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Determination of Trinexapac in Wheat by Liquid Chromatography−Electrospray Ionization Tandem Mass Spectrometry

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A quantitative and confirmatory method for the analysis of trinexapac (free acid metabolite of trinexapac-ethyl) in wheat is described. Residues were extracted from wheat with acetonitrile in aqueous phosphate buffer (pH 7) overnight. The extract was directly injected into the HPLC system. Chromatographic separation was achieved on an octadecylsilica column, and detection was performed by negative ion electrospray ionization tandem mass spectrometry. The precursor ion of trinexapac $[M - H]^-$ at *m*/*z* 223 was subjected to collisional fragmentation with argon to yield two intense diagnostic product ions at *m*/*z* 135 and 179, respectively. Accuracy and specificity for routine analysis of trinexapac were demonstrated. The validated concentration range was 10−200 μg/kg based on a 0.10 g/mL wheat sample extract. Recoveries were within the range of 71−94%, with associated relative standard deviations better than 10%. The limit of detection for trinexapac in wheat was estimated at 5 μg/kg. The method has been applied to a survey of 100 samples of wheat. In 46% of the samples analyzed, a quantifiable amount of trinexapac was detected, ranging from 10 to 110 μg/kg. It has been demonstrated that analyses of trinexapac accurately reflect the total amount of residues of the plant growth regulator, trinexapac-ethyl, in the wheat samples following field application. No residues of the parent compound, trinexapac-ethyl, in wheat were detected.

KEYWORDS: Trinexapac-ethyl; wheat; electrospray tandem mass spectrometry

INTRODUCTION

Besides chlormequat, novel plant growth regulators (PGR), such as trinexapac-ethyl and prohexadione-calcium, have been recently introduced. Trinexapac-ethyl, ethyl 4-cyclopropyl-(hydroxy)methylene-3,5-dioxocyclohexane carboxylate, is a PGR first reported by Kerber (*1*) and introduced by Ciba-Geigy (now Syngenta Crop Protection) under the tradenames of Moddus and Primo. Trinexapac-ethyl is an acylcyclohexadione PGR that inhibits the biosynthesis of gibberellin (GA_1). Gibberellin is a phytohormone that promotes the growth of various plant organs. The free acid form of trinexapac-ethyl, 4-cyclopropyl(hydroxy)methylene-3,5-dioxocyclohexanecarboxylic acid, inhibits the hydroxylation of GA_{20} to GA_1 by competitively inhibiting the regulatory enzyme 3-β-hydroxylase, leading to a reduction in the size of leaves and stems (*2*). Also, trinexapac-ethyl inhibits gibberellin production much later in the biosynthetic pathway than quaternary ammonium compounds (e.g., chlormequat). The PGR is used for the prevention of lodging, to increase yields of cereals, to reduce mowing of turf, and as a maturation promotor in sugar cane.

Trinexapac-ethyl is rapidly metabolized, yielding the corresponding free acid metabolite, which is assumed to possess biological activity. Metabolism of trinexapac-ethyl to its free acid form in wheat, soil, and the environment has been well documented for the registration of the PGR (*3*, *4*). Problems in analyzing the parent compound, trinexapac-ethyl, using gas chromatography−mass spectrometry (GC-MS) in selected ion monitoring mode (SIM) were reported by Syhre et al. (*5*). The authors concluded that standard solutions of trinexapac-ethyl degraded under direct sunlight within 3 days and that rapid decomposition of trinexapac-ethyl occurred after 1 day of the agent's application on wheat, as determined by two field experiments. Within 48 h after application, trinexapac-ethyl is almost completely converted to its free acid form, which remains by far the predominant metabolite, and residues of the parent compound could not be detected in the wheat samples.

An analytical method for the determination of trinexapac was developed by Ciba-Geigy (*6*). The method includes extraction with acetonitrile−aqueous phosphate buffer, cleanup with C_{18} and strong anion-exchange solid-phase extraction material, partitioning into dichloromethane, and determination with single-column HPLC using UV detection. The limit of quantification of trinexapac in wheat was 50 μg/kg. The scope of the method

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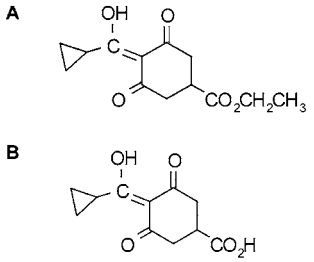

Figure 1. Structures of trinexapac-ethyl (A) and its free acid metabolite, trinexapac (B).

was expanded for animal produce (tissue, milk, and eggs) using a column-switching HPLC system (*7*).

