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Residue determination of glyphosate in environmental water samples with high-performance liquid chromatography and UV detection after derivatization with 4-chloro-3,5-dinitrobenzotrifluoride

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ABSTRACT

A pre-column derivatization high-performance liquid chromatographic method for glyphosate analysis has been developed. Derivatization of glyphosate was performed with 4-chloro-3,5-dinitrobenzotrifluoride (CNBF). In pH 9.5 H_3BO_3–$Na_2B_4O_7$ media, the reaction of glyphosate with CNBF completed at 60 °C for 30 min. The labeled glyphosate was separated on a Kromasil C18 column (250 mm × 4.6 mm, 5 μm) at room temperature and UV detection was applied at 360 nm. The separation of labeled glyphosate was achieved within 15 min by gradient elution mode. Compared to other pre-column derivatization, this derivatization was performed more mildly, the derivative was more stable, and the detection limits of a few reagents were higher than CNBF, except 9-fluorenylmethyl chloroformate (FMOC-Cl) using fluorescence and mass spectrometry, however, this reagent avoid to be removed after derivatization like FMOC-Cl. The detection limit of glyphosate was 0.009 mg L^{-1} (S/N = 3) without preconcentration and reach MRL, which is set at the level of 0.1 mg L^{-1} in China. The method linearity correlation coefficient was 0.9999, in concentrations ranging from 0.3 to 48.5 mg L^{-1}. The proposed method has been applied to the quantitative determination of glyphosate in environmental water with recoveries of 91.80–100.20% and R.S.D. of 2.27–6.80, depending on the sample investigated.

1. Introduction

Glyphosate [*N*-(phosphonomethyl)glycine] is a nonselective, post-emergent herbicide used for the control of a wide range of weeds [1]. It has excellent performance and effect in weed control, and by increasing the introduction of transgenic plant with anti-glyphosate capability, it has been extensively used in the agricultural field during the past years [2–4]. Due to its strong retention on soil components, high solubility in water and long half-life in the environment (about 47 days), glyphosate may still be detected long after application or even far from the site of application [5]. For decades, the long-term environmental and ecological effects of glyphosate have been the target of research and discussion [6–10]. A reliable method for the determination of glyphosate in environmental samples is therefore a must for this research and environmental monitoring.

Analytical methods for the analysis of glyphosate include thin-layer chromatography (TLC) [11], hydrophilic interaction chromatography (HILIC) [12], capillary electrophoresis (CE) [13–16], gas chromatography (GC) [17] and liquid chromatography (LC) [18]. LC is preferred over GC because of the ionic character of glyphosate. However, for LC with conventional detection systems, such as UV–vis or fluorescence detectors, glyphosate needs to be derivatized because of the lack of chromophore or fluorophore. For these reasons, chemical derivatization or labeling becomes a necessary procedure to transform the analytes into derivatives that can be more easily isolated, separated and detected [19]. Thus for sensitive determination of glyphosate, many derivatization agents have been developed including 4-chloro-7-nitrobenzofurazan (NBD-Cl) [20], 2,2-dihydroxy-1h-indene-1,3-(2h)-dione (Ninhydrin) [21–23], l-fluoro-2,4-dinitrobenzene (DNP) [24], 9-fluorenylmethyl chloroformate (FMOC-Cl) [25–34], *o*-phthalaldehyde-mercaptoethanol (OPA-MERC) [35,36], *p*-toluenesulphonyl chloride (TsCl) [37,38].

4-Chloro-3,5-dinitrobenzotrifluoride (CNBF) is an important fine chemical, which has been known to react with primary or secondary amines in presence of base to produce stable *N*-substituted-2,6-dinitro-4-(trifluoromethyl)-benzamine derivatives which are satisfactory ultraviolet absorption [39–43].

In the present work, we proposed a simple, sensitive and selective HPLC method for the determination of glyphosate by pre-column derivatization with CNBF. The derivatization process is fast and requires minimal consumption of solvents. This reagent avoid to be removed after derivatization like FMOC-Cl, and the samples in environmental water need not to be preconcentrated, and detec-

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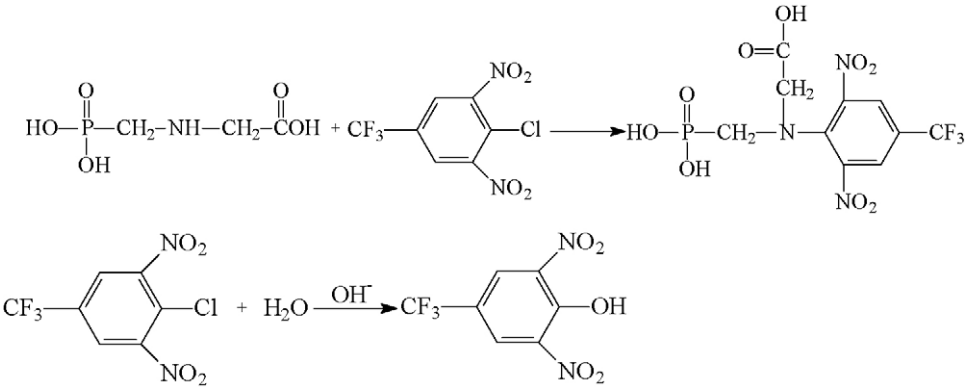

Fig. 1. The reaction scheme of CNBF with amino group on glyphosate molecule.

tion limit is 0.009 mg L^{-1} which is comparable or better than that reported by existing detection methods, and reach MRL in water, which is set at the level of 0.1 mg L^{-1} in China. To the best of our knowledge there is no method available describing the HPLC detection of glyphosate with CNBF derivatization.

