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Simultaneous determination of fenpropimorph and the corresponding metabolite fenpropimorphic acid in soil

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Summary. An analytical procedure for the simultaneous determination of fenpropimorph and its main metabolite fenpropimorphic acid in soil is reported. Extraction with acetone/water (2:1), liquid/liquid partition with dichloromethane and clean-up using gel permeation chromatography is employed to concentrate the analytes. Methylation of the polar metabolite is absolutely required for GC/MS and GC/NPD. The parent compound fenpropimorph is not influenced by this derivatization step. The detection limits are 0.005 mg/kg for fenpropimorph and 0.010 mg/kg for fenpropimorphic acid.

1 Introduction

The systemic morpholine fungicide Corbel[1] with fenpropimorph (4-[3-4-(1,1-dimethylethylphenyl)-2-methyl]-propyl-2,6-(cis)-dimethylmorpholine) as the active substance is frequently applied in cereal cropping to control *Erysiphe graminis*, *Puccinia striiformis* and *Puccinia recondita*. Due to uncontrolled drift during spray application and leaching out of the crop stand, the leaf fungicide contaminates the soil surface [1]. There, fenpropimorph metabolizes rapidly to fenpropimorph acid (4-[3-[4-(2-carboxypropyl)-phenyl]-2-methyl]-propyl-2,6-(cis)-dimethylmorpholine) [2] (Fig. 1). To verify decreasing concentrations of fenpropimorph definitely as result of microbial degradation it is urgently recommended to determine the main degradation product as well. Further, the metabolite shows the same biological activity as the parent compound [3] and, because of its polarity, a higher leaching tendency in soil has to be considered. These were the reason for developing an analytical method for the simultaneous determination of both substances.

2 Materials and methods

2.1 Standard material

The reference substances fenpropimorph and dodemorph were purchased from Dr. Ehrenstorfer while fenpropimorphic acid was supplied from the Federal Biological Research Centre for Agriculture and Forestry (BBA), Braunschweig.

[1] tradename by BASF

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2.2 Reagents

All chemicals used were of analytical grade. Solvents: acetone, dichloromethane, cyclohexane, ethylacetate, methanol, diethylether (Baker). Sodium sulphate (anhydrous), sodium chloride (Merck). N-methyl-N′-nitro-N-nitrosoguanidine (MNNG) (Aldrich).

2.3 Equipment

Gel permeation chromatography (GPC), clean-up-system (Abimed), Bio Beads SX-8 (Biorad), cyclohexane/ethylacetate (1:1) with a flow of 5 ml/min); Aldrich MNNG Diazomethane Generation Kit.

Varian 3400 gas chromatograph equipped with an ion-trap detector ITD 800 (Finnigan) as well as a HP 5890 GC Series II with a nitrogen-phosphorus detector (NPD) (Hewlett Packard), both with split-splitless injectors.

2.4 GC determination

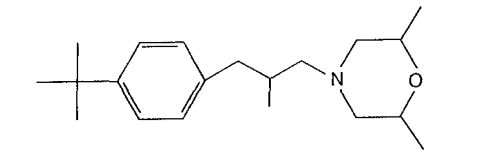
For the GC/MS determination a HT 5 fused silica capillary column (25 m, 0.22 mm i.d., 0.1 µm carborane-siloxane-copolymer, SGE) was used with helium as carrier gas (1.1 ml/min). Temperature settings were: injector: 280°C, temperature programme: 60°C (2 min) −20°C/min −200°C/min (1 min) −5°C/min −280°C (3 min). 1 µl was injected with the splitter closed for 0.75 min.

The ITD was connected using an open split interface with a transfer line temperature of 280°C and a flow of 1.1 ml helium/min. Electron impact ionization (70 eV) was carried out in a scan range of 76–449 amu and low pressure positive chemical ionization with methanol in a scan range of 100–449 amu.

For GC/NPD determination, a DB-17 fused silica capillary column (30 m, 0.25 mm i.d., 0.25 µm phenyl-methyl-polysiloxane, J & W) was used with helium as carrier gas (1.0 ml/min). Temperature settings were: injector: 250°C, detector: 280°C, temperature programme: 100°C (1 min) −10°C/min − 250°C (15 min). The detector gases were nitrogen for make-up (30 ml/min), synthetic air (100 ml/min) and hydrogen (3 ml/min). 1 µl was injected with the splitter closed for 0.75 min.

