SUPERCRITICAL CARBON DIOXIDE EXTRACTION OF ANDROGRAPHOLIDE FROM *ANDROGRAPHIS PANICULATA* LEAVES USING METHANOL AS CO-SOLVENT

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ABSTRACT

The optimisation and design of any separation processes, such as the extraction of bio molecules from their natural resources require mathematical modelling. The objective of this work is to develop a mathematical model based on first order desorption rate of solute into supercritical solvent containing a mixture of supercritical carbon dioxide and methanol in the extraction of andrographolide from *Andrographis paniculata* leaves. Numerical calculations to obtain the adjustable parameter of the model using experimental data obtained from the supercritical carbon dioxide extraction (SCDE) of andrographolide at different methanol concentration and temperature were done using MATLAB software. The calculation results show that this model agrees well with the experimental data. Therefore, this model is capable for the optimisation and design of SCDE of andrographolide from *Andrographis paniculata* leaves with the presence of methanol as co – solvent.

Keywords: *modelling, extraction, supercritical carbon dioxide, co – solvent, andrographolide, Andrographis paniculata, MATLAB*

INTRODUCTION

Andrographis paniculata NEES, locally known as Hempedu Bumi grows widely in the tropical areas of South East Asia, India and China with annual growth of 30 – 70 cm height. In Malaysia, this plant has been extensively used for traditional medicine and help against fever, dysentery, diarrhoea, inflammation, and sore throat. Furthermore, it is a promising new way for the treatment of many diseases, including HIV, AIDS, and numerous symptoms associated with immune disorders (Calabrese et al., 2000). Three main diterpenoid lactones identified in the *Andrographis paniculata* leaves were andrographolide, neo-andrographolide and deoxyandrographolide (Wongkittipong et al., 2000; Rajani et al., 2000; Choudhury et al., 1987). Andrographolide, which is grouped as an unsaturated trihydroxy lactone has molecular formula of $C_{20}H_{30}O_5$. The molecular structure of andrographolide is shown in Figure 1. Andrographolide is easily dissolved in methanol, ethanol, pyridine, acetic acid and acetone, but only slightly dissolved in ether and water. The melting point of this substance is 228^o – 230 ^oC, while its ultraviolet spectrum in ethanol, λ_{max} is 223 nm (Rajani et al., 2000). Hitherto, there are some techniques can be used for the analysis of andrographolide, such as thin layer chromatography (TLC) (Choudhury et al., 1987; Puri et al., 1993), high - performance liquid chromatography (HPLC) (Wongkittipong et al., 2000; Tang et al., 2000; Li et al., 2004) and crystallisation techniques (Rajani et al., 2000).

Extraction using organic solvent is the most common method of separating bioactive components from their natural hosts. However, since these liquid solvents are usually not able to be completely removed by the existing separation techniques and their traces may remain present in the final product, the extraction using only organic solvent is no longer attractive from clinical, environmental, energy and time consumption point of views (Luque de Castro and Garcia - Ayuso, 1998). Therefore, supercritical fluid extraction is a better alternative method to extract bioactive components from their natural hosts, since this method offers shorter extraction times, cheaper operating cost, higher extraction selectivity, safer condition (non toxic, non flammable, non hazardous) and adjustable solvating power (Taylor, 1996). However, if polar analytes are present in the sample matrix, the supercritical fluids may not have enough solvating power. With respect to such attractive properties of CO_2, extraction of highly polar analytes has usually been carried out using CO_2 containing a few percent of organic modifier (Nemoto et al., 1997, Langenfeld et al., 1994). The presence of modifier in supercritical fluid will lead to higher extraction efficiencies (Langenfeld et al., 1994). Common modifiers used are methanol, ethanol, propanol, etc. (Taylor, 1996). In general a modifier that has a lower critical temperature than the supercritical gas causes a decrease in the solubility of a low volatile analyte in gas, whereas a modifier of higher critical temperature causes an increase in solubility.

Mathematical formulation is necessary for the optimisation and scale - up of a separation unit such as the extraction of bio - molecules from their natural resources. The objective of this work is to develop a mathematical model based on first order desorption rate of solute into supercritical solvent containing a mixture of supercritical carbon dioxide and methanol in the extraction of andrographolide from *Andrographis paniculata* leaves.

