



HAL
open science

Comparison of matrix-solid phase dispersion and liquid-liquid extraction for the chromatographic determination of fenthion and its metabolites in olives and olive oils

Sara Cunha

► **To cite this version:**

Sara Cunha. Comparison of matrix-solid phase dispersion and liquid-liquid extraction for the chromatographic determination of fenthion and its metabolites in olives and olive oils. *Food Additives and Contaminants*, 2007, 24 (02), pp.156-164. 10.1080/02652030600970374 . hal-00577371

HAL Id: hal-00577371

<https://hal.science/hal-00577371>

Submitted on 17 Mar 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Comparison of matrix-solid phase dispersion and liquid-liquid extraction for the chromatographic determination of fenthion and its metabolites in olives and olive oils

Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2006-152.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	18-Aug-2006
Complete List of Authors:	Cunha, Sara; FFUP, Food Science
Methods/Techniques:	Chromatographic analysis, Chromatography - GC, Chromatography - GC/MS, Clean-up
Additives/Contaminants:	Environmental contaminants, Pesticide residues, Pesticides - organophosphorous
Food Types:	Oils and fats, Olive oil, Vegetables

SCHOLARONE™
Manuscripts

1
2
3 **Comparison of matrix-solid phase dispersion and liquid-liquid**
4 **extraction for the chromatographic determination of fenthion and its**
5 **metabolites in olives and olive oils**
6
7
8
9

10
11
12
13 Sara C. Cunha, José O. Fernandes, M. Beatriz P.P. Oliveira*
14

15
16
17
18 REQUIMTE/Serviço de Bromatologia, Faculdade de Farmácia, Universidade do Porto,
19
20 Rua Aníbal Cunha, 164, 4099-030 Porto, Portugal
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37

38 *Corresponding author: Tel: +351.222078927 Fax: +351.222003977
39

40 e-mail: beatoliv@ff.up.pt
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Abstract

Matrix-solid phase dispersion (MSPD) methodology has been developed to extract fenthion and its metabolites from olives and olive oils and this has been compared with conventional liquid-liquid extraction (LLE). The pesticide residues were analyzed by GC using a nitrogen-phosphorus detector. Both extraction methods gave linear response at least over the concentration range assayed (0.03-1 mg/kg for MSPD and 0.1-1 mg/kg for LLE). Recoveries and RSD (n=6) values in olives were 85-112% and 2-6% for MSPD and 68-108% and 4-16% for LLE, respectively. In the case of olive oil the recoveries and RSD (n=6) values were 67-98% and 5-11% for MSPD and 63-115% and 6-14% for LLE, respectively. When compared to LLE the newly developed MSPD method was at least twice as sensitive and required ten times less sample weight. The method was applied in olives and olives oil samples obtained from olives groves treated with fenthion.

Keywords: Fenthion, metabolites, matrix-solid phase dispersion, olive oil, GC/NPD

Intoduction

Fenthion is an organophosphorus insecticide commercialised since 1960, used in the control of several fruit flies. In olive trees, fenthion is employed to control olive fly (*Batrocera oleae*, Gmel.), a pest that widely decreases the quality and amount of oil produced (Lentza-Rizos 1999). Olive oil is a product obtained from olives solely by mechanical or other few physical means and which the presence of pesticides such as fenthion or other lipophilic organosphorus can occur.

The toxicity of fenthion and other pesticides has encouraged the establishment of MRLs (maximum residual levels) to control their presence in foods. Therefore, the European Commission (EC) and the *Codex Alimentarius* of the Food Agriculture Organization of the United Nation (FAO) have specified the MRL of 1 mg/kg of fenthion residues in olive oils and olives (EC 2001, FAO/WHO 1998). Moreover, pesticide metabolites show often higher toxicity than the parent molecule as occurs with fenthion. Fenthion is rapidly oxidised to sulfoxide and sulfone in plants, both with a lethal dose (LD₅₀) of 125 mg/kg, lower than the LD₅₀ of the parent compound: 220 mg/kg (Cabras *et al.* 1993). Another oxidative process caused by the plant enzymes can involve the other fenthion moiety, thus leading to another three substances, fenoxon, fenoxon sulfoxide and fenoxon sulfone, with a LD₅₀ of 125 mg/kg, 50 mg/kg and 30 mg/kg, respectively (FAO/WHO 1998).

Several studies carried out by Lentza-Rizos and Avramides (1990), Cabras *et al.* (1993), and Molinari *et al.* (1994), for chromatographic determination of fenthion and its metabolites in olive and olive oils, commonly used direct LLE as the sample preparation method. Organic solvents such as ethyl acetate, hexane saturated with acetonitrile, acetonitrile saturated with hexane, chloroform and acetone, are employed. These methods of sample preparation have the advantage to be simple and easy to execute, but they require the use of large sample sizes and appreciable amounts of solvents. Moreover lipids are always present in the final extracts at high amounts. These lipids are generally not volatile and tend to coat the column and internal parts of the GC injector, decreasing the chromatographic performance (Fong *et al.* 1999).

1
2
3 In order to minimise these problems a number of post-extraction cleanup methodologies
4 based on solid phase extraction (SPE), using adsorbents such as alumina (Molinari *et al.*
5 1994, 1998), Extralut (Di Muccio *et al.* 1999) and Florisil (Rotunno *et al.* 1997) have
6
7 been used for determination of fenthion and its oxidised metabolites in olive oils and
8
9 olives. Afterwards, more sophisticated instrumentation such as supercritical fluid
10
11 extraction (SFE) (Poustka *et al.* 2003), pressurised liquid extraction (PLE) and gel-
12
13 permeation chromatography (GPC) (Jongenotter *et al.* 1999), are also being applied in
14
15 samples with high fat content for multi-residue pesticides determination.
16
17

