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Article

Determination of avermectins residues in soybean, bean and maize using a QuEChERS-based method and ultra-high-performance liquid chromatography coupled to tandem mass spectrometry

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Abstract: Soybean, maize and bean are crops of great economic importance, but in the last years suffered with infestations of the caterpillar *Helicoverpa armigera*, being the main problem the resistance of this pest to most pesticides. Avermectin emamectin benzoate was recently released to control this pest. Other avermectins, like abamectin, doramectin, eprinomectin and ivermectin are used in large scale because they potent acaricidal, anthelmintic, and insecticidal activities. Thus, a simple and fast method for the determination of avermectins in these crops based on a quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction procedure and ultra-high performance liquid chromatography with tandem mass spectrometry (UHPLC-MS/MS) analysis was developed and validated. For extraction, water followed by acetonitrile:isopropanol and a partition step with salts was stablished. With the clean-up step using activated EMR-Lipid, limits of detection of 1.2 μg kg^{-1} for abamectin, doramectin, emamectin benzoate and ivermectin, and of 2.4 μg kg^{-1} for eprinomectin were achieved. Accuracy and precision evaluated at low levels presented satisfactory results. The method was successfully applied in commercial samples and is a good alternative for routine analysis.

Keywords: Macrocyclic lactones; agricultural crops; food; sample preparation; UHPLC-MS/MS

1. Introduction

Soybean (Glycine max) is among the most important crops in the world and their demand increases every year, as long as soybean may be applied for animal feed and biodiesel production to raw material for cosmetics [1]. Maize (Zea mays) also has its economic importance related to various forms of use, from animal feed to high technology industry. Common bean (Phaseolus vulgaris), an oleaginous crop such as soybean, is an important source of protein.

Avermectins are a family of natural products with a large macrocyclic lactone ring consisting of four major components (A1a, A2a, B1a and B2a) and four minor components (A1b, A2b, B1b and B2b) isolated from the fermentation broth of Streptomyces avermectinius. Abamectin, doramectin, emamectin benzoate, eprinomectin and ivermectin are avermectins used in large scale because they present potent acaricidal, anthelmintic, and insecticidal activities [2,3]. As an example, abamectin is a blend of

avermectin B1a (≥ 80%) and B1b (≤ 20%), and emamectin benzoate, a novel avermectin derivative developed as a pesticide, is a mixture of B1a (≥ 90%) and emamectin B1b (≤10%).

In 2013, the first attacks of Helicoverpa armigera were reported on soybean and bean crops [4]. This caterpillar affects several crops, such as soybean, bean, cotton, chickpea, tomato, eggplant, canola and sunflower. In general, this pest is controlled by the use of insecticides, especially carbamates, organochlorines, organophosphates and pyrethroids. However, due to extensive use of these pesticides, the caterpillar developed resistance against them, causing great damages in the crops [5]. Thus, a phytosanitary emergency was declared in Brazil, and the use of emamectin benzoate was released on an emergency basis. From the class of avermectins, only the use of abamectin as a pesticide was initially allowed in soybean, bean and maize crops [6]. The need for using emamectin benzoate led to the inclusion of this compound in the list of priority analysis pesticide for registration being officially authorized in Brazil in 2017 for use in soybean, bean, maize and cotton.

The maximum residue limits (MRL) values for abamectin and emamectin benzoate in soybean, maize and bean established in Brazil, European Union, USA and by the Codex Alimentarius are, in general, in the range of 5 to 15 μg kg^{-1}, with the exception of abamectin in beans (80 μg kg^{-1}) established by the Codex Alimentarius.

In recent years, ultra-high performance liquid chromatography coupled with mass spectrometry has been a preferred technique for the determination of pesticides and veterinary drugs in food samples [7,8]. However, the determination in complex matrices with high fat, sugar and protein content requires special attention in the sample preparation stage, in order to obtain extracts with low concentration of interferents and containing the analytes of interest [9].

As extraction techniques for determination of pesticide residues in cereals and oleaginous crops we can mention the QuEChERS method, which stands for quick, easy, cheap, effective, rugged and safe [10] and the matrix solid-phase dispersion (MSPD) [11]. Huang et al. [12] analyzed abamectin and ivermectin in olive, soybean, maize and peanut oils and in lard after liquid-liquid extraction (LLE). The extractive solvent used was n-hexane, followed by addition of acetonitrile while maintaining vortexing. Macedo et al. [13] analyzed abamectin, doramectin and ivermectin in butter using LLE at high temperatures and a mixture of acetonitrile, ethyl acetate and water (90:4:6, v/v/v) as solvent.

