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Review Article

Poisoning by organophosphorus nerve agents and pesticides: An overview of the principle strategies and current progress of mass spectrometry-based procedures for verification

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ABSTRACT

Intoxication by organophosphorus (OP) poisons, like nerve agents and pesticides, is characterized by the life-threatening inhibition of acetylcholinesterase (AChE) caused by covalent reaction with the serine residue of the active site of the enzyme (phosphylation). Similar reactions occur with butyrylcholinesterase (BChE) and serum albumin present in blood as dissolved proteins. For forensic purposes, products (adducts) with the latter proteins are highly valuable long-lived biomarkers of exposure to OP agents that are accessible by diverse mass spectrometric procedures. In addition, the evidence of poison incorporation might also succeed by the detection of remaining traces of the agent itself, but more likely its hydrolysis and/or enzymatic degradation products. These relatively short-lived molecules are distributed in blood and tissue, and excreted via urine. This review presents the mass spectrometry-based methods targeting the different groups of biomarkers in biological samples, which are already internationally accepted by the Organisation for the Prohibition of Chemical Weapons (OPCW), introduces novel approaches in the field of biomedical verification, and outlines the strict quality criteria that must be fulfilled for unambiguous forensic analysis.

Contents

Abbreviations: AChE, acetylcholinesterase; BChE, butyrylcholinesterase; CI, chemical ionization; CWC, chemical weapons convention; EI, electron ionization; ESI, electrospray ionization; GB, nerve agent sarin; GD, nerve agent soman; GC, gas chromatography; HR, high-resolution; HSA, human serum albumin; IMER, immobilized enzyme reactor; IMPA, isopropyl methyl phosphonic acid; IMS, immunomagnetic separation; LC, liquid chromatography; MS, mass spectrometry; MS/MS, tandem-mass spectrometry; MW, molecular weight; OP, organophosphorus; OPCW, Organisation for the Prohibition of Chemical Weapons; OPH, organophosphate hydrolase; RBC, red blood cells; SIM, selected ion monitoring; SPE, solid-phase extraction; SRM, selected reaction monitoring; UHPLC, ultra high-performance liquid chromatography.

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1. Introduction

Organophosphorus (OP) nerve agents and their chemically-related pesticides both act as inhibitors of acetylcholinesterase (AChE), which may induce a cholinergic crisis *in vivo* and, thus, might lead to death. OP nerve agents such as sarin, VX and a representative of the novichok group (Fig. 1) have been used within the last few years for chemical warfare in Syria [1], assassination in Japan [2] and Malaysia [3,4], and attempted murder in Japan [5–7] and in the UK [8–10]. At present, the case of the novichok poisoning of Alexei Navalny has attracted large amounts of public attention [11,12]. These incidents occurred even though the transport and deployment of nerve agents are banned by the chemical weapons convention (CWC) [13]. In consequence, there is a need for analytical methods to detect and identify these poisons for verification purposes (i.e., unambiguous evidence of the agent) and criminal prosecution. As abidance by the CWC is supervised by the Organisation for the Prohibition of Chemical Weapons (OPCW, Headquarter The Hague/The Netherlands, Nobel Peace Prize laureate 2013) this organisation has established an international network of a number of laboratories specializing in the analysis of either environmental or biomedical samples [14]. The majority of the relevant analytical methods established in these laboratories make use of mass spectrometry (MS) for qualitative analysis. This article focuses on the principles, strategies, methods and quality criteria applied to the verification of nerve agent poisoning, exclusively addressing biomedical samples.

2. Targets for biomedical verification

In general, most OP agents are highly reactive and readily hydrolyze following either an enzymatic or, more often, a non-enzymatic pathway and form covalent products (adducts) with endogenous proteins [15]. These chemical reactions may degrade the agent within hours to days, causing a rapid decrease in concentration of the circulating polar biotransformation products, due to elimination and excretion by the surviving organism. Accordingly, the detection window between the exposure event and sample draw is quite small making *in vivo* identification of these polar chemicals challenging, if not impossible [15]. In contrast, adducts with proteins are traceable up to several weeks after poisoning, as modified proteins generally exhibit similar protein turn-over rates as their non-modified natural variants [1,15–17]. Such adducts result from the phosphylation of nucleophilic moieties in the target molecules. Phosphylation denominates both the phosphorylation by derivatives of organophosphates, e.g. dimethyl- (DMP) and diethyl-phosphate pesticides (DEP), as well as phosphonylation by derivatives of organophosphonates, e.g. G- and V-type nerve agents (Fig. 1). During this reaction, the phosphorus atom of the agent binds to the nucleophile and the leaving group of the agent is cleaved. Accordingly, identification of exposure to an OP agent might be proven by detection of the remaining poison, its hydrolysis products, including the leaving group, enzymatic break-down products, or adducts with proteins [15,18].

Based on the distribution and elimination processes of nerve agents *in vivo*, the different markers are primarily detected in blood, plasma or serum, urine and tissues [1,15]. Due to the complexity of these biological matrices, sophisticated extraction procedures are often required to isolate, clean and concentrate the markers of interest from their specimens [18,19]. In contrast, analysis of hair contaminated by an agent may only require a simple wash to dissolve either the original agent or its hydrolyzed forms [1,18].

The following sections will outline the bioanalytical methods available to detect the different markers mentioned above, presenting the approaches for protein-adduct analysis in more detail.

2.1. Remaining agent

Due to their high chemical reactivity, immediate extraction of OP nerve agents from wet and aqueous biological sources is often strictly required to transfer the agent into a non-aqueous organic solvent, like dichloromethane, ethyl acetate or isopropanol, to sufficiently stabilize it [19]. Such isolation steps from plasma or urine, might include common simple liquid–liquid extraction (LLE), as well as solid-phase extraction (SPE) procedures [1,19]. The purified

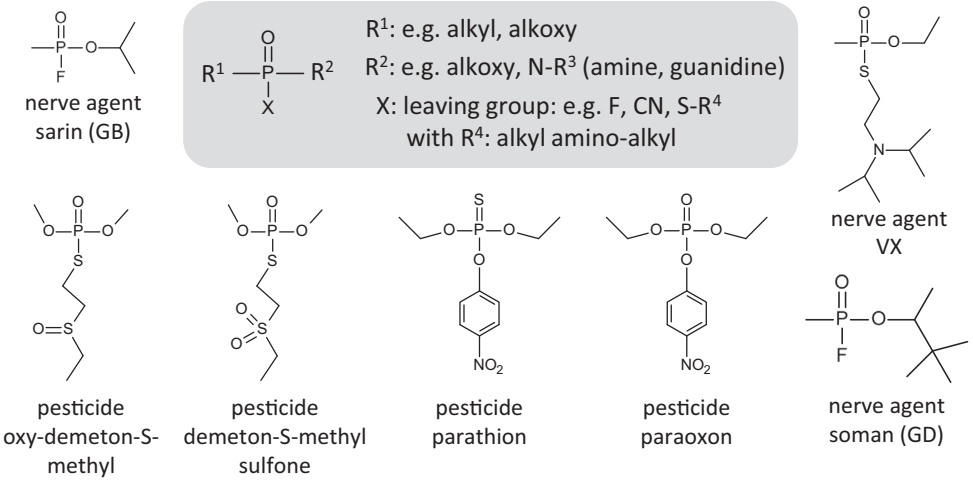

Fig. 1. Chemical structures of organophosphorus nerve agents and pesticides. The grey box presents the common structure of this class of cholinesterase inhibitors that cause phosphylation of the enzyme while losing the leaving group X.

agents (Fig. 1) are detected by gas (GC) or liquid chromatography (LC), typically online-coupled to MS operating in the highly selective tandem-MS (MS/MS) mode with selected reaction monitoring (SRM) [19].

In cases of human poisonings with low absolute amounts of nerve agents, the detection of the original poison is often unsuccessful due to its high reactivity and low concentration [1]. In contrast, in cases of massive poisoning with an OP pesticide, the original toxicant itself can be easily found in plasma, urine and tissue due to its lower chemical reactivity with proteins and its higher stability towards aqueous hydrolysis [19,20].

The differentiation of enantiomers and diastereoisomers of nerve agents may be of interest, thus demanding the chiral separation of the more toxic P(-)-agent molecules from their less toxic P(+)-variants [15]. Even though there is an academic interest in the differentiation of these molecules for toxicological and toxicokinetic reasons, there is only a minor need for forensic purposes, basically intending to document incorporation of such anthropogenic compounds. However, elaborate analytical methods were introduced to follow enantio-selective concentration–time profiles *in vivo* and *in vitro* of tabun [22], VX [23] and cyclosarin [24], but are not addressed herein in more detail.

2.2. Hydrolysis products

Hydrolysis products of nerve agents typically represent molecules in which the leaving group of the agent was exchanged with a hydroxyl-group (Fig. 2). In certain cases, hydrolysis might proceed, thus also cleaving an alkoxy-group bound to the phosphorus atom. Therefore, the organophosphoric and –phosphonic acids are prone to deprotonation and formation of salts, thus hindering direct GC analysis. In general, GC analysis requires the presence of less polar, non-ionic, volatile analytes exhibiting sufficient chemical stability at temperatures elevated above their boiling point. Accordingly, following extraction, hydroxyl-groups of hydrolyzed agents must be derivatized prior to GC analysis using reagents such as pentafluorobenzyl bromide to make the compounds non-ionic and less polar [1,19,25]. Mass spectrometric fragments of these derivatives resulting from the electron ionization (EI) or chemical ionization (CI) process are selectively detected either in the selected ion monitoring (SIM) or in MS/MS mode [25,26].

Less laborious sample preparation without the need for derivatization can be carried out when using LC, instead of GC, for separation. Nevertheless, the high polarity of the hydrolysis products can make the chromatographic separation from matrix components quite challenging. However, in cases of massive pesticide poisoning, such markers can easily be detected. When using sophisticated methods and modern instruments, hydrolysis products of nerve agents (Fig. 2B,C) might also be directly detected after negative electrospray ionization (ESI) [27–29]. Accordingly, isopropyl methyl phosphonic acid (IMPA), the hydrolysis product of the nerve agent sarin (GB) (Fig. 2B), was detected in serum samples of Japanese citizens exposed to GB during the Tokyo subway attack in 1995 [7]. In addition, IMPA was also found in a large number of different organ tissues including liver, heart and lung, as well as in the blood, skin and hair of a dead female victim of GB poisoning in 2013 in Syria [1]. Analyses were performed by LC-MS- as well as GC–MS-based procedures.

Detection of these hydrolysis products clearly documents the incorporation of an OP agent, but does not allow the identification of the leaving group. Therefore, these biomarkers are considered as secondary in plasma and as primary in urine [30]. Accordingly, after detection of these markers, the conclusion was that a "sarin-like" substance was incorporated, as published by the OPCW in 2017 reporting on the alleged use of GB in Syria [31,32]. However, the detection of the phosphyl-moiety provides essential evidence for the incorporation of an exogenous anthropogenic agent.

