

Multidimensional Planar Chromatography

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Biological matrices, e.g., plants can contain up to several thousand secondary metabolites



Therefore multidimensional separation techniques are to be used !

Topic of the lecture:

I. Criteria of multidimensionality

II. Characterisation of solvents

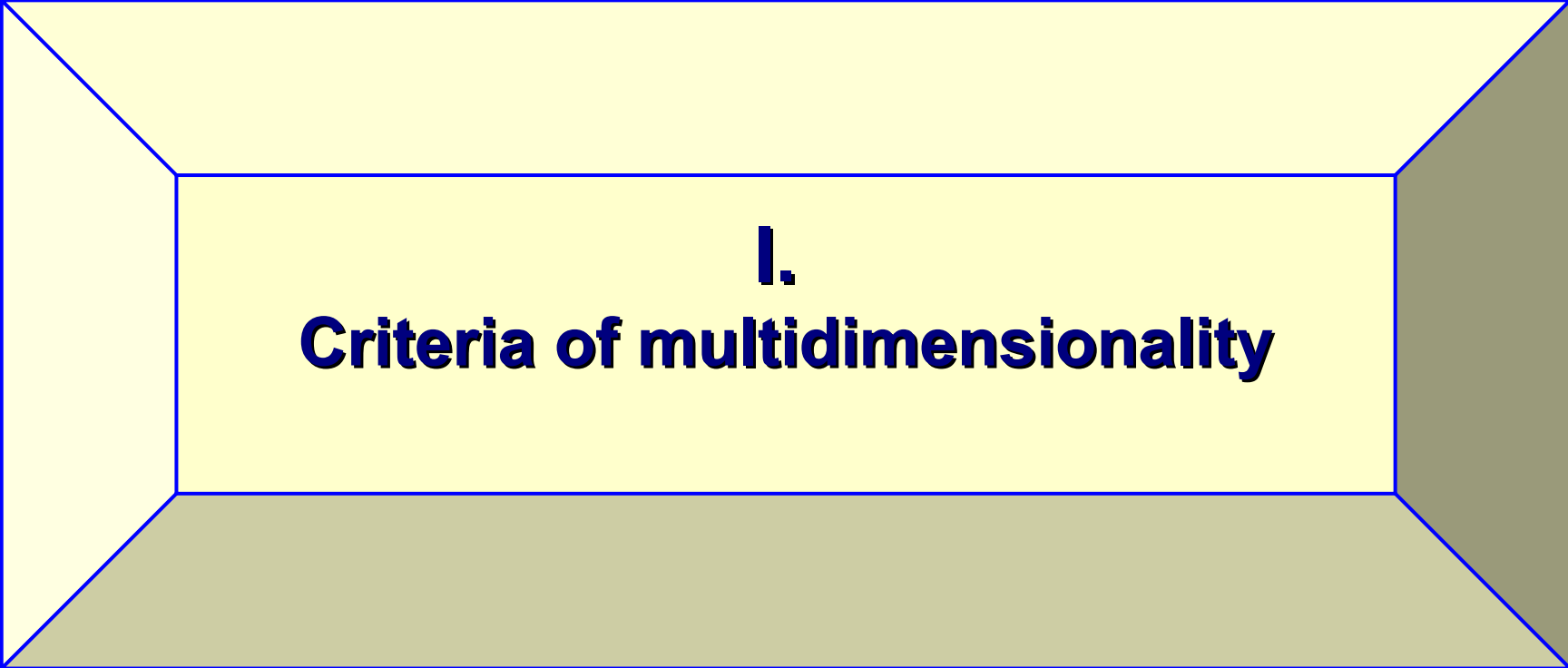
III. Comprehensive two-dimensional PC

IV. Targeted or selective two-dimensional PC

V. Modulated two-dimensional PC

VI. Coupled-layer PC

VII. Combined MD-PC methods



I.
Criteria of multidimensionality

Criteria of multidimensionality

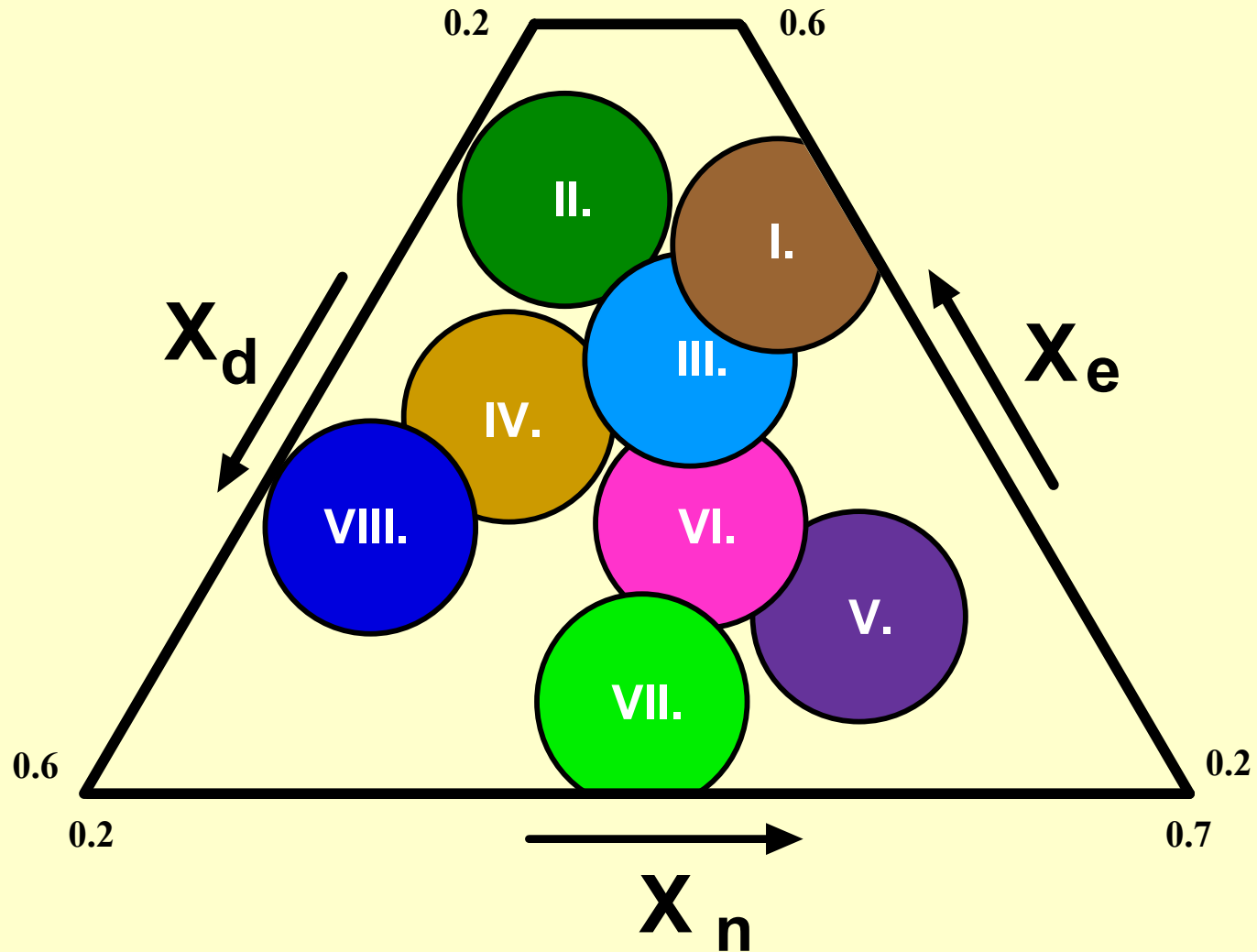
"First, it is one in which the components of a mixture are subjected to two or more separation steps in which their displacements depend on different factors.

