Poster Reprint

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# An Improved Method for Quantifying Glyphosine Impurity in Glyphosate API using LC/TQ via Standard Addition

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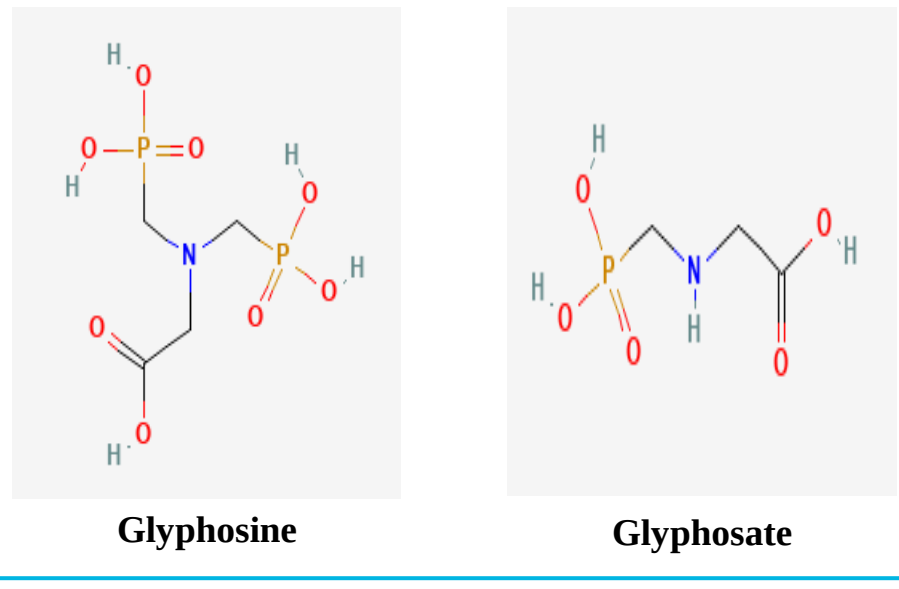
## Introduction

Glyphosate (CAS No. 1071-83-6) is a high efficiency, low toxicity and low residue herbicide. It is used throughout the world due to its superior performance. However, glyphosine (CAS No. 2439-99-8) is a by-product impurity in the glyphosate synthesis reaction. Quantifying the level of glyphosine impurity can provide technical guidance on process development and process parameter adjustment for glyphosate API (active pharmaceutical ingredient) synthesis. Here we present a novel and efficient method for rapidly and accurately quantifying glyphosine in glyphosate API using an Agilent 6470 LC/TQ and standard addition.



**Glyphosine** **Glyphosate**

Figure 1. Structure of Glyphosine and Glyphosate

## Experimental

### Instrument Conditions

| LC conditions | | | |
|---|---|---|---|
| HPLC system | Agilent 1290 Infinity II Binary Pump UHPLC | | |
| Column | Agilent Poroshell 120 CS-C18 (3.0 x 100mm, 2.7μm) | | |
| Column Temp. | 40℃ | | |
| Injection volume | 2μL | | |
| Needle Washing | Water (20s) | | |
| Mobile phase | Phase A: 100mMol/L ammonium acetate (pH=9): ultrapure water=10:90 (V:V, with 2.5μmol/L Agilent Deactivator Additive) | | |
| | Phase B: 100mMol/L ammonium acetate (pH=9): Acetonitrile=10:90 (V:V, with 2.5μmol/L Agilent Deactivator Additive) | | |
| Initial flow rate | 0.25mL/min | | |
| Gradient program | Time (min) | B% | Flow (mL/min) |
| | 0.0 | 5 | 0.25 |
| | 2.5 | 5 | 0.25 |
| | 2.6 | 90 | 0.4 |
| | 5.0 | 90 | 0.4 |
| | 5.1 | 5 | 0.4 |
| | 8.0 | 5 | 0.4 |
| Stop time | 8.0min | | |
| Post time | Off | | |

