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*Short Report*

# Direct Determination of *N*-Nitrosoglyphosate in Technical Glyphosate Using Ion Chromatography with UV Detection

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The official method for analyzing *N*-nitrosoglyphosate (NNG), a relevant impurity, in glyphosate matrix demands complicated instrumentation and operating conditions not available in many laboratories. In this work, we developed a direct, simple, selective and sensitive ion chromatography (IC) method with UV detection for the determination of NNG in samples of technical glyphosate. To separate NNG from the matrix we used an IC anionic column mounted in a high-performance liquid chromatography (HPLC) apparatus equipped with a photo-diode array detector. The system used a high ionic strength eluent. The method was validated, taking into account the following figures of merit: selectivity, linearity, repeatability, intermediate precision, recovery, limit of detection (LOD), and limit of quantification (LOQ). The proposed method is sensitive enough to quantify NNG below the maximum concentration determined by both the Food and Agricultural Organization of the United Nations (FAO) and the Brazilian regulation.

**Keywords:** pesticides, chemical analysis, ion chromatography, *N*-nitrosoglyphosate, glyphosate

## Introduction

The agriculture in Brazil is very dependent on the use of pesticides, and the country is one of the greatest pesticide consumers in the world.[1] Glyphosate (GLY) or [*N*-(phosphonomethyl)glycine], the active ingredient (AI) most often used in Brazil, is classified as a non-selective, systemic and post-emergent herbicide. It is highly effective in weed control, but its carcinogenicity in humans is in the spotlight since IARC (International Agency for Research on Cancer) panel classified glyphosate in Category 2A (probably carcinogenic to humans).[2-5]

*N*-Nitrosoglyphosate (NNG) is a controlled impurity of relatively high toxicity derived from the synthesis of GLY and, as any nitrosamine, it is known for its carcinogenic properties.[6-8] The maximum NNG acceptable level based on the Food and Agricultural Organization of the United Nations (FAO)[9] is 1 mg kg$^{-1}$, the same level imposed by INC 2/2008,[10] the Brazilian regulation about relevant impurities in technical pesticides products.

The motivation for this work was the arrival of twenty technical glyphosate samples in the forensic chemistry laboratory of Brazilian Federal Police. The samples were suspected of containing NNG in concentrations higher than the maximum level determined by INC 2/2008.[10]

The analytical methods proposed by the literature for the determination of NNG are often similar to the ones used to quantify GLY itself, due to their similarities in structures (Figure 1) and chemical properties. The FAO proposed determination method for NNG is based on a high-performance liquid chromatography (HPLC) system using strong anion exchange column, mobile phase with methanol/ammonium phosphate solution at pH 2.1 and post-column derivatization, where NNG reacts with HBr to form nitrosyl cation. The nitrosyl cation then reacts with *N*-(1-naphthyl)ethylenediamine and sulphanilamide to form a purple azo dye which is detected at 550 nm using UV-Visible detector.[11]

The main analytical challenge when quantifying NNG in technical GLY samples is the significant difference in their concentrations (high percentage for GLY and mg kg$^{-1}$ of NNG) resulting in co-elution of both compounds in the chromatographic system. The mobile phase pH at the HPLC

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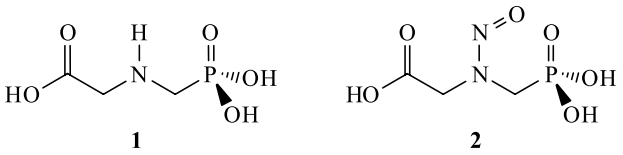

**Figure 1.** Structural formula of glyphosate (GLY, 1) and *N*-nitrosoglyphosate (NNG, 2).

system is an important parameter, since it modulates the hydrogen dissociations in both GLY and NNG, and can increase the selectivity of the chromatographic separation. On the other hand, the presence of the nitrous group in NNG allows the use of UV detection (absorbance peak at 244 nm),[12] while for GLY the absorption bands lower than 200 nm demand the use of special conditions (e.g. oxygen-free atmosphere or vacuum chambers).

