Toxichem Krimtech 2013;80(Special Issue):284

Determination of warfare agents (nerve agents, blister agents, saxitoxin and ricin) in food, water and on materials and articles

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Abstract

Different developed methods for determination of warfare agents allow the examination of water samples but also of complex matrices such as food, biological and environmental samples. The methods allow the estimation of toxicity of samples. Nerve agents are determined enzymatically on the inhibitory effect on acetylcholinesterase. Sulfur mustard can be determined photometrically after alkylation of a pyridine derivative. Lewisite is determined by gas chromatography after derivatization with ethanethiol. Ricin is determined immunologically by a lateral flow assay. The detection limit for all nerve agents in aqueous samples is 0.1 µg/l, for mustards 20 µg/l, for lewisite 50 µg/l. For ricin a detection limit of 50 µg/l can be achieved, with enrichment procedure a detection limit of 1 µg/l is possible.

1. Introduction

Nerve agents have a high acute oral and dermal toxicity as they irreversibly inhibit the action of acetylcholinesterase in the synaptic cleft, thus causing a cholinergic crisis. Their production requires sound knowledge in the field of organic synthesis. Small quantities of their individual components are commercially available. Sarin, soman and cyclosarin are mixed acid anhydrides of phosphonic acids and hydrofluoric acids. Sarin has low hydrolytic stability, while soman and cyclosarin remain stable for several days. VX is a phosphonothioic acid esterified with a tertiary alkanolamine. It also has a half-life of several days in water. All four nerve agents are organoleptically neutral in low concentrations.

Mustard gas and lewisite are highly toxic to skin and the mucous membranes including the intestinal mucosa. Again, sound knowledge in the field of organic synthesis is required for their production and small quantities of their individual components are commercially available. As sulfur mustard has low hydrolytic stability, it is unlikely to pose a threat, whereas nitrogen mustard remains stable for several days and lewisite forms stable, toxic hydrolysis products. Even in low concentrations, blister agents have a characteristic odour.
Ricin can easily be obtained from castor beans, even in larger quantities and has a very high pulmonary and oral toxicity. When administered orally, its toxicity is delayed for several hours and it cannot be detected organoleptically. Therefore it poses a severe threat. Saxitoxin, which is naturally produced by shellfish, is not available in significant quantities and cannot be synthesized. For this reason, it is unlikely to pose a threat (cf. [1]).

2. Material and Methods

2.1. Sample Preparation, Reference Substances

Certified material of chemical warfare agents was available as reference. Therefor the laboratory has a handling permit in accordance with the Chemical Weapons Convention. For sample preparation we used organic solvents, buffers and laboratory equipment such as filters, centrifuges and homogenizers.

Tab. 1. Estimated toxicity of chemical warfare agents (cf. [2]).

	LD 50 (oral) mg/person	min. toxic dose (oral) mg/person	min. toxic dosis (derm.)
Sarin	11	0,28	300 mg/person
Soman	11	0,008	15 mg/person
VX	5	0,006	1,75 mg/person
Tabun	311	1,1	no data known
Lewisite	3750	9	14 µg/cm²
Sulfur Mustard	1400	1,1	28 µg/cm²
Ricin	70	1	not toxic

2.2. Nerve Agents

The cleavage of acetylthiocholine, which serves as a substrate for the acetylcholinesterase from the electric eel (*Electrophorus electricus*), leads to the production of thiocholine. The thiocholine and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) form an adduct from which the yellow 2-nitro-5-mercaptobenzoic acid is hydrolytically released [3]. The enzyme-catalysed cleavage of acetylthiocholine is restrained by nerve agents which are acetylcholinesterase inhibitors. Consequently, after the addition of acetylthiocholine, considerably less yellow dye was produced with DTNB. An additional color reaction exists with Tillmann's reagent. In the presence of thiocholine the reagent is decolorized whereas in the presence of nerve agents it remains blue. The rapid test thus appears blue in poisoned water and green-yellow in non-toxic water (Fig. 1-3). In the laboratory the quantitative determination is carried out kinetically through the rate of the substrate conversion.

