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Multiresidue determination of pesticides in sweet peppers by LC-MS/MS using Si-Al(PMTDS) as a sorbent for extraction by MSPD

Determinação multirresíduo de agrotóxicos em pimentões por LC-MS/MS usando Si-Al(PMTDS) como dispersante para extração por MSPD

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Abstract

A fast, effective and environmentally safe method for multiresidue determination of pesticides in samples of sweet green peppers using matrix solid phase dispersion with a new dispersant, poly(methyltetradecylsiloxane) thermally immobilized onto alumina coated silica, Si-Al(PMTDS), with determination by liquid chromatography coupled to tandem mass spectrometry was developed and validated. The performance of the new dispersant was compared with others, such as C18 and SiO₂. Acetonitrile and ethyl acetate were evaluated as extraction solvents. The Si-Al(PMTDS)-based sorbent gave results similar to the commercial sorbents tested in relation to recovery. However, only the lab-made polymeric sorbent assured selectivity of the separation. With Si-Al(PMTDS) extraction, recoveries for almost all analytes were in the range of 70-120% with coefficients of variation less than 20%, acceptable values for the analysis of pesticide residues in complex samples, demonstrating accuracy and precision of results for these analytes. All quantification limits were below the listed maximum residues limits for the analyzed pesticides. The developed method was applied to sweet green pepper samples, in which some authorized pesticides were found, but at concentration levels lower than the maximum residue limits. However, several unauthorized pesticides were also encountered. The preparation of the sorbent Si-Al(PMTDS) is simple, reproducible, and the cost is compatible with commercially available sorbents used in matrix solid phase dispersion.

Keywords: liquid chromatography-tandem mass spectrometry; matrix solid phase dispersion; sweet peppers; pesticides; validation.

Resumo

Um método rápido, eficaz e ambientalmente seguro para determinação multirresíduo de agrotóxicos em amostras de pimentões verdes usando dispersão da matriz em fase sólida com um novo dispersante, poli(metil-tetradecil-siloxano), imobilizado termicamente em sílica recoberta com alumina, Si-Al(PMTDS), e determinação por cromatografia líquida acoplada à espectrometria de massas sequencial foi desenvolvido e validado. O desempenho do novo dispersante foi comparado com outros como C18 e SiO₂. Também foram avaliadas acetonitrila e acetato de etila como solventes de extração. O sorvente à base de Si-Al(PMTDS) forneceu resultados semelhantes aos sorventes comerciais testados em termos de recuperação. No entanto, apenas o sorvente polimérico preparado no laboratório garantiu a seletividade da separação. Usando Si-Al(PMTDS) como dispersante na extração, as recuperações para quase todos os analitos ficaram na faixa de 70-120%, com coeficientes de variação inferiores a 20%, valores recomendáveis para a análise de resíduos de agrotóxicos em amostras complexas, demonstrando exatidão e precisão dos resultados para todos os analitos. Todos os limites de quantificação foram inferiores aos limites máximos de resíduos para os agrotóxicos analisados. O método desenvolvido foi aplicado em análises de pimentões verdes adquiridos no comércio. Alguns agrotóxicos autorizados foram encontrados, mas todos em níveis de concentração inferiores aos limites máximos de resíduos. No entanto, vários agrotóxicos não autorizados também foram encontrados. A preparação do sorvente Si-Al(PMTDS) é simples, reprodutível e o custo é compatível com sorventes comercialmente disponíveis utilizados na dispersão de matriz em fase sólida.

Palavras-chave: cromatografia líquida acoplada à espectrometria de massas sequencial; dispersão da matriz em fase sólida; pimentões; agrotóxicos; validação.

1. Introduction

The application of pesticides for commercial production of fruits and vegetables is widely used for the control of diseases, disorders and weeds in various crops. However, their irregular use has been a concern to both government agencies and consumers, because it may cause food contamination and, consequently, possible adverse human health effects^[1,2].

In order to control the inappropriate use of pesticides and the concentration of these residues in foods such as sweet green pepper, *Capsicum annuum* L.^[3], national and international agencies have established the maximum residue limits (MRL) allowed for various pesticides in different cultures^[4,5]. The term MRL is understood as the maximum concentration of pesticide residues that may be present in foods consumed by the population without harming human health. In recent years sweet green peppers have shown irregularities due to pesticide residues^[6,7]. According to the Program for the Analysis of Pesticide Residues in Foods (PARA), conducted by the Brazilian Health Surveillance Agency (ANVISA) in 2014, 89% of the sweet peppers samples grown in Brazil were deemed inadequate, i.e., contained residues of pesticides not allowed or above the MRL^[6]. The Pesticide Residue Monitoring Program 2015, conducted by the U.S. Food and Drug Administration (FDA) and released in 2017^[7], revealed that 9.0% of samples obtained from diverse countries contained irregularities.

Due to negligence and the consequent exposure of the public to the pesticides, there is increased demand for food without these pesticides or food containing pesticide residues below the maximum allowed limits. This has instigated research to monitor and evaluate the risk of consuming contaminated foods^[8], such as sweet peppers.

Matrix solid phase dispersion (MSPD), introduced (and patented) by Barker et al. in 1989^[9] is an efficient technique for the extraction of analytes from solid and semi-solid matrices, overcoming limitations of other

techniques and being used for analysis of pesticides in vegetables since 1996^[10].

In the determination of analytes present in complex samples it is necessary to use a suitable dispersant and solvent(s) for elution to minimize the amount of matrix co-extractives that can interfere with the subsequent analysis, particularly the selectivity, as well as damage the equipment used. Simultaneously they must ensure high recovery of the analytes, that is, the type of sorbent (dispersant) and eluting solvent used depends on the polarity of the analytes and of the nature of the matrix. Classic applications of the technique employ reverse phase sorbents such as octadecylsilane, C18, and octylsilane, C8. For more lipophilic matrix analyses, normal phase sorbents such as alumina, florisil and silica are used to analyze more hydrophilic samples. Less polar solvents such as hexane are used to extract nonpolar analytes and more polar solvents such as acetonitrile, ethyl acetate and methanol are used for more polar compounds^[11,12].

