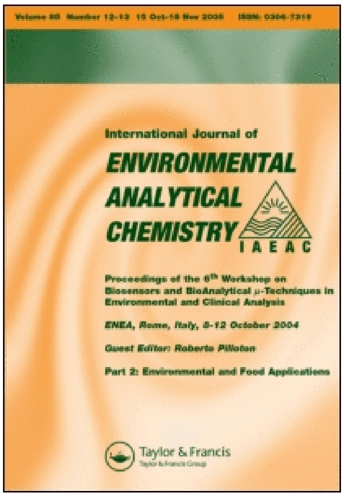

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# The Analysis and Fate of the Fungicide Fenpropimorph and Its Degradation Product Fenpropimorphic Acid in Soil by Liquid Chromatography Mass Spectrometry

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# THE ANALYSIS AND FATE OF THE FUNGICIDE FENPROPIMORPH AND ITS DEGRADATION PRODUCT FENPROPIMORPHIC ACID IN SOIL BY LIQUID CHROMATOGRAPHY MASS SPECTROMETRY

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This article describes a method for extraction of soil samples and analysis with liquid chromatography and mass spectrometric detection after atmospheric pressure chemical ionisation (APCI) of the fungicide fenpropimorph and its degradation product fenpropimorphic acid. The method gives high recovery rates (about 80 %) and detection limits below 1 µg/kg soil for both compounds. The article elucidates the difference between the parent compound and the degradation product with respect to adsorption properties, where fenpropimorph with a $K_d$ value about 161 kg/L in a sandy loam is expected to be immobile in soil while the $K_d$ value for fenpropimorphic acid is determined to 1.3 kg/L which means a much higher mobility.

Two studies on the degradation of fenpropimorph and occurrence of the degradation product are mentioned. The first experiment is a batch experiment where fenpropimorph is mixed with the soil while the second experiment is a field study with pesticide application in normal dosage and ten times normal dosage. Both experiments demonstrate the occurrence of the degradation product in the soil during months after application.

*Keywords:* Fenpropimorph; fenpropimorphic acid; pesticides; degradation; adsorption; liquid chromatography mass spectrometry

## INTRODUCTION

During the last decade many investigations and monitoring programmes have focused on the occurrence of pesticides in the environment. Many findings of pesticides in surface water and ground water have been reported[1,2]. However, most attention has been paid to active ingredients in formulated products while

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the occurrence of degradation products only has been elucidated to a minor degree. The parent compounds are commercially available, while only few degradation products appear in the chemical catalogues. Information about use, physical and chemical properties is accessible for the active ingredients, so it is possible to evaluate which compounds might be potential ground water contaminants. Each parent pesticide will degrade to a range of different products where the knowledge about their stability and mobility is sparse.

Chemical analyses have been developed to cover a broad range of pesticides using gas chromatographs or liquid chromatographs with different detector techniques[3]. In recent years, liquid chromatography with mass spectrometric detection has become very popular[4,5]. It is obvious that liquid chromatography is a good choice for pesticides which have potential for ground water contamination with respect to their polarity. The atmospheric pressure ionisation (API) mass spectrometric detection technique has become a robust and sensitive detection method in mass spectrometry[6].

Fenpropimorph (4-[3-4-(1,1-dimethylphenyl)-2-methyl]-propyl-2,6-(cis)-dimethylmorpholine) (Figure 1, A) is the active ingredient in the systemic fungicide Corbel (Novartis, BASF) and combined fungicide formulations like Tilt Top and Tilt Megaturbo (fenpropimorph and propiconazole, Novartis). Fenpropimorph is not expected to be a potential risk to ground water contamination, but Stockmaier et al.[7] have demonstrated that fenpropimorph degrades fast to fenpropimorphic acid (4-[3-[4-(2-carboxypropyl)-phenyl]-2-methyl]-propyl-2,6-(cis)-dimethylmorpholine) (Figure 1, C) which might be more mobile with respect to its acidic and more polar character. Dieckman et al.[8] have reported a method for the determination of fenpropimorph and its major degradation product in soil, based on gas chromatography with mass spectrometric detection after derivatisation with diazomethane.