The second criterion is that when two components are substantially separated in any single step, they always remain separated until the completion of the separative operation."



II.
Characterisation of solvents

Snyder's solvent characterisation



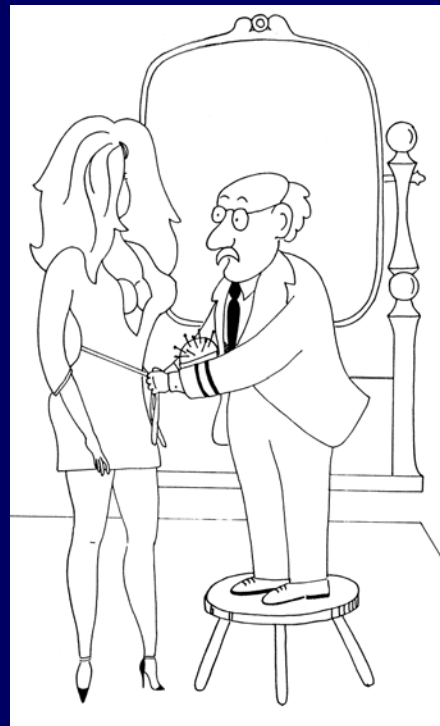
Characterisation of solvents based on Snyder's classification



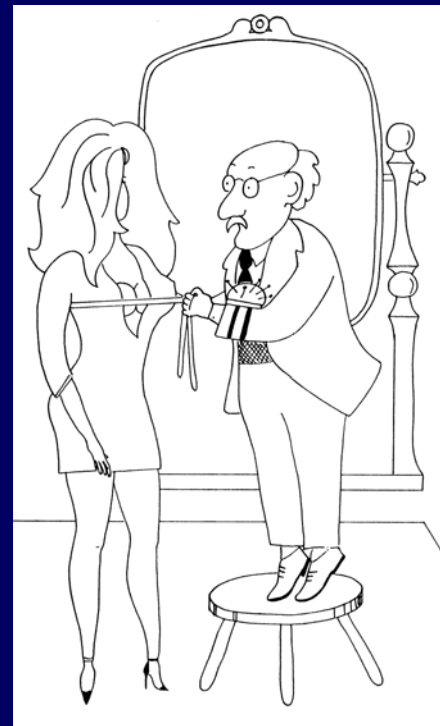
S_i



X_n



X_e



X_d

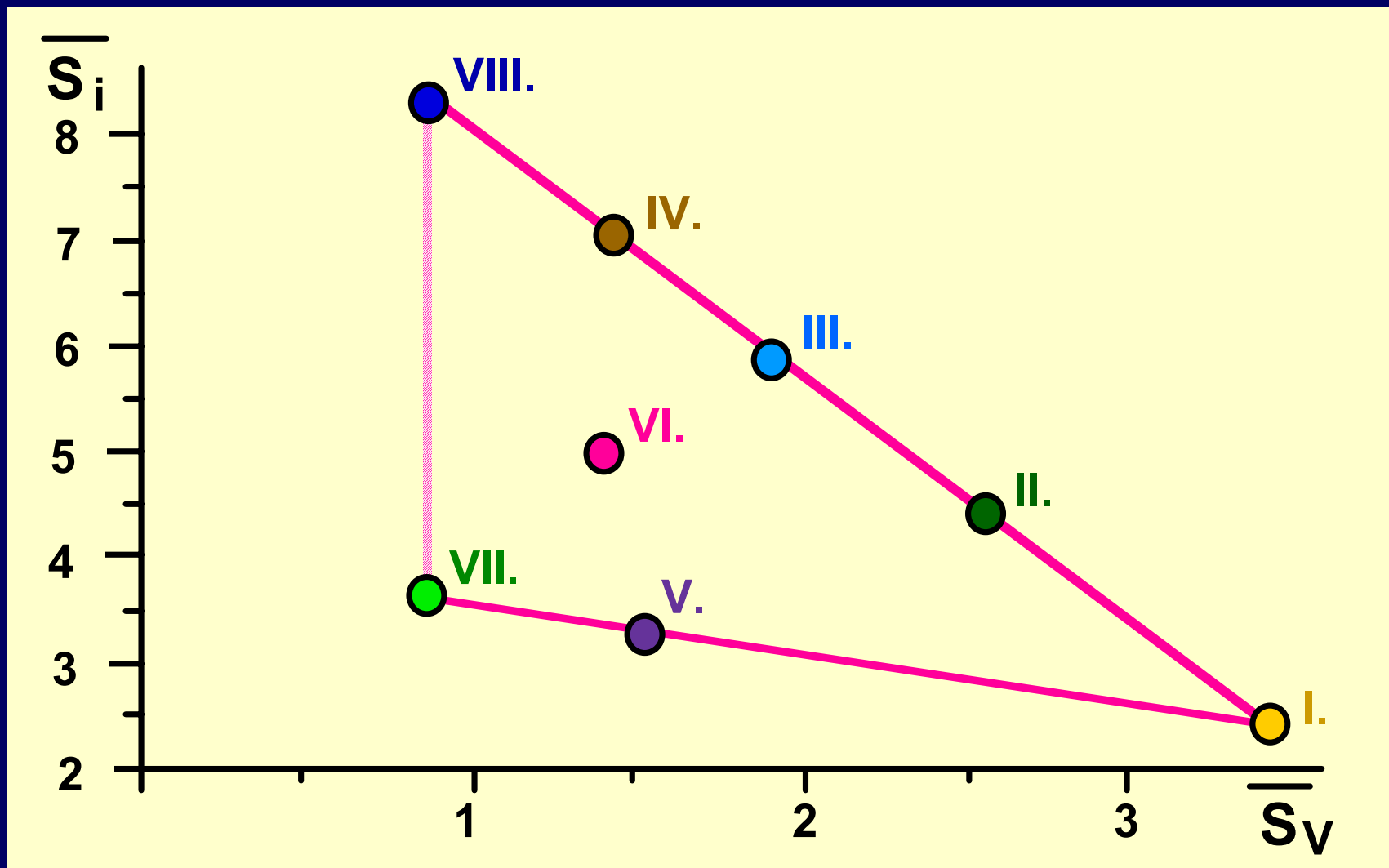
Characterisation of solvents based on Snyder's classification



