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# Determination of glyphosate and phosphate in water by ion chromatography—inductively coupled plasma mass spectrometry detection

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**Abstract**

Quantitative determination of trace glyphosate and phosphate in waters was achieved by coupling ion chromatography (IC) separation with inductively coupled plasma mass spectrometry (ICP–MS) detection. The separation of glyphosate and phosphate on a polymer anion-exchange column (Dionex IonPac AS16, 4.0 mm × 250 mm) was obtained by eluting them with 20 mM citric acid at 0.50 mL $min^{-1}$, and the analytes were detected directly and selectively by ICP–MS at $m/z = 31$. Parameters affecting their chromatographic behaviors and ICP–MS characteristics were systematically examined. Based on a 500-μL sample injection volume, the detection limits were 0.7 μg $L^{-1}$ for both glyphosate and phosphate, and the calibrations were linear up to 400 μg $L^{-1}$. Polyphosphates, aminomethylphosphonic acid (the major metabolite of glyphosate), non-polar and other polar phosphorus-containing pesticides showed different chromatographic behaviors from the analytes of interest and therefore did not interference. The determination was also interference free from the matrix anions (nitrate, nitrite, sulphate, chloride, etc.) and metallic ions. The analysis of certified reference material, drinking water, reservoir water and Newater yielded satisfactory results with spiked recoveries of 97.1–107.0% and relative standard deviations of ≤7.4% ($n = 3$). Compared to other reported methods for glyphosate and phosphate, the developed IC–ICP–MS method is sensitive and simple, and does not require any chemical derivatization, sample preconcentration and mobile phase conductivity suppression.


*Keywords:* Glyphosate; Phosphate; Ion chromatography; ICP–MS; Water analysis

## 1. Introduction

Glyphosate, [*N*-(phosphonomethyl)glycine], is a very broad spectrum, nonselective, post-emergence herbicide that is used worldwide to control the growth of long grasses and broad-leafed weeds. Glyphosate and its major metabolite aminomethylphosphonic acid (AMPA) are reportedly present in waters, soils, fruits, crops and even human sera. It is of low acute and chronic toxicity to mammals but its effect on non-target organisms and overall environmental impact is still not fully understood [1]. Currently, glyphosate is in the list of the United States national primary drinking water contaminants with a maximum contaminant level goal (MCLG) of 0.7 mg $L^{-1}$. In Europe, the drinking water standard for any pesticide has been set at 0.1 μg $L^{-1}$ regardless of its toxicological profile. Since glyphosate is used for different applications and has been widely spread around the world indicating potential toxicological risk to humans, it is necessary to develop rapid, easy and sensitive method to monitor glyphosate residue in the environment. However, encountered are the challenging aspects of analytical method development for glyphosate at μg $L^{-1}$ to sub-μg $L^{-1}$ residue level in environmental waters, which are mainly originated from its inherent properties: its strong polarity and in most cases ionic character, its insolubility in organic solvent and high solubility in water [2].

The reported methods for the determination of glyphosate mainly consisted of gas chromatography (GC), liquid chromatography (LC), capillary electrophoresis (CE) and enzyme-link immunosorbent assay (ELISA) [2]. A comprehensive review was presented on the analytical methods of glyphosate, the related pesticides and their metabolites in various

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environmental matrices [2]. In GC methods, glyphosate was usually derivatized to a less polar and sufficiently volatile compound to be subsequently chromatographed, and a number of derivatization procedures [2–7] were explored for this purpose. Several GC detectors were used to improve the sensitivity, and furthermore, tandem mass spectrometry (MS–MS) [4] and GC–MS [6,7] were employed.

LC methods for glyphosate were mostly based on cation- or anion-exchange separation coupled with pre- or post-column derivatization owing to the lack of a chromophore or fluorophore. Pre-column derivatization HPLC methods focused on derivatization with 9-fluorenylmethyl chloroformate (FMOC–Cl) [8] and their applications were relatively seldom. One example utilizing post-column derivatization technique was reported by Wigfield and Lanouette [9]. They used an anion-exchange column to preconcentrate glyphosate and AMPA in environmental waters, subsequently determined them by LC with a post-column reactor and a fluorescence detector. Detection limit of 1 $\mu g\,L^{-1}$ was achieved for glyphosate. The United States Environmental Protection Agency (USEPA)-promulgated standard method [10] utilized methanol-phosphate (pH 1.9) as mobile phase to elute the analyte from a cation-exchange column (at 65 °C). Subsequently glyphosate was oxidized with hypochlorite and coupled with *o*-phthalaldehyde-2-mercaptoethanol complex at 38 °C to form a product, which was detected by a fluorometer. The method detection limits (MDL) reported by USEPA were 6 $\mu g\,L^{-1}$ for drinking water and 9 $\mu g\,L^{-1}$ for ground water. One of its disadvantages was lack of an extraction procedure, since direct injection of sample with high amount of salts could damage the cation-exchange column. Therefore, some modifications to the standard method were reported [11,12] with a solid-phase extraction (SPE)-based procedure prior to HPLC. By an anion- and cation-exchange HPLC method glyphosate was detected by measuring the electrogenerated chemiluminescence signal at gold electrode after post-column addition of tris(2,2′-bipyridyl)ruthenium(II) [13]. The ion chromatography (IC) methods for glyphosate with suppressed conductivity detection [14], integrated pulsed amperometric detection [15] and condensation nucleation light scattering detection [16] avoided exhaustive derivatization step, but they were not sensitive enough (with detection limits of 42–53 $\mu g\,L^{-1}$). Analyses of glyphosate, AMPA, and EDTA in ground and surface waters were achieved by IC–electrospray ionization mass spectrometry (ESI–MS) without additional sample preparation at a concentration level of 1 $\mu g\,L^{-1}$ [17]. Vreeken et al. [18] developed a fully automated on-line SPE–HPLC–ESI–MS–MS method for selective analyses of glyphosate, AMPA and structurally similar herbicide glufosinate. Ion-exchange with LC–MS–MS was now popular for the determination of glyphosate [19,20]. Recently, Caruso and co-workers [21] employed ion-pairing reversed-phase LC–inductively coupled plasma mass spectrometry (ICP–MS) with octapole reaction cell (ORC) for the analyses of glyphosate, AMPA and glufosinate with detection limits at low ppt range (25–32 ng $L^{-1}$).

