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Enzyme-linked immunoassay for the detection of glyphosate in food samples using avian antibodies

A. Arul Selvi a, M.A. Sreenivasa b and H.K. Manonmani a*

aFermentation Technology and Bioengineering Department, Central Food Technological Research Institute, Mysore 570 020, Karnataka, India; bFood Safety and Analytical Quality Control Laboratory, Central Food Technological Research Institute, Mysore 570 020, Karnataka, India

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A simple competitive immunoassay was developed for the measurement of glyphosate in food samples. It employed the avian antibodies (IgY) that recognised glyphosate as a capture reagent and glyphosate-alkaline phosphatase conjugate as an enzyme label. The assay depended on the competitive binding between the antibody and glyphosate derived from food samples for binding sites with immobilised glyphosate-OVA conjugate. The concentration of glyphosate in the food samples was quantified by the ability of the pesticide present in food samples to inhibit the binding of the enzyme conjugate to the antibody and subsequently the colour formation in the assay. The assay was specific to glyphosate with a limit of detection of 2 ppb. Mean analytical recovery of glyphosate in different food samples was 9.00–134.00%. The precision of the assay was satisfactory. The assay compared favourably with high-pressure liquid chromatography (HPLC) in its ability to accurately measure glyphosate in the food samples.

Keywords: glyphosate; avian antibodies (IgY); enzyme-linked immunosorbent assays; herbicide; food samples; high-pressure liquid chromatography; immunology; hapten

Introduction

Glyphosate [N-(phosphonomethyl) glycine] is a broad-spectrum, non-selective, systemic, post-emergence herbicide used both agriculturally and domestically (Mensink & Janssen, 1994). It is an active ingredient in a number of commercial herbicides produced by Monsanto, Cheminova and Zeneca Corp. Various formulations are scheduled for use that include products with the trade names Clear It, Expedite, Ezject, Glyfos, Laredo, Renegade, Roundup, Touchdown 480, Vision and Wrangler. Glyphosate is a member of the amino acid herbicide family, and its mode of action is through inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (EPSP), an enzyme of the shikimic acid pathway. This enzyme is important in the biosynthesis of the aromatic amino acids – phenylalanine, tyrosine and tryptophan. A blockage of the shikimic acid pathway leads to a depletion of the free pool of aromatic amino acids in higher plants (Duke, 1988). Glyphosate translocates readily in the plant, making it effective for controlling perennial weeds and overwintering...
rhizomes and tubers. It is registered for preplant or postharvest treatment in crops and on non-crop land (Omafra staff, 2002). The continued use of glyphosate indiscriminately by agriculturalists raises the potential for residue accumulation in food and water commodities. The maximum residue limit (MRL) concentration for food crops has been set at 1.0 \( \mu g/ml \) by the Indian Prevention of Food Adulteration act (PFA), the Canadian Food and Drug Act as well as the US Food and Drug Regulations (USFDA). Glyphosate is persistent in soil with a half-life of 47 days (Wauchope, Buttler, Hornsby, Augustijn Beckers, & Burt, 1992). In many countries, toxicology studies have been conducted to enable evaluation of the potential health risk (Williams, Kroes, & Munro, 2000). Glyphosate's laboratory testing has discovered some negative short-term and long-term health effects. Additionally, the glyphosate-containing product Roundup has been known to be used in suicide cases in Japan and consumption of it results in symptoms such as intestinal pain, vomiting, excess fluid in the lungs, pneumonia, clouding of consciousness and destruction of red blood cells. Short-term exposure to glyphosate can cause breathing difficulties, loss of muscle control and convulsions. Some glyphosate-containing products belong to Toxicity Category I and II (acutely toxic) and are toxic to animals and humans, and some symptoms include eye and skin irritation, headache, nausea, numbness, elevated blood pressure and heart palpitations (Cox, 2000). Although glyphosate is an acid, it is commonly used as the isopropylamine or trimethylsulfonium salts and is usually distributed as water-soluble concentrates and powders.

