EXTRACTION OF CHEMICAL WARFARE AGENT FROM SOIL: CASE STUDY ON O-ethyl S-2-(diisopropylamino) ethyl methylphosphonothiolate (VX)

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Abstract: Extraction of chemical warfare agents (CWA's), *O*-ethyl S-2-(diisopropylamino)ethyl methylphosphonothiolate (VX) in soil sample has been done. The extraction was performed using six different solvents at ambient and cold temperature. Percent recovery of VX in soil samples was quantitatively determined by mean of LC-Tandem MS using selected reaction monitoring (SRM). The degradation products of the VX were screened by using multiple reactions monitoring (MRM). The storing effect investigation has shown the ability to extract the VX in soil samples and the application of LC-MS was most useful to detect the VX after cold water extraction, but not for its degradation products.

Prohibition of the development, production, stockpiling and use of chemical weapon came into force on 29 April 1997 under the Chemical Weapons Convention (CWC). To achieve the objectives and purposes, the convention is administrated by the Organization for the Prohibition of Chemical Weapons (OPCW) based in The Hague, The Netherlands (Carrick *et al.,* 2001; Wils, 2005). Part of this process involves the verification of chemical warfare agents, their precursors or their degradation products in samples collected from suspected production or storage sites or from the environment in the cases of alleged uses (OPCW, 1997). Nevertheless, in part owing to inadequate battlefield sampling and identification, the verification of the CWA's is often difficult (Black & Read, 2005).

Soil has often been used as a sampling medium for verification of alleged use of CW agents and was used as a medium in an international inter-laboratory comparison (Round-robin) test for the verification of chemical disarmament coordinated by the VERIFIN (Rautio, 1990; Hootjsschur *et al.*, 2002). The determination of CWA's in soil samples depends on effectiveness, reliability, and simplicity of methods of extraction. A number of works related to the extraction of CW agents from soil have been reported (Verweij & Boter, 1976; Tornes *et al.*, 1991). However, those studies revealed unsatisfactory result mainly for soil contaminated with *o*-ethyl S-2-(diisopropylamino)ethyl methylphosphonothiolate (VX).

Gronewold *et al.* (1999) has reported that this phenomenon due to characteristic structure of VX (Figure 1) containing alkaline behavior from diisopropylaminoetyl moiety. This moiety is responsible for strong adsorbent character and is also contribute to the compound's reputation for being difficult to detect. The objective of this study, therefore, was to develop of the extraction method for identification of VX in soil sample.
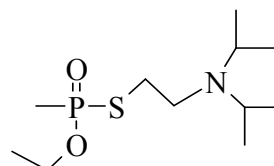

Figure 1.   Structure of VX

This report is based on the laboratory work focused on the extraction of VX in soil sample and identification of its degradation products. The ex-

traction of VX was carried out based on the Finnish Institute for Verification of Chemical Weapon Convention recommended operating procedure (VERIFIN ROP) (Rautio, 1994), and newly method developed during the laboratory work. The VX was spiked into the soil and then the spiked soil samples were extracted. LC-MS method was employed for quantitative analysis and the results of each method were compared.

The storing effect of spiked sample before analysis was evaluated. The spiked soil samples were stored at ambient and cold temperature for 2 and 4 weeks before analysis. The best method chosen during the laboratory work was used to extract the stored sample and the results were compared. The LC-MS was employed to screen the VX degradation products.

METHODS

Soil Sample

Fine sand soil was used as a soil matrix in the study. The soil was collected from the experimental farm of the Agricultural Research Center of Finland in Jokioinen. The soil was dried in air and sieved through a 2 mm screen to remove debris prior to use. The soil properties have been characterrized by the supplier as shown in Table 1.

