APPLICATION NOTE 000282

Determination of glyphosate and AMPA in oat flour by IC-MS

Authors: Jingli Hu and Jeffrey Rohrer

Keywords: Single quadrupole mass spectrometer, Dionex Integrion HPIC, Dionex IonPac AS19-4µm column, ISQ EC, Dionex ADRS 600 suppressor

Goal

To develop a method to determine glyphosate and AMPA in oat flour by coupling ion chromatography with single quadrupole mass spectrometry (IC-MS)

Introduction

Glyphosate is the active ingredient in the popular herbicide Roundup® and is widely used for weed control in cultivated and uncultivated areas. There are concerns about its potential adverse effects on human health, such as its potential carcinogenicity.[1] The bacteria in soil break down glyphosate into aminomethylphosphonic acid (AMPA), which may also be toxic to humans.[2] Due to its widespread use, trace amounts of glyphosate residues may be found in various fruits, vegetables, and cereals. The United States Environmental Protection Agency (EPA) set the maximum amount of glyphosate at 30 mg/kg for oats.[3] Glyphosate was reported to be present in oat-based cereals up to 2,837 µg/kg by the Environmental Working Group (EWG), a nonprofit organization.[4] Although the amounts of glyphosate found in oat products were well below the EPA tolerance, the EWG is petitioning the EPA to lower the acceptable amount of glyphosate in oats.

Determining glyphosate and AMPA is challenging due to their high polarity, low volatility, and lack of a chromophore. Glyphosate can be determined in food products by HPLC or GC. However, those methods require tedious and time-consuming derivatization. Ion chromatography with mass spectrometry (IC-MS) is more suitable for glyphosate determinations because these pesticides and their metabolites are ionic. As a result, a direct analysis with no derivatization is possible. An ion chromatography - tandem mass spectrometry (IC-MS/MS) method was developed for the determination of glyphosate, AMPA, and other polar pesticides in food products.[5]

An IC system coupled to an economical and simple-to-use single quadrupole mass spectrometer can be used to screen for the presence of ionic pesticides. The Thermo Scientific™ ISQ™ EC single quadrupole mass spectrometer seamlessly integrates IC with MS, taking advantage of the strengths of both techniques. Anion exchange chromatography using eluent generation and suppressed conductivity detection provides chromatographic selectivity, analytes in the ionic form, and compatibility with MS, which provides selectivity based on the mass-to-charge ratio of the analyte. Electrospray ionization (ESI) is used to introduce the liquid IC stream (after suppression) as a fine spray into the MS source. The heated electrospray ionization probe (HESI-II) probe improves the ESI interface by allowing the use of high temperatures and voltage to deliver better desolvation and enhanced sensitivity; thus, a make-up solvent is not needed.

The objective of the present work was to develop an IC-MS method to simultaneously determine glyphosate and AMPA in oat flour. Oat flour sample extracts were directly injected for analysis, and chromatographic separation was achieved in 30 min. The mass spectrometer was operated in selected ion monitoring (SIM) mode, allowing minimal sample cleanup and ensuring sensitive and selective quantification. Isotope-labeled AMPA (^{13}C, 99%; ^{15}N, 98%; methylene-D_2, 98%), and glyphosate (2-^{13}C, ^{15}N) were used as internal standards to ensure quantitation accuracy. Performance data were collected for the method: recovery, precision, sensitivity, and calibration range. Together these data show that the IC-MS can successfully determine the two targeted analytes in oat flour samples.

Experimental

Equipment

- Thermo Scientific™ Dionex™ Integrion™ HPIC™ system* (P/N 22153-60208) including:
  - Eluent generator
  - Pump
  - Degasser
  - Conductivity detector
  - Second 6-port injection valve (P/N 22153-62027) used as a diverter valve
  - Thermo Scientific™ Dionex™ IC PEEK Viper fitting kit (P/N 088798)
  - Column oven temperature control
  - Detector-suppressor compartment temperature control
  - Tablet control
- Thermo Scientific™ Dionex™ AS-AP Autosampler (P/N 074925), with 250 µL syringe (P/N 074306), 1,200 µL buffer line assembly (P/N 074998), 10 µL injection loop and 1.5 mL vial trays (P/N 074936)
- ISQ EC single quadrupole mass spectrometer (P/N ISQEC000IC) including Thermo Scientific™ HESI-II probe (P/N 70005-60155)
- Thermo Scientific™ Dionex™ AXP-MS auxiliary pump (used to deliver suppressor external water) (P/N 060684)
- Nitrogen generator with capacity for 3L/min flow at 100 psi (110 V: P/N 1R77606-1120; 230 V: P/N 1R77606-1230)

* This method can also be run on a Thermo Scientific™ Dionex™ ICS-5000+ dual IC system or Thermo Scientific™ Dionex™ ICS-6000 dual IC system using the second pump to deliver suppressor external water.