Glyphosate analysis in environmental matrices is problematic because it is a small molecule and has structural similarity to many naturally occurring plant materials such as amino acids and secondary plant compounds. It is highly soluble in water thereby making its extraction with solvents difficult. Therefore, glyphosate isolation and quantitation poses a challenge to the analytical chemist due to the necessity of removing matrix effects before analysis. In the last few years, many techniques have been tested for analysis of glyphosate, including ion chromatography with UV detection by Bedry and Parrot (1998), fluorescence detection (Mallat & Barcelo, 1998), conductivity detection (Zhu, Zhang, Tong, & Liu, 1999) or mass spectrometric detection (Bauer, Knepper, Maes, Schatz, & Voihsel, 1999). These methods are not, however, sufficiently sensitive to reach the quality threshold for drinking water. Gas chromatography continues to be of interest, although it suffers from problems such as tedious sample preparation to clean-up and convert the molecules into volatile derivatives (Borjesson & Torstensson, 2000). Another technique investigated is high-pressure liquid chromatography (HPLC) with post-column derivatisation with o-phthalaldehyde-mercaptoethanol and fluorescence detection (Reupert & Schlett, 1997; Winfield, 1990). The most difficult part of the analysis is, in fact, the extraction of the compounds from food materials, so derivatisation of glyphosate is usually conducted in this medium. The detection achieved using these methods is generally at higher concentrations than those typically obtained using enzyme-linked immunosorbent assays (ELISA) for most herbicides (Rubio et al., 2003).

ELISA is therefore said to be a valuable tool in residue analysis and complements conventional analytical methods (Johnson & Hall, 1996; Parnell & Hall, 1998). ELISA provides rapid sample testing and accurate results and is more cost-effective than conventional chromatographic analysis (Hall, Deschamps, & McDermott, 1990). ELISA has been used successfully for the quantitative analysis of numerous
pesticides in food matrices with little or no matrix interference (Lawruk et al., 1994; Rubio, Itak, Scutellaro, Selisker, & Herzog, 1991).

Previous publication on the indirect immunosorbent assay for the detection and quantitation of glyphosate described by Clegg, Stephenson, and Hall (1999) using mammalian antibodies (IgG) showed the limit of detection of 0.076 μg mL⁻¹ which indicates less sensitivity in comparison with the present studies which showed the detection limit of 2 ng mL⁻¹ using avian antibodies (IgY). This implies that the hen’s antibodies (IgY) in particular have clear advantages over mammalian immunoglobulins (Schade, Behn, Erhard, Hlinak, & Staak, 2001).

In this paper, we report the quantitative performance of ELISA for glyphosate detection and quantitation in environmental food samples using avian antibodies.

**Material and methods**

**Chemicals and reagents**

Bovine serum albumin (BSA), Freund’s complete adjuvant, Freund’s incomplete adjuvant, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), Ovalbumin (OVA), 2,4,6-Trinitrobenzenesulfonic acid (TNBS) and other pesticides standards were purchased from Sigma-Aldrich Chemical Company (Saint Louis, MO, USA). The analytical standard of glyphosate (99%) was obtained from Monsanto Co (St. Louis, MO, USA). Rabbit anti-chicken IgY conjugated to alkaline phosphatase (ALP), was purchased from Bangalore Genei (Bangalore, India). Microtiter plates were purchased from NUNC (Roskilde, Denmark). ELISA plates were analysed using a VERSAmax tunable microplate reader (Silicon valley, CA, USA). Dialysis cassettes were purchased from Thermo Scientific (Rockford, IL, USA). All the other chemicals used were of analytical grade and purchased from standard chemical companies. Food samples such as turmeric, chili, tea samples from different regions, coffee, coriander, ginger and rice were purchased from local market.

The hapten, OVA-pesticide conjugate and anti-glyphosate antibody for glyphosate assay were produced at CFTRI, Mysore, using 22-week-old single comb white leg horn poultry that were purchased from Kateel Poultry Farm (Mysore, India).

**Synthesis of pesticide–protein–hapten conjugates**

The synthetic route for the preparation of conjugate was devised. The glyphosate–BSA immunogen was prepared by dissolving the acid form of glyphosate (0.3 mM) and 100 mg of BSA in 10.0 mL of 0.1 M phosphate-buffered saline (PBS). This solution was mixed with 130 mg of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and allowed to react for 2 h at room temperature on a magnetic stirrer. The solution containing the glyphosate–protein (gly-BSA) conjugates was dialysed extensively using the dialysing cassettes (10K MW cutoff) against distilled water for 24 h at 4°C. The extent of conjugation was determined by using TNBS method (Plapp, Moore, & Stein, 1971). Coating conjugate of glyphosate (gly-OVA) was synthesised by conjugating glyphosate to ovalbumin (OVA). The conjugates thus obtained were lyophilised and stored at −20°C till further use.
**Estimation of the level of conjugation**

The TNBS reaction was carried out as follows. 200 µL of 0.6 M sodium borate buffer, pH 9.5, was mixed with 200 µL of a solution of conjugate in 0.05 M sodium acetate buffer, pH 4.7 and 50 µL of 0.2 M NaOH. The reaction was initiated by the addition of 50 µl of a freshly prepared solution of TNBS (7.2 mg mL⁻¹ of water). The reaction mixtures were kept at 25°C in dark for 60 min and then the absorbance at 367 nm was measured.

