Applications of Deactivated GC Columns for Analysis of Nitrogen-Containing Chemicals Related to the Chemical Weapons Convention

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Abstract

Nitrogen-containing chemicals are one of the important families of compounds relevant to the purposes of the Chemical Weapons Convention (CWC). Several applications, using various injection modes, of new deactivated columns specially designed for basic compounds are presented. These columns prove remarkably well-suited to the gas chromatographic (GC) analysis of the chemicals of interest, even to underivatized amino-alcohols, whose analysis on conventional GC columns is often difficult and hindered by poor resolution and high detection limits. Such a deactivated phase can even replace the typical GC phases used for CWC verification purposes.

Introduction

Nitrogen-containing chemicals represent the third most numerous family of compounds that enter in the scope of the Chemical Weapons Convention (CWC), the other being organophosphorus and organosulphur chemicals. They include relatively nonpolar and semivolatile compounds, such as nitrogen mustards and *N,N*-dialkylaminoethyl chlorides and polar, hydrophilic amino-alcohols such as *N*-methyl diethanolamine, *N*-ethyl diethanolamine, or triethanolamine (1). The latter are both precursors and degradation products of nitrogen mustards, and the former are key precursors in the synthesis of nerve agents such as VX.

Forensic analysis of complex samples that have been contaminated by CWC-related chemicals most often relies on gas chromatography (GC) with specific detectors or mass spectrometry (MS) (or both) (2), which are endowed with low limits of detection and generally afford sufficient information for unambiguous characterization of relevant chemicals. The use of specific detectors and the availability of electron impact spectral databases are a major advantage of GC or GC–MS versus liquid chromatography (LC)–MS analysis in this field.

However, several relevant compounds are relatively unsuited to GC analysis because of their high polarity or boiling point (or both), especially amino-alcohols, for which analytical procedures mainly rely on LC–MS (3). GC analysis of amino-alcohols at low levels using standard conditions, especially columns, such as those recommended by the Organization for the Prohibition of Chemical Weapons (OPCW) (4,5) or for more general purposes (6), may result in wide broadening of GC peaks and severe decrease of the corresponding signals, essentially because of strong interactions with residual silanol groups on the column. Therefore, derivatization is most often required prior to analyzing these chemicals.

A few years ago, several brands of deactivated nonpolar fused-silica GC columns claimed to be specially adapted to the analysis of amines and were marketed under the names Rtx5-Amine (7) (Restek Corporation, Evry, France), Optima5-Amine (Macherey-Nagel GmbH, Düren, Germany), or CPSil8 Amine (Varian, les Ulis, France). To our knowledge, only a few laboratories have reported applications of this type of column, mostly limited to the field of basic drug analysis [e.g., cold medications (8) and ephedrines (9)]. Comparison studies were performed at our laboratory, currently designated by the OPCW for the analysis of environmental samples under its responsibility, to ascertain if there was a benefit to using these columns for CWC-relevant nitrogen containing chemicals. These columns proved very suitable for the development of dedicated analytical methods and recently appeared to be extremely beneficial during an official proficiency test organized under the aegis of the OPCW (10).

Experimental

Columns

Base deactivated columns (further designated as "column A", 30-m × 0.25-mm i.d., 0.5-µm film thickness) were Rtx5-Amine

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provided by Restek and CPSil 8CB Amine from Varian (les Ulis, France). Liners (Gooseneck, 2-mm i.d, Siltek treatment) were also supplied by Restek. For comparison, VF-5MS (Varian) and Rtx-5 (Restek) columns were used based upon the same stationary phase (5% phenyl–95% dimethyl polysiloxane) but were non-base deactivated ("column X", 30-m × 0.25-mm i.d., 0.25-µm film thickness).

