Determination of the S-Methyl Isomer in Technical Grade Fenitrothion by Gas Chromatography and High Speed Liquid Chromatography

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Fenitrothion O,O-dimethyl-O-(4-nitro-m-tolyl) phosphorothioate is extensively used in Canadian forests for the control of spruce budworm. After aerial spraying, the analysis of washings from a glass plate, used to sample at ground level, revealed traces of a second organphosphorus compound. A mass spectrum of this material showed a base ion at m/e 125, a parent ion of m/e 277 and an ion at m/e 260 (m-17), which suggested that the compound was an isomer of fenitrothion. This was confirmed by comparison with an authentic sample of S-methyl fenitrothion. JOINER and BAETCKE (1973) attributed the finding of S-ethyl and S-nitrophenyl isomers of parathion on cotton leaves one week after treatment to the effect of light. Since the sample of fenitrothion was exposed to light for only a short period of time (1-2 hrs) after spraying, the S-isomer was thought not to arise from photolysis. The S-alkyl isomers can be formed by thermal isomerisation as was first shown by EMMETT and JONES (1911) and later by JAGLAN et. al. (1970b), by photolysis (COOK and PUGH 1957, and EL-RAFFAI and HOPKINS 1966), and by alkylation (BURN and CADOGAN 1961). Their presence in technical products is therefore not unexpected. The amount present will depend on the method of preparation of the sample and on the storage conditions. METCALF and MARCH (1953) have reported that the amount of S-ethyl isomer varied from 5-20% in some parathion samples.

S-methyl fenitrothion is a strong cholinesterase inhibitor like fenitrooxon, it is therefore important that fenitrothion samples be assayed prior to use. In this note, both GC and high speed LC are evaluated as a means of monitoring the purity of fenitrothion samples.

EXPERIMENTAL

GC Conditions-A Pye gas chromatograph fitted with an alkaki flame ionization detector (RbCl) was used. A glass column, 0.9 m x 4 mm ID, was packed with 100/120 mesh Gas Chrom Q coated with 3% OV-17 (Applied Science Labs, State College, Pa.). With a column flow of 40 ml/min nitrogen and a column temperature of 202°C, the retention time of fenitrothion was 3.4 min. and that of S-methyl fenitrothion 6.7 min.

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High Speed LC-A Waters automated liquid chromatograph, Model 201, equipped with a differential refractometer detector was used. The samples were injected directly on to a stainless steel column (0.6 m x 0.31 cm I.D.), containing Corasil II packing, by a stopped flow injection system (CASSIDY and FREI 1972). The eluant was ethyl acetate/hexane (21:79), and the flow rate was maintained at 1 ml/min. The retention time of S-methyl fenitrothion was 5.1 min.

TLC-Barker-flex silica gel precoated sheets, 2.4 x 7.5 cm were consisting of cyclohexane/ethyl acetate/acetone (70:20:10), Fenitrothion and S-methyl fenitrothion had Rf values of 0.45 and 0.21 respectively. The spots were visualised by either spraying with 10% alcoholic NaOH and heating for 5 min. at 100°C or using the enzyme spray as modified by MENDOZA (1972).

Chemicals-S-methyl fenitrothion was synthesised in the laboratories of the Research Institute of Agrochemical Technology (KOVAČIČOVA et. al. 1973). Samples of technical grade fenitrothion used in the assay study were obtained from various laboratories in the Ottawa area.

Mass Spectrometer-A Pye GC, Model 104 interfaced to a Du Pont mass spectrometer Model 490 with a jet separator was used.

RESULTS AND DISCUSSIONS

JAGLAN and GUNTHER (1970a) used GC to study the isomerisation reaction of methyl parathion. Similarly, the S-methyl isomer of fenitrothion can be resolved by GC, eluting later than fenitrothion on non polar columns (rel. ret. time on OV-17 is 1.79). Like most compounds possessing a P O bond, e.g. oxons, the GC response of S-methyl fenitrothion is less than that of fenitrothion with an alkali flame ionisation detector (AFID). This difference in sensitivity can be partly attributed to peak shape and partly due to combustion conditions. The calculated least detectable amount of S-methyl fenitrothion was found to be 2.5×10^{-12} g/sec. Hence in order to detect 0.1% S-methyl isomer in technical fenitrothion, samples of up to 500 ng would have to be injected.