MATHEMATICAL MODEL DEVELOPMENT

Consider a packed bed of leaf particles with an initial concentration of solute $S = S_0$. Fresh supercritical fluid is introduced into the bed, which is operated isothermally. The global mass balance of this condition can be represented by the following equation:

$$\alpha \frac{\partial C}{\partial t} + u \frac{\partial C}{\partial z} - \alpha \left(D_z \frac{\partial^2 C}{\partial z^2} + D_x \frac{\partial^2 C}{\partial x^2} \right) = -\left(1-\alpha\right) \frac{\partial S}{\partial t} \tag{1}$$

where C and S are the concentration of solute in the supercritical solvent and concentration of solute in the leaf particles in g/cm^3, respectively. While, α, t and u are the bed void fraction, time in minutes and supercritical fluid velocity in cm/s, and D_x and D_z are the radial and axial diffusion coefficients in cm^2/s.

Although several components exist in the sample matrix, the fitting parameter has been made by taking into account just a single one, called solute. This is a pseudo component, which represents the global behaviour of the analytes. The extraction system is considered as a fixed bed comprising of two phases:

(i) solid (static): matrix, which holds the solute.

(ii) fluid (mobile): supercritical solvent (solute concentration in the fluid phase is negligible due to solute's low solubility).

The solvent flow rate and physical properties are constant during the extraction course. Pressure losses, temperature gradients and heat of dissolution are neglected in the bed. Superficial velocity is constant and it is calculated from supercritical fluid flow rate, by neglecting extracted solute flow rate. The bed void fraction is constant. The time required by the supercritical solvent containing solute to flow from the exit of bed to the exit of separator is negligible. Axial dispersion can be ignored due to dominant axial mass transfer caused by bulk flow of supercritical solvent. In spite of extraction cell geometry ratio (L/D) = 10 <<100, radial dispersion is also plausible to be neglected. The mass balance in the bulk supercritical fluid phase in the column may now be written as:

$$\alpha \frac{\partial C}{\partial t} + u \frac{\partial C}{\partial z} = -(1-\alpha)\frac{\partial S}{\partial t} \tag{2}$$

The initial and boundary conditions are:

$$t = 0, \forall_z \rightarrow C = 0 \tag{3.a}$$

$$z = 0, \forall_t \rightarrow C = 0 \tag{3.b}$$

Because of the lack of information on supercritical fluid – solid mass transfer coefficient, effective diffusivity of andrographolide in supercritical carbon dioxide, and isotherms, the mass balance in the dried leaves is expressed in terms of linear or first order desorption kinetics, which may be written by:

$$\frac{\partial S}{\partial t} = -k_d S \tag{4}$$

where k_d is the desorption rate constant in 1/s.

The initial condition is:

$$t = 0, \forall_z \rightarrow S = S_0 \tag{5}$$

The concentration at the exit of the extraction column (C_e) then can be obtained by solving equations (2) to (5), and is expressed by:

$$C_e = \left(\frac{1-\alpha}{\alpha}\right) S_0 \left[\exp\left\{-k_d\left(t - \frac{\alpha L}{u}\right)\right\} - \exp\left\{-k_d t\right\}\right] \tag{6}$$

The total amount of extracted andrographolide can be then calculated by integrating C_e with respect with time. Therefore, the fraction of extracted andrographolide ($F(t)$) can be calculated using the following equation:

$$F(t) = -\left(\frac{1-\alpha}{k_d \alpha}\right) S_0 (1 - \exp\left\{-k_d t\right\}) + \exp\left\{-k_d\left(t - \frac{\alpha L}{u}\right)\right\} - \exp\left\{k_d\left(\frac{\alpha L}{u}\right)\right\} \tag{7}$$

The optimum values of k_d at each experiment were obtained by choosing the best or optimum value of k_d to minimise the sum of the squares of the difference between the experimental extraction yield (F_{exp}), and the estimated extraction yield (F_{calc}) or *SSE*, using the developed model as shown in equation (7):

$$Min(SSE) = \sum_{1}^{n} \left(F_{calc} - F_{\exp}\right)^2 \tag{8}$$

The inputs of the calculation are α (0.8455), S_0 (1.2×10^{-1} (g/100 g ground - dried leaves)), t (varied from 0 to 25200 seconds), L (21.3 cm) and u (2 cm/s) into the minimisation function of one variable using the MATLAB software to obtain minimum value of *SSE* in equation (8).

