Application Note

Food & Beverage Testing

Determination of Multiclass, Multiresidue Pesticides in Tree Nuts by Captiva EMR–LPD Passthrough Cleanup and LC/MS/MS

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Abstract

This application note presents the development and optimization of a multiresidue method for the analysis of pesticide residues in four common types of tree nuts: almonds, pecans, cashews, and hazelnuts. The method involves extraction with the Agilent Bond Elut QuEChERS AOAC extraction kit, followed by passthrough cleanup with Agilent Captiva Enhanced Matrix Removal–Low Pigment Dry (EMR–LPD), then LC/MS/MS. The newly developed method demonstrated efficient matrix removal, acceptable target quantitation results, and low failure rate for analysis of a large panel of pesticides in challenging tree nut matrices. Excellent method quantitation results were achieved, with >90% average recovery and <10% average RSD for a total of 125 LC-amenable pesticides, analyzed in four types of tree nuts. The matrix removal assessment by dried residue weight indicated that >64% of tree nut co-extractives were removed.

Introduction

Tree nuts are an important part of the human diet as they contain multiple heart-healthy nutrients; therefore, they are widely consumed. Typical tree nuts have a high fat content (>50%) that is low in saturated lipids and high in unsaturated fatty acids. Nuts also contain many nutrients and bioactive components, including plant sterols, dietary fiber, and antioxidants. Trace level pesticide analysis in a tree nut matrix can be challenging due to significant complexity and difficult cleanup of the matrix. Nut sample extraction usually involves premixing the sample with water followed by QuEChERS extraction. To clean the complex sample matrix co-extractives, complicated matrix cleanup strategies are used, such as dispersive solid phase extraction (dSPE) and freezing out. These methods could take significant time with multiple steps, but still be inefficient for matrix cleanup. A complicated sample cleanup procedure may also cause loss of targets and poor reproducibility, as well as a more significant matrix effect.

Captiva EMR–LPD cartridges contain the Agilent proprietary sorbents Carbon S and Captiva EMR–Lipid, blended with primary secondary amine (PSA) and C18 in an optimized formula. Captiva EMR–Lipid sorbent provides highly selective and efficient lipids removal, while PSA sorbent provides efficient fatty acid removal; other sorbents such as Carbon S and EC-C18 provide further matrix cleanup. The Captiva EMR–LPD formula was carefully developed and optimized to deliver the improved balance between matrix removal and target recovery for complex dry matrices with low or no pigments, using the simplified passthrough cleanup.

In this study, sample preparation using Captiva EMR–LPD cartridges for passthrough cleanup was optimized for the analysis of 125 common pesticides, by LC/MS/MS, in four typical tree nuts: almond, pecan, cashew, and hazelnut.

Experimental

Chemicals and reagents

Pesticide standards and internal standards (IS) were either obtained as the standard mix stock solutions from Agilent Technologies (part number 5190-0551) or as individual standard stock solutions or powder from Sigma-Aldrich (St Louis, MO, U.S.). HPLC grade acetonitrile (ACN) was from Honeywell (Muskegon, MI, U.S.). Reagent grade acetic acid, ammonium acetate, and ammonium fluoride were also from Sigma-Aldrich.

Solutions and standards

A combined standard spiking solution (125 pesticides) and a combined IS (two IS compounds) spiking solution were prepared at 10 µg/mL in ACN and stored at –20 °C in a freezer. The standard spiking solutions were warmed to room temperature, sonicated before use, and returned after use.

The ACN with 1% acetic acid extraction solvent was prepared by adding 10 mL of glacial acetic acid into 990 mL of ACN and stored at room temperature.

Equipment and material

The study was performed using an Agilent 1290 Infinity LC system coupled to an Agilent 6490 triple quadrupole LC/MS. The Agilent 1290 Infinity LC system consisted of an Agilent 1290 Infinity binary pump (G4220A), an Agilent 1290 Infinity autosampler (G4226A), and an Agilent 1290 Infinity thermostatted column compartment (G1316C). The coupled 6490 triple quadrupole LC/MS (G6490) was equipped with an Agilent Jet Stream electrospray ion source. Agilent MassHunter Workstation software was used for data acquisition and analysis.

Other equipment used for sample preparation included: a Centra CL3R centrifuge (Thermo IEC, MA, U.S.), a Geno/Grinder (SPEX, NJ, U.S.), a Multi Reax test tube shaker (Heidolph, Schwabach, Germany), pipettes and a repeater (Eppendorf, NY, U.S.), an Agilent positive pressure manifold 48 processor (PPM-48) (part number 5191-4101), the Agilent Bond Elut QuEChERS AOAC extraction kit (part number 5982-5755), and the Agilent Captiva EMR–LPD cartridge, 6 mL (part number 5610-2092).

Instrument conditions

Table 1 lists the LC/MS/MS conditions. For targets' dynamic multiple reaction monitoring (dMRM) parameters, please see the application note by Zhao and Wei.[1] Figure 1 shows a typical MRM chromatogram of targeted pesticides in the fortified almond sample at the level of 100 ng/g, prepared by QuEChERS AOAC extraction followed by Captiva EMR–LPD cleanup.

Table 1. Agilent 1290 Infinity LC and Agilent 6490 triple quadrupole LC/MS method conditions.

LC Conditions	
Columns	Agilent ZORBAX Eclipse Plus C18 column, 2.1 × 100 mm, 1.8 µm (p/n 959758-902) Agilent ZORBAX Eclipse Plus C18 column, UHPLC guard, 2.1 × 5 mm, 1.8 µm (p/n 821725-901)
Flow Rate	0.3 mL/min
Column Temperature	40 °C
Injection Volume	2 µL
Mobile Phase	A) 10 mM ammonium formate, 0.5 mM ammonium fluoride in water, 0.125% FA B) 10 mM ammonium formate, 0.5 mM ammonium fluoride in 95:5 ACN:water, 0.125% FA
Needle Wash	1:1:1:1 ACN:MeOH:IPA:water, 0.2% formic acid
Gradient	Time (min) %B Flow (mL/min) 0.0 15 0.3 6.0 95 0.3 8.01 100 0.3
Stop Time	10 min
Post Time	2.3 min
MS Conditions	
Ionization Mode	Electrospray ionization (ESI)
Gas Temperature	120 °C
Gas Flow	20 L/min
Nebulizer	40 psi
Sheath Gas Heater	225 °C
Sheath Gas Flow	11 L/min
Capillary Voltage	4,500 V (positive and negative)
Nozzle Voltage	0 V (both positive and negative)
iFunnel Parameters	High-pressure RF: 150 V (positive), 90 V (negative) Low-pressure RF: 60 V (positive), 60 V (negative)
Polarity	Positive and negative, see Table 4 from reference[1].

Sample preparation

The organic almond, pecan, cashew, and hazelnut samples were purchased from a local grocery store. Samples were homogenized with a grinder. Ground almond, pecan, and cashew were weighed at 7.5 g into 50 mL centrifuge tubes. For hazelnut, 3 g of ground sample was weighed. An aliquot of 10 mL water was added. Samples were then vortexed for 15 minutes for complete wetting and equilibrating of the dry matrix. The sample mixture was extracted following the QuEChERS AOAC method. After the extraction, 2.7 mL of crude extract was mixed with 0.3 mL of water. The mixed sample was then transferred into the Captiva EMR–LPD 6 mL cartridges for passthrough cleanup. A low level of positive pressure (1 to 3 psi) was applied to assist the consistent elution flow at 2 to 4 seconds per drop. The sample eluent was vortexed for 10 seconds for mixing, and a 250 µL aliquot of eluent was taken to mix with 750 µL of water in a 2 mL vial. The diluted sample was then ready for LC/MS/MS analysis. The detailed sample preparation procedure is shown in Figure 2. For a batch of ~30 samples, the entire procedure usually takes approximately 30 to 40 minutes.

