International Journal of Mass Spectrometry 430 (2018) 104–109

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Short communication

Rapid on-line microextraction method for the analysis of glyphosate in soy and pepper based on fiber-spray/mass spectrometry

Yea-Wenn Liou[a], Sing-Han Wang[a], Chih-Han Hsu[a], Yu-Ting Hsu[a], Chung-Hsuan Chen[a], Chien-Chung Chen[b], Cheng-Huang Lin[a,*]

[a] Department of Chemistry, National Taiwan Normal University, 88 Sec. 4, Tingchow Road, Taipei, Taiwan
[b] Graduate Institute of Biomedical Materials and Tissue Engineering, Taipei Medical University, 250 Wu-Hsing St., Taipei, Taiwan

ARTICLE INFO

Article history:
Received 26 February 2018
Received in revised form 7 May 2018
Accepted 8 May 2018

Keywords:
Glyphosate
Porous-polypropylene hollow fiber
Liquid phase microextraction
Fiber-spray/mass spectrometry

ABSTRACT

We report on the design and testing of a rapid, on-line microextraction kit based on fiber-spray/mass spectrometry. The kit consists of a section of a porous-polypropylene hollow fiber, a serum bottle, a seal cap with a gasket and an electromagnetic stirrer. The kit is placed between the mass inlet and the ESI needle. Using this configuration, glyphosate can be extracted from a very dilute solution and then evaporates and escapes from the fiber surface. When glyphosate molecules make contact with the ESI plume, which arises from the ESI needle tip, they are ionized and then detected by a mass spectrometer. Using the setup, it was possible to improve the limit of detection for glyphosate after microextraction by ~1000-fold, resulting in a limit of detection of 10 ppb.

1. Introduction

Most consumers have only limited knowledge of the types of pesticides that have been sprayed in their foods before they consume them. Labeling pesticides contained in foods may be needed. Such regulations would require suppliers of fruits and vegetables to list the pesticides used in their production in clear labeling, which would represent a new civil issue. Glyphosate, since its debut in 1974 under the trade name Roundup, has been very effective in killing a wide variety of weeds. It is now one of the most widely used herbicides in the agricultural sector, horticulture, viticulture, and silviculture, as well as garden maintenance (including home use). However, it has recently been proposed that glyphosate might be associated with certain diseases, including autism by oxidative damage and neurotoxicity [1,2], Parkinson's disease [3–5], Alzheimer's disease [6] and various types of cancer [7–9]. Thus, a simple and straightforward method for detecting low concentration levels of glyphosate would be highly desirable. Liquid chromatography-mass spectrometry (LC–MS) is a reliable and one of the most popular methods for the analysis of herbicides, including glyphosate [10,11]. A variety of ionization methods, including desorption electrospray ionization [12], easy ambient sonic spray ionization [13,14], electrospray-assisted laser desorption ionization and paper spray-mass spectrometry have been developed for this purpose [15–18]. These methods, so-called ambient ionization mass spectrometry, are currently in widespread used, because they permit the time required for a mass-spectrum analysis to be greatly reduced.

In many countries, LC–MS is an official method for the determination of glyphosate and its major metabolite (aminomethylphosphonic acid, AMPA). In Taiwan, the legal limits of glyphosate in fruits and vegetables range from 10 to 0.1 ppm (soybean, 10 ppm; pepper, 0.1 ppm, respectively), as required by the Taiwan Food and Drug Administration. Off-line sample pretreatment methods are recommended in determining such low concentrations of glyphosate residues. However, such off-line sample pretreatment methods typically lower analytical throughput to a considerable extent and can be time consuming.

In this study, we report on the development of a novel type of on-line microextraction method for the determination of glyphosate using soybeans and pepper as model samples. The procedure involves combining an on-line concentration technique with ambient ionization mass spectrometry. A commercially available, porous-polypropylene hollow fiber was used to achieve this. Details of the procedures used for on-line microextraction and the resulting improvement in the limit of detection are reported.

