

Determination of glyphosate and AMPA in oat flour using ion chromatography-mass spectrometry

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Abstract

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

Methods: Oat flour samples were extracted by following the modified Quick Polar Pesticides Extraction (QuPPE) sample preparation method. 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. Anion exchange chromatography using eluent generation and suppressed conductivity detection provides chromatographic selectivity, analytes in the ionic form, and compatibility with MS. Electrospray ionization (ESI) is used to introduce the liquid IC stream (after suppression) as a fine spray into the MS source.

Results: An IC-MS method was developed to simultaneously determine glyphosate and AMPA in oat flour. Using our method, 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.

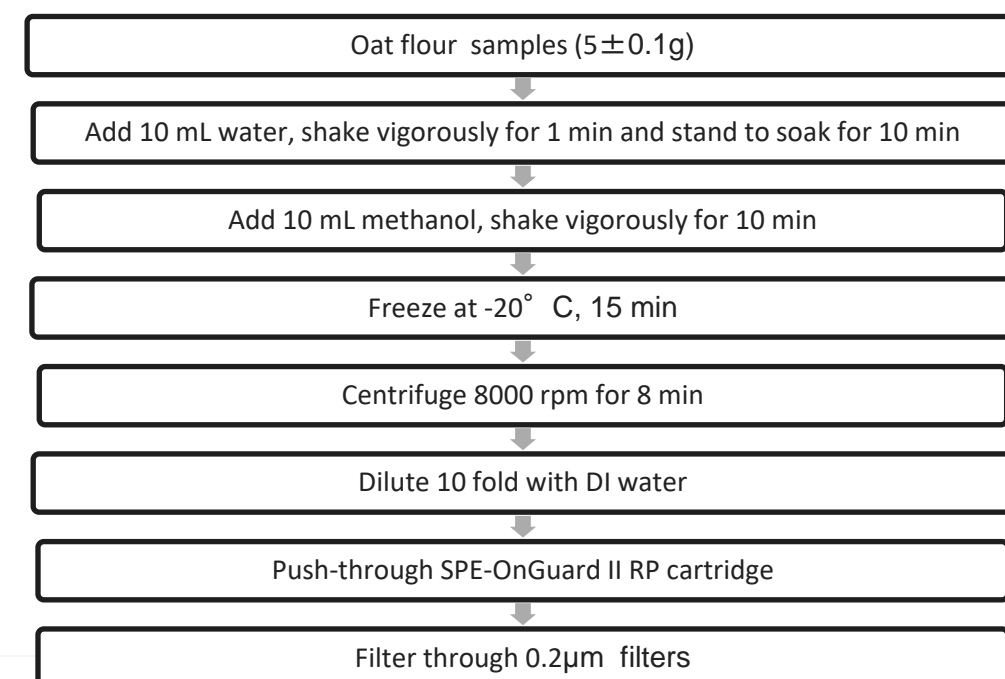
Introduction

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. The bacteria in soil break down glyphosate into aminomethylphosphonic acid (AMPA), which may also be toxic to humans. 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.¹ 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. 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. 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.

