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Determination of glyphosate mono-isopropylamine salt in process samples using flow injection analysis with tris(2,2′-bipyridyl)ruthenium(II) chemiluminescence detection

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Abstract

The mono-isopropylamine salt of glyphosate was selectively determined directly in industrial and commercial formulations using flow injection analysis with tris(2,2′-bipyridyl)ruthenium(II) chemiluminescence detection without the need for separation. Glyphosate and its mono-isopropylamine salt furnished detection limits of 7×10^{-9} and 3.5×10^{-10} M and relative standard deviations of 0.4% at 1×10^{-7} M and 0.8% at 5×10^{-8} M, respectively. The methodology is robust and reliable with samples subjected only to aqueous dilution prior to analysis.

1. Introduction

Glyphosate [*N*-(phosphonomethyl)glycine] (see Fig. 1), is a well known, broad-spectrum herbicide [1]. As the free amino acid exhibits poor aqueous solubility its ammonium, potassium and mono-isopropylamine salts are commonly used as the active ingredient in commercially available products [1]. Various ion exchange [2–5] and gas [6] chromatographic methods have been reported for the determination of glyphosate in environmental matrices and some representative examples of these [2–6] have been summarised in Table 1. To the best of our knowledge, the only previous use of chemiluminescence detection was that by Ridlen et al. [3] who used tris(2,2′-bipyridyl)ruthenium(III) to determine glyphosate and some related compounds in standard solutions. This type of chemiluminescence has also been employed for the sensitive and/or selective detection of various classes of analytes using either flow analysis or HPLC [7].

Nufarm Limited, produce proprietary glyphosate-based herbicide formulations and the quality assurance of these processes is currently monitored using standard HPLC methodology with conductivity detection [8]. This paper describes, for the first time, a simple approach to the determination of glyphosate mono-isopropylamine salt in commercial formulations using flow injection analysis with tris(2,2′-bipyridyl)ruthenium(II) chemiluminescence detection.

2. Experimental

2.1. Instrumentation and procedure

A two-line flow injection analysis manifold was used. A Gilson Minipuls 3 peristaltic pump (John Morris, Australia) with PVC pump tubing (1.85 mm i.d., A.I. Scientific, Australia) propelled the carrier and sample streams at a maximum total flow rate of 6 ml min^{-1}. The manifold tubing was PTFE (0.5 mm i.d., Chromalytic Technology, Australia). The reagent solution, tris(2,2′-bipyridyl)ruthenium(III)

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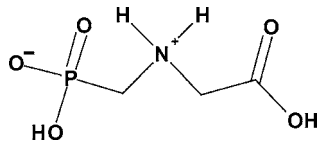

Fig. 1. Structure of glyphosate.

Table 1
Summary of chromatographic methodologies for the determination of glyphosate

Detection	LOD (M)	RSD (%)	Samples	Reference
Fluorescence	3×10^{-9}	11–20	Spiked lake water	[2]
Chemiluminescence	1×10^{-7}	–	Standard solutions	[3]
Suppressed conductivity	2.5×10^{-6}	1.99	Spiked lake water	[4]
Mass spectrometry	3×10^{-10}	–	Soil and water	[6]
Fluorescence	1×10^{-10}	–	Spiked river water	[5]

(1×10^{-3} M in 0.02 M sulphuric acid) was generated using a lead dioxide recirculating system [9] and injected (70 μL) into a phosphate buffer (pH 8) carrier stream, via a computer-controlled six port injection valve (Upchurch V.240, Activon, Australia). The reagent and analyte streams merged at a T-piece, positioned 20 mm from a coiled PTFE (0.5 mm i.d.) flow cell. The flow cell was mounted flush against the photomultiplier tube window (Thorn-EMI 9828SB, Australia) that was operated at 900 V, provided by a stable power supply (Thorn-EMI Model PM28B). The flow cell and photomultiplier tube were encased in a light tight housing. Output from the photomultiplier tube was monitored using a personal computer (Compaq, Armada 1592DT) running in house software [10] (LabVIEW 5.0, National Instruments, USA) to measure chemiluminescence response as peak area. All pH measurements were made using a Jenko pH metre (CHK Engineering, Australia). Serial dilutions of all standards and samples were performed using a 402 Dilutor-Dispenser auto dilutor (Gilson, USA).