In the present paper, a new method is described for the determination of fenpropimorph and its major degradation product fenpropimorphic acid in soil based on liquid chromatography mass spectrometry atmospheric pressure chemical ionisation (APCI) without derivatisation after a simple extraction procedure. The adsorption properties of the degradation product compared to the mother compound is determined expressed as $K_d$ values produced in accordance with the OECD Test Guideline 106[9]. The fate of fenpropimorph in soil and the occurrence of the degradation product fenpropimorphic acid is studied. The investigation has been part of a larger project which has focused on pesticides effects on agricultural soil ecosystems (PEASE), where other parts have been reported elsewhere[10,11].

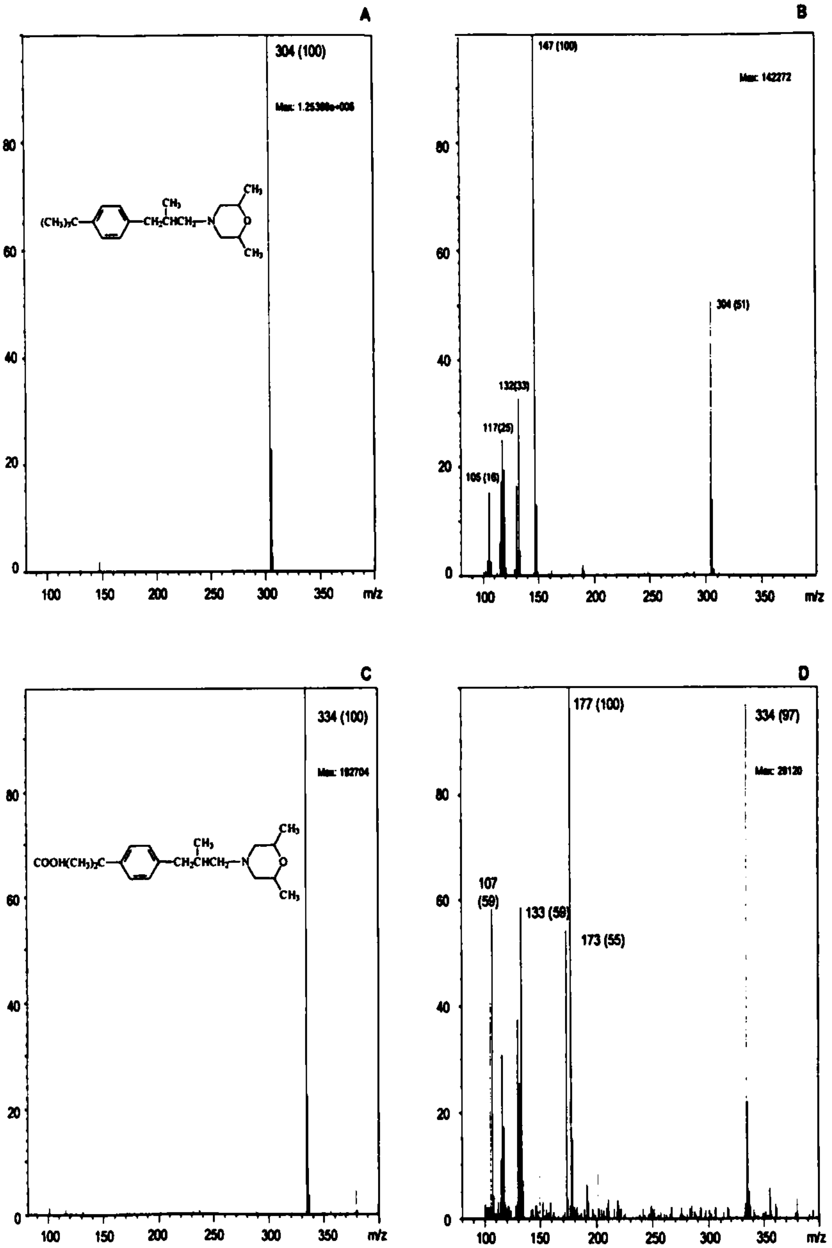

FIGURE 1 Chemical structure of fenpropimorph A), and fenpropimorphic acid C). A) and B) display mass spectre of fenpropimorph at fragmentation voltages of 100 and 200 V respectively. C) and D) display mass spectre of fenpropimorphic acid at the same voltages

## EXPERIMENTAL

### Soil type

The soil used for the experiments was sampled at the Royal Veterinary and Agricultural University experimental fields in Høje Taastrup, Denmark and is characterised as a sandy loam with coarse sand 23.7%, fine sand 26.2%, coarse silt 12.8%, fine silt 19%, clay 16% and organic matter 2.3% (dry weight).

