

Examination of an Internal Standard Substance for the Quantitative Analysis of Sarin Using ^{31}P -NMR

Yoshihiko Miyata* and Hiroaki Ando

Criminal Investigation Laboratory, Metropolitan Police Department, 2-1-1 Kasumigaseki, Chiyoda-ku, Tokyo 100-8929, Japan

(Received August 23, 2000; Accepted October 2, 2000)

The toxic solution used at the Tokyo Subway Sarin Incident was purified by vacuum distillation. We were thus able to obtain sarin with high purity. We conducted a quantitative analysis of sarin using ^{31}P -NMR spectrometry. High-purity sarin was examined by trimethyl phosphate as an internal standard. In comparison with ^1H nondecoupling, the relative intensity between sarin and the internal standard did not change in ^1H decoupling. Trimethyl phosphate is a good internal standard substance. A ^{31}P -NMR chemical shift of trimethyl phosphate is separate from those of sarin and related compounds. Trimethyl phosphate has one phosphorus atom in the molecule, as does sarin. Since it is advantageous in the conversion of quantitative value, the molecular weight of trimethyl phosphate (m.w. 140.08) is close to that of sarin (m.w. 140.09). By using trimethyl phosphate as an internal standard, it is possible to conduct a quantitative analysis of sarin through ^1H decoupled ^{31}P -NMR spectrometry.

Key words — sarin (isopropyl methylphosphonofluoridate), ^{31}P -NMR, quantitative analysis, internal standard substance, trimethyl phosphate

INTRODUCTION

NMR spectrometry is well suited for quantitation because the signal intensity is directly proportional to the amount of nuclei present when the measurement is performed under proper conditions.¹⁾

We have published ^{31}P chemical shifts of organophosphorus pesticides.^{2,3)} To date, we have identified sarin 35%, hydrogen methylphosphonofluoridate (MPF) 10%, diisopropyl

methylphosphonate (DIMP) 1% and diisopropyl phosphorofluoridate (DFP) 0.1% (w/w) in the original sample obtained at Kasumigaseki Station of the Chiyoda subway line by GC/MS, ^1H -decoupled ^{31}P -NMR, and chemical derivatization.⁴⁾ Now, we are examining the purity test and quantitative analysis of sarin using ^{31}P -NMR spectrometry. When a phosphorus compound as an internal standard was investigated, trimethyl phosphate was found to be chemically stable, and its chemical shift was separate from those of sarin and related compounds. Therefore, trimethyl phosphate is useful as an internal standard. It is known that the nuclear Overhauser effect (NOE) causes a change in integration intensity during ^1H decoupling. Then, the molecular weight of trimethyl phosphate is close to that of sarin. High-purity sarin was examined by trimethyl phosphate. In comparison with ^1H nondecoupling, the relative intensity between sarin and the internal standard did not change in ^1H decoupling. Therefore, we report that the relative quantitative analysis of sarin is possible.

MATERIALS AND METHODS

Chemicals — Trimethyl phosphate and methylphosphonic acid (MPA) were purchased from Tokyo Kasei (Tokyo, Japan); DFP from Aldrich Chem. Co. (Milwaukee, WI, U.S.A.); and acetonitrile- d_3 from Isotec, Inc. (U.S.A.). DIMP and isopropylhydrogenmethylphosphonate (IMPA) were synthesized in our laboratory according to the literature.⁵⁾ All other reagents used were of analytical reagent grade.

Sarin Sample — A liquid sample, obtained at Kasumigaseki Station of the Chiyoda subway line on March 20, 1995 at 8 a.m., was purified by vacuum distillation. The sample was distilled to give a major fraction at 60–61°C/25 mmHg. (Warning: In view of its toxicity, sarin should be handled only in a specialized laboratory.)

Sample Preparation for Quantitative Analysis

— The sample was prepared by mixing sarin with 0.406 g acetonitrile- d_3 with 0.330 g sarin sample (m.w. 140.09) and 0.183 g trimethyl phosphate (m.w. 140.08), followed by sealing in a sample tube.

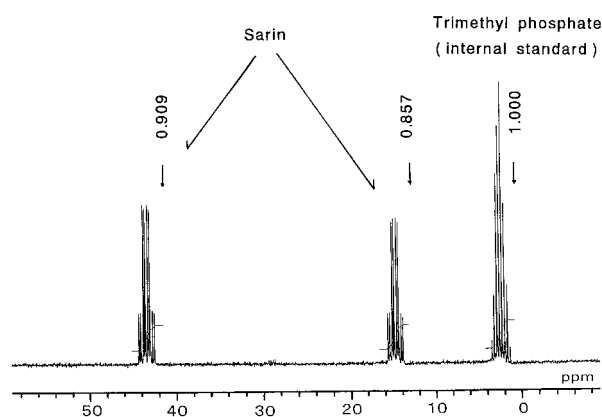
^{31}P -NMR Spectrometry — The ^{31}P -NMR spectrum was measured with a JNM-EX90A FT NMR spectrometer (Nihon Denshi, Tokyo, Japan) in the ^1H decoupling and nondecoupling mode at 36.25 MHz, using 85% phosphoric acid as an ex-

*To whom correspondence should be addressed: Criminal Investigation Laboratory, Metropolitan Police Department, 2-1-1 Kasumigaseki, Chiyoda-ku, Tokyo 100-8929, Japan. Tel.: +81-3-3581-4321, ext. 47451.