Over the past few years, liquid chromatography coupled with atmospheric pressure ionization interfaces to mass spectrometers (LC-API-MS) has gained in popularity for the analysis of pesticides. This promising technique combines the advantages of LC and MS for the separation and unequivocal identification of pesticides at low micrograms per kilogram levels in real matrices, such as food and environmental samples. Tandem mass spectrometry (MS-MS) for detection greatly reduces the need for excessive cleanup procedures and provides little chance of false positive findings, resulting in reduced analysis time and costs.

Due to the low doses used for typical application of trinexapac-ethyl, a highly sensitive method is required to detect residues of trinexapac in wheat. In this study, an LC-MS-MS method for the determination of trinexapac in wheat was developed and validated. The Dutch Pesticide Law has established a maximum residue limit (MRL) of 0.2 mg/kg trinexapac-ethyl, expressed as its free acid metabolite, for wheat, thereby emphasizing the need for its inclusion in enforcement programs. In view of the limited information available for trinexapac-ethyl in wheat for human consumption, the Inspectorate initiated an investigation as part of the Dutch National Pesticide Monitoring Program.

MATERIALS AND METHODS

Materials. HPLC grade acetonitrile, formic acid of analytical reagent grade, and ready-to-use aqueous phosphate buffer (pH 7) solution were purchased from Merck (Darmstadt, Germany). HPLC grade water was obtained by purifying demineralized water in a Milli-Q Plus ultrapure water system (Millipore SA, Molsheim, France). Reference pesticide standards of trinexapac-ethyl (purity = 98.3%) and trinexapac (purity = 99%) were gifts from Novartis Crop Protection (Münchwilen, Switzerland) and stored in a freezer at −18 °C. The structures of trinexapac-ethyl and trinexapac are shown in **Figure 1**. Organically grown wheat was used as blank control matrix in recovery experiments and for the preparation of matrix-matched calibration solutions. The organically grown wheat was previously determined to be free of residues of the PGR.

Sample Collection and Preparation. Wheat samples were randomly collected at wholesalers, granaries, and cereal-processing facilities as part of the Dutch National Pesticide Monitoring Program during the year 2001. Samples with a minimum weight of 1 kg were collected for the survey. For analysis, a laboratory subsample (100 g) was milled in a laboratory rotor mill (Retsch, Haan, Germany) to a typical particle size of 0.25 mm. Organically grown wheat and a QC wheat sample with a field-incurred residue of trinexapac (65 μg/kg) were treated in the same way.

Preparation of Matrix-Matched Calibration Solutions and Recovery Samples. Stock solutions of trinexapac-ethyl and trinexapac, both of 1 mg/mL, were prepared in acetonitrile. Separate intermediate standard solutions of 1 μg/mL were prepared by dilution in acetonitrile. For trinexapac, serial dilutions of the intermediate standard solution were performed to give five working solutions over the range of 10−200 ng/mL in 25% v/v acetonitrile−aqueous phosphate buffer (pH 7). All solutions were determined to be stable for up to 3 months at 4 °C in the dark.

For each injection sequence in the survey, a QC sample, a blank wheat extract from organically grown wheat, and matrix-matched calibration solutions of trinexapac over the range of 1.0−20 ng/mL, with a spiking volume of 1 mL working solution into 9 mL of blank wheat extract, were prepared. This working range is equivalent to residue concentrations between 10 and 200 μg of trinexapac/kg in wheat.

For validation experiments of trinexapac, recovery samples were prepared at 10, 20, and 200 μg/kg concentrations by adding 50, 100, and 1000 μL of intermediate standard solution (50, 100, and 1000 ng) trinexapac to a portion of 5 g of organically grown wheat. Recovery samples were allowed to stand under normal conditions (20 °C, light) for 1 h prior to extraction.

Extraction Procedure. A 5 g ground test portion was shaken with 50 mL of 25% v/v acetonitrile−aqueous phosphate buffer using an orbital laboratory shaker (GFL 3017, Gesellschaft für Labortech) for 1 h. The suspended material was allowed to settle at ambient temperature overnight. Subsequently, an aliquot of the supernatant was filtered through a 0.45 μm Spartan 30 RC membrane filter (Schleicher and Schuell, Dassel, Germany) into an autosampler vial.