## Experimental

| MS conditions | | | | | | |
|---|---|---|---|---|---|---|
| MS system | Agilent 6470 LC/TQ | | | | | |
| Ion Source | Agilent Jet Stream ESI | | | | | |
| Dry Gas Temp. | 250℃ | | | | | |
| Dry Gas Flow | 6L/min | | | | | |
| Nebulizer | 35psi | | | | | |
| Sheath Gas Temp. | 400℃ | | | | | |
| Sheath Gas Flow | 12L/min | | | | | |
| Capillary Voltage | 1500V | | | | | |
| Nozzle Voltage | 0V | | | | | |
| MRM Parameter | Name | Precursor Ion (*m/z*) | Fragmentor (V) | Product Ion (*m/z*) | Collision Energy (V) | Polarity |
| | Glyphosine | 262 | 90 | 244 | 12 | Negative |
| | | 262 | 90 | 79 | 52 | Negative |

### Sample Preparation

1) Accurately weighed 10mg glyphosine standard into a volumetric flask of 10mL. The volume was completed with ultrapure water, sonicated until complete dissolution and vortex mixed, prepared 1000mg/L glyphosine standard solution (solution A);
2) Accurately weighed 30mg glyphosate sample into a volumetric flask of 10mL, The volume was completed with ultrapure water, sonicated until complete dissolution and vortex mixed, prepared 3000mg/L glyphosate sample solution (Solution B);
3) Seven aliquots of 500μL Solution B were transferred into different vials. In each vial, except one (sample not spiked), it was spiked a different volume of the glyphosine standard using the Solution A (10,20,30,40,50 and 100μL,respectively) and water, completing 1 mL, prepared test sample solutions as described in Table 1 below:

Table 1. Glyphosine Fortification Concentration of Test Samples

| Glyphosine | Test samples | | | | | | |
|---|---|---|---|---|---|---|---|
| | Sample1 | Sample2 | Sample3 | Sample4 | Sample5 | Sample6 | Sample7 |
| Fortification concentration (mg/L) | 0 | 10 | 20 | 30 | 40 | 50 | 100 |
| Note: The test samples are analyzed in order from Sample1 to Sample7 | | | | | | | |

## Results and Discussion

### Optimization of Chromatographic Conditions

Glyphosine is a highly polar compound that is difficult to retain by reverse chromatography. Moreover, trace metal residues in the HPLC system can have adverse effects on the peak shape and method durability of glyphosine analysis[1]. To address these chromatographic challenges, this method adopts some solutions as follows:

1. Agilent InfinityLab Poroshell 120 CS-C18 column was selected following evaluation and screening of column chemistries.
2. The HPLC system and analytical column were both washed and deactivated overnight (12 hours minimum) by 0.5% phosphoric acid (in 90% ACN/ 10% water).
3. Agilent InfinityLab Deactivator Additive was added into the mobile phase.

Figure 2. Measures to Improve Chromatographic Retention and Peak Shape of Glyphosine

### Good Affect of Agilent InfinityLab Deactivator Additive on Glyphosine Analysis

Metal ions can cause decreased signal and poor peak shape for polar pesticides containing phosphate groups, whereas adding Agilent InfinityLab Deactivator Additive (medronic acid is its main ingredient) into the mobile phase can improve the negative effect of metal ions on pesticide analysis[1]. 2µg/L glyphosine standard solution had good signal and peak shape with Agilent InfinityLab Deactivator Additive, while no peak was observed without the Agilent InfinityLab Deactivator Additive (Figure 3).

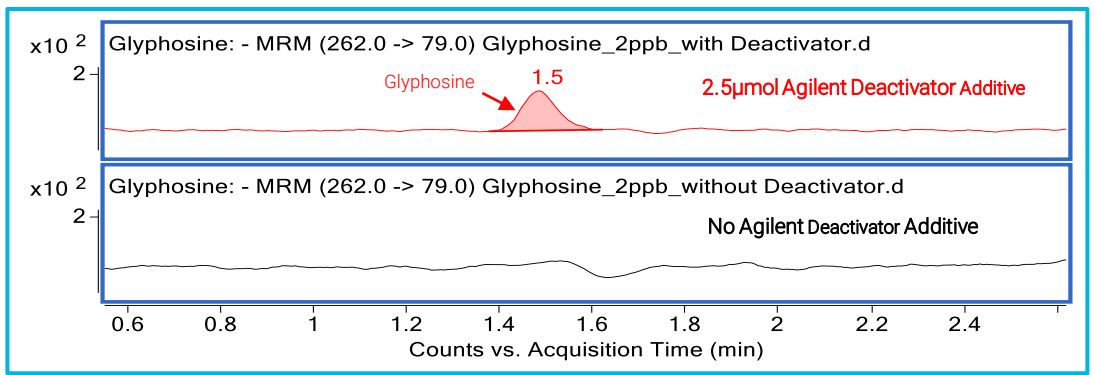



Figure 3. Good Affect of Agilent InfinityLab Deactivator Additive on Signal and Peak Shape of Glyphosine

