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Evaluation of Operating Conditions in Supercritical Fluid Chromatography Applications with Carbon Dioxide

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Introduction

Supercritical fluids are widely used as solvents for extraction in several applications because they are easy to separate from the extracted solute and leave no residue on the treated material. The advantage of using supercritical fluids in sorption processes is the opportunity to change the selectivity and capacity of the solvent simply by varying the pressure/ density, which influences the solid/solute and solvent/solute interactions.

Supercritical fluid chromatography (SFC) can be used as an analytical (quantative and qualitative determination) and preparative method mostly for separation and isolation of various nutraceuticals from natural materials and purifying of pharmaceutical substances.

Supercritical Fluid Chromatography (SFC)

The carbon dioxide (CO₂) is the most commonly used supercritical fluid(SCF). The use of sub- or supercritical CO₂ in chromatography has the some advantages over other solvents:

- ✓relatively low critical pressure (73,8 bar) and critical temperature (31°C),
- ✓is non corrosive, is less toxic and dangerous than other solvents,
- ✓is readily available in large quantities and high purity,
- ✓the solubility of many substances in CO₂ is very high,
- ✓relatively inexpensive solvents which can be continuously recycled,
- ✓it has good selectivity which can be increased by adding an entrained,
- ✓provides rapid separations without the use of organic solvents,
- ✓separations can be done faster than HPLC separations because the diffusion of solutes in supercritical fluids is about ten times greater than that in liquids and about three times less than in gases,
- ✓Compared with GC, capillary SFC can provide high resolution chromatography at much lower temperatures wh atallows fast analysis of thermo labile compounds.

And also some disadvantages:

- ≈strongly polar and ionic molecules are not dissolved by supercritical gases, which can be advantageously used in SFC.
- ≈elevated pressures required,
- ≈high compression costs,
- ≈relatively high capital investment for equipment.

The properties of SCFs are frequently described as being intermediate between those of a gas and a liquid. This phase retains the solvent power common to liquids as well as the transport properties common to gases. A comparison of typical values for density, viscosity and diffusivity of the gases, liquids and SCFs is present in Table 1.

Table 1: The properties of SCFs.

	Liquid	Vapor	Supercritical Fluid
Density (g/cm ³)	1.0	~10 ⁻³	0.3 - 0.7
Diffusivity (cm ² /sec.)	<10 ⁻⁵	~10 ⁻¹	10 ⁻² - 10 ⁻³
Viscosity (g/cm-sec.)	~10 ⁻²	~10 ⁻⁶	10 ⁻³ - 10 ⁻⁶

Experimental procedures

Method. Analytical chromatographic separations of the compounds from samples on a lab scale SFC were used for developing elution methods. Firstly, an analytical separation conditions (pressure, temperature, flow rate of mobile phase and stationary phase) are optimized, then under these conditions, separation with overloading is studied.

Apparatus. Flow scheme of lab scale SFC is presented in Figure 1. Components for delivering and controlling the mobile phase, comprised modules for pressurization, pressure control, and expansion.

Chromatographic conditions.
Column: LiChrospher Diol, Nukleosil C18, CN-HP, Hibar Si60, PrepSil 60
Mobile phase: CO₂, CO₂/MeOH, CO₂/EtOH
Temperature: 26 – 45°C
Pressure: 100 – 300 bar
Flow rate: 2.5 – 4 mL/min
Injection volume: 5, 20, 50 µL

