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Alkylsilyl derivatization of glyphosate and aminomethylphosphonic acid followed by gas chromatography mass spectrometry

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ABSTRACT

Prior to any chromatographic analysis of *N*-(phosphonomethyl)glycine, glyphosate (GLYP) and its principal metabolite aminomethylphosphonic acid (AMPA) – being particularly polar herbicides – first, derivatization needs to be the performed.

In this paper, for GLYP and AMPA for the time being not applied, alkylsilyl derivatization speciation is described to quantitate them with *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) reagent, resulting in fully labeled trimethylsilyl (TMS) derivatives, followed by gas chromatography mass spectrometry (GC-MS). MSTFA triggers the so far not recognized reactivity of these species: GLYP's equilibrium led to five active proton containing species, ready to form GLYP.5TMS (*m/z* 529), while AMPA show up as AMPA.4TMS (*m/z* 453). This recognition opens a new aspect of analyzing GLYP and AMPA. The equilibrium phenomenon of GLYP, proving the existence of the five active proton containing moiety, was confirmed by density functional theory (DFT) computation. Optimum analytical conditions both for GLYP selectively and for GLYP and AMPA simultaneously were highlighted. Impact of the solvents applied (pyridine, PYR, acetonitrile, ACN and ethyl acetate, ETAC) resulted in different distribution of GLYP.5TMS and GLYP.4TMS. In favor of GLYP alone, MSTFA/PYR proved to be the reagent of choice – while for the simultaneous quantification of GLYP and AMPA – the MSTFA/ACN composition had to be selected. The commonly used labeling with *N*-tert.-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA), based on self experiences, was documented and critically evaluated. LOQ values, expressed as ng/injection values proved to be for GLYP.4TMS + GLYP.5TMS: 1.41 ng (4.82%), AMPA.4TMS: 0.45 ng (3.4%). Practical utility of TMS derivatization was shown by the simultaneous GLYP and AMPA analysis of four herbicide market samples, characterized with relative standard deviation percentages (RSD%) of measurements: varying between 6.1 RSD% and 2.43 RSD%. All four herbicides contain AMPA impurity as follows: Glialka^{R} Star, 3.70 g/L (4.57 RSD%); Fozát^{R}, 2.68 g/L (4.16 RSD%); Medallon P, 1.56 g/L (4.19 RSD%) and Total Spray, 0.17 g/L (3.39 RSD%), respectively.

1. Introduction

The chromatographic quantitation of GLYP and its main metabolite (AMPA) was reviewed recently [1]. The increasing interest in the behavior of these herbicides can be attributed to GLYP's expected exponential production and use by 2020 [2]. This phenomenon was also manifested in the trend of a number of publications applying derivatization prior to GLYP's analysis. Following papers at the Scopus data basis obtained with the keywords glyphosate and chromatography, a continuous increase in the number of papers was found: in 2013 fifty-six, in 2014 sixty-seven and in 2015 – up to November 10 – hundred and two.

Coming back to literature premises, applying trialkylsilylation prior to the analysis of GLYP and AMPA by GC-MS [3–9], it turned out that out of seven publications in five MTBSTFA [3–5,7,8], in two *N,O*-bis(trimethylsilyl)-trifluoroacetamide (BSTFA) catalyzed with trimethylchlorosilane (TMCS) [6,9] were used. MSTFA, for the time being in GLYP or AMPA analysis was not exhausted.

MTBSTFA labeling – led to the *tert.*butyl(dimethylsilyl) (TBDMS) derivatives – was performed at 80 °C for 30 min [3,4], or at room temperature (rtp) [5,7,8] for 15 min [5] or vortexing for 15 s [8], or six times in every 10 min [7]. BSTFA was used at 90 °C for 150 min and at 60 °C for 30 min; comparing these time and temperature conditions they seem to be contradictory.

As to the reagent composition, in all cases in question, derivatizations were performed in mixture of solvents. MTBSTFA with dimethylformamide (DMF) [3,4] and ACN [5,7,8], in 1/1 volume ratios (*v*/*v*), while BSTFA in combinations, such as BSTFA (10%TMCS)/PYR = 1/1 [6] and BSTFA (1%TMCS)/PYR 0.6/1 [9] were used.