2.2. Reagents

All reagents were of analytical grade, dilutions being performed with deionised water (Millipore, MilliQ Water System, USA). A stock solution of glyphosate (1×10^{-3} M, Nufarm Limited, Australia) was prepared daily and diluted as required. The glyphosate mono-isopropylamine salt (40% (m/v), Aldrich, Australia) was diluted as required. Stock solutions of potassium dihydrogen orthophosphate (20 mM, BDH Chemicals Ltd., England) and disodium hydrogen orthophosphate (20 mM, Ajax Chemicals, Australia) were prepared daily and used to make all phosphate buffer solutions. Tris(2,2′-bipyridyl)ruthenium(II) chloride hexahydrate (1×10^{-3} M, Strem Chemicals, USA), was prepared in sulphuric acid (0.02 M, Univar, Ajax Chemicals, Australia). Lead dioxide (Ajax Chemicals, Australia).

Samples 1 and 2 were supplied by Nufarm Limited and Sample 3 was a commercially available herbicide concentrate; all underwent serial aqueous dilution prior to analysis.

3. Results and discussion

3.1. Analytical performance

As tris(2,2′-bipyridyl)ruthenium(II) chemiluminescence is sensitive to reaction pH [7], it was essential to determine what pH would afford the best signal to blank ratios for the two analytes. Therefore, standard solutions (1×10^{-7} M) of glyphosate and the mono-isopropylamine salt made up in six different phosphate buffers (20 mM) ranging from pH 4 to 9 were determined and the results are represented in Fig. 2. As expected [7], the blank responses increased dramatically as the reaction conditions became more alkaline. However, as can be seen in Fig. 2, the signal to blank ratios for both compounds were highest around pH 8 and as such that buffer was used for all further experiments. This result supported the findings of Ridlen et al. [3], who established their post-column reaction chemistry with flow injection analysis and observed that alkaline conditions gave the best detection limits for glyphosate. Likewise, a similar chemical environment was reported for the detection of the secondary amino acid proline using tris(2,2′-bipyridyl)ruthenium(II) chemiluminescence [11].

Standard solutions of glyphosate (five from 1×10^{-8} to 2×10^{-7} M) and the mono-isopropylamine salt (six from 8×10^{-10} to 7×10^{-8} M) were prepared in phosphate buffer (pH 8) and analysed using the conditions described above. Calibration functions and analytical figures of merit have been summarised in Table 2. The superior detectability attained for the mono-isopropylamine salt may have resulted from the presence of another amine functional group in the counter ion. However, primary amines generally afford poorer detection limits, with this form of chemiluminescence, than either secondary or tertiary [7]. Notwithstanding that, the limits of detection (three times the standard

Table 2
Analytical figures of merit

Analyte	Limit of detection (M)	Equation for line of best fit	R^2	RSD (%)
Glyphosate	7×10^{-9}	$y = 1.6 \times 10^{8}x - 0.9$	0.9982	0.4 (1×10^{-7})
Glyphosate salt	3.5×10^{-10}	$y = 1.7 \times 10^{8}x + 0.3$	0.9923	0.8 (5×10^{-8})

