衛生化学
EISEI KAGAKU
31(2) 141-144 (1985)

**Thin-Layer Chromatography with Flame Ionization Detector
for Determination of Methomyl in Serum and Urine
Using a Rapid Sep Pak Cartridge Extraction**

JUN IKEBUCHI,^a SUSUMU KOTOKU,^b MOTOMI OHTANI,^c
and KICHIRO OKADA^a

*Department of Legal Medicine, Tottori University School of Medicine,^a Department
of General Education, Tottori University College of Medical Care Technology,^b
and Department of Hospital Pharmacy, Tottori University School
of Medicine,^c Yonago 683, Japan*

(Received November 5, 1984)

A thin-layer chromatographic method with flame-ionization detector for the quantitative evaluation of methomyl (*S*-methyl *N*-[(methylcarbamoyl)oxy]thioacetimidate) in serum and urine is described. Methomyl in biological fluids was separated and purified simply and rapidly by passing the samples through a Sep Pak C₁₈ or Silica cartridge, in contrast to the complicated pretreatment required in conventional methods. The detection limit was 0.07 µg in the spotted volume using Chromarod A with acetone-hexane (1:4, v/v) as the solvent system. The recoveries were 104.0% in serum, and 98.5% in urine. Further, the method showed good precision; the coefficient of variation was 2.4% in serum, and 4.0% in urine. The reproducibility was satisfactory, and this method could be performed far more simply and rapidly than the conventional ones.

Keywords—pesticide; carbamate insecticide; methomyl; Sep Pak cartridge; TLC; flame ionization detection

Introduction

Carbamate pesticides are being used more and more in agriculture, replacing environmentally undesirable organophosphorus pesticides,¹⁾ and the incidence of poisoning by carbamates, especially methomyl, is increasing in Japan.²⁾

Methomyl, *S*-methyl *N*-[(methylcarbamoyl)oxy]thioacetimidate, is a broad-spectrum carbamate pesticide which is usually marketed as an aqueous solution, but it is also sold in solid form under the trade name Lannate (E.I. du Pont de Nemours & Co.). It is a representative of a group of extremely toxic carbamates with an acute oral LD₅₀ in male rats of 17 mg/kg body weight. Though it is rapidly metabolized and excreted, its toxicity cannot be neglected

because it causes reversible inhibition of cholinesterase.³⁾

Recently, flame ionization detectors (FIDs) have been widely applied to other types of chromatography as well as gas chromatography.⁴⁾ Cotgreave and Lynes⁵⁾ fed a vaporized sample from a narrow chromatoplate to the FID. Further approaches in this area were conceived by Padley,⁶⁾ Szakasits *et al.*⁷⁾ and Mukherjee *et al.*⁸⁾ Padley's idea of using a rod as a support instead of a plate was developed further by Okumura and Kadono.⁹⁾ The combined use of thin-layer chromatography with a flame ionization detector (TLC-FID) now represents a significant advance as a method applicable to screening in the pharmaceutical and biomedical fields.¹⁰⁾ This sen-

sitive, relatively simple and rapid procedure also offers advantages in analyses of heavy oil and food additives, as well as for industrial research analyses. Little work, however, has been reported on its application to the analysis of methomyl in biological materials.

The present paper describes a method for the determination of methomyl in serum and urine by TLC-FID in view of the development of analytical method for causative substances of pesticide poisoning. Furthermore, the procedure for the rapid extraction of methomyl in biological materials using a Sep Pak cartridge, which was established in the previous study, is reported in detail.

Materials and Methods

Methomyl Standard—Methomyl (Lannate®), *S*-methyl *N*-[(methylcarbamoyl) oxy] thioacetimide, was purchased from Sankyo Co., Ltd. (E.I. du Pont de Nemours & Co, USA; distributed by Sankyo Co. Ltd., Tokyo, Japan). Prior to use, methomyl was recrystallized from acetone.

Chemicals—Organic solvents used for the analysis were of special reagent grade. Other organic and inorganic reagents were of analytical grade or of the highest quality commercially available, and were used without further purification.

Serum and Urine Sample—Methomyl-free serum was collected from a cadaver at autopsy, and from the Department of Clinical Laboratory, Tottori University School of Medicine. Urine was collected from a volunteer. These samples were stored in a freezer (-20°C) prior to use.

Methomyl Analysis by TLC-FID—Internal Standard (IS): NAC Standard (1-Naphthyl *N*-Methylcarbamate), that was purchased from Wako Pure Chemical Industries Ltd., Osaka, Japan, was used as an internal standard.

