Carfentrazone-ethyl

Materials to be analyzed
Field corn grain, forage, stover and processed parts (grits, meal, flour, starch and oils); sweet corn ears, forage and stover; soybean seed and processed parts (meal, hulls and oil); wheat grain, forage, hay and straw; rice grain, straw and processed parts (hulls, bran and polished rice); sorghum grain, forage and stover; cotton seed, gin trash and processed parts (meal, hulls and oil); grape and raisins; and bovine milk, cream, liver, kidney, fat and muscle.

Instrumentation
Gas-chromatographic determination for plant and animal matrices.

1 Introduction

Table: Chemical properties of carfentrazone-ethyl

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical name</td>
<td>Ethyl α,2-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzene-propanoate</td>
</tr>
<tr>
<td>Empirical formula</td>
<td>C_{15}H_{15}N_{3}O_{3}F_{3}Cl</td>
</tr>
<tr>
<td>Molar mass</td>
<td>412.2</td>
</tr>
<tr>
<td>Boiling point</td>
<td>350–355 °C</td>
</tr>
<tr>
<td>Physical state/odor</td>
<td>Viscous yellow/orange liquid with a very faint petroleum-like odor</td>
</tr>
<tr>
<td>Vapor pressure</td>
<td>1.2 × 10^{-7} mmHg (25 °C)</td>
</tr>
<tr>
<td>Water solubility</td>
<td>22 mg L^{-1} (25 °C)</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>1.46 g mL^{-1} (20 °C)</td>
</tr>
<tr>
<td>Stability</td>
<td>Stable at pH 5, moderately stable at pH 7 and 9</td>
</tr>
<tr>
<td>Other properties</td>
<td>Undergoes hydrolysis rapidly. The half-life (t_{1/2}) of carfentrazone-ethyl in aqueous photolysis at pH 5 is 8.3 days of sunlight exposure.</td>
</tr>
</tbody>
</table>

Handbook of Residue Analytical Methods for Agrochemicals. © 2003 John Wiley & Sons Ltd.
Carfentrazone-ethyl is a rapid-acting, post-emergent contact herbicide that provides good control over broadleaf and sedge weeds in cereal grain crops. The product is also being developed for total vegetation control (TVC) as a potato desiccant and as a cotton defoliant. Currently, carfentrazone-ethyl is registered for agricultural use in the USA on soybeans and cereal grain crops and as a cotton defoliant, in Europe on small grain crops, and in Asia on wheat.

The metabolism of carfentrazone-ethyl in animals and plants is similar. The major plant metabolites are carfentrazone-chloropropionic acid (C-Cl-PAc), 3-desmethylcarfentrazone-chloropropionic acid (DM-C-Cl-PAc), and 3-hydroxymethylcarfentrazone-chloropropionic acid (HM-C-Cl-PAc). The major animal metabolites are carfentrazone-chloropropionic acid (C-Cl-PAc) and carfentrazone-propionic acid (C-PAc). The tolerance expression for livestock and plant commodities is carfentrazone-ethyl plus the ester hydrolysis product, C-Cl-PAc.

| Use pattern | Carfentrazone-ethyl is a rapid-acting, post-emergent contact herbicide that provides good control over broadleaf and sedge weeds in cereal grain crops. The product is also being developed for total vegetation control (TVC) as a potato desiccant and as a cotton defoliant. Currently, carfentrazone-ethyl is registered for agricultural use in the USA on soybeans and cereal grain crops and as a cotton defoliant, in Europe on small grain crops, and in Asia on wheat. |
| Regulatory position | The metabolism of carfentrazone-ethyl in animals and plants is similar. The major plant metabolites are carfentrazone-chloropropionic acid (C-Cl-PAc), 3-desmethylcarfentrazone-chloropropionic acid (DM-C-Cl-PAc), and 3-hydroxymethylcarfentrazone-chloropropionic acid (HM-C-Cl-PAc). The major animal metabolites are carfentrazone-chloropropionic acid (C-Cl-PAc) and carfentrazone-propionic acid (C-PAc). The tolerance expression for livestock and plant commodities is carfentrazone-ethyl plus the ester hydrolysis product, C-Cl-PAc. |

**Carfentrazone-chloropropanoic acid (C-Cl-PAc)**

\[
\begin{align*}
\alpha,2\text{-dichloro-5-}[4\text{-}(\text{difluoromethyl})\text{-}4,5\text{-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl}]\text{-4-fluorobenzenepropanoic acid}
\end{align*}
\]

**3-Desmethylcarfentrazone-chloropropanoic acid (DM-C-Cl-PAc)**

\[
\begin{align*}
\alpha,2\text{-dichloro-5-}[4\text{-}(\text{difluoromethyl})\text{-}4,5\text{-dihydro-5-oxo-1H-1,2,4-triazol-1-yl}]\text{-4-fluorobenzenepropanoic acid}
\end{align*}
\]

