

COLUMN COMPARISONS

1a. Engelhardt test, (E)

i. **Eluent:** methanol/water 49:51 (w/w) or 55:45 (v.v). Temperature 40°C.

Test compounds: uracil (t_0), aniline, phenol, N,N-dimethyl-aniline, p-ethylaniline, toluene and ethylbenzene.

Output: Hydrophobicity = $k_{ethylbenzene} / k_{toluene}$

Silanol activity = asymmetry of p-ethylaniline at 5% of peak height
(k = retention factor)

ii. **Eluent:** methanol/water, 75:25 (w/w) or 79:21 (v/v). Temperature 40°C

Test compounds: uracil (t_0), triphenylene and o-terphenyl.

Output: Shape selectivity = $k_{triphenylene} / k_{o-terphenyl}$

1b. Modified Engelhardt test, (E_m)

Eluent: methanol/aqueous 0.02M phosphate buffer, pH = 7.0, 49:51 (v/v) or 55:45 (w/w).
Temperature: 40°C

Output: Silanol activity = asymmetry of p-ethylaniline at 5% of peak height.

2. Walters test

Hydrophobicity test

Eluent: acetonitrile/water 65:35 (v/v). Temperature 40°C.

Test compounds: uracil (t_0), benzene, and anthracene.

Silanol activity test

Eluent: acetonitrile. Temperature 40°C.

Test compounds: N,N-diethyl-m-toluamide (DETA) and anthracene.

Output: Hydrophobicity = $k_{anthracene} / k_{benzene}$; Silanol activity = $k_{N,N-diethyltoluamide} / k_{anthracene}$

3. Tanaka test, (T)

Eluent 1: methanol/water: 80:20 (v/v)

Eluent 2: methanol/water: 30:70 (v/v)

Eluent 3: methanol/aqueous 0.02M phosphate buffer pH = 7.6, 30:70 (v/v)

Eluent 4: methanol/aqueous 0.02M phosphate buffer pH = 2.7, 20:70 (v/v)

Temperature 40°C.

Test compounds: uracil (t_0), thiourea (t_0), amylbenzene, butylbenzene, triphenylene, o-terphenyl, caffeine, phenol and benzylamine.

Output: Hydrophobicity = $k_{amylbenzene} / k_{butylbenzene}$, (Eluent 1).

Amount of alkyl chains = $k_{amylbenzene}$, (Eluent 1).

Steric selectivity = $k_{triphenylene} / k_{o-terphenyl}$, (Eluent 1).

Hydrogen bonding capacity = $k_{caffeine} / k_{phenol}$, = $\alpha_{c,p}$ (Eluent 2).

Ion exchange capacity (IEC) at pH>7 = $k_{benzylamine} / k_{phenol}$, = $\alpha_{a,p}$ (Eluent 3).

IEC at pH<3 = $k_{benzylamine} / k_{phenol}$, = $\alpha_{a,p}$ (Eluent 4).

4. Galushko test, (G)

Eluent: methanol/water 60:40 (v/v). Temperature 30°C.

Test compounds: uracil (t_0), aniline, phenol, benzene, toluene.

Output:

Hydrophobicity = $(k_{toluene} + k_{benzene}) / 2$

Hydrophobic selectivity: calculated from the phenol, toluene and benzene retention data.

Silanol activity = $1 + 3 [(k_{aniline} / k_{phenol}) - 1]$

Size selectivity: calculated from the retention data of benzene, phenol and toluene.

5. Column hydrophobicity by log k_w -measurements

Eluents: methanol/water mixtures in the range of 20% to 95% methanol (v/v). Temperature: 40°C.

Test compound: hexylbenzene.

By linear extrapolation of the k -values measured at several modifier concentrations on the different columns, the log retention of the test compound in pure water (log k_w) can be obtained