### Chemical analysis

#### *Solvents and pesticide substances*

Methanol was gradient grade from Merck (Darmstadt, Germany). Fenpropimorph was Pestanal quality from Riedel de Haën (Seelze, Germany), while fenpropimorphic acid was kindly supplied by Novartis. High purity water for the analysis was obtained in-house with a MilliQ system (Millipore Corporation, Bedford, MA, USA). Ammonium acetate was obtained from Merck (Darmstadt, Germany).

#### *Apparatus*

Soil extraction was performed on a Comfort Heto MasterMix type MMV 15 (Heto Holten, Allerød, Denmark). Centrifugation was performed on a Sorwall RT7 centrifuge (Newton, USA).

The analyses were performed with a Hewlett Packard LC-MSD 1100 system. Column for chromatographic separation was Hypersil (Cheshire, England) BDS 250 × 2.1 mm, C18 5 μm.

#### *Sample preparation for determination of the occurrence of the compounds in soil*

A 5 g soil sample was weighed into a Falcon 50 ml centrifuge tube (Becton Dickinson Labware, NJ. USA). 5 ml methanol and 0.5 ml 6 M hydrochloric acid was added for neutralisation of the fenpropimorphic carboxylic anion and the tube was ultrasonicated for 10 min. The soil was extracted for 4 hours with 25 revolutions/min on the Heto MasterMixer. The mixture was centrifuged at 4200 rpm for 10 min. 0.9 ml water was added to 1 ml of the supernatant for better peak performance and analysed on the LC-MS.

### *HPLC method*

The chromatography was performed with gradient elution at a flowrate of 0.5 ml/min at 40°C in a column oven. A-eluent was 10 mM ammoniumacetate:methanol (99:1). B-eluent was 10 mM ammoniumacetate:methanol (10:90). Eluent composition: 2 min. 50 % B, linear gradient in 6 min to 100 % B which was kept for 5 min. Back to 50 % B in 2 min. and 5 min. restabilisation with 50 % B, total runtime 20 min. Retention time was about 8 min. for fenpropimorphic acid and 13 min for fenpropimorph.

### *Mass spectrometric detection*

Detection was performed after atmospheric pressure chemical ionisation (APCI) with a MSD 1100 in positive selected ion monitoring mode (SIM). Capillary voltage was 2500 V, drying gas temperature 350°C, vaporizer temperature 425°C, drying gas flow 2.0 L/min, nebulizer pressure 20 psig. Fenpropimorphic acid was detected at *m/z* 334, Fenpropimorph at *m/z* 304, which were the mass to charge ratios for the protonated molecules. Dwell time for each ion was 460 msec.

The ionisation in positive and negative mode as well with atmospheric pressure chemical ionisation as with electrospray ionisation was investigated.

The influencence of fragmentor voltage on fragmentation of the molecules was investigated in a range from 40 volt to 240 volt. The fragmentor voltage determines the velocity of the ions in the front of the mass spectrometer and gives the energy in collisions between molecules.

## Adsorption – $K_d$ determination

Determination of $K_d$ values followed the OECD guideline[9]. 10 glass centrifuge tubes, each with 5 g soil, were equilibrated with 20 ml 10 mM $CaCl_2$ in MilliQ filtrated water for 24 hours. 9 of the tubes were spiked with 5 ml 2.25 mg/L fenpropimorph, giving final concentrations of about 0.45 mg/L. The last tube was used as an unspiked blank sample. 3 tubes for control were spiked with 5 ml 2.25 mg/L and 20 ml 10 mM $CaCl_2$ in MilliQ filtrated water without soil. The extraction was performed on the Heto MasterMixer. After 4, 8 and 24 hours, 3 tubes were centrifuged and the supernatant was analysed on the LC-MS as well as the blank samples and control samples. The study was repeated with fenpropimorphic acid.

## Fate of fenpropimorph and fenpropimorphic acid in soil

### *Degradation in batch experiment*

A plastic container was filled with sieved soil (360 g dry weight) and spiked to a fenpropimorph concentration of 1.3 mg/kg dry weight and incubated at 10°C. The water content was 25 % after spiking. 50 g soil samples were taken after 0, 3, 6, 9,17, 30 and 56 days and chemical analysis were performed in duplicate. The experiment was part of a study on effects of fenpropimorph on bacteria and fungi, which is described elsewhere[10].

