Chemicals Identification Related To The Chemical Weapons Convention During The 15th Interlabory Proficiency Test

Sri Sumartini, Harry Budiman, Nuryatini & Puspa Dewi
Pusat Penelitian Kimia –Lembaga Ilmu Pengetahuan Indonesia

ABSTRACT

Identification of some chemical weapons in the water and organic sample has been carried out during 15th proficiency testing hold by OPCW (Organisation Prohibition of Chemical Weapon). Sample preparation method was adopted from Recommended Operational Procedure (ROP) from OPCW and Helsinki University, Finlandia. Prepared sample was identified by gas chromatography (FID and FPD), gas chromatography mass spectrometry (EI and CI mode) and Liquid chromatography mass spectrometry method.. From 7 spiking chemical weapons introduced to water and organic sample, 3 spiking chemicals could be identified (propylphosphonic acid, isoprophylphosphonic acid and 1,4 Bis 2-chloroethyl (thio) butane). One chemical was reported as Butyl S-2-diisopropylaminoethyl propylphosphonothiolate but obviously this is not a spiking chemical (category false positive identification).

Keywords: 15th Proficiency testing, OPCW, Chemical Weapon, Gas Chromatography, Gas Chromatography Mass Spectrometry, Liquid Chromatography Mass Spectrometry

INTRODUCTION

Chemical weapons and efforts at chemical disarmament are not solely modern phenomena. Since the ancient times, humankind has been developing chemical means of waging war; in each generation, substances are developed that are even more dangerous and deadly that what had preceded them. Luckily, efforts to control the proliferation of chemical weapons and outlaw their use have also been a feature of the international system for as long as chemical weapons have been a threat to humans, animals, and plant life.

On 3rd September 1992, the conference of disamarment in Genewa successful concurred the Convention on the Prohibition of Development, Production, Stockpiling, and Use of Chemical Weapons and on Their Destruction (Chemical Weapons Convention or CWC. The Chemical Weapons Convention opened for signature with a ceremony in Paris, in January 1993, and was deposited with the UN Secretary-General in New York. The CWC was the first disarmament agreement negotiated within a multilateral framework that provides for the elimination of an entire category of weapons of mass destruction under universally applied international control. Organization was established with the responsibility to prepare detailed operation procedures and to put into place the necessary infrastructure for the permanent implementing agency provided for in the Convention: the Organisation for the Prohibition of Chemical Weapons (OPCW). The Chemical Weapons Convention entered into force on 29 April 1997, 180 days after deposit of the 65th instrument of ratification, by Hungary. At this time, 87 countries had ratified the CWC and became original States Parties to the Convention. With entry into force, the OPCW was formally established. Indonesia had ratified the CWC on 12 Nopember 1998 (browsing 1997).

Accredited laboratory for verification chemical weapon compounds was nessecarily established in Indonesia as state party. The advantage and benefit of the existence of accredited laboratory for verification chemical weapon compounds are presented in Figure 1. The laboratorium of state party must fullfil the several requirements, if it could be accredited as laboratory of identification of chemical weapon by OPCW. Laboratory of state party must pass at least three times in a row with score A on Proficiency Test held by OPCW. Score A means that laboratory identified all of the liberately introduce (spiking) chemical used for scoring and reported them with sufficient analytical data.

OPCW had carried out seventeen times Proficiency test until now, which was held two times in a year. State Party must participate at least once per calendar year on OPCW Proficiency Test.

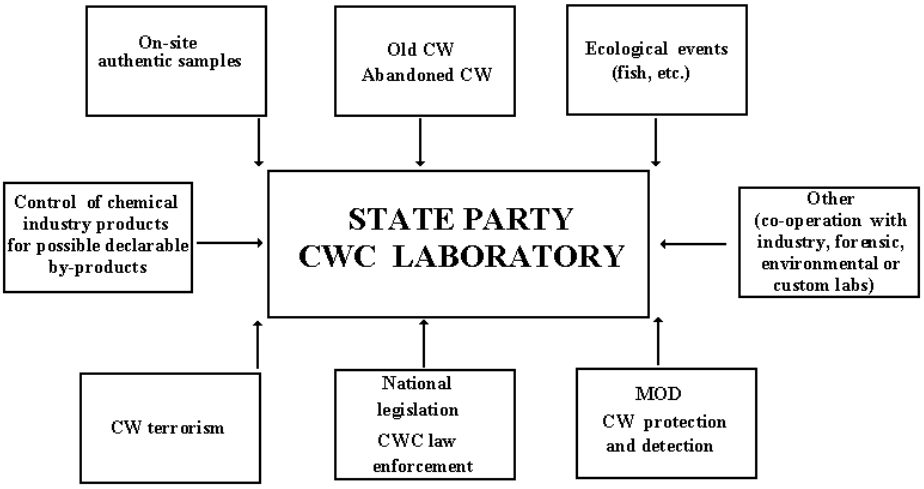


Figure 1. Scheme The benefit of Accredited Laboratory for Verification of Chemical Weapon

The requirements of this test are as follows: state party's laboratory must have at least three main analytical instruments to identified chemical weapon such as Gas Chromatography, Gas Chromatography Mass Spectrometry, Liquid Chromatography Mass Spectrometry, etc. List of all participating laboratory on First Proficiency Test until Seventeen Proficiency Test are presented in Table 1 (Straten *et al*. 2004)**.** Sample matrix such as water, soil, organic waste and paint is usually used in OPCW Proficiency Test.

