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Multi-residue method of pesticides by UPLC-MS/MS in bivalve mollusks samples as a tool for food quality and safety

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Abstract

Bivalve molluscs (BM) are filter animals and bioaccumulators of substances from the environment. This characteristic allows a great absorption of nutrients that makes them a source of protein-rich foods. On the other hand, if there are toxic contaminants in the environment, their absorption by animals may occur. This happens with pesticides coming from agricultural and livestock production systems that can migrate to areas of BM crops. Considering the high nutritional value of bivalve molluscs and their positive impact on the human diet, these products must be carefully evaluated for the possible presence of toxic substances in order to guarantee their safety.

Thus, the aim of this work was to implement and validate a multi-residue method using tandem mass spectrometry to evaluate pesticide residues commonly used in agricultural production systems present in these matrices. Extraction and cleaning steps were optimized and the method proved to be adequate to quantify 322 pesticides. The samples come from five different areas of culture of bivalve molluscs in the southeast, north and northeast regions of Brazil. The analysis of the mollusc samples showed the presence at the trace level of seven different pesticide residues in four of the five evaluated samples.

Keywords: mollusk bivalves; pesticides; UPLC-MS/MS; oyster; scallop.

Practical Application: Evaluation of the contamination by pesticides in samples of bivalve molluscs to guarantee food safety; to point out the presence of pesticides coming from areas far from the shellfish cultivations that may represent possible contamination for of the environment.

1 Introduction

In recent years, aquaculture has become one of the most developed food sources (Food and Agriculture Organization of the United Nations, 2018). The expansion of this type of activity is one of the most viable and sustainable alternatives for the production of high-protein foods for human consumption. In the future, this branch of activity may assume an even more important role capable of meeting global needs in terms of food production and nutrition.

The increase in fish consumption favored, at the same time, the development of oyster crops, which are considered a delicacy appreciated in several continents and have become an excellent option for genuinely protein food. Oysters are also an important source of minerals, amino acids, glycogen and essential fatty acids (Asha et al., 2014). This composition, rich in nutritional components, is essential to define its quality and commercial value.

On the other hand, the intensification of agricultural activities to increase food production and meet world demand has resulted in the intense use of pesticides in plantations to control diseases and parasites. The drift of pesticides to non-agricultural areas is a common problem in regions of intensive agriculture (Cech et al., 2022). Cui et al. (2020) showed that the pollution load of diffuse origin from agricultural activities depends on the amounts and frequency of irrigation and fertilization in the plantations. These pesticide residues are not restricted to the application site, but can be diverted to surrounding areas due to droplets that evaporate before reaching the target and travel with fine particles of spray, air movement and volatilization during application (European Food Safety Authority, 2008), or later by leaching and soil erosion (Linhart et al., 2019; Zivan et al., 2017). Burket et al. (2018) observed in samples of mussels and oysters collected in the outskirts of Hong Kong, the presence of pesticide substances at low levels (µg/kg). The presence of potentially toxic substances started to be commonly observed in surface waters as well as their accumulation in animals that make use of it. Its monitoring can point out possible contamination in food by pesticides that originate from their intense application in adjacent agricultural areas (Iliff et al., 2019; Bringer et al., 2021).

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The place where bivalve mollusks are cultivated is a very important factor that directly affects the quality of the product and also its characteristics as food. The risk associated with human exposure to pesticides occurs notably through ingestion. The diet of the population constitutes the main route of human exposure to pesticide residues, contributing with more than 90% of the total exposure (Fazal et al., 2022; Riaz et al., 2018). It should be noted that oysters are filtering animals and, admittedly, bioaccumulators of substances present in the environment in which they live, such as nutrients, minerals, microorganisms and also chemical contaminants. When these animals develop in polluted waters they can become vehicles of contamination for humans through the food route (Petrarca et al., 2022; Hussein et al., 2022). The health of these foods is essential and, as the cultivation of bivalve mollusks is intended to compose the human diet, these products must be carefully evaluated for the presence of contaminants in order to guarantee their safety and health (Onac et al., 2022). In order to carry out monitoring for the evaluation of contaminants in mollusks, it is essential to implement a validated analytical method for the matrices of interest capable of answering questions about the presence of toxic substances potentially harmful to human health.

The objective of this work was to validate a multiresidue method in order to investigate the occurrence of pesticide residues in samples of bivalve molluscs. For this, samples of molluscs from different regions of Brazil were evaluated. Four samples of *Crassostrea gasar* oysters and one sample of scallops (*Nodipecten nodosus*) were analyzed. The multiresidues method was implemented and validated for these matrices. The extraction and clean up conditions were optimized for the quantification of 322 active ingredients from pesticide residues of different chemical classes commonly used in agricultural production systems.

Residue analyzes were performed using the extraction method called QuEChERS (acronym for Quick, Easy, Cheap, Effective, Rugged, Safe) (Anastassiades et al., 2003). The complexity of the study matrix makes it difficult to quantify a large number of analytes, requiring a purification step (clean-up) of the extract and quantification of the active ingredients of pesticides using chromatographic methods coupled with sequential mass spectrometry (Lehotay et al., 2007).

