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Comprehensive gas chromatography with Time of Flight MS and large volume introduction for the detection of fluoride-induced regenerated nerve agent in biological samples☆,☆☆

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ABSTRACT

Recently, several methods have been developed to verify exposure to nerve agents. Most of these methods, such as the fluoride reactivation technique and the analysis of inhibited phosphonylated butyrylcholinesterase (BuChE), are based on mass spectrometry. The high specificity of the mass spectrometer might also imply a disadvantage, because the acquisition mass, i.e. the identity of the analyte must be known beforehand in order to direct the MS analysis in the most sensitive mode. In real cases, the identity of the nerve agent is not always known beforehand and the mass spectrometer should be operated in a scanning mode, with the consequence that sensitivity of the method will be lower. Comprehensive GC, or GC × GC, is a technique which offers enhanced separation. The implied larger selectivity of the GC separation allows mass spectrometry to be conducted in a less specific, scanning, mode. By the use of this configuration, the identity of the nerve agent does not have to be known beforehand but can be traced. In order to be able to detect lower concentrations and assess lower exposure levels, a large volume injection technique was developed allowing sample sizes up to 100 μL. The technique was tested with plasma samples that had been inhibited with various nerve agents. Subsequently, the cholinesterase-bound nerve agent was regenerated by the fluoride reactivation technique. Using the newly developed comprehensive GC–MS method it was possible to detect nerve agent at an exposure level of 1% BuChE inhibition, which is approximately 70 pg nerve agent/mL. These low exposure levels cannot be verified with a cholinesterase (ChE) activity assay. Moreover, the identity of the regenerated nerve agent was verified by the mass spectrum that was generated by the TOF mass spectrometer. This paper presents a technique able to deliver full-scan data on the analysis of nerve agents in biomedical samples at relevant exposure levels (1% BuChE inhibition). This full-scan data meets for a large part the forensic requirements that are in place for the analysis of biomedical samples in the context of alleged use of Chemical Warfare Agents.

1. Introduction

Although the Chemical Weapon Convention (CWC) prohibits the use, production and stockpiling of chemical warfare agents, it does not imply that chemical warfare agents have been expelled completely. Some countries have not (yet) signed the treaty of the CWC. Other countries have still several tons of chemical warfare agents waiting for destruction. In case of an incident with chemical weapons where the OPCW[1] is mobilized, or a terrorist attack, samples will be taken that can serve as forensic evidence. These samples can be environmental samples but also biomedical matrices such as blood and urine. In view of the high reactivity of chemical warfare agents and the time that is involved between exposure and sampling, intact compounds can often not be detected. More persistent biomarkers are urine metabolites or adducts to proteins and DNA. In this paper we will focus on the verification of exposure to nerve agents. The main metabolites of nerve agents are alkyl methylphosphonic acids that are rapidly excreted in urine. Depending on the seriousness of the exposure, these metabolites

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[1] OPCW is the Organization for the Prohibition of Chemical Weapons and is responsible for the implementation of the treaty of the Chemical Weapon Convention by the Member States. The OPCW is authorized to perform facility inspections and take samples that can be analyzed later.

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can be detected up to one week after exposure [1–6]. Additionally, nerve agents bind to essential enzymes such as AChE and BuChE. Although the concentration of BuChE adducts is lower than the concentration of metabolites in urine shortly after exposure, the life time of these adducts is much longer [1,3,7]. Another target protein for nerve agents is albumin [8,9]. However the reactivity of nerve agents with this protein is lower, which results in a low yield of adducts certainly at lower concentration of nerve agents. In the last decade several methodologies have been developed for the analysis of nerve agent adducts to BuChE [10–12]. Polhuijs et al. [13] described the fluoride reactivation technique, which implies the release of the nerve agent upon incubation with a large concentration of fluoride ions followed by analysis of the generated phosphofluoridate with GC–MS. Fidder et al. [14] described the analysis of the pepsin digest of BuChE with LC–MS. The resulting FGES(p)AGAAS peptide with the phosphyl moiety of the nerve agent attached to the active serine site is representative for nerve agent exposure.

