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# Versatile derivatization for GC-MS and LC-MS: alkylation with trialkyloxonium tetrafluoroborates for inorganic anions, chemical warfare agent degradation products, organic acids, and proteomics analysis

Enea Pagliano

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**Abstract** Analytical chemists resort to derivatization for improving the detection performance of certain categories of analytes. Within this context, alkylation reactions are regarded as an important asset for many methods based on GC-MS and LC-MS. Trialkyloxonium tetrafluoroborates ( $R_3O^+[BF_4]^-$ ) are powerful alkylating agents with ionic liquid properties: they are nonvolatile salts soluble in water which are easier and safer to handle with respect to common alkylating agents like diazomethane.  $R_3O^+[BF_4]^-$  can perform the alkylation in both organic and aqueous media at pH conditions ranging from acidic to alkaline. Recent analytical applications of trialkyloxonium derivatizations include the high precision determination of inorganic anions in complex matrices, the qualitative confirmation of chemical warfare agent degradation products in soils, the profiling of carboxylic acids in urine, and the detection of protein post-translational modifications induced by carbon dioxide. The common denominator for all methods presented can be found in the simplicity of the alkylation protocol which, in most of the cases, requires a single step addition of the reagent directly to the sample.

**Keywords** Derivatization; trimethyloxonium; triethyloxonium; inorganic anions; chemical warfare agents; organic acids; proteomics

## 1 Introduction

Since the commercialization of mass spectrometry (MS) instruments, the landscape of analytical laboratories has changed significantly. MS have found widespread applications in many fields, including food safety, environmental and biomedical analysis, metabolomics, proteomics, and genomics. Depending on the requirements, the analyst can choose between large varieties of MS configurations which may be interfaced with separation techniques like gas chromatography (GC) or high performance liquid chromatography (HPLC). Although such hybrid MS platforms allow broad detection of molecules in their native form, significant improvements of the performance characteristics of a method can be obtained upon chemical derivatization [1–4].

In GC-MS analysis, derivatizations are aimed to convert analytes into thermally stable, volatile molecules whereas in LC-MS the goal is to provide the derivatives with better retention and ionization efficiency with respect to the original analytes.

For sample preparation in GC-MS and LC-MS, alkylation reactions constitute an important part of sample preparation techniques [1–4]. For example, alkylation with diazomethane was recently used to methylate peptides and glycerophospholipids before LC-MS analysis [5, 6]. Using this approach, amino- and phosphate- groups were converted into cationic moieties permanently charged which could be detected by LC-MS with a tenfold enhancement in sensitivity. Similarly, countless applications of alkylation can be found for GC analysis of polar molecules including alcohols, phenols, amines, carboxylic acids, and amino acids.

Although detection of certain analytes is greatly improved after alkylation, two major shortcomings limit the widespread application of classical alkylating agents like diazomethane, alkyl-chloroformates, dimethyl sulfate, and pentafluorobenzyl bromide. First, most of these reagents are highly toxic and volatile, posing significant concern for the analyst. Furthermore, most of these chemicals work only in nonaqueous media, requiring preliminary extraction of the analyte from the matrix.

For these reasons, an alternative yet very convenient alkylation method based on trialkyloxonium tetrafluoroborates ( $R_3O^+[BF_4]^-$ ) has been recently proposed for analytical applications.  $R_3O^+[BF_4]^-$  salts are powerful alkylating agents but do not share the associated drawbacks of established alkylating agents [7, 8].  $R_3O^+[BF_4]^-$  owns the typical characteristics common to the ionic liquids lately used in green chemistry: they are water-soluble, nonvolatile, and are hydrolyzed in aqueous solution to benign by-products.

In analytical chemistry,  $R_3O^+[BF_4]^-$  was recently employed for the high-precision quantitation of inorganic anions

by isotope dilution headspace GC-MS [9, 10] with applications including complex matrices like food samples [11], crude oil [12], and biological fluids [13, 14]. Since 2016, the  $R_3O^+[BF_4]^-$  entered into the derivatization scheme for the identification of chemical warfare agents degradation by-products [15–17] and in 2018 Linthwaite et al. [18] employed  $R_3O^+[BF_4]^-$  to detect carbon dioxide mediated protein post-translational modifications (PTMs) by LC-MS/MS. Most of the relevant analytical literature was published after 2008 (Table 1) and this *Trend* paper is aimed to discuss milestones and future developments in this novel research area.