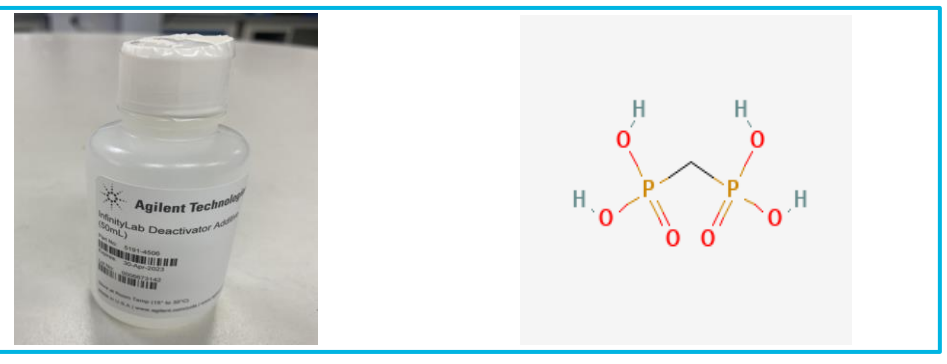



Figure 4. Agilent InfinityLab Deactivator Additive and the Structure of its Main Ingredient (Medronic Acid )

### Method Sensitivity and Precision

The sensitivity and precision of the 6470 LC/TQ were assessed by analyzing glyphosine standard prepared in ultrapure water. Figure 5 showed that glyphosine exhibited excellent sensitivity with LOQ=1µg/L. Figure 6 showed that glyphosine had good precision with RT RSD%=0% and peak area RSD%=3.5% for 10 replicate injections of 10µg/L glyphosine.

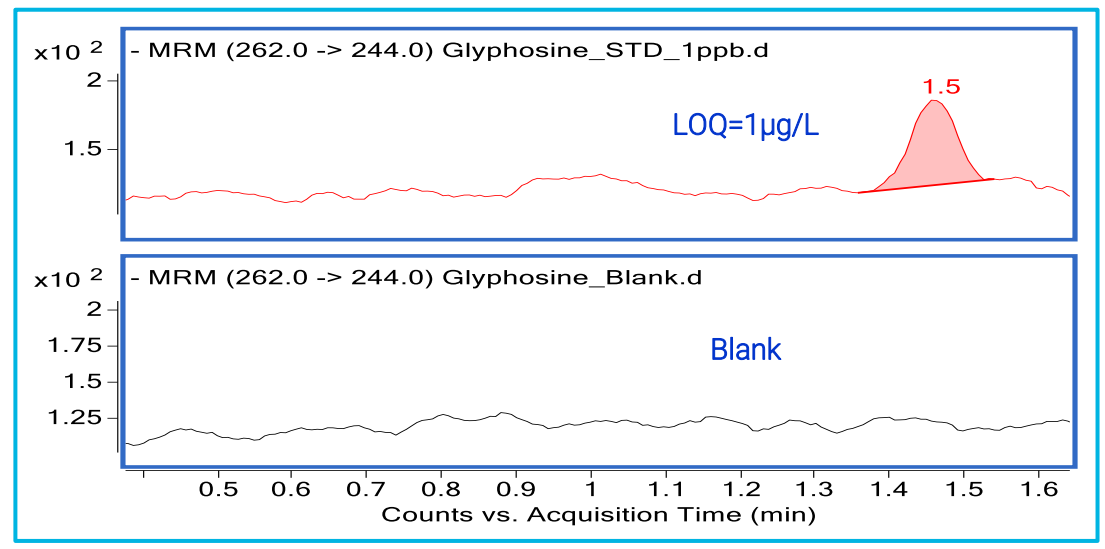



Figure 5. Chromatogram of Glyphosine at LOQ(1µg/L)

