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Original Article

Abamectin and emamectin in grapes of *Vitis vinifera* L. from a district of the Valley of Ica-Peru

[Abamectina y emamectina en uvas de la *Vitis vinifera* L. de un distrito del Valle de Ica-Perú]

Angel T. Alvarado[1*], Mario Pineda-Pérez[2], Gaby Moreno-Muñoz[3], Jaime Pérez-Montañez[3], Luis Sullón-Dextre[3], Ana María Muñoz[4], Mario Bolarte-Arteaga[5], Nesquen Tasayco-Yataco[6], Berta Loja-Herrera[7], María R. Bendezú[8], Jorge A. García[8], Felipe Surco-Laos[8], Doris Laos-Anchante[8], Haydee Chávez[8], Carmela Ferreyra-Paredes[8]

[1]International Research Unit in Molecular Pharmacology and Genomic Medicine (UNIPHARMAGEM), VRI San Ignacio de Loyola University, La Molina 15024, Lima, Peru.
[2]Pharmacy and Biochemistry, Faculty of Health Sciences, Scientific University of the South, UCSUR, 15067, Lima, Peru.
[3]Molecular Pharmacology Society of Peru, La Molina 15024, Lima, Peru.
[4]Institute of Food Science and Nutrition, ICAN, San Ignacio de Loyola University, La Molina 15024, Lima, Peru.
[5]Human Medicine, Continental University, Los Olivos 15304, Lima, Peru.
[6]Human Medicine, Norbert Wiener University, 15046, Lima, Peru.
[7]Environmental Engineering, San Ignacio de Loyola University, La Molina 15024, Lima, Peru.
[8]Faculty of Pharmacy and Biochemistry, San Luis Gonzaga National University of Ica, 11004, Ica, Peru.
*E-mail: angel.alvaradoy@usil.pe

Abstract

Context: In 14 districts of the valley of Ica-Peru, *Vitis vinifera* L. plants are cultivated that produce grapes for consumption as table grapes and raisins (dried grapes); at the same time, for the production of wines and Piscos.

Aims: To determine the levels of abamectin and emamectin in grapes of *Vitis vinifera* L from a district of the Valley of Ica-Peru.

Methods: 30 lots (30 kg) of Moscatel grape variety *V. vinifera* L. were collected from six countryside (artisanal and organic cultivation) of the San Juan Bautista district. The extraction of abamectin (ABM) and emamectin benzoate (EMB) from the grapes was carried out with acetonitrile; it was quantified by means of Liquid Chromatography coupled to Mass Spectrometry (HPLC-MS). The maximum permissible limit values (MRL) were established at 0.010 ppm for both insecticides.

Results: The determined levels of abamectin and emamectin in grapes were 0.0012-0.015 ppm and 0.0013-0.013 ppm, respectively. Values higher than the maximum permissible limits of abamectin were found in batches A2 (0.0102 ppm), C1 (0.015 ppm), C5 (0.0113 ppm), and F2 (0.012 ppm); emamectin benzoate in lots B1 (0.0113 ppm), B4 (0.013 ppm) and C4 (0.012 ppm). Using the Shapiro-Wilk, Anderson Darling and Student's t tests, it was found that the global means of the values of the two insecticides in grapes are lower than the MRL. According to the global analysis of variance, the means of the concentrations of both insecticides were not different between the six sampling zones (countryside).

Conclusions: The insecticides abamectin and emamectin are below the maximum permissible limit values (0.010 ppm) in Moscatel grapes of *Vitis vinifera* L., so the residual effect would not have implications for human health.

Keywords: abamectin; emamectin; insecticides; Peru; toxicity; *Vitis vinifera*.

Resumen

Contexto: En 14 distritos del valle de Ica-Perú se cultivan plantas de *Vitis vinífera* L. que producen uvas para el consumo como uva de mesa y pasas (uvas secas); a la vez para la producción de vinos y Piscos.

Objetivos: Determinar los niveles de abamectina y emamectina en uvas de la *Vitis vinifera* L de un distrito del Valle de Ica-Perú.

Métodos: Se colectaron 30 lotes (30 kg) de uva variedad Moscatel *V. vinifera* L. de seis campiñas (cultivo artesanal y orgánico) del distrito San Juan Bautista. La extracción de abamectina (ABM) y benzoato de emamectina (EMB) de las uvas, se realizó con acetonitrilo; se cuantifico por medio de Cromatografía Líquida acoplada a Espectrometría de Masas (HPLC-MS). Los valores límites máximos permisibles (MRL) se estableció en 0.010 ppm para ambos insecticidas.

Resultados: Los niveles determinados de abamectina y emamectina en uvas fueron de 0,0012-0,015 ppm y 0,0013-0,013 ppm, respectivamente. Se encontró valores mayores a los límites máximos permisibles de abamectina en lotes A2 (0,0102 ppm), C1 (0,015 ppm), C5 (0,0113 ppm) y F2 (0,012 ppm); benzoato de emamectina en lotes B1 (0,0113 ppm), B4 (0,013 ppm) y C4 (0,012 ppm). Mediante la Prueba de Shapiro-Wilk, Anderson Darling y la t de Student, se encontró que las medias globales de los valores de los dos insecticidas en uvas son menores a los MRL. Al análisis de varianza global las medias de las concentraciones de ambos insecticidas no fueron diferentes entre las seis zonas de muestreo (campiña).

Conclusiones: Los insecticidas abamectina y emamectina se encuentran por debajo de los valores límites máximos permisibles (0,010 ppm) en las uvas Moscatel de *Vitis vinífera* L., por lo que el efecto residual no tendría implicación en la salud humana.

Palabras Clave: abamectina; emamectina; insecticidas; Perú; toxicidad; *Vitis vinifera*.

