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New materials for solid-phase extraction and multiclass high-performance liquid chromatographic analysis of pesticides in grapes

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Abstract

Sample preparation procedures which included the use of new aminopropyl (NH_2) and octadecyl (C_{18}) solid-phase extraction (SPE) sorbents are proposed for the simultaneous multiclass determination of the fungicide benomyl and of the herbicides tebuthiuron, diuron, simazine, atrazine, and ametryn in grapes, using single wavelength high-performance liquid chromatography. Sorbent preparation uses a fast, easy, and effective procedure to obtain silica-based materials, made by depositing polysiloxanes on a silica support followed by thermal immobilization. Recovery results of the compounds, after elution from the SPE cartridges, indicate that the most efficient system employed silica loaded with 40% of an aminofunctional polydimethylsiloxane as sorbent, using dichloromethane:methanol (95:5, v/v) as eluent. Method validation, carried out in agreement with International Conference on Harmonization directives, was performed at three fortification levels (100, 200, and 1000 $\mu g\,kg^{-1}$). Limits of detection and quantification show that the method developed can be used to detect the pesticides at concentrations below the maximum residue levels established by Codex Alimentarius, the US Environmental Protection Agency, the European Union, and Brazilian legislation.

Keywords: Grapes; Food analysis; Solid-phase extraction; Validation; Pesticides

1. Introduction

Multiclass procedures for the determination of pesticides in fresh fruits are more and more required due to their importance in routines involving public health, environmental monitoring, and foreign trade aspects.

Solid-phase extraction (SPE) is one of the most popular techniques used in sample preparation prior to analysis by high-performance liquid chromatography (HPLC) and gas chromatography (GC), being used for environmental, food, pharmaceutical, and biological analyses [1,2]. SPE has many advantages over traditional liquid–liquid extraction, such as the use of smaller amounts of organic solvent, ease of automation, lower cost, and reduced volumes of toxic residues. SPE is used mainly to remove interferences, for pre-concentration and for sample storage and transport. Bonded phases having C_{18} on silica are the most used sorbents in SPE. Over the years, organochlorosilanes and organoalkoxysilanes have been used as silylating agents for the preparation of these bonded phases. The stability of the ≡Si–O–Si≡ bonds formed between the silylating agents and the hydroxyl groups on the silica surface is the main advantage of these phases [3]. On the other hand, this method has some limitations, such as high reagent cost, time consuming procedure, use of toxic solvents and reagents, and the need for an inert atmosphere and high temperatures to carry out the syntheses. A promising alternative method is the substitution of the chemical reaction by depositing a polymer on the support and then immobilizing the polymer using a thermal treatment or γ irradiation. This procedure has been successfully used to prepare several HPLC stationary phases, such as poly(methyloctylsiloxane) (PMOS) immobilized onto zirconized silica [4,5], titanium-grafted silica [6], and pure silica [7]. SPE sorbents based on poly(methyloctadecylsiloxane) (PMODS) immobilized onto silica by γ radiation or thermal treatment have also been described [8,9] and applied for the pre-concentration of pesticides in water and urine samples, respectively.

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The main advantages of this procedure are good performance, lower cost, simplicity, and reduction of toxic residues. Aminopropylsilicas are polar phases that exhibit both polar and non-polar interactions [2]. These materials can act as normal phase or weak anion-exchangers and have also been used in reversed-phase applications [2].

In recent years, many reports have been made describing the development of new SPE materials, such as these mixed-mode sorbents as well as restricted access sorbents, immunoaffinity extraction sorbents, molecularly imprinted polymers, and conductive polymers [2,10–17].

In Brazil, validation guidelines have been proposed in recent years by INMETRO (The National Institute of Metrology, Standardization and Industrial Quality) [18] and ANVISA (The Brazilian Sanitary Surveillance Agency) [19], based in the International Conference on Harmonization (ICH) directives [20,21] and in partnership with the Eurachem Group. Fiscalization of performance and the establishment of maximum residue levels (MRLs) are carried out by ANVISA while ISO17025 certification of laboratories is done by INMETRO.

