

Conference paper

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Novel analytical approaches to determination of chemical warfare agents and related compounds for verification of nonproliferation of chemical weapons

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Abstract: In this paper a summary of the author's approaches for investigation of the mass spectral behavior of some chemical warfare agents (CWAs), their degradation products and metabolites, as well as the results of development of analytical methods for confirmation of nerve and blister agents application are presented. Hydrolysis and oxidation metabolites of nerve agents, sulfur mustard and lewisite were used as biomarkers of the exposure. Sensitive analytical methods have been developed for their detection, based mainly on tandem mass spectrometry coupled with liquid chromatography. Several techniques for fast screening of CWAs degradation products based on capillary electrophoresis were also proposed. Some of developed approaches were successfully applied in the frame of the proficiency testing system of the Organization for the Prohibition of Chemical Weapons.

Keywords: biomonitoring; chemical warfare agents; lewisite; mass spectrometry; Mendeleev XX; nerve agent; sulfur mustard.

Introduction

Chemical warfare agents (CWAs) were employed several times in conflicts around the world. Commonly CWAs are divided into several categories according to their physiological effect. The first group, the vesicants or blister agents that affect the eyes, lungs and skin. Sulfur mustard (bis(2-chloroethyl)sulfide) was used in World War I and in the Iran–Iraq war. The second group, nerve agents, which cause the continuous cholinergic stimulation of the nervous system by inhibition of acetylcholinesterase. The first organophosphorus nerve agents, tabun (GA) and sarin (GB), were developed in the 1930s. Together with soman (GD) and VX these compounds are recognized as the major produced and weaponized nerve agents. Also several analogues of VX, such as VR (Russian VX), VE, VG, VM and VP are described by military science. The third group, blood agents, interfere with blood oxygen transport and may cause death by suffocation. The fourth group,

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incapacitating agents which have non-lethal physiological effects. Apart from this categorization there is a large group of possible chemical or biological warfare agents (BWAs), including bioregulators and toxins such as botulinium toxin, saxitoxin, abrin and ricin.

When the Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on their Destruction (the Chemical Weapons Convention; CWC) entered into force in 1997 the toxic chemicals, their precursors and degradation products essential for verification measures were listed in Schedules 1–3 of the Annex on Chemicals of the CWC [1]. These compounds have different chemical and physical properties: polar and less polar, neutral chemicals, acids, bases, volatiles and non-volatiles, with phosphorus, sulfur and arsenic heteroatoms as a part of the molecule.

For all above mentioned CWAs except of BWAs which are beyond the scope of this report the target analysis approach is commonly used for the unambiguous identification and confirmation of identity of the CWA. The major degradation products formed in case of hydrolysis and oxidation for sulfur mustard are thiodiglycol and thiodiglycol sulfoxide [2]. For nerve agents *N,N*-dialkylaminoethanesulfonic acids, methylphosphonic acid and its alkyl esters are produced. Also *S*-2-diethylaminoethylmethylphosphonothioate (DEMP, degradation product of VR-gas), *S*-2-diisopropylaminoethylmethylphosphonothioate (DIMP, degradation product of VX-gas), bis-2-*N,N*-diethylaminoethyldisulfide (DEAS, degradation product of VR-gas), and bis-2-*N,N*-diisopropylaminoethyldisulfide (DIAS, degradation product of VX-gas) are generated from V-gases in an aqueous medium [3]. For lewisite 2-chlorovinylarsonous acid (CVAA) and 2-chlorovinylarsonic (CVAOA) are monitored in water and biological fluids in order to confirm the fact of its release into environment. In living organisms β -lyase metabolites such as 1,1'-sulphonylbis[2-*S*(*N*-acetylcysteinyl)ethane] (SBSNAE) and 1,1'-sulphonylbis[2-methylsulphinyl]ethane] (SBMSE) or 1-methylsulphinyl-2-[2-(methylthio ethylsulphonyl)] ethane (MSMTESE) are determined in case of sulfur mustard exposure. While for nerve agents significant amount of the *N,N*-dialkylaminoethylthio leaving group appears to be bound to tyrosine in proteins such as albumin. Therefore, tyrosine adducts with alkyl methylphosphonic acids are important markers of this type of CWAs. A vast variety of analytical tools is developed for sample preparation and chromatographic separation of these target organic compounds. Screening methods include GC, HPLC-UV and capillary electrophoresis, while GC-MS and HPLC-MS techniques are utilized for determination of non-polar and less polar small organic biomarkers of CWAs with good retrospectivity and sensitivity. For polar compounds mainly GC-MS in combination with derivatization procedures is used. The prevalent use of MS detectors allowed minimization of the number of false positive and false negative results in the proficiency testing system held by the Organization for the Prohibition of Chemical Weapons (OPCW). A clear trend towards the increase of the performance and simplification of the analytical procedures employed for trace-level analysis of CWAs emerges from the reported studies. This paper summarizes our recent developments in the application of chromatographic, capillary electrophoretic and mass spectrometric techniques for the determination of degradation products of CWAs in environmental and biological samples.

