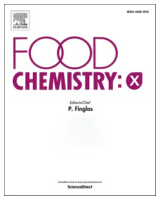
Food Chemistry: X 24 (2024) 101806

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Simultaneous determination of glyphosate, glufosinate and their metabolites in soybeans using solid-phase analytical derivatization and LC-MS/MS determination

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ARTICLE INFO

Chemical compounds studied in this article:
Glyphosate (PubChem CID: 3496)
Glufosinate (PubChem CID: 4794)
N-acetyl glyphosate (PubChem CID: 23510850)
N-acetyl glufosinate (PubChem CID: 129650895)
MPPA (3-(hydroxymethylphosphinyl) propanoic acid PubChem CID: 84788)

Keywords:
Glyphosate
N-acetyl glyphosate
N-acetyl glufosinate
N-(*tert*-butyldimethylsilyl)-*N*-methyl trifluoroacetamide
Derivatization
Solid-phase analytical derivatization
LC-MS/MS

ABSTRACT

Glyphosate and glufosinate are the most widely used herbicides worldwide. We developed a simple and rapid analytical method for detecting glyphosate, glufosinate, and their metabolites (*N*-acetyl glyphosate: Gly-A, *N*-acetyl glufosinate: Glu-A, and 3-(hydroxymethylphosphinyl)propanoic acid: MPPA) in soybeans. The method involved extraction with water, trapping in a mini-column containing polymer-based resin with strong anion exchange groups, dehydration with acetonitrile, and solid-phase analytical derivatization at ambient temperature for 1 min using *N*-(*tert*-butyldimethylsilyl)-*N*-methyl trifluoroacetamide (MTBSTFA), followed by Liquid chromatography-tandem mass spectrometry (LC-MS/MS) determination. This method offers a straightforward and rapid analysis, using on-solid phase dehydration and rapid derivatization at an ambient temperature with MTBSTFA, yielding reliable results for glyphosate, glufosinate, and their metabolites. The method was applied to both domestic and imported soybean samples. Glyphosate, glufosinate, and Glu-A were detected in imported feed soybeans and processed soybean meal for feed use, reflecting the current conditions of GM soybean cultivation.

1. Introduction

Glyphosate (*N*-phosphonomethyl glycine, PubChem CID: 3496) and glufosinate (2-amino-4-(hydroxy(methyl)phosphoryl) butanoic acid, PubChem CID: 4794) are the most widely used herbicides worldwide, and are used as non-selective herbicides for the agricultural control of a broad spectrum of grasses and broadleaf weed species (Benbrook, 2016; Xu et al., 2019). The global use of these herbicides has increased along with the increase in genetically modified crops (e.g., canola, maize, wheat, soybeans, and sugar beets) that are glyphosate and glufosinate resistant.

Maximum residue limit for soybeans are set in various countries around the world (CODEX, 2024; EU Pesticides Database - MRLs, 2024; Japan: Table of MRLs for Agricultural Chemicals, The Japan Food Chemical Research Foundation, 2024; USA: Code of federal regulations title40-part 180 glyphosate, 2024). The residue definition of glyphosate is the sum of glyphosate and *N*-acetyl glyphosate (Gly-A) (*N*-acetyl-*N*-(phosphonomethyl)glycine, PubChem CID: 23510850), with the total expressed as glyphosate. Glufosinate is defined as the sum of glufosinate and its metabolites *N*-acetyl glufosinate (Glu-A) (2-(acetylamino)-4-(hydroxymethyl phosphinyl) butanoic acid, PubChem CID: 129650895) and MPPA (3-(methyl phosphinico) propionic acid, PubChem CID: 84788), expressed as glufosinate. The structural formulas of these compounds are shown in Fig. 1. The widespread use of glyphosate and glufosinate has promoted studies on the detection of the residues of these compounds in many environmental and agricultural commodities

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https://doi.org/10.1016/j.fochx.2024.101806
Received 29 February 2024; Received in revised form 10 July 2024; Accepted 1 September 2024
Available online 2 September 2024

(Berg et al., 2018; Joshuva & He, 2018; Rubio et al., 2014; Sasano et al., 2023; Wang et al., 2023). The metabolites of glyphosate and glufosinate, produced by genetically modified soybeans, are likely to persist in food products. Consequently, there is a need for an analytical method to detect these compounds and their metabolites (Nagatomi et al., 2013).

There are two main methods for analyzing glyphosate, glufosinate, and their metabolites: direct measurement by LC-MS/MS and measurement by LC-MS/MS following derivatization. Several studies have reported the direct measurement of glyphosate and related compounds in soybeans using LC-MS/MS. (Botero-Coy et al., 2013; Chamkasem & Harmon, 2016; Wang et al., 2022). However, direct measurement by LC-MS/MS can be complicated by factors such as adsorption of the analytes to stainless steel components of the equipment used, poor peak shapes in chromatograms, and reduced sensitivity in the LC-MS/MS system. Furthermore, to prevent matrix interference, it is preferable to employ an absolute calibration curve method without relying on internal standards (Chamkasem & Vargo, 2017).