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AUTHOR INFO
ORCID:
0000-0001-8694-8924 (ATA)
0000-0001-6818-7797 (MPP)
0009-0005-8635-8277 (GMM)
0009-0002-7522-4431 (JPM)
0000-0001-6282-963X (LSD)
0000-0003-3080-9823 (AMM)
0000-0001-9939-8917 (MBA)
0000-0002-6775-4179 (NTY)
0000-0001-6560-0486 (BLH)
0000-0002-3053-3057 (MRB)
0000-0001-9880-7344 (JAG)
0000-0003-0805-5535 (FSL)
0000-0002-2454-7081 (DLA)
0000-0002-8717-4307 (HC)
0000-0001-8872-1555 (CFP)

INTRODUCTION

The cultivation valley of Ica is located on the south-central coast of the Peruvian coast, 299.3 km from the city of Lima-Peru. It has more than 7672 ha of cultivated land for *Vitis vinifera* L. (grape) distributed in the fields that are located in the Districts of San Juan Bautista, Santiago, Subtanjalla, Salas Guadalupe, Tate, Ica, La Tinguiña, Los Aquijes, Los Molinos, Ocucaje, Pachacutec, Parcona, Pueblo Nuevo and Yauca del Rosario; grape harvests are used mainly for the production of wines and Piscos, being the main economy of the Ica region, with a national production of Pisco between 60%-65% (Cáceres and Julca., 2018; Surco-Laos et al., 2023). *Vitis vinifera* L. (vine) belongs to the *Vitaceae* family, genus *Vitis* (flowering plant). It is a perennial plant that lives for decades, whose leaves and fruits (berries or grapes) are according to the variety of the vine (Cáceres et al., 2017; Surco-Laos et al., 2023). In Peru, eight strains of *V. vinifera* L. are cultivated for the production of Peruvian Pisco, such as Albilla, Italia, Mollar, Moscatel, Negra Criolla, Quebranta and Torontel. The Moscatel Vine family includes some 200 varieties, of which the pink Moscatel (red grape or Moscato Rosa del Trentino) is a spice adapted to the Ica Valley that is characterized by pentagonal-shaped leaves, spherical berry (grape) and red in color, which is used in addition to the production of Pisco and wine, as a table grape for its pronounced sweet floral aroma (Cáceres et al., 2017; Jambagi and Kambrekar, 2023; Surco-Laos et al., 2023). Most *V. vinifera* species are infested by fungi, bacteria and insect pests, including some non-arthropod pests, forcing the use of pesticides (insecticides, fungicides, herbicides and others) (Jambagi and Kambrekar, 2023; Martin-Culma and Arenas-Suarez, 2018; Schaaf, 2015), to protect the crops, which translates into increased yield and improved quality of the berries (Aktar et al., 2009; Jambagi and Kambrekar, 2023).

Abamectin is a mixture of two avermectins (80% B1a and 20% B1b). It is the natural avermectin that contains a macrocyclic lactone in its chemical structure (Palma et al., 2006; Salas et al., 2008; Sebestyen et al., 2021; Vázquez-Quintala et al., 2022). This insecticide has a spectrum of action on mobile mites, leaf miners, beetles (Salas et al., 2008), and on the fungi *Phaeomoniella chlamydospora* and *Phaeoacremonium minimal*, responsible for grapevine trunk disease (Sebestyen et al., 2021). Emamectin benzoate (4′-deoxy-4′-epimethylamino-4′-deoxyavermectin B1 benzoate) is a semi-synthetic abamectin insecticide that is produced by amination of C4″ of the disaccharide group of avermectin B1 that retains in its structure chemically a 16-membered macrocyclic lactone, and the derivative obtained is crystallized as a benzoate (Shoaib et al., 2018; Vázquez-Quintala et al., 2022; Wu et al., 2016; Yang et al., 2017; Zhang et al., 2022); It is commonly used by grape growers, and its application rate is 8.4-16.8 g/ha (Shoaib et al., 2018). It is a broad-spectrum insecticide that acts against lepidopteran and coleopteran insects, including lepidopteran larvae that feed on the leaves, whose activity is 1 to 3 times greater than avermectin, with a low residual effect (Shoaib et al., 2018; Zhang et al., 2022; Zhou et al., 2016), sensitive to light and temperature (Shoaib et al., 2018), and due to its low toxic effect on beneficial insects, it becomes a green line insecticide for the control of insect pests (Han et al., 2011; Lee et al., 2023; Yang et al., 2017; Zhou et al., 2016). The mechanism of action of abamectin and emamectin is to stimulate the γ-aminobutyric acid receptor associated with glutamate-regulated chloride channels ($GABA_A$), which increases the permeability of chloride ions in the membrane, generating hyperpolarization, which results in inhibition of the electrical activity of nerves and muscles paralyzing arthropods (Algethami et al., 2023; Salas et al., 2008). Fig. 1 shows the mechanism of action of insecticides and their effect on insects.

It is known that after the application of a certain amount of the pesticide, it is deposited on the plant products, and later they biodegrade. However, a residue of the pesticide or its metabolites remains in the fruits and foods that are detected at harvest or use and is expressed in parts per million (ppm) of the fresh weight of the product. The insecticide residue depends on the chemical structure that provides it with stability against environmental conditions (solar radiation, rain, wind) and on the plant species due to its metabolism, growth rate, and nature of the surface. Such a residue can be high, which has led governments and international organizations to establish maximum residue limits (MRLs) for foods (Algethami et al., 2023).

After carrying out a review in the PubMed-NCBI database on the studies of the levels (ppm) of abamectin and emamectin in fruits of *V. vinifera* from the Region of Ica and Peru, it has been shown that chemical-toxicological, pharmacological and human studies are scarce, despite the consumption of grapes directly (table grapes) or in the form of wine and Pisco, throughout Peru, for which it warrants carry out studies of this nature. The findings of this study are of special interest to the Peruvian and world population due to the possible impact it would have on Public Health. Therefore, the present study was designed to determine the maximum permissible limit values of abamectin and emamectin in grapes of *V. vinifera* from a field in the Valley of Ica-Peru and its implication of the residual effect on human health.

