New Generation Dynamic Supercritical Fluid System with Online Detection

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Abstract

The Supercritical Fluid Extraction (SFE) system has emerged as a leading technology in recent years, thanks to its benefits. This method avoids toxic chemical solvents when extracting bioactive compounds from plants, yields high-quality and efficient products, and facilitates quicker and more cost-effective production.

In classical SFE systems, the extraction period and the types and amounts of compounds found in the extracts obtained from the extraction process can usually be determined by analyses performed post-extraction. Since this situation does not permit any intervention during extraction, improving efficiency is only feasible through repeated extraction processes.

This study integrated the SFE system with flow injection analysis (FIA) and a diode array detector (DAD) by using directional valves. The extraction process's efficiency was evaluated over a 3-hour period.

Keywords: Bioactive compounds, SFE, DAD, FIA,

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1. Introduction

Classical separation methods used to obtain bioactive compounds from natural sources have many disadvantages such as the presence of chemical solvent residues in the products, the formation of oxidized products and impurities during the process, and low-yield product production. The SFE system is defined as the dissolution of the substance in a fluid under supercritical conditions to extract soluble components in a mixture and then the separation of the product from the fluid by reducing the ambient pressure. In this way, natural products with no chemical solvent residue can be obtained. Considering these features, the SFE system stands out as the most important extraction method [1,2].

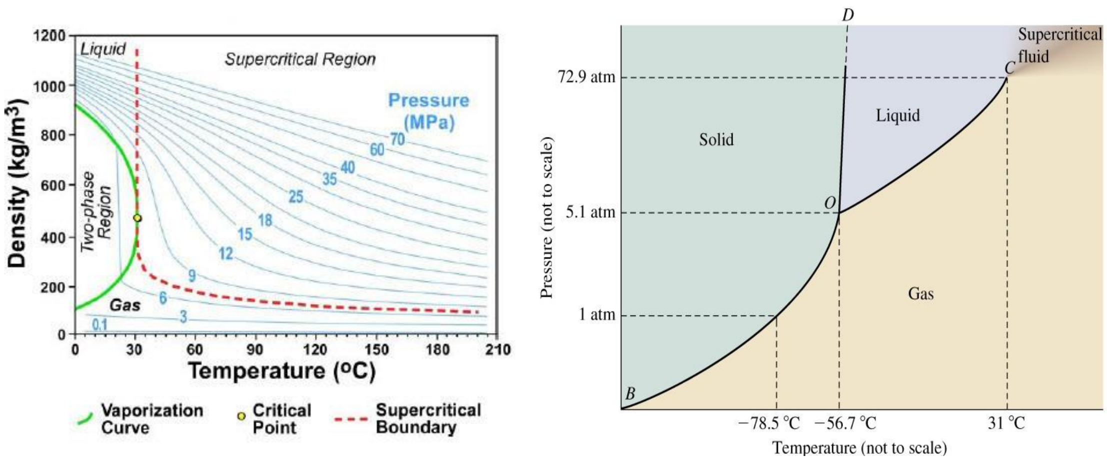

Figure 1. Temperature-pressure-density; Temperature-pressure, phase diagrams of CO_2 [3, 4]

The SFE system, called “green technology”, has become one of the new generation technologies that are becoming increasingly widespread, with significant advantages such as not using harmful chemical solvents during the extraction of bioactive compounds from plants, obtaining high quality, high yield products, faster extraction, and more economical production opportunities [5, 6]. To carry out an efficient extraction process with the SFE system, it is of great importance to optimise critical parameters such as the pressure and temperature of the extraction column, the amount of carbon dioxide and co-solvent used, flow rate, extraction cycle, and duration. Detailed examination of the efficiency and process of the SFE system is possible with the use of high-resolution detector systems that will determine the types and amounts of bioactive components obtained over time [7]. The online monitoring of the types and amounts of compounds passing into the solvent system during extraction, the optimisation of extraction parameters, and the collection of compounds with desired properties in desired locations with high purity and efficiency are only possible with a controlled combined extraction system.

In the study conducted by Abrahamsson et al., the properties of the products obtained and the system efficiencies depending on the extraction times were investigated using SFE-UV-Vis and SFE-ELSD combined systems. In these studies, the obtained extracts were passed through the detector and only monitored. Based on the information obtained, the extraction system efficiencies and general properties of the species were attempted to be determined. However, the group separation of the obtained extracts and the separation and detection of the compounds in the groups were not performed [7].

