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**Magnetic dispersive micro solid-phase extraction and gas chromatography  
determination of organophosphorus pesticides in strawberries**

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## **HIGHLIGHTS**

- An efficient method for OPPs analysis in strawberry samples was developed.
- The methodology is based on a QuEChERS, micro d-SPE cleanup, GC-FPD and GC-MS.
- A novel MNPs were synthesized, characterized and applied as sorbents in a d-SPE.
- MNP sorbents were successfully applied for the determination of OPP in strawberries.

**ABSTRACT**

Magnetic nanoparticles (MNPs) with different sizes and characteristics were synthesized to be used as a QuEChERS sorbents for the determination of seven organophosphorus pesticides (OPPs) in strawberries by gas chromatography analysis with flame photometric and mass spectrometry detection. To achieve the optimum conditions of modified QuEChERS procedure several parameters affecting the cleanup efficiency including the amount of the sorbents and cleanup time were investigated. The results were compared with classical QuEChERS methodologies and the modified QuEChERS procedure using MNPs showed the better performance. Under the optimum conditions of the new methodology, three spiking levels (25, 50 and 100  $\mu\text{g kg}^{-1}$ ) were evaluated in a strawberry sample. The results showed that the average recovery was 93% and the relative standard deviation was less than 12%. The enrichment factor ranged from 111-145%. The good linearity with coefficients of determination of 0.9904 – 0.9991 was obtained over the range of 25-250  $\mu\text{g kg}^{-1}$  for 7 OPPs. It was determined that the MNPs have an excellent function as sorbent when purified even using less amount of sorbents and the magnetic properties allowed non-use of the centrifugation in cleanup step. The new methodology was applied in strawberry samples from conventional and organic farming. The new sorbents were successfully applied for extraction and determination of OPPs in strawberries.

**KEYWORDS:** modified QuEChERS; organophosphorus pesticides; GC-FPD; GC-MS; magnetic nanoparticles

## 1. Introduction

Fruits and vegetables form the major component of food consumed by humans because of its high nutritional value and are therefore not surprising to know that many nutritional experts and other health professionals recommend the daily intake. [1]. However, at the same time, they can also turn out to be a source of toxic substances such as pesticides [2].

For millennia, agriculture has had far-reaching impacts on human society and natural systems [3-5]. However, the development of modern pesticides, alongside other technological advances, caused dramatic production increases but also concomitant increases in environmental and health concerns. The use of pesticides has resulted in the repeated and indiscriminate use of pesticides which has exposed farmers and consumers to health hazards due to their toxic residues that persist in foods after application [6-8][6, 9]. The increasing public concern about the possible health risk of pesticide residues in the diet has profoundly modified crop production strategies with the emphasis on food quality and safety. Apart from this, the widespread concern for the health of society has led to the strict regulation of maximum residue level (MRL) of pesticide residues in food [10]. Therefore, the concentration of pesticide residues must be monitored not only in various food commodities including fruits, vegetables, pulses, and cereals but also in all the three compartments of the environment, viz., soil, air and water to check the status [9, 11].

Among various pesticide classes, organophosphorus pesticide (OPPs) group is the most widely used class of agricultural pesticides [11-13]. The largest use of organophosphates is in agriculture but also have many domestic uses. Despite the non-persistent nature of organophosphates, most are found to exhibit toxic effects in mammals. Exposure to these chemicals by swallowing, inhalation or absorption through the skin can lead to immediate

health problems. In recent years, many studies have proved that OPPs are mutagenic, carcinogenic [14, 15], cytotoxic [16], genotoxic [17], teratogenic [18] and immunotoxic [19, 20].

Many scientists have analyzed the OPPs residues in several food samples reported the occurrence of these residues to be even more than MRL values [12, 21-29], recommended by European Union (EU), world health organization (WHO) or food and agricultural organization (FAO).

Scientists all over the world have adopted different extraction procedures and quantification methods for estimation of OPPs in food using gas chromatography (GC) [22-24, 26, 30-36]. The development of simple, rapid, selective and sensitive methodologies that allow their determination at very low levels still constitutes a special challenge for the scientific community [37-44]. In this sense, nanomaterials have produced a great impact on the improvement and development of Analytical Chemistry because of their properties [45-51]. In some cases, such nanomaterials are widely applied in electrochemistry but also used as sorbents in sample preparation, or as chromatographic stationary phases [52, 53].

In the field of sample preparation, several nanomaterials have been used as extraction sorbents such as different types of carbon nanotubes nanoparticles (NPs), metal-organic framework, graphene, etc. Among their different characteristics, the low resistance to diffusion, great surface area and the rapidity of the sorption kinetics are essential for the extraction of both organic and inorganic analytes from complex matrices [54]. In general, sorbents have been applied in different extraction techniques among which it can be highlighted solid-phase extraction (SPE) as well as solid-phase microextraction (SPME) due to the great number of publications present in the literature [52, 54], although they have also shown suitable extraction efficiency under other extraction procedures [56].

Regarding the nanomaterials that have been applied for the extraction of pesticides from food samples, there can also be highlighted the application in QuEChERS usually applied in cleanup step. A particular interest has arisen as a result of the use of MNPs which can be attracted by an external magnet simplifying the pretreatment procedures which constitute one of the most time-consuming steps in the analytical methodologies [52, 55]. The most recent applications in this field which have been mainly focused on their use as sorbents in extraction techniques, not only for analyte extraction but also for cleanup procedures to remove matrix interferences [52, 56]. Magnetic dispersive solid-phase extraction (Md-SPE) has been reported as a technique with very promising results. In Md-SPE, the type of magnetic sorbent plays a critical role for the effective extraction of the analytes [57-59]. Different types of pesticides have been extracted using different nanomaterials approaches from a wide range of food products of a high complexity. Among them, there can be found pyrethroids [46, 60], carbamates [61], organochlorine pesticides (OCPs) [62, 63] or OPPs [45, 64, 65], phenylurea [53] and sulfonylurea herbicides [66] although nanomaterials have also been suitably applied for pesticide multiresidues extraction [48, 49, 51].

This work is the first attempt to use specifically these MNPs fabricated by chemical synthesis. The resulting MNPs were used as a sorbent in the second step of a QuEChERS procedure. Experimental conditions had been optimized. In addition, the using of MNPs significantly simplifies the sample preparation procedure. The results attested that the proposed method for the analysis of OPPs in strawberries has the advantages of simplicity, rapidity, and high-efficiency.

## 2. Experimental

## 2.1 Reagents

Iron (II) chloride tetrahydrate ( $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ ), iron (III) chloride hexahydrate ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ), ammonium hydroxide ( $\text{NH}_4\text{OH}$ ), tetraethyl orthosilicate (TEOS), 3-(trimethoxysilyl)propyl methacrylate (MPS), toluene anhydrous, methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA) were purchased from Sigma-Aldrich (Darmstadt, Germany). Potassium bromide (KBr) was purchased from PanReac AppliChem (Darmstadt, Germany). Ethanol absolute anhydrous (EtOH) and acetonitrile (MeCN) were obtained from Carlo Erba and 2,2'-azobis(2-methylpropionitrile) (AIBN) from Fluka (Darmstadt, Germany). Ultra-pure water (resistivity 18.2  $\text{M}\Omega \cdot \text{cm}$ ) obtained from a Millipore water purification system (Simplicity 185) was used.

Standards of organophosphorus pesticides (dimethoate, diazinon, chlorpyrifos-methyl, parathion-methyl, malathion, chlorpyrifos, and chlorfenvinphos) were obtained from Sigma-Aldrich (St. Louis, MO, USA). The internal standard (IS), triphenyl phosphate (TPP) was also obtained from Sigma-Aldrich (St. Louis, MO, USA). All standards were of  $\geq 99\%$  purity, and all solvents were of chromatography grade. MeCN and n-hexane were from Merck (Darmstadt, Germany).

## 2.2. Preparation and characterization of modified MNPs as a sorbent

The MNPs used in the present work were prepared according to Antuña-Jiménez, et al. 2014 [67]: (1) synthesis of magnetic iron nanoparticles, followed by (2) functionalization of the magnetic core with TEOS, through the Stöber method, to obtain the  $\text{SiO}_2$  shell, and (3) subsequent modification with a silane coupling agent, MPS. The last step (4) consisted of the nanoparticle's surface polymerization in the presence of the functional monomer, the cross-linking monomer and the initiator (MAA, EGDMA, AIBN) (Figure 1).

