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Simultaneous analysis of quaternary ammonium cations and corresponding halide counterions by pyrolysis gas chromatography / mass spectrometry

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ABSTRACT

The use of quaternary ammonium compounds (QACs) as disinfectants has increased tremendously in the COVID-19 pandemic to inactivate Severe Acute Respiratory Syndrome Coronavirus type 2 (SARS-CoV2). Dialkyldimethylammonium halides represent a frequently used type among QACs. Different halide anions, each ionically linked to the same quaternary ammonium cation, show clear differences in biocidal activity, toxicity and allergic potential. Likewise, the alkyl chain length at the ammonium cation induces different biocidal efficacy and toxicology. Therefore, the object of this research was to develop a rapid and reliable method for the detection of ammonium cation and halide anion in a single analytical run. For that purpose, a gas chromatography mass spectrometry (GC/MS) method was developed for QACs of the dialkyldimethylammonium type. Pyrolytic conversion of the QACs in the injector port of the gas chromatograph into volatile molecule species allows fast and reliable subsequent GC/MS analysis. The developed method is suited for the determination of both the quaternary ammonium cation and the corresponding halide anion in a single gas chromatographic run. The application of this method to bulk material and standard material of explicitly specified didecyldimethylammonium chloride revealed deviations from the manufacturer’s specifications in a range up to four-fifths. Furthermore, didecyldimethylammonium chloride was detected in a disinfectant that does not comply with the labeling requirement for biocidal ingredients. With the method presented, results can be obtained for disinfectants with minimum effort within seven minutes.

1. Introduction

The outbreak of the COVID-19 pandemic has prompted health organizations around the world to urge for regular disinfection of hands and surfaces to impede the spread of the novel coronavirus. New health concepts have been created in many places, new hygiene procedures for regular disinfection of various premises have been initiated and implemented in a short time. An enormous use of disinfectants in many companies and health care facilities was the result [1,2]. The application of disinfectants with efficacy against enveloped viruses is sufficient according to the recommendation of the Austrian Agency for Health and Food Safety. Accordingly, for SARS-CoV-2 effective hand or surface disinfection is recommended, whereby the usage of disinfectants labeled as "limited virucidal" is acceptable [3].

Quaternary ammonium compounds (QACs) are seeing a notable increase in the application as disinfectants in the current COVID-19 pandemic, but QACs have already been an economically important class of industrial chemicals before [4]. In addition to their broad usage as disinfectants, they are also applied as fabric softeners, corrosion inhibitors in building materials, as preservatives or in a multitude of personal care products [4]. QACs are also found in everyday products often without being recognizable for the consumer [5]. QACs had received public attention in 2012 when didecyldimethylammonium chloride (DDAC-C10) was found in ice cream. It was traced as residue after cleaning of manufacturing equipment with products containing DDAC-C10 and lack of sufficient post-cleaning [6].

QACs pose a hazard to the user if concentrated products are handled improperly. Direct contact may cause skin irritation, injury to the eyes or even fibrosis and lung inflammation [7–9]. QACs are toxic to aquatic organisms, poorly degradable and therefore harmful to the environment [10–12]. Contrary there is currently an understandable recommendation for a wider use of this group of agents due to the virucidal properties [1].

Dialkyldimethylammonium halides represent a frequently used type among QACs. Major substances are DDAC-C10 and didecyldimethylammonium bromide (DDAB-C10). Diocetyl dimethyl ammonium chloride...
(DDAC-C8) and dioctydlydimethylammonium bromide (DDAB-C8) represent the shorter alkyl-chain molecules and the variant with an alkyl chain length of 12 carbon atoms are didodecyldimethylammonium chloride (DDAC-C12) and didodecyldimethylammonium bromide (DDAB-C12).

Despite considerable chemical similarity, the different chain lengths of the ammonium cations show differences in biocidal activity and allergic sensitization potential. Different halide anions ionically bound to the same quaternary ammonium cation also show clear differences in biocidal activity toxicity and allergic potential \[8,13-15\]. Although the diverse halide ions apparently induce divergences in terms of safety and hazards of QACs, the QAC cations are the main focus of interest \[11\].

Quaternary ammonium compounds as a group of charged molecules are readily analyzed by ion exchange chromatography and also by capillary electrophoresis (CE) \[16,17\]. The halide counter ion is easily determined separately by ion chromatography \[18\], but a determination of both the ammonium cation and the halide anion with one method is not simply realized in this way.