There are numerous reports on the analysis of glyphosate, glufosinate, and their metabolites following derivatization for sensitive and selective measurement. Thompson et al. (2019) developed a sensitive method for the determination of glyphosate, aminomethylphosphonic acid (AMPA), and glufosinate in honey using a combination of 9-fluorenylmethyl chloroformate (Fmoc-Cl) derivatization and LC–MS/MS for highly sensitive analysis (1 μg/kg). Derivatization methods have included Fmoc-CL (Sharma et al., 2015), 3,6-dimethoxy-9-phenyl-9H-carbazole1-sulfonyl chloride (DPCS-CL) (Zhang et al., 2013), trifluoroacetic acid anhydride (Alferness & Iwata, 1994), and trimethyl orthoacetate etc. as derivatization reagents (Sato et al., 2009). However, these derivatization methods involve long reaction times, and heating and evaporation of water and solvents.

Recently, solid-phase analytical derivatization (SPAD) has become popular for the sensitive and selective analysis of polar compounds in environmental and human samples. SPAD combines solid phase extraction (SPE) and simultaneous analytical derivatization in a single step (Takeo et al., 2017). The advantages of SPAD include effective sample preparation techniques, characterized by low organic solvent requirements, and ease of automation with Gas Chromatography/Mass Spectrometry (GC/MS) or LC/MS systems (Yamamoto et al., 2021). This method has enabled improved detection sensitivity and specificity, reduced organic solvent consumption, simplified process automation, increased throughput, and reduced costs.

Previously, we reported an analytical method for detecting residual glyphosate, glufosinate, and their metabolites (Gly-A, MPPA, and Glu-A) in honey using solid-phase extraction cleanup and direct determination by liquid chromatography-tandem mass spectrometry (LC-MS/MS) without derivatization (Sasano et al., 2023). However, challenges were encountered with sensitivity when measuring trace amounts, and with the stability of analytical operations. More recently, the use of *N*-(tert-butyl dimethylsilyl)-*N*-methyltrifluoroacetamide (MTBSTFA) as a derivatization reagent has been reported. This approach with MTBSTFA as a derivatization reagent enhances the sensitivity and speed of determining compounds with phosphate groups in an LC-MS method (Zhang et al., 2020).

In this study, we developed and evaluated a simple, sensitive, and rapid analytical method for glyphosate, glufosinate, and their metabolites using SPAD. The method uses MTBSTFA on a small-scale solid-phase resin (3 mg) combined with LC-MS/MS for simultaneous determination of residual glyphosate, glufosinate, Gly-A, Glu-A, and MPPA in soybeans.

2. Materials and methods

2.1. Chemicals and materials

Standard reagent grade glyphosate, glufosinate ammonium, and MPPA were purchased from FUJIFILM Wako Pure Chemicals Co. (Osaka, Japan). Gly-A and Glu-A were obtained from Toronto Research Chemicals Inc. (Toronto, ON, Canada). Acetonitrile (LC/MS grade, pesticide residue and PCB analysis grade, Japan), methanol (pesticide residue and PCB analysis grade, Japan), and acetic acid (special grade) were purchased from Kanto Chemical Co. (Tokyo, Japan). Ultrapure water was prepared using a PURIC-W system (ORGANO Co., Tokyo, Japan). The derivatization reagent MTBSTFA was purchased from Sigma-Aldrich Japan (Tokyo, Japan).

The Presh-SPE AXs cartridge (mini-column containing polymer-based resin with strong anion exchange groups, 3 mg) was purchased from AiSTI Science (Wakayama, Japan).

Soybean samples were obtained from a local market, Tsuji Oil Mills Co., Ltd., and Marubeni Nisshin Feed Co., Ltd. in Japan.

2.2. Preparation of standard solutions

Standard stock solutions of glyphosate, Gly-A, glufosinate, Glu-A, and MPPA were prepared by dissolving 10 mg of each standard powder in 10 mL of acetonitrile-water (1:9, v/v) to obtain a final concentration of approximately 1000 mg/L. All standard solutions were stored at 4 °C.

Calibration curves for the standards were prepared using solvent-based standards in the range of 0.1 to 2 μg/L for glyphosate and Gly-A, and 0.02 to 0.4 μg/L for glufosinate, Glu-A, and MPPA after derivatization.