EFFECT OF TEMPERATURE AND SUPERCRITICAL FLUID DENSITY ON THE DESORPTION RATE CONSTANT

In previous studies there have been found different desorption rate constant dependences on temperature. Tan and Liou (1988) reported that desorption efficiency of ethyl acetate from activated carbon using supercritical carbon dioxide decreased with temperature when pressure was fixed at 8.81 MPa, and an optimum temperature of about 40 °C was found when pressures were fixed and higher than 10.13 MPa. However, the desorption efficiency of toluene from activated carbon using supercritical carbon dioxide was found to increase with temperature by Tan and Liou (1989). If the desorption rate constant in this system follows the Arrhenius law, the following linear relation should exist:

$$\ln k_d = -\left(\frac{E}{RT}\right) + \ln k_{d0} \tag{9}$$

where E, R , T and k_{d0} are the apparent desorption activation energy in J/mol, universal gas constant in J/mol K, temperature in Kelvin, and standard desorption rate constant in 1/s.

In supercritical fluid technology, temperature and pressure have significant roles in adjusting supercritical fluid density, which is strongly related to its solvating power. If it is assumed that k_{d0} is function of supercritical fluid density, the following relationship is therefore proposed in this study:

$$k_{d0} = k_r \rho^n \tag{10}$$

where ρ is the supercritical fluid density (g/cm^3), and k_r and n are the constants of this equation.

RESULTS AND DISCUSSIONS

For the calculation in this work, the initial andrographolide content in the *Andrographis paniculata* leaf particles was 1.2×10^{-1} (g/100 g ground - dried leaves). It was determined by exhaustive standard soxhlet extraction of 5 grams of ground – dried leaves using 1.50 × 10 $^{-4}$ m^3 of pure methanol for 24 hours. Five random samples were analysed and an average value of initial andrographolide content was then taken. The data of semi batch supercritical carbon dioxide extraction of andrographolide from *Andrographis paniculata* leaves using methanol as co – solvent were obtained from the literature (Kumoro, 2006).

The effect of methanol concentration on the extraction rate was investigated by carrying out a set of experiments using 0.375 mm particle size at 40 ^{o}C and 10.00 MPa, while the total flow rate of supercritical fluid was set at 3.18 × 10^{-5} kg/s total supercritical solvent flow rate. Figure 2 was constructed to show the effect of time with methanol concentration as a parameter on cumulative yield of extract. The desorption model, which has only one adjustable parameter could explained the extraction profile fairly well, because it uses the integral bed system and a single order of desorption kinetic. Since the solubility of andrographolide in supercritical carbon dioxide and methanol mixture in the presence of leaf matrices is obviously low, the assumption of single order desorption kinetic is plausible.

The desorption rate constants obtained from this study are presented in Table 1. The desorption rate constants increased substantially with the increase of methanol content in supercritical solvent from 0 to 12.5 %. Further increase of methanol content in supercritical solvent did not increase the desorption rate constant, possibly due to the saturation of active sites on the leaf particles containing andrographolide by methanol molecules. Addition of more methanol into supercritical carbon dioxide also caused the leaf particles to agglomerate and reduced the effective contact between supercritical solvent and the active sites on the leaf particles, as indicated by reduction of desorption rate constants at high methanol content in supercritical solvent.

As can be seen in Table 2, addition of methanol into carbon dioxide increases the supercritical solvent density at 40 ^{o}C and 10.00 MPa, which lead to enhance the desorption rate constant. The desorption rate constant and the supercritical solvent density can be correlated by $k_d = 30.8674\,\rho^{2.5573}$ and the AARD was found to be 2.40 %.

The effect of temperature on andrographolide yield was carried out at 10.00 MPa using 0.375 mm particle size, 12.5% methanol in the supercritical carbon dioxide and total supercritical fluid flow rate of 3.18×10^{-5} kg/s total supercritical solvent flow rate. Figure 3 shows the andrographolide yield as function of time at various extraction temperatures. It can be seen that the yield of andrographolide increased when extraction temperatures were increased from 30° to 45 °C. However, further increase in temperature will lead to decrease the extract yield due to thermal degradation of andrographolide and drastic decrease in supercritical solvent density.