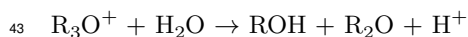
**Table 1:** Analytical methods based on alkylation with trialkyloxonium salts

Year	Matrix	Analyte	Reagent	Derivative	Instrument, notes	LOD (ng/g)	Run time (min)	Ref
1993	Urine	Phthalates	$Et_3OBF_4$	Ethyl phthalates	EI GC-MS on-column injection	<25	24	[19]
1998/99	Urine	Organic acids	$Me_3OBF_4$	Organic acid methyl esters	SPME EI GC-MS screening 50+ acids	N/A	140	[20, 21]
2008	Urine	Organic acids	$Me_3OBF_4$	Organic acid methyl esters	SPME GC-MS/MS EI and PCI	10-600	29	[22]
2008/12	Peptides	Peptides	$Et_3OBF_4$	-NH <sub>2</sub> conversion	MALDI-TOF/TOF 20-100 times signal increase	n/a	n/a	[23–25]
2011	Urine Fig. 1	THC COOH-THC	$Me_3OBF_4$	Full methylation	EI GC-MS	0.5-0.7	15.3	[26]
2013	Seawater urine	F <sup>-</sup>	$Et_3OFeCl_4$	EtF	HS EI GC-MS	3.2	15.5	[27]
2014	Seawater	NO <sub>2</sub> <sup>-</sup> NO <sub>3</sub> <sup>-</sup>	$Et_3OBF_4$	EtONO EtONO <sub>2</sub>	HS NCI GC-MS isotope dilution	low ng/g	19	[28]
2015	Saliva	SCN <sup>-</sup>	$Et_3OBF_4$	EtSCN	HS EI GC-MS isotope dilution	5	15	[13]
2016	FAMES Fig. 2	-POH -SOH	$Me_3OBF_4$	-POMe -SOMe	EI GC-MS chemical warfare agents	<10 µg/g	35.5	[15]
2016	Food	Geronic acid	$Me_3OBF_4$	Geronic acid methyl ester	EI GC-MS	<1.5	26	[29]
2017	Vegetables	NO <sub>3</sub> <sup>-</sup>	$Et_3OBF_4$	EtONO <sub>2</sub>	HS EI GC-MS isotope dilution	2 µg/g	1.8	[11]
2018	EBC	SCN <sup>-</sup>	$Et_3OBF_4$	EtSCN	HS EI GC-Orbitrap isotope dilution	0.29	15	[14]
2018	Seawater	Total N	$Et_3OBF_4$	EtONO <sub>2</sub>	HS NCI GC-MS persulfate digestion	30	6.4	[30]
2019	Cells Fig. 3	Protein-NH- COO <sup>-</sup>	$Et_3OBF_4$	Protein-NH- COOEt	LC-MS/MS CO <sub>2</sub> mediated PTMs	n/a	77	[18]
2018/19	Soil Fig. 2	-POH -SOH	$Me_3OBF_4$	-POMe -SOMe	EI GC-MS, GC-FPD chemical warfare agents	<10 µg/g	35.5	[16, 17]
2019	Crude oil	Cl <sup>-</sup>	$Et_3OBF_4$	EtCl	HS EI GC-MS/MS isotope dilution	200	6	[12]
2019	Wastewater	SeCN <sup>-</sup>	$Et_3OBF_4$	EtSeCN	GC-MS/MS isotope dilution	0.1	14.5	[31]

## 2 The trialkyloxonium salts: general properties

The first account on the chemistry of trialkyloxonium salts dates back to 1932 with the work of Meerwein and coworkers [32, 33]. This class of alkylating agents has found broad application in synthetic chemistry [7, 34] and

38 has supported notable research work up to this day [35]. Trimethyloxonium and triethyloxonium are commercially  
39 available under the form of the corresponding tetrafluoroborates:  $\text{Me}_3\text{O}^+[\text{BF}_4]^-$  and  $\text{Et}_3\text{O}^+[\text{BF}_4]^-$ . Although these  
40 compounds are powerful alkylating agents [34], the risks related to their manipulation are lower when compared to  
41 other alkylating agents: as reported by Perst [8],  $\text{R}_3\text{O}^+[\text{BF}_4]^-$  are nonvolatile solid salts which decompose within  
42 few hours in water solution:



44 The  $\text{R}_3\text{O}^+[\text{BF}_4]^-$  salts are therefore manipulated in a fume hood avoiding skin contact. As these substances are  
45 hygroscopic, they are stored at  $-20\text{ }^\circ\text{C}$  and are opened to air only when in use.

46 Trialkyloxonium salts can be used under a variety of conditions to perform methylation and ethylation. Being ionic  
47 compounds,  $\text{R}_3\text{O}^+[\text{BF}_4]^-$  salts work in water as well as in polar organic solvents. As the hydrolysis of  $\text{R}_3\text{O}^+[\text{BF}_4]^-$   
48 liberates  $\text{H}^+$  (in the form of  $\text{HBF}_4^-$ ), organic amines, bicarbonate, phosphates or ammonia are often used to remove  
49 the acid from the reaction media. Notably, aqueous derivatizations have been carried out under all pH conditions  
50 from strongly acidic to  $\text{pH}>10$  depending on the analysis and overall analyte stability.