A number of papers have been published in which the technique of solid phase matrix dispersion is used in sample preparation for the multiresidue analysis of pesticides in vegetables, fruits and other food matrices^[13-15], in veterinary residues^[16] and for mycotoxins in grains^[17,18]. Bogialli and Corcia^[19] have reviewed the technique for determination of contaminants such as pesticides, veterinary drugs, naturally occurring contaminants and persistent environmental chemicals in food.

In recent years, several innovations related to the technique have been the synthesis and use of new materials. In this context, there are reports of the use of highly selective sorbents such as molecularly imprinted polymers (MIP)^[20,21] and less specific ones such as multi-walled carbon nanotubes (MWCNT)^[22,23] for the determination of pesticides in different matrices. In both of these, using molecularly imprinted polymers or multi-walled carbon nanotubes, the results show good selectivity and sensitivity, and no problems are reported in blend preparation^[12].

The liquid chromatography coupled to tandem mass spectroscopy (LC-MS/MS) has shown great progress in terms of technological development and application^[24]. It provides increased selectivity and detectability, allowing the determination of low concentrations of pesticide residues belonging to different chemical groups in complex matrices in a single analysis, as well as permitting unequivocal analyte identification. Several recent papers report the use of liquid chromatography-tandem mass spectrometry as a technique for multiresidue determination of pesticides in food^[14,22,24,25], such as sweet pepper^[26,27].

Based on these considerations, and to the few complete works on multiresidue analysis of pesticides in sweet pepper, a method using LC-MS/MS and, for the first time, MSPD with a dispersant consisting of poly(methyltetradecylsiloxane) thermally immobilized on alumina coated silica, Si-Al(PMTDS), was developed, validated and applied for quantification of multiresidues of pesticides found in samples of sweet green peppers from the region of Campinas/SP/Brazil.

2. Experimental

2.1. Materials

Analytical standards for the pesticides clomazone (98.1% w/w), difenoconazole (97.0% w/w), ethion (97.8% w/w), methamidophos (98.5% w/w), methomyl (99.9% w/w), pyraclostrobin (99.9% w/w), pyriproxyfen (99.1% w/w), thiabendazole (99.8% w/w), thiacloprid (99.9% w/w), thiamethoxam (99.7% w/w), acephate (97.2% w/w), azoxystrobin (99.9% w/w), carbofuran (99% w/w), fenarimol (99.8% w/w), iprodione (99.3% w/w), metconazole (99.5% w/w) and tebuconazole (99.8% w/w) were from Fluka; carbendazim (99.1% w/w) and methiocarb (98.5% w/w) were from Dr. Ehrenstorfer GmbH, carbaryl (99.5% w/w) was from Chem Service, and imidacloprid (99.9% w/w) was from Riedel-de Haen. The pesticides were selected from those authorized by ANVISA, for application to sweet green pepper crops, as well as from those present, although

not authorized, that have been reported in the PARA assessment^[6,28]. Table 1 shows important information about the selected analytes and the conditions for their fragmentation and analysis.

Standard stock solutions of each pesticide in concentrations of 1000 mg L⁻¹ were prepared by solubilizing each analytical standard in methanol. The other solutions were prepared by diluting the stock solutions with the same solvent. All were stored in a refrigerator at a temperature of approximately 4 °C. Polyethylene membranes (0.45 µm, Millipore), a glass vacuum filtration system, vacuum pump (N° WP6111560, Millipore), water (Milli-Q, Millipore), formic acid (P.A., Synth) and methanol (chromatographic grade, J. T. Baker) were used to prepare the mobile phases, the last was also used in the preparation of standard solutions. Silica gel, SiO₂, with mean particle sizes of 35-70 µm and mean-pore diameter of 6 nm (Acros Organics), aluminum isopropoxide (98.0% w/w) (Aldrich), poly(methyltetradecylsiloxane) polymer, (PMTDS, United Chemical Technologies), anhydrous toluene (99.9%, J. T. Baker), nitric acid (P.A., Synth) and hexane (95.0%) (chromatographic grade, Tedia) were used in the preparation of the sorbent. Polypropylene cartridges (6 mL capacity) having octadecylsilane, C18, on silica, with a mean particle size of 50 µm and mean pore diameter of 6 nm (Agilent Technologies); silica gel, acetonitrile and methanol (chromatographic grade, J. T. Baker), ethyl acetate (Mallinckrodt Chemicals) and Millex filters of 0.22 µm porosity (Millipore) were also used in sample preparation.

2.2. Synthesis and characterization of the support Si-Al and of the sorbent Si-Al(PMTDS)

2.2.1. Preparation of support

The preparation of the alumina-coated silica, Si-Al, was adapted from the procedure described by Faria et al.^[30], for the synthesis of a zirconia-coated silica support. Briefly, for each synthesis 1.00000 g of chromatographic silica, measured on an analytical balance precise to 5 decimal places (CP225D, Sartorius),

Table 1. Information and conditions of fragmentation for analysis of the studied pesticides.