Liquid Chromatography—Mass Spectrometry. The chromatographic separation was performed using a Waters Alliance separations module model 2695 (Waters Corp., Milford, MA) equipped with a quaternary solvent delivery system, autosampler, and column heater. Chromatographic separations were performed using an Inertsil ODS 80A (150 × 3.0 mm i.d., 5 μm) analytical column, coupled with an Inertsil ODS (7.5 × 3.0 mm i.d., 5 μm) guard column (GL Sciences Inc., Tokyo, Japan). Isocratic elution was performed with 25% v/v acetonitrile−0.1% formic acid (pH 2.7). The flow rate was set at 0.3 mL/min, and the temperature of the column heater was maintained at 30 °C. The analysis time was 10 min. The injection volume was 20 μL and, to avoid carry-over, the autosampler was flushed between runs with mobile phase composition. A divert valve was placed between the LC column outlet and the mass spectrometer inlet, and the flow was diverted to waste during the first 6 min of the analytical run.

API-MS detection was achieved using the Quattro Ultima tandem mass spectrometer, fitted with a Z-spray electrospray interface (Micromass, Manchester, U.K.). The instrument was operated in negative ion electrospray (ESI−) mode with two MS-MS transitions monitored for the precursor ion using multiple reaction monitoring (MRM). Direct infusion of trinexapac for optimization procedures was achieved using a syringe pump (Harvard Apparatus, Kent, U.K.). Typical source conditions were as follows: capillary voltage, 3.0 kV; sample cone voltage, 50 V; source temperature, 100 °C; and drying gas temperature, 350 °C. Nebulizing gas and drying gas (nitrogen) flow rates were 100 and 600 L/h, respectively.

The MRM experiments were conducted with a dwell time and intrachannel delay of 0.05 s, a span (window) of 0 Da, and an acquisition time of 10 min. Ions were collisionally fragmented with argon at 1.5×10^{-3} mbar, with a collision energy of 15 eV. Two MRM transistions of m/z 223 → 135 and 223 → 179 were monitored for trinexapac.

Quantification and Confirmation. Quantitation data for trinexapac were obtained using the MRM transition m/z 223 → 135. Quantification was based on peak area measurements using QuanLynx version 3.5 software (Micromass). Data were smoothed with two passes of a moving average smoothing algorithm with a three-point window. For regulatory application of the method, matrix-matched calibration solutions at five concentrations were injected prior to analysis of the wheat samples and again after 10 sample injections. Calibration coefficients were calculated using data of the calibration analysis, bracketing each group of samples. The calibration curves (peak area versus concentration) were fitted to a first-order linear model with intercept and $1/x$ weighting. Confirmation of trinexapac was achieved by monitoring the MRM transition m/z 223 → 179.

Qualitative Analysis of Trinexapac-ethyl. For detection of the parent compound trinexapac-ethyl in wheat, a second qualitative method was applied. Gradient elution was performed by linearly increasing

the percentage of acetonitrile from 25 to 75% v/v in 0.1% formic acid in 15 min. The mass spectrometer was operated in time-scheduled electrospray ionization negative/positive (ESI−/+) mode with up to four MS-MS transitions monitored using MRM. During gradient elution, the electrospray interface was switched from negative to positive mode after 10 min of run time. During the first 10 min, the electrospray interface was set in the negative mode for monitoring two MRM transitions (*m*/*z* 223 → 135 and *m*/*z* 223 → 179) of trinexapac. Subsequently, the interface was set in the positive mode for monitoring two MRM transitions (*m*/*z* 253 → 69 and *m*/*z* 253 → 207) of the parent compound, trinexapac-ethyl. Experimental tuning parameters and other LC conditions were kept as described for the determination of trinexapac.

