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Determination of Acetonitrile-hexane Partition Coefficient of *O*,*O*'-dialkyl Methylphosphonates by NMR Spectroscopy for the Verification Analysis of Chemical Weapon Convention

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ABSTRACT

The extractability of the O,O'-dialkyl methylphosphonates, (the important markers of nerve agents) included in the schedule 2B4 of the Chemical Weapons Convention (CWC) has been studied by NMR, the aim being the optimisation of extraction protocols routinely used for the identification of Convention-related Chemicals (CRCs) during Official Proficiency Tests (OPTs) conducted by the Organization for Prohibition of Chemical Weapons (OPCW) and for the off-site analysis of real field samples. The technique is easy to optimise, fast, nondestructive, and shows excellent reproducibility.

Keywords: O,O'-dialkyl methylphosphonates, chemical warfare agents, partition-coefficient, official proficiency tests, NMR spectroscopy

1. INTRODUCTION

Chemical Weapon Convention (CWC) is an international treaty endorsed by 184 countries which prohibits the production, stockpiling, and use of chemical weapons[1]. The CWC is administered by The Organization for Prohibition of Chemical Weapons (OPCW) located at The Hague, The in Netherlands, through its verification regime[2,3]. Verification of CWC involves the detection and identification of chemical warfare agents (CWAs) and their environmental signatures. CWAs and their related chemicals (i.e., the starting materials, precursors and degradation products) are enlisted in three schedules of CWC[1,3]. These chemicals are also referred as convention convention-related chemicals (CRCs) and are required to be identified by the OPCW inspectors on-site to ascertain their deliberate or inadvertent spillage; in case of any ambiguity, the samples are sent to off-site laboratories designated by the OPCW[3,5-6].To become and maintain designation, the laboratories have to participate in and demonstrate high degree of analytical skills in official proficiency tests (OPTs) conducted by the OPCW[5-10]. The tests mimic the real field scenario[4,7,9] and it requires identification of CRCs spiked (usually at 1-10 μg/ml) in the environmental (soil, water, etc.) and/or synthetic matrices (viz., organic liquid, wipe, paint, concrete, etc.).

Detection and identification of CRCs in the samples during OPTs and real off-site analysis is performed by two main steps, namely sample preparation, and analysis by a suitable spectroscopic technique. Sample preparation and spectroscopic / spectrometric analytical protocols have recently been reviewed by Markku Mesilaakso[11]. This review is based on the recommended operating procedures published by Marjatta Rautio from extensive work done at Verifin, University of Helsinki, Finland, and outcome of the OPTs conducted by the OPCW[12].

Owing to the small sample size, low concentration of the analytes and complexity of the matrices, efficient sample preparation techniques are essential for the successful verification analysis of CRCs. This unit operation enriches the analytes by extracting these from the interferents and converts them into liquid, analysable by GC-MS, the most sought after technique for both on-site and off-site analyses. Of the variety of simple preparation methods used[13,14], liquid-liquid extraction methods are one of the simplest and highly effective of them all methods.

A variety of matrices have been provided in these tests. Organic liquid (hexane, dichloromethane, dodecane spiked with hydrophobic CRCs) is the most difficult to analyse. No recommended operating procedure (ROP) is available for this matrix and interferants (viz. diesel oil) are generally added to complicate the analysis. DRDE has successfully developed a simple but highly efficient solvent exchange method[15] involving the partitioning of analytes from non-polar organic solvent (viz., hexane) to polar organic solvent (acetonitrile) for the cleanup of such samples with concomitant extraction of the analytes. This technique is though although reliable and used by DRDE and others in the OPTs however, lacks the quantitative information on the partitioning of the CRCs between hexane and acetonitrile.

Considering the wide applicability of this technique to extract and enrich the analytes from organic liquid, and given the absence of published data on the partioning of the CRCs between these two phases. The partitioning

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behaviour of selected CRCs employing was investigated NMR as a monitoring technique. The liquid-liquid extraction efficiency of analytes is expected to vary for different homologs of the same class of CRCs, and effect is expected to be more pronounced in the presence of interfering agents. To investigate this problem, the isolation of *O,O'*-dialkyl methylphosphonates ranging from highly polar *O,O'*-dimethyl methylphosphonate to highly non-polar *O,O'*-didecyl methylphosphonate was studied. These compounds are considered to be the important markers of nerve agents and as included in the schedule 2B4 of the CWC and hence their detection and identification is of prime importance for verification of CWC. Quantitative $^{31}P\{^{1}H\}$ NMR was used to determine the partition behaviour of these compounds.