Definition of selectivity value:

$$S_v = \frac{X_e}{X_d}$$

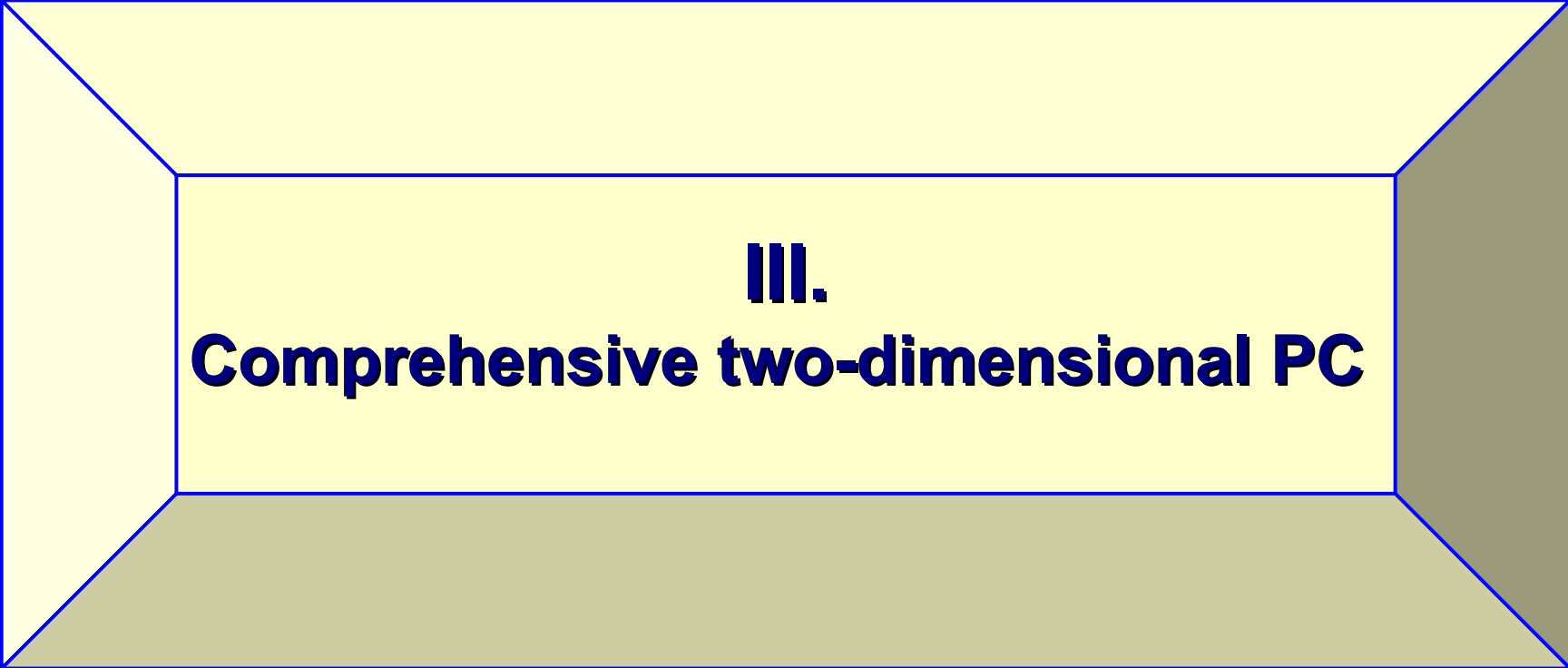
Connection between the average selectivity values and average solvent strength values



Orthogonality

**Orthogonal stationary phases:
completely independent retention mechanisms
(e.g., RP, NP phases) ensure the separation**

**Orthogonal solvent systems:
completely different total solvent strength and
total selectivity values characterises the
mobile phases**



III.
Comprehensive two-dimensional PC

Comprehensive two-dimensional PC (PC x PC)

MD-PC technique

**using the same monolayer stationary phase and
two developments with different (orthogonal)
solvent systems (mobile phases)**

