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# Evaluation of Supercritical Fluid Extraction/Aminopropyl Solid-Phase "In-Line" Cleanup for Analysis of Pesticide Residues in Rice

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Supercritical fluid extraction (SFE) and the use of aminopropyl solid-phase material for "in-line" cleanup was evaluated for residue analysis of 22 GC-amenable pesticides in wild- and white-rice samples with a fat content of 1.9 and 0.4%, respectively. After optimizing the extraction conditions on glass beads as inert material and evaluating the fat amount extracted from rice by SFE, the use of Florisil, Celite, Extrelut, Hydromatrix, and an aminopropyl material as fat-retention materials for SFE "in-line" cleanup was assessed, aminopropyl being the most suitable material for this cleanup of fat. Pesticide mean recoveries obtained from rice samples, at fortification levels around 0.5 mg/kg, by means of the SFE/in-line cleanup method finally proposed (15-mL $CO_2$ volume, 50 °C temperature, 200 atm pressure, 200 $\mu$L of methanol static modifier, and a 1-cm layer of aminopropyl at the bottom of the extraction vessel), ranged between 74 and 98%, except for captafol and dimethoate for which mean recoveries lower than 21% were determined.

**KEYWORDS: SFE; pesticides; rice; aminopropyl; in-line cleanup.**

## 1. INTRODUCTION

Supercritical fluids have unique solvating properties (*1*, *2*) and have been generally accepted as extraction media. In the past few years, a number of papers have described the extraction of pesticides from a variety of matrixes using supercritical $CO_2$ that is employed as the extraction agent primarily because of its low critical constants (31 °C and 73 atm), low toxicity and reactivity, and its availability in high purity at low cost. A number of supercritical methods to determine pesticide residues have been published for a variety of foods such as fruits and vegetables (*3*−*6*), eggs (*7*), meat (*8*), and cereals (*9*, *10*).

When SFE is applied to fruit and vegetable samples, it generally provides clean extracts that can be directly analyzed by gas chromatography without any further cleanup step (*11*, *12*). On the other hand, SFE has been demonstrated to be an excellent method for extracting fats from various matrixes (*13*, *14*). So, when SFE is used for pesticide analysis of lipid-containing samples, large quantities of lipid co-extractives can often accompany the target analytes of interest due to the substantially high solubility of lipids in supercritical carbon dioxide. Because of their high boiling points and molecular weights, many lipid species are difficult to elute under conventional gas chromatographic conditions. Hence, the lipid moieties tend to accumulate in a GC injector port, resulting in highly irregular chromatographic profiles. Various approaches can be selected to minimize the interference of lipid co-extractives in GC assays. Among these, general procedures for sample extract purification, like cleanup via gel permeation chromatography (GPC) or SPE columns packed with different sorbent materials such as Florisil, graphited carbon black, or aminopropyl (*15*, *16*), may be used. In fact, GPC and SPE on short columns are predominantly employed to purify SFE cereal extracts (*17*, *18*). However, these two generally add additional manipulation stages in the process and, mainly in the first case, they increase the total extraction time and solvent volume consumed.

Alternative cleanup techniques, such as matrix solid-phase dispersion (*19*) and even the use of binary gas mixtures for SFE (*20*), have been employed by researchers in SFE extraction of products of animal origin. Also, "in-line" trapping methods have been shown to be very selective (*21*, *22*). This technique was first applied for pesticides by France et al. (*23*) and involves placing a sorbent between the sample and restrictor so that the lipids are retained and the pesticides are eluted in supercritical $CO_2$; however, information on the applicability of the above-mentioned in-line techniques for removing fats from cereals using SFE is not extensive, and there are no literature reports.

The main objectives of this work were to study the extractibility of 22 GC-amenable pesticides from rice using supercritical carbon dioxide and to assess the applicability of an in-line cleanup technique to reduce the amount of lipid co-extractives to avoid the need for an additional sample cleanup step prior to GC. To carry out the study, the 22 pesticides were grouped in accordance with the detector used for its chromatographic detection. Eleven of these pesticides were detected by

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ECD (electron capture detector) and 11 pesticides were analyzed using TSD (thermoionic selective detector); some of them can even give a quantitative response in ECD.

## 2. EXPERIMENTAL PROCEDURES

**2.1. Reagents and Materials.** Ethyl acetate, cyclohexane, and methanol were pesticide residue grade from Panreac (Barcelona, Spain). Aminopropyl was supplied by Waters (Massachusetts). Florisil (60−100 mesh), Celite, Extrelut, and Hydromatrix were from Riedel de Häen (Seelze, Germany), J. T. Baker (Deventer, Holland), Merck (Darmstadt, Germany), and Varian (Harbor City, CA), respectively. All sorbents were analytical grade.

Certified standards of the 22 studied pesticides (purity > 96%, except for triazophos (79%)), were supplied by Dr. Ehrenstorfer (Augsburg, Germany).

An individual stock standard solution (about 1000 mg/L) was prepared in acetone for each pesticide. Two mixed pesticide standard solutions were prepared by suitable dilution of stock solutions with acetone. The first standard solution included pesticides that were determined using TSD; these pesticides were dichlorvos, dimethoate, diazinon, chlorpyrifos-methyl, parathion-methyl, pirimiphos-methyl; fenitrothion, chlorpyrifos, methidathion, triazophos, and carbophenothion. The second standard solution contained those pesticides that were determined using ECD; they include $\gamma$-HCH, chlorothalonil, vinclozolin, procymidone, p,p′-DDE, p,p′-DDT, captafol, iprodione, bromopropylate, permethrin, and deltamethrin. These pesticide standard solutions, which contained close to 50 mg/L of the corresponding pesticides, were used as spiking solutions ("ECD spiking solution" and "TSD spiking solution") during SFE experiments and to prepare solvent or matrix standards for chromatographic analysis. Standard solutions for GC analysis were prepared by dilution of spiking solutions with either ethyl acetate/cyclohexane (1:1) or blank rice samples. Pure standards and standard solutions were stored in the dark at −20 °C.

