

# Quantitation of Over 300 Pesticide Residues in Three Processed Cocoa Matrices

Using an Agilent 6475 triple quadrupole LC/MS

## Authors

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## Abstract

Analysis of over 300 pesticide residues in three cocoa matrices (powder, liquor, and butter) was accomplished through sample extraction with the Agilent Bond Elut QuEChERS AOAC extraction kit, followed by cleanup with the Agilent Captiva Enhanced Matrix Removal-General Pigmented Dry (EMR-GPD), and LC/MS/MS analysis. Samples used in the study were obtained from Malaysia, where an initiative to ramp up cocoa processing capacity through modern practices was launched in 2024. Sustainability, including safety assurance for the detection of pesticides residues, is part of the practices. This comprehensive analysis covers the measurement of a wide range of pesticides in multiple matrices to accelerate routine laboratory testing. Excellent recovery was achieved with > 90% of pesticides exhibiting 70–120% recovery at low (2 ng/mL) and medium (10 ng/mL) spiking concentration. Method precision was also proven with > 93% pesticides showing < 10% RSD in three cocoa matrices.

## Introduction

Despite low bean production locally, Malaysia ranks among the world's top cocoa grinders, transforming raw beans into powder, liquor, and butter (Malaysian Cocoa Board, 2023). These value-added products are major contributors to the country's exports. Traces of contamination, including pesticide residues, have been spotted in processed cocoa beans, necessitating thorough inspection.

Processed cocoa beans pose complicated challenges for sample extraction and matrix removal. Cocoa liquor is in the form of pure, ground paste made using roasted cocoa beans, which when pressed, separate into fat (cocoa butter) and solids (cocoa powder). These matrices, the most common forms of processed cocoa beans, are used to conduct a comprehensive screening of pesticide residues.

Extraction and cleanup methods were adopted from a cinnamon workflow<sup>1</sup>, as cinnamon and cocoa beans exhibit similarities in terms of their complex, dry matrices. Due to their dark brown color, pigment removal is essential for the sample preparation of processed cocoa beans. The Agilent Bond Elut QuEChERS AOAC extraction kit was implemented for sample extraction and the Agilent Captiva EMR-GPD was used for highly selective and efficient cleanup. The cartridge was packed with the following sorbents: Carbon S for pigment removal, primary secondary amine (PSA) for fatty acid removal, and C18 for additional hydrophobic matrix removal.

## Experimental

### Reagents and chemicals

Mixed stock solution pesticide standards (part number 5190-0551), 10 Agilent ULTRA custom standard mixes, and LC/MS-grade acetonitrile (part number 5191-5101) were obtained from Agilent Technologies. LC/MS-grade formic acid was obtained from Honeywell Fluka (Michigan, USA). Acetic acid was procured from Sigma-Aldrich (Missouri, USA).

### Standard and sample preparation

Spiking solution was prepared at 1 and 5 µg/mL from mixed stock solutions in 100% acetonitrile and stored at -20 °C prior to the evaluation of recovery. A matrix-matched calibration set was made up by spiking matrix blank with mixed standards to the concentration of 0.5, 2, 5, 10, and 50 ng/mL. Figure 1 shows sample preparation, which has been adopted from a previously published application note.<sup>1</sup>

### Equipment and materials

Other equipment and materials used for sample preparation are as follows:

- VWR DVX-2500 Multi-Tube Vortexer (Massachusetts, USA)
- 2010 Geno/Grinder (California, USA)
- Eppendorf Centrifuge 5804R (Leipzig, Germany)
- Agilent positive pressure manifold 48 processors (PPM-48) (part number 5191-4101)
- Eppendorf Centrifuge 5430R (Leipzig, Germany)
- Agilent Bond Elut QuEChERS AOAC extraction kit (part number 5982-5755)
- Agilent Captiva EMR-GPD cartridge, 6 mL (part number 5610-2091)
- Agilent Bond Elut QuEChERS EMR-Lipid polish pouch, 3.5 g anhydrous MgSO<sub>4</sub> (part number 5982-0102)
- Ceramic homogenizers, 50 mL tubes, 100/pk (part number 5982-9313)