The concentration of the adduct biomarker (i.e. the inhibited butyrylcholinesterase) is very low, typically 50 nM (in case of 100% inhibition), and the adduct is present in a complex matrix, i.e. a plasma extract in case of the fluoride reactivated sample or the filtrate of a pepsin digest [14,15]. When low concentrations have to be measured by chemical analysis, the instruments must be sensitive and selective. With a quadrupole mass spectrometer, selectivity can be obtained by directing the mass spectrometer to the specific acquisition masses for target compound analysis, i.e. selected ion monitoring (SIM) analysis. However, gained sensitivity with the use of SIM analysis, decreases the number of target components that can be analyzed in a single run. The number of variations in the alkyl chains of nerve agents can exceed several hundreds, which number indicates that an unbiased approach to analysis must be able to span a large class of compounds. In case of an assessment of suspected exposure to nerve agents, a too high specificity of the analysis method might lead to an unacceptably high likelihood of false negative results. In previous papers, some solutions for this problem have been presented in an approach that employed analysis of the pepsin digest of butyrylcholinesterase. One option was the use of the limited precursor ion scan of the nonapeptide [16]. By searching the masses of the precursor ion, the identity of the nerve agent was successfully elucidated, except for the leaving group of the nerve agent. A second option comprised the conversion of the phosphonyl moiety of the nerve agent by a mass generic tag [17]. Using the last method, an exposure to an organophosphate can be verified while the identity of the nerve agent cannot be elucidated. In this paper, we do focus on a more generic method for the analysis of the fluoride regenerated nerve agents. Although fluoride induced regeneration was developed over a decade ago, it is still very interesting because of the simplicity of the sample preparation and the sensitivity of analysis of the regenerated nerve agents. This approach affords verification of nerve agents at low degrees of BuChE inhibition. A GC–MS, operated in Electron Impact (EI) scan mode, is not sensitive and selective enough to detect the regenerated organophosphofluoridate at low concentrations, resulting from a low degree of BuChE inhibition. Therefore the preferred method for the detection of regenerated nerve agent comprises GC–MS with positive chemical ionization (PCI) combined with selected ion monitoring (SIM) mode to obtain sufficient sensitivity and selectivity [15,21,22]. For the use of these methods, information about the identity of the target component is needed for selecting target ions. This prevents the method from being generically applicable due to absence of library search possibilities.

Modifications of a single dimension GC separation offers limited results for selectivity improvement. In contrast, comprehensive GC (GC × GC)–MS employs a modified GC process to afford higher selectivity at high sensitivity while retaining generic applicability.

Briefly, GC × GC comprises the utilization of two GC columns with different stationary phases connected in series [18]. Components are first separated over the first column (first dimension), where upon they are focused and automatically injected on the second column (second dimension). Firstly, a major advantage of GC × GC is the enhanced selectivity offered by the two-dimensional separation. The automated transfer implies that all fractions that come from the first column are injected into the second column. That means that no components will be lost in the overall separation process. Secondly, the overall analytical method remains sensitive because of the focusing step just before injection into the second column. The use of comprehensive GC for the detection of CWA's in complex environmental matrices has been published by Reichenbach et al. [19]. The current paper describes the results obtained with the comprehensive GC–MS technology for the analysis of fluoride regenerated nerve agents in biomedical samples.

2. Materials and methods

2.1. Materials

Tabun (GA), sarin (GB), VX, fluorotabun (FT), ethyl sarin (ES) and d_7-sarin (d_7-GB, used as internal standard) were prepared and analyzed by means of gas chromatography (GC) and/or NMR according to standard procedures at TNO Defence Security and Safety. Purity of each chemical exceeded 98%. Stock solutions of these compounds were prepared in 2-propanol and/or ethyl acetate. Solvents were purchased from Biosolve (Valkenswaard, The Netherlands). Nexus Solid Phase Extraction cartridges were purchased from Varian (Middelburg, The Netherlands). Human plasma was purchased from Sanquin (Leiden, The Netherlands).

2.2. Sample preparation

Human plasma was inhibited with tabun, sarin or VX. The concentration of the OP in plasma was 4 μM, which is a 100-fold excess compared to the approximate concentration of HuBuChE in plasma (44 nM). Inhibition of the sample was allowed for 2 h at room temperature. As a blank, non-inhibited plasma (1.0 mL) was used. The plasma samples were further processed using the method described by Holland [20]. Briefly, human plasma samples (1 mL) were mixed with acetate buffer (3 mL, 0.189 M acetic acid and 10.8 mM sodium acetate, pH 3.5) and potassium fluoride (190 μL, 5.25 M), resulting in a final concentration of 0.25 M KF. After incubation for 30 min at 25 °C, the internal standard was then added, and the regenerated nerve agents were extracted using SPE.