Glass beads (3-mm diameter) were obtained from Scharlab (Barcelona, Spain) and glass microfiber filter disks from Whatman (Maidstone, England). Carbon dioxide (99.995% purity), helium and nitrogen (99.999% purity), and air (99.99% purity) were supplied by Air Liquide (Madrid, Spain). Analytical balance was a Mettler H54 (Columbus, USA).

**2.2. Apparatus.** *Gas Chromatography.* Pesticide levels were determined with a model 3800 gas chromatograph from Varian (Walnut Creek, CA) equipped with ECD and TSD detectors, with a model 1079 injection port, and a model 8200 CX autosampler (for split/splitless injections), fitted with fused capillary columns DB-5MS and DB-1701 for ECD and TSD, respectively, (J&W, Folson, CA) of 30-m length × 0.25-mm i.d., 0.25-$\mu$m film thickness. The operating conditions were injector temperature 250 °C, ECD temperature 300 °C, TSD temperature 300 °C, splitless time 0.75 min, and injection volume 2 $\mu$L. The columns temperature program was 90 °C for 1 min, 25 °C/min to 180 °C, 5 °C/min to 280 °C, and hold for 9 min. The carrier gas was helium with electronic flow control at 1.2 mL/min; the make-up gas was nitrogen and helium, at a flow rate of 30 mL/min, for ECD and TSD, respectively; TSD detector gases were hydrogen and air at flow rates of 4.25 and 175 mL/min, respectively. A Varian Star 4.5 Chromatography Workstation was used for chromatographic data processing.

**Gas Chromatography−Mass Spectrometry.** GC−MS was used, not to determine pesticide levels but to evaluate the cleaning of extracts in relation with its fat content. A Varian 3400 gas chromatograph-Saturn 3 ion trap mass spectrometer equipped with a model 1077 injection port and a model 8200 Cx autosampler fitted with a DB-5MS fused-silica capillary GC column (30- m length × 0.25-mm i.d., 0.25 $\mu$m film thickness) were used. Operating conditions for GC−MS were 9 psi helium column head pressure; 0.75 min splitless time; and 250 °C injector temperature, 60 °C initial oven temperature for 1 min, ramped to 180 °C at 25 °C/min, then to 280 °C at 5 °C/min, and held at 280 °C for 15 min; 280 °C transfer line temperature; and 220 °C ion-trap manifold temperature. MS measurements were performed with electron impact (EI) at 70 eV in the full scan mode over the mass range of *m*/*z* 60 to 650 at 1 scan/s from 6 to 35 min. Saturn GC-MS version 5.2 software was used for data collection.

*SFE System.* An Isco SFE system consisting of a model 260D syringe pump and controller able to operate in the pressure range of 0.6 to 510 atm (1 atm = 101.3 kPa), a SFX 2-10 extractor with restrictor heater set at 70 °C, and 10-mL stainless steel extraction cartridges with removable 2-$\mu$m frits was used in this study. An uncoated and deactivated fused silica capillary column, 30-cm length × 50-$\mu$m i.d., was used as restrictor, and a 10-mL graduated test tube (immersed in a 15−20 °C water bath) containing 5 mL of ethyl acetate was used as a collection system. In the equipment, the $CO_2$ flow is dependent on pressure and type of restrictor used. With restrictor selected and at 300 atm, the flow rates ranged between 1 and 1.2 mL/min. In all cases, $CO_2$ volume passed through the extraction cell was the parameter controlled.

**2.3. Cereal Samples.** White-rice (AUCHAN, Villeneuve d'Ascq, France) and wild-rice (NOMEN, Tortosa, Spain) samples were purchased from a local market. Whole grains were put in a container introduced in a desiccator overnight to improve the milling process. Then they were milled with an electric stone grain mill model F100 (SAMAP, Andolsheim, France). Water and total fat content of the milled rice samples were determined by applying the official methods of analysis established by the Spanish Ministry of Agriculture, Fisheries and Food (*29*): weighing by differences after heating at 130 °C (water) and Soxhlet extraction with ethyl ether (total fat). Water and total fat content values determined in the milled samples in our laboratory were found to be 12.7 and 0.43%, respectively, for white rice, and 9.9 and 1.91%, respectively, for wild rice. The fat content value obtained for wild rice seems to be too high according to the USDA rice composition database (www.nal.usda.gov/fnic/foodcomp/search/), but it is in agreement with the fat content value declared by different producers of this kind of rice, which usually is in the range 1.8−2.0%.

**2.4. SFE Recovery Studies on Glass Beads.** The first recovery tests were conducted on glass beads, as inert support, fortified with 2.5 $\mu$g of each ECD pesticide (50 $\mu$L of ECD spiking solution) or 3.5 $\mu$g of each TSD (70 $\mu$L of TSD spiking solution). Different SFE conditions were studied following the addition of ECD or TSD pesticides to 10-mL extraction cartridges filled with glass beads. The first studied parameter was the solvent strength of supercritical carbon dioxide, which can be modified by varying the temperature and pressure. The tests carried out were the following: pressure variation from 100 to 400 atm (using 15 mL of $CO_2$ at 50 °C), and temperature variation from 40 to 60 °C (using 15 mL of $CO_2$ at 300 or 200 atm). Also, the influence of the $CO_2$ volume passed through the thimble was studied, experiments being carried out with 5, 10, 15, 20, and 30 mL of $CO_2$ at 50 °C temperature and 200/300 atm pressure. In all cases, tests were done in duplicate, and extractions were performed in dynamic mode after a 5-min static equilibrium period. The volumes of the final SFE extracts were adjusted to 2.5 mL with ethyl acetate and then to 5 mL with cyclohexane. Pesticide levels in these extracts were determined by GC−ECD or GC−TSD using external standard calibration. Previous $CO_2$-bubbling experiments, conducted with empty extraction cartridges and collection of $CO_2$ in ethyl acetate solution containing the studied pesticides, demonstrated that the losses of these pesticides during the bubbling process were negligible.