Pesticide	log K _{ow} ^b	pK _a ^c	M ^d (g mol ⁻¹)	MRM ^e transition (m/z)	Cone voltage (V)	Collision energy (eV)	t _R ^f (min)	Window (min)	Dwell time (sec) ^g
methamidophos ^a	-0.79	–	141.1	141.9 > 93.1 141.9 > 124.5	20.0	12.0 12.0	4.6		
acephate	-0.85	8.35	183.2	184.0 > 142.5 184.0 > 124.5	10.0	10.0 18.0	4.6		
thiamethoxam	-0.13	–	291.7	292.1 > 210.9 292.1 > 180.7	15.0	14.0 24.0	5.2	0-7	0.5
methomyl ^a	0.09	–	162.2	163.0 > 87.3 163.0 > 105.5	10.0	12.0 12.0	5.2		
imidacloprid	0.57	–	255.7	256.2 > 174.8 256.2 > 209.0	20.0	18.0 12.0	5.9		
thiacloprid	1.26	–	252.7	253.1 > 125.4 253.1 > 89.4	30.0	24.0 40.0	7.4		
carbendazim ^a	1.48	4.20	191.2	192.1 > 159.8 192.1 > 131.8	25.0	16.0 30.0	7.6	6-16	0.5
thiabendazole	2.39	4.73 12.00	201.3	202.1 > 174.8 202.1 > 130.6	40.0	24.0 36.0	8.5		
carbofuran ^a	1.80	–	221.3	222.2 > 164.9 222.2 > 122.6	15.0	12.0 22.0	12.7		
carbaryl ^a	2.36	10.40	201.2	202.2 > 144.7 202.2 > 126.7	10.0	10.0 24.0	16.0	10-20	0.9
clomazone	2.54	–	239.7	240.2 > 124.5 240.2 > 88.5	25.0	20.0 48.0	21.3		
azoxystrobin	2.50	–	403.4	404.2 > 372.0 404.2 > 328.9	20.0	14.0 32.0	21.5	20-24	0.5
methiocarb	3.18	–	225.3	226.2 > 168.8 226.2 > 120.6	15.0	10.0 18.0	22.3		
fenarimol ^a	3.69	–	331.2	331.2 > 80.2 331.2 > 268.0	35.0	34.0 22.0	24.0		
iprodione	3.10	–	330.2	330.1 > 244.9 330.1 > 287.8	20.0	14.0 14.0	24.8		
tebuconazole	3.70	–	307.8	308.2 > 69.0 308.2 > 124.5	30.0	22.0 38.0	25.9	22-31	0,3
pyraclostrobin	3.99	–	387.8	388.2 > 193.9 388.2 > 162.9	20.0	12.0 24.0	26.7		
metconazole	3.85	11.38 1.08	319.8	320.3 > 69.0 320.3 > 124.5	30.0	22.0 38.0	27.0		
difenoconazole	4.36	1.07	406.3	406.2 > 250.9 406.2 > 336.9	35.0	24.0 16.0	28.4		
ethion	5.07	–	384.5	385.2 > 198.9 385.2 > 170.8	15.0	10.0 18.0	31.6	29-43	0.9
pyriproxyfen	5.37	6.87	321.4	322.2 > 95.3 322.2 > 76.6	20.0	14.0 48.0	32.4		

^aPesticides found in samples analyzed by PARA but not allowed for peppers, according to the ANVISA. ^blog K_{ow}: logarithm of the octanol-water partition coefficient (pH = 7, temperature = 20 °C). ^cpK_a: co-logarithm of the acidity constant (temperature = 25 °C). ^dM: molar mass. ^eMRM: multiple reaction monitoring. The first transition was used for quantification and the second for confirmation. ^ft_R: retention time. ^gDwell time: 15 points per peak. Source^[6,28,29].

was added to a solution containing 3.70000 g of aluminum isopropoxide dissolved in 15 mL of anhydrous toluene. This mixture was placed in a thermostated bath (MA120, Marconi) at 40 °C for 3 h. Afterwards, the solution was centrifuged (Rotofix 32 A, Analítica) for 15 min at 6000 revolutions per minute (rpm) at room temperature, the supernatant was discarded and the solid was washed with anhydrous toluene (repeated five times). The material was hydrolyzed with 15 mL of 10^{-3} mol/L aqueous nitric acid solution and centrifuged for 15 min at 6000 rpm, in room temperature, and then dried in a vacuum oven (ADP21, Yamato) at 100 °C for 12 h. The quantification of aluminum on the final material was determined by X-ray fluorescence (XRF) on a bench-top energy dispersive X-ray fluorescence spectrometer (EDX 700 Shimadzu) with rhodium X-ray source and Si(Li) detector, using micro sample holders^[31].

2.2.2. Sorption and thermal immobilization of PMTDS on the support

The sorbent poly(methyltetradecylsiloxane) thermally immobilized on alumina-coated silica was prepared with a 50% excess loading of PMTDS (1.00000 g PMTDS/g Si). To load the PMTDS into the Si-Al pore system, a 10% w/v solution of PMTDS in hexane was added to the appropriate quantity of Si-Al, previously dried at 140 °C for 12 h. The mixture was sonicated using an ultrasonic bath (T14, Thornton) for 10 min. Afterwards the mixture was slowly stirred for 3 h at room temperature and then placed in a fume hood for evaporation of the hexane at room temperature (6 days).

Portions (2.00000 g) of the Si-Al(PMTDS) obtained after the evaporation of solvent were placed in stainless steel tubes (150 mm × 10 mm of internal diameter) fitted with frits and connectors and subjected to thermal treatment in a tubular oven (10P-S, EDG) under a nitrogen atmosphere. The optimal temperature and time for immobilization of PMTDS onto the Si-Al were 100 °C for 12 h.

After immobilization, the stainless steel tube containing the immobilized PMTDS phase was

connected to a pump (510, Waters) and placed in an oven with temperature control (CH-150, Eldex Laboratories) for extraction of non-immobilized polymer by passing hexane at 0.5 mL/min at 50 °C for 4 h. After extraction, the sorbent contained in the tube was removed and dried with nitrogen.

2.2.3. Physicochemical characterizations of the support and of the sorbent

Physicochemical characterizations by spectroscopic methods of SiO₂, Si-Al and Si-Al(PMTDS) were performed with an infrared absorption spectrometer (IR) (FTIR 1600, Perkin-Elmer) and a nuclear magnetic resonance spectrometer (NMR) (INOVA 500, Varian).

2.3. LC-MS/MS

For the chromatographic analysis a system composed of a liquid chromatograph (Alliance 2695, Waters), a Nova-Pak C18 chromatographic column (150 x 3.9 mm, 4 μm) and a Nova-Pak C18 guard column (20 x 3.9 mm, 4 μm), all from Waters, were used with a flow rate of 0.3 mL min⁻¹. The column was maintained at (25 ± 2) °C and 17 μL of sample were injected automatically. The mobile phase was a 0.1% aqueous solution of formic acid (A) and methanol (B). Gradient elution was used and the amount of methanol was changed as follows: 0 min – 50%; 12 min – 50%; 13 min – 75%; 30 min – 90%; 33 min – 90%; 35 min – 50%; 43 min – 50%.