Table 3 shows the optimised desorption rate constant as the function of temperature. The desorption rate constants increased with temperature. With the present observation, the Arrhenius expression $\ln k_d = \ln k_0 - \left(\frac{E}{RT}\right)$ was obtained, where $k_{do} = 2.9108 \times 10^{-2}$ (1/s), $E = 1.1676 \times 10^{7}$ J/mol and 4.92 % AARD was generated. Tan and Liou (1988) obtained the desorption activation energies of toluene from activated carbon at different temperature were in the range of 1.45 to 3.95×10^{7} J/mol, which is in the same order with the *E* value obtained in this study.

Correlation of desorption rate constant with supercritical fluid density is presented in Table 4. The desorption rate constants were found increased with the decrease of supercritical fluid density. This fact indicated that desorption is more favourable at lower supercritical fluid density. The following correlation between desorption rate constant and supercritical fluid density is proposed: $k_{do} = k_r \rho^n$, with $k_r = 2.0720 \times 10^{-5}$ (1/min) and n = -2.25 and gives AARD of 6.62 %.

CONCLUSIONS

A mathematical model based on unsteady state first order solute desorption has been developed and tested for the calculation of SCDE profile of andrographolide from *Andrographis paniculata* leaves at different methanol concentrations and temperatures. The extraction profiles obtained from the calculation using this model were in a good agreement with the experimental data obtained from the literature. The desorption rate constants were also well correlated with temperature and supercritical solvent density. Thus, the modelling presented in this work can be used in the optimisation and scale - up of a supercritical fluid extraction unit. This model is capable for the modelling of SCDE of andrographolide from *Andrographis paniculata* leaves.

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ABBREVIATIONS AND NOTATIONS

Parameters	**Meaning**	**Unit**
$AARD$	= Average Absolute Relative Deviation	-
ARD	= Absolute Relative Deviation	-
C	= Solute concentration in the fluid phase in bed void volume	g/cm^3
C_e	= Solute concentration in exit stream of fluid	g/cm^3
D	= Diameter of extractor	cm
D_x	= Radial dispersion coefficient	cm^2/s
D_z	= Axial dispersion coefficient	cm^2/s
E	= Apparent desorption activation energy	J/mol
F_{calc}	= Calculated fractional extraction yield as a function of extraction time	-
F_{exp}	= Experimental fractional extraction yield as a function of extraction time	-
$F(t)$	= Fractional extraction yield as a function of extraction time	-
k_d	= Desorption rate constant	1/s
k_{dcalc}	= Calculated desorption rate constant	1/s
k_{d0}	= Standard desorption rate constant	1/s
k_r	= Density based desorption rate constant in equation (10)	1/s
L	= Length of extractor	cm
n	= Constant in equation (10)	-

N	= Number of data	-
R	= Universal gas constant	J/(mol.K)
S	= Concentration of solute in leaf particles	g/cm^3
S_0	= Initial concentration of solute in leaf particles	g/cm^3
SSE	= Sum of squares of errors	-
t	= Time	s
T	= Temperature	^{o}C, K
u	= Supercritical fluid velocity	cm/s
x	= Radial coordinate in the extractor	cm
z	= Axial coordinate in the extractor	cm
α	= Void bed fraction	-
ρ	= Supercritical fluid density	g/cm^3

Table 1

Optimised desorption rate constant for integral desorption model at various methanol concentrations in supercritical phase

Methanol Concentration, (%)	**$k_d \times 10000$, (1/s)**
0.0	1.18
5.0	2.25
7.5	2.80
10.0	2.94
12.5	3.34
15.0	3.35

Table 2

Effect of supercritical carbon dioxide-methanol mixture density on desorption rate constant for integral desorption model at various methanol concentrations in supercritical phase measured at 40 °C and 10.00 MPa

Methanol Concentration (%)	**Density (g/cm^3)**	**k_d × 10000, (1/s)**	**k_{dcalc} × 10000, (1/s)**	**ARD[1], %**
0.00	0.5613	1.18	1.18	0.73
5.00	0.7332	2.25	2.33	3.50
7.50	0.7789	2.80	2.72	3.12
10.00	0.8109	2.94	3.01	2.21
12.5	0.8335	3.34	3.23	3.49
15.00	0.8497	3.35	3.39	1.39
			AARD[2] =	**2.40**

[1] $ARD = ABS\left(\frac{k_d - k_{d_{calc}}}{k_d}\right)$

[2] $AARD = \frac{1}{N}\sum_{1}^{N} ABS\left(\frac{k_d - k_{d_{calc}}}{k_d}\right)$, and N is the number of data.