In 2001, Brazilian viticulture produced 1012×10^6 kg of grapes, of which 21×10^6 kg were exported [22]. The 20×10^6 kg of these grapes were produced in the São Francisco river valley [23]. For export purposes, the grapes have to be certified that they do not have pesticide residues above the internationally determined limits. Thus, the purpose of this work is to develop validated methodologies for the multiclass analysis of pesticides in grapes, involving SPE for sample preparation and HPLC for separation and quantification. This also involved preparing and characterizing new NH_2 and C_{18} SPE materials, to compare with similar commercial SPE phases.

2. Experimental

2.1. Chemicals and materials

The following solvents were used: *n*-hexane, methanol, and petroleum ether, all HPLC-grade (Mallinckrodt, Rio de Janeiro, Brazil); acetonitrile (ACN) and dichloromethane, both HPLC-grade from Mallinckrodt and Merck (Darmstadt, Germany); analytical reagent-grade *n*-pentane (Merck); Milli-Q water (Millipore, Bedford, USA); analytical reagent-grade ammonium hydroxide (Synth, Diadema, Brazil). The solvents used to prepare the mobile phases were filtered using a 0.45 μm poly(vinylidene difluoride) (PVDF) membrane Millipore (São Paulo, Brazil).

The pesticide standards atrazine (97.7%), simazine (98.3%), and ametryn (96.8%) were obtained from Novartis (Basel, Switzerland), tebuthiuron (99.8%) was acquired from Supelco (Bellefonte, PA, USA) and diuron (99.3%) and benomyl (99.1%) were obtained as a donation from DuPont (Paulínia, Brazil). Standard stock solutions of these pesticides were prepared in methanol.

The silicas for preparing the SPE materials were from Fluka (Buchs, Switzerland) or Acros (Geel, Belgium); particle size 0.035–0.070 mm (200–400 mesh) with 6 nm pore size, while the polymers poly(methyloctadecylsiloxane) and aminopropyl-terminated poly(dimethylsiloxane) (NH_2Pr-PDMS) were from United Chemical Technologies (Bristol, USA). The commercial cartridges for SPE were from Supelco (LC-NH_2 and LC-18) and Merck (LiChrolut NH_2 and LiChrolut RP-18), both 500 mg.

The pesticide-free grape samples were obtained from a domestic plantation, in the city of Piumhi, MG, Brazil.

Before all the sample preparation procedures, the laboratory glassware was washed with Extran (Merck).

2.2. Chromatographic instrumentation and conditions

The HPLC system consisted of a Waters (Milford, MA, USA) 510 pump, a SSI (State College, USA) 3XL injector with a 10 μl loop and a Waters UV-Vis absorbance detector (Model 481). Data acquisition and treatment was performed by ChromPerfect software, Version 3.5 (Justice Innovations, Denville, USA). A Purospher RP-18 5 μm column (125 mm × 3 mm i.d.) from Merck and a similar guard column (4 mm × 4 mm i.d.) were used for the separations. The mobile phase was acetonitrile: 0.01% aqueous ammonium hydroxide, pH 8.4 (35:65, v/v) at a flow rate of 0.7 ml min^{-1} with UV detection at 235 nm. All measurements were carried out at room temperature.

2.3. Preparation of SPE laboratory-made cartridges

The silica support was dried at 120 °C for 24 h. A sufficient quantity of support was added to a solution of polymer dissolved in *n*-pentane to give materials loaded with 10 or 40% of the aminopropyl polymer and with 40% of octadecyl polymer. The mixture was stirred gently for 3 h at room temperature, after which the solvent was slowly evaporated in a fume hood at room temperature.