* Corresponding author at: Department of Chemistry, National Taiwan Normal University, 88 Sec. 4, Tingchow Road, Taipei 11677, Taiwan.
E-mail address: chenglin@ntnu.edu.tw (C.-H. Lin).

https://doi.org/10.1016/j.ijms.2018.05.008
1387-3806/

2. Experimental

2.1. Reagents

Glyphosate standard (99%) and Roundup (41% glyphosate) were purchased from CHEM service (West Chester, USA) and the SINON Corporation (Taiwan), respectively. Analytical grade *n*-dodecane was obtained from ALFA Aesar (Heysham, England). Aliquat 336 and 1-octanol were purchased from Acros Organics (Geel, Belgium). Porous polypropylene hollow fibers (I.D., 0.6 mm; wall thickness, 0.2 mm; average pore size; 200 nm) were acquired from Membrana (Model, PPQ3/1; Wuppertal, Germany). The soybeans and a green pepper were purchased from a local supermarket.

2.2. Apparatus

The mass spectrometer (Finnigan LCQ Classic LC/MS/MS) used in this study was the same instrument that was used in our previous study [19,20]. The mass signal was recorded under the full scan mode (*m*/*z*, 50–1000) and an Xcalibur data system was used for data collection. The capillary temperature and spray voltage were set at 275 °C and ±5 kV, respectively. The tube lens offset and capillary voltage were set at −23 V and 25 V, respectively. Serum bottles (2 mL, glass) were purchased from Agilent. The electromagnetic stirrer (0.4 cm in length) and electromagnetic heating mixer (REXIM RS-4DN) were obtained from AS ONE. A microwave oven (SAMPO, RE-081M1) was obtained from a local shop. A pH meter (Horiba NaVi F-52), blender (WONDER WH-M01J) and centrifuge (EYELA CVE-1000), ultrasonic oscillator (Branson 3510) were also used in this experiment.

2.3. Sample preparation

The procedure for sample preparation was based on method TFDAP0006.00, as specified by the Food and Drug Administration (Taiwan) in 2017 (Method of Test for Pesticide Residues in Foods-Multiresidue Analysis of Polar Pesticides and their Metabolites).

2.3.1. Standard glyphosate solutions

A 0.1 g sample of the glyphosate standard was diluted in 100 mL of deionized water, which was then used to prepare a series of standard sample solutions to prepare calibration curves.

2.3.2. Roundup solution

A 0.083 g Roundup sample (glyphosate, 41%; in weight) was diluted in 34 mL of deionized water and served as a stock solution (1000 ppm).

2.3.3. Pepper stock solution

After adding 60 mL of deionized water to a blender, the green pepper (30 g) was added, and the resulting pepper sample was homogenized with the blender for 10 min. The upper layer (4 mL) was centrifuged (5 min) and the resulting supernatant filtered through a syringe filter (PTFE membrane, 0.22 μm pore size, 15 mm diameter). After filtering, the pH of this solution was adjusted to 12.0 by adding a NaOH solution (1 M).

2.3.4. Soybean solution

Using the same procedures as described above, 2 g of soybeans was processed in 20 mL deionized water. The upper layer (10 mL) was collected, centrifuged (5 min) and then filtered through a syringe filter. After filtering, the pH of this solution was adjusted to 12.0 by adding a NaOH solution (1 M).

2.4. On-line microextraction kit

The graphical abstract shows the on-line microextraction kit and the Taylor cone when an ESI experiment was performed. The left photo shows a SEM (scanning electron microscope) image of