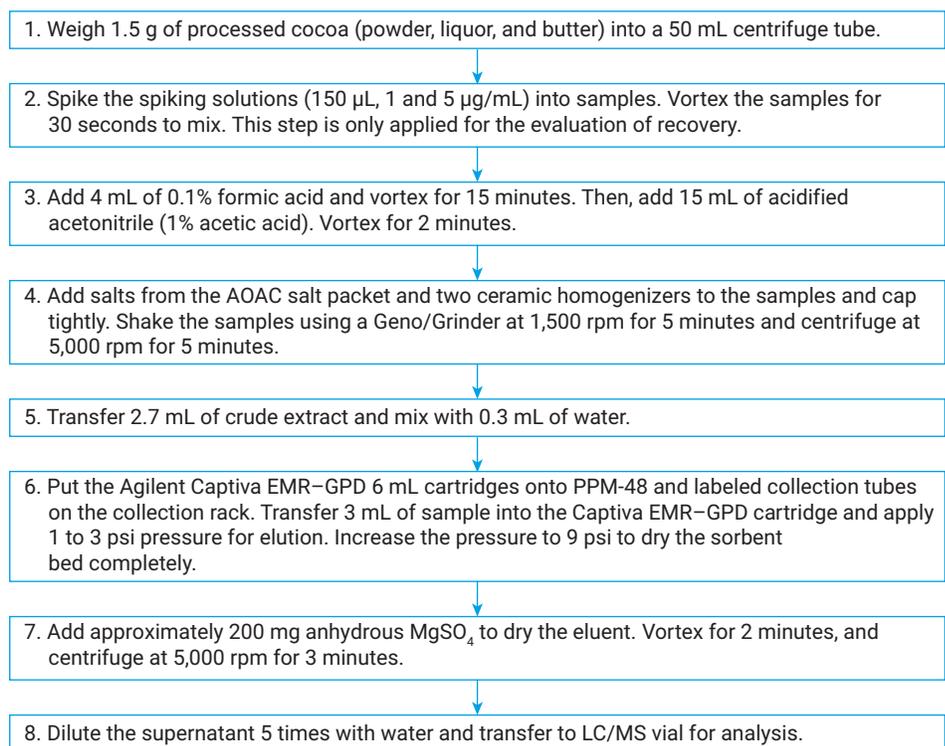


Figure 1. Sample preparation protocol.

## Method

The optimized dynamic multiple reaction monitoring (dMRM) transitions for 308 analytes were obtained from a previously published application note.<sup>2</sup> Data were processed using the Agilent MassHunter qualitative and quantitative analysis software version 12. Tables 1 and 2 list the LC/MS conditions.

## Results and discussion

The Bond Elut QuEChERS AOAC extraction kit provides prepackaged buffer salts for quick and hassle-free pesticide extraction. This kit has been proven with high recovery rates for fruits and vegetables. Therefore, it was used for the extraction of processed cocoa beans. Cocoa powder and liquor are considered to have high pigment content and are dark brown in color. Cocoa butter is a pale-yellow, edible fat that is obtained after pressing cocoa beans. The Captiva EMR–GPD cartridge is therefore deemed suitable for sample cleanup.

## Instrumentation

Liquid Chromatography System	
Module	Part Number
Agilent 1290 Infinity III High-Speed Pump	G7120A
Agilent 1290 Infinity III Multisampler	G7167B
Agilent 1290 Infinity III Multicolumn Thermostat	G7116B
Agilent InfinityLab Assist Hub	G7180A

Mass Spectrometry System	
Module	Part Number
Agilent 6475 Triple Quadrupole LC/MS	G6475AA
Agilent Jet Stream Technology Ion Source (AJS)	

**Table 1.** Agilent 1290 Infinity III LC parameters.

Parameter	Description										
Column	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 150 mm, 1.8 μm (part number 959759-902)										
Column Temperature	40 °C										
Mobile Phase	A) 5 mM ammonium formate + 0.1 % formic acid B) 5 mM ammonium formate + 0.1 % methanol										
Flow Rate	0.4 mL/min										
Gradient Program	<table border="1"><thead><tr><th>Time (min)</th><th>%B</th></tr></thead><tbody><tr><td>0</td><td>5</td></tr><tr><td>3</td><td>30</td></tr><tr><td>17</td><td>100</td></tr><tr><td>20</td><td>100</td></tr></tbody></table>	Time (min)	%B	0	5	3	30	17	100	20	100
Time (min)	%B										
0	5										
3	30										
17	100										
20	100										
Post Time	3 minutes										
Injection Volume	2 μL										