**2.5. Evaluation of Fat Co-extractives from Rice and In-Line Cleanup.** After performing the glass beads experiments, the percentage of fat extracted with supercritical $CO_2$ from rice and the influence of the use of a layer of different sorbent materials as in-line cleanup method were studied. Extractions were carried out on 7 g of milled white rice or wild rice in the experiments without sorbents. The experiments with sorbent materials were performed on 5.5 g of milled wild rice with a 1-cm layer of different sorbents placed in the outlet side of the extraction vessel (0.5 g of Celite, 0.5 g of Hydromatrix, 0.4 g of Extrelut, 0.8 g of Florisil, or 1 g of aminopropyl). In all cases, after filling the extraction vessel, any dead volume was filled with glass beads. Extractions were performed in dynamic mode after a 5-min static equilibrium period with 15 mL of $CO_2$ at 50 °C temperature and 200 atm pressure. All these extractions were carried out in duplicate, and the percentage of fat extracted was determined gravimetrically by weighing on the analytical balance, at room temperature, the extraction vessel before and after performing the extraction.

**2.6. SFE/Aminopropyl In-Line Cleanup Recovery Studies on Glass Beads and Rice.** Once aminopropyl was established as the best sorbent phase for in-line cleanup of fats, the influence of this sorbent on the extractability of the studied pesticides was assessed. For that, some recovery experiments on spiked glass beads and milled white-rice and wild-rice samples (5.5 g), with a 1-cm layer of aminopropyl (1 g) placed in the outlet side of the extraction vessel, were performed. Both, glass bead/aminopropyl and milled rice/aminopropyl samples were spiked in the extraction vessel with 50 $\mu$L of ECD spiking solution or 70 $\mu$L of TSD spiking solution, allowing the solvent to evaporate before sealing the vessel. In all cases, extractions were performed in dynamic mode after a 5-min static equilibrium period with 15 mL of $CO_2$ at 50 °C temperature and 200 atm pressure, assessing different static modifier conditions: (a) no modifier, (b) 200 $\mu$L of methanol, and (c) 200 $\mu$L of water (only in the experiments with glass beads). All these extractions were carried out in duplicate. The volumes of the final SFE extracts were adjusted to 2.5 mL with ethyl acetate and then to 5 mL with cyclohexane. In all cases, extracts were diluted (1:3) prior to ECD with ethyl acetate/cyclohexane, 1:1 (for glass beads experiments), or matrix blank extract (for recovery studies on rice samples). Pesticide levels in these extracts were determined by GC−ECD or GC−TSD using external matrix-matched standard calibration.

## 3. RESULTS AND DISCUSSION

**3.1. Optimization of SFE Conditions on Glass Beads.** Data obtained for recovery assays on glass beads carried out with 15 mL of $CO_2$ at 50 °C and pressures from 100 to 400 atm show that recovery percentages of the ECD pesticides are not clearly influenced by the pressure variation, although at 200 and 300 atm slightly higher recoveries were obtained for most of these pesticides, chlorothalonil being the ECD pesticide for which major differences can be observed (68−69% recovery at 100 and 400 atm versus 96−97% recovery at 200 and 300 atm). In the case of the TSD pesticides, recoveries of some pesticides are more dependent on pressure changes, but in all cases mean recoveries close to or higher than 80% were obtained at pressures of 200 and 300 atm. So, an increase of pressure from 100 to 200 atm has a marked influence on the recovery of dimethoate and triazophos, with recoveries that change from 31 and 48% to 75 and 80%, respectively. A similar behavior has also been observed by other authors (*9*, *24*) in the supercritical carbon dioxide extraction of some pesticides from wheat flour and Celite.

On the other hand, mean recovery values obtained for the 22 pesticides at the three studied temperatures (40, 50, and 60 °C), using 15 mL of $CO_2$ at 200 or 300 atm indicate that no pesticide seems to be clearly influenced by this parameter. Only in the case of triazophos were recoveries lower than 75% obtained in the experiments carried out at 40 and 60 °C. These results are in agreement with the results obtained by different authors in other SFE experiments on pesticides using similar temperatures, the recoveries of analytes being independent of temperature, probably due to the fact that solubility of all pesticides studied is adequate at the temperature range studied (*25*, *26*).

