

Supercritical Fluid Extraction of α -Methylene- γ -Butyrolactone from Alstroemeria: Optimization by Statistical Experimental Design

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ABSTRACT

As a process alternative to traditional organic solvent extraction, Supercritical Fluid Extraction (SCFE) has been demonstrated as a viable process for isolating a variety of natural products from plant materials. This work presents experimental and modeling results used to optimize the SCFE of α -methylene- γ -butyrolactone (MBL) from natural alstroemeria plants using ethanol-modified CO₂ as the extraction solvent.

The influence of temperature (T), pressure (P), modifier volume ([EtOH]), and solvent-to-feed ratio (S/F) on extraction efficiency in supercritical CO₂ is presented. This discussion is based on empirical model response surfaces determined from a series of statistically designed experiments performed at analytical scale. The resulting model was shown to accurately predict extraction performance as a function of these experimental factors. The optimum extraction conditions were determined from the response surfaces to be: T = 66 °C, P = 320 bar, [EtOH] = 20 vol%, and S/F = 140 mL /g alstroemeria (dry weight).

INTRODUCTION

The chemical industry continues to evaluate sustainable materials as alternatives to petrochemicals for chemical and pharmaceutical feedstocks. Extraction of desirable products from green plant matter and oil seeds is one approach under investigation. Physical pressing and conventional organic solvent extraction are commonly used for such product recovery processes, but safety, health, and environmental issues with the use of such solvents has prompted considerable interest in alternative separation processes such as SCFE with relatively benign solvents like carbon dioxide. Indeed, SCFE has been demonstrated as a viable process for selectively isolating a variety of natural products from plant materials [1-3].

One such natural product of interest is α -methylene- γ -butyrolactone (MBL) which is produced naturally in a number of plants including tulips and alstroemeria (Inca or Peruvian lily). In fact, the natural occurrence of this product in tulips gives rise to its common name, tulipalin. MBL is produced in alstroemeria as one of several glucoside analogs at concentrations typically on the order of 10-15 wt % on a dry weight basis. This product has fungicidal, insecticidal, and antimicrobial properties and is also a monomer that can be polymerized to produce amorphous acrylic homopolymers or copolymers having high thermal properties and low shrinkage characteristics. These polymers can also be blended with typical engineering polymers to impart such properties to these materials.

The purpose of this work was to scout the feasibility of using SCFE with CO₂ or ethanol-modified CO₂ as one separation process alternative for isolation of MBL from natural alstroemeria plants. Extraction data were obtained using a commercially available analytical instrument, and a statistical experimental design was incorporated to efficiently optimize the

extraction conditions and to develop simple empirical models for subsequent use in process economic evaluations.

EXPERIMENTAL

Equipment

Figure 1 shows a schematic diagram of the commercially available SCFE instrument (ISCO Model SFX 3560) used in the extraction experiments. The analytical-scale instrument is equipped with two ISCO syringe pumps used for metering solvent and cosolvent, a pressure cell accommodating an extraction sample cartridge, and a 24-position automated sampling system. About 4.5 g of mechanically reduced alstroemeria plant mass is loaded into a 10-mL extraction cartridge and packed between glass wool plugs. The cartridge is loaded into the extractor's pressure cell and preheated to operating temperature, then solvent and cosolvent are supplied to the extractor by the two syringe pumps. The solvents are metered as a liquid at 8°C and a flow rate of 1 mL/min. The relative solvent concentration is controlled by the instrument, and the solvents are mixed in a standard tee tube fitting immediately downstream of the pumps. The solvent mixture is first preheated to extraction temperature and then passes through the extraction cartridge at the specified operating pressure. The solvent/extract mixture leaves the extraction chamber through the analyte valve, passes through a filter, and then flashes across a variable restrictor which controls the flow rate through the extraction chamber. The pressure is reduced across the restrictor, and the extract is collected in glass vials for off-line HPLC analysis.

The extract samples were analyzed on an Agilent model 1100 HPLC equipped with a UV detector and two columns for separate analyses. The first analysis used a 10 μ L sample injection on a Waters (Shodex) SH1011 column at 50 °C, and a 0.01 N H₂SO₄ solution at a constant flow rate of 1.0 mL/min was used as the mobile phase. The second analysis used a 5 μ L sample injection on a Zorbax SB-C18 column at 25 °C, and purified water at a constant flow rate of 0.25 mL/min was used as the mobile phase. The duplicate analysis was used to quantify different compounds eluting in the chromatographic spectrum. Quantification was based on a multipoint calibration with standards.

