

System Peaks in Liquid Chromatography

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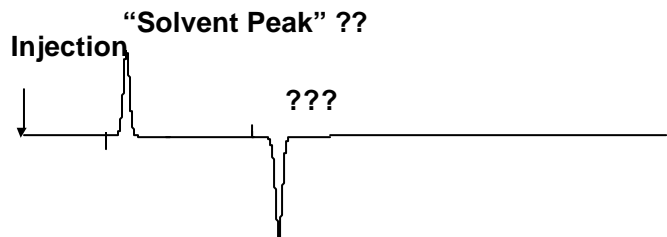
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INJECTION OF PURE SOLVENT



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Separation of Free Amino Acids by RP HPLC

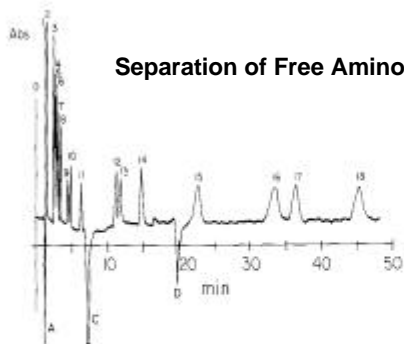


Fig. 8. Chromatogram obtained at 45°C. Conditions and peaks as in Fig. 7.

Separation of Free Amino Acids by RP HPLC

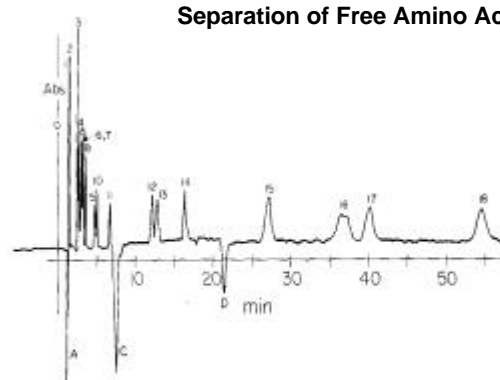
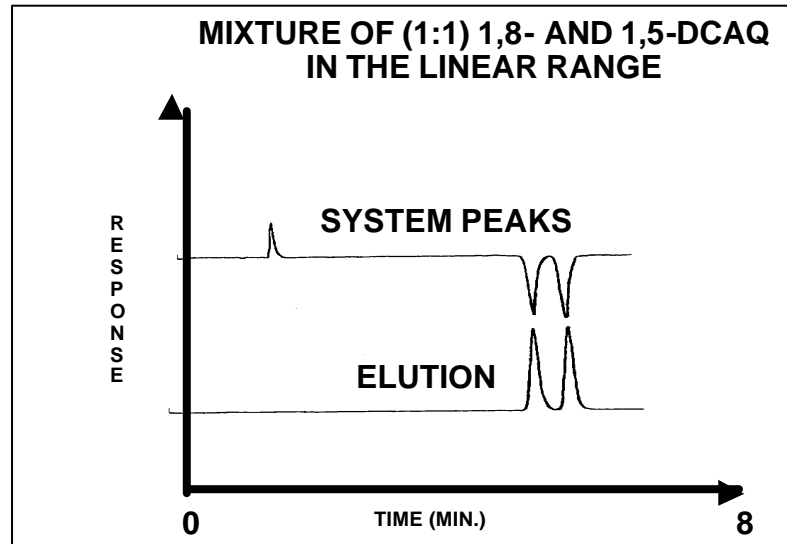
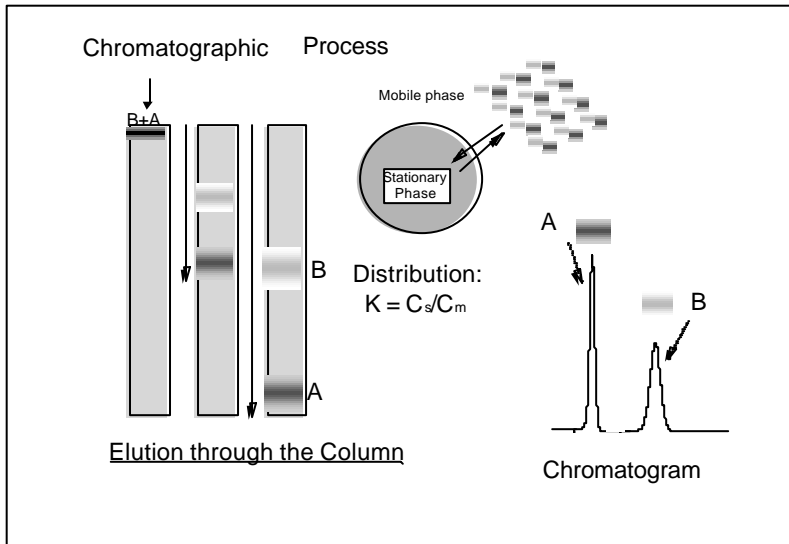