### 2.2.1. Synthesis of $\text{Fe}_3\text{O}_4$ magnetic nanoparticles ( $\text{Fe}_3\text{O}_4$ MNPs)

$\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$  (2 g) and  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  (5.4 g) were dissolved in  $\text{H}_2\text{O}$  (80 mL). The solution was deoxygenated by purging with nitrogen for 15 min in a reflux system heated to 80 °C. Then,  $\text{NH}_4\text{OH}$  (10 mL) was added, dropwise, and the reaction proceeded for 30 min, under stirring. Thereafter, the solution was removed from the system and placed in an ice bath to stop the reaction. The synthesized  $\text{Fe}_3\text{O}_4$  MNPs were washed 3x with  $\text{H}_2\text{O}$  (20 mL) and 3x with EtOH (20 mL). A magnet was used to efficiently wash the nanoparticles, which were then totally dried under nitrogen before proceeding.

#### 2.2.2. Functionalization of $\text{Fe}_3\text{O}_4$ MNPs with TEOS

For the core/shell synthesis, the  $\text{Fe}_3\text{O}_4$  MNPs (1 g) were dispersed in a mixture of EtOH- $\text{H}_2\text{O}$  (6:1, v/v) and sonicated for 15 min before slowly adding  $\text{NH}_4\text{OH}$  (5 mL) and TEOS (6 mL), respectively. The mixture was left overnight, under stirring, at room temperature. The obtained  $\text{Fe}_3\text{O}_4$  MNPs@TEOS were washed 5x with EtOH (20 mL) using a magnet for efficient attraction and dried with a nitrogen flow.

#### 2.2.3. Modification of $\text{Fe}_3\text{O}_4$ MNPs@TEOS with MPS

To modify the synthesized core/shell NPs, the  $\text{Fe}_3\text{O}_4$  MNPs@TEOS (1 g) were dispersed in toluene (50 mL), followed by the addition of MPS (10 mL). The solution was left for 10 min under a nitrogen flow and then the solution was allowed to react for 7 h, under stirring, at 70 °C. The modified NPs were washed 5x with EtOH (20 mL) using a magnet for efficient attraction and dried with nitrogen.

#### 2.2.4. Surface polymerization of $\text{Fe}_3\text{O}_4$ MNPs@TEOS@MPS with PMAA

The  $\text{Fe}_3\text{O}_4$  MNPs@TEOS@MPS (0.250 g) were mixed with the functional monomer MAA (21  $\mu\text{L}$ , 0.4 mmol), the cross-linking monomer EGDMA (470  $\mu\text{L}$ , 1 mmol), the initiator AIBN (250 mg, 1.5 mmol) and MeCN (20 mL). The solution was deoxygenated by purging with nitrogen flow and left to react during 4h, under stirring, at 60 °C. The



resulting MNPs were washed 3x with MeCN (20 mL) using a magnet for efficient attraction and dried with nitrogen.

Figure 1

#### 2.2.5. Apparatus for MNPs characterization

The obtained nanoparticles were characterized by Fourier transform infrared (FTIR) spectrometry and Scanning Electron Microscopy (SEM). The FTIR spectra were obtained using a Nicolet 6700 FTIR spectrometer (ThermoFisher Scientific, USA) between 4000 and 400  $\text{cm}^{-1}$ , using the KBr pellets method. SEM and EDS images were obtained at the “Centro de Materiais da Universidade do Porto (CEMUP)” using FEI Quanta 400FEG ESEM / EDAX Genesis X4M equipment. The MNPs sizes were determined using ImageJ open source software, and the histograms were obtained using SPSS software, version 20.0 (SPSS Inc., Chicago, Illinois).

#### 2.3. Standard solution

Stock solutions of each pesticide were prepared at 5000  $\mu\text{g L}^{-1}$  concentrations in n-hexane and stored at  $-18\text{ }^{\circ}\text{C}$ . Working standard mixture solutions of different desired concentrations were prepared in n-hexane and were used as spiking, calibration, and control solutions. For validation studies, matrix-matched standard calibration curves with six solutions (between 25 and 250  $\mu\text{g kg}^{-1}$ ) and three spiking levels (25, 50 and 100  $\mu\text{g kg}^{-1}$ ) were prepared. The IS solution was added in all experiments to have a final concentration of 100  $\mu\text{g kg}^{-1}$ .

#### 2.4. Strawberry samples

Strawberry samples were purchased from different markets across the north of Portugal. Ten samples from conventional agricultural practices and ten from organic farming were collected and stored at  $-18^{\circ}\text{C}$ .

#### 2.4.1. Sample preparation method

Strawberry samples were cut into small portions with a knife and placed in a chopper. The homogenized samples were stored in the freezer before being thawed just prior to extraction. Three versions of classic QuEChERS were evaluated in the study, which were based on the original version, AOAC Official Method 2007.01 and European version EN 15662 (Table 1). Different types of classical clean-up procedures using dispersive solid phase extraction (d-SPE), micro d-SPE and disposable pipette extraction (DPX) were evaluated (Table 1 and 2). In d-SPE and micro d-SPE, the extracts are mixed with loose sorbent(s) contained in centrifuge tubes, and in DPX, the sorbents are contained in a pipette tip fitted with a 2  $\mu\text{m}$  pore-size metal screen, which allows the extract to mix with the sorbent(s) (further dispersed with air bubbles using a syringe) and be dispensed but does not allow the sorbent(s) to pass through. After the classic procedures, the best composition was chosen. The selected one was used for further tests with micro d-SPE. For micro d-SPE, eight different sets of sorbents were evaluated (in each case, 10 mg of each synthesized  $\text{MNPs}(\text{Fe}_3\text{O}_4)$ ,  $\text{Fe}_3\text{O}_4@\text{TEOS}$ ,  $\text{Fe}_3\text{O}_4@\text{TEOS}@\text{MPS}$  and  $\text{Fe}_3\text{O}_4@\text{TEOS}@\text{MPS}@\text{PMAA}$ ) in combination with 10 mg of PSA and 10 mg of PSA + C18). Furthermore, eight more sets of sorbents were tested with the same composition but with different quantity (15 mg) (Table 2). The sample preparation procedure entailed the following steps:

- (1) weigh 10 g sample into a 50 mL QuEChERS polypropylene centrifuge tube (Table 1);
- (2) add 50, 100 and 200  $\mu\text{L}$  of the spiking standard ( $5000\mu\text{g L}^{-1}$ ), and shake the tubes vigorously by hand for 30 s and then left to stand for 20 min;
- (3) dispense 10 mL MeCN to the samples and shake the tubes with vigorously by hand for 15 s;
- (4) vortex for 1 min (avoiding formation of oversized  $\text{MgSO}_4$  agglomerates);
- (5) centrifuge the tubes at 4500 rpm for 4 min;
- (6) transfer 1 mL of MeCN extract (upper layer) to the d-SPE tubes for

classical cleanup studies (Table 1) and 0.5 mL for micro d-SPE cleanups (Table 2); (7) vortex the cleanup tubes for 30 s; centrifuge at 4500 rpm for 4 min the classical cleanups and separated the supernatant by an external magnet in case of micro d-SPE cleanups; (8) transfer 0.5 mL of the final extracts into the labeled vials or 0.25 mL into the insert vials for micro d-SPE cleanup; (9) add 10  $\mu$ L of IS standard solution. For DPX, steps (6) and (7) in the protocol were substituted with the following: (a) place the 5 mL DPX tips (Table 1) in the syringe; (b) transfer 1 mL of MeCN extract from step (5) into a 15 mL centrifuge tube; (c) use the piston to draw the 1 mL extract in and out from the bottom of the DPX tip twice, being sure to aspirate air into the tip for proper mixing of the sorbents with the extracts; and (d) dispense the final extract back into the same centrifuge tube. Continue with steps (8) and (9) as described previously.

Figure 2

Table 1

Table 2

#### 2.4.2. Method validation

A set of experiments regarding the limit of detection (LOD), limit of quantification (LOQ), linearity and reproducibility were performed to validate the proposed method. The linear regression analysis was performed from the matrix-matched standard calibration solutions by plotting the peak area versus concentrations of the respective analytes. The LODs and LOQs were calculated by considering the slope of the calibration line and the residual standard deviation of the regression line [4]. The absolute recoveries of targeted analytes were calculated by comparing the peak areas from the spiked strawberry sample to those obtained from the working standard solution at the same concentration.