Chemicals and Equipments

The chemicals and equipments used were provided by VERIFIN. The VX used was property of the VERIFIN laboratory and was an analytical reference standard. The VX was received and used as a 47.6 µg/mL (acetone) standard solution. *VX, CAS Number. 50782-69-9, is regulated and highly toxic compounds that must be handled in approved facilities with appropriate safety precaution to avoid risk to personnel.*

All other chemicals were purchased by VERIFIN from the commercial sources: aceton (Merck-Darmstadt, Germany), amoniumhydroxide (Merck-Dramstadt, Germany), triethylamine (Fluka chemie GmbH, Switzerland), methanol HPLC gradient grade (J.T Baker, Holland), silylation grade acetonitrile (Piere, USA), N,O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) (Pierce, USA), and formic acid (Merck-Darmstadt, Germany). Ultra high quality (UHQ) water was obtained from VERIFIN laboratory.

The equipment used included pH universal paper (Macherey-Nagel, Germany), screw-capped bottles 10 and 25 mL (Pyrex), universal centrifuge (Hettich, Germany), qualitative filter paper 90mm DIAx100 circle (Whatman), disposable plastic syringe equipped disposal filter PTFE millipore 0.45 Millex LCR (Millex, Irrish), Bond elut SCX cation exchanger (Varian, USA), sonicator Branson 3210 (Branson, Netherland), cooling system (Lauda, Wobser GmbH & Co, KG), and a rotary evaporator (Buchi Labortechnique, Switzerland).

Spiking Procedure

A soil sample (5 g find sand soil) was weighed into 25 mL screw-capped glass bottle and was spiked with VX at concentration level of 5 ppm. The standard solutions of VX were injected in soil that was shacked vigorously by hand and allowed to stand for 60 min at ambient temperature prior to extraction.

Extraction Procedure

Six different solvents were tested for the extraction including: 1% triethylamine in methanol (TEA/MeOH) [1], 1% ammoniumhydroxide in methanol (NH_4OH/MeOH) [2], water pH 2/ambient temperature [3], water pH 2/4 ^{o}C [4], water pH 7/ambient temperature [5], and water pH 7/4 ^{o}C [6].

Three spiked soil samples and a blank were extracted in parallel with 5 ml of each solvent using ultrasonic vibration for 10 min and then centrifuged for 3 min at 2000 g. The supernatants were quickly filtered through Whatman Number 4 filter paper to remove particulate matter. The extraction was performed twice and then supernatant was combined into 10 ml volumetric flask. The supernatant was filtered through disposal filter PTFE millipore 0.45 Millex LCR. The prepared extracts were stored in freezer at –20 ^{o}C prior to analysis. An amount of 10 µl of 25% NH_4OH was introduced in 1 mL of each water extract at pH 2 just before analysis for tuning extract solutions alkaline.

Storing Effect Evaluation

Sets of spiked soil sample were stored at ambient temperature for 2 and 4 weeks. The samples were extracted using the best method chosen during the laboratory work. Evaluation of storing effect was made by analyzing the recoveries of VX obtained after the different storing periods and the screening of some degradation products of VX was then performed.
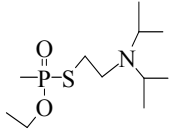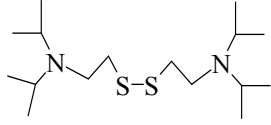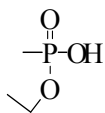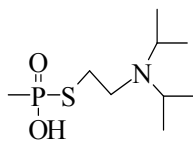
Instrumental

Analysis of samples were performed using the LC-MSMS applying selected reaction monitoring (SRM) for quantification of analyte and multiple reaction monitoring (MRM) for screening of degradation products.

Table 1.   Properties of Fine Sand Soil Used in This Study

Soil type	Humus (%)	Organic C (%)	pH (H_2O)	Mineral (mg/L)					
				Ca	K	Mg	P	Na	S
Fine sand	4.57	2.65	5.65	1565	123	60	31.7	42	23.6

Table 2.   Selected Reaction for Analysis of Different Chemicals

Chemical	Analysis	SRM/MRM	Dwell Time [s]
VX	Quantitation (APCI⁺)	m/z 268 > 128	0.5
Bis(diisopropylaminoethyl)disulfide	screening (AP-CI⁺)	m/z 321 > 128	0.2
Ethyl methylphosphonate	screening (AP-CI⁻)	m/z 123 > 95	0.2
S-2-diisopropylmetylphosphono thiolate	screening (AP-CI⁻)	m/z 238 > 160	0.2