When analyzing ionic compounds, gas chromatography (GC) is not the analytical technique of first choice due to the unmet primary requirement of component volatility. Nevertheless, if GC is feasible, it may be superior to other techniques due to a number of aspects: GC capillary columns compared to high performance liquid chromatography (HPLC) columns may have significantly higher plate numbers which results in a higher peak capacity. GC can be operated with less effort without the need of preparing liquid mobile phases. Furthermore, when hyphenated with mass spectrometry GC may be more robust compared to HPLC methods. So, in general, GC provides a result very quickly with short run times.

In case of quaternary ammonium compounds, gas chromatography may be possible by pyrolytic conversion of the ammonium salt in the injector port of the gas chromatograph to volatile pyrolysis products \[19-22\]. Previous work on the gas chromatographic analysis of pyrolysis products of quaternary ammonium compounds varies in terms of the analytes selected, the matrices, and also the place of pyrolysis (in situ in the injector \[19-22\] or offline prior to injection \[23\]). Above all, however, the published investigations differ regarding their objectives, thus a direct comparability is only partly possible. Some work focused on fundamental aspects of the pyrolysis of selected compounds, which were tested as aqueous solutions \[19,20\]. Nevertheless, injector port pyrolysis of QACs in aqueous solutions has the disadvantage that water significantly stresses the surface of the gas chromatographic separation column. To overcome this problem, in a recent work the pyrolysis of aromatic substituted quaternary ammonium compounds by headspace GC/MS was performed \[23\]. However, that method requires a total analysis time for a single measurement (equilibration time and GC run time) of more than one hour.

Contrary to published work, the goal of the present investigations should be an easy-to-perform in situ pyrolysis GC/MS method with short analysis times for QACs of the dialkyldimethylammonium type in disinfectants. The problem of the water content in the samples should be solved by diluting samples with an organic solvent, thus avoiding negative effects on the gas chromatographic column.

For QACs used as disinfectants a simultaneous quantitation of both the cation and the halide counter ion is desirable since the halide counter ions also play an important role in terms of allergic potential as mentioned above. GC methods applied so far for QAC analysis have not yet achieved the simultaneous detection of both the ammonium cation and the halide anion in one run. Therefore, in addition to the goal of easy-to-perform and fast to execute, the method should also allow to quickly obtain information on the cationic and anionic composition of common QACs in disinfectants. These products typically represent a rather simple matrix, so that major challenges regarding the separation of analytes and matrix peaks were not to be expected.

2. Materials and methods

2.1. Chemicals

Isopropanol, methanol and acetone analytical grade were obtained from VWR (Fontenay-sous-Bois, France). Ethanol analytical grade was acquired from Supelco Sigma Aldrich (St. Louis, MO, USA). Didecyldimethylammonium chloride (DDAC-C10) was purchased from Supelco Sigma Aldrich (St. Louis, MO, USA). Dioctydlydimethylammonium bromide (DDAB-C8) was obtained from ABCR (Karlsruhe, Germany); didecyldimethylammonium bromide (DDAB-C10) and dioctydlydimethylammonium bromide (DDAB-C12) were acquired from Fluka (Buchs, Switzerland). The general structure of these quaternary ammonium compounds is given in Fig. 1. Didecylmethylamine, decyldimethylamine, dioctylmethylamine and didodecylmethylamine were obtained from ABCR (Karlsruhe, Germany); decylchloride and decylbromide were purchased from Sigma Aldrich (St. Louis, MO, USA).

Standard material of quaternary ammonium compounds, tertiary amines and alkyl halides was dissolved in isopropanol in the concentration range of 0.001–0.1% (w/v). In the case of bulk materials - goods (in large supply quantities) that are processed by companies into disinfectants for end-users - sample solutions were always prepared freshly by dissolving the quaternary compound in isopropanol to obtain a concentration range between 0.01% and 0.1% (w/v). After homogenization at room temperature for 1 min either in an ultrasonic bath or with a laboratory vortexer, the solutions were filtered through a membrane filter (0.45 μm). In the case of commercially available disinfectants, 100–500 mg were dissolved in 10 ml isopropanol.

2.2. Gas Chromatography / mass spectrometry (GC/MS)

Gas chromatographic analysis was carried out using a Focus GC coupled to a DSQII quadrupole MS from Thermo Fisher Scientific (Waltham, MA, USA). The injection (injection volume 1 μl) was performed in split mode 1:50 at an injection temperature of 300 °C. A liner containing glass wool from Thermo Fisher Scientific (Waltham, MA, USA) was used. In case of the pyrolysis experiment with solid standard material without any solvent a liner with a small volume (a solid phase micro extraction liner) was installed.

