APPLICATION OF SUBAMBIENT AND ELEVATED TEMPERATURES FOR TLC SEPARATION AND DETECTION PROTOCOLS

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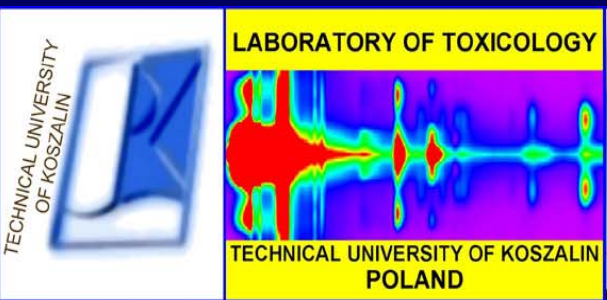
Berlin 2006

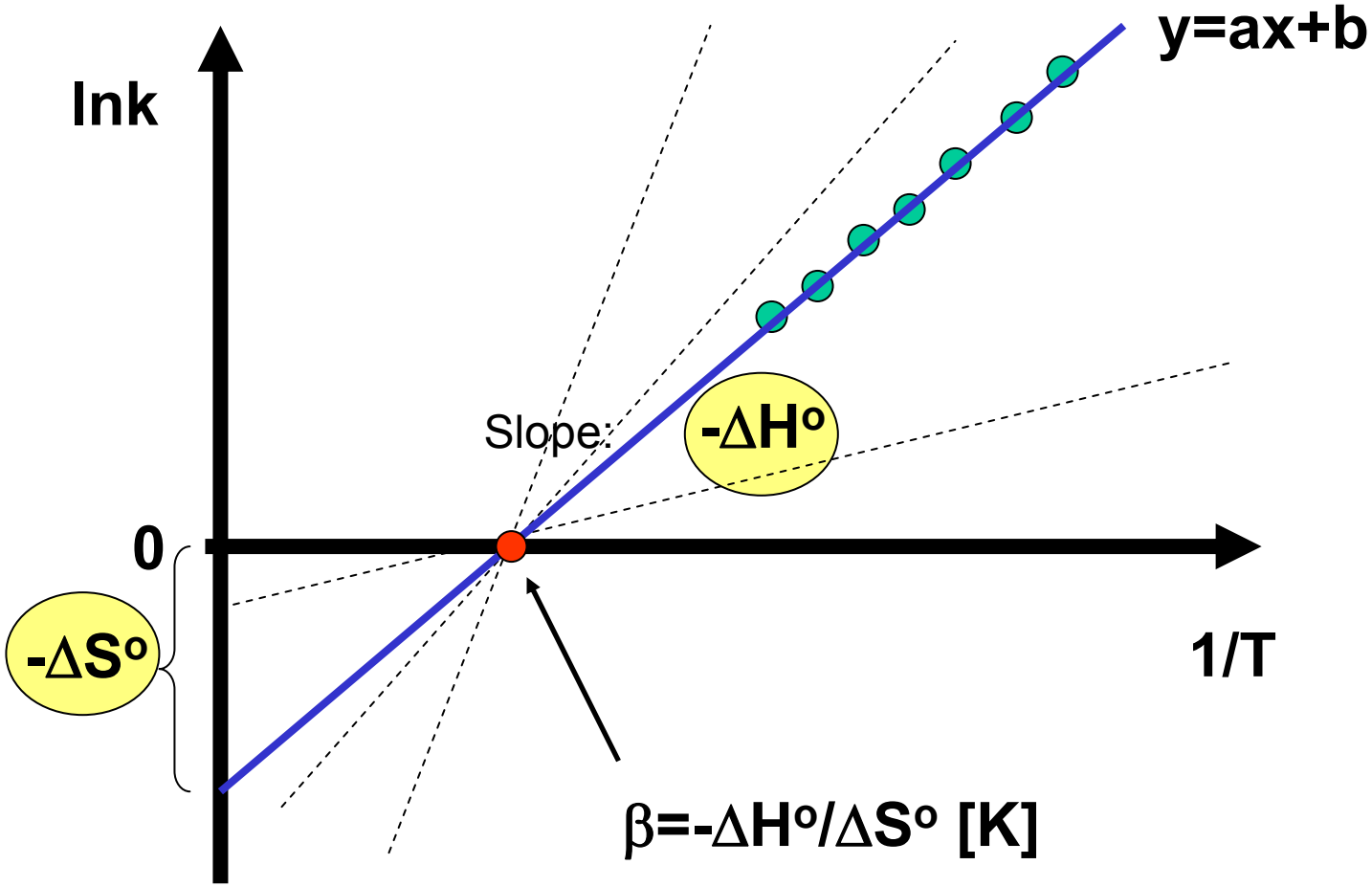


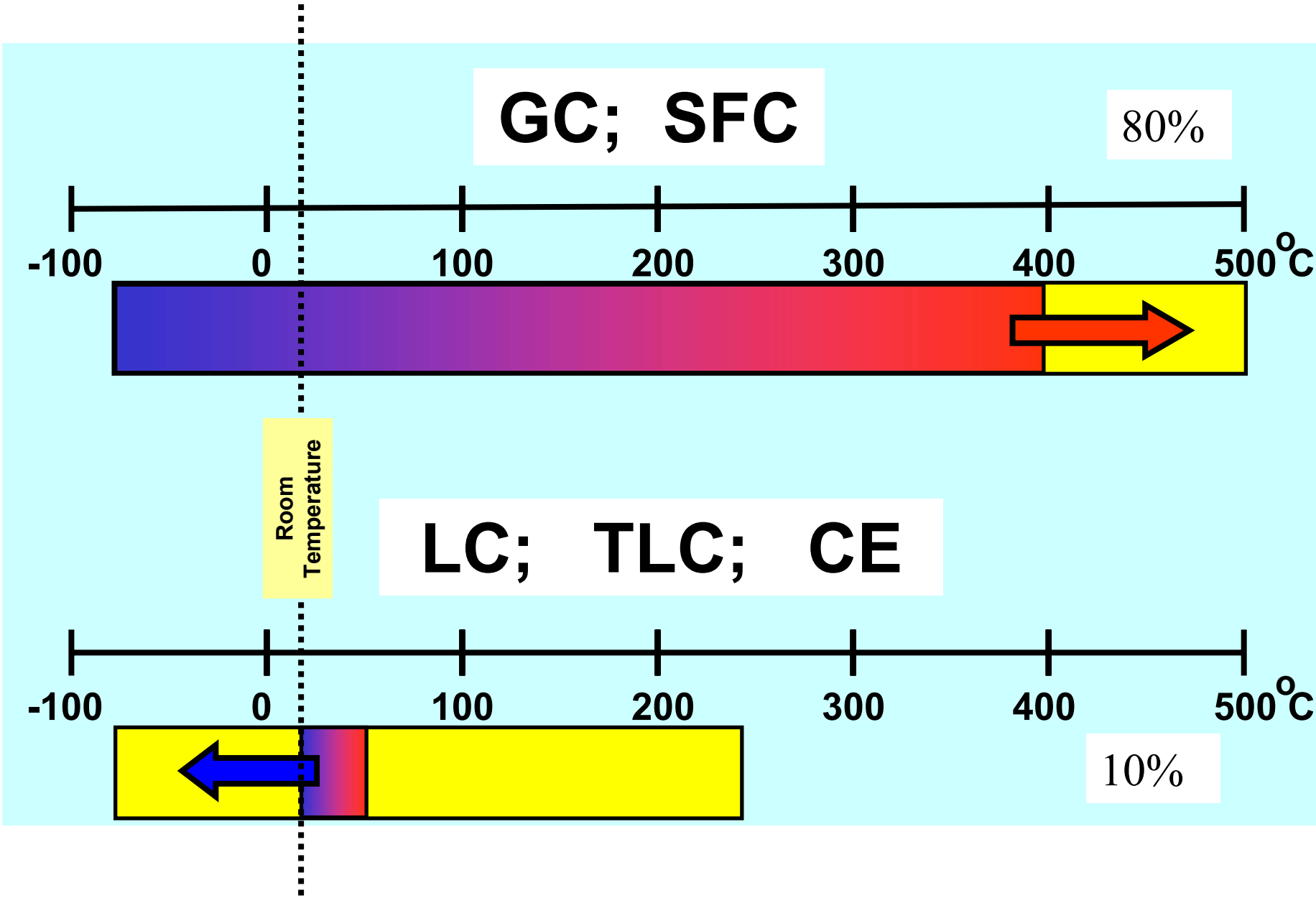
1. BASIC THEORY

2. EQUIPMENT

3. APPLICATIONS

$$\ln k = -\frac{\Delta H^o}{RT} + \frac{\Delta S^o}{R}$$




GC; SFC
80%
-100
0
100
200
300
400
500°C
Room Temperature
LC; TLC; CE
-100
0
100
200
300
400
500°C
10%

Giddings' equation

$$H = (C_k + C_{m2})u + 1/(1/A + 1/\Sigma C_{m1}u)$$

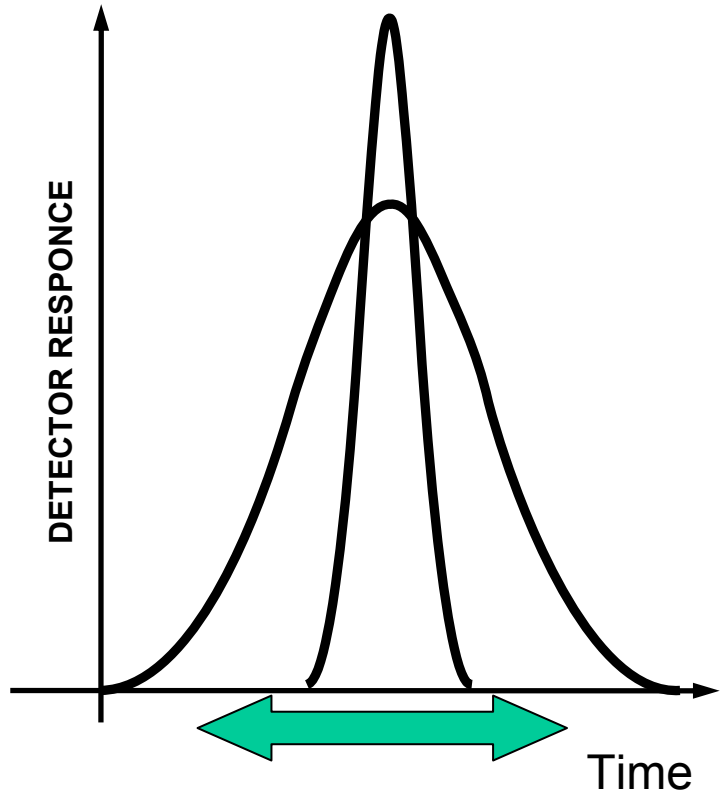
u linear velocity of the mobile phase

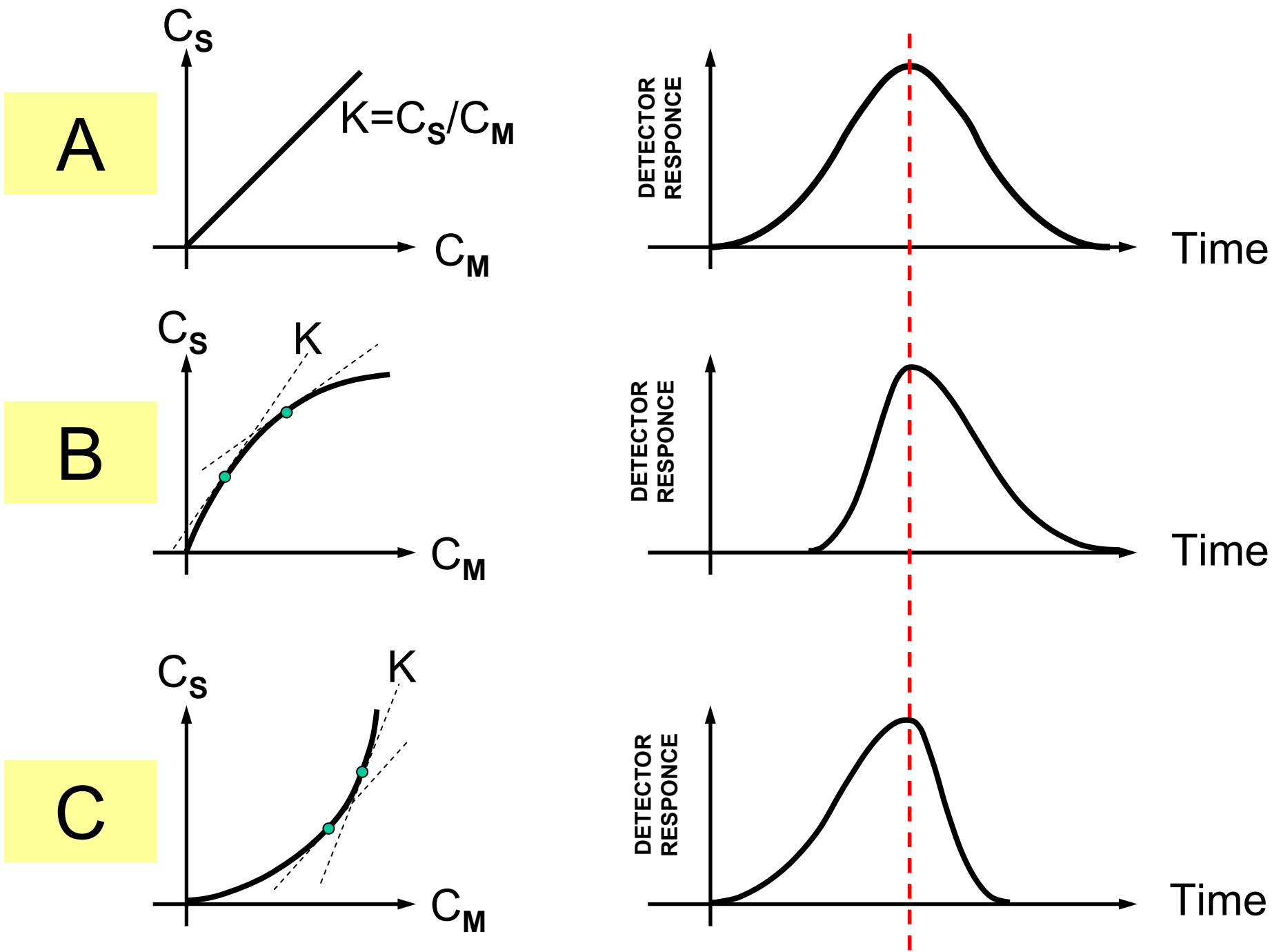
A eddy diffusion

C_k mass transfer resistance resulting from sorption-desorption kinetics

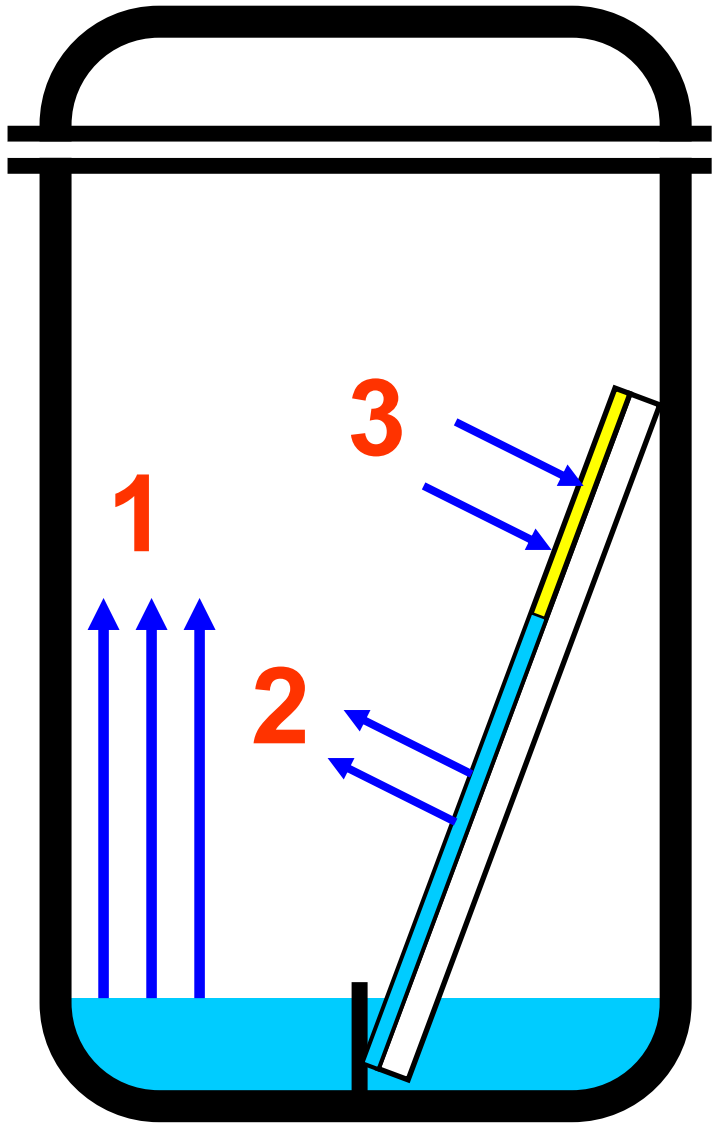
C_{m1} mass transfer resistance in the mobile phase

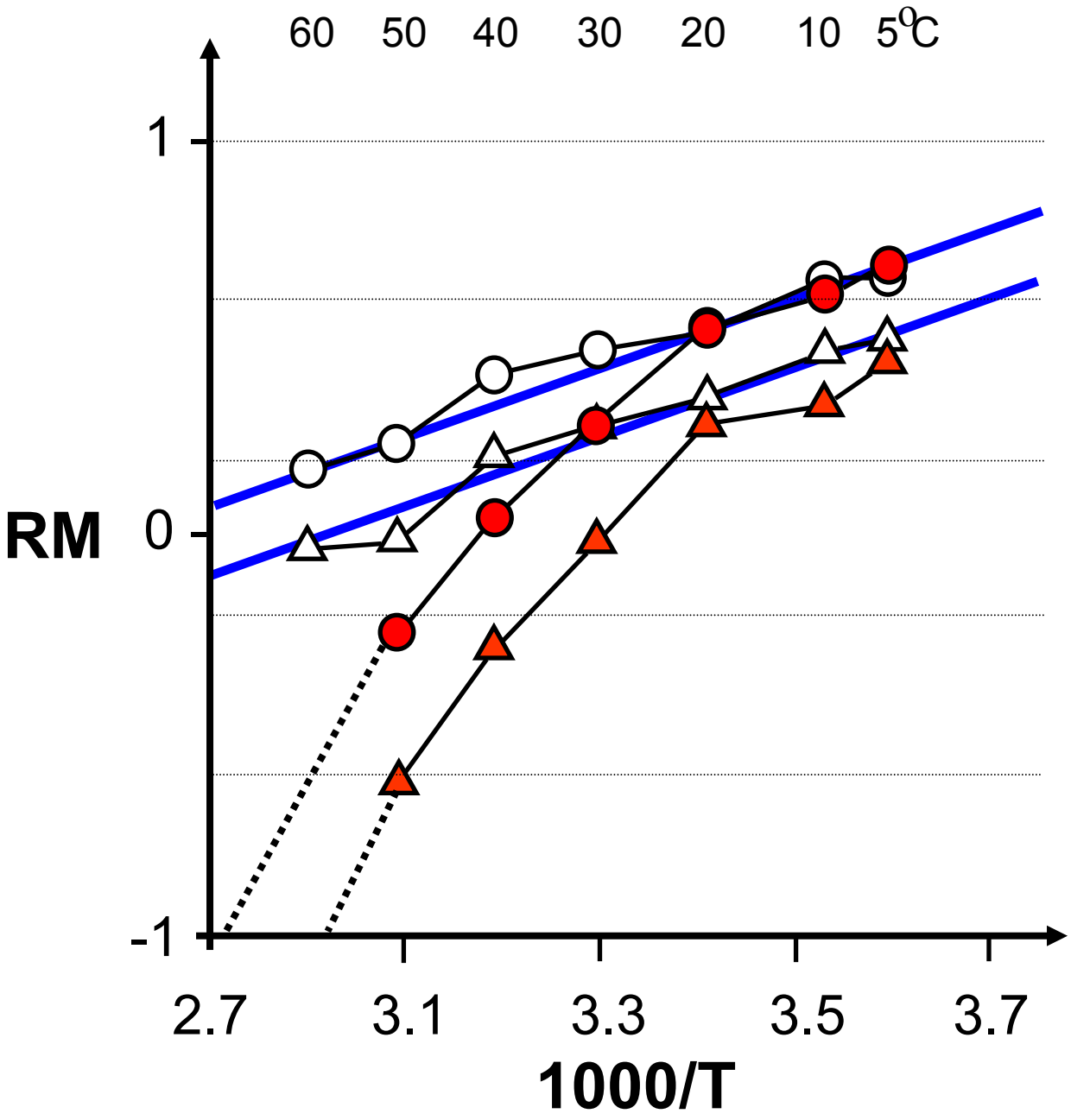
C_{m2} mass transfer resistance in the mobile phase deposited in the particles of the packing material

