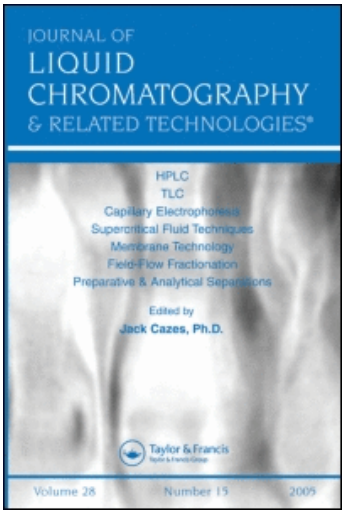

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A Comparison of UV and Fluorescence Detectors in the Liquid Chromatographic Analysis of Glyphosate Deposits After Post-Column Derivatization

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A COMPARISON OF UV AND FLUORESCENCE DETECTORS IN THE LIQUID CHROMATOGRAPHIC ANALYSIS OF GLYPHOSATE DEPOSITS AFTER POST-COLUMN DERIVATIZATION

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ABSTRACT

A sensitive and rapid liquid chromatographic method for the analysis of glyphosate using either post-column ninhydrin derivatization and UV detection (UV-D) or post-column reaction, fluorogenic labelling with *o*-phthalaldehyde in the presence of 2-mercaptoethanol and fluorescence detection (FD) is described. The sensitivity of the UV-D and two fluorescent detectors (HP-FD and Kratos-FD) were compared and evaluated to analyze glyphosate residues from glass fibre filter (GFF) discs used as deposit collectors in aerial application. The herbicide was extracted from the GFF discs using 30.0 mL of phosphate buffer adjusted to pH 2.1, derivatized and quantified. The method was tested initially, optimized and validated by using glyphosate standards. The average percent recoveries from fortified GFF samples were 93.7 (UV-D), 99.2 (HP-FD) and 94.9 (Kratos-FD). The corresponding standard deviation and coefficient of variation (percent) were 6.0 and 6.44; 1.6

and 1.67; and 4.4 and 4.59, respectively. The minimum quantifiable limits (ng/50 μL injection) for UV-D, HP-FD and Kratos-FD were 49, 16 and 28, respectively. The study showed that HP-FD was very sensitive compared to the other two detectors. The simplicity of the method allows high sample throughput for the routine analysis of glyphosate deposits.

INTRODUCTION

Glyphosate [N-(phosphonomethyl) glycine, $HOOC\text{-}CH_2\text{-}NH\text{-}CH_2\text{-}P(O)(OH)_2$] is a broad spectrum postemergence herbicide introduced by Monsanto Company, St. Louis, MO, USA in 1974 and is used extensively worldwide to control many annual and perennial weeds.[1] It translocates from the treated foliage to other plant parts inhibiting the biosynthesis of aromatic amino acids, thus causing the plant's death.[1,2] Vision®, a formulation concentrate containing 35.6% (w/v) of the acid equivalent of the isopropylamine salt $[(CH_3)_2HC\text{-}N^+H_3\text{-}O\text{-}P(O)(OH)\text{-}CH_2\text{-}NH\text{-}CH_2\text{-}COOH]$ of glyphosate is registered in Canada for forestry use. The aqueous formulation is sprayed aerially for conifer release and site preparations.

Deposits found on the target foliage during aerial application of pesticides over forests are extremely variable and the overall efficiency in application is low.[3,4] Pesticide deposition during aerial spray applications is influenced by a series of complex, interdependent processes, such as meteorological conditions, aircraft parameters, physicochemical properties of the active ingredient, type of formulation, dosage, drop size, target shape and size, terrain, canopy characteristics, etc. In addition, pilot error and inappropriate swath positioning could contribute to the variability in deposition. Therefore, to determine the spray efficiency and resultant efficacy, it has become necessary to measure and quantify the amount of material deposited on the biological target, namely the foliage, during glyphosate sprays. For such deposit accountability studies, 2-dimensional artificial deposit collectors, such as glass fibre filter (GFF) discs, Mylar® discs, etc., are generally used[5] to simulate the leaves of broad-leaved weeds instead of natural foliage. This simplifies the cumbersome and tedious analytical procedures required to analyze glyphosate residues from foliage.[1]