Indonesia represented by Indonesia Institute of Sciences, Research Centre for Chemistry was starting to participate on fourthteen Proficiecy Test with the result F. On the Fifthteen Proficiency Test, the samples was prepared by the Laboratory of Analytical Chemistry, Research Institute of Chemical Defense, Cina and evaluated by Protechnic Laboratory Ltd South Africa. There were two kinds of samples matrix used on Fifthteen Proficiency Test such as organic waste and water waste. The spiking chemicals, deliberately introduce to the sample matrix, were chemical weapon compounds or its precursor clasified in Schedule List (Schedule 1, 2, 3). The aim of this study (OPCW Proficiency testing) is evaluating the ability and competency of Research Centre of Chemistry (LIPI) laboratory in analyzing and verificating chemical weapons and to seek acreditation laboratory in the field of chemical weapon.

METHODS

Procedure for OPCW proficiency test

The criteria for acceptable performance and conduct of the OPCW Proficiency Tests have been summarized in Table 2. The number of samples should be between two and four, and matrix blank must be supplied. The test samples should reflect the situation given in a scenario, typically a site inspection, with respect to the matrix and with a certain concentration range of the analytes (1-10 ppm or higher) (OPPC 1997). Finally, the sample material should be as homogeneous as possible and the spiking compounds should be stable throughout the duration of the test. Laboratories are given 15 days to hand in and report their results.

Analytical procedure

Sample preparation for organic sample was conducted after Straten et al. (2004). The 100 mg silica cartridge was prepared, and then it was flashed with 1 ml hexane. The 100 µL sample was Inserted and eluted with acetone. Ready to inject. The eluate was injected to GC-FPD, GCMS (with EI and CI mode) and also inject to LCMS.

Sample preparation for water sample

Preparation for water sample (W) was done by ROP (Recommended Operation Procedure) standardized by OPCW and VERIFIN, Universitas Helsinki, Finland. 1.0 or 2,0 µl was injected to GC FID, GC FPD, GCEIMS, GCCIMS dan LCMS.

Table 1. List of all participating laboratory on First Proficiency Test until Seventeen Proficiency Test

No	State Party	Proficiency Test															
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
1	Argentina	C	F	D	C	D	C					F					
2	Austria	F	F		F												D
3	Belgium	C	D	C	C		C	A	A	D		A	A	F	A		A
4	Brazil							F		F		F					
5	Bulgaria	F	F	F													
6	Burkina Faso														F		
7	Cina	A	A	A		A	C				A		A		A	A	
8	Republik Chech	C	F	A	A	A	C	B	A		B		B		D		B
9	Denmark	F		F												C	C
10	Finland	A	A	A	A		A	A			A	A	A		A		A
11	France	A	B	A		A	B	A			A	A		A			A
12	Germany	C	C	A	A	A	B	A		A		A	A	A		A	
13	Greek						F										
14	Hunggaria	F															D
15	India	F		F	C	A	C	B	F	D	B	C	B	A	A	A	A
16	**Indonesia**														F	F	
17	Iran	C	F	D	C	F	D	D	F	D	D	F	D	F	F		
18	Italy				F	D	F	F		F							
19	Israel	A	A	A			F										
20	Japan	F	F	B	F	A											
21	Kenya					F											
22	South Korea	A		A		A	C	A		B		A	A		A	A	
23	Malawi															F	
24	Malaysia																D
25	Netherland	B	A	A	B		A	A	A	B	A		A		A		B
26	Norweg	B	D	C													
27	Oman											F					
28	Poland	B	A	B	A		A		A		A		C	A		A	
29	Portugal	D		D	F										F		
30	Rumania	F		F	F	F	C		D	D	F	F		C	C	D	C
31	Rusia	A	F	A	C	A	B	A		F	C	F	B	F	A	A	
32	South Africa	F		F	F	B		F		F	F	A		B		A	
33	Singapore		F	F	B	A	C		A	F	A	A	A		A	A	
34	Spain					F	D				B	A	A		B		A
35	Sweden	A	A	A	F	A	B		A		A		C		A		A
36	Swiss	A	A	A	A		B	A			A		A		A	A	
37	Turkey											F	D	D	F	F	
38	UK	A	F	A	A	A	B					A	A		A		A
39	Ukraine		F	F							F	F	F	F	D	C	F
40	USA	A	A	A	A		A	A		B	A	A	B	A	A	A	A
41	Zimbabwe				F												
42	Maroco																F

Notes see next page

Notes (continue..) :
A: laboratory identified all chemicals (maximum score), **B**: : laboratory identified all chemicals except one (maximum score, minus two), **C** : laboratory identifies more than half of the chemical (score between zero and two), **D**: laboratory misses more chemicals than it identified (negatif score), **F** : fail (no score)

Table 2. Criteria for acceptable performance of laboratories in proficiency testing (OPCW 1997)