During the development of modern analytical systems, interest in using supercritical fluids in chromatography systems has significantly increased. The ability of SFC to meet the requirements of good manufacturing practices (GMP) and to obtain verifiable methods has also been the focus of several recent studies based on the recommendations of the International Conference on Harmonization (ICH). It is known that SFC can yield excellent results in analyzing polar, non-polar, ionic, small, and large molecular weight chemical species [8-10]. To enhance the benefits of SFE systems in applications, new combined systems integrated with SFC have been explored. In recent years, studies on the SFE system's development considered the addition of various offline and online detector systems (DAD/ELSD/MS/IR) to the SFE-SFC system as a significant advancement [1, 10]. This study presents a combined system with numerous features, setting it apart from similar studies reported in the literature.

2. Materials and Method

SFE-FIA-SFC(DAD) combined system equipment.
SFE system; CO_2 tube, CO_2 pump (CO_2 sending pump used for SFE), extraction column, separators; used in extraction processes.
CO_2 pump: used to give SF character CO_2 to the system used in the analysis section.
Multi-way valve system: (6-channel/2-way (6PT/2PS)), valves, which are the main components responsible for directing the combined system, were used to collect samples, direct samples, and change the flow direction.
A flow injection system has been used to collect samples instantly during continuous extraction and to send collected samples to chromatography systems or detectors for rapid determination.
SFC Column has been used for the separation of alkaloids, phenolic compounds, flavonoids, vitamins, and fixed oils with C18, C8, and diol columns.
Modified DAD: It has been used to determine the characteristics of species in the supercritical fluid-extract mixture in high-pressure resistant FIA and SFC systems.

