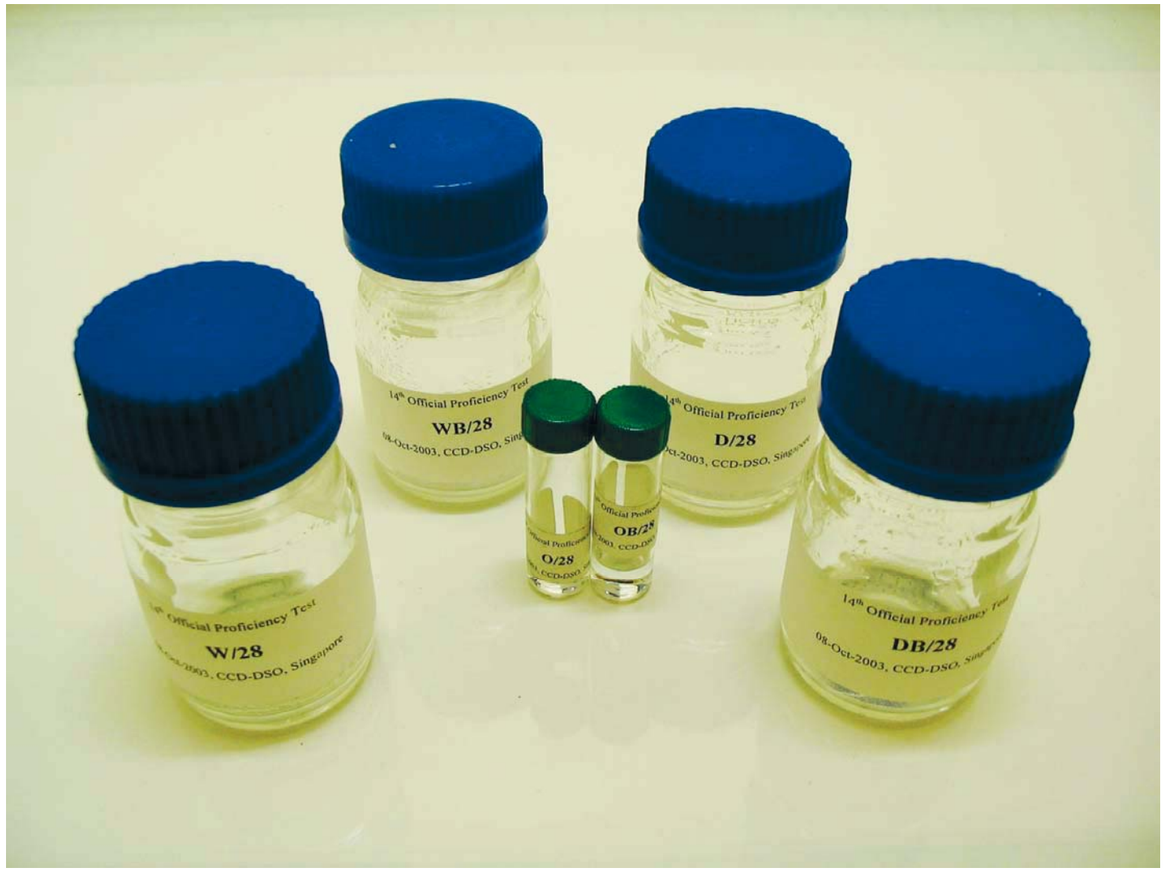
Participation in the 14th Official OPCW Proficiency Test

YVONNE NYGREN, CRISTER ÅSTOT, STEN-ÅKE FREDRIKSSON,
LARS-GUNNAR HAMMARSTRÖM, CALLE NILSSON



FOI is an assignment-based authority under the Ministry of Defence. The core activities are research, method and technology development, as well as studies for the use of defence and security. The organization employs around 1350 people of whom around 950 are researchers. This makes FOI the largest research institute in Sweden. FOI provides its customers with leading expertise in a large number of fields such as security-policy studies and analyses in defence and security, assessment of different types of threats, systems for control and management of crises, protection against and management of hazardous substances, IT-security an the potential of new sensors.

FOI
Defence Research Agency
NBC Defence
SE-901 82 Umeå

Phone: +46 90 10 66 00
Fax: +46 90 10 68 01

www.foi.se

FOI-R-- 1555 --SE User report
ISSN 1650-1942 December 2005

NBC Defence

Yvonne Nygren, Crister Åstot, Sten-Åke Fredriksson, Lars-Gunnar Hammarström, Calle Nilsson

Participation in the 14th Official OPCW Proficiency Test

Issuing organization	Report number, ISRN	Report type
FOI – Swedish Defence Research Agency NBC Defence SE-901 82 Umeå	FOI-R--1555--SE	User report
	Research area code 3. NBC Defence and other hazardous substances	
	Month year December 2005	**Project no.** A4934, A4855
	Sub area code 32 Biological and chemical defence research	
	Sub area code 2	

Author/s (editor/s)	Project manager
Yvonne Nygren Crister Åstot Sten-Åke Fredriksson Lars-Gunnar Hammarström Calle Nilsson	Crister Åstot, Calle Nilsson
	Approved by
	Sponsoring agency
	Scientifically and technically responsible Crister Åstot

Report title

Participation in the 14th Official OPCW Proficiency Test

Abstract

The 14th Official OPCW Proficiency Test was performed during October 2004. In this test, the Designated Laboratory at FOI assisted the OPCW as evaluators of the test results reported by 20 participating laboratories.

Prior to the evaluation three samples were received from the sample preparing laboratory; a decontamination solution, an organic liquid and a water sample, all with their corresponding blanks. They were analyzed in accordance with our "Recommended Operating Procedures" and seven spiking chemicals were identified by GC-MS(EI) and GC-MS(CI). The test included a water sample not spiked with any scheduled chemical, a new feature in the Proficiency Testing Programme. However, the main difficulty in this test was a severe mineral oil contamination of the organic sample, and hydrocarbons from the oil co-eluting with the listed chemicals resulting in interferences with their detection. This made it necessary to add a complementary clean-up step to the recommended procedure in order to acquire clean MS-spectra of the scheduled chemicals.

This report presents a compiled view of our own results and the results of the participating laboratories. We also present revisions of the reporting procedures at the FOI laboratory based on the experience of the evaluation.

Keywords

Inter-calibrations, OPCW, chemical warfare agents

Further bibliographic information	Language English

ISSN 1650-1942	Pages 19 p.
	Price acc. to pricelist

Utgivare	Rapportnummer, ISRN	Klassificering
FOI - Totalförsvarets forskningsinstitut NBC-skydd 901 82 Umeå	FOI-R--1555--SE	Användarrapport
	Forskningsområde 3. Skydd mot NBC och andra farliga ämnen	
	Månad, år December 2005	**Projektnummer** A4934, A4855
	Delområde 32 B- och C forskning	
	Delområde 2	

Författare/redaktör	Projektledare
Yvonne Nygren Crister Åstot Sten-Åke Fredriksson Lars-Gunnar Hammarström Calle Nilsson	Crister Åstot, Calle Nilsson
	Godkänd av
	Uppdragsgivare/kundbeteckning
	Tekniskt och/eller vetenskapligt ansvarig Crister Åstot

Rapportens titel

Deltagande i OPCW 14:e officiella interkalibrering avseende kemiska stridsmedel

Sammanfattning

The 14th Official OPCW Proficiency Test utfördes under oktober 2004. I detta interkalibreringstest, assisterades OPCW av FOI´s designerade laboratorium för utvärderingen av testresultaten från 20 deltagande laboratorier. Innan arbetet med utvärderingen påbörjades mottogs tre prover från det provpreparerande laboratoriet; en saneringslösning, ett organiskt prov och ett vattenprov, alla med sina tillhörande blankprover. De analyserades i enlighet med våra "Recommended Operating Procedures" och sju "spikkemikalier" identifierades med GC-MS(EI) and GC-MS(CI). Testets vattenprov innehöll inte någon listad kemikalie, en ny företeelse vad gäller OPCW Proficiency Tests. Den huvudsakliga svårigheten i det här testet var dock en kraftig bakgrund av mineralolja i det organiska provet och kolväten från oljan överlappade med vissa av spikkemikalierna och orsakade undertryckning av deras signaler. Detta gjorde att det var nödvändigt att lägga till ytterligare ett uppreningssteg till de rekommenderade proceduerna för att erhålla rena MS-spektra av de listade kemikalierna.
Denna rapport presenterar våra analytiska resultat och jämför dessa med resultaten från de deltagande laboratorierna. Vi presenterar också förändringar i FOI-laboratoriets rapporteringsrutiner som baserats på erfarenheterna från utvärderingen.

Nyckelord

Interkalibreringar, OPCW, kemiska stridsmedel

Övriga bibliografiska uppgifter	Språk Engelska
ISSN 1650-1942	**Antal sidor:** 19 s.
Distribution enligt missiv	**Pris: Enligt prislista**

Contents

Introduction

The Chemical Weapons Convention (CWC) was opened for signature in January 1993 and entered into force in 1997 after it had been ratified by 65 nations. The CWC calls for inspection and verification of the treaty, in cases of routine inspection, challenge inspection and alleged use. The analysis of Chemical Warfare Agents (CWAs), their precursors and degradation products is a key issue in these procedures and therefore the Organisation for the Prohibition of Chemical Weapons (OPCW) has appointed designated laboratories to undertake analysis of samples that may arise from these inspections. To gain the status as an "OPCW Designated Laboratory" a candidate laboratory must have a proper quality system and a national accreditation (ISO IEC 17025 or equivalent) for the analysis of CWAs. In addition, the laboratory must have performed successfully in three consecutive rounds in the OPCW's Official Proficiency Testing Programme. The laboratory at the Swedish Defence Research Agency (FOI) is accredited by the "Swedish Board for Accreditation and Conformity Assessment" (SWEDAC) and gained its designation in November 1998 after successful performance in three consecutive Proficiency Tests. The laboratory has since then participated in a Proficiency Tests annually to retain designation.

A Proficiency Test consists of samples artificially fortified with chemicals (spiking chemicals) relevant or non-relevant to the CWC. Relevant chemicals are to be reported to the OPCW while the non-relevant chemicals shall not. The report must be submitted within 15 days from the sample arrival day and the report, including all instrumental data, must fulfil well defined criteria for the identifications to be accepted.[1] The laboratory will be scored based on the number of accepted identifications. To retain designation all designated laboratories must each year participate and score successfully in a Proficiency Test, which are organised twice a year by the OPCW.

Each Proficiency Test is conducted by the OPCW with the assistance of two Designated Laboratories. Definition of the test scenario and the preparation of the test samples is a task for the "Preparatory Laboratory" after agreement with the OPCW. After the Proficiency test, the reports from the participating laboratories are evaluated by the "Evaluating Laboratory" and OPCW. The parallel evaluation ensures a thorough assessment according to the evaluation criteria and the compiled examination forms the basis for the performance scoring of the laboratories. During evaluation all presented analytical data is assessed. The presence of the "spiking chemical" in the sample and its absence in the blank must be shown and the quality of the data must fulfil the criteria. In addition, the link between the analytical data and the original sample must be clearly outlined (traceability) by a description of the sample preparation and sample aliquot codes for the purified fractions. All chemical structures and the nomenclature used must be correct. The assisting laboratories are credited with a maximum performance rating if their work and report meet the requirements in the Standard Operating Procedures for these tasks.

FOI assisted the OPCW as the Evaluating Laboratory in the 14th Official OPCW Proficiency Test (November 2003). On the eights of October 2003 three different artificially spiked samples were sent out to 24 laboratories for analysis of CWAs and related chemicals. Samples and a test scenario (Appendix 1) were supplied by the Sample Preparation Laboratory; DSO, Singapore. In order for us to gain experience from the test samples and to draw conclusions about the difficulties the participating laboratories might encounter, we analyzed the samples prior to the evaluation without knowledge of their spiking chemical content.

20 laboratories submitted reports to the OPCW and our evaluation of the results was published by the OPCW as a part of the documentation of the test.[2] All data submitted by the participants in the test was assessed and detailed comments were given. In the present report, the analytical data from our own analysis of the test samples is presented and the results are compared with the results of the participating laboratories. Different approaches used for sample preparation, chemical analyses and reporting will be assessed and conclusions are drawn concerning their efficiency for production of accepted data.

Materials and Methods

Chemicals

All solvents and chemicals used for analysis were of analytical grade. Prior to the analysis the purity of solvents was checked by GC-MS-analysis. *Solvents;* Dichloromethane (Merck), acetonitrile (LiChroSolv), n-hexane (Merck), deuterium oxide (Dr. Glaser AG). *Reagents and extraction cartridges;* $NaSO_4$-cartridge (Isolute 802-0250-M, Scantec Lab AB), BSTFA (Pierce, Boule Nordic AB), strong cat-ion exchanger (Alltech, Extract-Clean 209950, Scantec Lab AB), methylthioglycolate (Aldrich).
Reference chemicals were supplied by the Sample Preparation Laboratory; DSO, Singapore. Stock solutions were kept in a safe in our "Single Small Scale Facility" (SSSF). Sample analysis was performed without knowledge of the spiking chemicals and when a candidate compound was identified, a call was made to the manager of our SSSF for the corresponding reference chemical.

Test Samples

The samples were dispatched from Singapore on the 8th of October and arrived at the FOI laboratory on the 14th of October. As an Evaluating Laboratory two sample sets, numbered 28 and 29, were sent to us. Each sample set consisted of one water sample (W), one decontamination solution (D) and one organic sample (O), with their corresponding blanks (WB, DB and OB). Only one of the two identical sample sets (no 28) was used for the analyses discussed in this report.

Sample Preparation

Chemical Warfare Agents is a group of chemicals with widely varying properties ranging from water soluble, highly polar chemicals such as the degradation products of nerve gases to water immiscible non-polar chemicals such as sulphur mustards. The analytical strategies for determination of all scheduled chemicals are hence quite complex and consists of of many steps. The standard sample preparation procedures are described in the Recommended Operating Procedures (ROP's) in the quality system of our laboratory. However, when required, our quality system allows us to use alternative sample preparation methods, as long as they are carefully documented. In the Proficiency Test described in this report, the purification of the organic sample by silica solid phase extraction was applied when the recommended methods did not produce acceptable data. All samples handled in the accredited system are given an internal code describing the sample matrix. After the matrix code each sample is given an individual number. Each sub sample of the original sample is then given an extension; sub sample number and a letter combination corresponding to the analytical route making up the sample aliquot code, which together with the original code assures the traceability of each individual sample. Original codes and the corresponding internal codes for the 14th Official OPCW Proficiency Test are shown in Table 1.

Table 1. Sample aliquot coding system. OPCW codes; O = Organic sample , D = Decontamination solution, W = Water sample, FOI codes; L = Liquid sample, W = Water sample

OPCW Original Sample Code	FOI Sample Code	Aliquot codes from							
		Route NMR	Route A	Route B	Route C	Route D	Route E	Route F	Route G
O/28	L1	L1:4N	L1:1A	L1:2AS	L1:5AT	L1:7B	-	-	-
OB/28	LB1	LB1:5N	LB1:2A	LB1:3AS	LB1:6AT	LB1:8B	-	-	-
D/28	W1	W1:1N	W1:3A, W1:3AS	-	-	-	W1:2ES	W1:4FT	-
DB/28	WB1	WB1:1N	WB1:3A, WB1:3AS	-	-	-	WB1:2ES	WB1:4FT	-
W/28	W3	W3:3N	W3:4A, W3:4AS	-	W3:4CS	-	W3:2ES	W3:5FT	W3:6GS
WB/28	WB3	WB3:3N	WB3:4A, WB3:4AS	-	WB3:4CS	-	WB3:2ES	WB3:5FT	WB3:6GS

- = not analysed or not applicable

Decontamination solution (W1 and WB1)
The Recommended Procedure P2 was applied for the decontamination solution (Figure 1):

- 10 ml of sample and blank were extracted at prevailing pH (~8) with 2 x 5 ml dichloromethane (Route A, Fig. 1). The combined extract was dried by a $NaSO_4$-cartridge, evaporated under a gentle stream of nitrogen to approximately 1 ml before analysis.
- Route B, C, D and G (Fig 1) were not utilized in this test.
- A 5 ml portion of the sample (and blank) was subjected to ion exchange using a strong cation exchange cartridge in H^+-form to minimize the presence of cat-ions before derivatization (Route E, Fig. 1). The fraction from the cat ion exchange was evaporated to dryness, dissolved in 1 ml acetonitrile and derivatized as above.
- A 5 ml of the sample and blank respectively were made acidic (pH 1) using 1 M HCl and derivatized at room temperature for 1 h with methylthioglycolate for identification of Lewisite compounds (Route F, Fig. 1). The derivatives were extracted with 2 x 5 ml hexane, dried (using sodium sulphate) and concentrated to 1 ml.

All of the above described fractions were subjected to GC/MS-analysis using electron impact (EI), chemical (CI) ionisation, and GC-analysis using specific detectors (i.e. NPD and FPD in sulphur mode).

For the NMR analysis 0,1 ml of deuterium oxide was added to 0.6 ml of the sample and blank respectively (Route NMR-analysis, Fig. 1) .

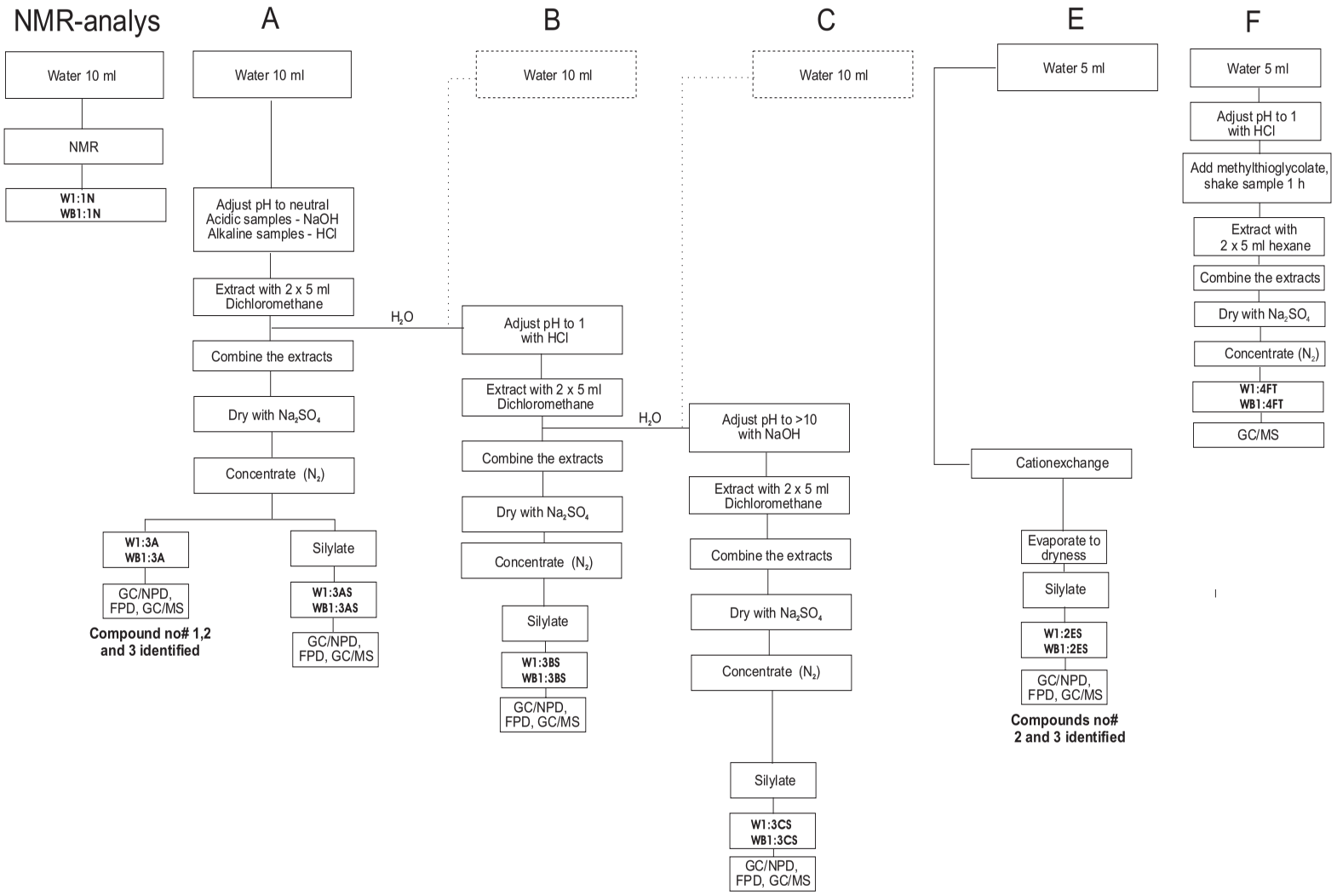


Figure 1. Recommended Operating Procedure flowchart for sample preparation of decontamination solutions (W1 and WB1)

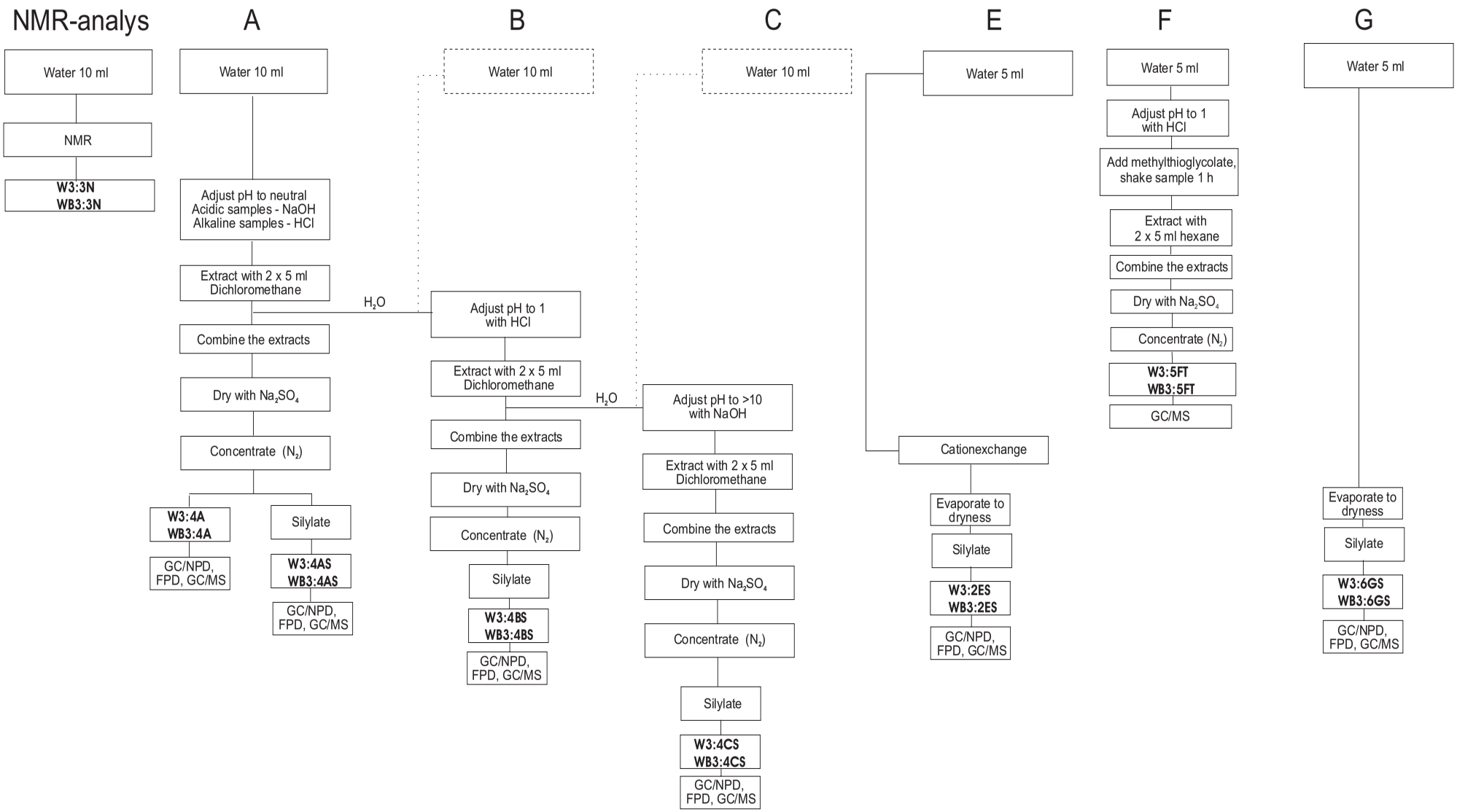


Figure 2. Recommended Operating Procedure flowchart for sample preparation of water samples (W3 and WB3)

Water sample (W3 and WB3)
The recommended procedure P2 was applied for the water sample (Fig. 2):

- 10 ml of sample and blank were extracted at neutral pH with 2 x 5 ml dichloromethane (Route A, Fig. 2). The combined extract was dried by a $NaSO_4$-cartridge, evaporated under a gentle stream of nitrogen to approximately 1 ml before analysis.
- Route B and D (Fig 2) were not utilized in this Proficiency Test.
- The water residue from route A was adjusted to pH 10 and extracted with 2 times 5 ml dichloromethane, dried and evaporated as above to approximately1 ml before analysis (Route C, Fig 2).
- 5 ml of the sample (and blank) was subjected to cation exchange as above before derivatization (Route E, Fig 2). The fraction from the cation exchange was evaporated to dryness, dissolved in acetonitrile and derivatized as above.
- 5 ml of the sample and blank respectively were made acidic with HCl (pH 1) and derivatized at room temperature for 1 h with methylthioglycolate for identification of Lewisite compounds as above (Route F, Fig. 2).
- 5 ml of the sample (and blank) was evaporated to dryness, dissolved in 1 ml acetonitrile and silylated using 100 µl BSTFA at 60 °C for 1 h. (Route G, Fig. 2)

All of the above described fractions were subjected to GC/MS-analysis using electron impact (EI) , chemical (CI) ionisation, and GC-analysis using specific detectors (i.e. NPD and FPD). For the NMR analysis 5 ml of the sample and blank were concentrated to approximately 0.5 ml, deuterium oxide was added and the samples were analyzed by NMR (Route NMR-analysis, Fig. 2).

Organic Sample (L and LB):
The recommended procedure P7 was applied on the organic sample (Fig. 3):

- 100 µl of the sample and blank respectively was analysed directly by GC/MS (Route A, Fig. 3).
- Another 500 µl portion was derivatized at 60 °C for 1 h with 100 µl BSTFA added to the sample (Route B, Fig. 3).
- A new 500 µl portion of sample (and blank) were derivatized for identification of Lewisite compounds at room temperature for 30 minutes using methylthioglycolate (Route C, Fig. 3).
- To remove the sample matrix consisting of diesel oil (saturated aliphatic hydrocarbons) an additional purification procedure was applied to the sample. A 100 µl aliquot of each the sample and blank were applied to columns packed with activated silica and conditioned with hexane. The slightly polar spiking chemicals were retained on the silica surface while the interfering alkanes were washed away by hexane. The spiking chemicals were subsequently eluted by methanol. (Route D, Fig. 3).

All fractions were subjected to GC/MS-analysis using electron impact (EI) , chemical (CI) ionisation, and GC-analysis using specific detectors (i.e. NPD and FPD in sulphur mode).

- A 1 ml portion of sample and blank were prepared by solvent exchange to deuteriochloroform and analyzed by NMR.(Route NMR-analysis, Fig 3).

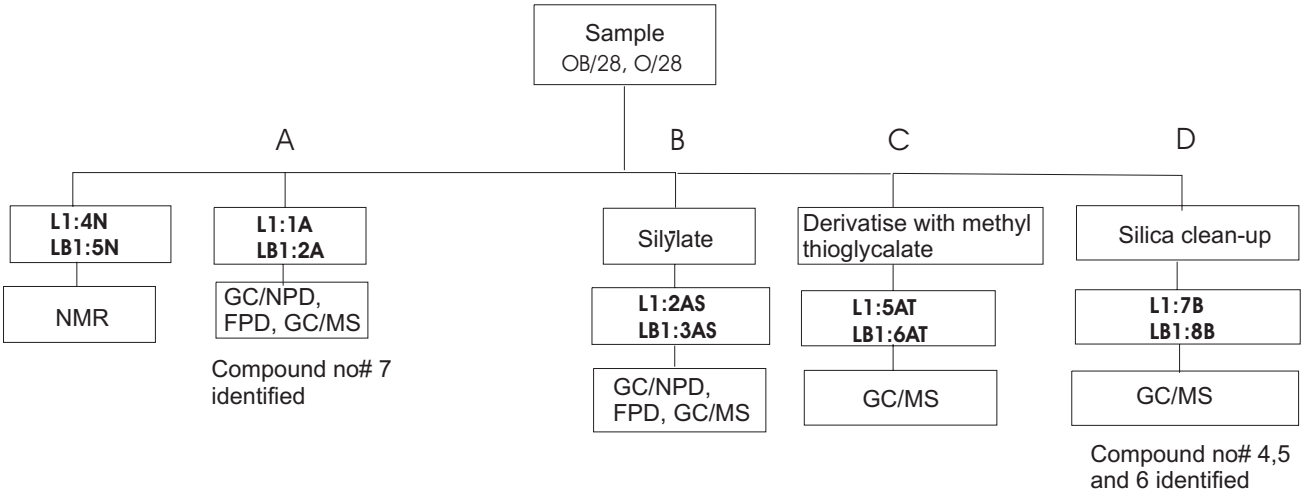


Figure 3. Recommended Operating Procedure flowchart for sample preparation of organic samples (L1, LB1)

Instrumental

Gas chromatography and mass spectrometry analysis

GC/MS (EI) analyses were performed using a Hewlett Packard 5890 GC coupled to a 5972 mass selective detector. Separations were accomplished on a J&W DB5MS capillary column programmed from 40 or 60 °C (depending on solvent) (1 min), 10°C/min to 280 °C (5 min). The injector temperature was 200 °C. 70 eV EI mass spectra were acquired over the range m/z 29 - 450 (3 scans/s) with a source temperature of 155 °C.

GC/MS (CI) analyses were performed using a Hewlett Packard 6890 GC coupled to a 5973N mass selective detector. GC conditions were the same as for GC-MS (EI). CI mass spectra were acquired over the range m/z 60 to 500 (3 scans/s) with a source temperature of 250 °C. Isobutane was used as chemical ionisation gas.

For screening of nitrogen and phosphorous containing compounds in the samples a Hewlett Packard 5890 GC with a Nitrogen-Phosphorous detector operating at 280 °C was used. GC conditions were the same as for GC-MS (EI).

For screening of sulphur containing compounds in the samples a Varian 3400CX GC-system with a PFPD-detector was used. GC conditions were the same as for GC-MS (EI).

The performance of GC/MS instruments was evaluated before analysing the samples. A compound mixture consisting of 8 chemicals (in retention order); deuterated dimethylmethylphosphonate (D_3-DMMP, RT = 5.81), decane, 2,6-dimethylphenol, 5-chloro-2-methylaniline, pentadecane, tributylphosphate, dibenzothiophene and malathion (Figure 4), was analysed and evaluated concerning GC-parameters; S/N, peak skewness and retention time, and for MS-parameters; isotope ratio and mass spectrum quality (Appendix 2).

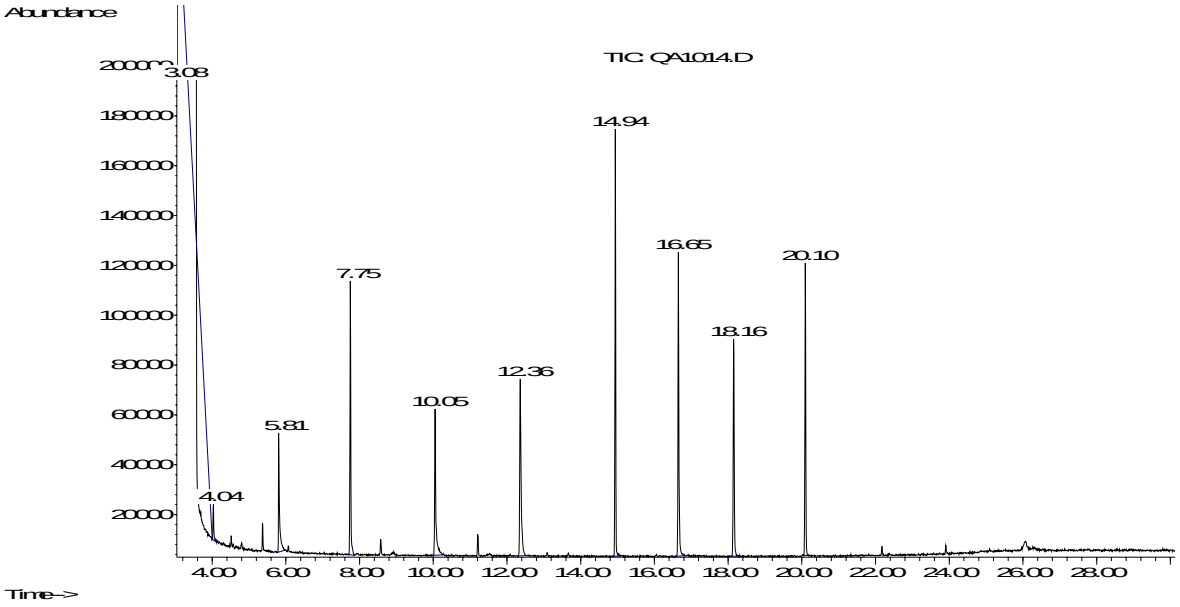


Figure 4. GC/MS total ion chromatogram (TIC) of an instrument performance quality assurance mixture.

NMR analysis

NMR analyses were performed on a Bruker Avance 500 instrument (500 MHz proton frequency) using a 5 mm QNP probe (^{1}H, ^{13}C, ^{31}P, ^{19}F) with Z-field gradient. Conventional proton NMR experiments as well as proton-decoupled phosphorus NMR experiments were performed. To study the structural environment around phosphorus, proton detected heteronuclear correlations were performed using gradient selected HMBC (Heteronuclear Multiple Bond Correlation) experiments. A polarization delay of 50-60 milliseconds was usually optimal to register proton-phosphorus correlations over 2-4 bonds.

Results and Discussion

General

The 14th Proficiency Test presented a new scenario with a water sample free from “spiked” chemicals relevant to the CWC. Although this is a likely scenario, it has not been applied in the Proficiency Testing Programme before. In the other two samples, seven chemicals relevant to the CWC were identified. The organic sample contained three highly toxic spiking chemicals; the nerve gas Tabun (chemical 4, Table 2) and two analogues related to the nerve gases VX and Cyclosarin, respectively (chemical 6 and 7). An expected by-product from Tabun synthesis was also present in the organic sample (chemical 5). The decontamination solution contained three expected degradation products from chemical 4 and 6 (chemical 1-3). The set of spiking chemicals matched the scenario of a challenge inspection of a synthesis facility.

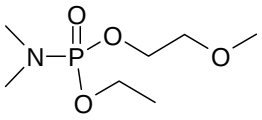
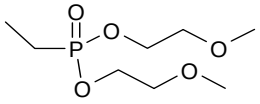
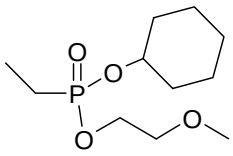
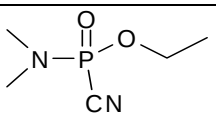
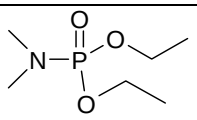
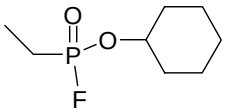
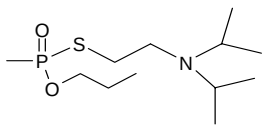
Sample code	Chem. No.	Chemical name	Chemical structure	Molecular formula	Schedule number
D28	1	O-ethyl, O-(2-methoxyethyl) N,N-dimethyl-phosphoramidate		$C_7H_{18}NO_4P$	-
D28	2	Bis-(2-methyoxyethyl) ethylphosphonate		$C_8H_{19}O_5P$	2.B.4
D28	3	O-cyclohexyl, O-(2-methoxyethyl) ethylphosphonate		$C_{11}H_{23}O_4P$	2.B.4
O28	4	O-ethyl N,N-dimethyl-phosphoro-amidocyanidate		$C_5H_{11}N_2O_2P$	1.A.2
O28	5	Diethyl N,N-dimethyl-phosphoramidate		$C_6H_{16}NO_3P$	2.B.6
O28	6	O-cyclohexyl ethylphosphono-flouridate		$C_8H_{16}FO_2P$	1.A.1
O28	7	O-propyl S-2-diisopropyl-aminoethyl methyl-phosphonothiolate		$C_{12}H_{28}NO_2PS$	1.A.3

Table 2. Identified and reported chemicals in the 14th Proficiency Test

Decontamination Sample

Screening of the original sample by Proton decoupled phosphorus NMR spectrum revealed three peaks which could be assigned to chemicals relevant to the CWC. Supplementary HMBC ^{1}H-^{31}P correlation assisted in the identification of a N,N-dimethylphosphoramidate and two ethylphosphonates.

