10.6.18A

AOAC Official Method 2000.05
Glyphosate and Aminomethylphosphonic Acid (AMPA) in Crops

Gas Chromatography with Mass-Selective Detection
First Action 2000
Final Action 2003

(Applicable to the determination of glyphosate and aminomethylphosphonic acid in crops at 0.050–2.0 mg/kg.)

See Tables **2000.05A** and **2000.05B** for results of the interlaboratory study supporting acceptance of the method.

A. Principle

Glyphosate and aminomethylphosphonic acid (AMPA) residues are extracted from crops by maceration with water. The crude extract is partitioned with methylene chloride and then subjected to a cation-exchange (CAX) procedure. The analytes in the purified extract are derivatized directly with a mixture of trifluoroacetic anhydride and heptafluorobutanol. The amine functional groups are derivatized to form the corresponding trifluoroacetyl derivatives. The carboxylic and phosphonic acid functional groups are derivatized to form the corresponding heptafluorobutyl esters. After derivatization, the excess reagents are evaporated, and the residue is dissolved in ethyl acetate. The concentrations of the analytes in the final extract are determined by capillary gas chromatography with mass-selective detection (GC/MSD). Quantitation is performed by the external standard method with calibration solutions derivatized concurrently with the extracts. In this method, a highly sensitive detection system allows small amounts of aqueous crop extract to be derivatized without evaporation, thereby overcoming difficulties associated with derivatization of aqueous extracts and analyte adsorption. The limit of quantitation (LOQ) is 0.05 mg/kg.

B. Apparatus

(**a**) *Gas chromatograph*.—Temperature-programmable for use with capillary columns; with data system for determination of peak measurement; and an autosampler/injector for automated analysis. Analytical column: 0.25 mm id 30 m, fused silica capillary with 0.50 m film thickness of cross-linked, 95% methyl–5% phenyl silicone phase [Restek XTI-5 (Restek Corp., 110 Benner Circle, Belleforte, PA 16823-8812, USA) or J&W Scientific DB 5.625 (J&W Scientific, 91 Blue Ravine Rd, Folsom, CA 95630-4714, USA); or equivalent]. A 4 mm id, single or double restrictor-type (1- or 2-piece) inlet liner, packed with a 1 cm long plug of silanized glass wool or fused silica wool, is positioned in the middle of the liner. Place the inlet end of the column within the inlet liner, 6–8 mm from the bottom restrictor of the inlet liner. He carrier gas flow rate: ca 30 cm/s at 180°C; equal to about 40–50 kPa (6–7 psi) at column head. Temperature program: initial temperature, hold at 90°C for 1.5 min, increase to 300°C at 30°C/min (20°C/min if limited by instrument capabilities), and hold at 300°C for 4 min. An alternative program for increased resolution is: initial temperature, hold at 60°C for 1.5 min, increase to 120°C at 10°C/min, hold at 120°C for 1.0 min, increase to 300°C at 30°C/min, and hold at 300°C for 4 min. Injection port temperature: 200°C. MSD interface temperature: 280°C. Injection volume: 2–5 L, depending on analyte detectability. Inlet system: operated in the splitless mode, with the inlet purged 1.0 min after injection. (*Note*: Sensitivity may be optimized by adjusting the initial oven temperature between 60 and 90°C. Adjusting other program variables such as intermediate hold temperature and duration may also be helpful.)

(**b**) *Mass-selective detector.*—A quadrupole instrument, capable of providing positive electron impact (EI) mass spectra with selected-ion monitoring (SIM) over an amu range up to *m/z* 650; operated in the SIM, low-resolution mode; manually tuned, **F**(**a**), for *m/z* 414, 502, and 614 by using perfluorotributylamine (PFTBA). Monitor the AMPA derivative at *m/z* 446; the glyphosate derivative, at *m/z* 611.5. Dwell time: 100 ms.

(**c**) *Pipets, air-displacement, single-channel (Eppendorf-type).*—Variable-volume pipets with disposable plastic tips. Used for handling underivatized aqueous standards, extracts, and derivatized analytes and standards. Volume ranges: 10–100, 200–1000, and 500–2500 L.