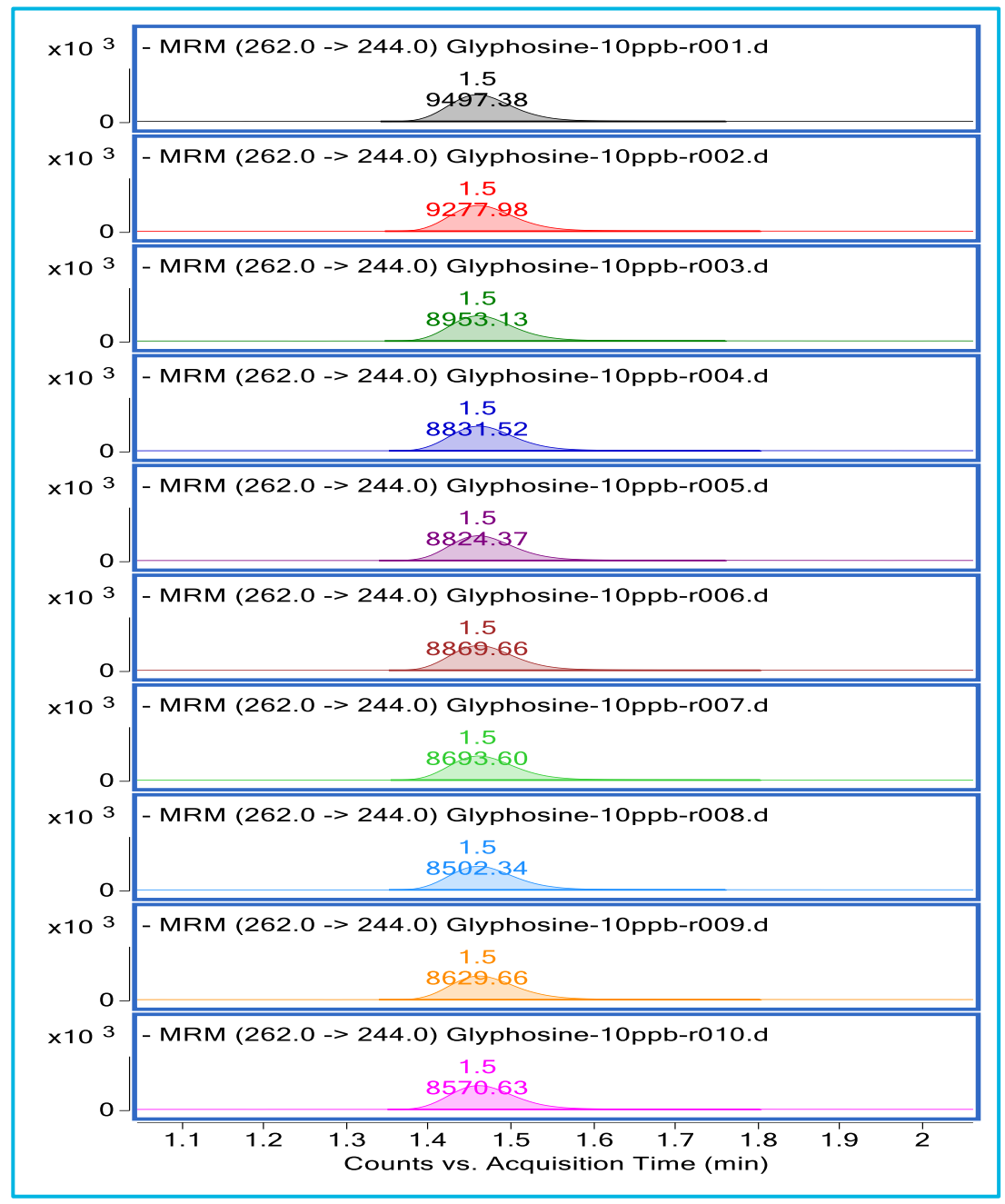



Figure 6. The Results of Retention Time and Peak Area for 10 Replicate Injections of 10µg/L Glyphosine

## Results and Discussion

### Co-Elution of Glyphosine and Glyphosate

Glyphosine and glyphosate are very similar in compound structure and properties, which makes separation by HPLC difficult (Figure 7). However, under the co-elution condition, the higher concentration of API (glyphosate) will cause a severe matrix effect on the analysis of trace or micro levels of a glyphosine impurity, which will compromise the reliable qualitative and accurate quantification of the glyphosine impurity.

