

# Determine the Content of Aquatic Glyphosate Efficiently and Quickly by Using the Method of MRM of Liquid Chromatogram-Polyionic Reaction

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## ABSTRACT

To establish HPLC (High Performance Liquid Chromatography)-trebling quadrupole rod tandem mass spectrometry to determine the content of domestic drinking water efficiently and quickly. Use water sample and 9-fluorenyl methyl trichloromethane acetone solution to have derivatization reaction. Through membrane filtration of 0.2 μm and by using the method of MRM to determine glyphosate, the method of determining the content of domestic drinking water efficiently and quickly. It has been proved that this method can have the lowest minimum mass concentration of glyphosate with the result of 0.015 mg/L (sample size 2 μl). Within the linearity range (0~0.8 mg/L), the coefficient of association is greater than 0.999 and the average recovery rate is within 93.7-102.4%. The ration is accurate, highly sensitive and the concentration range is wide. It is truly a good method to determine the content of aquatic glyphosate. The RSD is 0.59%~4.04%.

## Key Words

High performance liquid chromatography; Glyphosate; MRM; Derivative.

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## 1. Introduction

Glyphosate (common name and molecular formula is C<sub>3</sub>H<sub>8</sub>NO<sub>5</sub>P) is the most widely used and productive herbicide<sup>[1]</sup>. Recent researches have shown that environmental glyphosate have fortissimo toxicity to aquatic organism<sup>[2]</sup>.

Now the methods To determine the content of aquatic glyphosate are gas chromatography<sup>[3]</sup>, GC-MS<sup>[4]</sup>, HPLC-MS<sup>[5]</sup>, HPLC<sup>[6]</sup>, ion chromatography<sup>[7]</sup>, spectrophotometry<sup>[8]</sup>, CE<sup>[9]</sup> and American EPA547<sup>[10]</sup>, etc. All these methods have limitations. HPLC-MS is mostly used to analyse water and soil. HPLC to determine the content of aquatic glyphosate adopts precolumn derivatization fluorescence detection<sup>[11]</sup>, evaporation light scattering HPLC<sup>[12]</sup> and national standard technique<sup>[13-14]</sup>. Because that the water-solubility of the glyphosate is great and there is no conjugated double bond and the ultraviolet absorption wavelength in most organic compounds can produce absorbent end of ultraviolet wavelength (195 nm), which leads to an unsatisfied result that the determination of the content of aquatic glyphosate by using the method of MRM of Liquid Chromatogram-Polyionic Reaction. Glyphosate can be easily dissolved into water but hard to dissolved into organic solvent for it lacks chromophore fluorophore. The assay determination is hard<sup>[15]</sup> especially when there are other common compounds in the

environment and it is harder for trace-determination<sup>[16]</sup>.

This article adopts HPLC (High Performance Liquid Chromatography)-trebling quadrupole rod tandem mass spectrometry for analysis and the accuracy and reproducibility are improved.

## 2. Experimental Part

### 2.1 Reagent and Material

Standard sample of glyphosate (Environmental Protection of Ministry of Agricultur provides concentration 100 mg/L); 9-fluorenyl methyl trichloromethane (import chromatographically pure); ammonium acetate (import chromatographically pure); formic acid (Fisher Firm, chromatographically pure); anhydrous (import chromatographically pure); sodium borate (Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O); acetone (Liquid Chromatography); acetonitrile (Fisher Firm, chromatographically pure); carbinol (Fisher Firm, chromatographically pure); ultrapure water (distillation twice).

2.1.1 Moving Phase A: 0.1% acetonitrile solution of formic acid, blend 1 ml formic acid and 1000 ml acetonitrile solution.

2.1.2 Moving Phase B: dissolve 0.154 g anhydrous ammonium acetate into water and add 1ml formic acid to 1000 ml.

2.1.3 5% (volume fraction) borate buffered solution (pH=9): dissolved 5 g

sodium borate ( $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ ) into water to 1000 ml.

2.1.4 1.0 g/L 9-fluorenyl methyl trichloromethane: dissolve 99.0% 9-fluorenyl methyl trichloromethane 100 mg into acetone dissolution to 100 ml.

## 2.2 Instrument and Equipment

American Agilent HPLC (High Performance Liquid Chromatography) -trebling quadrupole rod tandem mass spectrometry combination instrument (G6420A); AgilentSB-C18 chromatographic column (1.8  $\mu\text{m}$  2.1 $\times$ 50 mm); 0.2  $\mu\text{m}$  millipore filter; suction filter.

