Structure-retention relationships for basic drugs separated on different GC phases

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Introduction

The analysis of semi-volatile drug residues in toxicology often uses gas chromatography with a variety of detectors. The added data dimension and deconvolution power of mass spectrometry has lessened the need for the column to provide complete separation of analytes or orthogonality of methods for dual column identification. However, trends towards fast and multi-dimensional techniques have rekindled interest in alternatives to GC using PDMS and 5 % phenyl-PDMS phases. We describe here the influence of phase aromaticity on the relative separation of a range of analytes and the way in which phase selection can influence drug analysis.

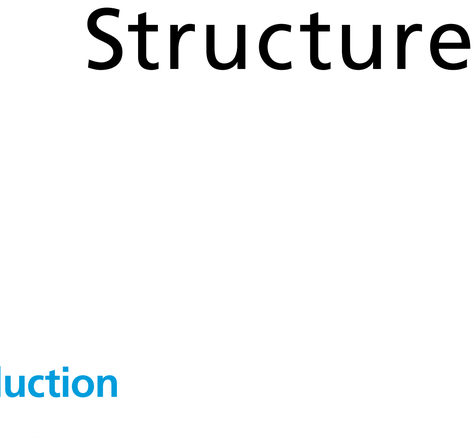

Figure 1. The key retentive chemistries of aromatic phases.

