

Validation and application of quick polar pesticide (QuPpe) extraction and ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) to analyze perchlorate in vegetables from Mexico

Validación y aplicación de la extracción rápida de plaguicidas polares (QuPpe) y cromatografía líquida de ultra rendimiento-espectrometría de masas en tándem (UPLC-MS/MS) para analizar perclorato en vegetales de México

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Please cite this article as/Como citar este artículo:

Leyva-Morales J.B., Calderón R., García-Hernández, J., Palma P., Bastidas-Bastidas, P.J., Zambrano-Soria, M., Olmeda-Rubio, C., Romo-Gómez, C., Acevedo-Sandoval, O.A., Camacho-López, C. (2024). Validation and application of quick polar pesticide (QuPpe) extraction and ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) to analyze perchlorate in vegetables from Mexico. *Revista Bio Ciencias*, 11, e1766. <https://doi.org/10.15741/revbio.11.e1766>

Article Info/Información del artículo

Received/Recibido: September 18th 2024.

Accepted/Aceptado: October 22th 2024.

Available on line/Publicado: October 24th 2024.

ABSTRACT

El perclorato Perchlorate is considered a contaminant that can cause endocrine disruption in people exposed through various foods, including vegetables. To address the lack of information on this health problem in Mexico, we developed an analytical method to quantify perchlorate in commonly consumed vegetables based on quick polar pesticide (QuPpe) extraction and ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS). According to SANTE guidelines, the method was validated through selectivity, accuracy, precision, linearity, matrix effect, limit of detection (LOD), and limit of quantification (LOQ). The developed method exhibited a linear range of 0.005–0.100 mg/kg with LOD and LOQ values of 0.001 and 0.003 mg/kg, respectively. Recovery in a fortified tomato matrix was 96.85 ± 12.90 % with a coefficient of variation (CV) of 11.55 ± 0.84 %. The method was applied to analyze 31 vegetable samples collected from different regions in Mexico. Perchlorate concentrations ranged from not detected (ND) to 0.016 mg/kg, fresh weight. This method generates robust scientific evidence to implement and strengthen perchlorate monitoring programs for food matrices, supporting efforts to regulate this analyte in Mexico.

KEY WORDS: UPLC-MS/MS, Perchlorate, Vegetables, Validation, Mexico

RESUMEN

El perclorato es considerado un contaminante que puede generar alteración endocrina en las personas expuestas a través de diversos alimentos, incluidos los vegetales. Para abordar la falta de información sobre este problema de salud en México, desarrollamos un método analítico para cuantificar el perclorato en vegetales de consumo común basado en la extracción rápida de plaguicidas polares (QuPPE) y cromatografía líquida de ultra rendimiento acoplado a espectrometría de masas en tándem (UPLC-MS/MS). El método fue validado mediante los parámetros de selectividad, exactitud, precisión, linealidad, efecto matriz, límite de detección (LOD) y límite de cuantificación (LOQ) según las pautas de SANTE. El método desarrollado exhibió un rango lineal de 0.005 a 0.100 mg/kg con valores LOD y LOQ de 0.001 y 0.003 mg/kg, respectivamente. La recuperación en la matriz de tomate fortificada fue de 96.85 ± 12.90 % con un coeficiente de variación (CV) de 11.55 ± 0.84 %. El método se aplicó para analizar 31 muestras de vegetales recolectadas de diferentes regiones de México. Las concentraciones de perclorato variaron desde no detectado (ND) hasta 0.016 mg/kg de peso fresco. Este método genera evidencia científica robusta para implementar y fortalecer programas de monitoreo de perclorato en matrices alimentarias, apoyando los esfuerzos para regular este analito en México.

PALABRAS CLAVE: UPLC-MS/MS, Perclorato, Vegetales, Validación, México.

Introduction

Perchlorate (ClO_4^-) is composed of a central chlorine atom bonded to four oxygen atoms (Motzer, 2001; Kumarathilaka *et al.*, 2016; Dong *et al.*, 2019). This strongly oxidizing substance has been primarily used in the aerospace industry and as an additive in fireworks, explosives, and rocket fuel (Seyfferth and Parker, 2006; Hepperle *et al.*, 2013; Leoterio *et al.*, 2017; Bauer, 2018; Constantinou *et al.*, 2019; Savini *et al.*, 2019; Calderón *et al.*, 2020a; Liao *et al.*, 2020; Panseri *et al.*, 2020; Huertas-Pérez *et al.*, 2022; Zhang *et al.*, 2022). The combination of its excessive use and a lack of final disposal processes has resulted in perchlorate becoming widely distributed in the environment and within food chains. Indeed, perchlorate has been reported in multiple water sources (drinking water, irrigation water, surface water, and groundwater), soil, plant-based foods, and animal products. Also, in the last decade, fertilizers have been identified as one of the main input sources of perchlorate in agri-food systems. The most relevant routes of exposure to perchlorate in humans are food and water consumption (Sánchez *et al.*, 2005; Seyfferth &

Parker, 2006; Yang *et al.*, 2011; Hepperle *et al.*, 2013; Calderón *et al.*, 2017; Bauer *et al.*, 2018; Chamkasem, 2018; Rajski *et al.*, 2018; Constantinou *et al.*, 2019; Dong *et al.*, 2019; Herrera-López *et al.*, 2019; Melton *et al.*, 2019; Savini *et al.*, 2019; Calderón *et al.*, 2020a,b,c; Garrido *et al.*, 2020; Liao *et al.*, 2020; Panseri *et al.*, 2020; Cutillas & Fernández-Alba, 2021; Calderón *et al.*, 2022a,b; Huertas-Pérez *et al.*, 2022; Zhang *et al.*, 2022; Muñoz-Arango *et al.*, 2023).

Constant exposure to perchlorate through water and food consumption poses a serious threat to public health. Perchlorate is a potent inhibitor that competes with the sodium iodide symporter (NIS), which lowers the active transport of iodine to the thyroid and decreases the production of thyroid hormones T₃ and T₄ and affects cellular homeostasis in the body (Leung *et al.*, 2010).

People who suffer from thyroid disorders, pregnant women, and babies are particularly vulnerable to perchlorate exposure (Calderón *et al.*, 2017; Leoterio *et al.*, 2017; Dong *et al.*, 2019; Huertas-Pérez *et al.*, 2022). Approximately 4–10 % of the global population has hypothyroidism (Flores-Rebollar *et al.*, 2015). In Mexico, 1.2 to 7.2 % of adults have overt hypothyroidism, and 5.6 to 15.4 % of adults have subclinical hypothyroidism (Flores-Rebollar *et al.*, 2015; Juárez-Cedillo *et al.*, 2017). Pregnant women are the most vulnerable group for hypothyroidism (12.8 % overt hypothyroidism and 21.1 % subclinical hypothyroidism) (Cruz-Cruz *et al.*, 2014).

Thus, robust analytical methods to evaluate perchlorate in plant-based foods in Mexico are urgently needed due to lack of available information, the absence of regulations for its use in the country and given how frequently this compound is detected in different environmental matrices in other countries of the world (Calderón *et al.*, 2017; Bauer *et al.*, 2018; Chamkasem 2018; Rajski *et al.*, 2018; Liao *et al.*, 2020; Cutillas *et al.*, 2021; Zhang *et al.*, 2022).

Currently, various analytical methods are available, that have low sensitivity, to analyze perchlorate in vegetables: X-ray fluorescence (Hatzistavros & Kallithrakas-Kontos, 2011); electrophoresis (Kiplagat *et al.*, 2011); surface enhanced Raman scattering (SERS) (Hu *et al.*, 2021); ion chromatography (IC) coupled to conductivity (USEPA, 2000), mass spectrometry (IC-MS) (Mathew *et al.*, 2005), tandem mass spectrometry (IC-MS/MS) (Bauer *et al.*, 2018; Panseri *et al.*, 2020), and inductively coupled plasma mass spectrometry (ICP/MS) (Voogt & Jackson, 2010) detectors; potentiometry with ion-selective electrodes (Leoterio *et al.*, 2017); and ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) (Lee *et al.*, 2012; Chamkasem, 2018; Herrera-López *et al.*, 2019; Huerta-Pérez *et al.*, 2022; Zhang *et al.*, 2022). According to Dong *et al.* (2019), X-ray fluorescence analysis, potentiometry with ion-selective electrodes, and electrophoresis methods show reduced sensitivity. In addition, IC coupled to a conductivity detector is not selective, leading to frequently reported false positives. Furthermore, IC-MS is susceptible to the presence of interference. However, UPLC-MS/MS has proven to be effective for determining polar analytes, including perchlorate in complex matrices, while also exhibiting adequate selectivity and sensitivity (Dong *et al.*, 2019; Zang *et al.*, 2022).