Separation was carried out on an Optima 5 MS Accent fused silica column (25 m x 0.20 mm i.d.; 0.20 μm film thickness) from Machery Nagel (Düren, Germany). The oven program started at 65 °C and the temperature was increased to 300 °C (held for 5 min) at 40 °C/min. Helium (4.6) was used as mobile phase with a column flow rate of 1.0 ml/min. The mass spectrometer was operated in the range of m/z 50–500 in the scan mode. The ion source temperature was adjusted to 220 °C and transfer line temperature configured to 300 °C. The temperature in the injection port of 300 °C was selected after experimentally
determining the temperature-dependent response optimum for the lead compound DDAC-C10 dissolved in isopropanol. For the quantitation of DDAC-10, the peak area of its pyrolysis product didecylmethylamine was determined using the extracted m/z 184.

3. Results and discussion

3.1. Mechanism

As reported in the literature [19,21,22], pyrolysis of quaternary ammonium compounds in the injector of the GC produces tertiary amines and organic halides. So far, this principle has been exploited for the quantitation of the quaternary ammonium cation without taking advantage of the possibility to identify the corresponding anion simultaneously. Pyrolysis of quaternary ammonium compounds of the dialkyl(dimethyl)ammonium halide type in the injector of the GC produces two tertiary amines, that is didecylmethylamine and dimethylalkylamine, and two organic halides namely alkyl halide and methyl halide. The pyrolysis reaction follows the chemical reaction type of a nucleophilic substitution. The negatively charged halide anion acts on the carbon atom adjacent to the nitrogen atom as an electron pair donor, thus replacing the nitrogen atom. The mechanisms are shown in Fig. 2 for DDAC-C10 as an example. The efficiency of the pyrolytic process depends on the temperature of the GC injector. For the molecule DDAC-C10 the response of the molecules generated by pyrolysis reaches a plateau at 280 °C. Fig. S1 in the Supplementary material shows the dependence of peak area of didecylmethylamine produced from DDAC-C10 upon pyrolysis temperature. Accordingly, in all subsequent analysis 300 °C injector port temperature was used. This observed plateau is in good agreement with the results from the literature [19], where the formation of the tertiary amine from the pyrolysis of QACs in dependence of the injector temperature likewise showed a plateau beginning at approximately 280 °C. Compared to the other pyrolysis products, the dialkylmethylamine species provides the highest response and was therefore used for quantitation in all analyses. The pyrolysis of DDAC-C10 for example yields didecylmethylamine as a product of highest response (compare Fig. 2).

The solvents tested for QAC dissolution gave similar responses. The use of isopropanol resulted in the highest response. The other solvents tested were methanol, ethanol and acetone that resulted in a reduced relative response of approximately 80%. In addition, bulk material and disinfectants are often on an isopropanol basis. Consequently, in all subsequent analysis isopropanol was used for dissolving standard material and all types of samples.

Fig. 3 shows a chromatogram of the quaternary ammonium compound DDAC-C10 (a) pyrolytically converted in the injector to the components decylchloride, decyldimethylamine and didecylmethylamine. In addition, the chromatograms of the standard materials decylchloride (b), the two tertiary amines decyldimethylamine (c) and didecylmethylamine (d) are shown.

Methylchloride cannot be seen in the chromatogram because its retention time lies in the unrecorded time range of the solvent peak. The formation of methylchloride (and methylbromide respectively) through pyrolytic cleavage can be confirmed by solid material introduction using a syringe without any solvent. Figs. S2 and S3 given in the Supporting material show chromatograms of DDAC-C10 and DDAB-C10 after pyrolysis of the solid compounds, and the corresponding mass spectra. The differentiation of QACs with different halides ionically bound to the same quaternary ammonium cation is possible due to the different gas chromatographic retention of the alkyl halides generated by pyrolysis. Fig. 4 shows chromatograms of pyrolytic converted DDAC-C10 (a) and DDAB-C10 (b). Regarding the generated molecules of the same alkyl chain length, as expected, the alkylhalides with lower boiling point reach the detector before the alkylbromides (Fig. 4). In general, alkylhalides fragment readily during electron impact ionization so that the molecular ion cannot be identified in the mass spectrum [24].
Nevertheless, even without the molecular ion peak, the use of the spectra library and the use of an authentic standard material has confirmed the molecular structure. Both molecules exhibit the typical mass spectrometric fragment patterns of chlorinated and brominated molecules, respectively.

