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Tandem capillary column gas chromatography–mass spectrometric determination of the organophosphonate nerve agent surrogate dimethyl methylphosphonate in gaseous phase

Les N. McDaniel[a,*], Nicholas A. Romero[a], Jonathan Boyd[b,1], Gopal Coimbatore[a], George P. Cobb[a]

[a] Texas Tech University, Department of Environmental Toxicology, The Institute of Environmental and Human Health, Box 41163, Lubbock, TX 79409-1163, USA
[b] Bennett Department of Chemistry, West Virginia University, Morgantown, WV 26506, USA

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ABSTRACT

A procedure based on capillary column gas chromatographic–mass spectrometric (GC–MS) confirmation was developed for the verification of the ubiquitous and versatile chemical and nerve agent simulant, dimethyl methyl phosphonate (DMMP; CAS# 756-79-6), from gaseous samples. This method was developed to verify low nanogram DMMP concentrations during testing of a nerve agent detection system. Standard solutions of 1, 5, 10, 50, 100, 500, and 1000 ng/ml DMMP in acetonitrile were employed. Through 15 calibration curves using the 5 lowest concentrations, coefficient of determination (r^2) values showed a mean of 0.998 (0.992–1.000). An additional 15 calibration curves likewise containing 5 concentrations of DMMP spanning 3 orders of magnitude (1, 50, 100, 500, and 1000 ng/ml) yielded a mean r^2 of 0.997 (0.991–1.000). Sixty-five nitrogen diluted gaseous samples varying from 1.0 to 10.0 μl in volume were analyzed and concentrations of DMMP ranging from 1 to 1000 ng/ml were confirmed. An additional 35 vapor samples in UHP N_2 ranging in DMMP concentration from 5.8 μg/m^3 to 1.0 mg/m^3 were analyzed by increasing sample volume range to between 10.0 and 100 μl. For gaseous samples with volumes > 1.0 μl, the lowest concentration observed was 5.8 μg/m^3. The method detection limit (Appendix B of Title 40 CFR, United States) for 1.0 μl autoinjected standards in acetonitrile was determined to be 0.331 ng/ml. Method precision for 15 independently analyzed standards of 25 ng/ml had a relative standard deviation of 1.168. This method demonstrated high linearity across a wide range of concentrations, as well as excellent sensitivity and repeatability, and proved applicable to other lower alkyl-phosphonates.

1. Introduction

Dimethyl methyl phosphate (DMMP) is primarily used as a flame retardant (Fyrol DMMP), in epoxy resins, acrylic latexes, unsaturated polyesters, urethane coatings, urethane rigid foam, and vinyl copolymers. DMMP is also used as a preignition additive in gasoline, an antifoam agent, a plasticizer and stabilizer, a textile conditioner and antistatic agent, as well as a solvent for low-temperature hydraulic fluids [1].

The Chemical Weapons Convention (CWC) regulates DMMP due to its listing as a chemical weapons precursor (Schedule 2; Dual-Use Goods List Item 1C350) [2]. Because of their structural similarities, DMMP is a physical and spectroscopic simulant for anticholinesterase agents (nerve gases) tabun (GA), sarin (GB), and soman (GD). Conversely, DMMP does not share the dangerous biological properties of nerve agents, which makes it a desirable surrogate for testing G-agent protective clothing, detection equipment, and developing analytical methods.

The ability to detect chemical warfare agents (CWAs) is of special concern for the protection of the modern day warfighter. Although chemical weapons use was prohibited by the 1925 Geneva Protocol and subsequently by the CWC in 1997 [2], these compounds continue to appear in modern military conflicts [3], in instances of ethnic cleansing, and in terrorist attacks such as the use of GB in the 1995 subway attack in Japan.

CWAs are considered unstable in the atmosphere and prone to simple hydrolysis. Many of these degradation compounds and precursors are characterized by low volatility, which makes them unsuitable for direct gas chromatographic analysis [4]. However, it is generally agreed that confirmation of CWAs or their hydrolysis products require identification by mass spectrometry because

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* Corresponding author. Tel.: +1 806 885 4567; fax: +1 806 885 4567.
E-mail addresses: les.mcdaniel@tiehh.ttu.edu (L.N. McDaniel), nicholas.romero@tiehh.ttu.edu (N.A. Romero), jonathan.boyd@mail.wvu.edu (J. Boyd), gopal.coimbatore@tiehh.ttu.edu (G. Coimbatore), george.cobb@tiehh.edu.ttu (G.P. Cobb).
[1] Tel.: +1 304 293 3435; fax: +1 304 293 4904.