It has been reported that the retention time of NNG[12] and GLY[13] decreases in the presence of a large excess glyphosate and also with increasing ionic strength of the eluent (e.g. with $Na_2CO_3$). This is in agreement with the behavior of an ion-exchange column, when using a matrix with large excess of ions. Considering that commercial samples of technical and formulated glyphosate products exhibit GLY at concentrations far higher than the concentration of NNG, an adequate analytical method that overcomes this difficulty should be available.

The aim of this work was to develop a direct (no need of derivatization), simple, selective, and sensitive method for the determination of *N*-nitrosoglyphosate in samples of technical glyphosate.

## Experimental

### Chemicals

Glyphosate (99.8%) was obtained from Monsanto Brazil Ltda., and glyphosate-*N*-nitroso mono sodium salt (98.5%) was purchased from the Dr. Ehrenstorfer GmbH brand. Other chemicals were of HPLC grade. The stock solutions, prepared every week, were stored in a refrigerator and diluted to a series of concentrations with deionized water (Milli-Q system) before use.

### Samples and standards preparation

Two grams of each questioned technical glyphosate product (samples seized by the Brazilian Federal Police) were diluted with 0.1 mol $L^{-1}$ NaOH until final volume of 10 mL. The solution was vigorously stirred for 10 minutes, sonicated for 15 minutes, and filtered (Titan®, 0.45 μm).

Standards stock solutions of glyphosate (10.0 g $L^{-1}$ in NaOH 0.1 mol $L^{-1}$) and *N*-nitrosoglyphosate (500 mg $L^{-1}$ in $H_2O$) were prepared. Working solutions were prepared daily by dilution of the stock solutions, and kept in the refrigerator during the experiments.

### Ion chromatography with UV detection

Ion chromatography was carried out using an anionic column (Metrosep A Supp 7 250/4.0 mm) and a pre-column (Metrosep A Supp 4/5 Guard) in an Agilent chromatographic system (1100 Series) equipped with a photo-diode array detector (named as IC-UV). The mobile phase was a high ionic strength solution containing $Na_2SO_4$ (0.01 mol $L^{-1}$) and NaOH (0.01 mol $L^{-1}$ at pH 10). Chromatographic conditions: detection was done at 244 nm; mobile flow rate of 0.8 mL $min^{-1}$; injection volume of 40 μL; run time of 27 min and 5 minutes of post-running time. All chromatography was performed at 25 °C but the vials tray was kept at 10 °C.

### Validation

The validation was performed studying the following figures of merit: selectivity, linearity, repeatability, intermediate precision, recovery, limit of detection, and limit of quantification.

The content of NNG in the commercial samples was calculated by the equation 1:

$$C_N = \frac{[NNG]}{[GLY]} \times 10^6 \quad (1)$$

where $C_N$: NNG content in the sample in ppm (mg $kg^{-1}$); [NNG]: determined concentration of NNG in solution (mg $L^{-1}$); [GLY]: nominal GLY concentration in solution (mg $L^{-1}$).

*N*-Nitrosoglyphosate was quantified using analytical curves generated from five matrix matched standards at 0.1, 0.25, 0.75, 2.0, and 4.0 mg $L^{-1}$ of NNG in a 10 g $L^{-1}$ glyphosate solution, used to simulate the actual concentrations present in technical GLY products. The signal appeared as two partially resolved peaks, whose areas were integrated for NNG quantification (Figure 2).