### *Degradation in field experiment*

In May 1997 three field plots grown with winter wheat were sprayed with Corbel in dosages corresponding to normal application rate (750 g fenpropimorph per hectare) and 10 times normal application rate. The third field plot was not treated with fenpropimorph, and was used as reference. Soil samples to 5 cm depth were taken 0, 1, 7, 19, 49 and 111 days after application. The experiment which primarily focused on the effects on protozoa in the soil is further described by Bjørnlund et al.[11].

## RESULTS AND DISCUSSION

## Chemical analysis

Figure 2 displays a chromatogram of a prepared soil sample spiked with fenpropimorph and fenpropmorphic acid to a concentration level in the soil about 800 μg/kg, which will be the order of magnitude of the pesticide concentration in the uppermost five cm of the soil, if all the applied pesticide was mixed with the soil. As it appears in the chromatogram, the soil showed no interferences and both compounds performed with sharp symmetrical peaks.

Recovery was 80 % for fenpropimorphic acid as well as for fenpropimorph. Method Detection Limits (MDL) were calculated as three times the standard deviation on seven samples, spiked to a concentration level 5 times above the expected detection limit as described in Glaser et al.[12]. The MDL determination was performed at a soil concentration level about 2 μg/kg. The determination resulted in calculated method detection limits below 1 μg/kg for both substances which are three orders of magnitude below the application rate. It would be possible to improve the limit of detection by injection of larger volumes (in this study 10 μL) or by evaporation of the methanol fraction and redissolution in a smaller volume.

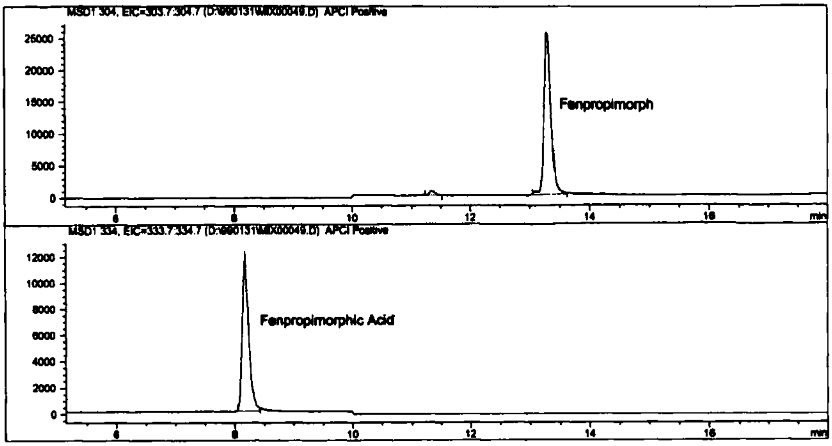

FIGURE 2 SIM chromatograms of fenpropimorph and fenpropimorphic acid in soil spiked to a concentration about 800 μg/kg

Linearity was studied in a concentration range from 5 to 2000 μg/L. Fenpropimorph fitted very well to a linear curve while fenpropimorphic acid gave the best fit with a quadratic curve.

## Mass spectrometric detection

With detection in APCI positive mode, the response factor for fenpropimorph is 2–3 times higher than for the acid. The signals were insignificant in negative mode as well as with the electrospray positive and negative modes, which are weaker ionisation techniques.

The influence of fragmentor voltage on signal and fragmentation was determined for both compounds. A fragmentor voltage about 100 to 120 V gave the highest signal for fenpropimorph while the optimum voltage was 20 V higher for the acid. At higher voltages decreased the signal and at 240 V the total ion current (TIC) was below one tenth of the TIC at optimum fragmentation voltage. Fig. 1 displays mass spectra for fenpropimorph and fenpropimorphic acid at 100 and 200 V. At 100 V the protonated molecular ions are totally dominant. At 200 V the molecules are fragmented into a number of ions. This means that quantification with highest sensitivity is possible at 100 V while 200 V might be chosen when qualifier ions are needed for identification and verification.

## Determination of adsorption to soil, $K_d$-values

Table I gives the results from the determination of $K_d$ values. The $K_d$ values were determined after 4, 8 and 24 hours extraction time. 4 hours extraction gave $K_d$-values close to the values determined after 24 hours, so equilibrium was reached within a few hours.

