Advances in Military Technology
Vol. 18, No. 1, 2023, pp. 133-150
ISSN 1802-2308, eISSN 2533-4123
DOI 10.3849/aimt.01809

Use of a Handheld Raman Spectrometer for Identification of Toxic Agents in Clandestine Laboratories

T. Rozsypal[1*] and N. Greplova[2]

[1] *NBC Defence Institute, University of Defence, Brno, Czech Republic*
[2] *26th Air Command, Control and Surveillance Regiment, Stara Boleslav, Czech Republic*

The manuscript was received on 17 February 2023 and was accepted after revision for publication as technical information on 26 June 2023.

Abstract:

Handheld Raman spectrometers are commonly used as fast detectors for preliminary noncontact analysis of a number of chemicals. The article deals with their possible use for military identification of chemical warfare agents and their precursors. Spectra of 29 chemical substances were recorded. Based on the similarity of the spectra in the library, the device was able to automatically detect 20 substances in all measurement methods, 1 substance only in the most transparent containers. By means of external software and the creation of a user's library, the vibrations of functional groups in the respective molecules were assigned to the individual Raman bands, thereby creating a database that enables the identification of toxic substances.

Keywords:

AEP-66, chemical warfare agent, deployable laboratory, chemical weapons, sampling and identification, precursors

1 Introduction

From the point of view of NATO norms, chemical warfare agents (CWAs) can be identified on preliminary, confirmed or unambiguous level [1, 2]. A unit composed of a sampling team and a deployable laboratory (DLAB) should have a confirmed identification capability. For this, it is necessary to confirm the presence of the substance in the sample, in addition to chromatographic retention data, also by spectrometric technique [3]. Methods that are not included in the AEP-66 standard [2] do not achieve the

* *Corresponding author: Nuclear, Biological and Chemical Defence Institute, University of Defence, Víta Nejedlého No. 1, CZ-682 01 Vyškov, Czech Republic. Phone: +420 973 45 23 38, Fax: +420 973 45 23 30, E-mail: tomas.rozsypal@unob.cz. ORCID 0000-0002-0872-0876.*

output information quality for identification and, from a terminological point of view, only allow detection. Identification methods must produce outputs that allow tracing information leading to the raw data. At the same time, the operator should be well informed about and interpret the measured data (e.g. spectrum), which in principle excludes fast detectors, where the output is limited to displaying the name of the compound in the library whose characters match the analyzed substance.

Raman spectroscopy is not explicitly listed in NATO standards, but it is also an optical (spectrometric) analytical technique, moreover fast, sensitive and contactless. It enables the analysis of the substance in an unopened container, which, however, must be transparent. Raman spectroscopy works on the principle of Raman scattering. When a monochromatic radiation incident occurs on the sample, the radiation gets reflected, absorbed, or scattered. The scattered radiation has a different frequency than the incident radiation. The difference in frequency is known as the Raman shift which is characteristic of the vibrational modes of the sample [4]. In the Raman spectrum, there is a unique, so-called fingerprint area, which is specific for each chemical substance, and is thus able to identify the chemical quite accurately (not in the NATO terminology).

Mass spectrometry, nuclear magnetic resonance and infrared spectrometry are listed as spectrometric techniques for identification level in AEP-66 [2]. The absence of Raman spectroscopy among the named techniques is probably because, unlike the others, it is not used as a hyphenated technique (mainly in combination with chromatography). The Raman signal of chemical substances is also very weak compared to other signals (only about one in 10 million scattered photons are Raman scattered). The Raman spectrometer is thus relatively expensive due to the necessity of a very sensitive detector. However, in recent years, due to the miniaturization of devices, hand-held detectors have been developed, but their parameters and software security do not reach the level of benchtop devices.

Handheld Raman spectrometers are commonly used for rapid detection of explosive substances [5, 6]. Raman spectrometry of chemical warfare agents (CWAs) has also been investigated by many authors. Miniaturization of detectors for the possibility of on-site analysis of toxic substances is a trend of recent years [7]. Handheld Raman spectrometers for standoff detection at short distances (units of meters) are also being developed [8]. Kondo et al. [9] used handheld Raman spectrometer Xantus-2 (Rigaku) with two excitation wavelengths to identify 22 CWAs. A wavelength of 785 nm was evaluated as suitable. However, some CWAs could not be identified, as proved by Satoh et al. [10] who were able to identify only 32 % of the tested substances using Rapid-ID (DeltaNu). Wiktelius et al [11] tested a number of sulfur mustard samples with different compositions referring to different synthetic routes. They confirmed the capability of handheld Raman spectrometers for rapid identification and recommended their use for forensic purposes. Raman spectroscopy was also used for the detection of chemical weapons contents [12]. The authors draw attention to possible interference of degradation products and other ballast substances in the resulting spectrum. The advantage of Raman spectroscopy is the no-preparation “point-and-shoot” identification system in units to tens of seconds [13]. Nan et al. [14] investigated the possibility of using a handheld Raman spectrometer for rapid screening of toxic substances in a sample. They recommend combining the technique with suitable software for mathematical analysis. A common problem is fluorescence, which interferes with the Raman signal and makes identification impossible. The solution is to use higher wavelengths of the excitation laser. On the other hand, this reduces the excitation energy

and thus the sensitivity. Wilcox and Guicheteau [15] mention the need to extract the substance from poorly transparent containers as a drawback due to the difficult penetration of the laser.

The aim of the paper is to demonstrate a possible use of handheld detectors for the identification of chemical warfare agents and their precursors by interpreting Raman spectra and to create a library of substances of interest. Part of this is the verification of the effect of the sample box type and its transparency on the quality of the resulting spectrum and the effect of the purity of the investigated substance.

2 Chemicals and Equipment

A pack of 29 representatives from individual groups of chemical warfare agents and their precursors were selected to carry out the experimental part (Tab. 1, Fig. 1).

Tab. 1 Chemical warfare agents and precursors investigated in the study

Group	Code or abbreviation	Chemical name	Purity	Manufacturer*
choking	DP	trichloromethyl chloroformate	95	ZK
blood	KCN	potassium cyanide	99	LachNer
blister	HD	bis(2-chloroethyl)sulfide	95	ZK
	HN-1	ethyl bis(2-chloroethyl)amine hydrochloride	99	VVU
	HN-2	methyl bis(2-chloroethyl)amine hydrochloride	99	VVU
	HN-3	tris(2-chloroethyl)amine hydrochloride	99	VVU
	L-1	2-(chlorovinyl)dichloroarsane	83	ZK
	TDG	thiodiglycol	99	Sigma
	HN1OH	*N*-ethyldiethanolamine	98	Sigma
	HN2OH	*N*-methyldiethanolamine	99	Sigma
	HN3OH	triethanolamine	99	Sigma
	As_2O_3	arsenic oxide	99	Sigma
nerve	GB	isopropyl methylphosphonofluoridate	99	ZK
	GD	pinacolyl methylphosphonofluoridate	89	ZK
	GA	ethyl (dimethylphosphoramido)cyanidate	95	ZK
	GF	cyclohexyl methylfluorophosphonate	99	VVU
	VX	[2-(diisopropylamino)ethyl] ethyl methylphosphonothiolate	78	ZK
	DIAET	2-diisopropylaminoethanethiol	95	ZK
	DIAE	2-diisopropylaminoethanol	99	Sigma
	DMAET	dimethylaminoethanethiol	95	ZK
	COH	cyclohexanol	99	Sigma
	IOH	isopropyl alcohol	99	Sigma
	POH	pinacolyl alcohol	99	Sigma
irritant	CN	chloroacetophenone	95	ZK
	CR	dibenzo[b,f][1,4]oxazepine	98	ZK
	CS	2-chlorobenzylidenemalononitrile	94	ZK
psychoactive	BZ	3-quinuclidinyl benzilate	98	ZK
	QOL	3-quinuclidinol	99	Sigma

