INTERNAL STANDARDS FOR QUANTITATIVE ANALYSIS OF CHEMICAL WARFARE AGENTS BY THE GC/MS METHOD: BLISTER AGENTS

Tomáš ČAPOUN, Jana KRYKORKOVÁ
tomas.capoun@ioolb.izscr.cz

Abstract

Analysis of hazardous chemical substances in the Czech Republic is the main task of special chemical laboratories of the Fire and Rescue Service (FRS). General conditions and requirements for an internal standard useful in the determination of chemical warfare agents (CWAs) by the method of gas chromatography coupled with mass detection (GC/MS) were defined. The determination is based on a GC/MS analysis of a mixture of a CWA with an internal standard, conversion of the total ion current (TIC) chromatogram to extracted-ion chromatogram (EIC) at a particular m/z ratio, and calculation of the CWA concentration from the internal standard concentration, response factor and chromatographic peak areas. Available internal standards were identified, and they were verified for four blister agents. Corresponding response factors were determined as a ratio of slopes of the linear functions of peak area and compound concentration. Linearity, repeatability, and accuracy of the measurements were evaluated. The determination can be performed on all GC/MS systems of the FRS, where no CWA standards are available.

Key words

Gas chromatograph with mass detector, chemical warfare agent, blister agent, internal standard, response factor.

1 INTRODUCTION

Blister agents (for example sulfur mustard, nitrogen mustard, lewisite) are chemicals that cause tissue blistering. Their toxic activity is, however, not limited to the skin and their mode of action is complex. These cytotoxic alkylating agents were initially developed as CWAs used to induce ocular, dermal, and respiratory damage resulting in immediate casualties, reduction in fighting efficiency, and demoralization [1]. Even today, great amounts of these CWAs are stored, making them a relevant issue.

According to the Czech Republic law, competences of the FRS include chemical countermeasures in case of CWAs spills or abuse. The countermeasures include chemical reconnaissance, detection, identification, and determination of CWAs. This activity is ensured by specialised FRS chemical laboratories. All laboratories are equipped by gas chromatography with mass detection (GC/MS) as most frequent analytical systems. Three types of systems are used in the FRS: GC/MS 7890A/5975 (Agilent), GC/MS Intuvo 9000/5977B of the same manufacturer and EM 640 (Bruker Daltonik). Usually, quantitative analysis is performed by the absolute calibration method on these instruments, but only for those analytes where a standard of corresponding purity is available.

The fundamental issue is that pure and certified CWA standards are not available in the Czech Republic. Therefore, we had to turn our attention to a procedure based on an internal standard, i.e. addition of a known amount of a substance different from the analyte into the sample, to determine these substances. This method is based on the fact that within a certain

concentration range, the ratio of chromatographic peak areas and concentrations is constant. This ratio is called the response or calibration factor [2]:

$$F_R = \frac{A_{CWA}:c_{CWA}}{A_{ISTD}:c_{ISTD}} = \frac{A_{CWA} \times c_{ISTD}}{A_{ISTD} \times c_{CWA}} \quad (1)$$

where F_R is the response factor,
A_{CWA} the CWA chromatographic peak area,
A_{ISTD} internal standard chromatographic peak area,
c_{CWA} the CWA concentration in the solution, and
c_{ISTD} internal standard concentration in the solution.

The internal standard method has several significant advantages. Unlike the absolute calibration and standard addition methods, pure analyte standard is not required. Moreover, the latter method includes analysis of two separate samples which often introduces significant errors into the results [2, 3]. The same applies to the external standard method.

When using the internal standard method, the response factor value needs to be known in order to arrive at reliable analytical results. Additionally, the concentration range where the analyte and internal standard chromatographic peak area is a linear function must be known, as only then constant value of the response factor is ensured [2, 4, 5]. In case the sample is modified before the analysis (extraction, distillation, solvent evaporation, etc.), maximum similarity of chemical and physical characteristics of the analyte and of the internal standard should be ensured [2, 4]. Close retention times of the analyte and the internal standard are also required in order to eliminate peak area discrimination at varying temperatures under temperature programmed conditions [2, 4]. On top of these requirements for the internal standard selection, one needs to consider the requirement that the internal standard must not react or interact with the analyte, nor constitute a decomposition product or other admixture of the analysed compounds [5].