Results

Study of mass spectral behavior of CWAs

For the series of nerve agents negative ion electron ionization (EI) mass spectra have been obtained in the authors laboratory and theoretical treatment of negative ion fragmentation processes has been carried out [4]. Using *O*-isopropyl- and *O*-pinacolylmethylphosphonofluoridates as examples, the values of electron affinity of molecules and their fragments have been estimated with the aid of quantum chemical approach (UB3LYP/6-311+G(d,p)), and energetically most expedient and characteristic dissociative electron attachment routes have been revealed including the processes of simple bond cleavage and rearrangements. Basing on the obtained experimental and theoretical data the hypothetical fragmentation schemes (Fig. 1) have been drawn up and special algorithm has been compiled aimed at prediction of negative

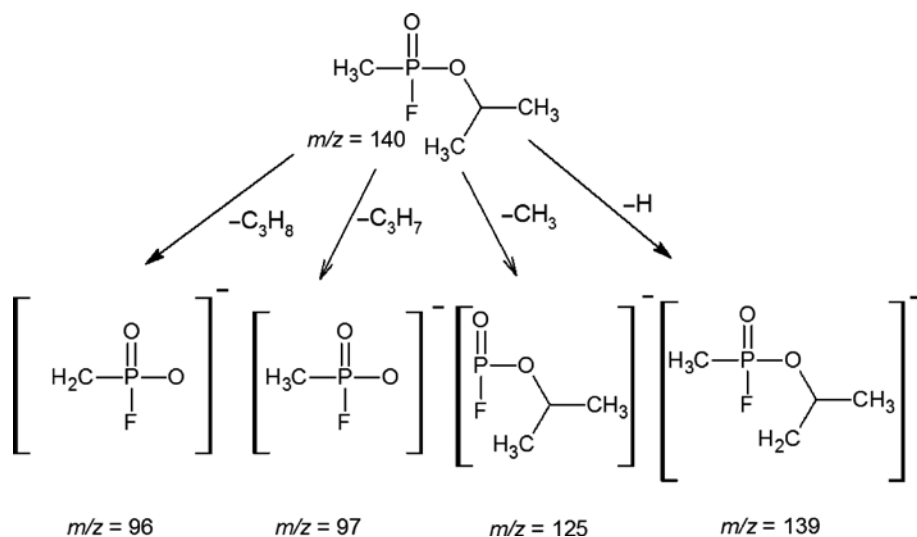


Fig. 1: Proposed scheme of *O*-isopropylmethylphosphonofluoridate negative ion fragmentation.

ion mass spectra of some groups of phosphorous organic compounds such as *O*-alkylmethylphosphonofluoridates, *O,O*-alkylphosphonodichloridates, and *O,O'*-dialkylphosphonochloridates. Simulated mass spectra revealed a good agreement with experimental ones, testifying the reasonable reliability of suggested algorithm.

It is believed that information about the molecular structure of highly toxic *O*-alkyl-*S*-2-*N,N*-dialkylaminoethylalkylthiophosphonates (V-gases), obtained from their EI mass spectra is insufficient. In particular, determination of molecular weight and structure of radicals at phosphorus and oxygen atoms causes great difficulties. In our paper [5], solutions of these problems are proposed. Fragmentation characteristics (Fig. 2) of V gases with branching at α -carbon atom of the alkoxy radical were studied. It was shown that using the information of EI mass spectra of *N,N*-dimethyl derivatives of such compounds in positive ion mode the number of carbon atoms in the radical at the phosphorus and ether oxygen atoms may be set.

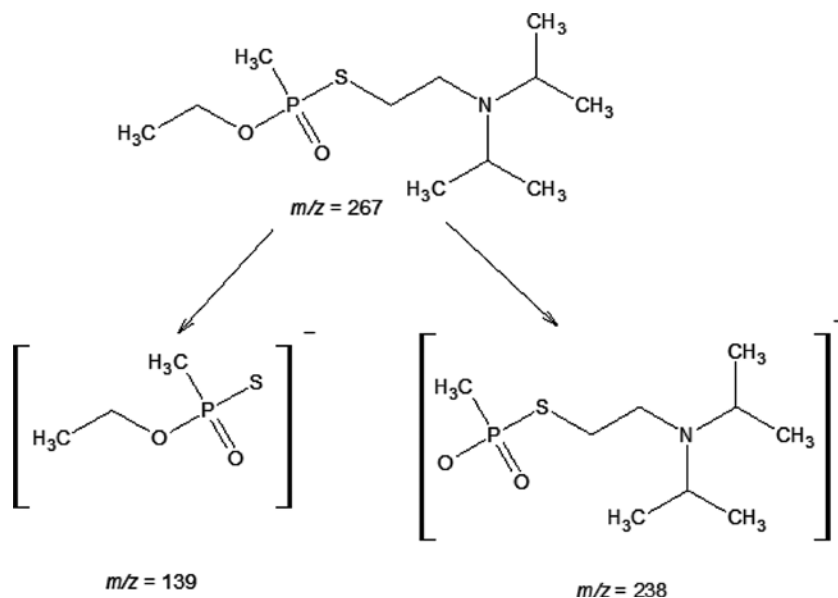


Fig. 2: Proposed scheme of *O*-ethyl-*S*-2-(*N,N*-diisopropylamino)ethylmethylthiophosphonate negative ion fragmentation.

The combination of chromatography and mass spectrometry has been applied to investigate technical lewisite containing α - and β -isomers, each containing the respective stereoisomers [6]. The mass spectra of positive ions of the stereoisomers have been practically identical, whereas substantial differences have been observed in the negative ion mass spectra. Using quantum-chemical simulation at the B3LYP/6-311+G(d,p) level, the relative stability of the isomers, bonds dissociation energy, electron affinity, and ionization energy have been estimated. The formation of characteristic negative ions (Fig. 3) from the *cis*-isomer of α -lewisite and their absence for the *trans*-isomer is shown to be driven by the steric factors affecting the rearrangement processes.