51 As reported in Table 1,  $\text{Me}_3\text{O}^+[\text{BF}_4]^-$  and  $\text{Et}_3\text{O}^+[\text{BF}_4]^-$  have been employed in aqueous media for the alkylation of  
52 inorganic anions, organic acids, and functional groups on proteins. As most of the samples of analytical interest are  
53 water based, the possibility of using  $\text{R}_3\text{O}^+[\text{BF}_4]^-$  directly in an aqueous environment has procedural advantages and  
54 avoids a pre-extraction step of the analyte during the analysis. However, as it is expected in these cases, the overall  
55 derivatization power of the oxonium salt will experience a marked decrease in efficiency, most notably with low-level  
56 concentration analytes. Nevertheless, Valdez et al. [15] found that dissolution of the oxonium salts in the reaction  
57 medium is not necessary for the derivatization to take place as they were able to successfully methylate sulfonic and  
58 phosphonic acids in  $\text{CH}_2\text{Cl}_2$ .  $\text{CH}_2\text{Cl}_2$  is a unique solvent in these applications, as its inertness towards the oxonium  
59 salts does not provide products that may interfere with the GC analysis and its immiscibility with water made the  
60 overall protocol a biphasic process. In this vein, the choice of the solvent may be used to modulate the alkylation  
61 power of  $\text{R}_3\text{O}^+[\text{BF}_4]^-$ .

62 Within the analytical methods which make use of  $\text{R}_3\text{O}^+[\text{BF}_4]^-$  derivatization, different approaches for transferring  
63 the reagent into the sample have been proposed. For  $\text{Me}_3\text{O}^+[\text{BF}_4]^-$ , which is the most reactive, the preparation of  
64 water solutions is penalized by the fast hydrolysis of the reagent [34]. Valdez et al. [15] tried dissolving  $\text{Me}_3\text{O}^+[\text{BF}_4]^-$   
65 in acetonitrile, but the solution was not stable. In most analytical work,  $\text{Me}_3\text{O}^+[\text{BF}_4]^-$  has been added directly to

66 the samples with a spatula. The  $\text{Et}_3\text{O}^+[\text{BF}_4]^-$  is not as reactive as the  $\text{Me}_3\text{O}^+[\text{BF}_4]^-$  and it can be handled in water  
67 as its complete hydrolysis occurs within 80 min at 18 °C [34]. Furthermore,  $\text{Et}_3\text{O}^+[\text{BF}_4]^-$  solutions in acetonitrile  
68 are stable for over a month when prepared and stored at -20 °C [10]. Both water and acetonitrile solutions could be  
69 easily pipetted into the samples, thus minimizing the efforts for reagent transfer.

70 As summarized in Table 1, the trialkyloxonium salt has recently found applications ranging from the determination  
71 of simple inorganic anions to proteomics analysis. The following sections aim to expand more in detail work involving  
72 the use of  $\text{R}_3\text{O}^+[\text{BF}_4]^-$  salts in the derivatization of analytes for their subsequent analysis by GC- and LC-based  
73 methods.

### 74 3 Inorganic anions

75 Triethyloxonium tetrafluoroborate has found applicability in the determination of several inorganic anions by GC-  
76 MS in complex matrices that have proven difficult to analyze by conventional methods [10]. In an aqueous medium  
77 at room temperature,  $\text{Et}_3\text{O}^+$  converts  $\text{F}^-$ ,  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$ ,  $\text{CN}^-$ ,  $\text{SCN}^-$ ,  $\text{SeCN}^-$ ,  $\text{S}^{2-}$ ,  $\text{NO}_2^-$ , and  $\text{NO}_3^-$  into the volatile  
78 ethyl derivatives: EtF (bp -37.2 °C), EtCl (bp 15.6 °C), EtBr (bp 38.4 °C), EtI (bp 72.4 °C), EtCN (bp 96.9 °C),  
79 EtSCN (bp 146.1 °C), EtSeCN, Et<sub>2</sub>S (bp 91.9 °C), EtONO (bp 17 °C), and EtONO<sub>2</sub> (bp 87.3 °C) [9, 10, 27, 31]. With  
80 the exception of EtSeCN, all other molecules are sufficiently volatile for efficient detection by headspace GC-MS.  
81 The matrix simplification obtained by headspace analysis offers remarkable benefits. Sample preparation and pu-  
82 rification are minimized: often a simple dilution followed by reaction with  $\text{Et}_3\text{O}^+$  is sufficient and avoids single-use  
83 plastic labware for sample cleaning, preconcentration and filtration. Furthermore, headspace analysis reduces the  
84 need for instrumental maintenance, allows the analytical column to be used for several years, and generates clean  
85 and interference-free GC-MS chromatograms even for complex samples.