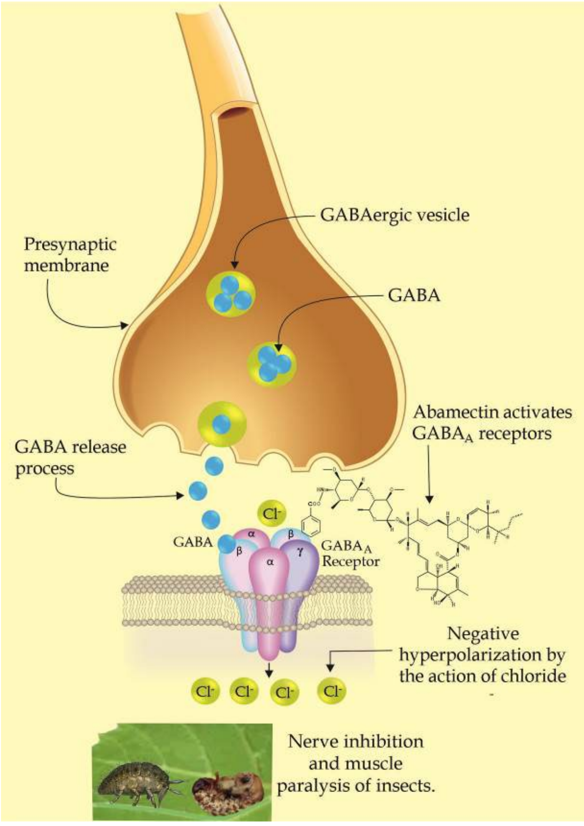

Figure 1. Mechanism of action of abamectin/emamectin on GABA$_A$ receptors of mites and insects.

The GABAergic vesicle is observed in the axoplasm, and within it, the neurotransmitter ϒ-aminobutyric acid (GABA); then, the process of GABA release at the presynaptic level and the activation of the GABA$_A$ receptor are proposed. It is also observed that abamectin activates the GABA$_A$ receptor, generating the opening of the chloride channel for the entry of the chloride ion (Cl^-), which causes negative hyperpolarization. This inhibits the nerves and produces muscular paralysis of the insects. Figure made by the authors.

MATERIAL AND METHODS

Type of study and sampling time

An experimental and cross-sectional study was carried out from January to March 2022.

Chemical and reagents

Certified reference standards of abamectin (lote 718501, CPAchem brand, Bulgarian-French company) and emamectin (lote 718800, CPAchem brand, Bulgarian-French company) were used, with a purity of 97.1% and 99.5%. Acetonitrile, water and methanol, in HPLC grade (Merck Brand, Germany); 98% formic acid and ammonium formate, LC-MS grade, sodium hydroxide (NaOH), anhydrous magnesium sulfate ($MgSO_4$), sodium chloride (NaCl), sodium citrate dihydrate (Merck brand, Germany).

Location area, study population, and sampling methodology

The samples of berries (grapes) of the Moscatel variety *V. vinifera* were collected from six countryside (cultivated in an artisanal way, without pesticides and considered organic grapes) on the road of kings in the San Juan Bautista district (14°00'41" S, 75°44'06" W at 426 m.a.s.l), Ica Province, Ica Region, Peru. The study population consisted of 30 lots of grape fruits (30 kg), which corresponded to six countrysides, which were identified as countryside A, B, C, D, E and F, and 5 lots were sampled from each countryside of grapes (each batch of 1 kg) and assigned the letters A (A1, A2, A3, A4 and A5), B (B1, B2, B3, B4 and B5), C (C1, C2, C3, C4 and C5), D (D1, D2, D3, D4 and D5), E (E1, E1, E3, E4 and E5) and F (F1, F2, F3, F4 and F5). The collected samples were transported to the Instrumental Analysis laboratory of the Faculty of Pharmacy and Biochemistry, San Luis Gonzaga National University of Ica, maintaining a cold chain and under quality criteria to avoid decomposition and contamination of the grape fruits. The organic grape (white matrix) was purchased at the Ica City Supermarket, 1 lot of 5 kg. The grape fruits were frozen until the moment of analysis (Carrasco et al., 2013; García-Ceccarelli et al., 2023; Huaccho et al., 2012; Ramos-Escudero et al., 2012; Surco-Laos et al., 2022a; 2022b).

Standard and reagent solutions

From the stock solution (1000 mg/L) of abamectin and emamectin, it was diluted with acetonitrile, obtaining a mixed solution of insecticides at a concentration of 10 mg/L. To generate the calibration curves, the standard mixtures were diluted with acetonitrile in the concentration range of 1-250 µg/L (seven concentration points). Standard solutions were protected from light and stored at -20°C. Fig. 2 shows the two calibration curves of the standards.