Determination of nerve agent degradation products

Nerve agents are one of the most dangerous type of chemical weapons. They irreversibly bind acetylcholinesterase, which is responsible for the metabolism of acetylcholine. It is mediator that regulates nervous system. The most well-known nerve agents are sarin, soman, VX and VR. Sarin was used during combat operations against the Kurdish population of Iraq in 1988, terrorist attacks in Japan in 1994, in the Tokyo subway in 1995, as well as in Guta, Syria, in 2013. Nerve agents are sufficiently volatile, so GC-MS and GC-MS-MS are most commonly used methods of its determination. However, in aqueous environment objects and living organisms organophosphorus nerve agents undergo rapid hydrolysis to the characteristic non-toxic compounds containing C-P bonds that are rarely found in nature. One of the most important nerve agent hydrolysis products are alkyl methylphosphonic acids (AMPAs), which are unique and correspond to the appropriate nerve agent. Further AMPAs slowly hydrolyze to the methylphosphonic acid (MPA), which is the most stable hydrolysis product (Fig. 4). AMPAs correspond to the compounds of medium polarity and MPA to the compound of

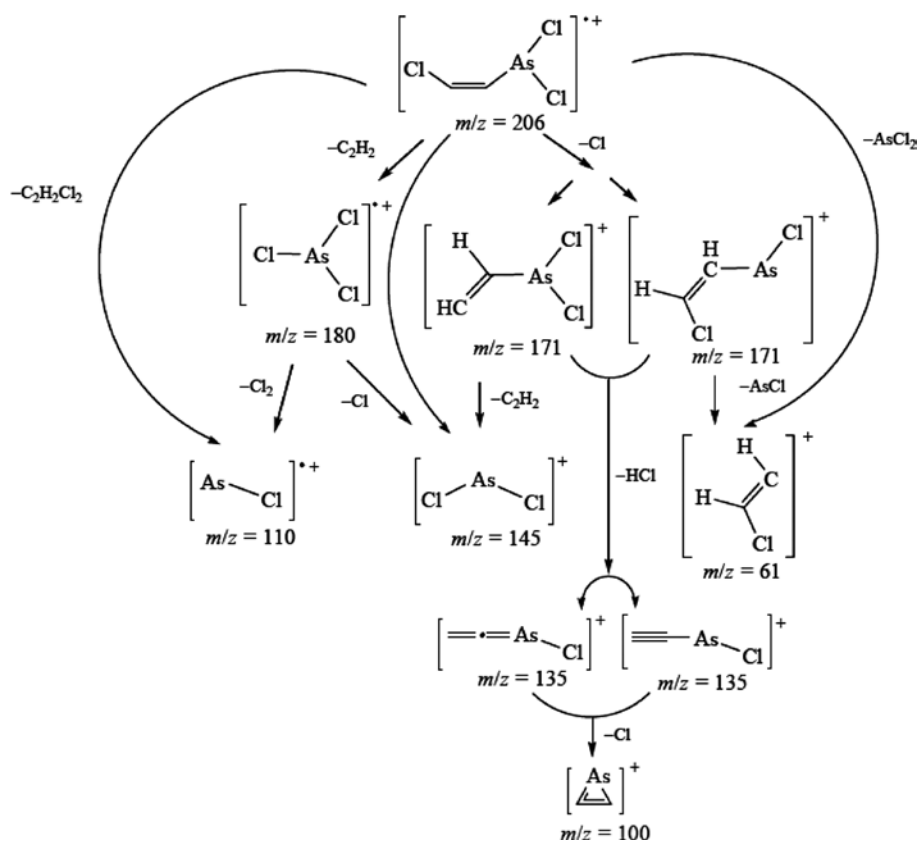


Fig. 3: Fragmentation scheme of α -lewisite.

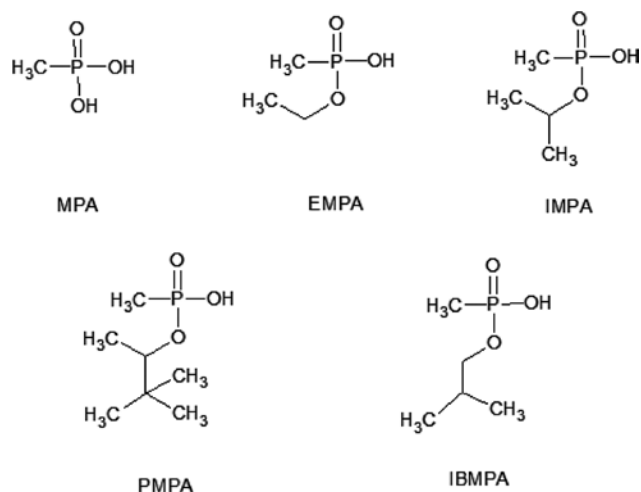


Fig. 4: Chemical structure of MPA and AMPAs. MPA, Methylphosphonic acid; EMPA, ethyl methylphosphonic acid; IMPA, isopropyl methylphosphonic acid; PMPA, pinacolyl methylphosphonic acid; IBMPA, isobutyl methylphosphonic acid.

high polarity. Detection of AMPAs may be the evidence of production, storage or application of the exact type of nerve agent. At the same time determination of MPA may not give information about the type of applied nerve agent, but may indicate that organophosphorus nerve agents were applied after long period of time.