**Immunisation of poultry**

Twenty-two-week old poultry were injected intramuscularly, with 1 mg of immunogen (Gly-BSA conjugate) in 0.5 mL of a 50 mM PBS, pH 7.5 and 0.5 mL Freund’s complete adjuvant. The secondary immunisations were repeated once in 15 days for 6 months with 1 mg of immunogen in 0.5 mL of a 50 mM PBS, pH 7.5 and 0.5 mL Freund’s incomplete adjuvant. The eggs were collected everyday for the isolation of antibodies.

**Anti-glyphosate antibody isolation**

Yolk was separated from egg white and suspended in PBS. The egg yolk was broken and made into uniform suspension by mixing well in a magnetic stirrer for 10 min. Mixing was continued for 30 more minutes with the addition of chloroform at room temperature. The mixture was centrifuged at 10,000 g, 4°C for 10 min. The supernatant was decanted and Polyethylene Glycol 6000 was added at 14% (W/V) level. Mixing was continued for 30 min at room temperature. Antibody was separated by centrifuging at 10,000 g, 4°C for 10 min. The precipitate (IgY) obtained was dissolved in required quantity of PBS and stored at −20°C (Schade et al., 2001).

**Immunoassay procedure**

Glyphosate-specific antibody titres were monitored as described by Campbell, Burdon, and Knippenberg (1984) and Gee, Miyamoto, Goodrow, Buster, and Hammock (1988). The protein concentration of IgY was determined by Bradford method (1976). The optimum antibody concentration for coating onto the microwell plates and the best working concentration of the enzyme conjugate were determined by checkerboard titration. The coating conjugate (glyphosate–OVA conjugate) was diluted in carbonate buffer (50 mM, pH 9.6) at concentrations of 3.125 mg mL⁻¹ through 625 pg mL⁻¹/ml and added to the wells of the microwell plates (100 µL). The plates were incubated at 4°C overnight and were washed with 0.1% Tween 20 in PBS (137 mM NaCl, 3 mM KCl and 10 mM sodium phosphate, pH 7.4) (PBS-T), and the wells were blocked with 0.2% gelatine for 1 h. After a wash with PBS-T, anti-glyphosate antibody (IgY) diluted in carbonate buffer at concentrations of 34.9 mg mL⁻¹ through 69.5 ng mL⁻¹ was coated onto microwell plates (100 µL). The plates were incubated for 1 h at 37°C. After a thorough wash in PBS-T, anti-chicken rabbit antibody (1: 10,000) conjugated with ALP was added (100 µL). After 1 h at 37°C, the plates were washed well in PBS-T. Colour was developed using p-nitrophenyl phosphate (pNPP) (1 mg mL⁻¹) in 1% diethanolamine buffer, pH 9.8 (150 µL). The
reaction was stopped by the addition of 50 µL of 3 M NaOH. The absorbance was measured in an ELISA reader at 405 nm. Concentrations of antibody and enzyme conjugate that yielded a signal between 0.8 and 1.2 absorbance units were used for further testing of the competitive immunoassay procedures.

**Optimisation of glyphosate concentration by ELISA**

Coating conjugate was loaded to microwell titre plates (100 µL/well) and allowed to incubate overnight at 4°C. The plates were washed well with PBS-T and patted dry on paper towels. Sites not containing coating conjugate were blocked with 100 µL/well of 0.2% gelatin. After 60 min incubation, the plates were washed and dried as previously described. Glyphosate standards or samples containing glyphosate were prepared in carbonate buffer. Anti-glyphosate antibody and sample or standard solutions were mixed 1:1 (v/v) and allowed to incubate in test tubes for 60 min. The pre-incubated mixtures were transferred to the plates (100 µL/well). The plates were incubated for another 60 min at room temperature before being washed with PBS-T. The secondary antibody tagged with ALP was added to each well (100 µL) and incubated at 37°C for 60 min. The wells were then washed as described earlier. About 150 µL of pNPP in diethanolamine buffer was added and incubated for 30 min. Optical density (OD) at 405 nm was recorded after stopping the reaction with 50 µL of 3 M NaOH.