Given the high rate of vegetable consumption in Mexico (Pérez-Lizaur *et al.*, 2014) and that vegetable intake is the primary route of exposure to this compound, the population is at a

potentially high risk of perchlorate exposure, its maximum residue limit in vegetables is 0.05 mg/kg (Calderón *et al.*, 2017, Calderón *et al.*, 2020a,b; European Commission, 2020; Calderón *et al.*, 2022a,b). Thus, this study aimed to validate and apply the extraction method for quick polar pesticide (QuPPE) (Anastassiades *et al.*, 2023) and subsequent quantification using UPLC-MS/MS for perchlorate determination in vegetables that are highly consumed in Mexico (tomato, green pepper, spinach, and lettuce) (Pérez-Lizaur *et al.*, 2014; Calderón *et al.*, 2022b; Calderón *et al.*, 2023).

Materials and Methods

Reagents and chemicals

Accustandard Inc. (New Haven, USA) supplied the perchlorate analytical standard (IC-PER-10X-1) (purity 99 %). Control Técnico y Representaciones S.A. de C.V. (Monterrey, Mexico) provided the solvents acetonitrile and methanol (MS grade, JT Baker) and acetonitrile and water (HPLC grade, JT Baker). Ammonium formate and formic acid (ACS grade) were obtained from Sigma Aldrich (St. Louis, USA).

Equipment

We employed a 12-L JR industrial blender, EBA21 centrifuge (Hettich GmbH & Co., Kirchlengern, Germany), CPX8800H ultrasonic bath (Branson Ultrasonics, Danbury, USA), M16715 vortex (Thermo Fisher Scientific, Waltham, USA), Practum 2102-1S granataria scale (Sartorius AG, Göttingen, Germany), AX224 analytical balance (Sartorius), R215 Digital rotary evaporator (Büchi, Flawil, Switzerland), and a vacuum pump (Welch-Ilmvac, Seattle, USA).

Stock solutions and fortified sample preparations

From the acquired perchlorate standard, a solution was prepared (1.0 ng/μL concentration) to fortify the blank samples and prepare the working solutions (system linearity test). Both stock and working solutions were stored in non-silanized amber vials at 4 °C in the dark. Before each use, the standard solutions were equilibrated at room temperature (Leyva-Morales *et al.*, 2015; Leyva-Morales *et al.*, 2023; Bastidas-Bastidas *et al.*, 2024).

As we did not have a reference material or blank matrix, control samples of fresh tomato fruit (1.5 kg) were taken as a representative matrix for validation. We collected the samples from an organic farm in Culiacan, Sinaloa, Mexico certified as an organic producer by Agricert Mexico. First, the samples were analyzed in triplicate to demonstrate that they were free of perchlorate and that no co-extractions (interfering compounds) were present that could interfere with the subsequent analysis. After confirming that the samples met these criteria, they were used in the validation tests.

We established the concentration levels so that the lowest was close to the limit of detection

(LOD), a second level was equal to 0.05 mg/kg, the maximum permitted residue limit (MRL) according to European regulations (as no regulation is currently available for Mexico), and the rest of the levels were higher than the MRL. Blank tomato sample (10 g) was weighed and fortified by adding (in quintuplicate) the standard solution at the five concentration levels directly to the prepared fruit sample. To fortify the samples, 50, 100, 250, 500 and 1000 μL of the solution were added to the control tomato samples at a concentration of 1.0 ng/mL for level 1 (0.005 mg/kg), level 2 (0.010 mg/kg), level 3 (0.025 mg/kg), and level 4 (0.0500 mg/kg) and level 5 (0.100 mg/kg), respectively. Subsequently, they were analyzed using the proposed method.

Sample preparation and extraction

The edible part of the total sample of the matrix deemed representative of high-humidity vegetable matrices (fresh tomato fruit) was crushed and homogenized in an industrial blender according to the protocols of the Pesticide Analytical Manual (PAM), specifically section 102-A, which refers to sample preparation (Bastidas-Bastidas *et al.*, 2019). A portion was taken as a representative sample for analysis.

Subsamples of the control material (10 g \pm 0.05 g) were placed in 50-mL polypropylene centrifuge tubes, and the fortification standard was added at the concentration levels reported in section Stock solutions and fortified sample preparations. Subsequently, the fortified samples were vortexed for approximately 30 s and kept at rest at room temperature for 30 min. Then, 10 mL of methanol acidified to 1 % with formic acid was added and shaken for 15 min in an ultrasonic bath, allowed to rest at $-18\text{ }^{\circ}\text{C}$ for 90 min, and centrifuged at 10,000 rpm for 10 min. After this, 1.0 mL of the supernatant was filtered through a 0.22- μm nylon membrane into a 1.5-mL polypropylene vial for UPLC-MS/MS analysis (Anastassiades *et al.*, 2023).

Instrumental analysis

The chromatographic system consisted of an Acquity UPLC H-class system coupled to a Xevo TQD triple quadrupole mass spectrometer equipped with a Torus DEA column (1.7 x 100 x 3.0 mm; pore size of 130 Å) (Waters Corporation, Milford, USA). The system employed a flow rate of 0.5 mL/min and an oven temperature of 50 $^{\circ}\text{C}$. Each sample was automatically injected through an Acquity sample manager with a flow-through needle autosampler system (Waters Corporation) in a volume of 10 μL . The mobile phases were mobile phase A (50 mM ammonium formate, pH 2.9) and mobile phase B (acetonitrile + 0.9 % formic acid) with the following gradient: (1) 0 min, 10 % A; (2) 0–4.5 min, 60 % A; (3) 4.5–8.5 min, 60 % A; and (4) 8.5–16 min, 10 % A. After reaching the initial conditions, the column was reequilibrated for 4 min before the subsequent injection. The total running time was 16 min.

Electrospray ionization was employed in negative ionization mode with a capillary voltage of 2,500 V. Nitrogen was used as a nebulizer (flow rate of 300 L/h) and desolvation gas (flow rate of 1000 L/h). The desolvation temperature was 600 $^{\circ}\text{C}$, and the source temperature was 150 $^{\circ}\text{C}$. Data acquisition and analysis were performed with the MassLynx 4.1 workstation (Waters Corporation) using multiple reaction monitoring (MRM) during at least two transitions that were

used as quantitative and confirmative transition pairs employing the tandem mass conditions shown in Table 1 and Fig. 1.

Table 1. Tandem mass conditions for perchlorate analysis.

Analyte	Parent m/z	Daughter m/z	Dwell (s)	Cone (V)	Collision (V)
Perchlorate	99.00	67.00	0.085	20	18
		83.00	0.085	20	45

Source: Own elaboration.

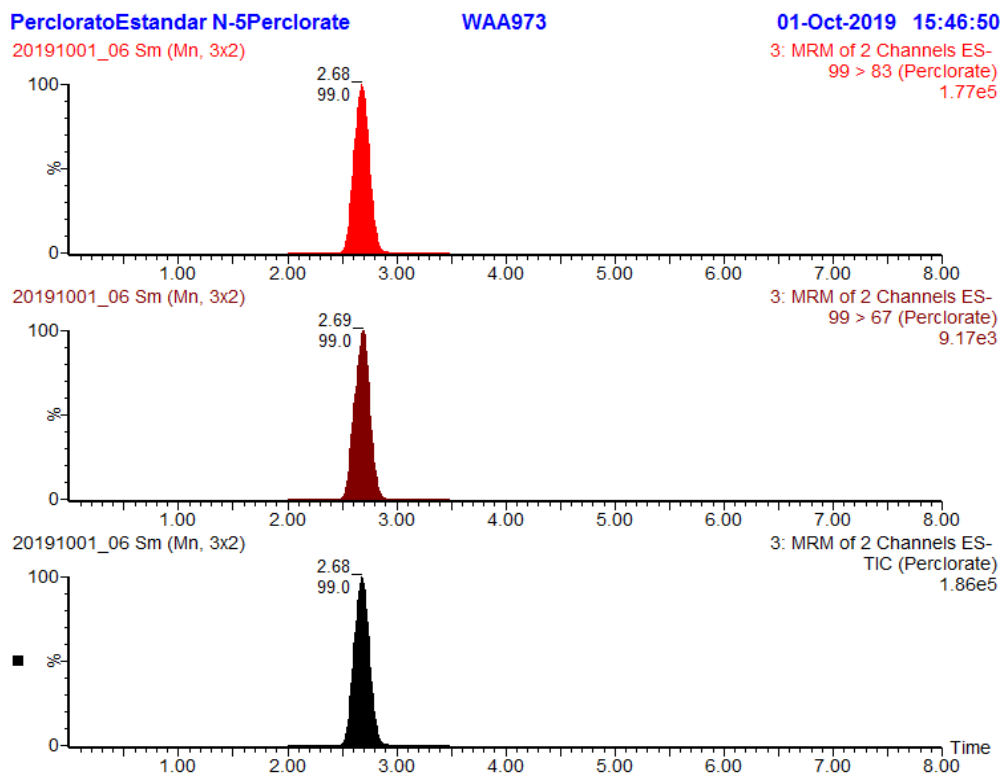


Figure 1. Monitored transitions for perchlorate analysis.