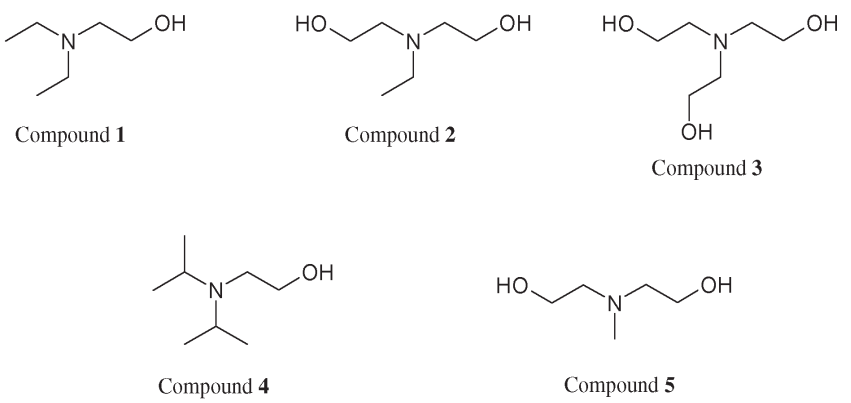

Figure 1. Structures of the amino-alcohols (compounds **1** to **5**) used in this study.

Table I. Desorption Parameters

Turbomatrix parameters	Values
Transfer line temperature	250°C
Valve temperature	220°C
Primary desorption temperature	300°C
Primary desorption time	10 min
Trap temperature	–30°C
Secondary desorption temperature	280°C
Secondary desorption time	10 min
Helium pressure	16 psi
Purge time	1 min
Desorption flow	50 mL/min
Inlet split	No
Outlet split	20 mL/min

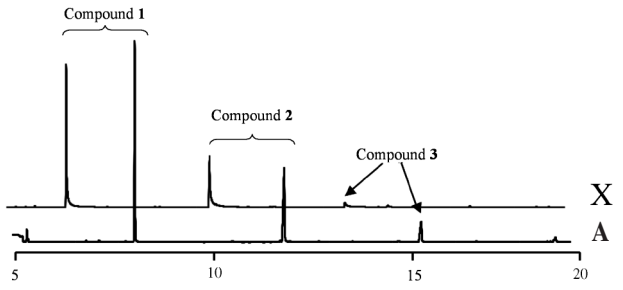

Figure 2. Chromatograms resulting of the GC–FID analysis of a mixture of three amino-alcohols (50 µg/mL) using columns A and X.

Chemicals

O-Alkyl (methyl to tetradecyl) *O*-methyl methylphosphonates (purity checked by ^{31}P NMR, > 98%) were synthesized in-house. All other reference chemicals (compounds **1** to **5**, presented in Figure 1) were purchased from commercial sources (Sigma-Aldrich, Saint-Quentin Fallavier, France) and had the highest purity available.

Operating conditions

Solid-phase microextraction

Experiments were performed on a GC Agilent 5890/flame ionization detector (FID) (Massy, France) equipped with a Gerstel MPS2 multipurpose sampler (Berlin, Germany) in solid-phase microextraction (SPME) configuration. The fiber was a 75-µm carboxen–polydimethylsiloxane (PDMS) provided by Supelco (Bellefonte, PA).

Extractions were performed under saturated salt conditions (277 g/L of NaCl) with an extraction time of 120 min. Desorption of the fiber into the injection port was carried out in the splitless mode at 250°C. The inner diameter of the inlet liner for SPME was 0.75 mm. The exposure time of the fiber was 5 min.

The oven temperature program was as follows: the initial temperature was 40°C (held for 1 min), then increased 10°C/min to 270°C and held for 5 min.

The carrier gas (He) flow rate was 1.2 mL/min. The detector temperature was 280°C.

Thermal desorption–GC–MS

The sorbent tubes were desorbed with an automated thermal desorber (Model Turbomatrix, PerkinElmer, Boston, MA). Desorption parameters are presented in Table I. Subsequent analysis was done on a PerkinElmer Autosystem XL GC/Turbomass MS.

The GC oven temperature was held at 40°C for 1 min and then was increased at 10°C/min to 280°C in 10 min. The mass selective detector with electron impact ionization was operated in full scan mode with a range of *m/z* 30 to 400.

GC–atomic emission detector

Analyses were performed with a 6890 series GC coupled to a G2350A atomic emission detector (Agilent Technologies). The GC was equipped with an Agilent Technologies 7683 series autosampler, a split/splitless injector with a deactivated Siltek Gooseneck 4-mm splitless liner (Restek).

The splitless injector was maintained at 250°C with the purge valve off for 45 s. The splitless injection volume was 1 µL. The GC oven temperature was held at 40°C for 0.75 min and then was taken to 280°C at 10°C/min, where the temperature was held for 2 min. Transfer line and cavity block of the atomic emission detector were both heated to 250°C. A solvent delay from 0.1 to 4.5 min was applied in order to reduce solvent effects on the discharge tube. The detector was operated at

an emission wavelength of 193 nm for carbon element.