Software

Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) version 7.2.9

Consumables

- Thermo Scientific™ Dionex™ EGC 500 KOH cartridge (P/N 075778)
- Thermo Scientific™ Dionex™ CR-ATC 600 continuously regenerated anion trap column (P/N 088662)
- Thermo Scientific™ Dionex™ ADRS 600 anion dynamically regenerated suppressor, 2 mm (P/N 088667)
- Thermo Scientific™ Dionex™ On Guard II RP 2.5 cc cartridge (P/N 057084)
- Dionex AS-AP autosampler vials 10 mL (P/N 074228)
- Dionex AS-AP autosampler vials 1.5 mL (P/N 079812)
- Fisherbrand™ narrow-mouth field sample bottles, high density polyethylene (HDPE), 125 mL, 250 mL sizes for storage of standards and samples (Fisher Scientific P/N 02-895A, B)
- Thermo Fisher™ Nalgene™ syringe filter PES 0.2 µm (Fisher Scientific P/N 09-740-113)

Reagents and standards

- Deionized (DI) water, Type I reagent grade, 18 MΩ·cm resistivity or better
- Sodium and potassium salts, A.C.S. reagent grade or better, for preparing anions standards
- Glyphosate, 1,000 µg/mL in DI water (Restek P/N 32426)
- Aminomethylphosphonic acid (AMPA), (Alfa Aesar P/N L09833, 250 mg)
- Glyphosate (96 chemical purity) (2-^{13}C, 99%; ^{15}N, 98+%) 100 µg/mL in H_2O (Cambridge Isotope Laboratories P/N CNLM-4666-1.2)
- Aminomethylphosphonic acid (AMPA) (^{13}C, 99%; ^{15}N, 98%; methylene-D_2, 98%) 100 µg/mL H_2O (Cambridge Isotope Laboratories P/N CDNLM-6786-1.2)
- Methanol, HPLC grade, (Fisher Scientific P/N A452SK-4)

Samples

Three oat flour samples were analyzed in this study. S1 and S2 were provided by the NIST collaborative study organizer. S3 was an organic oat flour from a local grocery store.

System preparation and setup

Figure 1 shows the flow diagram of the IC-MS system. The Integrion HPIC system is plumbed as a Reagent-Free ion chromatography (RFIC™) system using eluent generation following the Dionex Integrion installation and operator manuals.[6] Install the suppressor in external water mode using a Dionex AXP-MS pump to provide the DI water regenerant.[7] The Dionex AXP-MS pump can be added in the instrument configuration, and thus can be controlled by the Chromeleon chromatography workstation software. The ISQ EC is installed according to its installation guide.[8]

Chromatographic conditions

Parameter	Value
Columns	Thermo Scientific™ Dionex™ IonPac™ AG19-4µm guard column, 2 × 50 mm (P/N 083225) Thermo Scientific™ Dionex™ IonPac™ AS19-4µm analytical column, 2 × 250 mm (P/N 083223)
Eluent	14 mM KOH from 0 to 10 min, 14–60 mM KOH from 10 to 14 min, 60 mM from 14 to 25 min, 14 mM from 25.1 to 30 min
Eluent source	Thermo Scientific™ Dionex™ EGC 500 KOH cartridge with Thermo Scientific™ Dionex™ CR-ATC 600
Flow rate	0.35 mL/min
Injection volume	25 µL in push-full injection mode
Column temperature:	30 °C
Detection 1	**Suppressed conductivity**
Suppressor	Thermo Scientific™ Dionex™ ADRS 600 (2 mm) suppressor, external water mode (flow 0.35 mL/min), 52 mA current
Detection/Suppressor compartment	20 °C
Cell temperature	35 °C
Background conductance	<1 µS/cm
System backpressure	~4,500 psi (100 psi = 689.5 kPa)
Noise	<1 nS/cm
Run time	30 min
Detection 2	**Mass spectrometry**
MS detector	ISQ EC single quadrupole MS
Ionization interface	Electrospray ionization (ESI), negative mode
Diverter valve switch time	0–8 min to waste, 8–25 min to MS
Sheath gas pressure	40 psi
Other parameters	
Aux gas pressure	2 psi
Sweep gas pressure	1 psi
Source voltage	-2,500 V
Vaporizer temp.	350 °C
Ion transfer tube temp.	350 °C
Chrom. filter peak width	Off
Scan mode	Table 1

