Purification of Parthenolide in Feverfew by Supercritical Fluid Extraction and Supercritical Fluid Chromatography with Evaporative Light Scattering Detection

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1. Introduction

Evaporative light scattering detector (ELSD) can be used for the detection of the compounds which do not have any specific properties such as UV absorption due to its wide range of applicability except for volatile compounds. However, when ELSD is used in supercritical fluid chromatography (SFC), the detection parameters in ELSD should be optimized because the chromatogram shows specific behavior in SFC.

In this presentation, we measured the behavior of chromatogram by changing those parameters. parameters.

Feverfew (*Tanacetum parthenium*, Figure1) is a medicinal herb and contains sesquiterpene lactone Parthenolide (Figure 2) which is reported to be active principle[1)].

In this presentation, we tried to purify Parthenolide in feverfew by Supercritical fluid extraction (SFE) and the subsequent SFC with ELS detection

Figure 1. Feverfew

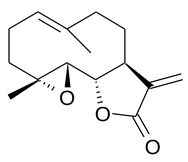


Figure 2. Structure of Parthenolide

2. Experimental

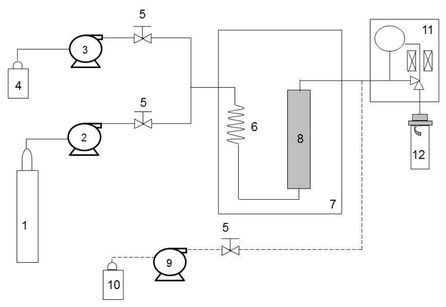
2.1 Apparatus

Apparatus. Figure 3 shows the appearance of JASCO Semi-preparative SFC/PDA/ELSD system (all from JASCO Co., Tokyo, Japan) used in this experiment.
Figure 4 shows the schematic diagram of the SFC/PDA/ELSD System.
Figure 5 shows the flow diagram of the SFE System.
Figure 6 shows the Micro Cyclone Separator (MCS).

Materials and Chemicals. . Columns, SFCpak SIL-5, 5µm, 4.6 mm ID x 250 mmL and SFCpak SIL-5SP, 5µm, 10.0 mm ID x 250 mmL were purchased from JASCO Co., Tokyo, Japan.

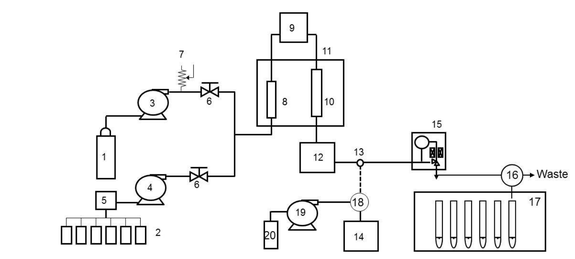
Carbon dioxide (99.98 %) was supplied by TAIYO NIPPON SANSO Co., Ltd, Tokyo, Japan. HPLC-grade methanol which was used as modifier and make up solvent and Caffeine were purchased from Wako Pure Chemicals, Osaka, Japan. Ethy p-Hydroxybenzoate (Ethylparaben) was purchased from TOKYO CHEMICAL INDUSTRY Co., Tokyo, Japan. Parthenolide was purchased from Sigma-Aldrich Co., USA. Air-dried Feverfew was made in USA.

Figure 3 JASCO Semi-preparative SFC/PDA/ELSD System



1. CO_2 Cylinder
2. PU-2080-CO_2 CO_2 Delivery Pump
3. PU-2085 Modifier Delivery Pump
4. Modifier Solvent
5. ST-500 Stop Valve
6. Preheater Coil
7. CO-2060 Column Oven
8. EV-2 Extraction Vessel
9. PU-2080 Solvent Delivery Pump
10. Make up Solvent
11. BP-2080 Back Pressure Regulator
12. MCS-1 Micro Cyclone Separator (MCS)