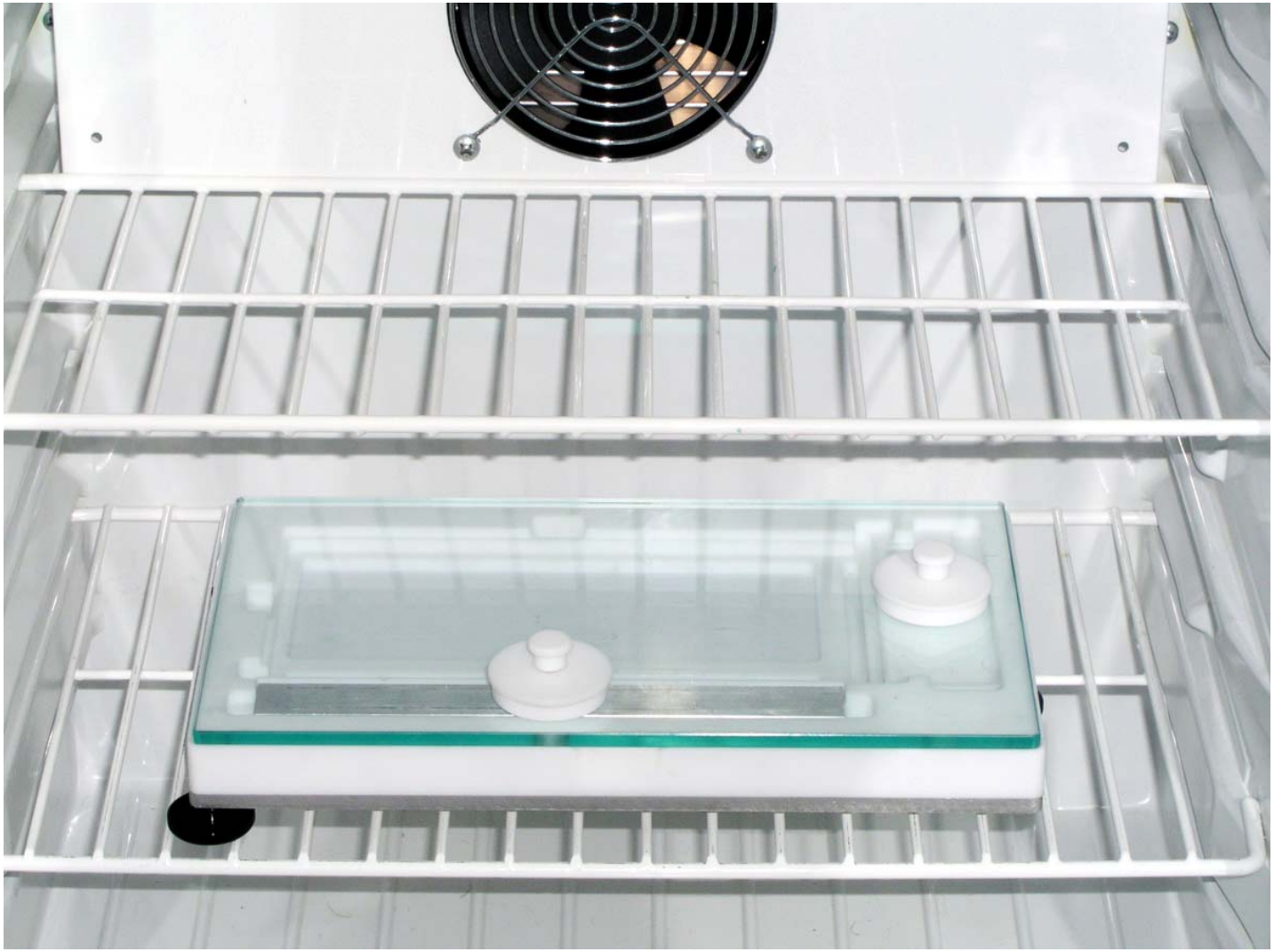


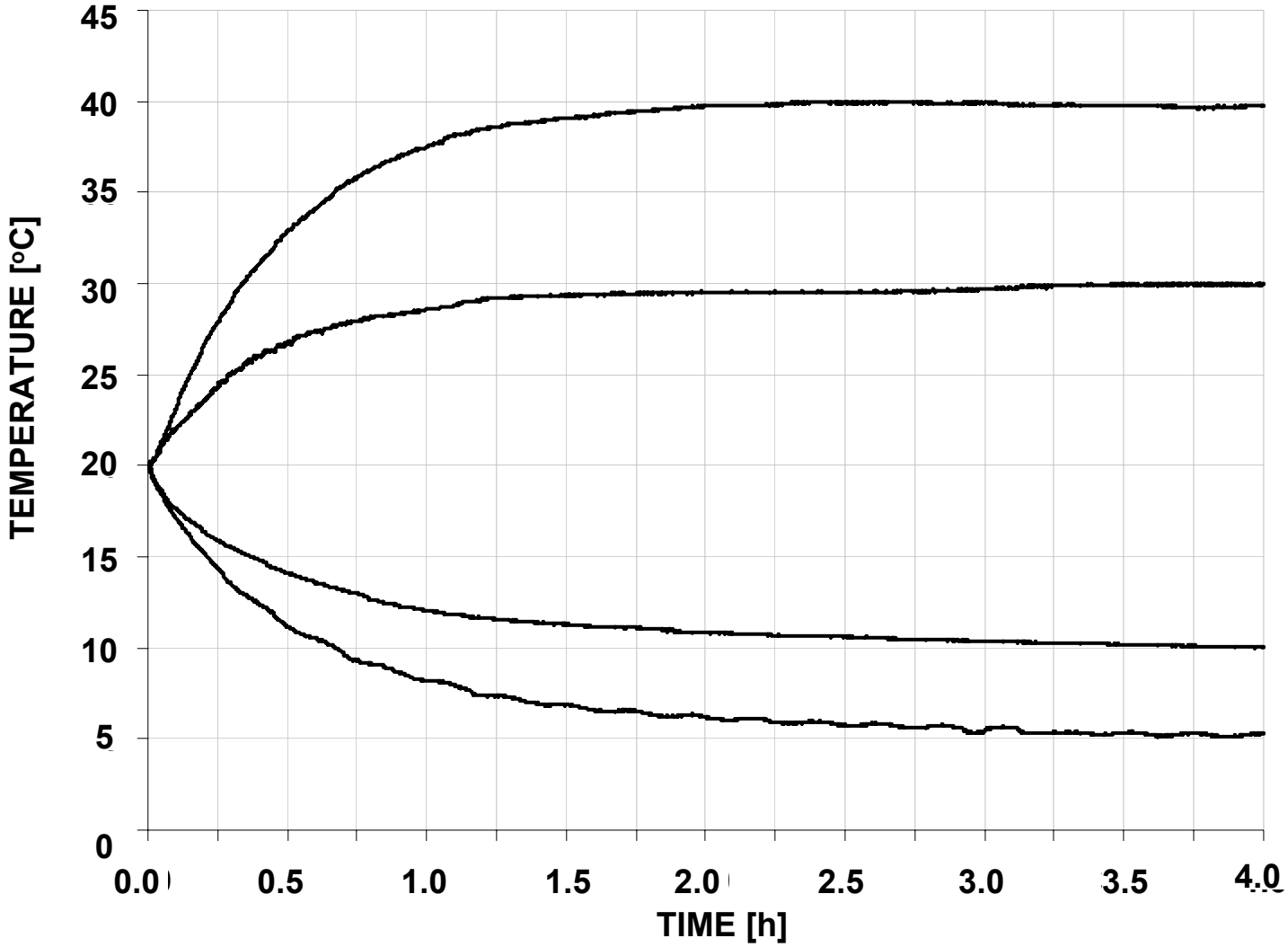

A
C_S
$K=C_S/C_M$
C_M
DETECTOR RESPONCE
Time
B
C_S
K
C_M
DETECTOR RESPONCE
Time
C
C_S
K
C_M
DETECTOR RESPONCE
Time


BECKMAN
BECKMAN


3
1
2


60
50
40
30
20
10
5°C
1
0
-1
RM
2.7
3.1
3.3
3.5
3.7
1000/T




45
40
35
30
25
20
15
10
5
0
TEMPERATURE [°C]
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
TIME [h]

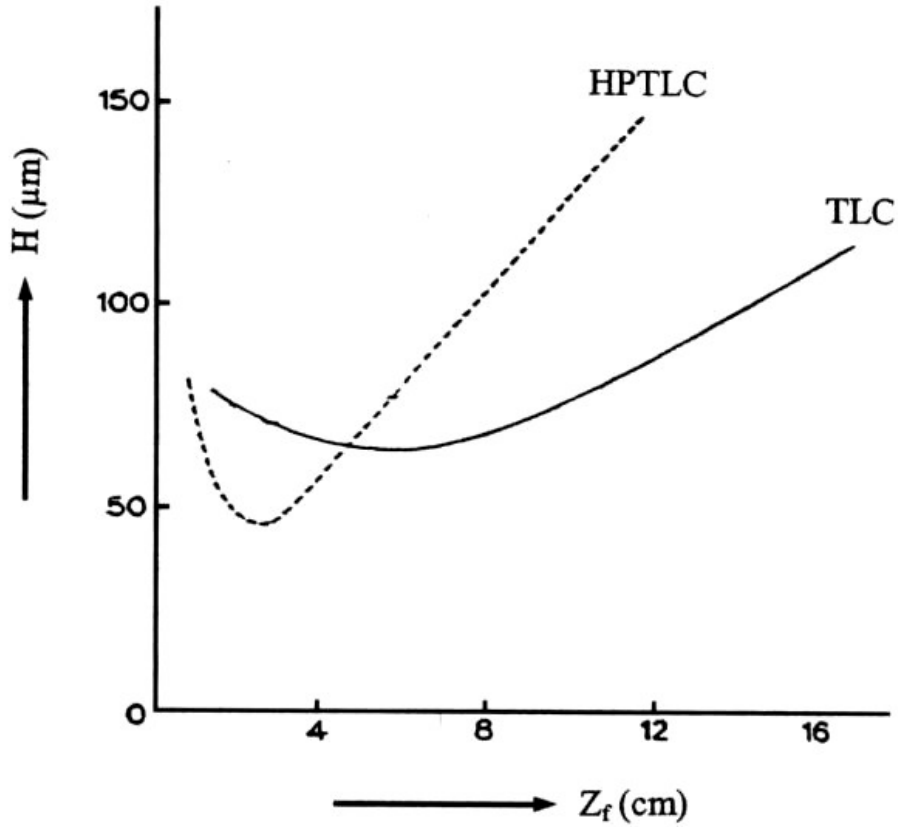


Figure 41 *Plate height (H) versus solvent migration distance (Z_f) comparison between silica gel HPTLC and TLC. Typically demonstrated with a chloroaniline (R_f 0.35) as a standard and using toluene as the mobile phase. As the graph shows, at low Z_f (short development distances) improved resolution is observed for the HPTLC layers, but the effect diminishes with increased development distances*

Source:
Peter E. Walls Thin-layer chromatography. A modern practical approach RSC, Cambridge, 2005; page 47.

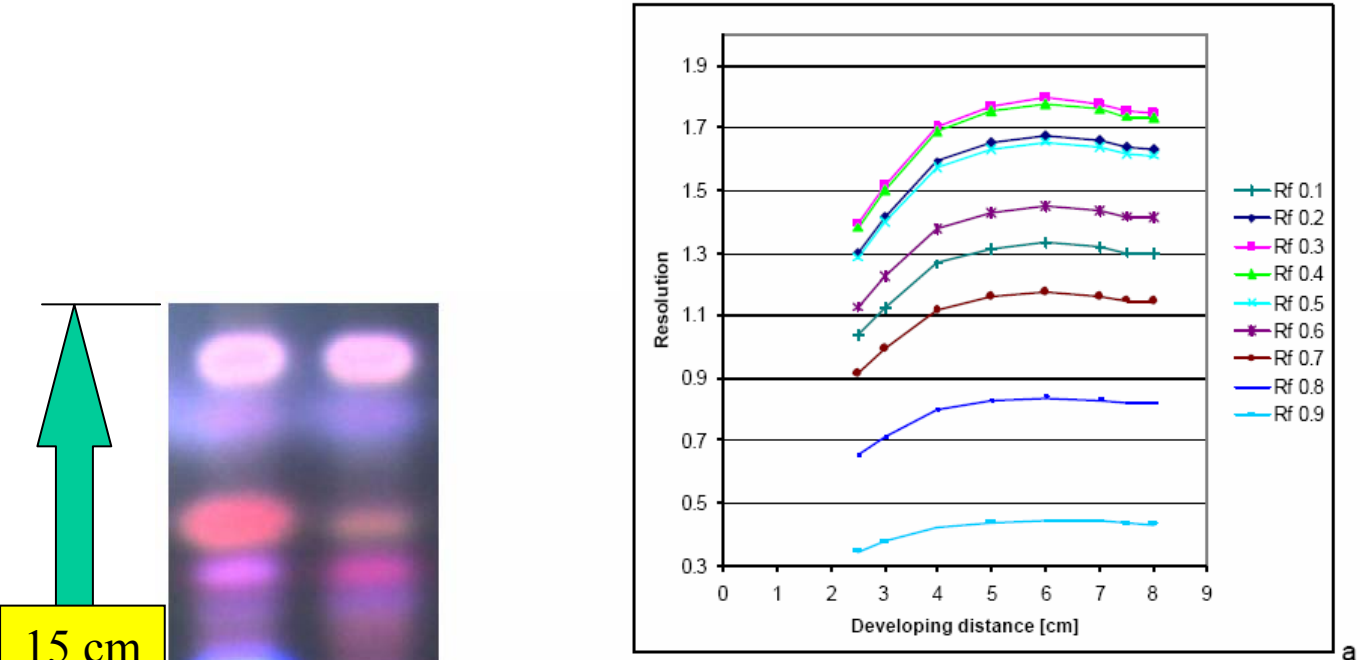
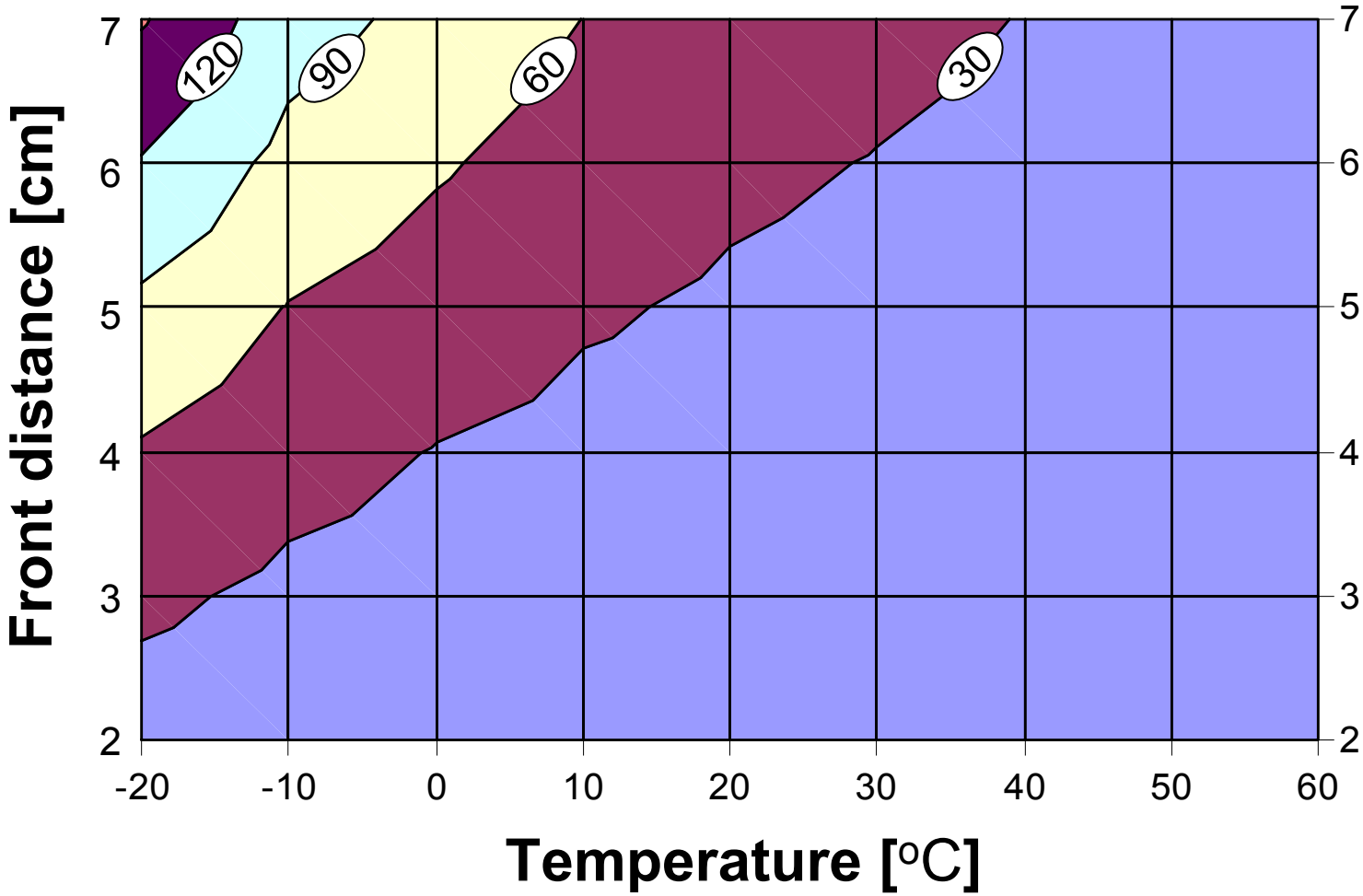


Figure 6 - Influence of the developing distance (a) and R-values (b) on the resolution: $R_s = {}^1/_4(\alpha-1)(R_f N)^{1/2}(1-R_f)$, selectivity ($\alpha$) 1.5, plate numbers (N) taken from [3, p. 666]

Figure 1 - Separation of sweet (left track) and bitter orange oil (right track) on TLC and HPTLC plates. Development on the TLC plate (left) over 15 cm requires 45 min, separation over 5 cm on HPTLC material (right) is achieved in 7 min. Mobile phase: ethyl acetate, toluene (15:85 V/V), visualization at 366 nm after derivatisation with anisaldehyde reagent.

Modified from: Eike Reich, Anne Blatter and Beat Meier; "TLC for the Analysis of Herbal Drugs A Critical Review of the Status and Proposal for Improvement of Monographs"; Camag Scientific Note available online through Camag WebPages.


120
90
60
30
Front distance [cm]
7
6
5
4
3
2
-20
-10
0
10
20
30
40
50
60
Temperature [°C]