(**d**) *CAX columns*.—Prepacked, disposable analyte preparation columns filled with 2 mL bed volume of strongly acidic cation exchanger, 8% crosslinked styrene divinylbenzene resin (AG 50W-X8), H^+ form, 200–400 mesh [Bio-Rad Poly-Prep No. 731-6214 (Bio-Rad Laboratories, 1000 Alfred Nobel Dr, Hercules, CA 94547, USA), or equivalent, is suitable].

(**e**) *Caps and derivatization vial.*—Phenolic plastic, polytetrafluoroethylene (PTFE)-lined, with 13–425 threads [Wheaton No. 240408 (Wheaton Scientific Products, 1501 N. 10th St, Millville, NJ 08332-2093, USA), Supelco No. 27141 (Supelco, Inc., Supelco Park, Bellefonte, PA 16823, USA), or equivalent are suitable]. Caps made from nonphenolic plastic such as polypropylene are softer and may loosen during derivatization.

(**f**) *Vials, derivatization.*—4.0 mL, screw-top, glass [Wheaton No. 224702; Fisher No. 03-340-5N (Fisher Scientific Co., 2000 Park Ln Dr, Pittsburgh, PA 15275-1126, USA), or equivalent is suitable].

(**g**) *Evaporation manifold, with heating block.*—Manifold capable of delivering a gentle stream of N to multiple 4 mL vials maintained at 40 –50°C [Evap-O-Rac (Chemical Research Supplies, PO Box 888, Addison, IL 60101-0888, USA), Techne sample concentrator (Techne, Inc., 743 Alexander Rd, University Park Plaza, Princeton, NJ 08540, USA), or N-Vap (Organomation Associates, Inc., 266 River Rd W, Berlin, MA 01513, USA) is suitable].

(**h**) *Laboratory homogenizer (rotor/stator-type).*—Laboratory homogenizer/macerator capable of having the blade mechanism disassembled for cleaning; or (**i**) below.

(**i**) *Laboratory blender (Waring type).*—Laboratory blender capable of having the blade mechanism disassembled for cleaning, with ca 1 L (1 qt) capacity.

(**j**) *Disposable polypropylene centrifuge tubes*.—50 mL. Graduated, with screw cap, 28 115 mm [Corning No. 430291 (Corning, Inc., 45 Nagog Park, Acton, MA 01720, USA), Fisher No. 05-539-9, or equivalent is suitable].

C. Reagents

(**a**) *Solvents*.—Ethyl acetate, methanol, and methylene chloride; all of high purity suitable for pesticide residue analysis.

(**b**) *CAX mobile phase solution.*—Combine 160 mL water, 2.7 mL HCl, and 40 mL methanol.

(**c**) *Acidic modifier solution.*—Combine 16 g KH_2PO_4, 160 mL water, 40 mL methanol, and 13.4 mL HCl.

(**d**) *2,2,3,3,4,4,4-Heptafluoro-1-butanol.*—98% (Aldrich Chemical Co., PO Box 355, Milwaukee, WI 53201-0355, USA, No. H160-4, or equivalent).

(**e**) *Trifluoroacetic anhydride.*—99%.

Table 2000.05A. Interlaboratory study results for determination of glyphosate in crops by GC/MSD

Material pair ID	$\bar{x}$, mg/kg[a]	No. of labs[b]	s_r	RSD_r, %	s_R	RSD_R, %	HorRat[c]	Recovery, %[a]
C1	0.045	11 (1)	0.0067	15.0	0.0067	15.0	1.1	84.5
C2	0.368	12 (0)	0.0413	11.2	0.0680	18.5	1.0	87.5
C3	1.706	12 (0)	0.1809	10.6	0.3716	21.8	1.5	89.7
S1	0.046	12 (0)	0.0079	17.0	0.0091	19.7	0.8	88.1
S2	0.407	12 (0)	0.0427	10.5	0.0617	15.2	0.8	96.6
S3	1.774	12 (0)	0.1451	8.2	0.2905	16.4	1.1	93.3
W1	0.051	10 (1)	0.0046	9.0	0.0046	9.0	0.7	97.3
W2	0.386	10 (1)	0.0235	6.1	0.0441	11.5	1.3	91.7
W3	1.767	9 (0)	0.1970	11.2	0.3519	19.9	1.4	93.1

[a] Corrected by subtraction of the amount found in the control.

[b] Each value is the number of laboratories retained after the elimination of outliers; each value in parentheses is the number of laboratories removed as outliers.

