American Journal of Analytical Chemistry, **2013, 4, 647-652**
Published Online November 2013 (http://www.scirp.org/journal/ajac)
http://dx.doi.org/10.4236/ajac.2013.411077

Study of the Reaction Derivatization Glyphosate and Aminomethylphosphonic Acid (AMPA) with *N*,O-Bis(trimethylsilyl)trifluoroacetamide

Tereza Cristina Pimenta Gonçalves Catrinck[1], Maria Clara Santana Aguiar[2], Amanda Dias[2], Flaviano Oliveira Silvério[2], Paulo Henrique Fidêncio[1*], Gevany Paulino de Pinho[2]
[1]Department of Chemistry, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina, Brazil
[2]Institute of Agricultural Sciences, Universidade Federal de Minas Gerais, Montes Claros, Brazil
Email: *paulo.fidencio@ufvjm.edu.br

Received September 15, 2013; revised October 25, 2013; accepted November 5, 2013

ABSTRACT

This work aimed to study the derivatization unprecedented of glyphosate and AMPA solutions using *N*,O-bis (trimethylsilyl)trifluoroacetamide (BSTFA) combined with trimethylchlorosilane (TMCS), evaluating the composition of the reaction medium, use of ultrasound, volume of BSTFA:pyridine and pH of the reaction medium. From this study it was inferred that the reaction medium was composed of BSTFA:pyridine in ratio 60:100, respectively, without ultrasonic vibration and pH adjustment that provide optimal conditions for analysis by GC-MS. Furthermore, the methodology used was simple and fast, and that was the most practical method commonly used.

Keywords: Glyphosate; GC-MS; Derivatization; BSTFA

1. Introduction

Chemical control of weeds was adopted in the second half of the twentieth century leading to a significant development in the industry of herbicides [1]. Among these substances, glyphosate (*N*-phosphonomethylglycine) has been widely used due to its excellent performance and effective pest control [2]. This compound is presented as a polar molecule, post-emergent, non-selective and systemic action [3]. It may be degraded by two catabolic routes (**Figure 1**), producing aminomethylphosphonic acid (AMPA) as the major metabolite and sarcosine as an intermediary in the alternative route [4].

Glyphosate (GLY) has been worldwide used in different cultures, however, their potential toxicological risks to human health [5] and environmental pollution [6] have demonstrated the need to develop simple methodologies, fast and sensitive to monitor GLY residues and their metabolites in the environment [5]. Some techniques have been used, including high performance liquid chromatography (HPLC) [6,7], capillary electrophoresis (CE) [8] and spectrophotometry in the visible region.

Among the proposed techniques, the gas chromatography is frequently used due to their high selectivity and sensitivity [9]. However, the low volatility of GLY and AMPA molecules makes the determination of these analytes difficult [10], requiring the use of derivatization techniques (pre- or post-column) [8].

The derivatization procedure consists in chemically modifying a compound, to increase the sensitivity and/or make it volatilizable [11]. In the analysis by gas chroma-

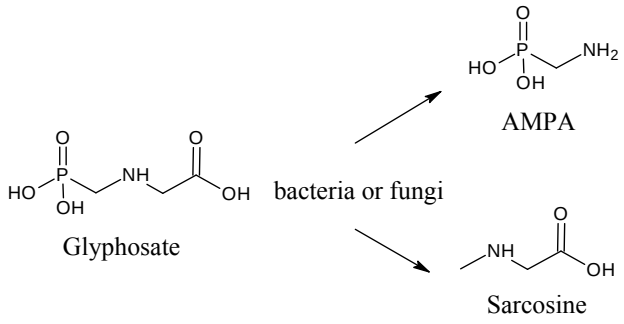

Figure 1. Degradation scheme of glyphosate with the production of aminomethylphosphonic acid (AMPA) and sarcosine [4].

*Corresponding author.

tography, substances containing functional groups-OH and -NH, as the GLY and AMPA may form hydrogen bonds with each other and/or matrix components, making their volatilization difficult [11]. Thus, some reagents may be used to reduce the polarity of the compound replacing labile hydrogens by aliphatic groups [11]. Among the reagents, trifluoroacetic anhydride (TFAA), trifluoroethanol (TFE) [12] and *N*-methyl-*N*-tert-butyldimethylsilylfluoracetamide (MTBSTFA) [13] have been used for the derivatization of the GLY.