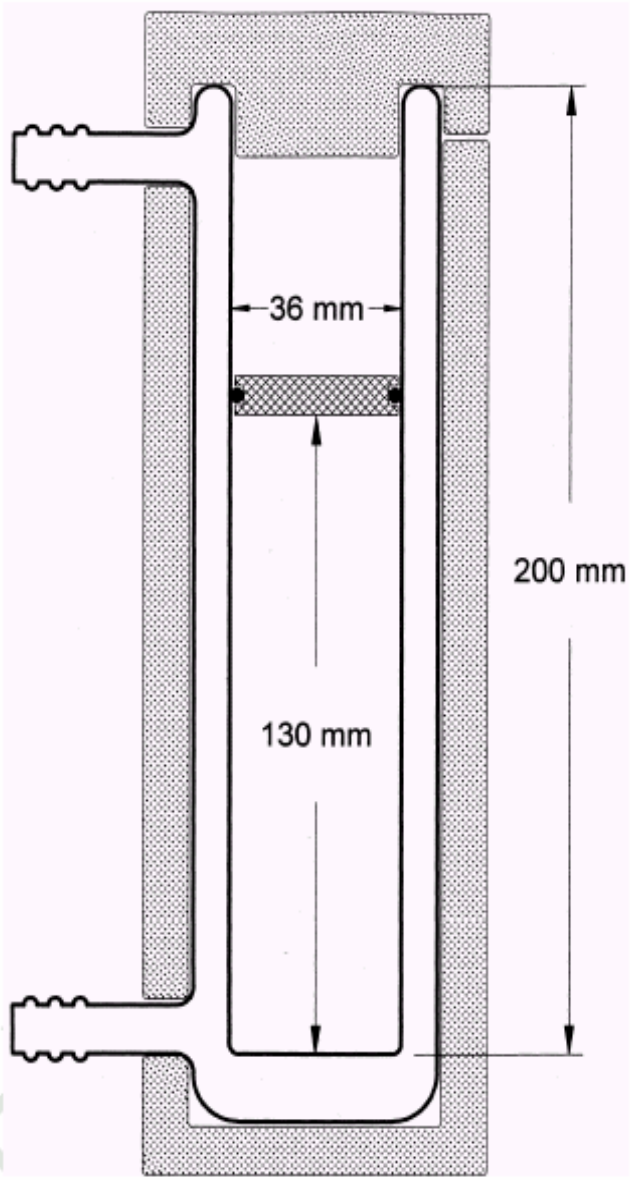
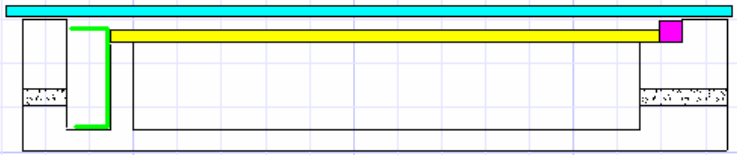
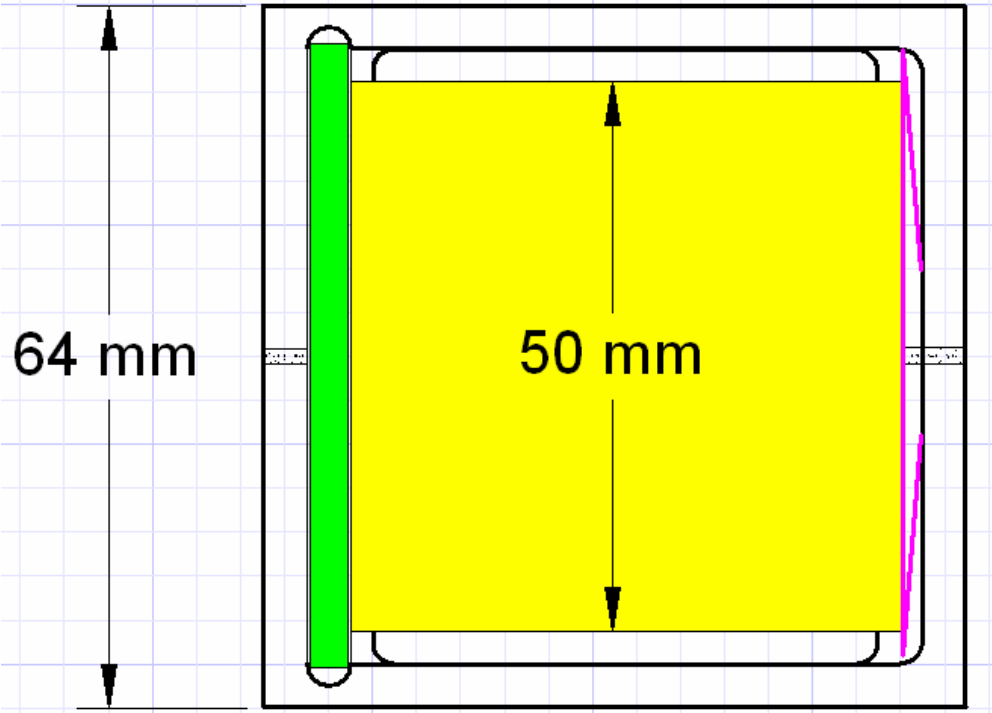
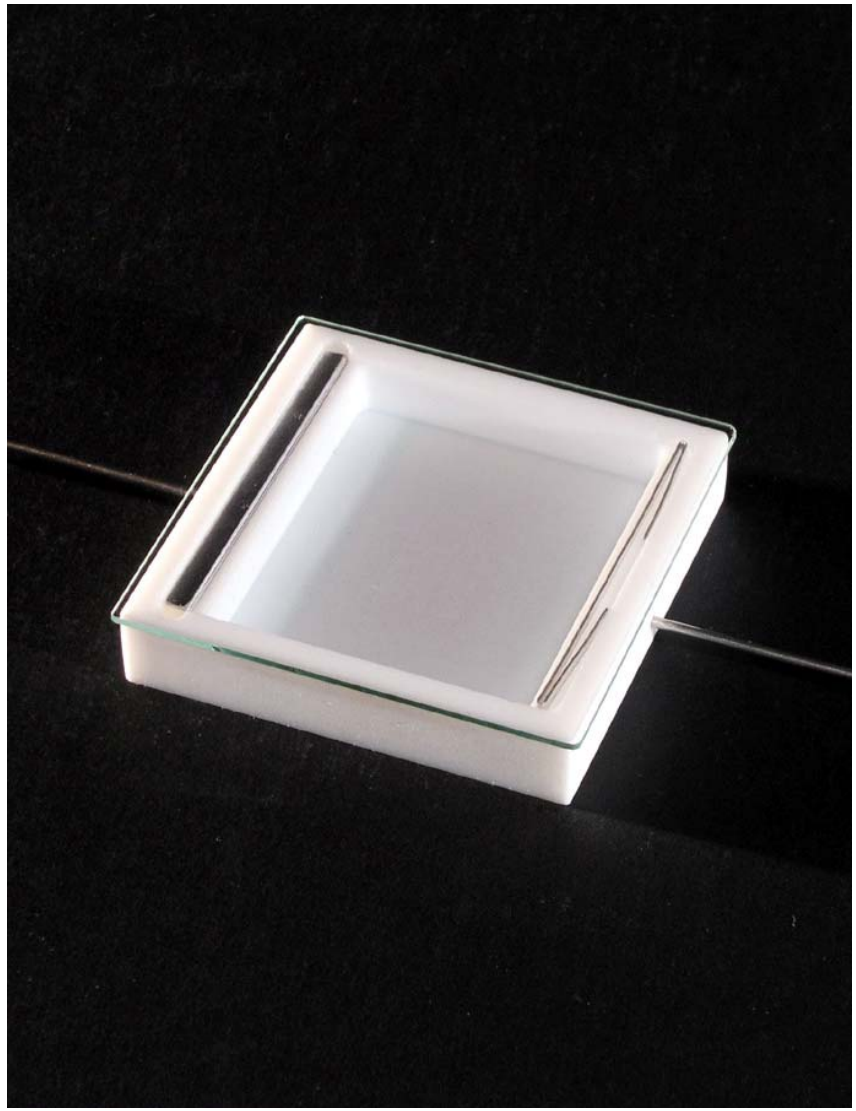


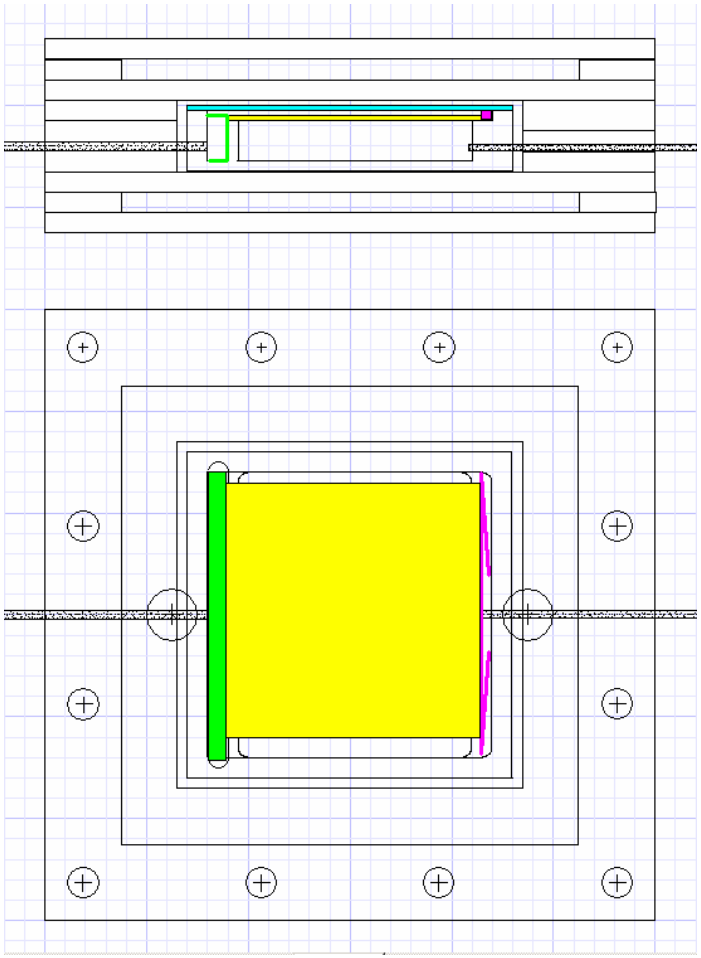
Fig. 2. Section drawing of the chamber unit.

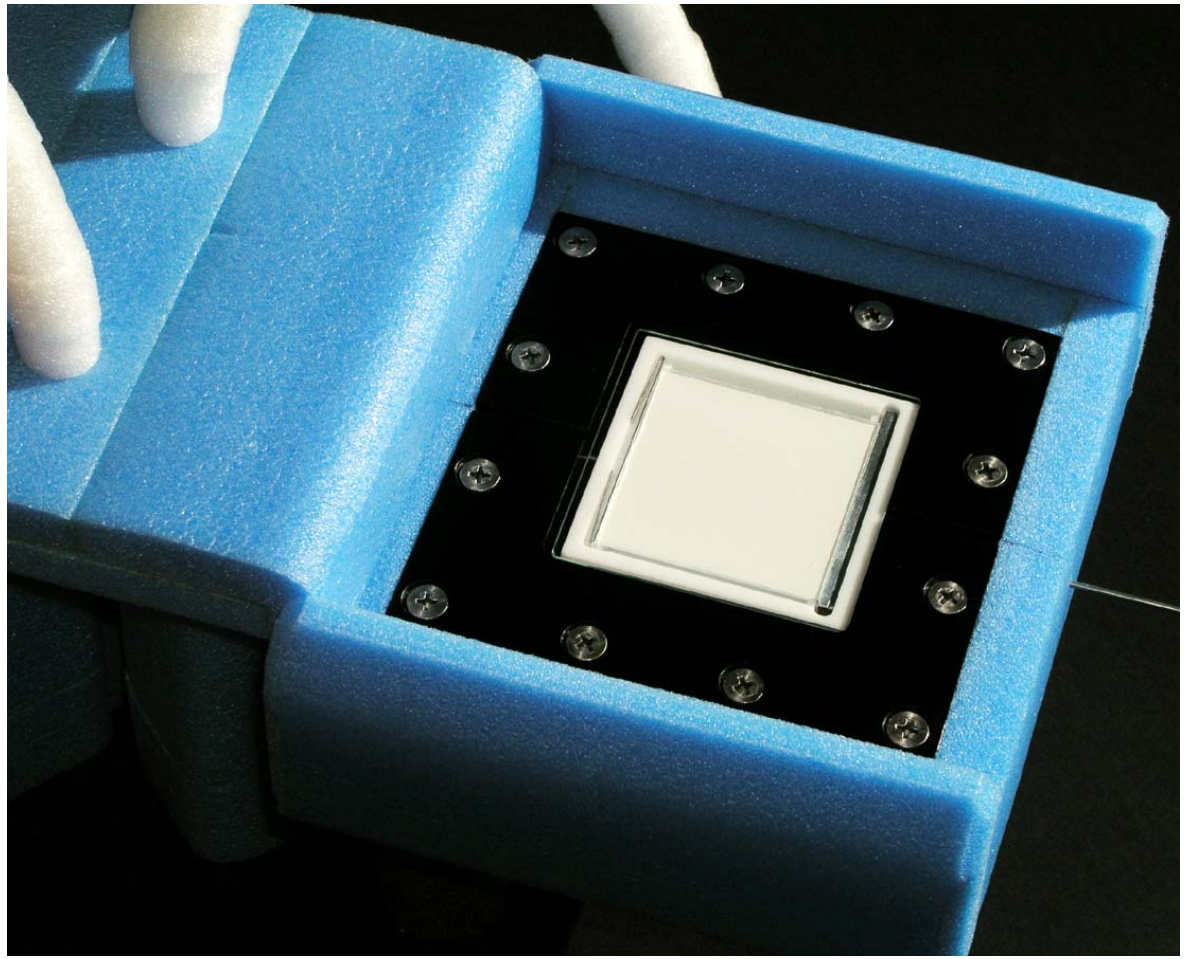
Adapted from:
P. K. Zarzycki. "Simple chamber for temperature-controlled planar chromatography" J. Chromatogr. A, 971 (2002) 193-197.

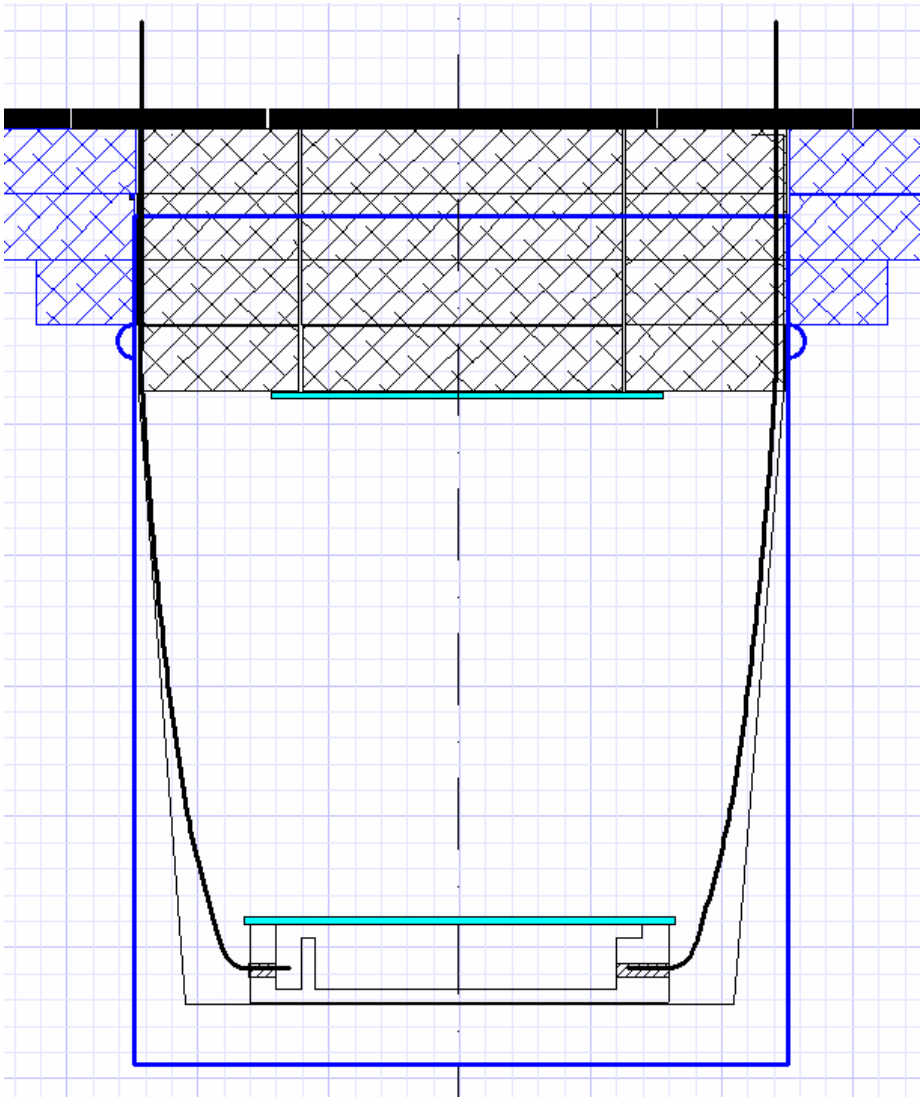


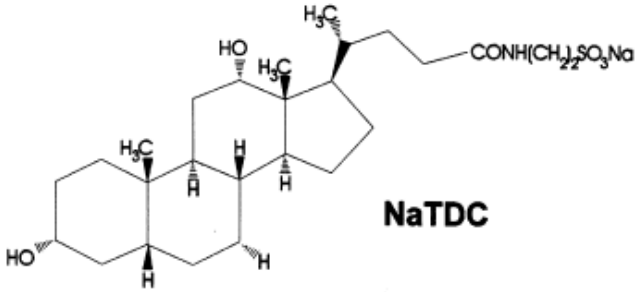

64 mm
50 mm



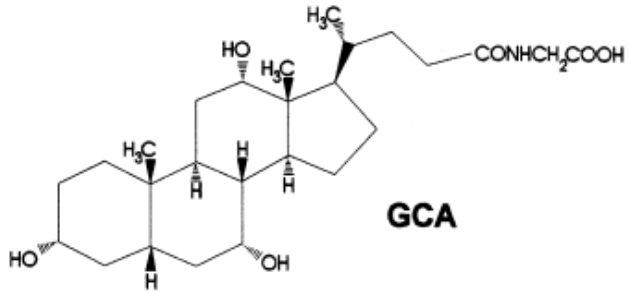




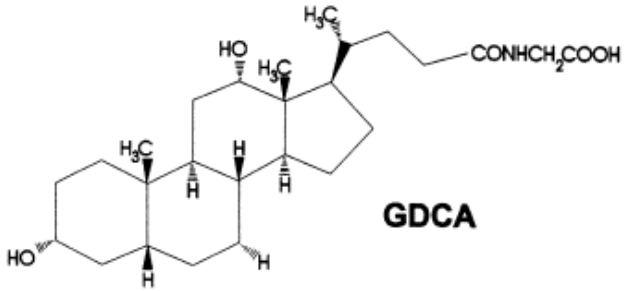




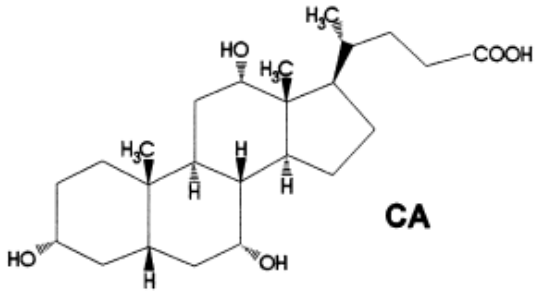
NaTDC



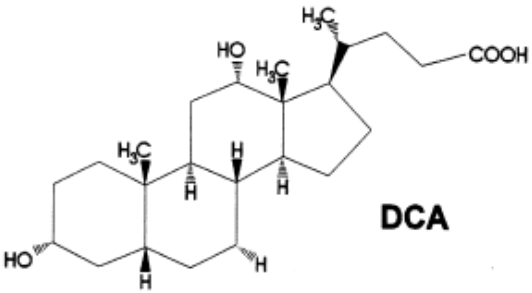
GCA



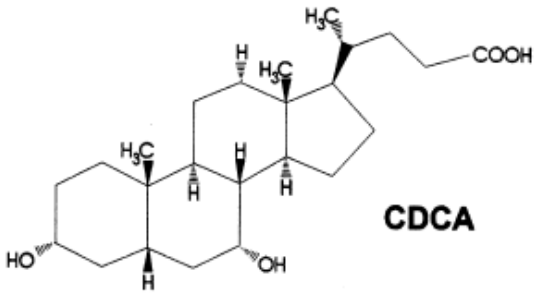
GDCA



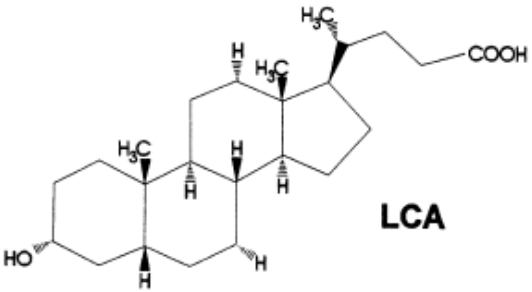
CA



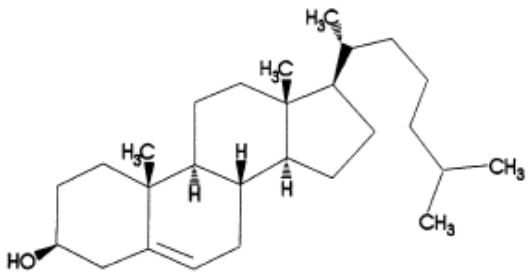
DCA



CDCA



LCA



Cholesterol

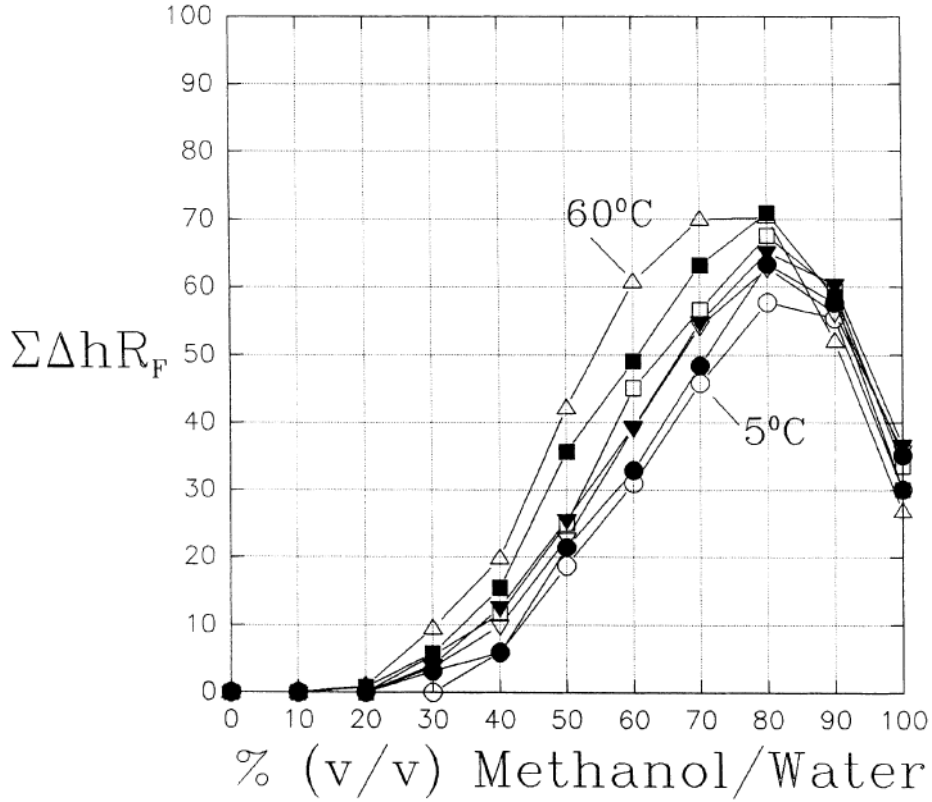


Fig. 3. Relationships between $\Sigma\Delta hR_F$ values and composition of mobile phases at different temperatures, obtained for mixtures consisting of eight steroids. 5°C (○), 10°C (●), 20°C (▽), 30°C (▼), 40°C (□), 50°C (■), 60°C (△).