Liquid Chromatography Conditions

A HP 1100 LC pump with a C_{18} reverse phased column (Waters XTerra™ MS; 150 x 4,6 mm, 5 µm) was applied for separation of the analytes by applying the isocratic elution using 80% of methanol (B eluent) of and 20% of 20 mM NH_4Ac/pH 8.6 (A eluent) as eluent components. A flow rate of 1 mL/min with an injection volume 20 µL, was applied. A gradient elution (5% (B) – 100% (B)/0 –5 min+5min isocratic) or isocratic elution (50%:50%; A:B) were used for screening of degradation products of VX.

Mass Spectrometric Conditions

A Micromass Quattro II triple stage quadrupole mass spectrometer with positive and negative APCI ionization modes was used. Cone voltage 20 V and corona voltages 3.5 kV and 2.0 kV in positive mode and negative modes, respectively, were applied. Source temperature was 150 ºC and probe temperature was 450 ºC. Nitrogen was used as APCI sheath gas and drying gas with flow rates of 75 L/h and 350 L/h, respectively. Argon was used as the collision gas with a pressure 1.0×10^{-3} mBar. Collision energies applied were 15 eV. Selected reactions (SRM, MRM) for different chemicals are collected in Table 2. A quasi-molecular ion of each analyte was used as parent ion and a most intense reaction was applied for analysis. One example reaction (m/z 268 > 128 for VX) is presented in Figure 2.
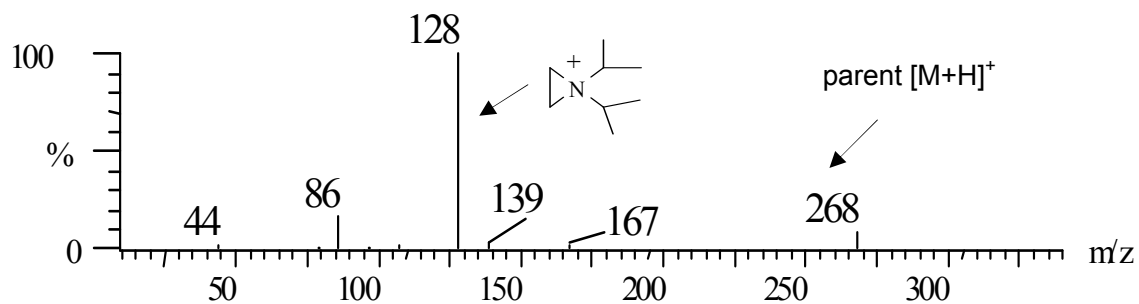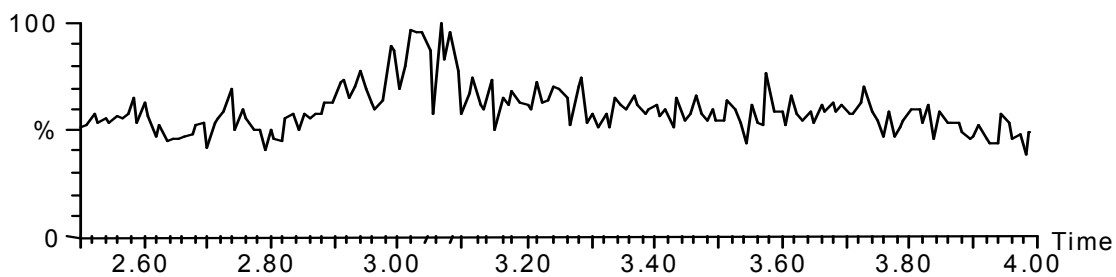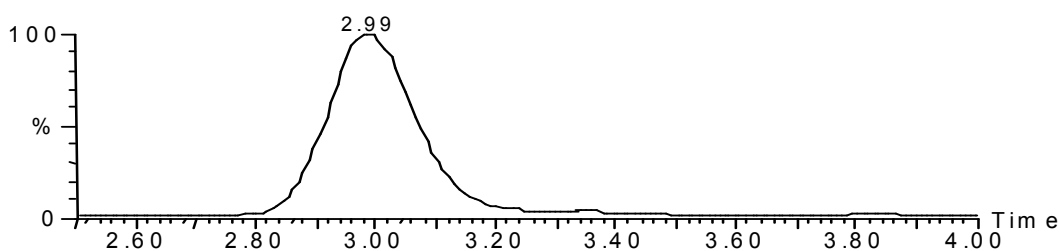
Figure 2. The LC-MSMS Spectrum of VX

