A MULTIDIMENSIONAL APPROACH TO ANALYSIS BASED ON GC PHASE FUNCTIONALITY: ORGANOPHOSPHATES

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Introduction

Understanding the factors that determine the dominant retention mechanism between an analyte and phase is an important step in selecting capillary columns for phase orthogonality rather than 'tuned' separation. Such mechanisms are important in the design of robust multidimensional experiments.

In this study we examine the separation of organophosphoate and phosphothioate pesticides (OPs, Table 1) by gas chromatography-mass spectrometry (GCMS) using BPX90 and BPX5 capillary columns. The OPs are a useful class because they are well characterized and the family is comprised of a large number of structural variants that allows for the study of structure-retention relationships. The OPs may be subdivided on the basis of functionality into aliphatic, planar, coplanar and complex or polycyclic types. These divisions are made on the basis of the degree of unsaturation in the pendant group, the stabilizing influence of through conjugation and the extent to which steric hindrance is able to influence the conformation of the molecule. The relative contribution of the pendant groups to the molecule's overall steric volume is also important.

aspon	dicrotophos	isomalathion	pyridaphenthion
butamifos	dimethoate	isoprothiolane	Ronnel
chlorfenvinfos (cis)	dioxathion	isoxaoxon	sulfotepp
chlorfenvinfos (trans)	disulfoton	isoxathion	sulphrofos
chlorpyrifos ethyl	EPN	leptophos	TEPP
chlorpyrifos Methyl	ethion	malaoxon	terbufos
coumaphos	ethoprop	malathion	tetrachlorvinphos
cyodrin (Crotoxyphos)	famphur	merphos	thionazin
DEF(oxymerphos)	fenitrothion	mevinfos	tokuthion
demeton-O	fenitroxon	monocrotophos	tolclofos Methyl
demeton-S	fensulfothion	parathion ethyl	trichloronate
diazinon	fenthion	parathion methyl	trimethyl phosphothioate
dichlofenthion	fonofos	phorate	trithion
dichlorvos	isofenphos	phosphamidone	-

Table 1: OP compounds included in the study.

Results and Discussion

Factors affecting the retention of compounds by different phases may be studied in either a side by side comparison of data sets acquired under identical conditions (an arrayed experiment) or by measuring the effect of variables on the retention time difference for each analyte chromatographed on different phases. Both techniques have merit when elucidating mechanisms by pattern recognition.

Following the isothermal elution of OPs from BPX5 and BPX90 columns, a plot of retention time difference against analyte molecular weight was prepared (Fig 1). The plot shows clustering of OP types suggesting the sub-grouping of analytes is significant but that the size of the analyte is not important to differences in retention mechanism. Using octanol-water partition coefficient as a de facto measure of surface presentation of the OP, this parameter was plotted against corrected retention times for both columns (Fig 2a and 2b). Changes in retention behavior between columns are apparent for complex polycyclic and planar OPs but less clear for coplanar and aliphatic types. For BPX90, planar analytes show increasing retention as a function of decreasing partition coefficient. When retention time differences between the two phases are plotted (Fig 3), a clear correlation with partition coefficient is observed.

The same influence on retention is not observed for BPX90. The shift in OP retention between BPX5 and BPX90 shows structure dependent correlation with the octanol-water partition coefficient (log K_{ow}) (Fig 3). Log K_{ow} is a useful measure of partitioning as it gauges the surface chemistry of the analyte by measuring interactions in a polar environment. The correlation is linear for planar and out of plane conformed OPs. The out of plane group is further divided with unsaturated and aromatic OPs showing different shifts in retention to those OPs having aliphatic substituents. Rotation of each series away from the vertical (i.e. no difference between phases) to horizontal (i.e. orthogonal retention that is independent of K_{ow}) is a measure of phase orthogonality.

Dioxathion also fits this series because it shows the same dominance of substituent group over P=S interaction with the phase. Fensulfothion is an outlier to all types and shows retention somewhere between planar and complex.

Conclusion

Retention on a BPX90 column is determined by steric access to the π- electron structure, the presence of hard or soft double bonds (e.g. phosphoates and phosphothioates), planarity, free rotation and lipophilicity. In contrast, non-polar retention shows a clear association between molecular weight which, under isothermal conditions, is attributable to both conventional Henry's Law behavior and to mobility.

Non-polar retention of OPs may be sub-divided on the basis of intramolecular mobility (free rotational barriers and through conjugation). In contrast, retention of the same target group by BPX90 is complex and influenced by functionality rather than molecular size. The correlation of retention differences between the phases with octanol-water partition coefficient reflects the influence of steric and lipophilic surface chemistry on the ability of BPX90 to interact with analytes. The specificity of the BPX90 phase make it highly orthogonal to the non-polar phases and so it is suitable for not only primary separation of some analytes but also a suitable choice as a second column in multidimensional techniques.

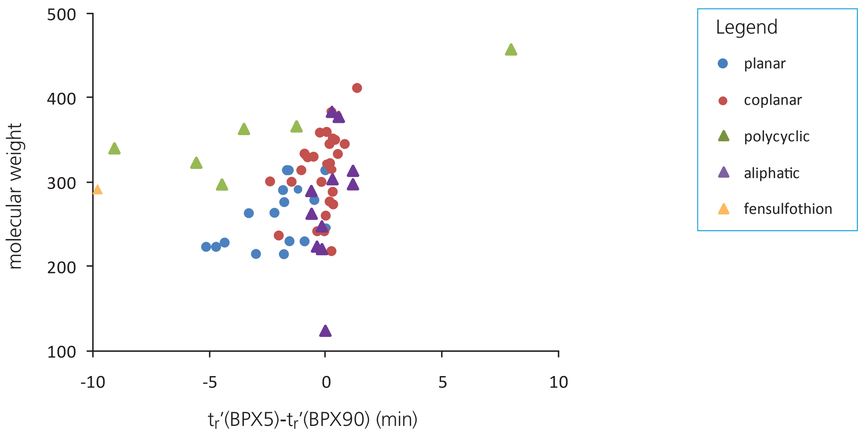


Figure 1: The difference in isothermal retention time between BPX5 and BPX90 columns operated under identical conditions (t_r'(BPX5)-t_r'(BPX90)) versus analyte molecular weight. The population is divided on the basis of functionality (steric volumes and through conjugation).