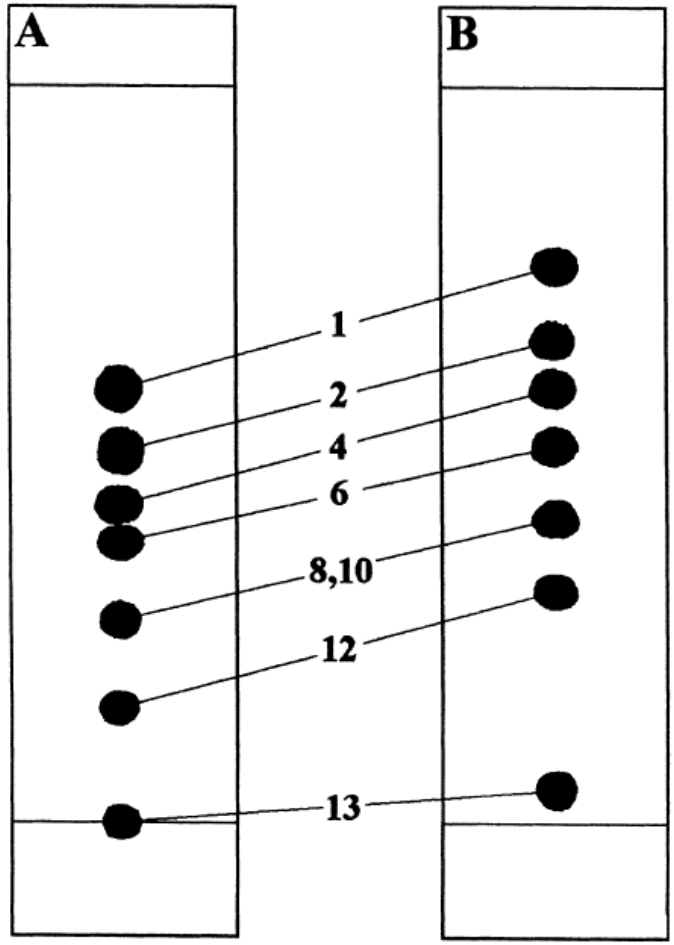
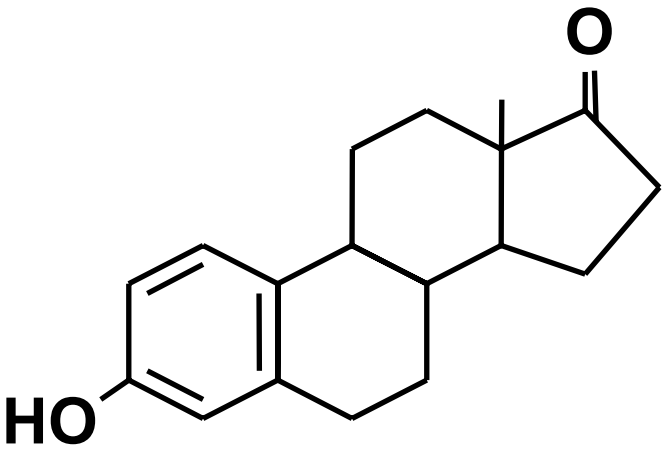
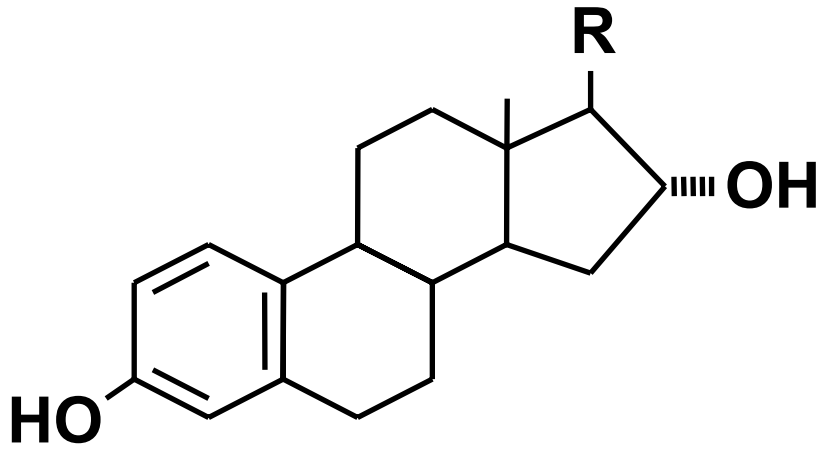


Fig. 4. Chromatographic separation of steroids at 5°C (A) and 50°C (B) using RP-18W plates and methanol–water (80:20, v/v) mobile phase. Spot numbers correspond to steroids numbers listed in Table 1.

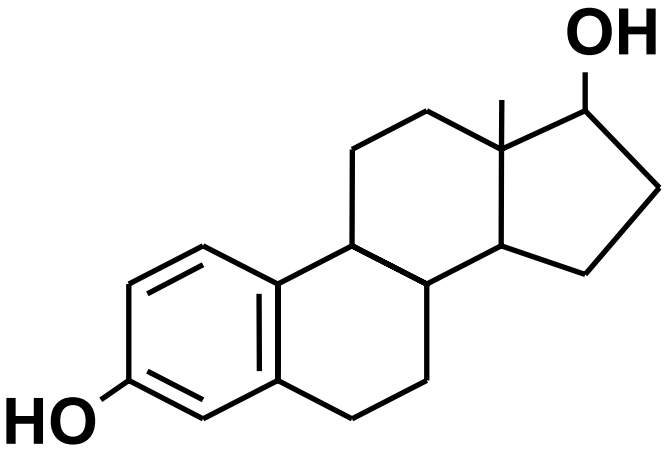
Adapted from: P.K. Zarzycki, M. Wierzbowska, H. Lamparczyk; „Retention and separation studies of cholesterol and bile acids using thermostated thin-layer chromatography", *J. Chromatogr. A*, 857 (1999) 255-262.



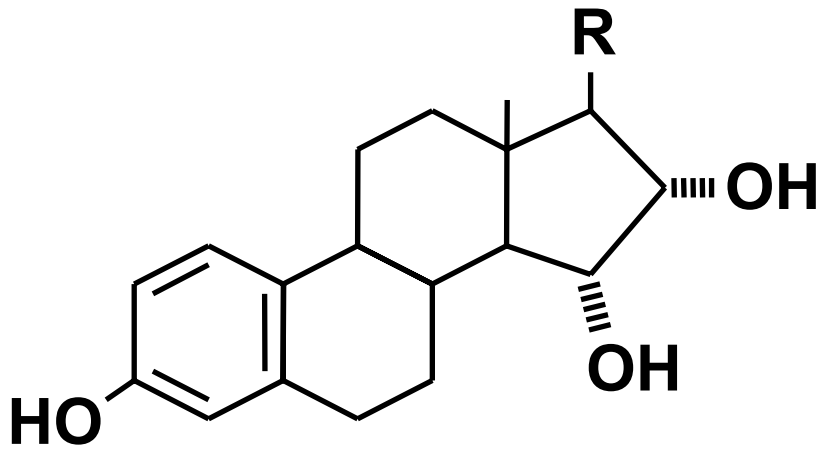
Estrone; E1



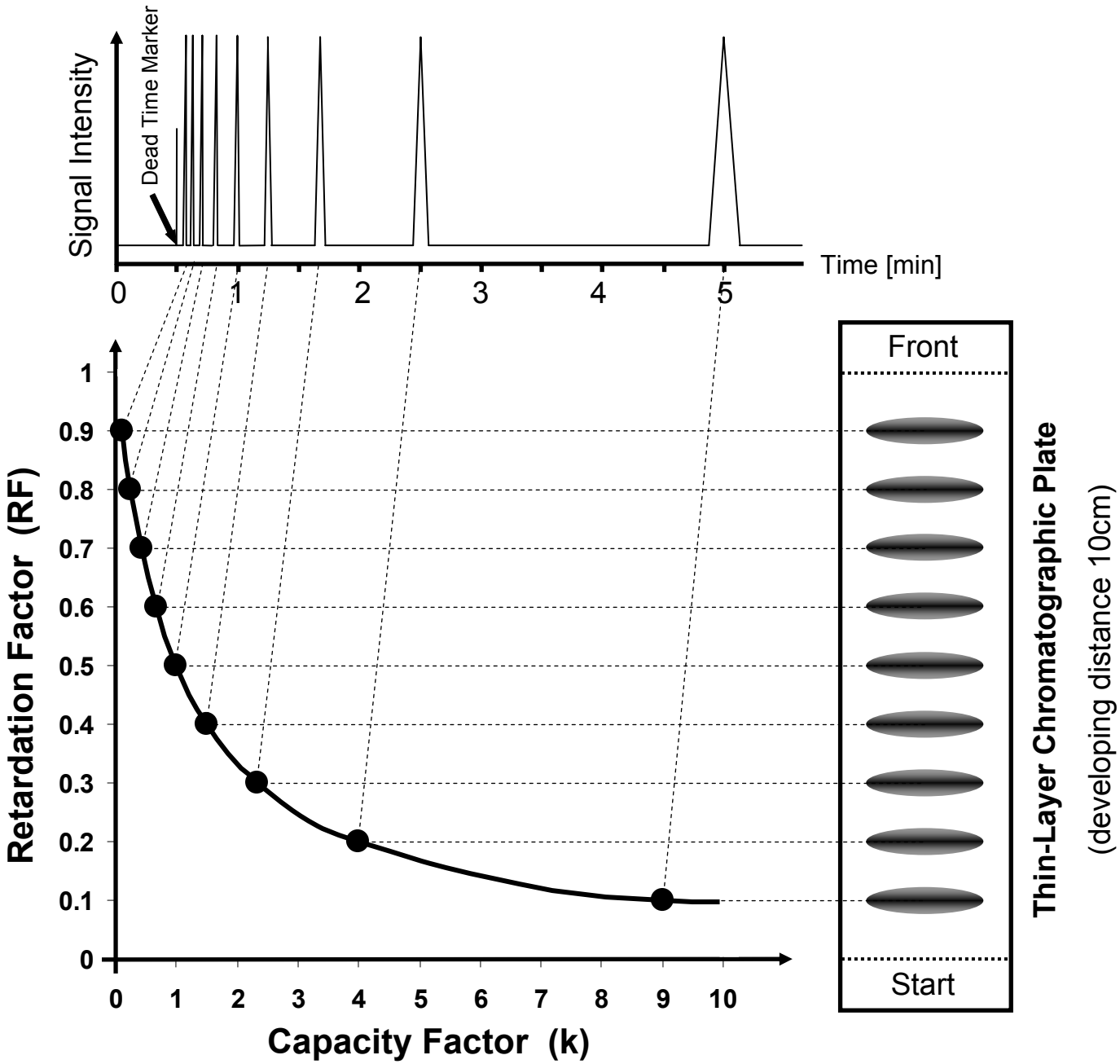
Estriol; E3

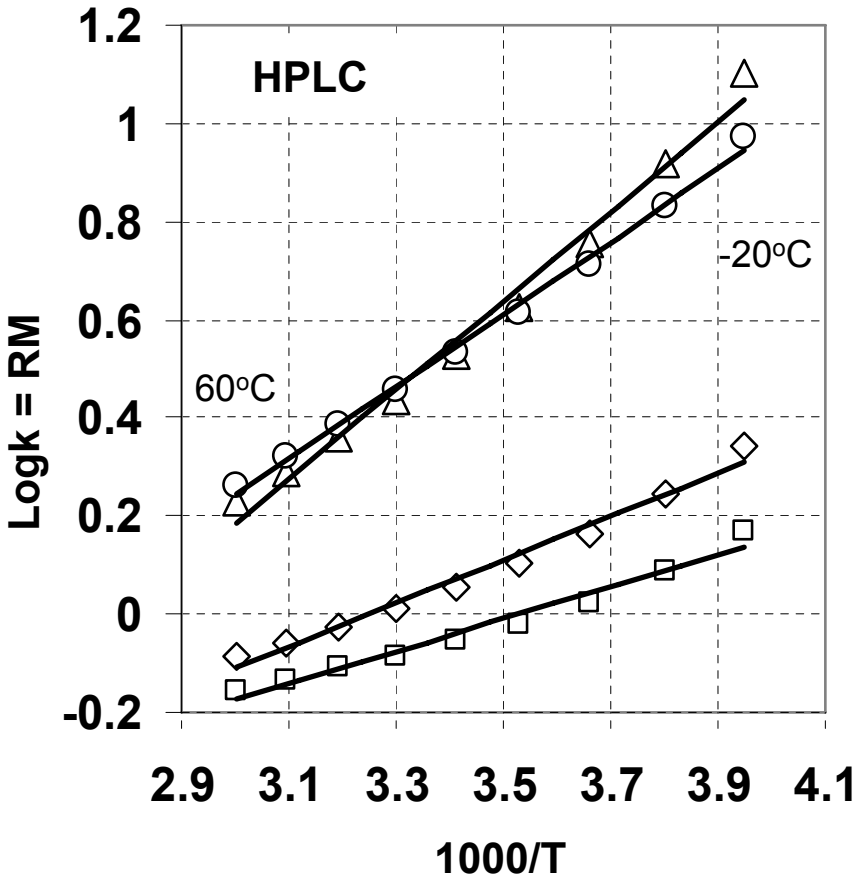


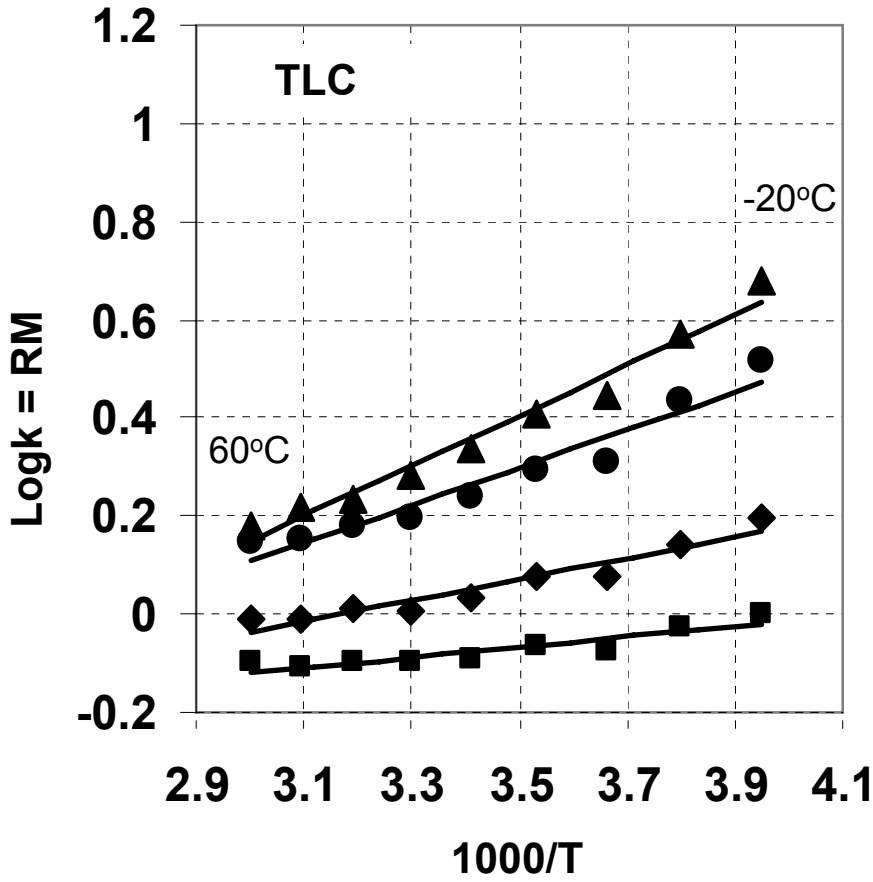
17β-Estradiol; E2



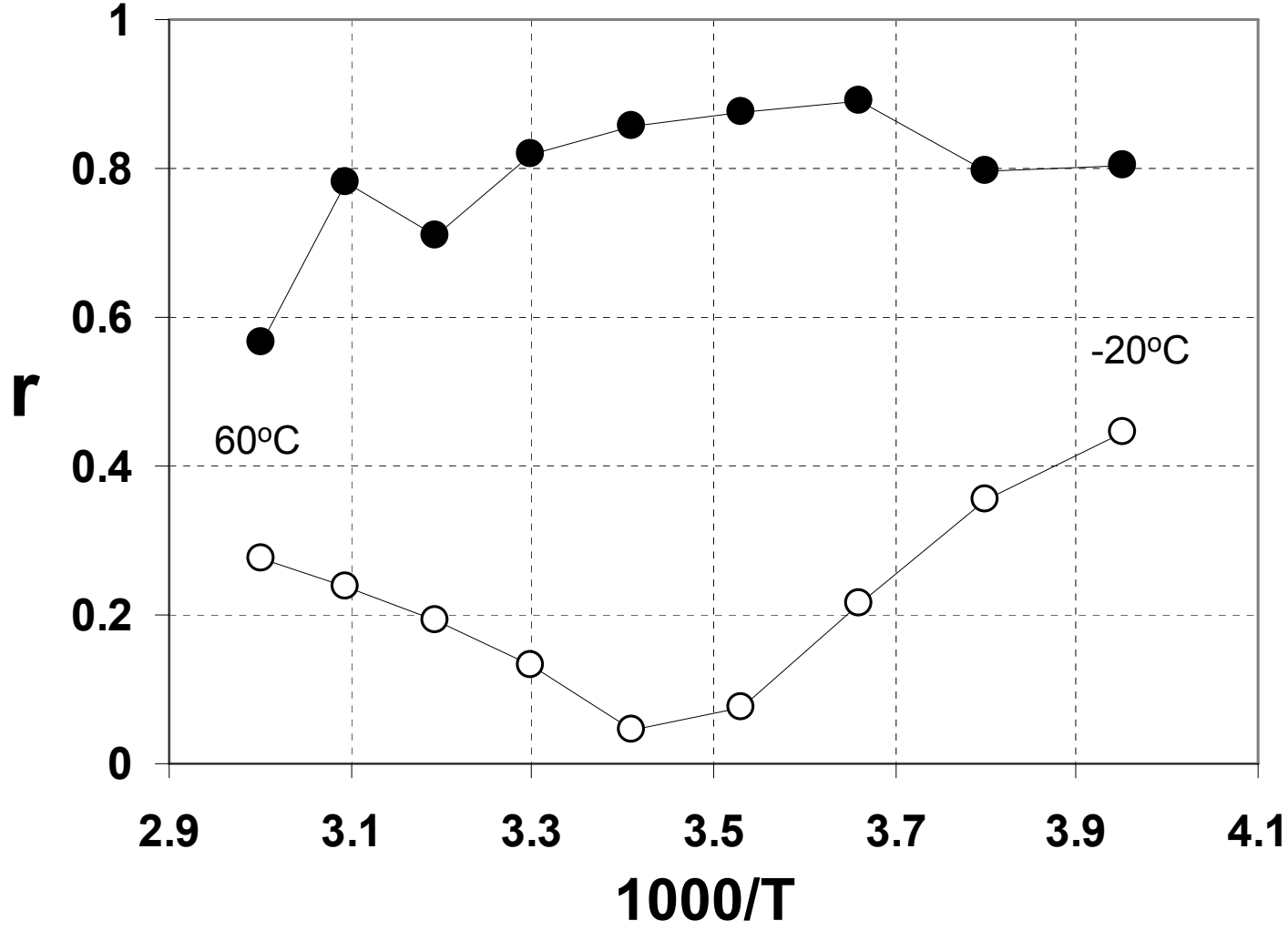
Estetrol; E4

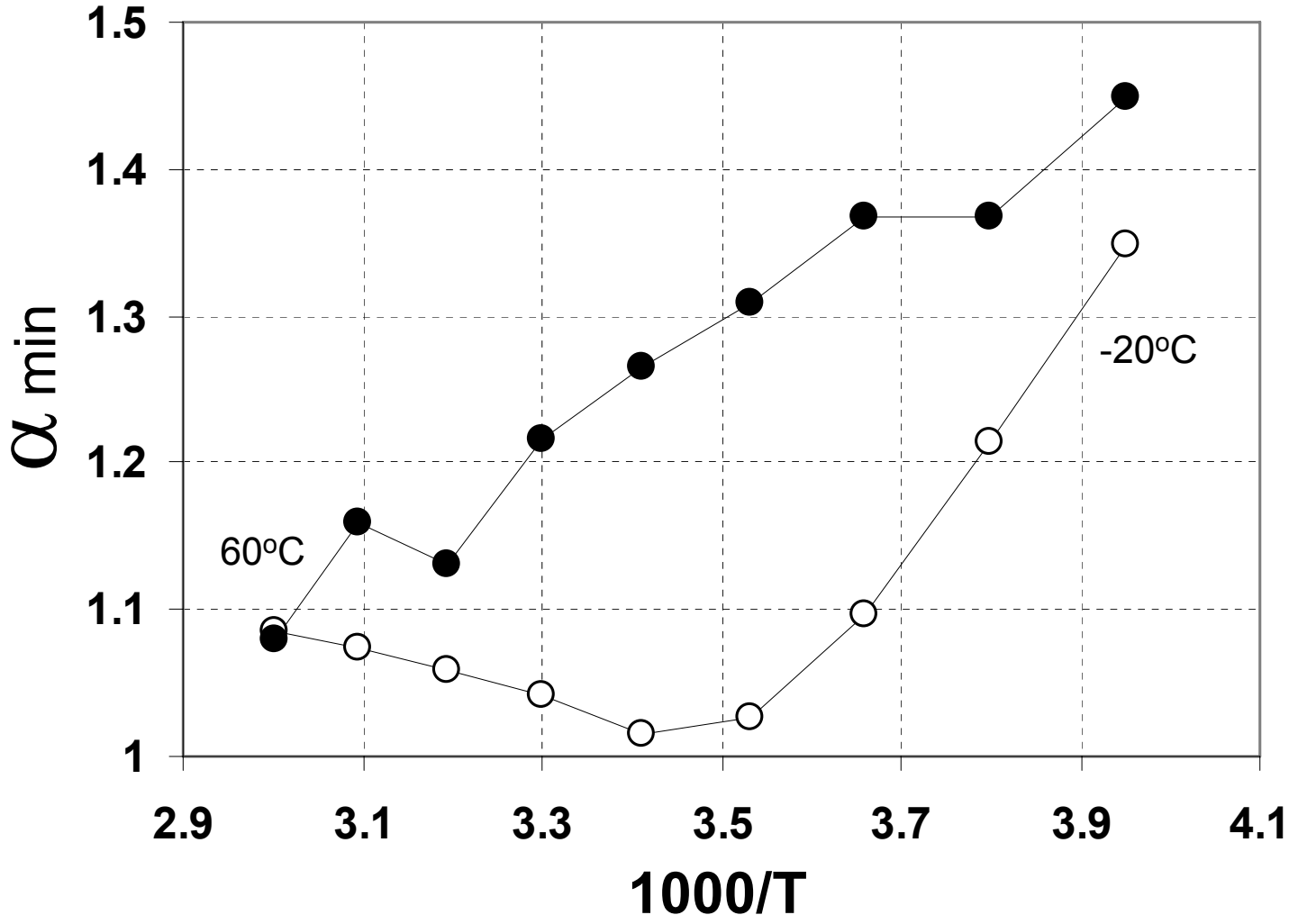

Signal Intensity
Dead Time Marker
Time [min]
0
1
2
3
4
5
Retardation Factor (RF)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
5
6
7
8
9
10
Capacity Factor (k)
Front
Start
Thin-Layer Chromatographic Plate
(developing distance 10cm)