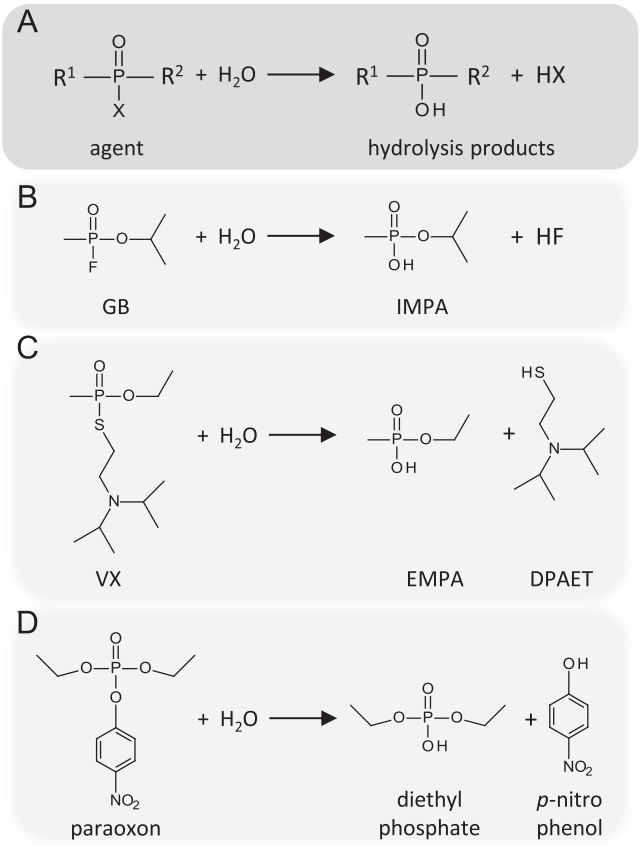

Fig. 2. Hydrolysis of OP agents. Hydrolysis products generated by the nucleophilic substitution of the leaving group represent common biomarkers of poisoning. The common reaction scheme (A), as well as hydrolysis of the nerve agents sarin (B) and VX (C) and the pesticide paraoxon (D) are shown. Phosphyl-moieties as well as larger leaving groups are detectable by MS coupled to either GC or LC. DPAET, 2-(diisopropylamino)ethanethiol; EMPA, ethyl methyl phosphonic acid; GB, sarin; IMPA, isopropyl methyl phosphonic acid; R^1 and R^2 are organic substituents as illustrated in Fig. 1.

In some special cases the released leaving group can also serve as a marker of exposure. The leaving group of the agent molecule is detected either in its free form, e.g. para-nitrophenol derived from the pesticides parathion and paraoxon (Fig. 2D), or trichloropyridinol from chlorpyrifos and chlorpyrifos-oxon [33], or in a bound form, e.g. thiol-containing leaving groups of V-type nerve agents and certain pesticides, as addressed in Section 3 in more detail [34–37].

2.3. Enzymatic biotransformation products

Based on the current literature, the degradation of OP nerve agents typically results from non-enzymatic aqueous hydrolysis. In contrast, OP pesticides, especially the thiono-toxicants that comprise a P = S moiety like parathion (Fig. 1), dimethoate and chlorpyrifos, require essential enzymatic conversion for both activation and degradation [19,21,37]. Different cytochrome P450 (CYP450) enzymes initiate the desulfuration and oxidation of the thiono-variants, thus producing the much more reactive and toxic oxono-forms, like paraoxon (Figs. 1, 2D), omethoate and chlorpyrifos-oxon [19]. In addition, the leaving group of such pesticides might also be oxidized, as shown for oxydemeton-S-methyl (Fig. 1) [38]. However, all the different products might serve as reliable biomarkers for poisoning, as described recently [19,21,38]. Apart from these CYP450-enzyme-targeting pesticides, little is

known about other natural mammalian enzymes involved in nerve agent biotransformation [15]. Even though the natural forms of the circulating enzyme paraoxonase (PON1, EC 3.1.8.1) are known to hydrolyze, e.g. paraoxon by cleaving the *para*-nitro phenol moiety (Fig. 2D), their enzymatic activity towards G- and V-type nerve agents is negligible [15]. Other endogenous enzymes present in mammalian organisms that catalyze nerve agent hydrolysis to a relevant extent are not yet known; therefore, genetically-modified and bacterially-produced enzymes are under investigation for use as antidotes or decontamination, via catalyzing nerve agent hydrolysis [39–42]. Organophosphate hydrolases (OPH) and mutants of diisopropyl fluorophosphatase (DFPase) are currently under development [39–43]. Aforementioned enzymatic biotransformation products can be detected by respective GC- and LC-MS(/MS) techniques as described above (Section 2.2).

2.4. Adducts with proteins

As outlined above, OP nerve agents, as well as OP pesticides, cause phosphylation of nucleophilic moieties of amino acid side chains, thus producing highly stable adducts that can be targeted by appropriate bioanalytical methods for verification. Reactive nucleophilic groups may include the hydroxyl-functions in the side chains of the amino acid serine, as relevant in AChE and butyrylcholinesterase (BChE) (Fig. 3), or tyrosine, as relevant in human serum albumin (HSA) (Section 2.4.3) [15]. In addition, amine-groups in lysine are also prone to phosphylation as shown for ubiquitin, albumin, keratin, tubulin, actin and transferrin [44–46].

OP-induced modifications of large proteins like BChE (molecular weight (MW), of monomer: 85 kDa) or HSA (MW of monomer 68 kDa) only cause slight changes of the MW (increase of about 0.115 kDa per phosphyl-group attached) or of the physicochemical properties (slightest alteration of the isoelectric point, pI, or hydrophobicity). Therefore, OP-protein adducts are infrequently used to separate and discriminate their non-adducted natural forms. Direct MS-based unambiguous identification of OP adduction of proteins can also be hampered due to the highly variable extent of point mutations or post-translational modifications (e.g. sulfuration, phosphorylation, glycosylation, oxidation, or adduct formation with endogenous or exogenous molecules), thus causing a complex mixture of different masses all correlating to just one protein (e.g., UniProtKB database entries for BChE, P06276, and HSA, P02768). Accordingly, bioanalytical procedures were developed that i) extract the adducted and non-adducted target proteins from complex biological matrices like serum or plasma, ii) subject extracted proteins to proteolysis, iii) separate the resulting peptide cleavage products by LC, and iv) monitor and discriminate modified and non-modified peptides by mass-selective MS detection (Figs. 3 and 4) [15,19,20].

Based on this bioanalytical strategy, several procedures were introduced in the literature that enable evidence of OP-poisoning, in human and other mammalian organisms, to be detected by targeting specifically modified peptides and single amino acids derived from AChE, BChE (Sections 2.4.1 and 2.4.2), albumin (Section 2.4.3) and other proteins (Section 2.4.4) [1,6,15,20,47]. As such adducts document that an OP agent capable of protein phosphylation was incorporated, they are considered primary biomarkers according to the OPCW guidelines [30].

In the following sections, the related procedures will be introduced in more detail to underline the relevance of these modern, important, and valuable approaches that are indispensable for biomedical verification.

2.4.1. Butyrylcholinesterase

The detection of BChE-adducts represents the most prominent way to prove OP-exposure. Due to the high reactivity between BChE (EC 3.1.1.8) and OP agents, corresponding adducts are easily and effectively produced *in vivo,* thus allowing evidence of small amounts of an agent [6,15,48–51]. BChE-adducts are considered to be detectable for up to 3 weeks after exposure ($\tau_{1/2}$ 6–10 d [47]) either in their intact or aged variant forms [6,15]. Ageing of BChE-adducts denominates the hydrolysis of a P-bound alkoxy-substituent resulting in the exchange to a hydroxyl-function bound to the P-atom (Fig. 3B) [15].

Based on the original report of the working group of Noort [6], BChE is initially extracted from plasma or serum where it can be found in concentrations of about 4 μg/ml [15,52]. This extraction is done by subjecting the biofluid to affinity chromatography using immobilized procainamide as the stationary phase. Procainamide is a small molecule acting as a reversible, non-covalent inhibitor of BChE. It interacts with the active center of the enzyme, allowing binding of BChE to the stationary phase causing its retention,

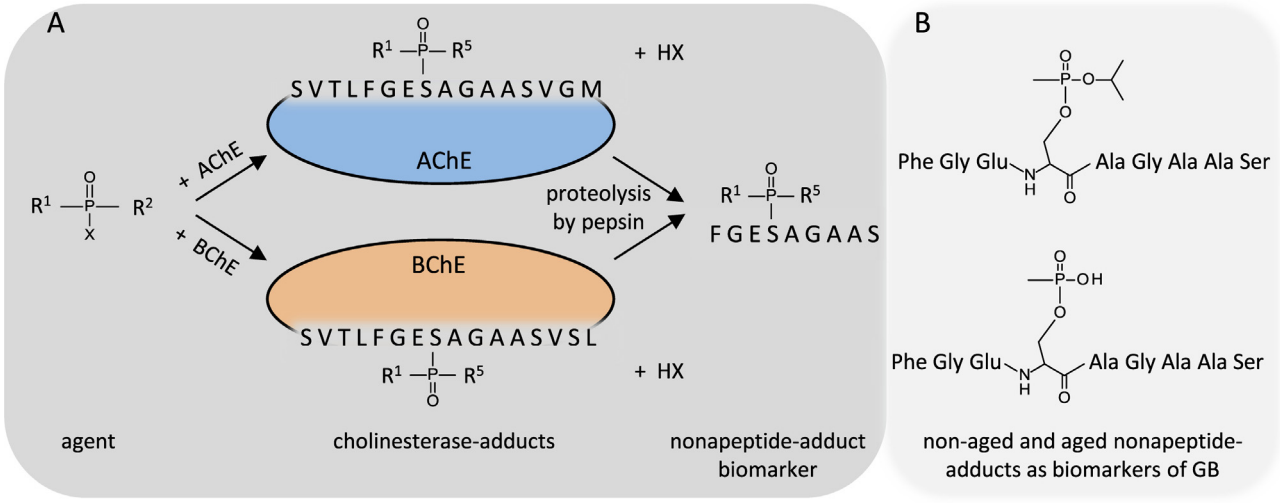

Fig. 3. Phosphylated nonapeptide biomarkers derived from inhibited AChE and BChE A: The OP agent phosphylates the serine residue of the active centers of AChE and BChE. Following the isolation from biological matrices these enzyme-adducts are subjected to proteolysis with pepsin generating a phosphylated nonapeptide potentially present in its non-aged and aged (hydrolyzed) form. B: Examples of adducted nonapeptides in its non-aged and aged form derived from the nerve agent sarin. R^1 and R^2 are organic substituents as illustrated in Fig. 1. R^5 represents either R^2 or OH.

whereas the majority of the other plasma constituents flow through freely [6,53,54]. Immobilized BChE can easily be eluted from the column by adding aqueous buffers with high NaCl concentrations to increase the ionic strength of the mobile phase. The resulting high-salt BChE solution is subjected to ultrafiltration and washed with dilute formic acid to concentrate and desalt the dissolved BChE. Even though the extraction procedure using procainamide is reliable and allowed real sample analysis [6], its selectivity and efficiency is limited [49]. Often large volumes of plasma (1–3 ml) were required and a huge number of protein contaminants were also extracted from plasma, thus resulting in complex protein solutions that deteriorated the sensitivity of subsequent LC-MS/MS analysis [49]. However, by combining procainamide extraction with ultra high-performance liquid chromatography (UHPLC) and MS/MS detection, Liu *et al.* succeeded in the detection of BChE-derived biomarkers when using a plasma volume as low as 100 μL [49].