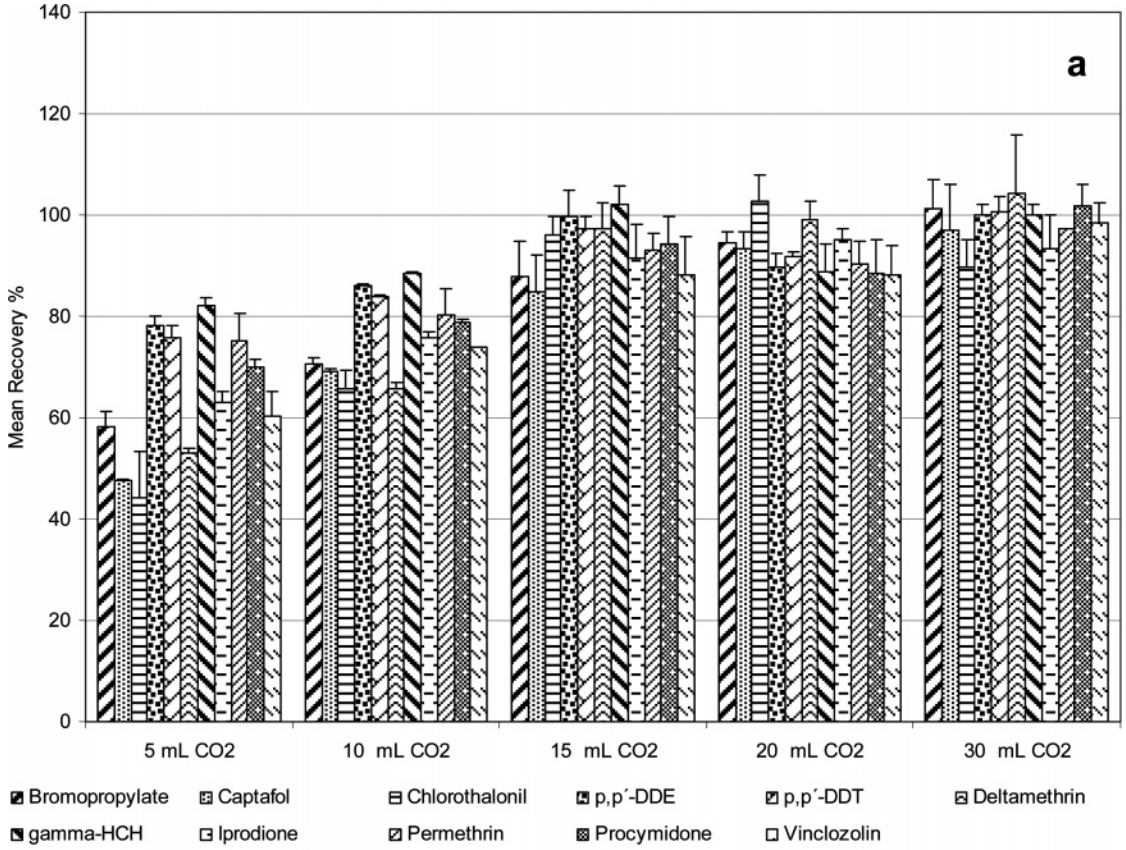
The results obtained in the extractions carried out to evaluate the influence of the $CO_2$ volume on the recovery of the 22 studied pesticides from glass beads (5−30 mL of $CO_2$, 50 °C, 200 and 300 atm) indicate that most of the pesticides were acceptably recovered with only 10 mL of $CO_2$, but all pesticides were well recovered using 15 mL of $CO_2$ (recoveries > 75%) and that an increase of supercritical fluid volume passed through the cell up to 30 mL did not produce a great difference in recoveries. In **Figure 1** are included, as an example, the recovery data obtained at a pressure of 200 atm for (a) ECD and (b) TSD pesticides, respectively. Recoveries obtained at 300 atm were very similar. These results are in agreement with the reported SFE behavior for many pesticides which require only 1−2 elution-vessel-size volumes of supercritical $CO_2$ to give acceptable recoveries (*27*, *28*).

From the data obtained in these experiments on glass beads, a temperature of 50 °C, a $CO_2$ volume of 15 mL, and a pressure of 200 or 300 atm were selected to perform the next assays.

**3.2. Fat Co-extractives from Rice and In-Line Cleanup.** The amounts of fat per 100-g sample extracted from wild rice and white rice with 15 mL of $CO_2$ at 200 atm and 50 °C were 1.86 and 0.39 g, respectively, these values being similar to those determined by Soxhlet extraction with ethyl ether. In the case of wild rice, additional experiments at 300 atm were carried out, and the amount of fat extracted per 100-g sample was 2.04 g. So, it is evident that the use of both pressures produces an appreciable fat extraction from rice and that the use of higher pressures may also increase the extraction of fat and other non-fat material from this matrix (*30*).

Results obtained in the experiments carried out to evaluate the effectiveness of different sorbent materials to retain the fat from wild rice during the extraction with 15 mL of $CO_2$ at 200 atm and 50 °C showed that Celite, Extrelut, and Hydromatrix are not suitable materials for in-line SFE cleanup of fat (the amounts of fat extracted per 100 g of wild rice using Celite, Extrelut, or Hydromatrix were 1.84, 1.80, or 1.62 g, respectively). With the use of Florisil, the amount of fat extracted per 100 g of wild rice was reduced up to 0.36 g, and fat-free SFE extracts were obtained only when the in-line cleanup was carried out using a 1-g layer of aminopropyl. **Figure 2** shows the full-scan MS chromatograms obtained for SFE wild-rice extracts without in-line cleanup, with Florisil in-line cleanup, and aminopropyl in-line cleanup. The two big chromatographic peaks in the GC−MS chromatogram obtained for the SFE extract of wild rice without any cleanup (**Figure 2a**), which are partially or totally eliminated with Florisil or aminopropyl, respectively, were identified as 9-octadecenoic acid (peak at 14.5 min) and 9,12-octadecadienoic acid (peak at 18.4 min) by matching with the NIST92 MS spectra library. There it is evident that the cleanest SFE extract was that obtained by using aminopropyl in-line cleanup.

**3.3. SFE Pesticide Recoveries from Glass Beads/Aminopropyl. Figure 3** includes an ECD (a) or TSD (b) chromatogram for a matrix-matched standard solution prepared with a wild rice/aminopropyl SFE extract, and in **Figure 4** are shown the results obtained in the recovery tests carried out on spiked glass beads with a 1-cm layer of aminopropyl placed at the bottom of the vessel. Recovery values previously obtained from glass beads (without the aminopropyl layer and no modifier) using the same extraction conditions (15 mL of $CO_2$, 50 °C, 200 atm) are also included in this figure to show clearly the effect of the aminopropyl layer on the recoveries from glass beads. In the experiments carried out without modifier, 0% recoveries were obtained for bromopropylate, captafol, dimethoate, iprodione, and triazophos; it is to say, these five pesticides were completely retained in the aminopropyl layer. In addition, it is very difficult to elute carbophenothion, deltamethrin, diazinon, dichlorvos, methidathion, and procymidone through the aminopropyl layer when extractions are carried out with pure $CO_2$ (recoveries lesser than 40%). For the other eleven pesticides, recoveries higher than 60% were obtained, but there are only six pesticides with mean recoveries over 75% (p,p′-DDE, p,p′-DDT, $\gamma$-HCH, permethrin, vinclozoline, and chlorpyrifos-methyl).