Concerning analytical performance characteristics, in spite of selective acquisition processes – (GC-MS [3,6,7,9] and GC-MS-SIM [4,5,8]) – because of the methods' limited sensitivity they have been poorly documented [3–9]. In three papers [3,6,9] neither LOD nor LOQ data were given.

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To investigate the characteristic properties of our novel trimethylsilylation process and in order to optimize it as a quantitative method, a many-sided research was undertaken. Reagent composition, impact of solvent, as well as, time and temperature for optimum analytical conditions were determined. In parallel with GC/MS studies, DFT modeling computation was performed to confirm that the favored equilibrium of GLYP led to the GLYP.5TMS formation. In addition also literature documentation regarding MTBSTFA derivatizations of GLYP and AMPA was critically commented based on our own experiences.

2. Experimental

2.1. Materials and methods

GLYP standard was obtained from Molecula Fine Chemicals (distributor: France, Europe; manufacturer: Jiang SU Feng Shan Group, CO. LTD, China). AMPA (≥99%), MSTFA (97%), BSTFA (≥99%), MTBSTFA (≥95%), PYR, ACN, and ETAC were products of Sigma-Aldrich Ltd. (St. Louis, MO, USA), in highest purity available. Herbicide market samples, as Glialka^R Star (Glialka), Medallon Premium (Medallon) and Fozát^R 480 (Fozát), all three contained 360 g/L GLYP as salts; Glialka (K salt) and Medallon (diammonium salt), products of Monsanto Europe, S.A., Brussels, Belgium (Glialka) and Syngenta, AG Suisse (Medallon). Fozát^R 480 was from Agro-Chemie Kft, Budapest, Hungary, while Total Spray containing 7.2 g/L GLYP (isopropyl amine salt), was obtained from Sinon Corporation (Taiwan, Republic of China; distributor: Cresco Chemical Kft, Budapest, Hungary).

2.2. Sample preparation

GLYP and AMPA (20–22 mg/50 mL), weighed with ±0.01 mg uncertainty, were dissolved in distilled water and stored in the refrigerator, at 4 °C for up to one year. Dilutions – from these solutions – (2 μL–20 μL) were rotary evaporated in triplicates to dryness at 30–40 °C. Residues for trialkylsilylation were treated with 60 μL PYR (or ACN, or ETAC) and 120 μL MSTFA or MTBSTFA, mixed and treated at room temperature (rtp) or heated in oven from up to 100 °C in the time range of 15–120 min, respectively. For trimethylsilylation with MSTFA, as optimum condition, 80 °C, 30 min was selected. Derivatized solutions were transferred into the autosampler vial and 1 μL was injected.

2.3. Instrumentation

The apparatus consisted of a Varian 4000 GC-MS/MS system (Varian, Walnut Creek, CA, USA), a Varian CP-8400 Autosampler, and a Septum Programmable Injector (SPI). The column used was product of SGE (Victoria, Australia); SGE forte capillary BPX5: 30 m × 0.25 mm; df = 0.25 μm. Temperatures of transfer line, ion trap and manifold were 300 °C, 210 °C and 80 °C. Optimized temperature programs: injections were made at 100 °C (0.5 min hold), heated to 300 °C (4 min hold), then cooled down to 100 °C (100 °C/min); column temperature profile was: 100 °C, held for 1.00 min, heated to 300 °C for 20 °C/min (4 min hold). Total elution time: 16 min. Helium purity: 6.0, 99.9999%, flow rate: 1 mL/min. General MS parameters were: Fil/Mul delay: 3.00 min; electron energy: 70 eV.

Blank test was performed with all prepared series in the same manner as the GLYP and AMPA containing tests: taking into account contaminations/impurities.