**Standard curve**

The absorbance at 405 nm is inversely proportional to the concentration of glyphosate in the standards and samples. To normalise the absorbance, the background absorbance was subtracted from each value followed by division of each value by the positive control (0 ng ml⁻¹ glyphosate). The standard curves were constructed by plotting the normalised absorbance values (A/A₀) against the log values of the glyphosate concentration. Glyphosate concentrations in the food samples were interpolated from the standard curve.

**Cross-reactivity**

Few pesticides were tested for cross-reactivity to the anti-glyphosate antibody. Glyphosine [N, N-bis (phosphonomethyl) glycine], a related herbicide, and few structurally related smaller molecules were tested for their cross-reactivity. A 1000 µg mL⁻¹ standard of each test chemicals was prepared using the carbonate buffer and tested against the anti-glyphosate antibodies. The IC₅₀ value, per cent cross-reactivity and least detectable dose for each of the compounds were determined.

**High-pressure liquid chromatography (HPLC) analysis**

Sample preparation was carried out by weighing one gram of food sample into a 50 mL polypropylene centrifuge tube with stopper. About 10 mL of ultrapure water and 5 ml dichloromethane were added. The tube was sonicated for 10 min and shaken thoroughly for 1 min that was followed again by 10 min of sonication. After that the tube was centrifuged for 10 min at 8000 g. The upper water phase,
approximately 9 mL, was transferred to another polypropylene tube. Evaporation to dryness with stream of N\textsubscript{2} at 55\degree C on water bath was followed, and the sample was redissolved in 1 mL of carbonate buffer prepared using ultrapure distilled water.

Glyphosate was detected by HPLC using a cation exchange analytical column. The compound was hydrolysed at 36\degree C (Clegg & Ripley, 1996) with sodium hypochlorite to form glycine. The glycine was then reacted with o-phthalaldehyde (OPA) in the presence of mercaptoethanol at 55\degree C to produce a highly fluorescent isoindole, which was detected fluorometrically (excitation 330 nm, emission 465 nm). All glyphosate standards were prepared in distilled water and serially diluted in potassium dihydrogen phosphate buffer to the concentration range required for analysis. A volume of 10 \mu L of standard/sample was injected into the liquid chromatograph, which had a mobile phase of potassium dihydrogen phosphate buffer (0.005 M) with a flow rate set at 1.0 ml/min. The total chromatographic run was 30 min. The mobile phase of potassium dihydrogen phosphate buffer was isocratic for 17 min, column regenerant (potassium hydroxide) for 2 min and finishing the chromatographic run with an 11 min continuation of potassium dihydrogen phosphate buffer mobile phase. Peak areas of the standards were plotted against the concentration of glyphosate, and the resulting standard curve was used to interpolate glyphosate concentrations in the food samples.

**Results and discussion**

This study describes a sensitive enzyme immunoassay that quantifies glyphosate in food samples using avian antibodies. The food sample containing glyphosate competes with anti-glyphosate IgY which will be recognised by immobilised glyphosate–OVA antigen (coating conjugate) in microwell plates. After removal of unbound reagents, the amount of antibody bound to the immobilised glyphosate–OVA conjugate was determined by using a chromogenic substrate. The concentration of glyphosate in a food sample was quantified by the ability of the glyphosate in food material to inhibit binding of anti-glyphosate antibody to the conjugate, and the colour development was inversely proportional to the concentration of glyphosate in the original sample.

It was observed that with the glyphosate–BSA conjugate, out of total 28 available lysine molecules in BSA, 19 lysine molecules were found to be bound to glyphosate molecule, i.e. the extent of conjugation was 68%.

**Optimum assay conditions**

The optimum concentrations of anti-glyphosate antibody required and the best working concentration of the conjugate required for coating on to the microwell plates were determined by checkerboard analysis. The analysis indicated that antigen (glyphosate–OVA conjugate) dilution of 1:2.5 lakhs with carbonate buffer (125 pg protein) and antibody dilution of 1:25 K (139 ng protein) gave the optimum readings (data not shown). These concentrations were chosen for further work.
Stability of anti-glyphosate antibody

Antibody stored at 4°C and −20°C was checked at different intervals of time for the amount of antibody activity. As shown in Figure 1, the antibody could be stored for nearly six months at 4°C. The loss in activity was 37% by the end of six months of storage period at 4°C. Thus the antibody (IgY) could be stored at 4°C with little activity loss up to six months. This reduces the cost of antibody production if stored properly. The loss in activity for antibody stored at −20°C was ~2% by the end of six months. Chicken egg yolk antibodies have been shown to have good activity up to 12 years (Schade et al., 2001) at −40°C. It is clear that avian antibodies have longer half-life than mammalian antibodies (IgG). These antibodies would be better choice to mammalian counterparts (Schade et al., 2001).