2.3. LC-MS/MS quantification

The analyte concentrations were determined using an LC-30 CE high-performance liquid chromatograph with a SIL-30 AC sampler and CTO-

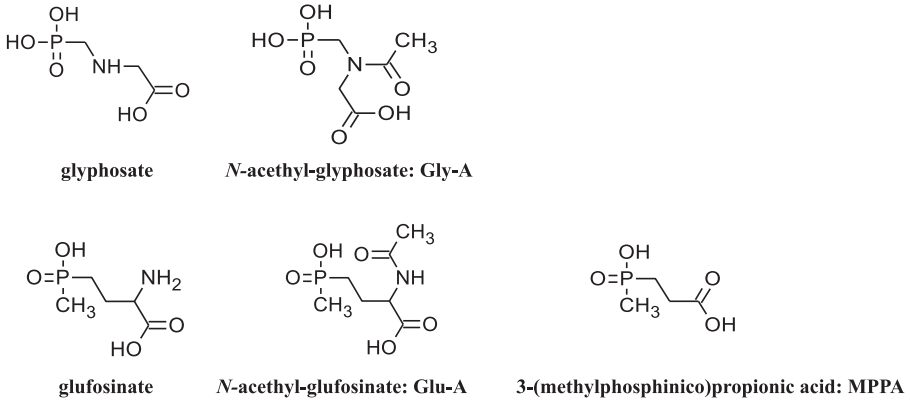

Fig. 1. Chemical structures of glyphosate, *N*-acetyl glyphosate, glufosinate, *N*-acetyl glufosinate, and MPPA.

20 AC column oven (Shimadzu Co., Kyoto, Japan). The separation of analytes was accomplished using an Inert Sustain C18 column (2.1 mm i.d. × 150 mm, 3 μm; GL Sciences, Tokyo, Japan), with operation of the column oven at 30 °C and the sample cooler at 4 °C. LabSolutions software (Shimadzu Co.) was used for instrument control and data processing. The mobile phase was composed of 0.2 mM ammonium acetate in MeOH. The injection volume was 10 μL. The flow rate was set at 0.2 mL/min under an isocratic condition and the total chromatographic run time (injection-to-injection) was 6 min. A LC-MS-8060 triple quadrupole mass spectroscopy system (Shimadzu Co.) was used with the ESI source in positive mode. The operating parameters were optimized according to the following conditions: nebulizing gas flow, 3 L/min; drying gas flow, 10 L/min; heating gas flow, 10 L/min; interface temperature, 400 °C; desolvation line temperature, 150 °C; heat block temperature, 350 °C. Data for quantification and confirmation were acquired in multiple reaction monitoring (MRM) mode. The MRM transitions for quantification and qualification of derivatized compounds were as follows: glyphosate; 512 > 311, 295; Gly-A; 554 > 311, 352; glufosinate; 410 > 195, 179; Glu-A; 452 > 195, 250; and MPPA; 381 > 249, 151.

2.4. Sample preparation

The concentrations of target compounds were determined by solid-phase analytical derivatization (Presh SPE AXs) coupled with LC-MS/MS. The operation procedure for this method is shown in Fig. 2. A 1.0 g sample of homogenized soybean was weighed into a 50-mL polypropylene centrifuge, to which 5 mL of water was added, and the mixture was shaken for 5 min. Subsequently, 5 mL of 50 % aqueous acetonitrile was added, and the mixture was shaken again for 5 min before centrifugation at 1929 ×*g* (3500 rpm) for 5 min. To an aliquot (400 μL) of the supernatant, 600 μL of acetonitrile was added for deproteination, and the sample was allowed to stand for 5 min before centrifugation under the same conditions. A 25-μL aliquot of the supernatant was diluted with 975 μL of water. Then, a 50-μL aliquot of the sample solution was loaded onto the Presh SPE AXs column, which had been prewashed with 100 μL of 10 % acetonitrile aqueous solution. The column was then washed with 100 μL of a 0.02 % acetic acid aqueous solution, and dehydration was achieved by passing 100 μL of acetonitrile through the column. A 100-μL portion of MTBSTFA-acetonitrile: 1/9 (v/v) was impregnated into the solid phase taking care to prevent flow-through, and the cartridge was allowed to stand for 1 min at ambient temperature to facilitate derivatization of the target material on the solid phase. Finally, the derivatized compounds were eluted with the remaining reagent solution into a vial containing 400 μL of acetonitrile. The eluate was mixed using a vortex mixer for 10 s and a 10-μL aliquot of the sample solution was injected into the LC-MS/MS for analysis. The analytical flow chart showing the processing of glyphosate, glufosinate, and their metabolites in soybean is shown in Supplemental Fig. 1.

2.5. Validation test

A validation test was conducted by two analysts, who performed duplicate analyses in parallel over 3 days, in accordance with the "Guidelines for the Validation of Analytical Methods for Testing Agricultural Chemical Residues in Food in Japan" (Abbreviated as Guidelines in Japan) (Guidelines for the validation of analytical methods for testing agricultural chemical residues in food in Japan, 2024).