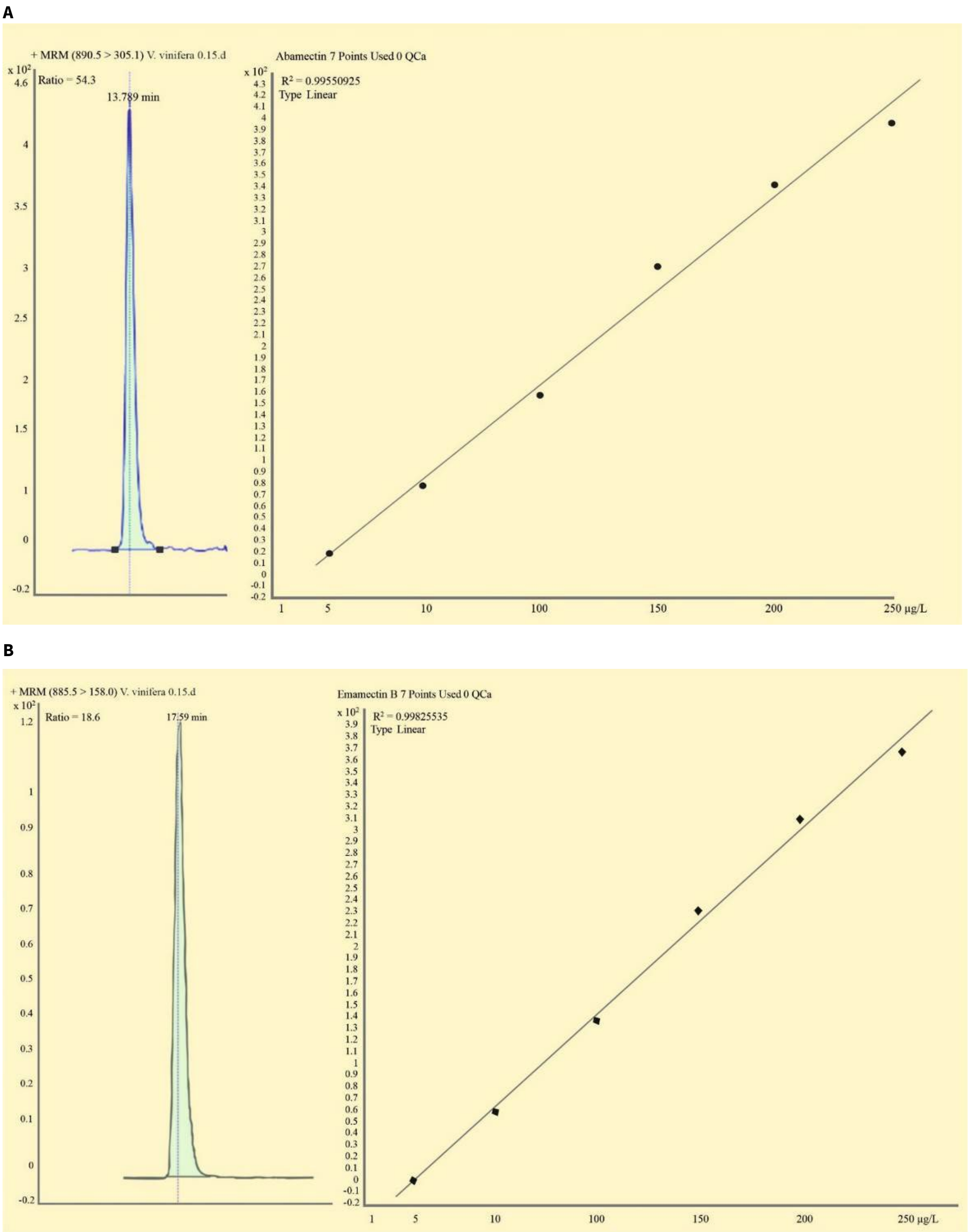

Figure 2. Chromatograms and calibration curves of abamectin standard and emamectin benzoate diluted in acetonitrile.

(A) Chromatogram of the abamectin standard and its calibration curve; **(B)** Chromatogram of the emamectin benzoate standard is indicated. Both chromatograms were determined at 10 µg/L, and both calibration curves were analyzed at 1, 5, 10, 100, 150, 200, 250 µg/L concentrations. All analyses were performed in triplicate (n = 3).

Sample preparation

The frozen grape fruit samples were crushed, then 10 g of the homogenized sample was weighed in a 50 m**L** polypropylene centrifuge tube and centrifuged at 1500 rpm for 1 min**.** Then a mixture of 4 g $MgSO_4$, 1 g NaCl, 1 g sodium citrate dihydrate was added. It was shaken manually for 2 min and immediately centrifuged at 3000 rpm for 5 min. The obtained supernatant was filtered through syringes with 0.22 µm filters (Whatman, WA, USA). 200 µL of the filtered extract was measured**,** and 200 µL of acetonitrile (to extract the insecticides) + 600 µL (methanol/water) + 10 µL of 5% formic acid were added. The final extract was analyzed by Liquid Chromatography (LC 1290 Infinity from Agilent) coupled with Mass Spectrometry (Agilent 6495 triple Quadrupole Mass Spectrometer), quantifying with triphenyl phosphate (internal standard) that helps to correct the area of the analyte. To obtain the white matrix solution (pesticide-free organic grapes), we proceeded in the same way as the sample. The quantification analysis was performed in triplicate.

Processing techniques

The injecting process in the equipment was carried out sequentially for 20 samples: solvent blank, the calibration curve solution, matrix blank, sample and internal standard (curve control). Two mobile phases were used: mobile phase 1 (5 mM 0.1% formic acid in water for HPLC) was added 5 mL of 1 M ammonium formate and 1 mL of formic acid to 1 liter of water. Mobile phase 2 (5 mM 0.1% formic acid in methanol for HPLC MS-MS) was added 5 mL of 1 M ammonium formate and 1 mL of formic acid to 1 liter of methanol. The 1 M ammonium formate solution was obtained by dissolving 6.31 g of ammonium formate in 100 mL of water. Twenty samples per day were analyzed in the Liquid Chromatography coupled to Mass Spectrometry (LC-MS) equipment. Data processing was performed using the Masshunter Workstation Software.

LC-MS equipment and analysis conditions

The conditions of the mass detector equipment were programmed as follows: 120°C (gas temperature), 25 psi (nebulizer), 11 L/min (shet gas flow), and capillary in positive mode 3500V and negative mode 3000V.

Analytical studies

System aptitude, linearity, precision, accuracy, and specificity of the quantification method by Liquid Chromatography coupled to Mass Spectrometry (LC-MS: Agilent 6495 triple Quadrupole Mass Spectrometer) were evaluated. Regarding the aptitude of the system, it was found that the reproducibility of the retention time (RSD ≤1% acceptance criteria) and area reproducibility (RSD ≤2%) for abamectin and emamectin were within the acceptance criteria. Linearity was analyzed in a range of 1-250 µg/mL, with a correlation coefficient (R) of 0.9998 and 0.9999 and a coefficient of determination (R^2) of 0.9955 and 0.9982 for abamectin and emamectin, respectively. Precision was assessed by the relative standard deviation of repeatability (RSD ≤2%), and relative standard deviation of intermediate precision (RSD ≤2%); accuracy by percent recovery (98-102%) and relative standard deviation of recovery (RSD ≤2%), both parameters were within the acceptance criteria.