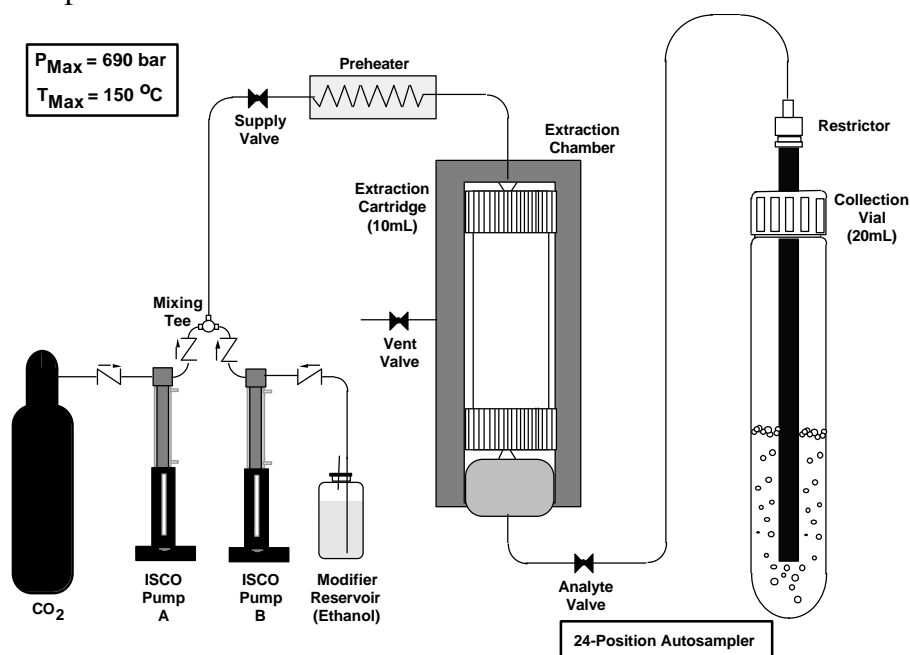


Figure 1. Schematic diagram of ISCO Model SFX 3560 supercritical fluid extractor.

Materials

The solvents used in this study included supercritical-grade carbon dioxide (MG Industries) and reagent-grade ethanol (Aldrich). Standard nursery-stock alstroemeria was used for the plant substrate, and the plants were grown in a green house. The plant mass was harvested by cutting the main stalk and combining the leaves, stems, and stalks, thus simulating simple commercial harvesting by clear cutting. Freshly harvested plant mass was combined and hammer milled at ambient temperature and a rotational speed of 20,000 rpm on a Fritsch Pulverisette mill equipped with a 0.08 mm screen. A preliminary study on various grinding techniques showed that this method gave reproducible results and high yields. Hammer milling can also be implemented at commercial scale for such applications. The milled material was frozen until immediately before use in extraction experiments.

RESULTS AND DISCUSSION

A statistical design of experiments (DOE) was developed to efficiently optimize the extraction conditions using a limited number of experiments and to develop an empirical model of the extraction yield as a function of selected key operating parameters. This empirical model could then be incorporated into process economic evaluations for cost estimation. A face-centered central composite design was selected as a representative response surface DOE to determine the effect of multiple experimental factors on the extraction yield, including factor interaction effects [4]. The response was defined as the extracted yield of MBL normalized by the sample weight of alstroemeria (dry-weight basis).

Figure 2 shows an example of a face-centered central composite design in three experimental factors. The axes X_i represent the independent variables, and each circle represents an experimental point having the indicated combination of these experimental factors. The total number of experiments n required for this design is given by

$$n = 2^k + 2k + m \quad (1)$$

where k is the number of experimental factors and m is the number of replicates at the central point (middle point value for all factors). Figure 2 illustrates that this design consists of experiments at the boundary points (2^k), face-centered points ($2k$) and the middle point (m).

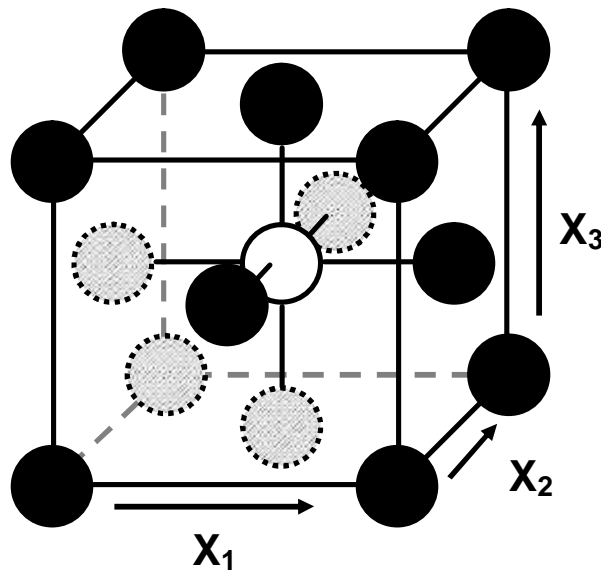


Figure 2. Schematic diagram illustrating face-centered central composite design in three experimental factors.

