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Determination of Trace Amounts of Russian Toxic Agent VX

L. K. Gustyleva and E. I. Savel'eva

Research Institute of Hygiene, Occupational Pathology and Human Ecology, Federal Medical Biological Agency,
st. Kapitolovo, korp. 93, p.g.t. Kuz'molovskii, Leningradskaya oblast, 188663 Russia
e-mail: gustyleva@mail.ru

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Abstract—Method was developed for detection of residual amounts of Russian toxic agent RVX on metallic surface. The method is based on RVX conversion into *O*-isobutyl ester of methylphosphonic acid fluoride on a filter impregnated with silver fluoride, followed by detection of the formed derivative using a flame photometer. The method sensitivity has been enhanced by application of a large volume injection (0.20 mL). The method allows determination of RVX levels corresponding to the sanitary regulations and is intended for control measures at the toxic agents elimination establishments and for determination of the Danger grade of metallic waste.

Keywords: *O*-isobutylmethylphosphonic acid fluoride, toxic agent, VX, large volume injection, thermodesorption, gas chromatography

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Elimination of chemical weapon arsenals in the frame of governmental programs is carried out at the specialized establishments that should be decommissioned at the final stage. This stage is subject to risk of environmental pollution and should be therefore accompanied with comprehensive ecological examination including determination of residual concentration of chemical weapon components in various technological media at level comparable to maximum permissible concentration, MPC.

Organophosphorus compounds, especially these of VX group, are the most toxic of the known chemical weapons, and their chemical neutralization is extremely complicated. Due to the high toxicity of organophosphorus compounds, the MPC values stated in the sanitary regulations are extremely low, and determination of these compounds is classified as ultra-trace analysis. Isolation and detection of the VX group compounds is fairly complicated under conditions of chromatographic analysis. Due to the presence of many polar groups, these compounds are readily adsorbed by different materials, therefore their extraction from complex objects (soil, building waste, polymers, etc) is even more complicated.

O-Isobutyl-S-[(2-diethylamino)ethyl]methylthiophosphonate (hereafter denotes as RVX), one of typical representatives of the VX group, was produced exclusively in the former USSR. This compound has been scarcely studied so far as compared with the isomeric VX compound produced in foreign countries, *O*-ethyl-*S*-[(2-diisopropylamino)ethyl]methylthiophosphonate. These compounds are not identical with regard to their physicochemical properties.

To date, new methods of V-agents determination in different objects have been developed, aiming to improve the available procedures sensitivity. For example, mechanism of VX decomposition at concrete surfaces was studied in [1]. It was demonstrated that relatively small amounts of VX were half decomposed within 2–3 h at room temperature, via the rupture of P–S and S–C bonds (mass spectrometry of secondary ions). The decomposition was assumed to occur via basic hydrolysis in the presence of water layer adsorbed at the sample surface.

A method to determine low VX concentration (~10–7 g/L, ~0.1 MPC) in the working area air was developed in [2]. In particular, air to be analyzed was passed through a tube filled with glass beads; VX was efficiently sorbed at the beads surface. The sorbate was then extracted with isopropyl alcohol, the deuterated VX-d_5 internal reference was introduced into the extract, and the latter was analyzed by liquid chromatography method using an HPLC–MS–MS quadrupole mass spectrometer. The linearity range of VX determination using the said method was of 0.5–2 MPC provided that 24 L of air was sampled during 1 h (0.4 L/min). The major advantages of the method were high selectivity and sensitivity. However, the use of expensive HPLC–MS–MS equipment is a restriction for wide application of the described method.

Yet many laboratories at the chemical weapon elimination facilities are equipped with gas chromatographs combined with thermodesorbers, therefore, chromatography methods of RVX determination have been widely applied. These methods are essentially variations of the parent procedure proposed in [3]: concentration of the V-agent at the sorption tube accompanied with conversion into the corresponding ester of methylfluorophosphonate induced by silver fluoride; thermodesorption of the prepared derivative; and its detection by means of gas chromatograph equipped with flame photometer.