18
19 An attractive alternative introduced recently for sample preparation of complex matrixes
20
21 is the so called matrix solid-phase dispersion (MSPD) (Barker 2000a; 2000b). This
22
23 technique was successfully applied for the determination of pesticides in several food
24
25 samples such as honey (Fernández *et al.* 2002), fruit (Torres *et al.* 1996), vegetables
26
27 (Kristenson *et al.* 2003, Chu *et al.* 2005) and olive oil (Ferrer *et al.* 2005). MSPD is a
28
29 sample preparation methodology that combines aspects of several techniques for sample
30
31 disruption, whilst generating a material with unique dispersed character for extraction of
32
33 compounds (Barker 2000 a). MSPD also helps to prevent emulsion in liquid distribution
34
35 and to reduce organic solvents disposals and analysis time.
36

37 The application of MSPD involves the mixture of the sample with a solid support, such
38
39 as octadecylsilyl (C₁₈), octyl (C₈) or aminopropyl (NH₂), packing the extraction/cleanup
40
41 column and eluting the target compounds with solvents. The extraction and cleanup can
42
43 be completed in one step simplifying the sample preparation procedure (Barker 2000b).
44

45
46 In general, the analysis of organophosphorus residues is carried out by gas
47
48 chromatography coupled with selective detection of analytes, such as flame photometric
49
50 detector (FPD), mass spectrometry (MS), atomic emission detector (AED) and nitrogen
51
52 phosphorus detector (NPD) (Poustka *et al.* 2003).
53

54
55 The aim of this study was to develop an analytical procedure that combines MSPD and
56
57 GC/NPD for the determination of the fenthion and its metabolites in olive oils and
58
59 olives. The effects of several extraction parameters, such as sorbent adsorption, solvent
60
elution and additional clean-up procedures have been tested. The MSPD was evaluated
and compared with conventional LLE procedures. The method was successfully applied

1
2
3 to determine the levels of fenthion residues and its metabolites during the pre-harvest
4 interval in olive fruits.
5
6
7

8 9 **Experimental**

10 *Reagents*

11 Acetonitrile, methanol, chloroform, acetone, *n*-hexane, toluene, acetyl acetate were high
12 purity grade solvents for pesticide residue analysis from Fluka (Neu-Ulm, Germany).
13 Anhydrous MgSO₄, anhydrous Na₂SO₄ and NaCl were analytical grade from Riedel-de
14 Haën (Buchs, SG, Schweiz). To ensure efficient removal of phthalates and residual
15 water, MgSO₄ and Na₂SO₄ were submitted for 5 h at 500 °C in a muffle furnace.
16
17

18 Pesticide reference standards fenthion-I, fenthion sulfoxide-II, fenthion sulfone-III,
19 fenoxon-IV, fenoxon sulfoxide-V, fenoxon sulfone-VI were obtained from Dr
20 Ehrenstorfer (Augsburg, Germany). Internal standards triphenylphosphate (TPP) and
21 tributylphosphate (TBP) were obtained from Fluka.
22
23
24
25
26
27
28
29

30 Sorbents tested for MSPD included C₁₈-bonded silica (pore size 55-105 µm) from
31 Waters (Milford, MA, US), primary secondary amine-PSA (pore size 40-120 µm) from
32 Varian (Harbor City, CA, US) and aminopropyl silica gel-NH₂ (pore size 15-35 µm)
33 from Fluka. Sorbents tested for dispersive SPE included Florisil (pore size 60-100
34 mesh) and graphitized carbon black (pore size 100-400 mesh) obtained from Sigma (St.
35 Louis, MO, US), silica (pore size 45 µm) and alumina (pore size 60-325 mesh) from
36 Supelco (Bellefonte, PA, US) and Extralut 3 from Merck (Darmstadt, Germany).
37
38
39
40
41
42
43

44 *Standards*

45 Stock solutions of 20 mg/l of each analyte were prepared in acetonitrile and keep at -20
46 °C. Working standard pesticide mixtures of 5 mg/l was prepared in acetone from the
47 stock standard solution. Internal standard solutions of TPP (4 mg/l in acetone) and TBP
48 (0.5 mg/l in acetone) were used in quantification and in monitorization of
49 chromatographic conditions, respectively.
50
51
52
53
54
55
56

57 *Samples*

58 The trial was carried out in 2 pilot Cv. Cobrançosa olive groves with 2.4 ha and 1.9 ha,
59 respectively, located in the northeast of Portugal. One olive grove had no phytosanitary
60

1
2
3 treatment and the other was treated with Lebaycid (containing 550 g/l of fenthion, 167
4 g/l of xylene and 395 g/l of other ingredients, mostly emulsifiers). Sampling started
5 immediately after the treatment on October 15th and was repeated weekly during the
6 preharvest interval (42 days). Air and soil temperatures, solar radiation, wind and rain
7 were registered every day during this period (data not shown). Samples of 50 fruits per
8 tree were collected and stored at -20 °C until analysis.
9

10
11
12 Olive oils were obtained following the method described by Pereira *et al.* (2002).
13 Briefly, olive fruits were hand-picked and processed through a mill, a thermo beater and
14 a pulp centrifuge, after which the oil was separated from the pulp by decantation, and
15 kept in dark glass bottles, at 4°C, in the absence of light.
16
17

18 *Sample extraction*

19 *MSPD extraction*

20
21 Olives: 25 g of olive pulp were homogenized in Ultra-turrax (2 min at 8000 rpm). An
22 aliquot (1 g) was placed in a mortar with 1 g NH₂, added with 25 µl of TPP solution (4
23 mg/l) and gently blended for a few minutes using a pestle to obtain a dry-powder-like
24 homogeneous mixture. Then, the mixture was introduced into a column containing 1 g
25 of Florisil. This column was attached to a vacuum manifold (Visaprep, Supelco, PA,
26 US) coupled with a small vacuum pump, and the flow adjusted to 3 ml/min. The elution
27 step was carried out with 3 x 5 ml of acetonitrile. The eluent was dried with anhydrous
28 MgSO₄, centrifuged at 3500 g for 1 min and decanted. The extracts were evaporated
29 until dryness, then re-dissolved in 1 ml of acetone containing TBP (0.5 mg/l).
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45

46 Olive oil: 0.2 g of sample were placed in a mortar and gently blended with 1 g NH₂ and
47 25 µl of TPP solution (4 mg/l) using a pestle. The other steps of the method were the
48 same described above for olives.
49
50
51