In a recent glyphosate field trial, we used GFFs mounted onto sampling units, to collect spray deposits. The residues were solvent extracted, derivatized in a post-column reactor (PCR) using ninhydrin and analyzed by high performance liquid chromatography (HPLC) using ultraviolet detection (UV-D).[1,6] We also used the method of fluorogenic labelling with fluorescence detection (FD) by post-column oxidation of glyphosate to glycine using calcium hypochlorite $[Ca(OCl)_2]$ followed by derivatization with *o*-phthalaldehyde (OPA) in the presence of 2-mercaptoethanol

(MERC) and detecting the fluorophore by Kratos and Hewlett-Packard fluorometers connected in series.[1,7-10] The influence of chromatographic conditions on performance and sensitivity between the two fluorometers and between the UV-D and FD methods were evaluated and compared. The methods and experimental conditions used in this study were validated initially and optimized for performance enhancement by analyzing the pure glyphosate standard under different experimental conditions by using the HPLC-PCR-ninhydrin and HPLC-PCR-fluorogenic labelling and the corresponding detection methods. Results are reported in this paper.

MATERIALS AND METHODS

Solvents

Methanol (BDH OmniSolv®, available from VWRCanlab, Mississauga, ON), water (deionized and purified by Milli-Q® Water System, Millipore Ltd., Mississauga, ON) and dimethyl sulfoxide (DMSO) (HPLC/spectrograde, available from Pierce Chemical Co., Rockford, IL) were used in the study after filtering using Nylaflo filter of 0.20 μm pore size (available from Gelman Sciences Inc., Rexdale, ON).

Reagents

Potassium dihydrogen phosphate (KH_2PO_4), concentrated phosphoric acid (85%) (H_3PO_4), sodium chloride (NaCl), sodium hydroxide (NaOH), sodium acetate solution (4.0 M, CH_3COONa), 2-mercaptoethanol (MERC) and *o*-phthalaldehyde (OPA) were of primary standard grade obtained from Fisher Scientific Ltd. (Unionville, ON). Ninhydrin was obtained from Sigma Chemical Co. (St. Louis, MO). Hydrindantin (dihydrate), calcium hypochlorite [$Ca(OCl)_2$] (65% available chlorine) and disodium tetraborate ($Na_2B_4O_7.10H_2O$) were analytical grade supplied by VWRCanlab. Pure glyphosate, to prepare standard solutions for fortification and HPLC calibration, was supplied by Monsanto Company. Glass fibre filter (GFF) discs (9 cm diameter, 63.62 cm^2), used as deposit collectors, were purchased from Gelman Sciences.

HPLC Instrumentation

The liquid chromatograph system consisted of a Hewlett-Packard (HP) 1090M HPLC, HP-9000/310 computer work station operated by HP-7995R software, HP-automatic sampler, variable volume auto-injector fitted with a 250-μL syringe, DR5

binary solvent delivery system with a helium-purge degassing system, two dual-syringe metering pumps which gave stable and reproducible flows and a diode-array detector (UV-D) with wavelength range of 190 to 600 nm. For the detection and quantification of the derivative formed between ninhydrin and glyphosate, the wavelength of UV-D was set at 570 nm.

A Kratos (Kratos Co., Ramsey, NJ) post-column derivatization system, URS 051, equipped with dual reagent pumps for delivering ninhydrin (single pump only) or $Ca(OCl)_2$ and OPA (both pumps) was used. The reagents were degassed with helium prior to use and kept under a helium atmosphere. The reaction between glyphosate and ninhydrin, at 100°C, and the oxidation of glyphosate to glycine, at 43°C, were carried out in a temperature controlled Pierce Reacti-Therm Heating Module (Pierce Chemical Company) consisting of 284 x 0.02 cm id coil. The OPA was allowed to react with glycine in the Kratos 1-mL reaction coil, to form the fluorophore.