Capillary electrophoresis methods for glyphosate [22–24] provided high resolutions and efficiency, but some of them suffered low sensitivity owing to the limited sample injection volume. Although ELISA methods may offer high sensitivity and selectivity [25,26], an obvious drawback was the high cost and currently difficult commercial availability of the test kit.

As well known, phosphate is an indicator of water quality. For its analysis at $\mu g\,L^{-1}$ level, the most widely used methods were automated colorimetry [27] and IC with conductivity detection (CD) [28]. With widespread application of ICP–MS, there were some reports on phosphorus determination using quadrupole or sector-field ICP–MS systems [29,30]. With ICP–MS as a sensitive and selective LC detector, phosphate at $\mu g\,L^{-1}$ or lower levels can be determined without the need of sample preconcentration or pre- or post-column derivatization. For example, Jiang and Houk [31] reported an ion-pairing LC method for both inorganic phosphates and adenosine phosphates. The reported detection limit for phosphate was 0.4 ng P (50 μL injection) and the method was interference free from pyrophosphate and tripolyphosphate. The anion-exchange IC–ICP–MS method with aqueous 11 mM ammonium carbonate (pH 11.2) as eluent could detect phosphate in the presence of common anions, but the detection limit was higher (36 $\mu g\,L^{-1}$) [32]. Yang et al. [33] developed a procedure for determination of dissolved phosphate in sea-water by ion-exclusion chromatography–ICP–MS with detection limit of 2 $\mu g\,L^{-1}$ as P (100 μL injection). It required no sample pretreatment. A size-exclusion chromatography–ICP–MS method was reported to determine phosphorus and trace elements in agricultural products [34]. Instead of ICP–MS, Morton et al. [35] recently combined inductively coupled plasma optical emission spectrometry (ICP–OES) with IC to determine reduced phosphorus species in a wide range of environmental samples.

The present study aimed to develop a simple, sensitive and reliable method for the simultaneous determination of glyphosate and phosphate in waters. The separation was achieved on an anion-exchange column with a simple mobile phase, and followed by quadrupole ICP–MS detection.

## 2. Experimental

### 2.1. Chemicals and materials

All chemicals were of analytical reagent grade or better and used without further purification, and deionized water (18.2 MΩ cm) prepared by a Milli-Q Plus system (Millipore, Bedford, USA) was used as reagent water for aqueous solution preparation. Phosphate standard stock of 1000 mg $L^{-1}$, purchased from SPEX CertiPrep (Metuchen, NJ, USA), was refrigerated at 4 °C. Glyphosate, a product of Riedel-deHaën with a certified purity of 99.2%, was used to prepare the standard stock (refrigerated at 4 °C) by dissolving it in water. The working solutions of glyphosate and phosphate were freshly diluted from their stocks.

Citric acid solution of 20 mM was prepared by dissolving 3.842 g of citric acid (≥99%, Aldrich, WI, USA) in 1000 mL water. The solutions prepared from tartaric acid (99%, Aldrich), phthalic acid (98%, Aldrich), oxalic acid dehydrate (Merck), sulfuric acid (Merck), ammonium sulfate (Merck),

respectively, were examined as potential eluents. AMPA (>99%, Sigma, St. Louis, USA), ethephon (Riedel-deHaën), glufosinate-ammonium (certified purity 93.7%, Riedel-deHaën), fosamine-ammonium (Chem Service, West Chester, PA, USA) and ampropylfos (Dr. Ehrenstorfer) were directly dissolved and diluted in water for interference examination, while acephate (Riedel-deHaën), glyphosine (10 mg $L^{-1}$ in methanol, Dr. Ehrenstorfer), malathion (Riedel-deHaën) and methyl parathion were first dissolved in methanol (HPLC solvent grade, Merck), then diluted at least 100-fold in water. Pyrophosphate solution was prepared from tetrasodium pyrophosphate decahydrate (92–96%, Sigma). Nylon filter of 0.45 μm pore size (Whatman, Clifton, NJ, USA) was employed to remove insoluble particles from water sample prior to its injection into the chromatography column.