## 2.3 Analyse the Procedures

2.3.1 Stock solution 100 mg/L glyphosate good merchantable quality methyl alcohol as preparation 1 mg/L, store it in the refrigerator of 4°C

2.3.2 Working solution dissolve 0.25 ml, 0.5 ml, 1 ml, 2 ml, 4 ml, 8 ml glyphosate into water in the volumetric flask, making the preparation 25  $\mu\text{g/L}$ , 50  $\mu\text{g/L}$ , 100  $\mu\text{g/L}$ , 200  $\mu\text{g/L}$ , 400  $\mu\text{g/L}$ , 800  $\mu\text{g/L}$  standard series.

## 2.4 Sample Preparation

2.4.1 Lay the suction filtration device to the millipore filter to get rid of the impurity and gas.

2.4.2 Add 0.2ml 1.0 g/L 9-fluorenyl methyl trichloromethane respectively to standard solution and refined water sample, bleng it and wait for the derivatization reaction under room temperature for a night and filter the solution through 0.2  $\mu\text{m}$  filter membrane, waiting for determination.

## 2.5 Chromatography Mass Spectrum Condition

Chromatographic column: AgilentSB-C18 chromatographic column (1.8 $\mu\text{m}$ 2.1 $\times$ 50mm); flow velocity: 0.2 ml/min; sample size: 3  $\mu\text{L}$ ; column temperature: 35°C; ionization: ESI; capillary voltage (Positive) 4000 V; solvent temperature: 350°C; gas flow velocity: 11 L/min; ionization source temperature: 100°C.

Moving phase gradient elution program Table 1. Ionic reaction monitor (MRM) conversion condition Table 2.

## 3. Results and Discussion

### 3.1 Choosing of the Constant Volume Liquid

Make a attenuation of glyphosate standard solution 90%, 80%, 50%, 20%, 0% acetonitrile aqueous solution to 100  $\mu\text{g/L}$  solution and successive sample introduction. After observing the chromatographic peak and separating effect, we find peak width is relatively narrow when using pure water as constant volume with no trailing, so we use pure water as constant volume liquid.

### 3.2 Optimizing of Chromatographic Condition

3.2.1 Selection of chromatographic column LC adopts chromatography padding whose grain size is 1.8  $\mu\text{m}$  C18 can obtain greater specific surface area, make column efficiency remarkably improve, deplete the time of chromatograph 4.2 min, compared with convention 5 m C18 chromatographic column; the flow velocity is 0.2~0.4 ml/min, which matches the electric mist spray ionization. Therefore LC liquid phase system is an ideal choice.

#### 3.2.2 Choosing of moving phase

Glyphosate can be easily dissolved into acetonitrile and methyl alcohol. Considering the chromatograph, the elution of sample impurity and the match with mass spectrum condition. Choosing acetonitrile and water as moving phase and adding 0.1% formic acid can enhance the ionization efficiency and the detection sensitivity will quickly improved.

### 3.3 Optimizing of Mass Spectrum Condition

According to molecular structure of glyphosate, we choose ESI(+) as ionization pattern, prepare glyphosate

Table 1. Moving Phase Gradient Elution Program.

Time (min)	Flow Velocity (ml/min)	Acetonitrile (1000 ml) + Formic Acid (1 ml)	Water+Formic Acid +Anhydrous Acetic Acid (total volume 1000 ml)
0	0.2	20%	80%
5	0.2	70%	30%
8	0.2	95%	5%
12	0.2	95%	5%
13	0.2	20%	80%
20	0.2	20%	80%

Table 2. Ionic Reaction Monitor(MRM) Conversion Condition.

Project Name	Parention	Daughterion	Dwell	Fragmentor	Collision Energy	Cell Accelerator Voltage	Polarity
Glyphosate	392	214	200	80V	5V	3V	Positive
		88	200	80V	20V	3V	Positive

Table 3. Method Degree of Precision and Recovery Test.

Name	Addition ( $\mu\text{g/L}$ )	Measured quality ( $\mu\text{g/L}$ )	Range of recovery(%)	average recovery rate (%)	RSD(%)
Glyphosate	500	503.4	96.6~101.8	98.9	3.21
	100	95.7	97.2~102.0	99.3	0.59
	30	29.8	93.7~102.4	97.1	4.04

standard solution to 10 mg/L aqueous solution and then add 0.2 ml 9-fluorenyl methyl trichloromethane to have derivatization reaction through the aperture of 0.2 μm filter membrane and then have a full scan to find out parent ion. Use Product Ion scanning method to choose characteristic daughter ion gradually increase induce collision energy, with the decrease of the abundance of parent ion, and the abundance of daughter ion will increase. Choose glyphosate abundance of two major ions as ration assist qualitative ions and confirm the range of the best induce collision energy. Finally, by using RMR to adjust collision energy to gain the peak response of glyphosate. The final chosen parent ion and characteristic ion collision energy can be seen in Table 2.

