Supercritical Fluid Adsorption and Desorption of Lipids on Various Adsorbents

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Dedicated to the memory of professor Vojko Ozim

Abstract

In present work the feasibility of using supercritical fluid chromatography (SFC) for separation of free fatty acids (FFA) and triglycerides has been examined. Lipids such as oleic acid and sunflower oil (containing 96% of triglycerides) were used; adsorption and desorption on adsorbents such as silica gel and neusilin using supercritical CO_2 as mobile phase was investigated. The experiments were performed at 40 °C and in the pressure range from 13 to 30 MPa. Adsorption kinetic lines were determined by analysing breakthrough curves. Several parameters such as rate of adsorption and desorption, loading, desorbed quantity and diffusion coefficients in solid phase were calculated.

Keywords: Supercritical fluid chromatography, lipids, oleic acid, triglycerides, adsorption, desorption

1. Introduction

Supercritical fluid extraction (SFE) and subsequent separation and fractionation of solute from supercritical fluid mixture by simply changing the temperature or/and pressure was extensively studied over the past years and several advantages over conventional processes were verified due to favourable transport and solvent properties of supercritical fluids. However, it was also observed, that often the separation factors achieved with supercritical fluids, even with added modifiers, are not high enough to enable a separation.[1] Lately, there is a growing interest in applying adsorption from supercritical fluid phases in downstream process. In this way better separation can be achieved due to additional surface interactions. Furthermore, a solvent cycle at nearly constant pressure is feasible, which is, considering energy consumtion, the most effective operating mode.[1] The combination of an adsorption with a desorption step can be used for separating multicomponent mixtures into fractions of similar molecular weight and polarity.[1] Fundamentals of adsorption from a high pressure gaseous phase, adsorption equilibria and experimental techniques for determination of adsorption isotherms are presented by Brunner and Johannsen.[1] Compared to preparative-LC, preparative SFC is an environmentally clean technology that has several advantages,

such as fast elution time, yielding at least a three-fold increase in the throughput, easy recovery of products by simple decompression, low consumption of organic solvents and wider range of applicability.[2–4] Some of the most latest researches are dealing with the use of SFC in chiral separations of racemic mixtures,[5–16] separation of bioactive compounds from natural sources such as tocopherols and sterols[1] and separation of natural antioxidant and antimicrobial substances from aromatic compounds.[4,17,18] The influence of the parameters (e.g. pressure, temperature, composition of supercritical fluid mixture and the ratio of adsorbent to adsorbate amount) on the adsorption and desorption processes have to be determined experimentally for each system.

The aim of this work was to examine the feasibility of using SFC for separation of free fatty acids and triglycerides. For this purpose, the adsorption and desorption of oleic acid and sunflower oil containing 96% of triglycerides on selected adsorbents (silica gel and neusilin) using supercritical CO_2 as mobile phase was investigated. Adsorption kinetic lines were determined by analysing breakthrough curves at pressure 13 MPa and temperature 40 °C. Desorption was performed at pressure 30 MPa and temperature 40 °C. From experimental results parameters such as concentration of adsorbing components on the adsorbent (loading), rate of adsorption and desorption and

desorbed quantity were determined for each compound. Desorption curves were modelled by using an unsteady state mass transfer model and the diffusion coefficient of compounds in the solid phase were calculated.

2. Experimental

2. 1. Materials

Silica gel 60 was obtained from Merck (Germany) and Neusilin S2 from Fuji Chemicals Industry (Japan). The oleic acid was technical grade (90%) and was obtained from Aldrich (Germany). Sunflower oil was obtained from local market. Sodium hydroxide solution (0.1 N) was purchased from Merck (Germany). Phenolphthalein was from Kemika (Croatia). CO_2 was 99.5% pure and supplied by Messer (Slovenia).

2. 2. Analyses

The content of free fatty acids in sunflower oil was determined by volumetric method. 0.1 g of sunflower oil was diluted in 20 mL of 0.1 wt % phenolphthalein solution in absolute ethanol and then titrated with standardized sodium hydroxide solution of 0.1 mol/L in water.[19]

The mean particle diameter of adsorbents was determined by laser particle sizer Analysette 22 Compact (Fritsch, Germany).