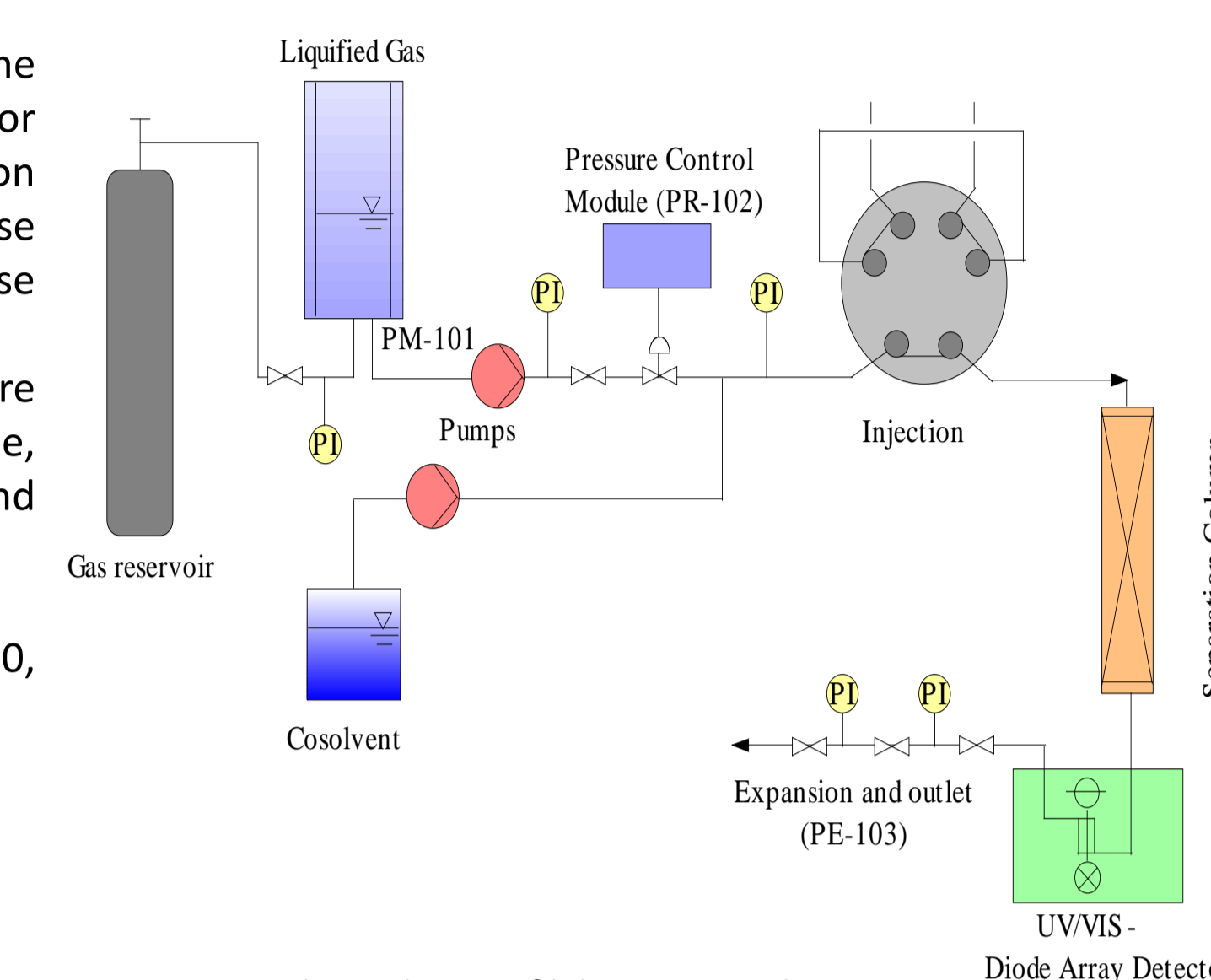


Figure 1: Flow scheme of laboratory scale SFC.