## 2.5. GC analysis

### 2.5.1. GC – flame photometric detector (FPD) analysis

The determination of the analytes was performed using a Shimadzu GC-2010 with a FPD with phosphorus filter. The separation was achieved on a capillary column with 30 m, ZB-XLB (0.25 mm i.d., 0.25  $\mu$ m film thickness, Zebron, Phenomenex). The GC oven temperature program was optimized to separate the OPPs as follows: 50 °C held for 1 min, ramped at 10 °C/min to 140 °C and held for 1 min, ramped at 5 °C/min to 160°C and held 1 min, ramped at 5 °C/min to 180°C and held for 1 min and finally ramped at 5°C/min to 270°C, at which it was held for 5 min. The FPD port was at 250 °C splitless mode, and the detection was carried out at 290 °C. Helium (purity 99.99% from Linde Sogás) was used as carrier gas at 83.6 KPa, 25.4 cm/s linear velocity and column flow was 1 mL/min. The injection volume was 1  $\mu$ L. The system was operated by GC Solution Shimadzu software.

### 2.5.2. GC/MS and GC/MS/MS analysis

In addition, the contaminated real samples with chlorpyrifos residue detected by GC-FPD were analyzed using a Thermo Trace-Ultra gas chromatograph coupled to an ion trap mass detector Thermo Polaris, operated in the electron impact ionization (EI) at 70 eV. The ion source temperature was 250 °C and the MS transfer temperature, 250 °C. The system was operated by Xcalibur v 1.3 software. Confirmation of chlorpyrifos residue was carried out by GC-MS/SIM and MS/MS using a Zebron column ZB-5MSi (30m x 0.25 mm, 0.25  $\mu$ m film thickness) column operating in the splitless mode; helium was used as carrier gas at a constant flow rate of 1.0 mL/min. The injector was maintained at 250 °C. The oven temperature was programmed starting at 50 °C held for 1 min, ramped at 10 °C/min to 140°C and held for 1 min, ramped at 5 °C/min to 180°C and held for 2 min and finally ramped at 5°C/min to 270°C, at which it was held for 5 min. For the

identification of the chlorpyrifos pesticide by GC-MS/SIM, the retention time, and six ions ( $m/z$  97, 197, 199, 258, 286, 314), the NIST and Wiley pesticide libraries were used. The confirmation was also performed by GC-MS/MS and MS/MS conditions were: precursor ion 314, for the isolation (wideband application = 1 and isolation time = 12 ms), for the fragmentation (excitation time = 15 ms, excitation voltage = 1V and factor “q” = 0.450).

### 3. Results and discussion

#### 3.1. Nanoparticle characterization

The MNPs were synthesized according to the description presented in section 2.2. In order to confirm the successful preparation of synthesized MNPs ( $\text{Fe}_3\text{O}_4$ ;  $\text{Fe}_3\text{O}_4@\text{TEOS}$ ;  $\text{Fe}_3\text{O}_4@\text{TEOS}@MPS$ ;  $\text{Fe}_3\text{O}_4@\text{TEOS}@MPS@PMAA$ ) were characterized by SEM and FTIR analysis.

##### 3.1.1. SEM analysis

SEM images (Figure 3) and EDS spectra (Figure S1) were used to characterize the synthesized nanoparticles. The histograms, the fitted distribution and the determination of the average particle size were based on the analysis of three distinct images of each type of synthesized nanoparticle and are presented in Figure S2.

The synthesized  $\text{Fe}_3\text{O}_4$  MNPs (1) formed agglomerates with asymmetric distributions, which is inherent to the iron oxide MNPs. In the EDS spectra, it is possible to observe the intense peaks related to the presence of iron (Fe) and oxygen (O) that are in concordance with the FTIR analysis (Figure 4). The presence of a silica (Si) peak can also be observed

which is due to the use of the Si substrate as support to obtain the SEM images. In the related histogram, an average nanoparticle diameter of  $17.6 \pm 2.7$  nm is observed.

Subsequently, the nanoparticles surface functionalization with silica is fundamental to protect the magnetic core from oxidation as well as to provide functional groups for the anchorage of the acrylic polymer. The coating of the iron nanoparticles core with TEOS leads to the formation of circular core/shell  $\text{Fe}_3\text{O}_4$  MNPs@TEOS (Figure 3-B), with an average diameter of  $199.2 \pm 46.4$  nm and an average layer thickness of  $11.7 \pm 3.6$  nm. The EDS spectra show a visible decreasing of the Fe peak and the increasing of the Si peak, related to the protective shell layer formation when compared with  $\text{Fe}_3\text{O}_4$  MNPs. Subsequently, the addition of MPS (Figure 3-C) provided an acrylic anchoring layer on the silica surface and dispersed nanoparticles with sizes in the range of  $232.6 \pm 37.7$  nm, with an average layer thickness of  $15.2 \pm 4.5$  nm. The formation of the polymeric layer (Figure 3-D) through the addition of the functional monomer MAA, the cross-linking monomer EGDMA, and the initiator AIBN, resulted in circular nanoparticles, with a visible layer formation. Particles with an average size of  $406.4 \pm 82.1$  nm and layer thickness of  $39.9 \pm 8.1$  nm were perfectly observed in the SEM images. In the EDS spectra, the presence of Si remains similar as observed in the  $\text{Fe}_3\text{O}_4$  MNPs@TEOS@MPS. However, with the polymeric layer formation, it is possible to observe the presence of carbon (C), due to the addition of the functional groups.

### 3.1.2. FTIR spectra of the synthesized MNPs

The FTIR spectra of the synthesized MNPs are shown in Figure 4. For the magnetic iron particles ( $\text{Fe}_3\text{O}_4$  MNPs), an intense and characteristic band at  $563\text{ cm}^{-1}$  attributed to the Fe–O–Fe stretching vibration is observed, confirming the formation of the core nanoparticle. The C–H peak at  $1393\text{ cm}^{-1}$  is related to the symmetric bending vibration of methyl groups. The FTIR spectrum of  $\text{Fe}_3\text{O}_4$ @TEOS shows the Fe–O–Fe vibration

peak at  $468\text{ cm}^{-1}$  and the protective layer of silica obtained by the addition of TEOS is confirmed by the absorption band at  $799\text{ cm}^{-1}$  characteristics of the Si–O bending vibration, and at  $956\text{ cm}^{-1}$  the peak of Si–OH. The strong peak observed at  $1086\text{ cm}^{-1}$  was attributed to the asymmetric stretching vibration of Si–O–Si bonds. The obtained results demonstrate that the  $\text{SiO}_2$  was effectively coated on the surface of the magnetic iron nanoparticles.

The acrylic anchoring layer on the protective  $\text{SiO}_2$  shell with the addition of MPS reveal the band at  $1702\text{ cm}^{-1}$ , characteristic of the carbonyl stretching of methacrylic compounds, at  $1393\text{ cm}^{-1}$  corresponding to the C–H symmetric bending vibration from methyl groups and at  $1629\text{ cm}^{-1}$  the N–H bending vibration. After adding the last layer, the previous absorption bands, characteristic of the acrylic groups that appear at  $1393\text{ cm}^{-1}$  and  $1629\text{ cm}^{-1}$  were attenuated due to the disappearance of these groups, because of the polymerization. The reported bands confirmed that the polymerization was successfully performed.

Figure 3

Figure 4

### 3.2. Optimization of sample preparation

Sample cleanup was necessary to remove co-extracted interferences. The QuEChERS procedure were tested for the seven investigated OPPs from strawberry samples. In the experiments, different classical types of QuEChERS compositions were tested according to different methods (Original, AOAC and EN15662) followed by different cleanups (Table 1).

Consider the advantages of a simple, effective and fast, a modified QuEChERS extraction method was developed and the parameters which affect the cleanup efficiency including

the type (MNPs, PSA and C18) and amount (10 and 15 mg) of the sorbent and cleanup time (30 s and 1 min) were investigated.

### 3.2.1 Extraction with and without MNPs sorbents- Comparison with classical QuEChERS method

The development of sample preparation procedures that effectively cleanup the sample and concentrate the analytes is a complex task. The methods may not sufficiently remove endogenous compounds and could result in significant matrix interference. Matrix components can be coextracted and later coeluted with the target compounds and interfere with analyte identification. To optimize the extraction efficiency of seven pesticides and reduce or eliminate interference in the matrix, different MNPs were explored.