[c] HorRat = RSD_R (found)/RSD_R (predicted from the Horwitz equation).

(**f**) *Citral*.—3,7-Dimethyl-2,6-octadienal, 95%. Purchase citral in small quantities, and assign a shelflife of 6 months; keep refrigerated. A yellow color indicates possible polymerization; replace discolored reagent.

(**g**) *Citral, 0.2% in ethyl acetate.*—Combine 200 L citral with 100 mL ethyl acetate. Prepare fresh monthly.

D. Preparation of Standard Solutions

(**a**) *Glyphosate stock standard solution.*—1000 g/mL. Place a known quantity (0.1 mg) of ca 50 mg glyphosate into a ca 118 mL (4 oz), narrow-mouth polyethylene or polypropylene bottle. Add to the bottle a known amount of water to produce a solution containing glyphosate at 1000 g/mL. Calculate W_W, weight of water needed (g), as follows:

$$W_W = \frac{W_S \quad P_S \quad D_W}{C_S}$$

where C_S = concentration of analyte in the final solution (1.0 mg/mL); W_S = weight of reference standard (mg); P_S = purity of the reference standard (100% = 1.00); and D_W = density of water (assume 1.00 g/mL).

(**b**) *AMPA stock standard solution.*—Prepare as described in (**a**), but use the AMPA reference standard.

(**c**) *Intermediate standard solutions*.—100, 10.0, and 1.0 g each analyte/mL. Prepare a single intermediate calibration solution containing both analytes, each at a concentration of 100 g/mL. Using disposable polypropylene transfer pipets, transfer 5.0 g aliquots of each stock standard solution, (**a**) and (**b**), into a single, tared, ca 118 mL (4 oz), narrow-mouth poly bottle. Dilute with water to a total weight of 50.0 g. In a similar manner, serially dilute the 100 g/mL intermediate standard to produce standards of 10.0 and 1.0 g/mL. Add 2–3 drops HCl to all stock and intermediate standard solutions, (**a**)–(**c**), as a preservative biocide. Store refrigerated at <5°C, and assign a shelflife of 6 months.

(**d**) *Working standard solutions*.—225, 36, and 4.5 ng each analyte/mL. Prepare by serial dilution, using automatic pipets with disposable tips, **B**(**c**). Use CAX mobile phase solution, **C**(**b**), as the diluent for all working standards. Prepare the 225 ng/mL standard by combining 1.8 mL 1.0 g/mL intermediate standard, (**c**), and 6.2 mL diluent. Combine 0.80 mL 225 ng/mL working standard with 4.20 mL diluent to produce the 36 ng/mL standard. Combine 1.0 mL 36 ng/mL standard with 7.0 mL diluent to produce the 4.5 ng/mL standard. Prepare fresh daily. These working standards will be derivatized, **E**(**e**), to produce calibration standard solutions at 50, 8.0, and 1.0 ng/mL.

Table 2000.05B. Interlaboratory study results for determination of AMPA in crops by GC/MSD

Material pair ID	$\bar{x}$, mg/kg[a]	No. of labs[b]	s_r	RSD_r, %	s_R	RSD_R, %	HorRat[c]	Recovery, %[a]
C1	0.046	11 (0)	0.0111	24.1	0.0111	24.1	1.0	87.5
C2	0.374	11 (0)	0.0413	11.1	0.0874	23.4	1.3	88.9
C3	1.699	11 (0)	0.2469	14.5	0.4215	24.8	1.7	89.4
S1	0.041	12 (0)	0.0097	23.7	0.0175	42.9	1.7	77.7
S2	0.364	12 (0)	0.0729	20.0	0.0872	24.0	1.3	86.6
S3	1.649	12 (0)	0.2501	15.2	0.3340	20.3	1.4	86.8
W1	0.048	11 (0)	0.0077	15.9	0.0135	27.9	1.1	92.4
W2	0.354	11 (1)	0.0400	11.3	0.0541	15.3	1.6	84.2
W3	1.671	9 (0)	0.1801	10.8	0.3883	23.2	1.6	88.0

[a–c] *See* footnotes in Table **2000.05A**.

