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Optimization and validation of a method of analysis for fenitrothion and its main metabolites in forestry air samples using sorbent tubes with thermal desorption cold trap injection and gas chromatography–mass spectrometry

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Abstract

An analytical methodology using thermal-desorption cold trap (TCT) and GC–MS was developed for the determination of the insecticide fenitrothion and its main metabolites, 3-methyl-4-nitrophenol and fenitrooxon, in forestry atmospheres. The sampled atmosphere was pumped through a glass tube containing 100 mg of Tenax adsorbent at a flow rate of 50 ml min^{-1}. Adsorption/thermal desorption and breakthrough experiments were performed to test the ability to quantitatively trap the compounds. The detection limits of method for these compounds ranged between 1.6 and 2.1 ng m^{-3}. This methodology was developed to evaluate the persistence of fenitrothion in forest atmospheres after treatment. Spray application at 21.5 mg active ingredient m^{-2} resulted in atmosphere levels of the insecticide of 78.3 ng m^{-3} (after 2 h of application). Within 2–4 days following treatment, the presence of fenitrooxon fell to 50–55%. During this period residues of metabolites began to appear, disappearing 19 days later.

Keywords: Fenitrothion; Forestry air samples; Thermal desorption; GC–MS

1. Introduction

Fenitrothion is a broad-spectrum organophosphorous insecticide widely used to control numerous forest and agriculture pests. It is applied in rainy areas in the pest control of *Thaumetopea pityocampa* (pine processionary caterpillar) and *Eriosoma lanigerum* (affecting black poplar forestry populations). Its persistence in forest ecosystems is not well known either due to its dependence on several factors, such as the applied dose and formulation, application parameters, the number of applications, climatic conditions and the forest characteristics. The fate and persistence of fenitrothion in various ecosystems have been a subject of interest for some years [1–4]. Most studies refer to the persistence of fenitrothion in plants, soil, and water after aerial application, being a potential risk to a number of living creatures in the ecosystem.

The determination of this insecticide in urban and rural atmospheres has also been studied by several authors [5–9]. In general the method of analysis for the airborne pesticides is determined by the trapping technique used. For example, if quartz fiber filters and XAD-2 or related sorbent traps are used, after sampling, the pesticides must be extracted with organic solvents [10,11], before subsequent GC or HPLC analysis. In order to accelerate the solvent extraction stage, solid-phase microextraction (SPME) [12] has recently been used as an alternative technique for trapping airbone pesticides. Other authors have used systems based on a combination of adsorption/thermal desorption followed by direct injection into a GC system [13–15]. This improves the detec-

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tion limits, reduces analysis time, reduces interfering peaks caused by solvents and opens the possibility of automation. To date there have been two types of system based on adsorption/thermal desorption: the automatic thermal-desorption units (ATD) and the thermal-desorption cold trap injectors (TCT). These use Tenax type trap resins, carbotrap, carboxen or carbopack and employ Peltier effect or liquid nitrogen top of the column cryofocussing systems, respectively. The advantages and drawbacks of each have been discussed in depth [16,17].

As far as we know, work has not been published that assesses the atmospheric level of fenitrothion and its main metabolites due to volatilization of post-application residues. To carry out this study, a suitable and sensitive TCT–GC–MS method for the simultaneous determination of the pesticide and its major metabolites in forestry atmosphere was developed. The method is focused on the application of air trap systems on polymeric resins, which permit the use of TCT type injectors, to reach the required sensitivities.

2. Experimental

2.1. Chemicals and adsorbents

Certified chemical pesticide and its metabolite: fenitrothion (98.5%) and 3-methyl-4-nitrophenol (99.0%) were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany) and fenitrooxon (99%) was obtained from ChemService (West Chester, PA, USA). The stock solutions and all dilutions were made using 2-propanol (Riedel-de Häen, Seelze, Germany).

Tenax TA (60–80 mesh) and treated glass wool DMCS were purchased from Varian (Lake Forest, CA, USA). Glass tubes (4 mm i.d. × 14 cm) were obtained from Varian. Each tube was hand-packed with the same amount of adsorbent, 200 mg, and the ends of the tubes were plugged with glass wool. Before use, the sampling tubes were conditioned by heating at 300 °C for 2 h and purged with helium at flow rate of 100 ml min^{-1}. After each use or storage, they were reconditioned at 300 °C for 30 min while being purged with helium al 100 ml min^{-1}. Each tube was used for a maximum of 10 applications.

