A Quantitative Lateral Flow Immunoassay for Measuring Glyphosate

Jianmin Liu and Lingyun Chen

Waters Corporation, VICAM, Milford, MA USA

ABSTRACT

Glyphosate (trade name Roundup™ herbicide) is one of the most used herbicides that control broadleaf weeds and grasses. The widespread use of glyphosate makes it ubiquitous in the environment. Researchers have found its presence in our food, soil, groundwater and surface waters. Recent studies have raised health concerns on glyphosate exposure even though the toxicity of glyphosate is still under debate. The international agency for research on Cancer (IARC) classified glyphosate as a probable carcinogen in 2015. The instrumental method for analysis of glyphosate is available including HPLC and LC-MS/MS. However, the sample preparation and testing procedure are relatively complicated and time-consuming. In this study, a simple quantitative strip test (Glyphosate-V™ Test) for measuring glyphosate was developed and evaluated with applications on water, wheat and oats. The samples were extracted by distilled water, filtered, derivatized and mixed with a diluent, then directly applied to the test strip device. The results generated from Glyphosate-V™ test (Quantitative Strip Test) showed a very high degree of linearity (r^2 = 0.999), with the test ranging from 2 to 1000 ppb for water, and 25 to 3000 ppb for wheat and oats. The limit of detection (LOD) is 2 ppb for water and 25 ppb for wheat and oats. Comparative study indicates that the results generated from Glyphosate-V™ testing correlated well with the results of LC-MS/MS. The time required from sample preparation to result is less than 20 minutes. In conclusion, the Glyphosate-V test (Quantitative Strip Test) can be used as a screening tool for glyphosate detection in food and environmental samples.

SAMPLE PREPARATION & TESTING PROCEDURES

Glyphosate standard was purchased from Restek (#32427), Distilled water, glyphosate no-detectable wheat and oats, glyphosate contaminated wheat and oats were obtained commercially. Samples extraction and test flow charts are shown below. Glyphosate-V kit and its accessories were obtained from VICAM, Waters Corp.

Test glyphosate in grain

3.0 g finely grounded sample + 30 mL distilled water in Vertu PREP Tube

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Blend 2 minutes on Vertu PREP Mixer

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Filter into cup filter

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Transfer 1 mL extract to 1.5 mL Strip Test Vial

Test glyphosate in water

Transfer 1 mL water to 1.5 mL Strip Test Vial

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Add 100 µL reagent A (Clear Bottle)

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Add 100 µL reagent B (Amber Bottle)

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Immediately vortex 10-15 seconds and incubate 5min

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Transfer 100 µL to strip sample well

↓

Develop 5 minutes

↓

Read result

RESULTS

Limit of Detection (LOD)

The LOD for Glyphosate-V test is defined as the least amount of glyphosate spiked in the distilled water, wheat and oats, which can be clearly detected by applying the testing procedure. Based on the definition, the LOD of current Glyphosate-V test for water is 2 ppb, for wheat and oats is approximate 25 ppb.

Linearity and Test Range

The test Linearity was done by spiking glyphosate into water, wheat and oats which were known glyphosate-free tested by LC-MS/MS. Results are shown in Fig 1 and 2. As shown, clearly the Glyphosate-V test can determine samples ranging from 0 to 1000ppb in water, and 0 to 3000ppb in wheat and oats.

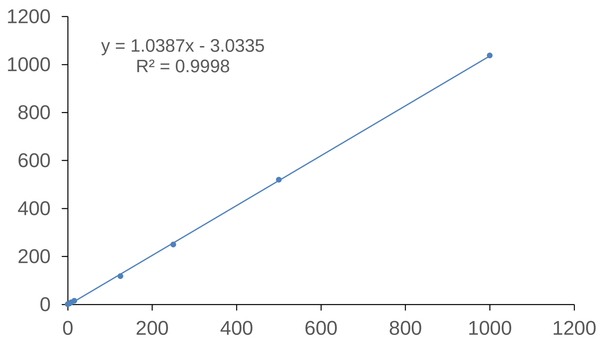

Fig 1. Glyphosate spiked in water ranging from 0 to 1000 ppb.

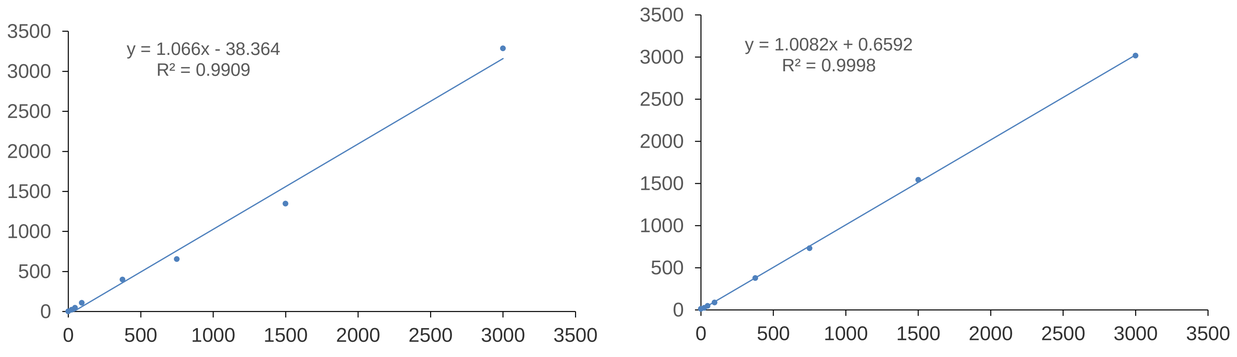

Fig 2. Glyphosate spiked in wheat (left) and oats (right) ranging from 0 to 3000 ppb.

Accuracy

Naturally contaminated samples and reference material were analyzed by using current Glyphosate-V testing procedure, the same samples were also determined by LC-MS/MS, the results were summarized in Table 1.

Table 1. Results from Glyphosate-V test and LC-MS/MS

Sample	Glyphosate-V (ppb)	LC-MS/MS (ppb)
Water	5.4	< 10
	56.1	56
Wheat	1.7	< 10
	157.5	153
Oat	1.8	16
	361.3	338
FAPAS Oats	405.1	467 (299-634)*

***** Reference material, test method unknown

Reproducibility

Reproducibility was done by different operators (A,B,C) testing the same samples according to the Glyphosate-V procedures described above. Water samples were spiked at 10 and 100 ppb. Wheat sample was predetermined by LC-MS/MS, oat sample was purchased from FAPAS. , the results were summarized in Table 2.

Table 2. Results from operators

	PPB	A	B	C
Water	10	10.3	10.8	10.5
	100	89.8	84.8	95.1
Wheat	153	153	194.3	144.9
Oats	467	405.1	481.3	349.7

CONCLUSIONS

- **The Glyphosate-V test (Quantitative Strip Test) is a rapid quantitative test. Results can be achieved within 20min including sample preparation.**
- **The result of Glyphosate-V test is accurate, reproducible, and comparable to the result of LC-MS/MS.**
- **The Glyphosate-V test provides a new screen tool for glyphosate determination in food and environmental samples.**