López-Blanco et al. [14] used a modified QuEChERS method for the multiresidue analysis of abamectin and other pesticides in avocado and olive oil. Several sorbents were tested for the clean-up step. Du et al. [15] established a QuEChERS method, with a clean-up step using primary secondary amine (PSA), to analyze residues of avermectins, pyriproxyfen and diflubenzuron in mushrooms. Liu et al. [16] developed a QuEChERS method for multiresidue determination of pesticides, including abamectin, in grains, with water addition before extraction with acetonitrile. Wang et al. [17] analyzed abamectin, emamectin benzoate and other pesticides in soybean applying a modified QuEChERS acetate method. Thus, giving the importance of avermectins residue analysis in soybean,

bean and maize crops, the aim of this work was to develop a suitable method for determination of residues of the avermectins abamectin, doramectin, emamectin benzoate, eprinomectin and ivermectin in soybean, maize and bean crops using the same sample preparation step in order to simplify the execution in routine analyses. To the best of our knowledge, there are no methods available in the literature for the determination of avermectins residues in more than one cereals or legumes matrix.

2. Materials and Methods

2.1 Standards, Chemicals and Materials

Analytical standards of abamectin, doramectin, emamectin benzoate, eprinomectin and ivermectin with purities from 95.0 to 98.3% were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Sulfadimetoxina-d6, used as surrogate standard (SS) and dimetridazol-d3 used as internal standard (IS) were purchased from Witega Laboratorien Berlin-Adlershof GmbH (Berlin, Germany). Individual stock standard solutions (1000 mg L^{-1}) of the analytes were prepared in acetonitrile HPLC grade considering the purity of solid standard. From the stock solutions, new individual solutions at 10 mg L^{-1} were prepared and kept at -20 °C. Methanol, acetonitrile and magnesium sulfate were obtained from JT Baker (Phillipsburg, USA). Isopropanol, ammonium formate, formic acid, trisodium citrate dihydrate, sodium hydrogencitrate sesquihydrate and sodium chloride were purchased from Sigma-Aldrich (St. Louis, USA). Ultrapurified water (18 MΩ cm) was obtained from a Millipore Milli-Q® system (Molsheim, France). Nylon filters (0.22 µm), silica, Florisil®, Primary Secondary Amine (PSA) and EMR-Lipid were from Agilent Technologies (Santa Clara, USA). The sorbents C18 and Z-Sep+ were from Supelco (Bellefonte, PA, USA).

For method development, soybean, bean and maize blank samples were obtained from controlled experiments without use of pesticides. None of the evaluated pesticides were detected. Sample were processed for 1 min in a IKA A11 Basic (Staufen, Germany) analytical mill.

2.2 UHPLC-MS/MS Parameters

Analysis were performed in a UHPLC-MS/MS system from Waters (Milford, USA) consisting in an Acquity UPLC™ binary pump liquid chromatography with Xevo TQ™ MS/MS triple quadrupole detector, autosampler and column temperature controller. The Quanpedia™ library of UHPLC-MS/MS was used, where the compounds with their respective precursor and product ions, as well as energies applied to cone and collision, were set. The aqueous phase (mobile phase A) with 10 mmol L^{-1} of ammonium formate was maintained in all tests. Two organic mobile phase B were evaluated: methanol 1% (v/v) formic acid and acetonitrile 0.1% (v/v) formic acid. Three chromatographic columns were tested: Acquity UPLC™ BEH C_{18} (50 × 2.1 mm, 1.7 µm) and Acquity UPLC™ HSST3 (100 × 2.1 mm i.d., 1.8 µm) from Waters (Wexford, Ireland), and Zorbax Eclipse Plus® C18 (100 x 2.1 mm, 1.8 µm) from Agilent Technologies (Santa Clara, USA). The mobile phase operated in gradient mode, started at 50% B and remained constant for

1 min, increasing for 2 min to reach 80% B and then increases to 100% B, remaining for 0.5 min, and returning to 50% B maintaining constant until the end of the analysis. The flow rate was 0.225 mL min^{-1}, with 10 µL of injection volume. The mass spectrometer was operated using selected reaction monitoring (SRM) mode. The electrospray ionization operated in positive mode (ESI+). The transition with the highest intensity was selected for quantification, and the transition with the second highest intensity was used for identification. The following conditions were used for the ESI source: desolvation and cone gas flow rate were set at 500 and 60 L N_2 h^{-1}, respectively; the capillary voltage at 2.5 V; desolvation temperature at 350 °C and source temperature at 150 °C. The collision gas was argon used at 0.15 mL min^{-1}. Partition coefficients of avermectins and UHPLC parameters for the determination of their residues in soybean, bean and maize are presented in Table 1.