For thermal immobilization, an amount of the loaded support was placed in an oven (120 °C) for 4 h. After immobilization the material was placed in a stainless steel tube, which was connected to a Waters 510 pump for extraction of the remaining soluble polymer by passing approximately 25 ml of *n*-hexane per gram of sorbent, at 2 ml min^{-1}, and then 15 ml of methanol per gram of material, at 3 ml min^{-1}. To make sure that all soluble residues were removed, a final extraction step was made using a high-pressure packing pump (Haskel, Burbank, USA, model 51769), at 1000 psi (6.9 MPa), in a proportion of 20 ml of methanol per gram of sorbent. After extraction, the solid-phase was removed from the tube and the solvent was evaporated at room temperature.

The cartridges were prepared using 0.5 g of sorbent packed into a 5 ml polypropylene syringe, retained by two polyethylene frits (20 μm pore size).

2.4. Solid-phase characterization

The SPE phases were characterized by elemental carbon and nitrogen analysis (Perkin-Elmer, Norwalk, CT, USA, model 2400 CHN analyzer), infrared spectroscopy (Perkin-Elmer 1600 FT-IR spectrophotometer), thermogravimetric analysis (TA Instruments, New Castle, USA, model 2050 TGA), and specific surface area, S_{BET}-N_2 (Micromeritics, Norcross, USA, Flowsorb II, model 2300).

2.5. Sample preparation

Spiked samples at three levels (100, 200, and 1000 μg kg^{-1}) were prepared by adding 100 μl of standard solutions of the pesticides to 5 g of pesticide-free grape sample and mixing thoroughly in a blender. The lowest spiking level was chosen to be close to the Codex Alimentarius [24], European Union [25], US Environmental Protection Agency (EPA) [26], and Brazilian ANVISA [27] MRL for these pesticides in fruit and vegetables.

The procedure for the aminopropyl SPE sample preparation was adapted from Hiemstra et al. [28] and that using the octadecyl cartridges from Torres et al. [29].

For solid-phase extraction with laboratory-made aminopropyl or commercial NH_2 cartridges, 7 ml of acetone were added to the sample/standard mixture and homogenized in a vortex mixer (Phoenix, Araraquara, Brazil; model A-250) for 30 s. The 7 ml of dichloromethane and 7 ml of petroleum ether (b.p. 40–60 °C) were added and the mixture was homogenized for another 30 s. The mixture was then centrifuged for 15 min at 838 rad s^{-1} (8000 rpm) (Fisher Scientific, Pittsburgh, USA, centrifuge) and the organic layer was decanted and concentrated under nitrogen. The residue was redissolved in 1 ml of dichloromethane and placed on a 500 mg aminopropyl cartridge, previously conditioned with 2 ml of dichloromethane. The cartridge was eluted in a 12-port SPE vacuum manifold (Supelco Visiprep) with two portions of 3 ml each of dichloromethane:methanol (95:5, v/v), and the eluent was concentrated to dryness and redissolved in 2 ml of methanol.

For solid-phase extraction with the laboratory-made octadecyl and commercial C_{18} cartridges, 20 ml of acetone:water (1:1, v/v) were added to the spiked grapes. The sample was mixed thoroughly for 15 min by sonication. The mixture was centrifuged for 15 min at 838 rad s^{-1} (8000 rpm) and 20 ml of water were added to the liquid phase. The 500 mg cartridges were previously conditioned with 5 ml of methanol and 5 ml of water, before applying the sample. The cartridge was eluted in a 12-port SPE vacuum manifold with 10 ml of dichloromethane and the eluent was concentrated to dryness and redissolved in 2 ml of methanol.

2.6. Method validation

Method validation was carried out using parameters proposed by the ICH directives [20,21].

Stock solutions of each pesticide were prepared in methanol at concentrations of 100 μg ml^{-1} and stored at 4 °C. The solutions for calibration and fortification were prepared in ACN:water (1:1, v/v). The analytical curves were made using six different concentrations (50 μg l^{-1} to 5 mg l^{-1}) for each analyte, with three replicates each. For recovery and precision evaluation, samples were spiked at three levels: 100, 200, and 1000 μg kg^{-1}.