A comparable but slightly more selective approach for BChE extraction from plasma or serum was introduced with the use of hupresin [55]. Hupresin is a hybrid of tacrine and huperzine representing a reversible non-covalent inhibitor of BChE [55]. This ligand is commercially available bound either to sepharose gel or to magnetic beads [55,56]. Nevertheless, to the best of our knowledge, application of this affinity material has not yet been reported for adduct extraction from *in vivo* samples.

The most accepted extraction procedure used today is immunomagnetic separation (IMS) [48,50,51,57]. IMS makes use of highly selective monoclonal anti-human BChE antibodies that are immobilized on paramagnetic beads. These beads are mixed with the liquid sample, thus enabling BChE binding. Application of a magnetic field allows easy separation of the solid BChE-containing bead phase from the liquid biological sample and buffer phase [48,50,51,57]. Following BChE immobilization the beads are washed and subsequently subjected to proteolysis. The major advantage of this extraction technique is its high selectivity, which minimizes the amount of protein impurity and reduces ion suppression effects during mass spectrometric ionization; thus improving the sensitivity and lower limits of detection. In addition, only small volumes of plasma, as low as 50–200 μL, are typically used for extraction [48,50,51,57].

Independent of the extraction technique applied, the concentrated and purified BChE is subsequently subjected to pepsin-catalzyed hydrolysis. This enzymatic cleavage produces either the non-phosphylated nonapeptide Phe-Gly-Glu-Ser-Ala-Gly-Ala-Ala-Ser (FGESAGAAS) derived from non-phosphylated BChE, or the phosphylated nonapeptide FGES*AGAAS either in its aged or non-aged variant derived from phosphylated BChE containing the modified serine residue (S*, Ser^{198}) of the active center of the cholinesterase (Fig. 3B) [15]. LC separation of the nonapeptide-adduct from other peptides is typically carried out on a reversed-phase (RP) column applying a binary mobile phase with a gradient of an organic modifier like acetonitrile or methanol [48,50,51,57]. The most selective detection is carried out by MS/MS enabling fragmentation of the nonapeptide-adduct and monitoring of a series of diagnostic product ions that prove the identity of the targeted biomarker peptide. As an example, Fig. 4 illustrates the structure, MS/MS spectrum and μLC- ESI MS/MS (SRM) analysis of the BChE-derived adduct produced by the nerve agent VX. Whereas the precursor mass of the intact adducted nonapeptide is quite selective for the OP agent (e.g., $[M + H]^+$ *m/z* 902.4 for VX, Fig. 4B) the resulting product ions at *m/z* 778.4, *m/z* 673.3, *m/z* 602.3, *m/z* 531.3 and *m/z* 474.2 are independent of the agent, but document the peptide backbone of the biomarker (Fig. 4A) [49]. This phenomenon represents a major benefit of this method as the simultaneous detection of at least two product ions enables a high degree of reliability

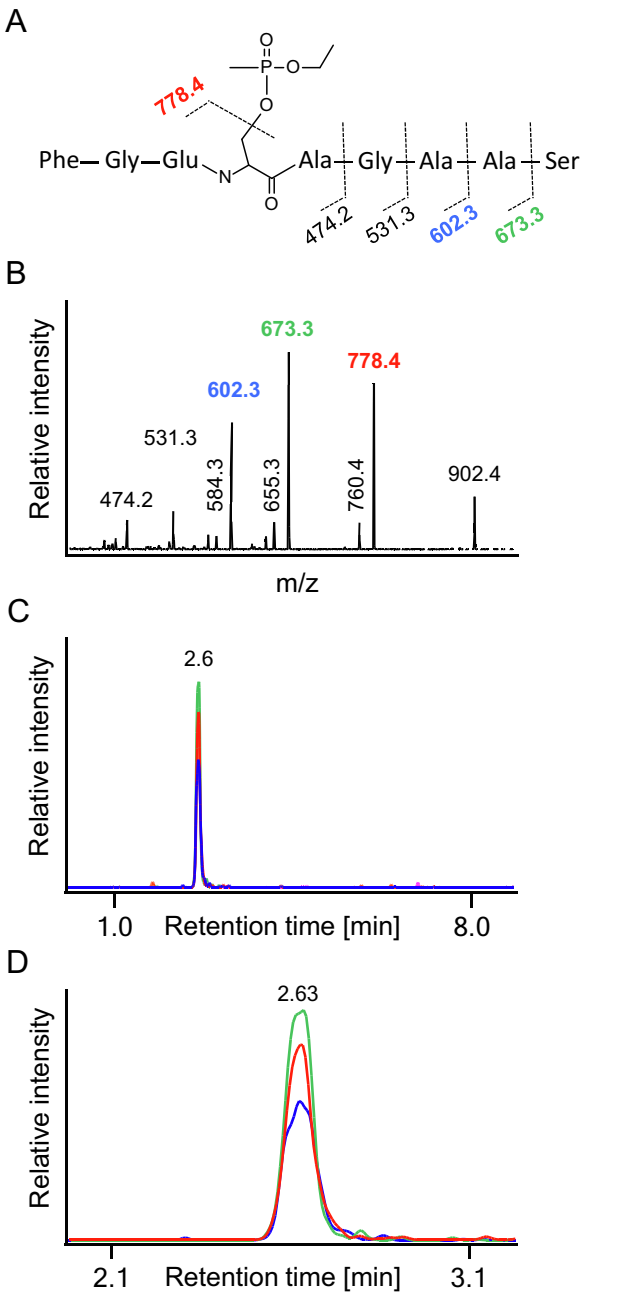

Fig. 4. Analysis of a phosphylated BChE-derived nonapeptide as a biomarker of exposure to the nerve agent VX. A: Chemical structure of the nonapeptide phosphylated at the hydroxyl-function of the serine residue side chain and sites of mass spectrometric fragmentation. B: MS/MS spectrum of the single protonated phosphylated nonapeptide ($[M+H]^+$ *m/z* 902.4) extracted from a μLC-ESI MS/MS run. C: Extracted ion chromatogram (XIC) of product ions obtained from the fragmentation of the the single protonated phosphylated nonapeptide ($[M+H]^+$ *m/z* 902.4) at *m/z* 673.3 (green trace), *m/z* 778.4 (red trace) and *m/z* 602.3 (blue trace). Chromatographic separation was carried out on a nanoEase M/Z HSS T3, C18 column (50 mm × 0.3 mm, 100 A, 1.8 μm) in gradient mode with acetonitrile as organic modifier in selected reaction monitoring mode. Monitoring of at least two product ions is essential to fulfill the quality criteria for selective detection of the biomarker. D: Zoom of the XIC shown in figure part C illustrating the relative peak areas of the corresponding product ions at *m/z* 673.3 (green trace), *m/z* 778.4 (red trace) and *m/z* 602.3 (blue trace). Peak area ratios are calculated to be compared with a reference of the same adduct. The corresponding ratios have to be identical within a tolerance interval as defined by the quality criteria of the OPCW [30]. The most intense peak (related to *m/z* 673.3) is set to 100% and peaks of ions at *m/z* 778.4 (86%) and at *m/z* 602.3 (59%) are related thereto. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

required to confirm the identity of the analyte. A more detailed discussion of the essential quality criteria required for biomedical verification will be provided below (Section 5).

2.4.2. Acetylcholinesterase

Even though the phosphylation of the serine residue in the active center of AChE represents the major reason for OP toxicity [15], bioanalytical evidence of these adducts is quite challenging. AChE present in the synaptic clefts is not easily accessible and, therefore, reliable methods for its isolation and detection of OP-modifications are lacking. In contrast, AChE expressed in red blood cells (RBC, erythrocytes), that are present in whole blood, are considerably easier to access. Nevertheless, preparation of RBC from whole blood samples and subsequent isolation of AChE is still laborious and complex [56,58]. Therefore, only two methods describing the AChE-OP adduct detection can be found openly in the literature, but it has not yet been applied to *in vivo* samples of real cases of poisoning [56,58]. Nevertheless, the procedures introduced by the working group of Lockridge should be outlined in brief [56,58], and will be done so, below.

AChE (EC 3.1.1.7) was isolated from frozen RBC after thawing and initial dilution with phosphate buffered saline (PBS) containing 1% Triton X-100 [56,58]. Subsequent centrifugation separated the cell debris from a red colored, but not viscous, solution of AChE and hemoglobin that the authors named "no-ghost RBC AChE" [58]. This solution was subjected to affinity extraction using sepharose material labelled either with hupresin (see Section 2.4.1) [56] or mouse monoclonal anti-human AChE antibodies [58]. Applying these procedures to RBC that were incubated previously with an OP model compound (resulting in 50% enzyme inhibition) produced AChE-adducts identical to those of the nerve agent soman (GD) and enabled isolation of AChE and its adducts. The purified adducted and non-adducted AChE was finally subjected to pepsin-catalyzed proteolysis, thus generating the same nonapeptide as obtained from BChE (Section 2.4.1, Fig. 3). Accordingly, LC-MS-based analysis was performed following the same principles as outlined above (Section 2.4.1). A major drawback of this interesting approach is the need for large volumes of RBC (6.5 ml [58] or 8 ml [56]) to allow identification of the aged GD-derived adducts. Nevertheless, these analytical methods may help to directly correlate pathophysiological symptoms of poisoning with molecular toxicology.

Whereas the procedures described above (Section 2.4.2) target adducted peptide biomarkers, McGuire *et al.* succeeded in the detection of phosphylated proteins from RBC by the method of fluoride-induced reactivation (Section 2.4.4) [59].