Nexus cartridges were conditioned by consecutive rinsing with *n*-hexane (4 mL), chloroform (2× 4 mL), and water (2× 5 mL). Each solvent was gently pushed dropwise through the cartridge until the cartridge was dry. The sample was then applied to the cartridge, and the regenerated nerve agents were eluted with chloroform (2 mL) and ethyl acetate (150 μL). The eluate was collected in a centrifuge tube and cooled in dry ice/acetone (−80 °C) to freeze the water. The organic layer was then transferred to a 4 mL GC vial by Pasteur pipette. A small stream of nitrogen gas was used to evaporate the chloroform part of the eluent until a final volume of approximately 150 μL. The ethyl acetate layer was then transferred to a GC vial fitted with an insert of 250 μL. All extracts were analyzed by GC × GC-TOF MS.

2.3. Gas chromatography–TOF MS conditions

For the analysis of large volume samples (100 μL) a 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA)

was equipped with an Optic 3 PTV injector and a CryoFocus module (ATAS GL, Veldhoven, The Netherlands), a Cryogenic modulator and a LECO Pegasus 4D GC × GC-TOF MS instrument (LECO Corporation, St. Joseph, MI, USA). The injector was fitted with packed liner type A for large volume injections (ATAS GL, Veldhoven, The Netherlands). A Combipal autosampler (CTC Analytics, Zwingen, Switzerland) equipped with a LINEX automated liner exchanger (ATAS GL, Veldhoven, The Netherlands) was used to automatically inject samples and regularly replace liners from the injector. A retention gap, 0.6 m × 0.53 mm id. methyl deactivated (Varian, Middelburg, The Netherlands), was installed in the CryoFocus module between the injector and the first dimension column (25 m × 0.25 mm × 0.25 μm CP Sil 8 CB (Varian, Middelburg, The Netherlands). The second dimension column was 1 m × 0.1 mm × 0.2 μm CB 52 Wax (Varian, Middelburg, The Netherlands). A retention gap (0.5 m × 0.1 mm methyl deactivated, Varian, Middelburg, The Netherlands) was used to connect the second dimension column to the detector. Helium was used as carrier gas, and cooled nitrogen gas was used for modulation. A sample of 100 μL was injected onto the packed liner in the injector. At a temperature of 35 °C the solvent was evaporated using a split flow of 100 mL/min during 140 s (result of analysis with solvent sensor connected to split line) and a column pressure of 25 kPa (0.1 mL/min). Desorption of the liner was performed by heating the injector at a rate of 8 °C/s to 200 °C in splitless mode (200 kPa). The cryo trap was kept at −100 °C during desorption and transfer of the components to the column and then heated to 200 °C at a rate of 15 °C/s. The first dimension oven was held at 45 °C for 5 min, then ramped at 10 °C/min to 200 °C and held for 5 min. The second dimension oven was held at 55 °C for 5 min, then ramped at 8.5 °C/min to 200 °C and held for 5 min. The total analysis time was 38.5 min. The modulator temperature offset is kept at 30 °C (relative to second dimension oven temperature). The modulation time was 8 s and the hot pulse time was 0.6 s. The transfer line set point was 200 °C, and the ion source was set to 150 °C. A solvent delay of 500 s was used to protect the detector for excessive solvent due to large volume injections. Mass spectra were collected from *m*/*z* 40 to 350 at a rate of 80 spectra/s. Data collection and processing were performed with ChromaTOF version 3.34 (LECO Corporation, St. Joseph, MI, USA).