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these compounds lack a sufficiently strong UV chromophore required for UV-HPLC [4,5]. Black and Muir [6] provide a concise review of the derivitisation reactions required for the chromatographic analyses of CWAs.

Since CWA are highly toxic compounds with low stabilities, the capability to confirm the presence of these compounds or their degradation products at low concentrations is paramount. Modern sensing equipment must detect these dangerous analytes in nanogram and picogram quantities, while laboratory equipment must be equally capable of verifying these concentrations. Simple methods for laboratory verification are also critical to maximize sample throughput in near real-time. Safe alternative surrogates for CWAs like DMMP have a significant role in this process.

Numerous chromatographic methods for laboratory quantification of G-agents and their surrogates have been developed; these include capillary gas chromatography (GC) with tandem mass spectrometry [5] (GC–MS–MS), GC with flame ionization detector [7,8] (GC-FID), atmospheric pressure ion mobility time of flight MS [9] and conventional GC–MS [10]. Additionally, liquid chromatographic (LC) methods using MS have been recently developed [4,11]. Thorough reviews of analytical methods for the quantification and detection of chemical weapons, their surrogates, and related compounds are available in literature [12–15].

The method described herein was developed for concentration verification of laboratory exposure of novel sensing devices developed to detect DMMP as a simulant for organophosphate nerve agents, and for the purposes of determining the sensitivity and effective ranges of those devices. An additional application for this method is calibration confirmation for commercial detectors by using commonly available analytical equipment and an uncomplicated methodology.

Three other common industrial alkyl-phosphonates were also analyzed with this method in order to determine applicability within this family of compounds: diethyl ethyl phosphonate (DEEP), diethyl phosphonate (DEP), and dimethyl phosphonate (DMP).

2. Experimental

2.1. Reagents and materials

DMMP (97.0%, CAS# 756-79-6) and DMP (98.0%, CAS# 868-85-9) were obtained from Sigma–Aldrich (St. Louis, MO 63103, USA). DEEP (98.0%, CAS# 78-38-6) and DEP (99.0%, CAS# 762-04-9) were obtained from Fluka (St. Louis, MO 63103, USA). Standard solutions were prepared with HPLC-grade acetonitrile (Fisher Scientific, Pittsburg, PA, USA). All stock and standard solutions were stored at 4 °C.

2.2. Gas handling and vapor dilutions

Ultra high purity (UHP) nitrogen (Airgas, Inc., USA) was the carrier gas for the effusion and dilution systems. Flow rates were controlled with stainless steel metering valves (Swagelok, Solon, OH 44139, USA) connected downstream of the tank regulator, followed by 150 mm direct reading panel mount stainless steel-lined flowmeters (Cole-Parmer, Vernon Hills, IL 60061, USA). Flows were adjusted to 20.0 SCCM and set with Digital Flow Check HR flow meter (Altech Corp., Flemington, NJ 08822, USA) calibrated for N_2 which was NIST traceable to 0.01 SCCM for rates below 10.0 SCCM, and 0.1 SCCM for rates above 10.0 SCCM. Vapor effusor system was built in-house, and all components were either stainless steel, PTFE, or glass, and were connected with 1/8″ ID PTFE or stainless steel tubing.

All components of the vapor dilution system (Fig. 1) were stainless steel or stainless steel-lined (Swagelok, Solon, OH 44139, USA). DMMP vapor concentrations were diluted by controlling the adjustable flow from output valves: by redirecting a small percentage of DMMP vapor through a shut-off valve into a parallel continuous line UHP N_2 stream and mixing loop, 10–100-fold dilutions were routinely performed. Dilutor design is capable of 2 successive dilutions and was capable of producing surrogate concentrations which exceeded the sensitivity of the instrumentation and method described herein.