2.4.3. Human serum albumin

In addition to ChE-adducts, modified amino acids in HSA can also be detected. Phosphylation at diverse tyrosine, serine and lysine residues has been reported [16,47,60–62]. For verification purposes HSA may be isolated from plasma or serum initially by affinity supports like HiTrap blue [57,63]. However, depending on the required sensitivity and the resolution of the MS-system used, initial protein isolation from plasma or serum might be omitted, since albumin is the most abundant protein in this biological fluid (40 mg/ml) [38,64,65]. Accordingly, the biological sample is directly subjected to pronase-catalyzed proteolysis for the generation of single tyrosine residues (Fig. 5) [16,57,60]. These Tyr residues, especially Tyr^{411} from HSA, might be phosphylated (Y*) in cases of exposure to OP poisons [16,36–38,66]. This biomarker can be monitored by LC-MS/MS and represents an important complementary analytical target in addition to BChE (Fig. 5B). The HSA-adduct is considered to be more stable and long-lived *in vivo* ($\tau_{1/2}$ 20 d [47]) than the corresponding BChE-adduct [57]. Evidence of the phosphylated Tyr-adduct of GB in blood was described for a

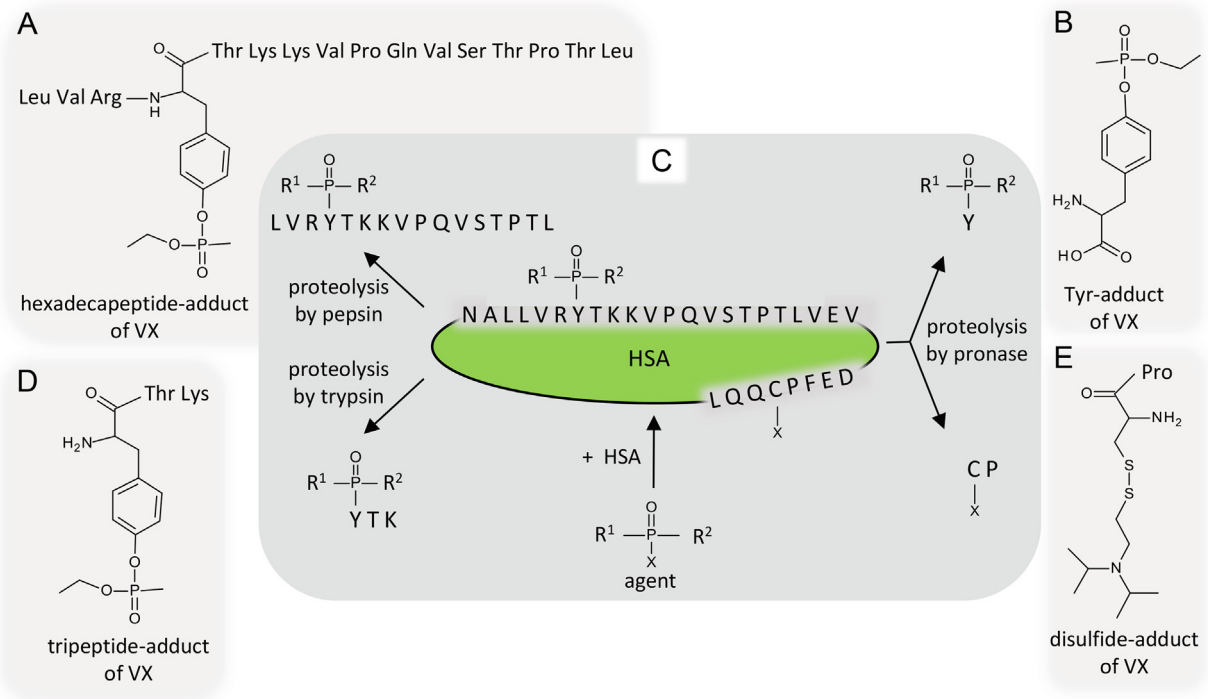

Fig. 5. Amino acid- and peptide-adduct biomarkers derived from HSA-adducts with OP agents. A: Hexadecapeptide-adduct of VX obtained after proteolysis with pepsin containing the phosphylated Tyr^{411} residue, B: single Tyr residue-adduct of VX obtained after proteolysis with pronase, C: proteolysis with either pepsin, trypsin or pronase liberates adducts suitable for detection by LC-MS/MS, D: tripeptide-adduct obtained from trypsin cleavage containing the phosphylated Tyr^{411} residue, E: disulfide-adduct between Cys^{34} and the leaving group of VX obtained after pronase treatment. R^1 and R^2 are organic substituents as illustrated in Fig. 1.

real case of nerve agent poisoning in Syria in 2013 [1] and diverse human poisonings with OP-pesticides [37,38,67]. Besides the single phosphylated tyrosine residue, additional phosphylated HSA-derived markers have also been introduced [66]. Following trypsin-mediated proteolysis the tripeptide Y*TK (Fig. 5D), and after the use of pepsin, the hexadecapeptide LVRY*TKKVPQVSTPTL (Fig. 5A), all contained the phosphylated Tyr^{411} residue [66]. These biomarkers were successfully used to prove human poisoning by diverse OP pesticides [66]. Even though the Y*-adduct is a well-established and internationally accepted primary biomarker, its traceability typically requires much higher concentrations of the agent than needed for BChE-adduct formation [68]. In addition, it must be supposed that certain toxic OP agents do not form adducts with HSA at all, due to their insufficient reactivity, thus limiting the suitability of HSA-OP adducts. In contrast, thiono-OP pesticides that possess a P = S, instead of a P = O, double bond (Fig. 1) form Y*-adducts, whereas adducts with BChE are not produced [66,67,69].

Apart from BChE and HSA one could expect that a large number of additional proteins are phosphylated *in vivo*, even though to a smaller extent [15]. These adducts might also possess potential as biomarkers as discussed for ubiquitin [44], ceruloplasmin [70] and α-chymotrypsin [71]. Therefore, a more general and non-protein selective procedure targeting phosphylated amino acids was introduced.

2.4.4. Proteins in general

The method of fluoride-induced reactivation is based on the incubation of proteins precipitated from plasma, serum or RBC with an aqueous solution of high potassium fluoride (KF) concentration [16,59]. Following nucleophilic substitution, a fluoride ion attacks the central phosphorus atom of the phosphyl-moiety attached to an amino acid side chain. Subsequently, the phosphyl-group is cleaved, thereby producing a fluoro-analogue of the original OP-poison (Fig. 6). This resulting small molecule is extracted from the aqueous reaction mixture by SPE and finally subjected to GC analysis [16,59,67,72]. This technique also belongs to the toolbox of bioanalytical standard methods accepted and recommended by the OPCW for producing a primary biomarker. The method of F^--induced reactivation is applicable until a substantial period after poisoning and was successfully applied to prove GB-poisoning in monkey plasma up to 35 days after *in vivo* exposure [73].

Following this approach, a large number of tissue extracts from a female dead body from Syria tested positive for the presence of GB (Fig. 6B) [1]. Evidence of GB poisoning was also reported by Polhuijs *et al.* analyzing specimens from Japanese victims of the Matsumoto attack in 1994 [74]. Some cases of human poisoning with the OP-pesticides, chlorpyrifos and diazinon, were investigated using this technique (Fig. 6C,E) [67].

An additional approach for the release of a bound phosphyl-moiety attached to an esterase and its subsequent detection was suggested by the OPCW in connection with the 5th bioproficiency test (5th BioPT) in 2020.

Following the proposed sample preparation, plasma is mixed with a highly concentrated solution of HI 6 (resulting in 243 μg/ml) for a 15 min incubation at 37 °C (Fig. 7) [75]. HI 6 is bispyridinium oxime currently under development as an antidote acting as a cholinesterase reactivator to treat nerve agent and pesticide poisoning in a similar way to the oximes obidoxime and pralidoxime [76]. These compounds induce the detachment of the phosphyl-moiety from the serine residue of the inhibited esterase following the mechanism of a nucleophilic substitution [77]. This reaction yields a reactivated enzyme, as well as a short-lived and highly reactive phosphylated oxime intermediate that undergoes hydrolysis producing the hydrolysis product of the original OP agent (Figs. 2, 7) [77]. The hydrolysis product can be extracted from the plasma mixture and analyzed either by GC (after derivatization) or by LC-MS techniques, as described in Section 2.2. This approach is suggested to be used to release bound phosphyl-moieties derived from GB, cyclosarin (GF) or GD. However, this procedure has not yet been published in the literature.

3. Novel targets and potential biomarkers

In 2014 the working group of Harald John reported on a novel kind of adducts between proteins and V-type nerve agents resulting from the initial phosphonylation of lysine residues originally reported for ubiquitin [44]. Due to spatial proximity to a glutamic acid residue, an isopeptide bond between the carboxyl-group of the side chain and the ε-amino group of the phosphonylated lysine residue was produced. This intramolecular cyclization was a consecutive reaction to the phosphylation not described previously, and its reaction products, in principle, might represent novel targets for adduct-analysis. Meanwhile, Schopfer and Lockridge also found these kind of adducts in aprotinin, casein, HSA, BChE and tubulin after exposure to chlorpyrifos-oxon [62]. Such intramolecular cyclizations or intermolecular cross-links may have an impact on the function of the protein and might also serve as biomarkers of exposure, although they have not yet been used in this way.

In addition to the well-known Y*-adducts (Section 2.4.3) in albumin, Fu *et al.* also reported on a number of lysine residues being phosphonylated by GD as observed after trypsin-catalyzed proteolysis of rabbit albumin [45]. Using nano-LC coupled to an Orbitrap MS system, phosphonylated peptides were identified after gel electrophoretic separation. However, the suitability of these

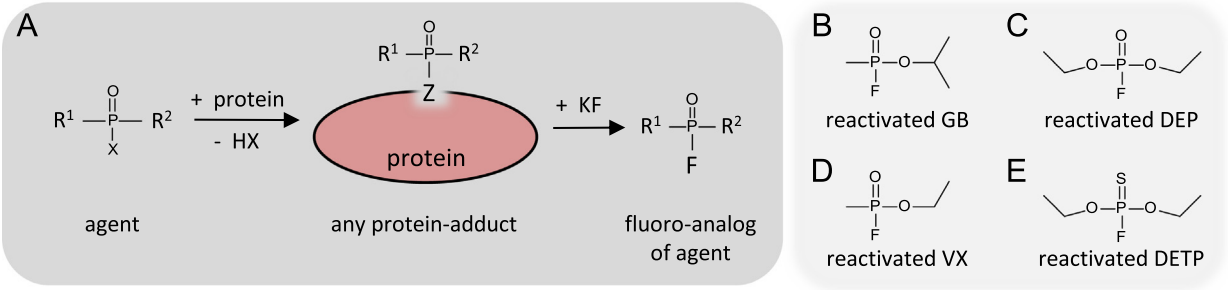

Fig. 6. Fluoride-induced reactivation for verification of poisoning by OP agents. A: Any protein not restricted to BChE or AChE is phosphylated at any amino acid (Z) as a consequence of exposure to an OP agent. Incubation with highly concentrated potassium fluoride induces the release of the fluoridated phosphyl-moiety suitable for GC–MS detection. Fluoro-analogs obtained from proteimns phosphylated by B: sarin, C: a diethyl pesticide (DEP), D: VX and E: a diethyl thiono pesticide (DETP). R^1 and R^2 are organic substituents as illustrated in Fig. 1.