Firstly, an approach to the detection of organophosphorus nerve agents, such as IMPA, PMPA and IBMPA in plasma samples using liquid chromatography and mass spectrometry was applied in our laboratory [7]. Mass spectrometric detection was performed with electrospray ionization (ESI) in the negative ion mode, using deprotonated molecules of AMPAs. Chromatographic separation was conducted by reversed phase chromatography using hydrophilic endcapped adsorbents. Solid phase extraction (SPE) on reversed phase adsorption cartridges containing a copolymer of styrene and divinylbenzene was proposed for sample preparation. Sufficiently low LODs (4, 0.6 and 1 ng/mL) were achieved for IMPA, PMPA and IBMPA, respectively. The curves of nerve agent metabolites excretion were obtained for plasma samples of rats exposed by toxic substances. Application of tandem mass spectrometry and ultra-performance liquid chromatography allowed to lower LODs for the respective metabolites in human urine samples analyzed [8]. For EMPA, IMPA, PMPA and IBMPA LODs of 0.8, 0.5, 0.1 and 0.4 ng/mL, respectively were achieved. Compounds were determined using ESI in negative ion mode and detected as deprotonated molecules. Analysis of urine samples was carried out using reversed-phase chromatography with C18 sorbent. SPE based on reversed-phase adsorption cartridges containing copolymer of sterol and divinylbenzene was employed in sample preparation procedure. The developed approach was applied to the analysis of human urine spiked with 5–50 ng/mL of the AMPAs during the world test of the Organization for the Prohibition of Chemical Weapons (OPCW).

MPA unlike AMPAs is a very polar substance. It does not retain on reversed phase C18 columns and sensitivity of its mass spectrometric detection is low due to the small molecular weight of MPA. To increase sensitivity of MPA detection derivatization with p-bromophenacyl bromide and hydrophilic interaction liquid chromatography (HILIC), as it is known that high concentration of acetonitrile in mobile phase positively effects on MS response, were used. In our study the fact that AMPAs unlike MPA did not react with p-bromophenacyl bromide under chosen conditions was discovered. This allowed simultaneous chromatographic separation and mass spectrometric detection of derivatized MPA and underivatized AMPAs using HILIC-MS/MS method. As a result, highly sensitive method has been developed for MPA and AMPAs determination in environmental waters as well as dust and ground mixed samples from the territory of the former chemical weapons plant [9, 10]. The approach successfully combined relatively fast derivatization and sample preparation with HILIC and tandem mass spectrometry which allowed to achieve the lowest limit of detection (0.1 ng/mL) for MPA in spiked environmental waters in comparison with other HPLC techniques described.

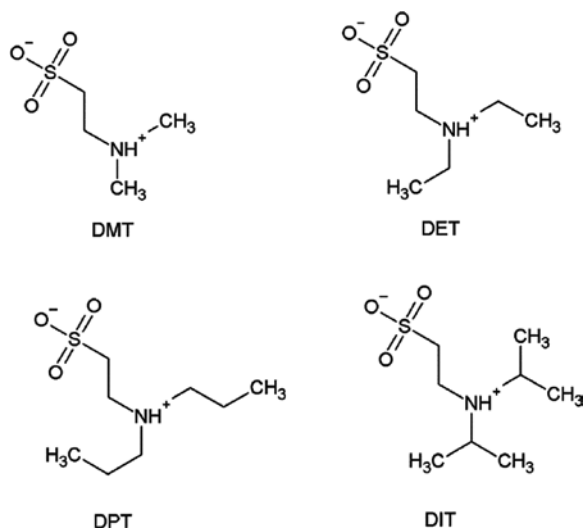


Fig. 5: Chemical structure of substituted derivatives of taurine – oxidative products of V-gases transformation. DMT, Dimethyl taurine; DET, diethyl taurine; DPT, dipropyl taurine; DIT, diisopropyl taurine.

The achieved limits of detection for AMPAs in the extracts of dust and ground mixed samples were ultra-low: 200 pg/mL for MPA, 70 pg/mL for EMPA, 8 pg/mL for IMPA, 8 pg/mL for IBMPA, 5 pg/mL for PMPA.

One of the poorly studied class of compounds which indirectly indicates the presence, application, storage and production of highly toxic organophosphorus nerve agents are 2-N,N-dialkylaminoethanesulfonic acids (dialkyl taurines) – substituted derivatives of taurine (Fig. 5). In contrast to taurine, 2-N,N-dialkylaminoethanesulfonic acids are not found in nature, and are products of oxidative transformation of V-gases.

Using capillary zone electrophoresis (CE) a rapid screening approach for determination number of 2-dialkylaminoethanesulfonic acids in water samples was developed [11]. During our study optimization of determination conditions, that include the choice of wavelength and detection mode, composition and pH of the working buffer, sample injection volume, diameter and the working program of capillary conditioning were made. The proposed approach has been tested in the analysis of various real water samples by the “added-found” method and showed good accuracy, reproducibility and selectivity of the determination. No matrix influence was observed. In the context of the proposed approach with direct spectrophotometric detection at 200 nm, detection limits for DMT, DET, DPT, DIT in water samples were 5, 3, 3 and 3 µg/mL, respectively. This approach is rather simple and rapid which is allowing to make a conclusion about the fact of application and type of nerve agents after the short period of time.