The limits of detection (LOD) and quantification (LOQ) were calculated based on the standard deviation of the response and the slope of the calibration curve [21].

The accuracy was determined as percent recovery, at three different fortification levels. Precision was evaluated in terms of repeatability and intermediate precision, also using three different fortification levels.

3. Results and discussion

3.1. Characterization of the new SPE cartridges

The polymers studied, aminopropyl-terminated poly(dimethylsiloxane) NH_2Pr-PDMS and poly(methyloctadecylsiloxane) (PMODS)—are both polysiloxanes (Fig. 1). PMODS has strong apolar character (useful in the extraction of apolar compounds), due to its repeating octadecyl units. The aminopropyl terminated polymer, however, has $(CH_2)_3NH_2$ groups at the chain ends; these groups have polar properties (useful in the extraction of polar compounds) and may act as a weak anion exchanger, depending on the medium, while the body of the polymer presents apolar dimethyl groups on the silicon atoms.

Table 1 shows some features of the new solid-phase sorbents prepared in this work as well as the properties of similar commercial materials.

The percent carbon for the laboratory-made aminopropyl materials with a 10% load and for the laboratory-made octadecyl sorbents were very similar to those of the commercial materials. A decrease in the amount of carbon in the new phases after extraction, compared to the carbon content before extraction, can be observed, demonstrating that

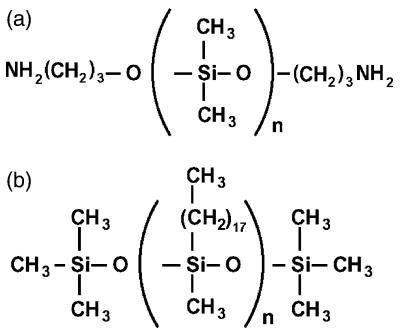

Fig. 1. (a) Aminopropyl-terminated poly(dimethylsiloxane) (NH_2Pr-PDMS) and (b) poly(methyloctadecylsiloxane) (PMODS).

Table 1
Characterization of the developed and commercial sorbents

Sorbent	Type of bond	% C	% N	End-capped	Pore size (nm)	Particle size (μm)
Commercial NH_2	$\equiv Si(CH_3)_2NH_2$	4.1 (5)*	2.0	Yes	6	45
Commercial C_{18}	$\equiv Si(CH_2)_{17}CH_3$	18 (17)*	0.20	Yes	6	45
Laboratory-made NH_2 40% load before extraction	NH_2Pr-PDMS immobilized onto silica	13	0.79	No	6	35–70
Laboratory-made NH_2 40% load after extraction	NH_2Pr-PDMS immobilized onto silica	12	0.51	No	6	35–70
Laboratory-made NH_2 10% load before extraction	NH_2Pr-PDMS immobilized onto silica	3.8	0.28	No	6	35–70
Laboratory-made NH_2 10% load after extraction	NH_2Pr-PDMS immobilized onto silica	3.5	0.26	No	6	35–70
Laboratory-made C_{18} 40% load before extraction	PMODS immobilized onto silica	22	0.17	No	6	35–70
Laboratory-made C_{18} 40% load after extraction	PMODS immobilized onto silica	15	0.15	No	6	35–70

* Values from manufacturer.

the extraction step eliminates non-immobilized polymer. For the aminopropyl phase, this decrease is on the order of 6%, while for the octadecyl phase, it is about 30%, indicating that the aminopropyl polymer has stronger interactions with the silica support than does PMODS. Infrared spectra of the silica support and of the sorbents confirms polymer incorporation onto the support even after extraction with strong solvents such *n*-hexane.

Thermogravimetric analyses (Table 2) showed that the prepared materials are thermally stable in the temperature range generally used for extraction while the specific surface area (Table 2) showed that the laboratory-made materials have larger surface areas than the commercial sorbents, a characteristic which is very desirable in SPE.

