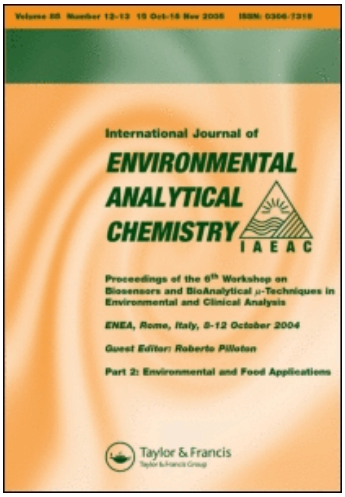


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# Gas Chromatography of Organophosphorus Compounds on Chiral Stationary Phases

Carla E. A. M. Degenhardt[a]; Albert Verweij[a]; Henk P. Benschop[a]
[a] Prins Maurits Laboratory TNO, AA Rijswijk, The Netherlands

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# Gas Chromatography of Organophosphorus Compounds on Chiral Stationary Phases†

CARLA E. A. M. DEGENHARDT, ALBERT VERWEIJ and HENK P. BENSCHOP

*Prins Maurits Laboratory TNO, P.O. Box 45, 2280 AA Rijswijk, The Netherlands*

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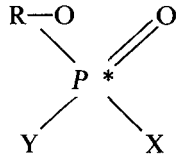
The gas chromatographic separation of the stereoisomers of several chiral organophosphorus compounds is described. Glass capillary columns coated with the non-chiral phases SE-30 and Carbowax 20M, or with the chiral phases Chirasil-Val and Ni(II)Bis[(1R)-3-(heptafluorobutyryl)camphorate] were used.

KEY WORDS: Organophosphorus compounds, gas chromatography, stereoisomers, chiral stationary phases.

## INTRODUCTION

Nerve agents are organophosphorus compounds which show strong cholinesterase-inhibiting properties. As a consequence these compounds are extremely toxic. In our institute the pharmacokinetics of nerve agents with the general formula




†Presented at the Workshop on Chemistry and Fate of Organophosphorus compounds, Amsterdam, Holland, June 18–20, 1986.

where $R = \text{alkyl}$ or $R = C_2H_5$

$Y = CH_3$ $Y = N(CH_3)_2$

$X = F$ $X = CN$

are studied.

All these organophosphorus compounds have a centre of asymmetry (a chiral centre) at the phosphorus atom, resulting in the existence of enantiomers. As enantiomers may differ in their pharmacokinetic properties, an analytical procedure had to be developed which would allow separate determination of the isomers. In this paper the application of gas chromatography with chiral and non-chiral capillary columns for the separation of enantiomeric and diastereoisomeric organophosphorus compounds will be shown.



## EXPERIMENTAL PART

Soman or 1,2,2-trimethylpropyl methylphosphonofluoridate (Figure 1) has been the principal test compound. Having chiral centres both at the P-atom and at the alpha-C-atom, four stereoisomers of Soman exist, designated as C(−)P(+), C(+)P(−), C(+)P(+) and C(−)P(−) in which C stands for the alpha-C-atom in the 1,2,2-trimethylpropylgroup and P for the phosphorus atom.

