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Note

Shelf-life of unused high-performance liquid chromatographic columns

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Manufacturers of prepacked high-performance liquid chromatographic (HPLC) columns claim that columns are prepared on a large scale with stringent controls that ensure uniformity and reproducible selectivity. They emphasize that all columns have a finite lifetime before their efficiency and selectivity diminish to unacceptable levels. When one considers lifetime, two aspects have to be considered: first the shlef-life of a column that is stored for later use and second the lifetime of a column in use.

Precautions are taken to protect a column during its use *e.g.*, the mobile phase must have a pH range compatible with the column and the mobile phase and sample solution must be free from particulate matter.

Overseas users often need to order more than one column at a time for economy and to safeguard the continuity of the work, as there is considerable batch-to-batch variation, as can be seen from the characteristics of two silica (10 μm) columns from one manufacturer given in Table I.

In order to establish the effect of column storage, we examined five different types of columns (A–E), all of 25 cm × 4.6 mm I.D. The stationary phase, mobile phase and flow-rate (according to the manufacturer's certificate), the non-retained solute employed for the determination of t_0 and the value of t_0 were as follows: (A) silica, 10 μm; heptane; 0.67 ml/min; hexane; 4.28 min; (B) octadecylsilane, 10 μm; methanol–water (7:3); 1 ml/min; KNO_3; 2.52 min; (C) strong anion exchanger, 10 μm; 0.05 *M* phosphate, pH 3.35; 0.67 ml/min; 0.01 *M* KH_2PO_4, 4.67 min; (D) strong cation exchanger, 10 μm; 0.02 *M* phosphate, pH 3.5; 0.67 ml/min; KNO_3; 3.78 min;

TABLE I

CHARACTERISTICS OF TWO COLUMNS SUPPLIED BY ONE MANUFACTURER

t_0 = Retention time of unretained sample; t_R = retention time; N = plates per column.

Column	t_0 *(min)*	*Carbon tetrachloride*		*Benzene*		*Naphthalene*	
		t_R *(min)*	N	t_R *(min)*	N	t_R *(min)*	N
1	4.25	4.68	6160	6.00	7520	7.32	7300
2	4.69	5.72	10 670	7.65	10 890	10.20	10 840

TABLE II

COMPARISON OF COLUMN CHARACTERISTICS REPORTED IN THE CERTIFICATE AND THOSE DETERMINED IN OUR LABORATORY

The dates and figures given in parentheses pertain to our work.

Column	*Date of testing*	*Test mixture**	*Column characteristics*			
			t_R *(min)*	*N*	*k'*	*R*
A	29.8.79	CCl_4	4.68 (4.97)	6160 (3109)	0.10 (0.14)	5.11 (3.76)
	(11.1.85)	Benzene	6.00 (6.32)	7520 (4579)	0.41 (0.45)	4.20 (4.93)
		Naphthalene	7.32 (5.30)	7300 (5646)	0.72 (0.91)	
B	11.2.80	Benzene	4.24 (4.00)	5990 (1883)	0.56 (0.59)	5.45 (2.55)
	(20.12.84)	Naphthalene	5.83 (5.00)	5110 (2443)	1.14 (0.97)	
		Biphenyl	7.17 (5.80)	4710 (2430)	1.64 (1.25)	3.41 (1.67)
C	4.6.80	CMP	5.82 (5.78)	3730 (2738)	0.39 (0.24)	2.96 (2.48)
	(14.6.84)	AMP	7.19 (6.95)	3170 (2898)	0.72 (0.48)	3.28 (2.14)
		UMP	8.97 (8.06)	4410 (3064)	1.14 (0.73)	6.68 (5.47)
		GMP	13.68 (12.44)	4410 (3660)	2.27 (1.66)	
D	1.2.79	Uracil	5.84 (5.29)	6800 (1843)	0.36 (0.40)	4.35 (4.10)
	(12.6.84)	Guanine	7.60 (8.12)	4630 (2283)	0.77 (1.15)	3.52 (5.20)
		Cytosine	9.54 (13.22)	4640 (2878)	1.22 (2.50)	
E	15.7.82	Benzyl	– (3.80)	13 799 (2885)	1.02 (0.64)	–
	(19.12.84)	alcohol				

* CMP = cytidine-, AMP = adenosine-, UMP = uridine- and GMP = guanosine-5'-monophosphate (disodium salt).

and (E) trimethylsilane, 6 μm; methanol–water (1:1); 1.3 ml/min; KNO_3; 2.32 min.

Using test mixtures similar to those mentioned in the manufacturer's certificate, the following column characteristics were determined[1]: retention time (t_R); number of theoretical plates (N) per unit column length; capacity factor (k'); and resolution (R). The results in Table II show that stored, unused columns (silica, reversed-phase, ion-exchange) undergo considerable decrease in efficiency and selectivity.

Manufacturers of HPLC columns should, therefore, advise customers about the shelflife of their columns and supply them only from stocks of recent manufacture.

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