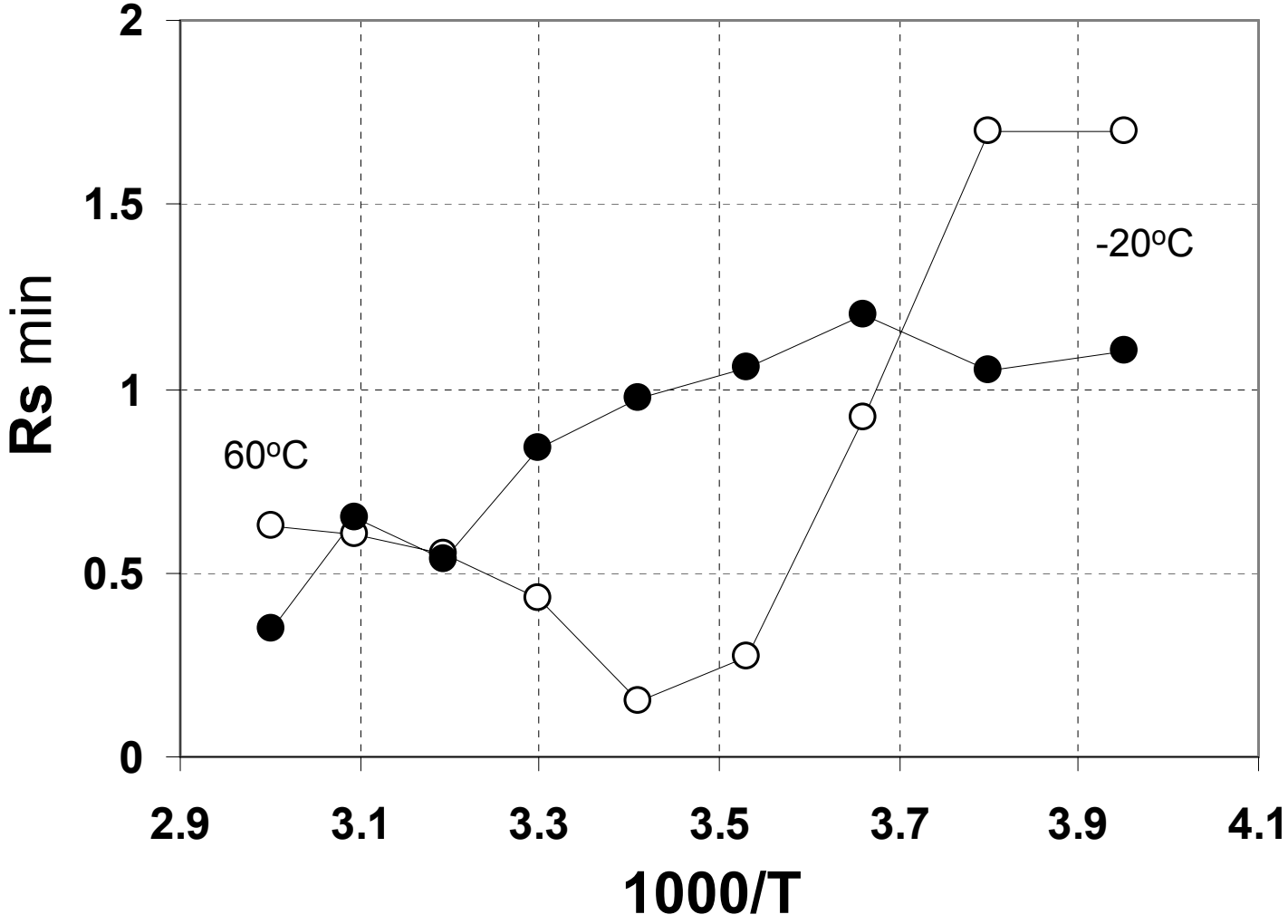


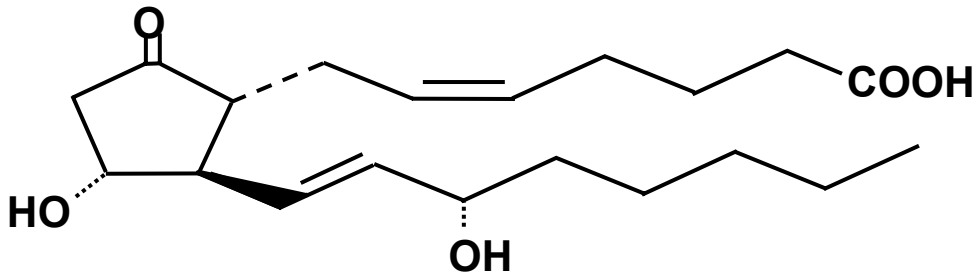


Adapted from P. K. Zarzycki, K. M. Kulhanek, R. Smith, M. A. Bartoszuk, H. Lamparczyk, "Planar Chromatography Versus Column Chromatography: A Performance Comparison"; *LCGC North America* 23 (2005) 286-300.

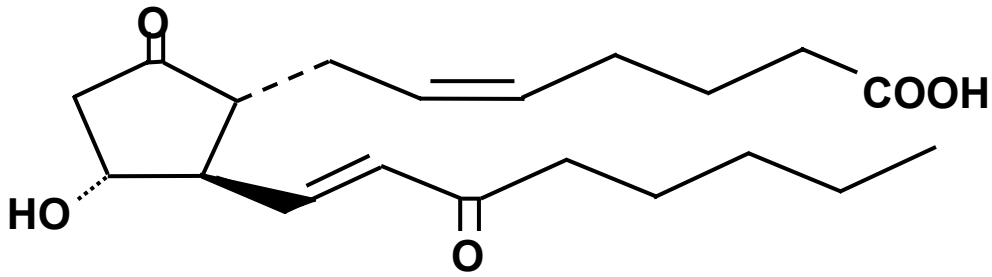

1
0.8
0.6
0.4
0.2
0
r
60°C
-20°C
2.9
3.1
3.3
3.5
3.7
3.9
4.1
1000/T


1.5
1.4
1.3
1.2
1.1
1
α min
60°C
-20°C
2.9
3.1
3.3
3.5
3.7
3.9
4.1
1000/T

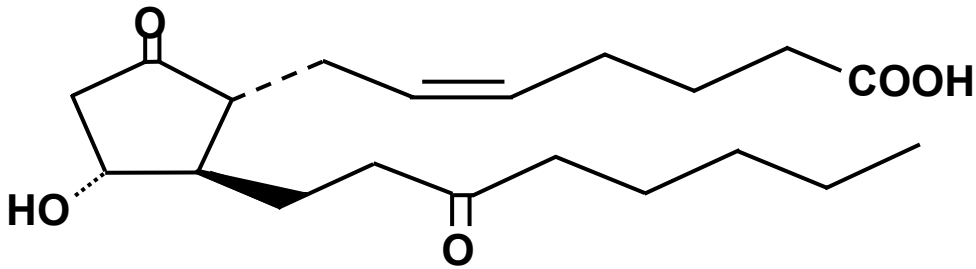

2
1.5
1
0.5
0
Rs min
60ºC
-20ºC
2.9
3.1
3.3
3.5
3.7
3.9
4.1
1000/T



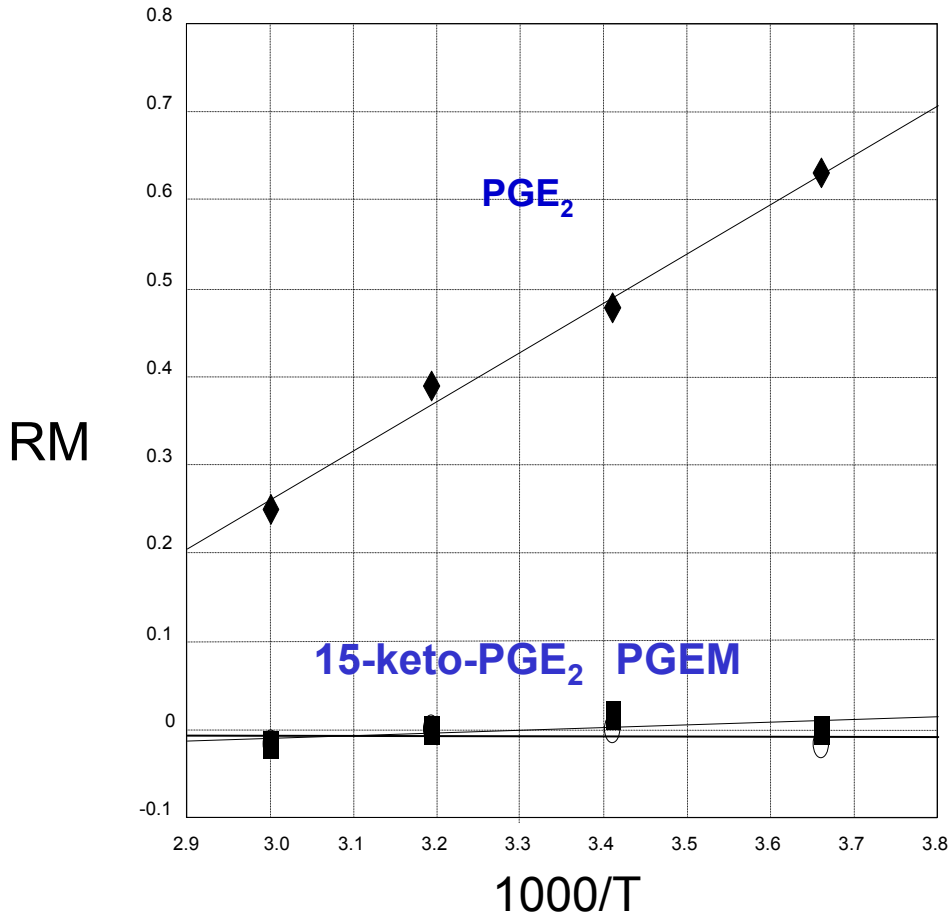
PGE_2

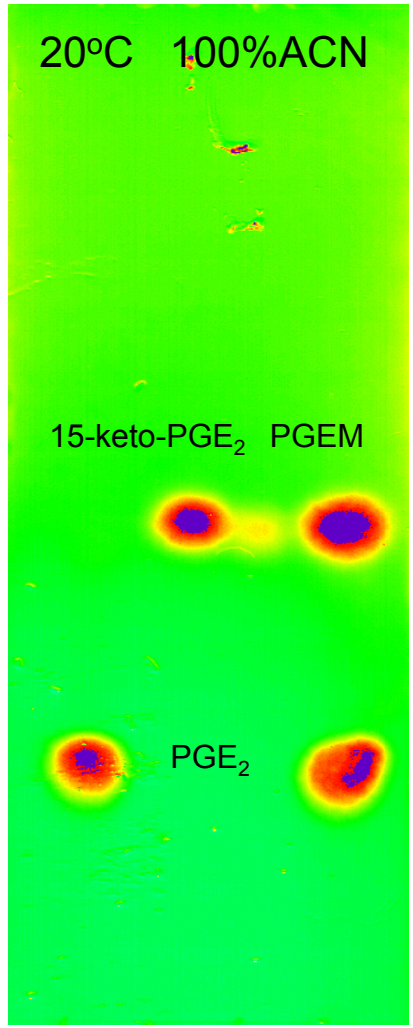


15-keto-PGE_2

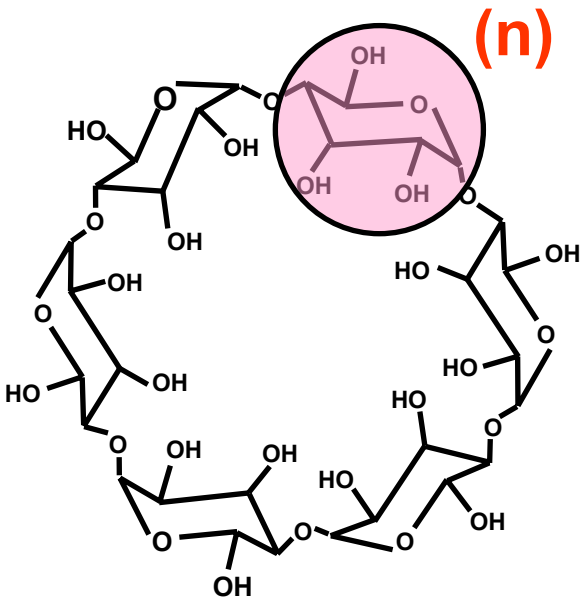


PGEM





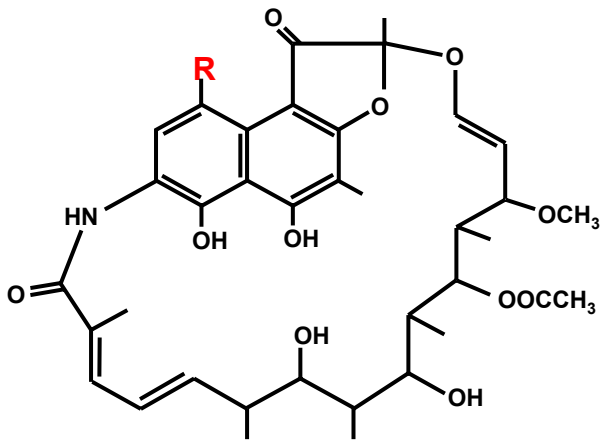
Adapted from:
T. Welsh, T. Zakar, S. Mesiano, P. K. Zarzycki; "Separation of Bioactive Prostaglandins and their Metabolites by Reversed-Phase Thin-Layer Chromatography", J. Planar Chromatogr., 16 (2003) 95-101.



α-Cyclodextrin (n=6)

β-Cyclodextrin (n=7)

γ-Cyclodextrin (n=8)



R = – OCH_3COOH

RIFAMYCIN B

R = – CH=N-N N-CH_3

RIFAMPICIN

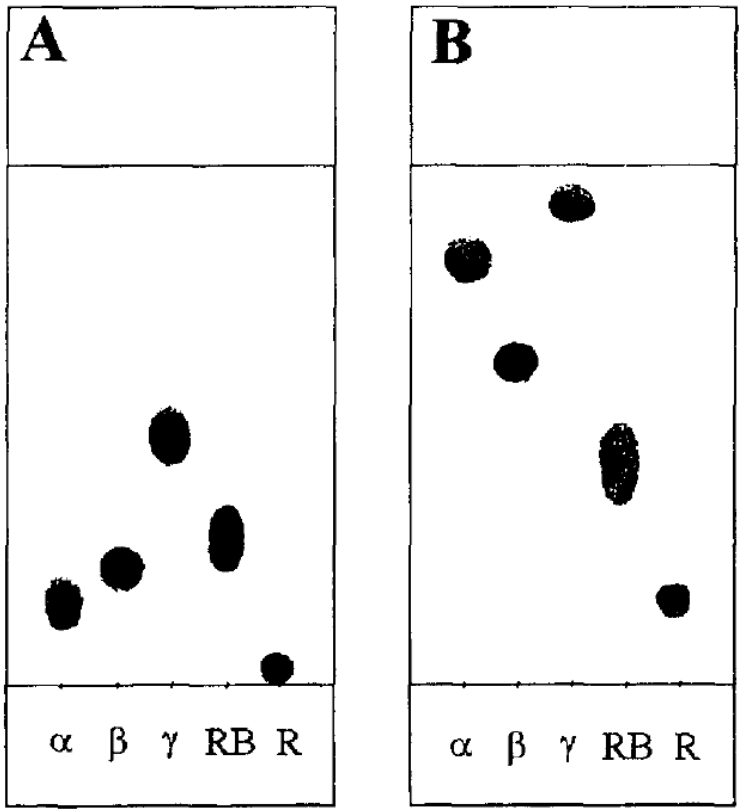


Fig. 2. Separation of studied macrocycles using methanol–water (50%, v/v) as mobile phase. The temperature of chromatographic process was 5 (A) and 50°C (B), respectively.

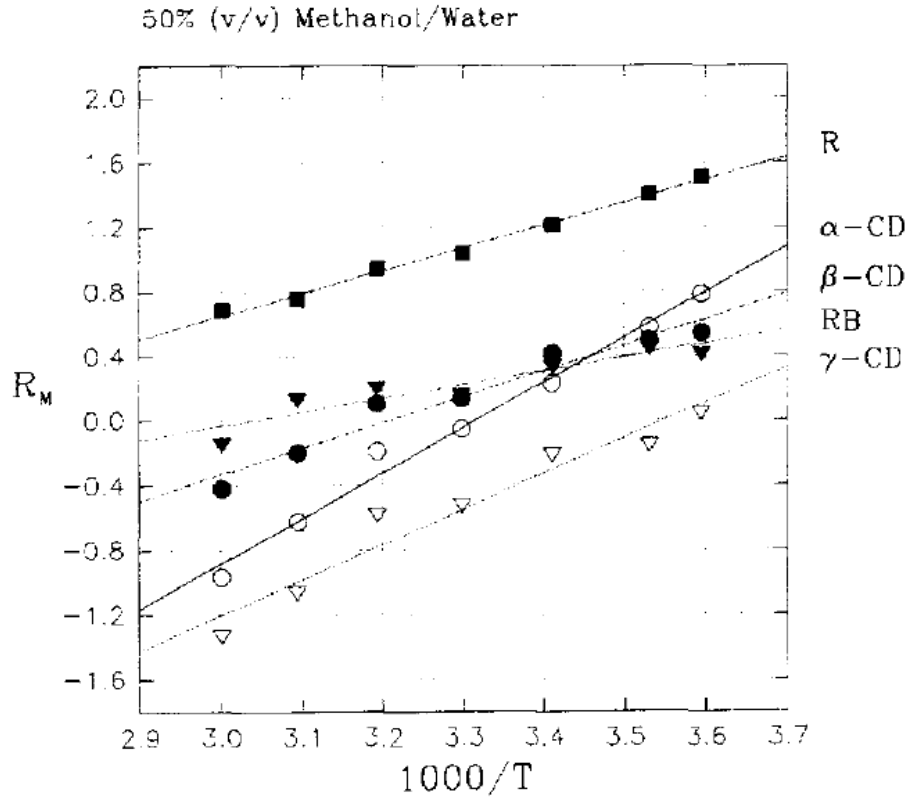


Fig. 3. Plots of R_M versus $1000/T$ for α- (○), β- (●), and γ-cyclodextrin (▽), rifamycin B (▼) and rifampicin (■). Mobile phase: methanol–water (50%, v/v).

Adapted from:
P. K. Zarzycki, J. Nowakowska, A. Chmielewska, M. Wierzbowska, H. Lamparczyk, "Thermodynamic study of retention of selected macrocycles using RP-HPTLC plates and methanol/water mobile phases", *J. Chromatogr A*., 787 (1997) 227-233.