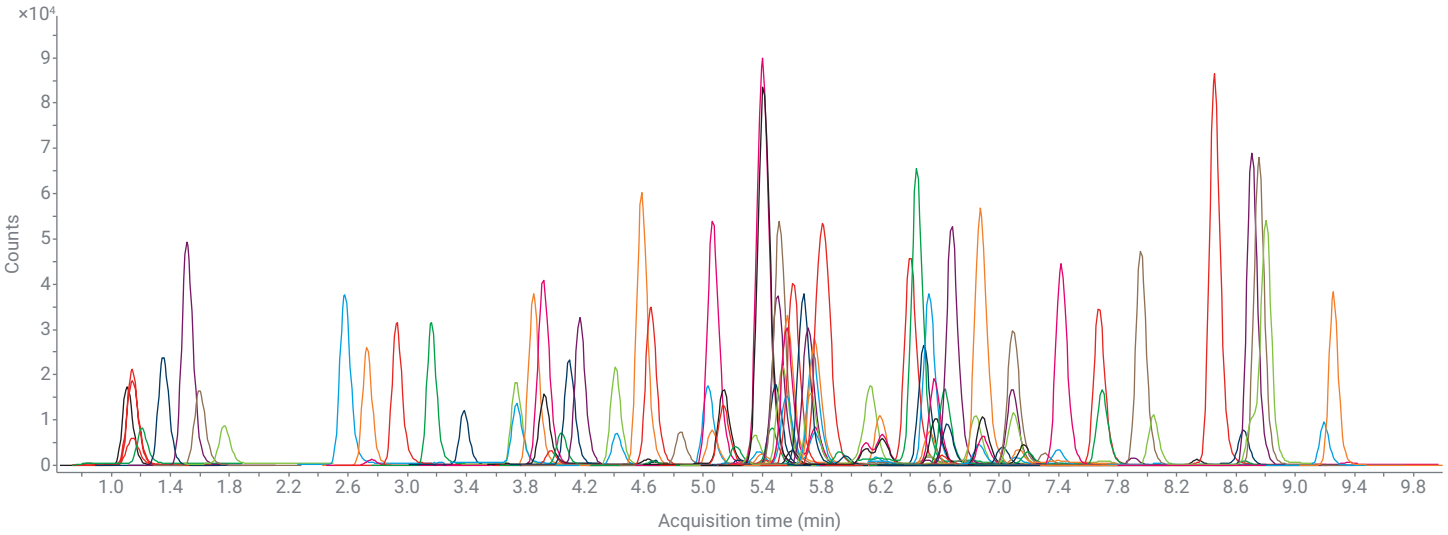

Figure 1. LC/MS/MS MRM chromatogram for an extracted almond sample fortified with 100 ng/g of 125 targeted pesticides. The sample was prepared using the Agilent Bond Elut QuEChERS AOAC extraction kit, followed by Agilent Captiva EMR–LPD cleanup.

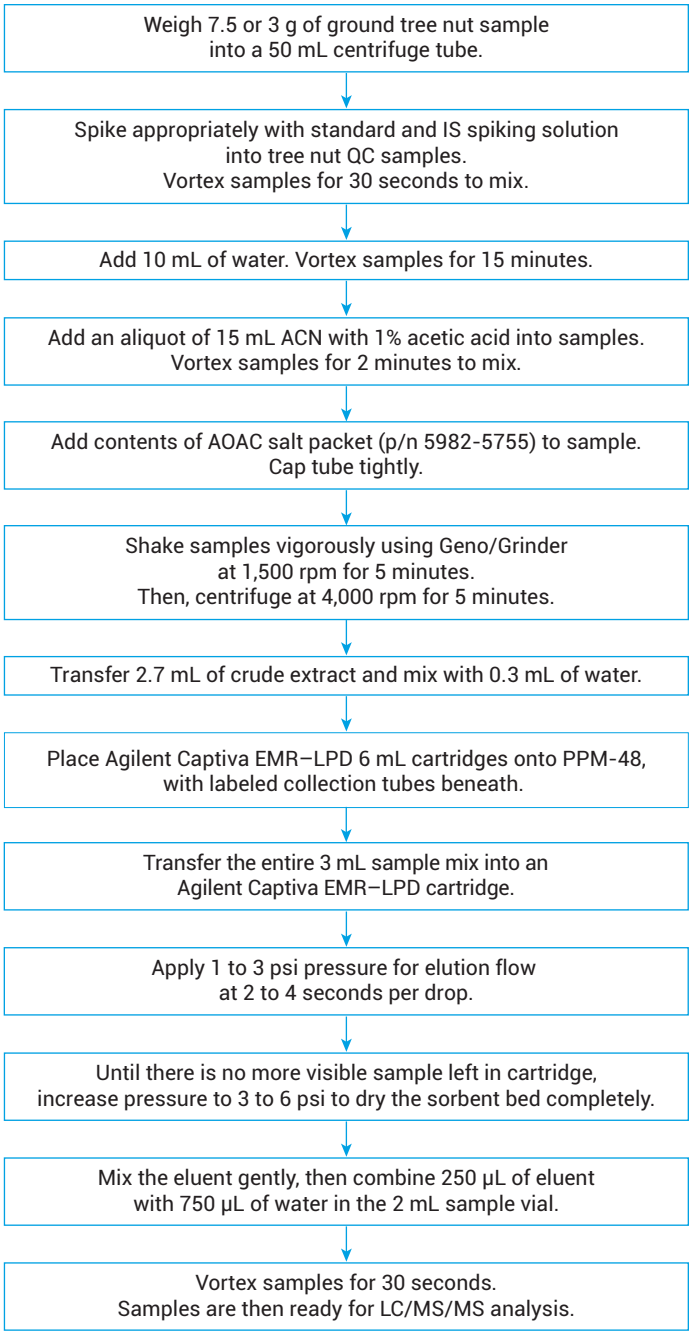

Figure 2. Sample preparation procedure for tree nut samples by Agilent Bond Elut QuEChERS AOAC extraction followed by Agilent Captiva EMR–LPD passthrough cleanup. For almond, pecan, and cashew, 7.5 g of ground sample was used. For hazelnut, 3 g of ground sample was used.

Method development

For QuEChERS extraction of the tree nut samples, both standard AOAC and EN extraction methods were evaluated based on a comparison of target recoveries after using each method. Both almond and pecan samples were used for this study at the spiking level of 10 ppb in the crude extract.

For Captiva EMR–LPD cleanup, the premixing with water was optimized by comparing the recovery results with 0, 10, and 20% water addition. Almond crude blank extract was spiked at 10 ppb and used for the parallel comparison.

Matrix removal was assessed based on the dried residue weight of the sample and a GC/MS full scan comparison of samples with and without the Captiva EMR–LPD cleanup.