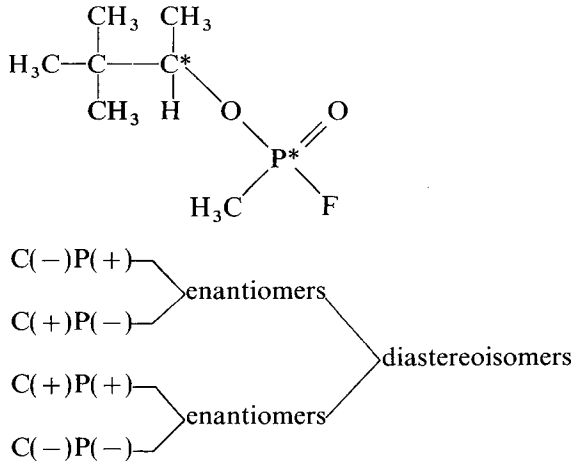



**Figure 1** Structure of 1,2,2-trimethylpropyl methylphosphonofluoridate (Soman).

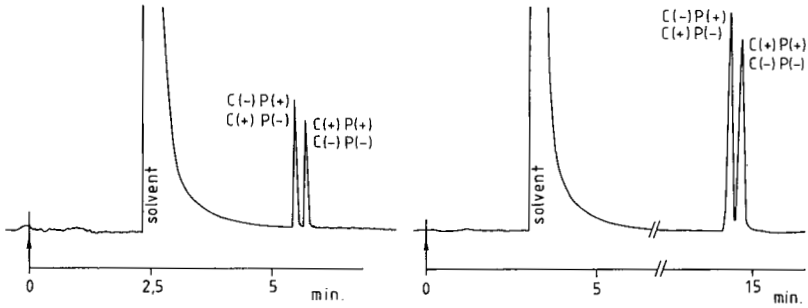
The four isomers show widely differing toxicological properties as published by Benschop *et al.*[1-4]

The diastereoisomeric pairs, having different physico-chemical properties, can be separated on non-chiral stationary phases. However, the enantiomers, having identical physico-chemical properties can only be separated on chiral stationary phases.

## GLC of diastereoisomers on non-chiral stationary phases

In 1971 Verweij *et al.*,[5] published the gas chromatographic separation of a number of diastereoisomeric alkyl methylphosphonofluoridates and related compounds on polar and apolar packed columns. Among other structural aspects it was demonstrated that the relative retention ($r$) i.e. the ratio of the adjusted retention times of the diastereoisomers increased both on lengthening and on branching the alkyl chain as well as on applying a polar column instead of an apolar column. With Soman the experiment was repeated on capillary columns, giving comparable relative retention values; the chromatograms are presented in Figure 2.

The (+) and (−) designations for C(±)P(±) Soman concerning the configuration were derived from reference experiments with synthesized or isolated optically pure compounds.[4] The peak area




Column: Carbowax 20 M
L = 23 m; i.d. = 0.25 mm
T oven = 80°C
r = 1.07

Column: SE-30
L = 32 m; i.d. = 0.27 mm
T oven = 90°C
r = 1.03

**Figure 2** GLC of the diastereoisomers of Soman on Carbowax 20 M and SE-30 capillary columns.

ratio of the diastereoisomers in synthesized C(±)P(±) Soman proved to be constantly amounting to 55:45.

As can be seen from Figures 5 and 6 each diastereoisomeric pair contains equal amounts of the respective enantiomers.

## GLC of stereoisomers on the Chirasil-Val chiral stationary phase

In 1978 Frank *et al.*,[6] introduced the chiral gas chromatographic phase Chirasil-Val consisting of L-valine-tert.-butylamide bonded to a polysiloxane skeleton (Figure 3).

