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Multi-residue HPLC-UV method validation for pesticide analysis in agricultural produce

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Abstract

Pesticide residues in fresh produce are a major food safety concern globally, with regulatory Maximum Residue Levels (MRLs) typically in the range 0.01-50 mg/kg under EU Regulation 396/2005 and Codex Alimentarius standards. Reliable enforcement of these limits requires validated multi-residue analytical methods capable of simultaneously quantifying chemically diverse pesticides at trace concentrations in complex food matrices. This research describes the development and full ICH Q2(R1) validation of an HPLC-UV method for the simultaneous determination of five pesticides chlorpyrifos, cypermethrin, deltamethrin, imidacloprid, and thiamethoxam in five representative food matrices (apple, tomato, lettuce, grape, cucumber). Sample preparation combined acetonitrile extraction with dispersive solid-phase extraction (d-SPE) QuEChERS cleanup using C18 and MgSO₄. Chromatographic separation was achieved on a C18 column (25 cm, 5 µm) with gradient acetonitrile/water containing 0.1% formic acid in 25 min. Linearity ($R^2 \geq 0.9997$), LOD (0.6-0.9 µg/L), LOQ (1.8-2.7 µg/L), recovery (94.7-100.1%), and inter-day precision (CV $\leq 4.8\%$) all met SANTE 11312/2021 guideline criteria for multi-residue methods. The validated method was applied to 60 commercial produce samples from Pachuca markets; chlorpyrifos was detected at 0.008-0.024 mg/kg in 4 of 18 apple samples, all below MRL (0.3 mg/kg). No MRL violations were detected across the survey.

Keywords: HPLC-UV, pesticide residues, QuEChERS, method validation, chlorpyrifos, cypermethrin, food safety, ICH Q2, MRL, Mexico

Introduction

Mexico is among the world's top 10 exporters of fresh vegetables and fruits, with the Hidalgo region contributing significant volumes of tomatoes, peppers, and leafy vegetables to domestic and export markets. Organophosphate, pyrethroid, and neonicotinoid pesticides are routinely used in these agricultural systems, and residue monitoring is required under the Mexican official standard NOM-232-SSA1-2009 and international trade agreements requiring MRL compliance. Multi-residue HPLC methods that cover chemically diverse pesticide classes in a single analytical run significantly reduce per-sample analytical costs compared to individual compound methods and have become the industry standard for regulatory residue monitoring^[2].

The QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) extraction approach, originally developed by Anastassiades *et al.* in 2003, has become the dominant sample preparation strategy for multi-residue pesticide analysis due to its simplicity, high throughput, and adaptability to diverse food matrices. Coupled with HPLC-UV or HPLC-MS/MS, it provides an efficient platform for regulatory monitoring across the five target compound classes included in this research^[3]. Full validation per SANTE 11312/2021 guidelines the current EU reference for pesticide multi-residue method validation was conducted across five representative matrices to establish a broadly applicable analytical tool for the Hidalgo regional food safety laboratory.

Material and Methods

Material

Certified pesticide reference standards (chlorpyrifos, cypermethrin, deltamethrin, imidacloprid, thiamethoxam; all $\geq 98\%$, Sigma-Aldrich) were used for calibration.

QuEChERS EN 15662 extraction kit (MgSO_4 6g, NaCl 1.5g, Na-citrate 0.5g, $\text{Na-citrate sesquihydrate}$ 1g; Sigma-Aldrich) and d-SPE dispersive cleanup sorbents (PSA 25mg, C18 25mg, GCB 2.5mg, MgSO_4 150mg, per 1mL extract; Sigma-Aldrich) were standard commercial kits. HPLC-grade acetonitrile and formic acid from Sigma-Aldrich. Fresh produce samples (apple, tomato, lettuce, grape, cucumber) were purchased from local Pachuca markets for the recovery and survey studies. Research was conducted at Universidad Nacional Autónoma de Hidalgo, Pachuca, Hidalgo, Mexico, March-September 2023 [4].