Method performance evaluation

The developed sample preparation method was evaluated in terms of matrix removal; target recovery, reproducibility, and matrix effect; matrix-matched calibration curve linearity and limits of quantitation (LOQs) in four kinds of tree nuts. To evaluate recovery, reproducibility, and matrix effect, prespiked quality control (PR-QC) samples were prepared at 20 ng/g in almond, pecan, and cashew powder, and 50 ng/g in hazelnut, in replicates of six, corresponding to 10 ng/mL in crude sample extract after extraction. The spiked samples and matrix blank samples were then prepared following the procedure. Postspiked QCs (PO-QC) were prepared in matrix blank before water dilution. It is important to use the appropriate sample volume adjustment for postspiking the corresponding concentration in the crude ACN extract at a 10 ng/mL level. Neat QCs were directly spiked at 10 ng/mL in reagent blank (extraction solvent), then diluted appropriately with water. Six replicates of each type of QC were prepared. The peak area ratios of corresponding targets in PR-QCs versus PO-QCs were used to calculate target recovery. The peak areas in PR-QCs were used for sample preparation method reproducibility RSD calculation. The peak area ratios of corresponding target in PO-QCs versus neat QCs were used for target matrix effect calculation. Matrix matched calibration curve linearity and LOQs were evaluated by postspiking at the levels of 0.5, 1, 5, 10, 50, 100, 250, 400, and 500 ng/mL in tree nut matrix blank extract, corresponding to 1 to 1,000 ng/g in almond, pecan, and cashew, and 2.5 to 2,500 ng/g in hazelnut. Analyte identification, confirmation, and quantitation were determined from retention times and MRM transitions.

Results and discussion

Method development and optimization

The QuEChERS extraction method was first evaluated for sample size, the extraction solvent, and sample partition salts. As tree nuts contain very low water but high fat, the different types of tree nut were firstly screened for matrix complexity and basic matrix removal by Captiva EMR–LPD cleanup. The preliminary matrix cleanup showed that Captiva EMR–LPD pass-through cleanup provided highly efficient nut matrix removal, which allowed the use of a relatively larger sample size, with less dilution during sample extraction. The matrix complexity screening of the different nut types indicated that hazelnut was the most complex matrix, containing a significantly higher abundance of fatty acids and lipids, while the other three types of tree nuts were less complicated. Given this preliminary understanding of the matrices' complexity, a larger sample size was used for almond, pecan, and cashew, with a 2x dilution factor, and a smaller sample size was used for hazelnut, with a 5x dilution factor.

For a large panel of pesticides with many sensitive targets included, it is important to use buffered extraction salts for sample partition. Therefore, only standard AOAC buffered salts and EN salts were evaluated. Originally, EN extraction was followed using the neutral ACN. However, neutral ACN extraction caused significant loss of some sensitive pesticides, such as pymetrozine, propamocarb, MCPA, and so on. As a result, the EN extraction method was modified using the acidified ACN extraction solvent (with 1% acetic acid). This modified EN extraction method was then compared with the standard AOAC extraction method.

Figure 3 shows the comparison results for the recoveries of sensitive pesticides using AOAC extraction versus modified EN extraction. The results show that the use of AOAC extraction generally improved the recovery of sensitive targets. For pymetrozine, fenpropidin, spiroxamine, and tralkoxydim, >30% increased recovery was achieved for both matrices when using AOAC extraction salts. As a result, the AOAC method was used for pesticide extraction in this application.

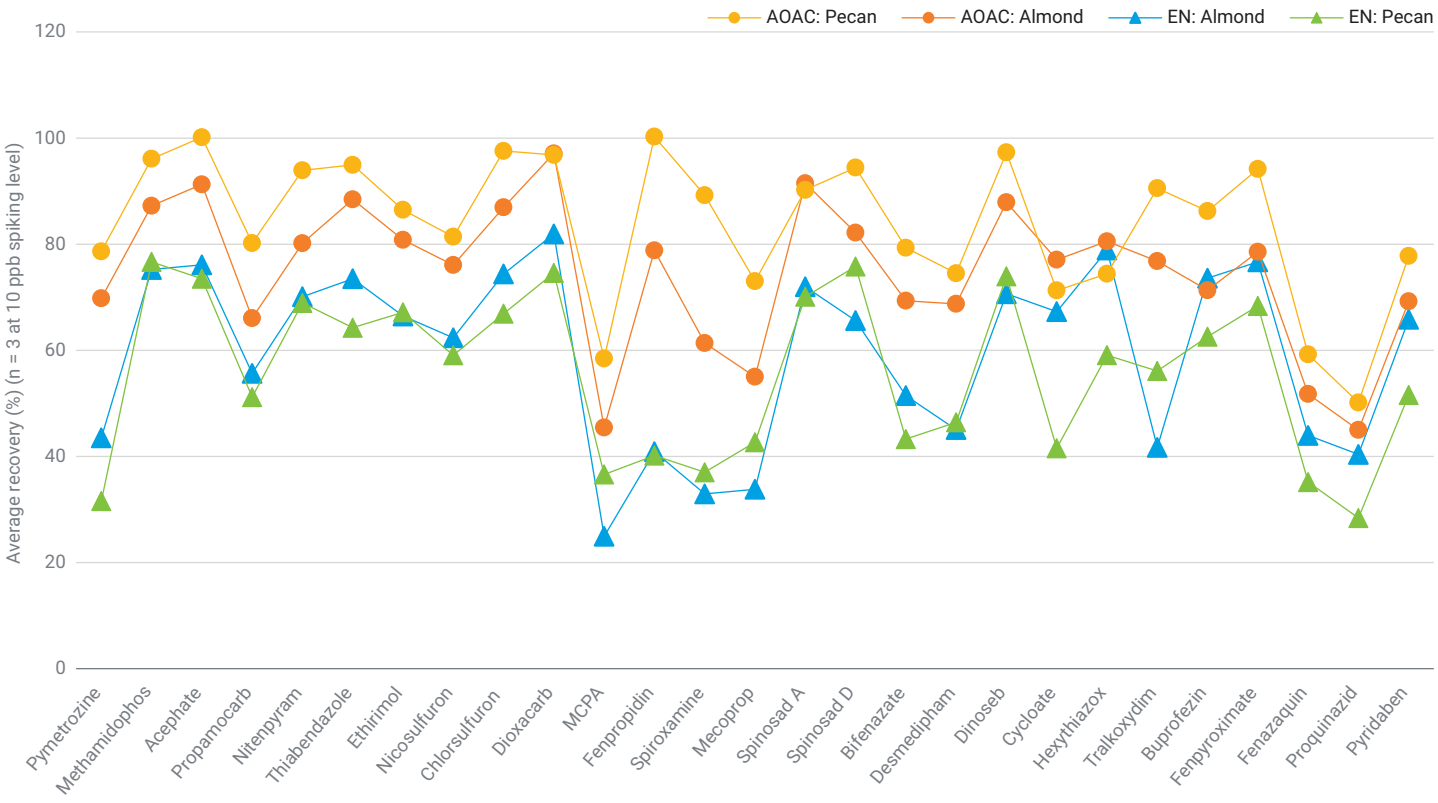

Figure 3. Agilent QuEChERS extraction method comparison: AOAC extraction versus modified EN extraction. Almond and pecan samples spiked at 20 ng/g level were used for the comparison.