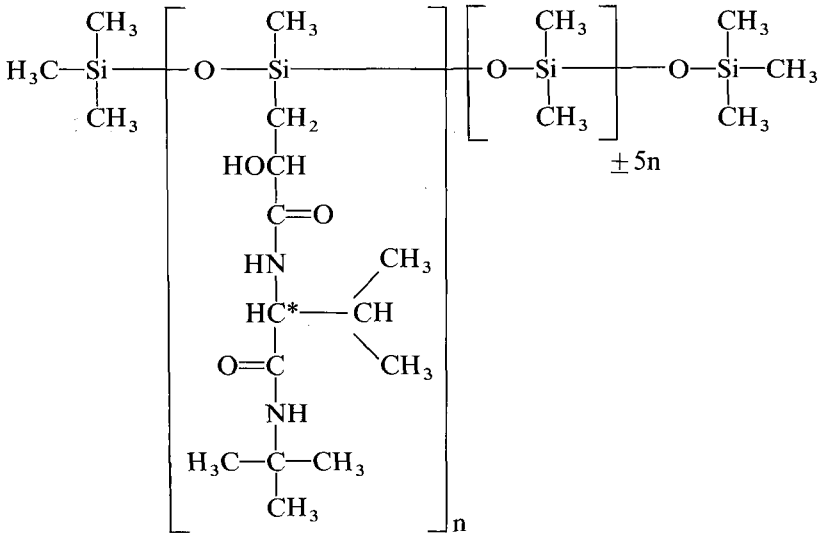



**Figure 3** Structure of Chirasil-Val.

In our laboratory the synthesis of Chirasil-Val led to two different qualities of Chirasil-Val, which were coded type I and type II. They show different GC separation capabilities for Soman as was found in experiments on the coated capillary columns. Until recently the cause for this difference in behaviour was unknown. However, now we know which, obviously, critical step in the synthesis of Chirasil-Val is responsible for the encountered differences. The separation of

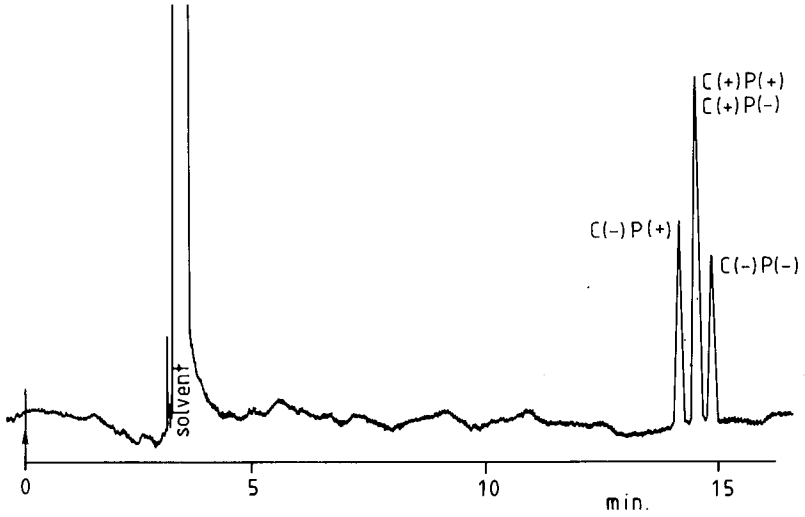



**Figure 4** GLC of the stereoisomers of Soman on Chirasil-Val type I. L = 48 m, i.d. = 0.44 mm, T oven = 75°C.

Soman isomers on Chirasil-Val type I is presented in Figure 4. Only three peaks were obtained which could be designated as C(−)P(+), C(+)P(−)+C(+)P(+), C(−)P(−) respectively. This chromatogram shows the ability of this chiral phase to separate the enantiomers.

As diastereoisomers can be separated on non-chiral columns, the Chirasil-Val type I column was extended with a Carbowax 20 M column, thus giving complete separation of the four Soman stereoisomers as can be seen in Figure 5. This chromatogram demonstrates again the typical peak area ratio amounting to 55:45 of the diestereoisomers. The peak area of the enantiomers are identical.

The separation of Soman isomers on Chirasil-Val type II is presented in Figure 6. This type of Chirasil-Val is able to separate Soman into four peaks without the assistance of an additional non-chiral column.

As can be derived from Figures 5 and 6 the elution order of the two inner peaks is inversed. This inversion is confirmed by the characteristic peak ratios of the diastereoisomers as well as by injection of the optically pure Soman components.

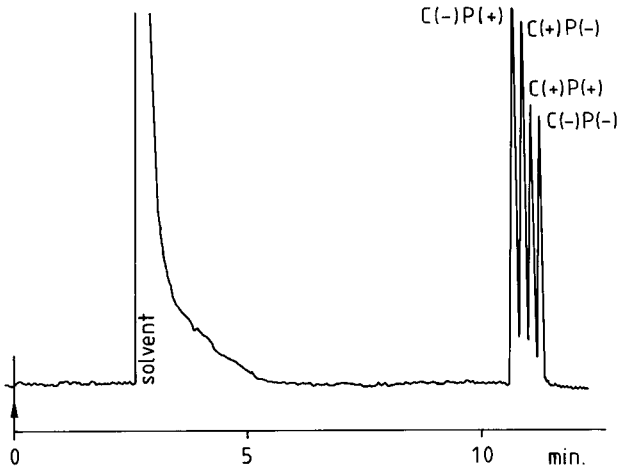



**Figure 5** GLC of stereoisomers of Soman on Chirasil-Val type I—Carbowax 20 M combination.
Chirasil-Val: L = 48 m, i.d. = 0.44 mm
Carbowax 20 M: L = 14 m, i.d. = 0.48 mm
T oven = 80°C.