Performance criteria
a) Analysis of test samples and reporting of test results should be carried out within 15 calendar days, starting from the day the samples arrive at the particular participant laboratory site.
b) Identification of chemicals should be based on, at least, two different, preferably spectrometric, techniques (e.g. EI-MS, CI-MS, LC-MS, IR, NMR), with mutually consistent results.
c) All analytical data supporting the identifications (chromatographic and spectrometric data) must be annexed to the participant laboratory report.
d) The laboratory must indicate on which basis the chemicals have been identified (i.e. by comparison with data on standard chemicals, with data in analytical databases or by interpretation of spectra).
e) The participant laboratory must describe sample preparation and analytical methods in detail or make reference to accessible Recommended Operating Procedures (ROPs), or Standard Operating Procedures (SOPs) or to the validated procedures according to the quality assurance / quality control (QA/QC) regime of the laboratory. All deviations from the procedures will have to be described in detail.
f) The identified chemicals must be reported with sufficient structure information, including at least structural formula, CAS registry number (if available) and chemical name (preferably IUPAC or CA name), and the CWC Schedule number. If IUPAC or CA names are not available, a name from the structure can be derived should be included.
g) Only chemicals relevant to the aims of the test should be reported.
h) False positive results must not occur. Any chemical that is not contained in or that could not conceivably be formed in the sample matrix will constitute a false positive result. Reporting any false positive result will constitute failure of the Proficiency Test.

GC-FPD condition
Detector: FPD→ P mode. Detector temperature: 250 ^oC. Carrier gas (Helium) was adjusted at 1,1 ml/minute. Injection mode: Splitless. Injector temperature: 250 ^oC. Column phase; RTX5MS. Column length = 30m; ID= 0.25mm; Film Thickness 0.25µm. Column temperature programme; 40 ^oC (2 minute); 10 ^oC/minute, 280 ^oC (4 minutes).

GC MS condition
Carrier gas (Helium) flow rate: 1,1 ml/minute. Injection mode: Splitless. Injector temperature: 250 ^oC. Column phase: DB5MS. Column length = 30m; ID= 0,25mm; Film Thickness 0.25 µm. Column temperature programme: 40 ^oC(2 minute); 10 ^oC/minute, 280 ^oC (4 minutes). Solvent delay time: 4 minute. Electron energy 70 ev. Ionisation polarity: Positive. Scan range: 40-450 m/z, scan time; 0.46 s.

LCMS (ESI MS)
Sample introduction: Direct infusion. Injection volume: 10 µl. Elution: Isocratic. Eluent composition: Methanol: Water (70: 30) with 0.3 % Acetic acid. Flow rate: 0.1 ml/minute. Column phase: C18. Column Length × ID × Particle size: 150mm × 2 mm × 5 µm. Column temperature: ambient. Ionisation polarity: positive.

RESULTS AND DISCUSSION

Analysis of test samples and reporting of test results should be carried out within 15 calendar days, starting from the day the samples arrive at the particular participant laboratory site. After sample had been received from OPCW, all samples were put in the refrigerator on 4^oC to avoid the damage of sample.

The samples were prepared and analysed in accordance with the principles describe in the work instruction for the preparation of test samples for OPCW proficiency test so the analyse result should be accurate. The extract of samples were analysed and identified by GC-EIMS, GC-CIMS, GC-FID, GC-FPD dan LC-MS. The List of compound identified in organic waste (O) and water waste (W) were presented in Table 3.

It can be seen from Table 3, 6 compounds suspected as spiking chemicals was found in Organic sample (O) and 2 suspected compounds was found in water sample (W). In Table 3 , also can be seen that Rentention Index (RI) was got from GC FID experiment was compared to RI from OCAD data. EI GCMS spectra from experiment were compared to spectra from NIST Library

Database, OCAD, and AMDIS . While its molecular weight was found from GC- CI -MS dan LC-MS experiment is compared to references and OCAD.

The blanko spectra also made in this experiment and then plot in the same intensity with sample spectra. In this Proficiency Test, the chemicals have been identified was not compared to that authentic reference chemicals (spectra chromatogram) because laboratory is not have authentic reference chemicals and not able to synthesize it.

From table 3 it can be derived that all RI data have been got from the experiment is similar (the diferrent are between 1 to10) to OCAD data except for compound no.6 (the different is 20). Also from table 3 it can be derived that all base peak data got from EI spectra from the experiment is similar to OCAD data.. All molecular weight can not found in any kind of compound except for compound no. 6. The presentation molecular weight of compound no.6 is presented in Figure 5, 6 and 7. Figure 5 is LC chromatogram for O sample blank , Figure 6 is chromatogram for sample O and Figure 7 is Mass spetrum of peak with retention time 2,5 to 3,5 minutes got from LCMS experiment GC-EIMS spectra of compound no.6 (Retention time 20,6) if compare to data base (NIST and AMDIS) and blanko spectra can be seen in Figure no 2 , 3, 4, 4a and 4b. From all data presented in Table 3, Butyl S-2-diisopropylaminoethyl propylphosphonothiolate (Compound no.6) was considered as one of the spiking chemical and was reported to OPCW.