For Captiva EMR–LPD cleanup, the water premixing was investigated using the following different ratios of water to crude almond extract: 0:100 (0% water addition), 10:90 (10% water addition), and 20:80 (20% water addition). The comparison was based on target recoveries and matrix removal efficiency. The matrix removal investigation using different conditions indicated a slight improvement from 0 to 10% of water addition, but then a decrease in removal from 10 to 20% of water addition. The target recovery comparison results are shown in Figure 4. The results show that: A) The addition of water and premixing with the crude extract significantly improved the recoveries of many sensitive targets. This can be attributed to the better buffering effect with water in the sample, as well as preventive interactions between water and PSA sorbent. The improved matrix removal was due to better lipid removal, provided by the Captiva EMR–Lipid sorbent with water in the sample mixture. B) Recoveries did not improve with increased water addition. For many sensitive targets, the recoveries using 20% water were not as good as those using 10% water. Matrix removal efficiency also decreased with 20% water addition. It is hypothesized that more water in the sample mixture compromised PSA sorbent interactions with acids and polar interferences, resulting in reduced matrix removal. Considering both matrix removal efficiency and target recoveries, 10% water addition and premixing was shown to be optimal for Captiva EMR–LPD cleanup.

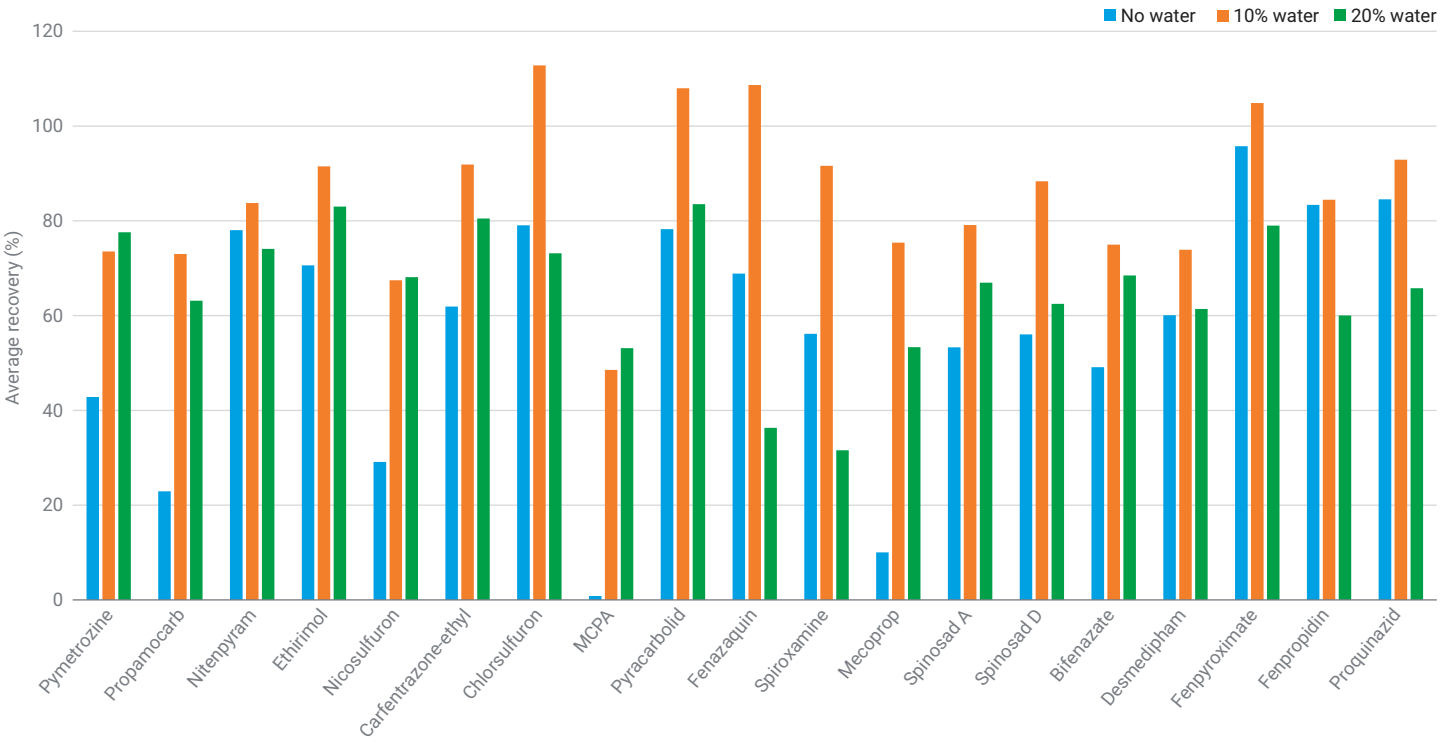

Figure 4. Optimization of water addition before Agilent Captiva EMR–LPD cleanup. Crude almond extract spiked with 10 ng/mL level was used for the comparison.

Sample matrix removal

The blended sorbents packed in Captiva EMR–LPD cartridges provided highly efficient matrix removal for all four types of tree nuts. Since tree nuts contain a high abundance of fats and other hydrophobic components, a GC/MS full scan test was used to evaluate sample cleanliness after Captiva EMR–LPD cleanup. In addition, the dried residue weight of sample extract was also used to indicate the complete matrix co-extractives, even those unable to be detected by the instrument. The evaluation results shown in Figure 5 and Table 2 demonstrate the highly efficient matrix removal provided by Captiva EMR–LPD.

Figure 5 shows the matrix background on GC/MS full scan comparison with the cleanup versus without cleanup for four tree nuts matrices. Table 2 shows the calculated matrix removal based on GC/MS full scan complete integrated peak area and dried residue weight.

Table 2. Sample matrix removal evaluation results.

	Matrix Removal	
	GC/MS FS Background	Dried Residue Weight
Almond	85%	81%
Pecan	81%	64%
Cashew	82%	82%
Hazelnut	56%	73%