To testify the feasibility of the proposed QuEChERS using MNPs with PSA and C18 in sample preparation it was compared with a classical QuEChERS methodology (with sorbents commercial available). The purification effect was better using MNPs sorbent with PSA for cleanup than using the classical sorbents only (MgSO<sub>4</sub>, PSA, C18, CARB). The chromatogram shows a baseline without interferents (Figure S3).

The use of MNPs for cleanup demonstrated to be a better choice for strawberries as it can remove more matrix components, such as sugars, organic acid and pigments (ex. mainly anthocyanins). These new micro d-SPE cleanups have the advantage of improved speed (non-use of the centrifugation), less solvent and lower amount of sorbents usage. In addition, the new QuEChERS method developed because of this extra sorbent capacity of MNPs cleanup, reduces the GC inlet/column contamination and chromatographic interference from the matrix.

### 3.2.2. Results from spiking recovery of the optimization experiments



The description of the experiments are given in detail in Experimental section. Figure 5 (A) showed the recoveries and variabilities for the different QuEChERS versions (original, AOAC and EN15662) averaged from 56 spikes (2 replicates of each QuEChERS version with 7 classic cleanups). The different types of classical sample preparations, worked equally well for strawberry matrix tested (overall average of 90.0% recoveries with 15% RSD maximum). None of the versions consistently worked well for dimethoate, but the original method gave better results for screening of those pesticides (average of 98.8% recoveries with 10% RSD) (Figure 5). The classical cleanup (150 mg  $\text{MgSO}_4$ , 50 mg PSA, 50 mg C18 and 50 mg CARB from Agilent) showed the best result. As the original version of QuEChERS exhibited advantages compared to the other tested methods, it was the chosen one for the MNPs cleanup studies.

The average recoveries obtained from the set of tests of original QuEChERS version with the four MNPs ( $\text{Fe}_3\text{O}_4$ ;  $\text{Fe}_3\text{O}_4@TEOS$ ;  $\text{Fe}_3\text{O}_4@TEOS@MPS$ ,  $\text{Fe}_3\text{O}_4@TEOS@MPS@PMAA$ ) with PSA and the four MNPs with PSA and C18 (48 spikes (3 replicates of each cleanup with 10 mg and 15 mg) are showed in Figure 5 (B)).

The comparison between Figure 5 A and B showed a higher variability in the results obtained with classical QuEChERS and cleanup methodologies, in certain cases with recovery values higher than those established as acceptable.

The new methodology based on QuEChERS methodology and cleanup with MNPs showed results closer to 100% and with less deviation, which means more accurate and precise results. The average recovery of all pesticides studied was better for the set experiments with  $\text{Fe}_3\text{O}_4@TEOS$  and  $\text{Fe}_3\text{O}_4@TEOS@MPS$ . These MNPs presented similar characteristics (average size 200nm and EDS spectra (Figure S1 and S2– support information) but different coating material. However, the MNPs that present the best result was the  $\text{Fe}_3\text{O}_4@TEOS@MPS$ .

Figure 5

### 3.2.2.1. The amounts of sorbents (MNPs, C18, and PSA) for micro d-SPE cleanup

The used of MNPs with PSA and MNPs with C18 and PSA in the cleanup step provided better results in terms of recovery of target analytes and the amount of isolated matrix co-extracts. The synthesized MNPs presented high surface areas, strong magnetism and high extraction efficiency. To assure sufficient cleanup efficiency towards the target analytes, the adsorbent amount was examined for 10 and 15 mg. The final strawberry extract became colorless with these adsorbent amounts. In the experiments, 4 types of sorbents,  $\text{Fe}_3\text{O}_4@$ ,  $\text{Fe}_3\text{O}_4@\text{TEOS}$ ,  $\text{Fe}_3\text{O}_4@\text{TEOS}@MPS$ , and  $\text{Fe}_3\text{O}_4@\text{TEOS}@MPS@PMAA$ , were tested for the seven investigated pesticides (dimethoate, diazinon, chlorpyrifos-methyl, parathion-methyl, malathion, chlorpyrifos and chlorfenvinphos) in strawberry samples. The recoveries of 7 pesticides in 2 different amounts (10 and 15 mg) of MNPs, PSA and C18 are shown in Figure 6 and Figure 7.

The results showed that the efficiency changed with the amount of the sorbents, the MNP type, the use of PSA or PSA and C18. The recoveries obtained for the modified MNPs cleanups using 10 mg of sorbents were between 70-120% and meet the requirements except for dimethoate in certain cases (Figure 6 and Figure 7). Using 15 mg of sorbents and C18 the matrix effects were observed (recoveries > 120%) for dimethoate, chlorpyrifos-methyl, parathion-methyl, and chlorpyrifos. The results suggest that the use of C18 does not show significant improvement in the efficiency of the methodology (Figure 7). Despite the use of small amounts of adsorbents than normal, the results for these new micro d-SPE cleanups were very promising.

Figure 6

Figure 7

### 3.2.2.2. Cleanup time

The effect of the cleanup time on the extraction efficiency was investigated from 30 s and 1 min and the results were shown in Figure 8. There is no obvious difference in the absorption intensity and color of the final strawberry extract in the tested range. The minimum, maximum and median recovery values obtained for all the pesticides were more adequate for cleanup time of 30 s. Considering the cleanup efficiency and time consumed in sample preparation process, 30 s of cleanup time was adopted.

Based on the discussions mentioned in section 3.2, the optimal cleanup conditions for the modified QuEChERS procedure are as follows: 10 mg of the sorbent ( $\text{Fe}_3\text{O}_4@\text{TEOS}@\text{MPS}$ ) and 10 mg of PSA and 30 s using the vortex in cleanup step.

Figure 8

### 3.2.3. Method validation

To verify the accuracy and precision of the modified method, several analytical parameters were evaluated, including recovery, linear range, LODs and LOQs based on SANTE/11813/2017 guidelines. The recovery values revealed that the determination of the seven organophosphorus pesticides was affected by the interferences from real samples. The coextracted components from complex matrix would influence the signals of analytes. Therefore, to provide reliable results, matrix-matched calibration curves were chosen as reference. Under the optimization conditions, the coefficients of determination ( $R^2$ ) are higher than 0.9904 in the range from 25-250  $\mu\text{g kg}^{-1}$ . The LODs and LOQs values were in range 3.64 – 10.38  $\mu\text{g kg}^{-1}$ , respectively. High enrichment factor (EF) were acquired with the method. The EF ranged from 111-145%. Recovery and repeatability studies were performed for 7 pesticides spiked into strawberries at concentrations of 25, 50 and 100  $\mu\text{g kg}^{-1}$  and were tested in three replicates. For the modified MNPs cleanup

using the optimum conditions, the average recovery obtained from the 3 levels was 93 % and the relative standard deviation was less than 12% (Table 3).

Table 3

#### 3.2.4. Analysis of real samples

To demonstrate the applicability of the developed method to real world samples, it was applied for the analysis of 20 strawberry samples from conventional and organic farming which were got from local markets and supermarkets in the north of Portugal. It was shown that chlorpyrifos was present in 3 samples of strawberries from conventional farming, but the concentration was lower than the maximum residue levels (MRL=200  $\mu\text{g kg}^{-1}$  for strawberries) of European requirements values (3.8 – 4.1  $\mu\text{g kg}^{-1}$ ). The positive samples were analyzed by GC-MS in SIM mode and GC-MS/MS for confirmation of chlorpyrifos. The most abundant precursor ion (m/z 314) was selected after injecting chlorpyrifos standard solution at 200  $\mu\text{g L}^{-1}$ .

#### 3.2.5. Comparison of micro-d-SPE with other sample preparation techniques

The proposed method was compared with some other reported methods using new sorbents for the determination of pesticides. As listed in table 4, the presented work showed satisfactory linearity, low RSDs and good recoveries. In general, the present methodology has comparable parameters with other extraction methods. The recoveries obtained in the micro d-SPE are better with those obtained in the previously published methods. The results show that the presented method has several advantages like being a micro extraction, sensitive, simple, efficient and less hazardous for the environmental.