The specificity was evaluated to detect interference of the mobile phase, blank and diluents with the insecticide, being specific the response for the abamectin standard at 13.789 m and for emamectin it was 12.59 m; A similar response was obtained with the samples enriched with both insecticides, no response was found with the mobile phase, blank, extractants or with the diluent. Table 1 specifies the values and acceptance criteria for the validation of the method.

Statistical analysis

The results were transcribed into an Excel spreadsheet, from where they were exported for statistical analysis. Data were expressed as mean ± standard deviation (SD), the Shapiro-Wilk test, Anderson-Darling test, Student's t-test, and one-factor analysis of variance (ANOVA) were calculated. A $p<0.05$ was considered statistically significant. The GraphPad Prism 9 Statistical Software was used. Version 9.1.2 (Carrasco et al., 2013; Chávez et al., 2021).

RESULTS AND DISCUSSION

It was determined that the levels of abamectin (ABM) and emamectin benzoate (EMB) of organic grapes (white matrix) were on average of 0.00554 ppm (SD 0.00203) and 0.00756 (SD 0.00083), respectively. Table 2 shows ABM levels above the maximum permissible limit values (MRL) in lots A2 (0.0102 ppm), C1 (0.015 ppm), C5 (0.0113 ppm), and F2 (0.012 ppm); and emamectin benzoate (EMB) in lots B1 (0.0113 ppm), B4 (0.013 ppm) and C4 (0.012 ppm). These values were found despite the fact that they are grapes from producing areas that do not use insecticides; however, as there are large extensions of vine plants that are cultivated by agro-exporters, it is likely that they use pesticides and these are carried by the wind to other cultivation areas. The Shapiro-Wilk Test was applied for a sample of less than 50 (study sample 30), whose results indicate that the grape lots analyzed for ABM and EMB present p = 0.328 and 0.849, respectively ($p>0.05$), that is, that the 30 batches analyzed come from a normal distribution, to corroborate this result, the Anderson Darling Test was applied (for n>20 and n <1000) with a 95% confidence interval, concluding that the grape samples (n = 30 lots) do come from a normal distribution. Based on the two previous statistical tests, the Student's t parametric test was applied. Despite the fact that there were 7 batches with values of both pesticides above the MRL, the Student's t-test confirmed that the global means of

Table 1. Validation criteria of the HPLC method for abamectin and emamectin.

Analytical performance parameters	Criteria of acceptance	Results	
		Abamectin	Emamectin
System aptitude			
Retention time reproducibility	RSD ≤ 1%	0.45%	0.23%
Area reproducibility	RSD ≤ 2%	0.78%	0.39%
Linearity			
Equation of the line	Y = bx ± a	y = 4.1968x + 0.8309	y = 4.592x + 0.8797
Correlation coefficient	R ≥ 0.995	R = 0.9998	R = 0.9999
Determination coefficient	R^2 = 0.990	R^2 = 0.9955	R^2 = 0.9982
Precision			
Relative standard deviation of repeatability	RSD ≤ 2%	RSD = 0.568%	RSD = 0.979%
Relative standard deviation of intermediate precision	RSD ≤ 2%	RSD ≤ 0.896%	RSD = 0.597%
Accuracy			
Recovery percentage	98-102 %	100.97%	101.54%
Relative standard deviation of recovery	RSD ≤ 2%	0.423%	0.645%
Specificity			
Standard response	There is answer	At 13.789 m	At 12.59 m
Sample response enriched	There is answer	At 13.789 m	At 12.59 m
Mobile phase response	There is no answer	At 13.789 m	At 12.59 m
Response of extractants and diluent	There is no answer	At 13.789 m	At 12.59 m
Blank sample response	There is no answer	At 13.789 m	At 12.59 m
Method interval			
Linearity	1-250 µg/mL	Linear	Linear
Precision	1-250 µg/mL	Accurate	Accurate
Accuracy	1-250 µg/mL	Exact	Exact

the ABM and EMB values in grapes is lower than the MRL (0.010 ppm = 0.010 mg/kg) established in the Sanitary Technical Standard of the General Directorate of Environmental Health (DIGESA) NTS No. 128-MINSA/2016/DIGESA approved by Resolution of the Ministry of Health of Peru, RM No. 1006-2016/MINSA (MINSA, 2016). There is a similar standard in the European Union in which an MRL of 0.010 ppm has been established for rice (Deng et al., 2020); for other crops, in Japan MRL 0.10 ppm EMB has been defined in cabbage and Chinese cabbage (Yoshii et al., 2000); in China, it is 0.05 ppm in cabbage and 0.10 ppm for savoy (Deng et al., 2020; Wang et al., 2012), as observed in Table 3.

Table 4 shows the average values of the concentration levels of the two insecticides analyzed. The global analysis of variance (ANOVA) of the ABM and EMB shows that the means of the values (ppm) among the 6 sampling countryside of the berries (grapes) are not statistically significant with a p = 0.373 and 0.154 respectively ($p>0.05$); in this sense, it is concluded that the means of the concentrations of ABM/EMB are not different between the six sampling zones (road of kings countryside) of the district of San Juan Bautista in Valle de Ica. While the means of the ABM/EMB (ppm) values in each field are statistically significant at the level of significance of 0.05 with a probability that the mean values of each sample lot are not above the maximum permissible limit values (MRL) ($p<0.05$).

The World Health Organization (WHO) classifies EMB as Category II (moderately hazardous pesticide) (Wang et al., 2012; WHO, 2020); In a study carried out by Antonenko et al. (2022), it is established that ABM and EMB belong to Class III (moderately persistent pesticides) and Class IV (non-persistent pesticides), respectively. It has also been reported that the acceptable daily intake (ADI) of EMB is 0.0005 mg/kg

Table 2. Concentration levels of insecticides in berries (grapes) in six countrysides and each one with five sampling lots.