**Table 2.** Agilent 6475 triple quadrupole LC/MS and source parameters.

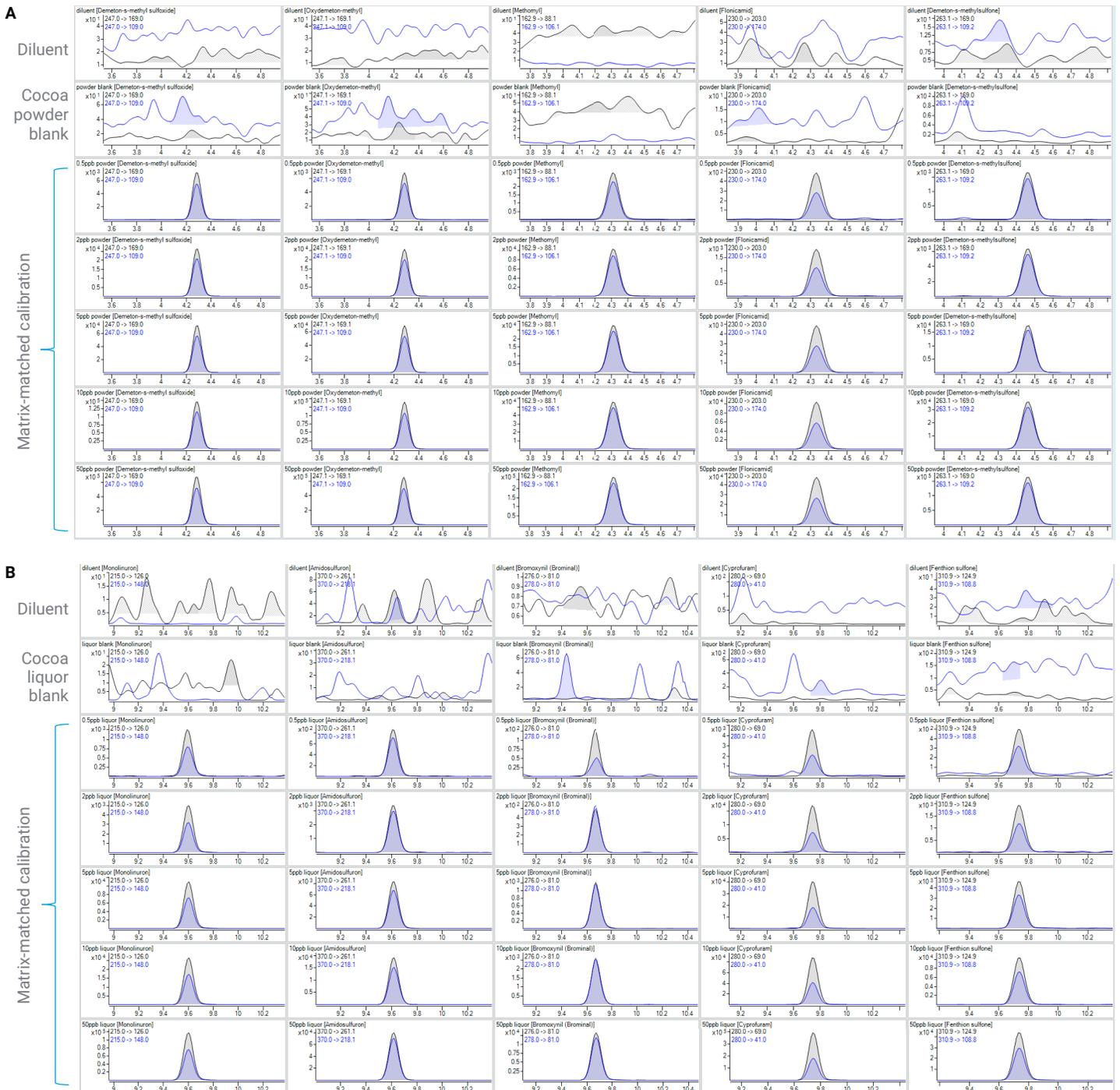
Parameter	Description
Drying Gas Temperature	200 °C
Drying Gas Flow	9 L/min
Sheath Gas Temperature	400 °C
Sheath Gas Flow	12 L/min
Nebulizer	35 psi
Capillary Voltage	2,500 V (+), 3,000 V (-)
Nozzle Voltage	0 V (+/-)
Measurement Mode and Polarity	dMRM, positive and negative