Figure 5. Flow diagram of the JASCO SFE System



1. CO_2 Cylinder
2. Modifier Solvent
3. PU-2086-CO_2 CO_2 Delivery Pump
4. PU-2086 Modifier Delivery Pump
5. LV-2080-06 Solvent Selector Valve
6. ST-500 Stop Valve
7. Safety Valve
8. Accumulator
9. AS-2059-SF SFC Auto Sampler
10. Column
11. CO-2060 Column Oven
12. MD-2018 PDA Detector
13. Splitter
14. ELS-2041 ELS Detector
15. BP-2080 Back Pressure Regulator
16. HV-2088-06 Fraction Valve Unit
17. Gilson 223 Sample Changer
18. HV-2080-01 Column Switching Valve
19. PU-2080 Solvent Delivery Pump
20. Make up Solvent

Figure 4. Schematic diagram of the JASCO Semi-preparative SFC/PDA/ELSD System

Model MCS-6
MCS kit for 6 fractions

Model MCS-1
MCS kit for 1 fraction

Figure 6. Micro Cyclone Separator

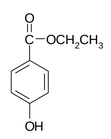


Figure 7. Structure of Ethylparaben

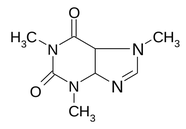


Figure 8. Structure of Caffeine

3. Results and Discussion

3.1 ELSD Parameters of SFC

First of all, the parameters for the gas flow rate, evaporator temperature and nebulizer temperature were optimized in SFC. These parameter are specific parameter for ELSD.
Ethylparaben (Figure 7) and caffeine (Figure 8) were used as standard sample.

3.1.1 Effect of Gas Flow Rate

Figure 9 shows the chromatograms of Ethylparaben and caffeine when the gas flow rate is changed.

Figure 10 shows the effect of gas flow rate on the areas for Ehylparaben and caffeine.

The areas for Ehylparaben and caffeine were decreased as the gas flow rate was increased. In the case of SFC, CO_2 enters in the detector probe, and the gas flow rate is the total of CO_2 and gas flow rates. For this reason, it is thought that the sensitivity becomes higher as the gas flow rate is small.

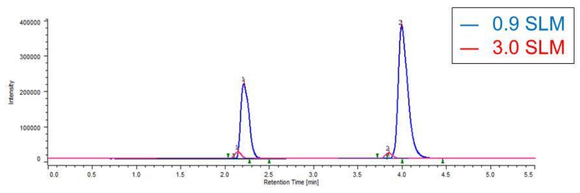


Figure 9. Chromatograms of Ethyparaben and Caffeine when the gas flow rate is changed
The separation conditions are: column; SFCPak SIL-5 (4.6 mm ID x 250 mmL, 5 µm); CO_2 flow rate: 2 mL/min at -10ºC; modifier: methanol @ flow rate 0.5 mL/min; make up solvent: methanol @ flow rate 0.5mL/min; pressure: 20 MPa; column temperature: 40 ºC; ELSD evaporator temperature: 40 ºC; nebulizer temperature: 40 ºC; Gas Flow 0.9, 3.0 SLM; injection volume: 5 µL; Splitter : I.D. 50µm x 505 mm; Sample: Ethylparaben and Caffeine (1mg/mL in MeOH.)

Figure 10. Effect of gas flow rate
The separation conditions are: column; SFCPak SIL-5 (4.6 mm ID x 250 mmL, 5 µm); CO_2 flow rate: 2 mL/min at -10ºC; modifier: methanol @ flow rate 0.5 mL/min; make up solvent: methanol @ flow rate 0.5mL/min; pressure: 20 MPa; column temperature: 40 ºC; ELSD evaporator temperature: 40 ºC; nebulizer temperature: 40 ºC; gas flow 0.9, 1.0, 1.2, 1.5, 2.0, 2.5, 3.0 SLM; injection volume: 5 µL; Splitter : I.D. 50µm x 505 mm; Sample: Ethylparaben and Caffeine (1mg/mL in MeOH.)

3.1.2 Effect of Evaporator Temperature

Figure 11 shows the chromatograms of Ethylparaben and caffeine when the evaporator temperature is changed.

Figure 12 shows the effect of evaporator temperature on the areas for Ethylparaben and caffeine.

The evaporator temperature depends on the volatility of sample. The area for Ethylparaben, which is a semi-volatile organic compound, was decreased as the temperature was increased. On the other hand, the area for caffeine, which has low volatility, was almost the same when the temperature was 30 ºC or more.