86 Recent headspace GC-MS methods allowed accurate quantitation of nitrite, nitrate and total dissolved nitrogen in  
87 seawater [28, 30], thiocyanate in saliva [13] and exhaled breath condensate (EBC) [14], nitrate in vegetable extracts  
88 [11], and inorganic chloride in crude oil [12] (Table 1). For all these applications, the detection limit was tailored  
89 for the requirements and the experimental conditions could be tuned to quantify from low ng/g  $\text{SCN}^-$  in ECB [14]  
90 up to mg/g  $\text{NO}_3^-$  in vegetables [11]. Headspace GC-MS allowed also for rapid determinations with a high sample  
91 throughput. For example, using a standard 30 m capillary DB 5.625 column, the measurement of  $\text{NO}_3^-$  in vegeta-  
92 bles and  $\text{Cl}^-$  in crude oil could be obtained within 1.8 and 6.0 min.

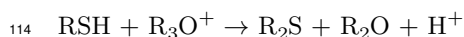
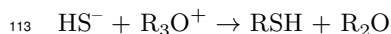
93 Recently,  $\text{Et}_3\text{O}^+[\text{BF}_4]^-$  derivatization was extended to the analysis of  $\text{SeCN}^-$ , which is a contaminant found in  
94 wastewater originating from coal, petroleum, and gold mining industries [31]. The  $\text{SeCN}^-$  was fully converted into  
95  $\text{EtSeCN}$  by aqueous reaction with  $\text{Et}_3\text{O}^+$ . The derivative was not sufficiently volatile for headspace sampling, there-  
96 fore its extraction was obtained in chloroform. As the  $\text{Et}_3\text{O}^+[\text{BF}_4]^-$  hydrolyzes in water to yield  $\text{EtOH}$ ,  $\text{Et}_2\text{O}$  and  
97  $\text{BF}_4^-(\text{aq})$ , the large reagent excess did not interfere with the GC-MS analysis of the chloroform extract, resulting in  
98 a clean chromatography with a stable baseline.

99 Methods for inorganic anions based on aqueous  $\text{Et}_3\text{O}^+$  derivatization are very simple to implement, fast, econom-  
100 ical, and make use of standard GC-MS instruments which are largely available in testing laboratory worldwide.  
101 Furthermore, MS also allows implementation of high-precision quantitation by isotope dilution mass spectrometry  
102 (IDMS). IDMS is regarded as a primary ratio method [36] and it is widely employed for metrological applications.  
103 Notably, some of the internal standards for anions analysis are available as affordable  $^{15}\text{N}$ ,  $^{13}\text{C}$ , and  $^{37}\text{Cl}$  enriched  
104 materials which could be used for routine analysis [37].

105 Most of the research developed for inorganic anions was devoted to achieve sensitive and specific detection within  
106 few minutes of analysis and with minimal sample preparation. Future investigations could explore experimental con-  
107 ditions aimed to enhance the ethylation yield with the use of preconcentration techniques for sub part-per-billion  
108 detection.

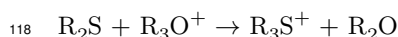
#### 109 **4 Inorganic sulfide, thiols and thioether: the sulfonium salt**

110 In 2009, we demonstrated that the reaction between a diluted aqueous solution of  $\text{Na}_2\text{S}$  with  $\text{Me}_3\text{O}^+[\text{BF}_4]^-$  and  
111  $\text{Et}_3\text{O}^+[\text{BF}_4]^-$  resulted in generation of  $\text{Me}_2\text{S}$  and  $\text{Et}_2\text{S}$ , both identified by headspace GC-MS [9]. In this case, two  
112 ethyl-groups were linked to the analyte in a step-wise process:

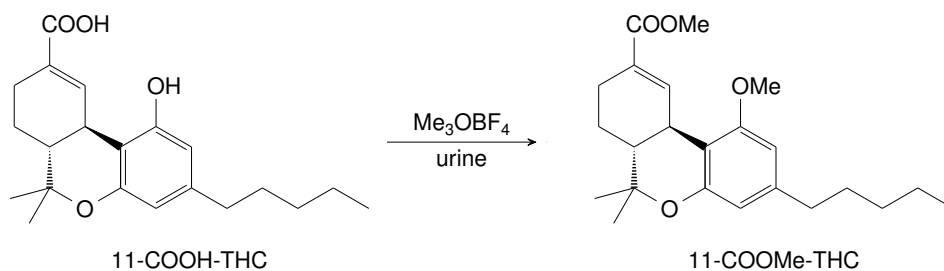


115 Although such chemistry was fit for sulfide determination by headspace GC-MS, no applications to real samples  
116 have been demonstrated so far.

117 Most notably, the thioethers have the disposition for a further alkylation [7, 34]:



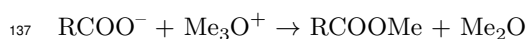
119 Kozlov et al. [38] made use of alkyloxonium salts for the generation of various  $R_3S^+$  cations. The preparation was  
120 obtained from reaction of the organic sulfides with  $Me_3O^+[BF_4]^-$  or  $Et_3O^+[BF_4]^-$  in  $CH_2Cl_2$  for 24 h at 30 °C. The  
121 generation of stable, permanently charged sulfonium cations was beneficial for detection by MALDI-MS and ESI-  
122 MS. Upon a better understanding of the reaction conditions necessary for formation of  $R_3S^+$  cations, this approach  
123 could witness further development for MS analysis of organic thiols.