Fig. 7. Chromatogram obtained at 40°C. Mobile phase, 10 mM acetate buffer-0.4 mM copper(II) acetate-0.8 mM heptanesulfonate. Other conditions as in Fig. 1. Peaks: 1 = Asp; 2 = Glu; 3 = Gly + Ser; 4 = Asn; 5 = Gln; 6 = Thr; 7 = Ala; 8 = Thr; 9 = α Abu; 10 = His; 11 = Pro; 12 = Val; 13 = Nvl; 14 = Met; 15 = Tyr; 16 = Ile; 17 = Leu; 18 = Arg.

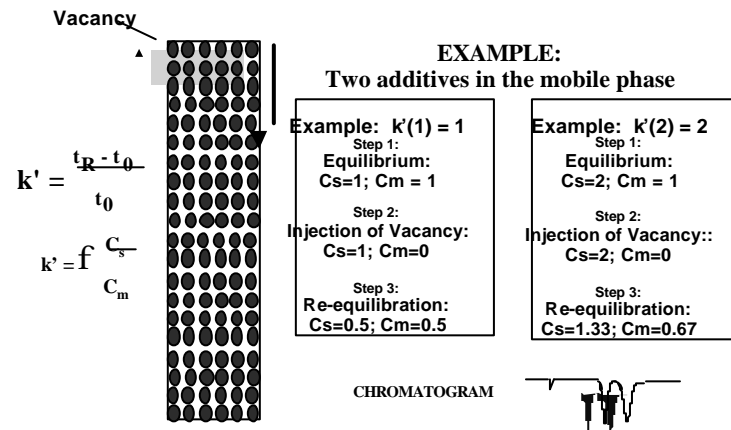
System Peaks in Liquid Chromatography



CONDITIONS FOR APPEARANCE OF SYSTEM PEAKS

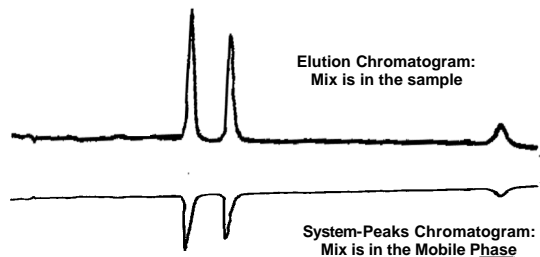
- Mobile phase is multicomponent ($n \geq 2$)
- Mobile phase contains adsorbable components
- Mobile phase's components respond to the detector (high background)
- Sample or sample diluent are different than the mobile phase, enough to create equilibrium perturbation.