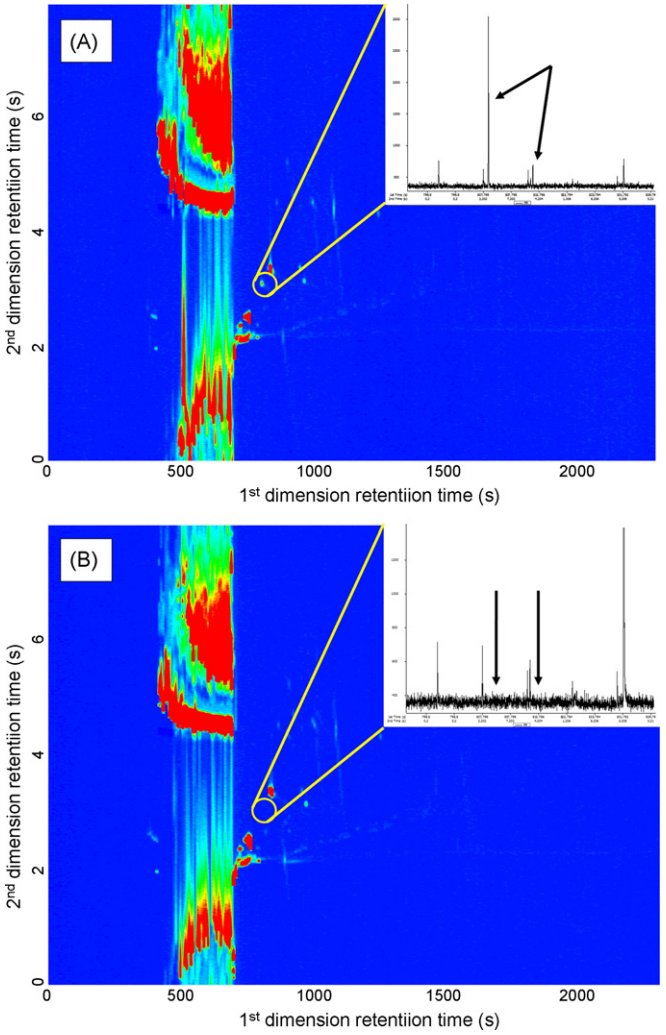

Fig. 1. GC × GC chromatogram (*m*/*z* 99) of fluoride reactivated plasma sample. (A) BuChE was fully inhibited with sarin. The peak of sarin is in the yellow circle. Inset shows the raw detector signal with the sarin peak indicated by an arrow. (B) Sample was not inhibited (blank). Injection volume was 1 μL. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

3. Results and discussion

In order to show the applicability of GC × GC-TOF MS towards fluoride reactivation of nerve agent inhibited butyrylcholinesterase, plasma was incubated with sarin. The plasma sample was processed according to the fluoride reactivation method and analyzed with GC × GC-TOF MS. Fig. 1 shows a representative example of a GC × GC chromatogram of regenerated sarin from plasma with the peak of sarin indicated in the circle. Fig. 1A shows the contour plot of ions *m*/*z* 99, which is a characteristic fragment ion, $[CH_3P(OH)_2F]^+$, for alkyl methylphosphonofluoridates. On the horizontal axis, the time of separation in the first dimension is shown. The vertical axis represents the separation in the second column, which takes only 8 s. Fig. 1B shows the contour plot of a blank sample, from which the peak of sarin is clearly absent. Since the *m*/*z* 99 signal is representative for alkyl methylphosphonofluoridates, peaks that appear in the chromatogram of an unknown sample but not in a blank sample are suspect and require further investigation. The TOF mass spectrometer was equipped with an electron ionization (EI) source, which affords recorded mass spectra that are comparable with the spectra in common spectrum libraries, for example the NIST library. Fig. 2 shows the EI spectrum as recorded by the TOF MS and the spectrum available from the NIST library. Searching for "differential peaks" in the contour plots of an exposed and non-exposed sample can be rather laborious. Alternatively, the software associated with the NIST and OPCW library can search the chromatogram for nerve agents by a match with the library mass spectrum. In this case, sarin was detected with a 92% hit, although there are small differences between the spectrum from the library and the deconvoluted detected spectrum. Database search for other compounds did not result in any additional hits (no false positives). A forward library search (OPCW library) was used with a minimum hit of 85%. In this case the use of a TOF type mass spectrometer was required to obtain a full EI mass spectrum with sufficient data points per second. The Mass Selective Detector (MSD) commonly used with GC–MS does not have the appropriate scan rates to detect the narrow peaks obtained with GC × GC. GC–MS by operating a MSD in full-scan EI spectrum mode ionizes many compounds almost equally. For complex samples, this results in very complex chromatograms, where multiple compounds may become hidden under a single GC peak. Increased selectivity can be offered by the use of GC combined with High Resolution MS or GC–MS/MS [2,15]. Also with these configurations, except High Resolution TOF-MS, a pre-selection of acquisition masses have to be made in order to achieve an analysis with sufficient sensitivity. Alternatively, the