* ZK = Military Repair Facility Zemianske Kostolany, Slovakia; LachNer = LachNer, Czech Republic; VVU = Military Research Institute, Czech Republic; Sigma = Sigma Aldrich, Germany

The purities of the substances were deducted from the manufacturer's certificate, or were verified by gas chromatography with a flame ionization detector. Non-additive diesel fuel (MOL, Czech Republic) and toluene p.a. (LachNer, Czech Republic) were

used as a contaminant in CWAs samples. The chemical warfare agents and other chemicals used were not purified or treated in any way.

FirstDefender RM Raman spectrometer (Thermo Scientific, USA) was used to capture Raman spectra. It is a handheld detector with a laser wavelength of 785 nm and a maximum power of 300 mW. For the purposes of monitoring the influence of the container on the quality of the scanned spectrum and automatic evaluation, universal 2 ml clear vials made of borosilicate glass with a screw thread with a diameter of 9 mm (container 1) and 2 ml amber vials (container 2) with the same properties were tested (both Chromservis, Czech Republic).

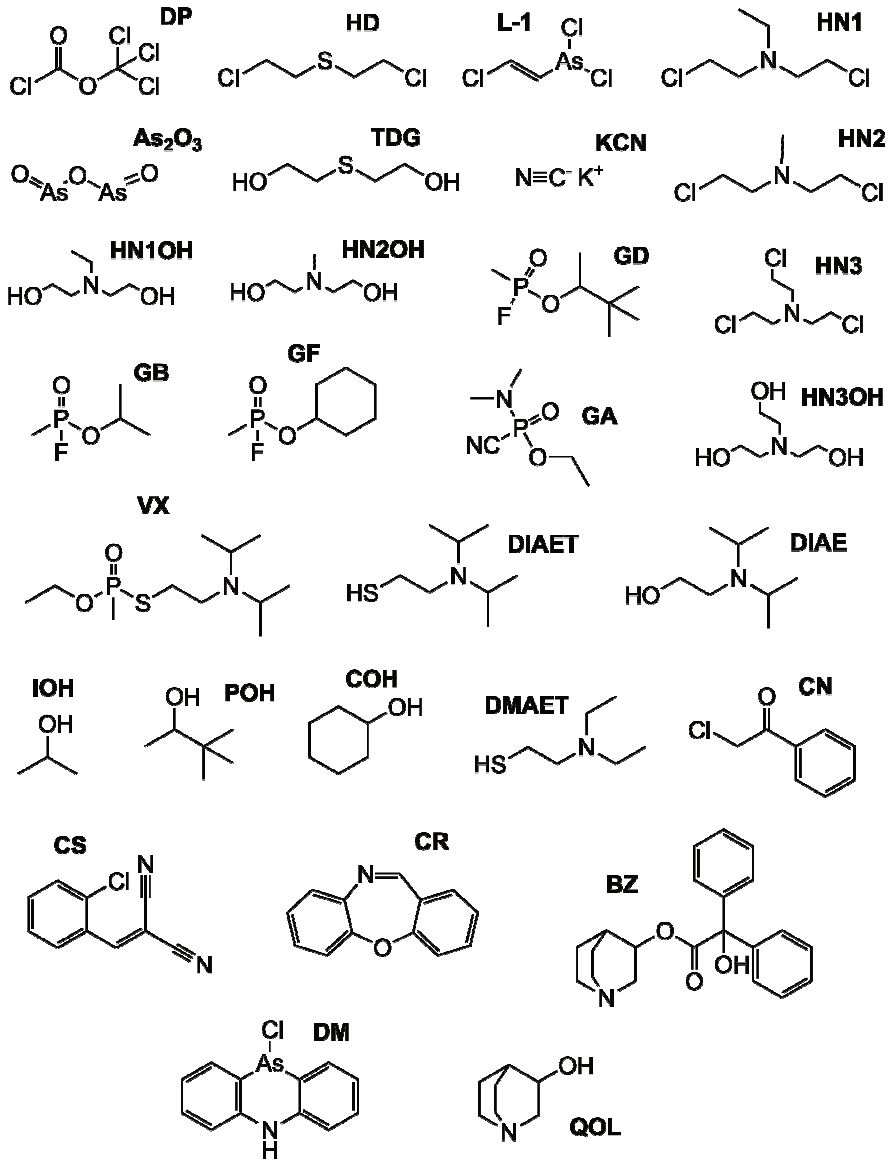

Fig. 1 Structures of chemical warfare agents and their precursors

Another type of sample containers tested were 4 ml borosilicate glass vials with alleged enhanced transmittance for Raman spectroscopy (container 3, RMI, Czech Republic), as well as polyethylene platic containers 100 ml (container 4, Kartell, Italy), a plastic laboratory container with a volume of 250 ml (container 5, Denios, Czech Republic) and a polyethylene bottle with a volume of 250 ml (container 6, VITLAB, Germany). Spectragryph software (Spectroscopy Ninja, Germany) and OriginPro 8.5 (OriginLab, USA) were used to evaluate Raman spectra after data export to a computer. A UV/VIS spectrophotometer Helios α (Thermo Electron Corporation, USA) was used to measure transmittance. A Sonorex (Bandelin, Germany) ultrasonic bath was used to homogenize the solutions.

3 Procedures

3.1 Characterization of Sample Containers

The containers were first visually assessed and their peculiarities noted. For an objective comparison of the transparency of the containers, their transmittance was measured using a two-beam UV/VIS spectrophotometer. Air (empty cuvette space) was used as a comparison blank, and the corresponding vial without liquid content was irradiated with the measuring beam. The measurement took place at a fixed wavelength of the rays of 785 nm (a value chosen identical to the wavelength of the excitation laser of the Raman spectrometer). The thickness of the material was determined with a caliper.

3.2 Raman Spectra Scanning

Three types of sample containers were prepared for each compound – container 1-3. Each sample container was filled with about 100 mg of solid (KCN, HN-1, HN-2, HN-3, As_2O_3, CN, CR, CS, DM, BZ or QOL) or 100 µl of liquid (DP, HD, L-1, TDG, HN1OH, HN2OH, HN3OH, GA, GB, GD, GF, VX, IOH, POH, COH, DIAE, DIAET or DMAET). The selected substances were also tested in containers 4-6 (KCN, HN-3, HD, POH, IOH).

Each prepared sample was scanned at 3 powers of the excitation laser (250, 125 or 75 mW). The scan delay was set to 0 s. The maximum scan time was set to 2 min. Each sample was then measured noncontact inside the unopened container. The recorded spectra were subsequently stored in the memory card database and exported.