Table 3

Desorption rate constant as function of temperature

(k_{do} = 1.7465, E = 1.3877 × 10^7J/mol and the value of SSE is: 4.979E-6)

Temperature, (^{o}C)	k_d **× 10000, (1/s)**	k_{dcalc} **× 10000, (1/s)**	**ARD[1], %**
30	2.52	2.83	11.18
40	3.34	3.28	1.78
45	3.71	3.52	5.40
50	3.72	3.77	1.33
		AARD[2]	**4.92**

[1] $ARD = ABS\left(\frac{k_d - k_{d_{calc}}}{k_d}\right)$

[2] $AARD = \frac{1}{N}\sum_{1}^{N} ABS\left(\frac{k_d - k_{d_{calc}}}{k_d}\right)$, and N is the number of data.

Table 4

Desorption rate constant as function of supercritical solvent density

(k_r = 0.0124331, n = -2.25 and the value of SSE is: 2.0164E-7)

Density × 100 (g/cm^3)	**k_d × 10000, (1/s)**	**k_{dcalc} × 10000, (1/s)**	**ARD[1], %**
0.8913	2.52	2.69	6.20
0.8335	3.34	3.12	7.06
0.7944	3.71	3.48	6.62
0.7482	3.72	3.98	6.58
		AARD[2]	**6.62**

[1] $ARD = ABS\left(\frac{k_d - k_{d_{calc}}}{k_d}\right)$

[2] $AARD = \frac{1}{N}\sum_{1}^{N} ABS\left(\frac{k_d - k_{d_{calc}}}{k_d}\right)$, and N is the number of data.

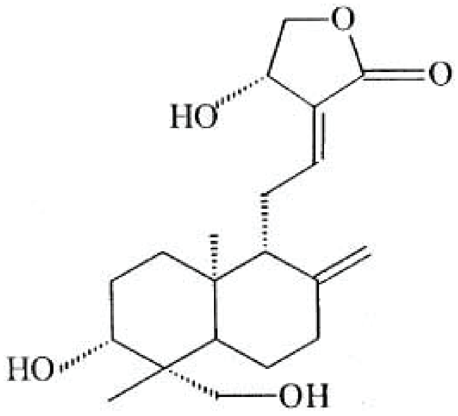

Figure 1: Molecular structure of andrographolide (Rajani et al., 2000)