Table 1. ^{31}P -NMR Chemical Shifts (^1H Decoupling Mode) of Trimethyl Phosphate, Sarin and Related Compounds

Compound	Chemical shifts in ppm ^{a)}	Coupling constants (Hz)
Trimethyl phosphate	2.39 ^{b)}	
DFP	-10.42 ^{b)}	$J_{\text{PF}} = 967$
DIMP	29.32 ^{b)}	
Sarin	29.62 ^{b)}	$J_{\text{PF}} = 1037$
MPA	31.51 ^{c)}	
IMPA	32.80 ^{c)}	

a) ^{31}P chemical shifts are reported downfield from external 85% phosphoric acid (0.00). b) ^{31}P -NMR spectrum was measured in CD_3CN . c) ^{31}P -NMR spectrum was measured in D_2O .

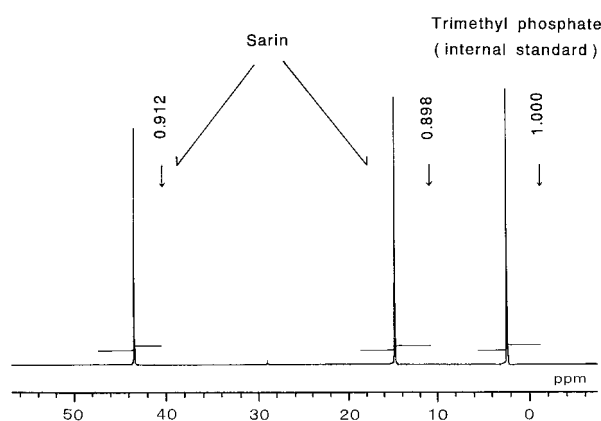
**Fig. 1.** ^{31}P -NMR Spectrum of Mixed Sample (Sarin and Trimethyl Phosphate) with Integrals (Result, Sarin : Trimethyl Phosphate = 1.77 : 1)

ternal standard (for chemical shifting) at 26°C . Other conditions used were data points 32 K, lock material acetonitrile- d_3 (solvent), sample tube 5 mm internal diameter, spectral width 8000.0 Hz, pulse width $12.6 \mu\text{sec}$ (pulse angle 90°), pulse delay time 30 sec (routine measurement 3 sec), and number of FID accumulation 256.

RESULTS AND DISCUSSION

^{31}P -NMR chemical shifts of trimethyl phosphate, sarin and related compounds, are shown in Table 1. The chemical shift of trimethyl phosphate is separate from those of sarin and related compounds (DFP, DIMP, MPA and IMPA).

Figures 1 and 2 show the ^{31}P -NMR spectra (non decoupling and ^1H -decoupling mode, respectively) for the quantitative analysis sample. Owing to the amount of sarin and trimethyl phosphate in the quantitative analysis sample, the theoretical ratio of inte-

**Fig. 2.** ^{31}P -NMR Spectrum of Mixed Sample (Sarin and Trimethyl Phosphate) Obtained by ^1H Decoupling with Integrals (Result, Sarin : Trimethyl Phosphate = 1.81 : 1)

grated intensity is 1.80 : 1. Figures 1 and 2 show that their integrated intensities almost agree with the theoretical value.

Trimethyl phosphate can be recommended as an internal standard substance for the following reasons. Trimethyl phosphate has one phosphorus atom in its molecule, the same as sarin does. Since it is advantageous for the conversion of a quantitative value, the molecular weight of trimethyl phosphate is close to that of sarin. In addition, trimethyl phosphate is chemically stable.

In FT NMR spectroscopy, the spin-lattice relaxation time (T_1) must keep pace with the exciting pulses.⁶⁾ Therefore, pulse delay time must be long enough to allow complete relaxation. We made trimethyl phosphate the internal standard substance. In this case, ^1H decoupled ^{31}P -NMR spectrometry may be applicable to the quantitative analysis of sarin.

Acknowledgements The authors acknowledge helpful discussions with Prof. Takemi Yoshida of the Department of Biochemical Toxicology, School of Pharmaceutical Sciences, Showa University, pertaining to this manuscript.

REFERENCES

- 1) Rautio, M. (ed.), (1994) *Recommended Operating Procedures for Sampling and Analysis in the Verification of Chemical Disarmament*, The Ministry for Foreign Affairs of Finland, Helsinki, pp. 1–8.
- 2) Miyata, Y., Takahashi, Y. and Ando, H. (1988) Analysis of organophosphorus pesticides using FT-

- NMR. *Kakeiken-Houkoku (Reports of the National Research Institute of Police Science)*, **41**, 159–163.
- 3) Miyata, Y. and Ando, H. (1994) Analysis of organophosphorus pesticides by using ^{31}P -NMR. *Jpn. J. Toxicol. Environ. Health*, **40**, 49–54.
 - 4) Miyata, Y., Nonaka, H., Yoshida, T. and Ando, H. (2000) Analyses of sarin and related compounds used in the Tokyo Subway Sarin Incident. *Jpn. J. Forensic Toxicol.*, **18**, 39–48.
 - 5) The Society of Synthetic Organic Chemistry, Japan (ed.), (1971) *Organophosphorus compound*, Gihoudo, Tokyo, pp. 330–351.
 - 6) Breitmaier, E. (1993) *Structure Elucidation by NMR in Organic Chemistry A Practical Guide*, John Wiley & Sons, Ltd., Chichester, England, pp. 1–12.