Later two more approaches for the sensitive determination of DMT, DET, DPT and DIT in seven different natural water samples using high performance liquid chromatography-mass spectrometry (HPLC-MS) and CE with direct ultraviolet detection (CE-UV) were developed in the authors laboratory [12]. Linear response ranges were approximately two orders of magnitude for these four compounds. LODs for these substances were between 0.015 and 0.025 mg/L for HPLC-MS and close to 1.5–2 mg/L for CE-UV determination. Successful development of the technique led to the opportunity of simple screening for V-gases destruction products. Analysis of DMT, DET, DPT, and DIT as specific markers of different types of V-gases performed by these two approaches is also allowing to track the source of the nerve agents released into environment.

In certain conditions, when the pH is in the range from 7 to 10, VR and VX may hydrolyze (Fig. 6) in the environment to the DEMP and DIMP. When pH > 10 VX and VR may hydrolyze to the corresponding 2-N,N-diisopropylaminoethanethiol and 2-N,N-diethylaminoethanethiol, which further undergo dimerization forming DEAS and DIAS. DEMP, DIMP, DEAS and DIAS also may be used as markers of nerve agents application.

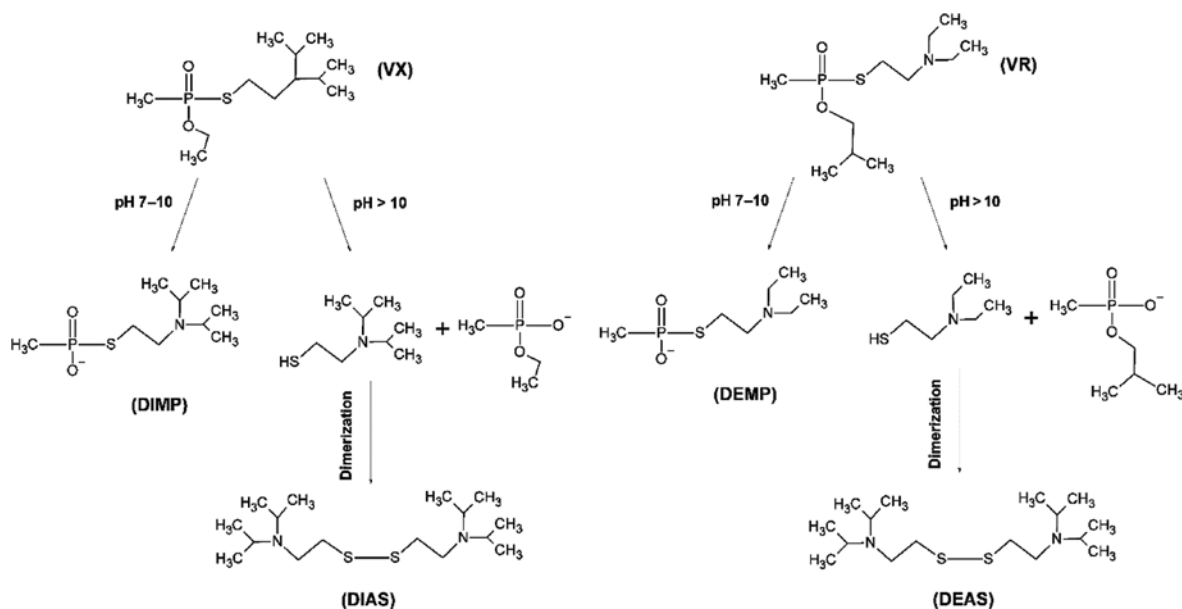


Fig. 6: Hydrolysis scheme of VX and VR with forming DEMP, DIMP, DEAS and DIAS.

An approach for the determination of four nerve agent markers DEMP, DIMP, DEAS and DIAS in aqueous environmental samples with use of HPLC with tandem mass spectrometric detection was proposed by the authors [13]. Optimal mass-spectrometric detection and LC separation conditions were chosen during the work. The proposed approach was applied to the analysis of real water samples and showed absence of matrix effect, good accuracy, reproducibility and selectivity. Detection limits achieved for DEMP, DIMP, DEAS and DIAS in water samples were 0.0003, 0.003, 0.3 and 0.05 mg/mL, respectively.

Determination of sulfur mustard degradation products

Sulfur mustard is one of the oldest and most well-known chemical warfare agents. In human or warm-blooded animals mustard undergo rapid biochemical degradation. As a result, shortly after intoxication there is no free mustard in the body, but there are large quantities of various metabolites, including reaction products with proteins. To detect the fact of mustard exposure it is necessary to perform determination of such transformation products as SBSNAE, SBMSE and MSMTESE. These compounds are formed during interaction of proteins with mustard in the presence of β -lyase (Fig. 7) and are contained in significant amounts in the

