Brief review analytical methods for the determination of glyphosate

Abstract

The quantification of levels of biological samples can be an important tool in human biomonitoring and can provide reliable estimates of the levels of human exposure to products of substances used in plant protection. Due to this, it is emphasized the importance in developing methods that are effective and quick to determine and quantify these molecules of interest. Thus, it performed a critical review of literature and data comparison studies already available on the development of analytical methods for determining the pesticide glyphosate.

Keywords: glyphosate, determination, chromatography, pesticides, analytical methodologies

Abbreviations: HPLC, high-performance liquid chromatography; GC, gas chromatography; LC, liquid chromatography; AMPA, aminomethylphosphonic acid

Introduction

Glyphosate is a non-selective herbicide widely used in agriculture, which accounts for over 60% in the global market, providing a wide spectrum of action which enables an optimal control danin has plants.1-4 Besides, this pesticide has low acute oral toxicity when compared to other herbicides.5-7 It is known that pesticide residues are widely used in chromatographic methods for quantifying the target analyte. However, many pesticides have high polarity, such as glyphosate, making it difficult to determine. Therefore, in many cases, it is used derivatization of these compounds, in order to make them suitable for a particular method in determining analítico.8-12 It is noteworthy that even with derivatization often these residues require significant laboratory resources, as well as treatment processes of long, complex and expensive samples. So there is a clear need to develop simple, fast and effective methodologies that are able to determine and analyze high polarity pesticides.13,14 The aim of this study was to develop brief review of the main analytical methodologies available in the analytical determination of glyphosate pesticide by chromatographic methods.

Analytical methodologies

Glyphosate has high polarity with the added feature of forming ionic species and no chromophores groups, determine their ends become more complexa.26 Thus, the determination of this pesticide often requires additional processes that may allow quantification by chromatographic methods. It is known that there are several studies on the determination of pesticide glyphosate in various types of samples, such as urine and human blood grain in breast milk and água.15,17 However, some of these studies vary widely depending on the sensitivity and recovery of the chromatographic unit and sample processing.

High-performance liquid chromatography (HPLC)

In general, the development of analytical methods for HPLC are common in determining glyphosate, although their properties hinder this process, necessitating often derivatization reactions so that there is a response from chromatographic techniques such as gas chromatography (GC);15-22 Liquid Chromatography (LC)23-25 and equipment with UV detection-vis.26-28 Determination of glyphosate by HPLC with fluorescence detection

It is known that glyphosate from determinations of fluorescence detectors have a high sensitivity and selectivity when compared to analysis by ultraviolet. Therefore, by derivatization procedures of this compound, it is possible to make it fluorescent allowing a proper determination. Another alternative to glyphosate analysis was performed by Sancho et al.,2 which held a simultaneous determination of glufosinate, glyphosate and AMPA samples in water using a C18 column for better efficiencies for the separation of analyte, coupled with an amino second chromatographic column of anion exchange for the separation of derivative compounds. In tentative to improve the sensitivity, the authors attempted to decrease the detection limit for the unit of mg/L concentrating a given volume by evaporation of water sample. However, this step resulted in lower recoveries than 20 and 30% of glyphosate and AMPA, respectively. However, they investigated the lowering of the detection limit for application of method focusing on individual determination for each analyte obtained where higher recoveries than 95%. In one of their studies Mallat & Barceló10 performed studies to determine and glyphosate degradation and its major metabolite aminomethylphosphonic acid (AMPA) in water samples from their derivatization using p-Phthalaldehyde (OPA) post-column reaction with wavelengths of excitation and emission 330nm and 465nm, respectively, and ion exchange chromatographic column 150x4mm in K+ form. His studies showed that 83% recoveries for glyphosate and AMPA to 90%. Another derivatizing widely described in the literature is the fluorenilmetilloxicarbonilo chloride (Fmoc-Cl). Higendoorn et al.,7 determined glyphosate in cereal samples per pre-column derivatization with FMOC-Cl. For the HPLC analyzes was used chromatographic column 30x4.6mm C18 Hypersil ODS 5μm DI and adsorption column 5μm 250x4.6mm DI NH2. Recoveries between 74 and 86% were obtained. It was given a limit of detection (LOD) of 2 and 4pb to glyphosate and AMPA, respectively.
Determination of glyphosate by HPLC with detection by UV-vis

Catrinck et al.30 sought in his optimization studies a quick, easy and efficient technique for derivation of glyphosate and AMPA by using FMOC-Cl analyzed by UV-vis. The reaction of derivatization with FMOC-CL excellent proved to be an alternative due to the high sensitivity this compound in the ultraviolet region. Moreover, the authors of this paper investigated the occurrence and increased sensitivity of this reaction from parameters such as spectral behavior derivatization effect of the concentration of borate buffer effect of homogenization time, effect of reaction time and effect solvent wash. Overall, the results showed that the optimal homogenization conditions were vortexed for 5 minutes using borate buffer 200mmol/L and FMOC-CL 500mg/L.