Note: Glyphosate (and to a lesser extent, AMPA) adsorbs onto glass surfaces. This phenomenon is especially pronounced with standard solutions, but less pronounced when matrix is present and after derivatization. Volumetric glassware cannot be used for the preparation of standards because glassware cannot be washed adequately. Prepare standard solutions on the basis of weight, using an analytical balance. Use automatic pipets (Eppendorf-type), with disposable plastic tips, for handling most aqueous test samples and standards. Calibrate automatic pipets as directed by the manufacturer. Devices for handling test samples are given in appropriate sections.

E. Preparation of Test Solutions

(**a**) *Crop extraction*.—Place a 25 g test portion of a homogeneous crop into a wide-mouth, ca 236 mL (8 oz) poly bottle. Estimate the moisture content to within 10–20%. (Values from nutrient tables are acceptable.) After taking into account the amount of water in the test matrix, add volume of water that would bring the total volume of water to 125 mL. For example, a fruit crop that contains 80% water would require addition of 105 mL water.

Macerate at high speed for 3–5 min, using a laboratory homogenizer, **B**(**h**). Centrifuge for 10 min at ca 2000 *g*. Use a disposable glass pipet to transfer 20 mL aqueous supernatant to a 50 mL disposable polypropylene centrifuge tube, **B**(**j**). Add 15 mL methylene chloride to the tube, and shake 2–3 min. Centrifuge for 10 min at ca 2000 *g*. Use a 5 mL disposable glass pipet to transfer a 4.5 mL aliquot of the aqueous layer to an 8 mL glass vial. Add 0.50 mL acidic modifier solution, **C**(**c**); cap and shake vial. Centrifuge for 10 min at 2000 *g*.

Alternatively, place test portion in a Waring-type laboratory blender jar, add water as above, and blend at high speed for 3–5 min. Transfer ca 40 mL crude extract to a 50 mL disposable polypropylene centrifuge tube. Centrifuge for 10 min at ca 2000 *g*. Transfer 20 mL supernatant to a clean 50 mL polypropylene tube, and continue with methylene chloride partitioning step as described above.

(**b**) *Matrix-specific modifications*.—(*1*) *Dry crops*.—For crops, such as straw, that adsorb large amounts of water, reduce the test portion size to 12.5 g, leaving the extraction volume unchanged. Adjust the other aliquot volumes as shown in Table **2000.05C**. Prepare working standards, **D**(**d**), at concentrations of 150, 24, and 3.0 ng/mL. Prepare calibration standards, derivatized in **E**(**e**), at 37.5, 6, and 0.75 ng/mL. (*2*) *High protein crops*.—For crops such as dried beans, add 100 L HCl to 20 mL aliquot of crude extract; cap and shake vial. Centrifuge at ca 2000 *g* for 10 min. Transfer 15 mL supernatant to a clean, 50 mL centrifuge tube, and continue with the methylene chloride partitioning step. (*3*) *High oil crops*.—For crops such as nuts, use a disposable glass pipet to remove the organic layer after the partitioning with methylene chloride. Add a second, 15 mL aliquot of methylene chloride, and repeat the partitioning.

(**c**) *CAX cleanup*.—Prepare the disposable CAX cleanup column, **B**(**d**), by shaking the capped column and allowing the resin to settle to the bottom. Let column drain, using gravity elution for this and all other elution steps. With gravity elution, the flow should stop as soon as the liquid reaches the top of the column bed without draining the column bed. Process test samples in a timely manner; do not let the resin become dry. Multiple test solutions may be prepared by using an appropriate solid-phase extraction (SPE) manifold, but do not use vacuum. Wash the resin with two 5 mL portions of water. Use an automatic pipet, **B**(**c**), with disposable plastic tips to transfer 1.0 mL extract (representing 0.18 g normal crop or 0.09 g dry crop) to the column reservoir. Because the extract may wet the surface of the pipet tip, equilibrate the tip by withdrawing and expelling extract before adding extract to the column. Use care to minimize disruptions to the column bed when making this and subsequent additions to the column reservoir. Elute to the top of the column bed; discard the eluate. Add 0.70 mL CAX mobile phase solution, **C**(**b**), to the reservoir and elute; again discard the eluate. Repeat with a second 0.70 mL portion. Elute the analytes with 12.0 mL CAX mobile phase solution, collecting the eluate in a 50 mL round-bottom flask. Evaporate just to dryness, using a rotary evaporator, with the water bath set at ≤40°C. Alternatively, collect the eluate in a 50 mL centrifuge tube, and evaporate using a vacuum Vortex-type evaporator. Dissolve the residue in 2.0 mL CAX mobile phase solution (for dry crops, use 1.5 mL). Dissolve the residue by swirling the flask to completely rinse the sides (only up to the neck); then stopper the flask, and shake well.