Materials and methods

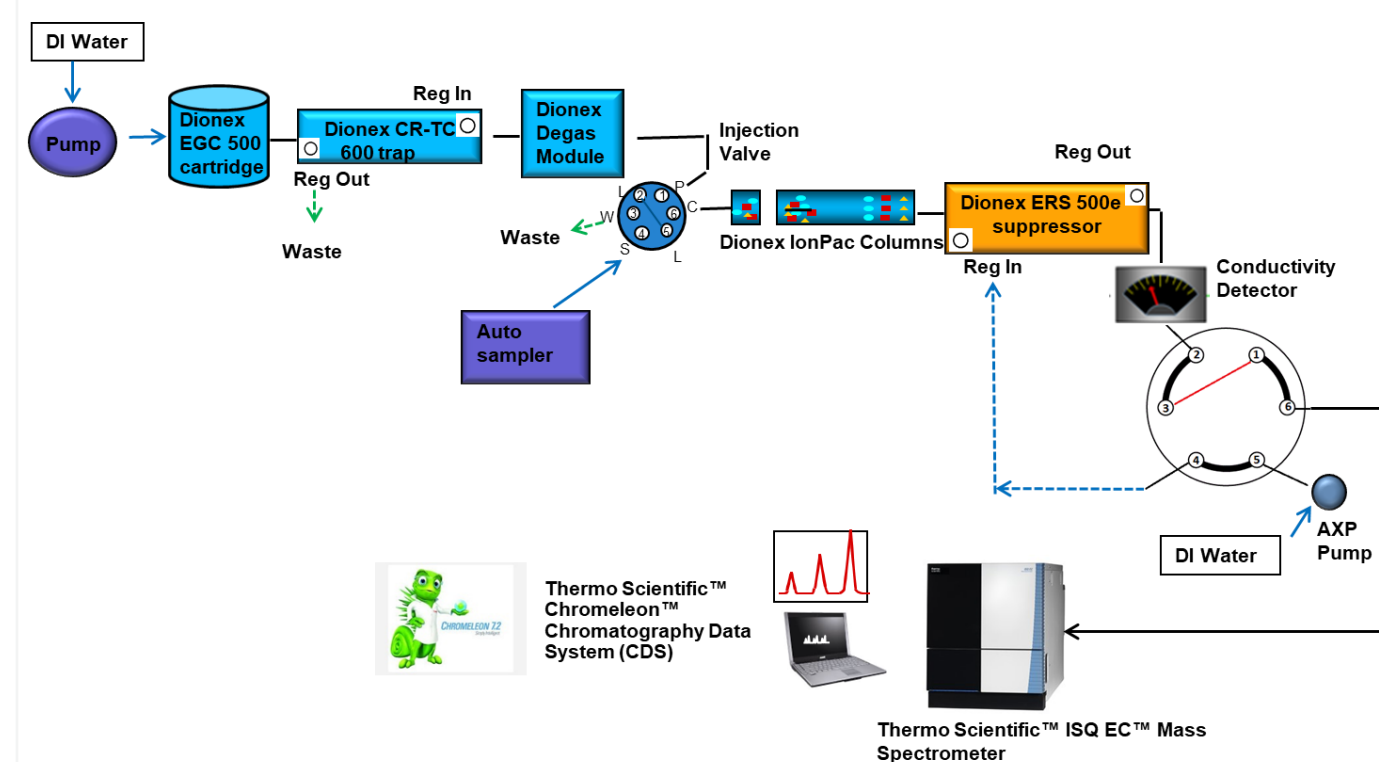
Sample Preparation



Test Method(s)

- Dionex™AS-AP Autosampler with sample syringe, 250 µL and 1200 µL buffer line
- Thermo Scientific™ ISQ EC™ single quadrupole mass spectrometer

Figure 1. IC-MS configuration with matrix diversion



Chromatographic Conditions

Columns:	Dionex IonPac™ AG19-4µm guard, 2 × 50 mm Dionex IonPac AS19-4µm, 2 × 250 mm
Eluent :	KOH Gradient (Refer to application note) ²
Eluent Source:	Dionex EGC 500 KOH Eluent Generator Cartridge with Dionex CR-ATC 600
Flow Rate:	0.35 mL/min
Column Temperature:	30 °C
Injection Volume:	25 µL
Detection:	Suppressed Conductivity, Dionex ADRS 600 Suppressor (2 mm),
Ionization interface	Electrospray ionization (ESI), negative mode
Gas control:	Sheath gas pressure: 40 psi; aux gas pressure: 2 psi; sweep gas pressure: 1 psi
Source voltage	-2500 V
Vaporizer temperature	350 °C
Ion transfer tube temperature	350 °C
Run time	30 min

Data Analysis

By Thermo Scientific™ Dionex™ Chromleon™ Chromatography Data System (CDS) software, version 7.2 SR9.

