nacalai tesque
The quality for certainty.

Research into the Retention Mechanism of SFC

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By comparing retention behavior in normal-phase high-performance liquid chromatography (NP-HPLC) and supercritical fluid chromatography (SFC), as well as evaluating the selectivity of various stationary phases, we attempted to elucidate the chromatographic properties of SFC.

Under NP-HPLC (ethanol/n-hexane) and SFC (ethanol/CO_2) conditions, the retention of hydrophobic, hydrophilic, and ionic compounds was compared. In addition, the hydrophobicity, hydrophilicity, π-π interaction, and dispersion force of 18 different stationary phases were evaluated under SFC conditions.

	(HPLC conditions)	(SFC conditions)
Column:	See figures below	See figures below
Column size:	4.6mmI.D.-250mm	4.6mmI.D.-250mm
Mobile phase:	See figures below	See figures below
Flow rate:	1.0 ml/min	3.0 ml/min
BPR:	--	10 MPa
Temperature:	40 °C	40 °C
Detection:	UV254nm	UV254nm
Equipment:	Prominence (Shimadzu)	Nexera UC (Shimadzu)

HPLC (NP) and SFC Comparison ①

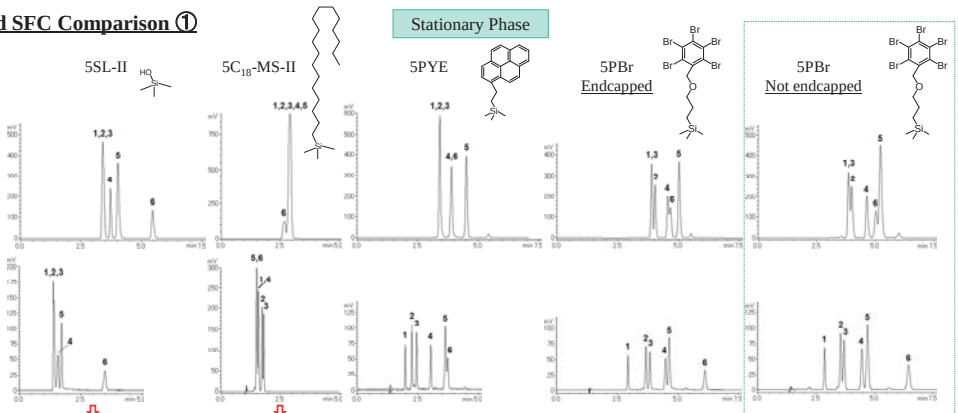
Stationary Phase

5SL-II | $5C_{18}$-MS-II | 5PYE | 5PBr Endcapped | 5PBr Not endcapped

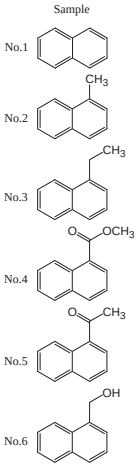
Mobile phase

(HPLC) Ethanol/ Hexane = 5/95 1ml/min

(SFC) Ethanol/ CO_2 = 5/95 3ml/min



Sample



3 Types

Same selectivity

Different selectivity Low retention

Different selectivity High retention

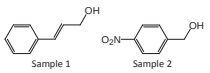
Endcapping has almost no effect on selectivity

HPLC(NP) and SFC Comparison ②-1

Mobile phase

(HPLC) Ethanol/ n-Hexane = 10/90

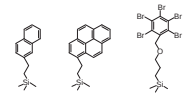
(SFC) Ethanol/ CO_2 = 10/90



Sample 1 Sample 2

Table 1: Retention Comparison

Column name	Stationary phase	k' (HPLC) Sample 1	k' (HPLC) Sample 2	k' (SFC) Sample 1	k' (SFC) Sample 2	SFC/HPLC Sample 1	SFC/HPLC Sample 2
COSMOSIL 5C18-MS-II	Octadecyl Group	0.00	0.00	0.07	0.01	**	**
COSMOSIL 5Cholester	Cholesteryl Group	0.00	0.08	0.15	0.20	**	**
COSMOSIL 5SL-II	none	0.65	1.34	0.77	1.51	118%	113%
COSMOSIL 5HILIC	Triazole	1.14	2.69	1.30	2.32	115%	86%
COSMOSIL 5HP	Hydroxyphenyl group	0.86	1.67	1.10	1.63	129%	97%
COSMOSIL 5PY	Pyridinyl group	0.82	2.26	0.92	2.07	112%	91%
2-Ethylpyridine (Not for sale)	Pyridinyl group	0.65	1.62	0.73	1.47	112%	90%
COSMOSIL 5 π NAP	Naphtyl Group	0.08	0.21	0.37	0.56	472%	261%
COSMOSIL 5PYE	Pyrenyl Group	0.13	0.51	0.67	1.46	531%	287%
COSMOSIL 5PBr	Pentabromophenyl group	0.09	0.26	0.75	1.04	815%	400%
						HPLC k' = 100%	