### 2.2. Instrumentation

The IC–ICP–MS system consisted of an Agilent 1100 Series liquid chromatograph module (Agilent Technologies, Waldbronn, Germany) and an Agilent 7500a quadrupole ICP–MS (Yokogawa Analytical System, Kyoto, Japan). The LC module was composed of an isocratic pump, a degasser, a handheld control module and a Rheodyne Series 7725i manual injection valve. The ICP–MS was equipped with a dual pass spray chamber, a quartz concentric nebulizer, a standard quartz torch (concentric tube of 2.5 mm i.d.), a nickel sampling cone and a nickel skimmer cone. The outlet of the separation column was directly connected to the nebulizer of the ICP–MS via a piece of PFA tubing (0.3 mm i.d., 10 cm length). Meanwhile, the ICP–MS and LC module were connected through a remote cable that enabled simultaneous starting on both instruments of the chromatographic running and detecting. The data acquisition and analysis were performed by the Agilent ICP–MS ChemStation software with plasma chromatographic option. The IC column chosen after a preliminary investigation was IonPac AS16 (4.0 mm × 250 mm) with its guard column (AG16, 4.0 mm × 50 mm) from Dionex (Sunnyvale, CA, USA). An ASX-100 autosampler (Cetac Technologies, Omaha, NE, USA) with Teflon vials were used to introduce the solution to ICP–MS for the optimization of ICP–MS parameters.

### 2.3. Procedure

Water samples were filtered through a 0.45 μm nylon filter disk and the total residual chlorine test was performed by using DR/4000 spectrometer and a test kit (Hach, Loveland, CO, USA). In the presence of chlorine, glyphosate was instable and degraded to AMPA. If chlorine was found in the sample and the sample was not analyzed immediately, the chlorine had to be removed by the addition of 100 mg/L sodium thiosulfate. A portion of the sample was filtered through a 0.45 μm filter and then injected into the LC module via a 500-μL sample loop. The separation and signal acquisition was carried out under the optimized conditions shown in Table 1. The whole study was performed at ambient temperature of 22–26 °C.

Table 1
Optimized IC-ICP–MS operating parameters

| | |
|---|---|
| ICP–MS parameters | |
| RF forward power (W) | 1350 |
| Plasma gas flow rate (L $min^{-1}$) | 15.0 |
| Auxiliary gas flow rate (L $min^{-1}$) | 0.9 |
| Carrier gas flow rate (L $min^{-1}$) | 1.07 |
| Makeup gas flow rate (L $min^{-1}$) | 0 |
| Sampling depth (mm) | 6.0 |
| Torch | Standard quartz, 2.5 mm |
| Nebulizer | Quartz concentric, Meinhard |
| Spray chamber | Double-pass quartz, Scott type, 2 °C |
| Sampling and skimmer cones | Nickel |
| Isotope monitored | $m/z = 31$ |
| Detector mode | Pulse |
| Dwell time (s per isotope) | 0.15 |
| IC parameters | |
| Separation column | Dionex IonPac AS16, 4.0 mm × 250 mm |
| Guard column | Dionex IonPac AG16, 4.0 mm × 50 mm |
| Mobile phase | 20 mM citric acid |
| Flow rate (mL $min^{-1}$) | 0.5 |
| Injection volume (μL) | 500 |

## 3. Results and discussion

### 3.1. Optimization of ICP–MS detection

In the present study, an ICP–MS system was applied as a detector of glyphosate and phosphate after their LC separation. Phosphorus in the effluent was therefore monitored. It is well known that phosphorus at a trace level is relatively difficult to be determined by ICP–MS. This is partially due to the reason that phosphorus has a high first ionization potential (10.49 eV) and is only about 35% ionized in the argon plasma. Furthermore, monitoring of the sole phosphorus isotope $^{31}P$ suffers isobaric interference, especially from molecular ions $^{15}N^{16}O^{+}$ and $^{14}N^{16}O^{1}H^{+}$ formed in argon plasma. The quadrupole ICP–MS has not enough mass resolution to separate $^{31}P^{+}$ with such isobaric ions. Some reports [30] therefore utilized high-resolution (double-focusing sector field) ICP–MS to distinguish P from polyatomic ions at nominal mass of $m/z = 31$, and others determined P at $m/z = 47$ based on the formation of $PO^{+}$ in the argon plasma [29,33,34]. Moreover, the application of the octapole reaction cell technique allowed a maximum signal-to-background ($S/B$) ratio and enabled a better sensitivity for phosphorus [21]. However, the ORC ICP–MS and HR-ICP–MS required higher cost and special hardware components, and the isobaric effect could be reduced to a minimum reasonable level by careful tuning of the ICP–MS parameters. A quadrupole ICP–MS without ORC was hence used to detect glyphosate and phosphate following their LC separation.

Radio frequency (RF) forward power and carrier gas flow rate are usually the most significant parameters affecting the detection sensitivity, background intensity, and precision. In the case of direct aspiration of deionized water (blank) and P standard solution into concentric nebulizer then into plasma, the intensity

of the blank (in nearly neutral medium) increased with increasing the carrier gas flow rate in the range of 1.08–1.25 L $min^{-1}$, and decreased with the increase of RF power in 1000–1500 W. For P, a maximum *S*/*B* ratio was obtained when the RF forward power was 1350 W and the carrier gas flowed at 1.12 L $min^{-1}$. Similar phenomena were observed for P and blank in diluted nitric acid or citric acid medium. However, with increasing nitric acid concentration, the blank intensity increased while the net intensity for phosphorus decreased significantly.