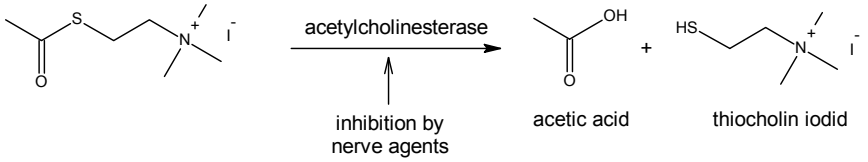


Fig. 1. Inhibition of the cleavage of acetylthiocholin by nerve agents.

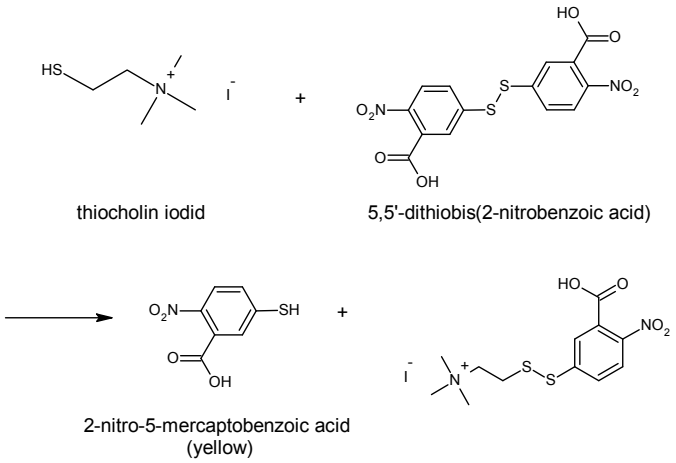


Fig. 2. Colour reaction of thiocholin with DTNB.

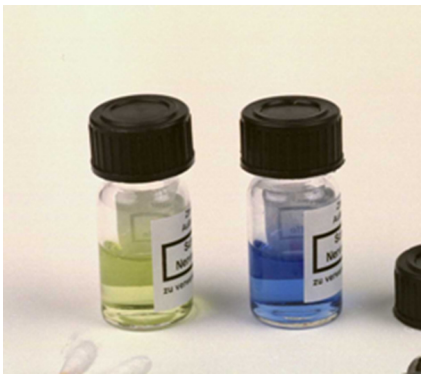


Fig. 3. Rapid test for nerve agents in water: yellow-green: negative; blue: positiv (detect. limit 4 µg/L).

2.3. Organic Arsenic Blister Agents (Lewisite)

Lewisite is poorly soluble in water and hydrolises rapidly, resulting in products which are highly water-soluble and still toxic. Lewisite and its hydrolysis products react with ethanethiol to a stable product, which can be determined by gas chromatography [4]. The rapid detection of lewisite and the hydrolisis products is only possible through determining arsenic by the Marsh test with a test strip in the gas phase, which shows a colour change to brown.

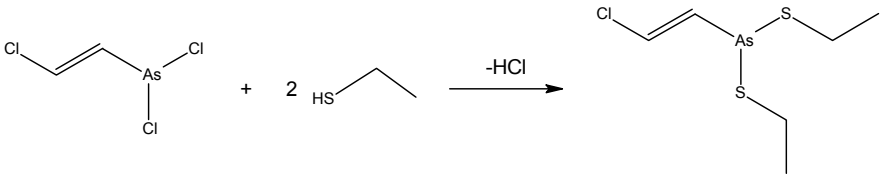
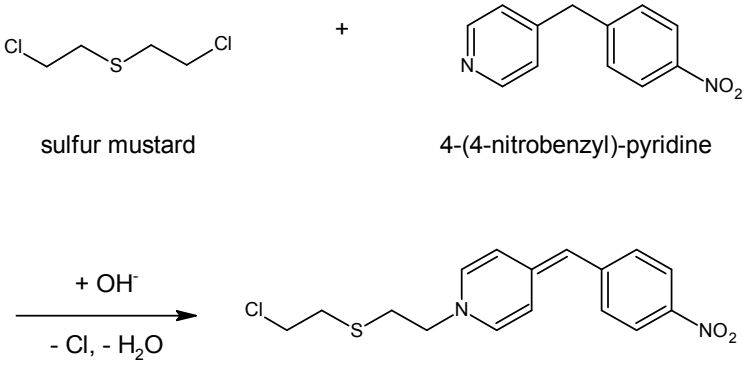