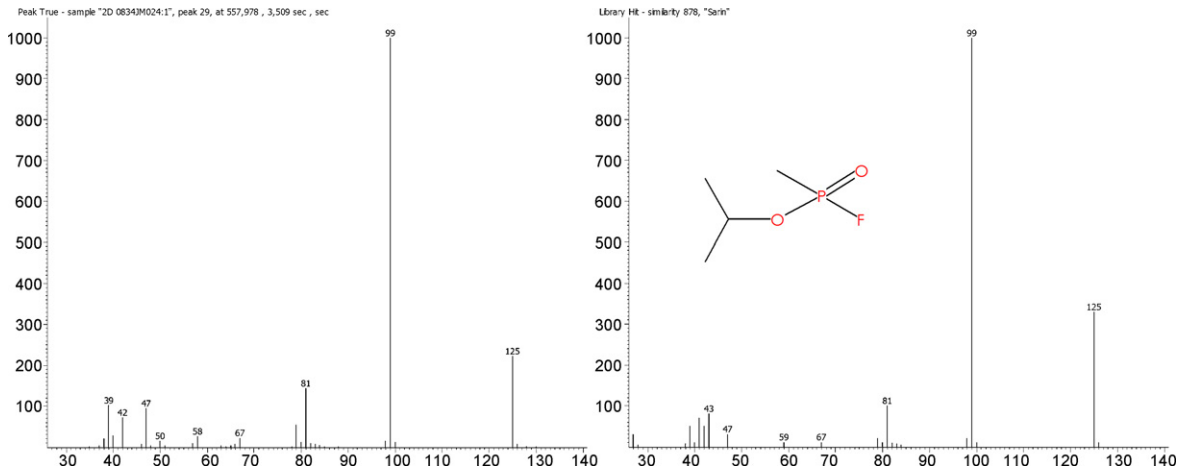

Fig. 2. Mass spectrum of sarin as recorded by the TOF mass spectrometer (left) and from the NIST library (right).

analysis of nerve agents with GC–MS could be operated in the positive chemical ionization (PCI) mode with ammonia as the reaction gas [15,21,22]. PCI decreases the level of fragmentation by protonation of molecules, instead of the electron removal in EI. Because phosphates and phosphonates have a high gas phase basicity, PCI affords sensitive detection, because many contaminants are less well protonated; for example alkanes will be suppressed in PCI mode. However the CI spectrum provides a limited number of ion signals for identification purposes because of less fragmentation.

The combination of GC × GC with EI TOF MS offers increased selectivity with retention of the sensitivity ensured by the peak focussing during the modulation. Based on the observed GC × GC-TOF MS peak intensity of sarin it was estimated that the regenerated compound can be detected at BuChE inhibition levels down to 15%. In order to decrease the lowest detectable concentration, a large volume introduction method was developed. This method encompasses a single injection, at once, of a 100 μL sample on the packed injection liner, a slow evaporation of the solvent, followed by thermal desorption of the analyte, cryo-focusing and subsequent injection into the column. Fig. 3A shows the chromatogram of a typical analysis result with an injection volume of 100 μL and a BuChE inhibition level of 100%.

As compared to the chromatograms in Fig. 1, obtained with smaller injection volumes, the background has increased. The solvent peak is absent because a solvent delay was employed with the large volume injection. Fig. 3A shows that sarin had sufficient retention on the second dimension column to be separated from the background. The choice of the polar stationary phase of the second dimension offered the needed selectivity that may be expected from a comprehensive GC system.

When introducing larger sample volumes, the analytical system is even more challenged and it must be investigated whether the following anticipated goals are achieved. The most important requirements are that:

(i) the chromatogram of the blank sample should be clean, i.e. that no false positives are detected;
(ii) retention times must be reproducible, because identification of a compound is partly based on retention times;
(iii) the injection of larger sample volumes should result in an increased signal of the target compounds and, therefore, in lower detectable concentrations.

Fig. 3B shows the chromatogram of a sample from a large volume injection experiment which was inhibited with tabun. After fluoride reactivation fluorotabun was generated and detected in the sample. The chromatogram showed no peaks at the location of sarin and can be considered as a true negative. Moreover, a computer search using all spectra of Schedule 1 compounds from the OPCW