In addition, Fig. 4 shows a comparison of the chromatograms of QACs with different alkyl chain length converted by pyrolysis: DDAC-C10 (b), DDAB-C8 (c), DDAB-C12 (d). These compounds are converted into two types of tertiary amines with a typical increasing retention order octyldimethylamine < decyldimethylamine < dodecyldimethylamine on the one hand, and dioctylmethylamine < didecylmethylamine < didodecylmethylamine on the other hand. Octyldimethylamine and dodecyldimethylamine were identified via the mass spectrometry library. All other tertiary amines were confirmed according to their retention time and mass spectrum with authentic standard materials.

3.2. Validation

The method performance was validated according to the ICH guidelines [25]. Detection/quantitation limit (LOD/LOQ) for DDAC-C10 when measuring the didecylmethylamine generated in the GC injector were defined according to [26] as 4.5 μg/ml and 1.5 μg/ml respectively. In a range from LOQ to 1000 μg/ml the relationship between peak area and concentration followed the polynomial equation $y = 65.53x^2 + 377528x - 5E+06$. A linear relationship could be observed within 50–1000 μg/ml ($R^2 = 0.9993$). The precision of the method was determined in the linear range considering 5 calibrators (50, 100, 250, 500, 1000 μg/ml) to be 3.9%. The intermediate precision was determined likewise as 7.4% considering two individual calibration series of five calibrators in the given range measured on individual days. The repeatability of a sixfold determination of a DDAC-C10 standard with the concentration of 1000 μg/ml showed a relative standard deviation of 2.9%. Accuracy was determined to be 104%, 105%, and 106%, respectively, with triplicate determinations of a disinfectant solution containing 200 μg/ml spiked with 50%, 100%, 150% of DDAC-C10. To test the robustness of the method, the injection temperature was varied by plus/minus 10 degrees. A triple determination of a standard solution (1000 μg/ml) at an injector temperature of 290 °C resulted in a deviation of -5.9% and at 310 °C in a deviation of +4.7%.

![Fig. 4. Pyrolysis GC chromatograms of DDAC-C10 (a), DDAB-C10 (b), DDAB-C8 (c), DDAB-C12 (d).]
3.3. Application to commercial products

3.3.1. Application to disinfectants

Disinfectant products of the dialkyldimethylammonium type on the market today are of simple composition, namely an aqueous/alcoholic matrix. Therefore, challenges regarding the separation of analytes and matrix peaks were not to be expected. Representative chromatograms of DDAC-10 in two commercially available disinfectants (denoted as disinfectant product 1 and disinfectant product 2) converted by pyrolysis are depicted in Fig. 5. The chromatograms (a, b) show the tertiary amines and the corresponding alkylchloride respectively.

While chromatogram (a) confirms the content of DDAC-C10 in accordance with the label, chromatogram (b) reveals the presence of QAC although it was not declared.

The concentration of DDAC-C10 in the disinfectants was quantified in the scan mode because it provides mass spectrometric information about other components possibly present. Lower quantitation limits that can be obtained in the selected ion monitoring mode were not an issue for the samples analyzed in this work.

In disinfectant product 1 a content of 7.8% was found, which agrees well with 8% given by the manufacturer. In the case of disinfectant product 2, 0.7% DDAC-C10 was determined although it was not indicated on the label. Preliminary tests in single ion monitoring (SIM) mode showed an improvement of the LOD/LOQ by a factor of 8 for DDAC-C10 with the selected ions m/z 311, 184 and 58. Considering the split flow of 1:50 used for the method presented here, there is a further possibility to increase the sensitivity by reducing the split flow in case this is needed.

The chromatograms obtained for commercial products demonstrate that the matrix plays no role, since in scan mode practically only baseline can be seen in addition to the pyrolysis products of the QACs. More sophisticated approaches like HPLC hyphenated with mass spectrometry (necessary for complex matrices such as environmental samples) were not required for the present application.