Table 4

#### 4. Conclusion

In the current study, different nanoparticles materials were synthesized and used as modified QuEChERS sorbent for the determination of OPPs in strawberry samples. Because the present materials have the magnetic property and adsorption ability toward pigment matrix and other co-extracts, the modified QuEChERS method has the advantages of simplicity, rapidity and high efficiency compared to the classical QuEChERS method. The developed micro d-SPE cleanups using MNPs with 200 nm average size were the ones that presented the better efficiency extraction. The new methodology provided significant removal of co-extractives materials and excellent recoveries for all analytes with exception of one (dimethoate), making the method applicable to food analysis. The present methodology provides an effective enrichment of the extract allowing the required sensitivity even using a FPD and a ion trap MS as a detectors. Summary, the novelty of this work was the use of new materials, namely MNPs, as a sorbent for pesticide analysis; the magnetic properties of the NPs allowed non-use of the centrifugation in cleanup step; the use of small amount of sorbents without loss efficiency and the application of the methodology in real samples. The organic farming strawberries were free of these 7 OPPs and the opposite happened with the conventional agricultural strawberry samples (three samples contaminated with chlorpyrifos under European MRLs).

Based on the results presented above, the modified methodology meets requirements for pesticide analysis in fruits and will have abroad advanced applications in food safety analysis.

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**Figure Captions:**

Figure 1. The preparation procedure of MNPs.

Figure 2. Sample preparation procedure.

Figure 3. SEM images of (A)  $\text{Fe}_3\text{O}_4$  MNPs, (B)  $\text{Fe}_3\text{O}_4@$ TEOS, (C)  $\text{Fe}_3\text{O}_4@$ TEOS@MPS, (D)  $\text{Fe}_3\text{O}_4@$ TEOS@MPS@PMAA.

Figure 4. The FTIR spectra of the distinct synthesized MNPs.

Figure 5. The comparison of average recovery results obtained from all the pesticides studied among different sample preparation methods (A - Classical QuEChERS and cleanups and B - modified QuEChERS with MNPs cleanups).

Figure 6. Recovery results (percentage) (n=3) for strawberry sample spiked at  $100 \mu\text{g kg}^{-1}$  obtained with 10 and 15 mg of four different MNPs sorbents and 10 and 15 mg of PSA, respectively.

Figure 7. Recovery results (percentage) (n=3) for strawberry sample spiked at  $100 \mu\text{g kg}^{-1}$  obtained with 10 and 15 mg of four different MNPs sorbents, 10 and 15 mg of PSA and 10 and 15 mg of C18, respectively.

Figure 8. The minimum, maximum and median recovery values obtained for all the pesticides at  $100 \mu\text{g kg}^{-1}$  during cleanup time studies (30 s and 1 min).

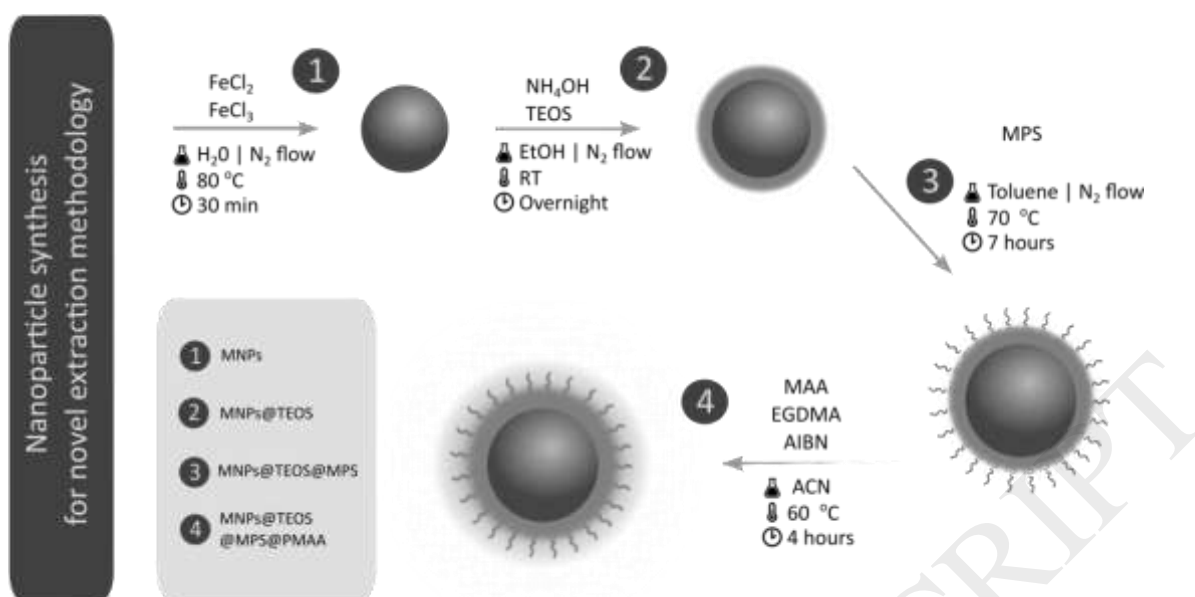
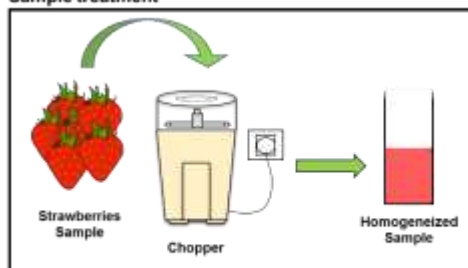


Figure 1. The preparation procedure of MNPs.

## Sample treatment



## QuEChERS extraction and clean-up steps (d-SPE and DPX)

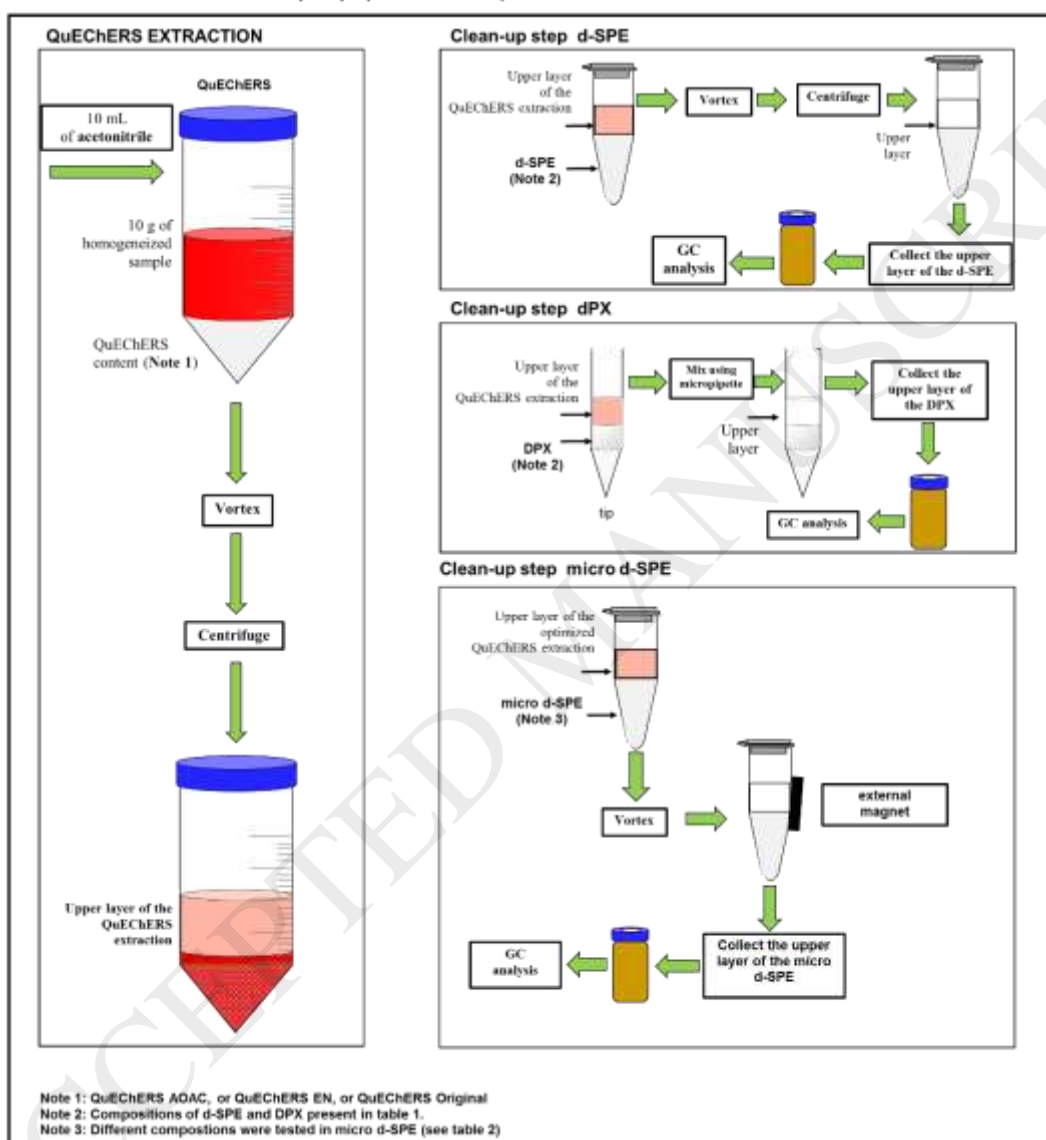


Figure 2. Sample preparation procedure.