3. Results and discussion

3.1. Optimization of extraction and deproteination

First, we optimized methods for extracting glyphosate, glufosinate,

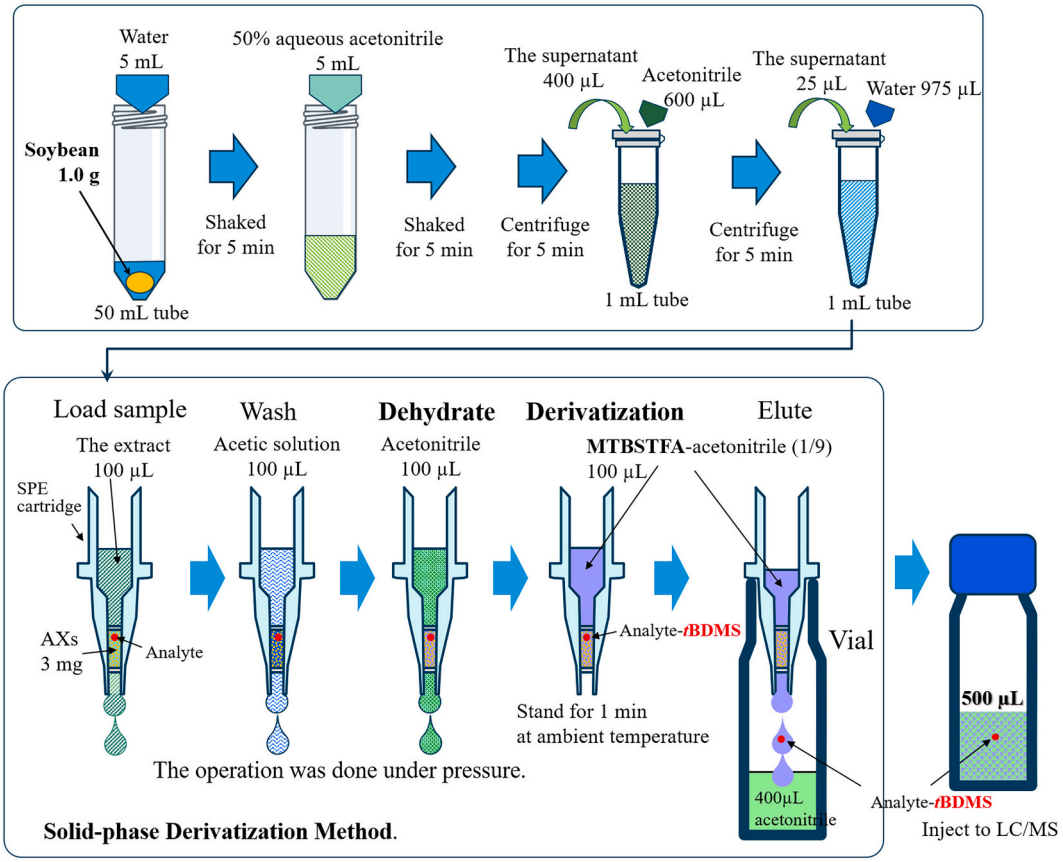

Fig. 2. Analysis operation method of glyphosate, glufosinate, and their metabolites in soybeans.

and their metabolites from soybeans, as well as techniques for removing interfering compounds, such as proteins. Approximately 85 % of the nutritional content of soybeans is composed of protein, carbohydrates, and fat. Soybean extract may cause ionization inhibition due to matrix interference in the LC-MS/MS analysis. To mitigate this, proteins were removed by first extracting with water, followed by the addition of acetonitrile and subsequent centrifugation. Following protein removal, the solution was diluted and passed through the anion exchange solid-phase cartridge, and then derivatized as described in the following section.

3.2. Optimization of solid-phase analytical derivatization

We examined the efficiency of SPAD for retaining and derivatizing glyphosate, glufosinate, and their metabolites on a solid phase. This derivatization step is essential for improving the sensitivity and selectivity of polar analytes such as glyphosate, glufosinate, and their metabolites. After trapping the sample on the anion exchange SPE cartridge (Presh SPE AXs), we tested various conditions to optimize the derivatization process. Prior to derivatization, the trapped solid phase was washed with an acidic aqueous solution to remove acidic and water-soluble compounds, and then washed with acetonitrile to remove any residual water in the solid phase. Washing with acetonitrile was effective for removing residual water and for performing stable derivatization and LC-MS/MS measurements.