Source: Own elaboration.

Quantification

To calculate the concentration of perchlorate in the samples, we employed the external standard method reported by Bastidas-Bastidas *et al.* (2019) using the following equation:

$$\text{ppm or mg/kg} = \frac{(\text{response of the sample peak})(\text{injected standard [mg]})}{(\text{response of the standard peak})(\text{equivalents of injected sample [mg]})} \quad \text{Eq. (1)}$$

Quality control of the method

Internal quality control was performed with each analytical batch to verify compliance with established continuous system suitability specifications and to evaluate various parameters of the method, including accuracy and precision. A blank-fortified sample at 0.025 mg/kg (level 3) was analyzed in each lot. For fortified blanks, a recovery percentage of 70–120 % perchlorate was required to ensure good quality control. The fortified blank samples exhibited 93–111 % recovery. Furthermore, the sample target did not show interference, demonstrating good performance (Pihlstrom *et al.*, 2021).

Method validation

The proposed method was validated per the guidelines established by the Analytical Quality Control and Method Validation Procedures for Pesticide Residue Analysis in Food and Feed SANTE 11312/2021 of the European Commission (Pihlstrom *et al.*, 2021). Method performance was evaluated based on the LOD, limit of quantification (LOQ), selectivity, linearity, accuracy, precision and matrix effect. All experiments were performed in quintuplicate, and the quality criteria were used to determine whether validation was successful. Selectivity was evaluated by qualitatively comparing the retention times of the peaks obtained with a blank sample with those obtained with a perchlorate analytical standard. The LOD and LOQ were determined based on the standard deviation of the blank sample response and the slope of the standard calibration curve. Linearity was evaluated via linear regression by analyzing calibration curves of the concentration levels indicated previously. Precision and accuracy were evaluated based on the recovery percentage. The percentage of the coefficient of variation (CV) of the observed overall recovery was taken as precision, and accuracy was calculated based on the percentage of the overall average recovery of the 25 replicates for perchlorate divided by the expected concentration (fortification level). Finally, the matrix effect (ME) was evaluated as the relationship between the analytical sensitivity of the curve prepared in matrix in relation to the sensitivity of the calibration curve prepared in solvent.

Vegetable sample collection and application of the method

After validating the method, samples of tomato and three other vegetables (green pepper, spinach, and lettuce) collected from markets and supermarkets in eight states of Mexico (Baja California, State of Mexico, Nayarit, Nuevo León, Sinaloa, Sonora, Tlaxcala, and Yucatán) which

were subjected to perchlorate analysis to evaluate the applicability of the method. The sampled vegetables were conventional agricultural products (n = 31; green pepper [8], tomato [8], lettuce [7], and spinach [8]) (Calderón *et al.*, 2022b; Calderón *et al.*, 2023).

Results and discussion

Method validation

The developed method clearly exhibited high sensitivity based on the obtained LOD value of 0.001 mg/kg and LOQ value of 0.003 mg/kg. Selectivity was adequate, as no interference was detected that could affect analyte determination (Table 2, Figure 2). The improved sensitivity and selectivity of the UPLC-MS/MS method make this technique appropriate for determining residue levels in polar and/or ionic compounds of various matrices (Leyva-Morales *et al.*, 2023; Bastidas-Bastidas *et al.*, 2024).

Furthermore, the developed method exhibited analyte linearity in the concentration range of 0.005 to 0.100 mg/kg, which was confirmed by the linear regression analysis (added versus recovered perchlorate concentrations; $R^2 > 0.99$) (Figure 3). In addition, the method was exact and precise, with recoveries of 94.08–103.31 % and CVs of 5.40–6.70 % (Table 2). In all these cases, the acceptance criteria established in the SANTE guidelines (70–130 %) and CVs (≤ 20 %) were met (Pihlstrom *et al.*, 2021).

Various validated methods for determining perchlorate in vegetables employ a tandem mass spectrometer as a detector, including the one proposed in the present study. For example, Bauer *et al.* (2018) optimized an analytical method for determining perchlorate in tomatoes using IC-MS/MS, analyzing the concentration levels of 0.010 and 0.100 mg/kg. The recovery percentages for the first and second concentration levels were 117 % and 102 %, respectively. In both cases, the CV was 1 % (Table 3). The method, as well as the one proposed here, was exact and precise according to the SANTE criteria (Pihlstrom *et al.*, 2021), demonstrating that tandem mass spectrometry is sufficiently sensitive to evaluate highly polar compounds at trace concentration levels (Leyva-Morales *et al.*, 2023; Bastidas-Bastidas *et al.*, 2024).

Table 2. Validation parameters for the determination of perchlorate in vegetables.

Analyte	LOD (mg/kg)	LOQ (mg/kg)	Accuracy (recovery percentage)	Precision (CV %)
Perchlorate	0.001	0.003	95.79 ± 14.61	15.25

Limit of detection (LOD), limit of quantification (LOQ), accuracy (criterion: recovery percentage of 70-120 %) and precision (criterion: coefficient of variation [CV] ≤ 20 %).

Source: Own elaboration.

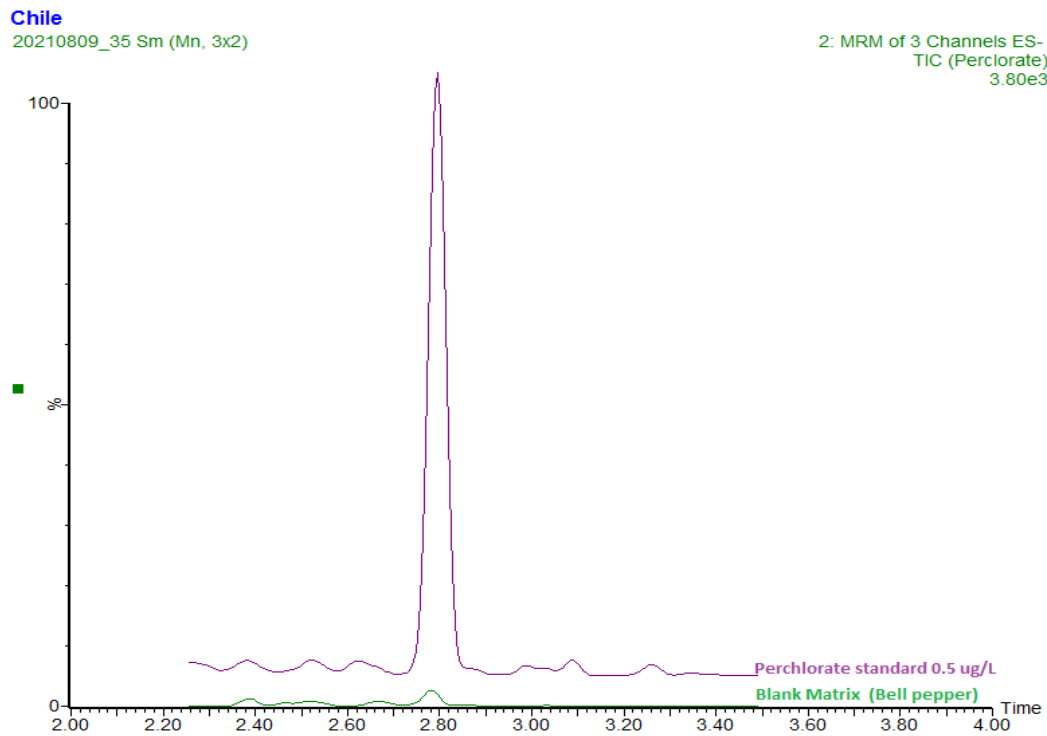


Figure 2. Multiple reaction monitoring (MRM) acquisition chromatogram for perchlorate (blank reagents vs. fortified samples at a peak level of 0.01 mg/kg).