After sampling, the tubes were removed from the sampling train and sealed with swagelock end caps to stop any further adsorption. Sample tubes were put into special plastic bags that were tightly closed and frozen at −45 °C prior to analysis. Uncapped blank Tenax tubes were also placed into the bags together with the used bags to detect any possible cross-contamination during storage. There were no cases of insecticide residues appearing in blank tubes.

2.2. Sampling devices

The sampling was done in duplicate. Two glass tubes were connected by a clear inert Tygon tube to low-flow sampling pumps, one tube to a Pocket Pump SKC 210 (SKC, P.A., USA) and the other one to a SKC 224-44EX. Air samples were collected at a flow of 50 ml min^{-1} during 8 h (9 a.m. to 5 p.m.). The flow rate of each air sampler was carefully calibrated each time prior to use to determine exactly the volume of air sampled. An EL-FLOW F-201C (Bronkhorst HighTech., The Netherlands) thermal mass flow meter and controller with a control module, Model E-7000 was used to calibrate the pumps, correct for pressure and temperature and obtain units of volume at the reference conditions (293 K and 101.3 kPa).

One hundred-litre capacity Tedlar sample bags, supplied by SKC (catalog No. 232.50) were used in the recovery studies. The sample bags are equipped with single polypropylene fitting. This fitting contains both a syribe port with PTFE-lined septum and a hose connection and acts as a shut-off valve for the hose connection.

2.3. TCT–GC–MS system

Analysis was carried out by using a thermal desorption cold trap injector Chrompack Model CP-4010 (Middelburg, The Netherlands), connected to a Hewlett-Packard (Palo Alto, CA, USA) model 5890 II gas chromatograph directly coupled to a HP-5972 quadrupole mass spectrometer. Analytes were separated using a HP-5MS column (30 m × 0.25 mm i.d., 0.25 μm) Hewlett-Packard and helium as carrier gas at a flow of 1.3 ml min^{-1}. The temperature program of the column started at 70 °C, and then increased at a rate of 40 °C min^{-1} up to 280 °C where it was held for 2 min. Detection was carried out using a MS detector with interface temperature at 280 °C employing electron ionization with an ionizing energy of 70 eV.

2.4. Location and characteristics of the sampling point

A plantation of poplar affected by a plant louse (*Phloemyzus paserinii*) was treated with fenitrothion pesticide. The plantation is in Trespuentes village (UTM, $x = 517\,072$, $y = 4\,743\,285$) 20 km from Vitoria-Gasteiz (Álava, Spain). The plot is about 1×10^4 m^2. One sampling point was located in the center of the plot at 1 m height. The first week after the application, air was sampled each day (6–11 November 2003), then 2 days a week (13 November–4 December) and the last month once a week (8–22 December).

2.5. Spray formulation and application conditions

The spray formulation used in operational forest treatment was Folithion 50 LE. A Tifone cannon spray (Cassana, Ferrara, Italy) was used, pulled by a Fiat tractor at an average speed of 28.8 m min^{-1}. The atomizer has 12 nozzles with a flow volume 5.16 l min^{-1} and a flow width of 50 m. The dose applied was 0.043 ml m^{-2}.

Table 1
Optimal conditions for thermal desoption unit

Parameter	Optimal conditions
Temperature of cold trap (°C)	−100
Temperature of desorption (°C)	300
Desorption time (min)	5
Desorption flow (ml min^{-1})	150
Trap temperature in the injection (°C)	260
Time for the injection (min)	1
Rod temperature (°C)	260

3. Results and discussion

3.1. Chomatographic conditions

All samples were thermally desorbed on the thermal desorption cold trap injector. The optimal conditions used to determine fenitrothion and its metabolites are summarized in Table 1. Analysis was performed with selected ion monitoring mode (SIM) based on the use of target and qualifier ions. Analytes were identified according to retention times, target ions and the qualifier-to-target ion ratios. The target and qualifier abundances were determined by injection of individual analyte standards under the same chromatographic conditions in full-scan from *m*/*z* 60 to 550. For each analyte three ions (target and two qualifier ions) were chosen for analysis in SIM: 109, 125 and 277 for fenitrothion; 109, 244 and 261 for fenitrooxon and 108, 136, 153 for 3M4NP. Calibration curves were obtained by direct injection on the Tenax tube of 2 μl of mixture of pesticide and its metabolites at concentrations between 0.05 and 2 mg l^{-1} which correspond to an injection between 0.1 and 4 ng of each pesticides.