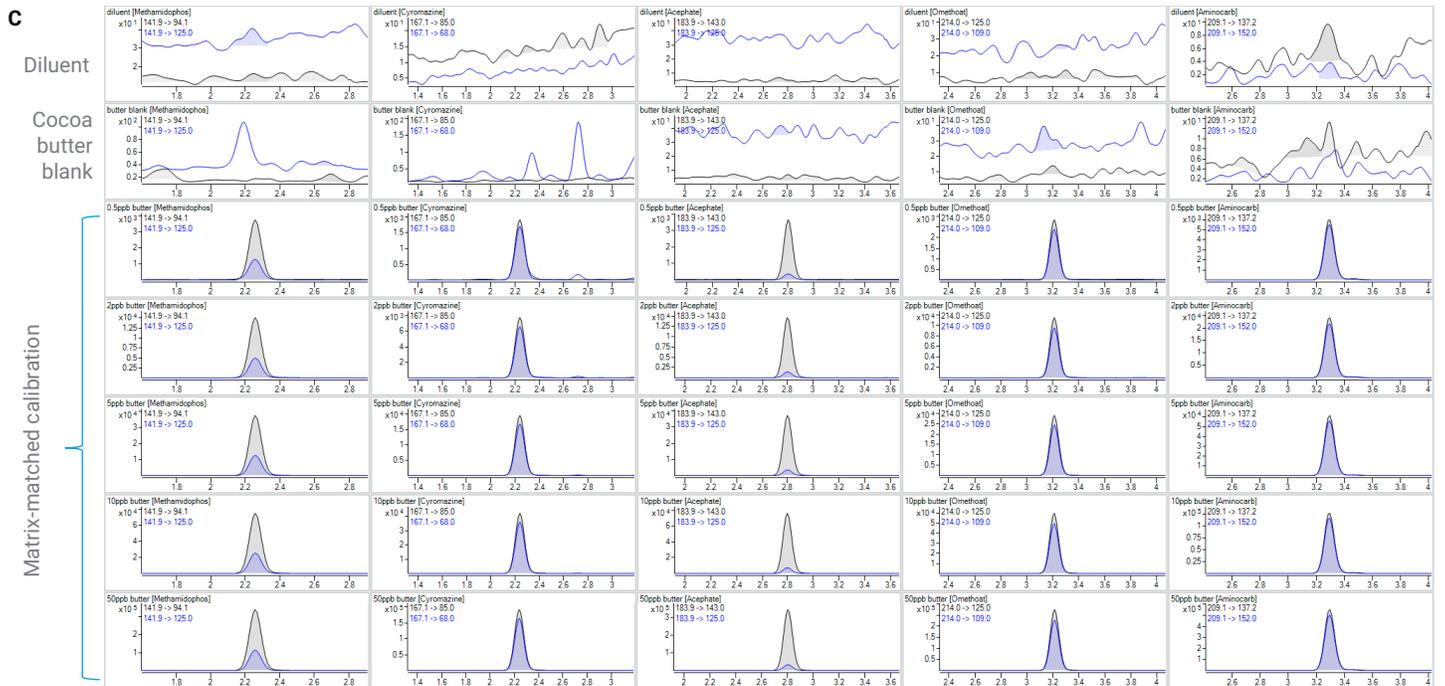
## Carryover and matrix interference

Pesticide screening analysis was verified based on the following criteria: carryover and matrix interference, linearity, precision, and extraction recovery.

Carryover and matrix interference were evaluated in diluent (50% acetonitrile) and cocoa matrix blank. No carryover or significant matrix or MRM transitions interference was observed for the 308 target pesticides (Figures 2A to 2C).

This confirms the effectiveness of sample cleanup and analytical methods to minimize unwanted interaction with matrix or analytes.

