

## 7.7.12

**AOAC Official Method 996.12**  
**Glyphosate in Water-Soluble**  
**Granular Formulations**  
**Liquid Chromatographic Method**  
**First Action 1996**  
**Final Action 2001**

See Table 996.12A for the results of the interlaboratory study supporting the acceptance of the method.

**A. Principle**

Formulations are dissolved in phosphate buffer mobile phase and injected into ion-exchange chromatographic system. Peak area response, as measured by UV detection, is quantitated by external standard technique.

**B. Apparatus**

(a) *Liquid chromatograph (LC)*.—Generating >3000 psi; with isocratic LC pump capable of accurately metering 2.3 mL/min; with UV detector set at 195 nm.

(b) *LC column*.—Anion exchange column. See F, System Suitability.

(c) *Injector*.—Fixed loop or autoinjector; accurately delivering 50  $\mu$ L; producing peak areas within 1% on the same glyphosate working standard or test solution. (Note: Some anion exchange columns require lower loading, 10–20  $\mu$ L, with the same reproducibility criteria. See Table 996.12B for ASTM column suitability parameters.)

(d) *Electronic integrator for chromatographic data*.—Data handling system may be used.

(e) *Analytical balance*.—Accuracy  $\pm$  0.1 mg.

(f) *Volumetric flasks*.—100 mL.

**C. Reagents**

(a) *Potassium dihydrogen phosphate*.—Primary standard grade.

(b) *Water*.—LC grade.

(c) *Phosphoric acid*.—85%, reagent grade.

(d) *Methanol*.—LC grade.

(e) *Glyphosate reference standard*.—99.9% Purity (available from Monsanto Co., 800 N. Lindbergh Blvd, St. Louis, MO 63167, USA). Reference standard is stable at least 2 years in closed, dry container at 0–30°C. Material of lesser purity is available commercially as *N*-(phosphonomethyl)glycine.

(f) *LC mobile phase*.—Dissolve 0.844 g  $\text{KH}_2\text{PO}_4$  in 960 mL  $\text{H}_2\text{O}$ . Add 40 mL methanol and mix well. Using pH meter (calibrated at pH 2.0), adjust pH to 2.1 with 85%  $\text{H}_3\text{PO}_4$  (v/v). Filter and degas be-

fore use. LC mobile phase is stable at least 2 weeks in closed containers under ambient conditions (20–30°C). When in use, lightly vent container into atmosphere.

**D. Preparation of Standard Solutions**

Prepare 0.2, 0.4, and 0.6% glyphosate working standard solutions as follows: Accurately weigh 0.2, 0.4, and 0.6 g glyphosate reference standard to the nearest 0.1 mg into separate flasks containing 100 mL LC mobile phase. Calculate exact concentrations of glyphosate taking into account purity of glyphosate reference standard.

**E. Calibration**

Equilibrate LC column with LC mobile phase ca 1 h at 2.3 mL/min.

Inject glyphosate working standard solutions from D onto LC column until reproducibility of injections is within 1%. To obtain glyphosate retention time 3.9–5.9 min, adjust pH of LC mobile phase and adjust flow rate (2.3 mL/min and pH 2.1 are typical). Assure linearity of standard injections.

Use practice test samples to assure baseline separation of peak from both surfactant peaks as well as small impurity immediately following glyphosate (see Figure 996.12). If necessary, adjust integration parameters to achieve the known analyses of formulated products.

**F. System Suitability**

The quality of LC column is a critical element of glyphosate analysis. The criterion for acceptable LC column is reliable integration of glyphosate peak in glyphosate formulations. Interference from surfactant (peak A; see Figure 996.12) with front end of glyphosate peak, or especially interference from impurity (peak C) with back end of glyphosate peak must be avoided.

Test LC columns for suitability, using performance criteria that include peak efficiency, resolution between glyphosate and peak C, and asymmetry (see Table 996.12B for ASTM column suitability parameters.)

The apparent mode of separation of glyphosate is based on ion suppression using ion exchange resin as partition media and not as ion exchange resin. This indicates that weaker exchangers or less efficient ion exchange resins may perform better in resolving glyphosate from its major impurities (see Table 996.12B).

The ideal criterion for adequate LC columns is performance on control charts with a typical formulation. Relative standard deviation should be  $\leq$  0.25%.

**G. Determination**

Into 100 mL volumetric flask, accurately weigh 0.4 g test portion to the nearest 0.1 mg and dilute to volume with LC mobile phase.

**Table 996.12A Interlaboratory study results for determination of glyphosate in water soluble granular formulations by liquid chromatography**

Glyphosate formulation <sup>a</sup>	No. of labs	$\bar{x}$ , %	$s_r$	$s_R$	RSD <sub>r</sub> , %	RSD <sub>R</sub> , %	$r^b$	$R^c$
60696	27	63.40	0.69	0.69	1.09	1.09	1.93	1.93
14445	28	73.07	0.84	0.92	1.14	1.26	2.35	2.58
14420G	28	68.90	0.65	0.94	0.94	1.37	1.82	2.63
8750	26	87.38	0.41	0.57	0.47	0.65	1.15	1.60
8750	26	87.35	0.67	0.67	0.77	0.77	1.88	1.88

<sup>a</sup> Youden pairs.

<sup>b</sup>  $r = 2.8 \times s_r$ .

<sup>c</sup>  $R = 2.8 \times s_R$ .