3.2. Selectivity

Fig. 2 shows chromatograms of the spiked grape extract, demonstrating the selectivity of the method developed. To illustrate the importance of the SPE step in the sample preparation, experiments were carried out in which chromatograms of fortified samples, prepared using the analytical routine developed, but excluding the SPE purification step, were obtained. The chromatographic profile (Fig. 3) shows the importance of this step for the removal of matrix interferents, while the low recoveries (Table 3) indicate the presence of matrix components, which were not properly extracted.

Table 2
Thermogravimetric analysis and specific surface areas of the sorbents

Sorbent	Thermogravimetric analysis: loss of mass (%) from 25 to 200 °C	Specific surface area S_{BET} ($m^2\ g^{-1}$)
Silica (support)	–	607
Commercial NH_2	2.21	254
Laboratory-made NH_2 (40%)	0.24	330
Laboratory-made NH_2 (10%)	1.60	379
Commercial C_{18}	0.65	95
Laboratory-made PMODS	0.60	190

3.3. Calibration and linearity

The analytical curves were obtained over three orders of magnitude of concentration and their linearities were evaluated by means of the ratio between signal (*S*) and concen-

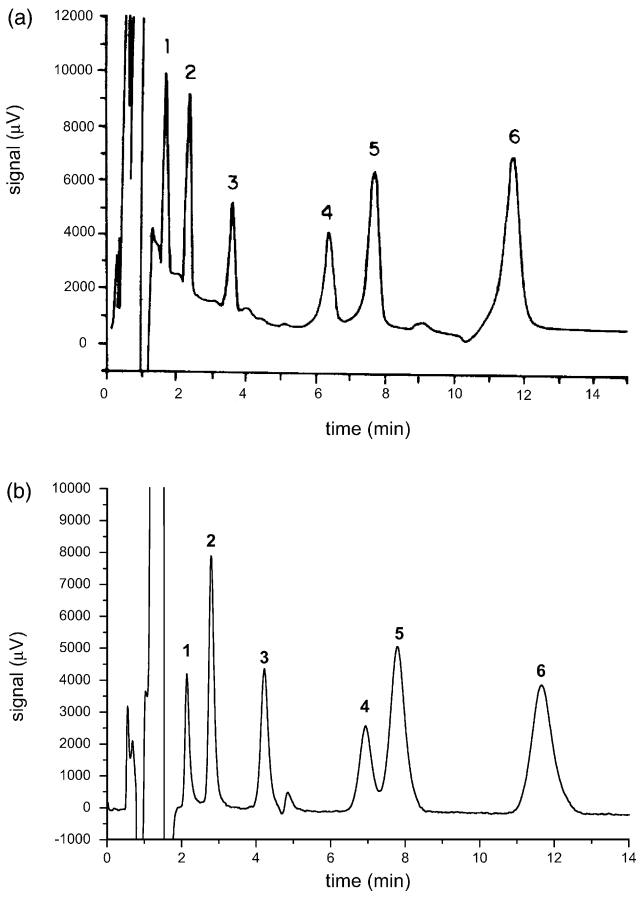

Fig. 2. Chromatograms of the extract from grapes spiked at fortification level F3 (1000 μg kg^{-1}), obtained using (a) laboratory-made 40% loaded-NH_2 and (b) commercial NH_2 cartridges. Chromatographic conditions: injection volume, 10 μl; column, Purospher RP-18 (125 mm × 4 mm), with pre-column (3 mm × 3 mm); mobile phase, CH_3CN: 0.01% aqueous NH_4OH pH 8.4 (35:65, v/v); flow rate, 0.7 ml min^{-1}; detection, 235 nm. Pesticides: (1) benomyl, (2) tebuthiuron, (3) simazine, (4) atrazine, (5) diuron, and (6) ametryn.