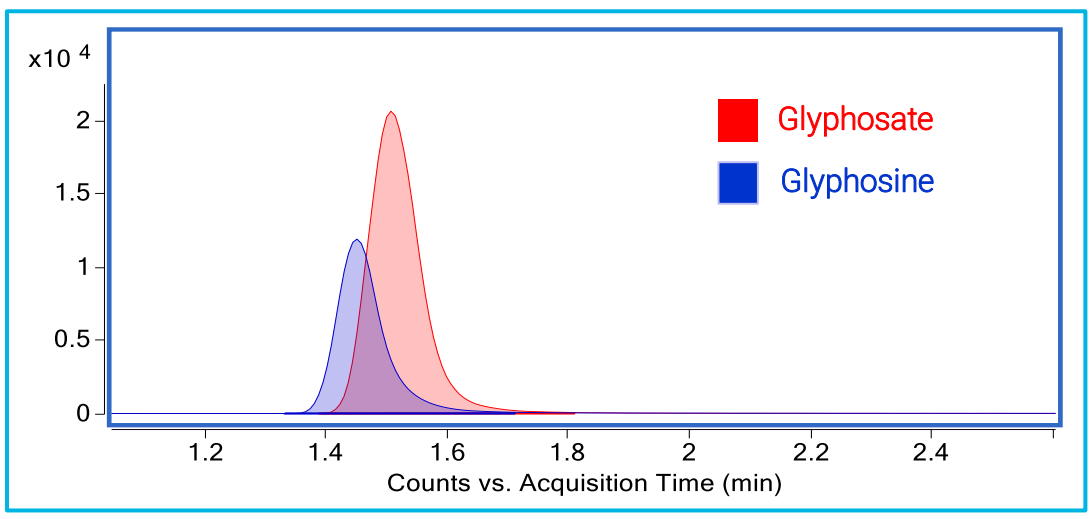



Figure 7. Chromatographic Overlay of the Co-Elution of Glyphosine and Glyphosate (both are 100µg/L)

### Matrix Effect Solved via Standard Addition

This study adopts standard addition to solve the difficult matrix effect problem under the condition of co-elution of the API and target impurity. A series of different concentrations of glyphosine standard were fortified into test samples with 1500mg/L glyphosate, respectively, and then the no fortified sample and the fortified samples were analyzed successively. "Standard Addition" option was selected in Agilent MassHunter Quantitative Analysis software, a calibration curve was set up with the fortified samples (Figure 8), and then the software would directly display the quantitative results of glyphosine for the no fortified sample.

