

INCREASING THE LEVEL OF TESTING STANDARDS FOR CAPILLARY COLUMNS

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INTRODUCTION

The majority of capillary GC column users assume that the test chromatogram supplied with their new column is either of limited significance to their particular application or otherwise irrelevant. Test mixes are rarely designed to be application specific as their normal function is to provide information about column phase, dimensions and activity characteristics.

To meet the most basic requirements, test mixes contain two retention time markers (normal alkanes) that are separated on the basis of boiling point and probes for acidity, basicity, non-specific or neutral hydrogen bonding and aromaticity. Assessing column characteristics on the basis of the test mixture has been well described for phases based on dimethyl or phenyl functionalized siloxanes.

Basic test mixes must be able to provide more specific information in the case of specialty phases where the important characteristics of the column may be either unique phase chemistry or a specific combination of column parameters. Selecting appropriate test components requires a sound understanding of the phase chemistries that might be at work for particular analytes.

This poster examines the application of test mixes to the specialty phases HT8 and BPX70 with particular emphasis on the true information content derived from test chromatograms.

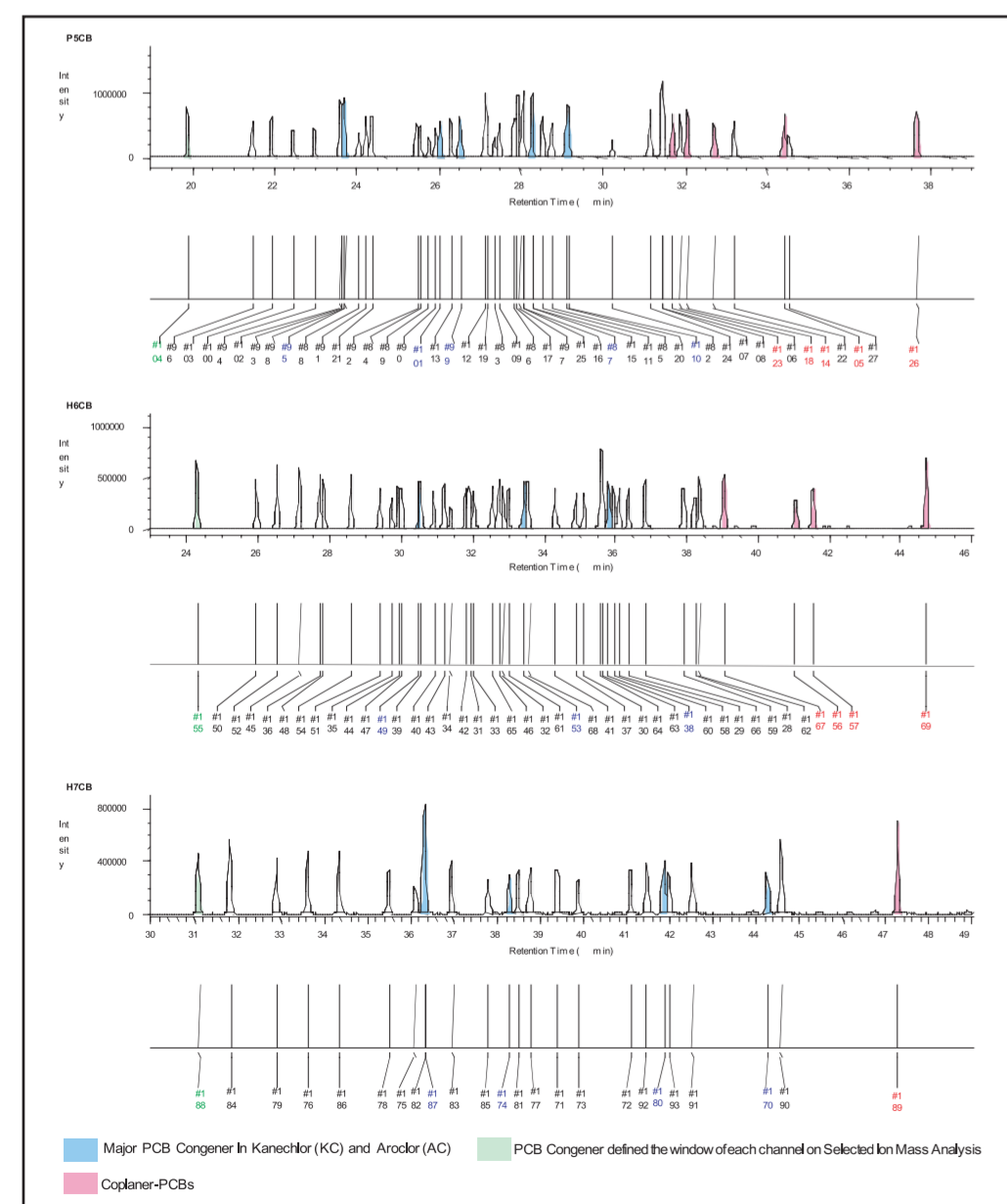


Figure 1. Extracted Ion Chromatograms for penta- hexa- and hepta- PCB congeners analyzed with a HT8-PCB column showing the selectivity of the phase towards coplanar analytes. The number shown under the chromatogram is the BZ number.

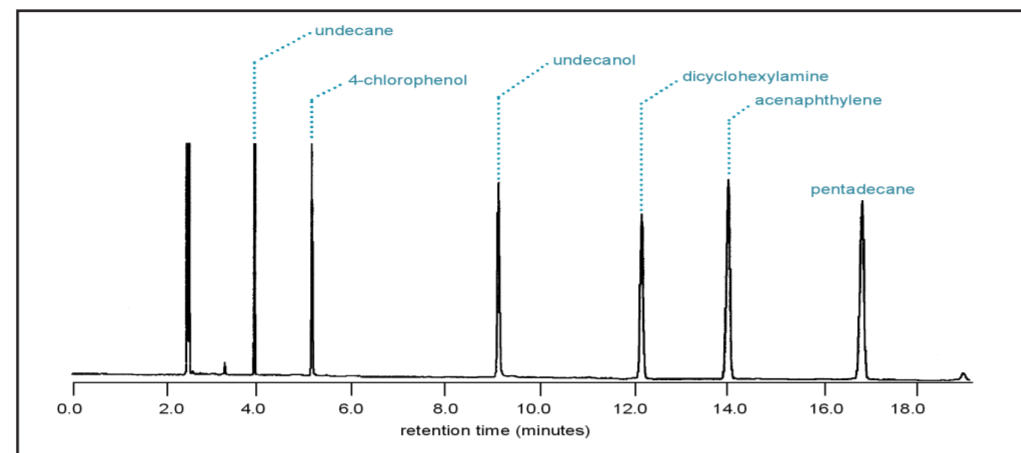