**3.4 Method- affirming Experiment**

**3.4.1 Drawing of standard curve**

Make a preparation of 25 μg/L, 50 μg/L, 100 μg/L, 200 μg/L, 400 μg/L, 800 μg/L standard series, under the set condition of chromatographic condition, get 2 μL sample introduction. See glyphosate standard series adjusting graph in Figure 1. acquisition of signal MRM (ration ion pair: 392>214, 392>88). The retention time is 4.2 min. Use peak area as ordinate, the concentration as abscissa, and get standard curve  $Y=15.02X-55.64$ . And the coefficient of association is  $R=0.99965$ . (Figure 2)

**3.4.2 Degree of Precision & Recovery Test**

Have water sample whose minimum proof mass concentration is lower than the content of glyphosate as pilot sample, add 500 μg/L, 100 μg/L, 30 μg/L glyphosate standard solution, and have the pretreatment in the way mentioned above. Use pure water to constant volume to 10 ml and then repeat sample introduction 10 times. Have high, medium and low concentration degree of precision and recovery experiments, and see the results in Table 3. It is thus clear that when the degree of precision is 0.59%~4.04%, recovery is 93.7%~102.4%, which meets the requirement.

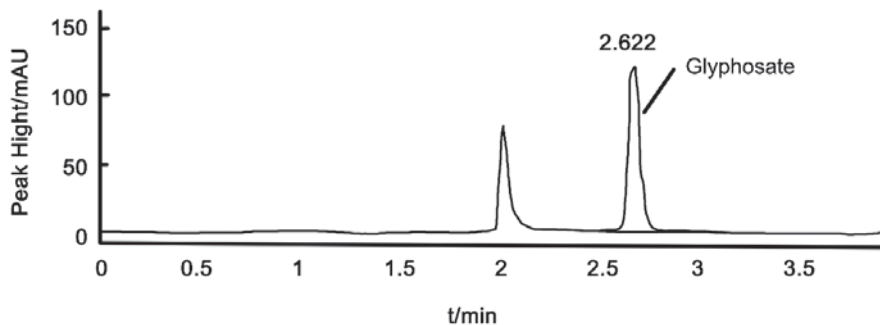


Fig. 1. Glyphosate Chromatogram.

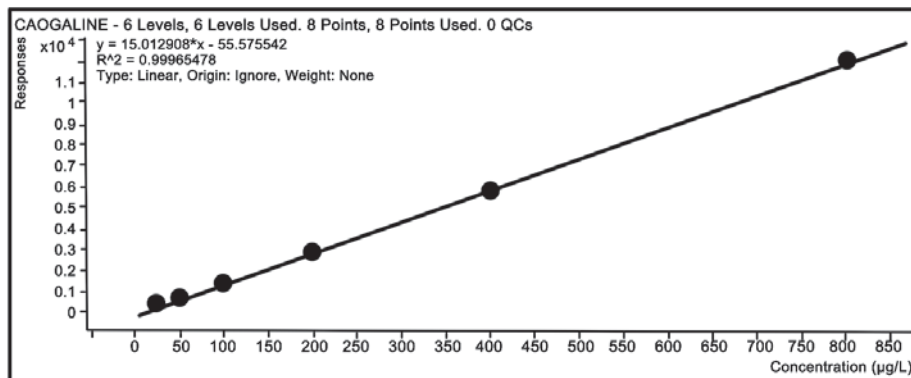


Fig. 2. Working Curve and Equation of Regression of Glyphosate.