52 *Liquid-liquid extraction (LLE)*

53
54 LLE was based on the method of Cabras *et al.* (1993) with minor modifications. Briefly,
55 in olives: 10 g of olive pulp were mixed with 10 ml of *n*-hexane, 25 µl of TPP solution
56 (4 mg/kg) and 1 g of anhydrous Na₂SO₄. The mixture was blended for 30 min and
57 centrifuged at 3500 g for 2 min. The decanted phase was evaporated until a final volume
58
59
60

1
2
3 of about 1.5 ml. For olive oil, the same described procedure was used with 1 g of
4 sample. But another method published by Ferrer *et al.* (2005) has also been adopted for
5 olive oil samples. The procedure consists in liquid-liquid partitioning with 15 ml of
6 ether petroleum saturated with acetonitrile followed by 35 ml acetonitrile saturated with
7 ether petroleum of sample (5 g) with 25 μ l of TPP solution (4 mg/l). Finally, an aliquot
8 (7 ml) of the acetonitrile fraction was evaporated until about 1 ml.
9
10
11
12
13
14

15 16 *Equipment and chromatographic conditions*

17 Chromatographic analysis was performed in a ThermoFinnigan Trace GC gas
18 chromatograph (Milan, Italy) equipped with a nitrogen phosphorus detector (NPD) and
19 a split-splitless injector. A fused silica capillary column, ZB-5 (30 m x 0.32 mm I.D.,
20 0.25 μ m film thickness- Phenomenex, Torrance, CA, US), was used for compounds
21 separation. GC conditions were as follows: splitless injection (period: 0.80 min), carrier
22 gas flow rate (helium) 1 ml/min (constant flow), injector temperature: 250 $^{\circ}$ C, detector
23 temperature: 300 $^{\circ}$ C, oven temperature: 70 $^{\circ}$ C (1 min held), 10 $^{\circ}$ C/min to 230 $^{\circ}$ C, 5
24 $^{\circ}$ C/min to 280 $^{\circ}$ C (2 min held). 1 μ l of the extract was injected, and quantification was
25 based on the internal standard method.
26
27
28
29
30
31
32
33
34

35 **Results and Discussion**

36 37 38 39 *1. MSPD procedure for olive fruits*

40 In sample preparation of olives several parameters were studied including, sample size,
41 sorbents, sample/sorbents ratio, solvents and sample/solvents ratio. The evaluation of
42 these parameters involved, in most of the cases, recovery determinations. With this
43 purpose blank samples were spiked with appropriate volumes to achieve 1 mg/kg of
44 analytes before extraction and compared with a mixture standard solution with the same
45 concentration.
46
47
48
49
50
51

52 53 a) Sample size

54 The sample preparation methods described in current published papers for
55 determination of fenthion and its metabolites often use large amounts of sample (i.e 25
56 to 50 g). However, the trend is clearly to reduce sample size to minimum amounts
57 providing statistically reliable results and to scale methods accordingly (Anastassiades
58
59
60

1
2
3 *et al.* 2003). Considering the published literature and taking into account that sample
4 size need to be representative and appropriate to sorbent amounts, amounts of olive pulp
5 ranging from 1 to 5 g, were tested. The best results were obtained with 1g.
6
7
8

9
10 b) Type of sorbent and sample/sorbent ratio

11 Fenthion is intimately associated with lipid components of the sample matrix.
12 Therefore, particular care should be taken during extraction to recover all pesticides
13 from the bulk of lipidic material. Table I shows the recoveries obtained using three
14 different types and amounts of sorbents: C₁₈, PSA and NH₂. Accordingly to the
15 literature these sorbents have been successfully used in extraction of pesticide residues
16 in fatty matrixes (Gillespie *et al.* 1995, Lehotay *et al.* 2005 b, Ferrer *et al.* 2005). To
17 select the most appropriate sorbent for fenthion and its metabolites, the elution was
18 performed with 15 ml of acetonitrile trough the extract/sorbent. C₁₈ was tested in this
19 study with and without previous activation, being washed with methanol and water to
20 activate. However, both experiments presented poor recoveries of all the compounds,
21 probably due to a strong apolar interaction with the alkyl chain of the sorbent. In
22 contrast, NH₂ and PSA interact with analytes by hydrogen bonding and dipole-dipole
23 interactions. In this study, NH₂ was found to be the best olive pulp dispersant, as can be
24 seen in Table I.
25
26
27
28
29
30
31
32
33
34
35

36 [Insert Table I about here]
37
38
39

40 Table I also shows the results concerning the influence of the amount of NH₂ (from 0.5
41 g to 2 g) in recovery of fenthion and its metabolites. The best recoveries are obtained
42 using 1 g of the solid phase.
43
44
45
46

47 The effect of several salts on phase separation using the so called QuEChERS (quick,
48 easy, cheap, effective, rugged and safe) method has been studied by Anastassiades *et al.*
49 (2003) for multiresidue determination. Their results have demonstrated that better
50 recoveries were obtained with a combination of anhydrous MgSO₄ and NaCl. Thus, the
51 applicability of different salts (MgSO₄, NaCl and Na₂SO₄) on MSPD sample
52 preparation was evaluated. As shown in Table II, the recoveries obtained without salts
53 were slightly higher than the obtained with any salt or salt combination. In conclusion,
54 these salts are not a good option to be used as dispersant for this kind of matrix.
55
56
57
58
59
60

[Insert Table II about here]

c) Type of solvent, sample/solvent ratio

Solvents are characterized by their polarity and elution strength for a specific sorbent. With polar sorbents, like NH₂, the eluting power increase with the solvent polarity (*n*-hexane < chloroform < toluene < ethyl acetate < acetone < acetonitrile) (Mitra 2003). The effects of these different solvents were compared in a series of experiments to determine their ability to elute adequately fenthion and its metabolites on NH₂. The only parameter changed was the type of solvent maintaining the volume constant (15 ml). Results were presented in Figure 1.

