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Determination of glyphosate and aminomethylphosphonic acid in soybean samples by high performance liquid chromatography using a novel fluorescent labeling reagent

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A highly sensitive pre-column derivatization HPLC method for simultaneous determination of glyphosate (GLYP) and its major metabolite aminomethylphosphonic acid (AMPA) in soybean samples was developed. The analytes were labeled with a novel fluorescent labeling reagent 3,6-dimethoxy-9-phenyl-9*H*-carbazole-1-sulfonyl chloride (DPCS-Cl) at 70 °C for 25 min. The optimized concentration of DPCS-Cl was 25 μg mL^{-1} and the molar ratio of analytes to DPCS-Cl was 1 : 4.2. The derivatives were separated on a reversed-phase column by gradient elution and were monitored with fluorescence detection at 318 nm (excitation) and 440 nm (emission). The method linearity, calculated for GLYP and AMPA, had a correlation coefficient greater than 0.999. The detection limits for GLYP and AMPA were 0.02 ng mL^{-1} and 0.01 ng mL^{-1} ($S/N = 3$), respectively. In addition, a simple sample pretreatment for the soybean samples was developed to extract GLYP and AMPA. The recovery of extraction was more than 95%. Then, this method gave the detection limits of 0.002 mg kg^{-1} for GLYP and 0.001 mg kg^{-1} for AMPA in soybean samples. This HPLC method was applied to the determination of glyphosate and AMPA in soybean samples with its merits of simplicity in pretreatment, rapidity in derivatization, stability of the derivatives and high sensitivity.

1. Introduction

Glyphosate (GLYP) as a nonselective and postemergence organophosphorus herbicide has been widely used in agriculture and forestry.[1] The mechanism of GLYP action is through inhibiting an enzyme that plays an important role in biosynthesizing aromatic acid precursors of many proteins.[2] GLYP products have been approved for weed control for more than 100 crops, which have extensive applications in agriculture, forestry and aquatic systems.

The development of glyphosate-resistant crops is one of the most important weed management innovations in the history of agriculture. Glyphosate-resistant crops represent more than 80% of the 120 million hectares of transgenic crops grown annually worldwide. Glyphosate-resistant soybean was one of the first major applications of genetic engineering.[3] Glyphosate-resistant soybean adoption was rapid in the United States, currently representing more than 90% of the area planted to soybean.[4] After government approval, adoption of glyphosate-resistant soybean has also been fast in other parts of the world. The utilization of glyphosate-resistant soybean crops is a factor encouraging an increase in glyphosate use. In China and Japan, soybean is a traditionally important crop for high-protein foods, although most soybeans are imported from North America. Consumers will ingest transgenic soybeans that will be grown on glyphosate-sprayed fields. Soybean quality monitoring for glyphosate is prudent especially when considering the current increasing trend in its agricultural use and therefore reliable methods are required for monitoring this herbicide in soybeans.

But another problem is that metabolites from GLYP degradation and GLYP itself have been certified as contaminants in the ecosystem.[5] It shows a teratogenic, mutagenic, carcinogenic effect on animals and also has a great impact on aquatic life.[2] Therefore, accurate determination of pesticides and their degradation products play an important role in monitoring and controlling environmental pollution. The maximum contamination level (MCL) of GLYP in drinking water is set at 0.7 μg mL^{-1} by the United States Environmental Protection Agency (EPA).[6] As for crops, different vegetables have different allowed levels which range from 0.1 to 20 mg kg^{-1}.[7] There is an increasing demand for studies on finding a sensitive and selective method for the determination of GLYP.

Determination of GLYP and its major metabolite aminomethylphosphonic acid (AMPA) at residue levels encountered

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difficulty, which mainly results from their properties such as small molecular size, insolubility in organic solvents, relatively high solubility in water, and high polarity. There have been many analytical methods for the analysis of GLYP and AMPA such as spectrophotometry,[8] gas chromatography (GC),[9,10] ion-exchange chromatography,[11] liquid chromatography (LC),[12–14] electrochemiluminescence,[15,16] capillary electrophoresis (CE),[17,18] electrochemical methods,[19,20] inductively coupled plasma-mass spectrometry (ICP-MS),[21] and the enzyme-linked immunosorbent assay (ELISA) method.[22] As for electrochemical methods, chemical sensors and biosensors are conventional tools. But their performance is limited by many uncertain factors and their accuracy cannot be guaranteed.[23] A spectrophotometer is used in spectrometry. Due to the fact that a spectrophotometer has no separation capability, it caused the problem that GLYP cannot be accurately measured. CE for GLYP offers high efficiency, but the injection volume is limited and it leads to low sensitivity.[21] Although the ELISA method can provide selectivity and high sensitivity, the significant shortcoming is the high cost and easily inactivated characteristics of the enzyme. So it is still difficult to commercialize the detection.[21] The ICP-MS method, which has been reported, can determine trace GLYP in water without a previous derivatization step.[21] However, this method needs more expensive instruments and the determination process is more cumbersome.