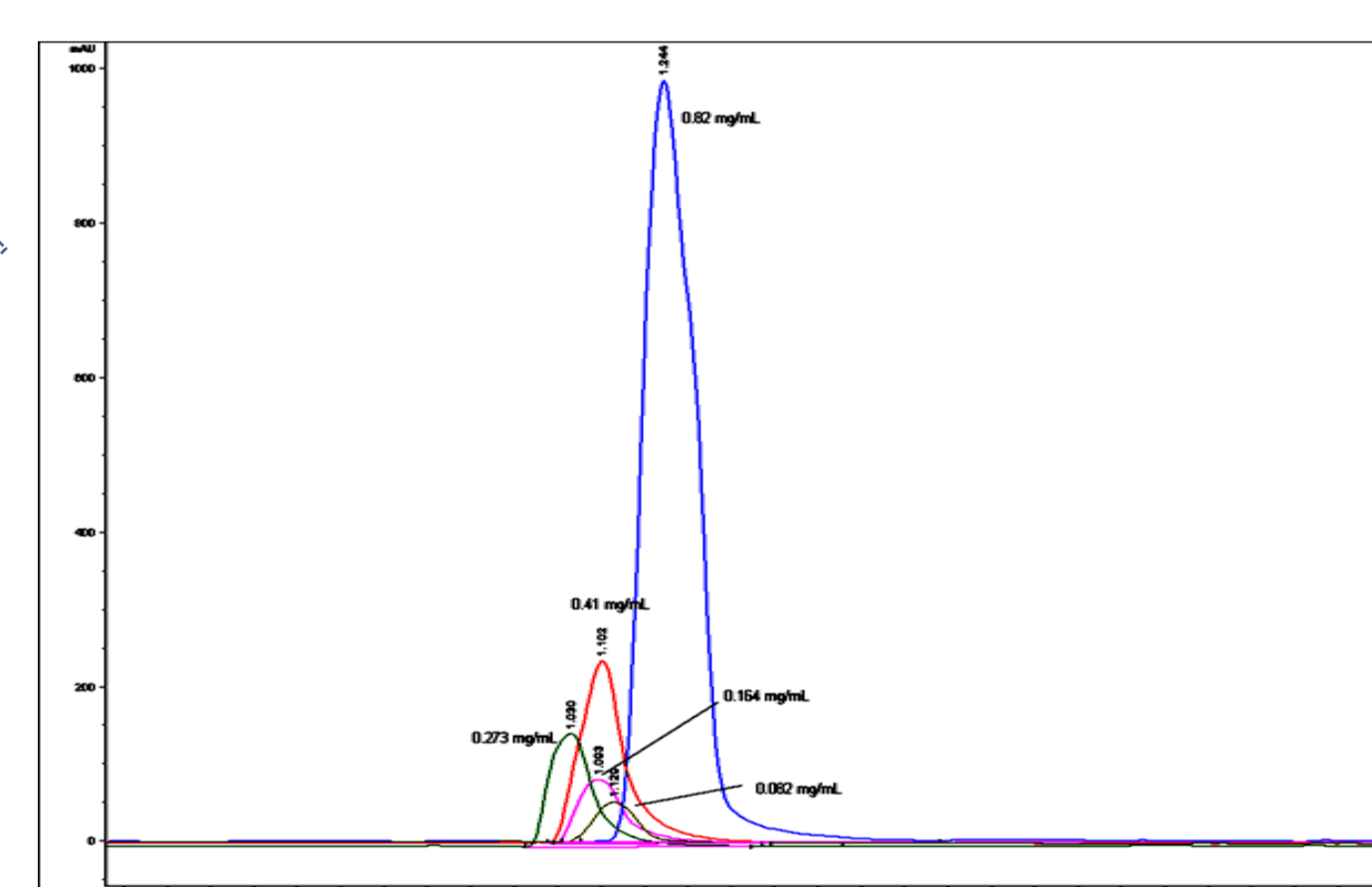
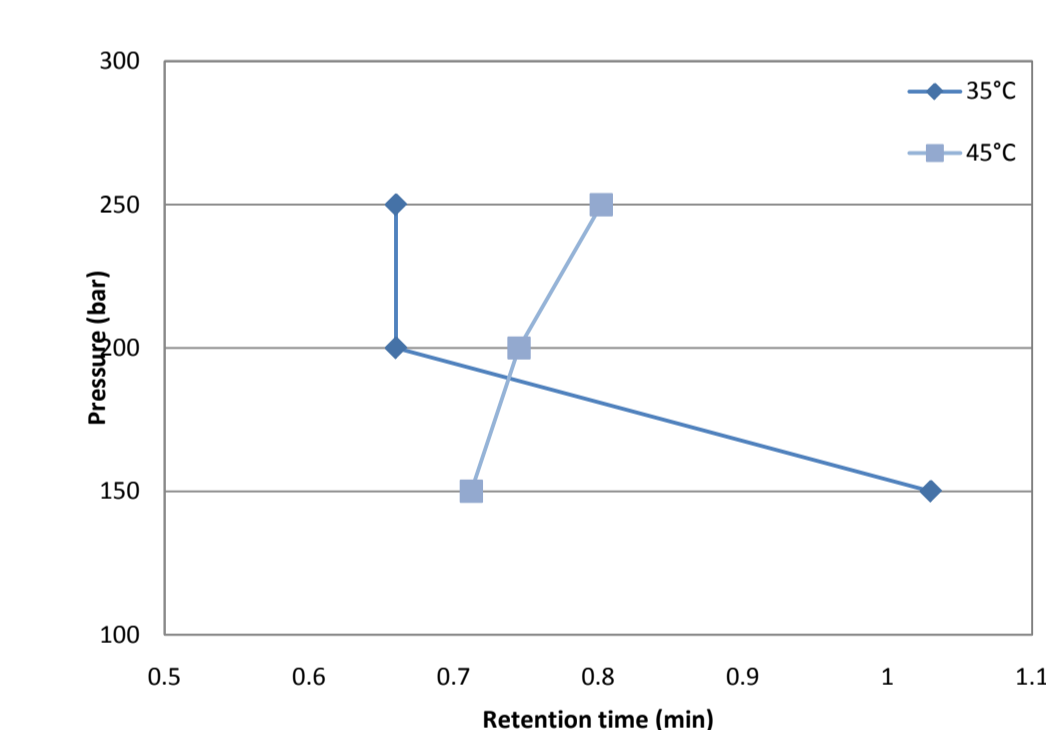
RESULTS