3.3 Interpretation of the Raman Spectrometer Outputs

The FirstDefender device enables matching of the measured spectrum to the substance spectrum stored in the library. However, it is not comprehensive, moreover, the assignment of the same substance in the library does not allow interpretation. The measured spectra were therefore exported and analyzed using Spectragryph and Origin software. Here, the wavenumber peaks of the Raman bands were identified in each spectrum, and with the use of libraries [4, 16], individual peaks were assigned to the characteristic bonds found in the molecule of the analyzed substance.

3.4 Effect of Contaminant Addition

Toluene and non-additive diesel fuel were used as contaminants of pure substances. These solvents come into consideration in clandestine laboratories as reaction media for synthesized substances, stabilizers, etc. The tested substances were liquid CWAs DP and HD, the spectra of which were recorded and stored according to the procedure described earlier. The measurement of the effect of contamination was started by scanning the Raman spectrum of pure CWA in a volume of 200 µl. Subsequently, 100 µl of the appropriate contaminant was added to the substance, the mixture was homogenized for 1 min in an ultrasonic bath, and its Raman spectrum was recorded. This was followed by additions in a volume of 100 µl of the contaminant until the phase when the device was unable to identify the analyte.

4 Results and Discussion

4.1 Characterization of Containers

Borosilicate glass appears to be a typical material for the potential container of a toxic substance. It is inert to the action of most chemicals and is used in laboratories (including clandestine) for synthesis, analysis and storage of substances. For the purposes of this research, universal laboratory clear vials made of borosilicate glass with a 9 mm diameter screw thread, a lid with a polytetrafluoroethylene (PTFE) septum and a flat bottom were used (container 1). Vials made of amber glass (container 2) differ only in the descriptive place on the outside and the color of the glass. These vials are mainly used when working with devices with autosamplers for sample analysis. As far as the third type is concerned, a sample box designed directly for Raman spectroscopy with alleged increased transparency, objectively clear borosilicate vials with a size of 15×45 mm, a volume of 4 ml with a PTFE silicone septum and a lined cap of green color was chosen. Container 4 was a 100 ml white opaque storage box for powdery substances made from polyethylene (PE). Container 5 consisted of a 300 ml wide-necked white low density PE (LDPE) bottle (Denios, Czech Republic) intended for sampling and storage of liquid and solid substances. Container 6 was a blue, slightly transparent 250 ml bottle used in the alcohol laboratory. The material is pro-dyed PE. The containers are depicted in Fig. 2.

Spectrophotometric verification of the transparency of the containers proved the suitable optical properties of the containers intended for Raman spectroscopy. Amber vials had half the transparency compared to clear vials with otherwise identical properties and volume. The lowest transparency was recorded for the opaque container 4. Transparency is objectified here in the form of transmittance, which expresses the ratio of the intensity of the light that passed through the sample and the intensity of the light that entered the sample (1):

$$\%T = \frac{I}{I_0} \cdot 100 \tag{1}$$

The results are summarized in Tab. 2. The material and color of the individual containers are also shown. In general, Raman spectroscopy is effective in transparent materials; however, coloring increases the proportion of fluorescence and thus reduces

the permeability of laser beams. Fluorescence is also an interference in Raman signal collection. Transparency also decreases with the thickness of the optical medium.

Fig. 2 Vessels used as containers for toxic substances analysis. Arranged from left to right as container 1-6

Tab. 2 Selected properties of used containers

Container	Material	Colour	Thickness, mm	%T
1	glass	clear	1.30	80.22
2	glass	amber	1.30	46.34
3	glass	clear	1.78	84.77
4	PE	white, opaque	1.68	14.09
5	LDPE	white	1.40	60.83
6	PE	blue	1.25	51.05

4.2 Choking and Blood Agents

Of the choking agents, DP, a liquid derivative of phosgene (carbonyl dichloride), was analyzed. A band of low intensity in the range 1 820-1 800 cm^{-1} was visible in the spectrum, which can be identified as the vibration of the C=O functional group (according to the literature [15, 16] 1 807 cm^{-1}). Furthermore, peaks with moderate Raman signal intensity were observed at 823 and 588 cm^{-1}, which corresponds to the C-Cl_3 group and the range of the Raman band 850-550 cm^{-1}. A C-Cl group (vibration at 800-600 cm^{-1}) was assigned to the 766 cm^{-1} peak. The most intense Raman scattering was observed at 494 and 398 cm^{-1}. The peak at 494 cm^{-1} was assigned the characteristic functional group O=C-Cl and the peak at 398 cm^{-1} the group C-Cl_3. The spectra measured at high, medium and low power of the device did not show significant changes in the individual peaks.

KCN is a simple polar molecule, the only bond visible in the Raman spectrum is N≡C (2 076 cm^{-1}). This is a strong signal and changing the laser power did not affect the quality of the spectrum. Tab. 3 summarizes the specific values of the wavenumbers of the Raman signals and the assigned chemical bonds of the individual investigated chemical warfare agents and precursors.

Tab. 3 Overview of Raman bands and assigned functional groups of chemical warfare agents and precursors

CWA	C-S	alkyl	C-C-O	C-OH	other
DP	—	—	—	—	398, 588, 823 (C-Cl_3), 494 (O=C-Cl), 766 (C-Cl), 1 807 (C=O)
KCN	—	—	—	—	2 076 (N≡C)
HD	650, 758	1 036, 1 295, 1 426	—	—	698, 733 (C=O)
HN-1		810-1 420			721 (C-Cl), 1 450 (C-N)
HN-2		805-1 440			728 (C-Cl), 1 447 (C-N)
HN-3		802-1 445			770 (C-Cl), 1 441 (C-N)
L-1		1 285	—	—	391, 1 553 (As-Cl)
TDG	657, 766	900-1 465	819	1 015	—
HN1OH		1 499			
HN2OH	—	348, 1 060, 1 459, 2 872	877	1 128	2 800 (CH_3-N)
HN3OH	—	352, 1 026-1 465, 2 868	877	—	—
As_2O_3	—	—	—	—	268, 470, 781 (As-O-As), 370, 560 (As=O)
GB	—	1 417, 1 452	—	—	505 (P-O), 721 (O-P-O), 882 (P-F), 1 273 (P=O)
GD	—	931, 1 447	—	—	540 (P-O), 729 (O-P-O), 760 (P-C), 869 (P-F)
GF	—	416, 640	—	—	534, 688 (P-O), 1 346 (P-CH_3)
VX	650	1 436, 1 465	—	—	503 (P-O), 762 (O-P=O), 889 (O-P-C)
IOH	—	952, 1 450, 2 873	819	—	1 026, 1 155 (C-O)
COH	—	556, 791, 1 260, 1 444, 2 854	—	1 026	1 350 (O-H)
POH	—	363, 1 219, 1 450	933	1 336	1 084 (C-O)
DIAET	—	880, 1 458	—	—	499 (N-C_3), 656, 2 571 (S-H)
DIAE	688	727, 869, 1 465, 2 872	929	1 110	490 (N-C3)
DMAET	296, 666	—	—	—	2 567 (S-H), 991 (N-C_3)
CN	—	621, 790, 1 210	—	—	1 000 (C-Cl), 1 597 (C=C), 1 695 (C=O)
CR	—	402, 715, 1 032	—	—	340 (C-CN), 1 201 (C-N), 1 395, 1 600 (C=C)
CS	—	623, 1 217, 1 295	—	—	340 (CH-Cl), 1 375, 1 586 (C=C), 2 228 (C=N)
DM	—	623, 1 217, 1 295	—	—	386, 1 571, 1 599 (As-Cl)
BZ	—	675, 1 190			830 (C-O-C), 1 003 (aromatic ring), 1 600 (hetero ring)
QOL	—	1 440, 2 867		1 025	