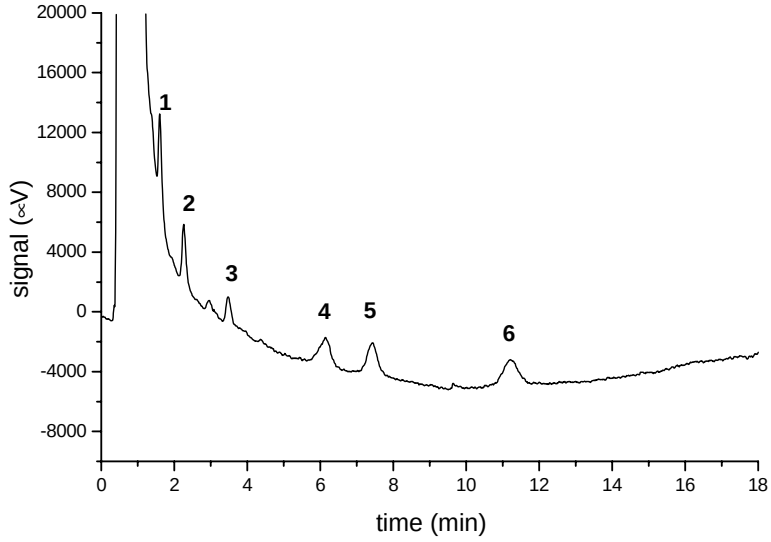

Fig. 3. Chromatogram of grapes spiked at fortification level F3 (1000 μg kg^{-1}), without the SPE step. Chromatographic conditions and peak identification as in Fig. 2.

tration (Q), defined by $(S_i/Q_i) = (S_i - a)/Q_i$, where the signal/concentration ratio for the ith point of the analytical curve, (S_i/Q_i), is calculated from the signal, S_i, at the corresponding concentration, Q_i, and the intercept of the analytical curve, a [30].

In the absence of random errors, i.e. with $r = 1$, and within the linear range, it can be shown that $(S_i/Q_i) = b$, where b is the slope of the curve, for all pairs of experimental values used to construct the curve. In the presence of random errors ($r < 1$), the real situation in the most experimental conditions, and within the linear range, $(S_i/Q_i) \approx b$. If $(S_i/Q_i) < b$ or $(S_i/Q_i) > b$, then the ratio is assumed to be out of the linear range. Based on IUPAC recommendations, points were considered to be in the linear range if their (S_i/Q_i) values did not differ by more than 5% from the slope [30].

The correlation coefficients (r) values were always above 0.996 and the linearities extended from 50 to 10000 μg l^{-1}.

3.4. LOD and LOQ

In this study LOD and LOQ were determined according to the definitions of ICH [20,21], as 3.3 or 10 times the ratio of the estimate of the standard deviation of the intercept of the curve and the slope of the curve, respectively.

The results of LOD and LOQ, before and after pre-concentrations, are presented in Table 4, showing LOQ after pre-concentration lower than 100 μg kg^{-1}, satisfying the European Union [25], EPA [26], Brazilian [27], and Codex Alimentarius [24] MRL. Considering these results, the method is adequate to determine these pesticides in grapes.

Table 3
Recoveries (%) for pesticides in grapes at fortification level F3 (1000 μg kg^{-1}) without the SPE step

Pesticide	Recovery (%)
Benomyl	32
Tebuthiuron	33
Simazine	28
Atrazine	49
Diuron	31
Ametryn	24

3.5. Recovery and precision (repeatability and intermediate precision)

Table 5 shows the recoveries (R) and precisions (repeatability and intermediate precision) for the methods developed, using laboratory-made sorbents and similar commercial sorbents for grapes spiked at several different levels. These parameters were calculated in agreement with the ICH definitions [21].

Repeatability expresses the precision under the same operating conditions over a short interval of time. Intermediate precision expresses within-laboratory variations on different days. Repeatability and intermediate precision are expressed as estimates of the relative standard deviation (R.S.D.) of a statistically significant number of samples.