Table 1. Partition coefficients of avermectins and UHPLC parameters for the determination of their residues in soybean, bean and maize.

Compounds	Log K_{ow}	t_R (min)	Precursor ion	Cone (eV)	SRM transitions, *m/z* (collision energy, eV)		Ion ratio
					Quantification	Identification	
Abamectin	4.0	2.83	$[M+NH_4]^+$	20	890.6 > 567.4 (11)	890.6 > 305.2 (25)	0.91
Doramectin	4.0	3.02	$[M+NH_4]^+$	15	916.6 > 331.2 (23)	916.6 > 219.1 (25)	0.81
Emamectin	3.0	2.23	$[M+H]^+$	40	886.6 > 158.0 (37)	886.6 > 126.0 (38)	0.62
Eprinomectin	5.4	2.72	$[M+H]^+$	15	914.5 > 186.0 (35)	914.5 > 144.0 (20)	0.67
Ivermectin	3.2	3.23	$[M+NH_4]^+$	15	892.6 > 569.4 (14)	892.6 > 551.4 (25)	0.42

K_{ow}= octanol/water partition coefficient; t_R= retention time; SRM= selected reaction monitoring

2.3 Optimized Sample Preparation Procedure

The proposed sample preparation method for determination of avermectin residues in soybean, bean and maize is based on the citrate QuEChERS procedure using 5 g of sample weighed in a 50-mL polypropylene (PP) tube followed by addition of 10 mL of water and homogenization for 1 min in ultra-turrax IKA T25 Digital (Staufen, Germany) at high speed to produce a slurry. Extraction was done with 10 mL of a mixture of acetonitrile:isopropanol 9:1 (v/v) vortexed for 1 min. Partition and salting-out effect were achieved by adding 2 g of $MgSO_4$, 0.5 g of NaCl, 0.5 g of $C_6H_5Na_3O_7.2H_2O$ and 0.25 g of $C_6H_6Na_2O_7.1.5H_2O$ followed by vortex shaking for 1 min. Tubes were then centrifuged at 4850*g* for 8 min at 5 °C. Then, 2 mL of supernatant was transferred to a 15-mL PP tube with 400 mg of EMR-Lipid previously activated with 2 mL ultrapure water according to Agilent Technologies Protocols [18], vortexed for 1 min, and centrifuged at 10,200*g* for 8 min at 5 °C. Afterthat, a final step to remove water exccess the extract was transfered to another tube containing 640 mg of $MgSO_4$ and 160 mg of NaCl, vortexed for 1 min, and centrifuged at 10,200*g* for 8 min at 5 °C. The cleaned extract was filtered in 0.22-µm nylon filters and diluted 1:1 (v/v) in the initial composition of the mobile phase for analysis by UHPLC-MS/MS.

2.4 Evaluation of the Different QuEChERS Procedures

The initial tests consisted in the evaluation of the three QuEChERS versions: original [19], citrate [20] and acetate [21] using amounts of sample, solvent and partition salts established in each version. Considering the low percentage of moisture, it was necessary to prepare a slurry using a ultra-turrax. Different ratio matrix:water (m/v) of 1:1; 1:1.5 and 1:2 were evaluated. Blank samples of soybean, bean and maize spiked at 20 μg kg^{-1} and matrix matched calibration curves at 1, 2, 5, 10 and 20 μg L^{-1}, corresponding to 4, 8, 20, 40 and 80 μg kg^{-1}, were used for evaluation of the different extraction procedures.