The limit of direct detection of P was estimated as 0.13 μg $L^{-1}$ in nearly neutral medium (reagent water) and 0.37 μg $L^{-1}$ in 2% nitric acid. The detection limit was lower than that of 18 μg $L^{-1}$ reported by Becker et al. [30], where a quadrupole-based ICP–MS was used for comparison with the sector field ICP–MS.

When IC column was connected with the ICP–MS concentric nebulizer, further optimization was carried out. The optimized carrier gas flow rate was 1.07 L $min^{-1}$ to ensure a stable baseline and a high *S*/*B* ratio. The Agilent 7500 ICP–MS was proved to be a fast signal detection system together with good long-term precision and stability, which was necessary for recording the chromatogram. The use of a concentric nebulizer in LC–ICP–MS system was often helpful to avoid the effluent diffusion that caused peak broadening.

### 3.2. Separation mode consideration

Among the existing techniques for the analysis of glyphosate in water samples, HPLC was more practical than GC. However, the absence of a chromophore or a fluorophore in the molecular structure of glyphosate made its derivatization essential for its detection in the effluent [2]. Thus, both pre- and post-column derivatization techniques were used in conventional HPLC methods, and post-column fluorescent derivatization was applied more widely [2,9–12]. If an ICP–MS was employed as a HPLC detector for glyphosate, there was no need for the derivatization thanks to the elemental specificity and high sensitivity of ICP–MS [31]. This could simplify the analytical procedure and save labor and chemical costs.

On the other hand, in the case of a normal ICP–MS configuration, organic solvent in HPLC mobile phase suppressed significantly both the net signal of the analyte and the blank intensity, and caused spectral interference and carbon deposit in the sampling and skimmer cones due to high carbon content in the matrix [36]. Furthermore, the introduction of organic solvent required solvent-resistant hardware such as instrument fittings, sample uptake tubing and peristaltic pump tubing. Therefore, followed by ICP–MS detection, reversed-phase or normal phase HPLC was less desirable for glyphosate than ion-exchange chromatography.

Glyphosate and phosphate are readily charged negatively in aqueous solution. If retained on anion-exchange column they could be eluted with simple aqueous eluent which benefits the ICP–MS detection. Therefore, anion-exchange chromatography mode was chosen for the separation of glyphosate and phosphate followed by ICP–MS detection.

### 3.3. Choice of the mobile phase

Retentivity of anions species on IC column is dependent on the p$K_a$ of the species and mobile phase variables (pH, ion strength, flow rate and temperature). Glyphosate has p$K_a$-values of 0.78 (first phosphonic), 2.09 (carboxylate), 5.96 (second phosphonic), 10.98 (amine) [16]. It is base or acid with negative or positive charges on the species depending on the solution pH/acidity. On the other hand, the acid dissociation constants for phosphoric acid are p$K_{a1}$ = 2.15, p$K_{a2}$ = 7.20, p$K_{a3}$ = 12.35 (25 °C). According to their p$K_a$ values, glyphosate and phosphate were partially dissociated and became monovalent anions at pH > 2.1, and completely dissociated in alkaline medium. For their anion-exchange chromatography separation, an aqueous solution of pH > 2.1 could be considered as an eluent.

For IC–ICP–MS, a mobile phase containing $Na^+$ was not desirable because a constant input of $Na^+$ could change the plasma condition and also cause clogging of the cone orifices. Ammonium-containing compounds can be easily converted to volatile compounds during the passage through the high temperature region of the plasma and could be one of the candidates as the mobile phase components. The experimental study indicated the elution with a solution of ammonium carbonate, ammonium nitrate or ammonium sulfate led to insufficient separation of glyphosate and phosphate on several anion separation columns, i.e., AS9, AS11, AS12 and AS16. Further study showed that diluted acidic solutions at pH 2.1–5.5 could elute glyphosate followed by phosphate from the separation column. Among them, citric acid (p$K_{a1}$ = 3.13, p$K_{a2}$ = 4.76, p$K_{a3}$ = 6.40), tartaric acid (p$K_{a1}$ = 2.98, p$K_{a2}$ = 4.34), oxalic acid (p$K_{a1}$ = 1.23, p$K_{a2}$ = 4.19), and phthalic acid (p$K_{a1}$ = 2.89, p$K_{a2}$ = 5.51) at 5–20 mM were investigated as eluents of glyphosate and phosphate (both at 100 μg $L^{-1}$) separation on AS16 column.

As shown in Table 2, the elution with 20 mM oxalic acid at 0.5 mL $min^{-1}$ showed slight co-elution, while with 20 mM citric acid, 20 mM tartaric acid, 5 mM oxalic acid or 5–10 mM phthalic acid the elution gave acceptable resolution between glyphosate and phosphate. When eluted with oxalic acid (5 or 20 mM), glyphosate had a wider peak than phosphate, and the peak area ratio of glyphosate to phosphate was 1:2.4, much lower than the theoretic value (reverse ratio of their formula weight, 1:1.76).