List of Spiking Chemicals compound introduced to organic solution (O) and water waste (W) during 15th Proficiency Testing compared to identification result have been done by Research Centre for Chemistry (LIPI), were presented in Table 4 and Table 5. From Table 4 and 5 it can be seen that a number of spiking chemicals are 7 (three are in organic samples and 2 are in the water sample).

The result of Research Centre for Chemistry (LIPI)'s Laboratory was categorized false positif on the fifthteen Proficiency Test by OPCW because Research Centre for Chemistry (LIPI)'s reported Butyl S-2-diisopropylaminoethyl propylphosphonothiolate (Compound no 6). Butyl S-2-diisopropylaminoethyl propylphosphonothiolate was neither spiked by the laboratory preparing the samples, nor was it found by other laboratories, including evaluating laboratory. Reporting of this compound was considered to be false positive identification. This false positif identification is appear because was not ignoring the a different of RI number Butyl S-2-diisopropylaminoethyl propylphosphonothiolate. Obviously the compound is not Butyl S-2-diisopropylaminoethyl propylphosphonothiolate but Isobutyl S-2-diisopropylaminoethylpropylphosphonothiolate 1,4-Bis(2-chloroethyl (thio)-n-butane, Propylphosphonic acid and isopropylphosphonic acid were spiking chemicals introduced to water and organic sample. These chemicals can be found in this experiment but could not reported because of the supporting analytical data is in sufficient (molecular weight data).

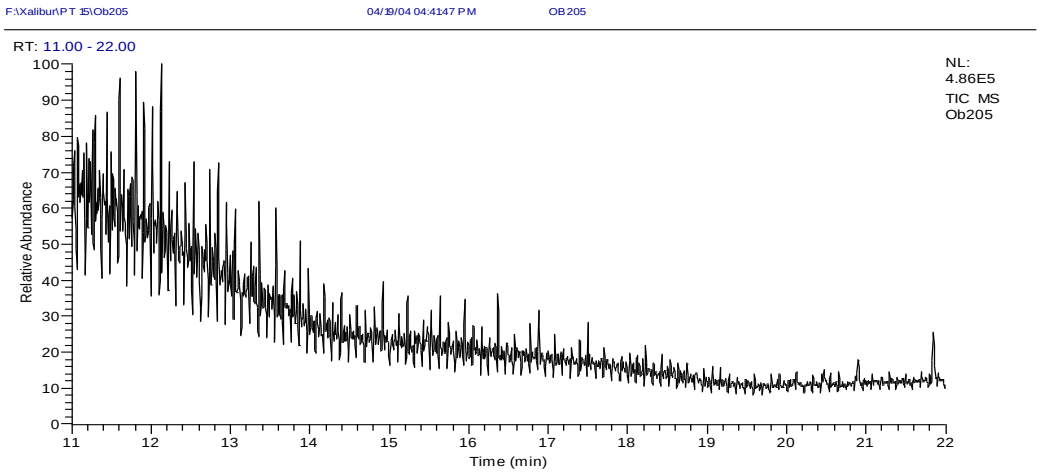


Figure. 2. EI TIC of organic waste blank

Table 3. Result of Organic (O1, O2) and waste water (W) identification by GC, GC MS and LCMS.before the 7 spiking chemicals were commenced to the participant.

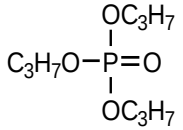
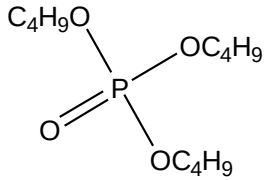
Sample code	No. of compound	Retention time GC-FPD (min)	Retention time GC-MS (min)	Base Peak by experiment / Base peak OCAD	Retention Index by experiment / Retention Index OCAD	Molecular weight from experiment (LC-MS)	Molecular weight	Name of chemicals sugested	Structure	Note
O1	1	14,257	13,86	99 / 99	1378 / -	224	224	Triiso propylphosphate		Was not reported, non schedule chemicals
O1	2	17,726	17,18 17,15	99 / 99	1646 / 1655	266	266	Tributyl phosphate		Was not reported, reference chemicals

Table 3. (continued)