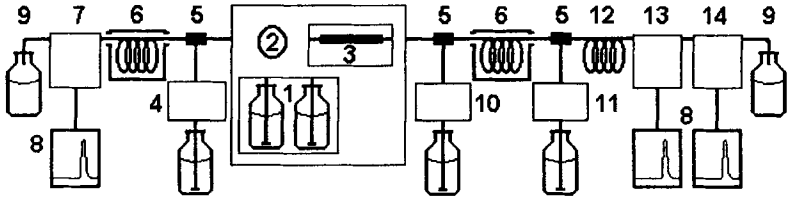
The FDs used were: a HP 1046A FD (HP-FD) equipped with a 5-µL flow-cell, variable excitation and emission wavelengths (range for both 190 to 800 nm and both can be scanned to optimize the maximum wavelength); and a Kratos FS970 HPLC fluorometer (Kratos-FD) equipped with a 10-µL flow-cell and automatic overload reset (FSA 986), with variable excitation wavelengths (190 to 700 nm; GM 970 monochromator) and fixed-wavelength emission filters.

The HPLC analytical column used was the Aminex A-9 cation exchange (10 x 0.46 cm id) preceded by Aminex A-9 cation exchange K^+ form cartridge guard column (Bio-Rad Laboratories, Richmond, CA), both thermostated at 50°C in the HPLC column compartment.

A schematic of the instrumental arrangement for the post-column derivatization using ninhydrin (UV-D) and OPA/MERC (FD with HP and Kratos fluorometers) is shown in Figure 1. The fluorescent derivatives formed in PCRs when glyphosate reacted with ninhydrin and with OPA/MERC after oxidation with $Ca(OCl)_2$ are given in Figures 2A and 2B, respectively.

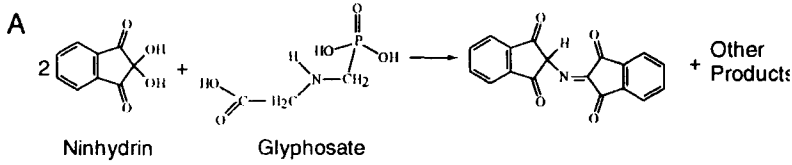
Procedure

The phosphate buffer, used as the mobile phase in the HPLC and as the extractant for glyphosate deposits from GFFs, was prepared by dissolving 0.006 M KH_2PO_4 (0.8437 g/L) in water with 4% methanol and adjusting the pH to 2.1 with concentrated H_3PO_4. The solution was filtered (0.20-µm Nylaflo filter) and degassed prior to use. The mobile phase was delivered to the Aminex A-9 column at a rate of 0.5 mL/min.



1 Solvents and pump
2 Autosampler
3 Heated column
4 Pump - ninhydrin
5 Vortex T mixer
6 Heated reaction coil
7 Diode array detector
8 Integrator
9 Waste
10 Pump - hypochlorite
11 Pump - OPA
12 Reaction coil
13 Fluorometer - Hewlett Packard
14 Fluorometer - Kratos

Figure 1. Configuration of HPLC-PCR-ninhydrin and HPLC-PCR-fluorogenic labelling systems for the analysis of glyphosate in glass fibre filters.