A tandem mass spectrometer (MS/MS) (Micromass Quattro Micro™ API, Waters) with a triple quadrupole and Z-spray interface using electrospray (ESI) (ESCI™ multi-mode ionization), operating in the positive mode with multiple reaction monitoring (MRM) data acquisition was coupled to the chromatographic system. Nitrogen was used as the cone and desolvation gas and argon as the collision gas at a constant pressure of 2.45×10^{-3} mbar). The parameters of the mass spectrometer for analysis were: capillary voltage: 2 kV, cone extractor voltage: 3 V, RF lens voltage: 0.2 V, source temperature: 120 °C, desolvation gas temperature:

400 °C, desolvation gas flow rate: 500 L h⁻¹ and cone gas flow rate: 50 L h⁻¹. The data acquisition and processing were performed using MassLynx software, V. 4.1.

2.4. Sample preparation by MSPD

2.4.1. Fortification and extraction of pesticides from samples of sweet green pepper

Sample preparation using MSPD was carried out as proposed by Rodrigues et al.^[25] for onion samples. The sweet green pepper, without washing, was cut into pieces and minced in a domestic multiprocessor (Facilic, Arno) until complete homogenization. Then, when desired, 0.50000 g of sweet pepper were placed in a container and fortified with 100 µL of a standard solution containing the pesticides under study, so that the final concentration of each analyte in the sample was equal to the method quantification limit (MQL). Shortly thereafter, the shredded sweet pepper sample was added to 1.00000 g of sorbent. The mixture was macerated to obtain complete dispersion of the sample (approximately five minutes) and then transferred to a 6 mL polyethylene cartridge. 10 mL of the extraction solvent or mixture (acetonitrile, ethyl acetate or acetonitrile/ethyl acetate 50:50 v/v) in portions of 5 mL, were added sequentially, and elution with the aid of a vacuum system (Supelco, manifold) occurred at a rate of approximately 1 mL min⁻¹. 50 µL of formic acid in 5% v/v acetonitrile was added to the extract and the solvent was evaporated under a stream of nitrogen gas. The solid was resuspended in 0.5 mL of methanol, filtered and stored in the freezer in a vial until analysis by LC-MS/MS.

2.4.2. Optimization of the MSPD sample preparation technique

Because sweet peppers have constituents that could interfere with the determination of the analytes, the standard solutions were prepared in a matrix extract free of the studied compounds (overlap matrix). Several factors which influence the recovery of analytes were evaluated: dispersant [C18, SiO₂ and Si-Al(PMTDS)] and solvent (acetonitrile and ethyl acetate), to establish optimal analysis conditions.

2.5. Evaluation of the matrix effect

The influence of the constituents of sweet green pepper on the determination of analytes by LC-MS/MS was evaluated by comparing analytical curves constructed by analysis of standard solutions containing pesticides at seven levels of concentration prepared in solvent to those prepared in extracts free from sample analytes.

2.6. Validation of the analytical method

The method was validated according to the international guideline SANTE/11945/2015^[32].

The selectivity was evaluated by comparing the chromatograms obtained after injection of extracts free of pesticides with those from fortified extracts. The analytical curves constructed from the adjusting the responses (peak areas corresponding to the pesticides) resulting from the analysis of extracts from spiked samples at seven concentration levels of the analytes (1; 1,2; 2,4; 4; 6; 10 and 12 x MQL) to a linear model were used to assess the linearity through the correlation coefficients (r) of the model and residue analysis. The method quantification limit, MQL, based on the visual method, was considered as the lowest concentration of the analyte in the matrix that could be determined with accuracy and precision, given the linear working range. For the evaluation of accuracy, recovery assays were performed in which sweet pepper samples were fortified at two concentration levels (MQL and 10 x MQL) in five replicates each, and the extracts analyzed. The accuracy was estimated in terms of recovery of the analytes obtained using the analytical curves and Equation 1.

$$\text{Recovery(\%)} = \left(\frac{\text{concentration of the analyte found in the extract}}{\text{concentration of the analyte added to the extract}} \right) \times 100$$

Precision, in terms of repeatability and intermediate precision, was evaluated by preparation, injection and analysis of five samples of sweet pepper extracts fortified with pesticides at concentrations equal to MQL and 10 x MQL on the same day and on different days (first and 14th day), respectively. For this evaluation