2. BASICS OF NMR ANALYSIS

Under quantitative conditions NMR analysis is considered to be a primary ratio method of measurement[16-17]. Hence, area under the NMR signal (I) of the analytes is directly proportional to the number of nuclei (N) evoking the signal. This linear relationship may be given by

$$I = k_s \times N$$

The spectrometer constant (k_s) depends on the spectrometer parameters. The value of N depends on the number of resonant nuclei in the irradiated or observed region of the NMR sample tube. If C and C' are molar concentrations of the analyte in the two fractions, and V and V' are volumes of the two layers of partitioning solvents in the field of view of the NMR probe, then

$$I/I' = N/N'$$

$$I/I' = C \times V/C' \times V'$$

Hence, using the same set of NMR tube and insert for analysis of the two fractions, one gets

$$I/I' = C/C'$$

where, I and I' represent the NMR signal areas of the analyte partitioned between two solvents, the ratio I/I' represents the partition coefficient of the analyte (under the same set of spectrometer conditions). This relationship may be expressed as

Partition coefficient = I/I'

3. EXPERIMENTAL SECTION

3.1 Materials

Tri-*n*-propyl phosphate, used as an external standard was purchased from Lancaster (Morecambe, UK) and the chemicals used for the synthesis of esters were purchased from Aldrich Chemical Company (St. Louis, USA). The compounds *O,O'*-dialkyl methylphosphonates were was synthesised as per standard procedures[18]. The purity of the compounds were tested by GC-MS and the compounds were found to be >99 per cent pure. HPLC grade solvents *n*-hexane and acetonitrile were procured from Merck Chemicals, India. The NMR lock solvents, dimethyl sulfoxide D-6 (degree of deuteration min 99.95%) and acetonitrile D-3 (degree of deuteration min 99.95%) were procured from Merck KGaA, Darmstadt, Germany. 2ml screw capped glass vials with PTFE-faced silicone rubber liner were procured from Wheaton Science Products, NJ. The 5 mm NMR tubes (Product no. 507-PP-7) and stem coaxial inserts (Product no. WGS-5BL) were purchased from Wilmad Glass (Buena, NJ).

3.2 Instrumentation and Spectroscopy

The experiments were carried out on Bruker AV-II, 400 MHz NMR spectrometer equipped with 5 mm broadband ATM probe (tuned to ^{31}P) and 2HTX module for gradient shimming. The following *q*NMR parameters were used: relaxation delay set at $5*T_1$ of the longest relaxing ^{31}P nucleus and a pulse program with inverse gated decoupling and 45° flip angle were used for the acquisitions. The spectral window of 55 ppm was used and transmitter offset was positioned at 17.5 ppm. 1024 scans with 4 dummy scans were acquired with 64 k data points for each sample with the receiver gain set automatically for the acquisition and samples were held at 20 +2 °C in a non-spinning mode during the experiments. Phase and baseline corrections were performed manually. Peak areas of the signals were determined by manual integration as well as using the inbuilt deconvolution routine of Topspin 1.3 program. A Shimadzu Corp. (Kyoto, Japan) model AUW220D electronic analytical balance with an accuracy of 0.01 mg was used for the mass determinations.

3.3 Sample Preparation and Analysis

3.3.1 Spiking of Organic Liquid

Before starting the partioning experiments the CH_3CN and hexane were mutually saturated by shaking at room temperature for 24 h and were let to stand long enough for separation of phases. A stock solution (100 mM) of spiking chemicals was made in hexane by weighing to precision and subsequently diluting to the required volume before dissolving them in the equilibrated hexane.

Hexane was added to 40 mg of diesel to make up the volume to 10 ml; 900 µl of this solution was added to 300 µl of the stock solution of the analytes to get 20 mM analytes and 3000 µg/ml diesel. Another set of solutions (without the diesel background) was prepared similarly.