2. 3. Apparatus

SCF chromatography system (Figure 1) consists of a reservoir for mobile phase (CO_2 tank), a unit for establis-hing, maintaining and controlling pressure (NWA PM-101 and PR-102), an optional unit for adding a modifier (High pressure syringe pump, Gilson 305, type 5SC), Rheodyne switching valve, adsorption column (4.6 mm ID, 100 mm length) with electrical heating, detector (UV-VIS diode array detector, Agilent 1100 with high pressure micro flowcell) for determining concentration of substance in the outlet stream, the unit for expansion of mobile phase (NWA PE-103) and flow meter.[20]

2. 4. Adsorption-Desorption Experimental Procedure

The CO_2 from the tank was cooled to a liquid state and pressurized. The pressure of CO_2 was controlled and maintained constant during the process by regulation valve. The flow of CO_2 through the column was regulated by expansion valve which was heated in order to avoid decrease of temperature. Adsorbent was filled into a steel column, which was weighed. Each experiment was performed in three steps; initial controlling step, adsorption and desorption step.

In the first controlling step the CO_2 was initially pumped over the bed of adsorbent for 15 min at 40 °C and 13 MPa after which the column was reweighed. No adsorption of CO_2 was detected. Afterwards the column was disconnected from the system by using Rheodyne valve and lipid was pumped into the stream of CO_2. The operating conditions were same as those used afterwards in the adsorption step: in this way the height of the signal, which corresponds to the initial concentration of the lipid-CO_2 mixture applied in experiment, was recorded. Finally, the system was purified by using pure CO_2.
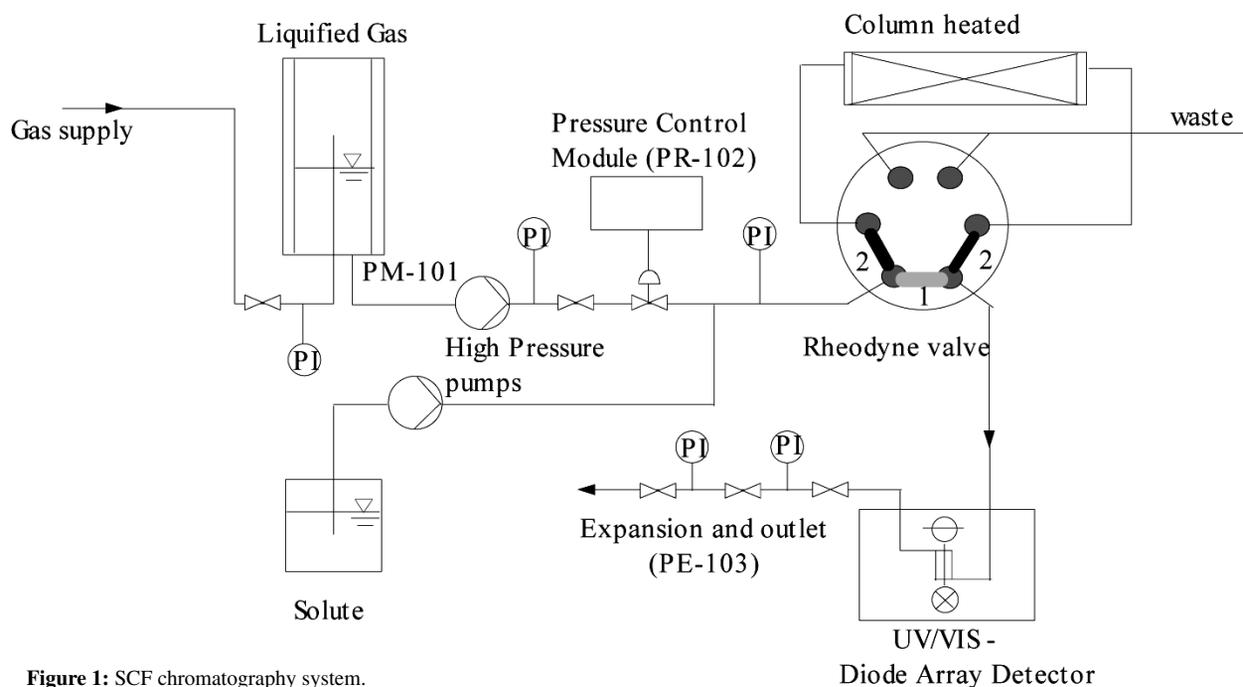

Figure 1: SCF chromatography system.