Notes: (*1*) The method may be interrupted before evaporation of the CAX eluate. Extracts may be stored refrigerated for ≤7 days before the method is resumed. Prederivatization extracts (after evaporation of eluate and redissolution of the residue) may also be similarly retained. Do not store dried residue without addition of solvent. (*2*) Low recoveries may be improved, or problematic interferences may be reduced, by altering the CAX elution scheme. To the CAX column, add an aliquot of an extract from a control matrix fortified with analyte at ca 2.0 mg/kg; elute with 15 mL CAX mobile phase solution. Collect the first 3–4 mL eluate in 0.5–1.0 mL fractions. Increase volume to 1–2 mL per fraction for the remaining

Table 2000.05C. Parameters for calculation of final crop-to-solvent ratio

Parameter	Section	Normal crops	Dry crops
C_{CS} = Final crop:solvent ratio, g/mL	**G**(**a**)	0.02	0.015
W_{test} = Wt of crop extracted, g	**E**(**a**), (**b**)	25	12.5
$V_{solvent}$ = Total volume of crop extract, mL	**E**(**a**)	125	125
V_{crude} = Volume of crude extract aliquot combined with acidic modifier, mL	**E**(**a**)	4.5	4.5
V_{mod} = Volume of acidic modifier added to crude extract, mL	**E**(**a**)	0.50	0.50
$V_{cax\text{-}aliquot}$ = Volume of extract aliquot subjected to CAX cleanup, mL	**E**(**c**)	1.0	1.0
$V_{cax\text{-}final}$ = Volume used to dissolve residual material after evaporation, mL	**E**(**c**)	2.0	1.5
V_{deriv} = Volume of purified extract subjected to derivatization, mL	**E**(**e**)	0.050	0.050
V_{final} = Final volume before analysis, mL	**E**(**e**)	0.225	0.200

eluate. Derivatize an aliquot from each fraction, and analyze to determine the elution pattern of analytes and interferences.

(**d**) *Preparation of chilled derivatization reagent*.—Prepare the derivatization reagent in a glass container of suitable size with a PTFE-lined cap by adding one volume of 2,2,3,3,4,4,4-heptafluoro-1-butanol to 2 volumes of trifluoroacetic anhydride. Do not fill the container to >75% of capacity. Cap the container, and gently invert container 3–4 times. Carefully loosen the cap to release any pressure. Prepare the reagent mixture fresh daily. Use a 2 mL disposable glass pipet to add 1.6 mL portions of the derivatization reagent mixture to 4.0 mL screw-top vials, **B**(**f**). Cap the vials, using phenolic plastic, PTFE-lined caps, **B**(**e**). Place the vials in an aluminum heating/cooling block with 15 or 16 mm diam. holes. Place the block on a slab of dry ice or in a pan containing crushed dry ice. Cool the vials to –40 to –60°C, as measured by a thermometer placed in the block.

(**e**) *Analyte derivatization*.—*Note*: Use caution when handling chilled vials; wear gloves. Use an automatic pipet, **B**(**c**), with disposable plastic tips to add a 50 L aliquot of extract or working standard solution to the prechilled derivatization reagent, (**d**), in the following manner. Withdraw 50 L extract or standard into the pipet tip. Place the pipet tip under the surface of the reagent, and slowly release the contents. Immediately rinse the pipet tip by repeatedly withdrawing (3–4 times) reagent into the tip and releasing it into the vial; always keep the pipet tip under the surface of the reagent.

Note: The volume of extract or working standard added to the reagent mixture must remain constant for all derivatizations within a set.