Large number of compounds were tested and verified as internal standards for the GC/MS quantitative analysis, see publication [5] for an overview. However, studies of internal standards for the determination of CWAs have been scarce. The use of some non-toxic compounds for the determination of seven nerve-paralysing agents using three different GC/MS systems was described [5]. Dipinacolyl methyl phosphonate was described as an internal standard for the determination of tabun, cyclosarin, VX agent and nitrogen mustard [6]. The TNO laboratory in Rijswijk, the Netherlands, focusing especially on the CWA analysis, uses deuterated sulphur mustard, [2H8]-bis(2-chloroethyl)sulfide, as internal standard for the determination of sulfur mustard [7].

The aim of the work was to develop a procedure for the FRS laboratories which would not only allow for the determination of CWAs in solutions, but could also be used for a fast and simple determination of the active substance in the CWA samples themselves. These samples are further used for the calibration of existing internal procedures for the determination of CWA, mostly by photometric methods.

2 MATERIAL AND METHODS

2.1 Chemicals

The quantitative analyses procedures by the GC/MS method were developed for the following blister agents: bis(2-chloroethyl)sulfide (sulfur mustard, HD, 90%), tris(2-chloroethyl)amine (nitrogen mustard, HN3, 74%), 2-chlorovinyldichloroarsine (Lewisite 1,

70%), bis(2-chlorovinyl)chloroarsine (Lewisite 2, 14%). All compounds were prepared in VOZ Zemianske Kostolany, Slovakia. Assay of blister agents was determined by potentiometric argentometric titration of chlorides by silver nitrate indicated by a silver electrode.

1,2-dichlorobenzene (ex. pur., 98 %, Acros Organics), acenaphthene (p.a., Merck), and 2-naphthylacetate (BIOSYNTH, Riedel-de Haën) were the internal standards used. Solutions of CWAs and internal standards were prepared in *n*-hexane (SupraSolv, for GC, Merck).

2.2 Measurement conditions and parameters

The measurements were performed on the following systems: system A – GC/MS 7890A/5975C (Agilent Technologies, Inc., Wilmington, USA); system B – GC/MS Intuvo 9000/5977B same manufacturer; system C – mobile GC/MS EM 640 (Bruker Daltonik GmbH, Bremen, Germany).

The analysis was performed at identical or comparable analytical parameters: Column HP-5MS 30 m × 0.25 mm, Carrier gas helium (nitrogen was used for system C), Injection volume 1 μL, Inlet 290 °C, Splitless mode, Purge flow 100 mL/min at 2 min, Detector Quadrupole MS, Scan mode, Transfer line 290 °C, Scan range 35–600 amu (50–550 amu for System C), GC gradient: 40 °C – 2 min, from 40 °C to 280 °C at dT/dt 10 °C /min, 280 °C – 10 min. Resulting chromatograms were evaluated employing the Agilent ChemStation GC/MSD – Data Analysis software, version E.02.02. (Agilent Technologies, Inc., 2011) on system A, by the MassHunter Workstation Software, version B.07.00 (Agilent Technologies, Inc., 2014) on system B, and by the Bruker Data Analysis software, version 1.1. (Bruker Daltonik GmbH, 2003) on system C.

Solutions of CWAs and the internal standard were mixed in a 1:1 (v/v) ratio, and the mixture was introduced into the injection inlet of the GC/MS system. An autosampler was used on systems A and B while manual injection was used on system C. The linearity range of the chromatographic peak area as a function of the compound concentration was studied in parallel both for the CWA and the corresponding internal standard. Hence, mixtures of CWAs and standards of the same concentration were injected. Three parallel measurements were performed for each concentration of the mixture of the blister agent and the standard.

2.3 Chromatogram evaluation

Peaks corresponding to the CWA and the internal standard were identified in the TIC chromatogram recorded in scan mode. Peak area was obtained by integration using the current software. Mass spectra libraries NIST use the software to identify substances. Generally, automatic integration was used, only tailing peaks were integrated manually. For further study, EIC chromatograms at particular *m/z* ratio were extracted from the TIC chromatograms; peak areas corresponding to the CWAs and the internal standards were obtained by an identical procedure.