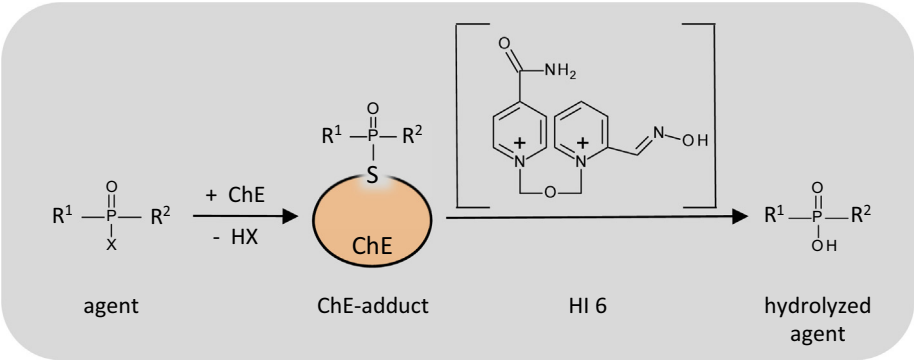

Fig. 7. Reaction scheme of HI 6-induced release of the hydrolyzed OP agent. HI 6 is a bispyridinium oxime currently under development as an antidote for the treatment of nerve agent poisoning. HI 6 reactivates the inhibited cholinesterases by nucleophilic substitution of the O-bound serine residue thus releasing the hydrolysis product of the agent. This biomarker can be detected either by GC- or LC-MS. R^1 and R^2 are organic substituents as illustrated in Fig. 1.

markers for HSA-adduct detection, especially in *in vivo* samples, has not been shown.

All procedures mentioned above for protein-adduct detection target phosphylated amino acid side chains. Detection of such phosphylations demonstrates the incorporation of an anthropogenic OP poison that acts as an AChE inhibitor. However, evidence of these adducts does not entirely allow the identification of the incorporated toxicant, as the identity of the leaving group remains unclear (Figs. 3, 5–7). Therefore, our working group has focused on additional methods that target protein-adducts of the leaving-group, especially of the V-type nerve agents (Fig. 1) [34–36] and some OP-pesticides, e.g. oxy-demeton-S-methyl (Fig. 1) [37,38]. We found that these thiol-containing leaving-groups produce a new class of protein-adducts that were introduced as disulfide-adducts (Fig. 5C,E) [34–36,38]. The free thiol group of the leaving group may be attached to the free thiol-group of the cysteine residue Cys^{34} in HSA. Following proteolysis with pronase, the dipeptide disulfide-adduct of Cys-Pro, C(X)P is produced (Fig. 5C,E). Together, with the phosphylated Y^* residue, it is now possible to deduce the original poison entirely as both the phosphyl-moiety, as well as the leaving group can be identified. This procedure was implemented by multiple designated laboratories within the course of the latest bioproficiency test (5th BioPT) in 2020 to prove exposure to Chinese VX.

Meanwhile, corresponding disulfide-adducts were also found to be produced in ceruloplasmin [71]. Fu *et al.* reported on four disulfide-adducts formed between neat human ceruloplasmin and V-type nerve agents *in vitro* [70]. Peptides containing the leaving group of the agent bound to the side chain of cysteine residues were obtained after trypsin-mediated cleavage, and detected by nano-LC coupled to a high-resolving Orbitrap system. Ceruloplasmin is present in plasma in concentrations as high as 300 μg/ml and exhibits a half-life of 5–6 days. However, whether these adducts may be suitable for the verification of an *in vivo* exposure scenario has not yet been shown.

As outlined above, there is growing interest in the scientific community regarding the benefit of long-lived protein-adducts for post-exposure analysis, not only for chemical warfare agents. Successful detection of such adducts requires elaborate technical procedures and modern instruments that allow trace analysis. A few of these aspects will be discussed in the next section.

4. Instrumental and methodological progress

Technical progress, in terms of instrumentation and procedures, has been achieved in the field of verification analysis. An important issue in plasma sample analysis is transport of the biological fluid from the sample draw site to the laboratory where the analysis will be performed. Such samples are typically categorized as infectious substances (hazard class 6, division 6.2) according to the International Air Transport Association (IATA) dangerous goods regulations (DGR) for transport by air [78]. These specimens are considered to potentially contain pathogens that do not cause life threatening effects (category B, shipping name UN 3373). The transport follows strict regulations with respect to sample packages requiring a primary receptacle, secondary packaging and an outer package. While shipment of liquid and frozen samples demands the aforementioned extensive efforts, these efforts can be avoided by sending dried blood and plasma samples. Dried material is considered non-infectious. Therefore, procedures and devices for the shipment of dried blood and plasma spotted onto filter paper were introduced that allow protein-adduct analysis [64,79–81]. The dried material showed excellent stability and the solid support did not cause any interference, thus highlighting an important alternative for simplified sample transport, storage and analysis.

To enhance throughput, 96-well plates were introduced [27,50,51]. This allows simultaneous preparation of a large number of plasma and serum samples when using IMS technology [50,51], or, alternately, of urine samples for SPE of hydrolysis products [27]. This is expected to be an important advantage in mass casualty scenarios, such as terroristic or military attacks. To further reduce the work-up time, columns containing immobilized enzyme, required for protein-adduct proteolysis, were introduced (immobilized enzyme reactor, IMER) [82]. These devices allow reduction of the incubation period with pepsin, as is often applied for conventional enzymatic cleavage, from 90 to 120 min [6,48,49] to about 20 min [82].

An approach for automation of sample preparation for BChE-adduct analysis was introduced by Bonichon *et al.* [83]. On-line immunoextraction using columns packed with sepharose, labelled with anti-BChE antibodies, were on-line coupled to IMER prior to LC-ESI MS/MS analysis, thus avoiding manual steps and increasing sample throughput [82,83]. Following this procedure, extraction and proteolysis of BChE took only 40 min [83].

To dramatically reduce the run time for LC-ESI MS/MS SRM analysis, Graham *et al.* introduced a UHPLC method that allows detection and quantification of five aged nonapeptide-adducts within only 3 min [84].

To improve the mass spectrometric response of GB generated by fluoride-induced reactivation, Blanca *et al.* presented a derivatization procedure using 2-[(dimethylamino)methyl]phenol to yield its corresponding phosphonylated derivative that was analyzed by LC-ESI MS/MS [85]. In comparison to GC–MS methods typically used for the measurement of reactivated GB, this novel procedure

turned out to be advantageous with respect to sensitivity, specificity and run time.

To improve the quality and reliability of protein-adduct analysis by MS/MS detection of their peptide biomarkers, high-resolving mass spectrometers may be used instead of conventional triple quadrupole systems for SRM analysis.

In addition, more generic mass spectrometric scan modes may be applied to monitor phosphylated nonapeptide-adducts. Approaches that use data-dependent scan mode may allow detection of unknown adducted nonapeptides [86]. Accordingly, any precursor ion detected in a predefined mass range is fragmented and all product ions are monitored. For data interpretation, the entire LC-MS/MS run is examined to identify any precursor ion that generated the diagnostic product ions of the nonapeptide-adduct at about *m*/*z* 778.3, *m*/*z* 673.3 and *m*/*z* 602.3 (Fig. 4, Section 2.4.1) [86]. This method does not require the targeted analysis of an SRM mode, which in principle limits the number of analytes to those previously known. Mathews *et al.* reported on such an approach, investigating a set of 96 samples for method validation resulting in neither false positive nor false negative identifications [86]. Therefore, the use of high-resolving instruments equipped with mass analyzers, like an Orbitrap or a time-of-flight (TOF) system, may be highly beneficial.

Even though the qualitative evidence of a biomarker for nerve agent poisoning is absolutely sufficient and unambiguous, to prove the incorporation of a poison, some approaches were introduced that also enabled quantification of the biomarkers. A few examples should be mentioned, representing a larger number of published methods. Swaim *et al.* reported on the determination of nerve agent hydrolysis products (Fig. 2, Section 2.2) in urine by LC-MS/MS [27]. Lin *et al.* applied isotope-dilution GC–MS/MS to quantify the derivatized hydrolysis products in the same matrix [25], whereas Rodin *et al.* simply used a "dilute-and-shoot" procedure for these analytes [28]. Salamo *et al.* quantified the fluoro-analog of VX from plasma samples after fluoride-induced reactivation by GC-HRMS (Fig. 6, Section 2.4.4) [71], and van der Meer *et al.* quantified IMPA (Fig. 2B) in serum samples from Japanese victims of the Tokyo GB-attack in 1995 [7]. However, without knowing the time of exposure and the route of administration, such quantitative data are of limited value and hardly suited to determine the dose of incorporated poison.

Finally, a highly interesting and innovative approach to detect nerve agent exposure should be presented that was originally introduced by Rubin *et al.* [87]. The authors succeeded in the LC-ESI MS/MS SRM-based detection of the hydrolyzed nerve agent VX (EMPA, ethyl methyl phosphonic acid, Fig. 2C) in the bones of mini-pigs after intramuscular and percutaneous challenge with VX *in vivo* [87]. The femoral cortical bone, not containing marrow or any other tissue, was prepared postmortem 24 h after exposure. The pulverized osseous tissue was found to be a pharmacokinetic compartment that is highly beneficial for forensic or toxicological analysis. The limits of detection were comparable to other biological matrices, such as plasma and urine. However, the application of these methods to the human bone tissue will be an interesting and important future effort.

5. Quality criteria of verification according to OPCW guidelines

For verification under the authority of the OPCW, samples are collected by an authorized inspection or fact finding team composed of inspectors of the OPCW. These politically independent persons have to carefully document sample draws and fulfill the requirements of chain of custody. Accordingly, the time, place and operator of the sample draw must be documented and traceable, specimens must be numbered individually, packed, sealed and compiled in a list, shipped to, and registered at, the OPCW laboratory, and further transported to designated laboratories of CWC member states for analysis.

Acquisition of bioanalytical data for verification analysis, according to the guidelines defined by the OPCW, requires strict compliance of high quality criteria and consideration of clear regulations for its reporting [30]. Some essential aspects should be mentioned here to exemplify the high standards and prerequisites required for forensic analysis of chemical warfare agent exposure.

In principle, verification of any agent demands the detection of two different biomarkers in one sample. Biomarkers can be categorized as primary or secondary. Primary biomarkers document that the agent underwent a chemical reaction with endogenous proteins, in contrast to simple hydrolysis products that are no longer able to phosphylate a protein. Well-accepted primary biomarkers for nerve agent exposure in plasma comprise adducted peptides or adducted amino acids derived from any aged or non-aged protein-adduct (Section 2.4), as well as nerve agent analogs obtained by fluoride-induced reactivation (Section 2.4.4). Corresponding secondary biomarkers represent either free hydrolysis products directly present in plasma, e.g. organophosphonic acids and organophosphoric acids (Section 2.2), or the same molecules obtained after their chemical release from proteins by the oxime HI-6 (Section 2.4.4). The primary biomarkers must be identified by an information-rich analytical technique, for example LC-MS/MS or GC–MS/MS, in contrast to non-MS techniques or simple SIM approaches for monitoring non-fragmented analytes.