Figure 2. Typical test chromatogram for HT8 phase showing probes for residual hydrogen bonding activity and carborane- π bonding relative to boiling point separation of alkanes.

HT8 CARBORANE PHASE

HT8 is a specialty phase based on a metacarborane modified dimethyl-diphenylpolysiloxane backbone. The thermal stability of the carborane phases makes them ideally suited to high temperature applications and subsequently they find application in the analysis of polycyclic aromatics including dioxins, PCBs and PBDEs.

The selectivity of HT8 for coplanar PCB congeners has been demonstrated by the excellent separation afforded to these analytes and to other polyhalogenated aromatics. The selectivity and resolving power of the phase for the analysis of penta-, hexa- and hepta- congeners of polychlorinated biphenyls is shown in **Figure 1**. While the analysis of PCBs is an important application for which HT8 is the most effective column available, a test of column performance must be a test of the primary retention mechanism and not of the application. The application does, however, illuminate mechanistic aspects of retention on the HT8 phase.

The *m*-carborane is a backbone component that is connected via carbon-silicon bonds but which, through stereochemistry, gives the polymer a non-linear structure (i.e. the backbone is kinked and exposes the (HB)_n structure). The unique delocalized electronic structure of the carborane makes it electrophilic but, as it does not expose planar π -orbital lobes like a phenyl or silphenylene phase, it does not provide the same opportunity for π - π interactions with planar aromatic analytes. The shape of the metacarborane does not make for a high "contact area" with aromatics and so electronic interactions are likely to be through-space rather than by formal overlap of orbitals.