Figure 3.A

Figure 3.B

Correlation coefficient: r = 0.999923, r^2 = 0.999846
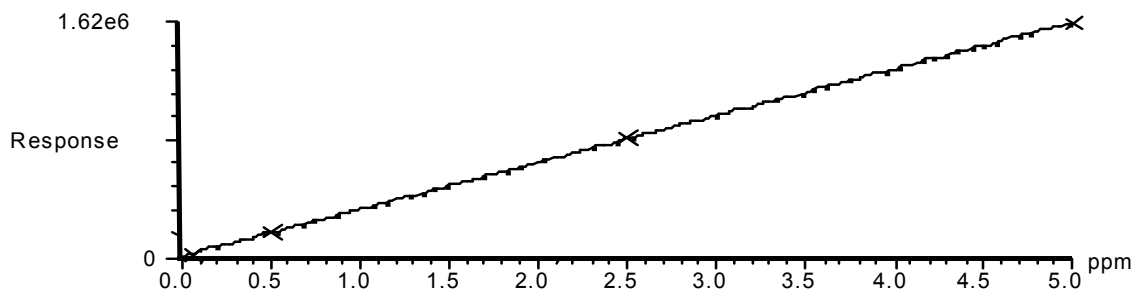Calibration curve: 321426 * x + 11009.4

Figure 3.C

Figure 3.   A Calibration of LC-MS Quantitation Method. SRM Chromatograms for Blank and 0.05 ppm of Standard, (A) and (B), Respectively and a Typical Calibration Curve (C).

RESULT AND DISCUSSION

Over the last decade, LC/MS has evolved from being primarily a research tool, available in number of analytical laboratories, to robust and widely available analytical and investigative technique (Dass, 1999; Niessen, 1999). At the time of my experimental work of this subject, few laboratories were using LC/MS in the analysis of chemical relevant to the CWC. This was due to a number of factors, two of which where the cost of instrumentation and a perception that the technique was difficult to implement in an analytical laboratory. In the intervening years, the cost of basic LC/MS have been reduced, and the instrumentation has become robust and easier to operate. LC/MS is now a mature technique that should be considered by all laboratories that are required to analyze chemicals related to CWC.

Calibration of Instrument

A set of quantification standards were obtained by spiking the analyte in each blank extract at concentration levels of 0.05, 1.0, 2.5, and 5 ppm corresponding recoveries 1, 20, 50, and 100%, respectively. A typical chromatogram for blank and a standard at 0.05 ppm and a calibration curve are presented in Figure 3.

Quantitative Analysis

CW agents in the environment are subject to hydrolysis and oxidation (Munro, *et al*., 1999). Both of these processes generally lead to more polar and less reactive molecules; example is the compound that was being investigated in this study. Although polar degradation products can be extracted from matrices such as soil with noaqueous polar solvent, water is often the solvent of choice and is the standard solvent included in the Recommended Operating Procedures for CWC-related analysis (Rautio, 1994).

The Technical Secretariat of the OPCW has provided rigid criteria for unambiguous identification of compound relevant to the CWC (OPCW, 2002). These are primarily aimed at the level used in OPCW Proficiency Tests, in which analytes are typically present at concentration from 5 to 50 ppm, and full scanning MS for identification. The reliable result of the quantitative analysis of chemical related to CWC depends essentially on the collection of good samples and well-planned, effective and reasonably simple preparation suitable to the method analysis. Proper preparation of samples requires a thorough understanding of the behavior of the various types of chemicals in different sample matrices, both before and after the sample preparation, and awareness of the limitation of the chosen instrumentation method of analysis.