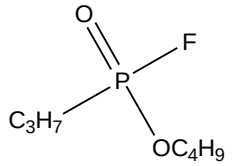
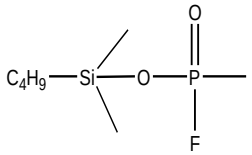
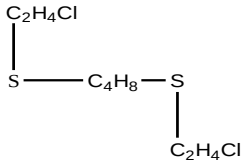
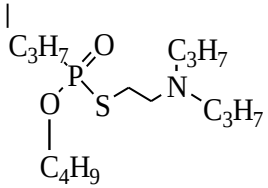
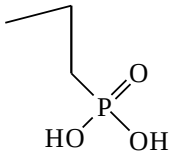
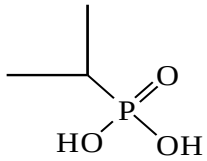
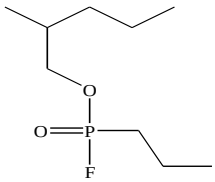
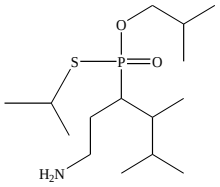
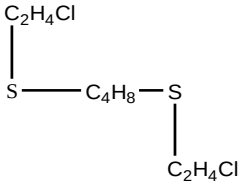
Sample code	No. of com-pound	Retention time GC-FPD (min)	Retention time GC-MS (min)	Base Peak by experiment / Base peak OCAD	Retention Index by experiment / Retention Index OCAD	Molecular weight from experiment (LC-MS)	Molecular weight	Name of chemicals sugested	Structure	Note
O2	3	12,91	12,655	127,1 / 127	1291 / 1111	Can not found	182,89	Isobutyl propylphosphonofluoridate		Was not reported (no MW data)
O2	4	TD	16,66	155 / 155	1600,09 / -	Can not found	212	Tertbuthyldimethylsilyl methyl phosphono Fluoridate		Was not reported (no MW data)
O2	5	TD	20,20	183,1 / 183	1927,8 / 1933	Can not found	246,01	1,4 Bis 2-chloroethyl (tio)butane		Was not reported (no MW data)
O2	6	21,073 21,033	20,561	114,1 / 114	1962 / 1982	323	323	Butyl S-2 diisopropylaminoethylpropylphosphonothiolate	Bhacument.5.0 	Was reported to OPCW, schedule 1.A.03

Table 3. (continued)

Sample code	No. of compound	Retention time GC-FPD (min)	Retention time GC-MS (min)	Base Peak by experiment	Retention Index by experiment	Molecular weight from experiment (LC-MS)	Molecular weight	Name of chemicals sugested	Structure	Note
				Base peak OCAD	Base peak OCAD					
W	7	12,25	12,01	253,1	1250	Could not found	248	Propyl phosphonic acid		Was not reported (no MW data)
				253	1255 1258					
W	8	TD	16,66	253,1	1278	Could not found	248	Isopropyl phosphonic acid		Was not reported (no MW data)
				253	1288 1284					

Note: OCAD : OPCW Central Analytical Database

Table 4. List of Spiking Chemicals compound introduced to organic solution (O) during 15th Proficiency Testing compared to identification result have been done by Resesearh Centre of Chemistry (LIPI).

Sample code	No. of Compoud	Compound name	Structure	Note
O2	A	2-methylpentyl propylphosp honofluoridate		Not found
O2	B	Isobutyl S-2-diisopropylamino Ethylpropyl phosphono thiolate		Not found
O2	C	1,4-Bis(2-chloroethyl thio)-n-butane		Found but not reported

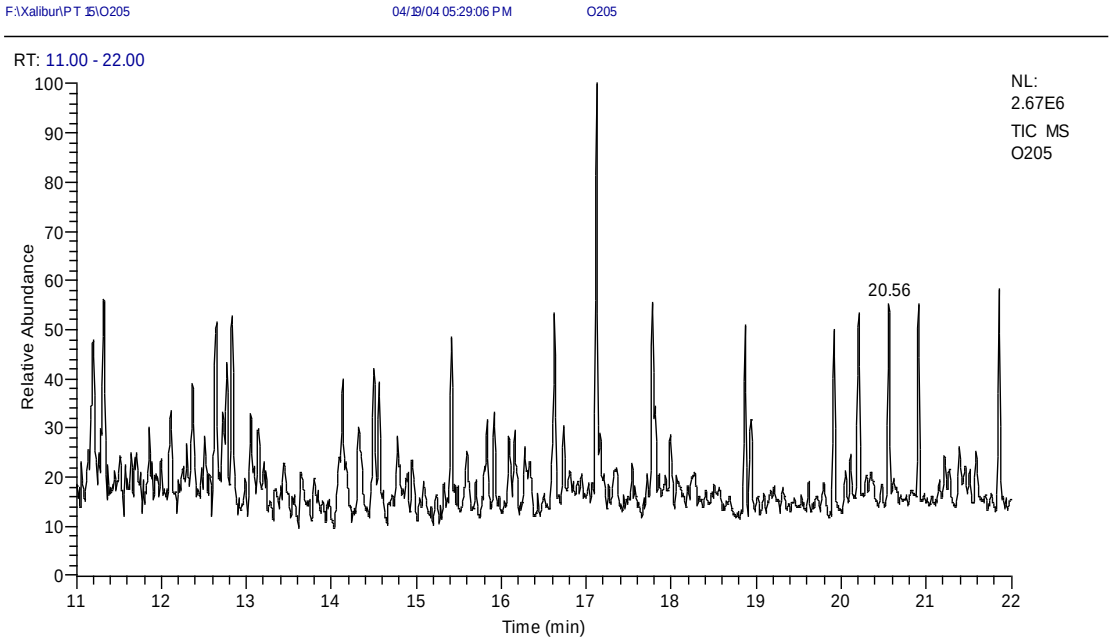
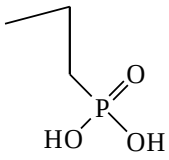
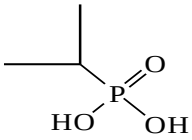
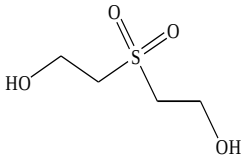


Figure 3. EI TIC of organic waste sample (Ret. Time : 20.56 minutes)

Table 5. List of Spiking Chemicals compound introduced to water sample (W) during 15th Proficiency Testing and identification result have been done by Research Centre for Chemistry (LIPI).