**Fig. 1:** Full methylation of THC and 11-COOH-THC directly in urine matrix was obtained using  $Me_3O^+[BF_4]^-$  at  $pH > 12$ . The derivatives were suitable for sensitive GC-MS analysis [26]

## 124 5 Carboxylic acids and phenols

125 The use of trialkyloxonium salts for esterification of carboxylic acids is long known to organic chemists. Already  
126 in 1979 Raber et al. [39] applied  $Me_3O^+[BF_4]^-$  and  $Et_3O^+[BF_4]^-$  for high-yield conversion of carboxylic acids into  
127 their corresponding methyl and ethyl esters. More importantly, sterically hindered and polyfunctional carboxylic  
128 acids could be efficiently converted within 24 h at room temperature by conducting the reaction in  $CH_2Cl_2$  in the  
129 presence of *N,N*-diisopropylethylamine (DIPEA). Such reactivity has been used for determination of organic acids  
130 by GC-MS. In 1993, Dirven et al. [19] applied trialkyloxonium chemistry for the derivatization of four metabolites  
131 in urine deriving from the degradation of bis(2-ethylhexyl) phthalate. After enzymatic hydrolysis of the samples, the  
132 metabolites were extracted in diethyl ether and derivatized with  $Et_3O^+[BF_4]^-$  in  $CH_2Cl_2$  with DIPEA. The  $CH_2Cl_2$   
133 was dried and reconstituted in *n*-hexane before on-column EI GC-MS analysis. A similar approach was undertaken  
134 by Liebich et al. [20, 21] and by Pacenti et al. [22] for the screening of organic acids in urine. The direct methylation  
135 of urine metabolites was carried out by treating 2 mL sample with excess  $Me_3O^+[BF_4]^-$  following neutralization  
136 with bicarbonate:

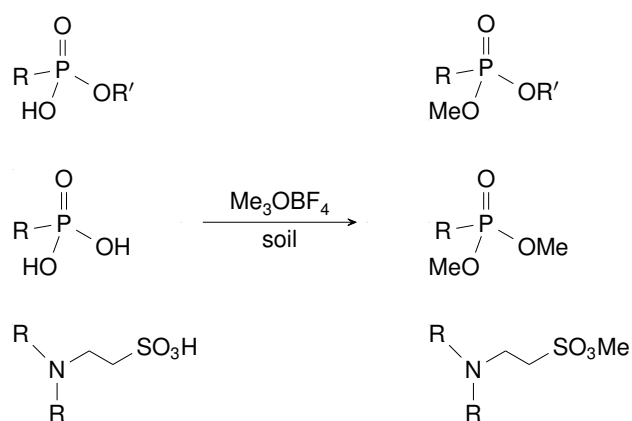


138 A  $\text{pH} > 7$  is required to shift the analyte acid-base equilibrium toward the carboxylate form which is readily reactive  
139 with  $\text{Me}_3\text{O}^+$ . For GC-MS applications, the use of  $\text{HCO}_3^-/\text{CO}_3^{2-}$  buffer is a better choice than DIPEA as this amine  
140 will result in a large and tailed GC peak which may overlap with the analytes in question. The methyl ester deriva-  
141 tives could be captured by 20 min immersion SPME following detection by EI GC-MS.  $\text{MeO}_3^+[\text{BF}_4]^-$  was effective  
142 in esterifying fatty acids, dicarboxylic acids, hydroxycarboxylic acids, and aromatic acids with similar performance  
143 with respect to the reference diazomethane method [21, 22]. In the conditions employed for aqueous alkylation,  
144  $\text{Me}_3\text{O}^+[\text{BF}_4]^-$  reacted also with phenolic alcohols but not with the aliphatic ones. Liebich et al. [20, 21] identified  
145 more than 50 organic acids in urine and Pacenti et al. [22] employed the method to quantify Biological Exposure  
146 Indices in urine including furoic acid, hippuric acid, methylhippuric acid, mandelic acid, phenylglyoxylic acid, and  
147 (*E,E*)-muconic acid. The yield of methylation in aqueous media was  $>95\%$  for all analytes.

148 Chericoni et al. [26] proposed another interesting application of the  $\text{Me}_3\text{O}^+[\text{BF}_4]^-$  derivatization for GC-MS deter-  
149 mination of  $\Delta^9$ -tetrahydrocannabinol (THC) and 11-nor- $\Delta^9$ -tetrahydrocannabinol carboxylic acid (11-COOH-THC)  
150 in urine. Since 11-COOH-THC is nonvolatile and decarboxylates at high temperature, its detection by GC-MS re-  
151 quires derivatization [40]. As reported in Fig. 1,  $\text{Me}_3\text{O}^+[\text{BF}_4]^-$  methylates both phenolic and carboxylic functions  
152 directly in the aqueous urine matrix adjusted at  $\text{pH} > 12$  by NaOH. The methyl derivatives were extracted in or-  
153 ganic solvent and detected by EI GC-MS with a LOD of 0.5-0.7 ng/mL.