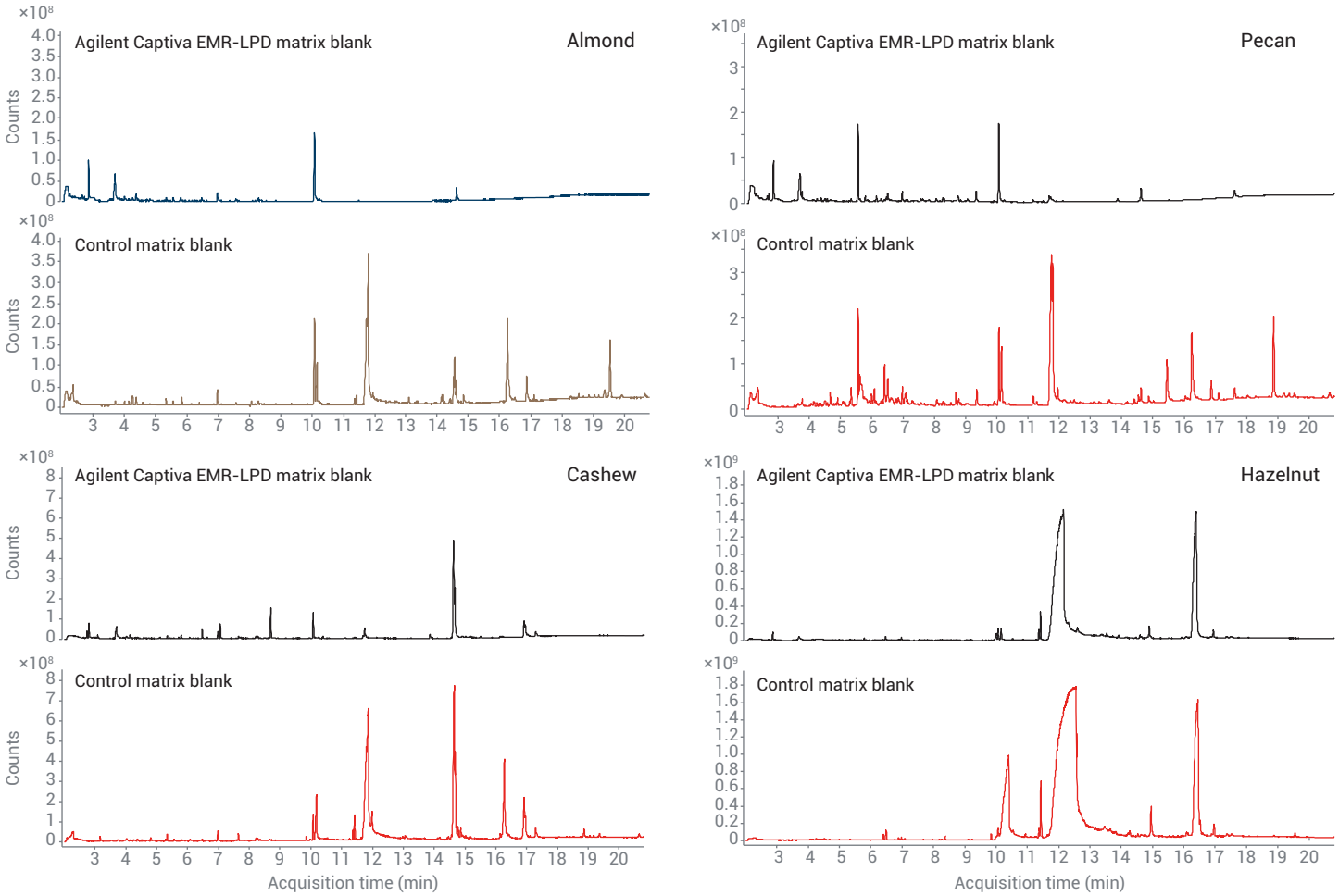

Figure 5. Tree nut matrix removal efficiency using Agilent Captiva EMR–LPD cleanup. GC/MS full scan (FS) background for each nut type, with Captiva EMR–LPD cleanup (top chromatogram) versus without cleanup (bottom chromatogram).

Method quantitation performance assessment

The method quantitation performance was evaluated by target recovery, reproducibility, and matrix effect, as well as matrix-matched calibration linearity and limits of quantitation (LOQs).

Target recovery, reproducibility, and matrix effect

These parameters are directly related to method quantitation accuracy and data quality. Therefore, it is important to use these parameters to demonstrate quantitation method performance. The SANTE/11312/2021 guideline was referred to for method performance assessment.[2] Figure 6 shows the method performance statistical results.

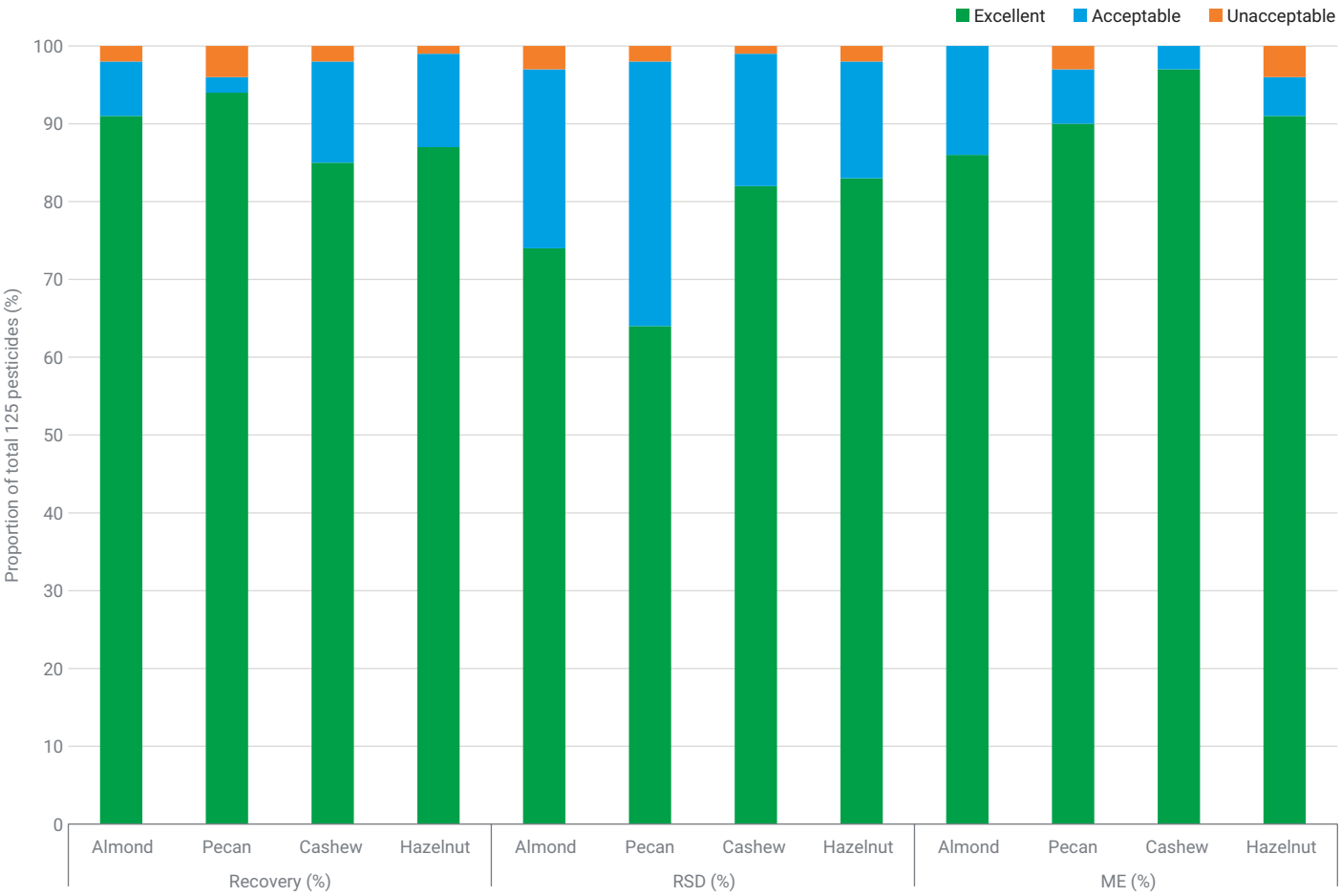

Figure 6. Method quantitation statistical results summary. The value ranges (criteria) corresponding to each category (excellent, acceptable, and unacceptable) are displayed in Table 2.