TABLE I Determination of $K_d$ values. Calculations after 4, 8 and 24 hours extraction

| *Extraction time (hours)* | *Kd, fenpropimorph (L/kg)* | *Kd, fenpropimorhic acid (L/kg)* |
|---|---|---|
| 4 | 134 ± 4[a] | 1.1 ± 0.1 |
| 8 | 148 ± 2 | 1.31 ± 0.02 |
| 24 | 161 ± 3[a] | 1.29 ± 0.03 |

The table gives the calculated $K_d$ values ± standard deviations based on triplicates after 3, 8 and 24 hours extraction time.

a. duplicates – one outlier.

The adsorption properties for fenpropimorph are so pronounced with a $K_d$ value above one hundred so the mobility in the investigated soil would be expected to be negligible. The mobility of the degradation product seems to be much more important with a $K_d$ value close to one. The $K_d$ determination for fenpropimorphic acid was repeated after the first determination with respect to the OECD guideline[9] which prescribes a second determination if the adsorption to soil is below 20 %. In the second run, the extraction volume was reduced from 25 to 10 ml and the $K_d$ value could be determined as it appears in Table I.

## Degradation in batch system

Figure 3 displays the degradation of fenpropimorph during 56 days after spike of the soil. All the determinations were made in duplicate. The figure displays the content of fenpropimorph and fenpropimorphic acid. An exponential trend line for the degradation of fenpropimorph is inserted in the figure.

The time for 50% disappearance of fenpropimorph is about 30 days in this experiment. After 17 days the occurrence of fenpropimorphic acid has reached a level about 120 µg/kg which is revealed in the rest of the study. This is about one tenth of the applied dosage. With respect to the low Kd value of the degradation product, occurrence during such a long time might give rise to leaching in a real situation with heavy rainfall. This study is further described in Thirup et al.[10].

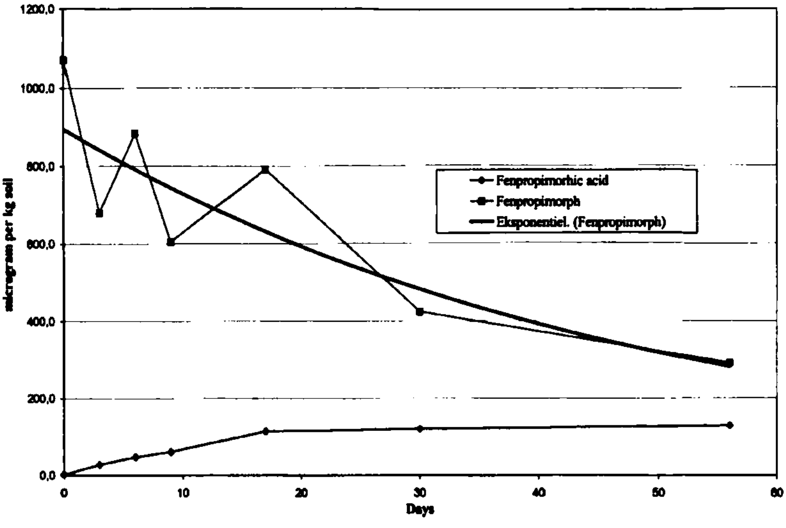

FIGURE 3 Degradation of fenpropimorph and occurrence of the degradation product in soil in the batch experiment

## Degradation of fenpropimorph in the field experiment

The chemical analysis of soil samples from the untreated field plot displayed no content of fenpropimorph and fenpropimorphic acid as expected.

Figure 4 A) displays the degradation of fenpropimorph in the field plot treated with fenpropimorph at normal application rate. The treated amount of the pesticide was recovered in the soil on the day of application which was not a surprise, because the application was followed by heavy rainfall which washed the pesticide from the leaves into the soil. After 7 days, more than 60 % of the treated amount was degraded. The maximum concentration of fenpropimorphic acid was detected after 19 days, with a concentration about 30 µg/kg soil. The degradation in the field experiment seems to go much faster than in the batch experiment. Referring to the high $K_d$ value, fenpropimorph is expected to be adsorbed in the uppermost soil surface with the highest microbiological activity. This might explain the faster degradation rate in the field experiment than in the batch experiment, where the pesticide was mixed with the soil sample. The experiment with ten times higher dosage displayed the same degradation profile where more than 50 % was degraded after 7 days, and the highest concentration of fenpropimorphic acid appeared after 19 days in a concentration about 200 µg/kg soil. This study is further described in Bjørnlund et al.[11].

