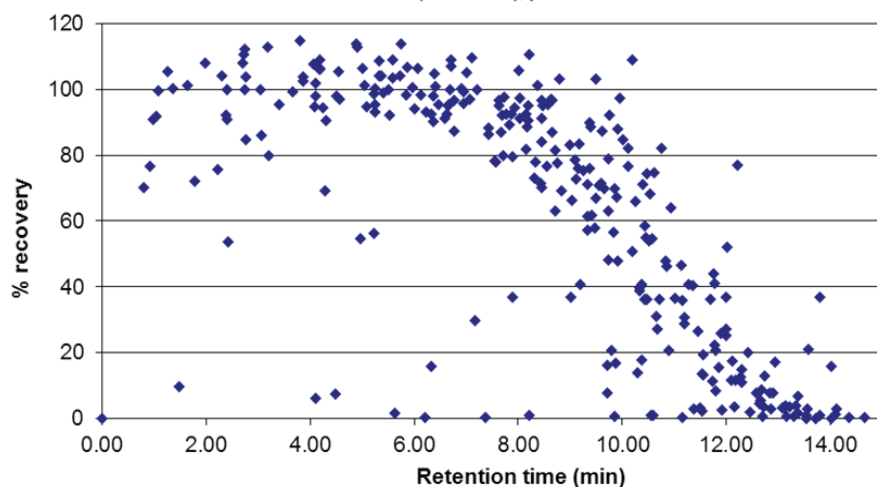


Figure 9: Percent pesticide recoveries versus LC-MS/MS retention times, for a 50 µg/kg spike on avocado, extracted by CEN QuEChERS, diluted 2-fold, cleaned up by dSPE (MgSO₄/PSA/C-18), diluted 5-fold with aqueous buffer, then filtered (0.4 µm cellulose syringe filter) before analysis. Calibrated against solvent-only standards.

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