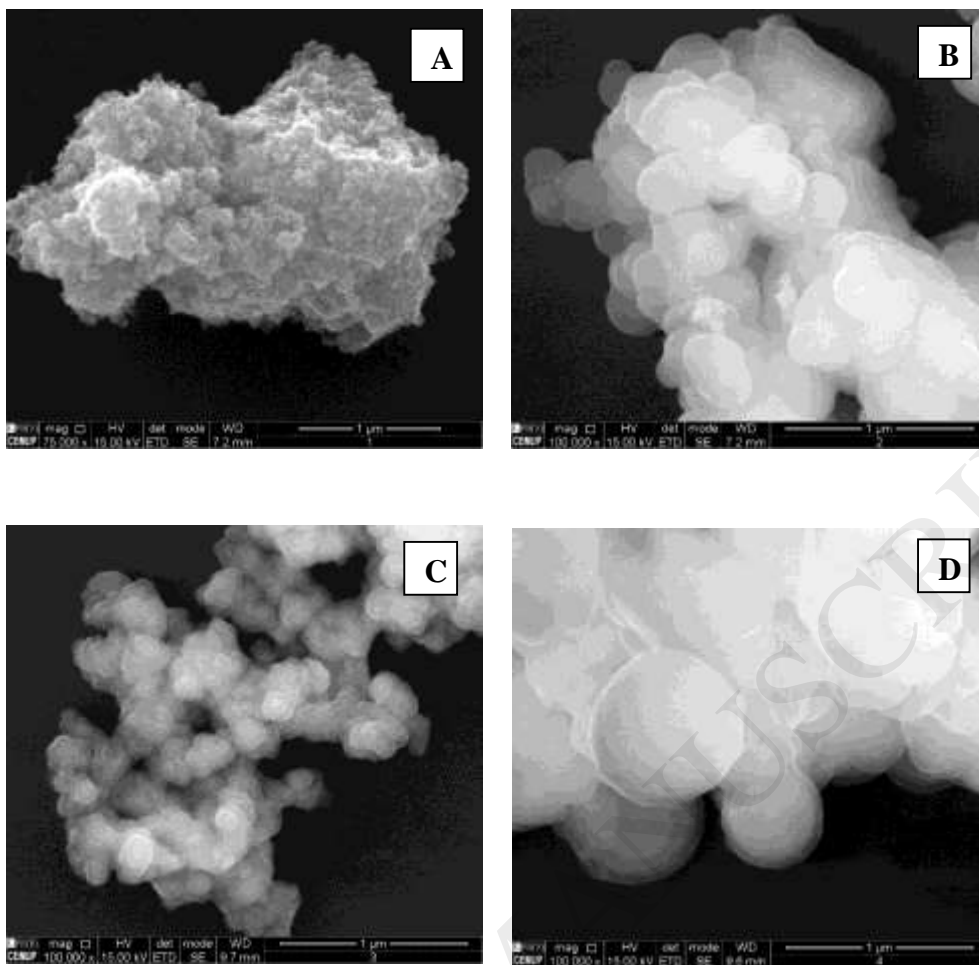


Figure 3. SEM images of (A) Fe<sub>3</sub>O<sub>4</sub> MNPs, (B) Fe<sub>3</sub>O<sub>4</sub>@TEOS, (C) Fe<sub>3</sub>O<sub>4</sub>@TEOS@MPS, (D) Fe<sub>3</sub>O<sub>4</sub>@TEOS@MPS@PMAA.

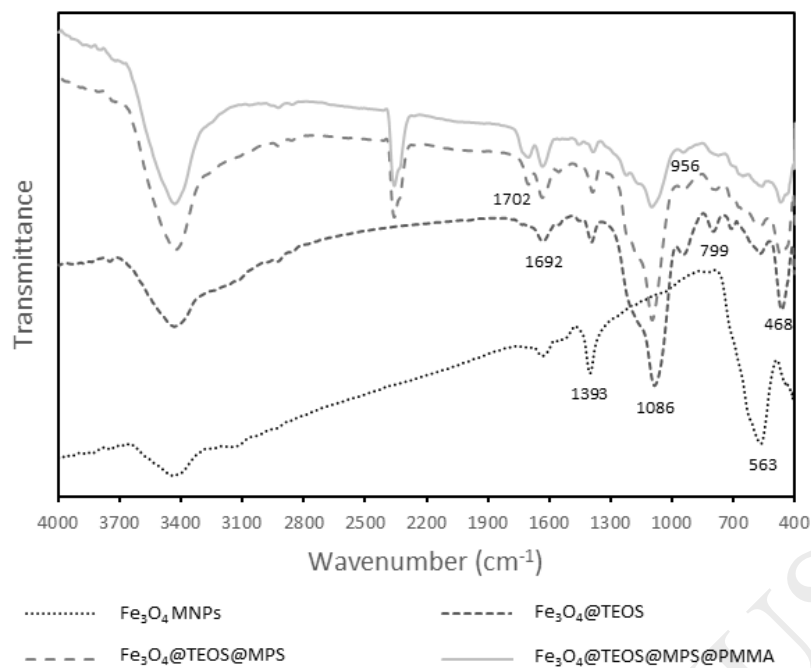


Figure 4. The FTIR spectra of the distinct synthesized MNPs.