To date, chromatographic methods with different clean-up techniques and a variety of separation and detection modes are most widely used in the analysis of GLYP and AMPA in water. GC for detecting GLYP should be under the condition that GLYP must be converted into a volatile and stable derivative through a previous derivatization step.[22] The preparation of the derivatives is time-consuming and complicated. Owing to the ionic character of GLYP and the availability of derivatization techniques, LC is an attractive technique for the measurement of GLYP compared with GC.[24] Recently, more attention has been paid to LC-MS in this field.[25] A few analytical methods that are based on the use of LC/MS have been reported, such as LC-ESI-MS without derivatization[26] and LC-ESI-MS-MS with a derivatization step.[27] These methods have shown good results with higher sensitivity, but require expensive equipment and trained personnel. For these reasons, it is difficult to achieve universal access to use these methods for determination of residues of GLYP.

In addition, detectors also play a very important role in the determination process. Until now, ultraviolet-visible (UV-Vis) detection,[12,28] fluorescence detection (FLD),[29,30] electrochemical (EC) detection,[31] and mass spectrometry (MS) detection have been applied for the determination of GLYP and AMPA.[32] An EC detector detects GLYP with poor stability. Although a MS detector shows a low limit of detection, high sensitivity and good accuracy, it requires specific equipment and the price of the instrument is quite high. In contrast to UV-Vis detection, FLD that has a lower detection limit and higher sensitivity may provide an efficient method for analysis of GLYP and AMPA. The lack of a chromophore or fluorophore in GLYP makes LC methods always include a pre- or post-column derivatization step.[33] Pre-column derivatization is preferred over post-column derivatization because it does not need complicated instruments and it is easy to control the reaction conditions.[34] Several labeling reagents for pre-column derivatization have been published in the literature for determination of GLYP and AMPA, including ninhydrin,[35] *p*-toluenesulphonyl chloride (TsCl),[28] 4-chloro-3,5-dinitrobenzotrifluoride (CNBF),[12] 4-methoxybenzenesulfonyl fluoride (MOBS-F),[34] l-fluoro-2,4-dinitrophenyl (DNP),[36] *o*-phthalaldehyde (OPA) and *o*-phthalaldehyde-2-mercaptoethanol (OPA-MERC),[37,38] 9-fluorenylmethyloxycarbonyl chloride (FMOC-Cl),[29,33,39,40] and 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-Cl).[8,41] Each of these reagents has its own specific advantages and limitations. For example, the OPA reagent offers greater sensitivity, but it can only react with primary amines.[42] GLYP is a secondary amine, so the OPA method requires a step to transform GLYP into a primary amine before the OPA derivatization.[43] FMOC-Cl can be used for pre-column derivatization with both primary and secondary amino groups. However, the main disadvantage of using FMOC-Cl is the interference of the product FMOC-OH, which is represented by a large peak in the GLYP chromatogram.[29] Therefore, we are committed to the study of a novel pre-column fluorescence derivatization reagent to avoid these shortcomings.

The aim of the present paper is to develop a rapid, sensitive and selective method for determination of residues of GLYP and its metabolite (AMPA). We employed HPLC with 3,6-dimethoxy-9-phenyl-9*H*-carbazole-1-sulfonyl chloride (DPCS-Cl) pre-column derivatization and fluorescence detection. DPCS-Cl is a stable, economical and novel fluorescent labeling reagent with chromophore and strong fluorescence absorption. This fluorescent derivatization reagent is easy to store and can react with a wide range of amino compounds under mild conditions. Its derived products are stable, easy to separate, and have high detection sensitivity. To our knowledge, it is the first time that DPCS-Cl has been used as a fluorescence derivatization reagent to detect GLYP and AMPA. In the meantime, we explore a new pretreatment for soybean samples, which is simple and effective. The detection limits of GLYP and AMPA in soybean samples for our method are 0.002 mg kg^{-1} and 0.001 mg kg^{-1}, reaching a maximum contaminant level of GLYP in crops at 0.1 mg kg^{-1}.[7] This method of detecting GLYP and AMPA is rapid, convenient with higher sensitivity and lower detection limits. It is hoped that this study could provide a new path for the determination of other amino compounds.