Countryside/ lot	Abamectin allowable value (ppm) Mean ± SD	Emamectin allowable value (ppm) Mean ± SD	Countryside/ lot	Abamectin allowable value (ppm) Mean ± SD	Emamectin allowable value (ppm) Mean ± SD
A1	0.0037 ± 0.0005	0.0083 ± 0.0002	D1	0.0043 ± 0.0001	0.0043 ± 0.0002
A2	0.0102 ± 0.0001	0.0063 ± 0.0001	D2	0.0055 ± 0.0001	0.0051 ± 0.0001
A3	0.0057 ± 0.0002	0.0069 ± 0.0002	D3	0.0094 ± 0.0001	0.0084 ± 0.0001
A4	0.0036 ± 0.0003	0.0078 ± 0.0001	D4	0.0088 ± 0.0001	0.0042 ± 0.0002
A5	0.0087 ± 0.0002	0.0083 ± 0.0001	D5	0.0052 ± 0.0002	0.0074 ± 0.0002
B1	0.0093 ± 0.0001	0.0113 ± 0.0006	E1	0.0068 ± 0.0001	0.0052 ± 0.0001
B2	0.0025 ± 0.0001	0.0024 ± 0.0001	E2	0.0049 ± 0.0002	0.0026 ± 0.0003
B3	0.0038 ± 0.0001	0.0089 ± 0.0001	E3	0.0059 ± 0.0002	0.0092 ± 0.0001
B4	0.0075 ± 0.0001	0.0130 ± 0.0015	E4	0.0076 ± 0.0001	0.0072 ± 0.0001
B5	0.0022 ± 0.0002	0.0023 ± 0.0003	E5	0.0053 ± 0.0001	0.0064 ± 0.0001
C1	0.0150 ± 0.0010	0.0096 ± 0.0001	F1	0.0045 ± 0.0001	0.0084 ± 0.0001
C2	0.0012 ± 0.0001	0.0013 ± 0.0001	F2	0.0120 ± 0.0010	0.0098 ± 0.0001
C3	0.0012 ± 0.0001	0.0073 ± 0.0015	F3	0.0058 ± 0.0001	0.0053 ± 0.0001
C4	0.0046 ± 0.0001	0.0120 ± 0.0006	F4	0.0096 ± 0.0001	0.0066 ± 0.0001
C5	0.0113 ± 0.0001	0.0084 ± 0.0001	F5	0.0053 ± 0.0001	0.0059 ± 0.0002

(0.5 µg/kg) (Jyot et al., 2014), that is, for a 70 kg adult, its ADI would be 0.035 mg (35 µg), which indicates that this value would be tolerated and safe, therefore, if we assume an average consumption of 100 g of berries (grapes) and multiply by the minimum (0.0013 ppm = mg/kg) and maximum (0.013 ppm = mg/kg) observed in the present study, the intake of the mentioned insecticide turns out to be 0.00013 mg (0.13 µg) and 0.0013 mg (13 µg), respectively, these calculated values would be safe compared to your ADI. Therefore, the values found in the present study would not have effects on human health, despite the fact that the reports of pesticides in grapes are limited or scarce, there are previous studies in other plant species, which indicate that the residual values EMB are not harmful to humans, as indicated by Zhou et al. (2016) in a study of the residual effect of EMB in tea. It was found that this insecticide at the recommended dose is negligible for humans (0.005 mg/kg in tea leaves and 0.0004 mg/L in infusion) according to the risk quotient (RQ) value, which was significantly less than 1, and it is concluded that it is safe to consume the tea in China. However, in other studies carried out in experimental animals and in humans, harmful effects have been reported. Zhang et al. (2017) demonstrated that EMB inhibits the viability of QSG7701 cells (normal human hepatocyte cell line) and are inducers of DNA damage. Chromatin condensation and DNA fragmentation, loss of mitochondrial membrane potential, releasing cytochrome-c, increasing Bax/Bcl-2 ratio, activating caspase-3/9, and apoptosis of QSG7701 cells were observed. In another study, Abou-Zeid et al. (2018) demonstrated that EMB induced high malondialdehyde (MDA) levels, suppressed reduced glutathione (GSH) levels, deficient catalase (CAT) and superoxide dismutase (SOD) activity, which leads to oxidative stress in liver and kidney of mice; At the brain level, an increase in the level of MDA and inhibition of SOD activity were observed. A significant increase in the *CYP2E1* and *GSTM1* genes was also observed. It is known that the *GSTM1* gene located on the short arm of chromosome 1 in region 13.3 (1p13.3) encodes different glutathione S-transferase (GST) isoenzymes. At the same time, said gene presents a homozygous *GSTM1*0* deletion that expresses an enzyme without activity. This generates genomic instability that predisposes to various types of cancer (Alvarado et al., 2021). Recently, Niu et al. (2020) have reported that EMB induces the release of cytochrome-c, activates caspase-3/9, and increases the Bax/Bcl-2 ratio, inducing cytotoxicity in cultured human bronchial epithelial cells (16HBE) associated with mitochondrial apoptosis and DNA damage. Based on these results, EMB would have a potential genotoxic effect on human lung cells. At high doses of ABM and other macrocyclic lactones consumed by humans, adverse effects such as hypotension progressing to respiratory failure, neuronal hyperarousal, coma, and even death have been observed (Chen-Chang, 2012).

Table 3. Maximum permissible limit values (MRL) in grapes and compared with other values established for cabbage and rice worldwide.

Type of insecticide	Maximum permissible limit values (MRL)			
	Technical standard in Peru (Grape ppm)	Technical standard in European Union (Rice ppm)	Technical standard in Japan (Cabbage ppm)	Technical standard in China (Cabbage ppm)
Emamectin benzoate	0.010	0.010	0.10	0.05
Abamectin	0.010	NR	NR	NR

NR: Not reported.

Table 4. Mean concentration values of abamectin and emamectin in berries (grapes) from six San Juan Bautista district countrysides.