Calibration curves and sensitivity

Calibration curve was generated using glyphosate (99% pure) at concentrations from 2 to 1000 ng mL⁻¹, prepared in carbonate buffer (Figure 2). The sensitivity of the assay was determined by identifying the limit of detection defined as the lowest measurable concentration of glyphosate that could be distinguishable from zero concentration. On the basis of eight replicate measurements, the limit of detection was 2 ng mL⁻¹. The IC₅₀ value of this avian antibody was 36.30 ng mL⁻¹. In the similar studies by Clegg et al. (1999) using IgG, the limit of detection was 0.076 μg mL⁻¹ with an IC₅₀ value of 1.54 μg mL⁻¹ with anti-glyphosate rabbit antibody showing slightly less sensitivity.

Analytical performance of the assay

The glyphosate at different concentrations (2–250 ng mL⁻¹) spiked to different food samples was extracted using ultrapure water, and immunoassay was carried as
described in the experimental section. Each sample was analysed in triplicate for its glyphosate content. The limit of detection of glyphosate in different food matrices varied (Figure 3). The background effect of the glyphosate extract from turmeric, tea and rice was very less. With chili and coffee as substrates for spiking, interference could be observed. Ginger had tremendous interference with immunoassay. Linear regression analysis of the results yielded a linear equation:

\[ y = 10.059x - 14.094 \]
\[ R^2 = 0.09649 \]

Figure 2. Immunoassay with different concentrations of glyphosate. Notes: The concentrations of the pesticide standard quoted on the X-axis (ng mL\(^{-1}\)) were diluted using carbonate buffer. Data were mean of eight independent experiments \((n = 8)\).

described in the experimental section. Each sample was analysed in triplicate for its glyphosate content. The limit of detection of glyphosate in different food matrices varied (Figure 3). The background effect of the glyphosate extract from turmeric, tea and rice was very less. With chili and coffee as substrates for spiking, interference could be observed. Ginger had tremendous interference with immunoassay. Linear regression analysis of the results yielded a linear equation: \( y = 0.9681x + 0.3409 \), \( R^2 = 0.9998 \) for turmeric, \( y = 0.6618x + 3.2148 \), \( R^2 = 0.9954 \) for chili, \( y = 0.9792x - 0.6683 \), \( R^2 = 0.9997 \) for tea (Assam), \( y = 0.9825x - 0.1669 \), \( R^2 = 0.9996 \) for coriander, \( y = 0.9638x - 0.6991 \), \( R^2 = 0.9996 \) for coffee, \( y = 0.9816x - 0.1925 \), \( R^2 = 0.9998 \) for tea (Ooty), \( y = 0.9725x - 0.6264 \), \( R^2 = 1 \) for rice and \( y = 0.6733x + 1.734 \), \( R^2 = 0.9895 \) for ginger.

Figure 3. Immunoassay of glyphosate spiked to different food samples. Notes: Data points represent the mean of three independent experiments \((n = 3)\). The standard deviation in the mean is depicted by the error bars.
Specificity of the assay

The specificity of the assay for glyphosate was studied. The presence of other pesticides at concentrations of 1000 ng mL\(^{-1}\) level was tested. Figure 4 shows the cross-reactivity of different individual pesticides added in the assay. These data were based upon triplicate determinations. The presence of any of the herbicide did not interfere in the assay, i.e. the cross-reactivity was very low and it ranged between 1 and 5%.Glyphosine, a structurally related herbicide cross-reacted by 22% with anti-glyphosate antibody.

Comparison of immunoassay with high-pressure liquid chromatography (HPLC)

The mean analytical recovery was calculated as the ratio between the glyphosate concentration found and the concentration added, and expressed as percentage.