The *N*,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) combined with trimethylchlorosilane (TMCS) is preferentially employed to promote trimetylsylation of alcohols, amines, carboxylic acids, among others. Being an alternative for the analysis of low volatile compounds by gas chromatography, the combination of these compounds favors the replacement of the amine and phosphonate groups which may be found in the structures of GLY and AMPA. TMCS acts as a catalyst, increasing the strength of the donor silyl (BSTFA) and assuring greater efficiency for the reaction. However, few reports have been found in the literature regarding the derivatization of GLY and AMPA using this combination of reagents.

With this study, we sought to develop and optimize a derivatization technique as from the silylation of GLY and AMPA using a combination of BSTFA and TMCS and analysis by gas chromatography, and detection by mass spectrometry (GC-MS).

2. Experimental

2.1. Reagents

Standard stock solutions of GLY (99.2% m/m) and AMPA (99.0% m/m) obtains of Sigma-Aldrich (St. Louis, MO, EUA) were prepared in deionized water with concentration of 500 $mg \cdot L^{-1}$ and stored at 4˚C. Working solutions were prepared from stock solutions at the concentrations of 15 e 50 $mg \cdot L^{-1}$ in the same solvent.

As solvents used Pyridine (99.8% v/v) and *N*,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% TMCS, both obtained from Sigma-Aldrich (St. Louis, MO, USA). Sodium hydroxide with a purity greater than 97.0% m/m (Dynamic, Brazil) and hydrochloric acid (37.0% v/v) acquired from Vetec (Rio de Janeiro, Brazil).

2.2. Instrumentation

For chromatographic analysis was used Agilent Technologies gas chromatograph (GC 7890A) coupled to a mass spectrometer (MS 5975). Was used a capillary column DB-5 MS (Agilent Technologies, stationary phase 5% phenyl and 95% methylpolysiloxane, 30 m × 0.25 mm d.i. × 0.25 μm film thickness). Helium (99.9999%) was used as carrier gas at a rate of 3.0 $mL \cdot min^{-1}$. The injector was maintained at 280˚C. The system initially at 100˚C increased the temperature at a rate of $8^{\circ}C \cdot min^{-1}$ to 300˚C. The sample volume introduced was 1μL in injection mode without flow divider, splitless, using an injector Combi PAL. The mass spectrometer was operated in electron ionization at 70 eV, and a quadrupole mass analyzer, operated in selective ion monitoring (SIM) (m/z 232, 312 e 340 for GLY and m/z 102, 298 e 312 for AMPA). The interface was kept at 300˚C and the ion source to 280˚C.

2.3. Sample Preparation

The optimized parameters of the reaction derivatization of GLY and AMPA are described afterwards. Samples of 10.6 μL of GLY and AMPA standard solution at 15 $mg \cdot L^{-1}$ respectively, were transferred for a derivatization vial (0.3 mL) and heated to dryness (60˚C). Then, was added 60 μL of pyridine and, after five minutes, 100 μL of the reagent derivatizing (BSTFA + TMCS 1%). The mixture was heated at 60˚C for 30 minutes, previously the analysis by GC-MS.

2.4. Optimized Parameters

To optimize the derivatization reaction, the following parameters were evaluated: the composition of the reaction medium, homogenization, volume of BSTFA:Pyridine and pH of the reaction medium according to **Table 1**.

The pH of the reaction medium was adjusted using concentrated hydrochloric acid and solution of sodium hydroxide with pH 10 and 13 (0.1 and 1.0 $mol \cdot L^{-1}$ respectively). The pH values were obtained in the pH meter micro processed of Quimis (São Paulo, Brazil). The best conditions were determined based on the mass spectra obtained for the studied compounds.

3. Results and Discussion

3.1. Structural Characterization of AMPA and GLY Derivatized

Representative mass spectra with the major ions proposed for GLY and AMPA derivatized may be observed in **Figure 2**. The identification of derivatives of these compounds was performed by interpretation of their mass spectra with respect to their molecular mass and expected elution order.

