Determination of Glyphosate In Water Samples by IC

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Abstract

Agricultural development and its associated impacts on the environment are resulting in increasingly restrictive guidelines and legislation concerning the use of chemicals in agro-ecosystems. The herbicide glyphosate is widely used for weed control in both cultivated and uncultivated areas and is considered to show low toxicity to mammals. It is highly water-soluble, and its monitoring in surface, underground, and potable waters is recommended by the United States Environmental Protection Agency. This work presents a method for the inclusion of glyphosate determination within routine anion analysis using ion chromatography in water sampler without any kind of extraction, clean-up, or preconcentration step. The equipment used was a Dionex Model ICS-3000 ion chromatograph fitted with a 25-μL loop, Ion Pac AG19 guard and AS19 analytical columns, ASRS-300 (2 mm) suppressor, and conductivity detector. The method showed a linear response to glyphosate between 0.05–0.75 mg/L with a correlation coefficient of 0.999, and a detection limit below the maximum levels permitted by Brazilian legislation. Recoveries in the range 90–105% were achieved in tests using surface, well, potable, and ultrapure water samples.

Introduction

Increasing concern over the impact of human activity on the environment has required the area of analytical chemistry to develop fast and efficient methods for the detection of species including trace metals, pesticides, and other chemical pollutants. Aquatic ecosystems may be the temporary or final receptors of a wide variety of such materials; and in rural areas increased use of herbicides and pesticides designed to enhance crop yields by prevention or reduction of pests and diseases is an important factor that can lead to contamination of surface or subterranean waters (1). As a result, it has been necessary to create regulatory agencies responsible for pollution control and to establish maximum permissible pollutant concentrations in natural and potable waters, as the U.S. Environmental Protection Agency (U.S. EPA, 0.700 mg/L) and the Council of the European Communities (Directive 91/414/EEC, 0.010 mg/L) have (2,3). In Brazil, both the National Environment Council (CONAMA) (0.067 mg/L) and the Ministry of Health (0.500 mg/L) have published standards for industrial pollutants in water resources (4,5).

Globally, glyphosate is the commonest herbicide used to control weeds in agriculture, forestry, and gardens due to its widespread applicability and low toxicity to mammals (6). Nonetheless, recent research has identified the occurrence of secondary effects in animals including reproductive dysfunction (7). Recently, a study proved the genotoxicity of glyphosate in cell and in mice (8). In Brazil, the herbicide is authorized for unselective post-emergence control in various cultivations, including direct planting, pastures, uncultivated areas, and non-agricultural applications (9,10).

Glyphosate is a foliar absorption herbicide, which penetrates the cuticle by diffusion and is rapidly transported throughout the plant tissue. In the soil it is efficiently absorbed by colloidal material, a feature that allows sowing of crops soon after application–within 28 days around 50% of the original molecule is metabolized to aminomethylphosphonic acid (AMPA) by microbial degradation (9,10). An analytical tool is therefore needed to measure possible environmental contamination arising from the use of glyphosate in regions close to springs and other surface water systems.

The first analytical techniques for detection of glyphosate employed thin layer chromatography, while later methods used gas (GC) or liquid (LC) chromatography, which required analyte derivatization (11). To be analyzed by GC, herbicides based on phosphonic aminoacids need to be derivatized in order to convert them to less polar and more volatile forms (11,12). In LC, derivatization improves detection. Other techniques, such as immunoassay (enzyme-linked immunosorbent assay, ELISA) and capillary electrophoresis, have also been used for the determination of glyphosate, gluphosinate, bialaphos, and their metabolites (11,16). The glyphosate molecule is highly polar and lacks the chromophores or fluorescent moieties that would be needed for detection using colorimetric, UV (> 200 nm), or fluorimetric techniques (11,13,14,15).

Zhu et al. reported a simple separation using ion chromatography (IC) for glyphosate (17). The technique was found to be suitable and offered simple and sensitive determination of glyphosate. Recently, several chromatographic methods to analyze glyphosate were developed for improved detection (18–22).

Within this context, the objective of the present work was to develop an IC technique that would allow simultaneous measurement of glyphosate alongside the other routinely measured anions fluoride (F^-), chloride (Cl^-), nitrite (NO_2^-), bromide (Br^-), nitrate (NO_3^-), phosphate (PO_4^{3-}), sulphate (SO_4^{2-}), thiocyanate (CSN^-), and arsenate (AsO_4^{3-}) in a single analysis.