2.3. Instrumental analysis

Seven working DMMP standards were prepared in acetonitrile at 1, 5, 10, 50, 100, 500, and 1000.0 ng/ml. Separate standards of DEEP, DEP, and DMP were likewise prepared in acetonitrile at 100 ng/ml. Standards and samples were analyzed using an HP 6890 series GC with an HP 5973 MS (Agilent, Palo Alto, CA, USA). For standards, splitless injections (1.0 μl) were made by autosampler. Manual injections (1.0–100 μl) were performed for vapor phase samples produced with effusor and dilutor. Separations were performed in a 30.0 m × 320.0 μm × 1.0 μm film thickness DB-1701 column (Agilent). Helium carrier gas was maintained at a constant linear velocity of 59.0 cm/s. The temperature program began at 100 °C and increased to 180 °C at a rate of 10 °C/min for 5 min, and then increased to 300 °C at a rate of 18.0 °C/min. Inlet temperature was maintained at 180 °C. The MS source and quadrupole temperatures were 230 and 150 °C, respectively. The MS was operated in the electron impact (EI) mode. The septa and inlet liners were replaced

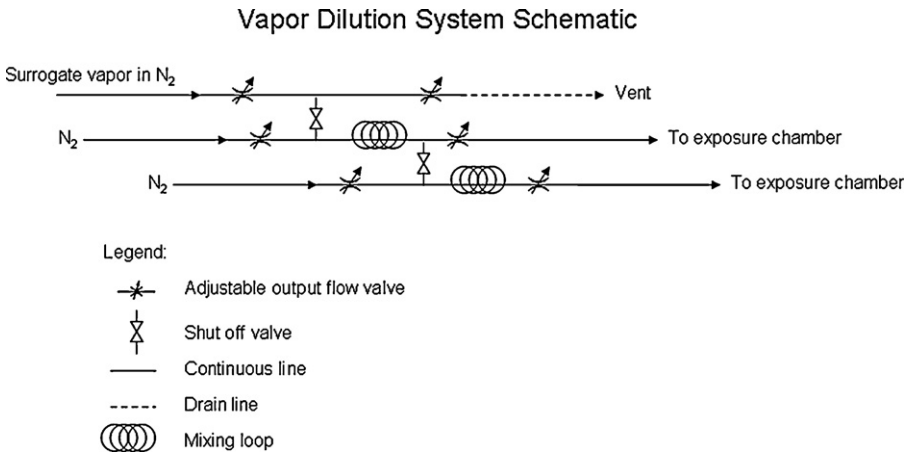

Fig. 1. International Organization for Standardization (IOS) schematic of vapor dilution system.

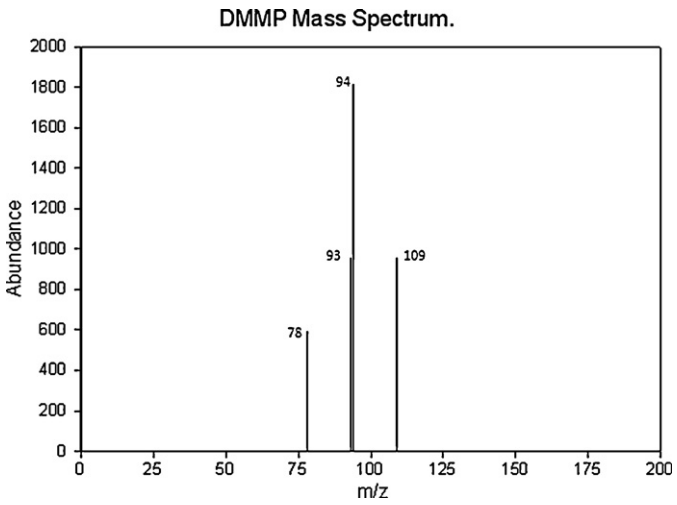

Fig. 2. DMMP mass spectrum showing relative abundances of qualifier ions.