Table 1. MS scan mode

Scan name	Mass list (amu)	Dwell or scan time (s)	SIM width (amu)	Ion polarity	Spectrum type	Source CID voltage (V)
AMPA	110	0.37	0.3	Negative	Centroid	10
AMPA IS	114	0.37	0.3	Negative	Centroid	10
Glyphosate	168	0.37	0.3	Negative	Centroid	10
Glyphosate IS	170.1	0.37	0.3	Negative	Centroid	10

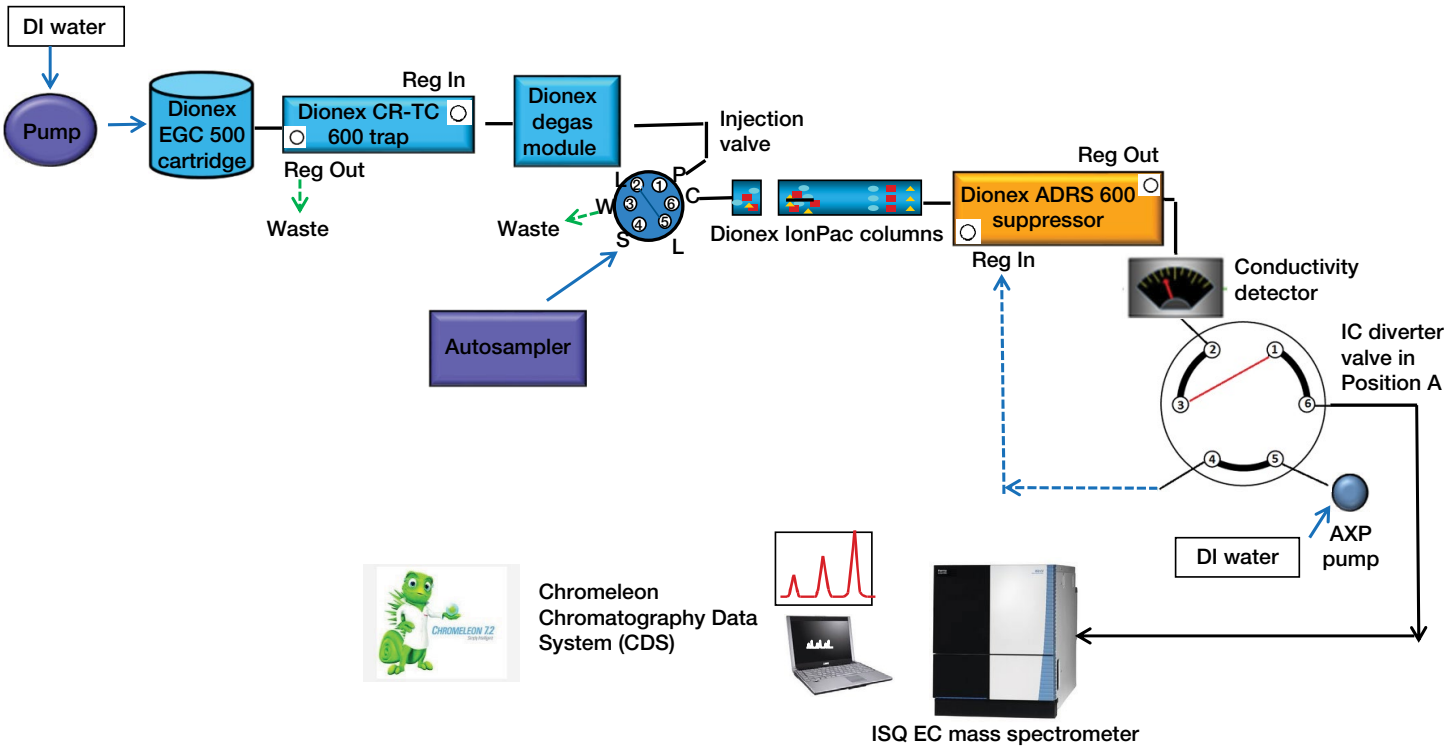


Figure 1. Flow diagram for IC-CD/MS with diverter valve in "A" position

A 6-port diverter valve is placed between the conductivity detector (CD) and the mass spectrometer. The diverter valve can be operated in two positions (Figure 2). A small piece of red PEEK tubing called a "jumper" is installed in the IC diverter valve connecting port 1 to port 3. In position A, eluent flows from the CD to the mass spectrometer, and the AXP delivers water to the suppressor Regen In. In position B, eluent flow is in recycle mode for the suppressor, and the AXP delivers water to the mass spectrometer. Configure the diverter valve in the instrument method script editor to divert everything to waste except the compounds of interest. Detailed instructions for configuring the IC-MS system are in Technical Note 72611[9].

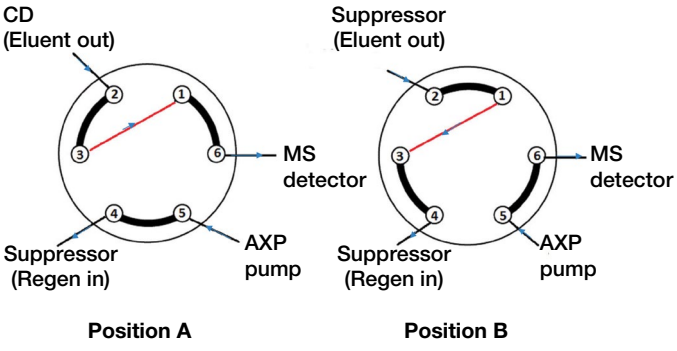


Figure 2. Diverter valve position

Precautions

1. Allow the system to equilibrate until the total conductivity is <1.5 µS/cm, and then it is safe to connect the IC flow to an operating mass spectrometer. In other words, keep the divert valve at position B, with flow from the Dionex AXP-MS auxiliary pump to the mass spectrometer until the background conductivity is below 1.5 µS/cm. This can prevent the non-volatile eluent from precipitating inside the ESI capillary.

2. The column used in this application has an inner diameter of 2 mm. Red PEEK tubing (0.005 in. i.d.) from the CD to the MS detector can be used to improve MS sensitivity. However, keep this tubing as short as possible to minimize backpressure on the suppressor. High backpressure can cause irreversible damage to the suppressor.