Source: Own elaboration.

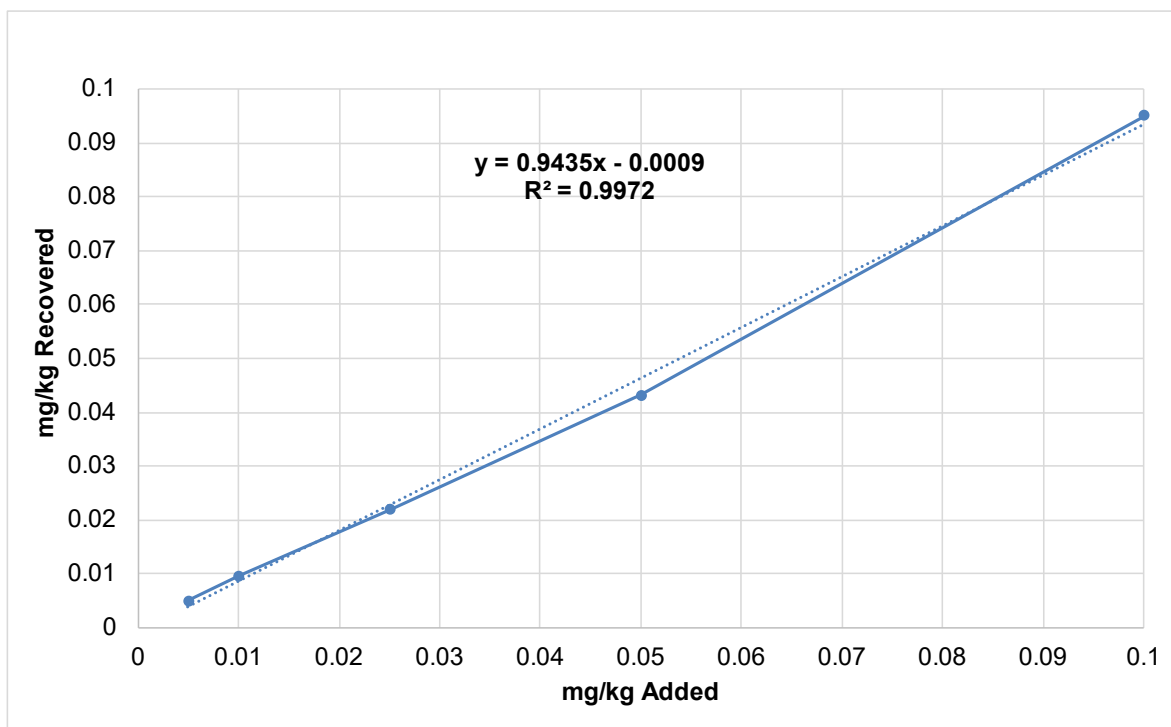


Figure 3. Linearity of the developed method to analyze perchlorate in vegetables.

Source: Own elaboration.

On the other hand, the developed method is more accurate and precise than the analytical method proposed by Chamkasem (2018) for the determination of perchlorate in carrot, apple, and green bean using liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Table 3).

Constantinou *et al.* (2019) also evaluated perchlorate in plant foods (strawberry, grape, apple, rye, orange, and rice) and employed the same method as the one proposed in this study (Table 3). Unlike the method proposed here, the one reported did not evaluate tomato as a matrix, and the LOD (0.02 mg/kg) and LOQ (0.05 mg/kg) were less sensitive. In addition, the isotopically labeled internal standard employed in that study increased the analysis cost without notably affecting the process (Leoterio *et al.*, 2017).

Hepperle *et al.* (2013) validated an analytical method for determining perchlorate in apple and barley using QuPPE extraction and UPLC-MS/MS quantification (Table 3). The LOQ reported by these authors (0.002 mg/kg) was slightly higher than the LOQ of the present study. Herrera-López *et al.* (2019) validated an analytical method for determining perchlorate in five matrices: grape, lettuce, orange, oat, and soybean. The LOQ values were 0.02 mg/kg for grape, lettuce, oat, and soybean, while the LOQ for orange was 0.05 mg/kg (Table 3). Thus, the LOQ values reported

in that study indicated that the method was less sensitive than the method proposed in the present study (0.003 mg/kg).

Huertas-Pérez *et al.* (2022) validated the QuPPE-UPLC-MS/MS analytical method for determining perchlorate in a wide range of food products (mango puree, applesauce, broccoli, fruits and vegetables-based baby food, prune, beet, and juice apple concentrate) (Table 3). Their method can handle a greater diversity of matrices than the method developed in the present study, and the range of work evaluated is broader. However, their LOQ values were similar or less sensitive than ours (0.010 mg/kg for all matrices evaluated).

Finally, regarding the matrix effect (ME), it was evaluated as the relationship between the analytical sensitivity of the curve prepared in matrix in relation to the sensitivity of the calibration curve prepared in solvent ($ME (\%) = [(slope \text{ in matrix}/slope \text{ in solvent}) - 1] \times 100$). According to the SANTE guide, values $< \pm 20 \%$ are interpreted as a low effect, values $> \pm 20$ and $< \pm 50 \%$ are considered a moderate effect, while values $> \pm 50 \%$ are considered a high effect (Pihlstrom *et al.*, 2021). In this sense, negative values of matrix effects mean signal suppression, while positive values represent an enhancement. Once the matrix effect is known, the pertinent correction is made in the calculations of the analytes evaluated. According to Leyva-Morales *et al.* (2023) an important aspect of the ME is that, for each type of matrix, the effect produced should be studied independently. In this sense, a negative effect matrix was observed in all the vegetables evaluated; in the case of tomato, it was -57.45% ; while, in the case of spinach, lettuce, and bell pepper it was of -42.98% , -26.44% , and -11.56% , respectively (Figure 4).

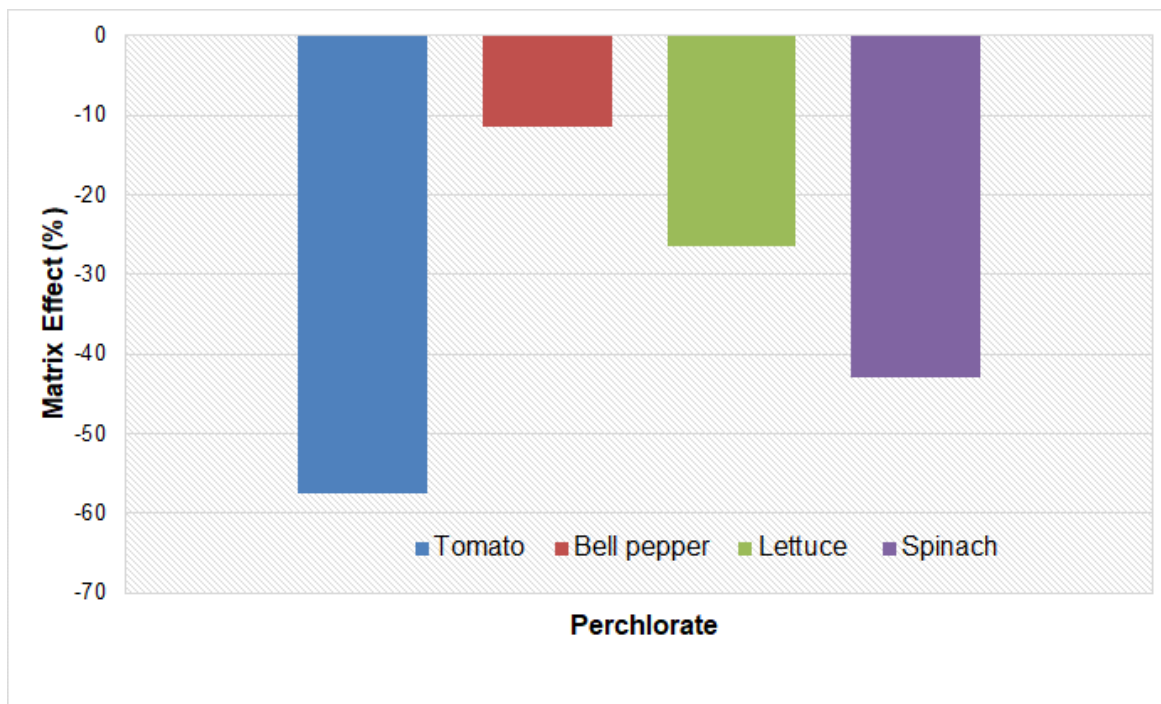


Figure 4. Matrix effect (ME %) for perchlorate in the evaluated vegetables.