After some tests, the QuEChERS citrate was selected for evaluation of diferrent extraction solvents: acetonitrile and acetonitrile:isopropanol in the proportions 9:1, 8:2 and 7:3 (v/v). To perform the evaluations, 5 g of sample were weighed in 50-mL PP tube with 10 mL of ultrapurified water and the content was homogenized in ultra-turrax for 1 min. Then, 10 mL of the different extraction solvents was added and tubes were vortexed for 1 min. Afterthat, 2 g of $MgSO_4$, 0.5 g of NaCl, 0.5 g of $C_6H_5Na_3O_7.2H_2O$ and 0.25 g of $C_6H_6Na_2O_7.1.5H_2O$ were added, tubes were vortexed for 1 min and centrifuged at 4850*g* for 8 min at 5 °C. The supernatant (2 mL) was submitted to a clean-up step by d-SPE with 250 mg of C18, 50 mg of PSA and 300 mg of $MgSO_4$ followed by centrifugation at 10,200*g* for 8 min at 5 °C.

2.5 Evaluation of Different Clean-up Conditions

An aliquot of 2 mL of extract was submitted to different d-SPE clean-up conditions with the following materials: 250 mg of C18, 50 mg of PSA; 100 mg Z-Sep+; 400 mg silica; 400 mg Florisil®; 400 mg EMR-Lipid activated with 2 mL ultrapurified water. The samples were centrifuged at 10,200*g* for 8 min at 5 °C. Only for the assay using EMR-Lipid, a complementar step was included where 2 mL of supernatant was transferred to another tube with 640 mg of $MgSO_4$ and 160 mg of NaCl as recommended by the product protocol. Extracts were centrifuged again at 10,200*g* for 8 min at 5 °C, filtred through 0.22-μm nylon filters and diluted 1:1 (v/v) in mobile phase for analysis.

2.6 Validation Procedure

After evaluating the sample preparation procedure for determination of avermectin residues in soybean, bean and maize, the method was validated according to SANTE guidelines [22]. The following parameters were evaluated: selectivity, matrix effect, analytical curve and linearity, limits of detection (LOD) and quantification (LOQ), precision (repeatability and intermediate precision) and accuracy. The selectivity of the method was evaluated by comparing the chromatograms obtained in the UHPLC-MS/MS system by injections of the blank sample extracts and spiked blank samples for each of the studied matrices. Calibration curves were prepared in acetonitrile, blank sample extract and blank matrix at 1.0; 2.0; 5.0; 10.0 and 20.0 μg L^{-1} for each of the studied matrices. The LOQ was considered to be the lowest spiked concentration, which presented signal/noise ratio higher than 10, recoveries between 70-120%, with relative standard deviation (RSD) ≤ 20%. The LOD was established dividing the LOQ by 3.33. The accuracy and precision

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(repeatability and intermediate precision) of the method was evaluated through recovery assays. Spiked blank samples of each studied matrix at 4, 8, 20, 40 and 80 μg kg^{-1} in replicates (n= 5) were prepared to evaluate accuracy and precision. The matrix effect was evaluated for the three matrices under study comparing the slopes of the analytical curves in solvent and in the blank matrix extract.

2.7 Application in Real Samples

The developed and validated method was applied for the determination of residues of the avermectins under study in 18 soybean samples, 12 bean samples and 15 maize samples obtained from supermarkets from the Rio Grande do Sul State, Brazil. Each commercial sample of at least 1 kg was collected, milled and stored in a freezer at -20 °C.

3. Results

3.1 UHPLC-MS/MS Analysis

The ionization of avermectins was performed with electrospray in positive mode (ESI+). The composition of the mobile phase may significantly influence the analytical signal and the proper separation of the analytes [23]. The mobile phase (A) aqueous solution ammonium formate 10 mmol L^{-1} and (B) methanol 0.1% formic acid was chosen due to the higher analytical signal obtained for the compounds under study. The selected mobile phase additives favored the formation of $[M+NH_4]^+$ adducts used for the detection of abamectin, doramectin and ivermectin after the chromatographic separation. The formation of the ion $[M+H]^+$, used for emamectin benzoate and eprinomectin, was favored by the addition of formic acid. The addition of acids also reduce the formation of $[M+Na]^+$ adducts. The sodium adducts are avoided due to its high stability and poor fragmentation response [24]. The column Acquity UPLC™ BEH C_{18} provided high resolution and good peak shape for the compounds under study. Still, the chromatographic column chosen is the same used in routine analysis in our laboratory.