Table 2
Comparison of organic acids as eluent for the separation of glyphosate and phosphate[a]

| Eluent (at 0.5 mL $min^{-1}$) | Glyphosate | | Phosphate | | $R_s$ |
|---|---|---|---|---|---|
| | RT (min) | $W_{1/2}$ (min) | RT (min) | $W_{1/2}$ (min) | |
| 5 mM oxalic acid | 13.76 | 0.413 | 16.02 | 0.194 | 4.38 |
| 20 mM oxalic acid | 11.36 | 0.231 | 11.80 | 0.152 | 1.35 |
| 5 mM phthalic acid | 18.69 | 0.223 | 20.48 | 0.380 | 3.49 |
| 10 mM phthalic acid | 13.42 | 0.184 | 15.47 | 0.342 | 4.59 |
| 20 mM citric acid | 13.95 | 0.389 | 21.04 | 0.589 | 8.53 |
| 20 mM tartaric acid | 12.78 | 0.371 | 19.56 | 0.507 | 9.09 |

[a] RT, $W_{1/2}$ and *R* refer to retention time, width at half peak and resolution for target analytes, respectively. The resolution calculation was based on the assumption of Gaussian peaks.

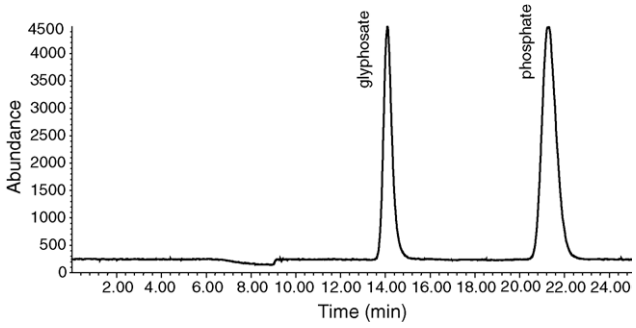


Fig. 1. Chromatogram of the glyphosate and phosphate fortified reagent water. Condition: Dionex IonPac AS16 column, 20 mM citric acid as the eluent at the flow rate of 0.5 mL $min^{-1}$, both analytes at 100 μg $L^{-1}$.

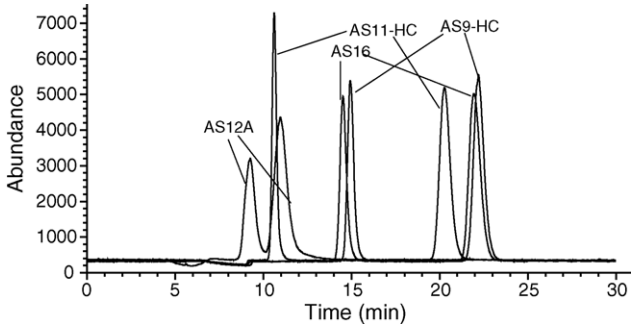


Fig. 2. Chromatograms of mixture of glyphosate and phosphate standards (100 μg $L^{-1}$ each) on different anion-exchange columns. Conditions were the same as those described in Table 1 except the separation column and related guard column.

This phenomenon was different from the case of elution with any other acids examined. Among the acids examined, citric acid and tartaric acid of 20 mM were the most suitable eluents. A typical chromatogram of the mixture of glyphosate and phosphate with 20 mM citric acid as eluent was displayed in Fig. 1. The experimental phenomenon could be explained in terms of the analyte retentivity dependent on the pH, p$K_a$ values of these polyvalent acids as a mobile phase. The acids have different p$K_a$ values and charge, and consequently different elution power. As for citric acid and tartaric acid, they had close p$K_{a1}$ and p$K_{a2}$ values, and their solutions of 20 mM were pH close (pH 2.45 and 2.35, respectively). Therefore, citric acid and tartaric acid functioned similarly as eluent. In such a case, both glyphosate and phosphate were almost completely present as their individual negatively charged species (monovalent) in the media.

The effect of citric acid of different concentrations was investigated on the separation and detection. Since the solutions were not pH buffered, this was a total impact of concentration and pH. The elution with 10 mM solution (pH 2.62) increased the retention time and peak width of the target species and the whole analytical period, although good peak shapes were obtained. In the case of elution with 80 mM solution (pH 2.13), glyphosate and phosphate appeared earlier and more sharply in the chromatogram, which resulted in a shorter turnaround. Meanwhile, the baseline intensity was increased, and carbon deposit was found in the sampling and skimmer cones after running several days. Citric acid of 15–50 mM (pH 2.24–2.53) was suitable as eluent. The impact of pH of the eluent at wider range was not examined here, since the bases commonly used to adjust pH of citric acid solution caused other problem. For example, as discussed above, constant input of $Na^+$ (from sodium hydroxide would change plasma condition and cause cone clogging, and N (from ammonia) would transform into interfering isobaric species $^{15}N^{16}O^+$ and $^{14}N^{16}O^1H^+$.

### 3.4. Effect of the flow rate of the mobile phase

With increasing flow rate of the eluent, the retention time, peak width and peak area of each analyte became shorter or smaller, while the resolution for two neighboring peaks became poorer. However, too slow flow rates caused peak tailoring and long analytical period. For LC with a detector of ICP–MS, the flow rate was normally below 2 mL $min^{-1}$. In the flow rate range of 0.40–1.50 mL $min^{-1}$ examined, both the peaks for glyphosate and phosphate were good in symmetry without obvious tailoring. A flow rate of 0.50 mL $min^{-1}$ was applied for all the subsequent experiments.