3. The mass spectrometer needs to be "baked out" when the system is idle for over a day or when the MS peak area reproducibility becomes poor. To bake out the mass spectrometer, set the vaporizer temperature to 500 °C, ion transfer temperature to 400 °C, sheath gas to 50 psi, aux gas to 10 psi, and sweep gas to 1 psi, and then deliver DI water to the mass spectrometer at 0.1 mL/min with the Dionex AXP-MS pump. Allow the system to bake out for at least 2 h.

Preparation of solutions and reagents

AMPA stock standard solution (1,000 mg/L): AMPA Stock standard solution can be prepared by dissolving 10 mg of AMPA in 10 mL of DI water.

Glyphosate stock solution (1,000 mg/L) is commercially available.

AMPA and glyphosate working solution mixture (100 µg/L): Prepare 100 µg/L of working standard solution mixture (glyphosate, AMPA) by diluting the standard stock solution with DI water. (Table 2)

Table 2. Volume of stock used to prepare 100 mL of a two pesticides mixture (100 µg/L)

Analyte	Stock concentration (mg/L)	Stock source	Stock volume (µL)
Glyphosate	1,000	Commercially available	10
AMPA	1,000	Prepare from powder	10

AMPA and glyphosate internal standard working solutions (ISTD) mixture (1 mg/L): Prepare the internal standard working solution mixture ((AMPA) (^{13}C, 99%; ^{15}N, 98%; methylene-D_2, glyphosate (2-^{13}C, ^{15}N) by diluting the internal standard stock solutions with DI water. (Table 3)

Table 3. Volume of stock used to prepare 10 mL of a two pesticides isotope internal standard mixture (1 mg/L)

Analyte	Stock concentration (mg/L)	Stock source	Stock volume (µL)
(AMPA) (^{13}C, 99%; ^{15}N, 98%; methylene-D_2)	100	Commercially available	100
Glyphosate (2-^{13}C, ^{15}N)	100	Commercially available	100