[Insert Figure 1 about here]

The best results were obtained with acetonitrile. This is in according with Kirivanta (1983) which referred that the best solvents for extraction of pesticide residues are simultaneously miscible with water and with apolar solvents. The solvents that follow this premise are acetone and acetonitrile. Compared with acetone, acetonitrile extracts less lipophilic material, e.g. waxes, fat and lipophilic pigments. Advantages and disadvantages of these and other solvents in extraction of pesticides are well explained by Maštovská *et al.* (2004). The large vaporisation expansion volume of acetonitrile that tends to limit amount that can be injected in GC injection can also lead to the deterioration of the NPD. Thus we re-dissolved the extract in acetone after elution with acetonitrile.

The dependence of recoveries upon solvent volume was also evaluated (Figure 2). Starting from the initial conditions mentioned above (addition of 1 g of sample and 1g NH₂) recoveries were carried out at several elution volumes, i.e. 5, 10, 15 and 20 ml. When elution volumes of 20 ml were applied no significant increase in the peak area of the compounds was observed. Decreasing the elution volumes to 10 ml or less had an adverse effect on the recovery. Thus, 15 ml was the elution volume adopted.

[Insert Figure 2 about here]

d) Clean-up

1
2
3 In order to eliminate some of the lipid material that co-eluted with the compounds in
4 study, the extract were submitted to a further clean-up procedure before analysis by
5 GC/NPD. In this study, several types of clean-up were used namely, graphitized carbon
6 black (GCB), alumina, silica, Extralut 3 and Florisil. GCB has resulted very efficient
7 from standpoint of visual appearance, but unfortunately it tends to remove the analytes
8 studied. In fact, GCB strongly retains some chemicals namely planar pesticides as
9 observed by Lehotay *et al.* (2005 a, b). Silica, alumina and Extralut 3 were discarded,
10 due to low recoveries and low clean-up efficiencies. Thus, a cartridge column of 1 g of
11 Florisil was found to be the best system to retain interfering compounds without
12 significant losses of the compounds in study as can seen in Figure 3.
13
14
15
16
17
18
19
20
21
22

23 [Insert Figure 3 about here]
24
25

26 To prevent negative effects in chromatographic separation caused by the presence of
27 water traces in the eluate, MgSO₄ was added before injection in GC/NPD.
28
29
30

31 2. MSPD procedure for olive oils 32

33 Fenthion and its metabolites are efficiently extracted from olive oil with the MSPD
34 procedure developed for olive fruits. However, taking into account that to obtain one
35 litre of olive oil, 5 kg of olives were usually required, the sample size was reduced to an
36 aliquot of 0.2 g, which showed to be a adequate.
37
38
39
40
41

42 3. Comparison between MSPD and LLE procedures 43

44 a) Olive fruits 45

46 Table III summarises the limits of detection, linearity and reproducibility of the method
47 to determine fenthion and its metabolites in olives, by MSPD under the established
48 conditions and by LLE. Linearity experiments were carried out with blank olive fruits.
49 The extraction procedures presented good linearity at least over the investigated
50 concentration ranging from 0.03 to 1 mg/kg by MSPD and from 0.1 to 1 mg/kg in LLE.
51 The detection limit was estimated on the basis of signal-to-noise (3:1). The MSPD
52 procedure presented higher sensitivity for all the compounds than LLE with detection
53 limits from 0.002 to 0.01 mg/kg and from 0.006 to 0.04 mg/kg for MSPD and LLE
54 respectively (Table III). This advantage is especially useful for the determination of
55 sample compounds present in small amounts, such as the metabolites of fenthion. The
56
57
58
59
60

1
2
3 levels herein reported for each type of procedure were well-below of MRLs set by
4 legislation.
5
6

7
8 To determine the reproducibility, the proposed extractions were applied six times on the
9 same blank sample spiked with working standard solutions (to achieve 1 mg/kg of each
10 analyte). As can be seen in Table III, repeatability values (measured by the relative
11 standard deviation) ranged from 1.7 % to 15.6 % in MSPD and from 4.3 % to 15.9 % in
12 LLE, which are considered acceptable for most validation procedures.
13
14
15
16

17
18
19 [Insert Table III about here]
20
21

22
23 The recoveries of the fenthion and its metabolites in olive fruits at different spiking
24 levels (0.2, 0.3 and 0.5 mg/kg), obtained by the MSPD and LLE procedures, are
25 summarized in Table IV. The results for the MSPD procedure were slightly higher to
26 those of LLE procedure. The recoveries for all compounds were higher than 85% and
27 68% on the MSPD and LLE procedures, respectively. As can be observed in Table
28 IV, when the spiking level was lower than 0.3 mg/kg the recoveries for LLE were
29 significantly worse than those of the MSPD procedure.
30
31
32
33
34

35
36
37 [Insert Table IV about here]
38
39

40 *b) Olive oils*

41
42 For olive oil, the MSPD procedure was compared with two different LLE procedures
43 used by Ferrer *et al.* (2005) and Cabras *et al.* (1993). The determinations were similar to
44 the described for the olive fruits. Thus, blank samples were spiked with appropriate
45 volumes to achieve 1 mg/kg of analytes before extraction and compared with a mixture
46 standard solution with the same concentration. Both LLE procedures assayed, presented
47 satisfactory recoveries (Table V). However, they involve the consumption of large
48 amounts of solvents and large samples size, becoming more expensive and unclean. As
49 shown in the Table V best recovery results were again obtained with MSPD, as
50 previously observed for olives. Linearity in MSPD method was checked with
51 calibration curves in a range of concentrations from 0.05 to 1 mg/kg. Regression
52 analysis was performed to generate the linear equations and coefficients of
53 determination (R^2) that ranged from 0.9640 to 0.9951.
54
55
56
57
58
59
60

[Insert Table V about here]

Method application

The proposed method has been applied in the analysis of olive pulp obtained a previously described in Experimental section. All samples (along the pre-harvest interval) had a common qualitative pattern, showing four identified compounds, the levels are depicted in Table VI. The fenthion residue levels herein reported were lower than the established by legislation (1 mg/kg) ranging from 0.01 to 0.03 mg/kg. Fenthion sulfoxide ranged from 0.03 to 0.01 mg/ kg, fenoxon ranged from 0.01 to 0.02 mg/ kg, and fenoxon sulfoxide ranged from 0.03 to 0.05 mg/ kg. Notwithstanding the concentration values, all compounds had a decreasing tendency along the pre-harvest interval. Fenthion and three metabolites were still present in the olives at harvest time. Cabras *et al.* (1993) described the existence of other metabolites of fenthion in olives namely fenthion sulfone and fenoxon sulfone, but those compounds were not detected in this study. The observed difference could be ascribed to the number of phytosanitary treatments used in that work, which was three times higher than was the applied in this research. In addition, fenthion showed a slow degradation in olive fruits as previously referred in literature (Cabras *et al.* 1993, Lentza-Rizos and Avramides 1990, Tsatsakis *et al.* 2003) which contributed to the different levels of fenthion and its metabolites.