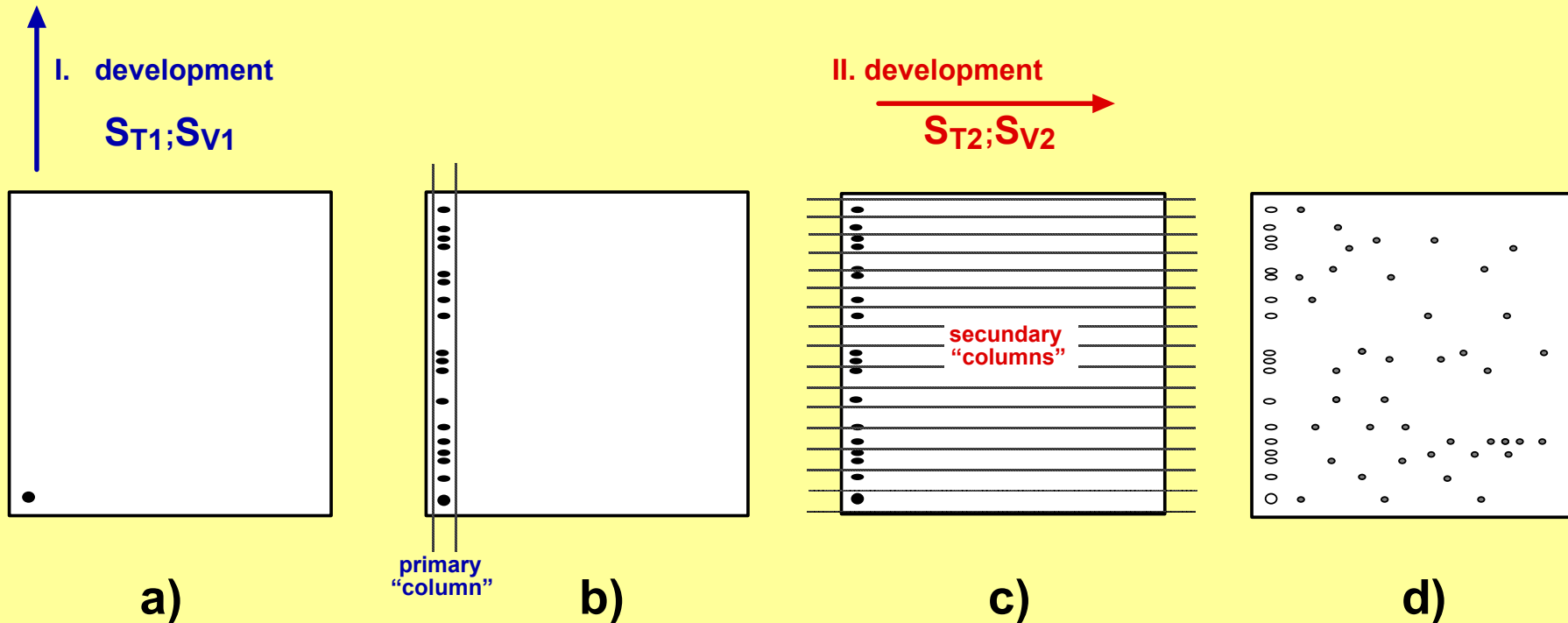
characterised by different

solvent strength (S_T) and selectivity values (S_V),

or

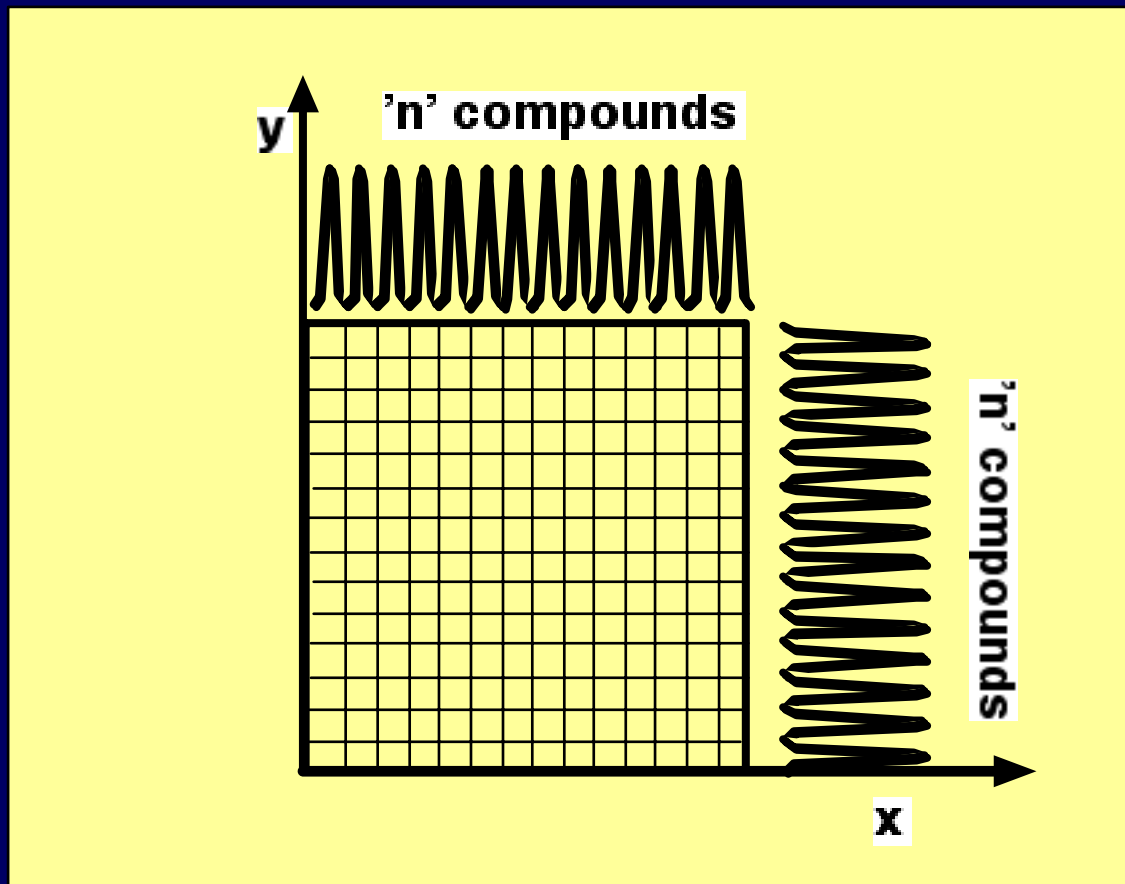
**using a bilayer (orthogonal) stationary phase and
two developments with the same or different
solvent systems /mobile phases**

Schematic illustration of a comprehensive two-dimensional PC (PC x PC) separation

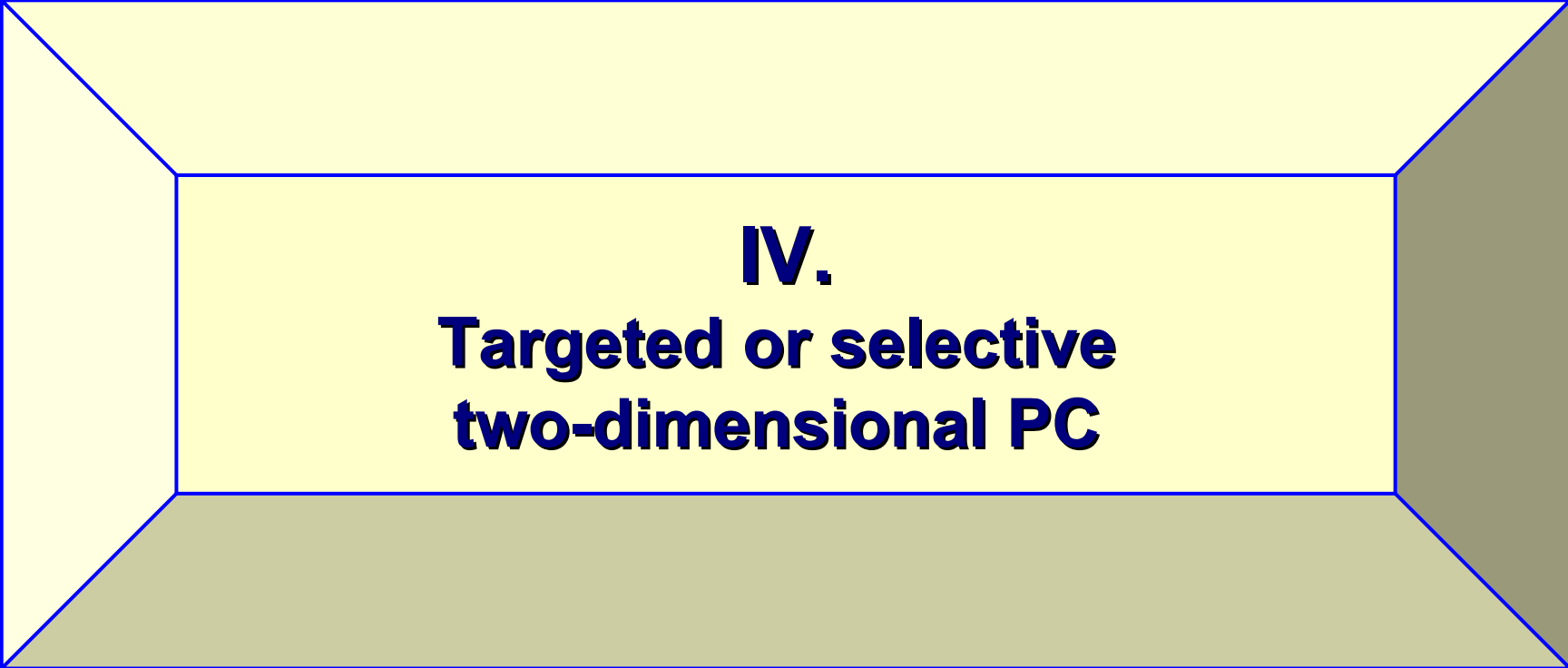


The number of "secondary columns" is unlimited, demonstrating the multidimensionality of PC

Schematic illustration of the peak capacity (n^2) of a PC x PC separation



The number of squares represents the number of compounds which can theoretically be separated