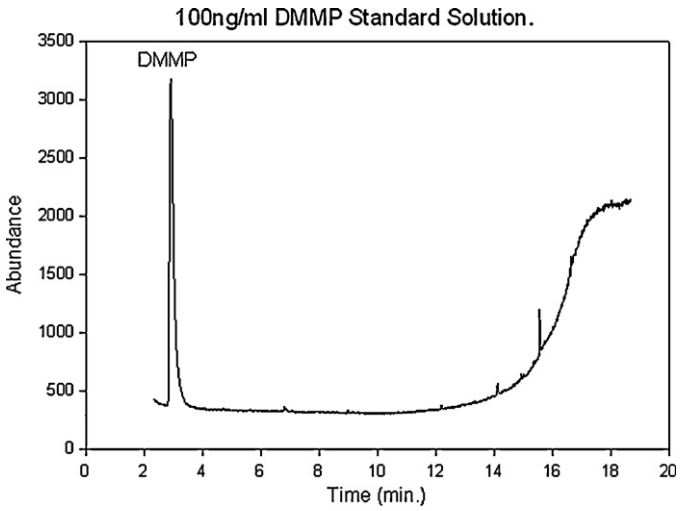

Fig. 3. Representative total ion current chromatogram from DMMP analysis via the novel method reported herein showing 1.0 μl injection of 100 ng/ml standard solution in acetonitrile.

every 25 and 50 injections, respectively.

The mass selective detector (MSD) was operated in single ion mode (SIM) with qualifier ions (target, Q1, Q2, Q3) of 94, 109, 93, and 78 amu. (The most relatively abundant target ion of 94 amu was selected over the molecular ion of 124 amu due to the sensitivity demonstrated under the conditions and equipment described herein; Fig. 2). Electron multiplier voltage was set to 200 V relative to most recent autotune. Solvent delay was set at 2.25 min to prolong MSD filament lifetime and allow full development of DMMP peak.

Each analysis sequence began with 5 calibration standards that spanned the applicable calibration range. Continuing calibration standards and blanks were analyzed daily. If response to continuing calibration standards changed by ≥15%, then a new standard curve was developed. This frequency of standard analysis ensured that analyte and detector stability were maintained during instrumental analysis.

2.4. Vapor samples

Samples of DMMP in nitrogen were taken directly downstream of the dilutor system using Pressure-Lok™ precision analytical syringes (Vici Precision Sampling, Inc., Baton Rouge, LA, USA). Syringe and needle combinations were rinsed with acetonitrile and dried with vacuum in between samples, and confirmed free of residual DMMP contamination via GC–MS blank (UHP N_2 only) injections daily.

Sample volumes varied depending on target concentration of DMMP: for liquid sample whose concentration fell within the calibration curve (1.0–100 ng/ml), 1.0 μl samples were taken and injected. For vapor samples with concentrations below 1.0 μg/m^3, larger volumes (≤100 μl) were injected so that peak areas fell within calibration range. Gaseous sample concentrations were calculated by dividing large volume injections (>1.0 μl) by their respective injection volumes.

3. Results

3.1. Calibration curves and detection limits of GC–MS

Standard solutions of 1, 5, 10, 50, and 100 ng/ml DMMP in acetonitrile were employed in conjunction with daily continuing calibration standards. Through 15 calibration curves spanning 5 concentrations across 2 orders of magnitude, coefficient of determination (r^2) values were consistent with a mean value of 0.998 (0.992–1.000). An additional 15 calibration curves likewise containing 5 concentrations and spanning 3 orders of magnitude (1, 50, 100, 500, and 1000 ng/ml) yielded a mean r^2 of 0.997 (0.991–1.000).

Sixty-five nitrogen diluted gaseous samples varying from 1.0 to 10.0 μl in volume were analyzed and concentrations of DMMP ranging from 1 ng/ml to 1 μg/ml were recorded. An additional 35 gaseous samples ranging in DMMP concentration from 1.0 pg/ml to 1.0 μg/ml were analyzed by increasing sample volume range to between 10.0 and 100.0 μl. The lower detection limit (LDL) for 1.0 μl autoinjected samples in acetonitrile was determined to be 1.0 ng/ml. For gaseous samples with sample volumes >1.0 μg/ml, LDL was 5.8 pg/ml.

Method detection limit (MDL) of 0.331 ng/ml was estimated as outlined by Appendix B of Title 40 Code of Federal Register (CFR, United States) 136. Method precision for 15 independently analyzed standards of 25 ng/ml was determined to have a relative standard deviation (RSD) of 1.168.

100 ng/ml standards of DEEP and DMP in acetonitrile yielded clean peaks with retention times of 4.327 and 3.044, respectively. Analysis of 100 ng/ml DEP standards yielded no peaks discernable from the acetonitrile solvent peak (retention time <2.25 min).