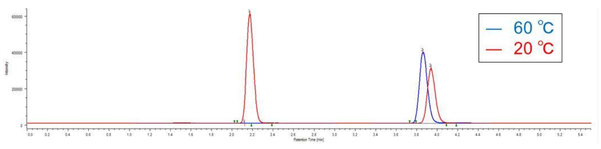


Figure 11. Chromatograms of Ethyparaben and Caffeine when the evaporator temperature is changed
The separation conditions are: column; SFCPak SIL-5 (4.6 mm ID x 250 mmL, 5 µm); CO_2 flow rate: 2 mL/min at -10ºC; modifier: methanol @ flow rate 0.5 mL/min; make up solvent: methanol @ flow rate 0.5mL/min; pressure: 20 MPa; column temperature: 40 ºC; ELSD evaporator temperature: 20, 60 ºC; nebulizer temperature: 40 ºC; Gas Flow 0.9 SLM; injection volume: 5 µL; Splitter : I.D. 50µm x 505 mm; Sample: Ethylparaben and Caffeine (1mg/mL in MeOH.)

3.1.3 Effect of Nebulizer Temperature

Figure 13 shows the effect nebulizer temperature on the areas for Ethylparaben and caffeine.

The areas were not changed as the temperature was changed. This shows that the nebulizer temperature is not effected on the areas for these samples.

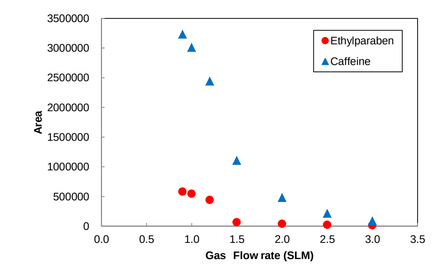


Figure 12. Effect of evaporate temperature
The separation conditions are: column; SFCPak SIL-5 (4.6 mm ID x 250 mmL, 5 µm); CO_2 flow rate: 2 mL/min at -10ºC; modifier: methanol @ flow rate 0.5 mL/min; make up solvent: methanol @ flow rate 0.5mL/min; pressure: 20 MPa; column temperature: 40 ºC; ELSD evaporator temperature: 20, 30, 40, 50, 60 ºC; nebulizer temperature : 40 ºC; Gas Flow 0.9 SLM; injection volume: 5 µL; Splitter : I.D. 50µm x 505 mm; Sample: Ethylparaben and Caffeine (1mg/mL in MeOH.)

3.2 Effect of Splitter Length

In the case of ELSD used for SFC, a splitter is used. Because all volume of sample stream cannot be entered into the detector probe. A typical splitter is made by capillary tube. A part of sample stream is split by this and introduced into the detector probe. The split ratio is decided by the inner diameter and length of capillary tube. However, it varies with the temperature, pressure and flow rate at that time.

Figure 14 shows the effect of splitter length on the areas for Ethylparaben and caffeine.

The area for caffeine became smaller as the length of the splitter was longer. This comes from the increase of tubing resistance.
On the other hand, the area for Ethylparaben became small when the length was 280mm. It is assumed that the CO_2 flow rate is increased and the sensitivity becomes smaller as shown in Section 3.1.1.

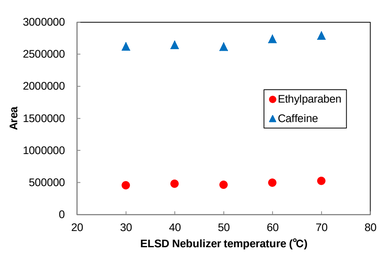


Figure 13. Effect of Neblizer temperature
The separation conditions are: column; SFCPak SIL-5 (4.6 mm ID x 250 mmL, 5 µm); CO_2 flow rate: 2 mL/min at -10ºC; modifier: methanol @ flow rate 0.5 mL/min; make up solvent: methanol @ flow rate 0.5mL/min; pressure: 20 MPa; column temperature: 40 ºC; ELSD evaporator temperature: 40ºC; nebulizer temperature: 30, 40, 50, 60, 70 ºC; Gas Flow 0.9 SLM; injection volume: 5 µL; Splitter : I.D. 50µm x 505 mm; Sample: Ethylparaben and Caffeine (1mg/mL in MeOH.)