RESULTS AND DISCUSSION

Liquid Chromatography—Mass Spectrometry. In this study, using ESI−, inlet conditions were optimized to achieve maximum formation (sensitivity) for the deprotonized molecular ion $[M - H]^-$ of trinexapac. Trinexapac gave a deprotonated parent ion $[M - H]^-$ at *m*/*z* 223. Fragmentation of the parent ion using collision-induced dissociation (CID) with argon, yielded two intense product ions at *m*/*z* 135 and 179, corresponding to loss of carbon dioxide $[M - H - CO_2]^-$ for *m*/*z* 179. The product ion with *m*/*z* 135 is probably the result of a ring breakage and a neutral loss of propane $[M - H - CO_2 - C_3H_8]^-$ or loss of a second carbon dioxide moiety $[M - H - 2CO_2]^-$. The complete product ion mass spectrum was investigated by infusing trinexapac in running mobile phase solution at cone voltages between 20 and 120 V. Maximum intensity of the product ions was achieved with a cone voltage of 50 V. The use of CID by varying the collision energy influences both the sensitivity and fragmentation. The collision energy was adjusted to obtain the best compromise between fragmentation for identification purposes (confirmation) and sensitivity. The collision energy was set at 15 eV, giving almost identical responses for *m*/*z* 223 → 135 and *m*/*z* 223 → 179. Conditions for interfacing the LC system to the MS were also evaluated. Modifications of nebulizer gas flow rate, drying gas flow rate, and source temperature did not drastically improve the sensitivity. MRM of the two transitions allowed selective detection of trinexapac, showing no detectable interferences in wheat samples with the analyte MRM signals. **Figure 2** shows representative ion chromatograms of the MRM transitions *m*/*z* 223 → 135 and *m*/*z* 223 → 179 of blankk and spiked organically grown wheat extract at the limit of quantification (10 μg/kg) under optimum MS-MS conditions, demonstrating the sensitivity and specificity of the method.

Because no cleanup was applied, the aqueous acetonitrile extracts of wheat contained substantial concentrations of coextractives and phosphate buffer. With the Z-spray interface fitted to the Quattro Ultima, a total of not more than 15 wheat extracts, including matrix-matched calibration solutions, and QC samples could be analyzed before loss of sensitivity necessitated ion source cleaning. Washing of the sample cone with a water−methanol mixture to remove deposits was normally sufficient to restore the sensitivity of the MS detector. Although the Z-spray configuration prolonged the operational time of the instrument considerably, the sensitivity was too important for the survey. Therefore, a switching valve controlled by the mass spectrometer was used to direct the LC flow to waste during the initial period of the analytical run and switched to the MS detector before the expected elution of the analyte peak. The switching valve eliminated the early eluting coextractives and the phosphate content of the extraction solvent from entering the ion source. This setup proved to be reliable and allowed

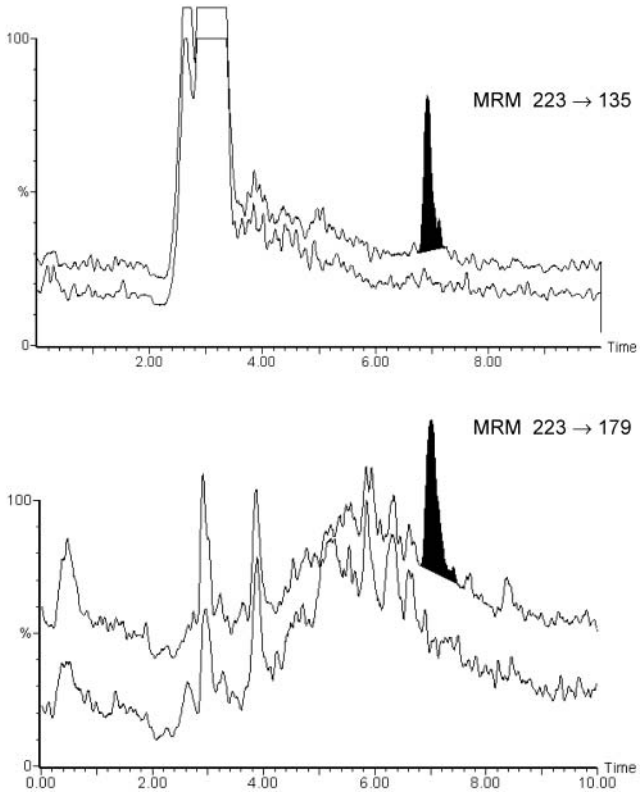

Figure 2. Chromatograms of a wheat sample, fortified with trinexapac at the limit of quantification (10 μg/kg). The ion chromatograms show the two MRM transitions *m*/*z* 223 → 135 and *m*/*z* 223 → 179, respectively. Switching of the divert valve was omitted, demonstrating the specificity of the analytical method.

continuous trouble-free operation overnight without loss of sensitivity, thereby improving method ruggedness.