Source: Own elaboration.

The ME results observed in the present work agreed with others previously reported where it is indicated that components present in various matrices can reduce the signal response of the analytes in the detector due to ionic suppression (Pizzutti *et al.*, 2016). This behavior has been reported to be very common in LC-MS/MS, due to the competition that is generated in the ion source during the ionization process, between the co-eluted components of the matrix with the analytes of interest, thus causing the suppression of ions and therefore, a reduced response signal. This problem, at the time of determination of the analytes, can be omitted by preparing matrix-matched standards, which was presented in this work (Pizzutti *et al.*, 2016; Pano-Farias *et al.*, 2017; Fei *et al.*, 2024).

Application of the method for vegetable analysis

The analysis of vegetables from different regions of Mexico revealed the presence of perchlorate in 71 % (22/31) of the analyzed samples. However, only 13 samples contained perchlorate concentrations above the LOQ, nine showed concentrations between the LOD and LOQ, and seven contained trace amounts (i.e., below the LOD). The maximum concentration (0.016 mg/kg) was detected in spinach from a local market in Tlaxcala. The frequency of detection

of perchlorate in agricultural products exhibited the following behavior: lettuce > spinach > tomato > green pepper (Table 4). Some authors have reported that leafy vegetables tend to be more susceptible to perchlorate accumulation (Sánchez *et al.*, 2005; USDA, 2005; Seyfferth & Parker, 2006; Garrido *et al.*, 2020; Calderón *et al.*, 2022a; Zhang *et al.*, 2022), which agrees with the results of this study. Figure 5 shows perchlorate in a tomato sample from Culiacan, Sinaloa, Mexico. None of the samples exceeded the MRL established by European regulations (0.05 mg/kg).

There are no reports of perchlorate presence in foods in Mexico. Nonetheless, when agricultural products are exported to other countries, they undergo strict review to ensure safety. During 2004–2005, the United States Department of Agriculture (USDA) monitored the presence of perchlorates in 27 foods, including vegetables produced domestically and imported from Mexico. The results of this effort indicated that perchlorate was present in all vegetables, with the highest frequency of detection occurring in lettuce and the highest accumulation (0.927 mg/kg) occurring in spinach. Interestingly, the maximum perchlorate concentration in spinach imported from Mexico (0.252 mg/kg) was generally higher than the concentrations in spinach produced in the United States (0.006–0.154 mg/kg), except for spinach produced in California, particularly in Brawley (0.927 mg/kg), Long Beach (0.266 mg/kg), Moorpark (0.259 mg/kg), and Riverside (0.680 mg/kg). In the case of tomato, the same behavior was observed. A maximum concentration was observed in domestic production from Somis, California (0.039 mg/kg), whereas tomatoes imported from Mexico exhibited approximately seven times more perchlorate (0.286 mg/kg). However, the maximum concentration in lettuce was detected in produce from Yuma, Arizona (0.129 mg/kg), with no reports for lettuce of Mexican origin (USDA, 2005). The concentrations reported by the USDA for vegetables imported from Mexico (lettuce and tomato) were higher than those detected in vegetables from supermarkets in different regions of Mexico analyzed in this study.

Finally, although there are no regulations in Mexico that establish maximum allowed limits for perchlorate in vegetables, the method developed in this study provides an alternative determination method and resulted in LOD and LOQ values that were lower than those of the regulations established by the European Commission (0.05 mg/kg) (European Commission, 2020).

Table 3. Comparison of the developed method with previously reported methods for determining perchlorate in various food matrices.

Extraction/ determination method	Matrix	Fortification level (mg/ kg)	Recovery percentage (%)	Coefficient of variation (%)	Limit of detection (mg/kg)	Limit of quantification (mg/kg)	Reference	
QuPPE/Ion Chromatography- Tandem Mass Spectrometry (IC-MS/MS)	Tomato	0.01	117	1				
		0.1	102	1				
	Apple	0.01	97	1				
		0.1	97	1				
	Lemon	0.01	106	2				
		0.1	94	1				
	Raisin	0.01	119	4		Not Reported (NR)	Not Reported	Bauer <i>et al.</i> (2018)
		0.1	97	1				
	Avocado	0.01	93	2				
		0.1	95	1				
	Wheat	0.01	109	1				
		0.1	99	1				
	Carrot	0.02	70	29				
	0.04	95	9					
	0.25	90	22					
Solvent extraction and centrifugation/ Liquid chromatography- tandem mass spectrometry (LC-MS/MS)	Apple	0.02	74	4	Not Reported	0.001	Chamkasem (2018)	
		0.04	94	8				
		0.25	85	2	(NR)			
	Green bean	0.02	92	7				
		0.04	113	2				
		0.25	88	2				

Continuation

Table 3. Comparison of the developed method with previously reported methods for determining perchlorate in various food matrices.

Extraction/ determination method	Matrix	Fortification level (mg/ kg)	Recovery percentage (%)	Coefficient of variation (%)	Limit of detection (mg/kg)	Limit of quantification (mg/kg)	Reference
QuPPE/Liquid Chromatography with Electrospray Ionization- Tandem Mass Spectrometry (LC/ESI-MS/MS)	Strawberry	0.05	96	14.9	0.02	0.05	Constantinou <i>et al.</i> (2019)
		0.2	104.6	5.5			
	Grape	0.05	87.2	12			
		0.2	97.5	4.5			
	Apple	0.05	87.5	6.6			
		0.2	97.4	2.8			
	Rye	0.05	89.1	2.2			
	Orange	0.2	87.4	3.5			
		0.05	110.5	9.8			
	0.2	100.7	12.6				
QuPPE/UPLC- MS/MS	Rice	0.05	104.1	6.1	Not Reported	0.002	Hepperle <i>et al.</i> (2013)
		0.2	110.5	9.8			
	Apple	0.01	108	2.6			
		0.1	98	2.2			
	Barley	0.01	106	2.3			
		0.1	93	5			

Continuation

Table 3. Comparison of the developed method with previously reported methods for determining perchlorate in various food matrices.

Extraction/ determination method	Matrix	Fortification level (mg/ kg)	Recovery percentage (%)	Coefficient of variation (%)	Limit of detection (mg/kg)	Limit of quantification (mg/kg)	Reference
Methanol-based extraction method with simple cleanup/Liquid Chromatography/ Electrospray Ionization-QTrap Mass Spectrometry (LC/ESI-Qtrap MS)	Grape	0.02	93	4	0.01	0.02	Herrera- López <i>et al.</i> (2019)
		0.05	97	11			
		0.10	97	5			
		0.50	97	1			
	Lettuce	0.02	113	3	0.01	0.02	
		0.05	107	5			
		0.10	105	2			
	Orange	0.50	105	2	0.01	0.05	
		0.02	NR	NR			
		0.05	108	7			
		0.10	103	11			
		0.50	102	6			
	Oat	0.02	101	8	0.01	0.02	
		0.05	102	3			
	Soy	0.10	102	7	0.01	0.05	
		0.50	101	2			
		0.02	NR	NR			
		0.05	114	19			
		0.10	116	5			
0.50	110	6					

Continuation

Table 3. Comparison of the developed method with previously reported methods for determining perchlorate in various food matrices.

Extraction/ determination method	Matrix	Fortification level (mg/ kg)	Recovery percentage (%)	Coefficient of variation (%)	Limit of detection (mg/kg)	Limit of quantification (mg/kg)	Reference
QuPpe/UPLC- MS/MS	Mango puree	0.002	103.6	2	Not Reported (NR)	0.01	Huertas- Pérez <i>et al.</i> (2022)
		0.020	99.7	0.4			
	Apple puree	0.002	93.3	5.4			
		0.500	99	1			
	Fruit-based baby food	0.002	107.3	2.8			
		0.500	99.3	1			
	Vegetable- based baby food	0.002	102.1	5.9			
		0.500	97.5	1			
	Broccoli	0.002	100.6	5			
		0.500	98.8	1			
	Prune	0.010	98.7	5.1			
		1.250	105.2	2.9			
	Dried beet	0.010	110.1	3.6			
		1.250	105.6	2.8			
Apple juice concentrate	0.002	95.5	5.3				
	1.250	106.9	3.7				
QuPpe/UPLC- MS/MS	Tomato	0.005	111.7	10.6	0.001	0.003	This study
		0.010	88.4	11.9			
		0.050	90.5	12.12			

Source: Own elaboration.