Initially, we tested traditional derivatization using trimethylsilyl (TMS) derivatization for glyphosate, glufosinate, and related compounds, which contain functional groups such as phosphate, hydroxy, and amino groups. These functional groups were transformed into their TMS derivatives. However, the TMS derivatives were not stable and decomposed over time. Next, we tested tert-butyl dimethyl silyl (*t*BDMS) derivatization using MTBSTFA. Various conditions were tested to optimize derivatization under ambient temperature, including reagent concentration (1, 10, and 50 %), reaction time (1 and 5 min), and using an elution solvent composed of acetonitrile or methanol after derivatization. Since we did not observe a significant difference in reagent concentration, 10 % MTBSTFA was selected, which is relatively stable. The derivatization reaction times showed similar results. Acetonitrile elution gave better stability of the derivatized compounds after 90 min than methanol, especially with respect to glyphosate and Gly-A. We finally established the optimal conditions for derivatization as follows: a 10 % reagent concentration, a reaction time of 1 min, and acetonitrile for elution, all conducted at ambient temperature. This configuration facilitated efficient on-column derivatization within a 1-min timeframe at ambient temperature. The derivatized compounds were confirmed to have undergone tert-butyl dimethyl silylation, in which the *t*BDMS moiety was substituted to hydroxy and carboxyl functional groups of each compound as follows: glyphosate-tri *t*BDMS (MW 511), *N*-acetyl glyphosate-tri *t*BDMS (MW 553), glufosinate-di *t*BDMS (MW 409), *N*-acetyl glufosinate-di *t*BDMS (MW 451), and MPPA-di *t*BDMS (MW 380).

The sensitivity ratio of the original compounds following derivatization was as follows: glyphosate, 3208 times; Gly-A, 308 times; glufosinate, 3 times; Glu-A, 75 times; and MPPA, 23 times. The data indicate a marked increase in sensitivity, particularly for glyphosate and Gly-A. In a comparison of the reaction time of other conventional derivatization conditions for glyphosates, Fmoc-Cl requires from 30 min to several hours at room temperature, trimethyl orthoacetate requires 30 min at 100 °C, and DPCS-Cl requires 25 min at 75 °C. Therefore, compared to other derivatization methods, the optimized derivatization conditions using MTBSTFA on a solid phase represent an excellent method for achieving sufficient reaction within 1 min at ambient temperature without heating.

3.3. Optimization of LC-MS/MS conditions

We investigated the optimal mobile phase using acetonitrile, methanol, and ammonium acetate-methanol. No increase in sensitivity was observed for any of the compounds using methanol. Using acetonitrile as the mobile phase, the sensitivity of glyphosate and Gly-A was sufficient for the determination, but that of glufosinate, Glu-A, and MPPA was insufficient. The addition of 0.2 mM ammonium acetate to methanol showed good sensitivity for all compounds; thus, 0.2 mM ammonium acetate-methanol was adopted as the mobile phase. Sensitivity and retention time were examined using a C18 column (carbon content 14 %). Optimal conditions, characterized by a rapid retention time, excellent peak shape, and high sensitivity, were achieved using this column.

Notably, to prevent water-induced degradation of derivatized products, water was omitted from the mobile phase and the cleaning solution of the LC autosampler.

3.4. Calibration curve of derivatized compounds

The coefficient of determination (R^2) of the calibration curve was calculated in the range of 0.1–2 μg/L for glyphosate and Gly-A, and 0.02–0.4 μg/L for glufosinate, Glu-A, and MPPA. All five compounds showed good linearity, with an R^2 of 0.994 or higher.

3.5. Validation result

Maximum residue levels (MRLs) for glyphosate and glufosinate have been established by regulatory agencies around the world. These levels are detailed in the following sources: Codex Alimentarius [CODEX: Pesticide Detail | CODEXALIMENTARIUS FAO-WHO] (CODEX, 2024), the EU Pesticides Database [EU Pesticides Database: Pesticide residue(s) and maximum residue levels (mg/kg) (europa.eu)] (EU Pesticides Database - MRLs, 2024), the United States Code of Federal Regulations [USA: Code of Federal Regulations Title40-part 180] (USA: Code of federal regulations title40-part 180 glyphosate, 2024), and the Japanese Table of MRLs for Agricultural Chemicals [Japan: Table of MRLs for Agricultural Chemicals, The Japan Food Chemical Research] (Japan: Table of MRLs for Agricultural Chemicals, The Japan Food Chemical Research Foundation, 2024).