Chromatographic separation of trinexapac ($pK_{a1} = 5.32$ and $pK_{a2} = 3.93$) was achieved on an Inertsil ODS 80A analytical column under isocratic elution with 25% v/v acetonitrile−0.1% formic acid (pH 2.7) in 10 min. Under these HPLC conditions, trinexapac eluted at 7.0 min. Lowering the concentration of formic acid in the mobile phase resulted in a badly tailing peak with less retention. Nowadays, good separation efficiency of acidic compounds is obtained using modern octadecylsilica columns with an acidic mobile phase. In LC-MS, postcolumn addition of a neutralization buffer (containing, e.g., an amine) is often recommended to form ions in solution and to facilitate charging of droplets. However, some studies (*8*, *9*) reported a decrease in detection sensitivity of acidic herbicides after postcolumn neutralization of the mobile phase. In our case, postcolumn addition of triethylamine did not provide significant improvement in sensitivity for the trinexapac product ions. Therefore, we have employed a simple setup with the LC column outlet directly interfacing the ESI-MS.

Extraction Efficiency and Method Performance. Extraction efficiency was investigated using a wheat sample field-incurred with trinexapac (65 μg/kg). Both trinexapac-ethyl and trinexapac are highly soluble in aqueous buffer solutions at elevated pH values. The solubilities (25 °C) of trinexapac-ethyl and trinexapac at a pH value of 6.8 are 20 and 200 g/L, respectively (*2*). Test portions of field-incurred wheat sample were extracted with 25% v/v acetonitrile−aqueous phosphate buffer (pH 7) using mechanical shaking, and maximum extraction efficiency was achieved after 1 h.

Matrix effects, due to ion suppression, are notoriously variable in occurrence and intensity. The best way to compensate for

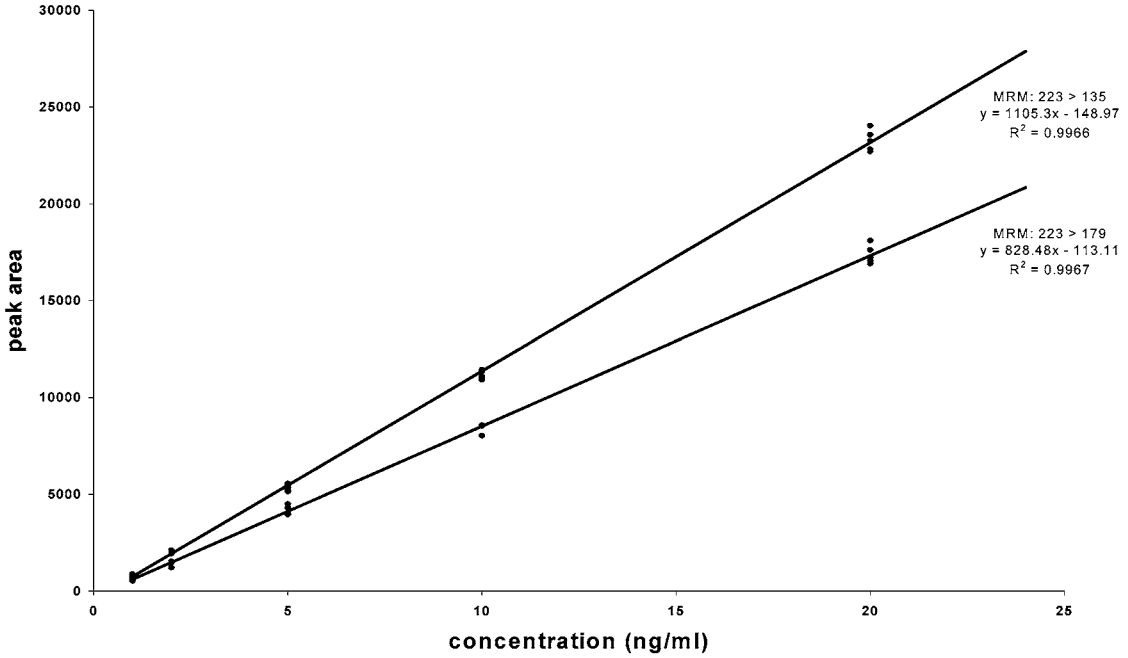

Figure 3. Calibration curves of trinexapac in organically grown wheat at two MRM transitions (*m*/*z* 223 → 135 and *m*/*z* 223 → 179) using matrix-matched calibration solutions. Each calibration level was injected five times.

ion suppression is the use of an isotopically labeled internal standard solution, added both to wheat samples and to calibration solutions. An isotopically labeled derivative of trinexapac is not commercially available. Several workers (*10*−*12*) have compensated for matrix effects by preparing calibration solutions in appropriate matrices, as recommended in EU guidelines (*13*).