2. Materials and methods

2.1 Equipment

The following equipment was used in this work.

(a) An Agilent 1200 series HPLC system (Agilent Technologies Inc.) with the following modules: a G1322A online vacuum degasser, a G1311A high-pressure gradient quaternary pump, a G1329A light-tight autosampler unit, a G1316A thermostatic column compartment and a G1321A fluorescence detection (FLD) system; (b) an analytical column: a reversed-phase ODS column (150 × 4.6 mm I.D., 5 μm, Shimadzu); (c) a Mettler-Toledo FE20 pH meter (Shanghai, China); (d) a TGL-16C Table-Top high-speed centrifuge; (e) a DK-S22 CNC constant temperature water bath (Shanghai, China); (f) a KQ-300 high-power digital control ultrasonic cleaner (Suzhou, China); (g) a

DHG series electric thermostat blast oven (Shanghai, China); (h) a Mettler Toledo EL104 electronic balance (Hangzhou, China); (i) an RE-5285A vacuum rotary evaporator.

2.2 Chemicals and reagents

All chemicals were of analytical reagent grade, unless stated otherwise. GLYP and AMPA were obtained from J & K Chemical Technology Co., Ltd (Shanghai, China). Hydrochloric acid (HCl), potassium dihydrogen phosphate, sodium dihydrogen phosphate dihydrate, sodium phosphate dodecahydrate, disodium hydrogen phosphate dodecahydrate, phosphoric acid, methanol, methylene chloride, acetonitrile (HPLC-grade) and 732 cation exchange resin were provided by Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). 3,6-Dimethoxy-9-phenyl-9*H*-carbazole-1-sulfonyl chloride (DPCS-Cl) was synthesized in our laboratory. DPCS-Cl was synthesized in four steps and the process of synthesis is described in ref. 44. Ultrapure water was obtained using a Milli-Q system (Millipore, Milford, MA, USA). The resistivity of ultrapure water is 18.2 MΩ cm.

2.3 Sample preparation and derivatization procedure

The preparation of the acidity regulator is as follows: 16 g potassium dihydrogen phosphate was dissolved in 160 mL of water, and then mixed with 13.5 mL of hydrochloric acid and 40 mL of methanol. The preparation of the cation exchange column eluate is as follows: 160 mL of ultrapure water, 2.18 mL of concentrated hydrochloric acid and 40 mL of methanol were fully mixed. A cation exchange column (1.8 cm × 7 cm) with 732 cation exchange resin was used to treat samples without a vacuum pump.

An aliquot of 50 mL of ultrapure water was added to soybean samples of 5.0 g which were ground to powders. Then, the mixture solution was shaken for 5 min and ultrasounded for 10 min. 4 mL of the above solution was taken and mixed with 4 mL of methylene chloride in a centrifuge tube. In order to mix well, the solution was shaken for 5 min, and then centrifuged for 10 min (9000 r min^{-1}). The upper water layer of centrifuge tubes was transferred and mixed in a plastic test tube. And 0.5 mL of acidity regulator was added to 4.5 mL of the above extract. After fully mixing, we take 3.00 mL of solution to purify with cation exchange resin (activated by 20 mL ultrapure water before using). After adding the samples, 45 mL of eluent was used to rinse. The eluent was collected in a round-bottom flask while the previous 7 mL was discarded. The collected eluent was evaporated to dryness using a rotary vacuum evaporator at 60 °C. The obtained solid was reconstituted in 5 mL of ultrapure water. The extract was mixed with buffer solution of pH = 10.5. Then the extract was derivatised with DPCS-Cl for 25 min at 70 °C. Before injection on the HPLC, the sample was filtered through a 0.22 μm membrane filter after cooling.

2.4 HPLC analysis

2.4.1 Mobile phase preparation. The mobile phase for HPLC analyses consisted of (A) phosphate buffer (50 mmol L^{-1}, pH = 3.0) and (B) acetonitrile. All solutions were filtered through a 0.22 μm membrane filter before being used.