Sampling zone	Abamectin			Emamectin benzoate		
	Mean ± SD	95%CI	p-value	Mean ± SD	95%CI	p-value
A	0.00638 ± 0.0029	0.0027-0.0101	2.17×10^{-10}	0.00752 ± 0.0009	0.0064-0.0086	4.78×10^{-8}
B	0.00506 ± 0.0029	0.0029-0.0072	9.80×10^{-15}	0.00758 ± 0.0047	0.0042-0.0109	1.34×10^{-8}
C	0.00666 ± 0.0058	0.0035-0.0099	1.05×10^{-11}	0.00772 ± 0.0037	0.0057-0.0098	1.12×10^{-7}
D	0.00664 ± 0.0021	0.0057-0.0076	1.12×10^{-13}	0.00588 ± 0.0018	0.0051-0.0067	2.94×10^{-11}
E	0.00610 ± 0.0010	0.0057-0.0065	2.12×10^{-9}	0.00612 ± 0.0022	0.0052-0.0070	1.21×10^{-12}
F	0.00744 ± 0.0029	0.0063-0.0085	6.65×10^{-9}	0.00720 ± 0.0017	0.0066-0.0078	1.56×10^{-12}

In a study reported by Abdu-Allah and Pittendrigh (2017), the topical LD_{50} of EMB was established at 0.00006 µg/bee with a potency 133.3 times more toxic than ABM (LD_{50} 0.008 µg/bee). Orally, the LD_{50} was established at 7.65 µg/bee for the two insecticides. According to the results, the residual concentration levels of the two insecticides analyzed in the berries (grapes) would not have an impact on the environment, especially on bees, which are key to biodiversity, due to the function of pollinating more than 70% of the berries plants, that is, more than 4,000 species of plants survive thanks to bees.

The limitations of the present study are in the number and variety of grapes studied. The other limitations that can lead to bias or confusion are having carried out the study in only one district of the Ica Valley, despite the fact that there are 14 districts with their respective fields that grow organic grapes, not having taken blood samples from the inhabitants of the countryside to know the critical concentrations of insecticides in cells and organs, at the same time to know the cumulative effect during the three months of grape consumption (January-March), for which reason the research team is considering evaluating all of this in a future study (January-March 2024). Notwithstanding the foregoing, we believe that this study is relevant, as it assesses levels of insecticides in the berries (grapes) that can be considered as part of quality control and as evidence for the Agriculture Sector and Ministry of Health of Peru.

CONCLUSION

It is concluded that the insecticides abamectin and emamectin are below the maximum permissible limit values (0.010 ppm) in *Vitis vinifera* Moscatel grapes. In this sense, its residual effect would not have implications for human health.

It is important to continue studying, especially to know the cumulative effect on human organs after three months or more.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Alvarado AT	Pineda M	Moreno G	Pérez J	Sullón L	Muñoz AM	Bolarte M	Tasayco N	Loja B	Bendezú MR	García JA	Surco F	Laos D	Chávez H	Ferreyra C
Concepts or ideas	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Design	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Definition of intellectual content	x	x	x	x	x	x									
Literature search							x	x	x	x	x	x	x	x	x
Experimental studies	x	x	x	x						x	x	x	x	x	x
Data acquisition	x	x	x	x			x	x							
Data analysis		x			x	x	x	x	x						
Statistical analysis		x	x	x					x						
Manuscript preparation	x	x	x	x		x									
Manuscript editing	x	x					x				x			x	
Manuscript review	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

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Supplementary data

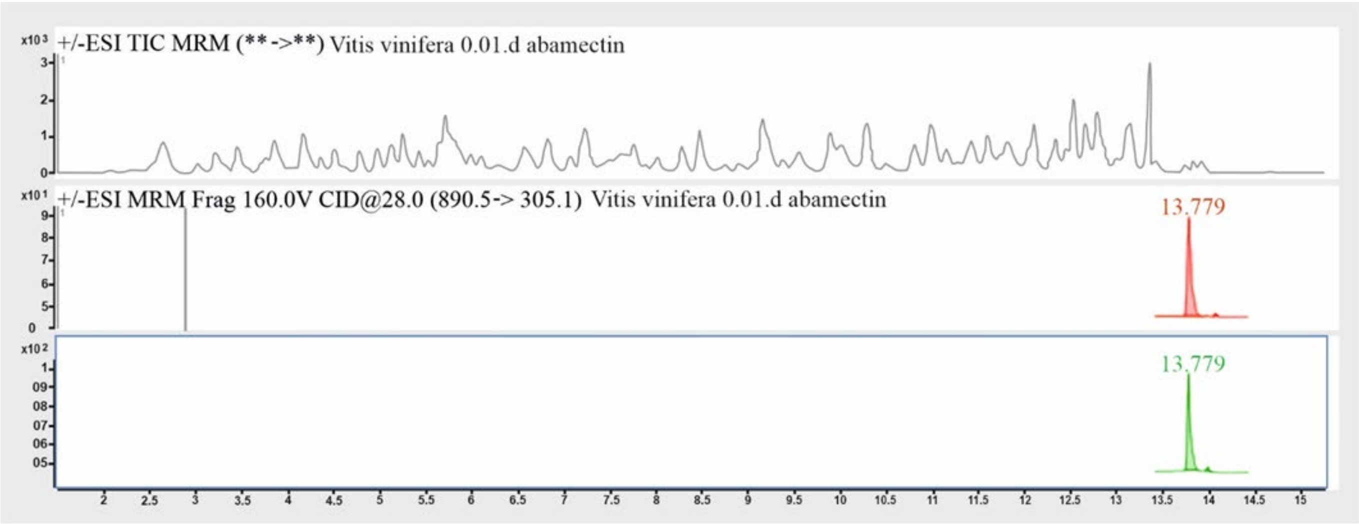

Figure 1S. Abamectin standard chromatogram.