**3-Hydroxymethylcarfentrazone-chloropropanoic acid (HM-C-Cl-PAc)**

\[
\begin{align*}
\alpha,2\text{-dichloro-5-}[4\text{-}(\text{difluoromethyl})\text{-}4,5\text{-dihydro-3-hydroxymethyl-5-oxo-1H-1,2,4-triazol-1-yl}]\text{-4-fluorobenzenepropanoic acid}
\end{align*}
\]

2 **Outline of method**

The analytical method for carfentrazone-ethyl and its major metabolites in/on corn grain, grits, meal, flour, and starch (nonoil matrices) consists of extractions with acetone and deionized water, followed by a partition with hexane, which allowed the separation of the parent carfentrazone-ethyl from the acid metabolites. The hexane fraction, containing the carfentrazone-ethyl, is cleaned up with a silica gel (SI) solid
phase extraction (SPE) cartridge. The aqueous phase, containing the acid metabolites, is acidified (1 N HCl), boiled under reflux, partitioned with methylene chloride, derivatized using boron trifluoride in methanol (BF$_3$-MeOH) and acetic anhydride, and cleaned up with an SI SPE cartridge. The carfentrazone-ethyl is quantitated in a gas chromatograph equipped with a DB-17 Megabore capillary column and an electron capture detector. The acid metabolite derivatives are quantitated using a gas chromatograph equipped with a DB-5 narrow-bore capillary column and a mass-selective detector.

This enforcement method has been validated on the (raw agricultural commodities) (RAC) and processed parts of various crops. The method limit of quantification (LOQ) was validated at 0.05 ppm and the method limit of detection (LOD) was set at 0.01 mg kg$^{-1}$ for all of the crop matrices. The method flow chart is presented in Figure 1.

### 3 Apparatus

AccessChrom or TurboChrom data acquisition software, running on a MicroVax Balance, Analytical PM 2000, Mettler Balance, top loading, Mettler Blender, Omni, equipped with a macro generator (20-mm diameter × 145-mm long w/sawteeth, part No. 15401, cat. No. 17105) or equivalent such as a Tekmar Tissuemizer Boiling stones, Hengar Buchner filter funnels, porcelain, 10.5-cm i.d., Coors Capillary column, DB-35, 15 m × 0.25-mm i.d., 0.25-µm, J&W Scientific Capillary column, DB-17, 30 m × 0.53-mm i.d., 1.0-µm, J&W Scientific Centrifuge tubes, 15-mL, graduated, Pyrex, 0.1-mL Centrifuge tubes, 50-mL, graduated, polypropylene, VWR (cat. No. 21008-714) Condensers, Graham coil, Pyrex, 41 × 500-mm with T 24/40 joint Cylinders, graduated, 10, 50, 100, 250-mL Cylinders, mixing, 250-mL, graduated Filtration tubes (6-mL capacity) containing a (20-µm pore size) polyethylene frit, VWR (cat. No. JT7121-6) Filter paper, Whatman 934-AH, 7-cm diameter, VWR (cat. No. 28496-955) Flasks, vacuum filter, Pyrex, 500-mL Flasks, round-bottom boiling, Kontes, 50-mL, T 45/50 joint Gas chromatograph, Hewlett-Packard (HP) 5890 equipped with an HP 7673A autosampler and an electron capture detector Gas chromatograph, HP 5890 equipped with an HP 7673A autosampler and an HP 5972 mass-selective detector Gas chromatograph injector liner [for gas chromatography/electron capture detection (GC/ECD)], cyclo-union liner insert, Restek (cat. No. 20337) Gas chromatograph injector liner [for gas chromatography/mass spectrometry (GC/MS)], cyclo-double gooseneck, 2 mm, Restek (cat. No. 20907) Heating mantles, 500-mL, Glas-Col Injection vials, 2-mL, Wheaton
Figure 1  Method flow chart for carfentrazone-ethyl determination
Injection vial crimps, 11-mm, Teflon/silicone/Teflon, Sun Brokers
Microsyringes, (25-, 50-, 100-, 250-, 500-µL), Hamilton
Mill, Hobart
Mill, Wiley (Model ED-5)
N-EVAP evaporator, Organomation
Single-tube vortexer, VWR
Pipets, disposable (5.75- and 9-in lengths)
Pipets, volumetric pipette bulbs
Reducing adapters (SPE), plastic, Supelco
Reservoirs, plastic, 75-mL
Screw-capped glass tubes, 50 × 150-mm
SPE cartridge, C<sub>18</sub> (1-g), Bakerbond, VWR (cat. No. JT7020-07)
SPE cartridge, SCX (1-g), Varian (part No. 1225-6011)
SPE cartridge, silica gel (1-g), J.T. Baker, VWR (cat. No. JT7086-07)
Test-tubes, glass, 25 × 150-mm
Stainless-steel blending cups, 400-mL capacity, Omni (cat. No. 17079)
TurboVap evaporator, Zymark
TurboVap centrifuge tube support rack, Zymark
TurboVap vessels, 200-mL, Zymark
TurboVap vessel support rack, Zymark
Visiprep vacuum manifold, Supelco
Visidry vacuum manifold drying attachment, Supelco