In order to assess the dependency of the chromatographic peak area on concentration of the given compound in the solution, calibration curves were constructed. The linearity range was determined using statistical software [8] based on the correlation coefficient R and quality coefficient QC values. Coefficient values of R_{CRIT} 0.99 and QC_{CRIT} 5.00 were considered as critical for the testing. The slope, and y-range were evaluated by software [8] in the identified linearity range.

In order to assess the accuracy of CWA determination, the results were compared to the known concentration. The t-test was used for statistical evaluation [8], comparing the value of t criteria to the critical value. Based on the results from parallel measurements, the precision of the determination was tested by calculation of relative standard deviation [8].

3 RESULTS AND DISCUSSION

3.1 Study of chromatographic peak area dependence on concentration

The primary aim of this work was to find a suitable internal standard, applicable on all GC/MS systems across the FRS chemical laboratories in a universal procedure. The procedure would be used especially for a quick and simple determination of the active ingredient of own CWA samples which are then used for the calibration of existing determination procedures. The mixture of the CWA and the internal standard is analysed without modification. Hence, similarities of chemical properties and physical characteristics of the analyte and the internal standard are not as important. On the other hand, this requires highly reliable determination which is closely related to the linearity of the chromatographic peak area as a function of the compound concentration.

Assuming a linear function of chromatographic peak area and concentration, the relation can be described by the following equation:

$$A = k \times c + q \tag{2}$$

where A is the chromatographic peak area,
k is the slope,
c is concentration, and
q is the intercept on the peak area axis.

This equation can be combined with the response factor equation (1):

$$F_R = \frac{(k_{\mathrm{CWA}} \times c_{\mathrm{CWA}} + q_{\mathrm{CWA}}) \times c_{ISTD}}{(k_{\mathrm{ISTD}} \times c_{\mathrm{ISTD}} + q_{\mathrm{ISTD}}) \times c_{\mathrm{CWA}}} \tag{3}$$

Assuming the intercept on the peak area axis is negligible compared to the product of slope and concentration, i.e., the linear function of CWA and internal standard peak area and concentration passes through the origin, the response factor equals the ratio of slopes of the two linear functions of CWA and internal standard peak area and concentration:

$$F_{\mathrm{R}} = \frac{k_{\mathrm{CWA}}}{k_{\mathrm{ISTD}}} \tag{4}$$

Use of equation (4) for the determination of the response factor has two fundamental prerequisites. First, the response factor can only be applied in the concentration range where the function of CWA and internal standard peak area is linear. We found that the linearity ranges significantly differ between the GC/MS systems tested. For example, the chromatographic peak area of sulfur mustard as a function of its concentration in the solution is shown in Figure 1.

In this particular sulfur mustard example, the EM 640 GC/MS system provides broadest linearity range, but lowest sensitivity due to lowest slope of the function. Agilent 7890A/5975C provides broad linear range; however, the function is significantly supralinear above 40 mg/L. The Intuvo 9000/5977 behaves in the opposite fashion: it provides a linear range from 2 to 15 mg/L, and the peak area increase with increasing concentration is negligible at higher concentrations.

Second, the intercept on the peak area axis must be negligible compared to the product of gradient and concentration both for the CWA and for the internal standard. We have set the

intercept must be lower than 10% of the product of the gradient and the lower concentration limit of the linearity range.