The high selectivity for coplanar PCB congeners is related to the break in through-conjugation when the biphenyl is twisted out of plane. That the corresponding para-substituted species do not yield the same result suggests that the competing σ/π substituent effects of the pendant halogens on the isolated aromatic π -systems is a less important effect. Selectivity may therefore be electronic, steric or a combination of both.

HT8 combines both the complimentary mechanisms of the phenyl and metacarborane phases and so maximizes the opportunity for separating complex mixtures. Probing the electrophilic nature of the phase is effectively achieved with the use of the planar aromatic acenaphthylene. The

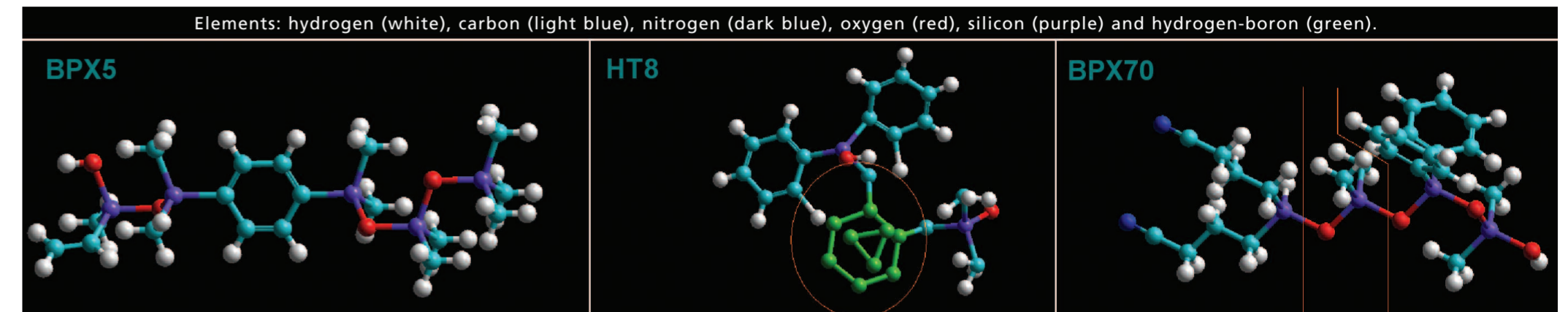


Figure 3. Ball and stick representations of HT8 phase and BPX-70 phase in comparison with the 'universal' BPX5 phase (5% phenyl-95%dimethyl polysiloxane). Understanding the mechanistic aspects of retention is an important step in selecting appropriate test compounds.

chromatographic characteristics of this compound relative to normal alkanes (which are separated by boiling point) provide a sensitive test of the electrophilic properties of the phase. A typical HT8 test chromatogram is shown in **Figure 2**. Aliphatic probes for basicity, acidity and non-specific hydrogen bonding are also included in the test mixture as indicators of secondary column characteristics. These substances are selected on the basis that they show little selectivity for the metacarborane or phenyl components of the phase.

BPX70

BPX70 is a specialty phase of the biscyanopropylsiloxane type. In addition to its performance as a polar phase, it has significant appeal because of its unique selectivity in the separation of fatty acid methyl esters (FAME). Appropriate testing of the phase is not simply based on the separation of a standard FAME test mixture, although such a separation is likely to be of interest in certain applications.

On a non-polar phase such as BP1, separation is in boiling point order and all FAME are separated into groups on the basis of carbon number. Introducing some aromatic character to the phase, for example with a BPX5 phase, still gives separation on the basis of carbon number but some selectivity by π - π interactions. On a BPX70 phase, the dominant chemistry for polyunsaturated FAME becomes π - π interaction while saturated elute on the basis of boiling point. The two different mechanisms of separation result in overlap of carbon number groups (e.g. C20:4 approaches the retention time of C22:0 as shown in **Figure 4**). Separation of FAME is measured using Equivalent Chain Length (ECL) numbers instead of Kovat's indices.