Table 2 shows the retention time of the compounds, relative standard deviations (R.S.D.) and the detection limits of the method. The limit-of-detection (LOD) for each analyte was expressed as the concentration of analyte which gives a signal that is 3σ above the mean blank signal (where σ is the standard deviation of the blank signal). The regression coefficients are higher than 0.995 and the detection limits range from 1.7 and 2.5 ng m^{-3} which proves the accuracy and sensitivity of this TCT–GC–MS analytical methodology in quantifying the fenitrothion and its metabolites. Fig. 1 shows a chromatogram obtained when 2 μl of a standard of 2 mg l^{-1} are injected in optimal conditions.

Table 3
Retention studies at three levels of concentration

	Injected values		
	2 ng	5 ng	20 ng
3M4NP	1.91 ± 0.22	4.87 ± 0.25	20.01 ± 0.11
Fenitooxon	2.00 ± 0.06	4.90 ± 0.08	19.98 ± 0.07
Fenitrothion	1.94 ± 0.17	4.92 ± 0.13	19.92 ± 0.12

Target compounds placed in 100 l sample bags were trapped under optimal experimental conditions. The values in the table are observed values for $n = 5$.

3.2. Optimization of the sampling process

In order to demonstrate the efficiency of the sampling process, three experiments were carried out to show: (i) that the analytes present in the air sample are completely retained by the Tenax sorbent, (ii) that the traps shows no losses of analytes due to elution phenomena, (iii) that during the thermal desorption process, the analytes absorbed in the resin are totally desorbed and injected in the chromatographic system.

3.2.1. Retention studies

For the first of the studies 100 l Tedlar sample bags equipped with an injection valve with septum were used. In separate tests, 100 μl of solutions containing 20, 50 and 200 μl^{-1} of each of the compounds were injected and synthetic air was introduced in the bag by negative pressure. The injection point was heated to volatize the compounds and the sample was left to homogenize for an hour. Finally, the air from the bag was sampled into Tenax TA sorbent tubes attached to a sampling pump. The resulting data in Table 3 shows that at the three levels of airbone concentration tested, recoveries close to 100% have been achieved.

3.2.2. Breakthrough volume

The sampling protocol was designed having taken into account the *breakthrough volume* (B_v) of the analytes, defined as theoretical volume of gas per gram of Tenax TA, which will elute each compound from the adsorbent at 20 °C. When collecting samples onto the adsorbent material bed, the safe sample volume ($B_s = B_v \times 0.5$) should not be exceeded in order to trap all the analyte on the adsorbent material bed.

In order to accurately determine the breakthrough volume as a function of temperature, a modified system based on other previously mentioned papers [18–20] was used. The

Table 2
Retention times, linear regression analysis, correlation coefficients, standards deviations intraday and interday at two different levels (0.2 ng and 2 ng) and the detections limits of TCT–GC–MS determination of fenitrothion, fenitrooxon and 3-methyl-4-nitrophenol

Compound	Retention time (min)	Slope (abundance s ng^{-1}) × 10^5	Intercept (abundance s) × 10^5	Correlation coefficient	LOD (ng m^{-3})	R.S.D. (%) intraday		R.S.D. (%) interday	
						0.2 ng	2 ng	0.2 ng	2 ng
3M4NP	5.12	2.5 ± 0.1	0.1 ± 0.2	0.995	2.08	10.43	6.46	10.12	8.05
Fenitrooxon	6.11	1.2 ± 0.0	−0.1 ± 0.0	0.999	2.50	10.53	11.89	10.38	10.75
Fenitrothion	6.27	5.5 ± 0.2	0.1 ± 0.3	0.997	1.67	5.23	6.33	7.39	10.89

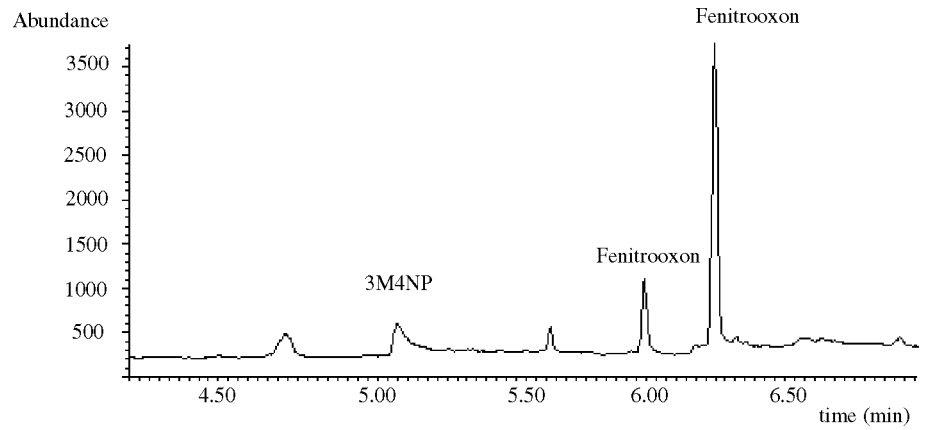

Fig. 1. Gas chromatogram obtained when 2 μl of a standard of 2 mg l^{-1} are injected in optimal conditions.