In the following adsorption step, the lipid-CO_2 mixture was pumped over the column. Adsorption experiments were performed at 13 MPa and 40 °C. The concentration of the lipid in CO_2 was approximately constant in all experiments and was below the saturated concentration.[21–23] The breakthrough of lipid, i.e. the end of adsorption process, was detected by the UV-VIS detector and the column was weighed.

In the third step the desorption was performed at 30 MPa and 40 °C and at the end of the process the column was reweighed. The amount of lipid in the outlet stream during the entire process was monitored with on-line UV-VIS detector at 230 nm.

2. 5. Modelling Desorption Curves

For modelling desorption curves the unsteady state mass transfer model was used. With this model it was assumed that diffusion inside the solid phase controls the rate of process and can be derived from Fick´s second law of diffusion. The diffusion equation given by Hong et al. was used to perform the calculation:[24,25]

$$\frac{m(t)}{m_0} = A \cdot e^{(-K \cdot t)} \quad (1)$$

$$K \cong \frac{F_0}{t} \quad (2)$$

$$F_0 = \frac{D_s \cdot t}{d^2} \quad (3)$$

where K and A are the slope and the intercept obtained from the plot $\ln\frac{m(t)}{m_0}$ vs. t, respectively. $m(t)$ and m_0 are the amounts of sample (in g) in the solid at time t and time zero, respectively, F_0 is the Fourier number, d is the particle diameter (m), and D_s diffusivity in the solid phase (in m^2/s).

3. Results and Discussion

The content of FFA in sunflower oil was 3.7 ±0.2%. The mean particle size of silica gel and neusilin were 102.14 and 20.16 μm, respectively. Each adsorption and desorption experiment was performed 3 times and results are presented as average values.

Operating parameters (i.e. pressure P, temperature T, flow rates of CO_2 and oleic acid or oil Q_{CO_2} and Q_{Lipid}, respectively) during the experiments of adsorption and desorption of oleic acid and sunflower oil are listed in Table 1.

In Table 2 the results of the adsorption and desorption experiments of oleic acid and sunflower oil are listed. Figs. 2 and 3 present the kinetic curves of adsorption and desorption of oleic acid and sunflower oil on silicagel. Results show that the loading and rate of adsorption of both, oleic acid and sunflower oil, on neusilin is much higher as on silica gel. The desorption quantity of solutes is generally high and is above 85%, except for sunflower oil desorbed from silica gel, where it is somewhat lower (73.2%).

Table 1: Operating parameters of adsorption-desorption experiments of oleic acid and sunflower oil using supercritical CO_2.

Adsorbent	Adsorption				Desorption		
	P (MPa)	T (°C)	Q_{CO_2} (L/min)	Q_{Lipid} (mL/min)	P (MPa)	T (°C)	Q_{CO2} (L/min)
Oleic acid							
Silica Gel	13	40	0.778 ± 0.079	0.095 ± 0.002	30	40	1.115 ± 0.135
Neusilin	13	40	0.524 ± 0.001	0.097 ± 0.005	30	40	0.743 ± 0.197
Sunflower oil							
Silica Gel	13	40	0.968 ± 0.206	0.096 ± 0.004	30	40	1.018 ± 0.225
Neusilin	13	40	0.524 ± 0.381	0.103 ± 0.003	30	40	0.790 ± 0.080

Table 2: Results of adsorption and desorption experiments of oleic acid and sunflower oil using supercritical CO_2.