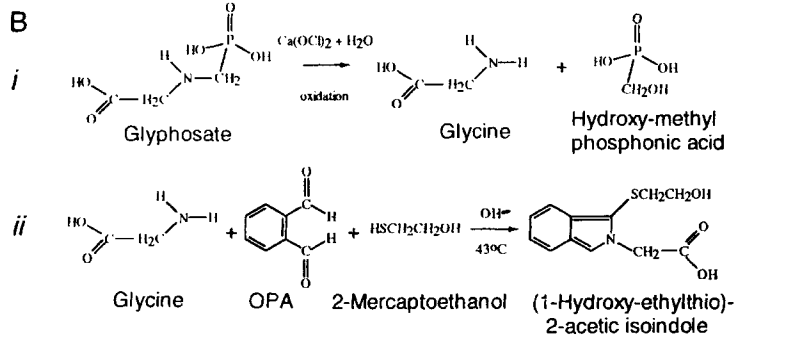


Figure 2. Reaction scheme of glyphosate with (A) ninhydrin, (B*i*) oxidation of glyphosate to glycine and (B*ii*) derivatization of glycine to isoindole derivative.

The ninhydrin solution was prepared as described by Thompson *et al.*[6] Briefly, 83.3 mL of 4 M sodium acetate and 13.33 g of ninhydrin were added successively with constant stirring under a nitrogen atmosphere to 833.4 mL of solution containing 1:1 ratio (volume) of deionized water and DMSO. To this, 0.461 g of hydrindantin dihydrate dissolved in 83.3 mL of DMSO was added. The solution was stored under nitrogen at 4°C in a tightly sealed bottle covered with aluminum foil. The ninhydrin solution was mixed with the column eluent at the rate of 0.5 mL/min.

The phosphate-hypochlorite reagent used in the oxidation of glyphosate was prepared by dissolving 1.36 g of KH_2PO_4, 11.6 g of NaCl and 0.4 g of NaOH in 0.5L water. A clear solution containing 0.060 g $Ca(OCl)_2$ in 50 mL water was then added to the above and the volume was brought to 1-L. The solution was shaken well, filtered (0.20-μm filter) and stored at 4°C in a tightly sealed bottle covered with aluminum foil. The oxidative solution was mixed with the column eluent at a rate of 0.1 mL/min.

The fluorogenic reagent was prepared daily by first dissolving 10.08 g of disodium tetraborate in 900 mL deionized water, under constant stirring. The pH was then brought up to 10.4 by adding concentrated NaOH dropwise. Subsequently 30 mL of a methanolic solution containing 1.75 g OPA and 4.0 mL MERC was added to the borate solution. The volume was adjusted to 1.0 L with water, mixed well, filtered and stored in a sealed bottle covered with aluminum foil. It was mixed with the oxidized effluent stream at a rate of 0.21 mL/min.

A stock standard solution of glyphosate was prepared by weighing exactly 10mg of pure glyphosate and dissolving in 100 mL of water. Analytical standards (0.01 to 10.0 μg/mL) for instrument calibration and fortification of GFF were prepared by serial dilution of the stock solution using the mobile phase. The concentrations of the analyte used for the calibration purposes were: 0.10, 0.50, 0.75, 1.0, 2.0, 5.0, 7.5 and 10.0 μg/mL. Fifty-μL volume of each standard was used during injections, which corresponded to the mass equivalent of glyphosate ranging from 5.0 to 500 ng.

GFF discs were fortified (n = 6 for each concentration) by placing droplets (*ca.* 300 μm in diameter) of known volume of glyphosate standards onto individual discs corresponding to 0.24 to 2.37 μg of glyphosate/cm^2. Samples were allowed to air-dry, cut into pieces, put in 50-mL polypropylene centrifuge tubes and allowed to equilibrate in darkness at 4°C for 24 h. Thirty mL of the mobile phase was then added to each tube and shaken in a mechanical shaker for 2 h. The extracts were filtered (0.20-μm), analyzed by HPLC using UV and FD and the percent recovery levels were computed.

During the field study, the GFF discs, fixed onto folding aluminum plates and mounted onto metallic stakes,[5] were placed at different locations in each spray block at 0.5 h prior to glyphosate spray application. They were collected at 1 h after application. Each GFF disc was placed in a polypropylene centrifuge tube containing 30 mL of the phosphate buffer and stored in a portable refrigerator at 4°C. The samples were brought to the residue laboratory, extracted and analyzed as discussed in the previous paragraph.