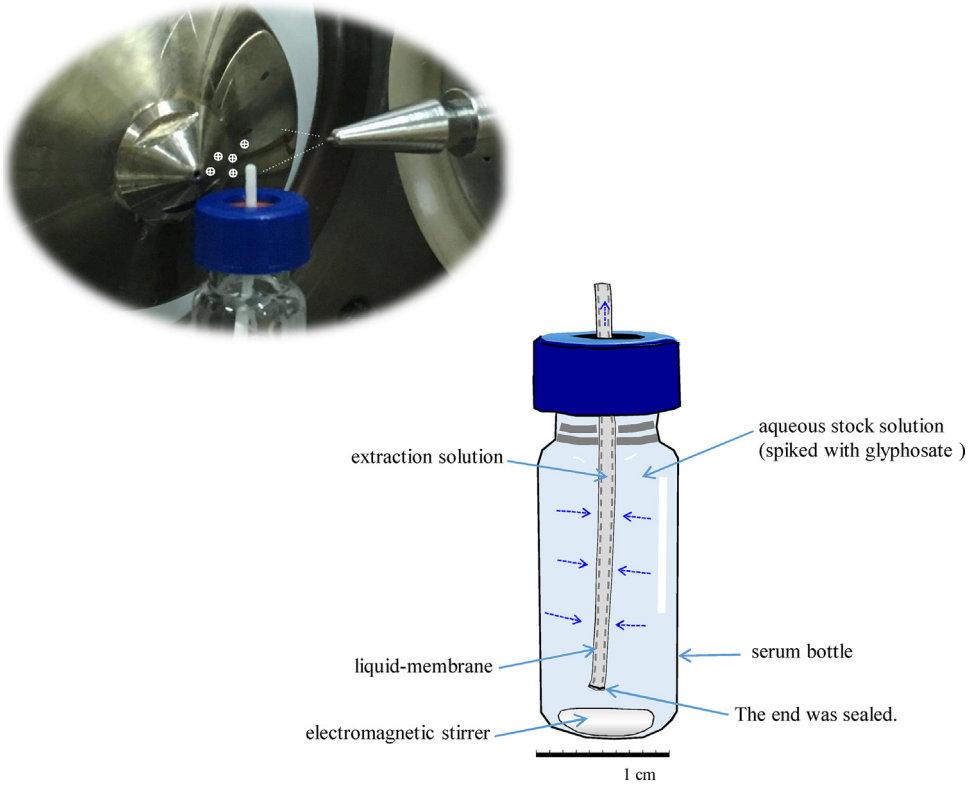

Fig. 1. Schematic diagram of the on-line microextraction kit. The insert shows the actual position of the kit, which is located between the ESI needle and the mass inlet.

top of the ends of the porous-polypropylene hollow fiber. Fig. 1 shows a schematic diagram of the on-line microextraction kit; the inset shows the actual position of the kit located between the ESI needle and the mass inlet. The kit consists of a section of a PP hollow fiber (3.5 cm in length), a serum bottle (glass, 2 mL), a seal cap with a gasket and an electromagnetic stirrer. The set up was initially modified by drilling a hole (1 mm, in diameter) in the gasket, and the PP hollow fiber was then passed through the hole, with the inside length maintained at 3.0 cm and an outside length of 0.5 cm. In order to prevent the extraction solvent from leaking, the end of the PP hollow fiber was sealed, using a needle-nose pliers. A 2 mL sample of a glyphosate solution was placed in the 2 mL serum bottle for extraction. In order to improve the efficiency of the liquid–liquid extraction, the pH of the solution was adjusted to 12.0 using a NaOH solution (1.0 M). The PP hollow fiber was ultrasonically cleaned in acetone for 5 min prior to use, and then air-dried by means of a syringe injector. A boundary solvent was prepared by mixing 0.4 mL of *1*-octanol, 1 mL of Aliquat 336 and 8.6 mL of *n*-dodecane [21,22] and the PP hollow fiber was then immersed in this mixed solvent. After several seconds, the inner mixed solvent was removed by blowing air through the interior of the fiber. Some of the mixed solvent was retained by the porous fiber-wall (liquid membrane). Following this, a 15 μL aliquot of an HCl solution (0.1 M) (the extraction solution) was injected into the lumen of the fiber. The liquid membrane plays a very important role, serving as a boundary between the sample solution and the extraction solution. Glyphosate is more soluble in acidic solutions than in alkaline solutions. Because of this, it readily moves into the acidic extraction solution. We compared the mass spectra from glyphosate solutions, which were prepared in 0.1 M HCl (extraction solution) and 0.1 M NaOH (sample solution), respectively. After microextraction process finished, the mass spectrum was the same as glyphosate in 0.1 M HCl. That means that glyphosate can be extracted from a very dilute solution using the so-called hollow fiber liquid phase microextraction process, and the resulting extract then moves to the outside portion of the fiber by diffusion. This process is very slow, usually requiring more than 1 h. However, in this study, we found that the process can be improved by simply stirring. For this reason, an electromagnetic stirrer was placed inside the bottle, as described below. The concentrated analytes are vaporized and escape, along with mixed solvent through the porous surface. Meanwhile, electro-sprayed/charged droplets are produced from a regular ESI stainless needle. Once the materials that evaporate from the surface of the hollow fiber (mostly neutral) meet the electrospray plume, ionization occurs under ambient conditions.