3.3.2 Liquid-Liquid Extraction Process

Three exclusive extractions were performed for each spiked sample matrix with CH_3CN (sample: CH_3CN were taken in the ratio of 1:1, 1:2, 2:1 v/v) and for each extraction 1 ml, 0.6 ml and 1.2 ml samples were extracted by shaking with 1 ml, 1.2 ml and 0.6 ml of CH_3CN respectively for 20 min. Sample preparations were run carried out in sample vials and analyzed by NMR after cooling the vialsthem at -20 °C for 5 h.

3.3.3 Linearity of Detector Response

For checking linearity of the detector response, a graph was plotted between concentrations of analytes in standard solutions and ratios of peak areas of analytes versus external standard. For this 0.1223 M stock solution

of tri-*n*-propyl phosphate (TPP) and 0.1484 M solutions of dimethyl methylphosphonate (DMMP) were prepared in CH_3CN. In the first series of experiments, 100 µl of the DMMP stock was taken in the stem coaxial insert and different dilutions of the TPP stock solution were taken in the NMR tube, whereas in the next series of experiments, the TPP stock solution was taken in the stem coaxial insert and different dilutions of the DMMP stock were taken in the NMR tube.

3.3.4 General Procedure

For the experiments, a 10 µl tri-*n*-propyl phosphate solution in 200 µl DMSO-d_6 was sealed in the stem coaxial insert and used as external standard. The extracted fractions (500 µl) were taken in the NMR tubes. The same set of NMR tube, and the stem coaxial insert was used for all the experiments. Two sets of experiments were performed: (i) In the absence diesel background, (ii) In the presence of 3000 µg/ml of diesel.

This was done to determine the effect of hydrocarbon background, and change in the size of the alkyl chain, on acetonitrile hexane partition coefficients.

Precision, expressed as per cent relative standard deviation (RSD) was ascertained from six replicate extraction followed by measurements for DMMP. For determining accuracy of the method, DMMP was partitioned and the layers were also analysed by GC by comparing the ratios of peak areas of analytes versus internal standard (in prepared samples) with peak areas of analytes versus external standard of standard solutions, to calculate the analytes in CH_3CN and hexane. Concentrations of analytes in standard solutions were 8.15µg/ml, 16.30 µg/ml, 32.58 µg/ml (or 65.6 µM, 131.30 µM, 262.60 mM) with a fixed concentration of internal standard (61.06 µg/ml or 62.5 µM). For GC analysis Chemito make GC with PFPD (P) detector and SGE BPX5 capillary column with 25 m length × 0.25 mm i.d. × 0.25 µm film thickness was used. The temperature program and the instrument settings were:

(i) Initial temperature 60 °C (1 min), 10 °C min^{-1} to 260 °C (2 min)
(ii) Injector temperature 220 °C and detector temperature 250 °C carrier gas was nitrogen, flow rate of 1.2 ml/min.

The partition coefficient values for DMMP was found to be 103.286 (with a standard deviation of 0.0213 for six replicate experiments).

4. RESULTS AND DISCUSSION

This investigation makes use of the quantitative NMR technique, for the determination of the extraction efficiency, owing to the fact that:

(i) NMR is a non-destructive, non-invasive and benign technique
(ii) The nucleus specificity of this technique makes it useful for studying the analytes of interest even in the presence of irrelevant background chemicals
(iii) Since the NMR is a primary ratio technique, i.e., the area under an NMR signal is directly proportional to the number of resonant nuclei/spins of a particular type under observation. Hence, the no need for intensity calibrations performed with one specific compound holds good for for different analytesothers.
(iv) Sample preparation is easy. Moreover, using $^{31}P\{^1H\}$ NMR experiments simplerifies the spectra and with lesser possibility of signal overlaps.

The distribution of target analytes between acetonitrile and hexane was determined after equilibrating the immiscible liquids with a given analyte at one time as per the details given in the experimental section. Two layers of the extracting mixture were separated and quantitative determinations of the analyte were made in each layer. The samples were prepared immediately before the experiments. The hexane extract and NMR probe were precooled to -10 °C prior to the NMR experiments, to minimise errors due to evaporation of hexane. The results of the extraction are enumerated

Table 1. Mean values for acetonitrile/hexane partition coefficients[a] of esters with their standard deviations[b]