Cap the vial, and return it to the cooling block. After all extracts are processed, remove all vials from the block, and let vials equilibrate to room temperature (25 C), checking to ensure the caps are tightened securely. Place the vials for 1 h in an aluminum heating block (15 or 16 mm holes) maintained at 85 –90°C. After 5 min of heating, check to ensure that all caps remained tightened securely. Shake vials every 15 min; ensure also that no evaporation is occurring by checking the level of reagent in the vials. After heating, remove the vials from the heating block, and let vials cool to room temperature (25 C). Evaporate excess derivatization reagent under a stream of N, using an evaporation manifold, **B**(**g**), to facilitate processing of multiple vials. The N delivery tube of the manifold should be placed just within the neck of each vial. During evaporation, the vials should be maintained at 40 –50 C. Once apparent dryness has been achieved, keep vials under the stream of N at 40 –50 C for an additional 30 min, because residual derivatization reagents or by-products can affect the chromatography of the analytes. After evaporation, cap the vials, and let vials cool to room temperature (25°C). Promptly add 225 L ethyl acetate containing 0.2% citral, **C**(**g**), using a 250 L glass syringe. (Add 200 L for dry crops.) Minimize exposure of residue to air. Cap the vial, and mix on a Vortex mixer for 20 s to dissolve contents. Use an automatic pipet (10–100 L) to transfer contents to a crimp-top autosampler vial containing a limited volume (250 L) insert.

F. Determination

(**a**) *Mass-selective detector tuning procedure*.—*Note*: In place of the standard tuning procedure, the following procedure is given as a method for increasing the sensitivity of the detector. In addition, optimum detectability of the high-mass fragments is achieved with a clean MSD electron source unit.

Use PFTBA as the calibration standard, and perform the tuning procedure recommended by the instrument manufacturer to ensure the proper functioning of the detector. After a successful standard tuning, perform the following manual tuning procedure. Select the tuning masses *m/z* 414, 502, and 614, with a scan range of *m/z* 350–650. Manually adjust the following parameters. Reduce the amu gain setting, thereby increasing the bandwidth of the 3 tuning masses from 0.5 amu to a range of 1.5–2.0 amu. Widening the bandwidth further increases the detectability at the expense of selectivity. Adjust the mass gain and mass offset to obtain the proper mass assignments, although the increased bandwidth helps to eliminate the need for precise adjustment. Operate the mass-selective detector in the SIM mode, using *m/z* 446 for the AMPA derivative and *m/z* 611.5 for the glyphosate derivative. Program the detector to switch from *m/z* 446 to 611.5 halfway between the elution of the AMPA derivative and the glyphosate derivative. Unless a confirmational analysis, **F**(**e**), is being performed, only one ion should be monitored at a time. Adjust the electron multiplier voltage as needed to obtain the optimum signal-to-noise ratio, while maintaining a linear response across the calibration range.

(**b**) *Equilibration of chromatographic system*.—The derivatized matrix/analyte combination requires that a thorough equilibration of the GC inlet system be performed before analysis so that active adsorption sites are deactivated. Including citral in the injection solvent also helps to maintain a deactivated inlet system. The injection of derivatized extracts containing high levels of analytes is also helpful. This is especially critical when a new column or inlet liner is installed. To equilibrate the system, make repeated injections, alternating between 2 injections of a derivatized extract and 1 injection of a 50 ng/mL calibration standard, until the standard provides a reproducible response. System deactivation/equilibration may be matrix dependent. As a result, conduct equilibration using derivatized extracts of the same matrix to be analyzed in the current run. If other matrixes are to be subsequently analyzed, re-equilibrate with a derivatized extract of that matrix. Do not analyze different matrixes within the same run.

(**c**) *System performance*.—The signal-to-noise (peak-to-peak) ratio for the 1.0 ng/mL standard should be ≥10:1. The AMPA derivative elutes at about 4–7 min, with the glyphosate derivative eluting about 0.5–1.0 min after the AMPA derivative. The peak width (at half height) of the analyte peaks should be from 0.6 to 2.0 s. The RSD of the response resulting from repeated injections of the 1.0 ng/mL standard should be <10%.

(**d**) *GC analysis*.—After equilibration of the instrument, make single injections of each of the calibration standard solutions: 50, 8.0, and 1.0 ng/mL. Make single injections of each of the test extracts. Reinject the standards after every 4–8 injections of extract. After injection of all extracts, inject each of the calibration standard solutions.

(**e**) *Confirmational analysis*.—Three major fragments can be used for the determination of the AMPA derivative: *m/z* 372, 446, and 502. Three major fragments exist for the determination of the glyphosate derivative: *m/z* 611.5, 584, and 460. Although *m/z* 446 and 611.5 provide the greatest response for the AMPA and glyphosate derivatives, respectively, the alternative ions can be used for confirmatory analyses. The alternative ions may also be helpful for eliminating or reducing problematic interferences.