3. Results and discussion

Since the position of the outside-fiber is very important in terms of acquiring sufficient ions for detection, ionization efficiencies were investigated when the kit was placed in various positions. Fig. 2 shows the relationship between ion intensities and the location of the microextraction kit. In this case, glyphosate was used as the test sample (concentration, 10 ppm) and the ESI voltage was −5 kV. It is clear that the position, indicated in red, i.e. at vertical and horizontal distances by ~3 mm and 2 mm relative to the mass inlet, respectively, is the optimized location. Hence, this position was used in the following experiments. Fig. 3A shows a typical mass spectrum obtained by an ESI–MS method, using an ESI voltage of +5 kV with Roundup at a concentration of 10 ppm. The inset chemical structures illustrate the pathway for the possible fragmentation of glyphosate. In fact, products that contain Roundup typically contain at least three components, including the active ingredient, *i.e.* glyphosate, water and a surfactant blend. Glyphosate ionizes with difficulty under a positive voltage; the ionization efficiency of surfactants is much higher than that of glyphosate. As can be seen, the surfactants produce complicated signals, making the signal corresponding to glyphosate difficult to observe. After expanding the mass region (*m*/*z*, 120–200), a tiny signal was found at *m*/*z* = 192, which corresponds to $[M+Na]^+$. This information is not sufficient to permit glyphosate to be identified. When the ESI mode was operated under a negative voltage, a mass peak, corresponding to $[M\text{-}H]^-$ was found. Furthermore, no interference by surfactants was observed (data not shown). When an acidic solution of glyphosate (0.1 M HCl) was examined, a clear mass signal was found at *m*/*z*, 125, i.e. $[M-PO_3+Cl]^-$, as shown in Fig. 3B. We selected this peak for the identification of glyphosate. Fig. 4A shows the relationship between ion-intensity and microextraction time, when glyphosate was used as the model sample (concentration level, 1 ppm; pH, 12). Herein, 6 containers of sample solution were prepared and processed for extraction (without stirring) at room temperature during 15, 30, 45, 60, 75 and 90 min, respectively. The results clearly show that the optimized extraction time was 60 min. Longer extraction time did not improve the results. This is because during extraction process, the concentrated analytes evaporate and escape along with the mixed solvent through the porous surface. The two effects (evaporation/concentration) cancel each other out, and as a result, an optimized timing was found. In order to shorten the extraction time, an electromagnetic stirrer was used; the stirring rates were set at 300, 400, 500, 600, 700, 800 rpm, respectively, for 10 min in each case. The findings indicated that the optimized rate was 600 rpm. Following this, 5 containers of sample solution were prepared and the stirring rate was set at 600 rpm for extraction at room temperature with stirring times of 5, 10, 15, 20 and 25 min, respectively. As can be seen in Fig. 4B, the rapid, on-line microextraction of glyphosate was achieved. The extraction time can be shortened to as short as 15 min. In order to further shorten the extraction time, a microwave assisted microextraction method was investigated. Under the same conditions, several different microwave-extraction times (10, 20 and 30 s) were examined. Using a microwave oven operating at 160 W, the extraction process can be completed within 20 s. However, when the time was increased to 30 s, the extracted solution began to boil, resulting in a decrease in ion intensity. Although the intensity obtained from stirring method was almost two-fold that for the microwave method (~3.1×10^5), we also conclude that

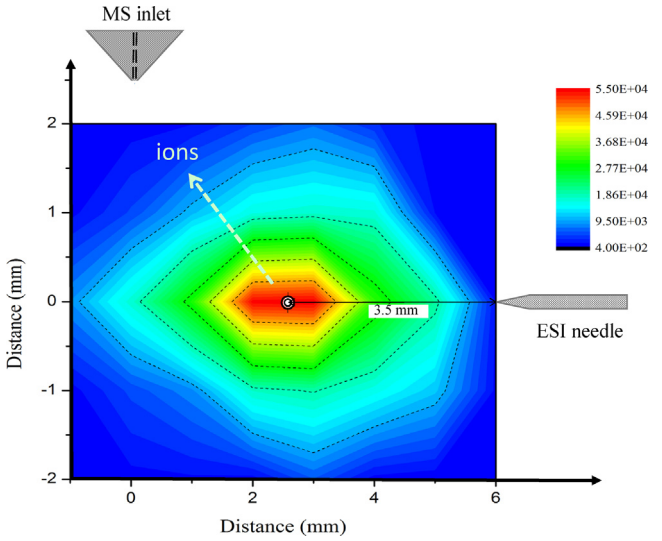

Fig. 2. Relationship between ion intensities and the location of the microextraction kit. It is clear that the position, indicated in red, is the optimized location. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