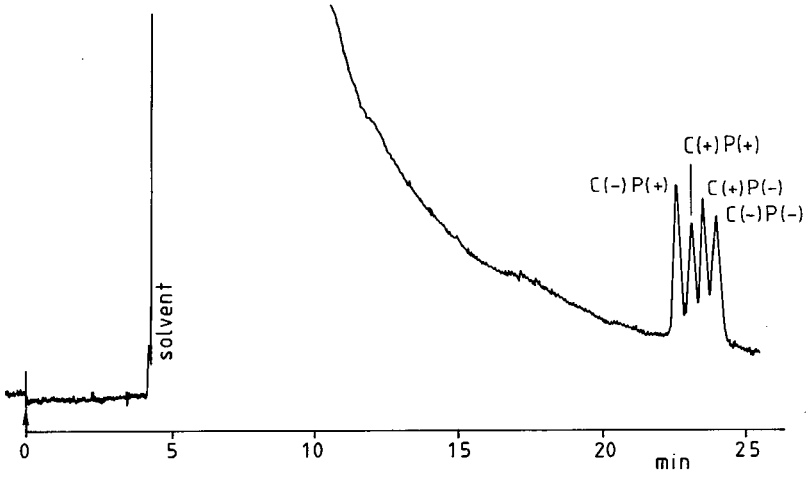
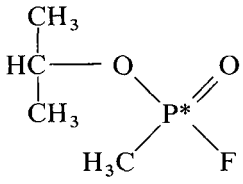
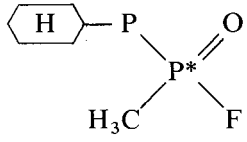



**Figure 6** GLC of the stereoisomers of Soman on Chirasil-Val type II.
L = 50 m, i.d. = 0.5 mm, T oven = 80°C.

Besides Soman, the enantiomers of isopropyl methylphosphonofluoridate (Sarin) and cyclohexyl methylphosphonofluoridate (cyclohexylsarin) were resolved as presented in Figure 7.




isopropyl methylphosphonofluoridate
(Sarin)




cyclohexyl
methylphosphonofluoridate

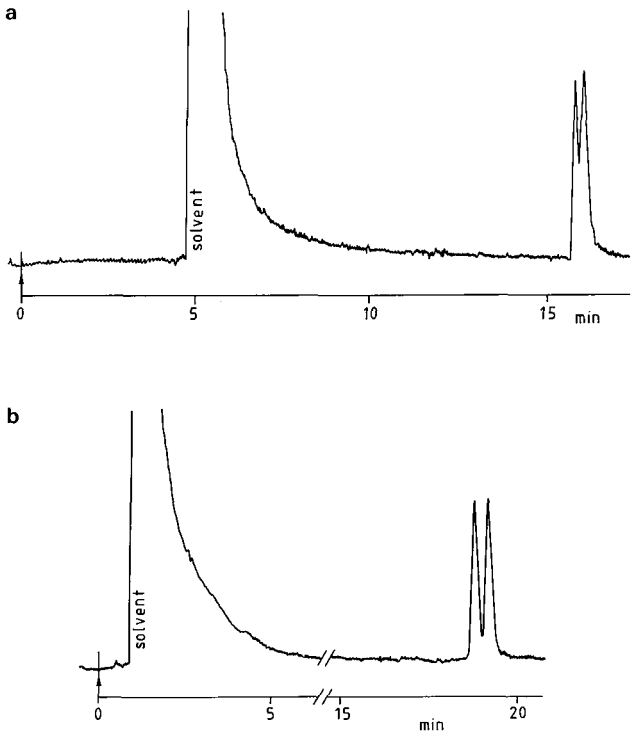



**Figure 7** GLC of the enantiomers of Sarin (Figure 7a, T oven = 60°C) and of cyclohexylsarin (Figure 7b, T oven = 90°C) on Chirasil-Val type II.
L = 28 mm, i.d. = 0.25 mm

The preparation and deactivation of the GLC columns have been described by Benschop *et al.*[7]

Proper deactivation of the Chirasil-Val columns proved to be important as can be seen from Figure 10.