Mechanism of System Peaks Formation



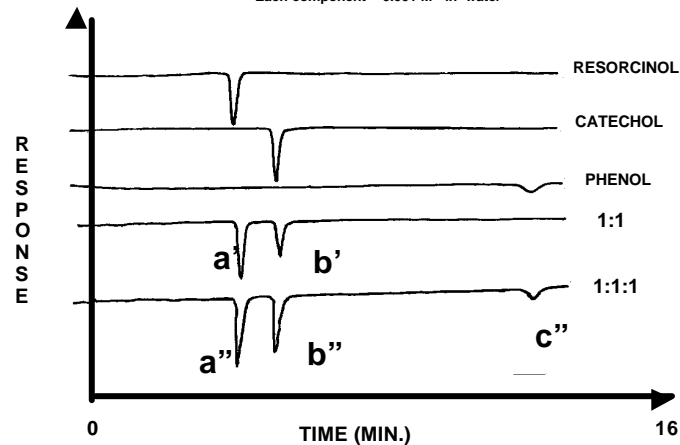
System Peaks in Liquid Chromatography

Resorcinol-Catechol-Phenol 1:1:1 Mixture in the Linear Range

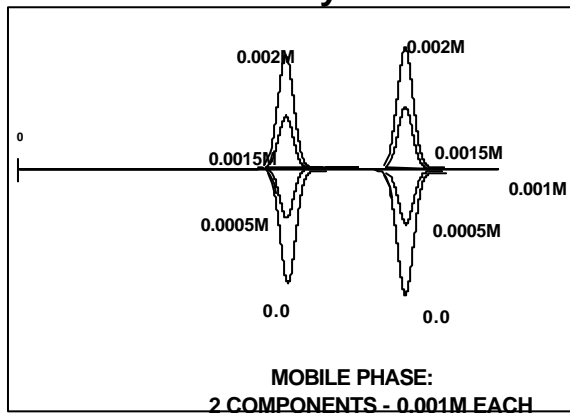


SYSTEM PEAKS (VACANCY) AT THE LINEAR RANGE