The use of such high sample loadings of phosphorothioates could be inductive to on-column rearrangement. Thermal isomerisation can occur at temperatures as low as 125°C in reactions (JAGLAN et. al. 1970b), also rearrangement of methyl parathion has been reported on co-injection with methanol and HCl when the injector block was heated (JAGLAN and GUNTHER 1970a). To investigate the possibility of on-column rearrangement at high sample loadings, fenitrothion was injected on an OV-17 column in amounts varying from 50-500 ng. Conditions thought to discourage thermal isomerisation were used including a non heated injector block and on-column injection. The variation

in the amount of S-methyl isomer in a technical sample was not more than 2% in this study, indicating that the S-methyl isomer was not formed at high sample loadings. It was essential however to thoroughly condition the column with S-methyl fenitrothion prior to starting the experiment and throughout the experiment in order to get reproducible results. A chromatogram of 149 ng of a technical sample of fenitrothion (≥95% pure) is shown in Fig. 1.

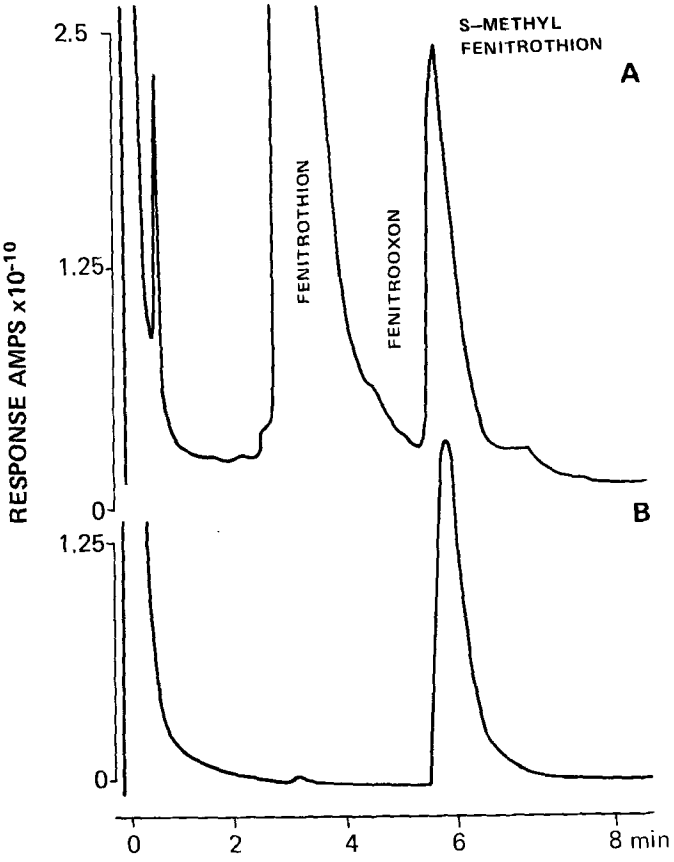

Figure 1. GC Chromatogram of a Technical grade sample of Fenitrothion.

(A) 149 ng Sample.

(B) 5.34 ng S-Methyl fenitrothion.

GC conditions - 0.9 m, 3% OV-17, Column flow-40 ml/min N_2

Column temperature - 202°C.

At a column temperature of 202°C the oxon was not resolved, but at lower temperatures this was possible. The peak immediately after the solvent corresponds to the cresol, detected by the AFID due to the presence of a nitrogen atom. The chromatogram for the S-methyl fenitrothion standard represents a 5.34 ng sample.

In order to confirm the absence of thermal isomerisation or on-column reaction, the sample was also analysed using high speed LC. S-methyl fenitrothion was chromatographed on a Corasil II column at 23°C with ethyl acetate/hexane as the eluant. With a differential refractometer detector, the least detectable amount of the isomer was 1.8×10^{-8} g/sec and the response was linear over the range examined, 5-55 µg (corr. coef. 0.99). Although much less sensitive than the GC method, high speed LC is quite adequate for technical samples. A chromatogram of the same sample as used for GC is shown below in Fig. II, here, a 1 mg sample was used and 31.4 µg of the S-methyl fenitrothion standard.

As can be seen, there is no interference from fenitrothion, cresol or fenitrooxon.

The amount of S-methyl fenitrothion present in the sample was determined by comparing peak areas relative to an external standard for both GC and LC. The values of $3.72 \pm 0.2\%$ and $3.36 \pm 0.2\%$ respectively, were obtained. The agreement of these two results is thought to be sufficiently close to warrant the assumption that under the conditions of the experiment, no thermal or on-column rearrangement occurs on GC.