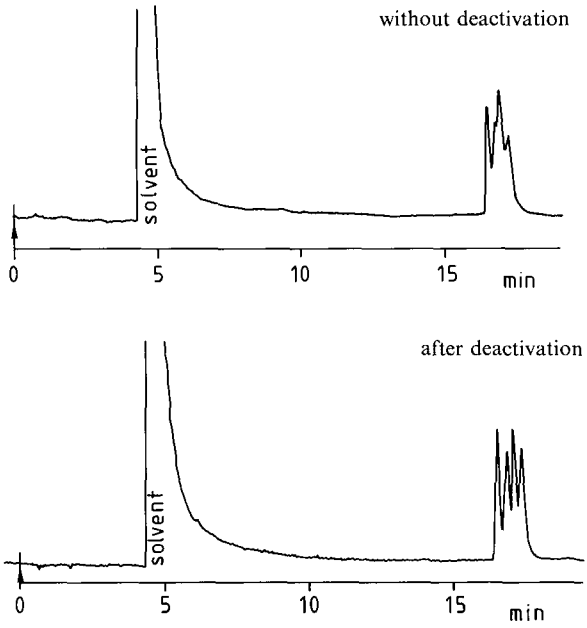



**Figure 10** Effect of deactivation on the separation of the stereoisomers of Soman on Chirasil-Val type II.
L = 50 m, i.d. = 0.5 mm, T oven = 90°C.

Additionally some commercially available Chirasil-Val-coated columns were tried, but none of these columns was able to separate Soman in more than three peaks. They proved to correspond with the Chirasil-Val type I-coated columns prepared in our laboratory.

## GLC of deuterated internal standards

The quantitative GLC analysis of trace amounts of Soman stereoisomers in biological material, in which these isomers are highly labile,

necessitates the use of internal standards with properties similar to those of the Soman stereoisomers. So, deuterated Soman stereoisomers were synthesized[3] in house, coded [$^2H_3$]-Soman (deuterated P-$CH_3$ group) or [$^2H_{13}$]-Soman (deuterated 1,2,2-trimethylpropyl group).

As the elution order of the Soman stereoisomers depends on the quality of the synthesized Chirasil-Val, the internal standard had to be adapted to the type of Chirasil-Val used.[7]

This means that, if the GC analyses were to be carried out on

a) a Chirasil-Val type I column combined with a Carbowaz 20 M column, then the internal standard used was C(−)P(+)−[$^2H_3$] Soman,

b) a Chirasil-Val type II column, then the internal standards used were C(±)P(+)−[$^2H_{13}$] Soman.

Figures 8 and 9 show the respective separation of the Soman stereoisomers and the internal standards on the two different Chirasil-Val types.



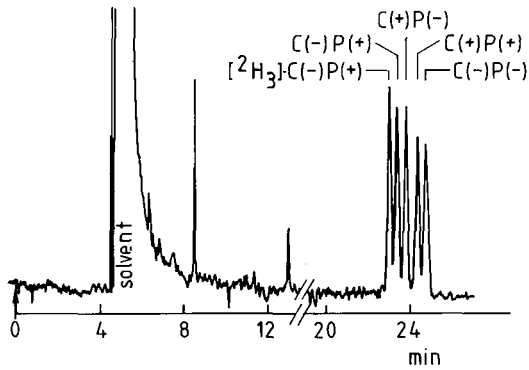



**Figure 8** GLC of C(−)P(+)[$^2H_3$] Soman and the stereoisomers of Soman on Chirasil-Val type I—Carbowax 20 M combination.
Chirasil-Val: L = 48 m, i.d. = 0.44 mm
Carbowax 20 M: L = 14 m, i.d. = 0.48 mm
T oven = 80°C.