Study Details

The HPLC-PCR-ninhydrin configuration (Figure 1) was used first to quantify glyphosate from the fortified and field GFF samples. The instrument system was turned on for about 0.5 h for stabilization; the pump, mixing tee, detector, etc. were washed with deionized water and methanol and the column was conditioned by flushing with the mobile phase. The flow rates for the buffer (0.5 mL/min) and ninhydrin (0.5 mL/min) and the temperature (100°C) of the heating module where the derivatizing reagent reacted with glyphosate to form the derivative (Figure 2A), were the same as Thompson *et al.*[6] and found to produce good response. Fifty-μL aliquots of glyphosate standards were injected in quadruplicate onto the Aminex A-9 column and the absorbance after the PCR with ninhydrin was measured at 570 nm. The area counts of the four injections agreed within 1.4%, showing good repeatability.

A calibration curve [average area count ± SD (n = 4) *vs* amount glyphosate (ng)] was prepared (Figure 3) and it was validated daily. It was used in the subsequent determination of glyphosate deposits on GFFs used for the fortification and in the analysis of field samples.

Similarly for the HPLC-PCR-fluorogenic studies, the flow rates of the oxidative and OPA solutions were optimized at 0.10 and 0.21 mL/min, respectively, to produce maximum response for the analyte. The cleaving temperature for the glyphosate to form glycine (Figure 2B) in the presence of $Ca(OCl)_2$ was set at 43°C. The excitation and emission wavelength for the HP-FD was selected by scanning and optimizing at 230 and 445 nm, respectively. The excitation wavelength for the Kratos-FD was set at 230 nm and the filter for the emission wavelength was chosen as close as possible to 445 nm by using a 440 nm filter.

Calibration curves (Figure 3) for both the detectors were prepared from the fluorogenic responses recorded for the standard solutions of glyphosate. These curves were used subsequently in the quantification of the analyte from the GFFs.

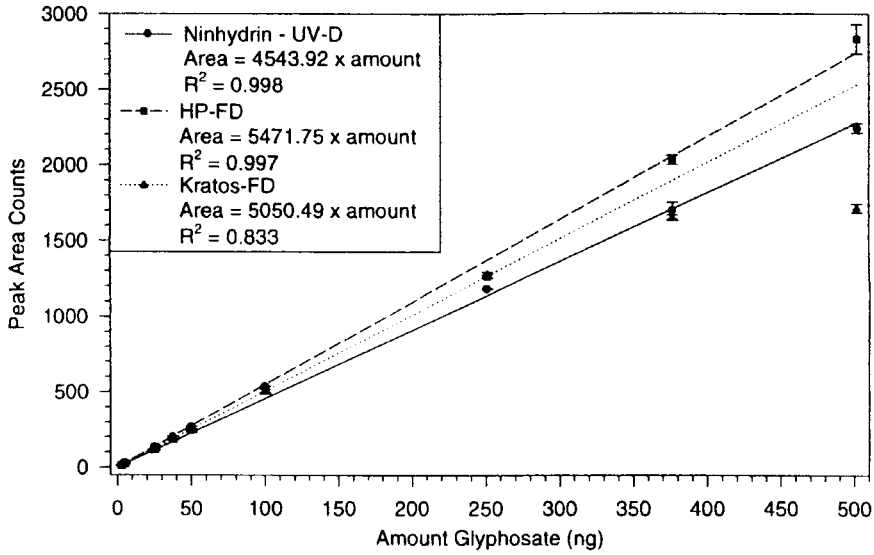


Figure 3. Calibration curves for glyphosate standards using UV and fluorescence detectors after post-column derivatization.

Response Optimization

Typical chromatograms obtained by the UV and two fluorescent detectors for glyphosate standard, GFF extract blank, and GFF extract from the field (glyphosate peak overlaid on the blank chromatogram) are given in Figure 4.