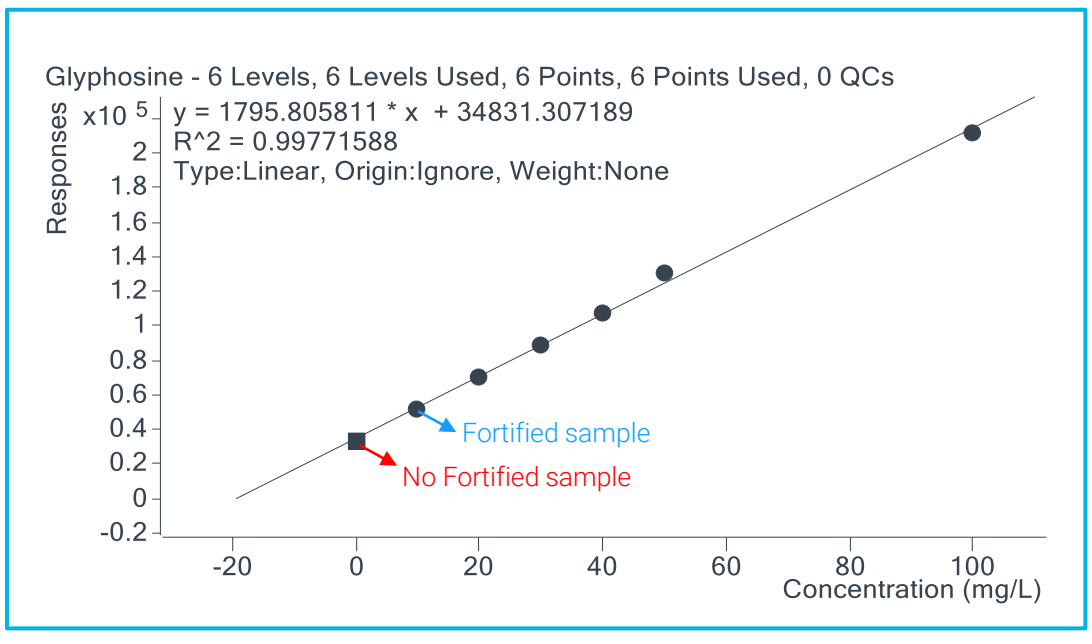



Figure 8. The Calibration Curve Obtained via Standard Addition

### Authentic Sample Test

Six batches of glyphosate API were obtained from pesticide manufacturers and tested using the described method. The linear correlation coefficient ($R^2$) of the six calibration curves was 0.997~0.999. The content of glyphosine impurity in the test samples was 1.16%~1.56%, which indicates that this method has good practicality.

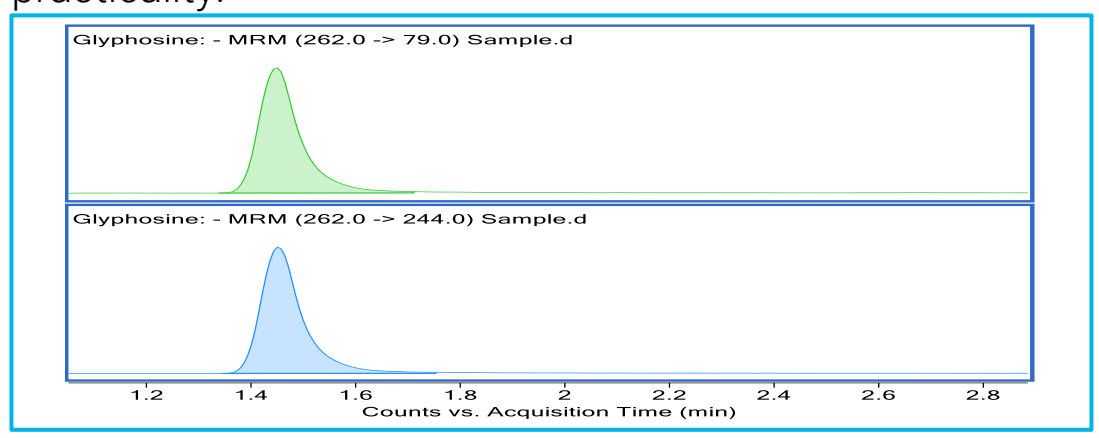



Figure 9. MRM Chromatogram of Glyphosine for One Authentic Sample

## Conclusions

We have demonstrated a rapid, highly sensitive and accurate UHPLC-MS/MS method for quantitative analysis of glyphosine impurity in glyphosate API using the Agilent 6470 LC/TQ via standard addition.

- Good peak shape and retention of glyphosine has been achieved by adopting optimized chromatographic conditions.
- For glyphosine, low sensitivity (LOQ=1µg/L) and high precision (RT RSD% = 0% and peak area RSD%=3.5% for 10 replicate injections of 10µg/L) have been achieved.
- In this method, standard addition has been adopted to effectively solve the adverse effects of the matrix effect on quantitative analysis caused by the co-elution of API (glyphosate) and the target impurity (glyphosine). Moreover, compared to the isotope internal standard, the cost of the standard addition is lower.
- This method has been applied to test authentic samples, and the test results show that it has good practicability.

## References

[1] Hsiao, J. J. et al. Improved LC/MS Methods for the Analysis of Metal-Sensitive Analytes Using Medronic Acid as a Mobile Phase Additive. Analytical Chemistry 2018, 90(15), 9457–9464.

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