Determination of glyphosate by HPLC coupled with mass spectrometry (MS/MS)

An analytical method was developed for the determination of glyphosate and AMPA in sewage samples. The method involving an alkaline extraction and purification sludge of a sufficiently strong anion exchange resin. Thus, while the remaining analytes fixed by ionic interactions, derivatization was performed by FMOC-CL. For the process of extraction, solid phase extraction cartridges were used (SPE) Oasis HLB. The analysis by HPLC-MS/MS was performed in positive mode obtaining average values of 70% glyphosate and AMPA recovery. In the same study, they were obtained LD 20 and 35 and 30ppb 50ppb and LQ of glyphosate and AMPA respectivamente.27

Cabrices et al., 31 also used by SPE extraction cartridges. Their method was able to evaluate concentration levels of up to 10mg/kg showing excellent reproducibility and values within the ±20% range. Moreover, we used the extraction method Quick Polar Pesticides (QuPPE) that has been compared to the results of the recovery of non-QuPPE extraction. Overall recoveries were between QuPPE using 70-120% for both analytes (glyphosate and AMPA receptors) in the array. However, the extraction recovery using non-QuPPE extraction were lower in all cases. Baker et al.26 Determined glyphosate and other pesticide residues by HPLC-MS/MS without derivatization evaluating various chromatographic columns and mobile phases, and evaluate the MS/MS parameters. For primero method, a ZIC-HILIC chromatography column was used (100x2.1mm, 3.5μm) and mobile phase with ammonium formate in water 20mmol/L with 0.3% formic acid and acetonitrile to evaluate the pesticides amitrole, chlorimquat, diminodize, diquat, kasugamycin, meipiquat, paraquat and trimesium. The presented method was linear with R²>0.998 at a concentration range from 0.005 to 0.2mg/kg (5-200ppb). The second method used a CMP Hypercarb column (100mmx2.1mm, 5μm), mobile phase water with 1% acetic acid and methanol with 1% acetic acid to determine the pesticide glufosinate, glyphosate, ethephon, fosetyl aluminum, maleic hydrazide, perchlorate, ETU, OCT and nicotine. All analytes in the method 2 showed excellent linearity with R²>0.999.

Gas chromatography (GC)

Also widely used for the determination of glyphosate, the technique for quantification by GC requires derivatization usually involves mixing two compounds; trifluoroacetic anhydride (TFAA) and trifluoroethanol (TFE) in excess. These compounds are known to convert glyphosate to AMPA and only a single reaction step, which derivatives are sufficiently volatile for GC analysis / MS.30-40

Determination of glyphosate by GC coupled to mass spectrometry (MS/MS)

Papers involved Development and validation of two independent analytical principles for the quantification of glyphosate in breast milk samples. The aim was to determine the most sensitive. One of the methods used HPLC-MS/MS without derivatization. The second is based on derivatization with TFAA and heptafluorobutanol (HFB) followed by GC-MS/MS for the determination of glyphosate. The results of this study showed recoveries higher for the method HPLC-MS/MS who were about 91 to 94% (at concentrations of 1 and 5ng/ml glyphosate in matrix, respectively), whereas GC-MS/MS the recovery remained values in the range 83-84% at the same concentrations. The LQ for both methods was 1ng/ml. Besides that, the method presented by GC was much slower due to the need of additional steps for removal of excess derivatizing, making this method more complicated and exhaustive. Therefore, it is important to consider the derivatization or not an analyte. In another approach, a method was developed using the same aforementioned derivatizing which demonstrated a 0,05μg LQ / L and average recovery values of 93 and 95% for glyphosate and AMPA, respectively. The separation of the analytes was performed with a 30mmx0.25mm id column with film thickness 5% phenyl and 95% dimetilpolisiloxano.30

Determination of glyphosate by GC with detection by flame photometry (DFC)

We conducted studies for simultaneous determination of glyphosate, glufosinate and AMPA receptors in environmental and food samples. The extraction procedure was done with water or sodium hydroxide solution, converting these compounds into n-isopropoxycarbonyl. The method used a capillary column DB-1701. This method was applied to samples of the river water, soil and carrot. The recovery of these compounds in the samples was 91 106%.21

Conclusion

While there are several and distinct chromatographic techniques for the determination of glyphosate, it is essential to find the most appropriate considering the peculiarities that this pesticide presents. The most common and widely utilize technique is HPLC, although often require derivatization to improve the sensitivity and selectivity, including detectors for UV-vis and fluorescence. Furthermore, these methods have higher recovery values when compared to other methods.

It was also found that determinations by CG despite having good sensitivity could become more complex due to the need to derivatize glyphosate in order to make it volatile.

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Conflict of interest
The author declares no conflict of interest.

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