Investigation of behavior of caffeine and hop active compounds (α- and β- acids)

The separation of compounds was investigated due to effect of pressure, temperature, sample loading volume and flow rate of mobile phase. Variation of retention time of active compounds can be observed as a consequence of different conditions of the process what is benefit in preparative scale.

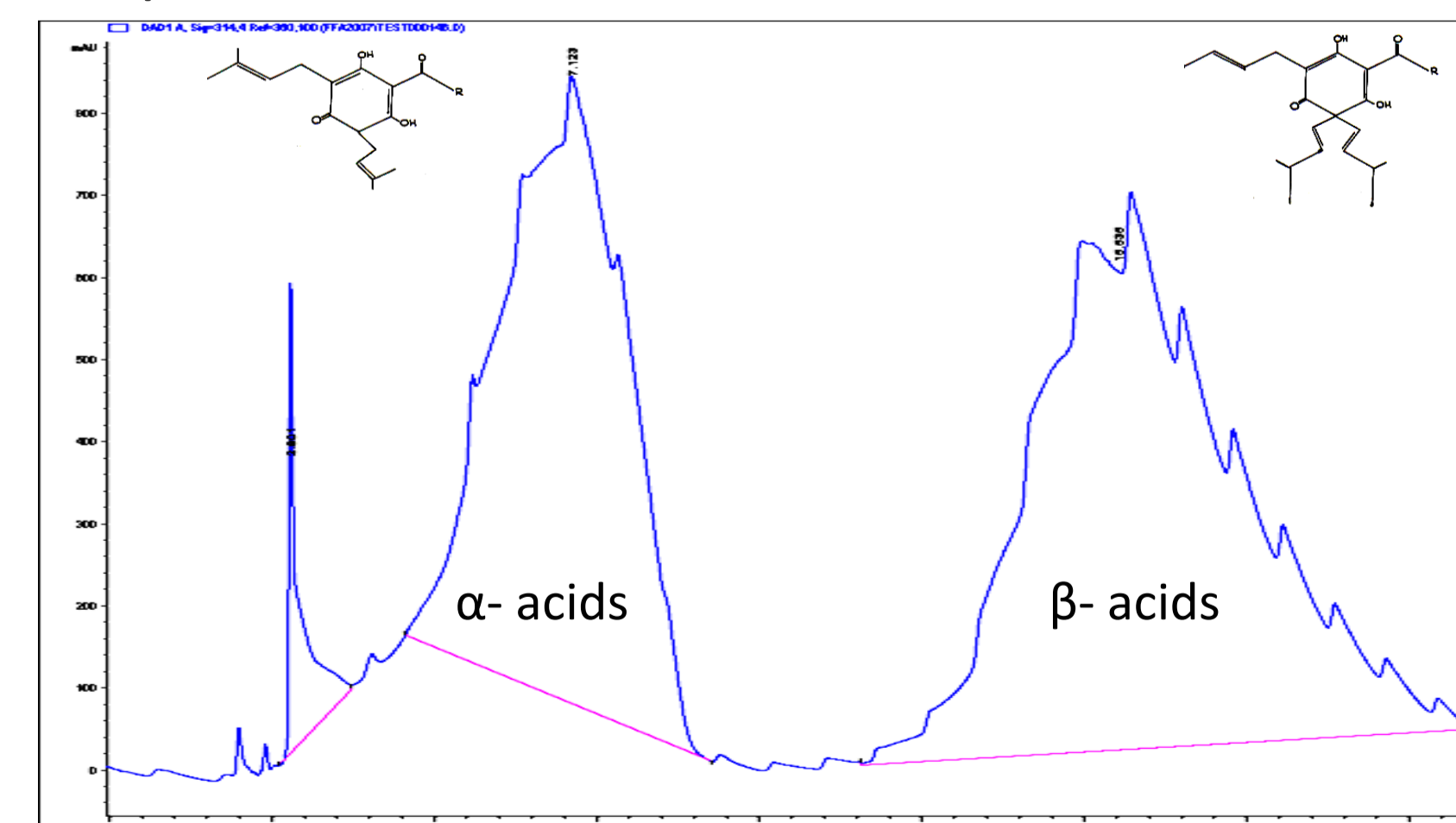
Caffeine

Effect of sample loading volume.