Each component = 0.001 M in water

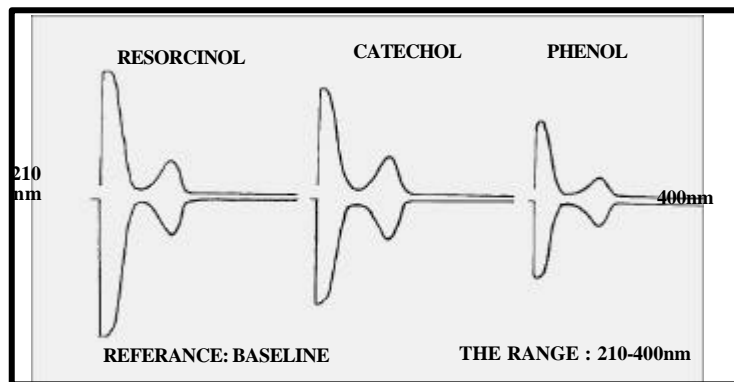


Detection of System Peaks

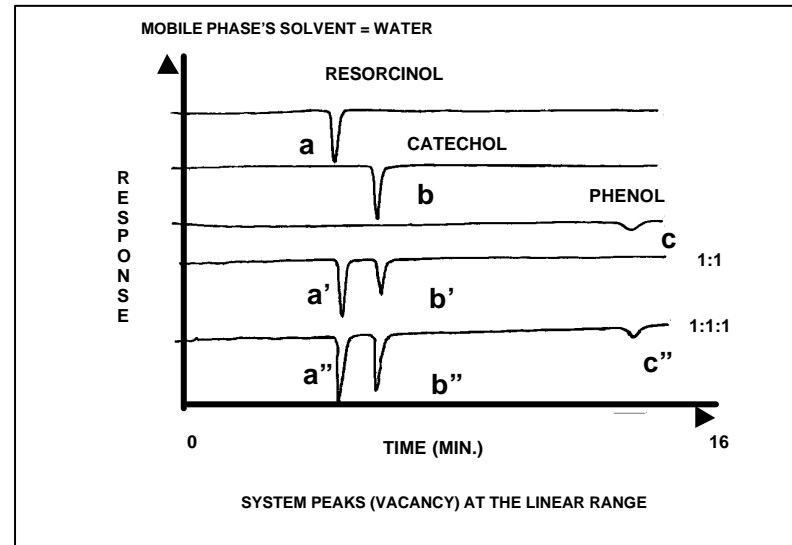
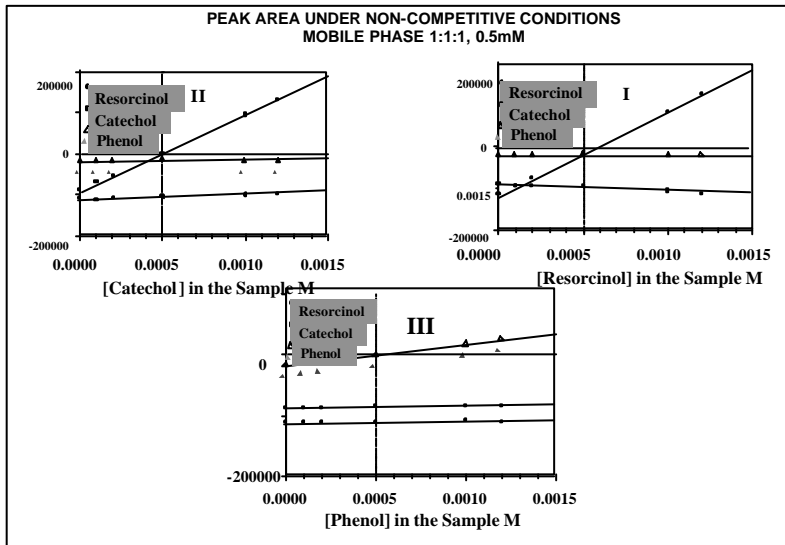
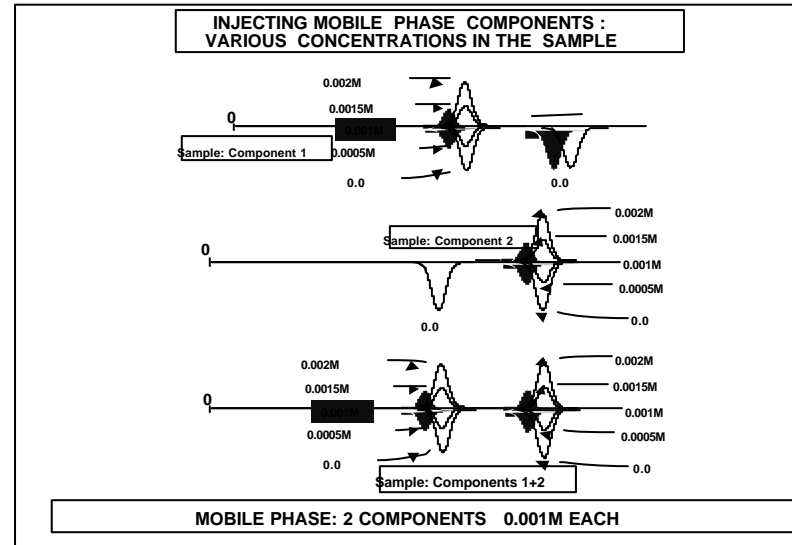
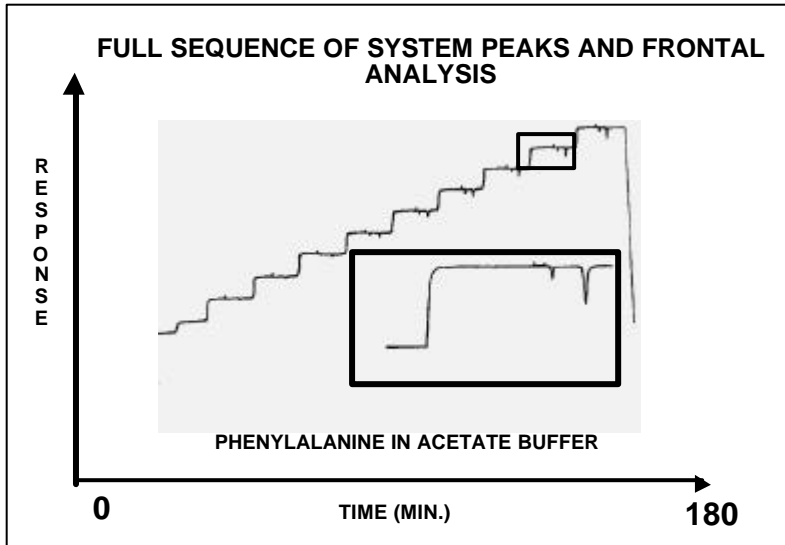