Extraction Procedure: For the extraction of methomyl in serum or urine samples a minor modification of the method previously described¹¹⁾ was introduced. A Sep Pak C₁₈ or Silica cartridge (Waters Associates Inc; Milford, USA) was connected to a 10 ml glass syringe. Prior to use, the

resin in the C₁₈ cartridge was activated by passing 2 ml of methanol and 5 ml of distilled water through the glass syringe. Serum or urine samples, aqueous solutions, and organic solvents were passed through the cartridge at a flow rate of approximately 5 ml/min using a glass syringe.

A 2 ml aliquot of urine was passed three times through a Sep Pak C₁₈ cartridge and then elution was carried out with 5 ml of methanol. After centrifugation at 3500 rpm for 5 min, the methanol layer was evaporated to dryness in a Dry Block (M & S Instruments Inc., Osaka, Japan). The residue was then dissolved in 25 μl of acetone and 25 μl of IS solution. A 5 μl portion of the mixture was used for TLC-FID analysis.

A 2 ml aliquot of serum was shaken with 10 ml of dichloromethane for 5 min. After centrifugation at 3500 rpm for 5 min, the dichloromethane layer was passed through a Sep Pak Silica cartridge and eluted with 4 ml of dichloromethane:methanol (9 : 1, v/v). The elution solvent was evaporated to dryness in a Dry Block. The residue was then dissolved in 25 μl of acetone and 25 μl of IS solution. A 5 μl portion of the mixture was used for TLC-FID analysis.

Apparatus and Operating Conditions: The instrument used was an Iatroscan TH-10 (Iatron Labs., Tokyo, Japan) and was then connected to a Chromatopac-EIA integrator system (Shimadzu Ltd., Kyoto, Japan) and a 056 recorder (Hitachi Ltd., Tokyo, Japan). Glass developing tanks (Iatron) were lined with Toyo No. 52 filter-paper which was pre-washed with mobile phase. The stationary phase was Chromarod-A (Iatron) and the mobile phase was acetone-hexane (1 : 4, v/v). The scanning speed was 30 sec/scan, the flow rate of hydrogen was 160 ml/min, and that of air was 2000 ml/min.

Spotting and Developing: The chromarods were stored in the rod holder provided with the instrument in groups of ten. All rods were activated in preliminary step by passing them through the FID three times just prior to use. A 5 μl aliquot of sample was then applied at the origin of the rod by means of a microdispenser. After the run, the

rods were air-dried for 5 min and transferred to the scanning frame for detection.

Results and Discussion

Separation and Internal Standard

Of the many developing solvents previously described,¹²⁾ the mobile phase of acetone-hexane (1:4, v/v) appeared to be most suitable. For the purpose of standardization, a number of compounds related to the carbamate pesticides were tested. These included XMC Standard (3,5-Xylyl *N*-Methylcarbamate), PHC Standard (*o*-Isopropoxyphenyl *N*-Methylcarbamate), NAC Standard (1-Naphthyl *N*-Methylcarbamate), MTMC Standard (*m*-Tolyl *N*-Methylcarbamate), and BPMC Standard (*o*-*sec*-Butylphenyl *N*-Methylcarbamate). Among the compounds tested, NAC Standard appeared to be the most suitable internal standard for determination of methomyl by TLC-FID. NAC Standard (20 mg) was dissolved in 25 ml of acetone. The compounds can be seen to show good peak shapes and resolution in the central zone of the chromatogram, all of which provided a realistic basis for good reproducibility of analyses. The TLC-FID separation of methomyl in the presence of NAC is shown in Fig. 1.

Calibration Curve

In order to draw the calibration curve, the ratio of the peak area of methomyl to that of the IS was calculated for several concentrations. The relationship was linear in the concentration range of 0.125–5.0 μg for methomyl. The equation parameters together with the correlation coefficient are shown in

Fig. 2. Under the above-mentioned experimental conditions, the detection limit was 0.07 μg in 2 ml of serum or urine sample.