3.2 Sample Preparation Evaluations

Soybean and bean are considered complex matrices due to the high amount of fats and proteins, besides having a low humidity, needing to add water for the extraction. Maize presents a low percentage of water and a particular characteristic that is the presence of starch, which increases its complexity. The preparation of slurry was uniformized for the proportion 1:2 of sample:water which produced suitable consistence. This proportion of sample:water was reported for low moisture matrices like maize [25], wheat and oat [26], and barley and wheat [27]. Lower protortions of water of 1:1 and 1:1.5 were not effective to have a homogeneous slurry.

Considering the main objective of developing an effective and unified sample preparation procedure for determination of avermectin residues in soybean, bean and maize, the evaluation of which of the QuEChERS procedures provides best results for the evaluated matrices was performed. The three most common QuEChERS procedures using acetonitrile as extraction solvent have not been effective for the extraction of the avermectins from the selected matrices presenting recoveries ranging from 35 to 101% and

RSD between 3 and 37%. There were no significant differences ($p < 0.05$) between most of the assays. The QuEChERS citrate was chosen for forder investigations due to present the best recovery (64%) for eprinomectin, that was the compound with lower recoveries in all cases. This result is closer to the acceptance criterion of 70-120% [22] but an inprovment is required. QuEChERS citrate also presented lower RSD values in comparison to the QuEChERS acetate and original.

As avermectins is a class of chemical compounds with high values of K_{ow}, isopropanol was evaluated in different proportions with acetonitrile trying to improve extraction efficiency. Isopropanol may be used as extraction solvent, besides being commonly used as mobile phase in liquid chromatography. Menezes Filho et al. [28] employed isopropanol in a mixture 8:2 (v/v) with water in the extraction of pesticides from mangoes with satisfactory results for most of the analytes. In addition, Seth et al. [29] considered isopropanol an efficient and advantageous extraction solvent for soybean grains and other oilseeds and an attractive alternative to the frequently used solvents. The extraction solvents acetonitrile and acetonitrile:isopropanol 9:1 (v/v) presented similar results for the extraction of abamectin, doramectin and ivermectin for all matrices, but for emamectin benzoate and eprinomectin the addition of isopropanol improved the extraction efficiency by about 25%. This was very important because emamectin benzoate, that in general presented lower recoveries, with this modification the recoveries were above 70% for all matrices. Then tests with acetonitrile containing different proportions of isopropanol (9:1; 8:2 and 7:3, v/v) were performed to evaluate whether there would be a significant increase in the recoveries of the compounds as the volume of isopropanol increased. No considerable variation in recoveries of avermectins for the three matrices were observed with the different proportions of the extraction solvents. However, it was observed that with higher volumes of isopropanol the analytical response decreased for abamectin and eprinomectin. Once, abamectin and eprinomectin are the two compounds with lowest sensitivity in UHPLC-MS/MS analysis, the ratio 9:1 (v/v) for acetonitrile:isopropanol was selected.

3.3 Clean-up Procedure Evaluations

In the clean-up step, it was decided to evaluate d-SPE, without considering SPE, in order to keep the QuEChERS advantages. Figure 1 shows recovery results for five sorbents evaluated for soybean. Recoveries above 70% for most of the compounds were obtained. Similar resuts were observed for bean and maize. The use of C18+PSA presented the lowest recoveries, with a mean recovery < 70% for abamectin (69%), ivermectin (63%) and doramectin (64%), while abamectin presented RSD > 20%. Regarding the EMR-Lipid and Florisil®, the five compounds presented adequate recoveries and RSD. Results for Z-Sep+ were satisfactory, except for eprinomectin in which presented recovery of 67%. Silica presented recoveries > 70% for all compounds, however the RSD for abamectin was >20%.