Sample code	No. of Compoud	Compound name	Structure	Note
W	D	Propylphosphonic acid		Found but not reported
W	E	Isopropylphosphonic acid		Found but not reported
W	F	1,4-Bis(2-hydroxyethyl sulfonyl)-n-butane		Not found
W	G	Bis(2-hydroxyethyl) Sulfone		Not found

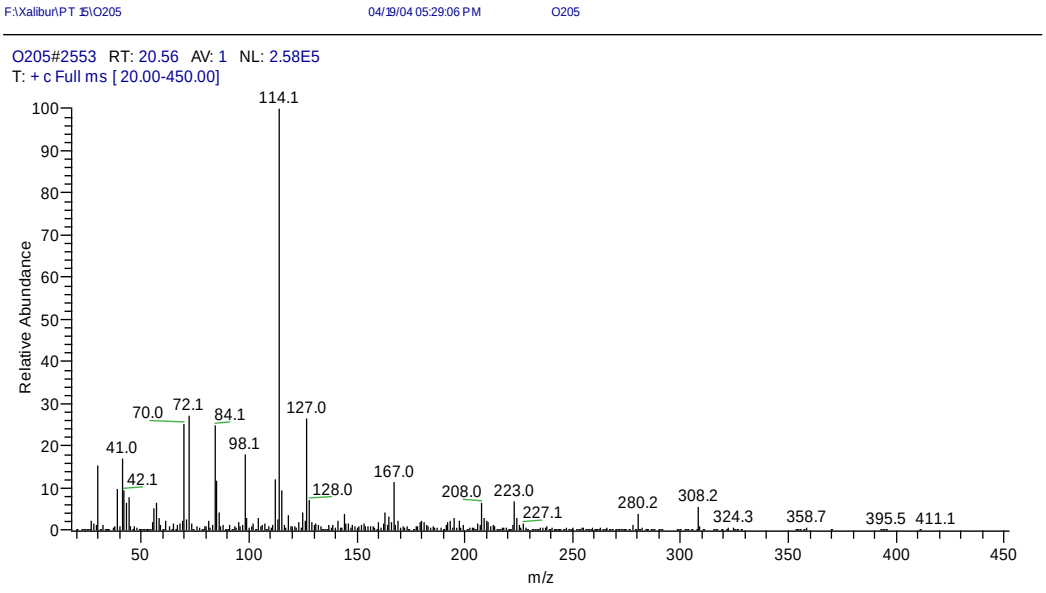


Figure 4. EI spectrum of organic waste sample compound no.6 (Ret. Time : 20.56 minutes)

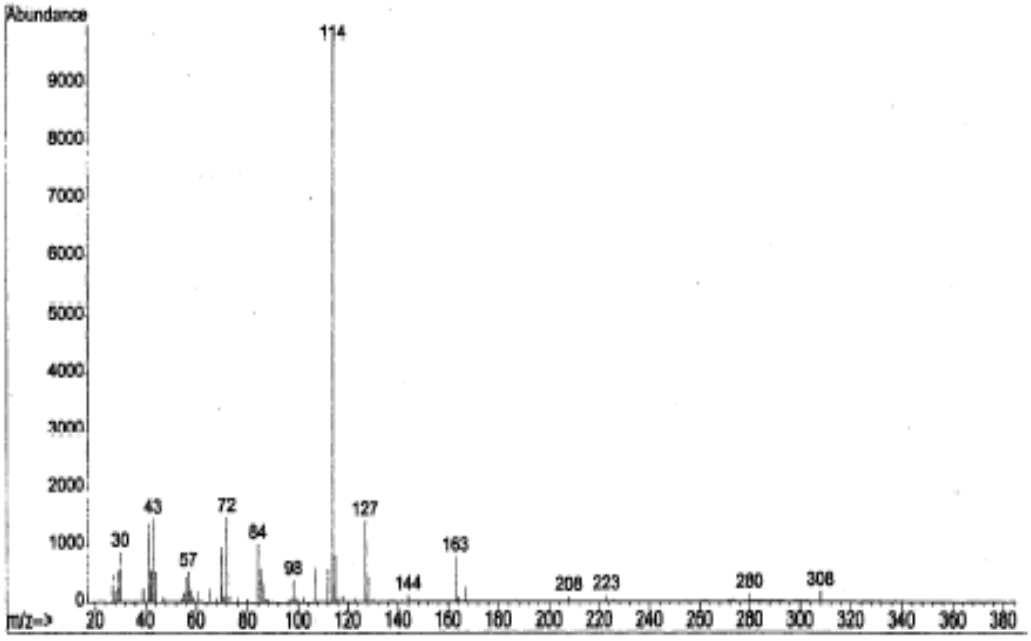
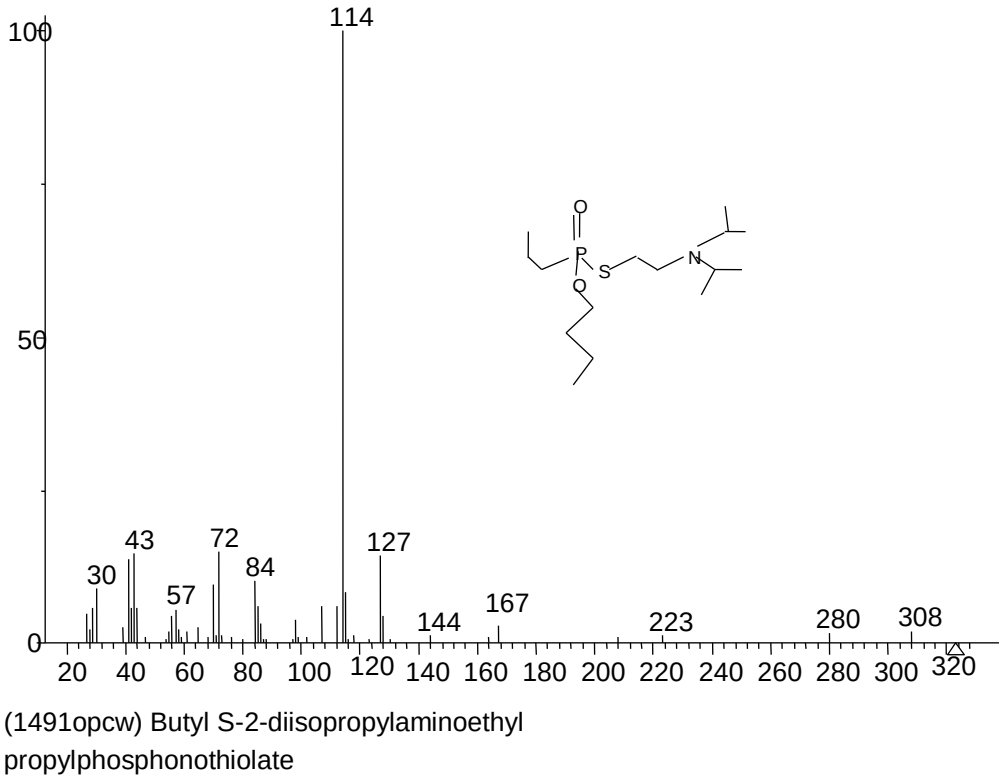