Accuracy and Reproducibility

The reproducibility of the TLC-FID method was examined by replicate analyses of 10 samples of serum and urine. The mean value, standard deviation and recovery are

Fig. 1. TLC-FID Separation of Methomyl in Serum

Fig. 2. Calibration Curve of Methomyl

$$Y = 0.243X - 0.009$$

$$r = 0.997$$

$$N = 18$$

TABLE I. Reproducibility of Methomyl Determination in Serum and Urine

Sample	Methomyl added (μg)	Number	Methomyl found (μg)	Recovery (%)	CV (%)
Serum	40.0	10	41.6 $\pm$ 0.98	104.0	2.4
Urine	40.0	10	39.4 $\pm$ 1.57	98.5	4.0

CV: Coefficient of variation.

summarized in Table I. These results show that the method is applicable for forensic analytical purposes.

Evaluation of the Analyses

In general, prior to methomyl analyses by gas chromatography, the samples have had to be passed through many complicated steps of pretreatment, namely, conversion to the oxime derivative by alkaline hydrolysis¹³⁾ or to the trimethylsilyl ether of the oxime,¹⁴⁾ so that the

procedures were time-consuming and laborious. In the method proposed here, however, methomyl in biological materials can be separated and purified simply and rapidly, by passing the sample through a Sep Pak C₁₈ or Silica cartridge, prior to TLC-FID. This method therefore compares favorably with conventional chemical analyses as regards extraction, precision, and the detection limit as a rapid analytical method for pesticide poisoning.

References

- 1) E. Grou and V. Rădulescu, *J. Chromatogr.*, **260**, 502 (1983); M. Araki, K. Yonemitsu, T. Kambe, D. Idaka, S. Tsunenari, M. Kanda and T. Kambara, *Jpn. J. Legal Med.*, **36**, 584 (1982).
- 2) National Research Institute of Police Science (ed.), "Circumstances of Drug and Toxic Chemical Poisoning in Japan" (in Japanese, translated by the authors), Vol. 21—23, National Research Institute of Police Science, Tokyo, 1979—1981.
- 3) J. Harvey, Jr., A.G. Jelinek and H. Sherman, *J. Agric. Food Chem.*, **21**, 769 (1973); J.A. Liddle, R.D. Kimbrough, L.L. Needham, R.E. Cline, A.L. Smrek, L.W. Yert and D.D. Bayse, *Clin. Toxicol.*, **15**, 159 (1979).
- 4) D. Vandamme, V. Blaton and H. Peeters, *J. Chromatogr.*, **145**, 151 (1978).
- 5) T. Cotgreave and A. Lynes, *J. Chromatogr.*, **30**, 117 (1967).
- 6) F.B. Padley, *J. Chromatogr.*, **39**, 37 (1969).
- 7) J. Szakasits, P. Feurifoy and L. Woods, *Anal. Chem.*, **42**, 351 (1970).
- 8) K.D. Mukherjee, H. Spaans and E. Haahti, *J. Chromatogr.*, **61**, 317 (1971).
- 9) T. Okumura and T. Kadoño, *Bunseki Kagaku*, **22**, 980 (1973).
- 10) T. Itoh, M. Tanaka and H. Kaneko, "Thin Layer Chromatography: Quantitative Environmental and Clinical Applications", ed. by J.C. Touchstone, John Wiley & Sons Inc., New York, 1980, pp. 536—552; T. Okumura, *J. Chromatogr.*, **184**, 37 (1980); R.G. Ackman, *Methods Enzymol.*, **72**, 205 (1981).
- 11) J. Ikebuchi, K. Susumu, T. Nishigaki and J. Kondo, *Jpn. J. Legal Med.*, **37**, 421 (1983).
- 12) D.C. Abbott, K.W. Blake, K.R. Tarrant and J. Thomson, *J. Chromatogr.*, **30**, 136 (1967); J. Askew, J.H. Ruzicka and B.B. Wheals, *Ibid.*, **37**, 369 (1968); C.E. Mendaza and J.B. Shields, *Ibid.*, **50**, 92 (1970); K. Nagasawa, H. Yoshidome and F. Kamata, *Ibid.*, **52**, 453 (1970); A. Ogamo, H. Kasahara and K. Nagasawa, *Eisei Kagaku*, **19**, 340 (1973).
- 13) H.L. Pease and J.J. Kirkland, *J. Agric. Food Chem.*, **16**, 554 (1968); K.K.H. Fung, *Ibid.*, **23**, 695 (1975).
- 14) R.A. Chapman and C.R. Harris, *J. Chromatogr.*, **171**, 249 (1979); M. Yashiki, I. Une, J. Yamauchi and T. Kojima, *Proc. Jpn. Soc. Med. Mass Spectrom.*, **7**, 131 (1982).