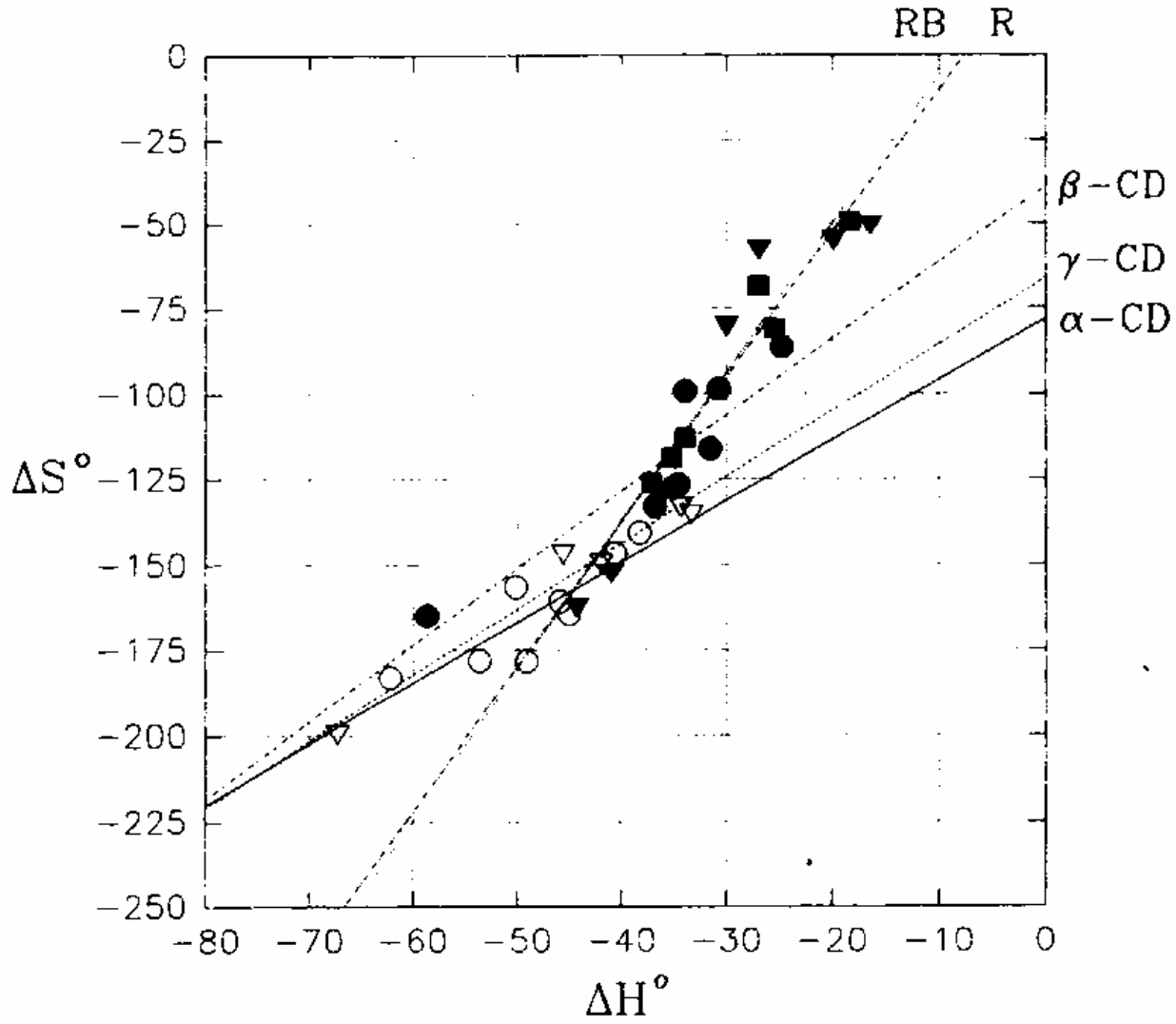
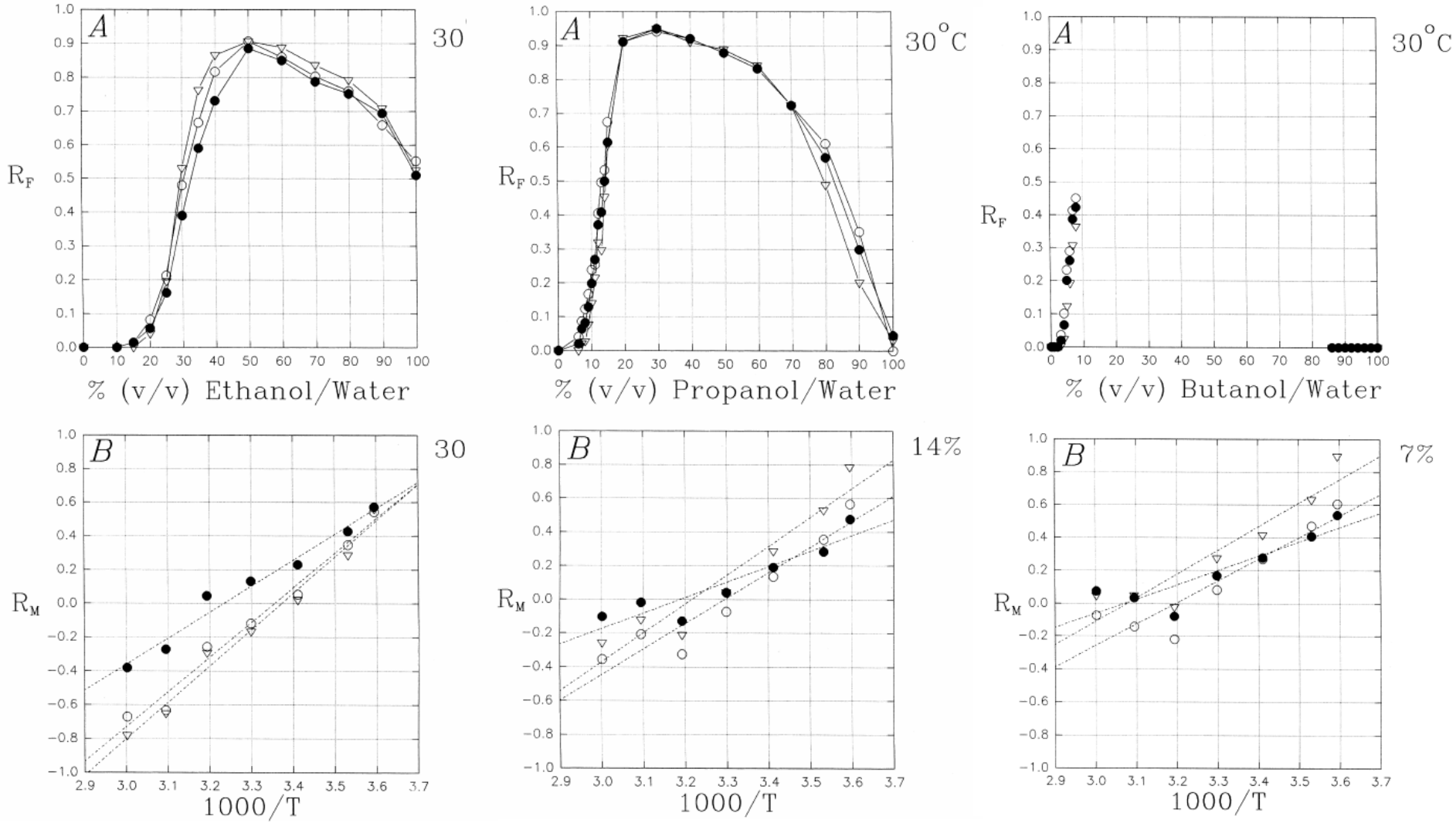
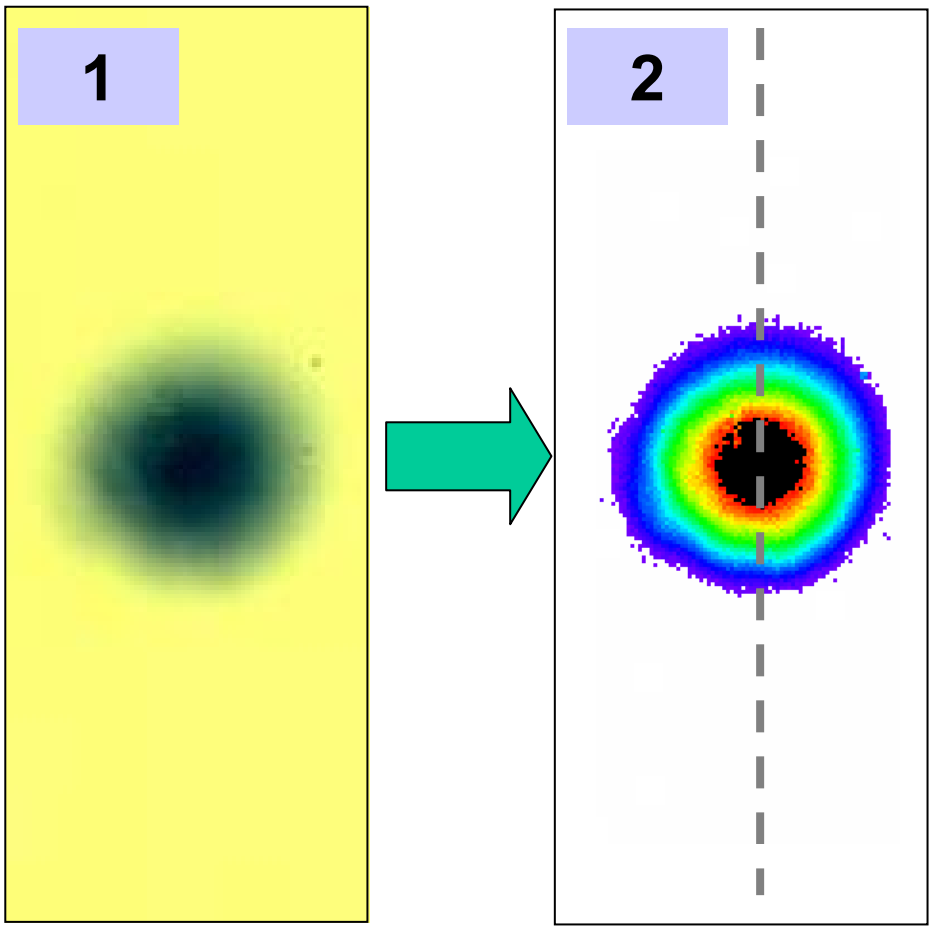
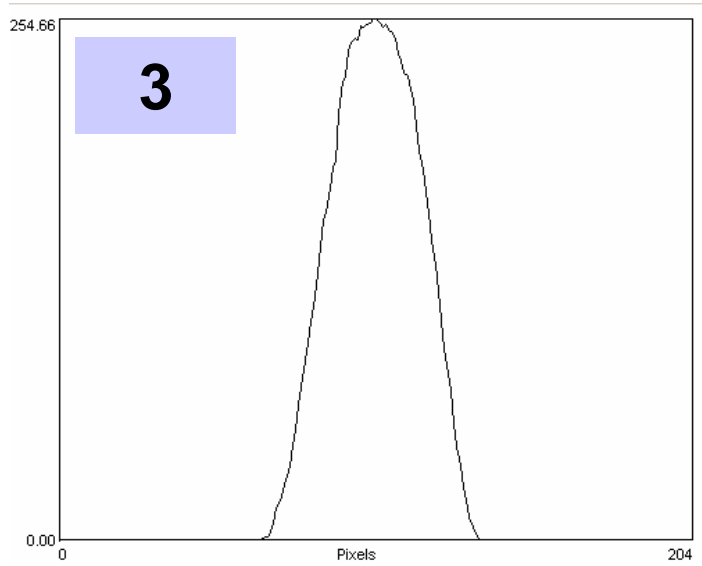


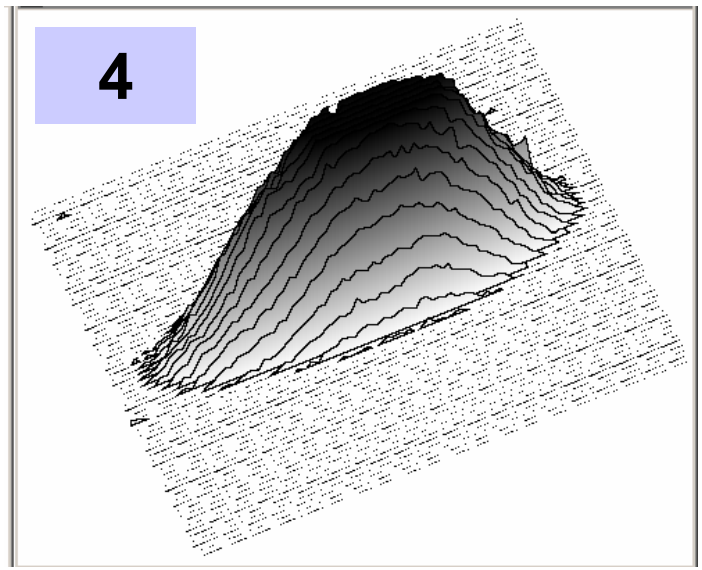
Fig. 4. Plot of enthalpy–entropy compensation for α-CD (○), β-CD (●), γ-CD (▽), rifamycin B (▼) and rifampicin (■).



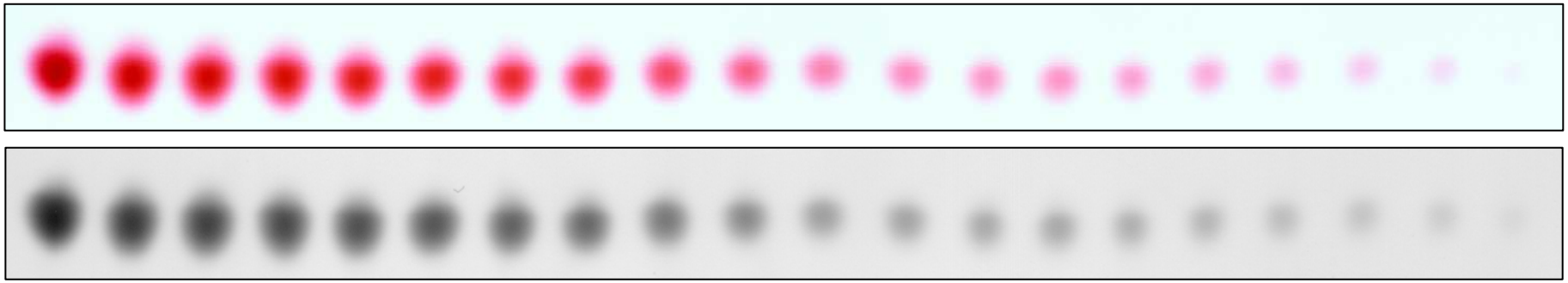
Adapted from P.K. Zarzycki, M. Wierzbowska, J. Nowakowska, A. Chmielewska, H. Lamparczyk; „Interactions between native cyclodextrins and *n*-alcohols studied using thermostated thin-layer chromatography", *J. Chromatogr. A*, 839 (1999) 149-156.


1
2

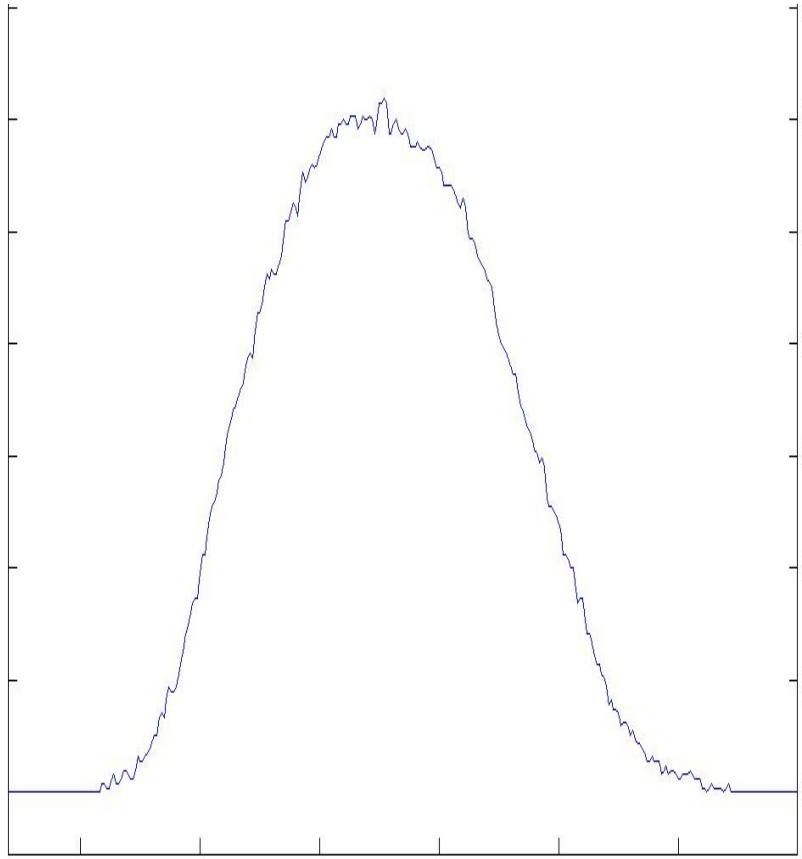

3
254.66
0.00
0
Pixels
204


4

2000 ng ← TLC Spot Mass Range → 10 ng

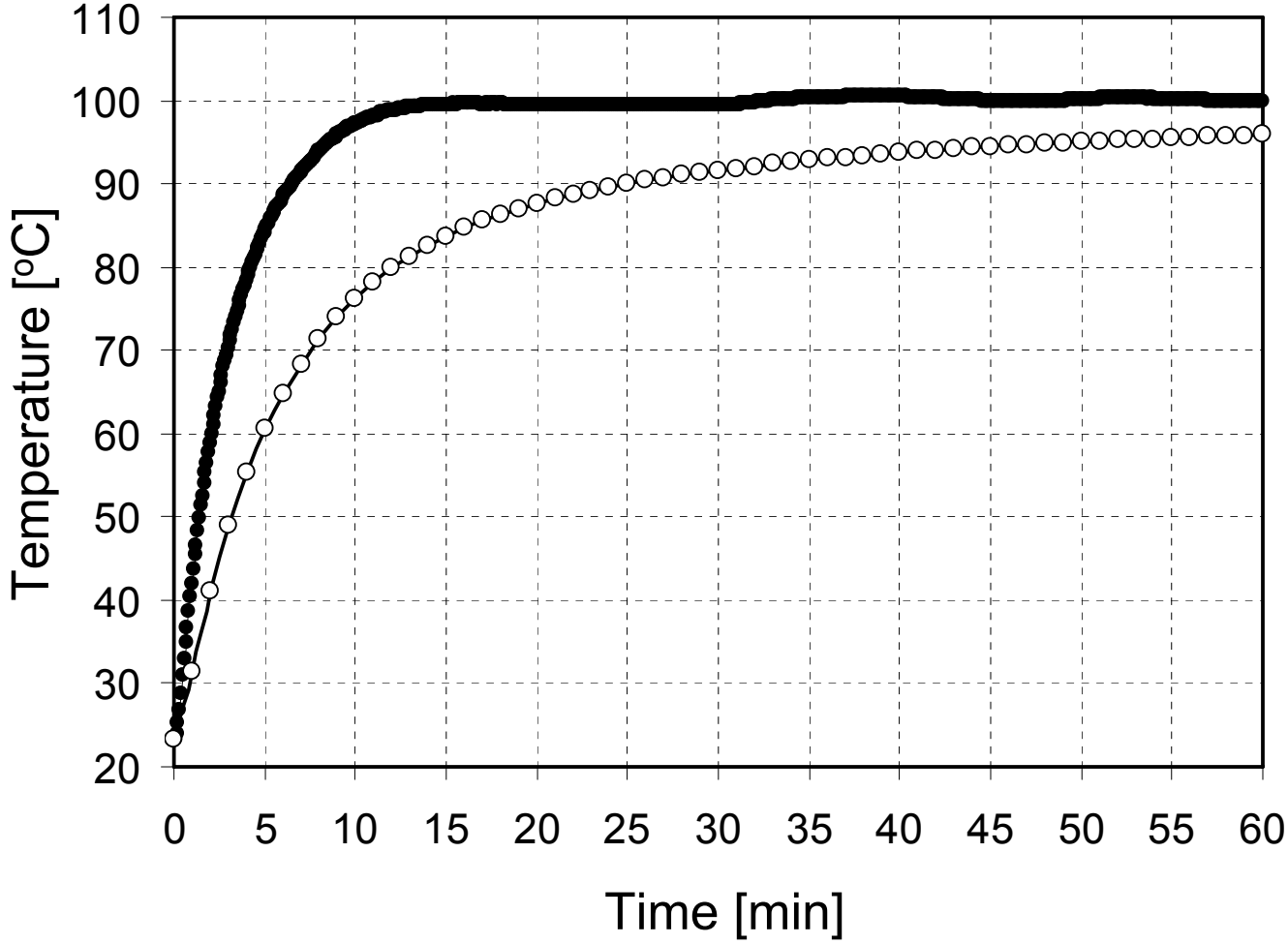


SCANNING MODE (RGB Channels)	
8 bits	16 bits
levels available theoretically	
2^8=256	2^{16}=65536
levels available in reality	
159	2817 (≈2^{11})