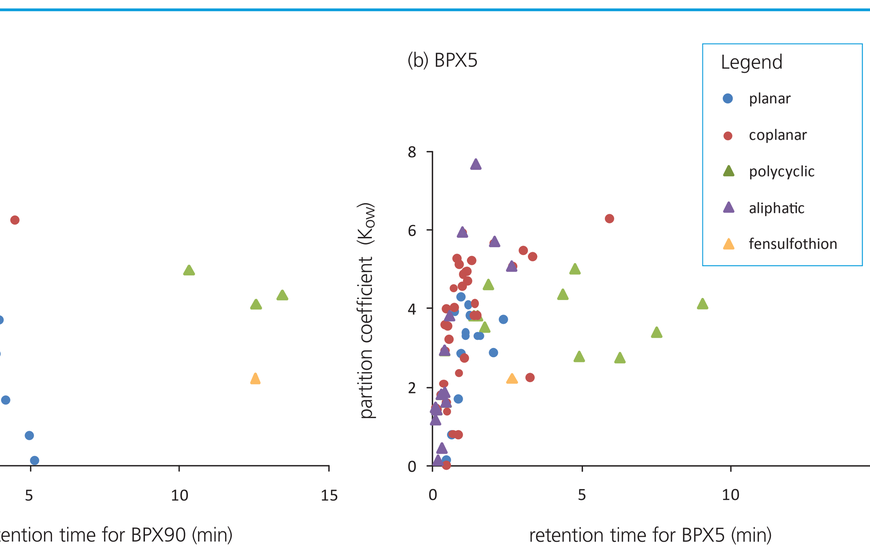


Figure 2: Isothermal retention time for BPX90 (a) and BPX5 (b) columns operated under identical conditions versus log K_{ow}. The population is divided on the basis of functionality (steric volumes and through conjugation).

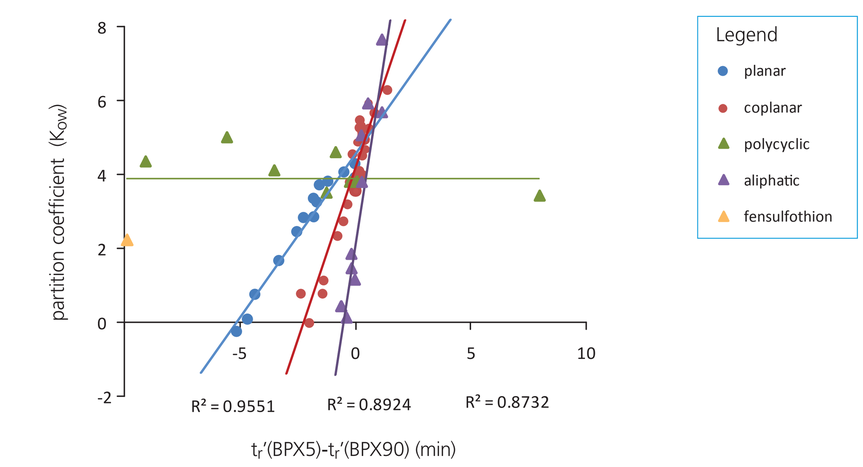


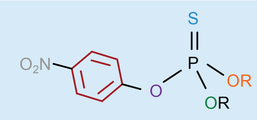
Figure 3: Difference in isothermal retention time between BPX5 and BPX90 columns operated under identical conditions (t_r'(BPX5)-t_r'(BPX90)) versus log K_{ow}. The population is divided on the basis of functionality (steric volumes and through conjugation).

Experimental detail

Mixed organophosphoate standards SPM-824, 834, 844 and 854 (Ultra Scientific, RI, USA) and single component standards of pesticides were dissolved in acetone at 200 µg/mL for use in this study.

Gas chromatography Mass spectrometry was performed on a 6890GC-5973N MSD (Agilent Technologies, CA, USA) equipped with a model 14642 electron multiplier (ETP, NSW, Australia) and either a BPX5 or a BPX90 column (30 m x 0.25 mm i.d., 0.25 µm film thickness, SGE Analytical Science, VIC, Australia). Injections of 0.1 µL standards in acetone were fast and split at a ratio of 60:1 and at a temperature of 275 °C. Purge flow was 50 mL/min and a nominal inlet pressure of 93 kPa. The oven temperature was 100 °C and the carrier gas was helium at a flow rate of 1.2 mL/min in constant flow mode. EI mass specta were collected over the range 40-500 Da at 2 scan/sec. The quadrapole temperature was 150 °C and the source was 230 °C.

Chromatographic data was acquired and processed using ChemStation software (Version 100 D.02.00.275, Agilent Technologies). Linear regression was performed using the data analysis pack from Excel 2007 (Microsoft Corp). Retention times are measured as single points and confirmed by reanalysis of the same sample under identical conditions on a second day.



Electronic effects and steric volume of substituent groups

Saturated, non-aromatic unsaturated, phenyl, heteroatomic or polycyclic aromatic pendant group

bridgehead atom type

thion or oxon

presence or ortho-substituents

connecting atom

substituent chain length

Figure 4: Many structural elements alter the conformation and electron density of the organophosphate pesticides.