[Insert Table VI about here]

Conclusions

MSPD is a good alternative to the LLE procedures for the analysis of fenthion and its metabolites in olive fruits and olive oils. The proposed method involves the use of NH₂ as dispersant followed by elution with acetonitrile, clean-up with Florisil and finally chromatographic analysis by GC/NPD. The results of this study show that the proposed method is simple and sensitive, requiring small volumes of solvents. Additionally, the proposed method offers good recovery and low detection limits when compared to other conventional methods.

1
2
3 The method was applied to evaluate fenthion and its metabolite residue levels during the
4 pre-harvest interval in olive pulp. Fenthion residual levels, in all periods, are lower than
5 the MRL established by legislation. Fenthion sulfone, fenoxon and fenoxon sulfoxide
6 were the only metabolites detected in the olive fruits.
7
8
9

10 11 12 **Acknowledgments**

13 S. C. Cunha is grateful to “Subprograma Ciência e Tecnologia do 3º Quadro
14 Comunitário de Apoio (BD 8822/2002)” and to “Acção 8.1. PO AGRO Nº 482” for
15 financial support.
16
17
18
19
20
21
22

23 **References**

24 Anastassiades M., Lehotay S. J., Štajnbaher D., Schenck. F. J., 2003, Fast and easy
25 multiresidue method employing acetonitrile extraction/partitioning and “Dispersive
26 Solid-Phase Extraction” for determination of pesticide residues in produce. Journal
27 AOAC International, **86**, 412-430.
28
29
30
31
32

33 Barker S.A, 2000 a, Applications of matrix solid-phase dispersion in food analysis.
34 Journal of Chromatography A, **880**, 63-68.
35
36
37

38 Barker S.A, 2000 b, Matrix solid-phase dispersion. Journal of Chromatography A, **885**
39 115-127.
40
41
42

43 Cabras P, Garau, V.L., Melis M., Pirisi F.M., Spanedda, L., 1993, Persistence of
44 insecticide residues in olives and olive oil. Journal of Agriculture and Food Chemistry,
45 **41**, 2431-2433.
46
47
48
49
50

51 Chu X.G., Hu X.Z., Yao H.Y., 2005, Determination of 266 pesticide residues in apple
52 juice by matrix solid-phase dispersion and gas chromatography-mass selective
53 detection. Journal of Chromatography A, **1063**, 201-210.
54
55
56
57

58 Di Muccio A., Pelosi P., Barbini D.A., Generali T., Girolimetti S., Stefanelli P.,
59 Leonelli A., Amendola G., Vergori L., Fresquet E.V., 1999, Determination of
60 pyrethroid pesticide residues in fatty materials by solid-matrix dispersion partition,

1
2
3 followed by mini-column size exclusion chromatography. *Journal of Chromatography*
4
5 A, **833**, 19-34.
6
7

8
9 European Commission, Brussels, 2001, Pesticide Residue,
10 http://europa.eu.int/comm/food/fs/ph_ps/pest/index_en.htm.
11
12

13
14 FAO/WHO Residues of pesticides in foods and animal feeds, 1998, CX/PR 98/6.
15
16

17
18 Fernández M., Picó Y., Mañes J., 2002, Rapid screening of organophosphorus
19 pesticides in honey and bees by liquid chromatography-mass spectrometry.
20 *Chromatographia*, **56**, 577-583.
21
22

23
24 Ferrer C., Gómez M.J., García-Reyes J.F., Ferrer I., Thurman E.M., Fernández-Alba
25 A.R., 2005, Determination of pesticide residues in olives and olive oil by matrix solid-
26 phase dispersion followed by gas chromatography/mass spectrometry and liquid
27 chromatography/tandem mass spectrometry. *Journal of Chromatography A*, **1069**, 183-
28 194.
29
30
31
32

33
34 Fong W.G., Moye H.A., Seiber J.N., Toth J.P., 1999. Extraction, clean-up, and
35 fractionation methods. In *Chemical Analysis*, Winefordner JD, editor. Pesticide residues
36 in foods. New Jersey: John Wiley Sons, Inc. p 41.
37
38
39
40
41

42
43 Gillespie A.M., Daly S.L., Gilvydis D.M., Schneider F., Walters S.M., 1995,
44 Multicolumn solid-phase extraction cleanup of organophosphorus and organochlorine
45 pesticide residues in vegetable oils and butterfat. *Journal AOAC International*, **78**, 431-
46 437.
47
48
49

50
51 Hercegová A., Dömötöröová M., Matisová E., Kirchner M., Otrekal R., Štefuca V., 2005,
52 Fast gas chromatography with solid phase extraction clean-up for ultra trace analysis of
53 pesticide residues in baby food. *Journal Chromatography A*, **1084**, 46-53.
54
55
56

57
58 Jongenotter G.A., Kerkhoff M.A.T., Knaap H.C.M., Vandeginste B.G.M., 1999,
59 Automated on-line GPC-GC-FPD involving co-solvent trapping and on-column
60

1
2
3 interface for the determination of organophosphorus pesticides in olives oils. Journal
4 High Resolution Chromatography, **22**, 17-23.
5
6

7
8 Kirivanta A., J. Miyamoto; P.C.Kearney Pesticide Chemistry: human welfare and the
9 Environment, Pergamon, Press, Oxford, Vol. 4, 117-122, 1983.
10
11