| Solute / Adsorbent	Loading (g solute/ g adsorbent)	Rate of adsorption (g/min)	Desorbed quantity (%)	K (min^{-1})	$D_s \cdot 10^{12}$ (m^2/s)
Oleic acid					
Silica gel	0.6317 ± 0.0345	0.0515 ± 0.0024	85.52 ± 12.18	0.031 ± 0.003	5.329 ± 0.443
Neusilin	2.2315 ± 0.0065	0.1768 ± 0.0388	87.97 ± 0.88	0.166 ± 0.015	1.124 ± 0.099
Sunflower oil					
Silica gel	0.6856 ± 0.0162	0.0578 ± 0.0021	73.20 ± 1.06	0.097 ± 0.013	16.81 ± 2.304
Neusilin	2.3708 ± 0.1241	0.3108 ± 0.1848	88.11 ± 7.79	0.044 ± 0.003	0.301 ± 0.022
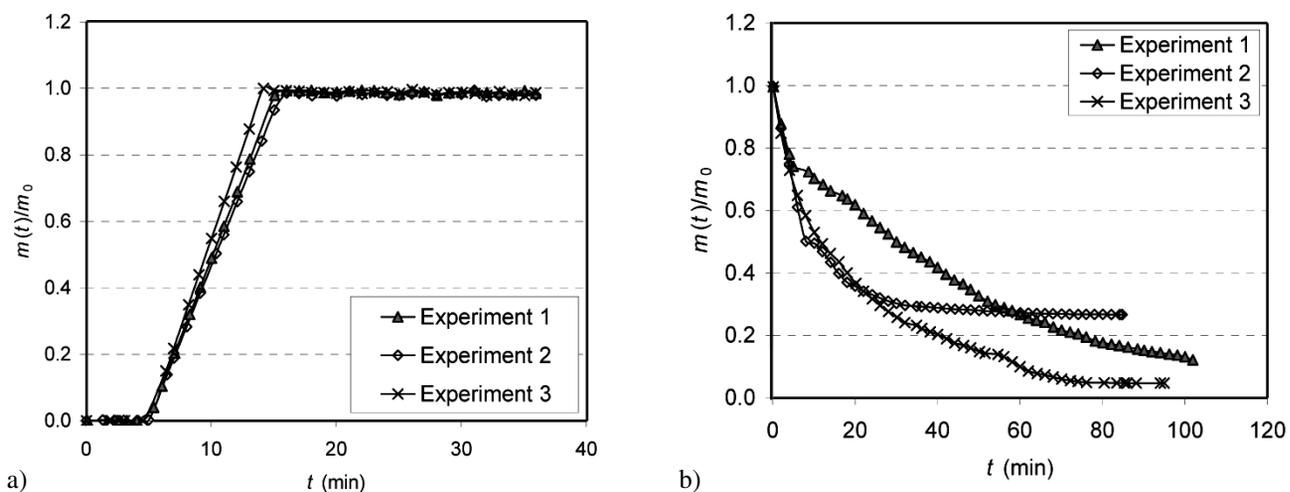
Figure 2: SFC of oleic acid using silicagel and supercritical CO_2: a) adsorption at 40 °C and 13 MPa, b) desorption at 40 °C and 30 MPa.
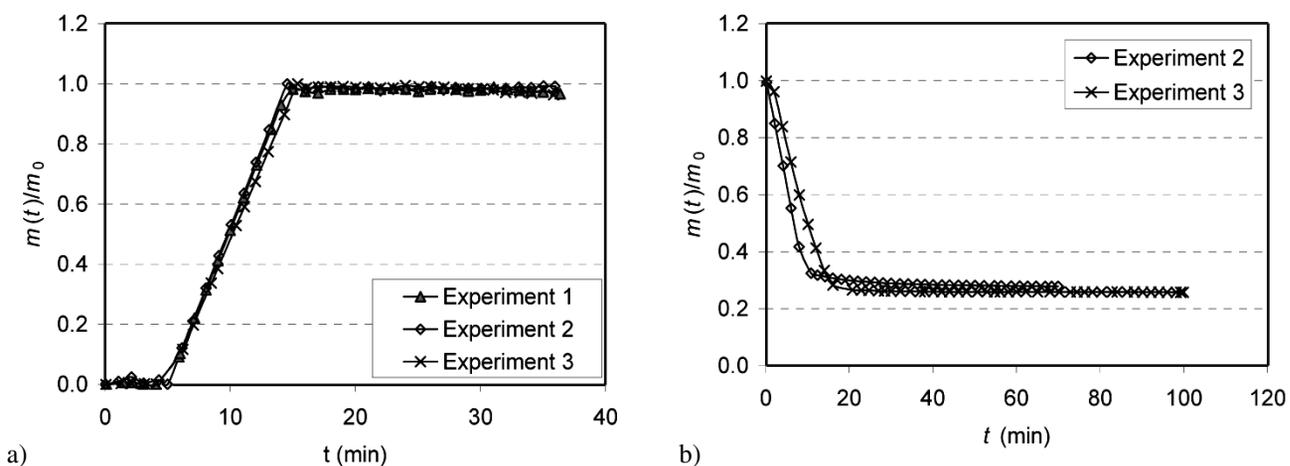