4.3 Blister Agents

The sensing of the spectra of nitrogen mustards was associated with high fluorescence. The molecular and fluorescent signals were at a similar level during the analysis. In these cases, the Raman spectrometer was unable to search for matching signatures of nitrogen mustards and compare them with the spectra stored in its library. Nevertheless, after data export, it was possible to identify a Raman shift characteristic of the alkyl function in all nitrogen mustards, followed by weak signals identifying C-Cl and C-N bonds. In addition, after storing the newly measured spectra in the library, the analytes were correctly identified by the instrument within a few seconds during reanalysis.

The HD structure is characterized by a thioether, methylene and C-Cl bond. The visible peak at 1 426 cm^{-1} was assigned to the Raman band 1 445-1 385 cm^{-1}, by the addition of the CH_2 functional group. The C-CH_2 bond was assigned to the following peak at 1 295 cm^{-1} falling within the range of 1 305-1 295 cm^{-1}. The observed peak at

1 036 cm^{-1} was assigned to the band with a span of 1 300-1 000 cm^{-1} given by the C-C bond. In the measured Raman spectrum, there are also characteristic peaks of the 800-600 cm^{-1} and 760-650 cm^{-1} bands, where the peaks at 733 and 698 cm^{-1} of the C-Cl bond and the 760-650 band were included in the first mentioned band peaks. Peaks at 758 and 650 cm^{-1} form the right and left boundaries of this Raman band. The C-S stretching mode with high Raman signal intensity was assigned to the peaks. The region 600-800 cm^{-1} can be considered as the fingerprint region by which HD was identified.

What the Raman spectrometer failed to evaluate were the results of the L-1 analysis through the manufacturer's library. Even though the measurements were performed repeatedly at each instrument power, the substance emitted a strong Raman signal, however, the spectrum of the substance did not show significant characteristic peaks identical to the substance in the library. When interpreting the spectra, the strong signal peaks at 1 553 and 391 cm^{-1} were assigned to the As-C group. The weak peak of the 1 295-1 190 cm^{-1} band at 1 285 cm^{-1} can be attributed to vibrations derived from the alkene group. Changing the laser energy did not result in variations of the recording of the peaks.

The Raman spectrum of TDG is dominated by a peak at 657 cm^{-1} with smaller peaks at 766, 819, 1 015, 1 421 and 1 465 cm^{-1}. The last two mentioned peaks were attributed to the CH_2 methyl group and the bands 1 475-1 450 cm^{-1}, representing scissor vibrations, and 1 445-1 385 cm^{-1}. With a very strong intensity of the Raman band 1 200-1 015 cm^{-1}, a peak at 1 015 cm^{-1} typical for alcohols was observed. The dominant peak at 657 cm^{-1} was preferentially assigned to the C-S-C stretching mode over C-Cl, as was the case with HD mainly because the chloride groups were replaced by hydroxyl groups. In the following comparison of individual TDG spectra, it was found that the reduced power of the laser and the different material of the containers did not affect the analysis of the substance. Fig. 3 illustrates a comparison of HD and TDG spectra.

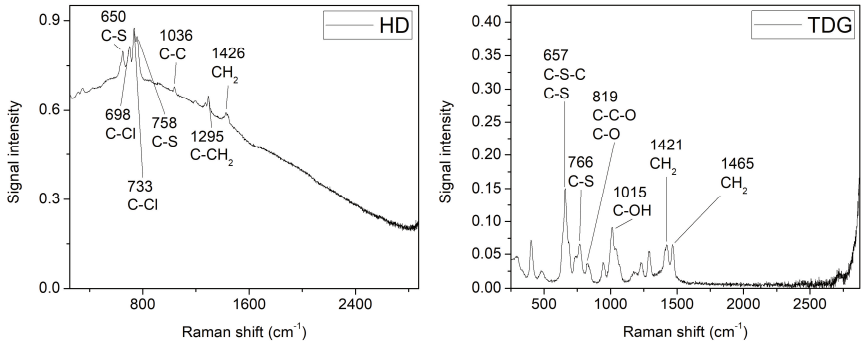

Fig. 3 Interpreted Raman spectra of sulfur mustard and its precursor thiodiglycol

The degradation products of nitrogen mustards were also measured. During the analysis of HN1OH, a high fluorescence exceeding the molecular Raman signal appeared, similar to that of nitrogen mustards, and identification was difficult, possible perhaps in clear containers only. In the spectrum, it was possible to record a particularly strong signal at 1 499 cm^{-1}, which is related to the alkyl functional group. There are strong band peaks at 2 872 and 2 800 cm^{-1} in the spectrum of HN2OH. The first peak of the 2 990-2 850 cm^{-1} band is given by the symmetric vibration of CH_2 or the anti-

symmetric vibration of CH_3. The second more significant peak was attributed to the CH_3-N functional group of the 2 850-2 700 cm^{-1} band. In the spectrum of HN3OH there are similar Raman lines as in the spectrum of HN2OH described above. The peak shown only at 1 465 cm^{-1} was attributed to CH_2 stretching vibration, and the peak at 1 295 cm^{-1} was assigned to the 1 305-1 295 cm^{-1} band, which is derived from the methylene chain.

The Raman spectrum of As_2O_3 showed five strong vibrations between 200-800 cm^{-1}. The peaks at 560 and 370 cm^{-1} were assigned to the out-of-phase vibrations of As=O and the bands 530-600 cm^{-1} and 335-380 cm^{-1}. The peaks at 781 and 470 cm^{-1} are due to stretching of the As-O-As bond. The peak at 268 cm^{-1} was assigned an As-O-As rotational mode. Unlike HN1OH, other substances could be identified in all sample boxes and using all laser powers.

4.4 Nerve Agents

Nerve agents, including their precursors, contain characteristic spectral peaks used to distinguish them from other CWAs. Strong band peaks at 700-800 cm^{-1} were observed for GB and GD substances, which are the result of vibrational modes involving the phosphorus atom. The strong band peaks at 1 452 and 1 417 cm^{-1} can be attributed to CH_3 bending vibrations, belonging to two overlapping Raman bands 1 465-1 440 cm^{-1} and 1 450-1 375 cm^{-1}. All G substances contain the functional group P-O, which according to previous studies [8, 9, 11] was always assigned to the Raman bands found approximately in the range of 1 280-1 240 cm^{-1}. A peak at 1 273 cm^{-1} of the P=O group was included in the 1 300-1 175 cm^{-1} band. The strongest GB line at 721 cm^{-1} can be assigned to a P-O-C or O-P-O symmetric stretch. The same was true for GD at 729 cm^{-1}. The P-F stretching vibrations were assigned to weaker peaks at 882 cm^{-1} and 869 cm^{-1} for GB and GD, respectively. Furthermore, a peak at 1 447 cm^{-1} belonging to the band 1 450-1 375 cm^{-1} was shown in the GD spectrum, which was assigned to the CH_3 methyl group, and a weak visible peak at 931 cm^{-1} characteristic of the C-C alkane chain was assigned to the band 955-900 cm^{-1}. Analogous to GB, it was possible to attribute the P-O vibration of the 540 cm^{-1} peak to the GD substance.