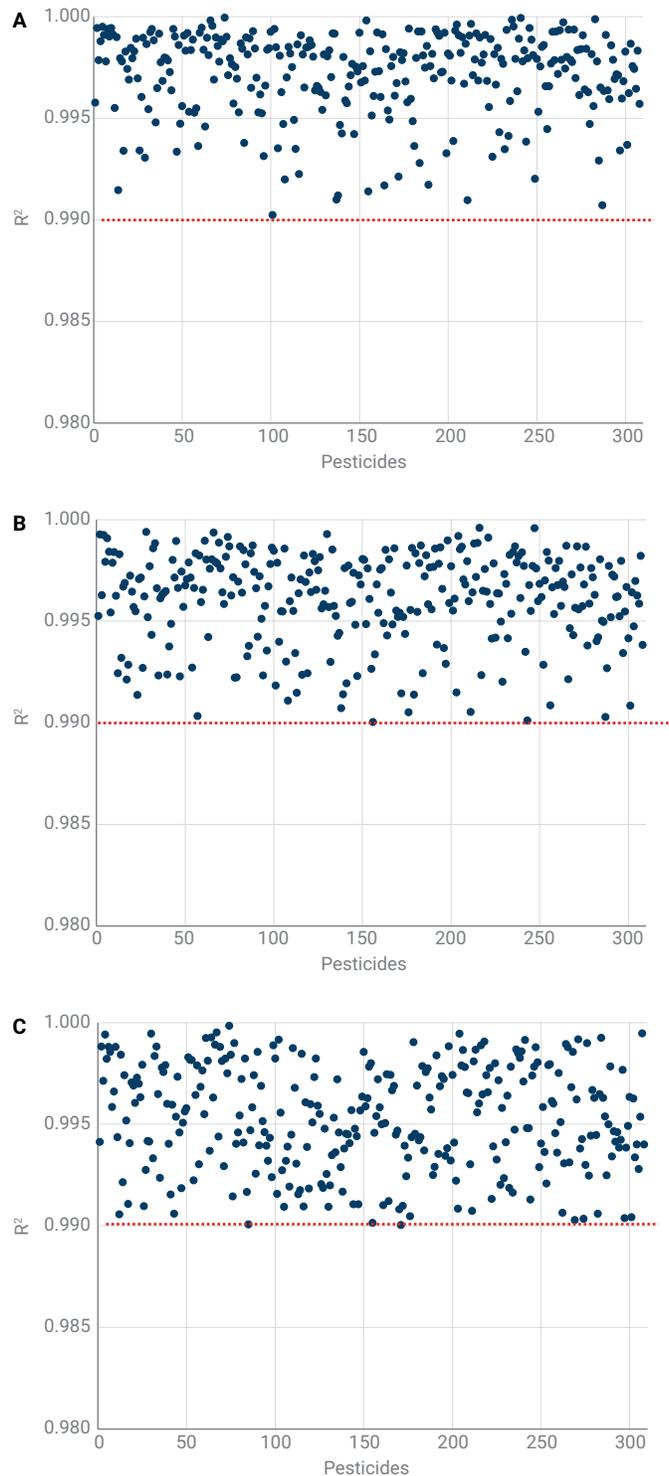




**Figure 2.** Evaluation of carryover and matrix interference in a few target pesticides in (A) cocoa powder, (B) cocoa liquor, and (C) cocoa butter.

### Linearity

The calibration set was prepared by spiking mixed standards into the cocoa matrix blank at five different concentrations. All 308 pesticides were successfully detected at the lowest spiking concentration—0.5 ng/mL—each exhibiting a signal-to-noise ratio (S/N) exceeding 10. Accordingly, 0.5 ng/mL was designated as the method limit of quantitation (LOQ). All pesticide residues demonstrated excellent linearity, with  $R^2$  values  $\geq 0.990$  (Figures 3A to 3C). The  $R^2$  values observed in cocoa butter were broadly distributed between 0.990 and 1.0. In cocoa liquor, the values shifted toward the upper end of this range. Cocoa powder exhibited the highest degree of linearity, with most pesticides achieving  $R^2$  values  $\geq 0.995$  (87.9% of total pesticides). In comparison, 59.7% and 77.3% of total pesticides showed  $R^2$  values  $\geq 0.995$  in cocoa butter and liquor, respectively.



**Figure 3.**  $R^2$  distribution of calibration curves for 308 pesticides in (A) cocoa powder, (B) cocoa liquor, and (C) cocoa butter.

### Method precision

Method precision was assessed by examining recovery repeatability (RSD) derived from the variation in recovery values across six replicates at 2 ng/mL. As illustrated in Figure 4, a vast number of pesticide residues showed RSD values < 20% in all cocoa matrices. Notably, 296 pesticides (96.1%) in cocoa powder, 291 pesticides (94.5%) in cocoa liquor, and 287 pesticides (93.2%) in cocoa butter exhibited RSD values < 10%.