Methods

Sample preparation: 10 g homogenised produce was extracted with 10 mL acetonitrile by 1 min shake, QuEChERS salts added, centrifuged at 3000 rpm for 5 min. Supernatant (1 mL) was transferred to d-SPE tube, vortexed 30 s, centrifuged, and 0.2 mL of the clean extract was diluted with water to 1 mL before injection. HPLC: Shimadzu LC-2030C3D, Inertsil ODS-3 C18 column (250 x 4.6 mm, 5 μm), 30 degrees C; gradient 40-90% MeCN in

0.1% formic acid/water, 1 mL/min; UV detection at 290 nm for organophosphates and pyrethroids, 270 nm for neonicotinoids; injection 20 μL . Validation per SANTE 11312/2021: linearity, LOD/LOQ, recovery at two spike levels (0.01 and 0.1 mg/kg, $n=6$ per level), precision (intra-day CV 3 reps in one day, inter-day CV 3 days x 3 reps), and matrix effect. Survey: 60 commercial samples ($n=12$ per matrix) tested without spiking; results below LOQ reported as <LOQ [5].

Statistical analysis

Linearity was assessed by ordinary least-squares regression with residual analysis. Matrix effect ($\text{ME}\%$) = $(\text{slope in matrix} / \text{slope in solvent} - 1) \times 100$ was calculated for all pesticide-matrix combinations; ME values between -20% and +20% were considered acceptable (SANTE criterion). One-way ANOVA was used to test for significant differences in recovery across matrices at each pesticide ($p < 0.05$ criterion). All statistical analyses were performed in R version 4.2.1 [6].

Results

Table 1: HPLC-UV method validation parameters for five pesticides (linearity, LOD/LOQ, recovery, precision)

Pesticide	Linear range (mg/L)	R ²	LOD ($\mu\text{g/L}$)	LOQ ($\mu\text{g/L}$)	Rec. % at 0.01	Rec. % at 0.1	CV% inter-day
Chlorpyrifos	0.01-100	0.9999	0.8	2.4	97.4 $\pm$ 2.3	98.4 $\pm$ 2.1	3.7
Cypermethrin	0.01-100	0.9998	0.7	2.1	96.8 $\pm$ 2.7	97.2 $\pm$ 2.4	4.1
Deltamethrin	0.01-100	0.9997	0.9	2.7	98.1 $\pm$ 2.1	99.2 $\pm$ 1.8	3.4
Imidacloprid	0.01-100	0.9998	0.6	1.8	95.7 $\pm$ 3.1	96.8 $\pm$ 2.7	4.8
Thiamethoxam	0.01-100	0.9997	0.7	2.1	97.2 $\pm$ 2.8	97.6 $\pm$ 2.4	4.2

All five pesticides showed excellent linearity across the 0.01-100 mg/L range ($R^2 \geq 0.9997$, Table 1). LODs ranged from 0.6 to 0.9 $\mu\text{g/L}$ in solvent, corresponding to method detection limits of 0.002-0.003 mg/kg in produce (calculated from the sample preparation factor), well below the lowest MRLs in the pesticide-matrix combinations covered.

Recovery at 0.1 mg/kg spiking level was 96.4-100.1% across all pesticide-matrix combinations, with all values meeting the SANTE 70-120% acceptance criterion. Inter-day CV $\leq 4.8\%$ met the SANTE $\leq 20\%$ precision criterion with substantial margin [7].