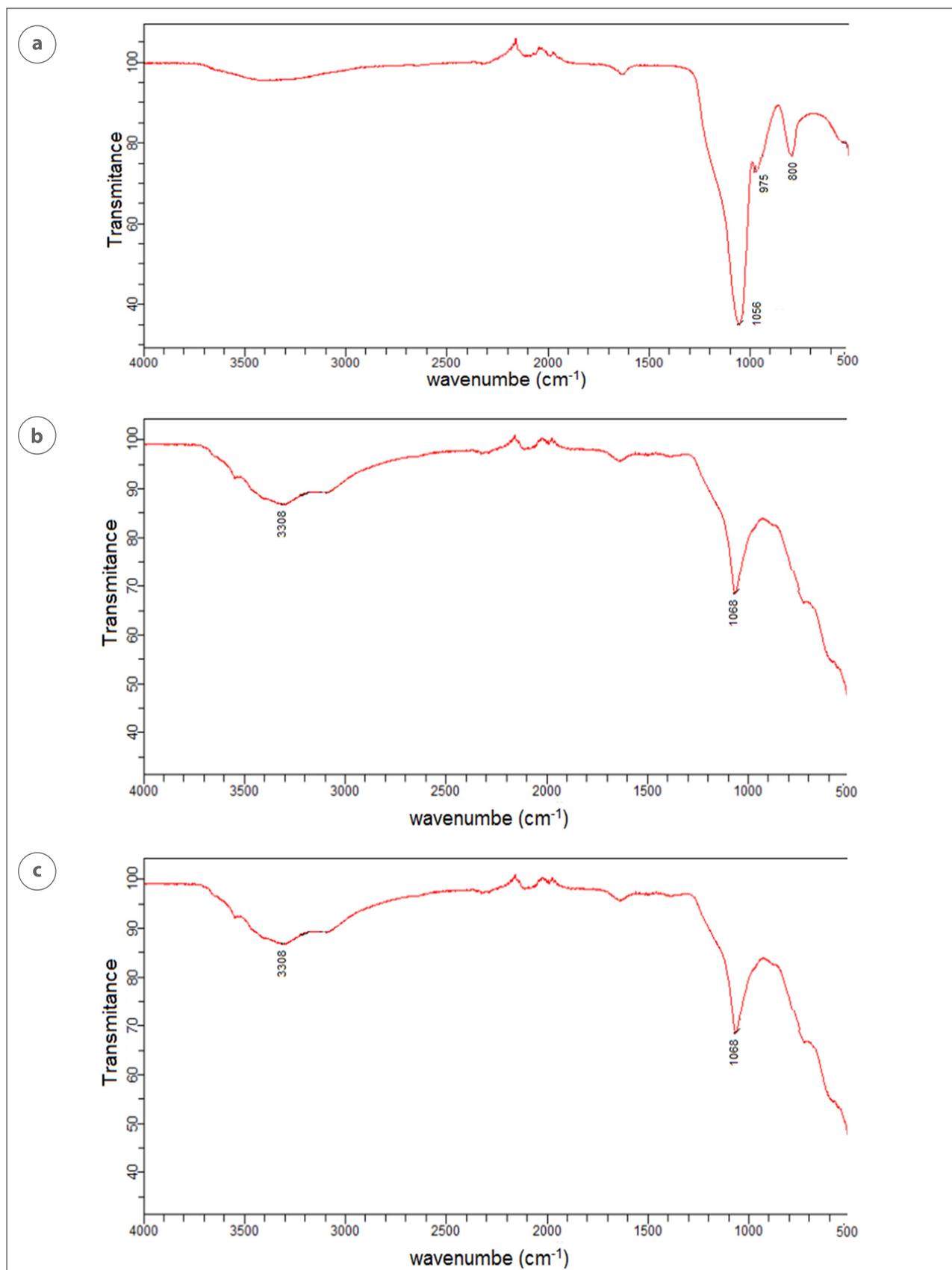


Figure 1. Infrared spectrum of (a) bare silica (SiO₂), (b) alumina coated silica (Si-Al) and (c) poly(methyltetradecylsiloxane) polymer phase thermally immobilized on alumina coated silica (Si-Al(PMTDS)).

the coefficients of variation (CV) for the recoveries of the analytes were determined.

2.7. Analysis of sweet green pepper

Samples of sweet green peppers were acquired from five different supermarkets in Campinas (SP, Brazil), three of these being described as selling organic produce. They were analyzed by the proposed MSPD-LC-MS/MS method. Three sweet peppers from each establishment were used to obtain the mixture for extraction, which was then analyzed.

3. Results and Discussion

3.1. Physicochemical characterizations of the support and of the sorbent

Initially, 7.5% (m/m) of aluminum was incorporated onto the silica, as determined by X-ray fluorescence. Physicochemical characterizations of SiO₂, Si-Al, and Si-Al(PMTDS) were carried out by several spectroscopic methods. Several different batches of the Si-Al support and of the Si-Al(PMTDS) sorbent were prepared and their physicochemical characterizations showed significant similarities. Infrared spectroscopy (FTIR) was used to compare bare silica, the modified support and the presence of PMTDS immobilized onto the Si-Al particles as well as the presence of residual silanol groups. These spectra are shown in Figure 1. Figure 1a presents a number of bands in the “skeletal region” (below 2500 cm⁻¹): the very weak and broad band at a 1635 cm⁻¹ (bending mode of H₂O), the broad band at 1056 cm⁻¹ with a shoulder at 1220 cm⁻¹, and the bands at 800 and 470 cm⁻¹ characteristic of SiO₂, corresponding to asymmetric and symmetric Si-O stretching, and Si-O-Si bending, respectively. The peak at 975 cm⁻¹ is characteristic of Si-OH vibrations. The only significant difference in Figure 1b is the disappearance of the small and weak signal at 975 cm⁻¹ that corresponds to free silanols of silica. The absence of this band in Si-Al indicates the successful insertion of the aluminum cation into the silica framework, resulting in the weakening of

the framework detected by the shift in the asymmetric and symmetric frequencies to lower wave numbers.

Evidence of thermal immobilization of PMTDS onto Si-Al is shown in Figure 1c by the appearance of C-H signals at 2856 cm⁻¹ and 2925 cm⁻¹, corresponding to CH₃ and CH₂ groups of the polymer^[33], as well as a decrease (when compared to the spectrum of the support) in the peak at 3500 cm⁻¹ attributed to silica hydroxyl groups and adsorbed surface water suggesting that most silanol groups are coated.

The support and sorbents were also analyzed by ²⁹Si NMR and alumina by ²⁷Al NMR employing magic angle rotation with crossed polarization (CP/MAS). Figure 2a and Figure 2b, respectively, show the ²⁹Si CP/MAS NMR spectra of bare silica, and the ²⁷Al CP/MAS NMR of bare alumina. The Qⁿ species, which is related to the number of Si-O-Si bonds, are identified as Q⁴ (siloxanes), Q³ (free and vicinal silanols) and Q² (geminal silanols) at -110, -101 and -91 ppm, respectively (Figure 2a). On the other hand, the two peaks at 60 ppm and 9 ppm shown in Fig. 2b are attributed to the two aluminum species present in alumina: Oh (octahedral aluminum) and Td (tetrahedral aluminum), related to the geometry of the coordination compounds formed between aluminum and oxygen.

Figure 2c shows that the Q² band has diminished after the reaction of silica with aluminum isopropoxide while Figure 2d shows the absence of the Q² band and a further decrease in the Q³/Q⁴ ratio for Si-Al(PMTDS), indicating that total quantity of free silanol groups was significantly reduced after immobilization. Figure 2d also shows peaks in the region of -6 to -18 ppm (D², D^{2'} and D_H¹ groups) due to the formation of new silicon species. These can be attributed to PMTDS adsorbed and chemically bonded onto the support particles.