Mass spectrum for GLY (**Figure 2(a)**) after substitution by groups TMS ($(CH3)_3Si$) showed fragmentation profile containing the main íons m/z 73 $[(CH3)_3Si]+$, 147 $[(CH_3)_3SiOSi(CH_3)_2]+$, 232 $[(CH_3)_3SiOCOCH_2N((CH_3)_3Si)CH_2]+$, 298 $[((CH_3)_3SiO)_2PO(CH_3)_3Si]$, 312 $[(CH_3)_3SiOPOO((CH_3)_3Si)CH_2NH(Si(CH_3)_2]+$ and 340 $[((CH_3)_3SiO)_2POCH_2N((CH_3)_3Si)CH_2]+$. The peak in m/z

Table 1. Variables evaluated in optimizing the derivatization reaction of GLY and AMPA.

Variables	Levels
Composition of the reaction medium (v/v)	Acetonitrile/BSTFA; Pyridine/BSTFA
Ultrasonic vibration (min)	0 e 2
Volume of BSTFA:Pyridine (μL)	20:200; 60:100
pH of the reaction medium	1, 6 and 13

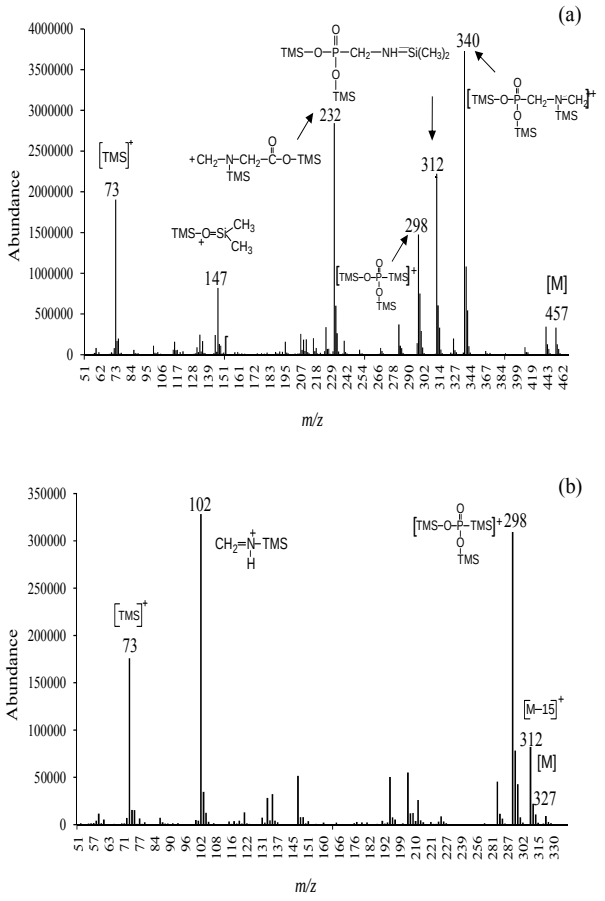

Figure 2. Mass spectrum showing the principal fragments of GLY (a) and AMPA (b) after derivatization with BSTFA.

73 represents the formed ion by trimethylsilane group. The peaks in m/z 147, 232, 298, 312 and 340 are cleavage and rearrangement products of the structure of GLY after the derivatization and electron impact at 70 eV. Among the major ions obtained for GLY, peaks at m/z 232, 312 and 340 were selected for selective ion monitoring (SIM), having greater abundance.

For AMPA derivatized, there is the following fragments common ionic m/z 73 $[(CH_3)_3Si]+$, 102 $[(CH_3)_3SiNHCH_2]+$, 298 $[((CH3)_3SiO)2PO(CH_3)_3Si]+$, 312 $[(CH_3)_3SiOPOO((CH_3)_3Si)CH_2NH(Si(CH_3)_2]+$. These ions were also proposed by Ngim and collaborators (2011) [14] to the optimize procedure for characterizing impurities in AMPA using the analyte in the solid state and analyzes by GC-MS.