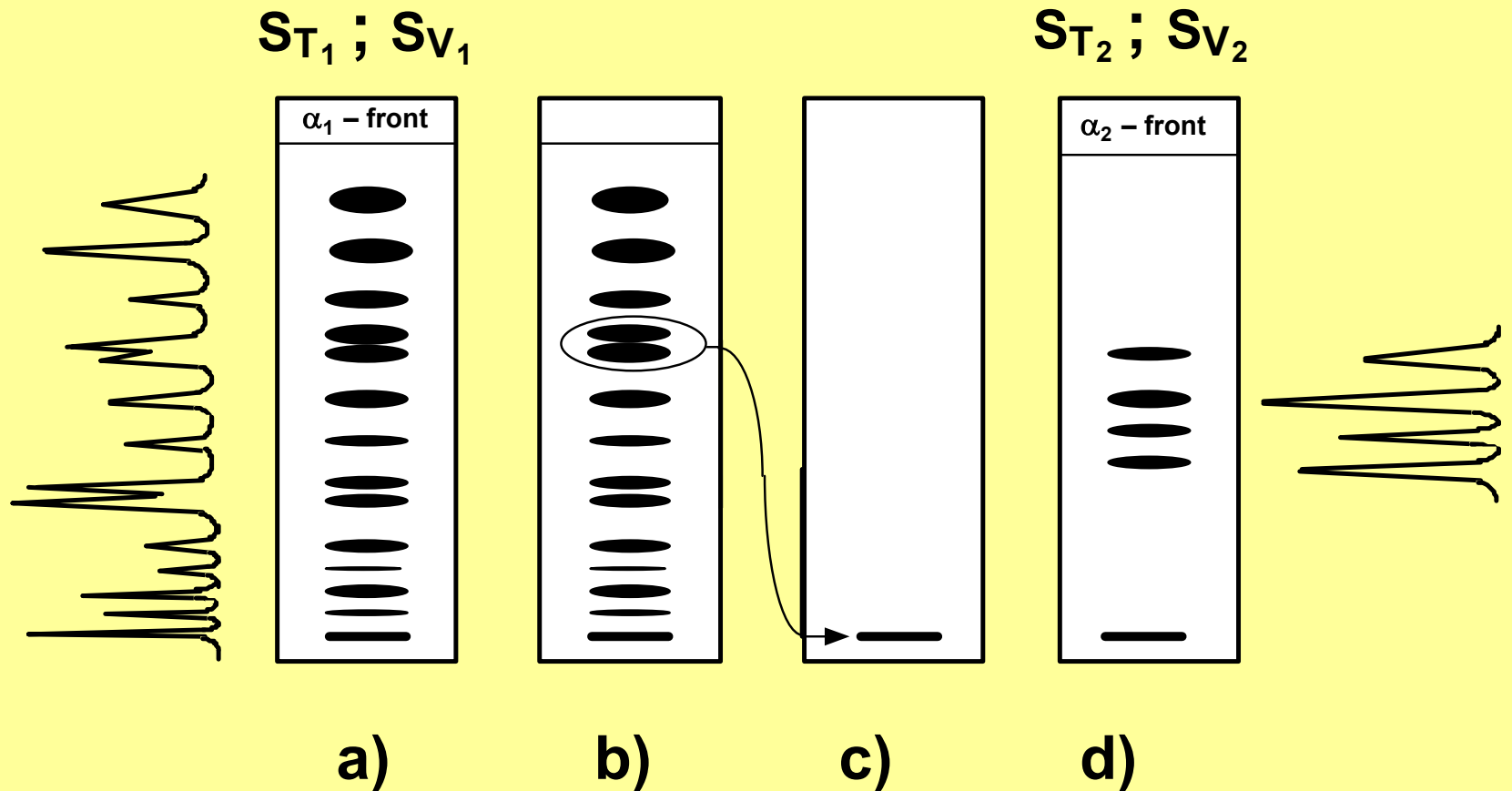


IV.
Targeted or selective
two-dimensional PC

Targeted (selective) two-dimensional PC ***(PC + PC)***

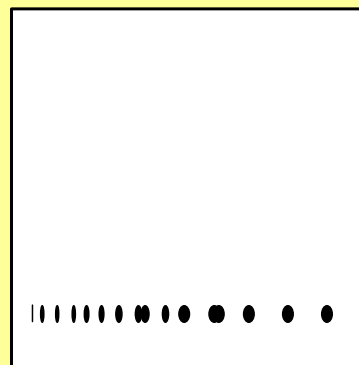
MD-PC technique, where after the first development from the stationary phase heart-cut spot applied to a second (the same or different type) stationary phase for subsequent development of the transferred compounds with different or the same solvent system/mobile phase composition, respectively

Schematic illustration of the steps of a targeted or selective two-dimensional PC (PC + PC) separation



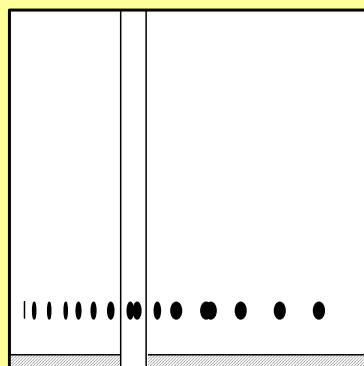
Schematic illustration of the steps of in-situ targeted or selective two-dimensional PC (PC + PC) separation

I. development
→
 $S_{T_1}; S_{V_1}$

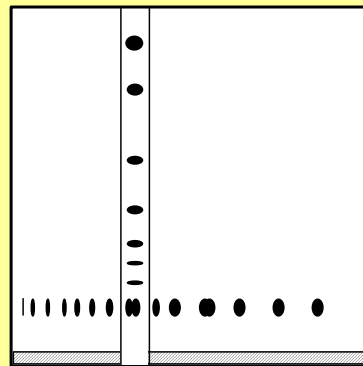


a)

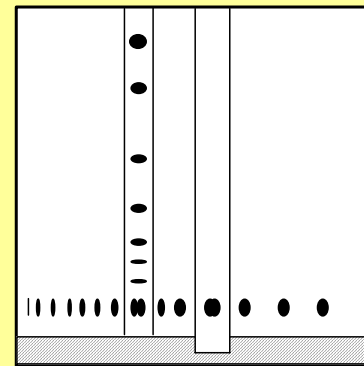
II. development
↑
 $S_{T_2}; S_{V_2}$



b)

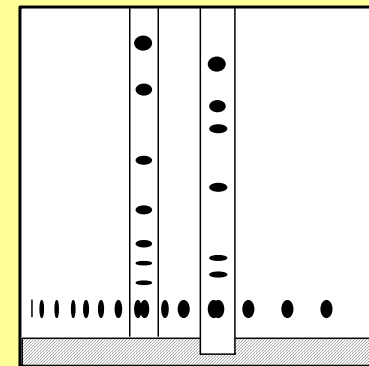


c)