Figure 3: SFC of sunflower oil using silicagel and supercritical CO_2: a) adsorption at 40 °C and 13 MPa, b) desorption at 40 °C and 30 MPa.
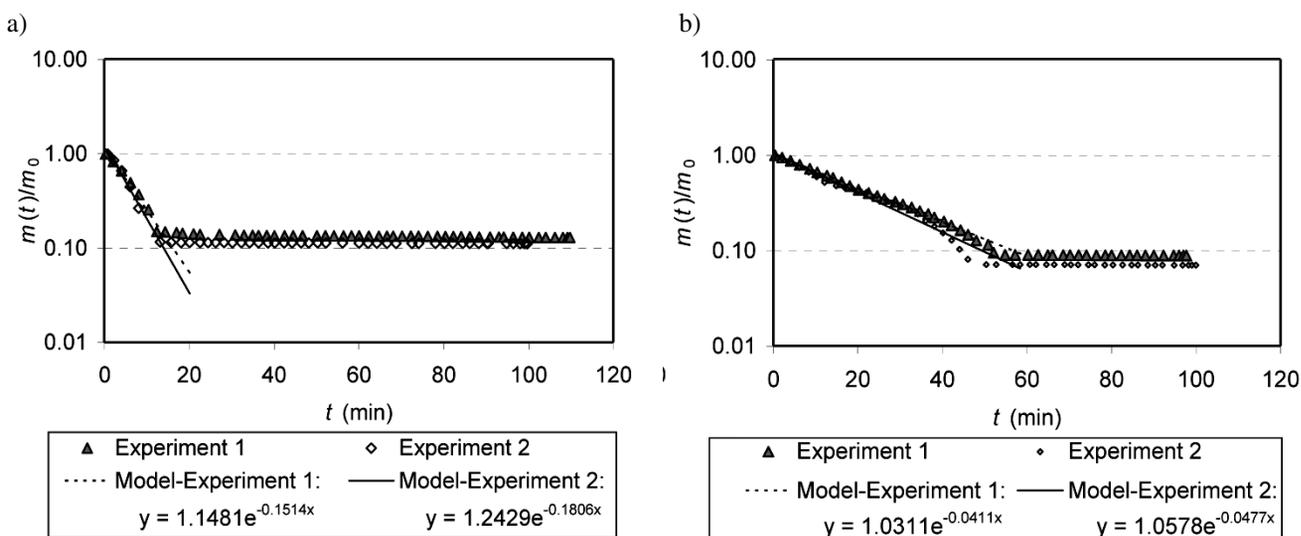

Figure 4: SFC desorption step from neusilin: plot $\ln\frac{m(t)}{m_0}$ vs. t.   a) oleic acid,   b) sunflower oil

Constant K obtained from the plot $\ln\frac{m(t)}{m_0}$ vs. t (Fig.

4) is indicating the rate of desorption; the higher is the value, the higher is the rate of desorption. Therefore different values of K have to be obtained in order to achieve separation of oleic acid and sunflower oil. The highest differences of K values are observed in case of neusilin, and the results indicate, that oleic acid is eluted faster than sunflower oil. Diffusion coefficients obtained by modeling desorption kinetic curves are for both solutes higher in case of silica gel.

4. Conclusion

Based on the results obtained by studying adsorption and desorption of pure oleic acid and sunflower oil containing 96% of triglycerides it can be assumed that the separation of solutes can be achieved by SFC using CO_2. In general, desorption of lipids can be performed successfully with pure CO_2 simply by increasing the pressure.

Loading of both solutes on neusilin was very high and was in the range from 2.2 to 2.4 g of solute per g of adsorbent. Rate of adsorption was somewhat higher for sunflower oil and oppositely, rate of desorption was approximately three times higher for oleic acid.

For designing the process, however, further experiments should be performed with multicomponent mixtures of FFA and triglycerides. Further studies should be performed in future in order to investigate and understand how process parameters such as pressure, temperature and concentration of the fluid phase are influencing the kinetic of adsorption and desorption processes and adsorption/desorption equilibria should be studied.

5. Acknowledgement

6. References

1. G. Brunner, M. Johannsen, *J. Supercrit. Fluids* **2006**, *38*, 181–200.

2. K. Yaku, F. Morishita, *J. Biochem. Biophys. Methods* **2000**, *43*, 59–76.

3. L. Toribio, M. J. Nozal, J. L. Bernal, E. M. Nieto, *J. Chromatogr. A* **2003**, *1011*, 155–161.

4. P. Ramirez, S. Santoyo, M. R. Garcia-Risco, F. Javier Senorans, E. Ibanez, G. Reglero, *J. Chromatogr. A* **2007**, *1143*, 234–242.