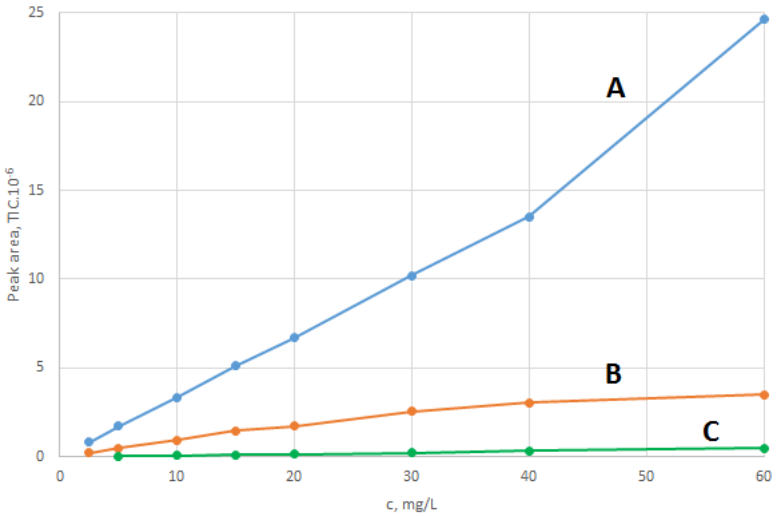


Figure 1
TIC chromatographic peak area of sulphur mustard as a function of its concentration in the solution, measured on the following GC/MS systems: 7890A/5975C (A), Intuvo 9000/5977B (B), EM 640 (C)

When applying the internal standard, it is always necessary to evaluate the intensity of the analyte and standard chromatographic peaks. In some cases the peak area of the particulate peak is used [3,9], in other cases the area of the TIC peak may be more useful [10]. This is discussed in publication [9].

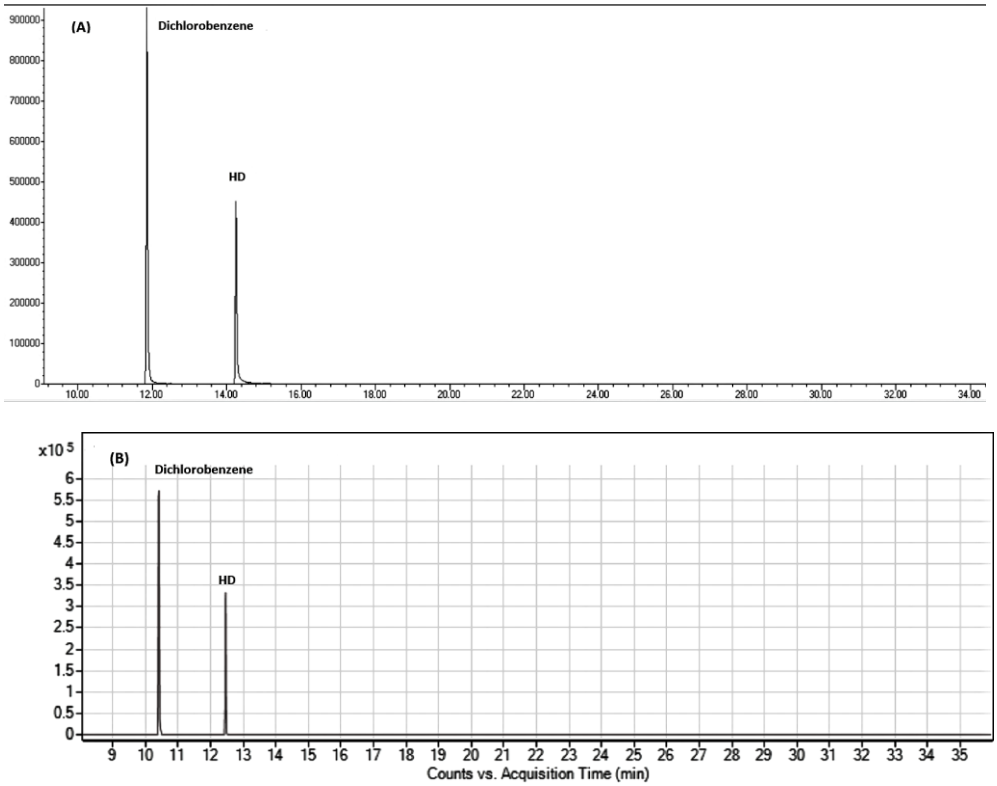
Previous research of internal standards for the determination of nerve-paralysing agents has clearly shown that the determination of response factor from TIC chromatograms recorded in the scan mode is not useful [5]. Use of EIC chromatograms of the extracted ion, evaluating the CWA and internal standard peak at the same *m/z* value, is the optimal procedure. Main reasons include significantly higher reproducibility, broader linearity ranges, and increased robustness and resistance against interference with compounds having similar retention times. [5] Hence, we have applied the procedure in this study, too.