## Results and Discussion

### Selectivity

Previous tests using traditional ion chromatography with conductivity detector was able to separate, with good resolution, and detect both GLY and NNG in mixed standard solutions. Nevertheless, when the matrix was

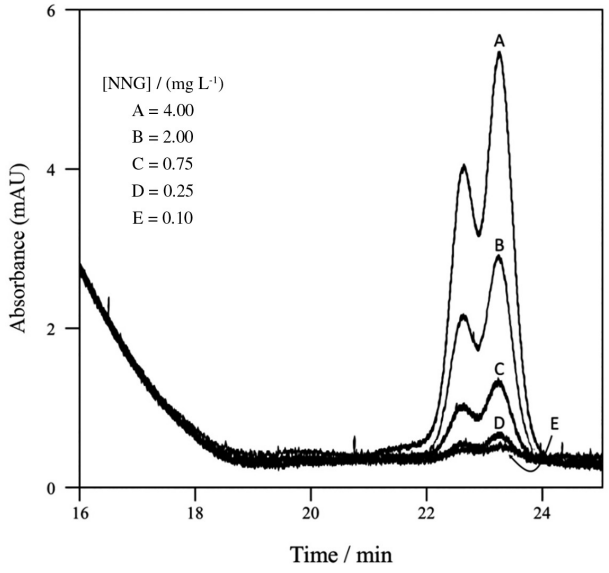


**Figure 2.** HPLC-UV chromatograms obtained from the calibration solutions of NNG (ranging from 0.10 and 4.00 mg $L^{-1}$) in matrix matched standards containing 10.0 g $L^{-1}$ of GLY.

composed of mainly GLY (as in technical products) the chromatograms presented a large GLY peak tail and no resolution for NNG. Based on these results, we moved the IC anion column from the ion chromatographic system and mounted it on the HPLC system coupled with a diode array detector (DAD). With this new arrangement an adequate resolution for NNG in a concentrated GLY matrix was obtained.

Injections of blanks prepared with the solvents/reagents used in the tests, and standard solutions of glyphosate between 10 and 40,000 mg $L^{-1}$ were performed. In all cases, there were no interfering peaks at the retention time of NNG.

Pastore *et al.*[12] fully characterized NNG using nuclear magnetic resonance (NMR), spectrophotometric and electrochemical analysis, proving the existence of two conformers, with a proportion in an equilibrium of 60:40. As shown in Figure 2, our results corroborate such finding, showing two peaks at the chromatogram ($t_r$ = 20.8 and 21.4 min), both with the same UV spectra. According to those authors, depending on the pH of the mobile phase, these peaks can elute separately, as observed in our results.

### HPLC-UV system accuracy (repeatability)

The accuracy was evaluated using nine injections of the same NNG solution, and by calculating the relative standard deviation or coefficient of variation (CV, %) of the peak areas. While the acceptable CV for an analyte with concentration around 1.0 mg $kg^{-1}$ is 15%,[14] this method resulted in a CV value of 0.9%, proving to be very accurate.

### Intermediate precision (method)

We investigated the intermediate precision through six determinations of one real sample, with an average concentration of NNG of 0.65 mg $kg^{-1}$, performed by two different analysts. The method once again showed good accuracy, with CV = 5.2%.

### Recovery

Two real samples were dissolved as previously described, in duplicate, with a concentration of NNG of 0.65 mg $kg^{-1}$ (dried weight). Those solutions were fortified with known concentrations of NNG, and they were analyzed in order to evaluate the recovery at two different NNG ranges, 0.2 and 0.4 mg $L^{-1}$. The results obtained were (recovery ± SD), respectively, 83.0 ± 0.9% and 105.3 ± 10.0%.

### Linearity and limits of detection (LOD) and quantification (LOQ)

The LOD and LOQ were calculated based on the slope of the calibration curve (S) and the standard deviation of the response (SD), according to the formula: LOD = 3.3SD / S, LOQ = 10SD / S, in which SD was obtained from the intercept of the calibration curve.[14]

Linearity (expressed by the regression coefficient values, $r^2$) was 0.9998 and the values obtained for the LOD and LOQ were, respectively, 0.016 mg $L^{-1}$ (corresponding to 0.08 mg $kg^{-1}$ in the sample) and 0.048 mg $L^{-1}$ (corresponding to 0.24 mg $kg^{-1}$ in the sample). Thus, the proposed method is sensitive enough to quantify NNG in GLY samples above the limits of Brazilian regulation.