2 Material and methods

2.1 Chemicals and reagents

Acetonitrile and methanol, LC-MS grade, were purchased from BIO-GRADE Chem (San Francisco, CA, USA); formic acid was obtained from Merck (Darmstadt, Germany) and ammonium formate was purchased from Fluka (Steinhein, Swirtzerland). $MgSO_4$ was obtained from Sigma-Aldrich (St. Louis, MO, USA); NaCl ACS grade were purchased from F. Maia (São Paulo, Brazil); citrate tribasic sodium dehydrate purchased from Vetec (Rio de Janeiro, Brazil); sodium hydrogen citrate sesquihydrate ACS grade was purchased from Sigma-Aldrich (St. Louis, MO, USA);Bondesil® PSA from Agilent Technologies (Santa Clara, CA, USA); ultrapure water was obtained from Milli-Q Advantage A10 Millipore System at 18.2 MΩ cm−1 (Molsheim, France); all the 346 pesticide reference standards were purchased from Dr. Ehrenstorfer Laboratory (Augsburg, Germany) and manufactured under ISO 17034.

2.2 Standards solutions

The standard solutions used in this study were prepared and stored at concentrations ranged from 1000 to 430 µg/mL and stored in a freezer at -25 °C. Calibration curves were constructed with a minimum of six points with concentration levels ranging from 0.005 to 0.08 µg/mL.

2.3 Bivalve samples

The culture of molluscs that generated the samples used in this work was structured after several stages that began with the domestication of native species such as *C. gasar.* The entire production process has been optimized from seed cultivation, animal handling and harvesting to obtain individuals in commercial size to add the correct value to the product.

Bivalve mollusc samples were acquired from five mollusc farms. A total of 5 samples were collected from four oyster farms and one scallop farm. Sample collection was carried out in the same way in the five regions. Each sample actually represents a total of 2.5-3.0 kg of oyster or scallop meat. This makes a considerable number of individuals.

The oysters came from four different regions located in the north/northeast of Brazil and a single sample of scallops (Nodipecten nodosus) grown on a farm in the southeastern region. These 5 samples were named A, B, C, D and E, respectively.

2.4 Fortified samples

The fortified oyster sample used in the recovery trials was prepared by mixing samples from each of the four cultures from the four different states in equal proportions. To this composite sample, 1.0 or 0.5 mL of a standard pesticide solution (MIX) of 346 pesticides of different functional classes was added to carry out the tests at two levels of fortification - 0.02 or 0.01 $mg.kg^{-1}$, respectively.

The fortified scallop sample was obtained by mixing the meat of all the animals collected and sent for analysis in the laboratory. In a sample containing about 10 g of this homogenized material, the Mix of 346 certified pesticide standards was added for the recovery tests at the same two levels of fortification.

2.5 Sample preparation

All samples were collected in a representative way in the different farms, properly packaged and kept at 4 °C until their arrival at the Residues and Contaminants Laboratory until the beginning of the analyses. Sample preparation consists of homogenizing the mollusk muscle tissue. The samples were properly ground in a food processor and stored in flasks in a freezer kept at -20 °C until the moment of analysis.

2.6 Extraction and clean-up method

The QuEChERS method was used (Anastassiades et al., 2003; Lehotay et al., 2007) as described below. About 10 ± 0.5 g

homogenized samples were weighed in a 50 mL polypropylene tube, 10 mL of extraction solvent (ACN) and 1 mL of ultrapure water were added. After vortexing for 1 min, 1 g NaCl, 4 g MgSO4, 1 g sodium citrate and 0,5 g sodium citrate sesquihydrate were added. The mixture was vortexed for another 1 min, subjected to ultrasound for 20 minutes and centrifuged at 4000 rpm for 5 min.

A 7 mL aliquot of the supernatant was transferred to a 10 mL test tube and left in the freezer at -20 °C for 2 hours so that the fat layer could decant. After centrifugation at 4000RPM for 5 min, a 5 mL aliquot of supernatant was transferred to another 15 mL test tube, containing 125 mg of Bondesil - PSA and 750 mg of MgSO4. After homogenation using vortex for 1 min, the tubes were centrifuged at 4000 rpm for 5 min. Finally, 2.0 mL of supernatant were transferred to a vial for UPLC/MS-MS analysis. All the analytical conditions of the method used in this work are summarized in Figure 1.

2.7 UPLC-ESI-MS/MS analysis

All pesticide residue analyzes were performed using an ultra-performance liquid chromatography system coupled with tandem mass spectrometry (UPLC-MS/MS).

The ultra-pure water was produced in the RiOs-Advantage A10 model water deionizer - Milli-Q® (Merck Millipore); The Waters ultra-efficiency liquid chromatograph, model Acquity UPLC® (Milford, USA) coupled to the Waters sequential mass spectrometer model Quattro Premier XE® (Milford, USA). The Waters Acquity UPLC® chromatograph has a binary pump system, automatic injector, degasser and column oven.

An elution gradient was used starting with mobile phase A (5 mmol L^{-1} ammonium formate in water with 10% methanol) with 82.5% (v/v) with a linear ramp until reaching 5.5% of the same. linear curve phase in 25 minutes.