2. Materials and methods

2.1. Instrumentation and conditions

A high-performance liquid chromatography system, which consisted of two LC-10ATvp pumps and an SPD-10Avp, ultraviolet detector (Shimadzu, Japan) was used for the analysis and separation. A reversed-phase Kromasil ODS C18 column (250 mm × 4.6 mm i.d., particle size 5 μm) was used for separation at ambient temperature and Chromato Solution Light Chemstation for LC system was employed to acquire and process chromatographic data.

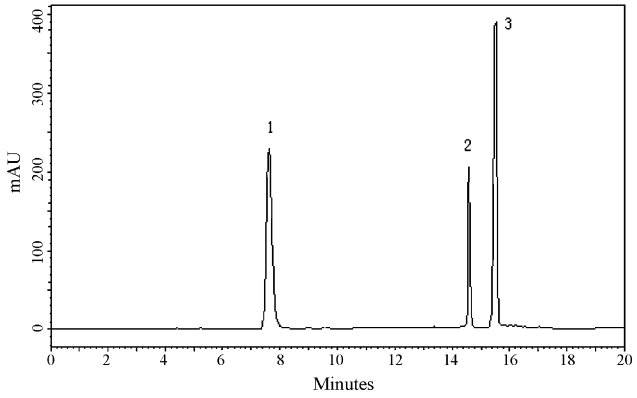

Fig. 2. HPLC chromatogram of the CNBF derivative of glyphosate standard solution. Chromatographic conditions: column, Kromasil C18 (250 mm × 4.6 mm, 5 μm); UV-DAD detection, λ = 360 nm; mobile phase, a linear gradient starting from 30% of acetonitrile–CTAB solution (5:5, v/v) to reach 100% of the solvent at 10 min; flow rate, 0.8 mL min^{-1}; room temperature.

2.2. Chemicals and reagents

Glyphosate was purchased from Nantong Jiangshan Agrochemical & Chemical Co., Ltd. (Jiangsu, China). A standard solution of 0.01 mol L^{-1} glyphosate was prepared in H_3BO_3–$Na_2B_4O_7$ buffer and further diluted to the required concentration when used. Working standards were prepared by mixing aliquots of the stock solutions and diluting with water. The stock and working standards were stored in dark at 4 °C when not in use. Acetonitrile and methanol were HPLC grade and purchased from J.T. Baker (USA). Ultrapure water was obtained in the laboratory using a Milli-Q water purification system (Millipore, Billerica, MA). CNBF was obtained from Alfa Aesar (Ward Hill, MA, USA) and its solution was prepared in methanol and filtered through a 0.45 μm nylon membrane filter and refrigerated when not in use. Cetyltrimethylammonium bromide (CTAB) was purchased from Sinopharm Chemical Reagent Beijing Co., Ltd. (Beijing, China). All other chemicals and solvents were analytical grade and from commercial sources. H_3BO_3–$Na_2B_4O_7$ buffer was prepared by mixing 0.2 mol L^{-1} H_3BO_3 solution with 0.05 mol L^{-1} $Na_2B_4O_7$ solution to the required pH value. The phosphate buffer was prepared by dissolving K_2HPO_4 in water, and then the pH was adjusted to the required value by adding concentrated H_3PO_4.

2.3. Chromatographic method

Before the analysis, the C18 column equipped with a guard column (4 mm × 3 mm i.d.) was pre-equilibrated with the mobile phase for 30 min. The HPLC separation of glyphosate derivative was carried out on Kromasil ODS C18 column. Acetonitrile–0.01 M CTAB solution (5:5, v/v, eluent A), phosphate buffer (50 mM, pH 2.5 with phosphoric acid) (eluent B) were used as mobile phase. All the solvents were filtered with a 0.45 μm membrane filter. The program was set for a linear gradient starting from 30% of solvent A to reach 100% of the solvent at 10 min. The injection volume was 20 μL, and detection wavelength 360 nm. The flow rate was con-

Table 1
Effect of glyphosate/CNBF ratio, derivatization pH value, derivatization reaction temperature, derivatization reaction time on the peak area of CNBF–glyphosate derivative.

[a]Glyphosate/CNBF ratio	Peak area (×10^6)	pH	Peak area (×10^6)	Temperature (°C)	Peak area (×10^6)	Time (min)	Peak area (×10^6)
1:1	3.2435	7.5	3.1864	40	2.2472	10	2.5465
1:1.5	3.3522	8.5	3.4165	50	3.3453	20	3.3546
1:2.5	3.4326	10.0	3.4289	60	3.4362	30	3.4384
1:3.5	3.4342	11.0	3.4062	70	3.4323	40	3.4453

[a] The glyphosate concentration value used was 1.0×10^{-3} mol L^{-1}; the CNBF concentration value selected was 2.5×10^{-3} mol L^{-1} for the effect of pH, temperature and reaction time on the peak area of CNBF–glyphosate derivative; each sample was determinated in triplicate injections.