### Questioned samples analysis

All 20 (twenty) questioned technical glyphosate product (samples seized by the Brazilian Federal Police) were prepared in duplicate and analyzed according to this article. As seen in Table 1, NNG levels in all samples were above the limit of quantification and below 1 mg $kg^{-1}$, the limit imposed by national and international regulation.

## Conclusions

The development of a simple and direct method (without derivatization), sensitive enough to quantify this impurity at levels below legislated limits, can facilitate NNG control in technical pesticide products by reducing the costs and time of analysis.

**Table 1.** *N*-Nitrosoglyphosate (NNG) determined by HPLC-UV in technical glyphosate (GLY) samples seized by the Brazilian Federal Police

| Sample | GLY[a] / (mg $L^{-1}$) | NNG 1[b] / (mg $L^{-1}$) | NNG 2[b] / (mg $L^{-1}$) | NNG[c] / (mg $kg^{-1}$) |
|---|---|---|---|---|
| 1 | 199,967.0 | 0.074 | 0.062 | 0.34 |
| 2 | 200,020.0 | 0.054 | 0.048 | 0.25 |
| 3 | 200,079.0 | 0.116 | 0.116 | 0.58 |
| 4 | 199,953.0 | 0.058 | 0.070 | 0.32 |
| 5 | 200,090.0 | 0.060 | 0.060 | 0.30 |
| 6 | 199,959.0 | 0.074 | 0.120 | 0.49 |
| 7 | 200,093.0 | 0.060 | 0.080 | 0.35 |
| 8 | 199,985.0 | 0.060 | 0.092 | 0.38 |
| 9 | 200,007.0 | 0.098 | 0.112 | 0.53 |
| 10 | 200,087.0 | 0.124 | 0.136 | 0.65 |
| 11 | 199,989.0 | 0.074 | 0.070 | 0.36 |
| 12 | 200,042.0 | 0.078 | 0.072 | 0.38 |
| 13 | 200,085.0 | 0.058 | 0.062 | 0.30 |
| 14 | 199,960.0 | 0.050 | 0.062 | 0.28 |
| 15 | 200,000.0 | 0.046 | 0.074 | 0.30 |
| 16 | 200,001.0 | 0.064 | 0.052 | 0.29 |
| 17 | 199,998.0 | 0.068 | 0.072 | 0.35 |
| 18 | 200,041.0 | 0.046 | 0.074 | 0.30 |
| 19 | 200,024.0 | 0.054 | 0.062 | 0.29 |
| 20 | 200,076.0 | 0.100 | – | 0.10 |

[a]Theoretical value informed by the producer; [b]NNG 1 and NNG 2 concentration are related to the technical product; [c]average value according to equation 1 (mass of GLY present in technical product). –: Value not available.

Most studies in the literature that describe the determination and quantification of NNG in glyphosate technical products demand sample preparation and special chromatographic conditions, such as post-column derivatization, which are not available in many laboratories. The development of a simple and direct method (without derivatization), sensitive enough to quantify this impurity at levels below legislated limits, can facilitate NNG control in technical pesticide products by reducing the costs and time of analysis.

In this work, we developed and validated a separation and detection method for NNG quantification in GLY technical products samples using an anion exchange column mounted in a HPLC system, equipped with a photo-diode array detector. The mobile phase was a high ionic strength solution of $Na_2SO_4$ and NaOH. We also analyzed 20 samples of technical glyphosate for NNG content and all samples were below 1 mg $kg^{-1}$, the limit imposed by Brazilian legislation.

This method was fit for the purpose, since it is sensitive enough to quantify NNG in a glyphosate technical products below the maximum legislated concentration.

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