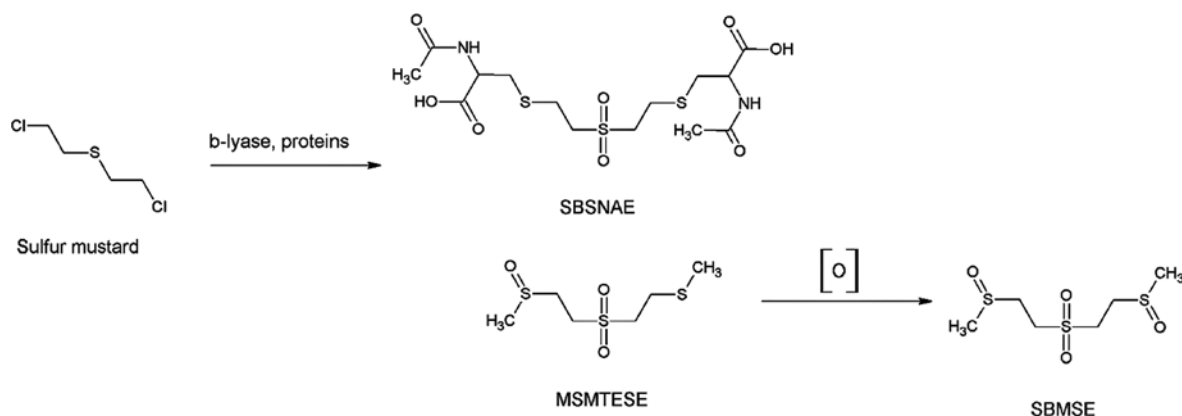


Fig. 7: Products of mustard interaction with proteins in the presence of β -lyase.

biological fluids of organisms, poisoned by mustard. These metabolites correspond to the compounds of medium polarity and are highly resistant. They have strictly exogenous nature (are formed in the body only in the case of mustard exposure) which eliminates false positive results of analysis. As seen from the Fig. 7 SBMSE is formed by further oxidation of MSMTESE, therefore development of rapid approach for simultaneous determination of SBMSE and MSMTESE in biological fluids will allow to study mustard metabolism in living organisms, and their total content will allow to estimate dose of toxic agents. The use of highly selective tandem mass spectrometric detection allows to significantly reduce LODs of the developed approaches.

An approach for the SBSNAE determination in human plasma and rat urine for quantification mustard exposure was proposed by the authors [14]. It employs solid-phase extraction sample preparation, followed by LC separation with negative ion electrospray ionization-tandem mass spectrometry (ESI-MS/MS). The method LOD for SBSNAE determination in rat urine samples is 0.05 ng/mL with relative standard deviation (RSD) value <10 %. Application of this procedure was demonstrated in the mustard animal exposure model. Rats were exposed intravenously with 5 mg/kg HD, and SBSNAE on different concentration levels in urine samples was observed for 22 days after exposure.

A sensitive rapid separation LC-MS/MS screening method for the simultaneous determination of another transformation products of mustard in urine – SBMSE and MSMTESE and degradation products of nerve agents – EMPA, IMPA and PMPA has been proposed by our group [15]. The analysis of these compounds is of interest because they are specific metabolites of different of CWAs: sulfur mustard, sarin, soman, VX and Russian VX. Application of “dilute-and-shoot” sample preparation and rapid separation LC and tandem mass spectrometry allowed to develop rapid, direct and sensitive method for determining CW exposure. Chromatographic separation of the metabolites was performed using a reverse phase column with gradient mobile phases consisting of 0.5 % formic acid in water and acetonitrile. Identification and quantification of species were achieved using ESI-MS/MS monitoring two precursor-to-product ion transitions for each compound. The method demonstrated linearity over at least two orders of magnitude and had detection limits of 0.5 ng/mL in urine.

There is another indirect metabolite of sulfur mustard which may be useful for its exposure identification – thiodiglycolic acid (TDGA). A Gram-negative bacterium, *Alcaligenes xylosoxydans* ssp. *xylosoxydans*, transforms thiodiglycol (TDG), the precursor and hydrolysis product of sulfur mustard, to TDGA [16, 17]. Allen et al. showed that when a site is testing for sulfur mustard contamination, it is important to determine not only sulfur mustard and thioglycolic acid but also for TDGA [18].

A sensitive fast LC-MS/MS approach for TDGA determination in aqueous samples and urine has been established in the authors laboratory [19, 20]. The use of “dilute-and-shoot” method helps to shorten sample preparation stage and provides a sensitive and direct approach for TDGA determination in aqueous samples and urine. Chromatographic separation of the analyte and other urine compounds was achieved using a reverse phase LC column with mobile phases consisting of 0.1 % formic acid in water and acetonitrile in gradient elution mode. For identification and quantification of TDGA ESI-MS/MS was used. The method demonstrates good linearity and has detection limit of 10 ng/mL in environmental water samples and 50 ng/mL in urine.

Determination of lewisite degradation products

Lewisite agent is usually found as a mixture of 1-chlorovinylarsonous chloride (“lewisite 1”, up to 90 % of mix), 2-chlorovinylarsonous dichloride (“lewisite 2”) and tris(2-chlorovinyl)arsine (“lewisite 3”). These compounds are strong vesicant and can act via inhalational, cutaneous and ocular routes of exposure [21]. After intoxication in mammals fast hydrolysis and oxidation take place. The primary metabolite of lewisite in animals is the hydrolysis product CVAA [22] shown in Fig. 8. CVAA further transforms to the CVAOA. These two metabolites may be used for determination of exposure to lewisite and give information about its transformation.