Figure 4a. EI spectrum of reference butyl S-2-diisopropylamino-ethyl propylphosphonothiolate (OCAD v.6)



(1491opcw) Butyl S-2-diisopropylaminoethyl propylphosphonothiolate

Figure 4b. EI spectrum of reference butyl S-2-diisopropylaminoethyl propylphosphonothiolate (NIST v.2.0)

Organic sample, Vol injection 10 ul, flow 0.1 ml/min, Eluent MeOH+Water = 90 + 10

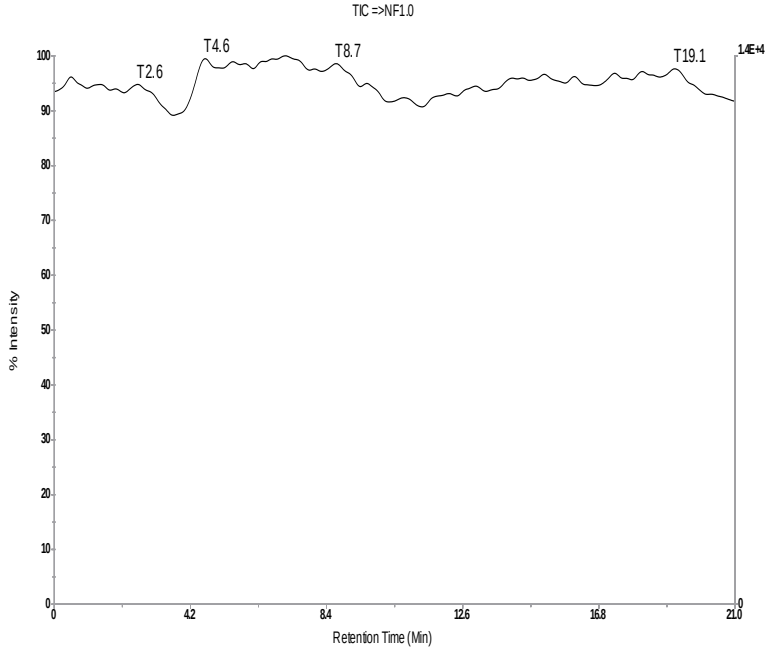


Figure 5. LCMS chromatogram of organic blank solution**.**

Organic sample, Vol injection 10 ul, Flow 0.1 ml/min, Eluent MeOH+Water = 90 + 10