154 For urine analysis, one method made use of  $\text{Et}_3\text{O}^+[\text{BF}_4]^-$  in  $\text{CH}_2\text{Cl}_2$ :DIPEA [19], whereas the other four [20-22, 26]  
155 performed the alkylation transferring solid  $\text{Me}_3\text{O}^+[\text{BF}_4]^-$  directly to the alkalized urine matrix, following extrac-  
156 tion of the derivatives in solvent or with SPME (Table 1). These procedures were very simple to implement and the  
157 alkylation tolerated complex urine matrix at  $\text{pH} > 7$ .

158 Recently, this derivatization chemistry was applied for the determination of geronic acid in food samples by GC-MS  
159 [29]. This carboxylic acid is a marker related to the spontaneous oxidation of carotenoids in food. The geronic acid  
160 was extracted from the samples and derivatized by  $\text{Me}_3\text{O}^+[\text{BF}_4]^-$  in  $\text{MeOH}:\text{H}_2\text{O}$  with  $\text{NaHCO}_3$  for maintaining  
161  $\text{pH} > 7$ . The method was successfully applied to 22 food samples where the analyte was quantified from 1.5 to over  
162 10,000 ng/g.

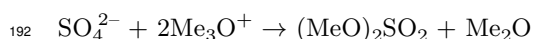


**Fig. 2:** Methylation of chemical warfare agents degradation byproducts directly in soil samples using  $\text{Me}_3\text{O}^+[\text{BF}_4]^-$  in  $\text{CH}_2\text{Cl}_2$ . The derivatives were confirmed by GC-MS and GC-FPD analysis. Successful identification was reported for: pinacolyl methylphosphonate, isopropyl methylphosphonate, cyclohexyl methylphosphonate, ethyl methylphosphonate, methylphosphonic acid, methyl methylphosphonate, ethylphosphonic acid, propylphosphonic acid, *N,N*-diethylaminoethanesulfonic acid, and *N,N*-diisopropylaminoethanesulfonic acid [15–17]

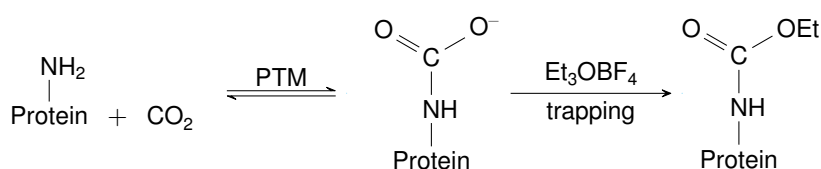
## 163 6 Chemical warfare agents forensic screening

164 Since 2016, Valdez et al. [15–17] introduced  $\text{Me}_3\text{O}^+[\text{BF}_4]^-$  as a derivatizing reagent for the identification of chemical  
 165 warfare agent (CWAs) degradation products by GC-MS. The group has focused on the analysis of organophosphorus-  
 166 based nerve agents classified as Schedule I chemicals by the Organization for the Prohibition of Chemical Weapon  
 167 (OPCW). Since the native nerve agents are short-lived in the environment, testing of environmental samples is aimed  
 168 to identify the phosphonic acids resulting from their degradation. Due to its diffusion, ease-of-use, and portability,  
 169 GC-MS is one of the standard techniques used for measurements in support to the Chemical Weapons Conven-  
 170 tion (CWC). Since the phosphonic acids are polar and nonvolatile, derivatization is required for their successful  
 171 detection by GC-MS means. Common strategies, including silylation and alkylation, are not without shortcom-  
 172 ings [41]. For example, the silyl derivatives are moisture sensitivity and alkylation is primarily performed with the  
 173 toxic and explosive diazomethane. For these reasons, Valdez et al. [15–17] demonstrated the efficacy of the much  
 174 safer  $\text{Me}_3\text{O}^+[\text{BF}_4]^-$  reagent for performing the methylation of a variety of phosphonic and sulfonic acids associated  
 175 with the organophosphorus-based nerve agents. Some of these target analytes included the ones in Fig. 2. Such  
 176 compounds were converted in the corresponding methyl ester by reaction with  $\text{Me}_3\text{O}^+[\text{BF}_4]^-$  in  $\text{CH}_2\text{Cl}_2$  at room  
 177 temperature. Although  $\text{Me}_3\text{O}^+[\text{BF}_4]^-$  is not soluble in  $\text{CH}_2\text{Cl}_2$ , the alkylation still proceeded to quantitative con-  
 178 version. The method was applied to the analysis of real samples including a fatty-acid ester organic matrix [15]  
 179 and several types of soil [16, 17] featured in recent OCPW proficiency tests. The sample preparation proposed by  
 180 Valdez et al. is noteworthy for its simplicity: 200-300 mg sample was suspended in 1-2 mL  $\text{CH}_2\text{Cl}_2$  with 20-150