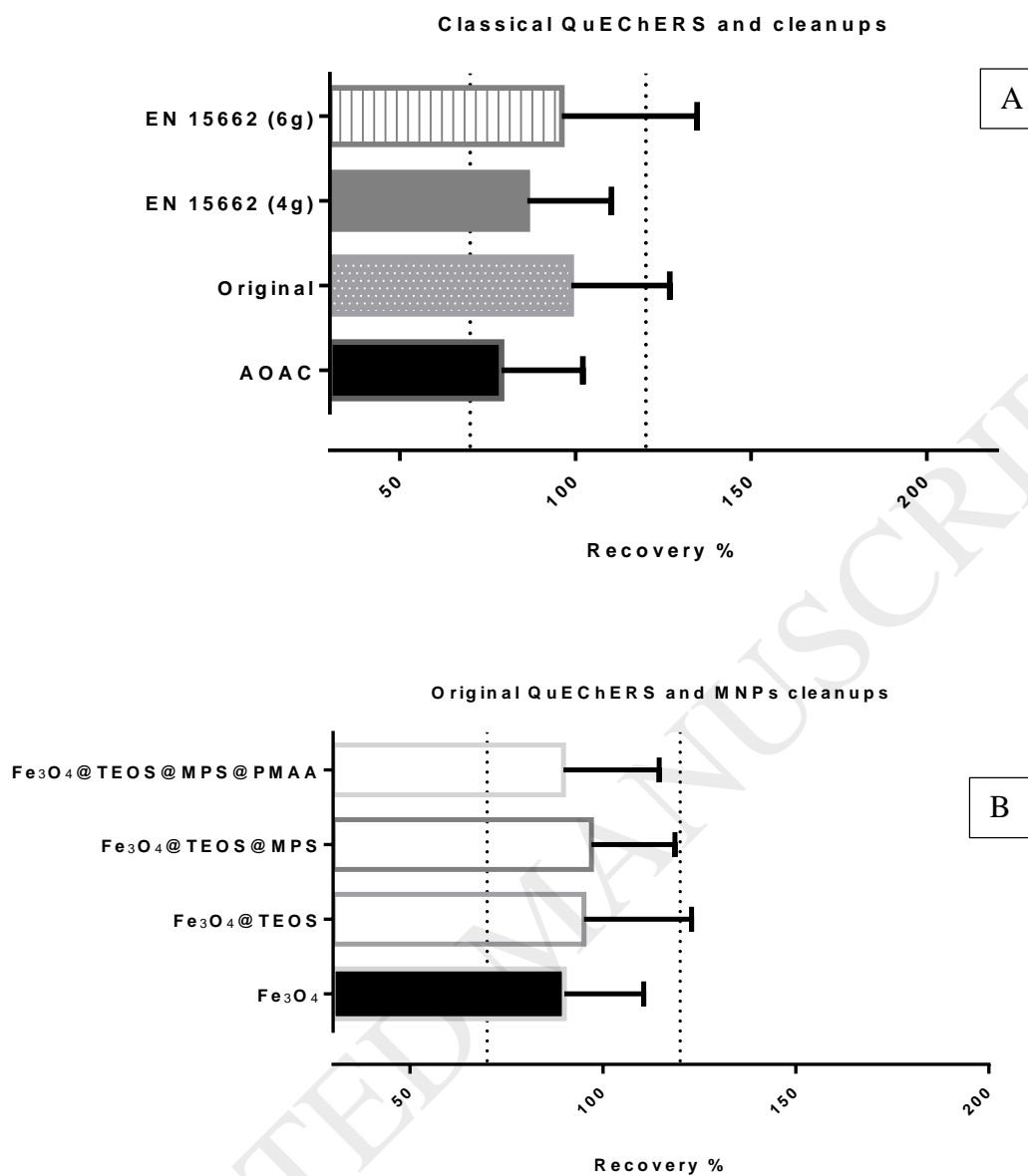


Figure 5. The comparison of average recovery results obtained from all the pesticides studied among different sample preparation methods (A - Classical QuEChERS and cleanups and B - modified QuEChERS with MNPs cleanups).

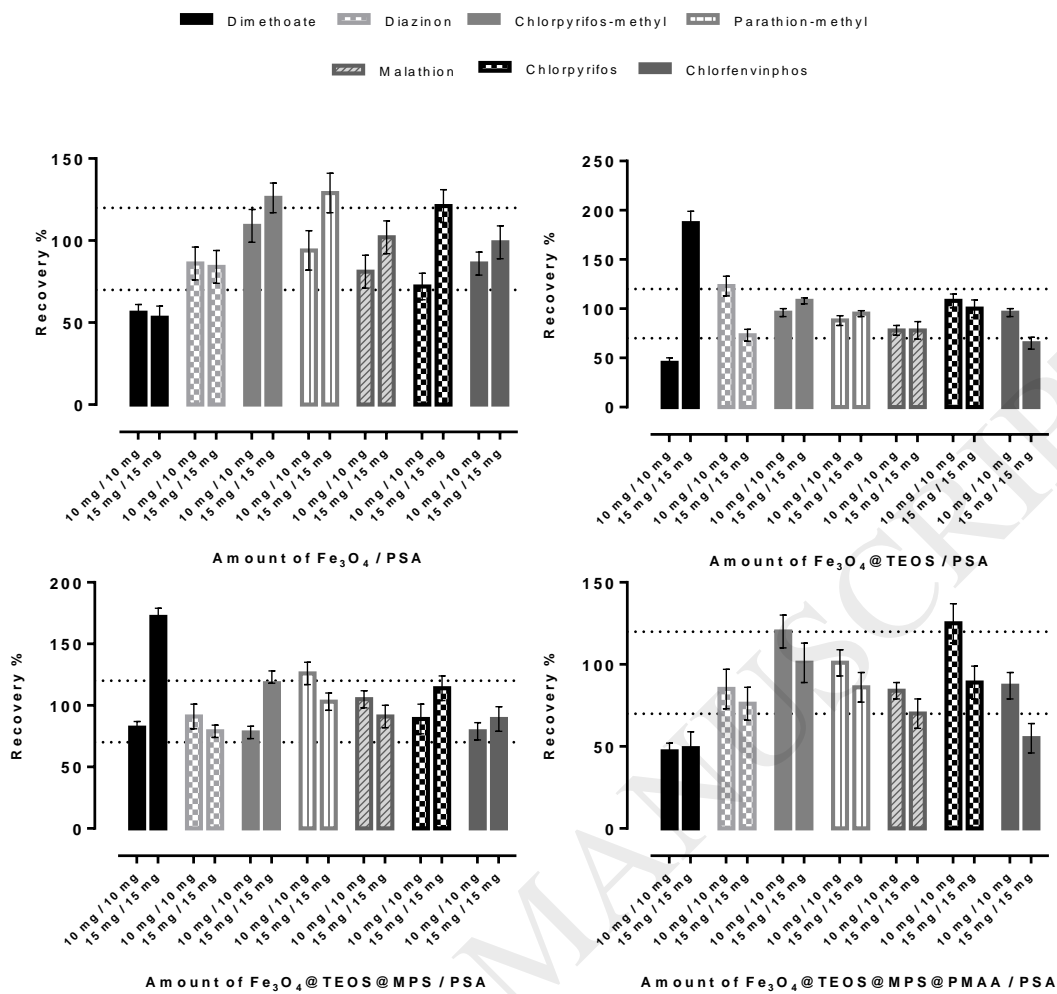


Figure 6. Recovery results (percentage) (n=3) for strawberry sample spiked at  $100 \mu\text{g kg}^{-1}$  obtained with 10 and 15 mg of four different MNPs sorbents and 10 and 15 mg of PSA, respectively.

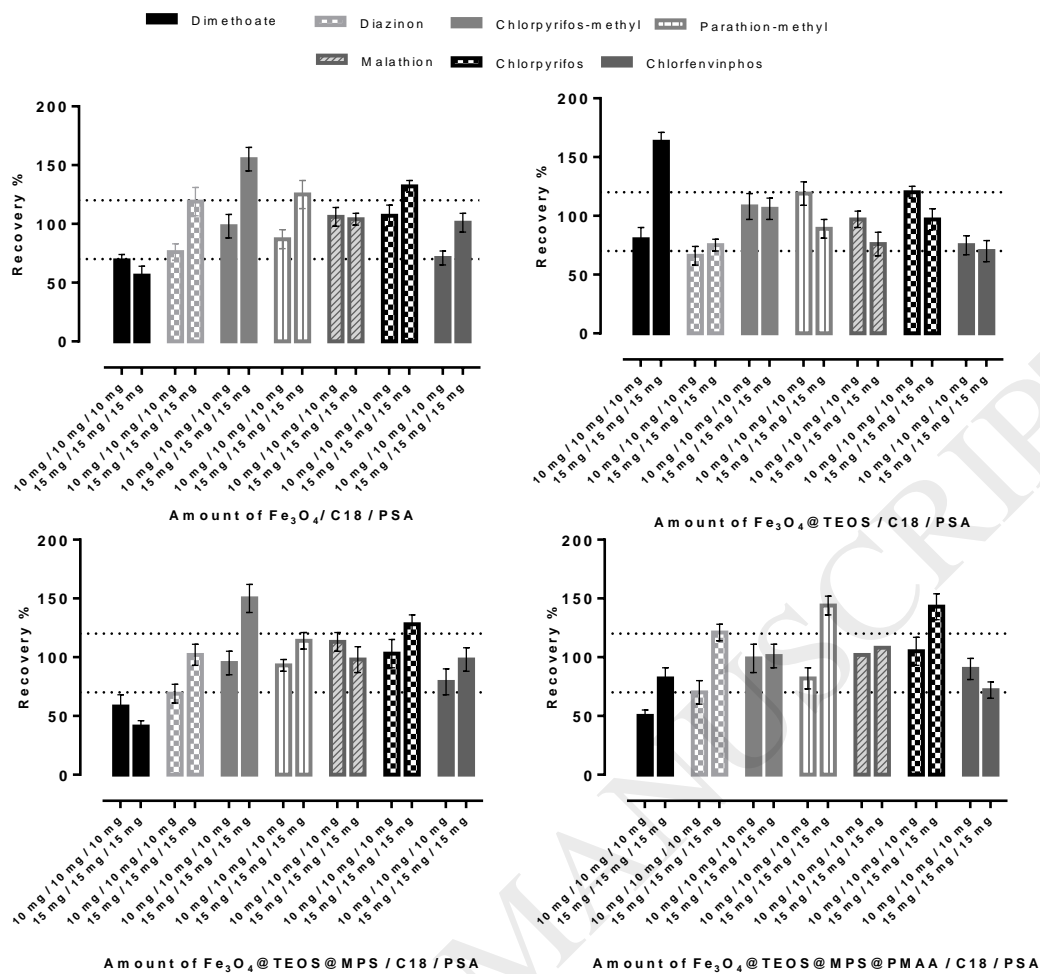


Figure 7. Recovery results (percentage) (n=3) for strawberry sample spiked at 100  $\mu\text{g kg}^{-1}$  obtained with 10 and 15 mg of four different MNPs sorbents, 10 and 15 mg of PSA and 10 and 15 mg of C18, respectively.