4 Reagents

Acetic anhydride, ACS Reagent Grade, Sigma Chemical (product No. A6404) or Aldrich (product No. 11,004-3)
Acetone, Resi-Analyzed, J.T. Baker
Acetonitrile, HPLC grade, J.T. Baker
Boron trifluoride (14% in methanol), Sigma Chemical (product No. 13-1127)
Ethyl acetate, Pesticide Grade, J.T. Baker
Hexane, Resi-Analyzed, J.T. Baker
Hydrochloric acid (HCl, 36.5–38.0%), J.T. Baker
Hydron pH buffer, VWR (cat. No. 34175-220)
Methanol, Resi-Analyzed, J.T. Baker
Methylene chloride, Resi-Analyzed, J.T. Baker
pH indicator strips (EM Science), VWR (cat. No. EM-9590-3)
Pyridine, Fisher (99.9%) or Sigma Chemical (product No. P-4036)
Sodium sulfate, anhydrous, J.T. Baker

Equivalent equipment and reagents may be substituted as appropriate, unless specified otherwise in the method.

5 Sampling and preparation

Prior to analysis, the samples were chopped and finely ground with liquid nitrogen using a large Hobart (forage, hay, fodder, straw and bovine tissue samples) or a Wiley
mill (grain and seed samples). Recently, frozen crop matrices were processed more effectively with Robot Coupe vertical cutter/mixer without liquid nitrogen.

6 Analytical procedures for nonoil crop matrices

6.1 Sample extraction, filtration and concentration

Weigh 2.5 or 5 g of crop matrix into a blending vessel. Fortify samples at this point with the appropriate analytical standards. Allow the solvent to evaporate. Add 100 mL of acetone–water (4:1, v/v) and blend the mixture using an Omni mixer equipped with a macro generator for 5 min at 6000–7000 rpm. Filter the sample through a Whatman 934 AH glass-fiber filter paper on a Buchner funnel/vacuum flask setup. Rinse the blending cup and filter cake with 100 mL of acetone. Transfer the filtrate into a 200-mL TurboVap vessel. Concentrate the sample (remove acetone) under nitrogen to ca 20–25 mL using a TurboVap (water-bath at 50 °C). Transfer the sample into a 50-mL polypropylene centrifuge tube. Rinse the TurboVap vessel with 5 or 10 mL of pH 6 buffer solution. The amount of pH 6 buffer required depends on the matrix being analyzed and should be determined as needed. All matrices need 5 mL of the buffer solution to adjust the sample to pH 6, except for sweet corn (ears, forage, and stover), which requires 10 mL. Add the rinse buffer to the sample. Rinse the TurboVap vessel with 10 mL of hexane and add the hexane to the sample.

6.2 Partition

Vigorously mix the aqueous and hexane fraction to partition carfentrazone-ethyl into the hexane fraction. Centrifugation may be necessary to break any emulsion that occurs. Remove and collect the hexane fraction for analysis of carfentrazone-ethyl. Partition the aqueous fraction with an additional 10 mL of hexane and add the hexane to the hexane from the first partition step. The aqueous fraction will be used for the analysis of the acid metabolites (see below).

6.3 Determination of carfentrazone-ethyl

6.3.1 Cleanup

Concentrate the hexane fraction (20 mL) from the previous hexane–aqueous partition to 3 mL in a TurboVap at ca 50 °C. For grain and forage matrices, condition a 1-g, 6-mL SI SPE cartridge with 1 cartridge volume (1 CV), (6 mL) of 10% (v/v) ethyl acetate in hexane followed by 1 CV of hexane (vacuum at 1 inHg). Load the 3-mL sample onto the cartridge, but do not elute the sample yet. Rinse the tube with 3 mL of hexane and also load this rinsate onto the cartridge. Drain the 6 mL of sample solution through the SI cartridge (vacuum at 1 inHg) and discard the eluate. Rinse the SI cartridge with 9 mL of 10% (v/v) ethyl acetate in hexane and discard the rinsate. Elute and collect the sample with an additional 12 mL of 10% (v/v) ethyl acetate in hexane (vacuum at 1 inHg).
For fodder, hay or straw matrices, in order to exclude an interference which only occurs in the dry matrices, a slightly less polar elution solvent (7.5% vs 10% ethyl acetate in hexane) and a larger volume (18 mL) are used.