For example, a method to determine the level of pollution of processing equipment surface with the VX compounds in the range of 1.0×10^{-6} mg/dm^2 to 2.0×10^{-4} mg/dm^2 [4] utilized a AgF-induced transformation of RVX into a volatile derivative followed by sorption of the acid fluoride in a tube equipped with Teflon attachment, thermodesorption, separation of the components at a capillary chromatography column, and the components detection with flame photometry and mass spectrometer. The RVX conversion into the acyl fluoride improved the method sensitivity as compared with direct RVX detection to reach the detection limit as low as 0.5 MPC.

Residual amount of RVX (1.0×10^{-3}–1.0×10^{-2} mg/mL) in the reaction mixtures after decontamination with RD4M could be determined using a method implying extraction of RVX with hexane, elimination of RVX decomposition products and excess of RD4M from the extract, and simultaneous concentration of the latter [5]. Separation and detection of the components was performed using a capillary chromatography column equipped with mass-selective detector. The reproducible results were obtained when using hexachlorobenzene as an internal reference.

A method to determine trace amounts (5.0×10^{-8}–50.0×10^{-8} mg/g) of RVX in ashy wastes has been developed, based on gas chromatography with flame photometry detection [6]. Despite the extraordinary laboriousness, no alternative method has been found to date.

Another method of gas chromatography determination of trace amounts (1.0×10^{-8}–10.0×10^{-8} mg/kg, 0.5–5 MPC) of RVX in the various combustion products and wastes was proposed in [7]. The procedure was based on RVX extraction from a specimen with alkaline aqueous solution of monoethanolamine, followed by re-extraction to hexane. After evaporating the solvent, RVX was converted into methyl ether of *O*-isobutylmethylphosphonic acid by HCl in the presence of $AgNO_3$. The formed derivative was detected by gas chromatograph equipped with a flame photometer. The procedure allowed determination of low concentration of RVX, of about 0.5 MPC.

Determination of trace amounts of RVX in complex materials requires enhancement of the sampling and the specimen preparation efficacy as well as improvement of detection accuracy. The enhancement of the components extraction from the decontaminated surfaces is often accompanied with co-extraction of other components that deteriorates the detection parameters. On the contrary, purification of the sample by repeated extraction or solid-phase extraction leads to losses of the detected components.

In order to improve the sensitivity of gas chromatographic determination of RVX in the washouts of the processing equipment, we tested the approach of large volume injection (LVI) into the capillary chromatograph column using temperature-programmable injectors of the PTV and MMI types. Such injectors are commonly applied in chromatographic analysis of thermally labile specimens or of the samples containing components with significantly different boiling points. A definite advantage of injection of large volume (50–100 μL) of the specimen over the conventional procedure to inject 1–3 μL is that the former method allows analysis of the whole sample rather than its aliquot part, thus improving the detection limit. Major features of the LVI approach have been discussed elsewhere [8].

Modern temperature-programmable injectors of the PTV and MMI types allow injection of a large volume either at once or in a series of portions (with a regular autosampler). The PTV and MMI injectors are efficiently cooled to below the solvent boiling point with carbon dioxide. Subsequent fast heating to a required temperature at 720 deg/min is ensured by high-power heaters. Chromatographic analysis with LVI allows for direct detection of RVX excluding the complicated stages of volatile derivatives formation.

Table 1. Analysis reproducibility with different methods of sample injection (100 μL, PTV)

Sample injection	Exp. no.	RVX retention time, min	Peak area, a.u.
Automated, 5 μL × 20	1	14.063	838073
	2	14.062	867389
	3	14.063	825706
	mean	14.063	$(84.4\pm2.5)\times10^4$
Manual, 100 μL × 1	1	14.064	525404
	2	14.069	575472
	3	14.067	584611
	mean	14.065	$(56\pm3)\times10^4$

Table 2. Metrological performance of methods MUK 4.1.37-2012 [9] and MUK 4.1.39-2012 [10]

Measurement range x, mg/dm^2	Reproducibility σ_R, %[a]	Correctness $\pm\delta_c$, %[b]	Accuracy $\pm\delta$, %[c]	Reproducibility limit R, %[d]
$10^{-6} < x \le 10^{-5}$	18	8	36	50
$10^{-5} < x \le 10^{-4}$	16	7	32	44
$10^{-6} < x \le 5\times10^{-5}$	19	8	38	53
$5\times10^{-5} < x \le 10^{-4}$	15	7	30	42