3.3.2. Application to bulk material

Furthermore, the method was applied to bulk material samples that were explicitly specified as DDAC-C10 with the CAS number 7173-51-5. Fig. 6 shows chromatograms of signals generated by pyrolysis of bulk material samples (a, b, c) and standard material (d). Some of the samples (b, c) showed surprisingly high amounts of impurities (up to four-fifths of quaternary ammonium impurities of deviating alkyl chain length). Further, the bulk material sample (b, c) shows the signals of octylchloride (1), octyldimethylamine (2), decylchloride (3), decyldimethylamine (4), dioctylmethylamine (5), octyldecylmethylamine (6), and didecylmethylamine (7). Moreover, the pattern of these tertiary amines can be seen in the chromatogram of a standard material of DDAC-C10 (d) from the fine chemical industry, but with different signal intensities. The identified tertiary amines dioctylmethylamine (5) octyldecylmethylamine (6) and didecylmethylamine (7) are derived from the QACs dioctyldimethylammonium chloride DDAC-C8 (CAS 5538–94–3) octyldecyldimethylammonium chloride DDAC-C8/C10 (CAS 32426–11–2) and DDAC-C10 (CAS 7173–51–5) respectively. The mass spectrum of octyldecylmethylamine (6) shows the molecular ion m/z 283 and fragments m/z 184 and m/z 156. In fact, tertiary amines lose an alkyl radical, resulting in the formation of a resonance-stabilized iminium radical [24]. Thus, fragment m/z 184 is the decyldimethyliminium radical and fragment m/z 156 is the octyldimethyliminium radical. Both fragments are apparent in the mass spectrum of octyldecylmethylamine (6). Mass spectra of dioctylmethylamine (5) and didecylmethylamine (7) contain only one of these two fragments (see Fig. 7).

Consequently, octyldecylmethylamine (6) is the pyrolytic product of the quaternary ammonium compound DDAC-C8/C10.

The quantitative analysis of bulk materials revealed a DDAC-C10 content of 48%, 18% and 23% (in case of a, b, c, respectively) and 84% for the standard material (d), opposing a designated purity of 99% in the latter case. These deviations may be attributed to the manufacturing process of the quaternary ammonium compound. They are produced by a reaction of amines with a surplus of an alkylating agent. Starting from decylamine contaminated with octylamine or from mixtures of decylamine and octylamine together with methylchloride may result in the three different molecules of quaternary ammonium compounds mentioned above.

4. Conclusion

The aim of the present work was to provide an easy-to-perform in situ pyrolysis GC/MS method with short analysis times for QACs of the dialkyldimethylammonium type in disinfectants. Pyrolytic conversion
of QACs in the injector of the gas chromatograph generates volatile compounds that can be measured by GC. The possibility of simultaneous determination of quaternary ammonium cations and the corresponding halide anions has not been pointed out or exploited so far.

A simple dilution of a disinfectant product with isopropanol prior to injection is sufficient to provide information on the nature of both the quaternary cation and halide anion of QACs.

This is the key advantage of the GC method presented here: a fast dilute and shoot approach allows to quantitatively assess standard materials, wholesale goods and commercially available disinfectants by one single GC run within 7 min.

Furthermore, in the present work it was revealed that materials clearly designated as DDAC-C10 show significant deviations in product quality. In fact, some of these samples contained up to four-fifths of quaternary ammonium impurities of deviating alkyl chain length. DDAC-C8 as well as DDAC-C8/C10 could be determined unambiguously in these samples and thus contradict the given CAS number of the samples. Even a standard material of DDAC-C10 was contaminated with these components and showed a content of only 84% instead of 99%.

Therefore, an ongoing evaluation of dialkyldimethylammonium compounds available on the market using the approach described here is desirable in order to distinguish the different existing product quality.

Fig. 6. Pyrolysis GC chromatograms of different DDAC-C10 bulk material samples (a-c) and a DDAC-C10 standard material (d). Although all samples are explicitly labeled with the CAS number of DDAC-C10, the results show for some samples that this is not correct. Peaks: 1 octylchloride, 2 octyldimethylamine, 3 decylchloride, 4 decyldimethylamine, 5 dioctylmethylamine, 6 octyldecylmethylamine, and 7 didecylmethylamine.
interests or personal relationships that could have appeared to influence this study. All authors have read and agreed to the published version of the manuscript.

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CRediT authorship contribution statement

B.T. mainly contributed to this manuscript including idea and conceptulization, method development and manuscript preparation. A.S.G. contributed in sample analysis and manuscript preparation. W.B. mainly contributed to this manuscript including idea and conceptualization, method development and manuscript preparation. A.S.G. contributed in sample analysis and manuscript preparation. W.B. supervised the work and contributed in manuscript editing. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jaap.2022.105447.

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