The regulatory MRLs for glyphosate and Gly-A in soybeans in Japan are set at 20 mg/kg, whereas MRLs for glufosinate, Glu-A, and MPPA, are established at 2 mg/kg. The Japanese guidelines recommend that the MRL value, or half of the MRL value, should be added for spiking validation. Therefore, glyphosate and Gly-A were spiked into soybeans at 5 mg/kg, while glufosinate, Glu-A, and MPPA were spiked at 0.5 mg/kg to assess the trueness and precision of the proposed method. Fig. 3 shows the chromatograms of recovery tests. The recovery rate was calculated based on the peak area value with respect to the prepared derivatization standard solution. As a result, the recovery rate, repeatability, and reproducibility all met the validity evaluation guideline criteria (recovery rate of 70–120 %, repeatability accuracy less than 10 %, and reproducibility accuracy less than 15 %) (Guidelines in Japan). The results confirmed the validity of the developed method in this study. As shown in Table 1, the trueness ranged from 97 to 108 %, repeatability ranged from 2 to 5 %, and reproducibility ranged from 4 to 9 %. These values were within the acceptable ranges of the criteria for trueness and precision established by Guidelines in Japan (Guidelines for the validation of analytical methods for testing agricultural chemical residues in food in Japan, 2024).

The limits of quantification (LOQ) and the limits of detection (LOD) were estimated using solutions prepared from soybean samples. For glyphosate and Gly-A, a concentration of 0.05 mg/kg was used, while for glufosinate, Glu-A, and MPPA, a concentration of 0.1 mg/kg was used. The resulting LOQ and LOD were as follows: 0.02 and 0.005 mg/kg for glyphosate and Gly-A; 0.04 and 0.01 mg/kg for glufosinate; 0.03 and 0.01 mg/kg for Glu-A; and 0.06 and 0.02 mg/kg for MPPA, respectively.

The derivatization of the phosphate group in the target compound, which is prone to adsorption, effectively prevented adsorption to the LC

