Problems in Analyzing Trinexapac-ethyl—a New Plant Growth Regulator

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A method for the determination of trinexapac-ethyl had proposed the use of HPLC with three-column switching. An unproved method, taking advantage of SPE cleanup and GC/MS for analyses, is described. Analyses of wheat and rape were used as experiments to evaluate the method. Rapid decomposition of trinexapac-ethyl was stated and must be confirmed. Therefore, spiked extract had to be used to determine the detection limit of the GC/MS method.

Keywords: *Plant growth regulator; trinexapac-ethyl; GC/MS*

INTRODUCTION

Trinexapac-ethyl (Figure 1) is a new plant growth regulator and, under the common name Moddus, licensed in Germany since 1995. It was successfully tested on various crops and plants (Amrein et al., 1989; Adams et al., 1992; Johnson, 1993). Not only the wide application range of trinexapac-ethyl with beneficial effects but also its rapid decomposition seem to be advantageous. Due to an agricultural government program in Saxonia (Germany), there is a restriction for **all** plant growth regulators in connection with the so-called "environmental agriculture". Therefore, the monitoring program demands the analysis of this plant growth regulator. A method, introduced by Ciba-Geigy, includes the extraction with acetonitrile/aqueous phosphate buffer, the reextraction into dichloromethane, an additional cleanup, and the final determination using a three-column HPLC switching system with UV detection (Ciba-Geigy, 1991). The amount of solvents, the required substitution of chlorinated solvents, and background problems in the chromatograms led to the development of the GC/MS method, described below. The use of 3 mL solid phase extraction (SPE) columns reduces the amount of solvents significantly. The advantages of GC/MS include the opportunity to ensure the results and to handle the high background in plant samples by monitoring the appropriate mass numbers only.

MATERIALS AND METHODS

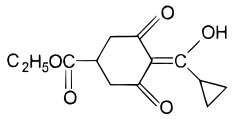
Reagents. A sample of Moddus with 25% trinexapac-ethyl was kindly provided by Ciba-Geigy, Frankfurt, Germany.

All solvents were obtained from Baker Gross-Gerau, Germany, and are used for organic residue analysis only. The cleanup SPE columns, filled with SDB 2 (a polystyrene adsorption resin), were also purchased from Baker. Phosphoric acid, Na_2HPO_4, and KH_2PO_4 were obtained from Fluka.

Field Experiments. As recommended by the producer, 0.4 L of Moddus/ha was applied to wheat. In each case four samples had been taken after 24, 48, and 72 h. (Unfortunately, there was no sample taken after 1 h.) Samples were stored at −22 °C until preparation.

The second experiment was carried out with rape on four microplots of 1 m^2. Again, 0.4 L of Moddus/ha was applied to rape and samples were collected (instantly and after 1, 3, 5, and 24 h). Processing of the samples carried out without delay.

Determination Methods. Twenty-five grams of sample was homogenized and extracted with 100 mL of methanol using a Büchi mixer B-400, (Büchi, Switzerland) for 5 min and filtered. Fifty milliliters of the extract was evaporated to a small residue and acidified with 1 mL of phosphoric acid (0.1 M). The 3 mL SPE columns, filled with 200 mg of SDB 2 resin, were conditioned with 2 × 3 mL tetrahydrofuran (THF) while keeping the sorbent bed wet. The acidified sample was transferred onto the prepared column and was allowed to pass through the resin with a flow rate of less than 5 mL/min. After washing with 3 mL of deionized water, 3 × 1 mL of THF was used to elute the retained analyte from the sorbent. No further treatment was carried out to avoid losses during evaporation. The eluate was injected directly into the GC/MS.



$C_{13}H_{16}O_5$ M= 252.3 g/mol

Figure 1. Structure of trinexapac-ethyl (other names are cimectacarb and CGA 163935).

The GC/MS analysis was performed on a QP 5000 from Shimadzu with AOC-1400 and SPL-17 injector: column, DB-5ms (30 m × 0.25 mm × 0.25 mm); injector/interface temperature, 250 °C; temperature program, 80 °C (2 min)−10 °C/min−280 °C (2 min); carrier gas, helium; pressure program, 4.9 kPa (2 min)−1 kPa/min−25 kPa (2 min); gain, 1.4 kV; SIM acquisition time, 17.96−19.6; selected ions *m/z*, 151.1/207/224.

RESULTS AND DISCUSSION

Standards. Several experiments with the standard mixture were carried out to optimize the cleanup procedure and the GC/MS conditions. The diluted standard solution was stable for up to 1 week at 4 °C in the dark. The trinexapac-ethyl concentration was degraded down to 50% under direct sunlight within 3 days. To determine method accuracy, recovery and reproducibility were tested by adding known amounts (0.5, 1.0, and 2.0 mg) of Moddus to untreated crop extracts. Trinexapac-ethyl recoveries were 98−101% (coefficient of variation 0.6−2.2%). In the described selected ion monitoring mode (SIM), concentrations below 1 pg (absolute) could be analyzed.

Real Samples. Only the four samples that had been taken 24 h after application showed a detectable concentration between 1 and 10 pg of trinexapac-ethyl (<2 mg/kg of crop).

To get more results during the very first period after application, a second experiment on microplots was carried out. Results are shown in Table 1. A rapid,

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Table 1. Degradation of Trinexapac-ethyl

h after applifcation	trinexapac-ethyl (mg/kg of crop)				
	M1	M2	M3	M4	av
0	11.6	13.2	12.0	13.2	12.5
1	9.3	11.2	10.3	12.3	10.8
3	9.3	11.7	11.5	12.7	11.3
5	7.1	7.9	8.1	8.7	7.9
24	3.2	1.3	<0.1	3.0	1.9
48	<0.1	<0.1	<0.1	<0.1	<0.1

significant decomposition occurred between 4 and 24 h after application. Therefore, there was no need for further field experiments and validation of the assay method.

Conclusions. The pure agent trinexapac-ethyl is very reliably and sensitively detectable by GC/MS after SPE cleanup. The use of adsorption resins simplifies the cleanup procedure enormously. However, this is only of limited use for monitoring purposes. With the help of the GC/MS method and samples gained in two field experiments, the rapid decomposition of trinexapac-ethyl after 1 day of the agent's application could be proved. There will not be a possibility to monitor the use of trinexapac-ethyl as a plant growth regulator after a certain period of time with chromatographic methods.

LITERATURE CITED

Adams, R.; Kerber, E.; Pfister, K.; Weiler, E. W. Studies on the action of the new growth retardant CGA 163935 (cimectacarb). *Curr. Plant Sci. Biotechnol. Agric.* **1992**, *13*, 818−827.

Amrein, J.; Rufener, J.; Quadrant, M. The use of CGA 163935 as a growth regulator in cereals and oilseed rape. *Brighton Crop Prot. Conf. Weeds* **1989**, (1), 89−94.

Ciba-Geigy. Agricultural Division; Liquid chromatographic determination of residues of parent compound, CGA 163935, 1991.

Johnson, B. J. Influence of plant growth regulators and mowing on two bermuda-grasses. *Agron. J.* **1993**, *85* (2), 276−280.

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