3.2 Determination of sulfur mustard

The *m/z* values of 109, 111, 63, 158, and 45 (in the order of decreasing abundance) dominate the mass spectrum of sulfur mustard. Naphthalenediamine and some phenols were tested as the internal standards. Naphthalenediamine at *m/z* 158 exhibited significant differences in the retention times. Aminophenols do not yield sufficient response at *m/z* 109 on the GC/MS systems tested. Broad linearity ranges could be obtained with methoxyphenol (*m/z*); however, with major differences in the response factors across the systems tested (F_R value reached 1.50, 0.80, and 2.00 for systems A, B, C, respectively). Eventually, *o*-dichlorobenzene at *m/z* 111 was selected for its most reproducible results in satisfactory linearity range as summarised in Table 1. Examples of extracted chromatograms are shown in Figure 2.

Table 1
Evaluation of the chromatographic peak area of sulfur mustard (HD) and 1,2-dichlorobenzene at m/z 111 as a linear function of their concentration (critical values of the correlation coefficient R_{CRIT} 0.99 and QC coefficient QC_{CRIT} 5.00)

GC/MS	**7890A/5975C**		**Intuvo 9000/5977B**		**EM 640**	
Compound	**HD**	**dichloro-benzene**	**HD**	**dichloro-benzene**	**HD**	**dichloro-benzene**
Retention time [min]	14.3	11.9	12.5	10.4	14.4	11.7
Linearity range [mg/L]	2–40	2–40	2–15	2–15	5–60	5–60
R	0.9985	0.9993	0.9979	0.9990	0.9994	0.9978
QC	4.25	2.77	3.98	4.12	3.25	3.45
Slope [$A\times10^{-6}\times$L/mg]	0.340	0.808	0.0961	0.229	0.00807	0.0188
Intercept [$A\times10^{-6}$]	–0.064	–0.14	0.0022	0.021	–0.0032	–0.0083
Response factor F_R	0.42		0.42		0.43	
Average value of F_R			**0.42**			



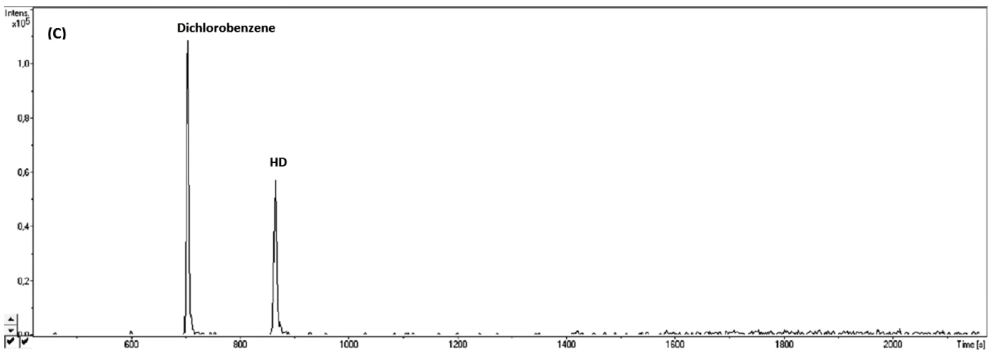


Figure 2
Examples of sulfur mustard and 1,2-dichlorobenzene chromatograms extracted at m/z 111 measured on the following GC/MS systems: (A) 7890A/5975C, c_{CWA} = 12.6 mg/L, c_{ISTD} = 13.4 mg/L, (B) Intuvo 9000/5977B, c_{CWA} = 5.9 mg/L, c_{ISTD} = 4.7 mg/L, (C) EM 640, c_{CWA} = 35.0 mg/L, c_{ISTD} = 37.0 mg/L

3.3 Determination of nitrogen mustard

The *m/z* values of 154, 156, 63, 56, and 42 dominate the mass spectrum of nitrogen mustard HN3. From the compounds available, we have tested bipyridine and trioctylamine at *m/z* 156. Bipyridine has retention time very close to the analyte; however, significant differences in the determined response factors were observed (F_R value reached 0.90, 0.40, and 1.05 for systems A, B, C, respectively). The same was observed for trioctylamine (F_R value reached 9.07, 2.43, and 3.37 for systems A, B, C, respectively). Moreover, the retention time of trioctylamine is 9 minutes longer than that of nitrogen mustard at the separation conditions used, and its linearity range is very narrow. Best results could be obtained with acenaphthene at *m/z* 154. Results obtained across the GC/MS systems are summarised in Table 2.