UV-SPECTRUM OF (1:1:1) MIXTURE

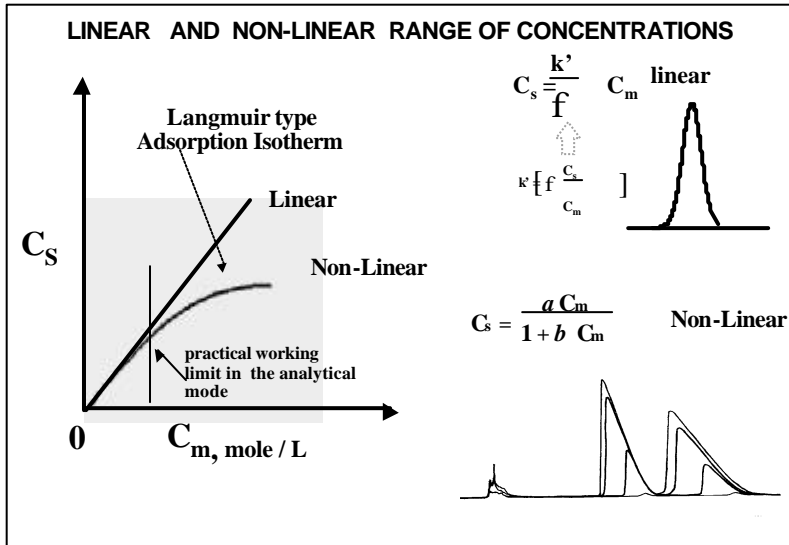
Apex of elution peak vs apex of system peak



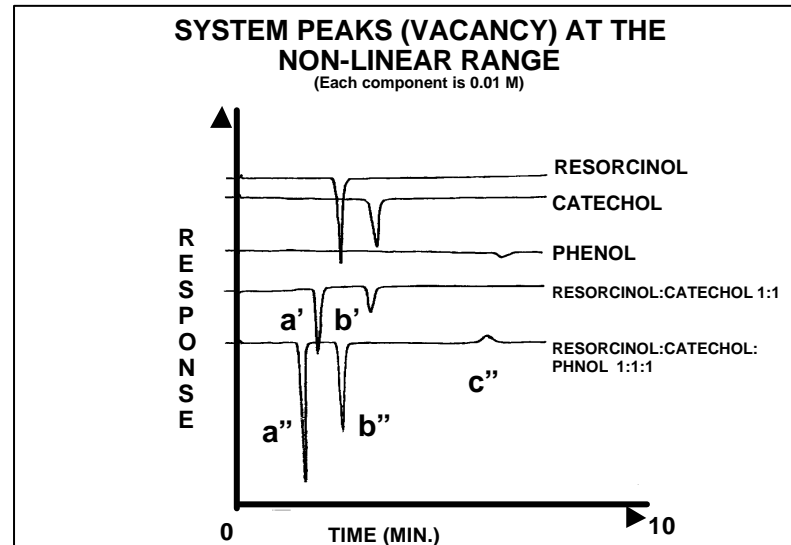
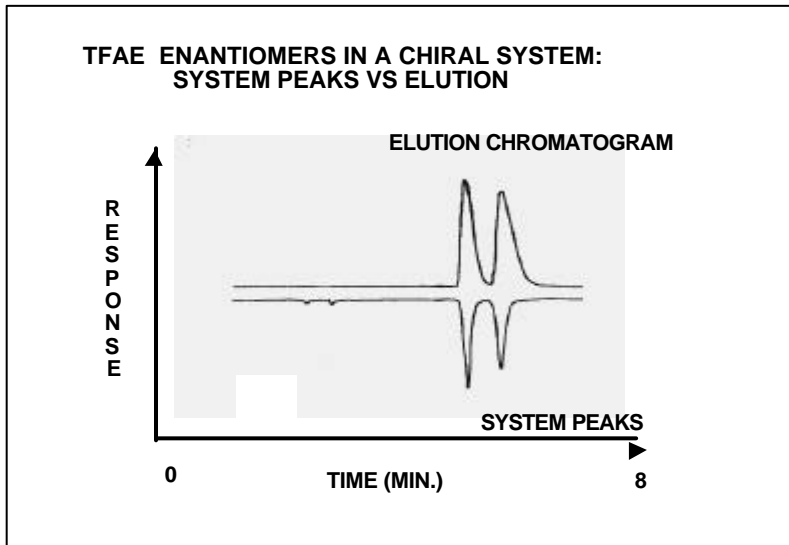
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System Peaks in Liquid Chromatography