5πNAP 5PYE 5PBr

In SFC, in addition to the retention properties of normal phase HPLC, secondary interactions, such as π-π interaction and dispersion force, are stronger compared to HPLC.
→ SFC has unique separation potential

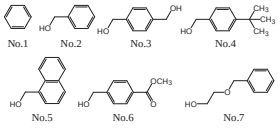
Stationary Phase Selectivity Evaluation

Table 4: Selectivity Evaluation

Column name	Stationary phase	(1) Retention 1	(2) Retention 2	(3) Hydrophilicity	(4) Hydrophobicity	(5) π-π interaction	(6) Polar group	(7) Dispersion force
COSMOSIL 5SL-II	none	0.00	1.06	9.83	1.09	1.74	2.26	1.17
COSMOSIL 5Diol-120-II	Diol Group	0.00	1.50	10.56	0.76	2.98	1.84	0.60
COSMOSIL 5PY	Pyridinyl group	0.00	1.10	7.70	0.96	2.58	2.35	0.63
COSMOSIL 5HP	Hydroxyphenyl group	0.00	1.31	10.33	1.07	2.28	2.06	0.90
COSMOSIL 5HILIC	Triazole	0.00	1.76	13.22	0.94	2.44	1.95	0.74
COSMOSIL 5PE-MS	Phenyl group	0.02	0.19	2.81	1.36	2.51	1.70	1.21
COSMOSIL 5 π NAP	Naphtyl Group	0.00	0.31	2.79	1.46	3.10	2.10	1.34
COSMOSIL 5PYE	Pyrenyl Group	0.00	0.52	3.54	1.53	3.66	2.59	1.45
COSMOSIL 5PBr	Pentabromophenyl group	0.04	0.71	3.34	1.45	5.19	2.34	1.29
COSMOSIL 5NPE	Nitrophenylethyl Group	0.00	0.50	4.70	1.23	2.54	2.22	1.23
COSMOSIL 5PFP	Pentafluorophenyl group	0.04	0.15	3.01	1.19	2.73	1.74	1.29
COSMOSIL 5Cholester	Cholesteryl Group	0.14	0.39	2.36	1.37	3.71	1.64	0.79
COSMOSIL 5C18-MS-II	Octadecyl Group (monomeric)	0.17	0.17	0.98	1.51	2.76	1.05	1.04
COSMOSIL 5C18-AR-II	Octadecyl Group (polymeric)	0.16	0.23	1.09	1.26	2.87	1.05	0.93
2-Ethylpyridine (Not for sale)	Pyridinyl group	0.00	0.91	6.08	1.02	2.30	2.15	0.57
Picolylamine (Not for sale)	2-Picolylamine	0.00	1.80	11.82	0.89	3.00	2.07	0.65
Aminoanthracene (Not for sale)	1-Aminoanthracene	0.00	1.24	9.81	0.92	2.57	2.03	0.76
DEA (Not for sale)	Diethylamine	0.00	2.06	13.65	0.76	2.88	1.76	0.44

Mobile phase: Ethanol/ CO_2 = 5/95

(1) Retention 1: Retention factor (k') of No.1
(2) Retention 2: k' of No.2
(3) Hydrophilicity: Separation factor (α) of No.3/No.2
(4) Hydrophobicity: α of No.4/No.2
(5) π-π interaction: α of No.5/No.2
(6) Polar group: α of No.6/No.2
(7) Dispersion force: α of No.7/No.2