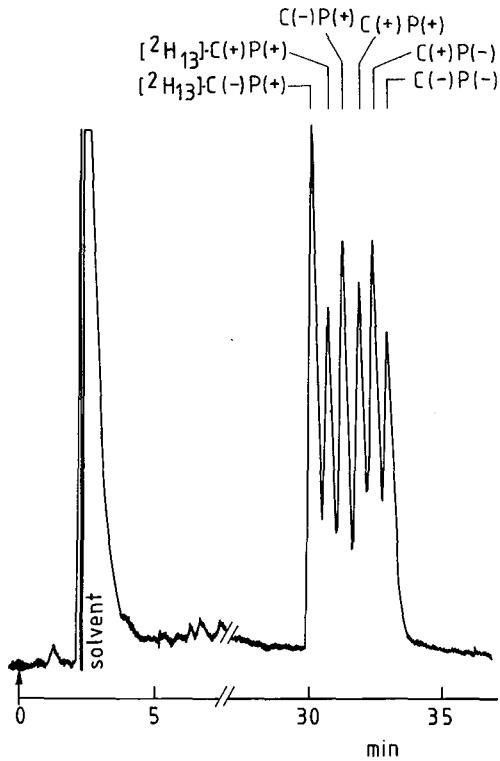



**Figure 9** GLC of the stereoisomers of C(±)P(+)[$^2H_{13}$] Soman and of Soman on Chirasil-Val type II.
L = 50 m, i.d. = 0.5 mm T oven = 80°C.

## GLC of stereoisomers on chiral complexation columns

Since 1977 Schurig *et al.*, have published[8-10] the separation of several classes of nonphosphorus chiral compounds by complexation gas chromatography on metal complexes with Ni(II)Bis[(1R)-3-(heptafluorobutyryl)camphorate] (Figure 11) as an efficient chiral stationary phase.

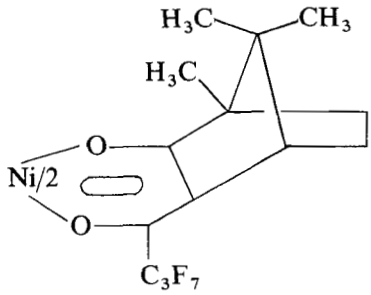
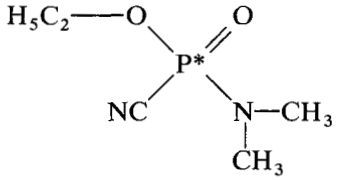



**Figure 11** Structure of the chiral metal chelate Ni(II)Bis[(IR)-3-(heptafluorobutyryl) camphorate].

A capillary column coated with this phase in 0V-101 separates the enantiomers of O-ethyl N,N-dimethylphosphoramidocyanidate (Tabun) as shown in Figure 12.[11] On Chirasil-Val columns (type I and type II) the enantiomers coincide.




O-ethyl N,N-dimethylphosphoramidocyanidate
(Tabun)

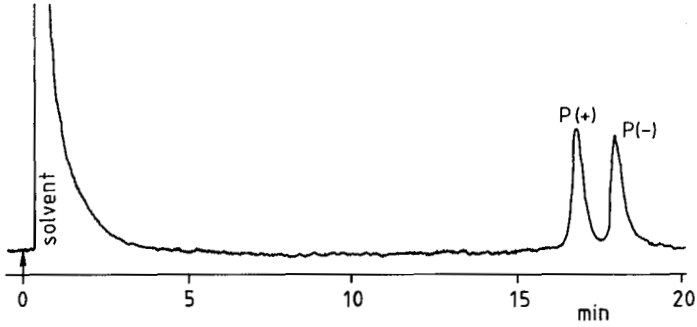



**Figure 12** Separation of Tabun enantiomers on an OV-101/Ni(HFB-1R-Cam)$_2$ complexation column.
L = 14 m, i.d. = 0.44 mm, T oven = 120°C