The Quattro Premier XE® mass spectrometer was operated in electrospray ionization (ESI) and multiple reaction monitoring (MRM) in positive and negative modes. The collision gas was argon and the desolvation gas was nitrogen. Table 1 presents the chromatographic parameters of the UPLC-MS/MS system.

Table 1. Conditions of multi-residue analysis of pesticides by UPLC-MS/MS.

UPLC	
Analytical column	BEH C_{18} (1.7 µm, 100 × 2,1 mm)
Pre-column	VanGuard® BEH C18 (Waters, USA)
Column temperature	35 °C
Mobile fase	A - 5 mmol L^{-1} ammonium formate in methanol 10%; B - Methanol
Injection volume	5 µL
Flow rate	0.3 mL min^{-1}
MS/MS	
Electronspray Source	ESI^+;ESI^-
Capillar voltage	0.98 kV
Source temperature	110 °C
Interface	Electrospray (Z-Spray)
Cone gas flow	Nitrogen (50 Lh^{-1})
Heated desolvation gas	Nitrogen; 400 °C
Colision gas	Argon (3.5 10^{-3} mbar)

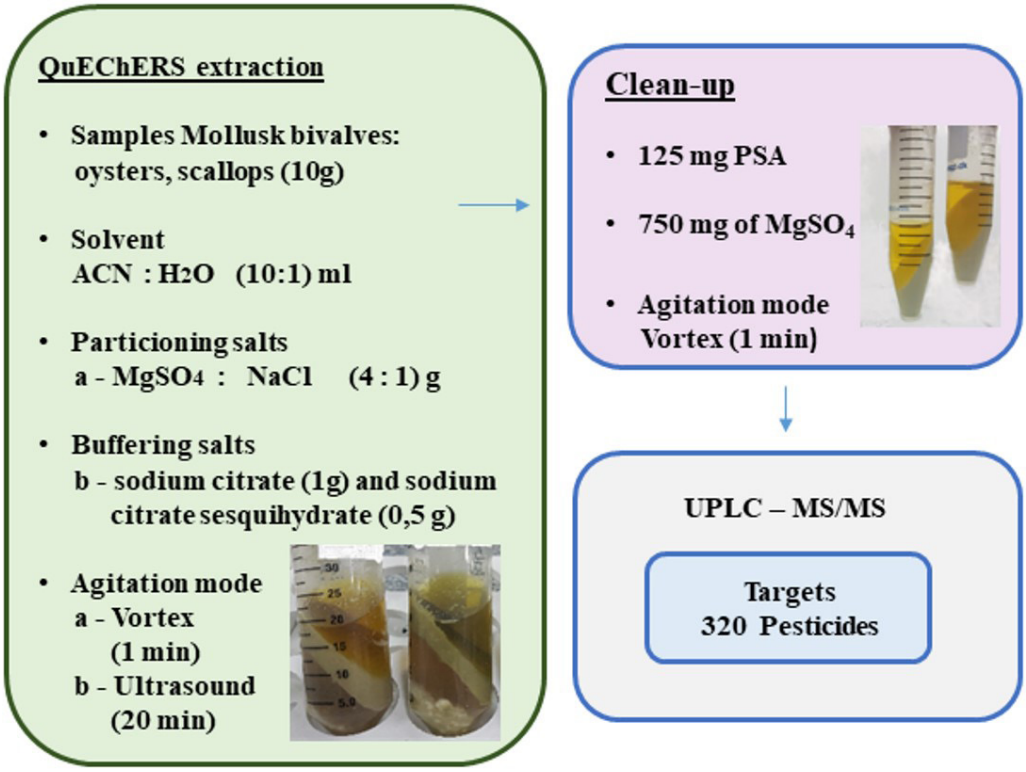

Figure 1. Analytical protocol used in the multi-residue monitoring of 320 pesticides in this work.

2.8 Method validation

The use of an analytical method to quantify pesticide residues in a matrix requires careful validation of all parameters involved and also the scope that must be achieved. The validation of the multi-residue method carried out in this study is suitable to the criteria described in SANTE (European Union, 2020) which specifically addresses the performance of methods for the analysis of contaminants in food.

The identity criteria specifically used in the sequential mass spectrometer to confirm the pesticide peaks in the samples were (1) Signal-to-noise ratio greater than or equal to 3; (2) Signals from the transitions between the precursor ion and its fragments (quantitation transition and confirmation transition) totally superimposed; (3) Sample retention time meeting a tolerance criterion of ± 0.1 minute in relation to the standard retention time; (4) Relative intensity of the ionic transitions detected in the evaluated sample and in the standard, is expressed as the ratio of the intensity of the most abundant transition and the corresponding transition of the standard. Assays are performed using the same concentrations and analytical conditions, meeting a tolerance criterion of up to 30% (European Union, 2020).

2.9 Pesticides monitored in this study

The parameters related to the mass spectrometer of all tested pesticides were individually determined in the UPLC-MS/MS system. The transitions used for the monitoring of pesticides are shown in Table 2, which contains only the active ingredients that were considered tested and approved by the validation of this method.