2.4.2 Chromatographic conditions. The temperature of the column was kept at 25 °C. The flow-rate of the mobile phase was 1.0 mL min^{-1}, and the fluorescence intensities were monitored at excitation and emission wavelengths of 318 nm and 440 nm, respectively. The injection volume is 10 μL. The gradient program is as follows: 30% of acetonitrile in the first 20 min, the proportion of acetonitrile increases to 40% in 5 min, then the proportion of acetonitrile increases to 70% in 5 min, then a decrease of acetonitrile to 40%, and finally, a stepwise decrease of acetonitrile to 30%, and the column was kept for 5 min to re-equilibrate.

3. Results and discussion

3.1 The principle of derivatization reaction and optimization of reaction conditions for the derivatization

GLYP and AMPA were labeled using DPCS-Cl under alkaline conditions, in order to obtain highly fluorescent derivatives (Fig. 1).

A standard solution of GLYP and AMPA (5 μg mL^{-1}) was used to optimize the derivatization conditions. Through experiments, we get the optimal conditions such as the reaction temperature, reaction time, pH value of the phosphate buffer, the volume of the reaction medium, the concentration of DPCS-Cl and the stability of the derivative product.

The effects of temperature on the derivatization reaction were investigated for GLYP and AMPA within the temperature

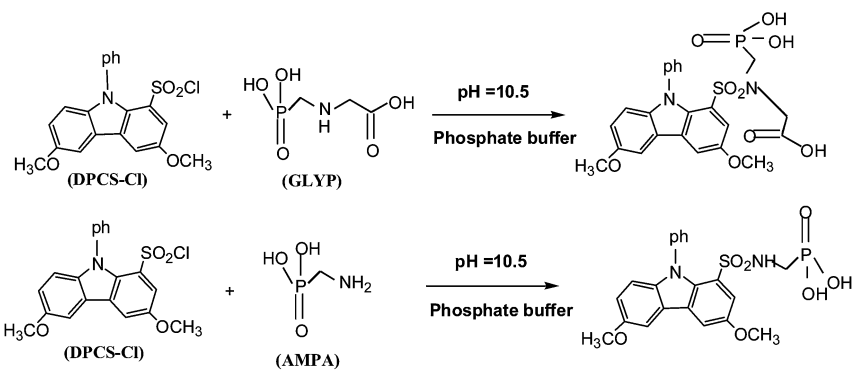

Fig. 1 Putative reaction scheme of the derivatization of GLYP and AMPA with DPCS-Cl as a derivative agent.

range of 20–80 °C with a 10 °C increment. Meanwhile, the pH value of the phosphate buffer is 11 and the amount of phosphate buffer is 100 μL, and the concentration of DPCS-Cl is 50 μg mL^{-1}. GLYP, AMPA and DPCS-Cl were mixed and reacted in a water bath for 30 min at different temperatures. Then the mixture was cooled to room temperature for HPLC separation. As a result, the optimum temperature for GLYP, AMPA and DPCS-Cl derivatization reactions was 70 °C, based on chromatographic peak area data.

The effect of the derivatization reaction time was examined. At 70 °C, we tried to investigate the statistical difference in derivatization time. We investigated the derivatization reaction within the time range of 5–40 min with a 5 min increment, while the pH value of the phosphate buffer is 11 and the amount of phosphate buffer is 100 μL, and the concentration of DPCS-Cl is 50 μg mL^{-1}. The maximum chromatographic peak area was obtained from the reactions performed above 25 min. It is indicated that all of the derivatization reactions were completed in 25 min. We thought that 25 minutes was sufficient for the derivatization reactions.

As the labeling reactions of GLYP and AMPA with DPCS-Cl proceeded under alkaline conditions, the effect of different pH values of the phosphate buffer on the derivatization reaction was examined. At 70 °C, we investigated the derivatization reaction for 25 minutes within the phosphate buffer pH value range of 9–12 with a 0.5 increment, while the amount of phosphate buffer is 100 μL, and the concentration of DPCS-Cl is 50 μg mL^{-1}. The results indicated that the derivatization reaction was incomplete when the pH value of the phosphate buffer was less than 10.5, since the chromatograph peak area of GLYP and AMPA is small.