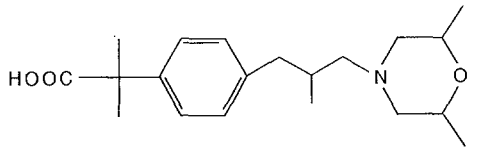
2.5 Standard solutions

Stock standard solutions with 1 µg/µl of the analytes and the internal standard were prepared. For the calibration



4-[3-[4-(1,1-dimethylethyl)phenyl]-2-methyl]-propyl-2,6-(cis)-dimethylmorpholine

Fenpropimorph



4-[3-[4-(2-carboxypropyl)phenyl]-2-methyl]-propyl-2,6-(cis)-dimethylmorpholine

Fenpropimorphic acid

Fig. 1. Chemical structures of fenpropimorph and fenpropimorphic acid

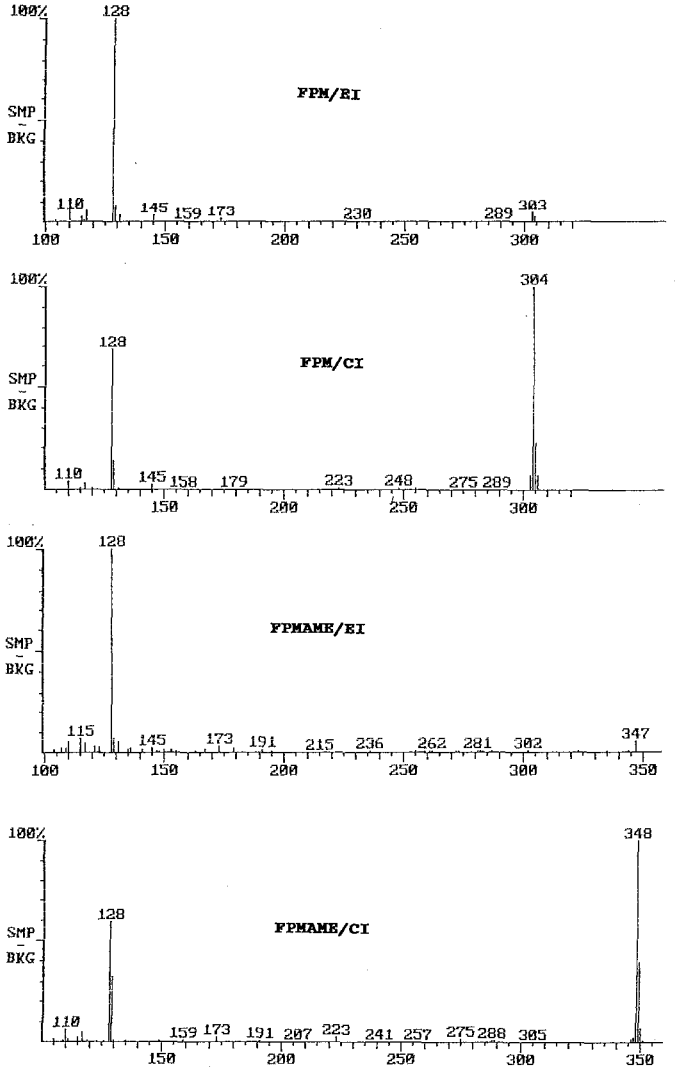


Fig. 2. Mass spectra of fenpropimorph (FPM) and fenpropimorphic acid methylester (FPMAME) detected by electron impact (EI) and positive chemical ionization (PCI)

fresh diluted standards at concentration levels of 0.5, 1, 2, 5 ng/µl were methylated with diazomethane [4]. Because of the instability of methylated standards, sample and standard preparation should be done simultaneously. The use of derivated methyl ester standards is, therefore, limited to a few days [5].

2.6 Sample preparation

The extraction procedure applied combines the principles of the DFG S19 multi method [6] and the on-line extraction method reported by Steinwandter [7]. Humid soil is extracted with acetone/water (2:1) on a horizontal shaker for 12 h. For a 50 g sample 100 ml acetone and (according to the water content of the soil) 50-x ml water are used. Then 15 g sodium chloride and 100 ml dichloromethane are added and the samples are again put on a shaker for another 2 h. The upper phase containing the organic solution is decanted and dried over sodium sulphate. An aliquot of the organic phase (100 – 150 ml) is concentrated on a rotary evaporator under vacuum at 40°C and dissolved in 5 ml cyclohexane/ethylacetate (1:1) for gel permeation chromatography (collecting fraction 100 – 200 ml). Next to the clean-up step, the extract is methylated with ethereal diazomethane solution (reaction time is 40 min at room temperature). For the preparation of diazomethane the precursor MNNG is decomposed by sodium hydroxide [4]. After derivatization the sample solution is concentrated under a gentle stream of nitrogen in a fume hood, then dissolved in methanol with 1.0 ng/µl dodemorph as internal standard and analyzed by GC/MS and GC/NPD, respectively.

3 Results and discussion

In the elaboration of this analytical method soil extraction procedures were tested at different pH-values due to the basic character of fenpropimorph and the acidic character of fenpropimorphic acid. According to approved analytical methods [8, 9, 10] the extraction of fenpropimorph was performed at pH 10, followed by extraction at pH 2 to optimize the efficiency for the metabolite. Because of comparable high recoveries of both compounds and low amounts of co-extracted substances from the soil matrix, an extraction procedure at neutral pH-value was preferred.