Making subtle changes in the polarity of the phase affects the ECL of unsaturated FAME as shown in **Figure 4**. This measure of π - π polarity is based on one chemistry and does not reliably measure column performance. Acenaphthylene is a planar polycyclic aromatic compound that chromatographs near methyl octadecanoate (C18:0-Me). The polycyclic

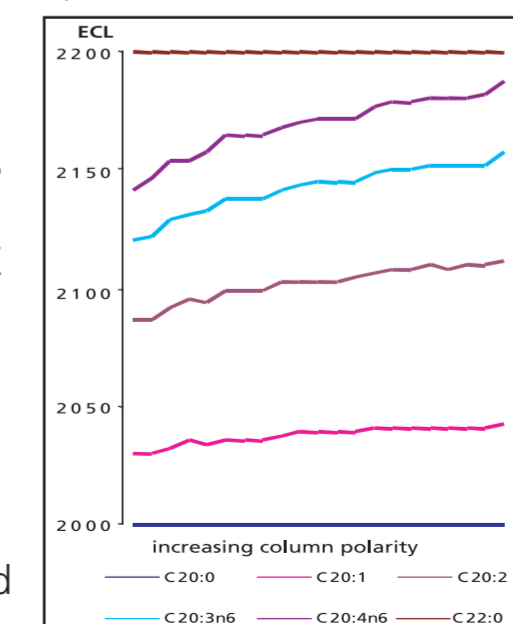


Figure 4. The change in ECL number for C20 FAMES with increasing column polarity.

aromatic is an important analyte for FAME applications as it may be found as a degradation product of polyunsaturated fatty acids. Acenaphthylene is retained by linear π - π interactions and not affected by free rotation or conformational effects as are the unsaturated FAME. Resolution of the aromatic from the boiling point separated ester is therefore a second test of column performance.

By testing the phase with a second and complimentary test, a more stringent performance standard is established as shown in **Figure 5**.

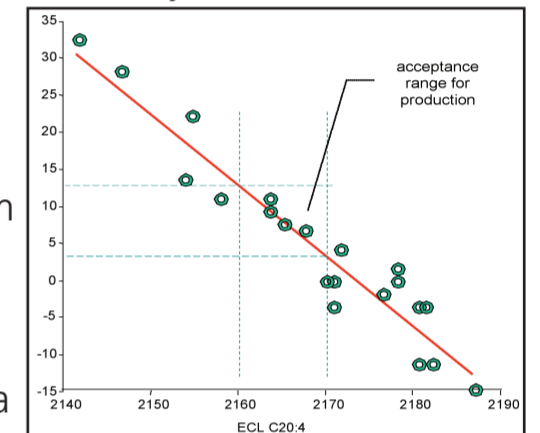


Figure 5. The relationship between ECL number for C20:4 FAMES and separation of acenaphthylene and methyl octanoate on a BPX70 column.

CONCLUSION

The selection of probes to test column performance must be based on an understanding of the primary retention mechanisms of the phase and tests for secondary or undesirable activity associated with the phase family.

For specialty phases, such as BPX70 and HT8, the test mixture must include probe compounds to test for specific phase chemistries in addition to those required to provide information about column phase, dimensions and activity characteristics.

Test mixtures only need to include appropriate probes to measure column characteristics. The inclusion of additional or application specific probes in the test mixture does not necessarily provide additional information. Additional substances included in the test mixture should not be misrepresented as demonstrating superior column performance.

References

Figure 1. Chisato Matsumura¹, Masahiro Tsurukawa¹, Takeshi Nakano¹, Tatsuya Ezaki² and Makoto Ohashi², ¹Hyogo Prefectural Institute of Public Health and Environmental Sciences, ²SGE Japan Inc., "Elution Orders of All 209 PCBs Congeners on Capillary Column HT8-PCB", *Journal of Environmental Chemistry*, Vol.12, 855-865 (2003).
Figure 2. The metacarborane is shown as a surface structure only and the substructure is representative only of steric volume. The electronic bonding of carboranes is described by K.Wade, *The key to cluster shapes*, *Chem. Brit.*, 11, 177 (1975).



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