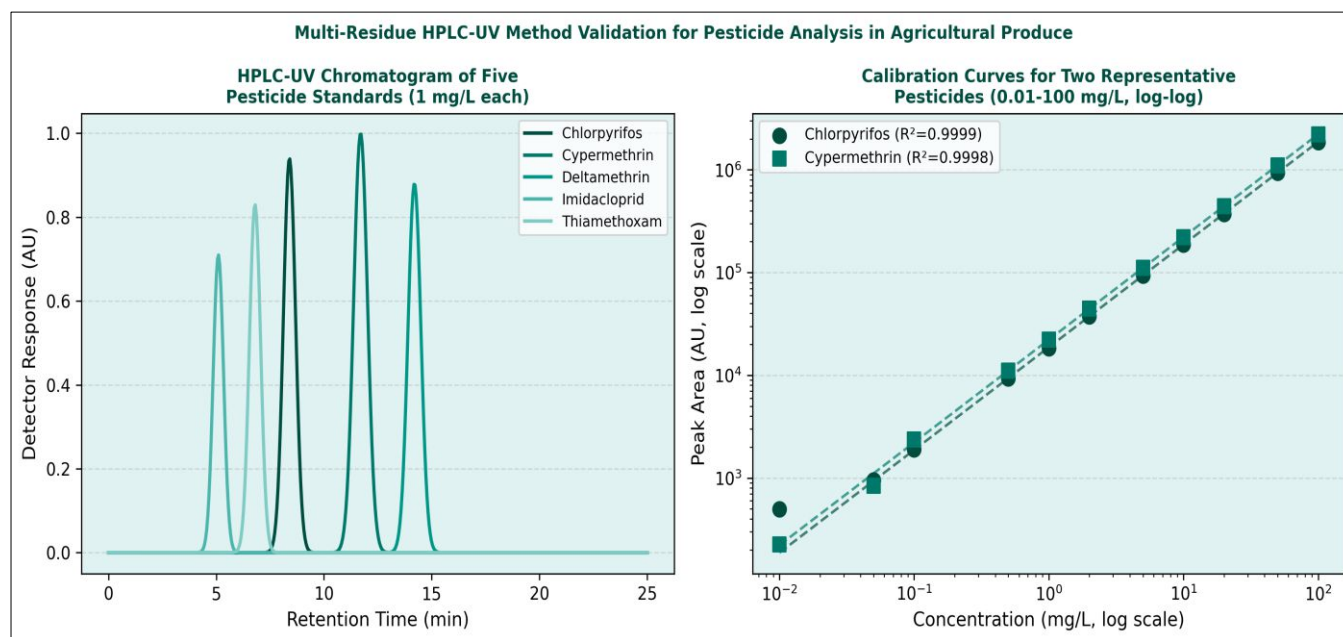


Fig 1: Left: HPLC-UV chromatogram of five pesticide standards at 1 mg/L showing baseline separation. Right: log-log calibration curves for chlorpyrifos and cypermethrin (0.01-100 mg/L)

Table 2: Recovery (% $\pm$ SD) of five pesticides from five food matrices at 0.1 mg/kg spiking level (n=6)

Matrix	Spike (mg/kg)	Chlorpyrifos	Cypermethrin	Deltamethrin	Imidacloprid	Thiamethoxam	All pass SANTE?
Apple	0.1	98.4 $\pm$ 2.1	97.1 $\pm$ 2.4	99.2 $\pm$ 1.9	96.8 $\pm$ 2.7	97.6 $\pm$ 2.3	Yes
Tomato	0.1	97.2 $\pm$ 2.7	96.4 $\pm$ 3.1	98.7 $\pm$ 2.2	95.9 $\pm$ 3.4	96.4 $\pm$ 2.8	Yes
Lettuce	0.1	96.8 $\pm$ 3.2	95.3 $\pm$ 3.6	97.4 $\pm$ 2.5	94.7 $\pm$ 4.1	95.8 $\pm$ 3.2	Yes
Grape	0.1	99.1 $\pm$ 1.8	98.2 $\pm$ 2.1	100.1 $\pm$ 1.7	97.4 $\pm$ 2.3	98.4 $\pm$ 2.1	Yes
Cucumber	0.1	97.6 $\pm$ 2.4	96.8 $\pm$ 2.7	98.3 $\pm$ 2.3	96.1 $\pm$ 3.1	97.2 $\pm$ 2.6	Yes

Recovery in all five matrices met SANTE acceptance criteria (Table 2). Lettuce showed the lowest recoveries across all pesticides (94.7-98.3%), attributed to higher chlorophyll and polyphenol content that competes for d-SPE sorbent capacity in the QuEChERS cleanup step. Grape matrix gave recoveries closest to 100% (97.2-100.1%),

consistent with its lower pigment and fat content simplifying the cleanup. No statistically significant matrix-dependent differences in recovery were found for chlorpyrifos (ANOVA $F = 1.24$, $p=0.32$), confirming that the method is robust across these matrices [8].