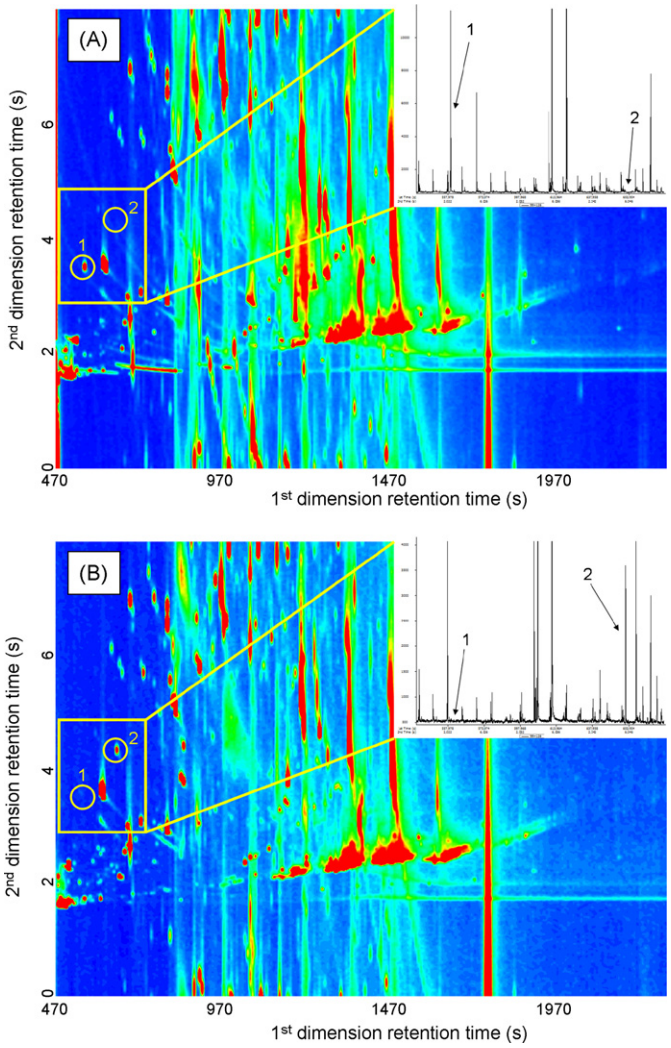

Fig. 3. Large volume injection GC × GC chromatogram (m/z 99 + 126) of fluoride reactivated plasma sample. (A) BuChE was fully inhibited with sarin (1) and was blank for tabun (2) and (B) sample was inhibited fully with tabun and was blank for sarin. Injection volume was 100 μL.

Table 1
Repeatability of retention times ($n = 10$) of nerve agents diluted in ethyl acetate.

Component	Retention time	
	First dimension (min)	Second dimension (s)
Ethyl sarin	8.71 ± 0.09	3.97 ± 0.06
Sarin	9.16 ± 0.08	3.59 ± 0.04
d_7-Sarin	9.15 ± 0.06	3.53 ± 0.03
Fluorotabun	10.9 ± 0.00	4.34 ± 0.00

Analytical Database library resulted only in a hit for fluorotabun (90%) with forward search. Besides fluorotabun, only peak hits with a poor probability match, lower than 50% and no hit matching any alkyl methylphosphonofluoridates at all were found. This result confirms that the large volume introduction did not induce any false positive detection.

The second requirement, essential for identification, concerns reproducibility of the retention time. In GC × GC the concept of 'retention time' is rather complex, since the retention of compounds on the columns must be expressed as coordinates in the first and second dimension.

Table 1 shows the repeatability of both these time coordinates of the different nerve agents that were analyzed, when a single standard mixture in ethyl acetate was analyzed by large volume injection. The standard deviation of the retention time in the first dimension was 5.4 s (<1%), which is smaller than the modulation time (8 s). As the deviation in GC × GC should be expressed as increments of modulations, the real deviation is less than 1 modulation for all of the tested target components. Table 2 shows the reproducibility results obtained from processed plasma samples. For this experiment, two plasma samples were inhibited with sarin and tabun to a level of 100% inhibition. Sarin inhibited plasma was diluted with tabun inhibited plasma (0, 1, 5, 10, 100% sarin inhibition) to generate a calibration curve. Due to solvent peak broadening presumably caused by small amounts of chloroform, the sarin peak shifted approximately two modulations compared to the analyses of nerve agents dissolved in ethyl acetate. Variation between the plasma samples was still not larger than one modulation in the first dimension. The variation of the retention time in the second dimension was less than 1.5% which is sufficient for partial identification by retention time. The system appears to be robust with respect to variations in solvent composition.

It must be emphasized that the GC × GC TOF MS configuration affords identification based on retention time and mass spectrum. When full-scan spectra are available, these make even stronger evidence for identification than the retention time. Nevertheless, the observed small variations in retention time indicate that the reproducibility requirement for large volume injection is fulfilled.

The third requirement concerns the achievement of lower detection levels. The calibration line used for this experiment simulated low levels of BuChE inhibition to a desired level of inhibition 1% of BuChE control values. Fig. 4 shows the chromatogram from large volume injection GC × GC-TOF MS analysis of a plasma sample after fluoride reactivation and clearly demonstrates that the

Table 2
Reproducibility of retention times ($n = 8$) of fluoride regenerated nerve agents.