Much experimentation was done, by adapting the methods used by Moye *et al.*,[11] to maximize the response of the detectors to glyphosate derivatives. The mobile phase pH (2.1), the flow rate (0.5 mL/min) and the temperature (100°C) chosen for the reaction to occur between ninhydrin and glyphosate were found to be optimum.

Similarly, hypochlorite concentration of 60 mg/L, its flow rate of 0.1 mL/min, and $Ca(OCl)_2$ effluent pH of about 9, conversion of glyphosate to glycine at 43°C, OPA/MERC flow rate of 0.21 mL/min and excitation (230 nm) and emission (445nm) wavelengths of the fluorometers were prime requirements to obtain maximum response (peak area counts) in fluorogenic labelling of glyphosate. Any deviation from these chosen parameters, yielded inconsistent results and lower sensitivity.

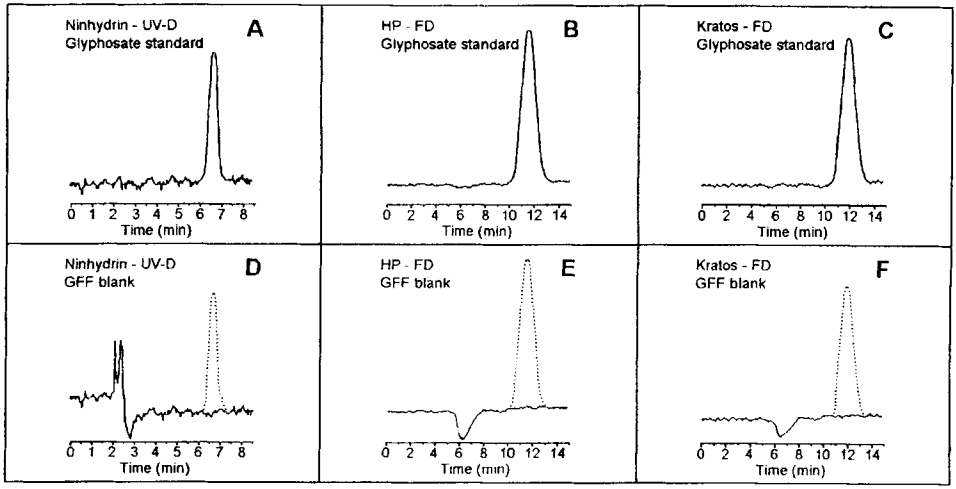


Figure 4. Representative chromatograms obtained from HPLC-PCR-ninhydrin (UV-D) and HPLC-PCR-fluorogenic (HP-FD and Kratos-FD) studies (glyphosate concentration injected 100 ng/50 µL) glyphosate peak in field extracts is shown by a dotted line over blank chromatograms in D to F.

RESULTS AND DISCUSSION

Injection of different concentrations of calibration solutions of glyphosate in the aminex A-9 column, followed by post-column derivatization and detection using the three detectors (UV-D, HP-FD and Kratos-FD), gave consistent results, indicating good repeatability. Plots of peak area count against amount of glyphosate (ng) (on the basis of underivatized material) (Figure 3) were linear for the UV-D from 50 to 500 ng, HP-FD from 5 to 375 ng and Kratos-FD from 5 to 250 ng mass ranges for 50 µL sample size. Beyond 250 ng glyphosate, the Kratos-FD showed deviation. In contrast, the UV-D did not show noticeable deviation for a wide range of concentrations (50 to 500 ng/50 µL) studied.

Excluding the points which were far off from the straight line for Kratos-FD, the response of the detectors was found to obey the linear regression equation: Y (peak area count) = b (slope) x c (amount glyphosate in ng) and the coefficient of determination (R^2) values were 0.998, 0.997 and 0.833 for UV-D, HP-FD and Kratos-FD, respectively. The values for 'b' are given in Figure 3. The higher R^2 values for UV-D and HP-FD indicate that these detectors are rugged and reliable in responding to a wide range of concentrations of glyphosate compared to Kratos-FD.