Considering the acceptability criteria to be recoveries between 50 and 120% with precisions up to 15% [31], a clear difference was observed between the methods using amino-based and octadecyl cartridges. In general, cartridges with amino-based material generate better results than the octadecyl sorbents. Within amino-based sorbents, the laboratory-made aminopropyl sorbents having a 40% loading generally presented the best performances.

The methodology using commercial NH_2 cartridges presents a low recovery for benomyl, which can be

Table 4
LOD, LOQ, and MRL values ($\mu g\,kg^{-1}$) from several agencies

Pesticide	LOD	LOQ	LOD*	LOQ*	MRL Brasil/ Codex [27,24]	MRL EPA [26]	MRL European Union [25]
Benomyl	54	163	22	65	10000	10000	2000
Tebuthiuron	45	138	18	55	n.d.	n.d.	n.d.
Simazine	37	113	15	45	n.a.	250	n.a.
Atrazine	36	108	14	43	n.d.	n.d.	100
Diuron	36	110	14	44	100	1000	n.d.
Ametryn	71	214	28	86	200	n.d.	n.d.
Other pesticides	–	–	–	–	>200	>100	>50

n.d.: not defined (MRL not defined); n.a.: not authorized (use of the pesticide not authorized).
* LOD and LOQ after 2.5-fold pre-concentration.

Table 5
Recoveries ($n = 6$) and precision (repeatability, $n = 6$, and intermediate precision, $n = 3$) for pesticides in grape; fortification levels: F1 ($100\,\mu g\,kg^{-1}$), F2 ($200\,\mu g\,kg^{-1}$), and F3 ($1000\,\mu g\,kg^{-1}$) using commercial and laboratory-made (40 and 10% polymer load) aminopropyl cartridges and commercial and laboratory-made (40% polymer load) octadecyl cartridges

	Recovery (%)			Repeatability (%)			Intermediate precision (%)		
	F1	F2	F3	F1	F2	F3	F1	F2	F3
Commercial NH_2 cartridges									
Benomyl	49	26	28	13	27	49	8.9	79	25
Tebuthiuron	87	78	72	12	10	10	4.9	50	12
Simazine	71	88	81	0.8	10	8.5	1.5	8.4	8.7
Atrazine	87	98	94	7.0	14	12	6.6	8.4	18
Diuron	105	93	81	3.7	10	11	15	9.1	12
Ametryn	84	88	93	11	9.3	11	18	3.9	4.3
Laboratory-made 40% loaded-NH_2 cartridges									
Benomyl	143	74	61	13	12	12	9.8	6.5	56
Tebuthiuron	84	81	80	7.1	9.9	14	20	3.5	14
Simazine	81	78	84	13	15	8.7	17	17	8.7
Atrazine	119	69	86	11	16	10	31	27	17
Diuron	96	69	82	19	1.8	1.4	7.4	5.9	13
Ametryn	109	55	94	4.5	11	3.8	10	1.2	15
Laboratory-made 10% loaded-NH_2 cartridges									
Benomyl	102	52	23	4.8	8.2	6.1	22	22	44
Tebuthiuron	94	52	26	4.6	5.4	5.4	11	15	44
Simazine	113	91	33	3.5	14	2.2	23	9.1	21
Atrazine	72	62	88	20	15	4.0	29	17	8.4
Diuron	83	69	78	17	23	4.6	13	17	11
Ametryn	101	68	90	14	16	7.9	20	18	8.9
Commercial C_{18} cartridges									
Benomyl	40	21	4.0	27	7.4	39	43	25	58
Tebuthiuron	73	22	13	44	13	20	14	18	11
Simazine	35	72	15	25	7.2	12	30	1.4	24
Atrazine	55	64	44	24	4.6	15	1.6	23	15
Diuron	86	75	62	5.3	4.9	7.0	5.2	6.2	4.4
Ametryn	59	65	54	15	7.6	9.5	8.3	25	10
Laboratory-made PMODS cartridges									
Benomyl	69	8.0	45	3.1	13	22	38	4.6	0.8
Tebuthiuron	75	15	33	1.9	0. 0	13	26	0.0	9.0
Simazine	60	20	27	14	7.8	0.0	18	4.6	0.0
Atrazine	76	37	36	18	8.8	27	24	1.6	28
Diuron	103	73	81	11	1.6	1.7	5.3	2.1	0.9
Ametryn	74	41	39	11	6.5	17	25	22	14