In the case of GF, no vibration was assigned to the peak at 1 541 cm^{-1} as no such functional group was found to match the structure of the substance. The peak could generally be derived from the NH vibration, assigned to secondary amines of the 1 580-1 490 cm^{-1} band. The bending vibrations of CH_3 fall in the band 1 450-1 375 cm^{-1}, where the peak at 1 346 cm^{-1} can be included. Peaks at 688 cm^{-1} (700-665 cm^{-1}) and 534 cm^{-1} (550-490 cm^{-1}) were attributed to characteristic P-O vibrations. The 710-605 cm^{-1} band peak was derived from the C-C bond, and the peak at 416 cm^{-1} was assigned to the stretching vibration of the hydrocarbon backbone of the 430-370 cm^{-1} band. The scanning itself and the subsequent evaluation of the sample with the spectrometer was done very quickly, but the spectrometer could not determine the identical features with the spectral library of substances on the basis of the scan. For interpretation, a scan of the substance at the highest power of the device was used, because at lower powers a mere line without the slightest peaks was displayed.

For VX, there was a problem with the evaluation of the scanned data, and the device was unable to match the measured spectrum to the VX in the library. After exporting the data from the spectrometer, the strong band peaks at 1 465 and 1 436 cm^{-1} were interpreted by assigning the vibrations of the CH_3 bond, the alkyl

function attached to the nitrogen belonging to the 1 465-1 440 cm^{-1} and 1 450-1 375 cm^{-1} bands. The significant peak at 899 cm^{-1} can be assigned an O-P-C or C-C-N vibrational mode. Similar to both G and V type agents, the strong band peak at 762 cm^{-1} generally observed in nerve agents was plotted in the Raman spectrum of VX. A weak band peak at 650 cm^{-1} was assigned a C-S functional group in the 775-650 cm^{-1} band range. Also, the peak at 503 cm^{-1}, which was already described for the GB agent, can be attributed to the P-O bending vibrations. Fig. 4 illustrates the interpretation of Raman spectra of nerve agents. The intensity of the respective Raman bands is weak for all nerve agents. In the case of GD and VX, there is also a significantly increased background, which can be attributed to the lower purity of the standards used.

In the case of IOH (GB precursor), two high intensity band peaks at 2 873 and 890 cm^{-1} were visible. The peak at 2 873 cm^{-1} was assigned CH_2 and CH_3 vibrations, since two bands of the range 2 880-2 830 cm^{-1} and 2 888-2 862 cm^{-1} intersect at this point. Due to the IOH structure, the CH_3 functional group was preferred. The second characteristic peak is typical for secondary alcohols with C-C-O band vibration of 900-800 cm^{-1}. The peak at 1 450 cm^{-1} occurring in the 1 450-1 375 cm^{-1} band was attributed to the CH_3 methyl group. The C-O vibrations were assigned to the peaks at 1 155 and 1 126 cm^{-1} and the C-C vibrations of the peak at 952 cm^{-1}. IOH showed a high Raman signal in all the measured spectra, which did not change when using a low power of the instrument or when measuring in containers made of a different material.

COH was analyzed as a precursor of GF. The first of the dominant Raman lines contained in the spectrum was made visible in the band 2 863-2 843 cm^{-1} at 2 854 cm^{-1}. This line was assigned the symmetric CH_2 vibration. Other peaks at 1 444 and 1 260 cm^{-1} were assigned to the CH_2 vibration, but of scissor type. The characteristic bond of COH is the -OH bond, which was assigned to the peak at 1 350 cm^{-1} in the 1 430-1 345 cm^{-1} Raman band. There is also a band with a span of 1 065-1 015 cm^{-1}, where a peak at 1 026 cm^{-1} with medium intensity Raman signal, typical for cyclic alcohols, was included. The dominant C-C strain ring vibration was assigned to a high signal intensity peak at 791 cm^{-1} and a peak at 556 cm^{-1}. Raman bands 1 300-600 cm^{-1} and 580-430 cm^{-1} correspond to these peaks. The resulting Raman spectra were not affected by different values of the device's laser power.

In the case of the Raman spectrum of POH (GD precursor), the right border of the 1 450-1 375 cm^{-1} band is formed by a relatively strong peak characteristic of the given compound. The peak at 1 336 cm^{-1} was assigned to the C-OH functional group of the 1 400-1 260 cm^{-1} band, where primary and secondary alcohols can be classified. The C-C bond assigned to secondary alcohols in the range of 1 300-1 000 cm^{-1} and 400-250 cm^{-1} was attributed to the peaks at 1 219 and 363 cm^{-1}. Subsequent peaks at 1 084 and 933 cm^{-1} were assigned to C-O (1 150-1 070 cm^{-1}) and C-C-O (935-925 cm^{-1}) vibrations.

One of the main hydrolysis products of VX is DIAET. The Raman spectrum of the substance was interpreted in terms of an alkanethiol and an alkyl-substituted tertiary amine. Peaks at 2 571 and 656 cm^{-1} were assigned to a characteristic thiol group, whose bands extend to 2 600-2 520 cm^{-1} and 750-570 cm^{-1}. The CH_3 methyl group was attributed to a strong peak at 1 458 cm^{-1} (1 465-1 440 cm^{-1}) and a peak at 880 cm^{-1} (890-810 cm^{-1}). The peak of the 1 075-1 200 cm^{-1} band was derived from the tertiary amine group, and the peak at 760 cm^{-1} belonging to the 785-750 cm^{-1} band

was assigned to the CH bending mode. Furthermore, the substance produced one N-C_3 mode in the region 500-400 cm^{-1}, where a peak at 499 cm^{-1} was included. When scanning this hydrolysis product, minor deviations were noted when measuring the sample in the Raman vial. At 250 mW, the instrument (based on the internal library) evaluated the contents of the vial as DIAET or 1,2,4,5-tetrafluorobenzene. Nevertheless, the problem was not identified in the other samples. After creating the user library with the newly measured spectrum, DIAET was rapidly identified when repeating the experiment.