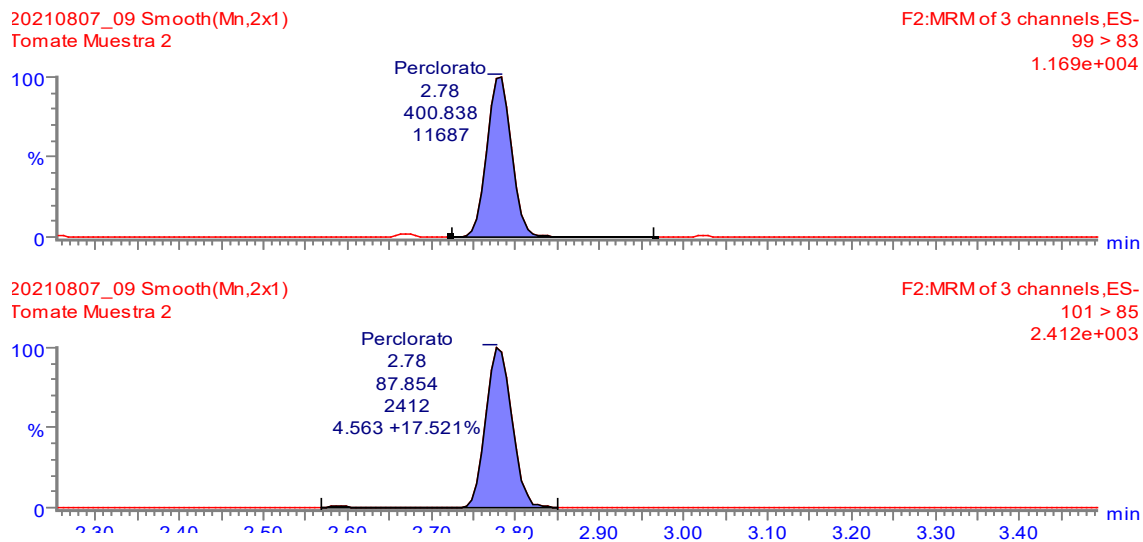


Figure 5. Presence of perchlorate in a tomato sample (0.010 mg/kg) from a local supermarket in Culiacan, Sinaloa, Mexico.

Source: Own elaboration.

Table 4. Perchlorate concentrations in vegetable samples from eight states of the Mexican Republic.

Sample number	Sample	Origin	Location	Perchlorate concentration (mg/kg)	European Commission MRL (mg/kg)*
1	Green pepper	Local supermarket	Culiacan, Sinaloa	0.004	
2	Tomato	Local supermarket	Culiacan, Sinaloa	0.010	
3	Lettuce	Local supermarket	Culiacan, Sinaloa	0.006	
4	Spinach	Local supermarket	Culiacan, Sinaloa	<LOQ	
5	Green pepper	Local supermarket	Tepic, Nayarit	<LOQ	
6	Tomato	Local supermarket	Tepic, Nayarit	<LOQ	
7	Lettuce	Local supermarket	Tepic, Nayarit	0.005	0.05
8	Spinach	Local supermarket	Tepic, Nayarit	<LOQ	
9	Green pepper	Local supermarket	Guaymas, Sonora	0.006	
10	Tomato	Local supermarket	Guaymas, Sonora	0.004	

Continuation

Table 4. Perchlorate concentrations in vegetable samples from eight states of the Mexican Republic.

Sample number	Sample	Origin	Location	Perchlorate concentration (mg/kg)	European Commission MRL (mg/kg)*
11	Lettuce	Local supermarket	Guaymas, Sonora	<LOQ	
12	Spinach	Local supermarket	Guaymas, Sonora	0.033	
13	Tomato	Local supermarket	Mexicali, Baja California	<LOD	
14	Lettuce	Local supermarket	Mexicali, Baja California	<LOD	
15	Spinach	Local supermarket	Mexicali, Baja California	0.004	
16	Green pepper	Local supermarket	Mexicali, Baja California	<LOQ	
17	Tomato	Food market	Monterrey, Nuevo León	0.006	
18	Spinach	Food market	Monterrey, Nuevo León	<LOD	
19	Green pepper	Food market	Monterrey, Nuevo León	0.005	
20	Lettuce	Chinampera producer	Xochimilco, State of Mexico	0.007	
21	Tomato	Local market	Xochimilco, State of Mexico	ND	0.05
22	Spinach	Chinampera producer	Xochimilco, State of Mexico	0.006	
23	Tomato	Local market	Tlaxcala, Tlaxacala	<LOD	
24	Green pepper	Local market	Tlaxcala, Tlaxacala	<LOD	
25	Lettuce	Local market	Tlaxcala, Tlaxacala	0.001	
26	Spinach	Local market	Tlaxcala, Tlaxacala	0.016	
27	Tomato	Local market	Mérida, Yucatán	<LOD	
28	Green pepper	Local market	Mérida, Yucatán	ND	
29	Lettuce	Central market	Mérida, Yucatán	<LOQ	
30	Spinach	Central market	Mérida, Yucatán	<LOQ	
31	Green pepper	Central market	Mérida, Yucatán	<LOD	

Limit of detection (LOD) = 0.001 mg/kg; Limit of quantification (LOQ) = 0.003 mg/kg; ND = Not detected;
*Regulation of the European Commission 2020/685 (European Commission, 2020); maximum permitted residue limit (MRL); values highlighted in bold are greater than the LOQ.

Source: Own elaboration.

Conclusions

An economical, simple, and rapid method was developed for quantifying perchlorate in vegetables using QuPPE extraction and UPLC-MS/MS. The developed method exhibited good linearity ($R^2 > 0.99$), selectivity, precision, and accuracy, with LOD and LOQ values lower than those required by European regulations.

Furthermore, this study is the first to report the presence of perchlorates in Mexico. Finally, the method was successfully applied to tomato, lettuce, green pepper, and spinach collected from different regions of Mexico, demonstrating that its sensitivity is either superior or equivalent to other methods reported in the literature to evaluate perchlorate in vegetables.

In terms of future perspectives, the developed method can be applied to other foods, and its scope can be extended to other analytes with similar properties. The developed method can be used to monitor this contaminant in agricultural products, setting the tone for creating public policies to regulate perchlorate concentrations in foods.

Authors contribution

Conceptualization, JBLM, RC, PJBB, MZS; methodology development, JBLM, RC, PJBB, MZS, COR; software management, PP, PJBB, OAAS, CCL; experimental validation, JBLM, RC, PP, PJBB, COR, CCL, OAAS; analysis of results, JBLM, RC, PP, CRG, OAAS, CCL; data management, JBLM, RC, JGH, PP, PJBB, CRG, OAAS, CCL; manuscript preparation and writing, JBLM, RC, JGH, PP, PJBB, CRG; drafting, revision, and edition, CCL, OAAS, CRG, MZS, JBLM; project administrator, JGH, RC, JBLM; funds acquisition, JGH, RC.

All manuscript authors have read and accepted its published version.

Funding

This Project (Fondo Conjunto de Cooperación Chile-México) was supported by the Agencia Chilena de Cooperación Internacional para el Desarrollo (AGCID) of Chile and Agencia Mexicana de Cooperación Internacional para el Desarrollo (AMEXCID) of México.

Conflict of interest

The authors declare that they hav

no conflicts of interest.