Concentrate the sample to 0.1 mL in a TurboVap at ca 50 °C and adjust the sample to a final volume of 1.0 mL with acetonitrile. Note: there is the potential for loss of analyte if the samples go to dryness at this step. Analyze the sample for parent carfentrazone-ethyl by GC/ECD.

6.4 Determination of acid metabolites

6.4.1 Acid reflux

Transfer the aqueous fraction from the hexane–aqueous partition (25–30 mL) into a 50-mL round-bottom flask. Add 3–3.5 mL of concentrated HCl (such that the final acid concentration is ≥1 N and several boiling chips to the round-bottom flask and reflux the sample for 1 h under a water-cooled condenser. This acid reflux step will cleave any conjugated acid metabolites in the crop matrices.

6.4.2 SCX/C₁₈ SPE cartridges

Allow the hydrolyzed sample to cool before handling. Assemble tandem SPE cartridges (SCX cartridge on top of the C₁₈ cartridge) and install them on the vacuum manifold. Condition both the SCX (Varian, 1-g), and the C₁₈ SPE cartridges (Bakerbond, 1-g) in series with methanol (1 CV) and then with 0.25 N HCl (1 CV) using 5 inHg of vacuum. After the 0.25 N HCl reaches the top of the column packing of the SCX cartridge, turn off the vacuum. Add an additional 0.5 CV of 0.25 N HCl and attach an SPE filtration cartridge with just a frit installed in the cartridge (no packing material) on top of the SCX cartridge. Attach a reducing adapter and a 75-mL reservoir to the top of the SPE cartridge containing the frit. Decant the hydrolyzed sample into the reservoir. Rinse the round-bottom flask with 40 mL of deionized water but do not add the rinsate to the hydrolyzed sample at this point. With the cartridge valve opened, apply a vacuum at 7–10 inHg and drain and discard the hydrolyzed sample. When the last of the hydrolyzed sample has passed through the SCX cartridge, add 40 mL of deionized water rinsate to the reservoir and drain the rinsate through all three cartridges. Discard the deionized water rinsate. Continue the vacuum of 7–10 inHg until all of the filtrate has eluted through all three cartridges.

Remove the reducing adaptor, reservoir, filtration cartridge, and the SCX cartridge and dry the C₁₈ SPE cartridge with nitrogen for at least 60 min using a drying manifold. Elute and collect the analytes from the C₁₈ SPE cartridge with 12 mL of 5% (v/v) methanol in dichloromethane. Concentrate the sample under nitrogen using the TurboVap to 0.1–0.25 mL (water-bath at 50 °C). Note: there is the potential for loss of analytes if the samples go to dryness at this step.

6.4.3 First derivatization (methyl esterification)

Add 1 mL of boron trifluoride in methanol (14% by weight) to the sample solution, vortex the solution and allow the sample to react for 45 min in a water-bath at 50 °C. After methylation, add 2 mL of water. If analysis of HM-C-Cl-PAc is not required,
extract the methylated analytes with 5 mL of hexane and proceed to clean up on the SI SPE cartridge.

Partition the sample in methanol twice with 2 mL of dichloromethane (DCM), remove the DCM after each partition step and pass the sample in DCM through a 6-mL filtration tube containing a polyethylene frit and packed with 1 g of anhydrous sodium sulfate. The use of the anhydrous sodium sulfate can be eliminated if great care is taken when removing the DCM from each partition step so that no water is included with the DCM. If water droplets are present in the DCM fraction, carefully remove them with a small pipet. The DCM is then concentrated in a Turbovap to 0.1 mL at 50°C. Note: there is the potential for loss of analytes if the samples go to dryness at this step.

6.4.4 Second derivatization (acylation)

Add 0.5 mL of acetic anhydride and 0.5 mL of pyridine to the sample solution, vortex the solution and allow the sample to react for 45 min in a water-bath at 50°C. This procedure acylates the hydroxyl group on the HM-C-Cl-Pac-methyl ester.

After acylation, add 2 mL of water to the sample and partition the sample twice with 2 mL of hexane. Retain the 4-mL hexane fraction. The aqueous fraction containing excess acetic anhydride and pyridine is discarded.