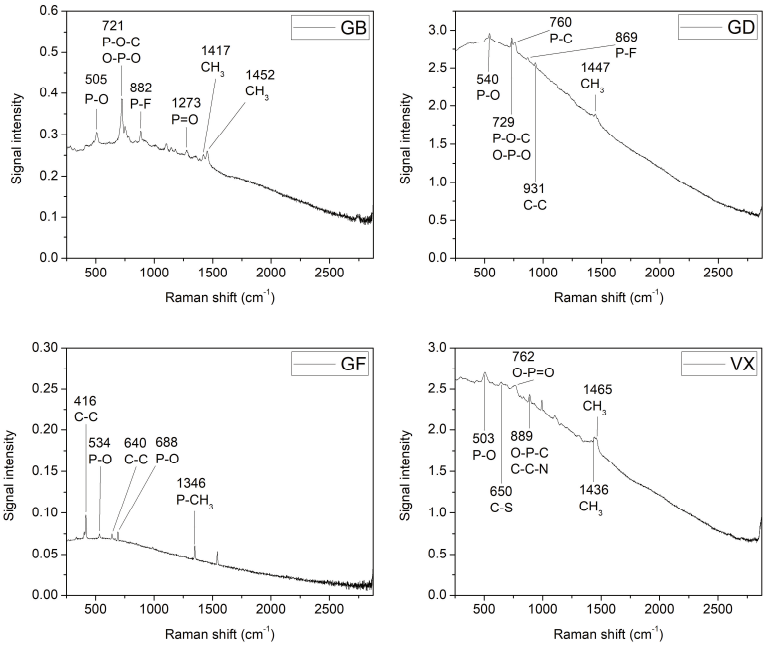

Fig. 4 Intepreted Raman spectra of nerve agents sarin (GB), soman (GD), cyclosarin (GF) and VX agent

Most of the peaks discovered in the DIAE spectrum were analogously assigned to the same vibrational bands as in DIAET, which differs structurally only in the substitution of a sulfur atom for oxygen. The difference was observed in the peak at 2 872 cm^{-1}, which was attributed to the symmetric vibration of CH_3. The peak at 1 110 cm^{-1} was assigned to functional groups C-OH (1 200-1 015 cm^{-1}) and C-C-N (1 230-1 110 cm^{-1}). However, this peak may also belong to the CN bond, as in DIAET, because the individual Raman bands for certain groups overlap. The peak at 929 cm^{-1} was attributed to the C-C-O vibration of the 935-925 cm^{-1} band and the peak at 688 cm^{-1} to the C-S stretching vibration of the 775-650 cm^{-1} band. The band in the range of 1 380-1 360 cm^{-1} determines the iso-propyl group contained in both DIAET and DIAE. When comparing the obtained DIAE spectra, no significant differences were found in the depiction of individual peaks. All spectra show a relatively low Raman lines.

The third analyzed product of V-type agents was DMAET. Characteristic band peaks for the substance at 2 774, 2 567 and 991 cm^{-1} were visible in the spectrum which can be assigned to symmetric CH_3 vibrations (2 895-2 840 cm^{-1}), thiol vibra-

tions in the 2 600-2 520 cm^{-1} band, and N-C_3 vibrations (1 015-980 cm^{-1}). The peak at 830 cm^{-1} was assigned the CH_3 vibrational mode, the band in the range 890-810 cm^{-1} and the peak at 749 cm^{-1} was assigned the CH bending vibration (785-750 cm^{-1}). The remaining peaks at 666 and 296 cm^{-1} were assigned to the C-S functional group spanning the bands 775-650 cm^{-1} and 410-200 cm^{-1}.

4.5 Irritants and Psychoactive Agents

The spectrum of CN contains a unique band peak at 1 695 cm^{-1} attributed to the C=O carbonyl group. The following relatively strong peak was derived from C=C aromatic hydrocarbon bonds, located in the band 1 630-1 590 cm^{-1}. The vibrations of the aromatic ring correspond to the peaks at 1 210, 790 and 621 cm^{-1} in the bands 1 290-1 130 cm^{-1} and 790-620 cm^{-1} and the peak at 1 000 cm^{-1} forming the right border of the band 1 400-1 000 cm^{-1} with a high Raman signal intensity was attributed to the C-Cl bond.

The CR Raman spectrum could only be identified after export, as no match was found with the spectra stored in the spectrometer library. The only spectrum that showed at least weak peaks at a low Raman signal intensity was measured in container 1. In the spectrum of CR, peaks derived from an aromatic hydrocarbon were found at 1 600 and 1 395 cm^{-1} belonging to the band 1 630-1 590 cm^{-1} and 1 440-1 340 cm^{-1}, respectively. Another, although weak but characteristic peak, appeared at 1 201 cm^{-1}, which was assigned to the functional group C-N determined for aromatic amines in the range of 1 280-1 180 cm^{-1}. The peaks at 1 032 cm^{-1} (1 040-990 cm^{-1}) and 715 cm^{-1} (730-660 cm^{-1}) were attributed to the CH group, the peak at 402 cm^{-1} to the C-C functional group of the 415-385 cm^{-1} band, and the peak at 340 cm^{-1} (390-340 cm^{-1}) was derived from the C-CN bond.

The structure of CS is characterized by an aromatic ring, a benzene-Cl bond, and an alkene and cyanide function. The cyanide CN functional group was assigned to a strong unique peak at 2 228 cm^{-1}. A strong band peak at 1 586 cm^{-1} was assigned to alkene C=C stretching vibrations in the 1 660-1 580 cm^{-1} band. The following peak at 1 375 cm^{-1} corresponds to multiple cyclic C=C bonds in the Raman band 1 440-1 340 cm^{-1}. The peaks at 1 295, 1 217 and 623 cm^{-1} located in the bands of 1 295-1 190 cm^{-1} and 790-620 cm^{-1} were attributed to the vibrations of the aromatic ring. The last visible peak in the spectrum at 1 041 cm^{-1} was derived from the benzene-Cl aromatic bond. There were no complications during the scanning of the CS substance that would affect its evaluation. The resulting spectra contained sharp and strong peaks characterizing the given compound.

The obtained DM spectrum was not able to be identified by the instrument. After exporting the data, it was found that the individual spectra of the substance do not show strong band peaks. When comparing the spectra, it was found that when measuring the sample in container 2, the quality of the recorded spectrum was the lowest. On the contrary, the sharpest peaks were observed when scanning the sample in container 3. Compared to the intense baseline visible in the Raman spectrum of DM, the peaks were very low and only two characteristic bonds occurring in its structure were found. Weak peaks at 1 599, 1 571 and 386 cm^{-1} were assigned to the characteristic As-Cl vibration. The peaks at 1 321 and 1 020 cm^{-1} can be attributed to vibrations derived from the aromatic structure, band 1 140-1 020 cm^{-1}. The obtained Raman bands differ from the results of Chrinstensen [17] and Kondo [9], probably due to the high fluorescence of our sample.

In the case of BZ and QOL as its degradation product, there were no identification problems. In the QOL spectrum, peaks 1 440 and 2 867 cm^{-1} were assigned to the alkyl function, and peak 1 025 to the alcohol C-OH bond. The substance BZ is formed by the reaction of benzylic acid and quinuclidinol by esterification, so it does not contain a C-OH bond. Instead, the 830 cm^{-1} peak was identified as a C-O-C ester bond. Furthermore, in addition to the peaks related to the alkyl function, it was possible to interpret the peak 1 003 cm^{-1} and 1 600 as related to the aromatic ring (2x phenyl group) and the heteroring (quinuclidinyloxy group).