The full structures of chemical 1-3 were subsequently identified by GC-MS (EI) and (CI) analyses of a dichloromethane extract from the sample (route A in Figure 1). The extraction removed polar components in the sample matrix such as amines and alkoxyethanols. In addition, the extraction concentrated the spiking chemicals and intense sample peaks were present in the GC-MS (EI) total ion chromatogram (TIC, Figure 5). The degradation products were identified based on their EI mass spectra (page 14, 20 and 26, appendix 3) and we verified their identities by comparison with the spectra of authentic reference chemicals. Most of the participating laboratories succeeded to correctly identify chemical 1-3 (Success rate: 67%).[2] The presence of reference EI spectra in the OPCW Central Analytical Database

made the identification easier and use of this database would probably have assisted three of the participating laboratories in identifying them correctly. Instead misinterpretation of the spectra resulted in the reporting of chemicals not present in the sample, which were categorized as false positive identifications.

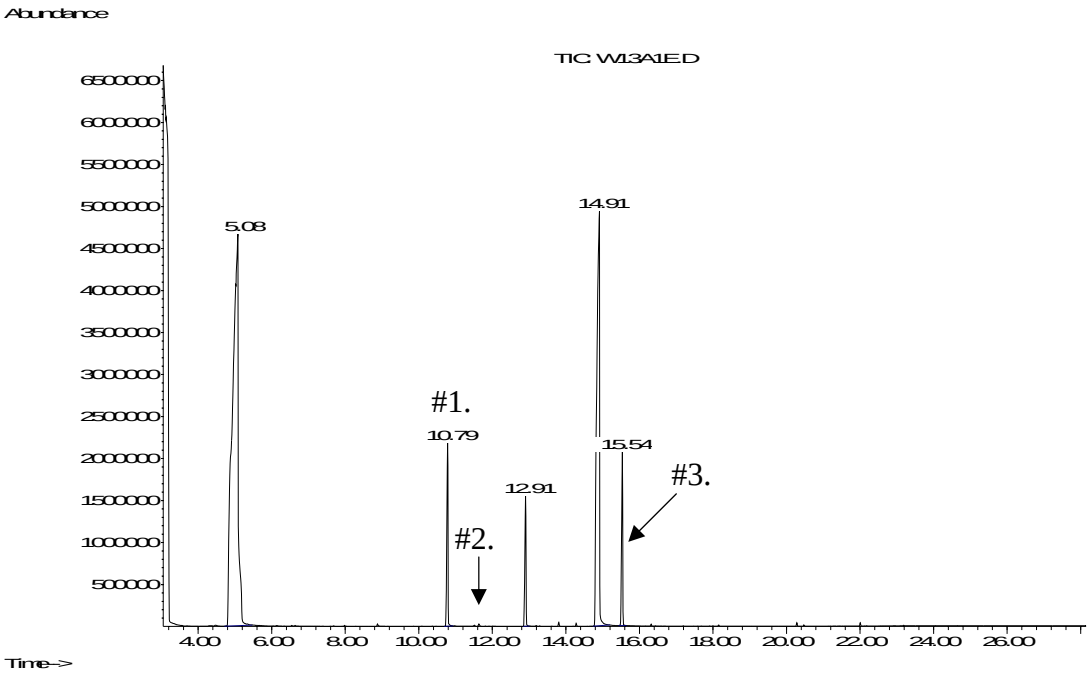


Figure 5. GC/MS EI TIC of sample aliquot W1:3A from sample D/28. Spiking chemicals #1, #2 and #3 are indicated.

On purification of an aliquot of the decontamination solution by strong cat ion exchange (route E in Figure 1) we observed that the acid labile chemical 1 was quantitatively hydrolyzed to its corresponding phosphate (chemical Q in reference 2). This unscheduled chemical gave a signal for phosphorus in the GC-NPD analysis that was not present in the blank (data not shown). Interpretation of the EI mass spectrum, which was not found in the database, together with results from NMR-analysis, lead us to the conclusion that this was an artefact caused by the sample preparation conditions. An extended study of this case is described in a separate report.[3] Chemical Q was reported by one laboratory and was initially categorized as a false positive identification causing a failure in the test. However, the chemical was re-categorized since it could not be excluded that partial degradation could have taken place, during the time from sample preparation to the time of analysis, in the sample analyzed by that laboratory.

Water Sample

According to the test scenario the Water sample was collected from the water treatment plant in the inspected facility. The sample was prepared and analyzed according to our Recommended Operating Procedures (see Figure 2) with sequential organic extractions at different pH, subsequent ion exchange treatment of the water residue before derivatization for GC and GC-MS analysis of polar chemicals such as phosphonic acids. However, no chemicals relevant to the CWC were found which, taken the scenario into account, was not surprising.

No phosphorus containing chemicals could be detected in the water sample by NMR analysis. Neither did proton NMR spectra show any evidence of CWC related chemicals.

Two laboratories incorrectly reported triethanolamine and thiodiglycol, respectively. These false positive identifications may have been due to contamination of the samples during sample preparation.

Organic Sample

A strong background (Figure 6) in combination with a relatively low spiking level made the identification of the spiking chemicals 4-7 difficult. The sample matrix contained diesel oil (hydrocarbons), p-xylene, chlorobenzene and tributyl phosphate in a hexane solvent. However, screening by proton decoupled phosphorus-31 NMR, showed peaks in the spectrum which indicated the presence of a dialkylphosphoramidate and a tabun analog, respectively. More information about the structural environment around the phosphor was obtained from a HMBC ^{1}H-^{31}P correlation experiment. From the results of this experiment tabun and diethyl N, N-dimethylphosphoramidate could be verified. It also showed that an ethylphosphonofluoridate was present in the sample.

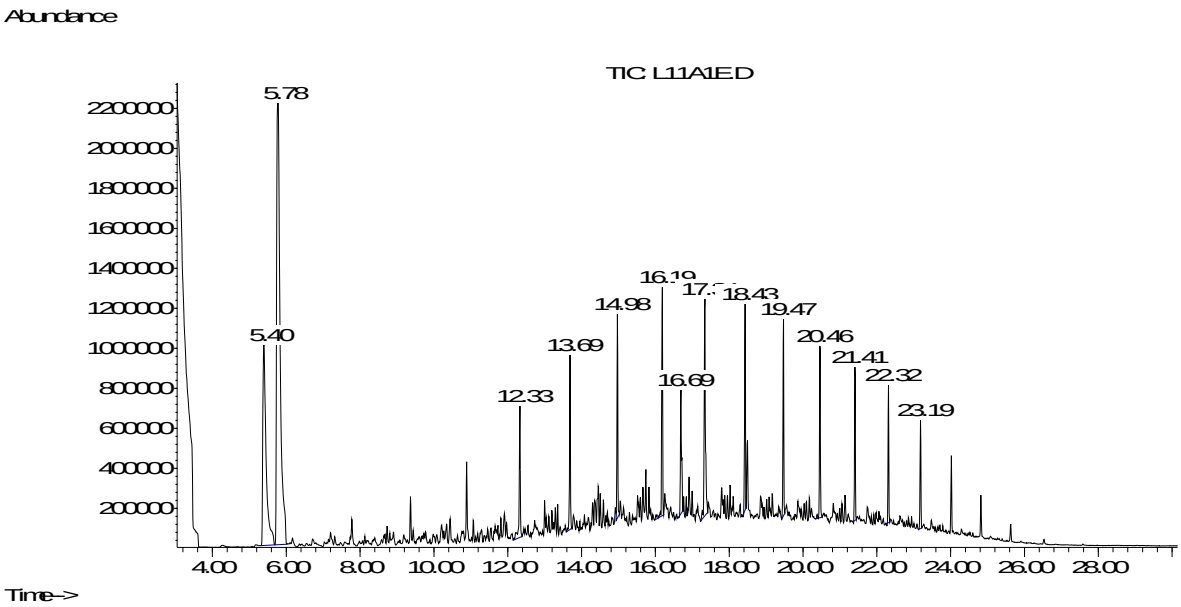


Figure 6. GC/MS EI TIC of the non-treated sample aliquot L1:1A from sample O/28. Signals from the spiking chemicals are covered by the high abundance peaks from p-xylene, chorobenzene and diesel oil.

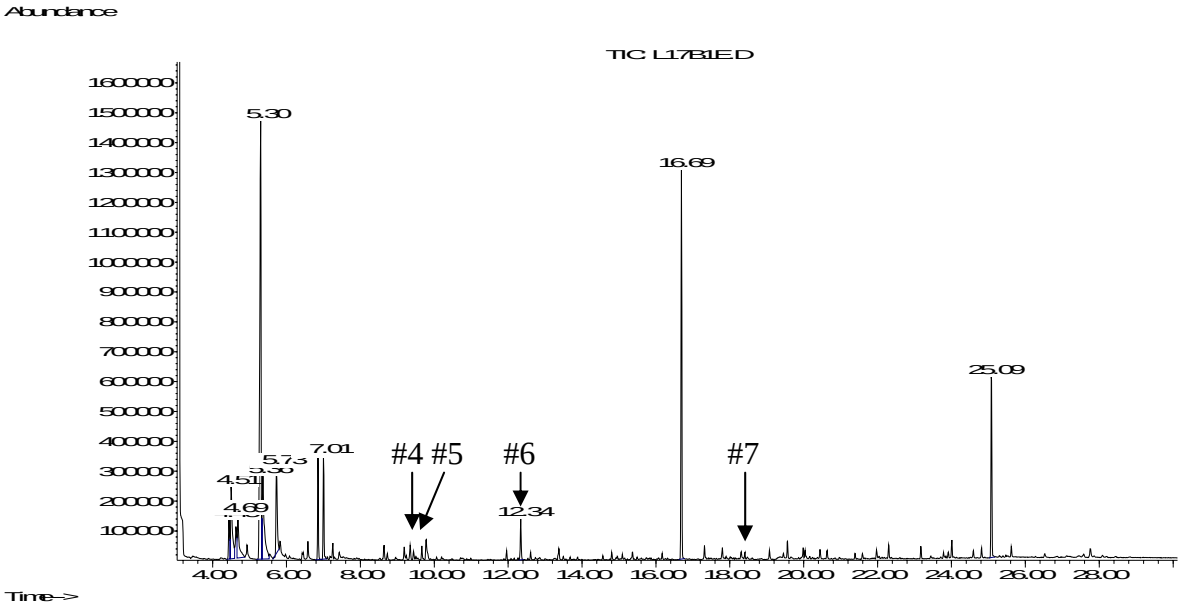


Figure 7. GC-MS EI TIC of a silica cleaned-up sample aliquot L1:7B from sample O/28. Spiking chemical #4, #5, #6 and #7 are indicated.

The presence of the spiking chemicals was indicated by the GC-MS data from the analysis of the untreated sample (Figure 6). However, sample purification by solid phase extraction on a silica cartridge was required to produce clean MS (EI)-spectra for correct identifications of chemical 4, 5 and 6 (route D in Figure 3)(data on page 34, 40 and 46 in appendix 3). In figure 7, the TIC from the analysis of the purified sample is shown. The peaks from the alkane series are absent in the purified sample and the purification efficiency is indicated by the strong peak at 16.69 min from tributylphosphate. In the TIC of the un-purified sample more than 10 peaks related to alkanes are more abundant than the tributylphosphate peak (Figure 6). In the sample purification procedure, the slightly polar spiking chemicals were retained on the silica surface while the interfering alkanes were washed away by hexane. The spiking chemicals were subsequently eluted by methanol. For the identification of chemical 6 (O-cyclohexyl ethylphosphonoflouridate) this step was crucial because of co-elution with alkanes in the GC-MS analysis of the un-purified sample. The spectrum of chemical 7 (O-propyl S-2-diisopropylaminoethyl methylphosphonothiolate) in the purified sample contained background peaks from an unknown compound originating from the silica column. Thus, the best data for this chemical was acquired from the un-purified sample aliquot L1:1A where only a few low abundant background ions were present in the spectrum. Four significant background GC-peaks were present in the extracted ion chromatogram (EIC) of the blank sample within the retention time window of chemical 7. Therefore, the spectra of those background chemicals were reported to show the absence of the test chemical in the blank (se discussion below). The participating laboratories used a wide range of purification procedures although the silica solid phase extraction was the preferred choice. Lack of screening techniques and less efficient methods to purify the sample were major reasons for a reduced success rate of 58%. Two laboratories reported false positive identifications based on misinterpretation of (EI) mass spectra.

Reporting of chromatographic data

The most common base for rejection of chromatographic data in the 14th Proficiency Test was the failure to demonstrate the absence of the reported chemicals in the blank. The blank data must be presented in such a way that the evaluators are able to assess the noise level in the blank. In the evaluation criteria for chromatographic data [1] the requirements for the data presentation are set:

> "The chromatograms of blanks must be presented with intensity scales that enable the evaluators to determine the signal-to-noise ratios of peaks…" Attachment 1, §1.3
>
> "The absence of a peak in the blank is considered to be demonstrated if the signal (peak height) of a peak in the respective retention time window in the blank is below 1% of the signal of the peak of the test chemical in the sample." Attachment 1, §1.3
>
> "The retention time of the GC peak must fall within a window of ±(5 seconds + 1% of the retention time from a reference chemical)." Attachment 1, §2.1.c
>
> "The signal-to-noise ratio of a chromatographic peak in GC-based techniques must be at least 5:1." Attachment 1, §2.1.d

Based on our experiences from the evaluation we have adopted the following reporting procedure:

- Display chromatograms with retention time (RT) range: test chemical peak RT ± 2 min.
- Intensity scale of sample chromatograms must be normalized to the test chemical peak.
- Intensity scale of blank chromatograms must be expanded at least 10 times compared to the intensity scale of the sample chromatogram in order to enable evaluation of the noise level. GC-MS: Always report EICs (m/z = base peak in test chemical mass spectrum).

The "Check List" below can be used to assess whether the reported data will show the absence of the test chemical in the blank down to the required level:

- Is there a peak in the blank chromatogram within the RT window of the test chemical?
- If **Yes**: Has the peak a signal to noise ratio > 5:1? If **No**: Data is sufficient.
- If **Yes**: Is the peak height > 1% of test chemical peak height in sample? If **No**: Data is sufficient.
- If **Yes (GC/MS)**: Mass spectrum of the peak in the blank must be reported. If **Yes (GC/ NPD or FPD)**: The absence of the test chemical in the blank can not be shown.

FOI Reporting Procedures

In previous tests, TIC and spectra from GC-MS (EI) and GC-MS (CI) was the only instrumental data reported by our laboratory. For an identification of a chemical to be accepted it has to be based on at least two different analytical techniques giving consistent results; at least one of these techniques must be a spectrometric technique. The evaluation of the 14th Proficiency Test has shown the importance of submission of data from complementary techniques to decrease the risk of having identifications rejected if one set of analytical data will not meet the evaluation criteria [1]. Thus, the probability of a good performance was improved by several laboratories submitting data from at least three techniques (e.g. GC-MS(EI), GC-MS(CI) and GC(NPD) or GC(FPD)). In future tests, our laboratory will submit GC-data using sulphur and phosphorous selective detectors in addition to the GC-MS-data.

During the evaluation we observed a range of different reporting strategies and the use of new macros in the Agilent MS-Chemstation software making it possible to reduce the number of pages in the report significantly. The macros were provided by Lawrence Livermore National Laboratory, CA, USA, and was used by our laboratory. The GC-MS data in appendix 3 has been reconstructed from the original files in order to increase the report quality (the original report was not evaluated or the basis for the score "A" in the test). In the new chromatogram display both TIC and EIC supporting the identification of the test chemicals are routinely shown and this is a significant improvement (se discussion above). The increased selectivity of EICs will improve signal to noise ratio of test chemical peaks in the samples and remove most of the peaks in the corresponding blank samples.

Revision of the Evaluation Criteria

Our laboratory suggested a number of revisions of the Evaluation Criteria [1] for the OPCW in order to improve the data quality of the reports and to facilitate the work of the Evaluators (3 a-f in ref. 2). The most important revision concerned the width of the retention time window in GC based techniques. The retention time window was much too wide compared to the reproducibility of modern GC instruments and the definition was unnecessary complicated: Test chemical RT ± (5 seconds + 1% of the retention time from a reference chemical). After a debate, the Final Conference of the 14th Proficiency Test could agree on a revision of the retention time window to; Test chemical peak RT ± 0.1 min.

Furthermore, a call was made for clarifications of the Evaluation Criteria concerning the "absence in the blank" of test chemicals and a revision of the NMR criteria. New revised criteria have since then been adopted. [4]

Concluding Remarks

The 14th Official OPCW Proficiency Test was a demanding exercise for the participating laboratories. The test highlighted a number of problems associated with the analysis of CWAs in environmental samples and the experiences from the test have improved the competence of the laboratories. As the evaluating laboratory we made an effort to achieve a thorough assessment of the reports and a fair evaluation. The experience we acquired by our own analysis of the samples was of great value in evaluating process. Our laboratory was awarded by the OPCW with the top score "A" for our contribution. However, the experiences from the evaluation are also of great value and they have made us better prepared for future tests.

References

1. Work Instruction for the Evaluation of Results of the OPCW Proficiency Tests. QDOC/LAB/WI/PT3. Organisation for the Prohibition of Chemical Weapons, The Hague, Netherland. Issue 1, Rev. 1, 1/9 2003.

2. Fredriksson S-Å., Hammarström L-G., Nygren Y. and Åstot C. (2004). Evaluation of the 14th Official OPCW Proficiency Test. (OPCW Technical Secretariat, Vol. 2., 2004) FOI-S--1285—SE

3. Åstot C., Nygren Y., Fredriksson S-Å., Hammarström L-G. and Nilsson C. (2006) Improved sample preparation for determination of phosphoramidates in water samples based on Na^+ cat-ion exchange. FOI-R-1988-SE

4. Work Instruction for the Evaluation of Results of OPCW Proficiency Tests. QDOC/LAB/WI/PT3. Organisation for the Prohibition of Chemical Weapons, The Hague, Netherland. Issue 1, Rev. 3, 17/9 2004

Appendix 1. Test scenario

TEST PLAN OF THE FOURTEENTH OFFICIAL OPCW PROFICIENCY TEST

Test name	**Fourteenth Official OPCW Proficiency Test**
Organising body	**Organisation for the Prohibition of Chemical Weapons** Johan de Wittlaan 32 2517 JR The Hague The Netherlands
Report delivery address	OPCW Laboratory Heulweg 28-30 2288 GN Rijswijk The Netherlands
Test co-ordinator	Mr. Stefan Mogl Head OPCW Laboratory Tel: +31 15 21 54 605 Fax: +31 15 28 40 679 Email: opcwrij@worldonline.nl
Laboratory preparing the samples	Verification Laboratory Centre for Chemical Defence (CCD) DSO National Laboratories Block 6, 11 Stockport Road Singapore 117605 Republic of Singapore
Laboratory evaluating the results	Swedish Defence Research Agency (FOI) Division of NBC Defence Cementvägen 20 SE-901 82 UMEÅ Sweden
Test start time	**Sample dispatch:** 8 October 2003
Test time for analysis and reporting	**15 calendar days** according to the following example: If samples arrive at the laboratory site on 9 October (day 1) the test period ends on 23 October (day 15). Please ascertain that the post or courier date stamp on the report envelope is readable.
Estimated time frame of the test	- Identity of spiking chemicals released: **17 November 2003**[1] - Meeting to discuss preliminary results: **28 February 2004**

[1] This date applies only if the test time for all participants has expired.

Test scenario	A State Party has presented in accordance with Article IX of the Convention an inspection request for a challenge inspection. The Executive Council did not decide against this request, and the Director-General in accordance with Part X of the Verification Annex sent a challenge inspection team to a certain facility that has been accused of producing chemical warfare agents. The facility is described by the inspection team as being a multi-purpose plant with a well equipped analytical and synthesis laboratory. The inspection team collected two samples inside the laboratory. One from a container labelled "Decon" and one from an unlabelled container of which the content seemed to be organic. The inspection team collected a third sample from the water treatment plant of the facility. The inspection team did not analyse the samples on-site and forwarded them directly for off-site analysis. Please analyse the samples for the presence of **any Scheduled chemicals and/or their degradation/reaction products**, taking into account the characteristics of the samples.
Samples	**Three samples coded as follows**: • **D:** Sample from container "Decon" • **O:** Sample appearing to be organic • **W:** Water sample from the water treatment plant **with their corresponding blanks :** • **DB** • **OB** • **WB**
Instructions and reporting	• Test Plan (this document) • Instructions (attached) • Electronic reporting-templates are forwarded to each participant **via email**. Please use **only these** templates for reporting.
Attachments	**Newly revised versions** of the proficiency test procedures. Please note the coding of the documents has changed from previously '.../PRO00x' to '.../PT1-3': 1. "Standard Operating Procedure (SOP) for the Organisation of OPCW Proficiency Tests", QDOC/LAB/SOP/PT1; 2. "Work Instruction for the Preparation of Test samples for OPCW Proficiency Tests", QDOC/LAB/WI/PT2; 3. "Work Instruction for the Evaluation of Results of OPCW Proficiency Tests", QDOC/LAB/WI/PT3.

Appendix 2. Instrument performance criteria form

Quality Control

Gaschromatography/Masspectrometri-EI

Date:	
Instrument:	
Signatur:	

GC-parameters

Column: ..... Serial no .....

S/N-test

Compound	S/N	Requirement	Passed
Pentadekan, C15 (5 ng)		100:1	

Column-test

Compound	Asymmetri at ½ height	Requirement	Passed
D_3MMP (10ng)		<5	
Dibensotiofen (5 ng)		<1,5	

Retention times

Compound	Today	Last control.....
Dibensotiofen		
Malation		

MS-parameters

Correct spectrum (compared to library) (5-klor-2metylanilin)

Library: .....Quality, Fit: .....

Compound	Isotopes	Isotope quota	Requirement	Passed
5-klor-2-metylanilin	141/143		33±10%	

ORGANISATION FOR THE PROHIBITION OF CHEMICAL WEAPONS

Report of the Fourteenth Official OPCW Proficiency Test

Laboratory code: 28, 29

Total number of pages:[1]

[1] Total number of pages including cover page and all attachments

TABLE OF CONTENTS

SUMMARY: PARTICIPATING LABORATORY

1. Participating laboratory

Laboratory code:	28, 29
Name of the laboratory/institute participating in the test:	Swedish Research Defence Agency
Contact person:	Dr. Crister Åstot
Address:	SE-901 82 Umeå, SWEDEN
Telephone number:	+46 90 106600
Fax number:	+46 90 106800
Email address:	crister.astot@foi.se

2. Analysts and authentication

	Name	Title	Pages*	Date**	Signature**
1	Yvonne Nygren	Research Engineer			
2	Lars-Gunnar Hammarström	Research Manager			
3	Sten-Åke Fredriksson	Research Manager			
4	Crister Åstot	Research Manager			
5					
6					
7					
8					
9					
10					

* Page numbers defining the responsibility area of the analyst;
** Date and signature of the responsible analyst;

SUMMARY: QUALITY ASSURANCE / QUALITY CONTROL (QA/QC)

1. Status of the laboratory (tick where applicable)

X Accreditation accepted:
Year 1997 Accreditation body: SWEDAC
Scope of accreditation: Analysis of Chemical Warfare Agents and Related Compounds

☐ Accreditation planned/pending:
Target year _____ Accreditation body: _____
Scope of accreditation: _____

☐ Not accredited.

2. Quality system (tick where applicable)

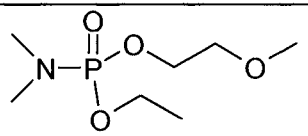
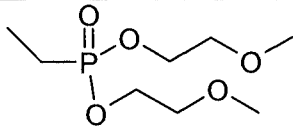
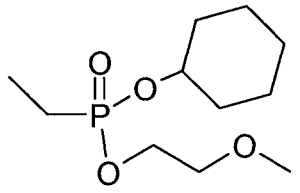
X Described in a Quality Assurance Manual/Handbook. Quality system in accordance with:
☐ ISO 900_____, ☐ EN 4500_____, X ISO Guide 17025, ☐ Other: _____

☐ No quality system. Please, fill in question number 3.

3. QA/QC Summary (Summary of the applied quality assurance and quality control (QA/QC) procedures concerning sample preparation, calibration, and analysis. Requested only from laboratories without a quality system).

SUMMARY: NAMES AND STRUCTURES OF ALL REPORTED COMPOUNDS

Laboratory code: 28, 29

Sample code	Compound number*	Compound name	Chemical Abstract Service number	Compound Structure	Molecular formula	Schedule number	Comments**
D28	1	O-ethyl, O-(2-methoxyethyl) N,N-dimethyl-phosphoramidate	-		$C_7H_{18}NO_4P$	2.B.6	
D28	2	Bis-(2-methoxyetyl) ethylphosphonate	170275-34-0		$C_8H_{19}O_5P$	2.B.4	
D28	3	O-cyclohexyl, O-(2-methoxyethyl) ethylphosphonate	-		$C_{11}H_{23}O_4P$	2.B.4	

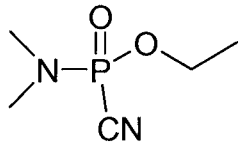
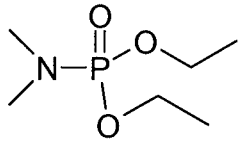
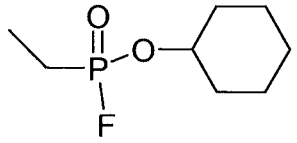
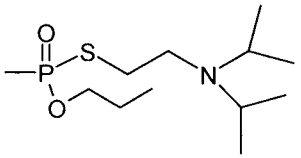
* Compound number defined by the participating laboratory and used throughout the report for the reported compound.

** Explanation for the reporting of non-scheduled compounds, details can be added in the comment section of the report.

Note: There must be an unbroken chain of evidence linking each reported chemical to the original sample from which an aliquot was prepared and analysed for the identification of this chemical.

SUMMARY: NAMES AND STRUCTURES OF ALL REPORTED COMPOUNDS

Laboratory code: 28, 29

Sample code	Compound number*	Compound name	Chemical Abstract Service number	Compound Structure	Molecular formula	Schedule number	Comments* *
O28	4	O-ethyl N,N-dimethylphosphoramido-cyanidate	77-81-6		$C_5H_{11}N_2O_2P$	1.A.2	
O28	5	Diethyl N,N-dimethylphosphoramidate	2404-03-7		$C_6H_{16}NO_3P$	2.B.6	
O28	6	O-cyclohexyl ethylphosphonoflouridate	7284-84-6		$C_8H_{16}FO_2P$	1.A.1	
O28	7	O-propyl S-2-diisopropylaminoethyl methylphosphonothiolate	52364-45-1		$C_{12}H_{28}NO_2PS$	1.A.3	

* Compound number defined by the participating laboratory and used throughout the report for the reported compound.

** Explanation for the reporting of non-scheduled compounds, details can be added in the comment section of the report.

Note: There must be an unbroken chain of evidence linking each reported chemical to the original sample from which an aliquot was prepared and analysed for the identification of this chemical.

SUMMARY: ANALYTICAL TECHNIQUES

Laboratory code: 28, 29 **Sample code(s): D28**

Compoundnumber*	Compound name	Compound analysed as	Analytical technique	Method name	Method page no.	Aliquot name
1	**O-ethyl, O-(2-methoxyethyl), dimethylphosphoramidate**	X original compound ☐ methylated ☐ silylated ☐ other:	GC-EIMS GC-CIMS	RRF60 RRD60_CI	12 15	W13A
2	**Bis-(2-methoxyetyl) ethylphosphonate**	X original compound ☐ methylated ☐ silylated ☐ other: _____	GC-EIMS GC-CIMS	RRF60 RRD60_CI	18 21	W13A
3	**O-cyclohexyl, O-(2-methoxyethyl) ethylphosphonate**	X original compound ☐ methylated ☐ silylated ☐ other: _____	GC-EIMS GC-CIMS	RRF60 RRD60_CI	24 27	W13A

* Compound number defined by the participating laboratory (see Summary: Names and Structures of All Reported Compounds);

SUMMARY: ANALYTICAL TECHNIQUES

Laboratory code: 28, 29 **Sample code(s):** O28

Compound number*	Compound name	Compound analysed as	Analytical technique	Method name	Method page no.	Aliquot name
4	**O-ethyl N,N-dimethylphosphoramido-cyanidate**	X original compound ☐ methylated ☐ silylated ☐ other:	GC-EIMS GC-CIMS	RRF40 RRD40_CI	32 35	L17B
5	**Diethyl N,N-dimethylphosphoramidate**	X original compound ☐ methylated ☐ silylated ☐ other:	GC-EIMS GC-CIMS	RRF40 RRD40_CI	38 41	L17B
6	**O-cyclohexyl ethylphosphonoflouridate**	X original compound ☐ methylated ☐ silylated ☐ other:	GC-EIMS GC-CIMS	RRF40 RRD40_CI	44 47	L17B
7	**O-propyl S-2-diisopropylaminoethyl methylphosphonothiolate**	X original compound ☐ methylated ☐ silylated ☐ other:	GC-EIMS GC-CIMS	RRF40 RRD40_CI	50 55	L11A

* Compound number defined by the participating laboratory (see Summary: Names and Structures of All Reported Compounds);

SAMPLE PREPARATION DESCRIPTION

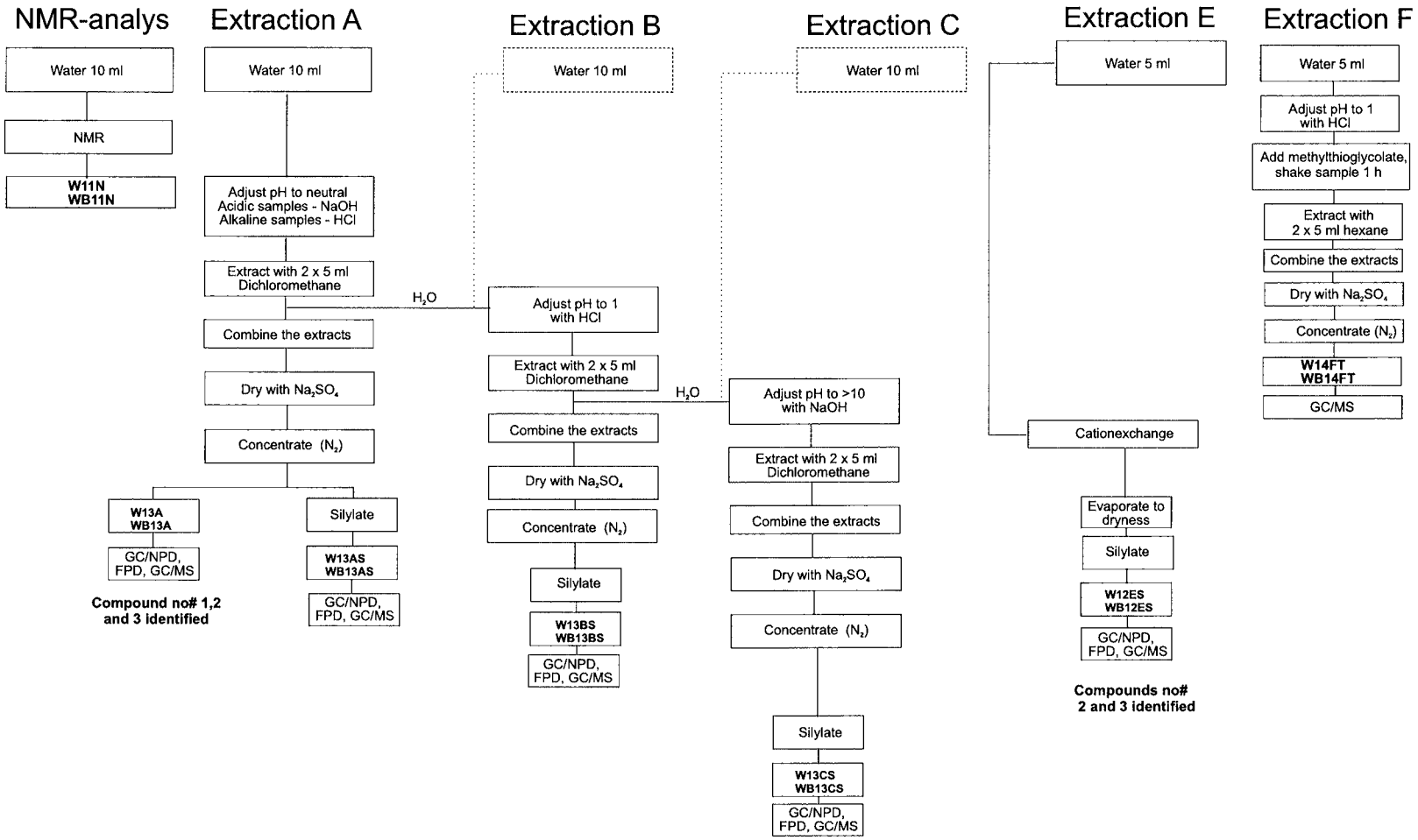
Laboratory code: 28,29 **Sample code:** D/28 **Blank code:** DB/28

1. Sample preparation

Sample/ Aliquot Code	Specification of Sample/ Type of Sample Preparation	Amount/ Volume	Sample Preparation Procedures	End Volume	Resulting Aliquot Code	Analytical Technique
WB12/W12	Evaporation and Derivatization	5 ml	Cation exchange (SCX;Extract-Clean), evaporation to dryness, resolvation in CH3CN, silylation with BSTFA	~1 ml	WB12ES/ W12ES	GC-EIMS GC-CIMS
WB13/W13	Extraction	10 ml	Methylenechloride 2*5 ml at pH 6, concentration with N2	~1.5 ml	WB13A /W13A	GC-EIMS GC-CIMS
			Silylation with BSTFA	~1 ml	WB13AS /W13AS	GC-EIMS GC-CIMS
	Extraction	10 ml	Methylenechloride 2*5 ml at pH 1, concentration with N2 silylation with BSTFA	~1 ml	WB13BS /W13BS	GC-EIMS GC-CIMS
	Extraction	10 ml	Methylenechloride 2*5 ml at ,pH 10, concentration with N2 silylation with BSTFA	~1 ml	WB13CS/ W13CS	GC-EIMS GC-CIMS
WB14/W14	Derivatization and Extraction	5 ml	Derivatization with methylthioglycolate at pH 1, extraction with 2*5 ml hexane, concentration with N2	~1 ml	WB14FT/W14FT	GC-EIMS GC-CIMS