2.1. SFE-FIA-SFC(DAD) Combined System

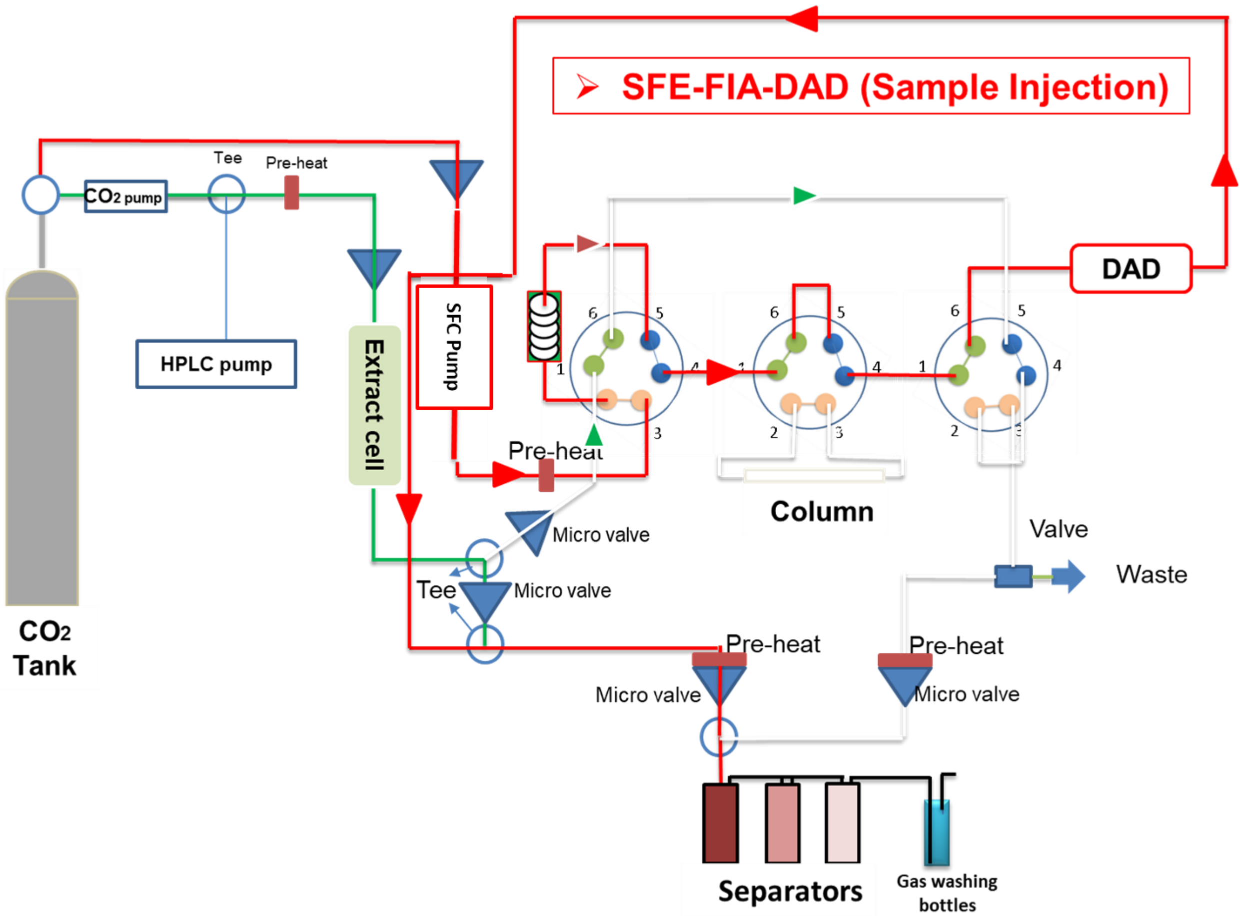

Figure 2. SFE-FIA (DAD)) system flow chart

2.2. Position for Valve Systems

Continuous extraction, system cleaning, DAD baseline, flow injection analysis, continuous signal monitoring with DAD, SFC, determination with 2D-GC were performed on a single system. Positions for valve systems are given in Figure 3.

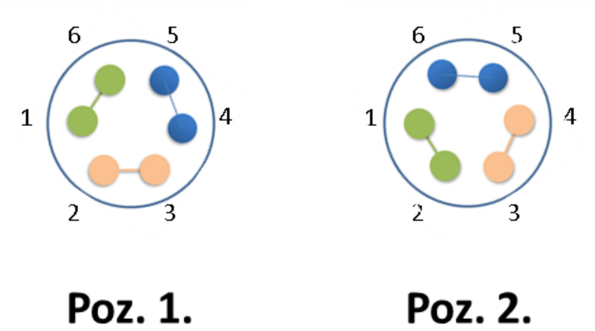

Figure 3. Positions for valve systems. Pos. 1 and Pos. 2.

Table 1. Valve positions of processes

Processes	1. Valve Position	2. Valve Position	3. Valve Position
SFE-DAD system baseline	**2**	**1**	**1**
SFE-DAD system	**1**	**1**	**2**
Loop Fill and Baseline (SFE-FIA-DAD)	**2**	**1**	**1**
SFE-FIA-DAD system	**1**	**1**	**1**
SFE-FIA-SFC-DAD system	**1**	**2**	**1**

3. Results

3.1. SFE System

To optimise the extraction time, a study was carried out until the point where the yield was highest according to the midpoint obtained from the central composite design. In the following studies, the plant seed to be used was brought to a diameter of 0.3-0.5 mm with the help of a grinder. 100 grams of plant seed was weighed for each study. Extraction time optimization study, 300 minutes extraction study was carried out at 200 bar pressure, 50 ^{o}C and 6.5 mL/min flow rate from the data obtained from the central composite design.

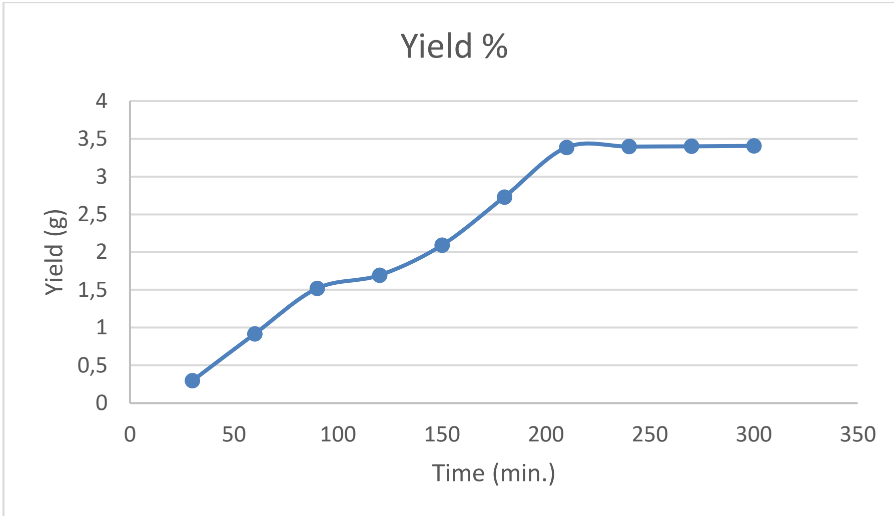

Figure 4. Amount of product obtained over time

3.2. SFE-FIA-DAD System

Mobile Phase; Supercritical CO_2
Extractor column pressure; 200 bar
Extractor column temperature; 50 ^{o}C
Flow rate; 2.5 mL/min
Loop volume; 20 uL
Wavelength: 210, 230, 254, 280, 325 nm