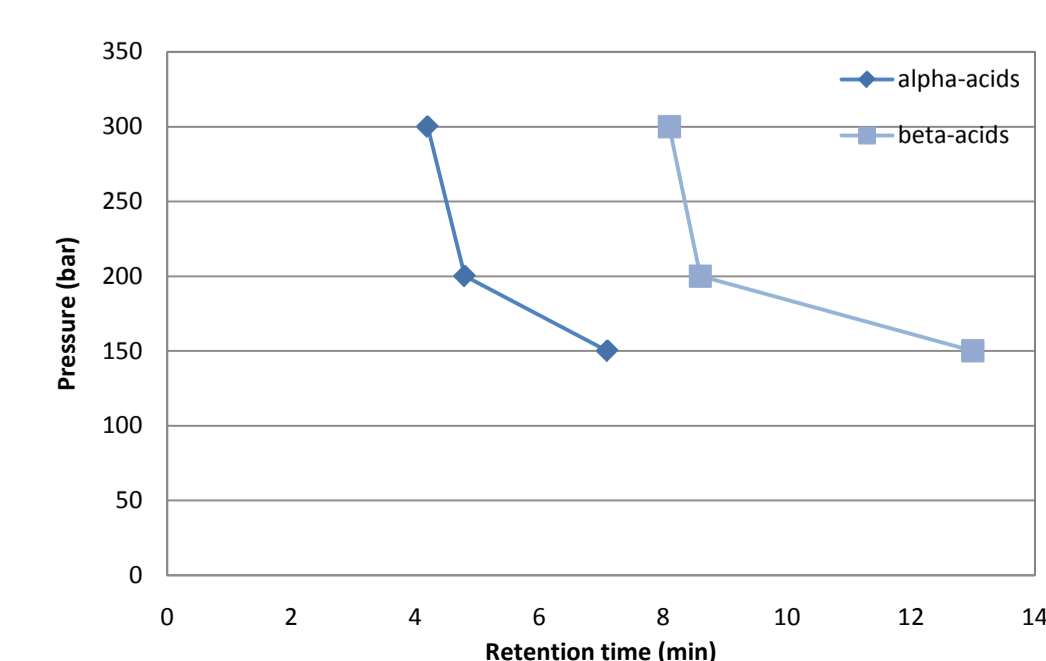
Effect of temperature and pressure on retention time of caffeine.

Hop extract



The separation of α- and β-acids was achieved at 200 bar and 35°C using ethanol as cosolvent with flow rate 0.05 mL/min.

Effect of sample loading volume.



Effect of pressure on retention time of compounds.

Separation of free fatty acids

Supercritical fluid chromatography was used for analysis of oils containing different amounts of free fatty acids. Free fatty acids were analyzed without derivatization

Separations were performed in pressure range from 100 to 300 bar and at temperatures 26, 29, 30, 32 and 35°C, the flow rate through the column was in range of 0.75 to 1.25 L/min STP. Better results were achieved by adding organic solvent, in our case methanol, to mobile phase – CO₂. UV detection was performed at wavelength 205 nm. Three different stationary phases were used as shown in Figure 2. Stationary phases LiChrospher 100 Diol and CN-HP were chosen as normal, with polar modified silica gel phases, which have better chromatographic properties for separation of substances than usually used silica or reverse phase columns. In Figure 2a can be seen that separation on stationary phase Nucleosil C-18 does not give any results. Unsatisfying results were obtained also with CN-HP column, what can be seen in Figure 2c. More experiments were done on column with stationary phase LiChrospher 100 Diol (Figure 2b), which was also used in HPLC method. The separation of palmitic, oleic and linolenic acid was achieved by using methanol as mobile phase modifier (Figure 2b). The ratio between CO₂ flow and modifier flow was 25:1.