3 Results and discussion

3.1 Method validation

The QuEChERS method was used (Anastassiades et al., 2003; Lehotay et al., 2007) to evaluate the pesticide residues in all mollusk bivalve samples. The validation of the multiresidue method constitutes the most important and critical part of this work and was carried out for the two matrices studied - oysters and scallops. All steps of the proposed method must meet the quality criteria to be considered valid.

Each step of the analytical procedure was separately optimized for all 346 pesticide standards surveyed in this study. The method validation was performed using the following parameters: accuracy (expressed as recovery), precision (expressed as RSD), linearity (expressed as R^2), limit of quantification and detection in accordance with the European Union (2020).

3.2 Optimization of extraction / clean-up

QuEChERS extraction parameters such as organic solvent (MeOH and/or ACN), amount of water and added acid were tested considering the response of samples fortified with Mix of standards in the UPLC-MS/MS system. Both organic solvents had a good response. However, the mixture of ACN:H_2O (10:1 v/v) presented better performance than the others.

The cleaning step using only PSA and MgSO4 produced extracts free of impurities for analysis. These results obtained with ACN and cleaning salts are compatible with previous studies using the QuEChERS method with fish samples.

3.3 Recovery and precision

One of the most important quality assessment parameters is accuracy. The recovery tests of all analytes in each matrix, native oyster Crassostrea gasar and scallop Nodipecten nodosus, were carried out at two recovery levels: 0.02 and 0.01 $mg.kg^{-1}$ of sample. The first recovery level corresponds to the first point on the curve and the second level to half of this value. These recovery tests were carried out on purpose at these low levels of concentration as we expected to find residues at really very low concentrations as is usually observed in the literature.

The study of the recovery and precision of all these 346 active principles was done through recovery tests in the matrix fortified with the MIX of standards as indicated in item 2.4.

Considering the linearity parameters (curves and the R^2 correlation coefficients), the accuracy and precision obtained for the 346 standards tested in this work, allowed proving that the vast majority (322 pesticides) among all the tested standards presented recovery in the range of 70 to 120%, recommended by the EU (European Union, 2020). In this specific case, the recovery was in the range of 76 to 106% and the accuracy was in the range of 3.5 to 13.6%. These values are fully consistent and within the criteria established by the EC. These results are very good and positively attest to the application of this method for the evaluation of pesticide residues in oyster and scallop meat.

3.4 Linearity, LOD and LOQ

The determination of the working ranges as well as the linearity of the calibration curves were evaluated by the correlation coefficients (R^2) of the 322 standards considered approved by the recovery tests. In addition, all calibration curves of pesticide standards provided R^2 values greater than 0.95.

The limits of detection and quantification (LOD and LOQ) in the UPLC-MS/MS system were calculated using the signal-to-noise (s/r) ratio. The default values in this s/r ratio correspond to 3 for the limit of detection (LOD, s/r = 3) and 10 for the limit of quantification (LOQ, s/r = 10). In our case, the concentration of the first point of the curves corresponds to the LOQ. Therefore the values of detection and quantification limits for almost all analytes correspond to LOD=2 ng/mL and LOQ=6 ng/mL or LOD=0.2 and LOQ=0.6 ng/g of the sample.

3.5 Analysis of oyster and scallop samples

The application of this multi-residue method of pesticides, validated for the two matrices in question, was carried out for the four samples of oysters and one of scallops produced in different cultures in the north / northeast / southeast of Brazil, being called A, B, C, D and E. All samples were fully evaluated following the analytical procedure described in the Material and Methods item.

Table 2. Monitored transitions of evaluated pesticides (to be continued).