Compound	MWt	BP1	BPX5	BPX35	BPX50	HT8
Nicotine	162	14.97	15.38	17.12	16.54	17.16
Ibuprofen	220	17.60	17.69	18.60	18.36	18.33
Amphetamine	177	18.09	17.46	19.67	19.76	18.94
Methamphetamine	191	18.14	18.49	20.71	20.09	20.07
Heptaminol	229	18.26	17.04	19.10	18.68	18.66
Metronidazole	213	18.49	19.43	21.66	21.83	21.08
Prolintane	217	18.65	17.46	20.03	19.09	19.64
Amylobarbitone	226	19.69	20.27	21.87	21.11	20.45
Pentobarbitone	226	19.95	20.56	21.49	21.55	20.88
Pseudoephedrine	249	20.30	20.89	23.22	22.71	22.36
Caffeine	195	20.60	21.61	23.89	24.04	23.02
Difluisal	264	21.20	21.54	22.92	22.68	22.76
Benzocaine	207	21.26	22.10	24.85	24.32	23.83
Lignocaine	234	21.30	21.68	23.72	22.92	18.66
Prilocaine	262	21.30	23.72	19.09	25.52	25.42
Diphenhydramine	255	21.45	21.77	22.92	22.94	22.55
Flufenamic Acid	295	21.52	21.70	22.79	22.49	22.85
Fenoprofen	256	21.55	21.94	23.55	23.63	22.74
Flurbiprofen	258	21.74	22.10	23.62	23.57	23.13
Niflumic Acid	296	22.24	22.43	23.65	23.41	23.87
Naproxen	244	22.62	23.06	24.81	24.86	24.30
Nefopam	253	22.80	23.43	25.77	25.30	24.58
Flunixin	310	22.90	23.09	24.37	24.17	24.42
Xylazine	220	22.90	23.45	26.12	25.58	24.85
Mepivicaine	246	23.02	23.75	26.07	25.35	25.66
Methylphenidate	275	23.13	23.81	26.44	25.96	25.38
Mefenamic Acid	255	23.47	23.81	25.46	25.49	25.00
Ketoprofen	268	23.65	24.15	26.11	26.18	25.47
Probenecid	299	24.13	24.51	26.09	25.87	26.37
Tolfenamic Acid	275	24.16	24.54	26.22	26.21	25.81
Diclofenac	310	24.44	24.83	26.78	26.93	26.12
Tiaprofenic Acid	274	24.48	25.13	27.18	27.25	26.67
Methandrostenolone	590	24.57	24.21	24.44	23.68	25.51
Eltenac	315	24.67	25.15	27.15	27.31	26.51
Imipramine	280	24.67	25.17	27.33	26.83	26.50
Methandriol	594	24.67	24.33	24.28	23.24	25.60
5(10),3β17βestrenediol	566	24.70	24.25	24.01	22.96	25.54
Trimipramine	294	24.86	25.21	26.36	26.37	26.36
Etodolac	301	24.86	25.10	26.68	26.72	26.47
Tolmetin	271	25.05	25.66	27.74	27.85	27.10
5β3β17βestranediol	568	25.05	25.07	24.64	23.25	26.71
Tetracaine	306	25.10	29.32	27.81	28.68	29.25
Meclofenamic Acid	310	25.24	25.63	27.48	27.63	27.06
Ketorolac	269	25.39	26.12	28.39	28.61	27.60
Norethandrolone	592	25.42	25.10	25.17	25.83	26.44
Ethacrynic Acid	317	25.45	25.92	27.81	27.81	27.65
Promazine	284	25.51	26.23	28.67	28.09	27.76
Phenytoin	252	25.52	26.64	29.14	29.42	27.64
Mestanolone	594	25.55	25.96	27.31	26.82	27.87
Zomepirac	305	25.74	26.32	28.37	28.48	27.76
Phenylbutazone	308	25.99	26.55	28.56	28.67	27.84
Benzydamine	309	26.05	26.78	29.24	28.68	27.85
Vedoprofen	296	26.17	26.62	28.40	28.45	27.91
1,4-androstadiene-3,17-dione	562	26.19	26.29	27.34	26.81	28.30
Methenolone	592	26.23	25.66	25.27	23.94	27.17
5β3β17βandrostanediol	582	26.26	25.76	25.40	24.07	27.41
Diazepam	284	26.30	27.23	30.20	29.74	29.20
Methyltestosterone	592	26.34	24.35	24.35	23.41	25.49
Diazepam	284	26.53	27.38	29.52	29.74	29.20
Fenspiride	260	26.68	27.90	31.33	30.79	30.08
Chloropromazine	318	26.92	27.64	30.10	29.41	29.25
Codeine	341	26.93	27.70	30.51	29.96	29.22
Nordiazepam	270	26.97	28.06	30.48	30.78	29.74
Mesterolone	304	27.35	27.41	28.20	27.35	29.62
Procaine	278	27.70	26.43	31.21	28.31	28.18
Morphine	369	27.85	28.64	30.88	31.22	30.29
Carprofen	287	28.07	28.93	31.46	31.69	30.82
Clanobutin	361	28.26	28.89	31.16	31.31	30.48
Acepromazine	326	28.49	29.32	32.60	31.65	31.31
Indoprofen	295	28.60	32.32	29.39	32.58	31.72
Butorphanol	411	29.24	29.89	32.97	32.35	32.02
Frusemide	344	29.74	30.74	35.84	36.89	33.82
Indomethacin	371	29.75	30.42	33.83	34.20	32.61
Sulindac	370	32.16	33.61	41.78	43.16	40.07
Diphenoxylate	452	35.50	37.63	47.60	48.76	44.77
Buprenorphine	551	35.60	44.61	49.07	44.61	45.20
nonane	128	7.90	7.92	7.72		8.02
decane	142	10.00	9.81	9.45		9.79
undecane	156	11.68	11.53	11.14		11.47
dodecane	170	13.23	13.12	12.74		13.05
tridecane	184	14.66	14.58	14.25		14.56
tetradecane	198	16.00	15.94	15.66		15.96
hexadecane	226	18.45	18.44	18.24	18.84	18.50
octadecane	252	20.65	20.68	20.54	19.89	20.77
nonadecane	268	21.67	21.72	21.61	20.89	21.83
eicosane	282	22.64	22.71	22.63	22.77	22.83
docosane	310	24.46	24.56	24.55	24.50	24.72
tetracosane	338	26.12	26.25	26.29	27.58	26.45
octacosane	394	29.10	29.26	29.42	30.27	29.55
dotriacontane (C32)	450	32.07	32.38	32.81	33.85	33.05
tetracontane (C40)	560	37.35	38.31	39.47	39.07	40.13

Results and Discussion

Probes were analysed under identical conditions on a series of columns selected for their aromaticity (Figure 1). Alkane retention on all phases was similar suggesting that increasing aromatic content does not reduce non-polar retention (See Table) but other analytes show a marked deviation from a simple van der Waals – molecular weight relationship (Figure 2). The aromatic character of many drugs resulted in greater retention with increasing phase polarity. The trend is associated with the extent of steric hindrance around the aromatic centre and forced coplanarity (e.g. lignocaine) versus stable planarity (e.g. sulindac) .