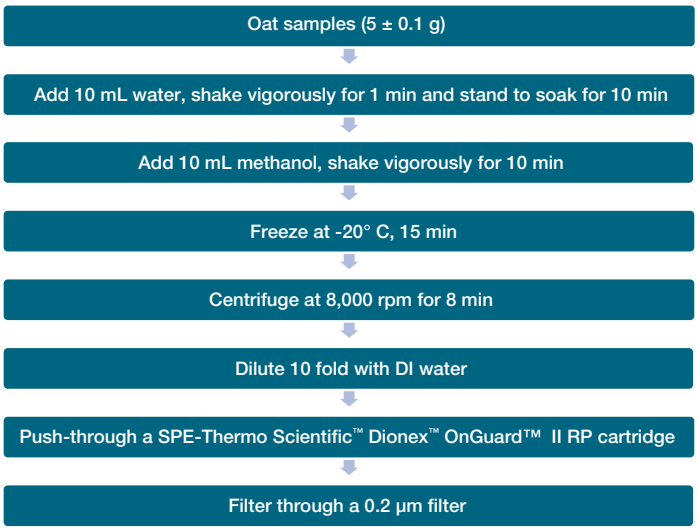
Working standard solutions: Diluted working standard solutions were prepared using the two pesticides working solution (100 µg/L). (Table 4). The mixed calibration standard solutions had concentrations of 0.5, 1, 2.5, 5, 10, 20, and 50 µg/L.

Table 4. Calibration standards

Calibration standard	Target concentration (µg/L)	Volume (µL) of glyphosate and AMPA standard mixture (100 µg/L)	DI H_20 (µL)
1	0.5	5	995
2	1	10	990
3	2.5	25	975
4	5	50	950
5	10	100	900
6	20	200	800
7	50	500	500

Sample preparation

Oat flour samples were extracted by following the modified Quick Polar Pesticides Extraction (QuPPE) sample preparation method.[10]



Standard and sample with ISTD

Add 10 µL of ISTD (1 mg/L) to each 1 mL of calibration standard or sample.

Results and discussion

Separation

The Dionex IonPac AS19-4µm hydroxide-selective anion-exchange column is a high capacity and high-resolution column. These factors are critical for the determination of pesticides at the low µg/L concentrations in samples containing high concentrations of common anions such as chloride, nitrate, and sulfate.[11] Figure 3 shows a separation of common anions, AMPA, and glyphosate within 30 min using a Dionex IonPac AS19-4µm column. The top chromatogram displays the CD profile of all anions. The bottom chromatogram displays the MS profile of the two analytes of interest, AMPA and glyphosate. As Figure 3 shows, AMPA and glyphosate were resolved from common inorganic anions.

A delay time of 0.17 min is applied to the MS channel to match the CD channel. The delay time is the time required for the analyte to travel from one detector to another when they are in series. Here, the analyte goes through the CD cell before going into the mass spectrometer. The delay time can be set in the Chromeleon software in: Processing Method-Advanced Setting-Delay Time.

Limit of detection (LOD) and limit of quantification (LOQ)

Several approaches for determining the detection limit are possible. The LOD method is based on the signal-to-noise (S/N) ratio. Determination of the S/N ratio is performed by comparing measured signal from a low concentration standard and establishing the minimum concentration at which the analyte can be reliably detected. A S/N=3 is used for estimating the limit of detection (LOD) and S/N=10 is used for estimating the limit of quantification (LOQ).[12] In this study, the baseline noise was first determined by measuring the peak-to-peak noise in a representative 1 min segment of the baseline where no peaks elute, but close to the peak of interest. The signal was determined from the average height of three injections of a 0.5 µg/L standard for AMPA, and 0.1 µg/L for glyphosate. The estimates of LOD for AMPA and glyphosate are summarized in Table 5. Figure 4 shows the chromatography of a 0.5 µg/L calibration standard. AMPA and glyphosate are detected.