4. Discussion

It is greatly advantageous to use DMMP as a simulant for G-agents because it elutes quickly from the GC capillary column relative to interfering molecules. In particular, GB and DMMP are detectable in complex matrices at relatively low concentrations because they elute prior to most sample extract components [5]. In this instance, DMMP peak elution at 2.9 min. is almost immediately after the acetonitrile peak and solvent delay of 2.25 min (Fig. 3). It is advantageous to utilize methods as presented here so as to increase sample throughput and reproducibility of results in near real-time.

The LDLs for previously published GC–MS method analysis of gaseous DMMP from headspace vapor samples were 0.1 ppm [10], 2 orders of magnitude higher than this method. The MDL for DMMP samples in acetonitrile (standards) for the method described here is 0.331 pg. GB standard solutions in solution (dichloromethane) were previously reported to be detectable at the 20 ng level with GC-FID [4] however, similar data for DMMP is not available. Comparable amounts of GB were detected via GC-EI-FID analysis in a matrix of diesel fuel fumes extracted from gas mask filter charcoal with dichloromethane, although quantitative data for detection in

clean samples were missing [5]. The GC–MS–MS method detection limit (S:N 5:1) for GB has been previously estimated to be 70 pg in the presence of diesel exhaust and estimated to be 5 pg in liquid matrices that do not contain these interferences [5]. The lowest concentration of DMMP in gaseous samples determined by our method was 5.8 pg, which was comparable to the estimated value of 5 pg when using GC–MS–MS [5].

Analysis of the 100 ng/ml standards of related alkyl-phosphonates DEEP, DEP, and DMP with this method demonstrated responses from DEEP and DMP which were comparable in height to the results seen with the DMMP analyses of the same concentration. Both DEEP and DMP had longer retention times than DMMP and, thus, can be analyzed simultaneously via this method as a general lower alkyl-phosphonate scan.

The inability of conventional capillary column GC–EI-MS to confirm trace levels of chemical warfare agents in complex environmental matrices has prompted investigation into application of GC–MS–MS and LC–MS instrumentation for the trace detection of chemical warfare agents [5]. The GC–MS method presented here is not intended for use with environmental samples and complex matrices containing DMMP. Rather it may be used to fill the need for laboratory testing of novel devices or for confirming calibration of commercial devices using commonly available analytical equipment and an uncomplicated method.

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References

[1] Technical Report Series No. 323 (1987) NIH Publication No. 88-2579.
[2] Convention on the prohibition of the development, production, stockpiling and use of chemical weapons and on their destruction, Technical Secretariat of the Organization for Prohibition of Chemical Weapons, The Hague, 1997.
[3] Report of the Mission Dispatched by the Secretary-General to Investigate Allegations of the Use of Chemical Weapons in the Conflict Between the Islamic Republic of Iran and Iraq, S 20060, United Nations Security Council, New York, 20 July 1988.
[4] J.R. Smith, M.L. Shih, J. Appl. Toxicol. 21 (2001) S27–S34.
[5] P.A. D'Agostino, L.R. Provost, J.F. Anacleto, P.W. Brooks, J. Chromatogr. 504 (1990) 259–268.
[6] R.M. Black, B. Muir, J. Chromatogr. A 1000 (2003) 253–281.
[7] P.A. D'Agostino, L.R. Provost, J. Chromatogr. 331 (1985) 47–54.
[8] P.A. D'Agostino, L.R. Provost, J. Chromatogr. 436 (1988) 399–411.
[9] W.E. Steiner, B.H. Clowers, P.E. Haigh, H.H. Hill, Anal. Chem. 75 (2003) 6068–6076.
[10] W. Kuang, M. Fingas, K. Li, Environ. Forensics 8 (2007) 383–390.
[11] P.A. D'Agostino, J.R. Hancock, L.R. Provost, J. Chromatogr. 912 (2001) 291–299.
[12] C.E. Kientz, J. Chromatogr. A 814 (1998) 1–23.
[13] D. Noort, H.P. Benschop, R.M. Black, Toxicol. Appl. Pharm. 184 (2002) 116–126.
[14] H.H. Hill Jr., S.J. Martin, Pure Appl. Chem. 74 (2002) 2281–2291.
[15] M. Mesilaaskso (Ed.), Chemical Weapons Convention Chemical Analysis: Sample Collection, Preparation, and Analytical Methods, John Wiley & Sons, Ltd., Chichester, UK, 2005.