There were no changes in the ²⁷Al NMR after reaction with silica or after the deposition and immobilization of the PMTDS.

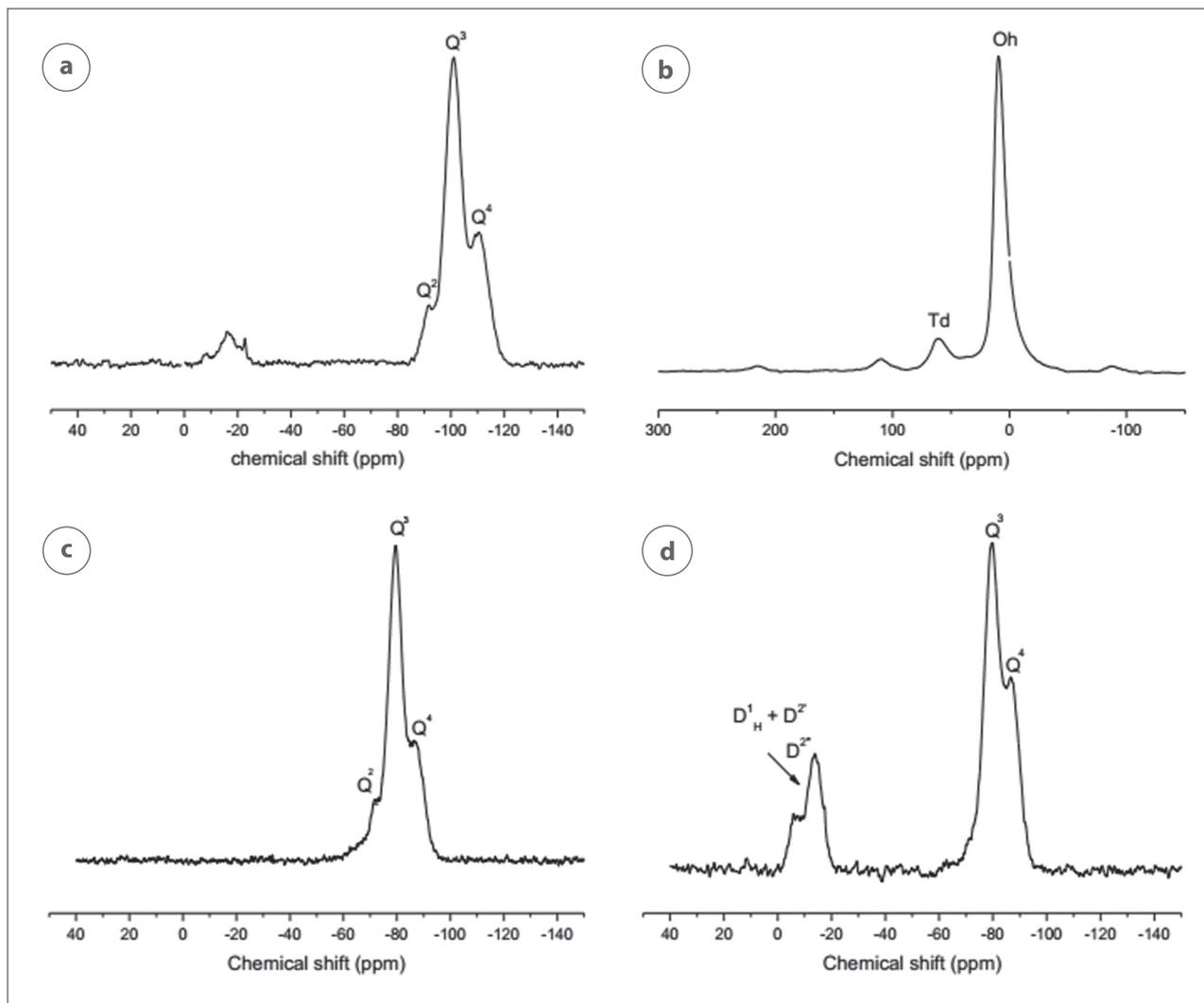


Figure 2. Nuclear magnetic resonance spectra of (a) ^{29}Si of bare silica (SiO_2), (b) ^{27}Al of bare alumina (Al_2O_3), (c) ^{29}Si of alumina coated silica (Si-Al) and (d) ^{29}Si of poly(methyltetradecylsiloxane) polymer phase thermally immobilized on alumina coated silica (Si-Al(PMTDS)).

3.2. Sample preparation by MSPD

3.2.1. Evaluation of clean up sorbents

To minimize the amount of matrix co-extractives that can interfere with the analyses in complex samples, it is necessary to use suitable clean up sorbents in a sample clean up step.

For this 1.00000 g of Si-Al(PMTDS) was used for each procedure, as this mass provided higher recoveries for most pesticides studied, as compared to use of a larger amount of sorbent, possibly due to less efficient elutions of some of the analyte molecules when a larger mass was used. This mass also minimized the quantity

of co-extractives in the extracts, minimizing the matrix effect, according Equation 2^[34], when compared to the use of 0.75000 of sorbent.

$$\text{Matrix effect (\%)} = \left(\frac{\text{peak area of analyte in the matrix extract}}{\text{peak area of analyte in the solvent}} - 1 \right) \times 100$$

Figure 3 shows the recoveries of the pesticides studied using the MSPD technique with different sorbents, using acetonitrile as extraction solvent. The use of either C18 or SiO_2 gave recoveries between 70 and 120% and coefficients of variation less than 20%, recommended values for multi-residue analyses of pesticides in foods^[32], but did not assure selectivity.