### 3.5. Comparison of separation column

Anion-exchange separation column is often packed with two types of packing: (1) ammonium-functionalized silane chemically bonded to particle through Si-O-Si bonds, such as Zorbax SAX column (product of Agilent Technologies) and (2) latex-agglomerated polymer resin. In this study, efforts were made in choosing an appropriate polymer column rather than a silane column. Four Dionex columns, i.e., IonPac AS9-HC, AS11-HC, AS16 (all 4.0 mm × 250 mm) and AS12A (4.0 mm × 200 mm), were compared for their separation efficiency with isocratic elution of 20 mM citric acid.

As shown in Fig. 2, the baseline separation of standard mixture of glyphosate and phosphate was achieved on AS9-HC, AS11-HC and AS16 column, while incomplete separation obtained on AS12A column. Obviously, these columns had different separation efficiency. This could be attributed to their difference in column packing composition. The packing substrates of all the columns were the same (bead diameter of 9.0 μm, and pore size of 2000 Å), but their latex layers had different latex diameter (70–140 nm) and crosslinking extent (0.2–15%). Further study showed that on AS11-HC column the determination of glyphosate suffered severe interference from glufosinate due to their co-elution with 20 mM citric acid. However, such an insufficient separation did not occur when AS9-HC or AS16 column was employed. Therefore, AS9-HC and AS16 columns were suitable and AS16 was chosen for this study. To be pointed out, different separation columns actually required different eluents, the poor resolution obtained from AS12A or AS11-HC column could be improved if the most suitable eluent was applied. However, further investigation of suitability of different eluents has to be performed.

### 3.6. Effect of sample injection volume

The retention time for glyphosate and phosphate became slightly longer as the sample loop size ($S_v$) increased from 20

to 2000 μL. The peak area for glyphosate versus $S_v$ was linear in the range of 20–1000 μL. For phosphate, the peak area was linear to $S_v$ in the range of 20–500 μL, and an injection volume of ≥ 1000 μL caused severe peak broadening. For analysis of sample without preconcentration, a volume of 500 μL was used.

### 3.7. Linear range and sensitivity

Peak area ($A$) of the analyte was used for quantification. The calibration graphs were linear at least up to 400 μg $L^{-1}$ for both glyphosate and phosphate in case of the ICP–MS in pulse detection mode. Linear regression equations in the range of 0–100 μg $L^{-1}$ under the optimized conditions were: $A = 1.17 \times 10^4 C + 129$ for glyphosate and $A = 1.95 \times 10^4 C - 462$ for phosphate (both $r = 0.9999$, $n = 5$). The detection limits for glyphosate and phosphate achieved by the procedure described above were both 0.7 μg $L^{-1}$ (three times the standard deviation of seven replicate analyses of a reagent water sample fortified with 3.0 μg $L^{-1}$ each of glyphosate and phosphate). The sensitivity for glyphosate of the proposed method was much higher than that of IC–CD [14], IC–condensation nucleation light scattering detection [15], and IC–post column derivatization with fluorescence detection [12]. The method was also competitive in sensitivity with that reported by Wigfield and Lanouette [9], and more importantly, it was much simpler and required no preconcentration. This method also had a higher sensitivity for phosphate than the prevalent automated colorimetry [27], IC–CD method [28] and the reported IC–ICP–MS methods [32,33].

### 3.8. Effect of coexisting species

As discussed earlier, the isobaric interference on P determination was mainly from $^{15}N^{16}O^+$ and $^{14}N^{16}O^1H^+$ in the plasma. These species contributed to the intensity at $m/z = 31$, i.e., the baseline in the chromatogram. Such interference resulted in a higher detection limit for glyphosate than that reported [21] which utilized ORC technique to minimize the isobaric interference. Under the optimized condition, the baseline was stable, so the interference from $^{15}N^{16}O^+$ and $^{14}N^{16}O^1H^+$ was tolerable.

Other potentially interfering species were polar and non-polar organophosphorus pesticides, other inorganic phosphorous species (polyphosphates), and other anions and cations in water matrix. Those species at different levels were examined with reference to their concentration ranges in water samples and their concentrations relative to glyphosate and phosphate. Table 3 includes the main results. With similar structures to glyphosate, glufosinate, fosamine and ethephon were negatively charged under the separation condition employed, but had different retention time from the analytes. The peaks of the three phosphorus-containing pesticides appeared at 10.92, 25.85 and 29.07 min, respectively) without any overlapping with those of glyphosate and phosphate. AMPA, the major metabolite of glyphosate, has p$K_a$ values of 2.4 (first phosphonic), 5.9 (second phosphonic), 10.8 (amine) [16]. AMPA and its analogue 2-aminoethylphosphonic acid (2-AEPA) were positively charged or uncharged at pH of the mobile phase, and thus were not retained on the anion-exchange column. As for non-polar organophosphorus pesticides, such as widely used malathion, methyl parathion and acephate, they were not retained on the column. Polyphosphates like pyrophosphate ($P_2O_7^{4-}$) has stronger interaction with the column packing, and could not be eluted by 20 mM citric acid within the elution time employed in this study. A chromatogram of a mixture of the analytes and other phosphorus-containing species is shown in Fig. 3.