The statistical results summary for target recovery, RSD, and matrix effect is shown in Figure 6, including the percentage distribution of passed/failed targets. The pass/fail criteria for target recovery, RSD, and matrix effect analyses are shown in Table 3. Overall, >95% of the 125 pesticides achieved excellent and acceptable results for target recovery, reproducibility, and matrix effect in four types of tree nuts when using the newly developed method. For target recovery, the method provided excellent recovery results (70 to 120%) for >85% of targets in tree nuts. For target reproducibility, the method provided excellent single-digit RSDs (<10%) for >64% of targets and acceptable RSDs (<20%) for >97% of targets in tree nuts. For matrix effect, the method provided insignificant matrix effect (80 to 120%) for >86% of targets in tree nuts. For the 125 targeted pesticides in tree nuts, the average target recovery was 90% or above, the average RSD was 10.2% or less, and the average matrix effect was within 94 to 105%. The average results are shown in Figure 7.

Table 3. Category criteria for the different performance analyses.

Evaluation Parameters	Category Criteria Value Range		
	Pass		Fail
	Excellent	Acceptable	Unacceptable
Target Recovery	70 to 120%	40 to 70%	<40% or >120%
RSD	<10%	10 to 20%	>20%
Matrix Effect	80 to 120%	40 to 80% and 120 to 130%	<40% or >130%

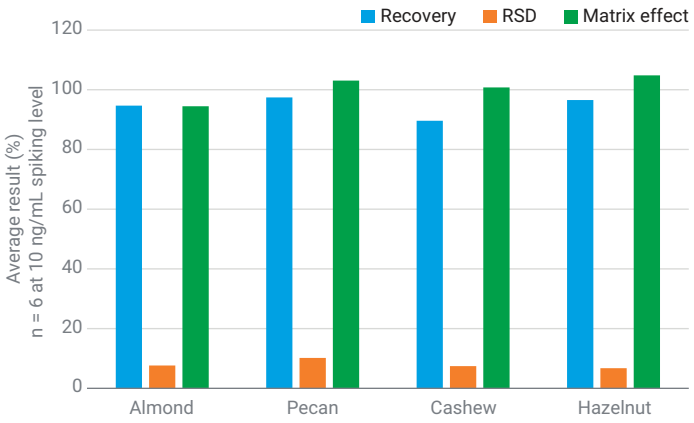

Figure 7. The average recovery, RSD, and matrix effect of 125 pesticides in four types of tree nut. Samples were tested with six replicates at a spiking level of 10 ng/mL.

Matrix-matched calibration and LOQ

Matrix-matched calibration standards were made by postspiking the standards into a final sample extract at the range of 0.5 to 500 ng/mL. Considering different dilution factors introduced during sample extraction, this corresponded to 1 to 1,000 ng/g in almond, pecan, and cashew, and 2.5 to 2,500 ng/g in hazelnut. Linear regression and $1/x^2$ weight were used for calibration curve generation, with quadratic regression or 1/x weight being used for some exceptions. The calibration dynamic range was determined based on LOQ sensitivity requirements and high concentration level alignment with the calibration curve. The results are shown in Table 4.

Table 4. Method matrix-matched calibration curve and detection limits results summary for 125 pesticides in four types of tree nuts.

	Almond			Pecan			Cashew			Hazelnut		
Target Name	LLOQ (ng/g)	HLOQ (ng/g)	R^2	LLOQ (ng/g)	HLOQ (ng/g)	R^2	LLOQ (ng/g)	HLOQ (ng/g)	R^2	LLOQ (ng/g)	HLOQ (ng/g)	R^2
Pymetrozine	1	1,000	0.9945	1	1,000	0.9965	1	1,000	0.9970	2.5	2,500	0.9956
Methamidophos	1	1,000	0.9930	1	1,000	0.9968	1	1,000	0.9970	2.5	2,500	0.9956
Acephate	1	1,000	0.9944	1	1,000	0.9963	1	1,000	0.9947	2.5	2,500	0.9929
Omethoate[2]	1	400	0.9939	1	1,000	0.9952	1	1,000	0.9900	2.5	2,500	0.9947
Aminocarb	1	1,000	0.9918	1	1,000	0.9949	1	1,000	0.9936	2.5	2,500	0.9908
Propamocarb[2]	1	400	0.9979	1	1,000	0.9949	1	1,000	0.9902	2.5	2,500	0.9916
Dinotefuran	1	1,000	0.9923	1	1,000	0.9951	1	1,000	0.9943	2.5	2,500	0.9956
Carbendazim	1	1,000	0.9970	1	1,000	0.9960	1	1,000	0.9930	2.5	2,500	0.9902
Monocrotophos	1	1,000	0.9914	1	1,000	0.9958	1	1,000	0.9924	2.5	2,500	0.9935
Nitenpyram	1	1,000	0.9940	1	1,000	0.9970	1	1,000	0.9937	2.5	2,500	0.9917
Thiabendazole	1	1,000	0.9937	1	1,000	0.9935	1	1,000	0.9917	2.5	2,500	0.9925
Fuberidazole	1	1,000	0.9937	1	1,000	0.9955	1	1,000	0.9930	2.5	2,500	0.9936
Thiamethoxam	1	1,000	0.9929	1	1,000	0.9975	1	1,000	0.9933	2.5	2,500	0.9961
Cymoxanil[2]	2	1,000	0.9929	2	1,000	0.9945	2	1,000	0.9965	5	2,500	0.9933
Mexacarbate	1	1,000	0.9958	1	1,000	0.9970	1	1,000	0.9926	2.5	2,500	0.9949
Ethirimol	1	1,000	0.9949	1	1,000	0.9959	1	1,000	0.9934	2.5	1,000	0.9914
Metamitron	1	1,000	0.9959	1	1,000	0.9931	1	1,000	0.9956	2.5	2,500	0.9959
Fenuron	1	1,000	0.9939	1	1,000	0.9944	1	1,000	0.9939	2.5	2,500	0.9922
Chloridazon	1	1,000	0.9933	1	1,000	0.9972	1	1,000	0.9969	2.5	2,500	0.9955
Imidacloprid	1	1,000	0.9973	1	1,000	0.9984	1	1,000	0.9947	2.5	2,500	0.9906
Cymiazol	1	1,000	0.9929	1	1,000	0.9945	1	1,000	0.9965	2.5	2,500	0.9933
Dimethoate	1	1,000	0.9947	1	1,000	0.9969	1	1,000	0.9920	2.5	2,500	0.9922
Fenobucarb	1	1,000	0. 9931	1	1,000	0.9931	1	1,000	0.9990	2.5	2,500	0.9906
Acetamiprid	1	1,000	0.9932	1	1,000	0.9985	1	1,000	0.9942	2.5	2,500	0.9901
Metsulfuron	1	1,000	0.99361	1	1,000	0.9940	1	1,000	0.9935	2.5	2,500	0.9940
Flumetsulam	1	1,000	0.9903	1	1,000	0.9924	1	1,000	0.9974	2.5	2,500	0.9936
Tebuthiuron	1	1,000	0.9986	1	1,000	0.9907	1	1,000	0.9978	2.5	2,500	0.9929
4-Nitrophenol[2]	2	1,000	0.9976	2	1,000	0.9915	2	1,000	0.9981	5	2,500	0.9978
Thiacloprid	1	1,000	0.9906	1	1,000	0.9935	1	1,000	0.9924	2.5	2,500	0.9905
Nicosulfuron	1	1,000	0.9946	1	1,000	0.9924	1	1,000	0.9945	2.5	2,500	0.9928
Thidiazuron	1	1,000	0.9961	1	1,000	0.9972	1	1,000	0.9955	2.5	2,500	0.9936
Secbumeton	1	1,000	0.9978	1	1,000	0.9907	1	1,000	0.9965	2.5	2,500	0.9892
Oxasulfuron	1	1,000	0.9989	1	1,000	0.9994	1	1,000	0.9938	2.5	2,500	0.9930
Bentazon[2]	2	1,000	0.9953	2	1,000	0.9975	2	1,000	0.9956	5	2,500	0.9927
Carfentrazone-ethyl	1	1,000	0.9950	1	1,000	0.9942	1	1,000	0.9980	2.5	2,500	0.9981
Imazalil	1	1,000	0.9938	1	1,000	0.9951	1	1,000	0.9945	2.5	2,500	0.9942
Lenacil[2]	2	1,000	0.9836	2	1,000	0.9919	2	1,000	0.9916	5	2,500	0.9918
Metribuzin	1	1,000	0.9946	1	1,000	0.9966	1	1,000	0.9933	2.5	2,500	0.9936
Cyazofamid[2]	2	1,000	0.9859	2	1,000	0.9936	2	1,000	0.9966	5	2,500	0.9909
Phenmedipham	1	1,000	0.99811	1	1,000	0.9924	1	1,000	0.9946	2.5	2,500	0.9867
Propoxur	1	1,000	0.9989	1	1,000	0.9950	1	1,000	0.9974	2.5	2,500	0.9916
Chlorsulfuron	1	1,000	0.9961	1	1,000	0.9969	1	1,000	0.9887	2.5	2,500	0.9965
Dioxacarb	1	1,000	0.9942	1	1,000	0.9922	1	1,000	0.9940	2.5	2,500	0.9915