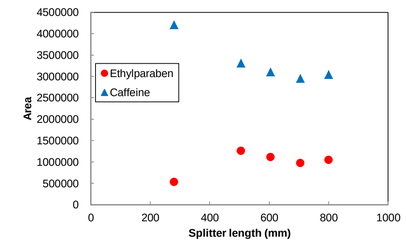


Figure 14. Effect of Splitter length
The separation conditions are: column; SFCPak SIL-5 (4.6 mm ID x 250 mmL, 5 µm); CO_2 flow rate: 2 mL/min at -10ºC; modifier: methanol @ flow rate 0.5 mL/min; make up solvent: methanol @ flow rate 0.5mL/min; pressure: 20 MPa; column temperature: 40 ºC; ELSD evaporator temperature: 40ºC; nebulizer temperature: 40 ºC; gas flow 0.9 SLM; injection volume: 5 µL; Splitter : I.D. 50µm x 280, 505, 605,705, 805 mm; Sample: Ethylparaben and Caffeine (1mg/mL in MeOH.)

3.3 Effect of Make up Pump Flow Rate

In the case of SFC, the solvent exhausted from the splitter outlet in the state of the aerosol because there is CO_2. The dissolubility of the sample falls, and there is a possibility that the sample blockades it in the tube because it becomes CO_2 of the gas from supercritical fluid. Therefore, protect the blockage of a high concentration sample at the time of preparative SFC and the stability of detection can be prevented by flowing the make up solvent. Figure 15 shows the relation of the area of the Ethylparaben and the caffeine when make up pump flow rate was changed. Flow rate went up at time when the make up solvent was not flowed and the sensitivity goes up. However, flow rate increased, the area decreased afterwards. It seems that flow rate is high, the liquid drop in ELSD has caused evaporation shortage.

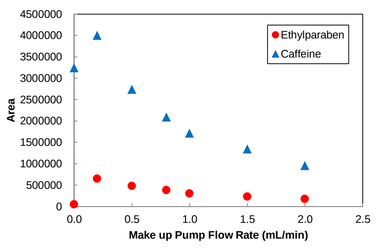


Figure 15. Effect of make up pump flow rate
The separation conditions are: column; SFCPak SIL-5 (4.6 mm ID x 250 mmL, 5 µm); CO_2 flow rate: 2 mL/min at -10ºC; modifier: methanol @ flow rate 0.5 mL/min; make up solvent: methanol @ flow rate 0, 0.2, 0.5, 0.8, 1.0, 1.5, 2.0 mL/min; pressure: 20 MPa; column temperature: 40 ºC; ELSD evaporator temperature: 40ºC; nebulizer temperature: 40 ºC; gas flow 0.9 SLM; injection volume: 5 µL; Sample: Ethylparaben and Caffeine (1mg/mL in MeOH.)

Summary of ELSD parameters with SFC

- Gas flow rate
  Low flow rate provides high sensitivity.
  Optimum gas flow rate: 0.8-1.0 SLM
- Evaporator Temperature
  The evaporator temperature depends on the volatility of the sample.
  In the case of ELS-2041, the sample with high volatility can detect.
  Optimum evaporator temperature 20-40 ºC
- Nebulizer temperature
  The nebulizer temperature does not affect on the sensitivity.
- Splitter length
  The sensitivity changes by changing the length of the splitter.
  However, it is decreased by the CO_2 flow rate. It depends on the sample.
- Make up pump flow rate
  Make up solvent should be added for Preparative SFC/ELSD to prevent clogging the tubing.

3.4 Purification of Parthenolide in Feverfew by Supercritical Fluid Extraction and Supercritical Fluid Chromatography

3.4.1 Feverfew by Supercritical fluid extraction

Feverfew was extracted using the SFE system shown in Figure 4, referring to the Reference[2)]. The SFE conditions are shown in Table I. The preparation after the extraction was shown in Figure 16.