Pesticide	MS/MS transitions (*m/z*)	Pesticide	MS/MS transitions (*m/z*)
2,4-D	219 > 161 | 221 > 163	Bromophos methyl	366 > 125 | 369 > 125
2,4-DB	247 > 161 | 247 > 125	Bromuconazole	376 > 159 | 376 > 70
2,6-Dichlorobenzamide	190 > 109 | 190 > 145	Bupirimate	317 > 108 | 317 > 272
3-Hydroxycarbofuran	238 > 163 | 238 > 181	Buprofezin	306 > 201 | 306 > 116
Abamectin	891 > 305 | 891 > 567	Butachlor	312 > 238 | 312 > 162
Acephate	184 > 143 | 184 > 95	Butocarboxim	213 > 75 | 213 > 116
Acetamiprid	223 > 126 | 223 > 90	Butocarboxim-sulfoxide	207 > 132 | 207 > 75
Acetochlor	270 > 224 | 270 > 148	Cadusafos	271 > 159 | 271 > 215
Acibenzolar-S-methyl	211 > 136 | 211 > 140	Carbaryl	219 > 145 | 219 > 127
Alachlor	270 > 238 | 270 > 162	Carbendazim	192 > 160 | 192 > 132
Alanycarb	400 > 238 | 400 > 91	Carbetamide	237 > 192 | 237 > 118
Aldicarb	191 > 116 | 191 > 89	Carbofuran	222 > 165 | 222 > 123
Aldicarb sulfone	223 > 86 | 223 > 76	Carbosulfan	381 > 118 | 381 > 160
Aldicarb sulfóxide	207 > 132 | 207 > 89	Carboxin	236 > 143 | 236 > 87
Ametryn	228 > 186 | 228 > 96	Carfentrazone-ethyl	412 > 346 | 412 > 266
Amicarbazone	242 > 143 | 242 > 85	Carpropamid	334 > 139 | 334 > 196
Aminocarb	209 > 137 | 209 > 152	Cartap	238 > 73 | 238 > 150
Atrazine	216 > 174 | 216 > 96	Chlorantraniliprole	484 > 453 | 484 > 286
Azaconazole	300 > 159 | 300 > 231	Chlorbromuron	294 > 206 | 294 > 182
Azadirachtin	719 > 687 | 719 > 491	Chlordimeform	197 > 46 | 197 > 117
Azamethiphos	325 > 112 | 325 > 139	Chlorfluazuron	540 > 383 | 540 > 158
Azinphos-ethyl	345 > 132 | 345 > 160	Chlorimuron-ethyl	415 > 186 | 415 > 83
Azinphos-methyl	318 > 132 | 318 > 104	Chloroxuron	291 > 72 | 291 > 164
Azocyclotin	369 > 205 | 369 > 287	Chlorpyrifos	350 > 98 | 350 > 97
Azoxystrobin	404 > 372 | 404 > 329	Chlorpyrifos-methyl	322 > 125 | 322 > 290
Benalaxyl	326 > 148 | 326 > 294	**Clethodim** *	358 > 238 | 358 > 268
Bendiocarb	224 > 167 | 224 > 109	Clofentezine	303 > 138 | 303 > 102
Benfuracarb	411 > 252 | 411 > 158	Clomazone	240 > 125 | 240 > 89
Bentazone *	239 > 132 | 239 > 197	Clorfenvinphos	359 > 99 | 359 > 127
Bifenazate	301 > 170 | 301 > 198	Clothianidin	250 > 169 | 250 > 132
Bitertanol	338 > 99 | 338 > 70	Coumaphos	363 > 307 | 363 > 289
Boscalid	343 > 307 | 343 > 271	Cumyluron	303 > 185 | 303 > 125
Pesticide	**MS/MS transitions (*m/z*)**	**Pesticide**	**MS/MS transitions (*m/z*)**
Cyazofamid	325 > 108 | 325 > 261	DMSA	201 > 92 | 201 > 137
Cycloxydim	326 > 280 | 326 > 180	DMST	215 > 106 | 215 > 79
Cyfçufenamid	413 > 203 | 413 > 295	Dodemorph	282 > 116 | 282 > 98
Cyfluthrin	451 > 191 | 451 > 127	Dodine	228 > 57 | 228 > 60
Cyhexatin	369 > 205 | 369 > 287	Doramectin	917 > 331 | 917 > 593
Cymoxanil	199 > 128 | 199 > 111	Emamectin benzoate	886 > 126 | 886 > 302
Cypermethrin	433 > 191 | 433 > 416	Epoxiconazole	330 > 121 | 330 > 123
Cyproconazole	292 > 70 | 292 > 125	Eprinomectin	915 > 186 | 915 > 144
Cyprodinil	226 > 93 | 226 > 108	EPTC	190 > 128 | 190 > 86
Cyromazine	167 > 60 | 167 > 125	Esfenvalerate	437 > 167 | 439 > 169
Daimuron	269 > 151 | 269 > 91	Esprocarb	266 > 91 | 266 > 71
Deltamethrin	523 > 281 | 523 > 506	Ethidimuron	265 > 208 | 265 > 114
Demeton-S-methyl	231 > 89 | 231 > 61	Ethiofencarb	226 > 107 | 226 > 169
Desmedipham	318 > 182 | 318 > 136	Ethiofencarb-sulfone	275 > 107 | 275 > 201
Diafenthiuron	385 > 329 | 385 > 278	Ethiofencarb-sulfoxide	242 > 107 | 242 > 185
Diazinon	305 > 169 | 305 > 97	Ethion	385 > 199 | 385 > 143
Dichlorvos	221 > 109 | 221 > 127	Ethirimol	210 > 140 | 210 > 98
Diclofuanid	350 > 123 | 350 > 224	Ethofumesate	287 > 121 | 287 > 259
Dicrotophos	238 > 112 | 238 > 72	Ethoprophos	243 > 131 | 243 > 97
Diethofencarb	268 > 226 | 268 > 124	Etiprole	414 > 351 | 414 > 255
Difenoconazole	406 > 251 | 406 > 188	Etobenzanid	340 > 179 | 340 > 149
Difenoxuron	287 > 122 | 287 > 71	Etofenprox	394 > 177 | 394 > 107
Diflubenzuron	311 > 158 | 311 > 113	Etoxazole	360 > 141 | 360 > 57
Dimethenamid	276 > 244 | 276 > 168	Etrimfos	293 > 125 | 293 > 265
Dimethoate	230 > 199 | 230 > 125	Famoxadone	392 > 331 | 392 > 238
Dimethomorph	388 > 301 | 388 > 165	Fenamidone	312 > 92 | 312 > 236

Caption: pesticides with * in bold are analyzed in ESI- mode; the others are analyzed in ESI+ mode.