6.4.5 Cleanup

Condition a 1-g, 6-mL SI SPE cartridge with 1 CV of 20% (v/v) ethyl acetate in hexane followed by 1 CV of hexane (vacuum at 1 inHg). Load the 4-mL sample onto the cartridge. Drain the hexane containing the sample through the SI cartridge (vacuum at 1 inHg) and discard the eluate. Rinse the cartridge with 3 mL of 20% (v/v) ethyl acetate in hexane. Discard the rinsate. Elute and collect the sample with an additional 12 mL of 20% (v/v) ethyl acetate in hexane. Concentrate the sample under nitrogen to 0.5 mL in a TurboVap (water-bath at 50°C), and adjust the sample to a final volume of 1.0 mL with hexane.

Analyze the sample by GC/MS, and monitor the ions at m/z 362 for C-Cl-Pac, 348 for DM-C-Cl-Pac, and 413 for HM-C-Cl-Pac.

6.5 Analytical procedures for crop refined oils

Crop refined oils should be dissolved in hexane and partitioned with deionized water in a separatory funnel. The hexane fraction containing the carfentrazone-ethyl should be further partitioned with acetonitrile, and the rest of the analytical procedures for the parent compound should be followed. Concentrated HCl is added to the aqueous fraction to make the solution 1 N and the samples are boiled under reflux for 1 h; the rest of the analytical procedures for the acid metabolites should be followed.
6.6 Analytical procedures for animal matrices

The analytical method to determine carfentrazone-ethyl and the major animal metabolites (C-Cl-Pac and C-Pac) in bovine matrices is similar to the method for crop matrices. The hexane–aqueous partition to separate carfentrazone-ethyl from the acid metabolites can be replaced by a C\textsubscript{18} SPE cartridge. After the SPE, use 12 mL of 30\% (v/v) acetonitrile in water to elute the metabolites and then use 12 mL of 20\% (v/v) ethyl acetate in hexane to elute carfentrazone-ethyl after drying the cartridge. Follow the rest of the respective analytical procedures for carfentrazone-ethyl and the acid metabolites described in Sections 6.3 and 6.4. However, no reflux under boiling is necessary for the analysis of acid metabolites based on a goat metabolism study, because no conjugated acid metabolites were detected. Also, since HM-C-Cl-Pac is not analyzed for in the bovine matrices, no acylation is needed in the method. Analyze the metabolites by GC/MS, and monitor the ions at $m/z$ 362 for C-Cl-Pac and 303 for C-Pac.

6.7 Instrumentation

Gas chromatography (GC) is used to analyze the sample extracts. Two detector systems are used, one for quantitation and the other for analyte confirmation and quantitation.

**Operating conditions for carfentrazone-ethyl determination**

- **Instrument**: HP 5890 or 6890 gas chromatograph
- **Column**: DB-17, phenyl/methyl (50:50) silicone gum, 30 m × 0.53-mm i.d., 1.0-µm film thickness
- **Inlet**: Splitless injection mode
- **Detector**: \textsuperscript{63}Ni electron capture
- **Temperatures**
  - **Injection port**: 250°C
  - **Oven**: 150°C/1 min (initial); 20°C min\textsuperscript{-1} (ramp 1); 200°C/0 min; 10°C min\textsuperscript{-1} (ramp 2); 260°C/10 min (final)
  - **Detector**: 300°C
- **Gas flow rate**: He carrier gas, 13 mL min\textsuperscript{-1}
  - Ar–methane, make-up gas, 40 mL min\textsuperscript{-1}
- **Injection volume**: 2 µL

**Operating conditions for carfentrazone-ethyl confirmation**

- **Instrument**: HP 5890 or 6890 gas chromatograph
- **Column**: DB-35MS, phenyl/methyl (35:65) silicone gum, 15 m × 0.25-mm i.d., 0.25-µm film thickness
- **Inlet**: Splitless injection mode (cyclo-double gooseneck insert)
- **Detector**: HP 5972 mass-selective detector
- **Temperatures**
  - **Injection port**: 250°C
  - **Oven**: 150°C/1 min (initial); 12.5°C min\textsuperscript{-1} (ramp); 280°C/10 min (final)
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Gas flow rate  He carrier gas, 1 mL min$^{-1}$
Injection volume  2 µL
Ions monitored  m/z 312, 340, and 411

Operating conditions for determination of acid metabolites
Instrument  HP 5890 gas chromatograph
Column  DB-35, phenyl/methyl (35:65) silicone gum, 15 m ×
0.25-mm i.d., 0.25-µm film thickness
Inlet  Splitless injection mode (cyclo-double gooseneck insert)
Detector  HP 5972 mass-selective detector
Temperatures
Injection port  250 ºC
Oven  150 ºC/1 min (initial); 15 ºC min$^{-1}$ (ramp); 280 ºC/18 min (final)
Gas flow rate  He carrier gas, 1 mL min$^{-1}$
Injection volume  2 µL
Ions monitored  m/z 348 (DM-C-Cl-PAc derivative); m/z 362 (C-Cl-PAc derivative); m/z 413 (HM-C-Cl-PAc derivative)

7  Method validation and quality control

7.1  Experimental design

The analytical method was validated at the LOQ (0.05 mg kg$^{-1}$) for each analyte by satisfactory recoveries of the respective analytes from control samples that were fortified at the initiation of each analysis set. The fortified control samples were carried through the procedure with each analysis set. An analysis set consisted of a minimum of one control sample, one laboratory-fortified control sample, and several treated samples.