The effect of different volumes of phosphate buffer on the derivatization reaction was investigated. Under the optimized conditions, we tried to investigate the statistical difference in the amount of phosphate buffer, while the concentration of DPCS-Cl is 50 μg mL^{-1}. The results show that when the amount of phosphate buffer is 20 μL, the chromatographic peak areas of GLYP and AMPA increase. However, when the amount of phosphate buffer is more than 20 μL, the chromatographic peak areas are on a declining curve. Thus, the amount of 20 μL was chosen as the optimum.

The effect of the concentration of labeling reagent DPCS-Cl was investigated by varying the concentration of DPCS-Cl to react with GLYP and AMPA. Under the optimized conditions, we tried to investigate the statistical difference in the concentration of DPCS-Cl, and the result indicates that when the concentration of DPCS-Cl is more than 25 μg mL^{-1}, maximum peak areas are obtained. That is, the reactions of DPCS-Cl with GLYP and AMPA are basically completed when the molar ratio of GLYP and AMPA to DPCS-Cl is 1 : 4.2.

In order to investigate the stability of derivative products, the derivatization reaction solutions were placed directly under light at room temperature. The derivative products were detected every day in the first week and once a week until the fifth week. From the dates, we can see that the peak areas of derivative products of GLYP and AMPA had no obvious changes after a month, which shows that the derivative products had good stability.

3.2 Reproducibility of HPLC analysis

The repeatability of the HPLC analysis can be assessed by measuring the retention time and peak area. In this experiment, the relative standard deviations (RSDs) for the reproducibility of HPLC analysis were obtained through replicate injections ($n = 5$) of different concentrations of derivatization solutions in intra-day and inter-day determination. RSDs were calculated and the results are listed in Table 1. It shows that RSDs for the retention time and for the peak area are quite low, which indicates that this method possesses good reproducibility.

3.3 Linearity and detection limit

To obtain calibration curves, standard solutions containing different concentrations of GLYP and AMPA were labeled with DPCS-Cl under the above optimum conditions. The relationships between the peak-area and the concentration of analytes are listed in Table 2. As we can see, the LODs of GLYP and AMPA were 0.02 ng mL^{-1} and 0.01 ng mL^{-1}, respectively. A comparison of DPCS-Cl with other commonly used labeling reagents for glyphosate determination is given in Table 3. As seen from the table, our method is attractive with less dosage of labeling reagent and lower detection limits.

3.4 Determination of the residue of GLYP and AMPA in soybean

3.4.1 Pretreatment of soybean samples. Due to their high polarity and high solubility in water, we extract the powder samples of GLYP and AMPA by ultrasounding with 10 times the

Table 1 Repeatability of the studied GLYP and AMPA by RP-HPLC ($n = 5$)[a]

	RSDs (%)											
	Retention time						Integrated area					
	Run-to-run			Day-to-day			Run-to-run			Day-to-day		
Compounds	A	B	C	A	B	C	A	B	C	A	B	C
DPCS–GLYP	0.37	0.36	0.64	0.89	1.2	1.1	1.9	2.2	3.0	3.4	3.9	3.7
DPCS–AMPA	0.22	0.35	0.54	0.84	0.86	0.80	2.1	1.9	2.6	3.5	3.7	3.6

[a] The results were obtained from 0.010 μg mL^{-1} (A) and 0.10 μg mL^{-1} (B) to 1.0 μg mL^{-1} (C) of GLYP and AMPA.

Table 2 Linear regression equations, correlation coefficients, linear ranges and detection limits of GLYP and AMPA labeled with DPCS-Cl

Derivatization reagent	Linear regression equation[a]	Related coefficient	Linear range (ng mL^{-1})	LODs[b] (ng mL^{-1})
GLYP	$Y = 1.33879x + 21.08541$	0.9991	1.0–3000	0.02
AMPA	$Y = 1.45385x + 17.96072$	0.9999	0.5–3000	0.01

[a] X: concentration of GLYP or AMPA (ng mL^{-1}); Y: peak area of GLYP or AMPA (arbitrary unit). [b] LODs: concentration detection limit.

volume of ultra-pure water. We add an equal volume of methylene chloride to remove impurities which are soluble in the organic phase in the sample. Next, the sample is purified by the cation column to remove the sugars and other impurities. The experimental results showed that when the sample was eluted, most of the sugars, derivative byproducts and other impurities were eluted in the initial 7 mL of eluate. For the calculation of recovery, we add standard solutions of GLYP and AMPA to samples. When the volume of eluate was between 7 and 35 mL, more than 95% GLYP was eluted. Meanwhile, when the volume of eluate was between 10 and 45 mL, more than 98% AMPA was eluted. Thus, we finalized that the initial 7 mL of eluate was discarded after the sample was added into the elution column. Next, we eluted with 38 mL of eluate and collected the eluate. Fig. 2 shows the elution curve. The subsequent steps are described in Section 2.3.