2. Additional information

Sample preparation of Water (Decon) DB/28 and D/28



GC-EI-MS TECHNIQUE METHOD AND ANALYSIS DESCRIPTION

Laboratory code: 28,29 **Sample code(s):** D/28, DB/28 **Compound number:** 1

Aliquot codes:W13A, WB13A			
Sample:	**W13A**	**Blank:**	**WB13A**

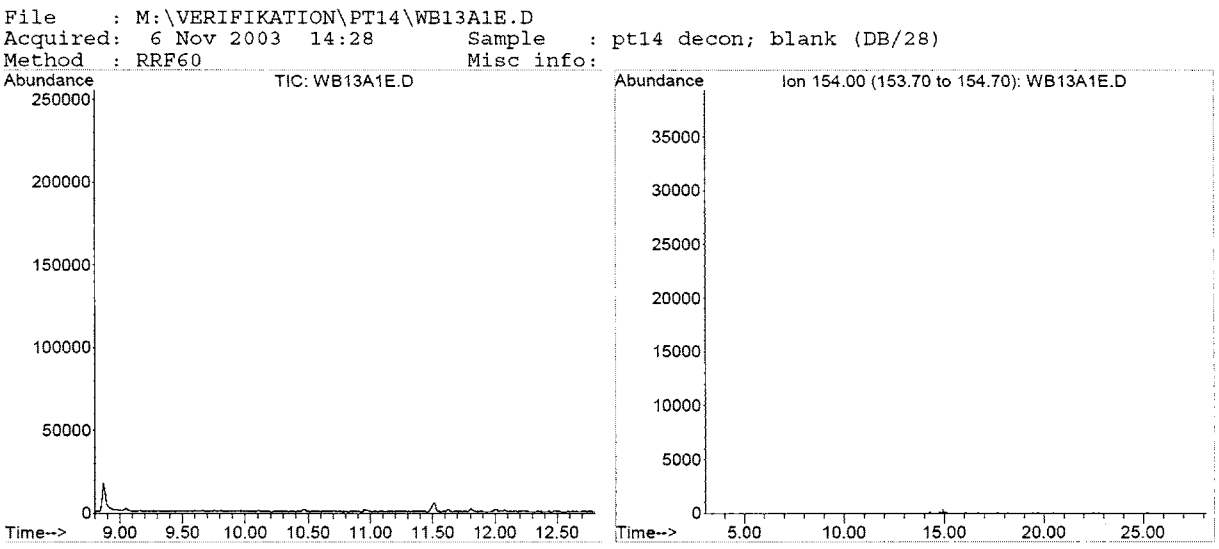
GC-EI-MS Method name:	**RRF60**

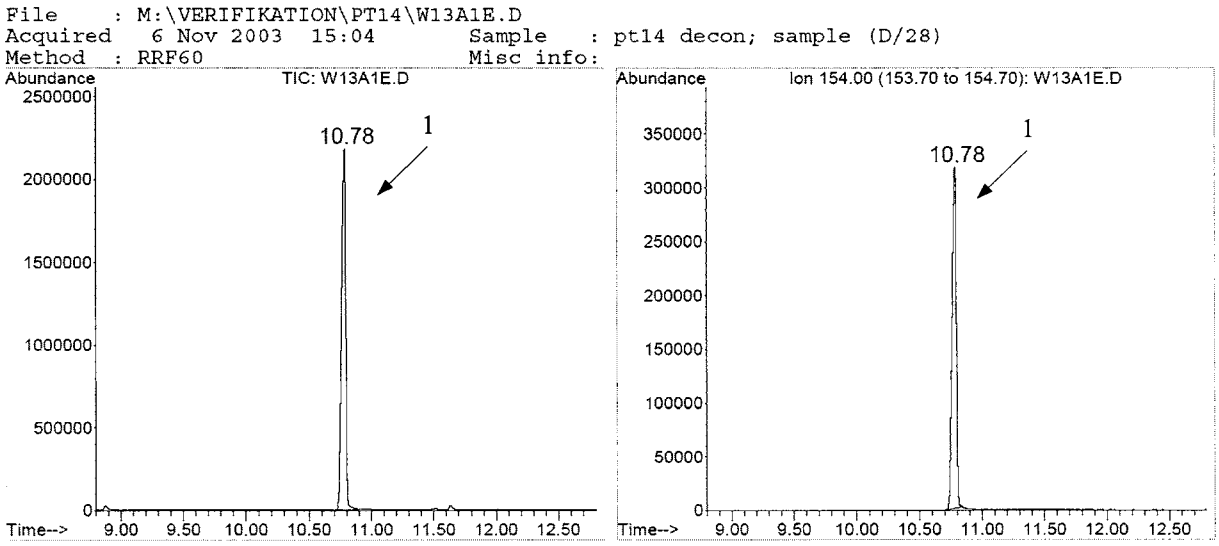
METHOD DESCRIPTION

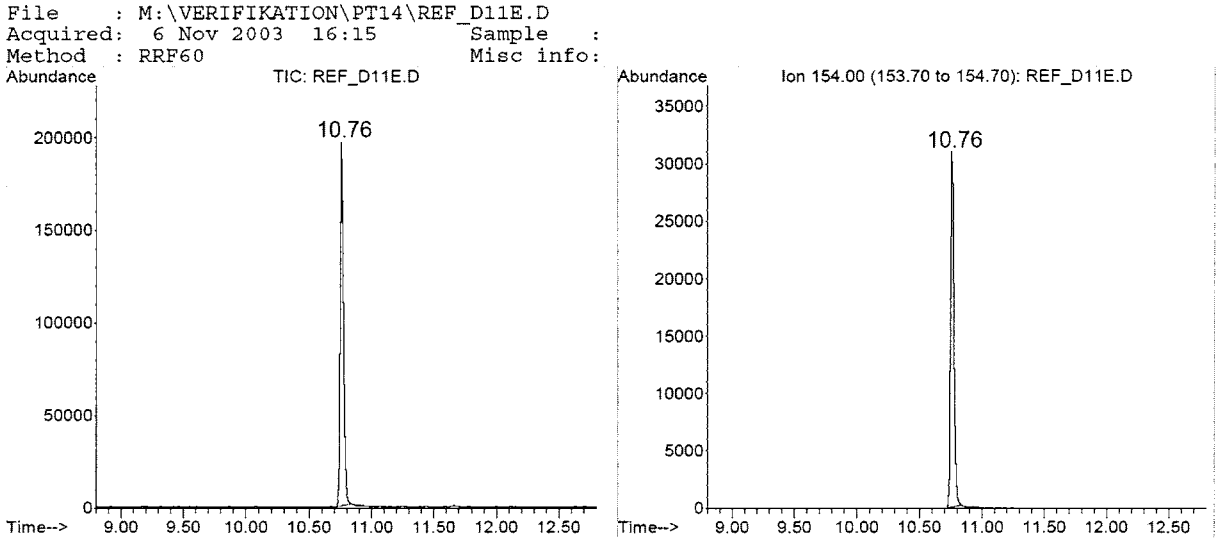
Instrument Manufacturer and Type:	HP 5890GC + HP5972 MSD		
Carrier gas:	X He ☐ N_2 ☐ H_2 ☐ Other:		
Flow rate:	☐ ml/min X 35 cm/s		
Flow control:	☐ Constant Pressure X Constant Flow		
Injection mode:	☐ Split → Split ratio = X Splitless → Splitless time = 1 min		
Injector temperature:	200 °C		
Column phase:	DB5MS		
Column Length x ID x Film thickness:	30 m x 0,25 mm x 0,25 μm		
GC temperature programme:	60 °C (1 min), 10 °C/min, 280 °C (5 min)		
Solvent delay time:	3 min	**Scan range:**	29-450 m/z
Electron energy:	70 eV	**Scan time:**	0,4 s
Ionisation polarity:	X Positive ☐ Negative	**Mass resolution:**	unit
Comments:			

ANALYSIS

Compound identified as:	X Original compound ☐ Methyl ester derivative ☐ TBDMS (t-Butyldimethylsilyl) derivative ☐ TMS (Trimethylsilyl) derivative ☐ Other derivative:
Retention parameter used for (peak) identification:	X Retention time (Rt) 10,76 ☐ Scan number
X Compared to reference chemical:	Source : ☐ Own Synthesis X DSO Singapore
☐ Compared to library spectrum:	Source : ☐ OCAD (code:) ☐ NIST ☐ Wiley ☐ Own ☐ Other:
☐ Not compared to reference chemical or library spectrum:	Intense ions in spectrum are explained; interpretation is supported by the spectral information derived from closely related chemical(s):
Comments:	



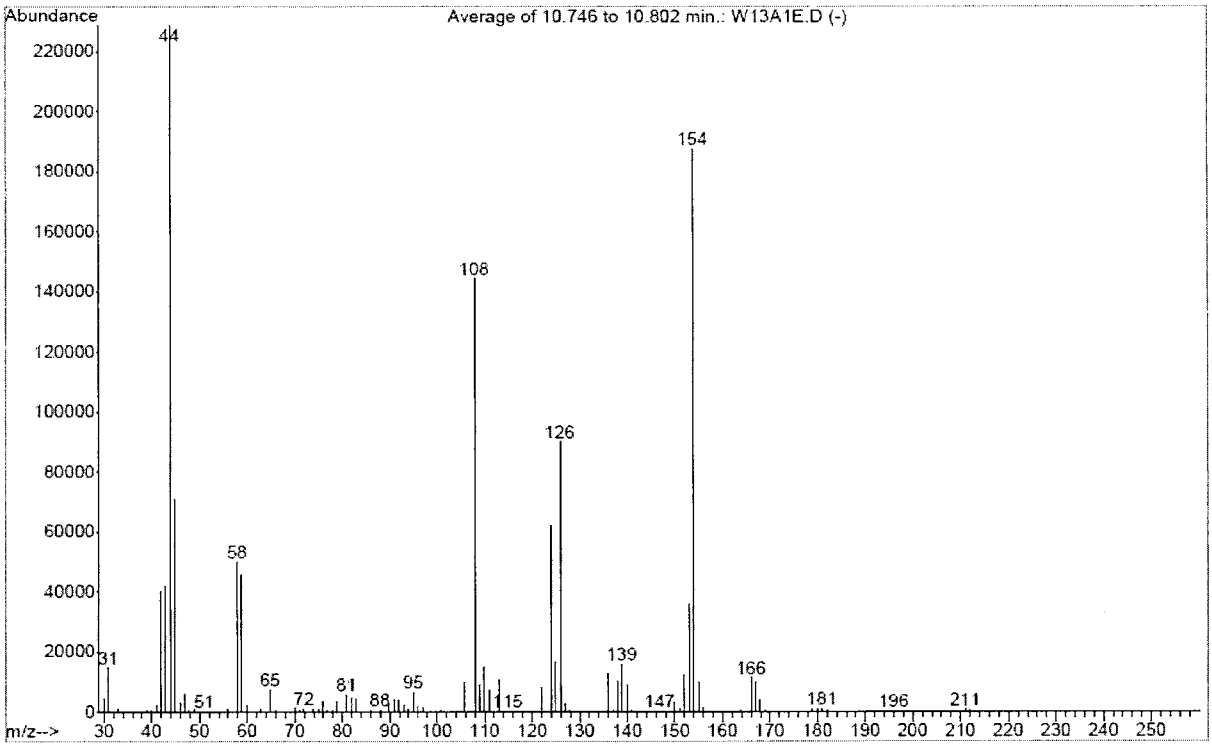




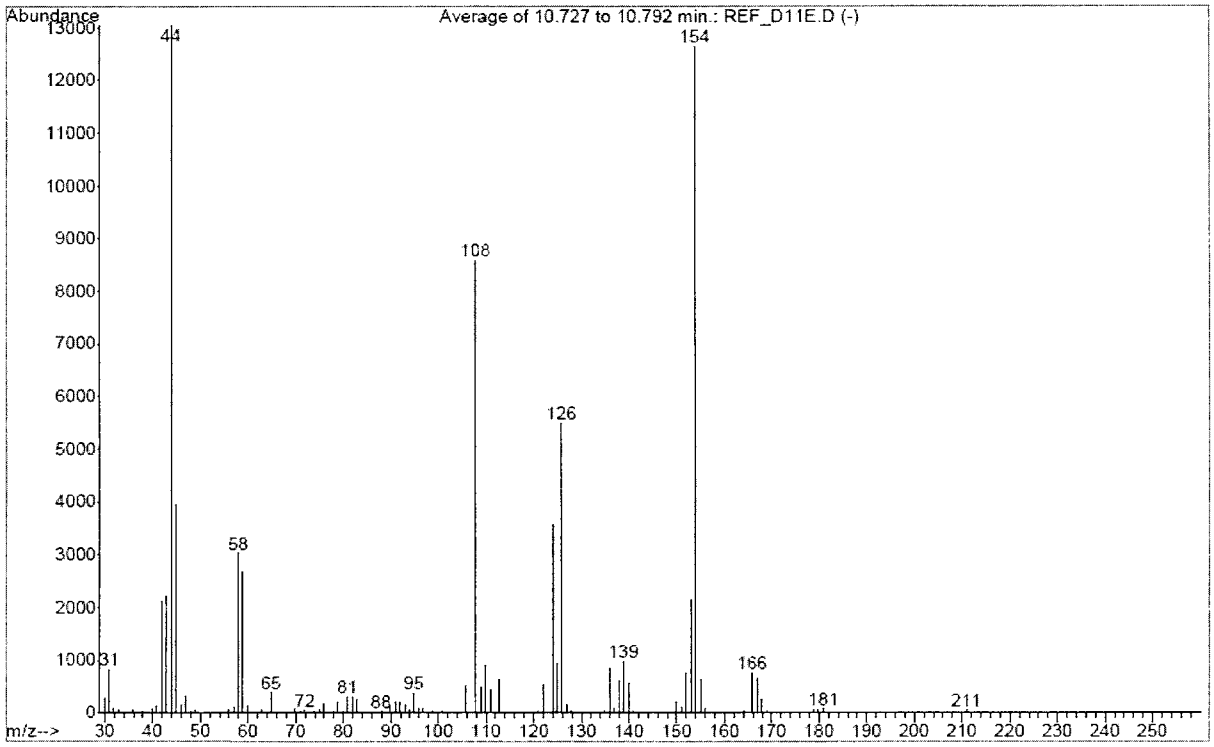
EI chromatograms supporting the identification of chemical 1; TIC on left; EIC (m/z 154) on right.

Top:	Chromatograms of Decon blank, aliquot WB13A from DB/28.
Center:	Chromatograms of Decon sample, aliquot W13A from D/28.
Bottom:	Chromatograms of authentic reference standard of O-ethyl, O-(2-methoxyethyl), dimethylphosphoramidate, retention time 10.76 min.

```
File        : D:\PT14 5972\PT14DATA\W13A1E.D
Acquired    :  6 Nov 2003  15:04      using AcqMethod RRF60
Sample Name: pt14 decon; sample (D/28)
Misc Info   :
```




```
File        : D:\PT14 5972\PT14DATA\REF D11E.D
Acquired    :  6 Nov 2003  16:15      using AcqMethod RRF60
Sample Name:
Misc Info   :
```



EI mass spectra (averaged and background subtracted) of:	
Top:	Chemical 1 in Decon sample, aliquot W13A from D/28
Bottom:	Authentic reference standard of O-ethyl, O-(2-methoxyethyl), dimethylphosphoramidate corresponding to chemical 1 (Monoisotopic MW 211)

GC-CI-MS TECHNIQUE
METHOD AND ANALYSIS DESCRIPTION

Laboratory code: 28, 29 **Sample code(s):** D/28, DB/28 **Compound number:** 1

Aliquot codes: W13A, WB13A			
Sample:	**W13A**	**Blank:**	**WB13A**

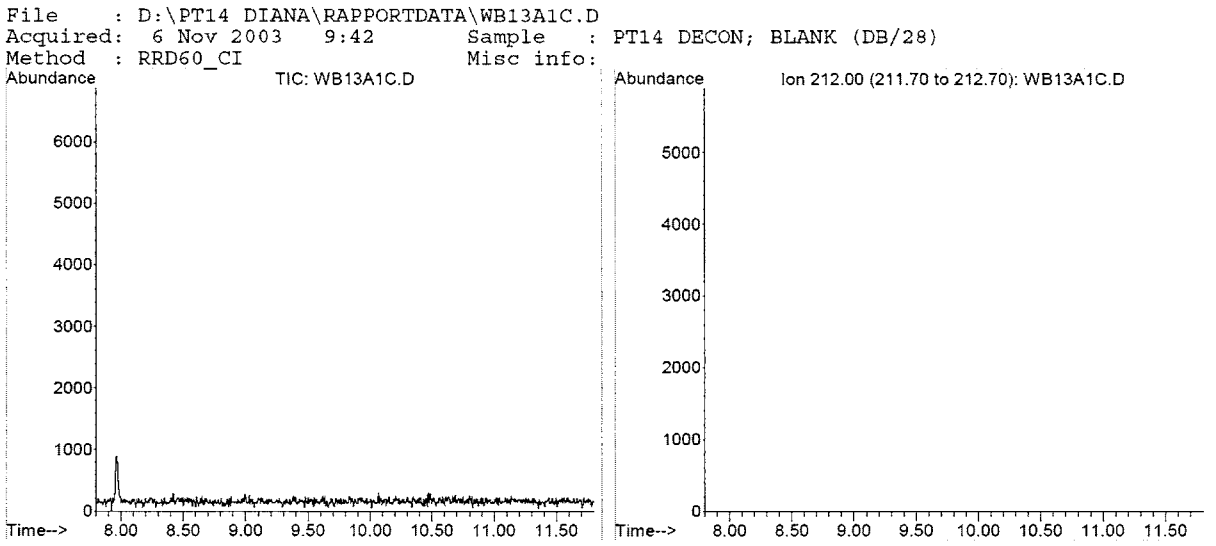
GC-CI-MS Method name:	**RRD60_CI**

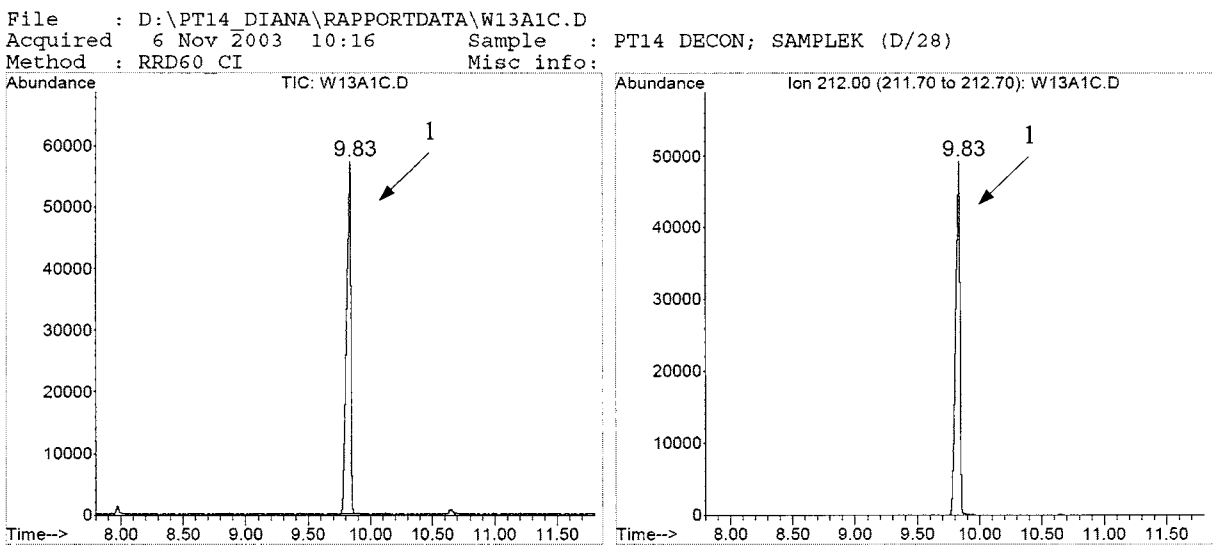
METHOD DESCRIPTION

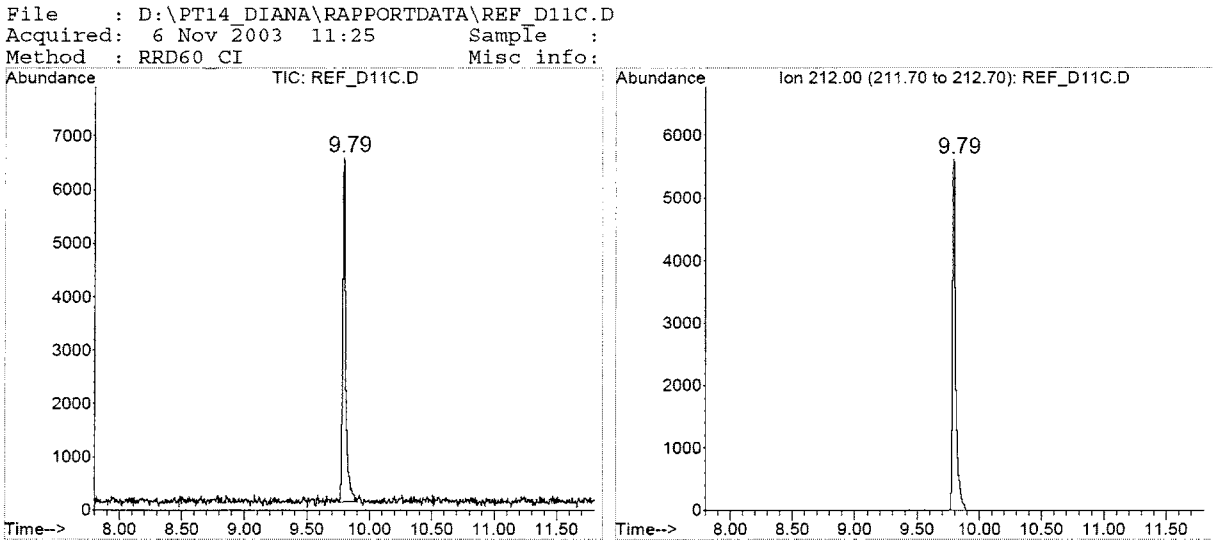
Instrument Manufacturer and Type:	HP6890 + HP 5973N (MSD)		
Carrier gas:	X He ☐ N_2 ☐ H_2 ☐ Other:		
Flow rate:	☐ ml/min X 35 cm/s		
Flow control:	☐ Constant Pressure X Constant Flow		
Injection mode:	☐ Split → Split ratio = X Splitless → Splitless time = 1 min		
Injector temperature:	200 °C		
Column phase:	DB5MS		
Column Length x ID x Film thickness:	30 m x 0,25 mm x 0,25 µm		
GC temperature programme:	60 °C (1 min), 10 °C/min, 280 °C (5 min)		
Reaction gas:	☐ Methane X Isobutane ☐ Ammonia ☐ Other:		
Solvent delay time:	4 min	**Scan range:**	60-500 m/z
Electron energy:	170 eV	**Scan time:**	0,6 s
Ionisation polarity:	X Positive ☐ Negative	**Mass resolution:**	Unit
Comments:			

ANALYSIS

Compound identified as:	X Original compound ☐ Methyl ester derivative ☐ TBDMS (t-Butyldimethylsilyl) derivative ☐ TMS (Trimethylsilyl) derivative ☐ Other derivative:
Retention parameter used for (peak) identification:	X Retention time (Rt) 9,83 ☐ Scan number
X Compared to reference chemical:	Source : ☐ Own Synthesis X DSO Singapore
☐ Not compared to reference chemical	Intense ions in spectrum are explained
Comments:	

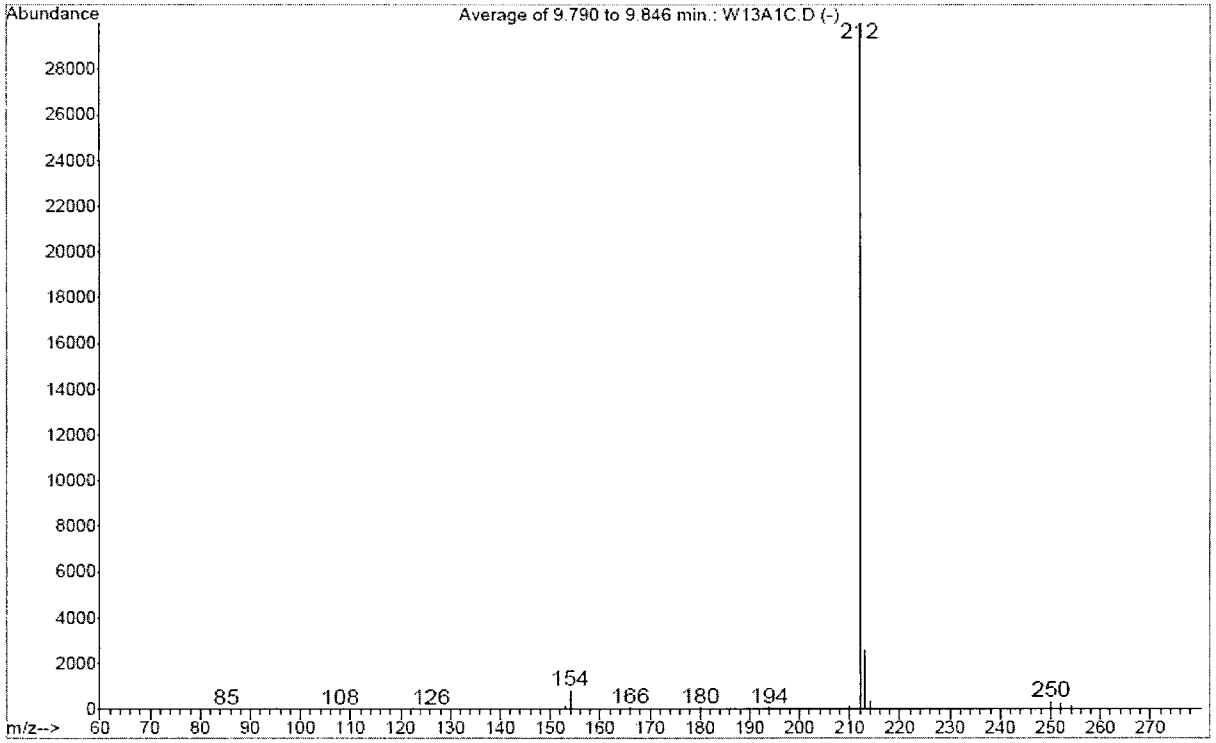




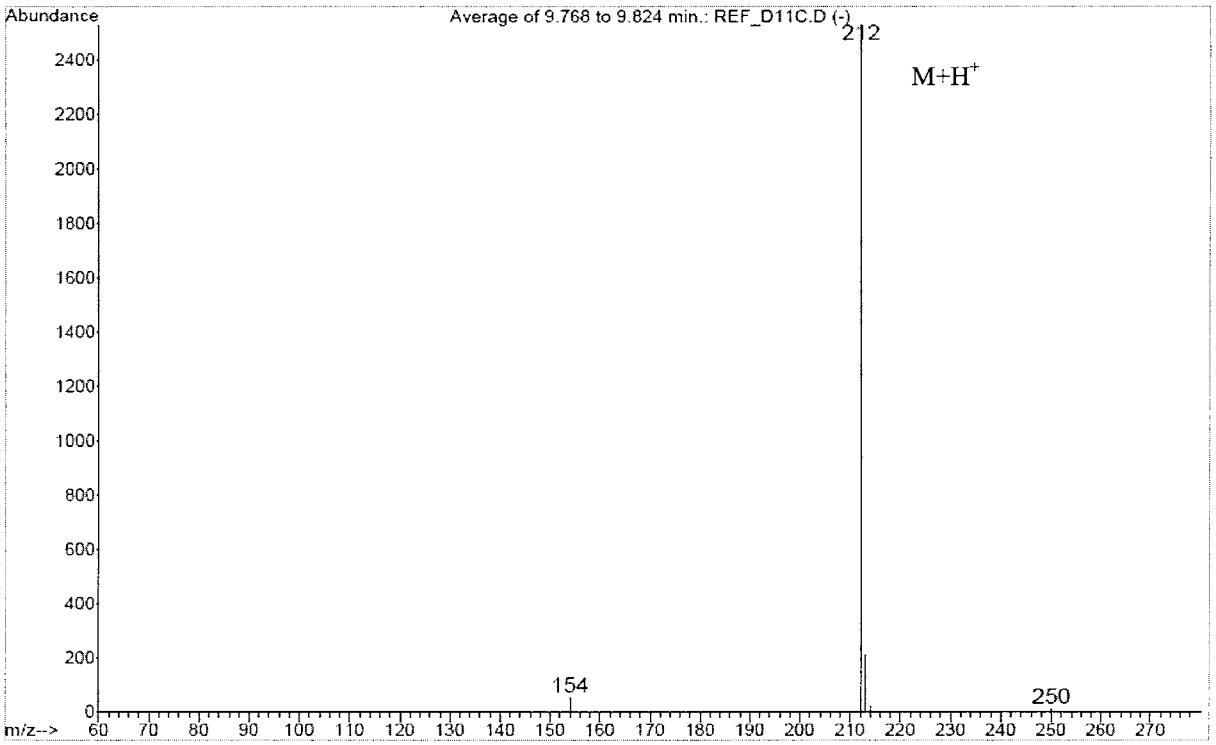


CI chromatograms supporting the identification of chemical 1; TIC on left; EIC (m/z 212 on right.	
Top:	Chromatograms of Decon blank, aliquot WB13A from DB/28.
Center:	Chromatograms of Decon sample, aliquot W13A from D/28.
Bottom:	Chromatograms of authentic reference standard of O-ethyl, O-(2-methoxyethyl), dimethylphosphoramidate, retention time 9.79 min.

```
File        : D:\PT14 DIANA\RAPPORTDATA\W13A1C.D
Acquired    :  6 Nov 2003  10:16      using AcqMethod RRD60 CI
Sample Name: PT14 DECON; SAMPLEK (D/28)
Misc Info   :
```




```
File        : D:\PT14 DIANA\RAPPORTDATA\REF D11C.D
Acquired    :  6 Nov 2003  11:25      using AcqMethod RRD60 CI
Sample Name:
Misc Info   :
```



CI mass spectra (averaged and background subtracted) of:	
Top:	Chemical 1 in Decon sample, aliquot W13A from D/28
Bottom:	Authentic reference standard of O-ethyl, O-(2-methoxyethyl), dimethylphosphoramidate corresponding to chemical 1 (monoisotopic MW:211)

GC-EI-MS TECHNIQUE METHOD AND ANALYSIS DESCRIPTION

Laboratory code: 28,29 **Sample code(s):** D/28, DB/28 **Compound number:** 2

Aliquot codes: W13A, WB13A			
Sample:	**W13A**	**Blank:**	**WB13A**

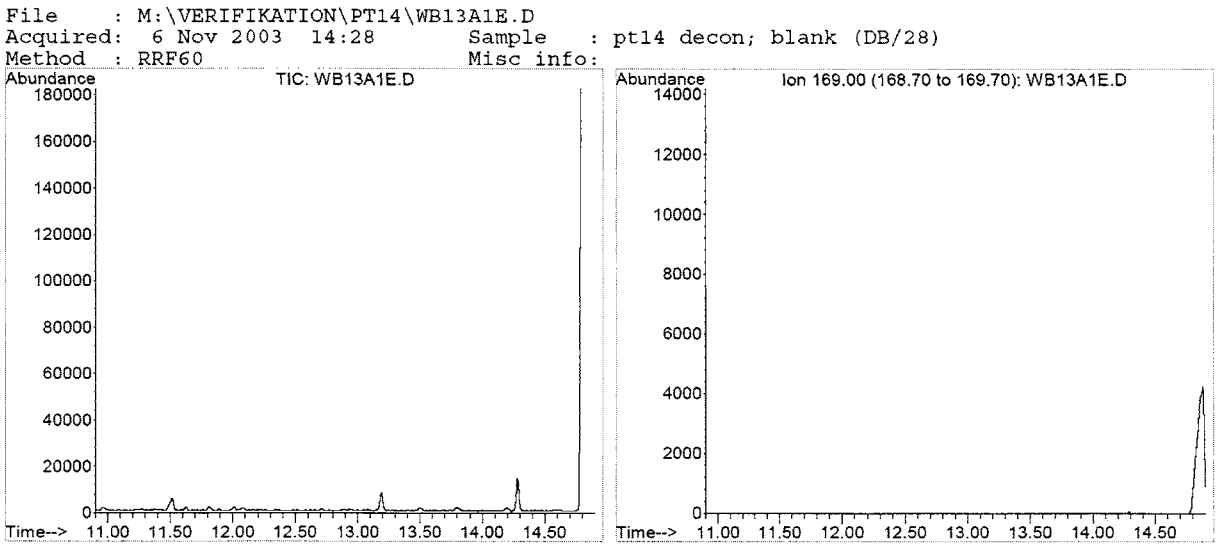
GC-EI-MS Method name:	**RRF60**

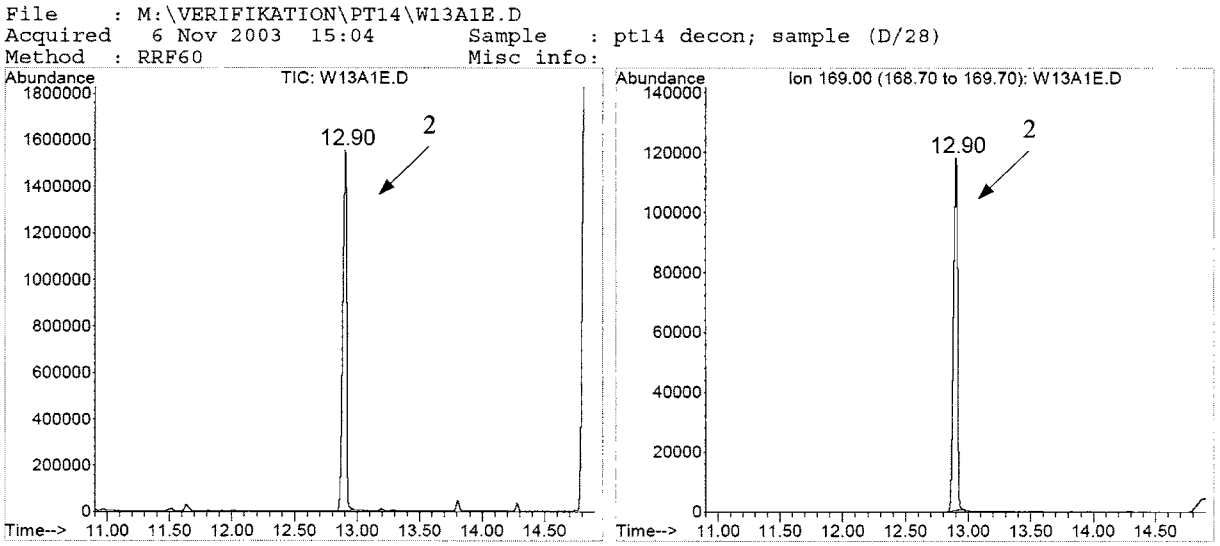
METHOD DESCRIPTION

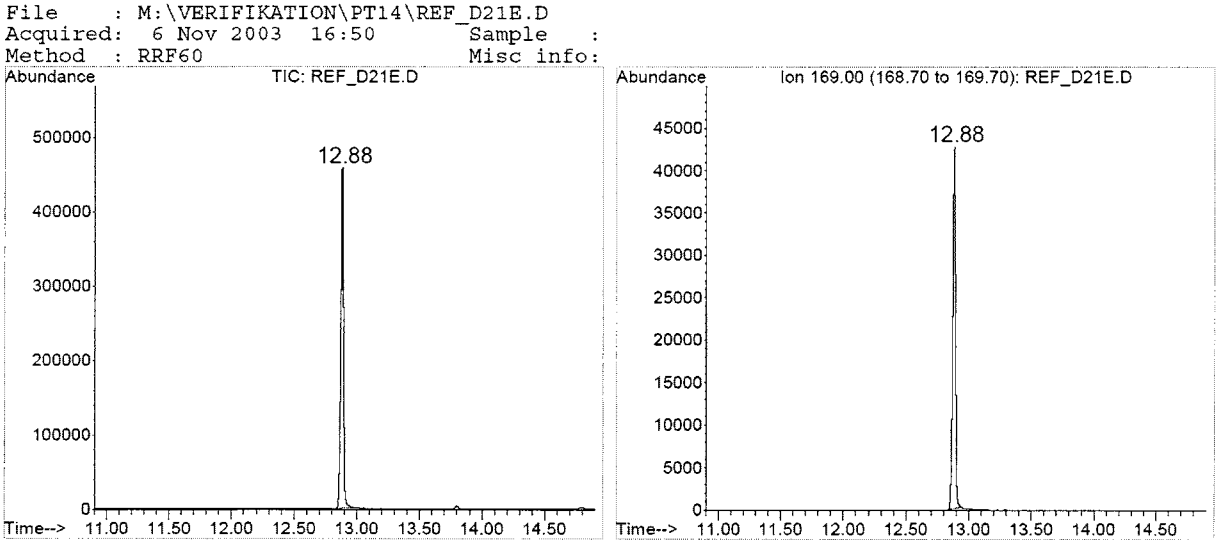
Instrument Manufacturer and Type:	HP 5890GC + HP5972 MSD		
Carrier gas:	X He ☐ N_2 ☐ H_2 ☐ Other:		
Flow rate:	☐ ml/min X 35 cm/s		
Flow control:	☐ Constant Pressure X Constant Flow		
Injection mode:	☐ Split → Split ratio = X Splitless → Splitless time = 1 min		
Injector temperature:	200 °C		
Column phase:	DB5MS		
Column Length x ID x Film thickness:	30 m x 0,25 mm x 0,25 μm		
GC temperature programme:	60 °C (1 min), 10 °C/min, 280 °C (5 min)		
Solvent delay time:	3 min	**Scan range:**	29-450 m/z
Electron energy:	70 eV	**Scan time:**	0,4 s
Ionisation polarity:	X Positive ☐ Negative	**Mass resolution:**	unit
Comments:			

ANALYSIS

Compound identified as:	X Original compound ☐ Methyl ester derivative ☐ TBDMS (t-Butyldimethylsilyl) derivative ☐ TMS (Trimethylsilyl) derivative ☐ Other derivative:
Retention parameter used for (peak) identification:	X Retention time (Rt) 12,88 ☐ Scan number
X Compared to reference chemical:	Source : ☐ Own Synthesis X DSO Singapore
☐ Compared to library spectrum:	Source : ☐ OCAD (code:) ☐ NIST ☐ Wiley ☐ Own ☐ Other:
☐ Not compared to reference chemical or library spectrum:	Intense ions in spectrum are explained; interpretation is supported by the spectral information derived from closely related chemical(s):
Comments:	



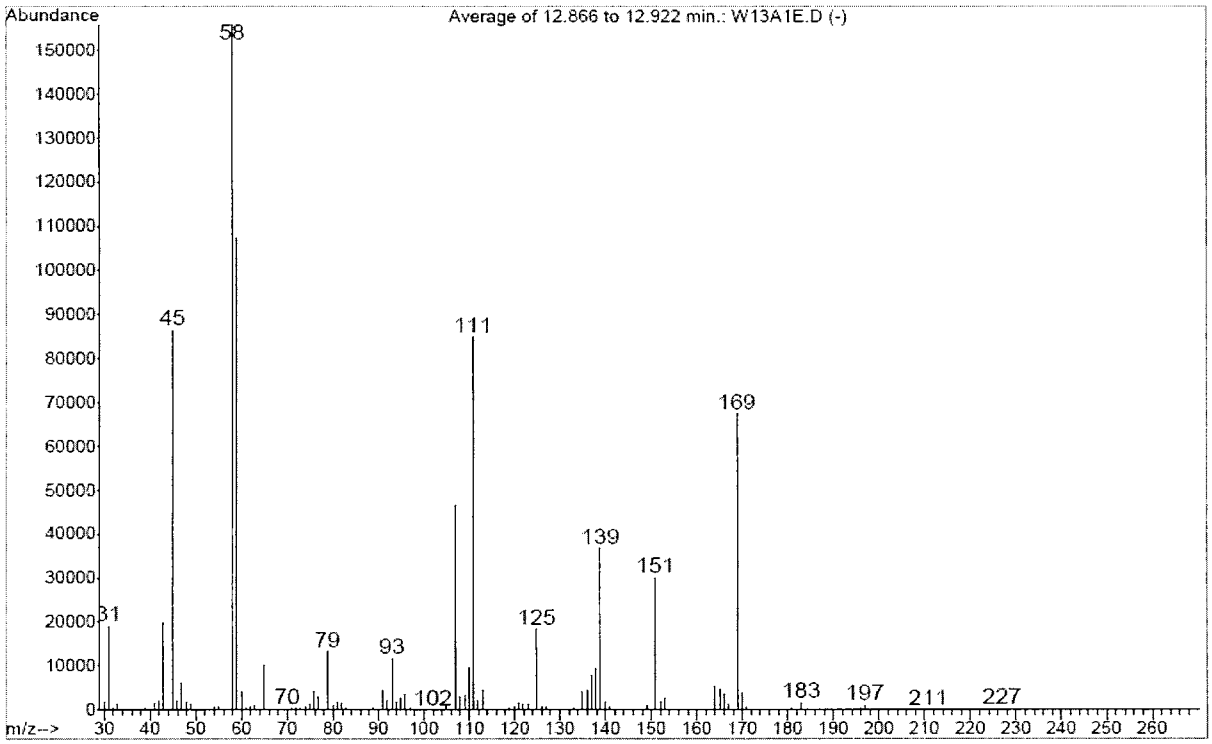




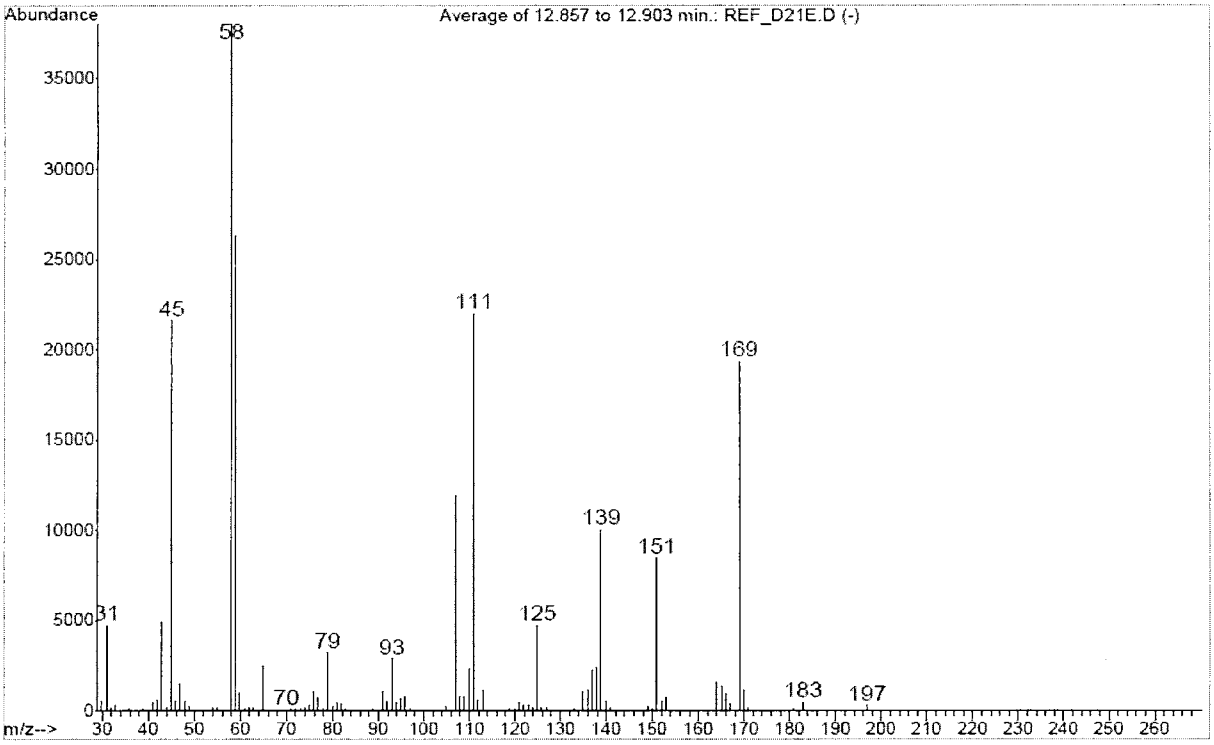
EI chromatograms supporting the identification of chemical 2; TIC on left; EIC (m/z 169) on right.