The identification of a biomarker in a sample requires an analytical behavior identical to that of a reference. This reference can be generated by spiking the suspected agent of interest into the relevant biological blank matrix, e.g. plasma, thus producing known protein-adducts or hydrolysis products under controlled laboratory conditions based on current scientific knowledge. Consistency must be documented with respect to the chromatographic retention time (±0.2 min in LC and ± 0.1 min in GC) and the relative peak area ratios (ion ratios) deduced from the extracted ion chromatograms of fragments or product ions of the biomarker resulting from the mass spectrometric fragmentation in the ion source or in the collision chamber. Each peak must be detected with a signal-to-noise ratio of at least 5. The peak area corresponding to the most intense product ion (e.g., the ion at *m*/*z* 673.3 in Fig. 4) is set to 100% and areas derived from additional qualifying product ions (e.g., ions at *m*/*z* 778.4 and *m*/*z* 602.3 in Fig. 4) are relative to this. Depending on the value of the relative peak area ratio of the reference, certain tolerance intervals are defined that the sample results must fit. If they do not fit, the respective traces may not be used for identification. For example, the illustrated detection of the VX-derived nonapeptide-adduct (Fig. 4D) yields the following ion ratios and allowed tolerance intervals: *m*/*z* 778.4 / *m*/*z* 673.3, 86% (tolerance 68.8%-103.2%) and *m*/*z* 602.3 / *m*/*z* 673.3, 59% (tolerance 47.2%-70.8%). Consideration of the ion ratios provides an effective tool for identifying interferences in the sample and, thus, helps avoid false positive interpretations.

In addition, it must be shown that the analysis of a blank matrix, which does not contain the biomarker, is free from interference that could hamper selective, sensitive and unambiguous detection. The absence of any interference should be shown by an analytical run prior to each sample analysis.

Depending on the technique used for biomarker detection, a certain number of identification points is scored. The higher the selectivity of the method, the more identification pointsawarded. A total of at least five points derived from not more than three different methods is required for the identification of each biomarker. Accordingly, the detection of two product ions using a high-resolution mass spectrometer (allowed mass deviation 2.5 ppm) yields four points. Three points are awarded for detection of two

product ions monitored in the SRM mode of a triple quadrupole system and only two points are given when analyzing in a simple LC-MS mode without fragmentation. In comparison, the use of non-MS techniques provides only one point.

In summary, the work protocol for analysis and reporting of biomedical samples ensures maximum quality and requires maximum reproducibility for qualitative analysis, thus providing data of highest confidence and reliability, which can have far reaching political and legal relevance.

6. Conclusions

The methods referred to herein represent either established and internationally accepted approaches that were successfully applied in the past to verify poisoning by OP nerve agents [1,2,6,7], or potential alternatives and extensions that might be beneficial in future investigations. The different groups of biomarkers are of common and general relevance for known and well-characterized agents, but are also adaptable to unknown agents. Even though, in most cases, a poisoning agent is identified, using individual biomarkers that are unambiguously detectable by selective masses of the precursor and product ions, modern mass spectrometric scan modes could also enable a more generic search to capture any protein modifications due to the exposure to any unknown structure. Adducts of OP agents with BChE, which will surely be produced *in vivo* in the course of acute poisoning, are highly valuable for verification due to the common fragmentation pathway of the adducted nonapeptide as outlined in Section 2.4.1. Further future technical improvement of mass spectrometers and accompanying software tools with high-resolution and sensitivity, as well as scan modes and data-processing, will allow more generic approaches for forensic analysis, which will be valuable additions to the toolbox recommended by the OPCW.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] H. John, M. van der Schans, M. Koller, H.E.T. Spruit, F. Worek, H. Thiermann, D. Noort, Fatal sarin poisoning in Syria 2013: forensic verification within an international laboratory network, Forensic. Toxicol. 36 (2018) (2013) 61–71, https://doi.org/10.1007/s11419-017-0376-7.

[2] J.B. Tucker, Chemical/biological terrorism: coping with a new threat, Politics Life Sci. 15 (2) (1996) 167–183.

[3] T. Nakagawa, A.T. Tu, Murders with VX: Aum Shinrikyo in Japan and the assassination of Kim Jong-Nam in Malaysia, Forensic Toxicol. 36 (2) (2018) 542–544, https://doi.org/10.1007/s11419-018-0426-9.

[4] Statement by H.E. Ambassador Kenneth D. ward permanent representative of the United States of America to the OPCW in response to the director-generaĺs presentation of the report of the OPCW investigation and identification team for Syria. https://www.opcw.org/sites/default/files/documents/2020/04/IIT%20Report%20USA%20National%20Statement%2004082020.pdf (accessed 23 October 2020). The Organisation for the Prohibition of Chemical Weapons (OPCW). The Hague. The Netherlands.

[5] H. Tsuchihashi, M. Katagi, M. Nishikawa, M. Tatsuno, Identification of metabolites of nerve agent VX in serum collected from a victim, J. Anal. Toxicol. 22 (5) (1998) 383–388.

[6] A. Fidder, A.G. Hulst, D. Noort, R. de Ruiter, M.J. van der Schans, H.P. Benschop, J.P. Langenberg, Retrospective detection of exposure to organophosphorus anti-cholinesterases: mass spectrometric analysis of phosphylated human butyrylcholinesterase, Chem. Res. Toxicol. 15 (2002) 582–590.

[7] J.A. van der Meer, H.C. Trap, D. Noort, M.J. van der Schans, Comprehensive gas chromatography with time-of-flight MS and large volume introduction for the detection of fluoride-induced regenerated nerve agent in biological samples, J. Chromatogr. B 878 (2010) 1320–1325.

[8] T.C.C. Franca, D.A.S. Kitagawa, S.F. de A. Cavalcante, J.A.V. da Silva, E. Nepovimova, K. Kuca, Novichoks: the dangerous fourth generation of chemical weapons, Int. J. Mol. Sci. 20 (2019) 1222. doi: 10.3390/ijms20051222.

[9] J.A. Vale, T.C. Marrs, R.L. Maynard, Novichok: a murderous nerve agent attack in the UK, Clin. Toxicol. 56 (2018) 1093–1097, https://doi.org/10.1080/15563650.2018.1469759.

[10] Summary of the report on activities carried out in support of a request for technical assistance by the United Kingdom of Great Britain and Northern Ireland (Technical Assistance Visit TAV/02/18) S/1612/2018. https://www.opcw.org/sites/default/files/documents/S_series/2018/en/s-1612-2018_e___1_.pdf. (accessed 23 October 2020). The Organisation for the Prohibition of Chemical Weapons (OPCW). The Hague. The Netherlands.

[11] R. Stone, How German military scientists likely identified the nerve agent used to attack Alexei Navalny, Science. https://www.sciencemag.org/news/2020/09/how-german-military-scientists-likely-identified-nerve-agent-used-attack-alexei-navalny, https://doi:10.1126/science.abe6561.

[12] Summary of the report on activities carried out in support of a request for technical assistance by Germany (Technical Assistance Visit – TAV/01/20) S/1906/2020. https://www.opcw.org/sites/default/files/documents/2020/10/s-1906-2020%28e%29.pdf. (accessed 23 October 2020). The Organisation for the Prohibition of Chemical Weapons (OPCW). The Hague. The Netherlands.

[13] Chemical Weapons Convention: Convention on the prohibition of the development, production, stockpiling and use of chemical weapons and on their destruction. (2005) https://www.opcw.org/sites/default/files/documents/CWC/CWC_en.pdf. (accessed 23 October 2020). The Organisation for the Prohibition of Chemical Weapons (OPCW). The Hague. The Netherlands.

[14] Organisation for the Prohibition of Chemical Weapons. www.opcw.org, 2020 (accessed 23 October 2020).

[15] H. John, F. Balszuweit, D. Steinritz, K. Kehe, F. Worek, H. Thiermann, Toxicokinetic aspects of nerve agents and vesicants, in: R. Gupta (Ed.), Handbook of Toxicology of Chemical Warfare Agents, thirrd ed., Elsevier Inc./Academic Press, San Diego, 2020, pp. 875–929.

[16] R.M. Black, R.W. Read, Biological markers of exposure to organophosphorus nerve agents, Arch. Toxicol. 87 (3) (2013) 421–437, https://doi.org/10.1007/s00204-012-1005-1.

[17] D. Steinritz, E. Striepling, K.D. Rudolf, C. Schröder-Kraft, K. Püschel, A. Hullard-Pulstinger, M. Koller, H. Thiermann, F. Gandor, M. Gawlik, H. John, Medical documentation, bioanalytical evidence of an accidental human exposure to sulfur mustard and general therapy recommendations, Toxicol. Lett. 244 (2016) 112–120, https://doi.org/10.1016/j.toxlet.2015.08.1105.

[18] J. Therkorn, D.G. Drewry III, O. Tiburzi, M. Astatke, C. Young, K. Rainwater-Lovett, Review of biomarkers and analytical methods for organophosphate pesticides and applicability to nerve agents, Mil. Med. 185 (2020) e414–e421, https://doi.org/10.1093/milmed/usz441.

[19] H. John, F. Worek, H. Thiermann, LC-MS based procedures for monitoring of toxic OP compounds and the verification of pesticide and nerve agent poisoning, Anal. Bioanal. Chem. 391 (2008) 97–116, https://doi.org/10.1007/s00216-008-1925-z.

[20] H. John, H. Thiermann, Relevance of bioanalytical methods for the medical chemical defense – a review, Challenge 1 (2012) 9–13.

[21] H. John, M. Eddleston, R.E. Clutton, F. Worek, H. Thiermann, Simultaneous quantification of the organophosphorus pesticides dimethoate and omethoate in porcine plasma and urine by LC-ESI MS/MS and flow-injection ESI-MS/MS, J. Chromatogr. B 878 (17-18) (2010) 1234–1245, https://doi.org/10.1016/j.jchromb.2010.01.003.

[22] O. Tenberken, J. Mikler, I. Hill, K. Weatherby, H. Thiermann, F. Worek, G. Reiter, Toxicokinetics of tabun enantiomers in anaesthetized swine after intravenous tabun administration, Toxicol. Lett. 198 (2) (2010) 177–181.

[23] G. Reiter, J. Mikler, I. Hill, Chromatographic resolution, characterization and quantification of VX enantiomers in hemolyzed swine blood samples, J. Chromatogr. B 873 (2008) 86–94.

[24] G. Reiter, M. Koller, H. Thiermann, F. Dorandeu, J. Mikler, F. Worek, Development and application of procedures for the highly sensitive quantification of cyclosarin enantiomers in hemolysed swine blood samples, J. Chromatogr. B 859 (1) (2007) 9–15.

[25] Y. Lin, J. Chen, L. Yan, L. Guo, B. Wu, C. Li, J. Feng, Q. Liu, J. Xie, Determination of nerve agent metabolites in human urine by isotope-dilution gas chromatography-tandem mass spectrometry after solid phase supported derivatization, Anal. Bioanal. Chem. 406 (21) (2014) 5213–5220, https://doi.org/10.1007/s00216-014-7695-x.