Table 1

Recoveries (%) of Glyphosate from Fortified Glass Fibre Filter Discs (Number of Replicates = 6)

Detector	Fortification Level (μg/cm²)[a]				Mean Recovery (%)	SD (±)	CV (%)
	0.24[b]	0.47[b]	0.95[b]	2.37[b]			
UV-D	NQ[c]	86.7	97.2	97.1	93.7	6.0	6.44
HP-FD	101.2	99.8	98.4	97.4	99.2	1.6	1.67
Kratos-FD	88.4	97.7	96.7	96.8	94.9	4.4	4.59

[a] Volume of buffer used in extraction = 30 mL.
[b] Amount injected in 50 μL at 0.24, 0.47, 0.95 and 2.37 μg/cm² were 25, 50, 100 and 250 ng, respectively. [c] NQ = not quantifiable below 0.46 μg/cm².

Detector sensitivity indicated by the slopes of straight lines in Figure 3 show that HP-FD is very sensitive among the three, and sensitivity decreased in the order HP-FD > Kratos-FD > UV-D. The corresponding sensitivity ratio computed from the slopes were 1.0 : 0.92 : 0.83. Together with slopes and accompanied sensitivity ratio, it is apparent that for the determination of glyphosate residues from GFF, HP-FD following PCR, would be preferable in comparison to the other two detectors if the deposit concentrations were low and higher analytical sensitivity was required.

The recovery, on the basis of underivatized glyphosate obtained from the GFF discs (area 63.64 cm²) fortified at 4 concentrations (range: 0.24 to 2.37 μg/cm² or 15.27 to 150.83 μg/disc) followed by extraction with 30 mL of buffer (mobile phase) and injection of 50 μL volumes (25 to 250 ng/injection, for the four fortification levels), is given in Table 1. The mean percentage recovery for each fortification level obtained from each detector with its standard deviation (± SD) and average coefficient of variation (CV), recorded in Table 1, were derived from multiple injections of the quadruplicate samples at each fortification level.

The mean percent recoveries observed in UV-D, HP-FD and Kratos-FD were 93.7, 99.2 and 94.9, respectively. However at the lowest fortification level (0.24 μg/cm²), the UV-D was not sensitive enough to quantify the amount of glyphosate present in the extract [minimum quantifiable limit (MQL) = 0.46 μg/cm²]. The SD (±) for UV-D, HP-FD and Kratos-FD were 6.0, 1.6 and

4.4, respectively. This indicates that the degree of reproducibility, as given by the SD values, decreased in the order HP-FD > Kratos-FD > UV-D. A similar trend among the detectors was also observed in the precision obtained for glyphosate measurements, as represented by the respective CV values. The precision was high (low % CV) in HP-FD (CV = 1.67 %), moderate in Kratos-FD (CV = 4.59 %) and low in UV-D (CV = 6.44 %). The minimum detection limit (MDL) was defined as three times the SD value[12] obtained for the four GFF discs fortified with glyphosate at 0.47 μg/cm^2 (29.91 μg/disc) and analyzed using the three detectors, after extraction with 30 mL of buffer and injecting 50 μL volume. The SD values found for UV-D, HP-FD and Kratos-FD were 4.9, 1.6 and 2.8, respectively, and the corresponding MDL values were 14.7, 4.8 and 8.4 ng per 50 μL injection. The MQL was defined as 10 times the SD[12] from the same four GFF samples and were 49, 16 and 28 ng per 50 μL injection for UV-D, HP-FD and Kratos-FD, respectively. These values are translatable to the MQL of 462, 151 and 264 ng/cm^2. The MQL values obtained in this study indicate that HP-FD is more sensitive, hence preferable, than the other two detectors to quantify glyphosate residues from GFF discs.