### Extraction recovery

Extraction recovery was evaluated at two spiking concentrations, 2 and 10 ng/mL (corresponding to 150 µL additions from the initial stock solutions of 1 and 5 µg/mL). Spiking was carried out both before and after the extraction procedure. Recovery was calculated from the analyte response ratios obtained by comparing pre-extraction spiked samples with post-extraction spiked samples. More than 93.8% of the pesticides—289 compounds—achieved recoveries within 70–120% in cocoa powder and cocoa liquor (Figure 5). Cocoa butter showed slightly fewer pesticides within this range, with 276 pesticides (90%) meeting the criterion. In addition, compared to cocoa powder and cocoa liquor, cocoa butter exhibited a higher number of pesticides with recoveries below 70% (32 or 10.4% pesticides at 2 ng/mL and 27 or 8.7% pesticides at 10 ng/mL). This observation is consistent with the broader and more scattered linearity ( $R^2$ ) distribution seen in cocoa butter, suggesting its higher lipid content and more complex matrix influence extraction recovery. Despite these complexities, the use of Captiva EMR–GPD in the cocoa butter workflow delivered encouraging cleanup performance.

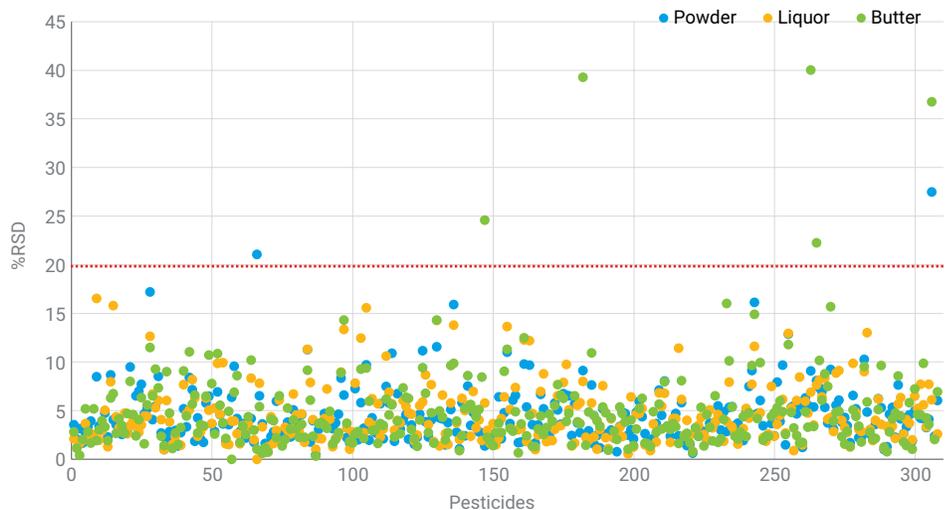


Figure 4. RSD of recovery rates at 2 ng/mL.

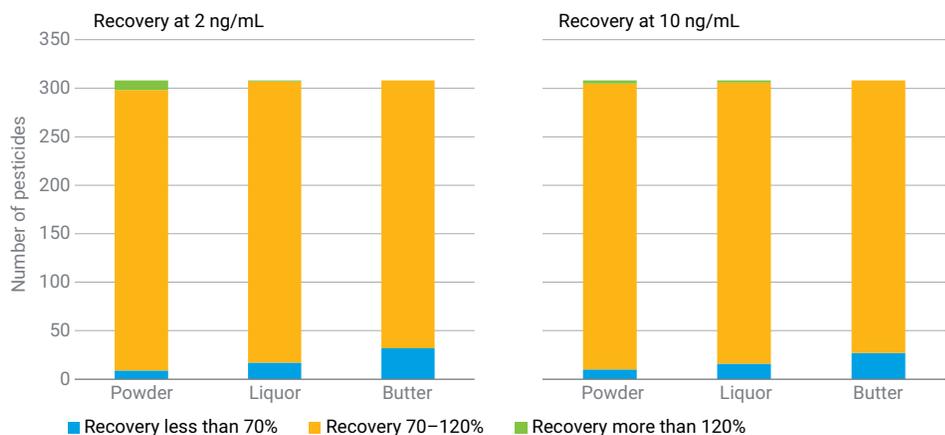


Figure 5. Recovery rates at two spiking concentrations for cocoa powder, liquor, and butter.

