

HPLC Column Classification

Introduction

1978 USP XIX Fourth supplement : L1 designation for C18 column

1980 USP XX : 7 columns were classified

-Classified HPLC column to 56 descriptions

-More than 220 columns currently available in the worldwide market can be classified as L1

-How to select column for a particular application ?

USP Approach

- Use NIST Standard Reference Material (SRM) 870 to evaluate C18 column
- SRM 870 : the mixture of 5 organic compounds in methanol (uracil, toluene, ethylbenzene, quinizarin and amitriptyline)
- The components in SRM 870 are provide a board characterization of column performance in a single, simple test

Parameters used in the characterization of the columns

1. Hydrophobicity (capacity factor of ethylbenzene)
2. Chelation (tailing factor of quinizarin)
3. Activity toward bases (silanol activity, capacity factor factor and tailing factor of amitriptyline)
4. Shape selectivity (bonding density)

Chromatographic condition

Mobile phase : 80% methanol and 20% phosphate buffer pH 7.0

Column temperature : $23^{\circ}\text{C} \pm 1^{\circ}\text{C}$

Chromatographic properties

- Peak width (efficiency: theoretical plates)
- Peak symmetry (A_s)
- Absolute retention (k')
- Selectivity factor (relative retention (k'_1 / k'_2))

Uracil

- Indicator of the void volume (unretained volume)
- To calculate the retention factor

Toluene/Ethylbenzene

- The selective factor $\alpha_{E/T} = k'_{\text{ethylbenzene}} / k'_{\text{toluene}}$: Characterize differences among C8 and C18 column
- Marker for calculation of column efficiency (theoretical plates, N)

Quinizarin

- Metal chelating agent
- Indicative of the present or absence of metals in HPLC system
- Low activity toward chelating reagent → symmetric peak shape
- High activity toward chelating reagent → tailing, asymmetric peak shape
- Embedded polar functional groups column, quinizarin elute last with good peak symmetry

Amitriptyline

-Basic cpd. (pKa 9.4)

-High silanol activity → elution of organic bases with severe peak tailing

Development of SRM870 with 41 commercial C18 columns

- No two columns exhibit identical retention behavior, similarities do exist among several columns
- k' of ethylbenzene : 0.2-2.8
- $\alpha_{E/T}$: 1.26-1.45
- Retention of quinizarin, k' : 1- 23.6 (peak asymmetry : 1.1-5.7)
- Retention of amitriptyline, k' : 1.4-72.9 (peak asymmetry : 1.0-11)

PQRI initiative

The Snyder/Dolan column test

-A series of standard mixtures

mixture 1 : thiourea, amitriptyline, 4-butylbenzoic acid

mixture 1a : N,N-diethylacetamide, 5-phenyl-1-pentanol,
ethylbenzene

mixture 2 : N,N-dimethylacetamide, 5,5-diphenylhydantoin,
toluene

mixture 2a : nortriptyline, acetophenone, mefenamic acid

mixture 3 : *p*-nitrophenol, anisole, 4-hexylaniline

mixture 3a : *cis/trans* chalcone, benzonitrile

mixture 4 : berberine

Chromatographic condition

Mobile phase : 50%acetonitrile/buffer: pH 2.8 and 7.0

Column temperature : 35°C

Column selectivity parameters

- relative retention (k_{EB})
- hydrophobicity (H)
- steric interaction (S^*)
- hydrogen-bond acidity (A)
- hydrogen-bond basicity (B)
- relative silanol ionization or cation-exchange capacity (C)

Column hydrophobicity (H)

- increase in total carbon \longrightarrow H increase

- Compression of the ends of the alkyl chains in small-pore packing



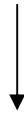
H increase

- Minor effect on column selectivity

Column steric interaction (S^*)

-Crowded bonded phase \longrightarrow S^* increase

-Increased chain length / concentration of bonded phase



S^* increase

- Compression of the ends of the alkyl chains in small-pore packing



S^* increase

-Significant effect on column selectivity, especially for molecules of different shape

Column hydrogen-bond acidity (A)

-non-ionized silanols increases with column acidity

-More acidic column, greater values of A

-For end-capped column, the number of accessible and unreacted silanols decrease



A decrease

-non-ionized basic molecules (amines, amides, aliphatic derivatives)

have significant A on column selectivity

Column hydrogen-bond basicity (B)

- Various function gr. Within the bonded phase \longrightarrow B increase
- Columns with larger values of B preferentially retain acidic cpd.

Silanol ionization (C)

-Results in a negative charge on column, attract ionized bases and repel ionized acids

-pH of mobile phase increase \longrightarrow C increase

-end-capping, decrease access to ionized silanols



large decrease in C

Equivalent columns

= similar values of the six column selectivity parameters

Column comparison function, F_s

$$F_s = \{[12.5(H_2-H_1)]^2 + [100(S_2^*-S_1^*)]^2 + [30(A_2-A_1)]^2 + [143(B_2-B_1)]^2 + [83(C_2-C_1)]^2\}^{1/2}$$

$F_s < 3$ = equivalent selectivity and band spacing

$F_s > 3$ = equivalent separation may still be achieved but less certain

- No acids or bases in sample, ignore term C_2-C_1



a much smaller value of F_s

- No carboxylic acid in sample, ignore term B_2-B_1



reduce in value of F_s

Conclusion

USP approach

- Provides column performance characterization (theoretical plate count, good peak symmetry, etc.)
- produces five data points to describe the column

PQRI approach

- provides selectivity characterization (relative retention times)
- produces a list of suitable columns ordered by the F_s factor