Table 2
Evaluation of the chromatographic peak area of nitrogen mustard (HN3) and acenaphthene at m/z 154 as a linear function of their concentration (critical values of the correlation coefficient R_{CRIT} 0.99 and QC coefficient QC_{CRIT} 5.00)

GC/MS	**7890A/5975C**		**Intuvo 9000/5977B**		**EM 640**	
Compound	**HN3**	**acenaph-thene**	**HN3**	**acenaph-thene**	**HN3**	**acenaph-thene**
Retention time [min]	18.0	19.4	15.6	16.8	18.8	20.5
Linearity range [mg/L]	5–40	1–40	2–15	1–15	5–40	5–40
R	0.9972	0.9975	0.9979	0.9978	0.9960	0.9960
QC	4.00	4.54	4.06	3.16	4.01	4.07
Slope [$A\times10^{-6}\times$L/mg]	1.33	2.48	0.343	0.591	0.00182	0.00331
Intercept [$A\times10^{-6}$]	–0.55	–0.23	0.027	0.036	0.00090	–0.0015
Response factor F_R	0.54		0.58		0.55	
Average value of F_R	**0.56**					

3.4 Determination of lewisites

As Lewisite 1 and Lewisite 2 occur together in technical samples [1], the determination procedure was developed for both lewisites in one injection. Both Lewisite 1 and Lewisite 2 exhibit intense peaks at *m/z* 145, 110, and 147 in the mass spectrum. Much effort was dedicated to the testing of phenols which are easily available as internal standards. They exhibit an intense peak at *m/z* 110 in the mass spectrum. In particular, we have tested resorcin, pyrocatechol, 1-naphthol, 2-naphthol, and ethoxyphenoles. Unfortunately, they were not suitable as the response of the mass spectrometers used was much lower than for the organoarsenic compounds studied. Value of the response factor exceeded 10, failing to guarantee sufficient precision of the determination method. Reproducible and acceptable results could be obtained with 2-naphthyl acetate at *m/z* 145 as the internal standard. The linearity ranges and resulting response factors across the systems testems are shown for Lewisite 1 and Lewisite 2 in Tables 3 and 4, respectively.

Table 3
Evaluation of the chromatographic peak area of 2-chlorovinyldichloroarsine (Lewisite 1) and 2-naphthylacetate at m/z 145 as a linear function of their concentration (critical values of the correlation coefficient R_{CRIT} 0.99 and QC coefficient QC_{CRIT} 5.00)

GC/MS	**7890A/5975C**		**Intuvo 9000/5977B**		**EM 640**	
Compound	**Lewisite 1**	**naphthyl-acetate**	**Lewisite 1**	**naphthyl-acetate**	**Lewisite 1**	**naphthyl-acetate**
Retention time [min]	11.6	19.9	9.8	16.8	10.9	20.7
Linearity range [mg/L]	20–50	5–50	5 –20	2–15	25–60	10–60
R	0.9994	0.9978	0.9999	0.9995	0.9989	0.9994
QC	2.61	4.77	0.30	2.30	3.92	2.75
Slope [$A\times10^{-6}\times$L/mg]	0,287	0.137	0.0290	0.0134	0.00267	0.00121
Intercept [$A\times10^{-6}$]	–0.22	–0.042	0.00066	0.00018	–0.0014	0.00097
Response factor F_R	2.09		2.16		2.21	
Average value of F_R			**2.15**			

Table 4
Evaluation of the chromatographic peak area of bis(2-chlorovinyl)chloroarsine (Lewisite 2) and 2-naphthylacetate at m/z 145 as a linear function of their concentration (critical values of the correlation coefficient R_{CRIT} 0.99 and QC coefficient QC_{CRIT} 5.00)