Target Name	Almond			Pecan			Cashew			Hazelnut		
	LLOQ (ng/g)	HLOQ (ng/g)	R^2	LLOQ (ng/g)	HLOQ (ng/g)	R^2	LLOQ (ng/g)	HLOQ (ng/g)	R^2	LLOQ (ng/g)	HLOQ (ng/g)	R^2
Carbofuran[2]	1	1,000	0.9937	1	400	0.9948	1	1,000	0.9967	2.5	2,500	0.9903
Methabenzthiazuron[2]	1	1,000	0.9932	1	400	0.9977	1	1,000	0.9950	2.5	1,000	0.9947
MCPA	1	1,000	0.99641	1	1,000	0.99721	1	1,000	0.99571	2.5	2,500	0.9990[1]
Amidosulfuron[2]	2	1,000	0.9817	2	1,000	0.9967	1	1,000	0.9919	2.5	2,500	0.9949
Cycluron	1	1,000	0.9874	1	1,000	0.9975	1	1,000	0.9942	2.5	2,500	0.9935
Chlorotoluron	1	1,000	0.9908	1	1,000	0.9945	1	1,000	0.9993	2.5	2,500	0.9980
Flutriafol[2]	1	1,000	0.9931	1	400	0.9990	1	1,000	0.9915	2.5	1,000	0.9937
Pyracarbolid[2]	1	1,000	0.99871	1	400	0.9960	1	400	0.9964	2.5	1,000	0.9927
Fluometuron	1	1,000	0.9919	1	1,000	0.9987	1	1,000	0.9987	2.5	2,500	0.9975
Forchlorfenuron	1	1,000	0.9954	1	1,000	0.9955	1	1,000	0.9915	2.5	2,500	0.9932
Carbaryl	1	1,000	0.9955	1	1,000	0.9990	1	1,000	0.9943	2.5	2,500	0.9987
Fosthiazate	1	1,000	0.9939	1	1,000	0.9920	1	1,000	0.9942	2.5	2,500	0.9975
Azaconazole[2]	1	1,000	0.9872	1	400	0.9937	1	1,000	0.9932	2.5	1,000	0.9937
Methoprotryne[2]	1	1,000	0.9944	1	1,000	0.9941	1	400	0.9987	2.5	2,500	0.9943
DEET	1	1,000	0.9967	1	1,000	0.9927	1	1,000	0.9866	2.5	2,500	0.9925
Fenpropidin[2]	1	400	0.9903	1	1,000	0.9957	1	1,000	0.9970	2.5	2,500	0.9940
Carboxin[2]	2	1,000	0.99881	1	1,000	0.9864	1	400	0.9937	2.5	2,500	0.9891
Diuron	1	1,000	0.9960	1	400	0.9934	1	1,000	0.9908	2.5	2,500	0.9918
Spiroxamine	1	1,000	0.9944	1	1,000	0.9927	1	1,000	0.9932	2.5	2,500	0.9901
Metobromuron	1	1,000	0.9932	1	1,000	0.9984	1	1,000	0.9959	2.5	2,500	0.9923
Mecoprop	1	1,000	0.99891	1	1,000	0.99471	1	1,000	0.99891	2.5	2,500	0.9963
Dimethomorph I[2]	1	1,000	0.9903	1	400	0.9932	1	1,000	0.9877	2.5	1,000	0.9900
Dimethachlor	1	1,000	0.9939	1	1,000	0.9958	1	1,000	0.9921	2.5	2,500	0.9913
Chlorantraniliprole	1	1,000	0.9926	1	1,000	0.9942	1	1,000	0.9916	2.5	2,500	0.9895
Clomazone	1	1,000	0.9908	1	1,000	0.9960	1	1,000	0.9948	2.5	2,500	0.9926
Dimethomorph II[2]	2	1,000	0.9915	1	1,000	0.9981	1	1,000	0.9921	2.5	2,500	0.9928
Cyproconazole	1	1,000	0.9911	1	1,000	0.9938	1	1,000	0.9943	2.5	2,500	0.9933
Furalaxyl	1	1,000	0.9908	1	1,000	0.9930	1	1,000	0.9955	2.5	2,500	0.9961
Chloroxuron	1	1,000	0.9981	1	1,000	0.9970	1	1,000	0.9961	2.5	2,500	0.9901
Spinosad A	1	1,000	0.9938	1	1,000	0.9928	1	1,000	0.9986	2.5	2,500	0.9917
Linuron2	1	1,000	0.9931	1	400	0.9926	1	1,000	0.9975	2.5	1,000	0.9946
Iprovalicarb	1	1,000	0.9917	1	1,000	0.9966	1	1,000	0.9929	2.5	2,500	0.9954
Halofenozide [2]	1	1,000	0.9975	1	1,000	0.9900	2	1,000	0.9968	2.5	2,500	0.9884
Pyridat[2]	1	1,000	0.9856	2	1,000	0.9955	1	1,000	0.9856	5	2,500	0.9955
Fenamiphos	1	1,000	0.9815	1	1,000	0.9908	1	1,000	0.9947	2.5	2,500	0.9920
Promecarb	1	1,000	0.9936	1	1,000	0.9985	1	1,000	0.9977	2.5	2,500	0.9873
Myclobutanil	1	1,000	0.9918	1	1,000	0.9918	1	1,000	0.9971	2.5	2,500	0.9914
Azoxystrobin	1	1,000	0.9986	1	1,000	0.9978	1	1,000	0.9914	2.5	2,500	0.9905
Mandipropamid	1	1,000	0.9924	1	1,000	0.9954	1	1,000	0.9958	2.5	2,500	0.9918
Fenamidone	1	1,000	0.9953	1	400	0.9963	1	1,000	0.9976	2.5	1,000	0.9921
Boscalid	1	1,000	0.9905	1	1,000	0.9907	1	1,000	0.9948	2.5	2,500	0.9952
Spinosad D	1	1,000	0.9970	1	1,000	0.9923	1	1,000	0.9917	2.5	2,500	0.9938
Fluopicolide	1	1,000	0.9905	1	1,000	0.9908	1	400	0.9924	2.5	2,500	0.9881
Isoxaben	1	1,000	0.9981	1	1,000	0.9858	1	1,000	0.9920	2.5	2,500	0.9910

