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Advances in the alkylsilyl derivatization of glyphosate and aminomethylphosphonic acid: A critical comeback to the *N-tert.*-butyldimethylsilyl-*N*-methyltrifluoroacetamide reagent

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ABSTRACT

A novel, full *tert.*-butyldimethylsilylation approach - derivatizing *N*-(phosphonomethyl) glycine (glyphosate, GLYP) and its principal metabolite aminomethylphosphonic acid (AMPA) - was noted. The full labeling with the *N-tert.*-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA) reagent was triggered, extending the mass acquisition up to the *m/z* 1000 values. Under this condition, the 4.TBDMS species - for GLYP and AMPA equally - were recognized and determined, at first. Following their mass fragmentation properties by gas chromatography mass spectrometry (GC/MS) the not yet described molecular ions (GLYP.4TBDMS: *m/z* 625, and AMPA.4TBDMS: *m/z* 567), along with their characteristic decomposition products were confirmed.

The complementary impact of solvents (acetonitrile, ACN, pyridine, PYR) was also studied. PYR, for the time being not yet applied with MTBSTFA proved to be also suitable in the *tert.*-butyldimethylsilyation process. Along with ACN both support the 4.TBDMS derivative formation: ensuring an improved selectivity and sensitivity of the protocol, for both species examined.

As to the analytical performance characteristics, under optimum conditions, LOQ values proved to be close to each other: in MTBSTFA/ACN medium for GLYP 1.18 ng/μL for AMPA 0.036 ng/μL was confirmed. In MTBSTFA/PYR medium for GLYP 2.36 ng/μL, for AMPA 0.036 ng/μL values were determined. It is worth to note that AMPA.4TBDMS ions in the MTBSTFA/ACN medium show up above 0.14 ng/μL while, in the MTBSTFA/PYR medium above 0.072 ng/μL: meaning that PYR favors their formation. Repeatability of model measurements, characterized with the relative standard deviation percentages (RSD%), varied from 0.39 RSD% up to 5.9 RSD% with an average of 2.97 RSD%.

Practical utility of the MTBSTFA/PYR method was confirmed by the analysis of the AMPA impurities in GLYP market samples, expressed as g/L AMPA: Glialka^{R} Star, 6.45 g/L (5.1 RSD%); Fozát^{R}, 6.89 g/L (4.34 RSD%); Medallon P, 7.15 g/L(4.28 RSD%) and Total Spray, 0.038 g/L (4.64 RSD%).

1. Introduction

Recently, the role of derivatization technique in the analysis of GLYP and AMPA by chromatography was reviewed [1]. Next, as a novelty to the field, the mechanism, quantitation and fully trimethylsilylation related labeling of these two compounds was described [2]. Based on our experiences [1,2] and due also to GLYP's continuously increased production [3], the reinvestigation/reconsideration of GLYP's *tert.*-butyldimethylsilylation seemed to be useful. Namely, in spite of the congruent literature premises [4–9, Table 1] - reporting for species as maximum labeling the 3TBDMS derivatives - we assumed that published data were prepared with missed analytical outlook.

As seen (data in Table 1), limited details of mass fragmentation properties are available, even for the 3TBDMS species: molecular ion for GLYP.3TBDMS is mentioned in a single case, only [4]. As abundant masses for both species, accordingly all papers published, the 3TBDMS-57 fragments were reported: as *m/z* 511-57 for GLYP and *m/z* 453-57 for AMPA. Independent of the solvent applied MTBSTFA derivatization was performed in 1/1 volume ratios with dimethylformamide (DMF) [4,5] or acetonitrile (ACN) [6–9]; the option using PYR was not tested.

This limited labeling of GLYP and AMPA served as the basis of reconsideration. Especially (i) being aware of the fact that in line of proton substitution readyness MTBSTFA is the reagent of choice [10], as well as (ii) in derivatization process of GLYP and AMPA sterical hindrance was not expected. Consequently, as a continuation of our basic studies [1,2] in this paper MTBSTFA derivation of GLYP and AMPA was reinvestigated by

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Table 1
MTBSTFA derivatization and mass fragmentation properties of GLYP (MW: 169.07) and AMPPA (MW: 111.07): literature data (Ref.).

Derivatized			Acquisition; *m/z* fragments (GLYP.3TBDMS: *m/z* 511, AMPA.3TBDMS: *m/z* 453)	LOD	LOQ	Ref.
with in v/v ratios	°C	min		ng/mL, ng/g		
MTBSTFA/DMF = 1/1	80	30	GC–MS; GLYP: 511, 496, **454**; AMPA: 438, **396**	–	–	[4]
MTBSTFA/DMF = 1/1	80	30	GC–MS; GLYP, 454; AMPA: 396	1–10 pg	–	[5]
MTBSTFA/ACN = 1/1	Room temp		GC–MS; GLYP, 454, 352, 253; AMPA: 396, 367, 144	3000	–	[6]
MTBSTFA/ACN = 1/1	Room temp		GC–MS·SIM; GLYP, 454, 352;, AMPA: 396, 367	10–15	–	[7]
MTBSTFA/ACN = 1/1	Room temp		GC–MS·SIM; GLYP, 454	–	–	[8]
MTBSTFA/ACN = 1/1	Room temp		GC–MS·SIM; GLYP, 511, **454**, 352, 253	10,000	50,000	[9]

Indications: MW = molecular weight; DMF = dimethylformamide; ACN = acetonitrile; room temp = room temperature; abundant *m/z* values are bold printed

1. Extending the acquisition range of the GC–MS protocol, ensuring the identification and quantification of masses up to the *m/z* values of 1000,
2. Selecting the optimum solvent for full derivatizations,
3. Defining the analytical performance characteristics of optimized working strategy, and
4. Presenting method's practical utility measuring AMPA impurities in GLYP market samples.