A calibration curve was generated for each analyte at the initiation of the analytical phase of the study. Standard solutions for injection contained carfentrazone-ethyl or derivatized acid metabolites. Standard solutions were injected at the beginning of each set of assays and after every two or three samples to gage the instrument response.

7.2  Preparation of standards

Carfentrazone-ethyl, C-Cl-PAc, C-PAc, DM-C-Cl-PAc and HM-C-Cl-PAc stock solutions of 1000 µg mL$^{-1}$ were prepared by dissolving the appropriate amounts of the analytical standards in acetonitrile. Working solutions were prepared in volumetric flasks by appropriate dilutions of the stock solutions for each analyte or combination of analytes. Working solutions containing the parent were prepared only in acetonitrile and working solutions containing acid metabolites were prepared in acetonitrile (underivatized) or hexane (derivatized). Underivatized solutions (containing the parent and/or metabolites in acetonitrile) were used for fortification. Solutions of derivatized esters were prepared simultaneously with the samples. Standard solutions
of carfentrazone-ethyl (in acetonitrile) and derivatized acid metabolites (in hexane) were used for analyte quantitation and instrument calibration.

### 7.3 Calculation

The amounts of carfentrazone-ethyl, C-Cl-PAc, C-PAc, DM-C-Cl-PAc and HM-C-Cl-PAc were quantitated by the external standard calibration method.

The amount of sample injected was determined using the following equation:

\[
\text{Amount of sample injected (mg)} = \frac{\text{initial aliquot weight (mg)}}{\text{final sample extract volume (µL)}} \times \text{sample extract volume injected (µL)}
\]

An equation representing area versus concentration was determined using a standard linear regression analysis applied to the injection standards, yielding a slope \(m\) and an intercept \(b\). The following equation was then used to calculate the concentration of the sample injected from the area measured:

\[
\text{Concentration of sample (ng µL}^{-1}) = \frac{\text{Area of sample} - b}{m}
\]

The amount of analyte (in nanograms) detected in a sample injection was calculated by multiplying the concentration calculated above by the injection volume. Then the concentration detected (in ppm) was determined by dividing this result by the amount of sample injected:

\[
\text{Detected or uncorrected ppm (ng mg}^{-1}) = \frac{\text{conc. of sample (ng µL}^{-1}) \times \text{inj. volume (µL)}}{\text{amount of sample injected (mg)}}
\]

No correction for molecular weights was necessary for the derivatized compounds since the injection standards were derivatized simultaneously with the analytes and all weights were based on the underivatized acids.

The uncorrected ppm of the fortified control samples was divided by the fortification level and multiplied by 100% to calculate the method recovery (%). The following equation was used:

\[
\text{Method recovery (%) } = \frac{\text{uncorrected mg kg}^{-1} - \text{control mg kg}^{-1}}{\text{fortification level (mg kg}^{-1})} \times 100
\]

The LOD was calculated as the concentration of analyte (ppm equivalent) at one-fifth the area of the LOQ level standard, or one-fifth the LOQ, whichever was larger.
7.4 Time required for analysis

For a set of 10 samples, the analytical method can be completed within 16 laboratory hours from the time of sample weighing to GC injection.

7.5 Accuracy and precision

The accuracy and precision of the analytical methods were determined by the average and standard deviation of individual method recoveries of the fortified-control samples in 50 different matrices (see Tables 1 and 2). These methods were also demonstrated to be very rugged based on the results of accuracy and precision for a variety of crop and animal matrices.

8 Important points

The extraction efficiencies using a blender and a shaker were compared and both methods gave similar results. A corn sample treated with radiolabeled carfentrazone-ethyl and collected from a metabolism study was used for comparison. Multiple samples can be extracted simultaneously if extraction is performed by shaking. In addition, since the extraction procedures in the residue study closely followed the extraction scheme in the metabolism study, the resulting extraction efficiencies from both studies were almost identical.

During the initial partition with hexane and water, the aqueous pH must not exceed 8. Carfentrazone-ethyl is extremely unstable under alkaline conditions and will rapidly degrade to C-Cl-PAc. At times, the workup of the crop samples, including the fortification step, should be completely separated for carfentrazone-ethyl and the acid metabolites, to avoid any possible interference from the parent compound.