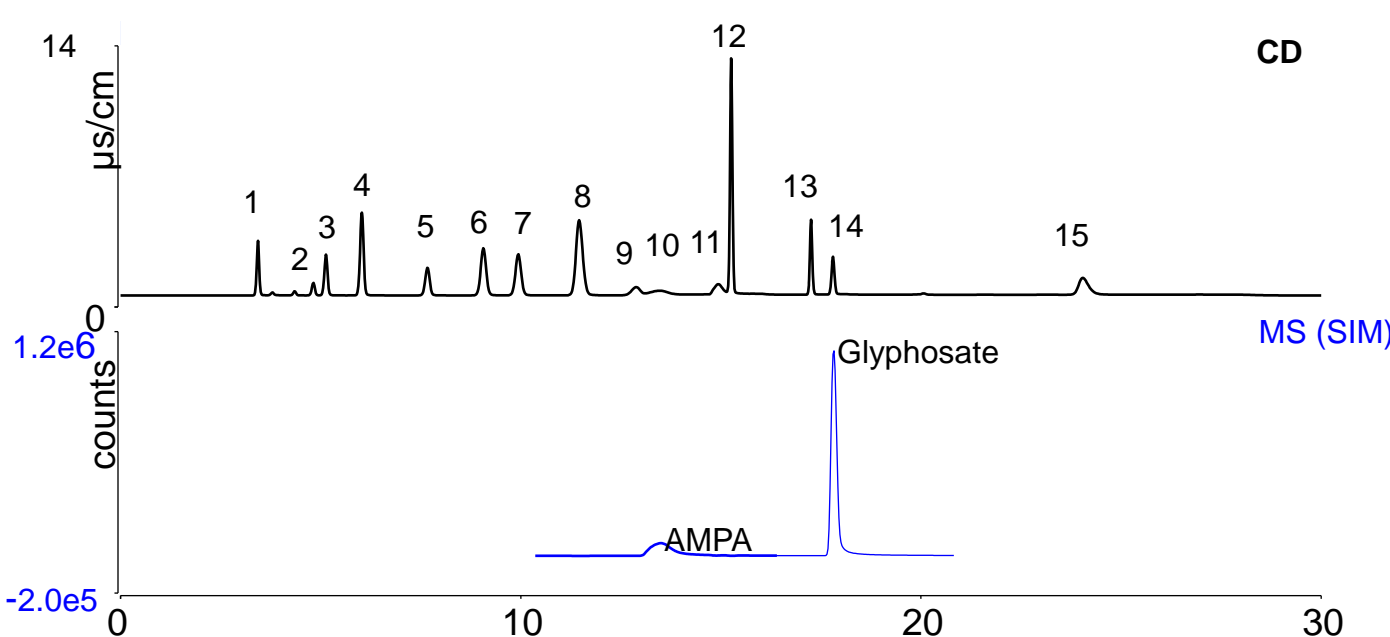
Results

Separation

The Dionex IonPac AS19-4µm hydroxide-selective anion-exchange column is a high capacity and high-resolution column, which are critical factors 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. Figure 2 shows a separation of common anions, AMPA, and glyphosate within 30 min using the Dionex IonPac AS19-4µm column. The top chromatogram displays the CD profiles of all anions. The bottom chromatogram displays the MS profile of the two analytes of interest, AMPA and glyphosate. As Figure 2 shows, AMPA and glyphosate were resolved from common inorganic anions.

Figure 2. Separation of common anions, glyphosate, and AMPA

Peaks	Analyte	Conc (ppm)	Peaks	Analyte	Conc (ppm)
1	Fluoride	0.15	9	Glufosinate	1
2	Chlorite	0.5	10	AMPA	1
3	Bromate	1	11	Carbonate	NA
4	Chloride	0.3	12	Sulfate	1.5
5	Nitrite	0.75	13	Phosphate	1
6	Chlorate	1.25	14	Glyphosate	1
7	Bromide	1.25	15	Perchlorate	1
8	Nitrate	1.25			



Limit of Detection (LOD)

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). 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 standard 0.5 µg/L for AMPA, and 0.1 µg/L for glyphosate. The estimates of LOD and LOQ for AMPA and glyphosate are summarized in Table 1.

Table 1. Calibration and Limit of Detection

	LOD (µg/L)	LOQ (µg/L)	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

Sample Analysis

Three oat flour samples were analyzed in this study. Each sample was extracted in duplicate by the modified Quick Polar Pesticides Extraction (QuPPE) method. AMPA was not detected in the three oat flour samples. Glyphosate was detected at concentrations ranging from 33.4 to 181 ng/g. Figure 3 and 4 show the sample and spiked samples.

Figure 3. sample #1 vs sample #1 spike 1 and 10 ppb standard (zoom for AMPA)

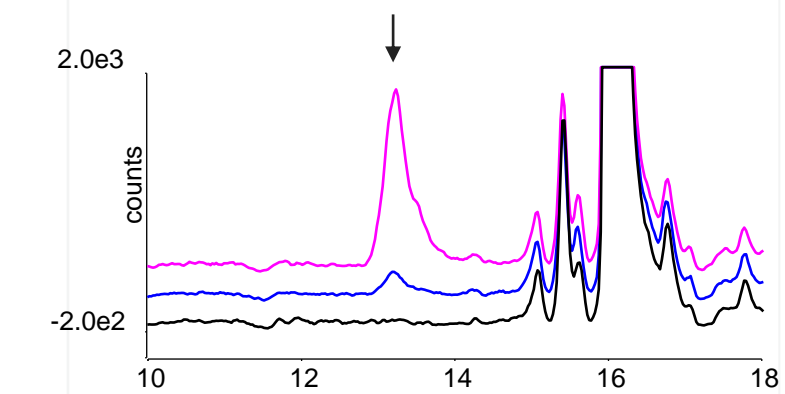
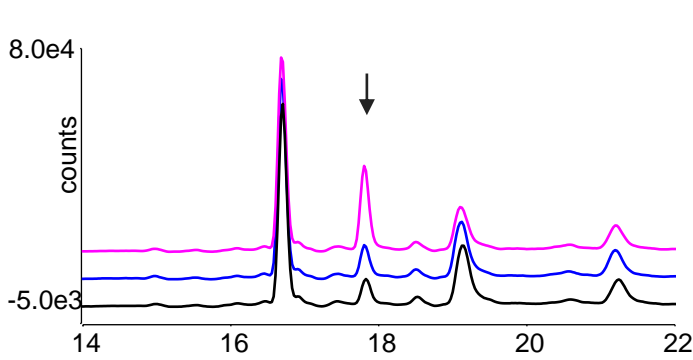


Figure 4. Sample #1 vs Sample #1 spike 1 and 10 ppb standard (Zoom for Glyphosate)



Conclusions

- This study described the simultaneous direct determination of glyphosate, 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 an ISQ EC single quadrupole mass spectrometer.
- The ion chromatography system with electrolytic eluent generation provides excellent reproducibility, thereby yielding greater quantification accuracy and consistently reliable results.

References

1. Electronic Code of Federal Regulations. (2019). eCFR — Code of Federal Regulations. <https://www.ecfr.gov/>
2. Thermo Scientific Application Note 000282: Determination of glyphosate and AMPA in oat flour by IC-MS

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