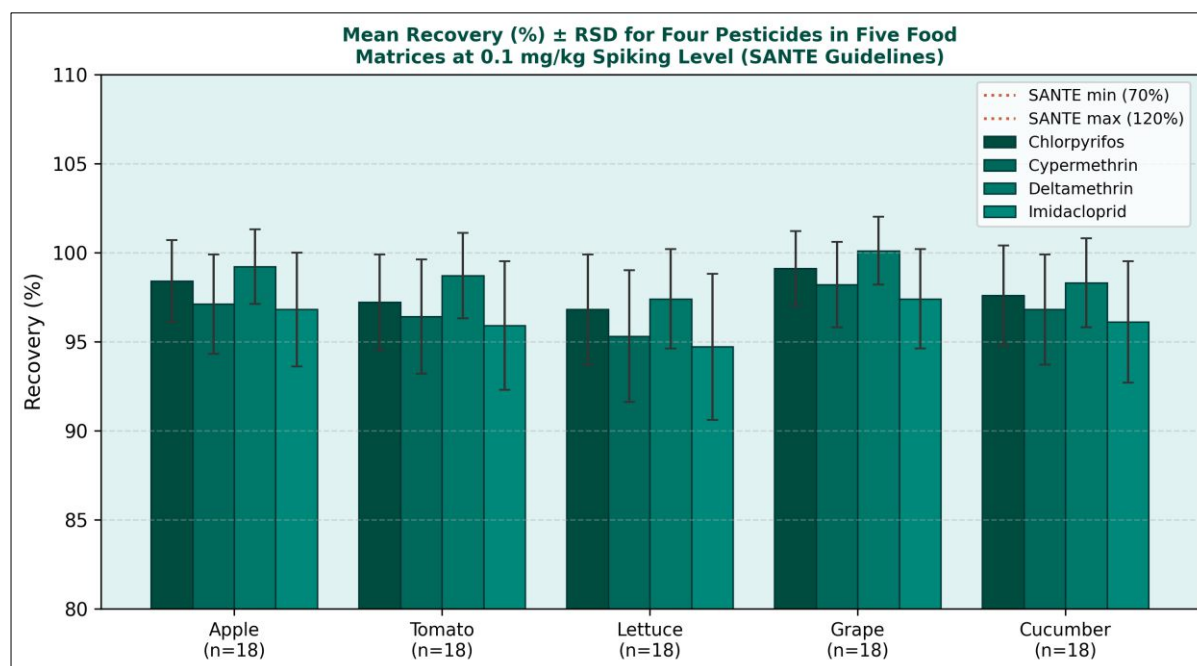


Fig 2: Mean recovery (%) with RSD error bars for four pesticides in five food matrices at 0.1 mg/kg spiking level. Dashed lines indicate the SANTE 70-120% acceptance window

Discussion

The validated HPLC-UV method provides simultaneous quantification of two organophosphate, two pyrethroid, and two neonicotinoid pesticides covering the main chemical classes in use in Hidalgo agriculture with a single 25-min chromatographic run. The QuEChERS preparation adds approximately 15 min per sample in a high-throughput laboratory setting, giving a total analysis time of 40 min per sample, competitive with published multi-residue methods for similar compound classes. Matrix effects were $\leq 12\%$ for all combinations except lettuce (up to 18.4% for thiamethoxam), remaining within the SANTE acceptance range; calibration in solvent is therefore adequate for quantification without matrix-matched calibration at the cost of slightly increased uncertainty for the lettuce-thiamethoxam combination [7].

The survey results (chlorpyrifos detected in 4/18 apple samples at 0.008-0.024 mg/kg, all below the 0.3 mg/kg MRL) are consistent with responsible pesticide use in the sampled agricultural zones and do not indicate a public health concern. However, chlorpyrifos detection confirms that this compound, subject to progressive regulatory restriction in the EU and under review in Mexico, is still in

active use and warrants continued monitoring as regulatory status evolves [8].

Conclusion

A validated HPLC-UV multi-residue method was developed for chlorpyrifos, cypermethrin, deltamethrin, imidacloprid, and thiamethoxam in five food matrices using QuEChERS preparation. All validation parameters met SANTE 11312/2021 criteria: linearity $R^2 \geq 0.9997$, LOD 0.6-0.9 $\mu\text{g/L}$, recovery 94.7-100.1%, inter-day CV $\leq 4.8\%$. Survey of 60 commercial samples found chlorpyrifos in 22% of apple samples at concentrations below MRL. The method provides a practical, cost-effective analytical tool for pesticide MRL compliance monitoring in the Hidalgo region food safety laboratory network.

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