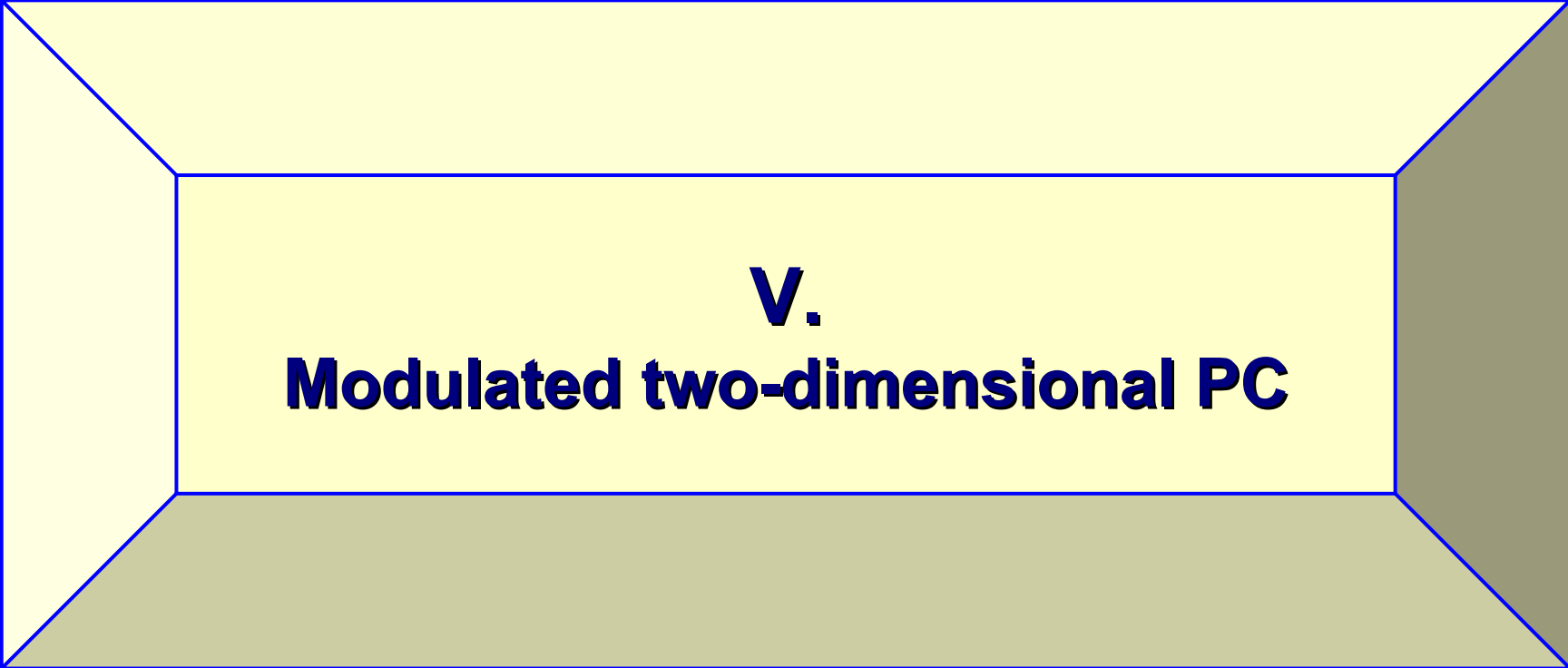


d)

III. development
↑
 $S_{T_3}; S_{V_3}$



e)

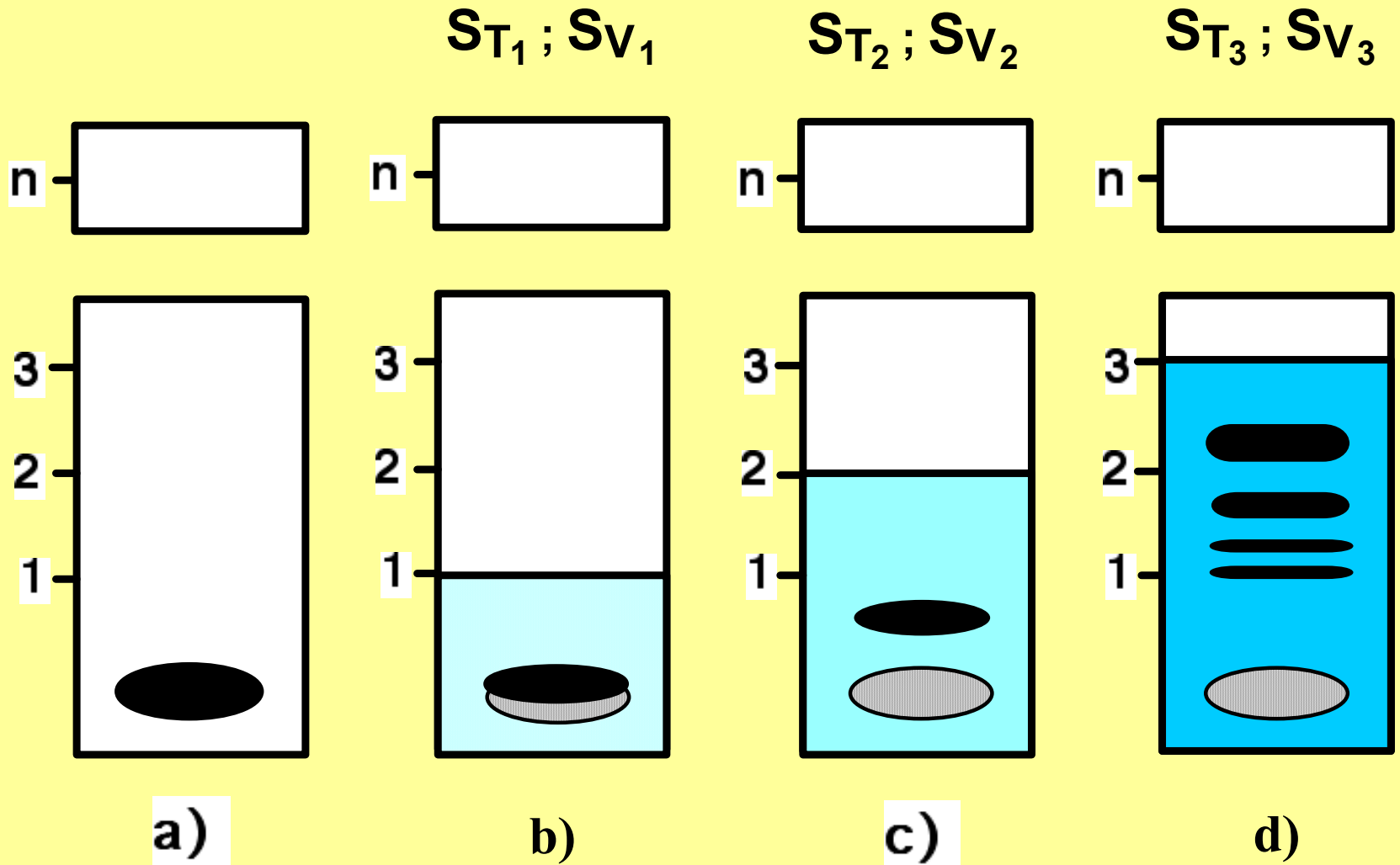


V.
Modulated two-dimensional PC

***Targeted or modulated
two-dimensional PC (ⁿPC)***

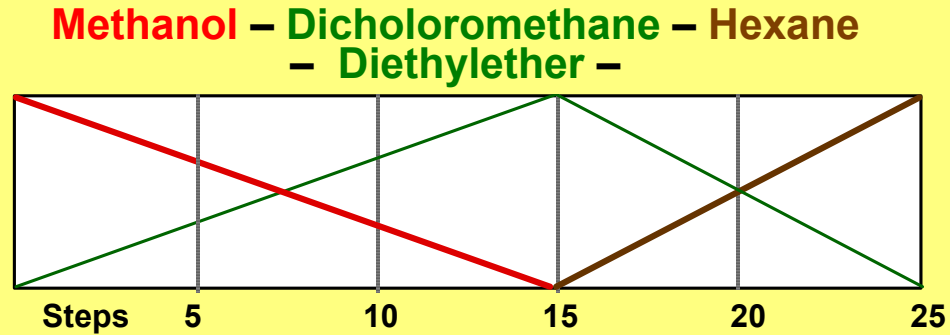
**MD-PC technique,
using the same stationary phase (in one, two,
or three geometrical dimensions) using solvent
systems/mobile phases with decreasing solvent
strength and different selectivity values**