Compound	Organic liquid (un-fortified)		Organic liquid (fortified, 3000 µg/ml of diesel)	
	log $P_{ACN/Hexane}$	$P_{ACN/Hexane}$	log $P_{ACN/Hexane}$	$P_{ACN/Hexane}$
O,O'-dimethyl methylphosphonate	2.0138	103.2222 (0.0103)	2.2271	168.6957 (0.0093)
O,O'-dipropyl methylphosphonate	1.4433	27.7524 (0.0016)	1.4889	31.6132 (0.0019)
O,O'-dibutyl methylphosphonate	1.1014	12.6311 (0.0009)	1.1912	15.5303 (0.0011)
O,O'-dipentyl methylphosphonate	0.7549	5.6867 (0.0010)	0.8349	6.8374 (0.0010)
O,O'-dihexyl methylphosphonate	0.5228	3.3328 (0.0009)	0.4624	2.9003 (0.0007)
O,O'-diheptyl methylphosphonate	0.1408	1.3828 (0.0009)	0.1278	1.3420 (0.0010)
O,O'-didecyl methylphosphonate	0.1099	1.2881 (0.0007)	-0.7449	0.1799 (0.0009)

[a] Using the same stem coaxial inserts having reference standard 5 µl tri-n-propyl phosphate in 200 µl DMSO-d_6 wrt 500 µl extracting solvent
[b] Mean values of six replicate measurements (SD)

in Table 1. It is evident from these data that the acetonitrile extrability of the esters vary widely.

The results indicate the lowest member *O,O'*-dimethyl methylphosphonate methylphosphonate is extracted is soluble in water in all proportions. It is extracted in CH_3CN of the order of hundred times more than the highestly hydrophobic member *O,O'*-didecyl methylphosphonate (entries 1 & 7 Table-1). The partitioning of the analytes into the acetonitrile layer can be attributed to the dipole-dipole interaction between acetonitrile and the analytes. The increase in hydrophobicity is due to increase in the alkyl chain length, which leads to increase in steric bulk around the polar oxygen atoms of phosphonates. Thus, extraction efficiency decreases drastically with the increase in the length of alkyl side chain. It was also observed that when the hexane was fortified with diesel the polar *O,O'*-dimethyl methylphosphonate was partitioned more into the acetonitrile layer whereas the most hydrophobic *O,O'*-didecyl methylphosphonate was retained more by the hexane layer (Fig. 1). This can be explained on the basis of interactions between the analyte and the extracting solvent. The former being less soluble in the diesel fortified hexane due to higher hydrophobicity of the medium, is partitioned more into the hexane layer, whereas for the later, shows just the opposite behaviour.

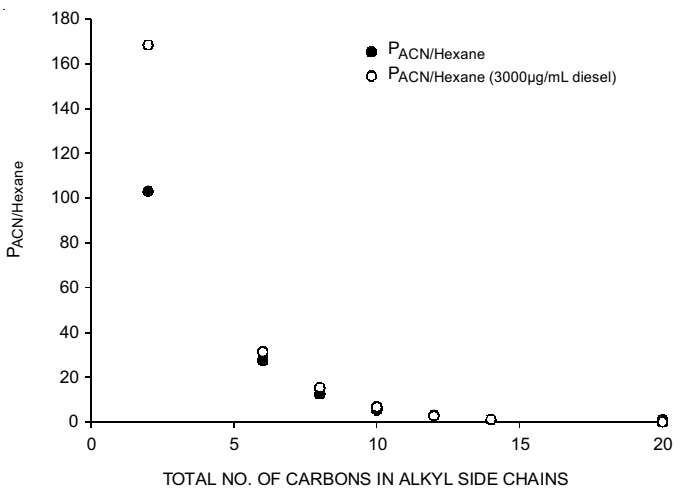

Figure 1. Shows extractability of the analytes from hexane to acetonitrile in the presence and in the absence of diesel background.

Trend of extractability shown by the *O,O'*-dialkyl methylphosphonates also indicates that the acetonitrile extraction protocol for the removal of hydrocarbon background and extraction of analyte must be used with caution keeping in view the nature of analytes and the detection limit of the analytical technique, the volume of the analyte solution being extracted and evaporative losses during further concentration prior to analysis.

This is the first investigation where NMR is used to determine the partition coefficient of CWC-related chemicals.

5. CONCLUSION

A extraction efficiency of CRCs has been determined based on quantitative NMR technique The method is simple, efficient, and non-destructive. Moreover, the method overcomes the problem of determination of detector response for the individual analytes and it compares well with the accuracy level attained by gas chromatographic technique.

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