The key operating parameters and suitable boundary values bracketing the useful range of these experimental factors were determined from practical considerations and preliminary screening experiments. The resulting ranges for these experimental factors incorporated into the DOE are summarized in Table 1.

Table 1: Experimental Factors and Ranges Utilized in Statistical DOE

Experimental Factor	Lower Limit	Mid-Point	Upper Limit
Temperature (°C)	40	60	80
Pressure (bar)	175	345	515
Ethanol Conc. (vol%)	0	10	20
S/F Ratio (mL/g DW)*	10	85	160

* Solvent-to-Feed Ratio (S/F) defined as the total liquid solvent (metered at a flow rate of 1.0 mL/min as measured at 8 °C and the specified pressure) normalized by the sample weight of alstroemeria on a dry weight basis.

The statistical DOE enables the construction of a simple, but balanced, multiple regression model describing the extraction yield over the ranges of the investigated parameters. A fourth-order model is possible with four factors, but in practice, the contribution of the third- and fourth-order terms are typically not statistically significant and can be deleted from the regression. Once the data have been fit to an empirical model incorporating only the statistically significant terms, the optimum operating conditions can then be found through analysis of the response surfaces of the desired objective function in terms of the independent factors. The statistical DOE and multiple regression fitting were conducted using a standard statistics software package [5].

Table 2 summarizes the experimental design and corresponding extraction yield at each experimental point. Seven replicates at the center point of the face-centered central composite design were included for the estimation of standard statistical metrics.

The data from Table 2 were fit to a fourth-order multiple regression model in each experimental factor. By principle component analysis for the resulting model, various statistically insignificant terms were eliminated, and the resulting model was refit to the experimental data. The final regressed model fitted the experimental data with a coefficient of multiple determination of $R^2 = 0.907$, indicating that 90.7 % of the variation in the extraction yield of MBL can be explained by the variation in the indicated experimental factors within the given ranges. Equation (2) presents the resulting empirical model, and Figure 3 illustrates the goodness-of-fit of the model to the experimental data.

$$\begin{aligned}
 \text{MBL Yield} = & -2.119 + 0.4350*T + 0.03665*P - 0.2576*[EtOH] + 0.03478*(S/F) \\
 & -0.005387*T^2 + 0.0003342*T*P + 0.005241*T*[EtOH] \\
 & + 0.001406*T*(S/F) - 0.000069*P^2 + 0.0000968*P*(S/F) \\
 & + 0.0006425*[EtOH]*(S/F) - 0.000383*(S/F)^2 - 0.000003*T*P*(S/F) \quad (2)
 \end{aligned}$$

The empirical model was used to generate response surfaces of the extraction yield versus the experimental factors to show the influence of the various process conditions. Figure 4 shows an example of one of the resulting response surface plots illustrating the extraction yield as a function of temperature and pressure. From analysis of a variety of such

plots, an optimum set of extraction conditions was determined to be $T = 66\text{ }^{\circ}\text{C}$, $P = 320\text{ bar}$, $[\text{EtOH}] = 20\text{ vol}\%$, and $S/F = 140\text{ mL/g DW}$ alstroemeria. The corresponding calculated extraction yield was 24.2 mg MBL/g DW . A series of five replicate runs at these extraction conditions was conducted to validate the empirical model. The corresponding experimental yield was $24.6 \pm 1.4\text{ mg MBL/g DW}$, which was in good agreement with the calculated value.