Table II Amino-Alcohol Detection Ranges Using Columns X and A*

Injection	1 ng	2 ng	5 ng	10 ng	15 ng	20 ng	25 ng	30 ng	40 ng	50 ng
Compound 1		A	A	A	X + A	X + A	X + A	X + A	X + A	X + A
Compound 2			A	A	A	A	X + A	X + A	X + A	X + A
Compound 3					A	A	A	A	A	X + A

* A = compound detected only when using column A; X + A = compound detected with both columns (signal-to-noise ratio > 3).
Operating conditions and compounds: see text.

Results and Discussion

Analysis of a standard mixture of amino-alcohols

Different mixtures of three amino-alcohols [*N,N*-diethylaminoethanol (compound **1**), *N*-ethyl diethanolamine (compound **2**), and triethanolamine (compound **3**), 50 ng of each injected] were analyzed, yielding the results presented in Figure 2. The excellent peak shape obtained using column A reveals the important decrease of interactions between amino-alcohols and the stationary phase. This phenomenon is also demonstrated by the important increase of the symmetry of the peak corresponding to *N,N*-diethylaminoethanol (0.35 on column X vs. 0.98 on column A).

The main interest of a base deactivated column is the capacity to detect amino-alcohols at trace levels. A comparison of detection ranges between the two columns is presented in Table II, which shows that column A affords a three-fold detection limit improvement.

Analysis of SPME extracts of water contaminated with an amino-alcohol

Column A was used within the context of a work on the SPME of *N,N*-diisopropylaminoethanol (compound **4**) (11). In the optimized operating conditions, the results were compared with those obtained using a classical column. Figure 3 shows the chromatograms resulting from SPME of a 9-mL solution of compound **4** at 20 and 50 ng/mL.

With column X, the compound response was particularly reduced at low concentrations, which is likely to be because of adsorption on the nonbase deactivated stationary phase. Conversely, column A provides higher peaks and an increase of the peak symmetry with the removal of tailing, leading to improvement of detection limits (< 10 ng/mL).

Thermal desorption of a soil sample

Following these studies, a soil sample was provided as a test sample during an official proficiency test. The analysis of this sample (150 mg) was performed by thermal desorption interfaced to GC–MS. The soil appeared to be heavily contaminated with hydrocarbons, as well as a number of other interfering contaminants. Because of this huge background noise, only the analysis with column A allowed the conventional identification of two amino-alcohols of interest in this soil sample [*N*-ethyl diethanolamine (compound **2**) and *N*-methyl diethanolamine (compound **5**)]. The laboratory that prepared the samples later stated that the contamination level was 10 µg/g for each chemical.

Figure 4 shows the chromatograms obtained with columns X and A. It was noteworthy that for column X, the peaks of the two amino-alcohols were hardly noticeable and presented broad shape, important tailing, and low response, which prob-

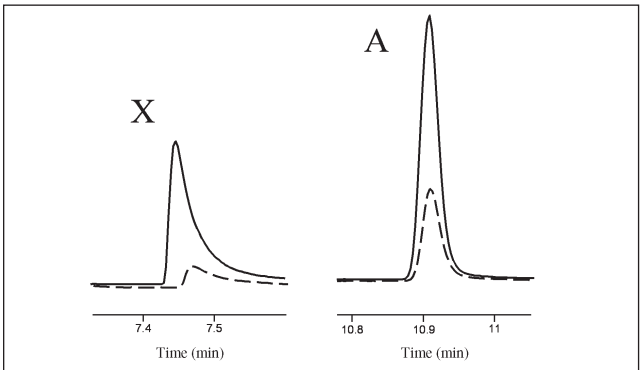

Figure 3. Chromatograms resulting of the GC–FID analysis with columns A and X of SPME extracts of water samples spiked with compound **4** (solid lines, 50 ng/mL and dashed lines, 20 ng/mL) [SPME conditions: carboxen–PDMS fiber, pH = 11.5; (NaCl) = 277 g/L ; 40°C, 120 min].