[26] S.-Åke. Fredriksson, L.-G. Hammarström, L. Henriksson, H.-Åke. Lakso, Trace determination of alkyl methylphosphonic acids in environmental and biological samples using gas chromatography/negative-ion chemical ionization mass spectrometry and tandem mass spectrometry, J. Mass Spectrom. 30 (8) (1995) 1133–1143.

[27] L.L. Swaim, R.C. Johnson, Y. Zhou, C. Sandlin, J.R. Barr, Quantification of organophosphorus nerve agent metabolites using a reduced-volume, high-throughput sample processing format and liquid chromatography–tandem mass spectrometry, J. Anal. Toxicol. 32 (2008) 774–777.

[28] I. Rodin, A. Braun, A. Stavrianidi, T. Baygildiev, O. Shpigun, D. Oreshkin, I. Rybalchenko, "Dilute-and-shoot" RSLC–MS-MS method for fast detection of nerve and vesicant chemical warfare agent metabolites in urine, J. Anal. Toxicol. 39 (2015) 69–74, https://doi.org/10.1093/jat/bku119.

[29] E.I. Hamelin, N.D. Schulze, R.L. Shaner, R.M. Coleman, R.J. Lawrence, B.S. Crow, E.M. Jakubowski, R.C. Johnson, Quantitation of five organophosphorus nerve agent metabolites in serum using hydrophilic interaction liquid chromatography and tandem mass spectrometry, Anal. Bioanal. Chem. 406 (2014) 5195–5202. Doi: 10.1007/s00216-014-7702-2.

[30] Work instruction for the reporting of the results of the OPCW biomedical proficiency tests. QDOC/LAB/WI/BioPT04. Issue 1, Revision 4, 6 January 2020. The Organisation for the Prohibition of Chemical Weapons (OPCW). The Hague. The Netherlands.
[31] Report of the OPCW fact-finding mission in Syria regarding an alleged incident in Khan Shaykhun, Syrian Arab Republic April 2017 (S/1510/2017). https://www.opcw.org/sites/default/files/documents/Fact_Finding_Mission/s-1510-2017_e_.pdf. (accessed 23 October 2020). The Organisation for the Prohibition of Chemical Weapons (OPCW). The Hague. The Netherlands.
[32] Status update of the OPCW fact-finding mission in Syria regarding a reported incident in Khan Shaykhun, 4 April 2017 (S/1497/2017). https://www.opcw.org/sites/default/files/documents/Fact_Finding_Mission/s-1497-2017_e_.pdf. (accessed 23 October 2020). The Organisation for the Prohibition of Chemical Weapons (OPCW). The Hague. The Netherlands.
[33] D.L. Sudakin, D.L. Stone, Dialkyl phosphates as biomarkers of organophosphates: the current divide between epidemiology and clinical toxicology, Clin. Toxicol. 49 (2011) 771–781.
[34] A. Kranawetvogl, F. Worek, H. Thiermann, H. John, Modification of human serum albumin by the nerve agent VX: microbore liquid chromatography/electrospray ionization high-resolution time-of-flight tandem mass spectrometry method for detection of phosphonylated tyrosine and novel cysteine containing disulfide adducts, Rapid Commun. Mass Spectrom. 30 (19) (2016) 2191–2200, https://doi.org/10.1002/rcm.7707.
[35] A. Kranawetvogl, J. Küppers, M. Gütschow, F. Worek, H. Thiermann, P.W. Elsinghorst, H. John, Identification of novel disulfide adducts between the thiol containing leaving group of the nerve agent VX and cysteine containing tripeptides derived from human serum albumin, Drug Test. Anal. 9 (8) (2017) 1192–1203, https://doi.org/10.1002/dta.v9.810.1002/dta.2144.
[36] A. Kranawetvogl, J. Küppers, M. Siegert, M. Gütschow, F. Worek, H. Thiermann, P.W. Elsinghorst, H. John, Bioanalytical verification of V-type nerve agent exposure: simultaneous detection of phosphonylated tyrosines and cysteine-containing disulfide-adducts derived from human albumin, Anal. Bioanal. Chem. 410 (5) (2018) 1463–1474, https://doi.org/10.1007/s00216-017-0787-7.
[37] A. Kranawetvogl, H. Thiermann, M. Siegert, F. Eyer, H. John, Verification of organophosphorus pesticide poisoning: Detection of phosphorylated tyrosines and a cysteine-proline disulfide-adduct from human serum albumin after intoxication with dimethoate/omethoate, Toxicol. Lett. 299 (2018) 11–20, https://doi.org/10.1016/j.toxlet.2018.08.013.
[38] H. John, M. Siegert, F. Eyer, F. Worek, H. Thiermann, A. Kranawetvogl, Novel cysteine- and albumin-adduct biomarkers to prove human poisoning with the pesticide oxydemeton-S-methyl, Toxicol. Lett. 294 (2018) 122–134, https://doi.org/10.1016/j.toxlet.2018.05.023.
[39] M. Eddleston, R.E. Clutton, M. Taylor, A. Thompson, F. Worek, H. John, H. Thiermann, C. Scott, Efficacy of an organophosphorus hydrolase enzyme (OpdA) in human serum and minipig models of organophosphorus insecticide poisoning, Clin. Toxicol. 58 (5) (2020) 397–405, https://doi.org/10.1080/15563650.2019.1655149.
[40] T. Wille, K. Neumaier, M. Koller, C. Ehinger, N. Aggarwal, Y. Ashani, M. Goldsmith, J.L. Sussman, D.S. Tawfik, H. Thiermann, F. Worek, Single treatment of VX poisoned guinea pigs with the phosphotriesterase mutant C23AL: intraosseous versus intravenous injection, Toxicol. Lett. 258 (2016) 198–206, https://doi.org/10.1016/j.toxlet.2016.07.004.
[41] M. Goldsmith, Y. Ashani, Catalytic bioscavengers as countermeasures against organophosphate nerve agents, Chem. Biol. Interact. 292 (2018) 50–64, https://doi.org/10.1016/j.cbi.2018.07.006.
[42] A.N. Bigley, F.M. Raushel, The evolution of phosphotriesterase for decontamination and detoxification of organophosphorus chemical warfare agents, Chem. Biol. Interact. 308 (2019) 80–88, https://doi.org/10.1016/j.cbi.2019.05.023.
[43] A. Richardt, M.M. Blum, Hydrolytic enzymes for chemical warfare agent decontamination, in: A. Richardt, M.M. Blum (Eds.), Decontamination of Warfare Agents, Wiley-VCH, Weinheim, 2008, pp. 135–162.
[44] C. Schmidt, F. Breyer, M.-M. Blum, H. Thiermann, F. Worek, H. John, V-type nerve agents phosphonylate ubiquitin at biologically relevant lysine residues and induce intramolecular cyclization by an isopeptide bond, Anal. Bioanal. Chem. 406 (21) (2014) 5171–5185, https://doi.org/10.1007/s00216-014-7706-y.
[45] F. Fu, R. Gao, R. Zhang, P. Zhao, X. Lu, L. Li, H. Wang, C. Pei, Verification of soman-related nerve agents via detection of phosphonylated adducts from rabbit albumin in vitro and in vivo, Arch. Toxicol. 93 (7) (2019) 1853–1863, https://doi.org/10.1007/s00204-019-02485-8.
[46] H. Grigoryan, B. Li, W. Xue, M. Grigoryan, L.M. Schopfer, O. Lockridge, Mass spectral characterization of organophosphate-labeled lysine in peptides, Anal. Biochem. 394 (1) (2009) 92–100, https://doi.org/10.1016/j.ab.2009.07.008.
[47] E.S. Peeples, L.M. Schopfer, E.G. Duysen, R. Spaulding, T. Voelker, C.M. Thompson, O. Lockridge, Albumin, a new biomarker of organophosphorus toxicant exposure, identified by mass spectrometry, Toxicol. Sci. 83 (2005) 303–312, https://doi.org/10.1093/toxsci/kfi023.
[48] J.L.S. Sporty, S.W. Lemire, E.M. Jakubowski, J.A. Renner, R.A. Evans, R.F. Williams, J.G. Schmidt, M.J. van der Schans, D. Noort, R.C. Johnson, Immunomagnetic separation and quantification of butyrylcholinesterase nerve agent adducts in human serum, Anal. Chem. 82 (2010) 6593–6600. doi: 10.1021/ac101024z.
[49] H. John, F. Breyer, C. Schmidt, B. Mizaikoff, F. Worek, H. Thiermann, Small scale purification of butyrylcholinesterase from human plasma and implementation of a μLC-UV/ESI MS/MS method to detect its organophosphorus adducts, Drug Test. Anal. 7 (2015) 947–956, https://doi.org/10.1002/dta.1792.
[50] J.S. Knaack, Y. Zhou, C.W. Abney, J.T. Jacob, S.M. Prezioso, K. Hardy, S.W. Lemire, J. Thomas, R.C. Johnson, A high-throughput diagnostic method for measuring human exposure to organophosphorus nerve agents, Anal. Chem. 84 (21) (2012) 9470–9477, https://doi.org/10.1021/ac302301w.
[51] B.G. Pantazides, C.M. Watson, M.D. Carter, B.S. Crow, J.W. Perez, T.A. Blake, J.D. Thomas, R.C. Johnson, An enhanced butyrylcholinesterase method to measure organophosphorus nerve agent exposure in humans, Anal. Bioanal. Chem. 406 (21) (2014) 5187–5194, https://doi.org/10.1007/s00216-014-7718-7.
[52] J. Grunwald, D. Marcus, Y. Papier, L. Raveh, Z. Pittel, Y. Ashani, Large-scale purification and long-term stability of human butyrylcholinesterase: a potential bioscavenger drug, J. Biochem. Biophys. Methods 34 (2) (1997) 123–135.
[53] C.C. Liu, G.L. Huang, H.L. Xi, S.L. Liu, J.Q. Liu, H.L. Yu, S.K. Zhou, L.H. Liang, L. Yuan, Simultaneous quantification of soman and VX adducts tobutyrylcholinesterase, their aged methylphosphonic acid adduct andbutyrylcholinesterase in plasma using an off-columnprocainamide-gel separation method combined with UHPLC–MS/MS, J. Chromatogr. B 1036 (2016) 57–65, https://doi.org/10.1016/j.jchromb.2016.09.044.
[54] M.J. van der Schans, A. Fidder, D. van Oeveren, A.G. Hulst, D. Noort, Verification of exposure to cholinesterase inhibitors: generic detection of OPCW schedule 1 nerve agent adducts to human butyrylcholinesterase, J. Anal. Toxicol. 32 (2008) 125–130.
[55] S. Onder, E. David, O. Tacal, L.M. Schopfer, O. Lockridge, Hupresin retains binding capacity for butyrylcholinesterase and acetylcholinesterase after sanitation with sodium hydroxide, Frontiers Pharmacol. 8 (2017) 713, https://doi.org/10.3389/fphar.2017.00713.
[56] S. Onder, L.M. Schopfer, J.R. Cashman, O. Tacal, R.C. Johnson, T.A. Blake, O. Lockridge, Use of hupresin to capture red blood cell acetylcholinesterase for detection of soman exposure, Anal. Chem. 90 (1) (2018) 974–979, https://doi.org/10.1021/acs.analchem.7b04160.
[57] J.Y. Lee, C. Kim, Y.H. Lee, Simultaneous time-concentration analysis of soman and VX adducts to butyrylcholinesterase and albumin by LC–MS-MS, J. Anal. Toxicol. 42 (2018) 293–299, https://doi.org/10.1093/jat/bkx066.
[58] A.J. Dafferner, L.M. Schopfer, G. Xiao, J.R. Cashman, U. Yerramalla, R.C. Johnson, T.A. Blake, O. Lockridge, Immunopurification of acetylcholinesterase from red blood cells for detection of nerve agent exposure, Chem. Res. Toxicol. 30 (10) (2017) 1897–1910, https://doi.org/10.1021/acs.chemrestox.7b00209.
[59] J.M. McGuire, J.T. Taylor, C.E. Byers, E.M. Jakubowski, S.A. Thomson, Determination of VX-G analogue in red blood cells via gas chromatography-tandem mass spectrometry following an accidental exposure to VX, J. Anal. Toxicol. 32 (2008) 73–77.
[60] R.M. Black, J.M. Harrison, R.W. Read, The interaction of sarin and soman with plasma proteins: the identification of a novel phosphonylation site, Arch. Toxicol. 73 (1999) 123–126.
[61] H. John, F. Breyer, Jörg.O. Thumfart, H. Höchstetter, H. Thiermann, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for detection and identification of albumin phosphylation by organophosphorus pesticides and G- and V-type nerve agents, Anal. Bioanal. Chem. 398 (6) (2010) 2677–2691, https://doi.org/10.1007/s00216-010-4076-y.
[62] L.M. Schopfer, O. Lockridge, Mass spectrometry identifies isopeptide cross-links promoted by diethylphosphorylated lysine in proteins treated with chlorpyrifos oxon, Chem. Res. Toxicol. 32 (4) (2019) 762–772, https://doi.org/10.1021/acs.chemrestox.9b0000110.1021/acs.chemrestox.9b00001.s001.
[63] Y. Bao, Q. Liu, J. Chen, Y. Lin, B. Wu, J. Xie, Quantification of nerve agent adducts with albumin in rat plasma using liquid chromatography–isotope dilution tandem mass spectrometry, J. Chromatogr. A 1229 (2012) 164–171, https://doi.org/10.1016/j.chroma.2012.01.032.
[64] E.I. Hamelin, T.A. Blake, J.W. Perez, B.S. Crow, R.L. Shaner, R.M. Coleman, R.C. Johnson, Bridging the gap between sample collection and laboratory analysis: using dried blood spots to identify human exposure to chemical agents, Proc. SPIE 9863 (2016) 98630P, https://doi.org/10.1117/12.2223796.
[65] H. John, M. Koller, F. Worek, H. Thiermann, M. Siegert, Forensic evidence of sulfur mustard exposure in real cases of human poisoning by detection of diverse albumin-derived protein-adducts, Arch. Toxicol. 93 (7) (2019) 1881–1891, https://doi.org/10.1007/s00204-019-02461-2.
[66] J. von der Wellen, P. Winterhalter, M. Siegert, F. Eyer, H. Thiermann, H. John, A toolbox for liquid chromatography-tandem-mass spectrometry analysis of albumin-adducts as biomarkers of organophosphorus pesticide poisoning, Toxicol. Lett. 292 (2018) 46–54, https://doi.org/10.1016/j.toxlet.2018.04.025.
[67] M.J. van der Schans, A.G. Hulst, D. van der Riet – van Oeveren, D. Noort, H.P. Benschop, C. Dishovsky, New tools in diagnosis and biomonitoring of intoxications with organophosphorothioates: case studies with chlorpyrifos and diazinon, Chem. Biol. Interact. 203 (1) (2013) 96–102, https://doi.org/10.1016/j.cbi.2012.10.014.
[68] B. Li, F. Nachon, M.-T. Froment, L. Verdier, J.-C. Debouzy, B. Brasme, E. Gillon, L. M. Schopfer, O. Lockridge, P. Masson, Binding and hydrolysis of soman by human serum albumin, Chem. Res. Toxicol. 21 (2) (2008) 421–431, https://doi.org/10.1021/tx700339m.
[69] D. Noort, A.G. Hulst, A. van Zuylen, E. van Rijssel, M.J. van der Schans, Covalent binding of organophosphorothioates to albumin: a new perspective for OP-pesticide biomonitoring?, Arch. Toxicol. 83 (11) (2009) 1031–1036, https://doi.org/10.1007/s00204-009-0456-5.