Table I. SFE Condition

Sample:	Feverfew 1.0 g
Extraction Vessel:	10mL, I.D.10 mm x 127 mm
CO_2 Flow Rate:	3.0 mL/min
Make up Solvent Flow Rate:	0.3 mL/min (MeOH)
Temperature:	45 ºC
Pressure:	30 MPa
Extraction Time:	60 min

Extracts in the sample was concentrated by evaporator.
↓
The concentrate diluted with MeOH in volumetric flask to 5.0mL.
↓
Filtration by the membrane filter (0.45µm).
↓
Injects to SFC

Figure.16 Preparation Flow Sheet

3.4.2 Purification of Parthenolide by Supercritical Fluid Chromatography

The sample extracted by SFE and pre-treated was injected to SFC and Parthenolide in the sample was fractionated by semi-preparative SFC. Figure 17 shows the chromatogram of the exacts in Feverfew. The peak shown in red rectangle was fractionated.

The fraction obtained in Figure 17 was analyzed and identified by analytical SFC. Figure 18 shows the chromatogram. By comparing the peak area with that for the standard sample of Parthenolide, we confirmed that 0.03mg/g of Parthenolide was collected.

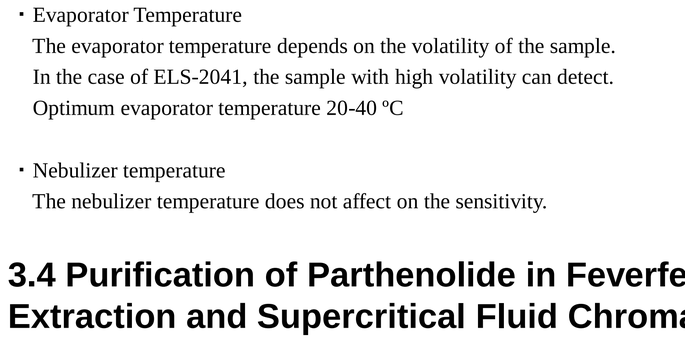


Figure 17. Chromatogram of the extract in Feverfew
The separation conditions are: column; SFCPak SIL-5SP (10 mm ID x 250 mmL, 5 µm); CO_2 flow rate: 10 mL/min at -10ºC;
modifier: methanol @ flow rates
0.3 0.73 1.5 mL/min
0 7.2 7.3 min
make up solvent: methanol @ flow rate 0.5 mL/min; pressure: 20 MPa; column temperature: 40 ºC; ELSD evaporator temperature: 40ºC; nebulizer temperature: 40 ºC; gas flow 0.9 SLM; Splitter : I.D. 25 µm x 505 mm; injection volume: 500 µL; Sample: the concentrated exact in Feverfew

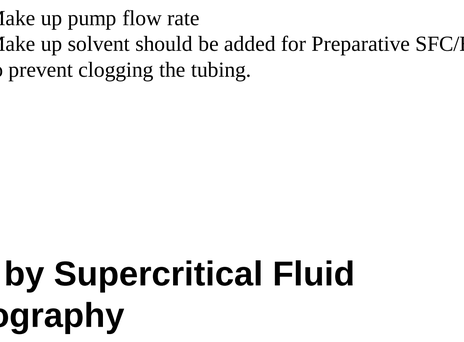


Figure 18. Chromatogram of the fraction
The separation conditions are: column; SFCPak SIL-5 (4.6 mm ID x 250 mmL, 5 µm); CO_2 flow rate: 3.0 mL/min at -10ºC;
modifier: methanol @ flow rates
0.2 0.38 mL/min
0 6.0 min
pressure: 20 MPa; column temperature: 40 ºC; injection volume: 5 µL; Sample: the fraction

4. Conclusion

1. We could optimized the ELS detection parameters for SFC because the chromatogram shows specific behavior in SFC.
2. Extracts from Feverfew was performed by Supercritical fluid extraction and Parthenolide was fractionated from the extracts by SFC with ELS detection.
   0.03mg/g. of Parthenolide was collected.

References

1) W. A. Groenewegen, D. W. Knight and S. Heptinstall, J. Pharm. Pharmacol., 38, 709 (1986)
2) R. M. Smith, M. D. Burford, J. Chromatography, 627, 255-261 (1992)