The extraction of VX was investigated in this study from soil using two different solvents (methanol and water). First the effectiveness of the methanol extraction was compared using two different modifiers: 1% TEA and 1% NH_4OH. Then, the research was continued with water extractions which were performed in two different pH values (pH 7 and pH 2) and temperatures (ambient and + 4 ºC). The yields of VX from find sand soil matrix with different solvents are presented in Table 3.

From Table 3 can be seen that ammonium hydroxide gave the best yield (60%) that was 9% higher when the extraction is corresponded with the VERIFIN ROP method where TEA is used as the alkaline modifier of methanol. A recovery comparable to the VERIFIN ROP method was obtained using cold water (+4 ºC) as extraction solvent at pH 2.

When the temperature water was increased to ambient at pH2, the recovery of VX was decreased 12%. The same increase of temperature at pH 7 had almost no effect for efficiency of water extraction. The decrease of pH in ambeint temperature increased the recovery 17% and in +4 ºC the increase of recovery was 27%. Declaration of the effect of pH is obvious. The VX exists in lowered pH as the ionized form and is then more watersoluble than as the neutral form (see Figure 4).

Storing Effect Evaluation

Recovery of the analyte, when spiked samples were let to stand in ambient temperature during two or four weeks was investigated. The decrease of the yield of the analyte when spiked samples were let to stand in ambient temperature for two or four weeks before water extraction/pH 2 and 4ºC is presented in Table 4. The recovery decreased about 40% during the period of the first two weeks and an additional about 10% during the second two weeks.

Screening of Degradation Products

In the several OPCW-designated laboratories, LC/MS is used as the initial screening procedure for aqueous extracts of matrices such as soil. This usually provides a tentative identification of polar analytes within half a day, in the basis of molecular mass, any fragment ions present, and retention

time. A second analysis, under LC/MS/MS condition, usually provides a firmer identification of the basis of a limited number of product ions, most of which result from simple neutral ions. The screening of VX degradation products have been reported previously by some authors (Bjork, 2003; United State/Finland, 2002). Degradation products were screened in this work from samples which were stood at ambient temperature two or four weeks. Three degradation products including ethyl methylphosphonate, S-2-(diisopropylamino)ethyl methylphosphonothiolate, and bis[2-(diisopropylamino) ethyl] disulfide) were screened from samples stored two weeks using LC-MS method. The degree of degradation of VX during the period was also estimated. The screening results are presented in Figure 5. By corresponding the height of the signal of ethyl methylphosphonate obtained from a sample with that obtained from 1 ppm standard sample it was estimated that the portion of 1-1.5 ppm of VX had hydrolyzed during the first two weeks. S-2-(diisopropylamino) ethyl methylphosphonothiolate could not to be detected in any samples.

CONCLUSION

A new procedure for verification chemical warfare agents VX in soil based on cold water extraction (water pH 2 at 4 $^{\circ}$C) has been achieved. Cold water extraction of soil spiked with VX has been performed, with a result that is comparable with that of the VERIFIN ROP method (Rautio, 1994), (the recovery using cold water is even greater than 1% TEA/MeOH).

The storing effect investigation has shown the ability to extract the VX in soil samples after 4 weeks and the application of LC-MS was most useful to detect the VX in soil after cold water extraction, but not for its degradation products. Therefore, further investigation of VX degradation after cold water extraction is highly recommended.