3. Results and discussion

3.1. Introductory studies derivatizing GLYP and AMPA with MSTFA

First the reagent composition of MSTFA/ACN, MSTFA/ETAC and MSTFA/PYR = 2/1 (*v*/*v*) was varied performing derivatizations at optimum conditions 80 °C for 30 min demonstrated in Fig. 1a–c. (Note:

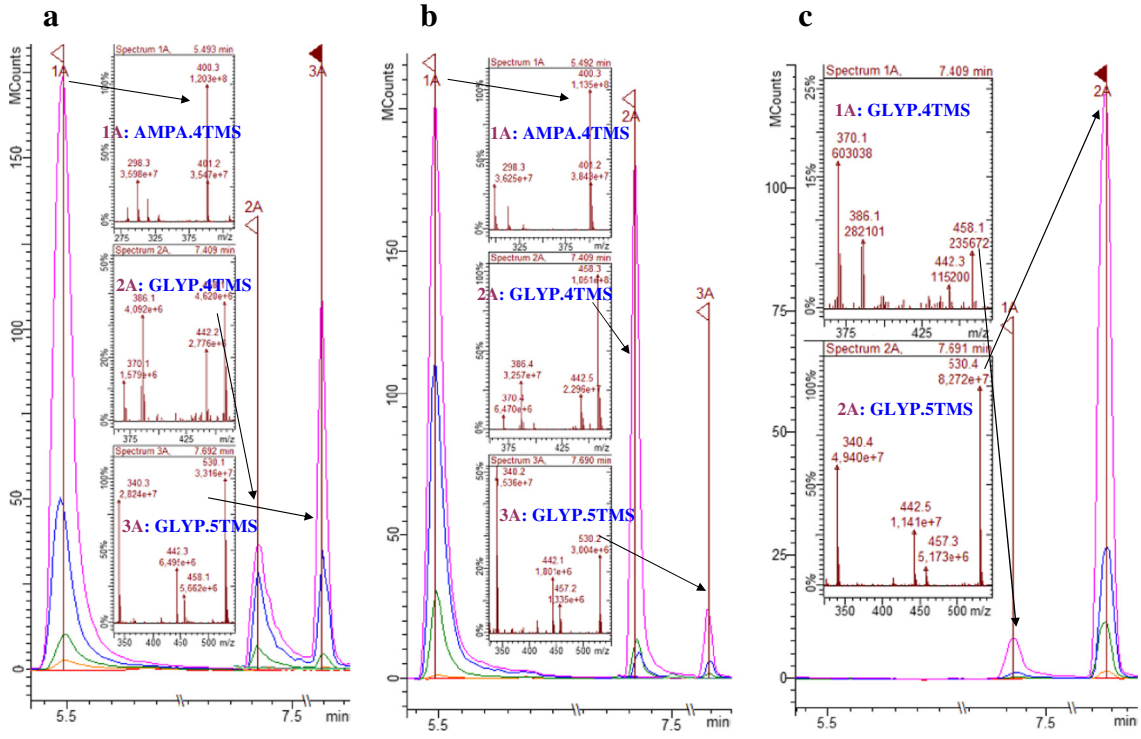

Fig. 1. a–c. Peak profiles and mass spectra of the MSTFA derivatized AMPA and GLYP (80 °C, 30 min), obtained with the MSTFA/ACN = 2/1 (**Fig. 1a**), MSTFA/ETAC = 2/1 (**Fig. 1b**) and MSTFA/PYR = 2/1 (**Fig. 1c**) (reagents all, in *v*/*v* ratios). *Indications*: t_R = retention time; peak profiles represent blanks (red lines) for AMPA.4TMS (t_R, 5.45 min), GLYP.4TMS (t_R, 7.41 min) and GLYP.5TMS (t_R, 7.69 min); injected amounts/1 μL: 2.8 ng (orange lines), 5.6 ng (green lines), 11.2 ng (blue lines) and 22.4 ng (violet-colored lines). *Selective fragment ions, m/z values*: AMPA.4TMS, spectra 1A: $[M + 1]^+$ 400.3; $[M + 1\text{-}(30 + TMS)]^+$ 298.2; GLYP.4TMS, spectra 2A: $[M + 1]^+$ 458.3; $[M\text{-}CH_3]^+$ 442.2; $[M + 1\text{-}TMS]^+$ 386.1; $[M\text{-}TMS\text{-}CH_3]^+$ 370; GLYP.5TMS, spectra 3A: $[M + 1]^+$ 530.3; $[M\text{-}TMS]^+$ 457; $[M + 1\text{-}Si(CH_3)_4]^+$ 442; $[M\text{-}(2TMS + 3CH_3)]^+$ 340.3.