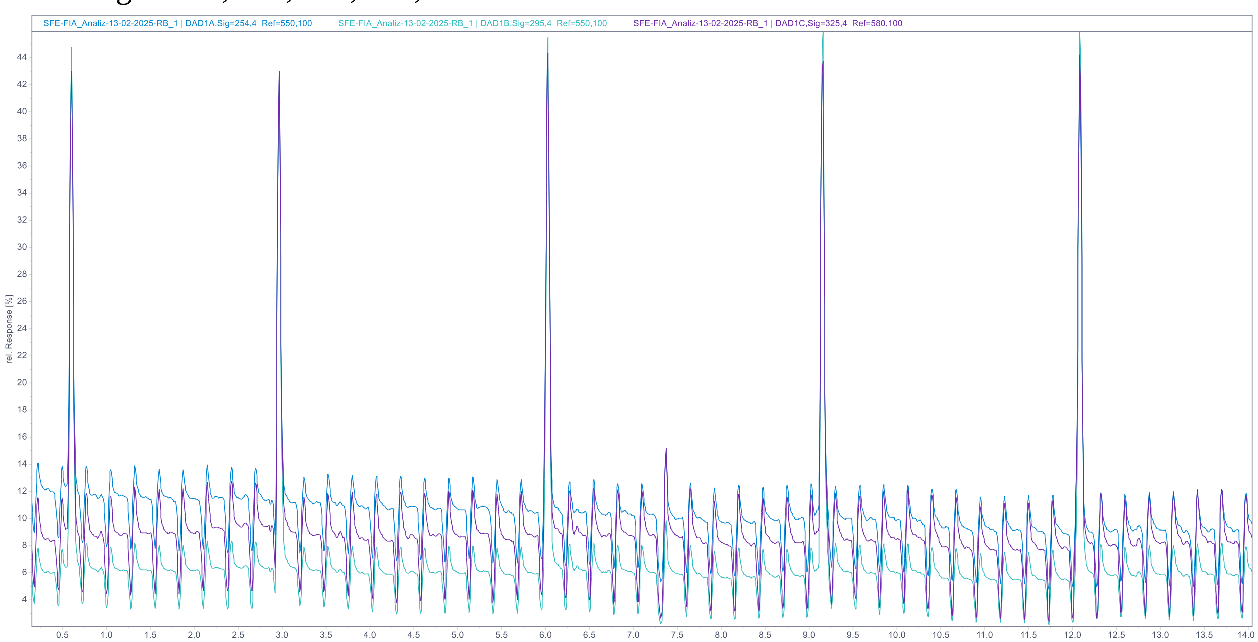

Figure 5. Graphic obtained with the SFE-FIA-DAD system

4. Discussion

The SFE system is connected to the analysis section with divider and valve systems, the extracted products are sent to both collection fractions and analysis sections, whose pressure and temperature can be controlled automatically, and the simultaneous extraction system is continuously monitored, and the obtained product properties are tried to be controlled. The SFE system is independent and can work separately from the analysis system. All products in the SFE system can be sent to the fractionation system without being divided by automatically adjusting the divider section if desired. The SFE system is designed to perform continuous extraction without being connected to the valve system. After the plant materials prepared by grinding are placed in the extraction column, the extraction column outlet valve is closed. CO_2 is sent to the system with the help of the SFE CO_2 pump, and the extraction process is started after the temperature and pressure are adjusted. The connection valves in the system are opened appropriately, the pressure and temperature are adjusted, and the system is brought to balance. After the pressure and temperature reach the desired level in the entire system, dynamic extraction is started in the system. The extraction solvent $SFCO_2$ was sent to the extraction column from above or below to increase efficiency and expand the solvent polarity as the types of solutes increased.

3 different separators were used in the system, whose pressures and temperatures could be controlled, and which could be operated at different pressures and pre-fractionation could be performed. The separators were used at various pressures and temperatures to perform the pre-separation processes of the species obtained during the extraction process. High molecular weight compounds (waxes, oleoresins) were collected in the first separator, medium molecular weight compounds (phenolic compounds, alkaloids, fixed oils) in the second separator, and smaller molecular weight compounds (volatile compounds) in the third separator. In this way, species with different properties were separated from each other and collected in different containers. For SFE-DAD system, with the start of the analysis process, the SF-extract mixture reached the detector within 30 seconds. The peak observed in the first minutes is due to the intense extract obtained during the equilibration of the pressure and temperature of the extractor column.

For the SFE-FIA-DAD system, with the initiation of the analysis process, the valve positions were changed at certain intervals, and the extract was collected in the loop and sent to the DAD system. During the 20-minute analysis process, the product was collected in the loop 9 times and transferred to the detector system.

Conclusions

With the study carried out, it was understood that the supercritical fluid extraction process could be better understood, and which compounds could be extracted at different times during the extraction process. It is thought that obtaining purer and more efficient products could increase industrial production.

Acknowledgements

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