Table 3. Results of Recoveries of VX Obtained by Different Extraction Methods

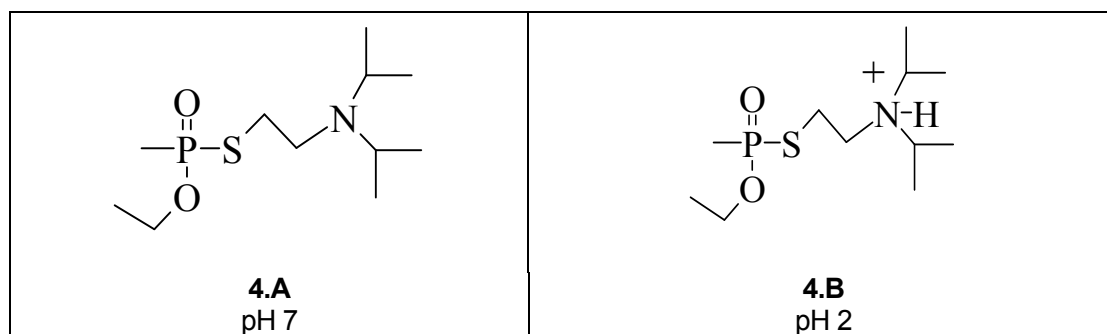| Solvent	pH	Temperature	Reg. coeff. (r^2)	Yield(%)
1% TEA/methanol (VERIFIN ROP method)	Alkaline	ambient	0.999	51 ± 4.8
1% NH$_4$OH/methanol	Alkaline	ambient	0.999	60 ± 2.5
Water	7	ambient	0.991	30 ± 1.2
Water	2	ambient	0.998	47 ± 1.9
Water	7	+ 4ºC	0.999	31 ± 1.9
Water	2	+ 4ºC	0.999	58 ± 2.6

4.A
pH 7

4.B
pH 2

Figure 4. Neutral (A) and Ionized (B) Structures of VX

Table 4. Effect of Storing for the Recovery of VX

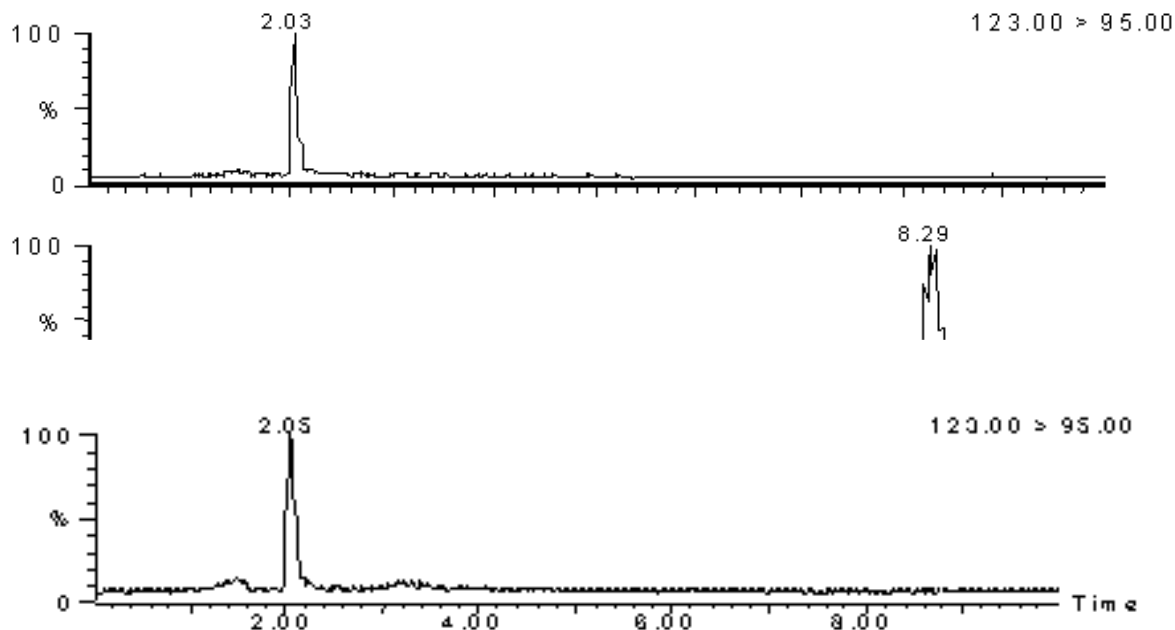| Storing time	Reg. Coefficient (r^2)	Yield (%)
One hour	0.999	58 ± 2.6
Two weeks	0.999	19 ± 5.0
Four weeks	0.989	12 ± 3.8

Figure 5. **LC-MS Screening Results of Degradation Products of VX. A SRM   Chromatogram of Ethyl Methylphosphonate (A) and a SRM Chromatogram of Bis(diisopropyl amino-ethyl)disulfide (B) Obtained from a Sample and at The Chromatogram Obtained from 1 ppm Standard Sample of Ethyl Methylphosphonate (C).**

REFERENCES

Bjork, H. 2003. *Screening and Characterization of O-alkyl S-2-dialkylaminoethyl alkylphosphono-thiolates Using LC-MS.* International Workshop on the Analysis of Chemicals Related to the Chemical Weapons Convention, September 8-9, VERIFIN, Helsinki University, Finland.