sorbent tube under test was connected to an injection port inside a GC oven, using 530 μm uncoated fused silica capillary tubing, and the other end of the sample tube was connected to a flame ionization detector. The capillary column was connected to both ends of the adsorbent resin bed using septum and swagelok union. Helium was used as the carrier gas and carrier gas flows were adjusted from 35 to 50 ml min^{-1} with a flow calibrator (Chrompack FP-meter). Similarly the temperature was controlled using the GC oven temperature controller. A correction was made for dead time of the packed adsorbent material and connecting lines. This dead time was determined by injecting a non-retained analyte (methane). For most temperatures this dead time was about 0.15 min. All samples were run in triplicate at each temperature. Then, breakthrough volumes can be obtained of the expression:

$$B_v = \frac{(R_t - D_t)\,F}{1000\,W_a} \quad (1)$$

where B_v is the breakthrough volume (in l/g adsorbent), R_t the retention time (min), D_t the dead time (min), W_a the adsorbent weight (g) and F is the carrier gas flow (ml min^{-1}). When collecting samples onto Tenax TA bed, the safe sample volume ($B_s = B_v \times 0.5$) should not be exceeded in order to trap all the analytes on the adsorbent material.

After calculating retention volumes at five different temperatures, usually higher than the trapping temperature, the breakthrough volume at 25 °C was obtained by linear extrapolation of the graph $\log B_v$ versus $1/T$. Values of $15\,000 \pm 400$, $12\,500 \pm 300$, and 7400 ± 250 l g^{-1}, for fenitrothion, fenitroxon and 3M4NP, respectively, were obtained once the uncertainty in the intercept was estimated. For the breakthrough experiments made using the experimental model described above, no breakthrough was observed for any of the compounds for an air volume of 1580 l (using 200 mg of TENAX TA). Thus, both trap volumes of between 24 and 50 l and those used in this paper do not exceed in any case the safe sample volume of any of the compounds (estimated at 790 l for 3M4NP as the worst case).

To confirm that the sampling volume used is suitable, 24 l, the maximum sampling volume during the air sampling, two adsorbent tubes were connected in series. Fenitrothion, fenitrooxon and 3M4NP (100 ng) 0.3 μg l^{-1} were injected in the head of the first tube. Synthetic air was pumped at a rate of 50 ml min^{-1} for 8 h. No analytes were observed on the second back-up tube.

3.2.3. Pesticides recoveries from Tenax

Pesticides recoveries from Tenax were calculated from Eq. (2) and this allows the optimization of the desorption time of the tube [21].

$$RE_i\,(\%) = \frac{A_{i,1} - A_{B,i}}{(A_{i,1} + A_{i,2}) - A_{B,i}} \times 100 \quad (2)$$

in which RE_i is the recovery efficiency for the analyte i, $A_{i,1}$ the peak area of the analyte i for the first desorption of the spiked tube, $A_{i,2}$ the peak area of the analyte i for the second desorption of the spiked tube and $A_{B,i}$ is the count of noise from the adsorbent blank.

Tubes spiked with 2 ng of each analyte ($n = 3$) were desorbed at same temperature (300 °C) and with the same desorption flow (140 ml min^{-1}) for different times (3, 4, 5, 6, 7 min). A desorption time of 5 min gives a recovery of 99% for all the compounds (99.6 ± 1.1, 99.3 ± 0.9 and 100.0 ± 0.6% for fenitrothion, fenitrooxon and 3M4NP, respectively).

Precool temperature was studied to determine the optimum cold trap temperature required to refocus all the compounds eluting from the sample tube. A temperature of −100 °C is enough to refocus the fenitrothion and its metabolites.

3.3. Field study

With the analytical method developed, it was possible to study the evolution of atmospheric fenitrothion and its metabolite levels on a treated plot of 1 ha. The sampling campaign was carried out between 6 November and 22 December 2003.