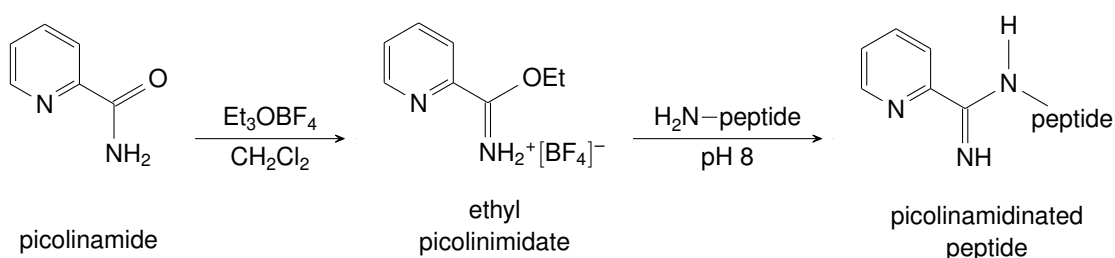
181 mg  $\text{Me}_3\text{O}^+[\text{BF}_4]^-$  [16, 17]. Full methylation was obtained in 3-5 hours at room temperature with constant stir-  
 182 ring. The  $\text{CH}_2\text{Cl}_2$  phase was washed with aqueous sodium bicarbonate, to remove/neutralize acids and unreacted  
 183  $\text{Me}_3\text{O}^+[\text{BF}_4]^-$  which would have damaged the analytical column. After drying on anhydrous sodium sulfate, the  
 184  $\text{CH}_2\text{Cl}_2$  extract was analyzed by GC-MS and GC-FPD (i.e. GC coupled to a flame photometric detector specific for  
 185 P- and S-based analytes). Most notably, the method was minimally affected by complex matrices and in all cases  
 186 allowed identification of phosphonic acids at the 10  $\mu\text{g/g}$  level. Since alkaline hydrolysis of  $\text{Me}_3\text{O}^+[\text{BF}_4]^-$  resulted in  
 187 MeOH,  $\text{Me}_2\text{O}$ , and  $\text{BF}_4^-(\text{aq})$ , a large excess of reagent did not interfere with the chromatography.  
 188 Beside esterification of phosphonic acids, Valdez et al. [15] demonstrated that  $\text{Me}_3\text{O}^+[\text{BF}_4]^-$  could perform methy-  
 189 lation also of sulfonic acids related to the CWC. Within the same derivatization scheme, *N,N*-diethylaminoethane-  
 190 sulfonic acid and *N,N*-diisopropylaminoethanesulfonic acid were converted to the corresponding methyl esters (Fig.  
 191 2). For a sample of Virginia type A soil, the GC-FPD chromatograms also showed a peak for  $(\text{MeO})_2\text{SO}_2$  [16]:



193 Within the research work performed on the alkylation of inorganic anions in water media, such reactivity was not  
 194 observed [9, 10], and the sulfate was retained quite inert to the alkylation [31]. The work of Valdez et al. underlined  
 195 that the alkylation power of  $\text{R}_3\text{O}^+$  can be increased by working in a nonaqueous media where successful conversion  
 196 of -P-OH and -S-OH moieties was obtained within a simple experimental design.



**Fig. 3:** The fleeting *N*-carboxylated intermediary PTMs can be successfully trapped via ethylation with  $\text{Et}_3\text{O}^+[\text{BF}_4]^-$  under physiological conditions. The derivatives were identified by downstream proteomics using LC-MS/MS [18]



**Fig. 4:** Ethylation of picolinamide with  $\text{Et}_3\text{O}^+[\text{BF}_4]^-$  furnished the highly reactive ethyl picolinimidate intermediate. Reaction between the imidate and available amino groups in peptide chains yields derivatives with enhanced sensitivity for detection by MALDI-TOF/TOF [23–25]

