# ANALYSIS OF FORMALDEHYDE IN GLYPHOSATE WETCAKE,GLYPHOSATE ISOPROPYLAMINE SALT AND ROUNDUP® SAMPLES

## 1. Principle of Method

This method describes a liquid chromatographic procedure for the selective determination of formaldehyde in glyphosate wetcake, glyphosate isopropylamine salt, and Roundup® samples

The Hantzch reagent is used to react with formaldehyde present in aqueous glyphosate solutions. The resulting derivative, diacetyldihydrolutidine or DDL, is determined by reversed phase HPLC with UV detection.

Quantitation is based on the area of the DDL peak. This response is compared to the response of external standards prepared in the same manner as the samples.

## 2. Safety

Several of the solvents and reagents for this method are hazardous chemicals and should be used only with proper ventilation.

## 3. Range and Sensitivity

### Range

This method has been validated for the range of 0.3 – 14 ug/mL formaldehyde in diluted samples of glyphosate wetcake, glyphosate isopropylamine salt, and Roundup® .

### Sensitivity

Analytical response was found to be linear over the range of 0.3 – 14 ug/mL. The detection limit of the method is 1 ppm under these conditions.

## 4. Interferences

Formaldehyde generating substances such as N-isopropylhexahydrotriazine will interfere with this method.
Any compound with the same retention time as DDL , that responds at 412 nm will interfere with this method.
The presence of formaldehyde in reagents will cause a high background level, visible in reagent blanks.
Formaldehyde contamination from Bakelite caps must be avoided.

## 5. Precision and Accuracy

### Precision

The pooled coefficient of variation of the analytical method in the range of 0.3-14 ug/mL is 0.222.

**Accuracy**

Average spiked recoveries for formaldehyde standard spikes in the range of 0.3-14 ug/mL in simulated glyphosate wetcake samples were 85.9 -106.2%. Simulated wetcake was prepared from pure glyphosate and water. Average recoveries for spikes in the range of 26 – 521 ug/g in glyphosate isopropylamine salt and 5.0 -371 ug/g in Roundup® samples (equivalent in each case to concentrations of 0.3-14 ug/g in solution) were 88.5- 106.7% and 97.9- 102.0%.

**6. Advantages and disadvantages**

**Advantages**

The method is sensitive and selective and is unaffected by the glyphosate matrix.

**Disadvantage**

Reaction time is at least 2 hours. Samples and standards should be prepared and analyzed on the same day due to the instability of DDL.

**7. Apparatus**

HPLC pump – Perkin Elmer series 3B
Injector – Perkin Elmer 420B autosampler
Column oven – Perkin Elmer LC – 100
Detector – Perkin Elmer LC-75 spectrophotometric detector
Recorder – Monsanto chromatography data system and strip chart recorder
Analytical column –Dupont Zorbax ODS 4.6mm i.d. X 15 cm
Assorted glasswares

**8. Reagents**

Formaldehyde – 37% solution Fisher F-79
Acetyl acetone – Fisher A-25
Ammonium acetate – Fisher A-637
Acetic acid – Glacial, Fisher A-38
Sodium hydroxide – Fisher S-318
HPLC water – Burdick and Jackson 365
Acetonitrile – Burdick and Jackson 015

**9. Calibration and Standardization**

A series of aqueous formaldehyde standards in the range of interest are derivatized and analyzed using the same HPLC conditions and on the same day as the unknown or spiked samples. Formaldehyde is quantitated by comparison with calibration data generated. Standards are prepared by appropriate dilution of a 37% w/w (40% w/w) formaldehyde solution to the working range of 0-14 ppm. These aqueous formaldehyde standards should be prepared fresh for every analysis.

**10. Procedure**

**Cleaning of Equipment**

All glassware used for this method should be washed with liquid detergent and rinsed thoroughly with deionized water.

**Collection and Shipping of Samples**

Samples should be placed in a tightly sealed container with minimal headspace to avoid drying.

**Sample Preparation**

The HPLC mobile phase is prepared by adding 800 mL of HPLC water to 200 mL of acetonitrile followed by mixing and degassing with helium.

The Hantzsch reagent is prepared by placing 150 g ammonium acetate, 3 mL acetic acid, and 2 mL acetyl acetone in a 1 L volumetric flask and diluting to volume with HPLC water.

The 11% NaOH solution is prepared by diluting 110 g NaOH to 1000 mL with HPLC water.

Prepare an aqueous solution of the glyphosate wetcake to be analyzed in the range of 1% - 20% w/v depending upon the anticipated formaldehyde level. A concentration of 1 ppm formaldehyde can be quantitated in a 20% wetcake. Add 3 mL 11% NaOH per gram of wetcake. Shake until all solids dissolve and dilute to volume with deionized water.

Samples of glyphosate salt and Roundup® should also be diluted such that the concentration of formaldehyde in the diluted samples falls within the range of 0.3 -14 ug/g. In all cases, at least slight dilution of these matrices is recommended to reduce potential viscosity issues associated with sample injection via an autosampler.

In a small vial, combine equal volumes of sample or standard solution, containing less than 14 ppm formaldehyde, and Hantzsch reagent. Shake well and allow to stand at ambient temperature for at least 2 hours.

**Analysis of Prepared Sample**

The derivatized samples and standards are injected onto the HPLC system alternately and the peak area of DDL is recorded. HPLC conditions are: flow = 1.0 mL/min, wavelength = 412 nm, column temperature = 60$^{o}$C, average retention time of DDL is 6.1 min under these conditions, injection volume = 50 ul.

The amount of formaldehyde in the samples is determined from the established calibration curve in the range of interest using linear regression

**Special Comments**

If too much formaldehyde is present in the original sample solution, DDL will eventually precipitate out of solution. The yellow color of DDL will fade with time, especially in sunlight.

**11. Calculations**

Quantitation is based upon comparison of the peak areas of the samples and standards. The concentration of formaldehyde in the original solution is determined from the established calibration curve in ppm. From this, the total weight of formaldehyde in the original sample is calculated and is then divided by the original sample weight to give ppm formaldehyde in the sample matrix

$$\text{ppm formaldehyde} = \frac{\text{formaldehyde found(ug)}}{\text{original sample weight(g)}}$$

**12. Discussion**

Due to inconsistent distribution of water content in glyphosate wetcake, there is some difficulty in weighing out representative samples. For validation purposes, glyphosate wetcake was simulated by weighing out dry recrystallized glyphosate and then adding 15% deionized water during spiking. This resulted in consistent samples containing 15% water.

A small background response was observed in the dry recrystallized glyphosate used to simulate wetcake and in the reagent blanks. This response was subtracted from the spike responses when calculating found concentrations. When quantitating low levels of formaldehyde, a reagent blank should always be run.

**13.References**

T. Nash, Biochem J. (London),55:416-421 (1953)

R. LaMonica, HPLC Assay for Formaldehyde in CMA, Unpublished Results, MAPC (1983)