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Experimental

The work was split into separate phases. Glyphosate was identified under the chromatographic conditions already optimized for determination of the seven anions, and the interference of these anions in detection of glyphosate was assessed. This was followed by recovery tests and verification of the linearity of the detector's response from the analytical curve. Finally, the method was applied to surface, well, and potable water samples. Experiments used potable water from the region of Bairro São José in Aracaju, which was provided by the Sergipe State water supply company (DESO), together with well water and samples of surface water from the Riacho Siri creek, Povoado de Moendas, Salgado, Sergipe State. The only pre-treatment required was filtration through a Millipore 0.45-μm membrane.

Equipment and reagents

The IC used was a Dionex Model ICS-3000 (Sunnyvale, CA) fitted with a conductivity cell detector, a 25-μL sample injection loop, Ion Pac AG19 (2 × 50 mm), AS19 (2 × 250 mm) guard and analytical columns maintained at 30°C, ASRS-300 (2 mm) self-regenerating suppressor, eluente generator, and AS40 autosampler.

The Ion Pac AS19 (2 × 250 mm) Analytical in combination with the AG19 Guard Column is designed for the analysis of inorganic anions and oxyhalides. The AS19 is compatible with pH 0–14 eluents and containing organic solvents from 0–100 % in concentration. The resin composition is supermacroporus polyvinylbenzyl ammonium polymer cross-linked with divinylbenzene. Its specifications are: 7.5-μm particle diameter, 5.5% substrate X – linking, column capacity 60 μeq/column, functional group is alkanol quaternary ammonium, and has low hydrophobicity (23).

Eluent generation technology allows automatic in-situ production of high-purity IC eluent. The pump delivers water to an eluent generator cartridge (EGC II KOH), which converts the water into the selected concentration of potassium hydroxide eluent using electrolysis. After separation on the column, the eluent enters the ASRS suppressor, which produces hydronium ions to exchange with potassium in the eluent, suppressing the conductivity of the mobile phase.

All reagents used were analytical-grade. Glyphosate standard (99 %) was obtained from Chemservice (Milano, Italy). Anion standards in deionised water was obtained from Dionex (Standard II). Sodium arsenate (Na_2HAsO_4, $7H_2O$) from Merck and sodium thiocyanate (NaSCN) from Reagen Quimibras Industria Química (Rio de Janeiro) were used. Stock solutions and dilutions were prepared using ultrapure deionized water obtained from a Millipore "Milli-Q" system (Milford, MA). Water samples were filtered through < 0.45-μm pore size membrane filters. All glassware was washed and decontaminated using deionized water.

Results and Discussion

Identification

The retention time of the glyphosate peak was determined by injecting standard solution under the routine chromatographic conditions employed for anions (Figure 1). The initial eluent concentration was 10 mM of KOH, isocratic for 5 min, followed by a gradient ramp to 35 mM KOH between 5–35 min and 10 mM KOH between 35–37 min. The eluent flow rate was 0.3 mL /min (giving an internal pressure of 2356 psi). Under these conditions, the glyphosate peak was well-defined, symmetrical, with good resolution, and had a retention time of ~ 27 min.

Interference of F^-, Cl^-, NO_2^-, Br^-, NO_3^-, SO_4^{2-}, PO_4^{3-} SCN^- and AsO_4^{3-}

Interference of the commonly determined anions such as F^-, Cl^-, NO_2^-, Br^-, NO_3^-, PO_4^{3-}, and SO_4^{2-} was assessed by analyzing a mixed standard solution, including glyphosate, under the same chromatographic conditions together with anions, such as thiocyanate (SCN^-) and arsenate (ASO_4^{3-}), that have strong retention times (Figure 2). There was no interference with all of the anions eluting before glyphosate.

Except arsenate whose the retention time was the closest to that of glyphosate (~ 26 min), their resolution is 1.4 when the baseline resolution requires $Rs \geq 1.5$. At this value, purity of the peak is 100%.

Linearity of response

The linear response range of the detector was determined under the same chromatographic conditions described previously by construction of a calibration curve using peak area as a function of analyte concentration in the range 0.05–0.75 mg/L (Table I). Good correlation was obtained between peak area and analyte concentration ($r = 0.999$). Limits of detection (DL) and quantification (QL) of the method were calculated according to equations 1 and 2 and were 0.01 and 0.05 mg/L, respectively.

$$LD = 3.3 \times s/S \qquad \text{Eq. 1}$$
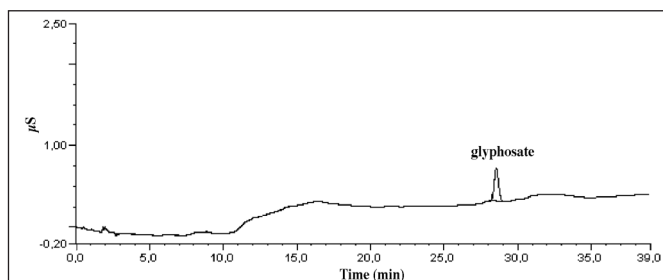$$LQ = 10 \times s/S \qquad \text{Eq. 2}$$