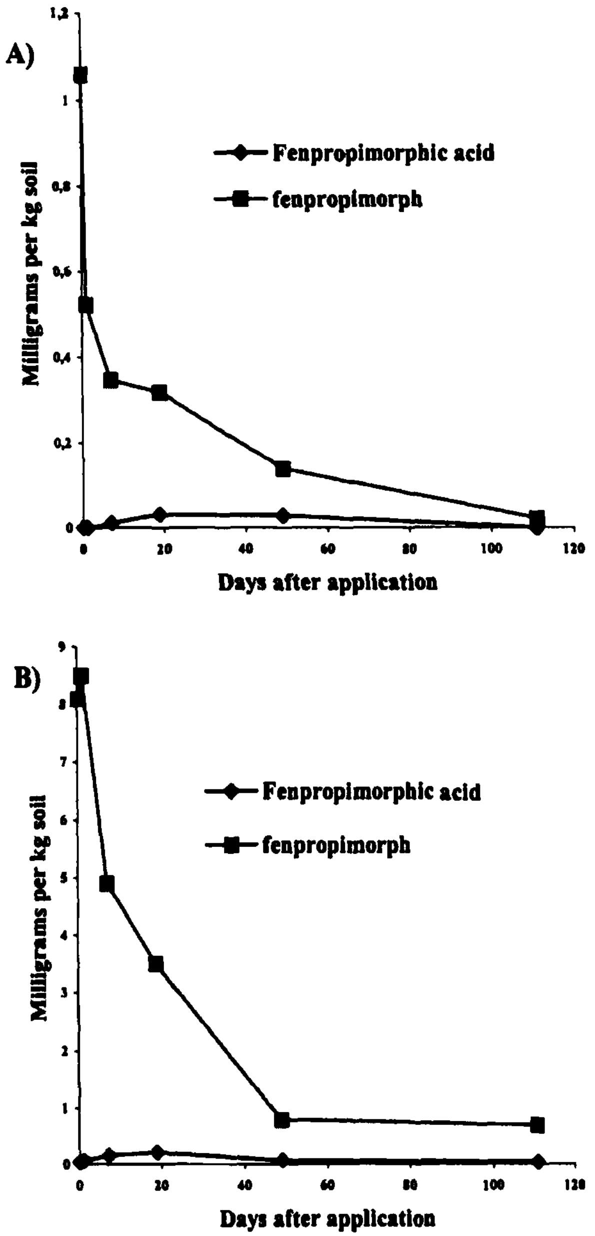

FIGURE 4 A). Degradation of fenpropimorph and appearance of fenpropimorphic acid in soil in a field experiment with normal application rate (750g fenpropimorph per hectare); B). Degradation of fenpropimorph and appearance of fenpropimorphic acid in soil in a field experiment with 10 times normal application rate (7.5 kg fenpropimorph per hectare)

## CONCLUSION

A specific, sensitive and simple method for determination of the fungicide fenpropimorph and its degradation product fenpropimorphic acid was developed, based on extraction of a 5 g soil sample with acidic methanol. The method displayed detection limits for both compounds below 1 µg/kg soil. The analysis was performed with liquid chromatography and mass spectrometric detection after atmospheric pressure chemical ionisation in positive mode. Negative mode and electrospray positive and negative modes gave insignificant signals. Quasi-molecular ions were formed and the highest sensitivity achieved for quantification with a fragmentor voltage at 100 to 140 V while the parent compounds were broken down to a number of fragment ions at 200 V. These fragment ions are useful as qualifiers for identification purposes.

The adsorption properties were determined expressed as $K_d$ values for fenpropimorph and the -acid in a sandy loam. The $K_d$ value for fenpropimorph was determined to 161 L/kg after 24 hours extraction time and the mobility of the compound in the soil is expected to be negligible, while the determination of the $K_d$ value for the degradation product resulted in a value about 1.3 L/kg after 24 hours extraction time. The low $K_d$ value of the degradation product combined with appearance in the soil during months after application of the parent compound might give risk of leaching which has to be further investigated in mobility studies and in monitoring programmes.

### *Acknowledgements*

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