Schematic illustration of modulated two-dimensional PC (n PC) separation



Universal used gradients during the stages of AMD separations

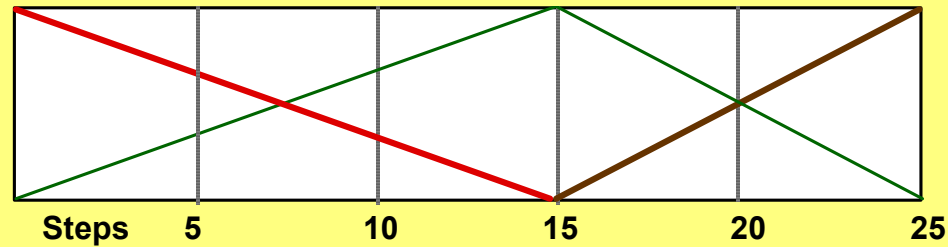
a)



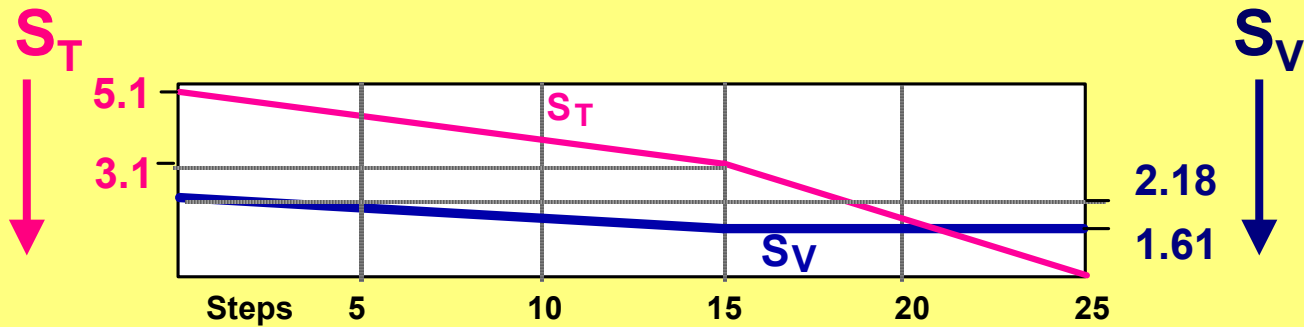
Universal used gradients during the stages of AMD separations

Methanol – Dicholoromethane – Hexane
– Diethylether –

a)



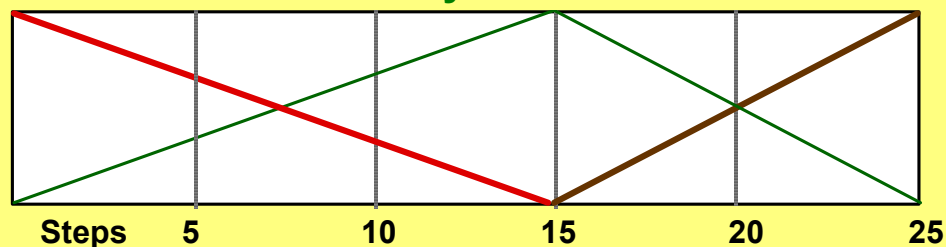
b)



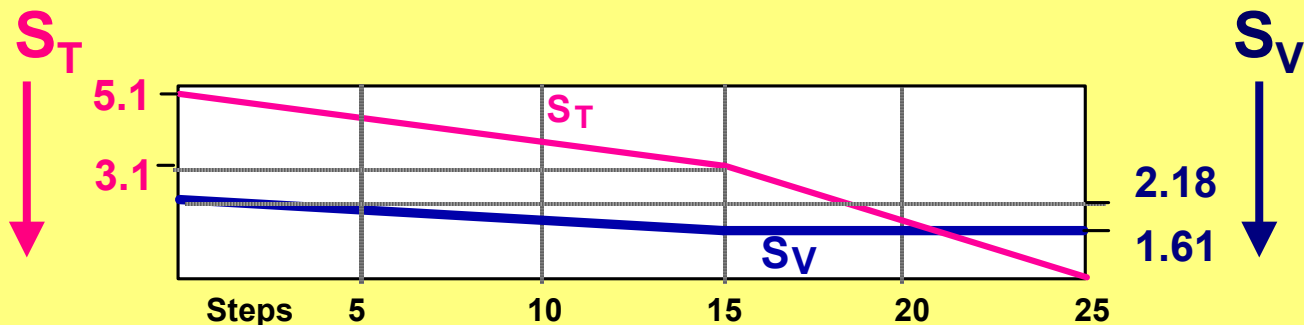
Universal used gradients during the stages of AMD separations

Methanol – Dichloromethane – Hexane
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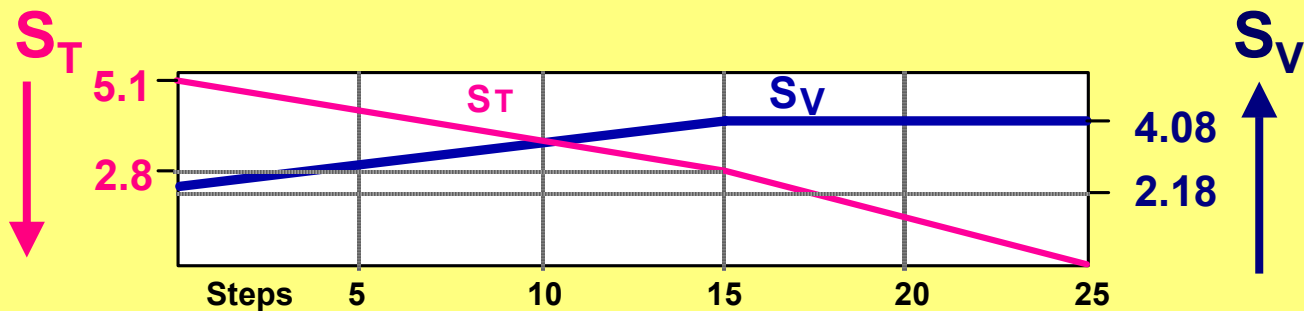
a)



b)



c)