Calibration curves for trinexapac were constructed for five different concentrations in the range of 1.0−20.0 ng/mL, both in organically grown wheat extracts and in extraction solvent (acetonitrile−aqueous phosphate buffer). Each calibration level was injected five times. For all calibration levels significant (∼20%) ion suppression in the matrix extracts was observed. The use of matrix-matched calibration solutions was necessary to compensate for signal suppression of the analyte in the matrix solution compared to the response in pure solvent, with that improving the accuracy of the method. Calibration curves for two MRM transitions (**Figure 3**), derived from ion chromatogram peak area measurements from matrix-matched calibration solutions, were obtained and displayed good linearity over the selected concentration range of 1.0−20 ng/mL with linear regression coefficients better than 0.995. Only 1 of 25 calibration standards had to be rejected due to a back-calculated response >20% from the true value.

Most wheat samples produced very low interfering peaks at the MRM transitions *m*/*z* 223 → 135 and *m*/*z* 223 → 179, as is evident in **Figure 2**. The latter transition, however, afforded the least sensitivity, and therefore *m*/*z* 223 → 135 was adopted as the primary quantification channel. On the basis of the S/N ratio of 3:1 using the MRM channel (*m*/*z* 223 → 179) providing the lowest signal response for trinexapac in wheat matrix, the calculated instrument detection limit is 0.5 ng/mL. This concentration corresponds with an estimated method limit of detection (LOD) in wheat of 5 μg/kg.

Method Validation. Recovery and precision of the analytical method were established to evaluate the method performance. The recovery of trinexapac was determined from the analysis of organically grown wheat spiked at three levels, 10, 20, and 200 μg/kg. The 10 and 200 μg/kg levels corresponded with the estimated limit of quantification (LOQ) and the maximum residue limit (MRL) for trinexapac-ethyl in wheat, respectively. The recoveries were calculated using matrix-matched calibration solutions at a concentration level corresponding with a recovery of 100%. **Table 1** shows detailed recovery data for trinexapac in wheat. Mean recoveries ($n = 6$) were >70% for all levels, with associated relative standard deviations (RSD) of <10%. The LOQ is defined as the lowest spiking level meeting the acceptability criteria of trueness between 70 and 110% and a precision, expressed as RSD, of <20%. At the lower spiking levels, the recoveries were within the range of 71−85%, which suggests some systematic error. On the basis of the acceptable trueness and precision demonstrated at the lowest spiking level, the 10 μg/kg level was determined to be the effective LOQ of trinexapac. The intraday precision (RSD_r, repeatability) was calculated from results of 10 replicate analyses of a QC wheat sample with field-incurred residue of trinexapac (65 μg/kg). The interday precision (RSD_R, reproducibility) was determined from analysis of the QC sample on five different days. The intraday and interday precisions were 5.9 and 13.4%, respectively.

Table 1. Overall Performance of the Analytical Method for Spiked and Field-Incurred Wheat Samples

	recovery of trinexapac			precision	
spike level (μg/kg)	range (%)	mean (%)	RSD (%), $n = 6$	intraday RSDr (%), $n = 10$	interday RSDR (%), $n = 5$
10	71–84	77.5	6.8		
20	72–85	77.5	6.4		
200	85–94	89.8	5.1		
65 (field-incurred)				5.9	13.4

In general, the validation data for trinexapac were in accordance with the EU guidelines for pesticide residue analysis (*13*, *14*), reflecting good method performance.

Qualitative Data of Trinexapac-ethyl. Using the optimized inlet source parameters for trinexapac in the ESI− mode, adequate analytical sensitivity could not be obtained to detect extremely low levels of trinexapac-ethyl in wheat. However, by switching to the ESI+ mode, trinexapac-ethyl gave an