3.2. Development and Optimization of the Silylation Procedure

3.2.1. Composition of the Reaction Medium

To favor the derivatization reaction with BSTFA one base was added to the medium. In this study, was used acetonitrile and pyridine, the latter is most often selected for derivatization reactions for analysis by GC [15,16]. The chromatograms obtained from the use of basic reagent: BSTFA in the proportion 20:200 μL may be observer in **Figure 3**.

In chromatograms was not observed sign of studied compounds. However this is the first measured parameter in the optimizating method allowing observing that the use of acetonitrile favored for derivatization of some impurities presents in the medium mainly represented by the compounds between 12 e 20 min (**Figure 3**). Already the use of pyridine gave a chromatogram with fewer interferences. These results differ from those found for amino acid analyzes that the use of acetonitrile favored for derivatization of the analyte [17]. Thus, the combination of pyridine and BSTFA was selected for the next experiments.

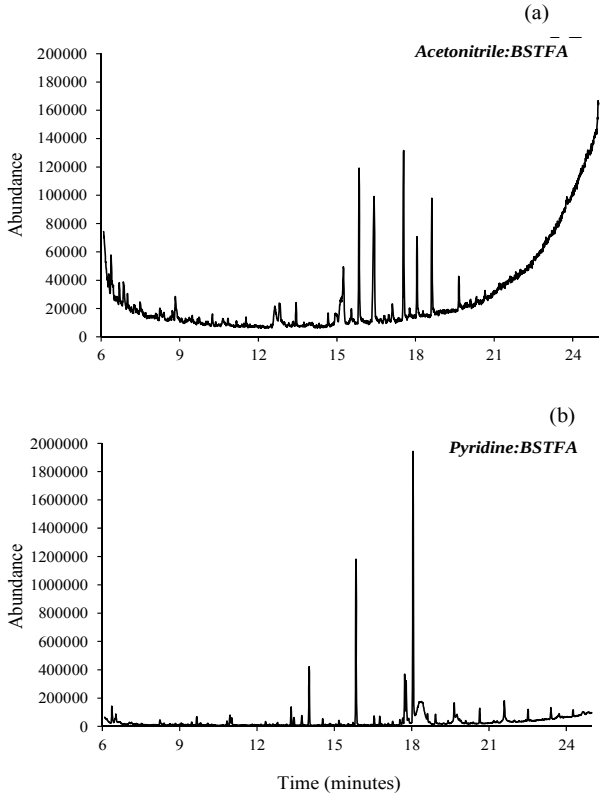

Figure 3. Part of the chromatogram of GLY e AMPA solution 1 mg·L^{-1} obtained by employing basic reagent: BSTFA in proportion 20:200 μL (acetonitrile (a) e pyridina (b)).

3.2.2. Ultrasonic Vibration

The ultrasonic waves create, increase and implode steam cavities and gases in a liquid, promoting activation in chemical reactions [18]. This process generates heat energy sufficient to favor homolytic cleavage of the compounds present [18]. To evaluate this parameter was used ultrasonic bath and two minutes as ultrasonic vibration time as shown in **Figure 4**.

The chromatograms showed that the ultrasonic vibrations have not favored in the derivatization of the analyte. This is because during the cavitation process, few radicalar species may be formed [18] interfering negatively in the derivatization process. However, this result differs from that found for derivatization with BSTFA of carboxylic acid wherein the homogenization and ultrasonic favored by 14% in the chromatographic response [19].

3.2.3. Volume of BSTFA:Pyridine

The relation between the basic reagent volume (Pyridine) and derivatizing reagent (BSTFA) was also evaluated and the results can be verified in **Figure 5**.

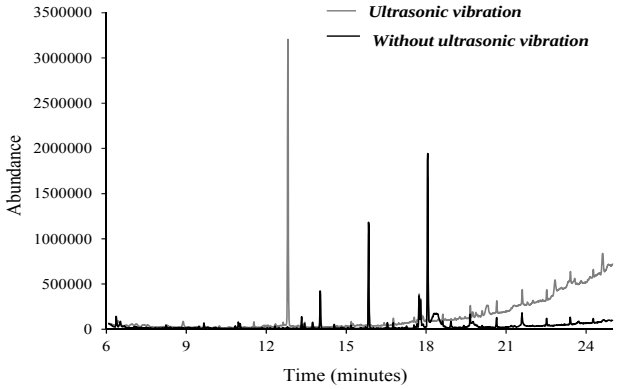

Figure 4. Part of the chromatogram of GLY e AMPA solution 1 mg·L^{-1} with and without ultrasonic vibration.