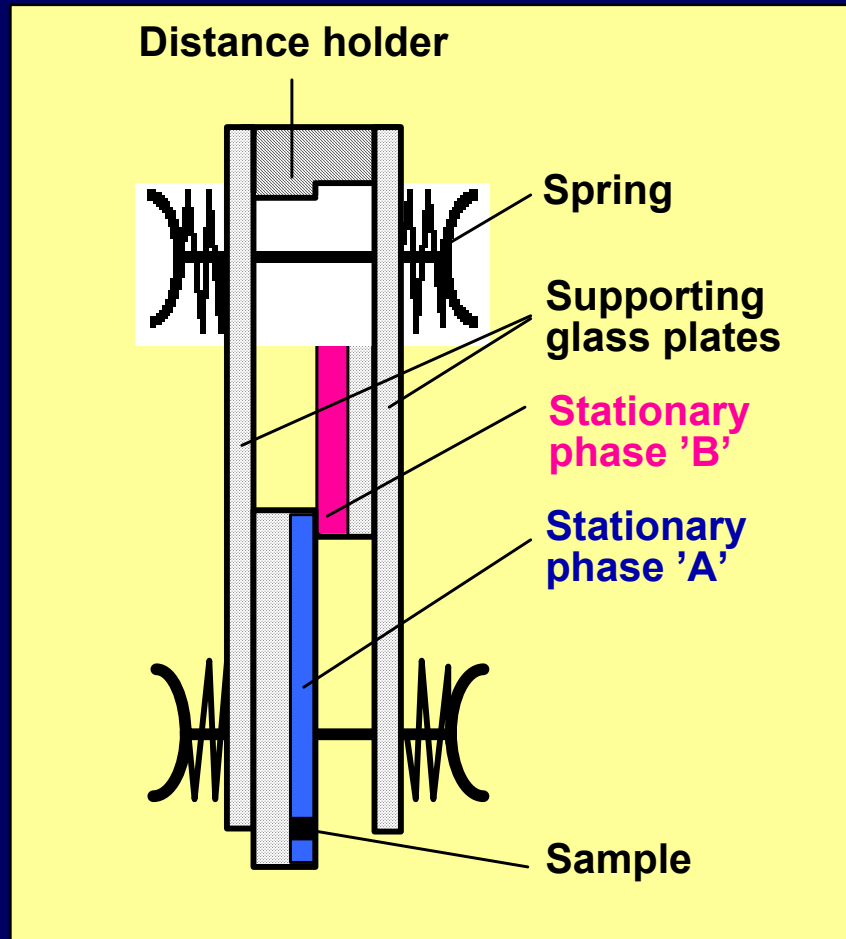


VI.
Coupled-layer PC

Coupled-layer PC ***(PC - PC)***

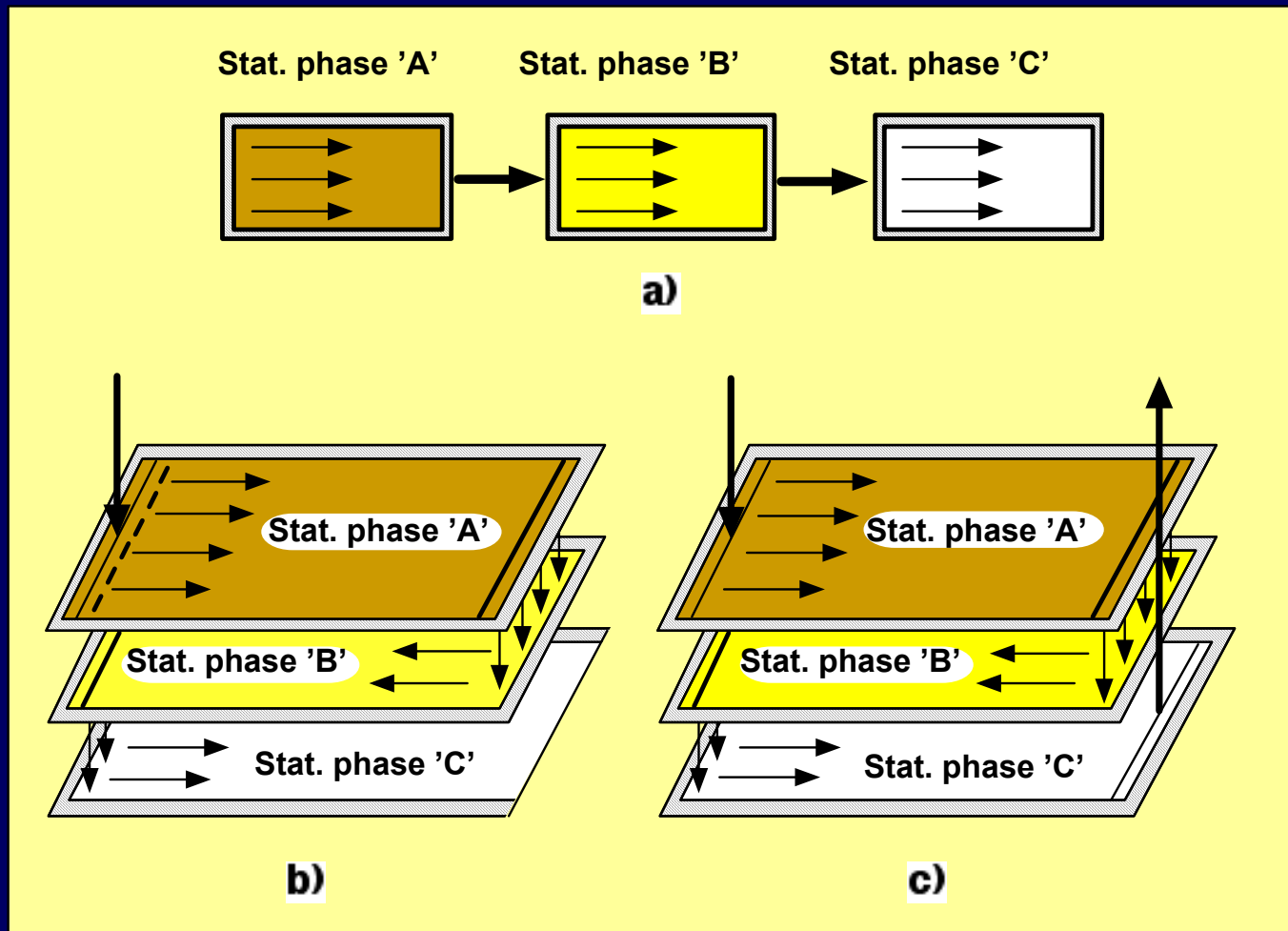
**MD-PC technique,
using coupled layers with orthogonal stationary
phases developed with a solvent system/mobile
phase of constant composition**

Schematic diagram of cross section of coupled layer PC (PC – PC) separation



The system ensure MD separation on stationary phases of decreasing polarity

Schematic illustration of coupled layer OPLC (OPLC – OPLC) separation



The system ensure MD separation on three stationary phases of decreasing polarity



VII.
Combined MD-PC methods

Combined MD–PC methods (cMD–PC)

A combination of at least two of the above mentioned modes, or coupling of two chromatographic techniques in which PC is used as the second dimension and another separation method, e.g., gas chromatography (GC), high-performance liquid chromatography (HPLC), etc., as the first.

Combined MD–PC methods (cMD–PC)

Some possible variations, where PC can be used as the second dimension are listed in the following:

GC x PC,
SFC x PC,
HPLC x PC,
CE x PC,
CCC x PC,
OPLC x PC,
RPC x PC

Conclusion

Scientists working with two-dimensional TLC have to use new terms like:

**COMPREHENSIVE,
ORTHOGONAL,
TARGETED,
MODULATED ,
COUPLED**

multi-dimensional planar chromatography

Nowadays, when there generally seems to be too much competition around, collaboration is a better strategy
(van Deemter)



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