Both washing solvent and the volume of it used during the SI cleanup step were critical to the method recovery. Generally, different volumes of wash solvents were needed in different methods to reduce the amount of co-extracts present without jeopardizing the recovery of the analytes. Silica gel cartridges from Varian were used to analyze the crop and animal matrices. When cartridges from other manufacturers were used, different elution patterns were observed. Therefore, the cartridge elution pattern should be evaluated prior to usage.

Pyridine and BF$_3$ in methanol are hazardous and must be used only in a well-ventilated hood. A solvent partition after acylation helps remove residual pyridine from the sample. Material Safety Data Sheets for the derivatizing agents should be reviewed and kept readily available.

The injection standards of carfentrazone-ethyl must be in acetonitrile. Other solvents (e.g., ethyl acetate) lead to poor chromatography following injection of matrix samples. This can lead to apparent enhanced recoveries of analyte in the fortified samples.

Conditioning the GC system with matrix samples before the actual run of the set is recommended to establish stable analytical conditions for the analytes. The GC
Carfentrazone-ethyl

Table 1  Recoveries from fortified samples

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Fortification level (mg kg(^{-1}))</th>
<th>No. of analyses</th>
<th>% Recovery (average ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field corn grain</td>
<td>0.05</td>
<td>23</td>
<td>88 ± 9</td>
</tr>
<tr>
<td>Field corn forage</td>
<td>0.05, 0.1, 0.15, 0.2, 0.3</td>
<td>14, 22, 23</td>
<td>98 ± 15</td>
</tr>
<tr>
<td>Field corn fodder</td>
<td>0.05, 0.1, 0.3</td>
<td>9, 21, 22</td>
<td>90 ± 15</td>
</tr>
<tr>
<td>Field corn grits</td>
<td>0.05</td>
<td>2</td>
<td>72</td>
</tr>
<tr>
<td>Field corn meal</td>
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<td>2</td>
<td>76</td>
</tr>
<tr>
<td>Field corn flour</td>
<td>0.05</td>
<td>2</td>
<td>95</td>
</tr>
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<td>Field corn starch</td>
<td>0.05</td>
<td>2</td>
<td>93</td>
</tr>
<tr>
<td>Field corn crude oil</td>
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<td>2</td>
<td>97</td>
</tr>
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<td>Field corn refined oil</td>
<td>0.05</td>
<td>5</td>
<td>92 ± 18</td>
</tr>
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<td>Sweet corn ears</td>
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<td>8</td>
<td>94 ± 9</td>
</tr>
<tr>
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<td>0.05, 0.1</td>
<td>8</td>
<td>86 ± 6</td>
</tr>
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<td>Sweet corn fodder</td>
<td>0.05, 0.2</td>
<td>8, 9</td>
<td>88 ± 8</td>
</tr>
<tr>
<td>Wheat grain</td>
<td>0.05</td>
<td>8</td>
<td>89 ± 14</td>
</tr>
<tr>
<td>Wheat forage</td>
<td>0.05, 0.25, 0.5</td>
<td>6</td>
<td>99 ± 4</td>
</tr>
<tr>
<td>Wheat hay</td>
<td>0.05, 0.25</td>
<td>3</td>
<td>99 ± 8</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>0.05, 0.25</td>
<td>5</td>
<td>104 ± 10</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>0.05</td>
<td>1</td>
<td>97</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>0.05</td>
<td>1</td>
<td>97</td>
</tr>
<tr>
<td>Wheat middlings</td>
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<td>68</td>
</tr>
<tr>
<td>Wheat shorts</td>
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<td>108</td>
</tr>
<tr>
<td>Wheat germ</td>
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<td>1</td>
<td>114</td>
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<td>0.05</td>
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<td>97 ± 16</td>
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<tr>
<td>Sorghum forage</td>
<td>0.05, 0.1</td>
<td>6</td>
<td>108 ± 10</td>
</tr>
<tr>
<td>Sorghum fodder</td>
<td>0.05</td>
<td>7</td>
<td>94 ± 10</td>
</tr>
<tr>
<td>Sorghum flour</td>
<td>0.05</td>
<td>2</td>
<td>116</td>
</tr>
<tr>
<td>Soybean seed</td>
<td>0.05</td>
<td>12</td>
<td>91 ± 10</td>
</tr>
<tr>
<td>Soybean forage</td>
<td>0.05, 0.25, 1.0</td>
<td>4, 5</td>
<td>105 ± 9</td>
</tr>
<tr>
<td>Soybean hulls</td>
<td>0.05</td>
<td>1</td>
<td>108</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>0.05</td>
<td>1</td>
<td>98</td>
</tr>
<tr>
<td>Soybean crude oil</td>
<td>0.05</td>
<td>1, 2</td>
<td>117</td>
</tr>
<tr>
<td>Soybean refined oil</td>
<td>0.05</td>
<td>2</td>
<td>117</td>
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<tr>
<td>Rice grain</td>
<td>0.05</td>
<td>21, 22</td>
<td>91 ± 11</td>
</tr>
<tr>
<td>Rice straw</td>
<td>0.05, 0.1, 1.0, 5.0</td>
<td>18, 21</td>
<td>98 ± 14</td>
</tr>
<tr>
<td>Rice hulls</td>
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<td>2</td>
<td>105</td>
</tr>
<tr>
<td>Rice bran</td>
<td>0.05</td>
<td>2</td>
<td>103</td>
</tr>
<tr>
<td>Rice, polished</td>
<td>0.05</td>
<td>2</td>
<td>110</td>
</tr>
<tr>
<td>Cotton seed</td>
<td>0.05, 0.1, 10</td>
<td>12, 14</td>
<td>94 ± 16</td>
</tr>
<tr>
<td>Cotton gin trash</td>
<td>0.05, 0.10</td>
<td>6, 7</td>
<td>89 ± 23</td>
</tr>
<tr>
<td>Cotton meal</td>
<td>0.05, 0.1</td>
<td>3</td>
<td>99 ± 9</td>
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<tr>
<td>Cotton hulls</td>
<td>0.05, 0.1</td>
<td>3</td>
<td>104 ± 7</td>
</tr>
<tr>
<td>Cotton refined oil</td>
<td>0.05, 0.1</td>
<td>3</td>
<td>125 ± 6</td>
</tr>
<tr>
<td>Grapes</td>
<td>0.05, 0.1</td>
<td>7</td>
<td>100 ± 10</td>
</tr>
<tr>
<td>Raisins</td>
<td>0.1</td>
<td>1</td>
<td>99</td>
</tr>
</tbody>
</table>