A number of commercial samples of technical grade fenitrothion ($\geq$ 95%) from various sources were then analyzed for the S-methyl fenitrothion. Thin layer chromatograms (TLC) indicated the presence of the isomer in all the samples, together with fenitrooxon. The level of oxon appeared to be less than that of the S-methyl fenitrothion. Quantitation of the results using both the GC and high speed LC is shown in Table 1.

The agreement between the two methods is reasonably good, indicating that both could be used for routine monitoring. The GC method is more sensitive, down to 0.1% S-methyl isomer, but to determine the oxon as well would require a longer time of analysis. The LC method has a limit of 0.3% for the S-methyl fenitrothion. In one case it was as much as 4%. The results obtained cannot be construed as a comparison of various manufacturers products, since the date of manufacture and the history of storage conditions of the samples was not reported.

Since the presence of 1% S-methyl isomer in fenitrothion increases the in vitro anticholinesterase activity of the mixture by a factor of 10 (KOVAČIČOVA et. al. 1973) it emphasises the necessity of assaying technical grade samples and formulations prior to use. The characterisation of the

S-methyl isomer in the ground samples following aerial spraying suggests it was present in the original formulation, which represents a potential hazard for field operators.

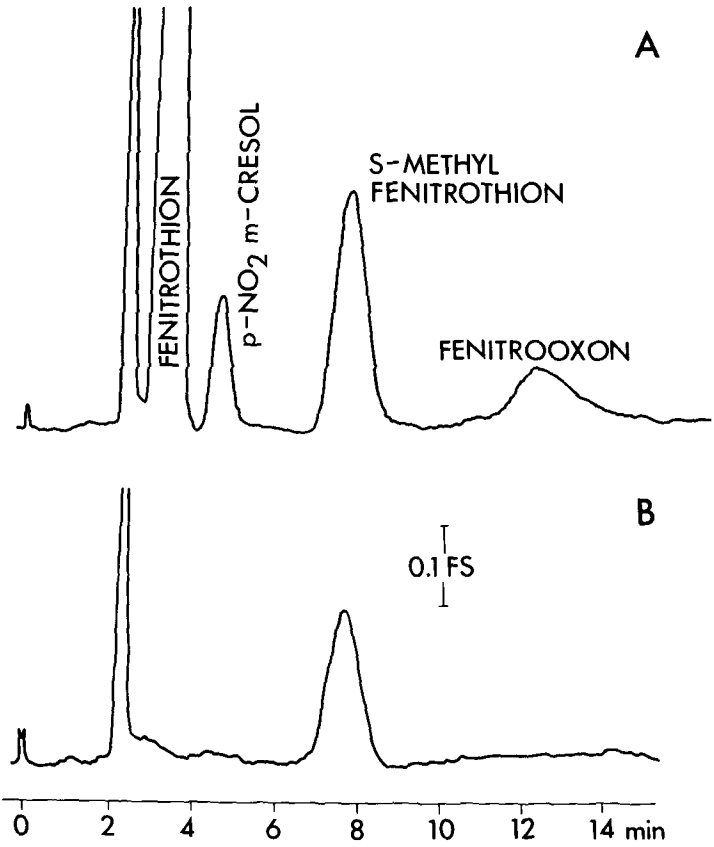

Figure 2. LC Chromatogram of a Technical grade sample of Fenitrothion

(A) 1 mg Sample.

(B) 21.4 µg S-Methyl fenitrothion

LC Condition - 0.6 m Corasil II, Column flow 1 ml/min, Et Ac/hexane (21:79)

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TABLE 1

S-Methyl fenitrothion content of Technical Grade Fenitrothion

Sample	% S-Methyl fenitrothion*	
	G.C.	L.C.
Accothion (Cyanamid)	1.89 ± 0.03	1.59 ± 0.1
Accothion (Cyanamid)	0.43 ± 0.01	0.49 ± 0.1
BASF (Canada)	4.24 ± 0.02	4.43 ± 0.2
Metation (Record. Chemicals)	3.02 ± 0.15	2.14 ± 0.2
Metation (CHJZD)	2.72 ± 0.04	2.38 ± 0.2
Novathion	3.72 ± 0.20	3.36 ± 0.2
Sumithion (Sumitomo)	0.67 ± 0.04	0.55 ± 0.1
Sumithion E-50 (Sumitamo)	1.22 ± 0.11	1.23 ± 0.1

analysis done in triplicate.*

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