An approach for rapid screening of CVAA and CVAOA in water samples by capillary zone electrophoresis has been developed by the authors [23]. Optimization of the detection conditions, which includes a choice of

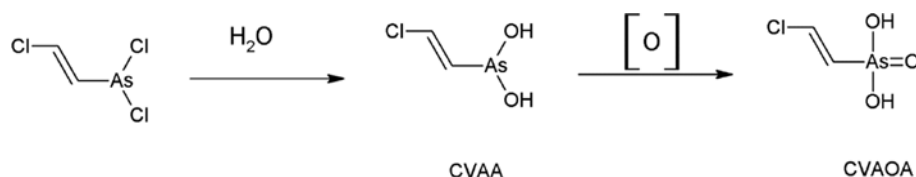


Fig. 8: Degradation of lewisite forming CVAA and CVAOA.

wavelength and detection mode, the composition of the working buffer, sample injection volume and conditioning program of working capillary were performed. The developed method is characterized by high accuracy, reproducibility, selectivity of determination and lack of interference by matrix components in natural water samples. The method was tested in the analysis of various real water samples. Achieved LODs for CVAA and CVAOA in water samples were 0.3 and 0.5 mg/L, respectively.

LC-MS approach for lewisite metabolite detection in human and rat urine samples: CVAA (detection limit – 10 ng/mL) was developed in the authors laboratory [24]. The validation of the approach on human urine samples spiked with CVAA and urine samples from rats intoxicated with lewisite was carried out. Compounds determination using atmospheric pressure chemical ionization in negative ion mode and applying deprotonated molecules as detecting ions was carried out. Biological samples analysis was carried out by reversed-phase chromatography using hydrophilic endcapped sorbent. It was shown that CVAA may be detected in rat urine during 13 days after intoxication. SPE based on reversed-phase adsorption cartridges containing copolymer of styrene and divinylbenzene was suggested as sample preparation procedure. To increase reliability of the approach, product of CVAA oxidation – CVAOA was determined simultaneously with CVAA. To lower LODs tandem mass-spectrometry was applied. A sensitive and simple method for the quantification and for the detection of CVAA and CVAOA was developed [25]. The developed assay was based on the use of SPE followed by LC coupled to negative ion-mode low-energy collision dissociation ESI-MS/MS. The method demonstrated linearity over at least three orders of magnitude and had LOD of 0.5 ng/mL for CVAA and 3 ng/mL for CVAOA. The RSD values for the quality control samples ranged from 6 to 11 %. Application of this procedure was demonstrated in the lewisite animals exposure model. Rats were exposed intravenously by non-lethal doses of lewisite and marker levels in urine samples were analyzed for 21 days post-exposure.

Summary and the comparison of the results

Decreasing limits of detection, chromatographic separation time and simplification of sample preparation were the main directions of our works. The developed approaches were applied to the analysis of environmental waters, dust and ground mixed samples from the former chemical weapons plant, plasma and urine samples of rats exposed by nerve agents. Analytical characteristics of the approaches are summarized in Table 1. Comparison of these techniques with another described in the literature by the moment of publication of each paper is summarized in Table 2. As it can be seen from the Table 2 the developed approaches in most cases exceed described in the literature in sensitivity. For taurine derivatives and DEMP, DEAS and DIAS analytical approaches were for the first time described in open sources by the authors.

Summary

Several presented works were conducted in order to broaden understanding of the CWA behavior under positive and negative EI conditions. Discovered fragmentation features will be helpful to gain the information about the structure of the toxic substances and to predict negative ion mass spectra of some groups of phosphorus organic compounds.

Table 1: Parameters of developed liquid chromatography methods.

Method of analysis	Degradation product	Limit of detection, ng/mL	Sample type	Characteristics of the column	Mobile phase	Reference
LC-MS	IMPA	4	Plasma	Kromasil 100 C18 column 250×1 mm, 5 µm and Sinerigi Hydro_RP column 150×2.1 mm, 5 µm	Mobile phase A – 0.1 vol % HCOOH in water, mobile phase B – 0.1 vol % HCOOH in methanol, gradient 0.2–0.3 mL/min	[7]
	PMPA	0.6				
	IBMPA	1				
LC-MS/MS	EMPA	0.8	Urine	Acclaim C18 150×2.1 mm, 2.2 µm	Mobile phase A – 0.5 vol % HCOOH in water, mobile phase B – 0.5 vol % HCOOH in methanol, gradient 0.45 mL/min	[8]
	IMPA	0.5				
	PMPA	0.1				
	IBMPA	0.4				
	EMPA	0.5				
LC-MS/MS	IMPA	0.5	Urine	Acclaim 120 C18 column 150×2.1 mm, 2.2 µm	Mobile phase A – 0.5 vol % HCOOH in water, mobile phase B – 0.5 vol % HCOOH in methanol, gradient 0.45 mL/min	[15]
	PMPA	0.5				
	IBMPA	0.5				
	MPA	0.1				
LC-MS/MS			Water samples	Luna HILIC 200A 150×4.60 mm, 5 µm	Solvent A – 10 mM ammonium, solvent B – acetonitrile 0.4 mL/min	[9]
LC-MS/MS	MPA	0.2	Dust and ground mixed samples	Luna HILIC 200A 150×4.60 mm, 5 µm	Solvent A – 10 mM ammonium solvent B – acetonitrile 1.5 mL/min	[10]
	EMPA	0.07				
	IMPA	0.008				
	PMPA	0.005				
	IBMPA	0.008				
CE-UV	DMT	5000	Water samples	Polyvinyl-coated silica unmodified capillary tubing, inner diameter of 50 and 75 µm (effective capillary length 50.5 cm, total – 60 cm)	20 mM borate buffer (sodium borate) set at pH 9.5 with 1.0 M NaOH	[11]
	DET	3000				
	DPT	3000				
	DIT	3000				
CE-UV	DMT	2000	Water samples	Polyacrylamide-coated silica capillary tubing, inner diameter of 75 µm (effective capillary length 50.5 cm, total – 60 cm)	20 mM borate buffer (sodium borate) set at pH 9.5 with 1.0 M NaOH	[12]
	DET	1500				
	DPT	1500				
	DIT	1500				
LC-MS	DMT	15	Water samples	Zorbax Stable Bond C-18E analytical column	Mobile phase A – an aqueous solution of 20 mM ammonium acetate, Mobile phase B acetonitrile or methanol, gradient 0.45 mL/min	[12]
	DET	15				
	DPT	25				
	DIT	25				
LC-MS	DEMP	0.3	Water samples	Zorbax SB-C18 150×4.6 mm, 1.8 µm	Mobile phase A – 0.5 vol % HCOOH in water, mobile phase B – acetonitrile, gradient 0.7 mL/min	[13]
	DIMP	0.3				
	DEAS	300				
	DIAS	50				