a NA, not analyzed.
Handbook of residue analytical methods for agrochemicals

Table 2  Recoveries from fortified samples

<table>
<thead>
<tr>
<th>Matrix</th>
<th>Fortification level (mg kg(^{-1}))</th>
<th>No. of analyses</th>
<th>% Recovery (average ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Carfentrazone-ethyl</td>
</tr>
<tr>
<td>Bovine milk</td>
<td>0.025, 0.25</td>
<td>12, 20</td>
<td>88 ± 11</td>
</tr>
<tr>
<td>Bovine milk cream</td>
<td>0.05</td>
<td>2</td>
<td>77</td>
</tr>
<tr>
<td>Bovine liver</td>
<td>0.05</td>
<td>2</td>
<td>NA(^a)</td>
</tr>
<tr>
<td>Bovine muscle</td>
<td>0.05</td>
<td>2</td>
<td>NA</td>
</tr>
<tr>
<td>Bovine kidney</td>
<td>0.05, 0.5</td>
<td>4, 6</td>
<td>91 ± 4</td>
</tr>
<tr>
<td>Bovine fat</td>
<td>0.05</td>
<td>2</td>
<td>102</td>
</tr>
</tbody>
</table>

\(^a\) NA, not analyzed.

An oven is programmed to a high final temperature after the analysis run to bake out any possible late eluting compounds.

More recently, liquid chromatography/mass spectrometry (LC/MS) and liquid chromatography/tandem mass spectrometry (LC/MS/MS) have been evaluated as possible alternative methods for carfentrazone-ethyl compounds in crop matrices. The LC/MS methods allow the chemical derivatization step for the acid metabolites to be avoided, reducing the analysis time. These new methods provide excellent sensitivity and method recovery for carfentrazone-ethyl. However, the final sample extracts, after being cleaned up extensively using three SPE cartridges, still exhibited ionization suppression due to the matrix background for the acid metabolites. Acceptable method recoveries (70–120%) of carfentrazone-ethyl metabolites have not yet been obtained.

9 Storage stability

Storage stability studies for carfentrazone-ethyl compounds on crop matrices have shown a pattern of stability for at least 7–24 months, depending on the study program or the maximum sample storage interval for the study. Carfentrazone-ethyl was not stable in field corn starch, potato tuber and bovine kidney. The residue results indicated that a significant portion of carfentrazone-ethyl was converted to C-Cl-PAc in these matrices; however, the total amount of carfentrazone-ethyl and C-Cl-PAc accounted for the original spiking level. Since both carfentrazone-ethyl and C-Cl-PAc were determined in these stability studies, the instability of carfentrazone-ethyl was not of any concern.

Acknowledgements


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Keywords: carfentrazone-ethyl; PPO herbicide; gas chromatography; raw agricultural commodity; processing fractions.