Table 2. Continued...

Pesticide	MS/MS transitions (*m/z*)	Pesticide	MS/MS transitions (*m/z*)
Dimoxystrobin	327 > 116 | 327 > 89	Fenamiphos	304 > 217 | 304 > 202
Diniconazol	326 > 70 | 326 > 159	Fenarimol	331 > 268 | 331 > 81
Dinotefuran	203 > 129 | 203 > 123	Fenazaquin	307 > 57 | 307 > 161
Dioxacarb	224 > 167 | 224 > 123	Fenbuconazole	337 > 125 | 337 > 70
Disulfoton	275 > 89 | 275 > 61	Fenhexamid	302 > 97 | 302 > 55
Diuron	233 > 72 | 233 > 160	Fenitrothion	278 >184 | 278 > 125
Pesticide	MS/MS transitions (*m/z*)	Pesticide	MS/MS transitions (*m/z*)
Fenobucarb	208 > 95 | 208 > 152	Heptenophos	251 > 127 | 251 > 109
Fenoxycarb	302 > 88 | 302 > 116	Hexaconazole	314 > 70 | 314 > 159
Fenpropathrin	367 > 125 | 367 > 250	Hexythiazox	353 > 228 | 353 > 168
Fenpropidin	274 > 147 | 274 > 86	Imazalil	297 > 159 | 297 > 69
Fenpropimorph	304 > 147 | 304 > 130	Imazapic	276 > 231 | 276 > 163
Fenpyroximate	422 > 366 | 422 > 138	Imazapyr	262 > 69 | 262 > 86
Fenthion	279 > 169 | 279 >105	Imazaquin	312 > 266 | 312 > 86
Fenthion-sulfoxide	295 > 109 | 295 > 79	Imazethapyr	290 > 245 | 290 > 86
Fenuron	165 > 72 | 165 > 46	Imazosulfuron	413 > 153 | 413 > 156
Fenvalerate	437 > 167 | 439 > 169	Imibenconazole	411 > 125 | 411 > 171
Fipronil	435 > 330 | 435 > 250	Imidacloprid	256 > 175 | 256 > 209
Flonicamid	230 > 203 | 230 > 148	Indoxacarb	528 > 203 | 528 > 218
Fluazifop-p-butyl	384 > 282 | 384 >328	Ioxynil	370 > 127 | 370 > 243
Fluazinam *	463 > 416 | 463 > 398	Iprovalicarb	321 > 119 | 321 > 203
Flubendiamide	683 > 274 | 683 > 408	Isocarbamid	186 > 87 | 186 > 130
Flufenacet	364 > 194 | 364 >152	Isocarbophos	291 > 231 | 291 > 121
Flufenoxuron	489 > 158 | 489 > 141	Isofenphos	346 > 245 | 346 > 217
Fluoxastrobin	459 > 427 | 459 > 188	Isoprocarb	194 > 95 | 194 > 137
Fluquinconazole	376 > 349 | 376 > 108	Isoprothiolane	291 > 231 | 291 > 189
Flusilasole	316 > 247 | 316 > 165	Isoproturon	207 > 72 | 207 > 46
Flusulfamide	413 > 171 | 413 >179	Isoxaflutole	359 > 251 | 359 > 220
Fluthiacet-methyl	404 > 274 | 404 > 215	Isoxathion	314 > 105 | 314 > 286
Flutolanil	324 > 262 | 324 > 65	Ivermectin	893 > 307 | 893 > 569
Flutriafol	302 > 70 | 302 > 123	Karbutilate	278 > 179 | 278 > 134
Fluxapyroxad	382 > 342 | 382 > 314	Kresoxim-methyl	314 > 116 | 314 > 267
Fomesafen *	437 > 195 | 437 > 286	Lactofen	479 > 344 | 479 > 462
Forchlorfenuron	248 > 129 | 248 > 93	Lambda-cyhalothrin	467 > 225 | 467 > 450
Formetanate	222 > 165 | 222 > 93	Linuron	249 > 160 | 249 > 182
Fuberidazole	185 > 157 | 185 > 156	**Lufenuron** *	509 > 323 | 509 > 339
Furalaxyl	302 > 95 | 302 > 242	Malathion	331 > 127 | 331 > 99
Furathiocarb	383 > 195 | 383 > 252	Mandipropamid	412 > 328 | 412 > 125
Halofenozide	331 > 275 | 331 > 105	Mefenacet	299 > 148 | 299 > 120
Pesticide	**MS/MS transitions (*m/z*)**	**Pesticide**	**MS/MS transitions (*m/z*)**
Mepanipyrim	224 > 106 | 224 > 77	Omethoate	214 > 183 | 214 > 125
Mephosfolan	270 > 140 | 270 > 196	Oxadiargyl	341 > 151 | 341 > 230
Mepronil	270 > 119 | 270 > 91	Oxadixyl	279 > 219 | 279 > 132
Mesotrione	340 > 228 | 340 > 104	Oxamyl	237 > 72 | 237 > 90
Metalaxyl-M	280 > 220 | 280 > 192	Oxamyl Oxime	163 > 72 | 163 > 90
Metamidophos	142 > 94 | 142 > 125	Oxycarboxin	268 > 175 | 268 > 147
Metconazole	320 > 70 | 320 > 125	Paclobutrazol	294 > 70 | 294 > 125
Methfuroxan	230 > 137 | 230 > 111	Penconazole	284 > 70 | 284 > 159
Methidation	303 > 145 | 303 > 85	Pencycuron	329 > 125 | 329 > 218
Methiocarb	226 > 169 | 226 > 121	Pendimethalin	282 > 212 | 282 > 194
Methiocarb-sulfone	275 > 122 | 275 > 201	Permethrin	408 > 183 | 408 > 335
Methiocarb-sulfoxide	242 > 185 | 242 > 122	Phenmedipham	301 > 168 | 301 > 136
Methomyl	163 > 88 | 163 > 106	Phentoato	321 > 247 | 321 > 163
Methoprene	311 > 279 | 311 > 191	Phosalone	368 > 182 | 368 > 111
Methoprotryne	272 > 198 | 272 > 170	Phosmet	318 > 160 | 318 > 133
Metobromuron	259 > 170 | 259 > 148	Phosphamidon	300 > 174 | 300 > 127
Metoxuron	229 > 72 | 229 > 156	Phoxim	300 > 129 | 300 > 125
Metoxyfenozide	369 > 149 | 369 > 313	Picoxystrobin	368 > 205 | 368 > 145
Metrafenone	409 > 209 | 409 > 227	Piperonyl butoxide	356 > 177 | 356 > 119