Target Name	Almond			Pecan			Cashew			Hazelnut		
	LLOQ (ng/g)	HLOQ (ng/g)	R^2	LLOQ (ng/g)	HLOQ (ng/g)	R^2	LLOQ (ng/g)	HLOQ (ng/g)	R^2	LLOQ (ng/g)	HLOQ (ng/g)	R^2
Bifenazate	1	1,000	0.9984	1	1,000	0.9841	1	1,000	0.9948	2.5	2,500	0.9951
Desmedipham	1	1,000	0.9978	1	1,000	0.9913	1	1,000	0.9930	2.5	2,500	0.9962
Diflubenzuron[2]	2	1,000	0.9929	1	1,000	0.9933	2	1,000	0.9916	2.5	2,500	0.9915
Penconazole	1	1,000	0.9914	1	1,000	0.9927	1	1,000	0.9928	2.5	1,000	0.9910
Prochloraz[2]	2	1,000	0.9933	1	1,000	0.9942	2	1,000	0.9907	2.5	2,500	0.9908
Fluoxastrobin	1	1,000	0.9897	1	1,000	0.9911	1	1,000	0.9917	2.5	2,500	0.9929
Isoprothiolane	1	1,000	0.9956	1	1,000	0.9931	1	1,000	0.9949	2.5	2,500	0.9922
Rotenone	1	1,000	0.9967	1	1,000	0.9924	1	1,000	0.9944	2.5	2,500	0.9922
Flufenacet	1	1,000	0.9938	1	1,000	0.9921	1	1,000	0.9960	2.5	2,500	0.9949
Dimoxystrobin	1	1,000	0.9917	1	400	0.9961	1	1,000	0.9940	2.5	2,500	0.9923
Cyprodinil[2]	1	400	0.9953	1	1,000	0.9940	1	400	0.9920	2.5	2,500	0.9954[1]
Moxidectin[2,3]	2	1,000	0.9980	2	1,000	0.9875	2	1,000	0.9915	50	2,500	0.9900
Azinphos-ethyl[2,3]	10	1,000	0.9907	2	1,000	0.9986	10	1,000	0.9861	5	2,500	0.9986
Tebufenozide	1	1,000	0.9995	1	1,000	0.9939	1	1,000	0.9910	2.5	2,500	0.9962
Flubendiamide	1	1,000	0.9935	1	1,000	0.9910	1	1,000	0.9924	2.5	2,500	0.9912
Beflubutamid	1	1,000	0.9982	1	1,000	0.9940	1	1,000	0.9942	2.5	2,500	0.9940
Dinoseb[2]	2	1,000	0.9969	2	1,000	0.9931	2	1,000	0.9940	5	2,500	0.9989
Kresoxim-methyl	1	1,000	0.9954	1	1,000	0.9916	1	1,000	0.9920	2.5	2,500	0.9912
Picoxystrobin[2]	1	1,000	0.9918	1	400	0.9964	1	1,000	0.9953	2.5	2,500	0.9944
Pyraclostrobin	1	1,000	0.9929	1	1,000	0.9934	1	1,000	0.9951	2.5	2,500	0.9916
Isofenphos-methyl	1	1,000	0.9905	1	1,000	0.9940	1	1,000	0.9947	2.5	2,500	0.9916
Diflufenican	1	1,000	0.9983	1	1,000	0.9932	1	1,000	0.9990	2.5	2,500	0.9980
Trifloxystrobin	1	1,000	0.9946	1	1,000	0.9930	1	1,000	0.9967	2.5	2,500	0.9932
Metrafenone	1	1,000	0.9924	1	1,000	0.9940	1	1,000	0.9947	2.5	2,500	0.9918
Cycloate[3]	20	1,000	0.9977	2	1,000	0.9903	10	1,000	0.9912	5	2,500	0.9924
Metaflumizone[2]	10	1,000	0.9974	2	1,000	0.9940	2	1,000	0.9940	5	2,500	0.9939
Fluazinam[2]	2	1,000	0.9944	2	1,000	0.9825	2	1,000	0.9972	5	2,500	0.9915
Temephos	1	1,000	0.9944	1	1,000	0.9936	1	1,000	0.9968	2.5	400	0.9934
Pyriproxyfen[2]	1	1,000	0.9934	1	400	0.9967	1	400	0.9993	2.5	1,000	0.9914
Hexythiazox	1	1,000	0.9970	1	1,000	0.9917	1	1,000	0.9959	2.5	2,500	0.9932
Tralkoxydim	1	1,000	0.9941	1	1,000	0.9948	1	1,000	0.9930	2.5	2,500	0.9956
Buprofezin2	1	1,000	0.9968	1	400	0.9952	1	1,000	0.9983	2.5	2,500	0.9933
Fenpyroximate[2]	2	1,000	0.9903	1	400	0.9966	1	1,000	0.9903	2.5	1,000	0.9930
Fenazaquin[2]	1	1,000	0.9918	1	400	0.9963	1	1,000	0.9952	2.5	1,000	0.9911
Proquinazid	1	1,000	0.9947	1	1,000	0.9929	1	1,000	0.9995	2.5	2,500	0.9962[1]
Pyridaben	1	1,000	0.9958	1	1,000	0.9957	1	1,000	0.9994	2.5	2,500	0.9829
Spirodiclofen[3]	10	1,000	0.99881	10	1,000	0.9938	10	1,000	0.9961	50	2,500	0.9907

[1] Quadratic regression fit.

[2] Modified dynamic calibration range either due to analyte sensitivity or selectivity at the low end in the matrix or failure of acceptance criteria at the high end.

[3] Raised LLOQ due to the positive contribution from matrix.

Conclusion

A simple, rapid, and reliable method using Agilent Bond Elut QuEChERS AOAC extraction followed by Agilent Captiva EMR–LPD cartridge passthrough cleanup was developed and verified for 125 LC-amenable pesticides in tree nuts by LC/MS/MS. The novel Captiva EMR–LPD cleanup method provides convenient and simplified sample passthrough cleanup, selective and efficient matrix removal from fatty tree nuts, and acceptable pesticide recovery, reproducibility, and matrix effect. The quantitation results demonstrated that a >95% pass rate was achieved for target recovery, RSD, and matrix effect, according to SANTE guidelines. Compared to Agilent Captiva EMR–Lipid cleanup, Captiva EMR–LPD cleanup improved complete lipid removal, especially for sample matrices enriched with fatty acids. The use of acidified ACN extraction and 10% water addition and premixing before Captiva EMR–LPD cleanup effectively prevented the loss of sensitive pesticides, due to unwanted interactions with sorbents such as PSA. Also, the complete sample preparation workflow is simple, saving time and effort, and eventually improving lab productivity.

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