## 197 **7 Bioanalytical applications: Proteomics**

198 In 2018, Linthwaite et al. [18] employed  $\text{Et}_3\text{O}^+[\text{BF}_4]^-$  in the derivatization and identification of carbon dioxide  
199 mediated post-translational modification (PTM) sites in proteins. Under physiological conditions, the  $\text{CO}_2$  can  
200 react with  $-\text{NH}_2$  moieties present on a protein, resulting in their carbamylation (Fig. 3). The  $\text{CO}_2$  mediated PTMs  
201 have a role in the modulation of protein activity [42, 43] and there is interest to study such PTMs within the context  
202 of protein regulation [18]. From an analytical point-of-view, detection of  $\text{CO}_2$  mediated PTMs is a challenging task  
203 as these carbamates easily revert to the native amines under non-physiological conditions (Fig. 3). For this reason,  
204 Linthwaite et al. used  $\text{Et}_3\text{O}^+[\text{BF}_4]^-$  to successfully trap the carbamate as a stable ethyl derivative (Fig. 3) [18]. The  
205 derivatization was demonstrated on free amino acids and small peptides, and then it was implemented to detect  $\text{CO}_2$   
206 mediated PTMs on proteins within intact red blood cells. The ethylation was carried out in an aqueous environment  
207 at pH 7.4 under relevant physiological conditions to ensure that the carbamylation equilibrium was not perturbed  
208 during sample preparation. For this purpose, acidity resulting from  $\text{Et}_3\text{O}^+$  hydrolysis was buffered by treating the  
209 reaction media with a 0.1 M NaOH solution. The authors demonstrated that in this environment the half-life of  
210  $\text{Et}_3\text{O}^+$  was 6 min, long enough for the ethylation to take place. The stable ethyl derivatives could be detected  
211 following downstream proteomics analysis by LC-MS/MS [18]. The potential of  $\text{Et}_3\text{O}^+[\text{BF}_4]^-$  to perform ethylation  
212 under mild physiological conditions was crucial for the successful trapping and identification of this notoriously  
213 labile PTM.

214 Within the context of peptides analysis, Kim et al. [23–25] made use of  $\text{Et}_3\text{O}^+[\text{BF}_4]^-$  in the picolinamidation of  
215 amino functionalities in peptides. The  $\text{Et}_3\text{O}^+[\text{BF}_4]^-$  could initially react to generate ethyl picolinimidate in  $\text{CH}_2\text{Cl}_2$   
216 at room temperature. Next, this intermediate was then used to derivatize *N*-terminal amino groups and the  $\epsilon$ -  
217 amino groups of lysine residues according to the reaction shown in Fig. 4. Upon derivatization, the sensitivity of the  
218 MALDI-TOF/TOF detection was enhanced by 20-35 fold with respect to the native peptide [23]. This chemistry was  
219 used for conversion of peptides with one, two and three amino groups: in all case, their picolinamidation was almost  
220 quantitative. The method was also employed for the derivatization of phosphopeptides, enhancing their MALDI-  
221 TOF/TOF response by two orders of magnitude [24]. As expected, the gain in sensitivity was higher in analytes  
222 displaying more than one  $-\text{NH}_2$  group [23]. In a more recent work, Kim et al. [25] employed this derivatization  
223 chemistry with a brominated ethyl picolinimidate able to add the characteristic Br isotopic signature to *N*-terminal  
224 sequences, facilitating their identification by MALDI-TOF/TOF.

## 225 8 Outlook

226 Trialkyloxonium salts are a valid alternative to common alkylating agents used for sample preparation in analytical  
227 chemistry. The salts are easy to handle and, in most cases, can alkylate the analytes directly within the sample  
228 matrix without the need of their dissolution in the reaction medium. The applications discussed in this *Trend*  
229 emphasize the versatility of these reagents which could be employed for a variety of analytes including inorganic  
230 anions, carboxylic acids, phosphonic acids and proteins. Overall, the methods relying on trialkyloxonium salts are  
231 very simple to implement, can easily be transferred between laboratories, and do not share the same level of risk  
232 associated to other alkylation protocols like those based on diazomethane. Furthermore,  $R_3O^+[BF_4]^-$  salts tolerate  
233 working in very complex matrices and do not interfere in GC-MS and LC-MS analysis despite their use in large  
234 molar excess. The  $R_3O^+[BF_4]^-$  could be employed under diverse conditions including aqueous and nonaqueous  
235 media, acidic and alkaline pH, and in a physiological environment for the derivatization of carbon dioxide induced  
236 protein post-translational modifications.

237 In the field of chemical derivatization, the use of trialkyloxonium salts for sample preparation is a novel area of  
238 investigation and most of the applications presented in this *Trend* were published within the last decade. Considering  
239 the many benefits of this chemistry, more analytical developments are expected in the future, starting from a  
240 systematic study of all the functional groups that could conceivably undergo the derivatization. Furthermore and in  
241 parallel fashion, studies aimed towards an understanding of the key factors governing the alkylation process would  
242 have to be implemented in order to identify the most efficient conditions for the transformation.

243 So far, applications of  $R_3O^+[BF_4]^-$  are limited to  $Me_3O^+[BF_4]^-$  and  $Et_3O^+[BF_4]^-$  which are commercially available  
244 reagents. In analytical chemistry is sometime useful converting the analytes using unique molecular signatures. In  
245 this vein, the preparation of halogenated (i.e. fluorinated or brominated) trialkyloxonium salts may further extend  
246 the interest of  $R_3O^+[BF_4]^-$  derivatization for both screening and quantitation purposes.

## 247 9 Compliance with Ethical Standards

248 The author declares no conflict of interest.

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