12
13 Kristenson E.M., Shahmiri S., Slooten C.J., Vreuls R.J.J., Brikman U.A.Th., 2004,
14 Matrix solid-phase dispersion micro-extraction of pesticides from singleinsects with
15 subsequent GC-MS analysis. Chromatographia, **59**, 315-3210.
16
17
18

19
20 Lehotay S.J., Mastovská K., Lightfield A.R., 2005 a, Use of buffering and other means
21 to improve results of problematic pesticides in a fast and easy method for residue
22 Analysis of fruits and vegetables. Journal AOAC International, **88**, 615-629.
23
24
25

26
27 Lehotay S.J., Matovská K., Yun S.J., 2005 b, Evaluation of two fast and easy methods
28 for pesticide residue analysis in fatty food matrices. Journal AOAC International, **88**,
29 630-638.
30
31
32

33
34 Lentza-Rizos Ch., 1999, Pesticide residues in olives and olive oil from a European
35 perspective. Proceedings of International Symposium, Chia, Laguna, Cagliari, Italy, p.
36 125-147.
37
38
39

40
41 Lentza-Rizos Ch., Avramides E.J., 1990, Determination of residues of fenthion and its
42 oxidative metabolites in olive oil. Analyst, **115**, 1037-1040.
43
44
45

46
47 Mitra S. 2003. Principles of extraction and the extraction of semivolatiles from
48 liquids. In Chemical Analysis, Winefordner JD, editor. Sample preparation techniques
49 in analytical chemistry. New Jersey: John Wiley Sons, Inc. p 105.
50
51
52

53
54 Molinari G.P., Cavanna, S., Fornara L., 1994, Trattamenti all' olivo con fenthion e
55 rischio di residui in olive ed olio. Atti Giornale Fitopatologiche, **1**, 39-43.
56
57
58
59
60

1
2
3 Molinari G.P., Cavanna, S., Fornara L., 1998, Determination of fenthion and its
4 oxidative metabolites in olives and olive oil. Errors caused by matrix effects. Food
5 Additives and Contaminants, **15**, 661-667.
6
7
8

9
10 Pereira J.A., Casal S., Bento A., Oliveira M.B.P.P., 2002, Influence of Olive Storage
11 Period on Oil Quality of Three Portuguese Cultivars of *Olea europea*, Cobrançosa,
12 Madural, and Verdeal Transmontana. Journal of Agriculture and Food Chemistry, **50**,
13 6335-6340.
14
15
16

17
18 Poustka J., Holadová K., Hajsová, J., 2003, Application of supercritical fluid extraction
19 in multi-residue pesticide analysis of plant matrices. European Food Research and
20 Technology, **216**, 68-74.
21
22
23

24
25 Rotunno T., Di Caterina R., Argenti, L., 1997, Decay of fenthion in green table olives.
26 Journal of Agriculture and Food Chemistry, **45**, 3957-3960.
27

28
29 The pesticide manual, Editor C D S Tomlin, Thirteenth edition, Hampshire, UK, 2003,
30 p. 423.
31
32

33
34 Maštovska K., Lehotay S., 2004, Evaluation of common organic solvents for gas
35 chromatographic analysis and stability of multiclass pesticide residues. Journal of
36 Chromatography A, **1040**, 259-272.
37
38
39

40
41 Tsatsakis A.M., Tsakiris I.N., Tzatzarakis M.N., Agourakis Z.B., Tutudaki M., Alegakis
42 A.K., 2003, Three-year study of fenthion and dimethoate pesticides in olive oil from
43 organic and conventional cultivation. Food Additives and Contaminants, **20**, 553-559.
44
45
46

47
48 Torres C.M., Picó Y., Redondo, M.J., Mañes, J., 1996, Matrix solid-phase dispersion
49 extraction procedure for multiresidue pesticide analysis in oranges. Journal of
50 Chromatography A, **719**, 95-103.
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9 Legend:

10 Figure 1 – Recoveries (%) of fenthion and its metabolites in olives samples extracted 1
11 mg/kg with different solvents in a MSPD method with 1 g NH_2 and 15 ml of eluent. The
12 errors bars represent relative standard deviation ($n= 3$).
13
14

15 Figure 2 – Recoveries (%) of fenthion and its metabolites spiked at 1 mg/kg in olives
16 samples extracted with different volumes of acetonitrile used in the MSPD proposed
17 method in. The errors bars represent relative standard deviations ($n= 3$).
18
19

20 Figure 3 – Chromatograms of olive extracts obtained with different types of clean-up
21 procedures after extraction with NH_2 and elution with acetonitrile.
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Figure 1