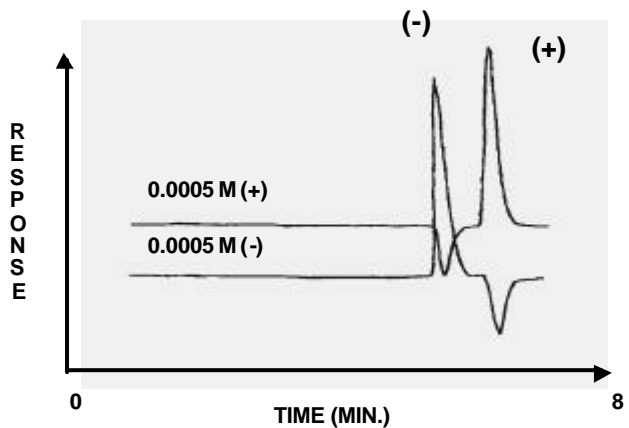


SYSTEM PEAKS (VACANCY) AT THE NON-LINEAR RANGE OF CHROMATOGRAPHY

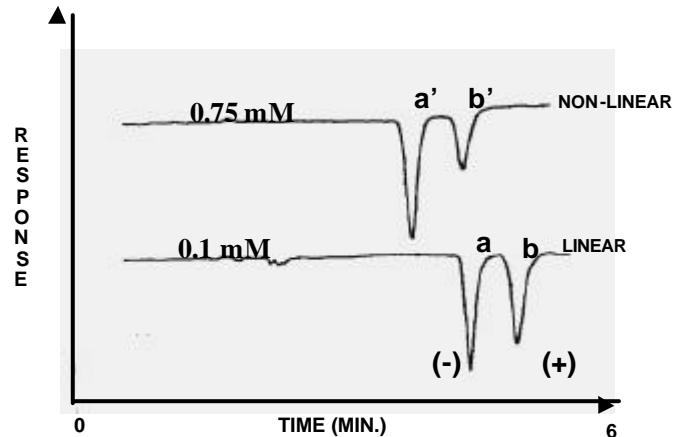


System Peaks in Liquid Chromatography

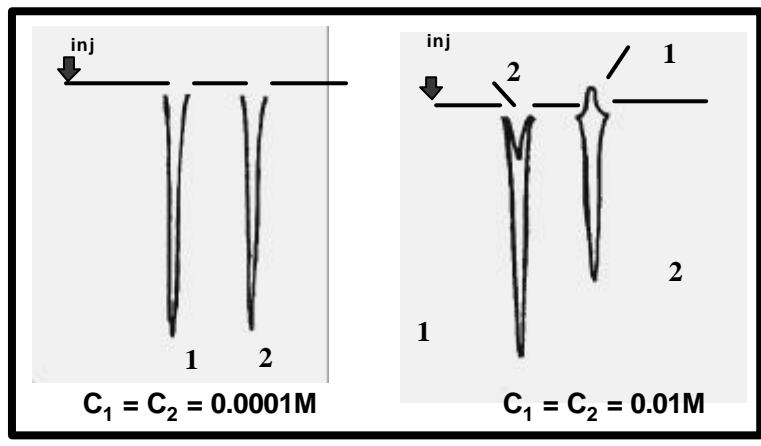
SAMPLE CONCENTRATION:
ABOVE [TFAE] IN THE MOBILE PHASE



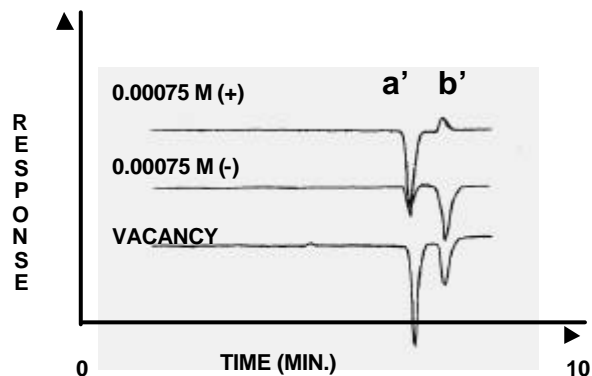
TFAE SYSTEM PEAKS AT LINEAR AND NON-LINEAR CONDITIONS