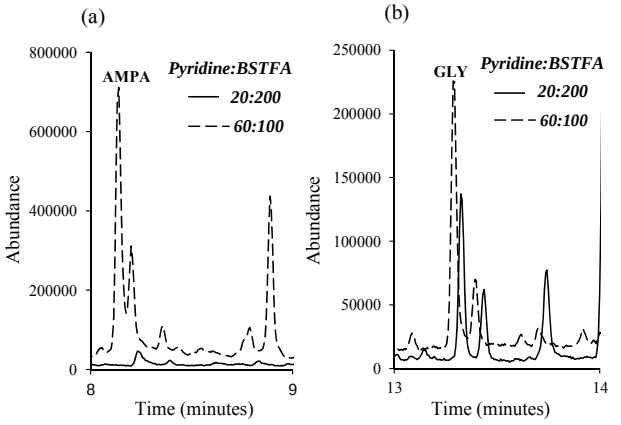

Figure 5. Part of the chromatogram of AMPA solution (a) and GLY (b) 1 mg·L^{-1} using differents volums of basic reagente (Pyridine) and derivatizing reagent (BSTFA) in proportions 20:200 and 60:100 µL.

Lower volumes of pyridine (20 µL) were not sufficient to basify the medium and favor the reaction of derivatization (**Figure 5**). It is observed that the derivatization reaction was promoted only when using proportion pyridine:BSTFA 60:100 µL. Under these conditions the AMPA eluted at 8.2 min and GLY 13.4 min. This ratio has been used for derivatization of plant extracts [20].

3.2.4. pH of the Reaction Medium

The GLY has secondary chemical equilibrium having its structure changed various forms in a certain medium pH [21]. In this work, pH 1.00 was used to ensure complete protonation of the molecule and pH 13.0 promoting complete desprotonation of the same.

The use of an acidic medium did not favor the derivavitization of the analytes and no signal was observed corresponding to the compounds obtained in the chromatograms. By using basic medium, occurred derivitization of GLY, however, the signal obtained in 13.4 min. showed lower intensity and in this condition the derivitization of AMPA was not favored (**Figure 6**). Thus, the step of adjusting the pH of the reaction medium was not inserted in the optimized methodology.

4. Conclusion

The optimization technique of derivitization of GLY and AMPA resulted in a rapid and simple method for the analysis of these compounds by GC-MS. It was observed that the process of derivitization occurred more favorably when using pyridine: BSTFA in proportions 60:100 µL, respectively, without the need to add steps to ultrasonic vibration or adjust the pH of the reaction medium (pH 6).

5. Acknowledgements

The authors thank the Conselho Nacional de Desenvol-

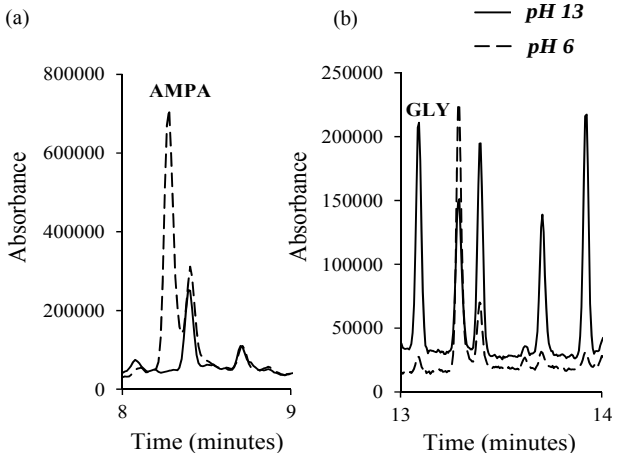

Figure 6. Part of the chromatogram of AMPA solution (a) and GLY (b) 1 mg·L^{-1} obtained by employing basic medium (pH 13) and no pH adjustment (pH 6).

vimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for their financial supports. At Universidade Federal de Minas Gerais for infrastructure available.