Component	Retention time	
	First dimension (min)	Second dimension (s)
Sarin	9.25 ± 0.06	3.52 ± 0.03
d_7-Sarin	9.20 ± 0.07	3.48 ± 0.05
Fluorotabun	10.8 ± 0.00	4.32 ± 0.01

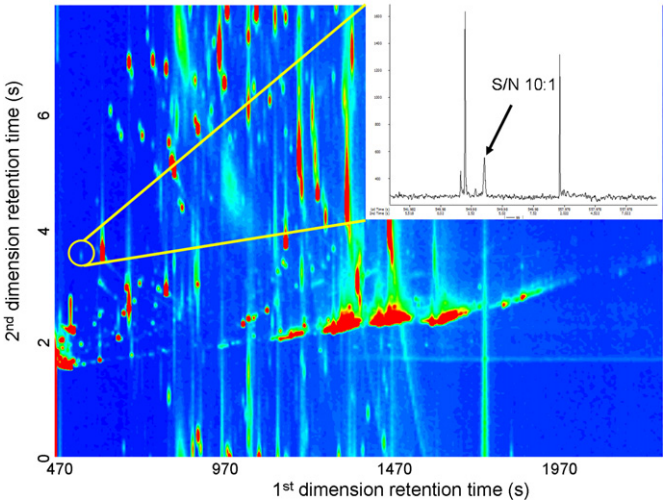

Fig. 4. Large volume injection GC × GC chromatogram of fluoride reactivated plasma sample, containing sarin inhibited BuChE equivalent to a 1% inhibition level. The peak representing sarin is in the yellow circle. The inset shows raw detection data with the peak of sarin indicated by the arrow. Injection volume was 100 μL. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

peak of sarin, at ~70 pg/mL plasma, was detected and was close to the detection limit (injection volume 100 μL). A peak was accepted when the signal-to-noise ratio exceeded 5:1. Although the signal-to-noise ratio of the mass spectrum was only 10:1, the software identified the sarin peak by comparing full-scan data with the OPCW Analytical Database library.

Plasma samples inhibited with VX and tabun were also prepared. The corresponding organophosphofluoridates, ethylsarin and fluorotabun, were detected and identified at the same BuChE inhibition levels of 1% inhibition. Chromatograms from the corresponding experiments are not shown here. These experiments with low levels of BuChE inhibition clearly demonstrate that detection of the adducts by large volume injection GC × GC-TOF MS is about ten times more sensitive than by common GC × GC-TOF MS.

For a final impression of the performance of large volume injection GC × GC-TOF MS, linearity of quantity-response in quantitative analysis was considered. Fig. 5 shows a calibration curve for the quantification of sarin in plasma samples. Although the number of data points over the range is somewhat limited, it may confidently be anticipated that the detector response is linear with the concentration. However, it must be emphasized that the aim of the present application is verification of exposure or non-exposure. Therefore, the experimental set-up is primarily intended for screening and not for accurate quantification.

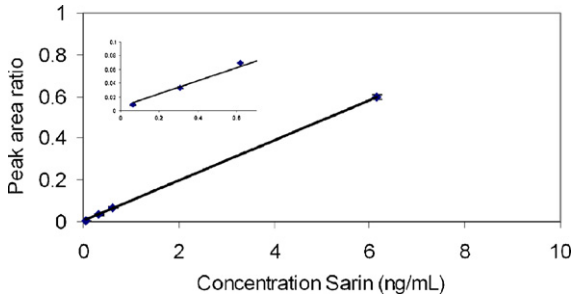

Fig. 5. Calibration curve with error bars for the quantification of sarin in plasma samples. Detector response is linear ($R^2 = 0.9998$) with the percentage of inhibition of BuChE ranging from 1% (62 pg/mL) to 100% (6.2 ng/mL).

4. Conclusion

GC × GC-TOF MS is a powerful tool for the analysis of fluoride-induced regenerated nerve agents in plasma. The separation capability of the GC × GC technology provides such a high selectivity that no pre-selection of acquisition masses for the mass spectrometer is required. Using conventional GC–MS pre-selection of masses is often required when very low concentrations need to be detected. Detection in SIM mode implies discarding of other mass signals and might result in false negative detection. The main advantage of GC × GC-TOF MS over conventional GC–MS is that the identity of the inhibitor does not have to be known beforehand, even when low concentrations (approximately 70 pg/ml) need to be detected. Additionally, TOF MS yields full-scan mass spectra that can be used for identification of the ChE inhibitor. With the full-scan spectra available, this identification meets the criteria of common forensic requirements [23]. In that respect, GC × GC-TOF MS can be used for the verification program of the OPCW for the analysis of biomedical samples. Using this method straightforwardly, nerve exposures equivalent to 15% BuChE inhibition were successfully detected.

In order to afford detection of lower concentrations, for verification of low exposure levels or of exposures that occurred in the past, a large volume injection technique was developed for use with GC × GC-TOF MS. This overall method afforded detection of regenerated sarin from BuChE at a virtual BuChE inhibition level of 1%. This level of BuChE cannot be detected with a ChE activity assay. The lowest detectable level of BuChE inhibition by GC × GC-TOF MS is still 10 times higher than the lowest detectable level of inhibition by GC–MSD with thermodesorption [15]. However, the MSD method requires prior knowledge of the identity of the nerve agent involved in the suspected exposure. Therefore, GC × GC-TOF MS is more suitable for screening.