[70] F. Fu, J. Chen, P. Zhao, X. Lu, R. Gao, D. Chen, H. Liu, H. Wang, C. Pei, Tracing and attribution of V-type nerve agents in human exposure by strategy of assessing the phosphonylated and disulfide adducts on ceruloplasmin, Toxicology 430 (2020) 152346, https://doi.org/10.1016/j.tox.2019.152346.
[71] K. Tsuge, Y. Seto, Analysis of organophosphorus compound adducts of serine proteases by liquid chromatography–tandem mass spectrometry, J. Chromatogr. B 776 (1) (2002) 79–88.
[72] M.I. Solano, J.D. Thomas, J.T. Taylor, J.M. McGuire, E.M. Jakubowski, S.A. Thomson, V.L. Maggio, K.E. Holland, J.R. Smith, B. Capacio, A.R. Woolfitt, D.L. Ashley, J.R. Barr, Quantification of nerve agent VX-butyrylcholinesterase adduct biomarker from an accidental exposure, J. Anal. Toxicol. 32 (2008) 68–72.
[73] C.E.A.M. Degenhardt, K. Pleijsier, M.J. van der Schans, J.P. Langenberg, K.E. Preston, M.I. Solano, V.L. Maggio, J.R. Barr, Improvements of the fluoride reactivation method for the verification of nerve agent exposure, J. Anal. Toxicol. 28 (5) (2004) 364–371.
[74] M. Polhuijs, J.P. Langenberg, H.P. Benschop, New method for retrospective detection of exposure to organophosphorus anticholinesterases: application to alleged sarin victims of Japanese terrorists, Toxicol. Appl. Pharmacol. 146 (1997) 156–161.
[75] N. Aurbek, H. Thiermann, F. Eyer, P. Eyer, F. Worek, Suitability of human butyrylcholinesterase as therapeutic marker and pseudo catalytic scavenger in organophosphate poisoning: a kinetic analysis, Toxicology 259 (3) (2009) 133–139, https://doi.org/10.1016/j.tox.2009.02.014.
[76] H. John, M. Eddleston, R.E. Clutton, F. Worek, H. Thiermann, Quantification of pralidoxime (2-PAM) in urine by ion pair chromatography-diode array detection: application to in vivo samples from minipig, Drug Test. Anal. 4 (2012) 169–178, https://doi.org/10.1002/dta.327.
[77] C. Becker, F. Worek, H. John, Chromatographic analysis of toxic phosphylated oximes (POX): a brief overview, Drug Test. Anal. 2 (10) (2010) 460–468, https://doi.org/10.1002/dta.167.
[78] International Air Transport Association (IATA). Dangerous goods regulation, 57th ed.; IATA: Montreal, Canada, 2016.
[79] J.W. Perez, B.G. Pantazides, C.M. Watson, J.D. Thomas, T.A. Blake, R.C. Johnson, Enhanced stability of blood matrices using a dried sample spot assay to measure human butyrylcholinesterase activity and nerve agent adducts, Anal. Chem. 87 (11) (2015) 5723–5729, https://doi.org/10.1021/acs.analchem.5b00893.
[80] L. Yishai Aviram, M. Magen, S. Chapman, A. Neufeld Cohen, S. Lazar, S. Dagan, Dry blood spot sample collection for post-exposure monitoring of chemical warfare agents – in vivo determination of phosphonic acids using LC-MS/MS, J. Chromatogr. B 1093-1094 (2018) 60–65, https://doi.org/10.1016/j.jchromb.2018.06.035.
[81] H. John, S. Willoh, P. Hörmann, M. Siegert, A. Vondran, H. Thiermann, Procedures for analysis of dried plasma using microsampling devices to detect sulfur mustard albumin adducts for verification of poisoning, Anal. Chem. 88 (2016) 8787–8794, https://doi.org/10.1021/acs.analchem.6b02199.
[82] M. Bonichon, A. Combès, C. Desoubries, A. Bossée, V. Pichon, Development of immunosorbents coupled on-line to immobilized pepsin reactor and micro liquid chromatography-tandem mass spectrometry for analysis of butyrylcholinesterase in human plasma, J. Chromatogr. A 1526 (2017) 70–81, https://doi.org/10.1016/j.chroma.2017.10.033.
[83] M. Bonichon, V. Valbi, A. Combès, C. Desoubries, A. Bossée, V. Pichon, Online coupling of immunoextraction, digestion, and microliquid chromatography-tandem mass spectrometry for the analysis of sarin and soman-butyrylcholinesterase adducts in human plasma, Anal. Bioanal. Chem. 410 (2018) 1039–1051, https://doi.org/10.1007/s00216-017-0640-z.
[84] L.A. Graham, D. Johnson, M.D. Carter, E.G. Stout, H.A. Erol, S.L. Isenberg, T.P. Mathews, J.D. Thomas, R.C. Johnson, A high-throughput UHPLC-MS/MS method for the quantification of five aged butyrylcholinesterase biomarkers from human exposure to organophosphorus nerve agents, Biomed. Chromatogr. 31 (2017), https://doi.org/10.1002/bmc.3830 e3830.
[85] M. Blanca, A. Shifrovitch, M. Madmon, M. Elgarisi, S. Dachir, S. Lazar, S. Baranes, I. Egoz, M. Avraham, H. Dekel Jaoui, S. Dagan, A. Weissberg, Retrospective determination of regenerated nerve agent sarin in human blood by liquid chromatography–mass spectrometry and in vivo implementation in rabbit, Arch. Toxicol. 94 (2020) 103–111, https://doi.org/10.1007/s00204-019-02622-3.
[86] T.P. Mathews, M.D. Carter, D. Johnson, S.L. Isenberg, L.A. Graham, J.D. Thomas, R.C. Johnson, High-confidence qualitative identification of organophosphorus nerve agent adducts to human butyrylcholinesterase, Anal. Chem. 89 (2017) 1955–1964, https://doi.org/10.1021/acs.analchem.6b04441.
[87] K.M. Rubin, B.A. Goldberger, T.J. Garrett, Detection of chemical weapon nerve agents in bone by liquid chromatography–mass spectrometry, J. Anal. Toxicol. 44 (2020) 391–401, https://doi.org/10.1093/jat/bkz118.