Top:	Chromatograms of Decon blank, aliquot WB13A from DB/28.
Center:	Chromatograms of Decon sample, aliquot W13A from D/28.
Bottom:	Chromatograms of authentic reference standard of Bis-(2-methoxyethyl)ethylphosphonate, retention time 12.88 min.

```
File       : D:\PT14 5972\PT14DATA\W13A1E.D
Acquired   :  6 Nov 2003  15:04     using AcqMethod RRF60
Sample Name: pt14 decon; sample (D/28)
Misc Info  :
```




```
File       : D:\PT14 5972\PT14DATA\REF D21E.D
Acquired   :  6 Nov 2003  16:50     using AcqMethod RRF60
Sample Name:
Misc Info  :
```



EI mass spectra (averaged and background subtracted) of:	
Top:	Chemical 2 in Decon sample, aliquot W13A from D/28
Bottom:	Authentic reference standard of Bis-(2-methoxyethyl)ethylphosphonate corresponding to chemical 2 (Monoisotopic MW:226)

GC-CI-MS TECHNIQUE METHOD AND ANALYSIS DESCRIPTION

Laboratory code: 28, 29 **Sample code(s):** D/28, DB/28 **Compound number:** 2

Aliquot codes: W13A, WB13A			
Sample:	**W13A**	**Blank:**	**WB13A**

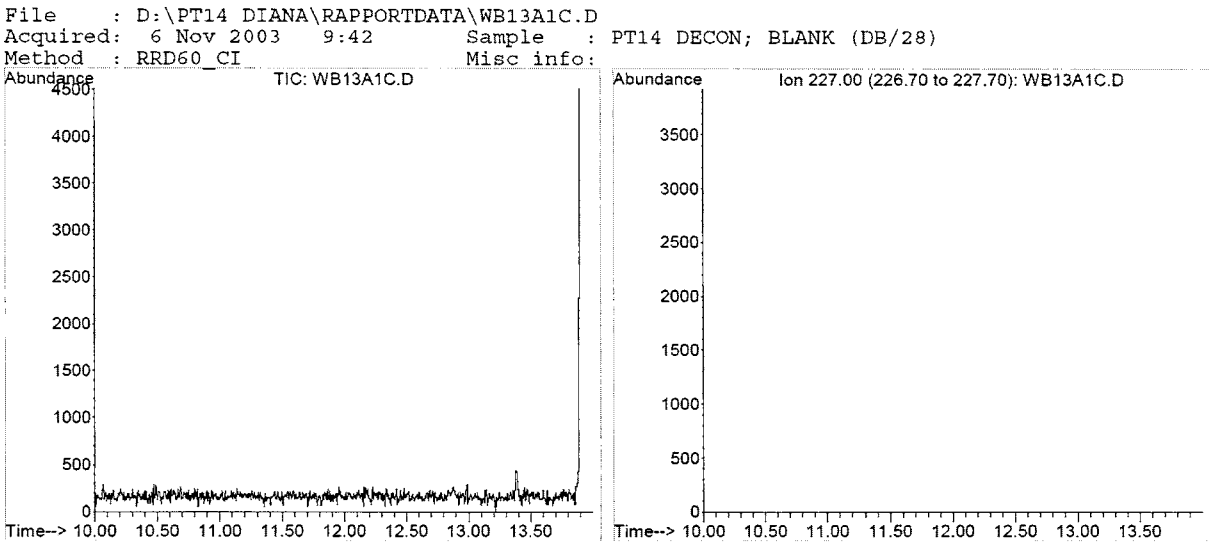
GC-CI-MS Method name:	**RRD60_CI**

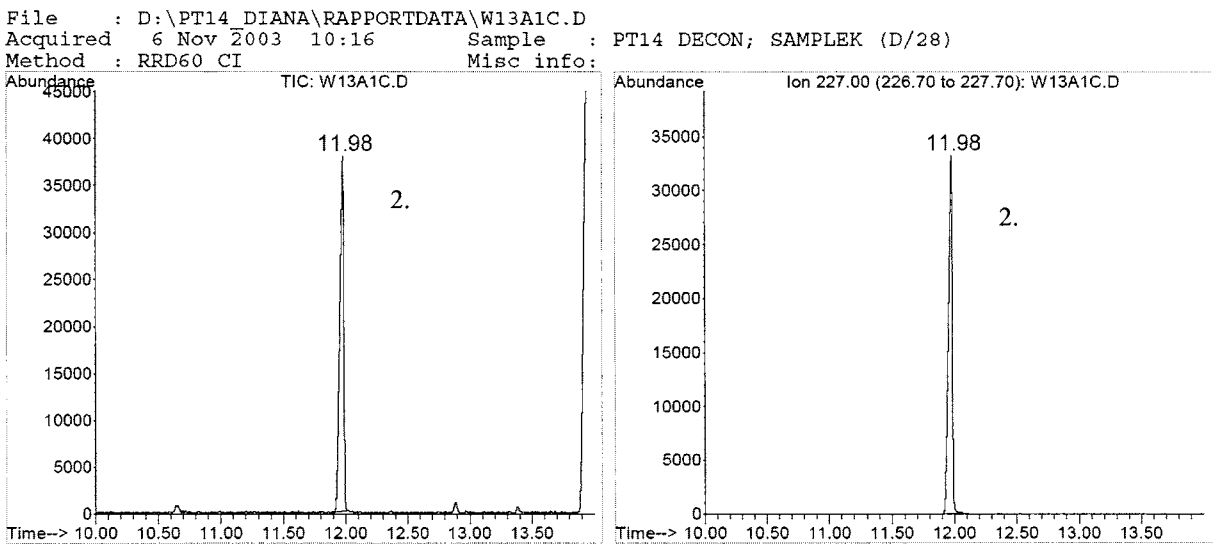
METHOD DESCRIPTION

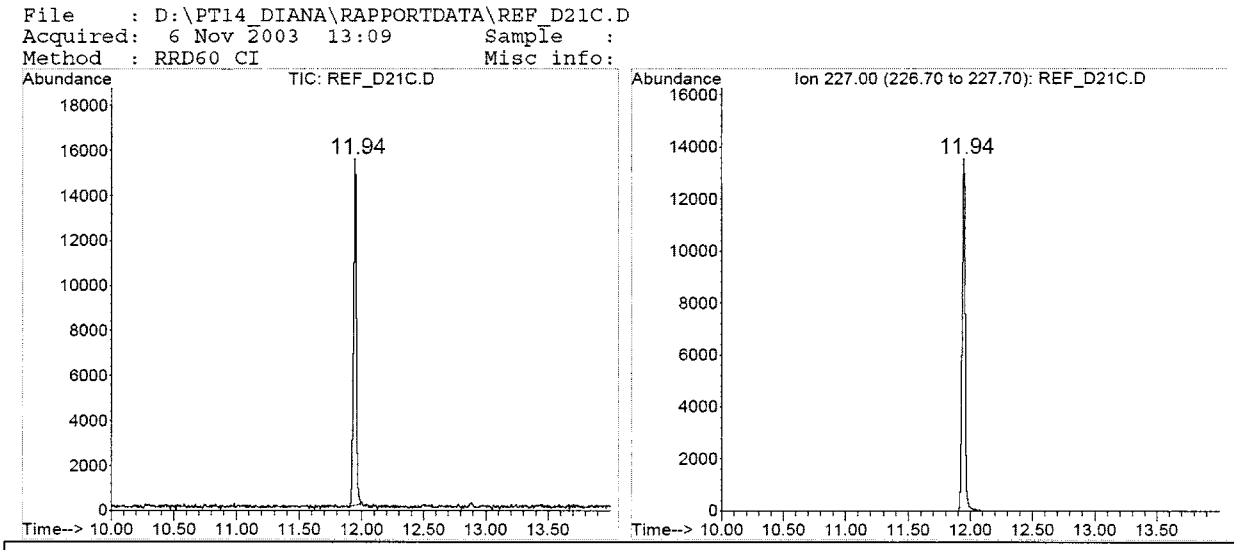
Instrument Manufacturer and Type:	HP6890 + HP 5973N (MSD)		
Carrier gas:	X He ☐ N_2 ☐ H_2 ☐ Other:		
Flow rate:	☐ ml/min X 35 cm/s		
Flow control:	☐ Constant Pressure X Constant Flow		
Injection mode:	☐ Split → Split ratio = X Splitless → Splitless time = 1 min		
Injector temperature:	200 °C		
Column phase:	DB5MS		
Column Length x ID x Film thickness:	30 m x 0,25 mm x 0,25 µm		
GC temperature programme:	60 °C (1 min), 10 °C/min, 280 °C (5 min)		
Reaction gas:	☐ Methane X Isobutane ☐ Ammonia ☐ Other:		
Solvent delay time:	4 min	**Scan range:**	60-500 m/z
Electron energy:	170 eV	**Scan time:**	0,6 s
Ionisation polarity:	X Positive ☐ Negative	**Mass resolution:**	Unit
Comments:			

ANALYSIS

Compound identified as:	X Original compound ☐ Methyl ester derivative ☐ TBDMS (t-Butyldimethylsilyl) derivative ☐ TMS (Trimethylsilyl) derivative ☐ Other derivative:
Retention parameter used for (peak) identification:	X Retention time (Rt) 11,98 ☐ Scan number
X Compared to reference chemical:	Source : ☐ Own Synthesis X DSO Singapore
☐ Not compared to reference chemical	Intense ions in spectrum are explained
Comments:	



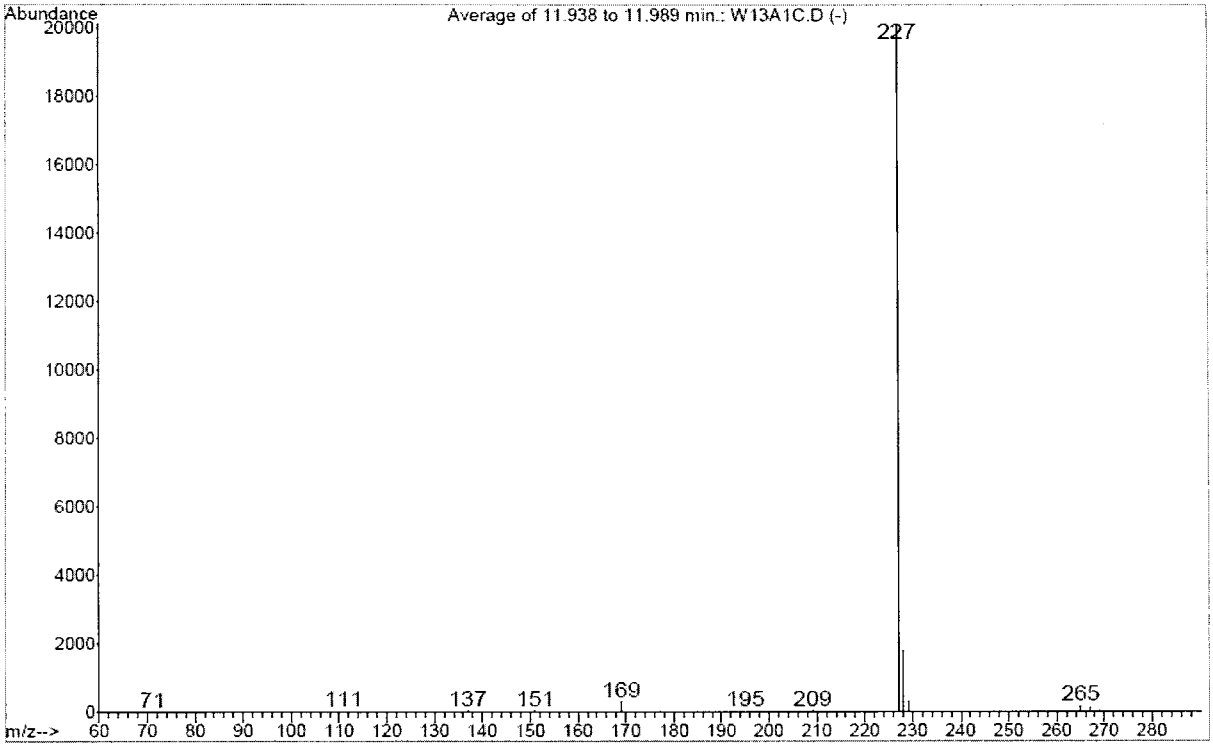




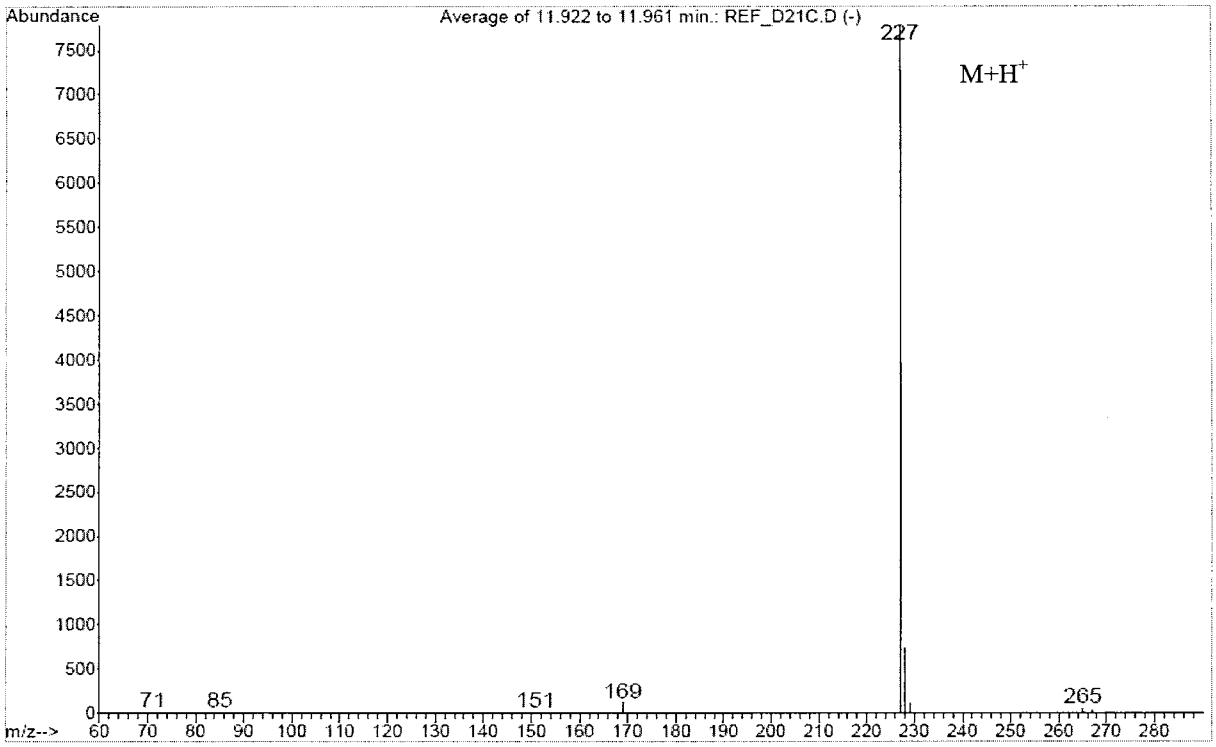
CI chromatograms supporting the identification of chemical 2; TIC on left; EIC (m/z 227 on right.

Top:	Chromatograms of Decon blank, aliquot WB13A from DB/28.
Center:	Chromatograms of Decon sample, aliquot W13A from D/28.
Bottom:	Chromatograms of silylated authentic reference standard of Bis-(2-methoxyethyl)ethylphosphonate, retention time 11.94 min.

```
File       : D:\PT14 DIANA\RAPPORTDATA\W13A1C.D
Acquired   :  6 Nov 2003  10:16     using AcqMethod RRD60 CI
Sample Name: PT14 DECON; SAMPLEK (D/28)
Misc Info  :
```




```
File       : D:\PT14 DIANA\RAPPORTDATA\REF D21C.D
Acquired   :  6 Nov 2003  13:09     using AcqMethod RRD60 CI
Sample Name:
Misc Info  :
```



CI mass spectra (averaged and background subtracted) of:	
Top:	Chemical 2 in Decon sample, aliquot W13A from D/28
Bottom:	Authentic reference standard of Bis-(2-methoxyethyl)ethylphosphonate corresponding to chemical 2 (monoisotopic MW:226)

GC-EI-MS TECHNIQUE METHOD AND ANALYSIS DESCRIPTION

Laboratory code: 28,29 **Sample code(s):** D/28, DB/28 **Compound number:** 3

Aliquot codes:W13A, WB13A			
Sample:	**W13A**	**Blank:**	**WB13A**

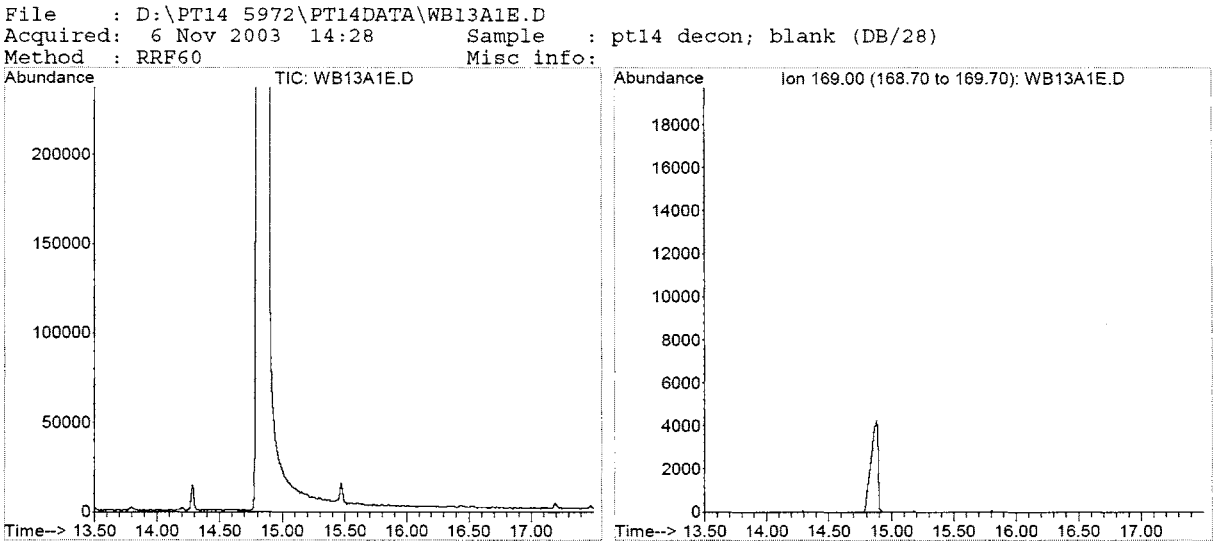
GC-EI-MS Method name:	**RRF60**

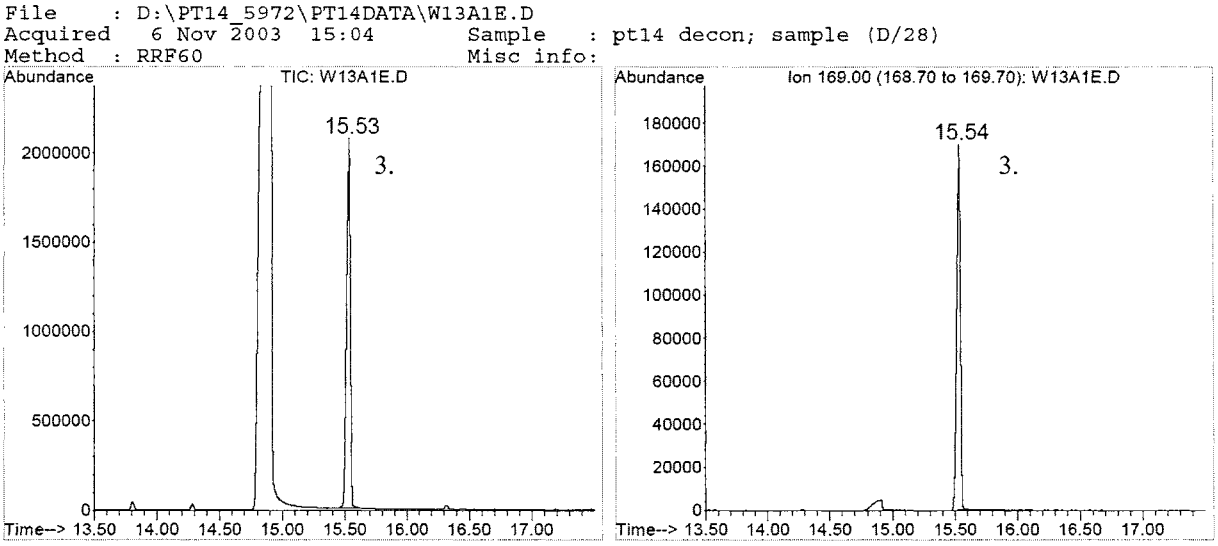
METHOD DESCRIPTION

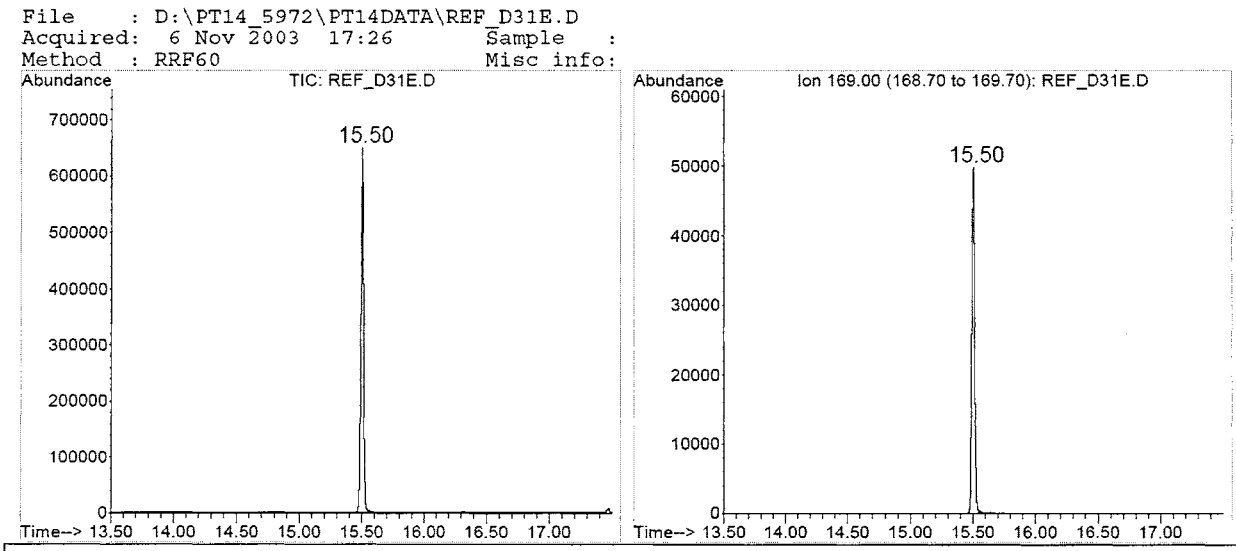
Instrument Manufacturer and Type:	HP 5890GC + HP5972 MSD		
Carrier gas:	x He ☐ N_2 ☐ H_2 ☐ Other:		
Flow rate:	☐ ml/min x 35 cm/s		
Flow control:	☐ Constant Pressure x Constant Flow		
Injection mode:	☐ Split → Split ratio = x Splitless → Splitless time = 1 min		
Injector temperature:	200 °C		
Column phase:	DB5MS		
Column Length x ID x Film thickness:	30 m x 0,25 mm x 0,25 μm		
GC temperature programme:	60 °C (1 min), 10 °C/min, 280 °C (5 min)		
Solvent delay time:	3 min	**Scan range:**	29-450 m/z
Electron energy:	70 eV	**Scan time:**	0,4 s
Ionisation polarity:	x Positive ☐ Negative	**Mass resolution:**	unit
Comments:			

ANALYSIS

Compound identified as:	x Original compound ☐ Methyl ester derivative ☐ TBDMS (t-Butyldimethylsilyl) derivative ☐ TMS (Trimethylsilyl) derivative ☐ Other derivative:
Retention parameter used for (peak) identification:	x Retention time (Rt) 15,50 ☐ Scan number
x Compared to reference chemical:	Source : ☐ Own Synthesis x DSO Singapore
☐ Compared to library spectrum:	Source : ☐ OCAD (code:) ☐ NIST ☐ Wiley ☐ Own ☐ Other:
☐ Not compared to reference chemical or library spectrum:	Intense ions in spectrum are explained; interpretation is supported by the spectral information derived from closely related chemical(s):
Comments:	

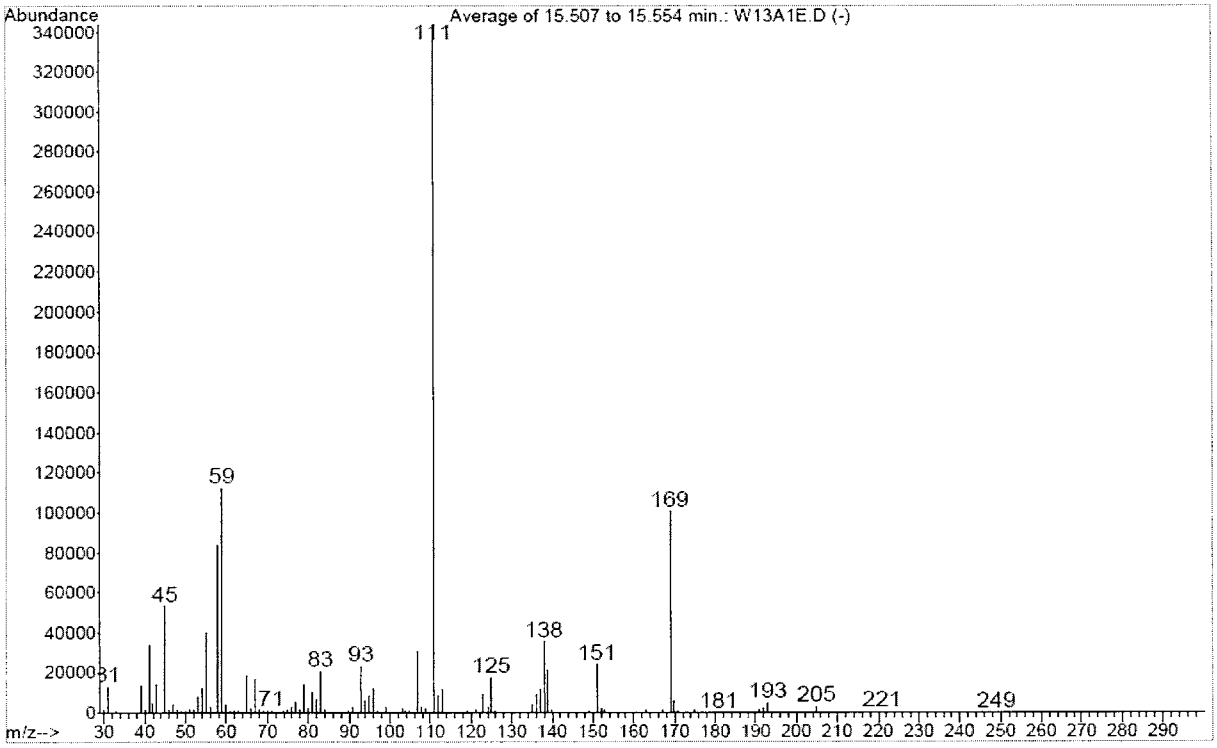




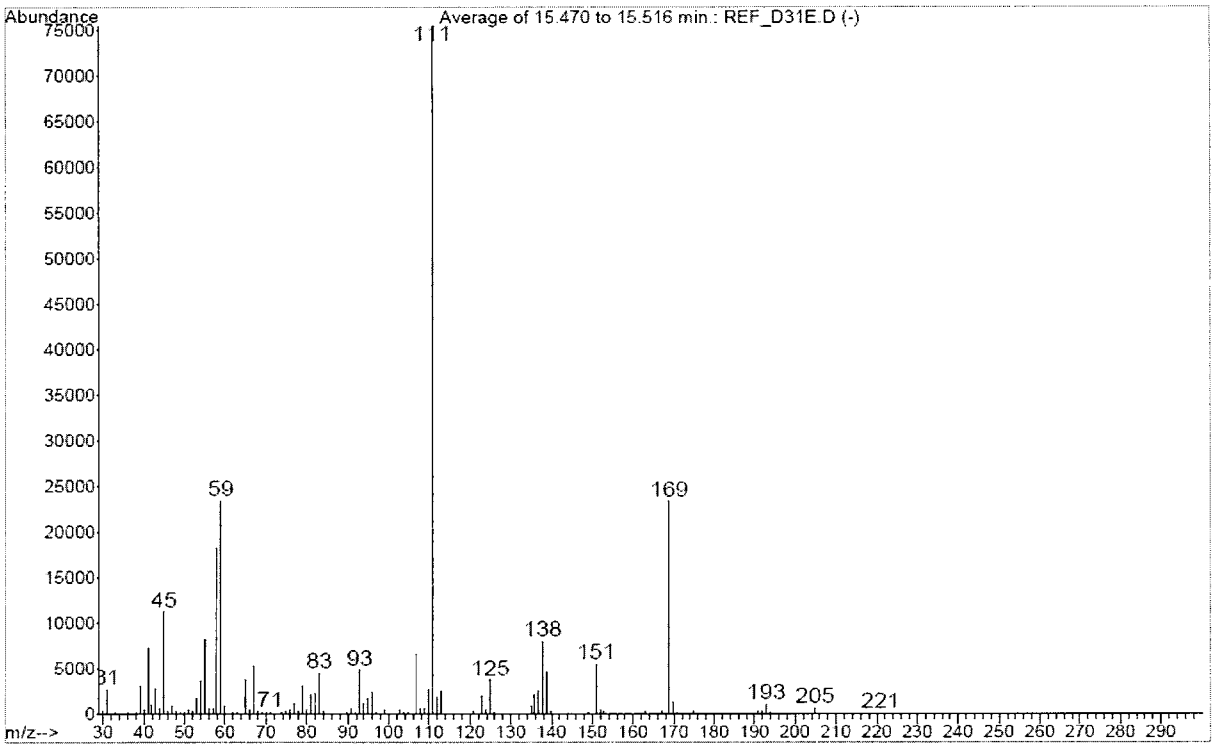


EI chromatograms supporting the identification of chemical 3; TIC on left; EIC (m/z 169) on right.
Top: Chromatograms of Decon blank, aliquot WB13A from DB/28.
Center: Chromatograms of Decon sample, aliquot W13A from D/28.
Bottom: Chromatograms of Authentic reference standard of O-cyclohexyl, O-(2-methoxyethyl)ethylphosphonate, retention time 15.50 min.

```
File       : D:\PT14 5972\PT14DATA\W13A1E.D
Acquired   :  6 Nov 2003  15:04     using AcqMethod RRF60
Sample Name: pt14 decon; sample (D/28)
Misc Info  :
```




```
File       : D:\PT14 5972\PT14DATA\REF D31E.D
Acquired   :  6 Nov 2003  17:26     using AcqMethod RRF60
Sample Name:
Misc Info  :
```



EI mass spectra (averaged and background subtracted) of:

Top:	Chemical 3 in Decon sample, aliquot W13A from D/28
Bottom:	Authentic reference standard of O-cyclohexyl, O-(2-methoxyethyl)ethylphosphonate corresponding to chemical 3 (Monoisotopic MW:250)