4.6 Effect of Contamination of the Analyzed Substance

The disadvantage of handheld Raman spectroscopy is its easy practical applicability only for pure or concentrated substances. It can thus be assumed that when a CWA is contaminated with a solvent or other substance, its contribution to the spectrum of the analyzed sample will increase with the increasing volume of the interferent until the moment when it will not be possible to distinguish the characteristic peaks of the bonds of the investigated toxic compound. This will be true if the contaminating interferent absorbs radiation and exhibits a Raman spectrum, which more complex organic compounds with polar and nonpolar bonds will do. When Christensen et al. [17] tested the contamination of HD and L-1 with chloroform, the detection limit was 0.5 %.

In the case of scanning of Raman spectra of HD contaminated with diesel fuel, after dosing the contaminant and subsequent homogenization, it was necessary to let the resulting solution stand for 0.5 min to eliminate turbidity and foaming, which reduced the ability of identification. In addition to HD, the contaminant (diesel) in the mixture was identified by the device when the HD concentration dropped to 66 %, i.e. immediately after dilution. All specific Raman bands (Tab. 3) were visible in the spectrum up to a HD concentration of 20 %. On further dilution, weak signals of HD bonds (CH_2, C-C) were drowned out by diesel. The substance was last identified by the device at a concentration of 14 %. For HD toluene contamination, the results were somewhat similar. Toluene was identified next to HD immediately after the first dilution of the CWA. The device was unable to identify HD next to toluene when the concentration dropped below 11 %. When user libraries measured earlier were used as the reference library instead of the manufacturer's spectra, HD was identified in diesel with a detection limit of 8.5 % and in toluene at 6.5 %.

Different results were noted in the case of DP. Diesel was initially detected only when DP was diluted to 33 %, when a peak of 1 450 cm^{-1} characteristic of diesel was identified. The analyte showed such intense Raman signals that it was previously impossible to identify the contaminating substance. At a 4 % concentration of DP in diesel, only two weak peaks defining C-Cl_3 and O=C=Cl bonds, which show a strong Raman signal, were visible in the spectrum of the mixture. The minimum detectable concentration of DP in diesel was 3.3 %. Compared to HD, this is a significant reduction of the detection limit, which occurred due to the presence of bonds with a strong Raman signal in areas where diesel does not have significant signals. In the case of toluene, DP was recorded with a detection limit of 8 %. In the case of using user libraries, the DP detection limit in diesel was 2 %, in toluene then 5 %.

The results indicate that it is not possible to estimate the detection limits of various CWAs in the mentioned matrices based only on the measurement of selected representatives. The Raman spectrum of two specific substances (e.g. DM + diesel fuel) may overlap in important Raman bands, or on the contrary, there may not be

interference. However, in the case of liquid samples, it is recommended to concentrate the sample after a negative detection to reduce its dilution. However, this activity will be related to physical contact with the sample.

4.7 Effect of Container Transparency

Raman spectroscopy is a method that allows contactless detection in closed containers. However, as an optical technique, it needs a transparent container to allow the beam to penetrate to the measured analyte and prevent its absorption [14]. In addition, the color and opacity of the container contribute to increased fluorescence in the spectrum. Fig. 5 illustrates the identification results of pinacolyl alcohol.

The device enabled identification of the investigated CWAs in all tested containers through the user library. The differences in the results of individual containers were the same for all substances. The results in container 1 and 3 are identical, which reflects their same color and transmittance (Tab. 1). These are light-permeable materials that are ideal containers for samples analyzed by Raman spectrometry. Due to the amber color, container 2 showed a lower signal intensity and the spectrum scan was 3× longer (about 9 seconds).

Container 4 was opaque and had the lowest transparency. This is related to the relatively high background value of the spectra. Furthermore, a shift of the Raman bands is noticeable, especially at higher wavenumber values. The instrument had problems identifying this sample when using an internal library that was scanned under optimal conditions. High fluorescence was also recorded during the scan and the measurement took about 25 seconds. The user library had no problem with this real sample. Container 5 showed a relatively high transmittance value despite its white color (Tab. 2). The intensity of the signals was high, the coloring of the container, and the associated fluorescence fraction, however, led to a shift of the Raman bands and increased the scanning time. Container 6 showed a high fluorescence value due to the distinctly blue color. The peaks were very weak, moving slightly above the noise values. The scan time was 30 seconds. However, all the investigated substances were successfully identified in all containers.

4.8 Discussion on the Novelty of the Topic

Kondo et al. [9] presented a method that did not record HN-3 and DM peaks at 785 nm excitation. When testing in gasoline, HD was then identified at a concentration of 20 % or more. Satoh et al. using a hand-held detector, identified only a third of the investigated substances [10]. The method presented by us identified all 29 investigated analytes. Those that could not be identified directly can be identified with a newly created library that was not presented by other authors. The importance of a user library that corresponds with Tab. 3 is also demonstrated by the significant reduction of the detection limit for the diluted HD sample below the values presented by other authors [9]. We also presented a study of this type with the largest amount of analytes. Validation of the method was performed by analyzing a substance in various containers, including opaque, where the use of the method for contactless identification in poorly translucent containers was demonstrated. Earlier studies focused on analysis in transparent materials [6, 9-11].

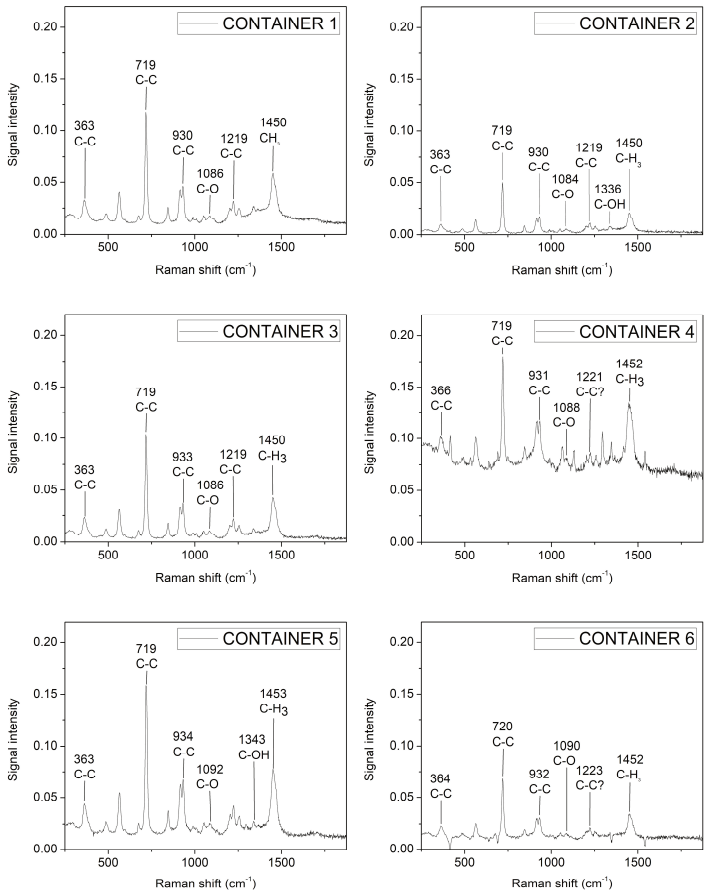

Fig. 5 Comparison of interpreted Raman spectra of pinacolyl alcohol in different containers

The presented results clearly demonstrate a possible use of Raman spectroscopy for the identification of CWAs, as it reliably differentiates between individual agents and no drawbacks were recorded compared to commonly accepted identification techniques. The method is suitable for pure substances, and significant dilution would result in the peaks of the analyte being sought being covered by the majority component of the mixture. However, with a suitable arrangement of the analytical system, a use in laboratory identification can be proposed. Raman spectroscopy can be used for the preliminary analysis of the crude sample, after which a sample preparation and chromatographic analysis will be performed. Raman spectroscopy can be used repeatedly on the same sample, for example after or during sample preparation, due to the low time requirement of non-contact analysis. In view of this fact and the results presented, it is possible to recommend the expansion of analytical identification methods within the framework of AEP-66 [2] by new spectroscopic techniques, or those which nowadays already fully meet the criteria for their inclusion.