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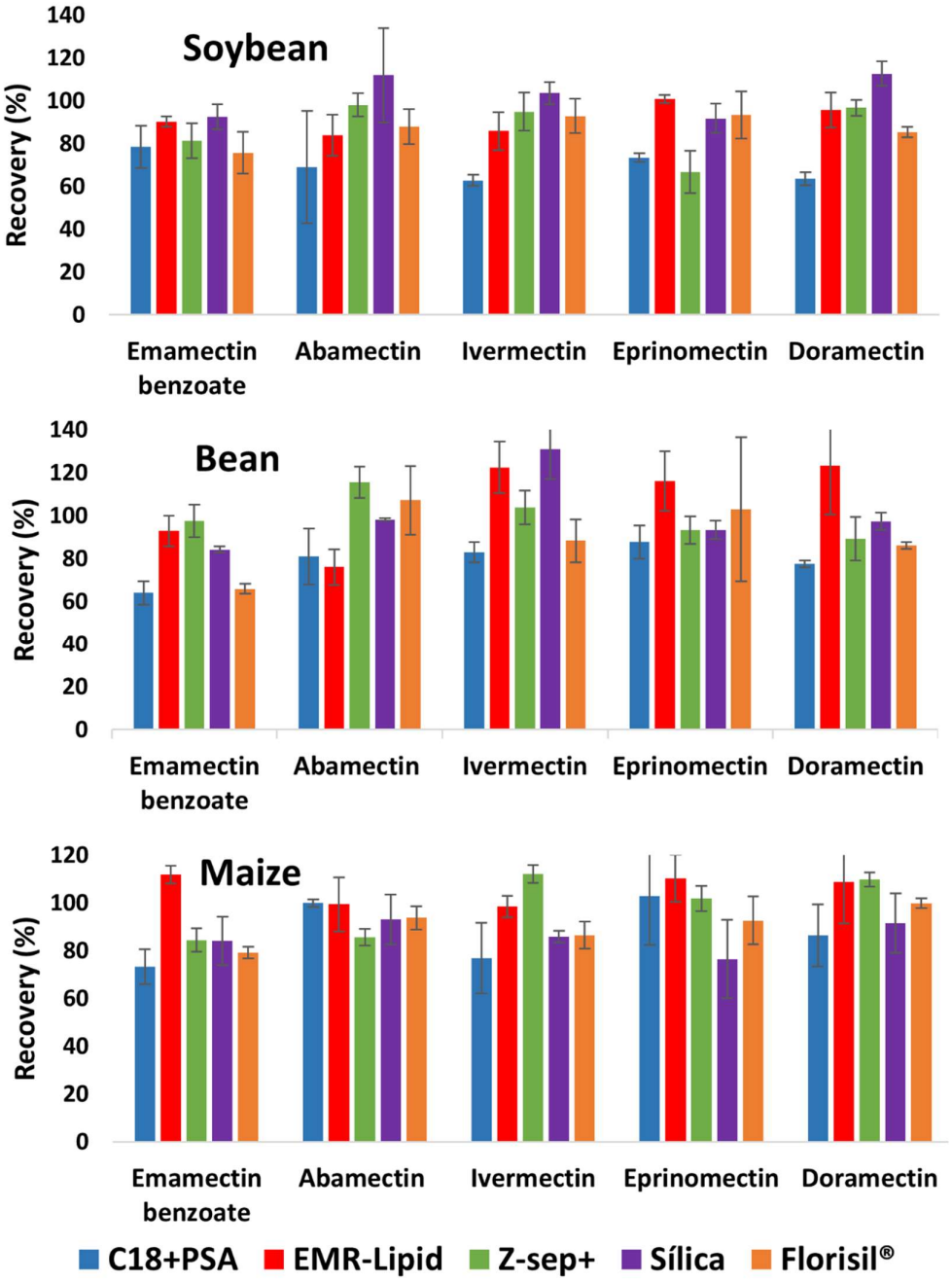

Figure 1. Recoveries obtained with d-SPE clean-up for soybean, bean and maize extracts using different sorbents

The clean-up with C18 + PSA, EMR-Lipid, Silica and Florisil® presented for some compounds recoveries < 70% and > 120%, especially for bean. The use of C18 + PSA resulted in the lowest recoveries in comparison to the other sorbents, and emamectin benzoate had only a recovery of 64%. EMR-Lipid showed all recoveries above 70%, with ivermectin and doramectin showing 122% and 123%, respectively. Silica had a mean recovery of 131% for ivermectin and Florisil® achieved a mean recovery of 66% for

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emamectin benzoate and a RSD of more than 30% for eprinomectin. Z-Sep+ was the only sorbent that presented recoveries between 70-120%. For maize, all sorbents evaluated presented satisfactory recoveries (70 to 120%). The RSD was satisfactory for all analytes, except for eprinomectin, when using C18 + PSA that presented RSD > 20%. As maize obtained recoveries between 70-120% for the five sorbents, the values obtained for soybean and bean were taken into account to select the most suitable sorbent. C18 + PSA was discarded because of the lowest recovery values in comparison to the other sorbents for both matrices. The claen-up that generated the best results for bean was Z-Sep+ and for soybean was EMR-Lipid. In general, EMR-Lipid presented lighter advantage in terms of recovery and RSD, as well the final extract presented less coextractives when evaluated by gas chromatography-mass spectrometry in full scan mode.