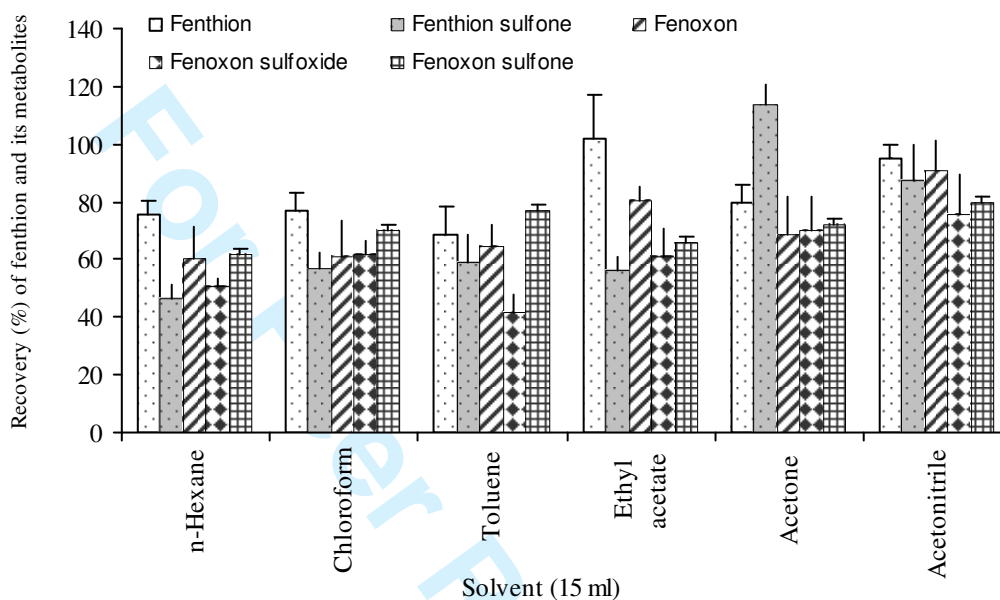


Figure 2

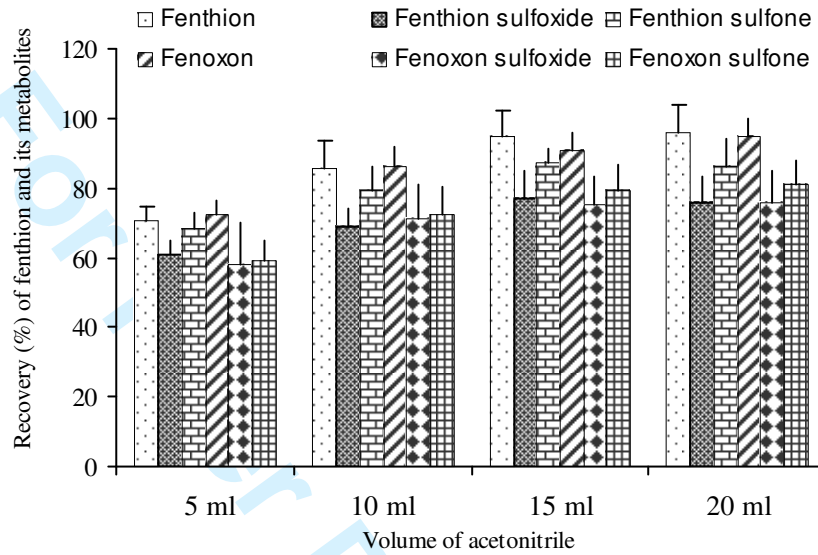


Figure 3

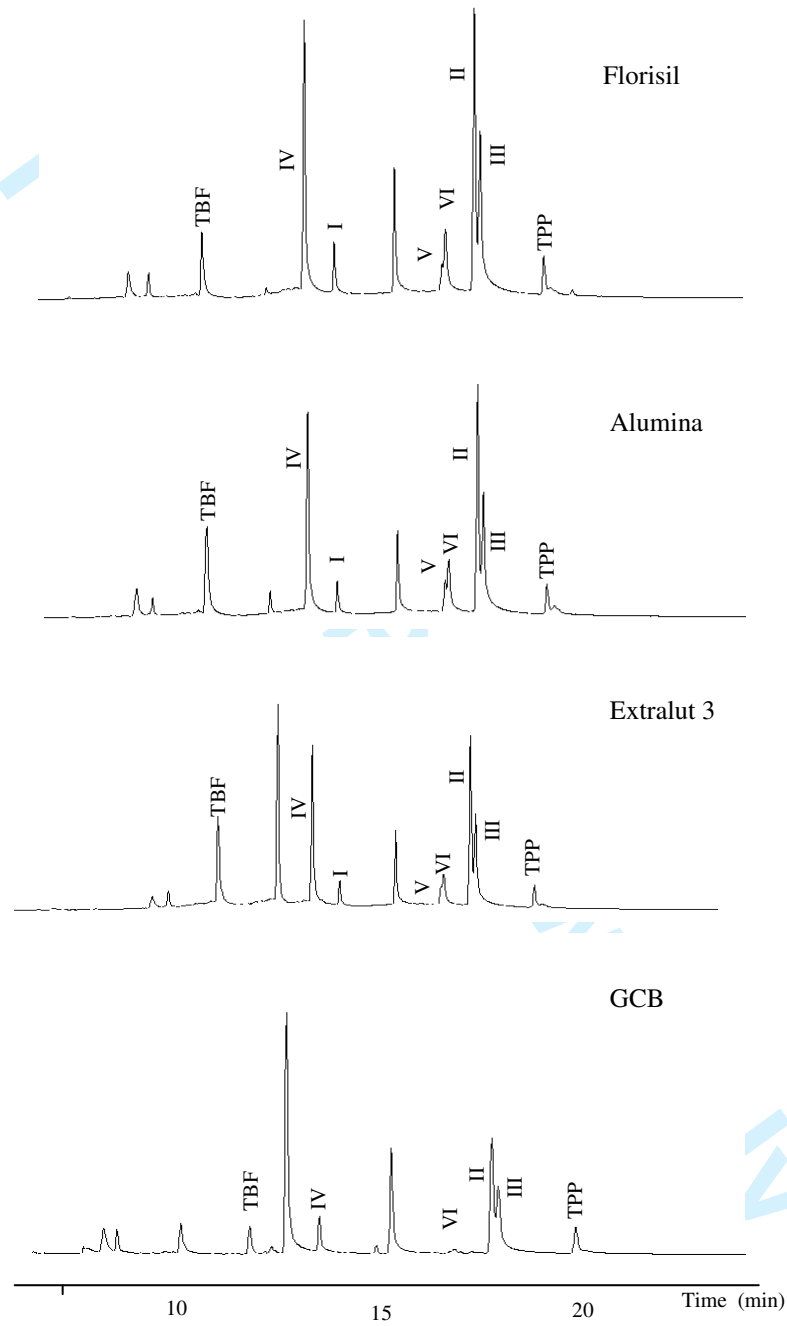


Table I - Recoveries (Rec. %) and relative standard deviations (RSD %) obtained with different sorbents in the extraction of fenthion and its metabolites, after elution of 15 ml of acetonitrile through extract/sorbent ($n=3$).