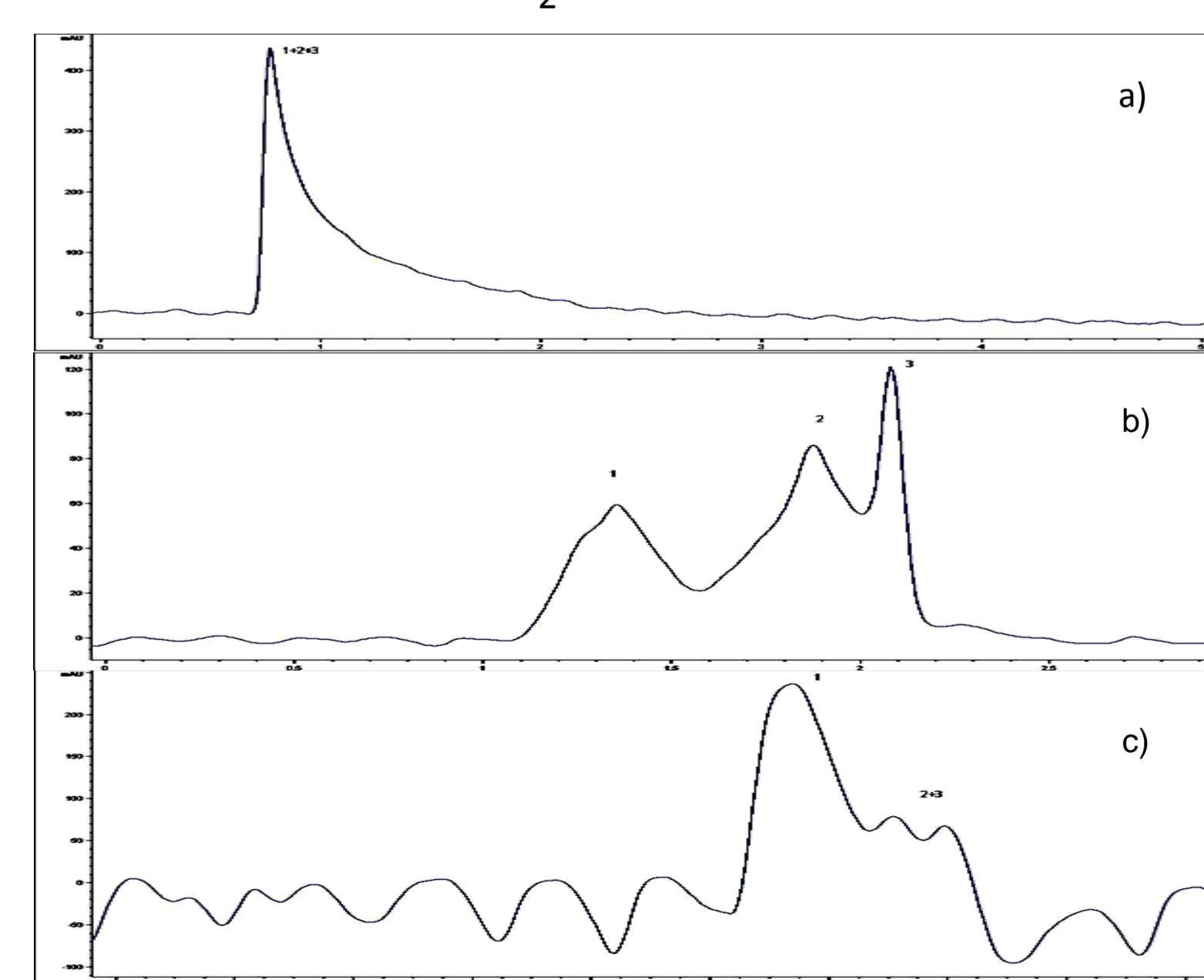


Figure 2: Comparison of fatty acids separation on SFC using different stationary phases of packed columns: (a) Nucleosil 100 C-18, (b) LiChrospher 100 Diol, (c) CN-HP. Separation of FFA in mixture of 1- palmitic acid, 2- oleic acid and 3- α-linolenic acid.