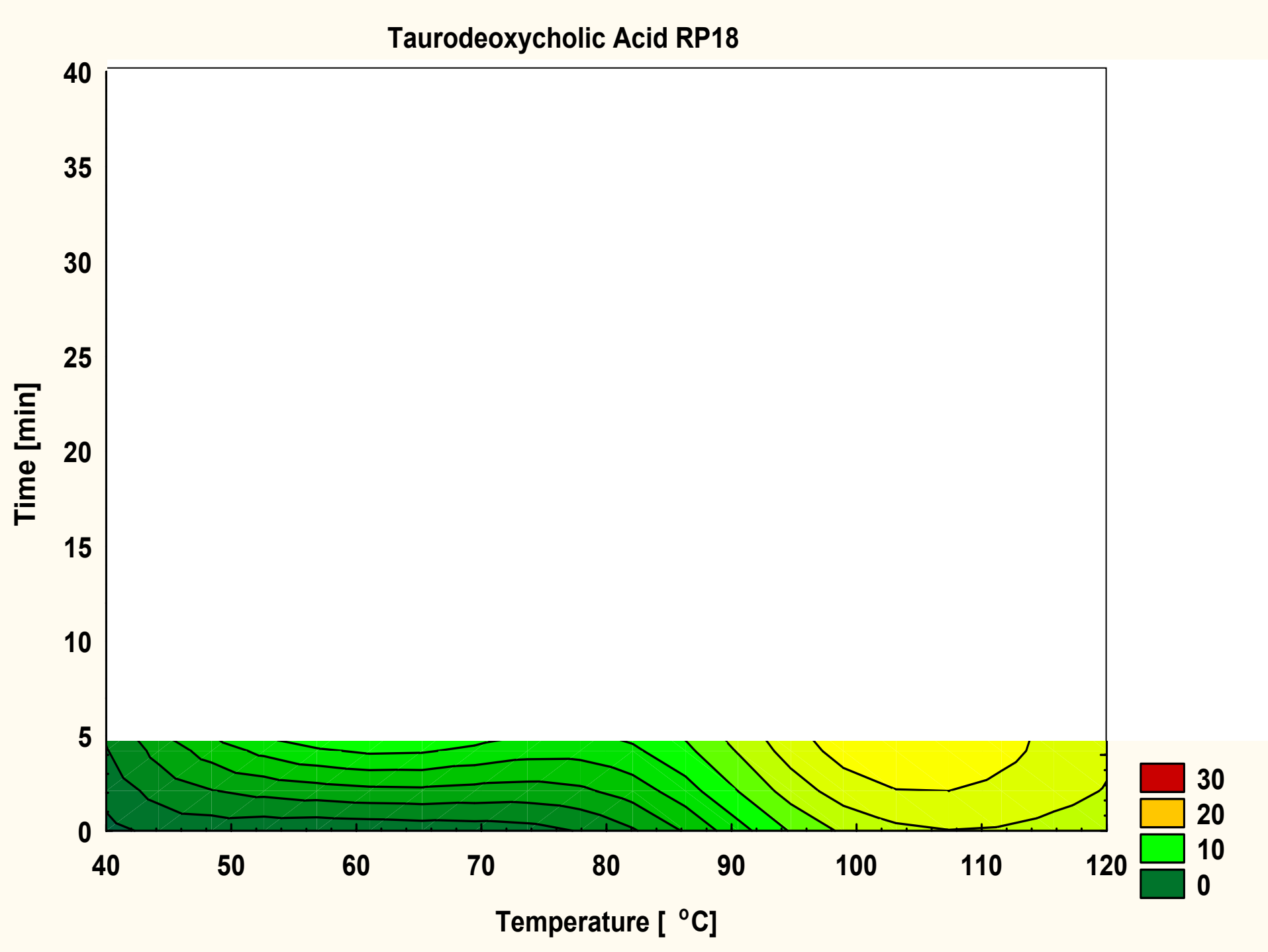


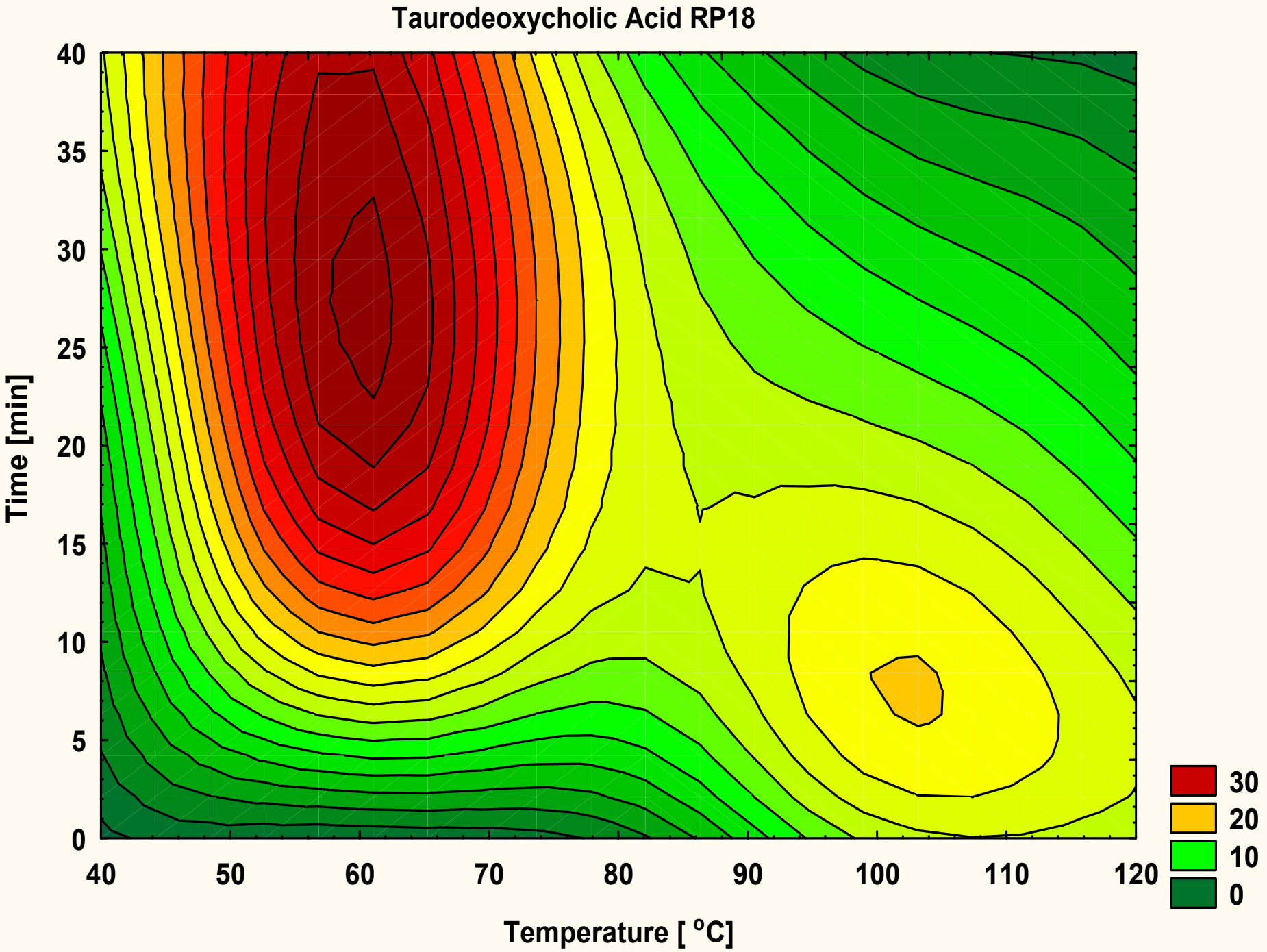
Common applications of the phosphomolybdic acid as the main component of the detection mixtures in planar chromatography.

Class of compounds	Ref.	PMA concentration	Temperature	Stationary phase
Lipids	**[19]**	5% in methanol	115°C for 15 min	Silica HPTLC
Neutral lipids and cholesterol	**[20]**	5% in ethanol	110-120°C for 5-10 min	Silica HPTLC
Cholesterol esters	**[21]**	10% in ethanol	100°C for 2 min	Silica HPTLC
Bile acids and cholesterol	**[22]**	10% in 2-propanol	120°C for 5-10 min.	HPTLC RP18W
Conjugated bile acids	**[23]**	3.5% in ethanol	70-80°C for 10 min.	Silica HPTLC
Saponins	**[24]**	5% in ethanol	110°C	Silica TLC
Prostaglandins	**[24]**	10% in ethanol	110°C for 3-6 min.	HPTLC RP18W
Peroxides and ketodienes from linoleic acid	**[26]**	5% in ethanol	110°C	Silica TLC
Olive oil components	**[27]**	20% in water	175°C for 60 min.	$AgNO_3$ impregnated silica TLC
Mammalian feces	**[28]**	5% in ethanol	120°C for 20 min.	Silica TLC
Aminophospholipids	**[29]**	5% in ethanol	60°C for 5 min.	Silica TLC
Triacylglycerols and phospholipids	**[30]**	5% in ethanol	110°C for 10 min.	Silica HPTLC
Terpenes	**[31]**	20% in ethanol	105°C for 15 min.	Silica TLC
Sesquiterpene lactones	**[32]**	10% in ethanol	100°C for 2 min	Silica OPTLC
Common sterols	**[33]**	10% in methanol or ethanol	110°C for 10 min.	Whatman No1 filter paper



Adapted from P.K. Zarzycki, M. A. Bartoszuk, A. I. Radziwon, „Optimization of TLC Detection by Phosphomolybdic Acid Staining for Robust Quantification of Cholesterol and Bile Acids", J. Planar Chromatogr., 19 (2006) 52-57.


Taurodeoxycholic Acid RP18
Time [min]
40
35
30
25
20
15
10
5
0
40
50
60
70
80
90
100
110
120
Temperature [°C]
30
20
10
0


Taurodeoxycholic Acid RP18
Time [min]
40
35
30
25
20
15
10
5
0
40
50
60
70
80
90
100
110
120
Temperature [°C]
30
20
10
0

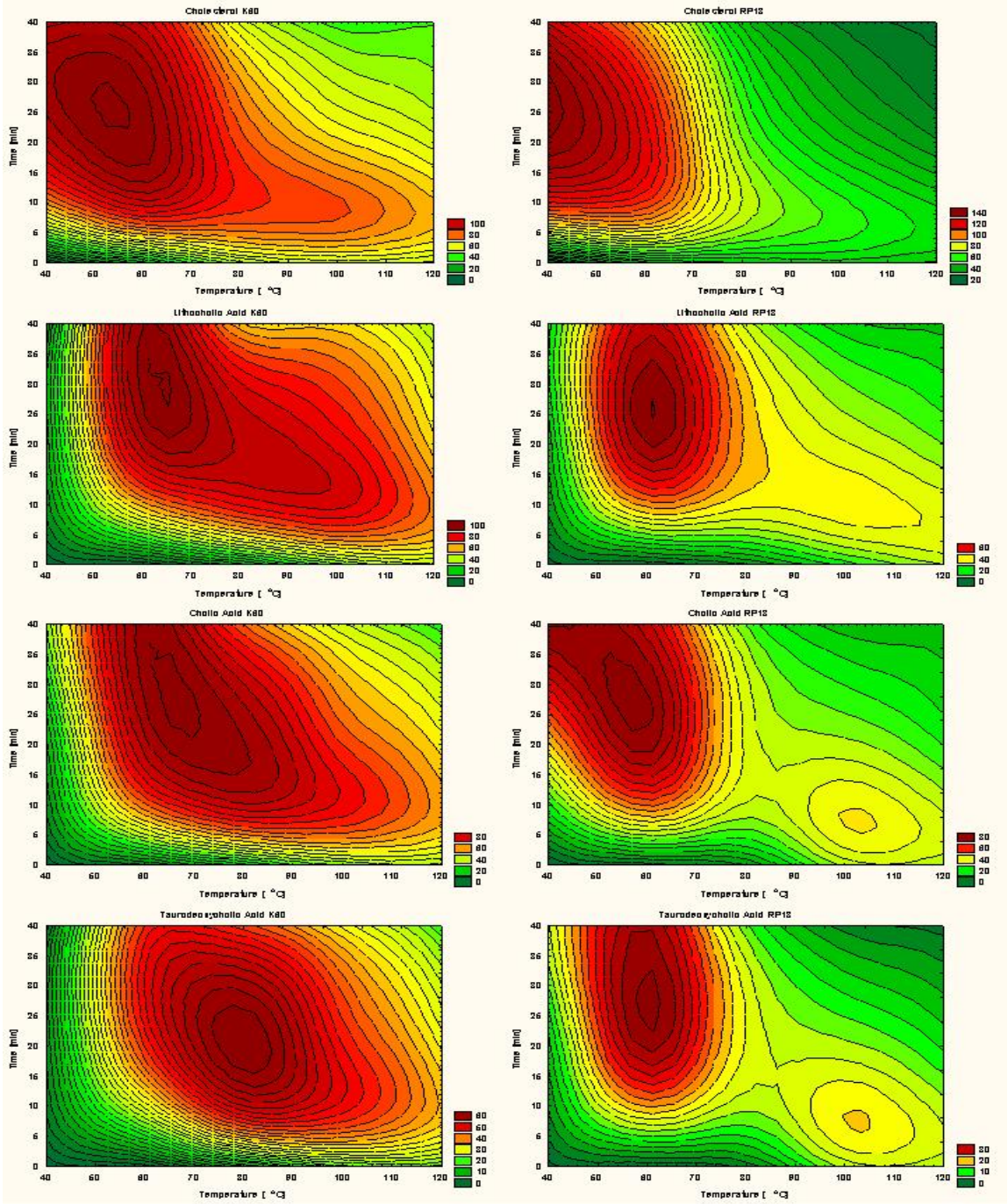

Cholesterol K60
Cholesterol RP18
Lithocholic Acid K60
Lithocholic Acid RP18
Cholic Acid K60
Cholic Acid RP18
Taurodeoxycholic Acid K60
Taurodeoxycholic Acid RP18
Time [min]
Temperature [°C]

Figure 2

CONCLUSION

For particular applications temperature-controlled planar chromatography shows potential to kick it's column counterpart out of the lime light.

APPLICATION OF SUBAMBIENT AND ELEVATED TEMPERATURES FOR TLC SEPARATION AND DETECTION PROTOCOLS

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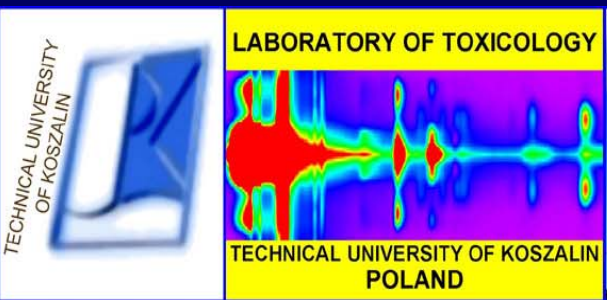
Technical University of Koszalin, Śniadeckich 2, 75-453 Koszalin, Poland

www.wbiis.tu.koszalin.pl/labtox

Berlin 2006



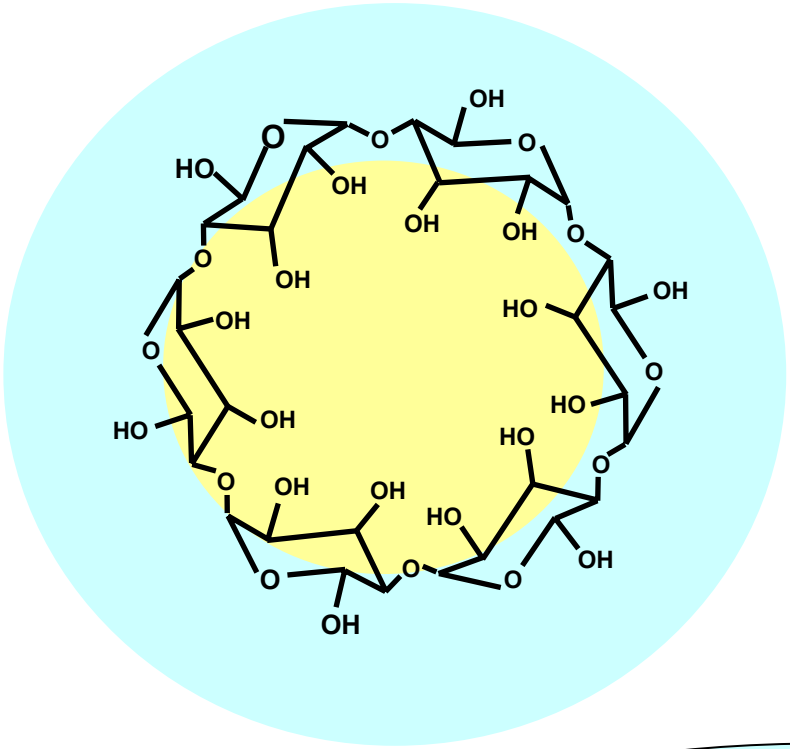
α-CYCLODEXTRIN

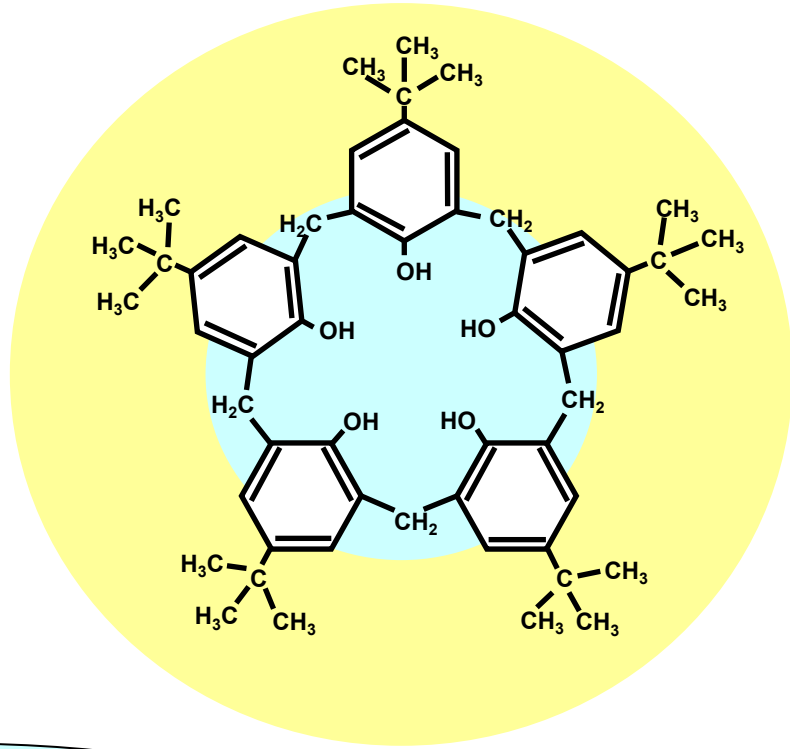
4-tert-BUTYLOKALIX[5]ARENE

Solvents:

Water, DMSO

Methanol, Ethanol, THF





Polar region

Non-polar area

SYSTEM I $(t_{R\,CD} \approx t_0)$

Macrocyclic modifier:

Cyclodextrin

Mobile Phase: 30% CH_3CN/H_2O

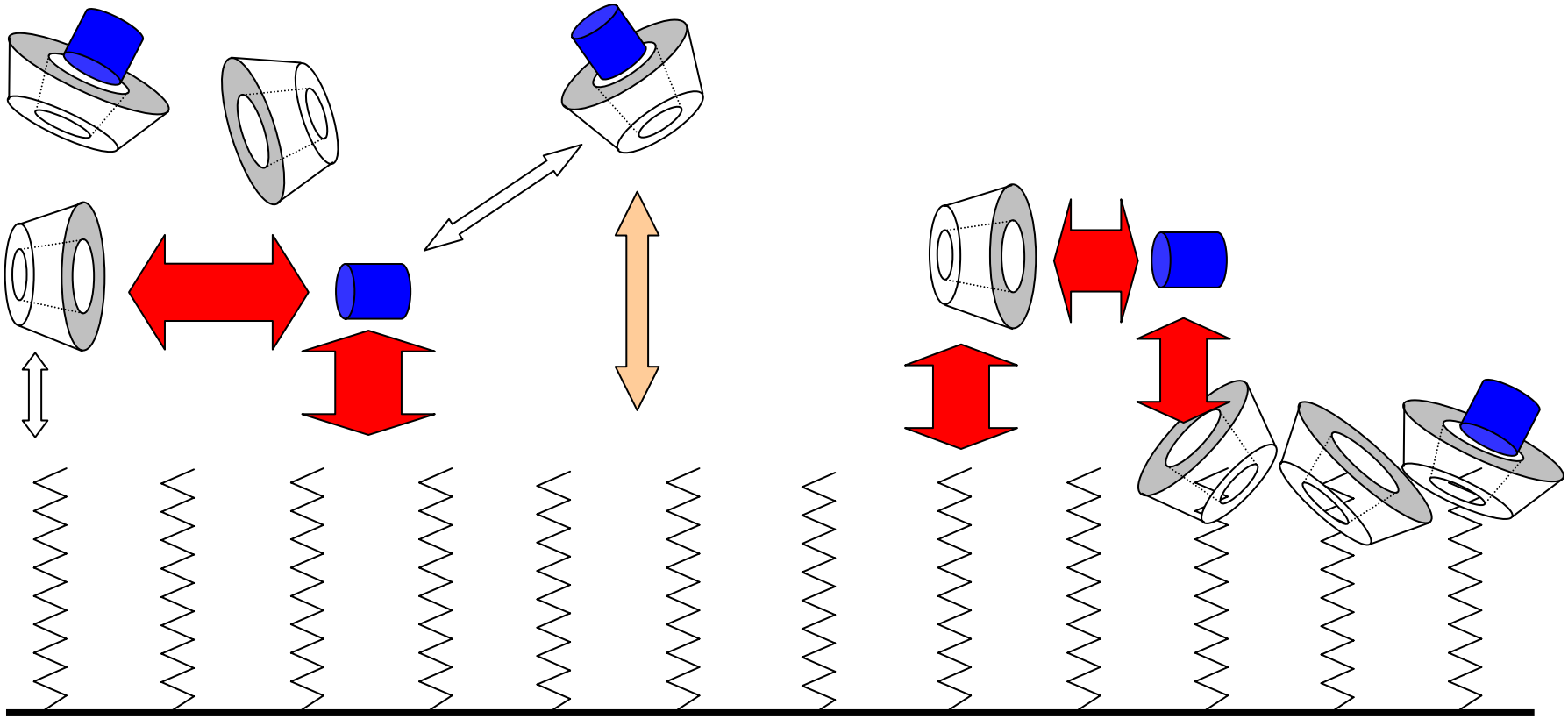
SYSTEM II $(t_{R\,CD} > t_0)$

Macrocyclic Modifier:

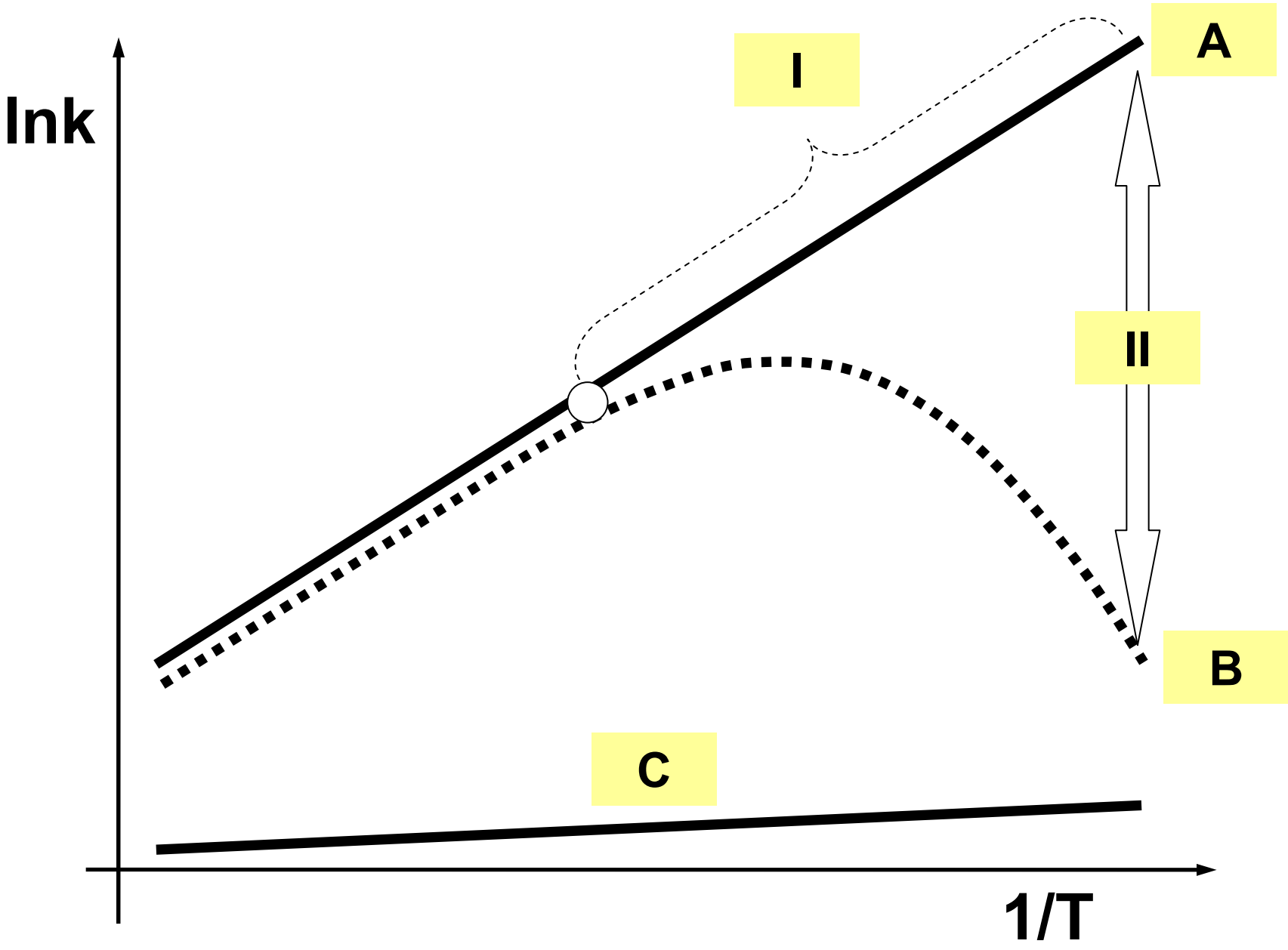
Cyclodextrin

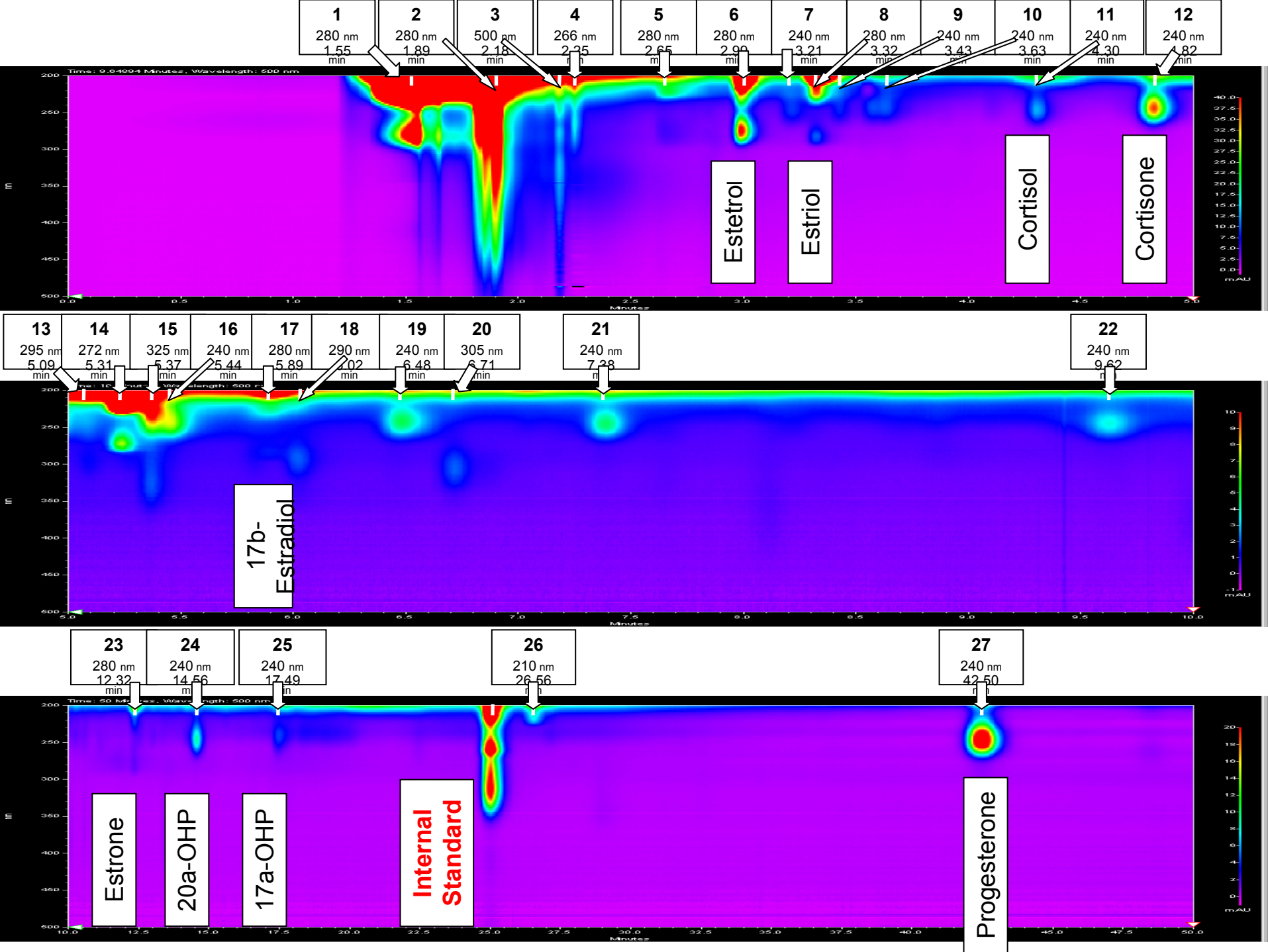
Mobile Phase: < 5% CH_3CN/H_2O

$C_{CD,\ System\ I} >>> C_{CD,\ System\ II}$

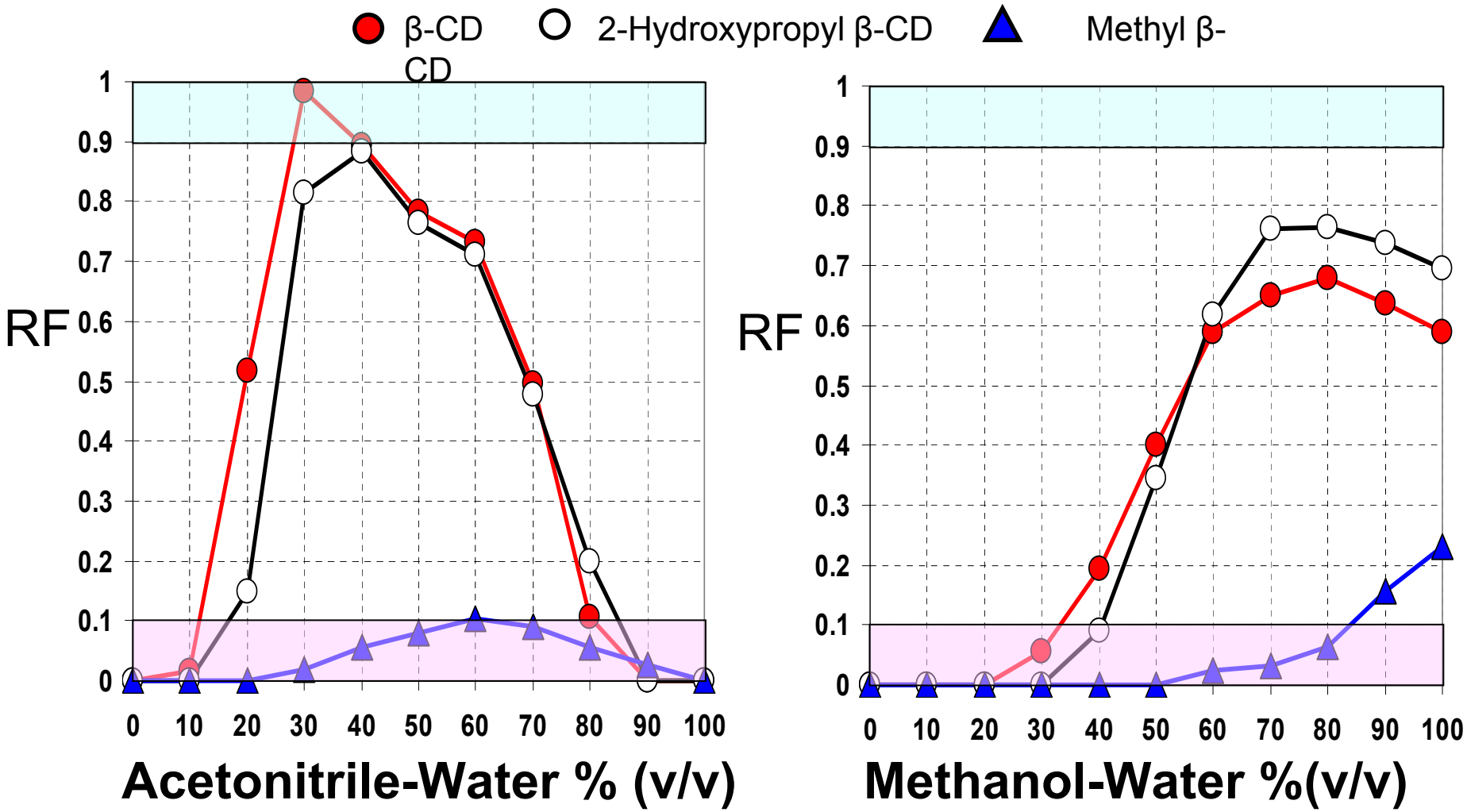


Stationary Phase: C-18


lnk
I
A
II
B
C
1/T


1 280 nm 1.55 min
2 280 nm 1.89 min
3 500 nm 2.18 min
4 266 nm 2.35 min
5 280 nm 2.65 min
6 280 nm 2.99 min
7 240 nm 3.21 min
8 280 nm 3.32 min
9 240 nm 3.43 min
10 240 nm 3.63 min
11 240 nm 4.30 min
12 240 nm 4.82 min
Estetrol
Estriol
Cortisol
Cortisone
13 295 nm 5.09 min
14 272 nm 5.31 min
15 325 nm 5.37 min
16 240 nm 5.44 min
17 280 nm 5.89 min
18 290 nm 6.02 min
19 240 nm 6.48 min
20 305 nm 6.71 min
21 240 nm 7.28 min
22 240 nm 9.62 min
17b-Estradiol
23 280 nm 12.32 min
24 240 nm 14.56 min
25 240 nm 17.49 min
26 210 nm 26.56 min
27 240 nm 42.50 min
Estrone
20a-OHP
17a-OHP
Internal Standard
Progesterone
Minutes
mAU

Chromatographic retention profiles of cyclodextrins under reversed-phase conditions



Stationary Phase: C-18 (HPTLC RP18W)

Table 2 Freezing temperatures (°C) of acetonitrile/water (35.6%, v/v) and methanol/water (30.0%, v/v) binary mobile phases unmodified and modified with β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin

	CD concentration/mM						
	0	0.1	1	5	10	50	100
Acetonitrile/Water							
β-Cyclodextrin	–10.08 (±0.01)	–10.09 (±0.01)	–10.09 (±0.01)	–10.10 (±0.01)	NA	NA	NA
2-Hydroxypropyl-β-CD	–10.08 (±0.01)	–10.07 (±0.05)	–10.06 (±0.04)	–10.08 (±0.02)	–10.12 (±0.03)	–10.295 (±0.007)	–10.532 (±0.005)
Methanol/Water							
β-Cyclodextrin	–23.4 (±0.5)	–24.3 (±0.4)	–23.6 (±0.8)	NA	NA	NA	NA
2-Hydroxypropyl-β-CD	–23.4 (±0.5)	–24.3 (±0.2)	–23.8 (±0.7)	–23.6 (±0.7)	–23.3 (±0.2)	–24.0 (±0.6)	–25.0 (±0.4)

NA: Non available. The numbers in parentheses correspond to the standard deviation values; number of samples, 5.

Selectivity

$$\alpha = k_2/k_1$$

Resolution

$$R_s = (t_{R2} - t_{R1})/[(w_{B1} - w_{B2})/2]$$

Relative Resolution Product

$$r = \frac{\prod_{i=1}^{n-1} R_{S_{i+1,i}}}{\left[\left(\sum_{i=1}^{n-1} R_{S_{i+1,i}}\right)/(n-1)\right]^{n-1}}$$

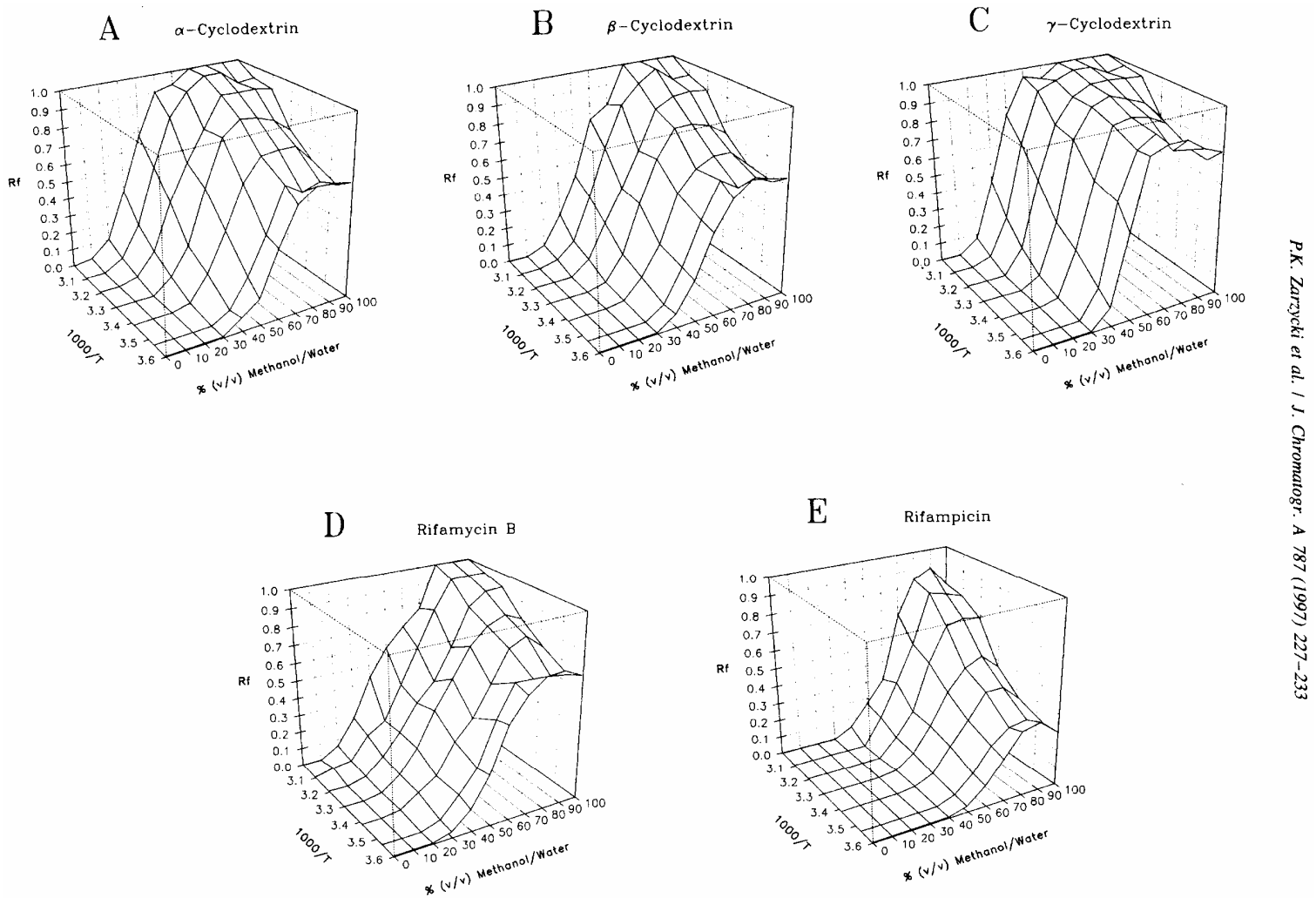
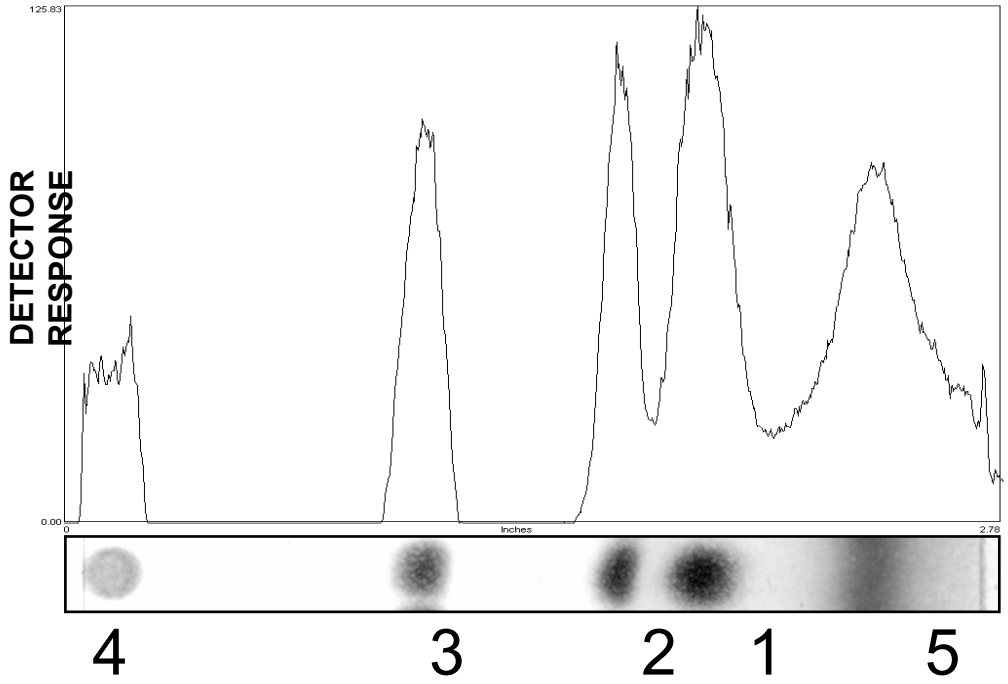


Fig. 1. Relationships between R_f values of α- (A), β- (B), and γ-cyclodextrin (C), rifamycin B (D) and rifampicin (E) versus different mobile-phase compositions and reciprocal of absolute temperature.

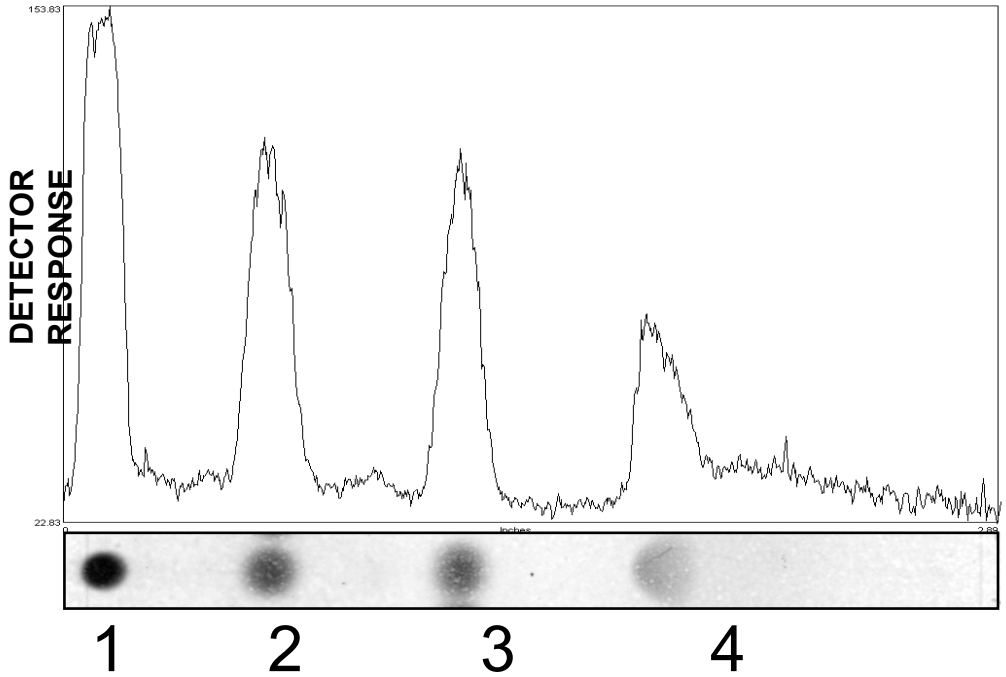
Adapted from:

P. K. Zarzycki, J. Nowakowska, A. Chmielewska, M. Wierzbowska, H. Lamparczyk, "Thermodynamic study of retention of selected macrocycles using RP-HPTLC plates and methanol/water mobile phases", *J. Chromatogr A*., 787 (1997) 227-233.

K60W


125.83
DETECTOR RESPONSE
0.00
0
Inches
2.78
4
3
2
1
5

RP18W


153.83
DETECTOR RESPONSE
22.83
Inches
1
2
3
4

Contribution of separation techniques for quantification, impurity tests and substance identification protocols used in European Pharmacopoeia monographs