Figures 4A, 4B and 4C illustrate the chromatographic response of glyphosate standard (100 ng/50 μL injection) obtained in the UV-D, HP-FD and Kratos-FD, respectively. The mean retention times (RT) (min) for the analyte in the three detectors were 6.7 (UV-D), 11.5 (HP-FD) and 11.8 (Kratos-FD). The percent CV in RT for UV-D, HP-FD and Kratos-FD were 2.9, 1.3 and 2.1, respectively. The analyte peak obtained from each detector, depending on its sensitivity, was sharp and well defined; however, the baseline stability in UV-D was poor and consequently the chromatographic efficiency was rather low, compared to the two FDs. The chromatograms of the blank GFF extracts (Figs. 4D, 4E and 4F) did not show any interfering peak corresponding to the RT of glyphosate.

Analysis of GFF Discs from Field after Glyphosate Spray

The PCR-ninhydrin-UV-D and PCR-fluorogenic labelling with FD methods were extended and validated to quantify the glyphosate deposits from GFF discs used in field efficiency studies. The results are presented in Table 2. The data show that the inter-assay variations were not much and that all the three methods were quite adequate and suitable to quantify the analyte from GFF discs as long as the concentration of glyphosate in the 30-mL extract was above the MQL (980 ng/mL or 462 ng/cm^2 on the disc, so that a 50 μL injection contained above 49 ng). Because of the lower sensitivity of UV-D compared to FD, if the deposit levels were below 462 ng/cm^2, then the use of FDs was preferred in glyphosate quantification. This is amply evident from the residue data recorded in Table 2.

Table 2

Analysis of GFF Deposit Collectors from Field for Glyphosate Residues[a] using UV-D, HP-FD and Kratos-FD

Sample No.	Glyphosate Concentration ($\mu g/cm^2$) UV-D	HP-FD	Kratos-FD
1	3.53 ± 0.19	3.89 ± 0.11	3.66 ± 0.22
2	NQ[b]	0.17 ± 0.06	NQ
3	1.93 ± 0.16	2.05 ± 0.13	2.11 ± 0.31
4	NQ	0.36 ± 0.09	0.29 ± 0.13
5	5.66 ± 0.21	5.79 ± 0.17	5.60 ±0.27
6	NQ	0.45 ± 0.16	0.38 ± 0.19

[a] See footnotes in Table 1.

[b] NQ - not quantifiable. MQL for UV-D 49 ng/50 µL, or 980 ng/mL or 462 ng/cm^2; for HP-FD 16 ng/50 µL, or 320 ng/mL or 151 ng/cm^2; for Kratos-FD 28 ng/50 µL, or 560 ng/mL or 264 ng/cm^2.

The data also reaffirmed the relatively higher sensitivity (*i.e.* lower MQL) of HP-FD over the Kratos-FD and it was a choice detector and eminently suitable to monitor and quantify glyphosate residues from GFF discs, if the deposit levels were around or just above the 151 ng/cm^2 level, which would normally be the case, if off-target drift studies are planned for glyphosate.

None of the chromatograms of the extracts obtained from either the GFF discs fortified with glyphosate or the GFF discs collected from the field after spray application (glyphosate peak shown as dotted line, Figure 4D to 4F) exhibited any peak that would interfere with the analysis of glyphosate.

CONCLUSIONS

The method for the determination of glyphosate residue from GFF deposit collectors reported in this paper, was simple and shorter than the method reported in literature[6] because no pre-concentration by anion-exchange chromatography was

necessary. The use of extremely acidic mobile phase, instead of aqueous ammonia,[6] to extract the glyphosate residues from GFF discs readily facilitated the removal of the analyte in its zwitterionic and monoanionic forms.[2] Although the use of UV-D and Kratos-FD are acceptable for the quantification of glyphosate, the use of HP-FD offers advantages such as its relatively high detection sensitivity and reproducibility, especially if the analyte levels are low in the deposit collectors.

The use of post-column fluorogenic labelling and detection by HP-FD would allow for relatively high sample throughput if the HPLC instrument is equipped with an autosampler and a computerized data acquisition system, and in such cases a large number of samples can be injected without much pretreatment.

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