Table 2: Face-Centered Central Composite Design and MBL Extraction Yield

Trial Number	Pattern	T ($^{\circ}\text{C}$)	P (bar)	[EtOH] (vol %)	S/F Ratio (mL/g DW)	MBL Yield (mg/ g DW)
1	----	40	175	0	10	13.9
2	---+	40	175	0	160	18.4
3	--+-	40	175	20	10	13.8
4	---+	40	175	20	160	17.9
5	-+--	40	515	0	10	14.1
6	-+++	40	515	0	160	18.4
7	-++-	40	515	20	10	14.6
8	-+++	40	515	20	160	17.0
9	+---	80	175	0	10	8.0
10	---+	80	175	0	160	17.5
11	+--+	80	175	20	10	12.3
12	++--	80	175	20	160	22.3
13	+++--	80	515	0	10	15.7
14	++++	80	515	0	160	13.8
15	+++--	80	515	20	10	13.9
16	++++	80	515	20	160	21.6
17	-000	40	345	10	85	20.2
18	+000	80	345	10	85	20.2
19	0-00	60	175	10	85	19.1
20	0+00	60	515	10	85	21.6
21	00-0	60	345	0	85	20.1
22	00+0	60	345	20	85	26.6
23	000-	60	345	10	10	17.3
24	000+	60	345	10	160	23.1
25-31	0000	60	345	10	85	21.1

CONCLUSIONS

A statistical DOE using a commercially available analytical extraction instrument provides an efficient means of measuring extraction data and optimizing the extraction yield as a function of experimental conditions. This technique was used to develop an empirical extraction model for the separation of MBL from natural alstroemeria plants. Good agreement between the empirical model and experimental results at predicted optimized conditions validated the model and verified that the statistical design and modeling gives a relatively accurate method for quantitatively determining the yield over a wide range of process conditions.

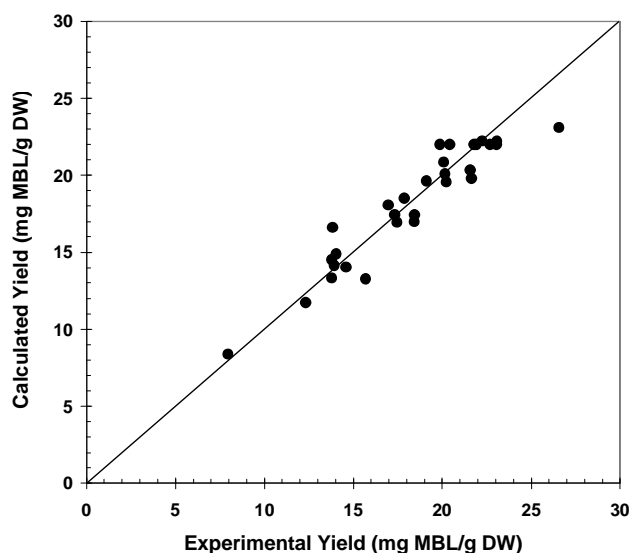


Figure 3. Parity plot illustrating goodness-of-fit of the empirical model.

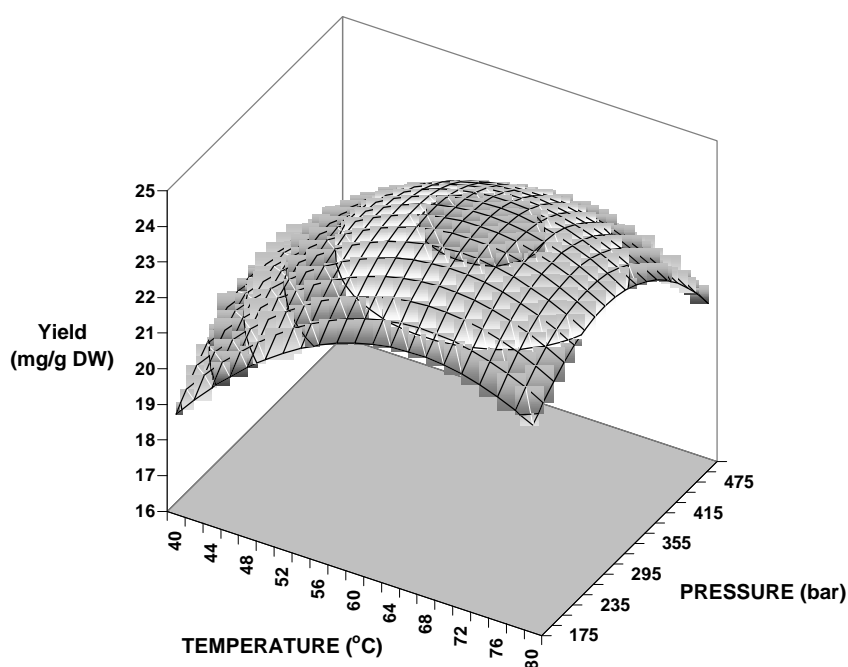


Figure 4. Response surface plot showing extraction yield as a function of temperature and pressure with fixed [EtOH] = 20 vol% and S/F = 140 mL/g DW.

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