Table 5. Limits of detection (LOD) and limits of quantification (LOQ)

	LOD (µg/L) in solution	LOQ (µg/L) in solution	LOD (ng/g) in oat flour	LOQ (ng/g) in oat flour
Glyphosate	0.0725	0.242	2.90	9.66
AMPA	0.284	0.947	11.4	37.9

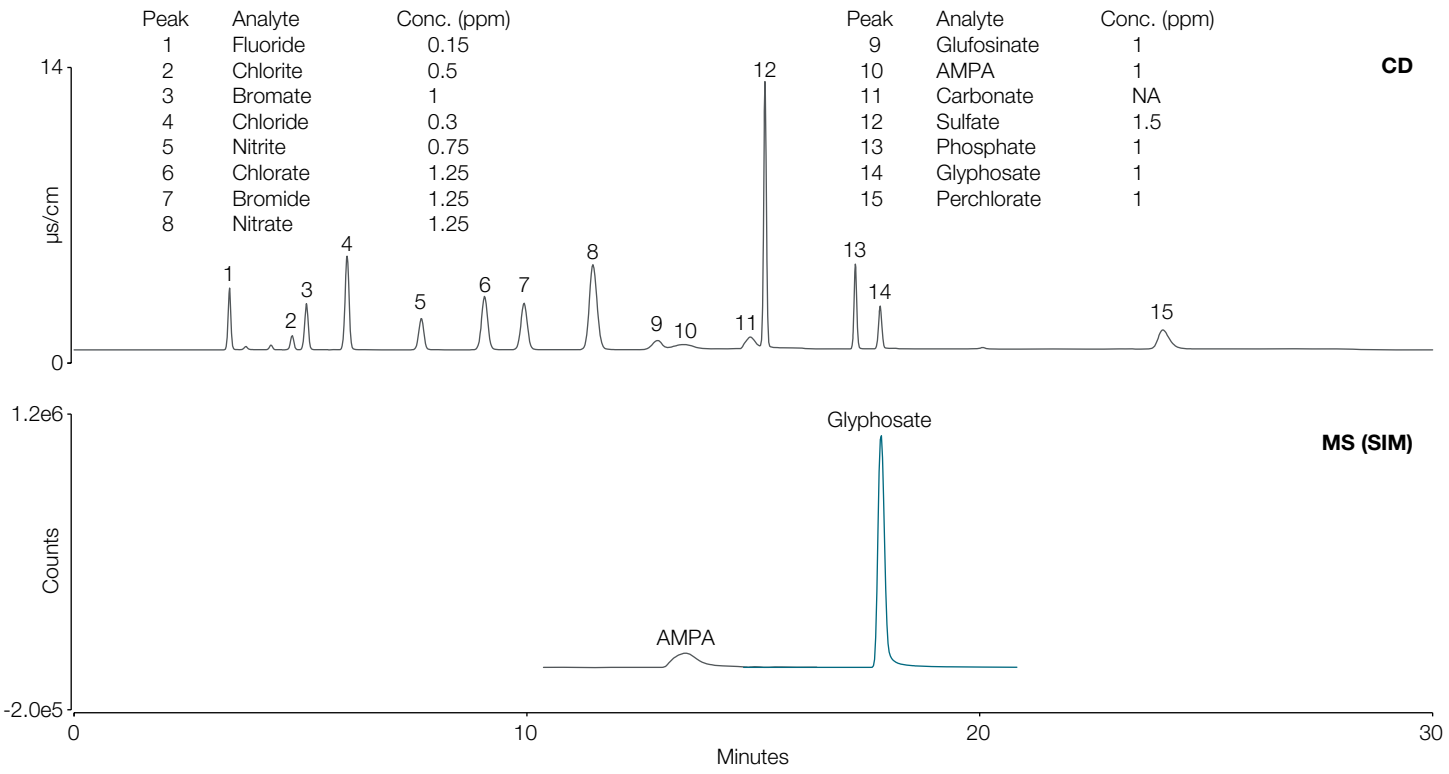


Figure 3. Separation of common anions, glyphosate, and AMPA

Calibration

Calibration standard mixtures (AMPA and glyphosate) in the range of 0.5–50 µg/L were prepared in DI water. The two ISTD mixture was spiked into each calibration standard at 10 µg/L. Each concentration level was injected three times. The internal standard method provides a means to account for losses in ionization efficiencies due to components of the matrix that may compete for ion formation in the source. The use of isotopically labeled internal standards ensures that both compound identification and compound quantification are of the highest degree of precision and accuracy possible. Table 6 summarizes the calibration results. Calibration curves were generated using internal standard calibration (Figure 5). The coefficient of determination is greater than 0.999 for all components.