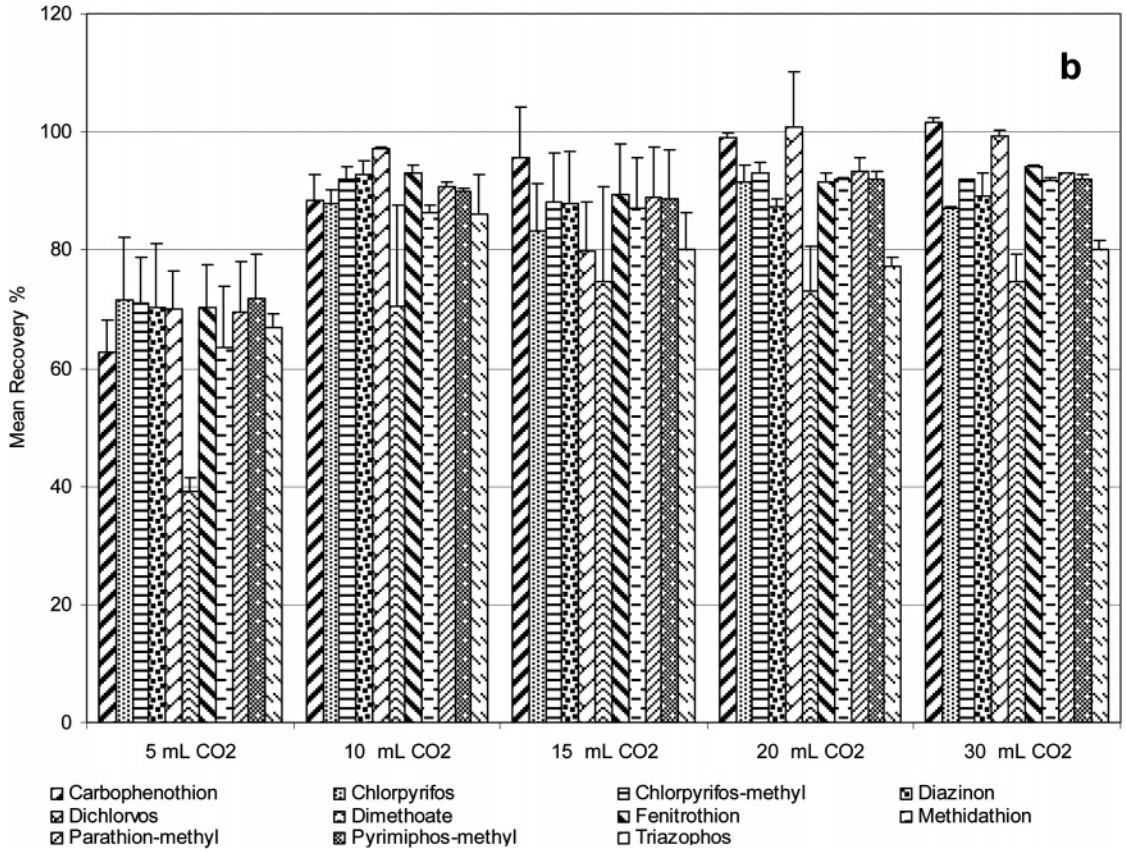




**Figure 1.** Mean recoveries obtained on glass beads fortified with (**a**) 2.5 $\mu$g or (**b**) 3.5 $\mu$g of pesticide at 200 atm and 50 °C and varying $CO_2$ volume from 5 to 30 mL.

In **Figure 4**, we can also see that when extractions are made with $CO_2$ modified with water, better recoveries are obtained for all pesticides, except captafol, dimethoate, iprodione, bromopropylate, and triazophos, which were again not recovered