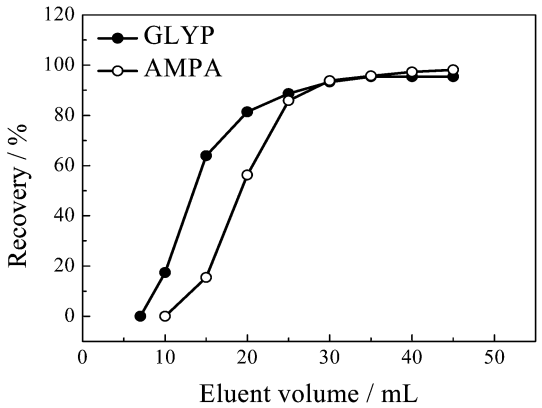

Fig. 2 Elution curves for GLYP and AMPA on a cation exchange column.

3.4.2 Chromatography analysis of samples. Following the steps of each soybean sample processing described in Section 2.3, under optimal conditions, we obtained the chromatograms of derivatization of GLYP, AMPA with DPCS-Cl and derivatization of soybean samples with DPCS-Cl. Then, chromatograms of soybean samples without derivatization and soybean samples labeled with DPCS-Cl were obtained (Fig. 3). The results showed that the derivative products of standard solutions of GLYP and AMPA were well separated. There are many differences between both chromatograms particularly at the beginning as shown in Fig. 3. We have confirmed that the peaks at the beginning resulted from some amino compounds in the soybean samples.

3.4.3 Recoveries and precision. Recovery testing was conducted by using three soybean samples spiked with various concentrations of standard GLYP and AMPA. The recoveries were calculated by using matrix matched calibration. The soybean samples which did not contain GLYP and AMPA were employed as matrices. Then, we calculated the relative standard deviations (RSDs). The results are shown in Table 4. The recoveries of DPCS–GLYP were between 85.4% and 94.1%, at the same time, the recoveries of DPCS–AMPA were between 87.3% and 95.2%. From the data of the recovery, we can see that the established method has acceptable accuracy in the determination of the content of DPCS–GLYP and DPCS–AMPA. We

Table 3 Comparison of the derivatization conditions and detection limit of the regents reported for glyphosate

Derivation reagent	Molar ratio[a]	Derivatization temperature	Derivatization time (min)	Detector	LOD (ng mL^{-1})	References
Ninhydrin	1 : 1355	100 °C	5	UV	40	35
CNBF	1 : 17.4	60 °C	30	UV	9	12
TsCl	1 : 70.3	50 °C	10	UV	10	28
MOBS-F	1 : 17.7	45 °C	10	UV	0.1	34
DNP	1 : 75.2	R.T.	60	UV	50–100	36
OPA-MERC	On column			FLD	2–4	37
	On column			FLD	0.02–0.1	38
FMOC-Cl	1 : 3000	R.T.	30	FLD	0.16	29
	Excess	R.T.	60	FLD	1.5–9.5	33
	Excess	R.T.	30	FLD	0.02	39
	Excess	R.T.	10	MS	20–30	40
NBD-Cl	1 : 1188	90 °C	5	FLD	2–5.4	8
	Excess	60 °C	60	FLD	0.1–1	41
DPCS-Cl	1 : 4.2	70 °C	25	FLD	0.01–0.02	This work

[a] The molar ratio of analytes to derivatization reagents.

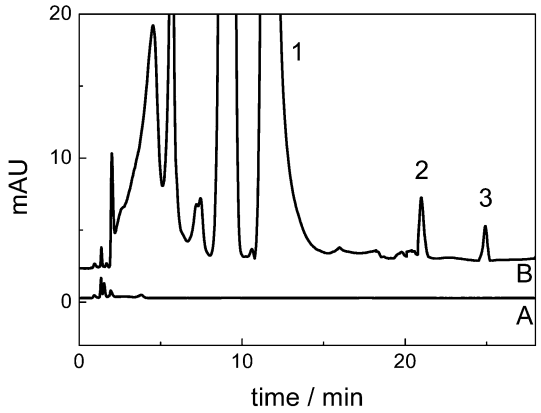

Fig. 3 Chromatograms obtained from a blank soybean sample (A) and a soybean sample labeled with DPCS-Cl (B) (peaks: 1 = DPCS–OH; 2 = DPCS–AMPA; 3 = DPCS–GLYP).