**Table 996.12B ASTM column suitability parameters for glyphosate separations using anion exchange columns (flow rate, 2.3 mL/min)**

LC column, type, and length	Injection volume, $\mu\text{L}$	Glyphosate retention time, min	Column efficiency (glyphosate) <sup>a</sup>	Resolution ratio between glyphosate and peak C <sup>b</sup>	Asymmetry ratio <sup>c</sup>
Whatman 4226 SAX, 250 mm <sup>d</sup>	50	4.05	1341	2.17	0.68
Hamilton PRPX 100 SAX, 100 mm	10	4.41	1776	1.33	0.90
Mac-Mod Zorbox SAX, 250 mm <sup>e</sup>	50	2.99	2842	1.74	0.69
Whatman MAX I WAX, 250 mm <sup>f</sup>	10	3.70	6228	4.71	1.02
Whatman MAX I WAX, 300 mm <sup>g</sup>	50	4.47	2784	4.78	0.38
Whatman MAX I WAX, 300 mm <sup>g</sup>	10	4.27	4387	5.21	0.58

<sup>a</sup> Column efficiency (glyphosate),  $N$ , calculated as theoretical plates as follows:  $N = 5.54 \times (RT/P_w)^2$ , where  $RT$  = retention time, min; and  $P_w$  = peak width, min.

<sup>b</sup> Ratio of retention time of peak C to glyphosate peak.

<sup>c</sup> Ratio of the length of the trailing half of the peak divided by the length of the leading half of the peak, measured on a line perpendicular to a line cropped from the peak maximum, drawn 10% of peak height above the baseline.

<sup>d</sup> Used in collaborative study.

<sup>e</sup> Limited applicability due to front end interference with peak A (surfactant, see Figure 996.12).

<sup>f</sup> Some front end interference observed at higher loading.

<sup>g</sup> Columns are based on weak anion exchange (WAX) and updated technology including 5  $\mu\text{m}$  silica rather than 10  $\mu\text{m}$ , and spherical rather than irregular packing.

Test measurements should be bracketed by 0.2 and 0.4% glyphosate working standard solutions.

Establish regression line of concentration vs response using glyphosate working standard solutions. Make duplicate determinations (i.e., 2 weighings, one injection/weighting) of each test bracketed between 0.2 and 0.4% glyphosate working standard solutions.

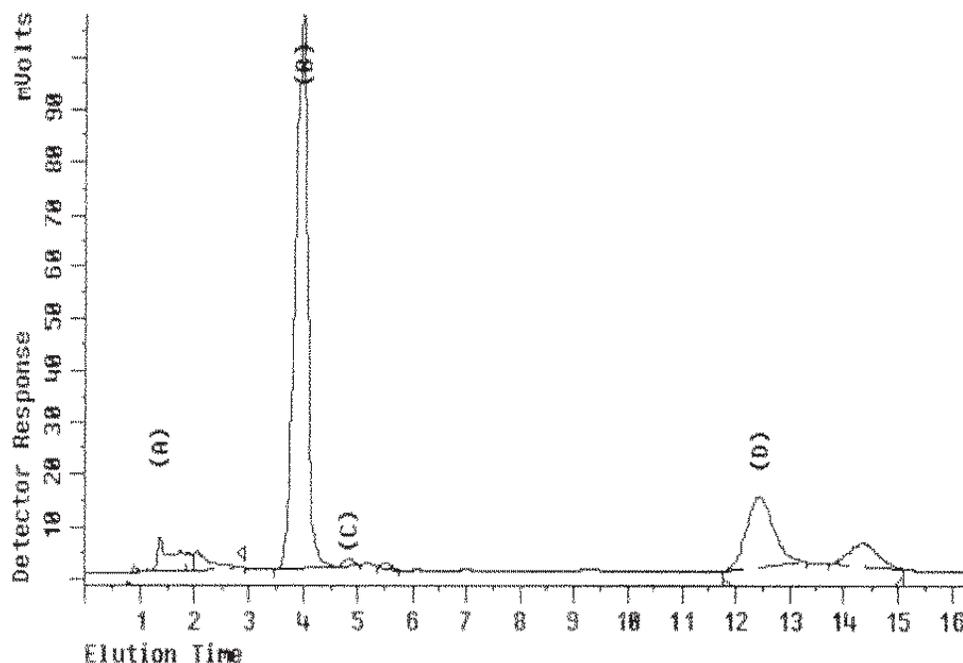
Most formulations contain a late eluting peak which approximately doubles the run time required for full elution. Injecting LC mobile phase after each analyte injection enables use of

autoinjectors to minimize analysis time for the required injections. Glyphosate working standard solutions bracketing tests should produce slopes and intercepts within control limits of established regression curve. With fixed volume loop injection, variability is dependent on fluctuations in laboratory temperature. Test determinations should agree within 1% relative basis.

Report average of 2 determinations.

#### H. Calculations

Calculate regression line as follows:



**Figure 996.12—Liquid chromatographic separation of surfactant (A), glyphosate (B), impurity 1 (C), and 2 isomers of late eluter peaks, impurity 2 (D) from glyphosate herbicide formulation.**

$$P_g = mx + b$$

where  $P_g$  = peak area of glyphosate in glyphosate working standard solution;  $m$  = slope of regression line;  $x$  = concentration of glyphosate in glyphosate working standard solution, %; and  $b$  = intercept of regression line.

Calculate glyphosate concentration, %, in pesticide formulation as follows:

$$\text{Glyphosate, \%} = \frac{(P_s - b) \times V}{m \times W}$$

where  $P_s$  = peak area of glyphosate in test portion;  $V$  = volume of diluted test solution, mL; and  $W$  = weight of test portion, g.

Reference: *J. AOAC Int.* **80**, 464(1997).

CAS-1071-83-6 (glyphosate) *N*-(phosphonomethyl)glycine

*Revised: March 2002*