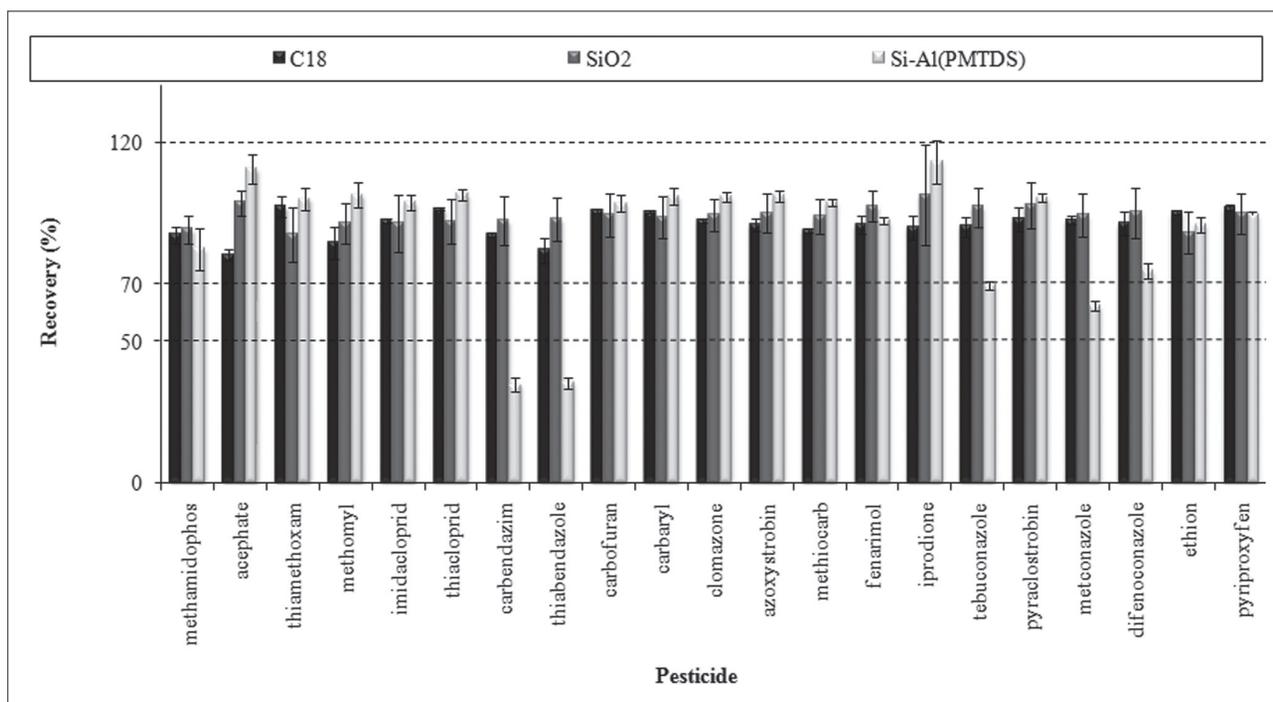


Figure 3. Influence of sorbents in recoveries of pesticides employed in the method using the MSPD.

Under these conditions the use of Si-Al (PMTDS) showed recoveries below 70% for four analytes. Thus it was decided, to vary some parameters to obtain more adequate recoveries with the new sorbent.

3.2.2. Evaluation of the extraction solvents

In order to improve the recoveries of pesticides from samples of sweet green peppers using the new sorbent, particularly carbendazim and thiabendazole, the influence of the extraction solvent was evaluated.

Ethyl acetate caused a significant increase in recoveries of the pesticides carbendazim, thiabendazole, tebuconazole, metconazole and difenoconazole. However, it led to a significant decrease in recoveries of the polar pesticides methamidophos and acephate. Considering these results, a mixture of intermediate polarity, consisting of equal volumes of acetonitrile and ethyl acetate was used. Recoveries between 70 and 120% and coefficients of variation less than 20% were obtained for most target compounds, with the exceptions of carbendazim and thiabendazole, which presented recoveries in the range of 50%, but with coefficients

of variation less than 15%, thus not compromising their quantification. The use of the different solvents in the extraction method resulted in MSPD extracts with similar amounts of the matrix constituents that influence the ionization of the compounds tested, as evidenced by the proximity effect of the matrix values related to the analyte.

Thus, the mixture of acetonitrile and ethyl acetate was used for extraction of the analytes.

3.3. Evaluation of the matrix effect

Analyzing the analytical curves, matrix effects were observed for all studied pesticides except for methomyl, since there are inclination differences between the curves fortified in the solvent and in the matrix extract. The chromatographic responses related to pesticides, from the injection of standard solutions prepared in pesticide-free matrix extract were smaller than those from the injection of standard solutions prepared in solvent. This demonstrates the influence of the co-extractives on the analyte signal.

Therefore, analytical curves using external standardization with superposition of matrix were used for quantification of the pesticides in real samples.

3.4. Validation of the analytical method

By comparing the chromatograms of the extracts free of the pesticides under study with the chromatograms of extracts fortified with these pesticides, there are no chromatographic peaks of coeluting compounds with the same retention times as those of the analytes, showing that the method is selective.

Table 2 presents the validation data of the analytical method developed.

Pesticides permitted for use in different cultures have established maximum residue limits, MRL. When applying these pesticides to these cultures the concentrations found in foods should be below the MRL. Therefore, we chose to evaluate a concentration range close to the maximum residue limit for pesticides that are allowed for sweet green peppers. For banned pesticides the linear concentration range included the method quantification limit, MQL.

The analytical curves constructed using injection of extracts obtained from spiked samples in seven concentration levels of the analytes between 5 and 24000 $\mu\text{g kg}^{-1}$ were used to evaluate linearity. Correlation coefficients greater than 0.99 indicate a good fit of

Table 2. Performance and validation data of the analytical methods developed.