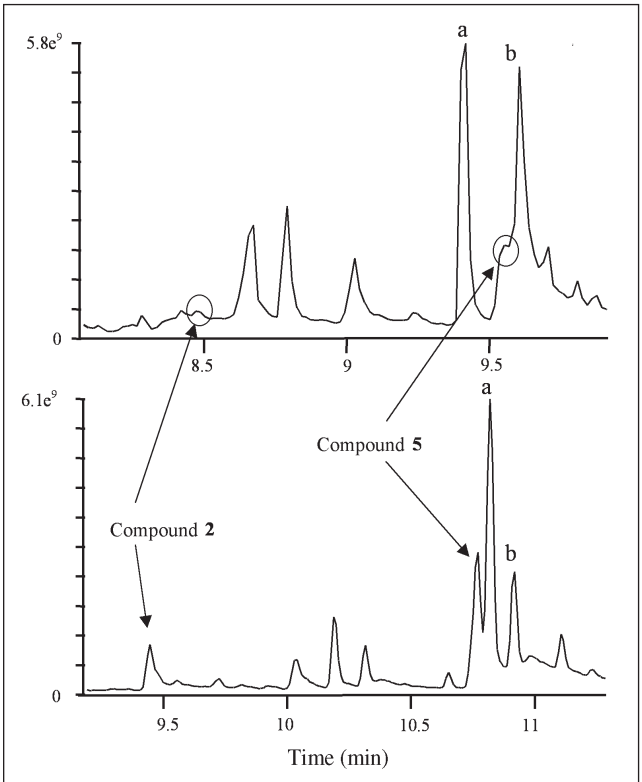

Figure 4. GC–MS analysis under electron impact conditions (constant He pressure, 16 psi) of a 100 mg soil sample spiked with compounds **2** and **5**, extracted by thermal desorption (280°C) using column X (top) and using column A (bottom) (a and b are interferents).

ably illustrates important interactions of the analytes of interest with the stationary phase. By comparison, peaks obtained on column A are much sharper and more symmetric, leading to better sensitivity and far easier visual detection. The superior performance of column A is particularly obvious when monitoring extracted ion chromatograms of the two compounds of interest (Figure 5), which show reduced peak height (by 25% to 40%) and extreme broadening of the signal corresponding to the major ions in the electron impact ionization spectrum.

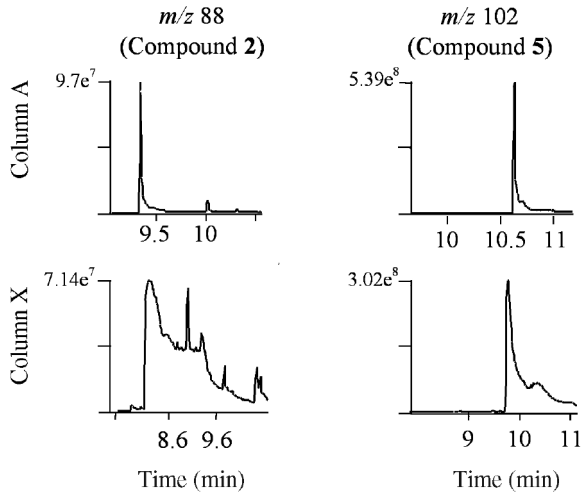

Figure 5. Extracted ion chromatograms for compound **2** (*m/z* 88) and compound **5** (*m/z* 102).

Column performance for the analysis of other CWC-related chemicals

To ascertain whether a deactivated phase would decrease analytical results obtained for other chemicals of interest, a standard mixture of phosphorus-containing chemicals was injected on both columns X and A. This series [*O*-alkyl (methyl to tetradecyl) *O*-methyl methylphosphonates] was designed in our laboratory for internal performance and quality control and is representative of the chromatographic behavior of organophosphorus chemicals, which are of the utmost importance in the field of CWC. Figure 6 clearly shows that columns A and X provide comparable separation and sensitivity, with column A only being superior because of a slightly increased peak width for the least volatile compounds of this series.

