Glyphosate in all its forms

S. Goscinny and V. Hanot
Scientific Institute for Public Health
Pesticides Unit

A breakthrough molecule

Since its first appearance on the market in 1974, glyphosate has become the most widely used phytosanitary compound worldwide (one million tons sold in 2010). Its growing popularity is closely linked to its agronomic efficiency, its low cost and, above all, its favourable toxicological profile (see CV). The main breakdown product of glyphosate is aminomethylphosphonic acid (AMPA), which has the same chemical and toxicological properties as the parent compound. In foodstuffs, AMPA was only detected in 2% of cases, which is why the maximum residue limit only concerns glyphosate. As they share the same analytical difficulties, the same methods for the determination of glyphosate are also implemented for AMPA. This not only provides access to more data for the risk assessment but also provides for better preparedness, in the event of any change to the residue definition in the future, without having to go through an additional development and validation phase.

Glyphosate

Curriculum Vitae

Chemical Properties

- **C₃H₈NO₅P** = N-(phosphonomethyl)glycine
- Molecular mass = 169.07
- pKa: 0.78-2.29-5.96-10.98
- Weak organic acid
- Water solubility: 12 g/L at 25°C

Mechanism of action

Inhibits the enzyme 5-enolpyruvoyl-shikimate-3-phosphate synthase (EPSPS) involved in the shikimic acid metabolic pathway. This pathway is absent in animals, which explains the high oral LD₅₀ values:

- Female goat 3 530 mg/kg
- Female rat 2 686 mg/kg

Agronomic Use

Broad spectrum systemic herbicide, which provides great flexibility of use: on all crop types and for the maintenance of urban spaces (green spaces, gardens, roads, railways).

Toxicology Breakdown

Moderately persistent in soil and with a half-life of 20 to 100 days, it undergoes microbial breakdown, which finally metabolises it into carbon dioxide and simple inorganic compounds. However, its widespread use calls into question the issue of its “relatively low toxicity” for the environment.

Exposure

Only slightly soluble in fats, thereby reducing the risks of bioaccumulation. It is incompletely absorbed (15 to 40%) after oral administration.
The “Prima Donna” of analytical chemistry

Compared to other herbicides, glyphosate is the most difficult to analyse. The source of these difficulties lies in its physicochemical properties, which complicate each stage of the analysis:

Sample preparation

Glyphosate is very polar, water soluble and insoluble in organic solvents, thus making the possibilities for its extraction limited. As a result, the use of water is essential for this stage of the analysis. However, within the extract, other water soluble matrix compounds can also be found (sugars, amino acids, salts, etc.) that may interfere with the determination of glyphosate. A column purification stage is problematic due to the amphoteric nature of glyphosate. A liquid-liquid extraction purification method is less problematic in terms of yield and is, as a result, the most common method referred to in literature. However, the latter requires more handling and extended analysis time.

Separation and detection

Not only are these compounds very polar and ionic, but in addition, they do not possess chromophore groups to allow fluorescence or ultraviolet absorption detection. At this stage of the analysis, a difficult choice has to be made: whether or not to derivatise the glyphosate.

The derivatisation technique allows for the analysis of compounds which cannot be analysed directly by gas chromatography (GC) or liquid chromatography (LC). Through a chemical reaction, an agent (chromophore) is grafted onto one or a number of sites of the target molecule. Changes in properties occur with the derivatised compound in favour of its analysis. In the case of glyphosate, derivatisation adds a chromophore, reduces its polarity and, depending on the chosen derivatising reagent, even allows for its GC analysis. However, this technique is not highly regarded by analysts as it requires the optimisation of a number of parameters (temperature, reaction time, concentration and purity of the reagents, laboratory handling time). If omitting this heavy task is deemed preferable, there are other viable options using liquid chromatography, such as hydrophilic interaction chromatography (HILIC), ion exchange chromatography (IC) or ion-pair chromatography suitable for very polar and ionic molecules. However, the robustness of these separation techniques is heavily dependent on enhanced purification or requires a high dilution factor of the extract and therefore a highly sensitive detection system. Derivatisation and direct analysis both have their advantages and disadvantages. The choice of technique should be made in a rational manner to ensure a cost-effective method. In both cases, mass spectrometric detection is possible and recommended in order to avoid the problems of interference and sensitivity that can occur with other detectors (UV, electrochemical etc.)
Background of the method

The analysis of glyphosate in cereal products has been mandatory since the beginning of 2011 for all laboratories involved in the European control plan. This obligation means that a robust analysis method must be developed and validated, which must be capable of being easily incorporated into the laboratory’s routine analyses. Within this framework, an analysis method for glyphosate and its metabolite AMPA was developed at the SIPH in partnership with the Austrian Agency for Health and Food Safety (AAHFS). Direct analysis methods (non derivatised) may be deemed attractive since they do not require management of the derivatisation stage. However, they impose chromatographic changes (mobile phases, columns, operation time) which may not be compatible with the routine analyses. We have favoured a protocol that allows the instrumental conditions of the multi-residue analyses to be maintained (conventional C18 column and mobile phase mixtures of methanol water buffered with ammonium acetate at pH ± 6). We have therefore opted for a method that incorporates a derivatisation stage. The chosen reagent is 9-fluorenylmethyl-chloroformate (FMOC) which allows, thanks to its hydrophobic grouping, the polarity of the target molecule to be reduced and thus its retention on a reverse phase C18 column to be increased (see chromatogram) as well as the molecular mass, which will facilitate MS-MS detection. FMOC binds to the amine functions by releasing a HCl molecule. The reaction must take place in an alkaline medium (a borate buffer is used-Na pH=9) in order to neutralise the acid formed and to help push the reaction towards the formation of glyphosate-FMOC. Unfortunately, the FMOC is not specific to glyphosate but reacts with all the amine functions and is highly reactive with water. The reagent concentration is of key importance in order to ensure a robust method, which is why the chosen concentration (1 mg/ml) is significantly high, thereby ensuring:

- that the surrogate and target compounds are not in competition with each other and reacting with the FMOC at the same time;
- the reaction with the water and amines from the extract is not harmful to the target compounds.

The excess of the reagent is removed by washing with dichloromethane and the product of the reaction is directly filtered before being injected.
Fig. 1: UPLC-ESI+-MS/MS chromatogram for glyphosate-FMOC and AMPA-FMOC in a calibration solution at 100 µg/kg.

Fig. 2: UPLC-ESI+-MS/MS chromatogram for glyphosate-FMOC and AMPA-FMOC in a spiked sample of oats at 10 x LOQ (200 µg/kg).
A “tailored” method

The method was validated in accordance with the guidelines set out in the SANCO/10684/2009 document. As a result of this:

• there is no matrix effect, the calibration curve is therefore prepared by derivatising the compounds outside of the matrix and covers a range of concentrations from 20 µg/kg to 500 µg/kg;
• the derivatisation conditions are well adapted since they achieve yields at the limit of quantification (20 µg/kg) of 101.1 % (RSD of 6.9 %) and 112.9 % (RSD of 11.9 %) for glyphosate and AMPA respectively.

The determination of glyphosate at trace level is unquestionably complicated. However, if the correct analytical method is chosen, rather effective results can be achieved. In our case, the choice of isotope dilution quantification has allowed us to achieve a good yield without having to worry about potential losses during the rapid stage (1 min) of simultaneous extraction-purification. Derivatisation has been a success since it allows us to easily incorporate the analysis of glyphosate into routine analyses without making any changes instrumentally, which therefore represents a considerable time saving benefit.

Severine.Gosciinny@wiv-isp.be
Vincent.Hanot@wiv-isp.be