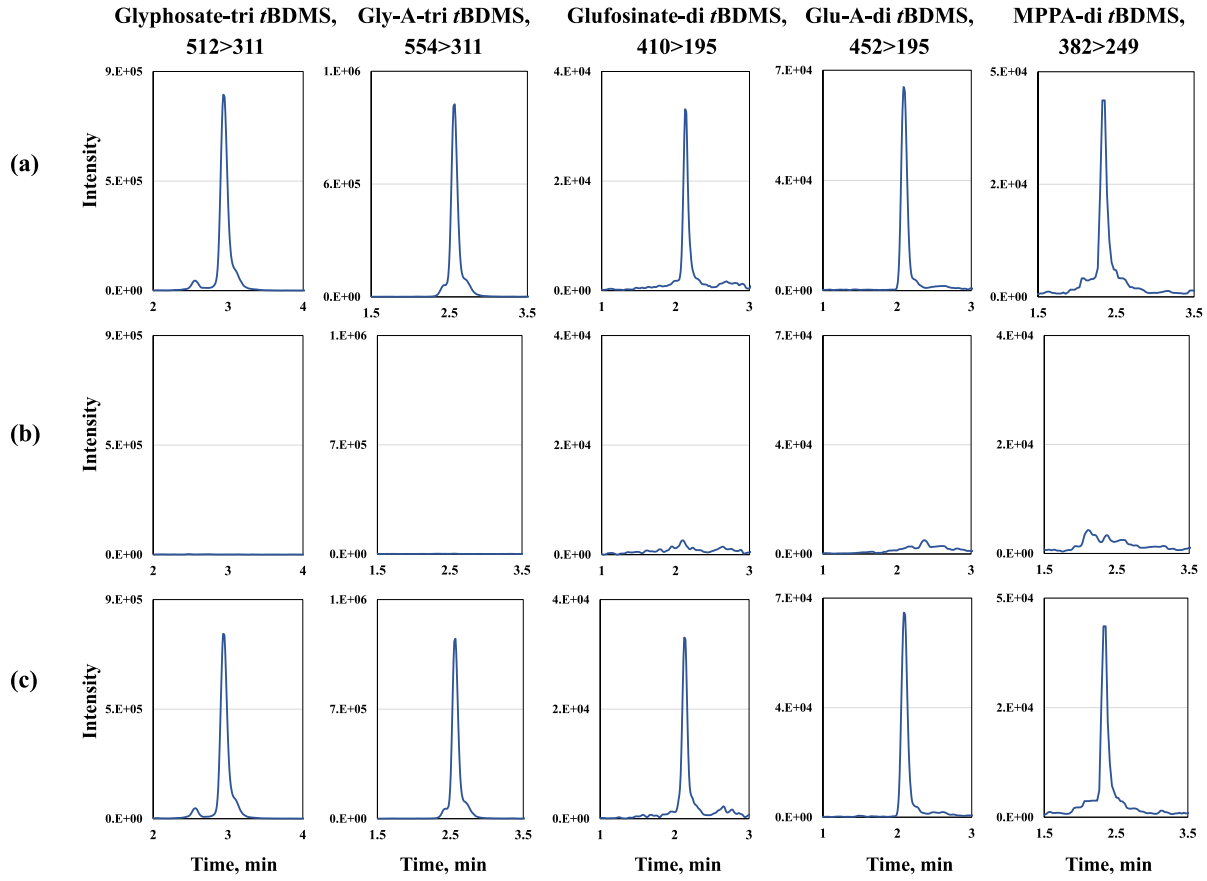

Fig. 3. MRM chromatograms of (a) standard solution, (b) soybean blank, and (c) spiked soybeans.
(a) Standard solutions were adjusted to 0.5 μg/L for glyphosate (−tri *t*BDMS) and Gly-A (−tri *t*BDMS), and to 0.05 μg/L for glufosinate (−di *t*BDMS), Glu-A (−di *t*BDMS), and MPPA (−di *t*BDMS).
(c) Spiked soybean samples were spiked at 5 mg/kg for glyphosate (−tri *t*BDMS) and Gly-A (−tri *t*BDMS), and at 0.5 mg/kg for glufosinate (−di *t*BDMS), Glu-A (−di *t*BDMS), and MPPA (−di *t*BDMS).

Table 1
Validation results in soybeans spiked Glyphosate, Gly-A, Glufosinate, Glu-A and MPPA.

Analytes	Spiked level mg/kg	Trueness [a] %	RSDr [b] %	RSDWR [c] %
Glyphosate	5	97	5	9
Gly-A	5	108	2	8
Glufosinate	0.5	99	4	9
Glu-A	0.5	98	2	4
MPPA	0.5	99	5	8

a Mean recovery.
b Relative standard deviation of repeatability.
c Relative standard deviation of within-laboratory reproducibility.

tube and LC column. This modification is believed to have increased peak intensity and improved the method's trueness, reproducibility, and the linearity of the calibration curve. Additionally, the method also incorporated a purification step using solid-phase extraction with an anion ion exchange resin, eliminating interference from foreign components in the soybeans during quantitative analysis. The increased sensitivity achieved through MTBSTFA derivatization enabled an increase in the dilution rate of the sample extract, thus reducing matrix effects in MS ionization. Consequently, quantification could be performed using the absolute calibration method, obviating the need for costly stable isotopes or internal standards, as well as avoiding matrix calibration or standard addition methods.

3.6. Application to a commercial soybean sample survey

Using the proposed analytical method, we analyzed glyphosate, glufosinate, and their metabolites in edible soybean samples, soybean meal samples for feed, and processed soybean meal for food or feed. We also evaluated the practical application of the developed method. Table 2 shows the results obtained for four edible soybean samples (Japan (Hokkaido), USA, Canada, China) and one processed edible soybean sample (produced in the USA). No peaks for the five substances were confirmed in all edible soybean samples, indicating that all five analytes were below the LOQ. Table 3 shows the results of soybean samples for feed. In the soybean meal for feed and processed soybeans for food, glyphosate, glufosinate, and Glu-A were detected, although the total values were within the Japanese MRLs. Thus, these findings showed that the developed method is applicable to real-world sample analysis. It should be noted that Glu-A was detected in the soybean samples imported from USA. Glu-A is produced by glufosinate-resistant soybeans, in which glufosinate is converted into an ineffective metabolite. Gly-A and MPPA were below the LOQ for all samples. A previous report (Soga et al., 2020) suggested that GTS40–3-2 (glyphosate-tolerant soybean introduced with a *cp4epsps* gene, expressing the CP4 EPSPS protein), MON89788 (glyphosate-tolerant soybean introduced with a modified *cp4epsps* gene, expressing the modified CP4 EPSPS protein), A2704–12, and A5547–127 (glufosinate-tolerant soybean introduced with a gene *pat*(*syn*), expressing the synthesis of phosphinothricin acetyltransferase) were mainly cultivated and distributed in the USA and Brazil in 2020. The present results suggest that MON89788

Table 2
Analytical results of distributed edible soybeans in Japan.

Producing area	type	Glyphosate	Gly-A	Glufosinate	Glu-A	MPPA
Japan (Hokkaido)	edible	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
U.S.A.	edible	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Canada	edible	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
China	edible	<LOQ	<LOQ	<LOQ	<LOQ	N.D.
U.S.A.	processed soybean	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

LOQ; Glyphosate and Gly-A 0.02 mg/kg, Glufosinate 0.04, Glu-A 0.03 and MPPA 0.06 mg/kg.

Table 3
Analytical results of feed-use soybean and soybean meal (mg/kg).