GC/MS	**7890A/5975C**		**Intuvo 9000/5977B**		**EM 640**	
Compound	**Lewisite 2**	**naphthyl-acetate**	**Lewisite 2**	**naphthyl-acetate**	**Lewisite 2**	**naphthyl-acetate**
Retention time [min]	15.2	19.9	12.7	16.8	15.4	20.7
Linearity range [mg/L]	20–50	5–50	5–20	2–15	25–60	10–60
R	0.9993	0.9978	0.9998	0.9995	0.9991	0.9994
QC	2.50	4.77	0.92	2.30	3.24	2.75
Slope [$A\times10^{-6}\times$L/mg]	0.355	0.137	0.0329	0.0134	0.00301	0.00121
Intercept [$A\times10^{-6}$]	–0.092	–0.042	–0.0015	0.00018	–0.0051	0.00097
Response factor F_R	2.59		2.46		2.49	
Average value of F_R			**2.51**			

3.5 Validation of the determination

Accuracy and precision of the determination were assessed by method validation. Accuracy was assessed by the statistical *t*-test. Analysed solutions were prepared from different CWA batches than those used for the determination of the response factors. Five parallel determinations were performed for each solution. The obtained set of concentration determinations was then evaluated by the statistical software [8] to assess the *t* criterion which was compared to the critical value. The procedure gives accurate results for $t < t_{CRIT}$.

Precision was statistically assessed by the method of concentration levels from parallel measurements and calculation of relative standard deviations [8]. Precision was evaluated for the abovementioned sets of five results.

The accuracy and precision assessment is summarised in Table 5, indicating that the method yields accurate results, and that relative standard deviation does not exceed 13% which generally corresponds to the precision of GC/MS-based determination procedures. The highest values of relative repeatability were achieved on the EM 640 system. On the 7890A/5975C GC/MS system, the maximum relative precision of CWAs determination reached 7%, on the Intuvo 9000/5977B 5%. Relative standard deviation values were determined for 95% confidence level.

Table 5

Assessment of blister agents determination accuracy and precision testing on the following GC/MS systems: (A) 7890A/5975C, (B) Intuvo 9000/5977B, (C) EM 640 (c_{ISTD} – internal standard concentration, t – t criterion value, S_R – relative standard deviation, number of measurements n = 5, t_{CRIT} = 2.776)

CWA / Internal standard	c_{ISTD}, mg/L	Known CWA concentration, mg/L	GC/MS system	Determined concentration, mg/L	*t*	S_R, %
Sulfur mustard / 1,2-dichlorobenzene	15.0	15.0	A	14.7	1.218	4.7
			B	14.3	0.815	2.2
			C	15.1	0.029	6.2
	37.0	35.0	A	33.7	1.616	4.7
			C	35.3	0.022	6.6
	4.7	5.9	B	6.1	1.325	3.8
Nitrogen mustard / acenaphthene	15.0	12.3	A	12.0	1.015	4.9
			B	11.5	2.354	5.0
			C	13.1	1.716	12.8
	25.0	31.5	A	32.5	0.556	6.4
			C	31.0	0.730	10.5
	7.0	11.4	B	10.8	1.224	4.9
Lewisite 1 / 2-naphthylacetate	45.0	39.7	A	41.2	1.542	6.8
			C	38.4	1.941	9.6
	10.0	15.0	B	15.0	0.021	4.2
Lewisite 2 / 2-naphthylacetate	45.0	27.8	A	28.5	0.994	6.0
			C	28.6	1.805	10.8
	10.0	9.3	B	9.5	0.923	3.6

The sulfur and nitrogen mustard determination methods could also be verified in a multilaboratory comparison performed as part of the FRS chemical laboratories proficiency

testing. The investigation was performed in 2017–2019 with five chemical laboratories equipped with three 7890A/5975C GC/MS systems, five Intuvo 9000/5977B systems, and two mobile EM 640 systems. Relative standard deviation of 12.5% was chosen for the accuracy evaluation.

In total, 45 determination results were evaluated. Out of those, two determinations of sulfur mustard and one determination of nitrogen mustard were inaccurate. Two inaccurate results were obtained on the 7890A/5975C GC/MS system, one on the Intuvo 9000/5977B system. Hence, the ratio of inaccurate results reached 7%. Relative difference of known and determined concentrations between the laboratories did not exceed 8%. The maximum relative standard deviation between laboratories reached 14%. Given that the participants in the interlaboratory comparison did not have the standards of all of the above-mentioned blister agents, these results can be considered very good.