Pesticide	MRL ^a	MRL ^a	MQL ^b	Concentration range ($\mu\text{g kg}^{-1}$)	Linearity (r) ^d	Accuracy		Repeatability		Intermediate precision	
	($\mu\text{g kg}^{-1}$) ANVISA	($\mu\text{g kg}^{-1}$) CODEX	(ALIMENTARIUS)			Recovery (%) ^e		Coefficient of variation (%) ^f			
	MQL	10xMQL				MQL	10xMQL	MQL	10xMQL		
methamidophos	– ^b	– ^b	500	500-6000	0.9976	70	120	5	1	4	1
acephate	1000	– ^b	500	500-6000	0.9925	75	119	6	1	7	1
thiamethoxam	200	– ^b	100	100-1200	0.9927	84	120	5	8	5	8
methomyl	– ^b	– ^b	50	50-600	0.9932	86	88	4	8	4	6
imidacloprid	500	– ^b	250	250-3000	0.9959	110	120	4	1	5	1
thiacloprid	200	1000	100	100-1200	0.9961	72	116	5	1	15	1
carbendazim	– ^b	– ^b	10	10-120	0.9991	50	69	1	4	6	6
thiabendazole	2000	– ^b	200	200-2400	0.9975	50	68	4	8	3	6
carbofuran	– ^b	– ^b	5	5-60	0.9949	85	114	12	14	14	12
carbaryl	– ^b	5000	50	50-600	0.9914	86	106	8	11	13	10
clomazone	50	– ^b	5	5-60	0.9983	81	92	9	7	10	6
azoxystrobin	500	– ^b	50	50-600	0.9956	80	98	8	8	9	8
methiocarb	50	2000	5	5-60	0.9986	70	87	6	4	5	4
fenarimol	– ^b	500	10	10-120	0.9974	70	92	8	6	7	6
iprodione	4000	– ^b	2000	2000-24000	0.9980	83	114	10	8	8	9
tebuconazole	100	1000	10	10-120	0.9943	78	96	9	8	11	8
pyraclostrobin	1000	– ^b	100	100-1200	0.9939	74	90	6	5	6	7
metconazole	100	– ^b	10	10-120	0.9968	78	94	6	8	6	7
difenoconazole	500	– ^b	50	50-600	0.9949	78	90	6	6	7	8
ethion	1000	– ^b	100	100-1200	0.9993	71	87	0	5	5	5
pyriproxyfen	500	– ^b	50	50-600	0.9958	90	98	6	7	12	8

^aMRL: maximum residue limit. ^bUnauthorized pesticides (ANVISA and/or CODEX ALIMENTARIUS). ^cMQL: method quantification limit. ^dr: correlation coefficient. ^eRecovery = 100 x (concentration of analyte found in the extract/concentration of the analyte add to the extract). ^fCoefficient of variation = 100 x (standard deviation/mean of recovery). Source^[28,35].

the model to the observed responses and the random distribution of residues left by adjusting the linear model, with deviation in the range of $\pm 20\%$, indicating the linearity of the method for the pesticides studied in concentration ranges considered.

The method developed can be applied to samples of sweet green peppers for the quantification of pesticides studied at concentrations at or lower than the maximum residue limit established by ANVISA^[28] and the CODEX ALIMENTARIUS^[35], since the limits of quantification considered were equal to or lower than the MRL of each compound.

Satisfactory recoveries between 50 and 120% and coefficients of variation less than 15% for all analytes indicate the accuracy and precision of the method.

3.5. Comparison of methods

The method using MSPD with the new sorbent Si-Al(PMTDS) and the dry ice-partitioning QuEChERS method, developed by Lee et al.^[26], were compared for the extraction of pesticide residues from sweet pepper followed by determination by liquid chromatography with electrospray ionization tandem mass spectrometry. The results show that both methods are efficient, precise and accurate for the determination of pesticide residues from sweet pepper. The matrix components using both methods affected the ionization of some analytes by LC-ESI-MS/MS, highlighting the importance of studying the matrix effect for different extraction procedures for pesticide determination in food. In these studies, external standardization with superposition of the matrix was used to compensate for the influence of matrix effects in the quantification of analytes. In general, the MSPD method is faster, requires less mass of sample, quantity of materials and was shown to be more environmentally friendly, due to the generation of smaller quantities of waste.

3.6. Application of the method

The method was applied to samples of sweet green peppers, acquired from supermarkets in Campinas. Three of these samples were classified as having organic cultivation. Each compound was identified using the two most prominent MS/MS transitions while quantification was made using the most intensive transition peak, as indicated in Table 1. The ratio of peak areas related to quantitation and confirmation were used for affirmation of the presence of the pesticides. One of the organic samples simultaneously showed the authorized pesticides imidacloprid, azoxystrobin, tebuconazole, pyraclostrobin and difenoconazole, all below the maximum residue limit, defined by ANVISA^[6], and the unauthorized pesticide carbendazim at a concentration of $23.2 \mu\text{g kg}^{-1}$ ($\text{CV} = 7.4\%$). Another sample had the pesticide azoxystrobin at a concentration lower the MRL. A third sample contained the pesticides tebuconazole, at a concentrations below the MRL, and carbendazim, an unauthorized pesticide, at $80.7 \mu\text{g kg}^{-1}$ ($\text{CV} = 3.2\%$).

4. Conclusions

A method was developed and validated for multiresidue determination of pesticides in sweet green peppers using liquid chromatography-tandem mass spectrometry after clean-up and concentration using a poly(methyltetradecylsiloxane) polymer phase thermally immobilized onto alumina-coated silica, Si-Al (PMTDS), as new dispersant for matrix solid phase dispersion. The preparation of the sorbent Si-Al(PMTDS) proved to be easy and reproducible, with a cost compatible with commercially available sorbents used in MSPD. However, the commercial sorbents did not assure selectivity in the analysis of some pesticide residues in sweet green pepper samples. The new technique proved to be fast, effective and environmentally safe due to the generation of small quantities of waste.

Moreover, the method showed selectivity, linear response, quantification limits lower than the limits

of maximum residue quantification, accuracy and precision, with recoveries in the range of 50 to 120%, and coefficients of variation less than 15% (acceptable values for complex samples). The method was applied to samples of sweet green peppers obtained locally for determination of the pesticides studied, and in two of the five samples analyzed, the pesticide carbendazim, unauthorized for this culture in Brazil, and banned in the United States and European countries, was detected.

These results prove the potentiality of using Si-Al(PMTDS) synthesized in the laboratory as a sorbent

for MSPD as the sample preparation technique for determination of pesticides in sweet green peppers.

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