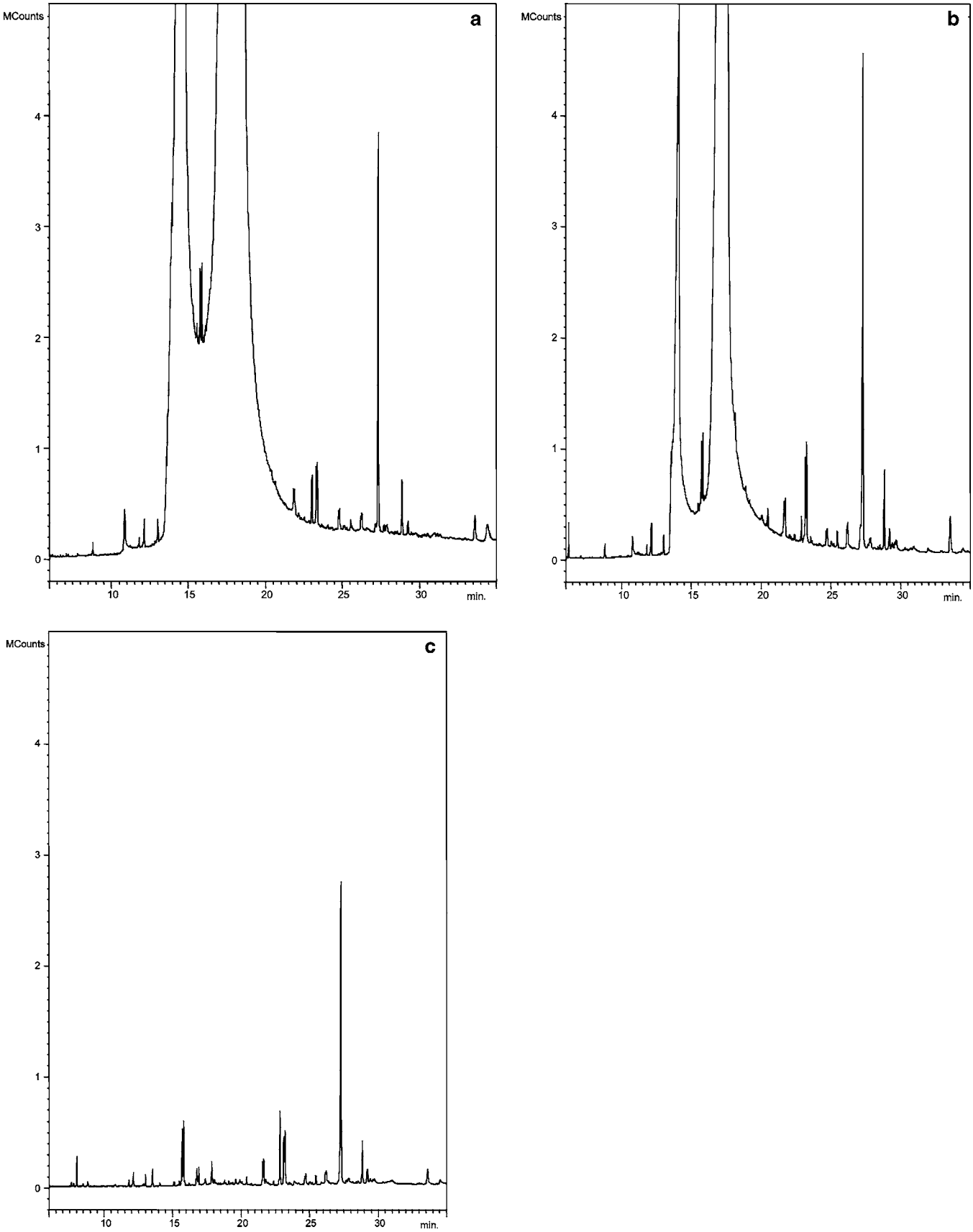



**Figure 2.** MS chromatograms obtained for a SFE extract of wild rice without any additional cleanup method (**a**), with in-line cleanup with Florisil (**b**), and with in-line cleanup with aminopropyl (**c**) (200 atm, 50 °C, 15 mL of $CO_2$).

at all. Deltamethrin, diazinon, dichlorvos, methidathion, and procymidone are still not completely recovered (recoveries between 21 and 61%) when extractions are carried out with $CO_2$ modified with water, but the other 13 pesticides are well eluted through the aminopropyl layer (recoveries higher than 80%; especially noticeable is the recovery of 96% obtained for carbophenothion, a pesticide which was not recovered with pure $CO_2$).