[a] Relative standard deviation of reproducibility as estimated in the interlaboratory experiment (*L* 2). [b] Limits of relative systematic error at *P* 0.95. [c] Limits of relative error at *P* 0.95, correspond to the extended uncertainty U_{rel} (r. u.) at coverage coefficient *k* 2. [d] Relative acceptable disagreement between a pair of measurement results under conditions of reproducibility at *P* 0.95.

This work aimed to optimize a method of determination of RVX traces at processing equipment surface. We compared the two ways of LVI: manual injection of 100 μL of the sample and the multifold injection of smaller portions (5 μL × 20 = 100 μL) using an autosampler. Other experimental conditions are given in the Experimental section.

Table 1 compares reproducibility of the measured peak area and retention time values after the manual and automated injection (PTV injector, 100 μL sample).

As evidenced by data in Table 1, automated injecttion was preferential, even though the manual injection gave high reproducibility as well. The experiment afforded linear dependences of the signal (peak area) on the mass of the analyte introduced. LVI allowed for direct quantitative detection of RVX in its solutions with concentration of 10 μg/L. Such sensitivity was never achieved before when using the GC equipment in combination with low resolution MS detector. The tested method can be used for analysis of the samples with the background signal from detector not exceeding the signal of the most dilute calibration sample. For instance, in the case of the equipment and calibrating samples set we used in this work the background signal should be below 50000 r.u. (mass-selective detector) or 450 nA (flame photometer). Noteworthily, the achieved sensitivity allowed for direct detection of RVX concentrations at the level stated in the sanitary regulations. The method can be used for analysis of atmospheric air, drinkable water, or pre-purified extracts.

Further experiments revealed that determination of RVX at contaminated processing equipment surfaces required taking into account the presence of various admixtures: products of RVX decomposition, components of decontaminating solutions (sulfonol, hydrogen peroxide, sodium hypochlorite, monoethanolamine, and phosphates) as well as inorganic salts, organic solvents, and other compounds used for the surface treatment. The admixtures increased both the mass spectrometer background signal (in particular, at, *m*/*z* 86 and 99) and the flame photometer background current during phosphorus detection. Therefore, the LVI method could not be used for direct detection of real life samples. Evidently, the admixtures effect could be less significant if RVX had any characteristic peaks with higher mass number.

In view of the above, we determined RVX content at non-absorbing metallic surfaces taking advantage of

Table 3. Operating mode of the PTV injector in the course of 100 μL sample injection with autosampler and the program of chromatographic separation

Injector type	PTV Inlet
Carrier gas	helium
Starting mode	PTV Solvent Vent (solvent blow-out)
Starting injector temperature	45°C
Pressure	2070 Pa
Total helium flow	63.5 mL/min
Flow of the spacer blow-off	3 mL/min
Injector temperature program	45°C (4 min), then heating to 250°C at 720 deg/min
Helium saving mode	20 mL/min after 8 min
Helium flow for blow	60 mL/min up to 6.5 min
Helium flow for injector blow-off	200 mL/min
Injector cooling temperature	to 25°C
Autosampling	5 μL × 20 = 100 μL
Column temperature program	45°C (5 min), then heating to 280°C at 20 deg/min, 280°C (15 min)
Mass-selective detector channels	*m*/*z* 86 and *m*/*z* 99
Mass-selective detector operating mode	electronic ionization
Ionizing electrons energy	70 eV

Table 4. Operating mode of the MMI injector in the course of 100 μL sample injection with autosampler and the program of chromatographic separation

Injector type	MMI Inlet
Carrier gas	nitrogen
Starting mode	MMI Solvent Vent (solvent blow-out)
Starting injector temperature	35°C
Starting flow	0.5 mL/min
Incubation at the starting flow	4.5 min
Increasing nitrogen flow rate	31 mL/min
Final nitrogen flow through injector	2 mL/min
Flow for spacer blow-off	3 mL/min
Injector temperature program	35°C (3 min), then heating to 280°C at 720 deg/min
Injector cooling temperature	to 30°C
Autosampling	5 μL × 20 = 100 μL
Column temperature program	35°C (5 min), then heating to 280°C at 20 deg/min, 280°C (20 min)