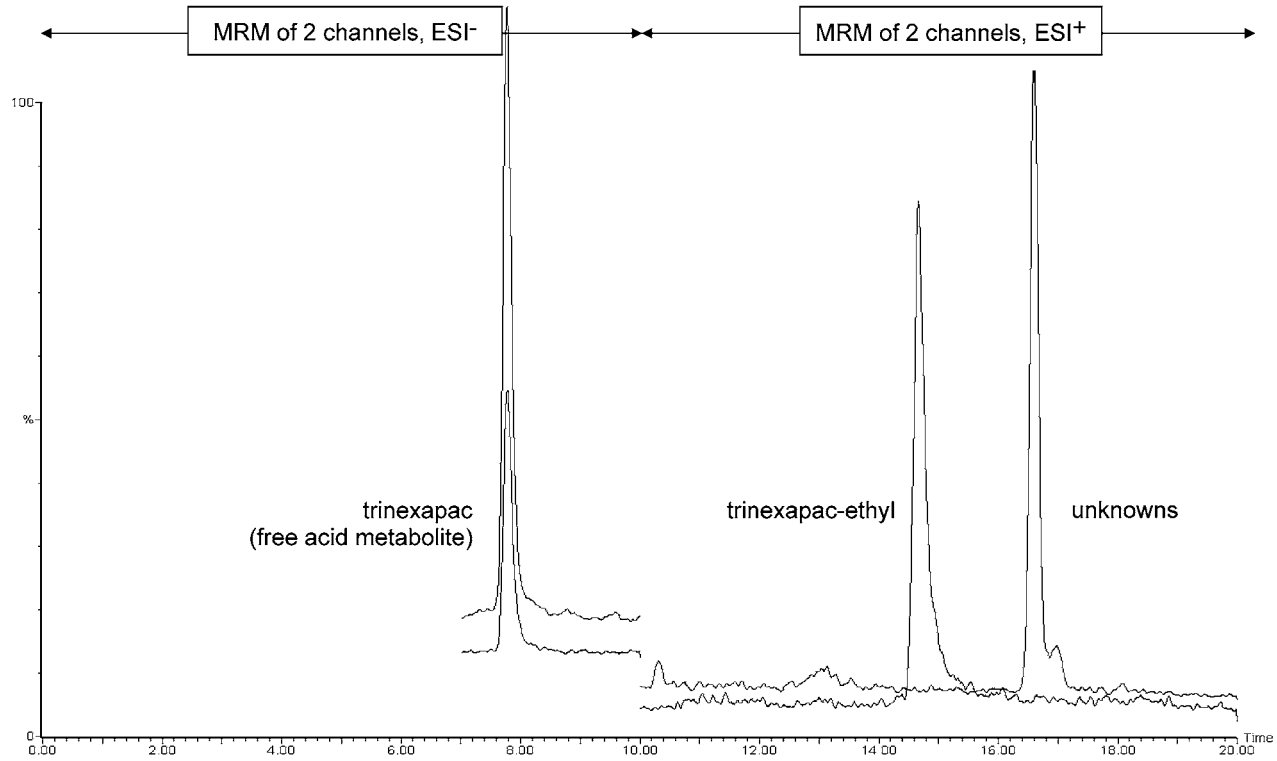

Figure 4. Normalized total ion chromatograms of a standard solution (bottom trace) containing trinexapac and trinexapac-ethyl (5 ng/mL) and of a wheat sample (top trace) with field-incurred residue of trinexapac (110 μg/kg) during gradient elution with time-scheduled ESI–/+ mode MS-MS, demonstrating the absence of the parent compound, trinexapac-ethyl.

abundant protonized parent ion $[M + H]^+$ at *m*/*z* 253. Fragmentation of this parent ion using CID with argon yielded two intense product ions at *m*/*z* 69 and 207. With these two MRM transistions monitored, the sensitivity was adequate to detect residues of trinexapac-ethyl at the low micrograms per kilogram levels. Time-scheduled ESI−/+ mode tandem mass spectrometry was used to detect residues of trinexapac-ethyl in wheat. Trinexapac was ionized in the negative mode and the ester in the positive mode. In the separation using gradient elution, the ester eluted much later than the free acid. Under gradient conditions, trinexapac and trinexapac-ethyl eluted at 7.8 and 14.7 min, respectively. By switching the MS detector from the negative to the positive ionization mode, after elution of trinexapac, trinexapac-ethyl could be detected simultaneously with excellent sensitivity.

An in vitro experiment was performed to determine the degradation of trinexapac-ethyl to its free acid metabolite expected during the given extraction process. Low (0.05 mg/kg) and high (1 mg/kg) spike concentrations of trinexapac-ethyl on one of the organically grown wheat composites were allowed to dry (daylight, 20 °C) for 8 h. Both samples were run through the extraction procedure (overnight) and analyzed for trinexapac-ethyl and its free acid metabolite (trinexapac). Trinexapac-ethyl was recovered at >95%, and analysis of the metabolite showed no response higher than the LOD of trinexapac in both experiments. The hydrolysis of trinexapac-ethyl probably takes place within the plant cells. The results demonstrated that detection of trinexapac-ethyl at the moment of sample extraction is feasible. The LOD of trinexapac-ethyl in wheat was estimated at 5 μg/kg in this case. Freshly prepared wheat extracts with positive findings for trinexapac were reanalyzed for detection of residues of the parent compound, trinexapac-ethyl.