VACANCY CHROMATOGRAPHY
IN THE CASE OF (1:1) MIXTURE

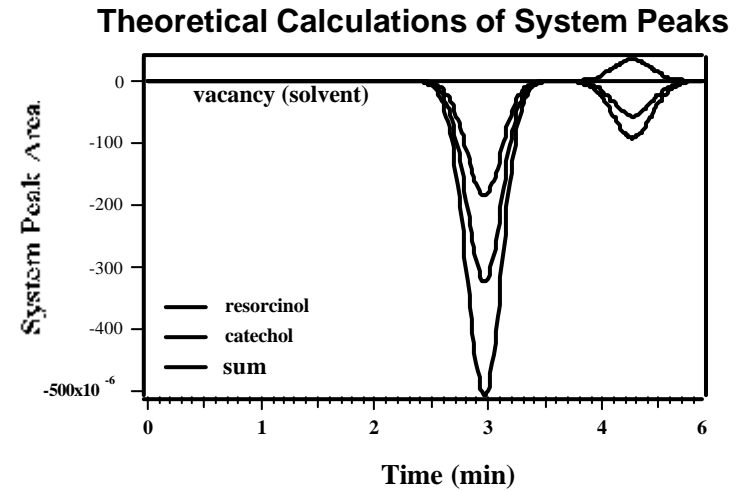


COMPETITIVE CONDITIONS



System Peaks in Liquid Chromatography

Practical Uses



Calculation of $C_{s,i}$:

System Peaks Analysis (SPA)

$$C_{s,i} = \frac{1}{\phi} \sum_0^{C_{m,i}} k'_i \Delta C_{m,i}$$

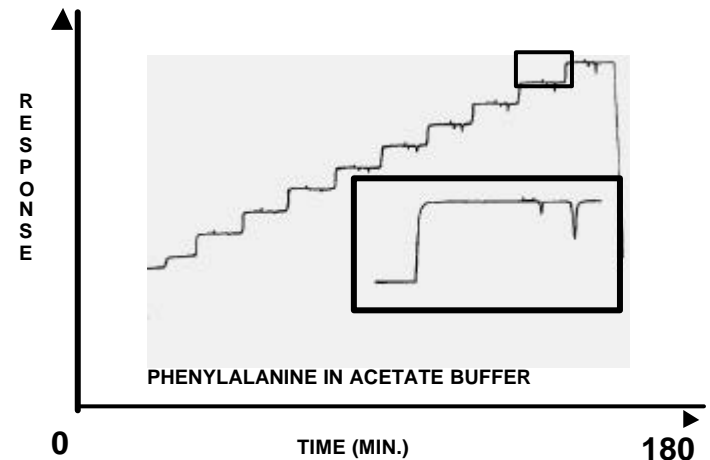
$C_{s,i}$ = concentration in the stationary phase

k'_i = capacity factor

ϕ = phase ratio

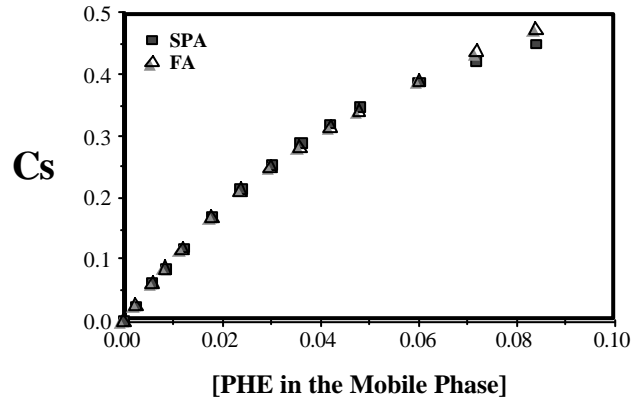
$\Delta C_{m,i}$ = difference in concentration between every two steps.

Measurements of Adsorption Isotherm Using both Methods: SYSTEM PEAKS AND FRONTAL ANALYSIS



System Peaks in Liquid Chromatography

ADSORPTION ISOTHERM OF PHENYLALANINE IN 0.1 M ACETATE BUFFER BY FA and SPA



Frontal Analysis (FA)

$$C_{s,i} = \frac{(V_{R,i} - V_0 - V_h)}{V_s} C_{m,i}$$



STEPWISE:

$$C_{s,i} = \sum_0^{C_{m,i}} \frac{(V_{R,i} - V_0 - V_h)}{V_s} dC_{m,i}$$

V_0 = column void volume

V_s = stationary phase volume

V_h = hold-up volume (from the pump to the detector)

DETECTION OF IRREVERSIBLE ADSORPTION VIA THE SYSTEM PEAKS OF THE RESIDUAL ENANTIOMERS