The non-activated EMR-Lipid was tested in the clean-up step to verify if it would have better results or similar to the activated one. As a result, the non-activated presented higher recoveries for abamectin, emamectin benzoate and ivermectin, compared to the activated EMR-Lipid. For eprinomectin the recovery was similar, but doramectin presented a much lower recovery. However, the RSD values for the five compounds increased (RSD >20%) with the use of non-activated EMR-Lipid. With activated EMR-Lipid the RSD were < 10% for all avermectins. Therefore, the activated EMR-Lipid was chosen for use in the clean-up step of the three matrices.

3.4 Validation Results of the Proposed Method

Matrix-matched calibration was used for all matrices, where linearity was obtained with a determination coefficient (r^2) > 0.99 for the five compounds and the levels 1, 2, 5, 10 and 20 μg L^{-1}. In order to evaluate the accuracy of the method, recovery tests were performed at five concentration levels (4, 8, 20, 40 and 80 μg kg^{-1}) for each matrix using the modified citrate QuEChERS method and the results were presented in Table 2. The lowest levels of concentration were chosen taking into account the established MRL values.

The proposed method presented adequate accuracy with recovery results between 70-120% for all compounds, matrices and spike levels, both in repeatability and intermediate precision assays. The precision was also very good, with RSD < 20% in all cases. The matrix effect is considered to have an influence on the analytical performance when the result is not between -20 and 20%. Therefore, for all compounds in soybean and bean the effect was not significant. For maize, doramectin presented a matrix effect of 22%. In order to compensate the matrix effect, matrix-matched calibration was selected for quantification. Considering that all spike levels presented recoveries from 70 to 120% and RSD ≤ 20% for all matrices, the method LOQ was established at 4 μg kg^{-1} and the corresponding method LOD at 1.2 μg kg^{-1}.

Table 2. Maximum Residues Limits, recovery (%) and relative standard deviation (RSD) (%) for repeatability and intermediate precision assays, LOQ (μg kg^{-1}) and matrix effects (%) for each compound in the different matrices

					Recovery (RSD) (%) for 5 spike levels (μg kg^{-1}) (n= 5)								
					4		8		20	40		80	
	MRL (μg kg^{-1})				Repeat.	Interm. prec.	Repeat.	Interm. prec.	Repeat.	Repeat.	Interm. prec.	Repeat.	ME (%)
Compounds	BR	CA	EU	USA									
								Soybean					
Abamectin	10		10	10	77 (11)	98 (17)	79 (5)	101 (13)	114 (14)	102 (12)	94 (13)	99 (10)	-7
Doramectin					109 (13)	100 (15)	101 (9)	100 (8)	99 (10)	99 (14)	102 (6)	100 (5)	-16
Emamectin	10		10		88 (7)	106 (9)	97 (5)	96 (6)	103 (7)	101 (9)	105 (5)	100 (9)	-4
Eprinomectin					102 (16)	87 (16)	98 (17)	117 (8)	113 (19)	91 (17)	105 (3)	101 (13)	-6
Ivermectin					105 (4)	98 (14)	104 (3)	89 (9)	104 (15)	94 (11)	102 (6)	101 (9)	-11
								Bean					
Abamectin	5	80	10	15	83 (5)	98 (13)	89 (6)	97 (4)	105 (10)	103 (10)	96 (11)	99 (9)	1
Doramectin					71 (2)	108 (7)	107 (3)	95 (10)	91 (5)	110 (6)	96 (4)	98 (7)	-11
Emamectin	10	10	10		105 (9)	108 (6)	94 (12)	95 (10)	105 (14)	104 (5)	103 (6)	99 (8)	1
Eprinomectin					112 (6)	89 (14)	103 (16)	106 (15)	90 (11)	96 (10)	95 (6)	101 (11)	0.2
Ivermectin					101 (5)	93 (12)	98 (3)	104 (19)	98 (7)	102 (4)	95 (9)	100 (11)	-4
								Maize					
Abamectin	5		10		71 (6)	74 (5)	106 (6)	109 (10)	92 (15)	107 (8)	97 (6)	99 (9)	-6
Doramectin					118 (3)	105 (11)	113 (7)	97 (5)	88 (7)	100 (6)	96 (6)	101 (11)	22
Emamectin	10		10		89 (16)	103 (12)	105 (1)	98 (6)	109 (3)	104 (4)	103 (3)	99 (3)	-10
Eprinomectin					96 (5)	94 (17)	117 (9)	99 (11)	89 (12)	103 (13)	98 (8)	100 (19)	-16
Ivermectin					102 (5)	115 (4)	102 (3)	92 (9)	87 (1)	109 (3)	101 (2)	99 (8)	14