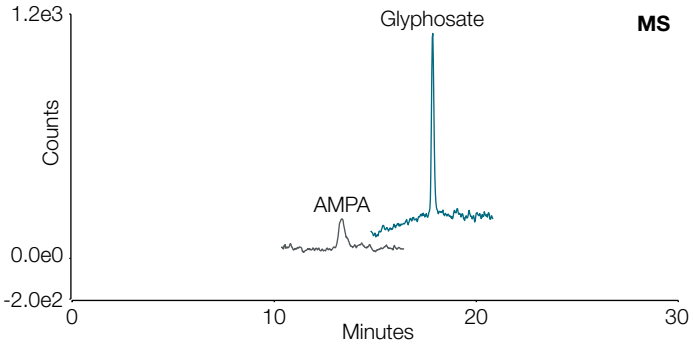


Figure 4. Calibration standard mixture (0.5 µg/L) in DI water

Sample analysis

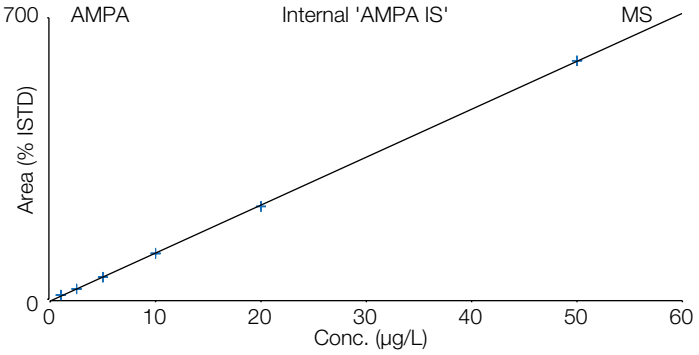
Three oat flour samples were analyzed in this study. Each sample was extracted in duplicate by the modified QuPPE method. Table 7 summarizes the amounts of AMPA and glyphosate in the three samples. AMPA was not detected in the three oat flour samples. Glyphosate was detected at concentrations ranging from 33.4 to 181 ng/g. Figure 6 shows the glyphosate chromatographic profile (CD and MS) of oat flour sample #1 (ISTD is not added to demonstrate the sensitivity difference.) Glyphosate was sensitively detected by MS; however, it was not detected by the CD.

Table 7. AMPA and glyphosate in oat flours (ng/g)

	AMPA		
	Extraction A	Extraction B	Average
Sample 1	ND	ND	ND
Sample 2	ND	ND	ND
Sample 3	ND	ND	ND
	Glyphosate		
Sample 1	181	182	181
Sample 2	54.3	54.0	54.1
Sample 3	33.8	33.0	33.4

Table 6. Calibrations

	Range (µg/L)	Calibration type	Internal standard (ISTD)	ISTD concentration (µg/L)	Coefficient of determination (r^2)
Glyphosate	0.5–50	Internal, linear	Glyphosate (2-^{13}C, ^{15}N)	10	0.9999
AMPA	1–50	Internal, linear	(AMPA) (^{13}C, 99%; ^{15}N, 98%; methylene-D_2	10	0.9999