GC-CI-MS TECHNIQUE METHOD AND ANALYSIS DESCRIPTION

Laboratory code: 28, 29 **Sample code(s):** D/28, DB/28 **Compound number:** 3

Aliquot codes: W13A, WB13A			
Sample:	**W13A**	**Blank:**	**WB13A**

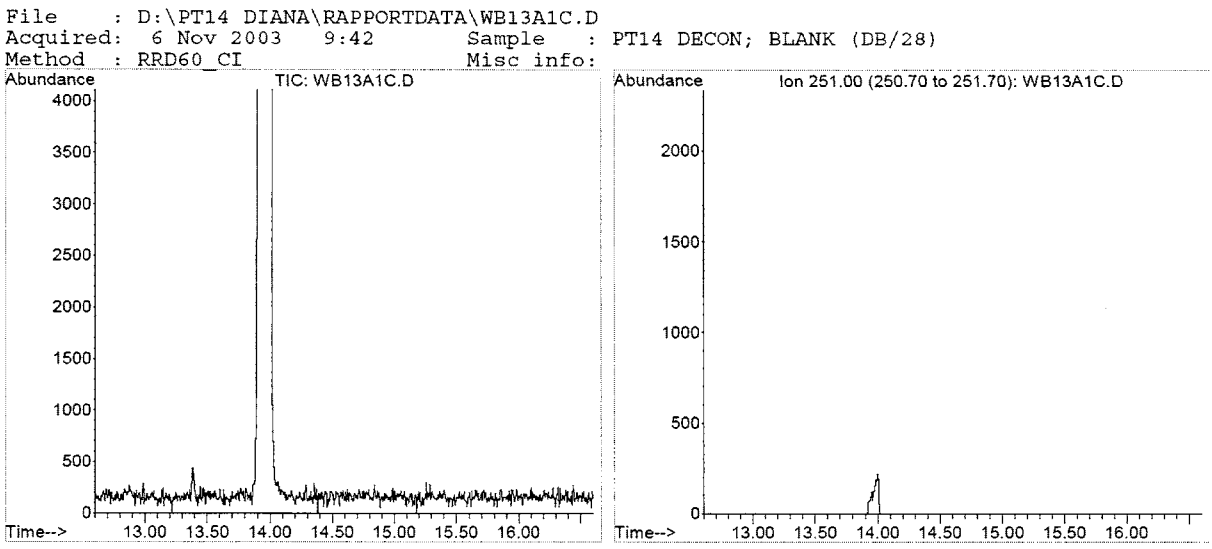
GC-CI-MS Method name:	**RRD60_CI**

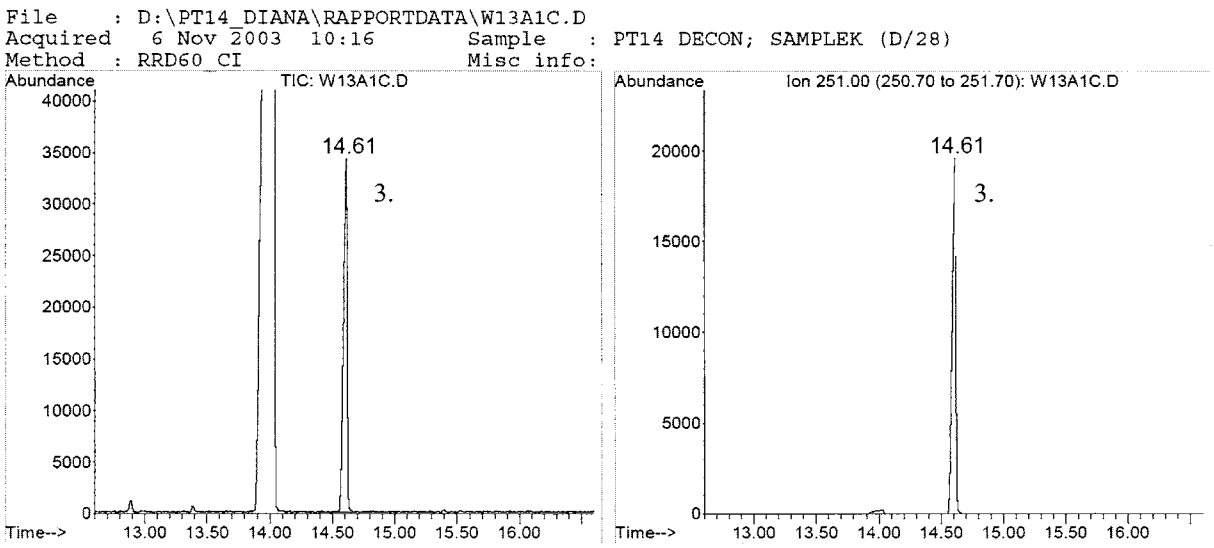
METHOD DESCRIPTION

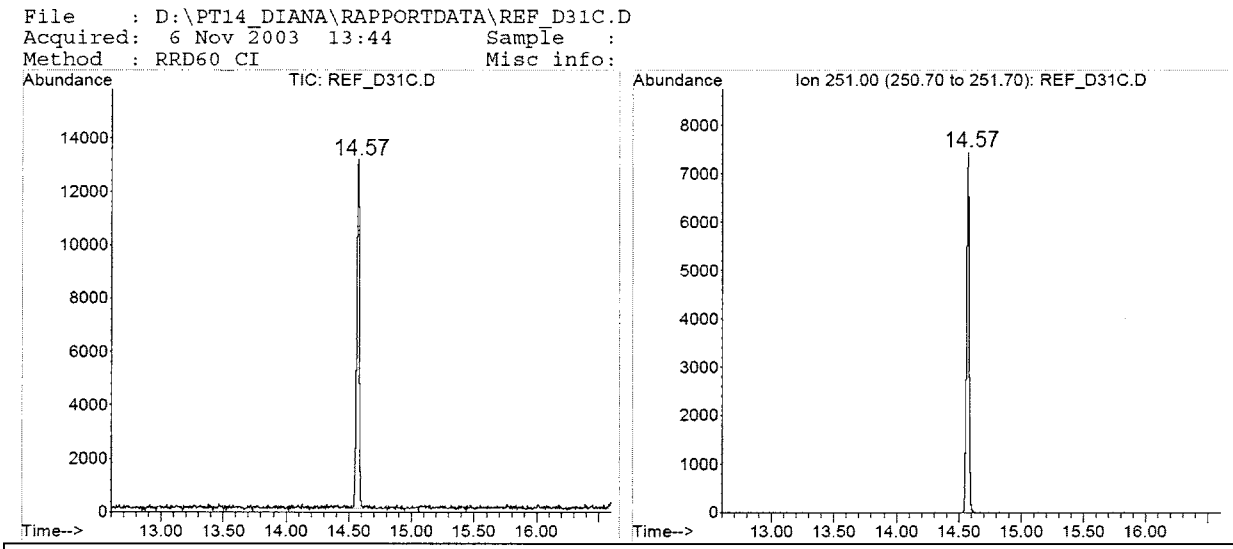
Instrument Manufacturer and Type:	HP6890 + HP 5973N (MSD)		
Carrier gas:	X He ☐ N_2 ☐ H_2 ☐ Other:		
Flow rate:	☐ ml/min X 35 cm/s		
Flow control:	☐ Constant Pressure X Constant Flow		
Injection mode:	☐ Split → Split ratio = X Splitless → Splitless time = 1 min		
Injector temperature:	200 °C		
Column phase:	DB5MS		
Column Length x ID x Film thickness:	30 m x 0,25 mm x 0,25 μm		
GC temperature programme:	60 °C (1 min), 10 °C/min, 280 °C (5 min)		
Reaction gas:	☐ Methane X Isobutane ☐ Ammonia ☐ Other:		
Solvent delay time:	4 min	**Scan range:**	60-500 m/z
Electron energy:	170 eV	**Scan time:**	0,6 s
Ionisation polarity:	X Positive ☐ Negative	**Mass resolution:**	Unit
Comments:			

ANALYSIS

Compound identified as:	X Original compound ☐ Methyl ester derivative ☐ TBDMS (t-Butyldimethylsilyl) derivative ☐ TMS (Trimethylsilyl) derivative ☐ Other derivative:
Retention parameter used for (peak) identification:	X Retention time (Rt) 14,61 ☐ Scan number
X Compared to reference chemical:	Source : ☐ Own Synthesis X DSO Singapore
☐ Not compared to reference chemical	Intense ions in spectrum are explained
Comments:	







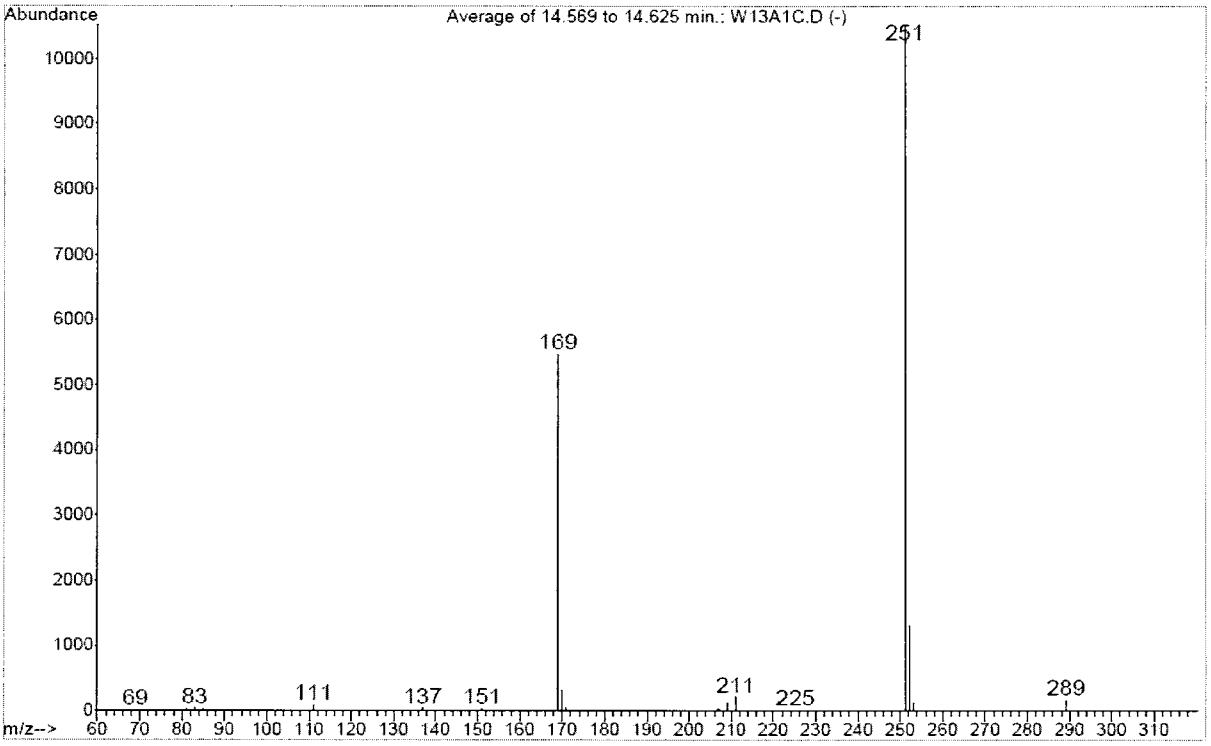
CI chromatograms supporting the identification of chemical 3; TIC on left; EIC (m/z 251) on right.

Top: Chromatograms of Decon blank, aliquot WB13A from DB/28.

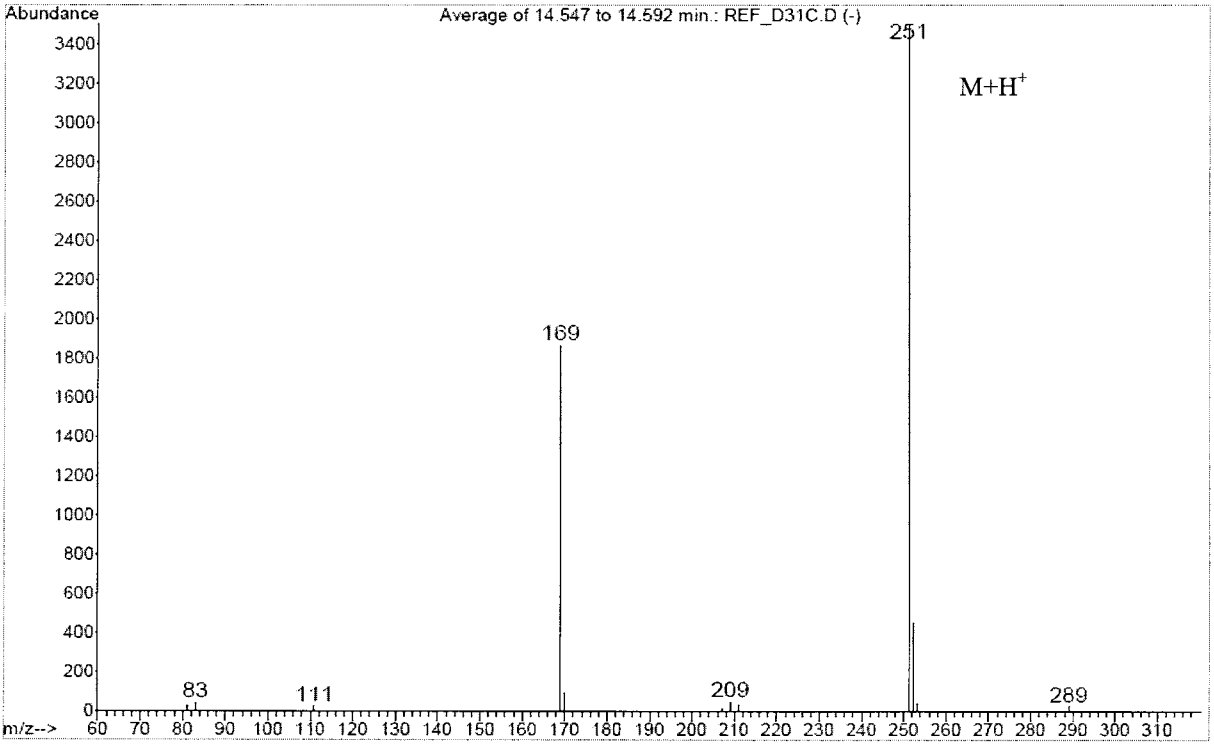
Center: Chromatograms of Decon sample, aliquot W13A from D/28.

Bottom: Chromatograms of authentic reference standard of O-cyclohexyl, O-(2-methoxyethyl)ethylphosphonate, retention time 14.57 min

```
File        : D:\PT14 DIANA\RAPPORTDATA\W13A1C.D
Acquired    :  6 Nov 2003  10:16     using AcqMethod RRD60 CI
Sample Name: PT14 DECON; SAMPLEK (D/28)
Misc Info   :
```




```
File        : D:\PT14 DIANA\RAPPORTDATA\REF D31C.D
Acquired    :  6 Nov 2003  13:44     using AcqMethod RRD60 CI
Sample Name:
Misc Info   :
```



CI mass spectra (averaged and background subtracted) of:	
Top:	Chemical 3 in Decon sample, aliquot W13A from D/28
Bottom:	Authentic reference standard of O-cyclohexyl, O-(2-methoxyethyl)ethylphosphonate corresponding to chemical 3 (monoisotopic MW:250)

SAMPLE PREPARATION DESCRIPTION

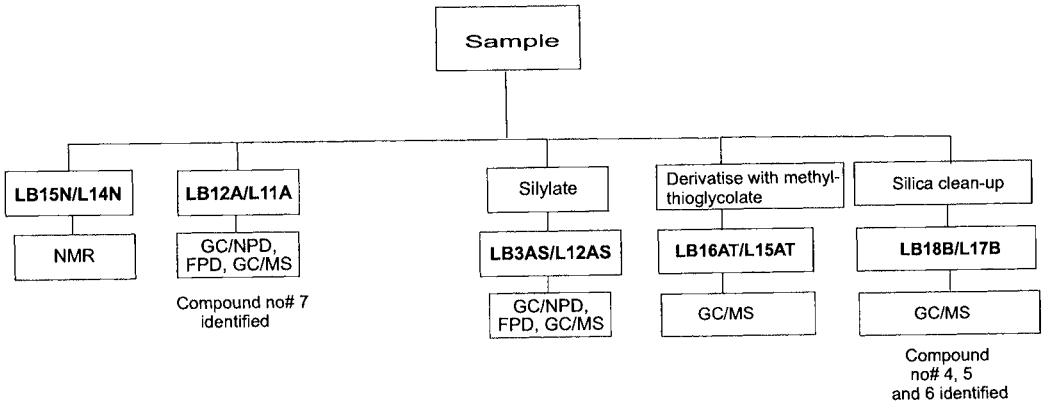
Laboratory code: 28,29 **Sample code:** O/28 **Blank code:** OB/28

1. Sample preparation

Sample/ Aliquot Code	Specification of Sample/ Type of Sample Preparation	Amount/ Volume	Sample Preparation Procedures	End Volume	Resulting Aliquot Code	Analytical Technique
LB12/L11	Direct analysis	100 ul	None	100 ul	LB12A/L11A	GC/EI-MS GC/CI-MS
LB13/L12	Derivatization	500 ul	Silylation with BSTFA	550 ul	LB13AS/L12AS	GC/EI-MS GC/CI-MS
LB16/L15	Derivatization	500 ul	Derivatization with methylthioglycolate	500 ul	LB16AT/L15AT	GC/EI-MS GC/CI-MS
LB18/L17	Clean-up	100 ul	Clean-up on silica gel	100 ul	LB18B/L17B	GC/EI-MS GC/CI-MS

2. Additional information

PREPARATION OF ORGANIC LIQUID OB/28 and O/28



GC-EI-MS TECHNIQUE METHOD AND ANALYSIS DESCRIPTION

Laboratory code: 28,29 **Sample code(s):** O/28, OB/28 **Compound number:** 4

Aliquot codes: L17B,LB18B			
Sample:	**L17B**	**Blank:**	**LB18B**

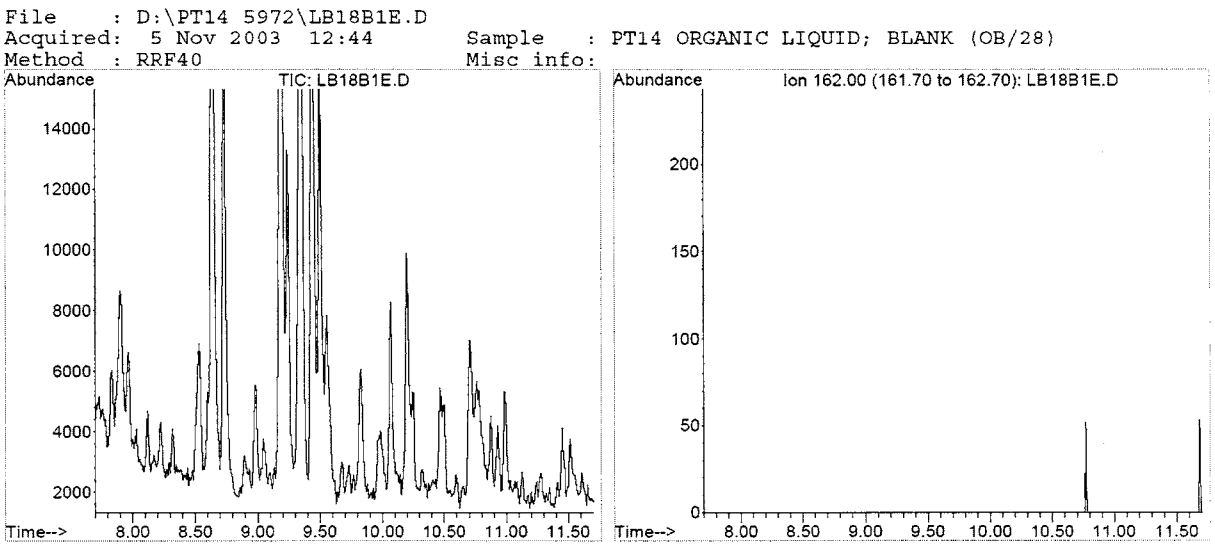
GC-EI-MS Method name:	**RRF40**

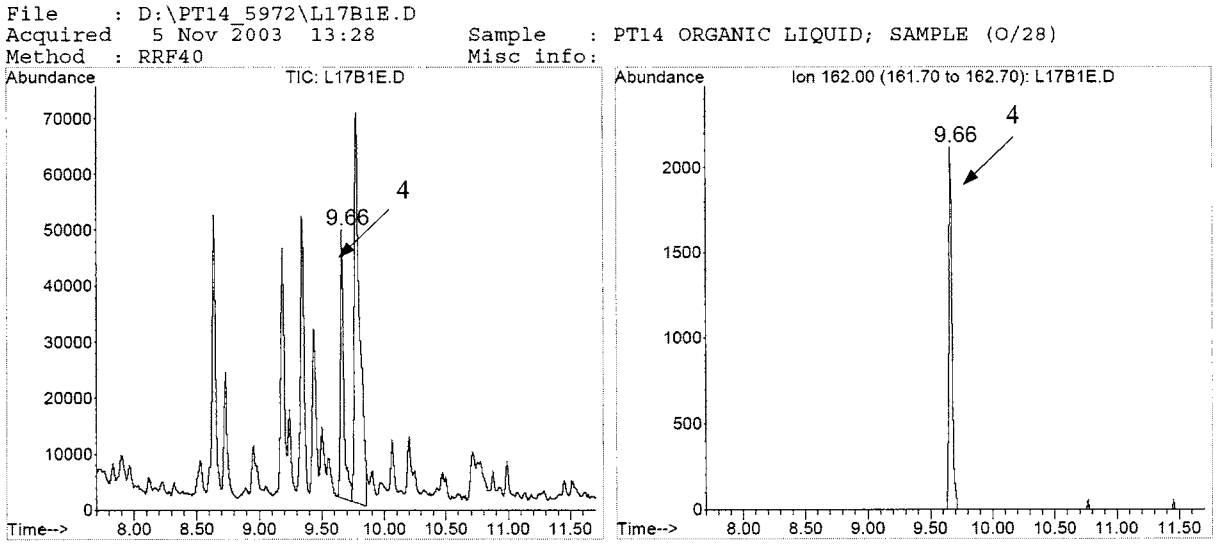
METHOD DESCRIPTION

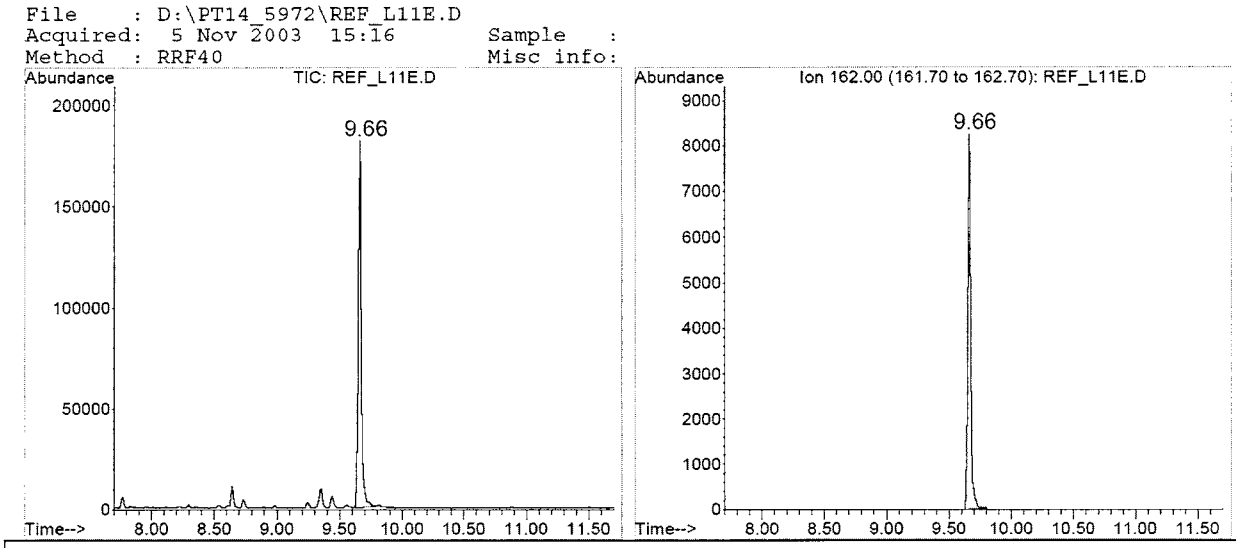
Instrument Manufacturer and Type:	HP 5890GC + HP5972 MSD		
Carrier gas:	x He ☐ N_2 ☐ H_2 ☐ Other:		
Flow rate:	☐ ml/min x 35 cm/s		
Flow control:	☐ Constant Pressure x Constant Flow		
Injection mode:	☐ Split → Split ratio = x Splitless → Splitless time = 1 min		
Injector temperature:	200 °C		
Column phase:	DB5MS		
Column Length x ID x Film thickness:	30 m x 0,25 mm x 0,25 μm		
GC temperature programme:	40 °C (1 min), 10 °C/min, 280 °C (5 min)		
Solvent delay time:	3 min	**Scan range:**	29-450 m/z
Electron energy:	70 eV	**Scan time:**	0,4 s
Ionisation polarity:	x Positive ☐ Negative	**Mass resolution:**	unit
Comments:			

ANALYSIS

Compound identified as:	x Original compound ☐ Methyl ester derivative ☐ TBDMS (t-Butyldimethylsilyl) derivative ☐ TMS (Trimethylsilyl) derivative ☐ Other derivative:
Retention parameter used for (peak) identification:	x Retention time (Rt) 9,66 ☐ Scan number
x Compared to reference chemical:	Source : ☐ Own Synthesis x DSO Singapore
☐ Compared to library spectrum:	Source : ☐ OCAD (code:) ☐ NIST ☐ Wiley ☐ Own ☐ Other:
☐ Not compared to reference chemical or library spectrum:	Intense ions in spectrum are explained; interpretation is supported by the spectral information derived from closely related chemical(s):
Comments:	







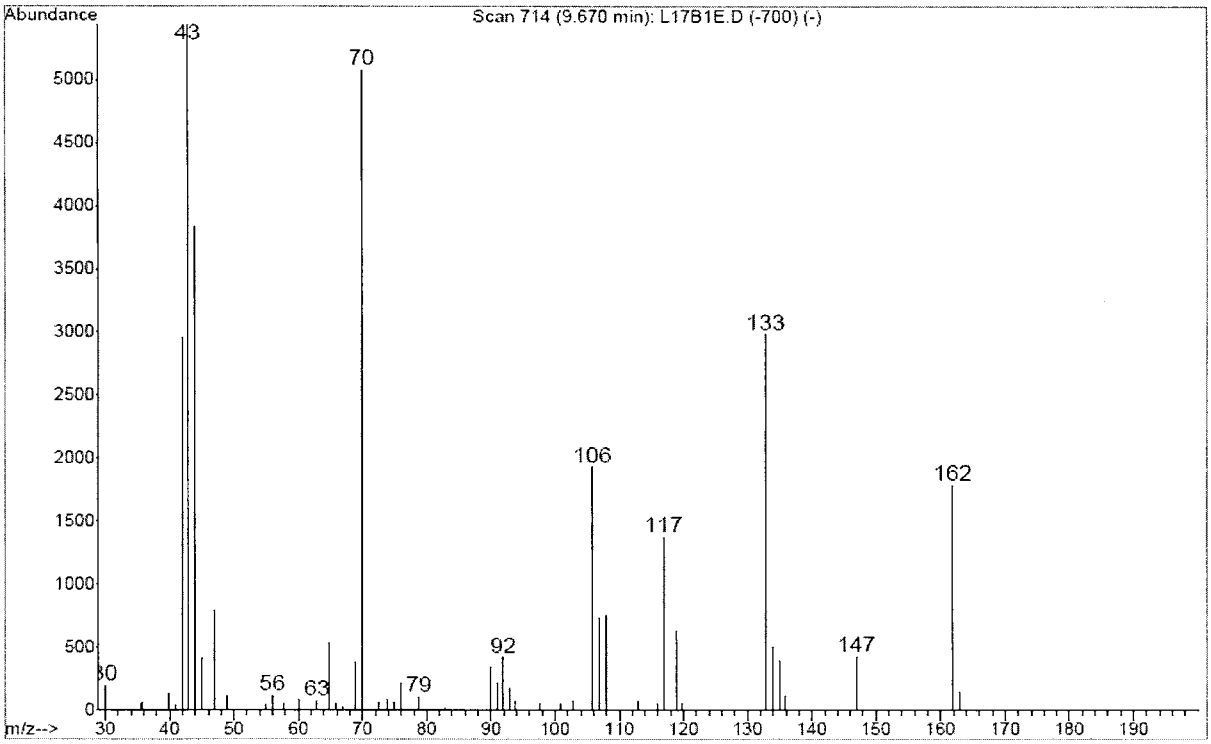
EI chromatograms supporting the identification of chemical 4; TIC on left; EIC (m/z 162) on right.

Top: Chromatograms of organic liquid blank, aliquot LB18B from OB/28.

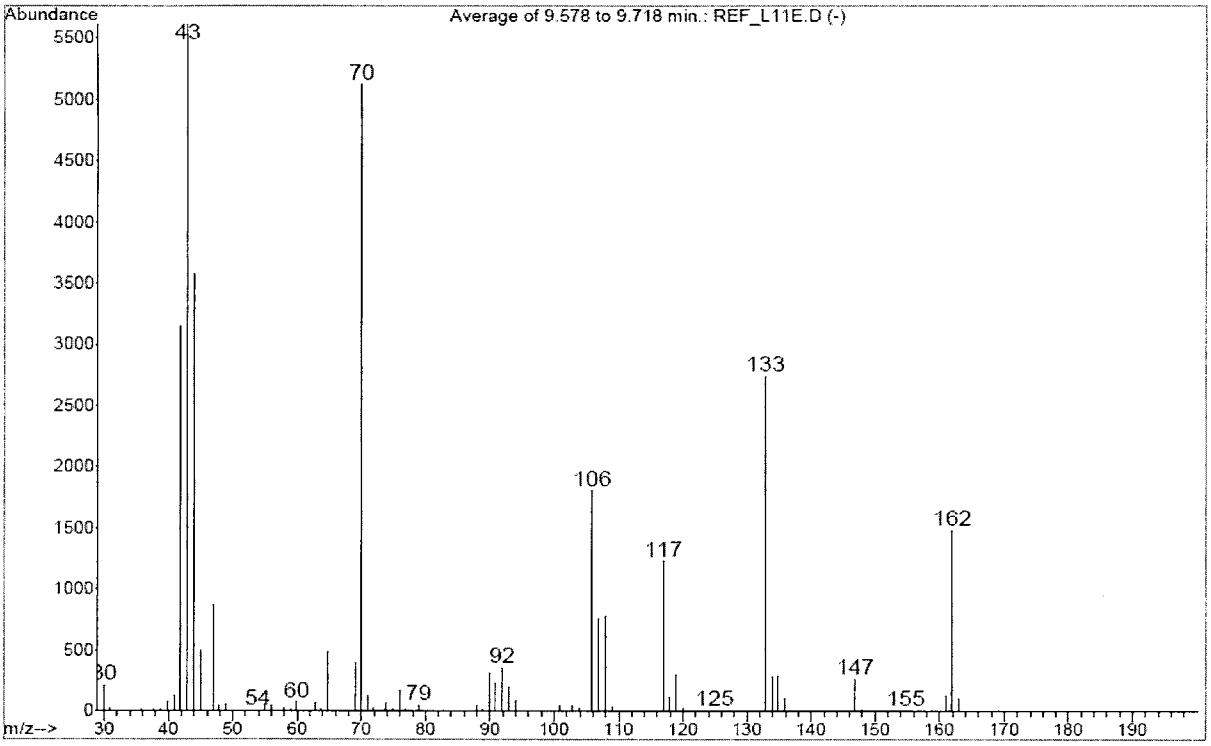
Center: Chromatograms of Organic liquid sample, aliquot L17B from O/28.

Bottom: Chromatograms of authentic reference standard of O.ethyl N,N-dimethylphosphoramidocyanidate, retention time 9.66 min.

```
File       : D:\PT14 5972\L17B1E.D
Acquired   :  5 Nov 2003  13:28     using AcqMethod RRF40
Sample Name: PT14 ORGANIC LIQUID; SAMPLE (O/28)
Misc Info  :
```




```
File       : D:\PT14 5972\REF L11E.D
Acquired   :  5 Nov 2003  15:16     using AcqMethod RRF40
Sample Name:
Misc Info  :
```



EI mass spectra (averaged and background subtracted) of:	
Top:	Chemical 4 in Organic liquid sample, aliquot L17B from O/28
Bottom:	Authentic reference standard of O.ethyl N,N-dimethylphosphoramidocyanidate corresponding to chemical 4 (Monoisotopic MW:162)

GC-CI-MS TECHNIQUE METHOD AND ANALYSIS DESCRIPTION

Laboratory code: 28, 29 **Sample code(s):** O/28, OB/28 **Compound number:** 4

Aliquot codes: L17B, LB18B			
Sample:	**: L17B**	**Blank:**	**LB18B**

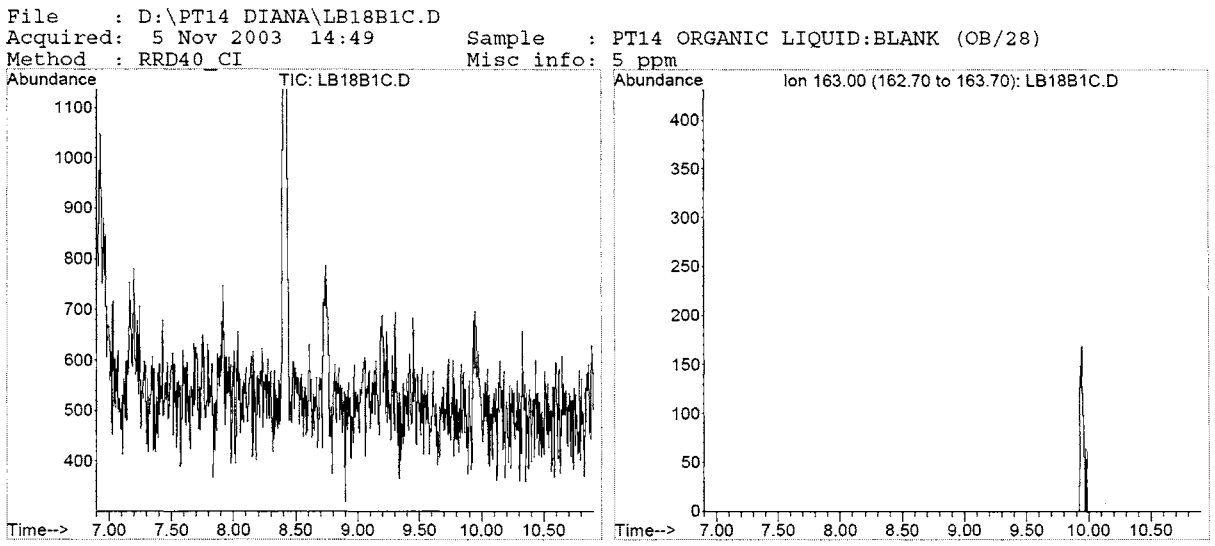
GC-CI-MS Method name:	**RRD40_CI**

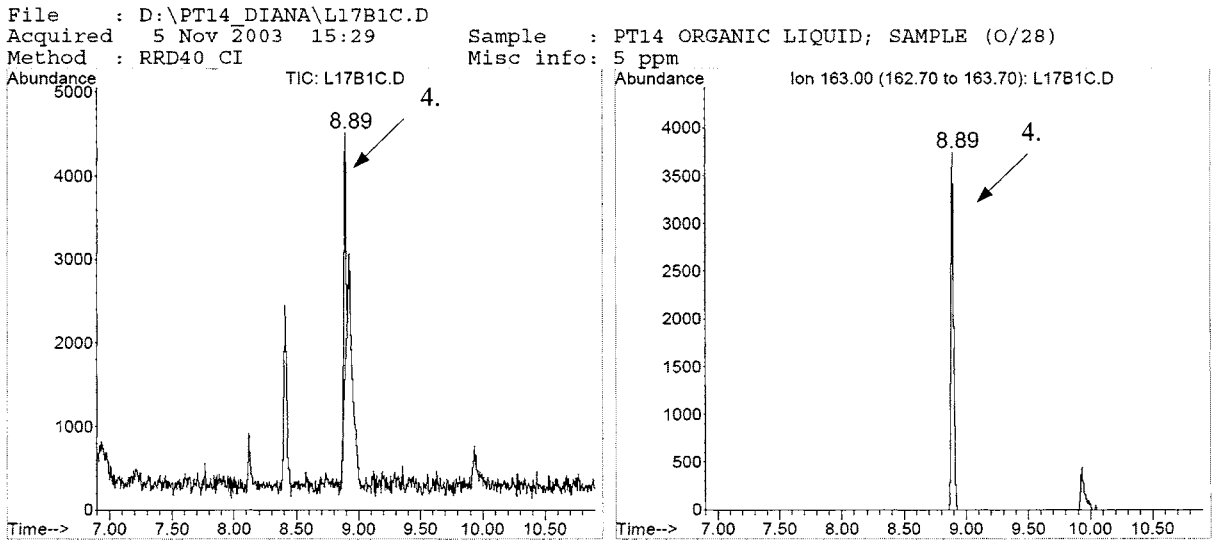
METHOD DESCRIPTION

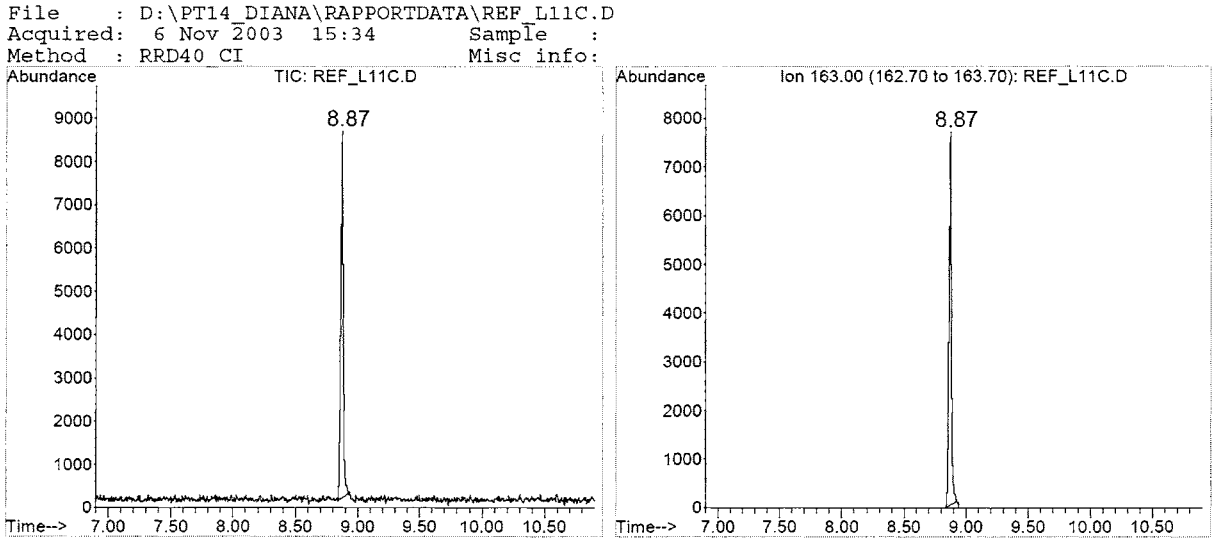
Instrument Manufacturer and Type:	HP6890 + HP 5973N (MSD)		
Carrier gas:	X He ☐ N_2 ☐ H_2 ☐ Other:		
Flow rate:	☐ ml/min X 35 cm/s		
Flow control:	☐ Constant Pressure X Constant Flow		
Injection mode:	☐ Split → Split ratio = X Splitless → Splitless time = 1 min		
Injector temperature:	200 °C		
Column phase:	DB5MS		
Column Length x ID x Film thickness:	30 m x 0,25 mm x 0,25 µm		
GC temperature programme:	40 °C (1 min), 10 °C/min, 280 °C (5 min)		
Reaction gas:	☐ Methane X Isobutane ☐ Ammonia ☐ Other:		
Solvent delay time:	4 min	**Scan range:**	60-500 m/z
Electron energy:	170 eV	**Scan time:**	0,6 s
Ionisation polarity:	X Positive ☐ Negative	**Mass resolution:**	Unit
Comments:			