REFERENCES

[1] S. M. Sanches, C. H. T. P. Silva, S. X. Campos and E. M. Vieira, "Pesticidas e seus Respectivos Riscos Associados à Contaminação da Água," *Pesticidas*: *R. Ecotoxicol. e Meio Ambiente*, Vol. 13, No. 0, 2003, pp. 53-58.

[2] B. Li, X. Deng, D. Guo and S. Jin, "Determination of Glyphosate and Aminomethylphosphonic Acid Residues in Foods Using High Performance Liquid Chromatography-Mass Spectrometry/Mass Spectrometry," *Chinese Journal of Chromatography*, Vol. 25, No. 4, 2007, pp. 486-490. http://dx.doi.org/10.1016/S1872-2059(07)60017-0

[3] M.-X. Chen, Z.-Y. Cao, Y. Jiang and 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," *Journal of Chromatography A*, Vol. 1272, 2013, pp. 90-99. http://dx.doi.org/10.1016/j.chroma.2012.11.069

[4] Y. V. Kryuchkova, G. L. Burygin, N. E. Gogoleva, Y. V. Gogolev, M. P. Chernyshova, O. E. Makarov, E. E. Fedorov and O. V. Turkovskaya, "Isolation and Characterization of a Glyphosate-Degrading Rhizosphere Stain, *Enterobacter cloacae* K7," *Microbiological Research*, 2013, in press. http://dx.doi.org/10.1016/j.micres.2013.03.002

[5] Z.-X, Guo, Q. Cai and Z. Yang, "Determination of Glyphosate and Phosphate in Water by Ion Chromatography-Inductively Coupled Plasma Mass Spectrometry Detection," *Journal of Chromatography A*, Vol. 1100, No. 2, 2005, pp. 160-167. http://dx.doi.org/10.1016/j.chroma.2005.09.034

[6] M. V. Khrolenko and P. P. Wieczorek, "Determination of Glyphosate and Its Metabolite Aminomethylphosphonic Acid in Fruit Juices Using Supported-Liquid Membrane Preconcentration method with High-Performance Liquid Chromatography and UV Detection after Derivatization with p-Toluenesulphonyl Chloride," *Journal of Chromatography A*, Vol. 1093, No. 1-2, 2005, pp. 111-117. http://dx.doi.org/10.1016/j.chroma.2005.07.062

[7] K. Q. Qian, T. Tang, T. Shi, F. Wang, J. Li and Y. Cao, "Residue Determination of Glyphosate in Environmental Water Samples with High-Performance Liquid Chromatography and UV Detection after Derivatization with 4-Chloro-3,5-dinitrobenzotrifluoride," *Analytica Chimica Acta*, Vol. 635, No. 2, 2009, pp. 222-226. http://dx.doi.org/10.1016/j.aca.2009.01.022

[8] H.-Y. Chiu, Z.-Y. Lin, H.-L. Tu and C.-W. Whang, "Analysis of Glyphosate and Aminomethylphosphonic Acid by Capillary Electrophoresis with Electrochemiluminescence Detection," *Journal of Chromatography A*, Vol. 1177, No. 1, 2008, pp. 195-198. http://dx.doi.org/10.1016/j.chroma.2007.11.042

[9] I. Hanke, H. Singer and J. Hollender, "Ultratrace-Level Determination of Glyphosate, Aminomethylphosphonic Acid and Glufosinate in Natural Waters by Solid-Phase Extraction Followed by Liquid Chromatography-Tandem Mass Spectrometry: Performance Tuning of Derivatization, Enrichment and Detection," *Analytical and Bioanalytical Chemistry*, Vol. 391, No. 6, 2008, pp. 2265-2276. http://dx.doi.org/10.1007/s00216-008-2134-5

[10] C. Druart, O. Delhomme, A. Vaufleuty, E. Ntcho and M. Millet, "Optimization of Extraction Procedure and Chromatographic Separation of Glyphosate, Glufosinate and Aminomethylphosphonic Acid in Soil," *Analytical and Bioanalytical Chemistry*, Vol. 399, No. 4, 2011, pp. 1725-1732. http://dx.doi.org/10.1007/s00216-010-4468-z