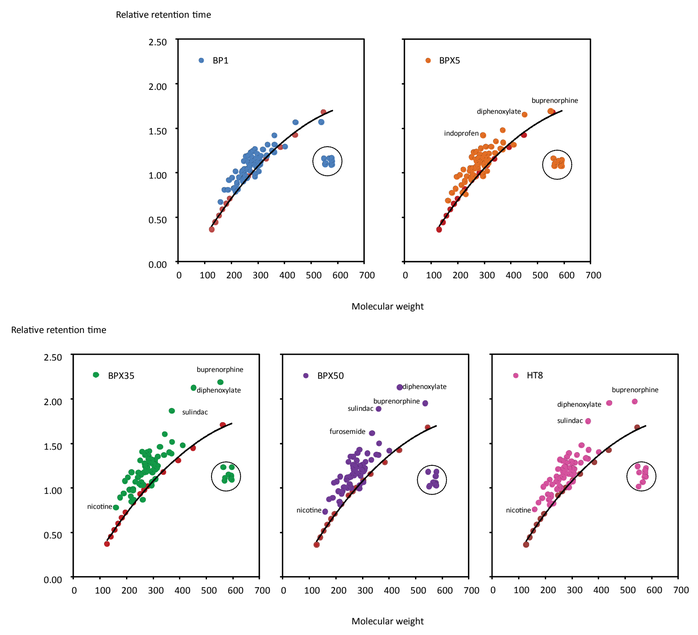

Figure 2. Retention relative to eicosane for all analytes on different columns. Alkane retention is shown as a van der Waals reference. Alkane retention shown by red markers and trend line, PFP-steroid retention circled with dotted line.

The perfluoroacylated steroids are clustered and less well retained on the basis of molecular weight than other analytes and show that reagents larger than methyl and acetoxy affect molecular weight, steric surface and volume and may also mask phase selective mechanisms. For PFPA derivatives, both π- and van der Waals type interactions are reduced by a perfluoro 'barrier-layer'.

A GCxGC space shows that increasing phase aromaticity is associated with deviation from equivalent retention (Figure 3). Orthogonality to BP1 was approached with BPX50 as elution orders were changed for several analyte pairs (Figure 4). HT8 was complimentary to BPX5 with enhanced selectivity observed for some hindered and planar analytes (e.g. diphenoxylate, sulindac and lignocaine). Retention of the aliphatic heptaminol is largely unaffected by polarity.

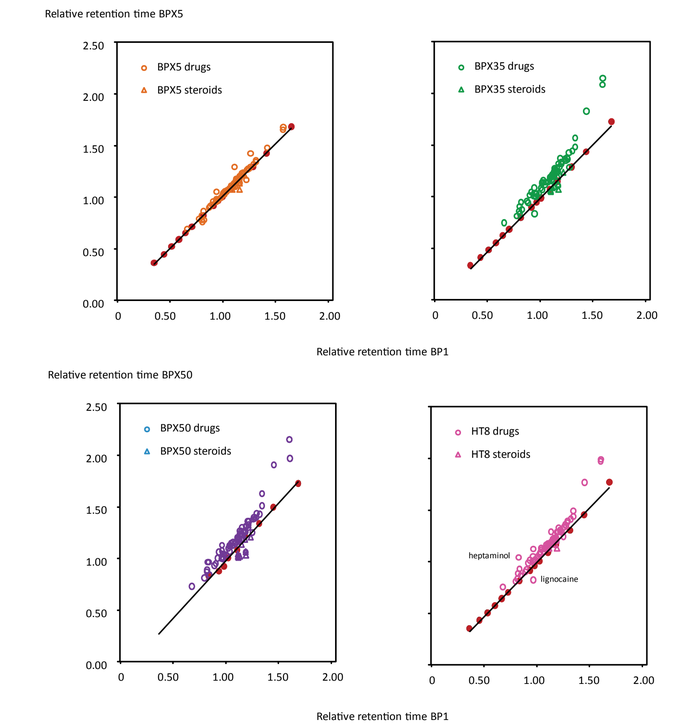

Figure 3. Reconstructed relative retention space for all analytes separated on BP1 and different aromatic phases. Alkane retention shown by red markers and trend line.