References

- Anastassiades, M., Kolberg, D. I., Benkenstein, A., Eichhorn, E., Zechmann, S., Mack, D., ... & Barth, A. (2023). Quick method for the analysis of numerous highly polar pesticides in foods of plant origin via LC-MS/MS involving simultaneous extraction with methanol (QuPPE-method). EU reference laboratory for pesticides requiring single residue methods (EURL-SRM). CVUA, Stuttgart, Germany. https://www.eurl-pesticides.eu/docs/public/tmpl_article.asp?CntID=887&LabID=200&Lang=EN
- Bastidas-Bastidas, P. D. J., Leyva-Morales, J. B., Olmeda-Rubio, C., Pineda-Landeros, J. M., & Martínez-Rodríguez, I. E. (2019). Comparación de dos métodos para el análisis multiresiduo de plaguicidas organofosforados en productos de origen agrícola con alto y bajo contenido de humedad. *Revista Bio Ciencias*, 6, e654. <https://doi.org/10.15741/revbio.06.nesp.e654>
- Bastidas-Bastidas, P., Cabrera, R., Valenzuela-Quintanar, A. I., Olmeda-Rubio, C., González-Mendoza, V. M., Perea-Domínguez, X. P., ... & Leyva-Morales, J. B. (2024). Validation and Application of UPLC-MS/MS Method to Analysis of Glyphosate and its Metabolites in Water. *Journal of Chromatographic Science*, 62(4), 364-371. <https://doi.org/10.1093/chromsci/bmad045>
- Bauer, A., Luetjohann, J., Rohn, S., Kuballa, J., & Jantzen, E. (2018). Ion chromatography tandem mass spectrometry (IC-MS/MS) multimethod for the determination of highly polar pesticides in plant-derived commodities. *Food Control*, 86, 71-76. <https://doi.org/10.1016/j.foodcont.2017.11.007>
- Calderón, R., Godoy, F., Escudey, M., & Palma, P. (2017). A review of perchlorate (ClO_4^-) occurrence in fruits and vegetables. *Environmental monitoring and assessment*, 189 (82), 1-13. <https://doi.org/10.1007/s10661-017-5793-x>
- Calderon, R., Rajendiran, K., Kim, U. J., Palma, P., Arancibia-Miranda, N., Silva-Moreno, E., & Corradini, F. (2020a). Sources and fates of perchlorate in soils in Chile: A case study of perchlorate dynamics in soil-crop systems using lettuce (*Lactuca sativa*) fields. *Environmental Pollution*, 264, 114682. <https://doi.org/10.1016/j.envpol.2020.114682>
- Calderón, R., Palma, P., Eltit, K., Arancibia-Miranda, N., Silva-Moreno, E., & Yu, W. (2020b). Field study on the uptake, accumulation and risk assessment of perchlorate in a soil-chard/spinach system: Impact of agronomic practices and fertilization. *Science of The Total Environment*, 719, 137411. <https://doi.org/10.1016/j.scitotenv.2020.137411>
- Calderón, R., Palma, P., Arancibia-Miranda, N., Kim, U. J., Silva-Moreno, E., & Kannan, K. (2020c). Occurrence, distribution and dynamics of perchlorate in soil, water, fertilizers, vegetables and fruits and associated human exposure in Chile. *Environmental Geochemistry and Health*, 1-9. <https://doi.org/10.1007/s10653-020-00680-6>
- Calderón, R., Jara, C., Albornoz, F., Palma, P., Arancibia-Miranda, N., Karthikraj, R., & Zhu, H. (2022a). Accumulation and distribution of perchlorate in spinach and chard growing under greenhouse: Implications for food safety in baby foods commodities. *Food Chemistry*, 370, 131101. <https://doi.org/10.1016/j.foodchem.2021.131101>
- Calderón, R., García-Hernández, J., Palma, P., Leyva-Morales, J. B., Zambrano-Soria, M., Bastidas-Bastidas, P. J., & Godoy, M. (2022b). Assessment of pesticide residues in vegetables commonly consumed in Chile and Mexico: Potential impacts for public health. *Journal of*

- Food Composition and Analysis*, 108, 104420. <https://doi.org/10.1016/j.jfca.2022.104420>
- Calderón, R., García-Hernández, J., Palma, P., Leyva-Morales, J. B., Godoy, M., Zambrano-Soria, M., ... & Valenzuela, G. (2023). Heavy metals and metalloids in organic and conventional vegetables from Chile and Mexico: Implications for human health. *Journal of Food Composition and Analysis*, 123, 105527. <https://doi.org/10.1016/j.jfca.2023.105527>
- Chamkasem, N. (2018). Rapid determination of polar pesticides and plant growth regulators in fruits and vegetables by liquid chromatography/tandem mass spectrometry. *Journal of Environmental Science and Health, Part B*, 53(9), 622-631. <https://doi.org/10.1080/03601234.2018.1473977>
- Constantinou, P., Louca-Christodoulou, D., & Agapiou, A. (2019). LC-ESI-MS/MS determination of oxyhalides (chlorate, perchlorate and bromate) in food and water samples, and chlorate on household water treatment devices along with perchlorate in plants. *Chemosphere*, 235, 757-766. <https://doi.org/10.1016/j.chemosphere.2019.06.180>
- Cruz-Cruz, E. A., Ramírez-Torres, A., Pimentel-Nieto, D., & Sánchez, A. M. R. (2014). Prevalence of clinical and subclinical hypothyroidism during pregnancy in a pregnant women population. *Ginecología y Obstetricia de México*, 82(11), 717-724.
- Cutillas, V., & Fernández-Alba, A. R. (2021). Analysis by LC-MS/MS of polar pesticides in fruits and vegetables using new hybrid stationary phase. *MethodsX*, 8, 101306 <https://doi.org/10.1016/j.mex.2021.101306>
- Dong, H., Xiao, K., Xian, Y., Wu, Y., & Zhu, L. (2019). A novel approach for simultaneous analysis of perchlorate (ClO₄⁻) and bromate (BrO₃⁻) in fruits and vegetables using modified QuEChERS combined with ultrahigh performance liquid chromatography-tandem mass spectrometry. *Food Chemistry*, 270, 196-203. <https://doi.org/10.1016/j.foodchem.2018.07.091>
- European Commission. (2020). Commission Regulation (EU) 2020/685 of 20 May 2020 amending Regulation (EC) No 1881/2006 as regards maximum levels of perchlorate in certain foods. <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32020R0685&rid=3>
- Fei, Z., Sun, M., Song, Q., Li, C., & Liu, Y. (2024). Freezing-assisted sugaring-out liquid-liquid extraction coupled with LC-MS/MS for quantitative determination of perchlorate in honey. *Food Chemistry*, 435, 137604. <https://doi.org/10.1016/j.foodchem.2023.137604>
- Flores-Rebollar, A., Moreno-Castañeda, L., Vega-Servín, N. S., López-Carrasco, G., & Ruiz-Juvera, A. (2015). Prevalence of autoimmune thyroiditis and thyroid dysfunction in healthy adult Mexicans with a slightly excessive iodine intake. *Nutrición Hospitalaria*, 32(2), 918-924. <https://doi.org/10.3305/nh.2015.32.2.9246>
- Garrido, Y., Marín, A., Tudela, J. A., Truchado, P., Allende, A., & Gil, M. I. (2020). Chlorate accumulation in commercial lettuce cultivated in open field and irrigated with reclaimed water. *Food Control*, 114, 107283. <https://doi.org/10.1016/j.foodcont.2020.107283>
- Hatzistavros, V. S., & Kallithrakas-Kontos, N. G. (2011). Determination of trace perchlorate concentrations by anion-selective membranes and total reflection X-ray fluorescence analysis. *Analytical chemistry*, 83(9), 3386-3391. <https://doi.org/10.1021/ac103295a>
- Hepperle, J., Wolheim, A., Kolberg, D., Wildgrube, C., Kaufmann-Horlacher, I., Anastasiades, M., & Scherbaum, E. (2013). Analysis of perchlorate in food samples of plant origin applying the QuPPE-method and LC-MS/MS. *Aspects of Food Control and Animal Health*, 2, 2–16. https://ejournal.cvuas.de/docs/cvuas_ejournal_201302.pdf