Caption: pesticides with * in bold are analyzed in ESI- mode; the others are analyzed in ESI+ mode.

Table 2. Continued...

Pesticide	MS/MS transitions (*m/z*)	Pesticide	MS/MS transitions (*m/z*)
Metribuzin	215 > 131 | 215 > 89	Pirimicarb	239 > 72 | 239 > 182
Metsulfuron-methyl	382 > 167 | 382 > 199	Pirimicarb-desmethyl	225 > 72 | 225 > 168
Mevinphos	225 > 127 | 225 > 193	Pirimiphos-ethyl	334 > 198 | 334 > 182
Molinate	188 > 126 | 188 > 55	Pirimiphos-methyl	306 > 108 | 306 > 67
Monalide	240 > 85 | 240 > 128	Prochloraz	376 > 308 | 376 > 266
Monocrotophos	224 > 127 | 224 > 98	Profenofos	375 > 305 | 375 > 347
Monolinuron	215 > 148 | 215 > 99	Prometon	226 > 184 | 226 > 86
Moxidectin	641 > 528 | 641 > 498	Prometryne	242 > 158 | 242 > 200
Myclobutanil	289 > 70 | 289 > 125	Propanil	218 > 162 | 218 > 127
Neburon	275 > 88 | 275 > 57	Propargite	368 > 231 | 368 > 175
Nitenpyram	271 > 225 | 271 > 126	Propazine	230 > 146 | 230 > 188
Norflurazon	304 > 284 | 304 > 160	Propham	180 > 120 | 180 > 138
Novaluron	493 > 158 | 493 > 141	Propiconazole	342 > 69 | 342 > 159
Nuarimol	315 > 252 | 315 > 81	Propoxur	210 > 111 | 210 > 93
Pesticide	**MS/MS transitions (*m/z*)**	**Pesticide**	**MS/MS transitions (*m/z*)**
Propyzamide	256 > 190 | 256 > 173	**Teflubenzuron ***	379 > 339 | 379 > 196
Proquinazid	373 > 289 | 373 > 331	Temephos	467 > 419 | 467 > 125
Prothioconazole	344 > 189 | 344 > 326	Tepraloxydim	342 > 250 | 342 > 166
Pymetrozin	218 > 105 | 218 > 78	Terbufos	289 > 103 | 289 > 57
Pyraclostrobin	388 > 194 | 388 > 163	Terbumeton	226 > 170 | 226 > 114
Pyrazophos	374 > 222 | 374 > 194	Terbutryn	242 > 186 | 242 > 91
Pyridaben	365 > 147 | 365 > 309	Tetraconazole	372 > 159 | 372 > 70
Pyridaphention	341 > 189 | 341 > 92	Thiacloprid	253 > 126 | 253 > 90
Pyrifenox	295 > 93 | 295 > 66	Thiobencarb	257 > 124 | 257 > 100
Pyrimethanil	200 > 107 | 200 > 82	Thiodicarb	355 > 88 | 355 > 108
Pyriproxyfen	322 > 96 | 322 > 185	Thiofanate-metyl	343 > 151 | 343 > 93
Quinalphos	299 > 163 | 299 > 147	Thiofanox	219 > 57 | 219 > 76
Quinoxyfen	308 > 197 | 308 > 162	Thiofanox-sulfone	268 > 57 | 268 > 76
Quizalofop-P-ethyl	379 > 211 | 379 > 115	Thiofanox-sulfoxide	252 > 235 | 252 > 178
Rotenone	395 > 213 | 395 > 192	Tiabendazole	202 > 175 | 202 > 131
Sebuthylazine	230 > 174 | 230 > 96	Tiamethoxam	292 > 211 | 292 > 181
Siduron	233 > 94 | 233 > 137	Tolclofos-metyl	301 > 269 | 301 > 175
Simazine	202 > 132 | 202 > 124	Tolylfluanide	363 > 238 | 363 > 137
Simetryn	214 > 124 | 214 > 96	Triadimefon	294 > 69 | 294 > 197
Spinetoram	749 > 142 | 749 > 98	Triadimenol	296 > 70 | 296 > 99
Spinosad A	733 > 142 | 733 > 98	Triazophos	314 > 162 | 314 > 119
Spinosad D	747 > 142 | 747 > 98	Triclhorfon	257 > 109 | 257 > 127
Spirodiclofen	411 > 71 | 411 > 313	Tricyclazole	190 > 162 | 190 > 136
Spiromesifen	371 > 273 | 371 > 255	Tridemorph	298 > 57 | 298 > 98
Spirotetramat	374 > 330 | 374 > 302	Trifloxystrobin	409 > 186 | 409 > 145
Spiroxamine	298 > 144 | 298 > 100	Triflumizole	346 > 278 | 346 > 73
Sulfentrazone	387 > 146 | 387 > 307	Triflumuron	359 > 156 | 359 > 139
Tebuconazole	308 > 70 | 308 > 125	Triflusulfuron-methyl	493 > 264 | 493 > 96
Tebufenozide	353 > 133 | 353 > 297	Triforine	435 > 390 | 435 > 215
Tebufenpyrad	334 > 117 | 334 > 145	Triticonazole	318 > 70 | 318 > 125
Tebupirimfos	319 > 276 | 319 > 153	Vamidothion	288 > 146 | 288 > 118
Tebuthiuron	229 > 172 | 229 > 116	Zoxamide	336 > 187 | 336 > 159