Across all matrices, a greater number of pesticides fell outside the 70–120% recovery range at the lower spiking concentration (2 ng/mL). As expected, recoveries improved at 10 ng/mL spiking concentration, with more pesticides meeting the 70–120% threshold in each cocoa matrix. Overall, the combination of the QuEChERS AOAC extraction kit and the Captiva EMR–GPD cartridge provided effective pesticide extraction and matrix cleanup for processed cocoa samples.

## Conclusion

Sensitive and robust analytical methods for screening over 300 pesticide residues in three processed cocoa matrices (powder, liquor, and butter) were developed using the Agilent 6475 triple quadrupole LC/MS. A fast and reliable extraction and passthrough cleanup method using the Agilent Bond Elut QuEChERS AOAC extraction kit and the Agilent Captiva EMR–GPD cartridge was evaluated for different cocoa matrices. A combination of effective extraction, selective matrix removal, and sensitive analytical measurement provides a comprehensive end-to-end solution for the downstream cocoa industry for screening pesticide residues in processed cocoa matrices.

## References

1. Zhao, L.; Andrianova, A. Determination of Over 300 Pesticides in Cinnamon Using Captiva EMR–GPD Passthrough Cleanup and LC/MS/MS and GC/MS/MS Detection. *Agilent Technologies application note*, publication number 5994-5671EN, **2023**.
2. Zou, A.; Pillai, S.; Kornas, P.; Schober, M.; Zhao, L.; Zanotti, M.; Gan, C. S. Comprehensive LC/MS/MS Workflow of Pesticide Residues in Food Using the Agilent 6470 Triple Quadrupole LC/MS System. *Agilent Technologies application note*, publication number 5994-2370EN, **2020**.

## Appendix

### List of 308 pesticide residues used in the study

2-(1-Naphthyl)acetamide	Chloroxuron	N-(Dimethylsulfamoyl)aniline (DMSA)
Acephate	Chlorpyrifos (Dursban)	N,N-dimethyl-N'-p-tolylsulfamide (DMST)
Acetamiprid	Chlorsulfuron	Ediphenphos
Aldicarb	Cinidon-ethyl	Ethyl p-nitrophenyl phenylphosphorothioate (EPN)
Aldicarb sulfone	Cinosulfuron	Epoxyconazole
Allidochlor	Clethodim	Ethiofencarb
Ametryn	Climbazole	Ethiofencarb sulfone
Amidosulfuron	Clodinafop-propargyl	Ethiofencarb sulfoxide
Aminocarb	Clomazone	Ethiprole
Atraton	Clothianidin	Ethirimol
Atrazine	Coumaphos	Ethofumesate
Azaconazole	Cyantraniliprole	Ethoprophos (Ethoprop)
Azamethiphos	Cyazofamid	Ethoxysulfuron
Azimsulfuron	Cycluron	Etofenprox
Azinphos-methyl (Guthion)	Cymiazol	Famphur (Famophos)
Azoxystrobin	Cymoxanil	Fenamidone
Beflubutamid	Cyproconazole	Fenamiphos
Benalaxyl	Cyprodinil	Fenamiphos sulfone
Bendiocarb	Cyprofuram	Fenamiphos sulfoxide
Benoxacor	Cyromazine	Fenbuconazole
Bensulfuron-methyl	Cythioate	Fenchlorazole-ethyl
Benthiavalicarb-isopropyl	Diethyltoluamide (DEET)	Fenchlorphos-oxon
Bifenazate	Demeton-S-methyl-sulfoxide	Fenfuram
Bispyribac	Demeton-S-methylsulfone	Fenhexamid
Boscalid (Nicobifen)	Desmedipham	Fenobucarb
Bromacil	Desmetryn	Fenoxaprop-ethyl
Bromfeninfos	Diazinon	Fenoxycarb
Bromoxynil (Brominal)	Dichlorvos	Fenpropimorph
Bromuconazole	Dicrotophos	Fensulfothion
Bupirimate	Diethofencarb	Fensulfothion oxon
Butafenacil	Difenoconazole	Fensulfothion oxon sulfone
Butocarboxim sulfoxide	Difenoaxuron	Fensulfothion
Butoxycarboxim	Diflubenzuron	Fenthion
Butralin	Dimefuron	Fenthion oxon
Buturon	Dimethachlor	Fenthion sulfone
Cadusafos	Dimethenamid	Fenthion sulfoxide
Carbaryl	Dimethoate	Fenuron
Carbetamide	Dimethomorph	Fipronil
Carbofuran	Dimethylvinphos	Flamprop-isopropyl
Carfentrazone-ethyl	Dimoxystrobin	Flazasulfuron
Carpropamid	Dinotefuran	Fonicamid
Chlorantraniliprole	Dioxacarb	Florasulam
Chlorbromuron	Diphenamid	Fluazinam
Chlorfenvinphos	Dipropetryn	Flubendiamide
Chloridazon (Pyrazon)	Disulfoton sulfone	
Chlorotoluron	Disulfoton sulfoxide	