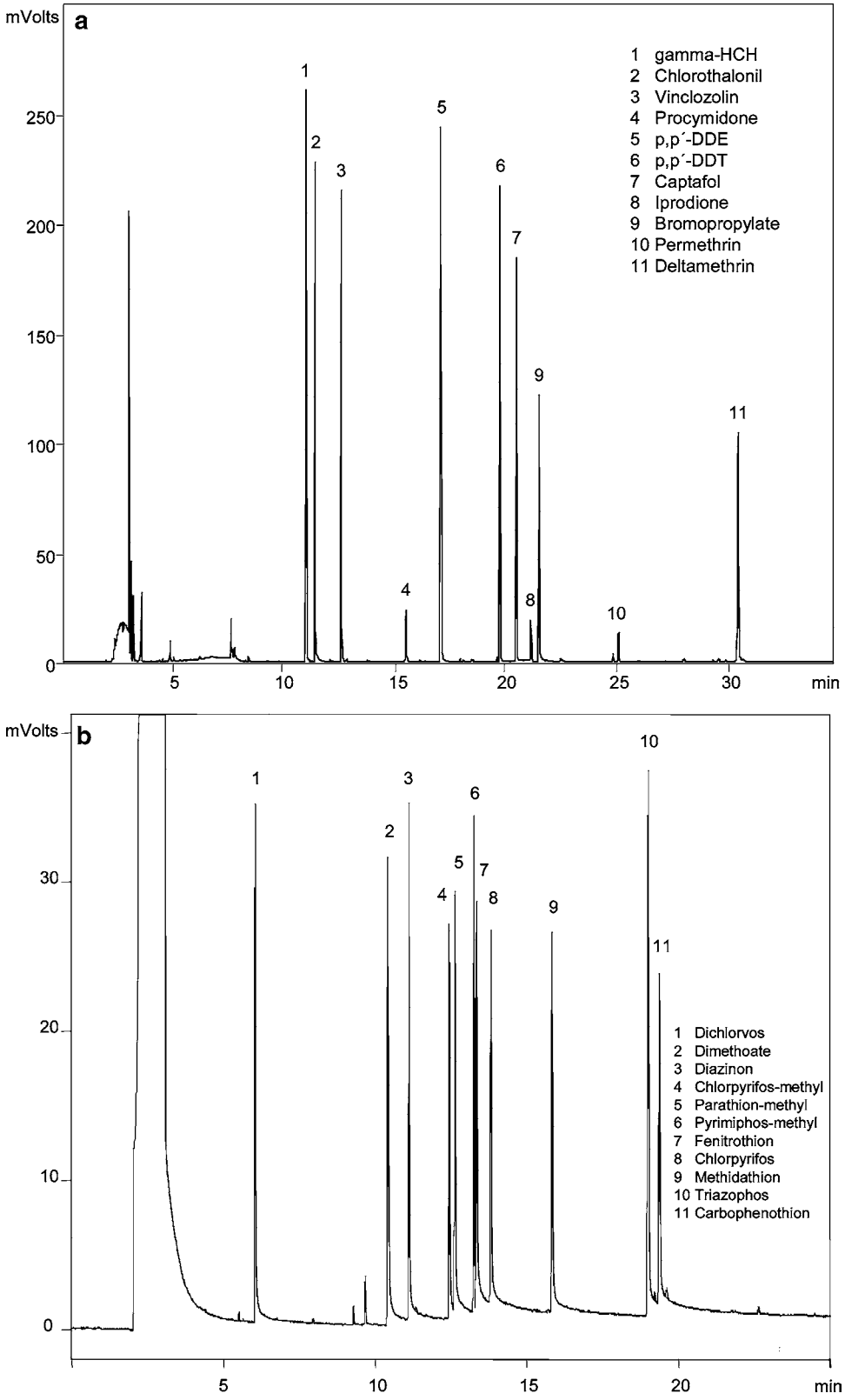



**Figure 3.** (**a**) ECD chromatogram for a matrix standard solution containing 0.168 $\mu$g/mL $\gamma$-HCH; 0.162 $\mu$g/mL chlorothalonil; 0.159 $\mu$g/mL vinclozolin; 0.173 $\mu$g/mL procymidone; 0.177 $\mu$g/mL p,p′-DDE; 0.172 $\mu$g/mL p,p′-DDT; 0.155 $\mu$g/ mL captafol; 0.176 $\mu$g/mL iprodione; 0.167 $\mu$g/mL bromopropylate; 0.180 $\mu$g/mL permethrin; and 0.166 $\mu$g/mL deltamethrin, prepared with a wild-rice extract obtained applying SFE and in-line cleanup with aminopropyl (200 atm, 50 °C, 15 mL of $CO_2$, 1 g of aminopropyl). (**b**) TSD chromatogram for a matrix standard solution containing 0.701 $\mu$g/mL dichlorvos; 0.710 $\mu$g/mL dimethoate; 0.709 $\mu$g/mL diazinon; 0.721 $\mu$g/mL chlorpyrifos-methyl; 0.706 $\mu$g/mL parathion-methyl; 0.694 $\mu$g/mL pyrimiphos-methyl; 0.678 $\mu$g/ mL fenitrothion; 0.661 $\mu$g/mL chlorpyrifos; 0.706 $\mu$g/mL methidathion; 0.723 $\mu$g/mL triazophos, and 0.698 $\mu$g/mL carbophenothion, prepared with a wild-rice extract obtained applying SFE and in-line cleanup with aminopropyl (200 atm, 50 °C, 15 mL of $CO_2$, 1 g of aminopropyl).

Finally, in **Figure 4**, we can also see that all the studied pesticides are well recovered from the glass beads/aminopropyl layer with $CO_2$ modified with methanol (81−106% recoveries), except captafol, carbophenothion, dimethoate, and triazophos, pesticides which were recovered but only partially (20−63% recoveries).