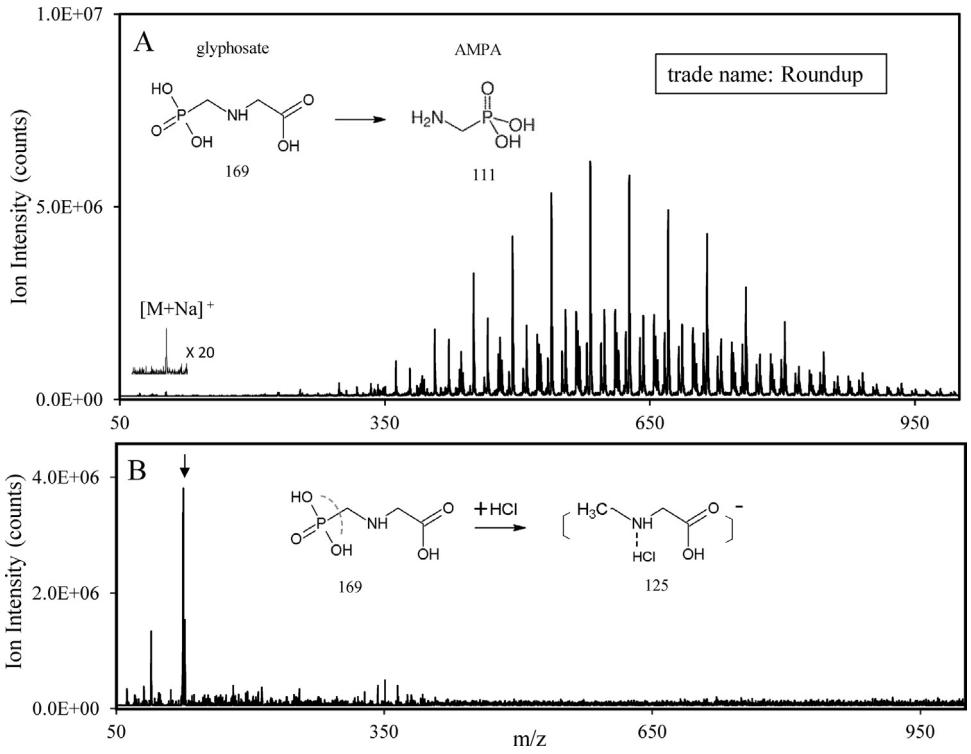

Fig. 3. Frame A, typical mass spectrum obtained by an ESI–MS method, in which the ESI voltage was +5 kV; the concentration level of Roundup was 10 ppm. Frame B, mass spectrum obtained by a fiber spray/mass spectrometry, in which the ESI voltage was − 5 kV and the pH value was adjusted to 12.0.

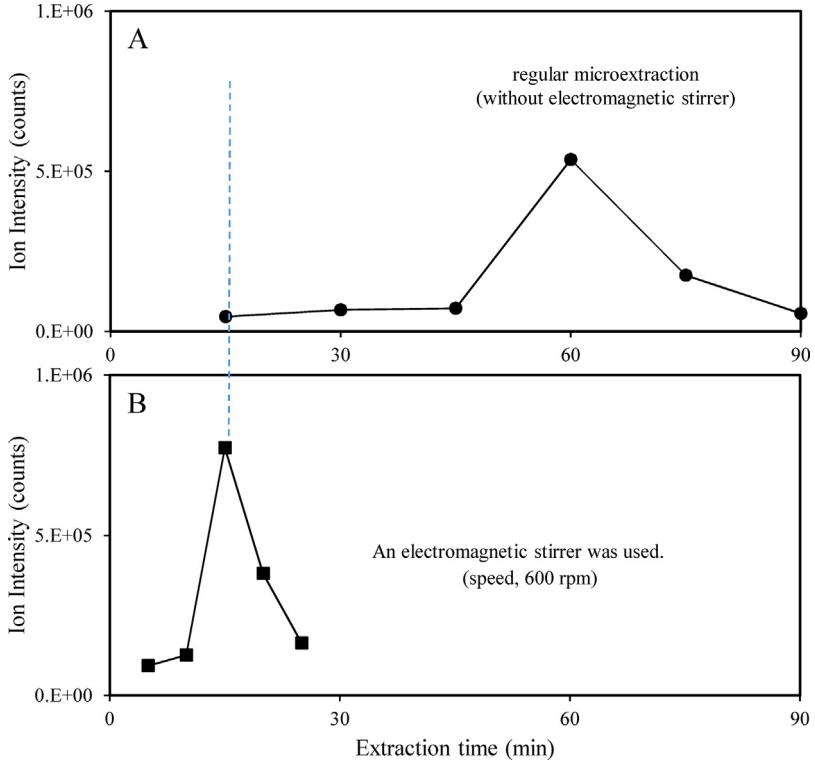

Fig. 4. Relationship between ion-intensity and microextraction time when glyphosate was used as the model sample (frame A, without electromagnetic stirrer; frame B, electromagnetic stirrer was used at 600 rpm).