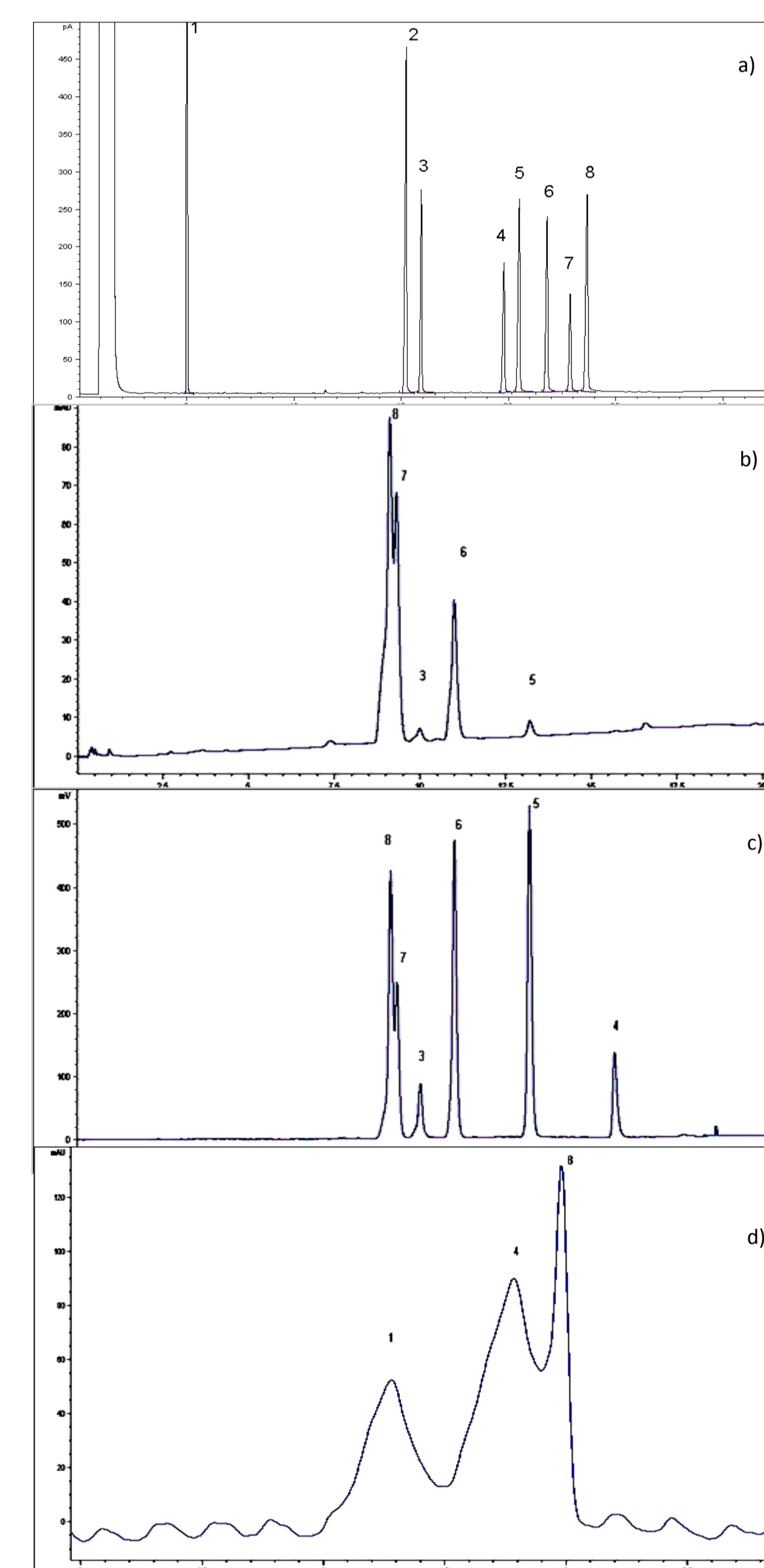


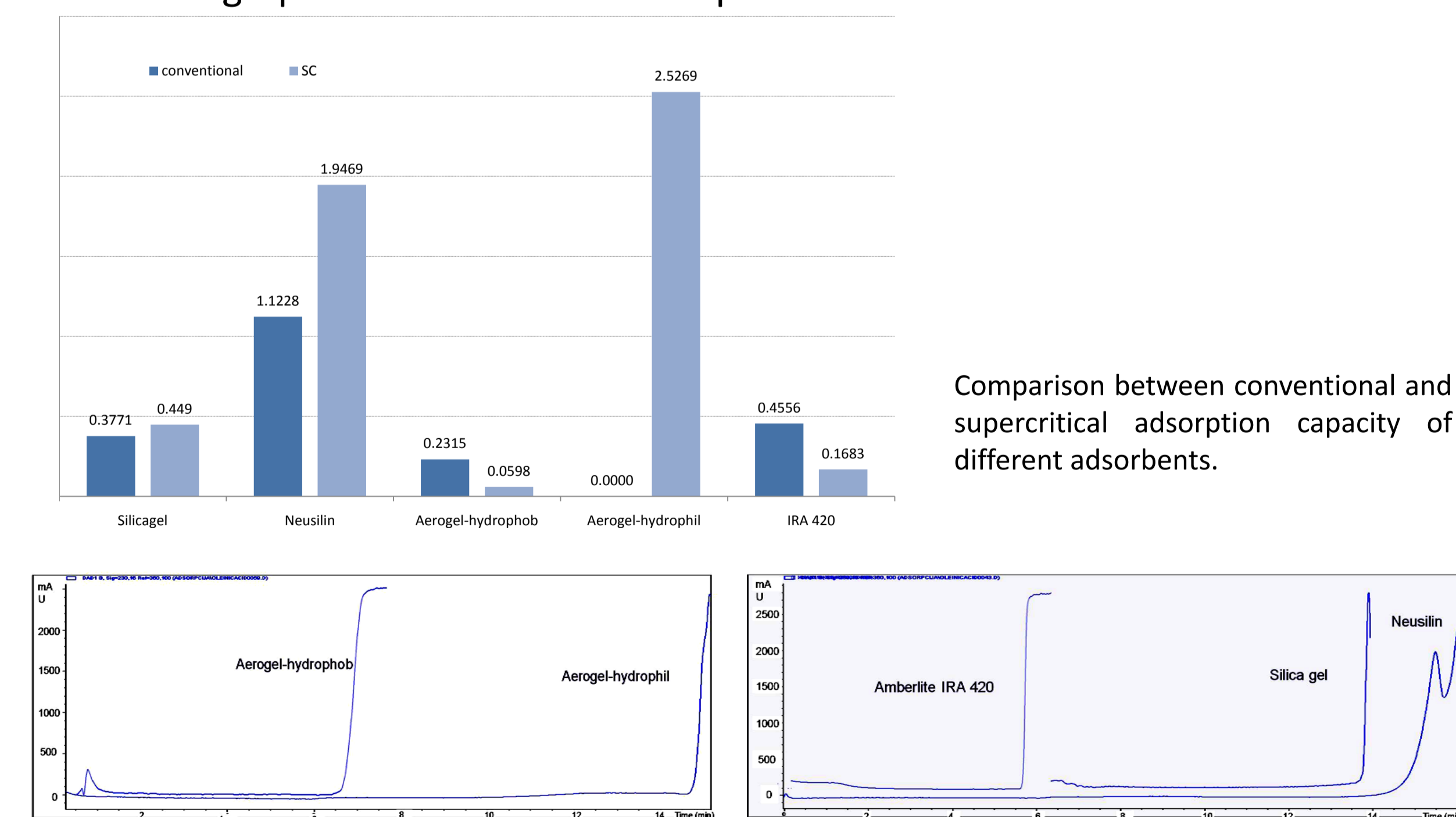
Figure 3: Separation of free fatty acid 1- heptanoic acid, 2- palmitic acid, 3- palmitoleic acid, 4- stearic acid, 5- oleic acid, 6- linoleic acid, 7- γ-linolenic acid, 8- α-linolenic acid, 9- eruric acid with (a) GC-FID, (b) HPLC-UV, (c) HPLC-ELSD, (d) SFC-UV.

According to obtained results with SFC, the separation of free fatty acids and also the quantitative and qualitative informations were compared with results obtained by gas and liquid chromatography (Figure 3).

Adsorption and desorption

Adsorption and desorption experiments, as another application of SFC, were carried out with liquid substance (oleic acid) as adsorbate in this process. The supercritical fluid adsorption-desorption process was compared with conventional adsorption-desorption process. The adsorption efficiency and capacity of solid matrices according to various adsorbates was investigated.

Adsorption and desorption experiments of oleic acid on selected adsorbents (silicagel, neusilin, two aerogels) and ion exchange resin (Amberlite IRA 420) were performed. The conventional adsorption-desorption process was performed on glass chromatographic column using gravity flow. Experiments were performed at room temperature using organic solvents (methanol, acetone, acetonitrile, methanol + acetic acid). The supercritical CO₂ adsorption-desorption experiments were performed on high pressure chromatographic column at 40°C and pressures 130 and 300 bar.



Comparison between conventional and supercritical adsorption capacity of different adsorbents.

Breakthrough curves for adsorption of oleic acid on various materials in SC CO₂

- ✓ capacity of silica gel, neusilin and silica aerogel hydrophil for oleic acid was higher in the case of supercritical CO₂ adsorption process at 130 bar and 40 °C compared to conventional adsorption of methanol solution under gravity flow.
- ✓ adsorption time is much lower for high pressure process.