Table 5. Operating parameters of Agilent 7890A chromatograph and ACEM-9300 thermodesorber

Extract application onto the reactive filter	
Volume of extract applied to the filter	20 μL
Volume rate of air through the filter after extract application	200±50 mL/min
Duration of the filter blow-off after extract application	1.0 min
Volume of hexane applied to the filter for additional washing	50 μL
Duration of the filter blow-off after hexane application	2 min
Thermodesorption	
Temperature of 8-position valve	200±1°C
Temperature of interface	230±1°C
Temperature of sorption tube during hexane evaporation	50±1°C
Operation temperature of sorption tube	210±1°C
Starting temperature of focusing tube	37±1°C
Operation temperature of focusing tube	220±1°C
Duration of hexane removal	3.0±0.1 min
Duration of sorption tube heating	4.0±0.1 min
Duration of sorption tube cooling	1.0±0.1 min
Duration of focusing tube heating	3.0±0.1 min
Volume rate of nitrogen	20.00±0.01 mL/min
Chromatographic separation	
Flow split	0.1:1
Injector temperature	250±1°C
Starting temperature of column	50±1°C
Starting isothermal exposure of column	1 min
Column heating rate	16.0±0.1 deg/min
Final column temperature	270±1°C
Final temperature isothermal exposure of column	10 min
Carrier gas	nitrogen
Carrier gas flow through column	2.00±0.01 mL/min
Flame photometer detection	
Nitrogen flow	60.0±0.1 mL/min
Hydrogen flow	75.0±0.1 mL/min
Air flow	100.0±0.1 mL/min
Phosphorus detection channel	λ 525 nm
Detector temperature	250±1°C

RVX conversion into *O*-isobutyl ether of methylfluorophosphonate **I** with silver fluoride. Such chemical transformation was earlier used in other methods of analysis of metallic surfaces washouts [4]; however, preparation of the volatile derivative led to the sample dilution.

In order to eliminate the said drawback, in this work the washouts extract (20 μL) was directly applied onto the modifying (reactive) filter that was installed in the Teflon attachment to the sorption tube filled with the SBTenax-TA/LBHaySepD sorbent. To do so the tube with the Teflon attachment was connected to the aspirator, the extract was applied onto the reactive filter, and the system was blown with air during 1 min (0.20 L/min) to transfer the formed derivative **I** into the sorption tube. After air-blowing, the reactive filter was thoroughly washed with 50 μL of hexane to wash residual RVX and compound **I** from the filter, and the air-blowing was continued for 2 min. At that stage the formed phosphonate **I** was concentrated in the sorption tube. The sorption tube was then disconnected from Teflon attachment and aspirator and installed at thermodesorber. The thermodesorption stage was performed as follows. First, hexane was blown out (50°C, 20 mL/min of nitrogen, 3 min). Second, the analyzed compounds were transferred (210°C, 4 min, nitrogen stream) to a focusing tube of thermodesorber (Tenax TA sorbent, 40°C). Then, the focusing tube was heated up to 220°C, and the desorbed compounds were carried to the capillary chromatography column with a carrier gas. Simultaneously, the temperature program of the oven of chromatograph was run and chromatographic separation of the mixture started, while the sorption and the focusing tubes were regenerated in the reverse nitrogen stream at 220°C.

The system was calibrated using a series of solutions with known concentration of RVX. The results revealed that certain correction should be used when analyzing the data obtained with the developed method. In particular, the coefficient accounting for RVX losses during sampling and the sample preparation was of 0.6 in the case of non-absorbing surfaces and 0.5 in the case of metallic waste. The proposed method of RVX determination is advantageous over other known methods, in particular:

– RVX concentrated in the extract is converted in the volatile compound **I**, the latter giving narrow symmetric peak in the chromatogram;

– less volatile matrix components are not desorbed at the temperatures applied, hence, their effect on compound **I** determination was significantly lowered;

– after the thermodesorption stage, the gas stream in the tubes is reversed, and at 220°C the matrix components are removed from the sorption and the focusing tubes; hence, after the analysis thermodesorber is ready for the next analysis cycle.