Fig. 4. Reaction of lewisite with ethanethiol.

2.4. Alkylating Blister Agents (Mustard Gas)

The colour reaction is caused by adding the toxic substance to the reagent 4-(4-nitrobenzyl)-pyridine and extraction of the product with toluene [5]. It can detect all toxic agents from this group and indicates only active toxic substances. The quantitative determination is carried out photometrically.



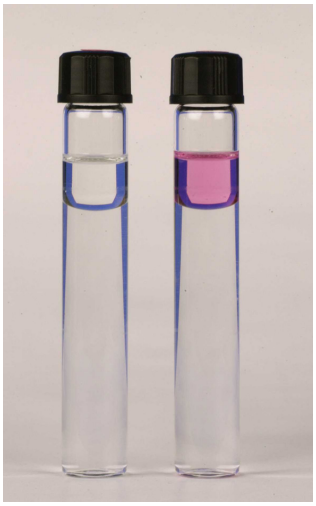
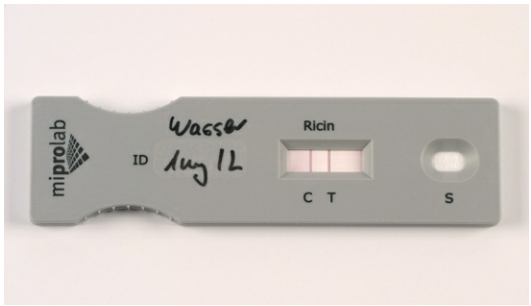


Fig. 5 and 6. Colour-reaction of mustard gas with 4-(4-nitrobenzyl)-pyridine.

2.5. Ricin



An available rapid immunoassay is based on the principle of the “pregnancy test”. The evaluation of the band intensity is performed with a densitometer.

Fig. 7. Ricin lateral flow assay carried out with water (1 mg/L ricin).

3. Results and Discussion

The developed methods allow the examination of water samples but also of complex matrices such as food, biological and environmental samples. The methods can be done without complex equipment and in a short time. The reached detection limit for all nerve agents in aqueous samples is 0.1 µg/ l, for mustards 20 µg/l, for lewisite 50 µg/l. For Ricin a detection limit of 50 µg/l can be achieved, with enrichment procedure performed in a laboratory environment, a detection limit of 1 µg/l is possible.

4. Conclusions

Methods were developed for the quantitative determination of chemical warfare agents in water, food and environmental samples that can be used in the investigation of crimes and terrorist attacks. The detection limits are significantly below the acute toxic doses. The procedures are part of the accredited methods according to DIN EN ISO 17025. In addition, rapid tests are available for the detection of chemical warfare agents without laboratory equipment.

5. References

[1] Russmann H. Toxine, Biogene Gifte und potenzielle Kampfstoffe. Bundesgesundheitsblatt - Gesundheitsforschung - Gesundheitsschutz 2003;46:989-996.

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[3] Ellmann GL, Courtney KD, Andres V jr, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961;7:88-95.

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[5] Epstein J, Rosenthal RW, Ess RJ. Use of γ-(4-nitrobenzyl) pyridine as analytical reagent for ethylenimines and alkylating agents. Anal Chem 1955;27(9):1435–1439.