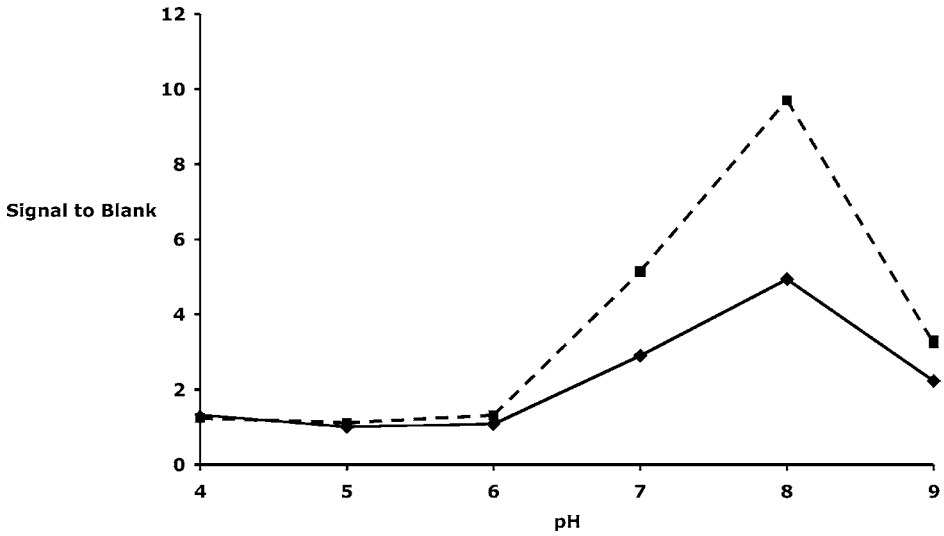

Fig. 2. The effect of pH on the chemiluminescence signal to blank response of glyphosate and the mono-isopropylamine salt. (—) glyphosate, (– – –) mono-isopropylamine salt.

deviation of the blank, 3σ) in Table 2 compared favourably with those achieved for various compounds containing amine moieties using this type of chemiluminescence [7] and also with other glyphosate methodologies that required sample preconcentration, and derivatisation procedures [2,4–6] as listed in Table 1.

3.2. Determination of glyphosate mono-isopropylamine salt in various formulations

The significant differences in chemiluminescence response between the glyphosate and its mono-isopropylamine salt meant that only standards of the latter could be used for calibration. Using the same conditions as were employed for the calibration standards, the concentration of the glyphosate mono-isopropylamine salt was determined in two proprietary formulations and one commercially available herbicide concentrate. The samples were diluted using an auto-diluter, to ensure that quantification was performed within the portion of the calibration function, which most closely approximated linearity. The inherent sensitivity of this methodology coupled with high analyte concentrations necessitated dilution factors of the order of one million. Despite these high dilution factors, it can be seen from Table 3, that the results for Samples 1 and 2 were in excellent agreement with the HPLC determinations provided by Nufarm Limited [8]. However, Sample 3 was somewhat lower than the concentration printed on the product container but we have no knowledge as to the quality of this figure. Additionally, the high dilution factors may have enhanced the selectivity of the methodology and thus facilitated the use of aqueous external calibration standards. This analytically beneficial situation has been observed previously in our laboratory with the determination of opiate pharmaceuticals in process samples using flow injection analysis with two different types of chemiluminescence detection [12,13].

Table 3
Determination of glyphosate mono-isopropylamine salt in various formulations

	Reported concentration ($g\,L^{-1}$)	Found concentration ($g\,L^{-1}$)
Sample 1	362	360 ± 5
Sample 2	445	440 ± 4
Sample 3	100	93 ± 2

4. Conclusions

A simple and rapid method has been developed for the determination of glyphosate mono-isopropylamine salt and applied successfully to three different industrial and commercial formulations. This approach has the selectivity, sensitivity and simplicity essential for on-line process monitoring.

References

[1] J.E. Franz, M.K. Mao, J.A. Sikorski, ACS Monograph 189, American Chemical Society, Washington, DC, 1997.
[2] M.E. Oppenhuizen, J.E. Cowell, J. Assoc. Official Anal. Chem. 74 (1991) 317.
[3] J.S. Ridlen, G.J. Klopf, T.A. Nieman, Anal. Chim. Acta 341 (1997) 195.
[4] Y. Zhu, F. Zhang, C. Tong, W. Liu, J. Chromatogr. A 850 (1999) 297.
[5] J. Patsias, A. Papadopoulou, E. Papadopoulou-Mourkidou, J. Chromatogr. A 932 (2001) 83.
[6] E. Börjesson, L. Torstensson, J. Chromatogr. A 886 (2000) 207.
[7] R.D. Gerardi, N.W. Barnett, S.W. Lewis, Anal. Chim. Acta 378 (1999) 1.
[8] R.D. Gerardi, Personal Communication, 2003.

[9] R.D. Gerardi, N.W. Barnett, P. Jones, Anal. Chim. Acta 388 (1999) 1.
[10] C.E. Lenehan, N.W. Barnett, S.W. Lewis, J. Automat. Meth. Manage. Chem. 24 (2002) 99.
[11] J.W. Costin, P.S. Francis, S.W. Lewis, Anal. Chim. Acta 480 (2003) 67.
[12] N.W. Barnett, T.A. Bowser, R.D. Gerardi, B. Smith, Anal. Chim. Acta 318 (1996) 309.
[13] N.W. Barnett, D.G. Rolfe, T.A. Bowser, T.W. Paton, Anal. Chim. Acta 282 (1993) 551.