ANALYSIS

Compound identified as:	X Original compound ☐ Methyl ester derivative ☐ TBDMS (t-Butyldimethylsilyl) derivative ☐ TMS (Trimethylsilyl) derivative ☐ Other derivative:
Retention parameter used for (peak) identification:	X Retention time (Rt) 8,89 ☐ Scan number
X Compared to reference chemical:	Source : ☐ Own Synthesis X DSO Singapore
☐ Not compared to reference chemical	Intense ions in spectrum are explained
Comments:	

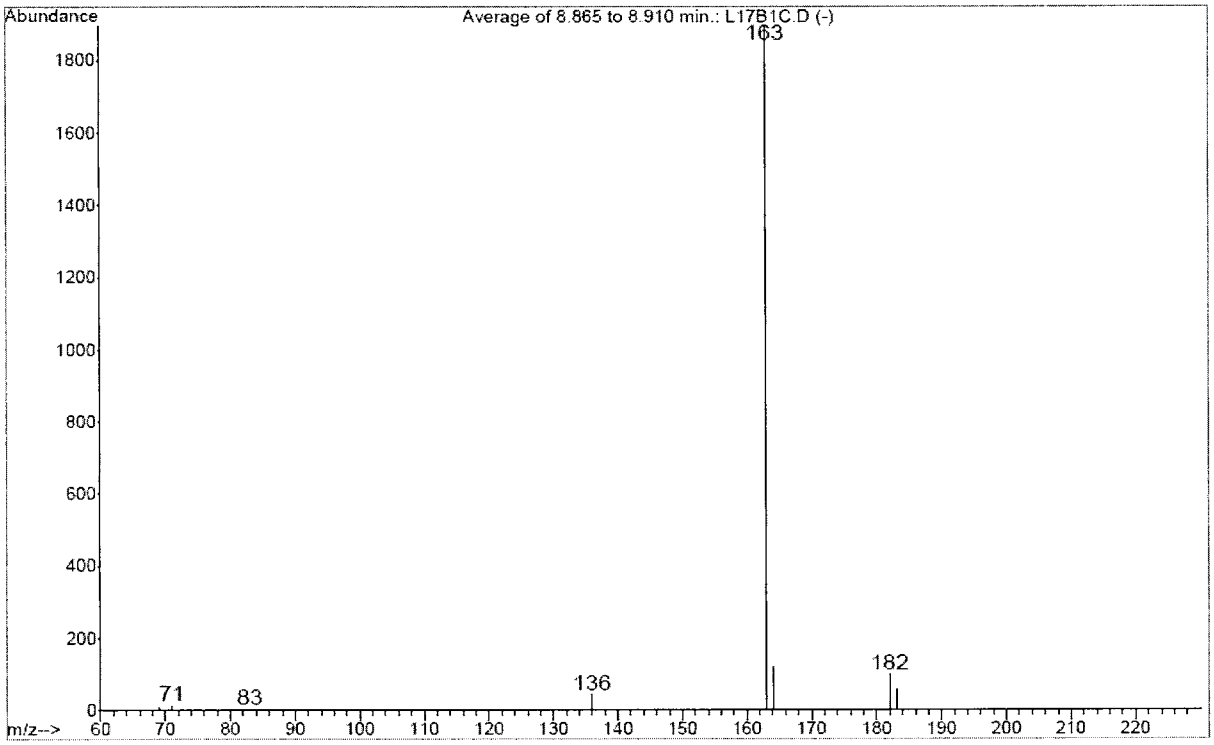




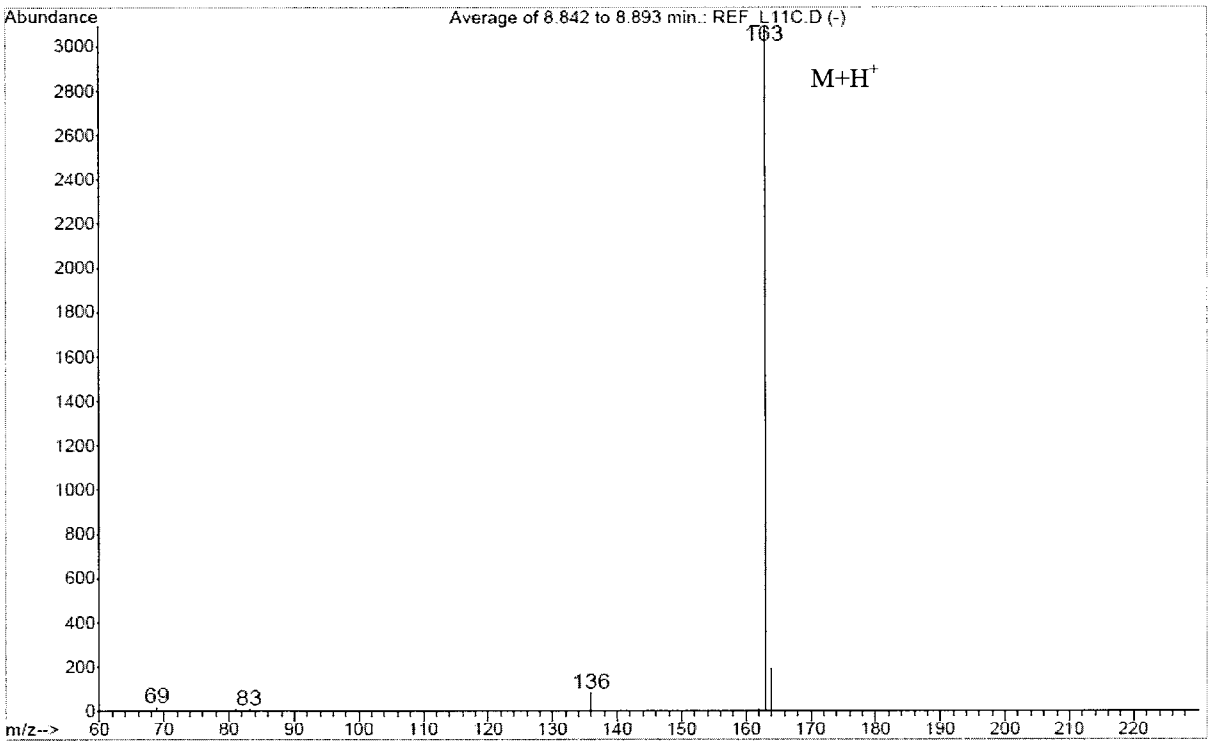


CI chromatograms supporting the identification of chemical 4; TIC on left; EIC (m/z 163) on right.
Top: Chromatograms of Organic liquid blank, aliquot LB18B from OB/14.
Center: Chromatograms of Organic liquid sample, aliquot L17B from O/28.
Bottom: Chromatograms of authentic reference standard of O.ethyl N,N-dimethylphosphoramidocyanidate, retention time 8.87 min.

```
File        : D:\PT14 DIANA\L17B1C.D
Acquired    :  5 Nov 2003  15:29      using AcqMethod RRD40 CI
Sample Name: PT14 ORGANIC LIQUID; SAMPLE (O/28)
Misc Info   : 5 ppm
```




```
File        : D:\PT14 DIANA\RAPPORTDATA\REF L11C.D
Acquired    :  6 Nov 2003  15:34      using AcqMethod RRD40 CI
Sample Name:
Misc Info   :
```



CI mass spectra (averaged and background subtracted) of:

Top:	Chemical 4 in Organic liquid sample, aliquot L17B from O/28
Bottom:	Authentic reference standard of O.ethyl N,N-dimethylphosphoramidocyanidate corresponding to chemical 4 (monoisotopic MW:162)

GC-EI-MS TECHNIQUE METHOD AND ANALYSIS DESCRIPTION

Laboratory code: 28,29 **Sample code(s):** O/28, OB/28 **Compound number:** 5

Aliquot codes: L17B,LB18B			
Sample:	**L17B**	**Blank:**	**LB18B**

GC-EI-MS Method name:	**RRF40**

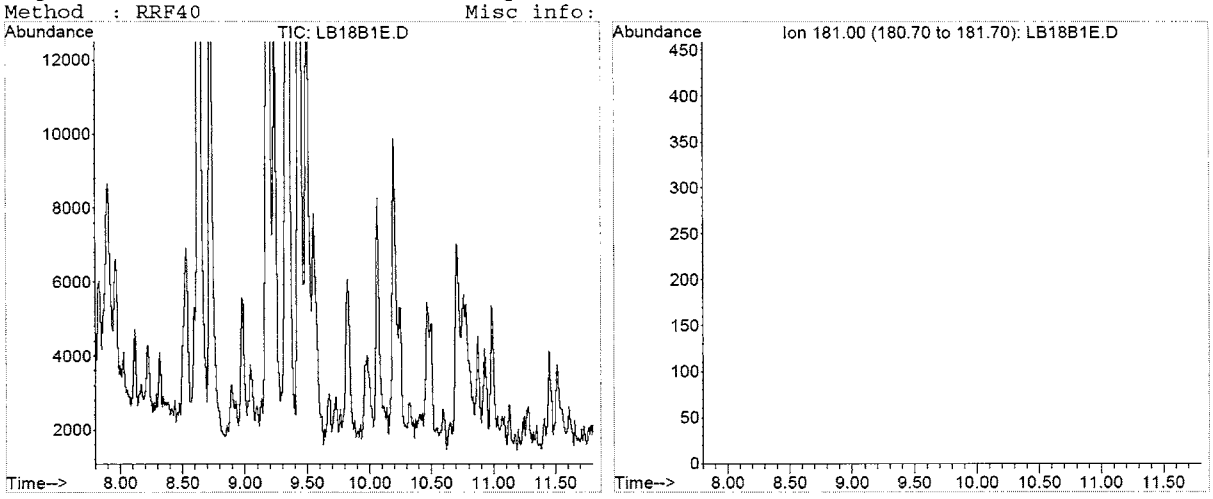
METHOD DESCRIPTION

Instrument Manufacturer and Type:	HP 5890GC + HP5972 MSD		
Carrier gas:	X He ☐ N_2 ☐ H_2 ☐ Other:		
Flow rate:	☐ ml/min X 35 cm/s		
Flow control:	☐ Constant Pressure X Constant Flow		
Injection mode:	☐ Split → Split ratio = X Splitless → Splitless time = 1 min		
Injector temperature:	200 °C		
Column phase:	DB5MS		
Column Length x ID x Film thickness:	30 m x 0,25 mm x 0,25 μm		
GC temperature programme:	40 °C (1 min), 10 °C/min, 280 °C (5 min)		
Solvent delay time:	3 min	**Scan range:**	29-450 m/z
Electron energy:	70 eV	**Scan time:**	0,4 s
Ionisation polarity:	X Positive ☐ Negative	**Mass resolution:**	unit
Comments:			

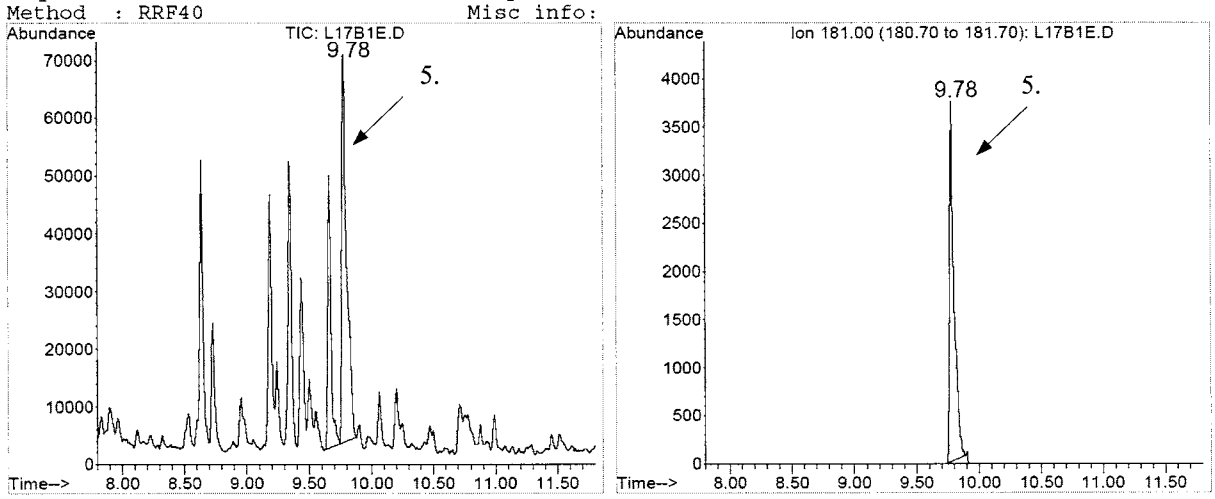
ANALYSIS

Compound identified as:	X Original compound ☐ Methyl ester derivative ☐ TBDMS (t-Butyldimethylsilyl) derivative ☐ TMS (Trimethylsilyl) derivative ☐ Other derivative:
Retention parameter used for (peak) identification:	X Retention time (Rt) 9,71 ☐ Scan number
X Compared to reference chemical:	Source : ☐ Own Synthesis X DSO Singapore
☐ Compared to library spectrum:	Source : ☐ OCAD (code:) ☐ NIST ☐ Wiley ☐ Own ☐ Other:
☐ Not compared to reference chemical or library spectrum:	Intense ions in spectrum are explained; interpretation is supported by the spectral information derived from closely related chemical(s):
Comments:	

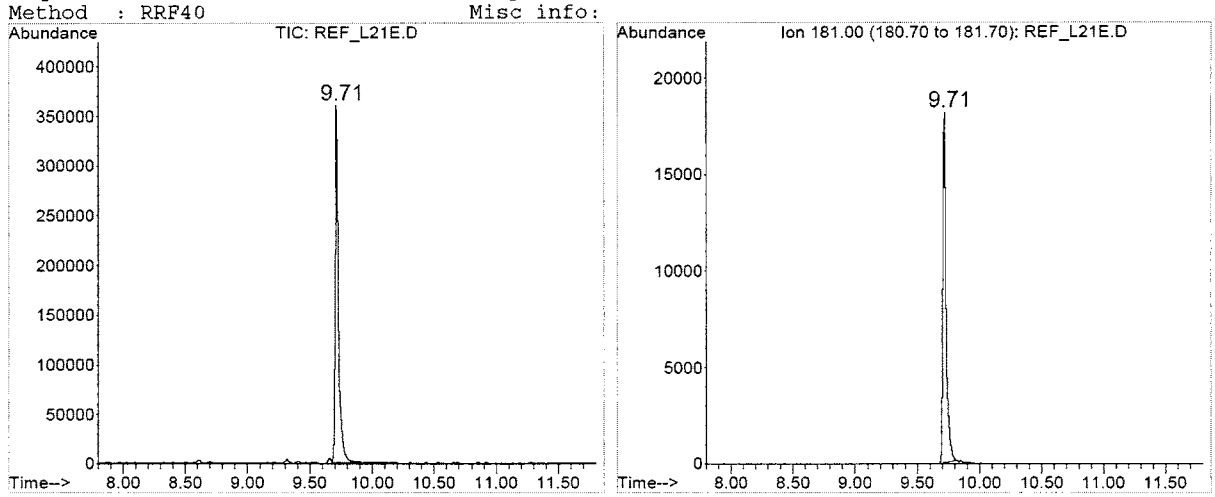
File : D:\PT14_5972\LB18B1E.D
Acquired: 5 Nov 2003 12:44 Sample : PT14 ORGANIC LIQUID; BLANK (OB/28)
Method : RRF40 Misc info:



File : D:\PT14_5972\L17B1E.D
Acquired 5 Nov 2003 13:28 Sample : PT14 ORGANIC LIQUID; SAMPLE (O/28)
Method : RRF40 Misc info:



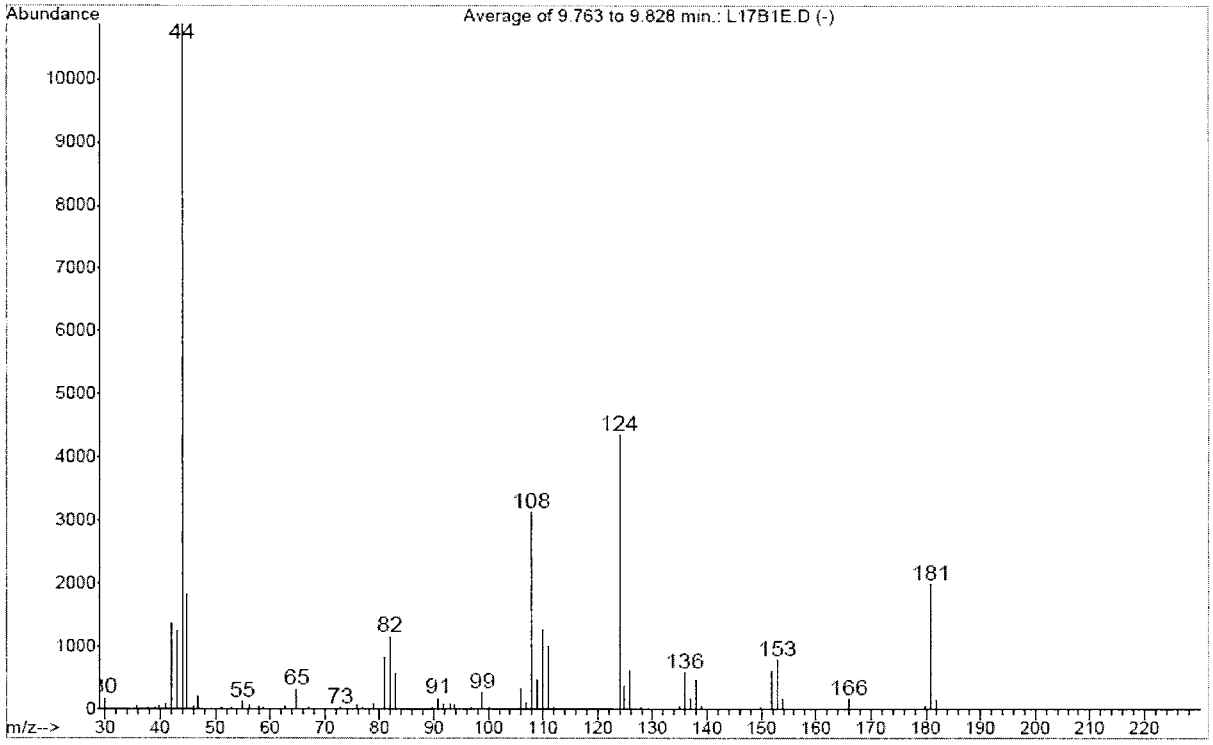
File : D:\PT14_5972\PT14DATA\REF_L21E.D
Acquired: 7 Nov 2003 10:56 Sample :
Method : RRF40 Misc info:



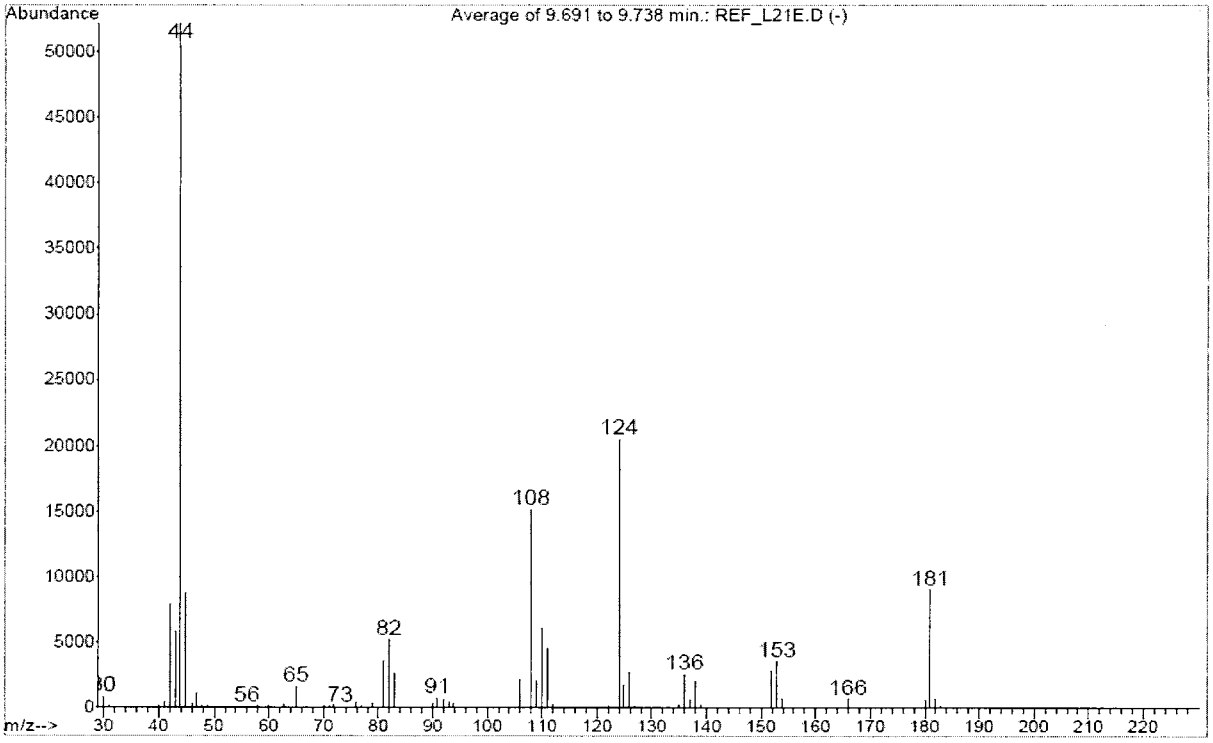
EI chromatograms supporting the identification of chemical 5; TIC on left; EIC (m/z 181) on right.

Top:	Chromatograms of Organic liquid blank, aliquot LB18B from OB/28.
Center:	Chromatograms of Organic liquid sample, aliquot L17B from O/28.
Bottom:	Chromatograms of authentic reference standard of Diethyl N,N-dimethylphosphoramidate, retention time 9.71 min.

```
File       : D:\PT14 5972\L17B1E.D
Acquired   :  5 Nov 2003  13:28      using AcqMethod RRF40
Sample Name: PT14 ORGANIC LIQUID; SAMPLE (O/28)
Misc Info  :
```




```
File       : D:\PT14 5972\PT14DATA\REF L21E.D
Acquired   :  7 Nov 2003  10:56      using AcqMethod RRF40
Sample Name:
Misc Info  :
```



EI mass spectra (averaged and background subtracted) of:

Top: Chemical 5 in Organic liquid sample, aliquot L17B from O/28

Bottom: Authentic reference standard of Diethyl N,N-dimethylphosphoramidate corresponding to chemical 5 (Monoisotopic MW 181)

GC-CI-MS TECHNIQUE METHOD AND ANALYSIS DESCRIPTION

Laboratory code: 28, 29 **Sample code(s):** O/28, OB/28 **Compound number:** 5

Aliquot codes: L17B, LB18B			
Sample:	**L17B**	**Blank:**	**LB18B**

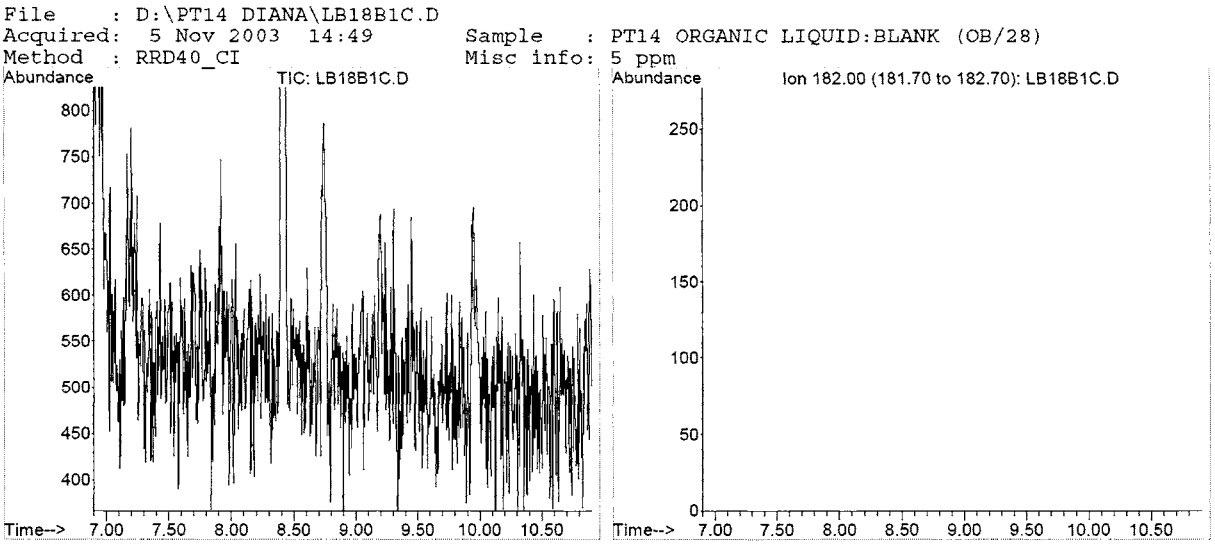
GC-CI-MS Method name:	**RRD40_CI**

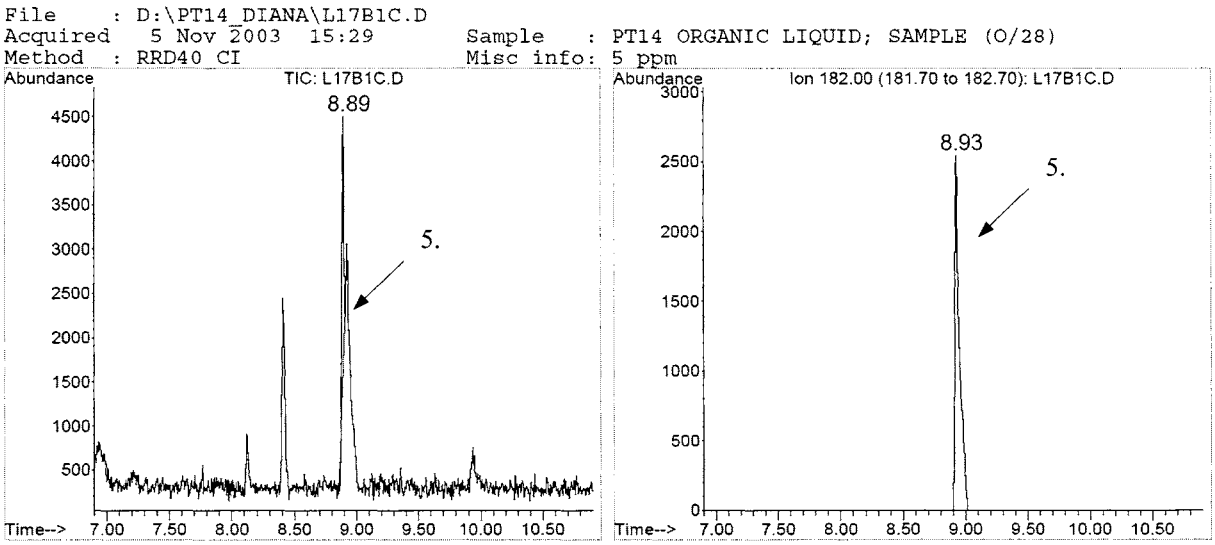
METHOD DESCRIPTION

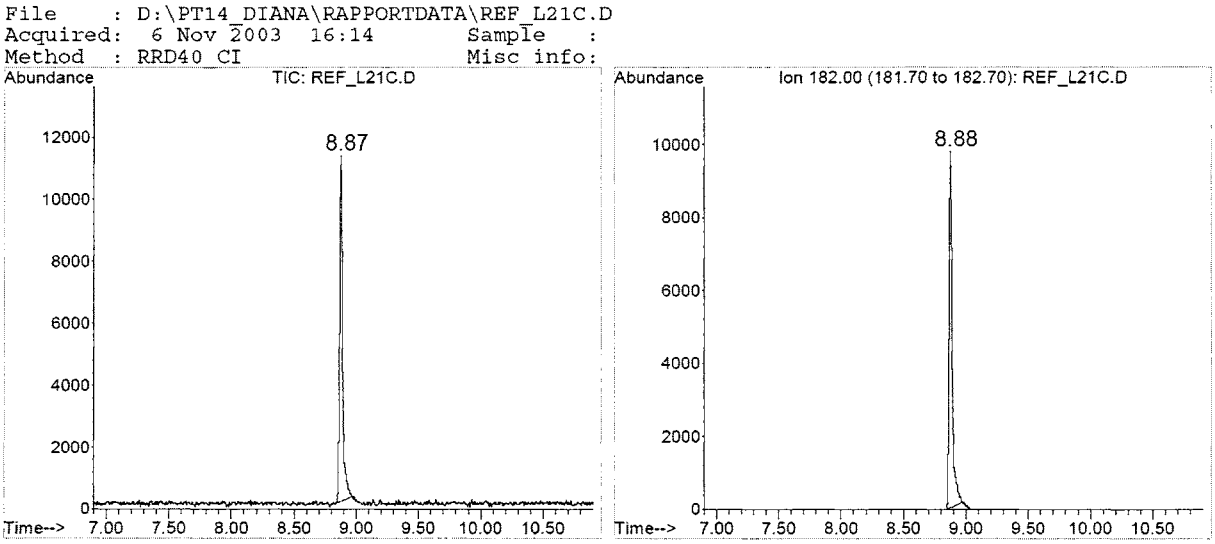
Instrument Manufacturer and Type:	HP6890 + HP 5973N (MSD)		
Carrier gas:	X He ☐ N_2 ☐ H_2 ☐ Other:		
Flow rate:	☐ ml/min X 35 cm/s		
Flow control:	☐ Constant Pressure X Constant Flow		
Injection mode:	☐ Split → Split ratio = X Splitless → Splitless time = 1 min		
Injector temperature:	200 °C		
Column phase:	DB5MS		
Column Length x ID x Film thickness:	30 m x 0,25 mm x 0,25 µm		
GC temperature programme:	40 °C (1 min), 10 °C/min, 280 °C (5 min)		
Reaction gas:	☐ Methane X Isobutane ☐ Ammonia ☐ Other:		
Solvent delay time:	4 min	**Scan range:**	60-500 m/z
Electron energy:	170 eV	**Scan time:**	0,6 s
Ionisation polarity:	X Positive ☐ Negative	**Mass resolution:**	Unit
Comments:			

ANALYSIS

Compound identified as:	X Original compound ☐ Methyl ester derivative ☐ TBDMS (t-Butyldimethylsilyl) derivative ☐ TMS (Trimethylsilyl) derivative ☐ Other derivative:
Retention parameter used for (peak) identification:	X Retention time (Rt) 8,93 ☐ Scan number
X Compared to reference chemical:	Source : ☐ Own Synthesis X DSO Singapore
☐ Not compared to reference chemical	Intense ions in spectrum are explained
Comments:	



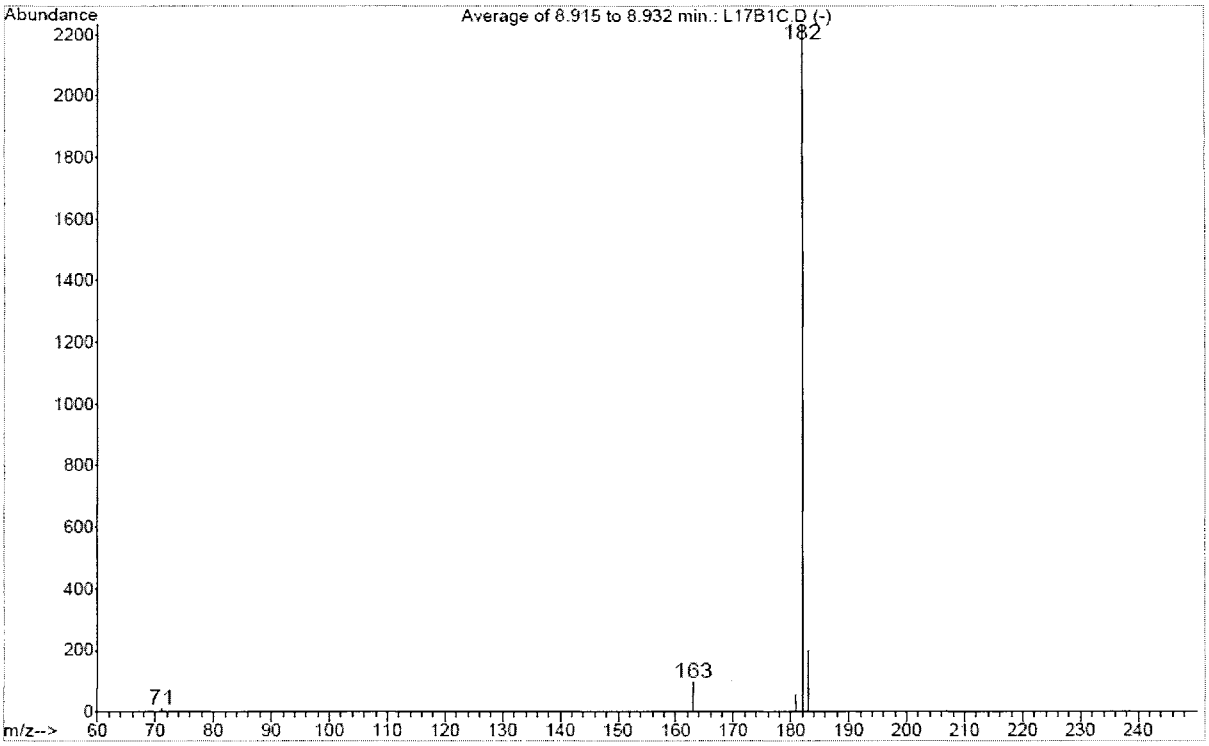




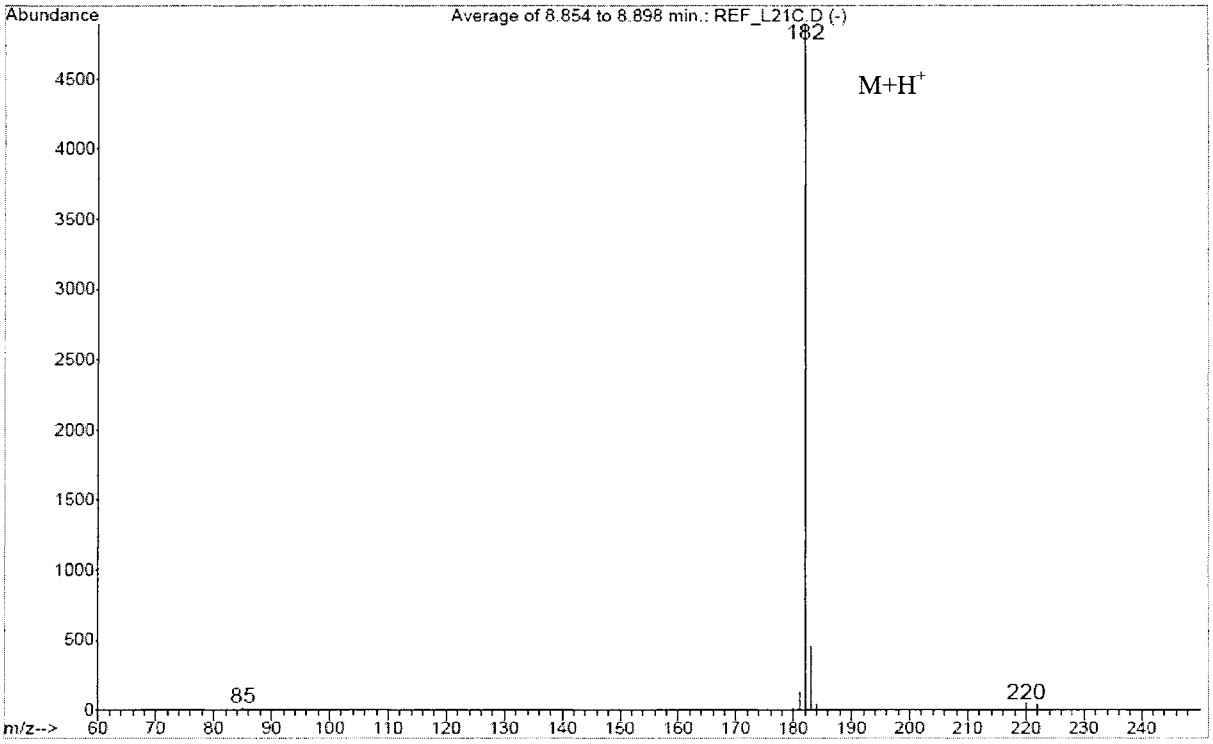
CI chromatograms supporting the identification of chemical 5; TIC on left; EIC (m/z 182) on right.