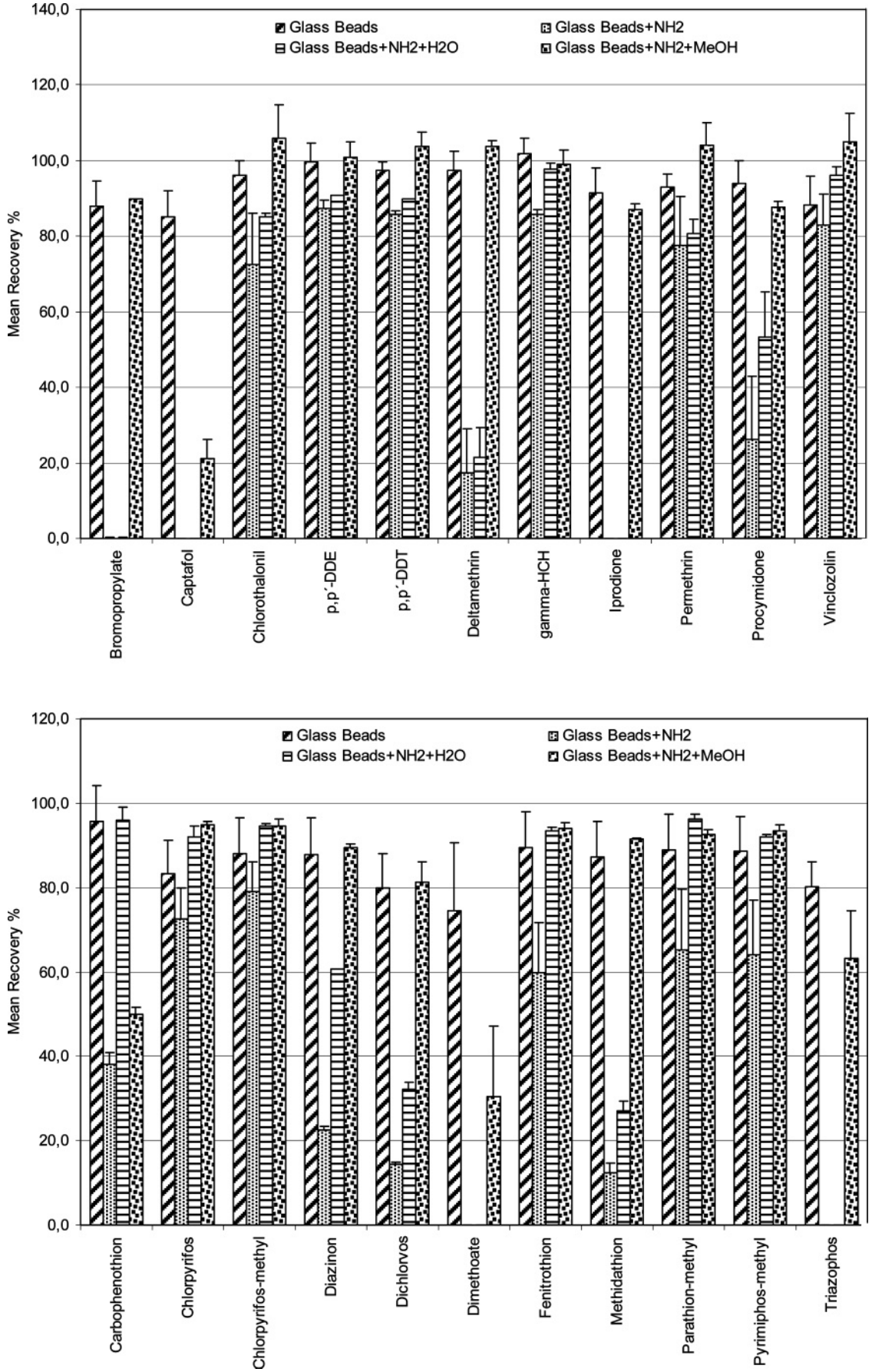



**Figure 4.** Recovery values (mean of two replicates) obtained on glass beads fortified with 2.5 $\mu$g (**a**) or 3.5 $\mu$g (**b**) of pesticide with 200 atm, 50 mL and 15 mL of $CO_2$. Results are from experiments on glass beads; on glass beads and aminopropyl layer ($NH_2$); and on glass beads and aminopropyl layer using 200 $\mu$L of water or 200 $\mu$L of methanol as static modifier.

**Table 1.** Pesticide Mean Recoveries Obtained for Rice Samples by Applying the SFE Method Assessed (200 atm, 50 °C, 15 mL of $CO_2$, 1 g of aminopropyl) with or without the Addition of 200 $\mu$L of Methanol as Static Modifier

| | | mean recovery (RSD), % $n = 4$ | |
|---|---|---|---|
| pesticide | spike level ($\mu$g/g) | no modifier | modifier |
| bromopropylate | 0.46 | 0 | 87 (9) |
| captafol | 0.42 | 0 | 21 (86) |
| carbophenothion | 0.63 | 44 (13) | 76 (8) |
| chlorpyrifos | 0.60 | 73 (7) | 85 (9) |
| chlorpyrifos-methyl | 0.66 | 73 (11) | 81 (10) |
| chlorothalonil | 0.44 | 75 (8) | 90 (12) |
| deltamethrin | 0.45 | 16 (29) | 89 (4) |
| diazinon | 0.64 | 48 (17) | 93 (15) |
| dichlorvos | 0.64 | 25 (37) | 84 (14) |
| dimethoate | 0.65 | 0 | 0 |
| fenitrothion | 0.62 | 72 (7) | 87 (13) |
| $\gamma$-HCH | 0.46 | 94 (5) | 94 (7) |
| iprodione | 0.48 | 0 | 86 (6) |
| methidathion | 0.64 | 16 (38) | 86 (15) |
| p,p-DDE | 0.48 | 93 (7) | 92 (5) |
| p,p-DDT | 0.47 | 90 (9) | 92 (5) |
| parathion-methyl | 0.64 | 71 (4) | 88 (10) |
| permethrin | 0.49 | 96 (4) | 98 (3) |
| pirimiphos-methyl | 0.63 | 75 (5) | 88 (12) |
| procymidone | 0.47 | 75 (8) | 86 (13) |
| triazophos | 0.66 | 0 | 74 (9) |
| vinclozolin | 0.43 | 90 (4) | 90 (14) |

As a summary of the results presented in **Figure 4**, we can conclude that, when aminopropyl solid-phase material is used for in-line SFE cleanup, the extractibility of many of the studied pesticides is very poor if the extraction is performed without a modifier and that the use of methanol as static modifier seems to be a critical parameter in obtaining acceptable recoveries of some pesticides which are difficult to elute through the aminopropyl layer. Also, the water content of the rice samples could be an effective "nonadded" modifier to improve the elution of some pesticides through the aminopropyl layer.

**3.4. SFE Pesticide Recoveries from Milled Rice/Aminopropyl.** Results obtained in the last experiments, which were carried out on spiked white-rice and wild-rice samples with a 1-cm layer of aminopropyl at the bottom of the extraction vessel, are indicated in **Table 1**. The assessed extraction conditions were the same as those used in the above-described glass bead experiments, but in this case only methanol was evaluated as a static modifier.

As expected from the results previously obtained on glass beads, bromopropylate, captafol, dimethoate, iprodione, and triazophos were not recovered at all from the rice samples when the extractions were performed with pure $CO_2$. Likewise, those pesticides partially recovered from glass beads/aminopropyl with pure $CO_2$ were also poorly recovered from rice, except procymidone, which shows a better recovery in rice.

Methanol addition was again demonstrated to be a critical parameter in achieving acceptable recoveries of almost all of the studied pesticides and especially for bromopropylate, deltamethrin, iprodione, methidathion, and triazophos. With its use, all the pesticides, except captafol and dimethoate, are acceptably extracted from rice samples, showing recovery percentages higher than 70%, with mean values between 98% for permethrin and 74% for triazophos.

In conclusion, from the above results it can be seen that the in-line cleanup with aminopropyl is an effective method for obtaining fat-free SFE extracts of rice samples, but it is not suitable for some pesticides such as captafol or dimethoate. On the contrary, the SFE/in-line cleanup method proposed has been demonstrated to be suitable for the other 20 studied pesticides.

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