Table 4 Recoveries of GLYP and AMPA in soybean ($n = 5$)

Compounds		Amount added (mg kg^{-1})	Average recovery (%)	RSD[a] (%)
GLYP	Soybean 1	0.05	85.4	4.7
	Soybean 2	0.10	91.6	3.6
	Soybean 3	0.50	94.1	3.1
AMPA	Soybean 1	0.05	87.3	4.4
	Soybean 2	0.10	92.6	3.4
	Soybean 3	0.50	95.2	3.0

[a] RSD = Relative standard deviation.

Table 5 Precision of determination of GLYP and AMPA in soybean

		RSD[a] (%)	
		GLYP	AMPA
$n = 7$	Soybean 1	4.6	4.1
	Soybean 2	3.8	3.3
	Soybean 3	3.4	3.2
$n = 5$	Soybean 1	4.7	4.3
	Soybean 2	3.9	3.5
	Soybean 3	3.5	3.3

[a] RSD = Relative standard deviation.

investigated the intra-day precision by repeating measurements of samples 7 times a day and the inter-day precision by repeating measurements of samples within five days. The results are shown in Table 5. We can see that RSDs of intra-day were less than 4.6% and RSDs of inter-day were less than 4.7% when this method was used for determination of soybean samples. It was shown that this method had satisfactory precision.

3.4.4 Determination of GLYP and AMPA residues in soybean samples. The allowed levels of different vegetables ranged from 0.1 to 20 mg kg^{-1}.[7] We determined the residues of GLYP and its metabolite AMPA in several soybeans. After the steps of each soybean sample processing described in Section 2.3, GLYP and AMPA were derivatized. Then we determined the derivatives under chromatographic conditions described in Section 2.4.2. This method gave the method detection limits of 0.002 mg kg^{-1} for GLYP and 0.001 mg kg^{-1} for AMPA in soybean samples. We detected residues of GLYP and AMPA in genetically modified soybeans. Our results show that there is a certain amount of GLYP and AMPA in genetically modified soybeans. It is possibly because of the extensive use of GLYP in the transgenic soybean growth process due to their nature of glyphosate-resistance. Then, the concentrations of GLYP and AMPA obtained from our experiments were compared with those measured by LC-MS. The results are listed in Table 6. The RSD of each sample is less than 4.0%. The comparison clearly shows that our results agree satisfactorily with those obtained by LC-MS/MS.[45] HPLC-MS/MS operation conditions: (1) electrospray voltage: 3500 V; (2) pressure of atomization gas: 50 Pa; (3) pressure of curtain gas: 20 Pa; (4) pressure of pilot gas: 25 Pa; (5) ion source temperature: 250 °C.

4. Conclusion

In this paper, a method for determination of glyphosate and AMPA was developed, and the simple extraction was successfully applied to soybean samples. 3,6-Dimethoxy-9-phenyl-9*H*-carbazole-1-sulfonyl chloride (DPCS-Cl) as a novel fluorescent labeling reagent was used for the first time in the determination of GLYP and AMPA. DPCS-Cl seemed to be an attractive choice owing to high fluorescence absorption and stable derivatives, simple derivatization procedure and no multiple derivatives or by-products. The present method showed good repeatability, excellent linearity and low detection limits, and it is hoped to provide a new method for the detection and quantification of amino compounds.

Table 6 Comparison between the values obtained by LC-MS/MS and those obtained by our HPLC-FLD

Samples		Determined by LC-MS/MS (mg kg^{-1})		Determined by our HPLC-FLD (mg kg^{-1})		Er (%)	
		GLYP	AMPA	GLYP	AMPA	GLYP	AMPA
Soybean (genetically modified)	[a]	0.052	0.063	0.050	0.064	4.0	−1.2
	[b]	0.069	0.061	0.071	0.063	−2.9	−3.3
	[c]	0.044	0.071	0.045	0.069	−2.3	2.8

[a] American Soybean, origin USA. [b] Argentina Soybean, origin Argentina. [c] Brazil Soybean, origin Brazil.

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