5. T. Szczerba, P. Wrezel, *LC GC N. Am.* **2007,** *19*, Suppl. S.

6. L. Toribio, C. Alonso C, M. J. del Nozal, J. L. Bernal, M. T. Martin, *J. Chromatogr. A* **2006**, *1137*, 30–35.

7. D. R. Wu, L. Leith, B. Balasubramanian, T. Palcic, D Wang-Iverson, *Am. Lab.* **2006**, *38*, 24–33.

8. D. R. Wu, *Abstr. Pap. Am. Chem. S.* **2005**, *230*, 282–289.

9. T. E. Beesley, *LC GC N. Am.* **2006**, *24*, 28–31.

10. L. M. Nogle, C. W. Mann, W. L. Watts, Y. R. Zhang, *J. Pharmaceut. Biomed.* **2006**, *40*, 901–909.

11. C. M. Kraml, D. H. Zhou, N. Byrne, O. McConnell, *J Chromatogr. A* **2005**, *1100*, 108–115.

12. M. Maftouh, C. Granier-Loyaux, E. Chavana, J. Marini, A. Pradines, Y. Vander Heyden, C. Picard, *J. Chromatogr. A* **2005**, *1088*, 67–81.

13. C. J. Welch, W. R. Leonard, J. O. DaSilva, M. Biba, J. Albaneze-Walker, D. W. Henderson, B. Laing B, D. J. Mathre, *LC GC Eur.* **2005**, *18*, 264–272.

14. K. W. Phinney, *Anal. Bioanal. Chem.* **2005**, *382*, 639–645.

15. C. White, *J. Chromatogr. A* **2005**, *1074*, 163–173.

16. R. E. Majors, *LC GC N. Am.* **2005**, *23*, 1248–1255.

17. P. Ramirez, M. R. Garcia-Risco, S. Santoyo, F. J. Senorans, E. Ibanez, G. Reglero, *J. Pharmaceut. Biomed.* **2006**, *41*, 1606–1613.

18. S. Li, T. Lambros, Z. Wang, R. Goodnow, C.-T. Ho, *J. Chromatogr. B* **2007**, *846*, 291–297.

19. M. Habulin, Ž. Knez, *J. Am. Oil Chem. Soc.* **1990**, *67*, 775–778.

20. M. Škerget, P. Kotnik, Ž. Knez, *Supercritical carbon dioxide sorption processes on various sorbent materials*, in: M. Perrut (Ed.), Proceedings of the 10th Europen meeting on supercritical fluids, International Society for the Advancement of Supercritical Fluids, Colmar (France), **2005**, Proceedings on CD, Pv9.

21. M. Škerget, Ž. Knez, M. Habulin, *Flud Phase Equilib* **1995**, *109*, 131–138.

22. P. Mahesshwari, Z. L. Nikolov, T. M. White, R. Hartel, *Am. Oil Chem. Soc.* **1992**, *69*, 1069–1076.

23. M. Zou, Z. R. Yu, P. Kashulines, S. S. H. Rizvi, J. A. Zollweg, *J. Supercrit. Fluids* **1990**, *3*, 23–38.

24. J. W. King, M. Cygnarowicz-Provost, F. Favati, *Ital. J. Food Sci.* **1997**, *9*, 193–204

25. I. K. Hong, S. W. Rho, K. S. Lee, W. H. Lee, K. P. Yoo, *Korean J. Chem. Eng.* **1990**, *7*, 40–46.

Povzetek

Raziskali smo uporabnost postopka superkritične kromatografije (SFC) za separacijo prostih maščobnih kislin (FFA) in trigliceridov. V eksperimentih adsorpcije in desorpcije na silikagel in neusilin smo uporabili oleinsko kislino in sončnično olje, ki je vsebovalo 96 % trigliceridov. Kot mobilno fazo smo uporabili superkritični CO_2 pri 40 °C in v območju tlakov od 13 do 30 MPa. Študirali smo kinetiko adsorpcije in desorpcije in določili parametre kot so adsorpcijska in desorpcijska hitrost, adsorbirana in desorbirana množina substance ter difuzijske koeficiente substanc v trdni fazi (adsorbentu).