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References

[1] R.M. Black, D. Noort, in: T.C. Marrs, R.L. Maynard, F.R. Sidell (Eds.), Chemical Warfare Agents, Toxicology and Treatment, John Wiley & Sons, Chichester, UK, 2007, p. 127.
[2] J. Riches, I. Morton, R.W. Read, R.M. Black, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 816 (2005) 251.
[3] B. Capacio, J.R. Smith, R.K. Gordon, J.R. Haigh, J.R. Barr, B.J. Lukey, in: J.A. Romano, B.J. Lukey, H. Salem (Eds.), Chemical Warfare Agents, Chemistry, Pharmacology and Toxicology, CRC Press, Baton Rouge, FL, USA, 2008, p. 501.
[4] D.B. Mawhinney, E.I. Hamelin, R. Fraser, S.S. Silva, A.J. Pavlopoulos, R.J. Kobelski, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 852 (2007) 235.
[5] L.L. Swaim, R.C. Johnson, Y. Zhou, C. Dandlin, J.R. Barr, J. Anal. Toxicol. 32 (2008) 774.
[6] F.L. Ciner, C.E. McCord, R.W. Plunkett, M.F. Martin, T.R. Coley, J. Chromatogr. B 846 (2006) 42.
[7] M.J. van der Schans, M. Polhuijs, C. van Dijk, C.E.A.M. Degenhardt, K. Pleijsier, J.P. Langenberg, H.P. Benschop, Arch. Toxicol. 78 (2004) 508.
[8] N.H. Williams, J.M. Harrison, R.W. Read, R.M. Black, Arch. Toxicol. 81 (2007) 627.
[9] B. Li, L.M. Schopfer, S.H. Hinrichs, P. Masson, O. Lockridge, Anal. Biochem. 361 (2007) 263.
[10] R.M. Black, D. Noort, in: M. Mesilaakso (Ed.), Chemical Weapons Convention Chemicals Analysis; Sample Collection, Preparation and Analytical Methods, John Wiley & Sons, Chichester, UK, 2005, p. 403.
[11] D. Noort, R.M. Black, in: M. Mesilaakso (Ed.), Chemical Weapons Convention Chemicals Analysis; Sample Collection, Preparation and Analytical Methods, John Wiley & Sons, Chichester, UK, 2005, p. 433.
[12] D. Noort, H.P. Benschop, R.M. Black, Toxicol. Appl. Pharmacol. 184 (2002) 116.
[13] M. Polhuijs, J.P. Langenberg, H.P. Benschop, Toxicol. Appl. Pharmacol. 146 (1997) 156.
[14] A. Fidder, A.G. Hulst, D. Noort, R. de Ruiter, M.J. van der Schans, H.P. Benschop, J.P. Langenberg, Chem. Res. Toxicol. 15 (2002) 582.
[15] C.E.A.M. Degenhardt, K. Pleijsier, M.J. van der Schans, J.P. Langenberg, K.E. Preston, M.I. Solano, V.L. Maggio, J.R. Barr, J. Anal. Toxicol. 28 (2004) 364.
[16] M.J. van der Schans, A. Fidder, D. van Oeveren, A.G. Hulst, D. Noort, J. Anal. Toxicol. 32 (2008) 125.
[17] D. Noort, A. Fidder, M.J. van der Schans, A.G. Hulst, Anal. Chem. 15 (2006) 6640.
[18] J. Dallüge, J. Beens, U.A.T. Brinkman, J. Chromatogr. A 1000 (2003) 69.
[19] S. Reichenbach, M. Ni, V. Kottapalli, A. Visvanathan, E. Ledford, J. Oostdijk, H. Trap, Chemical and Biological Sensing IV, Proc. SPIE, 5085, 2003, p. 28.
[20] K.E. Holland, M.I. Solano, R.C. Johnson, V.L. Maggio, J.R. Barr, J. Anal. Toxicol. 32 (2008) 116.
[21] P.A. Agostinho, L.R. Provost, Biomed. Environ. Mass Spectrom. 13 (1986) 231.
[22] E.M. Jakubowski, J.M. McGuire, R.A. Evans, J.L. Edwards, S.W. Hulet, B.J. Benton, J.S. Forster, D.C. Burnett, W.T. Muse, K. Matson, C.L. Crouse, R.J. Miodeszewski, S.A. Thompson, J. Anal. Toxicol. 28 (2004) 357.
[23] R.M. Black, J. Anal. Toxicol. 32 (2008) 2.