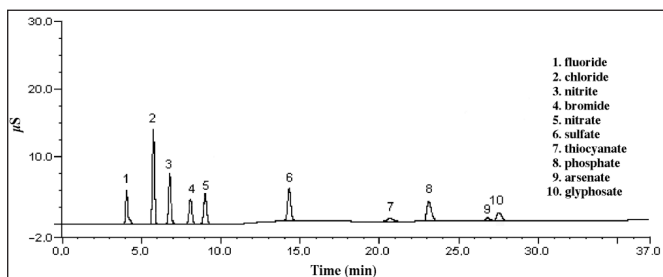**Figure 1.** Chromatogram of glyphosate standard with concentration 0.50 mg/L.

Figure 2. Chromatogram of glyphosate with anions at the following concentrations (mg/L): 1, fluoride 0.40; 2, chloride 2.00; 3, nitrite 0.61; 4, bromide 2.00; 5, nitrate 0.45; 6, sulphate 2.00; 7, thiocyanate 1.31; 8, phosphate 1.31; 9, arsenate 1.34; and 10, glyphosate 0.75.

Table I. Analytical Performance for the Proposed Method

Peak name	Retention time (min)	Linear range (mg/L)	r^2	Linear regression	LD (mg/L) ($n = 3$)	LQ (mg/L) ($n = 3$)	RSD (%) ($n = 7$)
Fluoride	4.07	0.09–4.00	0.9993	$y = 1.503x$	0.0258	0.0859	3.04
Chloride	5.76	0.05–20.00	0.9996	$y = 1.038x$	0.0042	0.0141	2.59
Nitrite	6.79	0.02–6.09	0.9993	$y = 1.988x$	0.0032	0.0107	3.06
Bromide	8.10	0.05–20.00	0.9994	$y = 0.458x$	0.0110	0.0366	3.05
Nitrate	9.02	0.03–4.52	0.9995	$y = 2.6107x - 0.132$	0.0092	0.0308	2.84
Sulphate	14.3	0.20–20.00	0.9995	$y = 0.765x - 0.269$	0.0465	0.1551	2.57
Thiocyanate	20.69	0.36–2.87	0.9991	$y = 0.125x$	0.0408	0.1361	2.41
Phosphate	23.16	0.03–13.05	0.9996	$y = 1.002x$	0.0066	0.0220	2.51
Arsenate	26.86	1.03–2.76	0.9583	$y = 0.083x$	0.5503	1.8343	9.72
Glyphosate	27.57	0.05–0.75	0.9995	$y = 0.477x + 0.002$	0.0154	0.0515	1.95

Table II. Recoveries of Glyphosate Using Samples of Surface, Well, Potable, and Ultrapure Water and a Mixed Anion Standard Solution

Water sample	n	Expected conc. (mg/L)	Range of conc. obtained (mg/L)	Range of recoveries (%)
Surface	8	0.30	0.296–0.306	99–102
Surface	3	0.05	0.048	94
Well	3	0.30	0.31	103
Potable	4	0.30	0.284–0.304	95–102
Ultrapure	3	0.50	0.524	104
Ultrapure	4	0.05	0.047–0.049	90–99
Anion standard solution	3	0.30	0.307	102

where s is standard deviation of the blank sample, and S is slope of the regression line equation (24). The relative standard deviation (RSD) values on the ratios of the peak area ($n = 7$), linear regression, linear range, retention time, coefficient of determination (r^2), LD and LQ ($n = 3$) for all anions studied were listed in Table I.

The analytical data presented in Table II shows that the method is not efficient for the determination of arsenate and thiocyanate, but they are present only to study their interference in the determination of glyphosate.

Application of the method and recovery tests

Glyphosate was not detected in these unadulterated samples. Precision and recovery were then measured by analysis of these samples and ultrapure water, to which known concentrations of glyphosate had been added. Recoveries were between 90–105% (Table II).

Conclusions

IC is shown to be highly effective for the quantitative determination of glyphosate in the presence of various anion species. Trace levels can be detected without any need for prior enrichment, using a fast, simple procedure that does not interfere with existing laboratory routines. The method is sufficiently sensitive to ensure a LQ below the maximum values permitted by U.S. EPA (0.70 mg/L) and Brazilian legislation, including those cited in the Brazilian National Environment Council Resolution 357/05 (0.067 mg/L) and in Ministry of Health Statute 518/MS/04 (0.50 mg/L) (2,3).

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