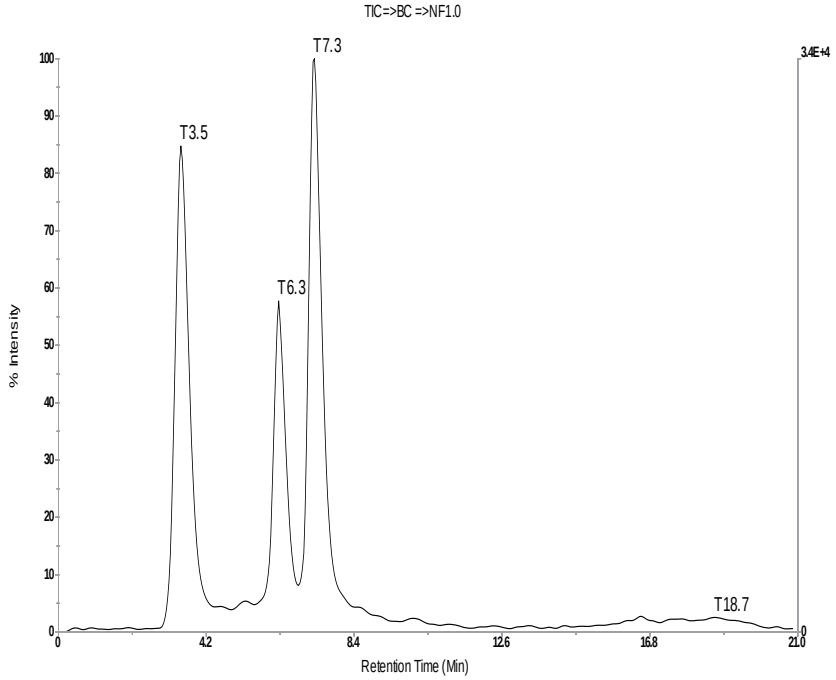


Figure 6. LCMS chromatogram of organic solution**.**

1. Rt = 3.40-3.52

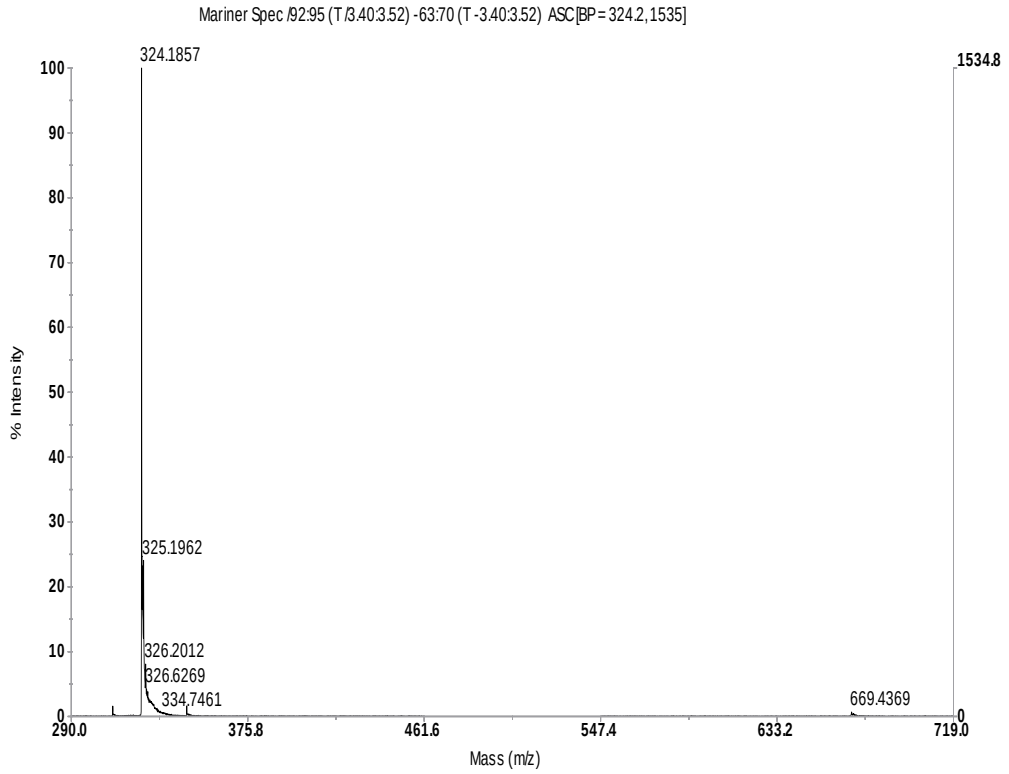


Figure 7. LCMS spectra of peak in the retention time 3,40- 3,52 minutes

CONCLUSION

The result of the 15th PT could be reported on time. Three compounds of spiking chemicals introduced to W and O samples could be found and one chemical suspected as Butyl S-2-diisopropylaminoethyl propylphosphonothiolate was reported as false positive identification.

REFFERENCES

www.opcw.nl/chemhaz/nerve.htm, 1997, Lethal Organo-phosphorus Compounds Inhibiting Cholinesterase, A FOA Briefing Book on Chemical Weapons, ICA Division OPCW.

Van Straten, F., Hildebrand,T., Pienaar, E., Seemane, P. 2004. Preliminary evaluation results fifteenth Official OPCW proficiency test. Volume 1. Technical Secretariat OPCW.

Van Straten, F., Hildebrand,T., Pienaar, E., Seemane, P. 2004. Preliminary evaluation results fifteenth Official OPCW proficiency test. Volume 2. Technical Secretariat.

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. 1997. Criteria for Acceptable Performance of Laboratories in Proficiency Testing, Conference of the State Parties, OPCW, The Hague, C-I/DEC.62, 22 May.