G. Calculations

(**a**) *Final crop-to-solvent ratio*.—Using the parameters in Table **2000.05C**, calculate the amount of crop represented in the final extract, C_{CS}, as follows:

$$C_{CS} = \frac{W_{test}}{V_{solvent}} \quad \frac{V_{crude}}{V_{crude}} \quad \frac{V_{cax\ aliquot}}{V_{mod}} \quad \frac{V_{deriv}}{V_{cax\ final}} \quad \frac{V_{deriv}}{V_{final}}$$

(**b**) *Calibration, linear*.—Calculate the response factor, $RF_{1–3}$, at each calibration level, $C_{1–3}$, by using the average response, $R_{1–3}$, resulting from all injections of the calibration solution at that level.

$$RF_{1–3} = \frac{C_{1\ 3}}{R_{1\ 3}}$$

Average the response factors, $RF_{1–3}$, from all calibration levels to obtain the average response factor, RF_{avg}. Calculate the percent deviation, $D_{1–3}$, of the individual response factors from the average as follows:

$$D_{1–3} = \frac{RF_{1\ 3} \quad RF_{avg}}{RF_{avg}} \quad 100$$

If the deviation of the response factor at any level is >20%, follow the recalibration instructions, (**d**). Calculate the concentration of analyte in the final extract, C_E, using single-point calibration. Use the *RF* of the standard with the response closest to that of the unknown, if the test sample response, R_S, is within 50–200% of the standard response. If the test sample response is not within 50–200% of the average standard response at any calibration level, use the average *RF* from the 2 standards with responses that bracket the response of the test sample. Use the *RF* of the low-level calibration solution, equivalent to the LOQ, to determine the background concentrations in controls.

$$C_E = RF \quad R_S$$

Calculate crop residue levels in the original test sample, C_R, as follows:

$$C_R = \frac{C_E}{C_{CS}}$$

(**c**) *Calibration, nonlinear*.—The linear response range may vary by instrument. Deviations from linearity are more common when the calibration range is extended. A second-order polynomial curve fit is the recommended alternative calibration to calculate C_E for nonlinear responses. When using nonlinear calibration methods, use a forced-zero *y*-intercept.

(**d**) *Recalibration*.—If recalibration is required, follow the procedure outlined for equilibration of the GC system, **F**(**b**). If subsequent calibrations are not improved, prepare new working standard solutions, **D**(**d**), and rederivatize those standards to produce new derivatized calibration solutions, **E**(**e**). Concurrently, rederivatize extracts, taking aliquots from the retained purified extracts, **E**(**c**). Recalculate the values of $D_{1–3}$, **G**(**b**); if values still remain >20%, use the nonlinear calibration, **G**(**c**).

H. Quality Control

Minimum quality control requirements include initial demonstration of laboratory capability and analysis of calibration standards, reagent blanks, control samples, and laboratory-fortified controls, as described below.

(**a**) *Calibration standards*.—Initial determination of instrument performance includes the preparation and analysis of calibration standards covering the expected range of use. The primary consideration is the detectability of the analytes. The signal-to-noise ratio obtained from the injection of a calibration standard at a concentration equivalent to the concentration of analyte in a test sample at the LOQ should be >10:1. Replicate injections of this standard should provide responses with an RSD of <10%.

(**b**) *Reagent blanks and control matrix materials*.—Initial reagent blanks should be run to demonstrate that all reagents and glassware are free from interferences. *Note:* This is extremely important because of the adsorptive properties of the analytes. Run at least one unfortified control matrix with each set of test samples. Control matrixes are required when determinations must be corrected for the amount of the analyte or the presence of background in the matrix, or when recovery data are required. For recovery determinations at 1–2 times the LOQ, the calculated concentration of background should be less than the LOQ.

(**c**) *Laboratory-fortified control*.—At least one laboratory-fortified control is required for each set to demonstrate method recovery. Fortify a known amount of blank matrix at a level equal to 1–2 times the LOQ, using the intermediate standard solutions, **D**(**c**). If multiple test matrixes are used, run one laboratory-fortified control for each matrix. Calculate percent recovery for each analyte, correcting for the background concentrations found in the control sample, (**b**). Recovery should be 70–130% of the fortification level.

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