4 CONCLUSIONS

A simple procedure for the determination of CWAs, comprising the preparation of CWA and internal standard solutions, their mixing and injection into the GC/MS system, was developed. The analysis is followed by identification of the components as usual, extraction of the chromatogram at particular *m/z* value, integration and reading of the chromatographic peak area of the analyte and the internal standard. The CWA concentration is then calculated based on the internal standard concentration, peak areas and response factor.

We were able to identify and verify standards which exhibit a pronounced peak corresponding to the same ion present in the CWA mass spectrum, and verified the useful concentration range for all blister agents in scope of this work. The response factors for individual CWAs and internal standards were calculated as ratios of the slope of linear functions of peak area and concentration. The response factors are valid across all GC/MS systems within the FRS laboratories. The described procedure is fully usable in other laboratories dealing with the analysis of CWAs.

The most important and decisive asset of this work is that the developed procedure enables the chemical laboratories to determine the concentration of CWAs in solution in the absence of standards. This is especially important for the determination of purity of own CWA samples which are then in turn used for the preparation of calibration solutions for other methods employed.

ACKNOWLEDGMENT
This study was supported by the Ministry of Interior of the Czech Republic (Security Research and Development Project No. VI20152020009).

References

[1] YOUNG, R. A., C. BAST. Mustards and vesicants. In: R. C. GUPTA, ed. *Handbook of Toxicology Chemical Warfare Agents*. 1st ed. London, UK: Academic Press Elsevier, 2009, pp. 93–108. ISBN 978-0-12-374484-5.

[2] ZENKEVICH, I. G., E. D. Makarov, I. Yu. Makarova. Quantitative Chromatographic Analysis under Changes in the Composition of Samples in the Course of Sample Preparation: A Modification of the Double Internal Standard Method. *J. Anal. Chem.* 2007, **62**(8), 748–755. doi: 10.1134/S1061934807080102

[3] SIEBERT, T. E., C. Wood, G. M. Elsey, A. P. Pollnitz. Determination of Rotundone. the Pepper Aroma Impact Compound. in Grapes and Wine. *J. Agric. Food Chem.* 2008, **56**(10), 3745–3748. doi: 10.1021/jf800184t

[4] ZENKEVICH, I. G., A. Yu. Eshchenko, I. O. Klimova. Characterization of the interlaboratory reproducibility of results in quantitative gas-chromatographic analysis using the internal normalization method. *J. Anal. Chem.* 2005, **60**(2), 119–124. doi: 10.1007/s10809-005-0034-9

[5] CAPOUN, Tomas, Jana KRYKORKOVA. Internal Standards for Quantitative Analysis of Chemical Warfare Agents by the GC/MS Method: Nerve Agents. *Journal of Analytical Methods in Chemistry*. 2020. Article ID 8857210. 11 p. doi: 10.1155/2020/8857210

[6] IWAI, T., K. KAKEGAWA, M. AIDA, H. NAGASHIMA, T. NAGOYA, M. KANAMORI-KATAOKA, H. MIYAHARA, Y. SETO, A. OKINO. Development of a Gas-Cylinder-Free Plasma Desorption/Ionization System for On-Site Detection of Chemical Warfare Agents. *Anal. Chem.* 2015, **87**(11), 5707–5715. doi: 10.1021/acs.analchem.5b00874

[7] KONING, M. C. Decontamination of CWAs by Hvezda – as summary of results. *Earth, Environmental and Life Sciences*. Rijswijk, Netherlands, 2013.

[8] *Statistical software EffiValidation.* Version 3.0. EffiChem: Oulehla. Czech Republic, 2002.

[9] CHEN, B. G., C. D. CHANG, C. T. WANG, Y. J. CHEN, W. T. CHANG, S. M. WANG, H. R. LIU. A Novel Approach to Evaluate the Extent and the Effect of Cross-Contribution to the Intensity of Ions Designating the Analyte and the Internal Standard in Quantitative GC-MS Analysis. *Soc. Mass Spectr.* 2008, **19**(4), 598–608. doi: 10.1016/j.jasms.2008.01.004

[10] SORIA, A. C., I. Martinez-Castro, J. Sanz. Study of the precision in the purge-and-trap–gas chromatography–mass spectrometry analysis of volatile compounds in honey. *J. Chrom. A.* 2009, **1216**(15), 3300–3304. doi: 10.1016/j.chroma.2009.01.065