the microwave-extraction method could still be further improved. For convenience, we selected the stirring method for the following experiments, *i.e.* extracting glyphosate from pepper (legal limit, 0.1 ppm) and soy (legal limit, 10 ppm), respectively. Fig. 5A-a shows the mass spectrum obtained from the green pepper stock solution (before adding glyphosate) by an ESI–MS method, (ESI voltage, − 5 kV), where the sample solution was directly injected using a syringe injector. As can be seen, some peaks including Vitamin

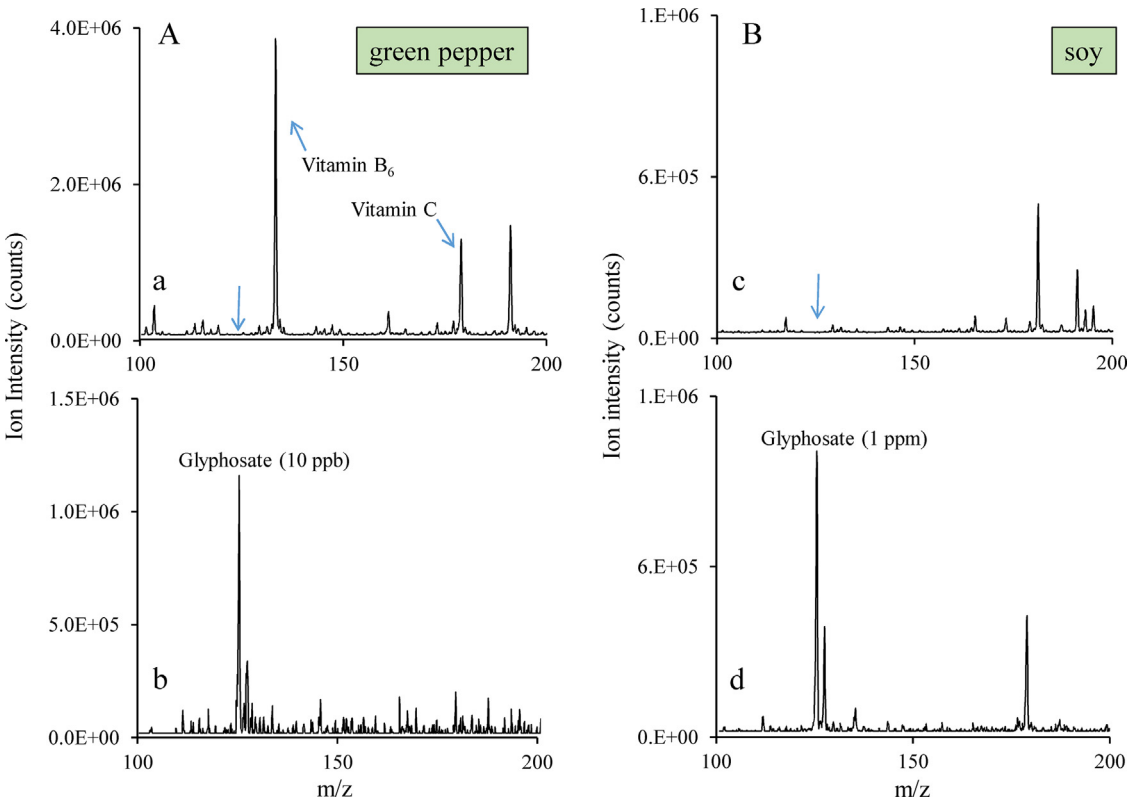

Fig. 5. Typical mass spectra obtained using an ESI–MS method (spectra a and c) and on-line micro extraction fiber-spray/mass spectrometry (spectra b and d), respectively. Frame A shows the results obtained from the green pepper stock solution before (spectrum a) and after (spectrum b) spiking with glyphosate (10 ppb); frame B shows the results obtained from the soybean stock solution before (spectrum c) and after (spectrum d) spiking with glyphosate (1 ppm), respectively.