The method developed in this work differs from that described in [4] in that the larger extract volume (20 μL) was applied onto the AgF-containing reactive filter, and the analysis sensitivity was significantly improved.

Basing on the results, we developed and certified the methods [9, 10]; the corresponding metrological performance parameters are collected in Table 2. RVX detection limit following the methods was 0.2 MPC.

Method [9] is intended to estimate the completeness of decontamination of the surface of processing equipment to be transported out of the chemical weapon elimination facility.

Method [10] is intended to determine the danger grade of the metal waste that underwent decontamination and is to be further utilized.

EXPERIMENTAL

The following equipment and materials were used:

– quadrupole chromato-mass spectrometer Agilent 5975C (Agilent, USA) with ChemStation software, equipped with an HP-5ms quartz capillary column (no. 19091S-433) with length of 25 m, inner diameter of 0.25 mm, and the stationary phase layer thickness of 0.25 μm; the instrument was equipped with an injector for split and splitless sample injection, a temperature-programmable PTV detector, and an autosampler;

– gas chromatograph 7890A (Agilent, USA) with HP-GC ChemStation software, equipped with an HP-5ms quartz capillary column (no. 123-5532 30 m long, inner diameter 0.32 mm, and the stationary phase layer thickness of 0.25 μm; the instrument was equipped with an injector for split and splitless sample injection, a multifunctional temperature-programmable MMI detector, an autosampler, and a flame photometer;

– thermodesorber ACEM-9300 (DYNATHERM, USA) equipped with a Focusing Trap 4-1/12 focusing tube (sorbent Tenax TA, 60–80 mesh) and a sorption tube (combined sorbent SB Tenax-TA/LB HaySep D).

RVX conversion into the volatile derivative **I** was performed using AgF-impregnated filters placed into a special Teflon attachment that was directly connected to the sorption tube inlet.

10, 100, and 250 μL GC microsyringes (Hamilton, USA) were used for sample injection.

The calibration was performed using the State Standard Reference Sample of VX analog (GSO 8249-2004) with mass fraction of the main component of 91.0–95.0%, the certified value accuracy being of ±1% at P 0.95.

Operating parameters of the PTV and MMI injectors are collected in Tables 3 and 4 along with the conditions of chromatographic separation and detection (LVI method development). Operating parameters of the Agilent 7890A gas chromatograph and the ACEM-9300 thermodesorber during determination of RVX in the washouts extract under LVI conditions are summarized in Table 5.

REFERENCES

1. Williams, J.M., Rowland, B., Jeffery, M.T., Groenewold, G.S., Appelhans, A.D., Gresham, G.L., and Olson, J.E., *Langmuir*., 2005, vol. 21, no. 6, p. 2386.
2. Evans, R.A., Smith, W.L., Nguyen, N.P., Crouse, K.L., Crouse, C.L., Norman, S.D., and Jakubowski, E.M., *Anal. Chem*., 2011, vol. 83, p. 1315.
3. Fowler, W.K. and Smith, J.E., *J. Chromatogr*., 1989, vol. 478, p. 51.
4. Standart MVI 031-04-222-07. Saratov, 2007.
5. Standart MVI 031-02-125-05, Saratov, 2005.
6. Standart MVI 031-09-325-2010, Moscow, 2010.
7. Stan’kov, I.N., Sergeeva, A.A., Sitnikov, V.B., Morozova, O.T., Derevyagina, I.D., Mylova, S.N., and Shchukin, V.M., *J. Analyt. Chem.*, 2008, vol. 63, no. 7, p. 668.
8. Mol Hans, G.J., Janssen, Hans-Gerd, Cramers, C.A., *J. High Resol. Chromatogr*., 1995, vol. 18, p. 19.
9. Standart MUK 4.1.37-2012.
10. Standart MUK 4.1.39-2012.