No.1 No.2 No.3 No.4

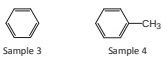
No.5 No.6 No.7

HPLC(NP) and SFC Comparison ②-2

Mobile phase

(HPLC) Ethanol/ n-Hexane = 1/99

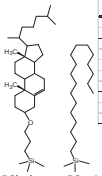
(SFC) Ethanol/ CO_2 = 1/99



Sample 3 Sample 4

Table 2: Comparison of Retention for Non-Polar Compounds

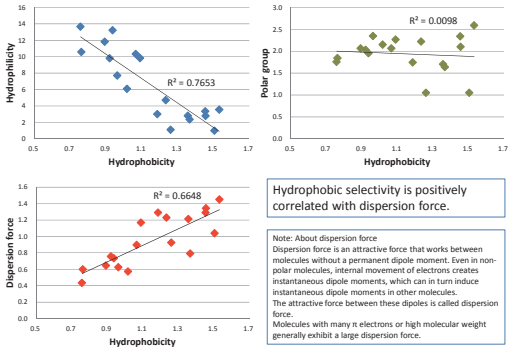
Column name	Stationary phase	k' (HPLC) Sample 3	k' (HPLC) Sample 4	k' (SFC) Sample 3	k' (SFC) Sample 4
COSMOSIL 5C18-MS-II	Octadecyl Group	0.02	0.01	0.17	0.22
COSMOSIL 5Cholester	Cholesteryl Group	0.02	0.01	0.11	0.17
COSMOSIL 5SL-II	none	0.02	0.00	0.00	0.01
COSMOSIL 5HILIC	Triazole	0.03	0.01	0.00	0.01
COSMOSIL 5HP	Hydroxyphenyl group	0.02	0.01	0.00	0.02
COSMOSIL 5PY	Pyridinyl group	0.02	0.01	0.00	0.02
2-Ethylpyridine (Not for sale)	Pyridinyl group	0.02	0.01	0.00	0.02
COSMOSIL 5 π NAP	Naphtyl Group	0.00	0.00	0.03	0.07
COSMOSIL 5PYE	Pyrenyl Group	0.01	0.00	0.03	0.07
COSMOSIL 5PBr	Pentabromophenyl group	0.01	0.01	0.12	0.23



5Cholester $5C_{18}$-MS-II

Low-polarity samples were not retained in HPLC (NP). However, slight retention was achieved in SFC with low-polarity stationary phases.

Hydrophobic Selectivity



Hydrophobic selectivity is positively correlated with dispersion force.

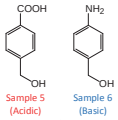
Note: About dispersion force
Dispersion force is an attractive force that works between molecules without a permanent dipole moment. Even in non-polar molecules, internal movement of electrons creates instantaneous dipole moments, which can in turn induce instantaneous dipole moments in other molecules.
The attractive force between these dipoles is called dispersion force.
Molecules with many π electrons or high molecular weight generally exhibit a large dispersion force.

HPLC(NP) and SFC Comparison ②-3

Mobile phase

(HPLC) 0.5%Formic Acid-Ethanol/ n-Hexane = 15/85

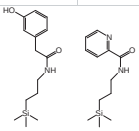
(SFC) 0.5%Formic Acid-Ethanol/ CO_2 = 15/85



Sample 5 (Acidic) Sample 6 (Basic)

Table 3: Comparison of Retention for Dissociative Compounds

Column name	Stationary phase	k' (HPLC) Sample 5	k' (HPLC) Sample 6	k' (SFC) Sample 5	k' (SFC) Sample 6	SFC/HPLC Sample 5	SFC/HPLC Sample 6
COSMOSIL 5SL-II	none	0.66	1.24	0.81	1.32	123%	106%
COSMOSIL 5HP	Hydroxyphenyl group	2.19	3.31	2.24	3.27	102%	99%
COSMOSIL 5PY	Pyridinyl group	3.06	2.28	2.83	1.50	93%	66%
COSMOSIL 5HILIC	Triazole	8.46	5.09	6.06	4.98	72%	98%
						HPLC k' = 100%	



5HP (Acidic) 5PY (Basic)

Both HPLC (NP) and SFC displayed comparatively high retention for acidic compounds with basic stationary phases and basic compounds with acidic stationary phases

Results and discussion

For hydrophilic and ionic compounds, silica (SL) showed similar retention in NP-HPLC and SFC, while the pyrenylethyl (PYE) and pentabromobenzyl (PBr) phases retained the compounds several times more strongly in SFC compared to NP-HPLC. On the other hand, while hydrophobic compounds were not retained by any of the stationary phases in NP-HPLC, C_{18}, PYE, and PBr retained them slightly in SFC. The hydrophobicity parameters, obtained from evaluating the selectivity of the stationary phases, were, unsurprisingly, negatively correlated with hydrophilicity. On the other hand, dispersion force was positively correlated with hydrophobicity, suggesting that dispersion force is involved in the retention of hydrophobic compounds.

From the above, we can surmise that retention in SFC is caused by secondary interactions, such as dispersion force and π - π interaction, working more strongly than in HPLC, in addition to the interactions at work in NP-HPLC.