B_6 and Vitamin C, but none correspond to glyphosate. We conclude from this that this green pepper was not contaminated with glyphosate. After spiking the green pepper stock solution with glyphosate (10 ppb), same microextraction procedure was performed (stirring rate, 600 rpm; extraction time 15 min.). Fig. 5A-b shows the mass spectrum obtained from the green pepper stock solution after spiking. The spectrum now contains a major peak corresponding to glyphosate. Hence, the method developed in this study is very useful for the detection of glyphosate. The limit of detection (10 ppb) was one order lower than the legal limit (0.1 ppm). Fig. 5B-c and −d show the mass spectra obtained from the soybean sample. Since the legal limit of glyphosate in soybean is 10 ppm, herein, the concentration level of glyphosate in spectrum d was 1 ppm. We also detected a major peak belonging to glyphosate. It should be noted that the intensity of the peak at m/z 125 is not only reflecting the concentrations of glyphosate, but also reflecting the extraction efficiencies of glyphosate in the two different samples. The soybean sample solution is denser than pepper sample solution, resulting to poorer extraction efficiency, resulting different limit of detection of glyphosate in different sample. It should be noted that the types of extraction solvents, pH values of the sample solution and extraction times are all important variables and all of these parameters should be optimized. Compared to the data obtained using standard glyphosate solutions, we therefore conclude that, in this case, the ion intensity can be improved by ~1000-fold by using on-line microextraction. The limit of detection was about 10 ppb for glyphosate. A combination of on-line microextraction using a hollow fiber with fiber spray-mass spectrometry was developed in this study, and the results indicate that it represents a potentially useful new methodology for use in the field of mass spectrometric analysis.

4. Conclusions

The development of an on-line concentration technique is described. A fiber-spray ionization source that involves the use of a liquid-phase microextraction hollow fiber with a regular electrospray source was developed and tested. The methodology is simple and economical, and is suitable for use in low-level drug screening. While the results show that the methodology is feasible, extensive work needs to be done to verify that the method can be effectively used for drug screening in biofluids. Further applications are currently being explored.

Acknowledgment

This work was supported by grants from the Ministry of Science and Technology (Taiwan) under Contract 106-2113-M-003-009.

References

[1] E.Y. Hsiao, S.W. McBride, S. Hsien, G. Sharon, E.R. Hyde, T. McCue, J.A. Codelli, J. Chow, S.E. Reisman, J.F. Petrosino, P.H. Patterson, S.K. Mazmanian, The microbiota modulates gut physiology and behavioral abnormalities associated with autism, Cell 155 (2013) 1451–1463.
[2] D. Cattani, V.L. de L. O. Cavalli, C.E.H. Rieg, J.T. Domingues, T. Dal-Cim, C.I. Tasca, F.R.M.B. Silva, A. Zamoner, Mechanisms underlying the neurotoxicity induced by glyphosate-based herbicide in immature rat hippocampus: involvement of glutamate excitotoxicity, Toxicology 320 (2014) 34–45.
[3] T.R. Sampson, J.W. Debelius, T. Thron, S. Janssen, G.G. Shastri, Z.E. Ilhan, C. Challis, C.E. Schretter, S. Rocha, V. Gradinaru, M.F. Chesselet, A. Keshavarzian, K.M. Shannon, R. Krajmalnik-Brown, P. Wittung-Stafshede, R. Knight, S.K. Mazmanian, Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease, Cell 167 (2016) 1469–1480.
[4] E.R. Barbosa, M.D. Leiros da Costa, L.A. Bacheschi, M. Schff, C.C. Leite, Parkinsonism after glycin-derivate exposure, Mov. Disord. 16 (2001) 565–568.