Producing area	type	Glyphosate	Gly-A	Glufosinate	Glu-A	MPPA
U.S.A	Feed 1	0.97	<LOQ	<LOQ	2.00	<LOQ
U.S.A	Feed 2	1.50	<LOQ	<LOQ	1.22	<LOQ
U.S.A	Feed 3	1.25	<LOQ	<LOQ	1.91	<LOQ
U.S.A	Feed 4	0.41	<LOQ	<LOQ	0.31	<LOQ
U.S.A	Feed 5	0.37	<LOQ	<LOQ	0.57	<LOQ
U.S.A	Feed 6	0.44	<LOQ	<LOQ	0.85	<LOQ
U.S.A	Feed 7	0.36	<LOQ	<LOQ	0.58	<LOQ
U.S.A	Feed 8	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Brazil	Feed 1	3.39	<LOQ	<LOQ	<LOQ	<LOQ
Brazil	Feed 2	1.05	<LOQ	<LOQ	<LOQ	<LOQ
Brazil	Feed 3	1.42	<LOQ	<LOQ	<LOQ	<LOQ
U.S.A/ Brazil	Soybean meal	1.73	<LOQ	<LOQ.	0.28	<LOQ.
U.S.A	NonGM-1	<LOQ	<LOQ	0.26	<LOQ	<LOQ
U.S.A	NonGM-2	<LOQ	<LOQ	0.29	<LOQ	<LOQ
U.S.A	NonGM-3	<LOQ	<LOQ	0.24	<LOQ	<LOQ

NonGM; non genetically modified.
LOQ; Glyphosate and Gly-A 0.02 mg/kg, Glufosinate 0.04, Glu-A 0.03 and MPPA 0.06 mg/kg.

and A2704–12 or A5547–127 are the main GM soybeans cultivated in the USA, since glyphosate and Glu-A were detected. GTS40–3-2 and/or MON89788 are the main GM soybeans cultivated in Brazil (see Soga et al., 2020), since only glyphosate was detected was detected in these samples. These qualitative findings from soybean samples imported from USA and Brazil likely reflect the prevailing conditions of GM soybean cultivation in these countries. To the best of our knowledge, this is the first report of Glu-A detection in a real soybean sample, although its presence was previously identified in a honey sample imported from Canada (Sasano et al., 2023). Further genomic DNA analyses of the soybean samples could be conducted using a kernel detection system that combines DNA preparation from individual soybean kernels and event-specific real-time PCR. Such an approach would potentially clarify the identification of the GM event present in those samples. It would be interesting to investigate the relationship between pesticide residues and GM soybean events in the field of food chemistry.

This method provides sufficient detection sensitivity for MRL screening surveys. However, to detect the Japanese default MRL of 0.01 mg/kg, which applies to foods without specified MRLs, it is necessary to explore methods to enhance sensitivity for glufosinate, Glu-A, and MPPA. One potential strategy could be to adjust the dilution rate, potentially to as high as 10,000.

We will expand this method for the analysis of glyphosate, glufosinate, and their metabolites in cereals and other genetically modified crops. In the future, we aim to perform fully automated system analysis using solid-phase analytical derivatization and LC-MS/MS determination.

4. Conclusions

We developed a simple and rapid analytical method for detecting glyphosate, glufosinate, and their metabolites, specifically *N*-acetyl glyphosate (Gly-A), *N*-acetyl glufosinate (Glu-A), and 3-hydroxymethylphosphinylpropanoic acid (MPPA), in soybeans. The method involves water extraction, trapping on a Presh-SPE AX3 (3 mg) column, dehydration with acetonitrile, and solid-phase analytical derivatization at ambient temperature for 1 min using *N*-(*tert*-butyldimethylsilyl)-*N*-methyl trifluoroacetamide (MTBSTFA), followed by LC-MS/MS determination. This method features on-column dehydration and rapid derivatization, demonstrating effective results for glyphosate, glufosinate, and their metabolites.

The developed analytical method was applied to various soybean products, including edible soybeans, feed-use soybean meal, and processed soybeans. We detected glyphosate, glufosinate, and Glu-A in soybean meal for feed and processed soybeans for feed. These findings provide insights into the current state of soybean distribution. This method is rapid, simple, and reliable, and is expected to be useful for monitoring soybeans in a variety of distribution channels.

CRediT authorship contribution statement

Ryoichi Sasano: Writing – original draft, Methodology, Conceptualization. **Junpei Sekizawa:** Validation, Investigation. **Isao Saito:** Writing – review & editing. **Mikihisa Harano:** Validation, Investigation. **Kyoka Katsumoto:** Validation, Investigation. **Rie Ito:** Validation, Formal analysis. **Yusuke Iwasaki:** Validation, Investigation. **Takaaki Taguchi:** Validation, Investigation. **Tomoaki Tsutsumi:** Validation, Investigation. **Hiroshi Akiyama:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This work was supported in part by the Ministry of Health, Labour and Welfare of Japan (Grant No. 22KA1009) and the Japan Food Chemical Research Foundation (Grant No.R05-007). The authors gratefully acknowledge Tsuji Oil Mills Co., Ltd. and Marubeni Nisshin Feed Co., Ltd. for providing soybean meal samples for feed, as well as for processed soybean meal for feed samples.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101806.

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