[11] C. Shummer, O. Delhomme, B. M. R. Appenzeller, R. Wennig and M. Millet, "Comparison of MTBSTFA and BSTFA in Derivatization Reactions of Polar Compounds Prior to GC/MS Analysis," *Talanta*, Vol. 77, No. 4, 2009, pp. 1473-1482. http://dx.doi.org/10.1016/j.talanta.2008.09.043

[12] T. A. Souza, M. H. R. Matta, É. Montagner and A. B. G. Abreu, "Estudo de Recuperação de Glifosato e AMPA Derivados em Solo Utilizando-se Resinas Nacionais," *Química Nova*, Vol. 29, No. 6, 2006, pp. 1372-1376. http://dx.doi.org/10.1590/S0100-40422006000600037

[13] M. Motojyuku, T. Saito, K. Akieda, H. Otsuka, I. Yamamoto and S. Inokuchi, "Glyphosate Metabolites, and Glufosinate in Human Serum by Chromatography-Mass Spectrometry," *Journal of Chromatography B*, Vol. 875, No. 2, 2008, pp. 509-514. http://dx.doi.org/10.1016/j.jchromb.2008.10.003

[14] K. K. Ngim, J. Green, J. Cuzzi, M. Ocampo and Z. Gu, "Optimizad Derivatization Procedure for Characterizing (Aminomethyl)phosphonic Acid Impurities by GC-MS," *Journal of Chromatographic Science*, Vol. 49, No. 1, 2011, pp. 8-14. http://dx.doi.org/10.1093/chrsci/49.1.8

[15] M. B. Pessuto, I. C. Costa, A. B. Souza, F. M. Nicoli, J. C. P. Mello, F. Petereit and H. Luftmann, "Atividade Antioxidante de Extratos e Taninos Condensados das Folhas *Maytenus ilicifolia* Mart. ex Reiss," *Química Nova*, Vol. 32, No. 2, 2009, pp. 412-416. http://dx.doi.org/10.1590/S0100-40422009000200027

[16] J. R. Silva, L. T. Silva and J. I. Druzian, "Otimização e Validação Intralaboratorial de Método para Análise de Resíduos de Cloranfenicol em Leite Caprino por Cromatografia Gasosa com Detecção por Captura de Elétrons (CG/DCE)," *Química Nova*, Vol. 33, No. 1, 2010, pp. 90-96. http://dx.doi.org/10.1590/S0100-40422010000100017

[17] X. Shen, C. Deng, B. Wang and L. Dong, "Quantification of Trimetilsilyl Derivatives of Amino Acid Disease Biomarkers in Neonatal Blood Samples by Gas Chromatography-Mass Spectrometry," *Analytical and Bioanalytical Chemistry*, Vol. 384, No. 4, 2006, pp. 931-938. http://dx.doi.org/10.1007/s00216-005-0241-0

[18] G. S. Garbellini, G. R. Salazar-Banda and L. A. Avaca, "Aplicação do Ultra-Som em Sistemas Eletroquímicos: Considerações Teóricas e Experimentais," *Química Nova*, Vol. 31, No. 1, 2008, pp. 123-133. http://dx.doi.org/10.1590/S0100-40422008000100024

[19] V. M. Prata, E. S. Emídio and H. S. Dorea, "New Cata-

lytic Ultrasound Method for Derivatization of 11-Nor-Δ^9-tetrahydrocannabinol-9-carboxylic Acid in Urine, with Analysis by GC-MS/MS," *Analytical and Bioanalytical Chemistry*, Vol. 403, No. 2, 2012, pp. 625-632. http://dx.doi.org/10.1007/s00216-012-5827-8

[20] I. M. C. Rodrigues, A. P. S. Souza Filho, F. A. Ferreira and A. J. Demuner, "Prospecção Química de Compostos Produzidos por Senna Alata com Atividade Alelopática," *Planta Daninha*, Vol. 28, No. 1, 2010, pp. 1-12.

[21] C. F. B. Coutinho and L. H. Mazo, "Complexos Metálicos com o Herbicida Glyphosate: Revisão," *Química Nova*, Vol. 28, No. 6, 2005, pp. 1038-1045. http://dx.doi.org/10.1590/S0100-40422005000600019