Survey Results. Monitoring of food is necessary to enforce MRLs, to provide information on the intake of pesticides by consumers, and to give the impetus for further toxicological studies if the occurrence of pesticides becomes a concern. To comply with legislation, the Inspectorate samples lots of domestically grown and imported crops for analysis of pesticide residues. Within the framework of this survey, 100 wheat samples used for human consumption were collected for the Dutch National Pesticide Monitoring Program during the year 2001. Wheat was randomly sampled at wholesalers, granaries, and cereal-processing facilities by field inspectors. The wheat samples were primarily of western European origin (The Netherlands, Germany, France, and Great Britain). Among the wheat samples collected, 15 samples were organically grown. The validated method was used to determine residue concentrations of trinexapac in wheat samples. Of the wheat samples analyzed, 46% contained a residue above the limit of quantification (10 μg/kg). The average concentration found was 23 μg/kg. The highest concentration of trinexapac found was 110 μg/kg in wheat of Dutch origin. All results were well below the MRL of 0.2 mg/kg, as stated in the Dutch Pesticide Act.

As part of the enforcement program, the wheat samples were also analyzed for compliance with the MRL (2 mg/kg), set for the PGR chlormequat using an LC-MS method developed in-house (*15*). Of the wheat samples analyzed, 80% contained a residue of chlormequat above the LOQ (50 μg/kg). The concentrations of chlormequat found ranged from 50 to 560 μg/kg, and the results were in good agreement with surveys conducted elsewhere in Europe (*16*, *17*). All wheat samples that contained a residue of trinexapac also gave a positive finding for chlormequat, indicating wheat sprayed with trinexapac-ethyl had previously been treated with a product containing chlormequat. Another possibility is that the wheat fields have been sprayed with a tank mix of chlormequat plus trinexapac-ethyl, thereby improving growth regulatory performance. None of the organically grown wheat samples contained any detectable residue of trinexapac or chlormequat. If the organically grown wheat samples are left out of consideration, 55% of the wheat

samples held a residue of chlormequat plus trinexapac-ethyl and 95% a residue of chlormequat.

Residues of the Parent Compound, Trinexapac-ethyl, in Wheat. Field trials have proved the rapid decomposition of trinexapac-ethyl in wheat after 1 day of the agent's application (*3*−*5*). Generally, plant growth regulators are applied to wheat in spring (April). Wheat samples taken at normal harvest time (June−July) are very unlikely to have residues of the parent compound, trinexapac-ethyl. Nevertheless, qualitative analyses to detect possible residues of the parent compound in the wheat samples were performed. Sixty-four wheat samples with positive findings for trinexapac were reanalyzed. As was expected, residues of the parent compound, trinexapac-ethyl, in the survey samples could not be detected. Therefore, analysis of trinexapac accurately reflects the amount of total residues of PGR in wheat samples following field application. As a typical example, **Figure 4** depicts normalized total ion chromatograms of four MRM channels for trinexapac and trinexapac-ethyl during gradient elution with time-scheduled ESI−/+ mode MS-MS, demonstrating the absence of the parent compound, trinexapac-ethyl, in wheat samples, which contained a residue of trinexapac.

Conclusions. The proposed method for the determination of trinexapac is suitable for the application in pesticide residue enforcement programs, because it fulfills appropriate analytical criteria and has the particular advantage of enabling high-throughput quantitation and confirmation of the PGR in wheat. The validation results have shown the LC-MS-MS method for trinexapac in wheat to have a trueness within 71−94% and a precision of better than 13.4% RSD based on the interday QC results. Sensitivity (10 μg/kg) is sufficient to demonstrate quantifiable amounts of trinexapac following field application of the PGR in wheat. A survey has revealed that the PGR trinexapac-ethyl is frequently applied in European wheat-growing. Negative acute health effects cannot be expected, because the MRL is not exceeded. The frequency of residue findings, however, suggests that periodical monitoring for residues of trinexapac-ethyl in wheat samples for human consumption is recommendable.

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