Flufenacet
Flumetsulam
Fluopicolide
Fluopyram
Flurprimidol
Flurtamone
Flusilazole
Flutolanil
Flutriafol
Foramsulfuron
Forchlorfenuron
Formetanate
Fosthiazate
Fuberidazole
Furalaxyl
Furathiocarb
Furmecyclox
Halofenozide
Halosulfuron-methyl
Haloxypop-methyl
Heptenophos
Hexaconazole
Hexazinone
Imazamethabenz methyl (para)
Imazosulfuron
Imidacloprid
Ioxynil
Isazofos
Isocarbamide
Isocarbophos
Isomethiozin
Isoprocarb
Isoprothiolane
Isoproturon
Isoxaben
Isoxadifen-ethyl
Isoxaflutole
Karbutylate
Kresoxim-methyl
Lenacil
Linuron
Malaoxon
Mandipropamid

Mecarbam
Mefenacet
Mepanipyrim
Mephosfolan
Mepronil
Mesosulfuron-methyl
Metalaxyl
Metamitron
Metazachlor
Metconazole
Methabenzthiazuron
Methamidophos
Methiocarb
Methiocarb sulfoxide
Methomyl
Methoprotryne
Metobromuron
Metolachlor
Metolcarb
Metosulam
Metoxuron
Metrafenone
Metribuzin
Metsulfuron-methyl
Mevinphos
Mexacarbate
Monocrotophos
Monolinuron
Monuron
Myclobutanil
Napropamide
Neburon
Nicosulfuron
Nitenpyram
Norflurazon
Ofurace
Omethoate
Orbencarb
Oxamyl
Oxasulfuron
Oxycarboxin
Oxydemeton-methyl
Paclobutrazol

Paraoxon
Paraoxon methyl
Penconazole
Pencycuron
Pethoxamid
Phenmedipham
Phenthoate
Phorate sulfone
Phorate sulfoxide
Phosmet
Phosphamidon
Picoxystrobin
Piperophos
Pirimicarb
Pirimicarb-desmethyl
Pirimicarb-desmethyl-formamido
Pirimifos-ethyl
Pirimiphos methyl
Pirimiphos-methyl-N-desethyl
Prochloraz
Procymidone
Profenofos
Profoxydim
Promecarb
Prometon
Prometryn
Pronamide
Propachlor
Propanil
Propaphos
Propiconazole
Propoxur
Propoxycarbazone-sodium
Propyzamide
Prosulfocarb
Prothioconazole-desthio
Pymetrozine
Pyracarbolid
Pyraclostrobin
Pyridafenthion
Pyridalyl
Pyrifenox
Pyrimethanil

Quinalphos
Quinoclamine
Quinoxyfen
Quizalofop-ethyl
Rotenone
Secbumeton
Sethoxydim
Siduron
Silthiofam
Simazine
Simetryn
Sulfodiazol (Ethidimuron)
Sulfotep
Tebuconazole
Tebutam
Tebuthiuron
Tepraloxymid
Terbufos sulfone
Terbufos sulfoxide
Terbutylazine
Terbutylazine-desethyl
Tetrachlorvinphos (Dietreen)
Tetraconazole
Thiacloprid
Thiazafluron
Thifensulfuron-methyl
Thiodicarb
Tiocarbazil
Tralkoxydim
Triadimefon
Triazamate
Triazophos
Tribenuron-methyl
Trichlorfon
Trietazine
Trifloxystrobin
Trifloxysulfuron
Triflusaluron-methyl
Trimethacarb
Triticonazole
Uniconazole-P
Vamidothion
Zoxamide

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