During liquid/liquid partition with dichloromethane, a nearly salt saturated aqueous phase is required to transfer even polar pesticides into the organic phase [6]. Column chromatographic clean-up steps on silicagel and alumina in addition to the GPC procedure do not improve the cleaning effects, neither before nor after derivatization. Because of similar polarities, no separation of the metabolite and most of the co-extracted substances could be achieved. The derivatization with ethereal diazomethane solution generates the methyl ester as a volatile derivative of fenpropimorphic acid. The reaction conditions of methylation are so gentle that fenpropimorph as the parent compound is not affected. Without any additional sample preparation, the simultaneous determination of fenpropimorph and fenpropimorphic acid facilitates the integration of both analytes into approved multi-residue analysis methods.

Recovery rates were determined by spiking soil samples at various concentration levels between 0.01 mg/kg and 0.1 mg/kg. For fenpropimorph the average recovery is 99%,

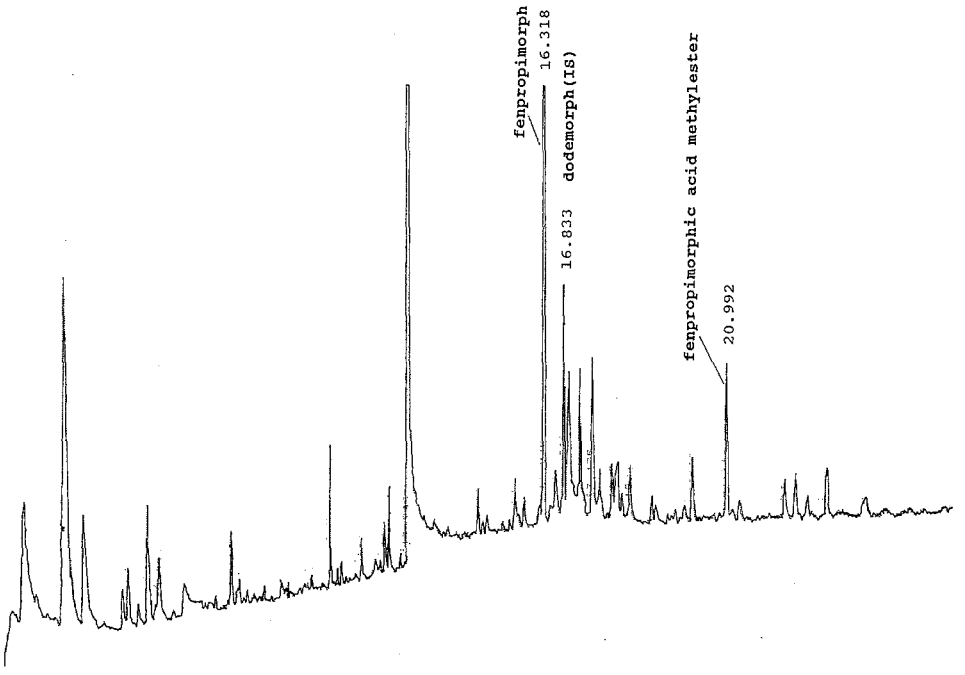


Fig. 3. GC/NPD chromatogram of a soil sample with 0.356 mg fenpropimorph/kg soil and 0.043 mg fenpropimorphic acid/kg soil

whereas for fenpropimorphic acid 97% is achieved by GC/MS determination. For electron impact ionization the detection limits are 0.005 mg/kg for fenpropimorph and 0.010 mg/kg for fenpropimorphic acid.

When screening soil samples with unknown environmental fate, GC/MS full scan mass spectra are preferred, although fenpropimorph as well as fenpropimorphic acid show only less fragmentation with m/z 128 as the base peak and m/z 303 and m/z 347 as molecule ions. Particularly in case of less intensive molecule ions, GC/MS with positive chemical ionization (PCI) emphasizes the higher mass ranges, which ensures the identification by detecting the typical $[M + H]^+$ ions (Fig. 2).

In routine residue investigations, the analytes are also detectable by the sensitive and selective NPD because of the nitrogen containing morpholine ring. Applying a DB-17 capillary column for high resolution GC, a sufficient separation of the analytes fenpropimorph, fenpropimorphic acid and dodemorph as the internal standard is possible (Fig. 3). These three detection methods mentioned above show comparable detection limits. But in case of GC/NPD analysis, verification of the results with another column of different polarity is necessary.

Soil samples of batch experiments and field investigations were analyzed with this elaborated method. Both compounds, fenpropimorph and fenpropimorphic acid could be determined. During the investigation period, increasing concentrations of the main metabolite verified definitely the degradation of fenpropimorph.

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