- Herrera- López, S. H., Scholten, J., Kiedrowska, B., & de Kok, A. (2019). Method validation and application of a selective multiresidue analysis of highly polar pesticides in food matrices using hydrophilic interaction liquid chromatography and mass spectrometry. *Journal of Chromatography A*, 1594, 93-104. <https://doi.org/10.1016/j.chroma.2019.02.024>
- Hu, J., Xian, Y., Wu, Y., Chen, R., Dong, H., Hou, X., ... & Wang, L. (2021). Perchlorate occurrence in foodstuffs and water: Analytical methods and techniques for removal from water—A review. *Food Chemistry*, 360, 130146. <https://doi.org/10.1016/j.foodchem.2021.130146>
- Huertas-Perez, J. F., Mottier, P., Baslé, Q., Tan, S. Y., Kopeć-Durska, M., Zawada, P., ... & Sánchez-Calderón, M. G. (2022). Chlorate and perchlorate—LC-MS/MS analytical method validation in a broad range of food commodities. *Microchemical Journal*, 177, 107281. <https://doi.org/10.1016/j.microc.2022.107281>
- Juárez-Cedillo, T., Basurto-Acevedo, L., Vega-García, S., Sánchez-Rodríguez Martha, A., Retana-Ugalde, R., Juárez-Cedillo, E., ... & Escobedo-de-la-Peña, J. (2017). Prevalence of thyroid dysfunction and its impact on cognition in older mexican adults:(SADEM study). *Journal of endocrinological investigation*, 40 (9), 945-952. <https://doi.org/10.1007/s40618-017-0654-6>
- Kiplagat, I. K., Doan, T. K. O., Kubáň, P., & Boček, P. (2011). Trace determination of perchlorate using electromembrane extraction and capillary electrophoresis with capacitively coupled contactless conductivity detection. *Electrophoresis*, 32(21), 3008-3015. <https://doi.org/10.1002/elps.201100279>
- Kumarathilaka, P., Oze, C., Indraratne, S. P., & Vithanage, M. (2016). Perchlorate as an emerging contaminant in soil, water and food. *Chemosphere*, 150, 667-677. <https://doi.org/10.1016/j.chemosphere.2016.01.109>
- Lee, J. W., Oh, S. H., & Oh, J. E. (2012). Monitoring of perchlorate in diverse foods and its estimated dietary exposure for Korea populations. *Journal of hazardous materials*, 243, 52-58. <https://doi.org/10.1016/j.jhazmat.2012.09.037>
- Leoterio, D. M., Paim, A. P. S., Belian, M. F., Galembeck, A., Lavorante, A. F., Pinto, E., ... & Montenegro, M. C. (2017). Potentiometric perchlorate determination at nanomolar concentrations in vegetables. *Food chemistry*, 227, 166-172. <https://doi.org/10.1016/j.foodchem.2017.01.088>
- Leung, A. M., Pearce, E. N., & Braverman, L. E. (2010). Perchlorate, iodine and the thyroid. *Best Practice & Research Clinical Endocrinology & Metabolism*, 24(1), 133-141. <https://doi.org/10.1016/j.beem.2009.08.009>
- Leyva-Morales, J. B., Valdez-Torres, J. B., Bastidas-Bastidas, P. J., & Betancourt-Lozano, M. (2015). Validation and application of a multi-residue method, using accelerated solvent extraction followed by gas chromatography, for pesticides quantification in soil. *Journal of Chromatographic Science*, 53(10), 1623-1630. <https://doi.org/10.1093/chromsci/bmv063>
- Leyva-Morales, J. B., Cabrera, R., Bastidas-Bastidas, P. D. J., Valenzuela-Quintanar, A. I., Pérez-Camarillo, J. P., González-Mendoza, V. M., ... & Cruz-Acevedo, E. (2023). Validation and application of liquid chromatography coupled with tandem mass spectrometry method for the analysis of glyphosate, aminomethylphosphonic acid (AMPA), and glufosinate in soil. *Agriculture*, 13(6), 1131. <https://doi.org/10.3390/agriculture13061131>
- Liao, Z., Cao, D., Gao, Z., & Zhang, S. (2020). Occurrence of perchlorate in processed foods manufactured in China. *Food Control*, 107, 106813. <https://doi.org/10.1016/j.>

[foodcont.2019.106813](https://doi.org/10.1016/j.foodchem.2019.125028)

- Mathew, J., Gandhi, J., & Hedrick, J. (2005). Trace level perchlorate analysis by ion chromatography–mass spectrometry. *Journal of Chromatography A*, 1085(1), 54-59. <https://doi.org/10.1016/j.chroma.2005.05.015>
- Melton, L. M., Taylor, M. J., & Flynn, E. E. (2019). The utilisation of ion chromatography and tandem mass spectrometry (IC-MS/MS) for the multi-residue simultaneous determination of highly polar anionic pesticides in fruit and vegetables. *Food chemistry*, 298, 125028. <https://doi.org/10.1016/j.foodchem.2019.125028>
- Motzer, W. E. (2001). Perchlorate: problems, detection, and solutions. *Environmental Forensics*, 2(4), 301-311. <https://doi.org/10.1006/enfo.2001.0059>
- Muñoz-Arango, D., Torres-Rojas, F., Tapia, N., Vega, M., Alvear, C., Pizarro, G., ... & Vargas, I. T. (2023). Perchlorate and chlorate assessment in drinking water in northern Chilean cities. *Environmental Research*, 233, 116450. <https://doi.org/10.1016/j.envres.2023.116450>
- Pano-Farias, N. S., Ceballos-Magaña, S. G., Muniz-Valencia, R., & González, J. (2017). Validation and assessment of matrix effect and uncertainty of a gas chromatography coupled to mass spectrometry method for pesticides in papaya and avocado samples. *Journal of food and drug analysis*, 25(3), 501-509. <https://doi.org/10.1016/j.jfda.2016.09.005>
- Panseri, S., Nobile, M., Arioli, F., Biolatti, C., Pavlovic, R., & Chiesa, L. M. (2020). Occurrence of perchlorate, chlorate and polar herbicides in different baby food commodities. *Food chemistry*, 330, 127205. <https://doi.org/10.1016/j.foodchem.2020.127205>
- Pérez-Lizaur, A.B., Palacios-González, B., Castro-Becerra, A.L., & Flores-Galicia, I. (2014) Sistema mexicano de alimentos equivalentes. 3ra ed., *Fomento de Nutrición y Salud*, A.C. México, 63 pp.
- Pihlstrom, T., Fernandez-Alba, A.R., Ferrer Amate, C., Erecius Poulsen, M., Lippold, R., Carrasco Cabrera, L., Pelosi, P., Valverde, A., Mol, H., Jezussek, M., Malato, O., & Stepan, R. (2021). Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis. *in Food and Feed SANTE*, 11312, 2.
- Pizzutti, I. R., Vela, G. M.E., de Kok, A., Scholten, J. M., Dias, J. V., Cardoso, C. D., ... & Vivian, R. (2016). Determination of paraquat and diquat: LC-MS method optimization and validation. *Food chemistry*, 209, 248-255. <https://doi.org/10.1016/j.foodchem.2016.04.069>
- Rajski, Ł., Díaz Galiano, F. J., Cutillas, V., & Fernández-Alba, A. R. (2018). Coupling ion chromatography to Q-orbitrap for the fast and robust analysis of anionic pesticides in fruits and vegetables. *Journal of AOAC International*, 101(2), 352-359. <https://doi.org/10.5740/jaoacint.17-0410>
- Sanchez, C. A., Crump, K. S., Krieger, R. I., Khandaker, N. R., & Gibbs, J. P. (2005). Perchlorate and nitrate in leafy vegetables of North America. *Environmental science & technology*, 39(24), 9391-9397. <https://doi.org/10.1021/es050804k>
- Savini, S., Bandini, M., & Sannino, A. (2019). An improved, rapid, and sensitive ultra-high-performance liquid chromatography-high-resolution orbitrap mass spectrometry analysis for the determination of highly polar pesticides and contaminants in processed fruits and vegetables. *Journal of agricultural and food chemistry*, 67(9), 2716-2722. <https://doi.org/10.1021/acs.jafc.8b06483>
- Seyfferth, A. L., & Parker, D. R. (2006). Determination of low levels of perchlorate in lettuce and spinach using ion chromatography– electrospray ionization mass spectrometry (IC-ESI-

- MS). *Journal of agricultural and food chemistry*, 54(6), 2012-2017. <https://doi.org/10.1021/jf052897v>
- United States Food and Drug Administration [USDA]. (2005). Exploratory Survey Data on Perchlorate in Food 2004-2005. <http://www.fda.gov/Food/FoodbornellnessContaminants/ChemicalContaminants/ucm077685.htm>
- US-EPA. (2000). US-EPA Method 9058. Determination of perchlorate using ion chromatography with chemical suppression conductivity detection. <https://hwbdocs.env.nm.gov/Waste%20Isolation%20Pilot%20Plant/200318.2/U.S.%20EPA.%201996.%20SW-846.pdf>
- Voogt, W., & Jackson, W. A. (2010). Perchlorate, nitrate, and iodine uptake and distribution in lettuce (*Lactuca sativa* L.) and potential impact on background levels in humans. *Journal of agricultural and food chemistry*, 58(23), 12192-12198. <https://doi.org/10.1021/jf101227d>
- Yang, M. J., Kim, J. S., Han, J. H., & Her, N. G. (2011). Development of Enhanced Analytical Method for Perchlorate in Food by IC-MS/MS with an Internal Standard. *Korean Journal of Food Science and Technology*, 43(5), 532-536. <https://doi.org/10.9721/KJFST.2011.43.5.532>
- Zhang, W., Lu, Q., Zheng, R., Qiu, W., Hua, Y., & Li, Y. (2022). Simultaneous determination of chlorate and perchlorate in drinking water and foods using ultra performance liquid chromatography-tandem mass spectrometry. *Research square*, 1-11. <https://doi.org/10.21203/rs.3.rs-2201547/v1>