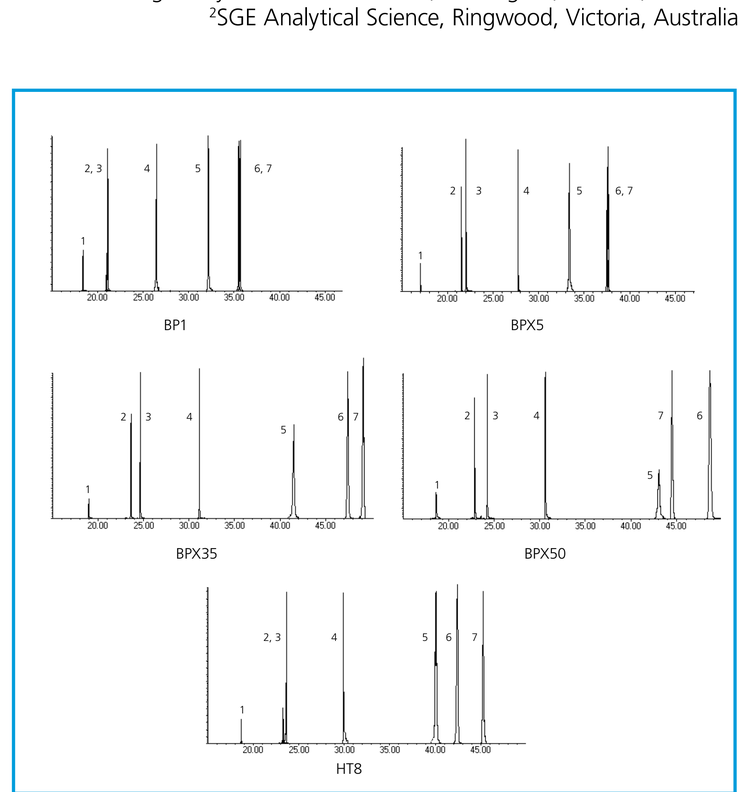

Figure 4. Extracted ion chromatograms showing changes in retention for selected drugs on columns with different polarity. Analytes: Diacetyl heptaminol (1), lignocaine (2), benzocaine (3), fenspiride (4), sulindac (5), diphenoxylate (6) and buprenorphine (7).

Mechanistically, HT8 can interact with aromatics in the presence of ring substituents (e.g. halogens and sulphur substituents) that might hinder their approach to phenyl or silphenylene phases. Purely phenyl phases are restricted and are selective towards terminal phenyl groups (e.g. fenspiride).

Like the carboranes, silphenylene based phases are capable of interacting with planar and mid-mounted aromatics (e.g. promazines, benzodiazepines) but are hindered by steric and free rotational barriers generated by terminal aliphatic groups (c.f. benzocaine and lignocaine). Similarly, the aromaticity of buprenorphine is masked but exposed for diphenoxylate and so the retention of the two compounds on different phases changes with the balance of non-polar, sterically hindered aromatic and planar aromatic moieties. The reversal of retention order for this pair on BPX50 is more correctly observed as greater affinity for diphenoxylate and decreased capacity for non-polar shape adaption in the rigid BPX50 'baskets'.

Conclusion

Increasing the aromatic content of the GC phase alters selectivity and may offer increased separation without increasing run time by spreading peaks across the chromatographic space. Unclustering of analytes on BPX35 and 50 is based on steric assessibility to pendant aromatic rings over core unsaturation. Both BPX35 and HT8 offer selectivity towards many drug classes without significant changes in elution order. Highly aromatic phases such as BPX50 offer a degree of orthogonality to 5 % phenyl equivalent phases and may be useful in multi-dimensional or confirmatory strategies.

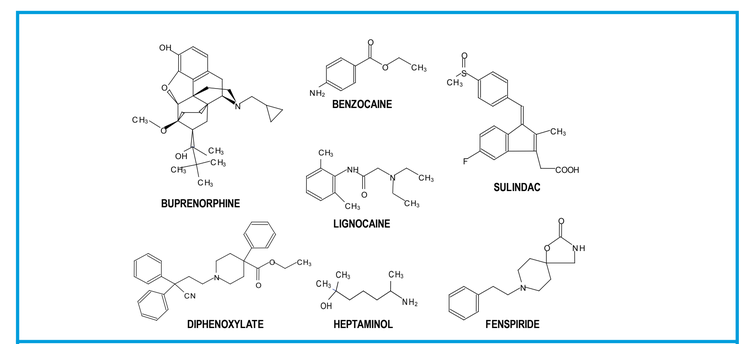

Figure 5. Structures of selected analytes referred to in text.

Experimental

A collection of 75 drugs and steroids and 15 n-alkanes were derivatised (pentafluoropropanoylation of anabolic steroids, acetylation of bases and methylation of acids) and dissolved in ethyl acetate at concentrations of 1 µg/mL. EI-GCMS was performed on a Hewlett Packard 6890 GC - 5975 MSD equipped with a 7683B Autoinjector (Palo Alto, USA). The GC was equipped with either a BP1, BPX5, BPX35, BPX50 or HT8 column (30 m x 0.25 mm x 0.25 µm, SGE, Melbourne, Australia) and used helium as the carrier gas with a constant flow of 1.2 mL/min. The oven temperature was held at 40 °C for 2 minutes then heated at 10 °C/min to 300 °C with a final holding time of 20 minutes. Injections of 2 µL were splitless at 250 °C with a nominal head pressure of 66 kPa. A scan range of 50-550 Da at 2.48 scan/sec was used.

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