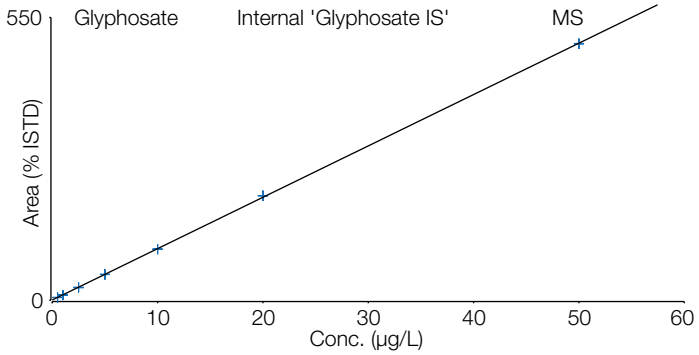


Figure 5. MS calibration curve

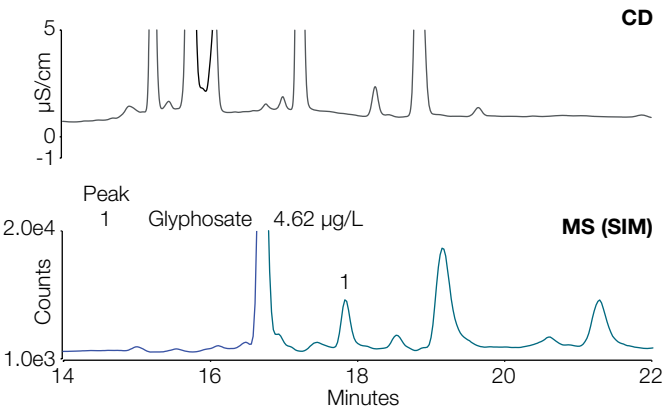


Figure 6. Sample #1 CD vs MS channel

Method accuracy

Method accuracy was evaluated through recovery studies using the oat flour sample extracts. AMPA and glyphosate were spiked into the sample extracts at 1, 2, and 10 µg/L. Figures 7 and 8 and Table 8 show the results of this study. The recoveries for AMPA and glyphosate in the three samples were in the range of 96 to 101%, suggesting that the method is accurate.

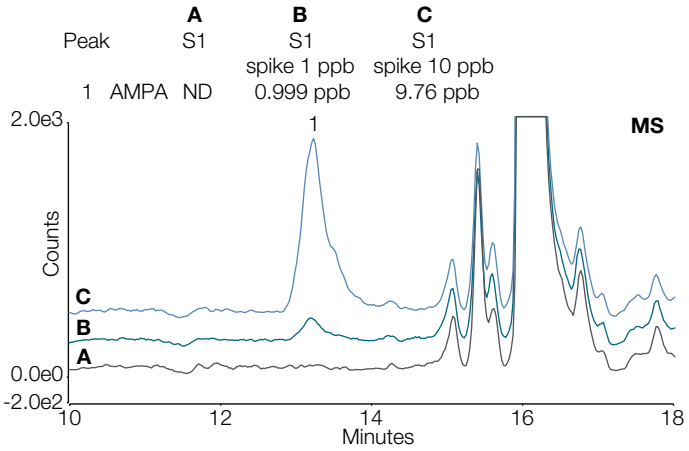


Figure 7. Sample #1 vs. sample #1 spike 1 and 10 ppb (zoom for AMPA)

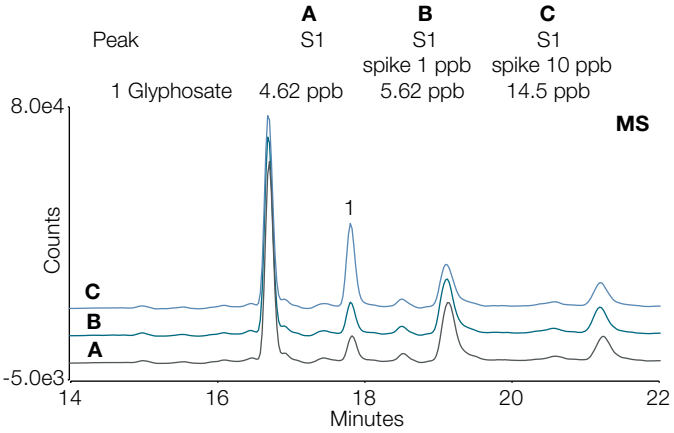


Figure 8. Sample #1 vs. sample #1 spike 1 and 10 ppb (zoom for glyphosate)

Table 8. Recoveries of AMPA and glyphosate spiked in oat flour sample extracts

Sample	Spike level (µg/L)	AMPA	Glyphosate
#1	1	99.9	99.5
	2	97.7	99.0
	10	97.6	99.2
#2	1	96.2	98.6
	2	98.6	97.9
	10	98.6	97.2
#3	1	99.6	99.8
	2	96.3	101
	10	97.1	100

Precision

Method precision was determined by triplicate injections of the 10 µg/L calibration standard on three separate days. As shown in Table 9, the calculated peak area precision varied from 0.87 to 1.22% with retention time precision <0.39% for the target anions. The high precision of this method is consistent with results typically found with an RFIC system.

Table 9. Retention time and peak area precisions

Component	Retention time RSD	MS relative peak area to ITSD RSD
AMPA	0.39	1.22
Glyphosate	0.06	0.87

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Conclusion

This study described the simultaneous direct determination of glyphosate and AMPA in oat flour by IC-MS. Glyphosate and AMPA can be determined sensitively and accurately using a Dionex IonPac AS19-4 µm column and ISQ EC single quadrupole mass spectrometer. The Reagent-Free ion chromatography system provides excellent reproducibility, thereby yielding greater quantification accuracy and consistently reliable results.

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