Table 1 (continued)

Method of analysis	Degradation product	Limit of detection, ng/mL	Sample type	Characteristics of the column	Mobile phase	Reference
LC-MS/MS	SBSNAE	0.05	Urine	Synergi Polar RP 250×2 mm, 4 μm	Mobile phase A – 20 mm ammonium acetate in water, mobile phase B – acetonitrile, gradient 0.3 mL/min	[14]
LC-MS/MS	SBMSE MSMTESE	0.5 0.5	Urine	Acclaim 120 C18 column 150×2.1 mm, 2.2 μm	Mobile phase A – 0.5 vol % HCOOH in water, mobile phase B – 0.5 vol % HCOOH in methanol, gradient 0.45 mL/min	[15]
LC-MS/MS	TDGA	10	Water samples	Acclaim RSLC 120 C18 column 150×2.1 mm, 2.2 μm	Mobile phase A – 0.1 vol % HCOOH in water, mobile phase B – acetonitrile, gradient 0.4 mL/min	[20]
LC-MS/MS	TDGA	50	Urine	Acclaim RSLC 120 C18 column 150×2.1 mm, 2.2 μm	Mobile phase A – 0.1 vol % HCOOH in water, mobile phase B – acetonitrile, gradient 0.4 mL/min	[21]
CE-UV	CVAA CVAOA	300 500	Water samples	The polyvinyl-coated silica unmodified capillary tubing with inner diameter of 50 and 75 μm (effective capillary length 50.5 cm, total – 60 cm)	30 mM borate buffer (sodium borate) set at pH 8.9 with 1.0 M NaOH	[23]
LC-MS	CVAA	10	Plasma and urine	Synergi-Polar 250×2.1 mm, 4 μm	Mobile phase A – an aqueous solution of 20 mM ammonium acetate set at a pH 5.4, Mobile phase B acetonitrile, gradient 0.5 mL/min	[24]
LC-MS/MS	CVAA CVAOA	0.5 3	Urine	Acclaim 120 C18 column 150×2 mm, 4 μm	Mobile phase A – 0.5 vol % HCOOH in water, mobile phase B – acetonitrile, gradient 0.3 mL/min	[25]

Table 2: Comparison of the obtained results with the literature.

Compound	Proposed method of analysis – LODs, ng/mL	Method of analysis – LODs, ng/mL from the literature/reference
MPA	LC-MS/MS – 0.100	IP-LC-ICP-MS – 0.139/[26]
EMPA	LC-MS/MS – 0.06	LC-MS/MS – 0.07/[27]
IMPA	LC-MS/MS – 0.008	LC-MS/MS – 0.04/[27]
PMPA	LC-MS/MS – 0.005	LC-MS/MS – 0.01/[27]
DIT	LC-MS – 25	–
DPT	LC-MS – 25	–
DET	LC-MS – 15	–
DMT	LC-MS – 15	–
DEMP	LC-MS – 0.3	–
DIMP	LC-MS – 3	LC-MS – 760/[28]
DEAS	LC-MS – 300	–
DIAS	LC-MS – 50	–
SBSNAE	LC-MS/MS – 0.05	LC-MS/MS – 0.5/[29]
SBMSE	LC-MS/MS – 2	LC-MS/MS – 4/[30]
MSMTESE	LC-MS/MS – 1	LC-MS/MS – 4/[30]
TDGA	LC-MS/MS – 10	GC-MS – 20/[31] GC-MS – 50/[32]
CVAA	LC-MS/MS – 0.5	GC-MS – 1/[33]
CVAOA	LC-MS/MS – 3	GC-MS – 20/[34]

A wide range of approaches, which allow comprehensive solution of the problem for establishing the fact of using nerve and blister agents, has been developed. The approaches based on electrophoresis, available in a vast variety of laboratories allow to perform rapid and simple screening of CWA degradation products. Sensitivity of developed LC-MS approaches were comparable with the most sensitive existing GC-MS approaches and did not require derivatization, reducing the time of the analysis and the number of false negative results. The developed complex of approaches was designed for sensitive LC-MS determination of the majority of known chemical weapon metabolites on the same equipment and has been tested in the frame of OPCW proficiency testing system.

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