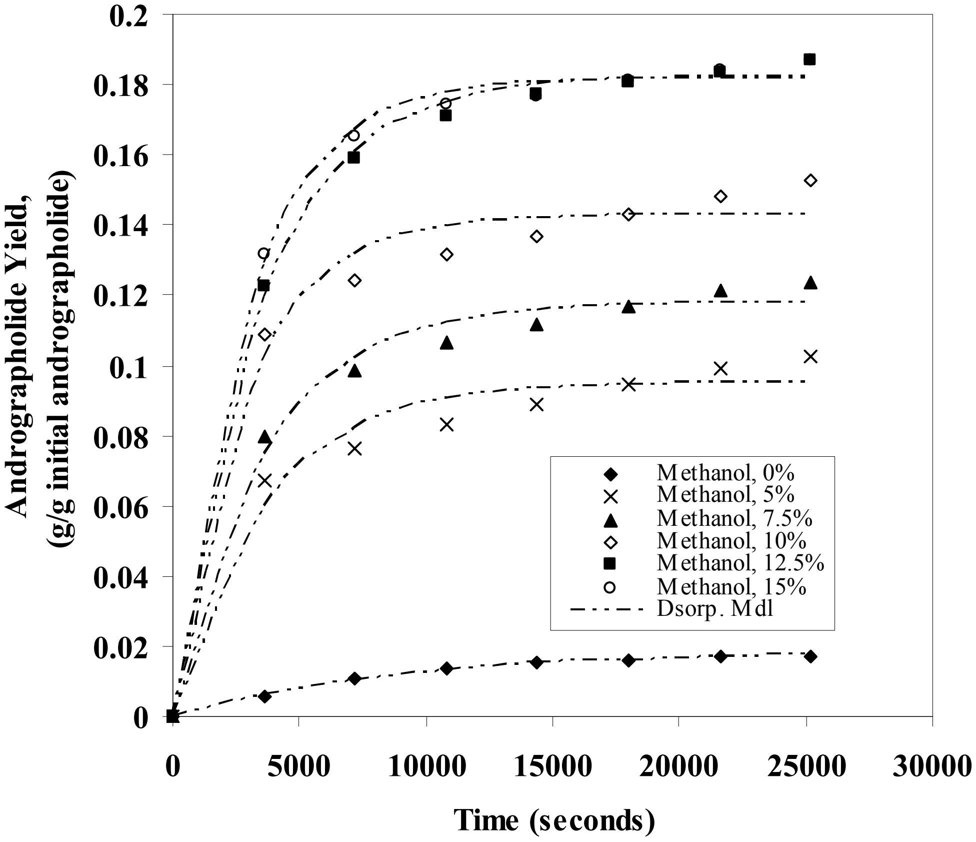

Figure 2. Comparison of calculated and experimental extract yield of andrographolide as function of methanol concentration at 10.00 MPa, 40 °C and 3.18×10^{-5} kg/s total supercritical solvent flow rate.

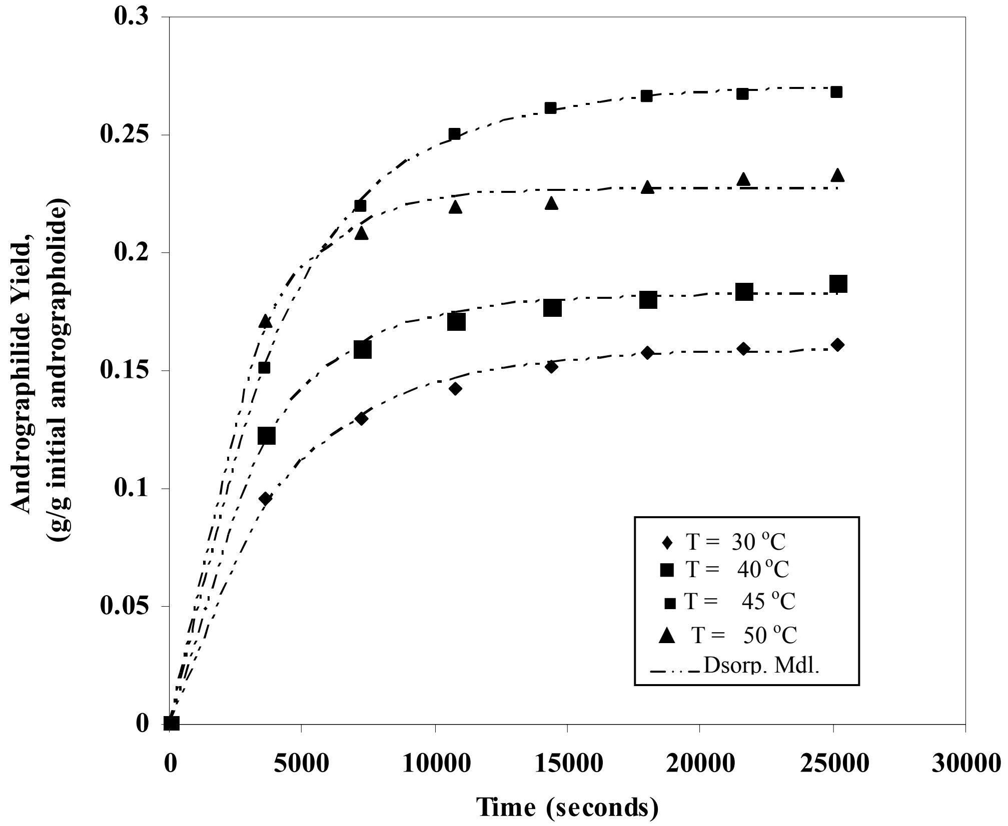

Figure 3. Comparison of calculated and experimental extract yield of andrographolide as function of temperature at 10.00 MPa, 12.5 % methanol concentration and 3.18×10^{-5} kg/s total supercritical solvent flow rate.