5 Conclusions

A handheld Raman spectrometer is a relatively simple and fast tool that can be upgraded to an identification device with creation of user libraries. Having done this modification, we were able to identify all 29 investigated compounds from all groups of highly toxic chemical warfare agents and their precursors. It was found that, despite the general hypothesis of the necessity of highly transparent container, its high value is not completely necessary. It is thus possible to use the method in clandestine laboratories, where there are often a number of containers with unknown liquids and solids.

The non-contact method of analysis greatly facilitates the work of sampling teams and, in the case of identification, speeds up the entire process from entering the incident site to passing on information to the commander about the risk at the sampling site. It goes without saying that the subsequent physical collection of the sample and its transport to the laboratory will carry out the confirmatory identification, however, the initial provisional identification using Raman spectroscopy can already play a significant role in the creation of follow-up precautions.

References

[1] STANAG 4632: 2005, *Deployable NBC Analytical Laboratory*.

[2] AEP-66: 2015, *NATO Handbook for Sampling and Identification of Biological, Chemical and Radiological Agents*.

[3] ROZSYPAL, T. and R. ZAHRADNÍČEK. Comparative Analysis of NATO Requirements for Laboratory Identification of Chemical Agents and Military Technical Means for Its Implementation. *Advances in Military Technology*, 2021, **16**(1), pp. 5-17. DOI 10.3849/aimt.01455.

[4] LARKIN, P.J. *IR and Raman Spectroscopy: Principles and Spectral Interpretation*. New York: Elsevier, 2011. ISBN 0-12-386984-6.

[5] DE ARAUJO, W.R., T.M.G. CARDOSO, R.G. DA ROCHA, M.H.P. SANTANA, R.A.A. MUÑOZ, E.M. RICHTER, T.R.L.C. PAIXÃO and W.K.T. COLTRO. Portable Analytical Platforms for Forensic Chemistry: A review. *Analytica Chimica Acta*, 2018, **1034**, pp. 1-21. DOI 10.1016/j.aca.2018.06.014.

[6] HAKONEN, A., P.O. ANDERSSON, M.S. SCHMIDT, T. RINDZEVICIUS and M. KÄLL. Explosive and Chemical Threat Detection by Surface-Enhanced Raman Scattering: A Review. *Analytica Chimica Acta*, 2015, **893**, pp. 1-13. DOI 10.1016/j.aca.2015.04.010.

[7] PACSIAL-ONG, E.J. and Z.P. AGUILAR. Chemical Warfare Agent Detection: A Review of Current Trends and Future Perspective. *Frontiers in Bioscience*, 2013, **S5**(2), pp. 516-543. DOI 10.2741/S387.

[8] WATERBURY, R.D., T. CONGHUYENTONNU, H. HARDY, T. MOLNER, R. ROBINS, M. SCOTT, J.A. GUICHETEAU and C.R. HOWLE. Recent Development of a New Handheld UV Raman Sensor for Standoff Detection. In: *Chemical, Biological, Radiological, Nuclear, and Explosives (CBRNE) Sensing XXII*. Society of Photo-Optical Instrumentation Engineers, 2021, **117490I**. DOI 10.1117/12.2587656.

[9] KONDO, T., R. HASHIMOTO, Y. OHRUI, R. SEKIOKA, T. NAGOMI, F. MUTA and Y. SETO. Analysis of Chemical Warfare Agents by Portable Ra-

man Spectrometer with both 785 nm and 1064 nm Excitation. *Forensic Science International*, 2018, **291**, pp. 23-38. DOI 10.1016/j.forsciint.2018.07.032.

[10] SATOH, T., R. SEKIOKA, Y. SETO, H. MATSUMIYA and H. NAKAGAWA. Analysis of Chemical Warfare Agents by Portable Raman Spectrometer. *Analysis Chemistry*, 2016, **65**(9), pp. 539-545. DOI 10.2116/bunsekikagaku.65.539.

[11] WIKTELIUS, D., L. AHLINDER, A. LARSSON, K.H. HOLMGREN, R. NORLIN and P.O. ANDERSSON. On the Use of Spectra from Portable Raman and ATR-IR Instruments in Synthesis Route Attribution of a Chemical Warfare Agent by Multivariate Modeling. *Talanta*, 2018, **186**, pp. 622-627. DOI 10.1016/j.talanta.2018.02.108.

[12] ROY, E., P.G. WILCOX, S. HOFFLAND and I. PARDOE. Detection of Munitions Grade G-Series Nerve Agents Using Raman Excitation at 1064 nm. In: *Proceedings of the SPIE.* Toulouse: Society of Photo-Optical Instrumentation Engineers, 2015, **9455**. DOI 10.1117/12.2194027.

[13] BRADY, J.J. and P.M. PELLEGRINO. Next Generation Hazard Detection via Ultrafast Coherent Anti-Stokes Raman Spectroscopy. In: *Chemical, Biological, Radiological, Nuclear, and Explosives (CBRNE) Sensing XIV*. Baltimore: Society of Photo-Optical Instrumentation Engineers, 2013, **8710**. DOI 10.1117/12.2015838.

[14] NAN, D.-N., L.-Q. DONG, W.-X. FU, W.-W. LIU and J.-L. KONG. Fast Identification of Hazardous Liquids Based on Raman Spectroscopy. *Spectroscopy and Spectral Analysis*, 2021, **41**(6), pp. 1806-1810. DOI 10.3964/j.issn.1000-0593(2021)06-1806-05.

[15] WILCOX, P.G., J.A. GUICHETEAU, A.W. FOUNTAIN, J.A. GUICHETEAU and C.R. HOWLE. Comparison of Handheld Raman Sensors through Opaque Containers. In: *Chemical, Biological, Radiological, Nuclear, and Explosives (CBRNE) Sensing XIX*. Orlando: Society of Photo-Optical Instrumentation Engineers, 2018, **10629**. DOI 10.1117/12.2303968.

[16] LIN-VIEN, D., N.B. COLTHUP, W.G. FATELEY and J.G. GRASSELLI. *The Handbook of Infrared and Raman Characteristic Frequencies of Organic Molecules*. New York: Elsevier, 1991. ISBN 978-0-12-451160-6.

[17] CHRISTESEN, S., B.M ACIVER, L. PROCELL, D. SORRICK, M. CARRABBA and J. BELLO. Nonintrusive Analysis of Chemical Agent Identification Sets Using a Portable Fiber-Optic Raman Spectrometer. *Applied Spectroscopy*, 1999, **53**(7), pp. 850-855. DOI 10.1366/0003702991947432.