Top:	Chromatograms of Organic liquid blank, aliquot LB18B from OB/28.
Center:	Chromatograms of Organic liquid sample, aliquot L17B from O/28.
Bottom:	Chromatograms of authentic reference standard of Diethyl N,N-dimethylphosphoramidate, retention time 8.88 min.

```
File        : D:\PT14 DIANA\L17B1C.D
Acquired    :  5 Nov 2003  15:29      using AcqMethod RRD40 CI
Sample Name: PT14 ORGANIC LIQUID; SAMPLE (O/28)
Misc Info   : 5 ppm
```




```
File        : D:\PT14 DIANA\RAPPORTDATA\REF L21C.D
Acquired    :  6 Nov 2003  16:14      using AcqMethod RRD40 CI
Sample Name:
Misc Info   :
```



CI mass spectra (averaged and background subtracted) of:	
Top:	Chemical 5 in Organic liquid sample, aliquot L17B from O/28
Bottom:	Authentic reference standard of Diethyl N,N-dimethylphosphoramidate corresponding to chemical 5 (monoisotopic MW:181)

GC-EI-MS TECHNIQUE METHOD AND ANALYSIS DESCRIPTION

Laboratory code: 28,29 **Sample code(s):** O/28, OB/28 **Compound number:** 6

Aliquot codes: L17B,LB18B			
Sample:	**L17B**	**Blank:**	**LB18B**

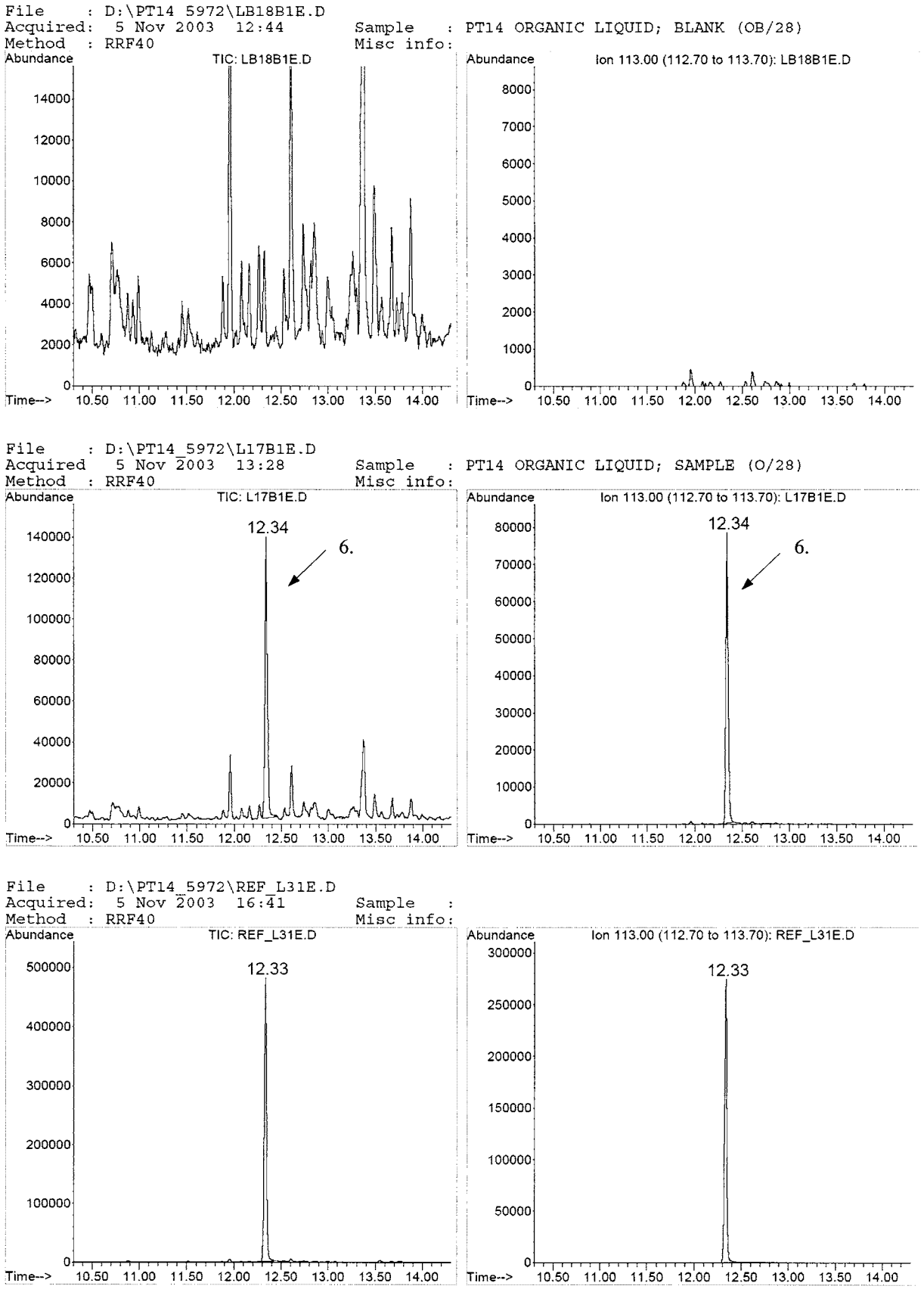
GC-EI-MS Method name:	**RRF40**

METHOD DESCRIPTION

Instrument Manufacturer and Type:	HP 5890GC + HP5972 MSD		
Carrier gas:	X He ☐ N_2 ☐ H_2 ☐ Other:		
Flow rate:	☐ ml/min X 35 cm/s		
Flow control:	☐ Constant Pressure X Constant Flow		
Injection mode:	☐ Split → Split ratio = X Splitless → Splitless time = 1 min		
Injector temperature:	200 °C		
Column phase:	DB5MS		
Column Length x ID x Film thickness:	30 m x 0,25 mm x 0,25 μm		
GC temperature programme:	40 °C (1 min), 10 °C/min, 280 °C (5 min)		
Solvent delay time:	3 min	**Scan range:**	29-450 m/z
Electron energy:	70 eV	**Scan time:**	0,4 s
Ionisation polarity:	X Positive ☐ Negative	**Mass resolution:**	unit
Comments:			

ANALYSIS

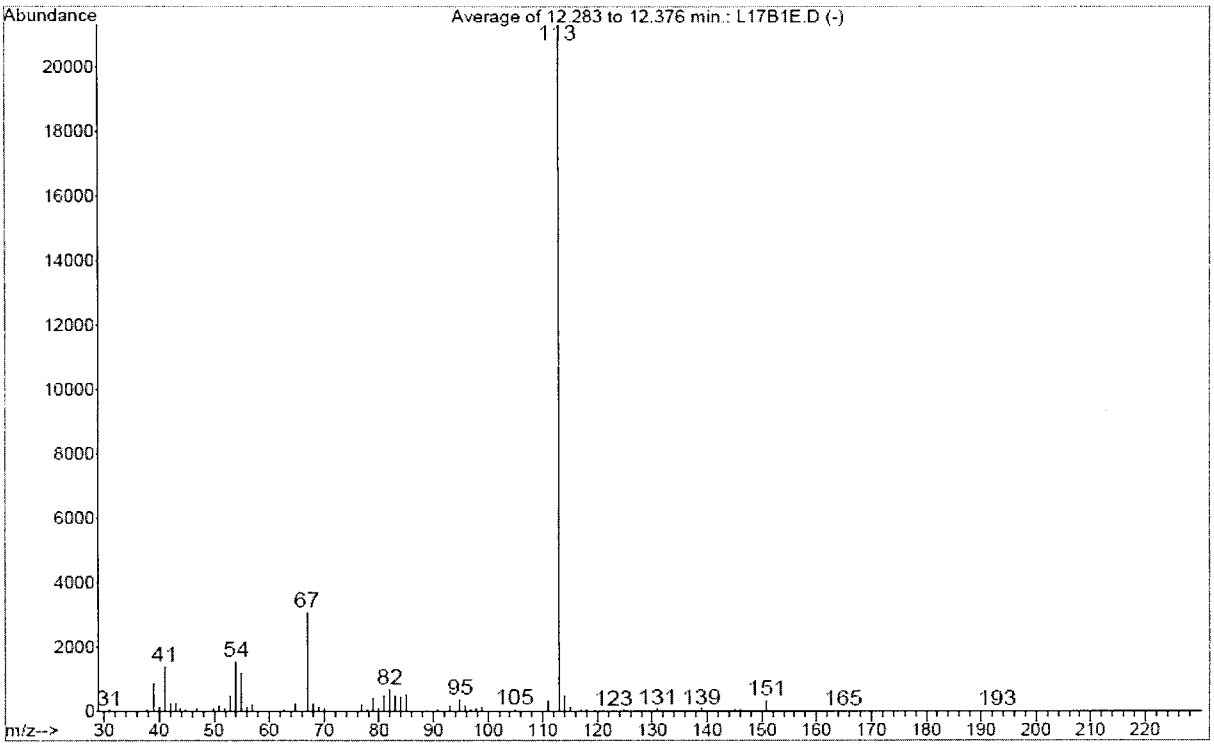
Compound identified as:	X Original compound ☐ Methyl ester derivative ☐ TBDMS (t-Butyldimethylsilyl) derivative ☐ TMS (Trimethylsilyl) derivative ☐ Other derivative:
Retention parameter used for (peak) identification:	X Retention time (Rt) 12,33 ☐ Scan number
X Compared to reference chemical:	Source : ☐ Own Synthesis X DSO Singapore
☐ Compared to library spectrum:	Source : ☐ OCAD (code:) ☐ NIST ☐ Wiley ☐ Own ☐ Other:
☐ Not compared to reference chemical or library spectrum:	Intense ions in spectrum are explained; interpretation is supported by the spectral information derived from closely related chemical(s):
Comments:	



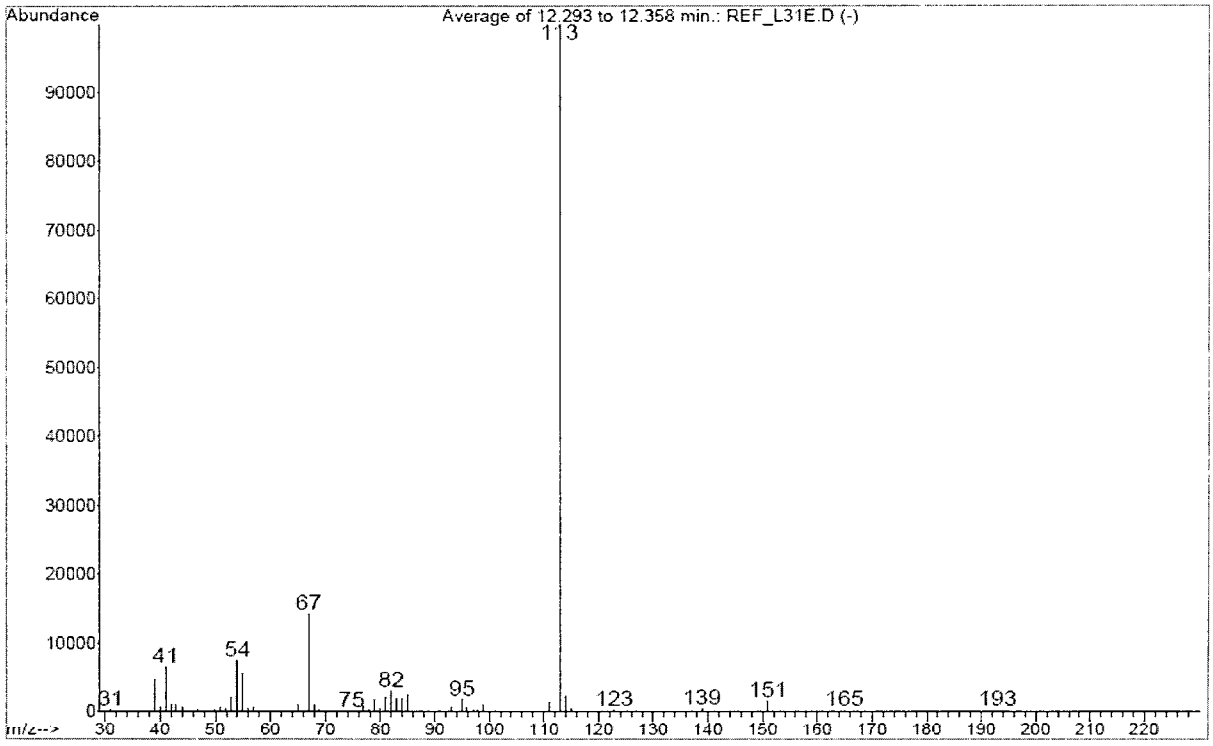
EI chromatograms supporting the identification of chemical 6; TIC on left; EIC (m/z 113) on right.

Top:	Chromatograms of Organic liquid blank, aliquot LB18B from OB/28.
Center:	Chromatograms of Organic liquid sample, aliquot L17B from O/28.
Bottom:	Chromatograms of authentic reference standard of O-cyclohexyl ethylphosphonoflouridate, retention time 12.33 min.

```
File       : D:\PT14 5972\L17B1E.D
Acquired   :  5 Nov 2003  13:28      using AcqMethod RRF40
Sample Name: PT14 ORGANIC LIQUID; SAMPLE (O/28)
Misc Info  :
```




```
File       : D:\PT14 5972\REF L31E.D
Acquired   :  5 Nov 2003  16:41      using AcqMethod RRF40
Sample Name:
Misc Info  :
```



EI mass spectra (averaged and background subtracted) of:

Top: Silylated chemical 6 in Organic liquid sample, aliquot L17B from O/28

Bottom: Aauthentic reference standard of O-cyclohexyl ethylphosphonoflouridate corresponding to chemical 6 (Monoisotopic MW:194)

GC-CI-MS TECHNIQUE METHOD AND ANALYSIS DESCRIPTION

Laboratory code: 28, 29 **Sample code(s):** O/28, OB/28 **Compound number:** 6

Aliquot codes: L17B, LB18B			
Sample:	**L17B**	**Blank:**	**LB18B**

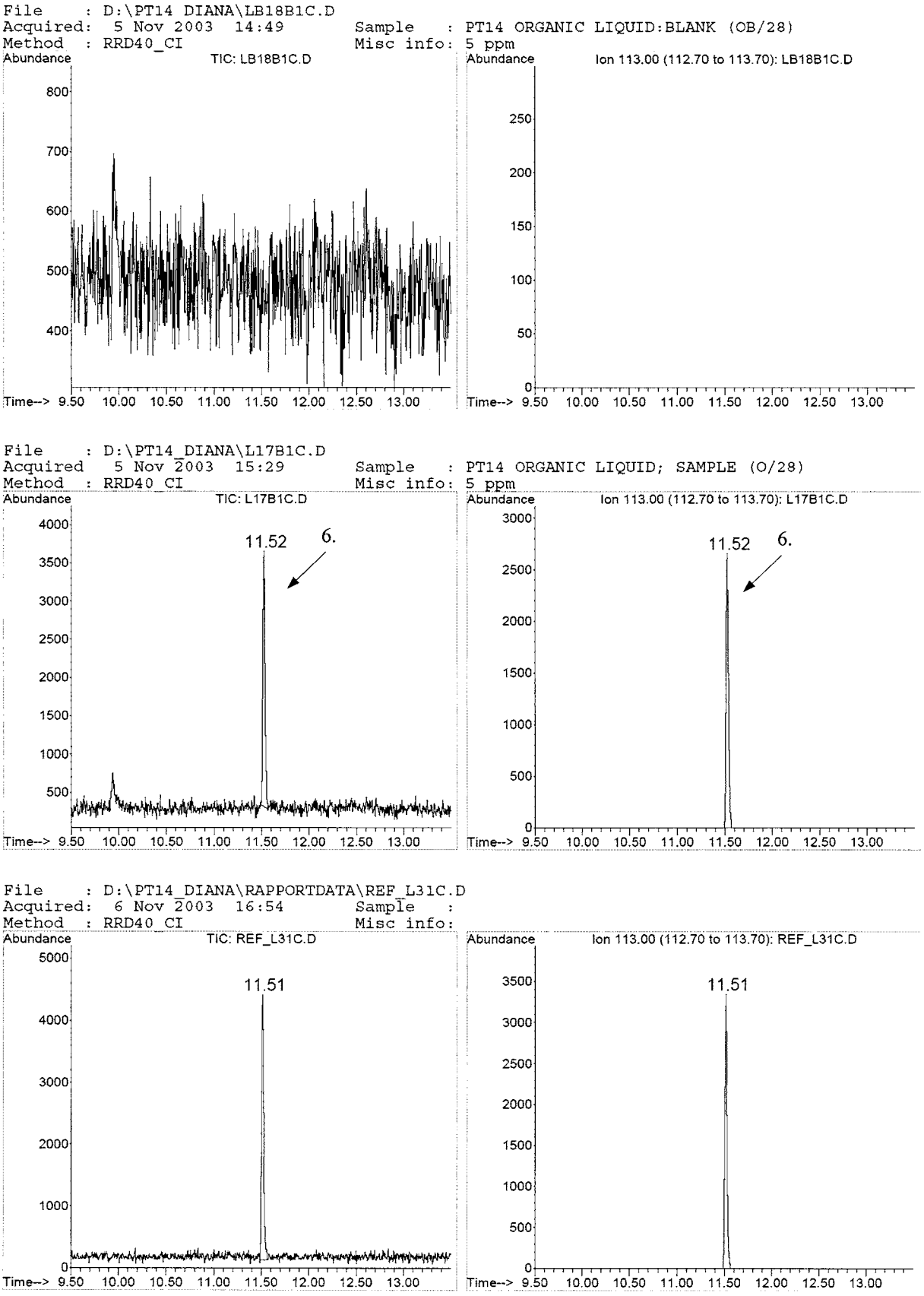
GC-CI-MS Method name:	**RRD40_CI**

METHOD DESCRIPTION

Instrument Manufacturer and Type:	HP6890 + HP 5973N (MSD)		
Carrier gas:	X He ☐ N_2 ☐ H_2 ☐ Other:		
Flow rate:	☐ ml/min X 35 cm/s		
Flow control:	☐ Constant Pressure X Constant Flow		
Injection mode:	☐ Split → Split ratio = X Splitless → Splitless time = 1 min		
Injector temperature:	200 °C		
Column phase:	DB5MS		
Column Length x ID x Film thickness:	30 m x 0,25 mm x 0,25 μm		
GC temperature programme:	40 °C (1 min), 10 °C/min, 280 °C (5 min)		
Reaction gas:	☐ Methane X Isobutane ☐ Ammonia ☐ Other:		
Solvent delay time:	4 min	**Scan range:**	60-500 m/z
Electron energy:	170 eV	**Scan time:**	0,6 s
Ionisation polarity:	X Positive ☐ Negative	**Mass resolution:**	Unit
Comments:			

ANALYSIS

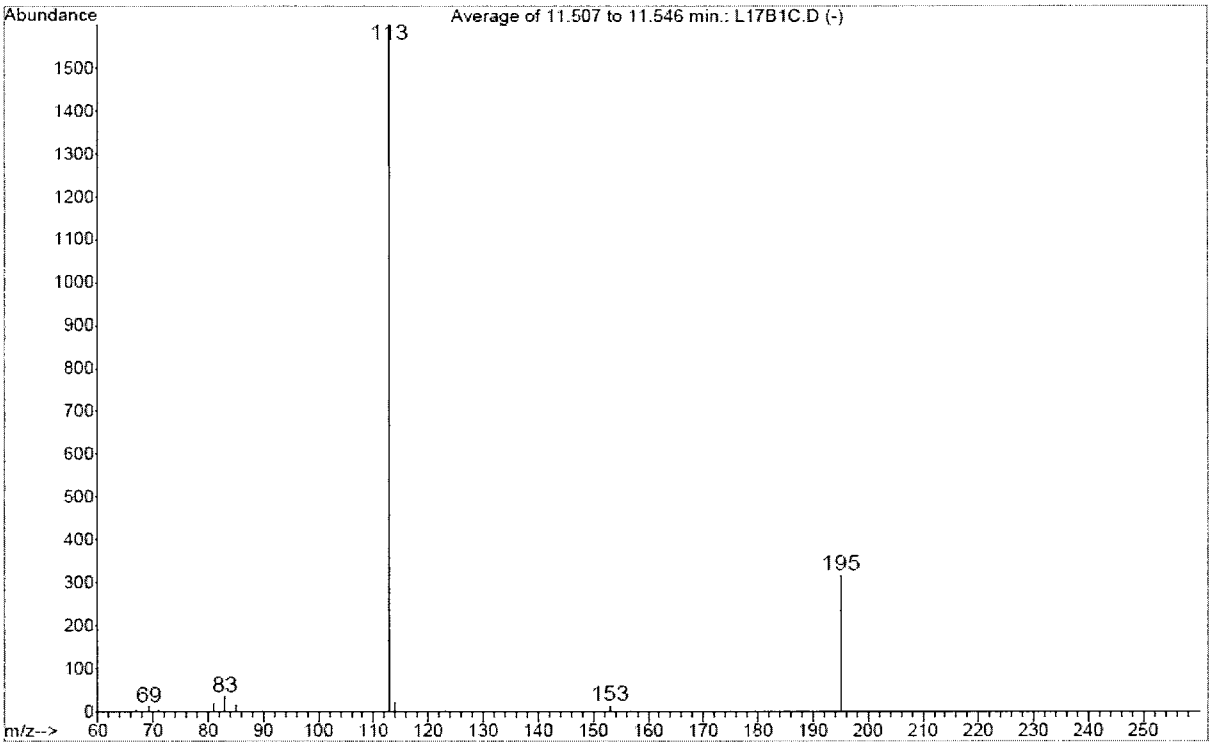
Compound identified as:	X Original compound ☐ Methyl ester derivative ☐ TBDMS (t-Butyldimethylsilyl) derivative ☐ TMS (Trimethylsilyl) derivative ☐ Other derivative:
Retention parameter used for (peak) identification:	X Retention time (Rt) 11,52 ☐ Scan number
X Compared to reference chemical:	Source : ☐ Own Synthesis X DSO Singapore
☐ Not compared to reference chemical	Intense ions in spectrum are explained
Comments:	



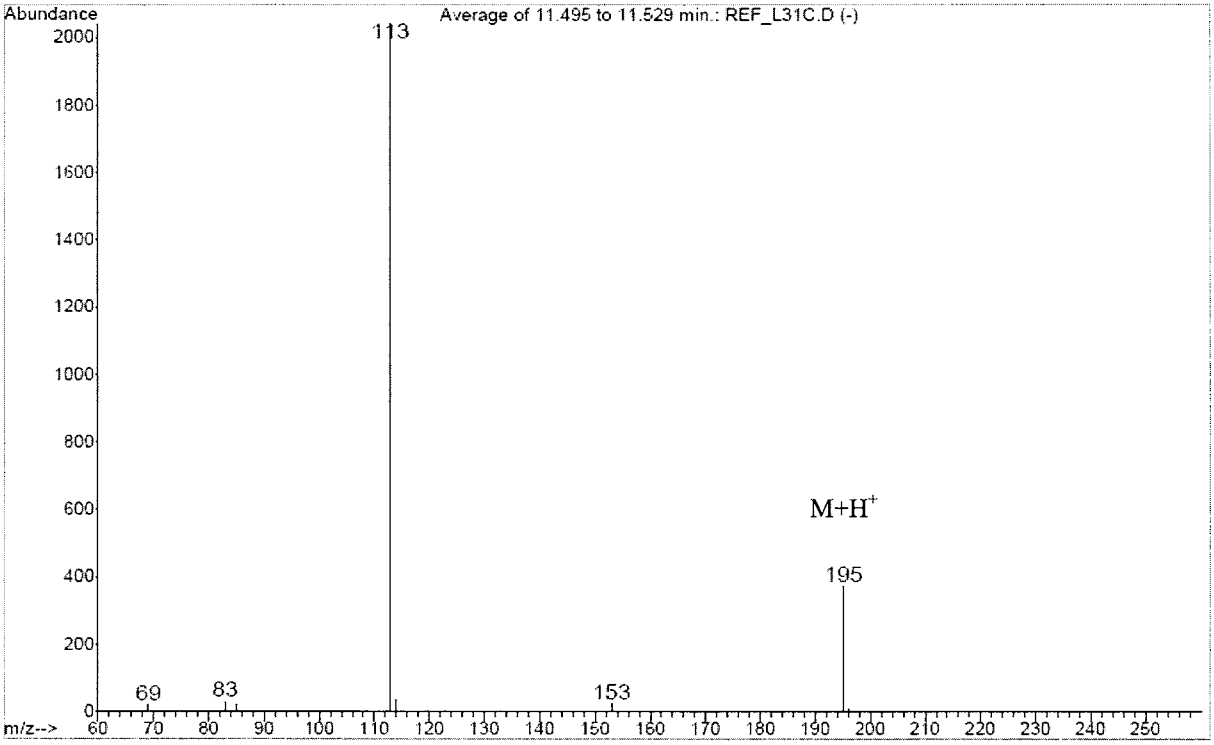
CI chromatograms supporting the identification of chemical 6; TIC on left; EIC (m/z 113) on right.

Top:	Chromatograms of Organic liquid blank, aliquot LB18B from OB/28.
Center:	Chromatograms of Organic liquid sample, aliquot L17B from O/28.
Bottom:	Chromatograms of authentic reference standard of O-cyclohexyl ethylphosphonoflouridate, retention time 11.51 min.

```
File       : D:\PT14 DIANA\L17B1C.D
Acquired   :  5 Nov 2003  15:29     using AcqMethod RRD40 CI
Sample Name: PT14 ORGANIC LIQUID; SAMPLE (O/28)
Misc Info  : 5 ppm
```




```
File       : D:\PT14 DIANA\RAPPORTDATA\REF_L31C.D
Acquired   :  6 Nov 2003  16:54     using AcqMethod RRD40 CI
Sample Name:
Misc Info  :
```



CI mass spectra (averaged and background subtracted) of:

Top: Chemical 6 in Organic liquid sample, aliquot L17B from O/28

Bottom: Authentic reference standard of O-cyclohexyl ethylphosphonoflouridate corresponding to chemical 6 (monoisotopic MW:194)

GC-EI-MS TECHNIQUE METHOD AND ANALYSIS DESCRIPTION

Laboratory code: 28,29 **Sample code(s):** O/28, OB/28 **Compound number:** 7

Aliquot codes: L11A,LB12A			
Sample:	**L11A**	**Blank:**	**LB12A**

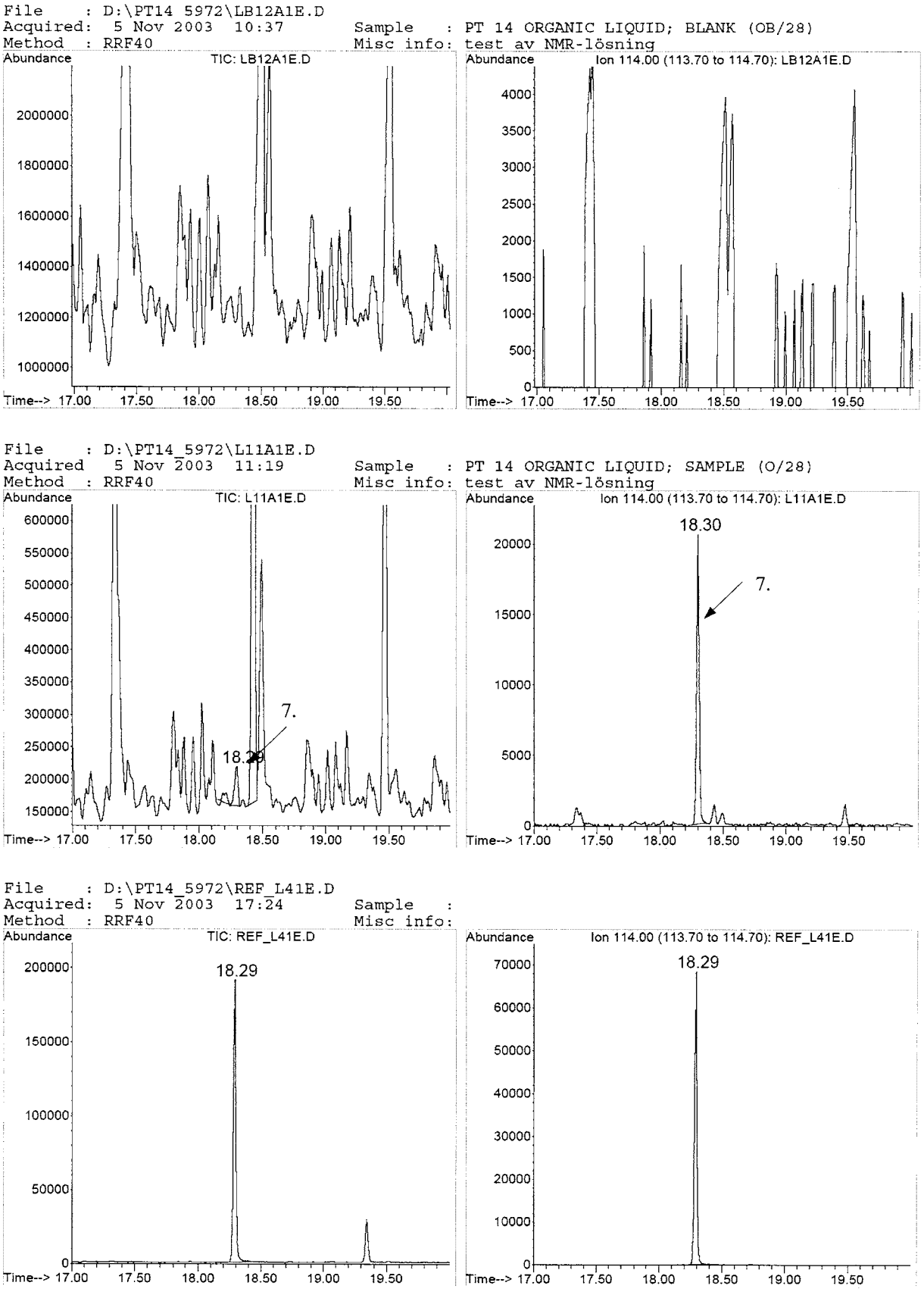
GC-EI-MS Method name:	**RRF40**

METHOD DESCRIPTION

Instrument Manufacturer and Type:	HP 5890GC + HP5972 MSD		
Carrier gas:	X He ☐ N_2 ☐ H_2 ☐ Other:		
Flow rate:	☐ ml/min X 35 cm/s		
Flow control:	☐ Constant Pressure X Constant Flow		
Injection mode:	☐ Split → Split ratio = X Splitless → Splitless time = 1 min		
Injector temperature:	200 °C		
Column phase:	DB5MS		
Column Length x ID x Film thickness:	30 m x 0,25 mm x 0,25 μm		
GC temperature programme:	40 °C (1 min), 10 °C/min, 280 °C (5 min)		
Solvent delay time:	3 min	**Scan range:**	29-450 m/z
Electron energy:	70 eV	**Scan time:**	0,4 s
Ionisation polarity:	X Positive ☐ Negative	**Mass resolution:**	unit
Comments:			

ANALYSIS

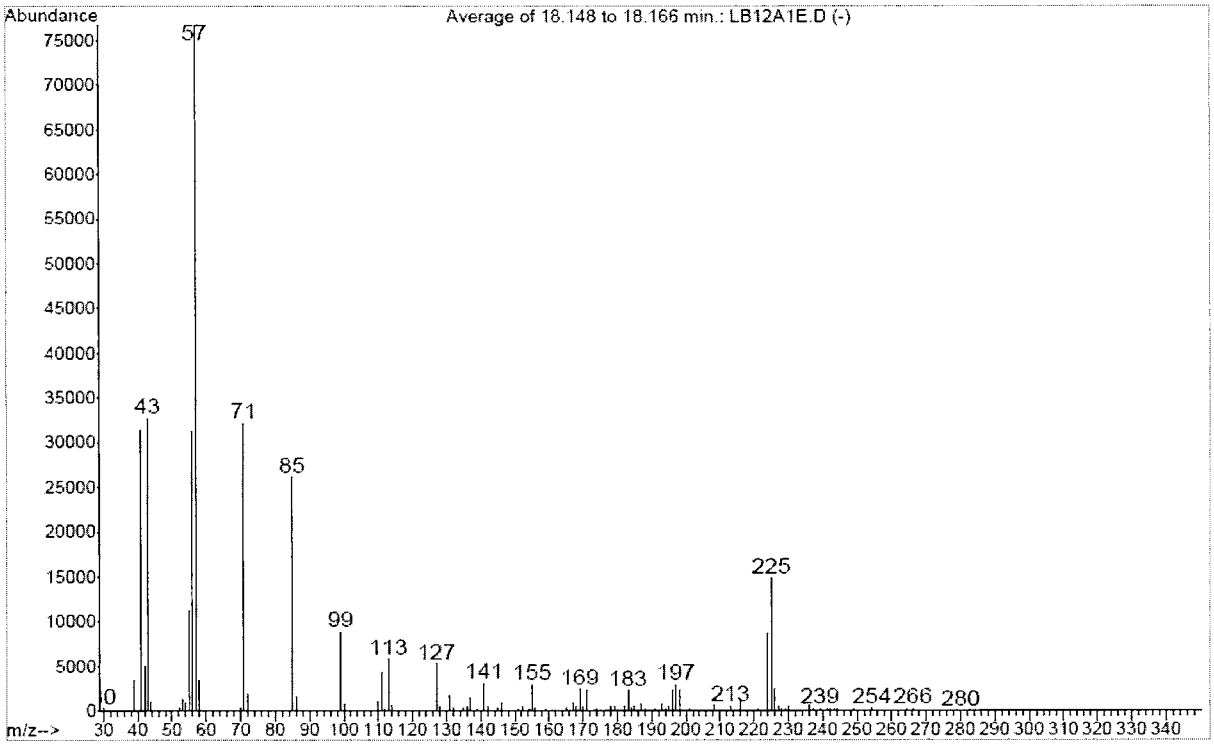
Compound identified as:	X Original compound ☐ Methyl ester derivative ☐ TBDMS (t-Butyldimethylsilyl) derivative ☐ TMS (Trimethylsilyl) derivative ☐ Other derivative:
Retention parameter used for (peak) identification:	X Retention time (Rt) 18,29 ☐ Scan number
X Compared to reference chemical:	Source : ☐ Own Synthesis X DSO Singapore
☐ Compared to library spectrum:	Source : ☐ OCAD (code:) ☐ NIST ☐ Wiley ☐ Own ☐ Other:
☐ Not compared to reference chemical or library spectrum:	Intense ions in spectrum are explained; interpretation is supported by the spectral information derived from closely related chemical(s):
Comments:	



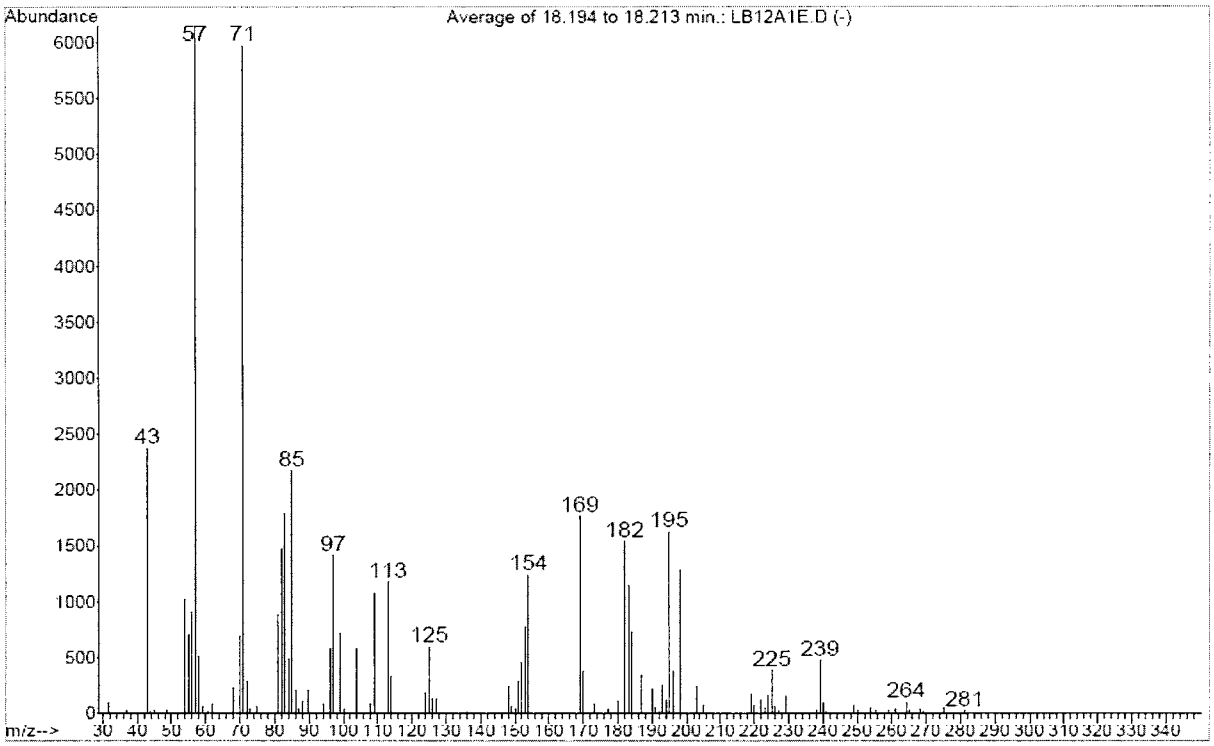
EI chromatograms supporting the identification of chemical 7; TIC on left; EIC (m/z 114) on right.