Black, R.M. & Read, R.W. 2005. *Chemical Weapons Convention Chemicals Analysis: Gas Chromatography/mass Spectrometry in Analysis of Chemicals Related to Chemical Weapons Convention.* Edit. Markku Mesilaakso. John Wiley & Sons. ISBN: 0-470-84756-5, p 284-315.

Carrick, W.A., Cooper, D.B. & Muir, B. 2001. Retrospective Identification of Chemical Warfare Agents by High Temperature Automatic Thermal Desorption-gas Chromatography-mass Spectrometry. *J. of Chromatography A.*, (925):241-249.

Dass, C. 1999. Recent Developments and Applications of High Performanceliquid Chromatography Electrospray Ionization Mass Spectrometry. *Curr. Org. Chem*, (3):193-209.

Gronewold, G.S., Appelhans, A.D., Gresham, G.L., Olson, J.E., Jeffery, J. & Wright, J.B. 1999. Analysis of VX on Soil Particles Using Ion Trap Secondary Mass Spectrometry. *J. of Anal. Chem.*, (71):2318-2323.

Hootjsschur, E.W.J., Hults, A., de Jong A.L., de Reuver, L.P, van Krimpen, S.H., van Baar, B.L.M., Wils, E.R.J., Kientz, C.E. & Brinkman, U.A. 2002. Identification of Chemical Related to the Chemical Weapons Convention During an Interlabora-tory Proficiency Test. *Trend in Analytical Chemistry*, (21):116-130.

Munro, N.B., Talmage, S.S., Griffin, G.D., Waters, L.C., Wtason, A.P., King, J.F. & Hauschild, V. 1999. The Source, Fate and Toxicity of Chemical Warfare Agent Degradation Products. *Environ. Health Perspect*, (107):933-974.

Niessen, W.M.A. 1999. Liquid Chromatography-mass Spectrometry, 2nd Edition. *Chromatographic Science Series*, Vol. 79. Marcel Dekker, New York.

OPCW. 1997. *Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on their Destruction.* The Hague, The Netherlands.

OPCW. 2002. *Work Instruction for the Evaluation of the Results of OPCW Proficiency Tests*, Quality System Document No.: QDOC/LAB/W1/PRO003, OPCW The Hague, 12 August.

Rautio, M. (Coordinator). 1990. *International Interlaboratory Comparison (Round-robin) Test for the Verification of Chemical Disarmament: F.1. Testing of existing procedures*, The Ministry of Foreign Affair of Finland, Helsinki, ISBN 951-47-3872-1.

Rautio, M. (Editor), 1994. *Recommended Operating Procedures for Sampling and Analysis in the Verification of Chemical Disarmament*, 1994 Edition, The Ministry of Foreign Affair of Finland, Helsinki, ISBN 951-724-008-2.

United State/Finland. 2002. *Sample Preparation Method for GC/MS Analysis on Sit*e. Joint document, Vol. 2 January 1998-December 2002.

Verweij, A. & Boter, H.L. 1976. Degradation of S-2-Di-isopropylaminoethyl O-Ethyl Methylphosphono-tioate in Soil: Phosphorus Containing Products. *J. of Pest. Sci.*, (7):355-362.

Wils, E.R.J. 2005. *Chemical Weapons Convention Chemicals Analysis: Gas Chromatography/mass Spectrometry in Analysis of Chemicals Related to Chemical Weapons Convention.* Edit. Markku Mesilaakso. John Wiley & Sons. ISBN: 0-470-8-4756-5, p 249-279.