Table 3
Effect of coexisting species on the determination of glyphosate and phosphate (100 μg $L^{-1}$ each)

| Coexisting species[a] | Glyphosate (μg $L^{-1}$) | Phosphate (μg $L^{-1}$) |
|---|---|---|
| Glufosinate (100 μg $L^{-1}$) | 102.1 | 101.5 |
| Ethephon (200 μg $L^{-1}$) | 101.7 | 104.8 |
| Aminomethylphosphonic acid (AMPA) (200 μg $L^{-1}$) | 97.6 | 96.5 |
| 2-Aminoethylphosphonic acid (2-AEPA) (200 μg $L^{-1}$) | 101.4 | 100.8 |
| Malathion (200 μg $L^{-1}$) | 104.2 | 105.2 |
| Methyl parathion (200 μg $L^{-1}$) | 97.0 | 97.2 |
| Acephate (40 μg $L^{-1}$) | 108.8 | 99.1 |
| Pyrophosphate (400 μg $L^{-1}$) | 100.7 | 105.0 |
| Acephate (20 μg $L^{-1}$); AMPA (30 μg $L^{-1}$); 2-AEP, malathion, methyl parathion (50 μg $L^{-1}$) each; glufosinate, ethephon, pyrophosphate (100 μg $L^{-1}$) each | 98.7 | 103.2 |
| $Br^-$, 10.0; $Cl^-$, 100; $NH_4^+$, 2.2; $Na^+$, 64.9 | 96.9 | 97.4 |
| $NO_3^-$, 20.0; $Na^+$, 7.4 | 96.5 | 99.8 |
| $NO_2^-$, 20.0; $Na^+$, 10.0 | 96.1 | 98.3 |
| $ClO_2^-$, 20.0; $Na^+$, 6.8 | 100.7 | 106.6 |
| $Cl^-$, 39.4; $NH_4^+$, 20.0 | 106.4 | 104.7 |
| $ClO_3^-$, 20.0; $K^+$, 9.4 | 97.7 | 95.5 |
| $ClO_4^-$, 5.0; $K^+$, 2.0 | 105.5 | 101.1 |
| $SO_4^{2-}$, 20.0; $CO_3^{2-}$, 24.0; $NH_4^+$, 18.0 | 106.7 | 98.9 |
| $F^-$, 10.0; $HCOO^-$, 10.0; $Ac^-$, 11.8; $NH_4^+$, 3.6; $Na^+$, 17.6 | 103.5 | 101.0 |
| $CO_3^{2-}$, 24.0; $Cl^-$, $SO_4^{2-}$, $ClO_2^-$, 20.0 each; $Ac^-$, 11.8; $NO_3^-$, 10.0; $Br^-$, $F^-$, $NO_2^-$, $ClO_3^-$, $HCOO^-$, $ClO_4^-$, 5.0 each | 102.2 | 102.5 |
| $Na^+$, 10.2; $Mg^{2+}$, 10.2; B, 10.0; $K^+$, $Ba^{2+}$, $Al^{3+}$, $Fe^{3+}$, $Cd^{2+}$, 5.2 each; Si, 2.0; other element (As, Ag, Be, Bi, Cu, Cs, Cr, Co, Ce, Ca, Ga, In, Pb, Mn, Ni, Rb, Se, Sr, Tl, U, V, Zn), 0.20 each | 103.6 | 101.4 |

[a] The concentration unit of coexisting species was mg $L^{-1}$ unless otherwise stated.

Metal ions and $NH_4^+$ in the sample showed no significant effects on the determination. Various inorganic anions prevalently and possibly coexisting in water matrix, such as sulfate, nitrate, carbonate, chloride, bromide, fluoride, nitrite, chlorite, chlorate, perchlorate, formate and acetate, did not interfere with the chromatographic separation and ICP–MS detection of the analytes, although they could be retained in the column and subsequently eluted along with the analytes. The analytical column AS16 had a capacity of 170 microequivalent (μeq) (its guard column AG16 has a 3.5-μeq capacity packed with a low capacity microporous resin), which enabled the separation of anions at mg $L^{-1}$ levels without overloading in case of hydroxide or carbonate elution. When 20 mM citric acid served as the