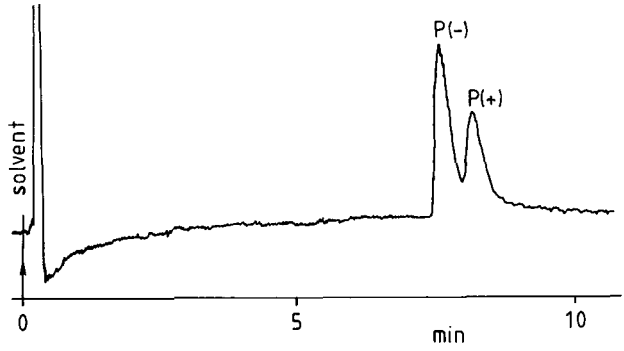



**Figure 13** Separation of Sarin enantiomers on an OV-101/Ni(HFB-1R-Cam)$_2$ complexation column.
L = 9 m, i.d. = 0.44 mm, T oven = 100°C.
An excess of the (−) Sarin had been added to a racemic mixture.



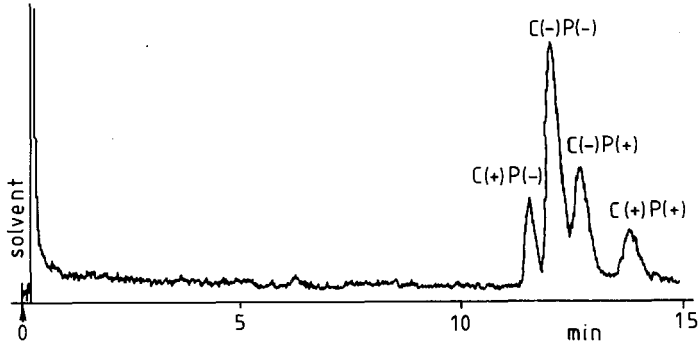



**Figure 14** Separation of Soman stereoisomers on an OV-101/Ni(HFB-1R-Cam)$_2$ complexation column.
L = 9 m, i.d. = 0.44 mm, T oven = 120°C.
An excess of C(−)P(−) Soman had been added to C(±)P(±) Soman.

Figures 13 and 14 show the separation of the Sarin and Soman stereoisomers respectively.

The elution order of the Soman stereoisomers on the complexation phase differs from that on the Chirasil-Val phase. On a Chirasil-Val column (type I and type II) the first peak belongs to C(−)P(+)

**Table I** Separation capability of some gas chromatographic stationary phases for Soman

| Stationary phase | Separation capability, number of peaks | Elution order |
|---|---|---|
| SE-30; Carbowax 20 M | 2 | C(−)P(+) C(+)P(+)<br>C(+)P(−) C(−)P(−) |
| Chirasil-Val type I | 3 | C(−)P(+) C(+)P(+) C(−)P(−) C(+)P(−) |
| Chirasil-Val type I + Carbowax 20 M | 4 | C(−)P(+) C(+)P(−) C(+)P(+) C(−)P(−) |
| Chirasil-Val type II | 4 | C(−)P(+) C(+)P(+) C(+)P(−) C(−)P(−) |
| OV-101/Ni $(HFB\text{-}1R\text{-}Cam)_2$ | 4 | C(+)P(−) C(−)P(−) C(−)P(+) C(+)P(+) |

Soman, whereas on the complexation column the first peak belongs to its enantiomer C(+)P(−) Soman.

The synthesis of the metal chelate and the preparation of the glass capillary columns were carried out in our laboratory according to Schurig *et al.*[9,10]

## SUMMARY

Table I summarizes gas chromatographic separation of some chiral organophosphorus compounds on two different chiral stationary phases and on polar and apolar non-chiral stationary phases. The results presented are focussed on the elution order of the peaks of the four stereoisomers of Soman.



## Acknowledgement

The authors gratefully acknowledge George R. van den Berg and Matthijs F. Otto for the Synthesis of Chirasil-Val, and Roland P. E. van Damme and Ger W. H. Moes for the synthesis of the nickel chelate.

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