attributed to its high polarity, resulting in loss of the analyte during the sample preparation (low affinity for the apolar solvents dichloromethane and petroleum ether). The recovery at fortification level F1 is higher than at levels F2 and F3, suggesting that there is a limit to the mass of analyte that is dissolved in the initial solvents or retained by the organic phase.

For laboratory-made, 40% loaded-NH_2 cartridges, the benomyl recovery shows acceptable values at the F2 and F3 levels. The only recovery result out of the acceptable interval is for benomyl at fortification level F1. For commercial NH_2 cartridges, the high recovery values can be attributed to difficulty in peak integration, since it appears at the beginning of the chromatogram.

The laboratory-made, 10% loaded-NH_2 cartridges show lower recoveries for the polar pesticides (benomyl, tebuthiuron, and simazine) than do the laboratory-made 40% loaded-NH_2 sorbents, at the fortification level F3 although higher values of precision are seen in several cases. However, overall, the laboratory-made 10% loaded-NH_2 cartridges were not considered appropriate, probably due to the low polymeric covering, resulting in irreversible retention of the most polar pesticides by the silanol groups of the support.

Methodologies using commercial and laboratory-made C_{18} cartridges had poorer performances than did the NH_2 cartridges. Commercial C_{18} cartridges presented acceptable results only for diuron. This can be explained by the high affinity of diuron for the apolar material of this solid-phase. For laboratory-made PMODS cartridges, the recoveries are appropriate for diuron at all fortification levels and also in multirresidue analysis at the F1 level.

Similarities among results for commercial and laboratory-made C_{18} cartridges can be attributed to the high amount of carbon in both sorbents (Table 1), which make them highly apolar materials, with few residual silanol groups available to promote interaction with polar pesticides.

The polarity order of the laboratory-made sorbents is: 10% loaded-NH_2 > 40% loaded-NH_2 > PMODS (confirmed by the results of the carbon analysis, Table 1). Considering only polarity, the expected results for application in retaining polar analytes also follow this sequence. However, the better results with a 40% load of NH_2 material indicate that the effect of the number of amino groups also contributes to sorbent performance.

Conventional aminopropyl sorbents are on the border line between polar (normal phase) and ionic exchanger materials. They can act as normal phase sorbents for extraction of polar compounds, phenolic pigments, drugs and metabolites and as weak anionic exchanger for carbohydrates, weak anions and organic acids, when in appropriate solvents [2].

Our results suggest that the laboratory-made NH_2 sorbents can also function as a mixed-mode sorbent: possessing apolar characteristics due to the dimethylsiloxane chains and also polar characteristics because of the aminopropyl terminations, presenting both polar and apolar interactions and adapting well to different chemical ambients.

4. Conclusions

New materials for solid-phase extraction were developed, characterized, and tested in the multiclass analysis of pesticides in grapes. The analytical methodologies were validated and presented satisfactory results. These methodologies involved two different SPE modes (clean-up and pre-concentration), using both commercial and laboratory-made materials.

The preparation of NH_2-type material from an aminofunctional siloxane polymer immobilized on a silica surface was tested and presents a fast, easy, and effective procedure to obtain silica-based NH_2-type sorbents for use in SPE. The main advantages of this procedure are good performance, lower cost, simplicity and, in common with other SPE procedures, reduction of toxic residues.

The very good performance of the laboratory-made 40% loaded-NH_2 materials can be attributed to its mixed-mode sorbent effect.

The limits of quantification (LOQ) show that the methods developed can be used to detect the pesticides at concentrations below the maximum residue levels (MRLs) established by Codex Alimentarius, Brazilian legislation, and other recommendations.

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