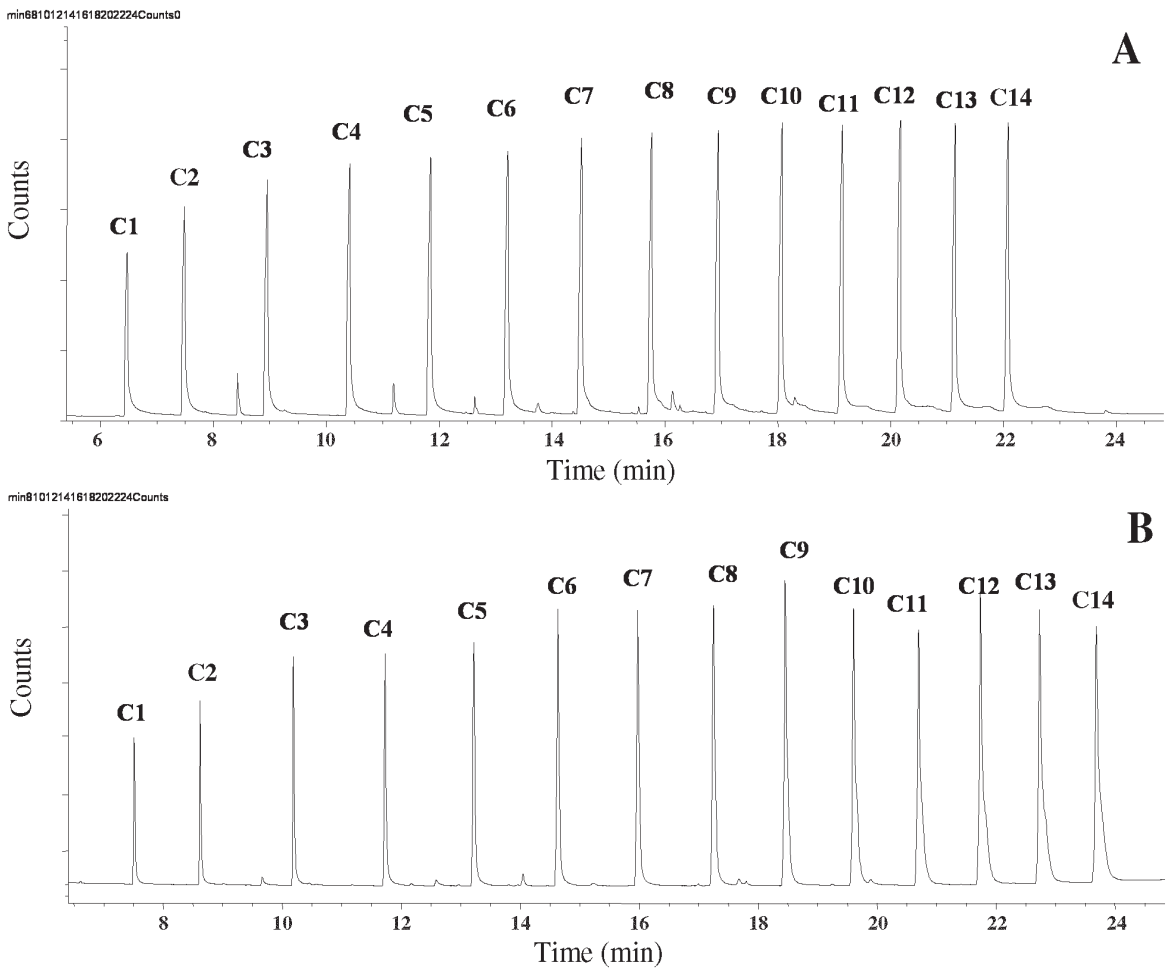

Figure 6. Comparison of atomic emission detector (C-193 nm-line) chromatograms obtained after injection of a series of 14 *O*-alkyl (methyl to tetradecyl) *O*-methyl methylphosphonates (50 µg/mL each in ethyl acetate) on column X (top) and column A (bottom) [1 µL splitless injection, 40°C (0.75 min) then 10°C/min to 280°C] (both chromatograms have the same vertical scale).

Conclusion

The results presented here illustrate the efficiency and diversity of applications of deactivated GC columns in the field of chemical warfare agent analysis. Various nitrogen-containing chemicals related to the CWC have been analyzed in several conditions (liquid injection, SPME, thermal desorption, FID, and MS analysis), which all show very significant improvements in peak shape and limits of detection for the compounds studied. No significant differences were observed between the various brands of "Amine" columns on the market.

Moreover, no degradation of performance was noticed for a series of phosphonates representative of a wide range of other compounds of interest such as sarin or soman. Taken together, these results suggest that deactivated columns could usefully replace conventional GC columns as a standard for analytical work in the scope of the CWC.

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