Caption: pesticides with * in bold are analyzed in ESI- mode; the others are analyzed in ESI+ mode.

These analyzes produced very interesting results. The data obtained clearly showed residues of some pesticides at the trace level for four of the five samples evaluated. One of the oyster samples (C) did not show any residue from any of the 322 agrochemicals monitored in this study.

This result, at first, surprised us, but proved total adherence with the fact that this sample comes from an organic crop considered as standard.

The other four samples evaluated, A, B, D and E, showed the presence of pesticide residues in very low concentrations, at the trace level, as shown in Table 3.

The application of the pesticide multi-residue method proposed in this study in the evaluation of bivalve molluscs allowed the presence of some pesticides to be evidenced. If only routine analyzes were carried out for these samples, this occurrence would not be pointed out because they are not yet part of the mollusc production

Table 3. Pesticides found in BM samples through multiresidue analysis.

Sample		Pesticide
oysters	A	Methyl pyrimiphos
	B	Methyl pyrimiphos
		Clomazone
	C	-
	D	Sprocarb
		Picoxystrobin
		Promethrin
		Tebupirinphos
		Tricyclazole
scallops	E	Piperonyl butoxide
		Methyl pyrimiphos
		Thiabendazole

process and, therefore, would not be monitored. In the context of food safety, it is important to consider the danger associated with contamination of the environment in which molluscs live.

Pesticide residues from areas adjacent to the cultivations may be present in the muscle of molluscs, indicating that these toxic substances from other regions are contributing to the increase of contamination in areas where shellfish are cultivated. Careful analysis of data on food contamination associated with environmental characteristics around mollusc cultivation areas may generate solutions to this problem through the application of corrective measures.

It should be considered that the quality of a food is intrinsically linked to both its phytosanitary nature and the presence of contaminants. Pesticides affect human health in many ways and the increase in levels of contamination in food can drastically affect its quality and even make it unsuitable for consumption, greatly reducing its commercial value.

Comprehensive and effective control of all possible sources of contamination can provide a complete and more accurate assessment of food quality. In this context, the application of a multi-residue method with 322 analytes, commonly used in agricultural activities as proposed in this work, is extremely valuable.

4 Conclusion

The multi-residue pesticide method validated for oyster (*Crassostrea gasar*) and scallop (*Nodipecten nodosus*) matrices according to the requirements recommended by the European Union (2020) proved to be adequate to quantify 322 pesticides listed in Table 2.

The results of the pesticide residue analysis of all five samples of bivalve mollusks in this study clearly showed that they all meet the level of healthiness and fully meet the quality standard of the legislation.

Among the five samples of this work (A, B, C, D and E), three of the four samples of oysters and one of scallops (named A, B, D and E) showed the presence of residues only at the trace level and not causes damage to health. This result attests to the quality of oyster and scallop meat from these crops, guaranteeing its safety as food.

Among the oyster samples, only the sample C from Rio Grande do Norte did not present any pesticide residue, not even at the trace level. This sample was produced in an "organic area" and this fact can be proven because it did not present abiotic stresses.

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