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%), reagents like MTBSTFA (≥95%), and solvents like PYR and ACN were Sigma-Aldrich Ltd. (St. Louis, MO, USA) products, in highest purity available. Herbicide market samples, as Glialka^{R} Star, Medallon Premium and Fozát^{R} 480, 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), 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) and 60 μL

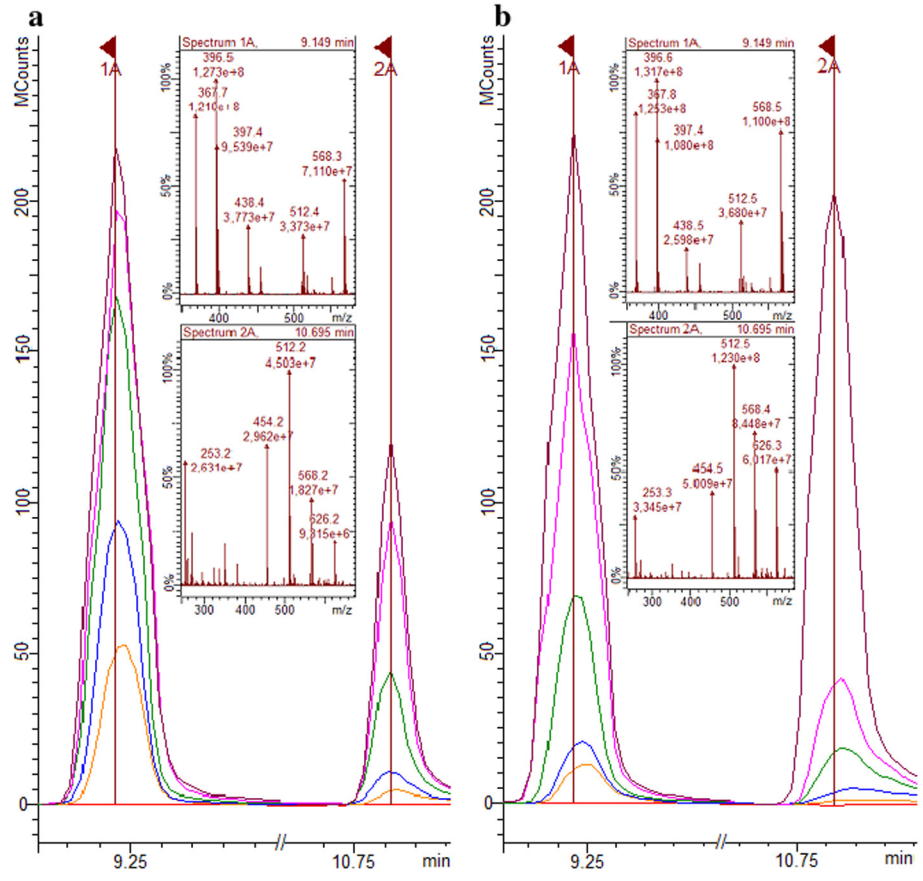

Fig. 1. a, b Peak profiles and mass spectra of the MTBSTFA derivatized AMPA and GLYP (80 °C, 30 min), obtained with MTBSTFA/ACN = 1/1 (Fig. 1a) and MTBSTFA/PYR = 1/1 (Fig. 1b) (reagents all, in v/v ratios). *Indications*: t_R = retention time; peak profiles represent blanks (red lines), spectra 1A = AMPA (t_R, 9.14 min) and spectra 2A = GLYP (t_R, 10.69 min); injected amounts/1 μL, 2.4 ng (orange lines), 4.7 ng (blue lines), 11.9 ng (green lines), 18.9 ng (pink lines) and 21.3 ng (violet-coloured lines). *Selective fragment ions*, *m/z* (±1) values: AMPA, spectra 1A: $[4TBDMS + 1]^+$ 567; $[4TBDMS + 1–57]^+$ 512; $[3TBDMS -15]^+$ 438; $[3TBDMS-(15 + 57)]^+$ 397; $[3TBDMS-(3 \times 15 + 57)]^+$ 367 GLYP, spectra **2A**: $[4TBDMS + 1]^+$ 626; $[4TBDMS - 57]^+$ 568; $[3TBDMS + 1]^+$ 512; [3TBDMS-57] 454; $[TBDMS-(2 \times 15)]^+$ 253. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)