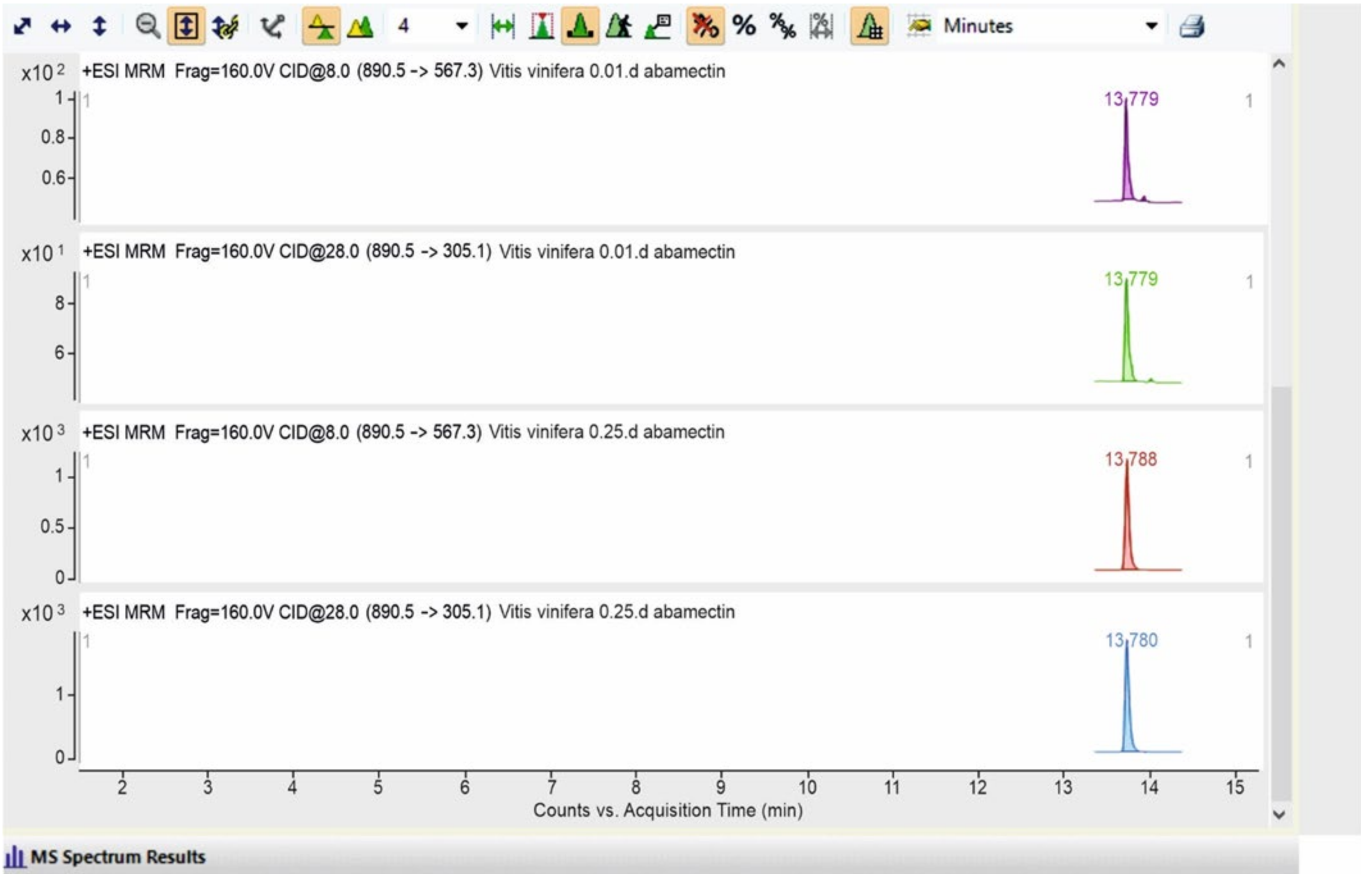

Figure 2S. Chromatogram of the standard and samples spiked with abamectin.

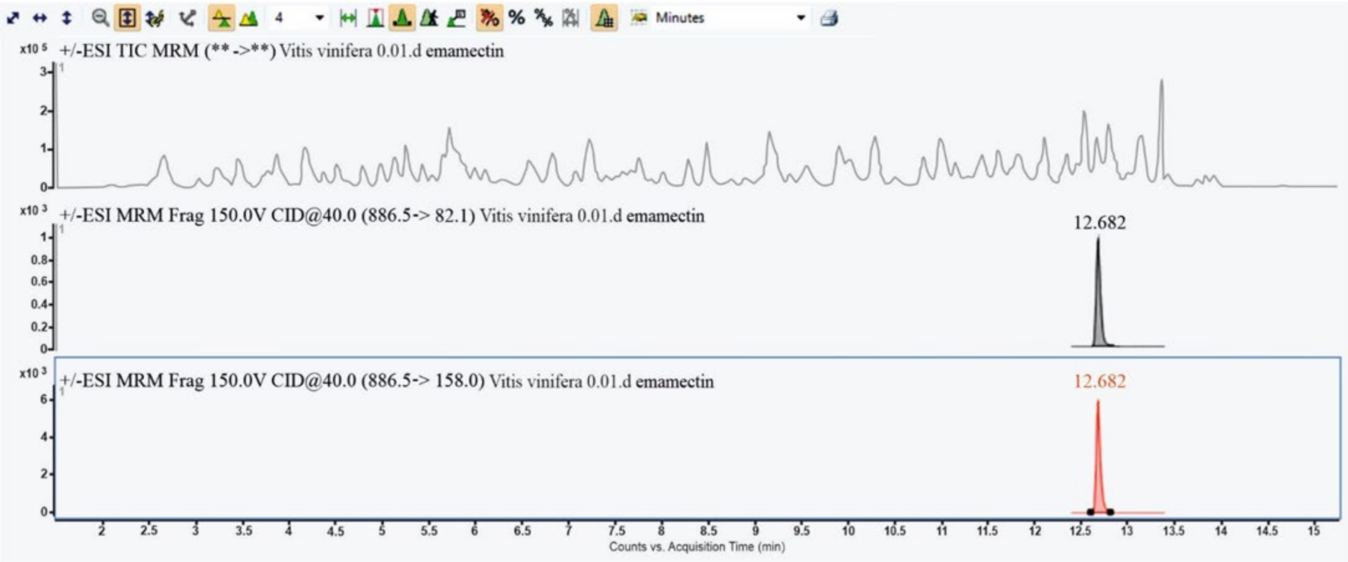

Figure 3S. Emamectin standard chromatogram.