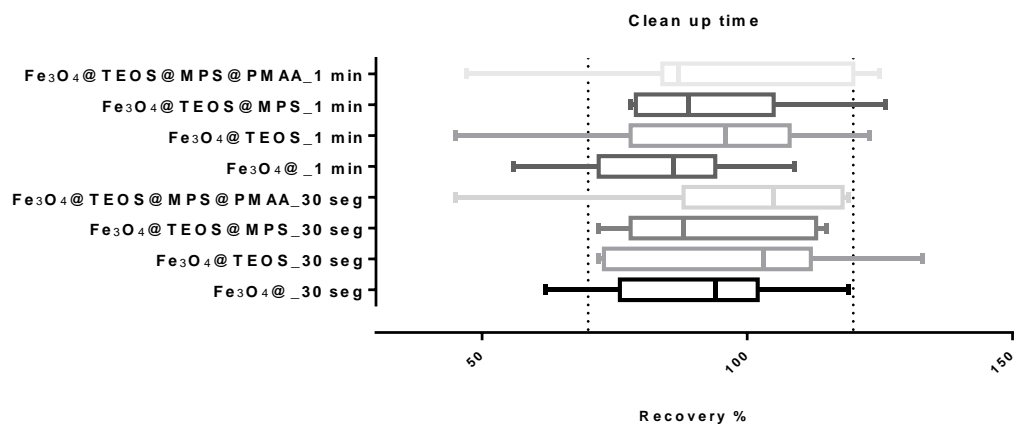


Figure 8. The minimum, maximum and median recovery values obtained for all the pesticides at 100  $\mu\text{g kg}^{-1}$  during cleanup time studies (30 s and 1 min).

**Table captions:**

Table 1. Classical QuEChERS and cleanup compositions.

Table 2. Different micro d-SPE cleanup compositions tested using 10 and 15 mg amount of 4 synthesized MNPs sorbents ( $\text{Fe}_3\text{O}_4$ ,  $\text{Fe}_3\text{O}_4@\text{TEOS}$ ,  $\text{Fe}_3\text{O}_4@\text{TEOS}@\text{MPS}$  and  $\text{Fe}_3\text{O}_4@\text{TEOS}@\text{MPS}@\text{PMAA}$ ) PSA and C18.

Table 3. Recoveries and relative standard deviations for strawberry samples fortified at 3 levels using a modified QuEChERS methodology.

Table 4. Comparison of the presented method with some other reported methods using new sorbents for the extraction and determination of pesticides.

Table 1

<b>QuEChERS</b>									
		<b>Composition (mg)</b>							
<b>Company</b>	<b>Method</b>	<b>MgSO<sub>4</sub></b>	<b>NaCl</b>	<b>AcetNa</b>	<b>NaCit</b>	<b>Na<sub>2</sub>Cit</b>	<b>PSA</b>	<b>C18</b>	<b>CARB</b>
Agilent Technologies (California, USA)	Original	4	1						
	AOAC 2007.01	6	-	1.5					
	EN 15662	4	1		1	0.5			
UCT® (Pennsylvania, USA)	EN 15662	6	1.5		1.5	0.75			
<b>d-SPE and DPX cleanup</b>									
UCT® (Pennsylvania, USA).		150					150	50	
Thermo Fisher Scientific (Massachusetts, EUA)	AOAC 2007.01	150					50		50
United Science (USA)	AOAC 2007.01	150					50	50	50
Agilent Technologies (California, USA)	AOAC 2007.01	150					50	50	50
	-	150					150	50	50
	-	50					50	50	
DPX labs (Columbia,USA)	AOAC 2007.01	150					50	50	7.5

Table 2.

Micro d-SPE cleanup		
Compositions (mg)		
4 synthesized MNPs sorbents*	Commercial sorbents	
	PSA	C18
10	10	
10	10	10
15	15	
15	15	15

\*The different compositions were tested for each MNPs ( $\text{Fe}_3\text{O}_4$ ;  $\text{Fe}_3\text{O}_4@TEOS$ ;  $\text{Fe}_3\text{O}_4@TEOS@MPS$ ;  $\text{Fe}_3\text{O}_4@TEOS@MPS@PMAA$ ).

Table 3.

Pesticides	Average recovery $\pm$ RSD, % (n=3)		
	Level 25 $\mu\text{g kg}^{-1}$	Level 50 $\mu\text{g kg}^{-1}$	Level 100 $\mu\text{g kg}^{-1}$
Dimethoate	79 $\pm$ 12	75 $\pm$ 11	72 $\pm$ 11
Diazinon	95 $\pm$ 8	90 $\pm$ 9	81 $\pm$ 9
Chlorpyrifos-methyl	105 $\pm$ 11	111 $\pm$ 7	115 $\pm$ 7
Parathion-methyl	95 $\pm$ 1	101 $\pm$ 10	113 $\pm$ 10
Malathion	80 $\pm$ 10	85 $\pm$ 9	88 $\pm$ 9
Chlorpyrifos	89 $\pm$ 11	82 $\pm$ 9	78 $\pm$ 9
Chlorfenvinphos	101 $\pm$ 8	109 $\pm$ 10	104 $\pm$ 10

Table 4.

Pesticides	Sample	Recoveries	Limits	Method Sorbents	Detection	Ref
Multiresidues	Fruit juices	49-75%	LOD:0.15- 0.36 $\mu\text{g L}^{-1}$	Magnetic d-SPE  Sorbents: toner powder	GC-FID GC-MS	[57]
Multiresidues	Fruits	60-130%	LOQ:10 $\text{ng kg}^{-1}$	QuEChERS and d-SPE  Sorbents: magnetite ( $\text{Fe}_3\text{O}_4$ ) nanoparticles (NPs) modified with 3- (N,N-diethylamino) propyltrimethoxysilane ( $\text{Fe}_3\text{O}_4$ -PSA NPs)	ultra- HPLC- MS/MS	[68]
Benzoylureas	Water Tangerine	92-108%	LOD:0.10- 0.23 $\text{ng mL}^{-1}$	D-SPE Sorbents: nanoporous carbon using a metal-organic framework (MOF) as a template and furfuryl alcohol as the source for carbon.	HPLC	[69]
Organochlorines	Tobacco	64-126%	LOD:0.013- 3.15 $\text{ng g}^{-1}$	QuEChERS and d-SPE  Sorbents: magnetic graphene (G/PSA/ $\text{Fe}_3\text{O}_4$ )	on-line GPC- GC-MS <sup>2</sup> )	[70]
OPPs	Fruit Vegetables Water	90-108%	LOD: 0.02- 0.1 $\mu\text{g L}^{-1}$	Magnetic solid phase extraction  Sorbents: silica coated magnetic microparticles ( $\text{Fe}_3\text{O}_4@SiO_2$ ) and	GC-NPD	[65]

				graphene oxide (GO) functionalized with phenylethyl amine (PEA)		
OPPs	Fruit Vegetables Water	79-99%	LOD: 0.005-0.020 $\mu\text{gL}^{-1}$	SPME  Sorbents: fiber coated with porous carbon nanotubes–silicon dioxide (CNTs–SiO <sub>2</sub> ) nanohybrids	GC-CD-IMS	[64]
Fenitrothion Chlorpyrifos Hexaconazole	Tomato Grape	83-113%	LOD: 0.23- 0.30 $\mu\text{gkg}^{-1}$	Magnetic solid phase extraction (MSPE)  Sorbents: magnetic graphene based hybrid silica-N-[3-(trimethoxysilyl)propyl]ethylenediamine (MG@SiO <sub>2</sub> -TMSPED) nanocomposite	GC- $\mu\text{ECD}$	[51]
OPPs	Strawberries	72-115%	LOD:3.6- 3.8 $\mu\text{gkg}^{-1}$	Magnetic micro d-SPE  Sorbents:	GC-ECD GC-MS	This method