Table 4
Analytical results of water samples and spiked recoveries

| Sample | Determination result | | | | Spiked recovery | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Glyphosate | | Phosphate | | Glyphosate | | | Phosphate | | |
| | Found ($\mu g\,L^{-1}$) | RSD (%) | Found ($\mu g\,L^{-1}$) | RSD (%) | Added ($\mu g\,L^{-1}$) | Recovery (%) | RSD (%) | Added ($\mu g\,L^{-1}$) | Recovery (%) | RSD (%) |
| Raw water 1 | <0.7 | | 1.81 ± 0.13 | 7.1 | | | | | | |
| Raw water 2 | <0.7 | | 2.99 ± 0.04 | 1.4 | 25.0 | 103.2 ± 0.1 | 0.1 | 25.0 | 103.0 ± 1.0 | 0.9 |
| Fortified raw water | 5.65 ± 0.08 | 1.4 | 12.54 ± 0.30 | 2.4 | 10.0 | 97.1 ± 7.2 | 7.4 | 20.0 | 107.0 ± 5.1 | 4.8 |
| Tap water | <0.7 | | 2.28 ± 0.10 | 4.2 | 4.00 | 99.6 ± 5.7 | 5.7 | 40.0 | 97.9 ± 3.2 | 3.3 |
| Treated water 1 | <0.7 | | 1.52 ± 0.09 | 6.0 | | | | | | |
| Treated water 2 | <0.7 | | 1.79 ± 0.08 | 4.3 | | | | | | |
| Fortified treated water | 4.32 ± 0.11 | 2.5 | 5.53 ± 0.30 | 5.4 | 5.00 | 101.8 ± 6.0 | 5.8 | 5.00 | 104.9 ± 5.1 | 4.9 |
| Plant Newater 1 | <0.7 | | 3.06 ± 0.07 | 2.2 | | | | | | |
| Plant Newater 2 | <0.7 | | 7.07 ± 0.11 | 1.6 | 40.0 | 98.7 ± 0.4 | 0.4 | 10.0 | 103.1 ± 3.7 | 3.6 |

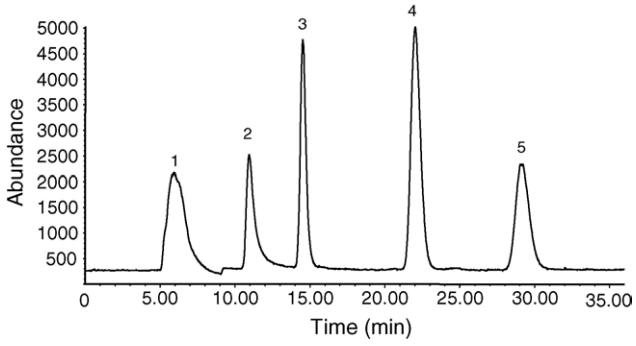


Fig. 3. Chromatogram of phosphorus-containing species fortified reagent water. Species ($\mu g\,L^{-1}$): AMPA, 30; 2-AEP, 50; malathion, 50; methyl parathion, 50; acephate, 20; glufosinate, 100; glyphosate, 100, phosphate, 100; ethephon, 100; pyrophosphate, 100. Peaks: (1) mixture of AMPA, 2-AEP, malathion, methyl parathion and acephate; (2) glufosinate; (3) glyphosate; (4) phosphate; and (5) ethephon.

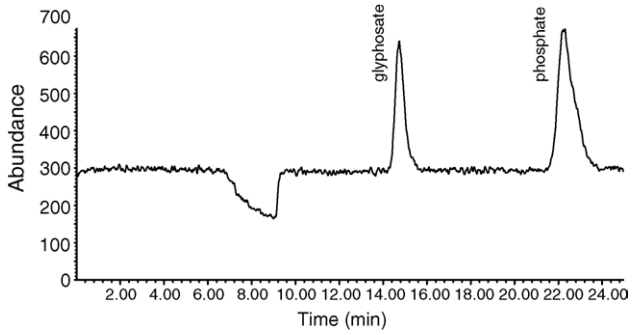


Fig. 4. Chromatogram of a spiked treated water sample 2 preliminarily fortified with glyphosate and phosphate at 4.00 $\mu g\,L^{-1}$ each). The analytes spiked were both 5.00 $\mu g\,L^{-1}$ (refer to Table 3).

eluent here, the overloading did not appear for typical water samples.

### *3.9. Determination and spike recovery of glyphosate and phosphate in waters*

A certified reference material for diquat, endothall, glyphosate and paraquat from R.T. Corporation (Laramie, WY, USA) was analyzed by the proposed method. Glyphosate was found to be 765 $\mu g\,L^{-1}$ (average of three measurements), identical to its certified value of 748 $\mu g\,L^{-1}$ and acceptance range of 624–872 $\mu g\,L^{-1}$. The relative standard deviation (RSD) of the measurement was 0.4%. Water samples of different matrices were collected from reservoirs and water plants and analyzed for glyphosate and phosphate under the optimized conditions. The results are shown in Table 4. Meanwhile, a typical chromatogram for spiked recovery test of a treated water sample is also shown in Fig. 4. Glyphosate was not found in the tested raw water (reservoir water here), treated water (for safe drinking purpose), tap water, and the Newater (recycled from wastewater) samples, and phosphate was at $\mu g\,L^{-1}$ level in same samples. Glyphosate and phosphate at different concentrations (4.0–40.0 $\mu g\,L^{-1}$) were spiked into raw water, tap water, plant treated water and Newater. The recoveries for glyphosate and phosphate (Table 4) were in the ranges of 97.1–103.2% and 97.9–107.0%, respectively, while the RSDs were ≤7.4% ($n = 3$) in all cases.

## 4. Conclusions

An IC–ICP–MS method was described to separate glyphosate and phosphate followed by element-specific and highly sensitive detection. Efforts were made to optimize the ICP–MS operating parameters and compare the efficiencies of the separation columns and mobile phases. The method is highly sensitive, selective and free from tedious sample preparation and chemical derivatization. The successful determinations and spike recoveries of the analytes in different water samples have shown the feasibility of this proposed method to water analysis.

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