Glyphosate-spiked food samples at 2–250 ng mL\(^{-1}\) levels were checked by both immunoassay and HPLC as described in the experimental section. The comparisons of these results are shown in Table 1. The recovery of glyphosate by immunoassay (Table 1a) with turmeric as matrix was 85 and 95%, respectively, at 2 and 250 ng mL\(^{-1}\). Chili showed 88 and 60% recovery at 2 and 250 ng mL\(^{-1}\) level. This may be due to the matrix effect of the sample. Glyphosate in tea could be recovered at 74 and 82%, respectively, at 2 and 250 ng mL\(^{-1}\). Glyphosate spiked to coriander could be recovered by 53 and 80%, respectively, at 2 and 250 ng mL\(^{-1}\). Coffee-spiked glyphosate had very low recovery of 9 and 56%, respectively, at 2 and 250 ng mL\(^{-1}\). About 71 and 96% of the added glyphosate could be recovered from rice-spiked samples. Ginger had the lowest recovery of 11 and 5%, respectively, at 2 and 250 ng mL\(^{-1}\). This may be due to the interference of the matrix effect. The recovery of glyphosate by HPLC (Table 1b) in turmeric was 91 and 96% at 2 and 250 ng mL\(^{-1}\) levels, respectively. In chili, it was 61 and 66%, respectively, for 2 and 250 ng mL\(^{-1}\). In tea, coriander, coffee and rice, the recovery was almost complete. In ginger, only 52 and 66% of the added glyphosate could be recovered. The immunoassay method
compared well with HPLC, with few food materials indicating the ability of immunoassay to accurately determine glyphosate in certain food samples.

### Conclusion

A sensitive immunoassay for the determination of glyphosate in food samples has been developed. The assay was made possible by using avian antibodies with high-binding affinity for glyphosate. The matrix effect was found with few food samples. Microwells immobilised with antigen and blocked could be stored at 4°C. These microwells could be used for routine assay. The assay involved two incubation steps after which the plate was ready to generate the signal. The assay is very easy to perform in a microwell plate, the number of wells to be chosen being dependent on the number of samples. It is not time-consuming and an operator can analyse and

### Table 1a. Comparison of immunoassay with HPLC – immunoassay data.

<table>
<thead>
<tr>
<th>Immunoassay with food matrix (ng/ml)</th>
<th>Concentration of glyphosate (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Turmeric</td>
<td>1.70 ± 0.20</td>
</tr>
<tr>
<td>Chili</td>
<td>1.76 ± 1.74</td>
</tr>
<tr>
<td>Tea (Assam)</td>
<td>1.49 ± 2.14</td>
</tr>
<tr>
<td>Coriander</td>
<td>1.07 ± 0.52</td>
</tr>
<tr>
<td>Coffee</td>
<td>0.18 ± 0.22</td>
</tr>
<tr>
<td>Tea (Ooty)</td>
<td>1.01 ± 0.22</td>
</tr>
<tr>
<td>Rice</td>
<td>1.43 ± 3.20</td>
</tr>
<tr>
<td>Ginger</td>
<td>0.22 ± 1.50</td>
</tr>
</tbody>
</table>

Notes: Samples were collected as described in experimental sections. Values were a mean of triplicate determinations ± SD. Competitive immunoassays were performed as described in the experimental section.

### Table 1b. Comparison of immunoassay with HPLC – HPLC data.

<table>
<thead>
<tr>
<th>HPLC with food matrix (ng/ml)</th>
<th>Concentration of glyphosate (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Turmeric</td>
<td>1.83 ± 0.16</td>
</tr>
<tr>
<td>Chili</td>
<td>1.22 ± 3.20</td>
</tr>
<tr>
<td>Tea (Assam)</td>
<td>2.00 ± 0.22</td>
</tr>
<tr>
<td>Coriander</td>
<td>1.83 ± 1.50</td>
</tr>
<tr>
<td>Coffee</td>
<td>2.09 ± 2.10</td>
</tr>
<tr>
<td>Tea (Ooty)</td>
<td>1.91 ± 2.20</td>
</tr>
<tr>
<td>Rice</td>
<td>1.83 ± 1.70</td>
</tr>
<tr>
<td>Ginger</td>
<td>1.04 ± 0.31</td>
</tr>
</tbody>
</table>

Notes: Samples were collected as described in experimental sections. Values were a mean of triplicate determinations ± SD. Competitive immunoassays were performed as described in the experimental section.
obtain results in less than two hours. It is able to detect glyphosate in food samples at a concentration as low as 2 ng mL\(^{-1}\). The assay was comparable with HPLC and future studies will include the exploitation of this approach to other food samples in the presence of other contaminating pesticides. The stability of IgY being very good at \(-20^\circ C\), single batch preparation of antibodies could be used for a longer time.

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