[5] A. Moretto, C. Colosio, Biochemical and toxicological evidence of neurological effects of pesticides: the example of Parkinson's disease, Neurotoxicology 32 (2011) 383–391.
[6] D. Yan, Y. Zhang, L. Liu, H. Yan, Pesticide exposure and risk of Alzheimer's disease: a systematic review and meta-analysis, Sci. Rep. 6 (2016) 32222.
[7] G.E. Seralini, E. Clair, R. Mesnage, S. Gress, N. Defarge, M. Malatesta, D. Hennequin, J.S. de Vendomois, Long term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize, Food Chem. Toxicol. 50 (2012) 4221–4231.
[8] G.E. Seralini, E. Clair, R. Mesnage, S. Gress, N. Defarge, M. Malatesta, D. Hennequin, J.S. de Vendomois, Retraction notice to Long term toxicity of a Roundup herbicide and a roundup-tolerant genetically modified maize [Food Chem. Toxicol. 50(2012) 4221–4231], Food Chem. Toxicol. 63 (2014) 244.
[9] S. Thongprakaisang, A. Thiantanawat, N. Rangkadilok, T. Suriyo, J. Satayavivad, Glyphosate induces human breast cancer cells growth via estrogen receptors, Food Chem. Toxicol. 59 (2013) 129–136.
[10] M.X. Chen, Z.Y. Cao, Y. Jiang, Z.W. Zhu, Direct determination of glyphosate and its major metabolite, aminomethylphosphonic acid, in fruits and vegetables by mixed-mode hydrophilic interaction/weak anion-exchange liquid chromatography coupled with electrospray tandem mass spectrometry, J. Chromatogr. A 1272 (2013) 90–99.
[11] H.A. Martins-Junior, D.T. Lebre, A.Y. Wang, M.A.F. Pires, O.V. Bustillos, Residue analysis of glyphosate and aminomethylphosphonic acid (AMPA) in soybean using liquid chromatography coupled with tandem mass spectrometry, soybean-biochemistry, Chem. Physiol. 27 (2016) 495–506.
[12] Z. Tak'ts, J.M. Wiseman, B. Gologan, R.G. Cooks, Mass spectrometry sampling under ambient conditions with desorption electrospray ionization, Science 306 (2004) 471–473.
[13] R. Haddad, R. Sparrapan, T. Kotiaho, M.N. Eberlin, Easy ambient sonic-spray ionization-membrane interface mass spectrometry for direct analysis of solution constituents, Anal. Chem. 80 (2008) 898–903.
[14] E.T. Jansson, M.T. Dulay, R.N. Zare, Monitoring enzymatic reactions in real time using venturi easy ambient sonic-spray ionization mass spectrometry, Anal. Chem. 88 (2016) 6195–6198.
[15] C.Y. Cheng, C.H. Yuan, S.C. Cheng, M.Z. Huang, H.C. Chang, T.L. Cheng, C.S. Yeh, J. Shiea, Electrospray-assisted laser desorption/ionization mass spectrometry for continuously monitoring the states of ongoing chemical reactions in organic or aqueous solution under ambient conditions, Anal. Chem. 80 (2008) 7699–7705.
[16] X.C. Lin, X.N. Wang, L. Liu, Q. Wen, R.Q. Yu, J.H. Jiang, Surface enhanced laser desorption ionization of phospholipids on gold nanoparticles for mass spectrometric immunoassay, Anal. Chem. 88 (2016) 9881–9884.
[17] S.Y. Lin, M.Z. Huang, H.C. Chang, J. Shiea, Using electrospray-assisted laser desorption/ionization mass spectrometry to characterize organic compounds separated on thin-layer chromatography plates, Anal. Chem. 79 (2007) 8789–8795.
[18] J. Liu, H. Wang, N.E. Manicke, J.M. Lin, R.G. Cooks, Z. Ouyang, Development characterization, and application of paper spray ionization, Anal. Chem. 82 (2010) 2463–2471.
[19] Y.W. Liou, J.S. Wang, C.C. Chen, C.H. Lin, Development of an on-line microextraction method for use in fiber-spray/mass spectrometry, Int. J. Mass spectrom. 421 (2017) 178–183.
[20] Y.W. Liou, K.Y. Chang, Cheng-Huang Lin, Sampling and profiling caffeine and its metabolites from an eyelid using a watercolor pen based on electrospray ionization/mass spectrometry, Int. J. Mass spectrom. 422 (2017) 51–55.
[21] C. Rios, V. Salvadó, M. Hidalgo, Facilitated transport and preconcentration of the herbicide glyphosate and its metabolite AMPA through a solid supported liquid-membrane, J. Membr. Sci. 203 (2002) 201–208.
[22] L. Goodwin, J.R. Startin, D.M. Goodall, B.J. Keely, Negative ion electrospray mass spectrometry of aminomethylphosphonic acid and glyphosate: elucidation of fragmentation mechanisms by multistage mass spectrometry incorporating in-source deuterium labeling, Rapid Commum. Mass Spectrom. 18 (2004) 37–43.