In the forest where the study of the persistence of fenitrothion and its metabolites was performed, the air was clean and

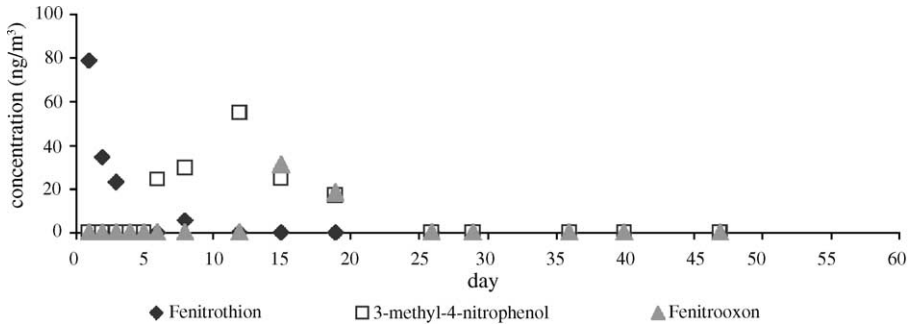

Fig. 2. Evolution of atmospheric fenitrothion, fenitrooxon and 3-methyl-4-nitrophenol concentrations after the application day. Each value represents the average of two independent measurements.

not many interfering compounds were expected. Nonetheless, the adsorbent Tenax TA was tested for its ability to trap possible interfering compounds. A number of air samples were collected a few days before the forest sprayed with fenitrothion. In the analysis of these samples no interfering peaks were found.

The first week after the treatment a sample was made each day. Then twice a week and finally once a week. The fenitrothion and the two metabolites studied were detected in any of the sampling days. In Fig. 2 the evolution of the fenitrothion and its metabolites atmospheric concentration in the plantation is presented. This figure gives a representation of fenitrothion and its metabolites evolution in the atmosphere. The day of the application of the pesticide the concentration level in the air was $78.3 \pm 21.6\,\mathrm{ng\,m^{-3}}$. The atmospheric level of fenitrothion remained during the first 3 days following the treatment and decreased rapidly to values under detection limits until the end of the experiment. Fig. 3 shows a chromatogram in which fenitrothion appears in this field study. These results are in agreement with the observations of Clément et al. [17] when the levels of atrazine concentration evolution after treatment were studied.

As for the metabolites, the first days after the applications they were not detected in the atmosphere. The sixth day after the application, 3M4NP was detected with a concentration of $24.1 \pm 6.6\,\mathrm{ng\,m^{-3}}$. The level of the 3M4NP increased during the second week until to reach $54.7 \pm 11.3\,\mathrm{ng\,m^{-3}}$, because of the gradual degradation of the fenitrothion, and the evaporation of the metabolite. Then the concentration decreased gradually. Two weeks after the application fenitrooxon was detected at a concentration of $30.8 \pm 4.9\,\mathrm{ng\,m^{-3}}$ and the concentration was decreased similarly to 3M4NP.

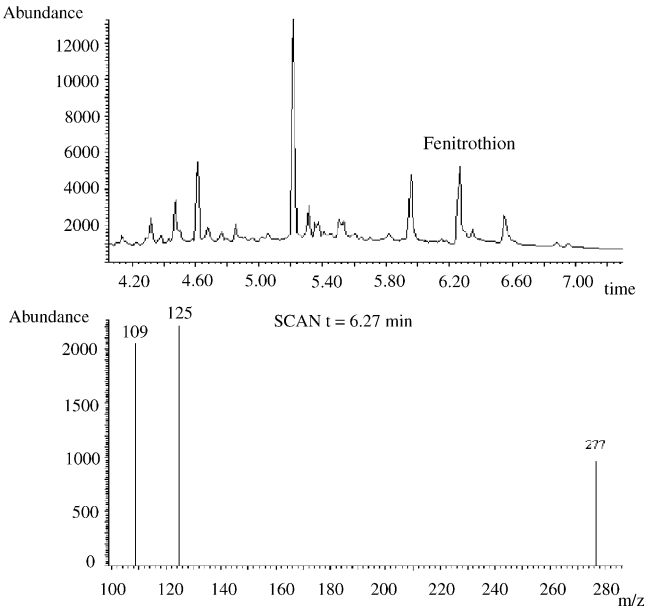

Fig. 3. Chromatogram obtained for the analysis of the air sample (11 November, 5 days after the application) and the mass spectrum in SIM mode (100% of 125, 90% of 109 and 37% of 277).

4. Conclusions

An analytical method using thermal-desorption/gas chromatography–mass spectrometry was developed for the analysis of fenitrothion and two of its metabolites (fenitrooxon and 3-methyl-4-nitrophenol) in the atmosphere after field application. This method appears to be rapid, accurate and sensitive, since no extraction and concentration steps between sampling and analysis are needed.

The method was applied for the study of the evolution of atmospheric concentration of fenitrothion and its metabolites after application.

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