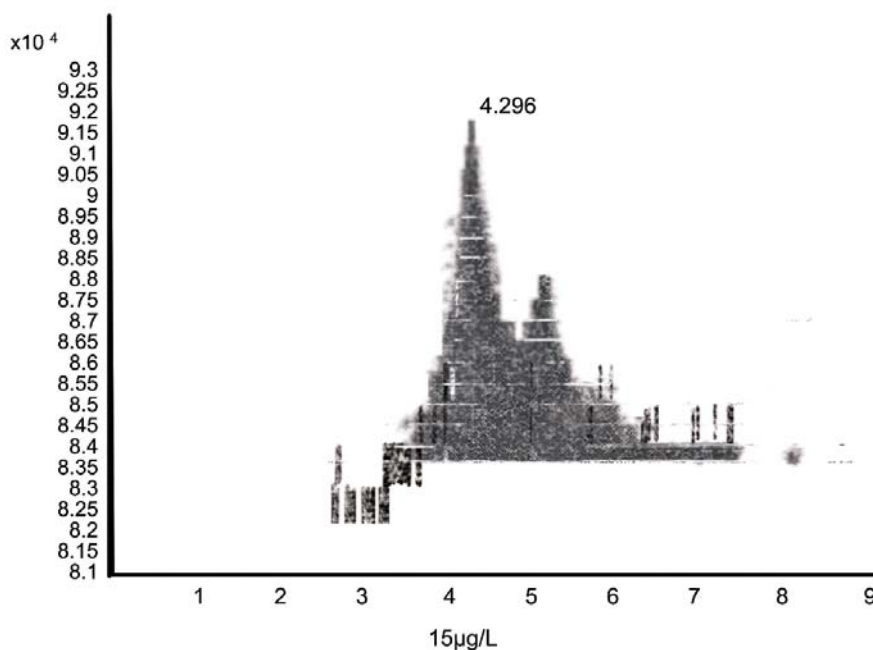


Fig. 3. Titer of Mass Concentration of Minimum Detection.

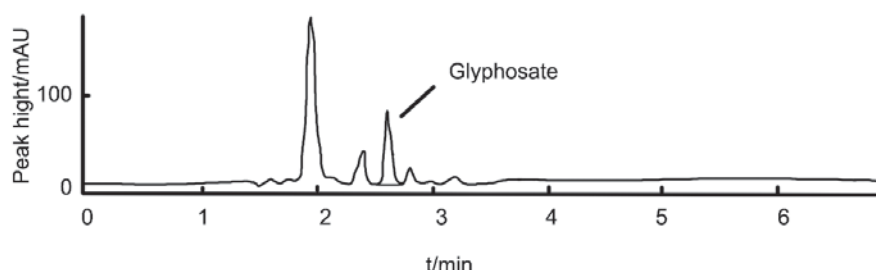


Fig. 4. The Atlas of the Measure of Aquatic Glyphosate.

### 3.5 Measure of Practical Samples and Discussion of Interference Factors

As to the measure of domestic water, we fetch domestic drinking water and impounding reservoir raw water of several sections of a highway or railway in main urban area and we detected no glyphosate.

As to the measure of waste water, we fetch rinsing water of some workshop section in the manufacturing shops to detect glyphosate in the same way. The result is as in Figure 4 and we can see that the spike and the separating effect are favorable.

However in practical work, because the manufacturing techniques are different, the interfering substance in glyphosate waste water is different from that in pure water. According to the principle of nitrosation and based on the analysis by synthesis of process route of all kinds of waste water<sup>[17]</sup>, a conclusion is made that the practical analysis procedure of glyphosate waste water, there may be some nitrogenous interfering substance:

(1) Tertiary Amine: Glyphosine [N,N-double (phosphinecarboxylmethyl) glycine], pmida [N-(phosphinecarboxylmethyl) iminodiacetic acid], triethylamine;

(2) Primary Amine: glycine.

According to the discussion of literature<sup>[18]</sup>, we get to a conclusion that although under acidic condition, primary amine (primary amine) and nitrite may have diazo-reaction<sup>[19]</sup>, the reaction condition are often rigorous (reaction temperature is below 5°C) and the diazonium persad is hardly stable and can have a easy hydrolysis. Therefore if there are primary amine, tertiary amine (tertiary amine doesn't react with nitrite) etc. in the test water sample of glyphosate, there is no remarkable effect on the test result.

## 4. Conclusion

This article adopts MRM of LC-MS/MS to detect aquatic glyphosate, uses 9- fluorenyl methyl

trichloromethane to have derivatization reaction, has analysis through 0.2 μm filter membrane, the lowest proof mass concentration of glyphosate is 0.015 mg/L, within the experimental concentration range, the coefficient of association  $R > 0.999$ , degree of precision  $RSD < 5\%$ , recovery 92.2%~102.4%, which meets the requirement of the water quality monitoring of domestic drinking water and source water. The method is quick and simple and the result is accurate and it has practical value of the contents check of glyphosate in waste water.

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