Top:	Chromatograms of Organic liquid blank, aliquot LB12A from OB/28.
Center:	Chromatograms of Organic liquid sample, aliquot L11A from O/28.
Bottom:	Chromatograms of authentic reference standard of O-propyl s-2-diisopropylaminoethyl methylphosphonothiolate, retention time 18.29 min.

```
File        : D:\PT14 5972\LB12A1E.D
Acquired    :  5 Nov 2003  10:37     using AcqMethod RRF40
Sample Name: PT 14 ORGANIC LIQUID; BLANK (OB/28)
Misc Info   : test av NMR-lösning
```

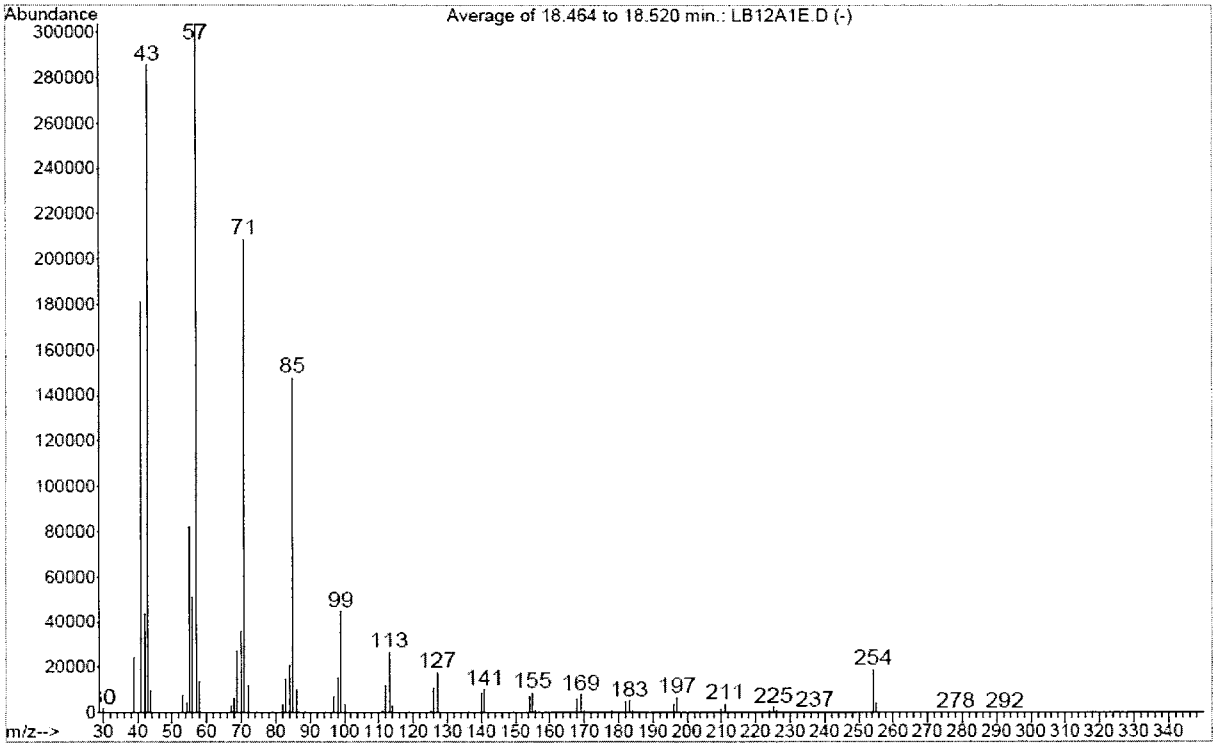



```
File        : D:\PT14 5972\LB12A1E.D
Acquired    :  5 Nov 2003  10:37     using AcqMethod RRF40
Sample Name: PT 14 ORGANIC LIQUID; BLANK (OB/28)
Misc Info   : test av NMR-lösning
```

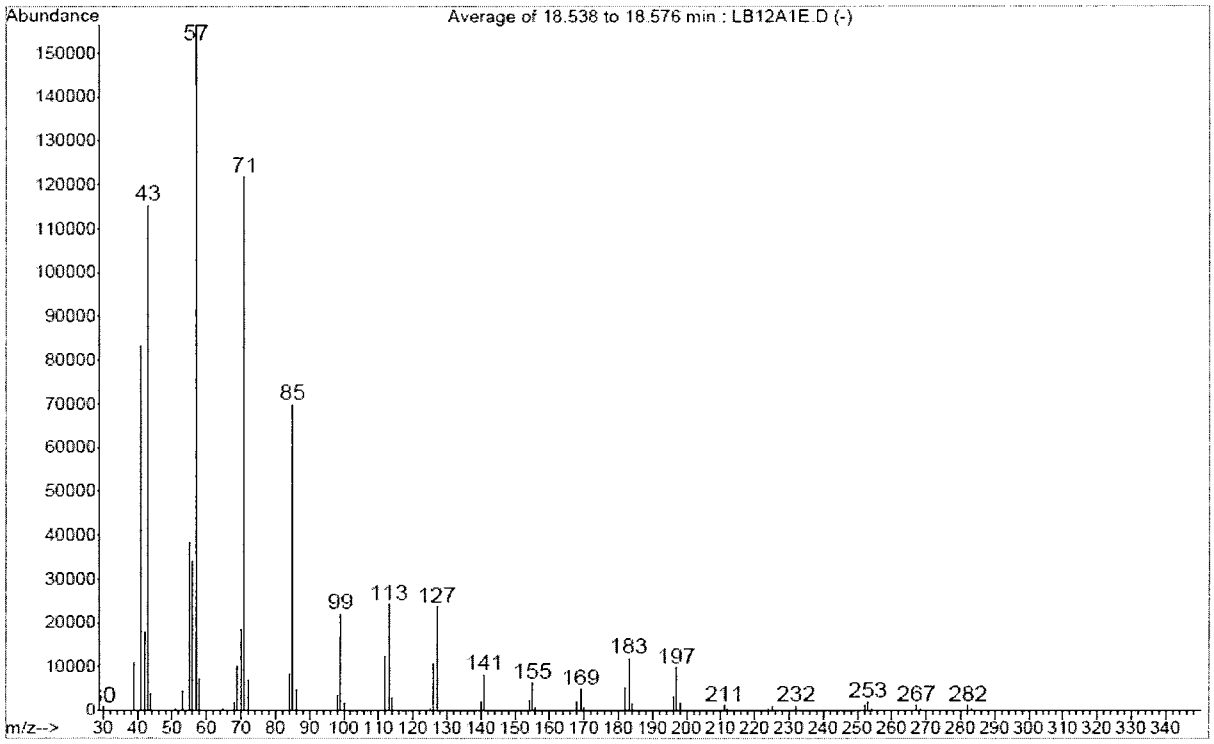


EI mass spectra (averaged and background subtracted) of:	
Top:	Peak present in blank, aliquot LB12A from OB/28 within the retention time window of chemical 7 at RT 18.15
Bottom:	Peak present in blank, aliquot LB12A from OB/28 within the retention time window of chemical 7 at RT 18.20

```
File       : D:\PT14 5972\LB12A1E.D
Acquired   :  5 Nov 2003  10:37     using AcqMethod RRF40
Sample Name: PT 14 ORGANIC LIQUID; BLANK (OB/28)
Misc Info  : test av NMR-lösning
```

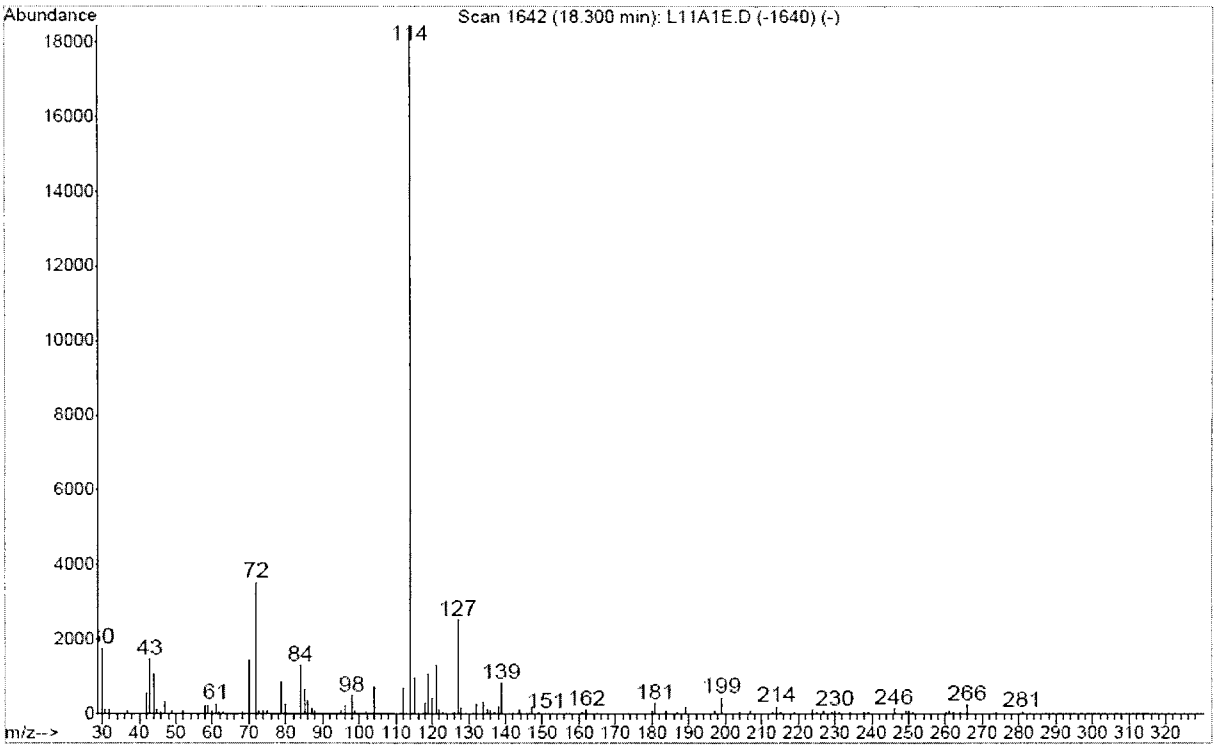



```
File       : D:\PT14 5972\LB12A1E.D
Acquired   :  5 Nov 2003  10:37     using AcqMethod RRF40
Sample Name: PT 14 ORGANIC LIQUID; BLANK (OB/28)
Misc Info  : test av NMR-lösning
```

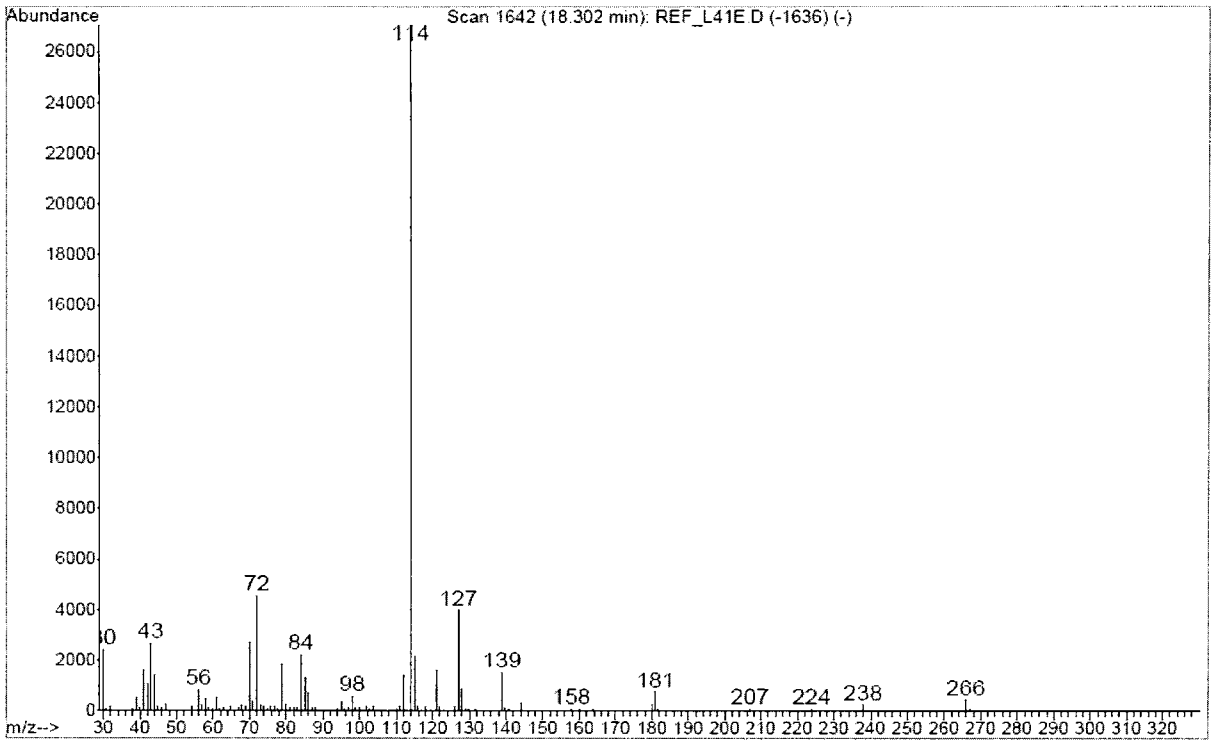


EI mass spectra (averaged and background subtracted) of:	
Top:	Peak present in blank, aliquot LB12A from OB/28 within the retention time window of chemical 7 at RT 18.50
Bottom:	Peak present in blank, aliquot LB12A from OB/28 within the retention time window of chemical 7 at RT 18.55.

```
File       : D:\PT14 5972\L11A1E.D
Acquired   :  5 Nov 2003  11:19     using AcqMethod RRF40
Sample Name: PT 14 ORGANIC LIQUID; SAMPLE (O/28)
Misc Info  : test av NMR-lösning
```




```
File       : D:\PT14 5972\REF L41E.D
Acquired   :  5 Nov 2003  17:24     using AcqMethod RRF40
Sample Name:
Misc Info  :
```



EI mass spectra (averaged and background subtracted) of:	
Top:	Chemical 7 in Organic liquid sample, aliquot L11A from O/28
Bottom:	Authentic reference standard of O-propyl s-2-diisopropylaminoethyl methylphosphonothiolate corresponding to chemical 7 (Monoisotopic MW:281)

GC-CI-MS TECHNIQUE METHOD AND ANALYSIS DESCRIPTION

Laboratory code: 28, 29 **Sample code(s):** O/28, OB/28 **Compound number:** 7

Aliquot codes: L11A, LB12A			
Sample:	**L11A**	**Blank:**	**LB12A**

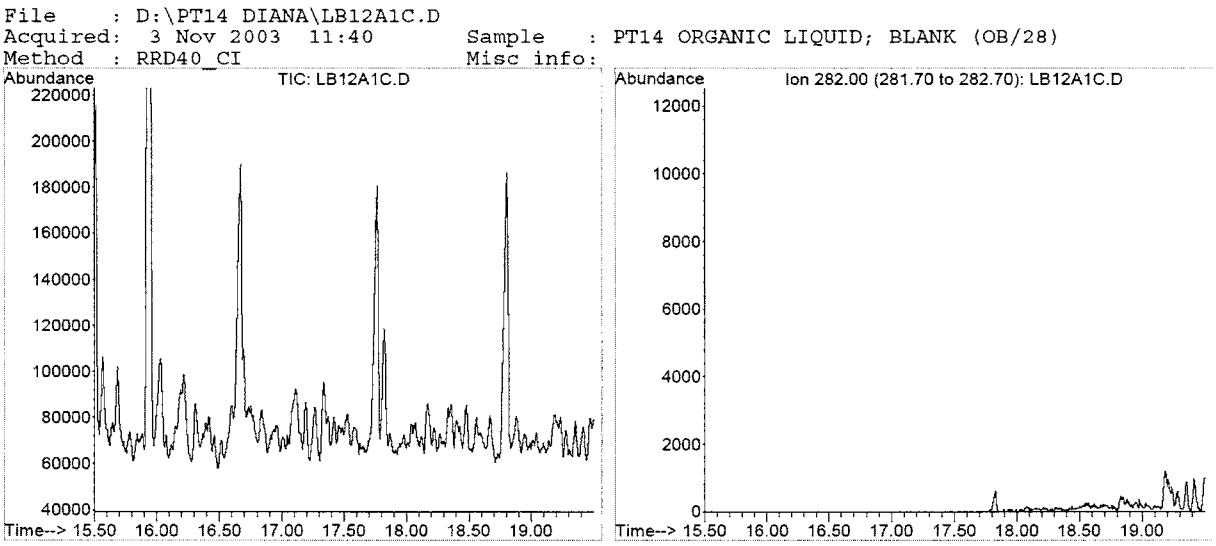
GC-CI-MS Method name:	**RRD40_CI**

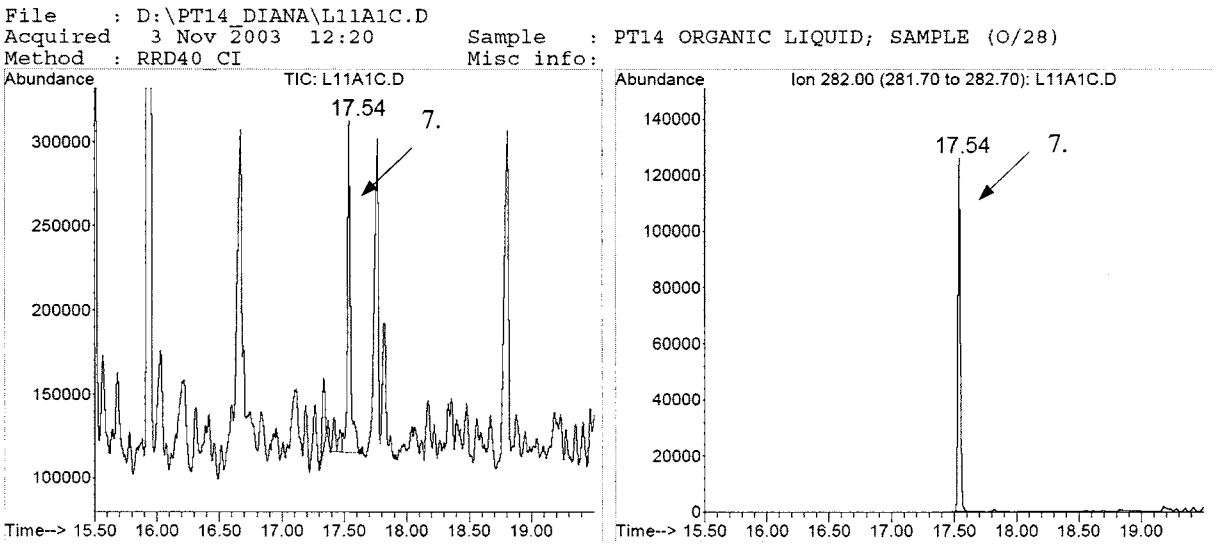
METHOD DESCRIPTION

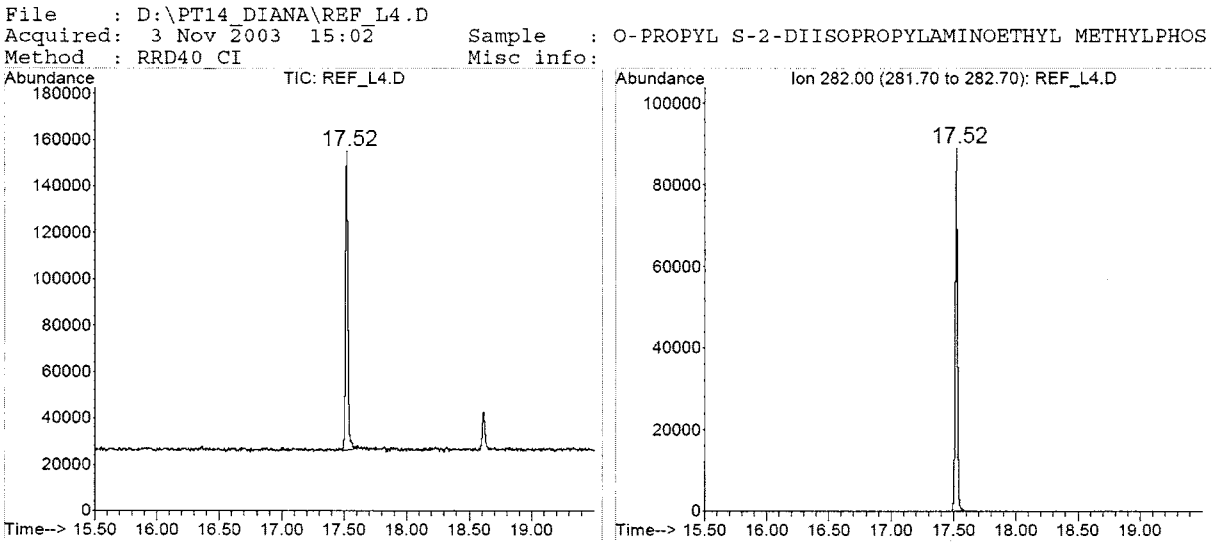
Instrument Manufacturer and Type:	HP6890 + HP 5973N (MSD)		
Carrier gas:	X He ☐ N_2 ☐ H_2 ☐ Other:		
Flow rate:	☐ ml/min X 35 cm/s		
Flow control:	☐ Constant Pressure X Constant Flow		
Injection mode:	☐ Split → Split ratio = X Splitless → Splitless time = 1 min		
Injector temperature:	200 °C		
Column phase:	DB5MS		
Column Length x ID x Film thickness:	30 m x 0,25 mm x 0,25 µm		
GC temperature programme:	40 °C (1 min), 10 °C/min, 280 °C (5 min)		
Reaction gas:	☐ Methane X Isobutane ☐ Ammonia ☐ Other:		
Solvent delay time:	4 min	**Scan range:**	60-500 m/z
Electron energy:	170 eV	**Scan time:**	0,6 s
Ionisation polarity:	X Positive ☐ Negative	**Mass resolution:**	Unit
Comments:			

ANALYSIS

Compound identified as:	X Original compound ☐ Methyl ester derivative ☐ TBDMS (t-Butyldimethylsilyl) derivative ☐ TMS (Trimethylsilyl) derivative ☐ Other derivative:
Retention parameter used for (peak) identification:	X Retention time (Rt) 17,54 ☐ Scan number
X Compared to reference chemical:	Source : ☐ Own Synthesis X DSO Singapore
☐ Not compared to reference chemical	Intense ions in spectrum are explained
Comments:	

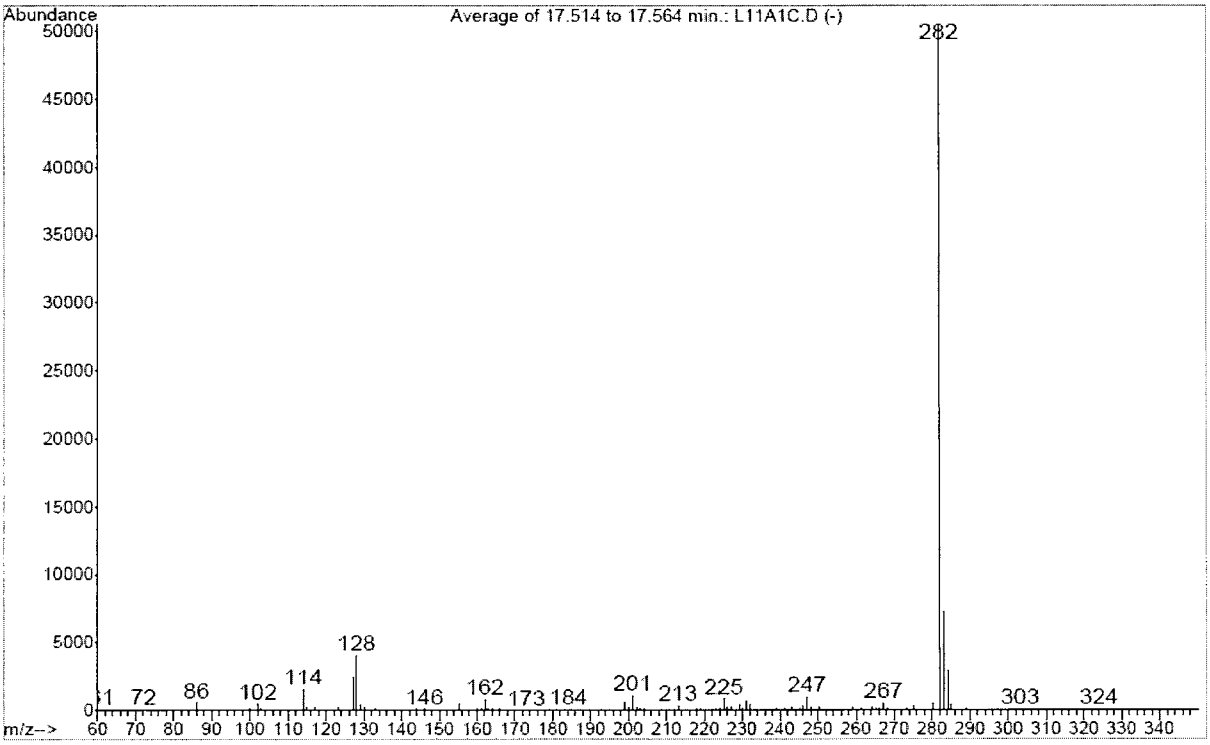




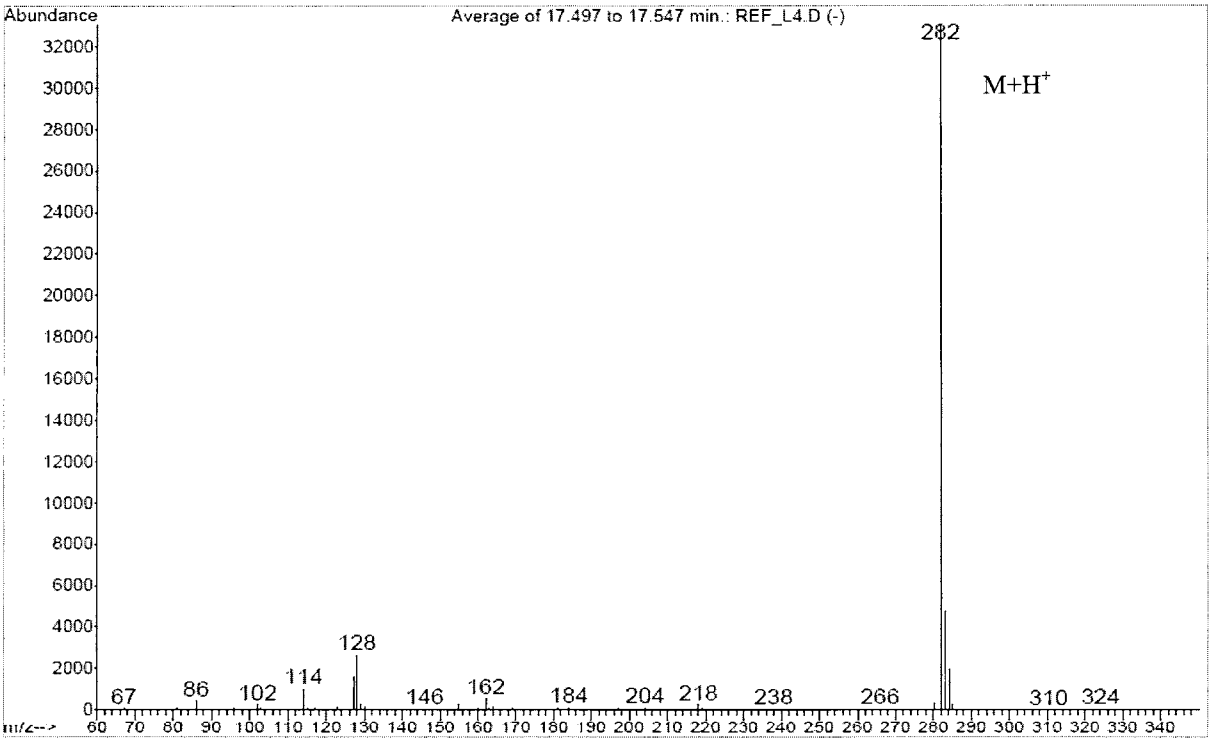


CI chromatograms supporting the identification of chemical 7; TIC on left; EIC (m/z 282) on right.
Top: Chromatograms of Organic liquid blank, aliqout LB12A from OB/28.
Center: Chromatograms of Organic liquid sample, aliquot L11A from O/28.
Bottom: Chromatograms of authentic reference standard of O-propyl s-2-diisopropylaminoethyl methylphosphonothiolate , retention time 17.52 min.

```
File        : D:\PT14 DIANA\L11A1C.D
Acquired    :  3 Nov 2003  12:20      using AcqMethod RRD40 CI
Sample Name: PT14 ORGANIC LIQUID; SAMPLE (O/28)
Misc Info   :
```




```
File        : D:\PT14 DIANA\REF L4.D
Acquired    :  3 Nov 2003  15:02      using AcqMethod RRD40 CI
Sample Name: O-PROPYL S-2-DIISOPROPYLAMINOETHYL METHYLPHOS
Misc Info   :
```



CI mass spectra (averaged and background subtracted) of:

Top: Chemical 7 in Organic liquid sample, aliquot L11A from O/28

Bottom: Authentic reference standard of O-propyl s-2-diisopropylaminoethyl methylphosphonothiolate corresponding to chemical 7 (monoisotopic MW:281)

SAMPLE PREPARATION DESCRIPTION

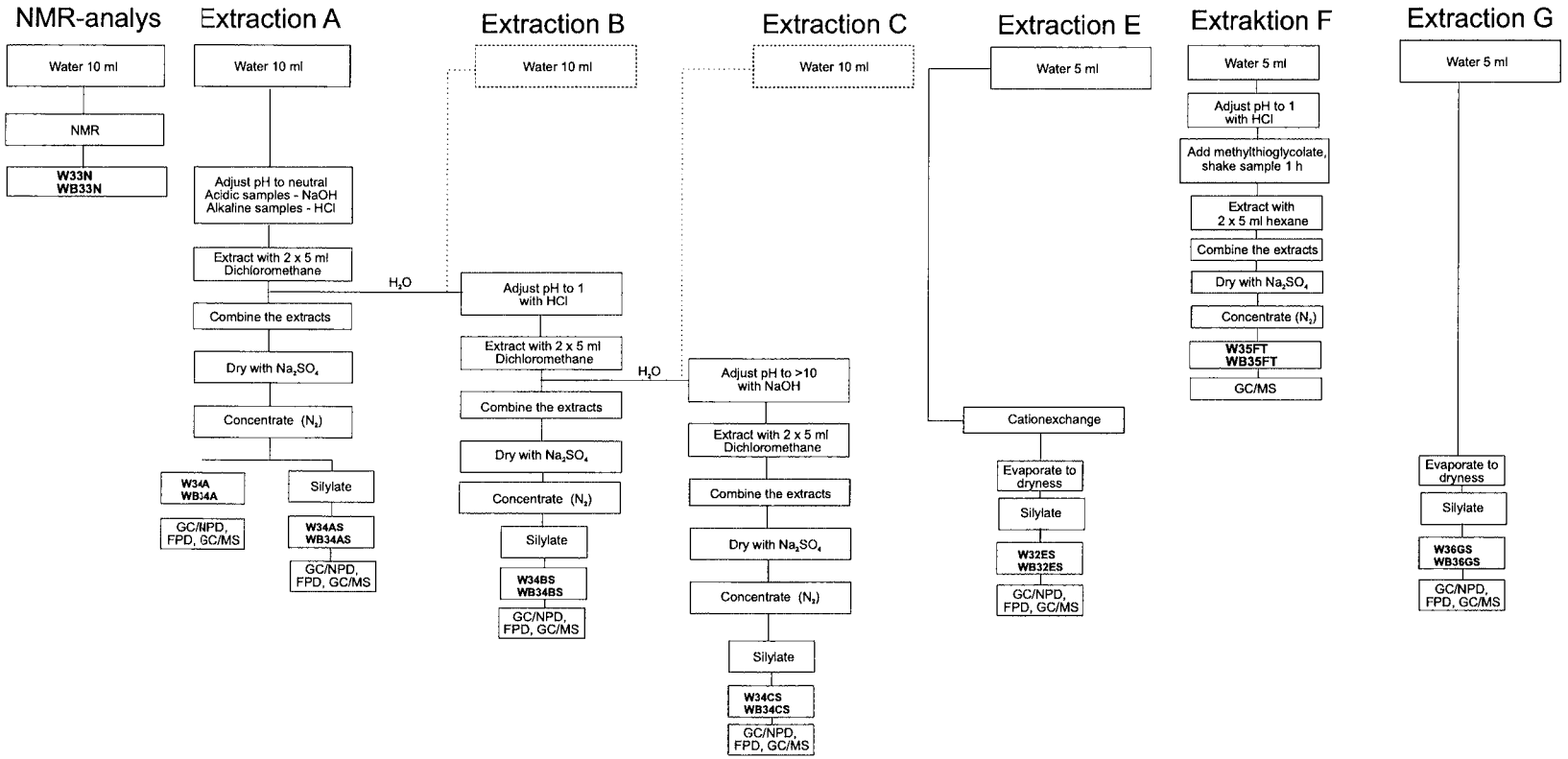
Laboratory code: 28,29 **Sample code:** W/28 **Blank code:** WB/28

1. Sample preparation

Sample/ Aliquot Code	Specification of Sample/ Type of Sample Preparation	Amount/ Volume	Sample Preparation Procedures	End Volume	Resulting Aliquot Code	Analytical Technique
WB34/W34	Extraction	10 ml	Methylenechloride 2*5 ml at pH 6, concentration with N2	~1.5 ml	WB34A/W34A	GC-EIMS GC-CIMS
			Silylation with BSTFA	~1 ml	WB34AS/W34AS	GC-EIMS GC-CIMS
	Extraction	10 ml	Methylenechloride 2*5 ml at pH 1, concentration with N2 silylation with BSTFA	~1 ml	WB34BS/W34BS	GC-EIMS GC-CIMS
	Extraction	10 ml	Methylenechloride 2*5 ml at ,pH 10, concentration with N2 silylation with BSTFA	~1 ml	WB34CS/W34CS	GC-EIMS GC-CIMS
WB35/W35	Derivatization and Extraction	5 ml	Derivatization with methylthioglycolate at pH 1, extraction with 2*5 ml hexane, concentration with N2	~1 ml	WB35FT/W35FT	GC-EIMS GC-CIMS
WB32/W32	Evaporation and Derivatization	5 ml	Cation exchange (SCX;Extract-Clean), evaporation to dryness, resolvation in CH3CN, silylation with BSTFA	~1 ml	WB32ES/W32ES	GC-EIMS GC-CIMS
WB36/W36	Evaporation and Derivatization	5 ml	Evaporation to dryness, resolvation in CH3CN, silylation with BSTFA	~1 ml	WB36GS/W36GS	GC-EIMS GC-CIMS

2. Additional information

Sample preparation of water WB/28 and W/28



No compounds found in any fraction !

COMMENTS

1. General

2. Sample preparation

3. Analysis

The extension from aliquot code of 1E respectively 1C in datafile name stands for order of injection and ionization teqnique.

Example:

1E = first injection, electron impact
1C = first injection, chemical ionization