Fenthion and its metabolites	C ₁₈ a)		C ₁₈ b)		PSA		NH ₂		NH ₂		NH ₂	
	1 g		1 g		1 g		1 g		0.5 g		2 g	
	Rec. (%)	RSD (%)	Rec. (%)	RSD (%)	Rec. (%)	RSD (%)	Rec. (%)	RSD (%)	Rec. (%)	RSD (%)	Rec. (%)	RSD (%)
Fenthion	93.2	± 6	72.4	± 7	100.8	± 8	95.0	± 6	82.0	± 6	88.4	± 8
Fenthion sulfoxide	53.3	± 9	31.2	± 10	67.6	± 9	77.0	± 8	65.3	± 7	70.8	± 9
Fenthion sulfone	54.2	± 7	64.2	± 8	77.5	± 9	87.5	± 7	68.2	± 6	71.6	± 8
Fenoxon	73.1	± 5	70.5	± 5	102.4	± 7	91.0	± 5	72.3	± 6	73.9	± 6
Fenoxon sulfoxide	39.6	± 12	35.5	± 16	57.5	± 18	75.2	± 12	55.7	± 15	65.8	± 14
Fenoxon sulfone	57.8	± 11	55.0	± 15	78.8	± 12	79.5	± 9	60.2	± 11	63.6	± 10

a) With previous activation with methanol/water

b) Without previous activation

Table II – Recoveries (Rec. %) and relative standard deviations (RSD %) obtained with 1 g NH₂ and with different salts in the extraction of fenthion and its metabolites in olives, after elution of 15 ml of acetonitrile through extract/sorbent (*n* = 3).

Fenthion and its metabolites	Without salt			NaCl		MgSO ₄		MgSO ₄ + NaCl		NaSO ₄ + NaCl	
				0.25 g		0.25 g		0.125 + 0.125 g		0.125 + 0.125 g	
	Rec. (%)	RSD (%)		Rec. (%)	RSD (%)	Rec. (%)	RSD (%)	Rec. (%)	RSD (%)	Rec. (%)	RSD (%)
Fenthion	95.0	± 6		70.6	± 8	74.1	± 7	84.1	± 7	61.8	± 9
Fenthion sulfoxide	77.0	± 8		58.4	± 8	109.2	± 5	76.6	± 8	75.0	± 8
Fenthion sulfone	87.5	± 7		48.6	± 8	54.8	± 7	75.5	± 6	79.6	± 7
Fenoxon	91.0	± 5		70.6	± 7	74.1	± 5	81.2	± 6	56.8	± 6
Fenoxon sulfoxide	75.2	± 12		46.4	± 11	51.8	± 13	64.8	± 11	50.2	± 12
Fenoxon sulfone	79.5	± 9		41.4	± 12	51.1	± 11	70.0	± 10	62.3	± 11

Table III - Limits of detection (LODs), linear range and repeatability (Relative standard deviations %) by MSPD and LLE procedures in olives.

Fenthion and its metabolites	MSPD			LLE		
	LODs (mg/kg)	Linear range (0.03 - 1 mg/kg)	Repeatability (<i>n</i> =6) RSD (%)	LODs (mg/kg)	Linear range (0.1 - 1 mg/kg)	Repeatability (<i>n</i> =6) RSD (%)
		R ²			R ²	
Fenthion	0.009	0.9959	8.51	0.02	0.9898	11.30
Fenthion sulfoxide	0.003	0.9977	1.66	0.009	0.9931	4.34
Fenthion sulfone	0.006	0.9940	2.92	0.02	0.9937	6.34
Fenoxon	0.002	0.9982	9.42	0.006	0.9884	9.52
Fenoxon sulfoxide	0.01	0.9953	14.08	0.04	0.9870	15.91
Fenoxon sulfone	0.009	0.9996	15.58	0.02	0.9962	12.51

Table IV – Recoveries (Rec. %) and relative standard deviations (RSD %) obtained with MSPD and LLE procedures in olive fruits ($n = 3$).

Fenthion and its metabolites	0.5 mg/kg				0.3 mg/kg				0.2 mg/kg			
	MSPD		LLE		MSPD		LLE		MSPD		LLE	
	Rec. (%)	RSD (%)	Rec. (%)	RSD (%)	Rec. (%)	RSD (%)	Rec. (%)	RSD (%)	Rec. (%)	RSD (%)	Rec. (%)	RSD (%)
Fenthion	110	± 4	108	± 6	101	± 6	102	± 7	95	± 5	84	± 7
Fenthion sulfoxide	98	± 7	91	± 7	92	± 8	83	± 9	88	± 8	79	± 8
Fenthion sulfone	112	± 8	102	± 8	105	± 9	97	± 10	102	± 7	85	± 8
Fenoxon	96	± 5	104	± 6	102	± 5	95	± 6	93	± 5	98	± 6
Fenoxon sulfoxide	85	± 10	88	± 15	87	± 11	68	± 12	86	± 13	73	± 13
Fenoxon sulfone	85	± 9	96	± 14	95	± 10	92	± 11	92	± 12	72	± 14

Table V – Recoveries (%) and relative standard deviations (RSD %) obtained with 3 extraction procedures applied to olive oil and linearity obtained with MSPD procedure ($n = 5$).

Fenthion and its metabolites	MSPD			LLE (1)		LLE (2)	
	Linearity (R^2)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
Fenthion	0.9945	79 ± 6	62 ± 7	68 ± 6			
Fenthion sulfoxide	0.9872	96 ± 8	91 ± 8	115 ± 8			
Fenthion sulfone	0.9910	90 ± 9	84 ± 8	105 ± 7			
Fenoxon	0.9951	78 ± 5	71 ± 6	65 ± 6			
Fenoxon sulfoxide	0.9640	67 ± 11	68 ± 13	63 ± 12			
Fenoxon sulfone	0.9798	98 ± 11	79 ± 10	81 ± 14			

(1) Method based in Cabras *et al.* (1993)

(2) Method based in Ferrer *et al.* (2005)

Table VI – Pesticide residues (mg/kg) determined olives, after treatment with the insecticide mixture.

Days after treatment	Residues of pesticides (mg/kg)					
	Fenthion	Fenthion sulfoxide	Fenthion sulfone	Fenoxon	Fenoxon sulfoxide	Fenoxon Sulfone
7	0.02	0.03	n.d.	n.d.	n.d.	n.d.
14	0.01	0.02	n.d.	0.02	0.03	n.d.
21	0.02	0.03	n.d.	0.01	0.05	n.d.
28	0.02	0.02	n.d.	0.01	0.05	n.d.
35	0.02	0.02	n.d.	0.01	0.04	n.d.
42	0.02	0.01	n.d.	0.01	0.03	n.d.