MRL: maximum residue limits; BR: Brazil; CA: Codex Alimentarius; EU: European Union; USA: United States of America; Repeat.: repeatability; Interm. prec.: intermediate precision; LOQ: limit of quantification; ME: Matrix effect.

3.5 Application of the Method in Real Samples

From the analysis of 18 soybean samples, one sample presented abamectin at 21.1 μg kg^{-1}, that is two times higher than the MRL (10 μg kg^{-1}) established in Brazil and USA. Another three samples also presented residues of abamectin, however below the method LOQ. Figure 2 demonstrates the determination of abamectin residues in a soybean sample at concentration higher than the method LOQ and the MRL established in Brazil and USA. For the 12 bean samples and 5 maize samples, no residues of the analyzed avermectins were detected. The absence of detectable residues may be due to the reduced number of samples analyzed. However, it is important to monitor these compounds in the crops under study and in other agricultural crops in order to guarantee the food safety and health of the population.

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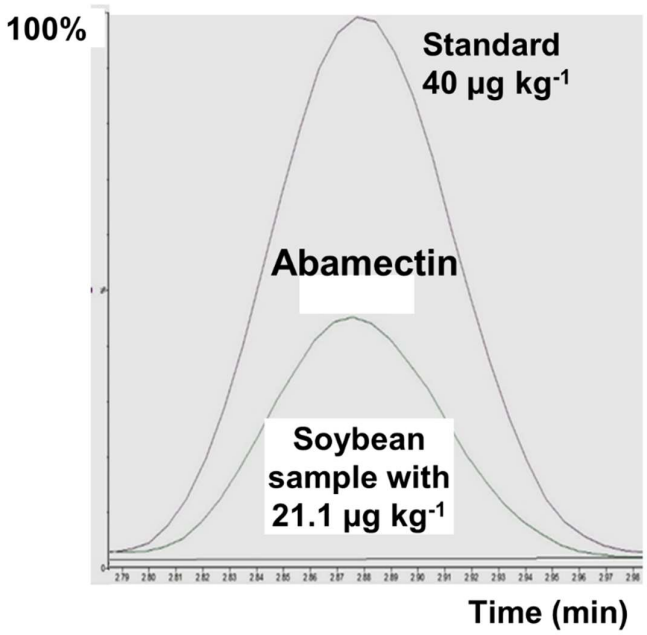

Figure 2. Overlapping of the signal obtained from a positive sample of soybean for abamectin (21.1 µg kg^{-1}) and a standard in blank matrix corresponding to 40 µg kg^{-1}.

4. Conclusions

The results obtained with the modifications of the QuEChERS citrate method indicate good efficiency of the proposed method for the determination of avermectins residues in soybean, bean and maize samples. The addition of isopropanol to the acetonitrile improved the extraction efficiency and the clean-up step using EMR-Lipid permitted to obtain clean extracts, avoiding the need of frequent maintenance of the UHPLC-MS/MS system. The use of UHPLC-MS/MS with additives in the mobile phase and specific conditions allowed the determination with method limits of quantification below the MRL values. The validation indicated very good results of accuracy and precision, proving that the proposed method is reliable. The method was efficiently applied to real samples with positive results, including with abamectin in a soybean at concentration higher than the MRL established in Brazil and USA. We emphasize that there are no available studies reporting the development of a method for the determination of avermectins in more than one agricultural crop, highlighting the relevance of this work. The proposed modified QuEChERS citrate method for determination of avermectins in soybean, bean and maize using UHPLC-MS/MS was effective and is a good alternative for routine laboratories.

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